Changing the Size and Surface Roughness of Polymer Nanospheres Formed Using a Microfluidic Technique

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Solid polymer nanospheres with contrasting size and surface roughness were prepared using a V-shaped microfluidic junction device. Polymethysilsesquioxane (PMSQ) polymer dissolved separately in either methanol, or ethanol, or propanol or butanol was used, and the nanospheres produced were collected in distilled water at two different temperatures (23°C and 100°C). Each polymer solution together with a volatile liquid, perfluorohexane, was fed into the inlet channels of the microfluidic device. The process of nanosphere generation was recorded by high-speed camera imaging. Only PMSQ solutions of ethanol and propanol generated well-defined nanospheres. The influence of the solvents and the nanosphere collection temperature on nanosphere size distribution and surface roughness was assessed using scanning electron microscopy, which showed that solvent selection was crucial in tailoring the size distribution of the nanospheres and that the nanospheres collected at 100°C had a noticeably rougher surface. The temperature also helped to vary the size distribution of the nanospheres. Nanospheres containing Evans blue dye were also prepared, and those with a rough surface exhibited a very different dye release profile compared with those having a smooth surface. In fact, the release profile changes due to a size differential can be largely compensated for by having a rough surface.

INTRODUCTION

Considerable attention has been paid to the microfluidic preparation of polymer nanospheres for drug delivery because of their ability to control drug release.[1–3] The advantages of using polymer nanospheres as drug delivery vehicles are primarily dependent on their favorable physical characteristics such as low density combined with high surface area.[4,5] Several techniques have been used to prepare polymer nanospheres and these include solvent evaporation, emulsion polymerization, electrohydrodynamic atomization, coacervation, nanoprecipitation and microfluidics.[6–12] Among these, microfluidic techniques including a V-shaped microfluidic junction device have enjoyed considerable success and interest in recent years because they are simple, cost-effective and enable the generation of relatively near-monodisperse nanospheres, opening up feasibility for preparing tailored commercially viable drug delivery systems.[13–16] In particular, polymer nanospheres can

deliver improvement in the bioavailibity of drugs via modification of dissolution rates.[17–20]

Although various polymers can be used to make nanospheres for drug delivery systems *in vitro*, those associated with human administration are limited to a few types.[21–24] Polymethylsilsesquioxane (PMSQ) which is a stable and biocompatible polymer is one of them.[25–27] There are several parameters such as polymer concentration, capillary size and device geometry which can influence the morphology, sphere size and size distribution in the microfluidic preparation process and many studies have been carried on the factors which influence the release profile of a drug present in polymer nanospheres.[22,28–34] However, the effect of solvents used in polymer solution preparation and the temperature at which the nanospheres produced are collected has received less attention and forms the basis of the present work.

Previously, we investigated the effect of polymer concentration and process parameters such as flow rates of solutions and the use of a volatile

liquid on the polymer nanosphere formation using a V-shaped microfluidic junction device.[35] In addition, we reported the usefulness of the V-shaped microfluidic junction device as a method of improving the bioavailability of drug, itraconazole.[14] Here, we attempted to prepare PMSQ nanospheres using various alcohols, i.e. methanol, ethanol, propanol and butanol, and demonstrated how solvent selection and the temperature at which the nanosphere produced are collected in water affects their size and surface features. In addition, using a dye, we examined how size and surface of the nanospheres produced affected their release profile.

MATERIALS AND METHODS

Materials

Polymethylsilsesquioxane (PMSQ) polymer was obtained from Wacker Chemie, Burghausen, Germany (average molecular weight: 7465 g mol^{-1}). Ethanol (98%), methanol (98%), butanol (95%), propanol (95%) and the dye Evans blue (dye content 85%) were purchased from SigmaAldrich (Poole, UK). Liquid perfluorohexane (PFH) was supplied by F2 Chemicals, Lea, UK (purity grade, 99.7–100%; density 1710 kg m^{-1}).

Methods

Solution Preparation

Amounts of 5, 10, 20, 30, 40 and 50 wt.% of polymer were dissolved in one of four different alcohols (methanol, ethanol, propanol and butanol). In addition, to investigate the release profile and encapsulation of active ingredient in the polymer nanospheres, a small amount of Evans blue dye dissolved in ethanol was used. Solutions were sealed and magnetically stirred in a conical flask for at least 1800 s at the ambient temperature ($\sim$23°C) until the polymer/dye was apparently fully dissolved.

Solution Characterization

The polymer solutions used were characterised by measuring their density, surface tension and viscosity. Density was measured using a standard 25-mL density bottle. Surface tension was measured using a tensiometer K9 (Kruss, Germany; standard Wilhelmy's plate method). Viscosity values were obtained using an Ostwald's U-tube viscometer and a VISCOEASY-L rotational viscometer (Schott Geräte, Germany). All experiments were performed at the ambient temperature ($\sim$23°C). Ethanol was
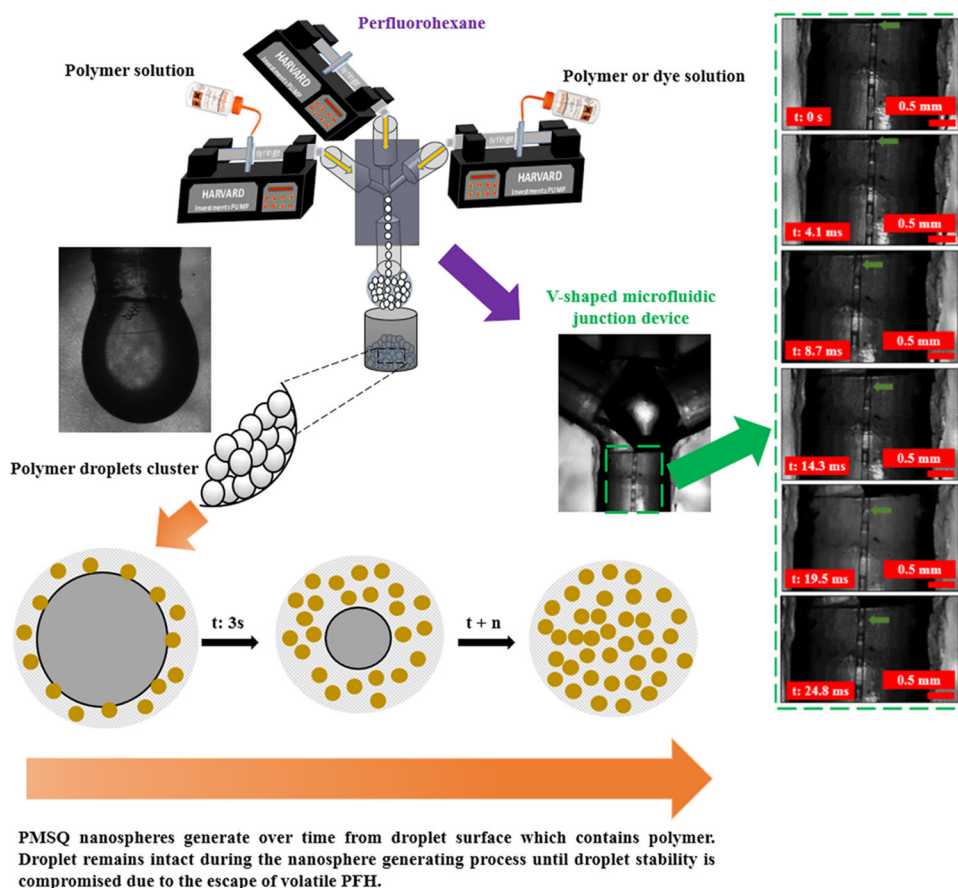

Fig. 1. Schematic illustration of preparation of polymer nanospheres using a V-shaped microfluidic junction device.

used as a calibration medium and a cleaning agent in all the experiments.

Microfluidic Device and Experimental Setup

A V-shaped microfluidic junction was used to prepare the polymer nanospheres. This device has been described in detail previously[14,35] and as shown in Fig. 1 comprises of four channels, two of which are for infusion of the polymer solutions and dye solution. The other placed alongside is for infusion of a gas or a volatile liquid and the remaining channel below the mixing area serves as the exit channel where droplets generated are collected. High-speed camera images showing details of nanosphere preparation are also shown in Fig. 1. Syringes filled with solutions and the volatile liquid were coupled to the V-shaped microfluidic junction device via calibrated high precision pumps to measure flow rate throughout the process, and are capable of delivering controlled infusion. The 30°-angled channels in the device is the infusion point of a known polymer solution, which can also be made to contain a small quantity of active ingredient (e.g. dye, drug). The selected polymer solutions were infused using 10-mL plastic syringes (Becton–Dickinson, Oxford, UK) using mechanical couplers and the rates of infusion were digitally controlled using Harvard pumps (PHD 4400; Apparatus, Edenbridge, UK) to deliver stable flow rates. Once processing conditions were optimised, droplets were generated at the junction of the device and collected as droplet clusters in insoluble media (distilled water) at the exit channel (Fig. 1). For electron microscopy investigations, droplet samples were collected in distilled water both at 23°C and at 100°C in a snapcap vial and allowed to generate polymer nanospheres. Subsequently, the nanopspheres produced were transferred to a glass slide.

High-Speed Imaging

High-speed images of droplets during the polymer nanospheres generating steps were captured using a Phantom V7 high-speed camera at 3082 frames per s with 1-ms delay and constant exposure time of 60 ms.

Characterisation of Polymer Nanospheres

Polymer nanospheres were examined using a scanning electron microscope (JEOL JSM 6301 F). Dried samples (48 h) were sputtered-coated with gold for 3 min prior to scanning electron microscopy at an accelerated voltage of 3–5 kV. ImageJ (1.47n; Wayne Rasband National Institute of Health, USA) image analysis software was used to assess approximately 200 polymer nanospheres.

Dye Release Study

In order to assess the encapsulation and release characteristics of PMSQ nanospheres, Evans blue dye (characteristic UV peak: 610 nm) solution was added to PMSQ nanospheres via V-shaped microfluidic junction device during preparation (see Fig. 1) before nanosphere collection in a snapcap vial (see "Microfluidic Device and Experimental Setup" section). Subsequently, nanosphere suspensions were centrifuged at 420 rpm for 30 min. The supernatant was removed and its absorbance was measured using a UV spectrometer (Lambda 35; Perkin Elmer, UK) to determine dye release from the PMSQ nanospheres. The UV spectrometer measurements were performed over a period of 1 h and this investigation was limited to nanospheres prepared using ethanol and propanol with a collection at two different temperatures (23°C and 100°C).

RESULTS AND DISCUSSION

Preparation Method

The experimental set up (Fig. 1) consists of immiscible liquids (PMSQ polymer solutions and PFH), a V-shaped microfluidic junction device, and collection liquid which was distilled water. Both polymer solutions and PFH are infused into the mixing area to generate droplets. Time-sequenced photographs of droplet formation were taken by a high-speed camera and they indicate that droplets generation takes 24.8 ms when both PMSQ solutions and PFH flowed in at 300 μL min^{-1} (Fig. 1). The resultant droplets moved down the outlet capillary to the channel exit, where they were gathered in water in which PMSQ is insoluble. A cluster of polymer nanospheres becomes evident when the collected groups of droplets make contact with the aqueous environment in the vial. A spontaneous high density of nanospheres appeared from bursting of the droplets much like in an explosion. Thus, the volatile liquid, PFH, evaporates and the PMSQ nanospheres shrink and adopt their morphology (Fig. 1). This is the solidification stage of polymer nanosphere formation in the collection liquid.

Influence of Solvents

Surface tension and viscosity are the two crucial physical properties which affect nanosphere generation.[36] These values are shown in Fig. 2. The solubility of PMSQ in alcohols is different and decreases as the molecular weight of the alcohol decreases.[32] The small amount of water in the alcohol is also important as PMSQ does not dissolve in water. As expected, both viscosity and surface tension values of all solutions show an increase with increasing amounts of PMSQ polymer. Both viscosity and surface tension can be used to predict the size of the polymer nanospheres generated. As depicted in Fig. 2, the lowest viscosity value of all solutions prepared was observed to be 0.9 mPa s for 5 wt.% PMSQ in methanol whereas the highest value observed was 6.8 mPa s for 50 wt.% PMSQ in
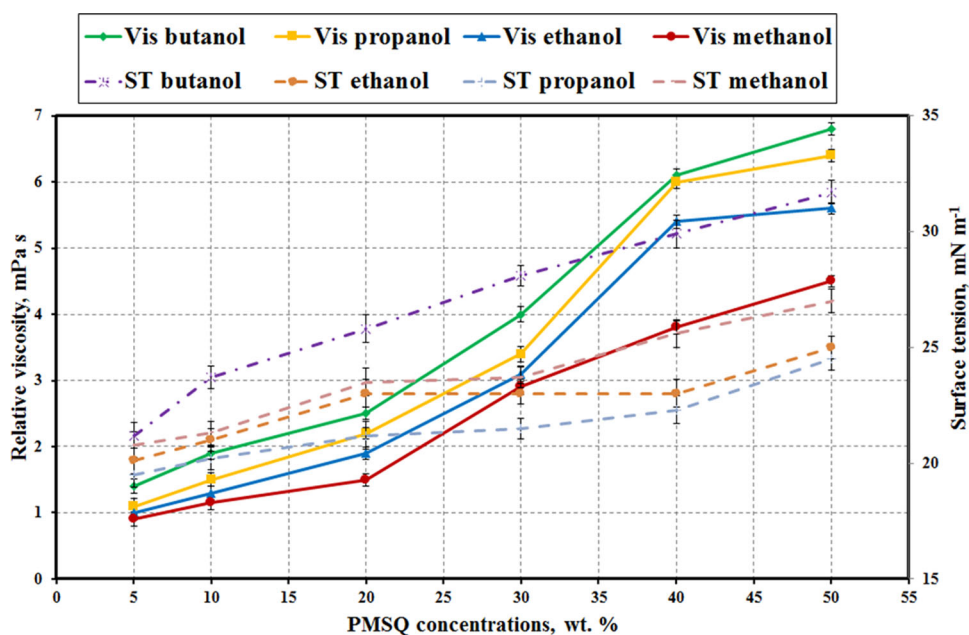
Fig. 2. Physical properties of PMSQ solutions obtained in this study. Error bars indicate standard deviation of the values. The viscosity and surface tension of PFH are 1.1 ± 0.11 mPa s and 12 ± 1.1 mN m^{-1}, respectively, calculated using five measurements. Vis and ST indicate viscosity and surface tension, respectively.
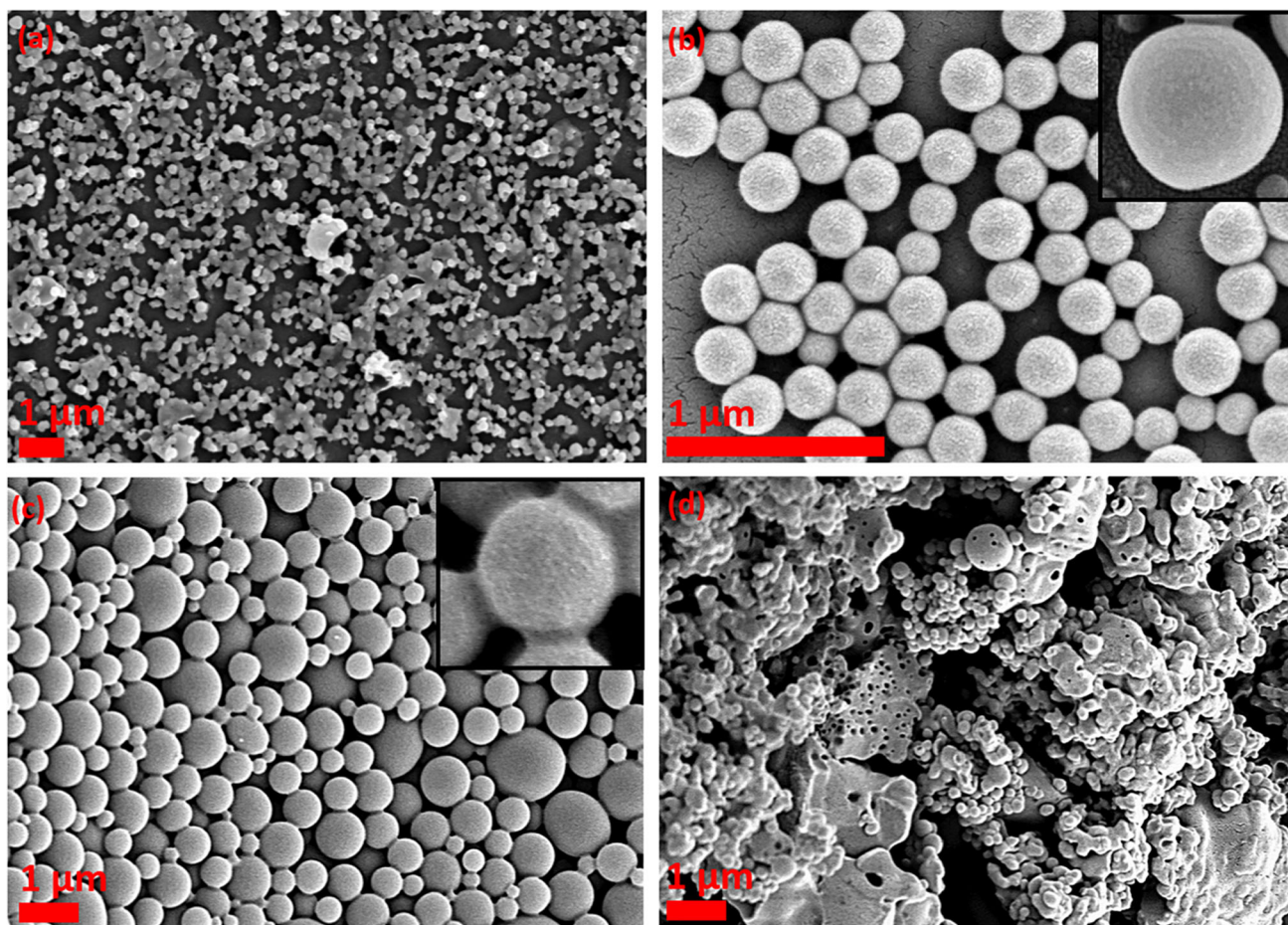

Fig. 3. SEM images of the PMSQ nanospheres prepared with (a) methanol, (b) ethanol, (c) propanol, and (d) butanol; 5 wt.% PMSQ concentration in all solutions. PMSQ solution and perfluorohexane flow rates were 300 μL min^{-1}.
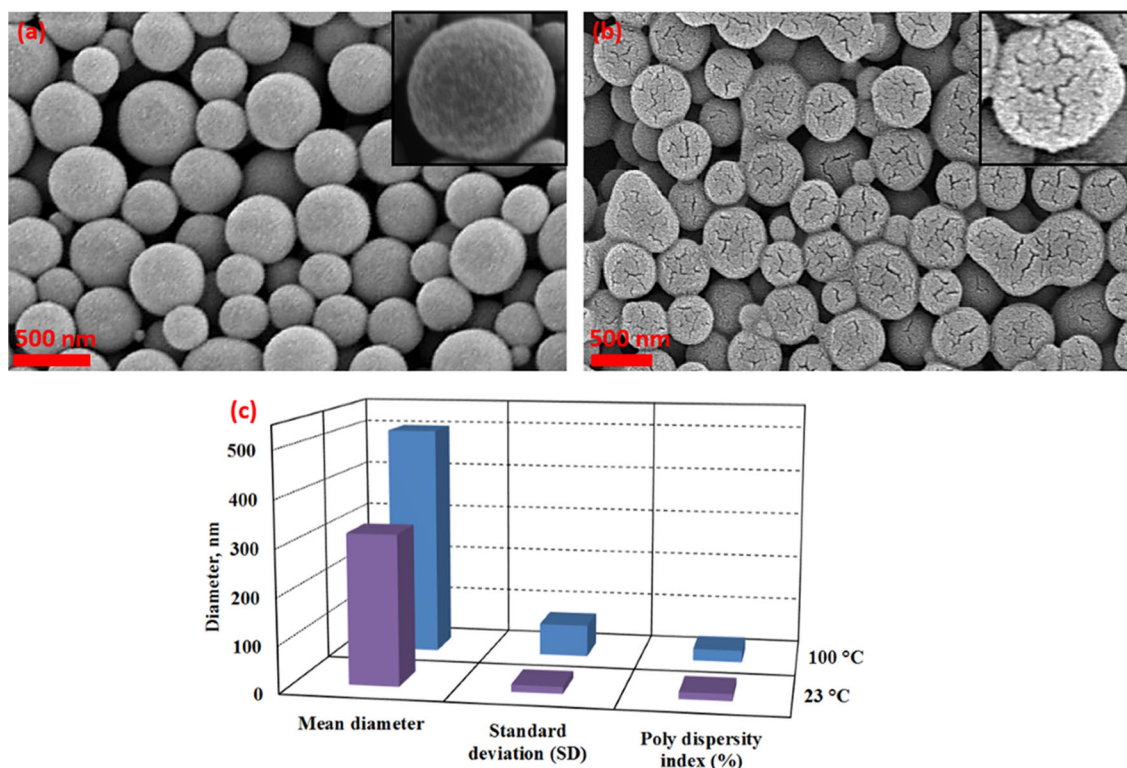
Fig. 4. SEM images of PMSQ nanospheres prepared under different collection temperatures, (a) 23°C ± 2°C, (b) 100°C ± 2°C. In both (a) and (b), inset shows a view of the PMSQ nanosphere surface (c) Corresponding size distributions of PMSQ nanospheres prepared. 5 wt.% PMSQ in ethanol was used, the flow rate of PMSQ solution and PFH was 300 μL min^{-1}.

butanol. PFH has a viscosity of 1.1 mPa s and the difference in viscosity will account for variations in nanosphere size, shape and distribution[37] as the evaporation of the volatile liquid, PFH, from the droplet core will be determined by this. The maximum value of surface tension was observed in the PMSQ-butanol solutions but much lower than that of water ($\sim$70 mN m^{-1}) and variations in surface tension will account for differences in characteristics of polymer nanospheres.[38,39]

Only 5 wt.% PMSQ in alcohol was used to prepare nanospheres and this is because the others were too viscous for the use of the device to generate nanospheres. From Fig. 3, it is clearly seen that only ethanol and propanol based polymer solutions produce well-defined nanospheres, while those prepared from methanol and butanol give mixed products, i.e. some nanospheres dispersed in flakes of polymer. However, the diameter of the PMSQ nanospheres formed from 5 wt.% PMSQ-ethanol solution were found to be about 320 nm with a polydispersity index of 15%, while those generated from the 5 wt.% PMSQ-propanol solution were about 650 nm with a polydispersity index of 26%, indicating that the solvent system can be used to control the polymer nanosphere size distribution. Yun et al.[40] reported that the average sphere size increased from 30 nm to 800 nm, when changing the alcohol from methanol to butanol using a sol–gel

method. The above-mentioned discussion refers to the nanospheres collected at 23°C. In addition, it can be seen from the SEM images (inset) that PMSQ nanospheres prepared using ethanol and propanol gave a relatively smooth surface.

Influence of Collection Temperature

SEM images were used to characterise the morphologies of both the PMSQ nanospheres obtained at 23°C ± 2°C (Fig. 4a) and at 100°C ± 2°C (Fig. 4b). Figure 4a shows that the PMSQ nanospheres exhibited a spherical shape and smooth surface. In comparison, Fig. 4b indicates that the nanospheres had near-spherical shape but rough surface. In addition, the size of PMSQ nanospheres were influenced by the collection temperature. As shown in Fig. 4c, when the temperature of distilled water was increased from 23°C to 100°C, the diameter of PMSQ nanospheres increased from 320 nm to 480 nm. Moreover, this increase of temperature of the distilled water affected the polydispersity of the PMSQ nanospheres, increasing from 15% to 26%. This could be due to the fact that a temperature change in the distilled water has an effect on its surface tension. An increase in the temperature of distilled water leads to a clear decrease in the surface tension of distilled water, decreasing from 70 mN to 60 mN m^{-1}.[41]
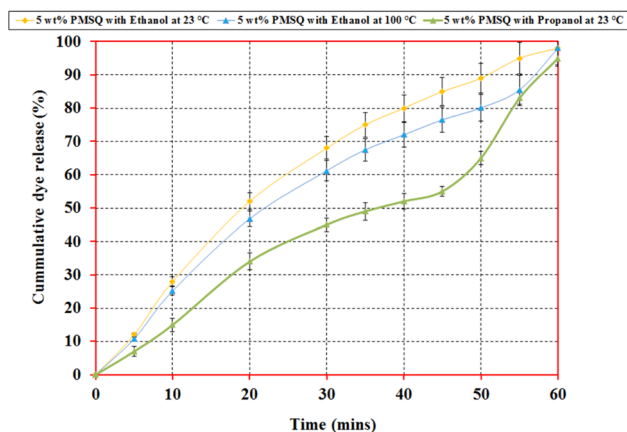
Fig. 5. Evans blue dye release profile from the PMSQ nanospheres prepared using different solutions and conditions. Error bars indicate standard deviations of the values.

In Vitro Dye Release

The encapsulated Evans blue dye was released from the PMSQ nanospheres over 60 min (Fig. 5). When PMSQ nanospheres prepared by using ethanol (320 nm) loaded with Evans blue were collected, it took ~20 min to release ~52% of dye, compared with 32% for PMSQ nanospheres prepared using propanol (650 nm) over the same time. However, this difference can be compensated for by having a rough surface. Release from PMSQ nanospheres (480 nm) with rough surface prepared using ethanol at 100°C was ~48% in 20 min (Fig. 5). Although the mean size of the nanospheres generated has also increased, this is probably due to the fact that in addition to dye encapsulated in the nanospheres; it is also trapped in the undulations resulting from the rough surface.

CONCLUSIONS

Microfluidic preparation of polymer nanospheres using various alcohols and at two different collection temperature in the V-shaped microfluidic junction device has been successfully achieved. By varying the alcohols and the collection temperature of the system, the mean diameter and surface morphology of the nanospheres were changed. Well-defined polymer nanospheres have been successfully prepared using ethanol and propanol. In addition, the sphere diameters obtained ranged from 320 nm to 650 nm, (polydispersity index: 15%–26%). The surface of the nanospheres can be changed from smooth to rough by collecting them at different temperatures, with a higher temperature achieving the formation of a rough surface. In addition, polymer nanospheres were encapsulated/coated with Evans blue dye using this microfluidic device. The release of dye from the nanospheres shows that surface roughness can compensate for size difference in normalising the release profile.

REFERENCES

1. A. Kumari, S.K. Yadav, and S.C. Yadav, *Colloids Surf. B* 75, 1–18 (2010).
2. A. Lenshof and T. Laurell, *Chem. Soc. Rev.* 39, 1203–1217 (2010).
3. J.P. Frampton, M.L. Shuler, W. Shain, and M.R. Hynd, *Cent. Nerv. Syst. Agents Med. Chem.* 8, 203–219 (2008).
4. A.-J. Wang, Y.-P. Lu, and R.-X. Sun, *Mater. Sci. Eng., A* 460–461, 1–6 (2007).
5. A.S. Karakoti, L.L. Hench, and S. Seal, *JOM* 58, 77–82 (2006).
6. B. Felice, M.P. Prabhakaran, A.P. Rodríguez, and S. Ramakrishna, *Mater. Sci. Eng. C* 41, 178–195 (2014).
7. N. Saito, Y. Kagari, and M. Okubo, *Langmuir* 22, 9397–9402 (2006).
8. Y. Zhang, H.F. Chan, and K.W. Leong, *Adv. Drug Deliv. Rev.* 65, 104–120 (2013).
9. A. Zvonar, J. Kristl, J. Kerc, and P.A. Grabnar, *J. Microencapsul.* 26, 748–759 (2009).
10. N.T.K. Thanh and L.A.W. Green, *Nano Today* 5, 213–230 (2010).
11. J.-M. Lim, N. Bertrand, P.M. Valencia, M. Rhee, R. Langer, S. Jon, O.C. Farokhzad, and R. Karnik, *Nanomedicine* 10, 401–409 (2014).
12. N. Nihant, S. Stassen, C. Grandfils, R. Jérome, P. Teyssié, and G. Goffinet, *Polym. Int.* 34, 289–299 (1994).
13. S. De Koker, R. Hoogenboom, and B.G. De Geest, *Chem. Soc. Rev.* 41, 2867–2884 (2012).
14. I. Kucuk, Z. Ahmad, M. Edirisinghe, and M. Orlu-Gul, *Int. J. Pharm.* 472, 339–346 (2014).
15. C.A. Serra and Z. Chang, *Chem. Eng. Technol.* 31, 1099–1115 (2008).
16. C.-H. Choi, J.-H. Jung, D.-W. Kim, Y.-M. Chung, and C.-S. Lee, *Lab Chip* 8, 1544–1551 (2008).
17. Y. Chen, G.G.Z. Zhang, J. Neilly, K. Marsh, D. Mawhinney, and Y.D. Sanzgiri, *Int. J. Pharm.* 286, 69–80 (2004).
18. C. Wu, X. Sun, Z. Zhao, Y. Zhao, Y. Hao, Y. Liu, and Y. Gao, *Mater. Sci. Eng. C* 44, 262–267 (2014).
19. F. Alexis, *Polym. Int.* 54, 36–46 (2005).
20. S. Adiga, L. Curtiss, J. Elam, M. Pellin, C.-C. Shih, C.-M. Shih, S.-J. Lin, Y.-Y. Su, S. Gittard, J. Zhang, and R. Narayan, *JOM* 60, 26–32 (2008).
21. M. Enayati, Z. Ahmad, E. Stride, and M. Edirisinghe, *J. R. Soc. Interface* 7, 667–675 (2010).
22. I. Armentano, M. Dottori, E. Fortunati, S. Mattioli, and J.M. Kenny, *Polym. Degrad. Stab.* 95, 2126–2146 (2010).
23. Y. Song, Q. Sun, T. Zhang, P. Jin, and L. Han, *J. Nanopart. Res.* 12, 2689–2697 (2010).
24. L.E. Murr, *JOM* 58, 23–33 (2006).
25. Z. Ahmad, E. Stride, and M. Edirisinghe, *J. Drug Target.* 17, 724–729 (2009).
26. M. Zou, S. Wang, F. Huang, Z. Zhang, and X. Ge, *Polym. Int.* 55, 305–311 (2006).
27. M.W. Chang, E. Stride, and M. Edirisinghe, *Langmuir* 26, 5115–5121 (2010).
28. C.-H. Yang, C.-Y. Wang, K.-S. Huang, C.-P. Kung, Y.-C. Chang, and J.-F. Shaw, *Int. J. Pharm.* 463, 155–160 (2014).
29. J. Michael Köhler, I. Kraus, J. Faerber, and C. Serra, *J Mater Sci* 48, 2158–2166 (2013).

30. E. Kumacheva, S. Xu, Z. Nie, M.S. Seo, P.C. Lewis, and H. Zhang, US Patent 8696952 (2014).

31. A. Jahn, J.E. Reiner, W.N. Vreeland, D.L. DeVoe, L.E. Locascio, and M. Gaitan, *J. Nanopart. Res.* 10, 925–934 (2008).

32. C.J. Luo, M. Nangrejo, and M. Edirisinghe, *Polymer* 51, 1654–1662 (2010).

33. K. Pancholi, E. Stride, and M. Edirisinghe, *Langmuir* 25, 10007–10013 (2009).

34. S.G. Kapsi and J.W. Ayres, *Int. J. Pharm.* 229, 193–203 (2001).

35. I. Kucuk and M. Edirisinghe, *J. Nanopart. Res.* 16, 1–9 (2014).

36. C.N. Baroud, F. Gallaire, and R. Dangla, *Lab Chip* 10, 2032–2045 (2010).

37. S.T. Knauert, J.F. Douglas, and F.W. Starr, *J. Polym. Sci. Part B Polym. Phys.* 45, 1882–1897 (2007).

38. M. Eltayeb, E. Stride, and M. Edirisinghe, *Nanotechnology* 24, 465604 (2013).

39. P. Sofokleous, E. Stride, W. Bonfield, and M. Edirisinghe, *Mater. Sci. Eng. C* 33, 213–223 (2013).

40. D.S. Yun, H.J. Kim, and J.W. Yoo, *Bull. Korean Chem. Soc.* 26, 1927 (2005).

41. J.M. Crane, G. Putz, and S.B. Hall, *Biophys. J.* 77, 3134–3143 (1999).