Cross-flow membrane emulsification technique for fabrication of drug-loaded particles

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Abstract
Cross-flow membrane emulsification is a new technique which was used in this study to achieve uniform and controllable emulsion systems. In this method, the droplet is individually formed at the pore on the surface of membrane in the more mild, controllable and efficient way as compared to traditional emulsification techniques. In this study, we used silicon nitride membranes of very precise parameters of pore size, shape and inter-pore distance in order to create curcumin loaded poly(d, l-lactic-co-glycolic acid) (PLGA) particles. It was demonstrated that more uniform and pore-size dependent particles was created by using different membrane pore sizes (ø200 nm, ø450 nm and ø2 µm). Other factors that could impact particle size and morphology such as membrane polarity, concentration and volume of two phases were investigated. Further tests on comparison to mechanical stirring method were also realized.

Keywords: cross-flow membrane emulsification, silicon nitride membrane, PLGA

Classification number: 2.05

1. Introduction
Since nanotechnology was applied to human life, it has been exploited in many small potential applications. In health diagnostics and treatment fields it is not exceptional. Drug delivery is especially attracting much attention from scientists as it can perform work that traditional drug treatment cannot meet. For example, nanoparticles can deliver drugs, heat and light to specific places in the body, called targeted drug delivery system. Besides, by controlling the comportments of drug-loaded materials we can also control drug release time, solubility, increase biocompatibility and decrease side effects [1].

Beside the advantages of nano/micro-engineered over traditional drugs, there is still one major factor that limits its development on the market, and that is production at industrial scale. Many drug delivery systems require specific manipulations that cannot be adjusted to larger scale production. Emulsification—evaporation of solvent technique—is one of the most common methods that has been used in research for fabrication of drug-loaded particles. It is a simple method and has a wide range of possibilities. In this method, emulsions are conventionally prepared by dispersion of a dispersed phase into a continuous phase by using mechanical methods [2]. Colloid mills, rotor stator systems, high-pressure homogenizers, ultrasonic homogenizers and magnetic stirrers are usually used for this purpose [3]. The principle of this method is as follows: droplets are broken and re-broken to make final expected droplet sizes. With each successive breaking and re-breaking of the droplets, the energy required increases because the smaller the droplet the more it resists deformation. Consequently, these methods have in common some disadvantages such as high energy requirement, high shear and stress forces created, poor control...
over droplet sizes and uniformity [4]. These disadvantages could reduce/remove original potential of a specified drug delivery system and limit application range, especially when using sensitive components as drugs and carriers. Especially, they may make the scale-up step become challenging and less efficient.

Cross-flow membrane emulsification (CME), first developed in Japan in the late 1980s, is one of the promising emulsification techniques that could be used to overcome the above difficulties. In this method, mono disperse emulsion could be obtained by forcing the disperse phase through membrane pores into immiscible continuous phase that is flowing under the membrane surface [5]. Droplet detachment is known to be dependent on four main forces: shear, interfacial tension, inertia/pressure from the flow through the membrane and buoyancy. CME method can be used for large-scale production and has two main advantages as compared to other conventional emulsification methods. Firstly, it requires less energy input for the emulsifying process, so it could reduce negative influences when using sensitive ingredients. Secondly, more uniform and controllable droplets size could be achieved [6]. Besides CME method, many ideas have been tried as shown in figure 1.

Choice of membrane characteristics is crucial in CME. The most frequently used membranes are microporous glass and Shirasu porous glass as they have been commercially available for several years [7]. These membranes are characterized by cylindrical, interconnected micro-pores. Ceramic membranes $\alpha$–Al$_2$O$_3$ uncoated or coated with titanium oxide/zirconia have also been investigated. Droplet size obtained is calculated and experimented to be usually 2–10 times larger than membrane pore size. Pore size, inter-pores distance and surface hydrophobicity are important factors that greatly affect the emulsification success [8,9]. In a study, a stirred dispersion cell apparatus provided by Micropore Technologies Ltd had been used. Pore sizes of 20 and 40 $\mu$m were used to prepare PLGA microspheres with controllable diameters within 40–140 $\mu$m [10]. Other factors that could influence final particle sizes and morphology are: drug components, solvents, surfactants, velocity of disperse and continuous phases.

This study concerns CME method using nano and micro pore sizes silicon nitride membrane, which has not been mentioned before in research for drug-loaded particles fabrication. In the following experiments, we used poly($\alpha$, $\beta$-lactic-co-glycolic acid) (PLGA) as drug delivery material, curcumin as drug ingredient, chloroform/H$_2$O as phases for emulsification and polyvinyl alcohol (PVA) as surfactant. The purpose was to fabricate nanoparticles by means of CME method using silicon nitride membranes of different pore sizes to evaluate this new aspect and to compare with traditional stirring methods.

2. Experimental

2.1. Materials

Poly($\alpha$, $\beta$-lactic-co-glycolic acid) with co-polymerization ratios between lactic and glycolic is 50:50, molecular weight $\sim$ 4036 (PLGA); curcumin and polyvinyl alcohol (PVA) (99% hydrolyzed), molecular weight $\sim$ 9000 (PVA) were purchased from Sigma-Aldrich. All other solvents were of analytical grade.

The CME module was designed and made from steel. Silicon nitride membranes ($5 \times 5$ mm$^2$) with active surface ($3 \times 3$ mm$^2$ size, 1 $\mu$m thickness) pierced with very regularly distributed pores ($\phi$200 nm, 450 nm and 2 $\mu$m) were supported from Nanosens$^\text{®}$ company. The membrane holder was made from Teflon. The solvent pump HF-8367 was purchased from Headon$^\text{®}$. Epoxy glue was used to glue membranes onto the holder.

2.2. Experimental setup

The CME module setup is shown in detail in figure 2. The disperse phase was pushed from conserver through membrane into continuous phase by a nitrogen gas pump. Continuous
phase velocity was controlled by a water pump. Cross-flow velocity under the membrane and disperse phase rate through the membrane were kept constant to maintain a constant pressure over the membrane and a stable flow-rate in the system.

2.2.1. Membrane pre-treatment. In order to optimize best shearing condition for the droplets formation at the membrane surface, polarizations of solvents and membrane surface characteristics are crucial factors to be considered. Thus, membrane should have polarization close to that of the continuous phase and opposite to that of the disperse phase. These elements enhance shearing force at the membrane surface and lower adhesion of adjacent droplets formation, respectively. Silicon nitride membranes used in this study were very hydrophobic so they were not theoretically suitable for oil/water emulsion. Pre-treatment with piranha had been realized to increase the polarity of membrane surface. Membranes were treated by piranha solvent in 15 min then washed with distilled water.

2.2.2. Membrane setup. Pretreated membrane was glued onto the holder using thermal epoxy which was then treated at 120°C for 15 min. Solidification of glue was required overnight. After each experiment, the membrane was removed and reconditioned. Due to their fragility, membranes before and after each experiment were examined by optical microscopy to confirm their primordial status.

2.3. Preparation of curcumin-loaded PLGA microspheres
Curcumin and PLGA were dissolved in chloroform to obtain the disperse phase. Continuous phase was PVA in distilled water. Set up the CME system, run the water pump to circulate the continuous phase. After 5 min, open nitrogen gas flow to push the disperse phase through membrane. Disperse flow rate and continuous flow velocity were kept constant at about $2 \mu l s^{-1}$ and $20 cm s^{-1}$, respectively. After emulsification was finished, the emulsion was gently stirred overnight. The suspension was then cold-centrifuged at 15,000 rpm, 4 °C for 15 min and washed by distilled water twice before being freeze-dried to obtain final products.

2.4. Mechanical stirring method
The microspheres were also prepared by the mechanical stirring emulsification method to compare with membrane methods. The same amounts of all ingredients were prepared. The disperse phase was slowly dropped into the continuous phase that was being agitated at high speed. Then evaporating, centrifuging and freeze-drying steps were realized as above.

2.5. Characterization of curcumin loaded PLGA particles
2.5.1. Scanning electron microscopy (SEM) observation.
The morphology of particles was observed by scanning electron microscopy (model JSM-6480 LV from JEOL, Japan). Suspension was dropped on an SEM holder, air dried at ambient atmosphere. The sample was then coated by platinum (30 mA in 40 s) before observation.

2.5.2. Particle size distribution.
Particle size distribution of the suspensions was measured by particle size analyzer (model LB550 from Horiba). This measurement was used to evaluate the uniformity of the particles obtained.

2.5.3. Curcumin loading efficiency.
Curcumin content in the particles was determined using a UV–Vis spectrophotometer (model UV-1800, Shimadzu, Japan). 2 mg of dry product was re-solubilized in 2 ml of acetone and the solution absorbance was recorded at 430 nm. Curcumin content was calculated according to the following equation:

$$\text{Loading efficiency} = \frac{m_{\text{curcumin}}}{(m_{\text{dry product}} - m_{\text{curcumin}})} \times 100\%.$$ 

2.5.4. Fourier transform infrared spectroscopy (FTIR).
FTIR spectra of curcumin, PLGA and curcumin loaded PLGA particles were recorded by a FTIR spectrometer (model Tensor™ 37 from Bruker). The scanning range used was 400–4000 cm$^{-1}$ with 32 scans and resolution was set at 4 cm$^{-1}$. FTIR results will give information about interactions between curcumin and PLGA in microspheres.

3. Results and discussion
3.1. Effect of surface polarity of the membrane
Two experiments were realized under the same condition using ø2 μm membrane, one before and one after piranha treatment. After treatment, water contact angle of the membrane was reduced from over 90° (untreated membrane) to 33° (treated membrane). Results in SEM photographs and particle size distributions are shown in figures 3 and 4.
respectively. Pre-treatment gave a slightly improved result in particles’ morphology but no obvious difference in size distributions was observed. This could be explained by very close inter-pores distribution distance that could not prevent adhesion of adjacent former droplets. It could also be explained by surface hydrophilicity gradual loss during emulsification. Later experiments were realized without surface treatment, for the simplicity and the convenience.

3.2. Effect of PLGA concentrations on uniformity of particles

PLGA was prepared at various concentrations in chloroform (0.75, 1.0, 1.5 and 2.0%). Curcumin concentration (0.1%) and other conditions of CME procedure were kept unchanged. The membranes used had 2 µm of pore diameter. Effect of PLGA concentrations on final particle diameter and productivity is shown in table 1. When PLGA concentration was augmented, it increased the viscosity of the disperse phase so the droplet formation required more shear force to be cut off. In consequence, it will create bigger particles than at lower PLGA concentrations.

3.3. Effect of phases condition on uniformity of particles

Disperse/continuous phase proportion and PVA concentration were important factors to be considered. A series of PVA concentrations (0.25, 0.5, 1.0 and 2.0%) and disperse/continuous phase proportions (1/5, 1/10, 1/20 and 1/40) were realized. Other conditions of CME procedure were kept unchanged. Effect of these factors on final particle diameter is shown in tables 2 and 3. PVA concentration as shown in table 2 had an important role in protecting emulsion during the emulsification and solidification process. PVA concentration of lower than 1% was not enough to protect emulsion from adhesion, and that higher than 2% had too high viscosity that could not provide shear force efficiently. Disperse/continuous phase proportion was varied to obtain the best proportion efficiency and solvents economy. The result in table 3 shows that final particle diameter was kept unchanged when 1/20 of disperse/continuous phase proportion was reached.

From the results obtained above, the optimum condition for CME method was as follows: PLGA concentration 1%, curcumin concentration 0.1% in chloroform, PVA concentration 1% in water and chloroform/PVA solution was 1/20.

3.4. Comparison of CME and stirring mechanical method

Two experiments were realized using the same optimum condition obtained above but one was by CME method (membrane pore size 450 nm), and the other by mechanical stirring method. Results in SEM photographs are shown in figure 5. As compared with membrane emulsification method, particles prepared by stirring method were less uniform and their diameter distribution was larger.

3.5. Effect of membrane pore size on final particle diameter

Membranes of 200 nm, 450 nm and 2 µm pore sizes were used to compare its abilities to control the final particle diameter.
The other CME conditions were kept unmodified. Figure 6 shows the difference in particle size distribution obtained. Uniform particles (average diameter 1 µm) were fabricated by sub-micron membrane pore sizes (200 and 450 nm). The difference in particle diameter fabricated with these two pore sizes could not be clarified in this study. Less uniform particles were fabricated by 2 µm pore size in comparison with 200 and 450 nm pore sizes, but with more uniform distribution and reduced diameter compared to stirring method. This could be explained by uncontrollability of disperse phase rate through
2 µm membrane pore size. In consequence, disperse phase rate was changed uncontrollably during emulsification and usually very fast.

3.6. FTIR and curcumin loading efficiency

FTIR spectrum and test of curcumin loading efficiency were realized using dried powder of particles which was prepared using the CME method with 450 nm pore size membrane. In FTIR spectrum, specific peaks of curcumin, PLGA and loaded particle are explained in figure 7. In the loaded particle spectrum, there were no more than peaks observed in PLGA and curcumin spectrum. This demonstrates that there was no interaction observed between PLGA and curcumin molecules, thus we got a curcumin encapsulation by PLGA.

A standard curve of curcumin UV–Vis absorption was built up from 0 to 10 ppm, λ = 430 nm. Best curcumin loading efficiency was obtained with this dried powder which was 2.0%.

4. Conclusions

The principal aim of this study was to compare cross-flow membrane emulsification method to mechanical stirring method in fabricating uniformly distributed particles. In this work, encapsulation of curcumin by PLGA was performed using silicon nitride membrane. Although this new method needs much more set up investigation and specific membranes, the results obtained showed much better results. Particles obtained by CME method were more uniform and smaller than those of the conventional method. Membranes of different pores sizes (200 nm, 450 nm and 2 µm) were used successfully to control final particle diameter. Furthermore, membranes with larger inter-pores distances if available will promise much better and controllable results that should be considered.

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