Research Article

DEVELOPMENT AND CHARACTERIZATION OF NANOSTRUCTURED LIPID CARRIERS OF ORAL HYPOGLYCEMIC AGENT: SELECTION OF SURFACTANTS

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ABSTRACT

A new generation of nanostructured lipid carriers (NLCs) consisting of a lipid matrix with a special nanostructure has been developed. This nanostructure improves drug loading and firmly incorporates the drug during storage. Surfactants play important roles in the formation and characterization of nanostructured lipid carriers. In order to investigate the influence of surfactants on properties of NLCs, three types of surfactants (Poloxamer 188, Soya lecithin, Sodium taurocholate) and their mixtures were used. Glipizide loaded NLCs were fabricated using compritol 888 ATO and Oleic acid as a solid lipid and liquid lipid respectively. Solvent diffusion method was employed to produce NLCs and the physicochemical properties of NLCs, such as particle size, zeta potential, entrapment efficiency, DSC analysis and in vitro release studies were investigated. The formulations that contain only single emulsifier exhibited larger particle size. The mean particle size decreased, when combination of surfactant used. The formulation (NL6) prepared with combination of above three surfactants exhibited smallest mean particle size and narrow size distribution. The formulation (NL3) that contains sodium taurocholate (ionic emulsifier) exhibited highest zeta potential leading to improved physical stability. The formulations, in which combinations of surfactant were used, exhibited good entrapment efficiency. TEM images revealed that NLCs are nearly spherical in shape with the particles size ranging from 200 nm to 300 nm that is almost closer to the particle size obtained by PCS. DSC studies indicate that Glipizide incorporated in NLCs was not in a crystalline state but in an amorphous or molecular state. In vitro release study revealed the biphasic release pattern of NLCs systems, initially burst release and then sustained release for prolong period of time. These results suggested that the combination of surfactants produces a stable system with good entrapment efficiency and desired particle size.

Keywords: Nanostructured lipid carriers, Glipizide, Solvent diffusion method, Surfactants.

INTRODUCTION

Solid lipid nanoparticles (SLNs) were developed at the beginning of 1990s as an alternative colloidal carrier system to emulsions, liposomes and polymeric nanoparticles. SLNs made from solid lipids show drug leakage after long term storage, resulting in low drug encapsulation efficiency. Drug expulsion occurs due to an ongoing crystallization process of the lipid towards a perfect crystal. NLCs were developed to overcome potential limitations associated with SLNs. NLCs composed of a mixture of solid lipids and liquid lipids. The inclusion of oil with solid lipid can produce imperfections in their lattice structure and create separation in the fatty acid chain packing, making more room for the drug.[1, 2, 3, 4]

Glipizide is an oral hypoglycemic agent, which is a most commonly used drug for the treatment of type II diabetes mellitus. The oral absorption of Glipizide is uniform, rapid and complete with a bioavailability of nearly 100% and an elimination half-life of 2–4 h. Glipizide is reported to have a short biological half-life (3.4 ± 0.7 h) requiring it to be administered in 2 to 3 doses of 2.5 to 10 mg per day.[5]

The current research was aimed at determining the effect of different surfactants on the production of stable and more efficient Glipizide loaded NLCs formulations for the effective treatment of type II diabetes mellitus.

In the present study, NLCs were produced by solvent diffusion method. Under this method, the NLCs can be obtained very easily, do not require any special equipment and can achieve the sustained drug release, but the use of organic solvent and lack of large scale production were the main drawbacks for this method.[6] In order to investigate the effect of surfactants on the development and characterization of NLCs and obtain NLCs formulation with excellent performance characteristics, lecithin, sodium taurocholate and poloxamer 188 and their mixtures were taken as an emulsifier. The effects of different surfactants on the physicochemical properties of NLCs such as particle size, size distribution, entrapment efficiency, crystal structure and in vitro release were determined.

MATERIALS AND METHODS

Materials

Glipizide was procured from Stadmed Pvt. Ltd. Kolkata (India). Compritol® ATO 888 was from Jubilant organosys, Noida (India). Poloxamer 188, Soya Lecithin and sodium taurocholate (STC) were purchased from Hi Media, Mumbai (India). Oleic acid (OA) was procured from Central drug house (CDH), Mumbai (India).

Preparation of NLCs

Glipizide loaded NLCs was prepared by solvent diffusion method reported elsewhere.[7] Compritol®ATO 888 and Oleic acid (OA) were used as the solid lipid materials and liquid lipid materials, respectively. Briefly, weighed

amount of Glipizide, Compritol ATO 888, Oleic acid, and lecithin (if it was used according to formulation in Table.1) were dissolved in mixed organic solvent ethanol and acetone (1:1 v/v) in a water bath at 60 °C. The resultant organic solution was quickly dispersed into aqueous solution of surfactants at room temperature (25 °C) under

mechanical agitation with 3000 rpm for 30 min until NLCs suspension were obtained. Prepared NLCs were placed in vacuum desiccators for 24 hrs at room temperature to evaporate the residual organic solvent. The formulations of NLCs were listed in Table 1.

Table 1: Formulations of NLCs systems

Sample	Lipid (% W/V) (Compritol 70%: OA 30%)	Drug (% W/V)	Lecithin (%W/V)	Poloxamer188 (% W/V)	STC (% W/V)
NL1	0.5	0.025	0.5	-	-
NL2	0.5	0.025	-	0.5	-
NL3	0.5	0.025	-	-	0.5
NL4	0.5	0.025	0.1	0.4	-
NL5	0.5	0.025	-	0.4	0.1
NL6	0.5	0.025	0.1	0.3	0.1

Particle size and zeta potential measurements

The average particle size and size distribution of the NLCs were determined by photon correlation spectroscopy using a Zetasizer DTS ver 4.10 (Malvern Instrument, UK). The samples of NLCs dispersions were diluted to 1:9 v/v with deionized water. The particles size and size distribution are represented by average (diameter) and the variance (polydispersity index) of the Gaussian distribution function in the logarithmic axis mode. The zeta potential determination was based on the particle electrophoretic mobility in aqueous medium. For measurement of zeta potential, Zetasizer (Malvern Instrument UK) was used. The field strength was 20 V/cm on a large bore measures cell. Samples were diluted with double distilled water adjusted to a conductivity of 50 µS/cm with a solution of 0.9% NaCl.

Transmission electron microscopy

Transmission electron microscopy was used to visualize particles morphology. Samples were examined after negative staining under a transmission electron microscope (Philips Morgangni 268, Eindhoven, and Netherland) at an acceleration voltage of 20 kv. A drop of the different formulations was placed on different carbon coated grids to leave a thin film on the grids. Then, the film was negatively stained with 1% phosphotungustic acid (PTA) by placing a drop of the staining solution and the excess of the solution was drained off with the filter paper. The grid was allowed to dry thoroughly, formulations were viewed under transmission electron microscope, and photographs were taken at suitable magnification.

Determination of drug entrapment efficiency

The NLCs were centrifuged for 20 min at 10,000 rpm (Remi Instruments Ltd. Mumbai India). The drug contents in the supernatant after centrifugation were measured spectrophotometrically at a wavelength of 223 nm. Prior to the measurement, the samples were filtered using a 0.22 nm pore size membrane filter. One milliliter of aliquot was withdrawn from the filtrate and added to a 25

ml volumetric flask. The flask was then filled with distilled water to make the final volume to be 25 ml. The drug entrapment efficiency in the NLCs was calculated using following equation.

$$\%EE = \left(\frac{W_{initial\ drug} - W_{free\ drug}}{W_{initial\ drug}} \right) \times 100$$

Where $W_{initial\ drug}$ is the weight of drug added to the system, while $W_{free\ drug}$ is the analyzed weight of free drug in the aqueous phase.[8]

Differential scanning calorimetry (DSC)

Thermograms were recorded with a differential scanning calorimeter (Jade DSC). For DSC measurements, standard aluminum pans with accurately weighed 2–5 mg samples were tightly sealed. An empty pan was used as reference. Samples were heated at the scanning rate of 10°C/min over a temperature range between 30 and 300°.

In vitro release study

The Glipizide-loaded NLCs suspension (2 ml) was transferred to a dialysis tube (molecular weight cutoff 12,000), and the tube was introduced into a conical flask containing 50 ml of a phosphate buffer solution (pH 6.8). The medium was stirred at 100 rpm at 37°C. At predetermined time intervals of 1, 2, 3, 4, 5, 6, 8 and 24 hours, a 2 ml sample of the medium was taken and replaced with the same amount of fresh medium. The amount of Glipizide released from the NLCs was determined by spectrophotometrically at 223 nm.[9]

RESULTS AND DISCUSSION

Particle size and zeta potential measurements

The stability of oil dispersions depends on the balance of emulsifiers at the oil-water interface and the nature of the oil phase. Therefore three types of surfactants were used as an emulsifier (Table 1 and Table 2); alone or in different combination to produce highly stable system. The particle size and zeta potential was taken as parameters to evaluate the formulations. The particle size and zeta potential of all the formulations were

determined immediately after production. It was found that the formulations that contain only single emulsifier exhibit larger particle size. The mean particle size decreased, when combination of surfactant used. NL6 prepared with combination of three surfactants exhibited smallest mean particle size and narrow size distribution. The combination of surfactants would not only better the system's stability and particle morphous, but also decrease the particle size because of its synergism.[10, 11]

Zeta potential is a key factor to evaluate the stability of colloidal dispersion. In general, particles could be dispersed stably when absolute value of zeta potential was above -30mV due to the electric repulsion between particles. Formulation NL3 exhibited a highest zeta potential due to presence of STC (ionic emulsifier). The reason might be after the ionization in water the emulsifier was adsorbed to the particle/water interface, and formed an electric double layer. The zeta potential of formulation NL2 with nonionic surfactant was around -20 mV. Non-ionic surfactant could not ionize into charging group like ionic, but demonstrated its zeta potential, the reason might be due to molecular polarization and the adsorption of emulsifier molecule on the charge in water, it was absorbed to the emulsifier layer of particle/water interface and electric double layer similar to ionic was formed. Formulation NL6 was added with ionic surfactant, its zeta potential was higher than all the formulations except NL3. The reason might be the uncharged soft chain of non-ionic emulsifiers of

Poloxamer 188 inserted among the charged polar group of ionic surfactants of STC and lecithin, the charges decreased resulting in the zeta potential of formulation NL6 decrease compared with the formulation NL3 only added with ionic surfactant SDC.[11, 12, 13, 14]

Transmission electron microscopy

TEM images were taken to investigate the morphology of optimized NLCs formulations (Fig. 1). TEM images revealed that NLCs are nearly spherical in shape with the particles size ranging from 200 nm to 300 nm that is almost closer to the particle size obtained by PCS.
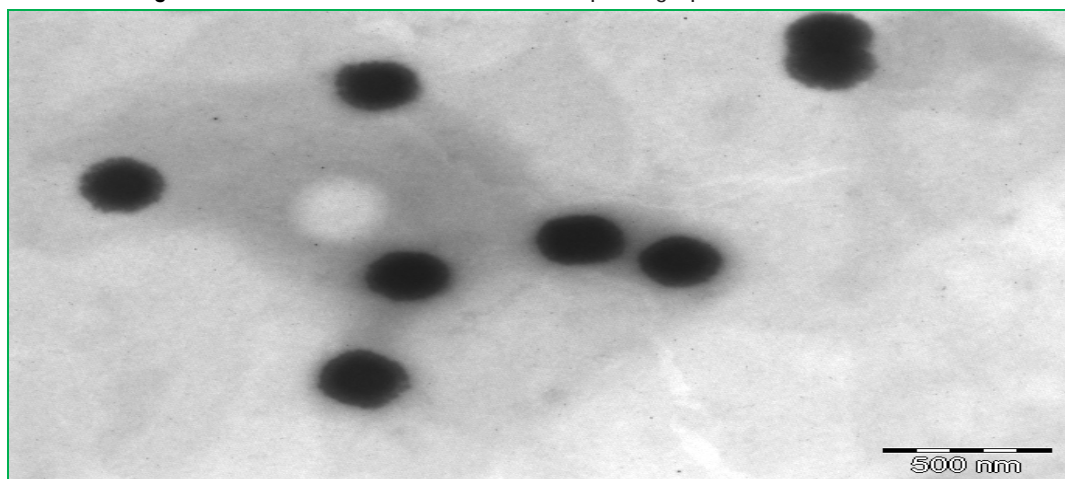
Entrapment efficiency

NLCs were prepared from blends of solid lipid (compritol ATO 888) and liquid lipid (OA) which were mixed in such a manner that the particle solidifies upon cooling but does not recrystallize and remains in the amorphous state. It has been reported that a second type of NLCs is formed when the lipid molecules are chemically very different, resulting in a structure with many imperfections leave sufficient space to accommodate the drug and thus higher loading capacity and entrapment efficiency. It was reported that entrapment efficiency and drug loading capacity of NLCs increases with increasing the percentage of liquid lipid (OA). Therefore 30% of OA was taken as an optimum concentration.

Table 2: Particle size, size distribution, zeta potential and entrapment efficiency of NLCs formulations

Sample	Particle size (nm) ± SD	PI ± SD	Zetapotential (mv)	% Entrapment efficiency
NL1	706±5.10	0.624±0.11	-28.3±1.6	45.4±1.8
NL2	356±3.58	0.311±0.05	-20.3±2.18	68.4±1.6
NL3	376±1.24	0.321±0.06	-33.2±1.4	61.3±1.7
NL4	254±3.21	0.218±0.05	-24.4±1.6	76.2±2.5
NL5	226±5.25	0.215±0.03	-27.3±0.56	71.5±2.2
NL6	197±2.09	0.212±0.02	-30.3±1.3	82.5±2.4

Figure 1: The Transmission electron microphotographs of NLCs formulation

The experimental results indicated that the different surfactants had critical effect on the entrapment efficiency. Formulation NL1 showed poor entrapment efficiency that may be due to poor formation of NLCs. The formulations, in which combinations of surfactant were used, exhibit good entrapment efficiency. As previously reported use of mixture of surfactant produced mixed surfactant film with sufficient viscosity to promote stability. [15, 16, 17, 18, 19] (Table 2 & Fig 2)

Figure 2: The drug entrapment efficiency values of NLCs formulations. The sample number was corresponding to the number listed in Table 1.
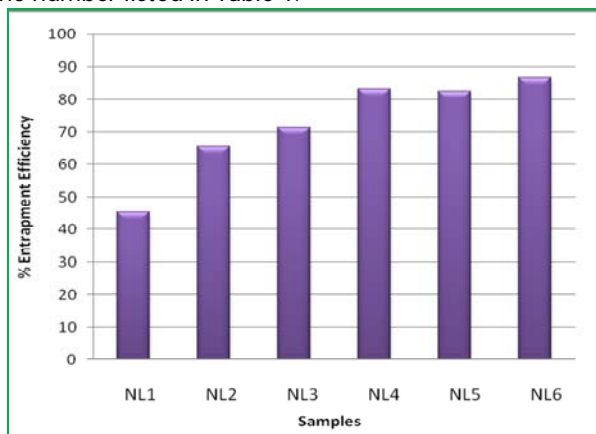
Differential scanning calorimetry (DSC)

DSC is a tool to investigate the melting and recrystallization behavior of crystalline materials like lipid nanoparticles.[20] Fig. 3 shows an over view of melting process of pure glipizide, compritol 888 ATO, blank NLCs formulation, drug loaded NLCs formulation and physical mixture.

Figure 3: DSC Thermogram (a) Pure Glipizide (b) Compritol 888 ATO (c) Physical Mixture (d) Blank NLCs (e) Drug loaded NLCs
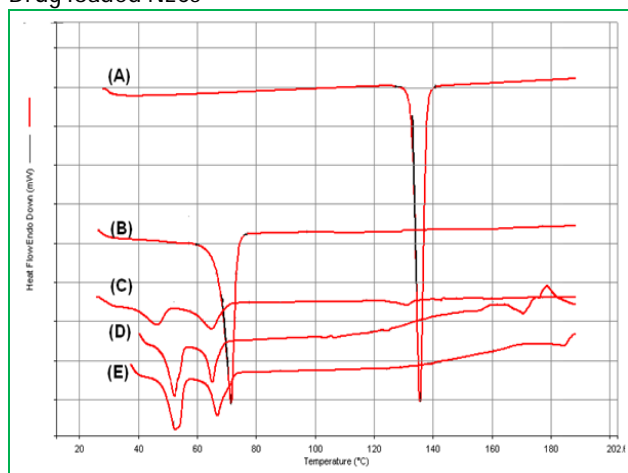

Glipizide exhibited a sharp endothermic peak at 135.61°C with an extrapolated onset of the melting peak at 132.90°C. Bulk compritol showed a sharp endothermic event, ascribing to the melting point around 74.33°C with an extrapolated onset of the melting peak at 69.82 °C. When the raw material was formulated as NLCs, the endothermic peak was broader and happened at a slightly lower temperature. The melting point of all developed formulations was lower than that of bulk compritol. However, there was a detectable melting event around 50°C confirming solid state of the lipid matrix at room temperature. The shift of melting point in NLCs may be ascribed to the interaction of solid lipid with the liquid lipid and the surfactants, or simply to the colloidal size range of the lipid particles.[21, 22] Depression of melting point and broadening of the peak of melting event are more pronounced in NLCs with higher content of the liquid lipid.[23, 24] A certain effect due to surfactant should also be taken in to consideration. [16] The drug loading did not show any significant changes in the melting peak, which indicates no effect of Glipizide on lipid matrix crystallinity. Although Glipizide had endothermic peak at 135.61°C and physical mixture had also exhibited a depressed endothermic peak at the same temperature. In addition no endothermic peak was found in the range of 100-200°C in the thermogram of Glipizide loaded NLCs formulation. These results showed that Glipizide incorporated in NLCs was not in a crystalline state but in an amorphous form. Similar results were also reported by other groups.[25, 26] The amorphous form was thought to have higher energy with increased surface area, subsequently higher solubility, dissolution rates and bioavailability.[27, 28]

In vitro release studies

The in vitro release study of NLCs formulations was carried out for 24 hours by using dialysis bag method in phosphate buffer (pH 6.8) at 37°C temperature and stirred by a magnetic bar at 100 rpm under sink condition. The release profile of all the NLCs formulations are shown in the fig. 4. The formulations NL1 and NL3 had shown 97% and 98% drug release within 4 and 5 hours respectively.

Figure 4: *In vitro* drug release study of NLCs formulations
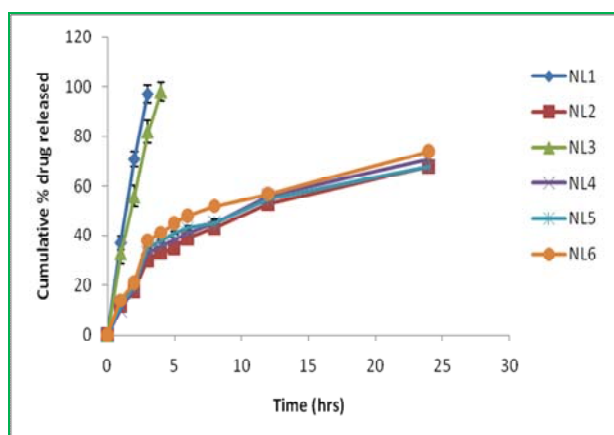
This may be due to poor formation of NLCs. On contrary, the rest of the NLCs formulations showed biphasic release pattern, which was drug burst release at initial stage and followed by sustained release at the constant rate. Possible explanation for this behavior is the difference in melting point of solid lipid and liquid lipid, and the solid

lipid which has a higher melting point could crystallize first forming a liquid lipid free or little lipid core. Finally, most of the liquid lipid located at the outer shell of the nanoparticles, led to drug enriched shell leading to drug burst release at the initial stage observed.[7] Regarding the formulations NL2, NL4, NL5, NL6 the value of drug release after 24 hours were found to be 68±1.863%, 71±2.256%, 68±1.956 and 74±0.246% respectively.

CONCLUSION

In the present study, we prepared NLCs formulation and investigated the effect of surfactant blend on development and characterization of NLCs. The formulations consisted of lecithin and sodium taurocholate alone showed poor performance where as the formulation in the study consisted of three types of surfactants including lecithin, Poloxamer 188 and Sodium taurocholate exhibited smallest particle size, narrow size distribution, better entrapment efficiency and good release profile.

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