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Nanosized Ethosomes Bearing *S. grandiflora* Leaves: A Comparative Assessment of Drug Loaded Ethosomal Gel and Non-Ethosomal Gel

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ABSTRACT

The number of products based on new drug delivery systems has considerably increased in the past few years, and this growth is projected to carry on in the future. These biopharmaceuticals present challenges to drug delivery scientists because of their different nature and difficulty in delivery through conventional routes. Therefore, further research will focus on the delivery of these complex molecules through different routes, including nasal, pulmonary, vaginal, rectal, etc. The intend of the study was to formulate and evaluate ethosomes of *Sesbania grandiflora* leaves which may transport the drug to the targeted site more efficiently than marketed gel preparation and also overcome the problems related to oral administration of a drug. Transdermal drug delivery is a technique that can be exploited to overcome the variables, which could affect the oral absorption of drugs such as pH, food intake, and gastrointestinal motility. As compared to the liposome or hydro-alcoholic solution ethosomal systems were much more capable of delivering a fluorescent probe to the skin in terms of quantity and depth. The formulations were prepared with ethanol, lecithin, propylene glycol, and glycerol and were evaluated. The lecithin (phospholipids) used as a vesicles forming component, polyglycol, and ethanol used as a skin penetration enhancer.

1. INTRODUCTION:

Ethosomes are lipid vesicles containing phospholipids and a high concentration of alcohol. Recurrently ethanol is used in the ethosomes as an alcoholic vehicle so it is named ethosomes. [6, 9] All components of the Ethosomal systems are well-thought-out as safe for pharmaceutical use. [24] The typical size of ethosomes can be through the range from 30 nm to few microns. As compared to liposome and too many commercial transdermal and dermal delivery systems ethosomal systems were found to be extensively better at delivering drugs through the skin in terms of both quantity and intensity.[14, 19]

Ethosomes are refined vesicular delivery carriers that are capable of delivering various chemical applications.[6] The penetration rate through the skin of ethosomes is higher as compared to liposomes and hence ethosomes can replace liposomes.[14, 19]The main reason suggested being responsible for deeper distribution and penetration in the skin was might be due to the combination of phospholipids and high concentration of ethanol in ethosomes.[24] *S. grandiflora* leaves are highly nutritive and have been shown to contain more amounts of proteins, fat, carbohydrates, fiber, and minerals such as iron, calcium, and phosphorus. [22]

The young leaves are eatable and are often used to supplement meals. The plant has the potency of antidote for tobacco and smoking-related diseases.[20] Many reports mention the isolation of sterols, saponin, and tannins from the leaves of the plant. [22] It has the property to treat smallpox and other eruptive fevers.[20] Leaves are chewed as it has disinfectant property.[22]These bioactive constituents have potential health benefits and possess important biological activities such as antibacterial, antifungal, antioxidant, antiurolithiatic, anticonvulsant and anxiolytic, and hepatoprotective properties.[20, 22] In the rural areas of Bangladesh, the villagers use a sweetened concentrated juice prepared from the leaves as a remedy for diarrhea. Oral administration of crude ethanol extracts of *Sesbania grandiflora* reduces the duration of defecation. Since we found to publish scientific investigation on topical preparation of *Sesbania grandiflora* leaves through developing the ethosomes.

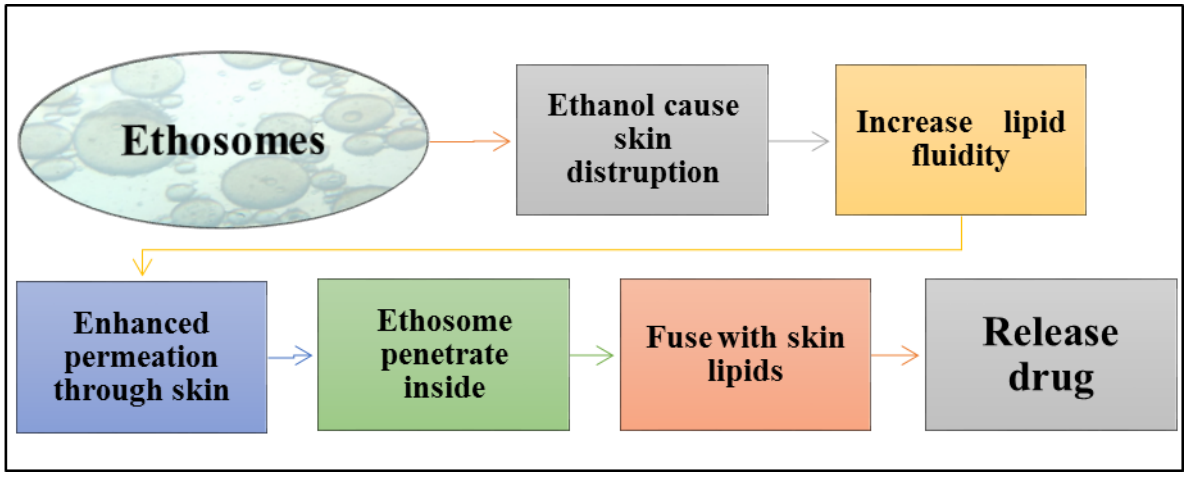


Figure No. 1: Schematic representation of drug release of ethosomal system. [6, 9, 24]

2. MATERIALS AND METHODS:

2.1 Collection and identification of plant:

The raw material (leaves of ***S. grandiflora***) was obtained from Empress Botanical Garden, Pune, Maharashtra, India. The authentication of the collected sample was done by comparing our sample with authentic herbarium specimens at the herbarium department of Empress Botanical Garden.

2.2 Material and Method:

2.2.1 Preparation of extract from leaves of *S. grandiflora*:

The leaves were air-dried at room temperature, after drying it was blended using bender and was converted into a fine powder. 10 g of powder was subjected to the soxhlet apparatus for 4 hrs for extraction purposes with 500 ml of ethyl acetate. The solvent was evaporated using the tray dryer at (60°C) and the dried extract was stored for further study.

2.2.2 Preparation of ethosomes:

a) Cold method:[6]

This method was most commonly used as the lecithin, extract, glycerol, and other lipid materials are dissolved together in the ethanol with vigorous stirring at room temperature. During stirring the polyethylene glycol was added at 40°C. This mixture was heated up to 30°C in the water bath. The stirring is continued for 5 min in the covered vessel and size

reduction was done using the sonicator. The lemon oil was added for flavor. The ethosomes were stored in a refrigerator.

b) Hot method:[6]

The lecithin in this method was first dispersed in water at 40°C. In another vessel take polyethylene glycol with the ethanol and heat up to 40°C. Mix the ethanol phase to the aqueous phase and add the drug to the mixture. The size reduction can be done by sonication. Lemon oil was added and the ethosomes are stored for further study.

Table No. 1: Excipient roles and material used in the formulation of ethosomes.[6, 12]

Excipient	Material used	Role
Phospholipids	Lecithin	Vesicle forming component
Alcohol	Ethanol	Penetration enhancer and give smoothening for vesicle membrane
Lipid base	Castor oil	Give stability to the vesicle membrane
Polyglycol	Polyethylene glycol	Penetration enhancer

c) Selection of method:[6, 15]

Overall four batches were prepared. The B1, B2, and B3 were prepared by using the Cold method and B4 by hot method. Accordingly, the following batches are formulated.

d) Formulation table of ethosomes:[4, 12]

Table No. 2: Composition of *S.grandiflora* ethosomes of various batches.

Material	B1	B2	B3	B4
Extract	1 gm	1gm	1gm	1gm
Lecithin	(2%) 5 ml	(10%)10ml	(10%)5ml	-----
Castor oil	5ml m 5 ml	-----	5ml	10ml
Polyethylene glycol	(50%)10ml	(70%)10ml	(70%)5ml	(50%)10ml
Glycerol	2.5ml	2.5ml	2.5ml	5ml
Ethanol	20ml	40ml	40ml	20ml
Lemon oil	***2ml***	**2ml**	**2ml**	**2ml**

2.2.3 Gel Preparation:[2, 3, 11]

The carbomer was soaked in dematerialized water overnight. The carbomer then was triturated in the mortar pestle. Triethanolamine was also triturated with the carbomer to form the homogenized mixture and the gel base was prepared. Then the ethosomes were added to the base. For non-ethosomal gel, the *S. grandiflora* extract was added to the gel base. The gels were homogenized for 15 min. The composition of gels is given in Table No. 3.

Table No. 3: Composition of various batches of ethosomal gel and non-ethosomal gel of *S.grandiflora.*

Ingredient	Concentration %(w/w)				
	EG1	**EG2**	**EG3**	**EG4**	**NEG**
Carbomer	0.5	0.5	0.5	0.5	0.5
Triethanolamine	0.08	0.08	0.08	0.08	0.08
Propylene glycol	8.5	8.5	8.5	8.5	8.5
Glycerin	-----	-----	-----	-----	1.5
Ethosomes of extract	ml. (Equal to 0.5 mg)	ml. (Equal to 0.5 mg)	ml. (Equal to 0.5 mg)	ml. (Equal to 0.5 mg)	———
Leaves extract	-----	-----	-----	-----	0.5mg
Water	q.s.	q.s.	q.s.	q.s.	q.s.

Formulation table of gel:[15]

3. EVALUATION OF ETHOSOMES AND ETHOSOMAL GEL:

3.1 Evaluation of ethosomes:

3.1.1 Vesicle shape and Size[8, 11, 17]

3.1.2 UV Spectroscopy Study[22]

3.1.3 FTIR analysis[22]

3.1.4 Measurement of drug loading capacity[20,21]

3.1.5 Measurement of entrapment efficiency[1,8,10, 16, 20]

3.1.6 pH[11, 21]

3.1.7 Viscosity[7, 11, 16, 21]

3.1.8 Spreadability[15, 17]

3.1.9 Zeta potential[10, 11, 19]

3.1.10 *In vitro* drug release study[8, 13, 20, 21]

3.1.11 Stability Study[13, 17, 20]

3.1.1 Vesicles size:[8, 11]

The vesicle size and size distribution of ethosomes were determined by nano-particle analyzer Horiba SZ-100 based on the laser light scattering principle. The diluted samples were used for the particle size analysis at 90° angle and 25°C temperature.

3.1.1 Vesicle shape:[8, 11, 15, 17]

The shapes of the ethosomes were examined under the Compound Microscope. The formulation was spread over the clean glass slide which was then covered with a coverslip without a single bubble in it. Then the slide was kept under the eyepiece of 100X and the sample was examined under the compound microscope. The photographs were collected.

3.1.2 UV Spectroscopy Study:[22]

Cyclohexane was used as a solvent for UV Spectroscopy study. The extract was dissolved in the cyclohexane to form a stock solution of 100 ppm. The dilutions of 2 ppm,4 ppm,6 ppm, 8 ppm, and 10 ppm were prepared by diluting the stock solution. The $\lambda_{max}$was measured from the spectral analysis.

3.1.3 FTIR analysis:[22]

The FTIR analysis was performed for the identification of various functional groups present in the extract of S. grandiflora leaves. This was performed by FOURIER TRANSFORM INFRARED SPECTROMETER(JASCO-4600 LE).

3.1.4 Measurement of drug loading capacity:[20, 21]

For measurement of drug loading capacity, the ethosomes were subjected to centrifugation (4000) at maximum rpm for 30min. The supernatant and the sediment were separated. The

dilution of supernatant and sediment was done by cyclohexane and diluted solutions were filtered. Both diluted supernatant and sediment were subjected to UV spectroscopy for assay.

$$\mathbf{D} = \frac{U}{W}\ \mathbf{100}$$

Where **D** is drug loading capacity,

U is the amount of drug in ethosomes,

W is the total amount of drug taken.

3.1.5 Measurement of entrapment efficiency:[1, 8, 10, 16, 20]

The procedure for entrapment efficiency was the same as the drug loading capacity. The entrapment efficiency was calculated by using the below formula.[10]

$$\mathbf{EE} = \frac{W-L}{W}\ \mathbf{100}$$

Where **W** is the total amount of drug taken.

L is the unentrapped drug

3.1.6 pH:[20, 21]

Skin compatibility was an important parameter for a good topical formulation. The pH study was carried out by simply using a digital pH meter (Sigma-27 DP). The pH meter was first calibrated using the buffer solutions of pH 9.2 and 7 and then the readings were taken in triplets.

3.1.7 Viscosity: [7, 11, 16, 21]

The viscosity was determined using the Brookfield viscometer. The rpm:30 and temperature was 25°C and at constant torque%. The readings were taken in triplicates using spindle no.62 (LV-2). The averages of three readings were recorded for more precise results.

3.1.8 Spreadability:[15, 16, 17]

Spreadability is a term expressed to denote the capability of media being spread on the application area. Spreadability was an important parameter for better therapeutic efficacy. A special apparatus has been designed to study the spreadability of the formulations in which

two glass slides of standard dimensions (6×2) were selected. The formulation was placed over one of the slides and the second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cm along with the slide. A 100 gram of weight was loaded over the slide so that the formulation spread equally over the slide and it forms a thin layer. Then the load was removed and the extra formulation was scraped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which a 20 gram load could be applied. The time taken by slide to travel the space of 6 cm and disconnect away from the lower slide under the weight was noted. Lesser the time taken for separation of slides better is the spreadability.

3.1.9 Zeta potential:[10, 11, 15]

Zeta potential provides a measurement of the net charge on the ethosomal surface. It was determined using a zeta potential analyzer (nanoparticle analyzer Horiba SZ-100) at 25.1°C. The measurement of Zeta potential gives electrophoretic mobility and means of zeta potential values were obtained directly from the measurement.

3.1.10 *In-vitro* drug release study:[8, 13, 20, 21]

The diffusion study of the formulation was studied using a cellophane membrane. The diffusion medium used was phosphate buffer (6.8). The receptor chamber was filled with the phosphate buffer 6.8. In the water jacket, the hot water was circulated to maintain the temperature up to 37°C ±0.5. The dilution medium was stirred continuously on the magnetic stirrer at the maximum rpm. The cellophane membrane was precisely kept on the top of the receptor chamber and above it, the drug chamber was kept. The clamp was attached to avoid the shifting of the membrane and leakage of the formulation. The sample was withdrawn through the sample port using a syringe in the time interval of 60 min.

3.1.11 Stabilitystudy: [13, 17, 20]

The stability study was an important parameter for the measurement of shelf life and storage temperature. It was mandatory to check the effect of environmental conditions on the product quality. The change in the quality of the product can lead to the therapeutic efficacy of the dosage form. The ethosomes were evaluated at the initial day, 30 days, and 90 days according to their Entrapment efficiency and pH.

3.2 Evaluations of ethosomal gel:

3.2.1 Organoleptic characterization[15, 16, 17]

3.2.2 Viscosity[7, 11, 18]

3.2.3 pH[11, 21]

3.2.4 Spreadability[15, 16,17]

3.2.5 Tube extruability[19]

3.2.6 Drug contain[11, 15, 21]

3.2.7 Drug released[2,7, 15, 23]

3.2.8 Irritation study

3.2.9 Stability study[17, 18, 23]

3.2.1 Organoleptic character: [15, 16, 17]

The organoleptic character of the ethosomal gel and non-ethosomal gel was determined. The colour and odour and homogeneity was checked by visual inspection

3.2.2 Viscosity:[7, 11, 18]

The procedure for gel was the same as for the simple ethosomes given above (3.1.7). The rpm set at 25 and temperature was 25°C, at constant torque%. The readings were taken in triplicates using spindle no.63 (LV-1).

3.2.3 pH:[11, 21]

The pH of topical preparation should be evaluated for the compatibility of the gel. To test the pH of formulated gels, the gels were first diluted by using distilled water in a dilution factor of 100 (gel: deionized water = 1:100). After that suspension was formed, the pH of each suspension was tested by using a pH meter (Sigma-27 DP).

3.2.4 Spreadability:[15, 16, 17]

The spreadability of both types of the gel was also measured in which the gel was sandwiched between the two slides across the length of 6 cm apart same as that of the ethosomes.

3.2.5 Tube extrudability:[19]

The gel was filled with collapsible metal or aluminum tubes. The weight of the tubes was taken. Then the load was given to the tube to extrude at least 0.5 cm ribbon of material in 10 sec. The test was performed in triplicates and the average was calculated. The extrudability was calculated by using the following formula.

$$Extruadability = \frac{\text{Weight applied to extrude gel from the tube (gram)}}{\text{Area (cm}^2\text{)}}$$

3.2.6 Drug content:[11, 15, 21]

The drug content was determined by UV spectroscopy. The standard solution, test solution was prepared and absorbance was measured at λmax. The drug content was calculated by using a standard plot.

3.2.7 Drug release:[2, 7, 15, and 23]

The drug release was studied on the Franz diffusion cell using the cellophane membrane. The procedure used was the same as that for the ethosomes. Diffusion apparatus: Franz diffusion cell; Volume of diffusion medium: 5.5ml; Speed: max. Rpm; Temperature: 37±0.5°C; Dissolution medium: Phosphate buffer (pH 6.8); Sampling interval: 60min; Quantity of sample withdrawn: 1ml; Samples analyzed: UV (λ_{max}) at 416 nm and absorbance; Cumulative percent drug release was calculated.

3.2.8 Irritation test:

The irritation test was done by simply spreading the gel on the skin and observing any changes on the skin.

3.2.9 Stability Study: [17, 18, 23]

The stability study was an important parameter for the measurement of shelf life and storage temperature. The stability study of the gels was performed after 30 days, 60 days, and 90 days. It was evaluated based on the pH, viscosity, and diffusion study.

4. RESULT AND DISCUSSION:

4.1 Ethosomal formulation:[12, 21]

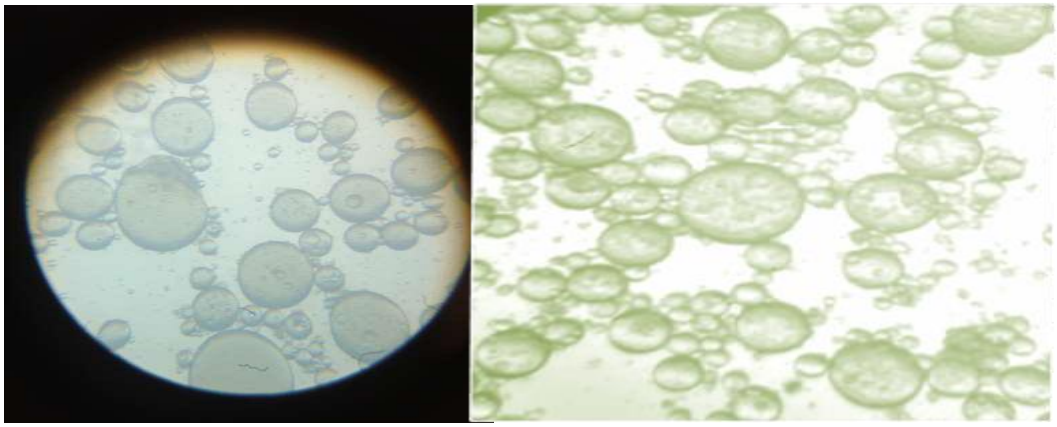
The ethosomes were made by using the lecithin and castor oil as phospholipids which is responsible for the formation of vesicles. Ethanol is used as a penetration enhancer. In B2 and B3 the concentration of lecithin was kept the same and in the B4 formulation, the lecithin was not added. In B4 the vesicle former added was castor oil which was not be used in the B2 formulation. The concentration of ethanol was changed in all formulations. The lemon oil was used as a flavoring agent.

4.1.1 Physicochemical properties of ethosomes:[2, 15, 17]

The physical properties like vesicle shape, vesicle size, spreadability, and chemical properties like pH of ethosomes were performed and the results are presented in Table No. 4. The vesicle size of all four batches was in the range and the shape was perfectly spherical as shown in the microscopical image of ethosomes. The spreadability found was good with pH ranging from 6.81 to 6.86 holding them most acceptable to avoid the risk of irritation after application on the skin.

Table No. 4: Characterization of ethosomal batches.

Batches	Vesicle Size(nm)	Vesicle Shape	Spreadability*(gm.cm/sec)[15]	pH*
B1	240-760	Spherical	39.1 ±0.28	6.86±0.02
B2	140-517	Spherical	57.3 ±0.36	6.81±0.01
B3	160-620	Spherical	44.9 ±0.31	6.83±0.04
B4	310-840	Oval	36.8 ±0.26	6.84±0.02
*** SD:** Standard deviation, (n=3).				



Microscopical vizualization of two batches. (B2 and B3)
Image clearly shows the entrapment of drug into vesicles ; Image under (100X)

Figure No. 2: Microscopical study of formulated batches.

4.1.2 FTIR Analysis:

The FTIR gives information about various functional groups in phytochemical constitutions of *S. grandiflora.*

Leaves extract. The stretching and bending types of peaks are observed in spectra.

Table No. 5: FTIR data analysis of ethosomal batch.

Phytochemicals	**Functional group**	**Observed transition%**	**Std. transition%**
Alkaloids	Amines (─NH)	3271 cm^{-1}, 3495cm^{-1}	3200 – 3600 cm^{-1}
Anthraquinones	Carbonyl (C=O)	1815 cm^{-1}	1670 – 1820 cm^{-1}
Flavonoid	Cyclic ketone	1742.9cm^{-1}	1745 cm^{-1}
Terpenoids	Alkenes(C=C)	1659.2cm^{-1}	1640 – 1680 cm^{-1}

4.1.3 Drug Loading Capacity: [21, 22]

The drug loading capacity was the same as the entrapment efficiency which gives the idea of the amount of drug-loaded per unit weight of the vesicle. It was an important parameter for nanomedicines. B2 had maximum loading capacity as the concentration of ethanol used was more. And loading capacity of B4 was minimum. The other two batches had almost the same drug loading capacity.

4.1.4 Entrapment efficiency: [1, 10]

The entrapment efficiency (EE) ranges from 42.5 ±1% to 88.5 ±2%. B2 batch had the maximum whereas B4 batch had the lowest % EE. The maximum % EE of the B2 batch was due to the average of more amounts of phospholipid, polyglycol, and ethanol. B4 had the lowest % EE due to the absence of the lecithin as phospholipids. If all batches are compared then the entrapment efficiency increased with an increase in the concentration of ethanol as ethanol is responsible for the penetration of the drug in the vesicles. However, it was also observed that the 70% of polyglycol also increased the % EE as it is also a penetration enhancer.

Table No. 6: Data of % Drug loading capacity and % Entrapment efficiency of ethosomal batches.

Batches	% Drug loading capacity	%Entrapment efficiency
B1	81.8 ±0.2 %	57.9 ±1%
B2	94.2 ±0.2 %	88.5 ±2%
B3	81.7 ±0.6 %	72.6 ±2%
B4	64.1 ±0.1 %	42.5 ±1%

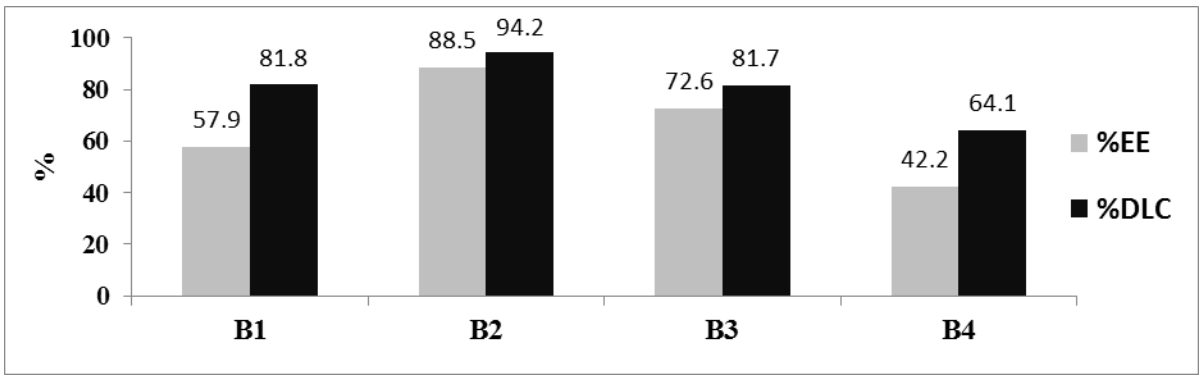


Figure No. 3: Comparative study of % DLC and % EE of ethosomal formulation.

4.1.5 Viscosity:[7, 11, 21]

Table No. 7: Rheological study of ethosomes of B1 to B4.

Batches	Samples	% Torque	Rpm	Viscosity (cps)	Mean(cps)
B1	a	17.2 ±0.5	30	194.7 ±2.18	193.56
	b	17.9 ±0.5		193.2 ±2.18	
	c	18.4 ±0.5		192.8 ±2.18	
B2	a	12.2 ±0.5	30	112.8 ±1.12	112.83
	b	13.2 ±0.5		112.3 ±1.12	
	c	12.8 ±0.5		113.4 ±1.12	
B3	a	9.8 ±0.5	30	94.2 ±0.96	96.23
	b	9.9 ±0.5		95.7 ±0.96	
	c	10.2 ±0.5		98.8 ±0.96	
B4	a	21.6±0.5	30	268.1±0.94	268.29
	b	21.0±0.5		266.9±0.52	
	c	21.9±0.5		268.7±0.83	

The viscosity measured is summarized in Table No.6. The readings were taken in triplicate and the average was calculated. The rpm was kept constant for every batch i.e. 30 rpm. B4 was found to be viscous than the other batches.

4.1.6 Zeta Potential:[2, 10]

The Zeta potential observed of B2 was -28.5mV. The value indicates that batches had good physical stability due to the electrostatic repulsion of the individual particle. The solution resists aggregation.

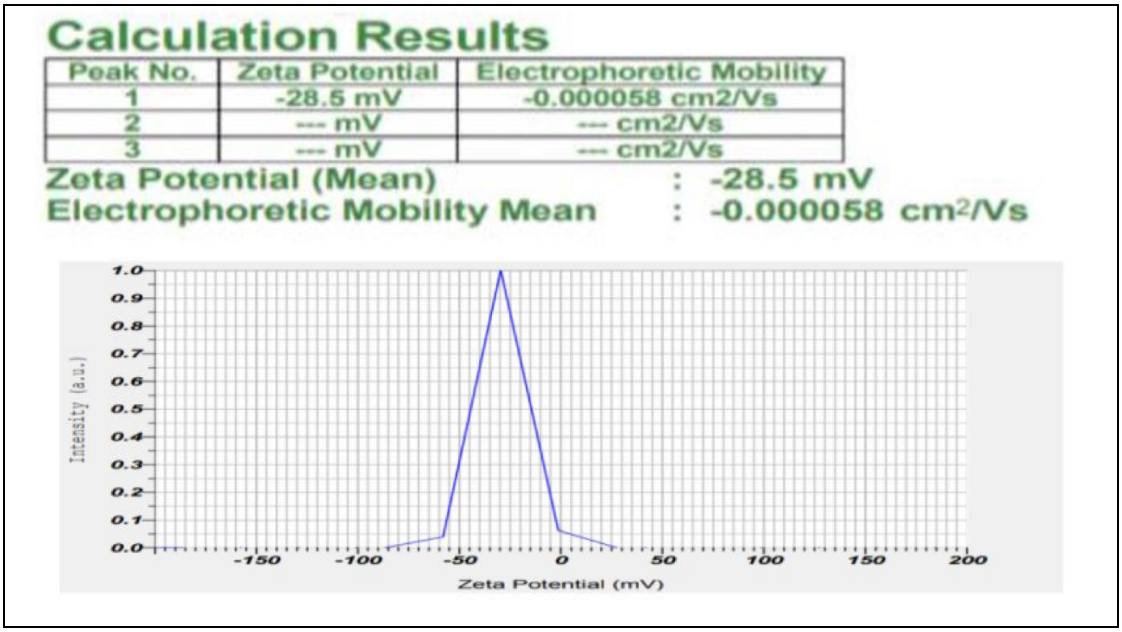


Figure No. 4: Zeta potential of optimized B2 ethosomes.

4.1.7 *In-vitro* drug release:[8, 13, 20]

The diffusion study of ethosomes gave promising results. The results were between 60 to 80 %. The B2 gave maximum release so we considered B2 as the optimized batch. B1 and B3 gave almost the same drug release. The B4 gave minimum drug release as compared to the other batches as the % EE, % CDR and other parameters of B4 were also not satisfying. It concludes that the presence of lecithin helps to prepare better ethosomes with promising results.

TIME (min)	**%CDR**			
	B1	**B2**	**B3**	**B4**
0	0	0	0	0
60	8.8	9.28	8.25	7.21
120	16.48	18.64	15.71	13.33
180	27.08	32.01	28.73	23.56
240	40.1	45.52	40.02	36.18
300	54.04	62.52	53.48	48.31
360	66.4	75.64	70.02	57.94
420	72.02	85.38	77.91	63.12
% CDR= % Cumulative drug release				

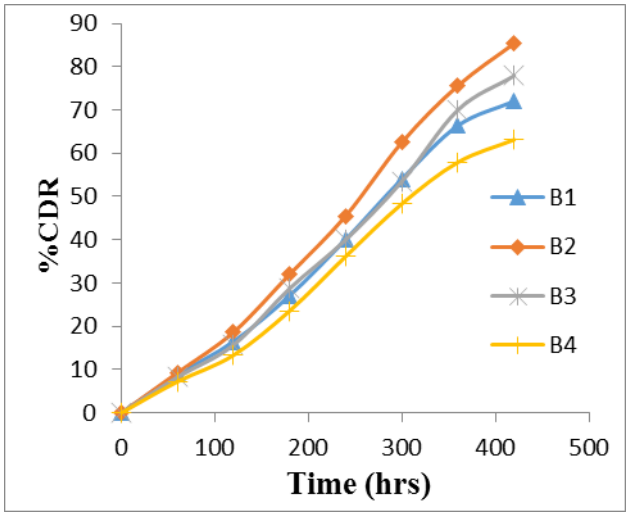


Figure No. 5: Comparison of *in-vitro* drug diffusion studies among the four batches of *S. grandiflora* ethosomes.

4.1.8 Stability study:[10, 13]

The stability study was performed according to the % EE and pH for three months. The changes in the results were negligible i.e. in few fractions. The % EE was unchanged during 90 days it means that the drug release will remain the same and the ethosomes will give a good therapeutic index till the end of the third month. The appearance and results of pH were stable i.e. the ethosomes were chemically and physically stable.

Table No. 8: Stability study of ethosomes.

	% Entrapment efficacy				**pH Study**			
	B1	**B2**	**B3**	**B4**	**B1**	**B2**	**B3**	**B4**
First day	57.9±1 %	88.5±2 %	72.6±2 %	42.5 ±1%	6.86	6.81	6.83	6.84
After 30 days	55.1±1 %	86.8±2 %	70.1±2 %	40.9±1 %	6.92	6.94	6.99	6.87
After 90 days	51.3±1 %	85.3±2 %	66.2±2 %	36. 8±1%	7.28	7.32	7.46	7.02

4.2 Gel Formulation:

4.2.1 Organoleptic characterization:[5, 15, 16, 17]

The colour of ethosomal gel (EG) observed was Chartreuse yellow while non-ethosomal gel (NEG) was yellow-green. The EG had a smooth texture while NEG was uneven. The homogeneity and consistency of EG were excellent whereas NEG was satisfactory. The picture of the gels is given below to know the physical appearance. It is given in Table No. 9.

4.2.2 Physicochemical properties: [11, 15, 17]

The viscosity of the EG was in the range of 43163.6 cps - 56289.4 cps and of NEG was 46521.6 cps. The viscosity depends on the type and concentration of polymer used in the formulation. Higher the concentration of the polymer more is the viscosity and less is the drug release. So, the viscosity of all the batches was at the limit. The pH of the gel observed was in the range of 6.43 - 6.76. It is compatible with the pH of the skin so irritation problems will not occur. The spreadability was also good i.e. 16.49 gm.cm/sec - 32.34 gm.cm/sec and tube extrudability measured was in the range of 21.91 g/cm^2 - 39.14 g/cm. [19]

Table No. 9: Physicochemical evaluation of ethosomal gel and non-ethosomal gel.

Batches	**Viscosity(cps) (mean**±SD***)**	**pH (mean**±SD***)**	**Spreadability(gm.cm/sec) (mean**±SD***)[15]**	**Tube extrudability (g/cm^2) (mean**±SD***)**
EG1	48251.3±5.128	6.73±0.05	22.68±0.9	27.21±0.89
EG2	38085.1±4.347	6.66±0.01	32.34±1.6	39.14±1.53
EG3	43163.6±5.412	6.53±0.04	27.10±1.3	33.16±1.41
EG 4	56289.4±7.641	6.76±0.02	16.49±0.7	21.91±0.85
NEG	46521.6±5.375	6.43±0.09	25.05±1.2	29.95±1.32
EG: Ethosomal Gel; **NEG:** Non-Ethosomal Gel; **SD:** Standard deviation,(n=3)				

Table No. 10: Physical characterization of ethosomal gel and non-ethosomal gel.

Batches	Colour	Odour	Texture	Homogeneity	Consistency	Appearance
EG1	Chartreuse yellow	Sweet	Smooth (no crystals observed)	Excellent (No phase separation)	Excellent	
EG2	Chartreuse yellow	Sweet	Smooth (no crystals observed)	Excellent (No phase separation)	Excellent	
EG3	Chartreuse yellow	Sweet	Smooth (no crystals observed)	Excellent (No phase separation)	Excellent	
EG4	Chartreuse yellow	Sweet	Smooth (no crystals observed)	Good (Uneven phase separation)	Fair	
NEG	Yellow-green	Sweet	Uneven (crystals observed)	Fair (No phase separation)	Satisfactory	

4.2.3 Drug Content:[11, 15, 21]

The drug content of EG was in the range of 98.93 % to 99.71 % and of NEG was 98.41 % EG had high drug content than NEG. EG2 had maximum drug content so the release will also be maximum and this batch can be considered as optimized.

Batches	EG1	EG2	EG3	EG4	NEG
% Drug Content	99.05±2.28	99.71±1.98	98.93±2.04	99.39±3.16	98.41±2.84

4.2.4 *In-vitro* Drug Release and Kinetic Study:[2, 7, 10, 15]

Table No.10 shows that the EG2 had maximum drug release than all the other batches.NEG had minimum drug release it means that the ethosomal gel is novel and had many advantages over the non-ethosomal gel. The drug release of EG ranges from 59.856 % - 81.065 % and of NEG was 25.836 %.

Table No.11: *In-vitro* drug release profile of ethosomal gel and non-ethosomal gel.

TIME (min)	% CDR				
	EG1	EG2	EG3	EG4	NEG5
0	0	0	0	0	0
15	8.628	8.955	7.836	6.445	2.814
30	14.068	14.089	14.256	11.961	5.330
60	18.325	24.125	26.612	21.162	9.432
120	26.902	37.635	38.143	33.126	14.762
210	38.656	59.833	50.357	44.167	19.680
330	51.358	73.349	66.519	52.448	23.372
480	64.229	81.065	73.155	59.856	25.836
% CDR: % Cumulative drug release; EG: Ethosomal gel; NEG: Non-ethosomal gel.					

Batches	R^2				Best Fitted Model
	Zero Order Kinetic	First Order Kinetic	Higuchi's Model	Peppa'sModel	
EG1	***0.967***	0.887	0.821	0.964	Zero Order
EG2	***0.971***	0.908	0.824	0.962	Zero Order
EG3	***0.988***	0.940	0.857	0.987	Zero Order
EG4	***0.990***	0.974	0.866	0.985	Zero Order
NEG	***0.989***	0.988	0.870	0.984	Zero Order

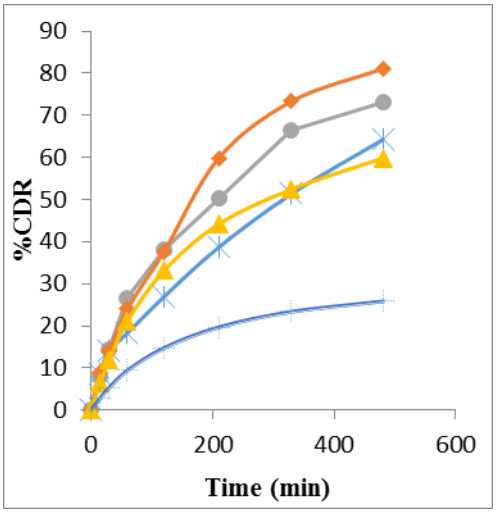


Figure No. 6: Comparison of in-vitro drug diffusion studies of ethosomal gel and non-ethosomal gel.

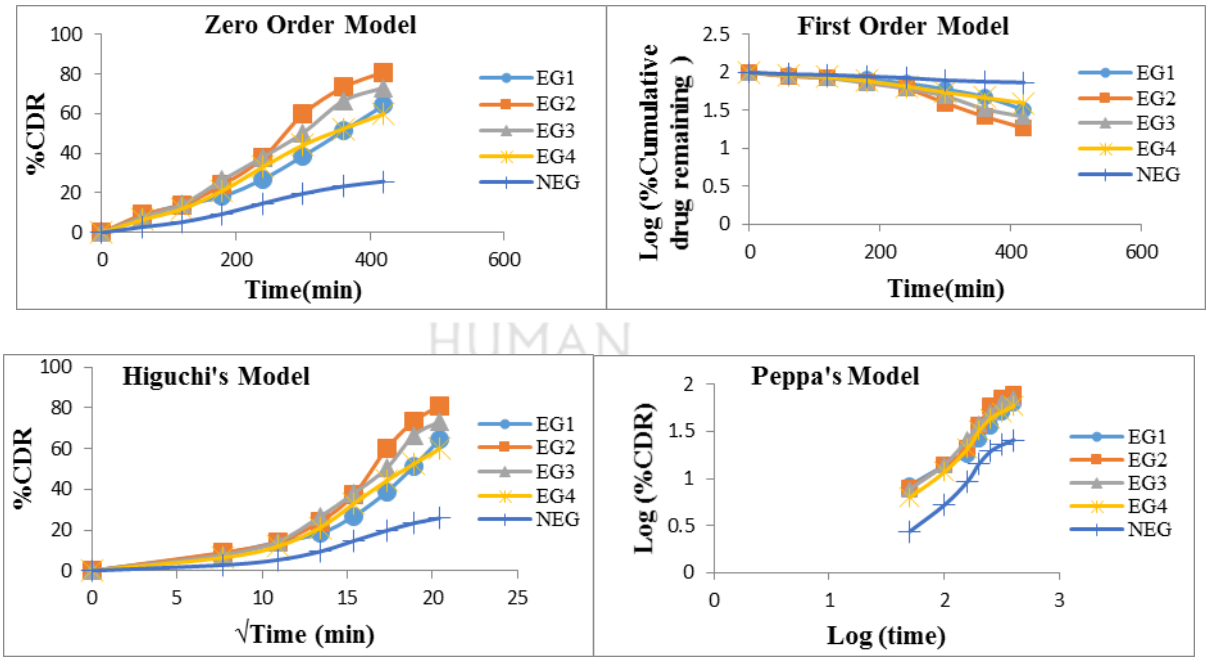


Figure No. 7: *In-vitro* diffusion kinetics of ethosomal gel and non-ethosomal gel.[10]

4.2.5 Stability Study:[3, 13, 17, 18]

The change in the results of the stability study was negligible. The fractional change in the viscosity indicated that the gel formulation was physically stable till 90 days. The pH remained unchanged which indicated its' chemically stable and nonirritant to the skin. The drug content remained the same and also the drug release didn't change. All the formulations gave good results so the gels prepared were stable till the end of 90 days**.** It was given in table no.11.

Table No. 12: Stability study of ethosomal gel and non-ethosomal gel.

Parameters	Batches	Initial Day	After 30 Days	After 90 Days
Viscosity(cps)	EG1	48251.3±5.128	48404.1±6.237	48889.5±5.468
	EG2	38085.1±4.347	38245.3±5.387	38605.9±4.356
	EG3	43163.6±5.412	43354.1±5.697	43736.6±6.986
	EG4	56289.4±7.641	56499.4±8.032	56846.1±8.465
	NEG	46521.6±5.375	46852.5±6.823	47012.5±6.548
pH	EG1	6.73±0.05	6.70±0.05	6.64±0.06
	EG2	6.66±0.01	6.61±0.06	6.57±0.02
	EG3	6.53±0.04	6.49±0.03	6.42±0.03
	EG4	6.76±0.02	6.71±0.01	6.62±0.02
	NEG	6.43±0.09	6.39±0.06	6.32±0.04
% Drug contain	EG1	99.05±2.28	98.19±3.48	97.85±3.79
	EG2	99.71±1.98	98.89±1.97	98.01±2.16
	EG3	98.93±2.04	98.13±3.78	97.23±3.98
	EG4	99.39±3.16	98.66±3.37	97.49±4.79
	NEG	98.41±2.84	97.86±3.46	96.91±4.69
Drug released (At 420 min)	EG1	64.229	63.657	60.594
	EG2	80.065	79.397	77.953
	EG3	71.155	69.974	66.879
	EG4	59.856	56.894	53.675
	NEG	25.836	23.352	21.275

EG: Ethosomal Gel;**NEG:** Non Ethosomal Gel

5. CONCLUSION:

The study revealed that ethosomes can be considered as a possible vesicular carrier for transdermal delivery of *S. grandiflora* leaves extract. The formulation was successfully prepared by loading leaves extract, phospholipids; ethanol, and ethosomal gel-based formulations were prepared with hydrophilic polymer carbomer. The ethosomes were spherical and discrete in shape. The size of the vesicle was found to be in the range and sensible for skin penetration.

The ethosomes showed the highest entrapment efficiency and rapidly penetrate through the skin because of their tiny vesicular size. The kinetic study of ethosomal gel shows that the formulations followed zero-order models means the drug is released at a constant rate. The viscosity of the gel was four times greater than the ethosomes. So, it can prevent the aggregation of ethosomes, stabilize the formulation and avoid increasing the size of vesicles which was analyzed by zeta potential result. As viscosity increases the spreadability decreases and the results obtained followed the same. The carbomer used for the gel is acidic and it can be quite irritating to the skin so we used triethanolamine to balance pH. The pH of the formulation was compatible with the skin.

The stability study carried out for 90 days showed negligible changes at room temperature. Ethosomal gel gave high *in-vitro* drug release than the non-ethosomal gel. The highest drug release leads to an increase in bioavailability which indicates that the ethosomes are advanced nanoparticles. Hence it is concluded that *S. grandiflora* leaves extract loaded ethosomal gel is advance over the non-ethosomal gel.

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