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Formulation and Evaluation of Nanostructured Lipid Carrier (NLC) For Glimepiride

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ABSTRACT

Nanostructured lipid carriers (NLC) of Glimepiride was prepared with the objective of treating type-2 diabetes. The formulated NLC consist of Compritol ATO 888 as solid lipid, medium chain triglycerides as liquid lipid and poloxamer 188 as surfactant. NLC's were prepared by using hot homogenization technique and characterized by FT-IR, DSC. All NLC had shown entrapment efficiency within a range of 78.52 to 90.38%. Both entrapment efficiency and release rate was effected by lipid concentration. Formulation F5 was considered as an optimized formulation based on its particle size and % drug release. Zeta potential value had suggested good particle stability. The optimized formulation did not show any physical/chemical changes when it is subjected to accelerated stability conditions. It was concluded that formulated NLC holds as a potential approach for controlled release of drug which may reduce the dose frequency and improves patient compliance.

Key words: Nanostructured lipid carriers; Glimepiride; type-2 diabetes; hot homogenization technique; Compritol ATO 888

INTRODUCTION

Nanostructured lipid carriers (NLC) and solid lipid nanoparticles (SLN) can be used to deliver the drug through various routes like parenteral, topical and oral routes by controlling the release of administered drug. SLN's have the potential to formulate for commercial use in the market [1]. But there are some disadvantages associated with SLN's like drug expulsion, particle concentration in the aqueous dispersion and drug loading due to less solubility of drug in the solid lipid [2]. These disadvantages can be overcomed by developing a nanoparticle with a controlled nanostructure can be termed as nanostructured lipid carrier (NLC). They possess a very low melting point due to the oil and maintain the particle state as solid at body temperature. It possess some advantages like enhanced drug loading, reduced burst release and better control over release of drug from the formulation [3].

SLN's are a typical oil in water emulsion where the oil is replaced with a solid lipid, which has a liquid to solid phase transition above the normal body temperature. Whereas NLC contains a certain amount of liquid lipid which leads imperfection in the lattice of the crystal. NLC's can be formulated by using various techniques like high pressure homogenization, microemulsion template, cold homogenization, solvent emulsification, solvent diffusion, reverse micelle double emulsion, homogenization followed by ultra-sonication and solvent injection etc [4].

Glimepiride comes under third generation of sulfonylurea class of drugs, which can be used to treat type 2 diabetes [5]. It releases insulin by intensification of insulin secretion by β-cells of pancreas, by closing potassium channels and depolarizing cells membrane which leads to initiation of metabolic process [6]. Physical state of glimepiride is white or off white crystalline powder, fairly insoluble in water, which causes huge differences in bioavailability [7].

The objective of this present research is to develop a simple approach for the Glimepiride NLC. Eight formulations where developed using various lipid concentrations, to study maximum entrapment efficiency (EE). In addition to this *Invitro* drug release, particle size, zeta potential, SEM, FT-IR and DSC were also determined.

MATERIALS AND METHODS

A. Materials

Glimepiride was obtained from. Compritol ATO 888 and Medium chain triglycerides from Gattefose, France. Polaxomer from Cipla Ltd., Goa, India.

B. Methods

1.Preparation of NLC

Different concentrations of glimepiride NLC dispersions were prepared by using hot homogenization technique which is mentioned in Table 1. In this technique lipids were melted to ten degrees above their melting point and glimepiride was added to the melted lipid. Both lipid and aqueous phases were prepared in separate ways.

Temperature was maintained until the dispersion becomes optically clear. Polaxomer was dissolved in water and it is also boiled at the same temperature as of lipid mixture. Using high shear homogenizer, the hot surfactant solution was added to lipid mixture at 2000rpm for 1 hour and the volume was made up to 100ml. then the mixture was removed from water bath and gently mixed at room temperature until it gets cooled. Then the dispersion is further used for various characterizations [8].

Table1. Formulation chart for preparation of NLC

Formulation	Drug (mg) (GMP)	Solid lipid Compritol ATO 888 (% w/v)	Liquid lipid Medium chain triglycerides (% w/v)	Polaxomer 188 (% w/v)	Water
F1	10	0.2	0.2	1	q.s.
F2	10	0.25	0.25	1	q.s.
F3	10	0.4	0.4	1	q.s.
F4	10	0.45	0.45	1	q.s.
F5	10	0.5	0.5	1	q.s.
F6	10	0.75	0.75	1	q.s.
F7	10	1	1	1	q.s.
F8	10	1.25	1.25	1	q.s.

2. Characterization of prepared NLC's

2.1 FT-IR Spectroscopy

FT-IR helps in detecting the presence of any interactions between the drug and carrier. RT-IR spectrums of pure Glimepiride drugand physical mixtures were taken at a specified temperature. Spectra's are scanned within range of 400 and 4000 cm^{-1}[9].

2.2 DSC analysis

Differential S canning Calorinetry (DSC) was performed using Shimadzu DSC-60, Japan. It was done by taking 5mg of sample into an aluminium pan and sealed. Scan was recorded within a temperature range of 30 to 300 ºC. Pure Glimepiride drug and physical mixture thermograms were recorded [10].

2.3 Particle size analysis

Distribution of size in the mean diameter of the nanoparticle is measured using Dynamic Light Scattering Particle size analyser [11, 12].

2.4 Polydispersability index

The polydispersability index (PDI) can also be measured from Dynamic Light Scattering. PDI can be termed as an index or difference with in the particle size distribution. It can be calculated by using the following equation [11, 12].

Polydispersability = [D90-D10]/D50

Where D50, D90 and D10 are the percentiles of the undesired particles.

2.5 Entrapment efficiency

Entrapment efficiency can be calculated by using this formula

Entrapment efficiency = $\frac{\text{Estimated \% drug content}}{\text{Theoretical \% drug content}} \times 100$

Estimated drug content can be obtained from analysis whereas theoretical drug content can be obtained from the ratio employed to formulate the NLC [13, 14].

2.6 Zeta potential

Zeta potential was measured using Malvern instrument 3000HSA, UK. Measurement of zeta potential is an easy process because when the suspension is placed between the electrodes the charge of potential moves with suspension and the velocity is directly proportional to the zeta potential [15].

2.7*In vitro* drug release

Dialysis method is used to carry out the in vitro studies for the formulated NLC. Dialysis membrane is obtained from (Hi-media, Mumbai, India) with molecular weight with in a range of 12,000 to 14,000 Dalton. Prior to conduct the experiment the dialysis membrane should be activated with 1% HCl for 12 hrs. 5ml of formulated NLC dispersion was placed into the assembly. The assembly was attached to a stand in 100 ml of pH 7.4 phosphate buffer at a temperature of 37 ±0.5 ºC so that the membrane should touch surface of the medium. An aliquot of 5ml was taken out periodically from the receptor medium and replaced it with fresh medium. Samples were analysed by using UV-Spectrophotometer at a wavelength of 236nm [16].

2.8 Stability studies

Formulated NLC's were subjected to accelerated stability conditions by the guidelines framed by ICH. The optimized formulation was sealed and stored at 25±2ºC, 60±5% RH and 40±2ºC, 75%±5% RH for a time period of three months. NLC was removed periodically and evaluated for physical characteristics [17].

RESULTS AND DISCUSSION

A. FT-IR Studies

Glimepiride had shown it significant peaks at 3323.07 (N-H), 2941.73 (C-H), 2853.82 (O-H), 1441.17, 1524.46 (N=O), 1156.37 (C-N) and 1031.83 (C-O). The spectra of the physical mixture with all other excipients had shown all the principle peaks of glimepiride drug, from which we can suggest the stable nature of drug during the process.

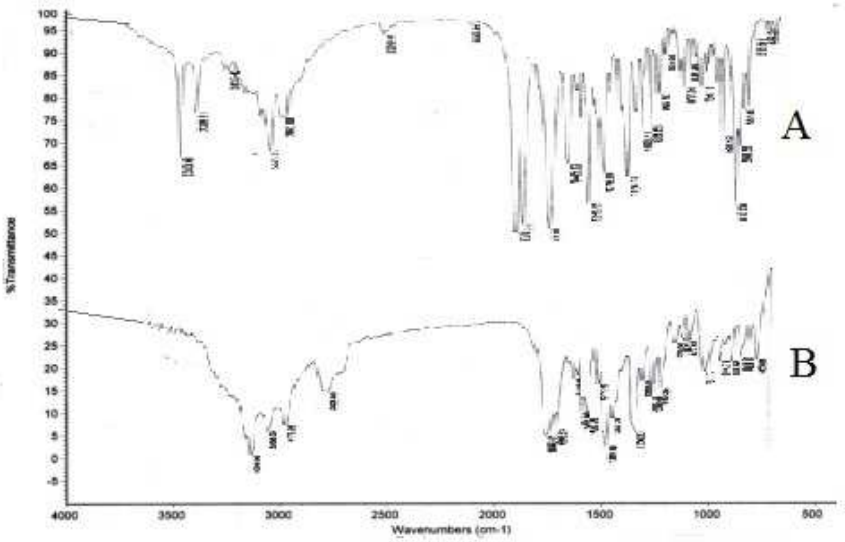

Figure 1. (A). FT-IR spectra of glimepiride, (B). FT-IR spectra of physical mixture

B. DSC analysis

Pure glimepiride showed a sharp endothermic point at 206.93ºC, which resembles with the melting point of pure glimepiride. The DSC curve of the physical mixture had shown a sharp endothermic peak at 73.49ºC which resembles Compritol ATO 888 followed by an exothermic peak of 206.97ºC of pure glimepiride. Both curves of pure drug and physical mixture had shown characteristic peaks of glimepiride, which indicates the glimepiride compatibility with Compritol ATO 888 when subjected to heat.

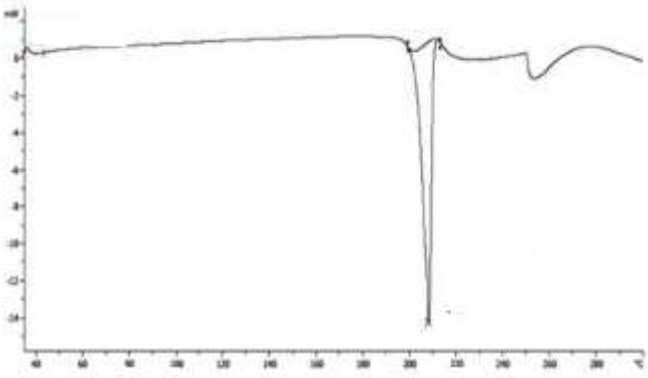

Figure 2(A). DSC of Pure Drug

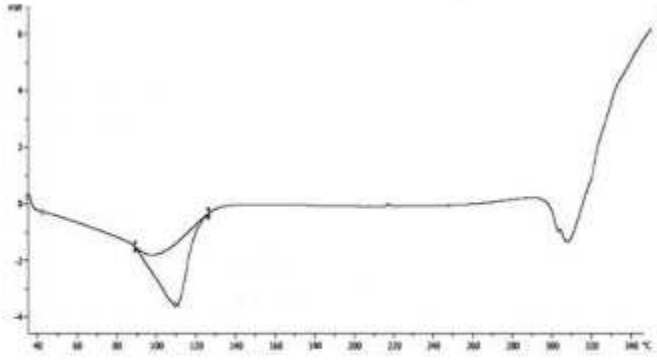

Figure 2(B). DSC of Physical mixture

C. Particle size analysis

Data consisting about particle size of the formulated NLC for glimepiride was given in table 2. From the table it is evident that particle size for formulations F1 to F8 was within a range of 62.25 to 443.32 nm. Lipid: surfactant ratio had played a significant role in obtaining NLC with desired particle size. NLC prepared with lipid: surfactant ratio of 1:1 had shown lowest particle size of 62.25, while NLC prepared with lipid: surfactant ratio of 2.5:1 had shown highest particle size of 443.32 nm. From this analysis it can be suggested that with increase in lipid concentration, by keeping surfactant concentration as constant there is an increase in particle size because there is lack of surfactant to coat all the lipid droplets which leads to aggregation of particle and hence increase the particle size.

D. Polydispersability Index (%)

Polydispersability is used to find the width of particle size distribution. In order to confirm the size distribution of the particles calculation of polydispersability index is essential. Polydispersability of all the drug loaded NLC's were given in table2. From the table it is evident that polydispersability index of all the formulations was within a range of 0.565 to 0.997, which indicates that all the formulations had shown polydispersability.

E. Entrapment efficiency (EE)

From the results it can be suggested that concentration of lipid has a major effect on Glimepiride entrapment efficiency. Entrapment efficiencies of drug loaded NLC's were given in table 2. From the table it is evident that entrapment efficiencies where found within a range of 78.52 to 90.38%. from the results we can infer that by increase in the concentration of lipid there is increase in the entrapment efficiency, this is due to higher concentration of lipid will increase the particle size that will affect the absorption drug present on the surface.

Table 2. Characterization of NLC of Glimepiride

Formulation	Mean Particle size (nm)	Drug content (mg)	Polydispersability Index (%)	% EE
F1	323.24±3.12	13.92±1.35	0.565	86.69
F2	279.21±2.95	13.76±1.26	0.634	86.21
F3	119.75±4.15	13.35±1.75	0.667	83.65
F4	82.34±7.25	12.79±1.27	0.912	79.95
F5	62.25±6.19	12.67±1.72	0.821	78.52
F6	116.73±3.14	12.98±1.22	0.614	82.37
F7	332.29±2.92	14.19±1.25	0.195	89.12
F8	443.32±513	14.34±1.82	0.997	90.38

F. Zeta potential

Zeta potential can be termed as an important to parameter to predict the physical stability of the prepared NLC. Higher the electrostatic repulsions between the particles higher the stability. NLC with a zeta potential more than +20 mV or less than -20 mV can be termed as physically stable dispersion. The zeta potential of the optimized formulation F5 was found to be -26.5 mV, from which it can be inferred that the dispersion has good physical stability which prevents aggregation with aging**.**

G.*In vitro* drug release

The in vitro profiles are biphasic with an initial burst which is associated with the drug present on the surface. Drug release profiles of formulated NLC'S were given in Figure 3. From the figure it was evident that particle size has a greater effect on the drug release. F5 with small particle size had shown a burst release of 24.92% after 2hrs and 93.85% after 24 hrs and F1, F8 which have high particle size had shown 17.31 and 17.79% after 2 hrs, 59.25 and 64.78% after 24hrs. From these findings it can be inferred that NLC's with small particle size have higher surface area which gives initial burst and sustained drug release.

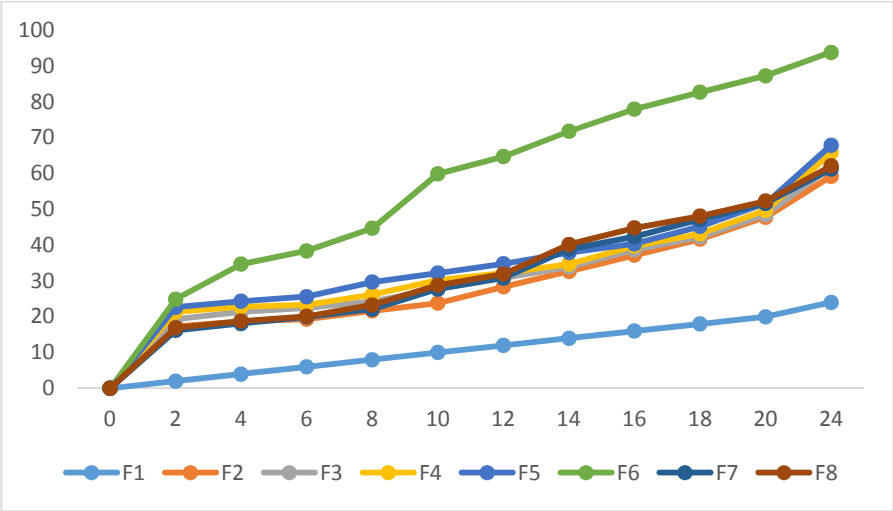

Figure 3. *In vitro* drug release profile

H. Stability studies

The optimized NLC was subjected to stability studies according to ICH guidelines by storing at 25±2ºC, 60±5% RH and 40±2ºC, 75%±5% for 90 days. There was no physical change in the formulation. The obtained data for data content is given in Table3. From the data it is evident that the formulation doesn't undergo any physical/chemical changes.

Table 3. Stability Data of Optimized Formulation

Stability condition	Sampling (days)	Drug content (%)
25±2ºC, 60±5% RH	0	98.25
	15	97.65
	30	98.27
	45	96.37
	90	98.21
40±2ºC, 75%±5%	0	97.27
	15	96.81
	30	98.29
	45	97.26
	90	96.37

CONCLUSION

Nanostructured lipid carriers were formulated using Compritol ATO 888, medium chain triglycerides and polaxomer 188. FT-IR and DSC studies revealed that there was no significant interactions between drug and excipients. Both particle size and entrapment efficiency was increased with increase in lipid concentration. Glimepiride release profile was completely dependent on the particle size. The larger particles had shown a slow release whereas smaller particles had shown a faster release. Formulation F5 was considered as an optimized formulation based on its particle size and % drug release when compared with other formulations. Zeta potential value had suggested good particle stability. The optimized formulation did not show any physical/chemical changes when it is subjected to accelerated stability conditions. Controlled release achieved by these formulations may reduce the dose frequency and improves patient compliance.

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