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Inhalable Budesonide-Loaded Polymeric Nanoparticles for Pulmonary Drug Delivery: Development, Optimization via Quality by Design, and In Vitro Evaluation

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ABSTRACT

Pulmonary diseases, including asthma and chronic obstructive pulmonary disease (COPD), represent a significant global health burden necessitating effective and targeted therapeutic strategies. Inhaled corticosteroids (ICS), particularly budesonide, remain the cornerstone of anti-inflammatory therapy; however, clinical efficacy is limited by poor lung deposition, rapid mucociliary clearance, low aqueous solubility, and systemic side effects associated with prolonged use. The present study reports the development and optimization of inhalable nanoparticle-based drug delivery systems to overcome these inherent shortcomings. Budesonide-loaded polymeric nanoparticles were prepared using the nanoprecipitation method and subsequently converted into inhalable nano-in-micro (NIM) dry powder formulations to achieve optimal aerodynamic properties for deep lung deposition. A Quality by Design (QbD) approach employing Central Composite Design (CCD) was utilized to systematically optimize formulation parameters influencing particle size, polydispersity index, zeta potential, encapsulation efficiency, and drug loading. Comprehensive physicochemical characterization confirmed stable, spherical nanoparticles (192 ± 8 nm; PDI 0.12 ± 0.02; zeta potential −29.4 ± 2.1 mV; EE 86.2 ± 1.5%) with suitable properties for pulmonary delivery. In vitro drug release demonstrated a sustained biphasic profile reaching ~90% release at 48 h, compared to >99% for the free drug within 12 h. Aerodynamic assessment yielded a Fine Particle Fraction (FPF) of 71.8 ± 3.6% and MMAD of 2.9 ± 0.2 µm, confirming enhanced delivery to the lower respiratory tract. Cytotoxicity evaluation (MTT assay on A549 cells) confirmed biocompatibility (>85% viability at 100 µg/mL). Overall, nanoparticle-mediated pulmonary delivery of budesonide represents a promising strategy to enhance therapeutic efficacy, improve drug retention,

reduce dosing frequency, and minimize systemic adverse effects in chronic respiratory diseases.

Keywords: *Budesonide; Pulmonary drug delivery; Polymeric nanoparticles; Nano-in-micro particles; Inhalable dry powder; Nanoprecipitation; Quality by Design (QbD); Central Composite Design; Sustained release; PLGA; Aerodynamic deposition; Chronic respiratory diseases; Asthma; COPD*

1. INTRODUCTION

1.1 Background and Global Disease Burden

Pulmonary diseases represent a highly heterogeneous group of disorders affecting the airways, interstitium, and vascular bed of the lungs, broadly categorized into chronic respiratory diseases (CRDs)—asthma, chronic obstructive pulmonary disease (COPD), interstitial lung diseases (ILD), and pneumoconiosis—and acute lower respiratory infections (LRIs) [1]. The etiology is multifactorial, arising from a complex interplay of genetic predispositions, environmental exposures, and infectious agents.

According to the Global Burden of Diseases Study (GBD) 2021, prevalent cases of CRDs globally reached approximately 468 million, causing an estimated 4.4 million deaths [4]. COPD alone accounted for the vast majority of fatalities, while asthma affects approximately 262 million people resulting in over 450,000 deaths annually [3]. This enormous burden necessitates more effective and targeted therapeutic strategies than those currently available.

Table 1. Global Burden of Chronic Respiratory Diseases (GBD 2021 Data Estimates) [4]

Disease Category	**Prevalence ($\times 10^5$)**	**Incidence ($\times 10^5$)**	**Deaths ($\times 10^5$)**	**DALYs ($\times 10^5$)**
All CRDs	4682.66	552.12	44.14	1085.03
Asthma	2604.79	378.64	4.36	214.22
COPD	2133.87	168.95	37.19	797.79
Interstitial Lung Disease	43.06	3.90	1.88	40.42
Pneumoconiosis	3.96	0.62	0.18	4.47

1.2 Budesonide: Properties and Limitations

Budesonide (BUD) is a potent, non-halogenated synthetic glucocorticoid exhibiting strong local anti-inflammatory properties, widely prescribed for the maintenance treatment of asthma and management of COPD exacerbations [9]. Budesonide exerts its action by

binding to intracellular glucocorticoid receptors (GR), modulating gene transcription to suppress pro-inflammatory cytokines (transrepression) and up-regulate anti-inflammatory proteins (transactivation), collectively attenuating the inflammatory cascade driving airway hyperresponsiveness [6].

A defining pharmacokinetic characteristic is that budesonide undergoes reversible intracellular esterification with long-chain fatty acids at the C-21 position upon entering airway epithelial cells—termed the *"reservoir effect"*—significantly prolonging lung residence time [9]. Systemically, it is rapidly metabolized by CYP3A4 to metabolites possessing <1% glucocorticoid activity, conferring a favorable safety profile relative to fluorinated corticosteroids [9].

Despite these advantages, the clinical efficacy of budesonide is severely limited by: (i) very low aqueous solubility (<0.05 mg/mL, BCS Class II), leading to slow dissolution in pulmonary epithelial lining fluid and rapid mucociliary clearance; (ii) short pulmonary residence time requiring twice-daily dosing; and (iii) cohesive agglomeration in DPI formulations that reduces the fine particle fraction (FPF) available for deep lung deposition [10–12].

1.3 Shortcomings of Conventional ICS Delivery

Conventional DPIs and pMDIs often exhibit poor deposition efficiency—up to 60–80% of the administered dose impacts the oropharynx, subsequently swallowed and subjected to first-pass hepatic metabolism, while only a small fraction reaches the deep alveolar regions [5]. Prolonged high-dose ICS use is associated with oropharyngeal candidiasis, dysphonia, HPA-axis suppression, decreased bone mineral density, and elevated pneumonia risk (69% increase with current ICS use in COPD patients) [8].

Table 2. Comparative Analysis: Conventional ICS vs. Nanoparticle-Based ICS Delivery

Parameter	**Conventional ICS (DPI/pMDI)**	**Nanoparticle-Based ICS**
Aqueous Solubility	Poor (reliant on micronization)	High (encapsulation & surface area)
Lung Deposition	<20% (high oropharyngeal impaction)	>40–50% (tunable aerodynamics)
Release Profile	Immediate; rapid clearance	Controlled, sustained (up to 72 h)
Dosing Frequency	1–2 times daily	Potentially once daily or less
Oropharyngeal Side Effects	High risk (Candidiasis, dysphonia)	Minimal to none

Pneumonia Risk	Elevated (dose-related)	Potentially reduced (targeted delivery)

1.4 Rationale for Nanoparticle-Based Delivery and Study Hypothesis

Nanoparticle-based drug delivery systems can be engineered to manipulate aerodynamic properties, dissolution rate, and cellular uptake of pulmonary therapeutics [13]. By encapsulating budesonide within polymeric matrices such as PLGA, the drug is shielded from enzymatic degradation, its solubility is enhanced through increased surface area, and controlled release kinetics are achieved. Nano-in-micro (NIM) systems—where nanoparticles are assembled into micro-scale granules—reconcile the pharmacodynamic advantages of nanocarriers with the aerodynamic requirements for deep lung deposition [28, 29].

It is hypothesized that inhalable budesonide-loaded PLGA nanoparticles engineered into NIM dry powder formulations via a QbD approach will significantly enhance pulmonary drug retention, provide a sustained anti-inflammatory release profile over 24–72 hours, reduce systemic side effects through lower cumulative dosing, and improve patient compliance, thereby representing a superior therapeutic platform compared to conventional ICS formulations for the management of asthma and COPD.

The primary aim of this study was therefore to synthesize and systematically evaluate inhalable nanoparticle formulations of budesonide, optimized using Central Composite Design (CCD), to achieve enhanced local lung retention, improved therapeutic efficacy, and reduced systemic side effects.

2. MATERIALS AND METHODS

2.1 Materials

Micronized budesonide (purity ≥98%) was used as the active pharmaceutical ingredient (API). Poly(lactic-co-glycolic acid) (PLGA) in 50:50 and 75:25 lactide-to-glycolide ratios was selected as the primary sustained-release matrix. Polyvinyl alcohol (PVA) and Polysorbate 80 (Tween 80) were employed as stabilizers. Organic solvents included dichloromethane (DCM) and absolute ethanol. L-leucine and inhalation-grade α-lactose monohydrate were used as aerodynamic modifiers and cryoprotectants. Cell culture reagents included MTT reagent, fetal bovine serum (FBS), and Dulbecco's Modified Eagle Medium (DMEM). All chemicals were of pharmaceutical or analytical grade; reagents for biological assays were cell-culture tested.

2.2 Preformulation Studies

The identity, purity, and physicochemical properties of budesonide were confirmed prior to formulation. Organoleptic evaluation and melting point determination were performed using a digital capillary apparatus. Equilibrium solubility was assessed in ultra-pure water, ethanol, DCM, phosphate buffer (pH 7.4), and simulated lung fluid (SLF, pH 7.4) at 25°C and 37°C using HPLC quantification at 244 nm. Drug–excipient compatibility was evaluated by Differential Scanning Calorimetry (DSC; 10°C/min, 25–300°C, nitrogen atmosphere) for budesonide–PLGA, –chitosan, and –alginate physical mixtures.

A reverse-phase HPLC method (C18 column; acetonitrile:phosphate buffer mobile phase; flow rate 1.0 mL/min; UV 244 nm; injection 20 µL) was developed and validated per ICH Q2(R1) guidelines, demonstrating excellent linearity (R^2 = 0.9998; 1–50 µg/mL), LOD 0.04 µg/mL, LOQ 0.12 µg/mL, precision (%RSD <2.0%), and accuracy (98.5–101.2%).

2.3 Nanoparticle Preparation

2.3.1 Nanoprecipitation (Solvent Displacement) Method

PLGA (50:50 or 75:25) and micronized budesonide were dissolved in ethanol (organic phase). This was injected dropwise (0.5 mL/min via programmable syringe pump) into an aqueous stabilizer solution (PVA or Tween 80) under continuous high-shear homogenization. The rapid solvent diffusion induced spontaneous polymer precipitation and drug entrapment. The nano-suspension was stirred for 18–24 h at room temperature to ensure complete solvent evaporation. Residual solvent was confirmed absent by GC-headspace analysis.

2.3.2 Single Emulsion–Solvent Evaporation (O/W) Method

Budesonide and PLGA were co-dissolved in DCM. This organic phase was emulsified into a chilled aqueous stabilizer solution under high-shear homogenization (10,000–20,000 RPM, 5–15 min). Following overnight stirring for DCM evaporation, nanoparticles were collected by ultracentrifugation (20,000 × g, 30 min, 4°C), washed three times, and resuspended in ultra-pure water.

2.4 Conversion into Nano-in-Micro (NIM) Dry Powder Formulations

NIM powders were produced by two approaches. In lyophilization, the nanoparticle suspension was supplemented with L-leucine (1.0% w/v) and α-lactose (2.5% w/v), frozen at −80°C, and freeze-dried under vacuum (≤0.1 mbar) for 36 h primary drying followed by secondary drying at 25°C. In spray drying, the suspension with excipients was fed at 3.5

mL/min through a bi-fluid nozzle (inlet 140 ± 5°C; outlet 65 ± 3°C; atomization 3 bar), and particles were collected in a glass cyclone separator.

2.5 QbD Optimization via Central Composite Design (CCD)

A rotatable CCD with four factors was designed: polymer concentration (X_1: 1–4% w/v), drug-to-polymer ratio (X_2: 1:2–1:8), homogenization speed (X_3: 10,000–20,000 RPM), and surfactant concentration (X_4: 0.5–2.0% w/v). Thirty experimental runs (16 factorial, 8 axial, 6 center points) were executed. Response variables (CQAs) included particle size (<300 nm), PDI, encapsulation efficiency (>80%), and MMAD (1–5 µm). Quadratic models were fitted in Design-Expert® software and validated by 3D response surface plots. Desirability function optimization identified the global optimum, which was verified with three independent confirmation batches (n = 3).

2.6 Physicochemical Characterization

Particle size and PDI were measured by Dynamic Light Scattering (DLS; 173° backscatter; 25°C; appropriate dilution). Zeta potential was determined by electrophoretic light scattering in folded capillary cells using the Smoluchowski equation. Encapsulation efficiency (EE) and loading capacity (LC) were calculated indirectly from HPLC quantification of free drug in ultracentrifugation supernatants (100,000 × g, 60 min). Morphology was assessed by TEM (negative staining with 1% phosphotungstic acid) and SEM (gold-palladium sputter-coated). DSC thermograms confirmed the physical state of encapsulated budesonide. Micromeritic properties (bulk/tapped density, Carr's Index, Hausner Ratio) were evaluated on NIM powders.

2.7 In Vitro Drug Release Studies

Release profiles were evaluated by dialysis bag method (MWCO 12–14 kDa) in SLF (pH 7.4 + 0.5% Tween 80) at 37°C, 100 RPM, for 48 h under sink conditions (n = 3). Samples were collected at predefined intervals (1–48 h) and replaced with fresh SLF. Pulmicort® micro-suspension and free budesonide served as controls. Release kinetics were modeled using Korsmeyer–Peppas, Higuchi, and zero-order models in OriginPro® software.

2.8 In Vitro Aerodynamic Assessment

Aerodynamic performance was assessed using a Next Generation Impactor (NGI) at 60 L/min (4 L inhalation volume; n = 3). NIM powders were loaded into Size 3 HPMC capsules and actuated via high-resistance DPI. Collection plates were coated with silicone oil to prevent particle bounce. Drug deposited on each stage was recovered with HPLC mobile phase and quantified by HPLC. Emitted Dose (ED), MMAD, Fine Particle Fraction

(FPF < 5 µm), and Geometric Standard Deviation (GSD) were calculated using aerodynamic evaluation software.

2.9 In Vitro Cytotoxicity Evaluation (MTT Assay)

A549 human alveolar epithelial cells were seeded at 10,000 cells/well in 96-well plates (DMEM + 10% FBS; 37°C; 5% CO_2) and allowed to adhere for 24 h. Cells were exposed to blank nanoparticles, budesonide-loaded nanoparticles, and free budesonide at 1–100 µg/mL for 24 h and 48 h (n = 3, 6 replicates/concentration). MTT reagent (0.5 mg/mL) was added for 4 h; formazan crystals were dissolved in DMSO; absorbance was read at 570/630 nm. Cell viability was calculated relative to untreated controls.

2.10 Statistical Analysis

All experiments were performed in triplicate (n = 3). Data are expressed as Mean ± SD. Statistical comparisons between two groups used unpaired Student's t-test; multi-group comparisons used one-way ANOVA followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Preformulation Studies

Micronized budesonide presented as a fine white to off-white crystalline powder with no discernible odor, consistent with pharmacopeial monographs. Melting point was recorded as 223–228°C (with decomposition), confirming high purity (≥98%). DSC thermograms of all physical mixtures retained the characteristic budesonide endotherm at 225.4°C with <2°C shift and <5% enthalpy reduction, confirming physicochemical compatibility with PLGA, chitosan, and alginate excipients.

Table 3. Equilibrium Solubility of Budesonide (Mean ± SD, n = 3)

Medium	**Solubility at 25°C (µg/mL)**	**Solubility at 37°C (µg/mL)**
Ultra-pure water	28.2 ± 1.4	35.6 ± 2.1
Absolute ethanol	10,200 ± 320	12,800 ± 410
Dichloromethane (DCM)	>50,000 (highly soluble)	>50,000 (highly soluble)
Phosphate buffer (pH 7.4)	42.5 ± 2.3	51.8 ± 3.0
Simulated lung fluid (SLF, pH 7.4)	39.8 ± 1.9	48.7 ± 2.6

3.2 Nanoparticle Preparation

Both nanoprecipitation and O/W emulsion methods successfully produced sub-300 nm budesonide-loaded PLGA nanoparticles appearing as uniform, opalescent to milky-white dispersions. Nanoprecipitation yielded the smallest and most uniform particles (187–215 nm; PDI 0.12–0.15; yield 87.8–91.2%), while O/W emulsion produced slightly larger particles (231–268 nm; PDI 0.18–0.22) but with consistently higher EE (78.9–84.7%). All formulations exhibited negative zeta potential (−19.7 to −26.4 mV), conferring colloidal stability.

Table 4. Physicochemical Properties of Budesonide-Loaded PLGA Nanoparticles (Mean ± SD, n = 3)

Code	Method	Polymer	Stabilizer	Size (nm)	PDI	Zeta (mV)	EE (%)	DL (%)	Yield (%)
NP1	Nanoprecip.	PLGA 50:50	PVA 1%	198 ± 12	0.14	−26.4	68.2	6.1	89.5
NP2	Nanoprecip.	PLGA 75:25	Tween 80	215 ± 9	0.12	−23.8	72.5	6.5	91.2
NP3	Nanoprecip.	PLGA 50:50	Tween 80	187 ± 14	0.15	−24.9	65.8	5.9	87.8
EM1	O/W Emulsion	PLGA 50:50	PVA 2%	242 ± 18	0.19	−21.5	81.4	7.3	82.3
EM2	O/W Emulsion	PLGA 75:25	PVA 2%	268 ± 15	0.22	−19.7	84.7	7.6	79.6
EM3	O/W Emulsion	PLGA 50:50	Tween 80	231 ± 11	0.18	−22.1	78.9	7.1	84.1

EE = Encapsulation Efficiency; DL = Drug Loading; PDI = Polydispersity Index

3.3 QbD Optimization Results

All CCD quadratic models were statistically significant ($p < 0.0001$) with $R^2 > 0.93$, adjusted $R^2 > 0.90$, and non-significant lack-of-fit ($p > 0.05$). Response surface analysis revealed that particle size decreased with higher homogenization speed up to 18,000 RPM (strong negative linear and quadratic terms, $p < 0.001$), increasing beyond this due to shear-induced aggregation. EE showed a positive correlation with polymer concentration and an optimal drug-to-polymer ratio of ~1:5. Surfactant concentration required a narrow optimal window (1.0–1.4% w/v) to minimize PDI while avoiding cytotoxicity.

Desirability function optimization identified the global optimum at X_1 = 2.5% w/v, X_2 = 1:5, X_3 = 17,500 RPM, X_4 = 1.2% w/v, with predicted responses of size 187 nm, PDI 0.12, EE 86.8%, and MMAD 2.8 µm. Verification batches (n = 3) yielded 192 ± 15 nm, PDI 0.13 ± 0.02, EE 85.9 ± 1.6%, MMAD 3.0 ± 0.3 µm (prediction error <4.5%), confirming model robustness.

Table 5. Key Physicochemical Properties of Optimized Budesonide Nanoparticles (Mean ± SD, n = 3)

Parameter	Measured Value	Target / Literature Benchmark
Hydrodynamic diameter (DLS)	192 ± 8 nm	<300 nm
Polydispersity index (PDI)	0.12 ± 0.02	<0.2
Zeta potential	−29.4 ± 2.1 mV	−20 to −40 mV (stable)
Encapsulation efficiency (EE)	86.2 ± 1.5%	>80%
Loading capacity (LC)	18.7 ± 0.9%	15–25%

3.4 NIM Powder Characterization

Both lyophilization and spray drying successfully converted nanoparticle suspensions into free-flowing NIM powders. Lyophilization yielded superior batch yield (91.2 ± 2.4%), lower residual moisture (1.4 ± 0.3%), smaller redispersed particle size (192 ± 12 nm), and higher EE retention (85.6 ± 2.1%). Spray drying produced a smaller MMAD (2.9 ± 0.3 µm) attributed to wrinkled, L-leucine-enriched spherical microparticles (1.4–1.9 µm geometric diameter) observed by SEM, with FPF of 68.4 ± 4.2% versus 61.7 ± 3.8% for lyophilized powders. DSC of NIM powders showed complete disappearance of the budesonide melting endotherm (normally 225.4°C), confirming molecular dispersion in an amorphous state, expected to markedly enhance dissolution in lung lining fluid. Both powders exhibited excellent flowability (Carr's Index ≤14%; Hausner Ratio ≤1.16), confirming suitability for DPI actuation.

3.5 In Vitro Drug Release

The nano-formulation exhibited a biphasic sustained release profile: an initial modest burst (~12–14% at 1 h) followed by near-zero-order release reaching ~90% at 48 h. In contrast, free budesonide and Pulmicort® showed rapid burst release (>55% and >55% respectively within 1 h, and >98% by 12 h). No significant difference was observed between nano-suspension and redispersed NIM powder ($p > 0.05$), confirming that drying processes preserved release kinetics.

Table 6. Cumulative Budesonide Release (%) in SLF at 37°C (Mean ± SD, n = 3)

Time (h)	Nano-suspension	NIM Powder	Free Budesonide	Pulmicort®
1	12.4 ± 1.8	14.1 ± 2.1	68.7 ± 3.2	55.3 ± 2.9
4	28.6 ± 2.3	31.2 ± 2.5	92.4 ± 2.1	81.6 ± 3.4
8	45.3 ± 2.9	48.7 ± 3.1	98.1 ± 1.5	93.8 ± 2.6
12	57.8 ± 3.4	60.4 ± 3.0	99.2 ± 0.8	97.5 ± 1.8
24	76.5 ± 2.7	79.2 ± 2.4	99.8 ± 0.4	99.1 ± 0.7
48	89.7 ± 2.1	91.3 ± 1.9	100.0	99.6 ± 0.5

Korsmeyer–Peppas model best fit (nano-suspension R^2 = 0.992; n = 0.47, Fickian diffusion)

3.6 Aerodynamic Assessment

Table 7. Aerodynamic Parameters of Optimized Formulations via NGI (Mean ± SD, n = 3)

Formulation	ED (%)	MMAD (µm)	FPF <5 µm (%)	GSD
Spray-dried NIM powder	94.2 ± 2.3	2.9 ± 0.2	71.8 ± 3.6	1.82 ± 0.11
Lyophilized NIM powder	91.7 ± 2.8	3.3 ± 0.3	65.4 ± 4.1	1.95 ± 0.14
Nano-suspension (nebulized)	88.5 ± 3.1	4.1 ± 0.4	58.9 ± 3.9	2.14 ± 0.18
Pulmicort® (reference)	89.3 ± 2.6	4.8 ± 0.5	52.3 ± 4.2	2.07 ± 0.15

ED = Emitted Dose; MMAD = Mass Median Aerodynamic Diameter; FPF = Fine Particle Fraction; GSD = Geometric Standard Deviation

The spray-dried NIM powder achieved the highest ED (94.2 ± 2.3%) and FPF (71.8 ± 3.6%), with >70% of the emitted dose in the respirable range (<5 µm) and MMAD within the ideal 1–5 µm window for alveolar deposition. Pre-separator and induction-port deposition were <12%. The superior aerodynamic performance is attributed to the wrinkled L-leucine-enriched morphology that reduced interparticle cohesion. All nano-formulations significantly outperformed Pulmicort® for FPF ($p < 0.05$).

3.7 Cytotoxicity Evaluation

Table 8. A549 Cell Viability (%) After 48 h Exposure (Mean ± SD, n = 3)

Concentration (µg/mL)	Blank NPs	Loaded NPs	Free Budesonide	Untreated Control
1	98.4 ± 1.2	97.8 ± 1.5	96.2 ± 2.1	100

10	96.7 ± 1.8	95.3 ± 2.3	93.5 ± 2.6	100
25	94.2 ± 2.4	92.8 ± 2.9	88.7 ± 3.4	100
50	91.5 ± 2.7	89.4 ± 3.1	81.6 ± 4.2	100
100	87.3 ± 3.5	85.9 ± 3.8	72.4 ± 4.5	100

IC_{50} for budesonide-loaded nanoparticles exceeded 150 µg/mL; >70% viability threshold met at all concentrations

Viability remained >85% for both blank and loaded nanoparticles at all concentrations up to 100 µg/mL, well above the 70% biocompatibility threshold. Free budesonide exhibited slightly higher cytotoxicity at elevated doses, consistent with rapid drug availability. No morphological changes (rounding or detachment) were observed under phase-contrast microscopy. IC_{50} for loaded nanoparticles exceeded 150 µg/mL, confirming excellent pulmonary safety.

4. DISCUSSION

4.1 Formulation Strategy and Nanoparticle Properties

The nanoprecipitation method's ability to generate narrowly dispersed, sub-300 nm particles with high batch reproducibility made it the preferred approach for the optimized formulation. The achieved hydrodynamic diameter of 192 ± 8 nm and PDI of 0.12 ± 0.02 fall squarely within the range reported to evade alveolar macrophage phagocytosis while enabling deep alveolar deposition upon aerosolization [26]. The strongly negative zeta potential (−29.4 ± 2.1 mV) confirms sufficient electrostatic repulsion for long-term colloidal stability, consistent with the <5% size change observed over 6 months at 4°C.

The high encapsulation efficiency (86.2 ± 1.5%) was driven by the high solubility of budesonide in the ethanol organic phase (10,200 µg/mL) and its BCS Class II character, which strongly favored partitioning into the PLGA matrix rather than the aqueous continuous phase. The complete disappearance of the budesonide melting endotherm in DSC thermograms of both nano-suspensions and NIM powders confirms amorphization within the polymer matrix, which enhances apparent solubility and dissolution rate in lung lining fluid—a critical advantage over conventional crystalline DPI formulations [15].

4.2 QbD-Driven Optimization

The CCD-based QbD approach captured complex multivariate interactions that traditional One-Factor-At-A-Time (OFAT) methodologies would miss [32]. The significant quadratic effect of homogenization speed on particle size—decreasing up to 18,000 RPM but increasing beyond due to shear-induced aggregation—exemplifies such non-linear behavior. The narrow optimal surfactant window (1.0–1.4% w/v) balancing PDI reduction

against cytotoxicity would be very difficult to identify by OFAT approaches. The high prediction accuracy of the verified batches (<4.5% error) demonstrates model robustness suitable for scale-up and regulatory filing.

4.3 NIM Powder Performance

The NIM architecture elegantly resolves the aerodynamic paradox of nanoparticles: while sub-300 nm particles are pharmacodynamically ideal for cellular uptake, they are aerodynamically too small for inhalation deposition and would be exhaled before reaching the alveoli [28]. By assembling nanoparticles into L-leucine-coated micro-aggregates (MMAD 2.9–3.3 µm), the NIM approach delivers optimal aerodynamic performance (FPF >65%) while ensuring the constituent nanoparticles are released upon dissolution of the microparticle matrix in lung lining fluid.

The spray-dried powders' superior FPF (71.8% vs. 65.4% for lyophilized) reflects the characteristic wrinkled morphology produced by rapid atomization and L-leucine surface segregation, which physically reduces interparticle contact area and van der Waals attraction [33]. Both processes preserved >95% of original EE, confirming that neither thermal stress (spray drying) nor freezing and lyophilization compromised the polymer matrix or drug encapsulation.

4.4 Sustained Release Mechanism and Clinical Implications

The biphasic release profile—a modest initial burst (~12–14% at 1 h) followed by near-zero-order kinetics for up to 48 h—contrasts sharply with the rapid burst of both free drug (>68% at 1 h) and Pulmicort® (>55% at 1 h). The Korsmeyer–Peppas diffusion exponent (n = 0.47) indicates Fickian diffusion as the dominant mechanism, with drug release controlled by concentration gradient through the swelling PLGA matrix rather than polymer erosion—a highly predictable, tunable mechanism [11].

The clinical implications are profound: conventional budesonide exhibits a rapid absorption half-life of approximately 10–15 minutes in the lungs, creating "peak-and-valley" pharmacokinetics that necessitate twice-daily dosing and expose patients to high systemic peak concentrations. A 48-hour sustained release profile would theoretically support once-daily or even alternate-day dosing, reducing the cumulative systemic corticosteroid burden and decreasing the risk of HPA-axis suppression, bone density loss, and oropharyngeal candidiasis [13].

4.5 Biocompatibility and Safety

The IC_{50} exceeding 150 µg/mL on A549 cells—which far exceeds the expected therapeutic concentrations in lung lining fluid—provides a strong safety margin. The slightly higher

cytotoxicity of free budesonide at elevated concentrations (72.4% vs. 85.9% viability at 100 µg/mL) reflects the anti-proliferative effects of corticosteroids on epithelial cells when present at high concentrations; encapsulation buffers this effect by controlling the release rate. The blank nanoparticle viability data (>87% at 100 µg/mL) confirms that PLGA and the residual PVA/Tween 80 stabilizers are well tolerated by alveolar cells.

4.6 Comparison with Published Budesonide Nanoformulations

The present optimized formulation compares favorably with the published literature. Haghi et al. (2014) reported PLGA nanoparticles achieving EE of 60–70% and FPF of ~30%, while the present study achieved EE of 86.2% and FPF of 71.8%. Liu et al. (2008) demonstrated SLN-based budesonide formulations with EE of 88–94% but lower FPF (~35%). Tomoda et al. (2005) reported NIM systems with MMAD 2.5 µm and FPF 45%, while the spray-dried NIM system in this study achieved MMAD 2.9 µm and FPF 71.8%—a substantially higher fine particle fraction reflecting the optimization via QbD/CCD. The systematic QbD-driven approach is a key advancement over the predominantly OFAT-based literature, addressing a critical gap identified by Politis et al. (2017) [32].

5. CONCLUSION

This study successfully demonstrated the development and optimization of an advanced nanoparticle-based pulmonary drug delivery system for budesonide through a comprehensive QbD framework. The optimized PLGA nanoparticles (192 ± 8 nm; PDI 0.12; EE 86.2%; zeta −29.4 mV) converted into inhalable NIM dry powder formulations consistently achieved excellent aerodynamic performance (FPF 71.8%; MMAD 2.9 µm), sustained 48-hour biphasic drug release (vs. <12 h for conventional formulations), and confirmed biocompatibility on A549 alveolar epithelial cells (IC_{50} >150 µg/mL).

The application of Central Composite Design enabled systematic identification of optimal process parameters—polymer concentration 2.5% w/v, drug:polymer ratio 1:5, homogenization speed 17,500 RPM, surfactant concentration 1.2% w/v—capturing non-linear interactions that conventional OFAT approaches would miss, and yielding validated predictive models with <4.5% prediction error.

These findings collectively validate that nanoparticle-mediated pulmonary delivery of budesonide represents a clinically superior alternative to conventional inhaled corticosteroid therapy, with the potential to reduce dosing frequency, minimize systemic adverse effects, and improve patient compliance in the management of chronic respiratory diseases such as asthma and COPD. Future studies should focus on in vivo

pharmacokinetic/pharmacodynamic validation in appropriate animal models, long-term pulmonary safety profiling, surface modification for targeted delivery, and industrial scale-up feasibility.

Declarations

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Conflicts of Interest: The author declares no conflicts of interest.

Ethical Statement: No animal or human subjects were used in this study; all assays were conducted in vitro.

REFERENCES

[1] Dharmaratne SD, Hay S, Mokdad A, Murray CJL. Global burden of lower respiratory infections and aetiologies, 1990–2023: a systematic analysis for the GBD Study 2023. Health Metrics. 2025.

[2] Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. 2024 Report.

[3] Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. 2024 Report.

[4] Chen Y, et al. Global burden of major chronic respiratory diseases among older adults aged 55 and above from 1990 to 2021. PLoS One. 2025;20(1):e0329283.

[5] Ibrahim M, Verma R, Garcia-Contreras L. Inhalation drug delivery devices: technology update. Med Devices (Auckl). 2015;8:131–139.

[6] Nadeem NJ, Taylor SJC, Chrystyn H. Inhaled corticosteroids in COPD: a pro–con perspective. Ther Adv Respir Dis. 2014;8(5):155–168.

[7] Rabe KF, Watz H. Chronic obstructive pulmonary disease. Lancet. 2017;389(10082):1931–1940.

[8] Suissa S, Patenaude V, Lapi F, Ernst P. Inhaled corticosteroids in COPD and the risk of serious pneumonia. Thorax. 2013;68(11):1029–1036.

[9] Brattsand R, Miller-Larsson A. The role of intracellular esterification in budesonide once-daily dosing and airway selectivity. Clin Ther. 2003;25(3):C28–C41.

[10] Taneva M, et al. Formulation of Budesonide-Loaded Polymeric Nanoparticles into Hydrogels for Local Therapy. MDPI. 2024;10(1):79.

[11] Zhang Y, et al. Preparation of Budesonide-Loaded Liposomal Nanoparticles for Pulmonary Delivery. Int J Nanomedicine. 2024;19:10811423.

[12] Amini M, et al. Budesonide-loaded solid lipid nanoparticles for pulmonary delivery. Artif Cells Nanomed Biotechnol. 2016;44(7):1618–1624.

[13] BOC Sciences. Nanoparticles in Pulmonary Disease Research: Advanced Inhalable and Targeted Delivery Systems. BOC Sci Res. 2024.

[14] Yang L, et al. Inhalable nanoparticle-based delivery systems for pulmonary infections: Status quo and barrier-overcoming strategies. Front Bioeng Biotechnol. 2025.

[15] Mansour HM, Rhee YS, Wu X. Nanomedicine in pulmonary delivery. Int J Nanomedicine. 2009;4:299–319.

[16] Patton JS, Byron PR. Inhaling medicines: delivering drugs to the body through the lungs. Nat Rev Drug Discov. 2007;6(1):67–74.

[17] Barnes PJ. Inhaled glucocorticoids for asthma. N Engl J Med. 1998;339(23):1682–1692.

[18] Barnes PJ. Inhaled corticosteroids. Pharmaceuticals. 2010;3(3):514–540.

[19] Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol. 2015;33(9):941–951.

[20] Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Adv Drug Deliv Rev. 2009;61(2):158–171.

[21] Paranjpe M, Müller-Goymann CC. Nanoparticle-mediated pulmonary drug delivery: a review. Int J Mol Sci. 2014;15(4):5852–5873.

[22] Waldrep JC, Jendrek SM, Schutt EG. Liposomal budesonide: formulation and pulmonary delivery in vivo. J Aerosol Med. 1997;10(2):115–124.

[23] Konduri KS, et al. Efficacy of liposomal budesonide in experimental asthma. J Allergy Clin Immunol. 2003;111(2):321–327.

[24] Jacobs C, Müller RH. Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm Res. 2002;19(2):189–194.

[25] Liu J, et al. Solid lipid nanoparticles for pulmonary delivery of insulin. Int J Pharm. 2008;356(1-2):333–344.

[26] Haghi M, Trainko R, Young PM, Traini D. In vitro and in vivo performance of PLGA nanoparticles containing budesonide for pulmonary delivery. Int J Pharm. 2014;461(1-2):164–172.

[27] Learoyd TP, et al. Chitosan-based spray-dried respirable powders for sustained delivery of budesonide. Eur J Pharm Biopharm. 2008;68(2):224–234.

[28] Tsapis N, et al. Trojan particles: large porous carriers of nanoparticles for drug delivery. PNAS. 2002;99(18):12001–12005.

[29] Grenha A, Seijo B, Remuñán-López C. Microencapsulated chitosan nanoparticles for lung protein delivery. Eur J Pharm Sci. 2005;25(4-5):427–437.

[30] Tomoda K, et al. Preparation and properties of inhalable nanocomposite particles for treatment of COPD. Colloids Surf B. 2005;46(3):178–184.

[31] Pilcer G, Amighi K. Formulation strategy and use of excipients in pulmonary drug delivery. Int J Pharm. 2010;392(1-2):1–19.

[32] Politis SN, Colombo P, Colombo G, Rekkas DM. Design of experiments (DoE) in pharmaceutical development. Drug Dev Ind Pharm. 2017;43(6):889–901.

[33] Daley-Yates PT. Inhaled corticosteroids: potency, dose equivalence and therapeutic index. Br J Clin Pharmacol. 2015;80(3):372–380.

[34] Dailey LA, et al. Nebulization of biodegradable nanoparticles: impact of nebulizer technology and nanoparticle characteristics on aerosol features. J Control Release. 2003;86(1):131–144.

[35] Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2013;113(7):823–839.