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Preparation of emulsion for nutrient delivery using 3D printed microfluidic chips

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Abstract

Microfluidic chip with T junction channel configuration was fabricated using stereo lithography 3D printing technology. The printed chip was used to prepare emulsion for nutrient delivery. This paper deals with the preliminary studies done to fabricate a delivery system for co-delivery of curcumin and resveratrol. The effect of ratio between flow rates of aqueous and oil phases on emulsion stability was studied and flow rates of the two phases were optimized at 6 ml/h and 0.2 ml/h respectively. Low flow rate ratio between the two phases resulted in lower size of droplets of oil phase. The prepared emulsion was studied for its characteristics such as morphology, particle size, zeta potential, color, and entrapment efficiency. Homogeneous monodisperse emulsions were formed with particle size 528 nm and low PDI of 0.151. The zeta potential was found to be 31.4 mV. The L^* , a^* , and b^* were 70.77 ± 0.01 , -13.78 ± 0.04 , 42.81 ± 0.12 respectively. The emulsion also exhibited good entrapment efficiency of 65.11% and 58.40% for curcumin and resveratrol respectively. Hence microfluidic emulsification serves as low energy technique for preparation of monodisperse emulsion delivery system for nutrients.

Keywords: Preparation, emulsion, nutrient delivery, microfluidic

1. Introduction

Emulsions are systems where droplets of one liquid are scattered in another immiscible liquid. These are utilized in variety of industries including pharmaceutical, food, cosmetic, and petroleum industries. Emulsion based delivery systems can be used in better entrapment of nutrients, controlled release and targeted delivery. Conventionally, the formation of droplets is achieved through mixing or mechanical disintegration. But these techniques give poor control over droplet size, shape and size distributions in emulsions resulting in low stability. These parameters if controlled can be used to prepare wide variety of microstructures that can impart specific characteristics to emulsions (Maan *et al.*, 2015) [13]. In nutrient delivery these can be used to achieve better release and digestion. Precise fabrication of emulsions with defined microstructures can be achieved using microfluidics.

Microfluidics is the science of systems that process micro-level amount of fluids (10^{-9} - 10^{-18} L), using channels with any one dimension less than one millimeter (Farré *et al.*, 2012) [6]. Microfluidic emulsification helps in fabrication of monodisperse emulsions that can produce microspheres, microcapsules and microgels which can be used in encapsulating nutrients. The use of microfluidic devices for emulsification decreases reagent exhaustion, increases throughput and reduces fabrication costs. The droplet generation during microfluidic emulsification is influenced by channel configurations and flow patterns. T junction, Y junction and flow focusing junction are the most common channel types used in nutrient encapsulation using microfluidics. In T junctions, the external phase applies force on the internal phase when the two phases intersect at the T shaped junction. The higher flow rate of the continuous phase destabilizes the interfacial tension force and as the pressure elevates the protruded droplet is pushed out. The T junction devices are easy to print and handle. These forces create a drift which eventually squeeze out the droplet (Gupta & Kumar, 2010) [7]. Over the past decade studies in microfluidic emulsification have increased tremendously.

Traditionally manufacturing of microfluidic devices were complex and time consuming. Soft lithography technique was used commonly in which first a master is manufactured using microfabrication in a cleanroom from a 2D photomask which is followed by soft lithography and binding. However this technique requires high expense related to infrastructure, equipment and maintenance.

Also for different configuration of channels, first different master should be created. This multi-step procedure hampers the research in microfluidics (Rogers *et al.*, 2015) [6]. The development in 3D printing technology and increase in printing resolution aids in one step printing of microfluidic chips. In an alternative approach the master mold can be created by 3D printing which can be followed by soft lithography (Chan *et al.*, 2015) [2]. This reduces the need of cleanroom facilities for microfabrication which significantly reduces the overall cost of operation. It increases the ease of operation where different configurations of microfluidic devices can be created rapidly (O'Neill *et al.*, 2014) [14]. There exist various types of 3D printing techniques including Fused Deposition Modeling (FDM), stereolithography (SLA), binder jetting, photopolymer jetting, material jetting, laser melting, laser sintering, electron beam melting and hybrid processes (Amin *et al.*, 2016) [1].

SLA is the first 3D printing technology that has been commercialized in 1988. Introduced by Chuck Hull, SLA uses UV light for curing photopolymers arranged in layer-by-layer manner on a built platform (Hull, 1984) [8]. There exist two types of configuration: free surface approach (bath configuration) and constrained surface approach (bat configuration). In both approaches, the liquid resin is photopolymerized in a layer-by-layer method using a scanning laser or a digital light projector. In bath configuration, a 2D cross-section is traced on to a substrate immersed in photoactive resin by UV radiation treatment. This 2D cross-section is further lowered into the resin and UV beam polymerizes the next layer over the first. In case of bat configuration, the object is fabricated by hanging it from a movable substrate which is placed over the resin tank. UV source located under the transparent tank cures the pattern (Waheed *et al.*, 2016) [20].

In this study we have fabricated a simple T junction using SLA 3D printing technology and prepared an emulsion system for curcumin and resveratrol delivery. Curcumin and resveratrol are important anti-cancer agents. These compounds exhibited synergistic effects on colon and breast cancer cells (Leena *et al.*, 2020) [11]. Zein colloidal particles were used in the experiment to produce stable, non-emulsifier oil-in water emulsions (De Folter *et al.*, 2012) [5]. The microfluidic chip manufacturing is discussed and the effect of flow rate on emulsion formation is studied. The paper also discusses the emulsion characterization.

2. Materials and Method

2.1. Materials

General purpose UV resin was used in fabrication of microfluidic chip. Curcumin (crystalline) was purchased from HiMedia, Resveratrol from TCI chemicals and zein from Sigma Aldrich. MCT oil (C₈ 52% and C₁₀ 45%) was purchased from Luxura Sciences, Amazon India.

2.2. Fabrication of microfluidic chip

The channel configuration of the T junction microfluidic chip was designed using AutoCAD 2017 software in 3D modeling mode and converted into STL file. The chip dimension was fixed at 60*30 mm. The inlet and outlet channel diameter was 2±0.2 mm and the inner channels diameter was 1±0.2 mm which tapers to 0.6±0.02 mm diameter at the T junction [Fig. 1]. The microfluidic chip was fabricated at Spaar3D Creative, Coimbatore using Proton SLA 3D printer [Fig. 2]. The printer was equipped with 405 nm, 150 mW laser with a print speed

up to 300 mm/s. It is installed with Cura 2.6.1 for slicing the model and XYZware software for support generation. Once the required supports were added using XYZware the STL file was imported to Cura 2.6.1 for slicing. The sliced model was saved in the SD card as g codes and inserted into printer to start printing. The resin exposure time was set up by controlling the laser power and laser moving speed. General purpose UV resin was used at laser setting 58 at 200 to 100 mm/s laser moving speed. After printing the microfluidic chip was subjected to IPA bath.

2.3. Preparation of emulsion

The aqueous phase consisting of zein colloidal nanoparticles was prepared by liquid-liquid dispersion method. 100 ml of 5% zein solution in 80% ethanol was prepared. Curcumin (1 mg/g) was incorporated into this solution. This solution was then dispersed in 150 ml distilled water (pH 4) undergoing high speed homogenization (IKA T18 Digital Ultra Turrax) at 20000 rpm. The prepared colloidal solution was then subjected to centrifugation (Elektrocraft RC 4100F) at 1200 rpm for 10 min. Ethanol was removed using rotary evaporator (IKA RV 10) at 65°C. The obtained solution was again centrifuged (Elektrocraft RC 4100F) at 1200 rpm for 10 min to remove larger particles. MCT oil was selected as the oil phase in which resveratrol (1 mg/g) was dissolved using magnetic stirrer at 1200 rpm. The inlet of the microfluidic chip was attached to two syringe pumps to pump the aqueous and oil phase separately. The oil phase flow rate was fixed at 0.2 ml/h whereas the aqueous phase flow rate was varied from 2 ml/h to 8 ml/h at flow rate ratios 0.1, 0.05, 0.033 and 0.025 (Ushikubo *et al.*, 2014) [19] (Table 1). The optimized condition for emulsion preparation at room temperature was standardized and the emulsion characteristics were studied.

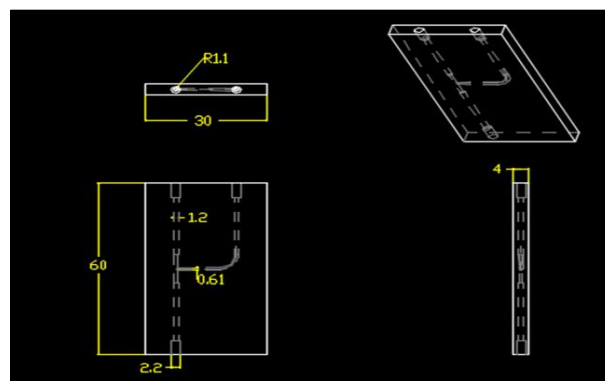


Fig 1: Auto CAD diagram of designed T junction

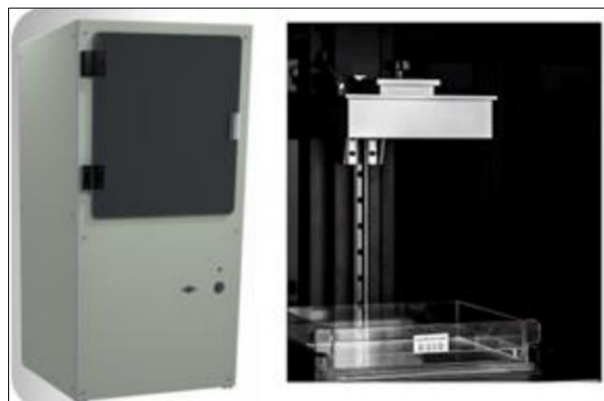


Fig 2: Proton SLA 3D printer

2.4. Emulsion characterization

2.4.1. Microscopic analysis

The microscopic view of emulsion was characterized using optical and fluorescent microscope (Radical Scientific RTC-7 Nx) at 485 nm. The sample was observed under 40X.

2.4.2. Color analysis

Color (CIE L^* a^* b^* ; where L^* -lightness, a^* -redness and b^* -yellowness) of the emulsion was evaluated using colorimeter (Hunter Lab Color Flex EZ). The high b^* values indicate that nutrient degradation was less in microfluidic emulsification (Zheng *et al.*, 2017) [21].

2.4.3. Particle size and zeta potential analysis

The particle size distribution and poly dispersity index (PDI) of optimized emulsion stored at room temperature was determined using particle size analyzer (AccuSizer, USA) by dynamic light scattering technology. The electrical charge (ζ potential) of the emulsion was determined using Zetasizer (Malvern Instruments, U.K.).

2.4.4. Entrapment efficiency

The entrapment efficiency (EE) of curcumin and resveratrol was determined as described by Chen *et al.*, (2020) [2, 3, 21] with slight modifications. 1 ml of emulsion was diluted in 80% ethanol solution. The curcumin and resveratrol was extracted by ultrasound assisted method (Labman PRO 650) and was then analyzed using a microplate reader (Molecular Devices SpectraMax iD3) at 426 nm and 307 nm. The EE was calculated using the following equations.

$$EE_{\text{curcumin}} = (\text{Encapsulated curcumin}/\text{Total curcumin}) \times 100 \quad (2.1)$$

$$EE_{\text{resveratrol}} = (\text{Encapsulated resveratrol}/\text{Total resveratrol}) \times 100 \quad (2.2)$$

3. Results and Discussions

3.1. Fabrication of microfluidic chip

The designed T junction microfluidic chip was printed using Proton SLA 3D printer by varying at different print speed from 200 mm/s to 100 mm/s. General purpose resin (red color) was used for printing. Printing speed of 100 mm/s was optimized as best considering the overall stability, channel size, and blockage [Fig. 3]. With higher resolution printers, microfluidic chips with lower (10-100 μm) channels can be fabricated. With lower channel sizes better fluid control and manipulation can be done to create complex structures of emulsions.

3.2. Emulsion preparation

The oil phase flow rate was fixed at 0.2 ml/h and the aqueous phase flow rate was varied from 2-8 ml/h. Table 2 gives the stability of emulsions in terms of phase separation at varied flow rates at three different storage conditions (29 ± 2 °C, 4 ± 1

°C, and 38 ± 2 °C) for Day 1. At flow rate ratio 0.033 with aqueous phase flow rate at 6 ml/h and oil phase flow rate 0.2 ml/h good stability was obtained. The oil content at flow rate ratio 0.025 was less; hence the flow rate ratio 0.033 was optimized as ideal.

At lower flow rate ratio the droplets formed at the T junction were larger than the droplets formed at higher flow rates. This higher size of droplets resulted in lower stability of emulsion. Ushikubo *et al.* (2014) [19] also observed similar results, where smaller droplet sizes were achieved at higher flow rate of continuous phase. At lower flow rate ratio, the friction between the two phases increases leading to droplet detachment. Low flow rate ratio is related to high relative velocity, this increases the stress between the phases in the surrounding.



Fig 3: T junction microfluidic chip

3.3. Emulsion characterization

3.3.1. Microscopy

The microscopic views of optimized emulsion at 0.033 flow rate ratio under optical and fluorescent microscopy at 485 nm at 40X magnification are given in Figure 4b and 4c. The morphology and size distribution of prepared emulsions is depicted in the images. The T junction produced homogeneous droplets with low PDI.

3.3.2. Color

The L^* , a^* and b^* values of the emulsion [Figure 4a] was found to be 70.77 ± 0.01 , -13.78 ± 0.04 , 42.81 ± 0.12 respectively.

Table 1: Phase separation in emulsions during three storage temperature conditions for day 1 storage

Flow rate (ml/r)		Phase separation		
Aqueous phase	Oil phase	29 ± 2 °C	4 ± 1 °C	38 ± 2 °C
8	0.2	-	-	-
6	0.2	-	-	-
4	0.2	-	-	+
2	0.2	+	+	+

+ indicates phase separation and – indicates no phase separation

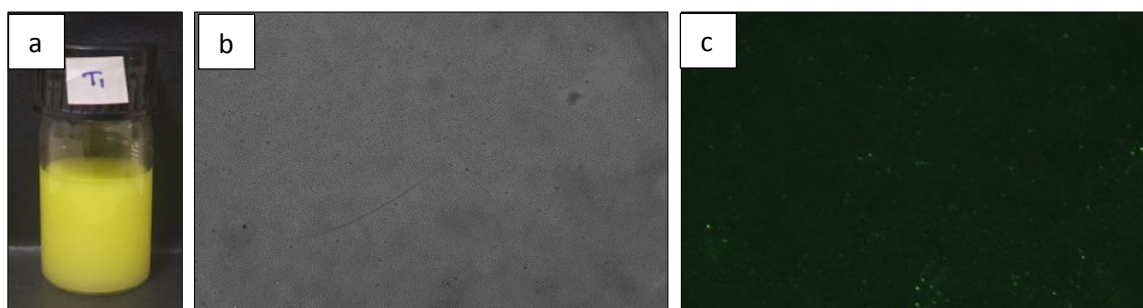


Fig 4: (a) Optimized emulsion at 0.033 flow rate ratio (b) Optical microscopy image (c) Fluorescent microscopy image

3.3.3. Particle size and zeta potential

The particle size of the emulsion was observed to be 528 nm with a PDI of 0.151 [Figure 5]. The low PDI of the emulsion is an important characteristic of microfluidic emulsification (Sugiura *et al.*, 2002) [18]. Other emulsifying techniques such as high speed homogenization, high pressure homogenization, ultrasound and microfluidization produces emulsions with higher PDI which affects its stability and nutrient release properties. Kobayashi *et al.* (2005) [9] prepared a water-in-oil-

in-water emulsion using two-step microfluidization and straight through microfluidics. The emulsions prepared with microfluidic system produced droplet sizes with less than 5% coefficient of variation whereas microfluidization produced droplet sizes with 45-53% coefficient of variation (Kobayashi *et al.*, 2005) [9]. The ζ potential of the emulsion was found to be 31.4 mV. Previous studies showed that zeta potential values around 30 mV indicates stable emulsions (Chen *et al.*, 2020; Dai *et al.*, 2018; Liu *et al.*, 2018) [2, 3, 21].

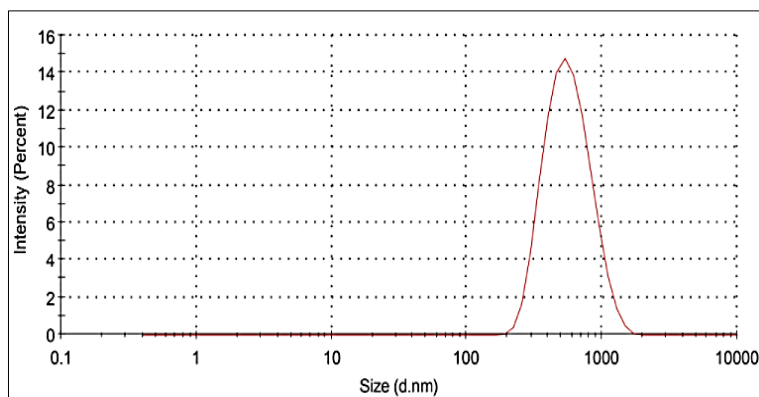


Fig 5: Droplet size distribution of prepared emulsion

3.3.4. Entrapment efficiency

The curcumin and resveratrol entrapment efficiency was found to be 65.11 and 58.40% respectively. This was in agreement with results obtained by Lababidi *et al.* (2019) [10]. The authors compared the encapsulation efficiency of curcumin loaded in PLGA using microfluidics, conventional syringe pump approach and hand injection. Microfluidic emulsification entrapped highest amount of curcumin (67%) followed by syringe pump (50%) and hand injection (30%) (Lababidi *et al.*, 2019) [10]. Further studies such as morphology, digestion studies, nutrient release studies, and storage studies should be analysed to prove the efficiency the emulsion prepared.

4. Conclusion

Microfluidics is an emerging branch of science that deals with small amount of fluids in sub millimetre range where surface forces are higher than volumetric forces. This can be used in precise control of fluids to produce emulsions for delivery of nutrients. Control of flow rates of the two phases helps in preparation of stable emulsions. High relative velocity of the two phases produces smaller droplets of dispersed phase in the emulsion resulting in better stability. The control of fluid flow in microfluidic emulsification helps in preparation of homogeneous emulsions with low poly dispersity index and high entrapment efficiency. Further this technique can be used in preparation of multi-layer emulsions with enhanced entrapment efficiency and release properties. With advanced 3D printing techniques with higher resolution of printing, the channel size can be further reduced and better control of droplet size and shape can be achieved. This study should be explored further to better understand the encapsulation and release of nutrients during microfluidic emulsification.

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