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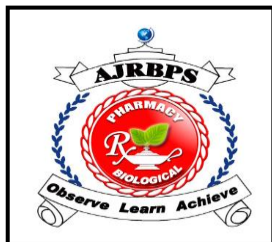

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DESIGN AND CHARACTERIZATION OF NABUMETONE ETHOSOMES FOR ENHANCED DRUG DELIVERY

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ABSTRACT

The present research aims to design and characterize the nabumetone ethosomes for enhanced drug delivery. The main objective is to improve the solubility of BCS class II drug nabumetone by ethosomal formulation and to improve bioavailability. Formulation of ethosomal gel by avoiding first-pass metabolism to overcome the side effects of gastrointestinal bleeding. Ethosomes can be formulated by hot method using ethanol, phospholipids, cholesterol and propylene glycol. Carbopol-934 acts as a gelling agent. The evaluation of ethosomal gel includes particle size determination, zeta potential, entrapment efficiency, drug content, *in vitro* drug release and kinetics release studies. The optimized formulation exhibits Z - an average of 390.6nm and -4.96nm of particle size and zeta potential respectively. *In vitro* drug release studies are performed using egg membranes in phosphate buffer, more than 90% of the drug can be released in 12 hours. The kinetics release studies of zero order, Higuchi model and Korsmeyer-Pappas model refer to the process of constant drug release from a drug delivery system independent of concentration. The regression value of this study is 0.99. Thus, the result of the current study indicates a promising potential of ethosomal gel as an alternative to the conventional form. Further, the studies are to avoid hepatic first-pass metabolism and enhance bioavailability and biocompatibility.

KEYWORDS

Ethosomes, Nabumetone, Hot method, Improve bioavailability and Ethosomal gel.

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INTRODUCTION NOVEL DRUG DELIVERY SYSTEM

A Novel Drug Delivery System (NDDS) can be defined as a new approach that combines innovative development, formulations, new technologies, and novel methodologies for delivering pharmaceutical compounds in the body as needed to safely achieve their desired pharmacological effects[1].

NOVEL DRUG DELIVERY APPROACHES

Various drug delivery and drug targeting systems are currently under development to minimize drug degradation and loss, prevent harmful side effects, and increase drug bioavailability and the fraction of the drug accumulated in the required zone. Among drug carriers, one can name soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles. The carriers can be made slowly degradable, stimuli-reactive (e.g., pH or temperature-sensitive), and even targeted (e.g., by conjugating them with specific antibodies against certain characteristic components of the area of interest). Targeting is the ability to direct the drug-loaded system to the site of interest.

Two major mechanisms can be distinguished for addressing the desired sites for drug release:

Passive and

Active targeting.

An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumours as a result of the enhanced vascular permeability of tumour tissues compared with healthy tissue. A strategy that could allow active targeting involves the surface functionalization of drug carriers with ligands that are selectively recognised by receptors on the surface of the cells of interest. Since ligand-receptor interactions can be highly selective, this could allow a more precise targeting of the site of interest[1].

ETHOSOMES

Ethosomes are the ethanolic phospholipid vesicles that are used mainly for the transdermal delivery of drugs. Ethosomes have a higher penetration rate through the skin as compared to liposomes hence these can be used widely in place of liposomes. The increased permeation of ethosomes is probably due to their ethanolic content. Ethanol increases the cell membrane lipid fluidity which results in increased skin penetrability of the ethosomes. Transdermal administration of drugs is generally limited by the barrier function of the skin. Vesicular systems are one of the most controversial methods for transdermal delivery of active substances. The interest in designing transdermal delivery systems was relaunched after the discovery of elastic vesicles: transferosomes and liposomes. This article reviews various aspects of ethosomes including their preparation, characterization, potential advantages and their applications in drug delivery[2].

Because of their unique structure, ethosomes can encapsulate and deliver through the skin highly lipophilic molecules such as cannabinoids, testosterone, and minoxidil, as well as cationic drugs such as propranolol, trihexyphenidyl, Cyclosporine A, insulin, salbutamol, etc. Ethosomes provide several important benefits including improving drug efficacy, enhancing patient compliance and comfort and reducing the total cost of treatment[2].

MAIN ROUTES OF PENETRATION

Under normal conditions, the main route is observed through the intercellular spaces or lipid bilayers. The diffusional path length is therefore much longer than the simple thickness of the stratum corneum (20-30mm). The penetration through the skin is also affected by several biological factors such as skin age, body size, skin condition and diseases, the water content of the skin, or hydration. The intercellular spaces contain structured lipids/proteins and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains before reaching the stratum corneum and viable epidermis junction. Although the nature of the barrier is very heterogeneous, the diffusion through the skin can be described by simple Fick's laws[3].

ETHOSOMES AS A NOVEL CARRIER

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are composed mainly of phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentrations of ethanol, and water. The high concentration of ethanol makes them unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid

membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improving drug distribution ability in stratum corneum lipids[4].

MECHANISM OF DRUG PENETRATION

The main advantage of ethosomes over liposomes is the increased permeation of the drug. The mechanism of the drug absorption from ethosomes is not clear. Drug absorption probably occurs in the following two phases.

Ethanol Effect

Ethosomal Effect[5]

METHODS OF PREPARATION OF ETHOSOMES

The literature reports various methods for the preparation of ethosomes and some commonly used methods have been compiled in the preceding text[6].

Hot method

The drug is dissolved in a mixture of ethanol and propylene glycol and the mixture is added to the phospholipid dispersion in water at 40°C. After mixing for five minutes the preparation is sonicated at 4°C for three cycles of five minutes, with a rest of five minutes between each cycle, using the Probe Sonicator. The formulation is then homogenized at 15,000 psi pressure, in three cycles, using a high-pressure homogenizer to get nano-sized ethosomes[6].

Advantages of Ethosomes

Enhanced permeation of drug through skin for transdermal drug delivery.

Delivery of large molecules (peptides, protein molecules) is possible.

High patient compliance the ethosomal drug is administered in semisolid form (gel or cream) hence producing high patient compliance.

Ethosomal system is passive and non-invasive[7].

Disadvantage of ethosomes

Ethosomes may clump together and get precipitated with poor shells and are not economical.

Adhesives will not stick to all kinds of skin.

They were designed for slow and sustained drug delivery.

Loss of product during transfer from organic to water media[8].

EXPERIMENTAL

Determination of FTIR spectrum

The IR spectra of the drug sample were recorded by using the KBr pellet method. The drug was triturated in a porcelain mortar pestle with dry potassium bromide in ratio (1:100). The pellet was prepared in KBr press at pressure 10 tones. The pellet was scanned over the range of 4000-400cm-1 on FTIR spectroscopy. The identified peaks were compared with the principal peaks of the reported IR spectrum of the drug and the samples were authenticated.

FTIR spectrum of nabumetone and a mixture of soya lecithin with excipients was recorded and it was found by the reported peak. There are no observed significant peak shifts and no generation of a new peak, although there might be no possible interaction between the drug and excipients of ethosomes. FTIR spectra were found to be pure, stable and u naltered.

RESEARCH ENVISAGED

Particle Size

Particle size was carried out by using Zetasizer version 6.32 (serial number: MAL1037088). The study was performed with water as a dispersant. The temperature of the assembly was maintained at 25ºC.

Zeta Potential

Zeta potential was carried out by using Zetasizer version 6.32. The study was performed with water as a dispersant. The temperature of the assembly was maintained at 25ºC.

Drug Content

About 500mg of ethosomes was taken and dissolved in 50ml of pH 5.8 PBS. The solution was then passed through the filter paper and 50µl of the filtrate was withdrawn. The filtrate was diluted by adding 3.5ml of distilled water, then the drug content was measured spectrophotometrically at 247nm against the corresponding ethosomes concentration.

Entrapment efficacy

Aliquots of ethosomes were subjected to centrifugation using a cooling centrifuge (REMI) at 5000 rpm. The clear supernatant was siphoned off carefully to separate the unentrapped extract and diluted with phosphate buffer saline (pH 5.8). The

EE was determined in terms of the percentage of drug content in the sediment. The percent entrapment was calculated using the formula

y = (total amount of drug - amount of free drug) / total amount of drug × 100

***In vitro* Studies**

The *In vitro* permeation study was carried out by using the donor and receptor compartment with dialysis membrane. The study was performed with phosphate buffer saline (pH 5.8). The formulation was placed (equivalent to 10mg of the drug) on the upper side of the skin in the donor compartment. The temperature of the assembly was maintained at 37±2º. Samples were withdrawn every hour from the receptor media through the sampling tube and at the same time, the same amount of fresh receptor media was added to make sink condition. Withdrawn samples were analyzed for a constant using a UV/Visible spectrophotometer.

***In vitro* anti-inflammatory activity**

The solution containing 1ml of different concentrations of the test (nabumetone ethosomes) and Standard (nabumetone) solution ranging from 1-10mg/ml was ned with 1ml egg albumin (5% aqueous solution), pH was calibrated at 6.3 in phosphate buffer. After preparation mixtures were incubated at 37°C for 15 min, subsequently heating at 70°C for 30 minutes. After cooling the samples absorbance was measured spectrophotometrically at 600nm and % inhibition of protein denaturation was calculated

Percentage inhibition = [Abs control – Abs treated/Abs control*100]

KINETIC STUDIES

Higuchi Model

This model helps to study the release mechanism of water-soluble and less water- soluble drugs incorporated in semi-solid and solid matrices. The mathematical expression for drug release is,

Q=[D(2C-Cs) Cs.t]1/2

Where Q-Cumulative % of drug released in time "t" per unit area, C = Initial drug concentration, Cs = Drug solubility in the matrix media, D = Diffusion coefficient

Assuming that the diffusion coefficient and other parameters remain constant during release, the above equation reduces to

Q=k.t1/2

Korsmeyer-Peppa's model:

To verify the fact that whether the diffusion follows Fick's law or not, the drug release data can be plotted against 'log time' according to Peppa's equation. The drug release can be expressed as,

Q = Kt n

Taking logs on both sides of the equation, log Q=log K + n log t

where, Q = cumulative % of drug release, t = Time, n = slope of linear plot of log Q Vs log t The "n" value can be used to characterize the diffusional release mechanism and the data is given[12].

RESULTS AND DISCUSSION PARTICLE SIZE

The particle size of nabumetone ethosomes was measured by a particle size analyzer (Malvern version 7.13). The particle size distribution of F8 is 390.6nm. The particle size data showed that ethosomes produced were of nano size and indicated a relatively broad particle size distribution.

ZETA POTENTIAL

The zeta potential report is shown in the above figure. The zeta potential of optimized nabumetone ethosomes (NMF8) was in the range of -11.4mV which indicates good stability.

DRUG CONTENT

The drug content was executed for all the formulations and the results are tabulated in the table. The drug content of nabumetone ethosomes was found to be in the range of 84.17% to 94.19%. The results obtained were within the pharmacopeial limits and indicated uniformity of mixing.

ENTRAPMENT EFFICIENCY

The percentage entrapment efficiency of nabumetone ethosomes for formulation F8 was maximum and minimum for formulation F1. It was observed that the use of phospholipids can change the entrapment efficiency of nabumetone. In the F8 formulation, the phospholipid concentration was decreased compared to the F1 formulation which increased the entrapment efficiency. Therefore, the concentration of phospholipids can change the percentage entrapment efficiency of the formulation.

IN VITRO DRUG RELEASE STUDY

Comparative *In vitro* drug release study of NMF1 to NMF10

The maximum amount of drug can be released in NMF8 formulation and it can be selected as an optimized formulation compare to all other formulation. It enhances the bioavailability and constant drug release in the systemic circulation.

The *In vitro* drug release of all the formulations F1 – F 10 was 44.17%, 57.25%, 56.32%, 63.28%, 55.08%, 73.24%, 77.36%, 91.32%, 80.48% and 84.34% respectively. In this formulation, F8 is selected as an optimized formulation of all other formulations. Because it satisfies all the parameters such as particle size, entrapment efficiency and *In vitro* drug release.

The slope of the curve gives the value of K in zero-order release kinetics. This is ideal behaviour for the dosage form. It refers to the process of constant drug release from a drug delivery system independent of concentration.

Table No.1: FTIR interpretation of drug nabumetone and polymers

S.No	Frequency	Group assigned
1	1705	C = O
2	1607	C = C
3	1640	C – H

Table No.2: Formulation of Nabumetone ethosomes

S.No	Drug (Mg)	Soya Lecithin (Mg)	Ethanol (Ml)	Propylene Glycol (Ml)	Cholesterol (Ml)	Water (Ml)
F1	10	50	20	5	5	q. s
F2	10	40	20	5	5	q. s
F3	10	30	20	5	5	q. s
F4	10	25	20	5	5	q. s
F5	10	20	20	5	2.5	q. s
F6	10	25	20	2.5	5	q. s
F7	10	25	20	2.5	2.5	q. s
F8	10	25	20	2.5	2.5	q. s
F9	10	25	20	1	2.5	q. s
F10	10	25	20	2	2.5	q. s

The particle size distribution

			Size (r. nm)	%Intensity	Width (r.nm)
Z-Average (r. nm)	390.6	Peak1	298.7	93.7	50.86
PDI	0.759	Peak 2	16.75	6.3	1.733
Intercept	0.864	Peak 3	0.000	0.0	0.000

Result quality: Refer to quality report

The zeta potential report

Zeta Potential (mV)	-11.4	Peak 1	Mean (mV)	Area (%)	Width (mV)
			-11.4	100.0	3.40
Zeta Deviation (mV)	3.40	Peak 2	0.00	0.0	0.00
Conductivity (mS/cm)	0.0324	Peak 3	0.00	0.0	0.00

Result quality: Good

Table No.3: Percentage Drug Content

S.No	Formulation Code	Drug Content
1	NMF1	84.17%
2	NMF2	87.92%
3	NMF3	88.62%
4	NMF4	89.13%
5	NMF5	85.71%
6	NMF6	89.79%
7	NMF7	91.81%
8	NMF8	94.19%
9	NMF9	93.92%
10	NMF10	93.15%

Table No.4: Percentage Entrapment Efficiency

S.No	Formulation code	Entrapment efficiency
1	NMF1	79.97%
2	NMF2	85.12%
3	NMF3	84.22%
4	NMF4	87.11%
5	NMF5	81.91%
6	NMF6	89.61%
7	NMF7	90.31%
8	NMF8	93.67%
9	NMF9	91.12%
10	NMF10	92.45%

Table No.5: Comparative *In vitro* drug release

Time (in hours)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	3.8%	4.8%	4.75%	5.33%	4.66%	6.16%	6.5%	7.6%	6.75%	7.08%
2	6.97%	8.69%	8.61%	10.18%	8.93%	13.35%	12.94%	14.4%	12.53%	13.2%
3	9.88%	13.53%	13.29%	15.03%	13.02%	17.56%	18.56%	22.06%	19.26%	20.25%
4	13.3%	18.19%	18.82%	20.38%	16.70%	23.7%	25.09%	29.17%	27.08%	27.42%
5	17.23%	22.25%	22.25%	25.76%	22.08%	30.24%	31.63%	37.44%	32.88%	34.55%
6	21.07%	28.09%	27.62%	30.23%	27.07%	36.13%	38.16%	45.18%	40.29%	41.67%
7	24.93%	32.07%	32.38%	36.48%	31.40%	42.34%	44.69%	52.88%	46.45%	48.8%
8	29.22%	37.80%	38.28%	41.84%	36.43%	48.53%	51.32%	60.58%	54.14%	55.85%
9	32.64%	42.68%	41.94%	47.2%	40.37%	54.65%	57.76%	68.24%	60.18%	63.02%
10	36.49%	47.54%	46.71%	52.59%	45.81%	60.43%	64.47%	75.95%	66.80%	70.23%
11	39.93%	52.39%	51.48%	57.89%	50.49%	67.12%	70.83%	83.70%	73.59%	77.26%
12	44.17%	57.25%	56.32%	63.28%	55.08%	73.24%	77.36%	91.32%	80.48%	84.34%

IN VITRO ANTI-INFLAMMATORY ACTIVITY

EFFECT OF NABUMETONE ON PROTEIN DENATURATION (STANDARD)

Table No.6: Protein Denaturation of Nabumetone

S.No	Concentration (Mg)	% Inhibition
1	10mg	96.62%

EFFECT OF NABUMETONE ETHOSOMES ON PROTEIN DENATURATION (TEST)

Table No.7: Protein Denaturation of Nabumetone Ethosomes

S.No	Concentration (mg)	% inhibition
1	10mg	88.78%

HIGUCHI MODEL

Table No.8: Higuchi Model

S.No	Cumulative % drug release	Square root of time in hours
1	7.6	1
2	14.4	1.414
3	22.06	1.732
4	29.17	2
5	37.44	2.236
6	45.18	2.449
7	52.88	2.645
8	60.58	2.828
9	68.24	3
10	75.95	3.162
11	83.07	3.316
12	91.32	3.464

Table No.9: Korsmeyer-Peppas model

S.No	Log cumulative % drug release	Log time in hours
1	0.88	0
2	1.15	0.301
3	1.343	0.477
4	1.464	0.602
5	1.572	0.698
6	1.654	0.778
7	1.723	0.845
8	1.782	0.903
9	1.834	0.954
10	1.880	1
11	1.919	1.041
12	1.960	1.079

ZERO ORDER KINETICS

Table No.10: Zero order Kinetics

Time in hours	Cumulative % drug release
1	7.6
2	14.4
3	22.06
4	29.17
5	37.44
6	45.18
7	52.88
8	60.58
9	68.24
10	75.95
11	83.07
12	91.32

Table No.11: Regression Coefficient

Formulation code	Regression		
	Zero-order	Higuchi	Korsmeyer Peppas
F8	0.9999	0.9969	0.9936

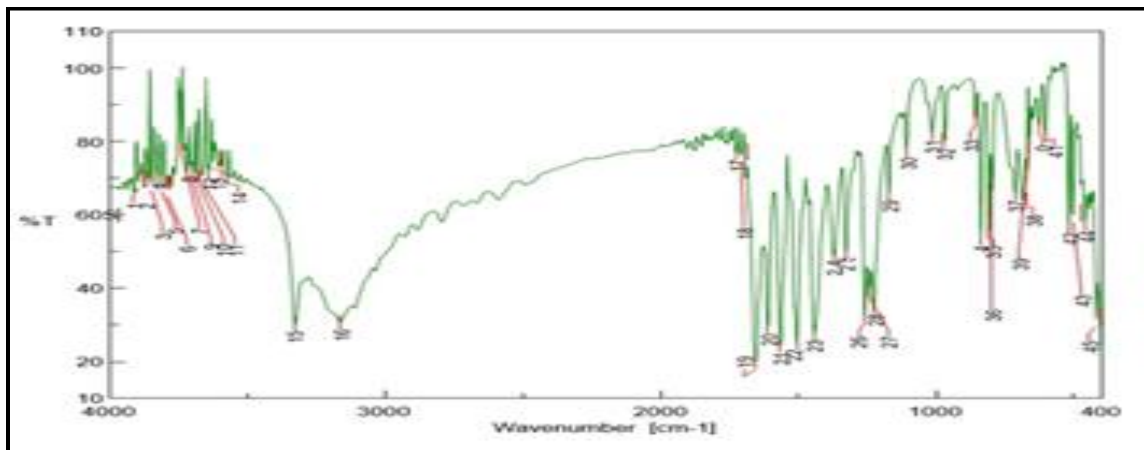

Figure No.1: FTIR Spectra of Nabumetone

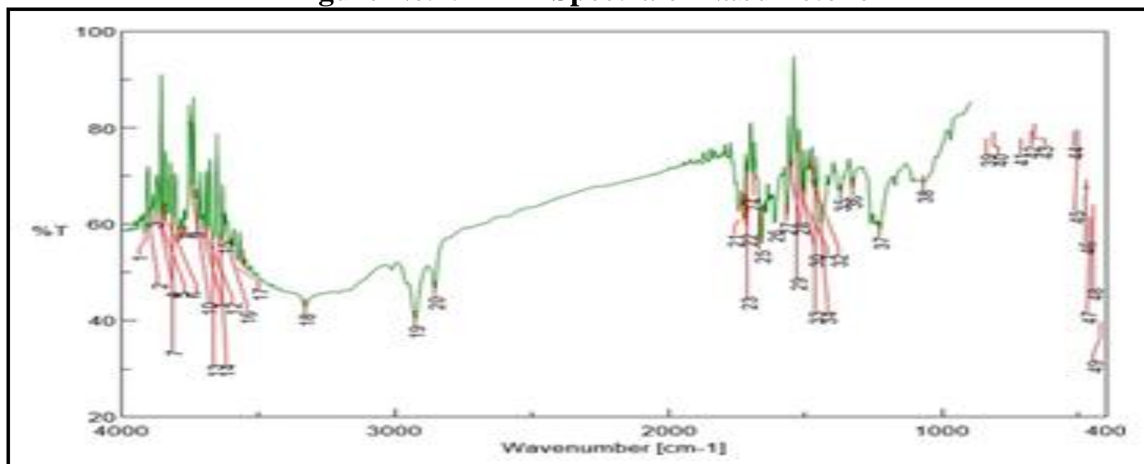

Figure No.2: FTIR Spectra of drug and excipients Composition of Nabumetone Ethosomes

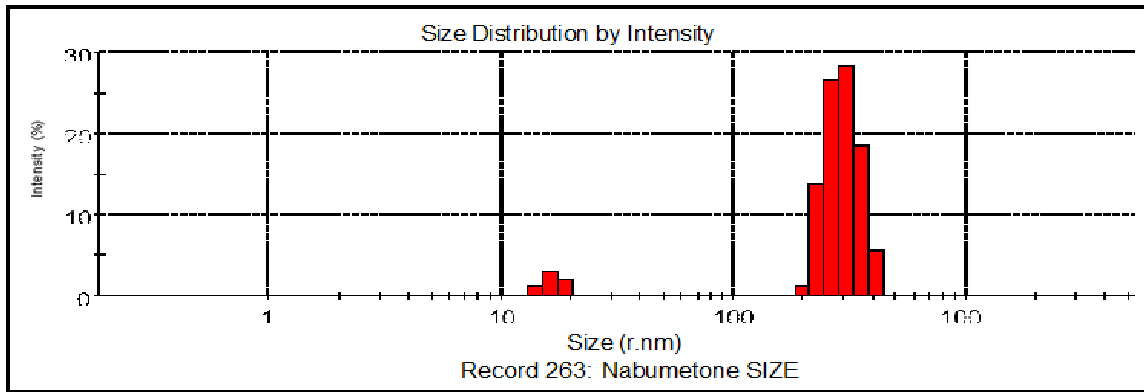

Figure No.3: Particle Size of Nabumetone

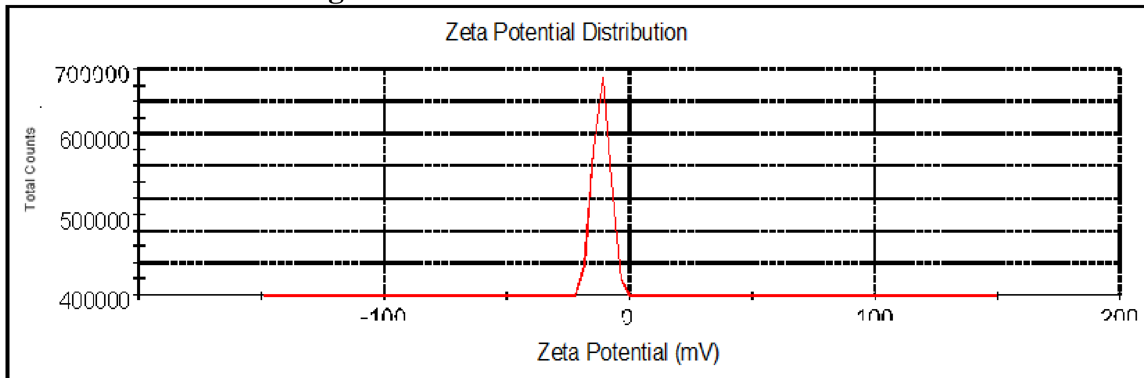

Figure No.4: Zeta potential of Nabumetone

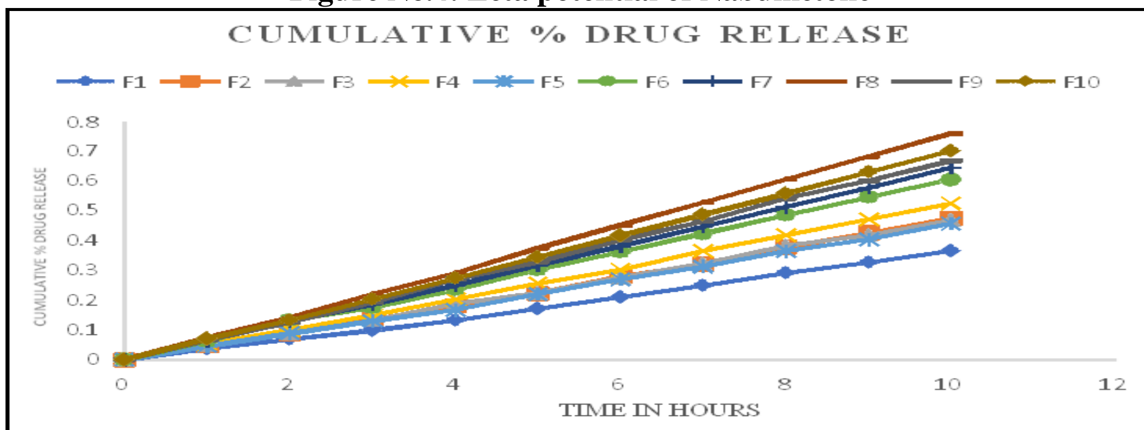

Figure No.5: Comparative of *In vitro* drug release

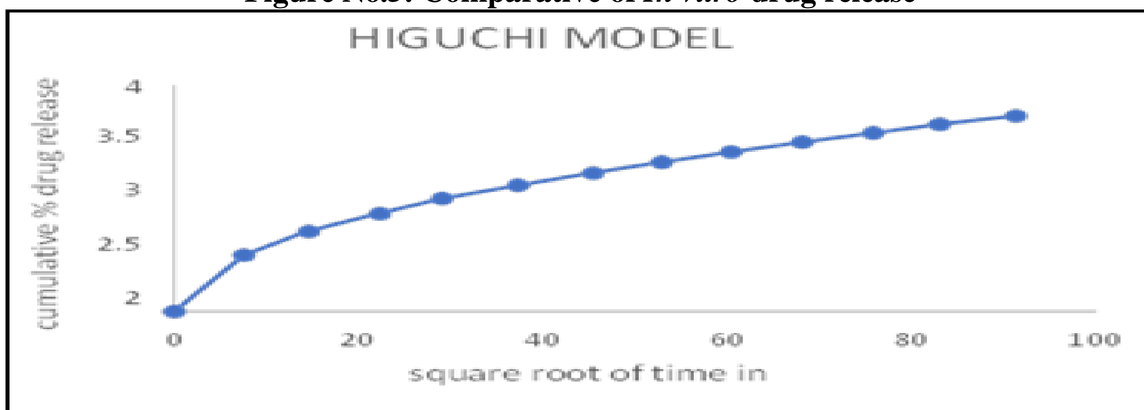

Figure No.6: Higuchi Model Korsmeyer-Peppas Model

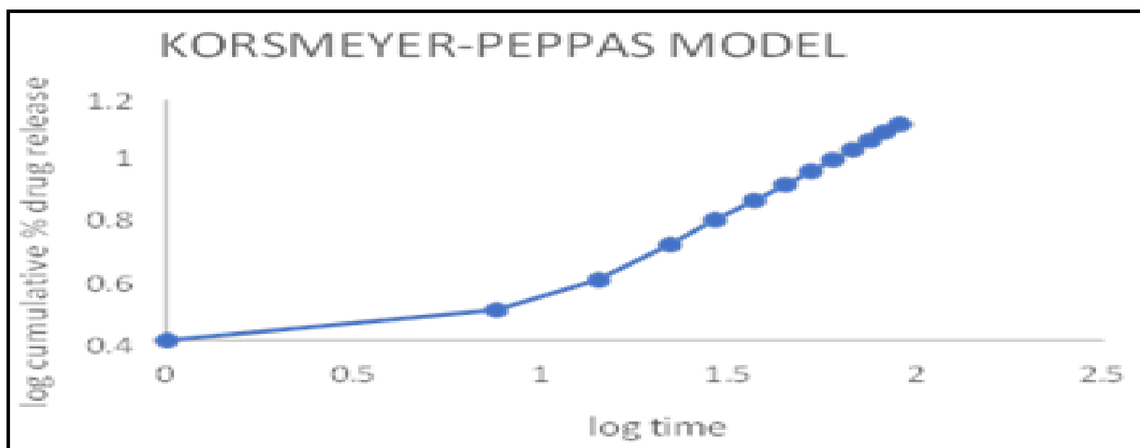

Figure No.7: Korsmeyer-Peppas Model

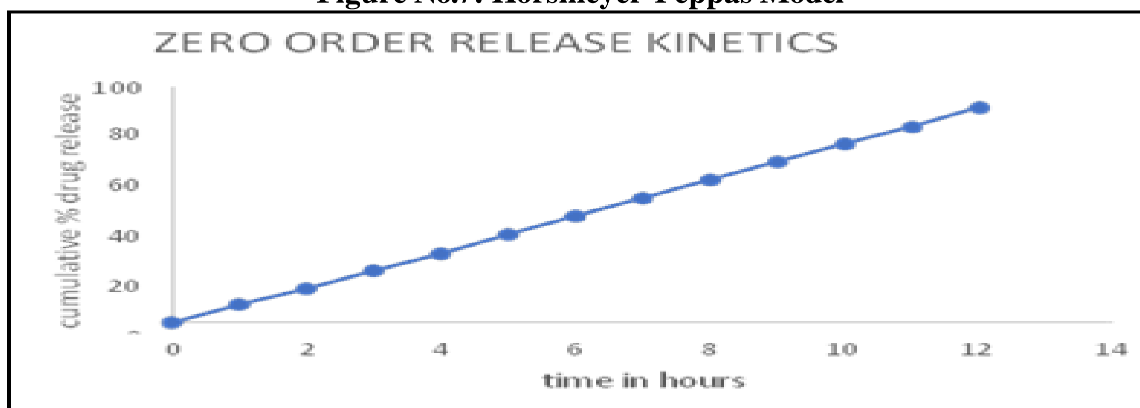

Figure No.8: Zero order kinetics

SUMMARY AND CONCLUSION

SUMMARY

In this study, we have taken an effort to formulate and evaluate the ethosomes containing nabumetone to increase the solubility of the drug through the transdermal route.

FT-IR spectroscopy approach was used to detect any possible chemical interaction between nabumetone and polymers. The study revealed that there was no major interaction between the nabumetone and other excipients used. Different formulations of Nabumetone ethosomes were prepared based on hot technique using polymers.

The formulation F8 was selected as an optimized formulation based on the results in terms of required particle size (390.6nm), zeta potential (-11.4mV), drug content (94.19%), entrapment efficiency (93.67%), *In vitro* drug release studies (91.32% - extended up to 12 hours), *In vitro* anti-inflammatory activity (88.78%) and kinetic drug release studies in a sustained release manner and also were found to be stable.

CONCLUSION

In this present work, ethosomes have been prepared to incorporate a low soluble anti- inflammatory drug nabumetone using polymers to enhance the solubility of drug.

From the results of the present experimental investigation, it may be concluded that the formulation of Nabumetone ethosomes showing better particle size, zeta potential, drug content and entrapment efficiency. The *In vitro* drug release and *In vitro* anti-inflammatory activity shown satisfactory results. The optimized formulation follows zero order kinetics and also appropriate diffusional release mechanism.

So, we can conclude that the result of the current study indicates a promising potential of ethosomes as an alternative to conventional dosage forms to enhance the bioavailability and biocompatibility.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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