INTERNATIONAL JOURNAL OF BIOPHARMACEUTICAL & TOXICOLOGICAL RESEARCH

FORMULATION DEVELOPMENT AND CHARACTERIZATION OF ORAL HYPOGLYCEMIC AGENT LOADED SOLID LIPID NANOPARTICLES

Jitendra Gupta[1]* Ajit Kumar Rajpoot,[1] Rajiv Soni[1].Pankaj Sharma[2]

1. Veerayatan Institute of Pharmacy Jakhaniya, Tal- Mandvi-Kutch, Gujarat.

2. Vidyasthali Institute of Technology, Science and Management (Pharmacy), Jaipur.

Keywords:
Solid lipid nanoparticles, Nateglinide, Cephalin, Diabetes, Stability studies

Corresponding Author-
Jitendra Gupta
Veerayatan Institute of Pharmacy Jakhaniya, Tal-Mandvi-Kutch, Gujarat Phone no:- +91-9409115430
Email id:-
ajitmpharma@gmail.com

ABSTRACT:

Solid Lipid Nanoparticles has been used as suitable carriers for delivery of drug with poor solubility. Nateglinide was taken as model antidiabetic drug was incorporated in Solid lipid Nanoparticles (SLNs) loaded with were prepared by a hot homogenization method using cephalin and lecithin as lipids and Tween 80 as stabilizer. Characterization and evaluation studies such as particle size measurement, poly dispersity index, Zeta potential, entrapment and loading capacity, Stability studies, in vitro release studies were done to ensure the quality Solid lipid Nanoparticles. Scanning Electron Microscopy showed the SLN particles were spherical shape in the size between 85 – 120 nm and the poly dispersity indexes were 0.148 to 0.227. The zeta potential was - 27.1 ± 2.5 to
−36.1 ± 2.1 mV. The entrapment efficiency (EE %) and drug loading capacity (DL %) determined were 80.4 ±
4.2 % to 92.3 ± 7.2 %. Differential scanning calorimetry (DSC) thermo grams revealed the stability of SLNs with no tendency of recrystallisation. in situ and externally sink method revealed the release pattern of drug was found to follow fick's and Higuchi equations. Results of stability evaluation showed a relatively long-term stability after storage at 5°C and 30°C for 12 weeks. In this formulation increase in concentration of lipid content has increased the entrapment efficiency of SLN. In conclusion, SLNs with small particle size, excellent physical stability, high entrapment efficiency, good loading capacity for diabetic drug can be maintaining blood glucose level normally for long time.

Introduction:

Solid lipids Nanoparticles4 (SLN) are a colloidal carrier system for controlled drug delivery and followed by the development of Emulsion, liposomes, Microparticles and Nanoparticles based on synthetic polymers since the beginning of the nineties. Compared to traditional carriers the SLN combine advantages of polymeric Nanoparticles and o/w fat

emulsions for drug delivery administration, such as a good tolerability compared with polyester Nanoparticles, a high bioavailability by oral and dermal administration of targeting effect on brain Due to the production by high pressure homogenization or Microemulsions they can be produced on large industrial scale.Nateglinide2 is an oral blood glucose lowering drug of meglitinide class used in the management of type 2 diabetes mellitus (NIDDM). Nateglinide, an amino acid derivative of D-phenylalanine, stimulates the secretion of insulin by binding to the ATP potassium channels in pancreatic beta cells. The result is an increase in beta-cell calcium influx, which leads to rapid, short-lived insulin release.

Nateglinide lowers blood glucose by stimulating the release of insulin from the pancreas. It achieves this by closing ATP-dependent potassium channels in the membrane of the β cells. This depolarizes the β cells and causes voltage-gated calcium channels to open. The resulting calcium influx induces fusion of insulin-containing vesicles with the cell membrane, and insulin secretion occurs.

The drug is rapidly and completely absorbed in the small intestine. The estimated bioavailability is 72%. Nateglinide is highly bound to plasma proteins, is metabolized extensively by the liver, and has an elimination half-life of 1.4 hours. Moreover it produces hypoglycemia after oral administration

Materials & Apparatus

Nateglinide obtained as gift sample from J.B.Chemicals and pharmaceuticals Ltd. Panoli, Gujarat. Phosphatidylethanolamine (cephalin), lecithin was obtained from Gujarat liqui pharmacaps Pvt. Ltd, Vadodara. Tween 80, poly ethylene glycol was obtained as gift sample from J.B.Chemicals and pharmaceuticals Ltd. Panoli, Gujarat. All reagents were of analytical grades.

Preparation of Nateglinide solid lipid Nanoparticles:

Solid lipid Nanoparticles dispersion was prepared by hot homogenization technique (table:1). In hot homogenization technique4, lipid was melted at temperature ten degrees above its melting point then Nateglinide was added to the melted lipid. The dispersion was kept at the same temperature until it appeared optically clear. Tween 80 (2.5 % w/w of total weight of SLN dispersion as stabilizer) was dissolved in distilled water and heated to the same temperature of lipid mixture. Hot surfactant solution was then added to the melted lipid-drug mixture and emulsified by a homogenizer at 12500 rpm for 2 hours. The formulation was then removed from water bath and the dispersion of SLN was mixed gently at room temperature until cooling.

Table no 1: Formulation of Repaglinide Solid Lipid Nanoparticles.

S.No	Ingredients	F1	F2	F3	F4	F5
1	Nateglinide	120	120 mg	120 mg	120mg	120 mg
2	Cephalin	12gm	6gm	-	-	6gm
3.	Lecithin	-	-	12	6mg	6gm
3	Tween 80	2.5%	2.5%	2.5%	2.5 %	2.5%
4	Water	Q.S	Q.S	Q.S	Q.S	Q.S

Table: 2 Entrapments Efficiency Mean ± sd (n=3)

S.No	Formulation no	Entrapment efficiency(%)
1	F1	92.10 ± 2.13
2	F2	90.30 ± 3.50
3	F3	92.70 ± 7.10
4	F4	82.07 ± 7.17
5	F5	80.14± 3.24

Particle shape and surface Morphology 4, 5, 6

Scanning Electron Microscopy:

Scanning electron microscopy is an excellent tool for physical observation of morphological features of particles both initially and degradation process. It is helpful to examine particles shape and surface characteristics such as surface area and bulk density. The SLN were sprinkled on to one side of the adhesive stub.

The stub was then coated with conductive gold with JOEL –JFC 1600 auto coater and was examined under JOEL –JFC 6360 scanning electron microscope for qualitative assessment of SLN morphology.

Zeta potential of Rosiglitazone SLN:

Zeta potential was determined by using Zetameter, about 1 mg of SLN was dispersed in 1 ml of distilled water by sonication and it was subjected to Zeta potential analyzer.

Polydispersity index:

Polydispersity of Solid lipid Nanoparticles non uniform size was calculated from the formula, Polydispersity = [D0.9 - D0.1] ÷ D0.5 Where D0.9, D0.1 and D0.5 particle diameters determined at 90th, 5oth and 10th percentile of undesired particles

respectively. Higher the Polydispersity index values indicate the high level of non uniformity and its value is used to characterize the nanoparticle as monodisperse, homogenous and heterogeneous systems.

Entrapment efficiency of SLN (% EE)

Nateglinide content of the Nanoparticles was determined by HPLC after drug extraction from SLN. Solid lipid Nanoparticles formulation was centrifuged at 19,000 rpm and 5ºC for 2 hr. Then supernatant was removed and the sediment was washed with distilled water. Then the sample was dried by freeze dryer for 8 hours. 10 mg of the freeze-dried formulation was dissolved in 1 ml dichloromethane and the volume was completed to 10 ml with ethanol. After cooling to room temperature the volume was then completed to 10 ml by water. After filtration through membrane filter a suitable dilution was performed. An aliquot of 10µl was injected onto HPLC column and assayed for drug content. The entrapment efficiency was calculated as the percentage ratio between the quantity entrapped in SLN and the amount of Repaglinide added to the melted lipid phase.

Table no 3: Permeation parameters of Repaglinide solid lipid Nanoparticles

S.No	Batch no	J(µg/cm²/h)± SD	P (cm/h) x 10^{-2} ± SD	D` (h)$^{-1}$ ± SD
1	F1	5.01±0.6	0.200±0.0002	0.064±0.016
2	F2	7.07±0.8	0.282±0.0003	0.330±0.200
3	F3	8.29±0.2	0.331±0.0001	0.241±0.035
4	F4	8.10± 0.4	0.304±0.0001	0.220±0.041
5	F5	8.78±0.1	0.351±0.00002	0.095±0.017

Mean ± sd (n =5)

Table No 4: In vitro release kinetic studies mean ± sd (n=3)

Sr.No	Batch no	R^2 (Fick's)	R^2 (Higuchi)
1	F1	0.9924	0.9944
2	F2	0.9909	0.9733
3	F3	0.9985	0.9888
4	F4	0.9980	0.9871
5	F5	0.9985	0.9832

Mean ± sd (n =3)

Table No 5: Particle size, poly dispensability Index, Zeta Potential of SLN

Sr.No	Batch no	Mean Size (nm)	PDI	Zeta potential(mV)
1	F1	120.00 ± 1.56	0.227 ± 0.07	-32.5±0.7
2	F2	101.00 ± 1.64	0.18 9 ± 0.05	-27.1± 2.5
3	F3	93.17 ± 3.21	0.243 ± 0.06	-34.5± 0.9
4	F4	87.40 ± 1.32	0.197 ± 0.02	-36.1± 2.1
5	F5	85.62 ± 2.67	0.148 ± 0.07	-36.0 ± 1.1

In- vitro release studies of Nateglinide[7, 8, 9]

In-vitro release across cellophane membrane was conducted using Franz diffusion cells (25 mm diameter orifice) the cells have 20 ml receptor volume. The area of diffusion was 5 cm². The cells were placed in a between the cell stirrer and maintain the water bath temperature to 32 ± 0.5°C. Cellophane membrane (molecular weight cut- off: 6000-8000) previously soaked in receptor medium was clamped in between the donor and the receptor chamber of diffusion cell. A suitable aliquot of the formulation (equivalent to 10 mg drug/g of SLN dispersion) was added to the donor chamber of the diffusion cell which was occluded with a paraffin film. The receptor medium was normal saline solution containing propylene glycol to maintain sink condition. The receptor medium was stirred by magnetic bar. 1 ml sample were withdrawn from the receptor compartment at the following time intervals: 2, 4, 6, 8, 18, 20, 22 and 24 h and were replaced by equal volume of the fresh receptor fluid. Samples were analyzed by HPLC after suitable dilution. The cumulative amount of drug released into the receptor medium was plotted versus time and steady state flux, J, (µg/cm2/h), was determined from the slope of the linear portion of the plot.

Stability Studies

To evaluate the physical stability of elected formulation (F5) containing 1:1 ratio of cephalin and lecithin. The release behavior and particles size measurement were evaluated after storage period of 12 week at 5° C and 30° C in the dark condition.

Results and discussion

Morphological of SLN

Scanning electron microscopic studies revealed the formation of spherical Nanoparticles with regular surface (fig:1). Surface of solid lipid Nanoparticles were smooth. So particles penetration was good compare to irregular particles. Here lecithin particles were regular surface but shrinkage was observed. In cephalin solid lipid Nanoparticles were observed rough. But in mixture of lipids (cephalin: lecithin) containing formulations surface as well as morphology was uniformity. It was observed in fig no. 1.

Particle size and Polydispersity index of SLN:

Particle size analysis of SLN indicated that increase in particle size were observed with increasing cephalin concentration (table:5) may be due to decrease in homogenization efficiency with increasing content of dispersed phase (lipid phase) in the formulation F1, F2 mean size were 101, 120 nm respectively.

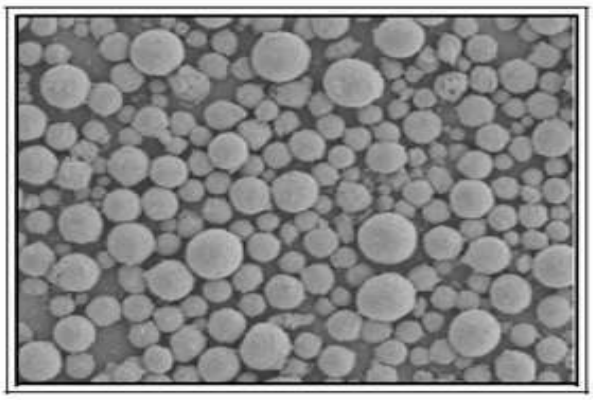

Fig:1 SEM image of Nateglinide SLN

Lecithin formulations were produced small particles 93.17 nm. But in case mixture of lipid has produced very small uniform size particles 85.62 nm (table no 5). So, the Polydispersity index was very low it reflects uniform size particles whereas remaining formulation has somewhat nonuniform. So, These SLN could minimize the uptake of macrophages giving long circulating systems in the blood stream that could potentially accumulated in receptors as a consequence of good effect due to their small size

Zeta potential:

The zeta potential of SLN was in the range of -27 to -38 mV which shown in table 5. Previous studies have shown that zeta potential affect the intracellular distribution of SLN

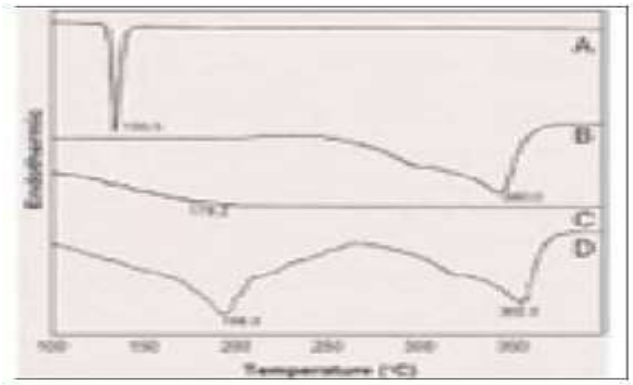

Fig:2 DSC image of (A) drug, (B) lipid 1 (C)lipid 2 (D) mixture

The negative charge present in the core of the Nanoparticles matrixes are involved in electrostatic interaction with weakly basic drugs molecules. Thus, negative charge on the surface of Nanoparticles could contribute to the negative zeta potential of SLN.

Entrapment efficiency of SLN (% EE):

Factors determining the % EE of drug in the lipid Nanoparticles are solubility of the drug in melted lipid, chemical and physical structure of solid matrix, and polymorphic state of lipid. The drug entrapment efficiency ranged from 81.2% - 91.7 % which indicated in table:2

Table no: 6 Stability studies of solid lipid Nanoparticles Mean ± sd (n =3)

S.No	Formulation no	Fresh particles (nm)	0° C (nm)	30°C (nm)
1	F1	120.00	143.00	150.00
2	F2	102.00	107.00	112.00
3	F3	93.17	102.00	107.00
4	F4	87.40	91.40	92.40
5	F5	85.62	87.80	88.10

The chemical nature of the lipid is important because lipid which forms highly crystalline10, 11, 12 particles with perfect lattice lead to drug expulsion Complex lipids which confirmed by DSC, containing free fatty acids of different chain length form less perfect crystals with many imperfections offering space to accommodate the drug. This could explain the high entrapment efficiency of SLN

Effect of lipid content on entrapment efficient13:

In the Nateglinide solid lipid Nanoparticles concerning the effect of lipid concentration on the entrapment efficient, Since the % EE decrease from 89.3 to 91.7% as when concentration of cephalin decreased from 10 gm to 5gm and % EE decrease from 92.3 to 82.2% as when concentration of lecithin decreased from 10 gm to 5gm which indicated that the increase in lipid concentration has improved entrapment efficiency (fig 4).

Invitro release studies of Nateglinide SLN:

The cumulative amounts of drug released across cellophane membrane after application of SLN formulations. The release data were fitted into Fick's and Higuchi equations. Almost all the SLN formulations followed Fick's law better than Higuchi model as indicated by the higher values of coefficient of determination, (R2>0.9944) as shown in Table (4)The in vitro release data were then treated in accordance to Fick's law to calculate the flux (J) which is the amount of drug permeated per unit area and per unit time (µg/cm²/h), the permeability coefficient (P,

cm/h) and diffusion parameter (D , h-1) as shown in Table 3. Statistical analysis of release data using ANOVA revealed the following order for the release of drug after application.

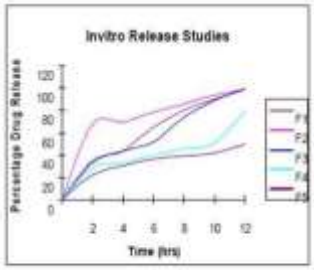

Fig No: 3 Invitro release studies of SLN different formulations: F1, F2,F3 F4

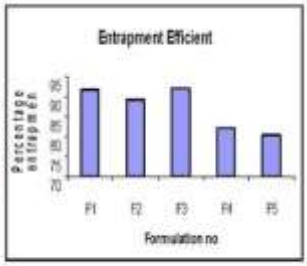

Fig No: 4 Entrapment efficient of SLN

Stability studies:

During the period of storage, the formulation showed no change in colour and creaming and phase separation. The cumulative amount of drug release significantly decreased on 10 and 12 week to correlate the change in permeation of Nateglinide with particles size. Statistically, the particle size increase significantly after storage of 12 week at 30°C. It was assumed that the high temperature (25° C) increase the kinetic energy of system, which could accelerate the collision of particles. Consequently increase the possibility of aggregations of particles. But there was no significant increase in particle size of SLN during stored at 5°C after 12 weeks. During studies, rearrangement of the lipid crystal lattice might occur in favour of thermodynamically stable configuration and this often connected with expulsion of the drug molecules. This could be resulted in decrease in drug release after storage of SLN which shown in table no. 6.

This effect was opposed by slight gelling of SLN that could be observed after 12 weeks at 30 C. Gelling phenomenon described the transformation of a low viscosity into gel. Gelling of SLN may lead to increases in micro viscosity and retard drug diffusion and decrease its release. The final consequences of the last mention effect may be decrease in overall amount of drug release.

Conclusion:

In this study the potential of SLNs dispersions as carriers for delivery of antidiabetic drugs was exploited. Solid Lipid Nanoparticles were prepared by the hot homogenization using bioacceptable lipids such as Cephalin and lecithin and Tween 80as a emulsifier. Drug loaded SLNs showed average diameters in the colloidal size range, a good loading capacity and drug release. Results strongly support the potential application of SLNs in Diabetic therapy as drug delivery system for the entrapment of all type of diabetic drugs.

Acknowledgements:

The authors are thankful to management of K.C. Reddy institute of pharmaceutical science for providing research facility. The authors are also thankful to Tri-Star formulations Pvt. Ltd, Pondicherry for providing Nateglinide as a gift sample.

References

1. Rendell., 2004. Advances in diabetes for the millennium, drug therapy of type 2 diabetes MedGenMed: Medscape general medicine 6 (3 Suppl): 9.PMIDV 15647714.
2. Culy, C.R., Jarvis, B., 2001. Nateglinide: a review of its therapeutic use in type II diabetes mellitus. Drugs 61, 1625–1660.
3. Rizzo MR, Barbieri M, Grella R., 2003. Nateglinide is more efficient than glimepiride on insulin secretion and post-prandial glucose excursions in patients with type 2 diabetes. A short term study. Diabetes Metab.30:81-9.
4. Mehnert, W., Mäder, K., 2001. Solid lipid nanoparticles production, characterization, and applications. Adv. Drug Deliv. Rev. 47, 165-196.
5. Trotta, M., Cavalli, R., Carlotti, M.E., 2005. Solid lipid microparticles carrying Insulin formed by solvent-in -water emulsion-diffusion technique. Int. J. Pharm. 288, 281- 288.
6. Bouwstra, J.A., Honeywell-Nguyen, P.L., 2002. Skin structure and mode of action of vesicles. Adv. Drug Deliv. Rev. 54, S41-S55.
7. 7. Yener, G., Incegul,T., Yener, N., 2003. Importance of using solid lipid microspheres as carriers for UV filters on the example of octyl methoxy cinnamate. Int. J. Pharm. 258, 203-207.
8. Souto, E.B., Wissing, S.A., Barbosa, C.M., 2004.Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int. J. Pharm. 278, 71-77.

9. Lim, S-J., Kim, C-K., 2002. Formulation parameters determining the physicochemical characteristics of solid lipid nanoparticles loaded with all Trans' retinoic acid. Int. J. Pharm. 243, 135-146.
10. Bunjes, H., Westesen, K., Koch, M.H., 1996. Crystallization tendency and polymorphic transition in triglyceride nanoparticles. Int. J. Pharm. 129, 159-173.
11. Wissing, S.A., Müller, R.H., 2002. Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. J. Control.Release. 81, 225-233.
12. Jenning, V., Gohla, S., 2000. Comparison of wax and glyceride solid lipid nanoparticles . Int. J. Pharm. 196, 219-222.
13. Jenning, V., Gohla, S., 2000. Comparison of wax and glyceride solid lipid nanoparticles. Int. J. Pharm. 196, 219-222