

Response Surface Optimization and *In-vitro* Evaluation of Sustained Release Topical Insulin Liposomal Spray for Wound Healing

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ABSTRACT

Chronic wounds are considered a major health care concern, that represents a life-threatening problem worldwide. Insulin has proven its great efficiency as a wound healing agent, especially with diabetic ulcers. However; insulin suffers degradation at the application site due to proteases, moreover; when wounds are painful the patient fails to apply any remedy frequently. In the present study, insulin has been formulated as a spray in liposomes, which protects it at the wound area and sustains its release and thus reducing the application frequency, furthermore; the spray reducing the direct contact of the applicator with the skin, thus, reducing the probability of infection. The full-factorial design has been applied in the preparation optimization of liposomes, where the effects of the cholesterol, method of preparation and sonication have been tested on the particle size and the entrapment efficiency. The present study shows how the thin film hydration method in the absence of cholesterol and sonication were the best conditions for insulin liposomal formulation, that satisfies the target of the study. Liposomes showed a sustained release of insulin up to 24 hours and were successfully formulated into a spray dosage form. In conclusion, topical insulin liposomal spray offers a protective method from insulin degradation with an expected increase in the patient compliance.

INTRODUCTION

Wounds are considered a major problem which may cost high and remain ineffectively treated, leading to the reduction in the quality of life of the patient (Mishra, 2014). Thus, the approach to close skin lesions with ideal results would be the goal of many clinical treatments (Mishra, 2014). Numerous therapies have developed in the recent years, resulting in new product introductions, as wound healing agents (Mishra, 2014).

Healing of diabetic ulcers has been a worldwide concern as there was a great difference in healing rates between diabetic wounds and non-diabetic wounds at the early of the 20th century (Gurd, 1937). In diabetics, infections aroused as wounds couldn't re-epithelialize normally thus, open wounds are exposed to the environment. Unfortunately; death would follow as the infection

spread to the blood (Falanga, 2005).

Many studies proved the effectiveness of using insulin in tissue regeneration, as insulin had a great role in regulating tissue repair either in wound healing (Thalhimer, 1923; Joseph, 1930; Hrynyk, 2013), or bone fracture (Stuck, 1932; Joslin, 1933). It was proven that insulin had a great role in nitrogen retention and the uptake of amino acids, synthesis of proteins and inhibition of protein catabolism, where it could be a potent growth factor in the absence of pituitary signaling molecules, and thus, in tissue repair (Lawrence *et al.*, 1954). Moreover; it may promote glucose utilization in tissues (Paul, 1966). Belfield *et al.* (1970) reported that "Insulin may help to normalize permeability, increase vascularization, reduce exudation, arrest bacterial growth, enhance phagocytosis, stimulate proliferation, decrease local tissue hypoxia, eliminate edema and increase wound contracture".

Thus, insulin delivery is considered as a good option in wound healing, as long as formulation and delivery are improving and developing. Furthermore; it might be very beneficial to the diabetic ulcer as in these ulcers, mostly there is a lack of the glycemic control which deteriorates the wound greatly thus; a

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great need to growth factors is required as healing impairment is caused by several factors, which may be intrinsic as, neuropathy, vascular insufficiency, etc. or extrinsic as, wound infection, callus formation, etc. (Falanga, 2005).

However; insulin suffers major limitations in its formulation, being a protein in nature due to its instability (Brange and Langkjoer, 1993), either shelf-life stability or its stability when inserted to the wound area due to the presence of proteases at the wound site (Lau and Kim, 2016). In an attempt to solve this problem; insulin may be encapsulated in a vesicular structure that not only protects the insulin from the external environment but also may sustain its release (Mourtas *et al.*, 2007). Vesicular systems as liposomes, offer number of advantages in drug delivery through the skin such as being biocompatible, non-toxic, and having the capability of incorporating hydrophilic and lipophilic drugs, controlling the rate and extent of drug delivery, in addition to acting as a depot for controlled release of drug (Shilakari *et al.*, 2013).

Many approaches have been settled in the recently, which allow growth factors (GF) to be delivered in an appropriate wound environment to improve healing (Gainza *et al.*, 2015). Natural agents as chitosan and hyaluronic acid (HA) or synthetic agents as Poly Ethylene Glycol (PEG) and Poly Lactic Acid (PLA), which are used alone or in combination to offer novel delivery systems for GFs (Gainza *et al.*, 2015). They have many advantages as being biodegradable, with low toxicity making them perfect candidates for wound healing therapy. These novel approaches to improve the stability of GF at the wound site, permitting their prolonged release and thus, lower doses and lower frequencies of administration are needed, therefore, improving treatment safety; and optimize the treatment effectiveness (Gainza *et al.*, 2015).

Sprays are considered patient-friendly, discrete and convenient, dosage form via topical administration, it offers many advantages over other topical preparations as being non-messy with little or nearly no skin irritation (Chavan *et al.*, 2016).

The aim of the present study is the formulation of certain delivery systems, such as insulin-loaded vesicular structures in optimized liposomal topical sprays may be a promising challenge to ensure controlled safe delivery into wound areas and ensures the high patient compliance, especially with diabetic ulcers.

MATERIALS AND METHODS

Materials

Insulin crystals were supplied as a gift from Sedico Company (Cairo, Egypt), Cholesterol was obtained from Bio Basic (Canada Inc.), L(α) Egg Phosphatidyl Choline (type IV-s) and Medium molecular weight Chitosan were purchased from Sigma Aldrich (St. Louis, MO, USA). All other chemicals and solvents were of pharmaceutical grade purchased from ADWIC Company.

Preparation of insulin-loaded liposomes

Dry thin film evaporation method (TFH)

Liposomes were prepared by dry thin film hydration protocol (Hülsermann and Hoffmann, 2009). Egg phosphatidylcholine together with cholesterol (3:1 molar ratio) were dissolved in a

mixture of organic solvent (2:1 Chloroform:Methanol) which was removed using rotary evaporation, under vacuum at 60°C (Heidolph 2, Schwabach, Germany). The formed film was then hydrated with 10 ml insulin solution in phosphate buffer saline (PBS) at pH 6.8. Some preparations were then sonicated, using bath type sonicator (Jiotech UC-10, Serangoon, Singapore). The preparations were left overnight and refrigerated for maturation of the vesicles.

Reverse phase evaporation method (REV)

Egg phosphatidylcholine together with cholesterol (3:1 molar ratio) were dissolved in diethyl ether, and then mixed with 4 ml of insulin solution in phosphate buffer saline (PBS) at pH 6.8 where, the ratio of the organic phase: aqueous phase was 3:1, v/v in a bath type sonicator for 5 minutes, to obtain a W/O emulsion. A gel was formed directly after organic solvent removal by rotary evaporator under reduced pressure. The gel evolved into a liposomal dispersion when the dispersion was mechanically agitated using vortex mixer. Some preparations were then sonicated. Finally, the preparations were sealed in glass containers and further, stored in darkness at 4°C (Maestrelli *et al.*, 2006).

In order to avoid false results; a negative control was run. Empty liposomes were additionally analyzed. Empty liposomes were exactly prepared without the addition of insulin.

Experimental design

To study the effect of formulation variables on physicochemical properties of insulin-loaded liposomes, a 2³ full-factorial design was applied using Design-Expert 10.0.1.0 software (Stat-Ease Inc., USA), where the full-factorial design enabled all the formulation variables to be varied simultaneously, effects produced may be quantified by these factors along with any possible interaction between them. The full factorial designs including investigated factors and responses were shown in Table 1. The effects of the cholesterol (X₁), the method of preparation (X₂) and application of sonication after preparation (X₃) each at two levels were all tested on the particle size (Y₁) and the entrapment efficiency (Y₂).

Physicochemical characterization of insulin-loaded liposomes

The insulin-loaded liposomes obtained from the 2³ full-factorial design were characterized in terms of their physicochemical properties to understand the effect of the studied factors on these physicochemical properties.

Determination of the particle size

Particle size measurement was performed using Zetasizer (Nano-ZS90, Malvern, Worcestershire, UK). The particle size of liposomal preparations was done after dilution with double distilled water. One ml of diluted dispersion was filled into polystyrene cuvettes at 25°C. All measurements were performed in triplicate and reported as “mean \pm SD width”.

Determination of insulin entrapment efficiency

The entrapment efficiency (EE%) of insulin in liposomes was detected by centrifugation of one ml of the dispersion at 20,160 g at 4°C for 60 minutes in a Cool centrifuge, (Megafuge 16R, Hanau, Germany), followed by washing the precipitate twice with PBS pH 6.8 so as to separate the entrapped drug from the free

one (Xu *et al.*, 2011). The free insulin concentration (C_f) in the resulting supernatant together with the resulting washing solution was assayed spectrophotometrically at 204 nm after filtration and suitable dilution. The EE% of the drug was calculated as follows:

$$EE\% = [(C_t - C_f)/C_t] \times 100, \quad \text{Equation 1}$$

where C_t is the total insulin concentration added in the liposomes preparation, and C_f is the untrapped insulin concentration (Aboelwafa *et al.*, 2010).

Morphology of the prepared liposomes

The structure of the formed optimized liposomal dispersion was determined using optical microscope at a magnification power of 400x (Leica Imaging Systems, Cambridge, UK) with a digital camera (JVC, Victor Co, Yokohama, Japan). A thin layer liposomal formulation was spread on a slide and examined after placing the coverslip. The average size of at least 100 particles was measured.

In-vitro release study

In-vitro release study was performed on the insulin dispersion and compared with the optimized insulin-loaded liposomal dispersion. Vertical type Franz diffusion cells (Hanson research, Los Angeles, California United States of America) was used for the *in-vitro* release studies with a diffusion area of 1 cm². Phosphate buffer saline (7 ml) with pH 6.8 was placed in the receptor compartment which was maintained at 37°C ± 0.5 and stirred for 2 minutes at 1000 rpm to remove air bubbles, which was then adjusted at 500 rpm for the rest of the experiment period. Cellophane membrane (Spectrum Medical Inc., Los Angeles, CA, USA cut-off 12,000–14,000) was used to separate the donor compartment from the receptor compartment which was soaked previously in the receptor medium overnight.

Four hundred µl samples were withdrawn at (0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 12 and 24 h), from the sampling port; which was replaced with fresh solvent to maintain a constant volume. Drug release was studied in triplicate at least, for each formula and the cumulative amount of drug released was determined.

The cumulative amount of drug released per unit area (µg/cm²) was plotted as a function of time (h) for each formula and for the insulin spray without liposomes. The slope of the linear portion of the graph was used to calculate the rate of drug release (Jain *et al.*, 2003; Gillet *et al.*, 2009; Aboelwafa *et al.*, 2010).

Data analysis

Generation and statistical analysis of the full factorial design were performed using Design-Expert 10.0.1.0 software (Stat-Ease Inc., USA). Responses were analyzed using ANOVA study, where the regression equations were analyzed. Statistically, linear, two factor interaction, quadratic or cubic models were calculated and the best fitting experimental model was chosen on the basis of comparison of several statistical parameters like multiple correlation coefficients (R^2), coefficient of variation (CV), predicted residual sum of square, adjusted multiple correlation coefficient (adjusted R^2), and graphically by 3D response surface plot provided by the program. A level of significance of p -value < 0.05 was considered. The linear regression plots (predicted versus observed value) and normal probability curves of responses were plotted.

Data optimization

The effect of each factor on the studied responses was analyzed for the establishment of design space. Data optimization was done by attaining largest particle size and highest EE%, to prevent it from being absorbed to the systemic circulation and enclosing highest amount of insulin, i.e., highest EE%. The desirability was the criteria used in the optimization of the full factorial design. Furthermore; the % bias between the observed and the expected results were calculated.

Preparation of insulin liposomal spray

Preparing 1.5% w/v chitosan in 1% glacial acetic acid in deionized water and glycerol, with constant stirring on a magnetic stirrer, where it was subjected for two cycles of sonication to expel out the entrapped air bubbles from the prepared spray. (Sezer *et al.*, 2007). The insulin liposomal spray was prepared by adding liposomal dispersion to the prepared chitosan solution and trituration to form the topical spray preparation.

Characterization of insulin-loaded liposomal spray

Assessment of spray pattern and spray angle

To examine the effects of formulation changes and pump design on spray-pattern behavior, spray-pattern studies were conducted (Dayal *et al.*, 2004).

The spray formula was mixed uniformly with Methylene blue, which was sprayed onto a Whatman filter paper. The distance separating the container from the target was kept constant, at 7 cm. Evaluation of the spray was based on the homogeneity of the spray, spherical shape and presence of stray droplets. Spray angle (θ) was calculated from the following equation:

$$\tan \theta = D/R, \quad \text{Equation 2}$$

where D is the distance from the spray was done and R is the radius of the circle (Ranade *et al.*, 2017).

The viscosity of insulin-loaded liposomal spray

The viscosity of the spray was measured using Brookfield viscometer at 25 ± 1°C. Viscosity was an important indicator to give the balance between the sprayability and the optimum viscosity for the spray to remain on the skin surface without falling and interact with it till drying (Ranade *et al.*, 2017).

pH of insulin-loaded liposomal spray

pH of the formulation was measured by digital pH meter (Jenway 3510, USA) (Mori *et al.*, 2017).

Determination of volume of preparation delivered upon each actuation

The volume of solution delivered from each actuation is calculated using Equation (2)

$$A_L = (W_t - W_o)/D_n, \quad \text{Equation 3}$$

where A_L is the solution volume delivered upon each actuation, W_t is the formulation weight after actuation, W_o is the initial formulation weight before actuation, and D_n is the density of the formulation. An average weight of five actuations was calculated (Mori *et al.*, 2017).

Table 1: Studied factors with their levels and layout of 2³ Full factorial design.

Formula code	Factors			Level 1	Level 2
	X ₁ (Cholesterol)	X ₂ (Method of Preparation)	X ₃ (Sonication)	Absent (A)	Present (P)
				Thin Film Hydration (TFH)	Reverse Phase Evaporation (REV)
			Absent (A)	Present (P)	
			Responses		
			Y ₁ ± S.D. (µm)	Y ₂ ± S.D. (%EE)	
F ₁	P	TFH	P	0.66 ± 0.24	56.95 ± 0.46
F ₂	A	TFH	A	1.02 ± 0.96	73.25 ± 0.78
F ₃	A	REV	A	2.21 ± 0.94	52.32 ± 0.09
F ₄	A	TFH	P	1.48 ± 1.27	59.92 ± 0.88
F ₅	P	REV	A	1.05 ± 2.65	58.38 ± 1.5
F ₆	P	REV	P	1.47 ± 0.99	37.87 ± 1.78
F ₇	P	TFH	A	0.67 ± 1.88	83.46 ± 0.94
F ₈	A	REV	P	2.88 ± 0.70	36.89 ± 0.24

The tackiness of the film after solvent evaporation

The current study was conducted on healthy volunteers at the age between 20 and 40 years, where a written informed consent was obtained from all participants in the study. The volunteers were conducted through the study at Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA) University. This work was approved by the research ethics committees of Cairo University and MSA University and it complied with the Helsinki Declaration Principles.

A placebo formulation was actuated on the forearm of three volunteers and allowed to dry for 60 seconds. To assess the tackiness of the film, a piece of cotton was lightly pressed over the films and the cotton fibers sticking to the film were visually inspected (Lu *et al.*, 2013).

Ex-vivo physical evaluation of the film after solvent evaporation

A placebo formulation was actuated on the left-hand palms of 3 healthy human volunteers, four times every 10 seconds from a distance of 15 cm. The time that was required for film formation, flexibility and appearance of the film, irritation potential, feeling of warmth, cooling sensation and water washability were all recorded. The appearance of the film was graded as dull and opaque (+++), or shiny and translucent (++) or shiny and transparent (+). Dermal adhesion, flexibility and water washability of the film were graded as good (+++), moderate (++) or poor (+).

The dermal adhesion of the film was tested by rotating the palms of each volunteer 10 min in anti-clockwise direction, where the palms were occasionally opened and closed of palm, after 8 min of actuation of the spray. During the period study; which was 720 min, the film nature was carefully assessed for any fracture, separation or removal. Moreover; the water washability of the film was checked (Gohel and Nagori, 2009).

Occlusion potential of the film formed

Fifty ml water was added to a beaker and covered with a Whatman paper filter. The spray was actuated over the filter paper. A film was formed over the filter paper. This test was carried out in triplicate to reduce the errors. The control beakers were 50

ml water only. All beakers were stored at room temperature and humidity for 48 h. For measuring the water evaporation through the membrane all the beakers were weighed before and after 48 h. The F value i.e. the occlusion factor was calculated using the following equation:

$$F = 100 \times [(A - B)/A], \quad \text{Equation 4}$$

where F is the occlusivity factor, A is the loss of water from the control beakers and B is the loss of water from the beakers with the film (Gohel and Nagori, 2009).

If the sample is identical to the control, F will be equal to 0, while the maximum occlusion factor is 100 (Wissing and Müller, 2001).

RESULTS AND DISCUSSION

Physicochemical results of insulin-loaded liposomes

Particle size analysis

The particle size of insulin-loaded liposomes was found to be from 0.66 to 2.88 µm as represented in Table 1. The full statistical data for the particle size was represented in Table 2, which shows that the particle size was significantly affected by all the studied factors. Table 3 shows the regression equation for the particle size, which shows that the particle size of insulin-loaded liposomes increased in the absence of cholesterol, which may be due to the increase in the packing density liposomes in the presence of cholesterol, where cholesterol increases the width of the phospholipid bilayers as well as overall size of the vesicles (Kaddah *et al.*, 2018). Whereas; thin film hydration method resulted in a larger particle size which may also be due to the formation of multilamellar vesicles, that may enclose more insulin and thus resulting in a larger particle size (Patil and Jadhav, 2014). Furthermore; application of sonication resulted in a smaller particle size which may be due to cavitation (bubble formation), as ultrasound mechanical waves generate cavitation bubbles in liquids. Bubbles whose size is near the resonant size for the applied frequency begins to oscillate nonlinearly and eventually collapse. As a result of such collapse, a violent implosion occurs

that produces extremely high temperatures, high pressures, and shock waves leading to reducing the size of the liposomal structure

(Essa, 2010).

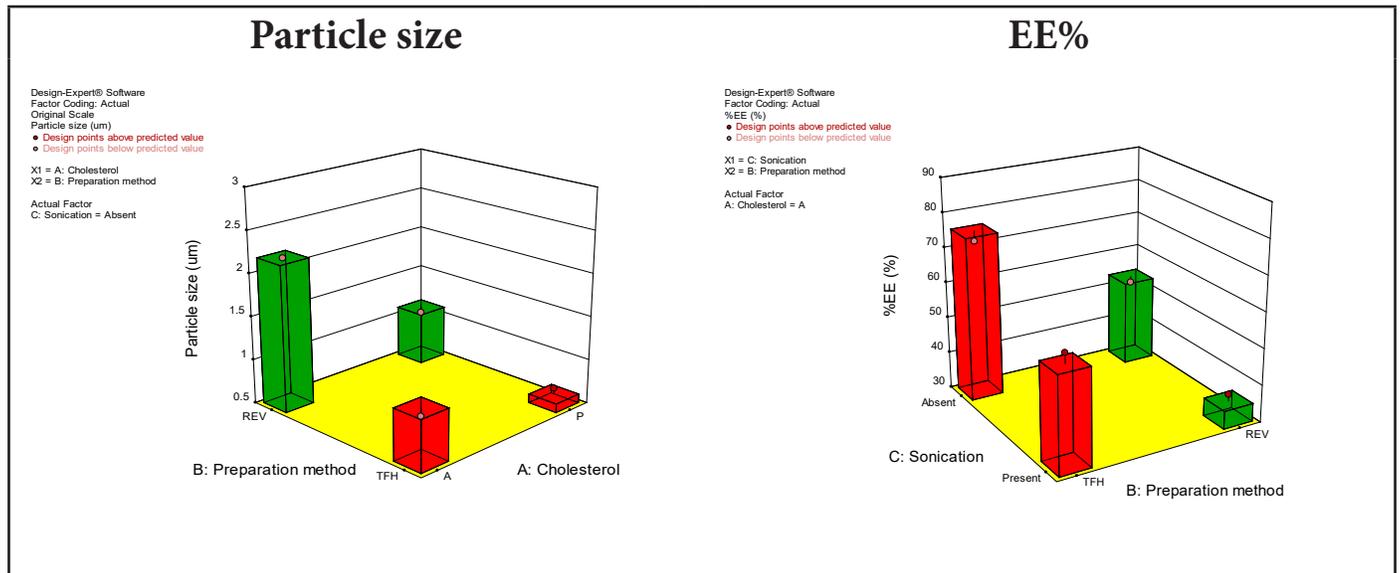


Fig. 1: Response surface plots for the studied responses.

As shown from Figure 1, the response surface plot of particle size of insulin-loaded liposomes increased when cholesterol was present and prepared by thin film hydration in the absence of sonication.

Entrapment Efficiency analysis

The entrapment efficiency (EE%) of insulin in liposomes was found to be from 36.89 to 83.46% as represented in Table 1. The full statistical data for the EE% was represented in Table 2, which shows that the EE% was significantly affected by the method of preparation and the presence of sonication. Table 3 shows the regression equation for the EE%, which shows that the EE% of insulin increased when thin film hydration was the method of preparation, which may be due to the formation of multilamellar vesicles, that may enclose more insulin (Patil and Jadhav, 2014). Moreover; application of sonication resulted in a significant decrease in the EE%, which may be due to the leakage of the entrapped drug by the effect of sonication (Ahad *et al.*, 2012).

Figure 1 illustrates the response surface plot, which shows how the EE% of insulin increased when prepared by thin film hydration in the absence of cholesterol and sonication.

Data analysis

Statistical analysis was represented in Tables 2 and 3. A level of significance of p -value < 0.05 was considered significant. The “Pred R-Squared” was in reasonable agreement with the “Adj R-Squared”; i.e. the difference is less than 0.2. Adequate precision indicates an adequate signal; thus, this model can be used to navigate the design space.

Data optimization

One optimized batch (O_1) was selected based on the desirability criteria, with desirability of 0.487, which was prepared

and characterized.

The composition of the prepared formula was outlined in Table 4, along with the predicted and observed responses. The optimized batch (O_1) was prepared without cholesterol, by thin film hydration method using a solvent mixture of chloroform and methanol in ratio 2:1. The particle size was found to be 1.267 μm and the EE% was 73.435%. The model prediction was in good agreement with the experimental observation thus, the validity of the model was established.

Morphology of the formed liposomes

A homogeneous, well-identified regular, spherical shape, with internal aqueous space, was observed from the photomicrograph of the optimized formula as illustrated in Figure 2.

In-vitro release analysis

A biphasic profile release was observed from the *in vitro* release of the drug from the formulae. The reason for this could be due to the presence of free and the entrapped drug together due to the limited capacity of the lipid to entrap large amounts of insulin resulting in the disposition of the free insulin at the surface (Jain *et al.*, 2003). As a result to this, an initial rapid release of the drug takes place due to the presence of the free insulin and the insulin present in the aqueous core, followed by a slower sustained release phase as the entrapped insulin diffuses through the lipid bilayers of the liposomes, which was found to be very effective in sustaining and controlling the release of insulin.

The release rate of the optimized insulin-loaded liposomes was compared with the insulin dispersion without liposomes as represented in Figure 3, showing how the release rate was sustained up to 24 h with the liposomal formula, while the insulin dispersion, released the insulin after 6 hours only.

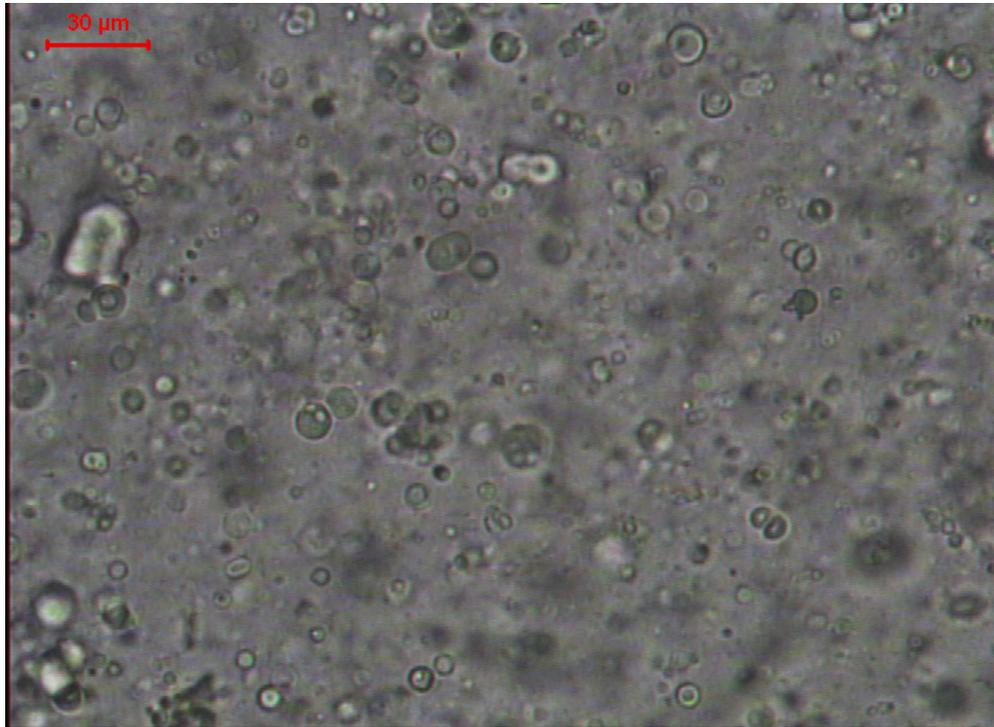


Fig. 2: Photomicrograph of the optimized formula.

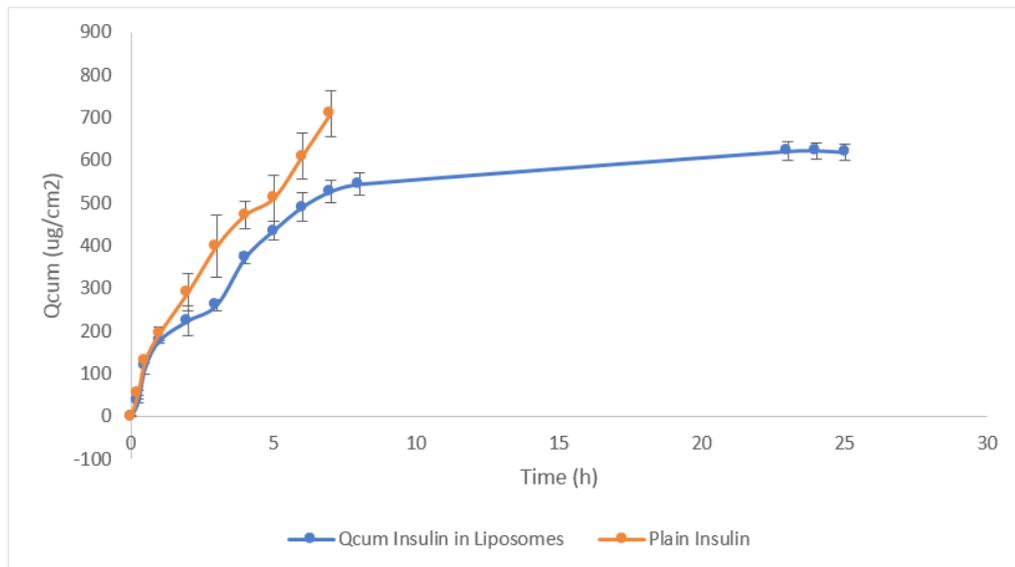


Fig. 3: Cumulative amount of insulin released from insulin dispersion and the optimized Insulin-loaded liposomes.

Characterization of insulin-loaded liposomal spray

Assessment of spray pattern and spray angle

The spray obtained was observed to be clear and uniform. The formulation showed good spray characteristics, clear film after application. Stray droplets were formed on the Whatman filter paper. The spray angle was $77.02^\circ \pm 0.346$. The acceptable spray angle of less than 85° was accepted for easy actuation of the solution from the container which may cover the maximum area

(Gohel and Nagori, 2009).

The viscosity of insulin liposomal spray

The viscosity of the spray dispersion range was found to be from 31 to 34 cps, which gave an optimum balance between the sprayability and the viscosity required for the spray to retain on the skin till drying (Mori *et al.*, 2017). It was found that increasing the viscosity may lead to a reduction in the spray pattern (Guo *et al.*, 2008).

Table 2: ANOVA statistical analysis for the studied responses according to the factorial design.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.075	3	0.025	47.84	0.0014	significant
A-Cholesterol	0.034	1	0.034	65.40	0.0013	
B-Preparation method	0.036	1	0.036	69.72	0.0011	
C-Sonication	4.361E-003	1	4.361E-003	8.40	0.0442	
Residual	2.078E-003	4	5.195E-004			
Cor Total	0.077	7				
			Particle Size			
Model	1714.27	3	571.42	44.08	0.0016	significant
A-Cholesterol	25.54	1	25.54	1.97	0.2331	
B-Preparation method	970.62	1	970.62	74.88	0.0010	
C-Sonication	718.11	1	718.11	55.40	0.0017	
Residual	51.85	4	12.96			
Cor Total	1766.12	7				
			EE%			

pH of insulin liposomal spray

pH of the formulation was found to be 4.9 ± 0.1 , which is considered as an excellent pH for the topical formulations as a pH above the isoelectric point (pI~4) of skin could be more appropriate for topical delivery (Nair *et al.*, 2013).

Determination of volume of preparation delivered from each actuation

The volume the formulation per actuation was found to be 158 ± 1 l; which shows a very narrow variation in the volume actuated.

The tackiness of the films formed after evaporation of the solvent

No cotton fibers stuck to the film, giving an indication to the spray to be not tacky or sticky. Thus, the validity of the topical film-forming spray formulation was performed.

Ex-vivo physical evaluation

The time required for film formation was found to be 30 seconds, which is acceptable for the *in-vivo* application. The film was found to be shiny and translucent (++). Dermal adhesion, water washability, and flexibility of the film were graded as moderate (++). The film formed was found to be free from any fractures, not easily removed from the hand by movement and easily washed by water. The film was found to be non-irritating with a slight feeling of cooling sensation.

Occlusion potential of film-forming spray

The occlusive property is considered to be very important to improve skin hydration and protection from infection from the external surroundings. At the same time, it can also cause irritation and bacterial growth below the patch or film surface. Thus, it is required to obtain a partially occlusive to non-occlusive film.

Table 3: Calculated R² values and regression equation models for the measured variables.

Response	Model	R ²	Adj. R ²	Pred. R ²	Adeq. precision	Reduced regression equation for the response
Particle size	Linear	0.9729	0.9525	0.8915	19.334	$Y_1 = +0.96 + 0.065 \times A - 0.067 \times B - 0.023 \times C$
EE%	Linear	0.9706	0.9486	0.8826	17.500	$Y_2 = +57.38 + 1.79 \times A - 11.01 \times B - 9.47 \times C$

The occlusion potential, F value, of the spray formulation was shown to be 26.7196, which is considered to be a small value indicating a partially occlusive formulation (Ranade *et al.*, 2017). With this value, the required target could be achieved.

CONCLUSION

Formulation of optimized insulin-loaded liposomes has been investigated in this study, using a full factorial design, with the aim of protecting the insulin at the application site and extending its release. It could be concluded that the best conditions for preparation of liposomal insulin were thin film hydration without cholesterol and sonication. The optimized formula successfully sustained the insulin release from 6 hours to up to 24 hours, thus the frequency of application could be reduced. Moreover, the spray with the required properties was formulated which reduces

the probability of messy contact, by avoiding rubbing of the wound which increases the patient compliance.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

Table 4: The predicted, observed and residual values of the two optimized formula O₁.

	Response	Predicted	Observed	% Biased*
O ₁	Particle size (μm)	1.095	1.267	-13.575
X ₁ = Absent				
X ₂ = TFH*				
X ₃ = Absent	EE (%)	76.084	73.435	3.607

*% Biased = (Predicted-Observed)/Observed.

*TFH: Thin Film Hydration Method.

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