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Development and Characterization of Curcumin Loaded Transfersome for Transdermal Delivery

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Abstract

The aim of the present cram was to investigate the potential of a tranfersomes formulation for transdermal delivery of Curcumin. Curcumin is widely used in potent anti-inflammatory herbal drug. Its activity is similar to the NSAIDs in inflammatory pain management but main problem with curcumin when given orally is its poor bioavailability due to less GI absorption. The preformulation study of drug was carried out initially in terms of identification (physical appearance, melting point and IR spectra), solubility study, and λ-max determination and results directed for the further course of formulation. Optimizations of the formulations were done by selecting various process variables such as effect of lecithin, surfactant ratio, effect of various solvents and effect of surfactants. The transfersomes were formulated by modified hand shaking method using surfactant such as Tween 80 and Span 80 in various concentrations. The entrapment efficiency was found to be PC (Lecithin): Edge Activator (Tween 80 & Span 80) ratio dependent. Higher entrapment was found to be 89.6±0.049 within T8 formulation. The average size of the vesicle also correlated with the entrapment efficiency of the formulation and found to be 339.9nm with formulation T8. Permeation which was also dependent on PC(Lecithin):Edge Activator ratio(Tween 80 & Span 80). The formulation T8, which showed higher entrapment efficiency, provides higher permeation of drug from transfersomal gel this fact confirms the above said. The present study conclude that transfersomes formed from PC:Span 80 in the ratio 85:15 (in mmol) is a promising approach to improve the permeability of Curcumin in period of time.

Key words: Curcumin, Transfersome, Lecithin, Surfactant.

Introduction

Transdermal delivery of drugs through the skin to the systemic circulation provides a convenient route of administration for a variety of clinical indications. Transdermal delivery systems currently available for the treatment of various diseases such as cardiovascular diseases, Parkinson's disease, Alzheimer's disease, depression, anxiety and attention deficit hyperactivity disorder (ADHD), skin cancer, female sexual dysfunction, post-menopausal bone loss and urinary incontinence. The application of transdermal delivery to a wider range of drugs is limited due to the significant barrier to penetration across the skin which is allied primarily with the outermost stratum corneum layer of the epidermis. Consequently the daily dose of drug that can be delivered from a transdermal patch is 5-10 mg, in point of fact limiting this route of administration to potent drugs [1]. A transfersome is an artificial vesicle designed to be like a cell vesicle, and used to deliver drugs or genetic material into a cell. Transfersome is composed of one natural amphipath (such as phosphatidylcholine) that tends to self-aggregate into vesicles. The later are then supplemented by at least one bilayer softener (e.g. a biocompatible surfactant). The vesicle-like transfersome thus normally possesses an aqueous core surrounded by a complex, very fluid and adaptable lipid bilayer. In its basic organization is broadly similar to a simple lipid vesicle (liposome), a transfersome differs from the later by its more flexible and permeable, 'softened' bilayer membrane [2]. Curcumin is chemically (1E, 6E)-1, 7-bis (4-hydroxy-3-methoxyphenyl) hepta-1, 6-diene-3, 5-Dione. Curcumin is used for the treatment of anti-cancer, anti-oxidant, anti-inflammatory, hyperlipidemic, anti-bacterial, wound healing and hepatoprotective activities. Apart from its pharmacological actions, it has also been investigated as photostabilizing agent to protect photo-labile drugs in solution, topical preparations and soft gelatin capsules. Despite the presence of large

number of pharmacological actions, the therapeutic efficacy of curcumin is limited due to its poor oral bioavailability. The poor oral bioavailability of curcumin has been attributed to its poor aqueous solubility as its partition coefficient 3.2 and extensive first pass metabolism. The focal intent of the present cram is to develop transdermal delivery system of curcumin in the form of transfersome to get enhanced bioavailability.

Materials and Methods

Materials:

Curcumin was procured as a gift sample from HIMEDIA Lab Mumbai. Tween 80 and Span 80 from Loba Chem Pvt. Ltd, Mumbai, Lecithin and Triton x-100 from HIMEDIA Laboratories Pvt. Ltd, Mumbai, 6-carboxyfluorescein, Acros, Organics, New Delhi. All other ingredients used were of analytical grade.

Methods:

Preparation of Transfersome:

The transfersome were prepared by modified hand shaking, lipid film hydration technique. The composition of formulation is in (Table a). Drug, lecithin (PC) and edge activator were dissolved in ethanol:chloroform (1:1) mixture. Organic solvent was removed by evaporation while hand shaking above lipid transition temperature (43°C). A thin lipid film was formed inside the flask wall with rotation. The thin film was kept overnight for complete evaporation of solvent. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minute at corresponding temperature. The transfersome suspension further hydrated up to 1 hour at 2-8^0 C [3,4].

Optimization of formulation:

There are various process variables which could affect the preparation and properties of the transfersomes. The preparation procedure was accordingly optimized and validated. The preparation of transfersomes containing curcumin involves various process variables such as effect of lecithin:surfactant ratio (95:05, 85:15), effect of various solvents (ethanol, isopropyl alcohol) and effect of various surfactants (Span80, Tween80), optimization was done by selecting entrapment efficiency of drug. During the preparation of a particular system, the other variables were kept constant.

Interference Study:

Interference study was carried out for any interference of drug-polymer, drug-diluents and drug-lubricant used in the formulation. The interference study was carried using FTIR.

Characterization of Transfersomes:

The Morphological characterization of transfersome vesicle such as shape and surface feature were projected by Photomicroscopy using a digital Labomed camera in 40x resolution and transmission electron microscopy using (TEM, FEI-Philips Tecnai 12) and photomicrograph was taken [5]. Chemical characterization includes those studies which established the purity and potency of various transfersomal constitutions.

Determination of Entrapment efficiency percentage:

Transfersome entrapped curcumin was estimated by centrifugation method. The prepared transfersome were placed in centrifugation tube and centrifuged at 14000 rpm for 30 minute. The supernatant (1ml) was withdrawn and diluted with phosphate buffer (pH 7.4). The unentrapped Curcumin was determined by UV spectrophotometer at 427.2nm. The samples from the supernatant were diluted 100 times before going for absorbance measurement. The free Curcumin in the supernatant gives us the total amount of unentrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped.

$$\% \text{ Entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100$$

Further, after fusing the vesicle with Triton X-100 and amount of trapped drug was estimated by UV spectrophotometer with

Table a: Composition of formulation

Edge activator	Formulation code	PC:EA*	Solvent
Tween 80	T-1	95:05	Ethanol
	T-2	85:15	
	T-3	95:05	Isopropyl alcohol
	T-4	85:15	
Span 80	T-5	95:05	Ethanol
	T-6	85:15	
	T-7	95:05	Isopropyl alcohol
	T-8	85:15	

PC indicates Lecithin, EA indicates Tween 80 & Span 80.

Table b: Formulation of topical hydrogel

Hydrogel ingredients	Topical Hydrogel formulations			
	H1	H2	H3	H4
Transfersome	Eqv. To 2% of drug	Eqv. To 2% of drug	Eqv. To 2% of drug	Eqv. To 2% of drug
Carbopol971P	0.5 %	1.0 %	1.5 %	2.0 %
Propylene glycol	10%	10%	10%	10%
Glycerol	30%	30%	30%	30%
Distilled water	q.s.			

Table c: Entrapment efficiency of different formulation

Molar ratio (PC:EA)*	Formulation Code	Entrapment efficiency* (%)
Tween 80		
95:05	T1	68.2±0.074
85:15	T2	71.6±0.021
95:05	T3	73.3±0.018
85:15	T4	76.3±0.086
Span 80		
95:05	T5	73.6±0.033
85:15	T6	78.9±0.045
95:05	T7	81.5±0.012
85:15	T4	89.6 ±0.049

***All values are mean ± S.D. for n=3, PC** indicates Lecithin, EA indicates Tween 80 & Span 80.

Table d: In vitro release study of topical hydrogel formulations (H1-H4)*

Time in hr	% Drug Permeated				
	H1	H2	H3	H4	Conventional gel
0	0	0	0	0	0
1	8.89±0.01	8.31±0.03	7.47±0.01	7.01±0.03	3.33±0.02
2	16.50±0.03	16.05±0.01	15.93±0.03	15.53±0.01	5.11±0.05
4	20.81±0.01	20.12±0.02	19.64±0.02	19.31±0.04	8.54±0.01
6	22.28±0.02	21.95±0.03	21.01±0.05	20.78±0.02	13.23±0.02
8	25.05±0.07	25.00±0.05	24.58±0.01	23.48±0.01	16.85±0.03
10	26.29±0.01	25.98±0.06	25.09±0.03	24.58±0.01	20.36±0.07
24	50.61±0.02	49.11±00.01	48.89±0.06	48.54±0/05	33.51±0.01

***All values are mean ± S.D. for n=3**

Table e: Kinetic assessment of dissolution data of topical hydrogel formulations (H1-H4)

Formulations	Zero order		First order		Higuchi		Korsmeyer and Peppas	
	r^2	f2	r^2	f2	r^2	f2	r^2	N
H1	0.9133	40.73	0.9786	11.66	0.9993	30.90	0.9485	0.49
H2	0.9112	40.22	0.9791	11.50	0.9990	33.47	0.9518	0.50
H3	0.9151	40.18	0.9722	11.32	0.9994	40.39	0.9368	0.52
H4	0.9190	40.0	0.9716	11.16	0.9893	39.81	0.9319	0.53
Conv. Hydrogel	0.9501	22.77	0.9769	8.46	0.9543	31.10	0.9915	0.76

r^2: correlation coefficient; **f2:** similarity factor and **n:** diffusional release exponent

suitable dilution by Phosphate buffer (pH 7.4). The vesicle was washed with firstly phosphate buffer (pH 7.4) and 3-4 times with distilled water and suspended in distilled water [6,7].

Vesicle size, size distribution and zeta potential analysis:

The average diameter and size distribution profile and zeta potential analysis of vesicles were determined by Malvern Zetasizer DTS version 5.03 (Malvern, UK). Zeta potential was analyzed to measure the permeation of transfersome by studying its colloidal property and stability of the vesicle [4-7].

***Preparation of topical hydrogel* :**

As a vehicle for incorporation of transfersomes for topical delivery, carbopol hydrogels were prepared. Optimized curcumin transfersomes aqueous dispersion was utilized for the formulation of topical hydrogel (Table b). Optimized transfersome dispersion equivalent to 200 mg of pure drug was taken. Hydrogel polymer such as carbopol 971P was utilized to obtain controlled release topical hydrogel. The appropriate

quantity of carbopol 971P powder (0.5, 1, 1.5, 2 g) was dispersed into vigorously stirred (stirred by magnetic stirrer) distilled water (taking care to avoid the formation of in dispersible lumps) and allowed to hydrate for 4 to 5 hrs. The dispersion was neutralized with 10% (w/v) aqueous solution of sodium hydroxide to adjust the pH [8].

Evaluation of Topical Hydrogel:

Determination of pH:

The value of pH of topical hydrogels was measured by using digital pH meter (ELICO.LI 610 pH meter) at the room temperature.

***In vitro* Skin permeation Studies:**

Modified Franz diffusion cell with a receiver compartment volume of 50ml and effective diffusion area of 2.50cm^2 was used for this study. *In vitro* drug study was performed by using goat skin in phosphate buffer solution (pH 7.4).

Fresh Abdominal skin of goat were collected from slaughterhouse and used in the permeation experiments. Abdominal skin hairs were removed and the skin was hydrated in normal saline solution. The adipose tissue layer of the skin was removed by rubbing with a cotton swab. Skin was kept in isopropyl alcohol solution and stored at 0-4^{0}C [9].

To perform skin permeation study, treated skin was mounted horizontally on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was 2.50cm^2 and capacity of receptor compartment was 50ml. The receptor compartment was filled with 50ml of phosphate buffer (pH 7.4) saline maintained at 37± 0.5^{o}C and stirred by a magnetic bar at 100rpm. Hydrogel formulation (equivalent to 10mg drug) was placed on the skin and the top of the diffusion cell was covered. At appropriate time intervals 1 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffers (pH 7.4) to maintain sink conditions. Correction factors for each aliquot were considered in calculation of release profile. The samples were analyzed spectrophotometrically at λ max 427.2nm Fig 1. [10-12].

Skin deposition studies of optimized formulation:

At the end of the permeation experiments (after 24hr), the skin surface was washed five times with ethanol: PBS pH 7.4 (1:1), then with water to remove excess drug from surface. The skin was then cut into small pieces. The tissue was further homogenized with ethanol: buffer solution pH 7.4 (1:1) and left for 6hr at room temperature. After shaking for 5 minutes and centrifuging for 5 minutes at 5000rpm, the curcumin content was analyzed by UV visible spectrophotometric method after appropriate dilutions with Phosphate buffer solution (pH 7.4) at 427.2nm. The result was compared with the control group using *student's t-test.*

Results and Discussion:

Transfersomes were used for non-invasive delivery of drugs into or across the skin. Transfersomes are also known as elastic liposomes or flexible vesicles which have better penetration ability than conventional liposomes. So in present vocation deformable lipid vesicles 'Transfersomes' were formulated. The proposed system is more stable, having higher entrapment efficiency, can be used as self penetration enhancer, easy to scale up & better for dermal delivery.

Characterization of transfersomes:

The surface morphology was studied by Optical Microscopy and transmission electron microscopy. The results were shown in the Fig 2 & 3.

Interference Study:

Interference study was carried out for any interference of drug-polymer, drug-diluents and drug-lubricant used in the formulation, which reveals that there is no interaction between drug and polymer used in formulation Fig 4.

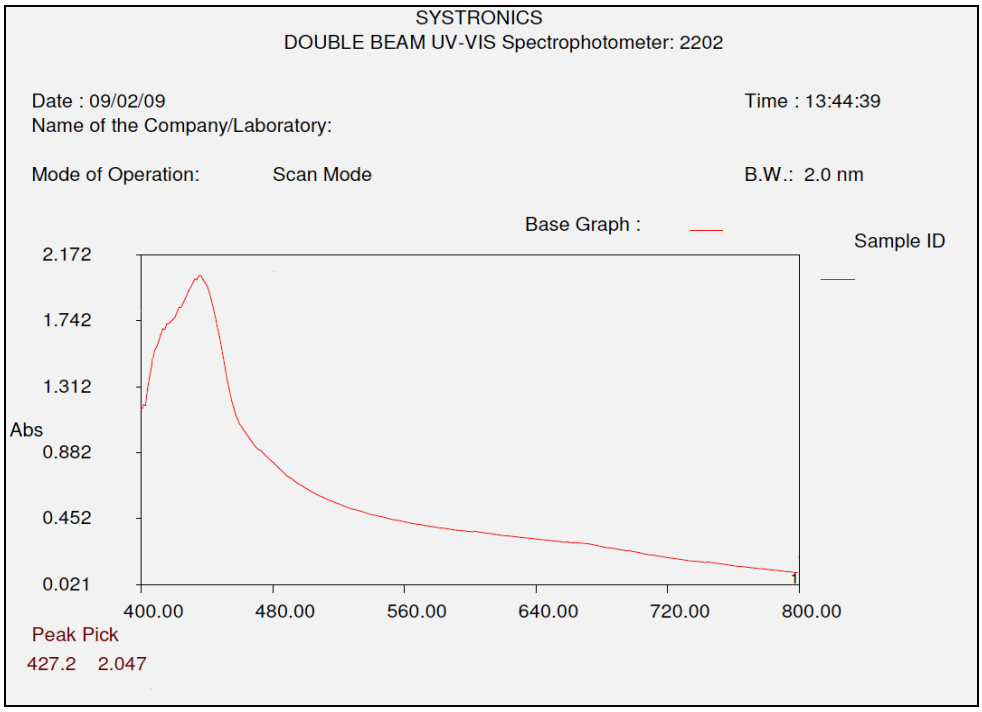

Fig. 1: Determination of absorption maxima (λmax) in PBS (pH 7.4)

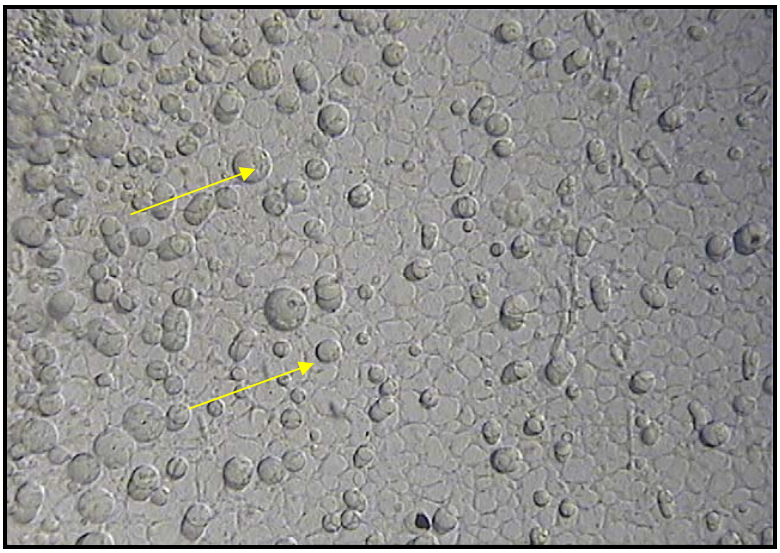

Fig. 2: Optical photomicrograph of curcumin loaded transfersome at 10×

Entrapment Efficiency:

The % entrapment efficiency of deformable vesicles formulations were found to be in the range of 68.2±0.074 to 89.6±0.049 (Table c). Entrapment efficiency of the T8 formulation was high (maximum 89.6±0.049 for T8) because of the increase in the ratio of lipid volume in the vesicles as compared to the encapsulated aqueous volume. The effect of phospholipids and edge activator ratio in the lipid components of vesicles on the entrapment efficiency of lipophilic drug, curcumin, the efficiency decreased with increasing surfactant concentration and thus increased with increasing PC

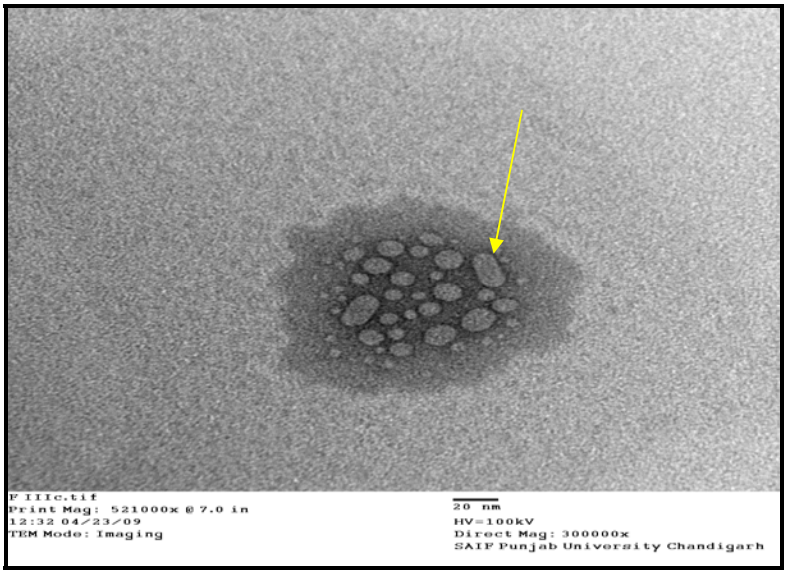

Fig. 3: TEM micrograph of transfersome

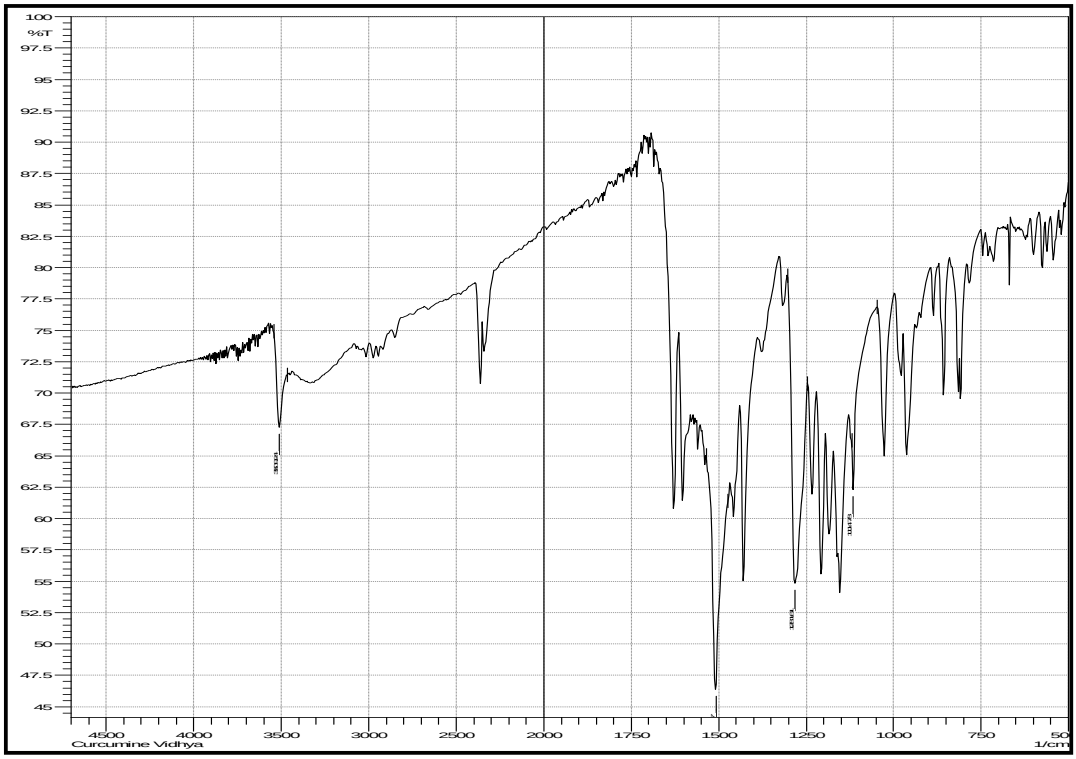

Fig. 4: IR spectra of curcumin with polymer (sample)

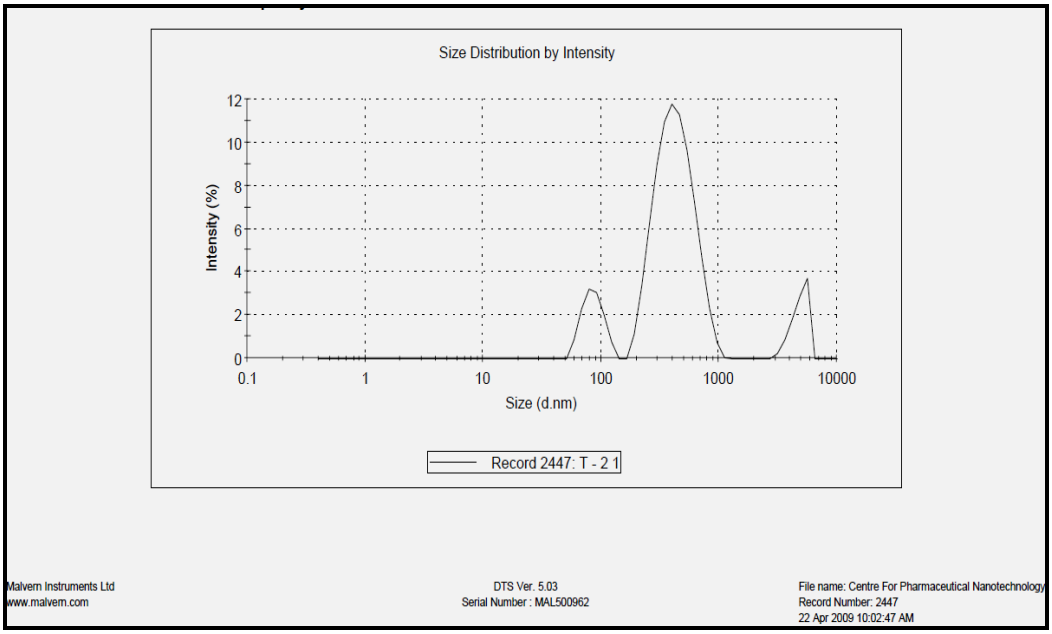

Fig. 5: Size statistics curve of optimized T8 formulation

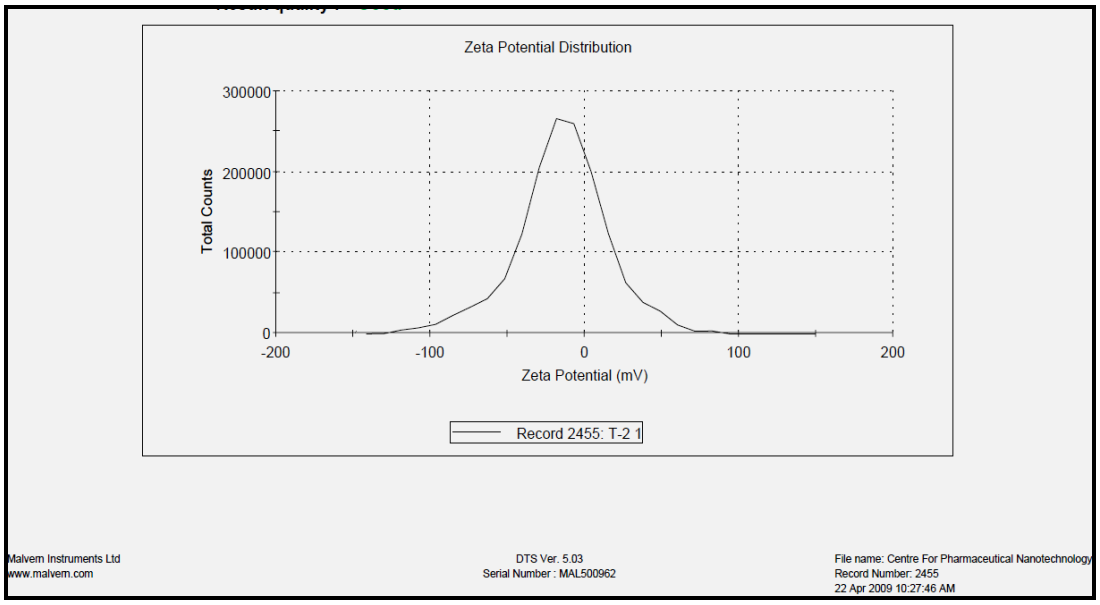

Fig. 6: Zeta potential of optimized T-8 formulation

concentration but have certain limit of PC: EA.

Vesicle size, size distribution and surface charge (zeta potential):

The vesicle size, size distribution and zeta potential were determined by light scattering method by Malvern Zetasizer (DTS version 5.03, Malvern, UK) of the optimized curcumin loaded formulation (T8). The mean vesicle diameter was found to be 339.3 nm Fig 5 & 6.

Size distribution curve confirms the normal size distribution of the vesicles. Transfersomal vesicles containing ethanol were larger in size compared with vesicles contained IPA because ethanol have greater solubility with water. The effect of edge activators on the size of vesicles that Span80 was small in size but no significant

difference with Tween80 when solvent system was similar.

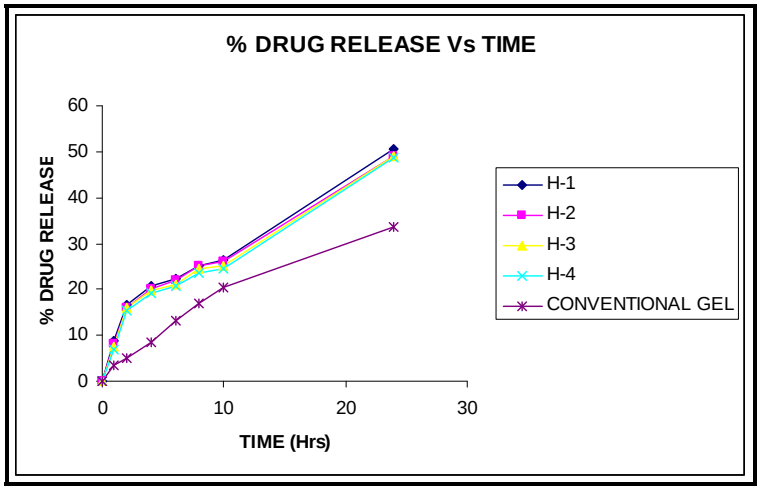

Fig. 7: Drug release study of topical Hydrogels

pH value of topical hydrogel:

The value of pH of topical hydrogels was measured by using digital pH meter (ELICO.LI 610 pH meter) at the room temperature. The value of pH of topical hydrogels H1, H2, H3 and H4 were found 6.6±0.12, 6.4±0.14, 6.6±0.02 and 6.8±0.25 respectively.

***In-vitro* release study of topical hydrogel:**

The dissolution rate studies for each of the formulations were performed in order to assess the effect of change in surfactant concentration on release profile. *In vitro* release study of topical hydrogel of curcumin transfersomes were carried out for 24 hours by using modified Franz diffusion cells in (pH 7.4) phosphate buffer maintained at 37±0.5^{o}C temperature and stirred by a magnetic bar at 100rpm under sink condition and the dissolution profile of all hydrogel formulations obtained from the dissolution data were shown in (Table d) and (figure 7). The result of dissolution profiles showed that the concentration of gelling agent (Carbopol 971P) in the range of 0.5% to 2.0% affects the release rate slightly. Regarding the formulation H1 to H4, the values of drug release after 24 hr were found to be 50.61%, 49.11%, 48.89% and 48.54% respectively. The values of drug release of the formulations H1 to H4 after 4 hour were found to be 20.81%, 20.21%, 19.64% and 19.31% respectively. This result of dissolution profile showed slight initial burst release. This is probably caused by the release of drug absorbed on the transfersome surface or precipitated from the superficial lipid layer. Prolonged release in the later stage can be attributed to the slow diffusion of the drug from the lipid vesicle.

Kinetic Analysis of Dissolution Data:

The drug release data were explored for the type of release mechanism followed. Release kinetic study of all formulation (H1 to H4) was studied (Table e) for different kinetic equation (zero order, first order and Higuchi equation). The best fit with higher correlation (r^2 > 0.99) was found with the Higuchi's equation for all the formulations, which means that release of curcumin from the lipid bilayer vesicles were due to diffusion. Release kinetic study was again verified by putting the values of release data in modern biopharmaceutics software MB-V6 and found that all the formulations follows Higuchi model (Table f). Hence, we can state that release of curcumin from the lipid bilayer system was mainly due to diffusion mechanism.

Conclusion

Curcumin is widely used as potent antiinflammatory herbal drug. Its activity is similar to the NSAIDs in inflammatory pain management. It is an herbal drug hence devoid of any side effect like bleeding in upper GIT, hepatotoxicity etc. but main problem with curcumin when given orally is its poor bioavailability due to less GI absorption. Nearly 25 to 85% of orally administered curcumin is eliminated unabsorbed by feaces. Difficulty is also associated with its topical application due to less permeability through skin. To alleviate this problem vesicular drug delivery system transfersomes is formulated to deliver Curcumin across skin.

Transfersomes formed from PC:Span80 in the ratio 85:15 (in mmol) is a promising approach to improve the permeability of Curcumin in period of time. Finally we conclude that transfersome entrapped curcumin gel gives better permeation as compare to plain drug gel. Transdermal delivery requires higher permeability because dose of the drug is very high. By this fact transfersomal formulation is better delivery system for transdermal delivery for the curcumin.

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