

**FORMULATION AND OPTIMIZATION OF ORAL POLYMERIC
COATED VILDAGLIPTIN NANOFORMULATIONS USING QUALITY
BY DESIGN CONCEPT**

Thesis submitted to
THE KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI
(KLE DEEMED UNIVERSITY)

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For the award of the degree of
DOCTOR OF PHILOSOPHY
IN
THE FACULTY OF PHARMACY

by
Mr. ANAND S. AMMANAGE M. Pharm.
(Registration No: KLEU/Ph.D./20-21 /DO1220033)



Under the Guidance of
PROF (Dr). VINAYAK MASTIHOLIMATH Ph. D.
Department of Pharmaceutical Quality Assurance
KLE College of Pharmacy,
KLE Academy of Higher Education and Research, Belagavi- 590010.

JANUARY - 2025

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KLE College of Pharmacy,
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Belagavi.

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Part time PhD Research Scholar

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Dept. of Pharmaceutical

Quality Assurance

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Date:

Signature

Prof (Dr). Sunil S. Jalalpure

Principal,

KLE College of Pharmacy,

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Place: Belagavi

Date:

Signature

Prof. (Dr.) Vinayak Mastiholimath

Dept. of Pharmaceutical Quality Assurance

KLE College of Pharmacy,

KAHER, Belagavi- 590010

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Date:

Mr. Anand S. Ammanage

Place:

TABLE OF CONTENTS

Sl.No.	Particulars	Page No.
1.	Introduction	1-25
1.1	Background	1-17
1.2	Literature Review	18-21
1.3	Justification	22-24
1.4	Objective and plane of work	25
1.5	Plan of work	25
2.	Material and Methods	26-42
3.	Results and Discussion	43-73
4.	Summary	74-75
5.	Conclusion	76
6.	Bibliographic references	77-88
7.	Annexure	89
	a. Animal Ethical Clearance letter	
	b. Publications	
	c. Oral presentations	
	d. NPTEL Certificate	

LIST OF ABBREVIATIONS USED (IN ALPHABETICAL ORDER)

ANOVA	:	Analysis of variance
AUC	:	Area Under Curve
BCS	:	Biopharmaceutical Classification System
BSA	:	Bovine serum albumin
Chitosan	:	CS
Cm	:	Centimeter
C _{max}	:	Maximum plasma concentration of a drug
cP	:	Centipoises
CCDs	:	Central Composite Designs
CPCSEA	:	Committee for the Purpose of Control and Supervision of Experiments on Animals
CMAs	:	Critical material attributes
CPPs	:	Critical process parameters
CQA	:	Critical Quality Attributes
DCM	:	Dichloromethane
DM	:	Diabetes mellitus
DMF	:	Dimethylformamide
DoE	:	Designs of Experiments
DPP-4-i	:	Dipeptidyl peptidase 4 inhibitors
EE	:	Entrapment Efficiency
Eudragit RL 100	:	Eud
FPG	:	Fasting plasma glucose
FT-IR	:	Fourier transform infrared spectroscopy
g	:	Gram
GLP-1	:	Glucagon-like peptide-1
GIP	:	Glucose-dependent insulintropic polypeptide
GIT	:	Gastrointestinal tract
Glutaraldehyde	:	GA
h	:	Hour
HED	:	Human equivalent dose
HCl	:	Hydrochloride
IAEC	:	Institutional animal ethics Committee

IDF	:	International Diabetes Federation
ICH	:	International Council for Harmonization
IP	:	Intra peritoneal
KDa	:	Kilo Dalton
LMW	:	Low molecular weight
LOD	:	Limit of detection
LOQ	:	Limit of quantification
min	:	Minute
mg	:	Milligram
μg	:	Microgram
ml	:	Mililiter
MPS NPs	:	Magnetic polystyrene nanoparticles
MW	:	Molecular weight
nm	:	Nanometer
N	:	Normality
Nanoparticles	:	NPs
NaoH	:	Sodium hydroxide
Opt	:	Optimized
P	:	Probability
PBS	:	Phosphate buffer solution
PDI	:	Polydispersity index
pH	:	Power of Hydrogen
PLGA	:	o-polymer poly(lactic-co-glycolic acid)
PNPs	:	Polymeric nanoparticles
QbD	:	Quality by Design
QTPP	:	Quality Target Product Profile
R^2	:	Correlation Coefficient
RH	:	Relative Humidity
rpm	:	Revolution per minute
RSD	:	Relative standard deviation
SD	:	Standard deviation
SGLT2-i	:	Sodium-glucose cotransporter 2 inhibitors
STZ	:	Streptozotocin
TEM	:	Transmission electron microscopy

$T_{\max}$	:	Time to peak drug concentration
USFDA	:	United states Food and Drug Administartion
UV	:	ultraviolet
Vildagliptin	:	VLD
Vs	:	Versus
v/v	:	Volume by volume
w/v	:	Weight by weight
w/w	:	Weight by weight
μg	:	Microgram
μm	:	micrometer
$^{\circ}\text{C}$	:	Degree centigrade

LIST OF TABLES

Sl. No.	Particulars	Page No.
1	List of instruments	26
2	List of chemicals	27
3	Formulation composition of VLD Eud NPs	32
4	Formulation composition of CS VLD NPs	33
5	Experimental design of VLD Eud NPs with their process variables and levels	34
6	Experimental design of CS VLD NPs with their process variables and levels	35
7	Dose dependent <i>In vivo</i> activity of optimized VLD Eud NPs F6	38
8	Standard calibration curve of Vildagliptin at 0.1N HCl and PBS pH 7.4	44
9	Standard calibration curve of Vildagliptin at 216nm	46
10	Results of accuracy	48
11	Results of precision	48
12	Results of robustness	49
13	Results of ruggedness	50
14	Results of LOD and LOQ	50
15	Regression analysis of Y_1 and Y_2 responses of VLD Eud NPs	58
16	ANOVA models for Y_1 and Y_2 of VLD Eud NPs	58
17	Regression analysis of Y_1 and Y_2 responses of CS VLD NPs	61
18	ANOVA models for Y_1 and Y_2 of CS VLD NPs	61

19	Results of particle size and entrapment efficiency of VLD Eud NPs	62
20	Results of particle size and entrapment efficiency of CS VLD NPs	62
21	PDI Values of VLD Eud NPs and CS VLD NPs	64
22	Percentage yield of VLD Eud NPs and CS VLD NPs	67
23	Cumulative drug release data of VLD-Eud NPs F1-F9	68
24	Cumulative drug release data of CS VLD NPs N3, N6 and N9	69
25	List of the drug release mechanism of VLD Eud NPs	70
26	Lists of the drug release mechanism of CS VLD NPs	70
27	Stability characteristics of optimized VLD Eud NPs F6	71
28	Stability characteristics of optimized CS VLD NPs N6	71

LIST OF FIGURES

Sl. No.	Particulars	Page No.
1	Pathologies of tissue and vascular in diabetes complications	1
2	Structure of Vildagliptin	3
3	Structure of Eudragit RL 100	6
4	Structure of Chitosan	6
5	An illustration of the nanoprecipitation process	10
6	A graphic representation of the emulsification-solvent evaporation	11
7	Diagrammatic illustration of the desolvation process	12
8	Flow chart of QbD Process	13
9	Pharmaceutical quality systems for quality by design	14
10	Cause-effect (Ishikawa diagram or fishbone) diagram	15
11	Quadratic, cubic, and linear relationships of a response	17
12	Preparation of VLD Eud NPs by solvent evaporation and nano-precipitation method	32
13	Preparation of CS VLD NPs by desolvation method	33
14	Animal experimental study plan	39
15	UV Spectrum of Vildagliptin at λ_{max} 208nm in 0.1N HCl	43
16	UV Spectrum of Vildagliptin at λ_{max} 208nm in PBS pH 7.4	44
17	Standard calibration curve of Vildagliptin in 0.1N HCl	45
18	Standard calibration curve of Vildagliptin in PBS pH 7.4	45

19	UV Spectrum of Vildagliptin at λ_{max} 216nm	46
20	Standard calibration curve of Vildagliptin in 0.1N NaOH	47
21	FT-IR spectra of Vildagliptin	51
22	FT-IR spectra of Eudragit RL 100	51
23	FT-IR spectra of Optimized VLD Eud NPs F6	52
24	Compiled FTIR images of a) VLD, b) Eud and c) Optimized VLD Eud NPs F6	52
25	FTIR spectra of Chitosan	54
26	FTIR spectra of Optimized CS VLD NPs N6	54
27	Compiled FTIR images of a) VLD, b) CS and c) Optimized CS VLD NPs N6	55
28	(a) 3D response surface plot and (b) 2D contour plot of PS of VLD Eud NPs	56
29	(a) 3D response surface plot and (b) 2D contour plot of % EE of VLD Eud NPs	57
30	(a) 2D contour plot and (b) 3D response surface plot for PS CS VLD NPs	59
31	(a) 2D contour plot and (b) 3D response surface plot for %EE of CS VLD NPs	60
32	TEM image of optimized VLD Eud NPs (F6)	66
33	TEM image of optimized CS VLD NPs (N6)	66
34	<i>In vitro</i> drug release profile of pure VLD and all formulated VLD Eud NPs	68
35	<i>In vitro</i> drug release profile formulation N3, N6 and N9 CS VLD NPs	69
36	Dose dependent <i>in vivo</i> activity of optimized VLD Eud NPs F6	72

ABSTRACT

Background

Diabetes mellitus is an alarming metabolic disorder, posing a global public health concern due to its association with high blood sugar levels. Vildagliptin, a DPP-4 inhibitor, is an antidiabetic agent used for the management of type 2 diabetes mellitus. The present research explores the formulation and optimization of two types of Vildagliptin-based nanoparticle systems—Vildagliptin-Eudragit RL 100 coated nanoparticles (VLD Eud NPs) and Chitosan-Vildagliptin nanoparticles (CS VLD NPs)—for sustained drug delivery using a QbD approach.

Objectives

In the present study, an attempt was made to formulate and optimize oral polymeric-coated Vildagliptin nanoformulations using the QbD approach and to evaluate their *in vivo* antidiabetic activity in Streptozotocin-induced diabetic Wistar rats.

Methodology

VLD Eud NPs were prepared by combining solvent evaporation and nano-precipitation techniques. CS VLD NPs were developed using a simple desolvation technique. Optimization was conducted via a 3^2 factorial design. The prepared nanoformulations were then characterized for particle size, PDI, morphology, and entrapment efficiency. FTIR studies were carried out to ascertain drug-polymer compatibility, and TEM studies to check the morphology of prepared nanoparticles. Further *in vitro* drug release and kinetics were studied. The accelerated stability studies for the optimized formulations were carried out for 3 months.

Results

VLD Eud NPs have shown particle sizes ranged from 58.5 ± 2.09 nm to 425.4 ± 4.71 nm, with PDI values between 0.27 ± 0.036 and 0.52 ± 0.086 . Entrapment efficiency varied from $32.29 \pm 1.16\%$ to $75.26 \pm 1.63\%$, with nanoparticles exhibiting cylindrical shapes. *In vitro* drug release studies demonstrated $>68\%$ release over 24 h, with the release following zero-order kinetics. CS VLD NPs exhibited particle sizes ranging between 118.24 ± 2.26 nm and 232.84 ± 6.79 nm, with PDI values from 0.24 ± 0.025 to 0.39 ± 0.037 . Entrapment efficiency ranged from $26.54 \pm 3.61\%$ to $51.57 \pm 1.52\%$ with nanoparticles exhibiting spherical-shapes. FTIR analysis confirmed drug-polymer compatibility. *In vitro* drug release showed sustained release over 24 h, with the release following Higuchi kinetics. Both the optimized VLD Eud NPs F6 and CS-VLD NPs N6 were found stable during the 3-month duration of the stability study. *In vivo* study of VLD Eud NPs F6, particularly at 7.5 mg/Kg (75 mg HED) and 10 mg/Kg (100 mg HED) doses, shown glycemic regulation compared to the standard VLD 10 mg/Kg (100 mg HED) dose.

Conclusion

Overall, both optimized formulations, VLD Eud NPs and CS VLD NPs, provide promising sustained-release delivery systems for VLD. VLD Eud NPs potentially enhancing therapeutic outcomes and minimizing dose and dosing frequency of the VLD for diabetes management over conventional drug delivery methods.

Keywords

Polymeric nanoparticles; Vildagliptin; Quality by Design; Diabetes mellitus; Solvent evaporation and nanoprecipitation method; Desolvation method.

1. INTRODUCTION

1.1 BACKGROUND

Diabetes mellitus

The term diabetes is often used to refer to diabetes mellitus (DM). A prolonged period of elevated blood sugar (glucose) levels is a sign of a potentially hazardous and chronic type of metabolic disorder.¹

Among diabetes, type 2 DM is most common, in which the body cells do not utilize insulin properly. It is most commonly developed in people with risk indicators including family history and being overweight (obesity).²

Treatment of type 2 DM leads to weight gain, which worsens risk factors for cardiovascular disease and is directly linked to the rise in the mortality rate of cardiovascular events.³ Other complications of DM include related tissues like the brain, heart, and parenchymal tissue, which affect organ's functional components, such as osteoarthritis, Alzheimer's disease, and cancers. (Figure 1).⁴

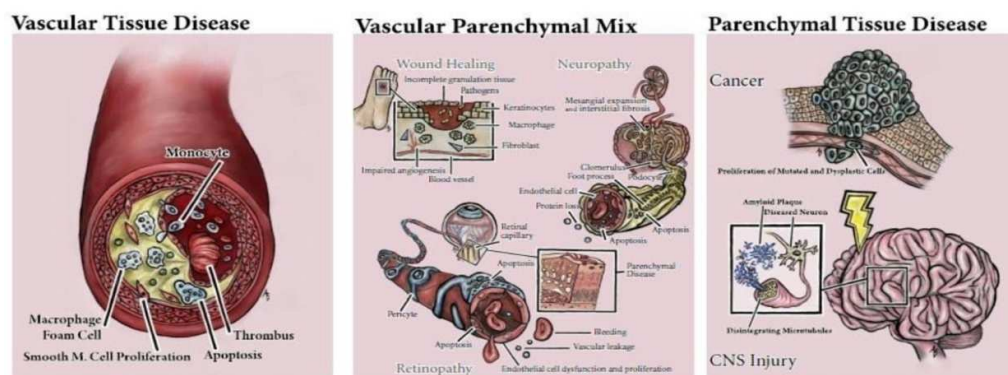


Figure 1: Pathologies of tissue and vascular in diabetes complications

Chronic hyperglycemic condition can result in multiple organ failure, which leads to various complications, such as diabetic kidney disorder and diabetic retinopathy.

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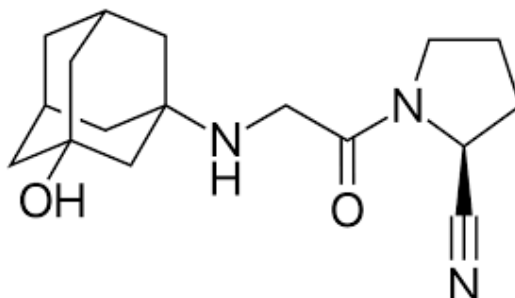
As per the International Diabetes Federation (IDF) report, approximately 536.6 million adults are affected by DM throughout the world, and this may cross 783.2 million by 2045 as expected.⁶

In recent decades, newer types of antidiabetic agents have been developed, like dipeptidyl peptidase 4 inhibitors (DPP-4-i), sodium-glucose co-transporter 2 inhibitors (SGLT2-i), and agonists of the glucagon-like peptide-1 (GLP-1) receptor. These agents act as game changers due to their non-hypoglycemic character, which is an added benefit in diabetic management.⁷

As of currently, Biguanides are the recommended first-line medication for type 2 diabetes. However, in Japan, DPP-4 inhibitors were used as first-line drugs, which were launched in the year 2009. The reason behind the frequent usage of DPP-4 inhibitors is their high safety and efficacy.⁸

Vildagliptin (VLD) is a newer class of DPP-4 inhibitor used in the treatment of type 2 DM.⁹ VLD is capable of increasing the postprandial incretin hormone levels—glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1).¹⁰ VLD increases insulin secretion (insulin dependent) of pancreatic β -cells and suppresses glucagon secretion from the α -cell.⁹

VLD is used both alone and in combination with other antidiabetic drugs. Oral administration of these drugs, either alone or in combination.¹¹

Drug and polymers profile**Vildagliptin:****Figure 2: Structure of Vildagliptin**

IUPAC name: (2S)-1-[2-[(3-hydroxy-1-adamantyl)amino]acetyl]pyrrolidine-2-carbonitrile

Molecular formula: C₁₇H₂₅N₃O₂

Molecular weight: 303.4g/mol

Brand Names: Galvus, Jalra, Xiliarx

Log P: 0.84

Physical properties: White in color and crystalline powder

Solubility:

- **Soluble:** Water, 0.1 N NaOH
- **Moderately Soluble:** Acetonitrile, ethanol
- **Readily Soluble:** Methanol, 0.1N HCl

CAS Registry No: 274901-16-5

Category: Treatment of Type 2 DM.

Half-life: 2-3 h

Dose: 50mg twice a day

Storage: Store Between 25-30°C, protect from moisture.^{12, 13}

Description:

VLD is a drug classified as class I in the Biopharmaceutical Classification System (BCS) that inhibits the DPP-4 enzyme. Type 2 DM is treated with it, particularly in individuals with glucose levels that are not managed solely via diet and exercise. VLD inhibits the breakdown of the three incretin hormones amylin (a 37-amino-acid peptide), the peptide GLP-1, which has 30 amino acids, and GIP, which has 42 amino acids.¹⁴

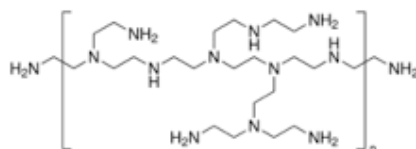
Pharmacodynamics:

Glycemic control in type II diabetes mellitus is improved by VLD, which enhances the glucose responses of β -cells in the pancreatic islets and boosts glucose-dependent insulin production. Alpha cells become more responsive to glucose when GLP-1 levels become elevated, which promotes the release of glucose.¹⁵

Vildagliptin lowers fasting as well as postprandial hepatic glucose production by increasing the amount of the incretin hormone, which raises the insulin-to-glucagon ratio. VLD has no effect on the gastric emptying. Additionally, in people with normal glycemic control, neither insulin secretion nor blood glucose levels are impacted. Clinical trials have demonstrated that VLD, at doses of 50–100 mg per day, significantly improves beta-cell responses, proinsulin to insulin ratio, and beta-cell responsiveness readings from the frequently sampled meal tolerance test in people with type 2 DM. VLD has been shown to improve glycated haemoglobin (HbA1c) and fasting plasma glucose (FPG) levels.¹⁶

Pharmacokinetics:

VLD is absorbed quickly after oral treatment when fasting. The peak concentrations of plasma are measured 1.7h after the dose. VLD plasma concentrations rise at a relatively dose-proportionate rate. The total exposure to the drug (AUC) is unaffected by food, but it does cause a 2.5 h delay in T_{max} and a 19% drop in C_{max}. Absolute bioavailability of VLD is 85%. Vildagliptin binds to plasma proteins with a 9.3% rate. VLD is distributed evenly in red blood cells and plasma. VLD is dispersed outside of blood vessels, indicating that, at steady-state following intravenous injection, its mean volume of distribution was 71L. VLD taken orally is excreted in approximately 69% of cases by metabolism that is not catalysed by cytochrome P450 enzymes. According to results from a study conducted on rats, DPP-4 contributes to VLD's hydrolysis activity. Vildagliptin undergoes renal metabolism, which yields the hydrolysis products amide (4%) and cyano (57%), which are inactive pharmacologically. Hydrolysis to cyano group forms LAY 151 (M20.7), a carboxylic acid that makes up a significant portion of the dose and is an inactive metabolite. VLD is eliminated through metabolic processes. After being taken orally, around 15% of the total dose of radio-labelled VLD was detected in faeces, and urine contained around 85% of the dose. About 23% of the urine's retrieved dosage was made up of the parent component that was unaltered. Approximately two hours is the average elimination half-life following intravenous administration. Its elimination half-life is around three hours after oral intake. VLD was administered intravenously to healthy individuals; its total plasma as well as renal clearance was 41 and 13L/h, respectively.¹⁷

Eudragit RL 100:¹⁸**Figure 3: Structure of Eudragit RL 100**

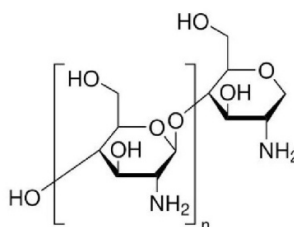
Eudragit RL 100 (Eud) is a copolymer containing methyl methacrylate, ethyl acrylate, and a small quantity of methacrylic acid ester and quaternary ammonium groups.

Physical properties: Powder appeared as clear to hazy, colorless granules that smell slightly like amine.

CAS Registry No: 33434-24-1

Applications:

Eudragit RL 100 is frequently utilized in the pharmaceutical sector, particularly in controlled drug delivery systems. This polymer provides controlled release properties due to its permeability and pH-independent swelling.

Chitosan (Low molecular weight):¹⁹**Figure 4: Structure of Chitosan**

Physical properties	:	Free-flowing, white to pale yellow color powder
Solubility	:	Soluble in 1%w/v solution of acetic acid
Viscosity	:	10 - 150 mPa.s at 20°C
Degree of Deacetylation	:	min. 90%

Applications:

Numerous applications for this biocompatible, antibacterial, and eco-friendly polyelectrolyte include treatment of water, chromatography, cosmetics (additives), textiles (antimicrobial activity), biomedical devices, photography (papers), films (biodegradable), and micro-capsule implants for controlled release in the delivery of drugs.

Oral polymeric nanoparticles:

Nanoparticles (NPs) are nanocarriers where the active ingredient is evenly distributed (nanospheres) or enclosed by a cavity that can be distinguished by a natural or synthetic polymeric barrier (nanocapsules).²⁰ The NPs drug delivery strategy is based on the potential to transport active compounds primarily to the targeted ill tissue, with a greater intracellular absorption than drugs that are free.²¹ This results in increased protection of healthy tissues as well as notable drug protection against systemic degradations.²²

The development of nanoparticulate drug delivery systems capable of delivering drugs to the correct location, at the right time, and at the right dose is one of the most promising areas currently being investigated for the oral delivery of drugs.²³

Many drug delivery methods, including liposomes, nanoparticles, nanoemulsions, and niosomes, have been developed to improve drugs for oral administration. Among the aforementioned strategies, polymeric nanoformulations have been investigated for enhancing the bioavailability of several drugs orally.^{24, 25}

Polymeric nanoparticles (PNPs) can efficiently alter the pharmacokinetic properties of the drug molecules; currently, when developing drugs for chronic disorders like diabetes, they are considered to be the best formulations.²⁶

In order to avoid premature drug degradation, reduce adverse effects, increase drug efficacy, and achieve targeted drug release rates, a number of biodegradable and biocompatible studies have been conducted on polymers as drug delivery methods. To obtain desirable particle sizes and shapes, controlled drug release, and a higher amount of drug entrapment efficiency, several types of synthesis approaches have been developed. Some of the most frequent synthesis techniques include the salting out method, solvent evaporation, and nanoprecipitation.²⁷

Methods for preparation of PNPs²⁸

The two primary types of PNPs preparation are preformed polymer dispersion and monomer polymerization. Among the several polymerization methods are mini-emulsion polymerization, micro-emulsion polymerization, surfactant-free emulsion polymerization, and emulsion polymerization. Techniques including salting-out, the emulsion solvent evaporation, emulsification solvent diffusion, and nanoprecipitation are frequently employed for preformed polymers. The type of polymers, size specifications, and intended use all influence the preparation technique selection, which has a big impact on the PNPs' characteristics. For example, PNPs meant for biological applications should stay away from reactive materials and additives. The

drug transport capacities of different polymers, such as poly(3-hydroxybutyrate) (P(3HB)) and PLGA, varied; P(3HB) has a greater loading efficiency than PLGA but a lower retention rate. Dissolution, solubility, cellular absorption, release of drugs, bio-distribution, and circulating half-life are important variables that impact PNPs performance, and they all become size-dependent. Since differences might impair reliable performance, maintaining uniform particle sizes is a significant problem in PNPs preparation. The production of PNPs from pre-formed polymers using a variety of techniques, such as salting-out, emulsification solvent evaporation, emulsification solvent diffusion, and nanoprecipitation. All of these techniques begin with the creation of an emulsification system, which is the same for all of them. However, the second step, which involves the production of PNPs, differs depending on the approach. The principles of this subsequent phase, which might happen via either precipitation or the organic solvent evaporating, are reflected in the method's name.

Nanoprecipitation method

A quick, easy, and energy-efficient way to produce nanoparticles is through nanoprecipitation (Figure 5).²⁹ Compared to other methods, it is more economical because it doesn't require a precursor emulsion.³⁰ This method is easily scalable, and by modifying the settings, the size of the nanoparticle may be regulated.³¹ The technique relies on interface deposition, where the solvent permeates non-solvents and causes the polymer to precipitate, producing narrowly distributed nanoparticles.³²

A polymer and an organic solvent that dissolves in water make up the organic phase of nanoprecipitation; these components need to get along with the aqueous medium in order to promote diffusion. A stabilizer, such as a surfactant, may be added to the polymer to make it insoluble in water and stop aggregation. Addition of organic

components progressively into the aqueous phase while being moderately stirred to create NPs. Additionally, the NPs are collected by ultracentrifugation and subsequently cleaned to eliminate surfactants. The NPs are collected by filtration, centrifugation, or freeze-drying after the organic solvent evaporates and hardens them.

Commonly used organic solvents include ethanol, acetone, hexane, and methylene chloride; binary solvent combinations can also be utilized. Differences in surface tension produce temperature gradients and interfacial turbulence, which cause solvent eddies at the interface. When these eddies and solvent diffusion combine, they cause nanocapsules to develop on hydrophobic drug surfaces.²⁸

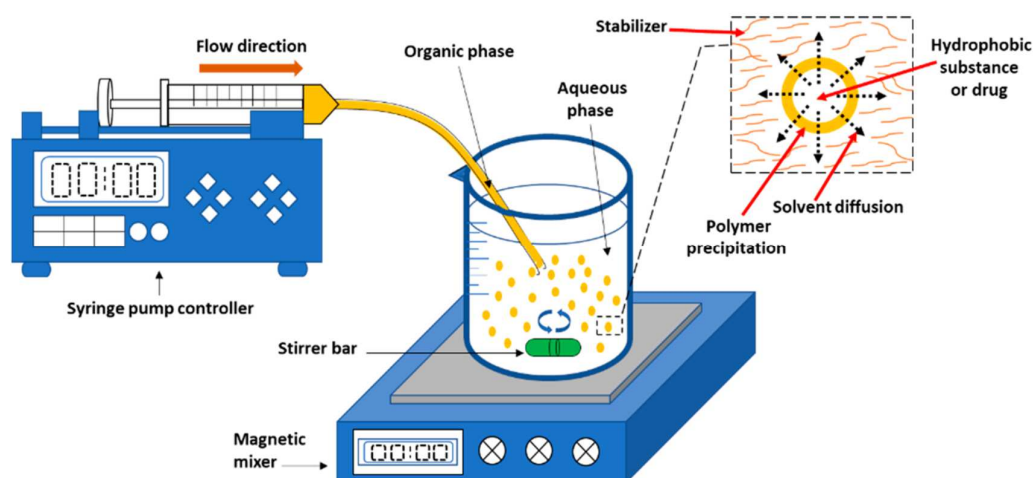


Figure 5: An illustration of the nanoprecipitation process

Emulsification-Solvent Evaporation²⁸

PNPs are widely synthesized using the emulsification-solvent evaporation process. This procedure starts with emulsifying a polymer mixture into an aqueous state. The precipitation of polymers is caused by subsequent solvent evaporation and the production of nanoparticles. Emulsions are formed using two main strategies: single-

emulsions (o/w) or double-emulsions (w/o/w or o/w/o). Primary emulsion (w/o) produced in double emulsions by dispersing a polymer in an organic solvent in an aqueous phase. After that, it is homogenized using an external aqueous phase that contains an emulsifier or surfactant to form nanoparticles.

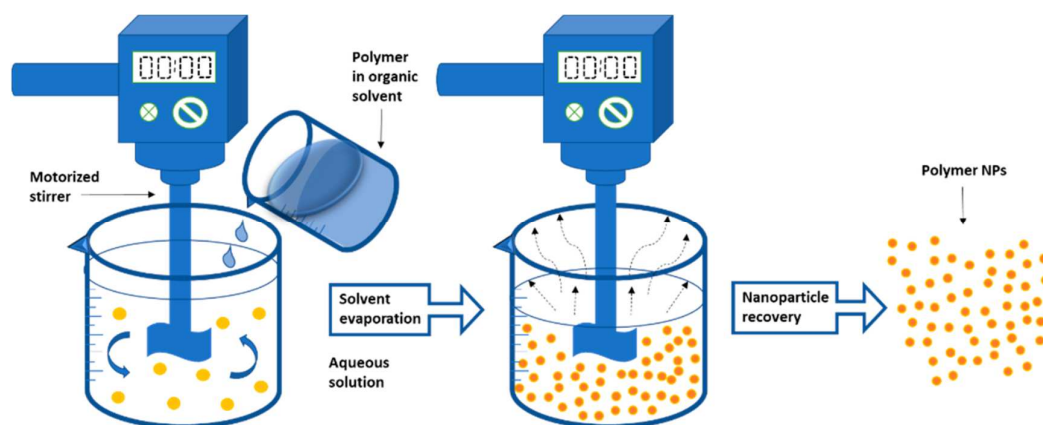


Figure 6: A graphic representation of the emulsification-solvent evaporation

Desolvation method³³

The desolvation process (Figure 7), sometimes referred to as simple coacervation or phase separation, partially desolvates fully solvated molecules to cause macromolecular aggregation. This technique was first utilized to make micron-sized carriers, but it is now frequently used to make chitosan nanoparticles. Commonly utilized precipitating agents include sodium sulfate, while water-miscible nonsolvents like acetone have also been employed. The procedure is straightforward: a chitosan solution is mixed with a desolvating agent (such as sodium sulfate) dropwise. Because of its greater affinity for water, the salt progressively eliminates the solvation water surrounding chitosan, leading to precipitation and polymer insolubilization. This causes chitosan molecules to interact more, resulting in the formation of nanoparticles.

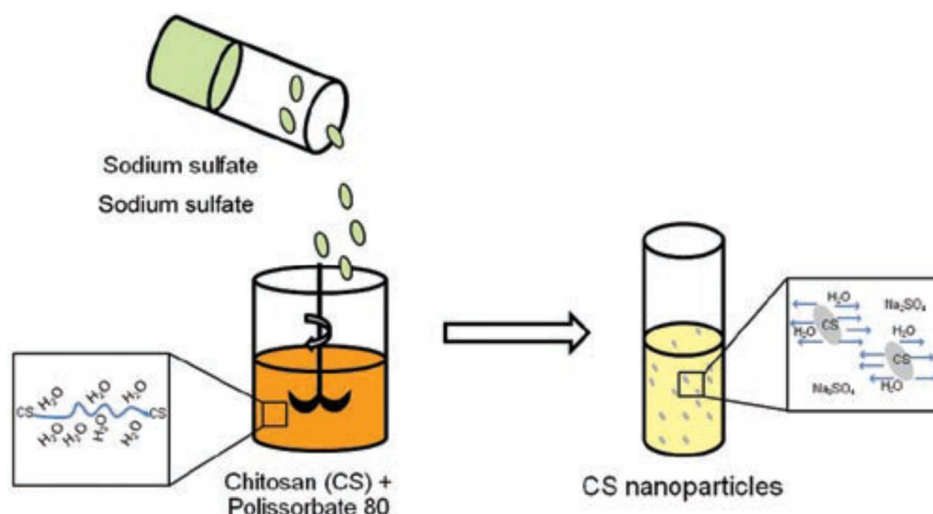


Figure 7: Diagrammatic illustration of the desolvation process

Quality by Design (QbD):

USFDA developed the QbD concept to direct pharmaceutical product development (FDA Guidelines for Industry, 2006). It involves employing systematic strategy to product design in order to assure successful commercialization.³⁴

The QbD approach is a risk-based quality improvement scientific and systematic holistic approach for the development of formulations with clear, predetermined goals that have a little financial, time, and effort commitment while improving process and product understanding. Designs of Experiments (DoE) have been recognized as an essential element of QbD, which aids in comprehending the cause-and-effect relationship. Numerous studies have documented the successful application of QbD along with DoE concepts in designing formulations to achieve strong product quality.³⁵

Four key components of QbD-centered principles as follows:

1. Defining Quality Targets: Establishing clear objectives for the product's quality profile.

2. Identifying Critical Attributes: Determining key product characteristics that impact quality.
3. Screening Process Parameters: Identifying and evaluating critical factors that affect product variability.
4. Estimating Impacts: Analyzing how these factors influence product attributes to optimize formulation and manufacturing processes.³⁶

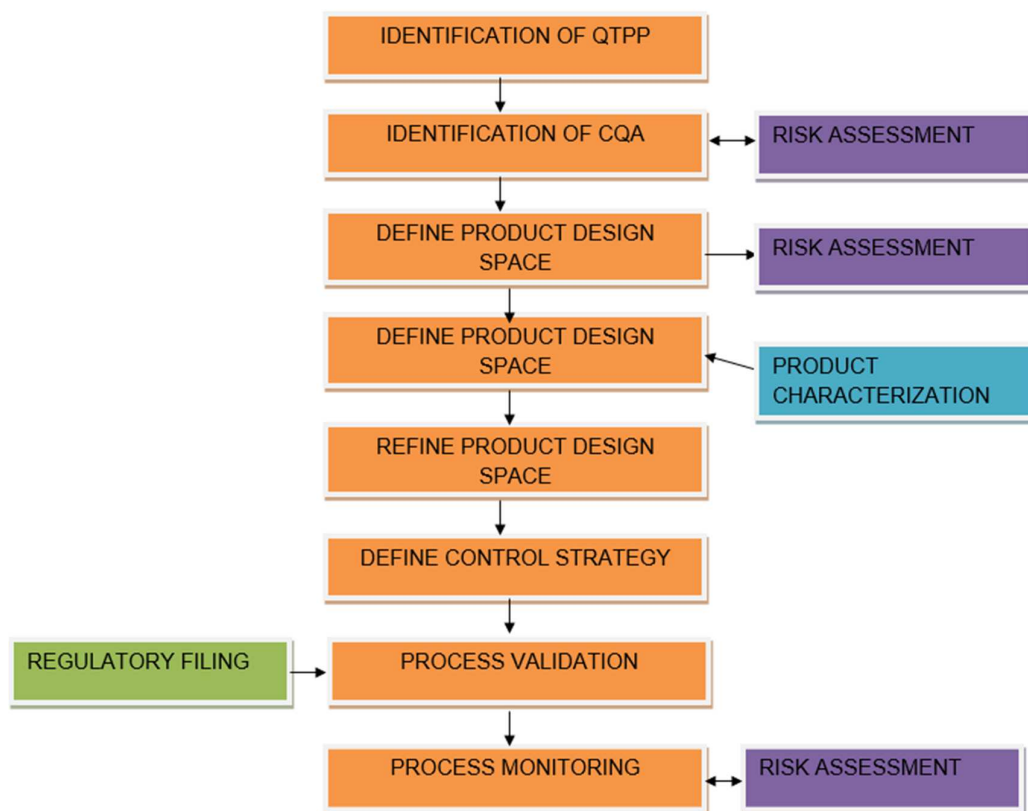


Figure 8: Flow chart of QbD process

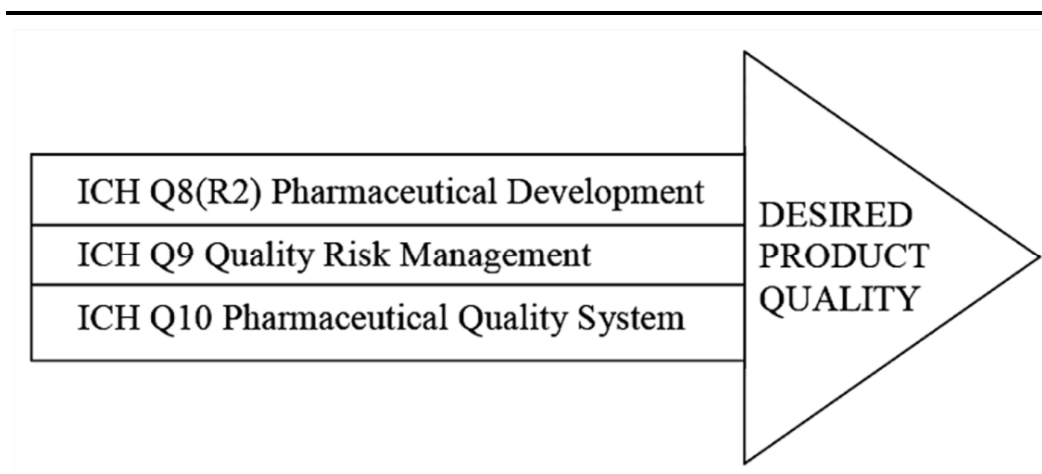


Figure 9: Pharmaceutical quality systems for quality by design³⁷

Objective of Integrating QbD Principles in Nanoformulation

There can be serious consequences from a lack of knowledge about how different pharmaceutical production steps affect quality and performance, as well as the influence of variables. These include rising development expenses for nanoparticulate drug delivery systems and a greater frequency of product failures, which eventually endanger the products' ultimate quality.³⁸ The goal of incorporating QbD principles into nanoformulation procedures is to enhance the effectiveness, safety, and quality of drug delivery systems using nanoparticulates. QbD aims to integrate quality into products from the outset of industrial planning to the finished product that is given to patients. This strategy optimizes the product's different quality features through a risk-based, holistic methodology that makes use of scientific tools.³⁶

Principal goals of incorporating QbD into nanoformulation

1. Assurance Product Safety and Quality: QbD lowers the risk of product failure by ensuring that the nanoformulation satisfies the necessary quality standards by comprehending and managing the critical material attributes (CMAs) and critical process parameters (CPPs) elements that affect the finished product.

2. Enhancement of Process Efficiency: QbD reduces process variability by offering a systematic approach to managing and enhancing variables such as drug loading, surface charge, entrapment efficiency (EE), polydispersity index (PDI), and particle size, all of which are critical to the functioning of nanoparticulate systems.^{36, 39}

3. Regulatory Compliance: The QbD methodology complies with regulations that stress the value of pharmaceutical quality systems and quality risk management in the development of drug delivery systems, including ICH Q8 (R2), Q9, and Q10.⁴⁰

4. Reduction of Risks and Costs: QbD helps to lower the risks and costs involved in developing and implementing nanoparticulate drug delivery systems by lowering the possibility of product failures and the accompanying legal penalties linked to poor quality.³⁶

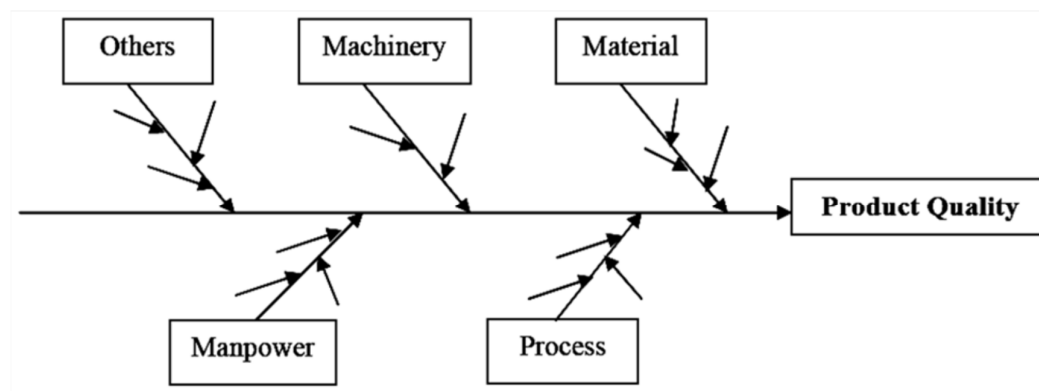


Figure 10: Cause-effect (Ishikawa diagram or fishbone) diagram³⁷

The stepwise progression of a QbD-based strategy involves^{36, 41}

1. Design and Analysis Tools: Define QTPP, identifies and emphasizes CQAs, and assess of risks.

2. Process Tools: Analysis of the impact of process parameters, screening of CPPs, and assessment of the impact of CMAs.

3. Control Tools: Defining Knowledge Space, Characterizing Space, Operating Space, Design Space, and Optimal Design Point. Develop strategies for comprehensive quality control and implement Life-Cycle Management.

4. Feedback-Based Continuous Improvement Tools: Focus on continuous improvement based on feedback and data analysis.

Design of experiments (DoE)³⁷

The DoE, a scientific approach employed to ascertain interactions among the variables influencing process and its outcomes. DoE assists in selecting and planning experiments to efficiently obtain trustworthy information, but it cannot take the place of experience or knowledge. Regression modeling, response surface modeling, screening, and comparison experiments are among its applications.

Types of Experiments:

1. Comparative Experiments: In order to determine which choice is superior, these compare the results of multiple options, such as selecting between various vendors.

2. Screening Experiments: By identifying the major variables influencing a result, this technique enables fewer experimental runs to concentrate on the most significant variables.

3. Response Surface Modeling (RSM): This technique is used to achieve certain goals, maximize or minimize responses, decrease variation, strengthen processes, or balance several objectives by modifying important variables.

4. Regression Modeling: This technique offers accurate models that specify the connections between inputs and response variables.

Common Experimental Designs:

- a. **Completely Randomized Designs:** Examine a primary element without taking other factors into consideration.
- b. **Randomized Block Designs:** By blocking, the impacts of controllable but irrelevant components are eliminated.
- c. **Full Factorial Designs:** Test every possible combination of factor levels; two-level factors require (2^n) runs.
- d. **Fractional Factorial Designs:** By sampling a subset from the full factorial design, you can cut down on the number of runs.
- e. **Plackett–Burman Designs:** Effective for determining the primary impacts when numerous factors are at play.
- f. **Response Surface Designs:** Response behavior is captured by cubic or quadratic models (Figure 11); for RSM, Box-Behnken Designs and Central Composite Designs (CCDs) are frequently employed.
- g. **Three-Level Full Factorial Designs:** Quadratic connections between (3^n) runs can be captured by using three levels for every element.
- h. **Visualization:** Plotting contours and responses provides a substitute to 3D surface plots by illustrating the links between causes and responses.

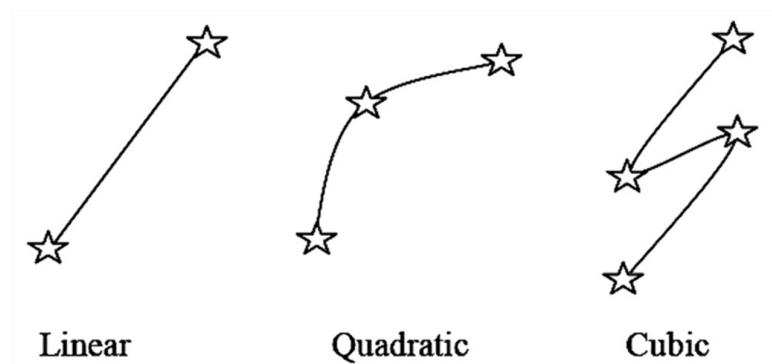


Figure 11: Quadratic, cubic, and linear relationships of a response

1.2 LITERATURE REVIEW

Ichimaru H *et al* (2024) have developed polystyrene magnetic nanoparticles (PSM NPs) by the solvent-evaporation method for clinical diagnosis (Chemiluminescent Immunoassay). PSM NPs were prepared by aqueous methanol (Me) solution 4% (v/v) and stearic acid (SA). 4MeSA–PSM NPs showed the largest concentration of magnetic particles. Moreover, 80pg/mL is the limit of detection for C-reactive protein, according to CLEIA analysis. With regard to clinical diagnosis, 4MeSA–PSM NPs thus seem promising.⁴²

Galiyeva Aet al (2023) have synthesized human serum albumin immobilized (rifampicin, isoniazid) nanoparticles by using the desolvation method. Optimization was done by using CCD. The particle size of optimized nanoparticles was 216.7 ± 3.7 nm with a spherical shape and a PDI value of 0.286 ± 4.9 . The optimized nanoparticles had loading percentages of 44% for rifampicin and 27% for isoniazid.⁴³

Todaro Bet al (2022) have formulated Pioglitazone-PLGA Nanoparticles by the single emulsification-solvent evaporation or nanoprecipitation methods. The best formulation produced through this procedure showed a very slow release of drug in PBS. The nanoprecipitation approach seems to be the easiest, fastest, and most dependable synthetic process for these drug nanocarriers based on its higher entrapment efficiency, improved storage time, and appropriate particle size.⁴⁴

Gonzalez-Melo C *et al* (2021) have suggested a very effective gelatin (type B) and chitosan (LMW) NPs production process. A double desolvation approach was used to produce gelatin nanoparticles (GNPs), and to develop chitosan nanoparticles (CNPs),

the ionic gelation method was employed. Zeta potentials were found to be $+36.60 \pm 3.25$ mV for CNPs and -13.42 ± 1.16 mV for GNPs, and their particle sizes were found to be 373 ± 71 nm for CNPs and 244 ± 67 nm for GNPs. They were examined for biocompatibility to ensure their prospective use as cell-penetrating compounds and carriers of bioactive molecules.⁴⁵

Yang Z *et al* (2020) prepared Doxorubicin loaded BSA nanoparticles by desolvation method. The findings demonstrated a highest loading capacity of drug (21.4%), a narrow PDI, a surface charge (-20.5mV), and a particle size of around 130nm. Overall, these results suggested that controlled release and drug distribution might find use in cancer treatment.⁴⁶

Ismail R. *et al* (2019) used QbD to produce PLGA nanoparticles loaded with Liraglutide using the solvent evaporation method (double emulsion). They then refined the process by using 7 factors and 2 levels of the Plackett Burman screening design. The resulting spherical nanoparticles showed a 188.95nm particle size with homogeneous dispersion and 51.81% encapsulation efficiency. This study illustrated the significance of applying DoE within the QbD concept at the beginning of the synthesis of nanoparticles containing Liraglutide because of the system's complexity.⁴⁷

Jahangir MA *et al* (2018) employed a combined technique of solvent evaporation and nano-precipitation to formulate Sitagliptin-loaded eudragit RL 100 nanoparticles. Formulations optimized using Box-Behnken Design. Optimized batch exhibited a 24 h sustained drug release period, a drug loading range of 6.36–8.76%, and a particle

size range of 135.86–193.45 nm. Both *in vitro* and *in vivo* anti-diabetic activity revealed the long-term reduction in blood sugar levels.⁴⁸

Jiang L *et al* (2018) developed Paclitaxel loaded chitosan NPs using a simple desolvation technique. In comparison to the ionotropic gelation method (0.43%), results indicated that adjusting the paclitaxel to chitosan ratio to 0.2 could result in a 9.8% loading efficiency of paclitaxel. An alternate method of synthesizing chitosan nanoparticles that has high processing yield, drug (hydrophobic) encapsulating capacity, and physical stability was demonstrated in this work.⁴⁹

Pandey AP *et al* (2017) prepared curcumin-loaded Lactoferrin nanoparticles by the desolvation technique using QbD approach for the screening investigation; the Plackett-Burman design was applied to eight components and two levels, and a custom design was chosen for further investigation. Particle sizes, yields, and polydispersity indexes of about 190 nm, 0.2, and 75%, respectively, were obtained from the optimized design space. The researchers came to the conclusion that curcumin served as a model polyphenol in order to evaluate the lactoferrin nanoparticles' ability to encapsulate curcumin.⁵⁰

Younis N *et al* (2016) formulated Eudragit RS 100-Silymarin-loaded nanoparticles (SMNPs) by the combined technique of solvent evaporation and nano-precipitation. The optimized formulation exhibited a drug EE% of $89.47 \pm 1.65\%$, with a particle size (mean) of 632.28 ± 12.15 nm and prolonged release of the drug over a 24 h period. Consequently, the developing of SMNPs could represent advancement in the production of anti-fibrotic drug.⁵¹

Sailaja AK *et al* (2012) have used acetone as a desolvating agent in their desolvation process to produce bovine serum albumin (BSA) nanoparticles. The outcome demonstrated that acetone was added periodically to obtain the size of BSA nanoparticles.⁵²

Xiong S *et al* (2011) synthesized PLGA NPs by employing a combined method of solvent emulsion evaporation and nanoprecipitation. Murine monocyte-derived macrophage (RAW264.7) and human bronchial epithelial cells (BEAS-2B) were used to measure the *in vitro* cell uptake efficiency. The absorption of RAW264.7 cells was higher than that of BEAS-2B cells. Additional research has yielded significant insights into the interactions between nanomaterials and cells and the potential effects of these interactions on toxicity or biocompatibility.²⁷

Agnihotri SA and Aminabhavi TM (2007) have prepared Timolol maleate-chitosan (TMCS) nanoparticles by the desolvation method. To investigate the percentage of drug release (response) at 4 hours, a 3² full factorial design and second-order regression models were employed. Particle size (mean) and zeta potential of TMCS NPs ranged between 118 to 203 nm and +17 to +22 mV, respectively. EE of TMCS NPs ranged between 47.6% to 63.0%. *In vitro* drug release of Timolol maleate in PBS pH 7.4 up to 24 h at a slow release rate was observed. Release kinetics suggested that Fickian pattern of drug release.⁵³

1.3 JUSTIFICATION

The most significant drawback of Vildagliptin is the fast metabolism and shorter half-life of two to three hours.⁵⁴

In a conventional oral dose of Vildagliptin, it is recommended to take 50mg twice a day due to its shorter half-life, which undergoes high fluctuations in drug plasma levels. Patients must strictly adhere to the dosage schedule to manage diabetic therapy.⁵⁵

Conventional dosage forms of VLD exhibit adverse effects like headache, cough, constipation, sweating, heartburn, nasopharyngitis, weakness, dizziness and hypoglycemia.⁵⁶

In this context, it is necessary to develop an alternative dosage form with sustained delivery of VLD, which would provide constant drug release in the GIT and maintain drug plasma levels in steady state for a longer period of time, which will improve treatment efficiency.

Recently, nanoparticles playing major role in the nanomedicine field for diagnosis and therapy. Nanocarriers facilitate drug release and protect the drug from degradation in a sustained or controlled manner. Furthermore, nanoparticles reduce side effects and improve therapeutic efficacy by decreasing clearance and improving accumulation of drug molecules in the diseased tissue.⁵⁷

In order to deliver drugs to the desired site at the right time in the right dose, researchers are currently focusing on developing oral nanosized drug delivery systems.⁵⁸

Currently, various nanoformulations like liposomes, polymeric nanoparticles, niosomes, and nanoemulsions are employed to assist with improving the oral delivery of several kinds of drugs. The methods outlined above include polymeric nanoformulations that were gaining more importance due to their inherent properties, like stability in the GIT region.⁴⁸

Nowadays, polymeric nanoparticles have been developed in order to minimize the dose and dosing frequency, thereby reducing adverse effects of the drug.

Polymeric nanoparticles are prepared from synthetic, semisynthetic, or natural polymers that improve drug delivery and targeting by reducing phagocytic absorption, inactivating, and extending their circulation half-life.⁵⁹

The FDA started the QbD concept in pharmaceutical drug development (FDA guidance to industry, 2006). The intended conception of a product for commercialization is a design effort.³⁴

The quality of the finished product may be affected by the physicochemical characteristics of the nanoparticles, which can be influenced by different formulation and processing conditions. To ensure the quality of nanoparticles, the QbD concept has become essential.⁴⁷

According to a literature search, no nanoformulations have been developed for oral administration of Vildagliptin using the solvent evaporation and nanoprecipitation and desolvation methods utilizing the QbD principle. Both the selected methods are simple, easy, and do not require any sophisticated equipment for the production of nanoparticles.

Therefore, in this work an effort is made to formulate and optimize the oral polymeric coated Vildagliptin nanoformulations using QbD concept.

1.4 OBJECTIVES

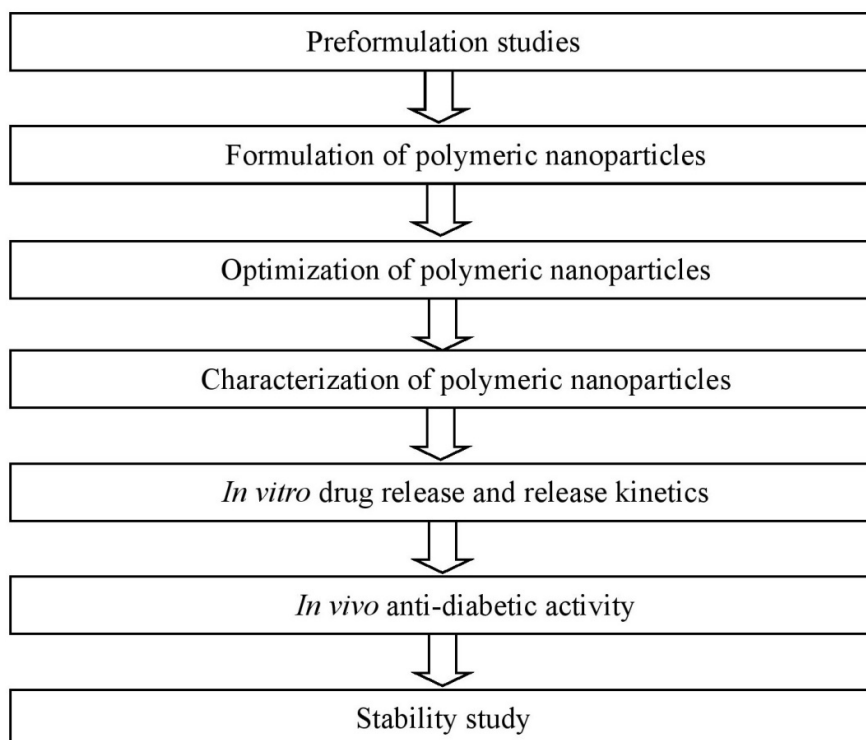
1.4.1 Primary objective:

To formulate and optimize oral polymeric coated Vildagliptin nanoformulations using QbD concept.

1.4.2 Secondary objective:

To study the antidiabetic activity of oral polymeric coated Vildagliptin nanoformulation in streptozotocin induced diabetic Wistar rats.

1.5 PLAN OF WORK



2. MATERIALS AND METHODS

Table 1: List of instruments

Sl. No.	EQUIPMENTS	MANUFACTURER
1	Centrifuge	KUBOTA 7000, Japan
2	ElectronicBalance	Sartorius
3	Milli Q water System	Merck Pvt. Ltd.
4	Zetasizer Nano ZS	Malvern Instruments Corporation, UK
5	MagneticStirrer	RemiMoors,Mumbai
6	Digital pH meter	Systronics, India
7	Dialysismembrane	HiMediaLtd,Mumbai
8	UV Visible spectrophotometer	UV -1900 Shimadzu Corporation, Japan.
9	Humiditycontrolchamber	M/sLabControlequipment,Mumbai
10	FourierTransformInfraRed(FTIR)	BrukerAlpha II,USA
11	Transmission Electron Microscopy	JPS 9030 JEOL ASIA Pvt. Ltd, Singapore TECNAI G2 SPIRIT BIOTWIN, Minnesota
12	USP dissolution apparatus	ELECTROLAB INDIA PVT. LTD. Mumbai
13	Lyophilizer	ALPHA 1-2LDplus, CHRIST. Germany
14	Glucometer	Morepen Labortories Ltd, Himachal Pradesh

Table 2: List of chemicals

Sl. No.	Chemical	Supplier
1	Vildagliptin	Ajanta Pharma Ltd. Mumbai
2	Eudragit RL 100	Centaur Pharma. Pune
3	Chitosan (LMW)	Yarrow chem products. Mumbai
4	Acetic acid	Loba Chemie Pvt. Ltd. Mumbai
5	Acetone	Research-lab fine chem industries Mumbai
6	Disodium hydrogen phosphate	Loba Chemie Pvt. Ltd. Mumbai
7	Dichloromethane	Yarrow chem products, Mumbai
8	Glutaraldehyde	Yarrow chem products, Mumbai
9	Hydrochloric acid	Loba Chemie Pvt. Ltd. Mumbai
10	Methanol	Loba Chemie Pvt. Ltd. Mumbai
11	Poloxamer 188	Yarrow chem products, Mumbai
12	Potassium dihydrogen phosphate	Loba Chemie Pvt. Ltd. Mumbai
13	Sodium hydroxide	Research-lab fine chem. industries, Mumbai
14	Sodium chloride	Loba Chemie Pvt. Ltd. Mumbai
15	Streptozotocin	Spectrochem Pvt. Ltd. Mumbai
16	Mannitol	Loba Chemie Pvt. Ltd. Mumbai
17	Citric acid	Loba Chemie Pvt. Ltd. Mumbai
18	Sodium citrate	Loba Chemie Pvt. Ltd. Mumbai
19	Sucrose	Loba Chemie Pvt. Ltd. Mumbai

2.1 Identification Tests

2.1.1 Solubility study

Solubility studies were conducted using 0.1N HCl, water, and PBS (pH 7.4).

2.1.2 Determination of Melting Point

Thiele's tube method was used to determine VLD's melting point. The experiment was conducted in three trials. Readings were noted as T₁- 149, T₂- 150 and T₃- 152.

2.2 Analytical methods for estimation of Vildagliptin

2.2.1 Determination of λ_{max} of Vildagliptin⁶⁰

The λ_{max} of VLD was determined by scanning the 20 μ g/ml solution of drug in HCl (0.1N) and PBS (pH 7.4) between 400-200nm in UV-Spectrophotometer.

2.2.2 Calibration curve of Vildagliptin in HCl (0.1N) and PBS (pH 7.4)

Weighed accurately, 20mg of VLD, then transferred into a 100ml volumetric flask containing 100ml 0.1N HCl and labeled as stock solution A. From this, 10ml solution was pipetted out and then transferred to a separate volumetric flask (100ml) further diluted it with up to the mark 100ml with 0.1N HCl, and labeled as stock solution B. Then, five separate 10ml volumetric flasks containing 1ml to 5ml of stock solution B further diluted them with 0.1N HCl until the volume was up to the mark of 10ml. Thus the concentrations of solution ranging from 2 μ g/ml to 10 μ g/ml were obtained. The above procedure was also repeated with PBS (pH 7.4). The UV spectrophotometer (Shimadzu 1900) was used to analyze various solution concentrations at 208nm.

2.2.3 Determination of λ_{max} of Vildagliptin in 0.1 N NaOH

The λ_{max} of Vildagliptin was determined by scanning the 40 μ g/ml solution of the drug in 0.1N NaOH between 400-200nm in UV-Spectrophotometer (Shimadzu 1900).

2.2.4 Standard calibration curve of Vildagliptin in 0.1 N NaOH⁶¹

Weighed accurately, 100mg of VLD was dissolved in 100ml volumetric flask containing 100ml 0.1N NaOH and labeled as stock solution A. Transfer 10ml of stock solution A with a pipette into a volumetric flask (100ml). Further, diluted it with 100ml of 0.1N NaOH and labeled as stock solution B. Then, five separate 10ml volumetric flasks containing 1ml, 2.75ml, 5ml, 7.5ml, and 10ml of stock solution B were filled, and each was diluted with 0.1N NaOH until it reached 10ml. Thus the concentrations of solution ranging from 10 μ g/ml to 100 μ g/ml were obtained. The UV spectrophotometer (Shimadzu 1900) was used to analyze various solution concentrations at 216nm.

2.2.5 Validation of UV-Visible spectroscopy⁶²

A UV-Visible (UV-Vis) spectroscopic method must be validated in order to guarantee that the analytical process is reliable, precise, and reproducible for the intended application. Guidelines such as those issued from ICH or other regulatory authorities are usually followed during the validation process. The following validation parameters were carried out for VLD in HCl (0.1N), PBS (pH 7.4) and NaOH (0.1N).

2.2.5.1 Linearity

By examining varying concentrations of the VLD standard solution, the method's linearity was assessed. The concentration range for beer lamberts was determined in

2–10 µg/ml (0.1N HCl and PBS (pH 7.4) at 208nm) and in 10-100 µg/ml (0.1N NaOH at 216nm). Plotting the absorbance against concentration data produced the calibration curve, which was then subjected to linear regression analysis.

2.2.5.2 Accuracy

Recovery studies were used to assess the accuracy of the suggested method. The accuracy of the method was assessed by computing the recovery percentage of a pure VLD sample at various levels (80%, 100%, and 120%), using the standard addition method. At 208nm (0.1N HCl and PBS pH 7.4) and at 216nm (0.1N NaOH) after measuring the appropriate solution's absorbance, the recovery percentage and the amount of drug added were calculated.

2.2.5.3 Precision

The accuracy of the procedure was examined by measuring intra-day variations in solution (6 µg/ml) absorbance at 208nm in 0.1N HCl and PBS pH 7.4 and (27.5 µg/ml) at 216nm in 0.1 N NaOH on the same day and inter-day variations on three separate days throughout a week. Relative standard deviation was used to compute the intra- and inter-day variations.

2.2.5.4 Robustness and Ruggedness

The developed method's robustness was assessed by making minor, deliberate adjustments to the process, such as altering the wavelength by $+2\lambda$ and -2λ . Changing the analyst assisted in the determination of the method's ruggedness. The relative standard deviation of the obtained variations was calculated.

2.2.5.5 Limit of detection and quantification (LOD and LOQ)

Response standard deviation and calibration curve slope were used to determine the LOD and LOQ using formulae that are as follows.

$$\text{LOD} = 3.3 \frac{\sigma}{s}$$

$$\text{LOQ} = 10 \frac{\sigma}{s}$$

2.3 FT-IR Spectroscopy

The FT-IR spectra of VLD, Eud, CS, VLD Eud NPs, and CS VLD NPs were obtained using an FT-IR spectrophotometer (DRS 8000, Shimadzu IR Affinity-1). The wavelength range in which the spectra were recorded was 4000-400 cm^{-1} . Additionally, the drug-polymer compatibility was established.⁹

2.4 Preparation of Vildagliptin Nanoformulation

2.4.1 Solvent Evaporation and Nano-precipitation Method

VLD Eud NPs were developed using a slightly modified method involving solvent evaporation and nano-precipitation (Figure 12).⁶³ Briefly, the combination of solvents of dichloromethane (DCM) and methanol was used to dissolve various drug:polymer (Eudragit RL 100) ratios, namely 1:2, 1:4, and 1:6, at three different ratios, namely 1:3, 1:1.66, and 1:1. Using a 22-gauge needle, the aforementioned organic mixture was then added drop by drop to 10ml of the 0.5% w/v aqueous solution of poloxamer 188 and rotated on a magnetic stirrer at 500rpm. After evaporating organic solvent for 2 h at room temperature, an aqueous dispersion was obtained. The nanoparticles were then collected by centrifuging the resulting aqueous dispersion at 35,000rpm for 60min at 4°C (KUBOTA 7000, Japan). The resulting sediment was centrifuged in triplicate under the same circumstances after washing, followed by resuspending it in

distilled water. Lyophilizer (ALPHA 1-2LDplus, CHRIST. Germany) was used to dry the finished product. A total of nine formulations were prepared and are presented in Table 3.

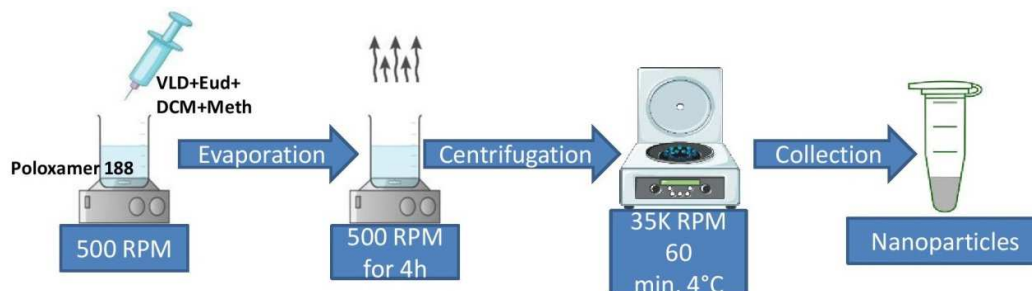


Figure 12: Preparation of VLD Eud NPs by solvent evaporation and nano-precipitation method

Table 3: Formulation composition of VLD Eud NPs

Formulation codes	Independent variables	
	X ₁ - Eud (mg)	X ₂ - DCM+Meth (ml)
F1	200	0.5+1.5
F2	400	0.5+1.5
F3	600	0.5+1.5
F4	200	0.75+1.25
F5	400	0.75+1.25
F6	600	0.75+1.25
F7	200	1+1
F8	400	1+1
F9	600	1+1

2.4.2 Desolvation technique

Vildagliptin-loaded chitosan nanoparticles (CS VLD NPs) were formulated by slight modification in a simple desolvation technique (Table 4).⁵³ Briefly, different concentrations of CS 200 mg, 400 mg, and 600 mg were dissolved individually in separate 2%v/v aqueous acetic acid solution (50ml) to produce a homogenous mixture, followed by 100 mg of VLD being added. After that, acetone was added drop

by drop while being constantly stirred resulting in slightly permanent turbidity, which was because of desolvation. After that, various amounts of glutaraldehyde (GA) were added as a cross-linking agent (50 μ L, 100 μ L, and 150 μ L), and a mechanical stirrer was used to stir the mixture overnight at 600 rpm in order to accelerate acetone evaporation. The resultant nanoparticles were collected using centrifugation (KUBOTA 7000, Japan) for one hour at 4°C and 20,000 rpm. Three consecutive washes of the sediment were performed under the same conditions. A total of nine formulations were prepared and are presented in Table 4.

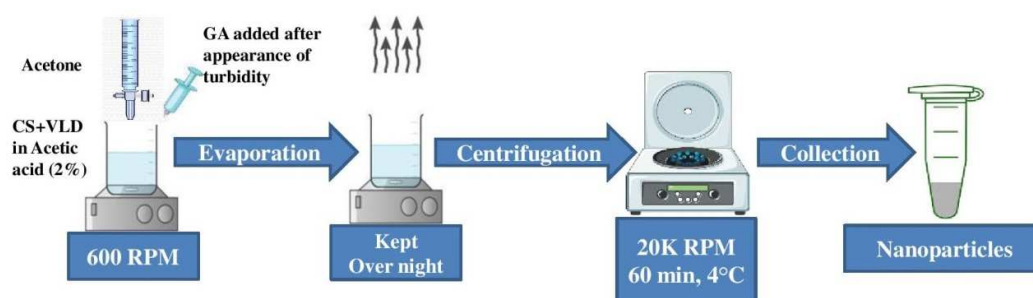


Figure 13: Preparation of CS VLD NPs by desolvation method

Table 4: Formulation composition of CS VLD NPs

Formulation codes	Independent variables	
	X ₁ - CS (mg)	X ₂ - GA (μ L)
N1	200	50
N2	200	100
N3	200	150
N4	400	50
N5	400	100
N6	400	150
N7	600	50
N8	600	100
N9	600	150

2.5 Statistical experimental design

Traditionally, one variable at a time had been altered when developing pharmaceutical formulations. It takes a lot of time and effort to use this strategy. Furthermore, the conventional approach does not account for the combined effects of individual components, which may make it challenging to develop an ideal formulation. Therefore, it is crucial to use reliable statistical techniques, such as factorial designs, in order to comprehend the complicated nature of pharmaceutical formulations.

In order to formulate VLD Eud NPs, 3^2 statistical designs for experiments were used (Design Expert 13). Two independent variables—the amount of (X_1) Eudragit RL 100 (mg) and (X_2) organic solvent's composition, i.e., DCM+methanol (ml)—were chosen for this design. In order to characterize the 3^2 factorial designs with nine different formulations, 3 levels for every independent variable were assigned codes as (-1) low, (0) middle, and (+1) high, as indicated in the Table 4. Two factors were examined at their three levels in relation to the dependent variables (Y_1) particle size (nm) and (Y_2) entrapment efficiency (%), as illustrated in Table 5.

Table 5: Experimental design of VLD Eud NPs with their process variables and levels

Levels	Independent variables		Dependent variables	
	X_1 =Eudragit RL 100 (mg)	X_2 =Volume of DCM and methanol (ml)	Y_1 =Particle size (nm)	Y_2 =Entrapment Efficiency (%)
-1	200	0.25+1.75	Goal to minimize	Goal to maximize
0	400	0.5+1.5		
+1	600	0.75+1.25		

CS VLD NPs were also developed using 3^2 statistical designs for experiments (Design Expert 13). The entire factorial design is shown in Table 6. Two independent factors were analyzed: the concentration of CS and the amount of GA utilized. Two dependent variables, namely particle size and entrapment efficiency, were explored in relation to three levels (low, medium, and high) of each variable.

Table 6: Experimental design of CS VLD NPs with their process variables and levels

Levels	Independent variables		Dependent variables	
	X ₁ CS (mg)	X ₂ GA (μL)	Y ₁ =Particle size (nm)	Y ₂ =Entrapment Efficiency (%)
-1	200	50	Goal to minimize	Goal to maximize
0	400	100		
+1	600	150		

2.6 Characterization of nanoformulations

2.6.1 Determination of Particle Size and PDI

Zetasizer (Malvern Nano ZS Ltd., UK) was used to examine the PS and PDI of the developed nanoformulations. Millipore water was used to appropriately dilute each sample. Samples were examined at 25°C with the refractive index set to 1.33.⁶⁴

2.6.2 Entrapment efficiency (EE)

The % EE of the Vildagliptin nanoformulations was determined using the indirect technique, which measures the amount of drug that is not entrapped. VLD Eud NPs in a known volume were put into 2ml centrifuge tubes and rotated for 60 min at 4°C and 35,000 rpm using an ultracentrifuge (KUBOTA 7000, Japan). After collecting the supernatant, 0.1N NaOH was used to produce an appropriate dilution. A UV

spectrophotometer (Shimadzu 1900, Japan) set to 216nm was used to evaluate the samples.⁶⁵

Similarly, using the same centrifuge model, the EE of the CS VLD NPs has been determined by centrifugation for 30 minutes at 4°C and 20,000 rpm. After collecting the supernatant, it was diluted with PBS at a pH of 7.4 as needed. The UV spectrophotometer was used to evaluate these samples at 208 nm.^{9, 66} In both cases, the following formula was used to calculate %EE.

$$\% \text{ EE} = \frac{\text{Amount of drug used} - \text{untrapped drug}}{\text{Amount of drug used}} \times 100$$

2.6.3 Transmission Electron Microscopy (TEM)⁶⁷

TEM study was conducted to investigate the morphological features of developed nanoformulations of optimized VLD Eud NPs F6 (JPS 9030 JEOL ASIA Pvt. Ltd. Singapore) and CS VLD NPs N6 (TECNAI G2 SPIRIT BIOTWIN, Minnesota). A drop of the nanoformulation was placed on a copper grid coated with carbon (mesh size 200). It was then stained for 2 min by adding phosphotungstic acid 2% w/v aqueous solution, allowed to air dry, and studied using a TEM set up at 200 kV at 40,000× magnification to take pictures of a randomly chosen region.

2.6.4 Determination of Percentage Yield⁵⁰

After determining the dry weight of all the VLD Eud NPs and CS VLD NPs then applying the following formula, the percentage yields of all the VLD Eud NPs and CS VLD NPs were calculated.

$$\% \text{ Yield} = \frac{\text{Weight of nanoformulation}}{\text{Weight of drug and polymer}} \times 100$$

2.6.5 *In vitro* drug release studies¹¹

In vitro drug release studies were conducted for both VLD Eud NPs and CS VLD NPs (N3, N6, and N9) dialysis bags with a molecular weight of 12KDa. A study was conducted for the VLD Eud NPs utilizing a USP dissolution apparatus (ELETROLAB TDT-06L) with a maintained $37 \pm 0.2^\circ\text{C}$ temperature, a volume of 500ml dissolution medium, and a stirring rate of 50rpm. VLD Eud NPs (20mg equivalent weight) were added to the dialysis bag that had been previously soaked. After two hours in 0.1N HCl, the dissolution study was conducted in PBS (pH 7.4). A 5ml samples were taken out at predetermined intervals for up to 24 h, replaced with fresh medium with an equivalent volume to keep constant sink conditions, and then spectrophotometrically examined at 208nm following the proper dilution and filtration.

For the CS VLD NPs, pre-treated dialysis bags with 10mg of VLD were sealed and submerged in 250ml of PBS (pH 7.4) in a beaker that was thermostatically kept at 37°C . A magnetic stirrer at a speed of 100rpm held the setup. A 2ml samples were taken out at predetermined times throughout the 24 h study, and the volume was then restored with PBS. Additionally, these samples were examined with a UV spectrophotometer set to 208nm.

2.6.7 Release kinetics⁶⁸

In vitro release data of all VLD Eud NPs and CS VLD NPs N3, N6, and N9 were fitted into several types of kinetic models, including the zero order, first order, Higuchi, and Korsmeyer-Peppas models, in order to understand the process of drug release. Often, these selected models are used to describe the release of drug data collected from the polymeric delivery system for drugs when its mechanism is unclear

or when there are several kinds of release events. The coefficient of correlation (R^2) was used to evaluate each model's fit accuracy.

2.6.8 Stability study⁶⁹

Both the optimized VLD Eud NPs F6 and CS VLD NPs N6 each were kept in three distinct amber-colored glass vials that were closely sealed for 3 months. In accordance with the guidelines provided by the ICH, nanoparticles' stability studies were conducted at $25^\circ\text{C} \pm 2^\circ\text{C}$ and RH 65%. Particle size, PDI, and %EE were evaluated for formulations F6 and N6 over the course of three months with a 1-month sample interval.

2.6.9 *In vivo* study⁷⁰

Dose-dependent *In vivo* activity of optimized VLD Eud NPs F6 was performed as per the study plan presented in Table 7 and shown in Figure 14.

Table 7: Dose dependent *In vivo* activity of optimized VLD Eud NPs F6

Groups	Animals	Treatment	No of animals (n)
I	Normal control group	Distilled water orally	6
II	Diabetic control group	STZ 40mg/kg Single dose by IP + Distilled water orally for 21 Days	6
III	Std VLD treatment group	STZ 40mg/kg Single dose by IP + VLD 10mg/kg/day orally 21 Days	6
IV	Nanoformulation treatment group I	STZ 40mg/kg Single dose by IP + Opt. VLD NPs 5mg/kg/day orally 21 Days	6
V	Nanoformulation treatment group II	STZ 40mg/kg Single dose by IP + Opt. VLD NPs 7.5mg/kg/day orally 21 Days	6
VI	Nanoformulation treatment group III	STZ 40mg/kg Single dose by IP + Opt. VLD NPs 10mg/kg/day orally 21 Days	6

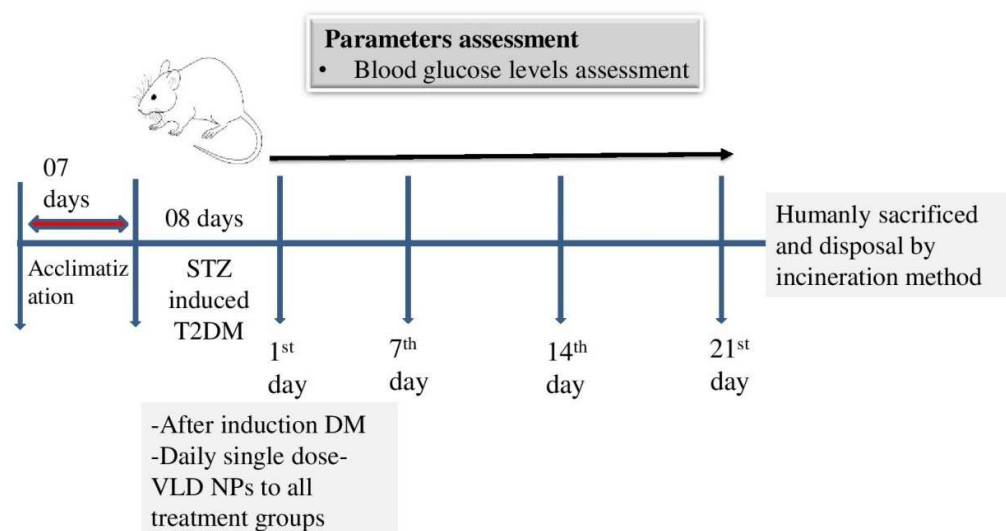


Figure 14: Animal experimental study plan

2.6.9.1 Approval from Institutional Animal Ethics Committee (IAEC)

The protocol for the study titled "Formulation and Optimization of Oral Polymeric Coated Vildagliptin Nanoformulations Using Quality by Design Concept" was submitted to the Institutional Animal Ethics Committee (IAEC) for review and approval. Following approval by the IAEC, the study was registered with the CPCSEA (Reg. No. 221/Po/Re/S/2000/CPCSEA, dated 19/12/2023), ensuring full compliance with ethical standards for animal research.

2.6.9.2 Selection of Animals⁷¹

Wistar rats (*Rattus norvegicus*) were selected as the experimental model for diabetes induction. Adult male rats, weighing between 200–250 grams and aged 4–6 weeks, were chosen based on their health status, being free from infectious diseases. The animals were obtained from a certified vendor. Prior to diabetes induction, the rats were housed in the laboratory animal facility for a one-week acclimatization period.

2.6.9.3 Housing and Animal Care⁷²

The Wistar rats were housed in polypropylene cages with wire mesh tops, ensuring proper ventilation. A 12 h light/dark cycle, a constant temperature of $22\pm 2^{\circ}\text{C}$, and RH $55\pm 5\%$ were all maintained in the housing environment. Throughout the experiment, the rats were provided with free access to water and standard rodent chow. All animals were monitored daily for signs of distress or health complications during the acclimatization and experimental phases. The cages were cleaned regularly to prevent infection and maintain hygiene.

2.6.9.4 Preparation of Streptozotocin solution⁷³

Streptozotocin (STZ), a hydrophilic and light-sensitive compound, was prepared just before administration to maintain its stability and efficacy. The preparation involved the use of 0.1M citrate buffer, which was obtained by dissolving sodium citrate and citric acid in distilled water in the proper proportions, and pH was adjusted to 4.5 with a calibrated pH meter to ensure optimal conditions for STZ stability. To minimize degradation, all the steps were performed under low light conditions. STZ powder was weighed accurately and dissolved in ice-cold citrate buffer with gentle mixing to ensure complete dissolution without introducing air bubbles. Throughout the preparation process, the solution was kept on ice to preserve its activity, as STZ is prone to degradation at higher temperatures. The freshly prepared solution was used within 10 to 15min to avoid any loss of potency and was quickly drawn into sterile syringes for administration.

2.6.9.5 Induction of Diabetes⁷⁴

Five days prior to the initiation of the study, male Wistar rats were acclimatized to the experimental conditions by housing them in groups of three per cage with ad libitum access to food and water. On the day preceding the induction of diabetes, the rats were fasted for 8 h, while water was provided ad libitum. This fasting period was implemented to stabilize their baseline metabolic state in preparation for STZ administration.

Diabetes (type 2 DM) was induced through a single dose of STZ injection by intra-peritoneal (IP) route. The STZ was dissolved in 0.1M citrate buffer (pH 4.5) to create a fresh STZ solution, which was then injected using a 1ml syringe fitted with a 23-G needle at a dose of 40mg/kg body weight. Following the STZ injection, the rats were provided with drinking water containing sucrose solution (10%) for first 24 h to prevent hypoglycemia, which can occur due to the initial release of insulin from the damaged β -cells. On the second day post-injection, the sucrose solution was replaced with regular drinking water to maintain hydration without affecting glucose metabolism.

The induction of diabetes was confirmed by measuring fasting blood glucose levels three days after the STZ injection. After an overnight fast, blood samples were drawn from the tail vein, and a glucometer (Morepen Laboratories Ltd, Himachal Pradesh) was used to measure the blood's glucose levels. Rats were included in the study if their fasting blood glucose levels were ≥ 250 mg/dL, which was known as diabetic. Additionally, clinical signs such as polyuria, polydipsia, and weight loss were monitored to corroborate the onset of diabetes.

2.6.9.6 Experimental Grouping^{75, 76}

The animals were randomly assigned to six experimental groups, each receiving different treatments. Group I (Control group) consisted of healthy animals that were administered distilled water orally for 21 days. Group II (Diabetic control) was induced with diabetes through a single dose of STZ 40mg/kg by IP injection, followed by oral administration of distilled water for 21 days. In Group III (Diabetic + Plain VLD), diabetes was induced by the same STZ treatment, and the animals received plain VLD at a dose of 10mg/kg/day orally for 21 days. Group IV (Diabetic + Optimized VLD Eud NPs 5mg/kg/day) also had diabetes induced by STZ, followed by treatment with Optimized VLD Eud NPs at a dose of 5mg/kg/day orally for 21 days. Group V (Diabetic + Optimized VLD Eud NPs 7.5mg/kg/day) followed a similar protocol with optimum Optimized VLD Eud NPs at a dose of 7.5mg/kg/day orally for 21 days. Lastly, Group VI (Diabetic + Optimized VLD Eud NPs 10mg/kg) consisted of diabetic animals induced with STZ and treated with Optimized VLD Eud NPs at a dose of 10mg/kg/day orally for 21 days. Fasting blood samples were collected after an 8 h fasting period on the initial day, followed by weekly collections for 3 weeks to assess blood glucose levels.

3. RESULTS AND DISCUSSION

3.1 Identification Tests

3.1.1 Solubility study

VLD was found soluble in HCl (0.1N), distilled water, and PBS (pH 7.4).

3.1.2 Melting point determination

VLD's average melting point was determined to be 150.33°C, which is in line with the melting range that has been reported in the literature (148–152°C).

3.2 Analytical methods for estimation of Vildagliptin

3.2.1 Determination of λ_{max} of Vildagliptin

The λ_{max} of VLD was found at 208nm in both 0.1N HCl and PBS (pH 7.4) which were found near to the reported value as per literature (210nm). The UV spectra are shown in Figure 15 (0.1N HCl) and Figure 16(PBS pH 7.4).

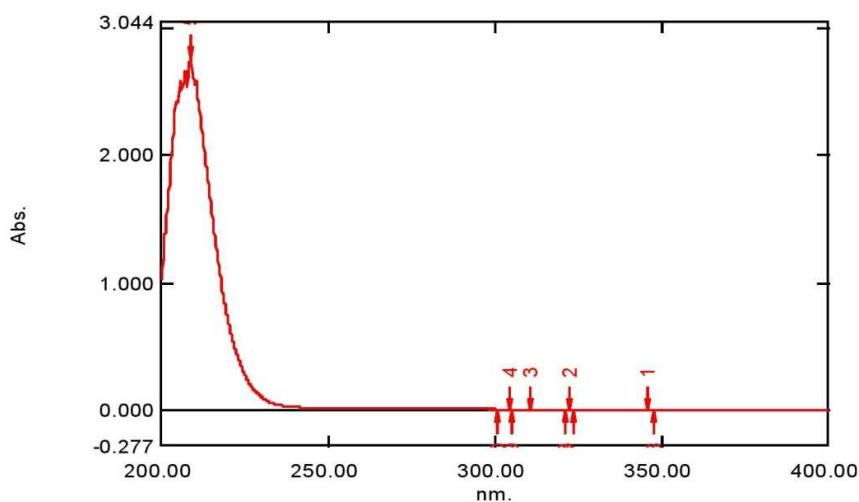


Figure 15: UV Spectrum of Vildagliptin at λ_{max} 208nm in 0.1N HCl

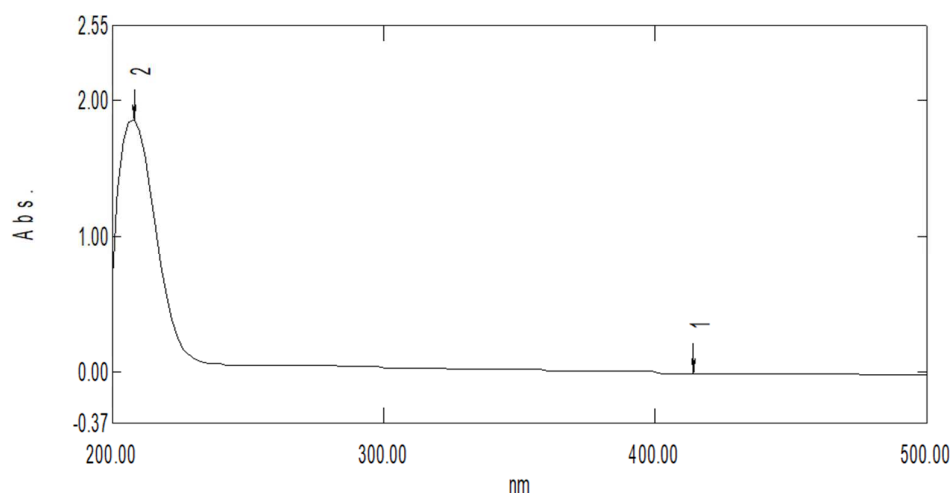


Figure 16: UV Spectrum of Vildagliptin at λ_{max} 208nm in PBS pH 7.4

3.2.2 Calibration curve of Vildagliptin in HCl (0.1N) and PBS (pH 7.4)

A standard calibration curve was plotted using concentration vs. absorbance obtained by each linear dilution of VLD. Each concentration showed a range of linear absorbance between 2 to 10 $\mu\text{g/ml}$ concentration with a regression equation of 0.993 in 0.1N HCl and 0.994 in PBS (pH 7.4). In Table 8, the absorbance of the corresponding concentrations was recorded. Standard calibration curve presented in Figure 17 (in 0.1N HCl) and Figure 18 (in PBS pH 7.4)

Table 8: Standard calibration curve of Vildagliptin at 0.1N HCl and PBS pH 7.4

Sr. No.	Concentration ($\mu\text{g/ml}$)	Absorbance at 208nm	
		0.1N HCl	PBS pH 7.4
1	2	0.056	0.060
2	4	0.093	0.078
3	6	0.134	0.098
4	8	0.172	0.120
5	10	0.202	0.142
Regression R^2		0.997	0.998

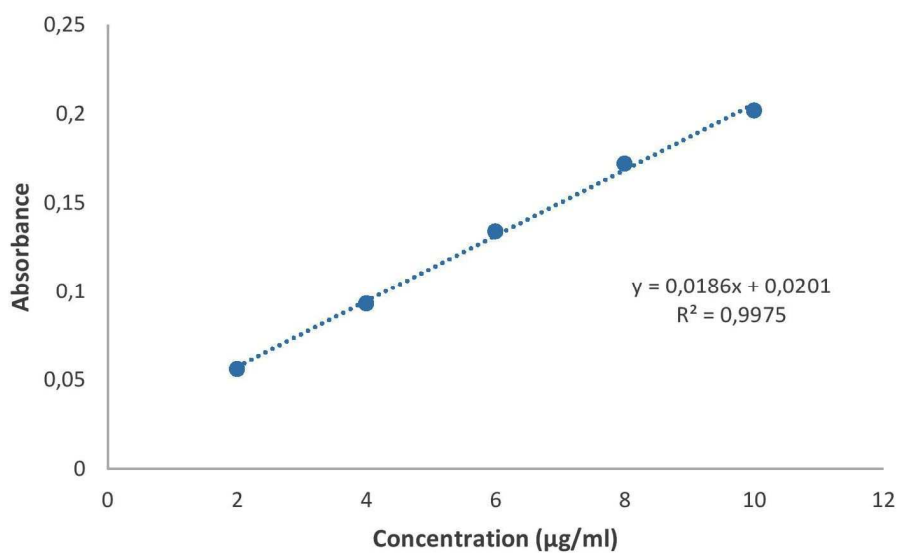


Figure 17: Standard calibration curve of Vildagliptin in 0.1N HCl

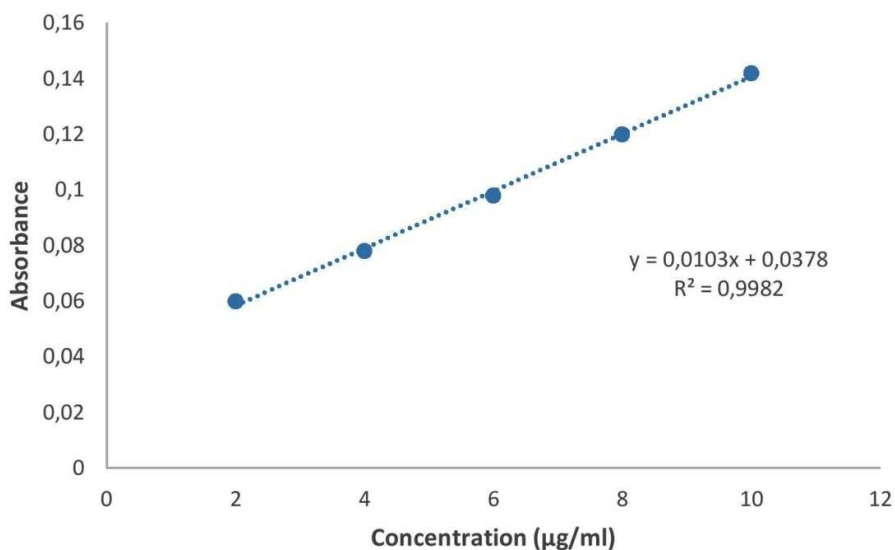


Figure 18: Standard calibration curve of Vildagliptin in PBS pH 7.4

3.2.3 Determination of λ_{max} of Vildagliptin in 0.1N NaOH

VLD's λ_{max} was measured at 216 nm in 0.1N NaOH which was found in line with the reported value as per literature (216nm). The UV spectrum is presented in Figure 19.

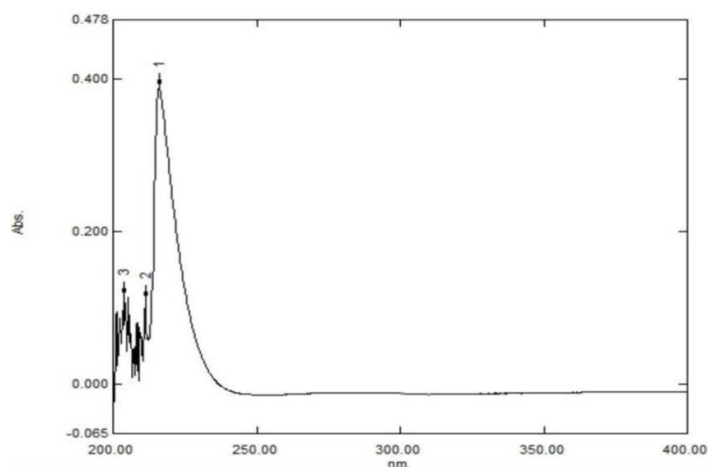


Figure 19: UV Spectrum of Vildagliptin at λ_{max} 216nm

3.2.4 Standard calibration curve of Vildagliptin in 0.1N NaOH

The standard calibration curve was plotted using concentration vs. absorbance obtained by each linear dilution of VLD. Each concentration showed a linear absorbance range between the concentration range of 10, 27.5, 50, 75, 100 $\mu\text{g/ml}$ with a regression equation of 0.996 in 0.1N NaOH. Absorbance of respective concentrations was tabulated in Table 9. Standard calibration curve presented in Figure 20.

Table 9: Standard calibration curve of Vildagliptin at 216nm

Sr. No.	Concentration ($\mu\text{g/ml}$)	Absorbance
1	10	0.156
2	27.5	0.304
3	50	0.451
4	75	0.616
5	100	0.792
Regression R^2		0.998

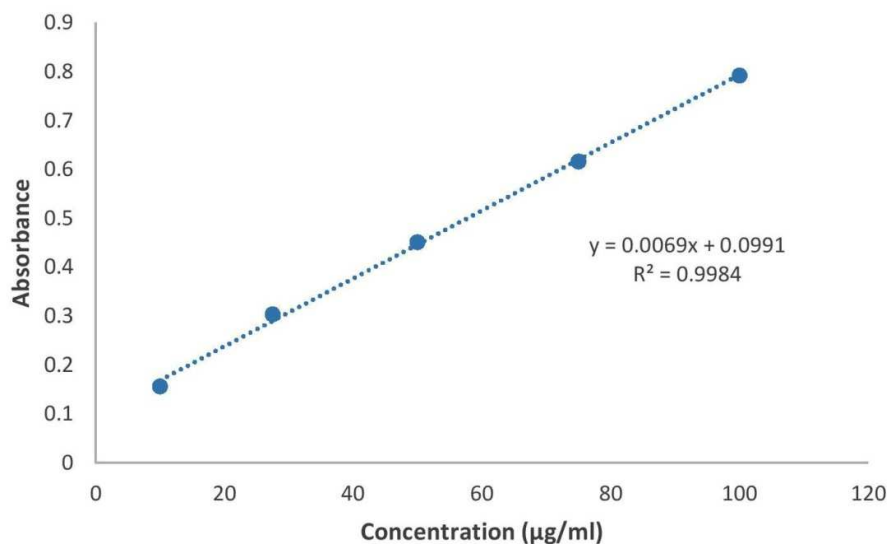


Figure 20: Standard calibration curve of Vildagliptin in 0.1 N NaOH

3.2.5 Validation of UV-Visible Spectroscopy

3.2.5.1 Linearity

The linearity of the Vildagliptin solution was examined under various conditions and at a range of concentrations and wavelengths. As mentioned below, the results showed good linearity:

Concentration range: 2–10µg/ml at 208nm:

- In 0.1N HCl, the calibration curve equation was $y=0.0186x+0.0201$ with a regression coefficient (R^2) of 0.997.
- In PBS (pH 7.4), the calibration curve equation was $y=0.0103x+0.0378$ with a regression coefficient (R^2) of 0.998.

Concentration range: 10–100µg/ml at 216nm:

- In 0.1N NaOH, the calibration curve equation was $y=0.0069x+0.0991$ with a regression coefficient (R^2) of 0.998.

Under the given conditions, these results validate the strong linearity of the method.

3.2.5.2 Accuracy

Determination of accuracy was performed by the percentage recovery method, and results are presented in Table 10. As per the results obtained by the accuracy method in 0.1N HCl, PBS (pH 7.4) and 0.1N NaOH, UV methods are found to be accurate, precise, and reproducible.

Table 10: Results of accuracy

API	Level of addition	Amount added	In 0.1 N HCl		In PBS pH 7.4		In 0.1 N NaOH	
			% recovery	Avg recovery (%)	% recovery	Avg recovery (%)	% recovery	Avg recovery (%)
VLD	80	80	98.6%	97.96%	99.02	99.12%	98.32%	98.96%
	100	100	97.3%		98.89		99.23%	
	120	120	98%		99.45		99.34%	

3.2.5.3 Precision

Both the intra-day and the inter-day were held on completely distinct days and at completely different times. The outcome demonstrates how much more consistent the suggested methodology is. The precision findings are shown in Table 11. The % RSD value for all the observations was found to be < 2% which witnesses the precision of the UV method.

Table 11: Results of precision

Sr. No	In 0.1 N HCl (at 6 µg/ml)		In PBS pH 7.4 (at 6 µg/ml)		In 0.1 N NaOH (at 27.5 µg/ml)	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
1	0.138	0.138	0.098	0.098	0.304	0.304
2	0.136	0.140	0.097	0.098	0.301	0.303
3	0.139	0.143	0.100	0.101	0.306	0.305
Avg	0.137	0.140	0.098	0.099	0.303	0.304
SD	0.0015	0.0025	0.00158	0.0017	0.00264	0.001
%RSD	1.1099	1.7935	1.607	1.7494	0.869	0.3289

3.2.5.4 Robustness and Ruggedness

In order to examine the method's robustness, the wavelength was intentionally altered by ± 2 nm; the results are displayed in Table 12. Additionally, the ruggedness was analyzed by altering the analyst, and the results are displayed in Table 13. It was found that the absorbance values fell within a range. The % RSD value for all the observations was found to be $< 2\%$, which witnesses the robustness and ruggedness of the UV method.

Table 12: Results of robustness

In 0.1 N HCl			In PBS pH 7.4			In 0.1 N NaOH		
λ (nm)	Condition	Abs	λ (nm)	Condition	Abs	λ (nm)	Condition	Abs
208	Normal	0.138	208	Normal	0.098	216	Normal	0.304
210	+2 λ	0.141	210	+2 λ	0.097	218	+2 λ	0.306
206	-2 λ	0.136	206	-2 λ	0.100	214	-2 λ	0.301
Avg	-	0.138	Avg	-	0.098	Avg	-	0.303
SD	-	0.00251	SD	-	0.00158	SD	-	0.0026
% RSD	-	1.819	% RSD	-	1.607	% RSD	-	0.869

Table 13: Results of ruggedness

In 0.1 N HCl			In PBS pH 7.4			In 0.1 N NaOH		
Analyst	λ (nm)	Abs	Analyst	λ (nm)	Abs	Analyst	λ (nm)	Abs
Analyst 1	208	0.138	Analyst 1	208	0.098	Analyst 1	216	0.304
Analyst 2	208	0.136	Analyst 2	208	0.101	Analyst 2	216	0.302
Avg	-	0.137	Avg	-	0.099	Avg	-	0.303
SD	-	0.0014	SD	-	0.0015	SD	-	0.001
% RSD	-	1.0321	% RSD	-	1.5890	% RSD	-	0.330

3.2.5.5 Limit of detection and quantification (LOD and LOQ)

Response's standard deviation and the calibration curve's slope were used to calculate the LOD and LOQ. Results are presented in Table 14. The LOQ values were found to be below 1.5 μ g/ml which indicates that UV method can be used for accurate analysis of minute concentration of drug.

Table 14: Results of LOD and LOQ

	In 0.1 N HCl	In PBS pH 7.4	In 0.1 N NaOH
LOD	0.280 μ g/ml	0.291 μ g/ml	0.478 μ g/ml
LOQ	0.850 μ g/ml	0.970 μ g/ml	1.449 μ g/ml

3. FT-IR Spectroscopy

Principle peaks of VLD were found at 3492.27 cm^{-1} and 2915.53 cm^{-1} which are responsible for O-H and N-H vibrations due to stretching, respectively. The $\text{C}\equiv\text{N}$ stretching vibrations were identified as responsible for the significant peak at 2849.95

cm^{-1} . The amide C=O and C–N stretching were attributed to the peaks at 1659.82 cm^{-1} and 1253.78 cm^{-1} , respectively. According to FT-IR interpretation, the drug under analysis is pure Vildagliptin. The FT-IR spectra of VLD, Eud, and VLD Eud NPs are displayed in Figures 21,22 and 23 respectively.

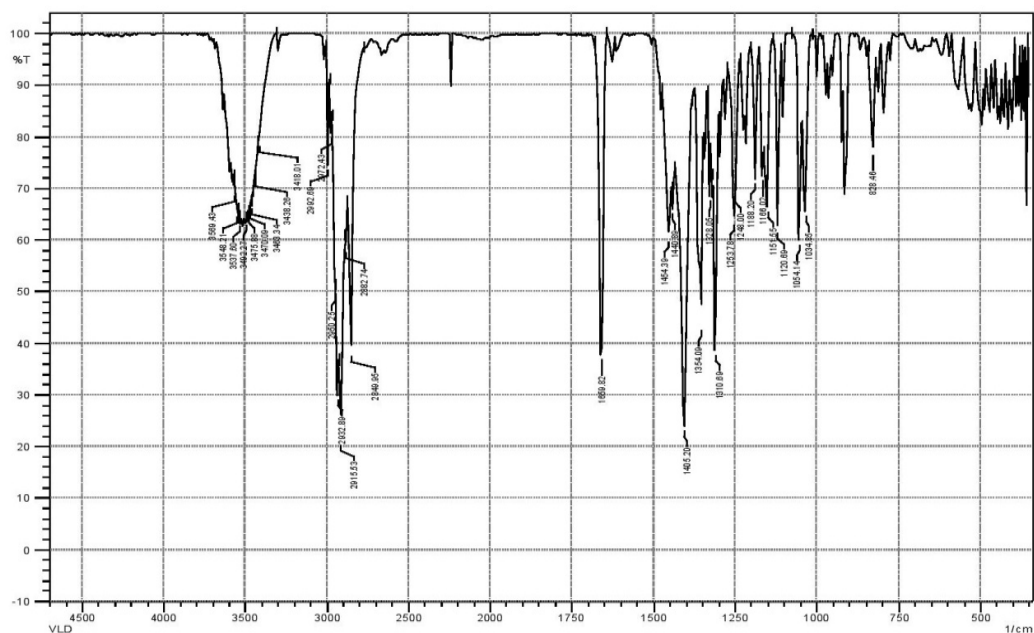


Figure 21: FT-IR spectra of Vildagliptin

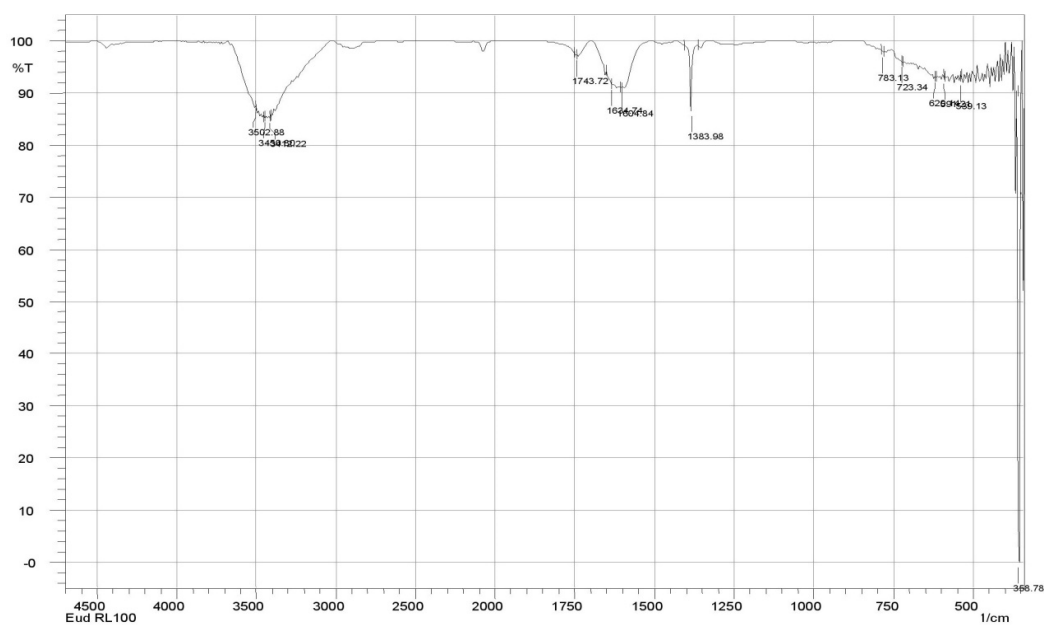


Figure 22: FT-IR spectra of Eudragit RL 100

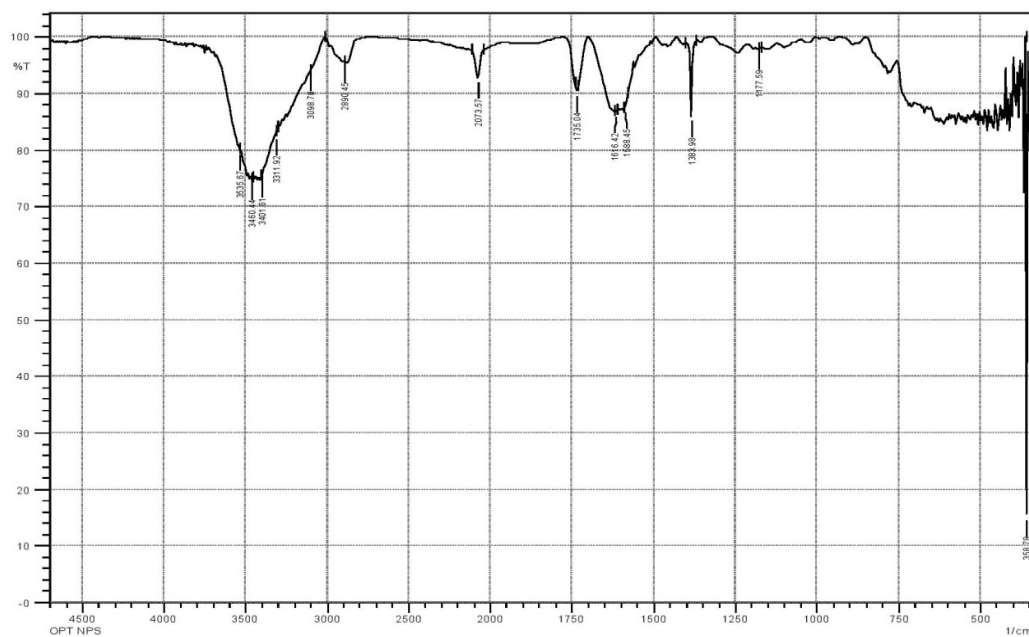


Figure 23: FT-IR spectra of Optimized VLD Eud NPs F6

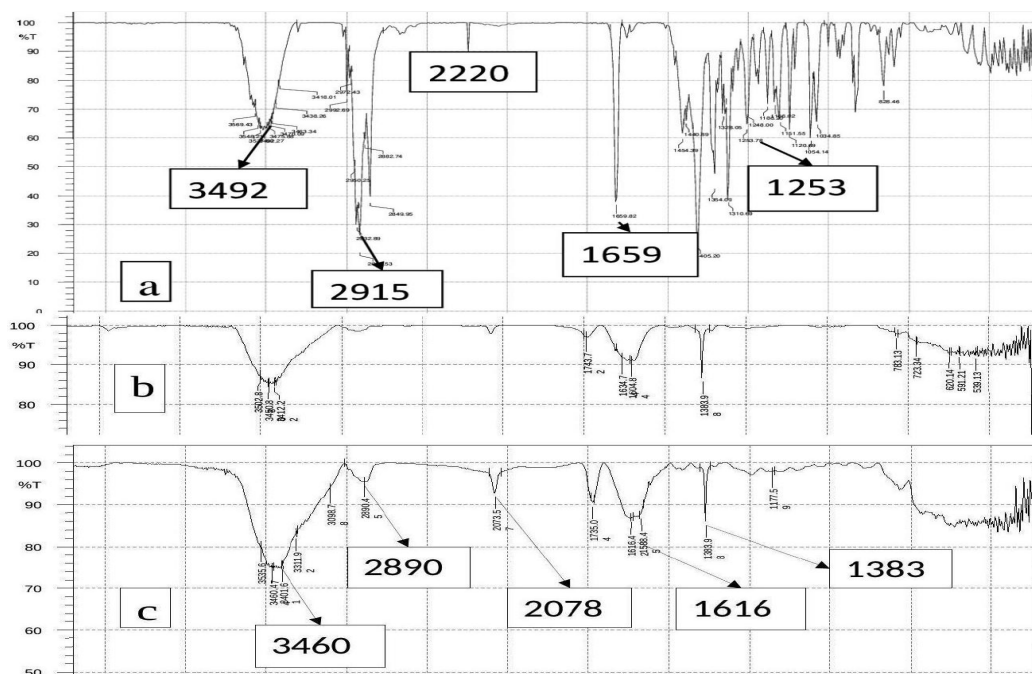


Figure 24: Compiled FTIR images of a) VLD, b) Eud and c) Optimized VLD Eud NPs F6

FT-IR spectra of VLD, CS, and CS VLD NPs are displayed in Figures 21,25, and 26 respectively. Characteristic peaks in the VLD spectrum are seen at 3,492 cm^{-1} and 2,915 cm^{-1} (O-H and N-H vibrations due to stretching), 2,125 cm^{-1} (stretching of $\text{C}\equiv\text{N}$), 1,659 cm^{-1} (amide $\text{C}=\text{O}$), and 1,253 cm^{-1} (stretching of C-N).

The VLD Eud NPs spectra showed that the polymer and pure drug had distinctive peaks of the functional groups. This suggested that there were no molecular interactions between polymer and drug, which confirmed drug-polymer compatibility.⁷¹

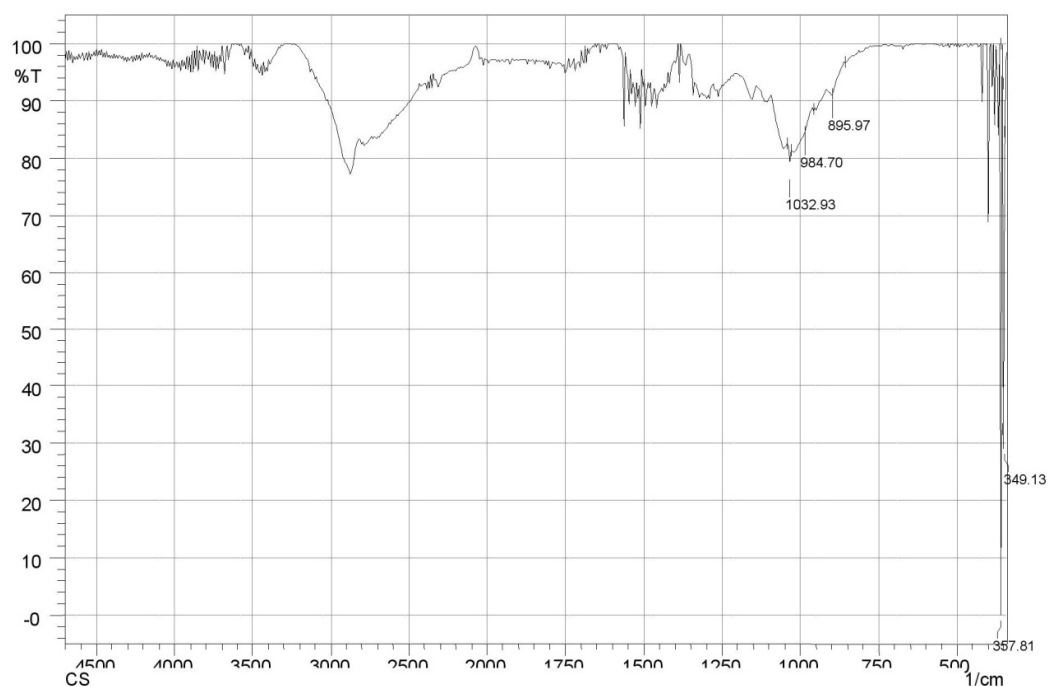


Figure 25: FTIR spectra of Chitosan

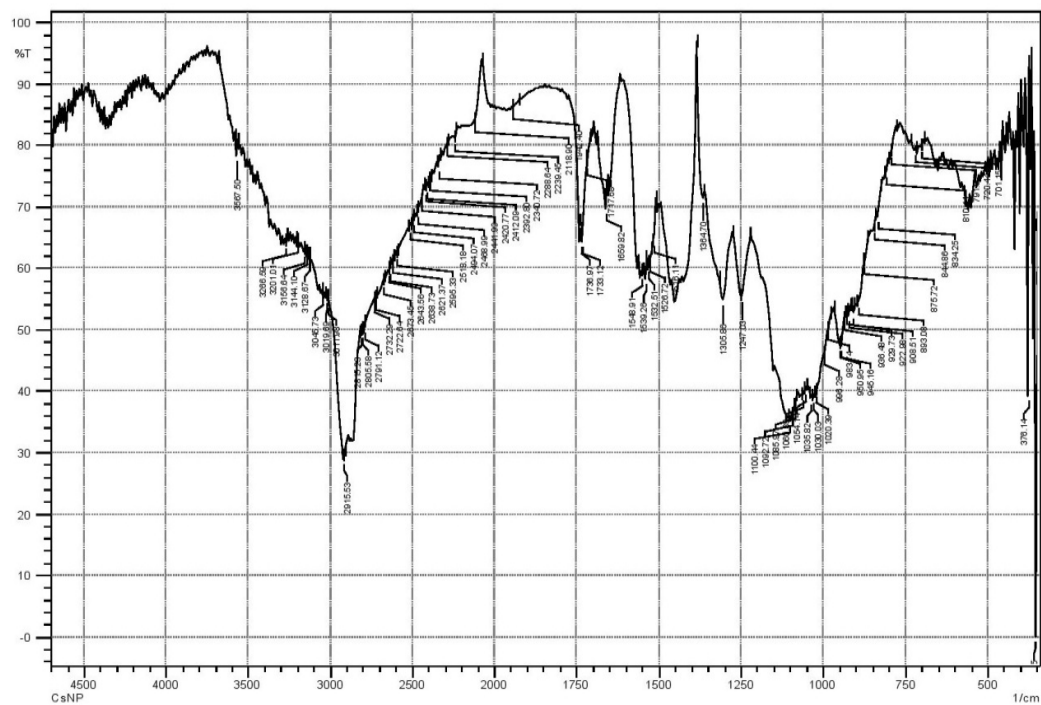


Figure 26: FTIR spectra of Optimized CS VLD NPs N6

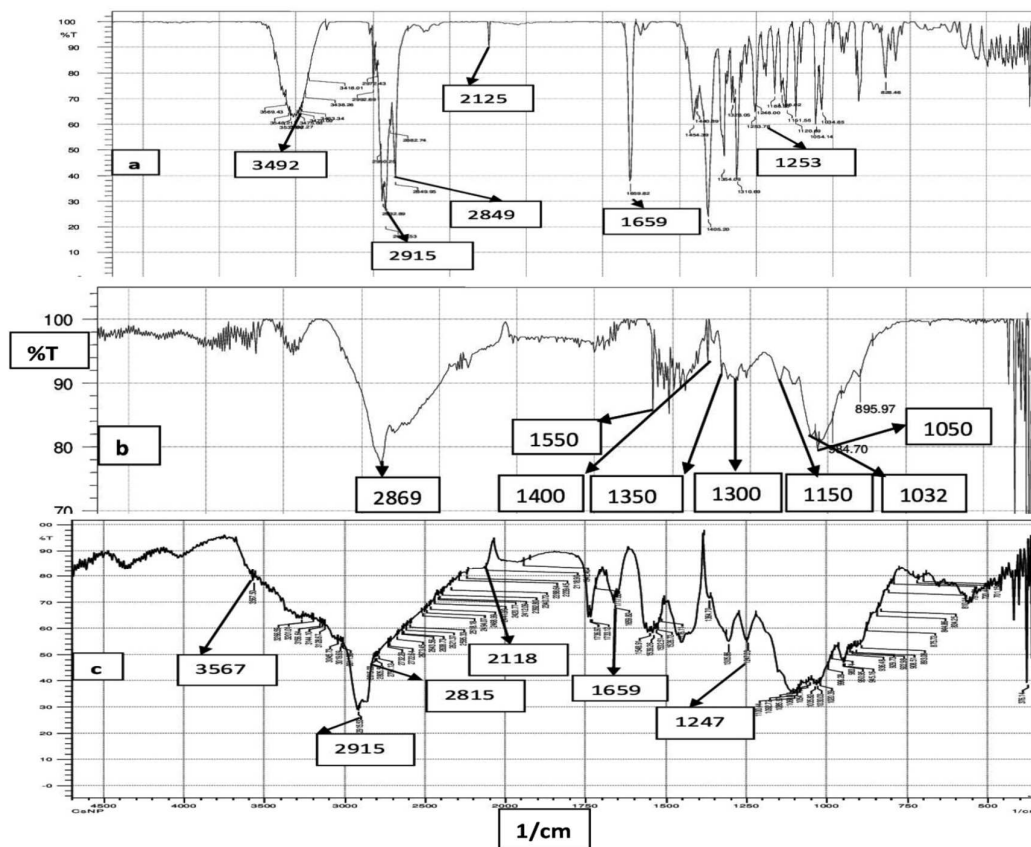


Figure 27: Compiled FTIR images of a) VLD, b) CS and c) Optimized CS VLD NPs N6

The FTIR analysis of Optimized CS-VLD NPs N6 shows that the characteristic peaks of both CS and the VLD are retained without significant shifts or new peaks. It indicates that the drug has been physically encapsulated in the matrix of chitosan polymers without strong chemical interactions, ensuring compatibility and preserving the drug's efficacy. The presence of broad bands around 3,400–3,500 cm^{-1} , consistent with O-H and N-H stretching, further supports the conclusion that the functional groups remain intact, confirming that the polymer and drug do not chemically interact significantly.⁷⁷

3.4 Statistical Optimization

3.4.1 Optimization of developed VLD Eud NPs⁷⁸

DoE software was used to optimize the formulated VLD Eud NPs utilizing two factors and three levels. The polymer eudragit and poloxamer 188 were used as stabilizers in a combination of solvent evaporation and nano-precipitation techniques to form nanoparticles. The methodology employed was a 3^2 response surface to ascertain the impact of independent factors (X_1 and X_2) on dependent variables (Y_1 and Y_2). To examine the effects of an independent variable, 3D response surface plots (Figures 28a and 29a) and 2D contour plots (Figures 28b and 29b) were constructed.

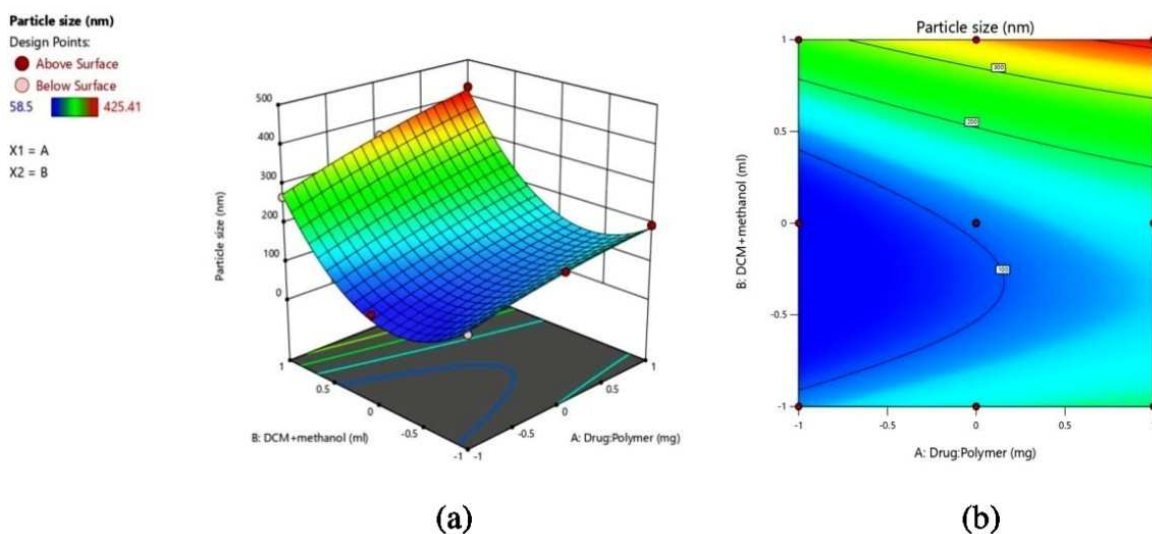


Figure 28: (a) 3D response surface plot and (b) 2D contour plot of PS of VLD Eud NPs

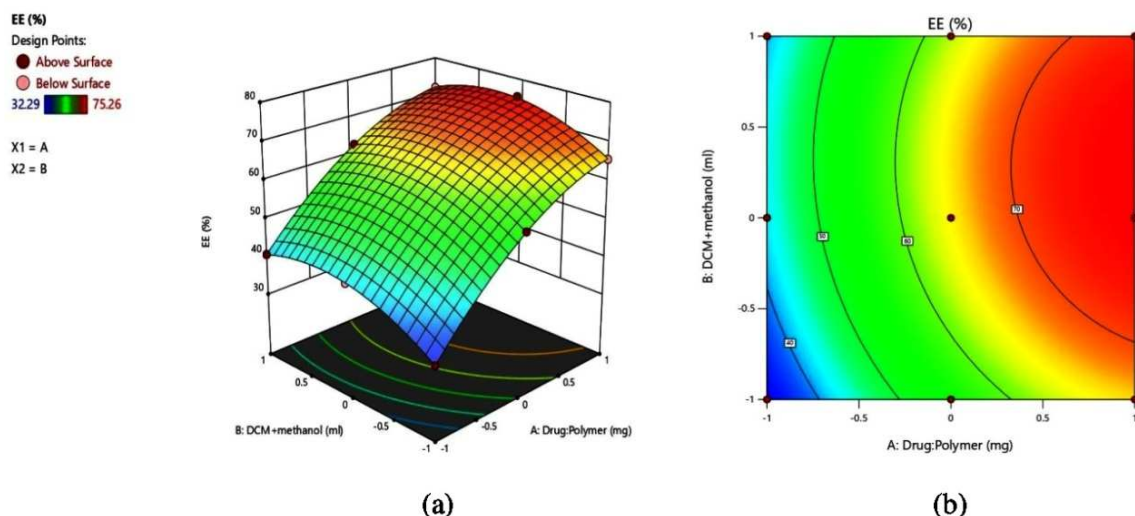


Figure 29: (a) 3D response surface plot and (b) 2D contour plot of % EE of VLD

Eud NPs

To analyze the independent and dependent variables' mathematical relationships, polynomial equations and counterplots are constructed. As shown in Table 15, the quadratic model's correlation coefficients (R^2) for the Y_1 and Y_2 responses were 0.9999 and 0.9998, respectively. The following equations were used to compute the responses for EE (Y_2) and particle size (Y_1). Y_1 and Y_2 ANOVA models are shown in Table 16.

$$\text{Particle size } (Y_1) = 107.9 + 53.25 X_1 + 96.22 X_2 + 18.4 X_1 X_2 - 10.45 X_1^2 + 153.16 X_2^2$$

$$\text{EE } (Y_2) = 64.88 + 16.2 X_1 + 3.6 X_2 - 0.56 X_1 X_2 - 6.13 X_1^2 - 6.12 X_2^2$$

According to the model fit study, the quadratic model, with a correlation coefficient (R^2) value >0.998 , is the model that best fits the results in the design domain space. An additional ANOVA analysis has demonstrated the model's significance with $p < 0.0002$.

Table 15: Regression analysis of Y₁ and Y₂ responses of VLD Eud NPs

Models	R ²	Adjusted R ²	Predicted R ²	Std dev.	Press	Remarks
Response Y₁ Particle size						
Linear	20.72	0.9824	0.9718	0.9315	8327.94	
2FI	8.59	0.9982	0.9951	0.9754	2990.92	
Quadratic	1.27	1	0.9999	0.9992	97.63	Suggested
Response Y₂ Entrapment Efficiency						
Linear	3.92	0.9575	0.932	0.8786	219.04	
2FI	5.01	0.9582	0.8886	0.4362	1017.13	
Quadratic	0.2317	0.9999	0.9998	0.9982	3.26	Suggested

Table 16: ANOVA models for Y₁ and Y₂ of VLD Eud NPs

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Response Y₁ Particle size						
Model	1.22E+05	6	20270.53	12613.85	< 0.0001	Significant
X ₁ -Polymer	17012.31	1	17012.31	10586.34	< 0.0001	
X ₂ -DCM+Methanol	1.03E+05	2	51233.75	31881.5	< 0.0001	
X ₁ X ₂	1925.05	2	962.53	598.96	0.0017	
X ₁ ²	218.34	1	218.34	135.86	0.0073	
Response Y₂ Entrapment Efficiency						
Model	1804.11	6	300.69	5598.77	0.0002	Significant
X ₁ -Polymer	1574.64	1	1574.64	29319.87	< 0.0001	
X ₂ -DCM+Methanol	152.84	2	76.42	1422.98	0.0007	
X ₁ X ₂	1.39	2	0.6959	12.96	0.0716	
X ₁ ²	75.24	1	75.24	1400.89	0.0007	

3.4.2 Optimization of formulated CS VLD NPs

DoEs software (Design Expert 13) was utilized to optimize the CS VLD NPs employing the three levels and two variables. After CS VLD NPs had been produced using the desolvation approach, GA was added as a cross-linking agent. Coacervation and precipitation are two processes that contribute to the creation of nanoparticles. A 3^2 RSM was utilized to ascertain the impact of independent factors (X_1 and X_2) on dependent variables (Y_1 and Y_2). 2D contour plots (Figures 30a and 31a) and 3D response surface graphs (Figures 30b and 31b) were constructed to examine the impacts of an independent variable.

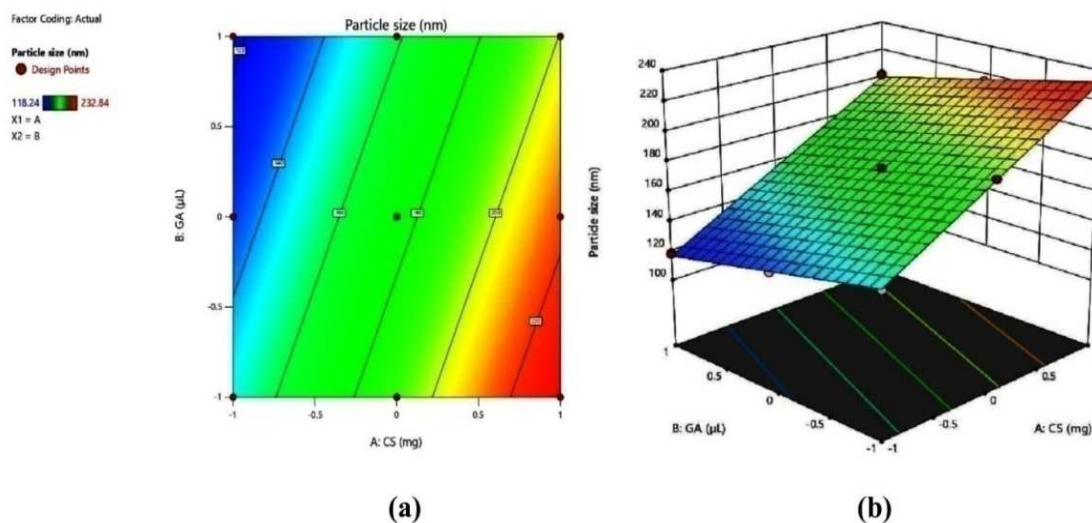


Figure 30: (a) 2D contour plot and (b) 3D response surface plot for PS CS VLD NPs

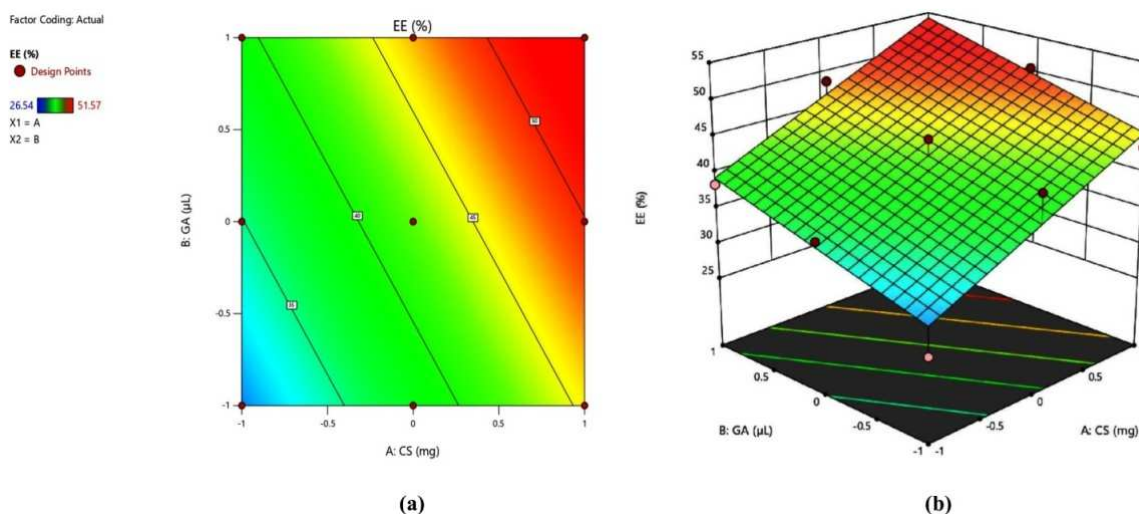


Figure 31: (a) 2D contour plot and (b) 3D response surface plot for %EE of CS
VLD NPs

Polynomial equations and counterplots have been generated in order to examine the mathematical relationship among independent and dependent variables. Table 17 displays the linear model's correlation coefficients (R^2) for the responses from Y_1 and Y_2 , which were 0.9981 and 0.892, respectively. Finally, Table 18 displays the ANOVA models for Y_1 and Y_2 . Using the following equations, the responses for EE (Y_2) and particle size (Y_1) were calculated.

$$\text{Particle size } (Y_1) = 174.72 + 41.88 X_1 - 16.17 X_2$$

$$\text{EE } (Y_2) = 42.38667 + 7.49000 X_1 + 4.38000 X_2$$

According to the findings of the regression analysis, the data fit a model that was linear for the two responses within the research domain space; for Y_1 and Y_2 , the R^2 values were 0.892 and 0.9991, respectively. The model is significant, according to the ANOVA research, with $p < 0.0001$ for Y_1 and $p < 0.0013$ for Y_2 .

Table 17: Regression analysis of Y₁ and Y₂ responses of CS VLD NPs

Models	R ²	Adjusted R ²	Predicted R ²	Std dev.	Press	Remarks
Response Y₁ Particle size						
Linear	0.9981	0.9974	0.9957	1.97	51.67	Suggested
2FI	0.9981	0.9969	0.9918	2.16	99.82	
Quadratic	0.9981	0.995	0.9807	2.76	233.71	
Cubic	0.9992	0.9939	0.8602	3.05	1693.53	Aliased
Response Y₂ Entrapment Efficiency						
Linear	0.892	0.856	0.7265	3.02	138.47	Suggested
2FI	0.8989	0.8382	0.3876	3.2	310.12	
Quadratic	0.9788	0.9436	0.803	1.89	99.74	
Cubic	0.9872	0.8973	-1.3403	2.55	1185.08	Aliased

Table 18: ANOVA models for Y₁ and Y₂ of CS VLD NPs

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Response Y₁ Particle size						
Model	12092.61	2	6046.31	1552.79	< 0.0001	Significant
A-CS	10524.44	1	10524.44	2702.86	< 0.0001	
B-GA	1568.17	1	1568.17	402.73	< 0.0001	
Response Y₂ Entrapment Efficiency						
Model	451.71	2	225.85	24.78	0.0013	Significant
A-CS	336.6	1	336.6	36.94	0.0009	
B-GA	115.11	1	115.11	12.63	0.012	

3.5 Characterization of nanoformulations

3.5.1 Particle size (PS) and Polydispersity index (PDI)

All developed VLD Eud NPs and CS VLD NPs were analyzed for PS and PDI employing a Zetasizer (Malvern Nano ZS Ltd., UK). PS of all the VLD Eud NPs and all CS VLD NPs were found in the range between $58.5 \pm 2.02 \text{ nm}$ to $425.4 \pm 4.71 \text{ nm}$ (Table 19) and $118.24 \pm 2.26 \text{ nm}$ to $232.84 \pm 6.79 \text{ nm}$ (Table 20) respectively.

Table 19: Results of particle size and entrapment efficiency of VLD Eud NPs

Formulation codes	Runs	Dependent variables					
		Y ₁ (nm)			Y ₂ (%)		
		Actual	Predicted	Residual	Actual	Predicted	Residual
F1	1	112.1±2.21	112.63	-0.5339	32.29±1.16	32.37	-0.0756
F2	2	165.9±1.87	164.83	1.07	55.31±3.16	55.16	0.1511
F3	3	195.6±3.73	196.13	-0.5339	65.61±0.87	65.69	-0.0756
F4	4	58.5±2.09	58	0.4994	42.45±0.98	42.34	0.1061
F5	5	106.9±3.48	107.9	-0.9989	64.67±2.01	64.88	-0.2122
F6	6	137.4±3.59	136.9	0.4994	75.26±1.63	75.15	0.1061
F7	7	268.3±3.17	268.29	0.0344	40.67±1.59	40.7	-0.0306
F8	8	357.2±2.78	357.28	-0.0689	62.43±1.86	62.37	0.0611
F9	9	425.4±4.71	425.38	0.0344	71.74±1.38	71.77	-0.0306

Y₁- Particle size (nm), Y₂- Entrapment efficiency (%) and all values are expressed in Mean±SD (n=3)

Table 20: Results of particle size and entrapment efficiency of CS VLD NPs

Formulation codes	Runs	Dependent variables					
		Y ₁ (nm)			Y ₂ (%)		
		Actual	Predicted	Residual	Actual	Predicted	Residual
N1	1	148.28±2.48	149	-0.7206	26.54±3.61	30.52	-3.98
N2	2	132.48±4.47	132.83	-0.35	42.18±2.78	38.01	4.17
N3	3	118.24±2.26	116.67	1.57	43.49±2.64	45.5	-2.01
N4	4	191.61±4.79	190.88	0.7278	35.69±3.03	34.9	0.793
N5	5	176.36±3.60	174.72	1.64	44.64±4.73	42.39	2.25
N6	6	155.18±3.09	158.55	-3.37	50.45±1.56	49.88	0.573
N7	7	232.84±6.79	232.76	0.0761	38.34±3.65	39.28	-0.94
N8	8	215.14±3.81	216.6	-1.46	48.58±2.93	46.77	1.81
N9	9	202.31±3.67	200.43	1.88	51.57±1.52	54.26	-2.69

Y₁- Particle size (nm), Y₂- Entrapment efficiency (%) and all values are expressed in Mean±SD (n=3)

The 2D contour plot and 3D response surface plot for the PS of VLD Eud NPs (Figure 28) showed that as the amount of eudragit increased, the particle size correspondingly increased. This is evident from the fact that a higher concentration of polymers causes more polymer molecules to deposit on one another, increasing their size.⁷⁹ In the case of solvent system composition (X_2), the size of the nanoparticle reduced as the DCM quantity raised from 25 to 37.5% (relative to the solvent system's total composition). Perhaps the solvent system's steric hindrance to the deposition of polymer molecules is the likely cause of this. The particle size has increased sharply as a result of further increasing its concentration to 50%.⁸⁰

The particle size CS VLD NPs' 2D and 3D response surface plots are shown in Figure 30. Whereas the line's directions show whether independent variables have a positive or negative impact on dependent variables, the slopes show how big the effect is. Comparing factor A (CS concentration) to factor B (amount of GA), the greater slope of the former shows a significant impact on PS. Because of strong intramolecular or intermolecular hydrogen interactions between CS molecules, which could lead to the nanoparticles aggregating and producing a high PS, PS raised as CS concentration increased.⁸¹ PS was able to somewhat decrease as a stiff matrix developed as the amount of cross-linking increased.⁵³

It is much desired for the formulations to have a reduced PDI value to achieve a uniform distribution of sizes.⁸² All developed VLD Eud NPs and CS VLD NPs had PDI values between 0.27 ± 0.036 to 0.52 ± 0.086 and 0.24 ± 0.025 to 0.39 ± 0.037 respectively, and results are presented in Table 21. PDI values of both the nanoformulations (VLD Eud NPs and CS VLD NPs) were shown have a narrow size distribution.⁸³

Table 21: PDI Values of VLD Eud NPs and CS VLD NPs

VLD Eud NPs	PDI Values	CS VLD NPs	PDI Values
F1	0.27±0.036	N1	0.24±0.025
F2	0.29±0.062	N2	0.29±0.041
F3	0.42±0.020	N3	0.38±0.015
F4	0.3±0.135	N4	0.31±0.041
F5	0.36±0.045	N5	0.35±0.052
F6	0.33±0.055	N6	0.32±0.06
F7	0.49±0.070	N7	0.33±0.026
F8	0.47±0.100	N8	0.37±0.005
F9	0.52±0.086	N9	0.39±0.037

3.5.2 Entrapment Efficiency (EE)

In the VLD Eud and CS VLD NPs, the EE ranged from 32.29±1.16 to 75.26±1.63 (Table 19) and 26.45± 3.61 to 51.57% ± 1.52% (Table 20), respectively. The 3D counter-plot from the Design-Expert Figure 29 predicts how the VLD to Eud ratio impacts the EE. The formulation F6 exhibits a higher drug:polymer (1:6) ratio of 75.26±1.63 EE. According to the results of EE, EE increases to a certain degree when eudragit content rises, but a further increase in eudragit content (400–600 mg) does not considerably raise EE. Higher EE was the result of greater space for drug entrapment due to a higher polymer concentration. After a certain threshold, the small change in EE could be caused by an overly dense polymer network that prevents drug molecules from entering the network.⁸⁴ Moreover, it has been noted that the solvent system's (X₂) composition has no discernible effect on the EE. The polymer concentration's synergistic influence on the size of particles and EE is further demonstrated by the polynomial equations' greater positive coefficient values for X₁. Furthermore, X₂ plays a negligible role in EE, as evidenced by the relatively small value of its coefficient in the EE equation.

The Design-Expert software data was used to create a 3D counter-plot (Figure 31b) that anticipated the impact on the VLD to CS ratio on the EE. A higher CS concentration was shown to improve EE; this finding could be explained via the observation that higher CS concentrations provide a greater capacity to retain drug. As the concentration of polymer increased to 400 mg of CS (N6), the EE of the chitosan nanoparticles also increased. Perhaps because all of the locations that could have been used for the drug's entrapment were completely saturated, the EE did not rise noticeably above this concentration.⁸² Nevertheless, a slight rise in EE has also been noted in nanoparticles as the degree of cross-linking has increased. This might be because of an increased crosslink density, which could prevent drug particles from escaping during the formation of the nanoparticles.⁵³

3.5.3 Transmission Electron Microscopy

The optimized VLD Eud NPs (F6) and CS VLD NPs (N6) were examined for morphology using High-Resolution Transmission Electron Microscopy (TEM); TEM pictures are displayed in Figures 32 and 33, respectively.

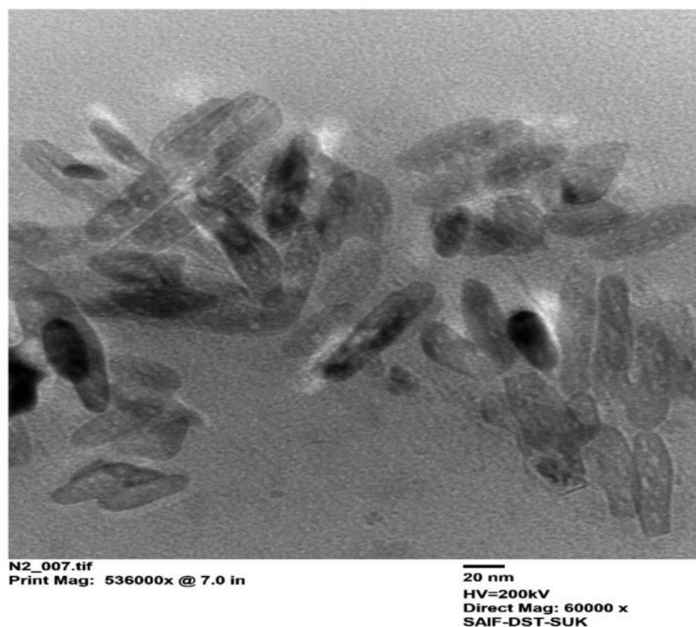


Figure 32: TEM image of optimized VLD Eud NPs (F6)

The TEM analysis of VLD Eud NPs (F6) revealed the presence of individual cylindrical-shaped nanoparticles.

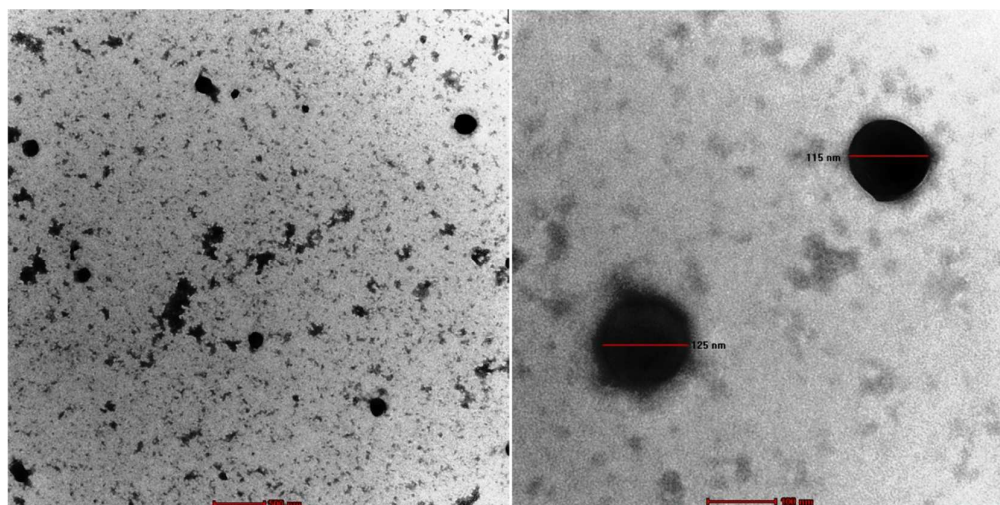


Figure 33: TEM image of optimized CS VLD NPs (N6)

The TEM analysis of CS VLD NPs (N6) revealed spherical-shaped nanoparticles that resembled distinct moieties.

3.5.4 Determination Percentage yield

It was determined that, at constant polymer concentration, the percentage production yield of all the VLD Eud NPs and CS VLD NPs ranged from 54.7 ± 3.45 to $84.4 \pm 1.51\%$ and 52.76 ± 1.81 to $81.73 \pm 1.10\%$ respectively, and results are presented in Table 22. As the concentration of polymers increased, it was discovered that the percentage production yield of all the VLD Eud NPs and CS VLD NPs increased as well.

Table 22: Percentage yield of VLD Eud NPs and CS VLD NPs

VLD Eud NPs	Percentage yield	CS VLD NPs	Percentage yield
F1	54.76 ± 3.45	N1	52.76 ± 1.81
F2	65.43 ± 1.53	N2	68.76 ± 1.35
F3	68.66 ± 1.92	N3	72.66 ± 2.66
F4	60.6 ± 0.85	N4	58.93 ± 0.40
F5	67.03 ± 2.36	N5	74.7 ± 0.60
F6	72.4 ± 1.96	N6	77.4 ± 1.08
F7	75.03 ± 2.15	N7	65.03 ± 2.15
F8	80.13 ± 1.45	N8	76.8 ± 1.60
F9	84.4 ± 1.51	N9	81.73 ± 1.10

3.5.5 *In vitro* drug release studies

The drug release from VLD Eud NPs was investigated by *in vitro* studies. Table 23 displays the cumulative drug release results for VLD Eud NPs F1–F9. Figure 34 displays the dissolution profiles of all formed VLD Eud NPs as well as pure VLD. During the first two hours in the 0.1N HCl, it was found that free drug solution released >90% of the drug, whereas the NPs only released 5% of the drug. Further study continues for the course of 24 h in PBS (pH7.4), all VLD Eud NPs showed a consistent, delayed release behavior. In the first 6 h, the nanoparticles released 15–25% of the drug; in the next 24 h, they released 68–89%. The polymer's pH-dependent solubility characteristic could be the cause of this. At acidic pH, Eudragit

RL 100 is insoluble, but the slight drug release may be due to an untrapped or adsorbed drug. Polymeric chain swelling may be the cause of extended drug release in the alkaline medium (PBS pH 7.4). Additionally, the results showed that when the concentration of polymer increased, the amount of VLD released through nanoparticles considerably decreased. These outcomes might be the consequence of a rise in polymer concentration, which might increase the polymer's thickness in the nanoparticles and, in turn, lengthen the corresponding polymeric membrane channel.⁸⁵

Table 23: Cumulative drug release data of VLD Eud NPs F1-F9

Time (h)	Free drug (%)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
2	91.46	4.92	3.26	2.76	4.28	3.61	2.42	3.51	3.43	2.69
4	98.27	16.89	17.54	15.34	18.48	14.38	15.34	10.56	9.36	8.76
6	98.27	23.53	25.64	22.41	25.58	22.59	22.78	21.24	19.41	15.63
8	98.27	30.86	31.64	29.42	32.48	29.13	29.58	34.36	30.42	24.18
10	98.27	38.27	39.54	35.45	39.51	36.89	36.58	44.57	39.08	28.46
12	98.27	43.34	46.19	42.25	46.84	43.26	42.26	49.07	48.93	34.21
24	98.27	86.61	82.67	79.54	89.55	86.77	87.9	76.74	71.67	68.92

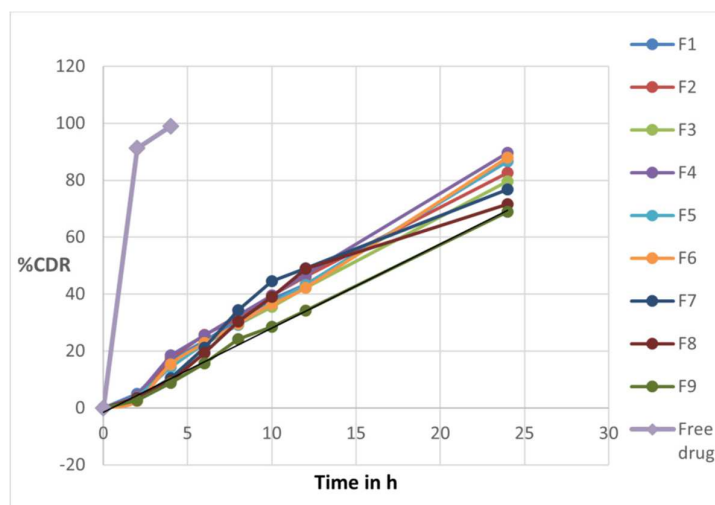


Figure 34: Dissolution profiles of pure VLD and all formulated VLD Eud NPs

The *in vitro* dialysis bag method was used to examine the drug release from CS VLD NPs N3, N6, and N9 for a duration of 24 h. Table 24 and Figure 35 present the cumulative drug release (%CDR) of pure VLD, N3, N6, and N9 CS VLD NPs. All three batches of CS VLD NPs displayed consistent delayed release characteristics over a 24 h period. The nanoparticles delivered approximately 55 to 70% of the drug in the first 6 h and 78% to 84% in the following 24 h. The free drug solution's rapid release property was shown by the fact that more than 90% of the drug was released in less than two hours. In the PBS (pH 7.4), a prolonged drug release was observed. In the first phase, nanoparticles' release of VLD started very quickly and then slowed down. In the initial fast-release phase, the drug that has stayed on the surface might dissolve rapidly. As the release medium enters the nanoparticles, the encapsulated drug dissolves in the subsequent phase.⁵³

Table 24: Cumulative drug release data of CS VLD NPs N3, N6 and N9

Formulation code/Time (h)	Time (h)						
	0	2	4	6	8	10	24
Free drug %	0	91.46	98.27	98.27	98.27	98.27	98.27
N3 (%)	0	42.16	61.25	70.24	76.43	77.37	84.74
N6 (%)	0	35.24	50.71	63.42	68.45	69.25	81.24
N9 (%)	0	32.34	42.78	57.58	64.58	66.26	78.92

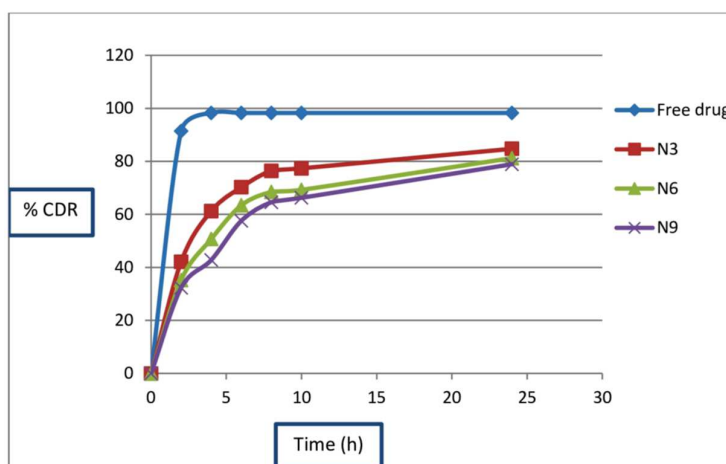


Figure 35: *In vitro* drug release profile of formulation N3, N6 and N9

3.5.6 Release kinetics

The drug release kinetics was ascertained by fitting the release data of all VLD Eud NPs and CS VLD NPs N3, N6, and N9 to different kinetic models. The results are shown in Tables 25 and 26, respectively.

Table 25: List of the drug release mechanism of VLD Eud NPs

Release mechanisms	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero order	0.936	0.956	0.998	0.996	0.985	0.992	0.949	0.949	0.996
First order	0.992	0.933	0.959	0.937	0.969	0.966	0.990	0.987	0.972
Higuchi	0.924	0.918	0.878	0.896	0.916	0.901	0.905	0.899	0.870
Korsmeyer-Peppas	0.950	0.961	0.974	0.952	0.948	0.957	0.967	0.972	0.987

The concentration independent release feature was demonstrated by the regression coefficient value (R^2) being at its maximum for zero order, as shown by the kinetic treatment for the release data of optimized VLD Eud NPs F6.⁸⁶

Table 26: Lists of the drug release mechanism of CS VLD NPs

Release mechanisms	N3	N6	N9
Zero order	0.496	0.589	0.639
First order	0.718	0.799	0.834
Higuchi	0.821	0.880	0.912
Korsmeyer-Peppas	0.596	0.628	0.650

The regression coefficient value (R^2), which is highest for the Higuchi model ($R^2 = 0.880$), indicated the release characteristic based on the kinetic release data of the optimized CS VLD NPs N6. From a matrix system, drug release is described using the Higuchi model, which mainly emphasizes diffusion as the governing mechanism.

Diffusion takes place one-dimensionally from the matrix into the surrounding media.⁸⁷

3.5.7 Stability study

To evaluate the stability of nanoformulations, PS, PDI, and %EE were considered as the primary parameters. Stability tests were carried out for optimized VLD Eud NPs (F6) and CS VLD NPs (N6). The findings of these stability studies are shown in Tables 27 and 28 respectively.

Table 27: Stability characteristics of optimized VLD Eud NPs F6

Stability parameter	Test period			
	0 month	1 month	2 months	3 months
Particle size (nm)	137.4±3.59	141.8±1.92	144.4±0.95	148.1±1.45
PDI	0.33±0.05	0.34±0.01	0.32±0.02	0.34±0.02
% EE	75.26±1.63	74.6±1.98	73.46±1.10	71.56±1.20

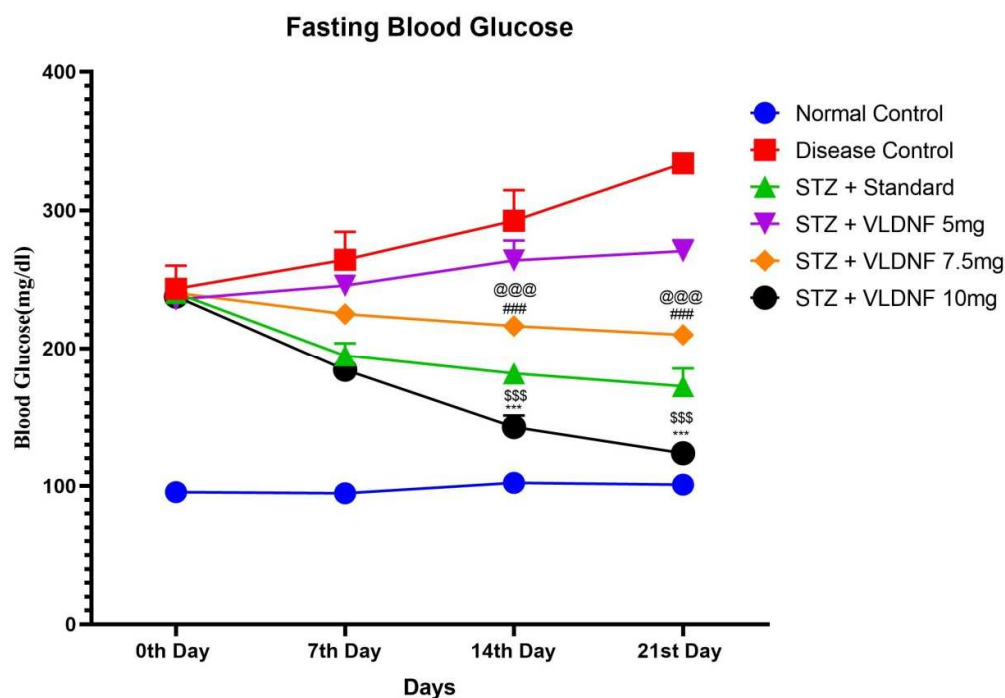
PS, PDI, and %EE of both optimized batches F6 and N6 did not significantly change throughout the course of the three-month accelerated stability trial, indicating the developed formulations' stability.

Table 28: Stability characteristics of optimized CS VLD NPs N6

Stability parameter	Test period			
	0 month	1 month	2 months	3 months
Particle size (nm)	155.18±3.09	156.2±2.74	158.06±1.46	159.83±0.77
PDI	0.32±0.06	0.34±0.01	0.33±0.02	0.35±0.02
% EE	50.45±1.56	47.29±2.40	48.94±2.24	46.88±0.80

3.6 *In vivo* study

Dose-dependent *In vivo* activity of optimized VLD Eud NPs F6 was performed and results are presented in Figure 36.



Note- All the values are expressed in Mean $\pm$ SEM; n=6; Analyzed by two-way ANOVA followed by Bonferroni multi- comparison test as post hoc test. Where, ***p<0.001 when compared to standard group, ###p<0.001 when compared to standard group, @@@p<0.001 when compared to normal group, \$\$\$p<0.001 when compared to normal.

Figure 36: Dose dependent *in vivo* activity of optimized VLD Eud NPs F6

This study evaluated the therapeutic efficacy of optimized VLD Eud NPs F6 (5, 7.5, and 10mg/Kg body weight of the animal) in comparison to the standard VLD dose (10mg/Kg body weight of the animal) in a STZ-induced diabetic Wistar rat model. Fasting blood glucose (FBG) levels were assessed at baseline (day 0) and subsequently on 7th, 14th, and 21st days, alongside normal and diseased control groups. On day 7, all treatment groups exhibited reductions in FBG levels; however, no

statistically significant difference was observed between the nanoformulation groups and the standard VLD group ($p>0.05$). By day 14, the 7.5mg/Kg and 10mg/Kg nanoformulation groups demonstrated a significant reduction in FBG levels compared to the standard formulation ($p=0.0001$), and this trend persisted through day 21.

The diseased control group consistently exhibited elevated glucose levels, validating the hyperglycemic state, whereas the normal control group maintained significantly lower and stable FBG levels throughout the experimental period ($p<0.0001$). Among the nanoformulations, the 7.5mg/Kg and 10mg/Kg (75mg and 100 mg Human equivalent dose (HED) respectively) doses exhibited superior glycemic control, with significant improvements observed over the 5mg dose ($p<0.05$) by day 21. These findings indicate that polymeric-coated Vildagliptin nanoformulations, particularly at 7.5mg/Kg (75mg HED) and 10mg/Kg (100mg HED) doses, offer enhanced glycemic regulation compared to the standard VLD 10mg/Kg (100mg HED) dose. This improvement is likely attributable to increased bioavailability and the sustained-release characteristics of the optimized VLD Eud NPs F6.

SUMMARY

Diabetes Mellitus is a chronic metabolic disorder requiring lifelong treatment. Current treatment with hypoglycemic agents has major drawbacks like poor bioavailability, frequent hypoglycemia, and weight gain. Vildagliptin (VLD) is a newer class of DPP4 inhibitor, gaining popularity due to increased patient adherence due to its good efficacy, favorable safety, and better tolerability. The main disadvantage of VLD is its quick metabolism and short half-life of two to three hours. Owing to VLD's short half-life, it is advised that patients should take 50 mg twice daily and pay meticulous attention to their dosages. Conventional dosage forms of VLD exhibit adverse effects like headache, cough, constipation, sweating, heartburn, weakness, and hypoglycemia. Nowadays, polymeric nanoparticles have been developed in order to minimize dose and dosing frequency, thereby reducing adverse effects of the drug. The present study successfully developed and optimized VLD Eud NPs and CS VLD NPs using the QbD approach to enhance the sustained release and therapeutic efficacy of VLD for diabetes management. Both the selected methods are simple, easy, and do not require any sophisticated equipment for the production of nanoparticles. The optimized VLD Eud NPs F6 was consisting of 600mg of Eudragit RL 100 and 0.75mL of DCM +1.25mL of Methanol, showing a PS of 137.4 ± 3.59 nm, the highest EE of $75.26 \pm 1.63\%$, and the desired PDI value of 0.33 ± 0.055 , with nanoparticles exhibiting cylindrical shapes and confirming monodispersity by TEM study. *In vitro* drug release studies demonstrated <5% release at acidic pH and >68% release over 24 h at basic pH, with the release following zero-order kinetics. The optimized CS VLD NPs N6, which utilized 400mg of Chitosan and 150 μ l of glutaraldehyde, showed a PS of 155.18 ± 3.09 nm, a high EE of $50.45\% \pm 1.56\%$, and a desired PDI value of 0.32 ± 0.06 , while TEM images indicated spherical-shaped nanoparticles with

confirmation of monodispersity by TEM study. *In vitro* drug release followed Higuchi kinetics and demonstrated a steady release over a 24h period. Both the optimized VLD Eud NPs F6 and CS-VLD NPs N6 were found stable during the 3-month duration of the study.

In vivo study findings indicate that VLD Eud NPs F6, particularly at 7.5mg/Kg (75mg HED) and 10mg/Kg (100mg HED) doses, offer enhanced glycemic regulation compared to the standard VLD 10mg/Kg (100mg HED) dose. This improvement is likely attributable to increased bioavailability and the sustained-release by minimizing dose and dosing frequency of the VLD Eud NPs F6.

These findings suggest that the optimized VLD Eud NPs and CS VLD NPs offer promising potential for sustained VLD delivery, providing improved therapeutic outcomes, patient adherence, and efficient diabetes therapy compared to conventional drug delivery approaches.

CONCLUSION

The solvent evaporation and nanoprecipitation combination technique with synthetic (Eudragit RL 100) polymer is more efficient in terms of entrapment efficiency (75%) compared to the desolvation method with natural (Chitosan) polymer (50%). Additionally, the combined method of solvent evaporation and the nanoprecipitation method require a smaller quantity (2ml) of organic solvent, making it potentially more economical and environmentally favorable. This suggests that for formulations prioritizing higher entrapment efficiency and lower solvent use, it may be the better choice. However, the choice of synthetic versus natural polymers might depend on biocompatibility, biodegradability, or other formulation-specific requirements. The *in vivo* investigations of VLD Eud NPs F6 likely indicate improved sustained release and enhanced bioavailability, leading to a reduction in both dosage and dosing frequency.

In the present study, antidiabetic potential of the prepared nanoformulations was checked by streptozotocin induced diabetic rats. The prolonged action of the formulation can be further ascertained by pharmacokinetic studies, wherein the plasma concentration of drug may justify the sustained release over time.

The prepared nanoformulations can be further developed into suitable dosage form for their ease of administration.

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KLE College of Pharmacy
A Constituent Unit of
KLE Academy of Higher Education and Research
(Deemed to be University)
DEPARTMENT OF PHARMACOLOGY
JNMC Campus, Nehru Nagar, Belagavi - 590 010, Karnataka, India
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DATE: 19/12/2023

CERTIFICATE

This is to certify that the project proposal No..... entitled," Formulation and Optimization of Oral Polymeric Coated Vildagliptin Nanoformulations using Quality by Design concept ", submitted by Dr./ Mr. / Ms Anand S. Ammanage under the guidance of Dr.Vinayak Mastiholimath has been approved/recommended by the IAEC of KLE College of Pharmacy, Belagavi, Reg.No.221/PO/Re/S/2000/CPCSEA in its meeting dated 19/12/2023 ; resolution No. 36 has been sanctioned 36..... Rats/ Mice/ Rabbits/Guinea pig (animals) sex male under this proposal for a duration of next.....months.

You are hereby informed to strictly adhere to the protocol submitted for approval. Further you are required to keep the account of animals used for the project in specified Performa, Form D.

Authorized by	Name	Signature	Date
Member Secretary:	<u>Dr. N.A. Khatib</u>		<u>19/12/2023</u>
MEMBER SECRETARY Institutional Animal Ethics Committee Reg. No. 221/PO/Rc/S/2000/CPCSEA KLE College of Pharmacy, BELAGAVI.			

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A Systematic Approach to Vildagliptin-Eudragit RL 100 Coated Nanoparticles by Combined Techniques of Solvent Evaporation and Nano-precipitation Using Quality by Design Concept

Anand Shripal Ammanage, Vinayak Shivamurthi Mastiholimath*

Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, INDIA.

ABSTRACT

Background: Diabetes is an endocrine disorder characterized by an abnormal increase in blood sugar level. Vildagliptin is a Dipeptidyl Peptidase-4 (DPP-4) inhibitor used in the treatment of type 2 diabetes mellitus. **Purpose:** The present work deals with the Quality by Design (QbD) approach based formulation and optimization of Vildagliptin-Eudragit RL 100 coated nanoparticles (VLD-EudNPs). **Materials and Methods:** VLD-Eud NPs were developed by combined technique of solvent evaporation and nano-precipitation. Optimization was done by 3^2 factorial designs. Prepared nanoformulations were characterized for percentage yield and morphology. Further *in vitro* drug release and kinetics were studied. The accelerated stability study of the optimized formulation was carried out for 3 months. **Results:** VLD-EudNPs ranged from 58.5 ± 2.02 nm to 425.4 ± 4.71 nm with a PDI of 0.27 ± 0.036 to 0.52 ± 0.086 . Entrapment efficiency (% EE) of nanoformulations F1 to F9 ranged between 32.29 ± 1.16 to $75.26 \pm 1.63\%$. The particles were found to be cylindrical-shaped, with a percentage yield of 54.7 ± 3.45 to $84.4 \pm 1.51\%$. During the *in vitro* drug release study, at acidic pH, <5% drug release, while delayed release at basic pH, at the end of 24 hr, all formulations have released >68% of the drug following zero order kinetics. The optimized formulation has proven its stability during stability study. **Conclusion:** The present work concludes that prepared and optimized VLD-Eud NPs could be a better approach for sustained delivery of Vildagliptin for the management of diabetes mellitus as compared to traditional drug delivery.

Keywords: Polymeric nanoparticles, Vildagliptin, Quality by Design, Diabetes.

Correspondence:

Vinayak Shivamurthi Mastiholimath

Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, INDIA.

Email: mastiholimath@rediffmail.com

Orcid Id: 0000-0002-8560-4705

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INTRODUCTION

Diabetes, an endocrine disorder characterized by an abnormal increase in blood sugar level, is one of the most prevalent illnesses throughout the world. According to a World Health Organization (WHO) estimate, diabetes affects 422 million people worldwide, predominantly in low- and middle-income nations. By 2045, it is expected to affect 693 million adults, a >50% increase from 2017. Among the Diabetes Mellitus (DM) population globally, ~95% of people suffer from type 2 DM due to a lack of physical activity and obesity.^{1,2} Existing medications are associated with side effects like hypoglycemic effects, gastric intolerance, and increased body weight. Hence, there is a need to focus on newer therapeutic agents to treat type 2 DM.³

Endogenous incretion hormones like Gastric Inhibitory Polypeptide (GIP) and Glucagon-Like Peptide-1 (GLP-1) are important in the pathophysiology of type 2 DM and in the physiology of glucose homeostasis by inducing the release of insulin from beta cells. GLP-1 is concentrated primarily on its ability to reduce blood sugar and body weight. Dipeptidyl Peptidase 4 (DPP-4) enzymes are found in our body and quickly inactivate incretin hormones. Hence, there is a necessity for DPP-4 inhibitors (gliptins) to manage the treatment of type 2 DM.^{4,5} Gliptins are attractive second-line anti-diabetic agents with good efficacy, favorable safety, and better tolerability. They are available orally, either as monotherapy or with various combination therapies. Nowadays, gliptins are gaining importance and increasing patient adherence over GLP-1 analogues, which are available as subcutaneous injections.⁶ Vildagliptin is a potential DPP-4 inhibitor that has a great molecular interaction with the DPP-4 enzyme.⁷ The major drawback of Vildagliptin is its short half-life of 2-3 hr and rapid metabolism.⁸ Due to the short half-life of Vildagliptin, it is



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recommended to take 50 mg twice a day, and patients need to adhere strictly to the dose intervals.⁹ Therefore, there is a need to develop a novel dosage form of Vildagliptin that will improve oral bioavailability.

Nowadays, Nanoparticulate Drug Delivery Systems (NDDSs) are most popular in the field of pharmaceutical research for the oral administration of drugs due to their capacity to deliver drugs to the proper site, dose, and timing.¹⁰ There are various NDDSs, like nanoemulsions, liposomes, niosomes, and nanoparticles, designed for oral delivery of various drugs.¹¹ In comparison to other novel delivery systems as aforementioned, polymeric nanoparticles have a number of advantages, including site-specific targeting, prevention of dose dumping through controlled and sustained release, and high surface-to-volume ratio. Additionally, the surface characteristics of nanoparticles offer mucoadhesive, cellular uptake, immune system interaction and cell targeting properties. These qualities assist in decreasing dose and frequency of administration in an indirect manner, which enhances patient compliance.^{12,13} For improving the oral bioavailability of numerous drugs, polymeric coated nanoparticles are the most promising of these strategies.

Synthetic, semisynthetic, or natural polymers are used to prepare polymeric nanoparticles that enhance the circulation half-life, reduce the phagocytic uptake, and inactivation of drug, by which improves delivery and targeting of drugs.¹⁴

Quality by Design (QbD) is initiated by the FDA for pharmaceutical product development (FDA guidance of industry, 2006). It is a design effort to deliberate product conceptualization for commercialization.¹⁵ The QbD concept is a systematic approach for pharmaceutical formulation development with specific predefined objectives that provides improved process and product understanding along with a limited investment of cost, effort, and time. Designs of Experiments (DoE) are taken into consideration as an integral part of QbD, which helps in understanding a relationship between cause and effect. Many literatures reported that effective utilization of QbD and DoE concepts for formulation development to attain robustness in product performance.¹⁶ Various formulation and processing parameters may affect the physicochemical properties of nanoparticles, which may affect the product quality. Hence, there is a necessity for the QbD concept to assure the quality of nanoparticles.¹⁷

In present work nanoformulations were prepared by solvent evaporation and nanoprecipitation technique using the QbD concept for oral delivery of Vildagliptin. This method of preparation is simple, reliable and not requires any sophisticated equipment. In this context, in the present study, an attempt is made to formulate and optimize VLD-Eud NPs using the QbD concept.

MATERIALS AND METHODS

Vildagliptin and Eudragit RL 100 were received as free gift sample from Ajanta Pharma Ltd., Mumbai, India, and Centaur Pharmaceuticals Pvt. Ltd., Pune, respectively. Poloxamer 188 and other solvents and excipients were obtained from authenticated sources.

Preparation of Vildagliptin nanoformulation

VLD-Eud NPs were formulated by the combined technique of solvent evaporation and nanoprecipitation with slight modification.¹⁸ Briefly, different drug: polymer (Eudragit RL 100) ratios, viz., 1:2, 1:4 and 1:6 were dissolved in the solvent mixture of dichloromethane (DCM) and methanol at three different levels, viz., 1:3, 1:1.66 and 1:1. The above organic solution was then added drop-wise with a 22-gauge needle to 10 mL of poloxamer 188 aqueous solution (0.5% w/v) on a magnetic stirrer at 500 rpm. The organic solution was then evaporated for 2 hr at room temperature to obtain an aqueous dispersion, which was finally centrifuged (KUBOTA 7000, Japan) at 35,000 rpm at 4°C for 60 min to collect the nanoparticles. The sediment obtained was washed by re-suspending in distilled water and centrifuged with the same conditions in triplicate. The final product was dried by using a freeze dryer.

Statistical experimental design

VLD-Eud NPs were formulated by adopting 3² statistical experimental designs (Design Expert 13). In this design, two independent variables, viz., the content of (X₁) Eudragit RL 100 (mg) and (X₂) the composition of the organic solvent, i.e., DCM+methanol (mL), were selected. Three levels for each independent variable were coded as: (-1) low, (0) medium, and (+1) high, characterizing the 3² factorial designs consisting of 9 different formulations as shown in Table 1. The effect of two factors at their three levels on dependent variables (Y₁) particle

Table 1: Experimental design with process variables and their levels.

Levels	Independent variables		Dependent variables	
	X ₁ =Eudragit RL 100 (mg)	X ₂ =Volume of DCM and methanol (mL)	Y ₁ =Particle size (nm)	Y ₂ =Entrapment Efficiency (%)
-1	200	0.5+1.5	Goal to minimize	Goal to maximize
0	400	0.75+1.25		
+ 1	600	1+1		

size (nm), and (Y_2) entrapment efficiency (%), were studied as shown in Table 2.

Characterization of Vildagliptin nanoparticles

Particle size (PS) and Polydispersity index (PDI)

PS and PDI of formulated nanoformulations were analyzed using Zetasizer (Nano ZS, Malvern Instruments Ltd., Worcestershire, WR14 1XZ, United Kingdom). All the samples were suitably diluted with Millipore water. The refractive index was set to 1.33, and samples were analyzed at 25°C.¹⁹

Entrapment Efficiency (EE)

The percentage EE of the formulated Vildagliptin nanoformulations was determined by the indirect method, i.e., the amount of untrapped drug was determined.²⁰ A known volume of Vildagliptin nanoformulations was transferred into 2 mL of centrifuge tubes and centrifuged (KUBOTA 7000, Japan) at 35,000 rpm at 4°C for 60 min. The supernatant was collected, and a suitable dilution was made with 0.1 N NaOH. The samples were analyzed using a UV-spectrophotometer (Shimadzu 1900, Japan) at 216 nm. Consequently, the % EE was calculated by the following formula:

$$\% \text{ EE} = \frac{\text{Amount of drug added} - \text{untrapped drug}}{\text{Amount of drug added}} \times 100$$

Percentage yield determination

The percentage yield of formulated nanoformulations was determined by taking the dry weight of the nanoformulation²¹ and using the following formula:

$$\% \text{ Yield} = \frac{\text{weight of nanoformulation}}{\text{weight of drug and polymer}} \times 100$$

FTIR Spectroscopy

A FTIR spectrophotometer (DRS 8000, Shimadzu IR Affinity-1) was used to obtain the FTIR spectra of VLD, Eudragit RL 100,

and VLD-Eud NPs. The spectra were recorded in the wavelength range of 4000-400 cm^{-1} . Further, the compatibility between drug and polymer was determined.²² Transmission Electron Microscopy

High-Resolution Transmission Electron Microscopy was used to study the morphology of optimized nanoparticles (F6). A drop of nanoformulation was deposited on a 200-mesh carbon-coated copper grid and stained with a 2% w/v phosphotungstic acid solution for 2 min. The grid was then air-dried and examined with a TEM (200 kV and 40,000 X) to capture images from a randomly selected area.²³

In vitro drug release studies

In vitro drug release from Vildagliptin nanoformulations was studied using the USP dissolution apparatus (ELETROLAB TDT-06L). Throughout the study, the stirring rate (50 rpm), volume of dissolution medium (500 mL), and temperature ($37 \pm 0.2^\circ\text{C}$) were maintained. The 20 mg equivalent weight of Vildagliptin nanoformulation was placed into a dialysis bag (previously soaked in dissolution media). The initial 2 hr dissolution experiment was conducted in 0.1 N HCl and then continued with pH-7.4 phosphate buffer. A 5 mL sample was withdrawn at predetermined time intervals up to 24 hr. The sink condition was maintained by adding an equal volume of fresh dissolution medium. The sample was appropriately diluted and filtered before being subjected to a UV spectrophotometer analysis at 208 nm.²⁴

Release kinetics

To comprehend the mechanism of drug release, *in vitro* release data were fitted into various kinetic models, such as the zero order, first order, Higuchi, and Korsmeyer-Peppas models. When the mechanism is not well understood or when multiple types of release phenomena are present, these chosen models are frequently utilized to represent the drug release data from

Table 2: Experimental runs and their observed values of dependent variables.

Runs	Independent variables		Dependent variables					
			Y_1 (nm)			Y_2 (%)		
	X_1	X_2	Actual	Predicted	Residual	Actual	Predicted	Residual
1	200	0.5+1.5	112.1±2.21	112.63	-0.5339	32.29±1.16	32.37	-0.0756
2	400	0.5+1.5	165.9±1.87	164.83	1.07	55.31±3.16	55.16	0.1511
3	600	0.5+1.5	195.6±3.73	196.13	-0.5339	65.61±0.87	65.69	-0.0756
4	200	0.75+1.25	58.5±2.09	58	0.4994	42.45±0.98	42.34	0.1061
5	400	0.75+1.25	106.9±3.48	107.9	-0.9989	64.67±2.01	64.88	-0.2122
6	600	0.75+1.25	137.4±3.59	136.9	0.4994	75.26±1.63	75.15	0.1061
7	200	1+1	268.3±3.17	268.29	0.0344	40.67±1.59	40.7	-0.0306
8	400	1+1	357.2±2.78	357.28	-0.0689	62.43±1.86	62.37	0.0611
9	600	1+1	425.4±4.71	425.38	0.0344	71.74±1.38	71.77	-0.0306

Values are expressed in Mean±SD ($n=3$).

the polymeric drug delivery system. The accuracy of the fit was assessed for each model using the coefficient of correlation (R^2).²⁵

Stability study

The optimized formulation (F6) was stored for 3 months in three different tightly sealed, amber-colored glass bottles. According to International Council for Harmonization (ICH) recommendations, the stability tests of the nanoparticles were carried out at $25^\circ\text{C} \pm 2^\circ\text{C}$ and RH 65%. Formulation (F6) was examined for % EE and particle size over a 3-month period with a one month sampling interval.²⁶

RESULTS

Optimization of formulated nanoformulations

Optimization of formulated VLD-Eud NPs was done by using two factors and three levels with the help of DoE software. The nanoparticles were prepared by combined technique of solvent evaporation and nanoprecipitation method using polymer eudragit and poloxamer 188 as stabilizer. To determine how independent factors (X_1 , and X_2) affect dependent variables (Y_1 and Y_2), a 3^2 response surface methodology was used. Figures 1a and 2a of 3D response surface plots and Figures 2b and 2b of 2D counter plots were created to investigate the impacts of an independent variable. The results of particle size and EE for 9 experimental runs are listed in Table 2 and are in the ranges of 58.5 ± 2.02 nm to 425.4 ± 4.71 nm and 32.29 ± 1.16 to $75.26 \pm 1.63\%$ respectively. Polynomial equations and counterplots are derived to study the mathematical correlation between independent and dependent variables. The correlation coefficients (R^2) value for the quadratic model for the Y_1 and Y_2 responses was 0.9999 and 0.9998 respectively and is presented in Table 3. The responses for particle size (Y_1) and EE (Y_2) were calculated using the following formulae. ANOVA models for Y_1 and Y_2 are presented in Table 4.

$$\text{Particle size } (Y_1) = 107.9 + 53.25 X_1 + 96.22 X_2 + 18.4 X_1 X_2 - 10.45 X_1^2 + 153.16 X_2^2$$

$$\text{EE } (Y_2) = 64.88 + 16.2 X_1 + 3.6 X_2 - 0.56 X_1 X_2 - 6.13 X_1^2 - 6.12 X_2^2$$

Characterization of nanoformulations

Particle size (PS) and Polydispersity index (PDI)

The PS and PDI of the formulated Vildagliptin nanoformulations F1 to F9 were analyzed by using a Zeta nano ZS (Malvern instrument Ltd). All the nanoformulations were found PS and PDI in the range between 58.5 ± 2.02 nm to 425.4 ± 4.71 nm and 0.27 ± 0.036 to 0.52 ± 0.086 respectively.

Entrapment Efficiency (EE)

The EE of the formulated nanoformulations was found in the range between 32.29 ± 1.16 to 75.26 ± 1.63 as presented in Table 2. The effects of the VLD to Eud ratio on the EE are predicted by the

3D counter-plot from Design-Expert Figure 2b. The formulation F6 shows 75.26 ± 1.63 EE at higher drug: polymer (1:6) ratio.

Percentage yield determination

The % production yield of all the nanoformulations was calculated, ranging from 54.7 ± 3.45 to $84.4 \pm 1.51\%$ at constant polymer concentration.

FTIR Spectroscopy

The FTIR spectra of (a) VLD, (b) Eudragit RL 100, and (c) VLD-EudNPs are shown in Figure 3. In the spectrum of VLD-Eud peaks, were appeared without any significant deviation from the VLD peaks.

Transmission Electron Microscopy

High-Resolution Transmission Electron Microscopy was used to study the morphology of optimized nanoparticles (F6), and its TEM image is shown in Figure 4.

In vitro drug release studies

In vitro drug release of formulated nanoformulations of the VLD-Eud NPs was studied for 24 hr. The dissolution profiles of pure VLD and all formulated VLD-Eud NPs are shown in Figure 5. Each batch of the developed VLD-Eud NPs exhibits a sustained, delayed release action over the course of 24 hr. The nanoparticles exhibited prolonged drug release patterns, with around 15-25% of the drug release during the first 6 h, followed by about 68-89% of the drug release in 24 hr, and from the free drug solution >90% of the drug release was found within 2 hr.

Release kinetics

To determine the drug release kinetics, release data were fitted to various kinetic models. The drug release mechanism for each batch is presented in Table 5.

Stability study

Stability studies were conducted for optimized VLD-Eud NPs (F6) using particle size and EE% as the main parameters.

DISCUSSION

The present study deals with the formulation of Vildagliptin-loaded nanoformulations and their evaluation. The nanoformulations were optimized by a 3^2 factorial design. The data obtained is subjected to analytical treatment by Design Expert 13 software. Further, equations were drawn for responses. In order to comprehend the primary effects and interactions of the independent variables, 3D response surface graphs were obtained (Figure 1a and 2a of 3D).^{25, 27}

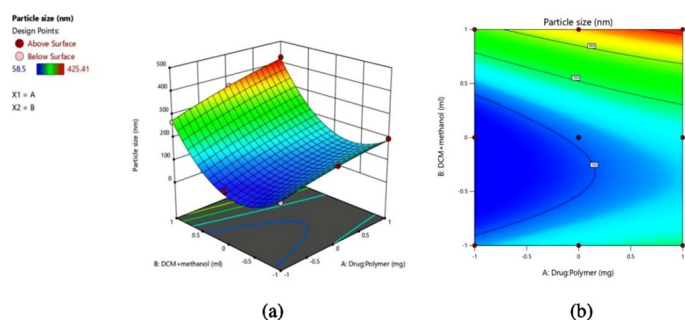
According to the 2D and 3D plots for particle size, it was observed that particle size increased when the content of eudragit was increased. This is obvious as the increased polymer concentration

Table 3: Summary of results of regression analysis for responses Y_1 and Y_2 .

Models	R^2	Adjusted R^2	Predicted R^2	Std dev.	Press	Remarks
Response Y_1 Particle size						
Linear	20.72	0.9824	0.9718	0.9315	8327.94	
2FI	8.59	0.9982	0.9951	0.9754	2990.92	
Quadratic	1.27	1	0.9999	0.9992	97.63	Suggested
Response Y_2 EE						
Linear	3.92	0.9575	0.932	0.8786	219.04	
2FI	5.01	0.9582	0.8886	0.4362	1017.13	
Quadratic	0.2317	0.9999	0.9998	0.9982	3.26	Suggested

Table 4: ANOVA models for Y_1 and Y_2 .

Response Y_1: Particle size						
Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Model	1.22E+05	6	20270.53	12613.85	<0.0001	Significant
X_1 -Polymer	17012.31	1	17012.31	10586.34	<0.0001	
X_2 -DCM+Methanol	1.03E+05	2	51233.75	31881.5	<0.0001	
$X_1 X_2$	1925.05	2	962.53	598.96	0.0017	
X_1^2	218.34	1	218.34	135.86	0.0073	
Response Y_2: EE						
Model	1804.11	6	300.69	5598.77	0.0002	Significant
X_1 -Polymer	1574.64	1	1574.64	29319.87	<0.0001	
X_2 -DCM+Methanol	152.84	2	76.42	1422.98	0.0007	
$X_1 X_2$	1.39	2	0.6959	12.96	0.0716	
X_1^2	75.24	1	75.24	1400.89	0.0007	

**Figure 1:** (a) 3D response surface plot and (b) 2D contour plot of particle size.

leads to the deposition of more polymer molecules on each other, which results in an increase in size. In the case of the composition of the solvent system (X_2), as the DCM content increased from 25% to 37.5% (respective to overall composition of mobile phase), the size of the nanoparticle decreased. The probable reason for this may be the steric hindrance provided by the solvent system for the deposition of polymer molecules. Further increase in its concentration to 50% has resulted into sharp rise in particle size. The result of EE has witnessed that, as the content of eudragit increases, the EE increases up to certain extent, further rise in content of eudragit (400 mg to 600 mg) do not increase the

EE significantly. The increased concentration of polymer has increased the space for entrapment of drug which resulted into higher EE. The insignificant change in EE after certain level may be attributable to excessive denser polymer network which hinders the entry of drug molecules into polymeric network. Further, it has been observed that the composition of the solvent system (X_2) does not play any significant role in the EE. This synergistic effect of polymer concentration on particle size and EE is also witnessed by higher positive values of coefficients for X_1 in polynomial equations. Further, the small value of the coefficient of X_2 in the EE equation is an indication of its insignificant role in EE. The model fit study has suggested that the quadratic model is the best fit model to obtain the results in design domain space with a correlation coefficient (R^2) value >0.998. A further ANOVA study has highlighted the significance of the model with $p < 0.0002$.

The heterogeneity of particle size in NPs is measured by PDI. The PDI values of all the nanoformulations were found in the range of 0.27 ± 0.036 to 0.52 ± 0.086 , showing the narrow size distribution.²⁸ For the formulations to have a uniform size distribution, a lower value of PDI is extremely desirable. The % production yield of all the nanoformulations was found to increase with an increase in polymer concentration.

VLD-Eud nanoparticle spectra revealed the presence of characteristic peaks of the functional groups in the polymer and pure drug, indicating the absence of molecular interactions between drug and polymer, which witnessed the compatibility between polymer and drug.²⁹ The TEM study has shown the cylindrical shaped nanoparticles that were present as individual moiety.

The *in vitro* drug release study was conducted for all the formulated nanoformulations, and for the initial 2 hr in the 0.1 N HCl, it found only a small amount of drug release (<5%) and >90% of the drug released from the free drug solution. This may be attributed to the pH dependent solubility behavior of the polymer used. The eudragit RL 100 is insoluble at acidic pH, so the small quantity of drug release may be associated with an untrapped or adsorbed drug. In the basic medium (phosphate buffer pH 7.4), prolonged drug release may be attributed by swelling of the polymeric chain. The findings also showed that when the polymer concentration increased, there

was a significant decrease in the amount of VLD released from nanoparticles. These outcomes might be the consequence of an increase in the polymer concentration causing an increase in the polymer thickness of the nanoparticles, which in turn caused an increase in the length of the polymer membrane pathway.³⁰

The kinetic treatment for the release data has depicted that the regression coefficient value (R^2) was maximum for zero

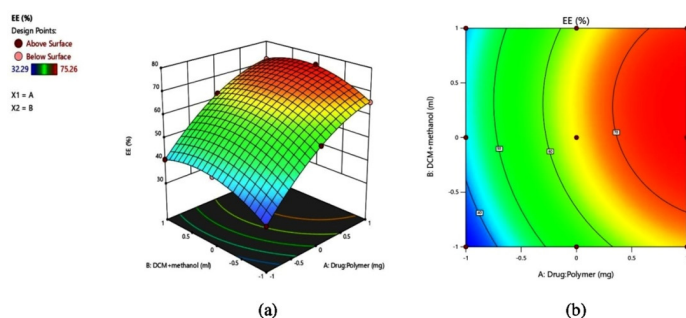


Figure 2: (a) 3D response surface plot and (b) 2D contour plot of % EE.

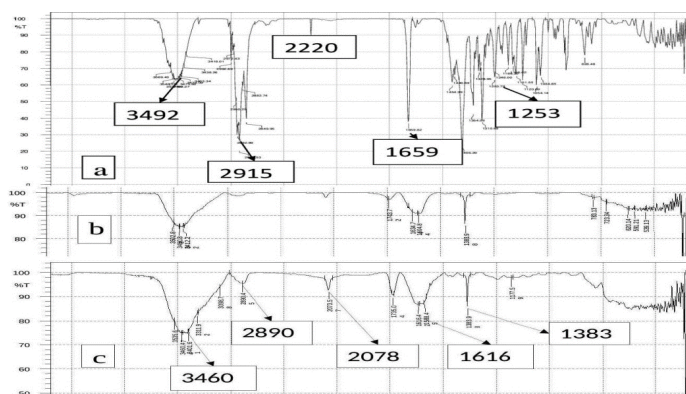


Figure 3: The FTIR spectra of (a) VLD, (b) Eud, (c) VLD-Eud NPs.

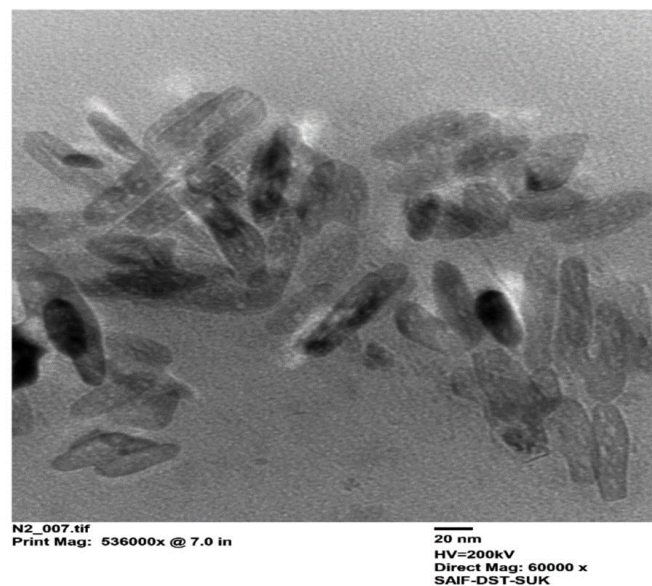


Figure 4: TEM image of optimized Vildagliptin nanoformulation.

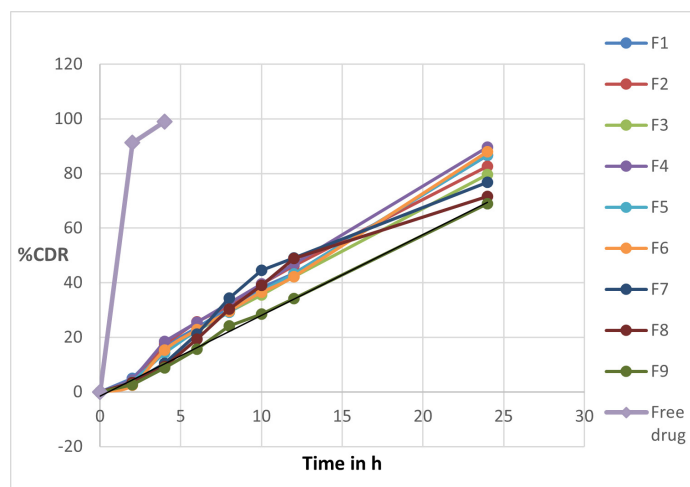


Figure 5: Dissolution profiles of pure VLD and all formulated VLD-Eud NPs.

Table 5: List of the drug release mechanism.

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero order	0.936	0.956	0.998	0.996	0.985	0.992	0.949	0.949	0.996
First order	0.992	0.933	0.959	0.937	0.969	0.966	0.990	0.987	0.972
Higuchi	0.924	0.918	0.878	0.896	0.916	0.901	0.905	0.899	0.870
Korsmeyer-Peppas	0.950	0.961	0.974	0.952	0.948	0.957	0.967	0.972	0.987

order, which indicated the concentration independent release characteristic of prepared nanoformulations.

During 3 months of accelerated stability study, the particle size and EE% of an optimized formulation (F6) was not shown any significant change, which depicts its stability of prepared formulations.

CONCLUSION

The VLD-Eud NPs were successfully prepared with solvent evaporation and nanoprecipitation method. The QbD approach was effectively utilized for the optimization of the nanoparticles. The study's findings indicate that the innovative formulations prepared exhibit sustained releasing activity for 24 hr; additionally, they solve the issue of frequent dosing of VLD for efficient diabetic therapy. Thus, the present work concludes that prepared and optimized VLD-Eud NPs could be a better approach for sustained delivery of Vildagliptin for the management of diabetes mellitus as compared to traditional drug delivery.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ANOVA: Analysis of variance; **DCM:** Dichloromethane; **DoE:** Designs of Experiments; **DM:** Diabetes mellitus; **DPP-4:** Dipeptidyl peptidase-4; **EE:** Entrapment efficiency; **GIP:** Gastric inhibitory polypeptide; **GLP-1:** Glucagon-like peptide-1; **mg:** Milligram; **mL:** Milliliter; **nm:** Nanometer; **NDDSs:** Nanoparticulate drug delivery systems; **QbD:** Quality by design; **R²:** Coefficient of correlation; **RH:** Relative humidity; **SD:** Standard deviation; **TEM:** Transmission electron microscopy; **USP:** United State Pharmacopeia; **VLD-Eud NPs:** Vildagliptin-Eudragit RL 100 coated nanoparticles; **X₁:** Eudragit RL 100 (mg); **X₂:** The composition of the organic solvent; **Y₁:** Particle size (nm); **Y₂:** Entrapment efficiency (%).

SUMMARY

Diabetes Mellitus is a chronic metabolic disorder. Treatment of DM requires continuous medication throughout the life. Vildagliptin is used in the treatment of type 2 diabetes mellitus. In this study we developed VLD-Eud NPs by combined technique of solvent evaporation and nano-precipitation. DoE with 3² Factorial design was used for optimization. The study's findings indicate that the innovative formulations prepared exhibit sustained releasing activity for 24 hr; additionally, they solve the issue

of frequent dosing of VLD for efficient diabetic therapy. Study concludes that prepared and optimized VLD-Eud NPs could be a better approach for sustained delivery of Vildagliptin for the management of diabetes mellitus as compared to traditional drug delivery.

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Quality by design approach assisted development and optimization of Chitosan–Vildagliptin nanoparticles using a simple desolvation technique

Anand Shripal Ammanage^{1,2}, Vinayak Shivamurthi Mastiholimath^{2*} 

¹Department of Pharmaceutics, KLES's College of Pharmacy Nipani, Rajiv Gandhi University of Health Sciences Bengaluru, Bengaluru, India.

²Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy Belagavi, KLE Academy of Higher Education and Research, Belagavi, India.

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ABSTRACT

Type 2 diabetes mellitus is a major metabolic condition that poses a serious risk and a significant public health concern worldwide. Vildagliptin is a new class of antidiabetic drugs used to treat type 2 diabetes mellitus. In the current work, Chitosan-Vildagliptin nanoparticles (CS-VLD NPs) were developed by simple desolvation technique. A 3² factorial design and response surface methodology were used for optimization. The prepared nanoparticles underwent characterization to determine their particle size (PS), polydispersity index (PDI), entrapment efficiency (EE), FTIR, transmission electron microscopy (TEM), *in vitro* drug release, and release kinetics. A 3-month accelerated stability study was performed for the optimized formulation (N6). The PDI value of CS-VLD NPs was varied from 0.24 ± 0.025 to 0.53 ± 0.031 , while the PSs were ranged from 118.24 ± 2.26 nm to 232.84 ± 6.79 nm. The EE values were varied from 20.54 ± 3.61 to $51.57\% \pm 1.52\%$. FTIR study had shown the compatibility of drug and polymer. TEM images were shown spherical-shaped nanoparticles. During the *in vitro* drug release study, sustained drug release was found up to 24 hours, followed by Higuchi kinetics. During the stability study, the optimized (N6) formulation proved its stability. The study concludes that the developed CS-VLD NPs may be a more effective approach than conventional drug delivery for the sustained release of Vildagliptin in the treatment of diabetes mellitus.

INTRODUCTION

Type 2 diabetes represents a significant metabolic disorder, posing a substantial threat as a major global health concern. This condition exerts direct or indirect adverse impacts on the overall quality of life [1]. The condition is largely caused by diminished sensitivity to insulin or insulin resistance, and the progressive degeneration of pancreatic beta cells [2]. Glucose absorption decreases when insulin sensitivity is reduced, which results in a diminished cellular response to glucose. Thus, hyperglycemia and the harmful consequences of glucotoxicity arise [3]. Diabetes was the cause of 1.6 million

deaths in 2016 alone. Predictions state that there will be an increase in the number of diabetes cases, with estimates of 643 million by 2030 and 783 million by 2045 [4].

Metformin and sulfonylureas are frequently prescribed drugs for managing Type 2 Diabetes Mellitus. However, there is a possibility that these oral hypoglycemic agents will cause side effects such as hypoglycemia, liver damage, pancreatitis, and stomach pain. Therefore, there is a critical need to develop innovative and effective anti-hyperglycemic drugs that can reach the appropriate glycemic levels without increasing the risk of hypoglycemia. Treatment for type II diabetes has advanced significantly with the use of incretin hormones. Notably, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) are two novel, potentially effective approaches to treat type 2 diabetes [5].

Significantly, the very short half-lives of GLP-1 and GIP are caused by the enzyme dipeptidyl peptidase-4 (DPP-

*Corresponding Author

Vinayak Shivamurthi Mastiholimath, Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy Belagavi, KLE Academy of Higher Education and Research, Belagavi, India.
E-mail: mastiholimath @ rediffmail.com

4). Thus, it is considered that inhibiting DPP-4 is a suitable approach for treating type 2 diabetes [6].

Vildagliptin is an inhibitor of the DPP-4 enzyme that has been studied extensively in the treatment of diabetes. Its molecular interactions with the DPP-4 enzyme have greatly advanced our understanding of this drug [7].

However, its glucose-lowering effects are transient due to a short half-life of 2–3 hours. As a result, diabetic patients are strongly advised to strictly adhere to the prescribed dosing schedule to maintain its efficacy [8].

Polymeric nanoparticles are pivotal in augmenting the efficacy of therapeutic agents by prolonging their circulation half-lives and diminishing phagocytic uptake and inactivation. These nanoparticles are prepared from synthetic or semi-synthetic polymeric blocks, incorporating biodegradable polymers to optimize tissue compatibility while minimizing cytotoxicity. Chitosan, a naturally occurring polymer, is produced by deacetylating chitin. It is nontoxic, biocompatible, biodegradable, and biologically safe [9].

Chitosan has been recognized as one of the most effective biopolymers for developing formulations that provide sustained ocular delivery [10]. Furthermore, some other research works have demonstrated that Chitosan nanocarriers interact with the ocular mucosa and extend their residence time [11–13]. In oral drug delivery, Chitosan's mucoadhesive properties are essential, as they enable it to stick to the mucosal lining of the gastrointestinal tract, thus enhancing localized drug delivery and absorption. The review by Mohammed *et al.* [14] examines how Chitosan can successfully overcome gastrointestinal barriers, boosting drug absorption and allowing for controlled release. The study highlights the importance of Chitosan's ability to adhere to the intestinal lining, which improves drug absorption and bioavailability [14].

In this instance, we used Chitosan (CS) as the starting material to prepare a typical oral nanoparticles drug delivery system of VLD by desolvation process. To avoid the complexity of the process adopted by earlier mentioned studies, in the current study, Chitosan having low molecular weight was used at three different levels to prepare nanoparticles.

Nowadays, there is an increasing focus on Quality by Design (QbD) as a systematic approach to ensure the quality of the products to accomplish certain goals, such as reduced costs, faster time-to-market, logical controls, and increased efficiency [15]. As QbD is useful in streamlining several processes and producing more stable and effective formulations, its application has grown significantly. Utilizing risk assessment also helps to improve comprehension of important techniques and product limitations [16]. Application of response surface methodology (RSM) and design of experiments (DoEs) is necessary to establish a design space for the input variables in the formulation [17]. DoE and RSM make it possible to collect data efficiently with fewer runs than with standard experimental designs, which change one variable at a time. This combination provides mathematical models that show output responses based on input variables and can be used to analyze response surfaces using linear or quadratic functions. Determining the ideal settings for these input variables becomes crucial to

achieving the expected performance of the formulation; in this regard, DoE and RSM are useful techniques [18].

In the present work, an attempt was made to develop Chitosan-loaded Vildagliptin nanoparticles by adopting a simple desolvation technique with QbD concept. The use of natural biodegradable polymers avoids the biocompatibility issue associated with nanoformulations. Furthermore, there is no any requirement for sophisticated equipment for the formulation thus it can be prepared at a small-scale laboratory and can be easily transferred at the production level.

MATERIALS AND METHODS

Materials

Vildagliptin was supplied as a complimentary sample from Ajanta Pharma Ltd. Mumbai, while low molecular weight Chitosan (CS), glutaraldehyde (GA), and acetone were procured from SD Fine Chemicals Ltd. Mumbai. Dialysis membrane (MW of 12 KDa) was brought from HiMedia, Mumbai, India. All other excipients and solvents used in the formulation were of analytical grade.

Preparation of Chitosan-Vildagliptin nanoparticles (CS-VLD NPs)

Vildagliptin-loaded Chitosan nanoparticles were developed using a simple desolvation method with a few small modifications [10]. Briefly, in a 50 ml aqueous acetic acid (2%) solution, various concentrations of CS 200 mg, 400 mg, and 600 mg were dissolved separately and then 100 mg of VLD was added to form a homogeneous solution. Desolvation was then induced by adding acetone drop-by-drop while continuously stirring until a slight permanent turbidity was achieved. Following that, different volumes (50 μ l, 100 μ l, and 150 μ l) of glutaraldehyde (GA) were incorporated as a cross-linking agent, and the solution was stirred overnight at 600 rpm using a mechanical stirrer to facilitate acetone evaporation. The obtained nanoparticles were collected by centrifugation (KUBOTA 7,000, Japan) at 4°C, 20,000 rpm for 1 hour. The sediment was washed with the same conditions in triplicate. A total of nine formulations were prepared and are presented in Table 1.

Statistical experimental design

Pharmaceutical formulations were traditionally developed by adjusting one variable at a time. This method requires an extensive amount of effort and time. Furthermore, it could be difficult to come up with an ideal formulation because the traditional method does not take the combined impacts of independent components into consideration. Thus, using well-established statistical tools like factorial designs is essential to understand the complexity of pharmaceutical compositions. The number of independent variables used in the study will determine how many experiments are required. Table 2 depicts the total factorial experimental design. The amount of GA used and the concentration of CS were the two independent variables that were examined. Three levels (low, medium, and high) of each variable were examined and their effects on two dependent

Table 1. Experimental runs and their observed values of dependent variables.

Runs	Formulation codes	Independent variables		Dependent variables					
				Y ₁ (nm)			Y ₂ (%)		
		X ₁	X ₂	Actual	Predicted	Residual	Actual	Predicted	Residual
1	N1	200	50	148.28 ± 2.48	149	-0.7206	26.54 ± 3.61	30.52	-3.98
2	N2	200	100	132.48 ± 4.47	132.83	-0.35	42.18 ± 2.78	38.01	4.17
3	N3	200	150	118.24 ± 2.26	116.67	1.57	43.49 ± 2.64	45.5	-2.01
4	N4	400	50	191.61 ± 4.79	190.88	0.7278	35.69 ± 3.03	34.9	0.7933
5	N5	400	100	176.36 ± 3.60	174.72	1.64	44.64 ± 4.73	42.39	2.25
6	N6	400	150	155.18 ± 3.09	158.55	-3.37	50.45 ± 1.56	49.88	0.5733
7	N7	600	50	232.84 ± 6.79	232.76	0.0761	38.34 ± 3.65	39.28	-0.9367
8	N8	600	100	215.14 ± 3.81	216.6	-1.46	48.58 ± 2.93	46.77	1.81
9	N9	600	150	202.31 ± 3.67	200.43	1.88	51.57 ± 1.52	54.26	-2.69

X₁- CS (mg), X₂- GA (μL), Y₁- Particle size (nm), Y₂- Entrapment efficiency (%) and all values are expressed in Mean ± SD (n = 3).

Table 2. Experimental design with process variables and their levels.

Levels	Independent variables		Dependent variables	
	X ₁ CS (mg)	X ₂ GA (μl)	Y ₁ =Particle size (nm)	Y ₂ =EE (%)
-1	200	50	Goal to minimize	Goal to maximize
0	400	100		
+1	600	150		

variables viz., particle size (PS) and entrapment efficiency (EE) were studied.

Determination of particle size and polydispersity index (PDI)

The PS and PDI of the developed nanoparticles were assessed using a Zetasizer (Nano ZS, Malvern Instruments Ltd., Worcestershire, WR14 1XZ, United Kingdom). Millipore water was used to properly dilute each sample and the refractive index was adjusted to 1.33. The temperature at which the analysis was performed was 25°C [19].

Entrapment efficiency

EE of the CS-VLD-NPs was determined through a centrifugation technique. These samples were centrifuged for 30 minutes at 4°C at 20,000 rpm (KUBOTA 7,000, Japan). The supernatant was collected, and the samples were diluted as needed with phosphate buffer at pH 7.4. These samples were then analyzed at 208 nm using a UV spectrophotometer (Shimadzu 1,900, Japan) [20,21]. The % EE was subsequently calculated using the following formula:

$$\% \text{ EE} = \frac{\text{Amount of drug added} - \text{unentrapped drug}}{\text{Amount of drug added}} \times 100 \quad (1)$$

FTIR spectroscopy

The interactions between Vildagliptin and Chitosan were investigated, and the structural alterations that resulted

from the conjugation of CS-VLD NPs were verified by using an FTIR spectrophotometer (Shimadzu DRS 8,000 IR Affinity-1, Japan), the FTIR spectra of VLD, CS, and CS-VLD NPs were captured. The objective of this investigation was to evaluate the stability of the drug and its interactions with the polymer [22].

Transmission electron microscopy (TEM)

A 0.5 μl sample was collected and diluted using 4 ml of purified water. A diluted sample of 0.2 μl was transferred and coated on a 200-mesh cu-carbon grid. It was then let to dry at room temperature and negatively stained with a 2% solution of phosphotungstic acid. TEM samples were examined at 100 kV using a TECNAI G2 SPIRIT BIOTWIN, MINNESOTA (Make: FEI) [23].

In vitro drug release study

A dialysis bag was used for the *in vitro* drug release study for formulations N3, N6, and N9. In order to investigate the release of VLD, pre-treated dialysis bags (MW of 12 KDa) were filled with CS-VLD-NPs containing 10 mg of VLD, and both ends were sealed. These dialysis bags were immersed in a beaker containing 250 ml of phosphate buffer solution (pH 7.4). A thermostat was used to maintain a 37°C temperature, and the apparatus was set up on a magnetic stirrer with a 100 rpm setting. For up to 24 hours, a 2 ml sample was removed, and a new phosphate buffer solution was added at different intervals.

Spectrophotometric examination of the samples was performed at 208 nm [24].

Kinetics study

Fitting data from *in vitro* releases into several kinetic models, such as the first order, zero order, Korsmeyer-Peppas, and Higuchi models, in order to develop an understanding of the drug release mechanism. The coefficient of correlation (R^2) was used for each model to evaluate the degree to which it fit [25].

Stability study

The CS-VLD-optimized batch (N6) was subjected to a stability study. They were kept for three months in three distinct amber-colored glass bottles that were well sealed. Following the recommendations of the International Council for Harmonization, stability testing of the nanoparticles was conducted at $25^\circ\text{C} \pm 2^\circ\text{C}$ and 65% relative humidity. Over a 3-month period with a 1-month sampling interval, PS, PDI, and % EE were determined [26].

RESULTS AND DISCUSSION

Optimization of CS-VLD NPs

The CS-VLD NPs were optimized utilizing the two variables and three levels using DoEs software (Design Expert 13). The desolvation method was used to produce CS-VLD NPs, which were then cross-linked with GA. Precipitation and coacervation are two mechanisms involved in the production of nanoparticles. In order to determine the effect of independent factors (X_1 and X_2) on dependent variables (Y_1 and Y_2), a 3^2 RSM was employed. To investigate the effects of an independent variable, Figures 1a and 2a of 2D counter plots and Figures 1b and 2b of 3D response surface graphs were constructed. To investigate the mathematical relationship between independent and dependent variables, counterplots and polynomial equations were constructed. The linear model's correlation coefficients (R^2) values for the responses from Y_1 and Y_2 were 0.9981 and 0.892, respectively, and are presented in Table 3. The ANOVA models for Y_1 and Y_2 are presented in Table 4. The responses for particle size (Y_1) and EE (Y_2) were computed using the following equations:

$$\text{Particle size } (Y_1) = 174.72 + 41.88 X_1 - 16.17 X_2 \quad (2)$$

$$\text{EE } (Y_2) = 42.38667 + 7.49000 X_1 + 4.38000 X_2 \quad (3)$$

When analyzing the relationship between particle size (Y_1) and the concentration of CS (X_1), it is evident from the positive integer that as the concentration of the polymer increases, the PS also increases. This outcome was expected because higher polymer concentrations lead to an increase in bulk, resulting in larger PSs. Furthermore, the negative integer for X_2 (the amount of GA) shows the negative effect of the cross-linking agent on PS.

With increasing concentrations of the cross-linking agent, there is a hindrance to the further swelling of the polymer, as it tightly holds the polymeric chains. This effect leads to a decrease in PS. The effect is more prominent for X_1

as compared to X_2 which is visible through the integer values (41.88 vs. 16.17). In the case of EE, both the variables (X_1 and X_2) have a synergistic effect, which is witnessed through their positive integers in Eqs. 2 and 3. Furthermore, the effect of polymer concentration is more as compared to cross linking agent, this is due to higher polymer concentrations generally lead to increased viscosity during the nanoparticle formation process, which can result in larger PSs and more densely packed polymer matrices. The glutaraldehyde crosslinking of the particle-matrix results in a drop in the isoelectric point of the nanoparticles; however, no discernible effect of the crosslinking conditions on the final PS was detected [27]. The regression analysis of the results obtained has shown that the data fit a linear model for both responses within the study domain space, with R^2 values of 0.9981 and 0.892 for Y_1 and Y_2 , respectively. ANOVA study has shown the significance of the model with $p < 0.0001$ (for Y_1) and $p < 0.0013$ for Y_2 .

Characterization of nanoparticles

PS and PDI

PS and PDI values for all the nanoformulations have been found in the range between 118.24 ± 2.26 nm to 232.84 ± 6.79 nm and from 0.24 ± 0.025 to 0.39 ± 0.037 , respectively. Figure 1 represents the 2D and 3D response surface plots for PS. The slopes of the line are indicative of the magnitude of the effect, while the directions of the line are indicative of the positive or negative impact of independent variables on dependent variables. The higher slope of factor A (concentration of CS) indicates its profound effect on PS as compared to factor B (amount of GA). PS increased as CS concentration increased; this could be due to strong intermolecular or intramolecular hydrogen bonds between CS molecules which may cause the nanoparticles to aggregate resulting in a large PS [28,29]. As the degree of cross-linking increased, a rigid matrix formed, enabling for a slight decrease in PS [10]. The PS heterogeneity in NPs was measured using PDI, which shows that the size distribution is narrow. A lower PDI value is highly desirable for the formulations that need a uniform size distribution [30].

Entrapment efficiency

The EE of the CS-VLD NPs was found in the range between 26.45 ± 3.61 and $51.57\% \pm 1.52\%$ as presented in Table 1. The influence of the VLD to CS ratio on the EE was predicted by the 3D counter-plot (Fig. 2b) using data from the Design-Expert software. EE was shown to increase with increasing CS concentration; finding may be explained by the fact that the increased concentrations of CS offer more capacity to hold drugs. With an increase in polymer concentration up to 400 mg Chitosan (N6), the EE of Chitosan nanoparticles increased. The EE did not significantly increase beyond this concentration, which may be due to the complete saturation of available sites for the entrapment of the drug [31]. However, there has also been a little increase in the EE observed in nanoparticles with an increase in the cross-linking extent. This could be the result of a high crosslink density, which could stop drug particles from leaking out as the nanoparticles are being formed [10].

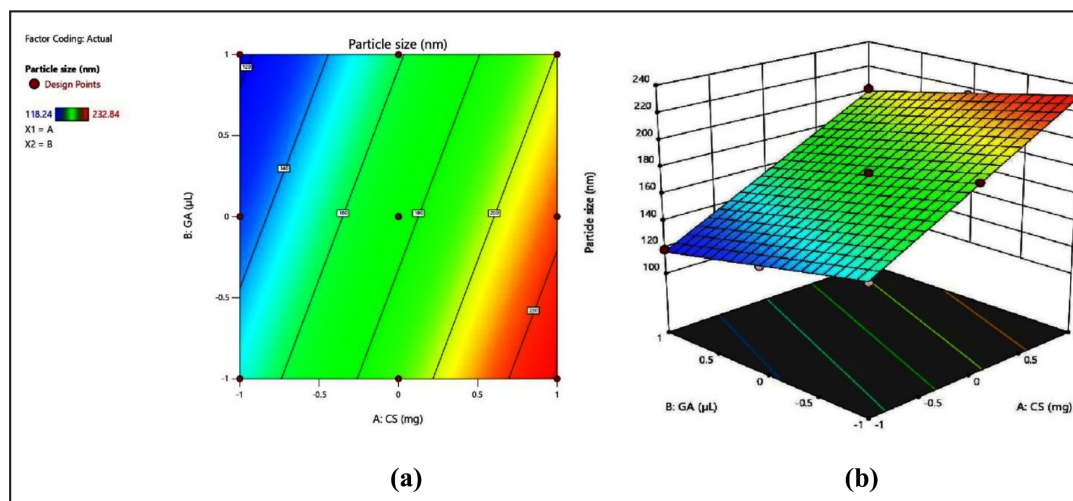


Figure 1. (a) 2D contour plot and (b) 3D response surface plot of PS.

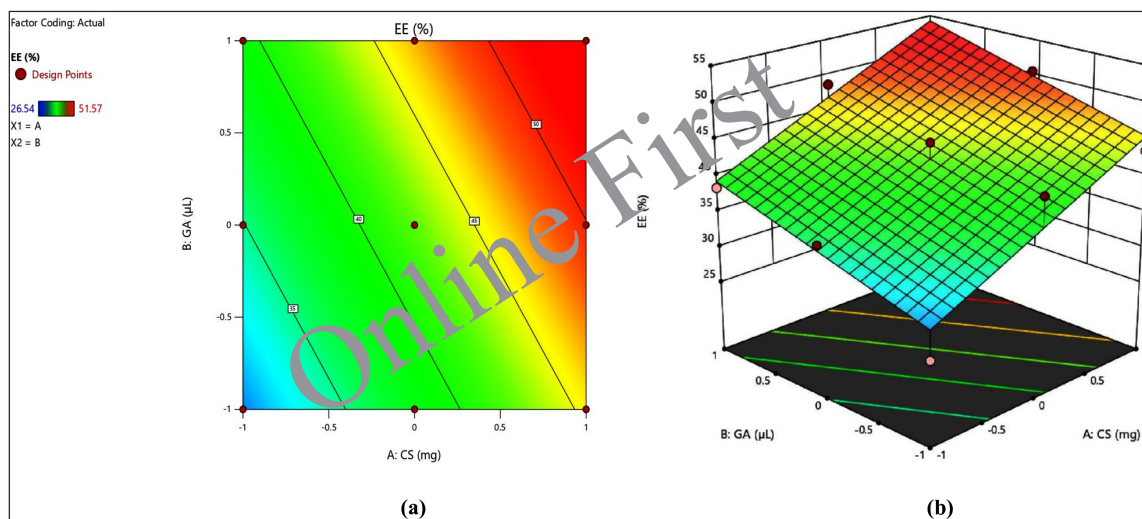


Figure 2. a) 2D contour plot and (b) 3D response surface plot of % EE.

FTIR spectroscopy

The FTIR spectra of (a) VLD, (b) Chitosan, and (c) CS-VLD-NPs are displayed in Figure 3a. Characteristic peaks in the VLD spectrum are seen at 3,492 cm^{-1} and 2,915 cm^{-1} (exhibiting O–H and N–H stretching vibrations), 2,125 cm^{-1} ($\text{C}\equiv\text{N}$ stretching), 1,659 cm^{-1} (amide $\text{C}=\text{O}$), and 1,253 cm^{-1} (C–N stretching). In Figure 3b, we can also observe the infrared spectrum of Chitosan. It is possible to attribute the absorption bands at around 2,869 cm^{-1} to C–H asymmetric stretching. The bands at about 1,550 cm^{-1} ($\text{C}=\text{O}$ stretching of amide I) and 1,350 cm^{-1} (C–N stretching of amide III), respectively, proved the presence of residual N-acetyl groups. The bands at approximately 1,400 and 1,300 cm^{-1} , respectively, confirmed the CH_2 bending and CH_3 symmetrical deformations. It is possible to explain the absorption band at 1,150 cm^{-1} to the asymmetric stretching of the C–O–C bridge. The bands corresponding to C–O stretching are located at 1,050 and 1,032 cm^{-1} [32]. In Figure

3c, The FTIR analysis of CS-VLD nanoparticles shows that the characteristic peaks of both Chitosan and the drug (VLD) are retained without significant shifts or new peaks. This indicates that the drug is physically encapsulated within the Chitosan polymer matrix without strong chemical interactions, ensuring compatibility and preserving the drug's efficacy. The presence of broad bands around 3,400–3,500 cm^{-1} , consistent with O–H and N–H stretching, further supports the conclusion that the functional groups remain intact, confirming that the polymer and drug do not chemically interact significantly.

Transmission electron microscopy

The shape of optimized nanoparticles (N6) has been examined using High-Resolution TEM, and its TEM image is shown in Figure 4. The spherical-shaped nanoparticles that appeared as individual moieties were seen in the TEM investigation.

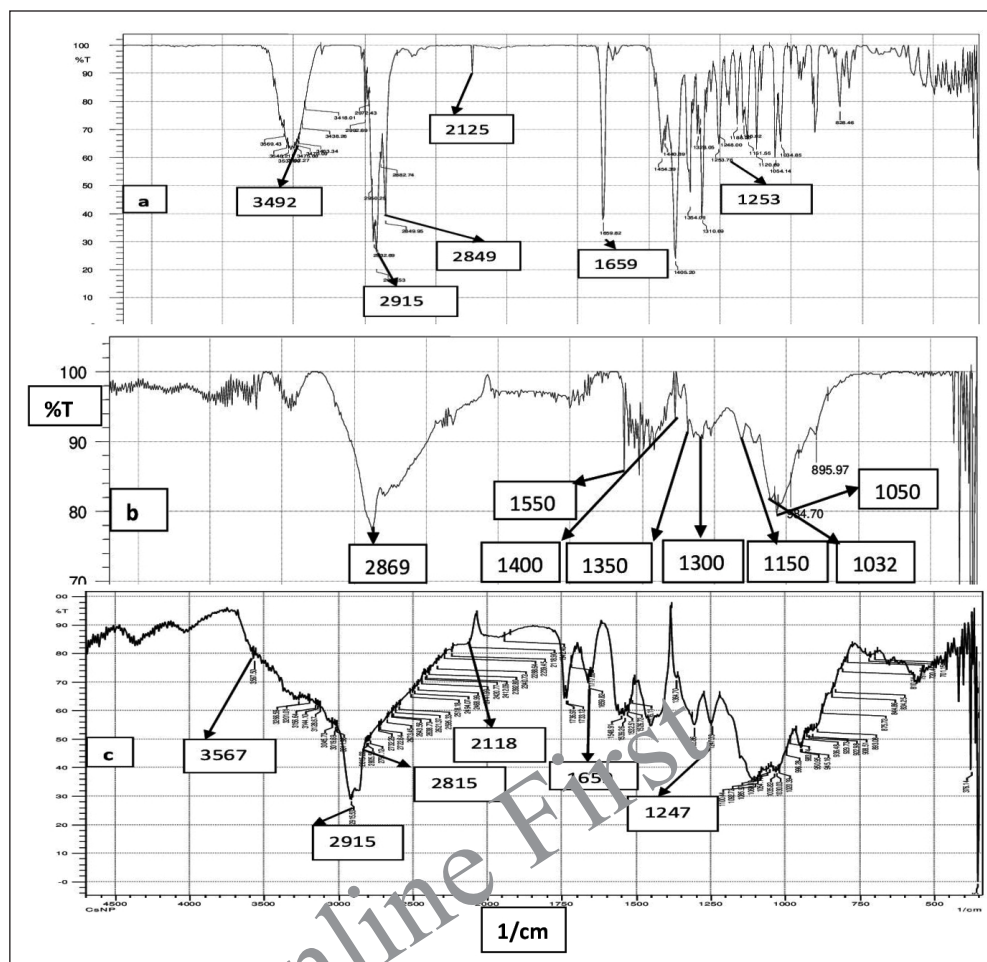


Figure 3. FTIR spectra of (a) VLD, (b) Chitosan, and (c) CS-VLD-NPs.

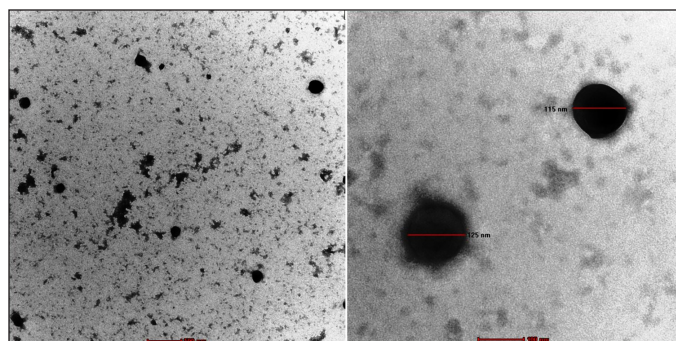


Figure 4. TEM image of optimized CS-VLD NPs.

In vitro drug release study

Drug release from CS-VLD NPs N3, N6, and N9 was studied by *in vitro* dialysis bag method for 24 hours. The cumulative drug release (%CDR) of pure VLD, N3, N6, and N9 CS-VLD NPs is shown in Figure 5. Over a period of 24 hours, all 3 batches of CS-VLD NPs showed a constant delayed release behavior. About 55%–70% of the drug was released by the nanoparticles in the first 6 hours, followed by 78%–84%

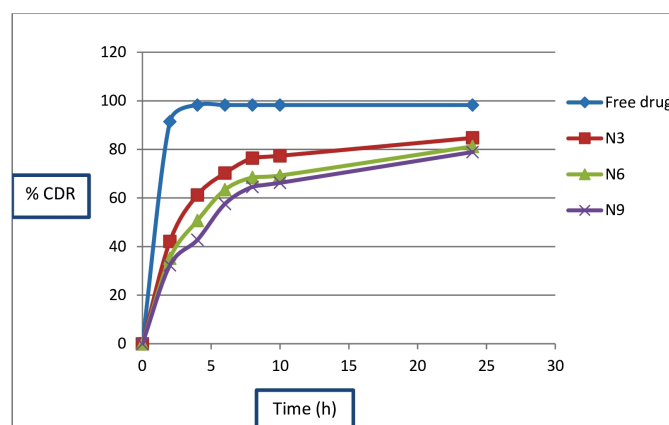


Figure 5. *In vitro* drug release profile of formulation N3, N6 and N9.

in the next 24 hours. The free drug solution exhibited a rapid release profile, with >90% of the drug being released within just 2 hours. Prolonged drug release in the phosphate buffer pH 7.4 was observed. The release of VLD from the nanoparticles started very rapidly in the initial phase which slowed down

Table 3. Summary of results of regression analysis for responses Y_1 and Y_2 .

Models	R^2	Adjusted R^2	Predicted R^2	Std dev.	Press	Remarks
Response Y_1: Particle size						
Linear	0.9,981	0.9,974	0.9,957	1.97	51.67	Suggested
2FI	0.9,981	0.9,969	0.9,918	2.16	99.82	
Quadratic	0.9,981	0.995	0.9,807	2.76	233.71	
Cubic	0.9,992	0.9,939	0.8,602	3.05	1,693.53	Aliased
Response Y_2: EE						
Linear	0.892	0.856	0.7,265	3.02	138.47	Suggested
2FI	0.8,989	0.8,382	0.3,876	3.2	310.12	
Quadratic	0.9,788	0.9,436	0.803	1.89	99.74	
Cubic	0.9,872	0.8,973	-1.3,403	2.55	1,185.08	Aliased

Table 4. ANOVA models for Y_1 and Y_2 .

Response Y_1: Particle size						
Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Model	12,092.61	2	6,046.31	1,552.79	<0.0001	significant
A-CS	10,524.44	1	10,524.44	2,702.86	<0.0001	
B-GA	1,568.17	1	1,568.17	402.73	<0.0001	
Response Y_2: EE						
Model	451.71	2	225.85	24.78	0.0013	significant
A-CS	336.6	1	336.6	36.94	0.0009	
B-GA	115.11	1	115.11	12.63	0.012	

Table 5. List of the drug release mechanisms.

	N3	N6	N9
Zero order	0.496	0.589	0.639
First order	0.718	0.790	0.834
Higuchi	0.821	0.880	0.912
Korsmeyer-Peppas	0.596	0.628	0.650

Table 6. Stability study of optimized CS-VLD NPs.

Stability parameter	Test period			
	0 month	1 month	2 months	3 months
Particle size (nm)	155.18 ± 3.09	156.2 ± 2.74	158.06 ± 1.46	159.83 ± 0.77
PDI	0.32 ± 0.06	0.34 ± 0.01	0.33 ± 0.02	0.35 ± 0.02
% EE	50.45 ± 1.56	47.29 ± 2.40	48.94 ± 2.24	46.88 ± 0.80

afterward. The drug that has remained on the surface may dissolve quickly in the first fast-release phase. The drug that has been entrapped is dissolved in the following phase when the release medium penetrates nanoparticles [10].

Kinetics study

The release data were subjected to fitting into various kinetic models to estimate the drug release kinetics. The drug release mechanism for N3, N6, and N9 batches are presented in Table 5. According to the kinetic release data of the N3, N6,

and N9 formulations, the release characteristic was revealed by the regression coefficient value (R^2) which being highest for the Higuchi model ($R^2 = 0.821$ 0.880 0.912 for N3, N6, and N9, respectively). The Higuchi model describes drug release from a matrix system, primarily focusing on diffusion as the controlling mechanism, which occurs one-dimensionally from the matrix into the surrounding medium [33].

Stability study

For optimized CS-VLD NPs (N6), stability tests were carried out with PS, PDI, and %EE as the main parameters. The stability study results are presented in Table 6. The PS, PDI, and %EE of an optimized formulation (N6) did not significantly alter throughout the course of a 3-month accelerated stability testing, indicating the formulations were stable.

CONCLUSION

In the present investigation, a simple desolvation method was adopted to prepare CS-VLD NPs. The nanoparticle optimization process was successfully carried out using the QbD technique. Based on QbD technique, the optimized formulation for making CS-VLD NPs can be achieved with 400 mg of Chitosan and 150 μ l of glutaraldehyde in formulation N6, which shows minimum PS (155.18 ± 3.09 nm) and maximum EE (50.45% ± 1.56%). The formulation's monodispersity is further supported by relatively low PDI (0.32 ± 0.06) and TEM investigations. Since nanoparticles address the issue of frequent VLD dosing for effective diabetes therapy, the observation of a

progressive release of VLD over a 24-hour period suggests the potential use of nanoparticles. Thus, the current investigation demonstrates that optimized CS-VLD NPs may provide improved therapeutic efficacy and sustained drug action, thereby enhancing patient outcomes and treatment adherence, in contrast to conventional drug delivery.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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