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Vildagliptin loaded polymeric nanoparticles using cross-linked polymer by ionotropic gelation method: development and characterization

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ABSTRACT: The aim of present work is to formulate and characterize the Vildagliptin (VLD) loaded polymeric nanoparticles for the treatment of type II diabetes mellitus. Polymeric nanoparticles were prepared by ionotropic gelation method with sodium tripolyphosphate (STPP) as a cross linkers. The obtained sample of VLD was evaluated by UV and FT-IR studies. Prepared polymeric nanoparticles were characterized in term of drug-polymer intraction, particle size and polydispersity index (PDI), zeta potential, % drug content (DC), % drug entrapment efficiency (DEE) and scanning electron microscopy (SEM). *In vitro* and *in vivo* drug release studies were also performed. FT-IR study showed that drug and polymers are compatible with each other and cross-linking was observed between chitosan and STPP. Particle size was range from 238.2 nm to 708.8nm for all the formulations. Zeta potential was range from-8.49mV to 11.1mV. %DC range from68.12% to 84.37%, and % DEE was range from 78.76% to 93.91%. SEM images showed that the prepared formulations were smooth and spherical. *In vitro* drug release evaluation in phosphate buffer pH 7.4 showed sustained release pattern, i.e. 55.17% to 62.89% within 12hours. The data were fitted to Korsmeyer-Peppas model which showed fickian diffusion as a predominant release mechanism. *In vivo* drug release evalutionshowed significant decrease in the blood glucose level. Thus, conclude that prepared polymeric nanoparticles by ionotropic gelation method have a sustain release pattern and incresesstabilty of drug.

KEYWORDS: Vildagliptin; Chitosan; STPP; Ionotropic gelation method; Polymeric nanoparticle; anti diabetic activity.

1. INTRODUCTION

In nanomedicine, particles used are 10 million times smaller than the human body, these particles are greatly lesser than the living cell. Due to this, nanomedicine holds great promise in the fight against cancer, neurodegenerative disorders and other diseases [1].

The size of polymeric nanoparticles is typically between 10 and 1000 nanometers. These polymeric nanoparticles are formulated from the polymers, which have the nature of bio-adaptability, bio-comptability and bio-degradable. Drugs are dissolved, entrapped, and encapsulated in nanoparticle. According to the preparation method, nanoparticles, nano-spheres, or nano-capsules are produced. As a technique, nanotechnology involves the design, production and application of materials on an atomic, molecular or macromolecular scale to produce new nanosized materials [2].

In recent years, researchers have focused on the preparation of nanoparticles from biodegradable hydrophilic polymers such as chitosan, gelatin, and sodium alginate. Ionotropic gelation method involves reaction of two aqueous phases: chitosan, a di-block co polymer ethylene oxide or propylene oxide (PEO-PPO), and poly anion sodium tripolyphosphate. As a result of this procedure, the positively charged amino group of chitosan reacts with the negatively charged tripolyphosphate to form nanometer-sized coacervates.

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Coacervation occurs as a result of electrostatic interactions between two aqueous phases, whereas ionic gelation occurs as a result of ionic proximity when a material undergoes the transition from liquid to gel under room-temperature conditions [3].

Many benefits can be obtained from nanoparticles over conventional dosage forms, includes reducing side effects, decreasing dosing frequency, and increasing patient compliance [4].

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both [5]. One therapeutic approach to decrease the hyperglycemia, especially after a meal, is to retard and reduce the digestion and absorption of ingested carbohydrates through the inhibition of carbohydrate hydrolyzing enzymes in the digestive organs. As a result, these inhibitors could decrease the postprandial rise in blood glucose concentration. It is now believed that inhibition of these enzymes involved in the digestion of carbohydrates can significantly decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet and therefore can be an important strategy in the management of type-II diabetes [6].

Vildagliptin is an antidiabetic drug used to treat type 2 diabetes mellitus. The mode of action of vildagliptin binds covalently to the catalytic site of DPP-4, eliciting prolonged enzyme inhibition. This raises intact GLP-1 levels, both after meal ingestion and in the fasting state [7].

Vildagliptin has been shown to stimulate insulin secretion and inhibit glucagon secretion in a glucose-dependent manner. Vildagliptin also inhibits hepatic glucose production, mainly through changes in islet hormone secretion, and improves insulin sensitivity. These effects willimprove glycaemia. Orally DPP-4 enzyme is inhibited by Vildagliptin which prolong the $t^{1/2}$ of endogenous GLP-1 and GLP thereby increasing insulin level. Biopharmaceutical classification system (BCS) of vildagliptinis class III [8].

Dipeptidyl peptidase-4 (DPP-4) inhibitors are the new class of drugs which exhibits great glycemic control in type-2 diabetes mellitus patient with great tolerance. Vildagliptin is selective and potent oral DPP-4 inhibitor efficiently lowers HbA1c in subjects with type 2 diabetes when used both in monotherapy and add-on to ongoing therapy with other drugs. Upper respiratory tract infections, diarrhea, nausea with hypoglycemia and poor tolerability during chronic treatment are common side effects with most of antidiabetic drugs available in the market. Long-term oral administration of high doses of these drugs in alone and in combination can lead to serious side effects. In addition, patient compliance is poor. The clinically recommended doses of vildagliptin for use in patients with type 2 diabetes are available as 50 mg twice in a day. One of the biggest drawbacks of using VLG is its short elimination half-life (2hours). Due to the shorter half-life, conventional oral dosage forms of VLG offer no control over drug delivery, leading to great fluctuations in plasma drug levels. For patients with type 2 diabetes, it is essential to follow the dosing intervals thoroughly. In order to achieve better treatment efficiency, formulation of a sustained drug delivery system for VLG can be most advantageous. This would release the drug constantly in the gastrointestinal tract (GIT) and maintain steady plasma drug levels for long periods of time which can achieve the better treatment efficiency [20].

2. RESULTS

2.1 Calibration curve of Vildagliptin in pH 7.4 phosphate buffer

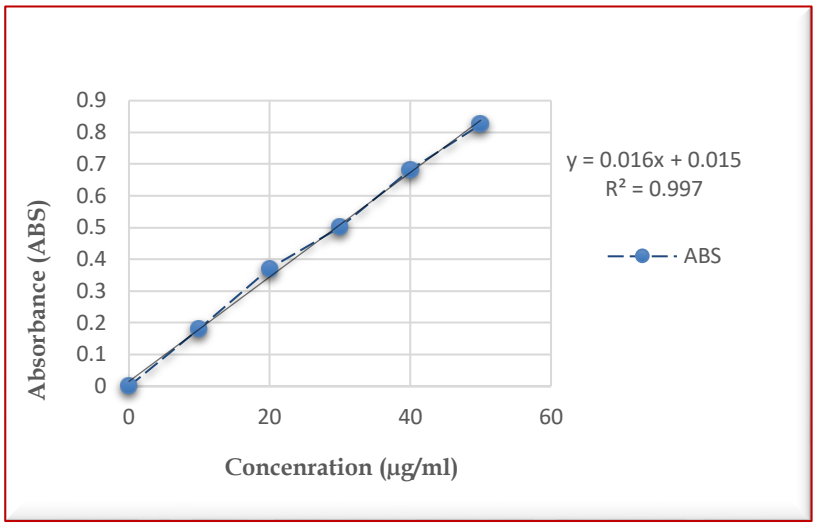
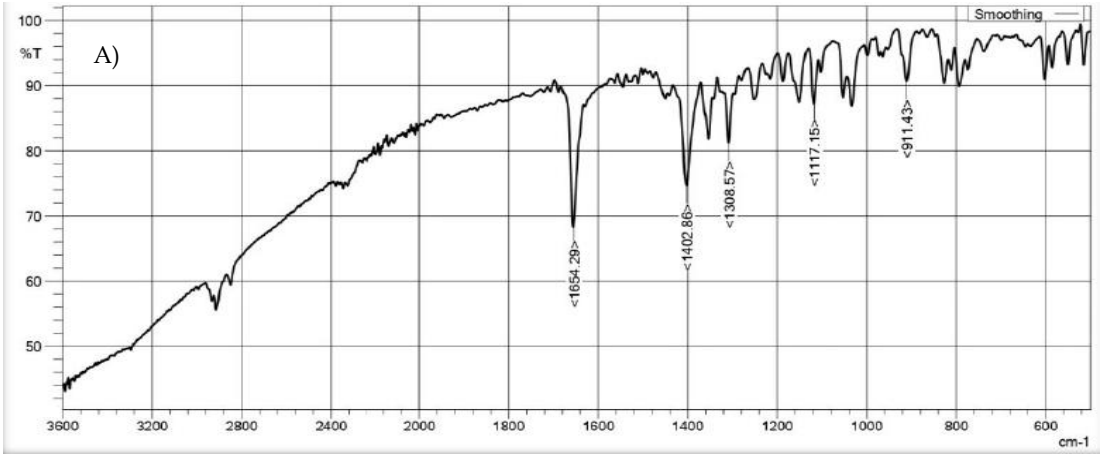
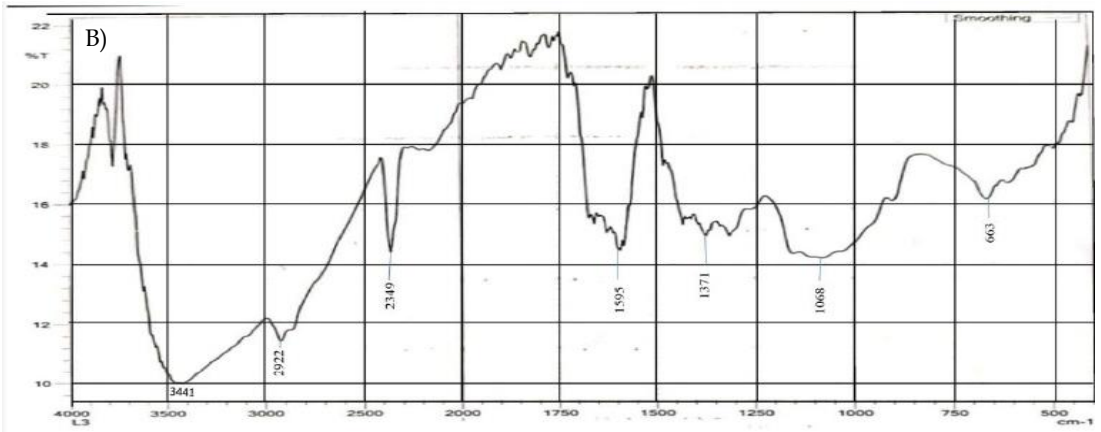


Figure 1: Graph of calibration curve of Vildagliptin in pH7.4 phosphate buffer at 210nm

2.2 FT-IR spectroscopy





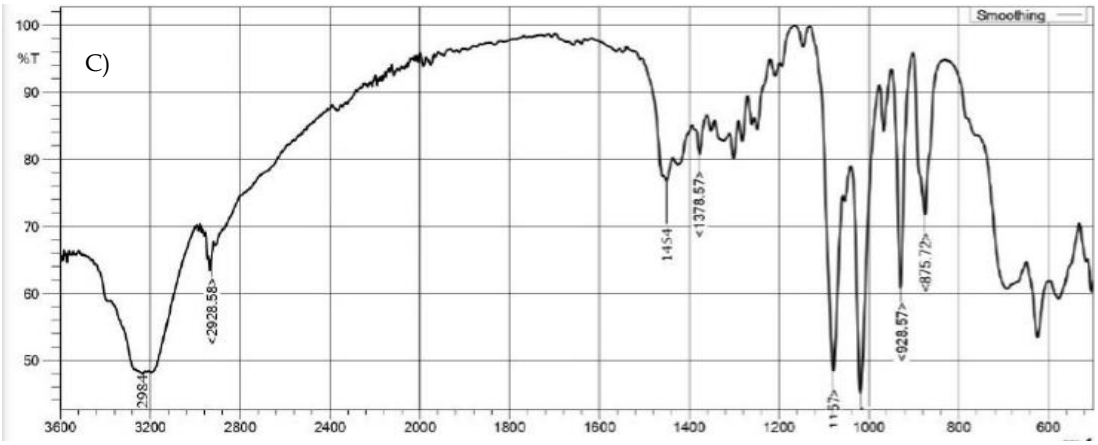


Figure 2: A)FT-IR spectrum of pure drug Vildagliptin B) FT-IR spectrum of Chitosan C) FT-IR spectrum of formulation F1

2.3 Percentage of drug content (%DC), percentage of drug entrapment efficiency (%DEE), particle size, polydispersity index (PDI) and zeta potential

Table 1: Measured values of %drug content (%DC), %drug entrapment efficiency(%DEE), particle size, polydispersity index(PDI) and zeta potential.

Sl.No.	Formulation	%DC	%DEE	Particle Size (nm)	PDI	Zeta Potential
1	F1	72.5%	89.65%	238.2	0.501	-8.49
2	F2	70.62%	84.4%	395.5	0.412	2.36
3	F3	68.12%	78.76%	516.7	0.813	5.8
4	F4	84.37%	93.91%	708.8	0.527	9.90
5	F5	73.12%	93.3%	385.6	0.801	4.60
6	F6	71.87%	86.32%	567.5	0.745	11.1

2.4Surface Morphology

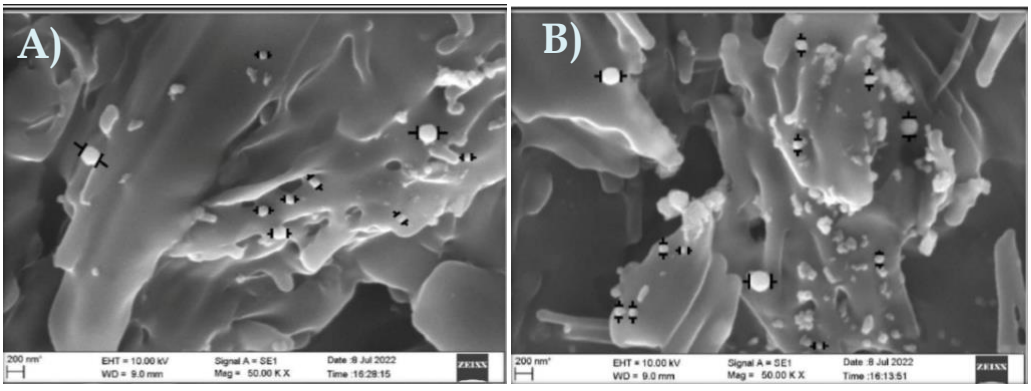


Figure 3: SEM photographs of A)Formulation F1 B) Formulation F5

2.5 *In vitro* drug release

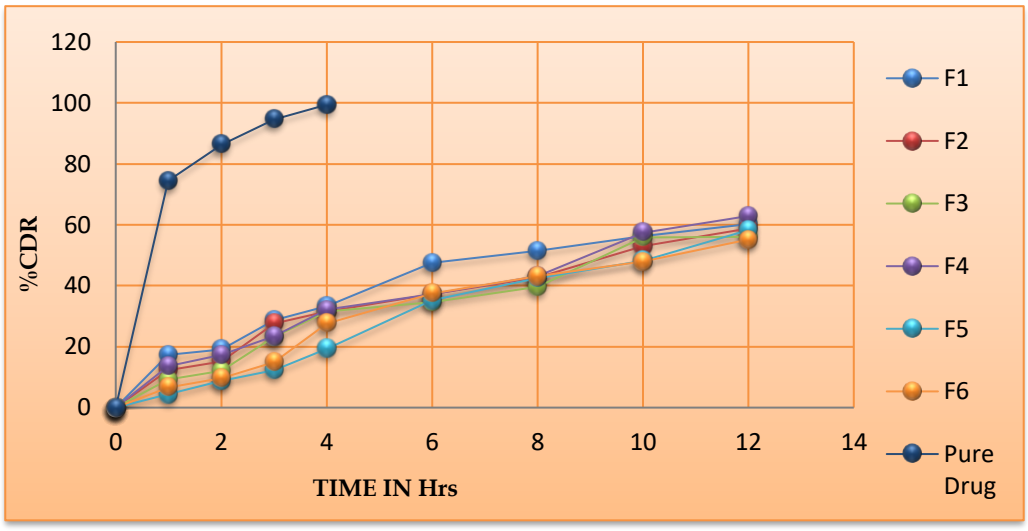


Figure 4: Graph of percentage cumulative drug release (CDR) for formulation F1-F6 and Pure drug

2.6 *In vitro* drug release kinetics study

Table 2: Obtained model for all formulation by *in vitro* drug release kinetics study

Formulation Code	Zero order (R^2Value)	First order (R^2Value)	Second order (R^2Value)	Korsmeyer-Peppas (R^2Value)	Higuchi (R^2Value)	Best Fit Model
F1	0.9422	0.8706	0.7712	0.9647	0.9610	Korsmeyer-Peppas
F2	0.9658	0.8574	0.7018	0.9670	0.9205	Korsmeyer-Peppas
F3	0.9448	0.8308	0.6575	0.9588	0.8179	Korsmeyer-Peppas
F4	0.9736	0.9234	0.7923	0.9890	0.8983	Korsmeyer-Peppas
F5	0.9826	0.8714	0.6133	0.9900	0.5670	Korsmeyer-Peppas
F6	0.9524	0.8350	0.6651	0.9669	0.7138	Korsmeyer-Peppas

2.7 *In-vivo* hypoglycemic activity

Table 3: *In vivo* hypoglycemic activity of formulation F1and F5

Treatment Groups	GLUCOSE (mg/dl)						
	0Hr	**0Hr-1Hr**	**0Hr-2Hrs**	**0Hr-4Hrs**	**0Hr-8Hrs**	**0Hr-12Hrs**	**0Hr-24Hrs**
Normal	74.3 ± 1.02	1.61 ± 0.27	4.54 ± 0.51	4.78 ± 0.54	7.18 ± 0.43	5.83 ± 0.59	2.18 ± 0.53
Standard	56.5 ± 5.73	14.52 ± 4.48	20.17 ± 4.18	26.40 ± 4.76	25.97 ± 5.49	19.1 ± 5.17	8.70 ± 3.97
F1	80.2 ± 2.25	2.317 ± 0.46	12.46 ± 1.32	31.72 ± 3.14	39.41 ± 3.17	47.72 ± 2.61	26.06 ± 2.47
F5	58.7 ± 3.75	10.22 ± 2.34	24.41 ± 3.31	25.59 ± 3.34	34.41 ± 4.37	36.62 ± 4.34	13.56 ± 4.52

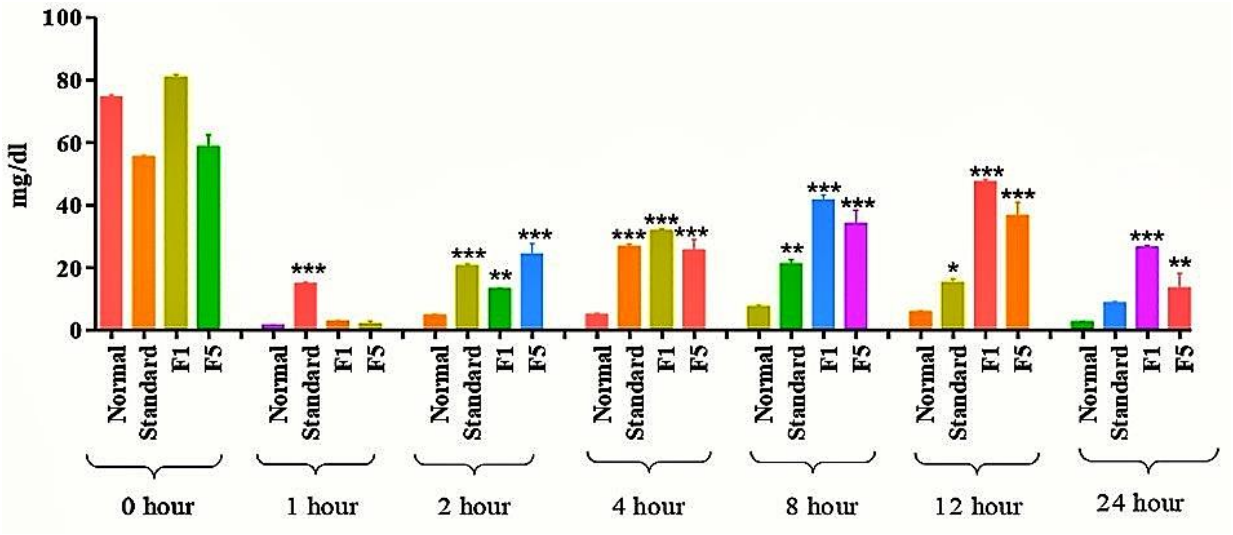


Figure 5: Graph of *in vivo* hypoglycemic activity obtained from graph Pad prism-5 software

3. DISCUSSION

3.1 Drug-polymer interaction study by FT-IR spectroscopy

FT-IR study was carried out for the drug, polymers and also final formulation for the drug polymer interaction. The obtained IR spectrum of the pure Vildagliptin was compared with functional group frequencies of standard. The results proved that there were no significant interactions between the drug and polymer.

3.2Percentage of drug content

Drug content results of all formulations were shown in Table 1. % drug content for the prepared formulation F1 showed (72.5%), F2(70.62%), F3(68.12%), F4(84.37%), F5(73.12%), F6(71.87%)among these formulation F4 showed better drug content compare to other formulations because of decrease in the concentration of chitosan in comparison with STPP. The drug content was decreased with increase in chitosan concentration. This may be due to loss of drug during manufacturing stage or increase in entrapment efficiency, so that drug is not available for estimation. This result indicated that there was no drug loss by manufacturing process or by excipients used in the formulation.

3.3 Percentage of drug entrapment efficiency

Entrapment efficiency of all the formulations were shown in Table 1. % DEE for the prepared formulation showed in the range of 78.76% to 93.91%. Drug entrapment efficiency was found to be greater in formulations F4(93.91%) because of having low concentration of chitosan in comparison with STPP compare to other formulations F1(89.65%), F2(84.4%), F3(78.86%), F5(93.3%), F6(86.32%). However, entrapment efficiency for all formulations was fairly good, indicating the fact that enough ionic interactions were present between positively charged chitosan and negatively charged STPP that resulted in efficient entrapment of drug within the formed nanoparticles.

3.4 Particle size analysis

The mean particle size of the prepared formulations was shown in Table 1. All prepared formulations had particle size range between 238.2 nm to 708.8 nm, Formulation F1(238.2nm), F2(395.5nm), F3(516.7nm), F4(708.8nm), F5(385.6nm), F6(567.5nm). The particles size of nanoparticles increased along with increasing concentration of polymer matrix density and this may be due to the increased viscosity of the inner phase and which leads to increased cross-linking.

3.5 Surface charge analysis

Zeta potentials results were found in the range of -8.49 mV to 11.1 mV for all formulations as mentioned in the table 1.Formulation F1 showed (-8.49mV), F2(2.36mV), F3(5.8mV), F4(9.90mV), F5(4.60mV), F6(11.1mV).Zeta potential increased proportionally by increasing the chitosan concentration due to neutralization of charge between negatively charged TPP and positive amine groups of chitosan.

3.6 Surface morphology

SEM was performed for the optimized formulations such as F1and F5 which is shown in the Figure 3. Various magnifications from 200 nm to 1 µm range photographs were taken and it founds that prepared polymeric nanoparticle found smooth with spherical shape showing different sizes of nonporous in nature.

3.7 *In vitro* drug release studies

All the formulations were subjected to *in vitro* drug release studies and data plotted in graph shown in the figure 4 in the range of 55.17 ± 0.53 % to 62.89 ± 0.78 %. Among these formulation F1(62.89±0.786) and F5(60.23±0.53) have shown a maximum percentage cumulative drug release at the end of 12 hrs. Whereas pure drug Vildagliptin showed 99.4±1.07 % of drug release at the end of 4 hours, an initial fast release suggests that some drug was localized on the surface of the Nanoparticles, later all the formulation have should sustain release action due to the swelling nature of chitosan in nanoparticle formulation.

3.8 *In vitro* drug release kinetics

Data obtained from *in vitro* release studies were subjected to various kinetic models such as zero order, first order, second order, Higuchi model and Korsmeyer-Peppas model using **Kinet DS-3** software. It was found that all the formulations showed a Korsmeyer-Peppas model, as study says korsmeyer-peppas model is used in pharmaceutical polymeric dosage form due to this polymeric nanoparticle showed this model. Thus, the results are lying between 0.5 to 1, which reports the mechanism was Fickian diffusion.

3.9 *In vivo* hypoglycemic activity

In this study, two optimized formulations were selected for activity such as F1 and F5. The results of the *in vivo* hypoglycemic activity were shown in figure 5. Both formulations have showed a significant decrease blood glucose level when compared with normal group. Among these optimized formulations, F1 showed potential hypoglycemic activity due smaller particle size and highest drug release with sustain release action.

4. CONCLUSION

In the current study, an attempt was made to develop and characterize Vildagliptin loaded polymeric nanoparticle by ionotropic gelation method. Polymeric nanoparticle was prepared successfully using chitosan and STPP. FTIR study concluded that no major interaction occurred between the drug and polymers used in the present study. Formulation F4 with low concentration of chitosan and STPP shows better percentage of drug content and percentage of drug entrapment efficiency. Particle size of polymeric nanoparticle was influenced by chitosan and STPP concentration ratio. Surface charge for all the formulation showed good and stable formulation. All the formulation showed a sustain release action for 12 hamong these formulations F1(62.89±0.786), F5(60.23±0.53) showed better drug release and for all formulations kinetic study showed a Korsmeyer-Peppas model which indicates the fickian flow. Our observation indicates that the optimized formulation F1, F5 showed a significant decrease in blood glucose level in *İn vivo* hypoglycemic activity. Thus, conclude that prepared formulations were stable and showed a sustain release action.

5. MATERIALS AND METHODS

5.1 Materials

Vildagliptin was obtained from Caplin Point Laboratories, Chennai, India as a gift sample. Chitosan was procured from Loba Chemie, Mumbai, India. Sodium tripolyphosphate and sodium hydroxide was procured from Fischer Scientific, Mumbai, India. Ethanol was procured from Changshu Hongsheng Fine Chemicals, Jiangsu, China. Glucose kit was procured from Erba Ltd, India.

5.2 Methods

5.2.1 Calibration curve of Vildagliptin by using pH 7.4 phosphate buffer

Preparation of stock solution

Stock 1: Accurately weighed 100 mg of Vildagliptin was dissolved in little quantity of pH 7.4 buffer solution and volume were made up to 100 ml to get concentration of 1 mg/ml.
Stock 2: From stock 1, pipette out 1ml of solution into 10 ml volumetric flask and volume was made up to 10 ml to get concentration of 100 µg/ml. Then appropriate aliquots were taken into different volumetric flask and volume was made up to 10 ml with pH 7.4 buffer solution so as to get drug concentration of 10, 20, 30, 40 and 50 µg/ml. The absorbance of prepared solutions of Vildagliptin in pH 7.4 buffer was measured at 210 nm using UV spectrophotometer against an appropriate blank (pH 7.4 buffer).
Experiment was performed in triplicate [9].

5.2.2 Formulation of Polymeric Nanoparticle

Table 4: Formulation Chart of Polymeric Nanoparticle

Sl. No.	INGREDIENTS	FORMULATION CODE					
		F1	F2	F3	F4	F5	F6
1	Vildagliptin (mg)	100	100	100	100	100	100
2	Chitosan (mg)	100	200	300	100	200	300
3	Acetic acid 1% v/v (ml)	100	100	100	100	100	100
4	Ethanol: Water (ml)	1:1	1:1	1:1	1:1	1:1	1:1
5	STPP (mg)	100	100	100	150	150	150
6	Distilled water(ml)	100	100	100	100	100	100

Preparation of chitosan solution:

0.1%, 0.2% and 0.3% of chitosan was dissolved in 1 % acetic acid solution and stirred for 2 hours and kept for overnight to get clear solution. If not, filter with muslin cloth. Then the solution having a pH 3.5 was adjusted to the pH up to 5 to 6 by using 0.1N sodium hydroxide solution. pH adjusted solution was sonicated for 30 minutes.

Preparation of sodium tripolyphosphate (STPP)solution:

0.1% and 0.15% of sodium tripolyphosphate was dissolved in deionized water and stirred for 30 minutes at 750 rpm.

Preparation of drug solution:

Vildagliptin drug solution was prepared by dissolving 1:1 of ethanol and water to obtain clear solution

Preparation of polymeric nanoparticles

Freshly prepared drug solution was added drop wise with #26 syringe (0.45 mm) to chitosan solution under sonication and sonicated for 1 hour cycle. Then to this mixture, STPP solution was added with a speed of 0.75 ml/min under stirring at 1000 rpm at 25°C for 30 minutes. The mixture solution was sonicated for 30 minutes and centrifuged at 9000 rpm for 1 hour to obtain the pellet. The obtained pellets were dispersed in 1% mannitol. Then the pre - frozen mixture was lyophilized at -80°C for 20 hours[10].

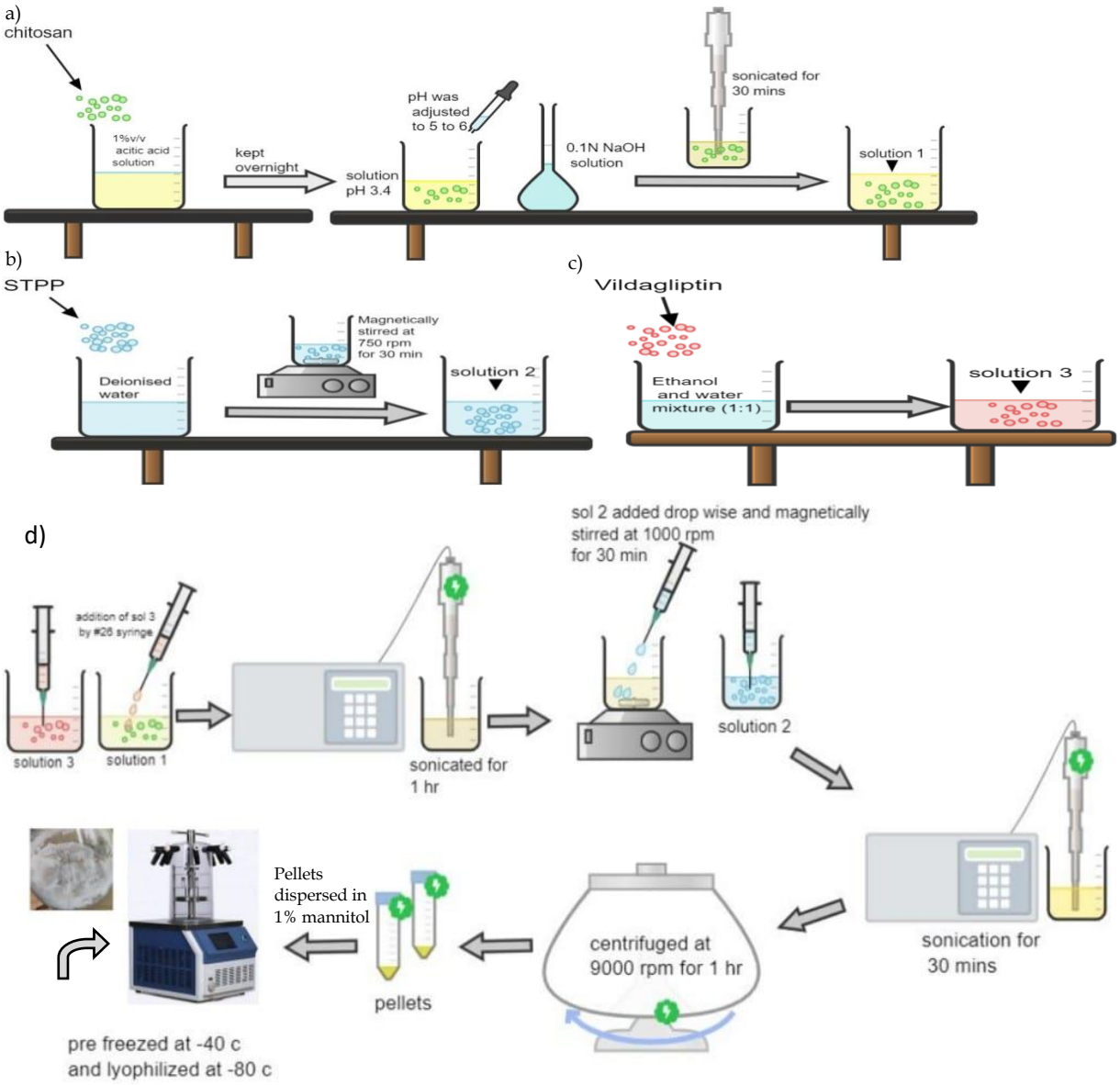


Figure 6:a) Chitosan solution b) Sodium tripolyphosphate (STPP) solution c) Drug solution) preparation of polymeric nanoparticles

5.3 Characterization of Polymeric Nanoparticles

5.3.1 Fourier transforms infrared spectroscopy (FT-IR) analysis

All the excipients individually and also formulation was subjected to FT-IR in Shimadzu IR Spirit kept at an ambient temperature of 25.0 ± 0.5°C. The spectra were recorded by placing the sample carefully and scanning the sample in region of 4000-400 cm^{-1} to determine various functional groups. All the obtained infrared spectrums were recorded [11].

5.3.2 Percentage of drug content (%DC)

10mg of Vildagliptin containing polymeric nanoparticle was equivalently weighed and dissolved in pH 7.4 phosphate buffer followed by continuous stirring, then the solution was sonicated for 10 mins and diluted to appropriate beer's range. Absorbance was taken at 210nm under UV spectrophotometer. %DC was calculated by using the formula:

(Eq. 1) $\%DC = \frac{AXdilutionfactorX\ 100}{1000}$

Where,

A = Absorbance at 210nm

5.3.3 Percentage of drug entrapment efficiency (%DEE)

10mg of Vildagliptin containing nanoparticle was equivalently weighed and dissolved in pH 7.4 phosphate buffer followed by continuous stirring. Then the solution was filtered and diluted to the appropriate beer's range and absorbance was taken at 210nm under UV spectrophotometer, then Percentage drug entrapment efficiency was calculated by using formula [12]:

(Eq. 2) $\%\text{DEE} = \frac{\text{Total drug content}-\text{unbound drug}}{\text{Total drug content}}$ X 100

5.3.4 Particle size, polydispersity index (PDI) and zeta potential

The mean particle size, polydispersity index and zeta potential of the prepared nanoparticle was determined by a particle size analyzer (Nano ZS, Malvern instruments, UK) using techniques of Dynamic light scattering (DLS) for size analysis and combination of Laser dopplervelocimetry (LDV) and phase analysis light scattering (PALS) for zeta potential. Average particle size, PDI and zeta potential were reported [13].

5.3.5 Surface morphology

Surface morphology was examined for optimized formulations by using scanning electron microscopy (Carl Zeiss, Germany). The sample was adhered to the aluminum slab with double-sided adhesive carbon tape, which was subsequently sputtered coated with gold under vacuum. The slab was placed in the sample chamber of the microscope at a working distance of 10 mm with the maintenance of inert ambience using liquid nitrogen of 99.99% purity with a low vacuum voltage of 30 kV and visualized in the microscope. Images were captured at different magnifications [14].

5.3.6 In vitro drug release study

Drug release study of polymeric nanoparticles was performed by using diffusion method i.e., equilibrium dialysis membrane method. Dialysis membrane used was dialysis membrane-50 (molecular weight of 12000 to 14000 Daltons, flat width 45mm and diameter 29mm) and diffusion was carried out in pH 7.4 phosphate buffer. Equivalently weighed 10 mg of drug containing polymeric nanoparticle was dissolved in 5 ml of pH 7.4 phosphate buffer. Then the obtained suspension/solution were poured on the equilibrium dialysis membrane and in the acceptor compartment 100 ml of pH 7.4 phosphate buffer was placed and magnetic stirrer was stirred at 100 rpm. At fixed intervals (for first 4 hours at 1 hour interval and next 8 hours at 2 interval) up to 12 hours, 1 ml of sample was taken from acceptor compartment, suitable dilution was made and absorbances were measured at 210 nm [15,16].

5.3.7 In vitro release kinetics

Drug release kinetic study was performed by using **Kinet DS3** software and all the polymeric nanoparticles formulations cumulative drug release were subjected to the zero order, first order, second order, Korsmeyer-Peppas, Higuchi model [17].

5.3.8 In vivo hypoglycemic activity

Hypoglycemic activity of Vildagliptin loaded polymeric nanoparticle was activity test done by using rat animal model. After obtained the approval from Institutional Animal Ethics Committee of BVVS Hanagal Shri Kumareshwar College of Pharmacy, Bagalkote [IAEC/HSKCOP/Aug 2021/PG15].In which four groups each were containing six animals, 1st group as a normal group which is administered normal saline, 2nd group as a standard group which administered with standard Vildagliptin drug with 10mg/kg body weight, 3rd and 4th group were treated with a formulation F1 and F5 respectively with Vildagliptin containing polymeric nanoparticle of 10mg/kg body weight. All the groups of wistar albino rats were fasted

for 18hours to the experiment with allowing access of water and during the experiment food and water were withdrawn. 0,1,2,4,8,12 and 24hours blood were withdrawn from the tip of tail and estimated for blood glucose level. At 0-hour blood was taken first then the dose was administered then following hours blood were taken and estimated [18,19].

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