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DEVELOPMENT AND CHARACTERIZATION OF TIZANIDINE TRANSFEROSOMES FOR TRANSDERMAL DRUG DELIVERY

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ABSTRACT

The novel drug delivery system is creating a new interest in development of drug deliveries. Vesicular drug delivery system is also a part of this novel drug delivery system which is designed according to the recent demands of treatment of drug in systemic circulation, definite dosing interval. Transferosomes are ultradeformable vesicles possessing an aqueous core surrounded by the complex lipid bilayer used to enhance skin permeation. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimizing. The present investigation was majorly aimed to develop transferosomes of tizanidine by reverse phase evaporation method. Various process variables were studied to optimize the formulation including lecithin: cholesterol: surfactant and different solvent (methanol and chloroform). The drug content varies from 78.36 – 89.47%. The percent entrapment efficiency was found between 65.12 – 82.03%. The transferosomes were characterized by SEM, FTIR and DSC studies. The physical stability was tested out for all formulations for the period of three months and *in-vitro* permeation studies was performed using saline phosphate buffer showed 16.39 µg/cm^2/hr and amount of tizanidine deposited in the skin was maximum 67.69 µg respectively. The data obtained from the study results evidenced that transferosomes are promising drug delivery carriers for the many potential drugs and was proved the potent muscle relaxant tizanidine.

Keywords: Surfactant, Lecithin, Permeation, Flux, Lipid bilayer, Reverse phase evaporation.

INTRODUCTION

Transdermal drug delivery system provides controlled and continuous delivery of the drug through the skin into systemic circulation. Topical application of drug transport through epidermal or dermal tissues for local therapeutic effect while major fraction of drug is transported into systemic circulation. Transdermal delivery advantageous over conventional oral and invasive methods avoids first pass metabolism, improve patient compliance and maintains steady state concentration, predictable, extended duration of action, utility of short half-life drugs, improves pharmacological and physiological response. Transdermal route is an interesting option because it is convenient and safe. Transdermal drug delivery system avoids fluctuations in drug levels, inter and intra patient variability provides patient convenience. Many physical and chemical approaches have been applied to increase the efficacy of drug transfer across the intact skin by use of the penetration enhancers, iontophoresis, sonophoresis and the use of colloidal carriers such as lipid vesicles (liposomes, proliposomes) and nonionic surfactants (niosomes)[1].

The permeability of skin is the major limitation of transdermal drug delivery systems it is permeable to small molecules lipohilic drugs and impermeable to large molecules hydrophilic drugs. Stratum corneum the outermost layer of the skin is the main barrier and rate limiting step for diffusion of drugs across the skin. [2]

Conventional systems require multidose therapy to deliver the drug at the right target site, becomes complicated if each medication delivered in an optimal and preferred manner to the individual patient. The conventional dosage forms whether it is in tablet form or injection or patch has to be delivered in an manner, becomes complicated [3][4].

Therapeutic outcome of drug required not only proper selection of drug but also effective drug delivery. Innovations in drug delivery systems include minimum frequency dosage, improved patient compliance reduced fluctuations of concentration and minimal side effects. The creation of transdermal drug delivery systems has been one of the most important of these innovations

The delivery systems which is controlled and continous release of medicament to target site and the delivery is convenient and safe into the systemic circulation is transdermal drug delivery systems.

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Pathway of transdermal permeation

- **Transcellular route**

Drugs entering the skin through the transcellular route passes corneocytes containing highly hydrate keratin provide an aqueous environment for which hydrophilic drugs can pass. The diffusion pathway requires number of partitioning wall diffusion steps.

- **Intercellular route**

Intercellular pathway involves drug diffusing through the continous lipid matrix.this route is significant

- **The appendegeal route**

Skin appendages act as continous channel across the stratum corneum. Their influence on drug penetration is hindered by several factors such as surface area by hair follicles and sweat ducts are small thus limiting the area available for direct contact of the drug. [2,3].

MATERIALS AND METHODS

Materials

Tizanidine is a gift sample from yarrow chemicals pvt.,ltd., Chennai. Cholesterol and lecithin is obtained from merck specialities ltd., Mumbai. Tween 80 and span 20 were obtained from S.D Chem.Ltd., Mumbai.

Methods

ROTARY EVAPORATION METHOD

1. A thin film is prepared from the mixture of vesicles forming ingredients phospholipids and surfactant by dissolving in volatile organic solvent (chloroform – methanol). Organic solvent is then evaporated above the lipid transition temperature (room temperature for pure pre vesicles, 50°c for dipalmitoyl phosphatidyl choline using rotary evaporator. Final traces of solvent were removed under vacuum overnight.
2. A prepared thin film is hydrated with buffer by rotating at 60 rpm for 1 hr at the corresponding temperature.
3. To prepare small sized vesicles they were sonicated at room temperature or 50 ° c for 30 min. the sonicated vesicles were homogenized by manual extrusion 10 times through a sand which of 200 and 100 nm.

MODIFIED HAND SHAKING METHOD

Drug, phosphatidyl choline, edge activators 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 film was formed inside the flask wall with rotation. The film was kept overnight for complete evaporation of solvent. Then the film is hydrated with phosphate buffer (6.4) with gentle shaking for 15 min at corresponding temperature. The transferosomes suspension further hydrated to 1 hr at 2-8° c [22, 23, 24].

FTIR-Spectroscopy

Drug excipients compatibility was analyzed by using ATR (Attenuated Total Reflectance) FTIR Spectrophotometer (Agilent cary 630 ATR.FTIR). The spectra of tizanidine, cholesterol, lecithin, mixture were recorded. A sample of material was placed on the diamond ATR Crystal and analyzed by using spectrum of sample average scans at a resolution of 4 cm^{-1}.

CHARACTERIZATIONS

Photo microscopy

The prepared transferosomal vesicles are mounted on a slide and focused under microscope and photomicrographs were taken and images are displayed in fig 6 & 7.

Drug content

10 mg equivalent formulation is taken and pour 10 ml saline phosphate buffer and kept in homogenizer for 24 hrs and dluted to10 ml two times and the absorbance was measured at 319nm by UV spectrophotometer.

Entrapment efficiency

Entrapped tizanidine was estimated by centrifugation method. Prepared vesicles were placed in micro centrifuge tube and centrifuged for 30 min. the supernatant 1ml was withdrawn and diluted with saline phosphate buffer (pH 7.4). The unentrapped drug was determined by UV Spectrophotometrically at 319.5 nm. Samples were diluted twice before taking absorbance measurement of unentrapped drug. Entrapment efficiency is expressed as the percent of drug entrapped.

Scanning electron microscopy

The samples were dried and sent to SV university for SEM analysis and the results were displayed in the fig 8-14.

Invitro skin permeation skin studies

Invitro drug study was performed by using goat skin in phosphate buffer (7.4). Modified franz diffusion cell with a receiver compartment volume of 50 ml and effective diffusion area of 2.5cm used for this study. Abdominal skin hair is removed and hydrated with saline solution. The adipose tissue layer removed by rubbing with a cotton swab.

To perform study, treated skin was mounted horizontally on receptor compartment with the stratum corneum facing upwards towards the donar compartment of franz diffusion cell. The area of donar compartment is 250 cm and capacity of receptor compartment 50 ml of phosphate buffer of 7.4) at 37± 5° C and stirred at a magnetic bar for 100 rpm. Formulation equivalent to 10 mg was placed on the skin and top was covered. At appropriate intervals 1ml aliquots were withdrawn and immediately replaced by fresh volumes. Analyzed by any instrumental technique.

Drug deposition studies

The skin which was employed for skin permeation studies were removed from diffusion cell and cut into small pieces and placed in 50% (v/v) ethanolic solution and kept

for 12 hrs and samples of 4ml were withdrawn and analyzed its absorbance spectrophotometrically and percentage of drug deposition can be estimated.

Invitro drug release

The invitro release of tizanidine transferosomes through cellophane membrane was determined by a simple dialysis method. [14] Place 250ml saline phosphate buffer in receptor medium and the temperature maintained to be 37± 0.2 ºC and stirred at 100 rpm in a magnetic stirrer and place 5ml of formulation in to the donar cell and the samples of 4ml are withdrawn from receptor compartment at appropriate time intervals and placed with equal volumes of phosphate buffer and the samples were analyzed spectrophotometrically at a wavelength of 319.5 nm against blank.

Release kinetics

To investigate the possible release mechanism of tizanidine from the prepared transferosomes, the drug release data were fitted to various models such as zero order, first order, Hixson crowell, higuchi and peppas models.

RESULTS AND DISCUSSION

Preformulation studies

FTIR-Spectroscopy

Tizanidine

The optimized transferosomes showed better images in the formulation prepared by using span 20 when compared to tween 80.

The drug content value range between 17.6-82.5% and the percentage of drug content has been determined more in CF8 formulation.

The entrapment efficiency was found between 24.2-97.8 % the more drug is entrapped in CF8 formulation.

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The entrapment efficiency was found between 24.2-97.8 % the more drug is entrapped in CF8 formulation.

Scanning electron microscopy

The SEM images of optimized formulation were been performed and displayed in fig 8-14.

Release kinetics

Release kinetics showed that all the formulations showed first order release and CF2, CF3,CF4,CF6,CF8 followed higuchi model and CF1,CF5,CF7 followed peppas model.

Table 1. Ftir compatability stretchings in tizanidine drug

S.No	IR-peak	Wave number(cm^{-1})
1	C=O-STRETCH	1735.486
2	C=S-stretch	1057.293
3	-C=H-CH stretch	2109.793
4	O-CH_3 Stretch	2921.506
5	-OCH_3	2851.971

Table 2. FTIR spectrum of soya lecithin

S.No	IR-peak	Wave number(cm^{-1})
1	C=O-STRETCH	1735.486
2	C=S-stretch	1057.293
3	-C=H-CH stretch	2109.793
4	O-CH_3 Stretch	2921.506
5	-OCH_3	2851.971

Table 3. FTIR spectrum of cholesterol

S.No	IR-peak	Wave number(cm^{-1})
1	C-N STRETCH	1049.324
2	-O-CH_2-stretch	1461.250
3	CO-CN_2 stretch	2085.476
4	-Se-HStretch	2321.071
5	-CH2-	2849.414
6	-Ar-CH_3	2923.693

Table 4. FTIR stretching of mixture

S.No	IR-peak	Wave number(cm^{-1})
1	C-N STRETCH	1184.110
2	-O-CH_2-stretch	1222.949

3	CO-CN_2 stretch	2853.512
4	-Se-HStretch	2917.243
5	-CH2-	3544.609

Drug content and entrapment efficiency

Table 5. Drug content and entrapment efficiency

Formulation code	%Drug content	%Entrapment efficiency
CF_1	**17.6**	**24.2**
CF_2	**21.3**	**39.7**
CF_3	**28.5**	**53.8**
CF_4	**35.4**	**68.6**
CF_5	**71.9**	**79.4**
CF_6	**45.8**	**83.6**
CF_7	**53.6**	**91.6**
CF_8	**82.5**	**97.8**

***Invitro* skin permeation studies**

Table 6. Cumulative amount of drug permeation profile

Time	F1	F2	F3	F4	F5	F6	F7	F8	Pure drug
0.5	19.8	18.7	19.6	21.1	20.9	23.6	25.4	27.9	29.6
1	33.4	31.6	32.5	33.6	36.1	42.1	39.6	46.1	38.7
2	41.5	42.3	43.4	39.4	40.9	48.9	44.5	50.1	53.2
3	52.6	51.7	52.8	45.8	44.3	53.3	49.9	56.7	72.4
4	59.8	58.6	57.7	49.7	51.6	61.5	51.6	62.8	83.8
5	62.6	63.5	66.8	52.1	59.9	69.2	62.3	68.9	95.3
6	71.5	70.6	71.2	58.9	63.8	73.5	68.9	73.5	
7	79.8	76.3	78.9	68.5	78.6	80.5	73.5	80.2	
8	84.6	81.4	83.2	79.8	81.9	88.3	89.4	93.5	

Drug deposition studies

Table 7. Drug deposition studies

Formulation	Permeation coefficient(k_p) (cm/ hr.10^{-2})	Enhancement ratio	Transdermal flux(J_{ss})(µg/cm^2.hr)
CF1	6.04	1.12	0.75
CF2	7.4	1.37	0.81
CF3	6.75	1.25	0.83
CF4	7.29	1.35	0.79
CF5	7.85	1.45	0.76
CF6	7.37	1.36	0.72
CF7	7.94	1.47	0.86
CF8	8.07	1.49	0.85
Pure Drug	5.39	-	0.81

Release kinetics

	CF1	CF2	CF3	CF4	CF5	CF6	CF7	CF8
Zero order								
r^2	0.9433	0.9616	0.9611	0.9261	0.9147	0.9128	0.9119	0.8997
k	4.15	4.23	4.34	3.94	4.09	4.8	4.45	5.01
Higuchi model								
r^2	0.9948	**0.9992**	**0.9991**	**0.9999**	0.984	**0.9831**	0.9827	0.9999
K	13.4646	13.5206	13.8787	12.9406	13.535	15.516	13.2568	18.6159
First order								
r^2	**0.9792**	0.9787	0.9766	0.9773	0.9678	0.9798	0.9735	0.9641
K	0.0536	0.055	0.0569	0.0501	0.526	0.0654	0.0589	0.0695
peppas								

r^2	**0.9999**	0.9956	0.9959	0.9897	**0.9921**	0.9803	**1**	0.97
k	0.3292	0.3563	0.3512	0.3014	0.2744	0.2444	0.2747	0.2172
Hixcrowell								
r^2	0.959	0.9719	0.9717	0.97	0.9457	0.9589	0.9615	0.9476
k	0.076	0.0777	0.0802	0.0714	0.0746	0.0909	0.0827	0.096

Fig 1. Digramatic representation of permeation routes

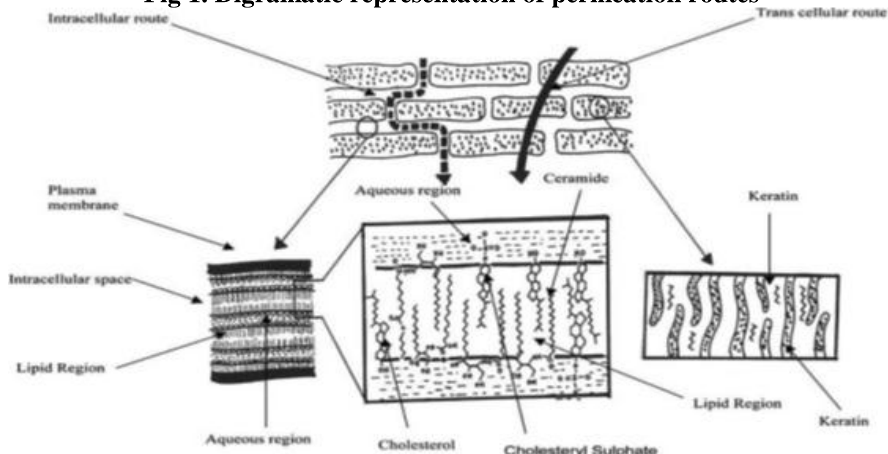


Fig 2. showing the FTIR spectrum of tizanidine

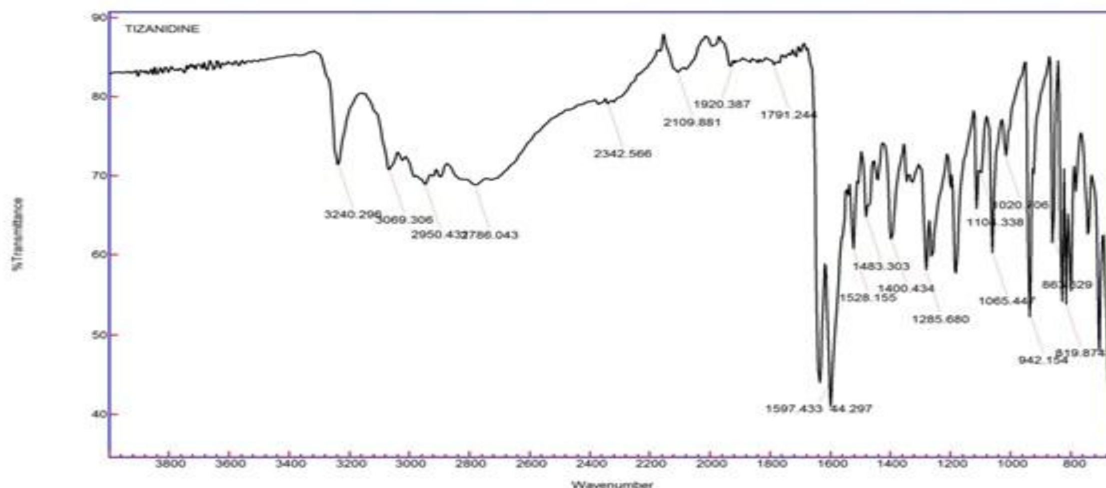


Fig 3. FTIR spectrum of lecithin

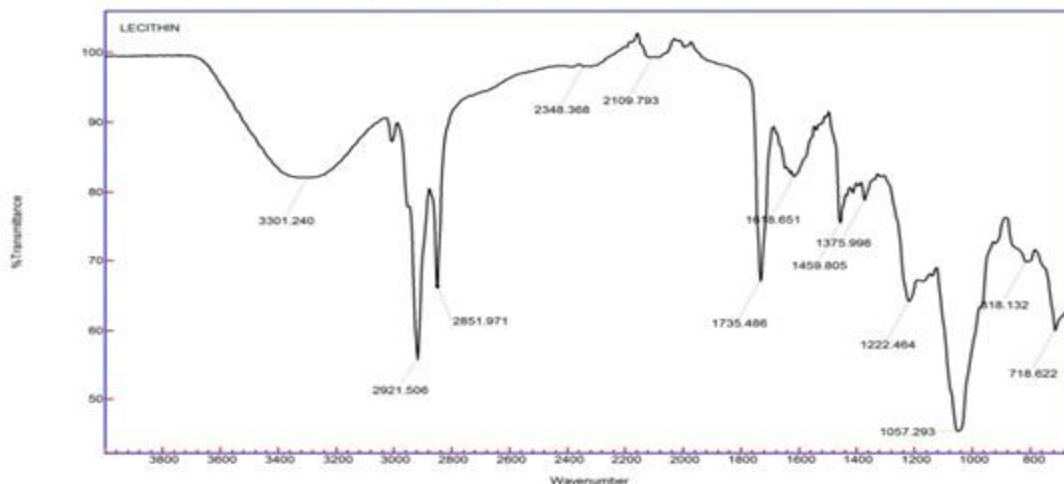


Fig 4. FTIR spectrum of cholesterol

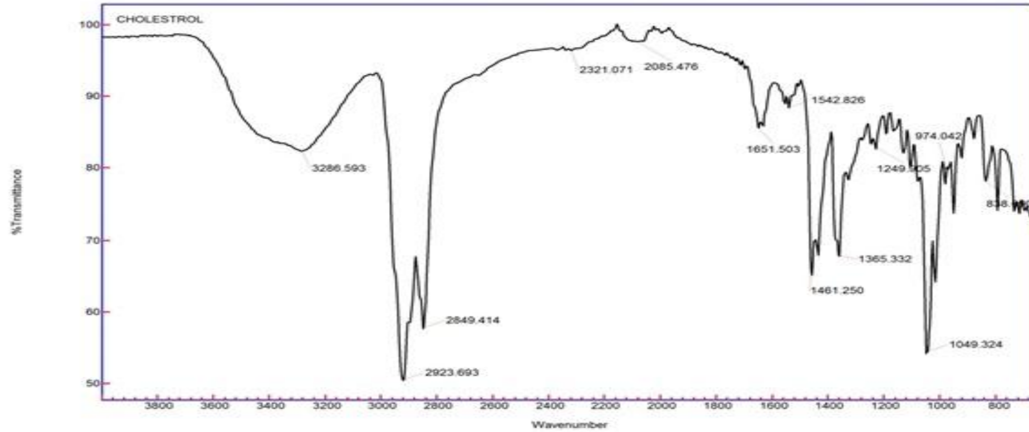


Fig 5. FTIR spectrum of mixture containing drug, lecithin, cholesterol

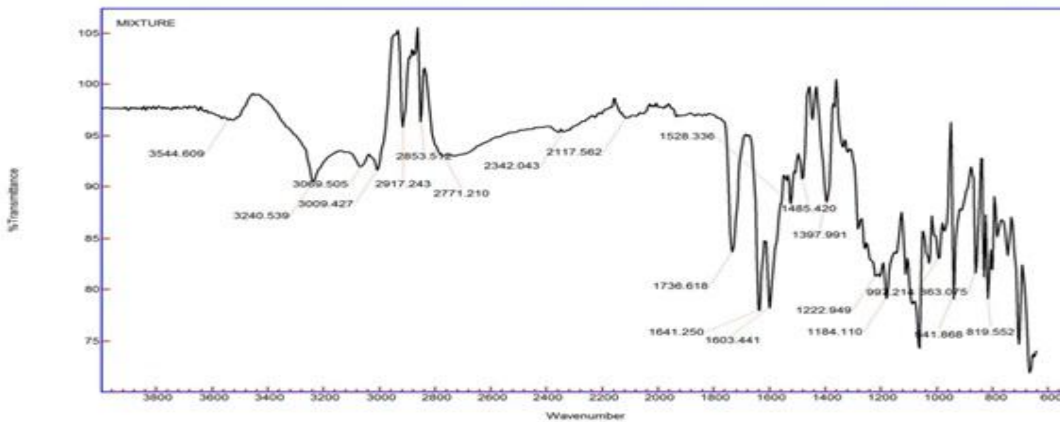


Fig 6. Photo morphology Figure 5 photo morphological images of tizanidine transferosomes using tween 80

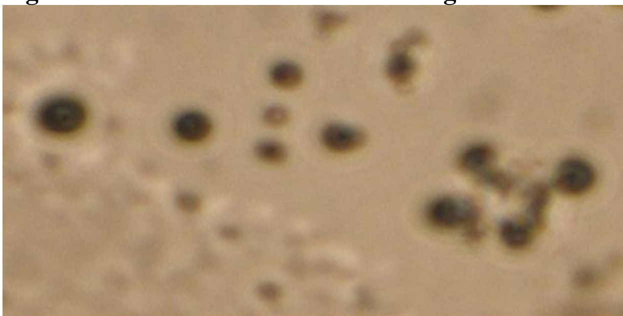


Fig 7. Photocontrast images of tizanidine transferosomes using span 20

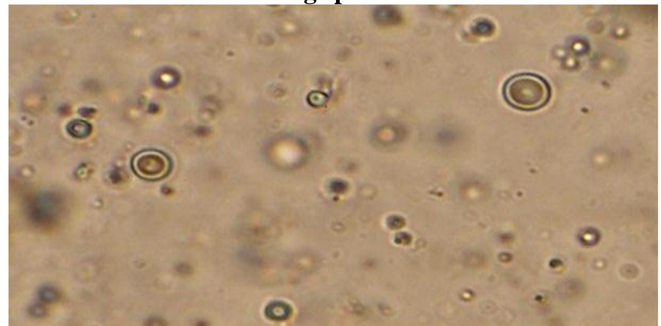


Fig 8. SEM image of optimized formulation under 500X magnification

Fig 9. SEM image of optimized formulation CF8 under 500X magnification

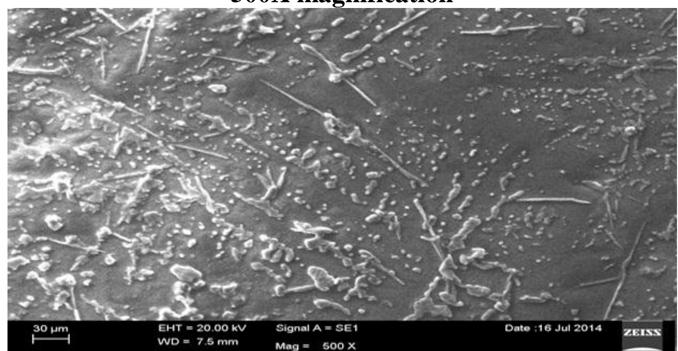


Fig 10. SEM image of optimized formulationCF8 under 1000X magnification

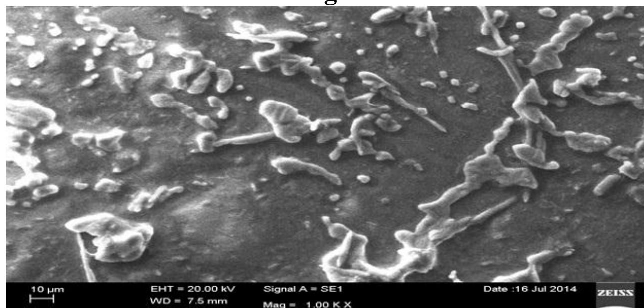


Fig 11. SEM image of optimized formulationCF8 under 2000X magnification

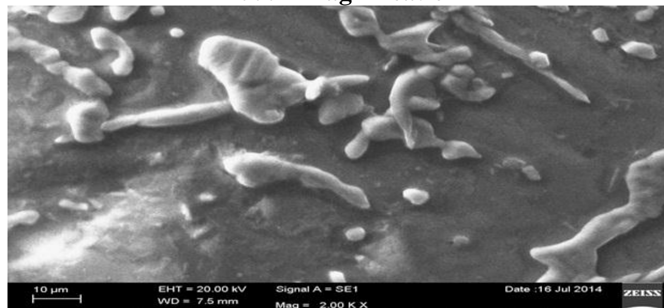


Fig 12. SEM image of optimized formulationCF8 under 3000X magnification

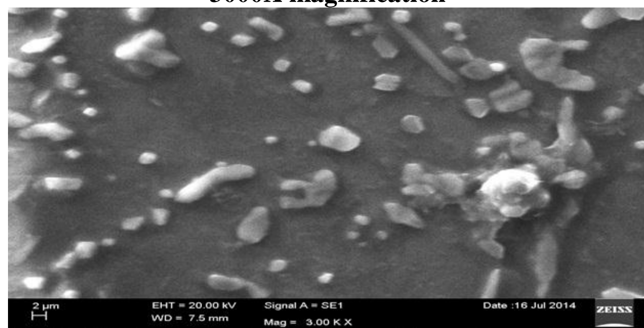


Fig 13. SEM image of optimized formulation CF8 under 5000X magnification

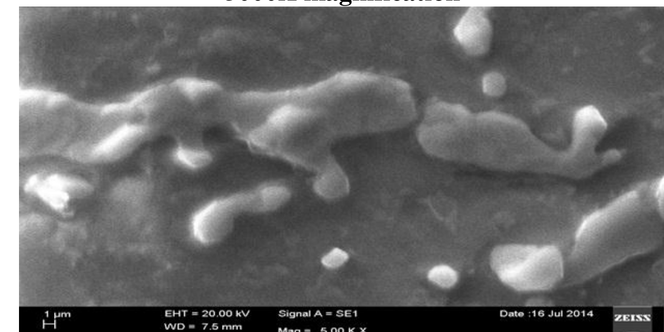
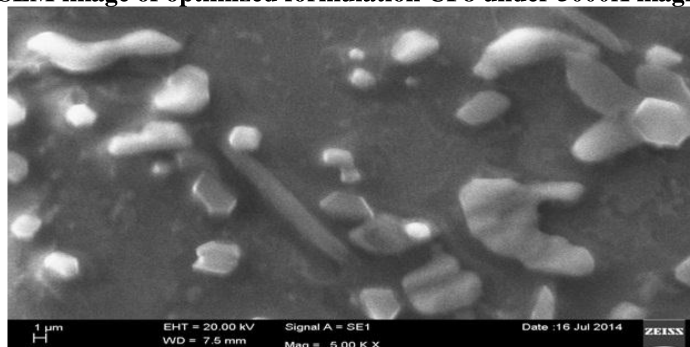


Fig 14. SEM image of optimized formulation CF8 under 5000X magnification



CONCLUSION

May prolong the release and increase the transdermal flux, improves the site specificity of bio active moieties. Due to their high deformability can incorporate large molecular weight drugs, both hydrohilic and lipophilic drugs. Tizanidine showed prolonged action when loaded in transferosomes compared to pure ones and can improve the muscle relaxant activity. Transferosomes are stable at low temperatures compared to high temperatures.

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