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RESEARCH PAPER

PLGA Microparticles Encapsulating Prostaglandin E_1-Hydroxypropyl-β-cyclodextrin (PGE_1-HPβCD) Complex for the Treatment of Pulmonary Arterial Hypertension (PAH)

Vivek Gupta • Marauo Davis • Louisa J. Hope-Weeks • Fakhrul Ahsan

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ABSTRACT

Purpose To test the efficacy and viability of poly (lactic-*co*-glycolic acid) (PLGA) microspheres encapsulating an inclusion complex of prostaglandin E_1 (PGE_1) and 2-hydroxypropyl-β-cyclodextrin (HPβCD) for pulmonary delivery of PGE_1 for treatment of pulmonary arterial hypertension (PAH), a disease of pulmonary circulation.

Methods PLGA-based microparticulate formulations of PGE_1-HPβCD inclusion complex or plain PGE_1 were prepared by a double-emulsion solvent evaporation method. HPβCD was used as a complexing agent to increase the aqueous solubility of PGE_1, act as a porosigen to produce large porous particles, and promote absorption of PGE_1. Particles were characterized for micromeritic properties, *in vivo* absorption, metabolic degradation, and acute safety.

Results Incorporation of HPβCD in the microparticles resulted in development of large particles with internal pores, which, despite large mean diameters, had aerodynamic diameters in the inhalable range of 1 to 5 μm. HPβCD incorporation also resulted in a significant increase in the amount of drug released *in vitro* in simulated interstitial lung fluid, showing a desirable burst release profile required for immediate hemodynamic effects. Compared to plain PLGA microparticles, entrapment efficiency was decreased upon complexation with HPβCD. *In vivo* absorption profile indicated prolonged availability of PGE_1 in circulation following pulmonary administration of the optimized microparticulate formulations, with an extended half-life of almost 4 hours. Metabolic degradation and acute toxicity studies suggested that microparticulate formulations were stable under physiological conditions and safe for the lungs and respiratory epithelium.

Conclusions This study demonstrates the feasibility of PGE_1-HPβCD complex encapsulated in PLGA microparticles as a potential delivery system for controlled release of inhaled PGE_1.

KEY WORDS microparticles. · poly (lactic-*co*-glycolic acid) · prostaglandin E_1 · pulmonary arterial hypertension · pulmonary delivery · 2-hydroxypropyl-β-cyclodextrin

INTRODUCTION

The absorption of a drug across biological membranes is governed primarily by its physicochemical properties, especially its aqueous solubility and distribution coefficient. Extremely hydrophobic drug substances exhibit low absorption and poor bioavailability, which often leads to the failure of many potential drug candidates to become a viable therapeutic agent. Indeed, the hydrophobicity of a molecule is a major determinant of its drug-like properties that influences absorption, distribution, protein binding and metabolism. A drug with an intermediate distribution coefficient, neither too hydrophobic nor hydrophilic, is most desirable for maximal absorption.

A variety of techniques and delivery systems have been investigated to increase the aqueous solubility of hydropho-

V. Gupta · F. Ahsan (✉)
Department of Pharmaceutical Sciences, School of Pharmacy
Texas Tech University Health Sciences Center
1300 Coulter Drive
Amarillo, Texas 79106, USA
e-mail: Fakhrul.ahsan@ttuhsc.edu

M. Davis · L. J. Hope-Weeks
Department of Chemistry and Biochemistry
Texas Tech University
Memorial Circle and Boston
Lubbock, Texas 79409, USA

bic molecules. Cyclodextrin complexation-based solubilization of drugs has attracted much attention over the past two decades (1–3). Cyclodextrins are cyclic oligosaccharides derived from amylose sugar and are composed of a varying number of α-1-4-glucose units linked together in a ring structure. Glucose chains in the cyclodextrin molecule form a toroid-like structure, with a characteristic arrangement of primary and secondary hydroxyl groups that results in the formation of a cone-like cavity that is less hydrophilic than the aqueous environment. This cone-like cavity provides a favorable environment for hydrophobic drug molecules to form noncovalent inclusion complexes, thereby modifying the physicochemical properties of the drug molecule (4, 5). Of the cyclodextrins tested as solubilizers, β-cyclodextrin (β-CD) has seven glucose units and exhibits an excellent absorption profile. But, unfortunately, use of β-CD is associated with hepatotoxicity and nephrotoxicity. This has led to the development of 2-hydropropyl-β-cyclodextrin (HPβCD), a hydroxylalkyl derivative with improved water solubility, which is relatively non-toxic irrespective of the route of administration (6).

Prostaglandin E_1 (PGE_1; Alprostadil) is a compound with vasodilatory, anti-inflammatory, anti-aggregatory, and anti-proliferative properties (7–9). PGE_1 is approved by the U.S. Food and Drug Administration (FDA) for the treatment of erectile dysfunction in men and patent ductus arteriosus in newborns. It has been investigated in the treatment of various cardiopulmonary disorders, especially pulmonary arterial hypertension (10–13). However, the clinical utility of PGE_1 has been limited by its chemical instability and extremely hydrophobic nature (log P=3.3), which results in low drug absorption and poor bioavailability. Several reports have suggested using complexes of cyclodextrin and PGE_1 to increase the aqueous solubility of PGE_1 (14–17). Gu *et al.* reported that a PGE_1-HPβCD inclusion complex resulted in increased aqueous solubility of PGE_1 and enhanced absorption following nasal administration in Wistar rats (14).

It has been reported that PGE_1, when administered via the pulmonary route, works as a selective pulmonary vasodilator (18). However, the main limitation of pulmonary delivery of PGE_1 is its very short half-life (3 to 4 minutes) as a result of first-pass metabolism in the lungs. We have shown recently that PLGA microparticles of PGE_1 release the drug over a prolonged period of time, improving the metabolic stability of the drug (19). The extreme hydrophobicity of PGE_1 limits its passage through the air-blood barrier. Many investigators have emphasized the effectiveness of cyclodextrins in enhancing drug absorption via modulation of tight junctions (20, 21). Cyclodextrins are also reported to alter the release properties of drug-encapsulated polymeric carriers (22, 23). In addition, cyclodextrins have also been investigated for their property to act as a porosigen, which is required for preparation of large porous microparticles that exhibit efficient deep lung deposition. Considering the properties of HPβCD to form an inclusion complex with PGE_1, enhance the drug absorption, modulate the drug release profile and act as a porosigen, we hypothesize that encapsulation of PGE_1-HPβCD complex in PLGA microparticles results in a microparticulate drug delivery system with improved drug release and superior absorption via the air-blood barrier than plain PGE_1 encapsulated in PLGA particles. This study tests the efficacy of PGE_1-HPβCD-loaded inhalable PLGA microparticles in providing sustained release of PGE_1 and improved absorption profile upon pulmonary administration in rodents.

MATERIALS AND METHODS

Materials

PGE_1 (Alprostadil) was purchased from Spectrum Chemicals (Gardena, CA, USA), and (2-hydroxypropyl)-β-cyclodextrin (HPβCD; Mw ≈ 1,380) was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). PLGA 85:15 polymer for the preparation of microparticulate formulations (inherent viscosity 0.55 to 0.75 dl/g; average Mw ≈ 85 kDa) was purchased from Lactel Absorbable Polymers (Pelham, AL, USA). Kits for various assays were purchased from the following vendors: PGE_1 ELISA kit from Assay Designs, Inc. (Ann Arbor, MI, USA); BCA protein assay kit from Pierce Biotechnology (Rockford, IL, USA); alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) kits from Pointe Scientific, Inc. (Canton, MI, USA). Polyvinyl alcohol (PVA), dichloromethane (DCM), and all other chemicals were of analytical/HPLC grade and were purchased from different commercial resources. Adult male Sprague-Dawley (SD) rats were procured from Charles River Laboratories (Wilmington, MA, USA).

Preparation of PGE_1-HPβCD Complex

PGE_1-HPβCD complexes were prepared in molar ratios of 1:4 and 1:10 by slightly modifying the freeze-drying procedure reported previously by Gu *et al.* (14). Molar ratios of 1:4 and 1:10 have been reported to be suitable for PGE_1 complex formulations with various cyclodextrins (16, 17). Briefly, PGE_1 was dissolved in a minimal amount of absolute ethanol, and the corresponding quantity of HPβCD was dissolved in double-distilled deionized water. The resulting solutions were mixed together, vortexed for 60 seconds, and freeze dried for 48 hours using a FreeZone 2.5 lyophilizer (Labconco Corporation, Kansas City, MO, USA). The freeze-dried complex of PGE_1 and HPβCD was

used for the preparation of polymeric microparticles and for *in vivo* absorption studies.

Preparation of PLGA Microspheres Encapsulating PGE_1-HPβCD Complex

Polymeric microparticles of PGE_1-HPβCD complex were prepared by water-in-oil-in-water (W/O/W) double emulsion/solvent evaporation, with slight modification to the previously described procedure (19). Briefly, PLGA 85:15 was dissolved in DCM (organic phase, OP) and emulsified (primary W/O emulsion) with deionized water containing 5 mg of PGE_1 alone (Plain-1, -2, and −3) or PGE_1-HPβCD complex (CD-1 – CD-6) equivalent to 5 mg active PGE_1 (internal aqueous phase, IAP) using a Branson Sonifier 450 (Branson Ultrasonics Corporation, Danbury, CT, USA). The primary W/O emulsion was emulsified again with an aqueous PVA solution (secondary emulsion) by homogenization at 10,000 rpm for 10 minutes (Ultra-Turrex T-25 basic, Ika, Wilmington, DE, USA). The resulting microparticulate formulations were stirred overnight (~50–100 rpm) for removal of the OP. The following day, the microparticulate formulations were washed three times by centrifugation at 50,000 g for 15 minutes at 4°C (Avanti J-25I, Beckman Coulter, Inc. Brea, CA, USA) to remove excess PVA. The washed microparticulate formulations were lyophilized for 48 h to obtain nine unique, free-flowing, powdered microparticulate formulations: Plain-1, Plain-2, Plain-3, CD-1, CD-2, CD-3, CD-4, CD-5, and CD-6. Formulation properties are listed in Table I.

Physical Characterization

The prepared microparticulate formulations were characterized for morphology, size, tapped density, mass median aerodynamic diameter, surface properties, drug loading, and entrapment efficiency.

Particle Morphology

To understand the morphology of the prepared particulate formulations, scanning electron microscopy (SEM) experiments were performed using a Hitachi S-3400 N SE microscope (Hitachi High Technologies America, Inc, Pleasanton, CA, USA). Briefly, a small amount of powdered microparticulate formulation was sprinkled onto double-sided adhesive tape attached to an aluminum stub and was sputter-coated with gold under argon (Emitech K550X; Quorum Technologies Ltd., Kent, UK) and viewed under SE microscope. Photographs were taken at varying magnifications so as to reveal surface characteristics of the microparticulate formulations.

Particle Size

Mean volume diameter and particle size distribution for all the microparticles were studied using Tri-laser diffraction technology (Microtrac S3500; Microtrac, Inc.; Largo, FL, USA). This instrument is capable of determining particle sizes in the range of 0.024–2,800 μm. Briefly, samples were prepared by dispersing 2 mg of powdered microparticulate formulations in 0.2% w/v tween-80 solution, which was poured into the wet sample port of the instrument for analysis. Mean volume diameter (M_v) was recorded. In addition, polydispersity indices (PDIs) of all the microparticulate formulations were also determined using the following equation:

$$Polydispersity\ Index = \frac{Volume\ averaged\ mean\ particle\ size\ (M_V)}{Number\ averaged\ mean\ particle\ size\ (M_N)} \quad (1)$$

Tapped Density, Carr's Index and Theoretical Mass Median Aerodynamic Diameter

The tapped density was measured to determine the bulkiness of the microparticulate formulations and to calculate theoret-

Table I Compositions of Various Microparticulate Formulations

Formulation	Polymer	IAP:OP:EAP	PVA Concentration (w/v)	PGE_1:HPβCD Molar Ratio	Polydispersity Index (PDI)
Plain-1		0.5:5:25	0.5%	PGE_1 alone	1.69 ± 0.005
CD-1		0.5:5:25	0.5%	1:10	1.41 ± 0.018
CD-2		0.5:5:25	0.5%	1:4	1.53 ± 0.02 6
Plain-2		0.5:5:25	1%	PGE_1 alone	1.61 ± 0.01
CD-3	PLGA 85:15 (0.55-0.75 dl/g)	0.5:5:25	1%	1:10	1.29 ± 0.001
CD-4		0.5:5:25	1%	1:4	1.68 ± 0.011
Plain-3		0.5:5:25	2%	PGE_1 alone	0.99 ± 0.16
CD-5		0.5:5:25	2%	1:10	1.536 ± 0.004
CD-6		0.5:5:25	2%	1:4	1.73 ± 0.029

ical mass median aerodynamic diameters ($MMAD_t$) as described previously (24). Briefly, 100 mg of particles were placed in a graduated cylinder (10±0.5 mL). The initial volume ($V_{initial}$) was recorded, and the cylinder was tapped 200 times onto the work station from a consistent height. The final volume (V_{Final}) was recorded and used to calculate tapped density (ρ) using the following equation 2 (25):

$$Tapped\ Density\ (\rho) = \frac{Sample\ weight\ (g)}{Final\ volume\ after\ 200\ taps\ (V_{final})} \quad (2)$$

Bulk density (ρ_i) was calculated using the following equation 3:

$$Bulk\ Density\ (\rho_i) = \frac{Sample\ weight(g)}{Initial\ core\ volume\ \ (V_{Initial})} \quad (3)$$

Thus, calculated tapped and bulk densities were used to determine the flow properties of the powdered microparticles by calculating the compressibility index (Carr's Index) using the following equation 4, as described in US Pharmacopoeia-National Formulary (USP33-NF28 S1 Reissue):

$$Carr's\ Index = \left(\frac{1-\rho_i}{\rho}\right) \times 100 \quad (4)$$

Theoretical mass median aerodynamic diameter ($MMAD_t$) was calculated using equation 5 (26),

$$MMAD_t = d\left(\frac{\rho}{\rho_0 X}\right)^{\frac{1}{2}} \quad (5)$$

where d = geometric mean diameter, ρ = tapped density (g/cm^3), ρ_0 = reference density for a sphere (1 g/cm^3), and X = shape factor (1 for sphere).

Actual Mass Median Aerodynamic Diameter ($MMAD_a$)

Actual mass median aerodynamic diameter ($MMAD_a$) was determined using an 8-stage Mark-II nonviable Anderson Cascade Impactor (ACI) (Westech Instruments, Marietta, GA, USA), according to a procedure listed in US Pharmacopoeia (USP 25). Briefly, a size 3 hard gelatin capsule was filled with 10 mg powdered microparticulate formulation and fired into the impactor at a constant flow rate of 28.3 L/min using a Spiriva Handihaler (Boehringer Ingelheim Pharmaceuticals). The amount of the microparticle deposited at each stage was determined gravimetrically by weighing glass fiber filters placed at each stage (0–7) on inverted collection plates (to prevent bounce or re-entrainment of the particles). Data were plotted on semi-log graph, and $MMAD_a$ was determined at 50% of total particle distribution. In addition to $MMAD_a$, the fine particle fraction (FPF) for each formulation was determined as the ratio of cumulative weight of particles <4.7 μM (stage 3 or lower) to total weight of particles recovered from the cascade impactor (27).

Entrapment Efficiency

The quantity of PGE_1 entrapped in the microparticulate formulations was determined indirectly by determining the amount of PGE_1 present in the supernatant recovered after the first washing of the microparticles. Briefly, the supernatant was diluted 10^4 to 10^5 times and was analyzed using a PGE_1 ELISA kit (Assay Designs, Ann Arbor, MI, USA), according to the manufacturer's protocol. Sample dilution was necessary due to the high sensitivity of the ELISA kit (limit of quantitation=4.88 to 5,000 pg/mL) and to minimize the matrix interference. All measurements were performed in triplicate, and data represent mean ± SD.

Determination of Surface Characteristics by Physioabsorption Isotherms

To determine the surface porosity and pore characteristics, we used nitrogen physiosorption isotherms obtained at 77 K using a Nova 3200e surface area analyzer (Quantachrome Instrument Corporation, Boynton Beach, FL, USA). Briefly, the microparticulate samples were degassed for 24 hours at room temperature to remove any dissolved components that could interfere with the analysis. The samples were run on a surface area analyzer in a sample cell placed in a liquid nitrogen-filled dewer to maintain equilibrium temperature. Each sample was run for 24 hours with a 150-second equilibrium interval to obtain Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) isotherms. Average surface areas (m^2/g) for all microparticulate formulations were determined by both BET and BJH isotherms, whereas average pore volumes (cm^3/g) and pore radii (Å) were determined using the desorption branch of the BJH isotherm (28).

In Vitro Release Studies in Simulated Interstitial Lung Fluid (SILF)

In vitro release studies were performed in simulated interstitial lung fluid (SILF; pH 7.2) prepared at 37°C according to the Moss Formula (Table II) (29). Briefly, 10 mg of the microparticulate formulation was dispersed in 1 mL of release medium (SILF) and incubated at 37±1°C with gentle shaking (~150 to 200 rpm). At predetermined time intervals ranging from 0 to 48 hours, samples were centrifuged at 16,000 × g for 15 minutes at 4°C, and 200 μL of the supernatant was collected for PGE_1 analysis using a commercially available PGE_1 ELISA kit (Correlate® ELISA Kit, Assay Designs, Ann Arbor, MI, USA). Collected sample volume was replaced with fresh release media. Data obtained were plotted against time (h) as percent cumulative drug release. Data represent mean ± SD of three simultaneous measurements.

Table II Composition of Simulated Interstitial Lung Fluid (SILF) (Moss Formula)

Chemical	Formula	Composition (mM)
Magnesium chloride	$MgCl_2.6H_2O$	1.0
Sodium chloride	NaCl	103.0
Potassium chloride	KCl	4.0
Sodium phosphate, dibasic	$Na_2HPO_4.7H_2O$	1.5
Sodium sulfate	Na_2SO_4	0.5
Calcium chloride	$CaCl_2.2H_2O$	2.45
Sodium acetate	$NaH_3C_2O_2.3H_2O$	7.0
Sodium bicarbonate	$NaHCO_3$	31.0
Sodium citrate	$Na_2H_5C_6O_7.2$	0.33

In Vivo Absorption Studies

To determine the effect of HPβCD complexation and encapsulation into PLGA microparticles on the circulation half-life ($t_{1/2}$) of PGE_1, *in vivo* absorption studies were performed in adult male Sprague-Dawley (SD) rats (250–300 g). All *in vivo* studies were performed under anesthesia, induced by an intramuscular injection of a ketamine / xylazine cocktail (90 mg/kg ketamine, 10 mg/kg xylazine). The anesthesia was maintained as needed during the experiment.

The rats ($n=42$) were divided into seven groups and were dosed with the equivalent of 120 μg/kg of PGE_1 via intravenous (IV) or intratracheal (IT) administration as follows: (i) saline, IT; (ii) PGE_1, IV; (iii) PGE_1, IT; (iv) PGE_1-HPβCD complex (1:4), IT; (v) PGE_1-HPβCD complex (1:10), IT; (vi) microparticulate formulation CD-3, IT; and (vii) microparticulate formulation CD-5, IT. Intravenous drug was delivered via the penile vein, and intratracheal delivery employed a Microsprayer (PennCentury, Philadelphia, PA) designed specifically for aerosol administration to small rodents. Blood samples were collected in citrated microcentrifuge tubes at predetermined time points (0 to 24 hours). While collecting the blood samples, 10 μg/mL indomethacin was added to each tube to inhibit prostaglandin synthase. Plasma was collected from blood samples by centrifuging at 2,300×g for 5 minutes and was stored at −20°C until analysis. Plasma PGE_1 concentrations were determined using ELISA as described above.

Metabolic Degradation Studies in Rat Lung Homogenates

Metabolic degradation studies were performed to determine the stability of optimized microparticulate formulations under physiological conditions. These studies were performed according to the protocol previously established in our lab (19) with slight modifications. Briefly, rat lungs were removed surgically and homogenized in 4 volumes of cold buffer (Bücher medium) using a Potter-Elvehjem glass homogenizer. The homogenate was centrifuged at 10,000 × *g* for 20 minutes at 4°C, and the supernatant was used for further studies. For degradation studies, PGE_1 (10 μg/mL) or optimized microparticles (CD-3 or CD-5) at a concentration of 10 μg/mL PGE_1 were incubated with lung homogenates at 37°C in the presence of 2 mM NAD^+. At predetermined time points (0–8 hours), the reaction was terminated by adding 200 μL 0.1 N HCl to the incubation mixture, and the PGE_1 concentration was determined by ELISA. To determine amount of PGE_1 remaining in the microparticles following incubation, PGE_1 was extracted from the microparticles by the procedure previously published (19), and quantified by ELISA, providing a measure of the controlled release properties for the PGE_1-HPβCD complex polymeric microparticles.

Bronchoalveolar Lavage (BAL) Fluid Analysis

To establish the safety profile of the optimized microparticulate formulations, various lung injury and toxicity markers were analyzed in the bronchoalveolar lavage (BAL) fluid, according to a published procedure (30, 31). Briefly, male SD rats (350 to 400 g; $n=16$) were divided into 4 groups and administered (i) saline, (ii) 0.1% sodium dodecyl sulfate (SDS), (iii) CD-3 equivalent to 120 μg/kg dose of PGE_1, and (iv) CD-5 equivalent to 120 μg/kg PGE_1 via the IT route. Lungs were removed surgically from anesthetized rats 12 hours after administration by exposing the respiratory system with a mid-level incision in the thoracic cavity. Lungs were cleaned of surrounding tissues/organs and weighed (g/100 g body weight) to assess the possibility of edema formation. To collect BAL fluid, 5 mL of normal saline was instilled into the lungs through trachea, left for 30 seconds, withdrawn and re-instilled for 30 seconds, and finally withdrawn. The collected BAL samples were centrifuged at 500 × *g* for 10 minutes. The supernatant was used to determine the levels of the injury markers ALP and LDH (IU/L; Pointe Scientific, Canton, MI), as well as total protein concentration (mg/mL; Pierce Biotechnology, Rockford, IL).

All animal studies were performed in accordance with NIH Guidelines for the care and use of Laboratory Animals under a protocol approved by TTUHSC Animal Care and Use Committee (AM-10012).

Cell Viability Studies

To access the *in vitro* safety profile, acute cytotoxicity studies were performed on Calu-3 lung epithelial cells using a propidium iodide exclusion fluorescence assay (32). Briefly, Calu-3 cells were seeded at 20,000 cells/well in a 96-well,

microtiter plate using EMEM cell culture media (+ 10% fetal bovine serum + 1% 200 mM L-glutamine + 1% penicillin/streptomycin solution) and allowed to attach overnight at 37°C in a 5% CO_2 incubator. Immediately prior to the experiment, the medium was removed, and cells were washed twice with KRH (Krebs-Ringer-HEPES) buffer (33) and were treated for 4 hours at 37°C with the following: (i) 20 μL saline; (ii) 20 μL 0.1% SDS (positive control); 0.5, 1.0, 2.0 or 5.0 mg/mL of test formulations (iii) CD-3, and (iv) CD-5.

After 4 hours the cells were washed twice with KRH buffer, and initial fluorescence (*Blank*) was measured with 100 μL of KRH buffer in each well. Propidium iodide (PI, 5 μM) was added to each well, and the plate was incubated at 37°C for 30 minutes. After 30 minutes, fluorescence (F) was measured and 375 μM of digitonin was added to permeabilize the cells and label all the nuclei with PI. Fluorescence was measured every 5 minutes up to 20 minutes. Fluorescence at 20 minutes (F_{max}) was used for all the calculations using following equation (34):

$$\%\ Cell\ Viability = 100 - \left(\frac{F - blank}{F_{max} - Blank}\right) \times 100 \qquad (6)$$

Fluorescence was measured at an excitation wavelength (Ex) of 546 nm and emission wavelength (Em) of 620 nm at a band pass of 20 nM, using a SynergyMX microplate reader (Biotek, Winnoski, VT).

Pharmacokinetic and Statistical Analyses

Pharmacokinetic parameters were determined by Kinetica Version 5.0 (Thermo Scientific, Waltham, MA, USA), using standard non-compartmental extravascular analysis. Data were analyzed for C_{max}, T_{max}, $t_{1/2}$, and area under concentration-time plot ($AUC_{0\text{-}24\ h}$). Data analysis was performed by using one-way ANOVA followed by appropriate post hoc analysis (GraphPad Prism 5.0, GraphPad Software, La Jolla, CA, USA). Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In this study, we have investigated the influence of HPβCD on the physical and biological properties of PGE_1-loaded PLGA microparticles. Toward this end, we first conducted a series of physical characterization studies to establish the feasibility of the proposed formulations for administration via the respiratory tract, and then we tested the optimized formulation for pharmacokinetic profiles, safety and metabolic stability upon pulmonary administration in rodents.

Physical Characterization

Physical characterization studies were performed to determine the effect of process variables and additives on the morphology and aerodynamic behavior of microparticulate formulations.

Particle Morphology

Scanning electron microscopy experiments were performed to determine the morphology of PGE_1-HPβCD complex-encapsulated microparticulate formulations. All the formulations had a relatively smooth outer surface and were spherical in shape (Figs. 1a–f). Despite the presence of few surface pores in all the microparticles, SEM images suggest that the incorporation of HPβCD as an excipient did not significantly affect particle morphology. As has been suggested in many reports, HPβCD works as an osmotic agent creating high osmotic pressure in the internal aqueous phase during particle hardening (35), which causes an influx of water and generally results in the development of a porous particle surface (36). Although relatively few surface pores are visible in the images, these formulations are more likely to produce internal particle pores not visible from the surface. We recently reported on the development of bulky and internally porous PLGA microparticles of PGE_1 by incorporation of 0.9% NaCl in the internal aqueous phase (19). Others have also reported the development of internal pores in microparticles with increased osmotic pressure in the IAP rendering influx of water from the EAP (37).

Particle Size

The mean volume diameter for all microparticulate formulations was dependent on the concentrations of HPβCD and PVA (Fig. 2a). Particle size increased significantly with increasing concentration of HPβCD (Plain-2 = 8.54 ± 0.055 μm; CD-4 = 10.09 ± 0.11 μm; and CD-3 = 14.54 ± 0.03 μm), an indication of the bulking and porosigenic properties of HPβCD. In addition, particle size decreased as the concentrations of PVA were increased from 0.5% to 2% (Fig. 2a). This decrease in particle size can be explained by the emulsion-stabilizing properties provided by the hydrophilic environment of PVA (38). All the microparticulate formulations were moderately dispersed, with PDI ranging from 0.99 to 1.73.

It is important to point out that a large variability in the particle size was observed when particles were examined under SEM (Fig. 1). But no such variability was observed when particle size was measured by Microtrac S350 that determines particle size based on the principle of the Tri-laser diffraction (Fig. 2a). This discrepancy may stem from

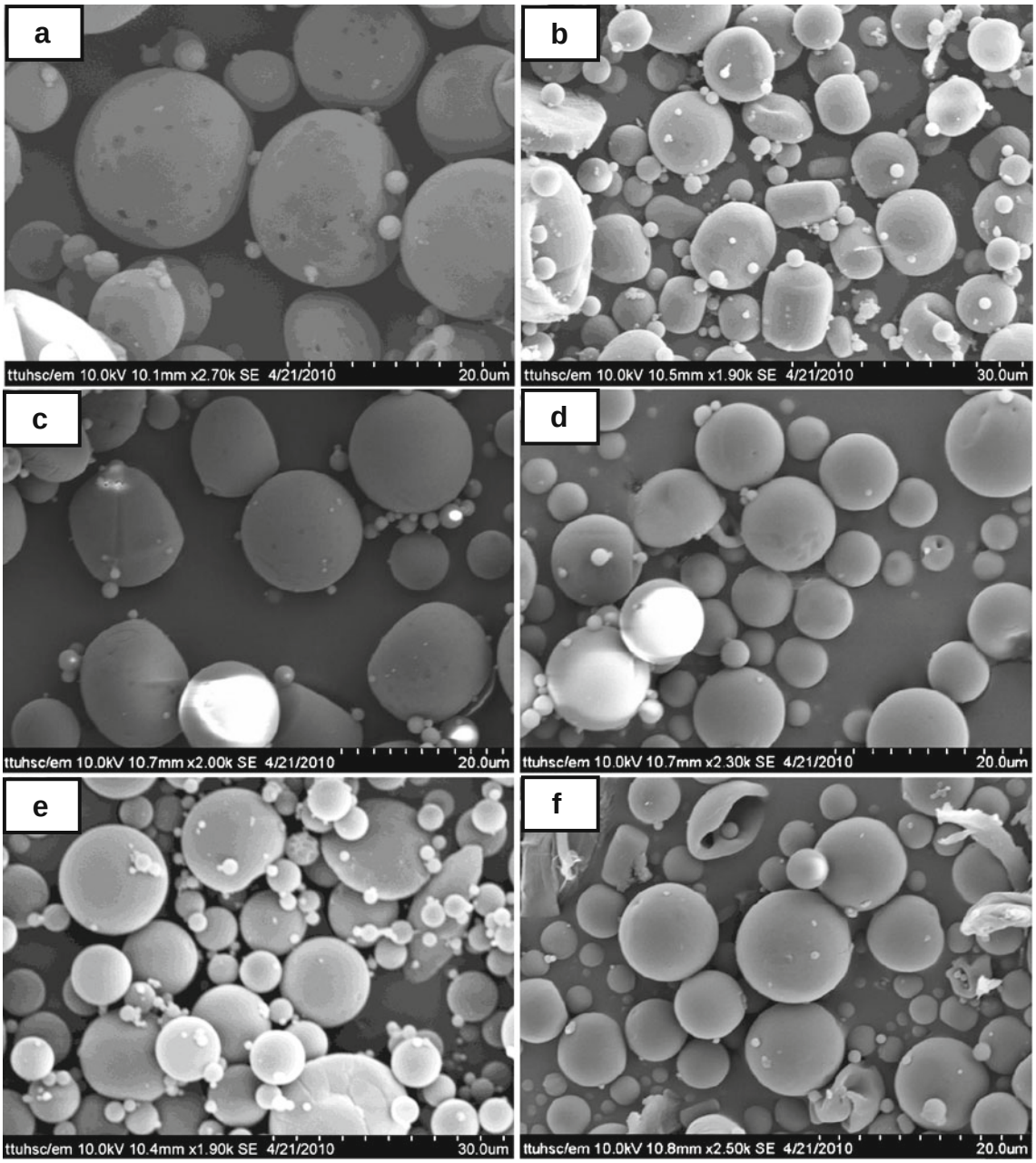

Fig. 1 Scanning electron microscopy (SEM) images of PGE_1-HPβCD-encapsulated PLGA microparticles: (**a**) CD-1; (**b**) CD-2; (**c**) CD-3; (**d**) CD-4; (**e**) CD-5; and (**f**) CD-6. Refer to Table I for composition of different formulations.

the fact that for microscopic examination a small amount of particle was fixed on an aluminum stub. Fixing of particles for SEM does not always give a uniform distribution of particles. It is also possible that the area focused during SEM study contained particles of variable sizes. However, particle size analysis was performed after dispersing the particles in deionized water, which provides a uniform dispersion of the particles. Moreover, a single SEM experiment does not give an accurate measurement of particle size.

Tapped Density and Carr's Index

Tapped density measurements provide important information regarding the flowability, porosity, and size distribution of the particulate formulations, as well as information regarding interparticulate forces (cohesive and adhesive) working in the system (24). The tapped densities, presented in Table III, of microparticulate formulations decreased with increasing HPβCD concentration (Plain-1 = 0.33 ± 0.04; CD-1 = 0.185 ± 0.03; and CD-2 = 0.188 ± 0.04). A

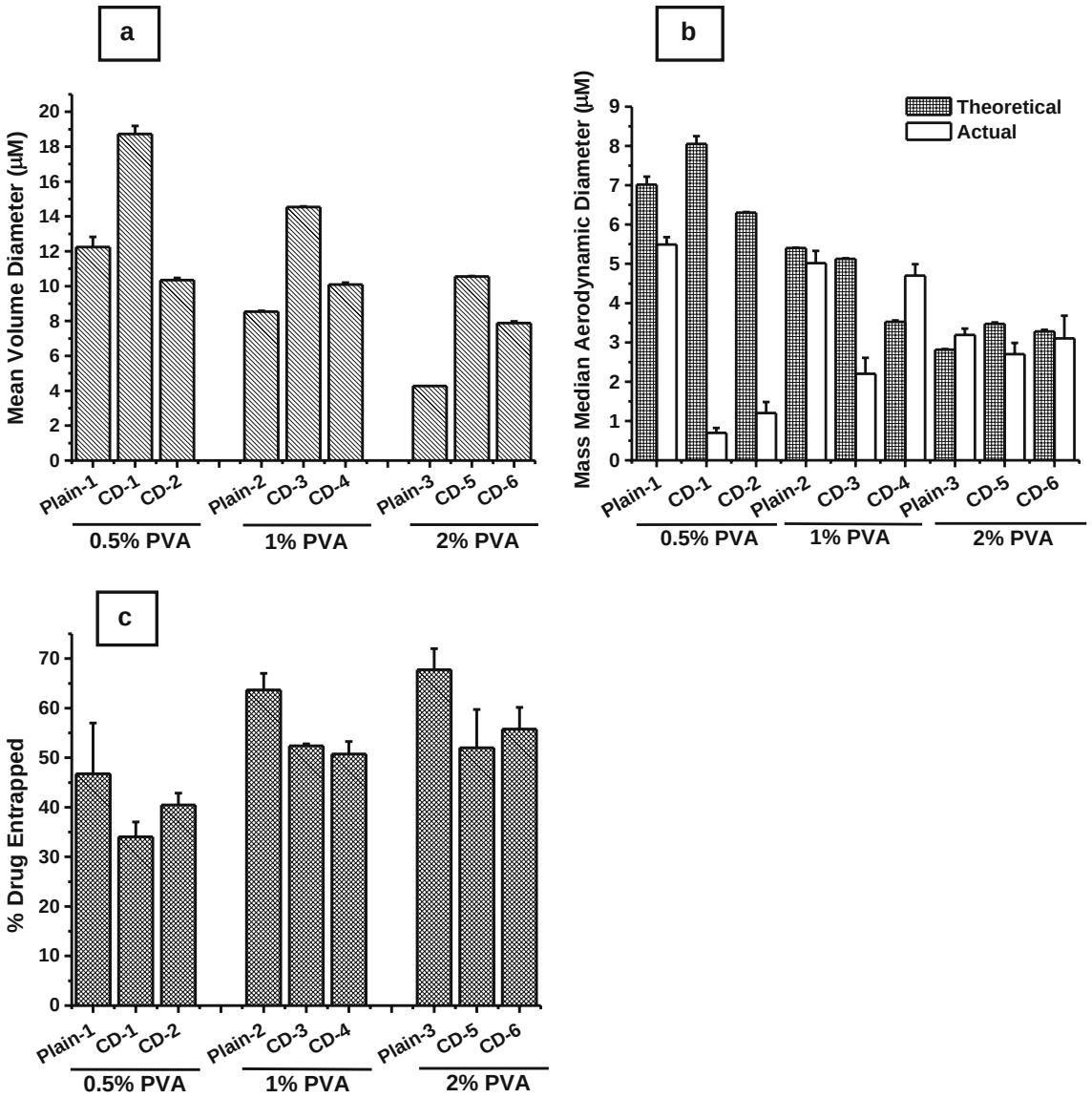

Fig. 2 Physical characterization of PGE_1-HPβCD-encapsulated PLGA microparticles. (**a**) Volume-based mean diameter (μm); (**b**) Theoretical and actual mass median aerodynamic diameter (MMADt and MMADa); and (**c**) Entrapment efficiencies of the formulations. Data represent mean ± standard deviation ($n = 3$).

Table III Physical Characteristics of PGE_1-HPβCD Complex Encapsulated Microparticles

Formulation	Tapped Density (g/cm^3)	Carr's Index (%)	Drug Loading (%)		Fine Particle Fraction (%)
			Theoretical	Actual	
Plain-1	0.329 ± 0.039	61.11 ± 2,43		1.016 ± 0.22	35.21 ± 6.71
CD-1	0.185 ± 0.028	30.0 ± 1.27		0.667 ± 0.059	91.82 ± 12.31
CD-2	0.188 ± 0.041	25.0 ± 2.19		0.793 ± 0.047	72.92 ± 10.13
Plain-2	0.40 ± 0.0812	62.5 ± 4.61		1.325 ± 0.07	45.11 ± 4.28
CD-3	0.236 ± 0.02	22.2 ± 2.92	1.96	1.027 ± 0.074	68.33 ± 7.28
CD-4	0.116 ± 0.016	28.9 ± 1.89		0.995 ± 0.049	48.89 ± 5.29
Plain-3	0.435 ± 0.11	64.28 ± 6.19		1.557 ± 0.098	48.24 ± 6.29
CD-5	0.118 ± 0.02	23.3 ± 3.16		1.019 ± 0.15	63.27 ± 7.01
CD-6	0.173 ± 0.018	40.6 ± 4.16		1.093 ± 0.086	54.55 ± 6.02

similar pattern was observed with the other formulations. Tapped densities also decreased with increasing PVA concentration (Table III). A decrease in tapped density is suggestive of an increase in the bulk and porosity of particles with HPβCD incorporation.

Carr's index (CI) provides an estimate of the flow properties of the particulate formulations. CI defines particle flowability: excellent (5 to 12%); good (12–18%); fair (18–25%); poor, cohesive (25–32%); very poor (32–38%); extremely poor (>40%). While CI has traditionally been used to determine the compressibility of powder for tablets, in this study we have used CI as a measure of powder flowability, which is important for optimizing the flow property of microparticulate formulations that are administered as a dry powder using an inhaler device. CI has previously been used to characterize inhalable dry powder formulations (25, 39). The CI values (Table III) of microparticles CD-1 to CD-5 were in the range of fair to poor flowability, with CD-3 (22.2±2.92%) and CD-5 (23.3±3.16%) exhibiting better flow properties compared to other CD based formulations. However, plain PLGA particles without HPβCD as a complexing agent exhibited CI values >40% and thus had extremely poor flow properties.

Theoretical and Actual Aerodynamic Diameter ($MMAD_t$ and $MMAD_a$)

Mass median aerodynamic diameter is the most important parameter determining particle deposition and distribution following pulmonary administration. Actual aerodynamic diameters ($MMAD_a$) for all the microparticulate formulations were determined by a nonviable eight-stage Anderson cascade impactor. $MMAD_t$ (Fig. 2b) for all the formulations containing HPβCD was <10 μm, which is similar to that of a published report (25). The values ranged from 0.7±0.12 μm (CD-1) to 4.7±0.294 μm (CD-4). Plain particles also showed $MMAD_a$ values between 3.19±0.162 μm (Plain-3) and 5.49±0.19 μm (Plain-1), which is within the respirable range. While the aerodynamic properties of HPβCD-based particles and plain particles were within the respirable range, HPβCD-based particles can be considered superior to plain particles because the latter showed better flow property. The fine particle fraction (FPF) for all microparticulate formulations was determined from the fraction of particles deposited at stage 3 or lower. FPF (Table III) values for all the CD microparticulate formulations were higher than those for the plain microparticulate formulations, indicating an overall improvement in aerodynamic properties with HPβCD complex. However, the FPF data did not follow any specific pattern. Large porous particles >5 μm in diameter and with densities <0.4 g/cm^3 ($MMAD_a$ between 1–5 μm) have been reported to exhibit desirable aerodynamic and deep lung deposition properties. Porous particles enhance bioavailability and prolong drug release (40). Thus, the microparticulate formulations developed in this project exhibited aerodynamic properties that are optimal for pulmonary delivery.

Entrapment Efficiency

Microparticles' entrapment efficiencies were determined indirectly by measuring drug content in the supernatant recovered after washing the microparticulate formulations. As can be seen in Fig. 2c, drug entrapment was comparable for all formulations, but did decrease somewhat with increasing HPβCD concentration (40.47±2.39% for CD-2 and 34.05±3.01 for CD-1, compared to 46.77±10.24% for plain particles). The same pattern was observed with other formulations as well, with higher entrapment efficiencies of 52.02±7.69% and 55.79±4.38% with CD-5 and CD-6, respectively. The increased PVA concentration was critical in increasing drug entrapment: CD-2=40.47±2.39% (0.5% PVA); CD-4=50.74±2.51% (1% PVA); and CD-6=55.79±4.38% (2% PVA). This result is incongruent with previous reports suggesting PVA works as an emulsion stabilizer (38, 41).

A decrease in entrapment efficiency with the incorporation of HPβCD in microparticles can be attributed to the bulk introduced by HPβCD in the internal aqueous core. As presented in Table I, a very high molar ratio of PGE_1:HPβCD was used to increase the hydrophilicity of PGE_1, which resulted in reduced drug entrapment due to (i) the increased water solubility of the PGE_1-HPβCD inclusion complex, which makes it more prone to leach out of microparticulate core, and (ii) increased bulk due to HPβCD incorporation. A similar pattern was observed in the calculated percent drug loading (Table III).

Surface Characteristics Determination

Surface characteristics of the prepared microparticulate formulations were determined by the physiosorption-isotherm-based method. As shown in Fig. 3a, the specific surface area of all microparticulate formulations increased as a function of HPβCD concentration, which agrees with our particle size data. BET and BJH isotherms used for surface area determination show a similar pattern. Various reports have suggested that HPβCD, when incorporated in the internal aqueous phase, helps in the development of porous particles (42). Due to the porous nature of the particles, the internal surface area is significantly higher than in nonporous particles, resulting in a porosity-dependent increase in specific surface area (43). As with surface area, pore volume also increased in an HPβCD concentration-dependent manner, with plain PLGA par-

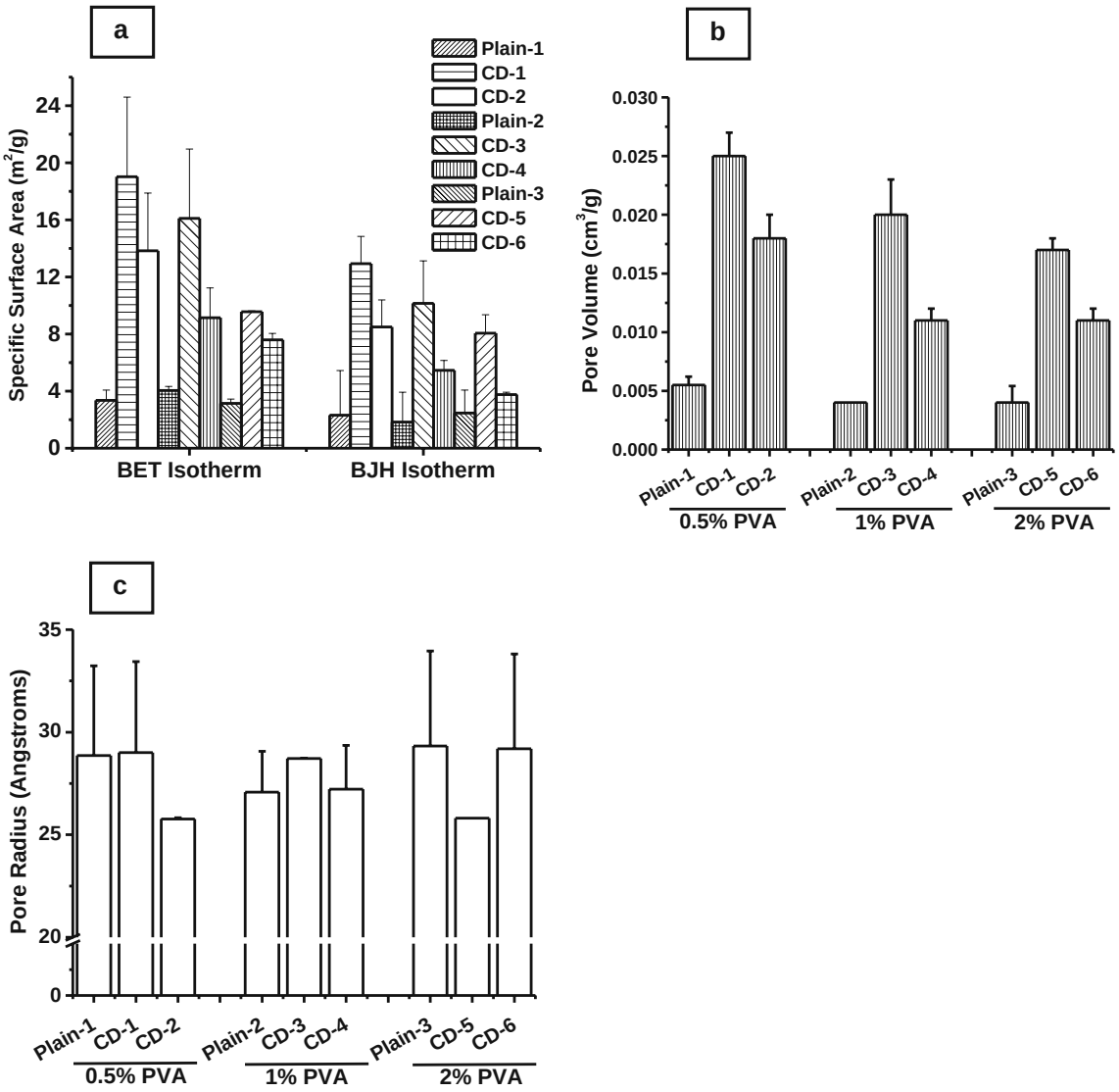

Fig. 3 Physiosorption-based surface characteristics of PGE_1-HPβCD-encapsulated PLGA microparticles. (**a**) Surface area as determined by BET and BJH adsorption isotherms; (**b**) Pore volume (cm^3/g); and (**c**) Pore radius (Angstroms). Data represent mean ± standard deviation ($n = 3$).

ticles being the least porous of them all (Fig. 3b). Similarly, both specific area and pore volume decreased with increasing PVA concentration, which is in agreement with the particle size data presented in Fig. 2. All the microparticulate formulations exhibited comparable pore sizes in the range of 26–30 Å (Fig. 3c), and no significant differences were observed with any of the formulation variables. As depicted in Fig. 1, there were not many surface pores visible in these formulations. Instead, internal pores contributed to the low density, porous nature of the current formulations, as is evident from the surface characteristics. Overall, the physical data reveal HPβCD to be a bulky, porosity-inducing agent with PVA acting as an emulsion stabilizer, both of which modify the physical properties of the microparticles in a concentration-dependent manner.

In Vitro Release Studies in SILF

The release profiles of all microparticulate formulations were studied in SILF, first described by Moss as a solution of electrolytes mimicking the concentrations of ions present in surfactant-rich human lung fluid secreted by Type II alveolar cells (29). This fluid is important in maintaining water surface tension in the lungs by filling the space between alveolar cells. In SILF, citrate ions are used to compensate for the ionic contribution from protein in human alveolar fluid. SILF was used as the release medium for *in vitro* release studies to provide a good estimate of drug released following *in vivo* pulmonary administration of the microparticulate formulations.

Figure 4 illustrates that the *in vitro* drug release profiles for the formulations were significantly different from one

another. Plain PLGA formulations without HPβCD showed very low percent cumulative release, which subsequently decreased with increase in PVA concentration ranging from 23.41±2.95 (Plain-1, 0.5% PVA) to 12.41±1.42% (Plain-3, 2% PVA) in agreement with previous reports. Incorporation of HPβCD resulted in a significant, concentration-dependent increase in percent cumulative release from the microparticulate formulations over a period of 48 hours (Fig. 4a, b, and c). A total of 62.48±6.73% of drug was released from CD-4 (1:4 molar ratio of PGE_1:HPβCD) over 48 hours, and this increased to 68.95±8.77% with CD-3 (1:10 molar ratio) (Fig. 4b). A similar pattern was seen within each pair of formulations having the same PVA concentration. Increase in drug release following HPβCD inclusion can be attributed to the increased hydrophilicity of PGE_1, which arises from its incorporation into a hydrophilic toroid cavity of HPβCD, resulting from the spatial arrangement of primary and secondary hydroxyl groups. As a result of the increase in aqueous solubility, the PGE_1-HPβCD inclusion complex tends to leach out of the microparticulate core into the external aqueous environment. In addition, all the microparticles showed a biphasic drug release, i.e., an initial burst release phase followed by a nearly zero-order drug release phase.

Drug release profiles were analyzed further to calculate (i) surface-associated drug release (drug released at 0 minutes) and (ii) burst release (drug release in first 30 minutes). As shown in Fig. 4d, a surface-associated drug release ranging from 5–10% was apparent for all formulations incorporating cyclodextrin, except CD-4 (19.87±

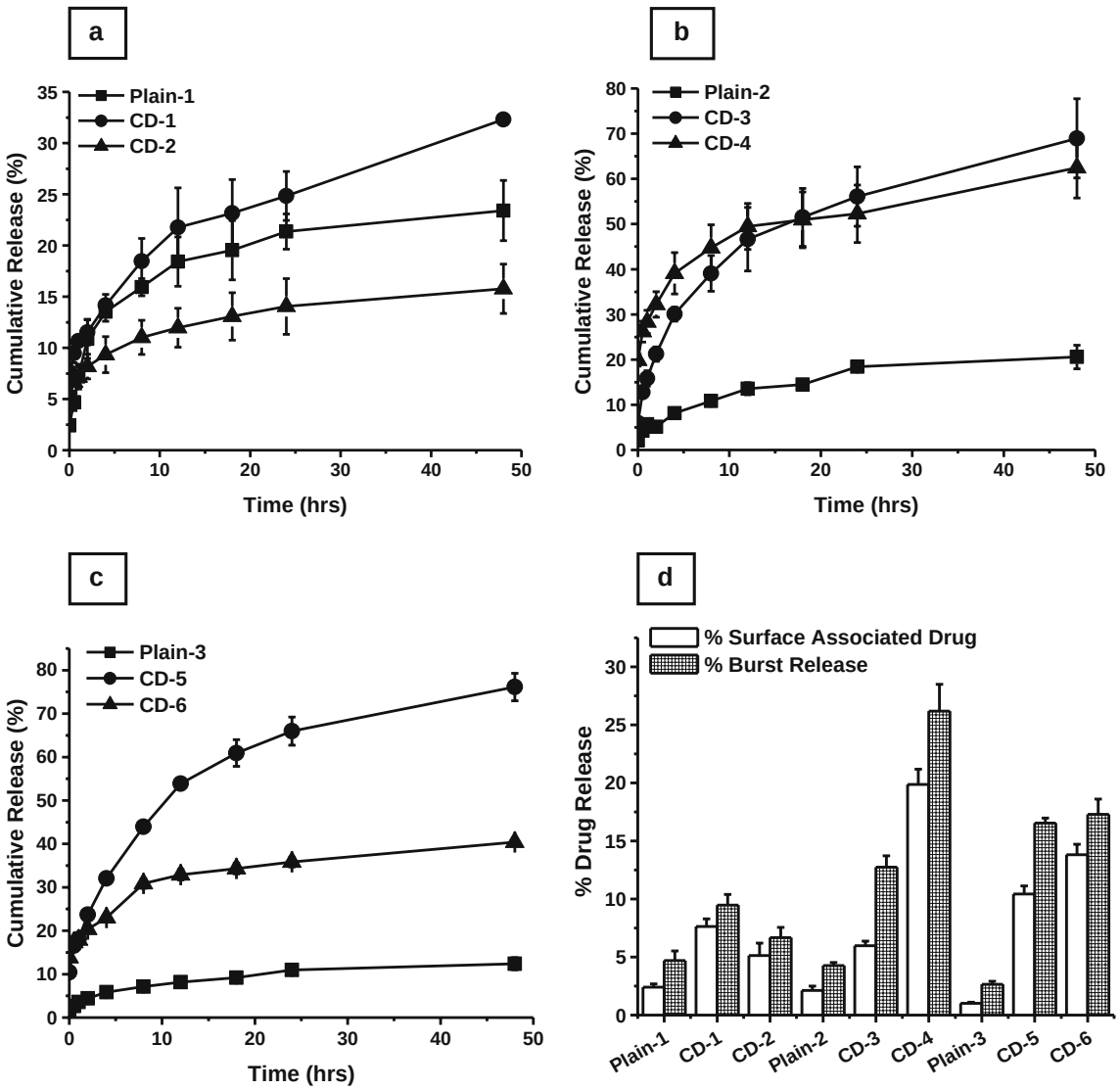

Fig. 4 *In vitro* release profiles of PGE_1-HPβCD-encapsulated PLGA microparticles in simulated interstitial lung fluid (SILF) up to 48 hours. Release profiles of formulations with (**a**) 0.5% w/v PVA; (**b**) 1.0% w/v PVA; and (**c**) 2.0% w/v PVA. (**d**) *In vitro* release kinetics showing release of surface-associated drug and burst release. Data represent mean ± standard deviation ($n=3$).

1.32%) and CD-6 (13.81±0.91%), which suggests the presence of more drug on the surface of CD-4 and CD-6 particles. Similarly, all the formulations showed favorable burst release profiles (ranging from 7–26%) (Fig. 4d). While there are concerns that burst release effect can lead to potential toxicity, such an effect is desirable in medical conditions that require both acute and chronic interventions. In this study, we prepared microparticles of PGE_1 with a goal to provide both immediate and sustained vasodilatory effects in pulmonary arteries. PGE_1 released during the burst release period will provide immediate vasodilation, while the drug entrapped in the core of the microparticles will provide sustained vasodilation of pulmonary arteries. Moreover, plain PGE_1 administered via the IV or pulmonary route is rapidly metabolized in the lungs and thus produces a very short-term vasodilation. However, PGE_1 encapsulated in particulate formulations is continuously released from the formulations over a prolonged period of time and thus replaces the amount of drug metabolized by various lung enzymes. The data obtained from the *in vitro* release studies are vital for characterizing the microparticles, because they were performed under conditions that mimic physiological conditions, providing a useful estimate of the correlation between *in vitro* release and *in vivo* absorption. Overall, microparticulate formulations CD-3 and CD-5 showed optimum flowability, aerodynamic properties, entrapment efficiencies, and favorable drug release with desired burst release effect. These formulations would be expected, therefore, to produce better lung deposition and prolonged release of drug following intratracheal administration.

In Vivo Absorption Studies

The absorption profiles of the optimized formulations (CD-3 and CD-5) were studied in adult male SD rats following intratracheal administration at a physiological dose of 120 μg/kg body weight. As is depicted in Fig. 5a, administration of PGE_1 via the IV or IT route resulted in a rapid increase in PGE_1 concentration in the circulation, with a C_{max} of 134.22±14.52 ng/mL (IV) and 96.85±5.33 ng/mL (IT), respectively. However, the drug disappeared from the circulation very quickly, exhibiting $t_{1/2}$ of 3.5±0.28 min (IV) and 6.49±0.41 min (IT), respectively. Intratracheal administration of PGE_1 resulted in a relative bioavailability of 0.67±0.078, indicating a rapid absorption of the drug in biologically active form into blood via the air-blood barrier (Table IV). When PGE_1-HPβCD complexes (molar ratio 1:4 and 1:10) were administered at equivalent dose via intratracheal route, we observed a significant decrease in C_{max} (1:4 complex=75.28±28.1 ng/mL; 1:10 complex=50.26±18.47 ng/mL), but a significant increase in $t_{1/2}$ for PGE_1 (1:4 complex=30.95±3.26 min; 1:10 complex=18.57±2.27 min). A significant increase in bioavailability was also observed (1:4 complex=0.91±0.12; 1:10 complex=0.86±0.095) relative to that observed with intratracheal administration of plain PGE_1 (0.67±0.078) (Table IV and Fig. 5b).

When PGE_1-HPβCD-encapsulated optimized formulations were administered via the pulmonary route, PGE_1 was measurable in the circulation for up to 8 hours (CD-3) or 12 hours (CD-5) after administration (Fig. 5c). T_{max} values (CD-3=60 minutes; CD-5=30 min) were increased and C_{max} values (CD-3=14.26±2.13 ng/mL; CD-5=22.05±2.4 ng/mL) were slightly lower for optimized microparticulate formulations relative to those for PGE_1 alone or for either PGE_1-HPβCD complex (Fig 5a–c). This was accompanied by a simultaneous and significant increase in $t_{1/2}$ for the particles relative to other formulations (CD-3=249.47±32.56 minutes; CD-5=136.23±15.63 min). Further, the relative bioavailabilities of the microparticulate formulations (CD-3=0.76±0.15; CD-5=0.79±0.14) were comparable to those of the cyclodextrin inclusion complexes (Table IV).

The data indicate formation of a PGE_1-HPβCD inclusion complex results in increased drug bioavailability, which can be attributed to the ability of HPβCD to increase the hydrophilicity of PGE_1, thus enhancing its bioavailability and distribution in blood. There may be several factors that contributed to increased $t_{1/2}$ and reduced C_{max} of PGE_1 in microparticulate formulations. Lungs have alveolar macrophages to clear foreign particles from the respiratory system via phagocytosis. Due to large particle size and low density, the CD-3 and CD-5 formulations were able to elude macrophages, thus prolonging release (40). In addition, time-dependent biodegradation of the PLGA polymeric core also contributes to the prolonged-release property of the formulations (44). However, HPβCD in the microspheric core is responsible for increasing the hydrophilicity of the drug, facilitating its absorption through the air-blood barrier. As we discussed recently (19), prolonged release can be achieved with plain PLGA microparticles of PGE_1, but the C_{max} achieved is significantly lower than that from microparticles encapsulating drug-cyclodextrin inclusion complexes. This phenomenon can be attributed to increased hydrophilicity of PGE_1-cyclodextrin complex, which results in increased absorption of available PGE_1 into the circulation. Overall, both the optimized formulations showed a significantly extended half-life and excellent bioavailability.

Metabolic Degradation Studies in Rat Lung Homogenates

Metabolic degradation studies in rat lung homogenates were used to establish the stability of optimized micro-

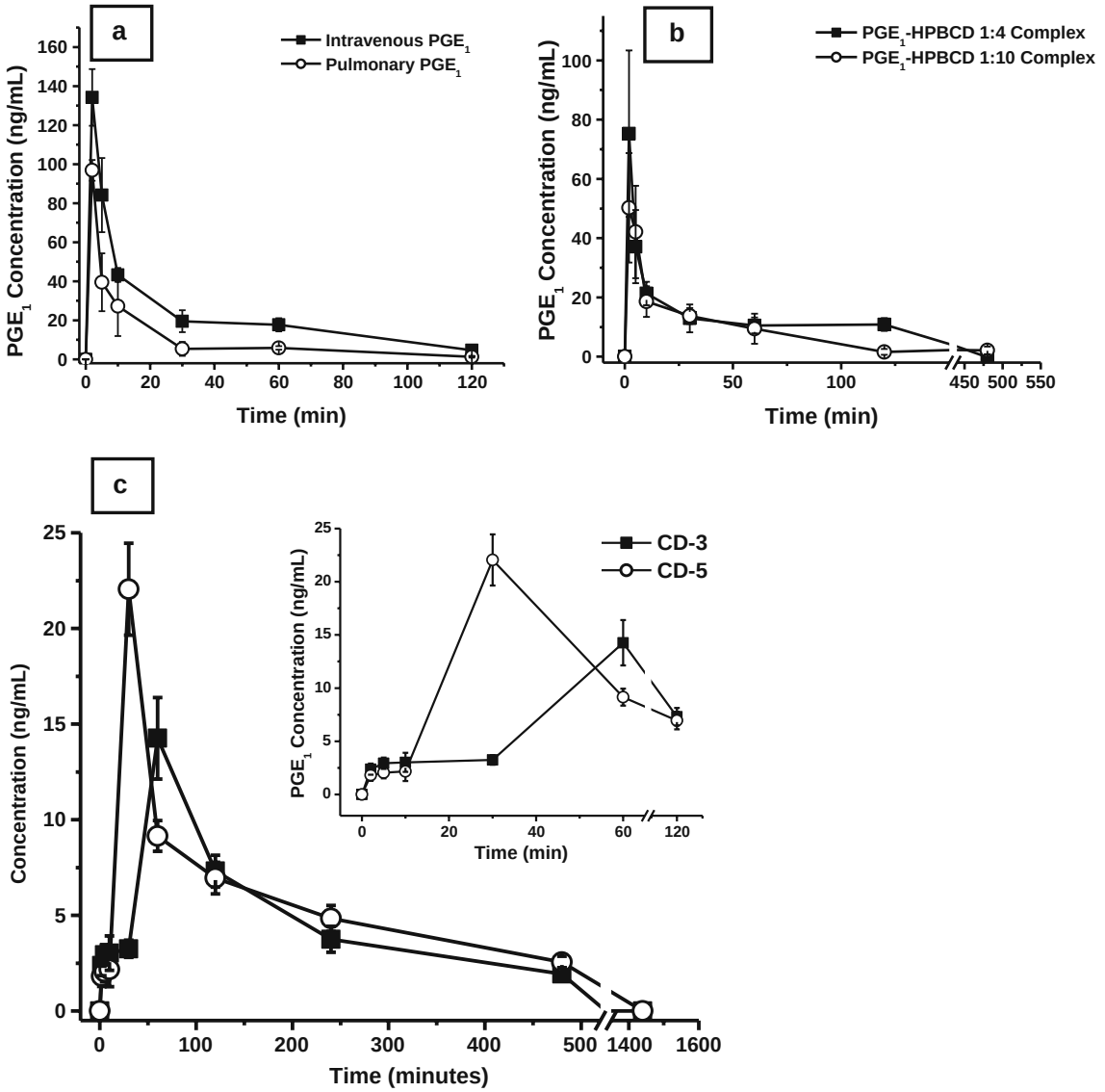

Fig. 5 *In vivo* absorption profiles of (**a**) PGE_1 following intravenous (IV) and intratracheal (IT) administration at a dose of 120 $\mu g/kg$; (**b**) PGE_1-HPβCD complex (molar ratio 1:4 and 1:10; 120 $\mu g/kg$ IT); and (**c**) optimized microspheric formulations of PGE_1-HPβCD complex (CD-3 and CD-5; 120 $\mu g/kg$, IT). Data represent mean ± standard deviation ($n = 6–8$).

Table IV Pharmacokinetic Parameters of PGE_1-HPβCD Complex-Loaded PLGA Microspheric Formulations. Data Represent Mean ± Standard Deviation ($n = 4–6$)

Formulation	C_{max} (ng/mL)	$t_{1/2}$ (min)	AUC_{0-24} (ng/mL*min)	Relative Bioavailability
Plain PGE_1 I.V.	134.22 ± 14.52	3.5 ± 0.28	2883.91 ± 286.4	–
Plain PGE_1 Pulmonary	96.85 ± 25.33	6.49 ± 0.41	1932.22 ± 150.1	0.67 ± 0.078
PGE_1-HPβCD 1:4	75.28 ± 28.1	30.95 ± 3.26	2607.78 ± 200.71	0.91 ± 0.12
PGE_1-HPβCD 1:10	50.264 ± 18.47	18.57 ± 2.27	2466.27 ± 289.71	0.86 ± 0.095
CD-3	14.26 ± 2.13	249.47 ± 32.56	2191.78 ± 178.1	0.76 ± .0.15
CD-5	22.05 ± 2.4	136.23 ± 15.63	2276.9 ± 190.2	0.79 ± 0.14

particulate formulations in a physiological environment. PGE_1 undergoes 70 to 80% oxidation as a result of first-pass metabolism in the lungs. PGE_1 is metabolized by NAD^+-dependent 15-hydroxy prostaglandin dehydrogenase into 13,14-dihydro-PGE_1, a stable PGE_1 metabolite with anti-aggregatory properties. This accounts for its rapid clearance from the lungs and circulation (45, 46). When incubated at a concentration of 10 μg/mL with rat lung homogenates, PGE_1 is rapidly cleared/degraded with almost no drug detectable after 2 hours of incubation at 37°C (8.5±1.28 μg/mL at time zero; 0.211±0.02 μg/mL after 120 minutes; Fig. 6a). However, when optimized PLGA microspheres encapsulating PGE_1-HPβCD inclusion complex were incubated at an equivalent dose, PGE_1 in the homogenate remained elevated for more than 8 hours, indicating a time-dependent release of PGE_1 from the core of polymeric microspheres. The mean PGE_1 level from CD-3 was 3.58±0.03 μg/mL at time zero, decreasing slightly to 2.81±0.13 μg/mL after 8 hours. PGE_1 from CD-5 increased from 2.86±0.14 μg/mL at time zero to 4.79±0.8 μg/mL after 8 hours (Fig. 6b).

The polymeric core of the microspheres is believed to degrade in a time-dependent manner to provide continuous availability of PGE_1 in the homogenate. To further confirm this hypothesis, we measured PGE_1 from microspheres incubated with lung homogenate. As shown in Fig. 6c, unreleased PGE_1 from microspheres decreased over the course of experiment, indicating a constant rate of release of PGE_1 from microspheres. For CD-3, the PGE_1 concentration in microspheres decreased from 6.42±0.64 μg/mL at time zero to 0.69±0.17 μg/mL at 8 hours. Similarly, PGE_1 concentration for CD-5 decreased from 5.61±1.28 μg/mL at time zero to 0.93±0.14 μg/mL at 8 hours (Fig. 6c). On the whole, the rat lung homogenate metabolic degradation data suggest that polymeric microspheres release PGE_1 continuously, supporting the view that the microspheres protect unreleased PGE_1 from first-pass metabolism in lungs. This finding is consistent with the

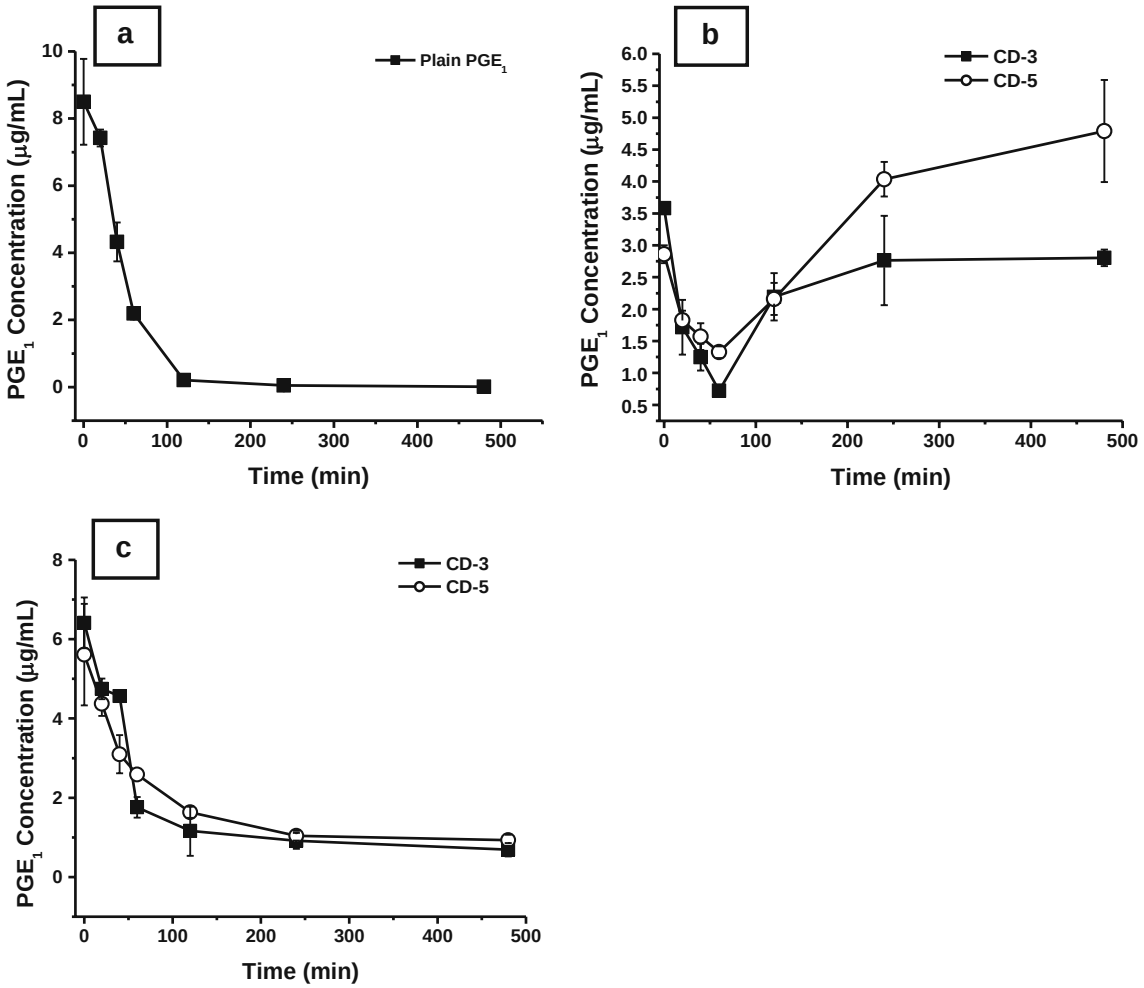

Fig. 6 Metabolic degradation studies of PGE_1 in rat lung homogenate. Concentration of PGE_1 in lung homogenate upon incubation of (**a**) 10 μg/mL PGE_1; (**b**) Optimized formulations CD-3 and CD-5 containing 10 μg/mL PGE_1; and (**c**) The amount of drug extracted from CD-3 and CD-5 microspheres incubated in lung homogenates. Data represent mean ± standard deviation ($n=3$).

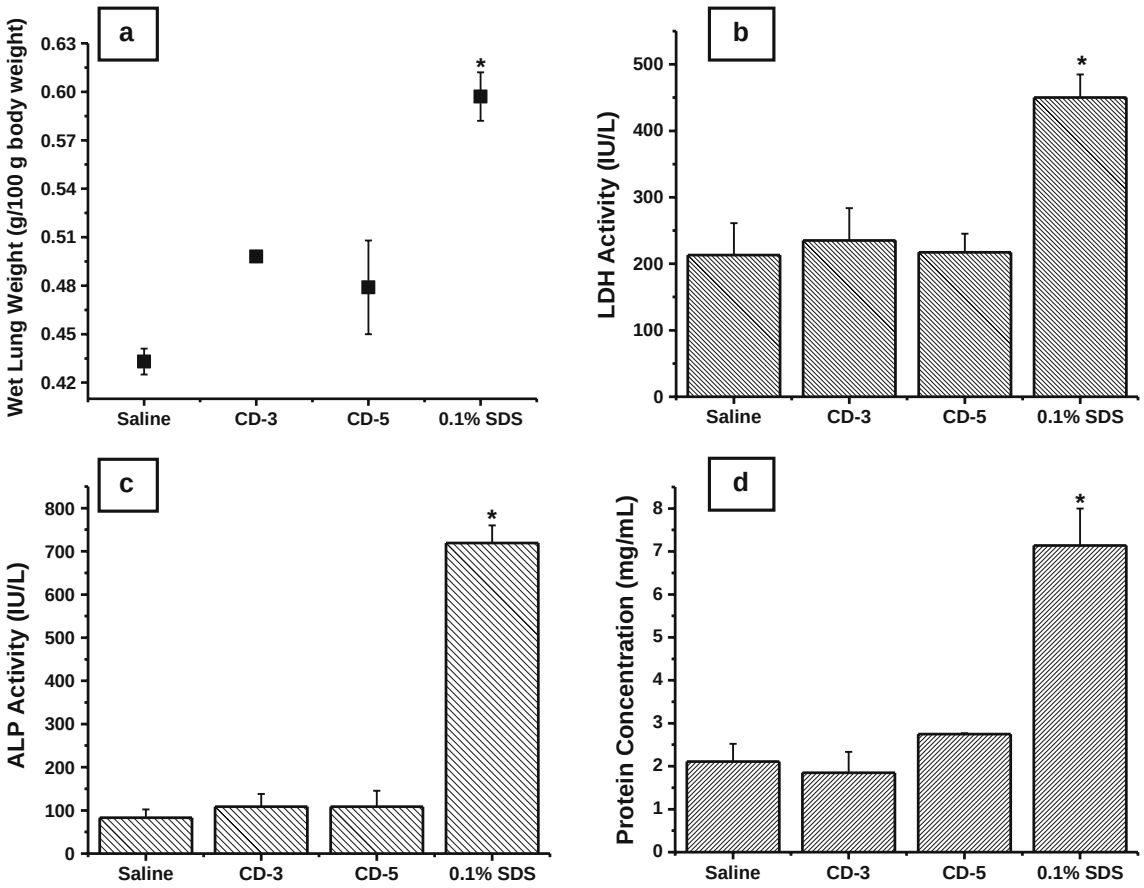

Fig. 7 Acute *in vivo* toxicity studies of CD-3 and CD-5 in bronchoalveolar lavage (BAL) fluid 12 hours after administration. (**a**) Corrected wet lung weights (g/100 g body weight); Levels of (**b**) LDH activity (IU/L); (**c**) ALP activity (IU/L); and (**d**) Total protein concentration (mg/mL). *Means are significantly different from each other ($p < .05$).

in vitro release data and *in vivo* absorption profiles of CD-3 and CD-5.

Bronchoalveolar Lavage (BAL) Fluid Analysis

We assessed the acute, *in vivo* safety of the optimized microparticulate formulations by analyzing lungs and BAL fluid collected from CD-3 and CD-5-treated rats for presence of edema, protein concentration, and levels of the lung injury markers LDH and ALP. Saline- and SDS-treated rats were used as negative and positive controls, respectively. An increase in the wet lung weight is an indication of extracellular fluid accumulation in the epithelial cell lining of the respiratory wall. The presence of LDH in BAL fluid is an indication of cell damage and lysis (47), and the presence of ALP represents alveolar type II cell proliferation due to type I cell damage (48). An increase in total protein concentration is indicative of inflammation and rupture of the alveolar capillary barrier (49).

As can be seen in Figs. 7a, the wet weights of lungs from CD-3- and CD-5-treated rats were higher than those from saline-treated rats, but were significantly lower those from

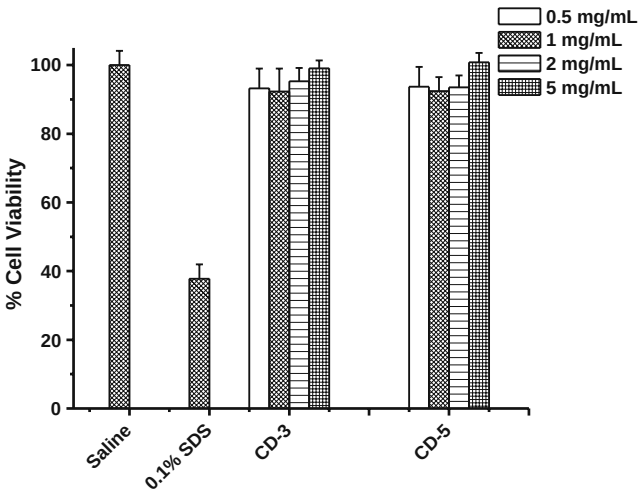

Fig. 8 Acute cytotoxicity studies to determine the safety of CD-3 and CD-5 microspheric formulations in Calu-3 lung epithelial cell lines. Data represent mean ± standard deviation ($n = 8$).

SDS-treated rats. Some of the edema can be attributed to stress related to intratracheal administration and anesthesia. No significant changes in the levels of either LDH (Fig. 7b), ALP (Fig. 7c), or total protein content (Fig. 7d) were observed with optimized microparticulate formulations. In contrast, SDS resulted in significant increase in the levels of all three. These data suggest that CD-3 and CD-5 may be safe for the noninvasive pulmonary delivery of PGE_1. However, long-term studies with both CD-3 and CD-5 are required to provide a better understanding of pathological changes occurring during repeated administration.

Cell Viability Studies

Cell viability studies were performed using Calu-3 human airway epithelial cells to determine the effects of microparticulate formulations and excipients on pulmonary epithelial lining following acute exposure. These studies used the propidium iodide fluorescence exclusion assay as described by Nieminen *et al.* (32). Propidium iodide is a fluorescent intercalating dye that binds double-stranded nucleic acids from dead cells and is excluded from viable cells. When bound to nucleic acid, it undergoes a red shift and a 20- to 30-fold increase in fluorescence intensity. As shown in Fig. 8, 100% cell viability was observed with saline treatment (negative control), whereas viability decreased to 37.79± 4.17% following SDS treatment (positive control). Neither optimized formulation resulted in any significant loss of cell viability at any concentration over the 4-hour exposure, with cell viability remaining within the 90-100% window (CD-3: 92.29±6.69% to 99.03±2.31%; CD-5: 92.41±4.11% to 100.81±2.74%). HPβCD is a relatively non-toxic derivative of β-CD, with a benign toxicity profile. PLGA has been approved by FDA as a safe polymer for drug delivery. It has been suggested that transport of cyclodextrins (height of toroid 0.79 nm) across the alveolar epithelium is mediated by an abundance of large equivalent pores of 6 nm radius, which facilitate passage of small hydrophilic molecules < 5 nm in size by passive diffusion without damaging the tight junctions (50). Moreover, it was reported recently that HPβCD is one of the safest cyclodextrins for pulmonary drug delivery applications due to its non-toxic interaction with airway epithelial cells (51). Hence, PGE_1-HPβCD-encapsulated PLGA microparticles can be considered suitable for the pulmonary delivery of PGE_1.

In summary, this study is one of the very few studies to investigate the suitability of PGE_1-HPβCD-encapsulated PLGA microparticles for the pulmonary delivery of PGE_1, as a potential medication for pulmonary arterial hypertension (PAH). Our data suggest that it is feasible to encapsulate PGE_1-HPβCD complex in PLGA microspheres to produce internally porous microspheres that exhibit favorable particulate properties and entrapment efficiencies and meet most of the micromeritical standards for noninvasive pulmonary administration. Microspheric formulations provided enhanced drug release from the particles under physiological conditions in simulated interstitial lung fluid (SILF) and also provided an extended biological half-life when tested *in-vivo* in rodents. Further, optimized microparticulate formulations offer protection against metabolic degradation of PGE_1 and appear to be safe for pulmonary administration on the basis of both *in vivo* and *in vitro* studies. Additional studies are currently underway in our laboratory to establish the efficacy of optimized microparticulate formulations in a rodent model of PAH.

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