

# Nanotechnology for Bioactives Delivery Systems

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## ABSTRACT

The properties of functional foods depend largely on their micro- and/or nano-scale structure. Conventionally, the preparation of functional foods is based on large-scale processing. As a consequence, the structure of the products cannot be precisely controlled. Recently, micro-/nano-technology have been developing and increasingly applied to the formulation of functional foods. This paper summarizes some applications of micro-/nano-processing of food materials into dispersions, foreseeing their application as bioactives delivery systems. Nanodispersions containing antioxidant food materials were formulated and evaluated using various top-down techniques or a bottom-up method, particularly the performance of various polyglycerol esters (PGE) of fatty acid in the preparation and characterization of  $\beta$ -carotene nanodispersions during *in vitro* gastric digestion. Microchannel (MC) and nanochannel (NC) emulsification for producing monodisperse emulsions were investigated. The development of NC emulsification devices for monodisperse fine emulsions is also presented.

Key words: food nanotechnology, micro-/nano-emulsification, monodisperse emulsions, bioactives, monosized droplets, microcapsules

## INTRODUCTION

The health benefits associated with functional lipids have been studied for many decades. However, most of these bioactive substances not only are water-insoluble, but also possess low solubility saturation in oil. The low solubility of the functional lipids impairs their bioavailability and limits their use in food formulations. On the other hand, the formulation of functional lipids into nanoparticles is expected to improve their bioavailability<sup>(1)</sup>. The properties of functional foods depend largely on their micro- and/or nano-scale structure. The conventional preparation for functional foods is generally done by applying large-scale processes. As a consequence, the structure of the products cannot be precisely controlled. Recently micro- /nano-technology have been under continuous development, as it has been applied in the formulation and characterization of functional foods<sup>(2)</sup>.

For instance, food nanotechnology may incorporate emulsification, dispersion, mixing; *i.e.*, micro-fabrication technology and microchannel (MC) and nanochannel (NC) emulsification, membrane emulsification<sup>(3)</sup>, micro-mixer, as well as the control of food rheology<sup>(4)</sup>. Application of CFD (Computational Fluid Dynamics) to food process has also received considerable attention<sup>(5),(6)</sup>. In addition, *in vitro* models have been applied to evaluate the digestion of lipids passing through gastro-intestinal tract digestion<sup>(7)</sup>.

The Ministry of Agriculture, Forestry and Fisheries, Japan, supported two consecutive 5-year projects entitled “*Development of Nanotechnology and Materials for Innovative Utilizations of Biological Functions*” launched in 2002, and “*Food Nanotechnology Project*” launched in 2007, respectively. The authors have been involved in both projects, the formulation and characterization of micro- /nano-scale emulsions<sup>(1,4)</sup>.

Emulsification is an important process for food, cosmetic, pharmaceutical and other chemical industries, where monodisperse emulsions are required for high quality and stability of the products. Conventional emulsification devices, such as high-pressure homogenizer and colloid mill, are commonly used to produce emulsions. However, the resultant emulsions with droplets diameters of 0.1 - 100  $\mu\text{m}$  exhibit considerable polydispersity. On the other hand, recent developments of micro-fabrication technology have enabled the precise fabrication of micrometer-sized channels on a microchip<sup>(4)</sup>.

A novel method for producing monodisperse emulsion droplets with a very narrow droplet size distribution using an MC array was proposed by our research group<sup>(8-11)</sup>. MC emulsification is capable of producing both monodisperse oil-in-water (O/W) and water-in-oil (W/O) emulsions, which have been applied in the formulation of monodisperse lipid microparticles<sup>(12)</sup>, and W/O/W emulsions<sup>(13)</sup>, among others. Recent works have shown that the stability of almost insoluble bioactive lipophilic compounds, such as

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carotenoids or polyunsaturated fatty acids (PUFAs), can be improved by MC emulsification<sup>(14-17)</sup>.

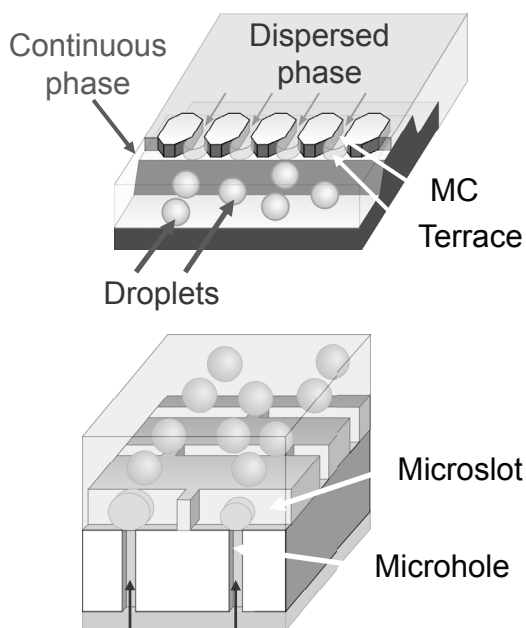
The authors have investigated a NC emulsification device, aiming to produce monodisperse sub-micron emulsions. In this study, we designed NC emulsification devices with smaller channels and investigated the effect of channel size on the droplet size and its distribution<sup>(18)</sup>.

The objectives of this research were to formulate and characterize O/W emulsions loaded with bioactive compounds, evaluating their physical and chemical stability. The effects of various types of polyglycerol esters of monolaurate (PGEs), different processes, the resulting droplet size, and storage temperature, on the stability of the resulting emulsions were also evaluated.

## MATERIALS AND METHODS

### I. Microchannel Emulsification

We have proposed MC emulsification in 1997, which can produce monodisperse emulsions using a grooved MC array with a slit-like terrace on a silicon chip<sup>(8)</sup>. Figure 1 illustrates the emulsification process using a grooved MC and an asymmetric straight-through MC. The emulsification setup consists of an MC plate, a module, apparatuses for supplying the two phases, and a microscope video system.



**Figure 1.** MC emulsification systems. Top: Grooved type; Bottom: Asymmetric straight-through type

### II. Nanochannel Emulsification

A silicon 8 x 22.5-mm NC emulsification chip with two NC arrays (14-mm length) was used in this study. Each NC array consists of 750 parallel NCs (320-nm depth and 3200-nm width) and terraces (320-nm depth

and 3200-nm length) formed outside the NCs. A channel for cross-flowing the continuous phase is fabricated between the NC arrays. To generate droplets by NC emulsification, the dispersed phase was pressurized to pass through an NC array into a well filled with the continuous phase. The size of the generated droplets was measured by a laser diffraction particle size analyzer (LS 13 320, Beckman Coulter Ltd., Fullerton, USA), with at least two repetitions for each measurement<sup>(18)</sup>.

### III. Nanoscale Antioxidant Oil-in-water Emulsion

**Materials:**  $\beta$ -Carotene, refined soybean oil and analytical-grade hexane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The nonionic emulsifiers (PGEs) were supplied by Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan). Disodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate dihydrate were purchased from Wako Pure Chem. Ind. Ltd.

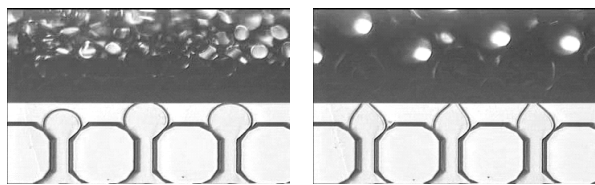
**Preparation of solutions:** 5 mM phosphate buffer (pH 7.0) in Milli-Q water. The emulsifier solution was prepared by dissolving 1.0 wt% PGE into phosphate buffer followed by filtration using a hydrophilic PVDF (polyvinylidene fluoride) filter (5.0  $\mu$ m, Millipore, Bedford, USA).

**Formulation of  $\beta$ -carotene nanodispersions:** The dispersed phase consisted of 0.1 wt%  $\beta$ -carotene dissolved in hexane, and the continuous phase consisted of different aqueous solutions of PGEs. Pre-mixtures containing 10 wt% oil dispersed into the aqueous phase were homogenized using a rotor-stator homogenizer (Polytron PT3000, Kinematica AG, Switzerland) at 5,000 rpm for 5 min, followed by microfluidization (M-110EH, Microfluidics Corporation, Newton, USA) at 100 MPa. Afterwards, 50 mL of emulsions were evaporated using a rotary evaporator (Eyela NE-1101, Tokyo Rikakikai Co., Ltd. Tokyo, Japan) at 45°C under programmed pressures, as described elsewhere<sup>(19)</sup>. Mean particle size and particle size distribution were measured by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments Ltd., UK), with at least three repetitions for each measurement.

## RESULTS AND DISCUSSION

### I. Microchannel emulsification

Figure 2 depicts an example of the emulsification behaviors using a grooved MC array. The dispersed phase that passed through the channels expands on the terrace with a distorted, disk-like shape, and then spontaneously transforms into a spherical droplet by interfacial tension<sup>(20)</sup>. MC emulsification process enables the generation of monosized droplets without applying external shear forces such as the continuous-phase flow. MC emulsification enables production of monodisperse emulsions with average droplet diameters of a few  $\mu$ m to hundreds of  $\mu$ m, and the minimum CV values of less than 5%<sup>(21)</sup>.

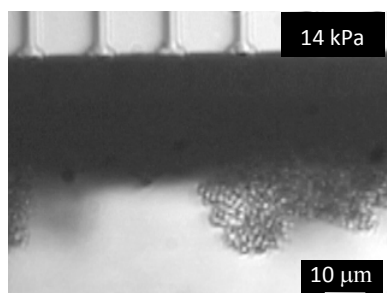


**Figure 2.** Optical micrographs of emulsification behaviors from a grooved MC array: regular droplet generation at lower dispersed phase flux (left), and continuous outflow at higher dispersed phase flux (right) for a triolein oil-in-water containing an emulsifier system.

A straight-through MC array chip was proposed as a solution of the major problem in a grooved MC array chip, which is the low throughput of monodisperse emulsion droplets (typically < 0.1 mL/h). The straight-through MC array chip of standard size had a maximum throughput capacity of monosized soybean oil droplets of 6 mL/h. A large straight-through MC array chip with a surface size of 40 mm × 40 mm achieved a maximum droplet throughput of 35 mL/h. Stable generation of monosized droplets were also obtained at very low viscosity using an asymmetric straight-through MC array with pairs of micro-slots and circular micro-holes<sup>(22-24)</sup>.

## II. Nanochannel Emulsification

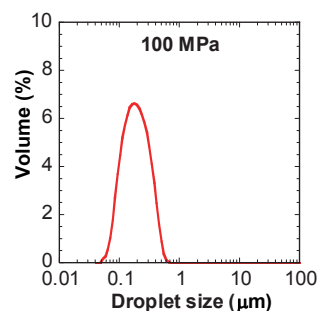
The micrograph in Figure 3 depicts a droplet generation result for NC emulsification. The dispersed phase began to pass through channels at the dispersed-phase pressure of 14 kPa. The dispersed phase that came out from the channel outlet expanded on the terrace with a disk-like shape, and then part of the expanding dispersed phase entered the well. Eventually, the oil-water interface was pinched off near the terrace outlet, leading to the generation of an oil droplet with a diameter of about 1 μm in the well. The preceding droplet generation process was periodically repeated during NC emulsification. The generated droplets moved randomly in the well due to their Brownian motion, demonstrating a difficulty in obtaining clear micrographs of the droplets. A monodisperse fine O/W emulsion with an average diameter of 1400 nm was stably produced using the NC emulsification chip<sup>(18)</sup>.



**Figure 3.** Optical micrograph of droplet generation from NCs.

## III. Nanoscale Antioxidant Oil-in-water Emulsion

As demonstrated in Figure 4, O/W  $\beta$ -carotene nanoemulsions were successfully formulated using high-pressure homogenization, and the resulting droplet size was dependent on the operating pressure<sup>(25)</sup>. Moreover, the final average particle size of  $\beta$ -carotene nanodispersions prepared using microfluidization and evaporation did not change considerably ranging between 40 nm and 47 nm, although increasing the operating pressure from 10 MPa to 100 MPa. These results suggest that the final particle size depended on the emulsion stability before evaporation. Moreover, it is suggested that in order to physically stabilize the particles formation, it is better to use PGEs with high polymerization degrees during the preparation of  $\beta$ -carotene nanodispersions<sup>(25)</sup>.



**Figure 4.** Droplet size distribution of  $\beta$ -carotene nanoemulsions prepared using microfluidization at various operating pressures, stabilized by PGE. Left: 10 MPa; Right: 100 MPa.

## CONCLUSIONS

This paper has outlined recent technologies for production of monodisperse emulsions using micro-/nano-devices. The micro-/nano-devices with well-defined channels enable successful production of size-controlled monodisperse emulsions with an average droplet diameter ranging from single-micrometer to several 100 μm by exploiting the flow characteristics and force balance of the two liquid phases inside the channels. The resultant monodisperse emulsions have been applied to preparation of monodisperse microparticles and microcapsules and to biological and chemical microreactor vessels. However, the present emulsification techniques using the micro-/nano-devices have considerable low throughput capacities for industrial uses. Actually, scale up is being tested for parallelization of MC chips and enlargement of chip size, foreseeing considerable increments on productivity.

Nanoscale anti-oxidant bioactives were formulated by high-pressure homogenizer and their bioavailability was investigated. For instance, we demonstrated that stable  $\beta$ -carotene nanodispersions can be prepared by emulsification-evaporation method using PGEs. The final particle size was affected by the stability of  $\beta$ -carotene dispersions before evaporation.  $\beta$ -carotene

nanodispersions stabilized by tetraglycerol ester of monolaurate resulted in the smallest average particle size of less than 50 nm.

### ACKNOWLEDGMENTS

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