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Poly(Glycerol Adipate-co- ω -Pentadecalactone) Spray-Dried Microparticles as Sustained Release Carriers for Pulmonary Delivery

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9		Organization	Liverpool John Moores University
10		Division	School of Pharmacy & Biomolecular Sciences
11		Address	James Parsons Building, Byrom Street, Liverpool L3 3AF, UK
12		e-mail	I.saleem@ljmu.ac.uk
13		Author	Family Name
14	Particle		
15	Given Name		Hesham
16	Suffix		
17	Organization		Liverpool John Moores University
18	Division		School of Pharmacy & Biomolecular Sciences
19	Address		James Parsons Building, Byrom Street, Liverpool L3 3AF, UK
20	Organization		Assiut University
21	Division		Department of Industrial Pharmacy, Faculty of Pharmacy
22	Address	Assiut , Egypt	
23	e-mail		
24	Author	Family Name	Khidr
25		Particle	
26		Given Name	Sayed
27		Suffix	
28		Organization	Assiut University
29		Division	Department of Industrial Pharmacy, Faculty of Pharmacy
30		Address	Assiut , Egypt
31		e-mail	

32		Family Name	Samy
33		Particle	
34		Given Name	Eman
35		Suffix	
36	Author	Organization	Assiut University
37		Division	Department of Industrial Pharmacy, Faculty of Pharmacy
38		Address	Assiut , Egypt
39		e-mail	
40		Family Name	Ahmed
41		Particle	
42		Given Name	Sayed
43		Suffix	
44	Author	Organization	Assiut University
45		Division	Department of Industrial Pharmacy, Faculty of Pharmacy
46		Address	Assiut , Egypt
47		e-mail	
48		Family Name	Murphy
49		Particle	
50		Given Name	Mark
51		Suffix	
52		Organization	Liverpool John Moores University
53	Author	Division	School of Pharmacy & Biomolecular Sciences
54		Address	James Parsons Building, Byrom Street, Liverpool L3 3AF, UK
55		Organization	Liverpool John Moores University
56		Division	General Engineering Research Institute
57		Address	Liverpool , UK
58		e-mail	
59		Family Name	Mohammed
60		Particle	
61		Given Name	Afzaal
62		Suffix	
63	Author	Organization	Aston University
64		Division	Aston Pharmacy School
65		Address	Birmingham , UK
66		e-mail	
67		Family Name	Shabir
68		Particle	
69		Given Name	Anjum

70		Suffix	
71		Organization	Aston University
72	Author	Division	Aston Pharmacy School
73		Address	Birmingham , UK
74		e-mail	
75		Family Name	Hutcheon
76		Particle	
77		Given Name	Gillian
78		Suffix	
79	Author	Organization	Liverpool John Moores University
80		Division	School of Pharmacy & Biomolecular Sciences
81		Address	James Parsons Building, Byrom Street, Liverpool L3 3AF, UK
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86	Abstract	<p>Purpose: The aim of this work was to optimize biodegradable polyester poly (glycerol adipate-co-ω-pentadecalactone), PGA-co-PDL, microparticles as sustained release (SR) carriers for pulmonary drug delivery.</p> <p>Methods: Microparticles were produced by spray drying directly from double emulsion with and without dispersibility enhancers (L-arginine and L-leucine) (0.5–1.5%w/w) using sodium fluorescein (SF) as a model hydrophilic drug.</p> <p>Results: Spray dried microparticles without dispersibility enhancers exhibited aggregated powders leading to low fine particle fraction (%FPF) (28.79 ± 3.24), fine particle dose (FPD) ($14.42 \pm 1.57 \mu\text{g}$), with a mass median aerodynamic diameter (MMAD) $2.86 \pm 0.24 \mu\text{m}$. However, L-leucine was significantly superior in enhancing the aerosolization performance (L-arginine:% FPF 27.61 ± 4.49–26.57 ± 1.85; FPD 12.40 ± 0.99–$19.54 \pm 0.16 \mu\text{g}$ and MMAD 2.18 ± 0.35–$2.98 \pm 0.25 \mu\text{m}$, L-leucine:%FPF 36.90 ± 3.6–43.38 ± 5.6; FPD 18.66 ± 2.90–$21.58 \pm 2.46 \mu\text{g}$ and MMAD 2.55 ± 0.03–$3.68 \pm 0.12 \mu\text{m}$). Furthermore, incorporating L-leucine (1.5%w/w) reduced the burst release ($24.04 \pm 3.87\%$) of SF compared to unmodified formulations ($41.87 \pm 2.46\%$), with both undergoing a square root of time (Higuchi's pattern) dependent release. Comparing the toxicity profiles of PGA-co-PDL with L-leucine (1.5%w/w) (5 mg/ml) and poly(lactide-co-glycolide), (5 mg/ml) spray dried microparticles in human bronchial epithelial 16HBE14o-cell lines, resulted in cell viability of 85.57 ± 5.44 and $60.66 \pm 6.75\%$ respectively, after 72 h treatment.</p> <p>Conclusion: The above data suggest that PGA-co-PDL may be a useful polymer for preparing SR microparticle carriers, together with dispersibility enhancers, for pulmonary delivery.</p>	
87	Keywords separated by ' - '	dry powder inhalation - microparticles - polyester polymers - pulmonary drug delivery - sustained drug release	
88	Foot note information		

Poly(Glycerol Adipate-co- ω -Pentadecalactone) Spray Dried Microparticles as Sustained Release Carriers for Pulmonary Delivery

Hesham Tawfeek · Sayed Khidr · Eman Samy · Sayed Ahmed · Mark Murphy · Afzaal Mohammed · Anjum Shabir · Gillian Hutcheon · Imran Saleem

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ABSTRACT

Purpose The aim of this work was to optimize biodegradable polyester poly(glycerol adipate-co- ω -pentadecalactone), PGA-co-PDL, microparticles as sustained release (SR) carriers for pulmonary drug delivery.

Methods Microparticles were produced by spray drying directly from double emulsion with and without dispersibility enhancers (L -arginine and L -leucine) (0.5–1.5%w/w) using sodium fluorescein (SF) as a model hydrophilic drug.

Results Spray dried microparticles without dispersibility enhancers exhibited aggregated powders leading to low fine particle fraction (%FPF) (28.79 ± 3.24), fine particle dose (FPD) ($14.42 \pm 1.57 \mu\text{g}$), with a mass median aerodynamic diameter (MMAD) $2.86 \pm 0.24 \mu\text{m}$. However, L -leucine was significantly superior in enhancing the aerosolization performance (L -arginine:%FPF 27.61 ± 4.49 – 26.57 ± 1.85 ; FPD 12.40 ± 0.99 – $19.54 \pm 0.16 \mu\text{g}$ and MMAD 2.18 ± 0.35 – $2.98 \pm 0.25 \mu\text{m}$, L -leucine:%FPF 36.90 ± 3.6 – 43.38 ± 5.6 ; FPD 18.66 ± 2.90 –

$21.58 \pm 2.46 \mu\text{g}$ and MMAD 2.55 ± 0.03 – $3.68 \pm 0.12 \mu\text{m}$). Furthermore, incorporating L -leucine (1.5%w/w) reduced the burst release ($24.04 \pm 3.87\%$) of SF compared to unmodified formulations ($41.87 \pm 2.46\%$), with both undergoing a square root of time (Higuchi's pattern) dependent release. Comparing the toxicity profiles of PGA-co-PDL with L -leucine (1.5%w/w) (5 mg/ml) and poly(lactide-co-glycolide), (5 mg/ml) spray dried microparticles in human bronchial epithelial 16HBE14o- cell lines, resulted in cell viability of 85.57 ± 5.44 and $60.66 \pm 6.75\%$ respectively, after 72 h treatment.

Conclusion The above data suggest that PGA-co-PDL may be a useful polymer for preparing SR microparticle carriers, together with dispersibility enhancers, for pulmonary delivery.

KEY WORDS dry powder inhalation · microparticles · polyester polymers · pulmonary drug delivery · sustained drug release

H. Tawfeek · M. Murphy · G. Hutcheon · I. Saleem (✉)
School of Pharmacy & Biomolecular Sciences
Liverpool John Moores University
James Parsons Building, Byrom Street
Liverpool L3 3AF, UK
e-mail: i.saleem@ljmu.ac.uk

H. Tawfeek · S. Khidr · E. Samy · S. Ahmed
Department of Industrial Pharmacy, Faculty of Pharmacy
Assiut University
Assiut, Egypt

M. Murphy
General Engineering Research Institute
Liverpool John Moores University
Liverpool, UK

A. Mohammed · A. Shabir
Aston Pharmacy School, Aston University
Birmingham, UK

INTRODUCTION

Poly(glycerol adipate-co- ω -pentadecalactone), PGA-co-PDL, is a biodegradable polyester polymer synthesized via lipase enzyme, ~~from candida albicans~~, catalyzed ring opening co-polymerization reaction of activated diacid, glycerol and lactone monomers (1). This polymer is synthesized by a one step reaction via a single non-biosynthetic pathway under mild reaction conditions (2), compared to fermentation and other chemical processes that have been extensively studied for the synthesis of biodegradable aliphatic polyesters (3). In addition, these polymers are designed to overcome the lack of chemical functionality associated with poly(lactic acid) (PLA) and its derivatives, due to the presence of pendant hydroxyl groups from the glycerol monomer in the PGA-co-PDL polymer, which permit the attachment of chemical

61 moieties such as pharmaceutically active drugs. Further- 114
62 more, the degree of hydrophilicity can be altered by 115
63 varying the backbone chemistry (4). Previously PGA-co- 116
64 PDL has been formulated as microparticles for delivery of 117
65 dexamethasone phosphate and ibuprofen (5,6), and inves- 118
66 tigated in our group for delivery of macromolecules using 119
67 α -chymotrypsin as a model protein (7). In the current 120
68 investigation, we propose using these polyester polymers as 121
69 pulmonary carriers for sustained delivery (SR) of therapeu- 122
70 tic agents to the lungs. 123

71 Pulmonary drug delivery is an attractive, convenient and 124
72 effective route for the administration of therapeutic drugs, 125
73 macromolecules (8) proteins and peptides (9), and is an 126
74 alternative for the treatment of many pulmonary disorders, 127
75 such as, lung cancer (10) and cystic fibrosis (11) enhancing 128
76 the pharmacokinetic effect of the therapeutic agent. Dry 129
77 powder inhalers (DPIs) are commonly used as they are 130
78 portable and less expensive compared to nebulizers, and 131
79 are considered to be environmentally friendly due to the 132
80 absence of propellant, as well as overcoming the synchron- 133
81 ization problems associated with pressurized metered dose 134
82 inhalers (pMDIs) (12,13). Furthermore, there is improved 135
83 stability in storage for therapeutic agents formulated as dry 136
84 powders (13). 137

85 Lately, research has focused on protecting the therapeu- 138
86 tic agent from degradation or premature clearance by a 139
87 suitable delivery system, and using the lungs as a portal for 140
88 sustained drug release and absorption over many hours to 141
89 days. SR therapeutic agents can reduce side effects and the 142
90 frequency of administration, hence increasing patient 143
91 acceptability and compliance (14,15). However, the clear- 144
92 ance mechanisms of the lung towards foreign particles are 145
93 likely to jeopardize the potential of a SR formulation to 146
94 release therapeutic agents over extended periods. Therefore 147
95 to achieve a SR effect, pulmonary formulations should 148
96 possess a small mass median aerodynamic diameter 149
97 (MMAD) and high fine particle fraction (%FPF) in order 150
98 to minimize central/tracheobronchial deposition and by- 151
99 pass the effects of mucociliary clearance (16). This has 152
100 generally been achieved using polymeric particles such as, 153
101 poly(ether-anhydride) and ~~poly(lactic-co-glycolic-acid)~~, PLGA, 154
102 as carriers for pulmonary delivery to achieve sustained or 155
103 controlled release of the intended therapeutic agent (17– 156
104 21). However, PLGA and PLA have many shortcomings, 157
105 such as, the polymer backbone cannot be chemically 158
106 functionalized, stability of macromolecules are affected 159
107 due to the degradation of PLGA and PLA polymers to its 160
108 acidic monomers, (22), and are often associated with drug 161
109 release in a triphasic manner (22,23). This is partly due to 162
110 the fact that PLGA and PLA were not specifically designed 163
111 for use in the lungs. Thus, a new polymer which overcomes 164
112 these problems is imperative in the formulation of carriers 165
113 for pulmonary delivery. 166

Previously; we investigated the aerosol performance of 114
PGA-co-PDL microparticles prepared via the emulsion 115
solvent evaporation technique (w/o/w) using sodium 116
fluorescein as a model drug (24). This study emphasized 117
the aggregated properties of the produced microparticles as 118
the %FPF did not exceed 15% (24). Consequently, this 119
investigation aims to enhance the respirable fraction and 120
maximize the drug deposition in the lung, using sodium 121
fluorescein (SF) as a model hydrophilic drug, via spray 122
drying from double emulsion (20,25). Furthermore, the 123
addition of various dispersing agents, such as L-arginine and 124
L-leucine amino acids, as potential dispersibility enhancers 125
(26,27) to improve the aerosol performance was investigat- 126
ed. In addition, to ensure the safety of PGA-co-PDL a 127
toxicity study was also performed in normal human 128
bronchial epithelium cell lines utilizing the MTT assay 129
with comparison to spray dried PLGA microparticles. 130

131 MATERIALS AND METHODS

132 Materials

Novozyme 435 (a lipase from ~~Candida antarctica~~, immobi- 133
lized on a microporous acrylic resin) was purchased from 134
Biocatalytics, USA. ω -pentadecalactone, sodium fluorescein 135
(SF), poly(vinyl alcohol) (PVA, 9–10 KMw, 80%), L-leucine 136
and L-arginine, RPMI-1640 medium with L-glutamine and 137
NaHCO₃, thiazoly blue tetrazolium bromide (MTT), poly 138
(DL-lactide-co-glycolide) (PLGA) (50:50) inherent viscosity 139
0.15–0.25, were obtained from Sigma-Aldrich, UK. 140
Dichloromethane (DCM) was purchased from BDH labo- 141
ratory supplies, UK. Tetrahydrofuran (THF), 75 cm²/ 142
tissue culture flask with vented cap, 24 well tissue culture 143
plates, 96 well flat bottom plates, Antibiotic/Antimycotic 144
Solution (100X) were purchased from Fisher Scientific, 145
UK. Divinyl adipate was obtained from Fluorochem, UK 146
and Foetal Calf Serum (FCS) heat inactivated was 147
purchased from Biosera UK. 16HBE14o- cells were 148
produced by Dr Dieter Gruenert from the California 149
Pacific Medical Center, University of California San 150
Francisco, USA. 151

152 Polymer Synthesis

The co-polymer PGA-co-PDL was synthesized via enzyme 153
catalyzed condensation and ring opening co-polymerization 154
reactions as described by Thompson *et al.* (28). The 155
synthesized polymer was characterized by gel permeation 156
chromatography, GPC (Viscotek TDA Model 300 using 157
OmniSEC3 operating software), calibrated with polysty- 158
rene standards (polystyrene standards kit, Supelco, USA), 159
and H¹-NMR spectroscopy (Bruker AVANCE 300, Inverse 160

161 probe with B-ACS 60, Autosampler with gradient chemm-
 162 ing) as described by Thompson *et al.* (28).

163 **Microparticles Preparation**

164 PGA-co-PDL microparticles were prepared by spray drying
 165 directly from double emulsion (w/o/w). Briefly, 5 mg SF
 166 was dissolved in 1.5 ml distilled water and homogenized
 167 (IKA yellowline DI 25 basic at 8000 rpm for 3 min) in
 168 13 ml DCM containing 390 mg polymer to form the first
 169 w/o emulsion. This was gradually added to the second
 170 aqueous phase, 135 ml distilled water containing 1%w/v
 171 PVA as an emulsifier, under moderate stirring conditions
 172 (Silverson LART mixer, 2000 rpm at room temperature,
 173 25°C) to form the w/o/w emulsion (PGA-co-PDL, control).
 174 L-arginine (0.5, 1, 1.5%w/w of polymer weight) (Repre-
 175 sented in text as: PGA-co-PDL, 0.5% Arg; PGA-co-PDL,
 176 1% Arg and PGA-co-PDL, 1.5% Arg) and L-leucine (0.5, 1,
 177 1.5%w/w of polymer weight) (Represented in text as: PGA-
 178 co-PDL, 0.5% Leu; PGA-co-PDL, 1% Leu and PGA-co-
 179 PDL, 1.5% Leu) were incorporated into the second
 180 aqueous phase in addition to PVA. The produced emulsion
 181 was spray dried at room temperature (25°C) utilizing a
 182 mini-spray dryer (Büchi, B-290 Flawil, Switzerland) with
 183 standard two-fluid nozzle (0.7 mm diameter), inlet and
 184 outlet temperature of 100 and 47°C respectively, a pump
 185 flow rate of 5–7 ml/min, aspirator at 38 m³/h and air flow
 186 at 600 L/h. Control spray dried PLGA microparticles
 187 incorporating L-leucine (1.5%w/w, PLGA, 1.5% Leu), for
 188 comparison to optimum PGA-co-PDL microparticles, were
 189 produced as above.

190 **Microparticles Characterization**

191 *Yield, Encapsulation Efficiency and Drug Loading*

192 10 mg of spray dried microparticle formulations were
 193 weighed and solubilized in DCM/water mixture (2:1) to
 194 dissolve the polymer and extract SF. The two phases were
 195 separated by centrifugation (5 min at 16200 X g, accuSpin
 196 Micro 17) and the aqueous layer analyzed for SF using
 197 spectroscopy at 273 nm. The yield of spray dried micro-
 198 particles was quantified as a percentage mass of expected
 199 total powder yield (n=6). The percentage encapsulation
 200 efficiency (EE) and drug loading were determined for all
 201 batches using Eqs. 1 and 2 respectively (n=6):

$$EE(\%) = \left(\frac{\text{actual weight of SF in sample}}{\text{theoretical weight of SF}} \right) \times 100 \quad (1)$$

204

$$Drug\ Loading = \frac{\text{weight of SF in microparticles}}{\text{microparticles sample weight}} \quad (2)$$

206

*Particle Size, Zeta Potential, Powder Density and Primary
 Aerodynamic Diameter*

207
208

100 μ l microparticle suspension was diluted to 5 ml using
 double distilled water and the measurements recorded at
 25°C (n=3) to determine the geometric particle size and
 zeta potential using a Zetaplus, Brookhaven Instruments,
 U.K. The poured density of spray dried microparticle
 powders were determined by adding approximately 0.5 g of
 powder to a 10 ml graduated cylinder and recording the
 volume. The tapped density was determined by tapped
 density measurements on the same samples in a 10 ml
 graduated measuring cylinder until constant volume was
 obtained (29) (n=3). Carr's Index values for each of the
 spray dried formulations were calculated according to Eq. 3
 (30), and can provide an indication of powder flow. Carr's
 Index flowability: 5–12%, excellent; 12–18%, good; 18–
 21%, fair; 21–25%, poor, fluid; 25–32%, poor, cohesive;
 32–38%, very poor; >40%, extremely poor. A value less
 than 25% indicates a fluid powder, whereas a value greater
 than 25% indicates a cohesive powder (31).

$$Carr's\ Index(\%) = \frac{\text{Tapped density} - \text{Poured density}}{\text{Tapped density}} \times 100 \quad (3)$$

Theoretical primary aerodynamic diameter (d_{ae}) was
 calculated using data acquired from geometric particle size
 (d) and tapped density (ρ) according to Eq. 4 (32).

$$d_{ae} = d \sqrt{\frac{\rho}{\rho_1}} \quad \rho_1 = 1\text{ g cm}^{-3} \quad (4)$$

Amorphous Nature and Water Content

The degree of amorphous material from the spray dried
 formulations were performed using differential scanning
 calorimetry (DSC, Perkin Elmer Pyris 1). Briefly, 3–5 mg of
 sample was placed into a hermetically sealed and crimped pan.
 The samples were subjected to two scanning programs in the
 DSC using a heating rate of 20°C/min purged with nitrogen at
 20 ml/min as described previously by Thompson *et al.* (6). The
 weight loss of the powders as a function of temperature
 was determined using a thermogravimetric analyser
 (TGA 2050-Thermogravimetric analyzer, UK). Approx-
 imately 6–8 mg of each sample was weighed in a
 platinum pan and heated at the temperature range 25–
 260°C using a scanning rate of 10°C/min purged under
 nitrogen at 20 ml/min (n=3).

Particle Morphology

The spray dried microparticles were visualized by scanning
 electron microscopy (FEI—Inspect S Low VAC Scanning

252 Electron Microscope). Particles were mounted on aluminium stubs (pin stubs, 13 mm) layered with a sticky conductive carbon tab and coated in gold (10–15 nm) using an EmiTech K 550X Gold Sputter Coater, 25 mA for 3 min. Confocal images were obtained using a Zeiss 510 Meta laser scanning microscope, mounted on a Axiovert 200 M BP computer-controlled inverted microscope. A small amount of spray dried microparticles were placed onto a cover glass chamber slide (Fisher Scientific, UK), and imaged by excitation with an argon ion laser at a wavelength of 488 nm and a Plan Neofluar 63×/0.30 numerical aperture (NA) objective lens. Image analysis was carried out using the Zeiss LSM software.

265 **In-Vitro Aerosolisation Studies**

266 The aerosol performance of spray dried microparticles was determined using a Next Generation Impactor (NGI). 267 Microparticle samples (~20 mg) were manually loaded into 268 hydroxypropyl methylcellulose capsules (HPMC size 2), and 269 placed in a HandiHaler® (Boehringer Ingelheim, Ingel- 270 heim, Germany). A pump (Copley Scientific, Nottingham, 271 UK) was operated at a flow rate of 60 L/min for 4 s and 272 the NGI plates were coated with 1% w/w glycerol/methanol solution. Following inhalation all parts of NGI were 273 washed with DCM/water (2:1), and analyzed as above. 274 The fine particle fraction (%FPF) (defined as the mass of 275 drug deposited ($d_{ac} < 4.6 \mu\text{m}$), expressed as a percentage of 276 the emitted dose), mass median aerodynamic diameter 277 (MMAD) (33), and the fine particle dose (FPD), expressed 278 as the mass of drug deposited in the NGI ($d_{ac} < 4.6 \mu\text{m}$), 279 was determined ($n=3$).

282 **In-Vitro Release Studies**

283 10 mg of spray dried microparticle formulations were 284 added to 1.5 ml microtubes, containing 1 ml phosphate 285 buffer saline pH 7.4 ($n=3$), and incubated at 37°C on an 286 orbital shaker (IKA KS 130) at 250 rpm. The supernatants 287 were collected to observe the release of SF over 24 h by 288 centrifugation (5 min at 16200 X g, accuSpin Micro 17) and 289 analysed using spectroscopy as above. The cumulative drug 290 release was assessed in different release models namely zero 291 order, first order and Higuchi's square root plot, and a 292 correlation coefficient close to unity was used as the 293 mechanism and order of release (34).

294 **Toxicity Study**

295 The toxicity profiles of PGA-co-PDL (control) and PGA-co- 296 PDL, 1.5% Leu were evaluated over 24 h in normal human 297 bronchial epithelial (16HBE14o-) cell line, and compared 298 to spray dried PLGA, 1.5% Leu microparticles.

16HBE14o- cells (passage No. 28) were cultured in 24 well 299 plates with 1 ml RPMI-1640 medium supplemented with 300 10% FCS/1% Antibiotic/Antimycotic solution for 24 h in 301 a humidified 5% CO₂/95% incubator at 37°C. The wells 302 were replaced with fresh medium (1 ml) containing PGA- 303 co-PDL, PGA-co-PDL, 1.5% Leu and PLGA, 1.5% Leu 304 (0–5 mg/ml) ($n=6$) and incubated for a further 24 h as 305 above, followed by the addition of 1 ml MTT solution 306 (0.5 mg/ml in PBS, pH 7.4) solution to each well. After 307 further 2 h incubation, the medium was removed and any 308 formazan crystals generated were solubilized with 500 μl of 309 isopropanol. Thereafter, aliquots of the resulting solutions 310 were transferred to 96 well plates and the absorbance was 311 measured using spectroscopy at 570 nm and corrected for 312 background absorbance. The relative cell viability (%) was 313 calculated using Eq. 5 as follows: 314

$$\text{Viability (\%)} = \frac{A - S}{CM - S} \times 100 \quad (5)$$

Where A is the absorbance of the test substance concentrations, S is the absorbance obtained for the (isopropanol) and CM is the absorbance obtained for untreated cells incubated with medium (control).

321 **Statistical Analysis**

Each formulation was compared with the control formulation by a one-way analysis of variance (ANOVA) with Dunnett multiple comparison test. The formulations were then compared with each other by means of a one-way ANOVA with the Tukey's comparison test. The statistical significance level was set at $p \leq 0.05$.

328 **RESULTS**

329 **Polymer Synthesis**

The PGA-co-PDL (equimolar monomer ratio, 1:1:1) prepared was a white solid powder, and the nature of the co-polymer was confirmed from the integration pattern of peaks obtained from H¹-NMR spectra (δ_{H} CDCl₃, 300 MHz): 1.34 (s, 22 H, H-g), 1.65 (m, 8 H, H-e, e', h), 2.32 (m, 6 H, H-d, d', i), 4.05 (q)-4.18 (m) (6 H, H-a, b, c, f), 5.2 (s, H, H-j) (Fig. 1). The molecular weight of PGA-co-PDL was 23.0 KDa, as determined by GPC.

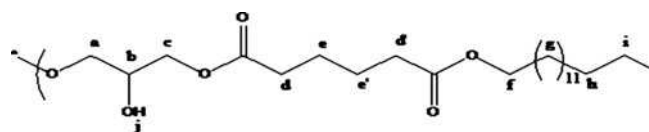


Fig. 1 Chemical structure of PGA-co-PDL polymer (MW 23 KDa).

338 Microparticles Characterization

339 A good yield of over 40% for the different formulations was
 340 obtained except for PGA-co-PDL, 1.5% Arg which had the
 341 lowest value of $16.4\% \pm 1.4$ (Table I). Furthermore, an
 342 inverse correlation between increasing arginine concentra-
 343 tion and yield was observed. There was no significant
 344 difference with addition of amino acids in encapsulation
 345 efficiency or drug loading when comparing spray dried
 346 formulations against control (PGA-co-PDL) ($p > 0.05$,
 347 ANOVA/Dunnett). In addition, all formulations had a
 348 negative surface charge, with higher values observed in
 349 L-leucine modified spray dried microparticles (PGA-co-
 350 PDL, 0.5,1, 1.5% Leu and PLGA 1.5% Leu), indicating a
 351 greater degree of colloidal stability within the dispersion
 352 medium (Table I). It is also worth noting that increasing the
 353 L-arginine concentration correlated with increased moisture
 354 content (Table I), while an inverse correlation was observed
 355 with L-leucine. However, the results for all formulations
 356 were within the range of moisture content obtained from
 357 spray dried powders (35,36). All formulations had a
 358 geometric particle size less than $2 \mu\text{m}$ (Table I) suitable for
 359 targeting the respiratory bronchioles. The tapped densities of
 360 all formulations were similar (0.24 ± 0.04 – $0.31 \pm 0.05 \text{ g cm}^{-3}$;
 361 Table I), and together with the geometric particle size,
 362 were used to calculate the theoretical aerodynamic
 363 diameter (d_{ae}). As shown in Table I, the d_{ae} for all
 364 formulations was between 0.50 ± 0.13 – 0.91 ± 0.11 . How-
 365 ever, the MMAD obtained from cascade impaction studies
 366 ranged from 2.18 ± 0.35 to $3.68 \pm 0.12 \mu\text{m}$, indicating
 367 particle aggregation (duplicate or triplicate) compared to
 368 geometric particle size. The aggregation was confirmed
 369 from Carr's index with values greater than 25 indicating
 370 poor and cohesive flowing powders (31).

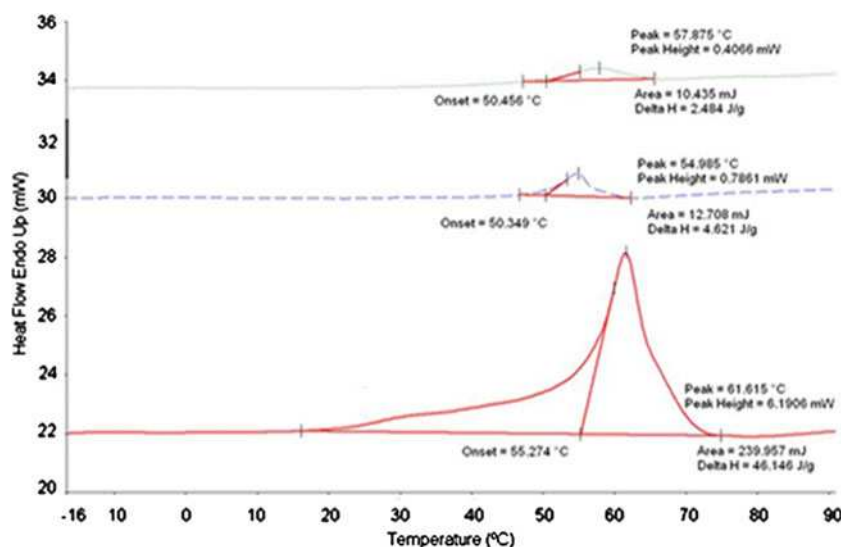
371 Figure 2 represents DSC thermograms of PGA-co-PDL
 372 polymer, spray dried PGA-co-PDL (control) and PGA-co-
 373 PDL, 1.5% Leu formulations respectively. The spray drying
 374 process changed the thermal properties of the polymer,
 375 resulting in a lower onset of melting, 50.46°C (PGA-co-
 376 PDL, control) and 50.35°C (PGA-co-PDL, 1.5% Leu)
 377 compared to 55.27°C for the polymer alone. In addition,
 378 the endothermic peaks became broader in shape with spray
 379 dried formulations coupled with a decrease in area under
 380 the endothermic curve and the heat of fusion (ΔH) (Fig. 2).
 381 Furthermore, PGA-co-PDL, 1.5% Leu had a broader
 382 melting peak and a lower ΔH (2.484 J/g) compared to
 383 control formulation (ΔH , 4.621 J/g). Scanning electron
 384 microscopy (SEM) confirmed PGA-co-PDL particles had a
 385 smooth surface, with no difference between the control
 386 (PGA-co-PDL) and amino-acid modified formulations
 387 (Fig. 3). However, L-arginine modified microparticles
 388 (PGA-co-PDL, 1.5% Arg) were aggregated and appeared
 389 to be fused together compared to unmodified control

Table I Physical Characteristics of Spray Dried Microparticles (Values are Means \pm SD)

Formulation	Yield (%)	EE (%)	Drug loading ($\mu\text{g}/\text{mg}$ particle)	Zeta potential (mV)	Particle size (μm)	Water content (%)	Tapped density (g cm^{-3})	Carr's Index (%)	Flowability	d_{ae} (μm)	MMAD (μm)
t1.1	46.2 \pm 1.9	20.26 \pm 0.01	2.60 \pm 0.17	-28.62 \pm 1.58	1.76 \pm 0.23	1.74 \pm 0.0	0.26 \pm 0.02	34.48	Very poor	0.90 \pm 0.10	2.86 \pm 0.24
t1.2	49.5 \pm 2.4	25.67 \pm 0.03	3.29 \pm 0.38	-25.92 \pm 0.76	1.85 \pm 0.13	3.40 \pm 0.0	0.24 \pm 0.04	30.00	Poor, cohesive	0.91 \pm 0.11	2.18 \pm 0.35
t1.3	43.9 \pm 7.7	21.56 \pm 0.01	2.76 \pm 0.04	-27.82 \pm 1.50	1.17 \pm 0.24	4.11 \pm 0.0	0.27 \pm 0.01	34.48	Very poor	0.61 \pm 0.09	2.58 \pm 0.19
t1.4	16.4 \pm 1.4	25.70 \pm 0.09	3.29 \pm 1.26	-25.39 \pm 0.67	0.89 \pm 0.17	5.09 \pm 0.0	0.31 \pm 0.05	30.43	Poor, cohesive	0.50 \pm 0.13	2.98 \pm 0.25
t1.5	41.1 \pm 4.9	21.42 \pm 0.01	2.74 \pm 0.14	-39.58 \pm 1.71	1.29 \pm 0.20	2.23 \pm 0.1	0.25 \pm 0.02	33.33	Very poor	0.65 \pm 0.10	3.68 \pm 0.12
t1.6	52.5 \pm 7.2	20.44 \pm 0.01	2.62 \pm 0.17	-29.95 \pm 1.57	1.09 \pm 0.20	1.71 \pm 0.2	0.28 \pm 0.02	33.33	Very poor	0.58 \pm 0.08	2.55 \pm 0.03
t1.7	54.7 \pm 2.6	18.94 \pm 0.01	2.42 \pm 0.09	-35.10 \pm 0.99	1.49 \pm 0.21	1.48 \pm 0.1	0.25 \pm 0.01	31.03	Poor, cohesive	0.75 \pm 0.12	3.43 \pm 0.58
t1.8	47.8 \pm 3.6	22.10 \pm 0.09	2.83 \pm 0.25	-31.24 \pm 1.69	1.08 \pm 0.17	1.89 \pm 0.23	0.26 \pm 0.01	32.12	Very poor	0.56 \pm 0.14	2.90 \pm 0.41

Yield, encapsulation efficiency (EE), and drug loading (n = 6). Zeta potential, particle size, water content, tapped density and mass median aerodynamic diameter (MMAD) (n = 3)

Fig. 2 Comparison of DSC thermograms of blank PGA-co-PDL polymer (bottom) and spray dried PGA-co-PDL (control) (middle) or PGA-co-PDL, 1.5% Leu (top).



390 microparticles (Fig. 3a and b respectively), whereas,
 391 L-leucine modified microparticles (PGA-co-PDL, 1.5%
 392 Leu) appeared spherical in shape, with no visual evidence
 393 of particle fusion (Fig. 3c). Confocal microscopy confirmed
 394 SF was homogenously distributed inside the microparticles
 395 in control formulation and PGA-co-PDL, 1.5% Leu (Fig. 4)
 396 during the emulsion/spray drying process.

397 **In-Vitro Aerosolisation Studies**

398 SF deposition data obtained from spray dried formulations
 399 indicated there was a difference in aerosolisation perfor-
 400 mance between the type and concentration of amino acids
 401 used (Fig.5). For example, PGA-co-PDL, 1.5% Arg showed
 402 significantly higher powder deposit in the capsule and
 403 inhaler compared to the other formulations, including
 404 control formulation (PGA-co-PDL) ($p < 0.05$, ANOVA/
 405 Dunnett) and PGA-co-PDL, 1.5% Leu ($p < 0.05$,

ANOVA/Tukey's). In addition, L-arginine modified for- 406
 mulations displayed a higher throat deposition in contrast 407
 to L-leucine modified microparticles, particularly PGA-co- 408
 PDL, 0.5% Arg and PGA-co-PDL, 1.5% Arg formulations, 409
 in comparison to control formulation ($p < 0.05$, ANOVA/
 410 Dunnett) and PGA-co-PDL, 1.5% Leu ($p < 0.05$, ANOVA/
 411 Tukey's). In addition, PGA-co-PDL, 1.5% Leu resulted in
 412 significantly lower powder deposits in capsule and inhaler
 413 ($p < 0.05$, ANOVA/Tukey's), and throat ($p < 0.05$,
 414 ANOVA/Tukey's) compared to PLGA, 1.5% Leu. Over-
 415 all, PGA-co-PDL, 1.5% Leu had the lowest powder
 416 deposit in the capsule and inhaler, and throat. 417

Addition of L-arginine (0.5–1.5%w/w) resulted in no
 418 significant change to %FPF ($p < 0.05$, ANOVA/Dunnett) 419
 compared to control formulation (PGA-co-PDL) (Fig. 6a). 420
 In contrast, L-Leucine modified microparticles (PGA-co-
 421 PDL, 1% Leu & PGA-co-PDL, 1.5% Leu) produced
 422 significantly higher %FPF compared to control formulation 423

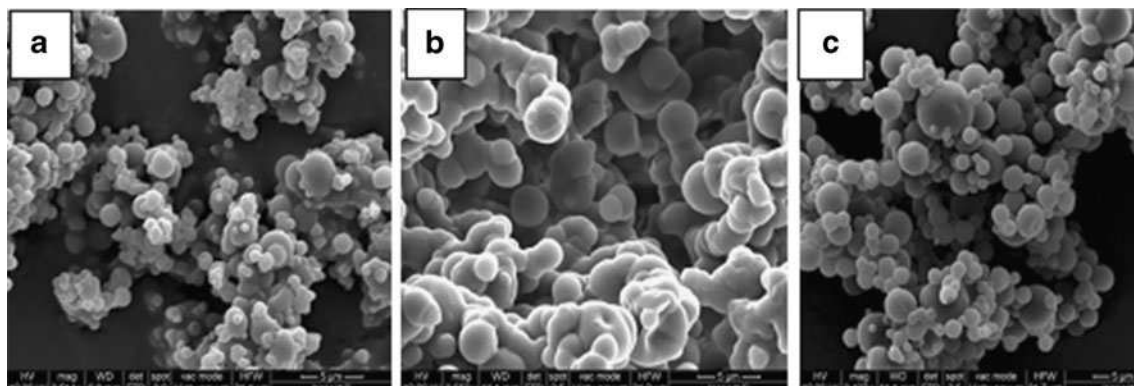
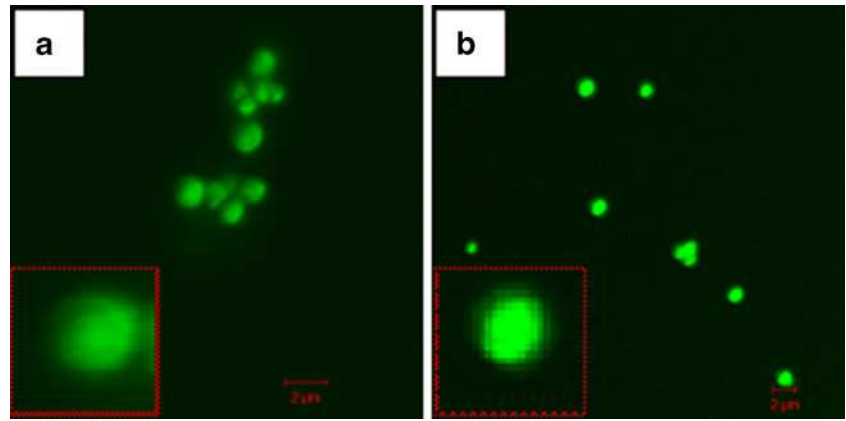


Fig. 3 SEM images comparing PGA-co-PDL (control formulation) (a) with PGA-co-PDL, 1.5% Arg (b) and PGA-co-PDL, 1.5% Leu (c). The scale bar represents 5 μm.

Fig. 4 Confocal laser scanning microscopy images comparing PGA-co-PDL (control formulation) (a) and PGA-co-PDL, 1.5% Leu (b). The scale bar represents 2 μ m.



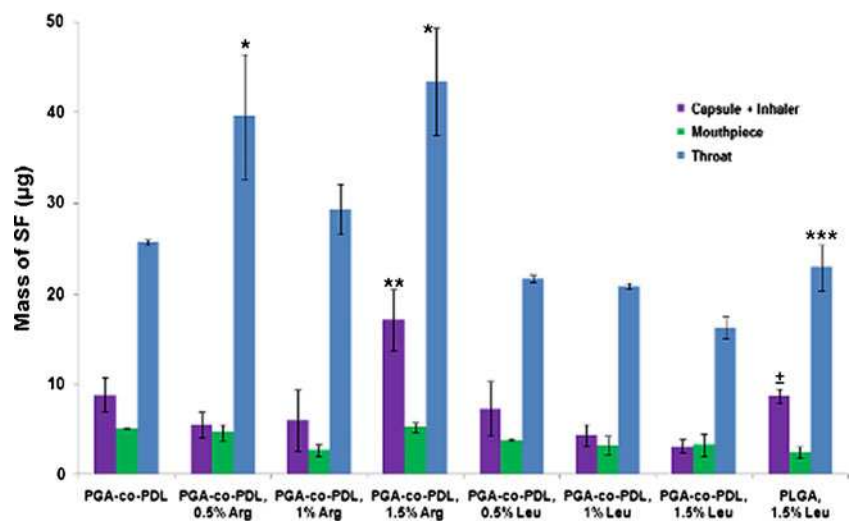
424 ($p < 0.05$, ANOVA/Dunnett) and L-arginine modified for-
 425 mulations (PGA-co-PDL, 1% Arg & PGA-co-PDL, 1.5%
 426 Arg) ($p < 0.05$, ANOVA, Tukey's). In fact, PGA-co-PDL,
 427 1.5% Leu produced the highest $\%FPF$ ($43.38 \pm 5.61\%$)
 428 which was more than 1.5 times greater than the value
 429 obtained with same concentration of L-arginine ($26.57 \pm$
 430 1.85%) ($p < 0.05$, ANOVA, Tukey's). However, increasing
 431 the L-leucine concentration from 1.0 to 1.5%w/w did not
 432 significantly enhance $\%FPF$ ($p > 0.05$, ANOVA/Tukey's)
 433 (Fig. 6a). Addition of amino acids resulted in no significant
 434 difference in FPD against control ($p > 0.05$, ANOVA/
 435 Dunnett) (Fig. 6b). However, incorporating L-leucine,
 436 PGA-co-PDL, 1% Leu ($21.58 \pm 1.21 \mu\text{g}$) and PGA-co-
 437 PDL, 1.5% Leu ($21.42 \pm 1.46 \mu\text{g}$), resulted in almost double
 438 the FPD compared to PGA-co-PDL, 1% Arg ($12.40 \pm$
 439 $0.99 \mu\text{g}$) ($p < 0.05$, ANOVA/Tukey's). Overall PGA-co-
 440 PDL, 1.5% Leu had the highest $\%FPF$ and FPD, but no
 441 significant difference was noted when compared to PLGA,
 442 1.5% Leu ($p > 0.05$, ANOVA/Tukey's).

In-vitro Release Studies

443

444 It was clear PGA-co-PDL, 1.5% Leu could be considered
 445 as an optimum delivery system based on the aerosolisation
 446 results (lowest throat deposition, highest FPD and $\%FPF$).
 447 Therefore, *in vitro* release studies comparing PGA-co-PDL
 448 (control), PGA-co-PDL, 1.5% Leu and PLGA, 1.5% Leu
 449 were performed and reported as cumulative percentage SF
 450 released over time (Fig. 7). Initially the SF adsorbed on the
 451 microparticles surface was removed by washing with 1 ml
 452 PBS buffer. A rapid burst release of SF was observed from
 453 all three formulations after 30 min, however the release of
 454 SF from PGA-co-PDL, 1.5% Leu ($24.54\% \pm 3.87$) and
 455 PLGA, 1.5% Leu ($24.04\% \pm 2.67$) was significantly less than
 456 PGA-co-PDL ($41.87\% \pm 2.46$) ($p < 0.05$, ANOVA/Dunnett).
 457 The rapid release continued for all three formulations up to
 458 5 h, where PGA-co-PDL, 1.5% Leu ($38.52\% \pm 3.27$)
 459 resulted in significantly less SF released compared to
 460 PGA-co-PDL ($54.90\% \pm 5.76$) and PLGA, 1.5% Leu

Fig. 5 Comparison of sodium fluo-
 rescein deposition in capsule and
 inhaler, mouthpiece and throat via
 different formulations. Data repre-
 sent mean \pm S.D., $n = 3$. * $p < 0.05$
 (Throat) PGA-co-PDL, 0.5% &
 1.5% Arg vs PGA-co-PDL
 (ANOVA/Dunnett) and PGA-co-
 PDL, 1.5% Leu (ANOVA/Tukey's),
 ** $p < 0.05$ (Capsule & Inhaler)
 PGA-co-PDL, 1.5% Arg vs PGA-
 co-PDL (ANOVA/Dunnett) and
 PGA-co-PDL, 1.5% Leu (ANOVA/
 Tukey's), *** $p < 0.05$ (Throat)
 PLGA, 1.5% Leu vs PGA-co-PDL,
 1.5% Leu (ANOVA/Tukey's), $\pm p <$
 0.05 (Capsule & Inhaler) PLGA,
 1.5% Leu vs PGA-co-PDL, 1.5%
 Leu (ANOVA/Tukey's).



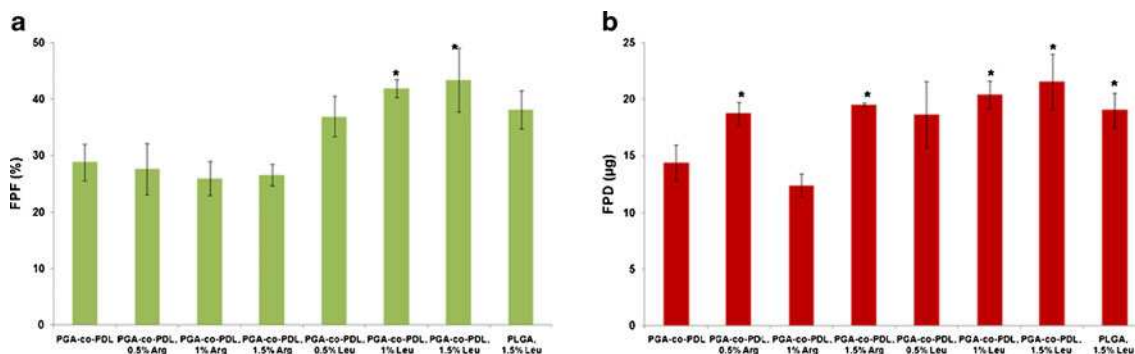


Fig. 6 **a** The percentage fine particle fraction of spray dried microparticles. Data represent mean \pm S.D., $n=3$. * $p < 0.05$ PGA-co-PDL, 1% & 1.5% Leu vs PGA-co-PDL (ANOVA/Dunnett) and PGA-co-PDL, 0.5%, 1% & 1.5% Arg (ANOVA/Tukey's). **b** The fine particle dose (μg) of

spray dried microparticles. Data represent mean \pm S.D., $n=3$. * $p < 0.05$ PGA-co-PDL, 0.5 & 1.5% Arg, PGA-co-PDL, 0.5%, 1% & 1.5% Leu and PLGA, 1.5% Leu vs PGA-co-PDL (ANOVA/Dunnett).

461 (54.20% \pm 4.67) ($p < 0.05$, ANOVA/Tukey's). After this
 462 time period the release of SF reached a plateau providing
 463 a slow continuous release phase up to 72 h, with PGA-co-
 464 PDL, 1.5% Leu (47.10% \pm 3.78) releasing significantly less
 465 SF compared to PGA-co-PDL (61.35% \pm 2.48) and PLGA,
 466 1.5% Leu (63.07% \pm 4.28) ($p < 0.05$, ANOVA/Tukey's). In
 467 this study SF was released from PGA-co-PDL, PGA-co-
 468 PDL, 1.5% Leu and PLGA, 1.5% Leu formulations
 469 according to Higuchi diffusion model (R^2 value of 0.890,
 470 0.924 and 0.832 respectively) and the release rate constant
 471 (K_h 2.13, 2.68 and 3.95 respectively) (Table II).

noted between PGA-co-PDL, 1.5% Leu and PLGA, 1.5% Leu microparticles at a concentration of 0.5 mg/ml (91.19 \pm 4.32, 82.72 \pm 2.58, respectively), 1 mg/ml (87.14 \pm 3.40, 74.20 \pm 3.13, respectively) and 5 mg/ml (85.57 \pm 1.44, 60.66 \pm 1.75, respectively) ($p < 0.05$, ANOVA/Tukey's). Furthermore, the addition of L-leucine, as a dispersibility enhancer, to the optimum formulation during the emulsion/spray drying process did not alter the cell viability, with values similar to PGA-co-PDL ($p > 0.05$, ANOVA/Dunnett) (Fig. 8).

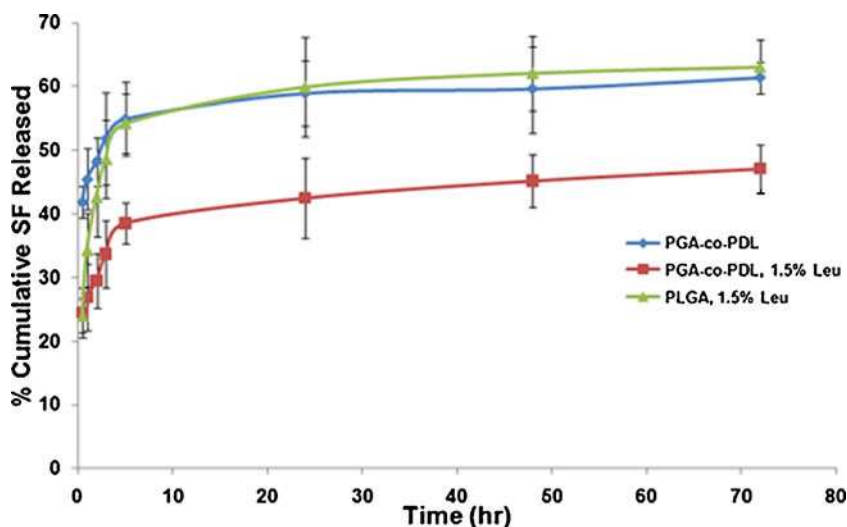
Cell Toxicity Study

473 Unmodified spray dried control formulation, PGA-co-PDL,
 474 and L-leucine modified formulation, PGA-co-PDL, 1.5%
 475 Leu appear to be well tolerated by normal lung bronchial
 476 epithelial cells *in vitro*, compared to PLGA, 1.5% Leu
 477 microparticles. Significant reduction in cell viability was

DISCUSSION

The aim of this study was to investigate the ability of a new family of polyesters, PGA-co-PDL, as SR carriers for pulmonary drug delivery, particularly as it had been investigated and shown promise as a delivery vehicle for both small molecular weight drugs and proteins (6,7). PGA-

Fig. 7 Cumulative *in-vitro* release of sodium fluorescein from spray dried microparticles in PBS buffer at 37°C. Data represent mean \pm S.D., $n=3$.



Poly(Glycerol Adipate-co- ω -Pentadecalactone) Spray Dried Microparticles

Table II Kinetic Analysis of Spray Dried Microparticle Formulations (n=3)

Formulation	Zero Order (R ²)	First Order (R ²)	Higuchi model (R ²)	Mechanism of Release	K _h
PGA-co-PDL	0.802	-0.828	0.890	Higuchi	2.13
PGA-co-PDL, 1.5% Leu	0.848	-0.869	0.924	Higuchi	2.68
PLGA, 1.5% Leu	0.732	-0.786	0.832	Higuchi	3.95

K_h = mg/cm².min^{1/2} is the release rate constant for Higuchi diffusion model

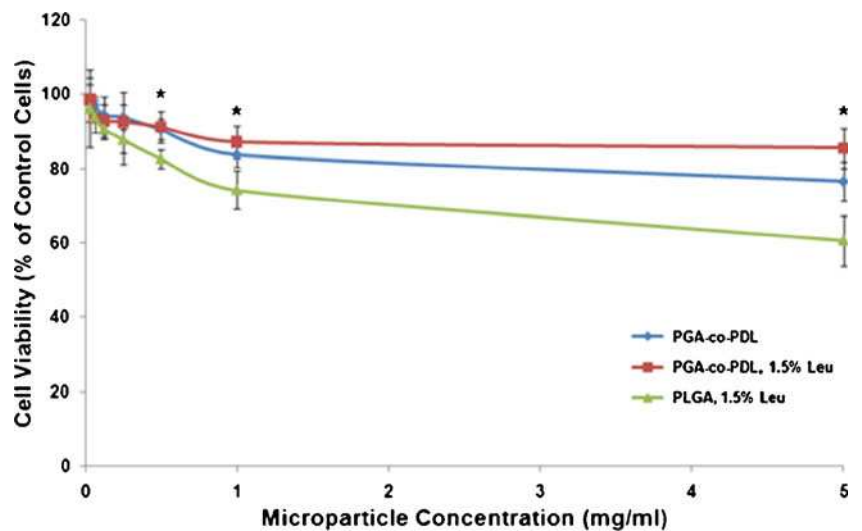
co-PDL microparticles were prepared utilizing double emulsion/spray drying technique as our previous investigations indicated preparation of these particles via double emulsion alone were highly aggregated and exhibited poor aerosolisation performance (24).

The spray drying parameters were set to preserve the outlet temperature in the range of 44–47°C, as DSC analysis indicated a low melting point for PGA-co-PDL polymer. Generally, the EE was low in all formulations possibly due to the hydrophilic nature of SF partitioning into the external aqueous phase and a lower concentration remaining in the organic phase of the double emulsion/spray drying process (37). The negative surface charge demonstrated the anionic nature of the produced microparticles, which may be associated with incomplete removal of the PVA emulsifier in the external aqueous phase of the double emulsion. It is accepted that spray drying products are mainly characterized by their amorphous nature or disordered crystalline phase due to rapid drying of droplets (38). This behavior was demonstrated in our study by the broadening of the melting endotherm peaks for spray dried formulations. It is also worth noting that the accumulation of L-leucine at the air-liquid interface and hence the surface of microparticles resulted in physicochemical modifications, such as, surface charge, water content and particle size,

which, additionally may have contributed to the enhanced broadening of the endothermic melting peak compared to control formulation (PGA-co-PDL). Furthermore, the shift to a lower temperature and intensity (peak height) indicated distribution of SF inside the PGA-co-PDL microparticles, which was confirmed from confocal microscopy images. Thus L-leucine treated formulations exist in a less crystalline state compared to untreated control formulation, and it is possible that incorporating L-leucine with these polymers may influence the encapsulation efficiency as the drug is mainly encapsulated in the amorphous region (6) and alter the physicochemical properties as noted above, which will inadvertently have an impact on the aerosolisation performance as observed in this study. However, further investigations are required to understand the influence of incorporating amino acids on the crystalline structure and the potential changes this may have on the physicochemical properties of generated spray dried particles.

The geometric particle size, particle shape and morphology are known to affect the aerodynamic properties and pulmonary deposition (39). The theoretical aerodynamic diameters calculated from tapped density indicate the spray dried particles generated are suitable for targeting the alveolar region. However, *in vitro* aerosolisation results from this investigation suggest the formulations did not

Fig. 8 Cell viability of human bronchial epithelium cell line (16HBE14o) measured by MTT cytotoxicity assay following 24 h exposure to different concentrations of PGA-co-PDL and PLGA microparticles suspension. Data represent mean \pm S.D., n=6. *p < 0.05 PGA-co-PDL, 1.5% Leu vs PLGA, 1.5% Leu (ANOVA/Tukey's).



544 aerosolize as individual particles, but rather as particle
545 aggregates, as indicated when comparing geometric particle
546 size with MMAD. This most likely occurred due to
547 incomplete powder de-aggregation as van der Waals forces
548 between particles were not completely overcome upon
549 inhalation. In addition, powder aggregation of all spray
550 dried powders generated was confirmed with a Carr's index
551 of ≥ 30 indicating the flow was very poor and/or cohesive.
552 Moreover, depending upon the addition and concentration
553 of amino acids, different deposition profiles were observed.
554 For example, L-arginine treated microparticles due to their
555 low zeta potential and high percentage of water content
556 were highly aggregated, which affected the deposition
557 pattern by incomplete powder release from the capsule
558 and device, and higher deposition in the throat region,
559 compared to control and L-leucine modified micropar-
560 ticles. Furthermore, increasing the L-arginine concentra-
561 tion resulted in a higher percentage of water content on
562 the surface of microparticles, possibly due to the hydro-
563 philic nature of L-arginine, which increased the tendency
564 of aggregation and consequently affected deposition.
565 Many researchers have indicated the formation of wrin-
566 kled surface morphology (40) due to excessive build up of
567 vapor pressure during solvent evaporation in the spray
568 drying process, especially with hydrophobic amino acids,
569 such as L-leucine, for improved aerosolization perfor-
570 mance (41). However, this behavior was not observed in
571 particles produced in this investigation, which had a
572 predominantly smooth surface morphology, and may be
573 related to little or no build up of vapor pressure within the
574 particles under spray drying operating conditions used in
575 this study.

576 The low yield associated with PGA-co-PDL, 1.5% Arg
577 primarily occurred due to production of highly cohesive
578 particles, as indicated from Carr's Index and the high water
579 content, resulting in powder adhesion to the wall of spray
580 drying chamber. Similar results have been reported where
581 enhancing the concentration of L-arginine resulted in
582 decreased spray drying powder yield and aerosol perfor-
583 mance, such as $\chi\%$ FPF (40). Furthermore, PGA-co-PDL,
584 1.5% Arg had the lowest zeta potential value, $-25.39 \pm$
585 0.67 , which provided an indication to the instability and
586 cohesiveness as the repulsion force could not exceed the
587 attraction forces between particles. Hence the aggregation,
588 low yield and poor aerosolisation performance (low $\chi\%$ FPF,
589 FPD and high powder deposits remaining in the inhaler
590 and capsule, mouthpiece and throat), compared to the
591 other formulations resulted, due to strong van der Waals
592 forces between particles. van der Waals forces are directly
593 proportional to the contact surface area of a particle, and
594 hence an increase in strength is observed with smaller
595 particle sizes due to larger surface area. However, similar
596 zeta potential values were achieved with the other L-argi-

597 nine modified formulations, but they possessed larger
598 geometric particle sizes resulting in decreased van der
599 Waals forces between particles.

600 Comparing all formulations, L-Leucine had the highest $\chi\%$
601 FPF and FPD values compared to control formulation
602 (PGA-co-PDL), L-arginine and PLGA modified formula-
603 tions. The possible mechanisms for the enhanced perfor-
604 mance might be related to the surface activity of the
605 relatively strong hydrophobic alkyl side chain of L-leucine
606 accumulating at the particle surface during spray drying
607 (40). Similar reports have also demonstrated the enhanced
608 aerosol performance with L-leucine containing formulations
609 compared to L-arginine and other investigated amino acids
610 (40,42,43). Comparing the three L-leucine formulations,
611 PGA-co-PDL, 1.5% Leu was considered to be the optimum
612 formulation as a carrier for pulmonary drug delivery, as it
613 exhibited the highest $\chi\%$ FPF and FPD. Hence, although the
614 powders generated had poor cohesive flow properties, the
615 high zeta potential values indicated good physical stability,
616 which together with the lowest tapped density, water
617 content and relatively large particle size compared to other
618 formulations resulted, in weak van der Waals forces
619 between particles. Consequently, inhalation provided suffi-
620 cient energy to de-aggregate the particles resulting in an
621 enhanced aerosolisation performance.

622 The results of this investigation indicate that L-Leucine
623 plays an important role not only in enhancement of the
624 aerosolisation properties of the microparticles but also in
625 sustaining drug release over 72 h, as indicated with PGA-
626 co-PDL, 1.5% Leu. Once again this could be attributed to
627 the surface activity of L-leucine coating the microparticles
628 during the spray drying process, resulting in reduced
629 surface adsorption of SF, which can be seen from confocal
630 images, and hence a decreased burst and continuous release
631 (44). Similar results have been reported for other surfac-
632 tants, such as polysorbate 20 and sodium dodecyl sulphate,
633 which reduced the surface accumulation of certain proteins
634 in a concentration dependant manner (41,45). As a result, it
635 is possible the high burst release associated with PGA-co-
636 PDL may be due to SF particles migrating towards the
637 microparticle surface by residual solvent during spray
638 drying. However; none of the formulations could be
639 considered an optimum SR pulmonary delivery system, as
640 PGA-co-PDL possessed a high burst release and although
641 PGA-co-PDL, 1.5% Leu had a lower burst release, it failed
642 to release its entire payload during 72 h, with similar
643 results obtained by Thompson *et al.* (6). The incomplete
644 release of SF may be associated with the slow hydrolyzation
645 of the ester linkages in the polymer backbone (46). Data
646 from our laboratory showed approximately 40% loss in
647 polymer molecular weight after 14 days incubation in PBS
648 buffer at 37°C (47) indicating the ester linkages between the
649 monomers were very stable. In this current investigation the

650 release of SF from control formulation (PGA-co-PDL) and
 651 PGA-co-PDL, 1.5% Leu was according to the Higuchi's
 652 model, and mediated through the diffusion process with
 653 very little contribution from degradation of the polymer.
 654 Hence, the controlled release of small molecular weight
 655 hydrophilic compounds from modified PGA-co-PDL spray
 656 dried particles appears to be a diffusion limited process.
 657 The more significant release of SF from PLGA, 1.5% Leu
 658 may be associated to the smaller particle size and hence a
 659 greater surface area. Future investigations are required to
 660 optimize the release profile, and may involve manipulating
 661 the polymer characteristics, such as decreasing the molec-
 662 ular weight or increasing its hydrophilic properties by
 663 incorporation of poly(ethylene) glycol, PEG, to the polymer
 664 backbone.

665 The results from this investigation indicate that PGA-
 666 co-PDL, 1.5% leu was an optimum pulmonary drug
 667 delivery carrier. However, the safety of the carrier used
 668 for pulmonary drug delivery is an important issue.
 669 Normal bronchial epithelial cells (16HBE14o-) were
 670 chosen in accordance with the aerosolization and
 671 particle size distribution (MMAD) results for the
 672 particles generated (48). The cytotoxicity profile data of
 673 PGA-co-PDL and PGA-co-PDL, 1.5% Leu was more
 674 superior to PLGA, 1.5% Leu spray dried microparticles
 675 at 0.5, 1 and 5 mg/ml concentrations. Consequently, this
 676 provides an indication about the feasibility of using PGA-
 677 co-PDL polymers as alternative safe carriers for pulmo-
 678 nary drug delivery.

679 CONCLUSIONS

680 The present investigation suggests that PGA-co-PDL could
 681 be considered as an alternative novel biodegradable carrier
 682 for pulmonary drug delivery having the ability to control
 683 the release of the encapsulated drug. In addition, incorpo-
 684 ration of L-leucine was found to enhance the aerosolisation
 685 performance and decrease both the burst and continues
 686 release of encapsulated drug. Toxicity studies revealed the
 687 safety of the spray dried PGA-co-PDL modified micro-
 688 particles compared to PLGA microparticles.

689 Future studies will be conducted to determine if the
 690 polymers elicit an immune response. In addition we will
 691 investigate enhancing the aerosolisation performance, en-
 692 capsulation efficiency and optimizing the release of thera-
 693 peutic agents from these polymers.

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