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Research Article

FORMULATION AND EVALUATION OF SEABUCKTHORN LEAF EXTRACT LOADED ETHOSOMAL GEL

NIMISHA*, KHUSHBOO SRIVASTAVA, AMRIT KUMAR SINGH

Amity Institute of Pharmacy, Amity University, Uttar Pradesh, Lucknow Campus, India.
Email: nsrivastava3@lko.amity.edu

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ABSTRACT

Objective: The objective of present research work is develop an ethosome as a carrier system for 75% ethanolic SBT leaf extract, its incorporation in to gel formulations and to characterize the prepared is to develop ethosomes and gel formulations using various parameters including estimation of total phenol content in terms of Gallic Acid Equivalents (GAE). Seabuckthorn (*Hippophae rhamnoids*. SBT) is a cold desert medicinal plant, and has high antioxidant content, especially phenol content. There are many reports revealing pharmacological potential of SBT extracts, however, only few literature reports highlight the conversion of these extract in to suitable dosage.

Methods: Twelve batches (F1 – F12) of ethosomes were prepared using Soyaphosphatidylcholine (SPC) (1-3%) and ethanol (10-40%). Carbopol 934P was used to prepare ethosomal gel. The range of entrapment efficiency (EE) of ethosomes was 51.05%-91.09%, polydispersity index (PDI) ranges between 0.041-0.392 and vesicle size from 96.98 nm to 395 nm. Four ethosomal batches were selected on the basis of EE and PDI to be used for further incorporation in to 4 gel formulations (G1, G2, G3 & G4, respectively). Prepared gels were then evaluated for their physicochemical properties, drug content and diffusion characteristics.

Results: The pH of the gel formulations was found to be in the range of 6.2 to 6.6. Viscosities of the gels were ranging between 4900 - 4550 centipoises. The drug content of the gels ranged between 46.47% - 82.47%. The cumulative release of ethosomal gel was maximum in G4 formulation (94.34%) and minimum in G1 (70.75%). These ethosomes, when converted in to gels, were found to show good physicochemical properties, drug content and diffusion pattern.

Conclusion: The present study revealed ethosomal gel as an efficient carrier for herbal extract.

Keywords: Ethanol, *Hippophae rhamnoides*, Soyaphosphatidylcholine, Total phenol content.

INTRODUCTION

Use of advanced lipid vesicles in transdermal drug delivery systems is a need of time since it is believed that conventional liposomal systems have limited applicability as carriers due to their confinement to the upper layer (Stratum corneum) of the skin, instead of its deep penetration. Permeation enhancers, such as Ethanol, are being used in conventional liposomal systems in order to produce an efficient permeation of the skin. However, ability of ethanol to create a finger-like interlocking of the lipid bilayers is a major drawback in using it in higher concentrations, as it destabilizes the entire system. Hence, ethanol can only be found in relatively low concentrations in liposome formulations. Moreover, researchers have been working on the development of liposomal systems with relatively high concentrations of ethanol and such vesicular systems are termed as ethosomes. [1]

Plant drugs are considered safe because of their natural origin. [2] Even after exhibiting promising therapeutic effects, most of the phytoconstituents fail to achieve bioavailability because of poor absorption. Large molecular sizes and low lipid solubilities are the prominent factors causing poor absorption of phytoconstituents resulting in to reduced bioavailability. Incorporation of these plant actives or extracts into vesicular carriers vastly improves their absorption and consequently bioavailability. The novel carriers have been exploited through almost all the routes of administration, topical route being one of them. Moreover, vesicular systems based on topical route of administration have already been used in medical treatments to improve the safety and bioavailability of drug and also to avoid first pass hepatic effect of oral administration. [3]

Seabuckthorn (*Hippophae rhamnoides*; SBT) is a high altitude medicinal plant having an excellent nutritional content along with a huge pharmacological profile. The pharmacological activities of this plant have been credited to its antioxidant effects. Both, the scientific investigations and the nutritional content of SBT leaf extracts also support the same. [4-8] In one of the studies by Singh et al (2012), 75% ethanolic leaf extract of this plant have been reported to have good antioxidant activity as well phenol content. [9]

Poor quality control profile is one of major lacunas in world wide acceptance of plant drugs. In the establishment of standard quality control profile of plant drugs, estimation of biomarker compound plays a crucial role. In the process of drug development also, biomarkers contribute majorly in standardization and validation of the developed drugs. In case of absence of biomarkers, chemical markers (compounds present in major quantities) can be utilized for quality control purposes. Like most of the plant drugs, SBT extracts also, do not have an identified marker compound. Since SBT extracts have been reported to possess excellent phenolic content and a linear correlation has also been observed between phenol content and antioxidant potential of SBT leaf extracts, quantitative analysis of phenolic compounds may be hypothesized as a marker for standardization of herbal formulations based on SBT extracts. [10]

In background of the above literature reports, it was decided to develop an efficient ethosome as a carrier system for 75% ethanolic SBT leaf extract, its incorporation in to gel formulations and to characterize the developed gel formulations using various parameters including estimation of total phenol content in terms of Gallic Acid Equivalents (GAE). The research work was performed keeping in mind that when SBT leaf extract

administered in novel vesicular carrier (ethosomes), it will solve the problem of large molecular size and poor lipid solubility of extract. It will show much better absorption profile which enables them to cross lipid rich biological membrane resulting in increased bioavailability.

MATERIALS & METHODS

Plant material

SBT leaves were collected from Leh, Ladakh, India. These were further authenticated by National Institute of Science Communication and Information Resources, New Delhi, India, and all other solvents and reagents were of analytical grade.

Preparation of extract

Cold percolation method was used for preparation of 75% ethanolic extract of dried SBT leaves. [5] The powdered leaves were extracted with 75% ethanol for 24 hours and filtered with 80 mesh nylon cloth. The raw material to solvent ratio used was 1:8. The extraction process was repeated 5 times. The filtrates obtained after each extraction were combined and stored at ambient temperature. The combined filtrates were again filtered with 250 mesh nylon cloth to get the liquid extract. This extract was then concentrated under reduced pressure till a solid mass was obtained.

Characterization of the extract

Total phenol content

Total phenol content was estimated in above prepared SBT extracts by Folin - Ciocalteu reagent (FCR) based assay. [11] To the aliquot (50µl) taken from stock solution (1mg/ml) of the extract, 3.5 ml distilled water and 250µl of FCR was added, the mixture was kept at room temperature for 1 – 8 min and 750µl of 20% sodium carbonate solution was added. Mixture was kept at room temperature for 2 hrs and absorbance of the color developed was recorded at 765nm with the help of a UV-Visible spectrophotometer against blank. Total phenolic content was determined using Gallic acid standard curve ($R^2 = 0.986$) and expressed in mg/gm as Gallic Acid Equivalents.

Preparation of ethosomes

Ethosomes were prepared by slight modification of cold method. [12] Initially Soyaphosphatidylcholine (SPC) was taken and dissolved in ethanol by use of magnetic stirrer in completely closed flask at 30°C. To this solution, 20 mg of the SBT extract dissolved in hot distilled water (30°C) was added as fine stream by the use of syringe very slowly. The volume was made up using distilled water (30°C) and then whole system was stirred for 15 min at 900 rpm. Further, it was sonicated for 5-15 min. Finally the formulations were stored under refrigeration. 12 batches of ethosomes were prepared using varying concentrations of SPC (1-3%) and ethanol (10-40%). (Table 1) The 4 most stabilized ethosomes with high entrapment efficiency and lower polydispersity index were selected for the preparation of 4 individual vesicle-incorporated gel formulations.

Table 1: Composition of various ethosomal vesicles containing SBT extract

Formulation	% SPC	Ethanol:Water
F1	1	10:90
F2	1	20:80
F3	1	30:70
F4	1	40:60
F5	2	10:90
F6	2	20:80
F7	2	30:70
F8	2	40:60
F9	3	10:90
F10	3	20:80
F11	3	30:70
F12	3	40:60

SPC: Soya phosphatidylcholine, SBT: Seabuckthorn *Hippophae rhamnoides*

Evaluation of the prepared ethosomes

The evaluation was done on the basis of vesicular size, polydispersity index and % entrapment efficiency.

Morphology

For visualization of sample by Scanning Electron Microscopy (SEM), one drop of ethosomal system was mounted on a stub covered with a clean glass. The drop was spread out on the glass homogenously.

Optical microscope observation

The ethosomal dispersion was spread on the glass slide using a glass rod. Formation of multilamellar vesicles was confirmed by examining the ethosomal suspension under an optical microscope with the magnification power of 100X (Olympus). Photographs of vesicles were taken using Olympus camera.

Entrapment efficiency

Aliquots of ethosomal dispersions were subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm. The clear supernatant was siphoned off carefully to separate the unentrapped extract. Sediment was treated with 1ml of 0.1% Triton X 100 to lyses the vesicles and then diluted to 100 ml with methanol. The entrapment efficiency was determined in terms of % GAE in sediment which was evaluated using the same method as was used for total phenol content determination of the SBT extract. [11]

The percent entrapment was calculated using the formula

% Entrapment efficiency =
(amount of GAE in sediment ÷ amount of
GAE in the extract added to ethosome) × 100

Production of hydrophilic gels

Carbopol 934P 0.75% w/v was soaked in minimum amount of water for an hour. 20 ml of ethosomal dispersion containing SBT extract (20 mg) was mixed to the swollen Carbopol 934P with continous stirring 700 rpm in a closed vessel and maintained at temperature 30°C until homogeneous ethosomal gels were achieved. Triethanolamine was then added to adjust the p□ to neutral and stirred slowly to obtain a gel. The last four formulations (F9, F10, F11 □ F12) were selected for incorporation into gel and the gels were renamed as G1, G2, G3 and G4 (Table 2).

Evaluation of gel

Viscosity

Viscosity of different formulation was measured using Brookfield viscometer (Model No DV-III ULTRA). The reading was taken at 100 RPM using spindle no 06.

pH measurement

The p□ measurements of the formulations were carried out using a p□ meter by dipping the glass electrode completely into the gel formulation as to cover the electrode.

Spreading diameter

The spreadability of gel formulation was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) after 1 min. The standard weight applied on upper plate was 125 gm.

Table 2: Formulation table of gels

S.N.	Formulation	Cabopol 934P (% w/v)	Ethanol: Water	Amount of extract (mg)
1.	G1	0.75	10:90	50
2.	G2	0.75	20:80	50
3.	G3	0.75	30:70	50
4.	G4	0.75	40:60	50

Drug content of the formed gels

A 500 mg of gel was taken and dissolved in 50 ml of pH 7.4 phosphate buffer (PBS). The volumetric flask was kept for 2 h and shaken well to mix it properly. The solution was passed through the filter paper and filtered. 50 µl of this solution was taken in 10 ml volumetric and 3.5 ml of distilled water was added to it. To this 250 µl of FCR was added and after 1-8 minutes 750 µl of sodium carbonate solution was added and it was then kept in dark for 2 hours. The drug content was measured spectrophotometrically at 765 nm against corresponding gel concentration as blank.

Drug release profile

The in-vitro studies were performed using Franz Diffusion Cell with egg membrane as the donor membrane. This semi permeable membrane was soaked in a buffer for10-15 minutes. It was clamped carefully to one end of the hollow glass tube of 1.5cm (area 1.76 cm^2). This acted as donor compartment. 50 ml of PBS 7.4 was taken in a beaker which was used as a receptor compartment. The known quantity of gel was spread uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37 ± 0.1°C. The donor compartment was closed from top in order to avoid evaporation of ethanol from the ethosomal gels. The solutions of the receptor side were stirred continuously by using magnetic stirrer placed inside the donor compartment. At predetermined time intervals, sample was withdrawn and replaced by 50 µl of PBS. The drug concentrations in the aliquot were determined at 765 nm against appropriate blank. The method for calculating the drug content involved the treatment of the samples withdrawn with FCR reagent and Sodium carbonate solution. [13-14]

RESULTS

Ethosomes

Morphology

Optical microscopy showed the surface morphology of ethosomes. The smooth surface of vesicles was further confirmed by SEM. (Figure 1)

Vesicle size

The size of ethosomes ranges from 96.98 - 395 nm. The sizes of twelve ethosomal formulations are presented in Table 3.

Entrapment efficiency

The range of entrapment efficiency (EE) of ethosomes was 51.05%-91.09%. Batch F1 showed minimum entrapment whereas batch F12 showed maximum entrapment of extract.

Polydispersity index

Polydispersity index (PDI) ranges 0.041-0.392.

Table 3: Entrapment efficiency, vesicle size and polydispersity index of the various ethosomal preparations

S.N.	Formulation	Entrapment efficiency	Vesicle size (in nanometers)	PDI
1.	F1	51.05±1.83	197.54±12	0.084±0.052
2.	F2	62.58±1.14	135.57±14	0.063±0.038
3.	F3	67.01±0.80	120.53±13	0.056±0.042
4.	F4	73.96±2.03	96.98±15	0.041±0.016
5.	F5	76.12±1.12	215.65±19	0.295±0.007
6.	F6	80.79±1.16	202.42±12	0.099±0.052
7.	F7	83.54±0.83	174.32±11	0.087±0.003
8.	F8	89.54±2.11	132.06±13	0.066±0.013
9.	F9	83.11±1.83	395.31±14	0.392±0.013
10.	F10	85.79±1.88	351.09±12	0.361±0.017
11.	F11	89.50±0.83	367.34±10	0.354±0.016
12.	F12	91.09±0.64	321.10±11	0.314±0.032

PDI: Polydispersity index The results are mean ± SD (n=3), SD: Standard Deviation

Physicochemical properties of gel [15-16]

Ethosomal gels were evaluated for their physicochemical properties, drug content and drug release profiles. The results of all the evaluation parameters for gels are presented in Table 4.

pH

The pH of the gel formulations was found to be in the range of 6.2 to 6.6.

Viscosities

Viscosities of the gels were ranging between 4900 - 4550 centipoises.

Drug content

The drug content of the gels ranged between 46.47% - 82.47%.

The cumulative release of ethosomal gel was maximum in G4 formulation (94.34%) and minimum in G1 (70.75%).

DISCUSSION

Drug should pass through the stratum corneum irrespective of whether transdermal absorption has a systemic action or a local effect. Most of the drugs when applied topically cannot give their required therapeutic effect due to the barrier effect of stratum corneum. In recent years ethosomes are widely used for transdermal delivery because they not only easily pass through the skin but also increase the accumulation of drug in the skin. According to one report, the steady state transdermal rate of trihexyphenidyl hydrochloride ethosomes composed of phospholipid, ethanol, and water increased by 87 fold compared with normal liposome (Dayan N et al 2000). In the present study also, to enhance the transdermal penetration of SBT extract, ethosomal formulations were prepared and they were further incorporated in to gels.

Ethosomal dispersions were produced with increasing amounts of ethanol (10 %, 20%, 30%, and 40%) and SPC concentration (1%, 2%, 3%). In all the dispersions, the use of different ethanol concentrations resulted in white milky suspensions with no significant differences in macroscopic view. Poly Dispersity Index (PDI) was included as one of the broad parameters for evaluation of quality of prepared ethosomes on the basis of their particle size distribution. A PDI value of 0-1 was set as reference in this study and the ethosomal preparations showing PDI in this range were further evaluated on the basis of their vesicular size and drug entrapment efficiency (Table 3).

Size and shape of the vesicle is a crucial factor in the therapeutic performance of transdermal drug delivery systems. In the present study, phase contrast microscopy showed the surface morphology of ethosomes. The smooth surface of vesicles was further confirmed by SEM. (Figure 1).

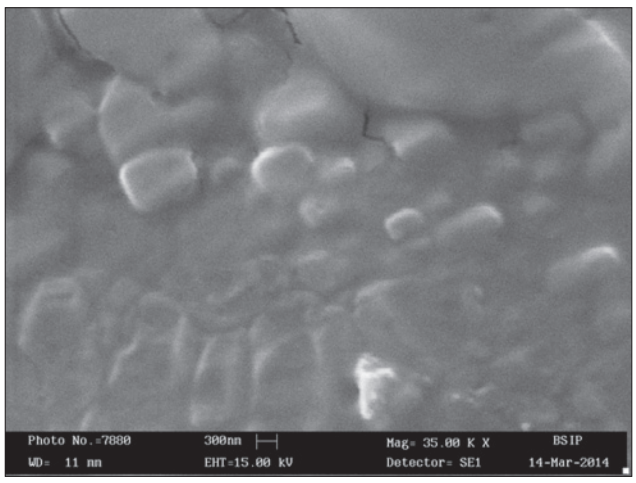

Fig. 1: SEM image of ethosomal dispersion

Table 4: Evaluation of physicochemical properties of gel formulations

Formulation	Color	Homogeneity	Texture	Viscosity (centipoise)	pH	Spreading diameter after 1 minute (mm)	Drug content (%)
G1	Black	Homogenous	Smooth	4900	6.2	55	46.47
G2	Black	Homogenous	Smooth	4500	6.7	48	75.73
G3	Black	Homogenous	Smooth	4700	7.0	42	65.27
G4	Black	Homogenous	Smooth	4550	6.6	44	82.47

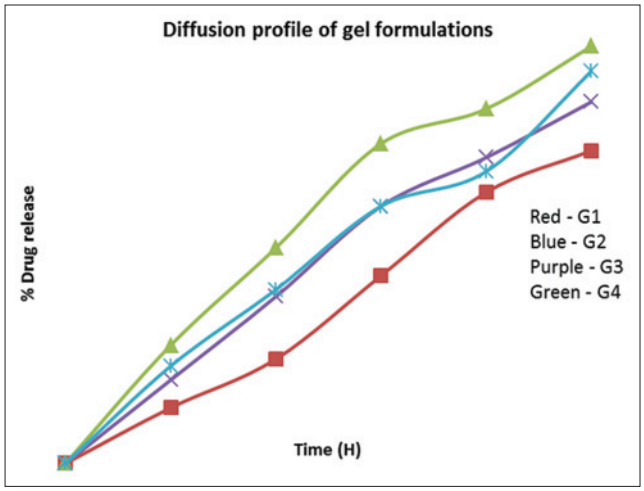

Fig. 2: Diffusion profiles of gel formulations

The minimum vesicle size was observed to be 96.98 nm whereas maximum was 395.31 nm depending on the concentration of SPC and ethanol. The size of the vesicles was found to be increased with increasing concentrations of SPC from 1–3% whereas concentration of alcohol affected the vesicle size inversely i.e. higher concentrations of alcohol produced lower vesicle size. This observation supports the findings of the Elsayed et al (2007) that higher concentration of ethanol confers a surface negative net charge to the vesicular systems by manipulating some surface characteristics, which causes the size of vesicles to decrease. When the amount of SPC was kept at 1% and the concentration of ethanol was increased from 10% to 40%, the size of the vesicles decreased from 197.54 to 96.98 nm. Similarly when the amount of SPC was kept at 2% and 3% and the ethanol concentration was increased in the same manner, the vesicle size was decreased from 215.65 to 132.06 nm and 395.31 to 321.10 nm, respectively (Table 3).

In order to determine the drug holding capacity of the prepared ethosomes, the entrapment efficiency was also evaluated. Moreover, it is a quality parameter that can directly influence the delivery potential of the vesicular systems. The entrapment efficiency of ethosomes was determined for all the formulations. Both, the amount of SPC and ethanol, influenced the entrapment of the herbal extract inside lipid vesicles in a positive way.

Entrapment efficiency of the formulations was observed to increase with increasing ethanol concentrations. Ethosomal formulation fabricated with 3% SPC and 20% ethanol (F10) exhibited 85.79% entrapment efficiency, which was increased to 89.5% and 91.09% when the concentration of alcohol was increased to 30% and 40%, respectively, keeping the amount of SPC unchanged at 3% (Table 3).

Higher entrapment efficiency with increased amount of ethanol is possibly due to increased solubility of SBT extract in water present in the ethosomal core.

The ethanol concentration in the ethosome system should not be too high, and generally should be kept below 45%. In this study also, increased drug entrapment efficiency was obtained with an increase in ethanol concentration, but when ethanol concentration exceeded 40%, drug started leaking from the lipid bilayer resulting in decreased percent drug entrapment. In view of the above, it was decided to keep the concentration of ethanol between 10-40%. Similarly, SPC concentration was also found to influence the entrapment efficiency in a positive manner up to 3% concentration. These data are in prefect agreement with previous finding by Paolino D et al, 2005.

After evaluation of prepared ethosomes, four of the formulations were converted further in to gels namely, G1, G2, G3 and G4, respectively. All the gel formulations were black in color, having good homogeneity and smooth texture. The pH of the gel formulations was found to be in the range of 6.2 to 6.6, which lies in the normal pH range of the skin. Viscosities of the gels were ranging between 4900 - 4550 centipoises. Spreadability of the prepared gel formulations were evaluated in terms of the spreading diameter after 1 minute. The results indicated that the gels were easily spreadable by small amount of shear.

The drug content of the gels ranged between 46.47% - 82.47%. The minimum drug content was found in G1 and maximum in G4. Gel formulations composed of vesicular systems with higher entrapment efficiency were found to exhibit higher drug content.

The in vitro drug release of various ethosomal gel formulations is shown in Figure 2. The cumulative release of ethosomal gel was maximum in G4 formulation (94.34%) and minimum in G1 (70.75%). The in vitro release efficiency of ethosomal gel shows that a significant amount of extract transported across the membrane when entrapped in ethosomes.

CONCLUSION

On the basis of observations of present study, it can be concluded that a combination of 40% ethanol, 3% SPC and 75% ethanolic SBT leaf extract (50 mg) can be used for preparation of ethosomes with good entrapment efficiency. The in vitro release efficiency of ethosomal gel was found upto 94.34% which support the potential of these carriers in penetrating the lipid rich biological membrane. The present study revealed ethosomal gel as an efficient carrier for herbal extract.

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