WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 7, 1319-1328. Research Article ISSN 2277– 7105

FORMULATION AND IN-VITRO EVALUATION OF NANOPARTICLES OF LINAGLIPTIN

K. Navaneetha*[1], A. Navya[2], B. Venkateswara Reddy[3], T. Saritha[2] and N. Jabili[2]

[1]Assistant Professor, Department of Pharmaceutics, School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad, Telangana.

[2]Department of Industrial pharmacy, St. Paul's College of Pharmacy, Hyderabad, Telangana.

[3]Professor, Department of Pharmaceutics, Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda, Telangana.

Article Received on
12 May 2017,

Revised on 01 June 2017,
Accepted on 22 June 2017

DOI: 10.20959/wjpr20177-8838

***Corresponding Author**
K. Navaneetha
Assistant Professor, Department of Pharmaceutics, School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad, Telangana.

ABSTRACT

The aim of the present work is to prepare and evaluate the sustained release nanoparticles of Linagliptin an anti diabetic in order to improve its therapeutic effect and reducing dosing frequency and its dose related side effects. Linagliptin containing Polycaprolactone and Polyvinyl Alcohol (PVA) nanoparticles were prepared by solvent evaporation method. The result showed that this method is reproducible and led to the efficient entrapment. Formulation had spherical particles in the particle range from 100 - 1500nm. Some process variables like effect of PVA concentration, Polycaprolactone concentration were also evaluated with respect to drug content and encapsulation efficiency. The maximum encapsulation efficiency is 89 ± 0.61%. The sustained release behaviour of nanoparticles were evaluated in phosphate buffer saline and results revealed that Linagliptin loaded nanoparticles are most suitable mode of delivery of drug for promising therapeutic action.

KEYWORDS: Linagliptin; Polycaprolctone; Polyvinyl Alcohol; solvent evaporation method.

INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle

matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period of time and target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes.[1-4]

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction in toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties.[5, 6]

Linagliptin is an oral antidiabetic drug used in the treatment of type-2 diabetes mellitus which acts by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4).[7] It belongs to BCS class-III drug ie., it has high solubility and low permeability and has a low bioavailability of 30%. Therefore the present research work is aimed to develop Linagliptin loaded nanoparticles to achieve a sustained release profile with maximum encapsulation efficiency by using different polymers. Nanoparticles improve permeability of drug and further decrease irritation potential due to entrapment.

MATERIALS AND METHODS

Materials: Linagliptin was obtained from KP labs, Hyderabad. Ethyl acetate was procured from S.D. Fine chemicals ltd, Polycaprolactone was procured from Pro prime Laboratories ltd and poly vinyl alcohol was procured from metro chemical industries, Hyd.

Methods

Preparation of Linagliptin Nanoparticles

Linagliptin nanoparticles were prepared by solvent evaporation method. First mutually saturated aqueous and organic phase were prepared. The saturated water contained 8.3% of ethyl acetate and the saturated solvent contained 3% of water. PVA was dissolved in saturated water at 50^{o}c for 2hr. Polycaprolactone was dissolved in saturated ethyl acetate at 50^{o}c and Capmul MCM oil was added when the solution has come back to room temperature.[8] The resulting organic solution was poured into the aqueous phase and emulsified with lab stirrer devise (Remi Motor Ltd, India) for 45 min at 4000 rpm and sonicated (Sartorius) for 15 min at 9cycle/min. After sonications the emulsion under gentle stirring with magnetic bar allowed the ethyl acetate to leave the droplets. The organic solvent and part of the water were evaporated under reduced pressure to afford a purified and concentrated suspension.[9] The prepared formulations were stored in cool and dry place. Nine different batches were prepared and labelled as LNP-1 to LNP-9 with composition as shown in Table-1.

Table 1: Formulation of Linagliptin Nanoparticles

Ingredients	LNP1	LNP2	LNP3	LNP4	LNP5	LNP6	LNP7	LNP8	LNP9
Linagliptin (mg)	50	50	50	50	50	50	50	50	50
PVA (%w/v)	2	1.75	1.5	1.25	1	0.75	0.5	0.25	---
Poly caprolactone (%w/v)	---	0.25	0.5	0.75	1	1.25	1.5	1.75	2
Water (ml)	40	40	40	40	40	40	40	40	40
Ethyl acetate (ml)	10	10	10	10	10	10	10	10	10

CHARACTERIZATION OF NANOPARTICLES

Particle Size Analysis and Zeta potential determination

The particle size distribution of the drug-entrapping nanoparticles was analyzed by a zeta sizer 3000 HSA with a photo correlation spectroscope (Malvern, Worcestershire, UK) at 25°C. This analysis assumed sphere-like LNPs without multiple scattering. The LNPs were immersed in 0.1 M trisbuffer with a particle concentration of 2 mg/ mL. The suspension was gradually injected into a quartz tube to avoid the formation and interference of bubbles. The duration of the detection was 120 sec.[10]

Surface Morphology

Shape and surface morphology of nanoparticles was studied using high-resolution scanning electron microscopy (SEM). The samples on conductive carbon paint were placed in a

specimen holder, vacuum-dried, and sputter-coated with platinum using accelerating voltage of 2 kV for 90 sec.

Drug Entrapment efficiency

The encapsulation efficiency of nanoparticles was determined by centrifuging the nanoparticles using ultracentrifuge at 10000 rpm for 30 min. The amount of free Linagliptin in the supernatant was measured by UV spectrophotometer at 241 nm. The Linagliptin entrapped in the nanoparticles was calculated as

Entrapment efficiency (%) = $(Tp - T_f)\ 100/Tp$

Where, Tp is the total Linagliptin used to prepare the nanoparticles and T_f is the total free Linagliptin in the supernatant.[11]

***In-vitro* Release studies**

In-vitro drug release studies from nanoparticles was carried out by dialysis method (Dialysis membrane-60 HI MEDIA, Mumbai). The donor chamber filled with 5ml of nanoparticles suspension, whereas reservoir chamber containing the PBS pH 7.2. This total setup was placed on a rotary shaker rotating at 50 rpm at 37°C ± 1°C. At pre-determined time intervals the content of receiver chamber was withdrawn and replaced with equal volume of fresh phosphate buffer, the amount of Linagliptin that diffused into the receiver chamber was quantified by UV- spectrophotometer at 241 nm.[12]

Stability studies[13]

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 25^0C/60% RH analyzed every month for period of three months.
2. 30^0C/75% RH analyzed every month for period of three months.
3. 40^0C/75% RH analyzed every month for period of three months.

The optimized formulation kept for stability at room temperature for 3 months.

RESULTS AND DISCUSSION

Particle Size Analysis: The size distributions along the volume mean diameter of the nanoparticles were measured by laser scattering light using Malvern Laser Analyzer Instruments. The obtained results are shown in Table-2 and in Figures 1.

Table 2: Particle size of nanoparticles of Linagliptin

Formulation code	Avg. Size of Particles
LNP 1	334.5 nm
LNP 2	256.4 nm
LNP 3	353.7 nm
LNP 4	248.4 nm
LNP 5	266.7 nm
LNP 6	299.6 nm
LNP 7	351.5 nm
LNP 8	214.7 nm
LNP 9	384.8 nm

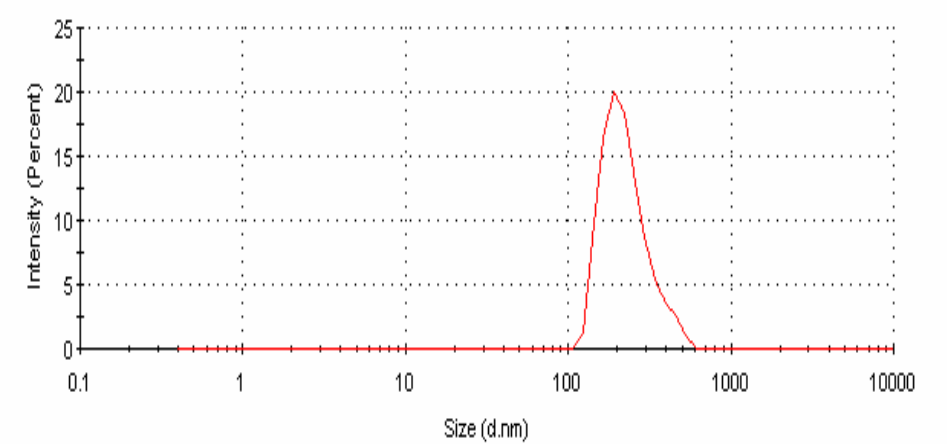

Figure 1: Particle size distribution of optimized formulation

Zeta potential and Poly dispersity index

The size of the nanoparticles decreased after sonication while zeta potential was unchanged. Zeta potential was over -20 mV for all formulations while polydispersity index was less than 0.3, which indicates homogeneous nature of the formulation. Sonication for 20 min reduced particle size to nano level; thereafter, there was no further change in particle size.

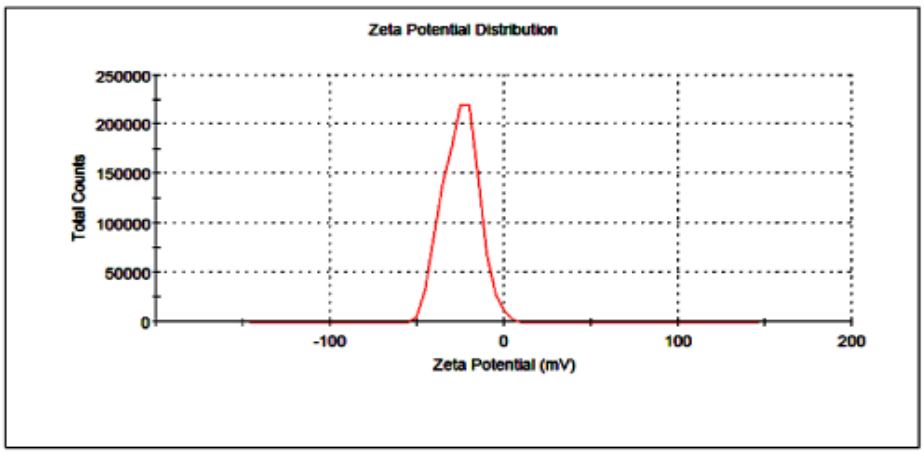

Figure 2: Zeta potential curve of linagliptin nanoparticles

Entrapment Efficiency

The entrapment efficiency of LNP gradually increased from LNP1 (55 ± 0.21%) to LNP3 (89 ± 0.61%) and decreased with formulations LNP6, LNP8 and LNP9. These results suggested that as the polymer concentration increases the entrapment efficiency of drug and after certain concentration it was decreased due to saturation capacity of the polymer.

Table 3: % Entrapment Efficiency of all formulations

Formulation Code	% Entrapment Efficiency
LNP1	55 ± 0.21%
LNP2	76 ± 0.13%
LNP3	89 ± 0.61%
LNP4	82 ± 0.46%
LNP5	79 ± 0.21%
LNP6	69 ± 0.75%
LNP7	81 ± 0.27%
LNP8	62 ± 0.53%
LNP9	78 ± 0.22%

Surface Morphology

The typical Scanning electron microscope image of optimized formulation LNP3 is shown in the figure-3, LNP exhibited discrete, uniform smooth surface and spheroidal feature. This spherical configuration suggested that the application of the Stokes–Einstein equation (linking the hydrodynamic and thermodynamic views on the diffusion of microspheres) could be reasonable to estimate the particle size of LNP. In addition, this drug carrier showed bright periphery, representing the coating of surfactants in the external layer. The polymer stabilized the nano-sized structure and prevented LNPs from coagulation. Their average diameter of LNP8 was found to be 214.7 n.

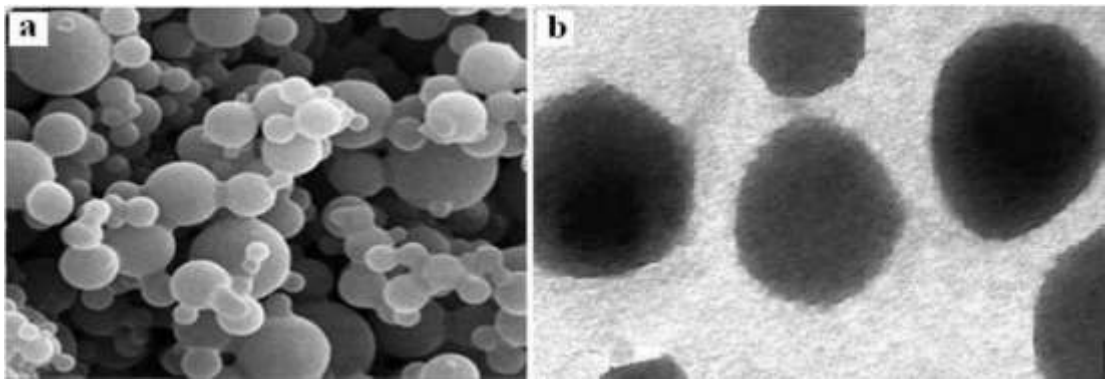

Figure 3: Scanning electron microphotograph of Linagliptin nanoparticles (A) At lower magnification (B) At higher magnification.

***In-vitro* Dissolution study of Linagliptin Nanoparticles**

From the drug release studies it was observed that as the concentration increases there was decrease in the drug release and an initial burst of release may be attributed to the drug that is adsorbed on to the surface of nanoparticles. The Linagliptin containing nanoparticles was constantly agitated during the dissolution test, collisions among LNP could first disintegrate the structure of the polymer layer. The subsequent fluidic shear devastated the particulate surfaces and caused the release of a drug. As indicated in the cumulative percentage of Linagliptin released in the initial 2-4 hr was minor. This suggested that the current formulation could prevent the drugs from the initial burst release. This suggested that the spatial distribution of the drugs confined could be quite homogeneous.

Table 4: Dissolution Profile of Linagliptin Nanoparticles

TIME (hrs)	LNP1	LNP2	LNP3	LNP4	LNP5	LNP6	LNP7	LNP8	LNP9
0	0	0	0	0	0	0	0	**0**	0
1	19.66	18.832	17.83	16.32	29.24	21.832	20.82	**22.6**	23.49
2	39.73	33.592	31.59	23.92	45.64	38.592	36.502	**48.68**	40.78
4	45.48	44.324	51.34	30.324	53.24	51.324	45.34	**63.2**	52.48
8	63.18	67.24	53.24	36.24	59.08	63.24	57.24	**75.56**	63.18
12	70.94	76.12	62.12	43.28	64.12	72.128	68.12	**83.44**	75.94
16	74.16	79.46	78.91	56.16	77.36	80.916	79.58	**94.91**	83.36
24	79.53	83.22	89.72	65.18	89.45	85.62	89.73	**99.53**	88.79

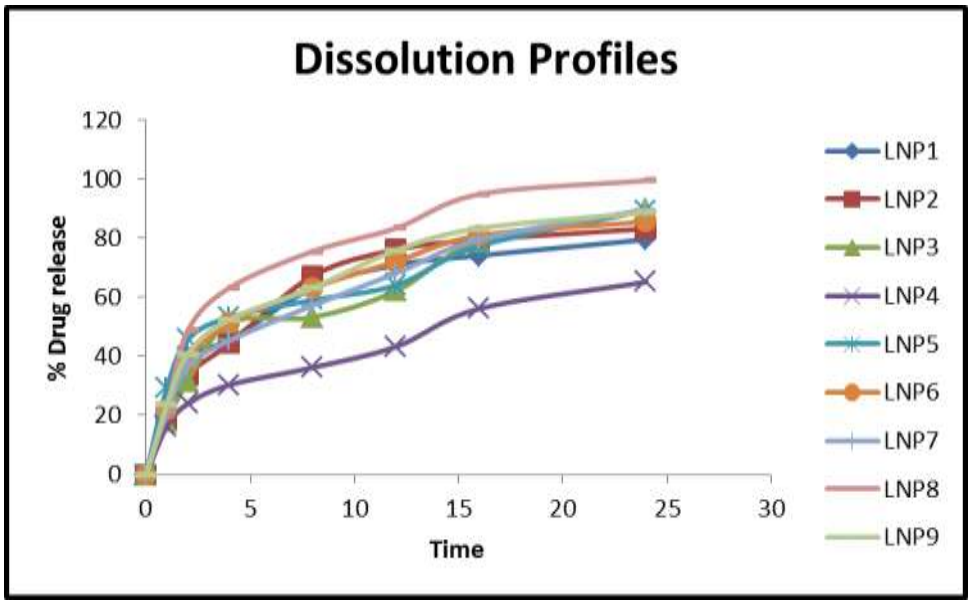

Figure 4: In-vitro Dissolution Profile of Nanoparticles

Stability study

The stability study was carried using the batch LNP8. The stability of drug loaded nanoparticles was evaluated in terms of its drug content. The stability of nanoparticles was

evaluated in PBS (pH 7.4). Nanoparticles formulation was incubated at 5-8° and 37 ± 1° for a period of 60 days. After specified time intervals, the suspension was centrifuged at 15,000 rpm for 1 h, supernatant was removed and nanoparticles were dissolved in dichloromethane. After adding of water and separation, the amount of drug was detected by UV-Vis spectro photo metric method at 241 nm.

Table 5: Results of stability studies of optimized Formulation LNP8

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
LNP8	25^0C/60%RH % Release	99.53	98.7	98.1	97.4	Not less than 85%
LNP8	30^0C/75% RH % Release	99.53	98.80	97.5	97.7	Not less than 85%
LNP8	40^0C/75% RH % Release	99.53	98.9	97.8	97.2	Not less than 85%

Table 6: Stability dissolution profile of LNP8 for 1st, 2nd & 3rd months

S.NO.	TIME(Hrs)	LNP8 1M	LNP8 2M	LNP8 3M
1	0	0	0	0
2	1	21.2	23.6	24.8
3	2	47.2	48.60	49.12
4	4	62.59	63.77	64.73
5	8	76.51	77.29	78.38
6	12	86.28	84.29	87.31
7	16	95.43	92.88	93.44
8	24	98.9	97.8	97.2

CONCLUSION

In present study nanoparticles of Linagliptin were prepared in ten different drugs to polymer ratio using solvent evaporation Technique. In present study PVA and Polycapro Lactone is used to achieve the controlled delivery of drug from polymer matrix. The particle size of various formulations varied due to variation in the composition of formulations and the mean particle size was in the range of 214nm- 384nm. The surface morphology of Linagliptin nanoparticles was seen by bifocal microscope and Scanning Electron Microscope. Surface of Linagliptin nanoparticles was found smooth and spherical. The percentage yield of different formulations were almost similar and in the range of 55 ± 0.21 − 89 ± 0.61%. The drug entrapment efficiency is decreased with the increase in drug content. This could be due to diffusion of a part of entrapped drug to the surrounding medium during preparation. The shape of the nanoparticles was found to be spherical by SEM study. Formulations with high

polymer content were observed to be fairly spherical and sphericity was decreasing with increasing drug content. Small pores and cavities were present on the surface of nanoparticles. Release of Linagliptin was evaluated in PBS (pH 7.4). Drug released within 8 to 9 hours of study. Formulation with increased polymer content showed slow drug release form nanoparticles which are up to 24 hours. No significant change was observed in the formulations during stability studies.

REFERENCES

1. Langer R. Biomaterials in drug delivery and tissue engineering: one laboratory's experience. Acc Chem Res., 2000; 33: 94-101.
2. Bhadra D, Bhadra S, Jain P, Jain NK. Pegnology: a review of PEG-ylated systems. Pharmazie 2002; 57: 5-29.
3. Kommareddy S, Tiwari SB, Amiji MM. Long-circulating polymeric nanovectors for tumor-selective gene delivery. Technol Cancer Res Treat., 2005; 4: 615 - 25.
4. Lee M, Kim SW. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. Pharm Res., 2005; 22: 1-10.
5. Vila A, Sanchez A, Tobio M, Calvo P, Alonso MJ. Design of biodegradable particles for protein delivery. J Control Release, 2002; 78: 15-24.
6. Mu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol(R): PLGA nanoparticles containing vitamin E TPGS. J Control Release, 2003; 86: 33-48.
7. Janet B. McGill. Linagliptin for type 2 diabetes mellitus: a review of the pivotal clinical trials. Therapeutic Advances in Endocrinology and Metabolism. 2012; 3(4): 113–124.
8. Eric A, Robert G, Eric D. Drug Loaded Nanoparticles-Preparation methods and Drug targeting issues. Eur. J. Pharm. Biopharm, 1993; 39(5): 173-91.
9. Sovan Lal Pal, Utpal Jana, P. K. Manna, G. P. Mohanta, R. Manavalan, Nanoparticle: An overview of preparation and characterization, Journal of Applied Pharmaceutical Science 2011; 1(6): 228-234.
10. Partha S, Amit K.G, Goutam R. Formulation and Evaluation of Chitosan-Based Ampicillin Trihydrate Nanoparticles. Tropical Journal Pharmaceutical Research Science, 2010; 9(5): 483-88.
11. Santhi K, Dhanraj S.A, Nagasamyvenkatesh D, Sangeetha S, Suresh B. Preparation and optimization of sodium alginate nanospheres of Methotrexate. Indian J. Pharm. Sci, 2005; 67: 691-696.

12. Soppimath K.S, Aminabhavi T.M, Kulkarni A.R, Rudzinski W.E. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release, 2001; 70: 1-20.
13. Amrita Panda, Sukhadakulkarni, Ravi Tiwari, Stability Studies: An Integral Part Of Drug Development Process, IJPRBS, 2013; 2(6): 69-80.