Preparation of porous microsphere-scaffolds by electrohydrodynamic forming and thermally induced phase separation

Hanif Ghanbar [a], C.J. Luo [a], Poonam Bakhshi [a], Richard Day [b], Mohan Edirisinghe [a],*

[a] Department of Mechanical Engineering, University College London, Torrington Place, London, WC1E 7JE, UK
[b] Division of Medicine, University College London, Rockefeller Building, 21 University Street, London, WC1E 6JJ, UK

ABSTRACT

The availability of forming technologies able to mass produce porous polymeric microspheres with diameters ranging from 150 to 300 μm is significant for some biomedical applications where tissue augmentation is required. Moreover, appropriate assembly of microspheres into scaffolds is an important challenge to enable direct usage of the as-formed structures in treatments. This work reports the production of poly (glycolic-co-lactic acid) and poly (ε-caprolactone) microspheres under ambient conditions using one-step electrohydrodynamic jetting (traditionally known as atomisation) and thermally induced phase separation (TIPS). To ensure robust production for practical uses, this work presents 12 comprehensive parametric mode mappings of the diameter distribution profiles of the microspheres obtained over a broad range of key processing parameters and correlating of this with the material parameters of 5 different polymer solutions of various concentrations. Poly (glycolic-co-lactic acid) (PLGA) in Dimethyl carbonate (DMC), a low toxicity solvent with moderate conductivity and low dielectric constant, generated microspheres within the targeted diameter range of 150–300 μm. The fabrication of the microspheres suitable for formation of the scaffold structure is achieved by changing the collection method from distilled water to liquid nitrogen and lyophilisation in a freeze dryer.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Microspheres have been widely used in medical and pharmaceutical applications as effective carriers of encapsulated drugs [1,2]. Spheres made of a biodegradable polymer enable the encapsulated drug to be released in a time-controlled manner, maintaining a constant therapeutic concentration in body fluids over a desirable period (hours or days) from the moment of administration [3]. Polymeric spheres are achievable with special characteristics such as high surface-to-volume ratio, low density, and low coefficient of thermal expansion [4]. Control of the internal and external morphology of the microspheres can be used to influence their interactions with the encapsulated drug as well as the microenvironment after their delivery into the body. Microspheres with surface porosity show a better rate of drug release compared with their smooth surface counterparts. This is because the porous membrane will lead to a slow homeostatic level of the encapsulated drug and prevent the spheres from initial sudden burst [4].

Common methods available for generating drug-encapsulated polymeric porous spheres include emulsion polymerization, thermal phase separation and spray-drying [5–8]. However, there are disadvantages with these methods. For example, emulsion polymerization produces polydisperse spheres with a broad size distribution profile [8]. Non-degradable additives such as surfactants or polymers are also typically required as emulsifiers [9]. Residual solvent toxicity is another issue and the purifying process to separate the spheres from the solvent is slow and costly for pharmaceutical applications [9]. Most importantly, due to exposure of drug for instance to elevated temperatures and high shear stresses in the emulsion method, the biological activity of the drug can be significantly reduced during processing [10–12]. A thermal phase separation technique can generate spherical particles with rigid outer surfaces and a long shelf life. However, the method requires time-consuming multiple processing stages, and suffers from poor control over the diameter distribution of the fabricated particles. In addition, the spheres often stick to each other during formation and before the completion of the processing stages, resulting in large aggregates [13]. Although this technique suffers from a number of disadvantages, the use of solvents with low-boiling point such as dimethyl carbonate (DMC) combined with freeze-drying can reduce the drawbacks in generation of therapeutic products. Thermally induced phase separation (TIPS) followed by freeze-drying has been widely adopted for fabrication of the porous drug vehicles for applications in chronic wound therapy, drug delivery and also tissue engineering [14–18]. Spray-drying is a robust sphere generation method and the processing conditions to control the generated particles diameters are relatively straightforward. However, solvent removal is an issue in spray-drying, which often produces large aggregates. In addition, a large number of spheres

are often lost during production due to the products sticking to the walls of the spray drier. [19–21].

Single-nozzle electrohydrodynamic atomisation (EHDA) can be used to generate near-monodisperse micro- and nano-spheres for applications in drug delivery systems [22,23]. It is a versatile method capable of processing a variety of solutions and emulsions of different polymers and/or therapeutic agents under ambient conditions via a single-stage production process, with flexibility to monitor the product quality at any time without delay during the production stage, without affecting the continuity of the process or having to wait for a multi-stage production process to complete before inspection [4,24,25].

An EHDA process subjects a liquid to a high electric field (in the range of kilovolts), which causes charges to build up within the liquid. When the applied electrostatic force overcomes the surface tension of the liquid, the meniscus of the liquid held at the tip of a nozzle elongates into a conical shape and a fine jet generates from the apex of the cone. The jet subsequently breaks up and deposits on an electrically grounded collector as fine polymeric droplets [26]. A stable cone-jet is the most desirable electrohydrodynamic jetting condition for near-monodisperse spherical particle generation [26]. The mean sphere diameter generated can be changed from the micrometre to the nanometre scale by varying EHDA processing parameters, especially flow rate and applied voltage as well as solution properties, e.g. concentration and physical properties of the polymer solution [27–30]. To maintain a stable cone-jet, the rate of mass transfer to the nozzle exit (controlled by the liquid flow rate) should be coupled and balanced by that out of the nozzle (controlled by the applied electric field, which is responsible for the force causing jet formation). Hence, the flow rate and the applied voltage used for each polymer liquid should be carefully coupled within a defined range [27].

A number of polymers with different properties have been investigated in EHDA studies for their potential application in drug delivery systems. However, only a few of them such as poly (ε-caprolactone) (PCL) and poly (lactic-co-glycolic acid) (PLGA) have been extensively used due to their biocompatibility, biodegradability and versatile degradation kinetics [31,32]. PLGA co-polymers have higher rate of biodegradation by hydrolysis under physiological conditions compared with PCL as they contain more ester groups per polymer molecular chain [33]. The biodegradation products of these two polymers have been shown to be non-toxic, non-immunogenic, non-teratogenic and non-carcinogenic [34]. Furthermore, the composition of these polymers can be varied in order to obtain a desirable release profile based on the rate of hydrolytic degradation. Because of their wide usage in the development of drug delivery systems [35], PLGA and PCL were selected for this investigation.

A suitable sphere diameter and morphology is one of the crucial requirements in a drug delivery system [25,36–38]. The diameter and morphology of the spheres (from the micrometre to the nanometre range) determines their surface area to volume ratio for biochemical reactions and physicochemical interactions with biological agents and cells [34,35]. The diameter, surface morphology and membrane porosity of the spheres influence the physical and chemical interactions as well as the anisotropy of the spheres in a physiological environment, and their ability to penetrate tissue structures in vivo. These characteristics also influence intercellular trafficking as well as drug release, endowing them additional promising advantages in different medical and pharmaceutical applications [39–41].

Microspheres are being considered for a number of biomedical applications where minimally invasive delivery combined with in-situ scaffold formation technology is required [14–18]. For example with chronic wounds, it is suggested that biodegradable microspheres with surface porosity and diameter range of 150–300 μm will provide a conformable structure capable of filling irregular shaped cavities caused by chronic wounds. The interstices formed between the packed microspheres need to be large enough to allow cell migration into the scaffold to facilitate wound healing. This study investigated the feasibility of producing microspheres using one-step fabrication

by single-nozzle EHDA and the subsequent assembly into scaffold structures with TIPS.

To ensure robust EHDA production, this work mapped EHDA parameters using a variety of polymer solutions for controlled generation of near-monodisperse microspheres of a targeted diameter. Comprehensive sets of data on the diameter distribution profile of the particles obtained over a broad range of flow rate and applied voltages are presented.

2. Materials and method

2.1. Materials

PLGA (copolymer 50:50, Resomer RG503H, number average molecular weight (Mn) = 33000 g/mol), was purchased from Boehringer Ingelheim (Ingelheim, Germany). PCL of two molecular weights, PCL10000 (Mn = 10000 g/mol) and PCL45000 (Mn = 45000 g/mol) were obtained from Sigma Aldrich (Poole, UK). Dimethyl acetomide (DMAc), dimethyl foramide (DMF), dimethyl carbonate (DMC) and toluene were obtained from Sigma Aldrich (Poole, UK). Liquid nitrogen was purchased from British Oxygon Company (London, UK). All materials were used as received.

2.2. Solution preparation

2.2.1. PLGA
5, 10 and 20%w/w PLGA solutions were prepared for two solvents of PLGA: DMAc and DMF. PLGA solutions of 5, 10 and 15%w/w were also prepared with combination of the polymer and DMC. Each solution was mechanically stirred for 900 s to ensure complete dissolution of PLGA.

2.2.2. PCL
15, 20 and 25%w/w PCL10000 and 5, 7 and 10%w/w concentrations of PCL45000 solutions were prepared by dissolving the respective polymer in toluene and mechanically stirring the solutions for 1800 s. Lower concentrations were used for PCL45000 to ensure the solutions remain in the dilute concentration regime for the higher molecular weight PCL polymer, to be able to generate spheres instead of fibres [42] during EHDA.

2.3. Polymer solution characteristics

The density, viscosity, surface tension, electrical conductivity and pH of each polymer solution were characterized at atmospheric pressure and ambient temperature (~20–24 °C). Density was measured with a 25 ml specific gravity bottle (VWR International, Lutterworth, UK). The mass of the empty bottle and the mass of the bottle filled with the solution were measured using an electronic balance (A&D HF-1200G A&D Instruments Ltd., Japan). Viscosity was measured using a rheometer (DV III Ultra Rheometer, Brookfield viscometers, USA). The electrical conductivity was estimated using a standard conductivity probe (pHenomenal PC 5000H, VWR, UK). The pH of the solution was measured by dipping a standard pH probe in the solution (pHenomenal CO11, VWR, UK). Surface tension was characterized using a Kruss tensiometer (plate method). Distilled and deionised water was used for calibrations of the instruments.

2.4. Droplet formation

Single-nozzle EHDA was used to prepare polymer droplets with different size ranges (Fig. 1). A stainless steel nozzle with 1.18 mm orifice (Stainless Tube & Needle Co Ltd, Tamworth UK) was coupled to a high-power voltage supply (Glassman Europe Ltd, Tadley, UK) to provide the applied voltage at the tip of the nozzle. The nozzle was supplied with the polymer solution by a silicon tube (with inner diameter
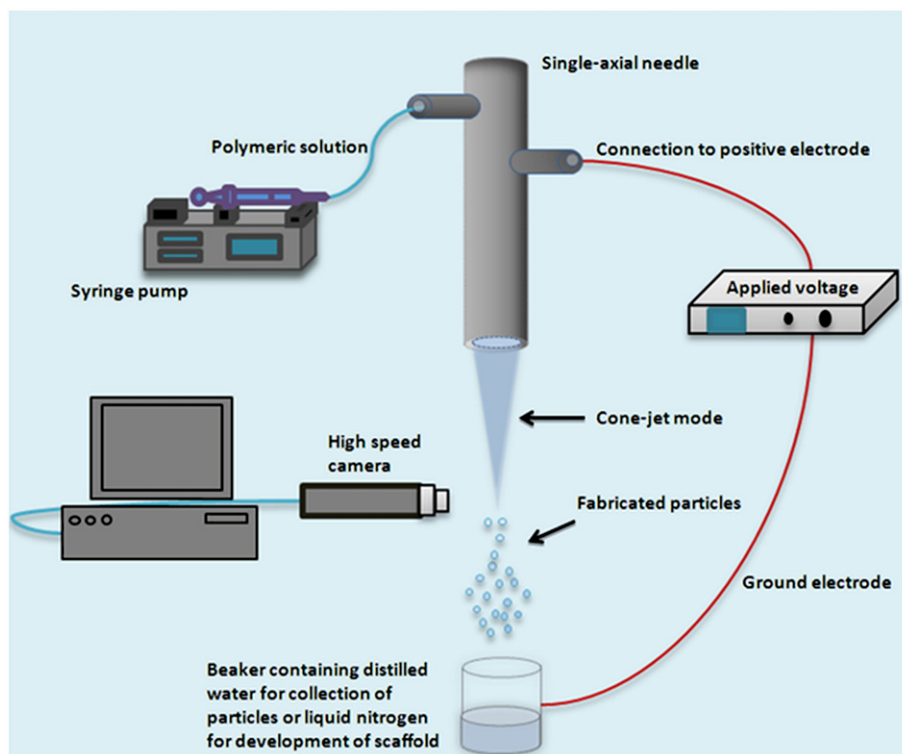
Fig. 1. Schematic diagram of single-nozzle EHDA.

1.0 mm, Sterilin Ltd, Newport, UK) connected to a precision syringe pump (PHD 4400, Harvard Apparatus Edenbridge, UK). Each polymer solution was delivered in a 5 ml syringe (VWR, UK) and pumped through the tubing to the nozzle (Fig. 1). To try and achieve the target microspheres with size range of 150–300 μm, flow rates were varied to change the rate of mass transfer at the tip of the nozzle, which in turn changes the droplet diameter of the EHDA product. Each solution was processed under EHDA at different flow rates, and the voltage at each specific flow rate was selected at the appropriate range to obtain a stable cone jet. The polymer droplets were collected for 300 s in a 10 ml glass vial filled with distilled water, kept 50 mm below the exit of the nozzle. The glass vials were placed on a stainless steel platform which was connected to the ground electrode. The collection distance was kept constant at 50 mm for all samples to ensure the applied electric field strength changed in proportion to the applied voltage values.

2.5. Microspheres formation for assembly into minimally invasive scaffold

15%w/w of PLGA solution in DMC was processed under EHDA for 1800 s and the resulting microspheres were collected in a stainless steel container filled with liquid nitrogen for the formation of scaffolds. Collection of the products in liquid nitrogen led to the generation of solid and near-monodisperse spherical particles which occurred upon thermally induced phase separation of polymer solution into a polymer rich and polymer lean phase [15]. The collection vessel was placed 50 mm below the exit of the nozzle with the ground electrode directly connected to it. The direct grounding of the electric field to the collector resulted in attraction of the charged microspheres and their deposition at the bottom of the metallic container. After 1800 s of microspheres collection in the container filled with liquid nitrogen, the samples were immediately placed in a freeze dryer (LTE Scientific Ltd, Oldham UK) and lyophilized for 12 h. After freeze drying, the free flowing scaffolds were produced by collecting the lyophilized microspheres in vials.

2.6. Characterization of microspheres

The morphology and structure of the prepared spheres were observed using optical microscopy (Nikon Eclipse ME-600 Nikon Co, Tokyo, Japan) and scanning electron microscopy (SEM, Jeol JSM-630 Field Emission). Prior to microscopic studies, five drops of each sample collected in a glass vial were dispersed on a glass slide using a pipette and subsequently placed in a desiccator for 12 h to dry. The SEM samples were initially sputtered coated with gold for 90 s (front and back) prior to analyses in the SEM chamber. The diameter distribution of 100 random microspheres of each sample at various flow rates and applied voltages was analysed using the micrographs obtained. For SEM analysis of the lyophilized microspheres collected in liquid nitrogen for scaffold formation, these were glued onto aluminium stubs via adhesive carbon tabs and then sputter-coated with gold for 180 s in an argon atmosphere.

3. Results and discussion

3.1. EHDA processing and jetting modes

The EHDA jetting modes generated for each polymer solution are determined by material parameters such as the properties of the flowing liquid solutions, and processing parameters such as the applied voltage, the flow rate of the liquid, the working distance between the nozzle tip (positive electrode) and the collecting medium connected to ground electrode [26,43]. As the applied electric field increases by either increasing the voltage or decreasing the working distance, the atomization mode can be transformed from dripping mode to cone-jet mode [26]. Given that the working distance between the positive and ground electrodes were kept constant in this study, the applied voltage values provided a direct indication of the strength of the applied electric field.

Fig. 2 shows the geometrical features of the EHDA jet and the various modes of jetting encountered in this study as a function of
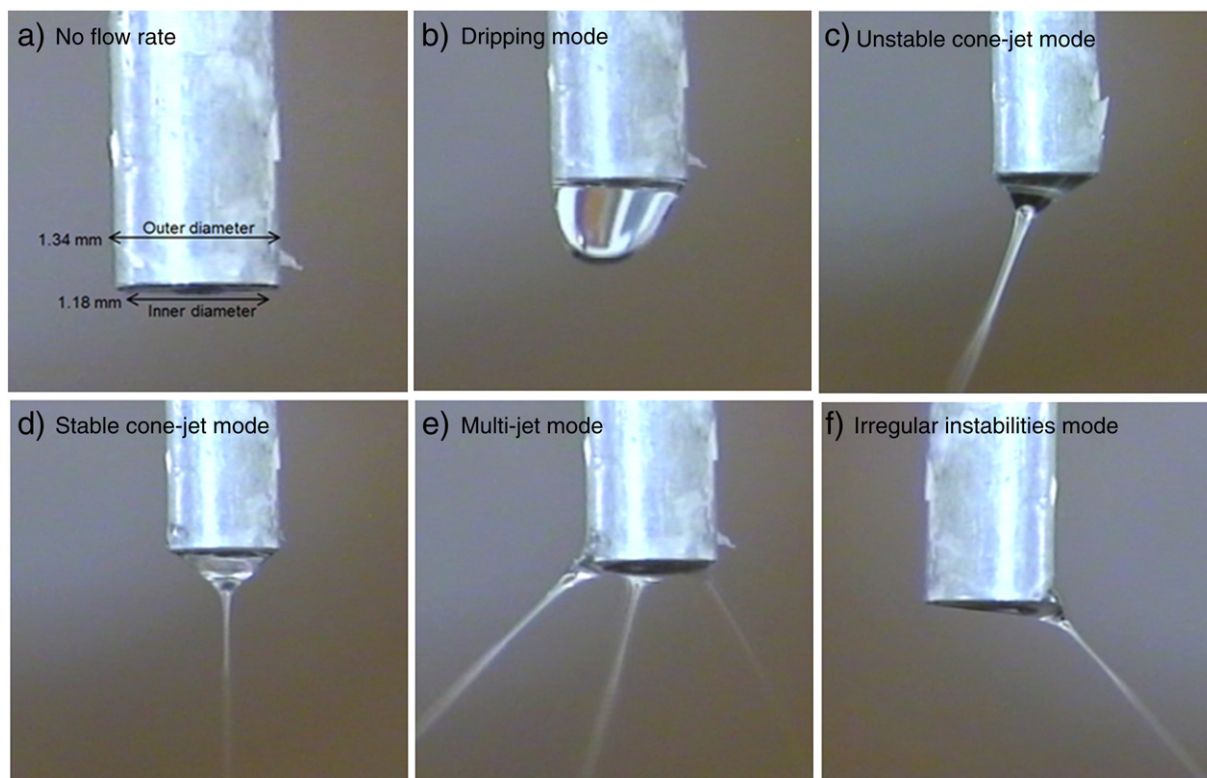
Fig. 2. Geometrical features a) needle size and jet modes: b) dripping, c) unstable cone-jet, d) stable cone-jet, e) multi-jet and f) irregular instabilities.

the operating parameters. Dripping mode (Fig. 2b), unstable cone-jet mode (Fig. 2c), stable cone-jet mode (Fig. 2d), multi-jet mode (Fig. 2e) and irregular instabilities mode (Fig. 2f) were observed. The stable cone-jet mode was the preferred atomization mode for collection of spheres, because it continuously generated near-monodisperse spherical particles [44–46]. A stable cone jet can be achieved when the applied voltage uniformly overcomes the surface tension of the liquid in all directions. This is influenced by the electrical conductivity and surface tension of the liquid [45]. Hence, depending on the inherent properties of the solution under EHDA, stable cone-jets can be obtained at different flow rates by tuning the applied voltage to a specific range, which is further illustrated in the later discussions (Table 1, Figs. 4–7). To obtain uniform and near-monodisperse microspheres for the formation of a polymeric scaffold, all the samples were collected under a stable cone-jet mode. An unstable cone-jet (Fig. 2c) was observed when the range of voltage is lower than the specific range of voltage for a particular material to obtain the stable cone-jet; whereas multi-jet mode (Fig. 2e) and irregular instabilities mode (Fig. 2f) occur when the applied voltage is greater than this specific range of stable cone-jet voltage for a particular material.

3.2. Stable cone-jet formation, microsphere morphology and parametric mapping

Table 1 provides the characterisation results of the polymer solutions. Here PCL (45) and PCL (10) are abbreviations for the two PCL types used. The four physical properties of viscosity, electrical conductivity, dielectric constant and surface tension determine the behaviour of solution in response to an applied electric field. With an increasing polymer solution concentration, surface tension and viscosity increased, whereas the electrical conductivity decreased. These changes in the solution properties due to changes in the polymer concentration led to different EHDA jetting modes, and the

ranges of flow rate and the applied voltage to achieve the stable cone-jet change for each solution.

An increase in the polymer concentration of the solution corresponds to an increase in the viscosity as well as the surface tension of the liquid (Table 1). This meant a stronger electrostatic force was required to form the stable cone-jet for a solution of a higher polymer concentration. For example, PLGA/DMF and PLGA/DMAc solutions had higher viscosity and surface tension compared with PCL/toluene and PLGA/DMC solutions, and higher applied voltages were found to be necessary to obtain cone-jetting during EHDA. However, in the case of PCL solutions, in particular PCL45000, due to the very low electrical conductivity and dielectric constant of the toluene, a much higher applied voltage was required to facilitate the stable cone-jet formation (Fig. 5).

Fig. 3 shows micrographs of the samples generated from EHDA of various polymer solutions. Placing the microspheres on the glass slides results in loss of their spherical shape, and the products appears concave (flattened) under the optical microscope. This is because the polymer particles collected in the vials are not fully solidified as the melting point of the water is well above to that of the solvents used in this study [47,48]. Therefore, diameters recorded show a 10–15% higher value compared with the actual diameter of the microspheres produced and stored in the vials. Polymeric spheres with smaller diameters were obtained from PLGA solutions in DMAc and DMF (Fig. 3a i–iii), compared with those collected from both PCL10000 and PCL45000 dissolved in toluene (Fig. 3b i–iii), and PLGA in DMC. Particles obtained from EHDA of PLGA solutions in DMF, DMAc and DMC showed spherical shapes with smooth surface (Fig. 3a & c); whereas PCL microspheres showed coarse surface morphology (Fig. 3b). It should also be noted that the electrohydrodynamic jetting can be used to generate flat surface particles which have their own significant biomedical applications [49].

Polymer solutions (PLGA or PCL, in different solvents) have different inherent physical properties, which required different ranges of

Table 1
Characterisation results of the polymer solutions and solvents.

Solution	Viscosity (mPa s)	Surface tension (mN m^{-1})	Conductivity/10^{-4} (Sm^{-1})	pH	Density/10^3 (kg m^{-3})	Dielectric constant
5% PLGA in DMAc	4.5	37.5	1.8	7.5	0.90	–
10% PLGA in DMAc	6.1	39.1	1.1	7.5	1.0	–
20% PLGA in DMAc	8.9	55.6	0.90	7.5	1.0	–
5% PLGA in DMF	4.0	36.9	2.0	7.5	0.90	–
10% PLGA in DMF	5.2	24.9	1.3	7.5	1.0	–
20% PLGA in DMF	8.5	49.1	1.0	7.5	1.0	–
5% PLGA in DMC	3.7	28.5	0.20	4.5	1.1	–
10% PLGA in DMC	6.7	32.8	0.10	4.0	1.1	–
15% PLGA in DMC	15	34.4	0.10	3.5	1.1	–
5%PCL (45) in toluene	3.0	26.5	–	7.0	0.90	–
7% PCL (45) in toluene	6.7	28.0	–	7.0	1.0	–
10% PCL (45) in toluene	11	28.5	–	7.0	1.1	–
15% PCL (10) in toluene	6.0	28.4	–	7.0	0.90	–
20% PCL (10) in toluene	16	29.6	–	7.0	0.90	–
25% PCL (10) in toluene	25	32.8	–	7.0	0.90	–
DMF	0.90	37.1	2.0	7.5	0.90	37.8 [47]
DMAc	2.1	36.1	1.8	4.0	0.90	36.7 [47]
DMC	0.60	30.7	0.20	6.0	1.1	3.1 [48]
Toluene	0.60	28.5	8.0×10^{-10} [47]	7.0	0.90	2.38 [47]

applied voltage and flow rate in order to obtain the stable cone-jet for generation of microspheres. Solutions with different concentrations will generate different size distribution ranges regardless of the flow rate and applied voltage. In general, when flow rate is increased from 20 µl min^{-1} to 450 µl min^{-1} during single-nozzle EHDA processing, the readiness of the liquid to form the cone-jet decreased

and the sphere diameter distribution broadened with increasing flow rate for all polymer solutions at all the concentrations tested.

For each liquid, a critical flow rate was observed, above which, the liquids showed difficulty achieving stable cone-jets, or the cone-jets remained stable for less than 5 min, and intermittent instability occurred. This critical flow rate was observed at 60 µl min^{-1} for 5%w/w PLGA in
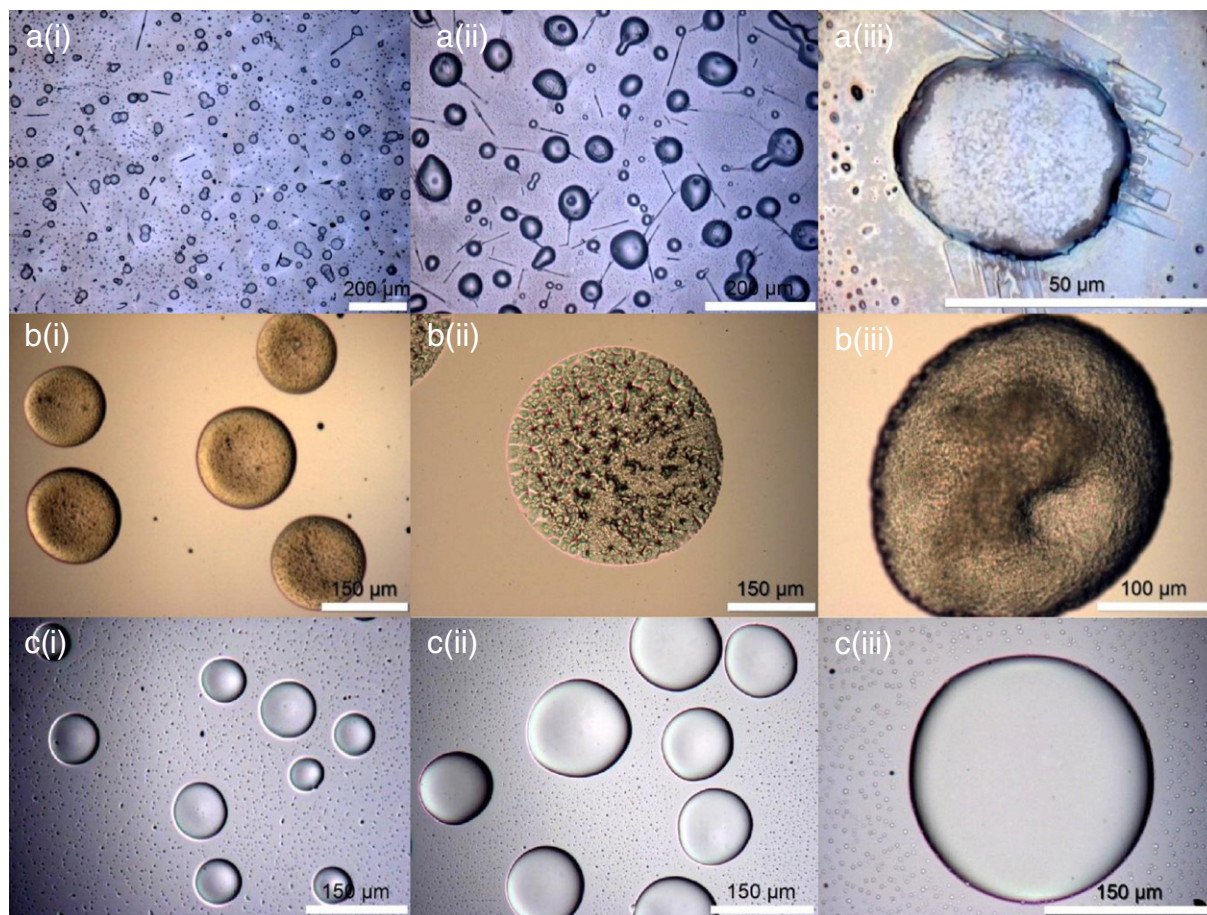

Fig. 3. Size distribution of microspheres pipetted onto glass slide a(i) 10% PLGA in DMAc at 150 µl min^{-1} (magnified ×5), a(ii) 20% PLGA in DMF at 200 µl min^{-1} (magnified ×5), a(iii) 20% PLGA dissolved in DMAc at 150 µl min^{-1} (magnified ×450), b(i) 5% PCL 45000 in toluene at 25 µl min^{-1} (magnified ×5), b(ii) 10% PCL 45000 in toluene at 30 µl min^{-1} (magnified ×50), b(iii) 20% PCL10000 in toluene at 50 µl min^{-1} (magnified ×450), c(i) 5% PLGA in DMC at 150 µl min^{-1} (magnified ×5), c(ii) 10% PLGA in DMC at 250 µl min^{-1} (magnified ×5), and c(iii) 10% PLGA in DMC at 300 µl min^{-1} (magnified ×450).

DMF and DMAc, 120 µl min^{-1} for 10%w/w and 15%w/w PLGA in DMF and DMAc, at 45 µl min^{-1} for PCL in toluene, and 400 µl min^{-1} for PLGA in DMC. This is attributed to the solvent's properties, in particular, the collective influence of electrical conductivity and dielectric constant (Table 1). Conductivity showed a stronger influence on stable cone-jet formation than dielectric constant of the solvents; whereas dielectric constant showed a greater influence on the product diameter distribution profile than the conductivity of the solvent. Polymer solutions in
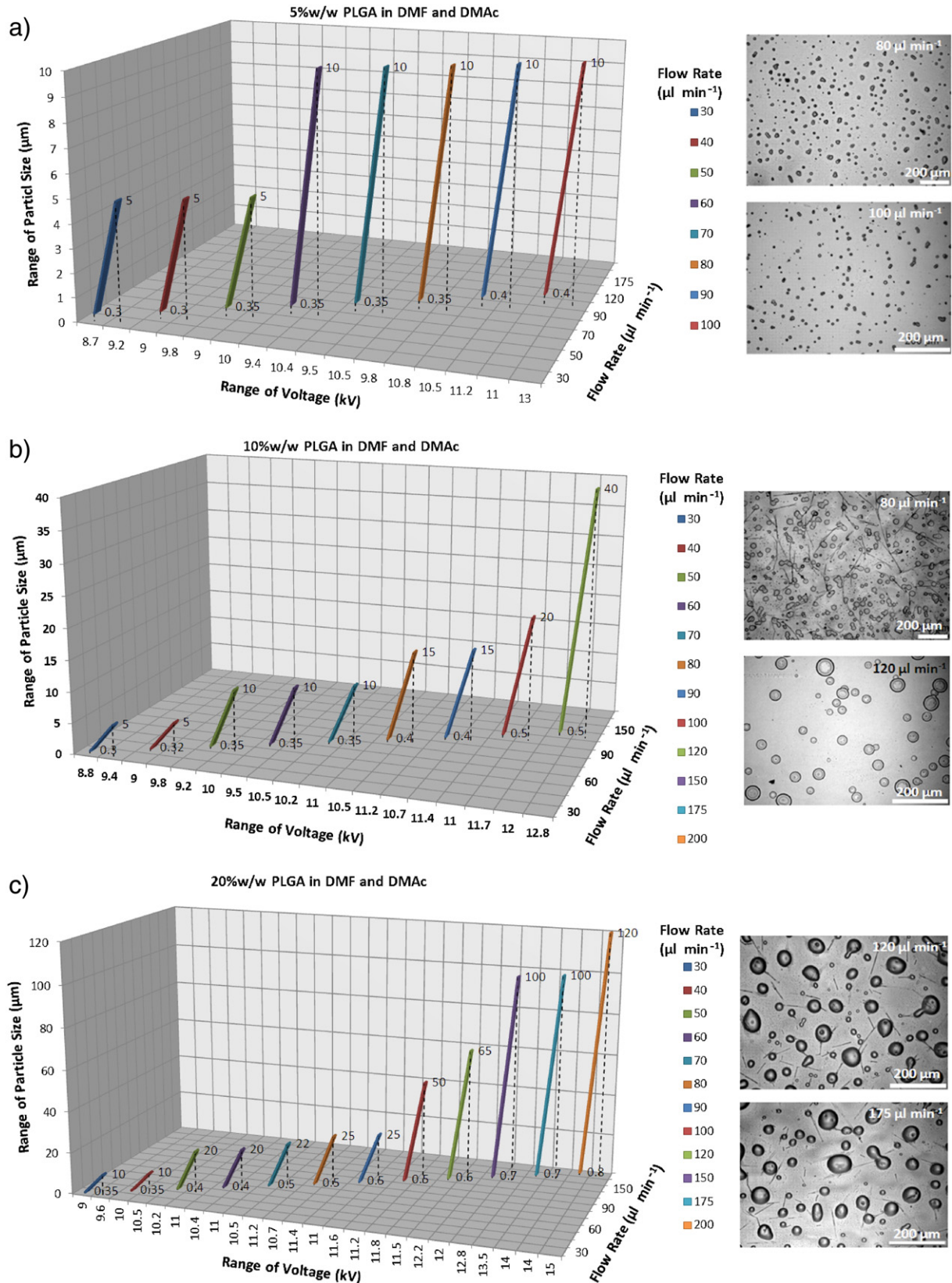

Fig. 4. Parametric mode mapping of microspheres pipetted onto glass slide (a) 5%w/w PLGA in DMAc/DMF; (b) 10%w/w PLGA in DMAc/DMF; and (c) 20%w/w PLGA in DMAc/DMF.

solvents with very low electrical conductivity (σ) (Table 1) as well as dielectric constant (ε) such as toluene (lowest conductivity among all solvents used in this work, $\sigma = 8.0 \times 10^{-14}$ S m^{-1} at 20 °C [47]; $\varepsilon = 2.38$ at 20 °C [47]) showed difficulty achieving stable cone-jets at flow rates above 45 μl min^{-1}, the lowest critical flow rate value among all samples.

Furthermore, polymer solutions in solvents with higher σ and ε, such as DMF ($\sigma = 2.0 \times 10^{-4}$ S m^{-1} at 20 °C (Table 1); $\varepsilon = 37.8$ at 20 °C [47]) and DMAc ($\sigma = 1.8 \times 10^{-4}$ S m^{-1} (Table 1); $\varepsilon = 36.7$ at

20 °C [47]) showed difficulty achieving stable cone-jets at relatively low flow rates (above 120 μl min^{-1}, Fig. 4). PLGA in DMF and DMAc also generated smaller products with a broad range of diameter distribution (Figs. 3a, 4) compared to that of PCL in toluene (Figs. 3b and 5–6). The diameter range distribution of PLGA in DMAc and DMF overlapped each other significantly and were presented together in Fig. 4. This is due to the similarity between the solvent properties of DMAc and DMF and their comparable
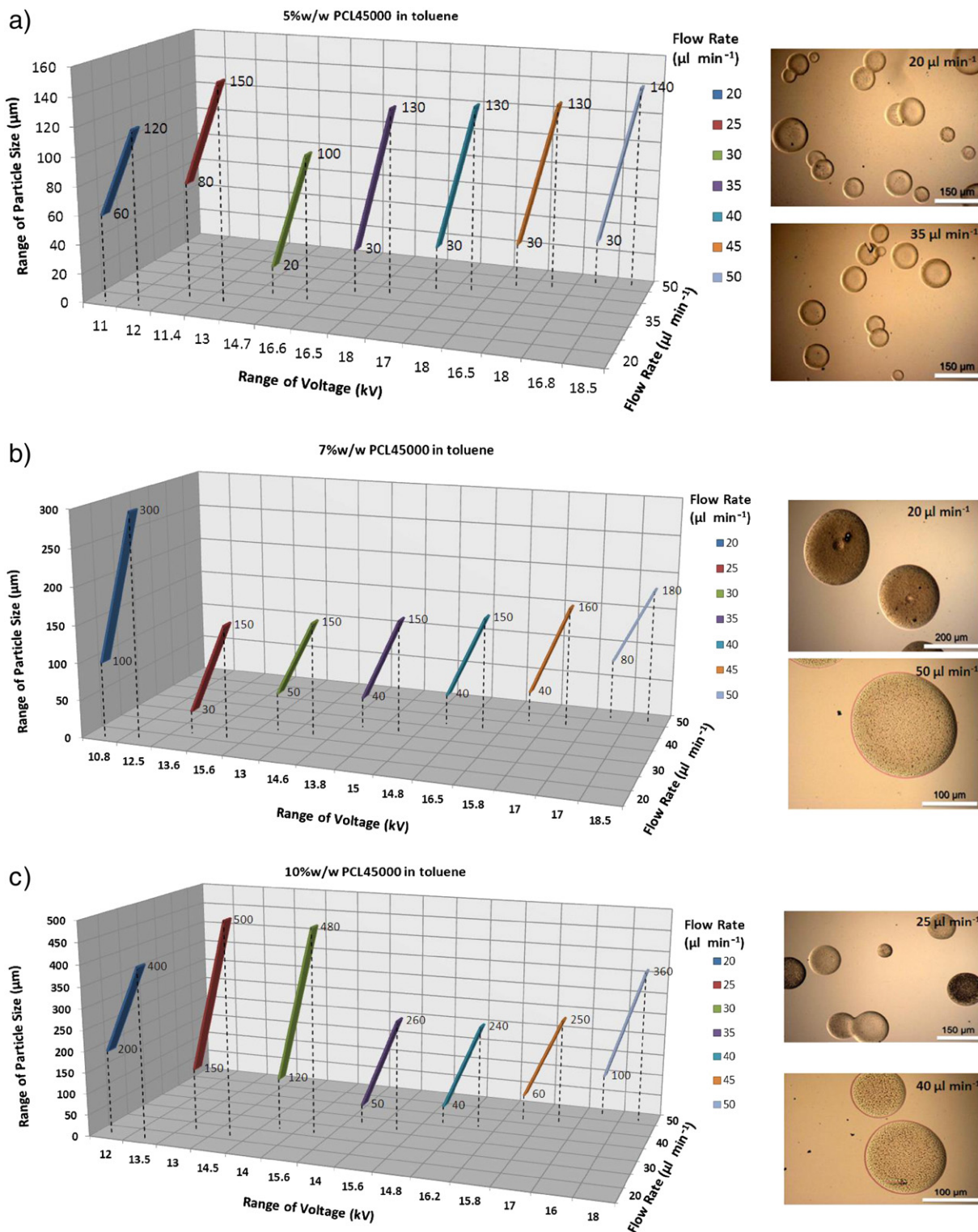

Fig. 5. Parametric mode mapping of microspheres pipetted onto glass slide (a) 5%w/w PCL45000 in toluene; (b) 7%w/w PCL45000 in toluene; and (c) 10%w/w PCL 45000 in toluene.
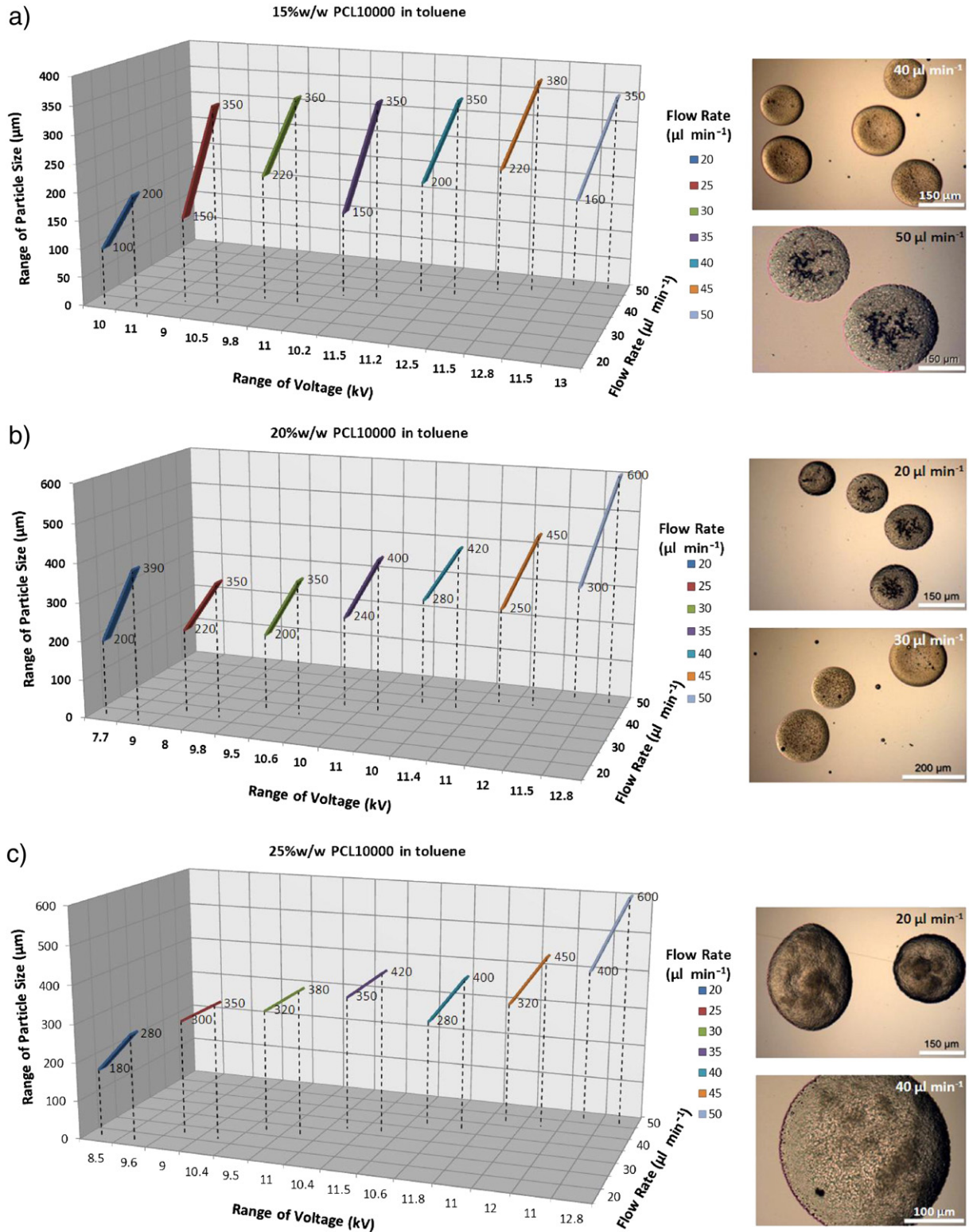
Fig. 6. Parametric mode mapping of microspheres pipetted onto glass slide (a) 15%w/w PCL10000 in toluene; (b) 20%w/w PCL10000 in toluene; and (c) 25%w/w PCL10000 in toluene.

strengths in all three of the major inter-molecular interactions of DMF and DMAc [48].

In comparison, DMC has moderate electrical conductivity and relatively low dielectric constant values ($\sigma = 0.2 \times 10^{-4}$ S m^{-1}; $\varepsilon = 3.1$ [48]) and PLGA solutions in DMC generated larger products in the desirable micrometre range (150–300 μm) for scaffold formation in this work (Fig. 7). Samples obtained from PLGA in DMF and DMAc

solutions showed higher polydispersity, with a lower minimum diameter range (0.3–0.35 μm, 5–20%w/w PLGA/DMF and PLGA/DMAc, Fig. 4) than samples obtained from both PCL polymers (PCL10000 and PCL45000) in toluene (>60 μm, Figs. 5 and 6) and PLGA in DMC (>80 μm, Fig. 7). The generation of significantly smaller products was attributed to the higher dielectric constant values of DMF and DMAc compared with that of toluene and DMC, based on an
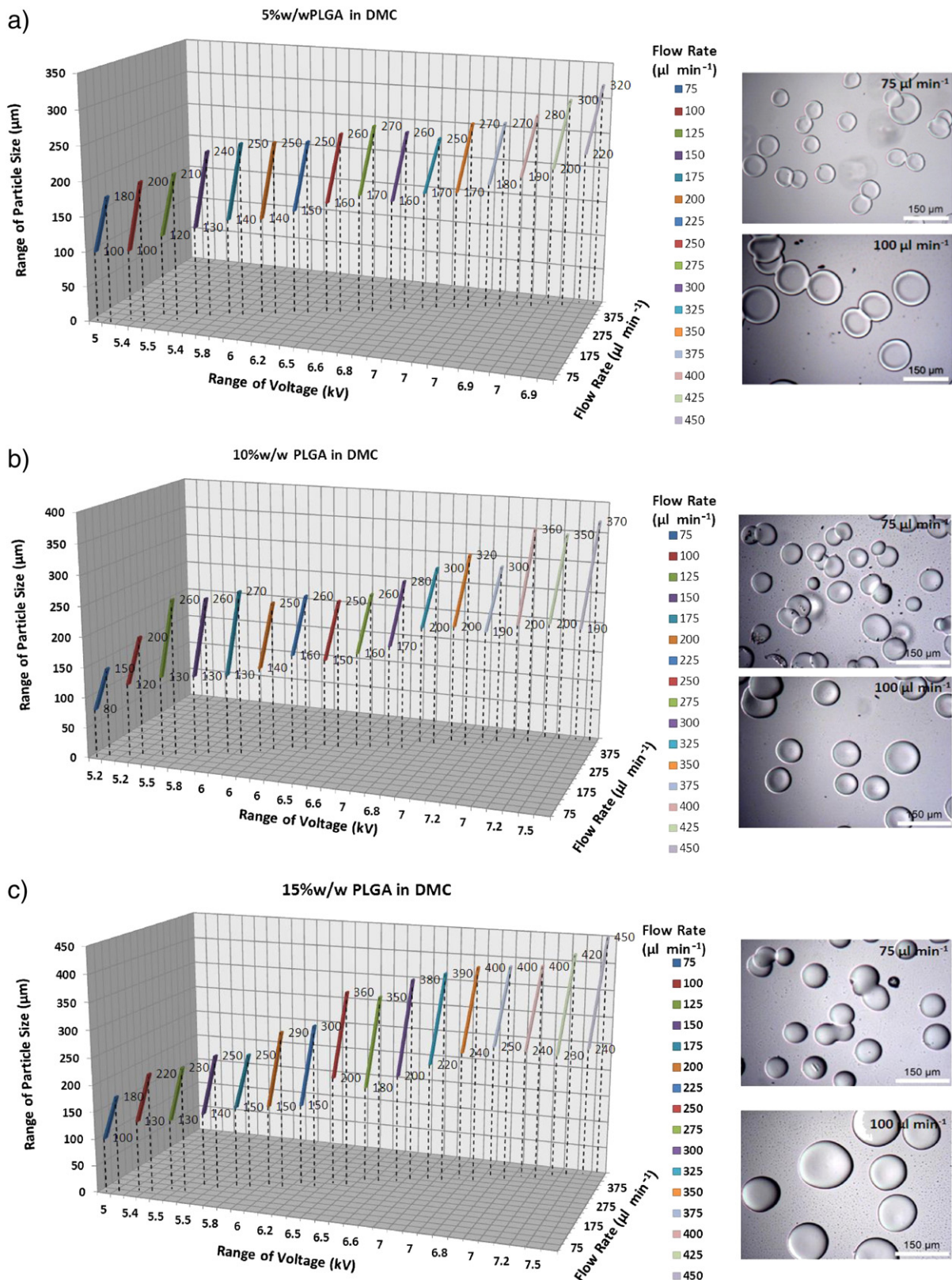
Fig. 7. Parametric mode mapping of microspheres pipetted onto glass slide (a) 5%w/w PLGA in DMC; (b) 10%w/w PLGA in DMC; and (c) 15%w/w PLGA in DMC.

earlier study mapping the influence of solvent dielectric constant on the diameters of EHDA products [50].

Hence, the EHDA parametric mode map for each liquid material under stable jetting was based on a minimum flow rate of 20–30 µl min^{-1} and the maximum flow rate used for each material

was adjusted based on the material's observed critical flow rate value for stable cone-jet formation. The range of flow rates was varied from 30 to a maximum of 200 µl min^{-1} for PLGA solutions in DMAc and DMF; between 20 and 50 µl min^{-1} for PCL solutions; and between 75 and 450 µl min^{-1} for PLGA solutions in DMC (Figs. 4–7). The

parametric maps showed that all solutions of PLGA in DMAc (5, 10, 15%w/w), PLGA in DMF (5, 10, 15%w/w) and PCL45000 in toluene (5, 7 and 10%w/w) did not achieve microspheres of diameters within the desirable range of 150–300 μm (Figs. 4 & 6); whereas 25%w/w PCL10000 under the cone-jet condition at 20 μl min⁻¹ flow rate generated desirable microsphere diameters ranging from 180 to 280 μm (Fig. 5c); and PLGA solutions in DMC were able to achieve microspheres within the desirable diameter requirement for a broad range of processing parameters for all of the concentrations tested (5, 10, 15%w/w, Fig. 7).

3.3. Microsphere-scaffold structure

PCL in toluene (low σ, low ε) was investigated together with PLGA in DMC (moderate σ, low ε), PLGA in DMF and DMAc (high σ, high ε) to understand the influence of liquid properties on the generation of

Fig. 8. a) Optical micrographs of microspheres formed into the scaffold structure with 15%w/w PLGA/DMC, magnified ×50; b) SEM image of one microsphere in the scaffold structure, magnified ×450; and c) SEM image of a microsphere showing the groove pattern on the surface, magnified ×800.

large microspheres by EHDA. PCL in toluene was not investigated for the development of a microsphere-scaffold for clinical use in treatments such as chronic wounds. Although PCL in toluene generated polymer microspheres with the desirable diameter distribution profile as well as surface porosity for the development of a scaffold structure, this material was tested purely for a proof-of-concept purpose to understand the solvent property as discussed in Section 3.2. Toluene's toxicity makes it unsuitable for clinical use, side effects include symptoms such as tiredness, confusion, weakness, memory loss, nausea, loss of appetite, and hearing and colour vision loss [51]. Although solutions of PLGA in DMF or DMAc are widely acceptable for clinical use at low dosage [52], these did not generate near-monodisperse spheres with the required diameter distribution in EHDA due the reasons discussed earlier in Section 3.2 (Fig. 3a(iii)). Therefore, this material was not investigated further for the development of a microsphere-scaffold structure.

PLGA in DMC, a low toxicity solvent [53], produced the appropriate size of microspheres over a broad range of processing parameters for all of the concentrations tested (5, 10, 15%w/w, Fig. 7). The microspheres selected for the formation of a scaffold structure was fabricated using 15% PLGA in DMC at a flow rate of 225 μl min⁻¹ and an applied voltage ranging 6.0–7.2 kV (Fig. 8). Collection in liquid nitrogen followed by freeze drying generated solid spherical particles with surface porosity (Fig. 8c). This is attributed to thermally induced liquid–liquid phase separation of the polymer solution upon quenching in liquid nitrogen and subsequent sublimation of the solvent via freeze drying of the sample. Combination of TIPS and freeze-dryer for formation of porous surface resulted in soft aggregation of microspheres that the subsequent separation of the products did not cause any morphological damage to the microsphere structure. Formation of these porous microspheres into scaffold will be further investigated for drug encapsulation.
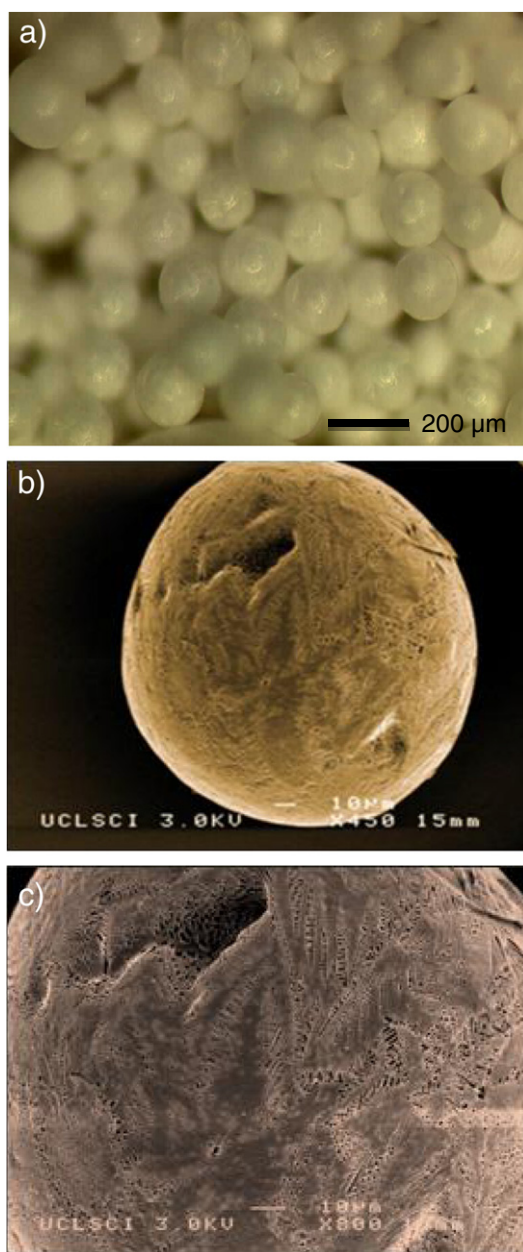
4. Conclusions

This study reports a new method for the fabrication of microspheres with surface porosity and a narrow diameter range (targeting 150–300 μm). Here we demonstrate the control of polymer microsphere size distribution within the required micrometre range using the technique. A detailed investigation was carried out on the diameter distribution profiles of the products obtained over a broad range of key processing parameters (flow rate and applied electric field strength) for 5 polymer solutions (PLGA in DMF, PLGA in DMAc, PLGA in DMC, PCL10000 in toluene and PCL45000 in toluene). The data were presented as parametric mode mappings to enable robust EHDA production and the appropriate selection of the required average diameter and diameter distribution of microspheres. Data analysis relating solvent properties of the various solutions and their respective parametric mode mappings revealed that electrical conductivity showed a stronger influence on stable cone-jet formation than dielectric constant of the solvents; whereas dielectric constant showed a greater influence on the spherical particle diameter distribution profile than the conductivity of the solvent. Electrospray of PLGA in DMC, a low toxicity solvent with moderate conductivity and low dielectric constant, followed by freeze drying generated porous microspheres within the required diameter range of 150–300 μm, suitable for use in a minimally invasive, in situ forming scaffold.

References

[1] C. Dai, B. Wang, H. Zhao, Colloids Surf. B Biointerfaces 41 (2005) 117–120.
[2] N. Qui, H. Yin, B. Ji, N. Klauke, A. Glidle, Y. Zhang, H. Song, L. Cai, L. Ma, G. Wang, L. Chen, W. Wang, Mater. Sci. Eng. C 32 (2012) 2634–2640.
[3] V.R. Sinha, A. Trehan, J. Control. Release 90 (2003) 261–280.
[4] M. Enayati, Z. Ahmad, E. Stride, M. Edirisinghe, J. R. Soc. Interface 7 (2009) 667–675.
[5] R. Arshady, Polym. Eng. Sci. 29 (1989) 1746–1758.
[6] M. Okubo, Y. Konishi, H. Minami, Colloid Polym. Sci. 276 (1998) 638–642.
[7] Y. Zhang, Y. Guan, S. Yang, J. Xu, C.C. Han, Adv. Mater. 15 (2003) 832–835.
[8] M. Fujiwara, K. Shiokawa, T. Kubota, Mater. Sci. Eng. C 32 (2012) 2484–2490.
[9] X. Luan, M. Skupin, J. Siepmann, R. Bodmeier, Int. J. Pharm. 324 (2006) 168–175.
[10] A. Rasiel, T. Sheskin, L. Bergelson, A.J. Domb, Adv. Technol. 13 (2002) 127–136.
[11] J. Vandervoort, A. Ludwig, Int. J. Pharm. 238 (2002) 77–92.
[12] D.S. Kohane, J.Y. Tse, Y. Yeo, R. Padera, M. Shubina, R. Langer, J. Biomed. Mater. Res. A 77A (2006) 351–361.
[13] K.G. Carrasquillo, A.M. Stanley, J.C. Aponte-Carro, P. De Jesus, H.R. Costantino, C.J. Bosques, K. Griebenow, J. Control. Release 76 (2001) 199–208.
[14] R. Ahmadi, N. Mordan, A. Forbes, R.M. Day, Acta Biomater. 7 (2011) 1542–1549.
[15] J.J. Blaker, J. Pratten, D. Ready, J.C. Knowles, A. Forbes, R.M. Day, J. Aliment. Pharmacol. Ther. 28 (2008) 614–622.
[16] H. Keshaw, N. Thapar, A.J. Burns, N. Mordan, J.C. Knowles, A. Forbes, R.M. Day, Acta Biomater. 6 (2010) 1158–1166.
[17] J.J. Blakcer, J.C. Knowles, R.M. Day, Acta Biomater. 4 (2008) 264–272.
[18] K.S. Foong, R. Patel, A. Forbes, R.M. Day, Tissue Eng. (2010) 1–10.
[19] M. Murillo, C. Gamazo, M. Goni, J. Irache, M. Blanco-Prieto, Int. J. Pharm. 242 (2002) 341–344.
[20] P.A. Burke, L.A. Klumb, J.D. Herberger, X.C. Nguyen, R.A. Harrell, M. Zordich, Pharm. Res. 21 (2004) 500–506.
[21] M.J. Blanco-Prieto, M.A. Campanero, K. Besseghir, F. Heimgatner, B. Gander, J. Control. Release 96 (2004) 437–448.
[22] L. Ding, T. Lee, C. Wang, J. Control. Release 102 (2005) 395–413.
[23] R. Pareta, M. Edirisinghe, J. R. Soc. Interface 3 (2006) 573–582.
[24] Y. Xu, M. Skotak, M. Hanna, J. Microencapsul. 23 (2006) 69–78.
[25] Y. Wu, J.A. Mackay, J.R. Mcdaniel, A. Chilkoti, R.L. Clark, Biomacromolecules 10 (2009) 19–24.
[26] C.J. Luo, S. Loh, E. Stride, M. Edirisinghe, Food Bioprocess Technol. 5 (2012) 2285–2300.
[27] J. Xie, W.N.L. Lee, C. Wang, J. Colloid Interface Sci. 317 (2008) 469–476.
[28] Y.L. Yeo, Z. Gagnon, H.C. Chang, Biomaterials 26 (2005) 6122–6128.
[29] Z. Ahmad, H.B. Zhang, U. Farook, M. Edirisinghe, E. Stride, P. Colombo, J. R. Soc. Interface 27 (2008) 1255–1261.
[30] S.R. Samarasinghe, K. Balasubramanian, M.J. Edirisinghe, J. Mater. Sci. Mater. Electron. 19 (2008) 33–38.
[31] K.W. Burton, M. Shameem, B.C. Thanoo, P.P. Deluca, J. Biomater. Sci. 11 (2005) 715–729.
[32] M. Ko̎rber, R. Bodmeier, Eur. J. Pharm. Sci. 35 (2008) 283–292.
[33] G. Chen, Degradation Behaviour of Aliphatic Biodegradable Polyesters, Plastics Research Online, Society of Plastics Engineers, 2010.
[34] H.B. Ravirapu, H. Lee, P.P. DeLuca, Pharm. Dev. Technol. 5 (2000) 287–296.
[35] A. Jain, Biomaterials 21 (2000) 2475–2490.
[36] C. Marambio-Jones, E.M.V. Hoek, J. Nanopart. Res. 12 (2010) 1531–1551.
[37] W.L. Monsky, D. Fukumura, T. Gohongi, M. Ancukiewcz, H.A. Weich, V.P. Torchilin, F. Yuan, R.K. Jain, Cancer Res. 59 (1999) 4129–4135.
[38] D.A. Edwards, J. Hanes, G. Caponetti, J. Hrkach, A. Ben-Jebria, M.L. Eskew, J. Mintzes, D. Deaver, N. Lotan, R. Langer, Science 276 (1997) 1868–1871.
[39] R. Sinha, G.J. Kim, S. Nie, D.M. Shin, Mol. Cancer Ther. 5 (2006) 1909–1917.
[40] H. Zhang, Y. Lu, G. Zhang, S. Gao, D. Sun, Y. Zhong, Int. J. Pharm. 351 (2008) 244–249.
[41] S. Mitragotri, J. Lahann, Nat. Mater. 8 (2009) 15–23.
[42] C.J. Luo, S.D. Stoyanov, E. Stride, E. Pelan, M. Edirisinghe, Chem. Soc. Rev. 41 (2012) 4708–4735.
[43] A. Jaworek, A. Krupa, J. Aerosol Sci. 30 (1999) 873–893.
[44] B.G. Amsden, M.F.A. Gossen, J. Control. Release 43 (1997) 183–196.
[45] J.M. Lopez-Herrera, A. Barrero, A. Lopez, I.G. Loscertales, M. Marqueze, J. Aerosol Sci. 34 (2003) 535–552.
[46] M. Cloupeau, B. Prunet-Foch, J. Electrost. 25 (1990) 165–184.
[47] I.M. Smallwood, Handbook of Organic Solvent Properties, Arnold, London, UK, 1996.
[48] ELECS Co., Ltd., Dimethyl Carbonate (DMC), http://www.elecs-korea.com/board/file/1135059729.pdf 2012, (last accessed 12th July 2012).
[49] K.J. Lee, J. Yoon, J. Lahann, Curr. Opin. Colloid Interface Sci. 16 (2011) 195–202.
[50] C.J. Luo, E. Stride, M. Edirisinghe, Macromolecules 45 (2012) 4669–4680.
[51] K. Foxall, Toluene toxicological overview, Health protection agency, http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1194947395545(2007), (Last accessed 23rd October 2012).
[52] New Jersey department health senior services, Right to know hazerdious substance fact sheet, Dimethylformamide, http://nj.gov/health/eoh/rtkweb/documents/fs/0759.pdf 2011, (last accessed 23rd October 2012).
[53] P. Tundo, M. Selva, Acc. Chem. Res. 35 (2002) 706–716.

Hanif Ghanbar graduated from the University of Surrey, UK, in Medical Engineering, in 2008. Subsequently, he obtained his master of science in Biomedical Engineering from the same university. Currently, he is a PhD student in the Department of Mechanical Engineering at University College London working in the biomaterials processing laboratory on drug delivery systems and chronic wound filling/healing, a joint project with the Division of Medicine in UCL.

Poonam Kaushik Bakhshi, is a post-doctoral researcher in the Biomaterials Laboratory, Mechanical Engineering Department in UCL. She joined UCL in 2009 as visiting researcher prior to which she was working as visiting researcher in the Royal Botanical Garden(Kew, UK). She attained her doctorate from the Indian Institute of Technology, Delhi, in the domain of formulation technology. She also worked as research fellow in Kraft Food Inc., USA, on a sponsored project on development of food formulations. Her research interest focuses on encapsulation technology and controlled release formulations for food, pharmaceutical and cosmetic applications.

C. J. Luo holds a BSc in Biochemistry and a PhD in Biomedical Engineering from UCL. She is a multidisciplinary scientist with wide-ranging knowledge and experience in production of polymeric nanofibres, nanospheres and bubbles for bioengineering, marine engineering, healthcare and food applications. Her research interests centre on processing and forming of sustainable biocompatible polymers for healthcare and environmental applications, emphasising technology transfer and translational research between life and physical sciences. Until 2012 she was a post-doctoral researcher in the Biomaterial Laboratory in the Department of Mechanical Engineering in UCL.

Richard Day is a Senior Lecturer in Biomedicine in the Division of Medicine at UCL. He leads a translational research programme on the application of biomedical engineering. His group's work includes developing novel biomaterials for tissue repair and methods for improving tissue function.

Mohan Edirisinghe DSc holds the Bonfield Chair of Biomaterials in the Department of Mechanical Engineering at University College London. He has published over 300 journal papers and his most recent research is on advanced jet techniques for the preparation of novel biostructures including microbubbles and drug delivery capsules. He has been awarded many prizes for his research including the 2012 UK Biomaterials Society President's prize.