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Formulation and in Vitro evaluation of Chlorzoxazoneethosomal Gel

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ABSTRACT
Chlorzoxazone USP is a muscle relaxant which belongs to BCS-II classification with logP – 1.3 and is well absorbed, making it better for local muscle relaxantwith low solubility and high permeability. The aim & objective of the present work is to formulate and evaluate Chlorzoxazone ethosomal gel and compare its permeability enhancement with that of normal topical gel. Topical ethosomal gels of Chlorzoxazone is formulated for better patient compliance and for its direct action on muscle spasm. The prepared ethosomal gels were subjected to physical evaluations, in vitro diffusion studies, pH determination, viscosity, spreadability, entrapment efficiency, zeta potential and SEM. All the formulations were found to be easily washable, spreadable and free from gritiness. The in vitro diffusion studies shows that the percentage drug release of optimized ethosomal gel formulation F2 after 6 hrs to be 83.1% and that of normal gel to be 43.2% . Ethosomal gel F2 with the entrapment efficiency 82.1%, zeta potential -63.4mv and percentage drug release of 83.1% after 6hrs is chosen as optimized formulation for its better drug entrapment stability and better release. Vesicular size and shape is confirmed by SEM (260nm). Stability studies of optimized ethosomal gels showed satisfactory results for a period of 6 months. The kinetics shows that the release follows zero order and mechanism of drug release follows Higuchi's model (diffusion). In the light of the data obtained from experimental work we can expect the ethosomal formulation to be safe, efficient as a drug carrier and better permeable drug delivery for topical drug delivery holding future in effective topical delivery.

Key words: Chlorzoxazone, Lipids, FTIR Studies, Ethosomal gel, In-vitro diffusion studies, Drug release kinetics.

I. INTRODUCTION

Gels for dermatological use have several advantageous properties such as thixotropic, greaseless, easily spreadable, easily removed, emollient, non-staining and compatible with many excipients and water soluble or miscible. [1] In ethosomes, ethanol interacts with lipid molecules in the polar head group region resulting in a reducing the rigidity of the stratum corneum lipids, increasing their fluidity. In addition to the effect of ethanol on stratum corneum structure, the ethosome itself may interact with the stratum corneum barrier.[2]Transdermal route offers several potential advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels.[3] Ethosomes can be defined as noninvasive delivery carriers that enable drugs to reach deep into the skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents.[4]Ethosomes vesicles are preferred as a carrier of isotretinoin due tothe absence of ethanol in liposomes. Besides, ethosomes are morepermeable to cations as compared to liposomes[4]. Ethosomalvesicles have more benefits in comparison to other transdermaldelivery systems [5]. Ethosomal vesicles enhance drug permeationthrough the skin and may deliver large molecules of compounds[6] Transdermal drug delivery systems are externally applied medicaments in the form of gels that deliver drugs for systemic effects at a predestined and controlled rate. Through a diffusion process, the drug enters the bloodstream directly through the skin. Since there is high concentration in ethosomal gel and low concentration in the blood, the drug will keep diffusing into the blood for an extended period of time, maintaining the constant concentration of drug in the blood flow.[7,8] Chlorzoxazone (CLZ) is a centrally acting musculoskeletal relaxant with sedative properties. CLZ inhibits muscle spasm by exerting an effect primarily at the level of the spinal cord and subcortical areas of the brain. Its effect begins within an hour after an oral dose and

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lasts for 3–4 h. CLZ belongs to bio pharmaceutics classification (BCS) class II, i.e. low solubility and high permeability. Thus making Chlorzoxazone ideal candidate from topical preparations. Common skeletal muscle relaxants have been approved for either treatment of spasticity or for treatment of musculoskeletal conditions.[9,10,11]The present study aims in designing ethosomal gel of Chlorzoxazone using phospholipids and evaluating the prepared ethosomal gel for various evaluation parameters.

II. MATERIALS AND METHODS

Chlorzoxazone was collected as a gift sample from Chiral drugs pvt.ltdSurat,Gujaratand various excipients likeSoya lecithin, Cholesterol, Triethanolamine, Carbopol 934, Sodium benzoate and other excipients were purchased from SD Fine chemicals, Hyderabad

2.1.METHODOLOGY

Drug-Excipients CompatibilityStudies:

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. Drug - Excipient Compatibility studies were done using ShimadzuFourier-transform infrared spectrophotometer. FTIR spectrum was taken for pure drug and physical mixture of excipients with drug by potassium bromide pellet method.The samples were analyzed between wave numbers 4000 and 400cm^{-1}

FORMULATIONDEVELOPMENT

Ethosomes of Chlorzoxazone were prepared using different compositions of phospholipid, ethanol, polyethylene glycol (PEG) and cholesterol. In this method, Chlorzoxazone API dissolved in polyethylene glycol and heated to 30^{0}C at 800 rpm. Lecithin and cholesterol dissolved in ethanol and added to the above mixture. Double distilled water was added slowly as a fine stream with constant mixing at 800 rpm. Mixing was continued for additional 5 minutes. The size of the ethosomes vesicles can be decreased using sonication or extrusion method. Ethosomes formulation was stored under refrigeration. Ethosomal vesicles suspension was incorporated into carbopol gel base. Tri ethanolamine was added to it drop wise and Water q.s was added with sodium benzoate as preservative with continuous stirring until homogenous formulation were achieved. [13]

Table-1: Formulation of Chlorzoxazone ethosomal gel

INGREDIENTS	F1	F2	F3	F4	F5
Chlorzoxazone	250mg	250mg	250mg	250mg	250mg
Ethanol	10ml	20ml	30ml	20ml	20ml
PEG 400	5ml	5ml	5ml	5ml	5ml
Soya lecithin	5ml	5ml	7.5ml	10ml	5ml
Cholesterol	50mg	75mg	100mg	50mg	50mg
Carbopol gel	1gm	1.5gm	2gm	2.5gm	3gm
Triethanolamine	2ml	2ml	2ml	2ml	2ml
Sodium benzoate	1mg	1mg	1mg	1mg	1mg
Distilled water	50ml	50ml	50ml	50ml	50ml

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Figure-1:EthosomalGel

Figure-2: Formulations

EVALUATION OF ETHOSOMALGEL

Surface morphology ofEthosomes

Scanning electron microscopy is used to determine the shape and size of formulated chlorzoxazone loaded ethosomes.[14, 15, 16]

Particle size distribution and zeta potentialdetermination

One millilitre of ethosomal suspension was diluted by 10 ml of double distilled water and Particle size distribution and zeta potential was determined by Dynamic light scattering method by particle size analyzer (nanopartica- Horiba Scientifics).

Physicalappearance

The prepared gel was examined for clarity, color, homogeneity and the presence of foreign particles.

Viscosity

Viscosity of all the formulated gels was measured using Brookfield DV-E viscometer. The samples weretaken in 100ml beaker and viscosity was measured at the rotation of 2 rpm shear rate at room temperature.

pHdetermination

The pH of formulated ethosomal gels was determined using pH meter. Required amount of gel was added to 10ml of distilled water to get a uniform suspension. The electrode was immersed in suspensions and readings were recorded on pH meter.

Spreadability

Spreadability can be determined by taking two glass slides. 500mg of prepared gel is placed on one of the glass slide and the next slide is kept on the top of first slide which is tied to 10g weight then it is allowed to spread for 1min and length of the spreaded gel on the glass slide was measured using scale. The time (seconds) required to separate the two slides was taken as a measure ofspreadability.

It was calculated using the formula,

$S = m.l / t$

Where, S - Spreadability in g.cm / sec m - Weight tied to upper slide

l - Length of glass slide t - Time in seconds

Drugcontent

1ml of chlorzoxazone loaded ethosomal suspension was taken and diluted with 6.8 phosphate buffer. It was ultracentrifuge (Remi-CM12plus) for 4000 rpm for 45min at 4^0C and after centrifugation, disruption of vesicles were done using appropriate quantity of methanol then 1ml of supernatant liquid was taken and suitable dilutions were made and analyzed by UV spectrophotometer at 255nm and % drug content can be calculated using following formula.

% drug content = practical drug content/theoretical drug content x 100

Entrapmentefficiency

1ml of chlorzoxazone ethosomal suspension was taken and diluted with 6.8 phosphate buffer and ultracentrifuge (Remi-CM12plus) for 4000 rpm for 45min at 4^0C, from that 1ml of supernatant liquid is taken and appropriate dilutions were made and analyzed using UV spectrophotometer at 255nm. % entrapment efficiency can calculate using following formula.

Entrapment efficiency = (Amount entrapped / Total amount added) ×100

In-vitro diffusionstudies[17]

A diffusion study of 5 formulations was carried out using Franz diffusion cell through dialysis membrane from Himedia. Dialysis membrane was soaked in distilled water for 24

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hours. Franz diffusion cell contain two compartments upperdonorand lower receptor compartment. The receptor compartment was filled with 6.8 phosphate buffer and donor compartment contain 200mgof ethosomal gel on dialysis membrane with exposure area of $2cm^2$ to receptor medium and whole assembly was kept on magnetic stirrer Samples were appropriately diluted with buffer and analyzed using UV spectrophotometer at 255nm.

Figure-3: Diffusion studies

RELEASE KINETIC PROFILE FOR ETHOSOMAL GEL OF OPTIMIZED FORMULATIONF2

The zero order rate Eq. (1) describes the systems where the drug release rate is independent of its concentration. The first order Eq. (2) describes the release from system where release rate is concentration dependent. Higuchi (1963) describedthe release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion.[18]

$C = K_0 t$ (1)

where, K_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$\text{Log}C = \text{Log}C_0 - K_1 t/2.303$ (2)

where, C_0 is the initial concentration of drug and K_1 is first order constant.

$Q = K_H t^{1/2}$ (3)

where, K_H is the constant reflecting the design variables of the system.

Korsmeyeret al (1983) derived a simple relationship which described drug release from a polymeric system Eq. (5). To find out the mechanism of drug release, first 60% drug release data was fitted in Korsmeyer–Peppas model.

$M_t / M_\infty = K_m t^n$ (4)

where M_t / M_∞ is fraction of drug released at time t, K is the release rate constant incorporating structural and geometric characteristics of the tablet, and n is the release exponent. The n value is used to characterize different release mechanisms.

A plot of log cumulative % drug release vs. log time was made. Slope of the line was n. The n value is used to characterize different release mechanisms, for the cylindrical shaped matrices. Case-II generally refers to the erosion of the polymeric chain and anomalous transport (Non-Fickian) refers to a combination of both diffusion and erosion controlled-drug release.

Table-2: Diffusion Exponent and Solute Release Mechanism

Diffusion exponent (n)	Overall solute diffusion mechanism
<0.45	Quasi Fickian
0.45	Fickian diffusion
0.45 < n < 0.89	Anomalous (non-Fickian) diffusion
0.89-1	Case-II relaxation or non-Fickiandiffusion
n >1	non-FickianSuper case-II

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STABILITYSTUDIES

It is vital for formulation development person to develop a stable product from formulation as well as regulatory point of view. The regulatory agencies around the globe have rhetoric guidelines of product stability studies. Stability study is performed to check physical and chemical integrity of the formulation. The optimised formulation of chlorzoxazone ethosomal gel was stored at ambient humidity conditions, at refrigerated temperature 4±2℃, room temperature 25 ± 2℃ / 60 ± 5% RH, and accelerated temperature 40 ± 20℃ / 75 ± 5%RH. The sample kept for stability was evaluated for homogeneity, pH, drug content, entrapment efficiency and in-vitro diffusion studies after 60days.

III. RESULTS & DISCUSSION

DRUG-EXCIPIENTS COMPATIBILITYSTUDIES

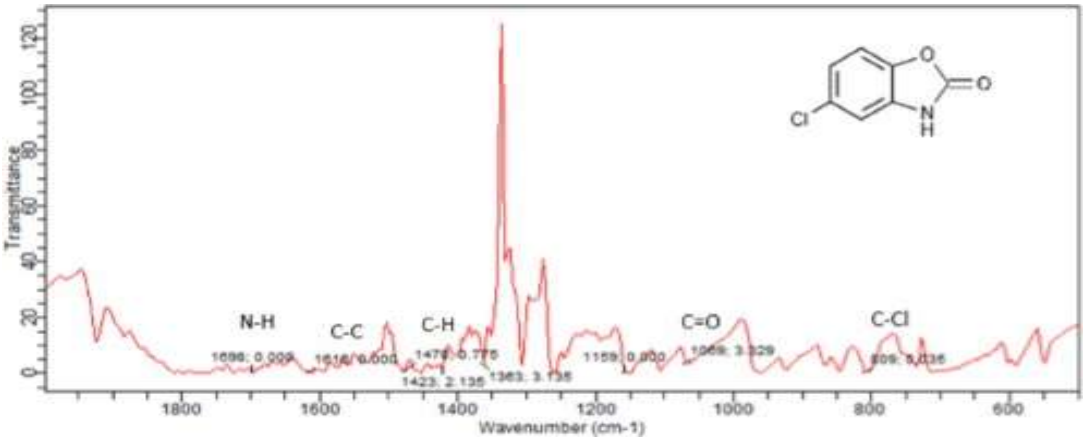

Figure-4: FTIR Spectrum of Chlorzoxazone

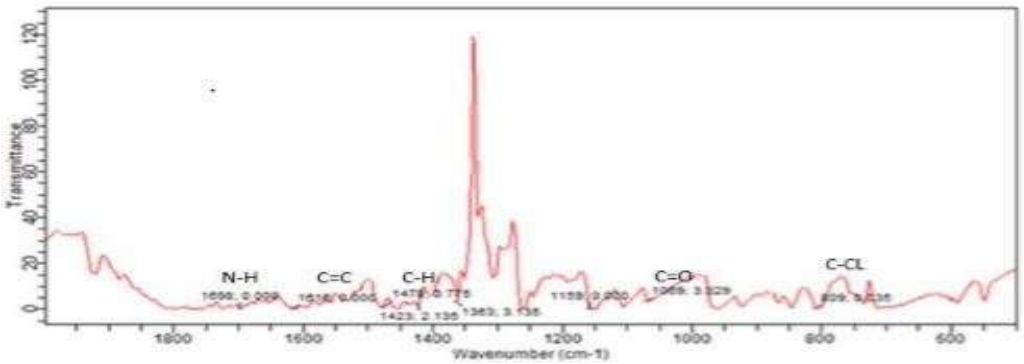

Figure-5: FTIR Spectrum of Drug and Excipients

Evaluation

Organoleptic properties (Colour, Odour and Appearance)

Table No-3: Showing Organoleptic Properties of Chlorzoxazone Ethosomal Gel

Colour	Odour	Appearance
Light brown	Odourless	No aggregates

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Characterization OfEthosomes

Surface Morphology Of (vesicle size) Of F2Formulation

Microscopic analysis was performed under different magnification to visualize the vesicular structure and to determine the size of topical preparation.

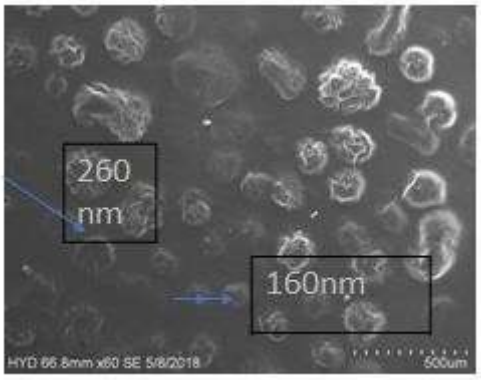

Figure-6: Scanning electron microscope image of chlorzoxazone ethosomal gel F2

Discussion: The scanning electron microscopy (SEM) of the prepared ethosomal gel formulation was performed and the shape of the ethosomal gel particles was found to be quite spherical and vesicle size of F2 formulation was found to be 260nm and 160nm.

Zeta potential: Zeta potential of F2 formulation was determined and it was found to be dispersed stable with the absolute value of -64.3 (mv) as shown in the figure 22

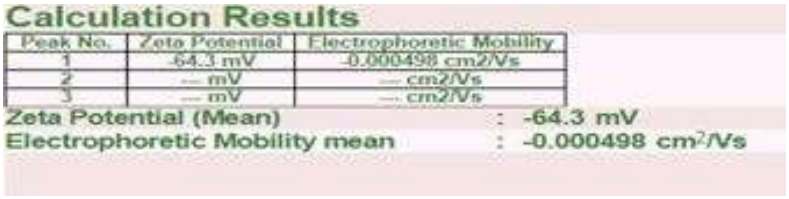

Figure-7: Results of zeta potential

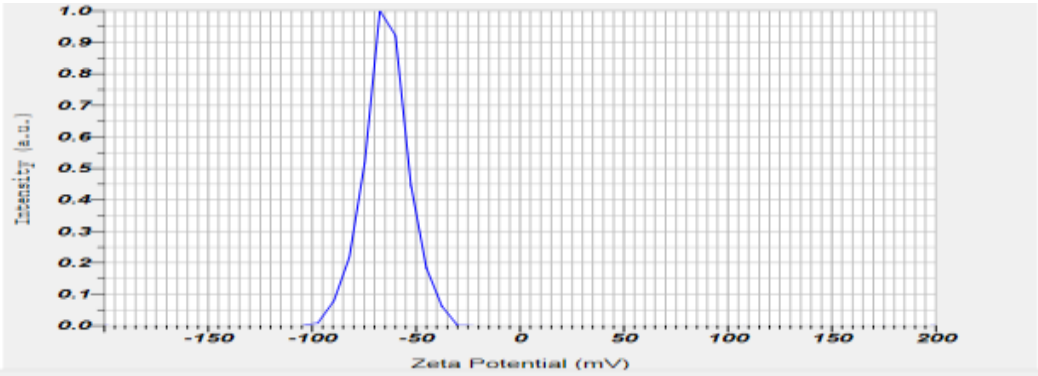

Figure-8: graph showing zeta potential

Discussion: The zeta potential of F2 formulation was found to be -64.3 mV and hence stable.

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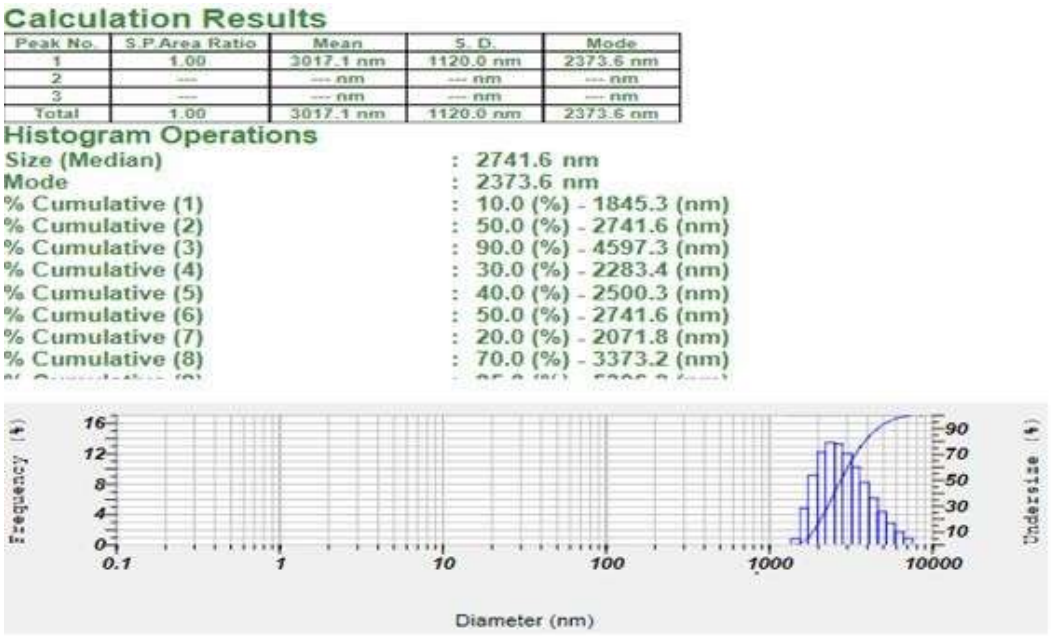

Figure-9: Particle size of f2 formulation

Particle size distribution and zeta potential determination: Particle size distribution and zeta potential was determined by Dynamic light scattering method by particle size analyzer (nanoparticle- HoribaScientifics).

Surface morphology of ethosomes: Scanning electron microscopy is used to determine the shape and size of formulated Chlorzoxazone loadedethosomes.

Physicalappearance: The prepared gel was examined for clarity, color, homogenosity andthe presence of foreignparticles.

Viscosity : Viscosity of all the formulated gels was measured using Brookfield DV-E viscometer

pH determination: The pH of formulated Ethosomal gels was determined using pH meter.

Spreadability: The spreadability of gel formulations was determined by measuring the spreading diameter of 1g of gel between two horizontal plates (20 cm × 20cm).

S = M/T

Where, S is the Spreadability in g/s,M is the mass in grams &T is the time in seconds.

DRUG CONTENT

1ml of Chlorzoxazone loaded ethosomal suspension was taken and diluted with 6.8 phosphate buffer. It was ultracentrifuge (Remi-CM12plus) for 4000 rpm for 45min at 4^0C and after centrifugation, disruption of vesicles were done using appropriate quantity of ethanol then 1ml of supernatant liquid was taken and suitable dilutions were made and analyzed by UV spectrophotometer at 244nm and % drug content can be calculated using following formula.

% drug content = practical drug content/theoretical drug content x 100

Table No-4: Drug Entrapment efficiency of all formulations

Formulation	**Drug content (%)**
F1	93.2
F2	95.6

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F3	94.1
F4	89.7
F5	90.1

ENTRAPMENT EFFICIENCY

1ml of chlorzaxazone ethosomal suspension was taken and diluted with 6.8 phosphate buffer and ultracentrifuge (Remi-CM12plus) for 4000 rpm for 45min at 4^0C, from that 1ml of supernatant liquid is taken and appropriate dilutions were made and analyzed using UV spectrophotometer at 244nm. % entrapment efficiency can calculate using following formula.

Entrapment efficiency = (Amount entrapped / Total amount added) ×100

Table No-5: Drug Entrapment efficiency of all formulations

Formulation	**Chlorzoxazone Entrapment efficiency (%)**
F1	81.4 ±0.12
F2	82.1± 0. 08
F3	76.8 ±0.02
F4	80.1± 0.02
F5	78.13±0.02

In-vitro diffusion:

Table no-6: In-vitro diffusion of all formulations

TIME INTERVAL (min)	**F1**	**F2**	**F3**	**F4**	**F5**
30	25.56	30.21	23.8	20.45	22.7
60	30.43	35.87	35.76	33.21	32.21
90	46.54	45.66	47.02	42.5	45.2
120	55.87	53.90	52.78	55.45	50.21
150	60.22	67.9	75.1	66.5	61.4

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225	75.61	77.9	82.3	73.21	73.87
300	81.83	83.1	86.3	80.3	79.4

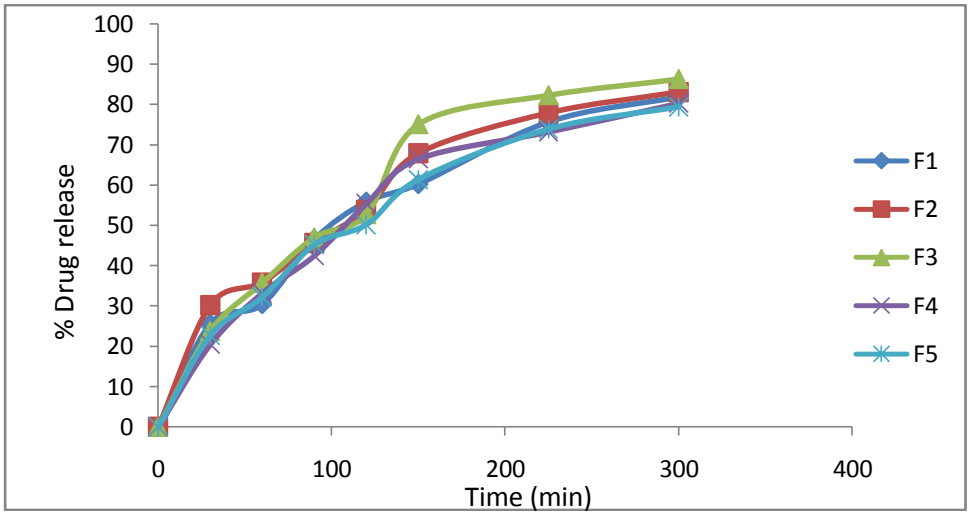

Figure-7: In vitro diffusion of all formulations

RELEASE KINETIC PROFILE FOR ETHOSOMAL GEL OF OPTIMISED FORMULATIONF2

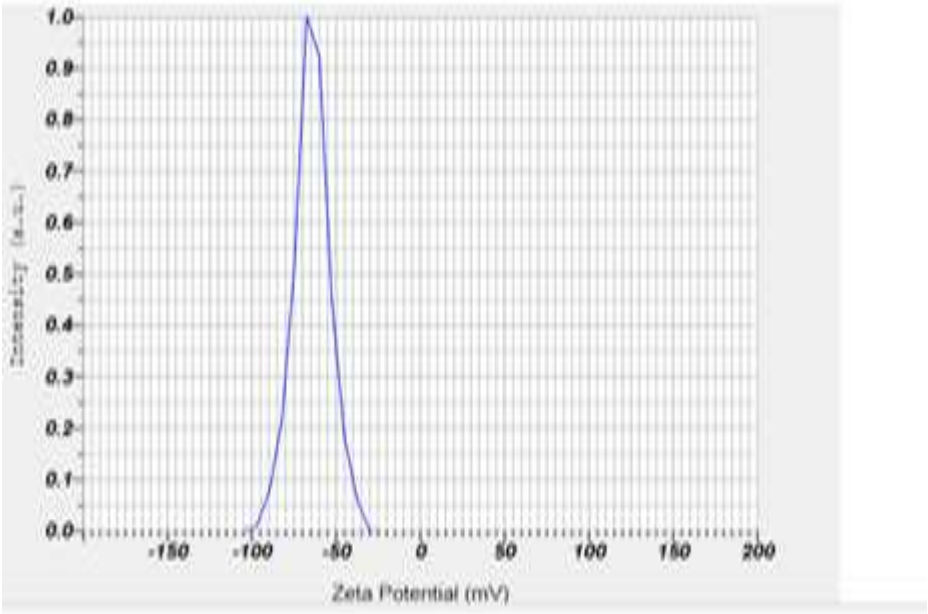

Figure-8: Zero order plot of Cumulative % drug release vs. time of optimized formulation

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LOG % DRUG REMAINED

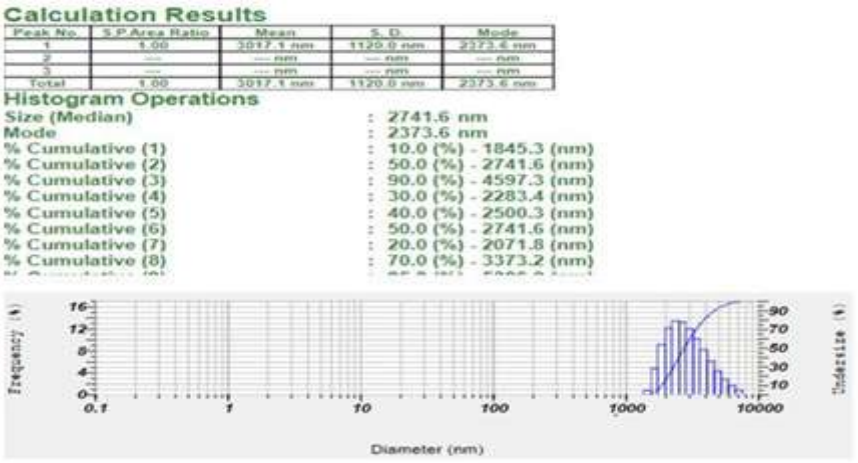

Figure-9: First order plot of Log Cumulative % drug remaining vs. time of optimized formulation

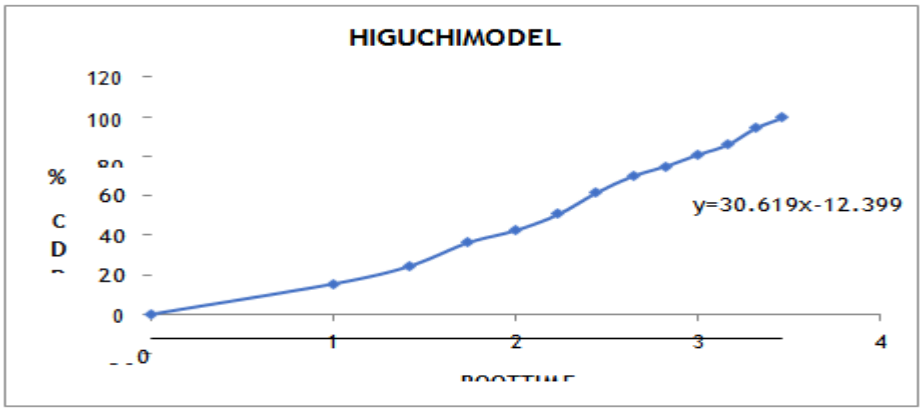

Figure-10: Plot of Cumulative % drug release vs. Root time of optimized formulation

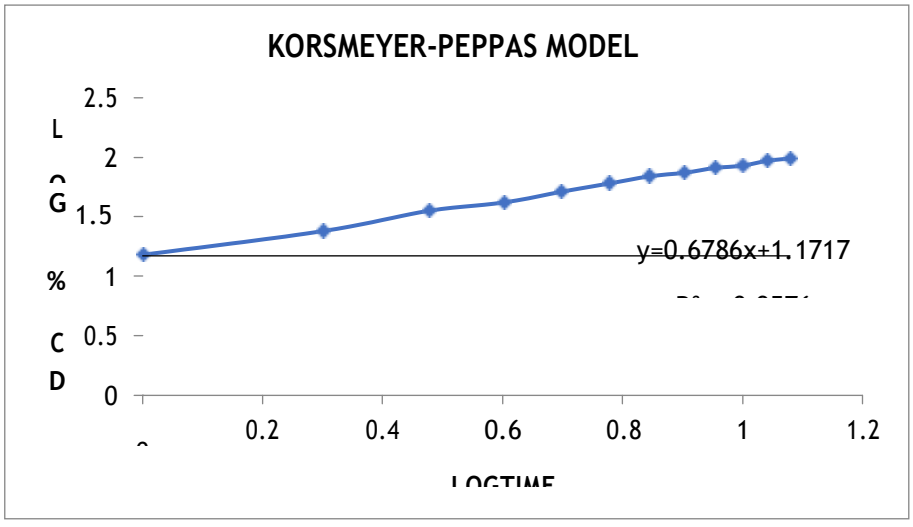

Figure-11: Plot of Log Cumulative % drug release vs. Log time of optimized formulation

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Discussion:

Kinetic studies of optimized formulation F2 follows Higuchi model with R^2 value 0.996 which is greater than Korsmeyer-Peppas Model R^2 value 0.957. Hence Drug release follows diffusionmechanism.

Stability Studies

Table-7: Stablity studies of optimized formulation

S.NO	DURATION	DRUG CONTENT			pH			VISUAL APPEARANCE		
		4℃ ±2	25±2℃ &RH 60% ±5%	A.T	4℃ ±2	R.T	A.T	4℃ ±2	R.T	A.T
1	30 days	94.4	86.73	83.87	6.3	6.7	6.4	No visual changes	No visual changes	No visual changes
2	60 days	93.5	85.7	82.8	6.5	6.8	6.3	No visual changes	No visual changes	No visual changes

Stability studies was conducted at room temperature. The optimised formulation of chlorzoxazone ethosomal gel was stored at ambient humidity conditions, at refrigerated temperature 4±2℃, room temperature 25 ± 2℃ / 60 ± 5% RH, and accelerated temperature 40 ± 2oC / 75 ± 5% RH. The sample kept for stability was evaluated for homogeneity, pH, drug content, entrapment efficiency and in-vitro diffusion studies after 60 daysThe results of the parameters after 2 months was found to be Drug content (93.5), pH (6.3), visual appearance, no visual changes were observed. All the parameters were found to be within limits after 2months.

IV. CONCLUSION

Chlorzoxazone is BCS-II classification muscle relaxant with low solubility and high permeability. 5 formulations were prepared and characterization of formulation were carried out. Among all formulation studied F2 is optimized for its better drug release (3hrs) and better stability.The entrapment efficiency of ethosomes containing 20% w/w ethanol has shown highest value with respect to all other formulation. The pH values for ethosomal gels formulations were in the range of 6.6 to 6.7 and the viscosity of the formulation was found to be 33,400cps. Stability studies were carried out for a period of 8 weeks andit showed no significance changes in the characteristics of ethosomalgel. Ethosomal gel of Chlorzoxazone with 20% ethanol concentration i.e; F2 has shown 83.1% drug release of Chlorzoxazone, showing zeta potential of -64.3 (mV), with specific vesicular size and shape confirmed bySEM. From the above observations it was concluded that ethosomal gel formulation F2 was found to be the better formulation among all the formulation of topicalgel. It showed high permeation and betterstability.

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