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QBD based Design and Characterization of Proniosomal Transdermal Delivery of Atenolol and Glibenclamide Combination: An Innovative Approach

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Abstract

There are numerous circumstances where chronic disease is associated with other disorders, especially in diseases like diabetes with noncommunicable disease risk factors, such as hypertension. This study shows a novel and innovative combinational proniosomal delivery of combination to beat the reactions by complex therapeutic regimen, and to improve patient compliance after controlling combinational transdermal delivery of Glibenclamide (GLB) and Atenolol (ATN) which have not been tried actually. To achieve the above reason, proniosomes were prepared and optimized utilizing Box-Behnken design. The ideal formulation was chosen by a point prediction method and formulation showed vesicle size of 562 ± 1.223 nm, entrapment efficiency of GLB & ATN $97.037 \pm 1.43\%$ and $96.230 \pm 1.62\%$ respectively which were found in concurrence with the predicted value. The optimized combinational proniosomal gel (OCPG) formulation was additionally assessed for *in vitro* drug release, *In vitro* drug permeation, and *in vivo* pharmacokinetic study. The OCPG formulation shows the greatest flux over the rabbit skin (128.609 ± 2.24 $\mu\text{g}/\text{cm}^2/\text{h}$ and 322.054 ± 1.53 $\mu\text{g}/\text{cm}^2/\text{h}$) of GLB and ATN respectively. The results indicated desired release and permeation profiles. OCPG showed significantly ($p < 0.001$) pharmacokinetic contemplate exhibited that transdermal proniosomal formulation demonstrated improvement in bioavailability of two drugs 129.30 and 174.62 times respectively as that of the oral formulation. Overall the results show that controlled release GLB and ATN provesicles offer a useful and promising transdermal delivery system for the treatment of type II diabetes and hypertension by using design. Henceforth this may be an achievement in treating diabetic hypertensive patient.

Keywords: Atenolol, Glibenclamide, Transdermal delivery, Proniosomes, Combination

1. Introduction

Medication administration remains the hallmark of drug therapy. A general aspiration of pharmacotherapy is to ensure a therapeutic drug concentration in specific areas of the body [1]. The Oral administration remains the most normally used routes for medicine. An oral medication generally becomes active when it

passes from the gastrointestinal tract and the liver into the blood, which reduces the bioavailability and other side effects. But for this combination medication, it may not be suitable because of the fact that the time of administration of the two medications is different, which assumes a vital role in ingestion of medications in GIT [2]. These side effects were overcome by one of the alternative routes transdermal drug delivery system

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(TDDS), which is a promising way of drug delivery to maintain a uniform plasma concentration, reduce dosing frequency associated with improved the patient compliance, and avoid the gastrointestinal action [3,4]. Transdermal Drug Delivery Systems (TDDS) rise the alternative choice for the administration of medications which permits self-administration and furthermore whenever required to terminate the medication exposure at any point of time [5,6].

Owing to this it exhibits a high level of patient compliance with low levels of subject variability [7]. Due to the realization of the factors like poor drug absorption due to permeability barrier (skin), TDDS is continuously looking into newer lane [8–11]. To overcome the skin barrier to a certain extent sophisticated technique [12,13] such as with self-regulating colloidal carriers [14,15]. Vesicular frameworks like niosomes show proof of noteworthy potential for success over such boundary. They collaborate with the stratum corneum layer to improve the permeation of drug and furthermore, it goes about as medication for controlling the release [16,17].

Diabetes mellitus (Type 2 diabetes) is associated with an increased risk of death due to cardiovascular disease (CVD) where hypertension is a major risk factor. Present medication systems for the treatment of such chronic coexisted diseases are inconvenient to overcome the side effects. Therefore, investigations are desired to deliver antidiabetics and antihypertensives using new delivery approaches. Around 285 million (6%) individuals worldwide is assessed to have diabetes and 972 million (26%) have hypertension. 40–80% of diabetes individuals likely to have hypertension [18–20].

Patients with these comorbid conditions about to take more medications per day. Instead of taking 2 or more medications they can take a single medication with this combination thereby they follow a full treatment regimen and furthermore to decrease high day by day costs [21]. Instead, Transdermal administration would provide a continuous stimulation of insulin production and reduce the blood pressure levels simultaneously and thereby help to reduce these complications. This has been proved for the administration of Antihypertensives with a rapid onset of action on awakening in the early morning seems to be a more rational and beneficial alternative than the conventional administration after breakfast [22].

Glibenclamide (GLB) is a prevalent medication and is generally utilized for treating type II diabetes [23]. Gastric aggravations like queasiness, vomiting,

heartburn, anorexia and increased hunger after oral treatment is the most well-known symptoms where it is directed for a long time. Atenolol (ATN), a β -blocker, is recommended broadly in various cardiovascular maladies. Organization of traditional tablets of ATN has been accounted for to display changes in the plasma sedate dimensions, bringing about either indication of reactions or decrease in medication focus at the receptor site [24]. From the literature, it is evident that β -blockers lessen irregular cardiovascular events when used in assistant evasion after Myocardial Infarction for both diabetic and nondiabetic patients. Evidence that diabetic patient may experience more prominent cardio protection with β -blockade than do nondiabetic patients [25].

This investigation exhibits a new combinational proniosomal transdermal delivery of ATN and GLB to conquer the symptoms by complex therapeutic regimen and to improve patient compliance after administering combinational transdermal delivery of GLB and ATN which have not been formulated so far.

2. Materials and methods

ATN and GLB is a gift sample obtained from Sun Pharma Ltd, Mumbai, India. Span 60 and cholesterol were purchased from SD Fine Chemicals (Mumbai, India). Phospholipid 90G is a gift sample obtained from Lipoid, Nattermannallee 1, D-50829 Köln and Germany. Potassium dihydrogen orthophosphate, Acetonitrile, Ethanol-AR, Cholesterol, Sodium hydroxide, Orthophosphoric acid, Carbopol® and Potassium dihydrogen orthophosphate were purchased from E Merck (Mumbai, India). All the reagents used are of analytical grade.

2.1. Analysis of GLB and ATN

The concentration of the combination of GLB and ATN in the samples was analyzed by using HPLC method (Water 2690 composed of PDA-2996 detector) with BDS C18 250 $\times$ 2.1 mm, 1.6 μ columns. Data acquisition, recording, and chromatographic integration were performed by Empower 2 software. The mobile phase consisting of 0.01N potassium dihydrogen orthophosphate (pH 4.8) and acetonitrile (ACN) taken in the ratio 55:45 with an injection volume 1.0 ml in gradient mode with column oven temperature maintained at 30 °C and elution monitored by a detector wavelength at 235.0 nm.

2.2. Differential scanning calorimetry (DSC)

DSC was performed using a DSC instrument (Horiba, Germany). About 5 mg of ATN, GLB, and

Optimized combination proniosomal gel (OCPG) were individually capped in the aluminum crucible. The crucible was kept under a dynamic atmosphere of nitrogen (50 ml/min) and a heat flow rate of 10 °C/min from 30 to 200 °C and the corresponding spectra between heat flow (w/g) on Y-axis and temperature on X-axis were obtained.

2.3. Fourier transform infrared (FTIR) spectroscopy

Infrared spectroscopy of ATN, GLB, span 60, Phospholipid 90G, Cholesterol and OCPG individually were analyzed by ALPHA-T (Brucker) FTIR Spectrophotometer and the spectra were recorded in the region of 4000–400 cm⁻¹.

2.4. Preparation of proniosomes

Proniosomes were prepared by the method reported by Perrett et al. [26] and Vora et al., [27]. The

Table 1. Independent and dependent variables used in Box–Behnken design for the development and optimization of proniosomal combination.

Variables	Levels		
Independent Variables	–1	0	+1
X1 = Span 60 (mg)	450.00	675	900.00
X2 = Cholesterol (mg)	150.00	200	250.00
X3 = Phospholipid (mg)	900.00	1050	1200.00
Dependent Variables	Constraints		
Vesicle Size (Y1)	Minimum		
EE% of GLB (Y2)	Maximum		
EE% of ATN (Y3)	Maximum		

compositions of different proniosomal formulations are listed in Table 2. Using a wide-mouth glass tube, précised amounts of drugs with surfactant, Phospholipid 90G, and cholesterol was mixed with 2.5 ml of absolute ethanol. The open end of the glass tube was covered with a lid to prevent loss of solvent, after mixing all ingredients and then warmed in a water bath at 60–70 °C for about 5min, until the surfactants were dissolved completely. The aqueous phase (pH 7.4 phosphate buffer) was then added and warmed in a water bath till clear solution was formed. The mixture was allowed to cool to room temperature until the dispersion was converted to proniosomal gel. Proniosomal gel was then mixed with 1% Carbopol® gel in 1:1 ratio. The gel obtained was preserved in dark until characterization.

2.5. Box Behnken experimental design

To assess the interaction impacts of surfactant, lipid, and Cholesterol in the formulations; 3-factor, 3-level Box Behnken configuration was utilized. An absolute 17 test runs were produced by design expert Version 11 software. The independent variables were surfactant (Span 60) (X1), Cholesterol (X2) and lipid (Phospholipid 90G) (X3) while Vesicle size (VZ) (Y1) entrapment efficiency of GLB (Y2) and entrapment efficiency of ATN (Y3) were the dependent variables. The scope of independent variables was selected based on the results of different initial trials and literature. The dependent and independent variables were tabulated in Table 1. Further, the optimum formulation was chosen by the numerical

Table 2. Observed response in Box–Behnken design for development and optimization of proniosomal combination generated by Design Expert Software.

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A: Surfactant	B: Cholesterol	C: Phospholipid	Vesicle Size	Entrapment Efficiency of GLB	Entrapment Efficiency of ATN
	mg	mg	mg	nm	%	%
1	–1	0	+1	487 ± 3.12	91.63 ± 0.56	90.32 ± 0.43
2	0	0	0	555 ± 2.98	95.98 ± 0.21	94.56 ± 0.32
3	0	–1	–1	564 ± 1.34	87.54 ± 0.43	86.43 ± 0.64
4	–1	–1	0	424 ± 3.21	83.24 ± 1.21	82.32 ± 0.76
5	0	0	0	564 ± 1.54	93.26 ± 0.22	92.23 ± 0.43
6	+1	0	–1	524 ± 2.43	98.26 ± 0.32	96.96 ± 0.21
7	0	0	0	560 ± 1.65	95.32 ± 0.25	94.64 ± 0.32
8	0	+1	–1	612 ± 2.76	78.65 ± 0.54	79.54 ± 0.54
9	+1	+1	0	534 ± 3.26	80.43 ± 0.32	80.35 ± 0.65
10	0	0	0	554 ± 2.34	95.34 ± 0.65	94.92 ± 0.43
11	0	–1	+1	543 ± 1.87	88.35 ± 0.78	87.47 ± 0.98
12	+1	–1	0	454 ± 1.32	90.54 ± 0.54	90.34 ± 0.76
13	+1	0	+1	544 ± 1.75	96.32 ± 0.45	95.99 ± 0.64
14	–1	+1	0	478 ± 2.34	81.54 ± 0.65	80.25 ± 0.43
15	–1	0	–1	520 ± 3.65	92.45 ± 0.76	91.43 ± 0.65
16	0	+1	+1	610 ± 2.87	78.43 ± 0.43	78.98 ± 0.21
17	0	0	0	556 ± 2.43	95.65 ± 0.98	96.87 ± 0.98

point prediction methodology utilizing the desirability function. At last, linear regression with ANOVA was utilized to pick the best-fitted model and p values below 0.05 were considered as statistically significant.

2.6. Characterization of the prepared proniosomes

2.6.1. Vesicle size and size distribution analysis

For all the batches of proniosomes vesicle size analysis was carried out using Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The freshly prepared hydrated niosomes were dispersed in double distilled water (DDW) and was used to characterize the vesicle size [28,29].

2.6.2. Encapsulation efficiency

A precisely weighed measure of a proniosomal gel was included 10 ml of pH 7.4 phosphate buffer in a glass vial and was sonicated in a sonicator. The medications containing niosomes were isolated from the untrapped drug by centrifugation at 25,000 rpm at 20 °C for 30 min. The supernatant was recuperated and measured by a UV method for the two drug content. The percentage of drug encapsulation (EE (%)) was calculated by the accompanying equation:

$$EE \% = [(C_t - C_r) / C_t] \times 100\%$$

where C_t is the concentration of total drug and C_r is the concentration of free drug [30,31].

2.6.3. Morphology

The shape, surface and size qualities of the prepared proniosomes were seen by scanning electron microscopy. A dainty layer of formed proniosomes was spread on a glass slide and watched the structure of the vesicle. Photomicrographs were taken utilizing Fujifilm Finepix F 40 fd 8.3 MP computerized camera with 3 optical zoom [32,33].

2.6.4. Zeta potential measurement

Zeta potential estimation was done by Malvern Zetasizer Nano Z (Malvern instrument, Malvern, Worcestershire, UK) device at 25 °C. The zeta potential was determined by applying an electric field to the particles of the colloidal scattering of particles which then move with a speed related to their zeta potential [34,35].

2.6.5. In vitro drug permeation studies

In-vitro drug permeation studies of the optimized proniosomal formulation were performed by Franz diffusion cells with a diffusional area of 3.14 cm². An

egg layer [36] was set among donor and receptor compartments. The OCPG was spread on egg film. The receptor compartment contained phosphate buffer pH 7.4 and was constantly mixed by remotely determined Teflon-coated magnetic bead. The temperature of the cell was kept up at 37 ± 1 °C, to mimic the physiological conditions. Aliquots (1 ml each) were gathered at regular time interval for 24 h and measured for the two drugs concentration utilizing HPLC technique as depicted previously. Each release study was performed in triplicate [37].

2.6.5.1. Method for egg membrane preparation. The contents of eggshells were removed and then it was dipped in the dilute hydrochloric acid for 30 min. The egg membrane was separated manually and washed thoroughly with distilled water [36].

2.6.5.2. Kinetic analysis of drug-release profiles. To consider the drug-release mechanism from proniosomes, the release data were fitted to the zero-order kinetics as cumulative percent of drug released versus time, the Higuchi model as cumulative percent drug released versus square root of time and the Korsmeyer Peppas's kinetics models as log cumulative percent drug released versus log of time [38].

2.7. Ex vivo permeation study

2.7.1. Preparation of rabbit skin

The preparation of skin was as per Xi et al. [39]. Rabbits weighing 2.0–2.5 kg were bought from the National center for laboratory animal sciences (NCLAS). This study convention was endorsed by the Animal Ethics Committee IAEC (1220/PO/Re/S/08/CPCSEA). After the rabbit was anesthetized with urethane (20%, w/v), the hair of abdominal area was carefully removed. The side of stratum corneum was extracted after the rabbit was yielded and the sub-dermal tissue was painstakingly expelled. The side of stratum corneum was cleaned delicately with refined water. The skin was washed with PBS, wrapped in aluminum foil and stored in a deep freezer at –20 °C till further use (used within 2 weeks of preparation) [40].

2.7.2. Skin permeation studies

A two-chamber horizontal Franz diffusion cell (volume of 15 ml and surface area of 3.14 cm²) was utilized and the system was kept up at 37 ± 0.5 °C with water bath. OCPG was spread on the stratum corneum side and two dissemination cells were clipped together. The receptor chamber was loaded up with phosphate buffer saline (pH 7.4), which was consistently mixed at 600 rpm. A sample of 2.0 ml

was withdrawn at the time points of 2, 4, 6, 8, 10, 12, 24 h and replaced with fresh receptor fluid quickly to keep up a constant volume. The sample was examined utilizing the HPLC for drug release [41].

Steady state flux (J_{ss}) = $Q/(t \times A)$

where,

Q = amount of drug permeated

t = time

A = area of membrane

2.8. *In-vivo* absorption studies

The *in-vivo* study protocol was affirmed by the Animal Ethics Committee IAEC (1220/PO/Re/S/08/CPCSEA). The rabbits ($n = 3$) were housed in an animal, place of the Institute. The entire animals used in the examination were confined and kept up according to the principles of CPCSEA and standards developed for care and use of research center animals [42]. Rabbits fasted for 18 h; but, water was given all through the examination. Rabbits were partitioned into 3 groups ($n = 3$). Group I: Control (pure drugs) given orally, Group II: treated with marketed preparations orally, Group III: Proniosomal gels (OCPG) applied as a gel to the skin (70). The portion was offered proportionately to body weight. Blood samples (0.7 ml) were collected from the marginal ear vein in the heparinized tube at the explicit time interims of 0, 1, 4, 8, 12, 16 and 24 h [43].

2.9. Pharmacokinetic analysis

The pharmacokinetic analysis of the GLB and ATN plasma concentration–time profile was assessed using Thermo Kinetica (ver. 5.0; Thermo Fischer scientific). Different pharmacokinetic parameters such as C_{max} (the maximum plasma concentration) and T_{max} (time to reach maximum plasma concentration) were directly obtained from the plasma concentration versus time curve [44]. The area under the curve from 0 to t (AUC_{0-t}), 0 to ∞ ($AUC_{0-\infty}$) were computed by trapezoid rule and elimination half-life ($t_{1/2}$) was calculated by using the formula $t_{1/2} = \log 2/K_{el}$ and elimination rate constant (K_{el}) was calculated from the slope of log plasma concentration versus time plot. The relative bioavailability of the drug after the test formulation transdermal Vs the oral application was calculated by using formula $F\% = AUC_{test}/AUC_{Control} \times 100$.

2.10. Stability studies

The OCPG formulation was subjected to stability studies to evaluate any physical or chemical changes

in storage. Gel formulation was kept at $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$ and $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$ for 3 months in aluminum foil-sealed glass vials throughout the study ICH Q1A (R2), [45,46]. The samples were analyzed for various parameters at 0, 1, 2 and 3 months after storage like Vesicle size, shape, Clarity and drug content.

3. Results and discussion

In the present investigation, the proniosomes of a combination of drugs were formulated, optimized and evaluated for its capability in transdermal delivery to overcome the major issues related to its oral delivery. The proniosomal gels were studied using particular non-toxic and biocompatible, non-ionic surfactants like spans with cholesterol and Phospholipid [47]. The proniosomes were set up by the coacervation phase separation method as stated by Vora et al. [27].

3.1. Analysis of GLB and ATN

Chromatogram A represents the blank mobile phase and chromatogram B represents with an average retention time of 2.322 min for GLB and 3.260 min for ATN and with no interfering peaks Fig. 1.

3.2. Differential scanning calorimetry (DSC)

DSC thermograms showed the characteristics endothermic peak for ATN and GLB at 146.39°C and 173.79°C , corresponding to their melting point. The result of DSC thermograms of pure drug, physical mixture and optimized s polymers showed an endothermic peak at 147°C and 170.07°C . In addition to this, additional declines in sharpness of endothermic peak in loaded with drug proniosomal formulation were notified. The drug-loaded proniosomes (OCPG) showed the peak at the same melting point which confirmed the absence of any chemical interactions between the Drug and proniosomal components as shown in Fig. 2 [48].

3.3. Infrared spectroscopy (FTIR)

The FTIR spectra of Combination of drugs span 60, Phospholipid, Cholesterol, blank and OCPG are shown in the spectra. FTIR spectra of pure Drugs showed the characteristic peak at 1515.29 cm^{-1} and 1521.99 cm^{-1} (N=N stretching), 1242.08 cm^{-1} and 1246.56 cm^{-1} (C=C stretching), 2924.46 cm^{-1} and 2931.19 cm^{-1} (CH_3 stretching), 1415.09 cm^{-1} and 1455.35 cm^{-1} (CH_3 deformation), 3356.86 cm^{-1} and

Fig. 1. HPLC chromatograms of blank mobile phase spike (chromatogram A) and HPLC chromatograms of mobile phase containing 10 $\mu\text{g/mL}$ GLB & 25 $\mu\text{g/mL}$ ATN (chromatogram B).

3367.54 cm^{-1} (O–H Stretching) for ATN and GLB respectively. Span 60 showed the characteristic peaks at 1220 cm^{-1} (aliphatic), 1755 cm^{-1} (cyclic 5-membered ring), 1400 cm^{-1} ($-\text{CH}_3$), 2928 cm^{-1} (aliphatic C–H stretching, asymmetric and

3400 cm^{-1} (O–H stretching). When the physical mixture of drugs, span 60, Cholesterol and Phospholipid were analyzed, the characteristics peaks of drugs were present in physical mixture similar to that of individual drugs spectrum and there were no

Fig. 2. DSC graphs for pure A) ATN, B) GLB, C) Cholesterol, D) Phospholipid 90G and E) OCPG.

Fig. 3. FTIR graphs for pure ATN, GLB, Span60, Cholesterol, Phospholipid 90G and OCPG.

detectable changes in FTIR spectra which confirmed the absence of any chemical interactions between them as shown in Fig. 3 [49,50].

3.4. Preliminary study

The preliminary study was conducted to evaluate the effect of various formulation factors such as vesicle size and entrapment efficiency on the release of both drugs from the gel as well as their permeation profile across an egg membrane. This study was conducted to evaluate one formulation variable at a time [29].

3.5. Box-Behnken design

Box-Behnken design, a 3-factor, 3-level was used to investigate quadratic response surfaces and constructing second order polynomial models with Design Expert 11. The Box-Behnken design was specifically selected since it requires fewer runs than a central composite design [51]. A design matrix comprising of 17 experimental runs generated by the Design expert software. The independent variables selected were the Span 60 (X1), Cholesterol (X2) and Phospholipid (X3), and; while vesicle size (Y1), entrapment efficiency of GLB (Y2), and entrapment

efficiency of ATN (Y3) were the chosen dependent responses. Table 2 shows the results of the prepared 15 formulations. Fig. 4 shows the three-dimensional plots for all the three responses for responses Y1, Y2, and Y3, respectively. These plots are known to study the interaction effects of the factors on the responses as well as being useful in studying the effects of two factors on the response at one time. Fig. 5 quantitatively compared the resultant experimental values of the responses with that of the predicted values. The regression analysis outcome of different responses is given in Table 3. The Quadratic nature relationships was affirmed by the larger values of the standard error for the coefficient.

3.5.1. Response 1 (Y1): effect of independent variables on vesicle size

For effective transdermal drug delivery, one of the essential criteria is small vesicle size [52]. The size of the vesicles was found to shift between 424 ± 3.21 to 612 ± 2.76 nm (Table 2). Primarily, Normal vesicle size increases with increment in the concentration of Span 60 yet further increase in the concentration of Span 60 quick decrease in the normal vesicle size. The incorporation of spans and cholesterol in the formulation interacts with the bilayer (lecithin present) and resulted in the increased vesicle size. As

Fig. 4. 3D-response surface plot showing effect of independent variables such as Span 60, Phospholipid and Cholesterol on (A) Vesicles size (B) Entrapment efficiency of GLB and (C) Entrapment efficiency of ATN.

the concentration of span 60 increased in the formulation, the vesicle size also increased from 424 ± 3.21 to 544 ± 1.75 nm, which is in agreement with the earlier findings [53]. Also the incorporation of lipophilic drugs into the hydrophobic domain of vesicle causes the bilayer molecules to move apart from each other, leading to an increase in vesicle size [54].

The vesicle size was also increased, when the amount of lecithin in the formulation was increased. In a similar way, when the amount of cholesterol was increased from 150 to 250 mg, the size of vesicle also increased. Elevated cholesterol in the formulation is likewise responsible for the enormous size of vesicles. It was accounted for that cholesterol increased the width of the lipid bilayer [27]. This

relationship is displayed by the accompanying equation.

$$\begin{aligned} \text{Vesicle Size (Y1): } &+557.80 + 18.38X_1 + 31.13X_2 - \\ &4.50X_3 + 6.50X_1 X_2 - 13.25X_1 \\ &X_3 + 4.75X_2 X_3 - 74.40X_1^2 \\ &- 10.90X_2^2 + 35.35X_3^2 \end{aligned}$$

The Model F-value of 151.25 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.05 indicated that the model terms are significant. In this case, X_1 , X_2 , X_3 , X_1X_2 , X_1X_3 , X_2X_3 , X_1^2 , X_2^2 , X_3^2 are significant model terms. There is an 18.66% chance that a Lack of Fit F-value this large could occur due to noise. The

Fig. 5. Linear correlation plots (A, C, E) between actual and predicted values and the corresponding residual plots (B, D, F) for various responses.

Predicted R^2 of 0.9430 is in reasonable agreement with the Adjusted R^2 of 0.9883; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your

ratio of 44.411 indicates an adequate signal. This model can be used to navigate the design space. There existed a direct relationship between the Span 60 concentration and cholesterol on the vesicle size.

Table 3. Summary of results of regression analysis for responses Y1, Y2 and Y3 for fitting to quadratic model.

Quadratic model	Lack of Fit p-value	Adjusted R^2	Predicted R^2
Response (Y1)	0.1866	0.9883	0.9430
Response (Y2)	0.1670	0.9548	0.7742
Response (Y3)	0.4187	0.9406	0.7821

Regression equation of the fitted quadratic model

Vesicle Size (Y1): $+557.80 + 18.38X_1 + 31.13X_2 - 4.50X_3 + 6.50X_1X_2 - 13.25X_1X_3 + 4.75X_2X_3 - 74.40X_1^2 - 10.90X_2^2 + 35.35X_3^2$

EE % of GLB (Y2): $+95.11 + 2.09X_1 - 3.83X_2 - 0.2713X_3 - 2.10X_1X_2 - 0.2800X_1X_3 - 0.2575X_2X_3 - 0.1250X_1^2 - 11.30X_2^2 - 0.5700X_3^2$

EE % ATN (Y3): $+95.86 + 1.91X_1 - 3.93X_2 - 0.2000X_3 - 1.48X_1X_2 - 0.4650X_1X_3 - 0.4000X_2X_3 - 0.4855X_1^2 - 11.06X_2^2 - 0.6955X_3^2$

Linear correlation plots (A) between actual and predicted values and the corresponding residual plots (B) for the responses are presented in Fig. 5.

3.5.2. Response 2 (Y2): effect of independent variables on EE% of GLB

Entrapment efficiency (%EE) is the percentage fraction of the total drug (GLB) entrapped into the vesicles (Table 2). The encapsulation efficiency was found to be higher in case of proniosomes prepared with spans with large saturated alkyl chain when compared to tweens with small saturated alkyl chain. This could be explained on the basis of their HLB values. Spans being lipophilic in nature, having low HLB value, formed a well closed uniform packed bilayer structure within which highly lipophilic GLB gets almost completely packed [55]. It can be observed clearly that when cholesterol was used in 150–200 mg concentration, there was a significant increase in entrapment efficiency, further increase in cholesterol concentration leads to a relative decrease in %EE. Phospholipid also plays a substantial role in the entrapment efficiency of GLB [43]. In addition, the nature of the drug also plays a significant role in the entrapment efficiency of drug because the drug is entrapped in the lipid phase. GLB is a lipophilic drug, so that the entrapment efficiency noticeably was found to be higher [48].

$$\begin{aligned} \text{EE \% of GLB (Y2)} = & +95.11 + 2.09X_1 - 3.83X_2 - 0.2713 \\ & X_3 - 2.10 X_1X_2 - 0.2800 X_1X_3 - \\ & 0.2575 X_2X_3 - 0.1250 X_1^2 - 11.30 \\ & X_2^2 - 0.5700X_3^2 \end{aligned}$$

The Model F-value of 38.58 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. The Predicted R^2 of 0.7742 is in reasonable agreement with the Adjusted R^2 of 0.9548; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 16.731 indicates an adequate signal. This model can be used to navigate the design space. The average Entrapment Efficiency of GLB was 88.79% with a minimum of 78.43% and a maximum of 98.26%. Linear correlation plots (C) between actual and predicted values and the corresponding residual plots (D) for the responses are presented in Fig. 5. According to Mokhtar et al. [56] the entrapment of drug happened in both bilayer and watery compartment of the vesicles. At the point when the lipid compartment and the aqueous compartment saturated with the drug, the vesicles gave constrained entrapment capacity, which is also mentioned by Lopes et al., [57].

3.5.3. Response 3 (Y3): effect of independent variables on EE% of ATN

Entrapment efficiency (%EE) is the percentage fraction of the total drug (ATN) entrapped into the vesicles (Table 2). It is observed from the experimental design that entrapment efficiency has a direct positive relationship with the concentration of Span 60, Cholesterol and Phospholipid also play a substantial role in the entrapment efficiency of ATN [43] as revealed by the following equation. In addition, the nature of the drug also plays a significant role in the entrapment efficiency of drug because the drug is entrapped in the lipid phase. Spans being lipophilic in nature, having low HLB value, they don't form a well closed uniform packed bilayer structure within which highly hydrophilic ATN, which exhibit a leakier and permeable bilayer structure resulted in low entrapment efficiency when compared to GLB [48].

$$\begin{aligned} \text{EE \% ATN (Y3)} = & + 95.86 + 1.91 X_1 - 3.93 X_2 - 0.2000 \\ & X_3 - 1.48 X_1 X_2 - 0.4650 X_1 X_3 - \\ & 0.4000 X_2 X_3 - 0.4855 X_1^2 - 11.06 X_2^2 \\ & - 0.6955 X_3^2 \end{aligned}$$

The Model F-value of 29.14 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. The Predicted R^2 of 0.7821 is in reasonable agreement with the Adjusted R^2 of 0.9406; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 14.206 indicates an adequate signal. This model can be used to navigate the design space. The average Entrapment Efficiency A was 83.86% with a minimum of 78.98% and a maximum of 96.96%. Linear correlation plots (E) between actual and predicted values and the corresponding residual plots (F) for the responses are presented in Fig. 5.

3.5.4. Numerical point prediction method

The optimum formulation of Proniosomal system was selected based on the criteria of attaining the reasonable value of vesicles size, entrapment efficiency of GLB and entrapment efficiency of ATN by applying numerical point prediction optimization method of the Design Expert software 11. The formulation composition with Surfactant (Span 60) (830.272 mg), Cholesterol (191.219 mg) and Phospholipid (900.00 mg) with desirability of 0.966 were found to fulfill the requisites of an optimized combination proniosomal Gel (OCPG) formulation. The morphology of the optimized formulation was observed by scanning electron microscopy. The picture demonstrated that the drug entangled in the

Fig. 6. SEM image of OCPG.

lipid network. They show well recognized round vesicles with uniform size, showing fixed nano-vesicular structure (Fig. 6). The OCPG formulation gives the experimentally observed values of vesicle size of 562 ± 2.13 nm with the entrapment efficiency of GLB is $97.037 \pm 1.43\%$ and entrapment efficiency of ATN is $96.230 \pm 1.62\%$. These investigative estimations of vesicles size, entrapment efficiency of GLB and entrapment efficiency of ATN yielded by the OCPG were found in agreement with the predicted value of vesicles size (562 ± 1.223 nm), entrapment efficiency of GLB ($97.13 \pm 1.47\%$) and entrapment efficiency of ATN ($97.42 \pm 1.85\%$) respectively created by design expert software, recommending that the optimized formulation was rational and reliable.

3.6. Evaluation of OCPG

The OCPG formulation was the smooth surface with uniform and consistent appearance. As per Ganeshpurkar et al. [58] the pH value of OCPG which was found at 7.2 ± 0.1 and it is viewed as satisfactory with no danger of aggravation on application to the skin. Formulated OCPG was

observed clear, free from particles and demonstrated great homogeneity with nonappearance of irregularities.

3.6.1. Vesicle size and particle size distribution

The results showed that OCPG dispersion effectively decreased the particle size with narrow size distribution. The average particle size of the OCPG was found to be 564 ± 2.13 nm. Vesicles size distribution of optimized formulation is presented in Fig. 7.

3.6.2. Zeta potential

Zeta potential is the measurement of electric charge present on the surface of the particles. Zeta potential of finally OCPG formulation was found to be -30.8 ± 1.13 mV (Fig. 7), the value of zeta-potential in a range of higher than ± 30 mV electrostatic charge indicates long term stability.

3.6.3. In vitro drug permeation studies

In drug release kinetics, the model revealing the highest value of R^2 (correlation coefficient) was inferred as the best model for drug release kinetics. The drug release from OCPG through rabbit skin was fitted into different mathematical models such as zero-order release kinetics, Higuchi's square root of time equation [59] and Korsmeyer Peppas'-power law equation [60,61]. The highest value of the correlation coefficient ($R^2 = 0.990$ for GLB and 0.996 for ATN) was observed in the Higuchi matrix model, followed by the zero order ($R^2 = 0.980$ for GLB and 0.985 for ATN) and Korsmeyer–Peppas ($R^2 = 0.945$ for GLB and 0.910 for ATN), models. In the greater part of the transdermal system, drug diffusion from the dosage form pursues the diffusion pattern shown in Fig. 8 [62]. Same release kinetics for proniosomes formulation was accounted for by previous researchers [29,52,63,64,65].

Fig. 7. Vesicles size distribution and Zeta potential of OCPG.

Fig. 8. In vitro and ex vivo drug permeation plot for OCPG.

Fig. 9. Plasma Concentration-time curve for OCPG.

3.6.4. Skin permeation studies

The release rate of OCPG over the egg layer and excised skin varies essentially, which demonstrates about the hindrance properties of skin which was shown in Fig. 8. The Combination of niosomes to the skin surface brought about a higher transition because of the immediate exchange of drug from the vesicles [66]. It is obvious that OCPG formulation demonstrated the greatest flux over the rabbit skin ($128.609 \pm 2.24 \mu\text{g}/\text{cm}^2/\text{h}$ and $322.054 \pm 1.53 \mu\text{g}/\text{cm}^2/\text{h}$) of GLB and ATN respectively.

Our findings were comparable as given by Imam et al. [53] where proniosomes containing Span indicated higher flux than with the other, which could be because of their less lipophilicity which drove them [67].

3.6.5. In vivo drug absorption studies

The peak area of GLB and ATN in plasma was linear with respect to the analyte concentration over the range 14–560 ng/ml and 10.5–420 ng/ml. Validated analytical method was found satisfactory and proved to be adequate for the determination of both drugs in plasma. The *In vivo* drug release studies were found in Fig. 9. The corresponding pharmacokinetic parameters of rabbit plasma were summarized in Table 4.

3.6.6. Pharmacokinetic analysis

The *in-vivo* study was performed on rabbits ($n = 3$) and the pharmacokinetic parameters for two API, marketed formulation and transdermal proniosomes (OCPG) were determined and listed in Table 4. The transdermal proniosomes exhibited the maximum plasma drug concentration (C_{max}) of $141.76 \pm 13.15 \text{ ng/ml}$ for GLB and $137.64 \pm 11.06 \text{ ng/ml}$ for ATN.

Table 4. Pharmacokinetic parameters of ATN and GLB in rabbits.

S.No	Pharmaco-kinetic Parameters	Control Formulation		Marketed Formulation		OCPG Formulation	
		GLB	ATN	GLB	ATN	GLB	ATN
1	T_{max} (h)	1 ± 0.16	1 ± 0.12	4 ± 0.53	4 ± 0.08	12.00 ± 0.23	12.00 ± 0.06
2	C_{max} (ng/ml)	273.45 ± 10.52	250.54 ± 12.16	241.58 ± 11.52	187.62 ± 12.14	141.76 ± 13.15	137.64 ± 11.06
3	AUC_{0-t} (ng h/ml)	1309.46 ± 1.13	1099.34 ± 1.18	2126.88 ± 2.41	1522.6 ± 2.68	2750.21 ± 3.15	2658.91 ± 1.41
4	$\text{AUC}_{0-\infty}$ (ng h/ml)	1408.25 ± 1.46	1152.59 ± 2.75	2397.83 ± 1.74	1676.28 ± 4.14	3382.17 ± 2.42	3222.55 ± 1.15
5	K_{el} (h^{-1})	0.098 ± 0.03	0.112 ± 0.006	0.0809 ± 0.02	0.1049 ± 0.003	0.099 ± 0.03	0.104 ± 0.003
6	$t_{1/2}$	7.05	6.18	8.562 ± 0.07	6.6025 ± 0.04	6.94107 ± 0.01	6.61 ± 0.23
7	MRT	8.04977	6.67152	11.347	10.7506	16.056	15.695
8	F%	-	-	-	-	129.30	174.62

ml for ATN ($p < 0.0001$) whereas two API's oral solution of GLB and ATN have C_{max} of 273.45 ± 10.52 ng/ml and 250.54 ± 12.16 ng/ml and the marketed oral solution of GLB and ATN have C_{max} of 241.58 ± 11.52 ng/ml and 187.62 ± 12.14 ng/ml respectively. The C_{max} of OCPG was lower (141.76 ± 13.15 ng/ml for GLB and 137.64 ± 11.06 ng/ml for ATN) than the oral formulation significantly lower than oral formulation (241.58 ± 11.52 ng/ml and 187.62 ± 12.14 ng/ml respectively) which can be allotted because of the control release in test formulation as against the variable assimilation (peak and valley design) which is incommensurable characteristic for oral marketed formulation which was mentioned in earlier findings for GLB in Mohammed Aslam et al. [68] and for ATN in Jun-Shik Choi et al. [69]. The low C_{max} and delayed T_{max} seen with the transdermal formulation as opposed to the oral can be ascribed to the effective boundary properties of the skin which was seen in past findings of M. Aqil et al. [70,71]. The area under the curve (AUC) represents the extent of drug absorption (bioavailability) from its dosage form. It was observed that Significantly high ($p < 0.0001$) $AUC_{(0-24)}$ and $AUC_{(0-\infty)}$ for proniosomes for GLB and ATN was 2750.21 ± 3.15 ng h/ml; 3382.17 ± 2.42 ng h/ml and 2658.91 ± 1.41 ng h/ml; 3222.55 ± 1.15 ng h/ml whereas this value for two API's is 1309.46 ± 1.13 ng h/ml; 1408.25 ± 1.46 ng h/ml and 1099.34 ± 1.18 ng h/ml; 1152.59 ± 2.75 for marketed preparation it is 2126.88 ± 2.41 ng h/ml; 2397.83 ± 1.74 ng h/ml and 1522.6 ± 2.68 ng h/ml; 1676.28 ± 4.14 ng h/ml. However, the AUC values were altogether higher due to the avoidance of hepatic metabolism by transdermal administration providing high systemic drug availability which is indicated by increased bioavailability of the drug from transdermal route as compared to oral administration. The $AUC_{0 \rightarrow \infty}$ of transdermal administration was 1.29-fold higher than that of oral administration alone. The relative bioavailability of proniosomes of GLB and ATN was found to be 126.85 and 174.30 times compared to marketed oral preparation which indicated that OCPG transdermal preparation can also be used as an alternative to oral route.

Time taken to achieve the peak plasma drug concentration (T_{max}) was fundamentally delayed to 12h in both instances of proniosomes which was 1h and 4 h in case of two API's and marketed preparation, which obviously demonstrated the boundary properties of the skin. These values were significantly higher as compared to control and marketed oral suspension ($p < 0.001$). Our results were in agreement to Sharda Sambhakar et al. [72] who

Table 5. Stability studies of formulation OCPG.

S. No.	Temperature	% Drug Content				Vesicle size and Shape			
		0	1	2	3	0	1	2	3
1.	At refrigerator	98.33 ± 0.3	98.23 ± 0.2	98.01 ± 0.2	97.98 ± 0.5	580 ± 15.4 nm and Round	580 ±12.76 nm and Round	582 ± 16.98 nm and Round	585 ± 15.87 nm and Round
2.	At Room temperature	98.65 ± 0.3	98.43 ± 0.3	98.31 ± 0.3	98.12 ± 0.5	560 ± 17.8 nm and Round	560 ± 15.87 nm and Round	560 ±15.87 nm and Round	564 ± 12.98 nm and Round

moreover reported the expansion in $t_{\max}$ when the proniosomes were given through transdermal route in rats. No huge changes in $t_{1/2}$ were seen with the proniosomal formulation with that of the API and marketed formulation [73,74].

3.7. Stability studies

The influence of elevated temperature and humidity conditions on the proniosomes gel formulation was performed in adherence to the ICH guidelines. There was no significant reduction in the drug content value of the OCPG formulation before and after three months of storage (Table 5). The proniosomal gel was also examined microscopically which showed round shaped vesicles. There was not much difference in the clarity which is clear and the size of the vesicles before and after storage. Therefore, the proniosomal gel formulations can be stored either at refrigeration or at room temperature [75,76].

4. Conclusion

This study presents here reports of fruitful design and evaluation of a combined proniosomal gel for transdermal delivery for treating concurrent disease especially diabetes with hypertension. We conveyed the widely used antidiabetic and antihypertensive since adherence with these medications is quite poor, and the utilization of this combination is to manage long-acting medicine, which releases drugs at the same time, simplifies complex therapeutic regimen, and to improve acceptability and adherence. This kind of system may give an alternative method of conveyance for patients who are taking multiple medications, following complex treatment regimens in concurrent diseases like diabetes with hypertension. If clinical benefits can be shown, then possibly health care providers would be willing to pay attention slightly more than they currently do for single oral medicines in such concurrent diseases. Combinational Proniosomal gel represents a promising technology that could be used for transdermal delivery of many other established drugs which are used for treating these types of concurrent diseases.

Consent for publication

Not applicable

Competing interests

The authors declare no conflicts of interest.

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