Research Article

Continuous Encapsulation of Vitamin E Using Polycaprolactone and Tween 20 in a Micro-Channel

Amaraporn Kaewchada and Rotsaman Chongcharoen
Department of Agro-Industrial Food and Environmental Technology, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

Preuk Tangpromphan
Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand

Khwanchanok Nakkong and Attasak Jaree*
Central of Excellence on Petrochemical and Materials Technology, Department of Chemical Engineering, Faculty of Engineering, Kasetsart University, Bangkok, Thailand

* Corresponding author. E-mail: fengasj@ku.ac.th DOI: 10.14416/j.asep.2022.01.001
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Abstract
Encapsulation of vitamin E is the preservation of the biological activities of vitamin E for various applications. In the first part of this research, factors affecting the batch encapsulation of vitamin E, including PCL concentration, the concentration of Tween 20, and the volumetric ratio of aqueous phase to organic phase were experimentally investigated. The Box-Behnken experimental design and response surface methodology were implemented to determine the optimal operating conditions of the batch encapsulation. At the optimal conditions, the percentage of vitamin E encapsulation (%EC) was 98.69%, using the PCL concentration, the Tween 20 concentration, and the volumetric ratio of aqueous phase to organic phase of 3.6 g/L, 0.6 g/L, and 0.9 mL: 1 mL, respectively. The second part is to enhance the productivity by applying the optimized formulation of vitamin E encapsulation in a continuous process using a micro-channel encapsulator. The effect of residence time was investigated. At the residence time of 1 s, the percentage of vitamin E encapsulation of 97.28% and the productivity of 153.61 mg/(mL·min) were achieved.

Keywords: Vitamin E, Micro-channel, Encapsulation

1 Introduction

Derivatives of vitamin E, such as α- ß- γ- δ- tocopherol and tocotrienol have been widely used in food supplements and pharmaceutical products. This was due to the strong bioactivity and anti-oxidation property, which can protect biomolecules and tissues from oxidation [1]–[3]. Vitamin E is also used in numerous cosmetic applications due to its outstanding anti-aging properties, reducing fine lines and wrinkles, and UV protection [4], [5]. Moreover, there have been reports on the health benefits of vitamin E associated with the prevention of carcinogenesis and neurological diseases, diabetes, and cardiovascular disease [2]–[4], [6], [7]. Among different derivatives, α- tocopherol has been the most wildly used, owing to the highest strength of bioactivity.

Vitamin E, a natural compound that can readily dissolve in fats, is sensitive to light, heat, and oxygen exposure. Its hydrophobicity hinders effective use, especially for applications involving the vascular system [2], [8], [9]. Therefore, the preservation of vitamin E from inappropriate conditions prior to the application is necessary. Various techniques involving emulsion

and encapsulation of vitamin E have been reported, such as micro-emulsion [6], [10], nano-emulsion [11]–[14], nano-dispersions [1], [8], nano-structured lipid particles [5], [15], oil-in-water emulsion [16], [17], and biopolymer-based nanoparticles [9], [18]. Efficient methods to preserve the bioactive compounds are crucial for developing food and pharmaceutical products. Some techniques can be used for the controlled release mechanism, in which the target compound can be precisely released under the appropriate conditions [9], [19], [20].

Several studies exploring the encapsulation of vitamin E have been reported. For example, Somchue *et al.* prepared the alginate coated-protein based-encapsulated α-tocopherol (α-TOC) using two different proteins including β-Lactoglobulin and hen egg white protein. The improvement of the encapsulation efficiency of 85.2% was achieved as compared to 31.9% for the case without alginate coating [18]. Luo *et al.* used chitosan as wall material for the preparation of α-TOC encapsulated in the form of nanoparticles. The encapsulation efficiency in the range of 76 to 86% was achieved and the zein/CS complex greatly improved the controlled release properties of α-TOC under gastrointestinal conditions [9]. Sharipova *et al.* encapsulated vitamin E using polymer–surfactant (anionic polyelectrolyte sodium polystyrene sulfonate, PSS, and cationic surfactant dodecyl trimethyl ammonium bromide, DoTAB) as an electrosteric emulsion stabilizer and as a charged precursor for the subsequent shell assembly. The encapsulation efficiency of 47% was obtained [6].

It is crucial for large-scale production that it must seek for new methods providing high efficiency and productivity to maintain the viability of process. Most of the techniques as mentioned above for encapsulation of vitamin E were regarded as batch processes, which are inherently associated with low productivity and difficulty for scaling up the production. It is conceivable that the application of microtube or microchannel as a continuous encapsulator can potentially improve the process of encapsulation of vitamin E in many aspects. The high efficiency of encapsulation can substantially reduce the footprint of process due to the excellent rate of diffusion (characteristics for microchannel). This also provides the flexibility for scaling out to suite the required production capacity. Successful demonstrations have been reported for various applications of microchannel, such as biodiesel production, gas absorption, etc. [21]–[24].

This work involved the parametric study for the encapsulation of vitamin E in a batch system. The effect of operating conditions, including PCL concentration, concentration of Tween 20, and the volumetric ratio of the aqueous phase and organic phase on the encapsulation efficiency was investigated. The Box-Behnken design (BBD) was applied for collecting the experimental data and the optimal operating condition was evaluated by using response surface methodology (RSM) [25]–[27]. The obtained optimal operating condition was adapted for the continuous encapsulation using a microchannel. The effect of residence was also studied as a possible means to boost the productivity.

2 Materials and Methods

2.1 *Materials*

α-tocopherol (>96%) and polycaprolactone (PCL) with a molecular weight of 14,000 were purchased from Sigma Aldrich (St. Louis, USA). Tween 20 was obtained from Merck (Darmstadt, Germany). Distilled deionized water (DDW) with a resistance of 18 MΩ was used as a solvent for Tween 20. Acetone (AR) and methanol (HPLC) were purchased from Merck (Darmstadt, Germany).

2.2 *Preparation of aqueous and organic phases*

The organic solution was prepared by dissolving α-tocopherol and PCL in acetone. The concentration of vitamin E was 5 g/L and the concentration of PCL was varied in the range of 1–6 g/L. The solution was analyzed by high-performance liquid chromatography (HPLC) to obtain the concentration of vitamin E. The aqueous solution was prepared by dissolving Tween 20 in DDW, with the concentration varying from 0.2–1 g/L using vortex mixer.

2.3 *Batch encapsulation*

The aqueous solution was mixed with the organic solution previously prepared, using a temperature-controlled shaker (Grant-Bio PHMT Thermoshaker, Cambridgeshire, United Kingdom). The volumetric

ratio of the aqueous phase to the organic phase was varied in the range of 0.25 : 1.00 to 1.00 : 1.00. Note that the total volume was kept at 1.50 mL. The shaker was operated at the rotational speed of 650 rpm and at the temperature of 40 °C. Each experiment was carried out for 5 min. The mixture was centrifuged at 15,000 rpm for 10 min and the solution was analyzed for the amount of vitamin E. This was used to evaluate the encapsulation efficiency as shown in Equation (1).

$$\%EC = \frac{C^I V^O - C^R V^S}{C^I V^O} \times 100 \quad (1)$$

C^I is the initial concentration of vitamin E in the organic solution (g/L) and V^O is the volume of organic phase (mL). C^R is the concentration of vitamin E in the supernatant liquid obtained after the centrifugal separation (g/L). V^S is the volume of supernatant liquid collected after centrifugal separation (mL). Encapsulated particles were purified by washing with DDW. The productivity of the encapsulation was calculated from Equation (2).

$$\text{Prod} = \frac{C^I V^O - C^R V^S}{V^W t_b} \quad (2)$$

V^W is the total volume of the mixture. t_b is the period time for the batch encapsulation (5 min). The Box-Behnken design was applied to investigate the effect of various parameters on the performance. The range of each parameter is shown in Table 1. All conditions were performed with a full replication. The software package used for the determination of batch optimal condition was MINITAB version 18.

Table 1: The ranges of parameters used for the optimization of operating conditions

Variables	Unit	Levels		
		–1	0	1
X_1	(g/L)	1.0	3.5	6.0
X_2	(g/L)	0.2	0.6	1.0
X_3	(mL/mL)	1:0.25	1:0.625	1:1
%EC	(%)	Optimize		

Concentration of PCL: X_1
Concentration of Tween 20: X_2
A/O ratio: X_3

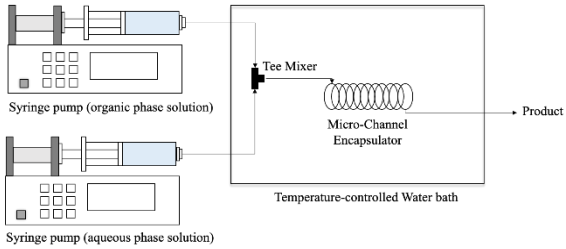

Figure 1: Schematic diagram of the continuous encapsulation of vitamin E in a microchannel.

2.4 *Continuous encapsulation*

The schematic diagram of our continuous encapsulator is shown in Figure 1. Two syringe pumps were used to individually introduce streams of organic phase and aqueous phase. The two streams combined at the T-mixer and the mixture propagated through the microchannel for a specific time called "residence time". The microchannel, made of polyether ether ketone or "PEEK" with an internal diameter of 0.5583 mm and the length of 90 cm, was placed in a water bath equipped with temperature control and internal circulation. The temperature was kept at 40 °C for all experiments. The flow rate of each phase was obtained from the optimal operating conditions of the batch encapsulation (section 2.3). In this set of experiments, the residence time was varied from 5 min to 1 s by simultaneously adjusting the flow rate of all input streams. Hence, the flow rate ratio was kept constant. At the steady state, the sample was collected for HPLC analysis. The encapsulation efficiency and productivity were evaluated by Equations (3) and (4), respectively.

$$\%EC = \frac{C^F Q^O - C^{RP} Q^P}{C^F Q^O} \times 100 \quad (3)$$

$$\text{Prod} = \frac{C^F Q^O - C^{RP} Q^P}{V^T} \quad (4)$$

C^F is the concentration of vitamin E in the organic solution (g/L). Q^O is the volumetric flow rate of the organic phase (mL/min). C^{PR} is the concentration of the supernatant liquid collected after the centrifugal separation (g/L). Q^P is the volumetric flow rate of the mixture exiting the microchannel (mL/min). %EC is the encapsulation efficiency (%). V^T is the volume of microchannel (mL). Again, the experiments were

performed with full replication.

2.5 *Analysis of vitamin E*

To quantify the amount of vitamin E in the samples obtained from batch and continuous encapsulation, an HPLC system (Knauer, Germany) consisting of S-1050 HPLC pump, S-3950 autosampler, column oven, and DAD detector was used. The analytical column was SUPELCOSILTM LC-18 (250 mm × 4.6 mm) with the particle size of 5 µm, purchased from SUPELCO Analytical (Pennsylvania, USA). The injection volume was 10 µL. Methanol was used as a mobile phase at a flow rate of 1 mL/min for isocratic elution. The temperature was kept constant at 40 °C in the oven and the detector wavelength was at 295 nm.

3 Results and Discussion

3.1 *Batch encapsulation*

According to the Box-Bhenken design, a total of 15 batch experiments for the encapsulation of vitamin E were performed and the results are summarized in Table 2, along with the calculated productivity for each experiment. The suspension of encapsulated particles was observed during the washing procedure, indicating the low solubility in water. However, the encapsulated particles released vitamin E to the solution upon exposure to the organic solvent i.e., acetone or methanol. This suggested that the shell was the material that can readily dissolve in the organic solvent. Hence, in this work, it can be postulated that PCL acted as a shell material for protecting the encapsulated vitamin E. Tween 20 was a co-chemical agent linking between PCL and vitamin E.

The effects of PCL concentration, Tween 20 concentration, and A/O ratio is presented in Figure 2. There was no apparent relationship of encapsulation efficiency on the plane of X_1 and X_2. Figures 2(b) and (c) also show that X_1 and X_2 did not cause significant changes in the encapsulation efficiency. On the contrary, it can be observed that high-performance encapsulation was obtained at the center or a high level of X_3. Increasing the A/O ratio significantly enhanced the encapsulation efficiency. We calculated the molar ratios of Tween 20 : PCL and Tween 20 : vitamin E to correlate with the encapsulation efficiency as shown in Figure 3. No distinct relationship between these ratios and the encapsulation performance was found except that the conditions providing low encapsulation efficiency were at the A/O ratio of 1 : 0.25. This was probable that, at small A/O ratios, the binding of Tween 20 was hindered by the relatively poor mixing compared to other conditions.

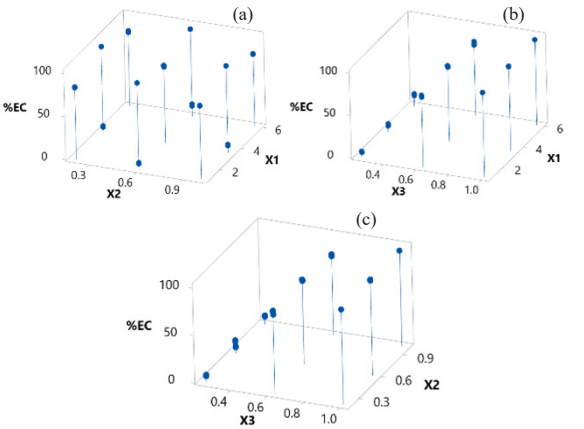

Figure 2: Effect of variable pairs on encapsulation efficiency (a) X_1–X_2 (b) X_1–X_3 and (c) X_2–X_3.

Table 2: The efficiency and productivity of batch encapsulation

X_1	X_2	X_3		% EC	Prod
		Org	Aq		(mg/mL· min)
1.0	0.2	1.0	0.625	81.59 ± 0.53	0.500
3.5	0.2	1.0	0.250	5.59 ± 0.95	0.045
3.5	0.2	1.0	1.000	98.16 ± 0.03	0.491
6.0	0.2	1.0	0.625	84.87 ± 1.03	0.521
1.0	0.6	1.0	0.250	4.90 ± 0.88	0.039
1.0	0.6	1.0	1.000	98.20 ± 0.08	0.491
3.5	0.6	1.0	0.625	87.57 ± 0.33	0.537
3.5	0.6	1.0	0.625	86.57 ± 0.81	0.531
3.5	0.6	1.0	0.625	87.55 ± 0.18	0.537
6.0	0.6	1.0	0.250	11.05 ± 1.27	0.088
6.0	0.6	1.0	1.000	99.36 ± 0.03	0.497
1.0	1.0	1.0	0.625	83.34 ± 0.16	0.511
3.5	1.0	1.0	0.250	6.80 ± 0.72	0.054
3.5	1.0	1.0	1.000	98.87 ± 0.01	0.494
6.0	1.0	1.0	0.625	81.70 ± 0.37	0.501

The response surface methodology was applied to analyze the effect of concentration of PCL, the concentration of Tween 20, and the volumetric

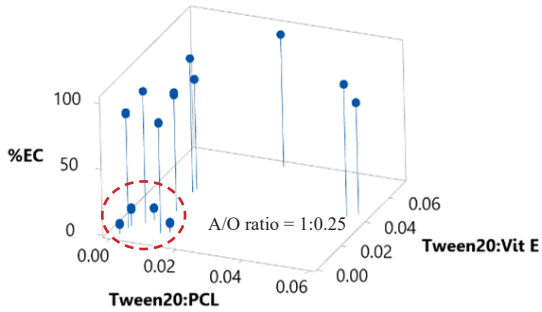

Figure 3: 3D plot of %EC.

ratio of the aqueous phase and organic phase on the encapsulation efficiency. Response surface regression was performed using linear model, linear + squares model, linear + interaction model, and full quadratic model. Among these models, the latter provided the highest r-squared and adjusted r-squared of 0.9995 and 0.9993, respectively. The model is described as Equation (5).

$$Y = -95.26 + 3.886\,X_1 + 25.13\,X_2 + 413.34\,X_3 - 0.2670\,X_1{*}X_1 - 16.79\,X_2{*}X_2 - 228.87\,X_3{*}X_3 - 1.230\,X_1{*}X_2 - 1.330\,X_1{*}X_3 - 0.84\,X_2{*}X_3 \quad (5)$$

Y is the encapsulation efficiency. This correlation can be used to determine the optimal operating conditions based on the encapsulation efficiency. The response surface plots of encapsulation efficiency and the operating parameters are shown in Figure 4. These results were in line with the 3D plots of the experimental data in Figure 2. The encapsulation efficiency strongly depended on the A/O ratio. The highest %EC was obtained at the upper boundary of A/O ratio (1.0), while PCL concentration slightly affected the %EC. Note that the %EC was above 98% at the A/O ratio of unity, which was considered as high efficiency in comparison with other studies [5], [6], [9], [15], [18], [21], [22]. The pareto plot as shown in Figure 5 indicates the magnitude and the importance of the effects, according to Equation (5). Factors crossing the vertical reference line are statistically significant at the 0.05 level and vice versa.

The predicted optimal operating conditions at the concentration of PCL of 3.6 g/L, concentration of Tween 20 of 0.6 g/L, and the A/O ratio of 0.9, providing the encapsulation of 100%, were experimentally validated. The results are summarized in Table 4.

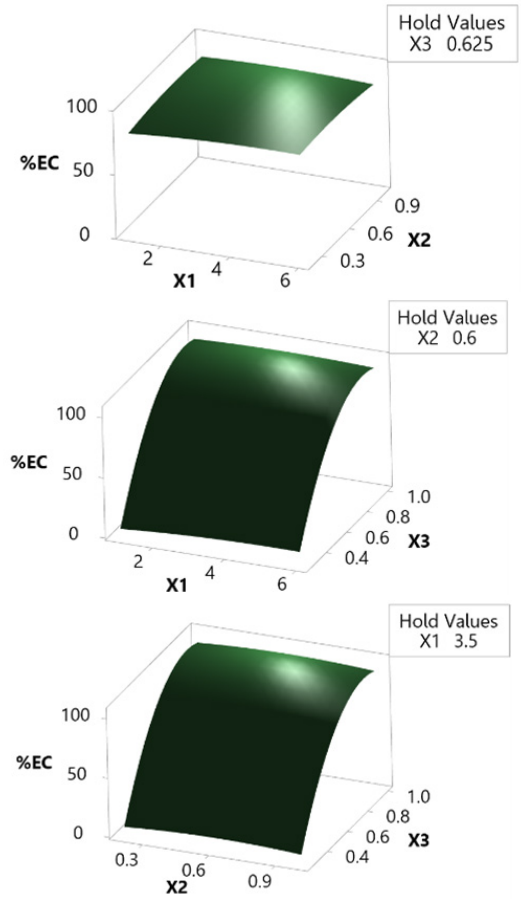

Figure 4: Response surface of encapsulation efficiency.

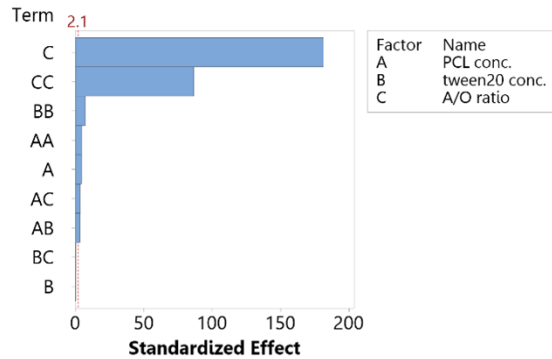

Figure 5: Pareto chart of the standardized effect.

Therefore, the model prediction for encapsulation efficiency was sufficiently accurate, with an error of 4.89%.

Table 4: Experimental verification of batch encapsulation at the optimal operating conditions

% EC		Productivity		
Experimental	Predicted	(mg/(mL·min))		
Average	SD	Average	Average	SD
98.69	0.0002	100.00	0.520	0.000001

3.2 *Continuous encapsulation*

The optimal operating conditions obtained from the batch encapsulation were used as a basis for determining the operating conditions of the continuous encapsulation using a T-microchannel. The residence time was varied from 5 min to 1 s to increase the process's productivity. The flow rates of organic solution and aqueous solution were simultaneously adjusted to maintain the A/O volumetric ratio. Table 5 summarizes the operating conditions for this set of experiments. The higher the flow rate, the shorter the residence time. The concentration of PCL was 3.6 g/L and the concentration of Tween 20 was 0.6 g/L. The first experiment was performed at the residence time of 5 min in order to compare the performance to that of the batch encapsulation. At this condition, the productivity of 0.52 mg/mL·min as well as high encapsulation efficiency of 98.12% were achieved. Then, the flow rates of the input stream were increased. It was found that all experiments yielded high encapsulation efficiency exceeding 93%, as shown in Figure 6. Decreasing the residence time from 5 min to 0.5 min increased the productivity to 5.15 mg/mL·min (more than tenfold of the productivity of the batch process) without a significant impact on the %EC. On the contrary, the productivity increased slightly according to Equation (4). The %EC markedly dropped to 95.74% upon decreasing the residence time to 0.25 min. This was attributed to the shorter residence time inside the microchannel. This effect was more pronounced for further decreasing the residence time to 0.083 min. The %EC of 93.97% and the much-increased productivity of 29.68 were obtained. For the residence time of 1s (0.017 min), the %EC was significantly improved to 97.28% due to the more intensified mixing as the two input streams at the T-mixer were fed at higher flow rates. At this condition, it was possible that the dispersed flow contained smaller droplets compared to other conditions with smaller feed rates. The productivity

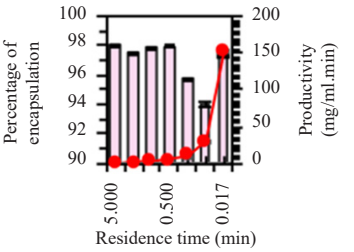

Figure 6: Effect of residence time on the encapsulation efficiency.

markedly increased to 153.61 mg/mL·min, which was approximately 295 times greater than that of the batch encapsulation. Therefore, the optimal residence time for our continuous encapsulation was 1 s.

Table 5: Operating conditions of the continuous encapsulation in the microchannel

Flow Rate (mL/min)			Residence Time (min)
Organic	Aqueous	Total	
0.023	0.021	0.044	5.000
0.039	0.035	0.073	3.000
0.116	0.104	0.220	1.000
0.232	0.209	0.441	0.500
0.464	0.417	0.881	0.250
1.392	1.252	2.644	0.083
6.958	6.262	13.220	0.017

3.3 *Comparison of production performance*

The encapsulation performance of various systems including our micro-channel encapsulator were compared as summarized in Table 6. Different formulas in terms of carrier and wall materials have been used to encapsulate vitamin E. Most of them involved batch processing and the preparation of vitamin E encapsulated particles required a considerably longer period of time compared to our continuous encapsulation system (1 s). The encapsulation efficiency achieved using our system was relatively higher than those obtained from other batch systems. For large-scale production, issues for scaling up the batch system must be considered such as mixing behavior, controllability of the operating parameters, turnaround time, etc. Alternatively, parallel processing can be applied to adjust the production capacity using our proposed method. A configuration micro-channels can be designed in the form of stacks, which can be relatively more compact compared to the batch process.

Table 6: Comparison of encapsulation performance among various systems

References	System	Formulae	Time	%Encapsulation (%EC)
This research	Continuous	Organic phase : α-tocopherol and PCL in acetone Aqueous phase : Tween 20 in DDW	1 s (residence time)	92.48
[18]	Batch	Organic phase : hen egg white protein and α-tocopherol Aqueous phase : ZnCl2 and alginate solution	30 min	85.2
[9]	Batch	Organic phase : α-tocopherol, zein solution (dissolved in ethanol), and chitosan (dissolved in acetic acid) Aqueous phase : Tween 20 (dissolved in ethanol)	> 2 h	84.3
[6]	Batch	Oil phase : α-tocopherol dissolved in neutral oil miglyol 812 Aqueous phase : dodecyl trimethyl ammonium bromide and anionic polyelectrolyte sodium polystyrene sulfonate	> 40 min	47

4 Conclusions

The encapsulation of vitamin E was performed by using PCL as a shell material and Tween 20 as a co-chemical reagent in both batch and continuous systems. The design of the experiment and response surface methodology were used to optimize the operating parameters of the batch encapsulation, including the concentration of PCL, concentration of Tween 20, and the A/O ratio. The encapsulation efficiency of 98.69% was achieved at the optimal conditions (concentration of PCL of 3.6 g/L, concentration of Tween 20 of 0.6 g/L, and A/O ratio of 0.9). These conditions were adapted for the continuous system using a T-micro-channel as an encapsulator. The effect of residence time was demonstrated in the range of 5 min to 1 s. The highest productivity of 153.61 mg/mL min (295 times greater than that of the batch encapsulation) was obtained at the residence time of 1 s. These results can be used as a foundation to scale up to the desired production capacity, by numbering up the microchannels for parallel processing.

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