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Research Article

FORMULATION DEVELOPMENT AND EVALUATION OF LIPOSOMAL GEL FOR THE TREATMENT OF ULCERATIVE COLITIS

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ABSTRACT

The present objective for the study as to prepare mesalazine liposomal gel intended for the treatment and management of ulcerative colitis. The aim of this project has been to develop liposomal drug carrier system, able to provide sustained and controlled release of Mesalazine for local rectal therapy. Various liposome formulation was prepared by the thin film hydration technique by using vacuum rotator evaporator by varying the lipid phase composition (lecithin/cholesterol) and evaluated drug content, Particle size analysis, Zeta (z) potential measurement, entrapment efficacy. Liposomal vesicles with good entrapment efficiencies were in corporated in carbopol gel base was evaluated for pH, viscosity, drug content, in-vitro drug release study. Results of all studies suggested that mesalazine liposomal gel formulation was therapeutically effective drug delivery system for treatment of ulcerative colitis.

Keywords: Mesalazine, Liposome, Anti-inflammatory, Ulcerative colitis.

INTRODUCTION

A liposome is defined as a self-forming structure consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartments Phospholipids are the backbone of these structures. Phosphatidylcholine (PC), also called lecithin (soya lecithin), is a biocompatible phospholipid that exists in animals and plants and used in liposomal preparation. Moreover, there are other molecules widely used in combination with phospholipids, such as cholesterol. Liposomes were first produced in England in 1961 by Alec D. Bangham. The size of a liposome ranges from some 20 nm up to several micrometers. Liposomes are usually classified according to their lamellarity and size. The following categories show the major types of liposomes[1,2].

Multilamellar vesicles (MLV): size range from 100 upto1000 nm. They have several bilayers and composed of a number of concentric lipid bilayers. Large unilamellar vesicles (LUV): The size of these vesicles is normally up to 1000nm. They have surrounded by a single lipid layer. LUV liposomes are delivery systems for nucleic acid drugs. Small unilamellar vesicles (SUV): The structure normally consists of single lamellae and the diameter of this population is below 100 nm[3-6].

Mesalamine is used as anti-inflammatory agent for treatment of inflammatory bowel diseases. Mesalamine is an amino salicylate (the common name of the compound 5-aminosalicylic acid or 5−ASA). This compound inhibits factors in the immune system, importantly, the cytokines that cause inflammation. Mesalamine preparations and formulations are very useful for treating active mild to moderate ulcerative colitis[7,8].

Inflammatory bowel disease (IBD) is a chronic relapsing inflammation any part of the entire bowel wall which can affect anywhere between the mouth to anus, e.g. oropharynx, esophagus, stomach and rectum. Inflammation of bowel disease that covers two disorders: Ulcerative colitis and Crohn's disease. Ulcerative colitis invariably affects the rectum and may extend proximally in a confluent pattern to involve a part of or the entire colon[13]. Ulcerative colitis is a chronic relapsing inflammatory disorder affecting colonic and rectal mucosa. Its origin and cause remain unclear[9].

Colon drug delivery refers to targeted delivery of drugs into the lower GI tract, which occurs primarily in the large intestine (i.e., colon). Single-unit dosage forms (tablets and capsules) for modified release colonic delivery suffer from problems such as unpredictable gastric emptying , GI transit variations resulting from inter subject variability in transit pattern , and incomplete drug delivery in GI tract due to the risk of not dissolving the polymer coat on the large, low surface area-coated tablets[10,11].

The main objective of the study is to develop and evaluated for its in-vitro drug release characteristics in an attempt to improve the drug efficacy, improve the patient compliance, to minimize the side effects associated with the of rectal delivery of mesalazine and also extend the drug release. Mesalazine liposomal gel for the treatment of ulcerative colitis[12].

MATERIALS AND METHODS

Materials

Mesalazine was obtained as gift sample from Wallace Pharmaceutical's Pvt. Ltd. India.
Soya lecithin (PC; phosphatidylcholine) was procured from VAV Life Sciences Pvt. Ltd. Mumbai, India as a gift sample. Cholesterol, Carbopol 940 and triethanolamine were purchased from Research-Lab Fine Chem. Industry Mumbai.
All other ingredients used in the study were of analytical grade. Distilled water was used throughout the experiments.

Equipment

Electronic balance (AY.220 Shimadzu corporation Kyoco, Japan), Rotary Flash evaporator (Kumar Sales Corporation, Mumbai), Centrifuge (Eltek centrifuge), Bath Sonicator (PCI Analytics, Mumbai), Brook Field Viscometer (DV II + pro, Brookfield engineering laboratories, Inc, USA), Digital pH meter (335, Systronics, Ahmadabad), UV Spectrophotometer (Jasco- V630, Japan), FT-IR spectrophotometer (Brukers, ECO-ATR).

Preparation of liposome

Two Solutions were prepared differently which were placed in the solvent mixture. Chloroform and methanol in 2:1 v/v ratio. And PC solution (soya lecithin and Cholesterol) both the solution was mixed in the RBF of 250ml. Also the drug solution was added. The same RBF was placed at the rotary vacuum evaporator with electrical water bath at temperature 40-50◦C until the solvent get completely evaporated. After the complete evaporation of solvent the RBF was removed with the formed thin dried film then hydrated with Phosphate buffer having pH 7.4. The RBF was then placed into the orbital shaker for next 2hrs until the multi lamellar vesicles (MLV) formed[2,13].

Preparation of Mesalazine-Loaded Liposomal Gel

The appropriate amount of carbapol 940 (1gm) was weighed and added slowly warm water (5ml) under constant stirring. After addition of the full amount of solid material, the gel was allowed to swell under moderate stirring for at least 24hrs or until fully swollen and transparent. Other ingredients such as glycerol (3ml) was added to obtain homogenous dispersion of gel. The mixture was stirred until thickening occurred and then neutralized by drop-wise addition of 0.5% w/w triethanolamine. Liposomal gel formulation is prepared by mixing the liposomal dispersion with gels in the ratio of 1:5 (w/w) (liposome dispersion/gel. The Carbopol 940 gel formulations were prepared using 0.5%, 1%, & 1.5%, Carbopol concentration[14-16].

Characterization of liposomal suspension formulation[4,16]

Particle Size Analysis

The formulations were determined for particle size by using Malvern Particle size Analyzer.

Zeta (z) Potential Measurement

The formulations were determined for zeta (z) potential by using Malvern Particle size Analyzer. Zeta (z) Potential was a measure of the particle charge. It was an index for particle stability.

Entrapment Efficiency

The entrapment efficiency measurements were performed on the Ultraviolet visible-spectrophotometer equipped with deuterium and tungsten lamp. In order to quantify the content of mesalazine in supernatant and pellets in samples, series of standard solutions were prepared. The known amounts of Mesalazine were dissolved in methanol and diluted to obtain a stock solution of 10µg/mL. The standard solutions were then prepared using the stock solution the respective concentrations (10, 20, 40, 60, 80 and 100 µg/mL). The absorbance was measured at 330nm based on the spectral analysis. A calibration curve of Mesalazine was developed by plotting absorbance versus concentration of standard solutions.
The supernatant and pellets were each dissolved in methanol. The measurements were done in triplicate. The entrapment efficiency was calculated using the following equation:

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

Where A is amount of Mesalazine in pellet and B is amount of Mesalazine in supernatant[13].

Physical Parameter of liposomal gel[16]

Clarity

The formulation was visually checked for the clarity.

Determination of pH

pH of each formulation was determined by using Digital pH meter. This was previously calibrated by pH 4 and pH 7. The pH values were recorded immediately after preparation.

Rheological Study

Viscosity

The rheological properties of gels were determined by the Brookfield viscometer; type DV-II + PRO using spindle SC4-18. Viscosity of the formulations were taken at room temperature and the 37°c with varying shear rate. Also the effect of pH condition on viscosity of gel was determined and the pH conditions were modified by using triethanolamine. The viscosity of the gel at respective pH range 5.9-6.5 and after adjusting the pH to 5.5 was determined.

In-Vitro drug release study

In-Vitro drug release study of the formulation in-situ gel was carried out by using diffusion cell through egg membrane as a biological membrane. Diffusion cell with inner diameter 1.4cm was used for the study. The formulation 1 ml were placed in donor compartment and Freshly prepared 100ml citrate buffer solution (pH 5.0) (citric acid 2.10gm, sodium citrate 2.941 gm. Distilled water q.s. 100ml) in receptor compartment. Egg membranes were mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°c ± 0.5°c. 2ml of sample is withdrawn from receiver compartment after 1, 2, 3, 4, 5, 6, 7, & 8 hrs and same volume of fresh medium is replaced. The withdrawn samples was diluted to 10 ml in a volumetric flask with 7.4 pH phosphate buffer and analyzed by UV spectrophotometer at 330nm[17-19].

RESULT AND DISCUSSION

Physico-chemical Characteristics

The liposomal formulation evaluated for their physico-chemical properties and found to be fine light tan to pink colored, needed-shaped crystals. Color may darken on expose to air. Odorless or may have a slight characteristic odor.

Table 1: Composition of optimized mesalazine liposome formulation

Formulation code	Mesalazine (mg)	Soya Lecithin(mg)	Cholesterol(mg)
F1	250	180	20
F2	250	270	20
F3	250	360	20
F4	250	180	30
F5	250	270	30
F6	250	360	30
F7	250	180	40
F8	250	270	40
F9	250	360	40

Table 2: Particle size, Zeta potential and Entrapment Efficiency of liposomal formulation

Formulation code	Particle size Analysis (in nm)	Zeta (z) Potential (mV)	Percent entrapment Efficiency
F1	640.6	-1.17	71.92%
F2	348.4	-0.84	65.88%
F3	441.9	-4.66	68.40%
F4	577.6	-16.0	74.46%
F5	348.5	-15.7	86.40%
F6	106.0	-0.502	72.50%
F7	754.5	-0.274	77.92%
F8	420.0	-3.45	70.00%
F9	915.8	-14.8	80.01%

Table 3: pH values of liposomal gel formulation in different concentration

Sr. No.	Concentration of Gel (w/v)	Formulation code	Observed
1	0.5%	F5	4.6
2	1%	F5	5.2
3	1.5%	F5	5.4

Table 4: Viscosity of formulation (n=3)

RPM	Viscosity (cp) at respective pH		
	Formulation code (F5 Batch) (±S.D.)		
	0.5%	1%	1.5%
5	521.1±0.200	752.2±0.004	854.1±0.030
10	407.4±0.014	434.1±0.016	682.1±0.016
15	384.3±0.014	420.4±0.012	574.2±0.013
20	298.4±0.010	327.7±0.030	451.1±0.030
25	245.2±0.010	266.2±0.020	386.1±0.020
30	232.3±0.020	240.2±0.010	256.3±0.010

Table 5: Cumulative Drug release of liposomal gel formulations (n=3)

Cumulative Drug Release (%) (±S. D.)			
Formulation Code (F5)			
Time (hr)	0.5%	1%	1.5%
0	0	0	0
1	12.94±0.20	5.11±0.08	17.18±0.17
2	20.66±0.26	18.14±0.09	30.14±0.17
3	30.16±0.26	24.98±0.09	44.14±0.14
4	44.16±0.17	35.34±0.18	54.54±0.09
5	52.54±0.30	45.74±0.15	62.32±0.19
6	70.30±0.91	58.72±0.03	70.10±0.13
7	74.32±0.90	72.70±0.04	80.52±0.09
8	80.50±0.16	77.92±0.14	84.70±0.11

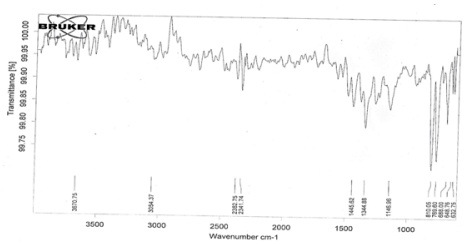

Figure 1: Infra-Red Spectrum of drug with Polymer

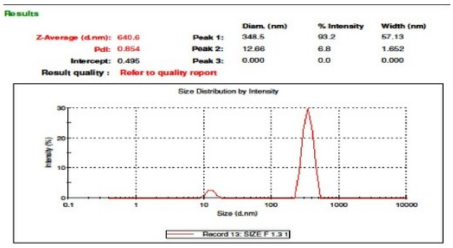

Figure 2: Particle size Analysis of Liposomal Suspension of Mesalazin

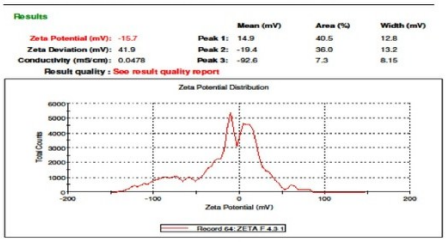

Figure 3: Zeta Potential of Liposomal Suspension of Mesalazine

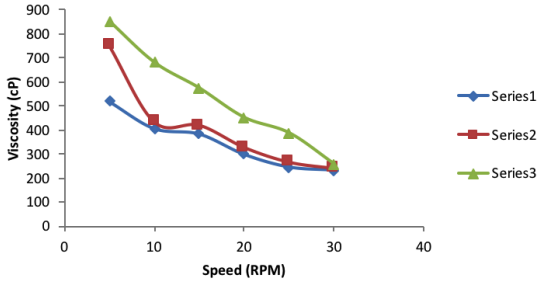

Figure 4: Viscosity of Liposomal gel formulation

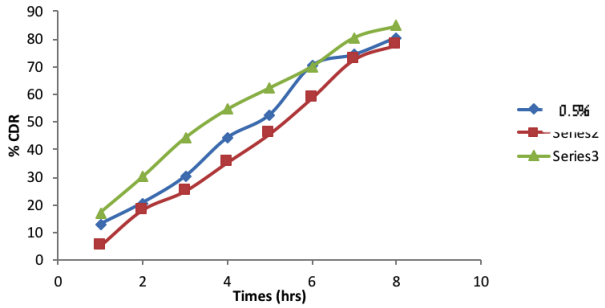

Figure 5: In-vitro drug release of formulation

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR studies of the complex systems results revealed the drug excipients compatibility. In IR spectra of mesalazine pure drug, many peaks were found prominently at various wave numbers indicating the presence of functional groups and substitutions peaks. Peak at 1486 cm^{-1} wave number due to O-H stretching, peak at 1446 cm^{-1} wave number due to stretching vibration of C-C, Peak at 1310 cm^{-1} wave number due to C-O stretching. Peak at 1263 cm^{-1} wave number due to plane bending, Peak at 808 cm^{-1} wave number due to C-H stretching vibration group.

Entrapment efficiency

The amount of drug entrapped into the liposome and in liposomal formulations was determined. The entrapment efficiency was in the range of 70.00% to 86.40%. A good amount of drug was entrapped in the liposome formulations prepared.

Particle size and Zeta potential

The mean particle size of the liposomal formulations was found to be 106.0±0.4 to 915.8 ±0.5 nm. The formulation F6 has low particle size. Multilamellar vesicles size should be under range of 300-5000nm and vesicle size of optimized formulation F5 was found to be 348.5 nm which is under limit.

The zeta potential of all the liposomal formulations were found to be in acceptable range (-0.27 mV to -16.0 mV). Zeta (z) potential should be less than -30mV which indicate the stability of the formulation. Zeta potential of optimized formulation was -15.7mV which indicate optimized formulation F5 is stable.

pH determination

The pH of the experimental gels with different percentage 0.5%, 1% and 1.5% of carbopol 940 was found to be range of 4.6 to 5.4 pH values of formulation.

Rheological study

During stress sweep, it was observed that there was linearity between stress and strain produced all over the applied stress range, which indicate that system was working in correct range. It was clear that gel containing 1.5% of carbopol 940 had shown low dependency on frequency for viscous moduli and phase degree. The viscosity values of all the formulation were affected by the concentration of carbopol concentration. The formulation containing the 1.5% w/v carbopol concentration showed the optimum viscosity.

In-vitro Drug release study

The in vitro diffusion studies in phosphate buffered saline (pH 7.4) were carried out using Franz diffusion cell according to the procedure explained in section.

Out of three formulation maximum release after 8 hrs was found for F5 1.5% strength formulation. This indicates release of 62.32% drug available for anti-inflammatory activity of the drug. F5 1.5% strength formulation showed steady state release up to 8 hrs which also indicates that this formulation would show good contact with biological membrane.

CONCLUSION

The present study was conducted with the view to formulate a topical liposomal gel formulation of Mesalazine for the effective anti-inflammatory of Ulcerative Colitis. In the present investigation, an attempt was made to develop the liposomes of Mesalazine using the soya lecithin and cholesterol as

phospholipid and lipophilic agent respectively. The prepared liposomes of mesalazine were evaluated by % entrapment efficiency, particle size analysis and zeta potential of the same. Further; the formed liposomes of mesalazine were formulated in the topical gel 0.5%, 1% and 1.5% for the using Carbopol as a gelling agent. The prepared topical gels were characterized by clarity, pH, drug content, cumulative drug release, viscosity, and stability studies.

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