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25 **Development of MPC-DPA polymeric nanoparticle systems for inhalation drug delivery applications**

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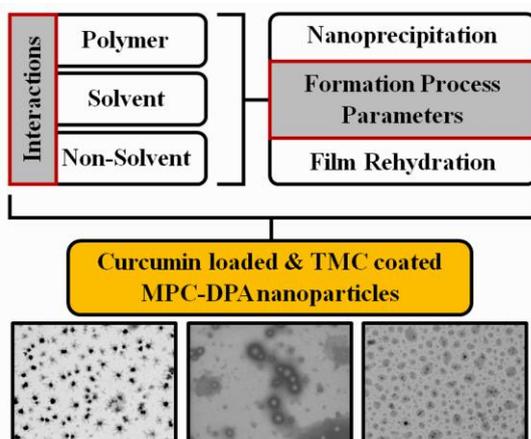
1 **Abstract**

2 Inhalation of nanoparticles for pulmonary drug delivery offers the potential to harness nanomedicine
3 formulations of emerging therapeutics, such as curcumin, for treatment of lung cancer. Biocompatible
4 nanoparticles composed of poly(2-methacryloyloxyethyl phosphorylcholine)-*b*-poly(2-(diisopropylamino)ethyl
5 methacrylate) (MPC-DPA) have been shown to be suitable nanocarriers for drugs, whilst N-trimethyl chitosan
6 chloride (TMC) coating of nanoparticles has been reported to further enhance their cellular delivery efficacy; the
7 combination of the two has not been previously investigated. Development of effective systems requires the
8 predictable, controllable, and reproducible ability to prepare nanosystems possessing particle sizes, and drug
9 loading capacities, appropriate for successful airway travel, lung tissue penetration, and tumour suppression.
10 Although a number of MPC-DPA based nanosystems have been described, a complete understanding of
11 parameters controlling nanoparticle formation, size, and morphology has not been reported; in particular the
12 effects of differing solvent phases remains unclear. In this current study a matrix of 31 solvent combinations
13 were examined to provide novel data pertaining to the formation of MPC-DPA nanoparticles, and in doing so
14 afforded the selection of systems with particle sizes appropriate for pulmonary delivery applications to be loaded
15 with curcumin, and coated with TMC. This paper presents the first report of novel data detailing the successful
16 preparation, characterisation, and optimisation of MPC-DPA nanoparticles of circa 150 – 180 nm diameter, with
17 low polydispersity, and a curcumin loading range of circa 2.5 – 115 μM , tunable by preparation parameters, with
18 and without TMC coating, and thus considered suitable candidates for inhalation drug delivery applications.

19

20 **Keywords:**

21 MPC-DPA; Nanoparticles; Curcumin; TMC; Inhalation; Pulmonary drug delivery.



22

23

1 **1. Introduction**

2 The pulmonary route for therapeutic administration, via inhalation, is a well-suited and effective way to deliver
3 therapeutic forms of hormones, peptides, and other drug molecules which are not easily absorbed through the
4 gastrointestinal tract, into systemic circulation (Barnett, 2004; Kuzmov and Minko, 2015; Mack, 2007).
5 Additionally, recent studies have highlighted that pulmonary delivery can be an effective method for the
6 treatment of lung cancer, compared to the limitations associated with of conventional treatments such as chemo
7 and radio therapy (Minko et al., 2013).

8 The phosphorylcholine (PC) containing copolymer poly(2-methacryloyloxyethyl phosphorylcholine)-*b*-poly(2-
9 (diisopropylamino)ethyl methacrylate) (MPC-DPA) has well proven biocompatibility (Giacomelli et al., 2006;
10 Porto et al., 2011; Yallapu et al., 2010; Yoshikawa et al., 2011), and self-assembles to form a range of
11 nanostructures, including micelles, polymersomes, and higher-order assemblies when the DPA block is
12 deprotonated at physiological pH 7.4. (Blanazs et al., 2009; Derry et al., 2016; Du et al., 2005; Pearson et al.,
13 2013; Porto et al., 2011; Robertson et al., 2014). Moreover, when subsequently exposed to an acidic
14 environment, for example tumor tissue, the DPA becomes protonated and hydrophilic, and the nanoparticles
15 disassemble releasing the therapeutic payload (Pearson et al., 2013; Pegoraro et al., 2013; Salvage et al., 2005).

16 Previous studies have highlighted that MPC-DPA diblock copolymers have the potential to be applied to a range
17 of nanoparticle based clinical therapies, which can utilise the stable nanoparticles for drug delivery and cell
18 targeting of hydrophobic and hydrophilic molecules, proteins, antibodies, and also genetic material (Canton et
19 al., 2013; Colley et al., 2014; Licciardi et al., 2008, 2006, 2005, Lomas et al., 2010, 2008, 2007, Mu et al., 2008;
20 Pegoraro et al., 2014, 2013; Salvage et al., 2005, 2015, 2016). However, copolymer size, the molecular weight
21 (Mw), can affect membrane properties of the nano-assemblies, including thickness, rigidity, stability, and the
22 mechanical properties of the resultant morphology formed (Ahmed et al., 2006; Discher et al., 2007; Discher and
23 Ahmed, 2006; Motornov et al., 2010). Additionally, the stability and morphology of MPC-DPA nanoparticles
24 depends on several key factors including the formation process, copolymer concentration, and the solvents used
25 during assembly (Pearson et al., 2013; Pegoraro et al., 2014; Salvage et al., 2015). The predominant processes
26 reported for the formation of MPC-DPA nanoparticles have been nanoprecipitation (Salvage et al., 2015, 2016)
27 and film rehydration (Canton et al., 2013; Colley et al., 2014; Robertson et al., 2014), both of which use solvents
28 within the process. However, there appears to be no single solvent combination suitable to dissolve the different
29 block length copolymers at the same concentration (Ye et al., 2015). It has also been reported previously that
30 MPC-DPA copolymers are selectively soluble in some organic solvents (Edmondson et al., 2010; Licciardi et al.,

1 2005; Porto et al., 2011), and that choice of solvent can influence the resultant self-assembled nanoparticle size
2 and polydispersity (Alexandridis and Spontak, 1999; Israelachvili, 2011; Meyer et al., 2006; Salvage et al.,
3 2015); however, to date, there has been no detailed and focused study of this effect

4 Curcumin is an active pharmaceutical ingredient (API) which has demonstrated anti-cancer effects against lung,
5 ovarian, pancreatic and colorectal cancers (Gou et al., 2011; Mock et al., 2015; Patil et al., 2015). However, the
6 therapeutic advantages are often attenuated after oral administration due to the physicochemical properties of
7 curcumin, such as low water solubility, $< 1\mu\text{g/mL}$, and a rapid half-life, with poor systemic bioavailability of < 1
8 %. (Betbeder et al., 2015; Nahar et al., 2015; Patil et al., 2015; Shanmugam et al., 2015; Tomren et al., 2007; Xie
9 et al., 2011; Zhang et al., 2013). To overcome these limitation, a number of approaches have been investigated
10 previously, such as solid dispersions (Paradkar et al., 2004), liposomes (Saengkrit et al., 2014), and polymeric
11 micro and nanoparticles (Kakran et al., 2012; Zhang et al., 2013), although none of these approaches yielded
12 significant improvements in bioavailability.

13 N-trimethyl chitosan chloride (TMC), like chitosan, has mucoadhesive properties which can help increase the
14 transit time of drug nano-carriers into the gastrointestinal tract (GIT) and the absorption rate of drugs (Amidi et
15 al., 2006; Zhang et al., 2013). Additionally, TMC can increase paracellular diffusion of nanoparticles, and drugs
16 including curcumin, via epithelial cells by opening the tight junctions of the cell membrane (Amidi et al., 2006;
17 El-Sherbiny and El-Baz, 2015; Guan et al., 2012; Sahni et al., 2008; Steed et al., 2010; Zhang et al., 2013).

18 Compared to chitosan, TMC is more soluble in aqueous solutions, especially at neutral physiological pH 7.4
19 (Sahni et al., 2008). It also has the ability to improve formulation stability and bioavailability, the stability of
20 liposomes after coating, and is an effective enhancer of hydrophilic drug and protein absorption (Chen et al.,
21 2016; Panya et al., 2010). Moreover, TMC has displayed strong electrostatic interactions between polymer
22 cationic amine groups and negatively charged tumor cells, thus providing targeted drug delivery properties
23 (Guan et al., 2012; Thanou et al., 2000). Indeed, TMC has been shown to have many promising micro and
24 nanoparticle based applications for oral, nasal, pulmonary, ocular, and intestinal delivery of drugs (Amidi et al.,
25 2006; El-Sherbiny and El-Baz, 2015; Guan et al., 2012; Sahni et al., 2008). Thus TMC coating of MPC-DPA
26 nanoparticles may enhance their efficacy further, and contribute towards developing curcumin formulations with
27 potential clinical applications for pulmonary delivered treatment of human lung cancers (Basnet and Skalko-
28 Basnet, 2011; Gou et al., 2011; Kakran et al., 2012; Mock et al., 2015; Nahar et al., 2015; Patil et al., 2015;
29 Prasad et al., 2014; Saengkrit et al., 2014; Wang et al., 2015).

1 The study reported herein investigated the development of MPC-DPA nanoparticles as potential pulmonary drug
2 delivery vehicles, based on controlling particle size such that effective airway travel and lung tissue penetration
3 would theoretically be achievable. Preparation and characterisation was undertaken using two contrasting
4 nanoparticle formation methods, nanoprecipitation and film rehydration, and the effects of 31 solvent mixes on
5 nanoparticle size and polydispersity studied. After this initial screening, nanoparticle formulations possessing
6 particle size and polydispersity values reported as suitable for pulmonary route applications, were selected for
7 subsequent curcumin drug loading, which has promising anticancer effects, and TMC surface modification,
8 which has tissue penetration enhancing properties. In summary, this paper details for the first time novel data
9 pertaining to the development of curcumin loaded, and TMC modified, MPC-DPA nanoparticle formulations for
10 inhalation based lung cancer therapies, and has also provided an extensive matrix of novel particle size
11 controllability data that will be of value for development of further novel MPC-DPA nanoparticle based
12 pharmaceutical delivery applications.

13

14 **2. Methods and materials**

15 *2.1. Materials*

16 The MPC₃₀-DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀ block copolymers were supplied by Prof Steven Armes (University of
17 Sheffield, UK) having been synthesised by atom transfer radical polymerisation (ATRP), as detailed previously
18 (Ma et al., 2003; Salvage et al., 2005). Methanol, ethanol, chloroform, isopropanol, acetonitrile, phosphate
19 buffered saline, phosphotungstic acid, hydrochloric acid, sodium hydroxide, sodium chloride, deuterium oxide,
20 curcumin 98%, methyl iodide 99%, sodium iodide 99%, 1-methyl-2-pyrrolidone 99.5%, 2 mL glass vials, 20 mL
21 glass vials, 13 mm diameter glass cover slips, 2 mL plastic syringes, 0.45 µm and 0.22 µm syringe filters, were
22 purchased from Fisher Scientific, UK. Transmission electron microscopy (TEM) grids were purchased from
23 Agar Scientific, UK. Chitosan (200 - 400 kDa) was purchased from Sigma-Aldrich, UK. Atomic force
24 microscopy (AFM) cantilever tips were purchased from Windsor Scientific, UK.

25

26 *2.2. NMR and GPC characterisation*

27 Block ratio composition and molecular weight of the received MPC-DPA block copolymers was confirmed
28 using ¹H nuclear magnetic resonance (NMR) spectroscopy and gel permeation chromatography (GPC) using
29 methods detailed previously (Salvage et al., 2016).

30

1 2.3. Solvent solubility

2 The solubility of the MPC-DPA copolymers in organic solvents of differing polarity values (Table 1) was
3 assessed for a matrix of 31 solvent combinations utilising methanol, ethanol, chloroform, isopropanol, and
4 acetonitrile, as shown in Table 2. For each solvent combination, copolymer solutions were prepared (40 mg/mL)
5 and allowed to equilibrate for two days, at room temperature, for dissolution to complete. The copolymer solutions
6 were then visually assessed, and solubility in each of the 31 solvents designated as one of three British
7 Pharmacopoeia based solubility classes: completely soluble, slightly soluble, and practically insoluble.

8

9 2.4. Nanoprecipitation formation of nanoparticle systems

10 Copolymer solutions (40 mg/mL) in the 31 solvents (Table 2) were prepared for MPC₃₀-DPA₁₀₀ and MPC₁₀₀-
11 DPA₁₀₀. The nanoprecipitation process was adapted from a published method (Salvage et al., 2015), whereby
12 aliquots (100 µL) of the copolymer solutions were added drop-wise to 10 mL of phosphate buffer saline (PBS)
13 (pH 7.4) and stirred (600 rpm) for 4 minutes with a magnetic stir bar (20 mm) to facilitate solvent evaporation.
14 Copolymer concentration of the prepared nanoparticle systems was 0.4 mg/mL. Independent (n = 3) triplicate
15 repeats of the experimental work were undertaken.

16

17 2.5. Film-rehydration formation of nanoparticle systems

18 Copolymer solutions (5 mg/mL) in 6 solvents selected from the 31 solvent matrix (Table 2), based on the
19 nanoprecipitation data, were prepared for MPC₃₀-DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀. Aliquots (10 mL) of these were
20 added to 20 mL glass vials, and the solvent removed via evaporation in a Fiestream digital vacuum oven (1000
21 mbar) at 50 °C for 8 hours. The copolymer films in the glass vials were then rehydrated using 10 mL of PBS (pH
22 7.4) with constant stirring (200 rpm), via a magnetic stir bar (15 mm), for 1 week at ambient room temperature,
23 and then sonicated for 15 minutes to reduce the average particle size and polydispersity (Battaglia et al., 2011;
24 Pegoraro et al., 2014; Sorrell et al., 2014). The resultant samples (5 mg/mL) were further diluted with PBS (pH
25 7.4) to provide 0.5 mg/mL copolymer nanoparticle systems. Independent (n = 3) triplicate repeats of the
26 experimental work were undertaken.

27

28 2.6. Nanoparticle system characterisation

1 The prepared MPC-DPA nanoparticle systems were assessed for particle size, polydispersity, zeta potential, and
2 particle morphology, using dynamic light scattering (DLS), laser Doppler electrophoresis (LDE), and scanning
3 transmission electron microscopy (STEM).

4 5 *2.6.1 Dynamic light scattering*

6 Particle size (Z_{Ave}) and polydispersity (Pd) of the nanoparticle systems were measured in triplicate at 25° C,
7 with a Malvern Zetasizer Nano ZS90 instrument, using the DLS method detailed previously (Salvage et al.,
8 2016). Samples were examined unfiltered, syringe filtered 0.45 μm , and syringe filtered 0.22 μm . The particle
9 size data are presented as intensity based hydrodynamic diameters (D_h), with average nanoparticle system
10 particle size (Z_{Ave}) determined via Cummulants analysis, and where multiple particle population peaks were
11 present within a sample, individual peak average diameters (D_h^1 , D_h^2 , D_h^3) determined by non-negative least
12 square (NNLS) analysis. Independent (n = 3) triplicate repeats of the experimental work were undertaken.

13 14 *2.6.2. Laser Doppler electrophoresis*

15 Zeta potential of the nanoparticles was determined via LDE, using a Malvern Zetasizer Nano ZS90 instrument at
16 25 °C, with Malvern DTS 1061 capillary cells and DTS 1235 zetapotential standards. Data consisted of the
17 average value of three consecutive, 120 seconds, measurements, composed of 12 runs. The LDE technique
18 measured the electrophoretic mobility of the nanoparticles, and then used the Henry equation ($U_E = 2\varepsilon z f(Ka) /$
19 3η), where U_E = electrophoretic mobility, ε = dielectric constant, z = zetapotential, $f(Ka)$ = Henry's function,
20 and η = viscosity, to calculate the zeta potential. Independent (n = 3) triplicate repeats of the experimental work
21 were undertaken.

22 23 *2.6.3. Scanning transmission electron microscopy*

24 Particle morphology was investigated via STEM, using a Zeiss SIGMA field emission gun scanning electron
25 microscope (FEG-SEM) equipped with a Zeiss STEM detector. Working conditions used were; 20 kV
26 accelerating voltage, 20 μm aperture, and 3 mm working distance. To prepare the STEM samples, 200 mesh
27 Formvar coated copper TEM grids were plasma treated, 40 seconds at 5 watts, in a Polaron PT7150 plasma
28 barrel etcher for 30 seconds, to improve surface wettability. Then 1 drop of sample (0.22 μm filtered) was
29 applied to the TEM grid for 60 seconds, excess wicked away, 1 drop of filtered (0.22 μm) 2% phophotungstic

1 acid (PTA) (pH 7.4) applied to the TEM grid for 60 seconds, excess wicked away, and then air dried at room
2 temperature.

3

4 2.7. Atomic force microscopy

5 Copolymer film morphology and roughness were examined via AFM, using a Digital Instruments Dimension
6 D3000 AFM, equipped with a Nanoscope 111A controller. The AFM was operated in tapping mode, at a scan
7 rate of 0.25 Hz, a scan size of 100 μm , and using Nanosensor PointProbe NCH-W cantilever AFM tips. The
8 copolymer films (5 mg/mL) were prepared by applying 1 drop of copolymer-solvent sample to a 13 mm
9 diameter glass coverslip.

10

11 2.8. TMC synthesis

12 The N-trimethyl chitosan chloride (TMC) synthesis was undertaken via nucleophilic substitution using methyl
13 iodide in a two-step reaction to produce a high degree of quarterisation (DQ %) in accordance with published
14 protocols (Patrulea et al., 2015; Sahni et al., 2008; Sieval et al., 1998). The chitosan degree of deacetylation
15 (DDA) (Lavertu et al., 2003), and the TMC degree of quaternisation (DQ) (Patrulea et al., 2015; Thanou et al.,
16 2000), were determined by ^1H NMR in deuterium oxide (D_2O) using a 400 MHz Bruker Ascend spectrometer
17 and were calculated according to equations 1 and 2, respectively:

18

$$19 \text{ DDA (\%)} = \left(\frac{H1D}{H1D + HAc/3} \right) \times 100 \dots\dots\dots (1)$$

20 Where: H1D is the value of the integral for the peak of proton H1 of deacetylation monomer at chemical shift
21 5.211 ppm; and HAc is the peak of the three protons of acetyl group at chemical shift 2.351 ppm.

22

$$23 \text{ DQ (\%)} = \left(\frac{[(\text{CH}_3)_3]}{[\text{H}]} \times \frac{1}{9} \right) \times 100 \dots\dots\dots (2)$$

24 Where: $[(\text{CH}_3)_3]$ is the value of the integral for the peak of trimethyl amino function $[-\text{N}+(\text{CH}_3)_3]$ at chemical
25 shift 3.3 ppm; and the $[\text{H}]$ is the value of the integral for the peaks of the proton H1 of chitosan at chemical shift
26 4.7 and 5.4 ppm and used as internal standards.

27

28 2.9. Calibration curves for curcumin solubility and loading

29 To determine the solubility of curcumin in the solvents (ethanol-isopropanol and methanol-ethanol-chloroform-
30 acetonitrile) used for nanoparticle loading (Section 2.10), standard curves were constructed using a PerkinElmer

1 Lambda 25 UV-Vis spectrophotometer. Curcumin in solvent solutions were prepared (0 – 12 mg/L), the λ max
2 measured, the absorbance of the dilutions recorded at the λ max, and the standard curves plotted. Saturated
3 solutions of curcumin in solvent were then prepared (Baka et al., 2008), filtered (0.22 μ m) to remove
4 undissolved curcumin, diluted to the linear range, the absorbance at λ max measured, and the curcumin solubility
5 calculated from the standard curves.

6 To determine curcumin encapsulation and loading efficiency, standard curves were also prepared for curcumin at
7 pH 1.6 in PBS-methanol (1:9 v/v), as curcumin has been shown to degrade in alkaline conditions (Liu et al.,
8 2012; Wang et al., 1997), and has greater stability in acidic solutions (Tomren et al., 2007). Aliquots (1 mL) of
9 the curcumin loaded MPC-DPA nanoparticle in PBS formulations (Section 2.10), were then dissolved in 9 mL of
10 methanol (pH 1.6), diluted to the linear range, the absorbance at λ max measured, the curcumin content
11 determined from the standard curve, and the encapsulation efficiency and loading capacity calculated from
12 equations 3 and 4. Independent (n = 3) triplicate repeats of the experimental work were undertaken.

13
14 Encapsulation efficiency (EE%) = $\left[\frac{\text{weight of curcumin in nanoparticles}}{\text{weight of feed curcumin}} \right] \times 100 \dots\dots\dots(3)$

15
16 Loading capacity (LC%) = $\left[\frac{\text{weight of curcumin in nanoparticles}}{\text{weight of feed copolymer}} \right] \times 100 \dots\dots\dots(4)$

17
18 *2.10. Curcumin loaded nanoparticle systems*

19 MPC-DPA nanoparticle systems loaded with curcumin were prepared for formulation 12 (MPC₁₀₀-DPA₁₀₀ in
20 ethanol-isopropanol) and formulation 26 (MPC₃₀-DPA₁₀₀ in methanol-ethanol-chloroform-acetonitrile) as per the
21 nanoprecipitation (Section 2.4) and film-rehydration (Section 2.5) samples, using curcumin saturated solvents.
22 The solubility of curcumin in the solvents used, and the curcumin encapsulation efficiency and loading capacity
23 of the prepared MPC-DPA nanosystems were determined using standard curves as per Section 2.9. Independent
24 (n = 3) triplicate repeats of the experimental work were undertaken.

25
26 *2.11. TMC coated MPC-DPA nanoparticles*

27 Coating of the MPC-DPA curcumin loaded nanoparticle systems with TMC polymer was undertaken by firstly
28 preparing samples as per Section 2.10. TMC was then added to the samples at 1 mg/mL with stirring (200 rpm)
29 for 24 hours to coat the nanoparticles. The TMC coated nanoparticle systems were characterised using the

1 methods described in Sections 2.6 and 2.9, for particle size and polydispersity, zeta potential, morphology, and
2 curcumin encapsulation efficiency and loading capacity. Independent (n = 3) triplicate repeats of the
3 experimental work were undertaken.

4 5 *2.12. Fourier transform infrared spectroscopy*

6 To investigate the chemical structure of curcumin, potential interactions between curcumin and the MPC-DPA
7 copolymers, the structure of MPC-DPA copolymers, chitosan, and the synthesised TMC polymers, Fourier
8 transform infrared spectroscopy (FTIR) was performed using a PerkinElmer Spectrum 65 FT-IR Spectrometer.
9 All transmission spectra were obtained at ambient temperature by recording the average of 32 scans in the region
10 between a wave number 4000 and 650 cm⁻¹ with a resolution of 4 cm⁻¹.

11 12 **3. Results and Discussion**

13 *3.1. NMR and GPC characterisation*

14 The composition of the MPC₃₀-DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀ diblock copolymers, confirmed via ¹H NMR and
15 GPC, and shown in Fig. 1a and 1b respectively, and Table 3, was consistent with previous reports (Ma et al.,
16 2003; Salvage et al., 2005), indicating good control of polymer synthesis and stability.

17 18 *3.2. Solvent solubility*

19 To explore the influence of organic solvent properties, polarity and miscibility in water, upon the formation of
20 nanoparticles, and the properties of particle size and polydispersity, a matrix comprising 31 organic solvent
21 combinations containing MPC₃₀-DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀ copolymers (40 mg/mL) were prepared. The
22 resultant copolymer solutions were visually classified for solubility in Table 4. The polarity value of the solvents
23 indicated the expected level of polymer solvent interaction, and will also have influenced the contact force,
24 surface tension, between the MPC-DPA copolymer and solvents, thus influencing the resultant aggregate
25 structure and homogeneity of the bulk solution (Ye et al., 2015). The data suggested that MPC₁₀₀-DPA₁₀₀
26 copolymer was more soluble in the 31 solvent combinations than MPC₃₀-DPA₁₀₀, possibly due to the higher ratio
27 of amphiphilic block copolymer MPC, and thus more hydrophilic nature.

28 29 *3.3. Nanoprecipitation formation of nanoparticle systems*

1 For MPC₃₀-DPA₁₀₀ nanoparticles in PBS (pH 7.4), formed via nanoprecipitation from the 31 solvent
2 combinations, DLS analysis revealed a wide range of average particle sizes (Z_{Ave}) and polydispersity (Pd)
3 values, as seen in Table 5. The particle sizes produced were consistent with those of micelles, polymersomes,
4 and high-order assemblies (Du et al., 2005; Pearson et al., 2013). Exceptions were formulations 3, 4, 13 and 14
5 which did not produce measurable nanoparticles, which correlated with the solvent solubility data (Table 4),
6 indicating polymer insolubility in those solvent combinations. Additionally, formulations 10, 17, 19, and 25
7 resulted in loss of nanoparticle presence upon filtration, principally due to exclusion of large particle aggregates.
8 Similar observation was noted for MPC₁₀₀-DPA₁₀₀ nanoparticle systems, except for formulations 3, 4 and 13 as
9 seen in Table 5, however there were no filtration associated total particle losses, possibly due to the more
10 hydrophilic nature of the MPC₁₀₀ block relative to the MPC₃₀ block. Regarding the filtration process, using 0.45
11 and 0.22 μm filters, the average particle size and polydispersity of the MPC-DPA nanoparticles was reduced
12 upon passing through the filter, which was in agreement with previous work (Salvage et al., 2015, 2016).
13 Without filtration MPC₃₀-DPA₁₀₀ nanoparticles displayed larger mean particle sizes compared to MPC₁₀₀-DPA₁₀₀
14 nanoparticles. However, after filtration (0.22 μm), MPC₁₀₀-DPA₁₀₀ produced larger nanoparticles with a higher
15 polydispersity compared to MPC₃₀-DPA₁₀₀. These results were not unexpected, as the MPC₁₀₀-DPA₁₀₀
16 copolymer structure has a higher degree of amphiphilic MPC block polymer compared to MPC₃₀-DPA₁₀₀, and
17 the findings were in agreement with previous reports of degree of polymerisation influencing particle size and
18 morphology when using the same organic phase (Patikarnmonthon, 2013). In summary, these data indicated that
19 particle size was dependent upon the type and nature of organic solvent combination used, could be refined with
20 filtration, and was in agreement with previous reports of solvent choice effects (Bilati et al., 2005; Salvage et al.,
21 2015).

22 In order to select suitable comparators against the film rehydration process, assessment of the post-filtration
23 (0.22 μm) data, (Table 5 and Table 6), indicated that nanoprecipitation formulations 15, 24, 26 for MPC₃₀-
24 DPA₁₀₀, and 12, 16, 29 for MPC₁₀₀-DPA₁₀₀, had the largest mean particle sizes combined with the lowest
25 polydispersity values, and were thus chosen.

26

27 *3.4. Film-rehydration formation of nanoparticle systems*

28 The MPC-DPA film rehydration samples for solvent combinations 15, 24, 26, (MPC₃₀-DPA₁₀₀) and 12, 16, 29
29 (MPC₁₀₀-DPA₁₀₀) were formed via solvent casting of the polymer film, followed by rehydration in PBS (pH 7.4),
30 with stirring, over a 7 day period. It can be seen from Figure 2 that there were visible differences in the rate of

1 film rehydration and polymer solubilisation between the polymers. Immediately after addition of the PBS ($t = 0$)
2 MPC₁₀₀-DPA₁₀₀ polymer films began to hydrate, whilst the less hydrophilic MPC₃₀-DPA₁₀₀ did not (Figure 2a).
3 This slower rate of rehydration was evident for MPC₃₀-DPA₁₀₀ throughout the subsequent experimental time
4 course (Figure 2b-d), until apparent full rehydration was reached at day 7 ($t = 7$) (Figure 2e). There was also
5 visual evidence suggesting that the solvent used for casting influenced rate of film rehydration. To investigate
6 this further, AFM analysis of the polymer films was undertaken.

7 The AFM data indicated that the film structure and surface morphology, of the copolymer films, were different
8 when using the selected solvent combinations, between and within the two copolymers, as shown in Table 7 and
9 Figure 3. In addition, it was evident that the surface roughness measurement data was greater for formulations
10 12, 15, 24 and 29 (Table 7), which contained isopropanol, but no chloroform, relative to formulations 16 and 26,
11 which contained chloroform, but no isopropanol. As seen in Table 1, Isopropanol had a higher viscosity and
12 boiling point than chloroform, and these data illustrated that solvent properties can influence copolymer
13 conformations when drying, and affect the resultant copolymer film topology. Furthermore, the higher level of
14 film surface roughness can lead to greater adhesive strength of the copolymer films (Bowen et al., 1998) , and
15 therefore rehydration of the film, and creation MPC-DPA nanoparticles, may require an increased stirring shear
16 rate or time, as seen in Figure 2.

17 Following the 7 day film rehydration, samples underwent sonication for 15 mins to reduce the heterogeneous
18 morphology of nanoparticles in the systems (Yealland, 2015), followed by DLS particle size measurement and
19 STEM imaging of the nanoparticles. As seen with the nanoprecipitation samples, MPC₃₀-DPA₁₀₀ nanoparticles
20 formed by film rehydration possessed larger average particle sizes, compared to the MPC₁₀₀-DPA₁₀₀, without
21 filtration as shown in Table 8. This may have been due to the hydrophobic effect of DPA₁₀₀ in the copolymer
22 being stronger than the amphiphilic effect of MPC₃₀, and thus consistent with the slower hydration observed
23 (Figure 2) and resultant larger diameter nano-assemblies. However, when samples were filtered (0.45 and 0.22
24 μm), the particle sizes and polydispersity values were greatly reduced for both MPC₃₀-DPA₁₀₀ and MPC₁₀₀-
25 DPA₁₀₀ samples, resulting in average diameters in the 120 – 150 nm size range, which was consistent with MPC-
26 DPA polymersome sizes (Du et al., 2005).

27 In summary, the DLS data (Table 8), indicated that filtration (0.22 μm) of MPC-DPA film rehydration samples
28 offers the potential to controllably produce nanoparticles of low polydispersity, with good reproducibility, and
29 the size viewed a suitable for inhalation delivery applications with maximum therapeutic efficacy (Cipolla et al.,

1 2013; Garbuzenko et al., 2014; Gratton et al., 2008; Hu et al., 2014; Koshkina et al., 2001; Kuzmov and Minko,
2 2015; Ungaro et al., 2012).

3

4 *3.5. Comparison of nanoprecipitation vs film rehydration*

5 The methods used to create MPC-DPA nanoparticles, with or without drug loading, were nanoprecipitation and
6 film rehydration, as described earlier, which have specific properties and requirements associated with each
7 (Battaglia and Ryan, 2006; Messenger et al., 2014). For example, the nanoprecipitation method is a bottom-up
8 procedure which assembles nanoparticles from copolymer molecules via the rapid injection of dissolved
9 copolymer organic phase into an aqueous phase. Due to using free moving molecules, this method is a fast
10 production procedure, capable of reproducibly forming nanoparticles of low polydispersity. In contrast, the film
11 rehydration method is a top-down approach, and depends upon transition of the bulk copolymer molecules from
12 dried, cast film, state, through a copolymer swelling phase, to formation of dispersed polymer nanoparticles,
13 which are primarily polymersomes. These often display, large, micron, particle scale, and require mechanical
14 energy input to form, such as high shearing and stirring rates, and sonication. This method has some
15 disadvantages, for example a long processing and formation time, high energy input, and in some cases is not
16 suitable for encapsulation of heat sensitive drugs (Bilati et al., 2005; Messenger et al., 2014; Sadeghi et al., 2016;
17 Schubert and Muller-Goymann, 2003)

18 In this current study, the nanoprecipitation data and film-rehydration data suggest that there were apparent
19 differences in the size and polydispersity of the nanoparticles formed via the contrasting methods. Thus, when
20 these parameters were compared for the MPC₃₀-DPA₁₀₀ formulations 15, 24, 26, and the MPC₁₀₀-DPA₁₀₀
21 formulations 12, 16, 29, that had been prepared via the two methods, there were indeed clear particle size and
22 polydispersity shifts evident, as shown in Figures 4a, 4b, and 4c. The more hydrophobic nature of MPC₃₀-
23 DPA₁₀₀ initially lead to the formation of larger nanoparticles, as the hydrophobicity can impede particle
24 aggregation in aqueous solutions (Bilati et al., 2005). However, following the 0.22 μm filtration process, the
25 MPC₁₀₀-DPA₁₀₀ nanoparticles also displayed a larger nanoparticle size, possibly due to a thicker outer corona on
26 the particles resulting from the MPC block length, with MPC₁₀₀ being longer than MPC₃₀ (Giacomelli et al.,
27 2006; Salvage et al., 2005).

28 Additionally, when the two methods of nanoparticle formation without filtration were compared, the film
29 rehydration method resulted in the formation of larger nanoparticles, with lower reproducibility, as seen in

1 Figure 4a, and also increased solution turbidity. This may have been the result of the copolymer concentration
2 being 5 mg/mL for film rehydration, in contrast to the lower 0.4 mg/mL concentration used for
3 nanoprecipitation. These results were consistent with Bilati et al. (2005) and Salvage et al. (2015, 2016) in that
4 they displayed an increase in the particle size, when polymer concentration was increased. To determine the
5 morphology of the nanoparticles formed, and establish the presence of either micelles or polymersomes, the
6 MPC₃₀-DPA₁₀₀ formulations (15, 24, 26) and MPC₁₀₀-DPA₁₀₀ formulations (12, 16, 29) prepared by the two
7 methods employed, nanoprecipitation and film-rehydration, were examined using STEM. As seen in Figures 5
8 and 6, both copolymers were successful in forming nanoparticles in the two formation processes. For the
9 nanoprecipitation samples, (Figure 5), STEM analysis indicated that these consisted predominately of small
10 unilamellar vesicles with a spherical morphology and size consistent with polymersomes. However, for the film
11 rehydration samples, a mixture of particle morphologies were observed, with polymersome sized nanoparticles
12 present, as evidenced by the DLS data (Figure 4c), together with larger polymer aggregates with irregular
13 structures and elongated edges, as shown in Figure 6. This was consistent with previous reports of film
14 rehydration particle population complexity (Robertson et al., 2016), where exploration and purification of film
15 rehydration particle populations was undertaken. The STEM data was largely consistent with the DLS data, in
16 that there was a visible difference in nanoparticle size between all formulations due to the different methods of
17 formation, and the copolymer compositions. Furthermore, the STEM images (Figures 5 and 6) suggested that
18 nanoprecipitation was more effective at forming a homogeneous population of polymersomes, compared with
19 film rehydration. This was possibly due to the copolymer being molecularly dissolved in the organic phase
20 immediately prior to nanoprecipitation, therefore the forces holding the copolymer chains together were weaker
21 (Ye et al., 2015) and thus allowed rapid nanoparticle formation. It appeared from Figure 9 that MPC₁₀₀-DPA₁₀₀
22 formed polymer aggregates with thicker particle coronas than MPC₃₀-DPA₁₀₀, which may have been the result of
23 MPC₁₀₀-DPA₁₀₀ having a higher degree of polymerisation MPC block (Du et al., 2005). The morphology of
24 MPC-DPA polymersomes, as well as aggregation style, can be influenced by several factors, including, the
25 formation process, nature of organic solvents used, the hydrophobic to hydrophilic balance of copolymer blocks,
26 polymer concentration, and stirring speed and duration (Ayen et al., 2011). According to the DLS data (Figures
27 4a, 4b, and 4c) and STEM images (Figures 5 and 6) for characterisation of the MPC-DPA nanoparticles, the
28 formulations 12 and 26, for both MPC₁₀₀-DPA₁₀₀ and MPC₃₀-DPA₁₀₀, possessed a large particle size together
29 with low polydispersity, and spherical morphology, and were therefore selected for subsequent curcumin
30 loading.

1 In summary, the solvent, non-solvent, and ratio used, together with copolymer composition, can affect the
2 resultant nanoparticle size and morphology. Additionally, STEM data (Figures 5 and 6) suggested that, in this
3 instance, nanoprecipitation was more effective at forming homogeneous populations of vesicle morphology
4 nanoparticles, polymersomes, than the film rehydration method which requires subsequent particle fractionation
5 and purification before use (Robertson et al 2016).

6

7 *3.6. TMC synthesis*

8 The physicochemical properties of chitosan can be affected by the degree of deacetylation, and the percentage of
9 deacetylated and acetylated monomer plays an important role in TMC synthesis efficacy. Therefore, before TMC
10 synthesis was undertaken, the degree of deacetylation (DDA%) of chitosan was calculated (Lavertu et al., 2003).
11 The ¹H NMR spectrum of the chitosan polymer (Figure 7a), which consisted of acetylated and deacetylated
12 monomers, was consistent with previously reported spectra (Lavertu et al., 2003). As such, the chemical shift at
13 2.351 ppm was attributed to the three protons of acetyl group (-CO-CH₃) in acetyl units (H-Ac), while the
14 chemical shifts at 4.918 and 5.211 ppm were assigned to protons H1 for both acetylated (H1-A) and deacetylated
15 (H1-D) units. Additionally, the chemical shifts between 3.916 and 4.225 ppm corresponded to protons H2, H3,
16 H4, H5, and H6 (H2-6) of glycosidic ring of chitosan, the chemical shift at 3.518 ppm was assigned to proton H2
17 in deacetylated units, and the shift at 4.733 ppm belonged to the solvent (HOD) proton (Lavertu et al., 2003).
18 According to the proposed shift assignments within the NMR spectra (Figure 7a) and the equation (1), the degree
19 of deacetylation (DDA) of chitosan used for TMC synthesis was 81%.

20 The TMC was synthesized from high molecular weight chitosan using the two step method (Polnok et al., 2004;
21 Sahni et al., 2008; Sieval et al., 1998) the resultant polymer was a white powder, which was soluble in water, the
22 ¹H NMR spectra for the TMC is shown in Figure 7b. The chemical shifts at 2.55, 3.05, and 3.8 ppm were
23 assigned to -NHCH₃, -N(CH₃)₂ and -N⁺(CH₃)₃ groups from the TMC structure, respectively, and was consistent
24 with previous reports (Hansson et al., 2012; Patrulea et al., 2016; Sieval et al., 1998). Additionally, the chemical
25 shifts at 4.8 and 5.25 ppm that were assigned to the H1 proton of chitosan and its derivatives, as described in
26 previous reports (Hansson et al., 2012; Patrulea et al., 2015; Thanou et al., 2000), had disappeared. According to
27 the proposed shift assignments, and using the equation (2), the degree of quaternization (DQ) of TMC after the
28 two step synthesis was high, at 75%, and this result corresponded well with other reports (Patrulea et al., 2015;
29 Polnok et al., 2004; Sieval et al., 1998; Thanou et al., 2000).

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3.7. Assessment the calibration curves and solubility of curcumin

The lambda max (λ max) of curcumin solutions was determined via UV-Vis spectroscopy as 428 nm, and used to measure the maximum solubility of curcumin, and also the encapsulation efficiency of drug loading with nanoparticles from formulations 12 and 26 for MPC₁₀₀-DPA₁₀₀ and MPC₃₀-DPA₁₀₀. Linearity was evident from 0 – 12 mg/L curcumin concentration in formulation 12, (ethanol-isopropanol) solvent mix ($R^2 = 0.9999$) and also in formulation 26, (methanol-ethanol-chloroform-acetonitrile) solvent mix ($R^2 = 0.9997$), and from 0 – 10 mg/L for the PBS-methanol (pH 1.6) mix ($R^2 = 0.9996$) where $y = 0.1462x + 0.0033$. Using the standard curves, the solubility of curcumin in formulation 12 (ethanol-isopropanol), $y = 0.1239x + 0.0114$, was determined as 3.49 ± 0.14 g/L, and in formulation 26 (methanol-ethanol-chloroform-acetonitrile), $y = 0.1315x + 0.0141$, as 24.54 ± 1.26 g/L, respectively, indicating solvent associated solubility differences.

3.8. Curcumin loading and TMC coating

As described earlier, two contrasting methods, nanoprecipitation and film rehydration, were used to prepare curcumin loaded MPC-DPA nanoparticles. The size and morphology of these nanoparticles were then assessed, together with the drug loading efficiency. The DLS data, as shown in Table 9, for nanoparticles prepared by nanoprecipitation, indicated that mean particle diameters were circa 154 nm and 188 nm when empty, and 142 nm and 175 nm after loading using curcumin saturated solvents, for formulations 26 (MPC₃₀-DPA₁₀₀) and 12 (MPC₁₀₀-DPA₁₀₀) respectively. These reductions in mean particle diameter may be the result of the hydrophobic curcumin acting as an increased focus of attraction for the hydrophobic DPA copolymer domains during the hydrophobic effect partitioning of curcumin into the nanoparticles, and hence causing nanoparticle diameter contraction. The addition of TMC (1 mg/mL) to the aqueous nanoprecipitated nanoparticle suspensions resulted in decreased mean particle diameters of circa 136 nm and 162 nm for formulations 26 and 12 respectively, possibly due to filtration (0.22 μ m) associated particle exclusion, extrusion, and average size reduction, or the TMC attenuating the previously fully hydrated, and nanoparticle surface extended, MPC copolymer branches. Polydispersity values ranged from 0.08 to 0.18, with formulation 26 (MPC₃₀-DPA₁₀₀) displaying the lower values. The film rehydration prepared samples were circa 139 nm and 154 nm when empty, and circa 153 nm and 164 nm when loaded with curcumin, for formulations 26 (MPC₃₀-DPA₁₀₀) and 12 (MPC₁₀₀-DPA₁₀₀) respectively, as seen in Table 10. This was the inverse of the effect seen with nanoprecipitation, with the nanoparticles becoming larger in this instance, however given the apparent complexity of film rehydration

1 nanoparticle morphologies and populations, the greater amount of curcumin loading achieved, and the possibility
2 that curcumin may have resulted in an increased level of copolymer nanoparticle aggregation, this could thus
3 explain the increased diameters observed. In a similar mode to the nanoprecipitation samples, addition of TMC
4 to the aqueous film rehydration prepared nanoparticle suspensions, again resulted in mean diameter size
5 reductions, to circa 132 nm and 157 nm. Polydispersity values for formulations 26 (MPC₃₀-DPA₁₀₀) and 12
6 (MPC₁₀₀-DPA₁₀₀) ranged from 0.14 to 0.34, with formulation 12 displaying the lower values.

7 Regarding the surface zeta potential of the unloaded and loaded nanoparticles, Tables 9 and 10, these were
8 initially slightly negative, and close to zero mV when the standard deviation was taken into consideration for
9 unloaded nanoparticles, due to the presence of the amphiphilic groups in MPC polymer structure (Goda et al.,
10 2007), and subsequent curcumin loading shifted the values further to negative. However, following TMC coating
11 of the nanoparticles the zeta potential values shifted and increased to positive values, as the TMC is a
12 polycationic polymer, and as such coated the MPC-DPA nanoparticles due to attractive electrostatic forces
13 between cationic amine groups in TMC polymer and amphiphilic phosphorylcholine groups in MPC (Patrulea et
14 al., 2015; Sahni et al., 2008; Sieval et al., 1998). The shift to positive zeta potential values was more pronounced
15 and consistent with the MPC₃₀-DPA₁₀₀ polymer, as the smaller MPC₃₀ group was unable to counter the positive
16 charge addition of the TMC, whilst the larger MPC₁₀₀ group polymer was able to more effectively reduce the
17 TMC addition effects on particle surface charge.

18 The curcumin encapsulation efficiency and loading capacity of the MPC-DPA nanoparticles were low, and
19 indeed undetectable via UV-Vis for MPC₃₀-DPA₁₀₀, as seen in Tables 9 and 10, possibly due to the differing
20 amounts of curcumin present in the saturated solutions used, 24.54 g/L and 3.49 g/L curcumin for MPC₃₀-
21 DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀, respectively, and resulting from the different levels of curcumin solubility in the
22 solvents used. When the organic solvent phase of formulation 26, containing MPC₃₀-DPA₁₀₀, was added to the
23 aqueous PBS phase, it may have precipitated due to formation of a solid dispersion of large micro particles. This
24 would have been the result of the high binding affinity hydrophobic forces between the concentrated curcumin
25 (24.54 g/L) and the DPA copolymer domain (Onoue et al., 2014, 2013; Suzuki et al., 2016). The curcumin
26 nanoparticles would then be lost by filtration (0.22 µm) immediately prior to UV-Vis measurement.

27 In contrast, the MPC₁₀₀-DPA₁₀₀ copolymer formed curcumin encapsulated nanoparticles, having used a lower
28 concentration of curcumin (3.49 g/L), with UV-Vis detectable and quantifiable levels of curcumin entrapped
29 (Tables 9 and 10). Previous reports have indicated that curcumin inhibits the migration, invasion, proliferation,

1 and viability of A549, CL1-5, and H1299 human lung cancer cell lines (Pillai et al., 2004; Chen et al., 2008; Lin
2 et al., 2009; Patil et al., 2015). For example, Chen et al., 2008 reported a curcumin concentration dependent
3 effective range of 1 – 20 μM against A549 and CL1-5 cells, and more recently Patil et al., 2015 found that
4 curcumin at 2.9 $\mu\text{g}/\text{mL}$ inhibited A549 cell growth. In this current study, the film rehydration prepared MPC₁₀₀-
5 DPA₁₀₀, formulation 12, nanoparticle data shown in Table 10 indicates that curcumin loading of 6.86 $\mu\text{g}/\text{mL}$ (18
6 μM) and 42.57 $\mu\text{g}/\text{mL}$ (115 μM) was achieved for MPC-DPA nanoparticles, and TMC coated MPC-DPA
7 nanoparticles respectively. Therefore both of these have sufficient curcumin loading to be considered potentially
8 effective against human lung cancer cells. The nanoprecipitation prepared MPC₁₀₀-DPA₁₀₀, formulation 12,
9 nanoparticle data shown in Table 9 indicates that curcumin loading at 0.92 $\mu\text{g}/\text{mL}$ (2.5 μM) and 0.85 $\mu\text{g}/\text{mL}$ (2.3
10 μM) were achieved for MPC-DPA nanoparticles, and TMC coated MPC-DPA nanoparticles respectively, and
11 these levels of curcumin loading are located at the lower end of the effective range (1 – 20 μM) suggested by
12 Chen et al., 2008. However, in this current study the nanoprecipitation was undertaken at a MPC-DPA
13 copolymer in PBS concentration of 0.4 mg/mL , whilst it has been reported previously (Salvage et al., 2016) that
14 MPC₁₀₀-DPA₁₀₀ nanoparticles successfully form in PBS at a copolymer concentration of 2 mg/mL . Therefore it
15 should be possible to increase the copolymer concentration, and thus increase curcumin loading, 5-fold, to circa
16 4.6 $\mu\text{g}/\text{mL}$ (12.5 μM) and 4.25 $\mu\text{g}/\text{mL}$ (11.5 μM) for MPC-DPA nanoparticles, and TMC coated MPC-DPA
17 nanoparticles respectively, both of which would be within the range reported by Chen et al., 2008 and Patil et al.,
18 2015, as effective against human lung cancer cells.

19 The morphological study of the curcumin loaded MPC-DPA nanoparticles, using STEM, confirmed that
20 MPC₁₀₀-DPA₁₀₀ copolymer successfully encapsulated curcumin and formed nanoparticles with an irregular
21 spherical morphology using curcumin at low concentration (3.49 g/L), as seen in Figures 8c and 9c, and were
22 consistent with previous reports of curcumin loaded polymer micelles (Patil et al., 2015). In contrast, the STEM
23 imaging (Figures 8 and 9) of MPC₃₀-DPA₁₀₀ copolymer nanoparticles and curcumin, revealed an apparent solid
24 dispersion formation when using curcumin at high concentration (24.54 g/L), for both the nanoprecipitation
25 (Figure 8a) and film rehydration (Figure 9a) preparation methods. Interestingly, the addition of TMC to all
26 formulations produced morphological changes in the nanoparticles, with the appearance of vesicle-like droplet
27 morphologies, as seen in Figures 8b, 8d, 9b and 9d. These were suggestive of the TMC polymer coating the
28 outside of the MPC-DPA nanoparticles, and were morphologically consistent with recent reports of
29 poly(ethylene oxide) based polymersomes (Bartenstein et al., 2016).

1 One of the key requirements for an effective nanoparticle based drug delivery system is the control of size and
2 drug loading, as these parameters are central to achieving good biodistribution and therapeutic efficacy after
3 administration. Four techniques, filtration, centrifugation, size-exclusion chromatography, and density gradient
4 centrifugation, have been reported as successful for purifying and separating heterogeneous nanoparticle
5 solutions into homogenous fractions (Robertson et al., 2016). Therefore, where necessary, the curcumin loaded
6 MPC-DPA nanoparticles developed in this current study, could be further separated and optimised into distinct
7 sizes and shape fractions in order to facilitate their future development for a range of biomedical applications,
8 including inhalation drug delivery. Additionally, the successful coating of the curcumin loaded MPC-DPA
9 nanoparticles with TMC polymer may provide key advantages such as, improved cell permeation and increased
10 curcumin absorption into human cells (Chen et al., 2016; Guan et al., 2012; Panya et al., 2010; Sahni et al., 2008;
11 Zhang et al., 2013). In summary, MPC₁₀₀-DPA₁₀₀ nanoparticles were formed via nanoprecipitation and film
12 rehydration that were of pulmonary delivery size, contained curcumin at anti-tumour concentrations, and could
13 be coated with TMC to further enhance cellular uptake and delivery.

14

15 3.9. Fourier transform infrared spectroscopy

16 The vibrational spectrum of the curcumin was consistent with previous reports (Darandale and Vavia, 2013;
17 Mohan et al., 2012; Zhang et al., 2013) and revealed characteristic main bands, as shown in Figure 10a. A sharp
18 peak at 3508 cm⁻¹ indicated the presence of the stretching vibration of the phenolic-OH group, and a sharp peak
19 at 1626 cm⁻¹ was attributed to vibration of the carbonyl C=O group. The peak at 1601 cm⁻¹ was assigned to the
20 enol C=O stretching, and the peak at 1504 cm⁻¹ was the symmetric stretching vibration of an aromatic ring (C=C
21 ring). Moreover, alkane C-H group bending vibration was at 1427 cm⁻¹, while aromatic C-O stretching vibration
22 was seen at 1272 cm⁻¹. C-O-C stretching vibration and aromatic ring-CH bending vibrations were found at 1024
23 and 961 cm⁻¹, respectively, and between 900 and 650 cm⁻¹ a forest of out of plane vibration for CH and CH₂
24 (Darandale and Vavia, 2013; Patil et al., 2015; Pecora et al., 2016).

25 The structure of MPC₃₀-DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀ copolymers were also characterised with FTIR. The main
26 bands in both are presented in Figure 10b, the broad peak at 3500-3000 cm⁻¹ may be stretching vibration of
27 nitrogen in primary and secondary amine groups (Gao et al., 2013; Zheng et al., 2013), it may also illustrate the
28 presence of residual moisture, bound or unbound water adsorption, in the copolymer as the carbonyl group in the
29 copolymer structure can strongly interact with water molecules and produce hydrogen bonding (Sammon et al.,
30 1998; Szakonyi and Zelkó, 2012). The peaks at 2963 and 2879 cm⁻¹ are the stretching vibration of C-H and -

1 CH₂, respectively (Furuzono et al., 2000; Gao et al., 2013), and the sharp peaks at 1724 and 1479 cm⁻¹ the
2 carbonyl C=O and C-H bonds. The stretching vibration of the C-N group and ester band C-O group were
3 observed at 1145 and 1064 cm⁻¹ (Faccia and Amalvy, 2013). To confirm the chemical groups in MPC and DPA
4 monomers, the peak of the methane group (CH₃)₂-CH- in poly DPA was observed at 2963 cm⁻¹ (Faccia and
5 Amalvy, 2013; Furuzono et al., 2000; Gao et al., 2013; Yan and Ishihara, 2008; Zheng et al., 2013), while peaks
6 at 1231, 964 and 786 cm⁻¹ were attributed to -POCH₂-, -N+(CH₃)₃ and C-O-P groups in poly MPC, respectively.
7 The FTIR spectra (Figure 10b) of MPC₃₀-DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀ copolymers were principally the same,
8 but the intensity of the peak around (3500-3000) cm⁻¹ was stronger for MPC₁₀₀-DPA₁₀₀ than MPC₃₀-DPA₁₀₀, due
9 to hygroscopic nature of MPC which over a broad solution pH range can hydrate with circa 15 water molecules
10 (Chen et al., 2007; Ishihara et al., 1992; Yang et al., 2016; Yaseen et al., 2006).

11 FTIR spectra of chitosan and TMC are presented in Figure 10c. For chitosan, the broad peak at the range (3500-
12 3000) cm⁻¹ corresponded to the combined stretching vibration bands for -OH and -NH₂ groups while the peak at
13 2872 cm⁻¹ was the asymmetrical and symmetrical stretching vibration of C-H in the glycosidic ring. Additionally,
14 the bands at 1650 and 1590 cm⁻¹ were attributed to -NHCOCH₃ group stretching vibration and N-H group
15 bending from chitosan structure, correspondingly. The strong band at 1024 cm⁻¹ was consistent with the C-O-C
16 peak (Nazar et al., 2011; Patrulea et al., 2016). Regarding the TMC polymer spectrum in Figure 10c, it was clear
17 that the insertion of trimethyl groups in chitosan structure lead to increased intensities of the main peaks such as
18 the intensity of methyl group C-H in two positions: the stretching bands (3000-2900 cm⁻¹) as well as the bending
19 bands at (1500-1470 cm⁻¹), and the amide stretching bands (1652 cm⁻¹) (de Britto et al., 2012; Hansson et al.,
20 2012; Nazar et al., 2011; Patrulea et al., 2016). The results of FTIR analysis of TMC polymer (Figure 10c)
21 corresponded with NMR analysis where all sets of bonds in the chemical structure were visible as evidence of
22 successful synthesis of TMC from chitosan.

23 When the FTIR data for all components were observed (Figure 10d) there were no discernible shifts of resonance
24 within the physical mixtures of either curcumin or TMC with the MPC-DPA copolymers. However, there were
25 apparent differences when the FTIR of pure curcumin was compared to that of curcumin mixed with MPC₃₀-
26 DPA₁₀₀ or MPC₁₀₀-DPA₁₀₀ copolymers in the organic solvents combinations used to prepare formulations 12 and
27 26, as shown in Figure 10e. In this instance, there were shifts in the majority of peak positions in all of the
28 mixtures examined. In addition, the sharp peak at 3509 cm⁻¹, assigned to phenolic-OH groups in curcumin,
29 disappeared in the both formulation 12 and 26 FTIR spectra. This suggested that there had been an interaction

1 when mixing curcumin at maximum solubility, 24.30 and 3.44 mg/mL, with MPC₃₀-DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀
2 copolymers at 40 mg/mL in the solvents for formulations 12 and 26 during nanoprecipitation and film
3 rehydration. This interaction was more distinct with MPC₃₀-DPA₁₀₀ in formulation 26, due the higher
4 concentration of curcumin (24.30 mg/mL) with the higher ratio of hydrophobic DPA polymer, compared to
5 formulation 12, which contained only 3.44 mg/mL of curcumin for the same ratio of MPC₁₀₀ and DPA₁₀₀
6 polymers. Furthermore, this interaction may be attributed to intermolecular hydrogen bonding between the
7 phenolic-OH groups in curcumin seen at 3508 cm⁻¹ and amine groups as well as carbonyl groups in MPC-DPA
8 copolymer structure at 3500-3000 and 1724 cm⁻¹, respectively.

9 The large amount of curcumin used in formulation 26 may have contributed to formation of a solid dispersion
10 between the MPC₃₀-DPA₁₀₀ copolymer and curcumin after evaporation of the organic solvents. This would be
11 due to high affinity binding between curcumin and the DPA monomer, via hydrophobic forces, and the high
12 concentration of curcumin used. The solid dispersion, being insoluble in water, was thus unable to form
13 polymersome morphologies when added to aqueous PBS solution (Gupta and Dixit, 2011; Paradkar et al., 2004).
14 Furthermore, when the FTIR spectra for the same mixture of curcumin and MPC-DPA copolymer were
15 compared, with the different concentrations used for nanoprecipitation and film rehydration, the interaction was
16 more detectable, with peak intensity reduced, in the mixture used for nanoprecipitation. This may be due to the
17 higher concentration of copolymer (40 mg/mL) used in nanoprecipitation process (Figures 10e (iv) and 10e (vi))
18 compared to the film rehydration (5 mg/mL) method (Figures 10e (v) and 10e (vii)). The FTIR data were
19 consistent with the STEM image (Figures 8 and 9), encapsulation efficiency, and drug loading capacity data
20 (Tables 9 and 10) for curcumin loaded nanoparticles, which suggested the formation of solid dispersions of
21 curcumin and MPC₃₀-DPA₁₀₀ copolymer, containing large insoluble particles. In summary, MPC₁₀₀-DPA₁₀₀
22 copolymer displayed a more effective ability to form curcumin encapsulated nanoparticles when using curcumin
23 at low concentration, and therefore the drug quantity used for entrapment has the potential to affect the
24 micellisation and nanoparticle formation processes with MPC-DPA copolymers.

25

26 **4. Conclusions**

27 In conclusion, this current work reported for the first time, by application of a 31 solvent matrix, a novel and
28 comprehensive investigation of solvent choice effects on nanoprecipitation assembled MPC-DPA nanoparticles.
29 The resultant novel data was used to select candidate systems deemed as potentially suitable for inhalation drug

1 delivery applications, based on particle size and polydispersity, for further assessment and comparison with
2 equivalent film-rehydration MPC-DPA assembled systems. Analysis of this second novel data set afforded
3 further selective testing in the form of curcumin loading and TMC particle coating, leading to the optimisation of
4 MPC-DPA nanoparticle systems with curcumin loading levels reported as effective against human lung cancer
5 cells. The systems prepared and optimised are thus considered suitable for progression to inhalation delivery
6 characterisation, and *in-vitro* human lung cancer cell drug delivery testing. It is believed that this is the first
7 report of curcumin loaded MPC-DPA nanoparticle systems, illustrating their potential to act as a delivery vehicle
8 for the emerging therapeutic compound curcumin. In doing so, this study contributed towards development of
9 novel curcumin nano-formulations with potential pharmaceutical applications for pulmonary delivered treatment
10 of human lung cancer, and is thus of societal interest, benefit, and significance.

11

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16

17 **References**

- 18 Ahmed, F., Pakunlu, R.I., Brannan, A., Bates, F., Minko, T., Discher, D.E., 2006. Biodegradable polymersomes
19 loaded with both paclitaxel and doxorubicin permeate and shrink tumors, inducing apoptosis in proportion
20 to accumulated drug. *J. Control. Release* 116, 150–158. doi:10.1016/j.jconrel.2006.07.012
- 21 Alexandridis, P., Spontak, R.J., 1999. Solvent-regulated ordering in block copolymers. *Curr. Opin. Colloid*
22 *Interface Sci.* 4, 130–139. doi:10.1016/S1359-0294(99)00022-9
- 23 Amidi, M., Romeijn, S.G., Borchard, G., Junginger, H.E., Hennink, W.E., Jiskoot, W., 2006. Preparation and
24 characterization of protein-loaded N-trimethyl chitosan nanoparticles as nasal delivery system. *J. Control.*
25 *Release* 111, 107–116. doi:10.1016/j.jconrel.2005.11.014
- 26 Ayen, W.Y., Garkhal, K., Kumar, N., 2011. Doxorubicin-loaded (PEG)3-PLA nanopolymersomes: Effect of
27 solvents and process parameters on formulation development and in vitro study. *Mol. Pharm.* 8, 466–478.
28 doi:10.1021/mp1003256
- 29 Baka, E., Comer, J.E.A., Takács-Novák, K., 2008. Study of equilibrium solubility measurement by saturation
30 shake-flask method using hydrochlorothiazide as model compound. *J. Pharm. Biomed. Anal.* 46, 335–341.

1 doi:10.1016/j.jpba.2007.10.030

2 Barnett, a H., 2004. Exubera inhaled insulin: a review. *Int. J. Clin. Pract.* 58, 394–401.

3 Basnet, P., Skalko-Basnet, N., 2011. Curcumin: An anti-inflammatory molecule from a curry spice on the path to
4 cancer treatment. *Molecules* 16, 4567–4598. doi:10.3390/molecules16064567

5 Battaglia, G., LoPresti, C., Massignani, M., Warren, N.J., Madsen, J., Forster, S., Vasilev, C., Hobbs, J.K.,
6 Armes, S.P., Chirasatitsin, S., Engler, A.J., 2011. Wet Nanoscale Imaging and Testing of Polymersomes.
7 *Small* 7, 2010–2015. doi:10.1002/sml.201100511

8 Battaglia, G., Ryan, A.J., 2006. Pathways of polymeric vesicle formation. *J. Phys. Chem. B* 110, 10272–10279.
9 doi:10.1021/jp060728n

10 Bertenstein, J.E., Robertson J., Battaglia, G., Briscoe, W.H., 2016. *Colloids and Surfaces A: Physicochemical
11 and Engineering Aspects.* 506, 739-746.

12 Betbeder, D., Lipka, E., Howsam, M., Carpentier, R., 2015. Evolution of availability of curcumin inside poly-
13 lactic-co-glycolic acid nanoparticles: impact on antioxidant and antinitrosant properties. *Int. J.
14 Nanomedicine* 10, 5355–5366.

15 Bilati, U., Allémann, E., Doelker, E., 2005. Development of a nanoprecipitation method intended for the
16 entrapment of hydrophilic drugs into nanoparticles. *Eur. J. Pharm. Sci.* 24, 67–75.
17 doi:10.1016/j.ejps.2004.09.011

18 Blanz, A., Armes, S.P., Ryan, A.J., 2009. Self-Assembled Block Copolymer Aggregates: From Micelles to
19 Vesicles and their Biological Applications. *Macromol. Rapid Commun.* 30, 267–277.
20 doi:10.1002/marc.200800713

21 Bowen, W.R., Hilal, N., Lovitt, R.W., Wright, C.J., 1998. A new technique for membrane characterisation: direct
22 measurement of the force of adhesion of a single particle using an atomic force microscope. *J. Memb. Sci.*
23 139, 269–274.

24 Canton, I., Massignani, M., Patikarnmonthon, N., Chierico, L., Robertson, J., Renshaw, S. a., Warren, N.J.,
25 Madsen, J.P., Armes, S.P., Lewis, A.L., Battaglia, G., 2013. Fully synthetic polymer vesicles for
26 intracellular delivery of antibodies in live cells. *FASEB J.* 27, 98–108. doi:10.1096/fj.12-212183

27 Chen, H.W, Lee, J.Y., Huang, J.Y., Wang, C.C., Chen, W.J., Su, S.F., Huang, C.W., Ho, C.C., Chen, J.J.W.,
28 Tsai, M.F., Yu, S.L., Yang, P.C., 2008. Curcumin inhibits lung cancer cell invasion and metastasis through
29 tumor suppressor HLJ1. *Cancer Research.* 68, 7428-7438.

30 Chen, M., Briscoe, W.H., Armes, S.P., Cohen, H., Klein, J., 2007. Robust, biomimetic polymer brush layers

1 grown directly from a planar mica surface. *ChemPhysChem* 8, 1303–1306. doi:10.1002/cphc.200700131

2 Chen, W., Yuan, Z.-Q., Liu, Y., Zhang, C., Li, J., Zhu, W., Li, F., Zhou, X., Lin, Y., Zhang, X., 2016.

3 Liposomes coated with N -trimethyl chitosan to improve the absorption of harmine in vivo and in vitro.

4 *Int. J. Nanomedicine* 11, 325–336. doi:10.2147/IJN.S95540

5 Cipolla, D., Gonda, I., Chan, H.-K., 2013. Liposomal formulations for inhalation. *Ther. Deliv.* 4, 1047–72.

6 doi:10.4155/tde.13.71

7 Colley, H.E., Hearnden, V., Avila-Olias, M., Cecchin, D., Canton, I., Madsen, J., Macneil, S., Warren, N., Hu,

8 K., McKeating, J. a., Armes, S.P., Murdoch, C., Thornhill, M.H., Battaglia, G., 2014. Polymersome-

9 mediated delivery of combination anticancer therapy to head and neck cancer cells: 2D and 3D in vitro

10 evaluation. *Mol. Pharm.* 11, 1176–1188. doi:10.1021/mp400610b

11 Darandale, S.S., Vavia, P.R., 2013. Cyclodextrin-based nanosponges of curcumin: Formulation and

12 physicochemical characterization. *J. Incl. Phenom. Macrocycl. Chem.* 75, 315–322. doi:10.1007/s10847-

13 012-0186-9

14 de Britto, D., de Moura, M.R., Aouada, F.A., Mattoso, L.H.C., Assis, O.B.G., 2012. N,N,N-trimethyl chitosan

15 nanoparticles as a vitamin carrier system. *Food Hydrocoll.* 27, 487–493.

16 doi:10.1016/j.foodhyd.2011.09.002

17 Derry, M.J., Fielding, L.A., Armes, S.P., 2016. Polymerization-induced self-assembly of block copolymer

18 nanoparticles via RAFT non-aqueous dispersion polymerization. *Prog. Polym. Sci.* 52, 1–18.

19 doi:10.1016/j.progpolymsci.2015.10.002

20 Discher, D.E., Ahmed, F., 2006. Polymersomes. *Annu. Rev. Biomed. Eng.* 8, 323–341.

21 Discher, D.E., Ortiz, V., Srinivas, G., Klein, M.L., Kim, Y., Christian, D., Cai, S., Photos, P., Ahmed, F., 2007.

22 Emerging applications of polymersomes in delivery: From molecular dynamics to shrinkage of tumors.

23 *Prog. Polym. Sci.* 32, 838–857. doi:10.1016/j.progpolymsci.2007.05.011

24 Du, J., Tang, Y., Lewis, A.L., Armes, S.P., 2005. pH-sensitive vesicles based on a biocompatible zwitterionic

25 diblock copolymer. *J. Am. Chem. Soc.* 127, 17982–17983. doi:10.1021/ja056514l

26 Edmondson, S., Nguyen, N.T., Lewis, A.L., Armes, S.P., 2010. Co-Nonsolvency Effects for Surface-Initiated

27 Poly(2-(methacryloyloxy)ethyl phosphorylcholine) Brushes in Alcohol/Water Mixtures. *Langmuir* 26,

28 7216–7226. doi:10.1021/la904346j

29 El-Sherbiny, I.M., El-Baz, N.M., 2015. A Review on Bionanocomposites Based on Chitosan and Its Derivatives

30 for Biomedical Applications, in: *Eco-Friendly Polymer Nanocomposites*. Springer India, pp. 173–208.

1 doi:10.1007/978-81-322-2473-0

2 Faccia, P.A., Amalvy, J.I., 2013. Synthesis, characterization, and swelling behavior of new pH-sensitive
3 hydrogels derived from copolymers of 2-hydroxyethyl methacrylate and 2-
4 (diisopropylamino)ethylmethacrylate. *J. Appl. Polym. Sci.* 127, 1974–1980. doi:10.1002/app.37576

5 Furuzono, T., Ishihara, K., Nakabayashi, N., Tamada, Y., 2000. Chemical modi “ cation of silk ” broin with 2-
6 methacryloyloxyethyl phosphorylcholine . II . Graft-polymerization onto fabric through 2-
7 methacryloyloxyethyl isocyanate and interaction between fabric and platelets 21, 327–333.

8 Gao, B., Feng, Y., Lu, J., Zhang, L., Zhao, M., Shi, C., Khan, M., Guo, J., 2013. Grafting of phosphorylcholine
9 functional groups on polycarbonate urethane surface for resisting platelet adhesion. *Mater. Sci. Eng. C* 33,
10 2871–2878. doi:10.1016/j.msec.2013.03.007

11 Garbuzenko, O.B., Mainelis, G., Taratula, O., Minko, T., 2014. Inhalation treatment of lung cancer: the influence
12 of composition, size and shape of nanocarriers on their lung accumulation and retention. *Cancer Biol.*
13 *Med.* 11, 44–55. doi:http://dx.doi.org/10.7497/j.issn.2095-3941.2014.01.004

14 Giacomelli, C., Le Men, L., Borsali, R., Lai-Kee-Him, J., Brisson, A., Armes, S.P., Lewis, A.L., 2006.
15 Phosphorylcholine-based pH-responsive diblock copolymer micelles as drug delivery vehicles: Light
16 scattering, electron microscopy and fluorescence experiments. *Biomacromolecules* 7, 817–828.
17 doi:10.1021/bm0508921

18 Goda, T., Konno, T., Takai, M., Ishihara, K., 2007. Photoinduced phospholipid polymer grafting on Parylene
19 film: Advanced lubrication and antibiofouling properties. *Colloids Surfaces B Biointerfaces* 54, 67–73.
20 doi:10.1016/j.colsurfb.2006.09.006

21 Gou, M., Men, K., Shi, H., Xiang, M., Zhang, J., Song, J., Long, J., Wan, Y., Luo, F., Zhao, X., Qian, Z., 2011.
22 Curcumin-loaded biodegradable polymeric micelles for colon cancer therapy in vitro and in vivo.
23 *Nanoscale* 3, 1558–67. doi:10.1039/c0nr00758g

24 Gratton, S.E. a, Ropp, P. a, Pohlhaus, P.D., Luft, J.C., Madden, V.J., Napier, M.E., DeSimone, J.M., 2008. The
25 effect of particle design on cellular internalization pathways. *Proc. Natl. Acad. Sci. U. S. A.* 105, 11613–
26 11618. doi:10.1073/pnas.0801763105

27 Guan, M., Zhou, Y., Zhu, Q.L., Liu, Y., Bei, Y.Y., Zhang, X.N., Zhang, Q., 2012. N-trimethyl chitosan
28 nanoparticle-encapsulated lactosyl-norcantharidin for liver cancer therapy with high targeting efficacy.
29 *Nanomedicine Nanotechnology, Biol. Med.* 8, 1172–1181. doi:10.1016/j.nano.2012.01.009

30 Gupta, N.K., Dixit, V.K., 2011. Bioavailability enhancement of curcumin by complexation with phosphatidyl

1 choline. *J. Pharm. Sci.* 100, 1987–1995. doi:10.1002/jps.22393

2 Hansson, A., Hashom, N., Falson, F., Rousselle, P., Jordan, O., Borchard, G., 2012. In vitro evaluation of an
3 RGD-functionalized chitosan derivative for enhanced cell adhesion. *Carbohydr. Polym.* 90, 1494–1500.
4 doi:10.1016/j.carbpol.2012.07.020

5 Hu, X., Yang, F.-F., Quan, L.-H., Liu, C.-Y., Liu, X.-M., Ehrhardt, C., Liao, Y.-H., 2014. Pulmonary delivered
6 polymeric micelles--pharmacokinetic evaluation and biodistribution studies. *Eur. J. Pharm. Biopharm.* 88,
7 1064–75. doi:10.1016/j.ejpb.2014.10.010

8 Ishihara, K., Oshida, H., Endo, Y., Ueda, T., Watanabe, A., Nakabayashi, N., 1992. Hemocompatibility of
9 human whole blood on polymers with a phospholipid polar group and its mechanism. *J. Biomed. Mater.*
10 *Res.* 26, 1543–1552. doi:10.1002/jbm.820261202

11 Israelachvili, J.N., 2011. Intermolecular and surface forces, in: Academic Press, USA.
12 doi:10.1002/elps.201000212

13 Kakran, M., Sahoo, N.G., Tan, I.L., Li, L., 2012. Preparation of nanoparticles of poorly water-soluble
14 antioxidant curcumin by antisolvent precipitation methods. *J. Nanoparticle Res.* 14. doi:10.1007/s11051-
15 012-0757-0

16 Koshkina, N. V, Waldrep, J.C., Roberts, L.E., Golunski, E., Melton, S., 2001. Paclitaxel liposome aerosol
17 treatment induces inhibition of pulmonary metastases in murine renal carcinoma model 1. *Clin Cancer*
18 *Res.* 7, 3258–3262.

19 Kuzmov, A., Minko, T., 2015. Nanotechnology approaches for inhalation treatment of lung diseases. *J. Control.*
20 *Release* 219, 500–518. doi:10.1016/j.jconrel.2015.07.024

21 Lavertu, M., Xia, Z., Serreqi, A.N., Berrada, M., Rodrigues, A., Wang, D., Buschmann, M.D., Gupta, A., 2003.
22 A validated ¹H NMR method for the determination of the degree of deacetylation of chitosan. *J. Pharm.*
23 *Biomed. Anal.* 32, 1149–1158. doi:10.1016/S0731-7085(03)00155-9

24 Licciardi, M., Craparo, E.F., Giammona, G., Armes, S.P., Tang, Y., Lewis, A.L., 2008. in Vitro Biological
25 Evaluation of Folate-Functionalized Block Copolymer Micelles for Selective Anti-Cancer Drug Delivery.
26 *Macromol. Biosci.* 8, 615–26. doi:10.1002/mabi.200800009

27 Licciardi, M., Giammona, G., Du, J.Z., Armes, S.P., Tang, Y.Q., Lewis, A.L., 2006. New folate-functionalized
28 biocompatible block copolymer micelles as potential anti-cancer drug delivery systems. *Polymer (Guildf).*
29 47, 2946–2955.

30 Licciardi, M., Tang, Y., Billingham, N.C., Armes, S.P., 2005. Synthesis of novel folate-functionalized

1 biocompatible block copolymers by atom transfer radical polymerization for gene delivery and
2 encapsulation of hydrophobic drugs. *Biomacromolecules* 6, 1085–1096.

3 Lin S.S., Lai, K.C., Hsu, S.C., Yang, J.S., Kuo, C.L., Lin, J.P., Ma, Y.S., Wu, C.C., Chung, J.G., 2009.
4 Curcumin inhibits the migration and invasion of human A549 lung cancer cells through the inhibition
5 matrix metalloproteinase-2 and -9 and vascular endothelial growth factor (VEGF). *Cancer Letters*. 285,
6 127-133.

7 Liu, K.N., Lai, C.M., Lee, Y.T., Wang, S.N., Chen, R.P.Y., Jan, J.S., Liu, H.S., Wang, S.S.S., 2012. Curcumin's
8 pre-incubation temperature affects its inhibitory potency toward amyloid fibrillation and fibril-induced
9 cytotoxicity of lysozyme. *Biochim. Biophys. Acta - Gen. Subj.* 1820, 1774–1786.
10 doi:10.1016/j.bbagen.2012.07.012

11 Lomas, H., Canton, I., MacNeil, S., Du, J., Armes, S.P., Ryan, A.J., Lewis, A.L., Battaglia, G., 2007.
12 Biomimetic pH sensitive polymersomes for efficient DNA encapsulation and delivery. *Adv. Mater.* 19,
13 4238–4243. doi:10.1002/adma.200700941

14 Lomas, H., Du, J., Canton, I., Madsen, J., Warren, N., Armes, S.P., Lewis, A.L., Battaglia, G., 2010. Efficient
15 encapsulation of plasmid DNA in pH-sensitive PMPC-PDPA polymersomes: study of the effect of PDPA
16 block length on copolymer-DNA binding affinity. *Macromol. Biosci.* 10, 513–530.
17 doi:10.1002/mabi.201000083

18 Lomas, H., Massignani, M., Abdullah, K. a, Canton, I., Lo Presti, C., MacNeil, S., Du, J., Blanazs, A., Madsen,
19 J., Armes, S.P., Lewis, A.L., Battaglia, G., 2008. Non-cytotoxic polymer vesicles for rapid and efficient
20 intracellular delivery. *Faraday Discuss.* 139, 143–159. doi:10.1039/b717431d

21 Ma, Y., Tang, Y., Billingham, N.C., Armes, S.P., Lewis, A.L., Lloyd, A.W., Salvage, J.P., 2003. Well-defined
22 biocompatible block copolymers via atom transfer radical polymerization of 2-methacryloyloxyethyl
23 phosphorylcholine in protic media. *Macromolecules* 36, 3475–3484. doi:10.1021/ma021762c

24 Mack, G.S., 2007. Pfizer dumps Exubera. *Nat. Biotechnol.* 25, 1331–1332. doi:10.1038/nbt1207-1331

25 Messenger, L., Gaitzsch, J., Chierico, L., Battaglia, G., 2014. Novel aspects of encapsulation and delivery using
26 polymersomes. *Curr Opin Pharmacol* 18, 104–111. doi:10.1016/j.coph.2014.09.017

27 Meyer, E.E., Rosenberg, K.J., Israelachvili, J., 2006. Recent progress in understanding hydrophobic interactions.
28 *Proc. Natl. Acad. Sci. U.S.A.* 103, 15739.

29 Minko, T., Rodriguez-Rodriguez, L., Pozharov, V., 2013. Nanotechnology approaches for personalized
30 treatment of multidrug resistant cancers. *Adv. Drug Deliv. Rev.* 65, 1880–1895.

1 doi:10.1016/j.addr.2013.09.017

2 Mock, C.D., Jordan, B.C., Selvam, C., 2015. Recent advances of curcumin and its analogues in breast cancer
3 prevention and treatment. *RSC Adv.* 5, 75575–75588. doi:10.1039/C5RA14925H

4 Mohan, P.R.K., Sreelakshmi, G., Muraleedharan, C. V., Joseph, R., 2012. Water soluble complexes of curcumin
5 with cyclodextrins: Characterization by FT-Raman spectroscopy. *Vib. Spectrosc.* 62, 77–84.
6 doi:10.1016/j.vibspec.2012.05.002

7 Motornov, M., Roiter, Y., Tokarev, I., Minko, S., 2010. Stimuli-responsive nanoparticles, nanogels and capsules
8 for integrated multifunctional intelligent systems. *Prog. Polym. Sci.* 35, 174–211.
9 doi:10.1016/j.progpolymsci.2009.10.004

10 Mu, Q.S., Zhao, X.B., Lu, J.R., Armes, S.P., Lewis, a L., Thomas, R.K., 2008. pH-responsive nanoaggregation
11 of diblock phosphorylcholine copolymers. *J. Phys. Chem. B* 112, 9652–9. doi:10.1021/jp710365u

12 Nahar, P.P., Slitt, A.L., Seeram, N.P., 2015. Anti-inflammatory effects of novel standardized solid lipid
13 curcumin formulations. *J. Med. Food* 18, 786–792. doi:10.1089/jmf.2014.0053

14 Nazar, H., Fatouros, D.G., Van Der Merwe, S.M., Bouropoulos, N., Avgouropoulos, G., Tsibouklis, J., Roldo,
15 M., 2011. Thermosensitive hydrogels for nasal drug delivery: The formulation and characterisation of
16 systems based on N-trimethyl chitosan chloride. *Eur. J. Pharm. Biopharm.* 77, 225–232.
17 doi:10.1016/j.ejpb.2010.11.022

18 Onoue, S., Kojo, Y., Suzuki, H., Yuminoki, K., Kou, K., Kawabata, Y., Yamauchi, Y., Hashimoto, N., Yamada,
19 S., 2013. Development of novel solid dispersion of tranilast using amphiphilic block copolymer for
20 improved oral bioavailability. *Int. J. Pharm.* 452, 220–226. doi:10.1016/j.ijpharm.2013.05.022

21 Onoue, S., Suzuki, H., Kojo, Y., Matsunaga, S., Sato, H., Mizumoto, T., Yuminoki, K., Hashimoto, N., Yamada,
22 S., 2014. Self-micellizing solid dispersion of cyclosporine A with improved dissolution and oral
23 bioavailability. *Eur. J. Pharm. Sci.* 62, 16–22. doi:10.1016/j.ejps.2014.05.006

24 Pillai, G.R., Srivastava, A.S., Hassanein, T.I., Chauhan, D.P., Carrier, E., 2004. Induction of apoptosis in human
25 lung cancer cells by curcumin. *Cancer Letters.* 208, 163-170.

26 Panya, A., Laguerre, M., Lecomte, J., Villeneuve, P., Weiss, J., McClements, D.J., Decker, E.A., 2010. Effects
27 of chitosan and rosmarinate esters on the physical and oxidative stability of liposomes. *J. Agric. Food*
28 *Chem.* 58, 5679–5684. doi:10.1021/jf100133b

29 Paradkar, A., Ambike, A.A., Jadhav, B.K., Mahadik, K.R., 2004. Characterization of curcumin-PVP solid
30 dispersion obtained by spray drying. *Int. J. Pharm.* 271, 281–286. doi:10.1016/j.ijpharm.2003.11.014

1 Patikarnmonthon, N., 2013. PMPC-PDPA polymersomes-mediated siRNA delivery. University of Sheffield.
2 doi:10.1007/978-1-4939-3112-5

3 Patil, S., Choudhary, B., Rathore, A., Roy, K., Mahadik, K., 2015. Enhanced oral bioavailability and anticancer
4 activity of novel curcumin loaded mixed micelles in human lung cancer cells. *Phytomedicine* 22, 1103–
5 1111. doi:10.1016/j.phymed.2015.08.006

6 Patrulea, V., Applegate, L.A., Ostafe, V., Jordan, O., Borchard, G., 2015. Optimized synthesis of O-
7 carboxymethyl-N,N,N-trimethyl chitosan. *Carbohydr. Polym.* 122, 46–52.
8 doi:10.1016/j.carbpol.2014.12.014

9 Patrulea, V., Hirt-Burri, N., Jeannerat, A., Applegate, L.A., Ostafe, V., Jordan, O., Borchard, G., 2016. Peptide-
10 decorated chitosan derivatives enhance fibroblast adhesion and proliferation in wound healing. *Carbohydr.*
11 *Polym.* 142, 114–123. doi:10.1016/j.carbpol.2016.01.045

12 Pearson, R.T., Warren, N.J., Lewis, A.L., Armes, S.P., 2013. Effect of pH and temperature on PMPC – PDPA
13 copolymer self- assembly. *Macromolecules* 46, 1400–1407.

14 Pecora, T.M.G., Cianciolo, S., Catalfo, A., De Guidi, G., Ruozzi, B., Cristiano, M.C., Paolino, D., Graziano,
15 A.C.E., Fresta, M., Pignatello, R., 2016. Preparation, characterization and photostability assessment of
16 curcumin microencapsulated within methacrylic copolymers. *J. Drug Deliv. Sci. Technol.* 33, 88–97.
17 doi:10.1016/j.jddst.2016.03.013

18 Pegoraro, C., Cecchin, D., Gracia, L.S., Warren, N., Madsen, J., Armes, S.P., Lewis, A., Macneil, S., Battaglia,
19 G., 2013. Enhanced drug delivery to melanoma cells using PMPC-PDPA polymersomes. *Cancer Lett.* 334,
20 328–37. doi:10.1016/j.canlet.2013.02.007

21 Pegoraro, C., Cecchin, D., Madsen, J., Warren, N., Armes, S.P., MacNeil, S., Lewis, A., Battaglia, G., 2014.
22 Translocation of flexible polymersomes across pores at the nanoscale. *Biomater. Sci.* 2, 680.
23 doi:10.1039/c3bm60294j

24 Polnok, A., Borchard, G., Verhoef, J.C., Sarisuta, N., Junginger, H.E., 2004. Influence of methylation process on
25 the degree of quaternization of N-trimethyl chitosan chloride. *Eur. J. Pharm. Biopharm.* 57, 77–83.
26 doi:10.1016/S0939-6411(03)00151-6

27 Porto, L.C., Aissou, K., Giacomelli, C., Baron, T., Rochas, C., Pignot-Paintrand, I., Armes, S.P., Lewis, A.L.,
28 Soldi, V., Borsali, R., 2011. Nanostructured films made from zwitterionic phosphorylcholine diblock
29 copolymer systems. *Macromolecules* 44, 2240–2244. doi:10.1021/ma1029554

30 Prasad, S., Gupta, S.C., Tyagi, A.K., Aggarwal, B.B., 2014. Curcumin, a component of golden spice: From

1 bedside to bench and back. *Biotechnol. Adv.* 32, 1053–1064. doi:10.1016/j.biotechadv.2014.04.004

2 Robertson, J.D., Rizzello, L., Avila-Olias, M., Gaitzsch, J., Contini, C., Magoń, M.S., Renshaw, S.A., Battaglia,
3 G., 2016. Purification of Nanoparticles by Size and Shape. *Sci. Rep.* 6, 27494. doi:10.1038/srep27494

4 Robertson, J.D., Yealland, G., Avila-Olias, M., Chierico, L., Bandmann, O., Renshaw, S. a, Battaglia, G., 2014.
5 pH-sensitive tubular polymersomes: formation and applications in cellular delivery. *ACS Nano* 8, 4650–
6 61. doi:10.1021/nn5004088

7 Sadeghi, F., Ashofteh, M., Homayouni, A., Abbaspour, M., Nokhodchi, A., Garekani, H.A., 2016. Antisolvent
8 precipitation technique: A very promising approach to crystallize curcumin in presence of polyvinyl
9 pyrrolidone for solubility and dissolution enhancement. *Colloids Surfaces B Biointerfaces* 147, 258–264.
10 doi:10.1016/j.colsurfb.2016.08.004

11 Saengkrit, N., Saesoo, S., Srinuanchai, W., Phunpee, S., Ruktanonchai, U.R., 2014. Influence of curcumin-
12 loaded cationic liposome on anticancer activity for cervical cancer therapy. *Colloids Surfaces B*
13 *Biointerfaces* 114, 349–356. doi:10.1016/j.colsurfb.2013.10.005

14 Sahni, J.K., Chopra, S., Ahmad, F.J., Khar, R.K., 2008. Potential prospects of chitosan derivative trimethyl
15 chitosan chloride (TMC) as a polymeric absorption enhancer: synthesis, characterization and applications.
16 *J. Pharm. Pharmacol.* 60, 1111–9. doi:10.1211/jpp.60.9.0001

17 Salvage, J.P., Rose, S.F., Phillips, G.J., Hanlon, G.W., Lloyd, A.W., Ma, I.Y., Armes, S.P., Billingham, N.C.,
18 Lewis, A.L., 2005. Novel biocompatible phosphorylcholine-based self-assembled nanoparticles for drug
19 delivery. *J. Control. Release* 104, 259–70. doi:10.1016/j.jconrel.2005.02.003

20 Salvage, J.P., Smith, T., Lu, T., Sanghera, A., Standen, G., Tang, Y., Lewis, A.L., 2016. Synthesis,
21 characterisation, and in vitro cellular uptake kinetics of nanoprecipitated poly(2-methacryloyloxyethyl
22 phosphorylcholine)-b-poly(2-(diisopropylamino)ethyl methacrylate) (MPC-DPA) polymeric nanoparticle
23 micelles for nanomedicine applications. *Appl. Nanosci.* 1–22. doi:10.1007/s13204-016-0520-4

24 Salvage, J.P., Thom, C., Lewis, A.L., Phillips, G.J., Lloyd, A.W., 2015. Nanoprecipitation of polymeric
25 nanoparticle micelles based on 2-methacryloyloxyethyl phosphorylcholine (MPC) with 2-
26 (diisopropylamino)ethyl methacrylate (DPA), for intracellular delivery applications. *J. Mater. Sci. Mater.*
27 *Med.* 26, 150. doi:10.1007/s10856-015-5480-9

28 Sammon, C., Mura, C., Yarwood, J., Everall, N., Swart, R., Hodge, D., Hallam, S., Uni, V., Campus, C.,
29 Sheffield, S., 1998. FTIR - ATR studies of the structure and dynamics of water molecules in polymeric
30 matrixes . A comparison of PET and PVC. *J. Phys. Chem. B* 102, 3402–3411.

1 Schubert, M.A., Muller-Goymann, C.C., 2003. Solvent injection as a new approach for manufacturing lipid
2 nanoparticles - Evaluation of the method and process parameters. *Eur. J. Pharm. Biopharm.* 55, 125–131.

3 Shanmugam, M.K., Rane, G., Kanchi, M.M., Arfuso, F., Chinnathambi, A., Zayed, M.E., Alharbi, S.A., Tan,
4 B.K.H., Kumar, A.P., Sethi, G., 2015. The multifaceted role of curcumin in cancer prevention and
5 treatment, *Molecules*. doi:10.3390/molecules20022728

6 Sieval, A., Thanoua, M., Kotze, A., Verhoef, J., Brussee, J., Junginger, H., 1998. Preparation and NMR
7 characterization of highly substituted N-trimethyl chitosan chloride. *Carbohydr. Polym.* 36, 157–165.

8 Smallwood, I.M., 1996. *Handbook of Organic Solvent Properties*, First. ed, Arnold, London. doi:10.1016/B978-
9 0-08-052378-1.50026-8

10 Sorrell, I., Shipley, R.J., Hearnden, V., Colley, H.E., Thornhill, M.H., Murdoch, C., Webb, S.D., 2014.
11 Combined mathematical modelling and experimentation to predict polymersome uptake by oral cancer
12 cells. *Nanomedicine* 10, 339–48. doi:10.1016/j.nano.2013.08.013

13 Steed, E., Balda, M.S., Matter, K., 2010. Dynamics and functions of tight junctions. *Trends Cell Biol.* 20, 142–
14 149. doi:10.1016/j.tcb.2009.12.002

15 Suzuki, H., Kojo, Y., Yakushiji, K., Yuminoki, K., Hashimoto, N., Onoue, S., 2016. Strategic application of self-
16 micellizing solid dispersion technology to respirable powder formulation of tranilast for improved
17 therapeutic potential. *Int. J. Pharm.* 499, 255–262. doi:10.1016/j.ijpharm.2015.12.065

18 Szakonyi, G., Zelkó, R., 2012. The effect of water on the solid state characteristics of pharmaceutical excipients :
19 Molecular mechanisms , measurement techniques , and quality aspects of final dosage form The effect of
20 water on the solid state characteristics of pharmaceutical excipie. doi:10.4103/2230-973X.96922

21 Thanou, M., Verhoef, J.C., Marbach, P., Junginger, H.E., 2000. Intestinal absorption of octreotide: N-trimethyl
22 chitosan chloride (TMC) ameliorates the permeability and absorption properties of the somatostatin
23 analogue in vitro and in vivo. *J. Pharm. Sci.* 89, 951–957. doi:10.1002/1520-6017(200007)89:7<951::AID-
24 JPS13>3.0.CO;2-1

25 Thanou, M.M., Kotzé, A.F., Scharringhausen, T., Lueßen, H.L., De Boer, A.G., Verhoef, J.C., Junginger, H.E.,
26 2000. Effect of degree of quaternization of N-trimethyl chitosan chloride for enhanced transport of
27 hydrophilic compounds across intestinal Caco-2 cell monolayers. *J. Control. Release* 64, 15–25.
28 doi:10.1016/S0168-3659(99)00131-5

29 Tomren, M.A., Måsson, M., Loftsson, T., Tønnesen, H.H., 2007. Studies on curcumin and curcuminoids. *Int. J.*
30 *Pharm.* 338, 27–34. doi:10.1016/j.ijpharm.2007.01.013

1 Ungaro, F., d'Angelo, I., Miro, A., La Rotonda, M.I., Quaglia, F., 2012. Engineered PLGA nano- and micro-
2 carriers for pulmonary delivery: challenges and promises. *J. Pharm. Pharmacol.* 64, 1217–1235.
3 doi:10.1111/j.2042-7158.2012.01486.x

4 Wang, J., Wang, H., Zhu, R., Liu, Q., Fei, J., Wang, S., 2015. Anti-inflammatory activity of curcumin-loaded
5 solid lipid nanoparticles in IL-1?? transgenic mice subjected to the lipopolysaccharide-induced sepsis.
6 *Biomaterials* 53, 475–483. doi:10.1016/j.biomaterials.2015.02.116

7 Wang, Y.J., Pan, M.H., Cheng, A.L., Lin, L.I., Ho, Y.S., Hsieh, C.Y., Lin, J.K., 1997. Stability of curcumin in
8 buffer solutions and characterization of its degradation products. *J. Pharm. Biomed. Anal.* 15, 1867–1876.
9 doi:10.1016/S0731-7085(96)02024-9

10 Xie, X., Tao, Q., Zou, Y., Zhang, F., Guo, M., Wang, Y., Wang, H., Zhou, Q., Yu, S., 2011. PLGA nanoparticles
11 improve the oral bioavailability of curcumin in rats: Characterizations and mechanisms. *J. Agric. Food*
12 *Chem.* 59, 9280–9289. doi:10.1021/jf202135j

13 Yallapu, M.M., Jaggi, M., Chauhan, S.C., 2010. Scope of nanotechnology in ovarian cancer therapeutics. *J.*
14 *Ovarian Res.* 3, 19. doi:10.1186/1757-2215-3-19

15 Yan, L., Ishihara, K., 2008. Graft copolymerization of 2-methacryloyloxyethyl phosphorylcholine to cellulose in
16 homogeneous media using atom transfer radical polymerization for providing new hemocompatible
17 coating materials. *J. Polym. Sci. Part A Polym. Chem.* doi:10.1002/pola

18 Yang, T., Choi, S.K., Lee, Y.R., Cho, Y., Kim, J.W., 2016. Novel associative nanoparticles grafted with
19 hydrophobically modified zwitterionic polymer brushes for the rheological control of aqueous polymer gel
20 fluids. *Polym. Chem.* doi:10.1039/C6PY00359A

21 Yaseen, M., Lu, J.R., Webster, J.R.P., Penfold, J., 2006. The structure of zwitterionic phosphocholine surfactant
22 monolayers. *Langmuir* 22, 5825–5832. doi:10.1021/la053316z

23 Ye, X., Niroomand, H., Hu, S., Khomami, B., 2015. Block copolymer micelle formation in a solvent good for all
24 the blocks. *Colloid Polym. Sci.* 293, 2799–2805. doi:10.1007/s00396-015-3658-9

25 Yealland, G., 2015. Polymersome Mediated Delivery of Mitochondrial Therapeutics to parkin Mutant
26 Fibroblasts by. PhD Thesis, Univ. Sheff.

27 Yoshikawa, H.Y., Rossetti, F.F., Kaufmann, S., Kaindl, T., Madsen, J., Engel, U., Lewis, A.L., Armes, S.P.,
28 Tanaka, M., 2011. Quantitative evaluation of mechanosensing of cells on dynamically tunable hydrogels.
29 *J. Am. Chem. Soc.* 133, 1367–1374. doi:10.1021/ja1060615

30 Zhang, J., Tang, Q., Xu, X., Li, N., 2013. Development and evaluation of a novel phytosome-loaded chitosan

1 microsphere system for curcumin delivery. Int. J. Pharm. 448, 168–174.

2 doi:10.1016/j.ijpharm.2013.03.021

3 Zheng, Z., Ren, L., Zhai, Z., Wang, Y., Hang, F., 2013. Surface modification on polyethylene terephthalate fi

4 lms with 2-methacryloyloxyethyl phosphorylcholine. Mater. Sci. Eng. C 33, 3041–3046.

5 doi:10.1016/j.msec.2013.03.036

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1 **Tables**

2

3 **Table 1**

4 Classification of organic solvent properties (adapted from (Smallwood, 1996)).

Solvent Name	Polarity Value	Solubility in Water (25 °C, % w/w)	Absolute Viscosity (25 °C, Cp)	Boiling Point (°C)
Methanol	76.2	Total	0.6	64
Ethanol	65.4	Total	1.08	78
Isopropanol	54.6	Total	2.0	82
Acetonitrile	46.0	Total	0.38	81.6
Chloroform	25.9	0.82	0.57	61

5

6 **Table 2**

7 The 31 solvent combination matrix used to assess MPC-DPA copolymer solubility at 40 mg/mL

Solvent combination ratios						
#	Methanol	Ethanol	Chloroform	Acetonitrile	Isopropanol	Ratio
1	1	0	0	0	0	1:0:0:0:0
2	0	1	0	0	0	0:1:0:0:0
3	0	0	1	0	0	0:0:1:0:0
4	0	0	0	1	0	0:0:0:1:0
5	0	0	0	0	1	0:0:0:0:1
6	1	1	0	0	0	1:1:0:0:0
7	1	0	1	0	0	1:0:1:0:0
8	1	0	0	1	0	1:0:0:1:0
9	1	0	0	0	1	1:0:0:0:1
10	0	1	1	0	0	0:1:1:0:0
11	0	1	0	1	0	0:1:0:1:0
12	0	1	0	0	1	0:1:0:0:1
13	0	0	1	1	0	0:0:1:1:0
14	0	0	1	0	1	0:0:1:0:1
15	0	0	0	1	1	0:0:0:1:1
16	1	1	1	0	0	1:1:1:0:0
17	1	1	0	1	0	1:1:0:1:0
18	1	1	0	0	1	1:1:0:0:1
19	0	1	1	1	0	0:1:1:1:0
20	1	0	1	1	0	1:0:1:1:0
21	1	0	1	0	1	1:0:1:0:1
22	1	0	0	1	1	1:0:0:1:1
23	0	1	1	0	1	0:1:1:0:1
24	0	1	0	1	1	0:1:0:1:1
25	0	0	1	1	1	0:0:1:1:1
26	1	1	1	1	0	1:1:1:1:0
27	0	1	1	1	1	0:1:1:1:1
28	1	0	1	1	1	1:0:1:1:1
29	1	1	0	1	1	1:1:0:1:1
30	1	1	1	0	1	1:1:1:0:1
31	1	1	1	1	1	1:1:1:1:1

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Table 3 Molecular weight (Mn and Mw) and polydispersity (Mw/Mn) of MPC-DPA diblock copolymers determined *via* ¹H NMR and organic GPC

MPC₃₀-DPA₁₀₀	Mn	Mw	Mw/Mn
Target (g/mol)	30190	-	-
¹ H NMR	30190	-	-
GPC	33050	33890	1.03

MPC₁₀₀-DPA₁₀₀	Mn	Mw	Mw/Mn
Target (g/mol)	50860	-	-
¹ H NMR	50860	-	-
GPC	50060	50500	1.01

Table 4
Visual classification of MPC-DPA copolymer solubility in solvents. See Table 2 for solvent formulation details.

Solubility visual classification of MPC-DPA copolymers			
#	Solvent	MPC ₁₀₀ – DPA ₁₀₀	MPC ₃₀ – DPA ₁₀₀
	Combination		
1	1:0:0:0	Completely soluble	Slightly soluble
2	0:1:0:0	Completely soluble	Slightly soluble
3	0:0:1:0	Practically insoluble	Practically insoluble
4	0:0:0:1	Practically insoluble	Practically insoluble
5	0:0:0:1	Completely soluble	Completely soluble
6	1:1:0:0	Completely soluble	Slightly soluble
7	1:0:1:0	Completely soluble	Completely soluble
8	1:0:0:1	Completely soluble	Slightly soluble
9	1:0:0:1	Completely soluble	Slightly soluble
10	0:1:1:0	Completely soluble	Completely soluble
11	0:1:0:1	Completely soluble	Slightly soluble
12	0:1:0:1	Completely soluble	Completely soluble
13	0:0:1:1	Practically insoluble	Practically insoluble
14	0:0:1:0	Completely soluble	Completely soluble
15	0:0:0:1	Completely soluble	Slightly soluble
16	1:1:1:0	Completely soluble	Completely soluble
17	1:1:0:1	Completely soluble	Completely soluble
18	1:1:0:1	Completely soluble	Slightly soluble
19	0:1:1:1	Completely soluble	Completely soluble
20	1:0:1:1	Completely soluble	Slightly soluble
21	1:0:1:1	Completely soluble	Completely soluble
22	1:0:0:1	Completely soluble	Slightly soluble
23	0:1:1:0	Completely soluble	Completely soluble
24	0:1:0:1	Completely soluble	Completely soluble
25	0:0:1:1	Completely soluble	Completely soluble
26	1:1:1:0	Completely soluble	Completely soluble
27	0:1:1:1	Completely soluble	Completely soluble
28	1:0:1:1	Completely soluble	Completely soluble
29	1:1:0:1	Completely soluble	Slightly soluble
30	1:1:1:0	Completely soluble	Completely soluble
31	1:1:1:1	Completely soluble	Completely soluble

1 **Table 5**
 2 Solvent effect on size and polydispersity of MPC₃₀-DPA₁₀₀ nanoparticle systems prepared via nanoprecipitation
 3 (Mean ± SD, n = 3). See Table 2 for solvent formulation details.
 4

Solvent Combination	Filter (µm)	Diameter (Z _{Ave}) (nm) (±SD)	Polydispersity (Pd) (±SD)	Dh ¹ (nm) (±SD)	Dh ² (nm) (±SD)	Dh ³ (nm) (±SD)
1 1:0:0:0:0	-	57.1 (0.2)	0.05 (0.02)	61.1 (0.8)	-	-
	0.45	56.7 (0.3)	0.04 (0.01)	60.4 (0.8)	-	-
	0.22	56.5 (0.1)	0.05 (0.00)	60.5 (0.4)	-	-
2 0:1:0:0:0	-	483.6 (8.4)	0.71 (0.06)	331.6 (115.3)	2180.3 (2588.4)	3459 (2480.9)
	0.45	135.2 (1.9)	0.15 (0.01)	160.1 (3.2)	-	-
	0.22	133.6 (2.9)	0.10 (0.00)	150.1 (5.0)	-	-
3 0:0:1:0:0	-	-	-	-	-	-
4 0:0:0:1:0	-	-	-	-	-	-
5 0:0:0:0:1	-	1099.0 (351.5)	0.93 (0.09)	233.2 (69.9)	1994.9 (3088.3)	-
	0.45	167.6 (0.6)	0.14 (0.02)	193.0 (9.2)	1747.7 (3027.0)	-
	0.22	150.2 (7.3)	0.12 (0.03)	169.3 (7.9)	1675 (2901.2)	-
6 1:1:0:0:0	-	177.6 (28.0)	0.51 (0.01)	46.9 (0.5)	346.1 (28.4)	1682.7 (2914.5)
	0.45	85.3 (8.7)	0.43 (0.03)	141.3 (154.8)	195.0 (110.9)	1657 (2870.0)
	0.22	50.6 (4.9)	0.13 (0.07)	55.2 (4.7)	418.7 (725.2)	1286.3 (2228)
7 1:0:1:0:0	-	415.4 (176.8)	0.68 (0.10)	218.7 (9.4)	1939.1 (2640.2)	2047.4 (3052.1)
	0.45	110.1 (95.6)	0.20 (0.14)	100.2 (87.3)	1791.3 (3102.7)	-
	0.22	102.3 (20.8)	0.41 (0.14)	9.9 (6.5)	128.2 (9.1)	3486.3 (3009.1)
8 1:0:0:1:0	-	55.7 (0.1)	0.05 (0.01)	59.5 (0.5)	-	-
	0.45	55.3 (0.3)	0.04 (0.00)	58.8 (0.7)	-	-
	0.22	55.6 (0.2)	0.03 (0.02)	58.9 (0.5)	-	-
9 1:0:0:0:1	-	379.6 (8.4)	0.61 (0.03)	292.1 (71.1)	2177.8(2282.2)	3224.3 (2805.0)
	0.45	130.9 (2.8)	0.15 (0.01)	157.5 (3.4)	-	-
	0.22	133.5 (7.6)	0.12 (0.01)	155.3 (10.7)	-	-
10 0:1:1:0:0	-	602.9 (299.1)	0.68 (0.17)	256.0 (61.3)	1919.4 (2217.0)	3540.9 (2979.1)
	0.45	172.96 (36.5)	0.51 (0.11)	44.6 (37.2)	166.8 (32.6)	175.1 (239.9)
	0.22	-	-	-	-	-
11 0:1:0:1:0	-	725.3 (49.6)	0.90 (0.08)	299.3 (31.6)	498.2 (802.5)	1723.5 (2882.3)
	0.45	155.1 (1.0)	0.15 (0.01)	184.5 (5.2)	-	-
	0.22	125.9 (3.5)	0.13 (0.01)	144.4 (2.9)	-	-
12 0:1:0:0:1	-	768.8 (248.7)	0.89 (0.13)	327.9 (151.1)	1737.7 (3009.7)	-
	0.45	160.5 (3.1)	0.12 (0.01)	183.1 (5.8)	-	-
	0.22	145.6 (3.2)	0.11 (0.00)	166.0 (4.2)	-	-
13 0:0:1:1:0	-	-	-	-	-	-
14 0:0:1:0:1	-	-	-	-	-	-
15 0:0:0:1:1	-	770.4 (141.8)	0.88 (0.10)	277.1 (26.0)	1853.3 (3210.1)	-
	0.45	174.0 (2.8)	0.11 (0.01)	196.8 (6.9)	-	-
	0.22	160.9 (1.3)	0.08 (0.01)	176.8 (1.9)	-	-
16 1:1:1:0:0	-	244.2 (12.1)	0.24 (0.00)	239.7 (19.2)	3597.3 (3115.4)	-
	0.45	157.1 (9.3)	0.15 (0.00)	167.2 (6.6)	1687.3 (2922.5)	-
	0.22	132.2 (3.9)	0.13 (0.02)	144.2 (1.2)	-	-

17	1:1:0:1:0	-	692.8 (167.7)	0.88 (0.08)	119.6 (199.1)	137.2 (140.3)	190.3 (38.0)
		0.45	-	-	-	-	-
		0.22	-	-	-	-	-
18	1:1:0:0:1	-	412.5 (25.6)	0.65 (0.05)	266.2 (33.3)	1768 (582.3)	4796.3 (247.6)
		0.45	135.1 (2.8)	0.15 (0.01)	11.8 (20.4)	160.3 (3.5)	1494.9 (2556.6)
		0.22	141.4 (6.2)	0.14 (0.00)	12.6 (21.8)	164.7 (5.9)	-
19	0:1:1:1:0	-	719.7 (42.9)	0.89 (0.03)	57.8 (50.1)	102.5 (176.3)	195.8 (37.1)
		0.45	-	-	-	-	-
		0.22	-	-	-	-	-
20	1:0:1:1:0	-	209.2 (1.6)	0.49 (0.00)	43.0 (0.8)	370.7 (6.4)	-
		0.45	74.5 (7.5)	0.41 (0.04)	128.6 (126.1)	251.2 (172.8)	-
		0.22	46.5 (0.2)	0.11 (0.00)	51.4 (0.5)	-	-
21	1:0:1:0:1	-	541.2 (167.6)	0.76 (0.10)	20.4 (33.5)	175.5 (37.2)	-
		0.45	147.9 (12.9)	0.34 (0.04)	5.2 (6.2)	121.8 (26.5)	-
		0.22	96.8 (13.6)	0.46 (0.14)	1.2 (0.2)	16.7 (22.9)	116.3 (7.1)
22	1:0:01:1	-	405.1 (25.2)	0.64 (0.04)	317.3 (57.5)	1463.0 (2534.0)	3679.8(2358.8)
		0.45	132.4 (1.5)	0.17 (0.01)	157.6 (2.4)	1684.5(2862.7)	-
		0.22	140.4 (3.9)	0.14 (0.01)	164.1 (1.9)	1579.2 (2706.2)	-
23	0:1:1:0:1	-	1095.8 (750.2)	0.83 (0.16)	98.4 (25.9)	183.6 (55.3)	379.4 (129.9)
		0.45	143.7 (25.1)	0.37 (0.03)	17.5 (22.7)	118.3 (20.3)	230.0 (374.3)
		0.22	114.6 (4.1)	0.33 (0.05)	1.6 (1.7)	12.4 (10.1)	131.7 (14.1)
24	0:1:0:1:1	-	860.2 (284.0)	0.91 (0.09)	122.2 (105.9)	306.5 (39.7)	1788.3 (3013.9)
		0.45	155.6 (1.8)	0.14 (0.00)	180.5 (6.2)	-	-
		0.22	148.3 (1.3)	0.11 (0.02)	165.5 (1.8)	-	-
25	0:0:1:1:1	-	651.7 (312.3)	0.81 (0.21)	193.6 (36.5)	668.9 (297.6)	1494.0 (2585.1)
		0.45	-	-	-	-	-
		0.22	-	-	-	-	-
26	1-1-1-1-0	-	269.9 (6.3)	0.18 (0.01)	294.7 (4.7)	5015.7 (238.1)	-
		0.45	164.8 (4.8)	0.09 (0.02)	179.2 (4.8)	-	-
		0.22	153.7 (1.4)	0.08 (0.01)	167.2 (3.2)	-	-
27	0-1-1-1-1	-	553.6 (68.3)	0.78 (0.04)	184.7 (37.0)	225.6 (105.2)	-
		0.45	159.9 (10.1)	0.182 (0.03)	163.4 (9.4)	1723.4 (2984.9)	-
		0.22	130.7 (3.3)	0.16 (0.05)	138.4 (7.9)	-	-
28	1-0-1-1-1	-	324.9 (30.6)	0.55 (0.07)	179.9 (16.2)	388 (672.0)	1695.7 (2937.0)
		0.45	145.4 (5.2)	0.25 (0.02)	143.8 (10.5)	-	-
		0.22	126.4 (14.2)	0.23 (0.023)	141.2 (5.1)	1724.7 (2969.9)	-
29	1-1-0-1-1	-	327.5 (17.2)	0.58 (0.04)	299.6 (44.2)	1374.7 (2381.0)	3612.3 (1691.1)
		0.45	133.0 (2.2)	0.15 (0.01)	160.5 (5.6)	-	-
		0.22	136.0 (1.6)	0.13 (0.00)	11.9 (20.6)	160.3 (1.3)	-
30	1:1:1:0:1	-	472.8 (80.3)	0.72 (0.08)	112.1 (122.7)	158.9 (19.2)	-
		0.45	144.7 (3.7)	0.18 (0.02)	149.9 (1.1)	-	-
		0.22	126.3 (1.2)	0.15 (0.01)	13.0 (22.5)	141.8 (3.5)	-
31	1:1:1:1:1	-	397.1 (105.8)	0.64 (0.16)	42.1 (72.9)	176.5 (25.9)	-
		0.45	146.2 (3.1)	0.13 (0.01)	157.3 (3.4)	-	-
		0.22	133.9 (2.4)	0.12 (0.00)	148.5 (3.4)	-	-

1 **Table 6**
 2 Solvent effect on size and polydispersity of MPC₁₀₀-DPA₁₀₀ nanoparticle systems prepared via nanoprecipitation
 3 (Mean ± SD, n = 3). See Table 2 for solvent formulation details.

Solvent Combination	Filter (µm)	Diameter (Z _{Ave}) (nm) (±SD)	Polydispersity (Pd) (±SD)	Dh ¹ (nm) (±SD)	Dh ² (nm) (±SD)	Dh ³ (nm) (±SD)
1 1:0:0:0:0	-	70.3 (0.6)	0.03 (0.01)	73.9 (0.7)	-	-
	0.45	69.6 (0.5)	0.04 (0.01)	73.4 (0.8)	-	-
	0.22	69.2 (0.2)	0.03 (0.00)	72.9 (0.4)	-	-
2 0:1:0:0:0	-	223.0 (16.7)	0.38 (0.02)	278.5 (33.0)	2047.7 (2391.0)	3190.3 (2768.2)
	0.45	135.8 (6.4)	0.17 (0.01)	161.6 (8.7)	1579.0 (2734.9)	-
	0.22	153.7 (5.2)	0.19 (0.01)	23.2 (20.5)	191.4 (4.9)	-
3 0:0:1:0:0	-	-	-	-	-	-
4 0:0:0:1:0	-	-	-	-	-	-
5 0:0:0:0:1	-	437.1 (47.2)	0.61 (0.03)	504.4 (70.67)	1760.3 (3049.0)	3357.4 (2743.2)
	0.45	137.8 (9.4)	0.17 (0.01)	165.1 (12.6)	-	-
	0.22	172.7 (3.9)	0.16 (0.01)	204.6 (5.5)	-	-
6 1:1:0:0:0	-	62.4 (0.7)	0.05 (0.01)	66.6 (0.7)	-	-
	0.45	61.3 (0.5)	0.05 (0.02)	65.4 (0.3)	-	-
	0.22	61.5 (0.4)	0.05 (0.01)	65.7 (0.7)	-	-
7 1:0:1:0:0	-	559.6 (266.6)	0.65 (0.22)	279.3 (86.4)	3480.7 (2782.1)	-
	0.45	131.0 (26.2)	0.52 (0.16)	15.7 (23.2)	190.3 (12.6)	1853.9 (3209.6)
	0.22	195.1 (22.4)	0.28 (0.02)	224.4 (25.4)	1733.1 (2972.2)	-
8 1:0:0:1:0	-	75.8 (0.7)	0.06 (0.00)	81.1 (0.6)	-	-
	0.45	74.9 (0.9)	0.05 (0.02)	79.8 (0.9)	-	-
	0.22	75.2 (0.5)	0.04 (0.01)	79.5 (0.5)	-	-
9 1:0:0:0:1	-	238.4 (0.6)	0.35 (0.02)	14.6 (25.2)	326.5 (13.8)	4690.0 (454.0)
	0.45	138.7 (6.3)	0.18 (0.01)	21.0 (18.4)	168.9 (9.8)	-
	0.22	159.3 (6.5)	0.19 (0.01)	194.6 (10.6)	3264.3 (2828.4)	-
10 0:1:1:0:0	-	309.3 (32.0)	0.45 (0.05)	336.8 (56.5)	574.3 (994.8)	4781.3 (490.2)
	0.45	172.2 (15.8)	0.28 (0.04)	4.9 (6.2)	22.7 (27.2)	197.7 (25.1)
	0.22	184.9 (20.5)	0.23 (0.08)	0.8 (1.3)	209.1 (16.9)	3293.3 (2829.3)
11 0:1:0:1:0	-	235.0 (10.8)	0.45 (0.02)	293.3 (27.0)	1613.4 (2763.2)	3846.0 (1377.6)
	0.45	131.5 (6.3)	0.13 (0.01)	149.13 (8.50)	-	-
	0.22	152.5 (5.8)	0.15 (0.01)	12.8 (22.2)	178.6 (7.9)	-
12 0:1:0:0:1	-	523.2 (21.7)	0.69 (0.02)	448.1 (146.3)	1977.6 (2525.2)	3412.7 (2960.7)
	0.45	164.0 (2.0)	0.17 (0.01)	196.4 (4.7)	-	-
	0.22	187.5 (3.9)	0.18 (0.01)	227.0 (10.3)	-	-
13 0:0:1:1:0	-	-	-	-	-	-
14 0:0:1:0:1	-	367.1 (156.9)	0.52 (0.17)	255.0 (61.2)	1710.7 (2962.7)	1869.5 (2861.0)
	0.45	195.1 (29.8)	0.42 (0.10)	0.2 (0.4)	274.9 (122.5)	1718.0 (2872.2)
	0.22	119.9 (61.3)	0.61 (0.34)	1.9 (2.6)	27.7 (37.7)	240.6 (36.4)
15 0:0:0:1:1	-	347.5 (24.3)	0.58 (0.05)	339.6 (19.0)	4800.7 (958.3)	-
	0.45	133.9 (4.0)	0.17 (0.01)	23.1 (20.1)	160.9 (5.1)	-
	0.22	161.6 (1.1)	0.15 (0.01)	187.5 (1.8)	1628.7 (2820.9)	-
16 1:1:1:0:0	-	461.8 (32.1)	0.50 (0.05)	486.4 (141.7)	2919.4 (2503.6)	-
	0.45	174.1 (6.4)	0.15 (0.03)	15.3 (26.5)	205.6 (9.5)	-

		0.22	184.6 (8.8)	0.16 (0.01)	213.4 (13.3)	-	-
17	1:1:0:1:0	-	461.5 (93.7)	0.58 (0.15)	334.3 (101.6)	1442.3 (2422.4)	-
		0.45	170.2 (13.5)	0.26 (0.04)	3.2 (5.5)	191.8 (20.9)	481.7 (834.3)
		0.22	199.6 (14.3)	0.21 (0.03)	221.3 (10.4)	1572.3 (2723.4)	-
18	1:1:0:0:1	-	245.2 (13.7)	0.41 (0.03)	356.3 (53.8)	4700.7 (335.8)	-
		0.45	145.3 (5.3)	0.18 (0.00)	177.47 (7.5)	-	-
		0.22	163.6 (5.9)	0.20 (0.01)	203.2 (8.5)	2964.3 (2575.8)	-
19	0:1:1:1:0	-	500.7 (32.5)	0.62 (0.03)	58.5 (65.0)	303.4 (372.2)	340.4 (7.1)
		0.45	158.7 (3.8)	0.18 (0.00)	179.6 (2.8)	1673.0 (2897.7)	-
		0.22	190.5 (5.2)	0.16 (0.00)	220.7 (1.6)	1604.3 (2778.8)	-
20	1:0:1:1:0	-	65.5 (2.2)	0.06 (0.01)	70.1 (2.9)	-	-
		0.45	64.8 (1.8)	0.05 (0.02)	69.30 (1.5)	-	-
		0.22	64.5 (1.5)	0.06 (0.01)	69.3 (2.1)	-	-
21	1:0:1:0:1	-	356.6 (12.3)	0.42 (0.03)	27.4 (47.5)	454.4 (76.0)	4599.7 (606.4)
		0.45	176.8 (2.2)	0.12 (0.01)	200.2 (3.9)	-	-
		0.22	188.0 (7.8)	0.14 (0.01)	217.6 (10.8)	-	-
22	1:0:0:1:1	-	205.8 (12.5)	0.39 (0.01)	10.8 (18.6)	306.1 (15.2)	4258.7 (314.5)
		0.45	131.6 (6.0)	0.16 (0.01)	156.0 (8.8)	3194.7 (2779.0)	-
		0.22	147.5 (11.8)	0.19 (0.02)	179.5 (19.7)	3047.3 (2639.1)	-
23	0:1:1:0:1	-	346.3 (27.6)	0.50 (0.02)	93.9 (24.6)	334.8 (23.9)	3522.3 (3051.2)
		0.45	158.5 (5.5)	0.14 (0.01)	177.3 (9.7)	1720.3 (2979.7)	-
		0.22	182.1 (5.7)	0.14 (0.01)	211.2 (6.6)	-	-
24	0:1:0:1:1	-	507.8 (68.7)	0.75 (0.03)	285.00 (99.5)	2356.37 (2786.3)	3664.3 (3174.0)
		0.45	134.0 (7.5)	0.18 (0.01)	163.3 (11.8)	-	-
		0.22	156.0 (1.5)	0.19 (0.01)	191.5 (6.7)	1694.3 (2874.9)	-
25	0:0:1:1:1	-	384.7 (133.9)	0.59 (0.16)	252.0 (23.5)	703.2 (1138.0)	2483.0 (2637.1)
		0.45	128.5 (37.5)	0.54 (0.15)	20.6 (28.2)	205.0 (32.2)	3078.1 (2679.9)
		0.22	179.1 (40.8)	0.28 (0.06)	218.5 (49.5)	1798.4 (3113.1)	-
26	1-1-1-0-0	-	521.3 (43.4)	0.69 (0.05)	142.4 (87.8)	377.0 (44.5)	2125.0 (2784.9)
		0.45	165.9 (3.4)	0.13 (0.01)	191.4 (5.2)	-	-
		0.22	183.7 (11.4)	0.15 (0.02)	213.8 (10.5)	-	-
27	0-1-1-1-1	-	545.2 (7.8)	0.69 (0.05)	239.2 (230.0)	352.5 (31.1)	462.6 (692.7)
		0.45	167.4 (1.0)	0.13 (0.00)	190.0 (5.2)	1610.0 (2788.6)	-
		0.22	182.9 (9.8)	0.15 (0.02)	16.0 (27.7)	211.3 (10.5)	-
28	1-0-1-1-1	-	506.5 (31.3)	0.65 (0.05)	47.2 (41.4)	312.0 (49.7)	437.6 (419.6)
		0.45	173.0 (3.6)	0.14 (0.00)	199.9 (4.7)	-	-
		0.22	189.4 (8.7)	0.15 (0.01)	222.4 (12.7)	-	-
29	1-1-0-1-1	-	242.5 (6.5)	0.43 (0.03)	13.1 (22.7)	370.7 (43.0)	4293.3 (192.0)
		0.45	136.9 (5.4)	0.16 (0.01)	162.9 (7.7)	-	-
		0.22	153.03 (9.1)	0.19 (0.03)	187.5 (13.7)	3147.6 (2708.8)	-
30	1:1:1:0:1	-	533.4 (78.5)	0.65 (0.10)	20.9 (36.2)	502.8 (77.9)	1657.4 (2627.3)
		0.45	168.4 (4.5)	0.14 (0.01)	194.3 (7.4)	-	-
		0.22	188.0 (10.0)	0.15 (0.02)	221.0 (5.8)	-	-
31	1:1:1:1:1	-	586.8 (79.6)	0.74 (0.09)	289.4 (98.9)	612.2 (702.6)	1889.0 (2431.2)
		0.45	154.7 (15.0)	0.14 (0.01)	12.9 (22.3)	179.4 (18.5)	-
		0.22	188.6 (7.0)	0.15 (0.03)	221.0 (5.6)	-	-

1 **Table 7**
 2 AFM measurements and roughness parameters (R) of MPC-DPA copolymer film surface roughness (Mean \pm
 3 SD, n = 6). Where Rq = root mean square roughness; Ra = average roughness; and Rz = ten point average
 4 roughness. See Table 2 for solvent formulation details.

#	MPC ₃₀ - DPA ₁₀₀	Rq (nm)	Ra (nm)	Rz (nm)
(a)	Solvent 15	231.5 (100.0)	183.6 (86.8)	867.3 (301.1)
(b)	Solvent 24	296.4 (72.0)	242.4 (61.0)	1167.0 (172.7)
(c)	Solvent 26	12.9 (2.3)	10.2 (1.8)	81.9 (6.3)
	MPC ₁₀₀ - DPA ₁₀₀	Rq (nm)	Ra (nm)	Rz (nm)
(d)	Solvent 12	80.0 (4.6)	61.1 (3.3)	529.1 (48.0)
(e)	Solvent 16	16.5 (1.7)	13.0 (1.6)	72.7 (5.9)
(f)	Solvent 29	70.7 (16.6)	52.9 (10.0)	284.1 (43.3)

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6 **Table 8**
 7 Solvent effect on size and polydispersity of MPC-DPA nanoparticle systems prepared via film-rehydration
 8 (Mean \pm SD, n = 3). See Table 2 for solvent formulation details.

MPC ₃₀ - DPA ₁₀₀							
Solvent Combination	Filter	Diameter (Z _{Ave})	Polydispersity	<i>Dh</i> ¹	<i>Dh</i> ²	<i>Dh</i> ³	
				(nm) (\pm SD)	(nm) (\pm SD)	(nm) (\pm SD)	(nm) (\pm SD)
15	0:0:0:1:1	-	2257.0 (743.8)	0.70 (0.28)	645.9 (508.9)	2024.0 (3062.5)	2788.4 (2224.6)
		0.45	151.4 (3.1)	0.22 (0.04)	169.2 (8.0)	4644.3 (570.5)	-
		0.22	139.1 (9.1)	0.17 (0.04)	160.3 (8.3)	-	-
24	0:1:0:1:1	-	3026.0 (1015.3)	0.48 (0.40)	308.9 (365.0)	1023.4 (493.5)	2587.5 (2243.2)
		0.45	149.2 (9.2)	0.20 (0.01)	165.2 (19.4)	1662.0 (2878.6)	3368.7 (1296.7)
		0.22	142.8 (7.8)	0.18 (0.00)	163.6 (5.7)	1768.3 (3062.9)	-
26	1:1:1:1:0	-	2871.3 (844.6)	0.59 (0.30)	1688.5 (1250.5)	1763.9 (2514.3)	2130.6 (2509.9)
		0.45	144.7 (9.9)	0.20 (0.03)	162.2 (11.5)	4427.0 (797.9)	-
		0.22	139.3 (2.5)	0.18 (0.03)	160.1 (3.4)	1741.0 (3015.5)	3190.4 (2734.6)
MPC ₁₀₀ - DPA ₁₀₀							
Solvent Combination	Filter	Diameter (Z _{Ave})	Polydispersity	<i>Dh</i> ¹	<i>Dh</i> ²	<i>Dh</i> ³	
				(nm) (\pm SD)	(nm) (\pm SD)	(nm) (\pm SD)	(nm) (\pm SD)
12	0:1:0:0:1	-	831.3 (343.2)	0.79 (0.27)	164.9 (144.3)	920.3 (437.2)	4609.7 (29.0)
		0.45	126.7 (16.4)	0.16 (0.04)	150.3 (12.9)	1525.7 (2642.5)	-
		0.22	153.8 (10.8)	0.14 (0.00)	178.5 (11.1)	-	-
16	1:1:1:0:0	-	552.2 (318.7)	0.78 (0.04)	288.1 (265.9)	1723.3 (1981.3)	1851.3 (2441.2)
		0.45	118.6 (6.1)	0.10 (0.03)	134.1 (11.4)	-	-
		0.22	136.6 (14.8)	0.13 (0.00)	155.9 (16.6)	-	-
29	1:1:0:1:1	-	574.0 (414.4)	0.69 (0.25)	302.8 (147.7)	2291.7 (1608.9)	2557.0 (2327.8)
		0.45	124.4 (14.4)	0.12 (0.03)	141.2 (16.2)	-	-
		0.22	148.3 (23.1)	0.12 (0.02)	168.5 (24.5)	1648.3 (2855.0)	-

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1 **Table 9**
 2 MPC-DPA nanoparticle size, polydispersity, zeta potential, and curcumin loading via nanoprecipitation. (Mean \pm
 3 SD, n=3). See Table 2 for solvent formulation details.
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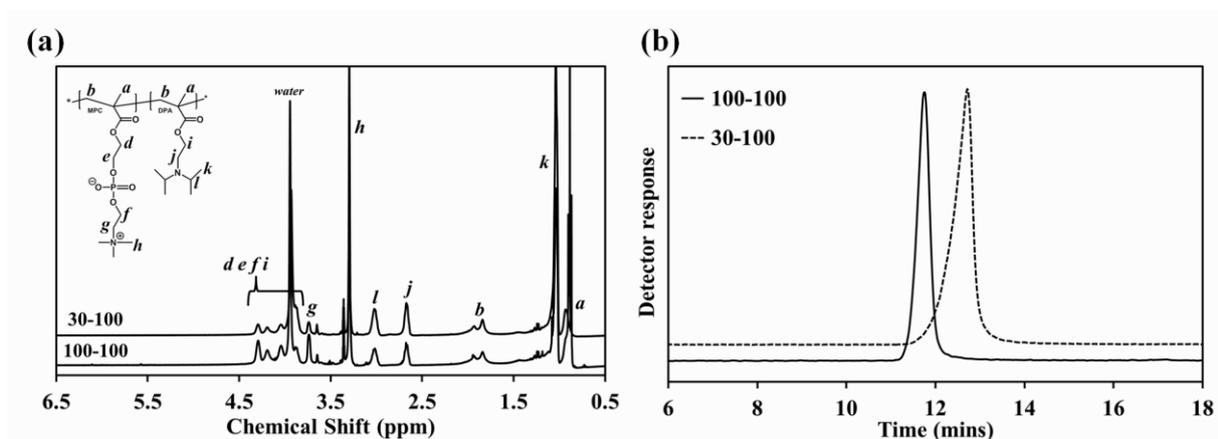
Formulation 26 MPC ₃₀ -DPA ₁₀₀	Parameters values after filtration (0.22 μ m): Mean \pm SD, n=3					Curcumin Loading (μ g ml ⁻¹)
	Diameter (nm)	Poly dispersity (Pd)	Zeta Potential (mV)	Encapsulation Efficiency (%)	Loading Capacity (%)	
Before loading with curcumin	153.7 (1.4)	0.08 (0.01)	-0.34 (1.10)	-	-	-
After loading with Curcumin ^a	141.9 (10.4)	0.10 (0.01)	-0.83 (0.97)	-	-	-
After coating with TMC polymer ^b	136.4 (3.7)	0.12 (0.02)	2.20 (0.09)	-	-	-
Formulation 12 MPC ₁₀₀ -DPA ₁₀₀	Parameters values after filtration (0.22 μ m): Mean \pm SD, n=3					Curcumin Loading (μ g ml ⁻¹)
Before loading with curcumin	187.5 (3.9)	0.18 (0.01)	-0.50 (0.14)	-	-	
After loading with Curcumin ^c	175.2 (8.1)	0.18 (0.00)	-0.55 (0.27)	2.63 (0.30)	0.22 (0.02)	0.92 (0.10)
After coating with TMC polymer ^b	162.4 (1.9)	0.16 (0.00)	-0.31 (0.49)	2.44 (0.19)	0.20 (0.01)	0.85 (0.06)

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 6 **Table 10**
 7 MPC-DPA nanoparticle size, polydispersity, zeta potential, and curcumin loading via film rehydration. (Mean \pm
 8 SD, n=3). See Table 2 for solvent formulation details.
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Formulation 26 MPC ₃₀ -DPA ₁₀₀	Parameters values after filtration (0.22 μ m): Mean \pm SD, n=3					Curcumin Loading (μ g ml ⁻¹)
	Diameter (nm)	Poly dispersity (Pd)	Zeta Potential (mV)	Encapsulation Efficiency (%)	Loading Capacity (%)	
Before loading with curcumin	139.3 (2.5)	0.18 (0.03)	-0.56 (1.74)	-	-	-
After loading with Curcumin ^a	152.5 (30.1)	0.34 (0.15)	-1.51 (0.11)	-	-	-
After coating with TMC polymer ^b	131.5 (11.6)	0.23 (0.01)	7.99 (3.15)	-	-	-
Formulation 12 MPC ₁₀₀ -DPA ₁₀₀	Parameters values after filtration (0.22 μ m): Mean \pm SD, n=3					Curcumin Loading (μ g ml ⁻¹)
Before loading with curcumin	153.8 (10.8)	0.14 (0.00)	-0.33 (0.20)	-	-	
After loading with Curcumin ^c	163.7 (3.1)	0.19 (0.01)	-0.83 (0.37)	1.96 (1.21)	1.35 (0.83)	6.86 (4.24)
After coating with TMC polymer ^b	156.8 (6.3)	0.17 (0.01)	1.04 (0.72)	12.19 (4.90)	8.39 (3.37)	42.57 (17.12)

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1 **Figures**



2

3 **Fig. 1.** (a) ¹H NMR spectra of MPC₃₀-DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀ diblock copolymers. (b) GPC elution profiles
4 of MPC₃₀-DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀ diblock copolymers.
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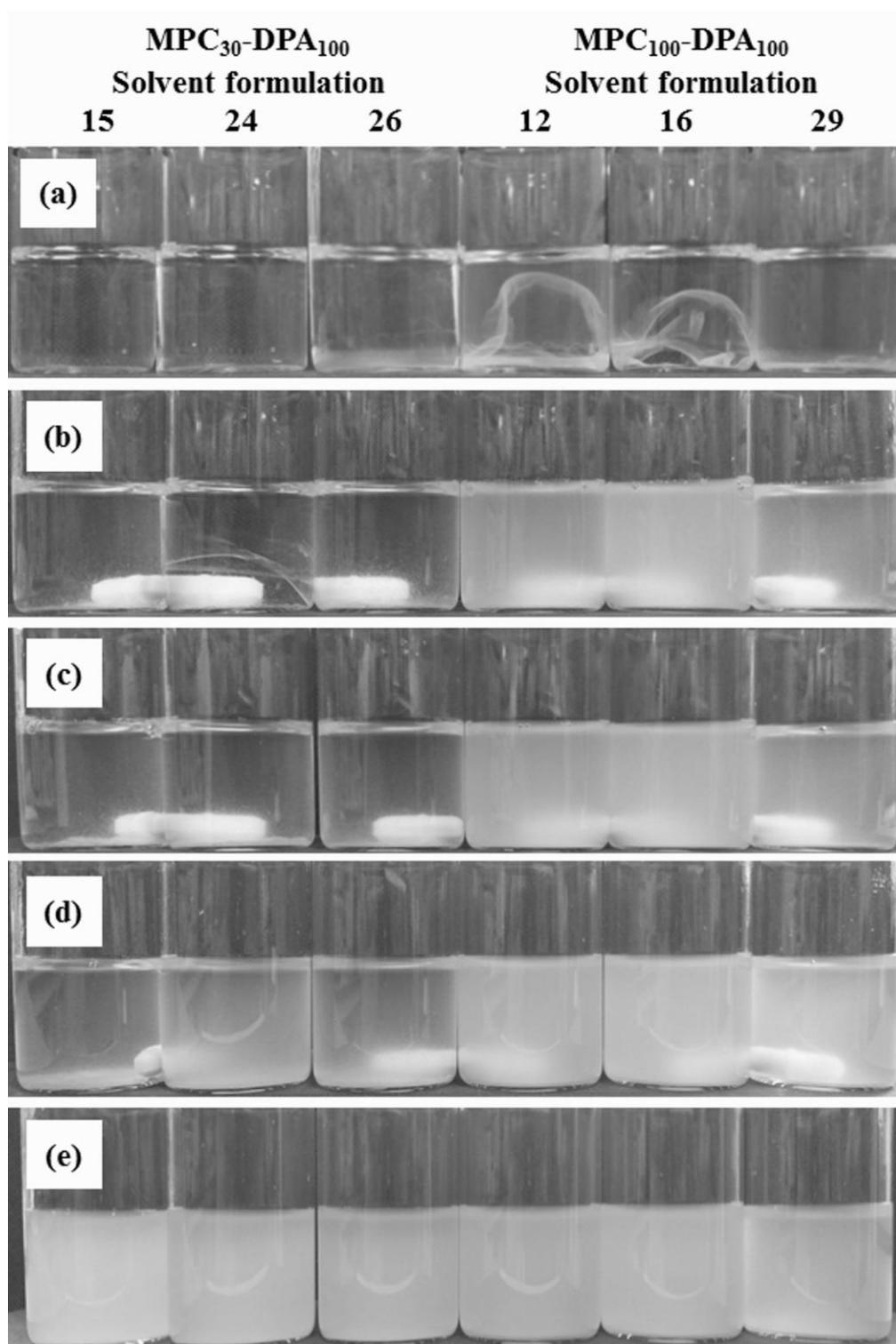
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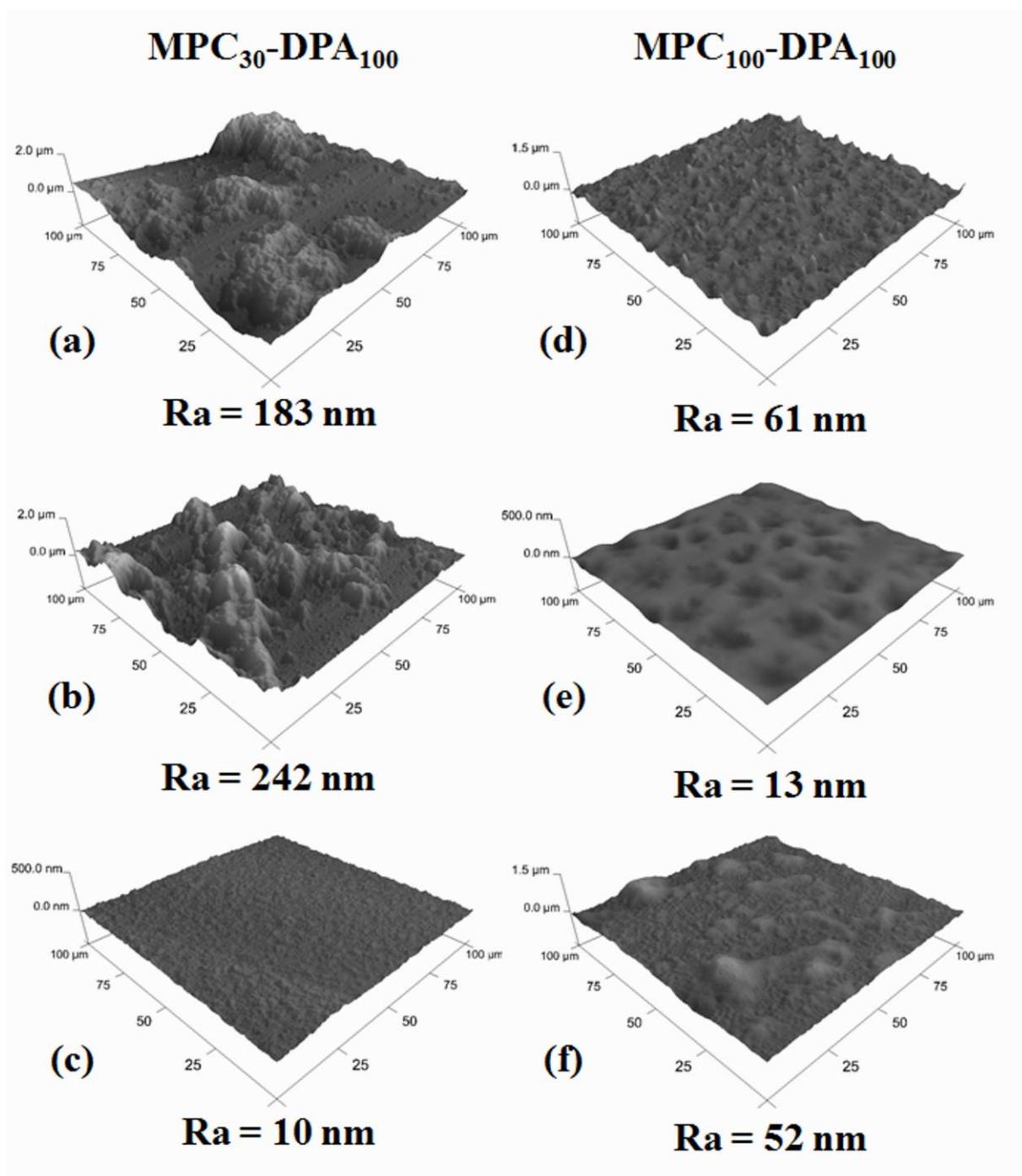
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4 **Fig. 2.** Film rehydration of MPC-DPA copolymers at (a) $t = 0$, (b) $t = 1$ day, (c) $t = 2$ days, (d) $t = 4$ days, (e) $t =$
5 7 days. See Table 2 for solvent formulation details.

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4 **Fig. 3.** Representative AFM images of MPC-DPA copolymer film surface topography; where Ra = average
5 roughness, for solvent combinations 15(a), 24(b), 26(c), 12(d), 16(e), and 29(f). See Table 2 for solvent
6 formulation details.

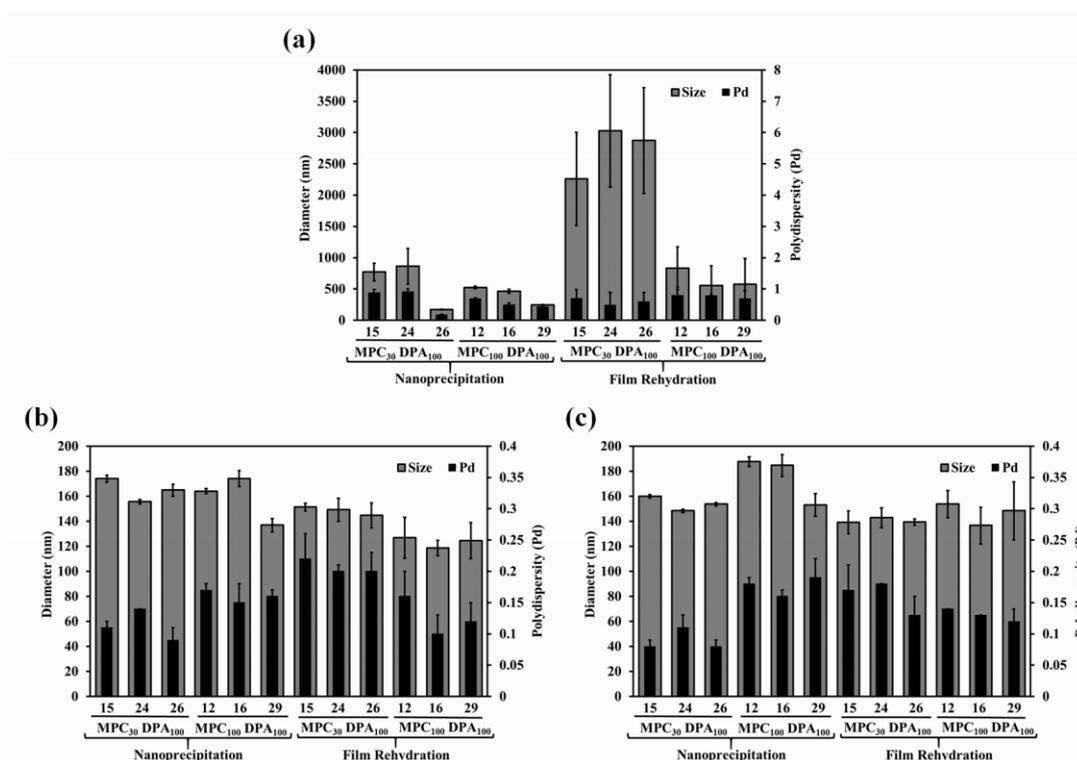
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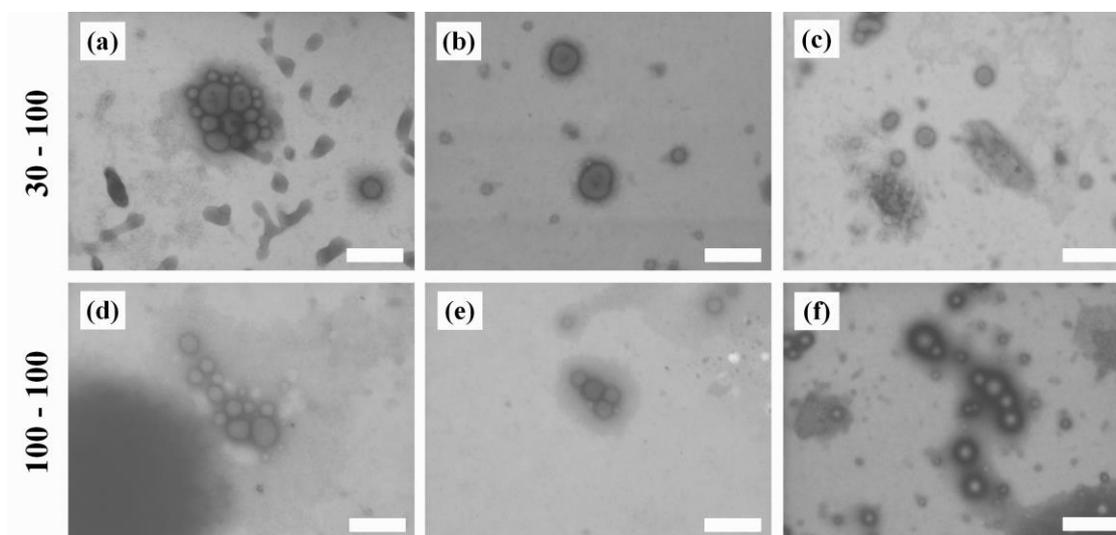
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3 **Fig. 4.** Comparison of preparation method, nanoprecipitation vs film-rehydration, effects on the particle size and
 4 polydispersity of MPC-DPA nanosystems measured using DLS (a) without filtration, (b) after filtration
 5 (0.45µm), and (c) after filtration (0.22µm). (Mean ± SD, n=3). See Table 2 for solvent formulation details.
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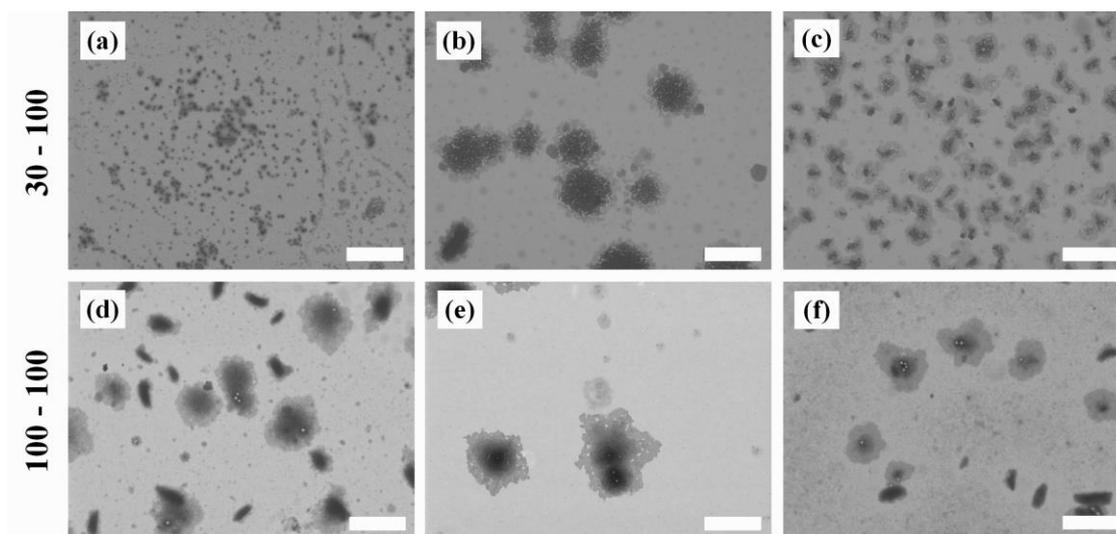
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9 **Fig. 5.** Scanning transmission electron microscopy (STEM) images of MPC₃₀-DPA₁₀₀ (a, b, c) and MPC₁₀₀-
 10 DPA₁₀₀ (d, e, f) nanoparticles formed via nanoprecipitation from solvent formulations 15 (a), 24 (b), 26 (c), 12
 11 (d), 16 (e), and 29 (f), as per Table 2. Scale bar = 200 nm. See Table 2 for solvent formulation details.
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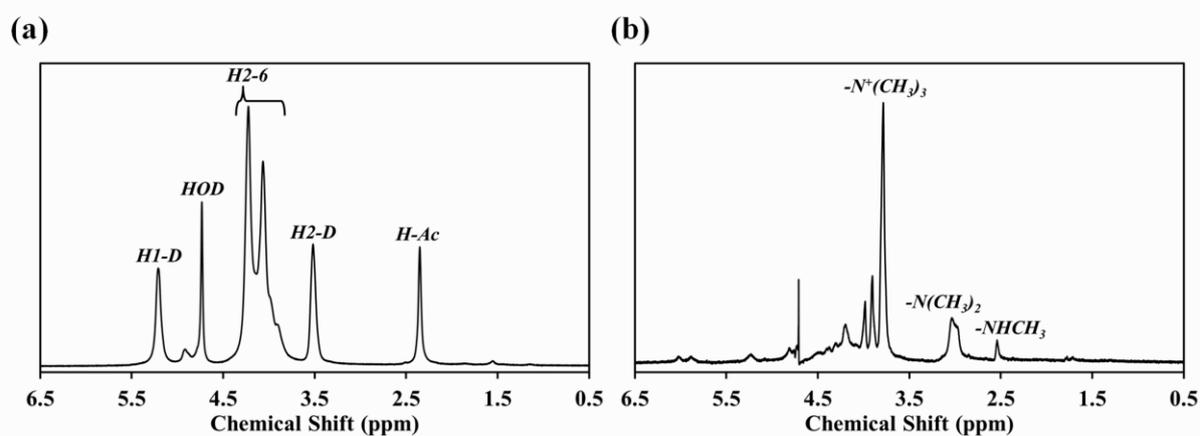


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4 **Fig. 6.** Scanning transmission electron microscopy (STEM) images of MPC₃₀-DPA₁₀₀ (a, b, c) and MPC₁₀₀-
 5 DPA₁₀₀ (d, e, f) nanoparticles formed via film rehydration cast from solvent formulations 15 (a), 24 (b), 26 (c),
 6 12 (d), 16 (e), and 29 (f), as per Table 2. Scale bar = 800 nm. See Table 2 for solvent formulation details.

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9 **Fig. 7.** ¹H NMR spectra of (a) chitosan; chemical shift (ppm) peak assignments: H-Ac = 2.35, H2-D = 3.52, H2-
 10 6 = 3.92 – 4.23, and H1-D = 5.21. NMR solvent peak: HOD = 4.73. (b) TMC; chemical shift (ppm) peak
 11 assignments: -NHCH₃ = 2.55, -N(CH₃)₂ = 3.05, and -N⁺(CH₃)₃ = 3.80.

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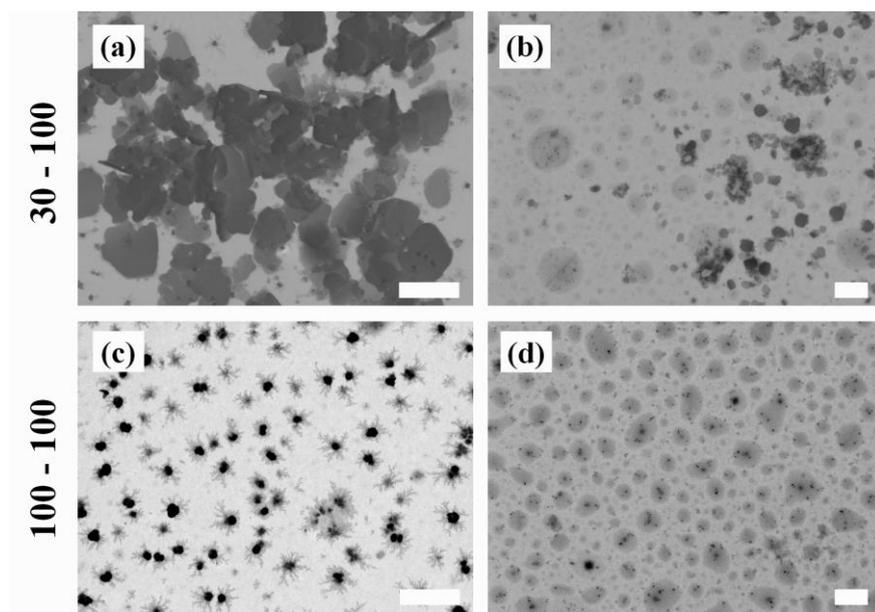
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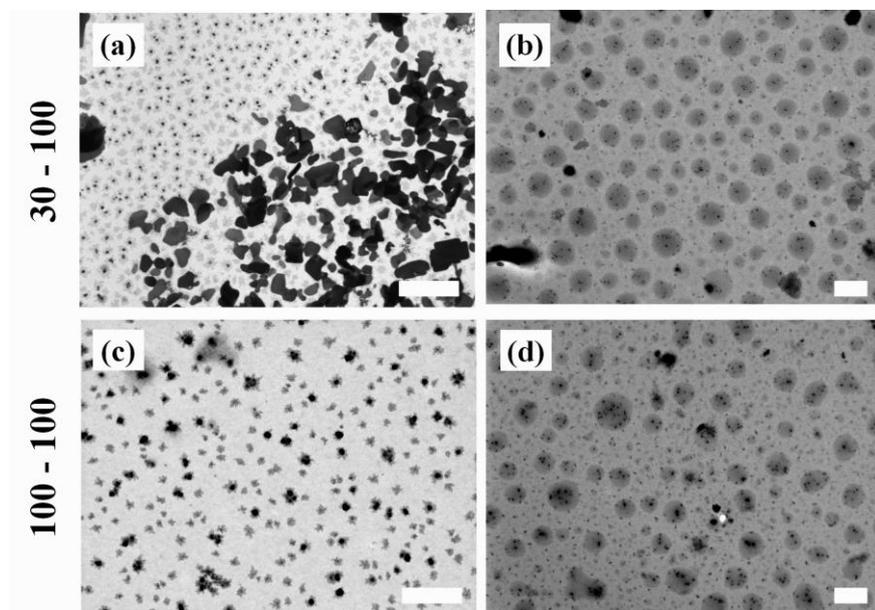


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4 **Fig. 8.** Scanning transmission electron microscopy (STEM) images of MPC₃₀-DPA₁₀₀ (a, b) and MPC₁₀₀-DPA₁₀₀
5 (c, d) nanoparticles formed via nanoprecipitation from solvent formulations 26 (a, b) and 12 (c, d), as per Table
6 2, and curcumin loaded (a, c) and TMC coated (b, d). Scale bar = 300 nm. See Table 2 for solvent formulation
7 details.
8

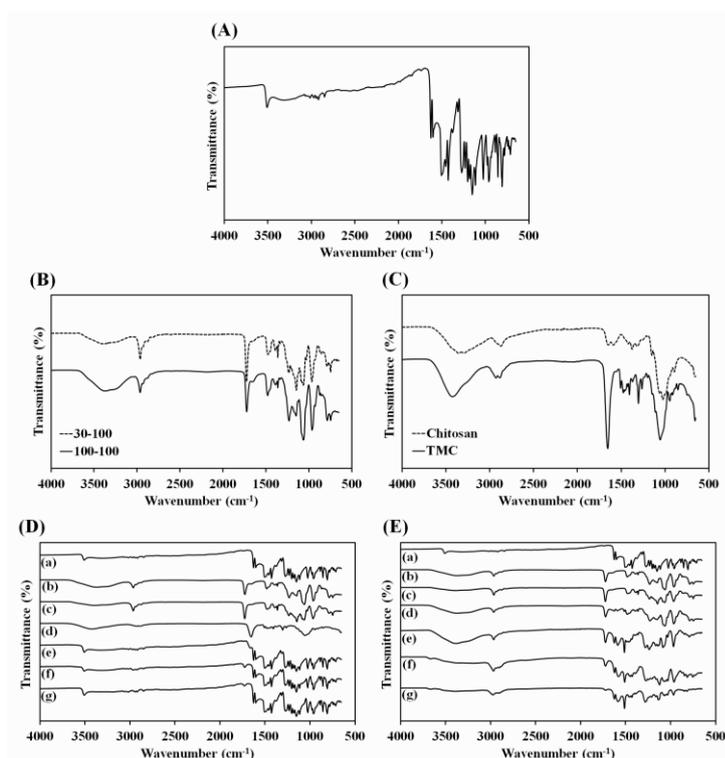
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11 **Fig. 9.** Scanning transmission electron microscopy (STEM) images of MPC₃₀-DPA₁₀₀ (a, b) and MPC₁₀₀-DPA₁₀₀
12 (c, d) nanoparticles formed via film rehydration cast from solvent formulations 26 (a, b) and 12 (c, d), as per
13 Table 2, and curcumin loaded (a, c) and TMC coated (b, d). Scale bar = 300 nm. See Table 2 for solvent
14 formulation details.
15

16



2

3 **Fig. 10.** FTIR spectra of **(10a)** curcumin, **(10b)** MPC₃₀-DPA₁₀₀ and MPC₁₀₀-DPA₁₀₀, **(10c)** chitosan and TMC,
 4 **(10d)** curcumin (i), MPC₁₀₀-DPA₁₀₀ (ii), MPC₃₀-DPA₁₀₀ (iii), TMC (iv), physical mix of curcumin and TMC (v),
 5 physical mix of curcumin and MPC₁₀₀-DPA₁₀₀ (vi), physical mix of curcumin and MPC₃₀-DPA₁₀₀ (vii) and **(10e)**
 6 pure curcumin (i), MPC₁₀₀-DPA₁₀₀ (ii), MPC₃₀-DPA₁₀₀ (iii), solvent mix of curcumin with MPC₁₀₀-DPA₁₀₀ from
 7 formulation 12 (iv and v), and solvent mix of curcumin with MPC₃₀-DPA₁₀₀ from formulation 26 (vi and vii).
 8 The organic solutions for **10e** (iv and vi) were those used for nanoprecipitation, and **10e** (v and vii) for film
 9 rehydration.

10

11 **This is the peer reviewed version of the following article: [Abdul Khaliq Elzhry Elyafi, Guy**
 12 **Standen, Steven T. Meikle, Andrew L. Lewis, Jonathan P. Salvage (2017), Development of MPC-**
 13 **DPA polymeric nanoparticle systems for inhalation drug delivery applications. European**
 14 **Journal of Pharmaceutical Sciences. doi.org/10.1016/j.ejps.2017.06.023], which has been**
 15 **published in final form at [https://doi.org/10.1016/j.ejps.2017.06.023].**

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