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Production of drug-releasing biodegradable microporous scaffold using a two-step micro-encapsulation/supercritical foaming process

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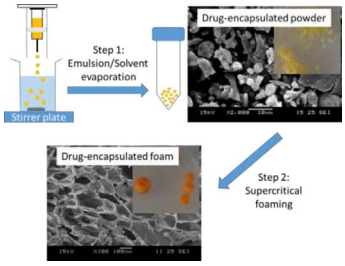
GRAPHICAL ABSTRACT



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ABSTRACT

A two-step fabrication process combining emulsification-solvent evaporation method for encapsulation of drug in PLGA microparticles followed by supercritical gas foaming was developed to produce drug-releasing biodegradable micro-porous foams. The encapsulation and release of model hydrophobic drug (Curcumin) and of model hydrophilic drug (Gentamicin) were investigated in this work. By utilizing a two-step fabrication process, a uniform dispersion of the drug in PLGA polymer matrix can be achieved and the method can be further adapted for the encapsulation of a wide range of active ingredient (both hydrophobic and hydrophilic) in biodegradable micro-porous scaffold.

The in vitro release profile of the drug-encapsulated PLGA foam was studied over a period of 2 weeks and it was observed that the drug release profile can be engineered by the selection of different PLGA polymer blend, varying lactic to glycolic ratio and molecular chain length of the polymer, and by addition of compatible biodegradable polymer such as Polyethylene Glycol (PEG) to the polymer matrix.

1. Introduction

Biodegradable polymer structures have important applications in biomedical and pharmaceutical applications as drug-releasing devices or implantable material in tissue engineering. Polylactic acid (PLA), polyglycolic acid PGA, and their copolymer poly (D,L-lactic-co-glycolic acid) (PLGA) were commonly used in various biomedical applications due to biocompatibility and ability to degrade into constituents that can be easily removed from the body [1,2].

Supercritical carbon dioxide processing has been applied in the development of various drug-delivery and biomedical materials using PLA and PLGA such as microparticles [3–6] and micro-porous biopolymer scaffold [7–14]. Microporous PLGA foams have potential applications as biodegradable and implantable drug delivery device [11], scaffold for DNA delivery [13] and tissue engineering [14]. Drug-releasing PLGA foams can be applied in tissue-engineering to support cell-growth as well as for implantable material for sustained release of medication. Microporous biopolymeric structures produced by

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supercritical CO_2 have high porosity (75–85%) [14], low residual organic solvent content [11], with good mechanical strength which allows easy handling without deformation [11].

Curcumin is a polyphenol compound derived from turmeric *(Curcumin longa)*. It has a distinct yellow-orange colour and has been found to display anti-inflammation, anti-oxidation, anticancer, antimicrobial and even anti-HIV [15–17] qualities. Current challenges in its biomedical application are mainly owing to its low aqueous solubility at physiological pH and low bioavailability [17]. Previous studies on formulation for curcumin using supercritical processing include using solution-enhanced dispersion via supercritical CO_2 (SEDS) to produce nano-curcumin [17,18], using atomized rapid injection solvent extraction (ARISE) system to produce inhalable curcumin with excipients such as polyvinylpyrrolidone (PVP) and hydroxypropyl-beta-cyclodextrin (HP-β-CD) [19], and co-precipitation of curcumin with PLGA to produce nanoparticles using a modified supercritical antisolvent (SAS) method [3].

On the other hand, Gentamicin is a commonly used highly hydrophilic antibiotic drug with antibacterial activity over a wide spectrum and excellent thermal stability [20–22]. Its application is limited by its hydrophilic properties which poses a challenge in developing prolonged antibiotic release implantable devices. Low encapsulation efficiency and short release times were some of the problems associated with its formulation. Gentamicin-loaded PLGA microspheres have been develop using water/oil/water emulsion [23,24] and also by spray drying methods [23]. Double-walled microspheres were also developed in an attempt to modify and prolong the release profile of gentamicin loaded samples for applications as implantable antibiotic treatment material [25].

Supercritical CO_2 foaming of biopolymers is an attractive method for production of microporous constructs for biomedical applications [7,9–11,13,14]. This is partly due to the non-toxic nature of CO_2 and its ability to effectively and cleanly remove residual organic solvents from the final product [11]. Encapsulation of active ingredient in microporous PLGA scaffold using supercritical foaming technique have been developed for compounds including chitosan [13], indomethacin [10], 5-fluorouracil [9] and paclitaxel [11]. Methods employed include a single-step impregnation process as presented by Cabezas et al. [9,10] and also a similar 2-step spray-drying/supercritical foaming process by Lee et al. [11] and Nie et al. [13].

In this work, drug encapsulation using established emulsification-solvent evaporation technique was performed to obtain a dispersion of drug compound in the PLGA polymer matrix. The 2-step process is adapted on both a model hydrophobic drug and a hydrophilic drug to demonstrate its potential application in a wide range of active ingredients that can be encapsulated in biodegradable foams using this technique. Due to the high affinity of supercritical CO_2 with organic solvents used in the microencapsulation step, the residual organic solvent content in the final product can be expected to be very low as shown in previous studies [11].

2. Materials and methods

2.1. Materials

Polymers poly(D,L-lactic-co-glycolic acid) PLGA 75:25 (lactide: glycolide = 75:25; Product number: P1941; MW = 66–107 kDa; Tg = 45–50 °C), PLGA 5050 with Low MW (lactide: glycolide = 50:50 acid terminated; Product number: Resomer RG502H; MW = 7–17 kDa; Tg = 42–46 °C), PLGA 50:50 (lactide: glycolide = 50:50 ester terminated; Product number: Resomer RG505; MW = 54–69 kDa; Tg = 48–52 °C), polyethylene glycol (PEG 8000; Mw 7–9 kDa) were purchased from Sigma Aldrich (Singapore). Phosphate buffered saline (PBS) tablet (0.01 M phosphate buffer, 0.027 M potassium chloride and 0.137 M sodium chloride, pH7.4 at 25 °C, Curcumin (CM) from Turmeric Powder, Gentamicin sulfate (GS), Dichloromethane (DCM) anhydrous and O-Phthaldialdehyde (OPA) Reagent were purchased from Sigma Aldrich (Singapore). Acetone was purchased from Tedia (Fairfield, OH, USA). Ethanol (HPLC grade) was purchased from Fisher Chemical. Compressed carbon dioxide (CO_2) was purchased from Soxal (Singapore Oxygen Air Liquide Pte Ltd, Singapore).

2.2. Micro-encapsulation of curcumin or gentamicin in PLGA

Drug-encapsulated PLGA powder were prepared using an oil/water emulsion method as described in Section 2.2.1 and 2.2.2 for curcumin encapsulation and gentamicin encapsulation respectively.

2.2.1. Curcumin (model hydrophobic drug)

5 mg of curcumin and 500 mg of polymer (~1% w/w) were dissolved in 10 ml acetone. The solution was added dropwise to 100 ml of DI water at a 1.0 ml/min using a syringe pump fitted with BD luer-lokTM syringe with Terumo® needle size 25G (500 μm ID) and size 21G (800 μm ID) for formulations with PLGA 50:50 and PLGA 75:25 respectively The distance from the needle tip to the beaker was 5 cm. The emulsion mixture was constantly stirred at 400 rpm for 4 h at 37 °C in the fume hood. The precipitated drug-encapsulated polymeric particles suspended in DI water were centrifuged for 10 min at 10000 rpm at 10 °C (KUBOTA High Speed Refrigerated Centrifuge). The supernatant was removed while the moist particles were lyophilized using freeze drying (Christ Alpha 1–2 LO plus) for 24 h under −43 °C and vacuum pressure.

2.2.2. Gentamicin (model hydrophilic drug)

20 mg of Gentamicin and 500 mg of PLGA (~4% w/w) were dissolved in 10 ml of acetone. As Gentamicin is only slightly soluble in acetone, sonication was carried out to disperse it in the solution. This is to ensure that PLGA is fully dissolved while gentamicin sulfate is well dispersed in the solution. Solution was added dropwise to 100 ml of DI water at 60 ml/hr using a syringe pump. The emulsion mixture was constantly stirred at 400 rpm for 2 h at 37 °C in the fume hood. The drug-encapsulating particles were collected via centrifugation and freeze drying as described in Section 2.2.1.

2.3. Supercritical foaming to obtain drug-encapsulated foam

Approximate 100 mg of drug-encapsulated particles were weighted and packed in a 1 cm diameter, custom-made cylindrical shaped mold (aluminum) and loaded in the supercritical foaming chamber. The chamber was connected in the supercritical CO_2 foaming set-up as shown in Fig. 1. Compressed CO_2 was first liquefied (Polyscience refrigerated circulator) before delivered to the high pressure chamber (Constructed from a Swagelok 1½" stainless steel bulkhead connector) using high pressure liquid pump (Eldex BBB-4-2). The supercritical foaming chamber is maintained at 35 °C (Polyscience 712 immersion circulator) and 120 bar (using Automatic Back Pressure Regulator, Thar Technologies Inc.) during the foaming process for 4 h. At the end of the experiments, the vessel was depressurized by setting the backpressure valve to $^1/_{10}$ opening till 90 bar pressure and subsequently increasing the backpressure valve opening to $^2/_{15}$ opening till atmospheric pressure.

2.4. Size and surface morphology analysis

The surface morphology of the drug-encapsulated microparticles and microporous foams generated in this study were evaluated using scanning electron microscopy (SEM, JEOL JSM-5600 LV, Japan). Platinum coating (Autofine Coater, JEOL JFC-1300, Japan) at a current of 40 mA for 60 s was applied to all samples prior to analysis. The characteristic pore size and size distribution of the foams were estimated by measuring the equivalent projected area diameter of micropores observed on SEM images using image processing software ImageJ [26].

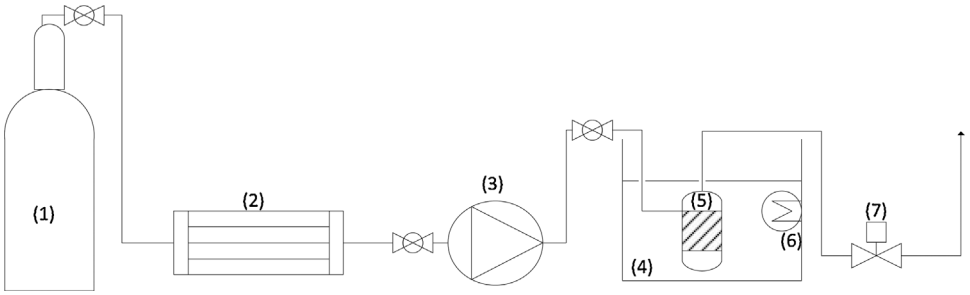

Fig. 1. Experimental setup for supercritical CO_2 foaming process: (1) Compressed CO_2 Cylinder, (2) Refrigerated Circulator, (3) High Pressure Liquid Pump, (4) Stainless Steel Water bath, (5) High Pressure Foaming Vessel, (6) Immersion bath heater, (7) Automatic Back Pressure Regulator.

2.5. Thermal analysis

Differential Scanning Calorimetry (DSC, 822 METTLER TOLEDO with STARe Default DB V9.10-STARe software) was used to study the physical state and glass transition temperature of the drug and polymer used in this study, respectively. Thermal analysis of the drug-loaded foams also provides information about the physical state of curcumin and gentamicin within the polymer matrix. Approximately 1–3 mg of sample weighed and placed in standard aluminum pans. A blank aluminum pan was used as reference. The samples were purged with pure dry nitrogen (50 ml/min) and analysed from 25 °C to 300 °C at a constant heating rate of 10 °C/min.

2.6. Encapsulation efficiency and in-vitro release

Encapsulation efficiency and in vitro release profile from each formulation was determined in triplicate. Typically, foam samples of approximately 3 mg each were dissolved in DCM and left in the fume hood for 8 h to remove the DCM. The drug released was then dissolved in ethanol or PBS to dissolve the curcumin or gentamicin respectively, before analysis using UV spectrophotometry (UV-1800 Shimadzu UV Spectrophotometer). Curcumin and Gentamicin samples were analysed at wavelength of 426 nm and 339 nm respectively. For gentamicin samples, derivatization was carried out with OPA reagent (purchased from Sigma Aldrich) and isopropanol in the absence of light for 1 h prior to analysis [22,27].

For in vitro release experiments, the drug-encapsulating foams were pre-cut into 3 mm diameter disc shaped with approximate mass of 3 mg each. Each sample was submerged in 4 ml PBS (pH = 7.4) and incubated at 37 °C (New Brunswick Scientific Excella E24 Incubator Shaker). At predetermined intervals, PBS with the released drug was removed and fresh PBS of 4 ml was added to provide sink condition for drug release.

3. Results and discussion

PLGA with different co-polymer ratio and molecular weight were used in this study to evaluate the effect on drug encapsulation and release for model hydrophobic drug (Curcumin) and model hydrophilic drug (Gentamicin). Table 1 summarize the formulations of drug-encapsulated particles and foams fabricated in this study. Fig. 2 shows sample images of the state of the drug (as-purchased) and drug-encapsulated powder and drug-encapsulated foams produced. Drug-releasing foam is cut out using standard stainless steel hole punch and surgical knife into 3 mm diameter disc shape for in vitro release evaluation (as illustrated in Fig. 2c). Scanning electron microscopy was used to analyse the microstructure of drug-encapsulated powders produced from using the first step emulsification-solvent evaporation technique (as shown in Fig. 3). For both Curcumin and Gentamicin PLGA formulations, it can be observed that the powdered precipitates sizes ranged from approximately 10 μm (Fig. 3b) to agglomerates in the sizes of few hundred micron. In this work, obtaining uniform size and morphology from emulsification-solvent evaporation step is not the main focus since the purpose of the micro-encapsulation step is to enhance a uniform dispersion of drug in the polymer matrix prior to foaming. The microencapsulated powders is an intermediate in the fabrication for the drug-loaded foams.

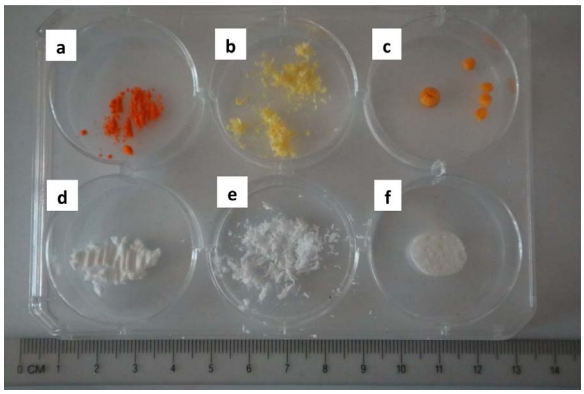

Fig. 2. Physical state of raw drug and drug-encapsulated formulation. (a) Raw Curcumin; (b) Curcumin-loaded PLGA powder (Step 1); (c) Curcumin-loaded PLGA foam (Step 2); (d) Raw Gentamicin; (e) Gentamicin-loaded PLGA powder; (f) Gentamicin-loaded PLGA foam.

3.1. Drug encapsulation in microporous foams

As shown in Table 1, the actual drug loading in all the polymeric

Table 1
Formulations of drug encapsulated particles.

Sample	Drug	Polymer	Molecular Weight (kDa)	Theoretical Drug loading (μg/mg)	Actual Drug loading (μg/mg)	Encapsulation Efficiency (%)
C1	Curcumin	PLGA 50:50 (Resomer RG502H)	7–17	9.90	5.16	52.2
C2	Curcumin	PLGA 50:50 (Resomer RG505)	54–69	9.90	5.77	58.3
C3	Curcumin	PLGA 50:50 (Resomer RG505) + 10% PEG	54–69 7–9	9.90	6.46	65.3
C4	Curcumin	PLGA 75:25 (P1941)	66–107	9.90	7.47	75.5
G1	Gentamicin	PLGA 50:50 (Resomer RG505)	54–69	38.5	8.07	21.2
G2	Gentamicin	PLGA 75:25 (P1941)	66–107	38.5	9.55	25.1

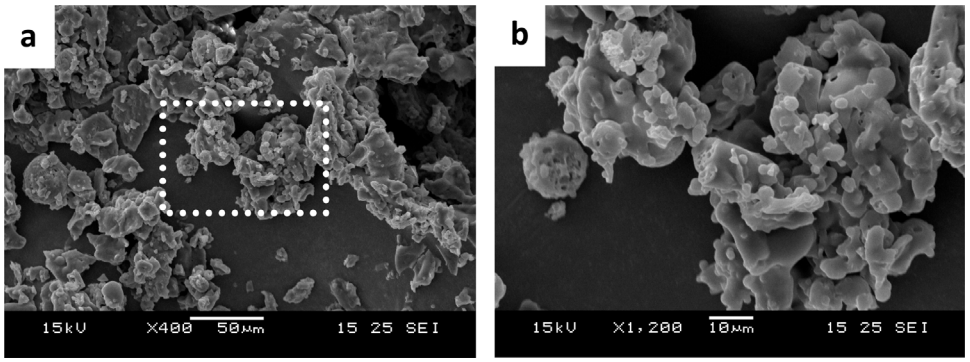

Fig. 3. Scanning electron micrographs of drug-loaded PLGA powders obtained from the emulsification/solvent evaporation technique. (a) Sample C1 powder (PLGA 50:50 low MW); (b) Close up of image in area outlined in (a).

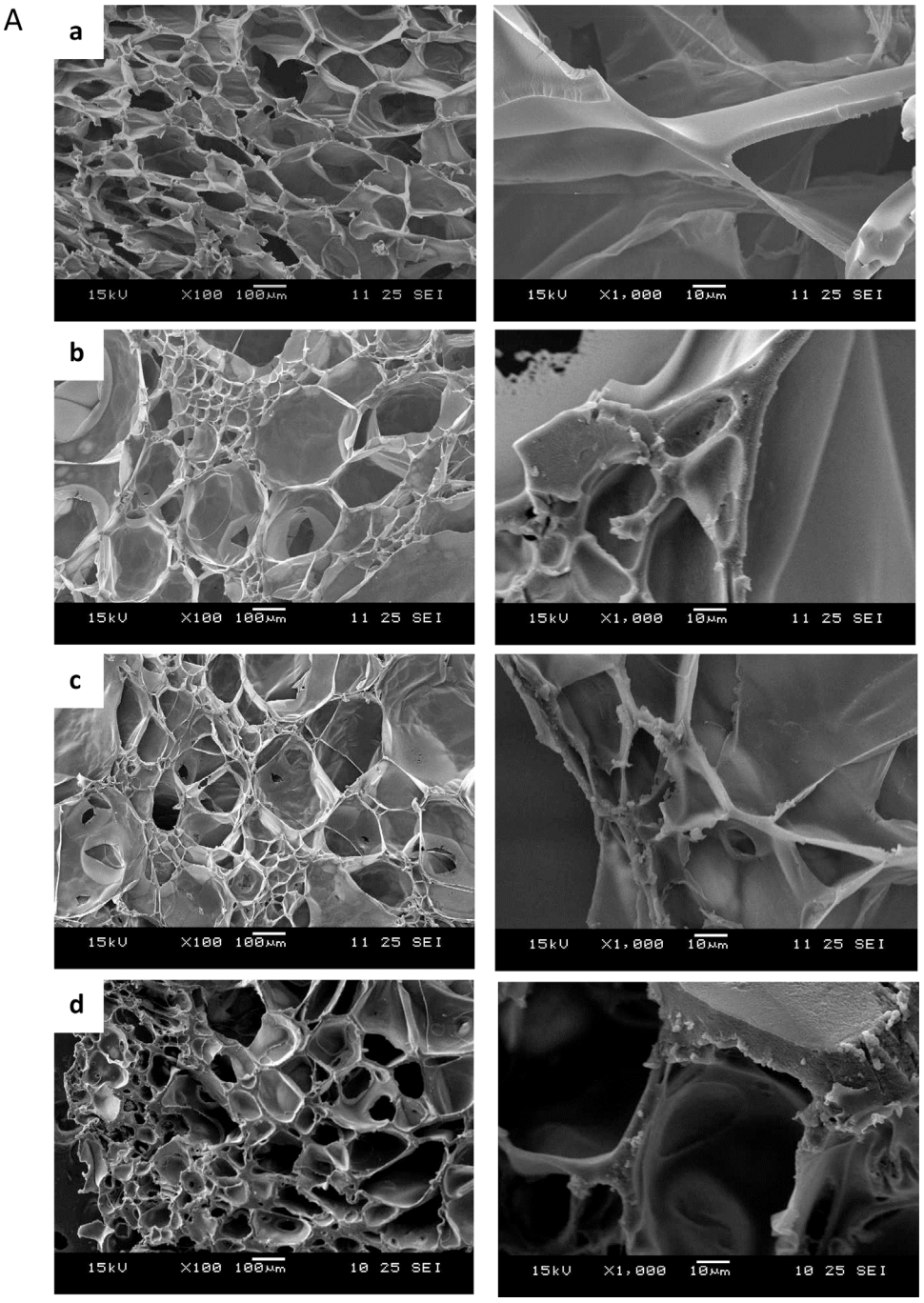

Fig. 4. (a) Scanning electron micrographs of Curcumin-loaded microporous foams (a) C1; (b) C2; (3) C3 and (4) C4; (Left: At 100× magnification; Right: At 1000× magnification). (b) Scanning electron micrographs of Gentamicin-loaded microporous foams (a) G1; (b) G2; (Left: At 200× magnification; Right: At 500× magnification).

foam formulations ranged from 5 to 10 μg/mg. The encapsulation efficiency for the 2-step encapsulation process is defined in Eq. (1) as:

$$\text{Encapsulation efficiency}(\%) = \frac{\text{Actual drug loading in foams}\left(\frac{\mu g}{mg}\right)}{\text{Theoretical drug loading in microparticles}\left(\frac{\mu g}{mg}\right)} \times 100\% \quad (1)$$

The encapsulation efficiency for the Curcumin loaded formulations C1 to C4 ranged from 53 to 75%. The encapsulation efficiency for the Gentamicin loaded formulations G1 and G2 were found to be significantly lower at 21% and 25% respectively. This can be attributed to the highly hydrophilic nature of Gentamicin, leading to loss a significant portion of the drug due to leaking out into the aqueous phase during the micro-encapsulation step.

The microstructure of drug-loaded microporous foams for all the formulations investigated in this work is shown in Fig. 4. The average and standard deviation of the pore sizes estimated by ImageJ software [26] is reported in Table 2. PLGA 50:50 tend to produce foam with non-uniform pore sizes and large size standard deviation. On the other hand, PLGA 75:25 foam has more homogenous pore size distribution and smaller size distribution. The thickness of the walls between pores is also found to be higher for PLGA with high lactic acid content (PLGA 75:25) compared to PLGA 50:50 which is consistent with observations from previous publication [11].

3.2. Thermal analysis

Thermal analysis was performed for the raw material and drug-loaded samples using DSC to determine the glass transition temperature (Tg) of the polymers and crystalline melting profile for the drug. Fig. 5a shows the thermal analysis of raw Gentamicin and Curcumin used in this study. The DSC profile clearly indicates a crystalline melting peak for Curcumin around 180 °C. Being a highly hydrophobic compound with a high tendency to form poorly soluble crystalline structures, it is therefore important to keep a low drug concentration in the polymer matrix to ensure that the drug is homogenously dispersed throughout the polymer during encapsulation and release. In previous studies with paclitaxel, low drug loading of 2% w/w in polymer was observed to be ideal for keeping the homogeneity of drug within the polymer matrix [6,11]. The DSC profile for Gentamicin do not show any distinct crystalline melting peak which is attributed to the amorphous nature of Gentamicin. Therefore a higher drug loading can be used for its formulation. DSC analysis also showed the higher thermal stability of Gentamicin up to approximately 230 °C. However, due to the hydrophilic nature and high aqueous solubility of Gentamicin, low encapsulation efficiency is observed due to the aqueous phase used during the micro-encapsulation step.

The thermal analysis of the raw PLGA pellets (Fig. 5b) showed glass transition temperatures (Tg) above physiological temperature of 37 °C. The lower molecular weight PLGA 5050 has a lower Tg (45–50 °C) compared with PLGA 5050 (Resomer RG505). This is consistent with reported trends that a Tg decreases with the molecular weight of polymer[28].

At higher drug loading, crystalline drug may be formed in the polymer matrix and this will affect the homogeneity of the drug-releasing foam. When crystalline drug is formed in the polymer matrix, the drug crystalline peak will be visible in the DSC scan as well as observable in the SEM images [6]. Fig. 5c shows the thermal analysis of foam sample C4 and G2 alongside raw polymer PLGA 7525. From this figure, it is observed that the samples after foaming displayed a higher T_g (~55–60 °C) compared with the raw polymer pellet (~45–50 °C). This suggests that the CO_2 foaming process and the incorporation of drug into the polymer matrix introduced a more rigid polymer chain structure in the PLGA [11]. From Fig. 5c, there was no observed crystalline peak in the DSC profile for sample C1 in the melting point region for curcumin which suggests that the formulation has homogenously dispersed drug molecules in the polymer matrix. This is supported by the SEM images in Fig. 4a where no drug crystal structures were observed in the cross-section of the Curcumin-loaded foam samples.

Table 2
Pore size and size distribution of microporous foams.

Foam Sample	C1	C2	C3	C4	G1	G2
Pore size (μm)	123	109	107	99	52	55
Standard Deviation (μm)	32	68	48	31	32	16

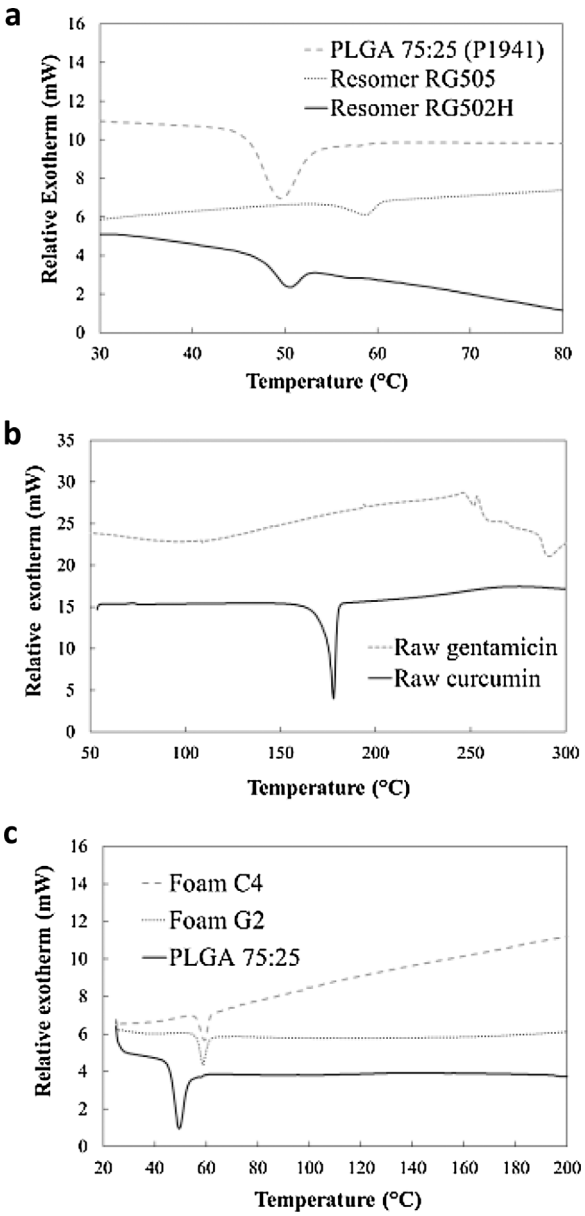

Fig. 5. Thermal analysis of **(a)** raw Gentamicin (broken line) and Curcumin (solid line); **(b)** raw PLGA pellets (PLGA75:25: broken line), (Resomer RG505: dotted line), (Resomer RG502H: solid line); and **(c)** Comparison of drug-loaded PLGA75:25 foam samples with original polymer pellets (Foam C4: broken line), (Foam G2: dotted line), (raw PLGA75:25: solid line).

3.3. In vitro release profiles

In vitro release studies were carried out for up to 2 weeks to

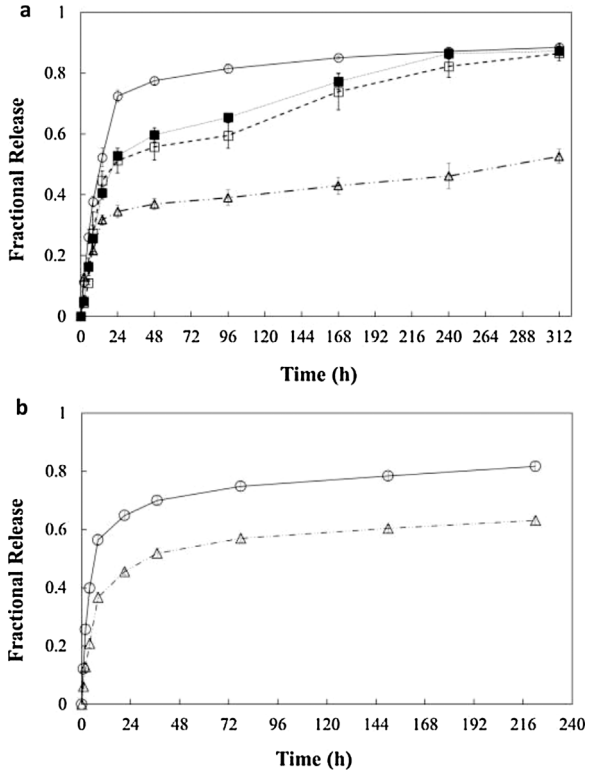

Fig. 6. In vitro release profile from **(a)** Curcumin-loaded PLGA microporous foams: C1 (blank circles, ○), C2 (filled squares, ■), C3 (blank squares,□), C4 (blank triangles, Δ) and **(b)** Gentamicin-loaded PLGA microporous foams: G1 (blank circles, ○), G2 (blank triangles, Δ).

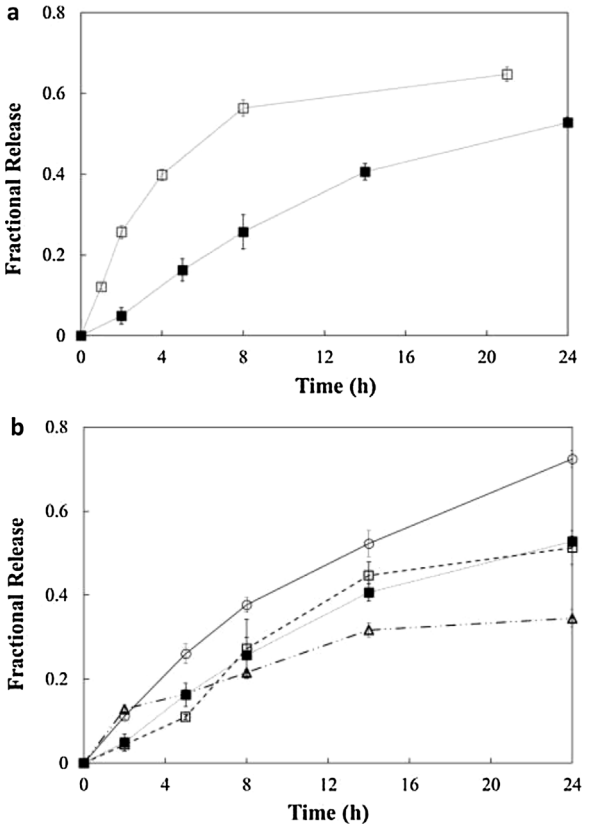

Fig. 7. Comparison of initial release profile from **(a)** PLGA 50:50 foam with curcumin (C2: filled squares, ■) and gentamicin (G1: blank squares,□); and from **(b)** Curcumin-loaded PLGA foams with low MW PLGA50:50 (C1: blank circles, ○), PLGA50:50 (C2: filled squares, ■), PLGA50:50 + 10% PEG (C3: blank squares,□) and PLGA75:25 (C4: blank triangles, Δ).

investigate the effect of using different co-polymer ratio, polymer molecular weight, and addition of additive to the release profile of drugs from these drug-loaded samples. The samples used for in vitro release were cut into standard 3 mm diameter by 1 mm height disc weighing approximate 3 mg each. Fig. 6a and 6b show the release profile of Curcumin-loaded (C1-C4) foam samples and Gentamicin-loaded (G1-G2) foam samples respectively. The release profile from all the samples suggests a diffusion-controlled model. As the polymeric foams do not undergo degradation until at least after 20 days in vitro[14], the release profile is not influenced by polymer degradation during the period of investigation for this work. The comparison of initial (1st 24 h) release of curcumin and gentamicin from PLGA 50:50. As gentamicin is more hydrophilic, its initial release rate can be expected to be faster than the CM-loaded samples. In this work, formulations for both hydrophobic drug and hydrophilic drug were designed with low drug loading to ensure uniform drug distribution throughout the polymer matrix and to maintain a sink condition during the in vitro release experiments, therefore release profile from the different drug-loaded formulation do not show significant difference.

Fig. 7b shows the comparison of samples C1-C4 at initial (1st 24 h) periods. Sample C1 with low MW PLGA 50:50 displayed the highest initial rate of release. Comparing C2 and C3, the addition of 10% w/w PEG to the PLGA 50:50 (Resomer RG505) do not present obvious differences in drug release profile. Overall, the PLGA 50:50 samples displayed typical diffusion-controlled release profile which suggests that there is good penetration of water into the polymer matrix and diffusion of drug out into the release medium. PLGA 50:50 is more hydrophilic than PLGA 75:25 due to the higher glycolic content in the co-polymer. From Fig. 7b, the drug release from PLGA 75:25 displayed a significantly lower initial burst in the first day. This is likely a result of the more hydrophobic properties of PLGA 75:25 and the thicker pore walls observed in the SEM image for sample C4, which may hinder both the water penetration into the foam and the drug diffusion from the foam matrix to the surrounding medium. For all the samples, the final release did not reach 100% after 2 weeks which is likely due to the complex porous network of the foam matrix and the water penetration into the core of the scaffold and the release of drug molecules through the scaffold to the surface may be subjected to the tortuosity and interconnectivity of the porous network.

The effect of different co-polymer on the release profile is similarly observed in Fig. 6b. Comparing sample G1 and G2 with PLGA 5050 and PLGA 7525 respectively, it was also observed that the drug release is faster and higher in sample G1 then G2. The SEM images in Fig. 4b provided evidence of similar thicker pore walls in sample G2.

4. Conclusions

In this work, it was demonstrated that an emulsification-solvent evaporation method can be a suitable alternative to spray drying as a precursor to produce drug-loaded biopolymer particles for supercritical CO_2 foaming. The experiments carried out in this study showed that the foaming condition of 120 bar, 35 °C and foaming time of 4 h are adequate in production of microporous PLGA foams of approximately 100 mg. Both the encapsulation and release of hydrophobic and hydrophilic drug is evaluated in this study. The SEM and thermal analysis of the Curcumin-loaded samples showed no signs of drug crystallization within the polymer matrix formed and the drug release profile examined over a 2-week period where suggested a diffusion-controlled type of release profile. However, due to the interconnecting porous network of the microporous foam, simple geometry models for diffusion controlled release (e.g. sphere, slab, cylinder, etc.) may not be adequate to describe the drug release profile. A lower release rate was observed for PLGA 75:25 due to higher polymer hydrophobicity and also due to a

thicker pore wall. Further studies for this work will be to demonstrate how specific drug release profile may be engineered using polymer blending or addition of suitable excipients such as chitosan, pH controlled release polymers, etc.

Acknowledgements

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