DESIGN, DEVELOPMENT AND CHARACTERIZATION OF LIPOSOMAL NEEM GEL

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ABSTRACT

Purpose: Liposomal formulations have been successfully used in the treatment of a number of dermatological diseases. Various synthetic as well as herbal drugs are incorporated into liposome to improve its efficacy. Incorporation of herbal extract into liposome reduces side effects which are associated with the synthetic ones. Azadirachta indica leaves possesse good anti bacterial activity, confirming the great potential of bioactive compounds of neem. Among aqueous extract and alcoholic extract, alcoholic leaf extracts of *A. indica* were found to be more active towards the bacterial species. Hence, this extract was incorporated into liposomes to enhance its activity in skin delivery. The objective of the present research work is to convert this age old miraculous herb into nanotechnology based formulations i.e. liposomes. An attempt has been made to prepare **liposomal Neem gel** for topical use for anti-microbial activity. Methods: Methanolic Neem Extract (MeNE) was incorporated into liposomes by thin film hydration method. The batch having lipid ratio i.e. Soya lecithin: Cholesterol (4:1); MeNE concentration 80 mg with entrapment efficiency 69.52 ±1.9% was finalized. Results and Conclusions: The vesicle size was found to be 3.2µm ± 0.67. *In vitro* drug diffusion and skin retention from liposomal gel was found to be 62.178% ± 0.91 and 20.03% ± 0.63 respectively. Stability studies indicated that formulation was stable over a period of 3 months when stored at 2-8°C.

Keywords: Azadirachta indica, Methanolic Neem Extract (MeNE), Thin film hydration, Soya lecithin, Cholesterol, *In vitro* drug diffusion.

INTRODUCTION

The aim of any drug delivery system is to modulate the pharmacokinetics and/or tissue distribution of the drug in a beneficial way. Among the variety of delivery systems that have been devised over the years are particulate carrier systems; for example microspheres, nanoparticles, lipoproteins, micellular systems and liposomes. Of these, most excitement has been liposomic drug delivery system. Because of the ability of liposomes to carry a wide variety of substances, their structural versatility and the innocuous nature of their components, liposomes have been studied for many different therapeutic situations [1]. Liposomes are the drug carrier loaded with great variety of molecules such as small drug molecules, proteins, nucleotides and even plasmids. Liposomes can be formulated and processed to differ in size, composition, charge and lamellarity [2].

When formulations/cosmetics containing liposomes is applied to the skin, the liposomes are deposited on the skin and begin to merge with the cellular membranes. In the process, the liposomes release their payload of active materials into the cells. Liposomal preparations reduce the skin roughness because of its interaction with the corneocytes and of the intercellular lipids resulting in skin softening and smoothening [3]. Liposomes are used for higher concentration of drugs in deeper layers of skin and a reduction in percutaneous absorption and unwanted side effects [4].

Various synthetic as well as herbal drugs are incorporated into liposome to improve its efficacy [5]. These are used as a good delivery vehicles for plant extract. Various extracts can be incorporated into it like turmeric [6], carrot extract, papaya extract, aloe-vera, green tea [7] extract etc. The incorporation of herbal extract into liposome reduces the side effects which are associated with the synthetic drugs [8].

The Neem tree has been known as the wonder tree for centuries in the Indian subcontinent. Neem has become important in the global context today for its variety of medicinal uses. The medical properties of neem have been known to Indians since time immemorial. The earliest Sanskrit medical writings refer to the benefits of neem's fruits, seeds, oil, leaves, roots and bark. Each has been used in the Indian Ayurvedic and Unani systems of medicines, and is now being used in the manufacture of modern day medicines, cosmetics, toiletries and pharmaceuticals. Neem found to be effective in fighting most epidermal dysfunction such as acne, psoriasis, and eczema. There has never been any report of the topical application of neem causing an adverse side effect. Neem also may provide antiviral treatment for smallpox, chicken pox and warts-especially when applied

directly to the skin [9]. In India it is said that, where there are large number of Neem trees, there are no diseases. The tree is still regarded as 'village dispensary' in India [10].

Azadirachta indica leaves possesse good anti bacterial activity, confirming the great potential of bioactive compounds of neem. Among the aqueous extract and alcoholic extract, alcoholic leaf extracts of *A. indica* were found to be more active towards the bacterial species [11].

The objective of the present research work is to convert this age old miraculous herb into nanotechnology based formulations i.e. Liposomal gel.

MATERIALS AND METHODS

Cholesterol and soya lecithin were purchased from Hi-media Laboratories Ltd (Mumbai). All materials and solvents (Acetone, Chloroform, Methanol) were used of AR Grade. The solvents were purchased from S.D.fine (Mumbai).

The dried leaves of the A. indicum were collected from Yucca Enterprises (Mumbai), was identified and authenticated from Department of Botany, Guru Nanak Khalsa College (Mumbai), having voucher specimen Number: **as 021113**. Quercetin (Marker) was obtained from Ultrapurity (Mumbai).

Preparation of extract

Total Methanolic Neem Extract (MeNE) was prepared by maceration technique. For this purpose, dry leaves (500 gm) of neem were extracted with 1.5 liter methanol and then evaporated by rotary evaporator (Superfit). The total methanol extract was preserved at 4°C until being analyzed.

Standardization of methanol extract of neem leaves by using marker compound (HPLC technique) [12],[13],[14],[15],[16]

HPLC condition:

Chromatographic analysis was carried out by using C18 column Phenomenex (250×4.60 mm), as the stationary phase and acetonitrile: water with 0.1ml of ortho-phospharic acid (40:60) as the mobile phase. Flow rate and injection volume were 1.0 ml/min and 20 µl respectively. The chromatographic peaks of the analytics were confirmed by comparing their retention time. The Spinchrom software was utilized. The system of HPLC was Shimadzu isocratic system with Rhyodyne manual injector. Detection was carried out by UV detector at 369nm. All chromatographic operations were carried out at ambient temperature. Determinations were performed after three separate extractions of each sample, and extract was injected in triplicate.

Preparation of sample solution:

To prepare stock solution of sample, 1 gm of accurately weighed MeNE were taken in a 100 ml volumetric flask and dissolved in the mobile phase and made upto the mark. From this, the working sample solution was prepared. The supernatant was filtered through a 0.45 µm membrane then 20 µl of the filtrate was injected to HPLC.

Procedure: After setting the instrument 20µl of standard solution and sample solutions was injected one by one and chromatograms were recorded.

Formulation of Liposomes [17]

A lipid phase was prepared by dissolving accurately weighed quantities of Methanolic Neem Extract (MeNE), Soya lecithin and Cholesterol in the chloroform- methanol mixture (2:1 v/v) in 250 ml round bottom flask containing glass beads. The solvent mixture was removed from the lipid phase by Rotary evaporation at 45-50°C (Superfit) under reduced pressure, to obtain a thin film of lipids on the wall of the flask and the surface of beads. The dry lipid film was hydrated with Phosphate buffer pH (6.5) at a temperature of 60± 2°C. Dispersion was left undisturbed at room temperature for 2-3 hours to allow complete swelling of the lipid film and hence to obtain vesicular suspension.

Optimization of formulation

The preparation of MeNE liposome includes optimization of various process variables such as ratio of Soya Lecithin: Cholesterol, various concentration of MeNE, and effect of sonication time.

Entrapment efficiency [16]

Entrapment efficiency measurements were performed on UV-spectrophotometer Systronics 2203 equipped with deuterium and tungsten lamp (Systronics). In order to quantify the content of MeNE in the supernatant and pellets in samples, series of standard solutions were prepared. To determine encapsulation efficiency of MeNE liposomes, Quercetin was chosen as standard. The known amounts of quercetin were dissolved in methanol and diluted to obtain a stock solution of 10µg/ml. Standard solutions were then prepared from stock solution. The absorbance was measured at 369 nm based on the spectral analysis. A calibration curve of quercetin was developed by plotting absorbance versus concentration of standard solutions. Liposomal dispersion was

centrifuged at 5000 rpm for 20 min. The supernatant and pellets were each dissolved in methanol. Measurements were done in triplicate. Entrapment efficiency was calculated using the following equation:

$$\textbf{Entrapment efficiency (EE\%)} = A/(A+B) \times 100$$

Where A is the amount of quercetin in the pellet and B is amount of quercetin in the supernatant. Based on the entrapment efficiency, final MeNE concentration was finalized.

Particle Size Reduction

Liposomal suspension was exposed to ultrasonic irradiation with duration of 30 min in continuous sonication bath. The sample was left to cool down and placed in the fridge at 4 °C for 1 day prior to further test. The liposomal suspension was then tested for particle size analysis by microscopy. Sample of liposomal suspension was evaluated for particle size after suitable dilution. Optical microscopy was used with oil immersion lens. Diameters of 50 liposomes were measured and mean geometric diameter and standard deviation were calculated.

Transmission Electron Microscopy

Transmission electron microscopy (PHILIPS, CM200) of liposome was done from Indian Institute of Technology, Mumbai. For Transmission Electron Microscopy (TEM), the samples were negatively stained with a 1% w/v aqueous solution of phosphotungustic acid (PTA) prior to use.

Preparation of Liposomal Gel

Topical MeNE liposomal gel formulations were prepared by incorporation of liposome dispersions into the structured vehicle of carbopol 940 (1%) and HPMC (0.5 %) with gentle mechanical mixing (25 rpm for 5 min.). Triethanolamine was added for neutralization. The same procedure was followed to prepare blank liposomal gel which does not contain MeNE.

Evaluation of liposomal gel

A. Physicochemical Evaluation

1) Physical examination: The MeNE Liposomal gel was prepared by the procedure mentioned and evaluated for colour, odor and transparency.

2) pH: The pH values of 1% aqueous solutions of the prepared gels were measured by pH meter which was calibrated using buffers of pH 4 and pH 7 before measurements.

3) Drug Content uniformity: The gel sample (100 mg) was withdrawn and drug (MeNE i.e. Quercetin as standard) content was determined using a UV spectrophotometer at 369 nm. Similarly, the content uniformity was determined by analyzing the drug concentration in gel taken from 3 to 4 different points from the container. In case of liposomal gel, it was shaken with sufficient quantity of methanol to extract the drug and then analyzed by using a UV spectrophotometer at 369 nm.

4) Spreadability: It was determined by wooden block and glass slide apparatus. Weights about 20g were added to the pan and the time were noted for upper slide (movable) to separate completely from the fixed slides. Spreadability was then calculated by using the formula:

$$S = M.L / T$$

Where,

S = Spreadability

M = Weight tide to upper slide

L = Length of glass slide

T = Time taken to separate the slide completely from each other

5) Homogenicity: Developed gel was tested for homogenicity by visual inspection after the gel has been set in the container. This was tested for their appearance and presence of any aggregates.

6) Viscosity Studies: Viscosity of prepared gel is measured by using Brookfield Viscometer. Apparent viscosity was measured at room temperature and rotating the spindle (64) at 100 rpm.

B. *In-vitro* drug diffusion study:

Preparation of skin

Rat abdominal skin was used for penetration studies. Subcutaneous fatty tissue was removed from the skin using a scalpel and surgical scissors. After the fatty tissue was removed, the surface of the skin was cleaned with saline solution. The skin was stored in saline solution at 4°C, and then used within one day.

Experiment

A Franz diffusion cell was used to perform the experiment. Full shaved abdominal skin was mounted between the donor and the receptor compartments with the stratum corneum side facing the donor compartment. The cell

provided a divisional area of 0.785cm^2, and the receptor compartment was 10 ml. The donor medium consisted of 1 gm liposomal gel. To maintain the sink condition Phosphate buffer pH (6.5): Ethanol (3:1) was used as receptor medium. Stirring rate and temperature were kept 400 rpm and 37°C respectively. At different intervals (1, 2, 4, 8, 12 and 24 h), the receptor samples were removed and replaced with fresh receptor medium. Receptor samples were then analyzed for drug (MeNE, as per quercetin) content spectrophotometrically at 369 nm wavelengh. Cumulative amount of drug release was determined as a function of time and the release rate was calculated.

Skin retention

After conducting permeation study, the skin mounted on the Franz diffusion cells was carefully removed. The remaining formulation adhering to the skin was scraped with a spatula, Cleaned with cotton, soaked in phosphate buffer (pH 6.5) and then gently dried by pressing between two tissue papers. The cleaned skin piece was mashed, and 50 ml of methanol was added to the meshed mass and mechanically shaken in a water shaker bath at 37 ºC for 1 hour for the complete extraction of the drug. The filtrate was removed and the drug (MeNE as per quercetin) content in filtrate was determined spectrophotometrically at 369 nm using a UV spectrophotometer. The measurement was done in triplicate.

Skin irritation test [18],[19]

Total 12 healthy Wistar rats of either sex having average weight 3.5 kg selected for the study. The dorsal skin (4cm^2) shaved carefully. The animals were divided into two equal groups. 1gm of MeNE liposomal gel was applied to shaved area of one group; same way control gel is applied to the second group for the determination of irritation characteristics. The visual observation were carried out at regular interval of 12, 24, 48 hours for various symptoms such as scaling, lesions and erythema. The registration number to perform test was OCP/CPCSEA/2013-14/OCP/10

Stability Studies of optimized formulation

The optimized formulation was subjected to stability studies. Optimized MeNE liposomal gel formulation was sealed in amber colored bottles with cap covered by aluminum foil and these packed formulations was stored in different temperature viz i) room temperature (R.T) ii) 2°-8°C and according to ICH guidelines, maintained at 40^0 C ± 2^0 C at for 3 month. The formulation was evaluated before and after periodic interval for change in appearance, pH, viscosity, drug content, and *in vitro* drug diffusion.

RESULTS AND DISCUSSIONS

The MeNE obtained by maceration technique is observed for color, odor and appearance. Results of description of MeNE were found to be similar as mentioned in literature. The extract is thick semisolid, dark green in color and having a leafy odor.

Determination of Solubility

The solubility values of MeNE in different solvents are checked. The extract is found to be soluble in methanol, Phosphate buffer pH (6.5): Ethanol in the ratio 3:1, ethanol, di-methyl sulfoxide.

Standardization of methanol extract of neem leaves by using marker compound (HPLC technique)

The HPLC chromatograms of MeNE and quercetin (Figure 1) were obtained after injecting the sample and standard solutions respectively. The chromatogram of MeNE (Figure 2) shows a sharp peak, at retention time of 6.133 min, and that of quercetin was at 6.153 min. The HPLC data of MeNE, was compared with that of standard quarcetin. It was found that the peak of qurcetin was eluted in the chromatogram of MeNE at 6.133 min. Hence, the methanolic extract of neem leaves was standardize, that it contains the quercetin potent marker.

Optimization of formulation

Amount of Soya Lecithin (SL) and cholesterol (CH) were found to be critical in the preparation and stabilization of liposomes. Cholesterol is essential for lowering permeability, and imparting stability. Without cholesterol, prepared liposome shows non rigidity and irregular shape. Therefore the Soya Lecithin and cholesterol were used in different molar ratio for preparation of liposomes. Before the addition of drug, placebo liposomes were prepared for selecting proper lipid ratio (Table 1) The batch of placebo liposomes having lipid ratio 4:1 i.e. SL (180mg): CH (45mg) was finalized as it forms the uniform multilamellar vesicles. Then the liposomes containing different concentrations of MeNE were prepared using SL: CH (4:1). Based on the entrapment efficiency finalized the concentration. Results are tabulated in (Table 2). Hence, on the basis of entrapment efficiency the MeNE concentration was finalized e.g. 80 mg.

It is well known that the sonication process may influence the size (size distribution) and entrapment efficiency of liposomes. In order to determine the optimal conditions for sonication, it was necessary to perform a number of trials and evaluate the impact of duration of sonication on the liposomal characteristics. Sonication parameters were evaluated in regard to vesicle size by using SL: CH (4:1) liposomal compositions. Liposomes

were sonicated in sonicator bath containing ice for different time duration. Vesicle size was reduced with increasing time duration. Results are tabulated in (Table 3)

Evaluation of liposomes

Vesicle size by microscopy

In optical microscopy, spherical lamellar vesicles with drug are observed under 100X oil immersion lens. The average particle size of MeNE liposomes was found to be 3.2 µm ± 0.67. (Figure 3)

Transmission Electron Microscopy

In TEM, spherical lamellar vesicles were observed in giving the liposomal suspension at 50 nm scale. TEM photographs shown in (Figure 4)

Evaluation of liposomal gel

A. Physicochemical Evaluation

This liposomal gel evaluated for colour, transparency, clarity, pH, drug content, viscosity, spreadability, homogenicity, in vitro diffusion and skin retention. The pH was found to be 6.5 with particle size of 3.2 µm ± 0.67. The spreadability of 13.5 ± 0.3 g.cm/sec indicates that the gel is easily spreadable by small amount of shear.

B. *In-vitro* drug diffusion study:

The in-vitro drug diffusion of optimized liposomal batch at the end of 24 hour was found to be 62.178 ± 0.91 %. (Table 4) The figure 5 shows the % drug release form liposomal gel batch. This release is also subjected to model fit shows in (figure 6)

Skin retention:

The skin retention is calculated from the calibration curve of Quercetin in methanol. The skin retention of optimized MeNE after 24 hrs was found to be 20.03 % ± 0.63. The skin retention effect of drug may be due to deposition of other components of liposomes with drug into the skin and it increases drug retention capacity of the skin.

Skin irritation test:

Liposome gel treted to check skin irritation in rat. There was no redness found on the skin of rat.

Stability Studies of optimized formulation:

The liposomal gel was unstable at room temperature and 40°C

Stability studies revealed that the physical appearance, rheological properties, drug release in the prepared liposomal gel remained unchanged upon storage at 2-8°C for 3 months. (Table 5)

CONCLUSION

The MeNE was incorporated into liposomal drug delivery system to increase the the rate of permeation into the skin and also to decrease the adverse side effects of extract of neem. The SL: CH ratio (4:1) is optimized for the preparation of liposomes. The batch having lipid ratio i.e. SL: CH (4:1); MeNE concentration 80 mg with entrapment efficiency 69.52 ±1.9% was finalized. This was evaluated for vesicle size by Optical microscopy and by Transmission electronic microscopy. The vesicle size was found 3.2µm ± 0.67. This was incorporated into gel. Carbopol 940 (1%), HPMC (0.5 %), was used for gel preparation. This gel was evaluated for colour, transparency, clarity, pH, drug content, Viscosity, Spredability, and Homogenicity. In vitro drug diffusion and skin retention from liposomal gel was found to be 62.178% ± 0.91 and 20.03% ± 0.63 respectively. Stability studies indicated that formulation is stable over a period of 3 months when stored at 2-8°C. The physical appearance, rheological properties, drug release in the prepared liposomal gel, remained unchanged upon storage at 2-8°C for 3 months.

REFERENCES

[1] Gert Storm, Daan J.A. Crommelin., Liposomes: quo vadis?; PSTT Vol. 1, No. 1 April 1998
[2] Vyas S.P. & Khar R.K. “Targeted & controlled drug delivery: Novel carrier system”. CBS publishers and distributors, 2007. Pg no.[173-248]
[3] Feingold K.R., Permeability barrier homoeostasis: Its biochemical basis and regulation. Cosmet Toilet, 1997; 112 (7), 49-59.
[4] Jain N. K (2007). Controlled and novel drug delivery. CBS publishers and distributors, New Delhi: [304-352]
[5] Ajazuddin, Saraf S., Applications of novel drug delivery system for herbal formulations; PubMed 2010 Oct; 81 (7):680-9.
[6] Patel NA, Patel NJ, Patel RP., Formulation and evaluation of curcumin gel for topical application. Pharm Dev Technol. 2009; 14(1): 80-9.
[7] Ranjit Singh, C.Sankar., Formulation of Herbal Liposomes Containing Green Tea And Gaultheria Procumbens For Anti Acne Activity; IJAPR / Oct. 2012/ Vol. 3 /Issue. 10 / 1211 – 1216.
[8] Sirirat Pinsuwan PhD, Thanaporn Amnuaikit PhD, Suwipa Ungphaiboon PhD, Arunporn Itharat PhD., Liposome-Containing Hibiscus sabdariffa Calyx Extract Formulations with Increased Antioxidant Activity, Improved Dermal Penetration and Reduced Dermal Toxicity; 2010 J Med Assoc Thai Vol. 93 Suppl. 7.
[9] Debjit Bhowmik, Chiranjib, Jitender Yadav, K. K. Tripathi, K. P. Sampath Kumar., Herbal Remedies of Azadirachta indica and its Medicinal Application; J. Chem. Pharm. Res., 2010, 2(1): 62-72

[10] P. Sudhir Kumar, Debasis Mishra, Goutam Ghosh and Chandra S. Panda., Biological action and medicinal properties of various constituent of Azadirachta indica (Meliaceae)" an Overview; Annals of Biological Research,2010,1 (3) :24-34
[11] Kausik Biswas, Ishita Chattopadhyay, Ranajit K., Banerjeeand Uday Bandyopadhyay., Biological activities and medicinal properties of neem (Azadirachta indica); Current Science June 2002, Vol. 82, No. 11, 10.
[12] Bharti Jain, Rashmi Raghuvanshi., Quantitative Analysis of Quercetin in Pueraria Tuberosa by Using High Performance Liquid Chromatography; J. Chem. Bio. Phy. Sci. Sec. A, 2012, Vol.2, No.4, 1688-1692.
[13] Yesi Desmiaty, Fikri Alatas., Determination of quercetin in Hibiscus sabdariffa L. calyces by High-Performance Liquid Chromatography (HPLC); Proceeding of The International Seminar on Chemistry 2008 (pp. 385-388) Jatinangor, 30-31.
[14] P.V. Rajalakshmi, K.Kalaiselvi Senthil., Direct hplc analysis of quercetin in exudates of abutilon indicum (linn). Malvaceae; Journal of Pharmaceutical Science and Technology Vol. 1 (2), 2009, 80-83.
[15] Moghaddasian, Behnaz, Eradatmand Asli, Davood, Alaghemand, Atena., Quantitative analysis of quercetin in different parts of Capparis spinosa by HPLC; Annals of Biological Research, 2012, 3 (12):5775-5778.
[16] Javad Asili, Navid Mosallaei, Ali Shaterzadeh, Bizhan Malaekeh-Nikouei., Preparation and characterization of liposomes containing methanol extract of aerial parts of Platycladus orientalis (L.) Franco; AJP, Vol. 2, No. 1, 2012. 17-23.
[17] Amit Bhatia, Rajiv Kumar, Om Prakash Katare., Tamoxifen in topical liposomes: development, characterization and in-vitro evaluation; J. Pharm Pharmaceut Sci 2004 7(2):252-259.
[18] Meena Devi, Murugesan Senthil Kumar, Nanjaian Mahadevan., Amphotericin-B Loaded Vesicular Systems for the Treatment of Topical Fungal Infection; International Journal of Recent Advances in Pharmaceutical Research; October 2011; 4:37-46.
[19] Anindita De, Amandeep Kaur Gill., Proniosomal Gel of Tretinoin for the Treatment of Acne Vulgaris; Journal of Applied Pharmaceutical Science Vol. 3 (07) 2013, pp. 081-086.

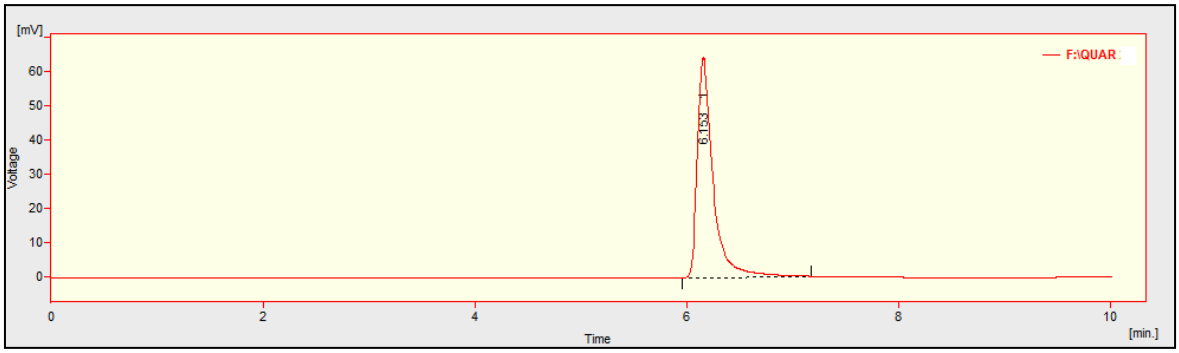

Figure 1: Chromatogram of standard Quercetin

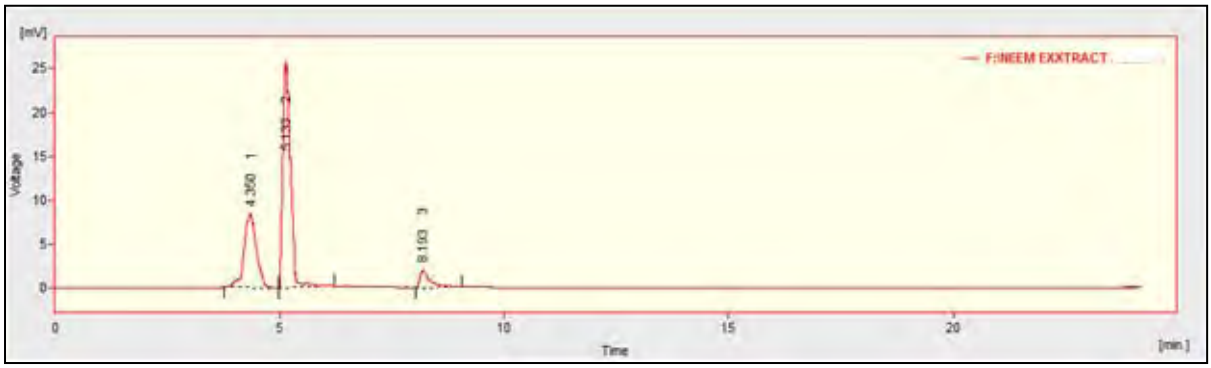

Figure 2: Chromatogram of MeNE

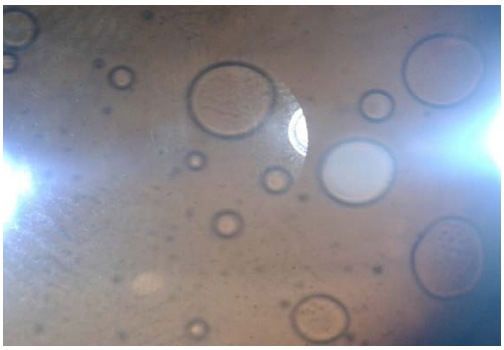

Figure 3: Image of liposome under 100X oil immersion lens

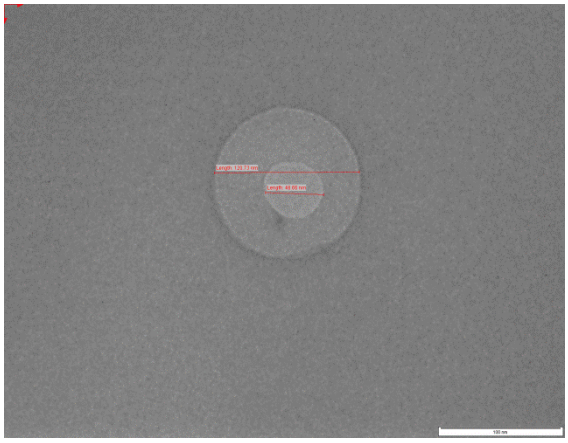

Figure 4: TEM image of liposome

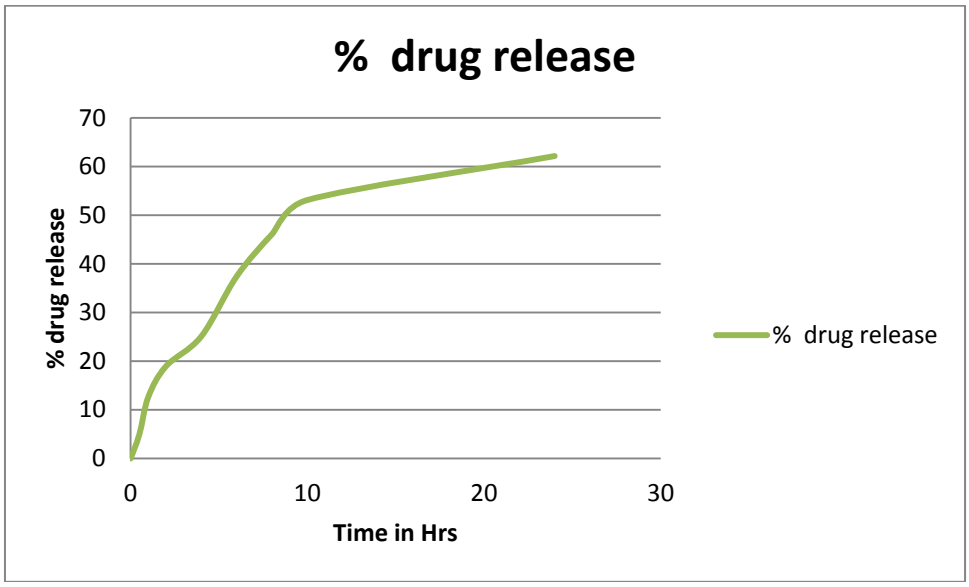

Figure 5: In-vitro drug diffusion of Liposomal gel

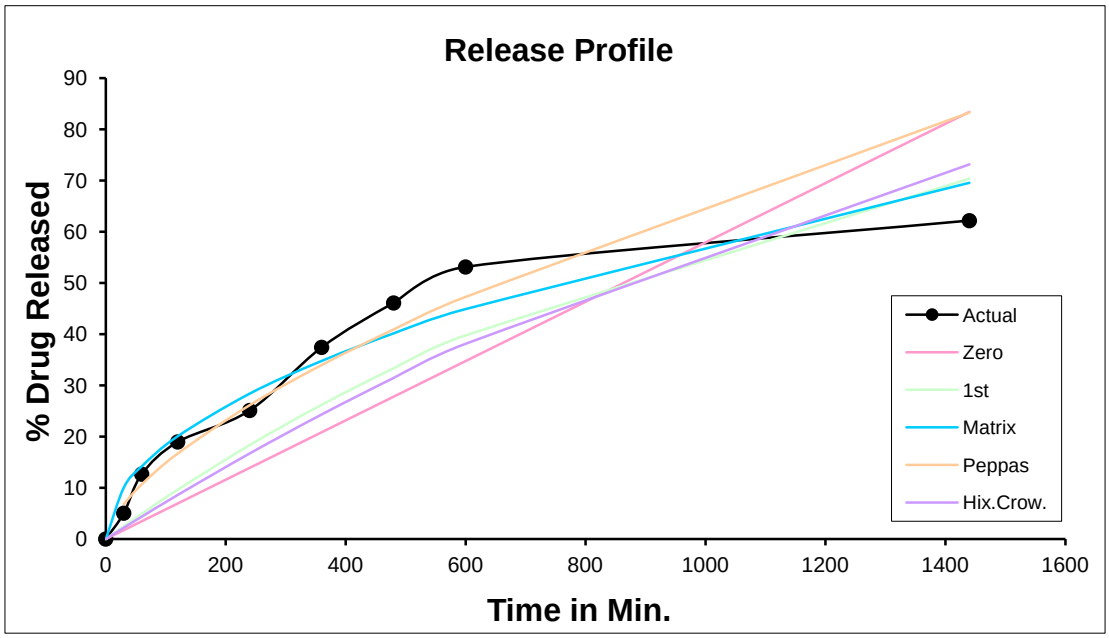

Figure 6: In vitro drug diffusion of liposomal gel with model Fitting

Table 1: Optimization of lipid concentration

Formulations	Molar ratio	Remarks
SL : CH	1:0	Non rigid Vesicles formed
SL : CH	1 :1	Aggregates formed
SL : CH	2 :1	Uniform vesicles were formed But aggregates are also seen.
SL : CH	4:1	Uniform, regular and rigid vesicles were formed
SL: CH	6:1	Irregular and non rigid vesicles were formed

Table 2: Optimization of MeNE concentration

Soya lecithin: cholesterol	Extract concentration (mg)	Entrapment efficiency (% ±SD)
4:1	10	33.1± 1.9
4:1	15	35.3± 2.6
4:1	20	40± 1.8
4:1	25	42.5± 0.3
4:1	50	50±0.3
4:1	75	62±1.5
4:1	**80**	**69.52±1.9**
4:1	100	60±2.1

Table 3: Effect of sonication on size of liposomes

Time in minutes	Particle size in (µm) ± SD
5	8.5± 1.0
10	6.8± 0.5
15	5.2± 1.4
30	3.2± 0.67

Table 4: In-vitro drug diffusion study of Liposomal gel

Time in Hrs	% drug release ± SD
0	0.000
0.5	5.050 ± 0.32
1	12.667 ± 0.31
2	18.964 ± 1.58
4	25.108 ± 0.67
6	37.435 ± 1.45
8	46.105 ± 0.30
10	53.138 ± 0.73
24	62.178 ± 0.91

Table 5: Stability of liposomal gel at 2- 8°C

Sr. No.	Evaluation parameter	After one month observation	After two month observation	After three month observations
1.	Colour	Green	Green	Green
2.	Transparency	Transparent	Transparent	Transparent
3.	pH	6.5	6.5	6.5
4.	Drug content (%)±SD	84±1.3	83.5±0.7	83±0.5
5.	Viscosity ±SD	4451 ± 0.6	4421 ± 0.2	4400 ± 1.2
6.	Spreadability ±SD	13.03 ± 0.41	13.01 ± 0.1	13.5± 0.252
7.	Homogenicity	homogenous	homogenous	homogenous