Development and In-Vitro Evaluation of Curcumin Proniosomes for Enhanced Stability and Antimicrobial Activity

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ABSTRACT

Curcumin is a natural polyphenolic compound derived from the rhizome of the turmeric plant. It has been reported to possess various biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. However, its low bioavailability limits its clinical applications. Proniosomes are an emerging drug delivery system that can improve the bioavailability of poorly soluble drugs, such as curcumin. In this study, proniosomal formulations of curcumin were prepared using different surfactants, including Tween 20, Tween 40, Tween 60, Span 20, Span 40, and Span 60. The vesicle size, drug entrapment efficiency, and stability of the formulations were evaluated. The results showed that Span 60-based proniosomes had the highest entrapment efficiency (78.77%) and stability at room temperature and refrigeration. This could be due to the high HLB value of Span 60, which is more suitable for forming niosomes than the lower HLB value Tween surfactants. The in vitro antimicrobial activity of curcumin proniosomes was evaluated against four strains of bacteria, including two Gram-positive (Bacillus subtilis and Staphylococcus aureus) and two Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria. The highest zone of inhibition was observed against Bacillus subtilis (19.3 mm) and Staphylococcus aureus (17.6 mm) when combined with PEG. This could be due to the synergistic effect of PEG and curcumin on the bacterial cell wall. In addition, the free radical scavenging activity of curcumin was evaluated using the DPPH assay. The results showed that curcumin was more potent than ascorbic acid at all concentrations tested, indicating its potential as a natural antioxidant. Overall, the findings suggest that Span 60-based curcumin proniosomes could be a promising option for enhancing the stability and antimicrobial activity of curcumin.

Section: Research Paper
Keywords: Proniosomes, Curcumin, Niosomes, DPPH assay, Antimicrobial actvity

1. Introduction

Curcumin, a polyphenolic compound known as diferuloylmethane, is the primary bioactive constituent found in the perennial herb Curcuma longa, commonly referred to as turmeric. For centuries, turmeric has been utilized in cuisine, cosmetics, and medicine, particularly in Ayurvedic and Asian medicinal practices (Anonymous, 2010; Hatcher et al., 2008). In Ayurveda, the use of turmeric to alleviate rheumatism, respiratory and hepatic disorders, and diabetic wounds has been well documented. Commercially available turmeric contains approximately 2-5% curcumin, with 77% being diferuloylmethane, 17% demethoxycurcumin, and 6% bisdemethoxycurcumin (Anand et al., 2007). Turmeric's yellow colour is a result of polyphenols called curcuminoids. You can apply or consume curcumin topically (Anonymous, 2010). Curcumin has a remarkable lack of toxicity and a low bioavailability. Curcumin shows significant promise as a medication (Hatcher et al., 2008). Curcumin has been shown to be biologically effective and safe (safe even at large dosages up to 12 g/day in people), but it hasn't yet been given the go-ahead to be used as a medicinal agent. During clinical trials in phase I, it has been observed that curcumin exhibits low bioavailability due to factors such as poor absorption, fast metabolism, and rapid systemic clearance. These factors have been identified as the primary causes of the low levels of curcumin observed in plasma and tissues.

Nonionic surfactants can be easily converted into proniosomes (gel) by dissolving them in the least amount of a suitable solvent (ethanol), followed by the least amount of water (water). Proniosomal gel has the potential to significantly improve therapeutic efficacy and decrease medication side effects (Sambhakar et al 2017). Drugs, both hydrophilic and hydrophobic, can be captured by pioniosomes (Saroha et al.) (Mokale et al 2016). Niosomes are approached pro-vesicularly by protoniosomes (Yadav et al, 2010). Niosomes are unilamellar or multilamellar spheroids made up of bilayers of amphiphilic molecules. They are regarded as rudimentary cell models, cell-like bioreactors, and bioencapsulation matrices. They are an alternative to liposomes since they are more stable and get rid of the issues with liposomes such chemical instability, inconsistent phospholipid purity, and high cost (Barry, 2001). We can argue that niosomes are liposomes based on non-ionic surfactants. Niosomes were first noted as a component of the cosmetic business in the 1970s, but they have subsequently been investigated as potential medication targeting agents. Niosomes can encapsulate significant amounts of active medicine in about smaller quantities of vesicles, are biocompatible, biodegradable, and cost-effective (Malhotra, 1994). Niosomes increase the epidermal penetration of medicines and the oral bioavailability of poorly soluble medications. You can direct them to the location of action orally, topically, or through your parents. Niosomes do, however, show good chemical stability when stored, however there may be issues with physical instability in niosomal dispersions. The shelf life of the dispersion is shortened by the possibility of niosome aggregation, fusion, drug leakage, or hydrolysis in aqueous solutions. The proniosome system is an improvement over niosomes and can be used in a variety of ways to transport active ingredients to the appropriate location. If applied topically, aqueous phase or the skin itself can easily hydrate proniosomes. They combine with additional substances to create a

cosmetic preparation when added to a base gel, cream, or ointment. These cosmetic compositions can be applied topically or subcutaneously for a variety of purposes (Yadav et al., 2010). Various studies suggest that vesicular formulations can enhance the cutaneous bioavailability of drugs compared to conventional dosage forms (Vora et al., 1998). Treatment with liposomes and niosomes can lead to a more porous intercellular lipid barrier in the stratum corneum, as reported by Alsarra et al. (2005) and Namdeo and Jain (1999). Both phospholipids and nonionic surfactants in niosomes can act as penetration enhancers (Ljeoma et al 1998), facilitating the permeation of different drugs. Direct transfer of the drug from the vesicles to the skin results in a greater drug flux due to the fusion of niosome vesicles to the skin surface (G. Bhavani Durga et al 2020).

2. Materials and methods

2.1 Materials

S.D. Fine-chem Ltd in India provided curcumin (95% purity). Moreover, S.D. Fine-chem. Ltd provided Span 20, Span 40, Span 60, Span 80, Tween 20, Tween 60, Tween 80, PEG-400, PEG-4000, and Absolute alcohol (99.9%). We bought soy lecthin powder from Rajasthan's Titan Biotech Ltd. (India). From Maharashtra's Loba Chemical Pvt. Ltd., cholesterol (AR) was received (India). We bought agar powder, a dialysis membrane, and 2, 2-diphenyl-1-picryl hydrazyl (DPPH) from Hi-Media Laboratories Pvt. Ltd. in Maharashtra. Borax, bees wax, stearic acid, liquid paraffin, and potassium hydroxide were purchased from Loba Chemie Pvt. Ltd. in Maharashtra (all of analytical grade). The microorganisms (Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa) used were obtained from MTCC in Chandigarh (India). Candida albicans, isolated from patients with ear infections in Kurukshetra. The other substances were analytical reagent grade and were applied exactly as they were given.

2.2 Preparation of Proniosomes

The coacervation phase separation technique was employed to prepare prososomes. The exact measured amounts of surfactant, lipid, and drug, along with 0.5 ml of alcohol, were added to a dry and clean 5 ml wide mouth glass vial. The components were thoroughly mixed using a glass rod after heating. The open end of the glass bottle was sealed with a lid to prevent solvent loss, and the mixture was heated over a water bath at 60–70°C for approximately 5 minutes, or until the surfactant combination was completely dissolved. Next, the aqueous phase (0.1% glycerol solution) was added and heated on a water bath until a clear solution was obtained. Proniosomes were formed when the clear solution cooled down (Gupta et al., 2007; Vora et al., 1998).

Several non-ionic surfactants, including sorbitan esters such as Span 20, Span 40, Span 60, and Span 80, and polyoxyethylene sorbitan esters such as Tween 20, Tween 60, and Tween 80, were utilised to optimise curcumin proniosomal formulations. Lecithin and cholesterol concentrations were also varied (Table 1). Four different water soluble bases, namely cold cream base, vanishing cream base,

PEG base, and aqueous gel base, were also adjusted for this in order to provide uniform application of formulation pronioosmes.

2.3 Hydration step and formation of niosomes

The gels made in the manner previously described were then hydrated to create niosomes. Each vial containing 500 mg of gel had about 5 ml of distilled water added to it before the formulations were vortexed for two minutes.

Characterization of formulation

2.4.1 Morphological Study

a) Light Microscopy

The niosomes produced were examined for vesicle structure and the presence of insoluble drug crystals by spreading small amounts on a glass slide and observing under a standard light microscope at different magnifications (10 and 40). Photomicrographs of the niosomes were captured using a Fujifilm Finepix F40fd 8.3MP digital camera with 3 optical zoom.

b) Scanning Electron Microscopy

A small quantity of the formulation was utilized to prepare the sample, which was subsequently coated with a thin layer of gold and mounted in a Hitachi scanning electron microscope for imaging analysis. The sample was then examined.

2.4.2 Size of Vesicles

The optical microscope was used to measure the vesicle diameter and the range of particle sizes. A 1000x magnification ocular and stage micrometre was used to measure the mean diameters of drug-loaded niosomes. The mean particle size and standard deviation were calculated (Madan et al 2016).

2.4.3 Entrapment efficiency

After hydrating Proniosomes (25 mg) in distilled water heated to 80°C and vortexing for 2 minutes, the drug entrapment percentage was determined. The resulting niosome dispersion was then centrifuged for 40 minutes at 5°C at 18000 rpm (Remi CPR-24 centrifuge) to measure the free drug using the clear fraction spectrophotometrically at 430 nm. The percentage encapsulation efficiency was determined using the formula.

$$PDE = [1 – (\text{un-encapsulated drug/total drug})] \times 100$$

***2.4.4 In-vitro* drug release study**

In vitro, Curcumin was released from niosomes using a dialysis membrane (Hi-Media). A flask (beaker) holding 50 ml of 50%v/v ethanol was filled with a dialysis membrane containing the required 1gm of Curcumin proniosomes. At 37 °C, the flask was heated to 100 rpm and swirled using a magnetic stirrer. At predefined intervals, aliquots of the dialysate were drawn, and the equivalent volume of release medium was added right away. At 430 nm, withdrawn samples were spectrophotometrically analysed.

2.4.5. Statistical treatment of in-vitro drug release data

The order and release mechanism for niosomes formed from proniosomes incorporated into the bases were explored using seven models in the current study (Costa and Sousa Lobo, 2001; Dash et al., 2010; Paulo et al., 2001).

2.4.6. Anti-Microbial activity

To evaluate the antibacterial activity of curcumin proniosomes, the agar well diffusion method was utilized. The bacterial strains were adjusted to the 0.5 McFarland standard, which appears like a suspension of 1.5 108 cfu/ml. The agar medium (20 ml) was placed on each petri plate and inoculated with 100 l of each test microbial strain, and left for 15 minutes to allow adsorption. Subsequently, 8mm-diameter holes were punched into the seeded agar plates, and 100 l volumes of 500 mg/ml proniosomes, proniosomes with bases (PEG), and 10 mg/ml curcumin reconstituted in dimethylsulphoxide (DMSO) were added. The plates were incubated at 37°C for 24 hours, and antimicrobial activity was assessed by measuring the diameter of the inhibition zone using a zone reader (Hi Antibiotic zone scale). The positive controls included ciprofloxacin (for bacteria) and amphotericin-B (for fungus), while DMSO served as the negative control. The experiment was conducted in triplicate, and the mean diameter of the inhibitory zone was calculated.

2.4.7. Anti-Oxidant Study

The antioxidant potential of curcumin was evaluated by assessing its ability to scavenge free radicals using the DPPH (2,2-diphenyl-1-pycrylhydrazyl) assay. In this assay, 100 µl of DPPH solution (400 µM in methanol) was mixed with 100 µl of curcumin solution (100, 300, and 500 µg/mL) in methanol. The reaction was allowed to proceed for 30 minutes at room temperature, and the absorbance of the solution was measured at 517 nm. The free radical scavenging activity at each concentration was determined by comparing the absorbance of each concentration to that of the control, which contained pure methanol instead of the drug solution.

The percentage inhibition was determined using:

$$\% \text{ inhibition} = \left(\left[\frac{A_{517}^{Control} - A_{517}^{Test}}{A_{517}^{Control}}\right]\right) \times 100$$

Curcumin's antioxidant activity was compared to that of ascorbic acid because ascorbic acid also has well-known antioxidant properties. Ascorbic acid samples were created in a similar manner and at the same concentration as curcumin samples.

2.4.8. Stability Studies

At room temperature (30–35°C) and refrigerated temperatures (2–6°C), respectively, the optimised proniosomal formulation and the proniosomal formulation integrated in base were kept in glass vials covered with aluminium foil. They were examined visually and under a microscope after 15, 30, and 60 days to check for changes in consistency and the presence of drug crystals. Niosomes were classified according to their size, shape, and degree of entrapment.

2.4.9. Statistical analysis

Prior to analysis, data were gathered and coded. Every piece of data was reported as mean with SD.

RESULTS AND DISCUSSION

3.1 Morphological Study

Light microscopy and scanning electron microscopy are two techniques used to analyse the morphology of vesicles. Through both, it is revealed that vesicles are nearly uniform and somewhat spherical in shape. (Fig.) .

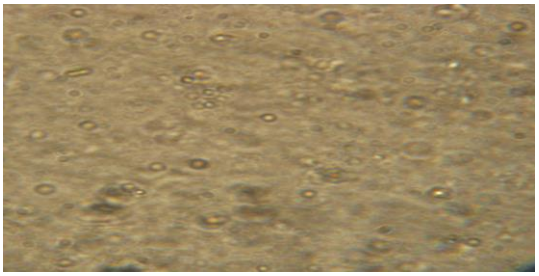


Figure 1: image light microscope

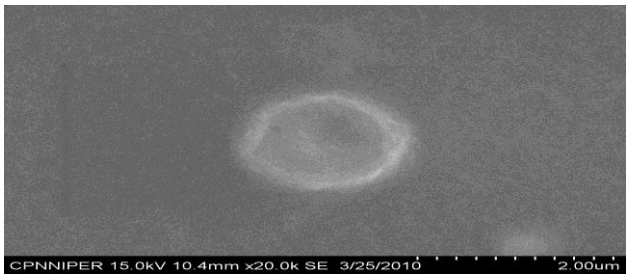


Figure 2 SEM image

4.2. Vesicle Size analysis

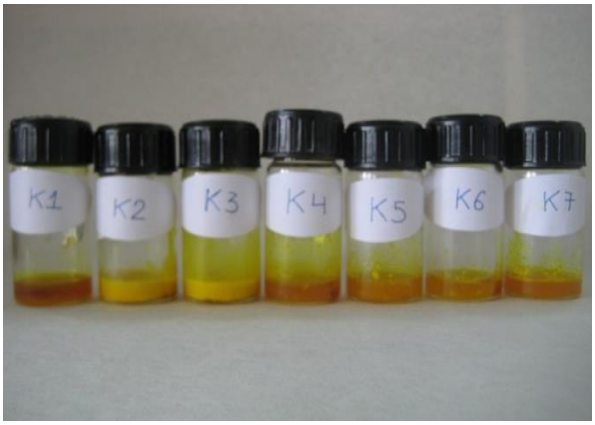
The range of vesicle sizes observed in this study ranged between 2 and 4 μm, which is within the desirable size range for drug delivery systems. This size range allows for enhanced cellular uptake and accumulation at the target site.

4.3.1. Effect of Surfactant type on entrapment efficiency and Vesicle size

The HLB (hydrophilic-lipophilic balance) values of tweens, which ranged between 14 and 17, were expected to generate niosomal vesicles due to their high hydrophilicity. However, contrary to this expectation, no niosomal vesicles were formed when they were used as surfactants. On the other hand, spans were able to generate niosomal vesicles, which is consistent with previous studies demonstrating that lower HLB values (around 8-9) are optimal for niosome formation.

Batch No.	Surfactant	Physical Appearance	Vesicle Size (nm)	Entrapment efficiency (%)
K1	Span 20	Liquid	3.5 ± 2.15	63.07 ± 1.05
K2	Span 40	Paste	3.0 ± 1.06	67.77 ± 0.80
K3	Span 60	Paste	1.75 ± 1.01	78.77 ± 0.89
K4	Span 80	Gel	1.52 ± 0.25	35.04 ± 1.00

K5	Tween 20	Gel	Not formed	0.007 ± 0.00
K6	Tween 60	Gel	Not formed	0.000 ± 0.00
K7	Tween 80	Gel	Not formed	0.004 ± 0.00

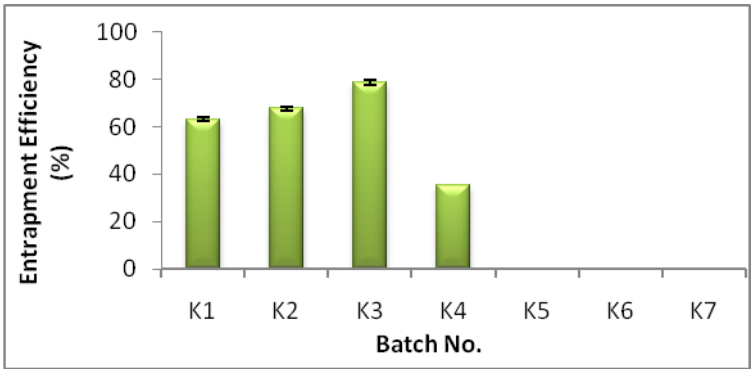


Span 20 (HLB 8.6) > Span 40 (HLB 6.7) > Span 60 (HLB 4.7) > Span 80 (HLB 4.3)

Vesicles with larger sizes were observed in formulations with higher HLB values, which could be attributed to a decrease in surface free energy. This facilitated the formation of vesicles with larger sizes and less exposed surface area to the surrounding medium. The percentage of entrapment efficiency data is illustrated in Figure 5.12. The graph indicates that batch K3 (containing span 60) exhibited the highest entrapment efficiency. The chemical composition of the surfactant could be a contributing factor. All span types share the same head group but have different alkyl chains. Previous studies by various researchers have shown that increasing the length of the alkyl chain results in better entrapment efficiency.

The efficiency of trapping followed the pattern:

Span 60 (C_{18}) > Span 40 (C_{16}) > Span 20 (C_{12}) > Span 80 (C_{18})



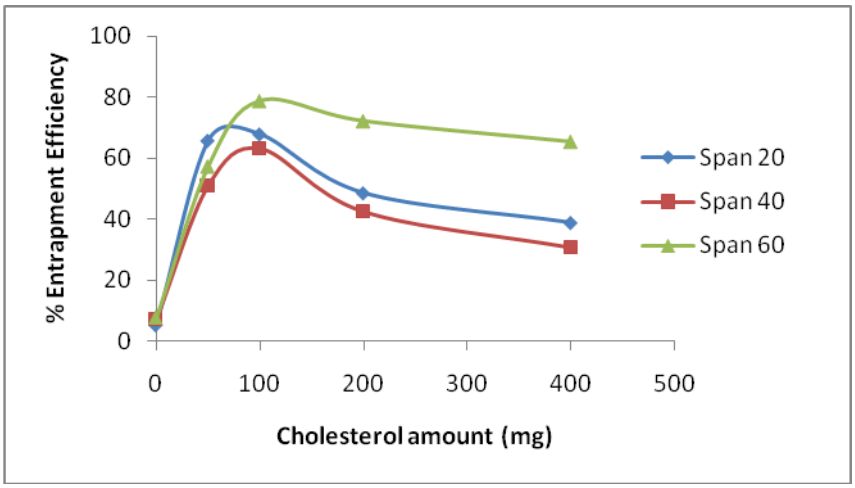
Uchegbu observed a similar trend, where the entrapment efficiency of Span 80 was lower than that of Span 60, despite having the same alkyl chain length (C18) but with an unsaturated alkyl chain. This could be attributed to the presence of double bonds in Span 80. Therefore, based on the findings mentioned above, Span 20, 40, and 60 were

found to produce proniosomes (Batches K1, K2, and K3) with high entrapment efficiency and were selected for further experimentation.

4.3.2. Effect of cholesterol Concentration on entrapment efficiency and Vesicle size

As the amount of cholesterol increased from 0 to 100 mg, the entrapment efficiency (%) of the proniosomes increased from 5 to 63% (for Span 20), 7 to 67% (for Span 40), and 7.61 to 79% (for Span 60). However, as the concentration of cholesterol continued to increase, the entrapment efficiency of all three surfactants decreased (as illustrated in Fig-). This trend can be attributed to the fact that cholesterol acts as a stabilizer for proniosomal vesicles, improving their entrapment efficiency at low concentrations. However, at high concentrations, cholesterol can destabilize the vesicles, leading to a decrease in entrapment efficiency.

Batch No.	**Surfactant**	**Cholesterol conc. (mg)**	**Physical Appearance**	**Entrapment efficiency (%)**
K8	Span 20	00	Liquid	5.295 ± 0.53
K9	Span 20	50	Liquid	59.08 ± 1.58
K10	Span 20	100	Liquid	63.07 ± 1.05
K11	Span 20	200	Liquid	48.48 ± 0.63
K12	Span 20	400	Gel	38.77 ± 0.76
K13	Span 40	00	Gel	7.15 ± 1.26
K14	Span 40	50	Paste	50.95 ± 0.79
K15	Span 40	100	Paste	67.77 ± 0.79
K16	Span 40	200	Paste	42.39 ± 0.99
K17	Span 40	400	Paste	30.63 ± 1.49
K18	Span 60	00	Paste	07.61 ± 1.59
K19	Span 60	50	Paste	57. 28 ± 0.39
K20	Span 60	100	Paste	78.77 ± 0.89
K21	Span 60	200	Paste	52.17 ± 0.90
K22	Span 60	400	Paste	45.39 ± 0.80



The presence of cholesterol has been shown to affect the stability and permeability of vesicles. Table 5.6 shows that the entrapment efficiency (%) of niosomes was dependent on the concentration of cholesterol. Previous studies have reported that an increase in cholesterol concentration leads to an increase in vesicle entrapment efficiency (%), followed by a decrease in drug entrapment efficiency as the cholesterol concentration continues to increase.

As cholesterol content was further raised, medication entrapment efficiency decreased primarily for two reasons that might be at odds with one another. As the level of cholesterol rises:

- As the amphiphiles come together to form vesicles, increased cholesterol levels can lead to competition for packing space within the bilayer, which can ultimately result in the drug being excluded from the vesicles.
- As the vesicle bilayer forms, there is an increase in hydrophobicity and stability, which enhances the ability to efficiently trap hydrophobic drugs within the bilayer.

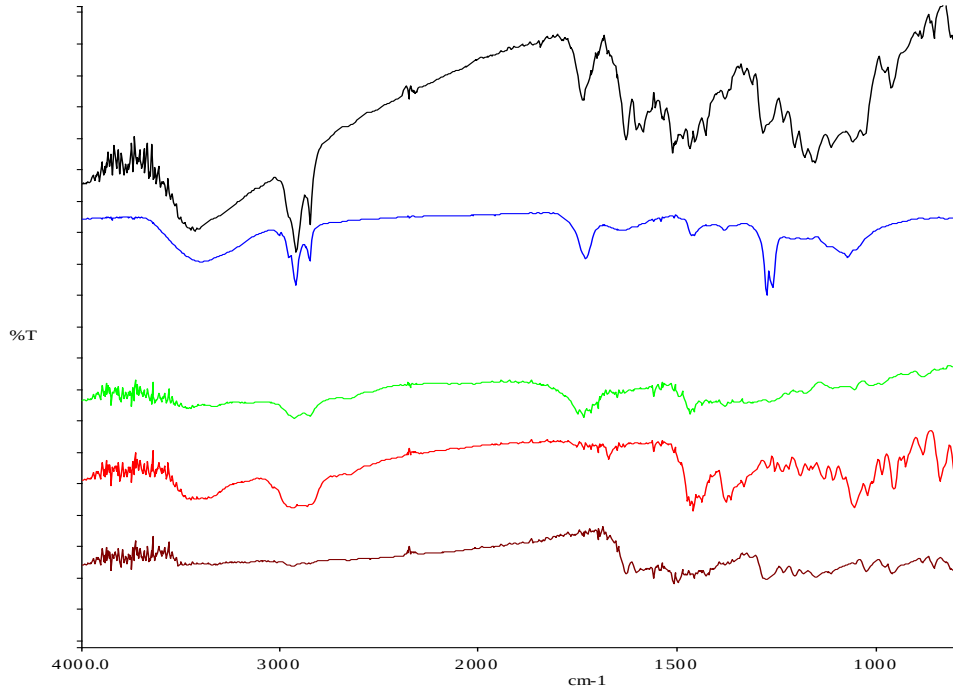
4.4. Interaction study

Fourier transform infrared (FT-IR) spectroscopy and differential scanning calorimetry were used to determine the compatibility of Curcumin with commonly used proniosome excipients like cholesterol, lecithin, and surfactants like span 60. (DSC)

4.5. FTIR

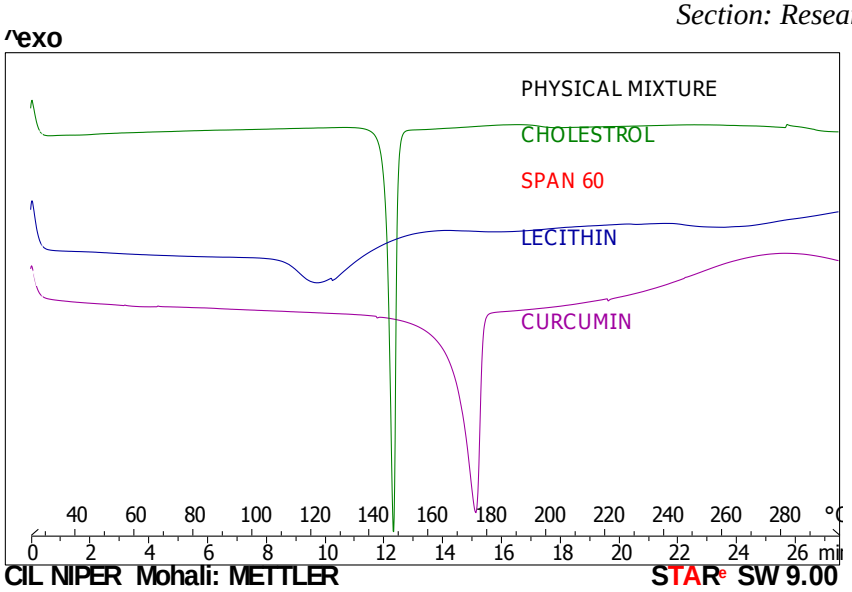
Fourier-transform infrared (FTIR) spectroscopy is a commonly used analytical technique in the pharmaceutical industry for the evaluation of drug-excipient interactions. The FTIR spectrum provides information on the chemical structure and functional groups of the sample.

In the case of the physical mixture of all formulation ingredients for proniosomes, the FTIR spectrum showed no additional peaks, indicating that there was no new chemical bond formation or incompatibility between the components. This is an important finding because any incompatibility between the drug and excipients could lead to instability of the formulation, reduced drug efficacy, and potential safety concerns. (Fig. 5.7).



4.5.1. DSC

The excipients and curcumin underwent DSC analysis with a heating rate of 10°C/min ranging from 25 to 300°C, resulting in individual melting endotherms as indicated by the DSC scans. Curcumin displayed an endotherm at 176°C, representing its melting point. On the other hand, lecithin was found to begin degradation after 120°C according to its DSC thermogram, while cholesterol showed a melting endotherm at 148°C and Span 60 displayed a melting endotherm at 59°C. The physical mixture of Curcumin and all of the formulation components was subjected to DSC analysis, revealing endothermic peaks for all of the constituents, including Curcumin at 176°C, cholesterol at 148°C, Span 60 at 59°C, and lecithin degradation beginning after 120°C (Fig. 5.8). Furthermore, no additional peak was observed, indicating that the DSC results do not indicate any incompatibility between the components used in the production of proniosomal drugs.



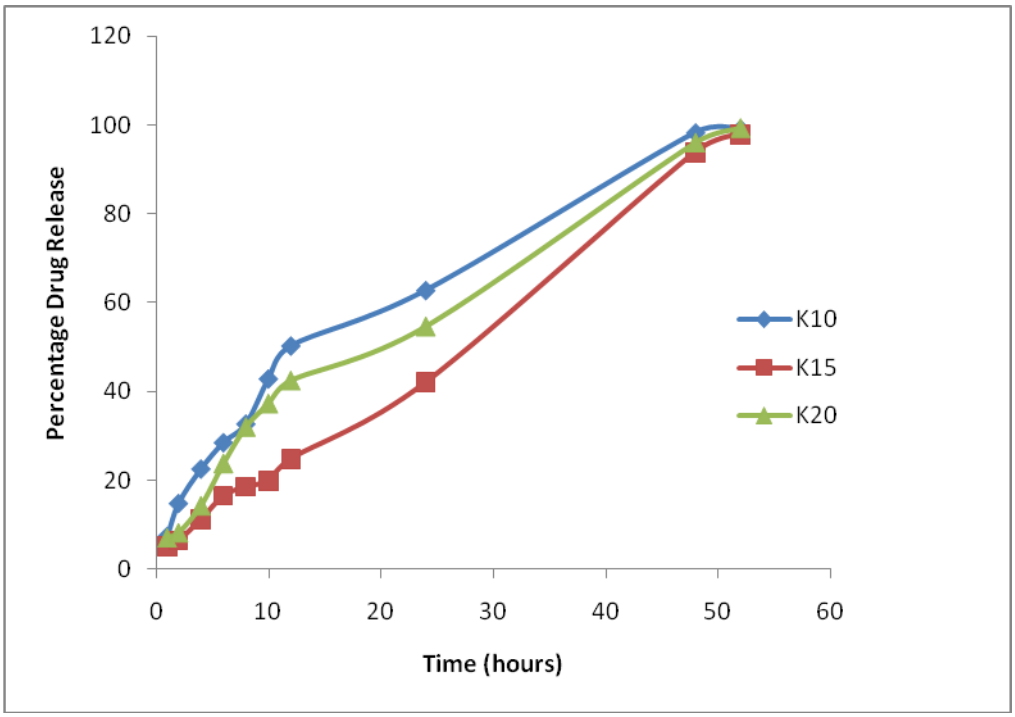
4.6. In-vitro release study

4.6.1. Release from

Based on the results of the aforementioned inquiry, it was determined that the surfactants span 20, span 40, and span 60 were adequate, and that the cholesterol concentration of 100 mg was the best concentration for the formulation of proniosomes. On the basis of in-vitro drug release experiments, the three surfactants were now further evaluated in order to choose the optimal one (Results shown in Table -5.7)

Time(hours)	Percentage Drug Release		
	BatchK10 (span 20)	**Batch K15 (span 40)**	**Batch K20 (span 60)**
1	7.38 ± 1.06	5.19 ± 0.60	7.16 ± 1.82
2	14.80 ± 2.38	6.54 ± 1.42	8.24 ± 0.56
4	22.55 ± 2.06	11.37 ± 1.17	14.36 ± 2.29
6	28.50 ± 1.65	16.64 ± 2.08	23.86 ± 1.52
8	32.68 ± 5.05	18.62 ± 0.45	32.09 ± 1.42
10	42.84 ± 3.26	20.05 ± 0.71	37.37 ± 2.11
12	50.26 ± 2.15	24.95 ± 1.32	42.53 ± 0.70
24	62.75 ± 1.79	42.12 ± 0.30	54.71 ± 0.87
48	98.26 ± 0.26	93.89 ± 1.47	96.10 ±1.98

52	99.09 ± 1.06	97.96 ± 3.56	99.36 ±1.79



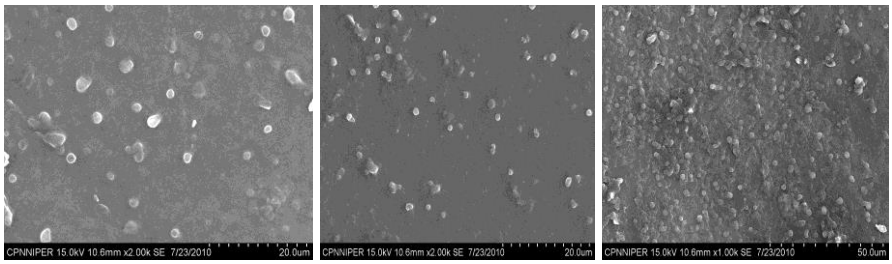
The drug release profiles in vitro for batches K10, K15, and K20 are depicted in Figure-5.14, employing 50 ml of v/v ethanol as the dissolution medium with agitation at 100 rpm. It can be observed that the drug release characteristics of all three batches are quite similar, with nearly 100% drug release after 52 hours. Thus, no surfactant outperforms the others concerning the drug release evaluation in vitro. Consequently, based on the entrapment efficiency, span 60 (with a percentage of 78.77) can be considered as the optimal surfactant for proniosome formulation. (Table-5.6).

4.6.2. Bases

The fundamental characteristics of all the bases were analyzed and evaluated for their suitability for formulation. It was found that all the cold cream bases showed attraction with the formulation and a shift in hue to reddish-brown. The literature suggests that curcumin and boric acid can combine to generate the red chemical rosocyanine, which could be the reason behind the color shift observed in the cold cream bases. However, since one of the components of cold cream base is boric acid, it cannot be used for the formulation of curcumin proniosomes due to the incompatibility between curcumin and boric acid.

Even though disappearing cream and aqueous gel were found to be compatible with curcumin and formed vesicles when hydrated, they were shown to be unstable when stored. Therefore, PEG ointment base was chosen for further research. This highlights the importance of carefully selecting the appropriate base for formulation to ensure the stability and effectiveness of the final product.

Properties	**Cold cream base**	**Vanishing cream base**	**Water misicible gel base**	**Water misicible ointment base**
Composition	borax, bees wax, liquid paraffin, water	Stearic acid, potassium hydroxide, glycerol, water	Water dispersion of Carbopol, glycerol, polyethylene glycol and propylene glycol	Polyethylene Glycols (PEGs)i.e. PEG 400, PEG 4000
Water content	Hydrous (64%)	Hydrous (40%)	Hydrous (99%)	anhydrous
Affinity for water	lipophillic	Hydrophilic	Hydrophilic	Hydrophilic
Spreadability	moderate	easy	easy	Moderate to easy
Washability	Poor washability	washable	washable	washable
Compatibility with active ingreadient	Incompatible	Compatible	Compatible	Compatible
Vesicle Formation (µm ± SD)	Not formed	2.25 ± 0.25	1.50 ± 0.40	2.00 ± 0.5
Stability	Unstable	Unstable	Unstable	Stable



4.7. Anti microbial activity of curcumin and curcumin proniosomes

The bacterial strains used to evaluate the antibacterial activity of Curcumin and Proniosomes on agar plates were diverse. Gram-negative bacteria did not demonstrate any effect, while Gram-positive bacteria only showed a reduction in growth, with the inhibition zone ranging from 13mm to 19mm.

Table 1 displays the zone of inhibition measurements for the antibacterial and antifungal activities of Curcumin and Proniosomes against various microorganisms. B. subtilis exhibited the greatest sensitivity to both Curcumin and Proniosomes, with maximum inhibition zones of 21.6 mm and 19.3 mm, respectively. S. aureus was the second most susceptible bacterium, with inhibition zones of 19.6 mm and 17.6 mm for Curcumin and Proniosomes, respectively. For antifungal activity, C. albicans was the most sensitive fungus, with an inhibition zone of 14.6 mm for Curcumin. It's important to note that Curcumin and Proniosomes did not exhibit any activity against Gram-negative bacteria.

Out of the three bases tested, PEG exhibited the best performance when combined with Proniosomes, exhibiting a zone of inhibition of 16.3 mm against B. subtilis and 14.6 mm against S. aureus.

Table-1. Antimicrobial activity of Proniosomes and curcumin

Extract concentration (mg/ml)	**Diameter of growth of inhibition zone (mm)**				
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida albicans***
Curcumin (10)	19.6	21.6	-	-	14.6
Proniosomes (500)	17.6	19.3	-	-	-
Proniosomes + PEG (500)	14.6	16.3	-	-	-
Ciprofloxacin (20 µg/ml)	27.3	26.3	25.6	25.0	ns
Amphotericin B (20 µg /ml)	ns	ns	ns	ns	16.3
DMSO	-	-	-	-	-

- No activity, ns –not studied

[a] Values, including diameter of the well (8mm), are means of three replicates

Table 2: MIC of Proniosomes and curcumin

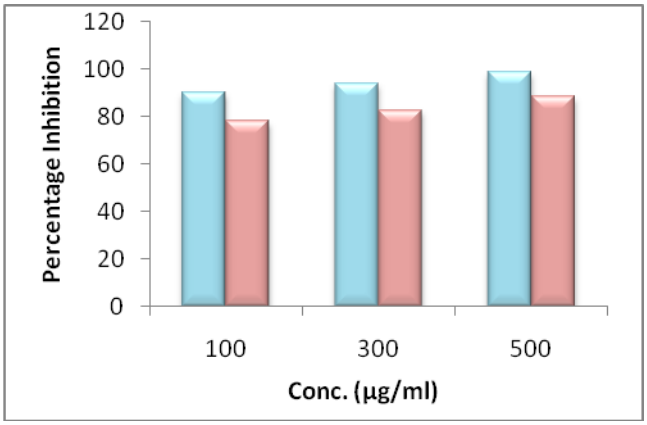
	MIC (mg/ml)		
	Staphylococcus aureus	***Bacillus subtilis***	***Candida albicans***
Curcumin (10mg/ml)	62.5	31.25	256
Proniosomes (500mg/ml)	128	62.5	nt
Proniosomes + PEG (500mg/ml)	256	128	nt
Ciprofloxacin (20 µg/ml)	5	5	nt
Amphotericin B (20 µg /ml)	nt	nt	16.6

The susceptibility of the extracts to Gram-positive bacteria was found to be higher than that of Gram-negative bacteria. These results are consistent with previous research that showed

plant extracts to be more effective against Gram-positive bacteria than Gram-negative bacteria (Jigna and Sumitra, 2006). Among the different Proniosome combinations, PEG and Proniosomes were found to be the most effective in inhibiting the growth of Gram-positive bacteria.

4.8. Anti-Oxidant activity of curcumin

The free radical scavenging potential of natural antioxidants is commonly evaluated using DPPH. In this method, stable DPPH is generated in a methanol solution, and allowed to react with antioxidants. By monitoring the decrease in its absorbance at a specific wavelength during the reaction, changes in DPPH concentration can be tracked. DPPH has an absorbance at 515 nm when it is in its radical form, but when reduced by an antioxidant or another radical species, the absorbance is reduced. The antioxidant's hydrogen donating capability determines the extent of the reaction. The DPPH free radical scavenging activity of curcumin at various concentrations is presented in Figure 5.27. It was found that curcumin exhibited more potent antioxidant activity than ascorbic acid at all tested concentrations.



Stability study

The stability of proniosomes is an essential factor to consider in the formulation of drugs. Studies have shown that proniosomal formulations are typically more stable at low temperatures, as compared to room temperature. In addition, it has been found that proniosomes can maintain their mean appearance, vesicle size, and drug entrapment efficiency percentage for up to 90 days of storage without any significant changes from the freshly prepared formulations.

This stability of proniosomes offers several advantages over conventional niosomes, which tend to suffer from issues such as hydrolysis or oxidation, sedimentation, aggregation, and fusion during storage. Proniosomes have been shown to be a more reliable system for drug delivery, especially for sensitive drugs that require stable environments. Moreover, the stable nature of proniosomes has significant implications in terms of reducing manufacturing costs, as the production of stable formulations reduces the need for frequent production runs.

Conclusion

In conclusion, the findings of this study indicate that proniosomes are a promising option for drug delivery as they have the ability to encapsulate a broad range of drugs. Additionally, they provide a stable drug delivery system that can overcome some of the issues associated with traditional niosomes. The entrapment efficiency of the proniosomes was found to be highest for Span 60, and its combination with PEG as a base was found to be most effective against Gram positive bacteria. Furthermore, curcumin exhibited potent antioxidant and antibacterial activity, with higher DPPH scavenging activity than ascorbic acid. Overall, this study provides valuable insights into the potential of proniosomes as a drug delivery system and the properties of curcumin as a natural antioxidant and antibacterial agent.

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