Original Article

Development Optimization and Cytotoxicity Evaluation of Glyburide Loaded Nanostructured Lipid Carriers

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ABSTRACT

Aim/Background: In the present study Glyburide, a hypoglycaemic agent was loaded into nanostructured lipid carriers. The solid and liquid lipid concentrations within the formulation were optimized using central composite design with two centre points augmented with a cluster of axial and factorial points (star points). **Materials and Methods:** The responses particle size and drug entrapment were used to optimize the concentration ratio by central composite design. The nanostructured lipid carriers were prepared by melt dispersion technique and the optimized formulations were evaluated for various physicochemical, morphological, histological and toxicity parameters. **Results:** Central composite design paradigm was successful in defining the best lipid concentrations. Scanning electron microscopy and atomic force microscopy revealed the morphological characteristics of the nanoparticle. The outcome of X- ray diffraction confirmed the molecular dispersibility of the drug in the lipid matrix. Histopathological study conceded the scope of formulating a transdermal delivery system using the optimized nano carrier. Cell viability study in baby hamster kidney-21 cell culture substantiated the non-toxicity of the nanoparticles. **Conclusion:** Study construed the suitability of the design model in optimization and assessment of the impact of the concentration of solid lipid as well as liquid lipid on the responses particle size and drug entrapment efficiency.

Key words: Glyburide, Central composite design, MTT assay, Cell viability study, BHK -21 cell-line, Histopathological study.

INTRODUCTION

Glyburide, an oral hypoglycaemic agent is a sulfonylurea, which increases the liberation of endogenous insulin together with increasing its peripheral effectiveness.[1] It acts by stimulating the beta cells to emanate insulin, thus reducing the blood glucose levels.[2] Glyburide due to its poor solubility and high permeability categorized as BCS class II drug.[3]

Frequent dosing, associated hypoglycaemia, gastrointestinal disturbance are the major drawbacks accompanying oral glyburide therapy.[4] Many alternates such as liposomes, niosomes, nanoparticles, were investigated to overcome these limitations.[5] Lipid nanoparticle- based delivery systems are also extensively explored to obtain a successful delivery of glyburide.

Among the various lipid based nano formulations, nano structured lipid carriers are considered as the most beneficial delivery scheme.[6] Amalgamation of solid and liquid lipid with surfactant gives NLC better stability when compared to the first-generation solid lipid nanoparticles.[7]

NLC incorporates hydropylic as well as lipophilic drugs owing to the presence of biocompatible solid and liquid lipids, also their exclusive nano size promises to achieve the goals of controlled drug delivery.[8,9] Decelerated polymeric transition and low index of crystallinity aids to the benefits of

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the spherical shape of NLC.[10] Restriction of the drug within the shambolic lipid core protects it from leakage and degradation.[11] The size also favours effective penetration through the skin, hence can be included as a useful vehicle in dermal formulations.[12]

In NLC, solid and liquid lipids assemble the inner nucleus and according to the literature, the ratio of solid lipid and liquid lipid assorted ranges from 70:30 to 99.9:0.1. Lipid concentrations plays a critical role in the success of the NLC formulation, as it affects the particle size, drug entrapment, drug release and stability.[13]

Hence, in the present work solid and liquid lipid concentrations in the glyburide-loaded NLC were optimized using central composite design (CCD) with two centre points and a set of axial and factorial points (star points) which can approximate the curvature effect. CCD was used, as it is useful in progressive experimentation and enables regular construction from former factorial experiments by tallying axial, and centre points.[14]

Among the different methods available for the fabrication of NLC, melt dispersion technique with ultrasonication was explored in the present study due to its feasibility and scalability. Optimized glyburide loaded NLC was prepared and evaluated for various characteristics. Histological evaluation of rat skin treated with optimised NLC was carried out to analyse the permeability and conveyance property of the formulation. Toxicity associated with the nanoparticle was determined using MTT assay on the BHK (baby hamster kidney)-21 cell lines.

MATERIALS AND METHODS

Glyburide was a generous gift of Mankind Pharma India. Stearic acid and oleic acid were furnished by Venus Ethoxyethers Pvt. Ltd India. Only analytical grade reagents and chemicals were used. Design expert 12.0.6.0 was used for optimization of the parameters.

Employment of central composite design

The central composite design possesses certain specified arrangement with a center point surrounded by corner points and extra points bounded from the sides.[15] By using the software design expert 12.0.6.0 the design generated 10 experimental trials. The design was evaluated with reference to particle size and drug entrapment to get the optimised concentration ratio of solid and liquid lipids, Table 1 gives the experimental trials generated by the design.

Table 1: Experimental trials.

Formulation	Space Type	Factor 1	Factor 2
		A:solid lipid %	B:liquid Lipid %
1	Axial	65.8	20
2	Factorial	70	10
3	Factorial	70	30
4	Axial	80	34
5	Axial	80	5.8
6	Factorial	90	30
7	Factorial	90	10
8	Center	80	20
9	Axial	94	20
10	Center	80	20

Preparation of glyburide nanostructured lipid carriers by melt dispersion technique

The melted stearic acid containing the drug and hot oleic acid was poured into the aqueous phase containing tween 20 maintained at the same temperature under continuous stirring to obtain oil in water emulsion. This hot dispersion was stabilized by stirring for a specified period of time. Then the dispersion was gradually dropped into ice-cold water, filtered and dried at room temperature to give Nano particles.[16,17] The NLCs were formulated according to the design, with previously defined high and low values for solid and liquid lipid.

Particle size analysis

Horiba-SZ-100 was used to determine the size of the formulated NLCs employing the principal of dynamic light scattering.[18] Suitable concentration for the examination was got by diluting the samples with double distilled water. The particle size analysis were made at an angle of 90° at room temperature.

Drug entrapment efficiency

Drug entrapment within the NLC, influences its release characteristics hence plays a very fundamental role.[19] Amount of drug encapsulated within the NLC was determined by measuring free glyburide concentration in the supernatant liquid. From this values encapsulation efficiency (EE) was determined indirectly. The entrapped and unentrapped drug from the NLC dispersion was separated by centrifugation at 4000 rpm for 45 min.[20,21] The concentration of the drug present in the supernatant was measured spectrophotometrically after suitable dilutions. The percentage entrapment efficiency of NLC was calculated as follows

$$EE\ \% = \left(\frac{W_a - W_b}{W_a} \right) \times 100$$

Where EE%= the percentage encapsulation efficiency, Wa = total drug content of the NLC taken for the study (equivalent to 5 mg) and Wb = the amount of unentrapped drug present in the supernatant fluid.

Formulation of the NLC using optimized parameters

GB-NLCs were formulated as mentioned in the above procedure using the optimised proportions of solid and liquid lipid obtained after the design evaluation. The formulated optimized GB-NLC were assessed for drug encapsulation, particle size and surface charge specifications.

Morphological study

Scanning electron microscopy and atomic force microscopy were employed to establish reliable morphological evidence and structural properties of the optimized nanoparticles.

Scanning electron microscopy (SEM)

High energy beam of electrons interact with the samples and produce SEM images of resolution up to 1 nm.[22] Scanning electron microscope; model EVO MA18 with Oxford EDS(X-act) was used to determine morphology of GB loaded NLC. The sample was placed on a metal stub with adhesive then made conductive by coating a thin layer of gold using sputter coater.

Atomic force microscopy (AFM)

The AFM image for optimized NLC was obtained using innova SPM atomic force microscope.[23] Tapping mode with an oscillating probe tip was used to obtain the topography. Flat and clean substrate was chosen and the sample was immobilized in the substrate. The tip of AFM interacts with the surface. Imaging and measuring of the surface was done by the laser positioned on the photodector.

X-ray diffraction (XRD)

The XRD patterns of pure glyburide and the optimized glyburide loaded NLC were recorded by X-ray diffractometer (RigakuMiniflex 600) equipped with Ni-filtered at 40 kV and 15 mA.[24] The samples placed on a sample holder and XRD scans were documented up at 2 theta plane at 5° to 50°.

Histopathological studies of NLC treated rat skin

In order to charge the prepared NLC into a transdermal delivery system, histopathological investigations were carried out on Wistar albino rat skin and the effect of NLC on the permeability of skin was studied. Optimized GB-NLC were placed on the excised skin samples for 12 hr, followed by washing and treating with 10% formalin. Excised skin sample treated with saline was used as a control.

The skin sections were grossed, taken for overnight processing with formalin in an automated tissue processor. Selected portions of the tissue were fixed, dehydrated, and then wax impregnated by the processor. To outline the tissue and cellular components hematoxylin/eosin stain was used.[25]

Cell viability study using MTT assay

The major concern in nanotechnology is the abrupt increase in the number as well as types of nanoparticles used in drug delivery systems. The reason for the growing concern is the toxicity associated with these nanoparticles. The size, structure, chemical composition are the major elements for the nano materials to show complex toxicity with respect to cell and organelles. Hence cell viability study was carried for the optimized NLC using MTT assay in BHK-21 cell culture.[26]

Sample preparation

The Stock solution of GB NLC was prepared in dimethylsulfoxide, followed by sonication for 10 min at 30Hz at 28°C. Then this solution was stored at 4°C until further use.

Cell line preparation

BHK -21 cell-line was cultivated and cultured within a bottle. Cell lines were harvested using trypsin versene solution after completion of merging. Part of the harvested cells were cultivated in Rosewell Park Memorial Institute (RPMI-1640) media. This media used for the cultivation had 10% bovine serum albumin which was incubated for 24 hr at 37°C. Small bottles of 2×10^5 cell/ml density were used to store the cells after the sub culture and used for analyzing the toxicity of the samples.[27]

Cytotoxicity assay

The toxic effects of new or obscure compounds are established *in vitro* by computing the number of cells viable when stained with a dye. The basic principle of this assay is built on the depletion of MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide), a yellow colored water-soluble tetrazolium dye, predominantly by the mitochondrial dehydrogenases, to formazan crystals which is purple in color. MTT yields a yellowish solution in a media or salt solutions which is phenol red deficient.

The viable mitochondrial dehydrogenase enzyme converts dissolved MTT to insoluble purple formazan.[28,29] Acidified isopropanol was used to solubilize the insoluble formazan and the resultant purple solution was measured spectrophotometrically. Quantity of formazan formed is proportionate to the number of animate cells from which degree of cytotoxicity can be Figured. An untreated sample (blank), control (Imidazole) and optimized glyburide loaded NLC were tested on the cell lines to determine the viability.

$$\% \text{ cell viability} = \frac{(\text{Sample} - \text{Blank})}{(\text{Control} - \text{Blank})} \times 100$$

RESULTS AND DISCUSSION

Design Evaluation

Design evaluation was done by estimating responses such as particle size and drug entrapment efficiency. All the ten formulations had particle size in range of 80±0.0871 nm to 500±0.031 nm and the entrapment efficiency was found to be in the range of 60±0.206 % to 95±0.128 %. This indicates the effect of change in the concentration of solid and liquid lipid on the responses.

Model fitting and statistical analysis

According to the CCD design using design expert software 12.0.6.0, two factors solid lipid concentration and liquid lipid concentration were opted in 2 levels (low and high) which accounted for 10 formulation trials. Ten formulations were prepared as specified by the design software and their responses are presented in Table 2. The design suggested quadratic and linear model for particle size and drug entrapment respectively and the model summary is given using ANOVA (Table 3 and 4).

Table 2: Results of experimental trials.

Formulation	Particle Size nm (*n*=3)	Drug Entrapment % (*n*=3)
F1	128±0.047	95±0.128
F2	80±0.087	91±0.236
F3	400±0.021	90±1.282
F4	500±0.031	64±1.282
F5	222±0.678	83±1.282
F6	201±0.950	70±0.358
F7	130±0.459	89±0.117
F8	290±0.098	87±0.110
F9	200±0.239	60±0.206
F10	350±0.658	80±0.110

Table 3: ANOVA for Particle size.

Source	Sum of Squares	Mean Square	*f*-value	*p*-value	significance
Model	1.437	28732.83	6.84	0.043	significant
A-solid lipid	278.20	278.20	0.0662	0.809	Not significant
B-liquid Lipid	76861.67	76861.67	18.30	0.0129	significant
AB	15500.25	15500.25	3.69	0.127	Not significant
A^2	39485.16	39485.16	9.40	0.037	significant
B^2	141.45	141.45	0.033	0.863	Not significant
Residual	16804.75	4201.19			
Lack of Fit	15004.75	5001.58	2.78	0.409	not significant

Table 4: ANOVA for drug entrapment.

Source	Sum of Squares	Mean Square	f-value	p-value	significance
Model	913.59	456.79	7.63	0.017	significant
A-solid lipid	638.99	638.99	10.67	0.013	significant
B-liquid Lipid	274.60	274.60	4.58	0.069	not significant
Residual	419.31	59.90			
Lack of Fit	394.81	65.80	2.69	0.435	not significant

Analysis of variance (ANOVA) for particle size

Design terms are considered significant if the p-values obtained are lesser than 0.05. The *F*-value of 6.84 infers that the model selected is significant.[30] The *f*-value obtained for the lack of fit was 2.78, which is not significant when compared to the pure error and there is only 40.91% chance that a lack of fit *f*-value this huge could occur due to noise. The mathematical equation obtained for the response particle size is given below.

*Particle size=-6750.61122-160.56029 solid lipid+57.37689 liquid Lipid-0.622500 solid lipid * liquid Lipid-0.929375 solid lipid ^{2} +0.055625 liquid Lipid^2*

In this quadratic equation; a positive value suggests that there will be increase in the response as the factor levels are increased, whereas a negative value specifies a converse relationship between the factor and the

Table 5: Predicted and actual values of particle size.

Formulation	Actual Value	Predicted Value	Residual
F1	128.00	142.46	−14.46
F2	80.00	78.25	1.75
F3	400.00	398.79	1.21
F4	500.00	469.74	30.26
F5	222.00	192.51	29.49
F6	201.00	262.50	−61.50
F7	130.00	190.96	−60.96
F8	290.00	320.00	−30.00
F9	200.00	125.79	74.21
F10	350.00	320.00	30.00

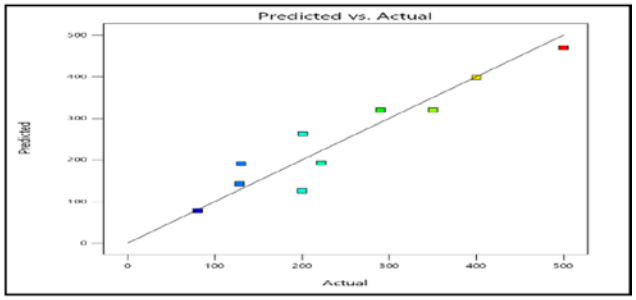

Figure 1: Predicted response verses actual response for particle size.

Table 6: Predicted and actual values of drug entrapment.

Formulation	Actual Value	Predicted Value	Residual
F1	95.00	93.54	1.46
F2	91.00	95.70	−4.70
F3	90.00	83.98	6.02
F4	64.00	72.61	−8.61
F5	83.00	89.19	−6.19
F6	70.00	66.10	3.90
F7	89.00	77.82	11.18
F8	87.00	80.90	6.10
F9	60.00	68.26	−8.26
F10	80.00	80.90	−0.90

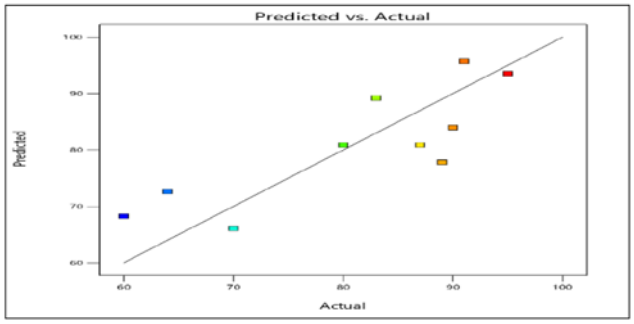

Figure 2: Predicted response verses actual response for drug entrapment.

response.[31] It is clear from the equation that liquid lipid concentration has a positive effect and solid lipid concentration has a negative effect on the particle size. From the equation it is evident that the association between factor and response is not always linear, when used at different levels it may produce diverse degrees of response. The actual vs. predicted values for particle size is given in Table 5 and in Figure 1.

Analysis of variance (ANOVA) for drug entrapment

The p-value of 0.0137 in case of solid lipid indicates that this term has significant effect on drug entrapment. Whereas liquid lipid concentration is considered statistically insignificant due to *p*-value above 0.05. The *f*-value of 2.69 implies that the lack of fit is not so significant relative to the pure error and the model predicted only 43.59% possibility of noteworthy lack of fit caused due to noise. The mathematical equation obtained for the response drug entrapment is given in the equation below

Drug entrapment: +164.11499-0.893718 solid lipid-0.585876 liquid lipid

From the above equation we can conclude that solid and liquid lipid has a negative influence on the drug

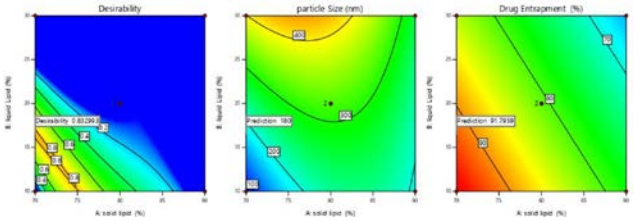

Figure 3: Contour Plot: (a) desirability (b) particle size (c) drug entrapment.

entrapment, hence increasing the lipid concentration may decrease the drug entrapment. The actual vs. predicted values for drug entrapment is given in Table 6 and in Figure 2.

Optimization

Optimum NLC formulation was achieved utilizing split-plot response surface approach taking into account the responses such as drug entrapment and particle size. The optimum condition specified by the design prototype was 70% solid lipid and 16.657% liquid lipid. The desirability function obtained was 0.833 and on the basis of this value, ideal conditions and predicted values were ascertained. Figure 3 gives the contour plot

Table 7: Optimum formulation conditions predicted by the model.

Sl No	solid lipid	liquid Lipid	particle Size(nm)	Drug Entrapment (%)	Desirability
1	70	16.657	180	91.796	0.833

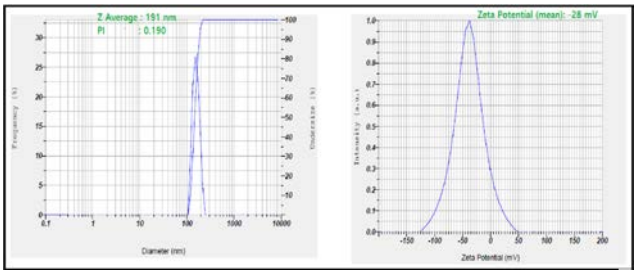

Figure 4: Particle size analysis and zeta potential of the optimized formulation F_{A1}.

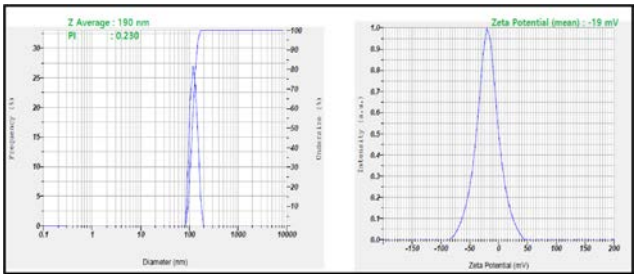

Figure 5: Particle size analysis and zeta potential of the optimized formulation F_{A2}.

predicting the optimum parameters in terms of particle size and drug entrapment using the desirability value. To substantiate the suitability of the model, the NLCs were formulated using the parameters generated by the model. Table 7 gives optimum formulation conditions predicted by the model.

Formulation and characterization of optimized glyburide nanostructured lipid carriers (GB-NLC)

In order to validate the precision of the design, GB-NLCs were formulated in triplicate with the ratio generated by the above central composite design which corresponds to 70 % stearic acid and 16.6 % of oleic acid. The formulations were evaluated for the particle size, zeta potential, and drug entrapment efficiency (Figures 4, 5 and 6) and all the three-formulation showed approximately similar results. The results are depicted in Table 8. Formulation F_{A3} exhibited comparatively better results hence was used for further evaluation studies.

Morphological study

Scanning electron microscopy (SEM)

Topical morphology, size and shape of solid materials and nano materials can be envisioned using SEM.[32] The

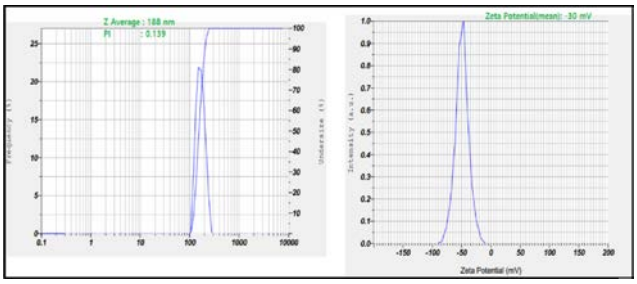

Figure 6: Particle size analysis and zeta potential of the optimized formulation F_{A3}.

Table 8: Characterization of optimized GB-NLC formulations.

Formulation	Particle size (nm)	polydispersity index	Zeta Potential	% Entrapment efficiency
F_{A1}	191±0.459	0.190	−28mV	93.5±1.29
F_{A2}	190±0.080	0.230	−19mV	93±1.100
F_{A3}	188 ±0.577	0.139	−30mV	92.6±0.456

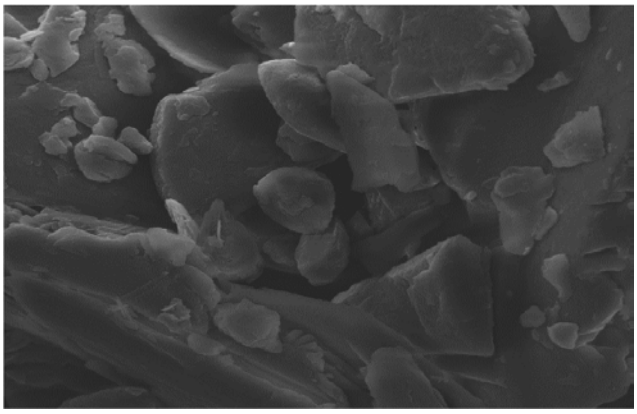

Figure 7: SEM of optimized GB-NLC.

topical surface morphology of optimized GB-NLC was visualized by scanning electron microscope and the particle size was found to be 220 nm. The NLCs were found to be discrete to an extent, nearly spherical with an even surface as depicted in the Figure 7.

Atomic force microscopy (AFM)

AFM has been extensively used to attain information on the surface morphology, size and shape of nanoparticles.[33] In the present sample, the separated lipid particles were nearly spherical with an even surface as depicted in the Figure 8. The particle size predicted by AFM was 173 nm which is closer to the size obtained by the DLS method.

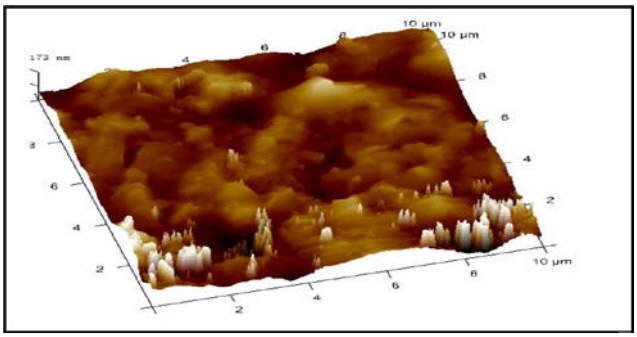

Figure 8: AFM image of optimized GB-NLC.

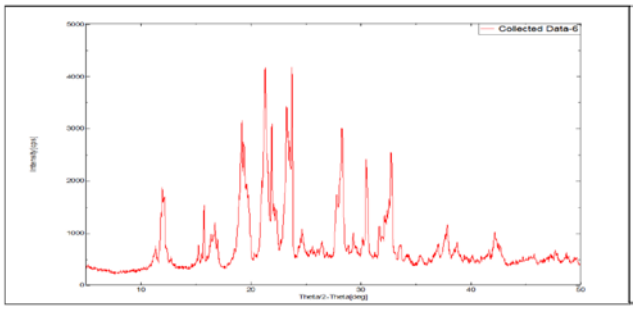

Figure 9a: X ray Diffraction of Pure Glyburide.

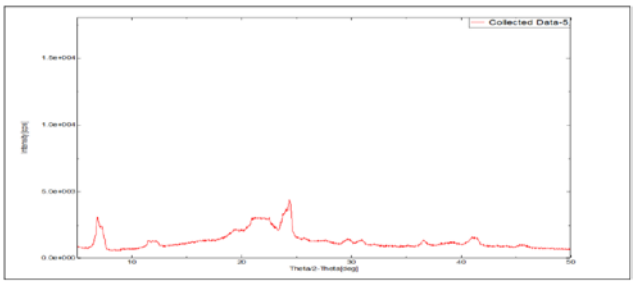

Figure 9b: X ray Diffraction of optimized GB-NLC.

Table 9: Predicted and observed responses of optimized formulation.

Characteristics	Predicted response	Observed response
Particle size(nm)	180	DLS:.188 ±0.577
		SEM: 220 ±1.00
		AFM: 173 ±0.08
Drug entrapment efficiency (%)	91.79	92.6±0.456

Table 9 gives a comparison between predicted and observed responses of optimized GB-NLC. It was observed that there was a slight difference between the forecasted and observed particle size of the optimized GB-NLC, which may be due to the difference in the sampling technique and other process variables, but the drug entrapment was very close to that of predicted response.

X-ray diffraction (XRD)

XRD studies are employed to explore the crystalline behavior and condition of the drug within the NLC.[34] The Figure 9a and 9b shows XRD spectra of the pure GB and optimized GB-NLC respectively. X-ray scans of NLC confirmed that all the entities lost their crystalline nature when incorporated into NLC. Powder glyburide was highly crystalline as depicted from the sharp peak pattern. XRD pattern of GB-NLC showed a randomized peak, which is a recommendation of reduction in crystallinity of glyburide when formulated into NLC. This confirms the absence of possible leakage of the drug from the formulation and guarantees a better loading competence.

Histopathological studies of NLC treated skin

The outer crust of the skin, stratum corneum acts as a prime barricade for the permeation of drug. It is a diverse system constituting keratin-filled corneocytes, entrenched in an intercellular lipid array.[35] This lipid array is systematized in lamellar bilayers. These lipid bilayers and corneocytes are considered to be the permeability barriers in the SC.

The Figure 10a is the specimen picture of excised rat skin treated with saline in which the SC is intact above the viable epidermis and has few layers of corneocytes. A single layer of basal cell is present at the base of epidermis and above this basal layer is a spinous layer which is shielded by a granular coat. All the associated skin appendages were within the normal limits.

Intercellular lipid bilayer disordering and cavitation in the keratin filled corneocytes are the key means of drug penetration. Figure 10b shows slackening and formation of tiny pores in surface of SC when treated with optimized nanostructured lipid carrier GB-NLC. Disarrangement in the lipoidal region, widened intercellular space are the major observation made from the histological images. Hence the possibility of formulating NLC into transdermal system can be acknowledged.

Cell viability study using MTT assay

Nanostructured lipid carriers are nanoparticles devised for localization within the cell and principally interact with cell essentials, in particular with the membrane, mitochondria, lysosomes, and nucleus.[36] In the present work, the lipids used; that is solid as well as liquid lipid are considered to be safe (GRAS category), but

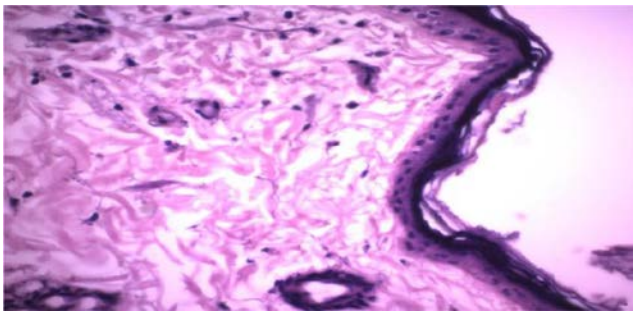

Figure 10a: Histopathological images of rat skin treated with saline.

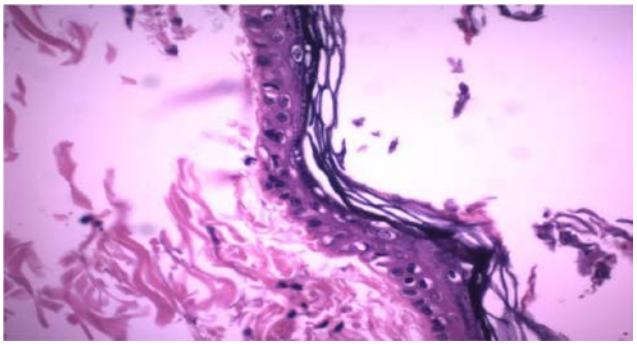

Figure 10b: Histopathological images of rat skin treated with optimized GB-NLC.

the particles with larger surface areas have the affinity to agglomerate and intermingle with vital protein components. These interactions may initiate oxidation or damage the DNA.[37,38] This induced oxidation may fast-track apoptosis or evoke mutagenesis or cause a lowering of the immune system.[39] Hence cell viability study is very essential to prove the non-toxicity, and biocompatibility of these nanoparticles.

The important tool to ascertain and evaluate the safety of nanostructured lipid carrier is using cell lines as an *in vitro* testing tool.

MTT assay is an effective and essential methodology to identify the cytotoxic potential of several newer chemical entity, drugs, nanoparticles, or herbal plant extracts.

The MTT assay measures the intensity of formazan colour due to the enzymes present in the living mitochondrial cells.

In the present investigation BHK-21 cell lines, are used to perform the MTT assay. BHK 21 cell lines are deliberated, as a significant member of mammalian expression systems useful in pharmaceutical fabrication. BHK 21 are genetically similar to the mammalian system and are steady and tranquil to transform and scale–up. It has decent culture bioreactor requirements and produce post-translational modification profiles similar to that of human proteins.

The cytotoxicity of the selected glyburide nanoparticles by MTT assay were verified in BHK-21 cell line. The viability of cells charged with nano lipids of Glyburide at different concentrations and at different duration of exposure were studied and the results are depicted in Figures 11 and 12 respectively.

The live cells after exposure to the sample for 24 hr metabolized the MTT to a purple-colored formazan which was spectrophotometrically analyzed.

After 24 hr of treatment, BHK-21 cells showed 99% viability with the lowest concentration (20 μg/ml) and with the increment in NLC concentration, cells exhibited exceptional viability even up to the concentration of 140 μg/ml i.e. the viability of the cells treated with GB-NLC was 83%.

In an attempt to determine the toxic effect of NLC with respect to time, the sustainability of the cells for up to 72 hr were observed, with highest concentration of 140 μg/ml. It was noted that after 72 hr of treatment the viability of the cells were around 70% which was appreciable. Hence time dependent toxicity can be ruled out.

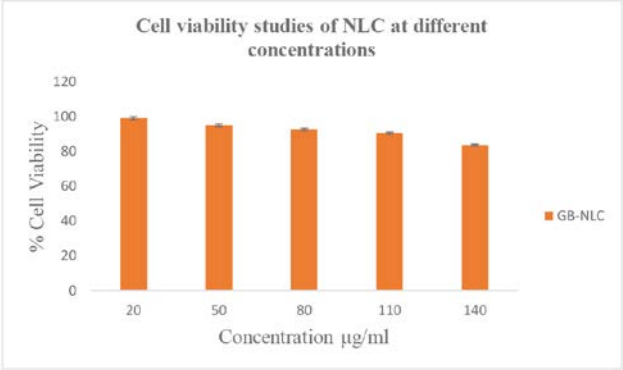

Figure 11: Cell viability of NLC at different concentrations.

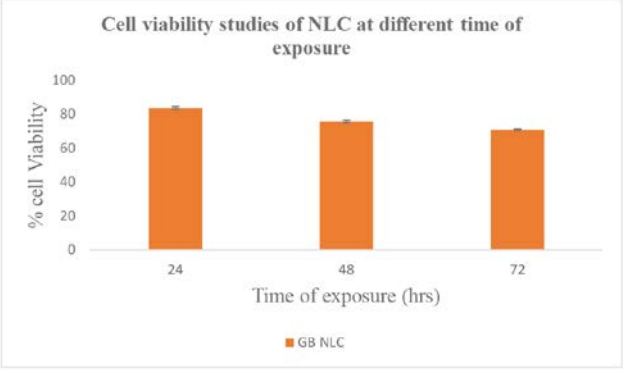

Figure 12: Cell viability studies of NLC at different time of exposure.

These results evidently established nontoxicity of the prepared nano lipids. The lack of any noticeable toxicity stabilized glyburide nanoparticles and offered a new prospects for the safe loading of these NLCs into suitable dosage form.

CONCLUSION

Optimization of the lipid concentration in NLC formulation has to be considered as an imperative stage. In the present study central composite design approach was sucessful in determining the best lipid concentrations. This design proved to be suitable, to optimize and assess the impact of the lipid concentrations on the response's particle size and drug entrapment efficiency. The morphological study by SEM and AFM disclosed almost spherical and even surface of the optimized NLC with particle size nearly closer to the design prediction. XRD demonstrated that the drug in the optimized NLC was molecularly dispersed within the lipid matrix. This would give the benefit of enhanced drug encapsulation and reduced drug eviction during storage. From the histopathological and cell toxicity study of the optimized NLCs it can be concluded that the GB-NLC can be proposed to be an assuring transdermal drug delivery scheme which is non-toxic, and biocompatible with a good biological performance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NLC: Nanostructured lipid carrier; **GRAS:** Generally recognized as safe; **PDI:** Polydispersity index; **EE:** Entrapment efficiency; **ANOVA:** Analysis of variance; **R^2:** Regression coefficient; **DSC:** Differential scanning calorimetry; **GB:** Glyburide; **GB-NLC:** Glyburide-loaded nanostructured lipid carrier.

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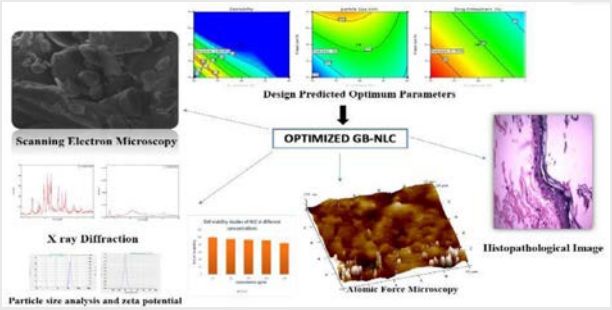
PICTORIAL ABSTRACT



SUMMARY

The solid and liquid lipid concentration in the glyburide loaded NLC fabricated using melt dispersion technique was established using statistical software design expert 12.0.6.0. Via central composite design (CCD). The optimized formulations were evaluated for various parameters and the constructive results proved the appropriateness of the design and the formulation.

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