Expert Opinion

Step emulsification: high-throughput production of monodisperse droplets

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Step emulsification is a promising method for the production of monodisperse droplets. Its main advantage is that its geometry allows massive parallelization of multiple nozzles to achieve high-throughput production of droplets. As the droplet generation process in step emulsification is mainly driven by interfacial tension rather than high energy shear stress systems, the droplet size is independent of the flow rates of both the continuous and the dispersed phases. Therefore, the high productivity of droplets (>100 l/h) and an excellent diameter coefficient of variation (<5%) can be guaranteed simultaneously, which makes step emulsification a potential tool for real industrial applications. This article provides an overview of step emulsification and discusses the existing challenges that can potentially impact the practical use of this tool; it concludes with perspectives and potential applications.

> *"Step emulsification can be useful for applications that need large-scale production of monodispersed droplets or encapsulating perishable samples."*

KEYWORDS

high throughput • microchannel arrays • microfluidics • monodisperse droplets • spontaneous transformation • step emulsification

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STEP EMULSIFICATION

The step emulsification was first proposed by Kawakatsu *et al.* in 1997 [1]. It is a droplet microfluidics technique that offers the detection and precise control of two incompatible fluids at the microscale. Step emulsification can produce monodispersed emulsion droplets, ranging from the submicron range to around 1000 μm [2,3], with a small coefficient of variation (CV). The use of the monodispersed emulsion droplets expands the applications of medicine [4], biotechnology [5–7] and biology [8,9]. Monodispersed droplets are also applied in the food [10], cosmetics [11] and chemical industries [12]. The CV of droplets is typically <5% in many step emulsification studies as the standard CV [13–15]. Using droplets with a small CV as miniature test tubes can improve the standardization and predictability of assays and increase the signal-to-noise ratio. Another merit of step emulsification is that it is relatively easy to parallelize to produce uniform droplets with high throughput [16–18]. At this time, a variety of encapsulated products can be produced using step emulsification, ranging from single to multiple emulsions, microcapsules, microspheres and many others [10,19–21].

DROPLET GENERATION IN STEP EMULSIFICATION

In step emulsification, droplets are generated by the spontaneous transformation of an oil–water interface [22], with the flow of the dispersed phase into the continuous phase through a rectangular microchannel [16–23], as shown in Figure 1. Microchannel arrays have been fabricated on a variety of substrates, such as a silicon-on-insulator and single silicon crystal, owing to the surface properties that can easily be modified using hydrophilic and hydrophobic treatments [24]. Microchannel arrays can also be produced with materials such as borosilicate glass, expanding the applicability to chemically aggressive fluids [17,25,26]. Because of the spontaneous droplet generation process, step emulsification is highly energy efficient, with typical energy input. However, the mild droplet generation in step emulsification, with no involvement of energy, makes it a preferable system to prevent denaturation of sensitive bioactive compounds [10,27]. Because the droplet generation process in step emulsification is mainly driven by interfacial tension, rather than high-energy shear stress systems, the droplet size is independent of the flow rates of both the continuous and the dispersed phases [14,28]. Therefore, there is no requirement for expensive gas regulators or precision pumps for preparing a monodispersed emulsion. The step emulsification devices can even be operated by manual injection of the dispersed phase to produce droplets with a small CV. Moreover, there are numerous papers on step emulsification with varied nozzle type and geometry. These nozzle variations significantly influence the degree and type of droplet size dependency on parameters such as volume flow rate. Although in most cases the mechanism of the droplet breakup itself is independent of dispersing and continuous volume flows, the droplet size can be influenced by the disperse flow rate. The dependency is smaller compared with other microfluidic droplet generation methods; however, it is significant [13]. The degree of dependency varies with type and the geometry of the step (straight/trapezoid/terraced/millipede etc.) [28,29]. Because of the importance of a stable and monodispersed droplet generation for industrial applications, these phenomena must be taken into consideration when designing new microreactors.

The droplet generation process in step emulsification has been evaluated using a variety of simulation methods such as

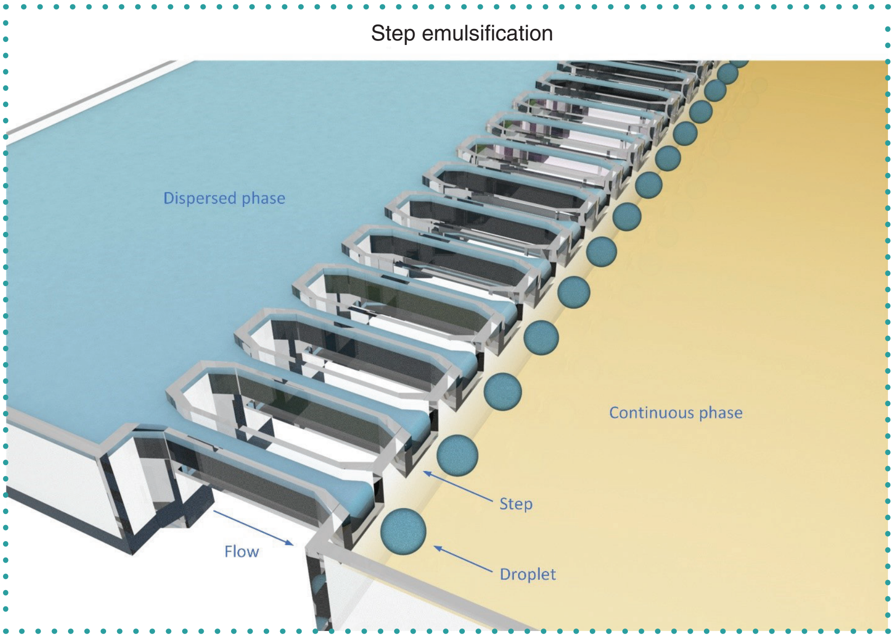

Figure 1. Step emulsification and parallelization: high-throughput production of monodisperse droplets.

computational fluid dynamics [30] and Lattice Boltzmann methods [31,32] and using the experimental approach with high-speed microscopy [17]. They all conclude that the nozzle height determines the maximum production rate and diameter of the monodisperse droplet in step emulsification device. Even though the nozzle height restricted the maximum production rate and the diameter of the droplets, numerous scientific works have already been done to address some of the challenges in increasing the production rate. Xu *et al.* reported a high aspect ratio (>3.5) step emulsification, which can produce 15,000 droplets per second with 2000 nozzles, with a CV below 3% [33]. Schuler *et al.* demonstrated a straightforward step emulsification system without any oil flow, which uses centrifugal forces to produce 500 droplets per nozzle (parallelization with 23 nozzles) with a CV between 2 and 4% [34]. Recently, Schuler *et al.* demonstrated a step emulsification system, which employed buoyancy in the centrifugal field to realize increased droplet generation of 2800 droplets per second and nozzle, with a CV below 5%. Droplet generation rates are about a factor eight above the critical capillary number. The main advantage is that a single nozzle is used in which manufacturing tolerances do not influence droplet generation rates [35]. Dangla *et al.* reported a droplet microfluidics system driven by gradients of confinement, single droplet generation as well as high throughput is demonstrated. Although the methodology is not strictly the step emulsification, the gradient confinement method is very close to it because droplet formation also depends on the geometry and very weakly on the flow rate. Droplet breakup is driven by surface tension. The only difference is that no step is employed but rather an inclined surface to change the surface energy [36]. Because of the profound engineering and scientific standards of step emulsification, to find more detailed descriptions and discussions about droplet formation mechanism, device fabrication and various applications, the reader is advised to study some of the excellent reviews and papers on the topic [37–44].

CHALLENGES & FUTURE PERSPECTIVE

Step emulsification can be useful for applications that need large-scale production of monodispersed droplets or encapsulating perishable samples. Despite the many compelling developments, there remains effort that needs to be put into bringing step emulsification out of the laboratory as an industrialized technology. First, researchers should focus on the basic research of step emulsification. Although droplet breakup dynamics have been explored [13], to our knowledge, no model exists to figure out precisely the diameter of droplets. More in-depth investigations of the relationship between droplet diameter and other parameters (flow rate, viscosity, microchannel geometry and temperature) are still expected. Second, mass production is a crucial issue. To lower the cost and to improve the throughput of the device simultaneously, the materials and the bonding approaches of the layers should be explored and prudently selected. Third, the nanodroplets (diameter of the droplet is nanoscale) can be used as small containers that will encapsulate one molecule per droplet, forming a high-throughput robust tool for single molecular studies. Fourth, step emulsification should not be separated from biomedical research, such as drug delivery [45], biomedical imaging [46,47], bioregeneration [48,49] and biosensing [50,51]. It can make full use of the advantage of high throughput when the functional biomaterials are encapsulated in droplets by step emulsification. Additionally, they can be integrated to synergetically promote real industrial applications, especially in the biomedical field. Therefore, more collaborative effort is still needed from researchers and entrepreneurs to achieve industrial-level high-throughput production of nanodroplet emulsions. We hope this article will stimulate researchers from different backgrounds to work on addressing the problems described previously and to make contributions to push step emulsification forward to become a robust industrial technology.

AUTHOR CONTRIBUTIONS

X Zhang and L Liu conceived and wrote the paper. N Xiang, Z Ni and X Huang provided the related references. J Zheng and Y Wang provided constructive discussions for the challenges and perspective section. X Zhang approved the final version of the manuscript.

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