International Journal of Advances in Pharmaceutical Analysis
ISSN: 2277-9353 (Online)
Journal DOI: https://doi.org/10.7439/ijapa **Research Article**

Development and characterization of Omeprazole loaded Ethosomes for treatment of ulcer

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Abstract

The current study aimed to design an oral coated solid dosage form to lower ulcer complications. The Omeprazole was incorporated an ion gelation technique, with sodium alginate as the gelling agent, calcium chloride as the cross-linking agent, and chitosan as the agent for sustained release. The optimized formulation of Omeprazole showed a diameter of 2.506 mm, a swelling rate of 838.2%, and an encapsulation efficiency of 93.8%. Scanning electron microscopy images revealed the microcapsules' spherical shape. The *in vitro* release of the o ml from the HGMC after two hours in a simulated gastric fluid was 13.2%1±0.08%, compared with the apparent solubility of the pure omeprazole under the same conditions (95.24%±3.2%). After 24 hours, the percent of omeprazole released from the optimized formula was 69.84±2.4%, which indicates a sustained release pattern. The results from the in vivo study demonstrated improved healing of the induced ulcers in rats when treated with the omeprazole formulation as compared to the standard omeprazole therapy; therefore, the obtained mucoadhesive was considered a potential drug delivery strategy for ulcer therapy.

Keywords: Anti-Ulcerogenic Effect, Box-Behnken design, Histopathological Analysis, In Vitro Release.

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***Article History:**
Received: 24/06/2026
Revised: 03/07/2026
Accepted: 04/07/2026
DOI: https://doi.org/10.7439/ijapa.v16i1.5919

How to cite: Mishra S. and Jain P. Development and characterization of Omeprazole loaded Ethosomes for treatment of ulcer. *International Journal of Advances in Pharmaceutical Analysis* 2026; 16(1): e5919. Doi: 10.7439/ijapa.v16i1.5919 Available from: https://ssjournals.co.in/index.php/ijapa/article/view/5919

1. Introduction

Nanotechnology is the science of the small; the very small. It is the use and manipulation of matter at a tiny scale. At this size, atoms and molecules work differently, and provide a variety of surprising and interesting uses [1]. Nanotechnology and Nanoscience studies have emerged rapidly during the past years in a broad range of product domains [2]. It provides opportunities for the development of materials, including those for medical applications, where conventional techniques may reach their limits [3]. Nanotechnology should not be viewed as a single technique that only affects specific areas. Although often referred to as the 'tiny science', nanotechnology does not simply mean very small structures and products [4].

Nanoscale features are often incorporated into bulk materials and large surfaces. Nanotechnology represents the design, production and application of materials at atomic, molecular and macromolecular scales, in order to produce new nanosized materials [5].

Pharmaceutical nanoparticles are defined as solid, submicron-sized (less than 100 nm in diameter) drug carrier that may or may not be biodegradable. The term nanoparticle is a combined name for both nanosphares and nanocapsules [6]. Nanospheres are matrix system in which drug is uniformly dispersed, while nanocapsules are the system in which the drug is surrounded by a unique polymeric membrane [7]. This systemic review focuses on Classification, method of preparation, Characterization, application, health prospective and Pharmacological aspects of nanoparticles [8].

Ethosomes

Ethosomes, soft vesicles consisting of biocompatible ingredients, phospholipids, ethanol and

water, were introduced by Touitou [9]. According to the suggested mechanism of skin permeation enhancement by ethosomes, ethanol fluidizes the lipid bilayers of the stratum corneum and of the ethosome, resulting in a more penetrable skin and less rigid vesicle [10]. Further the soft vesicle penetrates the disturbed skin bilayers and releases the drug in the skin layers along the penetration pathway [11]. It was shown that ethosomes are efficient carriers for drugs within a wide range of physicochemical proprieties: lipophilic, ionic, polar and high molecular weight molecules [12].

They represent a type of phospholipid vesicles developed by Touitou *et al*. for improved delivery of drug into or across the skin [13]. They are composed of phospholipids and water as the conventional liposomes, but in addition enclose high ratio of ethanol (usually 20-45%, v/v) Due to well-known skin permeation enhancing effect of ethanol, ethosomes are also described as skin permeation-enhancing vesicles [14]. They are prepared by dissolving phospholipids and lipophilic drug in ethanol and slow addition of aqueous phase during constant mixing [15]. Compared to conventional liposomes of the same phospholipid composition, ethosomes are of significantly smaller diameter that can be explained by high ethanol content [16]. Ethanol also contributes to the negative net surface charge of the vesicle, which additionally decreases the size of ethosomes. The encapsulation efficiency for lipophilic drugs in ethosomes is higher in comparison to conventional and especially to deformable liposomes [17]. This is a consequence of solubilizing effect of ethanol and the multilamellar morphology of ethosomes reported by Touitou and coworkers.

Most of the reports on ethosomes are related to transdermal drug delivery. However, they were also studied as drug carriers for improved skin therapy in dermatology providing increased drug solubility and enhancing its penetration through the stratum corneum [18]. Thus, incorporation of poorly soluble acyclovir into ethosomes led to improved skin bioavailability of the drug. Clinical investigation of ethosomal acyclovir in the treatment of recurrent herpes labialis demonstrated advantages of using ethosomes over commercial product; time necessary for crusting of the lesions and loss of crusts was significantly reduced by applying ethosomal formulation. Esposito and coworkers have studied ethosomes as carriers for skin delivery of azelaic acid. Generally, ethosomes were of smaller size and narrower size distribution than conventional liposomes. Among different ethosome preparations those prepared by the highest ethanol concentration (45%, v/v) were characterized by rapid release of azelaic acid.

Phospholipid vesicles containing 10-20% (v/v) of ethanol increased deposition of cyclosporin A into *stratum corneum* in comparison to conventional liposomes [19]. This study reported on the potential use of ethosomes as carriers to deliver the drugs which are skin-impermeable in the treatment of diseases such as psoriasis [20]. A few years later, Dubey *et al* explored ethosomes as a carrier for increased skin delivery of another potent drug for the therapy of psoriasis, methotrexate [21]. *In vitro* skin permeation studies using human cadaver skin demonstrated that ethosomes were able to enhance methotrexate flux and skin deposition in comparison to the control formulations [22].

2. Materials And Methods

2.1 Materials required

Table 1: Chemicals Used

Sr. No.	Chemicals	Company
1	Soya lecithin	Acros Organics, New Delhi, India
2	Omeprazole	Gift sample
3	Cholesterol	Himedia Laboratories Pvt. Ltd. Mumbai, India
4	Methanol	Merck India Ltd. Mumbai, India
5	propylene glycol	Merck India Ltd. Mumbai, India
6	Ethanol	Merck India Ltd. Mumbai, India

Table 2: Instruments Used

S. No	Instrument	Company made
1	Electronic Weighing Balance	A&D Company HR 200, Japan.
2	Mechanical Stirrer	Remi Motors, India.
3	Magnetic Stirrer	MC Dalal& Co India
4	UV Visible Spectrophotometer	Shimadzu, Japan.
5	Particle size analyser	CILAS – 1604L, France
6	TEM	FEI-Philips Tecnai 10
7	FTIR	Thermo
8	pH meter	Thermo
9	Ultracentrifuge	Eppendorf centrifuge, Germany
10	SEM	SEM Leo 430, England
11	Zeta sizer	Malvern Zetamaster ZEM 5002

2.2 Pre-formulation Studies

Pre-formulation may be described as a phase of the research and development process where the formulation scientist characterizes the physical, chemical and mechanical properties of new drug substances, in order to develop stable, safe and effective dosage forms. These studies are designed to determine the compatibility of initial Excipient with the active substance for a biopharmaceutical,

physicochemical and analytical investigation in support of promising experimental formulations.

The basic purpose of the pre-formulation activity are to provide a rational basis for the formulation approaches, to maximize the chances of success in formulating an acceptable product and to ultimately provide a basis for optimizing drug product quality and performance. Pre-formulation is defined as an investigation of physical & chemical properties of drug substance alone and when combined with excipient.

2.3 Organoleptic evaluation

Organoleptic properties were observed by visual observation. It is the initial evaluation during Pre-formulation studies which assess the colour, odour and appearance of the substance**.** The organoleptic studies of drug like general appearance like, colour, odour and appearance etc were observed.

2.4 Solubility study

Qualitative solubility of drug in different solvents was determined according to USP NF, 2007. Drug (1 mg) was accurately weighed and transferred into a 10 ml test tube; then, it was dissolved in the respective solvents (1 ml each) such as Methanol, Ethanol, Chloroform, N-hexane DMOS and water. The solubility (mg/ml) was observed by visual inspection.

2.5 Melting Point determination

Melting point was determined by open capillary method. A few amounts of the drug were placed in a thin-walled capillary tube 10-15 mm long, about 1mm inside diameter, and closed at one end. The capillary, which contains the sample, was suspended to heat the samples slowly and evenly and thermometer placed to check the temperature. The temperature range over where the sample is observed to melt is taken as the melting point of the drug**.**

2.6 pH determination

Electrochemical methods and Digital pH meter **(EI)** were used to determine the pH of drug. After the meter has been turned on, allowed to stabilize as necessary and properly calibrated, begin by rinsing the probe with deionized or distilled water and blotting the probe dry with lint-free tissue paper. Immerse the sensing tip of the probe in the sample and record the pH reading and Rinse the probe, blot dry and repeat step 2 on a fresh portion of sample. The two readings should agree to within the accuracy limits of the meter.

2.7 Partition coefficient

The partition coefficient of drug Omeprazole was examined in, n-Octanol: water system. It was determined by taking 5mg of Omeprazole (drug) in separating funnel containing, 20ml of n-Octanol and 20 ml water. The separating funnel was shaken for 2 hours in a wrist action shaker for equilibrium. Two phases were separated and the amount of drug in aqueous phase was analyzed spectrophotometrically at 294 nm. The partition coefficient of drug was calculated using the following formula-

Partition coefficient, $K = \frac{Amount\ of\ drug\ in\ organic\ phase}{Amount\ of\ drug\ in\ aqueous\ phase}$

2.8 Determinations of λ max (maximum absorbance)

2.8.1 Preparation of Omeprazole standard stock solution in water

Standard solution of Omeprazole was prepared by dissolving accurately weighed 10 mg of Omeprazole with 5 ml of water solvent, in a 10 ml volumetric flask. The volume was made up to 10 ml with water to obtain a stock solution of 1000 µg/ml. 1ml of this stock solution was taken and then diluted up to 10 ml using respective solvent to obtain a solution that has a concentration 100 µg/ml which is standard stock solution.

2.8.2 Lambda max

From the above stock solution 2 ml sample was transferred into a 10 ml volumetric flask and the volume was made up to mark with solvent to prepare a concentration of 20 µg /ml. The sample was scanned by UV-VIS Spectrophotometer in the range of 200- 400 nm, using respective solvent as a blank. The wavelength corresponding to the maximum absorbance (max) was found. This was further utilized to obtain a calibration curve.

2.8.3 Linearity or standard curve

Aliquots of 5, 10, 15, 20, 25, 30 and 35 µg/ml of 100 µg ml drug working standard solution were accurately transferred into a series of 5 ml calibrated flask and made up to the mark with drug. The absorbance of the resulting solution was measured 294 nm against solvent blank. Calibration curve was prepared by plotting the absorbance vs concentration of drug.

Seven points calibration curves were obtained in a concentration range from 5-35 µg/ml for drug. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was $y = 0.027x + 0.040$ with correlation coefficient 0.977.

2.8.4 Fourier transmission Infra-Red Spectroscopy

FT-IR spectrum of Drug was recorded over the range of 4000 to 400 cm−1 by KBr pellet method using a FT-IR spectrophotometer. The KBr disc was prepared using 1 mg of Drug and 100 mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and drug was mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT-IR chamber. Infrared spectrum was recorded in the 4000 - 400 cm-1 regions.

2.9 Preparation of Ethosomes

Ethosomes were prepared as reported. In brief the lecithin (1.5-3.5% w/v) was taken in a small round bottom flask and solubilized with ethanol (10-50% v/v) containing drug under mixing with a magnetic stirrer. The round bottom flask was covered to avoid ethanol evaporation. Distilled water was added slowly with continuous stirring to obtain the ethosomal colloidal suspensions. The final suspension of ethosomes was kept at room temperature for 30 min under continuous stirring. Formulations were stored in the refrigerator and evaluated for vesicle size, vesicular shape, surface morphology, entrapment efficiency, *in vitro* drug permeation study and stability study.

Table 3: Composition of formulation

S. No	Soya Lecithin (%)	Ethanol concentration (%)	Stirring Time (min)	Temperature (^{0}C)
1.	1.5	10	30 min	30^{0}C
2.	2.0	20	30 min	30^{0}C
3.	2.5	30	30 min	30^{0}C
4.	3.0	40	30 min	30^{0}C
5.	3.5	50	30 min	30^{0}C

2.10 Evaluation parameters of Drug loaded ethosomes formulation

2.10.1 Zeta potential

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the ethosome formulation was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. All samples were sonicated for 5-10 minutes before zeta potential measurements.

2.10.2 Particle size

The size of ethosomes was measured using Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25°C and sample was placed in disposable sizing cuvette. The size data is documented in Table 15**.**

2.10.3 Entrapment efficiency

To calculate the entrapment efficiency accurately weighed the quantity of ethosomes (10 ml) with 5 ml of ethanol in a volumetric flask was shaken for 1 min using vortex mixer. The volume was made up to 10 ml. Then the solution was filtered and diluted. The concentration of Omeprazole was determined spectrophotometrically at 294.0 nm.

Loading efficiency = Actual drug content in ethosome / Theoretical drug content × 100

2.10.4 Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the optimized Omeprazole loaded ethosome were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pretreated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography**.**

2.10.5 *In-vitro* drug release

The *in-vitro* drug release study of ethosome formulation was studied by dialysis bag diffusion method. Drug loaded ethosome was dispersed into dialysis bag and the dialysis bag was then kept in a beaker containing 100 ml of pH 7.4 phosphate buffer. The beaker was placed over a magnetic stirrer and the temperature of the assembly was maintained at 37 ± 2 °C throughout the experiment. During the experiment rpm was maintained at 100 rpm. Samples (2 ml) were withdrawn at a definite time intervals and replaced with equal amounts of fresh pH 7.4 phosphate buffers. After suitable dilutions the samples were analyzed using UV–Visible spectrophotometer at 294.0 nm. To analyze the *in vitro* drug release data various kinetic models were used to describe the release kinetics.

To analyze the *in vitro* release data various kinetic models were use to describe the release kinetics. The zero order rate Eq. (2) describes the systems where the drug release rate is independent of its concentration. The first order Eq. (3) describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion. The results of *in vitro* release profile obtained for all the formulations were plotted in modes of data treatment.

Zero - order kinetic model – Cumulative % drug released versus time.

First – order kinetic model – Log cumulative percent drug remaining versus time.

Higuchi's model – Cumulative percent drug released versus square root of time.

Korsmeyer- Peppas model - log cumulative % drug release vs log time (Kors-meyer–Peppas model)

Zero order kinetics

$$A_t = A_0 - K_0 t$$ -------- Eq. (2)

Zero order release would be predicted by the following equation:

Where

At=Drug release at time 't'

A0=Initial drug concentration.

K0=Zero-order rate constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero– order kinetics and its slope is equal to Zero order release constant K0.

First order kinetics

$$LogC=logC_0–K_t/2.303$$ -------- Eq. (3)

First-order release could be predicted by the following equation:

Where,

C=Amount of drug remained at time 't'

C0=Initial amount of drug.

K=First-order rate constant (hr^{-1}).

When the data plotted as log cumulative percent drug remaining versus time, yields astraight line, indicating that the release follow first order kinetics. The constant 'K1'can be obtained When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant 'K1' can be obtained by multiplying 2.303 with the slope value.

2.10.6 Higuchi's Model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

$$Q= [DЄ /\tau (2A–ЄCs) Cst]^{1/2}$$ .....eq (4)

Where,

Q= Amount of drug release at time 't'

D= Diffusion coefficient of the drug in the matrix.

A= Total amount of drug in unit volume of matrix.

Cs= Solubility of drug in the matrix

Є= Porosity of the matrix.

τ= Tortuosity.

t= Time (hrs at which q amount of drug is released.

Above equation can be simplified as if we assume, that 'D', 'Cs' and 'A' are constant. Then equation: becomes**.**

2.10.7 Korsmeyer- Peppas model

Korsmeyer *et al* (1983) derived a simple relationship which described drug release from a polymeric system equation (5). To find out the mechanism of drug release.

$$Mt / M\infty = Kt^n$$…….. (5)

Where Mt / M∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices.

2.11 Stability study

The Ethosomes formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing (25^0 C±2^0 C and 60 ± 5% RH) and (40^0 C±2^0 C and 70 ±5% RH) for 3 months. The formulation was checked for Physical appearance, particle size and entrapment efficiency 30, 45, 60, 90 days (3 month). The formulation was tested for stability under accelerated storage condition for 3 months in accordance to International Conference on Harmonization (ICH) guidelines. Formulation was analyzed for the change in Physical appearance particle size and entrapment efficiency studies. All Results were compared against final formulation of 0 days as the reference.

3. Result and discussion

3.1 Organoleptic properties

Table 4: Organoleptic properties of Omeprazole

Drug	Organoleptic properties	Observation
Omeprazole	Colour	White
	Odour	Characteristic
	State	Solid
	Appearance	Crystalline solid powder

Organoleptic properties of the Omeprazole including colour, odour, state and appearance were conducted. Omeprazole was discovered to have a white colour, characteristic odour and has a crystalline powder, according to research conducted on it. Omeprazole exhibited the same colour, state, odour and appearance as the I.P. requirements for these characteristics.

3.2 Solubility study

Table 5: Solubility study of Omeprazole

Drug	Solvents	Observation/Inference
Omeprazole	Water	Freely Soluble
	Ethanol	Soluble
	Methanol	Soluble
	N- hexane	Insoluble
	DMSO	Freely soluble

The solubility of Omeprazole was determined in various non-volatile or volatile liquid vehicles such as Dimethyl sulfoxide, methanol, ethanol, chloroform, n-hexane and water shown in Table 6. From the results, it was observed that the drug is freely soluble in Dimethyl

sulfoxide, water and soluble in ethanol and methanol and insoluble in n-hexane.

3.3 Melting point

Table 6: Melting point of Omeprazole

Drugs	Observed	Reference
Omeprazole	154^{0}C	145^{0}C to 155^{0}C

The capillary method is used to determine the melting point of a substance. The melting point of the Omeprazole was found to be 154ºC, which is well within the limits of the drug specification.

3.4 Determination of Partition coefficient

Table 7: Partition coefficient:

S. No.	Drug	Solvent	Partition coefficient
1	Omeprazole	n-Octanol: water	2.16

The partition coefficient of drug was examined in, n-Octanol: water system. It is defined as the ratio of unionized drug distributed between the organic and aqueous phases at equilibrium. Partition coefficient provides a means of characterizing the lipophilic/hydrophilic nature of the drug. The partition coefficients of the Omeprazole were found to be 2.16 shown in table 7.

3.5 Λmax:

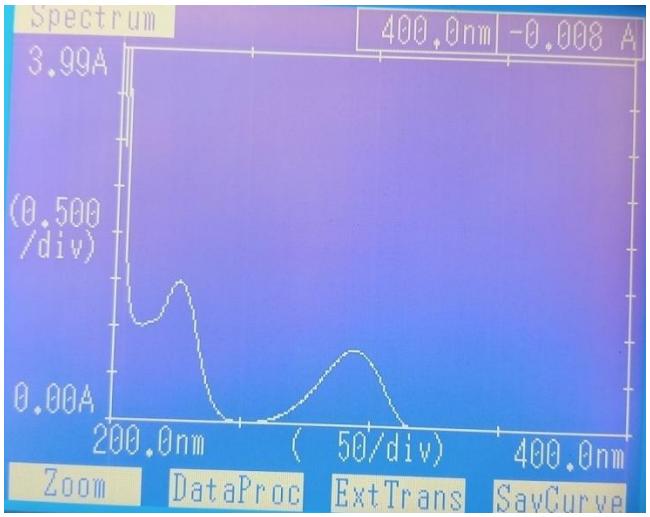

Figure 1: Lambda max of Omeprazole

Table 8: Lambda max

S. No	Drug	UV absorption maxima (Lambda max)
1.	Omeprazole	294.0 nm

Double beam UV visible spectrophotometer (Shimadzu- 1700) was used to determine the lambda max (absorption maxima) of a substance. The lambda max of the Omeprazole was found to be 294.0 nm. This is well within the limits of the drug specification

Calibration curve of Omeprazole

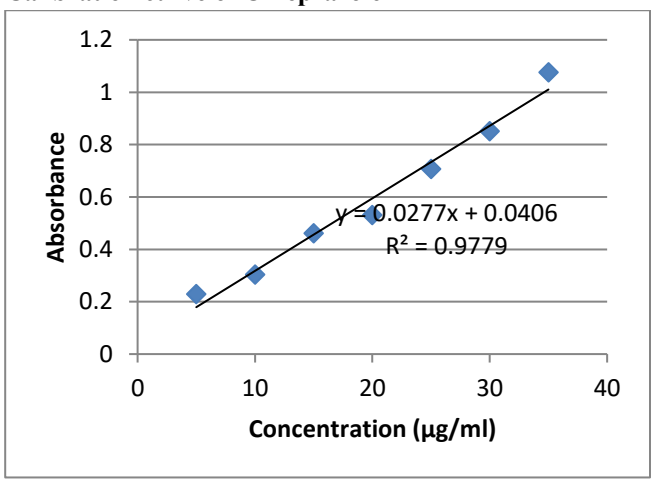

Figure 2: Calibration curve

The linearity of the proposed method was established by least squares linear regression analysis of the calibration curve. The regression equation for Omeprazole was obtained by plotting absorbance versus concentration of Omeprazole in the range of 5-35µg/ml.

Seven points calibration curve were obtained in a concentration range from 5-35 µg/ml for drug. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.027x + 0.040 with correlation coefficient R^2 = 0.977.

Functional group identification by FTIR

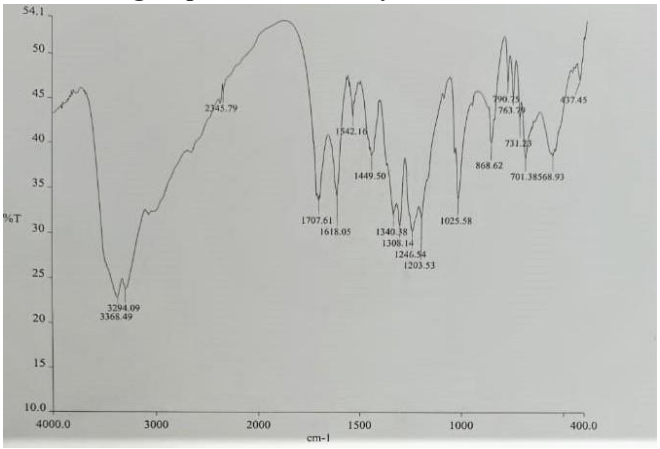

Figure 3: FTIR of Omeprazole

Sample Frequency	Reference Frequency Range	Functional group identified	Name of Functional group
3368.49	3500–3200	O-H stretch	Alcohol (Phenol) group
3294.09	3500–3200	O-H stretch	Alcohol (Phenol) group
1707.61	1760–1690	C=O stretch	Carboxylic acid
1542.16	1625 – 1440	C=C stretch	Aromatic alkene
1449.50	1470–1450	C-H bending	Alkane
1340.38	1320–1000	C-O stretch	Carboxylic acid
1308.14	1320–1000	C-O stretch	Carboxylic acid
1246.54	1320–1000	C-O stretch	Carboxylic acid
1203.53	1320–1000	C-O stretch	Carboxylic acid
1025.58	1000–650	=C–H bend	alkenes
868.62	860 – 800	C-H bending (Out of plane)	Para Di-substituted aromatic
731.23	770 – 735	C-H bending (Out of plane	Ortho Di-substituted aromatic
763.79-790.75	810 – 750	C-H bending (Out of plane	Meta Di-substituted aromatic

Fourier transform infrared (FTIR) spectroscopy is an emerging instrumentation technique in which molecular interaction via exposure to infrared electromagnetic radiation with the samples can be utilized to predict the structure of small molecules and also the functional groups present in it. So FTIR spectra were obtained on FTIR Spectrophotometer in a transmission mode with the wave number region of 4000-400 cm^{-1} in KBr Discs and the spectrum is compared with the standard values. In this FTIR spectrum, the Phenolic O-H stretching peak is observed at 3368.49 cm^{-1} and 3294.09 cm^{-1} which lie in the range of 3500-3200 cm^{-1}. The O-H of this phenolic group is hydrogen bonded, due to which it is showing the peak at 3368.49 cm^{-1} rather than in the range of 3640–3610 cm^{-1}. Carboxylic acid (C=O stretching) peak is observed at 1707 cm^{-1} which lie in the range of 1760-1690 cm^{-1}. The peak is observed at 1542 cm^{-1} which lies in the range (1625 – 1440 cm^{-1}) of aromatic C=C alkene stretching. Another functional group is observed at 1340.38, 1308.14, 1246.54, 1203.53 cm^{-1} which is carboxylic acid C-O stretching that comes under the standard range of 1320–1000 cm^{-1}. The compound containing para, ortho and meta di-substitutions that is the reason the graph is showing out of the plane C-H bending at 868.62, 731.23, 763.79-790.75 cm^{-1} which is confirmed by comparing with the standard values of 860 – 800, 770 – 735 and 810 – 750 cm^{-1} respectively.

Evaluation parameter of Omeprazole loaded ethosomes formulation

Particle size determination

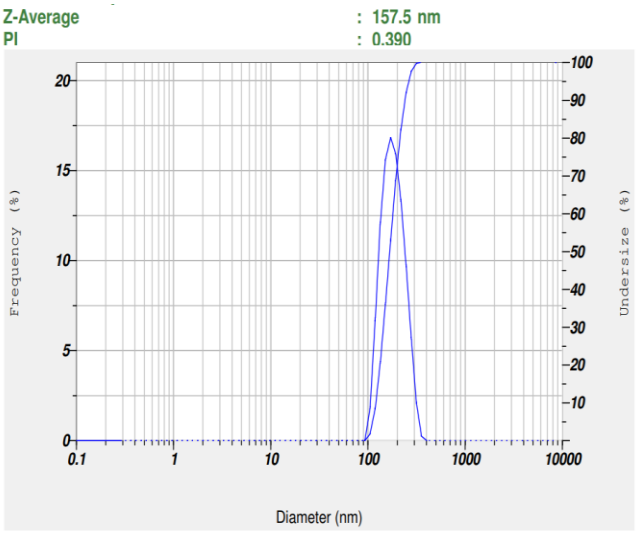

Figure 4.: Particle size (Formulation 1)

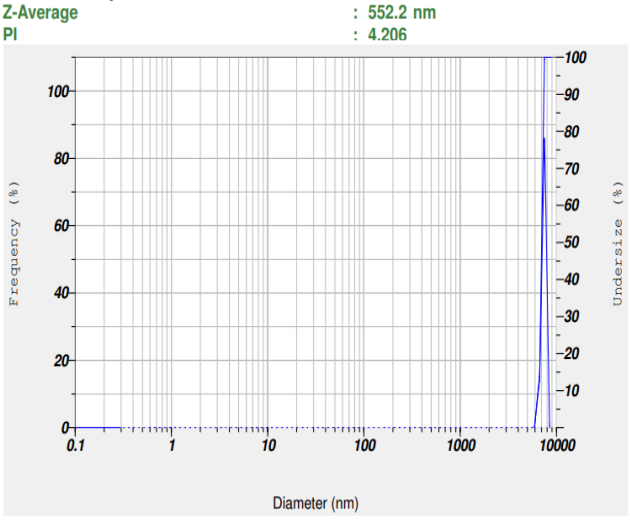

Figure 5: Particle size (Formulation 2)

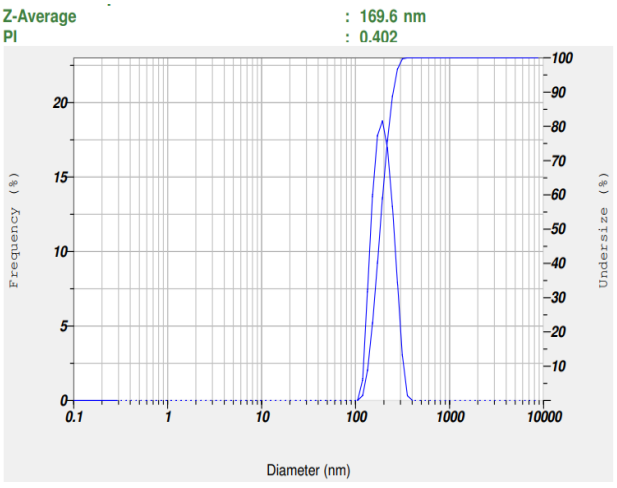

Figure 6: Particle size (Formulation 3)

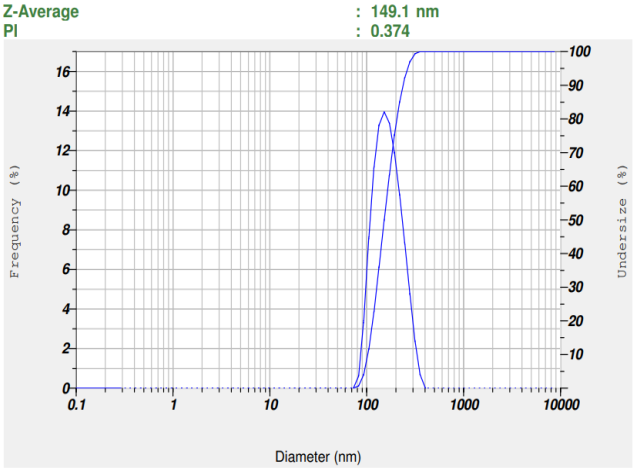

Figure 7: Particle size (Formulation 4)

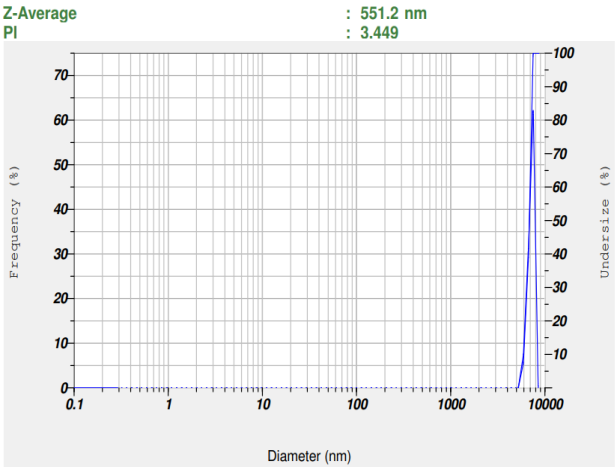

Figure 8: Particle size (Formulation 5)

Table 9: Particle size of Drug loaded Ethosomes

S. No	Formulation	Particle size	PI value
1	F1	157.5 nm	0.390
2	F2	552.2 nm	4.206
3	F3	169.6 nm	0.402
4	**F4**	**149.1 nm**	**0.374**
5	F5	551.2 nm	3.449

The particle size is one of the most important parameters for the characterization of ethosomes. The average particle sizes of the prepared drug loaded ethosomes formulation were measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of ethosomes was found to be range between 149.1 to 552.2 nm. These particle size values indicate that the all formulated ethosomes is under the range (Below 1000 nm) of ethosomes and F4 is the lowest particle size of all formulation shown in above table 9.

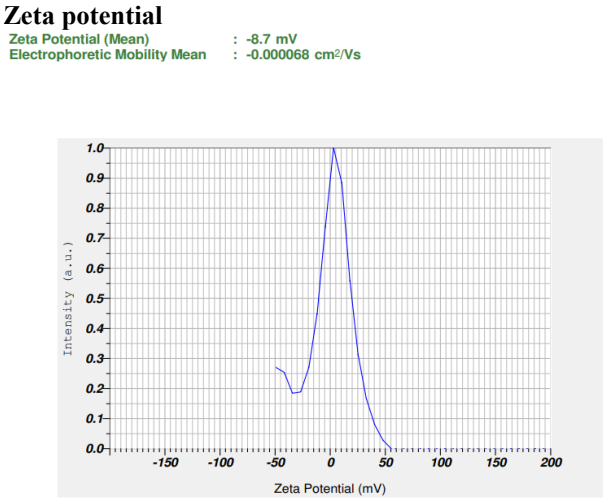

Figure 9: Zeta potential (Formulation 1)

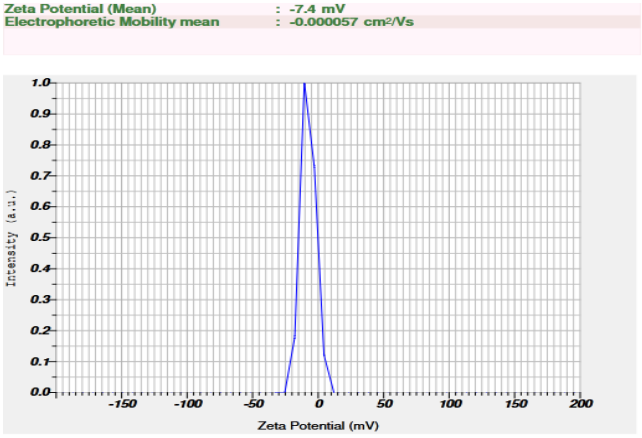

Figure 10: Zeta potential (Formulation 2)

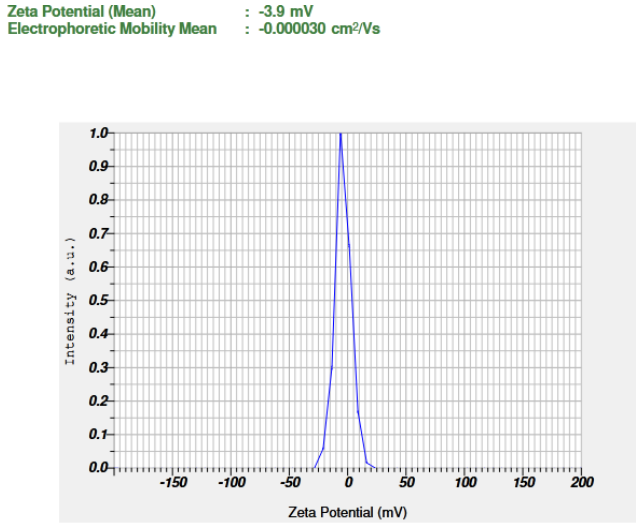

Figure 11: Zeta potential (Formulation 3)

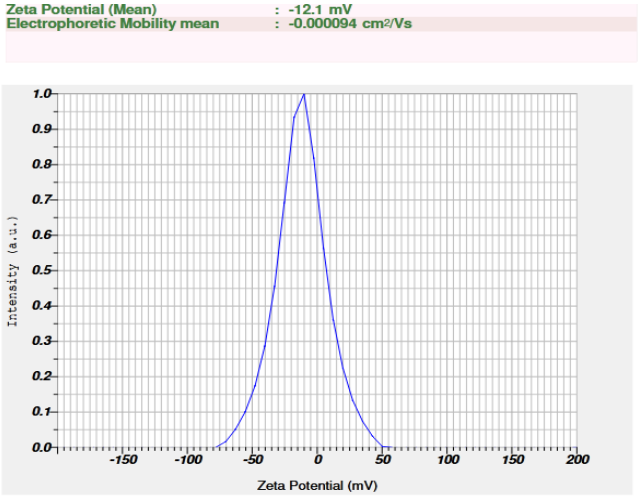

Figure 12: Zeta potential (Formulation 4)

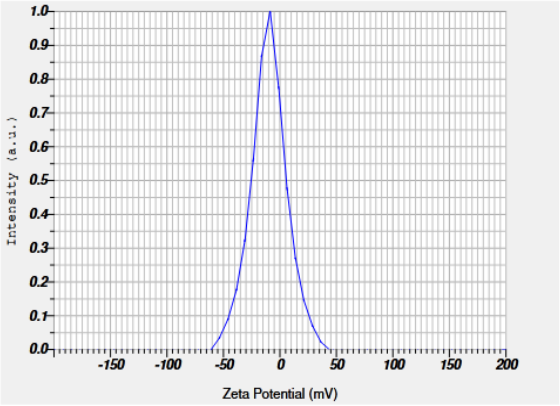

Figure 13: Zeta potential (Formulation 5)

Table 10: Results of Zeta potential

S. No	Formulation	Zeta potential
1	F1	-8.7 mV
2	F2	-7.4 mV
3	F3	-3.9 mV
4	**F4**	**-12.1mV**
5	F5	-9.8 mV

Zeta potential analysis is carried out to find the surface charge of the particles. The magnitude of zeta potential is predictive of the colloidal stability. Zeta potential was found to be all formulation range -3.9 to -12.1 mV with peak area of 100% intensity. These values indicate that the all-formulated ethanol are stable.

Scanning Electron Microscopic (SEM) of F4 formulation

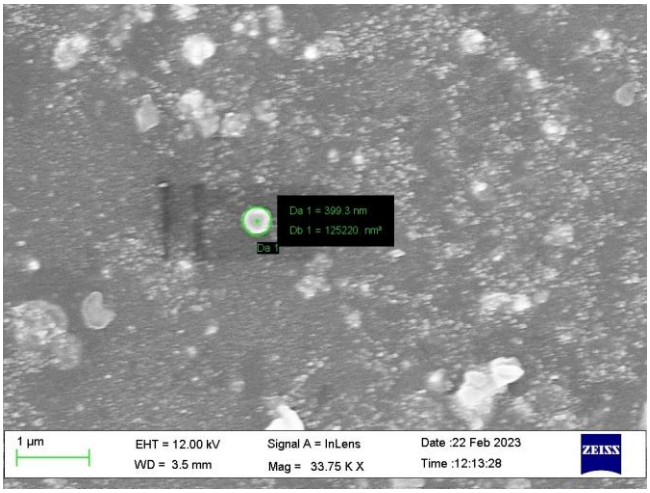

Figure.14: SEM (F4)

SEM analysis was performed to determine their ethosome characters (shape & morphology) of prepared drug loaded ethosomes. Omeprazole loaded ethosome were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared ethosome at 33.75 kx magnification showed that the ethosomes were smooth surface morphology and spherical shape. The smooth surface morphology and spherical shape of ethosome was clearly observed in the SEM images.

Entrapment efficiency

Table 11 Entrapment efficacy

S. No.	Formulations	Entrapment efficacy (%)
1.	F1	67.03 %
2.	F2	78.86 %
3.	F3	83.69 %
4.	**F4**	**89.63 %**
5.	F5	74.56 %

This might be due to the fact that the variation in entrapment efficiency was due to the changes in the polymer concentration. The prepared drug entrapped ethosomes possess drug entrapment range (67.03 to 89.63). The F4 Omeprazole loaded ethosomes possess high drug entrapment efficiency and were found to be in the range of 89.63%.

***In- vitro* drug release Study**

Table 12 : *In- vitro* drug release Study

S. No	Time (hr)	F1	F2	F3	F4	F5
1	0	0	0	0	0	0
2	1	23.27	16.00	19.32	16.13	21.01
3	2	33.97	28.48	35.12	28.18	42.71
4	4	41.04	39.90	47.67	39.05	58.83
5	6	53.78	43.51	58.24	50.64	64.25
6	8	69.11	59.80	63.11	66.87	70.88
7	10	77.20	68.18	79.17	77.23	81.11
8	12	86.09	77.11	85.10	83.62	88.69
9	14	93.25	89.63	90.57	96.83	92.06

Release kinetics study of Formulation 4

Table 13: Release kinetics study of optimized (F4) formulation

S. No	Time (hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	Log cumu % drug released
1.	0	0	100	0.000	2.000	0.000	0.000
2.	1	16.13	83.87	1.000	1.924	0.000	1.208
3.	2	28.18	71.82	1.414	1.856	0.301	1.450
4.	4	39.05	60.95	2.000	1.785	0.602	1.592
5.	6	50.64	49.36	2.449	1.693	0.778	1.704
6.	8	66.87	33.13	2.828	1.520	0.903	1.825
7.	10	77.23	22.77	3.162	1.357	1.000	1.888
8.	12	83.62	16.38	3.464	1.214	1.079	1.922
9.	14	96.83	3.17	3.742	0.501	1.146	1.986

Correlation value (R2 value)

Table 14: Correlation value (R^2 value)

Formulation	Model	Kinetic parameter values
Ethosomes	Zero Order	R^2 = 0. 976
	First Order	R^2 = 0.833
	Higuchi	R^2 = 0.979
	Korsmeyerpeppas	R^2 = 0.694

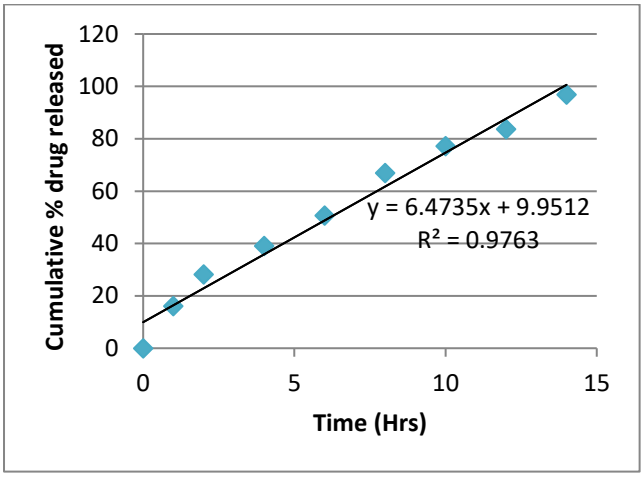

Figure 15: Zero order kinetic model

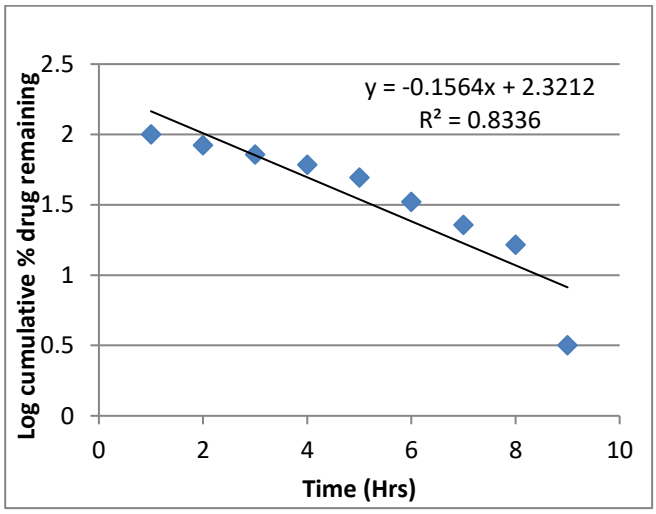

Figure 16: First Order kinetic model

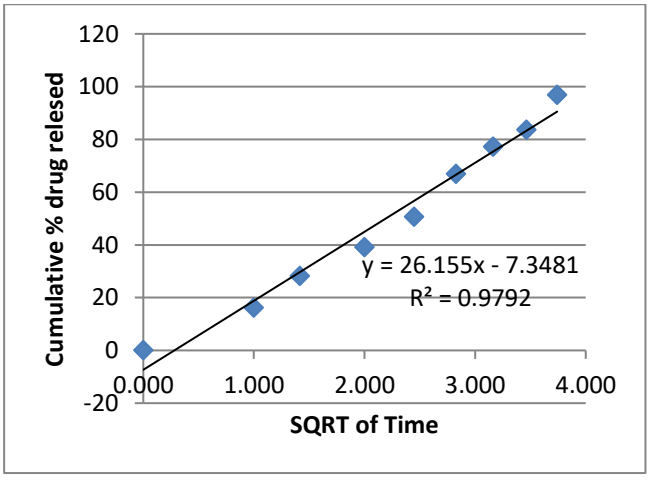

Figure 17: Higuchi model

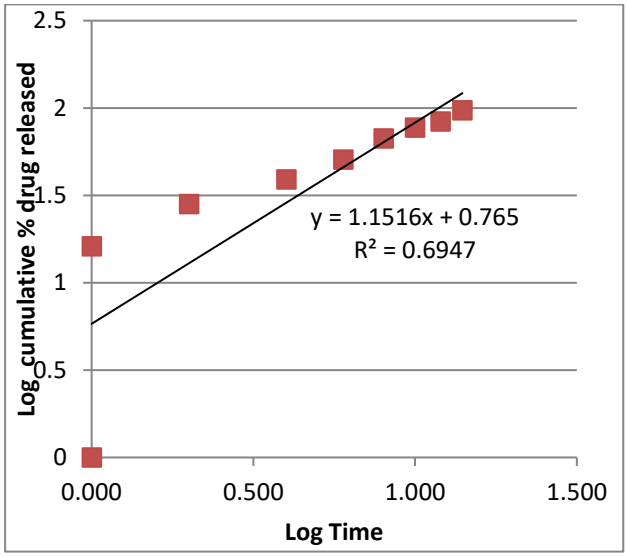

Figure 18: Korsmeyerpeppas

The data of percentage drug release formulation were shown in Figure. 8.14 to 8.17. For kinetic study following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative % drug remaining vs time (first order kinetic model); cumulative % drug release vs square root of time (Higuchi model); log cumulative % drug release vs log time (Korsmeyer–Peppas model). All Plots are shown in Figure. 18 to 21 and results are summarized in Table 8.11. Zero order kinetic models refer to the process of constant drug release from a drug delivery device independent of the concentration. The zero order graph of optimized formulation showed the constant drug release from the ethosome formulation, the results of the zero-order model was found to be y = 6.473 x + 9.951 R^2 = 0.976. The first order kinetic model describes the release from system where release rate is concentration dependent. The results of first order kinetic model was found to be y = -0.156 x + 2.321 R^2 = 0.833.

The Higuchi model is used to describe the limits for transport and drug release. The Higuchi model of formulation was found to be, y = 26.15x – 7.348 R^2 = 0.979. And the results of Korsmeyerpeppas kinetic model was found to be y = 1.151 x + 0.765 R^2 = 0.694. *In-vitro* drug release studies were carried out using dialysis bag. In the above table R2 is correlation value. On the basis of best fit with the highest correlation (R2) value it is concluded that in the optimized formulation of Formulation follow the Highuchi kinetic model.

Stability study

All Results were compared against final formulation of 0 days as the reference.

Table 15: Stability Study of (F4) formulation

S. No	Time (Days)	25^{0}C±2 ^{0}C and 60 ± 5% RH		40^{0}C±2 ^{0}C and 70 ±5% RH	
		Particle size (nm)	Entrapment efficiency (%)	Particle size (nm)	Entrapment efficiency (%)
1.	0	149.1 nm	89.63 %	149.1 nm	89.63 %
2.	30	149.9 nm	89.60 %	148.2 nm	89.50 %
3.	45	149.6 nm	89.59 %	148.5 nm	89.48 %
3.	60	148.0 nm	89.58 %	149.6 nm	88.97 %
4.	90	149.9 nm	89.61 %	149.8 nm	89.16 %

The selected optimized formulation was evaluated for stability studies which were stored at temperature of 25° ± 2°C and 60%± 5% RH and 40^{0}C±2 ^{0}C and 70 ±5% RH for 90 days (3 month) and were analyzed for their assay, Particle size and entrapment efficiency. There was no significant change in the physicochemical and evaluation properties of ethosome formulation during the stability period. There was a slight increase in particle size time for the stored formulation, but it was well within the acceptable limit. Results of assay and evaluation at periodic time points of stability studies are summarized in Table 16.

4. Summary and Conclusion

Novel penetration enhancer, ethosomes were prepared successfully by mechanical-dispersion method for prolonged as well as controlled release of omeprazole. The prepared formulation were characterized for various evolutionary parameters like vesicle size, entrapment efficiencies, vesicles zeta potential, in-vitro drug deposition (release) study.

From in-vitro drug release studies, it is concluded that by changing the ratio of soya lacithin and ethanol, Omeprazole release can be controlled for a prolonged period of time by reducing possible side effects occurred during conventional therapy. Ethosomes of different vesicle size, zeta potential and entrapment efficiency could be obtained by varying the ratio of soya lecithin and ethanol.

In our study, Omeprazole entrapped ethanolic ethosomes was successfully prepared and were showed reasonable entrapment efficiency, optimum vesicle size, and better stability. In vitro drug release studies through dialysis bag membrane showed sustained delivery of omeprazole from ethosomal formulation. Ethosomes substantially enhanced the permeation and bioavailability of omeprazole and also showed better skin accumulation of drug when compared to liposomes, hydroethanolic drug

solutions. In light of above findings, it can be a logical conclusion that ethosomal system can be a promising drug carrier for delivery of omeprazole.

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