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Formulation and Evaluation of Linagliptin Nanospheres

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Abstract
The success of the studies on the release of drugs in vitro recommends the product for further in vivo studies which may improve patient compliance. From the tests, formulation F9 containing Linagliptin Nanospheres using polymers combination evolved as the optimized formulation and releases over 98.9 percent drug in 24hrs.
IR spectroscopic experiments have shown that the optimized formulation does not interfere with drug-excipients. The optimized formulation F9 can be regarded as a Sustainable Linagliptin nano-sphere drug delivery system delivering almost zero-order drug release over a 24-hourspan.

INTRODUCTION:

The drug's therapeutic effectiveness depends on the bioavailability and ultimately on the solubility of drug molecules. Solubility is one of the essential criteria for achieving desired drug concentration in systemic circulation for desired pharmacological response currently, only 8 percent of new drug candidates are highly soluble and permeable. Drug aqueous solubility is also a constraining factor in the production of the most suitable dosage types. Many medicines and drug candidates are barely water soluble, reducing their chemical uses. 1A increasing number of newly formulated drugs are poorly water-soluble, and such low water-solubility creates major problems in developing formulations with reproducible effects that are sufficiently high in bioavailability.

Nanospheres in the range of 10-1000 nm are known as particulate dispersions or solid particles. The substance is dissolved, trapped in, encapsulated or bound to a matrix of nanospheres. Nanospheres, nano spheres, or nanocapsules can be obtained depending on the preparation process. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, whereas nanospheres are systems of matrix in which the drug is dispersed physically and evenly. 2 In recent years, biodegradable polymeric nanospheres, especially those covered with hydrophilic polymer such as poly(ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices due to their ability to circulate over a prolonged period of time targeting a specific organ, as gene therapy carriers of DNA, and their ability to deliver protein, pept, pept, pept,

The key goals in the design of nanospheres as a delivery method are to monitor particle size , surface properties, and release of pharmacologically active agents to achieve the drug's site-specific action at the therapeutically optimal rate and dosage regimen. 3 While liposomes have been used as potential carriers with specific benefits including shielding drugs from degradation, targeting to site of action and minimizing toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation capacity, rapid leakage of water-soluble drugs in the presence of blood components and poor storage stability. In comparison, polymeric nanospheres give other unique advantages over liposomes. We aid, for example, to improve drug / protein stability and possess valuable controlled release properties. The advantages of using nanospheres as a drug delivery system include:

Particle size and surface properties of nanospheres can be easily controlled after parenteral administration to achieve both passive and active drug targeting.4

1. During transportation and at the localization site, they monitor and manage the release of the drug, alter the organ delivery of the drug and eventual clearance of the drug in order to achieve improved drug therapeutic effectiveness and decreased side effects.
2. Controlled release and degradation characteristics of the particles can be easily modulated by choosing matrix constituents.

Drug loading is relatively small and drugs can be integrated in the systems without any chemical reaction; this is an significant factor in maintaining the drug activity

MATERIALS AND METHODS:

Works on the Preformulation:

The word preformulation is self-explaining, meaning the scientific study conducted before a dosage form is formulated to understand the properties of the drug and its interaction with excipients.

Preformulation preparation is the first step in designing the drug's dosage forms rationally. It can be defined as an investigation, alone and when combined with excipients, of the physical and chemical properties of drug substance. When designing safe and bioavailable dosage formulations, the ultimate goal of preformulation testing is to produce knowledge that is useful to the formulator.

Research on solubility:

Preformulation Analysis of solubility was carried out to select an effective solvent method to dissolve the drug as well as various excipients used for formulation and also to test drug solubility in the dissolution medium to be used.5

Spectroscopy of red infra:

The Linagliptin IR absorption spectrum was calculated using KBr dispersion method by FTIR spectrophotometer. The IR spectrum of the sample collected from manufactured nanospheres was compared with the normal pure drug IR spectra. FTIR spectra help to confirm the drug's identity and to detect the drug's interaction with polymers was performed to test drug-polymer compatibility.6

Preparation of regular Linagliptin graph

1 per cent Sodium lauryl solution preparation:

Accurately measured amount of 10 gm of sodium lauryl sulphate has been added to 1000 ml of distilled water to make up 1 per cent of sodium lauryl.

Basic graph preparation in 1 per cent SLS solution:

Linagliptin 10 mg was taken in a 1000 ml volumetric flask and dissolved in 1000 ml of water containing 10 gm of lauryl sodium solution. It was taken separately from stock 5, 10 , 15, 20, 25 and 30ml and made up to 10 ml with 1 per cent SLS solution to produce 5, 10 , 15, 20, 25 and 30 µg / ml respectively. This solution was found to be 275 nm for Linagliptin in 1 percent SLS as a blank in UV-Visible Spectrophotometer (Libra- Biochrome) when screened in the UV range i.e. from 200 nm to 800 nm. The absorption of these solutions was estimated at 238 nm, and a concentration vs. absorbance graph was plotted.7

Preparation Méthod

Emulsion prepared Linagliptin drug nanospheres followed by solvent evaporation process, and various polymer forms were used.8

Polymer and Drug Preparedness Solution:

1. Weighed the polymer needed, and put in a dry beaker.
2. Required solvent quantity (methanol) was taken from a measuring cylinder.
3. Now, gradually adding methanol to the beaker that contains polymer was applied.
4. Then, it was continuously stirred to form a polymer solution with glass pin.
5. Attach Linagliptin 300 mg, precisely measured, and blend thoroughly.

Aqueous solution prepared:

Weighed the necessary amount of SLS 1 g in 1000mL of water and then retained one side to eliminate air bubbles

A. Simple update:

Linagliptin Nanosphereswere prepared using the Emulsion technique followed by solvent evaporation as an efficient nanodrug preparation technology. Polymers dissolved in chloroform then 10 mg of Linagliptin drug was fully dispersed in polymer solution and 1% SLS solution applied to this under stirring at 400-500 rpm up to 20min then beaker put in sonicatoe probe for 15min after sonication held for continuous stirring by magnetic stirrer and temperature held at 10 rpm using ice bath. Nanospheres instantly emerged after mixing.

Nanospheric Characterisation

Assault

Weigh precisely around 0.3 g of Linagliptin (manufactured nano spheres), dissolve in exact 40 mL of methanol, and titrate with 0.1 mol / L of VS sodium hydroxide (potentiometric titration, Titrimetry Endpoint Detection Method).

Each mL of 0.1 mol / L Sodium Hydroxide VS = C16H13Cl2NO4 35.419 mg. When dried, Linagliptin contains no less than 99.0 percent and no more than 101.0 percent of Linagliptin

Changed Check Dissolution:

Studies on in vitro dissolution were performed using an open cut Boiling tube containing 25mL of a solution for nanospheres and a beaker containing 100mL of 1 percent sodium lauryl sulfate (SLS) solution in distilled water. The experiments were performed 24hrs. The dissolution medium was maintained at 37±0.05 ° C in thermostatically controlled water bath. Basket spin has been set to 50 rpm. At definite intervals, for drug release, 3 ml samples were extracted and spectro photo analysed at 301 nm metrically. In order to preserve sink condition, 3 ml of the corresponding fresh medium was inserted into the dissolution flask at each withdrawal period.

Spectroscopy of FT-IR:

Infrared (IR) spectral matching tests are used to identify any potential drug interaction with the polymers or excipients. The compatibility between the drug Linagliptin with different polymers and was tested with the aid of FT-IR (PERKIN ELMER FT-I Insf. USA) at present. The samples were scanned from an FT-IR spectrophotometer of 4000 to 400 cm -1 in. The IR spectra of all individual drugs and prepared nanocrystals were also reported in a similar manner. In order to reach any potential physical and chemical interaction, physical presence of the samples and presence or disappearances of peaks in the spectra were observed.10-12

Electron Microscopy scanning (SEM):

Scanning electron microscopy was used to describe the unprocessed drug's particle morphology as well as the nanospheres of the fabricated material. A small fraction of each sample of product powder was placed on a dual-sided conductive carbon tape and sputter-coated with 5 nm of a Pt – Pd alloy. Micrographs on a Zeiss DSM 982 Field Emission Weapon Scanning Electron Microscope were obtained (Carl Zeiss AG, Germany).13-15

Table 1: Different Formulations

Ingredients	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Linagliptin (mg)	300	300	300	300	300	300	300	300	300
HPMC K4M (mg)	75	150	225	-	-	-	75	150	225
Chitosan (mg)	-	-	-	75	150	225	75	150	225
Ethyl cellulose (mg)	75	150	225	75	150	225	-	-	-
Dichloromethane(ml)	10	10	10	10	10	10	10	10	10
Methanol (ml)	10	10	10	10	10	10	10	10	10
2% SLS (ml)	50	50	50	50	50	50	50	50	50

The distribution of particle sizes:
The size of drug nanospheres was measured by dynamic laser light scattering (Nanospheres scale analyser, Malvern) immediately after precipitation. The substance suspension was diluted to 0.2 mg / ml by filtered water before analyzation. The results of the study of the particle size were represented using graphical mean size (Mz) & measured surface area (Cs). **16-18**

Measuring Differential Calorimetry Scanning (DSC):
A DSC-41 apparatus (Shimadzu , Japan) has studied the thermal properties of the lyophilized powder samples. The temperature of scanning for each lyophilized powder sample was set at a heating rate of 10 C / min from 25 to 200 C / min. In an open aluminum pan 10 mg of each sample was analyzed, and magnesia was used as a reference. Thermal analysis was performed on Linagliptin & the excipients to assess the internal structure modifications after the nanosizing process.19-20

Potential Zeta
Zeta sizer (ZS 90 malvrn) analyzed the size, size distribution, and zeta potential of the nanospheres. The lyophilized samples were diluted on mg / ml and analyzed with PBS of 67 mm and ph 6.0. These samples were first placed in another clean cubet during size analysis and put on the zeta size analysis chamber to get different peaks and find their average zeta size next to it. Potential zeta-potential samples were held in the zeta sizer analysis chamber for its peak to collect zeta-potential data for analysis. In analyzing these results, monodispersic character is often taken into account instead of polydispersic character.21-22

Characterisation of Active pharmaceutical ingredients
Characterization of the API (appearance, FTIR recognition test, assay) was performed in preformulation studies and it was found that all are within the range defined in the pharmacopoeia.

Linagliptin standard graph at 0.1 per cent SLS solution
Linagliptin's regular graph was developed using 0.1 per cent SLS. Concentrations were prepared at 2 to 10 µg / ml. The absorbance of prepared concentrations was measured at 301 nm with blank sample change to zero. A graph was drawn by focusing on the x-axis and absorbing on the y-axis and the best fit side, and the regression value and equation were determined.

Table 2: Characterization of pharmaceutical active ingredient

Description	Specifications	Observations
Appearance	White Crystalline powder	White
Identification	FTIR	Complies
Assay	Not less than 99.0% w/w and not more than 101.0% w/w of Carvedilol	99.97%w/w

Table 3: Linagliptin standard graph values

Concentration (µg/ml)	Absorbance
0	0
4	0.2
8	0.39
12	0.55
16	0.72
20	0.89

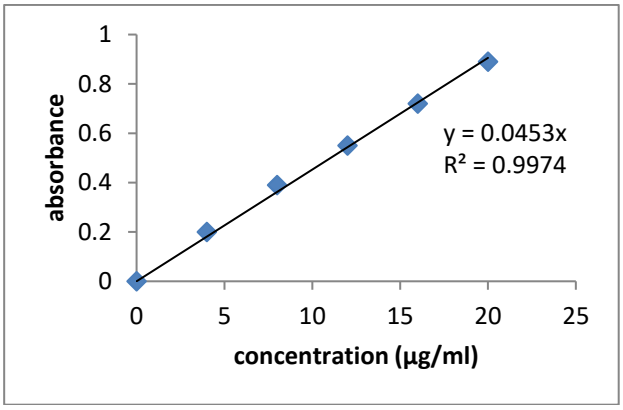


Figure 1: Linagliptin standard graph

Table 4: Evaluation of nano spheres:

Formulation code	Particle size (nm)	% yield	Entrapment efficiency	Drug content
F1	200.5	98.5	77.8	298.5
F2	210.2	80.7	87.5	297.8
F3	246.7	79.5	97.6	298.2
F4	198.2	96.2	75.2	298.0
F5	205.3	87.5	80.2	298.2
F6	226.7	79.8	91.8	297.4
F7	197.2	98.8	77.4	298.4
F8	220.2	84.2	83.4	296.3
F9	245.3	75.8	95.2	295.5

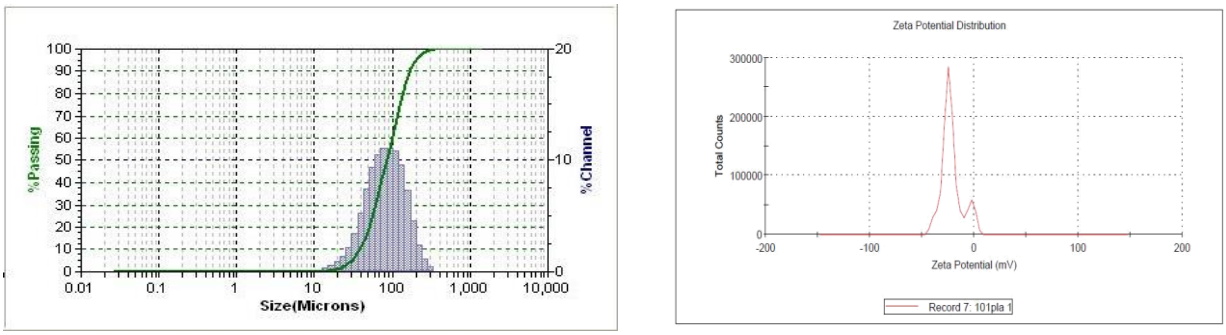


Figure 2: Particle size distribution and Zeta potential of drug

Table 5: Analysis of In Vitro Dissolution:

Time (hr)	% drug release								
	Formulation code								
	F1	F2	F3	F4	F5	F6	F9	F8	F9
1	15.2	12.5	10.8	20.8	17.8	15.2	25.4	22.8	9.5
2	38.9	25.9	21.6	28.9	25.9	21.8	38.9	30.2	17.8
4	46.4	34.5	30.8	35.4	31.8	29.6	46.2	42.7	28.3
6	54.8	46.4	42.7	48.9	43.6	40.9	54.8	51.8	39.4
8	68.9	58.5	55.8	56.1	50.7	49.4	61.7	59.7	47.5
10	82.5	67.5	63.7	69.8	59.8	56.8	76.8	71.5	54.5
12	96.7	85.4	81.6	84.7	76.8	71.2	89.5	85.3	67.6
14	-	94.8	90.8	96.8	87.8	83.5	97.6	94.5	79.7
16	-	-	96.2	-	95.5	93.7	-	97.8	87.2
20	-	-	-	-	-	-	-	-	98.9

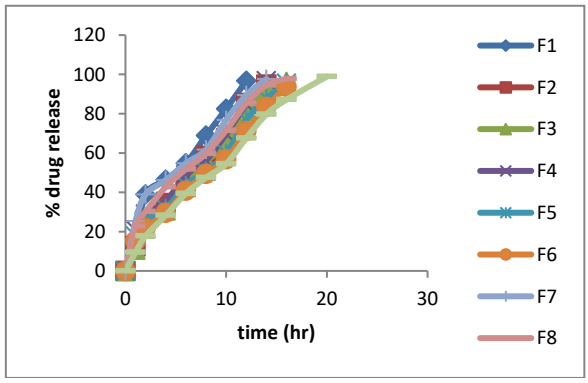


Figure 3: Dissolution Profile of formulation

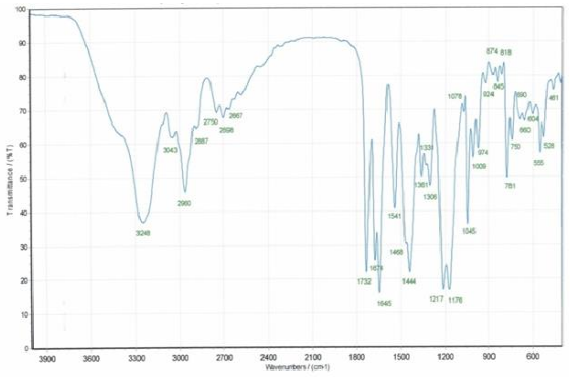
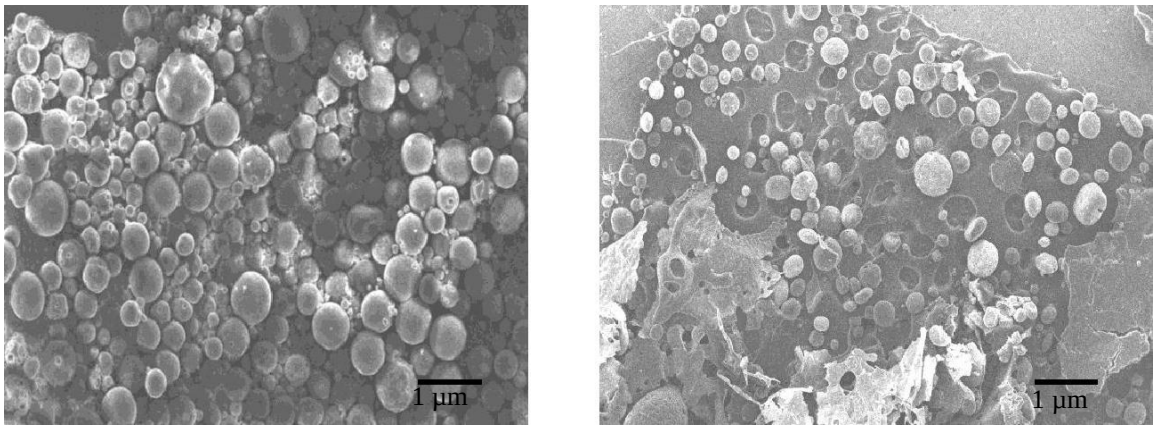


Figure 4: Linagliptin FT IR spectra



Linagliptin surface structure, with GMS(80 mg) Linagliptin surface structure, with GMS(100 mg)

Figure 5: SEM images

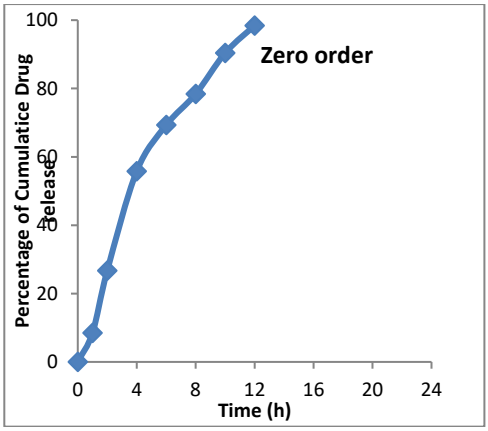
Through using scanning electron microscope (SEM), the morphology of these Linagliptin nano-particles is spherical structures as resolute. The particle surfaces were rugged and rounded. It has been stated that, as the polymer ratio increased, the relative pore sizes also lean to increase (Nayak et al . , 2009).

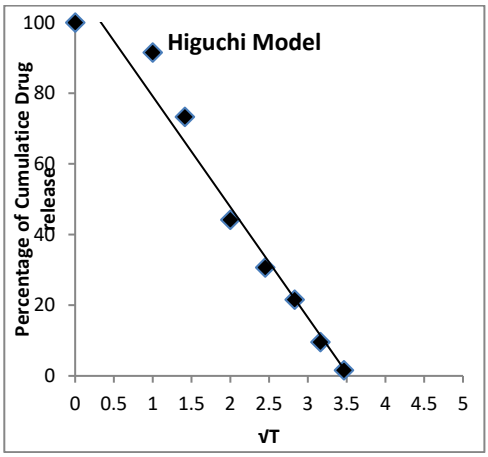
Dissolution data kinetic analysis:

The in-vitro release data was fitted into different release equations and kinetic models zero order, first order, Higuchi and Korsmeyer Peppasmodel to analyze the drug release mechanism.

Table 6: Dissolution data

Formulation code	Zero order	First order	Higuchi	Peppas	
	R2	R2	R2	R2	N
F9	0.99	0.8	0.96	0.99	0.8





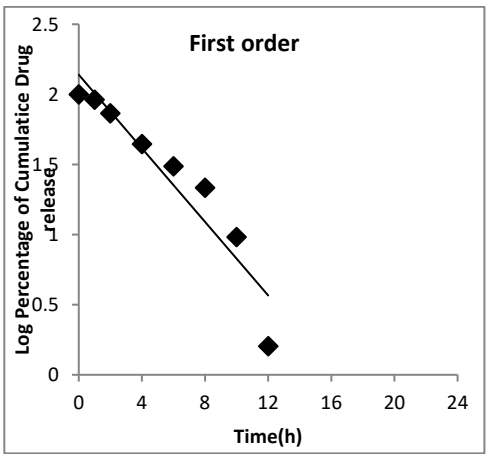


Figure 6: Kinetics models

DISCUSSION:

The present investigation was undertaken to formulate nano particles of Linagliptin solid lipid.

Nanospheres of strong lipids:

Together with other additives, they were prepared using various polymers GMS, Chitosan, PEG6000 SLN. Solvent evaporation process has been used for Nanospheres preparation. They prepared and tested a total number of 9 formulations.

Study of the Particle Size:

The analysis of particle size for the Linagliptin produced nanospheres using various polymers revealed that the particle size was influenced by the presence of the stabiliser. The results of the particle size analysis were represented using the graphical mean (Mz) & measured surface area (Cs). Graphic Mean produces a weighted mean particle size that is less coarse-particle than the mean volume diameter. Although it contains the median value, it may include a specific and probably better control value, as the measure involves both small particles and large particles. Smaller graphical mean (Mz) values were found by using GMS (F3) at 10 per cent. The maximum Mz value (369 nm) for Formulation F7 was found suggesting larger particles. The polymer concentration had affected the particle size. Increasing the concentration of most of the polymers studied decreased the particle size from 6 to 10 per cent.

Dissolution in Vitro:

In vitro dissolution experiments are carried out with solvent 0.1 percent SLS solution for Prepared Nanospheres using changed dissolution process apparatus. With growing polymer concentration the dissolution rate was found to increase linearly. The optimized formulations are (F9).Drug 98.9 was reported by Formulation in 24 hours, respectively.

Kinetics on Product Release:

In vitro drug release data of all Sustained formulations was subjected to fit testing goodness by linear regression analysis according to zero order and first order kinetic equations, Higuchi's and Korsmeyer – Peppas models to determine drug release mechanism. it can be seen that all the formulations showed kinetics of first order release ('r' values in the range from 0.900 to 0.965). From data from Higuchi and Peppas it is apparent that the drug is released by non-fickian diffusion process (n<0.5). It is evident from the kinetic data of factorial formulations, that the F9 formulation displayed drug release by zero order kinetics. The values of 'r' to formulation equation by Higuchi. These data show that drug release follows the Higuchi model of non-Fickian diffusion mechanism.

CONCLUSION:

The success of the studies on the release of drugs in vitro recommends the product for further in vivo studies which may improve patient compliance. From the tests, formulation F9 containing Linagliptin nanospheres using

polymers combination evolved as the optimized formulation and releases over 98.9 percent drug in 24hrs. IR spectroscopic experiments have shown that the optimized formulation does not interfere with drug-excipients. The optimized formulation F9 can be regarded as a Sustainable Linagliptin nano-sphere drug delivery system delivering almost zero-order drug release over a 24-hourspan.

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Conflict of Interest:
Authors declare no conflict of interest

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