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Utilization of microfluidic V-junction device to prepare surface itraconazole adsorbed nanospheres

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ABSTRACT

Itraconazole is widely used as an anti-fungal drug to treat infections. However, its poor aqueous solubility results in low bioavailability. The aim of the present study was to improve the drug release profile by preparing surface itraconazole adsorbed polymethylsilsesquioxane (PMSQ) nanospheres using a V-junction microfluidic (VJM) device. In order to generate nanospheres with rough surface, the process flow rate of perfluorohexane (PFH) was set between 50 and 300 μl min^{-1} while the flow rate of PMSQ and itraconazole solution were constant at 300 μl min^{-1}. Variations in the PFH flow rate enable the controlled size generation of nanospheres. PMSQ nanospheres adsorbing itraconazole were characterized by SEM, FTIR and Zetasizer. The release of itraconazole from PMSQ nanosphere surface was measured using UV spectroscopy. Nanosphere formulations with a range of sphere size (120, 320 and 800 nm diameter) were generated and drug release was studied. 120 nm itraconazole coated PMSQ nanospheres were found to present highest drug encapsulation efficiency and 13% drug loading in a more reproducible manner compared to 320 nm and 800 nm sized nanosphere formulations. Moreover, 120 nm itraconazole coated PMSQ nanospheres (encapsulation efficiency: 88%) showed higher encapsulation efficiency compared to 320 nm (encapsulation efficiency: 74%) and 800 nm (encapsulation efficiency: 62%) sized nanosphere formulations. The itraconazole coated PMSQ nanospheres were prepared continuously at the rate of 2.6×10^6 per minute *via* VJM device. Overall the VJM device enabled the preparation of monodisperse surface itraconazole adsorbed nanospheres with controlled *in vitro* drug release profile.

1. Introduction

Preparation of polymeric nanosphere drug delivery systems using microfluidic devices has received considerable attention due to their ability in controlling drug release from an encapsulated system (Kumari et al., 2010; Sudhamani, 2010; Wang et al., 2007). Favourable physical characteristics of low density combined with high surface area make polymeric nanospheres an ideal drug carrier system (Wang et al., 2007). There are several techniques including emulsion polymerization, solvent evaporation and electrohydrodynamic atomization to prepare nanospheres (Ahmad et al., 2009a; Gunduz et al., 2013; Saito et al., 2006). The flow focusing methods such as T-junction and V-junction microfluidic (VJM) devices have also gained considerable attention (De Koker et al., 2012; Serra and Chang, 2008). This is due to their simplicity, cost-effectiveness, relatively high monodispersity products and high feasibility in the preparation of particular drug delivery systems. Gunduz et al. reported the usage of a VJM device to prepare bubbles which disintegrated into spheres at nanoscale (Gunduz et al., 2013). Nanospheres offer enhanced dissolution rates hence enhancing the bioavailability of drugs (Chen et al., 2004; Chiou and Riegelman, 1971; Goldberg et al., 1965; Sekiguchi Keiji, 1961).

PMSQ is a polymer with high stability and biocompatibility. The applications of its nanosphere form have been demonstrated (Ahmad et al., 2009b; Chang et al., 2010; Enayati et al., 2010). Itraconazole is a potent broad-spectrum triazole antimcyotic drug with activity against fungal species such as *Cryptococcus*, *Aspergillus*, *Candida*, *Blastomyces* and *Histoplasma capsulatum* (Nakarani et al., 2010). Itraconazole prevents infection proliferation as a prophylactic agent (SuparnaDugal, 2011). It is

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administered orally but is practically insoluble in water. There were studies into improving its solubility by formulating using lipids (Fromtling, 1987) with an ionisation constant of 3.7 (Liu et al., 2006; Peeters et al., 2002; SuparnaDugal, 2011).

Drug release profile is influenced by various factors. A reduction in nanospheres size is accompanied by an increase in the surface area. The manipulation of drug crystal structure and pro-drug approach were used (Kapsi and Ayres, 2001). Water soluble carriers to form inclusion complexes and solubilisation in surfactant systems are also considered to be factors influencing the dissolution rate of drugs (Kapsi and Ayres, 2001). The VJM device, as a miniaturized and scalable process, was used to increase the dissolution rate (*via* size reduction) and hence control release behaviour (Gunduz et al., 2013).

The aim of the current study was to explore the preparation of PMSQ nanospheres adsorbed with itraconazole using a VJM device. In order to investigate process control parameters of the VJM device, three different perfluorohexane (PFH) flow rates (50, 150 and 300 $\mu l\,min^{-1}$) and various PMSQ concentrations of 5–50 wt.% were used during the microfluidic process. The drug release of itraconazole was also investigated using UV spectroscopy (Nakarani et al., 2010).

2. Materials and methods

2.1. Materials

Polymethylsilsesquioxane (PMSQ) powder (average molecular weight: 7465 g/mol, angular (Fig. 1a)) was used as the nanosphere matrix and purchased from Chemie AG (GmbH, Burghausen, Germany). Commercially available rod-shaped itraconazole powder (molecular weight: 705.633 g/mol, SMS Pharmaceuticals Ltd., Hyderabad, India) was used as an active pharmaceutical ingredient (Fig. 1b). Ethanol (purity grade, 98%; density, 789 $kg\,m^{-3}$) was acquired from BDH Laboratory supplies (London, UK). Perfluorohexane (PFH) was used for the volatile liquid and provided by F2 Chemicals Ltd., Lea, UK (purity grade, 99.7–100%; density, 1710 $kg\,m^{-3}$).

2.2. Preparation and characterization of solutions

Two solutions containing 5 and 20 wt.% PMSQ polymer were dissolved in ethanol by mechanical stirring (magnetic) for 20 min at the ambient temperature (23 ± 2 °C) until complete dissolution. Subsequently, for drug loaded samples 0.5 mg/ml of itraconazole–ethanol solution was prepared by stirring using a magnetic stirrer for 15 min whilst heating up to 70 °C in order for the itraconazole to dissolve in ethanol. All solutions, including PMSQ and itraconazole were characterized for the surface tension, viscosity and density at the ambient temperature.

2.3. Preparation of itraconazole coated nanospheres

Drug coated PMSQ nanospheres were prepared using a V-junction microfluidic device, where they were generated from droplets (Fig. 1c). The device arms are 30° apart and connected to mechanical syringe pumps (PHD 4400, Harvard Apparatus, Edenbridge, UK) which provide a high precision and adjustable flow rate. The V-junction device consisted of Teflon fluorinated ethylene polypropylene (TEP) capillaries, with outer diameter (OD) and internal diameter (ID) of ~1.6 mm and 100 μm, respectively, embedded in the polymethylmethacrylate (PMMA).

Once both solutions were dissolved, PFH was loaded into a 10 ml syringe. All solutions and solvents were transferred to the syringe pumps. The pumps then fed the liquid through TEP tubing and into the V-junction device chamber where mixing

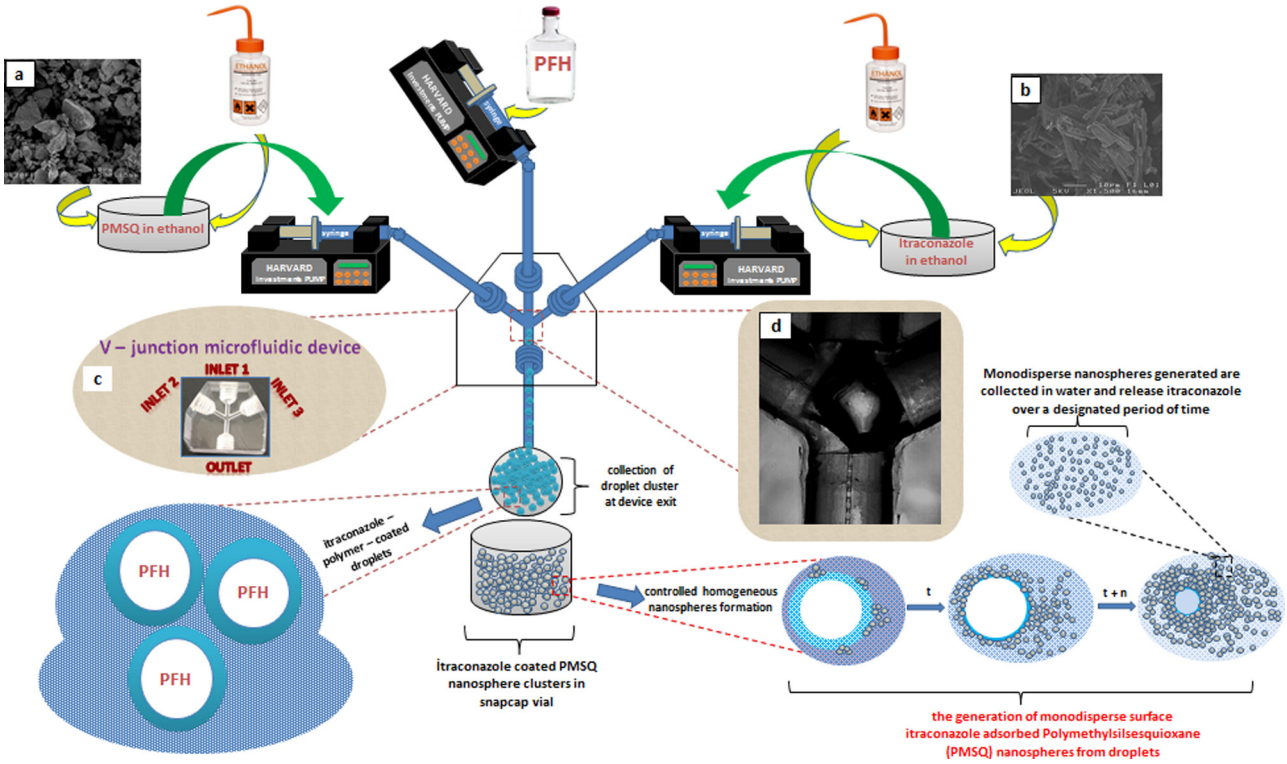

Fig. 1. Experimental set-up of preparation of itraconazole coated PMSQ nanospheres. SEM images of (a) raw PMSQ and (b) unprocessed itraconazole powder; (c) view of the VJM device, and (d) high-speed photograph showing the formation of droplets.

occurred and droplets were formed at the top of the outlet by manipulation of the flow rates (Fig. 1d). The obtained products streamed down through the outlet tube and collected at the exit channel. The system operation parameters such as the flow rates of liquids were used to partly control the production process. Samples were prepared at a various flow rates of 50–300 μl min^{-1} for inlet 1 (PFH), while inlet 2 (PMSQ solution) and inlet 3 (itraconazole solution) flow rates were kept constant at 300 μl min^{-1}. The nanospheres generated from the droplets were collected onto a snap cap vial containing distilled water. Nanospheres were also collected on glass slides and then left to dry in a desiccator under slight vacuum. A Phantom V7 high speed camera video was used to observe and control the process at all times.

2.4. Characterization of itraconazole coated nanospheres

The structural features of the nanospheres were assessed using scanning electron microscopy (SEM) (JEOL JSM 3600, Jeol Ltd., UK). For SEM, dried nanosphere samples were vacuum sputter coated with gold at 40 mA for 180 s, prior to observation, working at 5 kV. The mean diameter and polydispersity index (PDI) of the images obtained were determined, using ImageJ 1.47n software (Wayne Rasband National Institute of Health, USA). Approximately 200 nanospheres were measured for each set of different samples.

In order to measure the stability of the nanospheres, zeta potential measurements were conducted using Malvern Nano-ZS analyser at 25 °C. The measurements were repeated at least three times and were taken after 120 s (equilibrium time).

Fourier transform infrared spectrometer (FTIR) (PerkinElmer, 2000 FTIR spectrometer, MA, USA) was used to assess the chemical structure of the starting materials and products. 3 mg powdered samples of the pure PMSQ, dried PMSQ nanospheres, itraconazole and itraconazole coated PMSQ nanospheres were individually mixed with potassium bromide (KBr) and pelletized using a hydraulic press. These pellets were used for FTIR measurements. The spectra were recorded in the range of 400–4000 cm^{-1}, under ambient temperature. The FTIR spectra were obtained by averaging 265 scans at a resolution of 1 cm^{-1} for each run.

2.5. Drug encapsulation efficiency and yield

The itraconazole coated PMSQ nanospheres generated from the droplets were subjected to *in vitro* release studies. UV absorption spectra of itraconazole were measured on UV spectrometer (Lambda 35, PerkinElmer, UK) from the assessment of the absorbance of the solution with a known concentration. A calibration curve was prepared for known concentrations (1–40 ppm) of itraconazole at a wavelength of 268 nm (Fig. 2a and b). The encapsulation efficiency of the itraconazole coated PMSQ nanospheres was found by quantifying the coated itraconazole in polymeric matrix of PMSQ.

Measurement of itraconazole encapsulation efficiency (EE) and yield were performed by using 1 ml of PMSQ nanosphere solution.

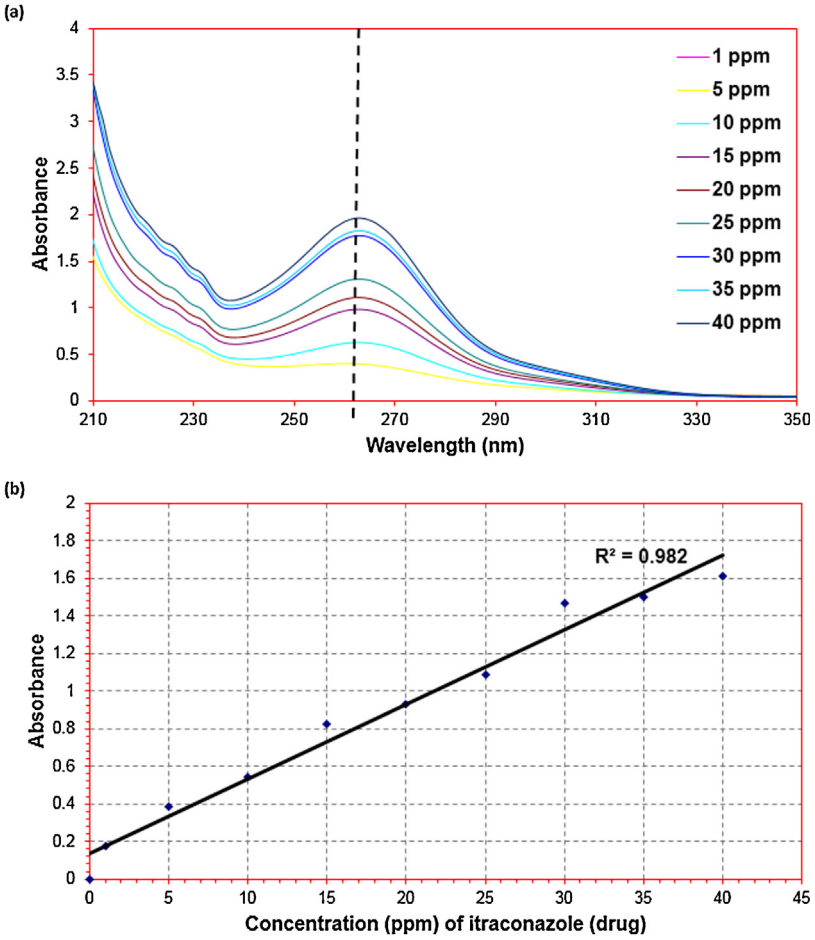

Fig. 2. UV spectra (210–350) of (a) itraconazole of different concentration (1–40 ppm) and (b) calibration curve.

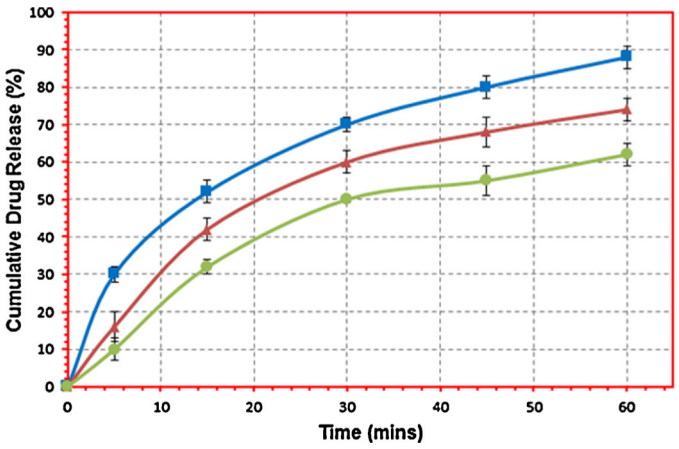

Fig. 3. Release profile of itraconazole coated PMSQ nanospheres with different particle sizes; 120 nm (square), 320 nm (triangle), and 800 nm (circle). Error bars show standard deviation of the values for drug release profiles.

The nanospheres were put in a centrifuge tube and dispersed in 10 ml double distilled water. The tube was put in a centrifuge (B4i Centrifuge, Jouan, St. Herblain, France) and processed at 4000 rpm for 20 min at 37 ± 0.5 °C. UV spectra were acquired in the region 210–350 nm. 1 ml supernatant from the samples was removed and its absorbance was measured using the calibration graph, based on the absorbance peak area at a wavelength of 268 nm. The mean absorbance value was found after four measurements were done for each nanosphere sample.

Knowing the initial amount of itraconazole used in preparing the nanospheres, the encapsulation efficiency of itraconazole on the PMSQ nanospheres was calculated by Eq. (1) and yield by Eq. (2) as described by previous workers (Jiang and Liao, 1993; Hu et al., 2011):

$$\%\text{EncapsulationEfficiency} = \frac{\text{actual itraconazole content}}{\text{the theoretical itraconazole content}} \times 100 \quad (1)$$

The PMSQ nanospheres prepared were dried at the ambient temperature, weighed and the percentage yield was calculated by:

$$\%\text{Yield} = \frac{\text{weight of nanospheres}}{\text{total weight of materials}} \times 100 \quad (2)$$

The release of itraconazole from the PMSQ nanospheres was characterized by fitting the Higuchi model (Eq. (3)) (Hu et al., 2011):

$$Q = k_H t^{\frac{1}{2}} \quad (3)$$

Q is the amount of drug released, k_H is the Higuchi dissolution constant and t is time. The error bars are the standard deviation of data from three repeat drug release experiments.

3. Results and discussion

3.1. In vitro *release kinetic studies of itraconazole coated PMSQ nanospheres*

The use of the V-junction device led to preparation of unagglomerated drug coated PMSQ nanospheres. UV spectroscopic analysis indicated the presence of itraconazole in the PMSQ matrix (Fig. 3). The total amount of itraconazole leached into the supernatant after centrifugation was calculated by the absorbance at 268 nm (Fig. 2a). The encapsulation efficiencies, respectively, were 62% for the 800 nm spheres, 74% for the 320 nm spheres, and 88% for the 120 nm spheres (Fig. 3). Also the yield percentages, respectively, were 61% for the 800 nm spheres, 72% for the 320 nm spheres, and 87.5% for the 120 nm spheres, indicating high encapsulation efficiency as well as a high yield. Furthermore, drug loading was found to be 13% for the 0.5 mg/ml of itraconazole used for 120 nm spheres.

In order to investigate the influence of PMSQ concentration and flow rates of PFH, 5 and 50 wt.% PMSQ concentrations, having respectively surface tensions of 21 ± 1.0 mN m^{-1} and 25 ± 0.9 mN m^{-1}, viscosities of 1 ± 0.10 mPa s and 5.6 ± 0.009 mPa s and densities of 762 ± 5.0 kg m^{-3} and 952 ± 4.4 kg m^{-3} were chosen. The experiments were run in triplicate. The drug release rates from

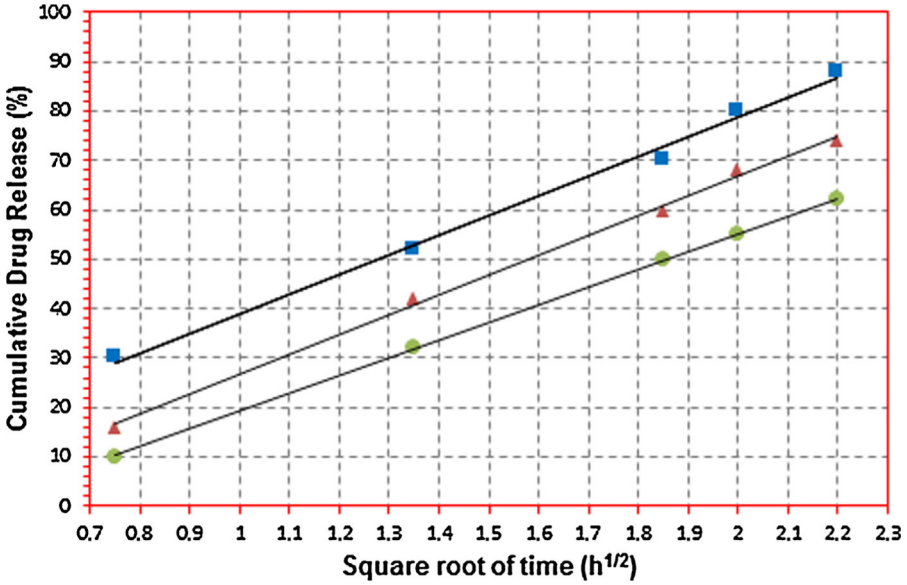

Fig. 4. Drug-release data in Fig. 3 fitted to the Higuchi model. Regression coefficient for $R^2 = 0.9941$ (square), $R^2 = 0.9978$ (triangle), and $R^2 = 0.9998$ (circle).

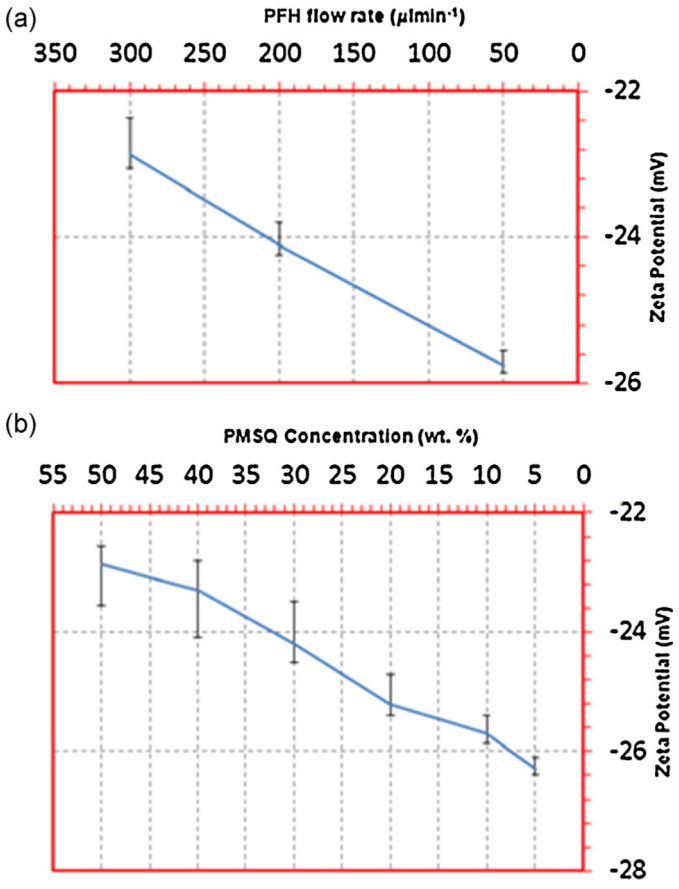

Fig. 5. Zeta potential results of nanospheres (a) prepared at the various flow rates between 50 and 300 μl min^{-1} for PFH when the itraconazole and PMSQ solution flow rates were constant at 300 μl min^{-1}, and (b) PMSQ concentrations between 5 and 50 wt.%. Error bars show standard deviation of the zeta potential values.

the resultant nanospheres were compared. Fig. 3 indicates the release profiles of itraconazole coated PMSQ nanospheres prepared with PFH flow rates of 50 and 300 μl min^{-1} when the itraconazole and PMSQ solutions were constant at 300 μl min^{-1}. The release profile (Fig. 3) also points to the release mechanism which is diffusion dominated, thus bringing about zero-order release of the itraconazole coated nanospheres.

From Fig. 3, it can be inferred that the total amount of itraconazole released corresponded almost exactly with the encapsulation efficiency. A reduction in diameter from 800 to 120 nm of nanospheres prepared with different PFH flow rates of 50 and 300 μl min^{-1} indicate that the flow rates of volatile liquid influenced the diameter of nanospheres. A reduction in diameter also shows the release rate increased with an increase in the flow of PFH. An increase of release rate is expected due to the rough surface associated with irregularities presenting on the surface – pores, fine cracks and undulations and large surface area to volume ratio of the smaller spheres. Xu et al. reported that smaller spheres tend to release drug more rapidly when compared with larger spheres (Xu et al., 2009). These findings show that there is an impact of PFH flow rate on the ultimate nanosphere size; confirms that the small nanospheres tend to release drug rapidly. When comparing the drug release profiles of the nanospheres in a range of diameters from 800 nm to 120 nm, an increase in release rate is obtained from a decrease in the flow rate of PFH during nanosphere preparation. Release of itraconazole from the PMSQ nanospheres was consistent with the Higuchi model (Fig. 4) and displays a burst release. The Higuchi model has been proposed for use with systems where drug diffusion occurs through the surface of the nanospheres. The k_H values were calculated as 0.113% h$^{-1/2}$, 0.093% h$^{-1/2}$ and 0.079% h$^{-1/2}$ from the results obtained at 120 nm, 320 nm and 800 nm, respectively, agreeing well with the literature (Overhoff et al., 2007). This points out that a determination of the release rate-controlling stage of PMSQ nanospheres was diffusion of the dissolved drug throughout the surface of the PMSQ nanospheres, which, in turn, describes why drug release occurs rapidly and displays burst release, consistent with similar findings in the literature (Chudasama et al., 2011).

3.2. Nanosphere stability

The zeta potential profiles in Fig. 5 shows the stability of nanospheres in various concentrations of PMSQ solutions and different flow rates of PFH. As the flow rates of PFH is increased, nanosphere size also increases and as a result, the stability of nanospheres is also increased. The values of zeta potential of the resultant PMSQ nanospheres increased from −25 to −23 mV for a range of PFH flow rates from 50 to 300 μl min^{-1} (Fig. 5a). Also, in Fig. 5b, it is clear that the zeta potential values of PMSQ nanospheres were influenced by the concentration of PMSQ solutions. The zeta potential values of PMSQ nanospheres slightly increased with an increase in the concentration of PMSQ. The zeta potential values of PMSQ nanospheres obtained using 5–50 wt.% PMSQ solutions, varied between −28 and −23 mV, respectively. This increase in the zeta potential value results in a greater electrostatic repulsion between nanospheres thus minimizing coagulation and flocculation of them and hence the PMSQ nanospheres are amenable to drug coating (Tripathy and De, 2006).

3.3. Chemical structure

In order to confirm the composition of pure PMSQ powder, PMSQ nanospheres, itraconazole and itraconazole coated PMSQ nanospheres, FTIR spectra were obtained over the range 400–4000 cm^{-1} (Fig. 6). From Fig. 6a, the spectra of pure PMSQ powders exhibited characteristic absorption bands for the C—H vibrations with —CH, —CH_2 and —CH_3 groups at around 2900 cm^{-1}. Less vital absorption was also detected at 2359 cm^{-1}, which may be identified with residual atmospheric carbon dioxide (C—O asymmetrical stretching vibration) (Chang et al., 2010). The absorption bands at 1410 and 1275 cm^{-1}, respectively, correspond to the methyl groups (CH_3). The spectra of resultant PMSQ nanospheres in Fig. 6b also demonstrated a representative absorption band of polysilsesquioxane at ∼1130 cm^{-1}. When comparing the spectrum of pure PMSQ powders and resultant PMSQ nanospheres, the spectrum of raw PMSQ powder is almost similar to that of the resultant PMSQ nanospheres excluding an additional absorption peak at 1119 cm^{-1}.

From Fig. 6c, it can be seen that the FTIR spectra of plain itraconazole was identical for the unprocessed powders. The principal peaks of itraconazole were observed at 3386, 1914, and 667 cm^{-1}. They probably arise from the stretching and vibration of functional groups such as the alkane, aromatic CH and amine groups (El Maghraby, 2009). Also, the spectra showed the characteristic peaks of itraconazole coated PMSQ nanospheres which occurred at 3438, 2979, 2365, 1740, 1274, 1124, 769, and 561 cm^{-1} (Fig. 6d). From Fig. 6d, the wave numbers observed at 2365 and 561 may be assigned to the C═O of the drug. This is in agreement with the previous recorded spectra's of the pure drug and PMSQ powders (El Maghraby, 2009; Prasad et al., 2010).

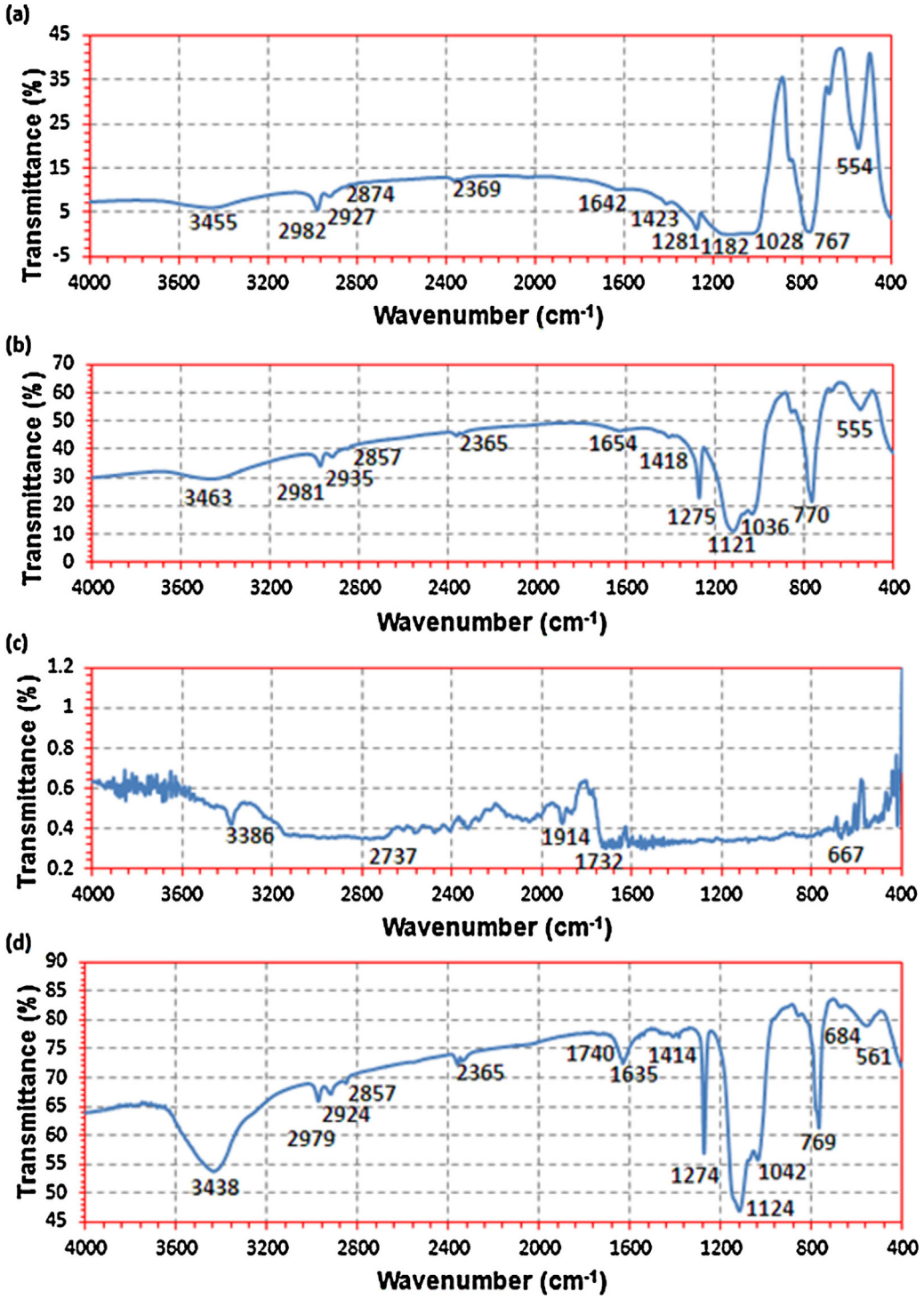

Fig. 6. FTIR spectra of: (a) pure PMSQ powders; (b) PMSQ nanospheres; (c) itraconazole (drug); (d) itraconazole coated PMSQ nanospheres.

3.4. Morphological characterization

SEM images were used to characterize the morphologies of both drug free and surface drug (itraconazole) adsorbed PMSQ nanospheres (Fig. 7). Representative images of the near-monodisperse polymeric PMSQ nanopheres are observed in Fig. 7a. Fig. 7a shows that the polymeric PMSQ nanospheres had spherical morphology and rough surface with an average diameter of 120 ± 20 nm ($n = 100$). In comparison to the SEM image of the unloaded PMSQ nanospheres in Fig. 7a, Fig. 7b displays an itraconazole coated layer on the outer surface of drug loaded PMSQ nanospheres.

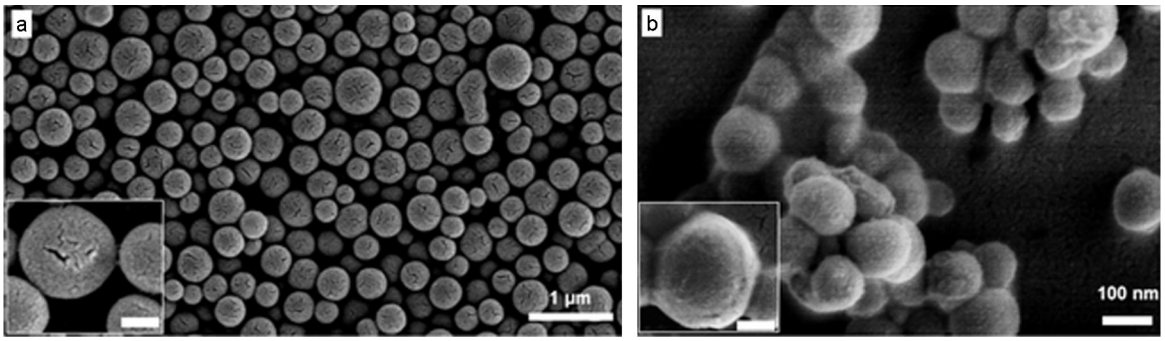

Fig. 7. SEM images showing (a) unloaded nanospheres with rough surface (inset: a view of polymeric PMSQ nanosphere (scale bar = 50 nm)) and (b) the surface of the drug adsorbed nanospheres prepared at a flow rate of 50 μl min^{-1} of PFH and 300 μl min^{-1} of PMSQ and itraconazole solution (inset: a view of itraconazole coated PMSQ nanosphere (scale bar = 50 nm)).

4. Conclusions

Polymeric nanospheres in this case formed using PMSQ, in the size range 120–800 nm, containing 0.5 mg/ml of itraconazole on the surface were prepared using a V-junction microfluidic (VJM) device. The data presented in this study indicated that these nanospheres have the potential to be used as nanocarriers for a range of hydrophobic drugs including itraconazole. By manipulating the processing parameters, such as the flow rates of the inlet solutions in the system, it is possible to control the final diameter of the nanospheres. Drug dissolution data displayed that different release profiles were obtained by changing the flow rates of PFH, and subsequently the final size of the nanospheres from 120 to 800 nm. Encapsulation efficiency and drug loading were in the range of 61–88% and 13%, respectively. The %yields of itraconazole coated PMSQ nanospheres were in the range of 61–88 indicating high values of yield accompanying high values of encapsulation efficiency. A characteristic burst release of itraconazole coated PMSQ nanospheres was modelled using a Higuchi model and values of k_H were calculated as 0.113% $h^{-1/2}$, 0.093% $h^{-1/2}$ and 0.079% $h^{-1/2}$ for the 120 nm, 320 nm and 800 nm diameter nanospheres, respectively. Zeta potential measurements showed the stability of the resultant PMSQ nanospheres change between −28 and −23 mV based on a flow rate of PFH and PMSQ concentrations. FTIR investigation confirmed the compositions of both pure PMSQ and itraconazole powders and nanosphere products. SEM images showed layered itraconazole on the surface of PMSQ nanospheres. In conclusion, the developed V-junction microfluidic device was found to be a promising method for the preparation of nanospheres enabling the controlled release of poorly soluble drugs such as itraconazole.

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