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Novel Sustained-Release Nateglinide Microspheres: In Vitro Evaluation for Improved Antidiabetic Therapy

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ABSTRACT

Nateglinide, a short-acting oral hypoglycemic agent, requires multiple daily administrations due to its short half-life, adversely impacting patient compliance. The purpose of this study was to develop and optimize a sustained-release microsphere formulation of Nateglinide to prolong drug release and improve therapeutic adherence in the management of type 2 diabetes. Nateglinide-loaded microspheres were successfully formulated using the solvent evaporation method with PLGA as the polymer and PVA as the stabilizer. Five formulations (F1–F5) with varying drug-to-polymer ratios (1:1 to 1:5) were developed, among which F4 (1:4 ratio) was optimized. F4 exhibited optimal particle size, excellent flow properties, high encapsulation efficiency (96.3%), and drug content (19.26%). SEM revealed spherical microspheres with moderate porosity, while DSC and FTIR confirmed drug–polymer compatibility and amorphous dispersion. The zeta potential (−23.7 mV) indicated stable dispersion. In vitro studies showed sustained drug release (97% over 24 hours), following first order and Higuchi models. Stability studies confirmed minimal degradation under storage conditions. The optimized Nateglinide microspheres provided sustained release, stable physicochemical properties, and effective kinetics, indicating potential to reduce dosing frequency and improve compliance in type 2 diabetes management.

Keywords: *Nateglinide, Sustained-release microspheres, PLGA, Diabetes management, Glycemic control.*

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1. INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by persistent hyperglycemia, continues to pose a significant public health challenge worldwide. Type 2 diabetes mellitus (T2DM), in particular, accounts for the majority of global diabetes cases and is associated with insulin resistance and progressive pancreatic β-cell dysfunction.[1,2] Conventional antidiabetic therapies often require frequent dosing, which can lead to poor patient adherence, suboptimal glycemic control, and increased risk of complications such as cardiovascular disease, neuropathy, nephropathy, and retinopathy. Therefore, there is an urgent need for advanced drug delivery systems that offer sustained therapeutic effects, improved pharmacokinetic profiles, and enhanced patient compliance.[3–6] Nateglinide, a meglitinide class insulin secretagogue, is widely used for controlling postprandial hyperglycemia in T2DM patients. It stimulates rapid insulin release by closing ATP-sensitive potassium channels in pancreatic β-cells, thereby enhancing glucose uptake. Type 2 diabetes mellitus (T2DM) is a worldwide metabolic disorder with ongoing hyperglycemia resulting from insulin resistance and impaired insulin secretion. Successful control of postprandial blood glucose is important in the prevention of long-term T2DM complications. [7] Nateglinide, a D-phenylalanine derivative of meglitinides, is popular for its fast onset and quick duration of action. It works through stimulation of insulin secretion from pancreatic β-cells, thus being useful for the management of postprandial peaks.[8] Yet, its therapeutic utility is compromised by a short half-life of about 1.5 hours, necessitating several daily administrations. This multiple-dose regimen has the potential to cause lower patient compliance and erratic glucose control.[9] To address these issues, controlled-release drug delivery systems, more specifically polymer-based microspheres, are a promising solution. Microspheres offer controlled release of the drug, enhanced bioavailability, and lower dosing intervals, all of which can lead to improved patient compliance and therapeutic responses.[10] While

microspheres have been successfully developed as formulations for several antidiabetic drugs, including metformin, pioglitazone, and glipizide, few studies have investigated the controlled-release delivery of Nateglinide using the microsphere concept. In addition, most available studies emphasize traditional immediate-release formulations rather than long-acting systems and do not assess pharmacodynamic efficacy.[11] Several analytical methods have been reported for the determination of Nateglinide in drug formulations, such as UV–visible spectrophotometry, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), liquid chromatography mass spectrometry (LC-MS/MS), fluorimetry, and capillary electrophoresis.[12–14] These methods are amenable to routine analysis of Nateglinide in bulk drug or simple matrices, but they are not optimized for complicated delivery systems like microspheres. The presence of polymeric materials and the necessity to analyse sustained drug release behaviour bring with them special challenges for analysis, e.g., matrix interference and drug polymer interactions that undermine assay accuracy and reliability.[15] There thus exists an evident requirement to create and check a new analytical technique, especially designed for the assessment of Nateglinide within microsphere formulations.The method should be sensitive, accurate, and rugged, per ICH guidelines, and capable of validating drug content, encapsulation efficiency, and in vitro release characteristics over prolonged periods.[16] Despite the clinical significance of Nateglinide, no integrated approach involving formulation, in vitro testing, method development, and pharmacodynamics evaluation has so far been described. [17] Some of the existing deficiencies in literature comprise the lack of validated microsphere-based Nateglinide delivery systems, inadequacies in analytical techniques specific to such drug-delivery systems, and the paucity of studies linking in vitro release with bioactivity. The current research fills the above limitations through the development and assessment of Nateglinide-loaded microspheres as an oral controlled-release delivery system. A new analytical procedure is developed and established for the quantitative determination of Nateglinide in the matrix of the microspheres.[18] The developed formulation is also characterized for particle size, surface morphology, drug loading, and release characteristics. Moreover, in vitro pharmacodynamics investigations are performed to determine the antidiabetic efficacy of the microspheres. This work is novel in the interdisciplinary approach that marries cutting-edge formulation design, analytical method development, and therapeutic assessment. Besides adding to the scientific knowledge of controlled-release systems for short-acting drugs such as Nateglinide, this study provides a starting point for future industrial development and clinical translation.

2. METHOD AND MATERIAL

Material

Nateglinide was obtained as a gift sample from Octavius Pharmaceuticals, Gujarat, India, for its proven efficacy in Type II diabetes management and suitability for sustained-release formulations. Microspheres were prepared using PLGA, Chitosan, and Ethylcellulose (Sigma-Aldrich) as biodegradable polymers. Polyvinyl alcohol served as a stabilizer, while dichloromethane (DCM, Sigma-Aldrich) acted as the organic solvent. Ethanol (Fine Chemicals) was used for washing and purification. Calcium chloride and phosphate-buffered saline for cross-linking and drug release studies were obtained from Sisco Research Laboratories (SRL), and distilled water was used in all aqueous preparations.

Preparation Method

Nateglinide-loaded microspheres were successfully prepared using the solvent evaporation technique, with process parameters optimized to achieve high encapsulation efficiency, sustained drug release, and stable morphology.[19–21] Drug-to-polymer ratios (1:1, 1:2, 1:3, 1:4, and 1:5) were evaluated to determine their effects on particle size, drug entrapment, and release characteristics. The resulting microspheres displayed spherical shapes with smooth surfaces, confirming effective emulsification and polymer solidification. Among all formulations, the optimized batch demonstrated superior encapsulation efficiency, uniform morphology, and a sustained drug release profile over 24 hours. This optimized formulation was prepared using 100 mg of Nateglinide, 400 mg of PLGA, and 1% PVA as a stabilizer, with continuous stirring at 1200 rpm for 5 hours and brief sonication (Table 1).

Table 1. Composition of Nateglinide-loaded microsphere formulations prepared with varying drug-to-polymer ratios.

Batch Code	Drug: Polymer Ratio	Nateglinide (mg)	PLGA (mg)	DCM (mL)	PVA Concentration (% w/v)	Aqueous Phase Volume (mL)	Stirring Speed (rpm)	Stirring Time (h)
F1	01:01	100	100	5	1%	100	1200	5
F2	01:02	100	200	5	1%	100	1200	5
F3	01:03	100	300	5	1%	100	1200	5
F4	01:04	100	400	5	1%	100	1200	5
F5	01:05	100	500	5	1%	100	1200	5

Microspheres Evalution parameter

Yield of Microspheres

The percentage yield of the formulated Nateglinide microspheres was evaluated to assess the efficiency of the preparation process. The yield represents the ratio of the actual weight of microspheres obtained to the theoretical total weight of the drug and polymer used in the formulation. .[22] The percentage yield (% yield) was calculated using the following equation:

$$Percentage\ Yield\ (\%) = \frac{Practical\ Yield\ of\ Microspheres\ (g)}{Theoretical\ Yield\ of\ Drug\ +\ Polymer\ (g)} \times 100$$

Flow Property of Nateglinide Microspheres

The flow properties of Nateglinide-loaded PLGA microspheres were evaluated using the angle of repose, Carr's Compressibility Index, and Hausner's Ratio, which are key indicators of powder flowability during formulation. A precisely weighed number of microspheres was transferred into a 10 mL graduated cylinder to measure the bulk volume before tapping. The cylinder was then placed on a tapped density tester and tapped 1000 times to obtain the tapped volume. From these values, bulk density and tapped density were calculated, followed by determination of Carr's Index and Hausner's Ratio using standard equations. The angle of repose was measured by allowing microspheres to flow through a funnel fixed at a specific height, forming a conical pile on a flat surface. The height (h) and radius (r) of the pile were recorded, and the angle of repose (θ) was calculated using:

$$\theta = \left(\frac{h}{r}\right)$$

$$\boldsymbol{Hausner's\ Ratio} = \frac{\boldsymbol{tapped\ density}}{\boldsymbol{Bulk\ density}}$$

$$\%Carr's\ index = \frac{Tapped\ density\ -\ Bulk\ density}{Tapped\ density}$$

Morphological Studies

The surface morphology and internal structure of Nateglinide-loaded microspheres were analyzed using scanning electron microscopy (SEM). Samples were gold-coated using a sputter coater prior to imaging. SEM observations were performed at various magnifications under an accelerating voltage of 17 kV to assess surface smoothness and structural integrity.

Particle Size Analysis

The mean particle size and size distribution were determined using a laser diffraction particle size analyzer. Microspheres were dispersed in distilled water, sonicated to ensure uniform dispersion, and analyzed using a Zetasizer (Model: Zetasizer Pro, Malvern Panalytical). Results were reported as mean particle diameter (d_0) and polydispersity index (PDI).

Zeta Potential Analysis

Surface charge measurements were performed with a Zeta Potential Analyzer (Model: Zetasizer Pro, Malvern Panalytical) using electrophoretic light scattering (ELS). Samples were suspended in distilled water, and zeta potential values were recorded. Absolute values above ±30 mV were considered indicative of good colloidal stability.

Encapsulation Efficiency and Drug Content

Encapsulation efficiency (EE%) and drug content (DC%) were determined by dissolving a known weight of microspheres in a suitable solvent and analyzing drug concentration via UV-visible spectrophotometry. The calculations were performed using the following equations:

$$EE\% = \frac{Actua\ \ l\ drug\ content}{Theoretical\ drug\ content} \times 100$$

$$DC\% = microspheres \times 100$$

Fourier Transform Infrared Spectroscopy

FTIR analysis was conducted to evaluate possible drug–polymer interactions and confirm compatibility. Spectra were recorded using an ALPHA II Compact FT-IR Spectrometer (BRUKER) in ATR mode with a diamond crystal. Samples of pure drug, polymer, and drug-loaded microspheres were scanned in the range of 4000–400 cm^{-1}. Any peak shifts, disappearance, or emergence of new peaks were examined to assess potential chemical interactions.

Differential Scanning Calorimetry (DSC)
DSC analysis was performed to investigate the thermal stability of microspheres. Measurements were carried out using a DSC 2500 (TA Instruments) by heating samples from 30 °C to 300 °C at a rate of 10 °C/min under a nitrogen atmosphere.

In Vitro Drug Release Studies
Drug release studies were conducted to evaluate the sustained release behaviour of Nateglinide from microspheres under physiological conditions. Microspheres containing 10 mg of drug were dispersed in 900 mL of PBS (pH 7.4) containing 0.1% Tween 80 and subjected to dissolution testing in a USP Apparatus II (LABINDIA DS 14000) at 37 ± 0.5 °C with a stirring speed of 100 rpm. Samples were withdrawn at predetermined intervals up to 24 hours, filtered (0.45 µm), and replaced with fresh medium. Drug concentration was determined using UV–Visible spectrophotometry at 216 nm based on a standard calibration curve. Cumulative drug release was calculated as:

Cumulative Drug Release (%)=Total Drug ContentAmount of Drug Released at Time t / total dug content ×100

Stability Studies
Stability testing of Nateglinide microspheres was performed in accordance with ICH guidelines to ensure long-term integrity. Samples were stored in sealed vials under accelerated (40 °C ± 2 °C/75 % ± 5 % RH), long-term (25 °C ± 2 °C/60 % ± 5 % RH), and refrigerated (4 °C ± 2 °C) conditions for up to 12 months. At specified intervals, drug content, morphology, and release profile were evaluated. Drug quantification was carried out using a UV–Visible Spectrophotometer (Evolution One, Thermo Fisher Scientific), and drug content (%) was calculated using:

$$Drug\ Content\ (\%) = \frac{Amount\ of\ drug\ retained\ in\ microspheres}{Initial\ drug\ content} \times 100$$

3. RESULTS AND DICUSSION

Yield of Microspheres
As shown in Table 2, the percentage yield of Nateglinide microspheres increased from 70.6% (1:1) to 89.5% (1:5), indicating improved matrix formation with higher polymer content.

Table 2. Percentage yield of Nateglinide-loaded microsphere formulations prepared with different drug-to-polymer ratios.

Batch Code	Drug: Polymer Ratio	Drug (g)	Polymer (g)	Theoretical Yield (g)	Practical Yield (Nateglinide) (g)	% Yield (Nateglinide)
F1	**01:01**	1	1	2	1.41	70.60%
F2	**01:02**	1	2	3	2.13	71.00%
F3	**01:03**	1	3	4	2.92	73.00%
F4	**01:04**	1	4	5	4.33	86.50%
F5	**01:05**	1	5	6	5.37	89.50%

Flow Property of Powder
The Carr's Index for Nateglinide microspheres ranged between 12.96% and 15.56%, indicating a slightly lower flowability. Hausner's Ratio values varied from 1.15 to 1.18, again falling within the moderate flowability category (Table 3). Higher polymer ratios (especially 1:4 and 1:5) showed increased cohesiveness, possibly due to enhanced particle aggregation and larger size. The flow properties of Nateglinide microspheres were assessed using the fixed funnel method. The angle of repose was determined by measuring the height and base radius of the conical pile formed by free-flowing microspheres. This parameter indicates the ease with which the microspheres can flow, where a lower angle suggests better flow. For the 1:1 drug-to-polymer ratio, the angle of repose was 37.8°, and it decreased to 30.1° at the 1:5 ratio, indicating improved flowability with increasing polymer content. Nateglinide microspheres demonstrated a gradual decrease in the angle of repose from 37.8° at the 1:1 ratio to 30.1° at the 1:5 ratio. This result suggests that higher polymer content contributed to the formation of microspheres with improved surface characteristics, reducing interparticle friction and promoting better flow. The 1:4 drug-to-polymer batch, with an angle of repose of 31.8°.

Table 3. Flow property measurements of Nateglinide-loaded microsphere formulations, including Carr's Index, Hausner's Ratio, and Angle of Repose, to evaluate powder flowability.

Batch Code	Polymer Ratio	[a]Carr's Index (Nateglinide) (%)	[B] Hauser's Ratio (Nateglinide)	[c]Angle of Repose (Nateglinide) (°)	Tapped Density (Nateglinide) (g/cm³)	Bulk Density (Nateglinide) (g/cm³)
F1	01:01	13.79	1.16	37.8°	0.58	0.5
F2	01:02	12.96	1.15	35.9°	0.54	0.47
F3	01:03	13.73	1.16	33.9°	0.51	0.44
F4	01:04	14.58	1.17	31.8°	0.48	0.41
F5	01:05	15.56	1.18	30.1°	0.45	0.38

[a] Carr's Index: 5–12%, Excellent; 12–18%, good; 18–21%, fair; 21–25%, poor, fuid; 25–32%, poor, cohesive; 32–38%, very poor;>40%, extremely poor

[b] Hausner ratio: 1.00–1.11, Excellent; 1.12–1.18, good; 1.19–1.25, fair; 1.26–1.34, passable; 1.35–1.45, poor; 1.46–159, very poor;>1.60, veryvery poor

[c]Angle of Repose: 25–30, Excellent; 31–35, good; 36–40, fair; 41–45, passable; 46–55, poor; 56–65, very poor;>66, very-very poor

Optimization of the Batch

Flow property analysis of Nateglinide microspheres showed improved flowability with increasing polymer concentration. The 1:4 batch exhibited balanced characteristics, with a Carr's Index of 14.58%, a Hausner's Ratio of 1.17, and an angle of repose of 31.8°, indicating good flow with manageable cohesiveness, making it the optimized batch for further evaluation.

Morphological Studies

Scanning electron microscopy was used to examine the surface morphology of the enhanced Nateglinide microsphere formulation; the results are shown in Figure 1. The microspheres were spherical and uniform in shape, with a surface that was moderately porous.. The pores observed were small and well distributed across the surface, indicating controlled solvent evaporation during microsphere formation. This fine porosity suggests the formation of a dense and coherent polymeric matrix, which is essential for sustaining drug release and minimizing the burst effect. Unlike lower polymer ratio batches, which may result in larger pores and irregular surfaces, the optimized formulation exhibited reduced surface defects and smooth contours. Such morphology is advantageous for maintaining physical stability and achieving consistent drug release kinetics. The moderate porosity also facilitates the gradual diffusion of the drug, aligning well with the intended sustained release profile of the formulation.

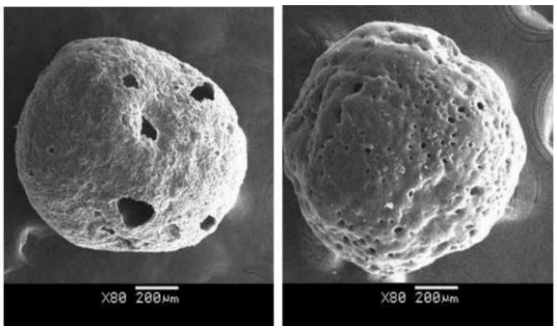

Figure 1. Scanning Electron Microscopy (SEM) images of Nateglinide-loaded microspheres captured at 80× magnification with a scale bar of 200 µm, showing spherical morphology and smooth surface characteristics.

Particle Size Analysis

Particle size analysis of Nateglinide-loaded microspheres was performed using laser diffraction after dispersion and sonication. Mean particle size increased with polymer concentration, from 115.3 µm (1:1) to 175.9 µm (1:5). All batches exhibited PDI < 0.3, indicating uniform size distribution. The 1:4 batch showed an optimal size of 160.7 µm with a PDI of 0.26, ensuring controlled drug release and high encapsulation efficiency. In contrast, smaller particles in the 1:1 and 1:2 batches could cause faster drug release, while the 1:5 batch displayed slightly broader distribution (PDI 0.29). Based on these findings, the 1:4 batch was selected for further studies. Particle size and PDI values for Nateglinide-loaded microsphere formulations prepared at different drug-to-polymer ratios. The optimized 1:4 batch exhibited a mean size of 160.7 µm with a PDI of 0.26, indicating uniformity and suitability for sustained release. (Table 4)

Table 4: Particle size of Nateglinide microspheres

Batch Code	Polymer Ratio	Mean Particle Size (Nateglinide) (µm)	PDI (Nateglinide)	Zeta Potential (Nateglinide) (mV)
F1	**01:01**	115.3	0.19	-8.97
F2	**01:02**	128.6	0.22	-13.8
F3	**01:03**	145.1	0.24	-17.4
F4	**01:04**	160.7	0.26	-23.7
F5	**01:05**	175.9	0.29	-25.2

Zeta Potential Analysis

Zeta potential analysis of Nateglinide microspheres was performed using the same technique, with the samples dispersed in distilled water and analyzed by ELS. The data showed a trend of increasingly negative surface charges with higher polymer concentrations, indicating improved stability of the colloidal system. Nateglinide microspheres at lower polymer ratios (1:1 and 1:2) displayed zeta potentials of −8.97 mV and −13.8 mV, indicating low electrostatic repulsion and a higher risk of aggregation. The 1:3 batch showed improved stability (- −17.4 mV). The 1:4 batch, with a zeta potential of −23.7 mV, achieved effective particle repulsion and minimized aggregation. The 1:5 batch had a slightly more negative value (- −25.2 mV) but may pose challenges in terms of formulation viscosity and processability. Thus, the 1:4 drug-to-polymer ratio was finalized as the optimal batch for Nateglinide microspheres, providing a reliable balance between stability and ease of formulation (Figure 2).

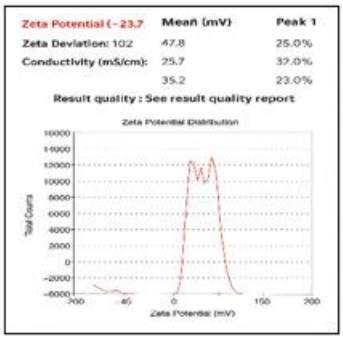

Figure 2. Zeta potential values of Nateglinide-loaded microsphere formulations, indicating surface charge characteristics and dispersion stability.

Loading Efficiency

The encapsulation efficiency and medication content of Nateglinide microspheres were determined similarly.[23] UV spectrophotometry was used to examine a 10 mg sample of each batch using the calibration equation y=0.0597x−0.0022. Increased polymer concentrations showed a rising trend in EE%, but increased polymer dilution caused the drug content (%) to progressively decrease (Table 5). The 1:4 formulation demonstrated excellent encapsulation efficiency (96.3%) while maintaining a drug content of 19.26%, making it the ideal batch for further studies. Although the 1:5 batch had slightly higher EE%, its drug load was lower (16.32%), which may not be sufficient for sustained therapeutic levels. The 1:1 batch had higher drug content but lower EE%, indicating possible drug leakage during formulation. As a result, the 1:4 batch was selected as the optimal formulation for Nateglinide microspheres.

Table 5. Encapsulation efficiency (EE%) and drug content (DC%) of Nateglinide-loaded microsphere formulations, highlighting the influence of varying drug-to-polymer ratios on entrapment and loading characteristics.

Batch Code	Polymer Ratio	Theoretical EE (%)	Theoretical Drug Content for both Formulations (10 mg formulation)	Nateglinide -		
				Actual Drug Content (mg) for 10 mg formulation	EE (%)	Drug Content (%)
F1	**01:01**	50	5	4.39	87.8	43.9
F2	**01:02**	33.33	3.333	2.9797	89.4	29.797
F3	**01:03**	25	2.5	2.2875	91.5	22.875

F4	**01:04**	20	2	1.926	96.3	19.26
F5	**01:05**	16.67	1.667	1.6319	97.9	16.3199

DSC

The DSC thermogram of Nateglinide microsphere formulation (Figure 03) shows distinct thermal transitions, confirming physical state and stability. An endothermic deviation near 50°C corresponds to PLGA's glass transition, indicating an amorphous matrix ideal for sustained release. A second Tg at ~85°C reflects PVA presence, confirming phase separation and thermal compatibility. A broad endothermic peak at 125°C indicates amorphization of Nateglinide, enhancing solubility and bioavailability. A minor peak at 139°C suggests minimal residual crystallinity. Decomposition around 300°C reflects thermal breakdown of excipients and drug, confirming the formulation's stability up to 270–280°C, adequate for pharmaceutical processing.

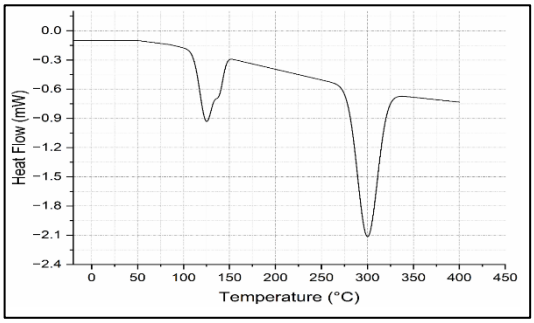

Figure 03: DSC Thermogram of Nateglinide microspheres formulation

FTIR

The FTIR spectrum of the Nateglinide microsphere formulation (Figure 4 & Table 6) confirms the successful encapsulation of the drug within the polymer matrix. Key peaks at 2922–2852 cm^{-1} (C–H stretching), 1735 cm^{-1} (ester C=O), and 1668 cm^{-1} (amide C=O) indicate retention of drug and polymer functional groups. Bands at 1565 cm^{-1}, 1340–1352 cm^{-1}, and 1174 cm^{-1} confirm C–N stretching, CH bending, and C–O vibrations, supporting drug–polymer interactions. Peaks at 723 and 503 cm^{-1} reflect the microsphere matrix. No new peaks or disappearance of key bands suggest physical encapsulation without chemical degradation. Slight shifts indicate weak hydrogen bonding, contributing to stable drug loading and sustained release.

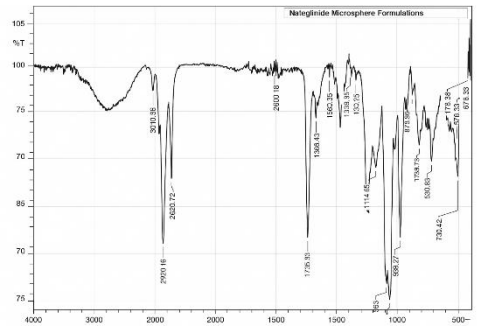

Figure 4: FTIR spectra of Nateglinide microspheres formulation

Table 6: FTIR Spectral Interpretation of Nateglinide Microspheres Formulation

Wavenumber (cm^{-1})	**Functional Group / Vibration**	**Assignment**
2922.16, 2852.72	Aliphatic C–H Stretching	Presence of polymer backbone (CH_2 and CH_3 groups)
3010.88	Aromatic/Amide C–H Stretching	Indicates the presence of the Nateglinide aromatic structure
2000.18	Combination band/overtone region	Minor overtone, no major assignment
1735.93	Ester Carbonyl (C=O) Stretching	Polymer ester bond (possible PLGA or excipient polymer)
1688.43	Amide Carbonyl (C=O) Stretching	Nateglinide drug amide group (drug integrity retained)
1565.55	N–H bending / C–N stretching	Amide II band from drug structure
1352.53, 1340.53	CH bending / C–N stretching	Amide functionalities from Nateglinide

1174.65	C–O stretching (Ether or Ester)	Polymer excipient or ester bond vibration
968.27	C–O / C–H bending	Polymer backbone/drug moiety fingerprint
878.85	C–H out-of-plane bending (Aromatic)	Confirms aromatic rings in Nateglinide
821.86, 723.31	CH_2 rocking / out-of-plane bending	Polymer backbone
503.42	Out-of-plane deformation	Polymer backbone and microsphere framework
416.62, 408.91	Low-frequency skeletal vibrations	Polymer or excipient lattice vibrations

Controlled Release Assessment

The in vitro drug release profile of Nateglinide microspheres was evaluated over 24 hours across various drug-to-polymer ratios and expressed as Mean ± SD (Table 9, Figure 5). The 1:4 batch consistently demonstrated superior drug release, beginning with 14.63% at 0.5 h and progressing to 25.43%, 37.53%, 66.33%, 86.27%, and 97.00% at 1, 2, 6, 12, and 24 hours, respectively. This outperformed other batches, especially the 1:1 (75.3%) and 1:5 (73.87%), indicating the 1:4 ratio achieved an ideal balance of drug diffusion, polymer erosion, and matrix stability. The sustained release and high cumulative percentage support the 1:4 formulation as the most optimized for extended release. Drug release kinetics were analyzed using Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models (Table 8). The First-order model showed the highest correlation ($R^2 = 0.996$ for the 1:4 batch), indicating concentration-dependent release. The Higuchi model ($R^2 = 0.936$) confirmed a diffusion-driven mechanism, while the Korsmeyer–Peppas analysis ($R^2 = 0.954$, $n = 0.393$) supported Fickian diffusion. Zero-order kinetics showed lower correlation ($R^2 = 0.769$), confirming that release was not uniform. These findings confirm that the 1:4 batch provides optimal sustained release characteristics with consistent, diffusion-controlled kinetics, making it a promising candidate for further in vivo or pharmacological studies.

Table 8. Kinetic modeling of drug release from Nateglinide-loaded microsphere formulations, comparing zero-order, first-order, Higuchi, and Korsmeyer–Peppas models.

Formulation	Zero Order	First Order	Higuchi	Korsmeyer-Peppas	N value
F1	0.761	0.973	0.927	0.948	0.419
F2	0.775	0.989	0.936	0.956	0.416
F3	0.771	0.997	0.934	0.956	0.407
F4	0.757	0.996	0.926	0.953	0.393
F5	0.772	0.97	0.933	0.949	0.433

Table 9. Cumulative percentage drug release of Nateglinide-loaded microsphere formulations under in vitro conditions, evaluated over 24 hours.

Time (h)	01:01 (Mean ± SD)	01:02 (Mean ± SD)	01:03 (Mean ± SD)	01:04 (Mean ± SD)	01:05 (Mean ± SD)
0.5	9.2 ± 0.24	10.57 ± 0.29	12.5 ± 0.16	14.63 ± 0.12	8.23 ± 0.12
1	17.57 ± 0.25	19.43 ± 0.21	21.77 ± 0.21	25.43 ± 0.17	16.3 ± 0.16
2	27.23 ± 0.29	30.27 ± 0.12	33.43 ± 0.29	37.53 ± 0.25	25.57 ± 0.17
4	39.3 ± 0.24	43.43 ± 0.21	47.5 ± 0.24	53.6 ± 0.24	36.9 ± 0.16
6	50.23 ± 0.21	54.87 ± 0.21	59.53 ± 0.25	66.33 ± 0.29	48.3 ± 0.24
8	59.4 ± 0.24	63.77 ± 0.21	69.1 ± 0.29	77.07 ± 0.21	57.27 ± 0.17
12	67.1 ± 0.24	71.67 ± 0.21	78.33 ± 0.21	86.27 ± 0.21	65.47 ± 0.21
24	75.3 ± 0.24	82.73 ± 0.21	89.03 ± 0.25	97.00 ± 0.16	73.87 ± 0.21

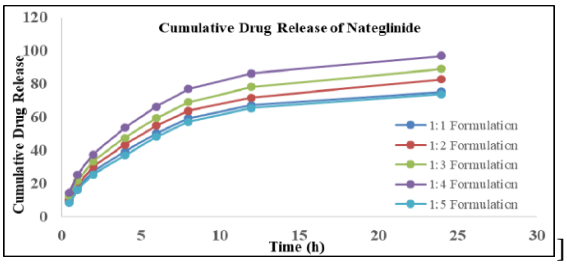

Figure 5. Cumulative percentage drug release profiles of Nateglinide-loaded microsphere formulations under in vitro conditions over 24 hours.

Stability Studies

The stability of Nateglinide microspheres was similarly evaluated under accelerated and refrigerated conditions for 6 months. Drug content was assessed at regular intervals using validated analytical methods. Under accelerated storage, Nateglinide exhibited a gradual decline in drug content from 97.0% to 92.1%, indicating a 4.9% reduction (Table 10). This suggests some degree of degradation, although the values remained within ICH-accepted limits for chemical stability. Under refrigerated conditions, drug content remained stable. A minor decline from 97.0% to 96.5% was observed, confirming the high chemical stability of the material at lower temperatures. Nateglinide microspheres demonstrated acceptable chemical stability under both conditions. The refrigerated samples retained over 96% of the original drug content after six months, confirming suitability for extended storage. While accelerated conditions led to minor drug loss, the formulation remained within stability criteria.

Table 10. Drug content analysis of Nateglinide-loaded microsphere formulations during stability studies under different storage conditions, as per ICH guidelines.

Time (Months)	Nateglinide	
	Accelerated (%)	Refrigerated (%)
0	97	97
1	95.8	96.9
3	94.2	96.7
6	92.1	96.5

4. CONCLUSION

This study successfully developed and optimized a sustained-release microsphere formulation of Nateglinide using PLGA and PVA via the solvent evaporation technique. Among the five formulations, the 1:4 drug-to-polymer ratio (F4) demonstrated optimal physicochemical properties, flowability, encapsulation efficiency, and controlled drug release. The optimized formulation exhibited high encapsulation yield (96.3%) and drug loading (19.26%), with stable particle size (160.7 µm) and low polydispersity index (0.26), ensuring uniform release. Flow properties (Carr's Index 14.58%, Hausner's Ratio 1.17, and angle of repose 31.8°) confirmed good handling characteristics for industrial processing. Zeta potential (−23.7 mV) indicated good colloidal stability, while in vitro studies showed a cumulative drug release of 97% over 24 hours, following first-order ($R^2 = 0.996$) and Higuchi ($R^2 = 0.936$) kinetics. Stability studies confirmed >96% drug retention under refrigerated conditions for six months and 92% under accelerated conditions, in compliance with ICH guidelines.This research provides a systematic approach for developing a pharmaceutically robust, scalable, and stable sustained-release system for Nateglinide, overcoming limitations of its short half-life and frequent dosing requirements. Analytical validations further support formulation integrity and performance.

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6. CONFLİCT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

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