

FORMULATION AND EVALUATION OF SAXAGLIPTIN-LOADED POLYMERIC NANOSPONGES FOR SUSTAINED ANTIDIABETIC THERAPY

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ABSTRACT

Saxagliptin is an effective oral antidiabetic drug used in the management of type 2 diabetes mellitus; however, its therapeutic efficacy is limited by rapid drug release and the need for sustained delivery. The present study aimed to formulate and evaluate Saxagliptin-loaded nanosponges to achieve controlled drug release and improve antidiabetic performance. Nanosponges were prepared by the emulsion solvent diffusion method using ethyl cellulose as the polymer, polyvinyl alcohol (PVA) as the stabilizer, and Pluronic F68 as the surfactant. Six formulations (F1–F6) were developed by varying the concentrations of polymer and stabilizers and were evaluated for percentage yield, entrapment efficiency, particle size, zeta potential, surface morphology, in vitro drug release, release kinetics, α -amylase inhibitory activity, and stability. Among all formulations, F5 exhibited the highest percentage yield ($88.56 \pm 1.14\%$) and entrapment efficiency ($86.65 \pm 1.45\%$). The optimized formulation showed a mean particle size of 157.2 nm with a zeta potential of +17.0 mV, indicating good colloidal stability, while scanning electron microscopy confirmed the formation of spherical porous nanosponges. The optimized formulation demonstrated sustained drug release of $97.36 \pm 0.41\%$ over 12 h compared with the rapid release of the plain drug. Drug release followed first-order kinetics ($R^2 = 0.9950$), suggesting concentration-dependent release. The α -amylase inhibition study revealed superior inhibitory activity of the optimized nanosponge formulation with an IC_{50} value of 15.68 $\mu\text{g/ml}$, compared with 19.24 $\mu\text{g/ml}$ for the standard drug acarbose. Stability studies indicated that the optimized formulation remained physically stable with minimal changes in particle size and entrapment efficiency over three months. The developed Saxagliptin-loaded nanosponge formulation demonstrated satisfactory physicochemical characteristics, sustained drug release, enhanced in vitro antidiabetic activity, and acceptable stability, indicating its potential as a promising controlled-release drug delivery system for the management of type 2 diabetes mellitus.

Keywords: Saxagliptin, Nanosponges, Emulsion solvent diffusion, Controlled drug delivery, Ethyl cellulose, Entrapment efficiency, In vitro drug release, α -Amylase inhibition, Stability studies, Type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most prevalent non-communicable diseases worldwide and is associated with serious microvascular and macrovascular complications, including nephropathy, retinopathy, neuropathy, cardiovascular diseases, and stroke¹. The increasing global prevalence of type 2 diabetes mellitus (T2DM), driven by sedentary lifestyles, obesity, and population aging, has made effective glycemic control a major public health priority. Early diagnosis and long-term management are essential to prevent disease progression and reduce diabetes-related complications².

Among the various classes of oral antidiabetic agents, dipeptidyl peptidase-4 (DPP-4) inhibitors have gained considerable attention because of their ability to improve glycemic control with a low risk of hypoglycemia and favorable tolerability. Saxagliptin is a potent and selective DPP-4 inhibitor that enhances endogenous incretin activity by preventing the degradation of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP)³. This results in increased glucose-dependent insulin secretion, reduced glucagon release, and improved postprandial glucose regulation. Owing to these pharmacological advantages, Saxagliptin is widely prescribed either as monotherapy or in combination with other antidiabetic agents for the treatment of T2DM⁴.

Despite its therapeutic efficacy, conventional oral Saxagliptin formulations exhibit limitations such as rapid drug dissolution, fluctuations in plasma drug concentration, and the requirement for repeated dosing to maintain therapeutic levels. Such limitations may reduce patient compliance and compromise long-term glycemic control. Therefore, the development of novel drug delivery systems capable of providing controlled and sustained drug release has become an important strategy to improve therapeutic effectiveness while minimizing dosing frequency and adverse effects⁵.

Nanotechnology-based drug delivery systems have emerged as promising approaches for enhancing the therapeutic performance of pharmaceutical agents. Among these, polymeric nanosponges have attracted significant interest because of their unique porous three-dimensional structure, high drug-loading capacity, controlled drug release characteristics, and excellent stability⁶. Nanosponges are submicron-sized porous particles capable of encapsulating both hydrophilic and hydrophobic drugs within their polymeric matrix. Their porous architecture enables gradual diffusion of the encapsulated drug, thereby prolonging drug release and maintaining therapeutic drug concentrations for extended periods. In addition, polymeric nanosponges improve drug stability, reduce drug degradation, enhance bioavailability, and minimize dose-related adverse effects⁷.

Several preparation techniques have been employed for nanosponge fabrication, including solvent evaporation, quasi-emulsion solvent diffusion, emulsion solvent diffusion, ultrasound-assisted synthesis, and melt methods. Among these, the emulsion solvent diffusion technique is widely preferred because of its simplicity, reproducibility, scalability, and ability to produce uniformly sized nanoparticles with high drug entrapment efficiency. The selection of suitable polymers and stabilizers plays a significant role in determining the particle size, entrapment efficiency, drug release behavior, and overall stability of the prepared nanosponges. Ethyl cellulose is commonly employed as the polymeric matrix because of its biocompatibility, hydrophobic nature, and sustained-release properties, whereas polyvinyl alcohol (PVA) and Pluronic F68 serve as effective stabilizers for producing physically stable nanosponge dispersions⁸.

Previous studies have demonstrated the successful application of polymeric nanosponges for improving the controlled delivery of several therapeutic agents. However, limited information is available regarding the formulation and evaluation of Saxagliptin-loaded polymeric nanosponges for sustained antidiabetic therapy. Developing such a delivery system may improve drug encapsulation, provide prolonged drug release, enhance antidiabetic efficacy, and improve patient compliance compared with conventional dosage forms.

Therefore, the present study was undertaken to formulate and evaluate Saxagliptin-loaded polymeric nanosponges using the emulsion solvent diffusion technique. Various formulations were prepared by varying the concentrations of ethyl cellulose, polyvinyl alcohol, and Pluronic F68 and were evaluated for percentage yield, entrapment efficiency, particle size, zeta potential, surface morphology, in vitro drug release, release kinetics, in vitro α -amylase inhibitory activity, and stability. The objective of the investigation was to develop an optimized nanosponge formulation capable of providing sustained drug release with improved antidiabetic performance and acceptable physicochemical stability.

MATERIAL AND METHODS

Material

Saxagliptin was obtained as a gift sample from a reputed pharmaceutical manufacturer. Ethyl cellulose was used as the polymer for nanosponge preparation, while polyvinyl alcohol (PVA) and Pluronic F68 served as stabilizers. Dichloromethane was employed as the organic solvent, and distilled water was used as the aqueous phase during formulation. Methanol, phosphate buffer (pH 6.9), soluble starch, 3,5-dinitrosalicylic acid (DNSA), and α -amylase enzyme were used for analytical and in vitro antidiabetic studies. Acarbose was used as the reference standard for enzyme inhibition studies. All chemicals and

reagents used in the study were of analytical reagent (AR) grade and were used without further purification.

Methods

Formulation and Development of Nanosponges

Saxagliptin-loaded nanosponges were prepared by the emulsion solvent diffusion technique. Accurately weighed 10 mg of Saxagliptin and the required quantity of ethyl cellulose (50–150 mg) were dissolved in 15 ml of dichloromethane to obtain the organic (dispersed) phase. Separately, the aqueous (continuous) phase was prepared by dissolving the required amounts of polyvinyl alcohol (PVA; 250–750 mg) and Pluronic F68 (50 or 100 mg) in 100 mL of distilled water with continuous stirring until a clear solution was obtained. The organic phase was added slowly, dropwise, into the aqueous phase under continuous magnetic stirring at 1000 rpm. Stirring was continued for 2 hours to allow diffusion and complete evaporation of the organic solvent, resulting in the formation of Saxagliptin-loaded nanosponges. The prepared nanosponges were collected by vacuum filtration, washed with distilled water to remove excess stabilizer, and dried in a hot air oven at 40°C for 24 hours. The dried nanosponges were stored in airtight containers for further characterization and evaluation.

This procedure corresponds to the formulation compositions shown in Table 1, where the concentrations of polyvinyl alcohol (250–750 mg), Pluronic F68 (50–100 mg), and ethyl cellulose (50–150 mg) were varied while maintaining constant amounts of Saxagliptin (10 mg), dichloromethane (15 mL), and distilled water (100 mL)⁹.

Table 1: Formulation of Saxagliptin loaded nanosponges

Ingredients	F1	F2	F3	F4	F5	F6
Saxagliptin (mg)	10	10	10	10	10	10
Polyvinyl alcohol (mg)	250	500	750	250	500	750
Pluronic F68 (mg)	50	50	50	100	100	100
Ethyl cellulose (mg)	50	100	150	50	100	150
Dichloromethane	15	15	15	15	15	15
Distilled water (ml)	100	100	100	100	100	100

Characterization of Nanosponges

Percentage yield

The nanosponges obtained after drying was weighed. Percentage yield value was calculated as follows:

$$\% \text{ yield} = \text{Weight of nanosponges} \times 100 / \text{Total solids weight}$$

Entrapment efficiency

UV spectrophotometric method was used to estimate entrapment efficiency of nanosponges¹⁰. A calibration curve was plotted for Saxagliptin in 0.1 N HCl in the range of 2-10 µg/mL (Beer's Lambert's range) at 210nm. 10 mg of nanosponges of each batch were selected, powdered in a mortar and placed in 10 mL of 0.1 N HCl. Saxagliptin was extracted by centrifuging at 1000 rpm for 30 min, filtered and analyzed concentration from calibration curve data after necessary dilution. Percentage entrapment was calculated as follows:

$$\% \text{ Entrapment efficiency} = \frac{\text{Actual drug}}{\text{Theoretical drug}} \times 100$$

Particle size, polydispersity index

Average particles size, polydispersity index (PDI) of prepared nanosponges was determined using Zetasizer (DTS were 4.10, Horriba instrument, India). The nanosponges formulation was diluted with deionized water (1:9 v/v) and analysed for average size and PDI¹¹.

Shape and surface morphology

The shape and surface morphology of the nanosponges were investigated using scanning electron microscopy. The nanosponges were fixed on supports with carbon-glue, and coated with gold using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the scanning electron microscope at 10 kV.

In vitro drug release of nanosponges

The test determines the time required for formulation to release percentage of drug under specified conditions¹². For the oral dosage forms the *in vitro* dissolution study must be conducted in the dissolution medium which simulate the *in-vivo* conditions (actual physiological conditions). The *in vitro* drug release studies for the prepared formulation were conducted for a period of 12 hrs using an Labindia DS 8000 model dissolution tester USP Type-2 apparatus (rotating paddle) set at 100 rpm and a temperature of 37± 0.5°C formulation was placed in the 900ml of the medium. At specified intervals 5ml samples were withdrawn from the dissolution medium and replaced with fresh medium to keep the volume constant. The absorbance of the sample solution was analyzed at 210nm for the presence of model drug, using a UV-visible spectrophotometer.

In-vitro anti-diabetic activity

Alpha amylase inhibition assay

The *in vitro* α-amylase inhibitory activity of the standard (acarbose) and nanosponge samples was evaluated using the dinitrosalicylic acid (DNSA) colorimetric method. Briefly, 500 µL of the test sample or standard solution (10–50 µg/mL) was mixed with 500 µL of 0.20 M phosphate buffer (pH 6.9)

containing α -amylase enzyme (0.5 mg/mL). The reaction mixture was incubated at 25°C for 10 min to allow interaction between the enzyme and inhibitor. Subsequently, 500 μ L of 1% soluble starch solution prepared in 0.02 M sodium phosphate buffer (pH 6.9) was added as the substrate, and the mixture was further incubated at 25°C for 10 min. The enzymatic reaction was terminated by adding 1.0 mL of 3,5-dinitrosalicylic acid (DNSA) reagent, followed by heating the reaction mixture in a boiling water bath for 5 min to develop the characteristic color. After cooling to room temperature, the reaction mixture was diluted with 10 mL of distilled water, and the absorbance was measured at 540 nm using a UV–Visible spectrophotometer. A control representing 100% enzyme activity was prepared similarly by replacing the test sample with the vehicle. The percentage inhibition of α -amylase activity was calculated by comparing the absorbance of the test samples with that of the control ¹³.

Stability studies of optimized nanosponges formulation (F3)

The prepared nanosponges subjected to stability studies at 40 $\pm$ 2°C/75 $\pm$ 5% RH and 30 $\pm$ 2°C/60 $\pm$ 5% RH as per ICH guidelines for a period of 3 months. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance and drug content.

RESULTS AND DISCUSSION

The present study successfully developed Saxagliptin-loaded nanosponges using the emulsion solvent diffusion technique by varying the concentrations of polyvinyl alcohol (PVA), Pluronic F68, and ethyl cellulose. The prepared formulations exhibited satisfactory physicochemical properties and sustained drug release characteristics, indicating the suitability of the selected formulation approach.

The percentage yield of the prepared nanosponges ranged from 71.85 $\pm$ 1.24% to 88.56 $\pm$ 1.14% (Table 2). Among all formulations, F5 showed the highest percentage yield (88.56 $\pm$ 1.14%), which may be attributed to the optimum concentration of polymer and stabilizers that minimized material loss during nanoparticle formation. Formulations containing either lower or higher polymer concentrations exhibited comparatively lower yields, suggesting that the appropriate polymer-to-drug ratio plays an important role in improving production efficiency.

The entrapment efficiency values varied from 70.32 $\pm$ 1.25% to 86.65 $\pm$ 1.45% (Table 3). The highest entrapment efficiency was obtained with formulation F5 (86.65 $\pm$ 1.45%), indicating effective encapsulation of Saxagliptin within the ethyl cellulose matrix. The improved drug entrapment observed in F5 may be due to the balanced concentrations of ethyl cellulose and stabilizers, which reduced drug diffusion into the external aqueous phase during nanoparticle formation.

The optimized formulation F5 exhibited a mean particle size of 157.2 nm, confirming successful preparation of nanosponges within the nanometer range (Figure 1). The small particle size is

advantageous because it provides a larger surface area for drug dissolution and facilitates improved drug release and absorption. The uniform particle size distribution also indicates the efficiency of the emulsion solvent diffusion technique in producing stable nanosized particles.

The zeta potential of the optimized formulation was found to be +17.0 mV (Figure 2). Although the value is below ± 30 mV, the presence of non-ionic stabilizers such as PVA and Pluronic F68 provides steric stabilization, which contributes to the physical stability of the nanosponge dispersion by preventing particle aggregation during storage.

The scanning electron microscopy (SEM) image of the optimized formulation (Figure 3) revealed that the nanosponges possessed a nearly spherical shape with a porous surface morphology. The porous structure is characteristic of nanosponges and is expected to provide a larger surface area for drug loading and controlled drug diffusion, thereby supporting sustained drug release.

The *in vitro* drug release study demonstrated a marked difference between the pure drug and the optimized nanosponge formulation (Table 4). The plain drug released approximately 88.24% within 2 h, whereas the optimized formulation (F5) exhibited a gradual release pattern, reaching $97.36 \pm 0.41\%$ over 12 h. This prolonged release profile confirms the ability of the nanosponge matrix to control drug diffusion and sustain drug release for an extended period.

The drug release kinetic analysis (Table 5) indicated that the release data best fitted the first-order kinetic model ($R^2 = 0.9950$) compared with the zero-order ($R^2 = 0.8795$), Higuchi ($R^2 = 0.9701$), and Korsmeyer–Peppas ($R^2 = 0.9753$) models. These findings suggest that the release of Saxagliptin from the nanosponge matrix is predominantly concentration-dependent while also involving diffusion through the porous polymeric network.

The *in vitro* α -amylase inhibitory activity of the optimized nanosponge formulation demonstrated enhanced antidiabetic potential compared with the standard drug acarbose (Table 6). The nanosponge formulation exhibited concentration-dependent enzyme inhibition, increasing from 40.56% at 10 $\mu\text{g/ml}$ to 93.42% at 50 $\mu\text{g/ml}$, whereas acarbose showed inhibition ranging from 35.82% to 90.18% over the same concentration range. Furthermore, the optimized formulation exhibited a lower IC_{50} value (15.68 $\mu\text{g/ml}$) than acarbose (19.24 $\mu\text{g/ml}$), indicating greater inhibitory potency against α -amylase. The enhanced enzyme inhibition may be attributed to improved drug dispersion and increased availability of Saxagliptin released from the porous nanosponge system.

The stability study of the optimized formulation (F5) demonstrated good physicochemical stability under both refrigerated ($4 \pm 0.2^\circ\text{C}$) and room temperature ($25\text{--}28 \pm 2^\circ\text{C}$) storage conditions over a period of three months (Table 7). Only minor changes were observed in particle size and entrapment efficiency,

while the physical appearance remained unchanged under refrigerated conditions. Slight aggregation and a marginal off-white coloration were observed after storage at room temperature for three months; however, these changes did not significantly affect the overall characteristics of the formulation. These findings indicate that the optimized nanosponge formulation possesses acceptable storage stability, particularly under refrigerated conditions.

Table 2: Percentage Yield of Saxagliptin-Loaded Nanosponge Formulations

Formulation	Percentage Yield (%)
F1	71.85 ± 1.24
F2	78.42 ± 1.18
F3	82.67 ± 1.36
F4	75.24 ± 1.27
F5	88.56 ± 1.14
F6	85.73 ± 1.29

*Average of three determinations (n=3)

Table 3: Entrapment Efficiency of Saxagliptin-Loaded Nanosponge Formulations

Formulation	Entrapment Efficiency (%)
F1	70.32±1.25
F2	76.32±0.98
F3	79.85±1.77
F4	74.65±1.36
F5	86.65±1.45
F6	80.23±1.36

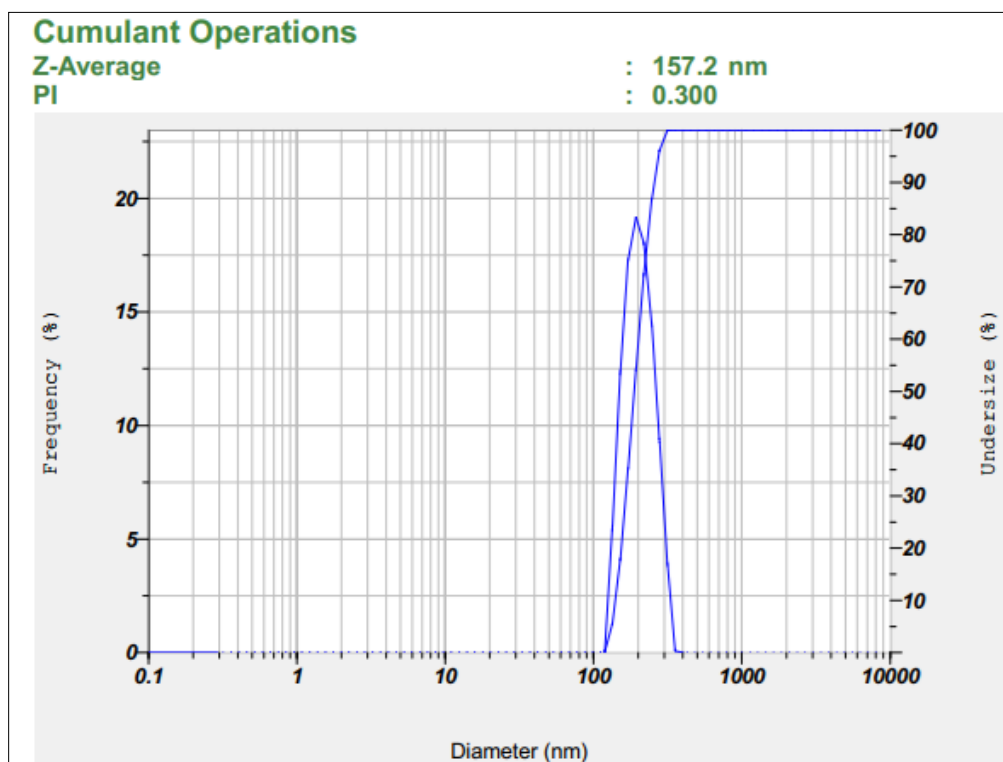


Figure 1: Measurement of mean particle size of Optimized formulation F5 (157.2nm)

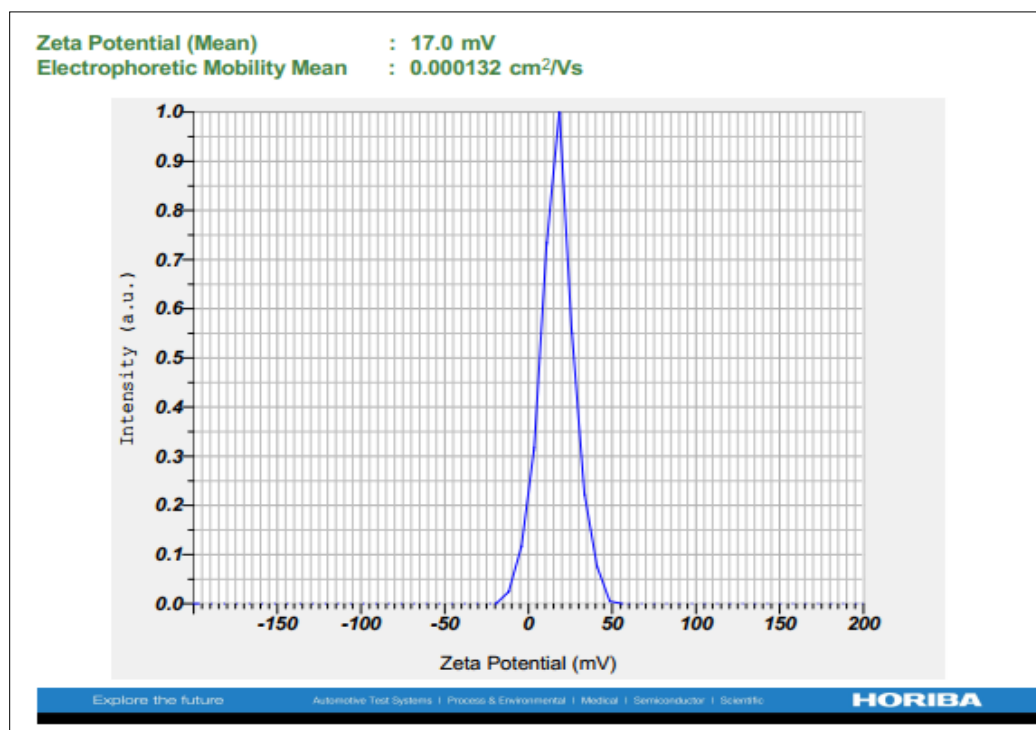


Figure 2: Graph of zeta potential of Optimized formulation F5 (17.0mV)

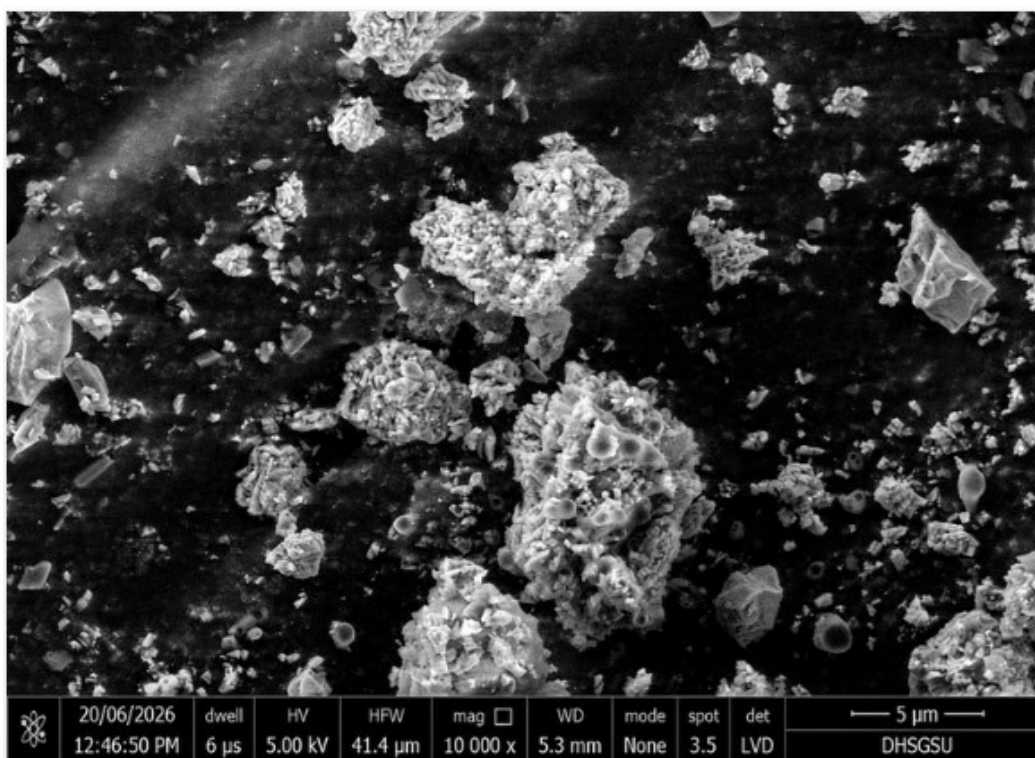


Figure 3: Scanning electronic microscopy of optimized formulation F5

Table 4: *In vitro* drug release study of Saxagliptin loaded nanosponges

S. No.	Time (hrs.)	Cumulative % Drug Release	
		Plain Drug	Nanosponges
			Optimized formulation F56
1.	0.5	28.45 ± 0.52	12.68 ± 0.34
2.	1	46.82 ± 0.61	20.74 ± 0.42
3.	1.5	59.36 ± 0.58	29.65 ± 0.48
4.	2	88.24 ± 0.63	38.92 ± 0.51
5.	3	-	52.48 ± 0.46
6.	4	-	64.83 ± 0.53
7.	6	-	78.94 ± 0.57
8.	8	-	89.58 ± 0.49
9.	12	-	97.36 ± 0.41

Table 5: Regression analysis data of Saxagliptin loaded nanosponges

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R ²	R ²	R ²	R ²
F5	0.8795	0.9950	0.9701	0.9753

Table 6: Results of % Inhibition of *in vitro* antidiabetic studies of standard Acarbose and Saxagliptin-loaded nanosponges formulation F5

S. No.	Concentration (µg/mL)	% Inhibition (Acarbose)	% Inhibition (Saxagliptin-Loaded Nanosponges F3)
1	10	35.82	40.56
2	20	50.64	54.88
3	30	64.93	68.75
4	40	78.45	82.64
5	50	90.18	93.42
	IC ₅₀ (µg/mL)	19.24	15.68

Table 7: Characterization of stability study of Optimized formulation (F5)

Characteristic	1 Month		2 Months		3 Months	
	4.0 ± 0.2°C	25–28 ± 2°C	4.0 ± 0.2°C	25–28 ± 2°C	4.0 ± 0.2°C	25–28 ± 2°C
Average	172.84 ± 1.26	174.62 ± 1.41	173.56 ± 1.34	176.85 ± 1.52	174.28 ± 1.48	179.43 ± 1.67
Particle Size (nm)						
Entrapment Efficiency (%)	89.48 ± 0.42	88.95 ± 0.47	89.12 ± 0.39	88.21 ± 0.51	88.74 ± 0.46	87.63 ± 0.58
Physical Appearance	No change	No change	No change	Slight aggregation	No change	Slight aggregation, off-white powder

CONCLUSION

Saxagliptin-loaded polymeric nanosponges were successfully formulated using the emulsion solvent diffusion technique. Among the prepared formulations, **F5** exhibited the highest percentage yield, entrapment efficiency, suitable particle size, and satisfactory physicochemical stability. The optimized formulation demonstrated sustained drug release for up to 12 hours, following first-order release kinetics, and showed enhanced **in vitro** α -amylase inhibitory activity compared with the standard drug. Stability studies confirmed that the formulation remained stable under the tested storage conditions. The developed polymeric nanosponge system represents a promising sustained-release carrier for Saxagliptin, with the potential to improve antidiabetic therapy by providing prolonged drug release and enhanced therapeutic efficacy.

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