

# Using a cross-flow microfluidic chip and external crosslinking reaction for monodisperse TPP-chitosan microparticles

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## Abstract

The purpose of this study is to generate monodisperse TPP-chitosan microparticles using a cross-flow microfluidic chip coupled with external crosslinking reaction. We have demonstrated that one can control the size of TPP-chitosan emulsions from 180 to 680  $\mu\text{m}$  in diameter (with a variation of less than 10%) by altering the relative sheath/sample flow rate ratio. Our strategy is based on the sheath effect (focusing) to form uniform self-assembling sphere structures, the so-called water-in-oil (w/o) emulsions, in the cross-junction microchannel. These fine emulsions, consisting of aqueous 1% (w/v) chitosan, are then dripped into a solution containing 10% (w/v) tripolyphosphate (TPP). They then undergo an ionic-crosslinking reaction and create water-insoluble TPP-chitosan microparticles in an efficient manner. In addition, the size distribution of the resulting TPP-chitosan microspheres is narrow (about polyindex = 1) which is suitable to provide the optimal release rate in the administration of controlled release drugs. The proposed microfluidic chip is capable of generating relatively uniform micro-droplets and has the advantages of actively controlling the droplet diameter, and having a simple and low cost process, with a high throughput.

*Keywords:* TPP-chitosan; Microfluidic; Monodisperse; Emulsion; Droplet; Drug carrier

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## 1. Introduction

Chitosan is currently gaining a great deal of attention for medical applications as well as for the controlled release of drugs [1–3]. The success of chitosan beads as carriers is due to the following features: (i) they can dissolve poorly soluble drugs and thus increase their bioavailability, (ii) they can stay in the body (in the blood) long enough to provide gradual accumulation in the required area, (iii) their size permits them to accumulate in body regions with leaky vasculature, (iv) they can be tailored to achieve targeting or other desired properties by attachment of a specific ligand to the outer surface, (v) they have low toxicity and a high loading capacity, as well as minimize drug degradation and loss, and (vi) they can be easily produced in large quantities [4–6]. Of critical importance to their successful implementation as a drug deliverer is their ability to control particle size and size distribution, as this influences the clearance rate from the body

which ultimately determines the drug dosage [7,8]. Basically, an ideal particle size can provide an optimal release rate.

To date, the production of chitosan microspheres has been accomplished mainly by the following: (i) spray-drying (atomization), (ii) coacervation (precipitation) (iii) emulsification and others [2,9–13]. However, the above techniques have well-known drawbacks such as unstable yield, tedious procedures and non-uniform particle sizes with a wide size distribution. It has become imperative for the pharmaceutical industry to develop a reproducible method for generating TPP-chitosan microparticles with a uniform particle size and a narrow size distribution in a controlled manner.

Several methodologies for the construction of size controlling microspheres with a narrow size distribution have been described in the literature, such as using: (i) supercritical fluid technologies for polymer microparticles [14], (ii) the precipitation reaction for manganese carbonate microparticles [15], (iii) a micro-nozzle array for alginate beads [16], (iv) electrostatics for polymer microbeads [17], (v) an acoustic excitation method for PLG microspheres [7], (vi) micro-nozzle channel emulsification for gelatin beads [18], (vii) a membrane emulsification

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method for alginate beads [19,20] and (viii) a micropipette and photopolymerization for polymer microparticles [21]. In contrast, the studies of monodisperse TPP-chitosan microspheres are rather scarce.

Microfluidic chip (containing cross-junction microchannel) emulsification is a relatively new technique for preparing water-in-oil (w/o) and oil-in-water (o/w) emulsions [22]. For example, Jahn et al. used a microfluidic channel for nano-scale liposome

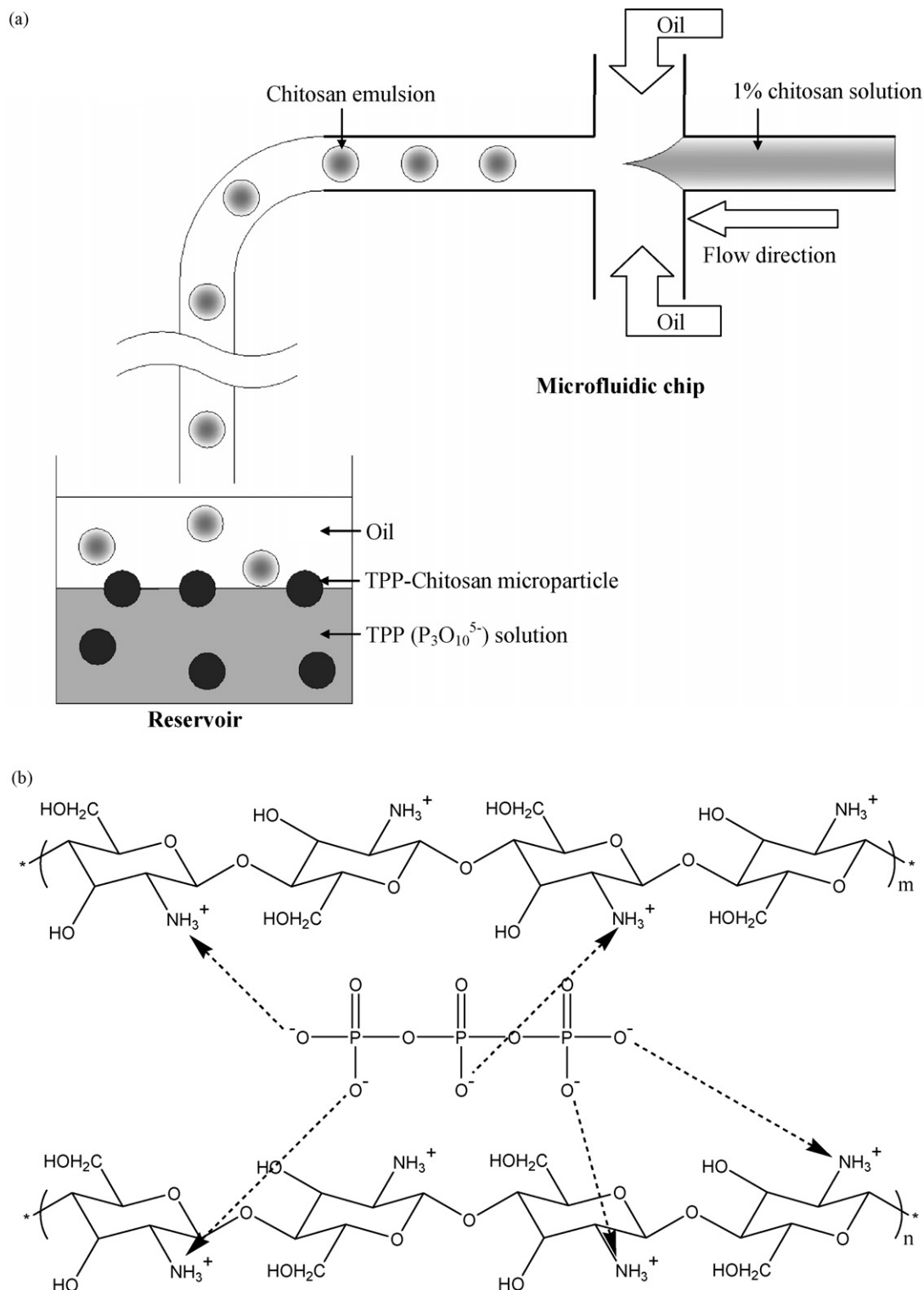


Fig. 1. Illustration of microfluidic chip emulsification coupled with the ionic-crosslinking reaction process. (a) Schematic drawing of the formation of chitosan emulsion in a cross-junction microchannel. Based on microfluidics to exert control over the focusing force, a large set of uniform self-assembling spheres can be obtained. The emulsions are gelled upon contact with 10% (w/v)  $P_3O_{10}^{5-}$ , and the chitosan molecules entrapped in the micro container (emulsion) are transformed into TPP-chitosan particles in the reservoir. (b) The mechanism of TPP-chitosan microspheres synthesis is based on the fact that in low pH (pH 2), TPP is dissociated into  $P_3O_{10}^{5-}$  anions. Therefore, TPP-chitosan microparticles prepared in the acidic TPP solution are completely ionic-crosslinking dominated [30].

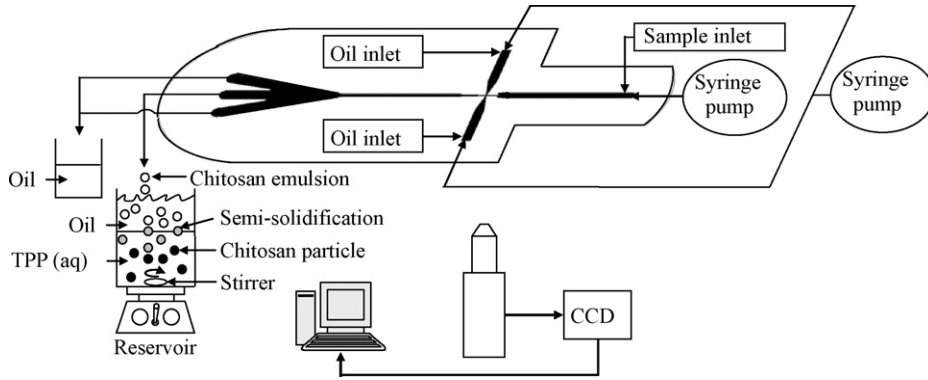


Fig. 2. Experimental setup of a microfluidic chip system for the generation of TPP-chitosan microparticles.

generation [23]. Abraham et al. used a T-junction microchannel for polymeric microcapsules generation [24]. Xu et al. used a microfluidic flow-focusing device for poly tripropyleneglycol diacrylate (polyTPGDA) particles [25]. In addition, recently we used a cross-junction microchannel of a microfluidic device

coupled with gelation reaction for the generation of uniform Ca-alginate microparticles [26]. The mechanism of this type of microfluidic chips in droplet-volume control is well represented in the recent literature [27–29]. However, none of these studies have attempted to apply the microfluidic chip to control the

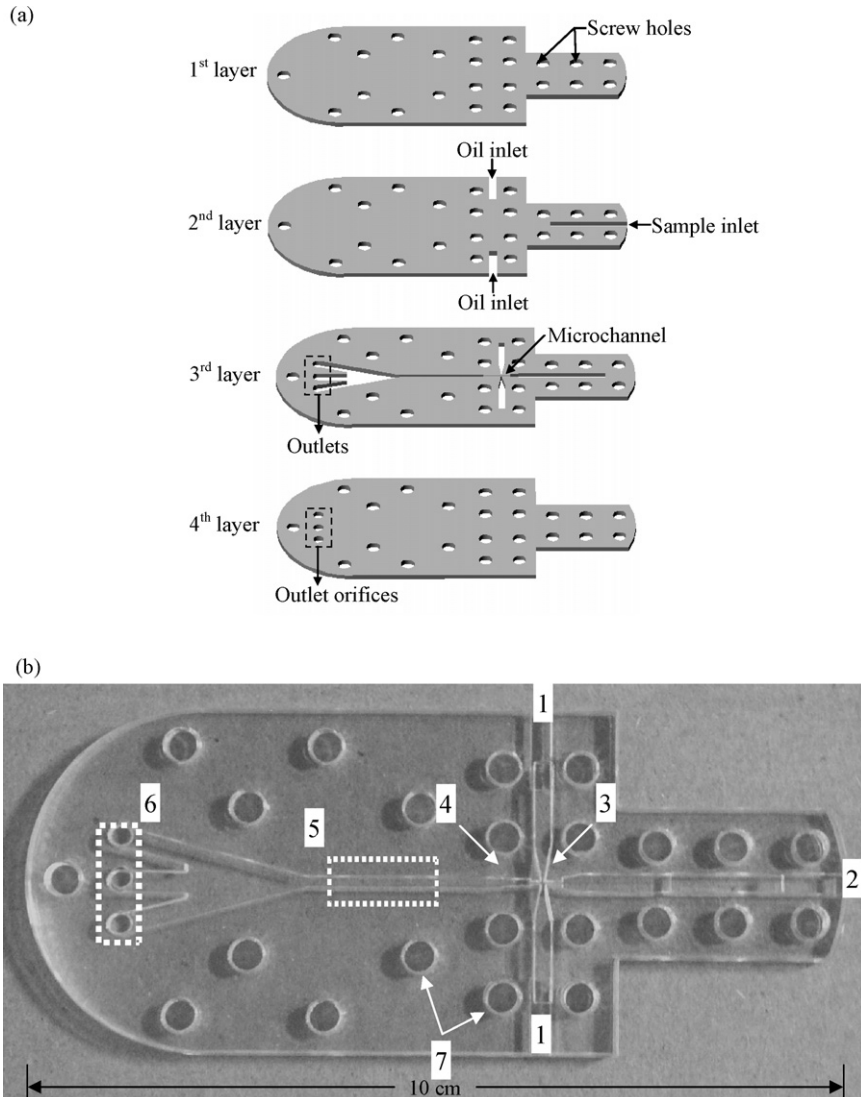


Fig. 3. Schematic drawing and photo image of our proposed microfluidic chip: (a) the chip in expanded view and (b) the photo image of the chip: 1, oil inlet; 2, sample inlet; 3, cross-junction channel; 4, broadened channel; 5, observation chamber (1200  $\mu\text{m}$  in width channel); 6, outlets; 7, screw holes for bonding.

performance of uniform TPP-chitosan microspheres. Therefore, the aim of this study is to investigate and compare the size of the TPP-chitosan microspheres obtained by a different ratio of flow rate in the side inlet channels to that in the center inlet channel. The developed microfluidic chip is easy to fabricate, easy to set up, and is easily programmed to generate a large set of monodisperse TPP-chitosan microspheres.

## 2. Materials and methods

### 2.1. Materials

Medium molecular weight chitosan ( $M_w$  40 kDa) and tripolyphosphate (TPP) were obtained from Sigma (Sigma

Chemical Co., St. Louis, MO). Distilled water (DI water) was filtered by a 0.22  $\mu\text{m}$  syringe filter (Millipore Inc., Clifton, NJ) before being used in the preparation process. All other reagents are commercially available and of the highest grade.

### 2.2. Principle of uniform chitosan microspheres generation

In this study, we report the use of microfluidics to exert control over the spontaneous self-assembly of w/o emulsions by means of a solution of dissolved chitosan. The mixture is then dripped into a solution containing TPP buffer, resulting in the instantaneous formation of TPP-chitosan microspheres (Fig. 1a). Our strategy is based on the sheath force at the cross-junction microchannel forming a narrow size distribution of

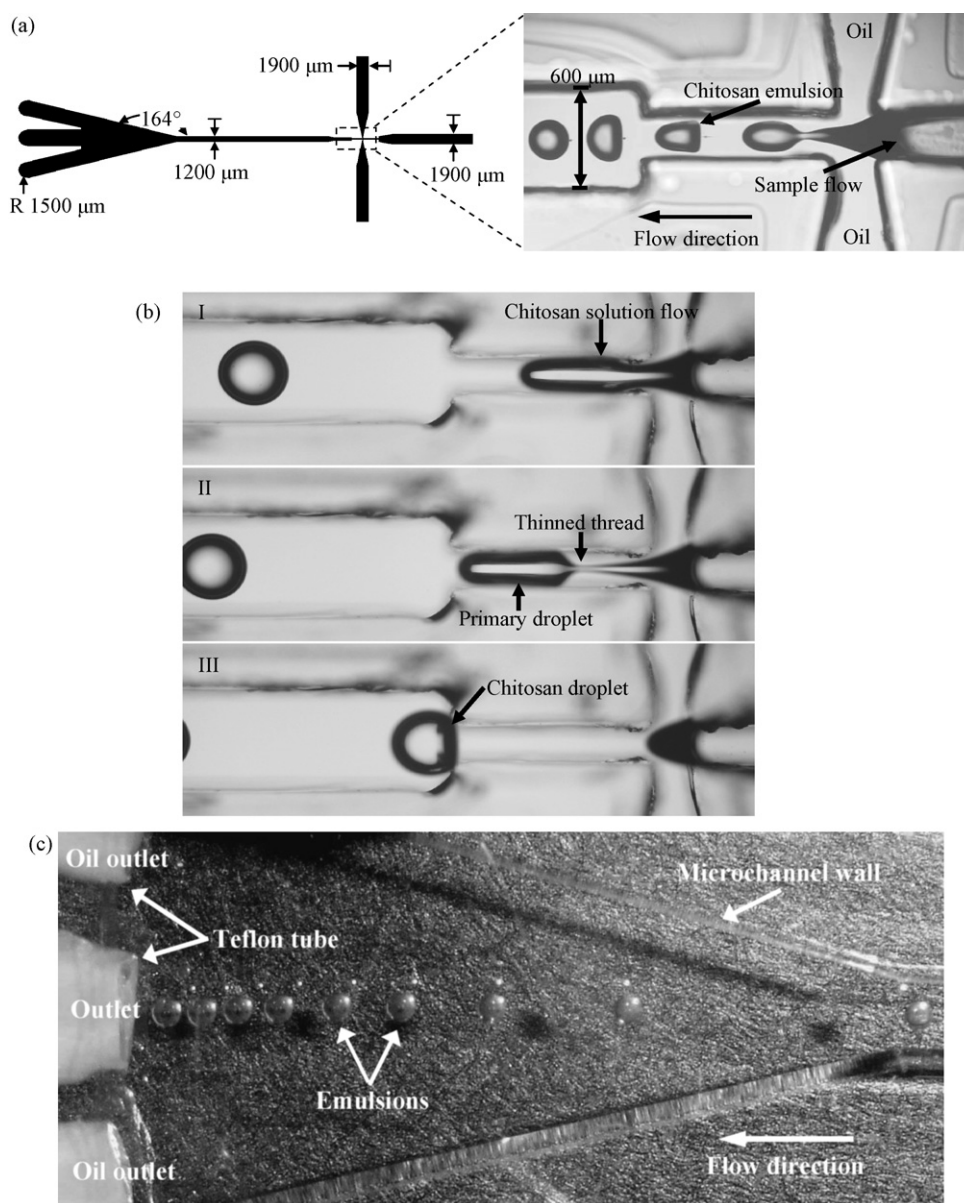


Fig. 4. (a) Monodispersed chitosan micro-emulsions are generated at the cross-junction with a sample flow at 0.012 mL/min and an oil flow at 1.800 mL/min. The arrow-shaped flow indicates the direction of the emulsion generation, and the scale bar is 600  $\mu\text{m}$ . (b) The time-series images of the generation of chitosan micro-emulsion under the condition of a sample flow at 0.012 mL/min and oil flow at 1.200 mL/min. (c) The photo image of uniform micro-emulsions were moved to a TPP solution through the middle outlet channel.

self-assembling sphere structures, the so-called chitosan micro-emulsions. When these emulsions are transported to a TPP solution through a teflon tube, they precipitate spontaneously at the bottom of the oil due to the fact that they have a higher density than oil. Therefore, chitosan micro-emulsions reacts with  $P_3O_{10}^{5-}$  ion at the interface between oil phase and water phase. After they have undergone ionic-cross-linking, TPP-chitosan microparticles are observed. The mechanism of this type of microfluidic chip in droplet-volume control has been well documented in recent literature [27–29]. Such as by varying the ratio between oil and water flow rates and/or the fluid viscosity a finer control of the droplet sizes can be obtained. Based on the outstanding performance of the microfluidic technique, we utilize it in this work for pharmaceutical use (e.g. TPP-chitosan microparticle generation).

TPP-chitosan microparticles are prepared by the ionic interaction between a positively charged amino group ( $NH_3^+$ ) of chitosan and  $P_3O_{10}^{5-}$  anions (Fig. 1b). The mechanism of TPP-chitosan microspheres synthesis is based on the fact that chitosan is a weak polybase, and as the pH of the solution decreases, the ionization of the amino group of chitosan increases. In low pH (pH 2), TPP is dissociated into  $P_3O_{10}^{5-}$  anions. Therefore, TPP-chitosan microparticles prepared in the acidic TPP solution are completely ionic-crosslinking dominated [30].

### 2.3. Experimental procedure

Fig. 2 shows an overview of the experimental set up. The procedure is as follows. First, set up the fluids of the center and side inlet channels with a chitosan solution and oil, respectively. Second, the fluids are then injected into the microfluidic chip by syringe pumps (Kdscientific KDS230) programmed by a PC. In this work, we hydrodynamically focus a stream of aqueous chitosan solution (dispersed phase) at a cross-junction microchannel by two oil streams (continuous phase), enabling the production of a w/o chitosan micro-emulsion in a microchannel. Finally, these emulsions undergo ionic-crosslinking by dripping them into a TPP solution. After 20 min of hardening, TPP-chitosan microparticles are formed.

### 2.4. Microspheres size measurement

A fluorescence microscope was used to observe the experimental results. The image and detection system consisted of an optical microscope (BX60, Olympus, Japan) and a digital camera (DP70, Olympus, Japan). The diameter of each microspheres was measured and a total of 50 microspheres were measured to provide an average size.

## 3. Results and discussion

### 3.1. Design and fabrication of a microfluidic chip

The developed microfluidic chip is laid out on a conventional poly methyl methacrylate (PMMA) substrate (length/width/depth: 270.0 mm/210.0 mm/1.5 mm) using a  $CO_2$  laser machine (LaserPro Venus, GCC, Taiwan). The microfluidic

chip (length/width/depth: 100 mm /43 mm/6 mm) consists of four layers (an expanded view is shown in Fig. 3a) which are, from top to bottom: the cover layer (containing 23 screw holes), the second layer (containing one sample inlet channel and two oil inlet channels), the main layer (containing the cross-junction channel) and the bottom layer (containing three outlet orifices). These four layers are integrated by screws (tightened at 1.0–1.2 Nm) to produce a microfluidic chip. This microfluidic chip has three inlet ports, three outlet ports, one cross-channel and an observation chamber, as shown in Fig. 3b. The broadened channel (600  $\mu m$  in width, near the outlet of the cross channel) is designed for slowing down the flow and enhancing the observation. In addition, the left and right outlets design helps to collect oil for re-use. This chip is low cost, easy to fabricate, easy to set up, as well as easy to organize and program.

### 3.2. Formation of monodisperse chitosan emulsions

For the generation of uniform w/o chitosan micro-emulsions, a pregel solution (25 mL of 1.0% (w/v) chitosan solution) and 200 mL of sunflower seed oil (55 mPa s (cP), Uni-President Enterprises Corp., Taiwan), are employed as the sample-phase fluid (dispersed flow) and oil-phase fluid (continuous flow), respectively. This pregel solution is fluidified by the shear forces in the microfluidic chip equipped with a cross-junction channel, resulting in uniform semi-products (chitosan micro-emulsions).

In the experiments, the flow rates of the sample-phase and the oil-phase fluids were set to 0.012 and 1.800 mL/min, respectively. We found that the sample-phase fluid was compressed by shear force into an arrow shape and then separated into emulsions of about 210  $\mu m$  in diameter (Fig. 4a). In addition, when we set 0.012 mL/min of sample flow and 1.200 mL/min of oil flow, similar monodisperse chitosan micro-emulsions were observed (Fig. 4b). The time-serial images show the generation of chitosan micro-emulsion in the microchannel. We found the diameter distribution of the emulsions formed to be quite uniform ( $390 \pm 15 \mu m$ ), and the gap between each emulsion stable

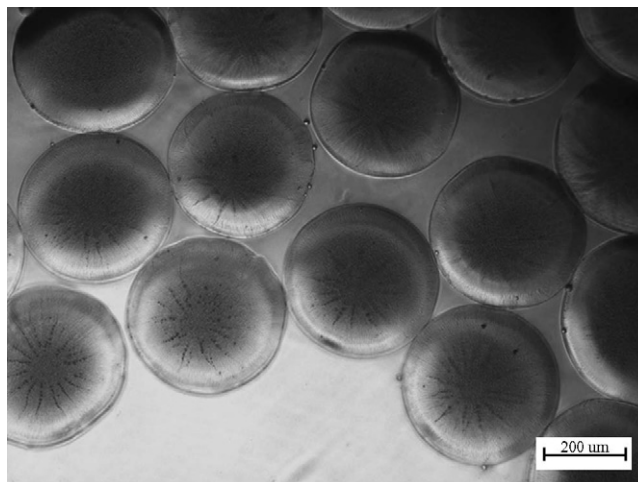


Fig. 5. The photo image of purified TPP-chitosan microparticles (scale bar 200  $\mu m$ ).



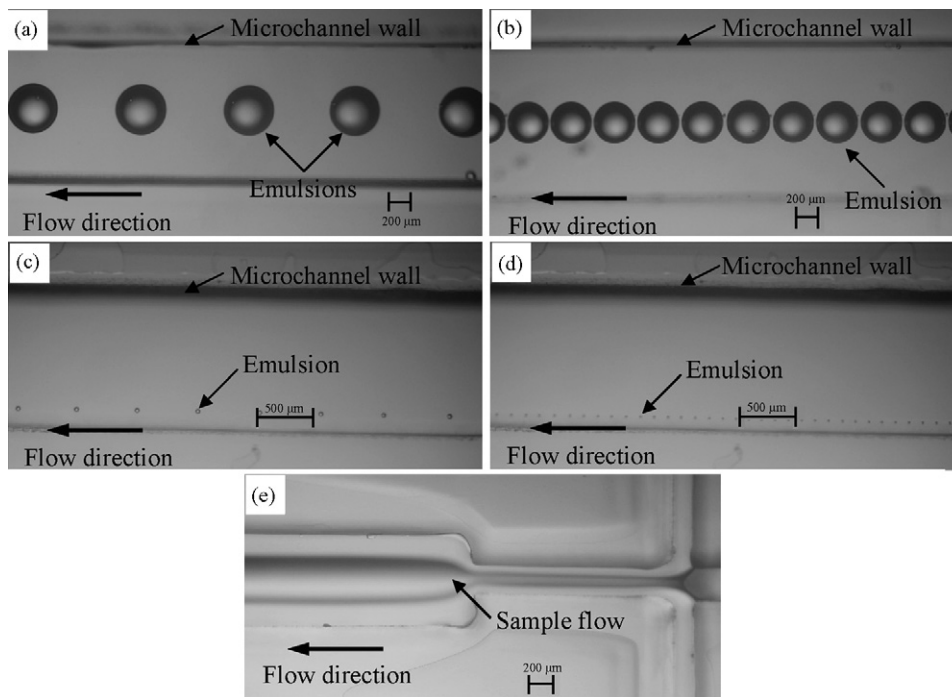


Fig. 6. The emulsion formation under the condition of (a) 0.006 mL/min of sample flow and 0.800 mL/min of oil flow (scale bar 200  $\mu\text{m}$ ), (b) 0.1 mL/min of sample flow and 1.6 mL/min of oil flow (scale bar 200  $\mu\text{m}$ ), (c) 40  $\mu\text{m}$ , (d) 20  $\mu\text{m}$  of chitosan emulsions are observed in the developed microfluidic chip (scale bar 500  $\mu\text{m}$ ) and (e) no emulsion generated under certain conditions.

(1100  $\pm$  50  $\mu\text{m}$ ). Fig. 4c shows the uniform droplets are moving to middle outlet channel before transporting to a TPP solution.

### 3.3. Formation of TPP-chitosan microparticles

The semi-products (chitosan micro-emulsions) are formed in the continuous oil flow. The continuous oil flow can prevent these semi-products from fusing together, and can transport them to a  $\text{P}_3\text{O}_{10}^{5-}$  ion pool (10% (w/v) TPP solution) through a teflon tube. The water-soluble chitosan micro-emulsions are gelled into solid spheres upon contact with  $\text{P}_3\text{O}_{10}^{5-}$  ion by ionic-crosslinking, resulting in water-insoluble TPP-chitosan microparticles. We find that the shapes of most TPP-chitosan microparticles remain spheroid after the ionic-crosslinking (Fig. 5).

### 3.4. Influence of flow rate

The emulsion size/gap is easily varied by changing the flow conditions in the microchannel. For example, Fig. 6a and b shows that both increasing sample flow rate and oil flow rate can result in smaller size (440  $\mu\text{m}$  change to 380  $\mu\text{m}$ ) and smaller gap (520  $\mu\text{m}$  change to 10  $\mu\text{m}$ ) micro-emulsions being obtained. The smallest size of emulsions generated in our microfluidic chip is approximately 20  $\mu\text{m}$  (Fig. 6c and d). At the same time, emulsions can not be generated when the flow speed ratio of the sample/oil is above 1:16 or below 1:100 (e.g. Fig. 6e).

Fig. 7 shows the relationship between the flow speed (average velocity) of the phases and the emulsion size (diameter). For a

given 0.1 mL/min of sample flow, the emulsion size decreases as the average velocity of the oil flow increases. The same tendency is observed in the range of 0.100–0.006 mL/min of the sample flow. On the other hand, when given a 1.0 mL/min of the oil flow, the emulsion size increases as the average velocity of the sample flow increases. The same tendency is also observed in the range of 0.8–1.6 mL/min of the oil flow. Based on the results shown in Figs. 6 and 7, it is evident that the size and the gap of the micro-emulsions, generated in the cross-junction, are controllable and reproducible by using our proposed microfluidic chip.

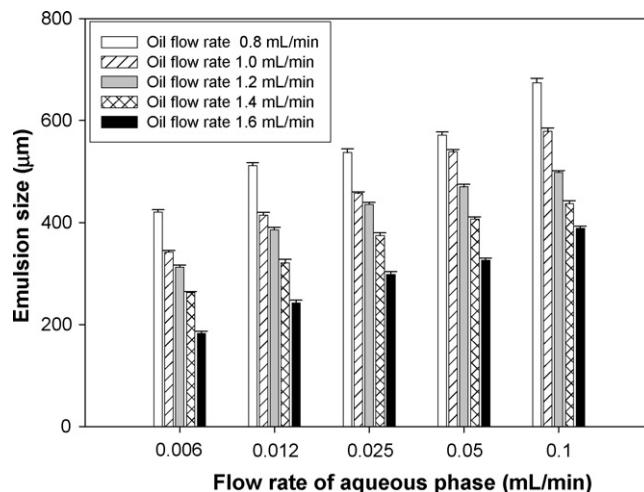


Fig. 7. The relationship between particle size and flow rate (sample/oil).

## 4. Conclusions

We have demonstrated that a microfluidic device utilizing a cross-junction microchannel, enabled the production of 180–680  $\mu\text{m}$  chitosan beads with a narrow size distribution (<10%). The strategy is based on a simple and cost-effective chip that manipulates chitosan microparticles by using the immiscible property of sample and oil solutions in the microchannel and the *in situ* ionic-crosslinking reaction. This method turns out to be one of the most efficient and cost-effective methods for the production of monodisperse chitosan microparticles. From a practical point of view, the microfluidic chip we developed is very attractive, since it emulsifies very easily and yields extremely uniform micro-emulsions. This approach in the manipulation of TPP-chitosan microparticles will have many potential usages for pharmaceutical applications.

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