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# Characterization and Aerosol Dispersion Performance of Spray-Dried Chemotherapeutic PEGylated Phospholipid Particles for Dry Powder Inhalation Delivery in Lung Cancer

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44 **ABSTRACT**

45 Pulmonary inhalation chemotherapeutic drug delivery offers many advantages for lung cancer  
46 patients in comparison to conventional systemic chemotherapy. Inhalable particles are  
47 advantageous in their ability to deliver drug deep in the lung by utilizing optimally sized  
48 particles and higher local drug dose delivery. In this work, spray-dried and co-spray dried  
49 inhalable lung surfactant-mimic PEGylated lipopolymers as microparticulate/nanoparticulate dry  
50 powders containing paclitaxel were rationally designed via organic solution advanced spray  
51 drying (no water) in closed-mode from dilute concentration feed solution.  
52 Dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylethanolamine  
53 poly(ethylene glycol) (DPPE-PEG) with varying PEG chain length were mixed with varying  
54 amounts of paclitaxel in methanol to produce co-spray dried microparticles and nanoparticles.  
55 Scanning electron microscopy showed the spherical particle morphology of the inhalable  
56 particles. Thermal analysis and X-ray powder diffraction confirmed the retention of the  
57 phospholipid bilayer structure in the solid-state following spray drying, the degree of solid-state  
58 molecular order, and solid-state phase transition behavior. The residual water content of the  
59 particles was very low as quantified analytically Karl Fisher titration. The amount of paclitaxel  
60 loaded into the particles was quantified which indicated high encapsulation efficiencies (43-  
61 99%). Dry powder aerosol dispersion performance was measure *in vitro* using the Next  
62 Generation Impactor™ (NGI™) coupled with the Handihaler® dry powder inhaler device and  
63 showed mass median aerodynamic diameters in the range of 3.4 – 7µm. These results  
64 demonstrate that this novel microparticulate/nanoparticulate chemotherapeutic PEGylated  
65 phospholipid inhalation aerosol platform has great potential in lung cancer drug delivery.

66

67 **KEYWORDS:**

68 Dry Powder Inhaler (DPI); Respiratory Drug Delivery; Biocompatible Biodegradable

69 Lipopolymers; Nanotechnology; Nanomedicine; Lung Surfactant; Self-assemblies; Paclitaxel;

70 anticancer; DPPC/DPPE-PEG; Particle Engineering

71 **1.1 INTRODUCTION**

72 Inhalation aerosol delivery dates back to ancient times (Hickey and Mansour, 2009; Patton and  
73 Byron, 2007) and aerosol formulations have been investigated for many pulmonary diseases  
74 including lung infections, cystic fibrosis, chronic obstructive pulmonary disease (COPD), and  
75 lung cancer (Arnold et al., 2007; Cartiera et al., 2010; Meenach et al., 2012a; Watts et al., 2008;  
76 Wu et al., 2010; Yang et al., 2009). The lung is an ideal target for drug delivery owing to the  
77 potential to avoid first-pass metabolism, enable a more rapid onset of therapeutic action, high  
78 local drug concentrations within the lung, and minimization of systemic absorption of the drug  
79 allowing for decreased side effects (Carvalho et al., 2011; Gill et al., 2007; Hickey and Mansour,  
80 2008; Mansour et al., 2009; Sharma et al., 2001; Vaughn et al., 2006). Additionally, for many  
81 drugs delivery via intravenous or oral administration routes often result in high systemic drug  
82 concentrations while a relatively low amount of the drug actually reaches the lung (Carvalho et  
83 al., 2011; Vaughn et al., 2006). Specifically for lung cancer, it has been shown that drug  
84 concentrations in lung tumors are often low after systemic administration of chemotherapeutics  
85 which could be a cause of treatment failure and in some cases, the initiation of chemotherapeutic  
86 resistance (Gagnadoux et al., 2008).

87 In addition to the general advantages of aerosolized chemotherapy formulations,  
88 inhalable dry powder formulations offer further improvements in the treatment of lung cancer.  
89 This includes the ability to design the particle size and amount of drug loaded into the system,  
90 enhance solubility of the drug, and improve dry state storage allowing for long-term stability  
91 (Mansour et al., 2009; Sung et al., 2007). In this study, dry powder nanoparticle/microparticle  
92 formulations were designed via dilute organic solution advanced spray drying in closed-mode  
93 which has been optimized by our group for the delivery of therapeutics to treat various lung

94 diseases (Hayes et al., 2011; Li et al., 2011; Li and Mansour, 2011; Meenach et al., 2012b).  
95 Spray drying (SD) is an advanced high-throughput pharmaceutical manufacturing process which  
96 can design and efficiently produce respirable particles in the solid-state (Hickey and Mansour,  
97 2008; Kikuchi et al., 1991; Mansour et al., 2009; Mansour et al., 2011). One of the advantages of  
98 using SD is that it can allow for the controlled production of particles in terms of their size,  
99 morphology, and aerosol performance characteristics. Particle engineering is particular important  
100 for pulmonary delivery where many factors impact the performance of a particle system  
101 including the aerodynamic diameter (MMAD), particle size distribution, dispersibility,  
102 morphology, and thermodynamic stability (Chow et al., 2007; Hickey et al., 2007b). In  
103 particular, previous research has shown the effect that both size and surface roughness has on  
104 particle performance where particles with MMADs 1-2  $\mu\text{m}$  deposit in the smaller (lower)  
105 airways and 5-10  $\mu\text{m}$  deposit in the larger (upper) airways (Vehring et al., 2007) and particles  
106 with increase surface roughness may have increased dispersibility properties due to decreased  
107 interparticulate interactions between the particles (Gilani et al., 2005).

108 While aerosol dry powder formulations utilizing polymers such as poly(lactic-co-  
109 glycolic) (PLGA) (Tomoda et al., 2009) and poly(ethylene glycol)-co-poly(sebacic acid) (PEG-  
110 PSA) (Tang et al., 2010) have been developed for lung cancer treatment applications, the  
111 introduction of foreign matter to the lung has the potential to induce complications. In this work,  
112 a first-line lung cancer chemotherapeutic drug, paclitaxel (PTX), was encapsulated in a  
113 PEGylated phospholipid microparticle/nanoparticle system comprised of  
114 dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylethanolamine-  
115 methoxy(polyethylene glycol) (DPPE-PEG). DPPC was chosen as the main excipient because it  
116 is the primary phospholipid component in lung surfactant (Mansour et al., 2011). In addition to

117 offering a natural excipient component to the formulated particles, the use of phospholipids as  
118 biocompatible biodegradable excipients can aid in the delivery of drugs to the lungs as they have  
119 been shown to improve particle migration to the lung periphery due to the reduction in surface  
120 tension provided by the surfactant (Ganguly et al., 2008; Mansour et al., 2001; Mansour and  
121 Zografi, 2007a, b). The use of PEGylated phospholipids, such as DPPE-PEG, can result in a  
122 formulation that could evade recognition and uptake of the immune system allowing for  
123 prolonged residence time in the lung (Ishihara et al., 1998; Labiris and Dolovich, 2003a, b;  
124 Mansour et al., 2011; Mansour et al., 2010), have mucopenetrating properties (Lai et al., 2009a;  
125 Lai et al., 2009b), and are used in marketed intravenous (IV) nanopharmaceutical products  
126 (Mansour et al., 2011; Mansour et al., 2010; Rhee and Mansour, 2011; Wu and Mansour, 2011).  
127 Also, for certain formulations, the relatively low clearance rate in the bronchioalveolar region  
128 may also allow for longer residence times (Carvalho et al., 2011). We have recently reported on  
129 the successful design and optimization of the novel DPI nanomedicine carrier platform  
130 consisting of DPPC/DPPE-PEG with varying PEG chain length and excellent aerosol dispersion  
131 performance as aerosolized dry powders (Meenach et al., 2012b).

132 Paclitaxel was chosen for this study since it is one of the most widely used drugs to treat  
133 lung cancer and is a first-line drug in the treatment of lung cancer (Carvalho et al., 2011; Eldar-  
134 Boock et al., 2011). Taxol<sup>®</sup>, the intravenous formulation of paclitaxel contains water-insoluble  
135 paclitaxel along with a mixture of Cremophor EL and dehydrated ethanol, and has been shown to  
136 cause adverse reactions such as hypersensitivity, muscle pain, and neurologic and cardiac  
137 toxicities (Marupudi et al., 2007). Paclitaxel is lipophilic, with high protein affinity, and also  
138 exhibits a volume of distribution much higher than the total water volume in the body, which  
139 causes it to have a low therapeutic index (Carvalho et al., 2011). The low solubility of paclitaxel



140 in water (0.7 to 30 µg/ml) (Liggins et al., 1997) can be overcome via encapsulation into a solid-  
141 state particle system helping to overcome this major hurdle.

142 The objective of this systematic study was to rationally develop and characterize an  
143 inhalable PEGylated phospholipid microparticulate/nanoparticulate dry powder aerosol platform  
144 containing paclitaxel with varying PEG chain lengths and paclitaxel content for the treatment of  
145 lung cancer. The organic solution advanced co-spray dried (co-SD) paclitaxel/PEGylated  
146 phospholipid dry powder inhalation aerosol microparticulate/nanoparticulate formulations were  
147 compared to one-component systems of spray dried paclitaxel. The formulated particles  
148 contained a fixed amount of DPPC to DPPE-PEG with varying PEG chain lengths of 2k, 3k, and  
149 5k and with varying paclitaxel ratios (5, 25, 50, and 75 mole % of paclitaxel overall). To the  
150 authors' knowledge, this is the first time to report on a comprehensive and systematic study on  
151 this novel anticancer lipopolymeric dry powder inhalation aerosol formulation platform  
152 engineered from organic solution advanced spray drying (i.e. no water) consisting of  
153 microparticles and nanoparticles of DPPC and DPPE-PEG with varying PEG chain lengths with  
154 various combinations of PTX for pulmonary chemotherapeutic delivery in lung cancer.

155

## 156 **2.1 MATERIALS AND METHODS**

### 157 **2.1.1 Materials**

158 Synthetic dipalmitoylphosphatidylcholine (DPPC, Molecular Weight: 734.039 g/mol; >99%  
159 purity) and dipalmitoylphosphatidylethanolamine-methoxy(polyethylene glycol) (DPPE-PEG,  
160 Molecular Weights: 2749.391 g/mol, 3716.304 g/mol, and 5741.510 g/mol which correspond to  
161 2000, 3000 and 5000 molecular weight poly(ethylene glycol) lengths per compound; >99%  
162 purity) were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Paclitaxel was obtained  
163 from LC Labs (Woburn, MA, USA; 99.5% purity; C<sub>47</sub>H<sub>51</sub>NO<sub>14</sub>·H<sub>2</sub>O). Methanol (HPLC grade,

164 ACS certified) and chloroform (HPLC grade, ACS certified) were obtained from Fisher  
165 Scientific (Pittsburg, PA, USA). HYDRANAL<sup>®</sup>-Coulomat AD was from Sigma-Aldrich (St.  
166 Louis, MO, USA). Ultra-high purity (UHP) dry nitrogen gas was from Scott-Gross (Lexington,  
167 KY, USA). All materials were used as received and stored at -20°C.

168

### 169 **2.1.2 Spray-Drying and Co-Spray Drying from Dilute Drug Feed Solution**

170 Advanced spray-drying of co-spray dried (co-SD) paclitaxel-loaded PEGylated phospholipid  
171 particles was performed using a B-290 Büchi Mini Spray Dryer coupled with a B-295 Inert Loop  
172 and high performance cyclone (all from Büchi Labortechnik AG, Switzerland) in closed-mode  
173 using UHP dry nitrogen as the atomizing gas. The nozzle diameter (composed of stainless steel)  
174 was 0.7 mm and the spray-drying (SD) particles were separated from the drying gas (using UHP  
175 dry nitrogen) in the high-performance cyclone and collected in a sample collector. The feed  
176 solutions were prepared by dissolving DPPC and DPPE-PEG (i.e. 95 mole % DPPC to 5 mole %  
177 DPPE-PEG) with different amounts of paclitaxel (PTX) ranging from 5 to 75 mole% paclitaxel  
178 to total DPPC/DPPE-PEG in methanol to form dilute concentration feed solutions of 0.1% w/v.  
179 Based on our previous work (Li et al., 2011; Li and Mansour, 2011; Meenach et al., 2012b), the  
180 following spray-drying conditions were used: atomization gas flow rate of 600 L/h, aspiration  
181 rate of 35 m<sup>3</sup>/h, inlet temperature of 150 °C (which represents the primary drying step), and  
182 pump rate of 30 mL/min (High P). Table I shows the various formulated particle systems and  
183 their corresponding PTX and DPPC/DPPE-PEG amounts and types and outlet temperatures  
184 (which represent the secondary drying process temperatures). For pure spray dried (SD)  
185 paclitaxel particles, the same atomization gas flow rate, aspiration rate, and inlet temperatures  
186 were used as for the PEGylated phospholipid systems. The pump rate was varied at 3 mL/min

187 (Low P), 15 mL/min (Med P), and 30 mL/min (High P). All SD and co-SD powders were stored  
188 in glass vials sealed with parafilm in desiccators over indicated Drierite™ desiccant at -23°C  
189 under ambient pressure.

190

### 191 **2.1.3 Scanning Electron Microscopy (SEM) for Morphology and Shape Analysis**

192 The shape and surface morphology of particles was evaluated by SEM, using a Hitachi S-4300  
193 microscope (Tokyo, Japan). Samples were placed on double-sided adhesive carbon tabs and were  
194 adhered to aluminum stubs (TedPella, Inc., Redding, CA, USA) which were coated with a  
195 gold/palladium alloy thin film using an Emscope SC400 sputter coating system at 20 μA for 1  
196 minute under Argon gas. The electron beam with an accelerating voltage of 5 - 10 kV was used  
197 at a working distance of 13.3 – 15.3 mm. Images were captured at several magnifications using  
198 similar conditions previously reported by the authors.(Meenach et al., 2012b)

199

### 200 **2.1.4 Particle Sizing and Size Distribution**

201 The mean size, standard deviation, and size range of the particles were determined digitally using  
202 SigmaScan™ 5.0 software (Systat, San Jose, CA, USA), as previously reported by the  
203 authors.(Meenach et al., 2012b) Representative micrographs for each particle sample at 5,000x  
204 magnification were analyzed by measuring the diameter of at least 100 particles per image.

205

### 206 **2.1.5 Karl Fisher (KF) Coulometric Titration**

207 The water content of all particle powders was chemically quantified by Karl Fisher (KF)  
208 coulometric titration, using similar conditions previously reported by the authors.(Li and  
209 Mansour, 2011; Meenach et al., 2012b) The measurements were performed with a 737 KF

210 Coulometer coupled with 703 Ti Stand (Metrohm Ltd., Antwerp, Belgium). Approximately 5 mg  
211 of powder was dissolved in a known volume of chloroform. The sample solution was injected  
212 into the reaction cell that contained HYDRANAL<sup>®</sup> KF reagent and the water content was then  
213 calculated from the resulting reading.

214

#### 215 **2.1.6 Differential Scanning Calorimetry (DSC)**

216 Thermal analysis and phase transition measurements were carried out using a TA Q200 DSC  
217 system (TA Instruments, New Castle, DE, USA) equipped with T-Zero<sup>®</sup> technology and an  
218 automated computer-controlled RSC-90 cooling accessory. Using similar conditions previously  
219 reported by the authors,(Li and Mansour, 2011; Meenach et al., 2012b) 1 - 3 mg of powder was  
220 weighed into hermetic anodized aluminum T-Zero<sup>®</sup> DSC pans and were sealed hermetically  
221 sealed with the T-Zero<sup>®</sup> hermetic sealer. UHP dry nitrogen gas was used as the purging gas at 50  
222 mL/min. The heating range was 0 – 250 °C at a heating scan rate of 5.00 °C/min.

223

#### 224 **2.1.7 Powder X-ray Diffraction (XRPD)**

225 XRPD patterns of powder samples were measured by a Rigaku Multiflex X-ray diffractometer  
226 (The Woodlands, TX, USA) with a slit-detector Cu K $\alpha$  radiation source (40 kV, 44 mA, and  $\lambda =$   
227 1.5406 Å). The scan range was 5 – 50° in 2 $\theta$  with a scan rate of 2°/min at ambient temperature.  
228 The sample was placed on a horizontal quartz glass sample holder plate.

229

#### 230 **2.1.8 Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)**

231 ATR-FTIR was performed using a Varian Inc. 7000e step-scan spectrometer (Agilent  
232 Technologies, Santa Clara, CA, USA). The particle powder was placed on the diamond ATR

233 crystal, covered with a glass cover slip, and held in place with a specialized clamp. ATR crystal  
234 and IR spectra were obtained at an 8 cm<sup>-1</sup> spectral resolution between 700 and 4000 cm<sup>-1</sup>. The  
235 data was collected and analyzed using Varian Resolutions software.

236

### 237 **2.1.9 Hot-Stage Microscopy (HSM)**

238 HSM studies were completed using an Olympus BX51 polarized microscope (Olympus, Japan)  
239 equipped with an Instec STC200 heating unit and S302 hot stage (Boulder, CO, USA). The  
240 polarized light was filtered by a  $\gamma$  530 nm U-TP530 filter lens. Powder samples were mounted on  
241 a cover glass and heated from 25 °C to 250 °C at a heating rate of 5 °C/min. The heating  
242 program was controlled by WinTemp software and images were digitally captured via a SPOT  
243 Insight digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI, USA).

244

### 245 **2.1.10 Paclitaxel Loading Analysis via UV-Vis Spectroscopy**

246 UV-Vis was used to determine the amount of paclitaxel loaded into the formulated particle  
247 systems. The particles were dissolved in known quantities of methanol prior to analysis. The  
248 absorbance intensity was measured at 227 nm using a UV-1800 UV-Vis Shimadzu  
249 spectrophotometer and a calibration curve of paclitaxel in methanol was used. The paclitaxel  
250 encapsulation efficiency (EE) and loading was calculated as follows:

251

$$252 \text{ Encapsulation Efficiency (EE)} = \frac{\text{Actual Mass of PTX}}{\text{Initial Mass of PTX}} \times 100\%$$

253

$$254 \text{ Drug Loading} = \frac{\text{Actual Mass of PTX}}{\text{Mass of Particles}}$$

255

### 256 **2.1.11 *In Vitro* Aerosol Dispersion Performance via Next Generation Impactor™ (NGI™)**

257 In accordance with United States Pharmacopeia (USP) Chapter <601> specifications on aerosols  
258 (2006) the *in vitro* aerosol dispersion properties of the dry powder particles were determined  
259 using the Next Generation Impactor™ (NGI™) with a stainless steel induction port (i.e. USP  
260 throat) attachment (NGI™ Model 170, MSP Corporation, Shoreview, MN, USA), equipped with  
261 specialized stainless steel NGI™ gravimetric insert cups (MSP Corporation, Shoreview, MN,  
262 USA). The NGI™ was coupled with a Copley TPK 2000 critical flow controller, which was  
263 connected to a Copley HCP5 vacuum pump (Copley Scientific, United Kingdom). The airflow  
264 rate, Q, was measured and adjusted prior to each experiment using a Copley DFM 2000 flow  
265 meter (Copley Scientific, United Kingdom).

266 The aerosolization studies were experimentally designed by Design Expert™ 8.0.7.1  
267 software (Stat-Ease Corp., MN, USA). Glass fiber filters (55 mm, Type A/E, Pall Life Sciences,  
268 Exton, PA, USA) were placed in the stainless steel NGI™ gravimetric insert cups for NGI™  
269 stages 1 through 7 to minimize bounce or re-entrapment (Edwards et al., 1998). Three  
270 hydroxypropyl methylcellulose hard capsules (size 3, Quali-V®, Qualicaps® Inc., Whitsett, NC,  
271 USA) were each loaded with 10 mg of powder which were then loaded into a high resistance (i.e.  
272 high sheer stress) FDA-approved human DPI device, the Handihaler® (Boehringer Ingelheim &  
273 Pfizer Ltd., USA), and tightly inserted into the induction port. The NGI™ was run at a controlled  
274 flow rate (Q) at 60 L/minute with a delay time of 10 seconds (NGI™ Flow controller) prior to  
275 the capsules being needle-pierced open by the Handihaler® mechanism, where the particles were  
276 then drawn into the impactor for 10 seconds. This was done with a total of 3 capsules per sample  
277 for a total of 30 mg total per run. For each 30 mg run, the amount of particles deposited onto  
278 each stage was determined gravimetrically by measuring the difference in mass of the glass

279 filters after particle deposition. For the NGI™ flow rate of 60 L/minute, the effective cutoff  
280 diameters for each impaction stage were calibrated by the manufacturer and stated as: Stage 1  
281 (8.06 µm); Stage 2 (4.46 µm); Stage 3 (2.82 µm); Stage 4 (1.66 µm); Stage 5 (0.94 µm); Stage 6  
282 (0.55 µm); and Stage 7 (0.34 µm). The fine particle dose (FPD), fine particle fraction (FPF),  
283 respirable fraction (RF), and emitted dose (ED) were calculated as follows:

284

285 Fine particle dose (FPD) = mass of particles < 4.4 µm (Stages 2 through 7)

286

287 Fine particle fraction (FPF) =  $\frac{\text{fine particle dose}}{\text{initial particle mass loaded into capsules}} \times 100\%$

288

289 Respirable fraction (RF) =  $\frac{\text{mass of particles} < 4.4 \mu\text{m (Stages 2 through 7)}}{\text{total particle mass on all stages}} \times 100 \%$

290

291 Emitted dose (ED) =  $\frac{\text{initial mass in capsules} - \text{final mass remaining in capsules}}{\text{initial mass in capsules}} \times 100 \%$

292

293 The mass mean aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were  
294 determined using a Mathematic program written by Dr. Warren Finlay.(Finlay, 2008) All  
295 experiments were triplicated (n = 3).

296

### 297 **2.1.12 Statistical Analysis**

298 All experiments were performed in at least triplicate. The aerosolization studies were  
299 experimentally designed by Design Expert™ 8.0.7.1 software (Stat-Ease Corp., MN, USA).  
300 MYSTAT 12 for Windows (12.01.00) was used for t-tests (paired t-test with unequal variances)

301 to determine any significance in observed data. A p-value of < 0.05 was considered statistically  
302 significant.

303

### 304 **3.1 RESULTS AND DISCUSSION**

#### 305 **3.1.1 SEM, Particle Sizing, and Size Distribution**

306 Formulated particle and surface morphologies were visualized and analyzed via SEM  
307 micrographs as seen in Figures 1 through 5. Their corresponding diameters are exhibited in  
308 Table I as determined via SigmaScan<sup>TM</sup> software. 5PTX:95DPPC, 5PTX:95DPPC/DPPE-  
309 PEG2k, and 5PTX:95DPPC/DPPE-PEG3k particles were smooth and spherical whereas  
310 5PTX:95DPPC:DPPE-PEG5k demonstrated characteristics of sintering between the particles.  
311 25PTX:75DPPC and 25PTX:75DPPC/DPPE-PEG2k were also smooth and spherical whereas  
312 25PTX:75DPPC/DPPE-PEG3k and 25PTX:75DPPC/DPPE-PEG5k demonstrated characteristics  
313 of sintering between the particles. The 50PTX and 75PTX systems (Figures 3 and 4,  
314 respectively) demonstrated an increase in corregation as seen in Figure 5. This phenomenon was  
315 seen in our previous systems comprised of pure SD PTX (Meenach et al., 2012b). As the amount  
316 of PTX in the particle systems increased, the degree of sintering and corregation of the particles  
317 also increased. The sintering for the particles with high PEG content is likely due to the low glass  
318 transition temperature for PEG, which is around -60°C (Törmälä, 1974). The diameter of all  
319 formulated dry powder systems ranged from 0.624 to 3.416 µm in diameter and there was a  
320 slight decrease in size with increasing amounts of paclitaxel, although this was not significant.  
321 There were no changes in the diameter due to the degree of PEGylation from DPPE-PEG.  
322 Overall, the particles were within the ideal size range necessary for inhalation into the deep lung



323 for both adults and children (Bosquillon et al., 2001; Coates and O'Callaghan, 2006) which is  
324 necessary for effective pulmonary delivery for treating lung cancer.

325

### 326 **3.1.2 Karl Fisher (KF) Coulometric Titration**

327 The residual water content of the formulated particles in the solid-state are shown in Table I. The  
328 water content for the co-SD formulated particles ranged from 1.47 % to 6.78 % (w/w) with no  
329 trends corresponding to excipient formulation or amount of paclitaxel present in the system. The  
330 pure SD PTX particles exhibited the lowest water content ranging from 0.44 % to 2.47 % (w/w).  
331 These values were slightly higher than those reported for the raw components (Meenach et al.,  
332 2012b), however, all of the values were low and well within the range previously reported by our  
333 group in other inhalable dry powder formulations. Low water content is a requirement for  
334 efficient dry powder aerosolization and effective particle delivery since water can significantly  
335 decrease the dispersion properties of dry powders during aerosolization due to the  
336 interparticulate capillary forces acting at the solid-solid interface between particles (Hickey et al.,  
337 2007a).

338

### 339 **3.1.3 Differential Scanning Calorimetry (DSC)**

340 As seen in Figure 6a for the 5PTX particles, an endothermic main phase transition peak ( $T_c$ ) was  
341 observed between 63°C and 65°C for all samples which corresponds to the gel-to-liquid self-  
342 assembly phase transition of raw DPPC, where the hydrophobic acyl chain core melts, indicating  
343 the presence of the phospholipid bilayer structure (Mansour and Zografi, 2007a, b; Pappalardo et  
344 al., 2005). The co-SD 5PTX samples with PEG3k and PEG5k also exhibited endothermic peaks  
345 at 41.5°C and 47.2°C, respectively, which corresponds to the presence of DPPE-PEG. These two

346 endothermic peaks are also present in raw DPPC and DPPE-PEG as we have shown previously  
347 (Meenach et al., 2012b). The enthalpy values for the 5PTX particles ranged from 22.1 to 25.7 J/g  
348 but there was no correlation due to the excipient used. 25PTX samples (Figure 6b) also exhibited  
349 strong transition peaks between 63°C and 66°C and 25PTX:DPPC/DPPE-PEG5k particles  
350 showed peaks near 45°C and 47°C. The enthalpy values for the 25PTX particles ranged from  
351 13.1 to 20.7 J/g but there was no correlation due to the excipient used. Both 5PTX and 25PTX  
352 systems underwent a metastable phase transition around 145°C which is likely the result of  
353 reorganization of the molecules within the particles. For the 50PTX formulated particles (Figure  
354 6c) there were no measurable transition peaks with the exception of 50PTX:50DPPC/DPPE-  
355 PEG5k, which exhibited small peaks at 49.2°C and 65.54°C with corresponding enthalpies of  
356 3.045 and 0.938 J/g. These correspond to the DPPC and DPPE-PEG bilayer phase transitions,  
357 respectively. In Figure 6d, the 75PTX samples indicate no measurable transition peaks in the  
358 phospholipid region (40°C to 70°C) but did exhibit a large exothermic peak around 212°C which  
359 corresponds to the degradation of the sample (also seen in panel e for raw PTX. The size of these  
360 peaks, and corresponding enthalpies, decrease with increasing PEG content at 88.4, 71.4, 59.0,  
361 and 15.9 J/g for DPPC, PEG2k, PEG3k, and PEG5k samples, respectively. Furthermore, the  
362 75PTX formulations exhibited a glass transition temperature ( $T_g$ ) near 146°C, which is a second-  
363 order phase transition for the amorphous glass-to-rubbery state as seen in Figure 6f. Raw PTX  
364 exhibited a small endothermic peak around 204°C which corresponds to the melting temperature  
365 ( $T_m$ ) of paclitaxel, which is a first-order phase transition whereas no measurable melting peaks  
366 were seen prior to degradation for SD samples. Both the  $T_g$  and  $T_m$  values were similar to those  
367 demonstrated by the spray dried and raw paclitaxel formulations as seen in Figure 6e and  
368 compares to what has previously been shown for raw paclitaxel (Lee et al., 2001; Liggins et al.,

369 1997). Overall, the limited presence of transition peaks from 40 to 70°C for both 50PTX and  
370 75PTX particles indicate limited multilamellar formation of the phospholipids within the particle  
371 matrix.

372

### 373 **3.1.4 X-ray Diffraction (XRPD)**

374 X-ray powder diffractograms (Figure 7) showed the a strong peak at 21° 2 $\Theta$  for all SD 5PTX  
375 and SD 25PTX formulated particles which corresponds to the presence of the solid-state  
376 phospholipid bilayer structure (Alves and Santana, 2004) indicating that the bilayer structure is  
377 preserved in the solid-state following organic solution advanced spray drying in closed-mode.  
378 These samples also exhibited strong peaks at 19 and 23° 2 $\Theta$  which designate the metastable  
379 phase of PEG as seen in previous results for PEG powders (Kang et al., 2007). The intensity of  
380 the 19 and 23° 2 $\Theta$  peaks increased with increasing PEG chain length for the SD 5PTX and SD  
381 25PTX systems. The intensities of the 19 and 23° 2 $\Theta$  peaks for the co-SD systems are lower  
382 overall in comparison to that seen for raw DPPC or DPPE-PEG (Meenach et al., 2012b). For the  
383 SD 50PTX and SD 75PTX formulated particles no strong peaks were present, indicating that  
384 PTX is amorphous within the particle matrix, likely with limited bilayer formation within the  
385 particles. The lack of characteristic peaks likely indicates that there is no detectable phospholipid  
386 bilayer structure for these powder systems within the detection limit of XRPD. The intensities of  
387 the peak corresponding to 21°C 2 $\Theta$  decreased with increasing paclitaxel content. Raw paclitaxel  
388 showed peaks throughout its diffractograms indicating its crystallinity prior to spray drying.

389

### 390 **3.1.5 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)**

391 Formulated particles and their raw counterparts underwent ATR-FTIR analysis to determine the  
392 functional groups present in the systems (Figure 8). For both the raw DPPC and raw DPPE-PEG  
393 powders, the spectra indicated the same characteristic peaks as previously reported in literature  
394 for DPPC and PEG, respectively (Lee et al., 2001; Meenach et al., 2012b). The formulated co-  
395 SD PTX:DPPC/DPPE-PEG3k particles exhibited sharp peaks at  $2916\text{ cm}^{-1}$  ( $-\text{CH}_2-$ -  
396 antisymmetrical stretching),  $2870\text{ cm}^{-1}$  ( $-\text{CH}_2-$  symmetrical stretching),  $1724\text{ cm}^{-1}$  ( $\text{C}=\text{O}$  ester  
397 stretching),  $1465\text{ cm}^{-1}$  ( $-\text{CH}_2-$  deformation),  $1060\text{ cm}^{-1}$  ( $-\text{C}-\text{C}-$ ), and  $965\text{ cm}^{-1}$  ( $(-\text{N}^+(\text{CH}_3)_3$ -  
398 antisymmetrical stretching) which all increased with increasing paclitaxel content. These peaks  
399 were present in both raw DPPC and DPPE-PEG3k but not raw or formulated paclitaxel. For the  
400 peak at  $1724\text{ cm}^{-1}$ , it was split for the raw PTX but not for the spray dried particles. Overall,  
401 ATR-FTIR analysis of the solid-state particles confirmed the presence of DPPC and DPPE-PEG  
402 where appropriate through the signature peaks of each component and the difference between SD  
403 paclitaxel and raw paclitaxel was confirmed by differing spectra between the two systems.

404

### 405 **3.1.6 Cross-Polarizing Light Hot-Stage Microscopy (HSM)**

406 Representative micrographs of co-SD 5PTX:95DPPC/DPPE-PEG3k and co-SD  
407 50PTX:50DPPC/DPPE-PEG3k are shown in Figure 9. The co-SD 5PTX:95DPPC/DPPE-PEG3k  
408 formulated particles showed dark agglomerates that lack birefringency between  $25^\circ\text{C}$  and  $60^\circ\text{C}$ ,  
409 which indicated a non-ordered, amorphous material. A phase transition was visualized near  
410  $65^\circ\text{C}$ , which likely corresponds to the gel-to-liquid self-assembly bilayer main phase transition  
411 as shown in the DSC thermogram at  $65^\circ\text{C}$  in Figure 6a. Melting was visualized starting at  $120^\circ\text{C}$   
412 with the formation of liquid droplets with decomposition occurring at  $250^\circ\text{C}$ . The micrographs  
413 for co-SD 50PTX:50DPPC/DPPE-PEG3k showed large agglomerates lacking birefringency

414 between 25°C and 80°C and a visible phase transition began starting around 90°C as shown by  
415 deformation of the particles. Melting started around 150°C and was complete by 155°C and  
416 decomposition occurred by 225°C. Pure SD paclitaxel (100PTX) showed dark agglomerates that  
417 lack birefringency between 25°C and 170°C. The particles began melting around 180°C as seen  
418 in Figure 10a. Raw paclitaxel had dark agglomerates lacking birefringency from 25°C to 180°C.  
419 Once the paclitaxel began melting at 190°C, it exhibited birefringency until decomposition  
420 around 240°C. Overall, HSM confirmed the amorphous nature of the formulated co-SD  
421 PTX:DPPC/DPPE-PEG systems as well as for SD 100PTX particles as no birefringency was  
422 observed. It also demonstrated the stability of the particles at room and physiological  
423 temperatures.

424

### 425 **3.1.7 Paclitaxel Loading**

426 Paclitaxel loading into the PEGylated phospholipid dry powder particles was determined by  
427 dissolving them in methanol and measuring the concentration of drug via UV-Vis spectroscopy.  
428 As shown in Table I, the PTX encapsulation efficiencies for 5PTX systems was the highest  
429 (ranging from 94.7% to 99.0%) and decreased with increasing PTX content. As expected, the  
430 paclitaxel loading increased with increasing PTX content where the SD 5PTX, 25PTX, 50PTX  
431 and 75PTX systems exhibited values in the range of 41.3 - 60.2 µg/mg, 191.4 - 242.6 µg/mg,  
432 316.8 - 511.0 µg/mg, and 571.2 - 679.9 µg/mg, respectively. Furthermore, the encapsulation  
433 values for the 25 – 75% PTX-loaded particles is high enough for the dose necessary for animal  
434 treatment. In particular, systems containing 100 – 500 µg/mg PTX contain enough paclitaxel to  
435 result in a dose to rats ranging from 2 – 4 mg/kg at 2 – 5 mg particles/rat, which has been

436 demonstrated as an effective dose range in the treatment of an orthotopic lung cancer model (Gill  
437 et al., 2011; Yang et al., 2007).

438

### 439 **3.1.8 *In Vitro* Aerosol Dispersion Performance via Next Generation Impactor™**

440 The aerosol dispersion properties of the co-SD particles and pure PTX SD particles were  
441 evaluated using the Next Generation Impactor™ (NGI™) coupled with a Handihaler® DPI  
442 device. As seen in Table II, the MMAD values for co-SD systems (regardless of PTX loading)  
443 increased with increasing PEG chain length and decreased with increasing PTX loading. The  
444 corresponding GSD also increased with increasing PEG chain length. Furthermore, for 100PTX  
445 particles, the MMAD values were approximately the same (ranging from 3.2  $\mu\text{m}$  to 3.4  $\mu\text{m}$ )  
446 whereas the GSD values were 2.3  $\mu\text{m}$  to 2.6  $\mu\text{m}$ . In general, fine particle fractions (FPF) and  
447 respirable fractions (RF) decreased with increasing PEG content, while the emitted dose (ED)  
448 increased. There was no discernible difference for FPF, RF, and ED with respect to the PTX  
449 loading. Figure 11 shows the aerosol dispersion performance of the dry powders as the %  
450 deposition on each NGI™ stage for both the co-SD and pure PTX systems. Aerosol deposition  
451 on each NGI™ stage is measurable and in particular, deposition on the lower stages of stage 2 all  
452 the way to stage 7 (lowest stage) is observed. In general, the % deposition on stage 1 increased  
453 with increasing PEG chain length. The exception to this was seen for the SD 50PTX dry powder  
454 aerosols where no difference was seen due to PEG chain length. This trend was opposite for  
455 stage 4 where the amount of powder deposited on this stage decreased with increasing PEG  
456 content for co-SD particles. For 100PTX SD dry powder aerosol, there was no difference  
457 between the amounts of powder deposited on each stage. In regards to paclitaxel loading, the  
458 amount of powder deposited on each stage decreased slightly with increasing PTX content. Since

459 there was significant particle deposition on Stages 5 – 7 (where the MMAD for these particular  
460 particles would be less than 1  $\mu\text{m}$ ), the particles on these stages should deposit in the lower  
461 airways of the lungs due to diffusion of the particles into this region via Brownian motion  
462 (Suarez and Hickey, 2000). Overall, the particles exhibit characteristics that will allow them to  
463 deposit in nearly all regions of the lung allowing for treatment throughout the entirety of the  
464 tissue.

465 The MMAD values for most of the particle systems were within the range necessary (1 –  
466 10  $\mu\text{m}$ ) for particles to deposit predominantly in the middle-to-deep lung regions and deposit by  
467 sedimentation due to gravitational settling (Carvalho et al., 2011; Edwards, 1995a, b; Hickey and  
468 Mansour, 2008; Hickey and Mansour, 2009; Suarez and Hickey, 2000). Furthermore, the ED  
469 values of co-SD particles remained approximately the same compared to systems without PTX  
470 (Meenach et al., 2012b). The RF values for the co-SD systems are lower than their corresponding  
471 drug-free systems reported by our group (45-50% compared to 60-70%) (Meenach et al., 2012b).  
472 While the RF values of the co-SD powders were lower than their respective values at a given  
473 PEG chain length for the PTX-free powders, their respective FPF values increased significantly  
474 (43-79% compared to 20-30%) (Meenach et al., 2012b).

475

#### 476 **4.1 CONCLUSIONS**

477 This systematic and comprehensive study demonstrated for the first time that organic solution  
478 advanced spray drying and co-spray drying in closed-mode of a dilute concentration feed  
479 solution can be successfully employed to formulate high performing DPI liposphere aerosols  
480 consisting of co-spray dried paclitaxel (a first-line chemotherapeutic lung cancer drug) into a  
481 biocompatible and biodegradable lipopolymeric system (DPPE-PEG) with varying PEG chain

482 length and containing the essential lung surfactant phospholipid, DPPC. The physicochemical  
483 characterization of these particles indicates that they would be suitable to deliver PTX to the  
484 middle and deep regions of the lungs to deliver the drug in a targeted fashion. For multilamellar  
485 particles, a lower paclitaxel loading will likely be optimal along with a medium PEGylation  
486 (using DPPE-PEG3k). The incorporation of DPPE-PEG lipopolymer of varying PEG chain  
487 length can potentially offer enhanced mucus penetration by phospholipid spreading and PEG  
488 penetration, controlled drug release, and “stealth” property of evasion of phagocytosis by  
489 immune cells.

490

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498

#### 499 **AUTHOR DISCLOSURE STATEMENT**

500 No conflicts of interest exist.

501

502



503 **REFERENCES**

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671

672 **LIST OF TABLE LEGENDS**

673 **Table I.** List of co-spray dried (co-SD) and single-component spray dried (SD) formulations and  
674 their corresponding outlet temperatures during spray drying, size, water content, and paclitaxel  
675 encapsulation efficiency (EE) and loading. (n = 3, Ave ± SD)

676

677 **Table II.** Next Generation Impactor<sup>TM</sup> results for co-spray dried (co-SD) and one-component  
678 spray dried (SD) aerosol systems including mass median aerodynamic diameter (MMAD),  
679 geometric standard deviation (GSD), fine particle fraction (FPF) below 4.4 μm, respirable  
680 fraction (RF) below 4.4 μm, and emitted dose (ED). (n = 3, Ave ± SD)

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682

683 **LIST OF FIGURE LEGENDS**

684 **Figure 1.** SEM micrographs of co-spray dried (co-SD) PEGylated phospholipid particles with  
685 varying PEG chain length containing 5% paclitaxel (PTX): (a) co-SD 5PTX:95DPPC; (b) co-SD  
686 5PTX:95DPPC/DPPE-PEG2k; (c) co-SD 5PTX:95DPPC/DPPE-PEG3k; and (d) co-SD  
687 5PTX:95DPPC:DPPE-PEG5k. Magnification for all samples was 10,000x.

688

689 **Figure 2.** SEM micrographs of co-spray dried (co-SD) PEGylated phospholipid particles with  
690 varying PEG chain length containing 25% paclitaxel (PTX): (a) co-SD 25PTX:75DPPC; (b) co-  
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692 25PTX:75DPPC/DPPE-PEG5k. Magnification for all samples was 10,000x.

693

694 **Figure 3.** SEM micrographs of co-spray dried (co-SD) PEGylated phospholipid particles with  
695 varying PEG chain length containing 50% paclitaxel (PTX): (a) co-SD 50PTX:50DPPC; (b) co-  
696 SD 50PTX:50DPPC/DPPE-PEG2k; (c) co-SD 50PTX:50DPPC/DPPE-PEG3k; and (d) co-SD  
697 50PTX:50DPPC/DPPE-PEG5k. Magnification for all samples was 10,000x.

698

699 **Figure 4.** SEM micrographs of co-spray dried (co-SD) PEGylated phospholipid particles with  
700 varying PEG chain length containing 75% paclitaxel (PTX): (a) co-SD 75PTX:25DPPC; (b) co-  
701 SD 75PTX:25DPPC/DPPE-PEG2k; (c) co-SD 75PTX:25DPPC/DPPE-PEG3k; and (d) co-SD  
702 75PTX:25DPPC/DPPE-PEG5k. Magnification for all samples was 10,000x.

703

704 **Figure 5.** SEM micrographs of spray-dried (SD) 100% paclitaxel particles (100PTX) following  
705 spray drying at three pump rates (Low P, Med P, and High P) for: (a) Raw paclitaxel (PTX); (b)  
706 SD 100PTX (Low P); (c) SD 100PTX (Med P); and (d) SD 100PTX (High P). Magnification for  
707 all samples was 10,000x.

708

709 **Figure 6.** DSC thermograms of spray-dried (SD) and co-spray-dried (co-SD) particles with  
710 varying PTX content and PEG chain lengths for: (a) co-SD 5PTX:95 DPPC vs. co-SD 5PTX:95  
711 DPPC/DPPE-PEG; (b) co-SD 25 PTX:75 DPPC vs. co-SD 25 PTX:75 DPPC/DPPE-PEG; (c)  
712 co-SD 50PTX:50 DPPC vs. co-SD 50 PTX:50 DPPC/DPPE-PEG; (d) co-SD 75PTX:25 DPPC

713 vs. co-SD 75PTX:25DPPC/DPPE-PEG; (e) SD 100PTX from three pump rates vs. raw PTX; and  
714 (f) insert of co-SD 75PTX:25 DPPC vs. co-SD 75PTX:25DPPC/DPPE-PEG for  $T_g$  transition  
715 visualization.

716  
717 **Figure 7.** X-ray powder diffractograms of spray-dried (SD) and co-spray-dried (co-SD)  
718 particles with varying PTX content and PEG chain lengths for: (a) co-SD 5PTX:95 DPPC vs.  
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720 DPPC/DPPE-PEG; (c) co-SD 50PTX:50 DPPC vs. co-SD 50 PTX:50 DPPC/DPPE-PEG; (d)  
721 co-SD 75PTX:25 DPPC vs. co-SD 75PTX:25DPPC/DPPE-PEG; and (e) SD 100PTX at three  
722 pump rates vs. raw PTX.

723  
724 **Figure 8.** Representative ATR-FTIR spectra of co-spray-dried (co-SD) PTX:DPPC/DPPE-  
725 PEG3k particles in comparison to raw DPPC and raw paclitaxel.

726  
727 **Figure 9.** Representative HSM micrographs of co-spray dried (co-SD) for: (a) co-SD  
728 5PTX:95DPPC/DPPE-PEG3k; and (b) co-SD 50PTX:50DPPC/DPPE-PEG3k particles (scale bar  
729 = 3 mm).

730  
731 **Figure 10.** Representative HSM micrographs of spray-dried (SD): (a) SD 100PTX (High P)  
732 particles; and (b) raw paclitaxel. (Scale bar = 3 mm).

733  
734 **Figure 11.** Aerosol dispersion performance as % deposited on each stage of the Next Generation  
735 Impactor<sup>TM</sup> (NGI<sup>TM</sup>) for spray-dried (SD) and co-spray-dried (co-SD) particles with varying  
736 PTX content and PEG chain lengths for: a) co-SD 5PTX:95 DPPC vs. co-SD 5PTX:95  
737 DPPC/DPPE-PEG; b) co-SD 25 PTX:75 DPPC vs. co-SD 25 PTX:75 DPPC/DPPE-PEG; c) co-  
738 SD 50PTX:50 DPPC vs. co-SD 50 PTX:50 DPPC/DPPE-PEG; d) co-SD 75PTX:25 DPPC vs.  
739 co-SD 75PTX:25DPPC/DPPE-PEG; and e) SD 100PTX particles spray-dried at three pump  
740 rates (Low P, Med P, and High P). For Q= 60 L/minute, the effective cutoff diameters for each  
741 NGI<sup>TM</sup> impaction stage are as follows: Stage 1 (8.06  $\mu\text{m}$ ); Stage 2 (4.46  $\mu\text{m}$ ); Stage 3 (2.82  $\mu\text{m}$ );

742 Stage 4 (1.66  $\mu\text{m}$ ); Stage 5 (0.94  $\mu\text{m}$ ); Stage 6 (0.55  $\mu\text{m}$ ); and Stage 7 (0.34  $\mu\text{m}$ ). (n = 3, Ave  $\pm$   
743 SD)



744 **Table I.** List of co-spray dried (co-SD) and single-component spray dried (SD) formulations  
 745 and their corresponding outlet temperatures during spray drying, size, water content, and  
 746 paclitaxel encapsulation efficiency (EE) and loading. (n = 3, Ave ± SD)

System	Outlet T (°C)	Size (µm)	Water (%)	PTX EE (%)	PTX Loading (µg PTX/mg particle)
5PTX:95DPPC	50	0.946 ± 0.427	3.60 ± 0.37	99.0 ± 0.3	60.2 ± 1.5
5PTX:95DPPC/DPPE-PEG2k	52	1.095 ± 0.458	4.30 ± 0.20	95.0 ± 0.2	48.4 ± 0.8
5PTX:95DPPC/DPPE-PEG3k	54	0.963 ± 0.431	2.44 ± 0.78	99.6 ± 0.2	48.2 ± 0.1
5PTX:95DPPC/DPPE-PEG5k	55	1.567 ± 0.673	2.25 ± 0.44	94.7 ± 0.2	41.3 ± 0.9
25PTX:75DPPC	56	1.539 ± 0.661	3.73 ± 0.96	88.5 ± 0.1	191.4 ± 0.3
25PTX:75DPPC/DPPE-PEG2k	55	3.416 ± 0.808	4.58 ± 1.31	82.3 ± 0.3	209.2 ± 0.9
25PTX:75DPPC/DPPE-PEG3k	55	Not measurable	2.21 ± 0.18	99.5 ± 0.4	242.6 ± 1.1
25PTX:75DPPC/DPPE-PEG5k	54	Not measurable	1.47 ± 0.18	95.0 ± 0.4	212.9 ± 0.9
50PTX:50DPPC	50	0.801 ± 0.230	3.85 ± 0.68	85.0 ± 0.4	511.0 ± 2.0
50PTX:50DPPC/DPPE-PEG2k	51	1.001 ± 0.316	6.78 ± 1.18	62.7 ± 0.4	316.8 ± 1.8
50PTX:50DPPC/DPPE-PEG3k	50	1.215 ± 0.394	3.27 ± 0.27	73.0 ± 0.4	359.1 ± 1.8
50PTX:50DPPC/DPPE-PEG5k	52	Not measurable	5.78 ± 0.40	71.2 ± 0.4	330.8 ± 1.8
75PTX:25DPPC	38	0.778 ± 0.306	3.78 ± 0.89	87.5 ± 0.4	679.9 ± 2.9
75PTX:25DPPC/DPPE-PEG2k	38	0.781 ± 0.307	5.86 ± 0.38	84.6 ± 0.4	638.2 ± 3.1
75PTX:25DPPC/DPPE-PEG3k	33	0.876 ± 0.337	1.60 ± 0.62	76.8 ± 0.4	571.2 ± 3.2
75PTX:25DPPC/DPPE-PEG5k	35	0.765 ± 0.377	4.60 ± 0.21	82.4 ± 0.2	644.7 ± 1.5
100PTX (Low P)	73	0.631 ± 0.265	2.28 ± 0.11	n/a	n/a
100PTX (Med P)	81	0.624 ± 0.247	1.41 ± 0.38	n/a	n/a
100PTX (High P)	41	0.672 ± 0.274	0.44 ± 0.39	n/a	n/a

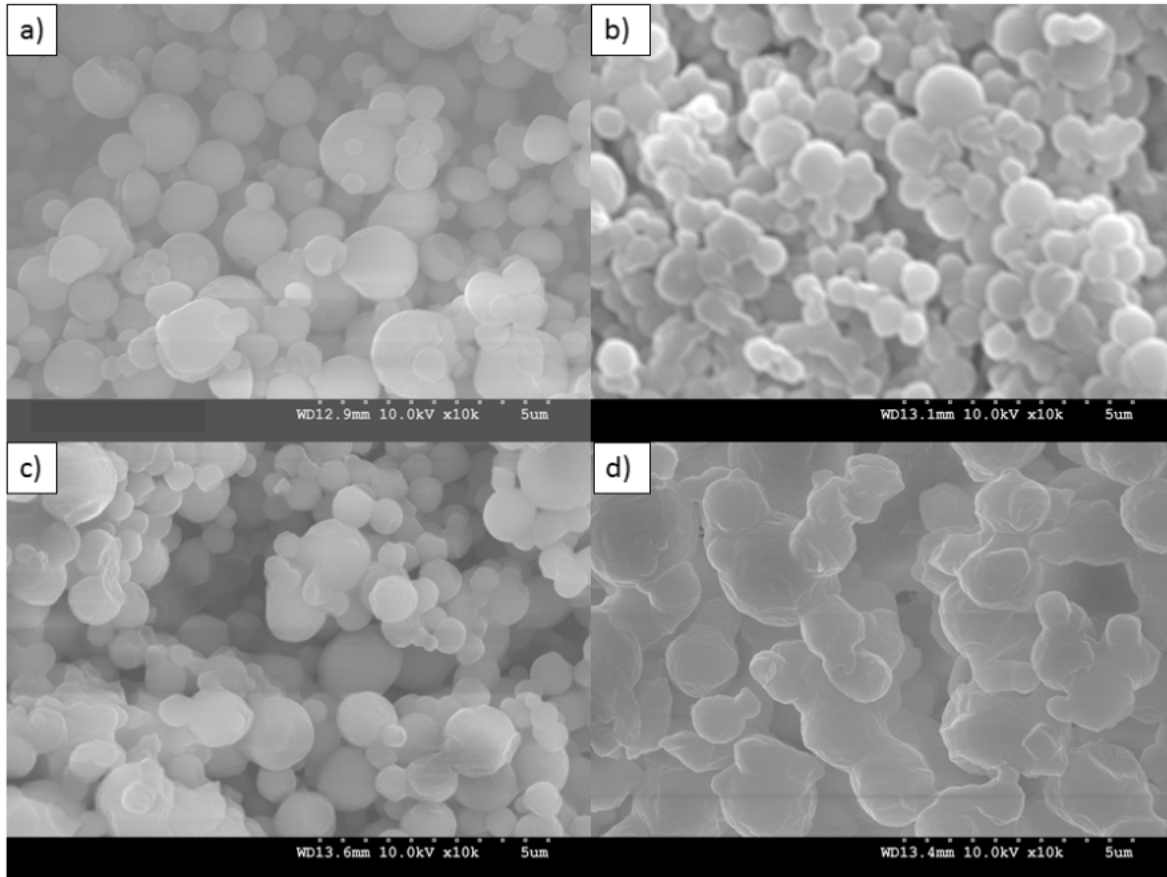
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748 **Table II.** Next Generation Impactor<sup>TM</sup> results for co-spray dried (co-SD) and one-component  
749 spray dried (SD) aerosol systems including mass median aerodynamic diameter (MMAD),  
750 geometric standard deviation (GSD), fine particle fraction (FPF) below 4.4  $\mu\text{m}$ , respirable  
751 fraction (RF) below 4.4  $\mu\text{m}$ , and emitted dose (ED). (n = 3, Ave  $\pm$  SD)

System	MMAD ( $\mu\text{m}$ )	GSD ( $\mu\text{m}$ )	Fine Particle Fraction (%)	Respirable Fraction (%)	Emitted Dose (%)
5PTX:95DPPC	3.4	2.4	77.9 $\pm$ 7.0	50.3 $\pm$ 0.5	80.0 $\pm$ 4.0
5PTX:95DPPC/DPPE-PEG2k	4.7	2.4	60.1 $\pm$ 8.0	49.2 $\pm$ 2.6	89.8 $\pm$ 1.7
5PTX:95DPPC/DPPE-PEG3k	4.9	2.9	64.1 $\pm$ 6.8	47.5 $\pm$ 6.7	90.8 $\pm$ 0.1
5PTX:95DPPC/DPPE-PEG5k	7.5	3.8	43.3 $\pm$ 2.2	44.0 $\pm$ 0.5	94.5 $\pm$ 1.0
25PTX:75DPPC	3.3	2.3	75.4 $\pm$ 4.0	52.4 $\pm$ 2.1	88.6 $\pm$ 6.1
25PTX:75DPPC/DPPE-PEG2k	4.0	2.6	64.5 $\pm$ 2.0	51.7 $\pm$ 4.5	95.2 $\pm$ 2.5
25PTX:75DPPC/DPPE-PEG3k	4.5	2.4	58.1 $\pm$ 8.0	49.1 $\pm$ 6.4	91.1 $\pm$ 3.4
25PTX:75DPPC/DPPE-PEG5k	6.8	3.1	55.4 $\pm$ 1.0	48.8 $\pm$ 7.1	92.2 $\pm$ 6.1
50PTX:50DPPC	3.1	2.5	65.8 $\pm$ 2.4	53.4 $\pm$ 1.3	90.1 $\pm$ 4.1
50PTX:50DPPC/DPPE-PEG2k	3.8	2.4	67.5 $\pm$ 2.7	54.4 $\pm$ 6.2	91.1 $\pm$ 4.1
50PTX:50DPPC/DPPE-PEG3k	4.2	2.8	62.7 $\pm$ 3.6	51.9 $\pm$ 2.5	94.5 $\pm$ 5.2
50PTX:50DPPC/DPPE-PEG5k	5.3	3.1	62.8 $\pm$ 7.2	49.9 $\pm$ 4.2	97.9 $\pm$ 3.2
75PTX:25DPPC	2.7	2.0	73.2 $\pm$ 6.3	52.5 $\pm$ 6.1	89.8 $\pm$ 3.3
75PTX:25DPPC/DPPE-PEG2k	3.9	2.3	72.3 $\pm$ 4.7	53.1 $\pm$ 1.1	91.5 $\pm$ 3.9
75PTX:25DPPC/DPPE-PEG3k	4.0	2.8	69.9 $\pm$ 2.9	54.5 $\pm$ 2.2	96.7 $\pm$ 7.1
75PTX:25DPPC/DPPE-PEG5k	4.8	3.4	63.2 $\pm$ 4.4	54.1 $\pm$ 2.0	94.3 $\pm$ 5.2
100PTX (Low)	3.2	2.3	70.6 $\pm$ 2.1	59.9 $\pm$ 0.8	90.2 $\pm$ 4.7
100PTX (Med)	3.3	2.5	64.6 $\pm$ 1.3	66.6 $\pm$ 3.1	85.1 $\pm$ 9.0
100PTX (High)	3.4	2.6	68.3 $\pm$ 1.1	65.7 $\pm$ 0.2	89.3 $\pm$ 3.2

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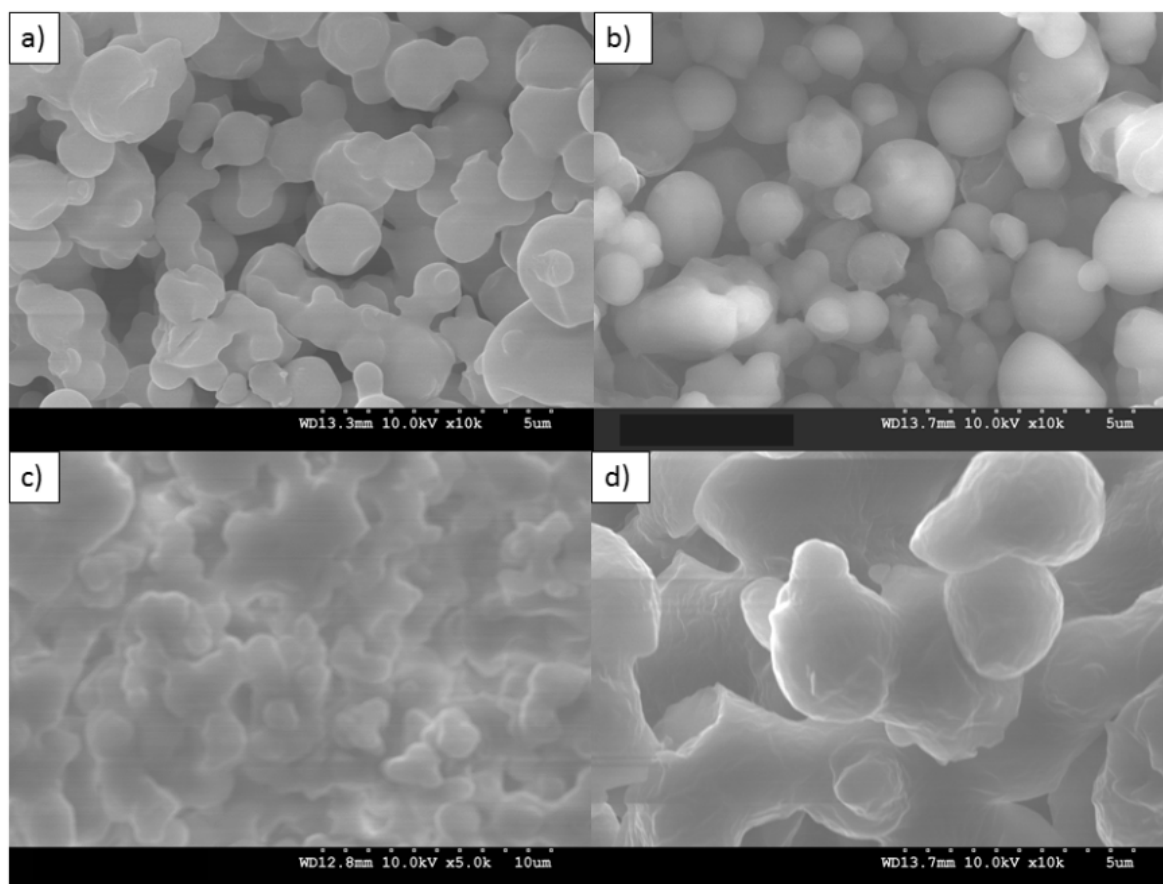
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758 5PTX:95DPPC:DPPE-PEG5k. Magnification for all samples was 10,000x.

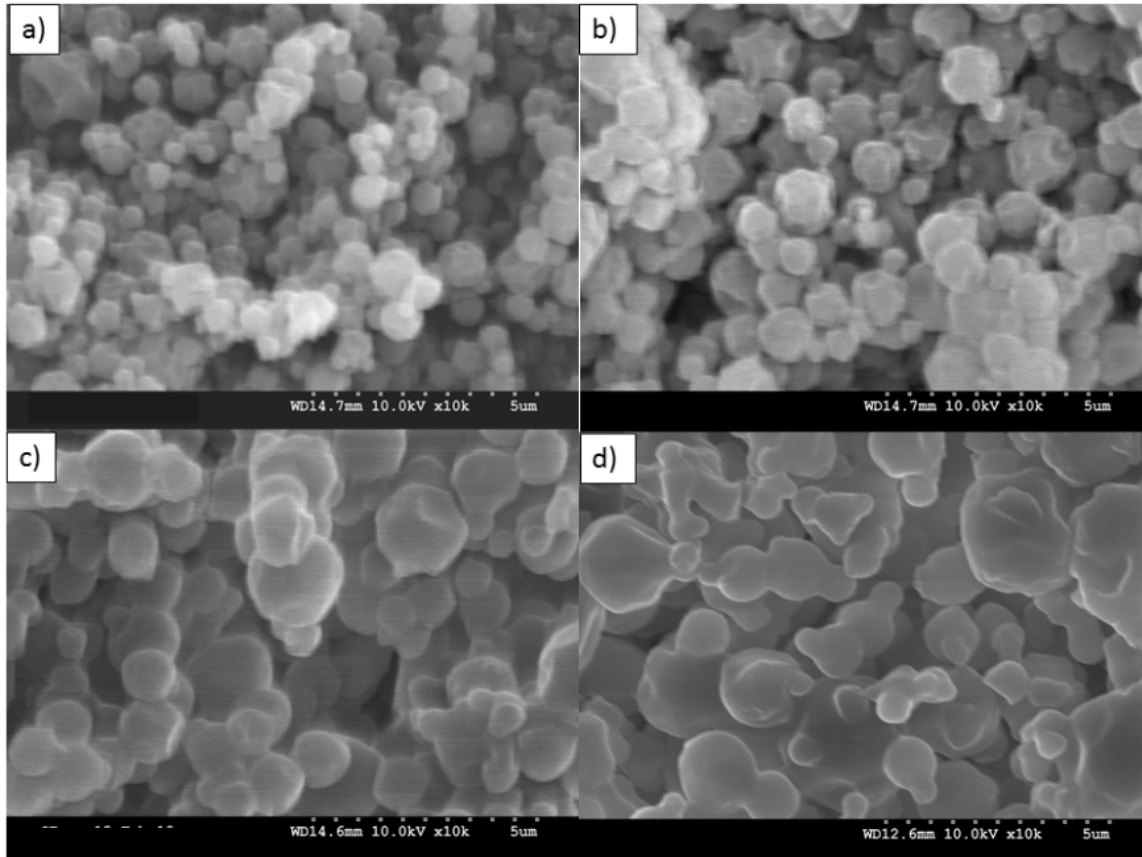
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761 **Figure 2.** SEM micrographs of co-spray dried (co-SD) PEGylated phospholipid particles with  
762 varying PEG chain length containing 25% paclitaxel (PTX): (a) co-SD 25PTX:75DPPC; (b) co-  
763 SD 25PTX:75DPPC/DPPE-PEG2k; (c) co-SD 25PTX:75DPPC/DPPE-PEG3k; and (d) co-SD  
764 25PTX:75DPPC/DPPE-PEG5k. Magnification for all samples was 10,000x.

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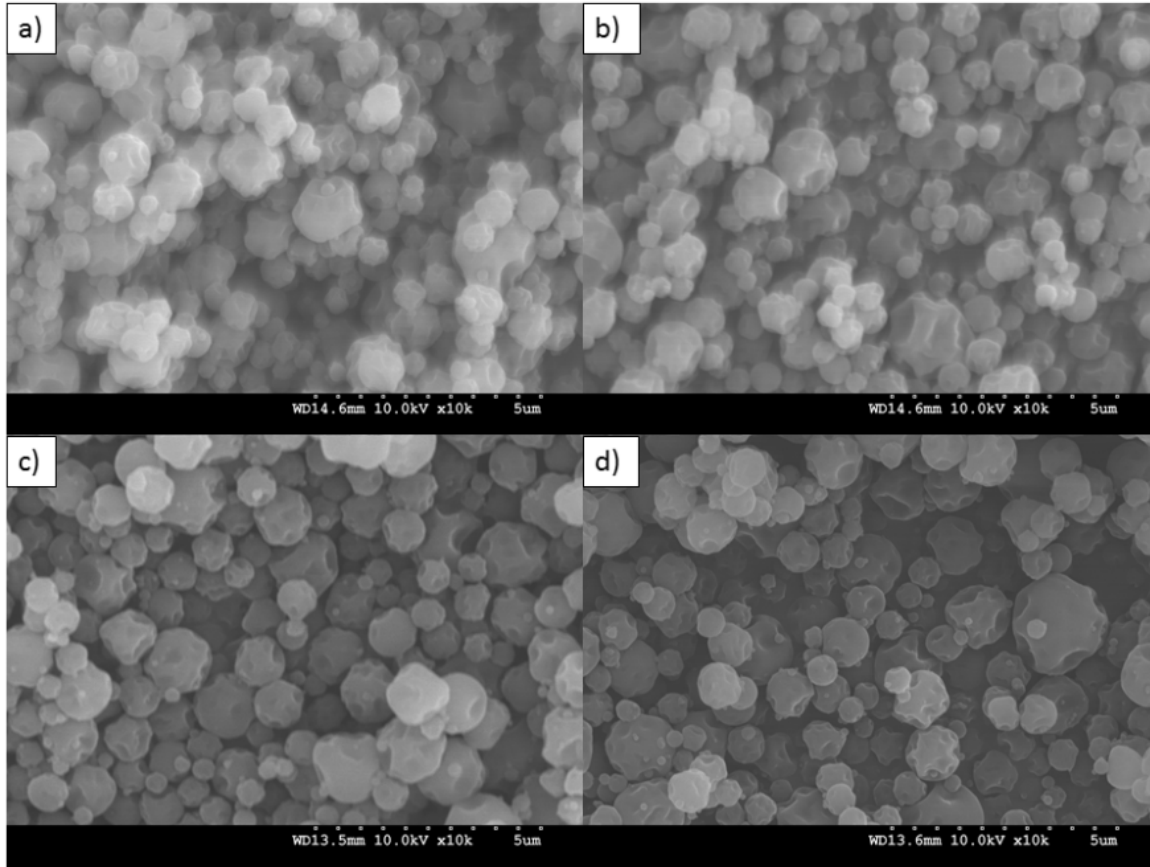


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767 **Figure 3.** SEM micrographs of co-spray dried (co-SD) PEGylated phospholipid particles with  
768 varying PEG chain length containing 50% paclitaxel (PTX): (a) co-SD 50PTX:50DPPC; (b) co-  
769 SD 50PTX:50DPPC/DPPE-PEG2k; (c) co-SD 50PTX:50DPPC/DPPE-PEG3k; and (d) co-SD  
770 50PTX:50DPPC/DPPE-PEG5k. Magnification for all samples was 10,000x.

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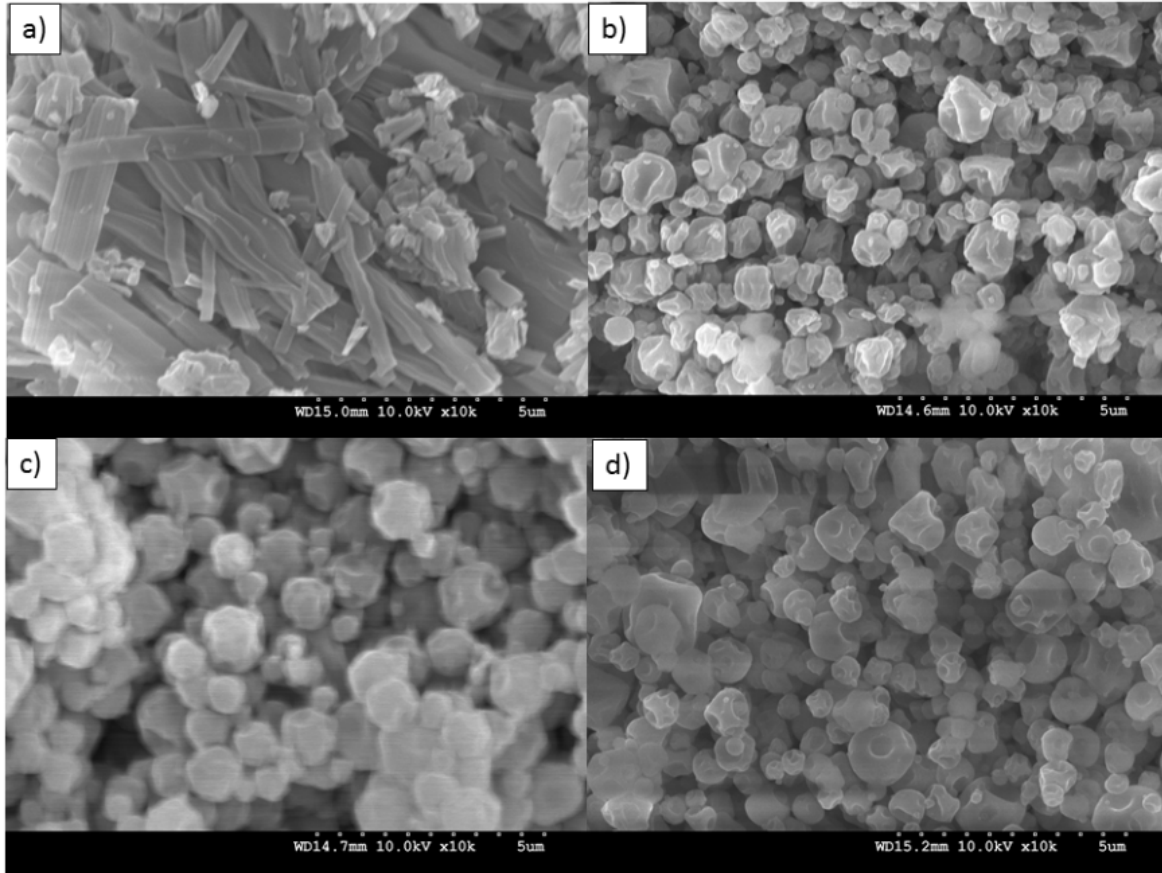
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**Figure 4.** SEM micrographs of co-spray dried (co-SD) PEGylated phospholipid particles with varying PEG chain length containing 75% paclitaxel (PTX): (a) co-SD 75PTX:25DPPC; (b) co-SD 75PTX:25DPPC/DPPE-PEG2k; (c) co-SD 75PTX:25DPPC/DPPE-PEG3k; and (d) co-SD 75PTX:25DPPC/DPPE-PEG5k. Magnification for all samples was 10,000x.



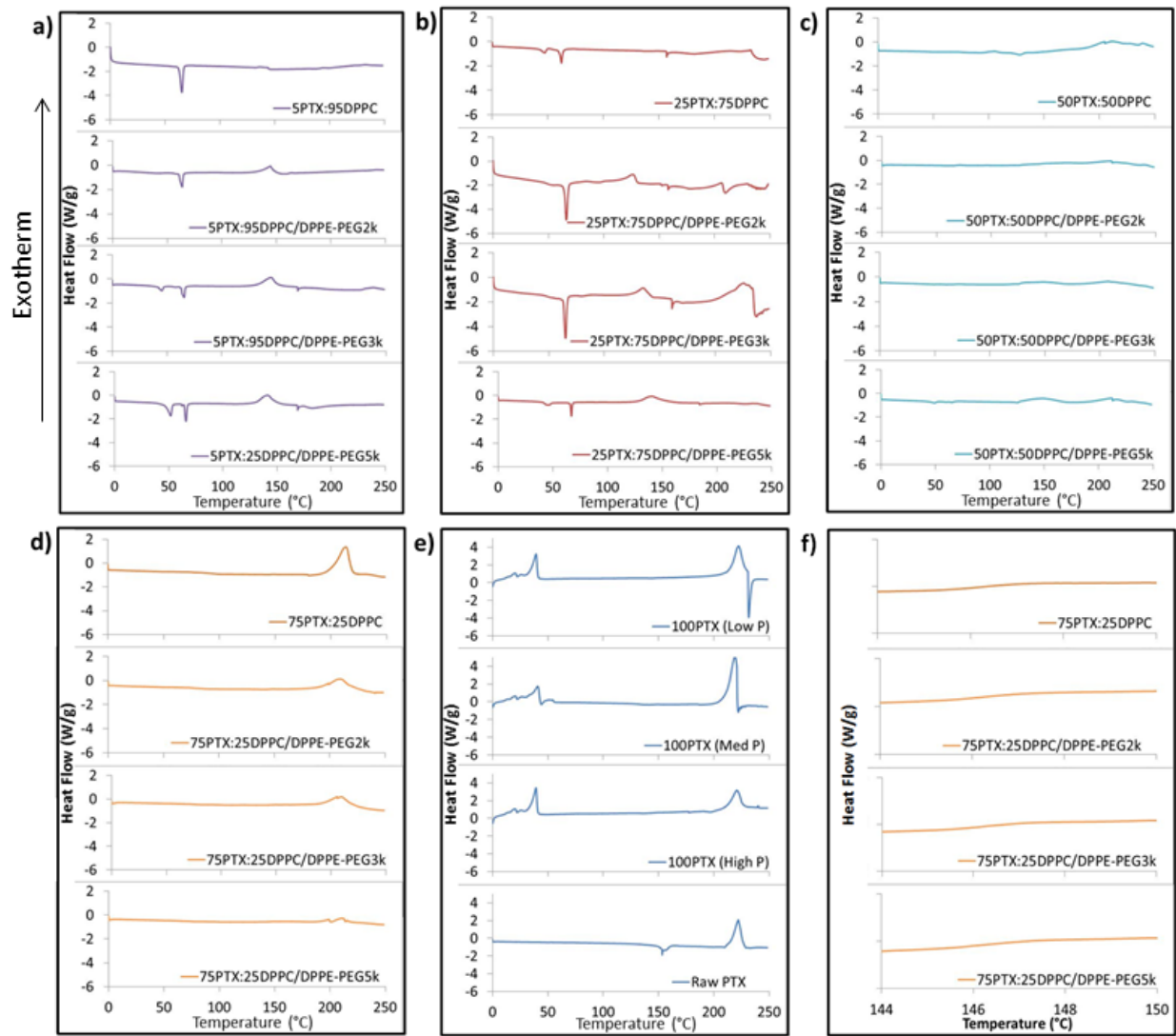
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780 **Figure 5.** SEM micrographs of spray-dried (SD) paclitaxel particles (100PTX) following spray  
781 drying at three pump rates (Low P, Med P, and High P): (a) Raw paclitaxel (PTX); (b) SD  
782 100PTX (Low P); (c) SD 100PTX (Med P); and (d) SD 100PTX (High P). Magnification for all  
783 samples was 10,000x.

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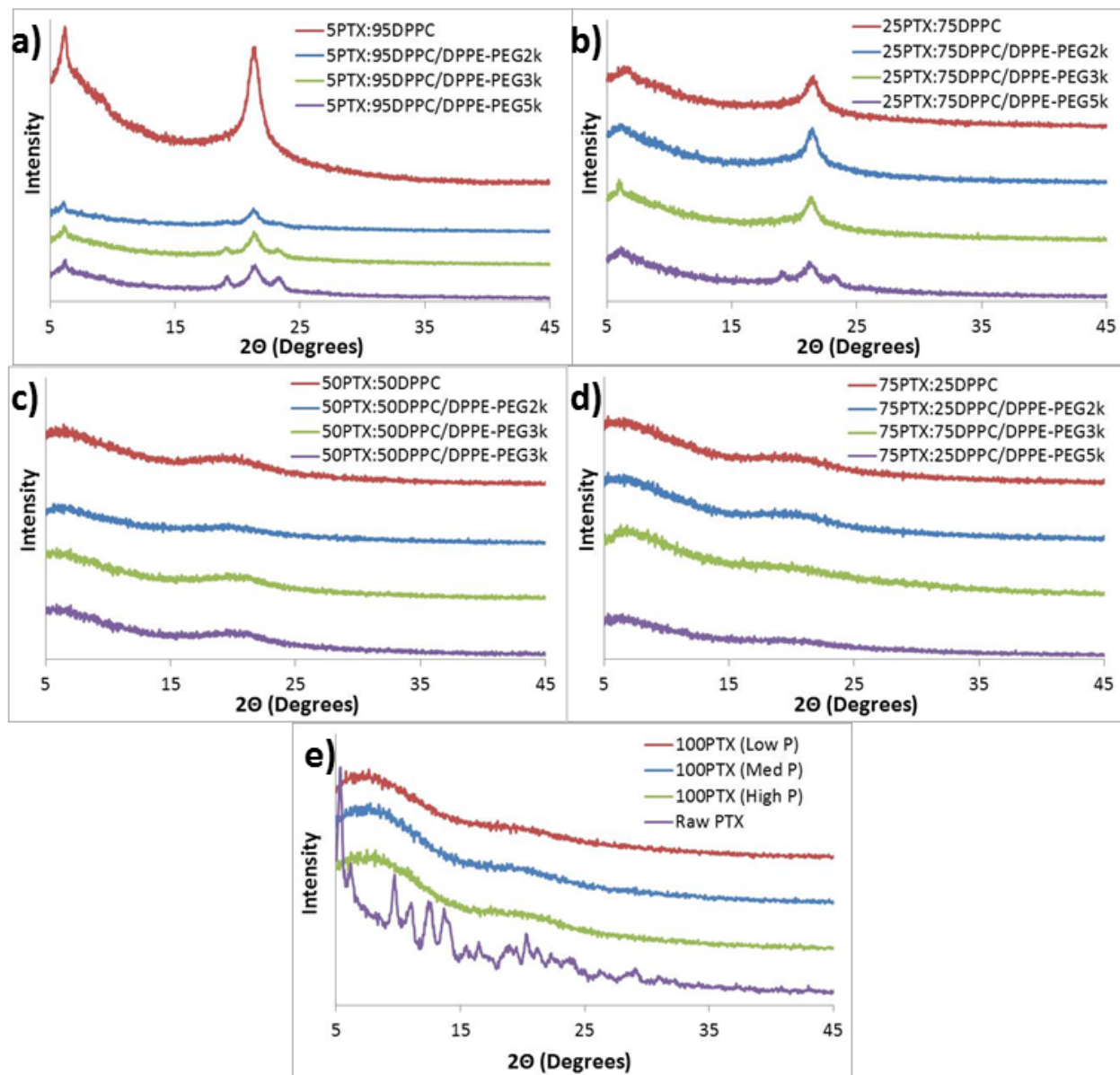
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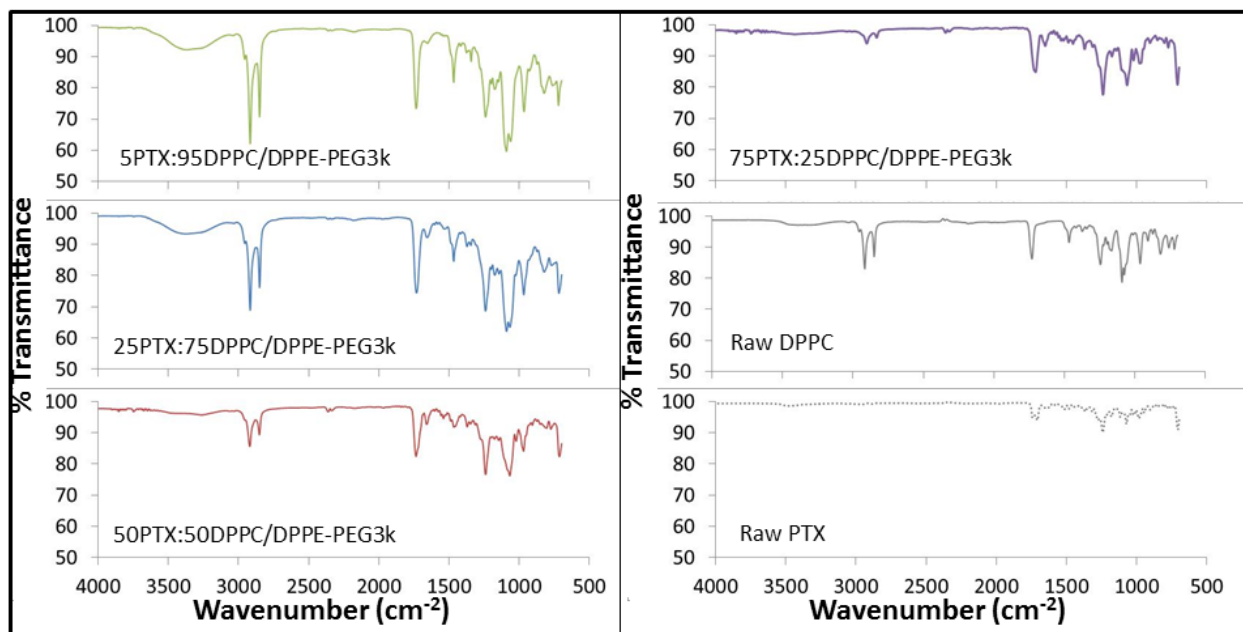
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 791 co-SD 50PTX:50 DPPC vs. co-SD 50 PTX:50 DPPC/DPPE-PEG; (d) co-SD 75PTX:25 DPPC  
 792 vs. co-SD 75PTX:25DPPC/DPPE-PEG; (e) SD 100PTX from three pump rates vs. raw PTX; and  
 793 (f) insert of co-SD 75PTX:25 DPPC vs. co-SD 75PTX:25DPPC/DPPE-PEG for  $T_g$  transition  
 794 visualization.

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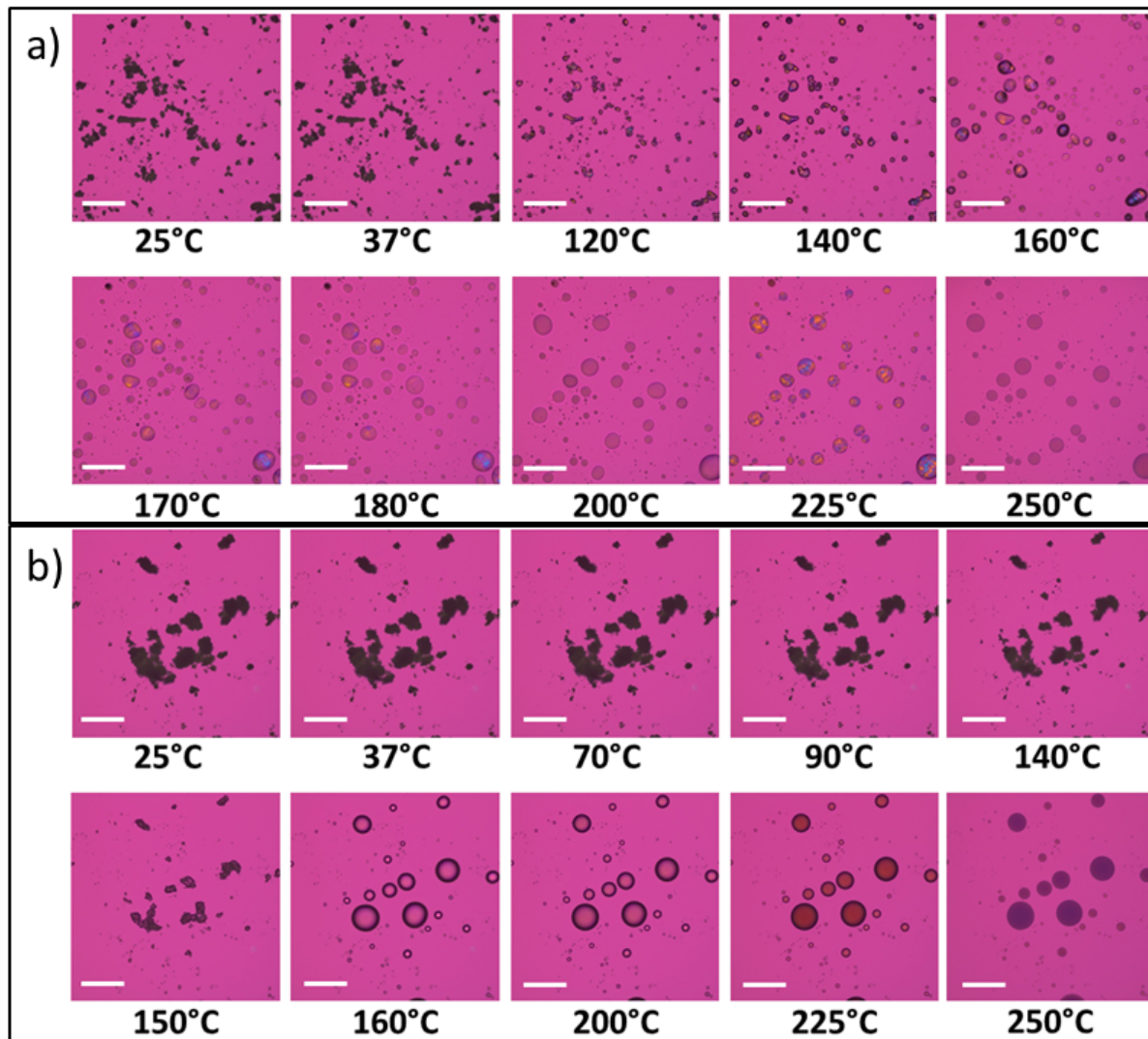
798  
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 801 co-SD 5PTX:95 DPPC/DPPE-PEG; (b) co-SD 25 PTX:75 DPPC vs. co-SD 25 PTX:75  
 802 DPPC/DPPE-PEG; (c) co-SD 50PTX:50 DPPC vs. co-SD 50 PTX:50 DPPC/DPPE-PEG; (d)  
 803 co-SD 75PTX:25 DPPC vs. co-SD 75PTX:25DPPC/DPPE-PEG; and (e) SD 100PTX from three  
 804 pump rates vs. raw PTX.  
 805



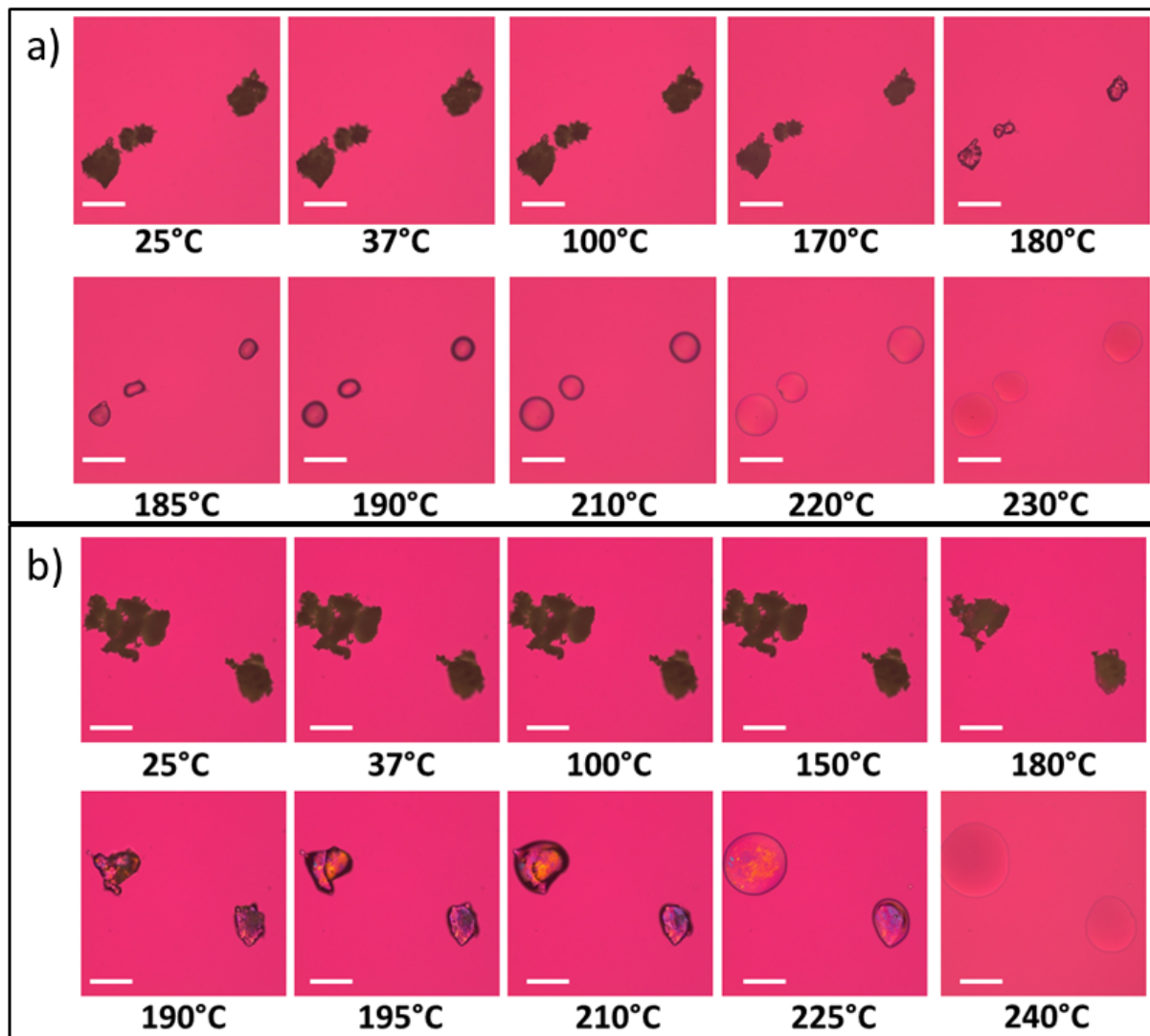
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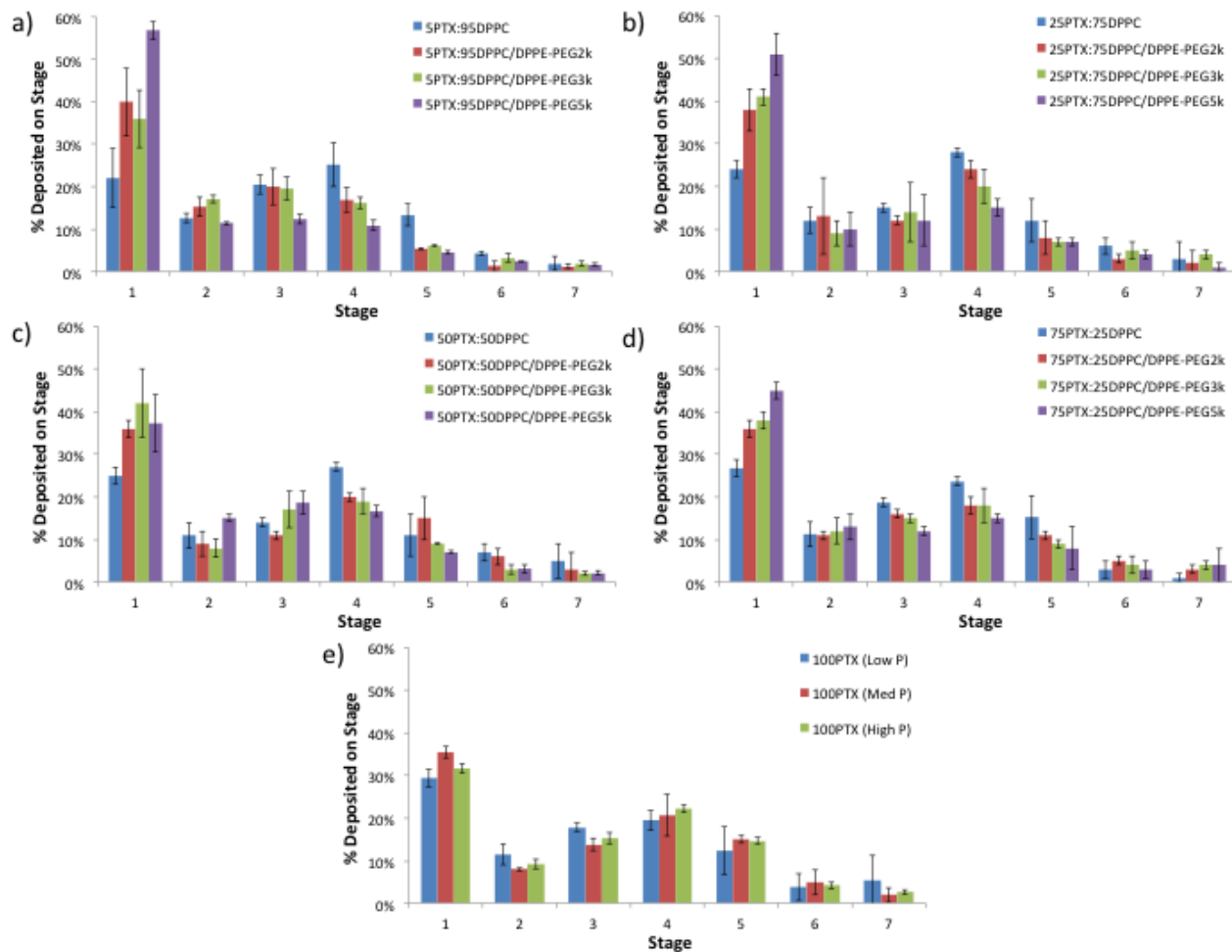
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810  
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814  
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 817



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823 SD 50PTX:50 DPPC vs. co-SD 50 PTX:50 DPPC/DPPE-PEG; d) co-SD 75PTX:25 DPPC vs.  
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828 SD)  
829