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DESIGN AND DEVELOPMENT OF BUDESONIDE PRONIOSOMES FOR TARGETED DRUG DELIVERY

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ABSTRACT: The aim of the present study was to formulate and evaluate Budesonide proniosomes for enhancement of solubility and bioavailability. To develop and evaluate budesonide proniosomes was the main objective of this research work. For the preparation of proniosomes slurry, a method was used using different ratios of (cholesterol: surfactant) (1:1.5) with the help of carriers. For optimization of formulation Box-Behnken Design was used in that concentration of span 60, cholesterol, and maltodextrin. The prepared proniosomes were evaluated for particle size, entrapment efficiency, and *in-vitro* drug release (up to 6 h). Particle size, entrapment efficiency, and drug release of optimized batch (F14) were found to be 220.3 nm, 81.42%, and 30.71%, respectively. The data were fitted into a zero order model, and the correlation value found to be 0.952, indicating controlled release. Transformation of niosomes from proniosomes and morphology study of the optimized batch was studied by Inverted Microscopy and Transmission Electron Microscopy, *etc.* Statistical analysis of *ex-vivo* permeation enhancement assessed from the flux, permeability coefficient, and enhancement ratio was significantly higher for proniosomes as compared to control. During the stability study of 30 days, it was found that no signs of instability like agglomeration, crystallization, and variation in vesicle size and entrapment efficiency. The preparation method of Budesonide proniosomes was more efficient and more effective. The optimized Budesonide proniosomes did improve the solubility and bioavailability of budesonide and offer a new approach to enhance the targeted drug delivery system of poorly water-soluble drugs.

INTRODUCTION: Budesonide is a locally acting glucocorticosteroid with an extensive, primarily hepatic, metabolism after oral administration. It is rapidly absorbed and biotransformed by cytochrome P450 (CYP) 3A to metabolites with negligible glucocorticoid activity [1.]

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Budesonide is an anti-inflammatory drug. It works by decreasing the body's natural defense response (immune response). The delivery of a drug can have a significant effect on its efficacy [2].

Some drugs have an optimum concentration range from which maximum benefit is derived, and concentrations above or below the range can produce a toxic effect or no therapeutic effect. Various drug delivery and drug targeting systems are currently under development [3]. The main goal for developing such delivery systems is to minimize drug degradation and loss, to prevent their harmful side effects and to increase their

bioavailability [4.] A number of novel drug delivery systems have emerged encompassing various routes of administration to achieved controlled and targeted drug delivery [5]. Niosomes are non-ionic surfactant vesicles with or without incorporation of cholesterol and other lipids. It can entrap a solute in a manner analogous to liposome. Vesicules having the ability to carry both hydrophilic and lipophilic drugs [6]. They are osmotically active while enhancing the stability of the entrapped drug.

The size of niosomes is microscopic and lies on nanometric scale. The particle size ranging from 10 nm to 100 nm. Niosomes have some problems like physical instability, aggregation, fusion, leaking of an entrapped drug, and some time hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion [7]. Provesicular concept has been developed to solve stability problems, generally shown in conventional vesicular systems, *i.e.*, liposomes and niosomes. In the composition of provesicular systems, it mainly contains a carrier, which is a water-soluble porous powder, and upon which phospholipids or nonionic surfactants may load and API (Active Pharmaceutical Ingredient). Organic solvents are also be used.

This new concept demonstrated the feasibility of proliposomes or proniosomes in improving the solubility, oral bioavailability and permeation of drugs across the cell membrane [8]. Based on the earlier research work it is clear that this system appear to be an alternate drug system for various routes of drug administration [9]. To overcome the problems of niosomes, proniosomes are prepared and reconstituted into niosomes. Proniosomes are dry formulated using a suitable carrier, which is coated with nonionic surfactants, and it can be converted into niosomes by hydration with aqueous media [10]. Proniosomes powder provides flexibility, unit drug dosing by fill in capsule, which could be beneficial. When proniosomes convert into niosomes, the surfactant molecules have the ability to orient themselves in such a way that the hydrophilic ends of the non-ionic surfactant point outwards, while the hydrophobic ends face each other to form the bilayer [11, 12].

MATERIALS AND METHODS:

Materials: Budesonide was purchased from the Cipla, Mumbai, India. Cholesterol and Mannitol purchased from SDFCL, Mumbai. Dialysis membrane (DM 70) was purchased from Hi-media, Mumbai, India. Span (20, 40, 60, and 80) was purchased from Hi-media. All other chemicals used were of analytical grade, and solvents were of HPLC grade. Freshly collected double distilled water was used all throughout the experiments.

Preparation of Proniosomes: The mixture of Budesonide, Cholesterol, and surfactant dissolve in Organic solvent in a Round bottom flask. Add Maltodextrin into the above Round bottom flask containing drug surfactant mixture, which forms a slurry. The flask and the rotary evaporator were filled with nitrogen then rotated under vacuum condition at 55 ºC for 15 min to remove the organic solvent. After that, the solid mixture was scraped from the flask and placed in a bowl to grind into powders. The powders were sieved with a 100 mesh screen, collected and transferred into a glass bottle, sealed, and stored at room temperature [25-27].

Optimization of Budesonide Proniosomes and Statistical Analysis: Response surface methodology (RSM) is a statistical method that uses quantitative data from appropriate experiments to fit regression model equations and operating conditions [28].

Box-Behnken design (BBD), which is a very common experimental design used in RSM was applied to evaluate the factors which affected the drug entrapment efficiency (EE) of the reconstituted niosomes. In this study, the BBD had 3 levels and three factors. The amount of Surfactant, cholesterol & Maltodextrin was the three major factors that influence the Entrapment Efficiency, particle size, and drug release. It is one quadratic response surface approach.

To demonstrate the effect parameters on pro-niosomes performance and characteristics, different batches were prepared using Box-Behnken design. The corresponding BBD was shown in **Table 1,** and the experiments were carried out in a randomized order. The concentration of Surfactant (X1), Concentration of Cholesterol (X2), and Concentration of carrier (X3) were selected as Independent variables. Dependent variables Particle sizes (Y1), % Entrapment efficiency (Y2), and drug release (Y3) were selected as critical

quality attributes. Drug concentration kept constant. A suitable polynomial model was selected based on the significant terms ($p < 0.05$), the least significant lack of fit, the multiple correlation coefficient (R^2). The data of the experiment were analyzed by the Design-Expert® 9.0.4.1 software. A total 15 models of proniosomes were randomly arranged. These included factorial points (high, medium, and low level from the constraints on overall center point). This design consisted 15 runs which are shown in **Table 2** with all actual and coded values. All batches were prepared and evaluated as per Box-Behnken design, three responses, particle size, entrapment efficiency, and drug release.

TABLE 1: LEVELS FOR BOX-BEHNKEN DESIGN

Independent variables	Levels		
	Low(-1)	Medium(0)	High(+1)
X_1 = Amount of Surfactant (mg)	32.3	64.5	96.88
X_2 = Amount of Cholesterol (mg)	9.66	38.66	67.67
X_3 = Amount of Maltodextrin (mg)	100	150	200

TABLE 2: CODED AND REAL LEVELS OF THE INDEPENDENT VARIABLES USED IN THE EXPERIMENTAL DESIGN OF ALL BATCHES

No. of Runs	Coded value			Real value		
	X1	X2	X3	X1	X2	X3
1	-1	-1	0	32.3	9.66	150
2	+1	-1	0	96.88	9.66	150
3	-1	+1	0	32.3	67.67	150
4	+1	+1	0	96.88	67.67	150
5	-1	0	-1	32.3	38.66	100
6	+1	0	-1	96.88	38.66	100
7	-1	0	+1	32.3	38.66	200
8	+1	0	+1	96.88	38.66	200
9	0	-1	-1	64.5	9.66	100
10	0	+1	-1	64.5	67.67	100
11	0	-1	+1	64.5	9.66	200
12	0	+1	+1	64.5	67.67	200
13	0	0	0	64.5	38.66	150
14	0	0	0	64.5	38.66	150
15	0	0	0	64.5	38.66	150

The checkpoint batch was also prepared and compared with the values obtained from the equations to confirm the validation of model. Graphical representation of factors influence of study was done with Contour plot and response surface plot. With the help of an overlay plot best-optimized formulation was found out by keeping the desired responses Y_1 (particle size), Y_2 (entrapment efficiency), and Y_3 (drug release).

The number of the experiment required for these studies is dependent on the number of the independent variable selected. The responses Y_1, Y_2, and Y_3 are measured for each trial.

$$Y= \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{12}3X_1X_2X_3 + \beta_{11}X_{12} + \beta_2 2X_{22} + \beta_{33}X_{32} + E$$

Where, Y = Measured response β_0= Intercept; regression coefficients β1 to β33; X_1, X_2, and X_3 are independent variable that was selected from the screening Experiments.

Formation of Vesicular Structures from Proniosome Powder: Small quantity of proniosomal powder was taken and placed on the glass slide; add few drops of distilled water were added dropwise with the help of a dropper, and a coverslip was placed over it. The slide was placed under the inverted microscope, observed at a magnification of 450X formation of vesicular structures were seen (Nikon), and micrographs of the formed niosomes were taken [29].

Surface Morphology: For surface morphological evaluation, the proniosome powder was hydrated with distilled water and agitated manually for 10-15 min, and Transmission Electron Microscopy observed and photographed was taken [30].

Determination of Particle Size Distribution of Reconstituted Budesonide Niosomes: The proniosome powder, were hydrated with 1 ml of distilled water (0.1 N HCl) under continuous

stirring for 45 min. To remove the carrier from the mixture, it was centrifuged for 3 min at 400 rpm .The supernatant containing niosomes and unentrapped drug was placed in a volumetric flask and diluted with media up to 10 ml. This niosomal suspension was used for particle size and zeta potential determinations [31].

Determination of Entrapment Efficiency and Drug Content of Budesonide Niosomes: The Entrapped Budesonide content was determined by hydrating 9 mg equivalents proniosomal powder in 20 ml of distilled water. Aliquots of sample was taken in micro centrifuge tubes and followed by refrigerated centrifugation at 25,000 rpm at 20 °C for 20 min. The supernatant was separated, suitably diluted with solvent and the sample was analyzed by compared it with blank niosomal suspension (without drug) using UV spectrophotometer at 247 nm. The absorbance was converted into drug concentration using standard curve [32]. The encapsulation efficiency was calculated as

Entrapment efficiency = Total amount of entrapped drug × 100 / Total amount of drug added

Drug content was estimated proniosome powder equivalents to 9 mg budesonide (drug) weighed and vesicles were lysed with 5 ml of methanol by bath sonication for 15 min to solubilizes the surfactant finally make up the volume up to 10 ml with 0.1 N HCl. Aliquots were withdrawn and drug content was calculated for Budesonide using UV-visible spectrophotometer at 247 nm. Blank (without drug) proniosomal dispersion was used as a reference standard.

***In-vitro* Dissolution Study:** *In-vitro* dissolution study of proniosome powder was performed carried out using dialysis bag method in simulated gastric fluid (0.1 N HCl), phosphate buffer pH 6.8 and phosphate buffer pH 7.4 as the dissolution medium. 9mg equivalent of budesonide niosomal suspension was taken in dialysis bag having 12,000-14,000 Da. cut off weight. Then, bag was placed in beaker containing 100 ml of dissolution medium. Beaker was placed over magnetic stirrer having 100 rpm stirring speed maintained at 37 ± 1 ºC. At predetermined time intervals a volume of 2 ml was withdrawn and replaced with fresh dissolution medium. At the end of the study, the samples were suitably diluted and the amount of drug was quantified by UV-visible spectro-photometer at 247 nm using dissolution medium as blank. The obtained data were fitted into mathematical equations (zero order, first order and Highuchi models) in order to describe the kinetics and mechanism of drug release from the proniosomal formulation [32-35].

Residual Solvent Determination by Gas Chromatography: Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients or in the preparation of drug products. The solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of drug substance may enhance the yield or determine characteristics such as crystal form, purity, and solubility.

Therefore, the solvent may sometimes be a critical parameter in the synthetic process. However, the content of solvents in such products should be evaluated and justified. Since there is no therapeutic benefit from residual solvents; all residual solvents should be removed to the extent possible to meet product specifications, good manufacturing practices, or other quality-based requirements.

Drug products should contain no higher levels of residual solvents than can be supported by safety data. Some solvents that are known to cause unacceptable toxicities (Class 1) should be avoided in the production of drug substances, excipients, or drug products unless their use can be strongly justified in a risk-benefit assessment.

Some solvents associated with less severe toxicity (Class 2) should be limited in order to protect patients from potential adverse effects. Ideally, less toxic solvents (Class 3) should be used where practical. The residual solvent to be detected here is Chloroform and ethanol, which was done by using GC. In this procedure, the liquid or solid sample was placed in a vial, closed with a septum, and thermostated until a thermodynamic equilibrium between the sample and the gas phase was reached. The time needed to reach this equilibrium depends strongly on the diffusion coefficient, which is at least three orders of magnitude lower than in

liquids. A known aliquot of gas phase is then transferred to a gas chromatograph and analysed [38].

***Ex-vivo* Permeation Study:** *Ex-vivo* permeation study was carried out using the non-everted gut sac technique. In this method, small intestine of freshly sacrificed chicken ileum was removed by cutting across the upper end of the duodenum and the lower end of the ileum. Then small intestine was carefully isolated, and approximately 10 cm length was cut and washed out with cold normal oxygenated saline solution (0.9%w/v NaCl) using a blunt end syringe. About 6 cm long clean intestinal tract was prepared into sacs. One end of the intestine was closed by tying with thread. From the other open end of the intestine, 1 ml drug in pH 7.4 phosphate buffer was filled with an oral syringe. Similarly, into another intestine of the same length, 1 ml suspension was added after reconstitution of optimized proniosomal formulation with pH 7.4 phosphate buffer equivalents to a single dose.

Each non everted intestinal sac was placed in a glass beaker containing pH 7.4 buffer. The entire system was supplied with aeration using a laboratory aerator. It was maintained at 37 °C ± 0.5 °C in a shaking water bath at 50 rpm. From media 2 ml samples were withdrawn for 2 h, at 15 min. intervals and replaced with fresh buffer solution. The amount of drug diffused through the membrane was calculated by analyzed using a UV-visible spectrophotometer. (UV 1800, Shimadzu, Japan).

Stability Study: Stability testing is to provide evidence of the effect of time and the influence of a variety of environmental factors such as humidity, light, the temperature on the quality of the formulation. It varies and enables storage conditions, re-test period, and shelf-lives.

The formulations were stored in glass vials covered with aluminum foil were kept at room temperature and kept in a refrigerator (2 °C – 8 °C) for 30 days.

A definite time intervals (10, 20, and 30 days), samples were withdrawn and hydrated with phosphate buffer pH 6.8 and observed for any sign of drug crystallization under an inverted microscope. Samples were also evaluated for particle size and Entrapment Efficiency before and after storage for 1 month.

RESULTS AND DISCUSSION: The Budesonide proniosomes were prepared successfully by using Slurry method. In the slurry method, surfactant and cholesterol dissolved in Ethanol & Chloroform mixture in round bottom flask (RBF). Maltodextrin powder was added into above solution, which forms slurry. Round Bottom flask attaches to rotary evaporator and applied vacuum for removing of organic solvent until the powder appears to be dry as shown in **Fig. 1**. Maltodextrin was chosen as the carrier because of its high Flowability.

FIG. 1: PREPARATION OF PRONIOSOMES BY ROTA EVAPORATOR

In this study, the temperature was fixed at 55 °C. The residual ethanol & chloroform was about 265 ppm & 12.95 ppm after the proniosomes powder was prepared. The analysis method was according to Indian Pharmacopoeia.

Optimization of the Budesonide Proniosomes and Statistical Analysis: The experiment data were fitted to a quadratic polynomial model using the software Design Expert, and the Equation for the EE, PS & Drug release was shown below in terms of coded factors:

EE = + 80.87 + 1.051A + 1.13B + 3.43C + 1.81AB + 0.82AC + 1.28BC - 4.89A2 - 9.802B2 8.49C2

DR= + 31.05 - 3.54A - 3.19B + 1.10C-5.18AB + 0.91AC - 0.68BC + 14.10A2 + 7.48B2 + 7.23C2

Box Behnken design with different 3 levels are used to study the effect of 3 independent variables over dependent variables. In this total 15 batches of proniosomes prepared and upon hydration, converted into niosomes and evaluated for the different parameters like Particle size, Entrapment efficiency, and drug release. BBD experimental design has a significant, less number of experiments than another experimental design.

Real values of batches with their results are shown in **Table 3**. Formulation 4, 8 13, 14 and had highest % Entrapment efficiency. **Table 3** shows the observed values with all the batches.

TABLE 3: OBSERVED VALUES FOR THE DIFFERENT LEVELS OF EXPERIMENTAL DESIGN

No. of Runs	Independent Variables			Dependent Variables		
	X_1	X_2	X_3	Particle Size (nm)	% EE	% DR
1	-1	-1	0	271.8	65.58	54.14
2	+1	-1	0	265.7	64.62	57.47
3	-1	+1	0	287.2	64.12	58.13
4	+1	+1	0	268.3	70.41	40.74
5	-1	0	-1	270.3	64.38	55.73
6	+1	0	-1	266.1	64.29	46.78
7	-1	0	+1	296.1	69.06	56.14
8	+1	0	+1	278.7	72.24	50.83
9	0	-1	-1	267.5	58.99	47.17
10	0	+1	-1	280.5	58.78	42.16
11	0	-1	+1	288.5	63.83	50.70
12	0	+1	+1	290.1	68.73	42.96
13	0	0	0	222.8	80.92	30.71
14	0	0	0	220.3	81.26	30.89
15	0	0	0	221.9	80.42	31.54

The %EE shows at different levels of the 3 independent variables X_1, X_2 and X_3 were subjected for multiple regression to yield a primary & secondary polynomial equation

EE = + 80.87 + 1.051A + 1.13B + 3.43C + 1.81AB + 0.82AC + 1.28BC - 4.89A2 - 9.802B2 8.49C2 ……..(1)

The correlation coefficient (r1) of equ.1 was found to be 0.9162, shows a perfect fit.

The %EE values for all batches showed variation from 64.12 to 81.62. The result clearly showed that selected variables for the study having an effect on the % EE.

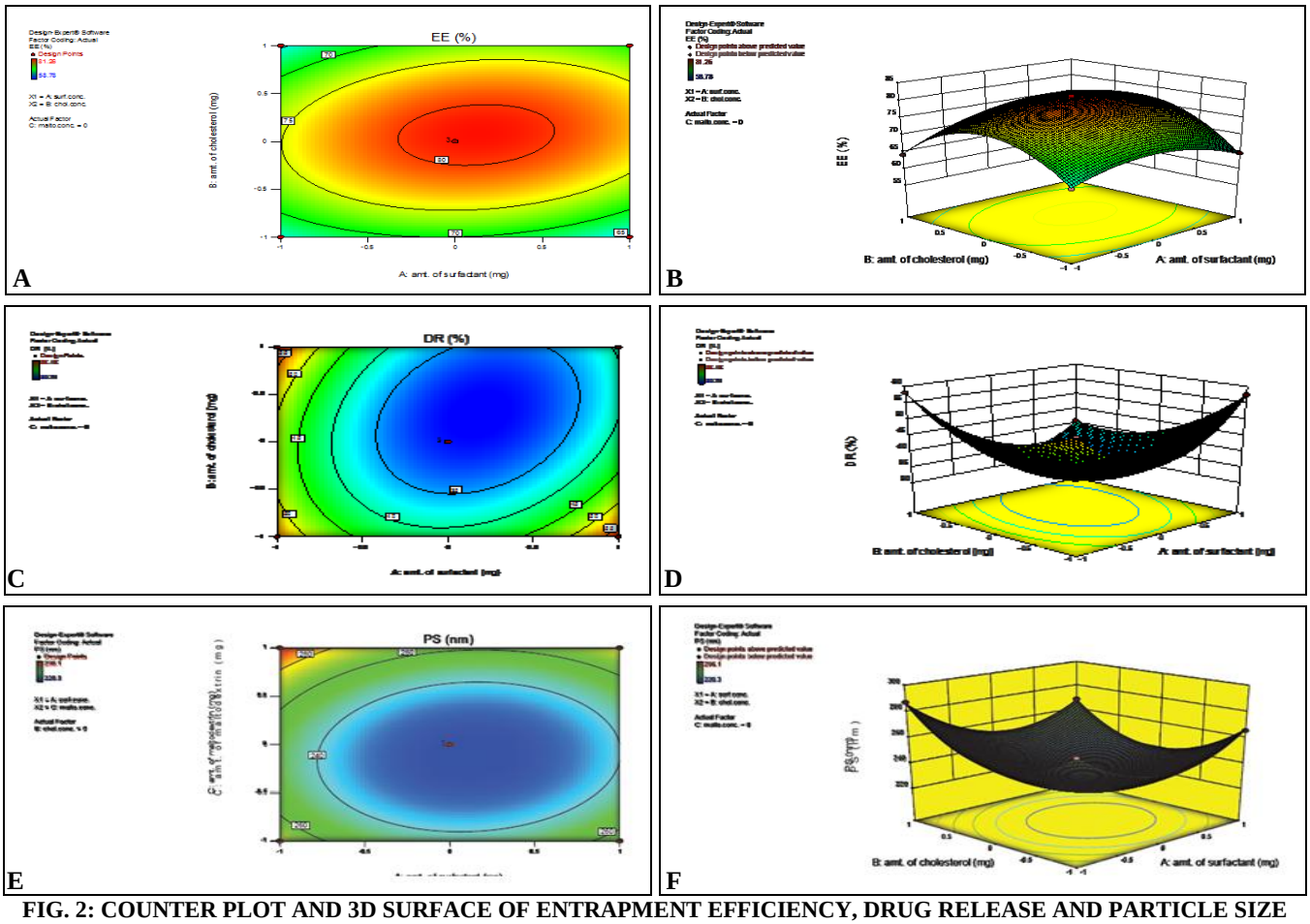

FIG. 2: COUNTER PLOT AND 3D SURFACE OF ENTRAPMENT EFFICIENCY, DRUG RELEASE AND PARTICLE SIZE

The contours plot (**Fig. 2A** & **B**) of %EE and curvilinear indicated that a higher value of %EE ((≥70%) can be resulted due to a combination of two different independent variables. It clearly indicated that as increasing the X_1 and X_2 variables, there is a clearly an increase in the %EE.

The contours plot (**Fig. 2C** & **D**) of % DR and curvilinear indicated that a higher value of %DR can be resulted due to combination of two independent variables. It clearly indicated that as increasing the X_1 and X_2 variables there is clearly increase in the %DR but up to optimum ratio it was controlled the release of the drug

DR = + 31.05 - 3.54A - 3.19B + 1.10C-5.18AB + 0.91AC - 0.68BC + 14.10A2 + 7.48B2 + 7.23C2 ……(2)

The contours plot (**Fig. 2E** & **F**) of PS and curvilinear indicated that reduction of vesicles size was obtained due to the combination of the two independent variables. It clearly indicated that as increasing the X_1 and X_2 variables there is clearly increase in the PS but up to optimum ratio it was decreasing the particle size. Rhodes *et al.*, [63]

PS = + 221.63 - 5.81A + 4.06B + 8.63C - 3.17AB -3.30AC-2.85BC + 23.85A2 + 27.70B2 + 32.28C2……. (3)

Formation of Vesicular Structures from Proniosome Powder: The proniosomes upon contact with hydration media derived to the formation of niosomes and were immediately suggesting a rapid conversion of niosomes on contact with physiological fluids in the body.

Results showed the formation of niosomes from proniosomes in **Fig. 3**. It is evidence that in the initial stages upon contact with water, the surfactant tends to form tubular vesicular structures and upon manual agitation they have deformed in to small vesicles having spherical shape.

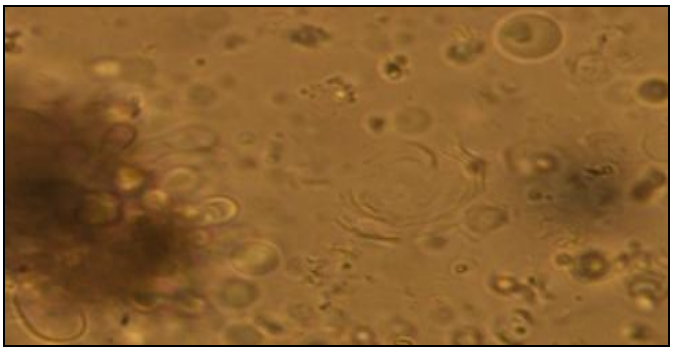

FIG. 3: PRONIOSOMES POWDER UNDER INVERTED MICROSCOPY IMMEDIATELY HYDRATION WITH DISTILLED WATER

Surface Morphology: Surface morphology of vesicles prepare from proniosome formulations were revealed by Transmission Electron Microscopy. TEM was carried out to determine the structure of proniosomal formulation.

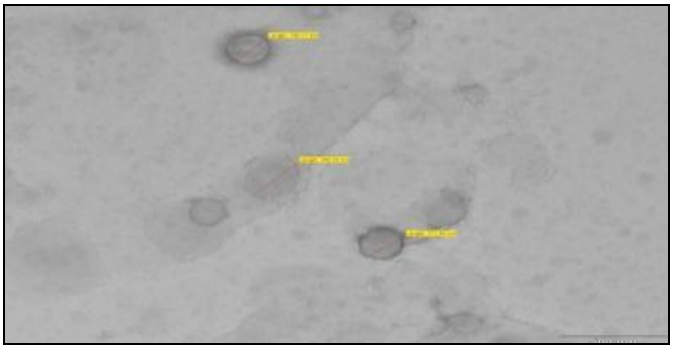

FIG. 4: TEM IMAGES OF HYDRATED PRONIOSOMES DISPERSION WATER

From **Fig. 4** it is confirmed that niosomes are in spherical morphology. From this, it could be concluded that most of the drug is entrapped in niosomes, and there were no crystals of the drug seen in the Transmission Electron Microscopy.

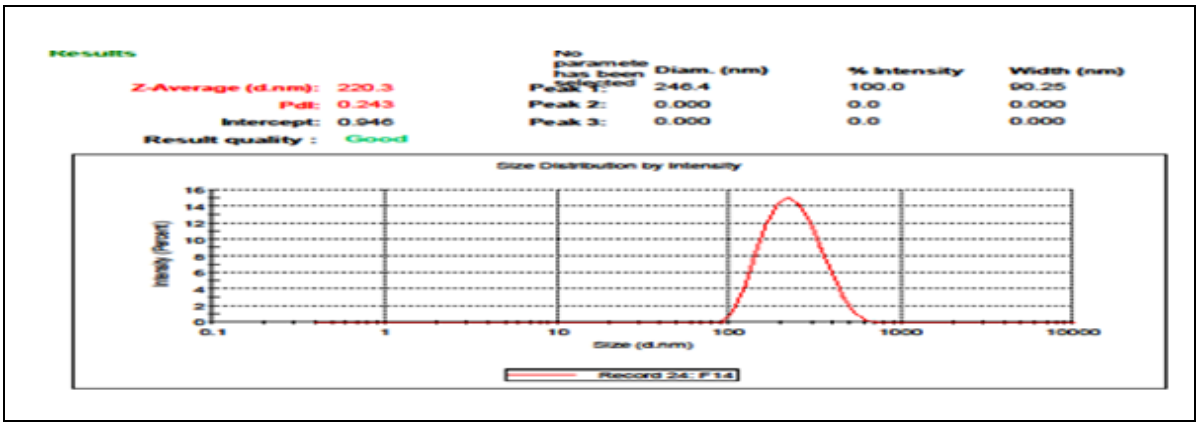

FIG. 5: PARTICLE SIZE GRAPH OF OPTIMIZED BATCH

Particle Size Distribution of Reconstituted Budesonide Niosomes: The average particle size analyzed by Malvern particle sizer analyzer was found to be in the range 220.3 to 296.1 nm shown in **Table 3**. This is in the desired range for oral preparation. The Optimized batch has a particle size of 220.3 nm shown in **Fig. 5**. The polydispersity index (PDI), is a measure of the distribution of molecular mass in a given polymer sample.

The PDI calculated is the weight average molecular weight divided by the number average molecular weight. It indicates the distribution of individual molecular masses in a batch of polymers. The optimized batch has a polydispersity index of 0.243 which is desirable.

Entrapment Efficiency and Drug Content of Budesonide Niosomes: Entrapment efficiency of Budesonide was found to be in the range of 58.78 to 81.42 %. Budesonide loading was increased with increases in the concentration of surfactant. The results obtained for all batches were given in **Table 3**. Entrapment efficiency of the optimized batch was found to be 81.42%. From the obtained data for entrapment efficiency, we can say that as the concentration of surfactant increases, the entrapment of the drug also increases up to optimum concentration for the hydrophobic drug.

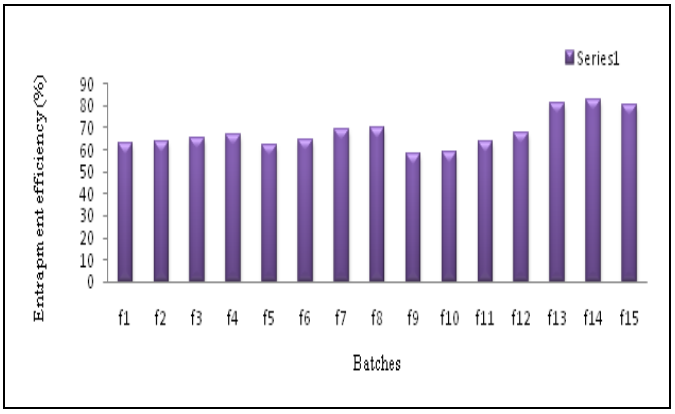

FIG. 6: ENTRAPMENT EFFICIENCY GRAPHS OF ALL FIFTEEN BATCHES

After reached to optimum concentration, there is a decrease in entrapment efficiency. Cholesterol also plays an important role in entrapment efficiency. As shown in **Fig. 6,** Entrapment Efficiency increases as the concentration of cholesterol increase up to a certain extent, but further increases in cholesterol cause a decrease in entrapment efficiency. F14 shows highest Entrapment Efficiency. The drug content of the optimized proniosomes powder (F14) was found to be 99% that is better drug content of proniosomes. It is under the limit of 98 to 102% as per IP.

***In-vitro* Dissolution Study:** Drug release of Budesonide was found to be in the range of 30.71 to 58.13%. Budesonide release was decreased with increases in the concentration of surfactant.

The results obtained for all batches were given in **Table 3.** Drug release of an optimized batch at 6 h was 30.89%.

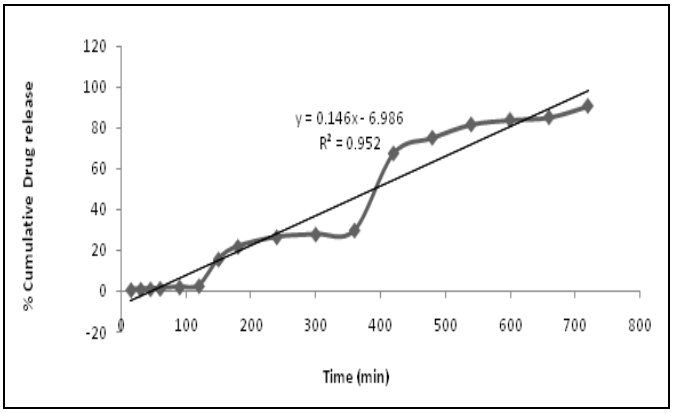

FIG. 7: *IN-VITRO* DRUG RELEASE OF OPTIMIZED BATCH

From the obtained data for drug release, we can say that as the concentration of surfactant increases, the release of the drug decreases at 6 h as shown in **Fig. 7**. Cholesterol also plays an important role in drug release. As the concentration of cholesterol increases, the drug release decreases up to a certain extent, but further increases in cholesterol cause an increase in drug release. F14 shows a very less amount of release at 6 h.

Residual Solvent Determination by Gas Chromatography: The result obtained from GC showed that the amount of Ethanol and chloroform was 265 ppm and 12.59 ppm, respectively, below the standard limit of Ethanol and chloroform as per IP as shown in **Fig. 8** and **9**.

***Ex-vivo* Permeation Study:** From the *ex-vivo* diffusion study, it was found that the optimized batch showed good steady-state flux and permeability coefficient as compared to the pure drug solution. The result of flux and permeability coefficient had shown in **Table 4** and **5**.

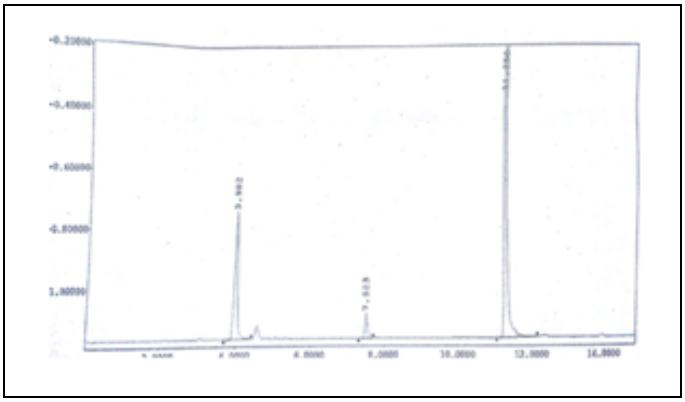

FIG. 8: PEAK OF STANDARD ETHANOL AND CHLOROFORM

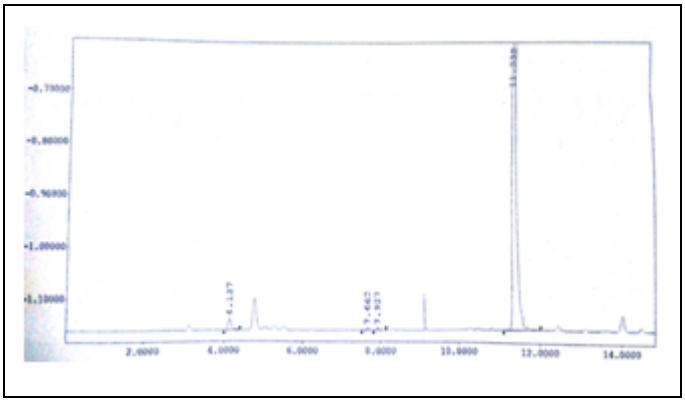

FIG. 9: PEAK OF ETHANOL AND CHLOROFORM IN OPTIMIZED BATCH

TABLE 4: COMPARISON OF *EX-VIVO* PERMEATION OF BUDESONIDE PRONIOSOME DISPERSION WITH PURE

Steady-state flux of Optimized Batch (F14)				Steady-state flux of pure Budesonide as controls		
Time (hr)	**% DR**	**%DR/cm^2**	**Steady-state Flux (Jss)**	**% DR**	**%DR/cm^2**	**Steady-state Flux (Jss)**
0	0	0		0	0	
0.25	26.287	2.796	2.772	11.924	1.268	0.871
0.5	37.831	4.024		14.428	1.534	
0.75	45.241	4.812		15.051	1.601	
1	52.558	5.591		16.905	1.798	
1.25	56.915	6.054		20.075	2.135	
1.5	63.696	6.776		21.745	2.313	
1.75	70.997	7.552		23.355	2.484	
2	72.254	7.686		26.796	2.850	

TABLE 5: COMPARISON OF FLUX BETWEEN OPTIMIZED AND PURE DRUG

Formulation	Steady-state Flux (Jss) mg/cm^2	Kp Permeability Coefficient (cm^2/h)	Enhancement Ratio Jss PL/ Pure Budesonide
Budesonide Proniosomes	2.772	0.308	3.182
Pure Budesonide	0.871	0.0967	

The comparison graph of permeation was shown in **Fig. 10**. The permeation enhancement ratio of proniosome formulation was found to be 3.182.

Stability Study: In stability study, particle size, entrapment efficiency, and drug crystallization of proniosomes were monitored for a period of 30 days at different storage conditions and then analyzed for different parameters. Proniosomes powder was prepared by using span 60, Cholesterol, and maltodextrin.

It is important to note the particle size, entrapment efficiency, and drug crystallization at the temperature 37 °C ± 5 and 2 °C - 8 °C the property of proniosomes powder was maintained.

TABLE 6: STABILITY STUDY DATA

S. no.	Parameter	Storage Condition	Before stability study	10 days	20 days	30 days
1	Particle size(nm)	Room Temperature	227.5	227.46	227.41	227.40
2	Entrapment Efficiency (%)	37 °C ± 5	83.59	83.58	83.46	82.23
3	Drug Crystallization		No	No	No	No
4	Particle size(nm)	Refrigerator	227.60	227.49	227.47	227.45
5	Entrapment Efficiency (%)	2°C – 8 °C	83.59	83.59	83.57	83.30
6	Drug Crystallization		No	No	No	No

The stability data showed that the optimized batch of proniosomes containing budesonide remained stable in terms of particle size, entrapment efficiency, and the drug crystallization at both Refrigerator and room temperature for 1 month. Results had shown in **Table 6**. There is no appreciable change in particle size and entrapment efficiency. No drug crystallization occurs after 30 days. It indicates that the optimized formulation was stable.

CONCLUSION: The proniosome drug carriers could be successfully developed by using the slurry method for the enhancement of solubility and bioavailability of Budesonide, a new drug delivery system with high potential for the targeted drug delivery system. The box-Behnken design was employed to get optimized proniosome formulation which entirely fills the criteria for targeted drug delivery.

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REFERENCES:

1. Manivannan R and Kugalur GP: Recent advances in novel drug delivery systems. Int J Res Ayur Pharm 2010; 1(2): 316-26.
2. Udasi TA, Wankhade VP, Ingle LM, Atram S and Tapar KK: proniosome: a novel approach to vesicular drug delivery system. Int J Pharm Pharmceu Sci Res 2013; 3(1): 1-6.
3. Jain NK: Controlled and Novel Drug Delivery. CBS publishers and Distributors, Delhi Edition 1, 1997; 292-01.
4. "Budesonide", September 2014, www.accessdata.fda.gov/ scripts/cder/drugsatfda/index.cfm
5. "Budesonide", September 2014, http://www.drugbank.ca/ drugs/DB01222
6. Vyas SP and Khar RK: Targeted & Controlled drug delivery. CBS publishers and Distributors, Delhi Edition 1, 2002; 249-77.
7. farroq U, Bashir I, Muhammad J and NA M: Niosomes: A unique drug delivery tool. World J Pharmceu Sci 2014; 3(12): 111-23.
8. Azeem A, Anwer K and Talegaonkar S: Niosomes in sustained and targeted drug delivery: some recent advances. Journal of Drug Targeting 2009; 17 (9): 671-89.
9. Chavda Y, Bhavin B, Upendra P and Daslaniya D: Niosome and proniosome- Vesicular structured Dosage form for targeted drug delivery system. J Adv Pharm Res 2011; 2(4): 175-84.
10. Singh S: Niosomes: a role in targeted drug delivery system. IJPSR. 2013; 4(2): 550-57.
11. Kamboj S, Saini V and Bala S: Formulation and characterization of drug-loaded non-ionic surfactant vesicles. The Scientific World Journal 2014; 1-8
12. Mahale NB, Thakkar PD, Mali RG, Walunj DR and Chaudhari SR: Niosomes: novel sustained-release nonionic stable vesicular systems - an overview. Advances in Colloid and Interface Science 2012; 183: 46-54.
13. Sharma R, Mahatma R, Bharkatiya M and Goyal A: Niosomes - as potential drug delivery system. Int J Drug Res Tech 2012; 2(6): 422-29.
14. Kazi KM, Mandal AS, Biswas N, Guha A, Chatterjee S, Behera M and Kuotsu K: Niosome: a future of targeted drug delivery systems. J Adv Pharm Technol Res 2010; 1(4): 374-80.
15. Patel DV and Misra M: Niosome: A magic targeted drug delivery system. Pharma Tutor 2014; 1-12.
16. Annakula D, Rao M, Jukanti R, Bandari S and Veerareddy P: Provesicular drug delivery systems: An overview and appraisal. Arch Appl Sci Res 2010; 2(4): 135-46.
17. Sudhamani T, Priyadarisini N and Radhakrishnan M, "Proniosomes: a promising drug carriers" Int J Pharm Tech Res 2010; 2(2): 1446-54.
18. Kalepun S, Mohanvarma M and Veerabhadhraswamy P: Oral lipid-based drug delivery systems – an overview. Acta Pharma Sinicab 2013; 3(6): 361-72.
19. Walker R and Edwards C: Clinical Pharmacy and Therapeutics. Churchill Livingstone, Publishing Elsevier Limited. Edition 3, 2003; 163-78.
20. Pawar PS and Saleem MA: Formulation and evaluation of oral colon targeted tablet of budesonide. Der Pharmacia Lettre 2013; 5 (3): 1-12.
21. Wakure BS, Yadav AV, Bhatia NM and Salunke MA: Development of chitosan capsule for colon specific delivery of budesonide. International Journal of Pharmaceutical Sciences and Research 2013; 4(8): 3239-49.
22. Amgaokar YM, Chikhale RV, Lade UB, Biyani DM and Umekar MJ: Design, formulation and evaluation of transdermal drug delivery system of budesonide. Dig J Nanomate Biostr 2011; 6(2): 475-97.
23. Liu Z, Liu Y, Wang Y, Wang Q, Ma Y and Gao X: Budesonide nano crystallizing preparation and preparation method thereof. Chinese patent. CN20101295644 20100929: 2011.
24. Rudolph M, Beckert T and Dressman J: Pharmaceutical formulation for the active ingredient budesonide, Indian patent. 2110/CHENP/2004: 2009.
25. Sahoo RK, Biswas N, Guha A and Kuotsu K: Maltodextrin based proniosomes of nateglinide: Bioavailability assessment. Int J Bio Macromol 2014; 6(9): 430-34.
26. Radha RV and Chowdary V: Formulation and evaluation of ornidazole proniosomal gel. Indo Ame J Pharmceu Res 2014; 4(4): 2657-65.
27. Veerareddy PR and Bobbala SK: Enhanced oral bioavailability of isradipine via proniosomal systems. Drug Dev Ind Pharm 2013; 39(6): 909-17.
28. Marwa HA, Omaima SA, El-ghamry HA and El-nahas HM: Optimizing proniosomes for controlled release of ketoprofen using box-behnken experimental design. International Journal of Pharmaceutical Sciences and Research 2011; 2(8): 2195-05.

29. Parthibarajn R, Rubinareichl C and Loganathan S: Formulation and evaluation of methotrexate proniosomal powder. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(1): 175-78.
30. Gurrapua A, Jukanti R, Bobbala SR, Kanuganti S and Jeevana JB: Improved oral delivery of valsartan from maltodextrin based proniosome powders. Advanced Powder Technology 2012; 23: 583-90.
31. Akhilesh D, Anoop VN and Rao BP: Formulation and evaluation of gliclazide loaded maltodextrin based proniosomes. Journal of Pharmaceutical and Biomedical Sciences 2011; 2(4): 1582-189.
32. Mohamed N: *In-vitro* and *in-vivo* evaluation of proniosomes containing celecoxib for oral administration. Ame Ass Pharm Sci 2010; 11(1): 85-89.
33. Tank CJ, Borkhataria CH, Baria AH, Patel RP, Tamizharasi S and Sureja DK: Formulation and evaluation of aceclofenac loaded maltodextrin based proniosome. Int J Chem Tech Res 2009; 1(3): 567-73.
34. Sandhya P, Fatima SM and Subrahmanyam CVS: Enhancement of permeability of losartan potassium by formulating as proniosomal gel. International Journal of Biological & Pharmaceutical Research 2013; 4(10): 743-48.
35. Wathoni N, Sriwidodo and Insani UC: Characterization and optimization of natural maltodextrin-based niosome. Journal of Applied Pharmaceutical Science 2013; 3(7): 68-71.
36. Sudhamani T, Ganesan V, Priyadarsini N and Radhakrishnan M: Formulation and evaluation of ibuprofen loaded maltodextrin based proniosome. Int J Bio Pharm 2010; 1(2): 75-81.
37. Liu, Xiao J, Liu L and Zhang B: Formulation of paeonol proniosomes and preparing method thereof. Chinese patent. CN 103340823 A; 2013.
38. Claudia W and Eric D: Residual solvents in pharmaceutical products: acceptable limits, influence on physicochemical properties, analytical methods and documented values. Eur J Pharma Biopharma 1997; 43: 215-42.
39. Carafa M: Niosomes, freeze-dried powder thereof and their use in treatment. United state Patent. WO 2011083428 A1; 2011.
40. Handjani RM, Ribier A, Vanlerberghe G, Zabotto A and Griat J: Cosmetic and pharmaceutical compositions containing niosomes and a water-soluble polyamide, and a process for preparing these compositions, United state Patent. US4830857A1; 1989.
41. Indian Pharmacopeia, The Indian Pharmacopeia commission, Edition 7, Ghaziabad, 2014; 1: 174.
42. Donald LP, Gary ML, George SK and James RV: In Spectroscopy, Cengage Learning India PVT. Limited, New Delhi, Edition 5, 2011: 30-45.
43. Gouda MM, Shabaraya RA, Shantakumar SM, Shyale SS and Putta RK: Development and validation of selective UV spectrophotometric analytical method for Budesonide pure Sample. Journal of Applied Pharmaceutical Science 2011; 01 (07): 158-61.
44. Lachman L, Lieberman HA and JL Kanig: The theory and Practice of Industrial Pharmacy, 3rd edition, Varghese publishing house 193: 57.
45. Sudhamani T, Priyadarisini N and Radhakrishnan M: Proniosomes: a promising drug carriers. International Journal Pharm Tech Research 2010; 2(2): 1446-54.
46. Chander PD, Singh SK, Kumar Sanjeev, Datusalia AK and Deep A: Development and characterization of nano-particles of glibenclamide by solvent displacement method. Acta Poloniae Pharm Drug Res 2010; 67(3): 283-90.
47. Singh R, Manirul H and Prem SA: Sensitive head space gas chromatographic method for the determination of residual solvents of cloxacillin sodium and dicloxacillin. Am J Sci Ind Res 2010; 1(2): 271-78.
48. Brahmankar DM and Jaiswal SB: Biopharmaceutics & pharmacokinetics; Vallabh prakashan, New Delhi, Eition 2nd, 2011; 431-33.

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