



## Formulation and Evaluation of Ethosomal Gel of Ciprofloxacin for Bacterial Infections

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### Abstract

The present study is to develop and evaluate an ethosomal gel formulation of ciprofloxacin. It aims to provide a topical treatment for many bacterial infections that affect the skin. Administration of medications topically having the facility of delivering a high concentration of the drug to the skin than would be possible with systemic therapy. Topical administration of drugs is better for local action and the efficiency of the topically administered drug is increased with liposome, proliposomes and ethosomes. Recently, it was found that ethosomal carriers were phospholipid vesicular systems having relatively high concentrations of alcohol, enhances dermal and transdermal delivery of both lipophilic as well as hydrophilic molecules. Ciprofloxacin hydrochloride is a second-generation antibiotic and a BCS class II drug. Ethosomes were formulated using phospholipid, cholesterol, ethanol, polyethylene glycol and purified water by cold method. Ethosomes were evaluated for vesicle size, shape, optical microscopy, entrapment efficiency and *in-vitro* release study. F4 have better drug entrapment efficiency than the other formulation. The best formulation (F4) was used to prepare gel by using carbopol 934 as a gelling agent. The ethosomes were entrapped in gel matrix of carbopol 980 in different concentration 0.5%, 1.00% and 2% w/w. FT-IR studies revealed no interaction between the drug and excipients. The formulated gel formulation was evaluated with parameter pH, viscosity, spreadability, *in-vitro* release test, wash ability, extrudability study and stability studies. The formulation EGF2 have better *in-vitro* drug release profile which contains carbopol 980 concentration 1% w/w. the stability studies performed (EGF2) at refrigeration temperature ( $4.0\pm 0.2^{\circ}\text{C}$ ), at room temperature ( $25-28\pm 2^{\circ}\text{C}$ ) and  $45\pm 1^{\circ}\text{C}$  for 45 days. These ethosomes were unstable at higher temperature like  $45\pm 2^{\circ}\text{C}$ . Percent efficiency of ethosomes also decrease at higher temperature like  $45\pm 2^{\circ}\text{C}$ . The present work also focuses on making the formulation more pharmaceutically acceptable.

**Keywords:** ciprofloxacin, ethosomal gel, bacterial infections, phospholipid, % entrapment efficiency, vesicle size

### Introduction

Topical drug delivery systems allow localized administration of the drug anywhere in the body through ophthalmic, vaginal, skin and rectal routes [1]. Topical formulations encompass a wide variety of formulations intended for cosmetic or dermatological application, to healthy as well as diseased skin. These formulations range in physicochemical nature from solid through semisolid to liquid [2]. Drug substances are infrequently administered alone, but rather as part of a formulation, in combination with one or more non-medicated agents that serve varied and specialized pharmaceutical function [3]. Drug absorption through the skin is enhanced if the drug substance is in solution, if it has a favorable lipid/water partition coefficient and if it is a nonelectrolyte [4]. For the most part, pharmaceutical preparations applied to the skin are intended to serve some local action and as such are formulated to provide prolonged local contact with minimal systemic drug absorption. Drugs that applied to the skin for their local action include antiseptics, antifungal agent, skin emollient, anti-inflammatory, analgesic and protectant [5]. Ethosomes are innovative nanovesicles containing the drug in a matrix of lipids, ethanol and water. The ethosomes are soft and a highly flexible vesicle efficiently penetrates through the skin and increases the drug delivery of drug molecules. Ethosomes are elastic vesicles made up of Phospholipids containing 20-45% ethanol. Ethanol also acts as a penetration enhancer by dissolving the skin lipids. The

ethosomes overcomes the disadvantages of liposomes and proliposomes such as less stability, scalability issues, leakage of drugs, fusion of vesicles and breaking of vesicles. Ethanol is a well-known permeation enhancer. Ethosomes are highly flexible which permits the elastic vesicles to squeeze themselves among the skin pores. Ethanol gives the net negative charge on the surface of ethosome vesicles due to which aggregation is avoided because of electrostatic repulsion. Ethosomes are much more stable than the liposomes and proliposomes. Topically administered ethosomes increases the residence time of the drug molecule in the different layers of skin such as stratum corneum, epidermis and reduces the systemic absorption. Because of all these properties, ethosomes get easily permeated in the deeper layer of skin and circulation. Ethanol in deeper layers of skin leads to disruption of the skin which increases the lipid fluidity that allows enhanced permeation of drug molecule through the skin. Ethosomes fuses with the skin lipids to release the drug into the deeper layers of skin [6-9]. Ciprofloxacin, a potent broad-spectrum antibiotic belonging to fluoroquinolones, has an *in vitro* antibacterial activity superior to other antibiotics [10, 11]. Chemically, it is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid hydrochloride monohydrate. Furthermore, ciprofloxacin has an efficient topical antibiotic activity and very low incidence of spontaneous resistance [12] making it a promising drug for the treatment of bacterial keratitis. Physically, ciprofloxacin hydrochloride has water

solubility of 36 mg/ml at 25°C and melting point of 318–320°C and has an elimination half-life of about 4 h. Ciprofloxacin has two pKa values of 6.09 and 8.74. It is soluble in 0.1N HCL. It is approved for use in the treatment of bone and joint infections, infectious diarrhea, lower respiratory tract infections, urinary tract infections, hospital-acquired infections and meningococcal prophylaxis [13]. Therefore, reliable drug delivery systems providing better drug penetration can result in better efficacy and also help in the prevention of development of resistance. The aim of the present study was to statistically optimize the ethosomal gel for enhanced skin delivery of ciprofloxacin, which was effective candidate for the treatment of bacterial infection.

## Materials and Methods

### Material

Ciprofloxacin hydrochloride was obtained as a gift sample from Nicholas Piramal (I) Ltd., Pithampur, Indore. Phospholipids, cholesterol, purchased from Himedia Laboratory, Mumbai. Ethanol, propylene glycol and carbopol-934 purchased from CDH chemical Pvt. Ltd. New Delhi. Dialysis membrane of Mol Wt cutoff 1200 was purchased from Himedia Laboratory, Mumbai. Double distilled water was prepared freshly and used whenever required. All other ingredients and chemicals used were of analytical grade.

### Determination of $\lambda_{\max}$ of ciprofloxacin

Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.2 pH buffer solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.2 pH buffer solution prepare suitable dilution to make it to a concentration range of 5-25 µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). A graph of concentration Vs absorbance was plotted.

### Preparation of ciprofloxacin ethosomes

Ethosomal formulations were prepared by using the cold method. The ethanolic vascular system was composed of phospholipid, cholesterol, ethanol, polyethylene glycol, drug and distilled water to 100% (V/V). Phospholipid was dissolved along with the drug in ethanol. This mixture was heated to 40°C ± 1°C and a fine stream of distilled water was added slowly, with constant mixing at 700 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 minutes, while maintaining the system at 40°C ± 1°C. The preparation was left to cool at room temperature for 30 min and then it was sonicated at 4°C for five cycles of 3 minutes each with a minute rest between cycles using a probe sonicator [14]. Six ethosomal formulae were presented in Table 1.

**Table 1:** Different composition of ethosomes formulation

F. Code	Drug(mg)	Phospholipid(mg)	Ethanol(ml)	Cholesterol(mg)	PEG(mg)	Water(ml)
F1	50	250	10	100	10	100
F2	50	250	20	100	10	100
F3	50	250	30	100	10	100
F4	50	500	10	100	10	100
F5	50	500	20	100	10	100
F6	50	500	30	100	10	100

In the ethosomes Formulations (F1 to F6), the Soya lecithin ratio was optimized by taking their different ratio such as 250 and 500mg and ethanol concentration 10, 20 and 30ml all other parameters were kept remain constant. The prepared formulations were optimized on the basis of average vesicle size and % entrapment efficiency.

### Evaluation of ciprofloxacin loaded ethosomes

#### Microscopic observation of prepared ethosomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared ethosomes formulation.

#### Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zeta master, ZEM 5002, Malvern, UK).

#### Zeta potential

The zeta potential was calculated according to Helmholtz–Smoluchowski from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 IS/cm.

### Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of untrapped free drug in aqueous medium. About 1 ml of the drug loaded ethosomes dispersion was placed in the Eppendorf tubes and centrifuge at 17000 rpm for 30 min. The ethosomes along with encapsulated drug were separated at the bottom of the tubes. Plain ethosomes without drug was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 270nm.

#### % Entrapment Efficiency

$$= \frac{\text{Theoretical drug content} - \text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

### Preparation of liposomal gels

The incorporation of the drug loaded ethosomes (equivalent to 0.3% ciprofloxacin ethosomes) into gels was achieved by slow mechanical mixing at 25rpm (REMI type BS stirrer) for 10 minutes. The optimized formulation was incorporated into three different carbopol gel concentration 0.5, 1 and 2% w/w for optimization of carbopol percentage.

## Evaluation of gel

### Physical characteristic

The Physical Characteristic was checked for gel formulations (homogeneity and texture).

### Determination of pH

The pH of the gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

### Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

### Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

### Spreadability

An important criterion for gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin. The therapeutic efficacy of a formulation also depends on its spreading value. A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability. It is determine by formula given below.

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 grams)

l = length of glass slide (6cms).

t = time taken in seconds.

### Viscosity

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25°C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25 ±1°C) before the measurements.

### In-vitro drug release studies using the prehydrated cellophane membrane

The cellophane membrane approximately 25 cm x 2cm was taken and washed in the running water. It was then soaked in distilled water for 24 hours, before used for diffusion

studies to remove glycerin present on it and was mounted on the diffusion cell for further studies. The drug release studies were carried out using modified franz diffusion cell. The dissolution study was carried out in 200 ml dissolution medium which was stirred at 50 rpm maintained at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 270 nm for ciprofloxacin and using UV visible spectrophotometer. The release of ciprofloxacin was calculated with the help of Standard curve of ciprofloxacin.

### Release kinetics

In-vitro diffusion has been recognized as an important element in drug development. Under certain conditions it can be used as a surrogate for the assessment of bioequivalence. Several theories/kinetic models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where it is the function of t (time) related to the amount of drug dissolved from the pharmaceutical dosage system. To compare dissolution profiles between two drug products model dependent (curve fitting), statistic analysis and model independent methods can be used.

In order to elucidate mode and mechanism of drug release, the *in vitro* data was transformed and interpreted at graphical interface constructed using various kinetic models. The zero order release Eq. (1) describes the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of transdermal systems, matrix tablets with low soluble drugs, coated forms, osmotic systems etc., where the drug release is independent of concentration.

$$Q_t = Q_0 + K_0 t \quad (1)$$

Where,  $Q_t$  is the amount of drug released in time t,  $Q_0$  is the initial amount of the drug in the solution and  $K_0$  is the zero order release constant

The first order Eq. (2) describes the release from the system where release is concentration dependent e.g. pharmaceutical dosage forms containing water soluble drugs in porous matrices.

$$\log Q_t = \log Q_0 + K_1 t / 2.303 \quad (2)$$

Where  $Q_t$  is the amount of drug released in time t,  $Q_0$  is the initial amount of drug in the solution and  $K_1$  is the first order release constant.

Higuchi described the release of drug from insoluble matrix as a square root of time as given in Eq. (3)

$$Q_t = K_H \sqrt{t} \quad (3)$$

Where,  $Q_t$  is the amount of drug released in time t,  $K_H$  is Higuchi's dissolution constant.

The following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative of % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (Higuchi model).

**Korsmeyer-Peppas**

The curves plotted may have different slopes, and hence it becomes difficult to exactly pin-point which curve follows perfect zero order release kinetics. Therefore, to confirm the kinetics of drug release, data were also analyzed using Korsmeyer’s equation.

$$Q_t/Q_\infty = k_{KP} \cdot t^n$$

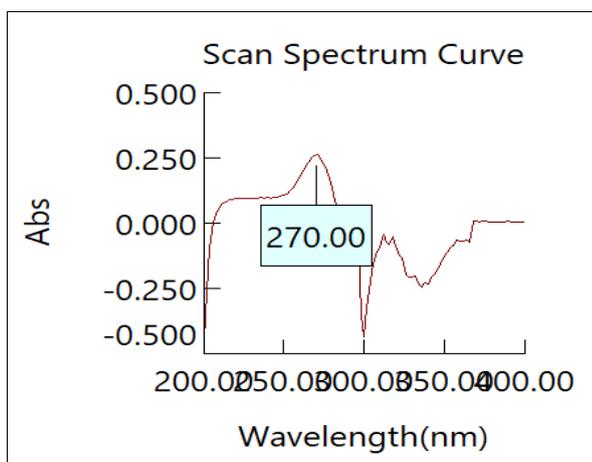
Where  $Q_t/Q_\infty$  is the fraction of drug released at time  $t$ ,  $k_{KP}$  constant comprising the structural and geometric characteristics of the device and  $n$  is the release exponent. The slope of the linear curve gives the ‘ $n$ ’ value. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of ‘ $n$ ’ gives an indication of the release mechanism. When  $n = 1$ , the release rate is independent of time (typical zero order release / case II transport);  $n = 0.5$  for Fickian release (diffusion/ case I transport); and when  $0.5 < n < 1$ , anomalous (non-Fickian or coupled diffusion/ relaxation) are implicated. Lastly, when  $n > 1.0$  super case II transport is apparent. ‘ $n$ ’ is the slope value of  $\log M_t/M_\infty$  versus  $\log$  time curve.

**Stability studies**

Stability study was carried out for drug loaded ethosomes at 3 different temperatures i.e. refrigeration temperature ( $4.0 \pm 0.2^\circ\text{C}$ ), at room temperature ( $25-28 \pm 2^\circ\text{C}$ ) and  $45 \pm 1^\circ\text{C}$  for 45 days. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The particle size of formulation was determined by optical microscopy using a calibrated ocular micrometer.

**Results and Discussions**

The absorption maxima of ciprofloxacin were determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer (Labindia UV 3000+) using concentration range of  $5-25 \mu\text{g/ml}$  ciprofloxacin in 7.2 phosphate buffers Fig. 1.



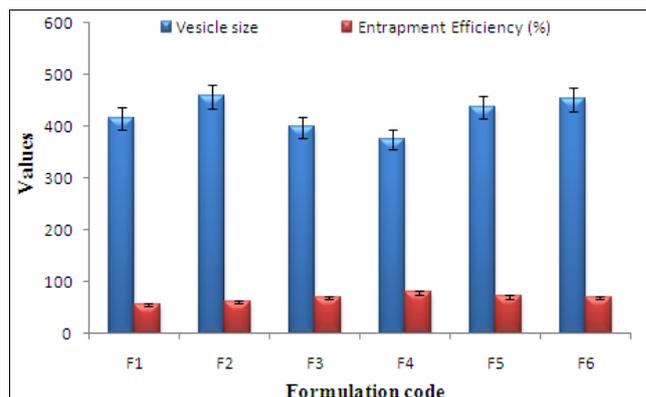
**Fig 1:** Wavelength maxima of ciprofloxacin in phosphate buffer pH 7.2

Vesicle size and zeta potential of the ethosomes were measured by photon correlation spectroscopy using a Malvern Zetasizer and entrapment efficiency was determined by measuring the concentration of untrapped

free drug in aqueous medium by UV spectrophotometer the results shown in Table 2 and Fig. 2.

**Table 2:** Result for vesicle size and entrapment efficiency of drug loaded ethosomes

Formulation Code	Vesicle size (nm)	Entrapment Efficiency (%)
F1	415.5±0.8	55.65±0.32
F2	457.8±0.5	62.25±0.45
F3	398.8±0.6	69.98±0.25
F4	375.3±0.4	80.14±0.65
F5	436.5±0.5	71.56±0.14
F6	452.1±0.7	69.89±0.45



**Fig 2:** Vesicle size and entrapment efficiency of drug loaded ethosomes

Results of evaluation of ethosomal gel formulation (EGF1-EGF3) of optimized formulation (F4) were incorporated into three different carbapol gel concentration 0.5, 1 and 2% w/w respectively. Formulation EGF2 was found to be good Table 3.

**Table 3:** Results of evaluation of gel formulation

Code	Homogeneity and Texture	pH	Spreadability (gm.cm/sec)	Viscosity (cps)	% Assay
EGF1	Good	7.2	12.25	3265	98.89
EGF2	Good	7.1	10.23	3150	99.25
EGF3	Good	6.9	9.89	3025	96.45

Results of *In-vitro* drug release from optimized formulation (EGF2) are given in Table 4 was found  $98.89 \pm 0.25$  after 8 hrs. The *in vitro* drug release data of the formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation and Korsmeyer’s -pappas models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that ‘ $r$ ’ values of formulation was maximum i.e 0.955 hence indicating drug release from formulations was found to follow zero order model of drug release kinetics. Table 5 & 6 and Fig. 3, 4.

**Table 4:** Cumulative % drug release of ciprofloxacin from optimized formulation

Time (hrs)	% Cumulative Drug Release*
0.5	26.65±0.22
1	42.25±0.15
2	55.65±0.35
4	70.25±0.14
6	82.23±0.23
8	98.89±0.25

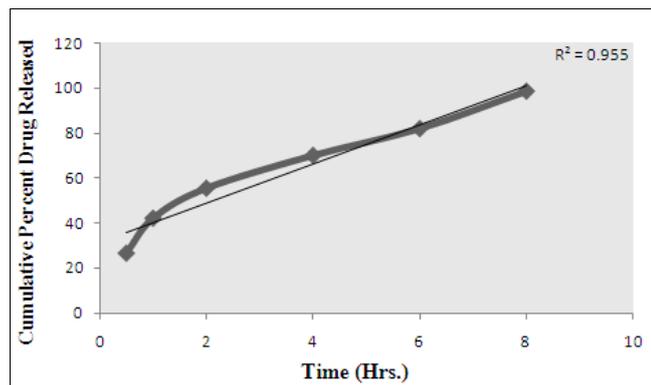
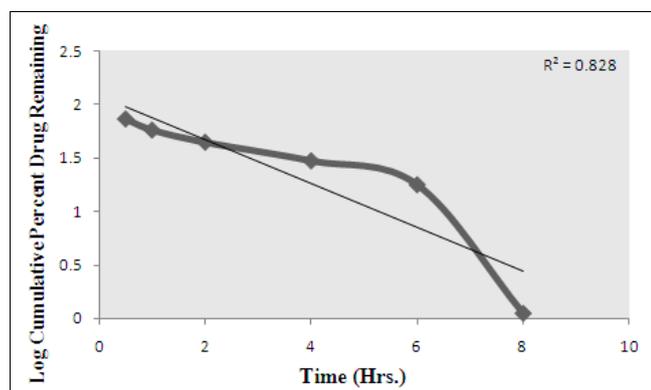
**Table 5:** *in Vitro* drug release data for EGF2

Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release $\pm$ SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
0.5	0.707	-0.301	26.65 $\pm$ 0.22	1.426	73.35	1.865
1	1	0	42.25 $\pm$ 0.15	1.626	57.75	1.762
2	1.414	0.30103	55.65 $\pm$ 0.35	1.745	44.35	1.647
4	2	0.60206	70.25 $\pm$ 0.14	1.847	29.75	1.473
6	2.449	0.77815	82.23 $\pm$ 0.23	1.915	17.77	1.250
8	2.828	0.90309	98.89 $\pm$ 0.25	1.995	1.11	0.045

\* Average of three determinations

**Table 6:** Regression analysis data of ethosomal gel formulation

Formulation	Zero order	First order
EGF2	R <sup>2</sup> = 0.955	R <sup>2</sup> = 0.828

**Fig 3:** Cumulative percent drug released vs time**Fig 4:** Log Cumulative percent drug remaining vs time

The particle size of the ethosomes was found to increase at RT, which may be attributed to the aggregation of ethosomes at higher temperature. At 45 $\pm$ 2 $^{\circ}$ C the aggregate i.e. these ethosomes were unstable at higher temperature like 45 $\pm$ 2 $^{\circ}$ C. Percent efficiency of ethosomes also decrease at higher temperature like 45 $\pm$ 2 $^{\circ}$ C.

### Conclusion

Ciprofloxacin loaded ethosomal formulation was successfully prepared by loading phospholipids and ethanol and ethosomal gel based formulations were prepared with hydrophilic polymer Carbopol 934. It can serve as a useful vehicle for the delivery of ciprofloxacin through the affected part of the skin for extended period of time. This study also revealed that ethosomal gel (EG2) resides at targeted site for a relatively longer period of time with a zero order release profile. It signifies the improved patient compliance.

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