

**FORMULATION AND EVALUATION OF ETHOSOMAL GEL OF
DICLOFENAC SODIUM AND CAPSAICIN****Komal Gupta***

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ABSTRACT

Ethosomes are ethanolic liposomes. Ethosomes are drug delivery carriers that are noninvasive and causes the drugs to transfer to the deep layers of skin and/or the systemic circulation. These are composed mainly of phospholipids such as phosphatidylcholine (PC), phosphatidyl serine (PS) and phosphatidic acid, high concentration of ethanol and water. The aim of the study was to improve the transdermal penetration of diclofenac sodium, poorly soluble drug. The purpose of the work was to formulate the vesicular carrier of combination of Diclofenac Sodium; an anti-inflammatory drug and Capsaicin; a powerful counterirritant in the form of ethosomes using phospholipids. The prepared ethosomes were characterized for

vesicular size distribution, vesicular shape and surface morphology, percent entrapment efficiency, drug excipient compatibility study and zeta potential. Topical gels were prepared by cold method using carbopol 934 and ethosomal suspensions were incorporated into carbopol gel. The ethosomal gels were evaluated for pH, drug content, viscosity and in vitro drug permeation studies. The release profile was evaluated using release studies of different developed ethosomal formulations and comparison with available brands in markets. Results revealed that ethosome formulations can act as drug reservoir in the skin and extend the pharmacologic effects of diclofenac sodium.

KEYWORDS: ethosomes, ethanol, vesicular, entrapment efficiency, pharmacologic, viscosity.

1. INTRODUCTION

Topical delivery can defined as the application of a drug containing formulation to the skin to

directly treat cutaneous disorder or general disease with the intent of possessing the therapeutic or other properties of the drug to the skin surface or within the skin. Topical preparations are frequently applied for the functioning at the site of their action with respect to penetration of drug into the underlying skin layers or mucous membranes. The wide range of topical products constitute semisolid preparations that comprise of ointment, cream, and gel.^[1]

Basic Principle of permeation

In the initial step, drugs molecules along the hair follicles or sweat ducts enter the skin and then absorption is done by the follicular epithelium and sebaceous glands. When a constant state has been achieved, diffusion by stratum corneum becomes the dominant pathway.

Drug Permeation incorporates the underlying steps

- A) Sorption by stratum corneum.
- B) Penetration of the drug through viable epidermis.
- C) Drug uptake in the dermal papillary layer by capillary network.

Gels

Gels are system of semisolid preparation in which a liquid phase is contrived within a natural or synthetic gums polymeric matrix of three dimensional in which a vast degree of cross linking has been produced.^[2] The USP defines gels as a semisolid system consisting of dispersion produced by either inorganic particles of small size or organic molecules that are of larger size incorporated and penetrated by liquid.^[3]

ETHOSOMES

“Ethosomes are ethanolic liposomes”.^[4]

Ethosomes are drug delivery carriers that are noninvasive and causes the drugs to transfer to the deep layers of skin and/or the systemic circulation. These are vesicles that are soft, malleable and are designed for increasing the delivery of pharmaceutical agents. The vesicles are greatly known for their application in cellular communication and transportation of particle for no of years. Vesicle permit the controlling of rate of drug release for an extended time, by protecting the drug from immune response or other systems and thus has the potential to deliver just the right quantity of drug and maintain that concentration constant for larger periods of time. The major advancement in research of vesicle include finding of a substitute of vesicle, which is also named as ethosomes.^[5,6] These are composed mainly of

phospholipids such as phosphatidylcholine (PC), phosphatidyl serine (PS) and phosphatidic acid, high concentration of ethanol and water.^[7] The use of lipid vesicles in delivery systems for curing the skin disorders has influenced enhanced attention. Further, it is mostly concluded that classic liposomes are of no value as transdermal drug delivery carriers because they do not deeply penetrate the skin, but rather remain limited to the superficial layer of the stratum corneum.^[8,9] Only uniquely designed vesicles were proven to allow drug through transdermal delivery.^[10] Ethanol is known as an efficient permeation enhancer.^[11]

2. EXPERIMENTAL WORK

A) Preformulation Studies

Pre-formulation studies on the obtained sample of drug were performed for identification and compatibility studies.

- i. Organoleptic properties:** The organoleptic properties like general appearance i.e. nature, color, odour etc. were evaluated by visual observations
- ii. Melting point:** Melting point was determined with the help of melting point apparatus by the capillary method.
- iii. Determination of absorption maxima (λ max) of drug, mixture of both drugs (i.e. combination of Diclofenac sodium and Capsaicin) by UV/VIS spectrophotometer**
UV scan of drugs: UV scans was done to know the λ max for preparation of standard curves in Acetonitrile (ACN): Water: Buffer (0.05% v/v Ortho phosphoric acid buffer): 75:10:15, ethanol and distilled water. For this purpose, Stock solutions of drug samples were prepared in three solvents. ACN: water: buffer was used as solvent for both drugs.

Preparation of standard curve of drugs using UV/VIS spectrophotometer: For determination of content of drug, standard plot of drug was prepared in ethanol and in distilled water, in a suitable concentration range. These are analyzed spectrophotometrically at 276 nm for diclofenac sodium and at 280 nm for capsaicin using UV/VIS spectrophotometer.^[12]

- iv. Solubility:** Solubility of drug was determined in various solvents such as water, methanol, ethanol, chloroform, dichloromethane, DMSO, 0.1 N HCl and phosphate buffer pH 6.8.
- v. Partition Coefficient:** The partition coefficient of a drug is usually referred to as the oil/water equilibrium partition coefficient and is therefore a measure of the drug lipid solubility. Partition coefficient of Diclofenac Sodium and Capsaicin were determined at $37 \pm 5^\circ\text{C}$ with 10 ml of octanol and saturate it with 10 ml of water by shaking with externally driven magnetic stirrer. After shaking, system remained undisturbed for 30 min.

drug about 10 mg of was incorporated into this solution and was shaken on wrist action mechanical stirrer. Two layers were separated through separating funnel and filtered through whatmann filter paper, and the amount of drug solubilized was determined by measuring the absorbance at 276 nm for Diclofenac Sodium and 280 nm for Capsaicin against reagent blank through double beam UV/VIS spectrophotometer in both solutions.

vi. Drug Excipient compatibility studies: The possible interaction between drugs and excipients were studied by FTIR spectroscopy.^[13]

B) Preparation of Ethosomes

Ethosomes were prepared by cold method using phospholipids, ethanol and drugs subsequently it was incorporated into topical gel which was prepared by using Carbopol-934. Soya phosphatidyl choline (SPC) was dissolved along with the drug in ethanol in a beaker. The beaker was sealed or covered with aluminum foil. Magnetic stirrer was used to stir the solution at 1500 rpm and Milli pore water was added at a constant rate of 1 ml/min at room temperature through a syringe pump. After addition of water stirring was continued for additional 30 min. The preparation was stored at 4° C. Ethosome prepared by the above procedure were subjected to sonication at 4°C causing probe sonicator in 3 cycles of 5 minutes with 5 minutes rest between the cycles. The optimization of formulations was carried out by varying SPC and alcohol concentration from 1-3% and 20–40%, respectively.

Ethosomal formulation was composed from drug, lipids and ethanol. Composition of ethosomal formulations and drug ratio were as shown in below table 2.1.

Table 2.1: Composition of ethosomal formulations and drug ratio

S.N	Name of Drug	Conc. Of drug	Lipid	Drug: lipid ratio
1.	Diclofenac Sodium	1 % w/w	Soya lecithin	1:3
2.	Capsaicin	0.05 % w/w	Soya lecithin	1:60

C) Evaluation of Ethosomes^[14]

The prepared ethosomes of anti-inflammatory drugs; Diclofenac Sodium and Capsaicin were evaluated for following parameters.

- Vesicular shape and surface morphology:** Vesicular shape of the ethosomal preparations were investigated by using a transmission electron microscope (TEM) with an accelerating voltage of 80KV
- Determination of percentage entrapment efficiency:** Percent entrapment efficiency of Ethosomal preparations of Diclofenac Sodium and Capsaicin were estimated by

ultracentrifugation technique.

Entrapment efficiency was calculated by using the formula.

$$\% EE = \frac{Q_t - Q_s}{Q_t} \times 100$$

Where % EE is the Percent entrapment efficiency.

Q_t is the theoretical amount of drugs (either Diclofenac sodium or Capsaicin) that was added and Q_s is the amount of drugs detected only in supernatant.^[15]

iii. Zeta potential determination: Zeta potential is defined as the difference in potential between the surface of the tightly bound layer and the electro-neutral region of the solution. Zeta potential of vesicles was determined using Zeta sizer at $25 \pm 1^\circ\text{C}$.^[16]

D) Preparation of carbopol gel base^[17]

Carbopol gel was prepared using Carbopol 934 as a gelling agent in 1% w/w concentration with distilled water using mechanical stirrer. Accurately weighed amount of Carbopol 934 was taken, dispersed in distilled water with mild stirring and allowed to swell for 24 hr. to obtain 1% gel. Other excipients were also added with continuous stirring.

Table 2.2: Composition of carbopol gel formulations.

S. No	Ingredients	Quantity (%w/w)
1.	Carbopol 980	1%
2.	Dimethylsulfoxide	5%
3..	Triethanolamine	0.5 %
4.	Methyl paraben	0.1%
5.	Propyl paraben	0.03%
6.	Water	q.s.

E) Incorporation of Ethosomal Suspension into Gel

Ethosomal suspension (10ml) containing DS (100 mg) and CAP (5 mg) was slowly added in the carbopol gel with continuous stirring at 700rpm in a closed vessel and maintained at temperature 30°C until homogeneous in order to achieve ethosomal gel. The pH was then adjusted to neutral using triethanolamine and stirred slowly till a gel was obtained.^[18]

F) Evaluation of Ethosomal Gel^[19]

The prepared ethosomal gels were evaluated for following parameters:

- **pH:** The pH measurement of formulations was carried out using a pH meter by dipping glass electrode completely into gel so as to cover the electrode.

- **Rheological studies:** The rheological analysis of prepared gels was measured using Brookfield Viscometer.
- **Drug content:** Gel formulations (100 mg) was dissolved in phosphate buffer (pH 6.8) and filtered and the volume was made to 100 ml with phosphate buffer. The resultant solution was suitably diluted with phosphate buffer and absorbance was measured at 281 nm for both drugs; Diclofenac Sodium and Capsaicin.^[20]
- **In-vitro drug permeation studies:** In-vitro drug release study of Diclofenac Sodium and Capsaicin from ethosomal gel formulation was studied using a Franz glass diffusion cell.

3. RESULT AND DISCUSSION

A) Preformulation studies

- Organoleptic properties of drug:** Diclofenac Sodium was found to be a white to slightly yellowish crystalline powder, slightly hygroscopic and odorless. Capsaicin was found to be pure dark red solid, highly volatile, pungent odor and burning taste detectable.
- Melting point:** The obtained sample of drug of diclofenac sodium and capsaicin were found to be in the range of 275-277°C and 62-65°C respectively. It complies with the standard thus indicating the purity of the drug sample.
- Determination of absorption maxima (λ max) of drug by UV spectrophotometer.** UV scans of Diclofenac Sodium.

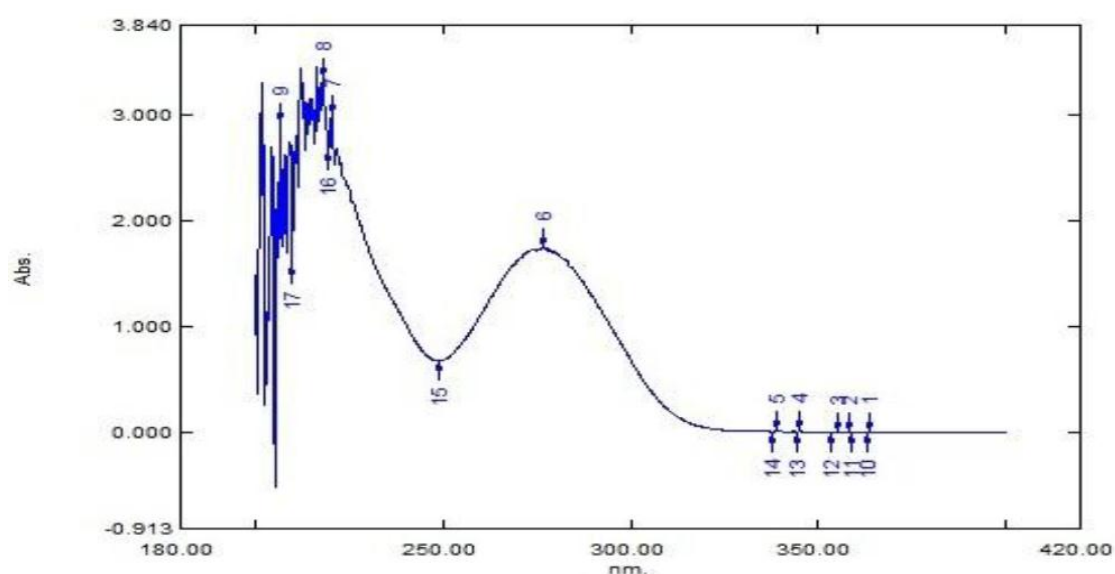


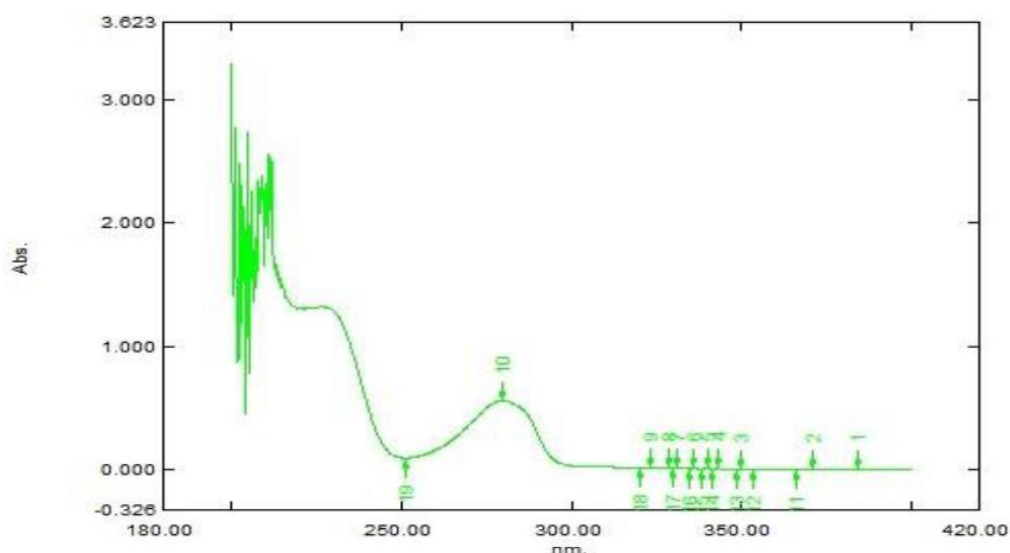
Figure 3.1: UV scan of DS in ACN: Water: Buffer.

Table 3.1: UV scans of Diclofenac sodium.

Sr. No.	Wavelength	Abs.
1	340	0.001
2	276	1.741
3	220.80	1.719

UV scans of Capsaicin

An absorption maximum (λ max) of drug was determined by UV/VIS spectrophotometer. UV spectra of drug in Ethanol and ACN: Water: Buffer showed maxima absorbance at 280 nm.

**Fig 3.2: UV scan of Capsaicin in ACN: Water: Buffer.****Table 3.2: UV scans of capsaicin.**

Sr. No.	Wavelength	Abs.
1	340	0.02
2	280	0.635
3	266	0.005

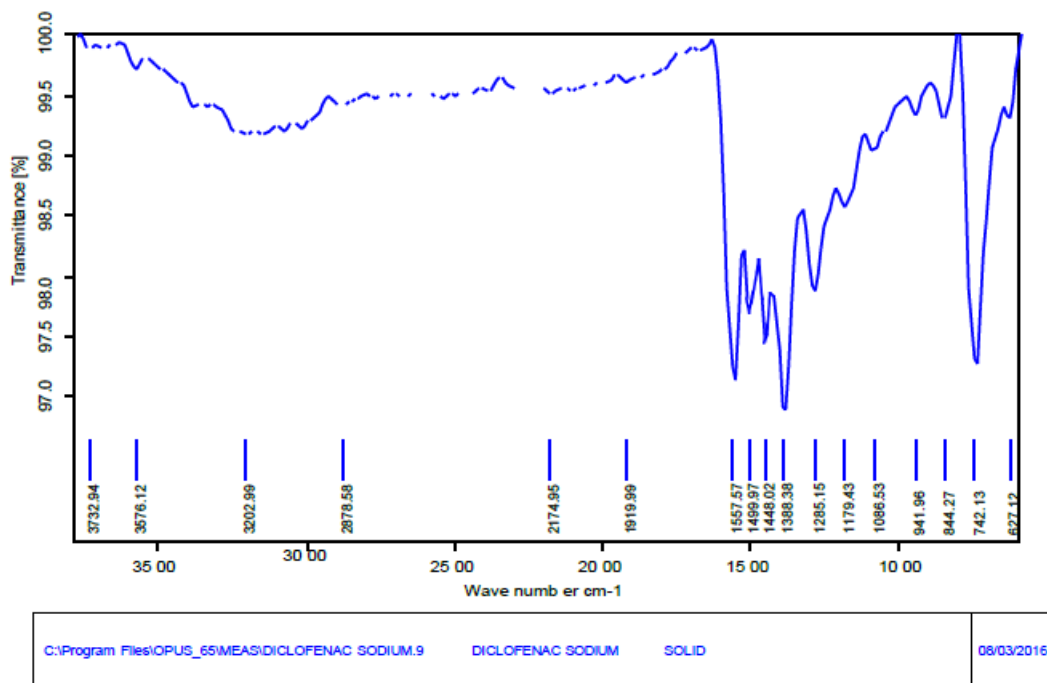
iv. Solubility: Diclofenac Sodium was found to be freely soluble in methanol, soluble in ethanol (95%), soluble in water, phosphate buffer (pH 7.2), slightly soluble in glacial acetic acid and acetone, practically insoluble in chloroform, ether and toluene. Capsaicin was found to be freely soluble in alcohol; soluble in ether, chloroform, benzene and DMSO; slightly soluble in HCL, petroleum; insoluble in water.

v. Partition coefficient: The partition coefficient of Diclofenac sodium was found to be 13.60 and partition coefficient of Capsaicin was found to be 4.73. The partition coefficient value of Diclofenac sodium and Capsaicin were more than zero indicating that both drugs are

lipophilic drugs.

vi. Drug Excipients compatibility study: The possible interaction between drugs and excipients were studied by FTIR spectroscopy.

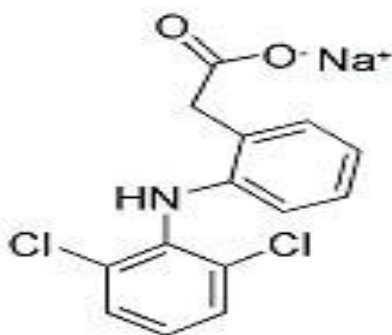
IR Spectrum of Diclofenac Sodium



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Fig 3.3: IR spectrum of Diclofenac Sodium.

Structure



In this IR spectrum, pure Diclofenac Sodium showed major peaks at 3202, 742, 1558, 1448 and 2878 cm^{-1} . The following peaks were observed which may be due to presence of different functional groups of Diclofenac Sodium.

Table 3.3: IR interpretation of Diclofenac sodium.

Functional group	Wave number (cm ⁻¹)
N-H stretch	3202cm ⁻¹
C-Cl stretch	742 cm ⁻¹
C=O stretch	1558cm ⁻¹
C=C stretch	1448cm ⁻¹
C-H stretch	2878cm ⁻¹

IR Spectrum of Capsaicin

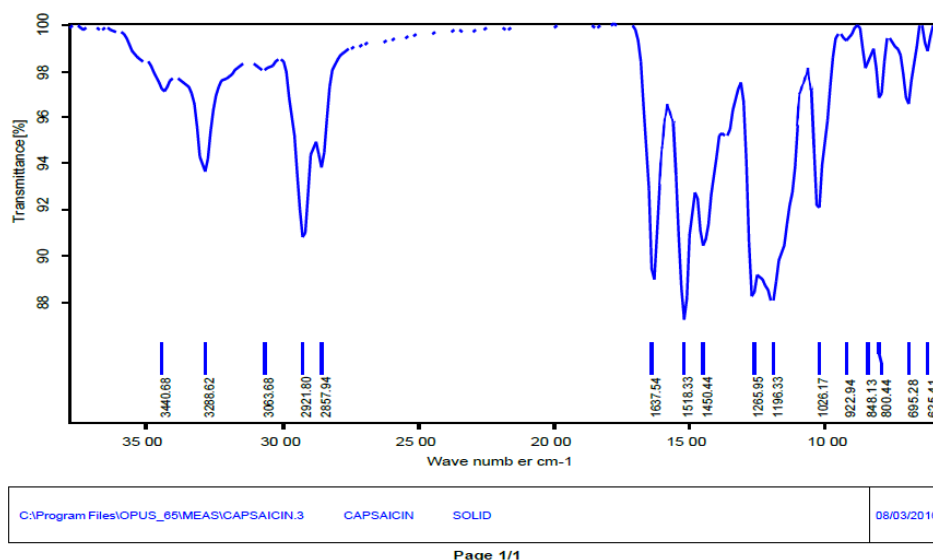
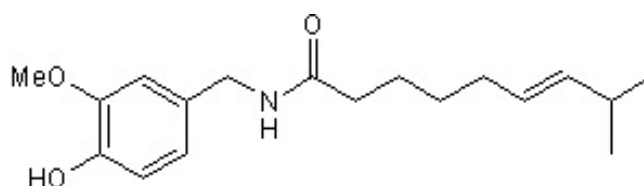


Fig 3.4: IR spectrum of Capsaicin.

Structure



In this IR spectrum, pure Capsaicin showed major peaks at 3440, 2921, 1637, 1518 and 1026 cm⁻¹. The following peaks were observed which may be due to presence of different functional groups of Capsaicin.

Table 3.4: IR interpretation of capsaicin.

Functional group	Wave number (cm ⁻¹)
N-H stretch	3440cm ⁻¹
C=O stretch	1637cm ⁻¹
C-O stretch	1026cm ⁻¹
C=C stretch	1518cm ⁻¹
C-H stretch	2921cm ⁻¹

FTIR Spectrum of Mixture of Diclofenac Sodium, Capsaicin and Phospholipid

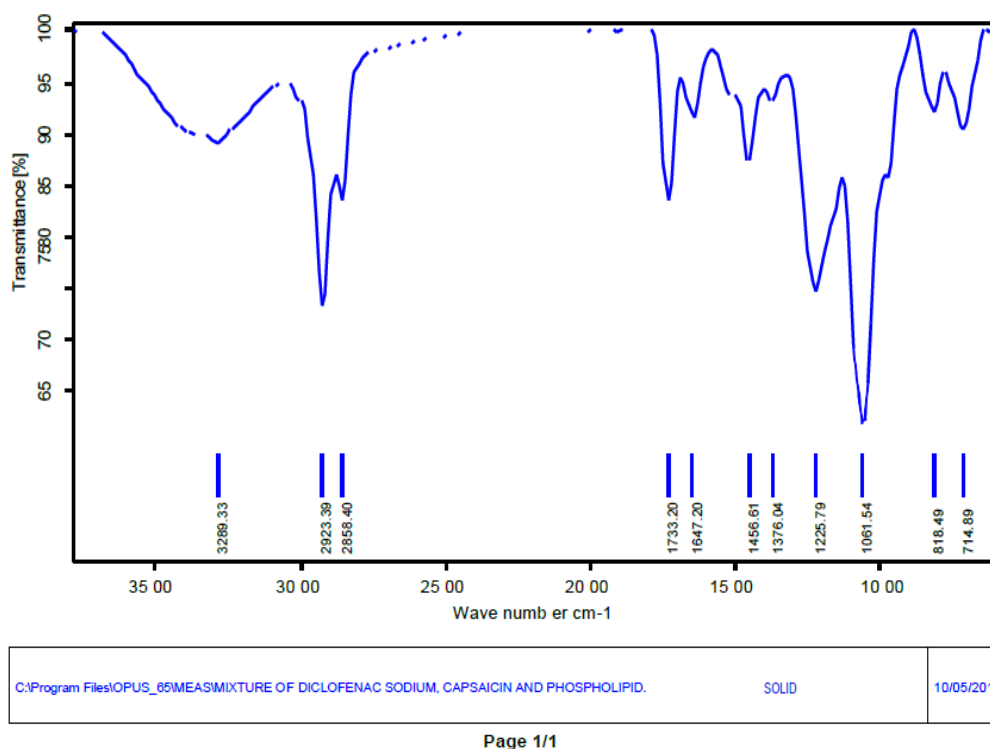


Fig 3.5: IR Spectrum Diclofenac Sodium, Capsaicin and Phospholipid

In this IR spectrum, Diclofenac Sodium, Capsaicin and Phospholipid showed major peaks at 3289, 2923, 2858, 1733, 1647, 1456, 1061, 818 and 714 cm^{-1} . The following peaks were observed which may be due to presence of different functional groups of mixture of drugs and phospholipid.

Table 3.5: IR interpretation of Diclofenac Sodium, Capsaicin and Phospholipid.

Functional group	Wave number (cm^{-1})
N-H stretch	3289 cm^{-1}
C-H stretch	2923 cm^{-1} and 2858 cm^{-1}
C=C stretch	1456 cm^{-1}
C-O stretch	1061 cm^{-1}
N-H rocking	818 cm^{-1}
C=O stretch	1733 cm^{-1} and 1647 cm^{-1}
C-Cl stretch	714 cm^{-1}

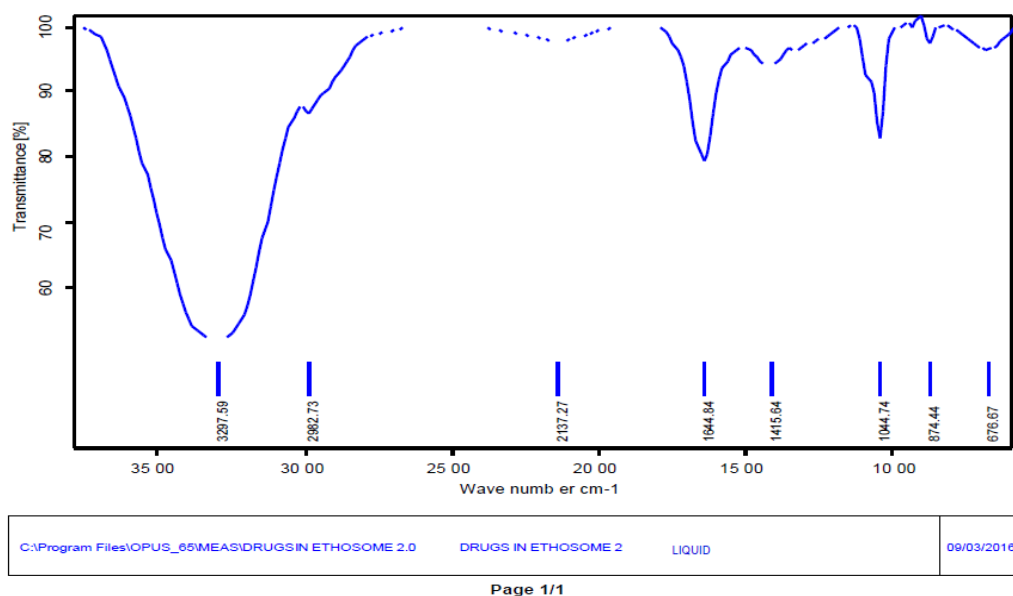


Fig 3.6: IR spectrum of Ethosomal formulation.

In this IR spectrum, Formulation Ethosome containing drugs showed major peaks at 3297, 2982, 1644, 1415, 1044 and 676 cm^{-1} . The following peaks are observed which may be due to presence of different groups of drugs and phospholipid.

Table 3.6: IR interpretation of Ethosomal formulation.

Functional group	Wave number (cm^{-1})
N-H stretch	3297 cm^{-1}
C-H stretch	2982 cm^{-1}
C=C stretch	1415 cm^{-1}
C-O stretch	1044 cm^{-1}
C-Cl stretch	676 cm^{-1}
C=O stretch	1644 cm^{-1}

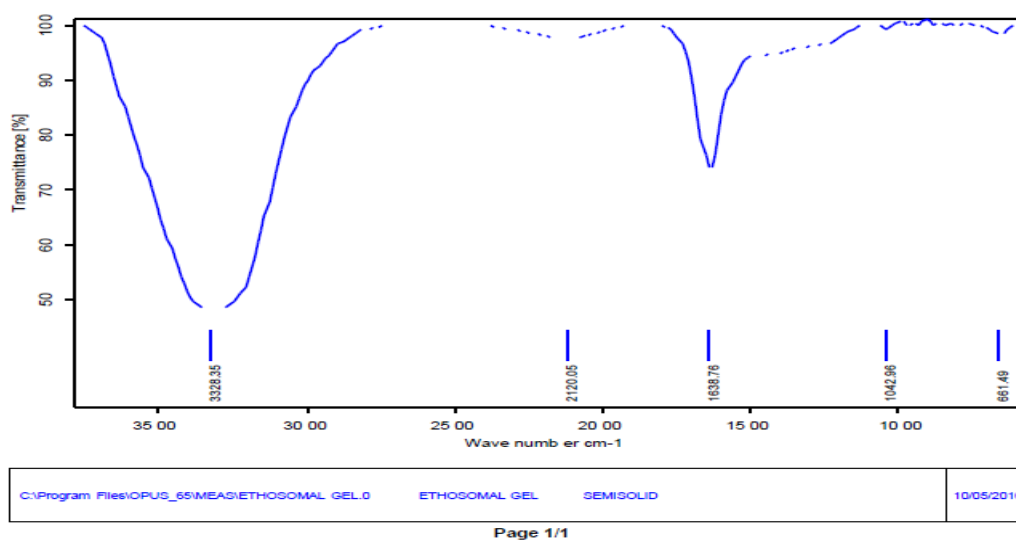


Fig 3.7: IR spectrum of Ethosomal Gel.

In this IR spectrum, Formulation of Ethosomal Gel which was containing drugs showed major peaks at 3328, 1638, 1044 and 661 cm^{-1} . The following peaks are observed which may be due to presence of different functional groups of drugs and excipients.

Table 3.7: IR interpretation of ethosomal gel.

Functional group	Wave number (cm^{-1})
N-H stretch	3328 cm^{-1}
C-O stretch	1044 cm^{-1}
C-Cl stretch	661 cm^{-1}
C=O stretch	1638 cm^{-1}

From IR data, it was observed that functionality of both drugs have remained unaffected, including intensities of the peak. This suggests that during development of formulation, excipients have not reacted with the drugs to give rise to reactant products. So there is no interaction between them which is in favor to keep for formulation.

B) Characterization of Ethosomes

i. Vesicular Shape and Surface Morphology: Investigation of formulations by TEM indicated spherical structure of vesicles with a smooth surface.

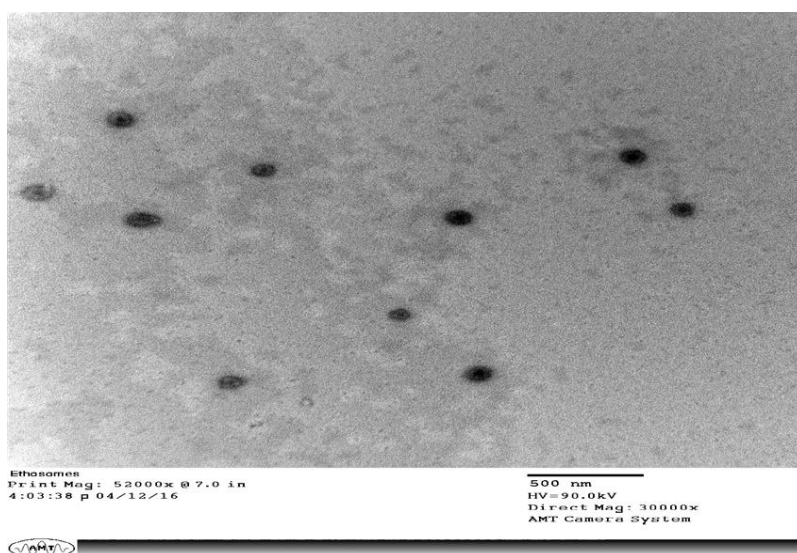


Fig 3.8: Transmission Electron Photomicrographs of Ethosomal formulation.

The prepared ethosomes were found to be spherical and discrete in shape. The vesicles size range was found to be 50 to 58.8 nm.

ii. Percentage Entrapment Efficiency (%EE): The % entrapment efficiency of ethosomal formulations of Diclofenac Sodium and Capsaicin was found to be 55.72 % of and 72.44 %

respectively.

iii. Zeta Potential: Zeta potential of ethosomal formulations of Diclofenac Sodium and Capsaicin was found to be -12.4 ± 2.85 . It was observed that zeta potential of prepared vesicles has sufficient charge to inhibit aggregation of vesicles and indicating stable vesicles.

C) Evaluation of Ethosomal Gel (EG)

- **pH**

Table 3.8: pH of ethosomal gel.

S. No.	Formulation code	pH
1	EG-C 1%	6.73 ± 0.30
2	EG-C 2%	5.23 ± 0.57
3	Marketed formulation	6.8 ± 0.35

- **Drug Content:** The drug content of prepared ethosomal gel of Diclofenac Sodium and Capsaicin were found to be 96.20 ± 0.86 % and 94.25 ± 0.76 % respectively.
- **Viscosity:** The viscosity of EG-C 1%, EG-C 2% and marketed formulation at 100 rpm of spindle was found to be 16100 ± 310 , 35400 ± 416 and 20400 ± 350 . The viscosity of formulation EG-C 1% was found to be optimum value.
- **In vitro drug permeation study:** Result revealed that % cumulative drug permeated from Diclofenac Sodium and Capsaicin ethosomal formulations were found to be 52.56 ± 0.54 and 64.76 ± 0.42 respectively. Drugs permeation from ethosomal gel was found to be higher as compared to the carbopol gel as well as in comparison with marketed formulation.

4. CONCLUSION

In this study ethosomal gel of Diclofenac Sodium, a NSAID and Capsaicin, a powerful counterirritant was formulated and evaluated. Ethosomes of the drugs were prepared by cold method using phospholipids, ethanol; subsequently this combination of ethosomal suspension was incorporate into topical gel which is prepared by using Carbopol-934.

The prepared ethosomes were characterized for vesicles size, vesicle shape and surface morphology, percentage entrapment efficiency by ultracentrifugation method, zeta potential and drug-drug, drug-excipients compatibility studies by IR spectroscopy. The prepared ethosomal gel was characterized for pH, drug content, viscosity and in vitro skin permeation

studies.

- IR study concluded that no major interaction occurred between drug, phospholipids and carbopol-934 used in the present study.
- Morphology of vesicles; the prepared ethosomes were spherical and discrete in shape. Average particle size of the prepared ethosomal formulations was found to be 55.13 nm.
- Percentage entrapment efficiency of prepared ethosomal formulations of DS and CAP were found to be $55.72 \pm 0.44 \%$ and $72.44 \pm 0.98 \%$ respectively.
- The pH value of prepared ethosomal gels (EG-C1 %, EG-C2 % and marketed formulation A) were found to be 6.73 ± 0.20 , 5.23 ± 0.057 and 6.8 ± 0.35 respectively. Adjustment of gel formulations pH nearby to skin pH will avoid skin irritation and other related problems. The result showed pH of formulations EG-C1% was found to be in range of skin pH.
- Drug content of prepared ethosomal gel DS and CAP was found to be $96.20 \pm 0.86 \%$ and $94.25.76 \pm 0.76 \%$ respectively. Viscosities of ethosomal gel formulation (EG-C 1%, EG-C2% and marketed formulation A) were found to be 16100 ± 310 , 35400 ± 416 and 20400 ± 350 c.p respectively at 100 rpm. The result showed that viscosity of formulation EG-C 2% was higher than formulation EG-C 1%. The viscosity of formulation EG-C 1% was found to optimum value.

The order of drug permeate was found to be:- Ethosomal gel formulation > marketed formulation > carbopol gel in phosphate buffer solution (pH 6.8)

- Result revealed that % cumulative drug permeate from ethosomal formulations loaded DS and CAP were found to be $52.56 \pm 0.54 \%$ and $64.76 \pm 0.42 \%$ respectively. Drugs permeate (i.e. combination of Diclofenac Sodium and Capsaicin) from ethosomal gel was higher as compared to the carbopol gel and marketed formulation of same drug.

From the above studies it was concluded that Ethosomal formulation is the best formulation for improving permeability of Diclofenac Sodium and Capsaicin.

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