JOURNAL OF PHARMACOLOGY AND BIOMEDICINE
E-ISSN: 2456-8244
www.rbscience.co.in

Formulation and evaluation of salicylic acid loaded ethosomes

Ravi Kumar*, Amit Jain

Institute of Professional Studies College of Pharmacy, Gwalior, Madhya Pradesh, India

*Corresponding author

Email- ravi1294kumar@gmail.com

Abstract

Ethosomes entrapping salicylic acid were prepared using cold method and the effect of varying concentration of ethanol was considered for obtaining an optimized formulation. Lecithin (2%w/w) was used as the phospholipid to provide the structure to the vesicles and propylene glycol (10%) was used as the permeating agent. The vesicles were found to be of spherical to irregular shape ranged from 1.4 µm to 1.8 µm in size. The drug entrapment in the ethosomes was studied by analyzing the unentrapped drug using UV spectrophotometry at 310.4 nm and it was found that the maximum entrapment efficiency was found to be 92.07% for formulation ET3 and minimum 58.68% for formulation ET5, respectively. The *in vitro* permeation study suggested that the maximum permeation in the egg membrane occurred in ET3 (0.38 mg/cm^2) with 30% ethanol concentration while ET5 exhibited the minimum permeation (0.28 mg/cm^2). It was observed that only about 2% degradation occurred at room temperature and all formulations were almost stable at 8° and 4° with only 1.3% degradation of SA thereby proving the stability of the developed system. The best ethosomal formulation (ET3) was incorporated into gel base to obtain gel formulations and the results revealed a good protection of the ethosomal gel when 2% carbopol was used as the gelling base.

Keywords: Ethosome, salicylic acid, gel, stability, lecithin, propylene glycol

Received 16/07/2021; Revised 10/08/2021; Accepted 13/08/2021

Scan QR Code to visit Website

Introduction

Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water. Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. The size range of ethosomes may vary from tens of nanometers to microns (Bhalaria et al., 2009). The ethosomes are composed on phospholipids as the vesicle forming component, alcohol and polyglycol to provide penetration enhancement across membrane, and gel forming vehicle (Kumar et al., 2010). Several studies on ethosomes for topical application have been reportedin literature (El-Shenawy et al., 2020; Fathalla et al., 2020; Chandra et al., 2019; Jain et al., 2018; Limsuwan et al., 2017; Ingebrigtsen et al., 2017; Shatalebi and Roostaei, 2015).

Salicylic acid, a therapeutic agent for many skin conditions, is known to induce concentration-related skin irritation. Literature evidence suggests that the topical administration of drugs encapsulated in liposomes may be advantageous in reducing the irritation and itching.

The literature revealed that liposome encapsulated salicylic acid combination formulations have been a topic of wide interest among the researchers and helps in improving the antibacterial efficacy and storage stability of the drug. It was further witnessed that ethosomes offered a higher advantage over the liposomes for transdermal delivery of drugs.

The method of preparation of the ethosomes influences the physicochemical characteristics of and hence the release kinetics and clinical efficacy of the drug.

The objective of the present work is to develop, optimize and characterize salicylic acid loaded ethosomes and to determine storage stability. The ethosomes will be characterized for size, size distribution, efficiency of drug loading, and *in vitro* drug release profile.

Material and Methods

Lecithin and Triton X-100 were purchased from HiMedia Laboratories, Mumbai; Salicylic acid (SA) was purchased from CDH Laboratories, New Delhi. LR/AR/HPLC grade methanol, ethanol and propylene glycol were procured from Oxford Fine Chemicals, Mumbai. All the chemicals were used as received.

Preformulation Study

The pure drug was observed for color, odor and other physical characteristics and its identification was carried out by FTIR spectrophotometry. Melting point was determined by open capillary method. Solubility was qualitatively determined in various solvents.

Standard calibration curve of Salicylic acid

Salicylic acid (SA) was quantified using UV spectrophotometric method (Jani and Patel, 2018). The analysis was conducted using Labtronic, LT-2201 double beam spectrophotometer. Standard stock solution was prepared by accurately weighing 10 mg of SA in 100 ml volumetric flask and making up the volume with methanol up to 100 ml to obtained a final concentration of 100 µg/ml. Accurately pipetted standard solutions of SA (0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml) were transferred to a marked series of 10 ml of volumetric flasks and diluted to the mark with methanol to obtain 5, 10, 20, 30, 40, 50, 60 µg/ml solutions of salicylic acid. The absorbance of each solution was determined at 310.4 nm.

Preparation of ethosomes (Gupta et al., 2014)

Ethosomes were prepared by cold method. In brief the lecithin (3% 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 1 Composition of ethosomal formulations

Formulation code	Lecithin concentration (%)	Ethanol concentration (%)	Polyethylene glycol concentration (%)
AET1	3	10	10
AET2	3	20	10
AET3	3	30	10
AET4	3	40	10
AET5	3	50	10

Evaluation of ethosomes

Shape and size

An optical microscope (Mkow) with a camera attachment was used to observe the shape of the prepared ethosomes formulation. Size and size distribution were determined by dynamic light scattering (DLS) using a computerized inspection system.

Entrapment efficiency

Aliquots of ethosomal dispersion were subjected to centrifugation using cooling ultracentrifuge (Remi) at 12000 rpm. The clear supernatant was siphoned off carefully to separate the unentrapped SA and the supernatant was analyzed by UV spectophotometry. Sediment was treated with 1 ml of 0.1% Triton X 100 to lyse the vesicles and then diluted to 100 ml with methanol and SA was analyzed by UV method. Amount of SA in supernatant and sediment gave a total amount of SA in 1 ml dispersion. The percent entrapment was calculated using the formula,

% entrapment= amount of SA in sediment/amount of SA added ×100

In vitro drug permeation study

The *in vitro* permeation study was carried out by using modified Franz diffusion cell with egg membrane. The study was performed with phosphate buffer saline (pH 7.4). The formulation was placed (equivalent to 2.5 mg of drug) on the upper side of skin in donor compartment. The temperature of the assembly was maintained at 37±2º. Samples were withdrawn after every hour from the receptor media through the sampling tube and at the same time, same amount of fresh receptor media was added to make sink condition. Withdrawn samples were analyzed for SA constant using UV spectrophotometric method.

Stability study

Optimized ethosomal formulations were selected for stability study. Formulations were stored at 4±2°, 8° and at room temperature. Percent drug entrapment was determined at different time intervalsS.

Formulation of ethosomal gel

Gel formulations were prepared by soaking varying concentration of Carbopol 934 in water for 24 h. The ethosomes equivalent to 2% w/w SA were dissolved in ethanol and was added to the gel with continuous stirring. The plasticizer and other ingredients were added and stirred to obtain the ethosome loaded gel formulation.

Table 2 Composition of gel formulation

Ingredients	EG1	EG2	EG3	EG4
SA ethosome (%)	2	2	2	2
Carbopol 934 (%w/w)	1	2	3	4
Propylene glycol (% w/w)	10	10	10	10
Ethanol (mL)	5	5	5	5
Triethanolamine (% w/w)	0.7	0.7	0.7	0.7
Water (g)	15	15	15	15

Evaluation of the ethosomal gel

pH of the formulation

Accurately weighed quantity of 5 g of each gel formulation was mixed separately with 45 mL of distilled water and the pH of the solution was determined with the help of digital pH meter.

Viscosity measurement

The viscosity of each formulation was measured at 10 rpm by using Brookfield DV-1 viscometer employing a S94 spindle.

Spreadability

Spreadability of the formulations was determined using indigenously developed apparatus. The apparatus consisted of a wooden block provided with a pulley at a one end. A rectangular ground glass was fixed on the block. An excess of cream (3-5 g) was placed on this plate sandwiched using another glass plate having the dimensions as that of fixed ground plate. A 1 kg weight was placed on the top of the plates for 5 minutes to expel air and to provide a

uniform film of the cream between the plates. Excess of the ointment was scrapped off from the edges. Weight of 80 g was hung on the hook of the top plate with the help of string attached to the hook and the time (in seconds) required by top plate to cover a distance of 10 cm was noted. Spreadability of the formulation was determined by the following formula:

$S = M * L/T$

where,

S – spreadability

L – distance travelled by the glass slide

T – time in seconds

M - weight in the pan

Tube extrudability

The formulations were filled in clean, lacquered aluminum collapsible tubes with nozzle of 5mm opening and pressure was applied on the tubes with the help of finger. Tube extrudability was determined by measuring amount of cream that extruded through the tip when the pressure was applied on tube.

Results and Discussion

Preformulation Study

The IR spectrum of SA was found to be similar to that of the standard spectrum of SA reported in literature. The spectrum shows the functional group peaks of OH (3240, alcohol), OH (3013, carboxylic acid), C=O (1662), C-O (1386) as shown in figure 1.

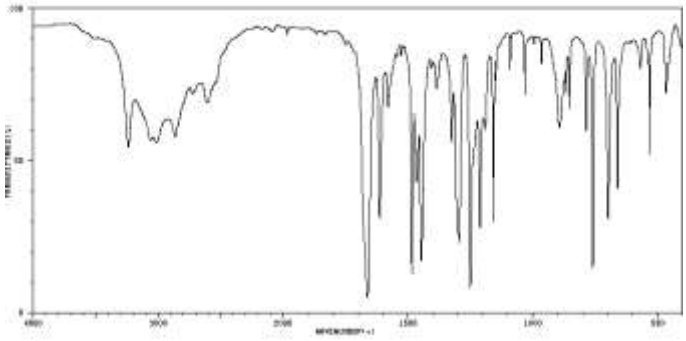

Figure 1 FTIR spectrum of SA

The procured salicylic acid was white fluffy powder with slightly acidic odor and acrid taste. It exhibited melting in the range of 155-157°C. It was found to be freely soluble in ethanol, acetone and chloroform with slight solubility in water.

The standard calibration curve of SA was obtained by measuring the absorbance of the working standards at 310.4 nm and plotting the graph of peak area v/s concentration. Estimation of drug content wherever applicable and the *in vitro* drug release studies are based on the calculations made using this standard curve.

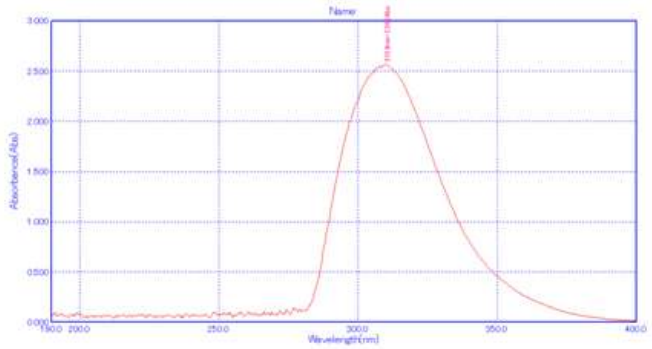

Figure 2 UV spectrum of SA

Evaluation of ethosomes

The ethosomes were evaluated for shape and size, entrapment efficiency and in vitro permeation through egg membrane. The results of the study are presented in the following sections.

Vesicle shape and size

The vesicles were found to be of spherical to irregular shape ranged from 1.4 µm to 1.8 µm in size. The smallest particle size was found to be the formulation ET2 whereas the largest size was found to be of ET1.

Entrapment and drug permeation

The entrapment efficiency of ethosomes was determined for all formulations. Effect of ethanol concentration was observed on percent drug entrapment of ethosomes. The maximum entrapment efficiency was found to be 92.07% for formulation ET3 and minimum 58.68% for formulation ET5, respectively. An increase in percent drug entrapment was observed with an increase in ethanol concentration, but when ethanol concentration exceeded 30%, a decrease in percent drug entrapment was observed. Improvement in aqueous solubility of SA was achieved with higher concentration of ethanol, which could be due to its co-solvent effect. Therefore, the more drug amount could be accommodated in the aqueous core of the vesicles however, as the concentration of ethanol increased above 30% resulting into leakage of drug from fluidized bilayer of vesicles.

Table 3 Evaluation parameters of ethosomes

Formulation Code	Vesicle Size (µm)	Shape	Drug Entrapment (%)	Cumulative amount of drug permeated (mg/cm²)
ET1	1.73	Irregular	84.31	0.31
ET2	1.44	Spherical	82.18	0.34
ET3	1.64	Spherical	92.07	0.38
ET4	1.69	Irregular	70.11	0.34
ET5	1.56	Irregular	58.68	0.28

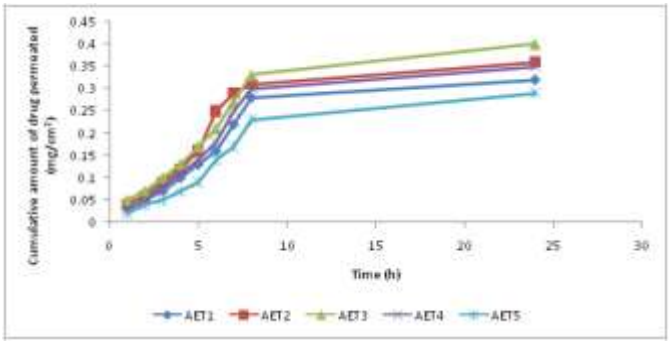

Figure 3 Drug permeation from SA ethosomes

Stability study

The optimized formulation (ET3) was selected for stability study of vesicles at different temperatures. The formulation was stored in amber glass container at different temperature. The drug content after treatment with triton X100 and % residue of SA was calculated as given in Table 4. It was observed that only about 2% degradation occurred at room temperature and all formulations were almost stable

at 8° and 4° with only 1.3% degradation of SA thereby proving the stability of the developed system.

Table 4 Stability of the ethosomes (ET3) on storage

Time (d)	Drug entrapment (%)		
	4°	8°	Room Temperature
1	88.23	89.06	88.07
15	87.91	88.46	87.75
30	87.86	88.17	87.41
45	87.76	87.89	87.02

Evaluation of the ethosomal gel

The gel formulations were prepared using four concentrations of the gelling agent and were evaluated for physical appearance, pH, viscosity, drug content and in vitro diffusion of the drug.

The gel formulations were found to be off-white in color, homogenous and sticky in feel. The pH of the all the formulations was between 6.4-6.7, rendering them suitable for topical application. The formulations were found to possess sufficient viscosity to make then suitable for application to the surface and extrusion from the collapsible tube in which they were packed.

The drug content in all the formulations ranged from 96.7 to 98.39 % confirming the incorporation of the ethosomes into the gel base. The results of the evaluation parameters are presented in Table 5.

Table 5 Evaluation of the gel formulations

Formulation code	Color	Appearance	pH	Viscosity (cps)	Drug content (%)	Spreadability (g.cm/sec)	Extrudability (%)
EG1	Off White	Sticky	6.44	7981	96.7	17.12	95
EG2	Off White	Sticky	6.71	8186	98.11	16.08	84
EG3	Off White	Sticky	6.41	8120	97.05	18.65	92
EG4	Off White	Sticky	6.45	8512	98.39	15.26	87

Acknowledgement

The authors are thankful to the management of IPS College of Pharmacy, Gwalior for providing necessary facilities to carry the research work.

References

Bhalaria MK, Naik S, Misra AN. Ethosomes: A novel delivery system for antifungal drugs in the treatment of topical fungal diseases. Indian J Exper Biol. 2009; 47:368-375.

Chandra A, Aggarwal G, Manchanda S, Narula A. Development of Topical Gel of Methotrexate Incorporated Ethosomes and Salicylic Acid for the Treatment of Psoriasis. Pharm Nanotech. 2019; 7(5): 362-374

El-Shenawy AA, Abdelhafez WA, Ismail A, Kassem AA. Formulation and Characterization of Nanosized Ethosomal Formulations of Antigout Model Drug (Febuxostat) Prepared by Cold Method: In Vitro/Ex

Vivo and In Vivo Assessment. AAPS PharmSciTech. 2020; 21: 31-43

Fathalla D, Youssef EMK, Soliman GM. Liposomal and Ethosomal Gels for the Topical Delivery of Anthralin: Preparation, Comparative Evaluation and Clinical Assessment in Psoriatic Patients. Pharmaceutics. 2020; 12: 446; doi:10.3390/pharmaceutics12050446

Gupta A, Singh S, Kotla NG, Webster TJ. Formulation and evaluation of a topical niosomal gel containing a combination of benzoyl peroxide and tretinoin for antiacne activity. Int J Nanomedicine 2014; 10: 171-182.

Ingebrigtsen SG, Škalko-Basnet N, de Albuquerque Cavalcanti Jacobsen C, Holsæter AM. Successful co-encapsulation of benzoyl peroxide and chloramphenicol in liposomes by a novel manufacturing method - dual asymmetric centrifugation. Eur J Pharm Sci 2017; 97: 192-199

Jain S, Kale DP, Swami R, Katiyar SS. Codelivery of benzoyl peroxide & adapalene using modified liposomal gel for improved acne therapy. Nanomedicine 2018; 13(12): 1481-1493

Jani DA, Patel SA. Spectrophotometric determination of salicylic acid from single as well as combined dosage form. World Journal of Pharmaceutical Research. 2018; 7(8): 938-947

Kumar KP, Radhika PR, Sivakumar T. Ethosomes-A Priority in Transdermal Drug Delivery. Int J Advances Pharm Sci. 2010; 1: 111-121.

Limsuwan T, Boonme P, Khongkow P, Amnuaikit T. Ethosomes of Phenylethyl Resorcinol as Vesicular Delivery System for Skin Lightening Applications. BioMed Res Int. 2017; doi.org/10.1155/2017/8310979

Shatalebi MA, Roostaei M, Preparation and physical evaluation of benzoyl peroxide 5% foamable emu oil emulsion, Jundishapur Journal of Natural Pharmaceutical Products, 10 (3), 2015, e16229-e16234.