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DEVELOPMENT AND EVALUATION OF TRANSDERMAL DRUG DELIVERY OF SALBUTAMOL SULPHATE *VIA* ETHOSOMES

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Keywords:

Salbutamol sulphate, Transdermal drug delivery, Ethosomes, Alcohol, Phosphatidylcholine, Carbopol 934

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ABSTRACT: Asthma is a chronic disease; there is a need for a drug delivery system that maintains adequate therapeutic concentrations for a longer duration of action to improve better clinical efficacy. Salbutamol sulphate (SS) is a widely prescribed drug to treat asthma though it has short plasma half-life and undergoes extensive first-pass metabolism. Thus, it necessitates frequent administration by oral route to maintain adequate plasma concentrations. Hence an attempt was made to formulate and evaluate transdermal ethosomal gel formulations of SS to improve therapeutic efficiency and to reduce the dosage regimen. Ethosomal suspensions of drugs were prepared using phosphatidylcholine, alcohol, propylene glycol, and cholesterol. Then, suspensions were used to develop gels by incorporating them into carbopol. Formulations were evaluated for vesicular size, size distribution, scanning electron microscopy, drug content estimation, drug entrapment efficiency, *in-vitro* diffusion studies, Z-Average, polydispersity index (PDI), and zeta potential measurement. Analytical studies confirmed the absence of drug and polymer interaction. Drug content of ethosomal suspensions and gel formulations was found uniform, and microscopic images displayed that vesicles are spherical shape. PDI values indicated a broad size distribution of ethosomes. Zeta potential values confirmed the dispersion stability of ethosomal vesicles in suspensions. The drug release rate from ethosomal gels extended up to 12 h, progressively increased with an increase in alcohol concentrations, and decreased with increased concentration of carbopol 934. The drug release rate followed zero-order and non-Fickian diffusion mechanism. The whole study disclosed that transdermal gel formulations of SS *via* ethosomes hold potential for providing controlled release by achieving adequate bioavailability and thus could enhance the overall life quality of asthmatic patients.

INTRODUCTION: The skin is a final frontier for drug delivery as well as a surprisingly well-organized barrier designed to keep 'our insides in and the outsides out'. Transdermal administration of many therapeutic agents is often precluded because of this barrier property of the skin.

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It is generally believed that the penetration of therapeutic agents through the skin is primarily regulated by the highly organized structure of stratum corneum (SC), the outmost layer of the epidermis [1].

For transdermal drug delivery systems (TDDS) to be effective, the drug must obviously be able to penetrate the skin tough barriers and reach the target site. Hence, significant effort has been devoted to overcoming the impermeability of intact human skin [2]. This is evident from the remarkable achievements of pharmaceutical technologists who have made TDDS the most successful non-oral

systemic drug delivery [3, 4]. However, judicious choice of drug as well as penetration enhancement techniques is critical to maximizing drug flux in order to make successful development of transdermal therapeutic systems [5]. Systemic drug delivery through the skin has several advantages over parenteral or oral drug therapy. They ensure sustained, controlled release and absorption, absence of the first-pass metabolism, improved bioavailability, and more uniform plasma drug concentrations for a longer period of time with decreased side effects [6]. Thus, improving convenience and patient compliance can be accomplished since the frequent intake of drugs by the oral route, or inhalation is no longer necessary, especially in the treatment of chronic conditions of asthma. Currently, ethosomes are an interesting novel lipid-based carrier (biocompatible component) as a backbone with ethanol for transdermal drug delivery systems.

They act as non-invasive, 'soft vesicles' designates as vesicular carriers tailored for enhanced delivery of active agents through the deep skin layers into the systemic circulation. They are a modified form of liposomes basically consists of phospholipids (Phosphotidyl choline), ethanol in relatively high concentration (20-50%), and water [7]. Several research reports confirmed the effectiveness of ethanol as a permeation enhancer in lipid vesicular systems [8]. Hence, ethosomes has prompted a new area in vesicular research for transdermal drug delivery in recent years. They have a higher permeation rate through the skin as compared to liposomes, improved drug delivery, increased drug entrapment efficiency, simple mode of preparation, and a lack of side effects, *etc.* [9, 10]. Salbutamol sulphate (SS) is one of the most widely prescribed drugs to treat asthma, including bronchial asthma, exercise-induced bronchoconstriction, and chronic obstructive pulmonary disease (COPD). The drug has a plasma half-life of about 4-6 h and undergoes extensive first-pass metabolism and thus necessitates frequent administration by oral route [11].

Consequently, multiple daily dosages of 2 to 4 mg and 3 to 4 times a day is necessary to maintain adequate plasma concentrations. Currently, it is available as a tablet, syrup, nebulizer solution, and intramuscular or intravenous injectable form. It is also available in the form of aerosols with a strength of 100-200 µg and 2 to 3 inhalations every 4 to 6 h is recommended for adults and children [12, 13]. However, frequent administration is not suggested by Fishwick and his research group based on the evidence of their studies [14]. It is usually given by inhalation or slow intravenous injections in the management of severe asthmatic attacks. Asthma is a chronic disease; there is a need for a drug delivery system that maintains adequate therapeutic concentrations for a longer duration of action to improve better clinical efficacy. Hence, the present work was aimed to formulate and evaluate gels of SS for exploring its applications as transdermal drug delivery *via* ethosomes to improve therapeutic efficiency and patient compliance in addition to reduce the dosage regimen. The purpose was to achieve a constant drug delivery profile, which is particularly important for those suffering from nocturnal asthma attacks and requires a longer duration of therapeutic action from a single application and thus could enhance the overall quality of life of asthmatic patients.

MATERIALS AND METHODS:

Materials: The pure drug salbutamol sulphate (SS), phospholipid, phosphatidylcholine (PC), and carbopol 934 were procured from Yarrow chemical products, Mumbai. Cholesterol and triethanolamine were purchased from Rolex Chemical Industries, Mumbai, and Qualigens fine chemicals, respectively. All other ingredients used throughout the study were of analytical grades.

Methodology:

Compatibility Study by Fourier Transforms Infrared Spectroscopy (FTIR): FTIR spectra were obtained using a Shimadzu FTIR-1700 spectrophotometer to detect compatibility between drug and polymers used in the present study. The spectra were recorded for pure drug SS, phosphatidylcholine (PC), carbopol 934, cholesterol, and then physical mixture (PM) of drug with carbopol 934 and PC.

The samples were prepared by the potassium bromide disc method, and the scanning range was kept from to 400 cm^{-1} to 4000 cm^{-1}. Further, selected transdermal ethosomal gel formulations were also studied by FTIR.

Formulation of Transdermal Ethosomal Gels: The first step consists of the preparation of ethosomal suspension containing SS by employing hot method. Accurately weighed quantities of PC were taken in a clean and dry beaker. Then required amount of distilled water was added & heated to 40 ºC until it dissolves completely [15, 16]. In another beaker, required quantity of drug, cholesterol, and propylene glycol was dissolved properly by heating to the same temperature of 40 ºC. Then, the organic phase was added to aqueous phase with continuous stirring at 40 ºC. The obtained homogenous ethosomal suspension was sonicated for about 3 to 5 min. Cholesterol 1% w/w was added to provide stability to a vesicle membrane, and propylene glycol was used as a skin penetration enhancer.

TABLE 1: FORMULATIONS OF ETHOSOMAL SUSPENSIONS OF SS

Composition of ethosomal suspensions of SS							
Formulation Code	**Drug:PC Ratio**	**Drug**	**PC**	**Alcohol (%)**	**Cholesterol (% w/w)**	**Propylene glycol (mL)**	**Distilled Water (mL)**
F1	1:9	10	90	30	1	0.12	qs
F2	1:9	10	90	50	1	0.12	qs
F3	1:9	10	90	70	1	0.12	qs
F4	1:19	10	190	30	1	0.12	qs
F5	1:19	10	190	50	1	0.12	qs
F6	1:19	10	190	70	1	0.12	qs

Table 1 shows the formulation of different batches of ethosomal suspensions with varying concentrations of drug: PC and alcohol. Then, second step was the formulation of transdermal ethosomal gels from prepared ethosomal suspensions. Previously prepared ethosomal suspensions containing SS was used to develop gels by incorporating into carbopol 934 at different concentrations of 0.75% w/v and 1% w/v. The suspension mixture was then converted into ethosomal gel formulations upon addition of triethanolamine as a neutralizer. Because some gelling agents like carbomers require a neutralizer or a pH adjusting chemical (triethanolamine) to create clear, uniform gel after the gelling agent has been wetted in the dispersing medium. The developed ethosomal gel formulations were stored properly in suitable containers for further studies. **Table 2** shows the composition of different batches of transdermal ethosomal gel formulations.

TABLE 2: FORMULATIONS OF TRANSDERMAL ETHOSOMAL GELS OF SS

	Composition of Transdermal Ethosomal Gels of SS				
Formulation Code	**Ethosomal Suspensions**	**Drug: PC**	**Alcohol%**	**Carbopol(% w/v)**	**Triethanolamine**
F1A	F1	1:9	30	0.75	qs
F1B	F1	1:9	30	1	qs
F2A	F2	1:9	50	0.75	qs
F2B	F2	1:9	50	1	qs
F3A	F3	1:9	70	0.75	qs
F3B	F3	1:9	70	1	qs
F4A	F4	1:19	30	0.75	qs
F4B	F4	1:19	30	1	qs
F5A	F5	1:19	50	0.75	qs
F5B	F5	1:19	50	1	qs
F6A	F6	1:19	70	0.75	qs
F6B	F6	1:19	70	1	qs

Characterization of Ethosomes:

Vesicle Size and Size Distribution by Optical Microscopy: To understand the effect of lipid and ethanol concentrations on vesicular size, prepared ethosomes were studied for vesicular size and size distribution by using optical microscope and photomicrographs were taken with the help of digital camera [17, 18]. A standard stage micrometer was used for calibration of eyepiece micrometer. After calibration, the eyepiece micrometer was used for particle size determination. Samples of all batches (ethosomal suspensions of F1 to F6) were suitably diluted with distilled water. Then a drop of suspension was mounted on a slide, and about 250 ethosomal vesicles were measured individually

with the help of eyepiece micrometer. The average of three trials (n=3) was taken, and their size distribution range, the average diameter was calculated.

Surface Morphology by Scanning Electron Microscopy (SEM): SEM analysis was used to characterize the surface morphology of the selected ethosomal vesicles (F1 and F2). 1-2 drops of vesicular suspension were mounted on a glass stub, then air-dried stubs were paste over grid by using double-sided carbon adhesive tape and sputter coated with conductive gold-palladium. A circular cover slip was gently placed over the stub to enable even distribution of the sample suspension. They were viewed with SEM (JEOL-JSM-6510, Japan) operating at an accelerating voltage of 200 kV, under a high vacuum. The vesicles were examined for surface characteristics like shape, vesicular size, and presence of aggregation.

Drug Content Estimation of Ethosomal Suspensions and Gels: Each batch of ethosomal suspensions equivalent to 3 mg of drug was taken into a standard 25 mL volumetric flask and volume was made up to 25 mL with distilled water and it is shaken for about 15 min. Then 1 mL of this solution was diluted suitably with distilled water and absorbance values were measured at 275.5 nm. Similarly, drug content estimation of all batches of ethosomal gels was carried, and an average of three readings was taken, and drug content was calculated from the calibration curve of the pure drug.

Drug Entrapment Efficiency: It was done by an exhaustive dialysis method using synthetic cellulose membranes. A measured quantity of ethosomal suspension was taken into a dialysis tube, which was securely attached with synthetic cellulose membrane on one side. Then, the dialysis tube was suspended in 200 mL of distilled water and the receiver mediums were stirred with a magnetic stirrer up to 6 h. The ethosomal suspension and unentrapped drug were separated from the medium through the synthetic cellulose membrane. After 6 h of exhaustive dialysis, optical density values were noted, and the estimation of the entrapped drug was estimated by UV spectrophotometric method. An average of three readings were taken and entrapment efficiency was calculated using the equation mentioned below,

Entrapment efficiency = Amount of drug entrapped / Total amount of drug

Z-Average, Polydispersity Index (PDI), and Zeta Potential Measurement: Vesicle size and PDI were determined by dynamic light scattering (DLS) using computerized Malvern Zetasizer Instrument at Manipal University, Manipal. Zeta potential was measured using the same instrument based on electrophoretic mobility. It is an important indicator of particle surface charge, which is used to predict the stability of ethosomal suspensions.

***In-vitro* Diffusion Studies of Transdermal Ethosomal Gels:** The release of drugs from transdermal ethosomal gel formulations was determined using membrane diffusion technique up to 12 h. The ethosomal gel containing equivalent to 3 mg of SS was taken in a glass tube having a diameter 2.5 cm with a length of 8cm, which was previously covered with soaked synthetic cellulose membrane, and this acts as a donor compartment. The glass tube was placed in a beaker containing 200 mL of distilled water, which acts as a receptor compartment.

The whole assembly was fixed in such a way that the lower end of the tube containing suspension was just touched the surface of the diffusion medium. The temperature of the receptor medium was maintained at 37 ± 10 0C and the medium was agitated at 100 rpm speed using a magnetic stirrer. Aliquots of 5 mL samples were withdrawn at fixed time intervals maintaining sink conditions. Then, samples were analyzed at 275.5 nm by means of a double beam UV spectrophotometer (Hitachi-U2000 spectrophotometer). Then in vitro drug release and kinetics of drug release from all transdermal ethosomal gel formulations were calculated using dissolution software PCP-DISSO V.3 and Graph pad PRISM.

Analysis of Release Kinetics and Mechanism of Drug Release: To understand *in-vitro* release data, various kinetic models such as zero-order model, first-order model, Higuchi model, Hixson-Crowell model, and Korsmeyer-Peppas model are used to describe the release kinetics and mechanism of transdermal ethosomal gel formulations. Among the various kinetic models studied for the data treatment, the model that best fits the release data is selected based on correlation coefficient (r) values.

Precisely, to know the mechanism of drug release, the *in-vitro* data also fitted according to the well known exponential equation, suggested by Korsmeyer and Peppas [19-21].

$$Mt / M_{\infty}=K_p t^n \text{ ……...(4)}$$

Where, M_t and M_{∞} is the amount of drug released at time‘t’ and at infinite time, respectively. Mt/M_{∞}= the fraction of drug released. Kp’= the release rate constant and ‘n’= the diffusional exponent for drug release. The value of ‘n’ calculated from the slope of the plot of log of fraction of drug released (Mt/M_{∞}) *vs.* log time (t) and used to characterize different drug release mechanisms.

RESULTS AND DISCUSSION:

FTIR Study: The IR spectrum of drug and comparative FTIR spectrum of drug and PMs are displayed in **Fig. 1** to **3**. No significant changes in functional groups of drug were observed in physical mixtures of drug and polymers. This ensured the compatibility between polymers and drug. IR spectra of ethosomal gel formulations F1A and F2B are shown in **Fig. 4** and **5** respectively. All characteristic peaks of drugs were observed in ethosomal gel formulations indicated that the drug exists in original form in ethosomal gel formulations and available for its therapeutic action.

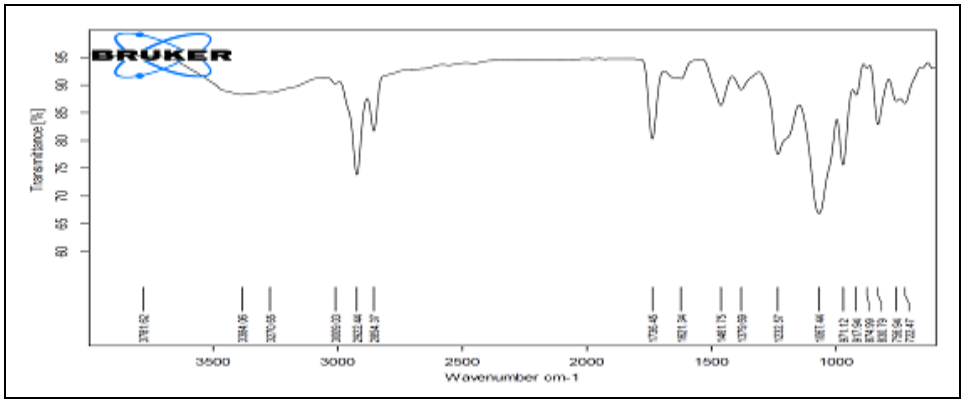

FIG. 1: FTIR SPECTRUM OF SALBUTAMOL SULPHATE

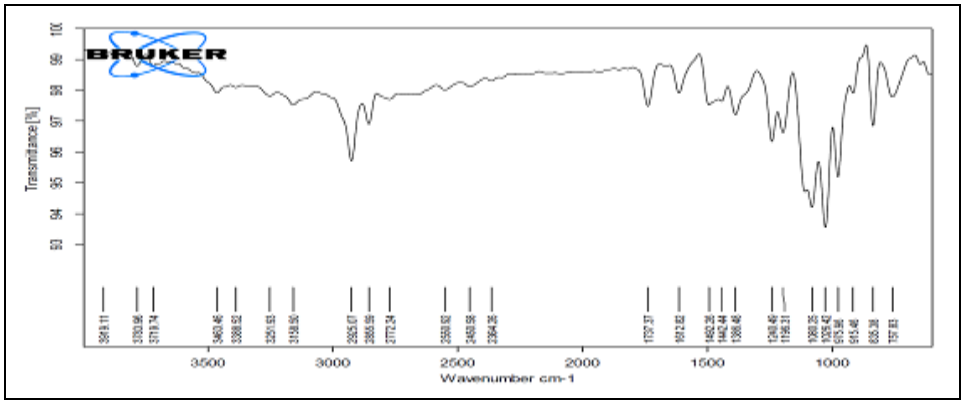

FIG. 2: FTIR SPECTRUM OF PHYSICAL MIXTURE OF DRUG AND PC

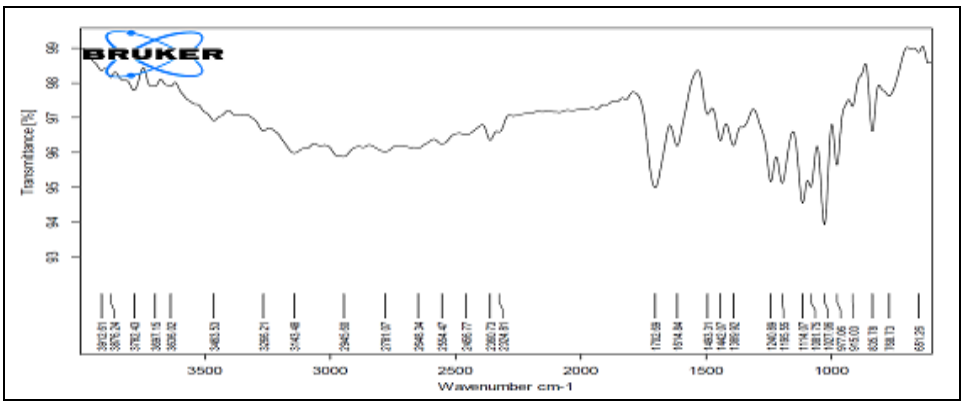

FIG. 3: FTIR SPECTRUM OF PHYSICAL MIXTURE OF DRUG AND CARBOPOL

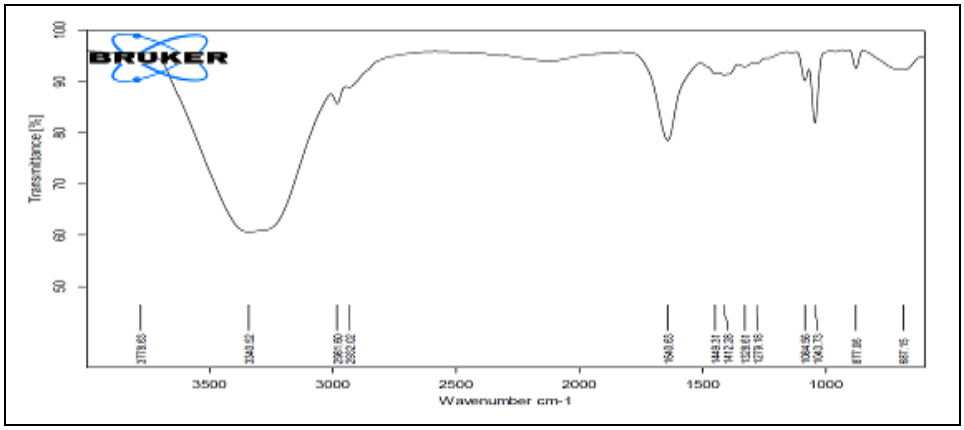

FIG. 4: FTIR SPECTRUM TRANSDERMAL ETHOSOMAL GEL (FIA)

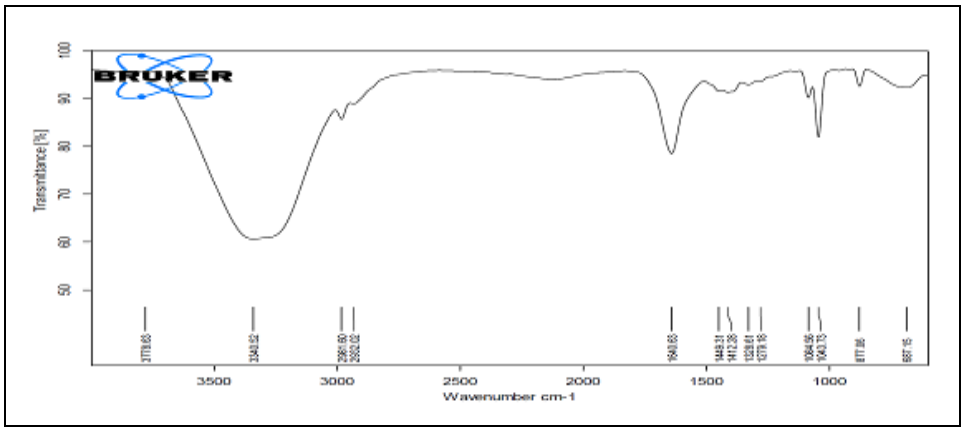

FIG. 5: FTIR SPECTRUM OF TRANSDERMAL ETHOSOMAL GEL (F4B)

Vesicular Size and Size Distribution by Optical Microscopy: The microphotographs of ethosomal formulations revealed spherical in their shape and well dispersed as shown in **Fig. 6.**

Average size of ethosomal vesicles in suspensions was measured by optical microscopic method and particle size distribution was shown in **Fig. 7.** About 250 ethosomes were measured individually for the diameter, then the average was calculated. The average vesicular size of ethosomes of F1 to F3 batches and F4 to F6 batches were in the range of 0.49 µm to 0.53 µm and 0.53 µm to 0.57 µm.

There was a slight increase in the size of vesicles when the concentration of PC was increased in ethosomal formulations.

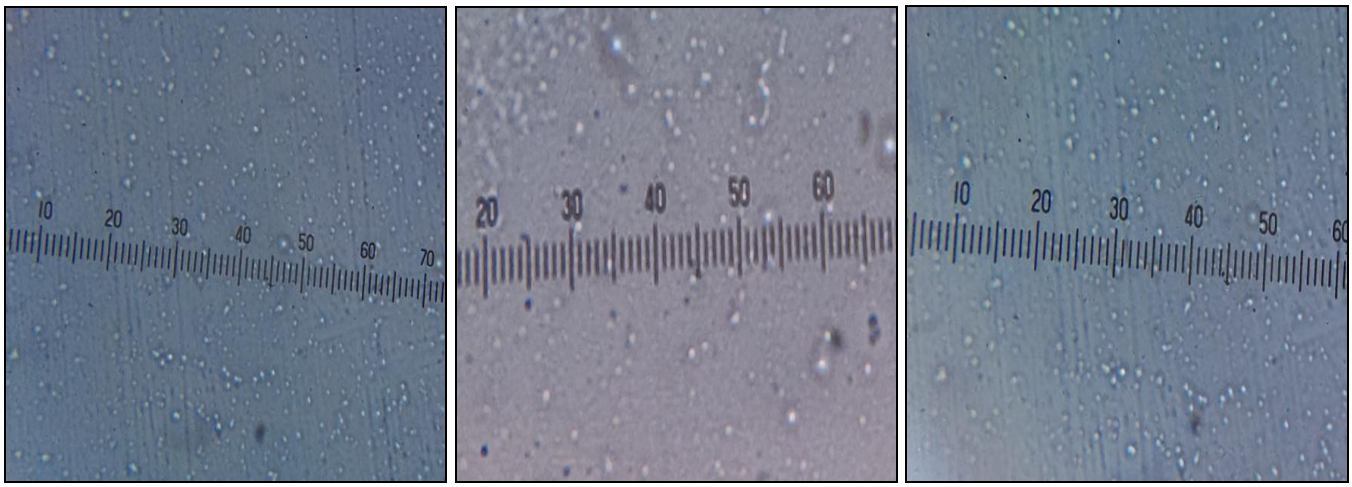

FIG. 6: MICROPHOTOGRAPHS OF SELECTED FORMULATIONS OF ETHOSOMAL SUSPENSIONS CONTAINING SS (F1, F2 AND F3)

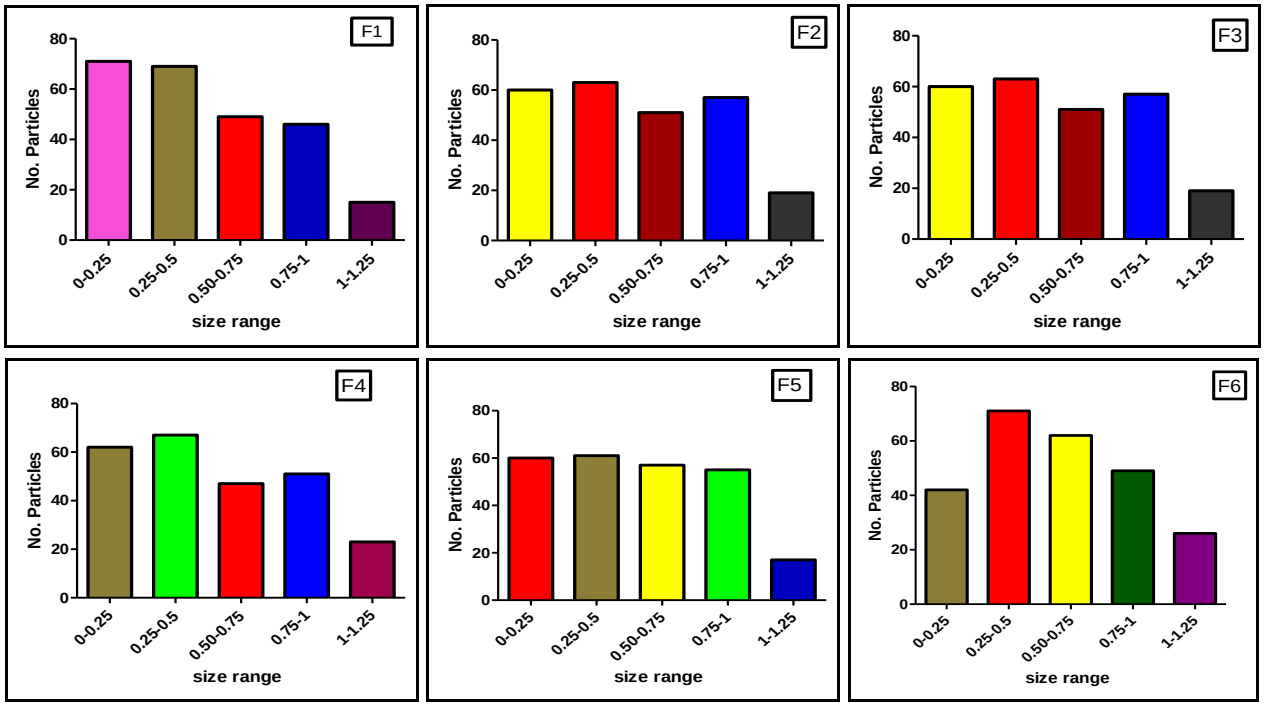

FIG. 7: PARTICLE SIZE DISTRIBUTION AND AVERAGE PARTICLE SIZE OF VARIOUS ETHOSOMAL SUSPENSIONS (F1 TO F6

Visualization by Scanning Electron Microscopy (SEM): SEM analysis was also conducted to characterize the surface morphology of selected ethosomal vesicles in suspension formulations (F1 and F4), and images are shown in **Fig. 8**. SEM images confirmed that ethosomal vesicles in spherical shape found no aggregation and well-dispersed suspensions. The images also revealed that ethosomal vesicles have particle size distribution from around 50 nm to 100 nm for F1 formulation and 125 nm to 250 nm for F4 formulation.

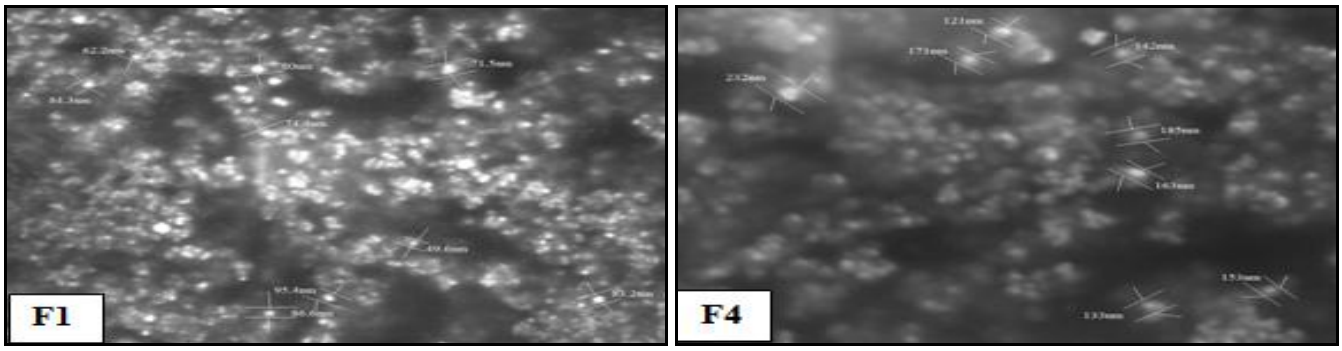

FIG. 8: SEM IMAGES OF ETHOSOMAL SUSPENSIONS (F1 AND F4)

Drug Content Estimation: Drug content was determined for all batches of ethosomal suspensions as well as transdermal ethosomal gel formulations and displayed in **Tables 3** and **4,** respectively. In the case of ethosomal suspensions, drug content was found in the range of 92.0 ± 0.005 to 97.3 ± 0.002 for F1 to F6.

TABLE 3: DRUG CONTENT OF ETHOSOMAL SUSPENSIONS CONTAINING SS

Formulation Codes	Amount of Drug Incorporated (mg)	Amount of Drug Recovered (mg)	% Drug Content ± SD
F1	3	2.82	94.0 ± 0.0057
F2	3	2.89	96.3 ± 0.0042
F3	3	2.78	92.6 ± 0.0021
F4	3	2.92	92.2 ± 0.0042
F5	3	2.88	97.3 ± 0.0021
F6	3	2.76	92.0 ± 0.0051

Whereas, drug content was found in the range of 92.0 ± 0.005 to 95.0 ± 0.005 and 90.0 ± 0.004 to 96.3 ± 0.002 for ethosomal gel formulations prepared with SS: PC at 1:9 and 1:19 concentrations respectively. The low SD values in all batches indicated uniform drug distribution in transdermal gel formulations. Uniform drug distribution is necessary for the gel systems to have the optimum activity of the drug.

TABLE 4: DRUG CONTENT OF TRANSDERMAL ETHOSOMAL GELS

Formulation Codes	Amount of Drug Incorporated (mg)	% of Carbopol	Amount of Drug Recovered (mg)	% Drug Content ± SD
F1A	3	0.75	2.76	92.0 ± 0.0057
F1B	3	1	2.79	93.0 ± 0.0023
F2A	3	0.75	2.81	93.6 ± 0.0034
F2B	3	1	2.85	95.0 ± 0.0051
F3A	3	0.75	2.69	89.6 ± 0.0052
F3B	3	1	2.71	90.3 ± 0.0015
F4A	3	0.75	2.89	96.3 ± 0.0023
F4B	3	1	2.83	94.3 ± 0.0032
F5A	3	0.75	2.78	92.6 ± 0.0051
F5B	3	1	2.73	91.0 ± 0.0057
F6A	3	0.75	2.70	90.0 ± 0.0042
F6B	3	1	2.72	90.6 ± 0.0050

Drug entrapment efficiency: The varied amount of ethanol and PC at different concentrations was found to have an influence on drug entrapment efficiency of ethosomal suspensions. Entrapment of drug in ethosomes was increased with an increase in the amount of PC (1:19 > 1:9).

Further, gradual decrease in drug entrapment efficiency was observed when the concentration of alcohol was increased from 30% to 50% and 70% with SS: PC ratios as shown in **Table 5**. The increase in drug entrapment efficiency in ethosomal suspensions prepared with SS: PC at 1: 9 and 1:19 ratios was found in the following order,

F4 > F1; F5 > F2; F6 > F3

Generally, hydrophilic drugs are entrapped in aqueous core inside the lipid layer, while the lipophilic drugs are retained in lipid lamella. Encapsulation of a hydrophilic drug depends on the captured aqueous volume, and that of the lipophilic drug depends on the number of bilayer of lipid [22].

As the salbutamol sulphate is hydrophilic in nature, more drugs could be accommodated in an aqueous core of the ethosomal vesicles. However, a decrease in drug entrapment efficiency with an increase in alcohol concentration above 30% might be resulted in drug leakage due to the greater fluidity effect of ethanol.

TABLE 5: DRUG ENTRAPMENT EFFICIENCY OF ETHOSOMAL SUSPENSIONS

Formulation Code	Entrapment Efficiency (%) ± SD
F1	57.4 ± 0.37
F2	52.7 ± 0.41
F3	43.8 ± 0.42
F4	64.3 ± 0.37
F5	59.3 ± 0.39
F6	42.4 ± 0.42

Z-Average, Polydispersity Index (PDI) and Zeta Potential Measurement: Dynamic light scattering (DLS) is a unique technique which provides the ability to measure and report particle size distribution of the sample. The primary result from DLS is typically the mean value from size distribution by intensity (called the Z-Average) and the polydispersity index (PDI) to describe the size distribution width. The term "polydispersity" (or "dispersity" as recommended by IUPAC) is used to describe the degree of non-uniformity of size distribution of particles [23, 24].

The charges on drug-loaded ethosomal vesicles and zeta potential were also determined for selected batches of F1 and F4 by analyzing the formulations for 60 sec and 90 sec, respectively. And PDI was also determined as a measurement of particle size homogeneity for the prepared ethosomal formulations. The Z-Average is an intensity-based calculated value and also known as the cumulants

mean. Ethosomal suspension formulations of F1 and F4 exhibited Z-Average of 889.9 nm **Fig. 9** and 1587 nm **Fig. 10** respectively. Comparatively, F1 ethosomal suspensions have small particle size than F4, indicated that an increase in amounts of PC in the formulations increased the particle size of ethosomes. Whereas, PDI is a dimensionless parameter which indicates particle size distribution.

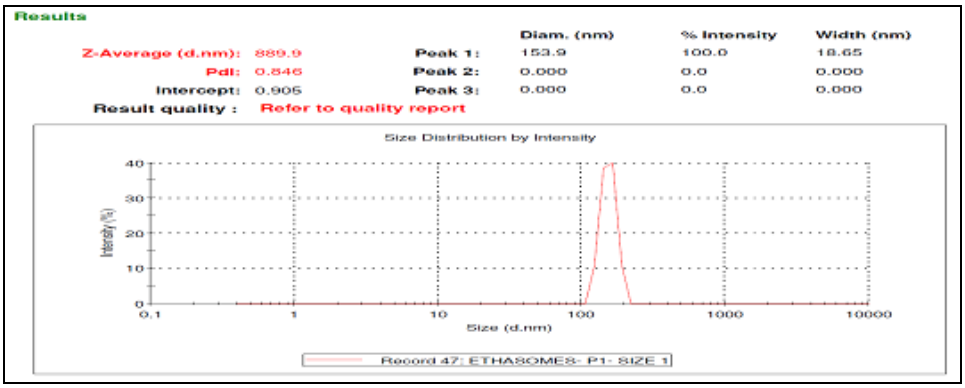

FIG. 9: SIZE DISTRIBUTION BY INTENSITY OF ETHOSOMAL SUSPENSION 'F1

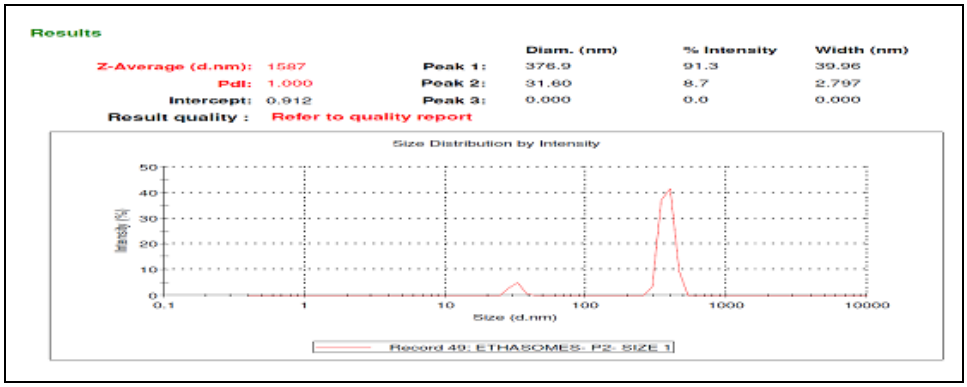

FIG. 10: SIZE DISTRIBUTION BY INTENSITY OF ETHOSOMAL SUSPENSION 'F4

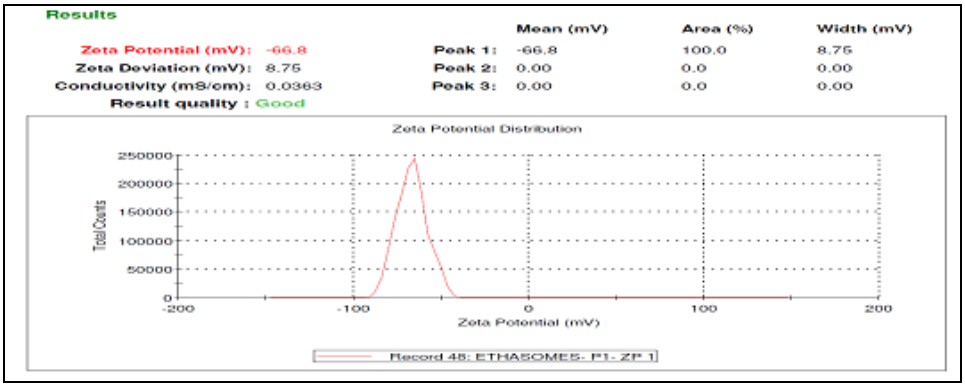

FIG. 11: ZETA POTENTIAL OF ETHOSOMAL SUSPENSION 'F1'

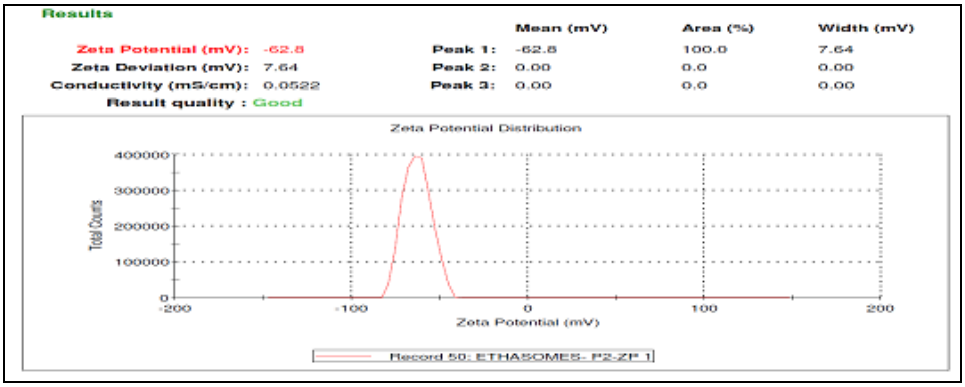

FIG. 12: ZETA POTENTIAL OF ETHOSOMAL SUSPENSION 'F4'

In general, PDI value within 0.03-0.06 is indicative of monodisperse system, while PDI within 0.10-0.20 suggests narrow size distribution and that PDI value within 0.25 to 0.50 and above 0.50 is considered to have broad size distribution [25]. In the present study, PDI value of F1 and F4 ethosomal suspensions was 0.846 and 1.0 which indicated broad size distribution of ethosomes. Zeta potential is a measure of the magnitude of the electrostatic, charge repulsion or attraction between particles in liquid medium. It gives the detailed insight into potential stability, aggregation, or flocculation of colloidal systems. If all the particles in suspension have a large negative or positive zeta potential, then they will tend to repel each other, and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values, then there will be no force to prevent the particles from coming together and flocculate. In general, particles with zeta potential values more than +30 mV or - 30 mV are normally considered stable. The zeta potential values of selected F1 and F4 ethosomal suspensions was found - 66.8 mV and - 62.8 mV, and shown in **Fig. 11,** and **Fig. 12** respectively**.** This indicated clearly that formulated ethosomes do not aggregate rapidly and further confirmed the dispersion stability of ethosomes in suspension formulations.

***In-vitro* Drug Diffusion Studies:** Release of the drug from the donor compartment to the receptor compartment is affected directly by the drug in the receptor solution in general as well as at the system-solution interface in particular. The "percent saturation" (also known as activity) in the receptor solution affects the drug release. The difference in concentration (concentration gradient) is the driving force for the diffusion process. The release mechanism are best predicted when the drug concentration in the solution are less than 10% saturation (sink condition) are maintained. Hence, sink conditions are maintained by replacing the dissolution medium with portions of fresh dissolution medium. In the present study, the drug salbutamol sulphate is soluble in four parts of water as per the USP [26]. Hence, distilled water was used as a dissolution medium by maintaining a constant temperature throughout in vitro diffusion studies.

TABLE 6: *IN-VITRO* DIFFUSION DATA OF TRANSDERMAL ETHOSOMAL GELS WITH SS AND PC (1:9 RATIO) USING CARBOPOL AT 1% AND 0.75% W/V

Time (h)	Cumulative Percent Drug Released (mg) ± SD					
	F1A	F1B	F2A	F2B	F3A	F3B
1	9.70 ± 0.85	10.19 ± 0.85	11.66 ± 0.85	13.13 ± 0.85	17.04 ± 0.85	15.57 ± 0.85
2	11.90 ± 0.83	13.38 ± 0.87	16.36 ± 0.87	20.80 ± 0.84	23.34 ± 0.87	20.86 ± 0.86
3	16.11 ± 0.85	16.65 ± 0.85	21.16 ± 0.95	24.24 ± 0.83	28.81 ± 0.84	27.24 ± 0.78
4	21.39 ± 0.83	22.92 ± 0.91	26.08 ± 0.91	28.74 ± 0.95	33.90 ± 0.89	33.28 ± 0.79
5	25.82 ± 0.85	27.87 ± 81	29.63 ± 0.93	32.85 ± 0.83	40.58 ± 0.82	38.48 ± 0.82
6	30.83 ± 0.84	31.47 ± 0.83	34.73 ± 0.80	39.49 ± 0.85	45.94 ± 0.84	43.78 ± 0.87
7	34.97 ± 0.84	36.60 ± 0.91	39.94 ± 0.85	44.81 ± 0.87	52.38 ± 0.74	49.69 ± 0.81
8	40.18 ± 0.85	43.31 ± 0.93	45.26 ± 0.91	51.22 ± 0.87	59.94 ± 0.84	55.23 ± 0.96
9	45.50 ± 0.83	48.71 ± 0.83	52.16 ± 0.83	55.80 ± 0.90	65.70 ± 0.76	60.88 ± 0.85
10	53.38 ± 0.85	55.68 ± 0.85	59.20 ± 0.75	61.45 ± 0.80	71.08 ± 0.79	66.64 ± 0.82
11	62.40 ± 0.83	60.84 ± 0.73	66.39 ± 0.93	67.21 ± 0.82	75.58 ± 0.84	71.04 ± 0.81
12	69.67 ± 0.84	67.07 ± 0.75	75.19 ± 0.95	72.59 ± 0.86	81.62 ± 0.79	78.45 ± 0.83

TABLE 7: *IN-VITRO* DIFFUSION DATA OF TRANSDERMAL ETHOSOMAL GELS WITH SS AND PC (1:19 RATIO) USING CARBOPOL AT 1% AND 0.75% W/V

Time (h)	Cumulative Percent Drug Released (mg) ± SD					
	F4A	F4B	F5A	F5B	F6A	F6B
1	9.70 ± 0.85	8.73 ± 0.85	14.11 ± 0.85	9.70 ± 0.85	14.11 ± 0.85	12.64 ± 0.85
2	13.37 ± 0.86	13.35 ± 0.84	19.35 ± 0.83	12.88 ± 0.84	18.86 ± 0.84	15.89 ± 0.87
3	18.10 ± 0.85	16.61 ± 0.86	24.23 ± 0.85	17.60 ± 0.86	23.73 ± 0.83	19.71 ± 0.87
4	22.94 ± 0.87	19.95 ± 0.85	28.73 ± 0.86	22.43 ± 0.85	28.71 ± 0.85	25.56 ± 0.81
5	27.90 ± 0.86	24.34 ± 0.89	33.82 ± 0.82	26.39 ± 0.89	34.28 ± 0.86	30.57 ± 0.83
6	33.94 ± 0.82	29.31 ± 0.91	38.03 ± 0.86	30.93 ± 0.78	41.94 ± 0.85	35.70 ± 0.85
7	38.15 ± 0.79	34.89 ± 0.76	44.29 ± 0.85	36.05 ± 0.87	50.74 ± 0.83	42.40 ± 0.81
8	44.42 ± 0.85	41.08 ± 0.82	51.67 ± 0.83	42.26 ± 0.86	56.32 ± 0.88	49.25 ± 0.86
9	50.32 ± 0.83	46.42 ± 0.84	58.22 ± 0.82	48.61 ± 0.84	62.98 ± 0.79	53.79 ± 0.85
10	55.86 ± 0.82	52.36 ± 0.90	62.95 ± 0.83	55.58 ± 0.86	67.33 ± 0.82	61.85 ± 0.83
11	61.51 ± 0.84	58.92 ± 0.87	68.75 ± 0.85	61.23 ± 0.88	73.23 ± 0.84	67.64 ± 0.82
12	67.27 ± 0.86	65.12 ± 0.93	73.19 ± 0.87	70.90 ± 0.80	79.23 ± 0.83	75.00 ± 0.84

The *in-vitro* diffusion studies of all batches of transdermal ethosomal gel formulations containing SS are performed through synthetic cellulose membrane up to 12 h in distilled water using a modified diffusion cell. Variable *in-vitro* release profiles of drug from different ethosomal gel formulations were observed from the studies and showed prolonged release of the drug over a period of 12 h. The *in-vitro* drug release data obtained from ethosomal gel formulations prepared with different concentrations of alcohol (30%, 50% and 70%) at 1:9 and 1:19 ratios of SS: PC using carbopol 934 (at 0.75% w/v and 1% w/v) are shown in **Table 6** and **7** and their respective graphical representations are shown in **Fig. 12** and **13.** The *in-vitro* data revealed that the drug release rate was progressively increased with an increase in concentrations of alcohol and found in the following order, F3A > F2A >F1A (70% > 50% > 30% and carbopol at 0.75%, SS: PC at 1:9 ratios). F6A > F5A > F4A (70% > 50% > 30% and carbopol at 0.75% SS: PC at 1:19 ratios). F3B > F2B > F1B (70% > 50% > 30% and carbopol at 1%, SS: PC at 1:9 ratios). F6B > F5B > F4B (70% > 50% > 30% and carbopol at 1%, SS: PC at 1:19 ratios). Additionally, it was observed that the drug release rate was decreased when the carbopol concentration from 0.75% to 1% was increased in all batches of ethosomal gel formulations. This observation can be explained on the basis of the concentration of the carbopol 934 polymer in the formulations. As the concentration of carbopol increases, which increases its viscosity and gel property in three-dimensional networks would restrict drug diffusion from the ethosomal gel formulations and hence more retardation of drug release. Furthermore, from the *in-vitro* diffusion studies, it was clear that the release of SS was decreased with increasing phospholipids concentration, whereas an increase in the amount of alcohol increased the drug release from ethosomal gel formulations. It might be due to increased fluidity of the bilayer membranes with an increasing concentration of ethanol.

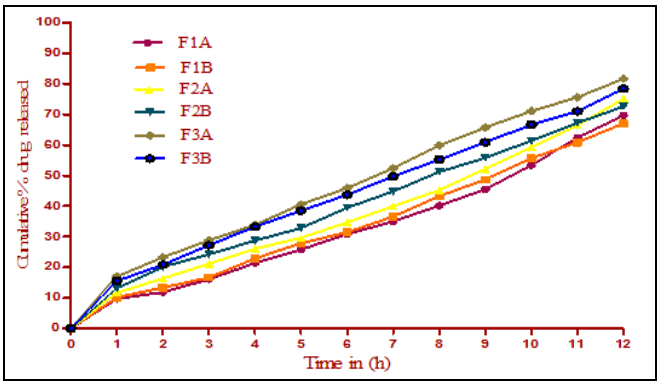

FIG. 13: COMPARATIVE *IN-VITRO* DIFFUSION PROFILE OF TRANSDERMAL ETHOSOMAL GELS PREPARED WITH SS AND PC (1:9 RATIO)

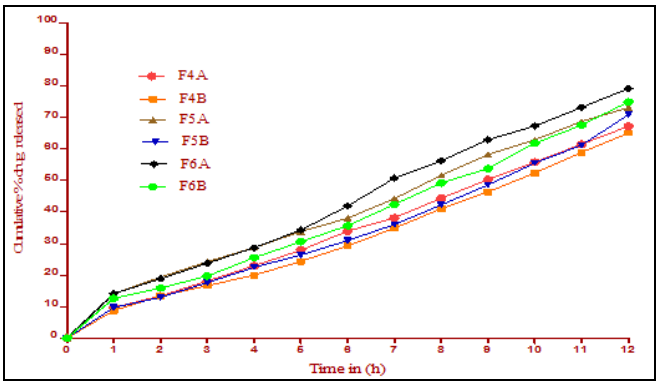

FIG. 14: COMPARATIVE *IN-VITRO* DIFFUSION PROFILE OF TRANSDERMAL ETHOSOMAL GELS PREPARED WITH SS AND PC (1:19 RATIO)

TABLE 8: MODEL FITTING VALUES OF TRANSDERMAL ETHOSOMAL GELS

Batches	Zero order Equation		1st Order Equation		Higuchi's Equation		Hixon Crowen Equation	
	K_o (%mg/h)	r	$K_1 \times 10^2$ (min^{-1})	r	K_h (%mg)	r	$K_{HC} \times 10^2$ ($mg^{1/3}.min^{-1}$)	r
F1A	5.408	0.9918	-0.0783	0.9518	15.2613	0.9110	-0.0229	0.9702
F1B	5.5062	0.9918	-0.0791	0.9518	15.6279	0.9110	-0.0232	0.9702
F2A	6.0073	0.9918	-0.0915	0.9518	17.0909	0.9110	-0.0262	0.9702
F2B	6.2991	0.9918	-0.0960	0.9518	18.1003	0.9110	-0.0275	0.9702
F3A	7.2669	0.9918	-0.1232	0.9518	20.9549	0.9110	-0.0339	0.9702
F3B	6.8527	0.9918	-0.1105	0.9518	19.7513	0.9110	-0.0310	0.9702
F4A	5.6057	0.9971	-0.0810	0.9802	15.9321	0.9363	-0.0237	0.9905
F4B	5.2410	0.9971	-0.0738	0.9802	14.8322	0.9363	-0.0218	0.9905
F5A	6.0839	0.9971	-0.0985	0.9802	18.3134	0.9363	-0.0281	0.9905
F5B	5.5445	0.9971	-0.0839	0.9802	15.6839	0.9363	-0.0236	0.9905
F6A	6.8667	0.9971	-0.1126	0.9802	19.6350	0.9363	-0.0314	0.9905
F6B	6.1702	0.9971	-0.0950	0.9802	17.5498	0.9363	-0.0271	0.9905

Mechanism of Drug Release: In order to find out the mechanism of drug release, the *in-vitro* release data of all transdermal ethosomal gel formulations were treated with various kinetic models such as Higuchi, first order and zero-order equations and given in **Table 8**. The release of the drug from all formulations followed predominantly zero-order kinetics. The value of diffusion release exponent for ethosomal gel-based formulations was found to be nearly '1', which indicated that the absorption of drugs through the skin follows zero-order release. Systems that obey zero-order release are ideal for transdermal drug delivery as they provide a constant release of the drug over an extended period of time and reflects improved therapeutic index [27]. To know precisely whether Fickian or non-Fickian diffusion, the data obtained were also put in Korsmeyer-Peppas model in order to find out the value of 'n', which is indicative of mechanism of drug release. In the present study, value of 'n' determined from ethosomal gel formulations prepared with different concentrations of alcohol at 1:19 ratios of SS:PC using carbopol 934 (0.75% w/w and 1% w/w) are ranged from 0.6533 to 0.8309 ($0.5 < n < 1$) with correlation coefficient values of 0.9783 to 0.9931.

In the case of ethosomal gel formulations with different concentrations of alcohol at 1:19 ratios of SS: PC using carbopol 934 (0.75% w/w and 1% w/w), the 'n' value was ranged from 0.6903 to 0.8270 with correlation coefficient values of 0.9798 to 0.9900. This indicated that the drug release mechanism from all ethosomal gel formulations followed non-Fickian diffusion controlled (anomalous diffusion) [28].

CONCLUSION: In the present study, an attempt was made to formulate and evaluate transdermal delivery of salbutamol sulphate *via* ethosomal gels to improve therapeutic efficiency and patient compliance in addition to reduce dosage regimen. The model drug was a logical candidate for transdermal delivery due to its short biological haf-life, first-pass metabolism and multiple oral dosing. Hence, transdermal ethosomal gel formulations containing drug were successfully prepared by hot method using alcohol, phosphatidylcholine (PC), and carbopol 934 as a gelling agents at different concentrations. *In-vitro* drug diffusion studies confirmed variable release profiles of drugs from different ethosomal gels and showed prolonged release of drug over a period of 12 h.

From the overall studies, it could be concluded that ethosomal gels of SS prepared with carbopol 934 holds the potential for transdermal delivery into systemic circulation, which provides slow and extended-release up to 12 h with a zero-order drug release profile. Developed transdermal ethosomal gels also provide an added advantage of circumventing the hepatic first-pass metabolism of the drug, consequently achieving higher systemic bioavailability, thus improving its therapeutic effectiveness. Thus, improving patient convenience and compliance as a frequent intake of salbutamol sulphate is no longer necessary, particularly in the treatment of chronic conditions like asthma.

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