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Effect of back pressure on emulsification of lipid nanodispersions in a high-pressure homogenizer

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ABSTRACT

We examined the effect of 0–20% back pressure, which functions as a resistance to emulsification in a high-pressure homogenizer, on emulsification of lipid nanodispersions (emulsion and liposomes) less than 100 nm in diameter. Back pressure in the range of 0.9–3.8% of the emulsification pressure enhanced the emulsification, and the particle diameter of lipid nanodispersion was the smallest at 2% back pressure. The back pressure effect was independent of the actual pressure, which was regarded as the difference between the emulsification and the back pressures. The mechanism of the back pressure effect was considered to be enhancement of emulsification by suppression of collapse cavitation in the high-pressure emulsification module. This back pressure effect appeared in emulsification of emulsion and liposomes, and was seen predominantly in the early emulsification phase (within 10 passages). The particles of lipid nanodispersions prepared at 2% back pressure with adequate re-circulation achieved physicochemically optimal diameter with a narrow size distribution, and were more stable at 60 °C for 7 days than particles prepared with 20% back pressure. Our results indicate that emulsification with a low level of back pressure is effective for production of stable lipid nanodispersions with narrow size distribution.

1. Introduction

Lipid dispersions, such as emulsions and liposomes, have been investigated as vehicles for drug delivery, and several products are in clinical use (Torchilin, 2007). Small particles with a size less than 100 nm in diameter are also required as parenteral carriers for intravenous administration. Such lipid nanodispersions offer two advantages. The first is ease of sterilization, because the particles of less than 100 nm in diameter can be sterilized by the use of a 0.2 μm filter, which is useful for heat-unstable active substances (e.g., proteins and polynucleotides). The second advantage is improved tissue distribution, because particles less than 100 nm in diameter show minimal accumulation in the lung (Litzinger et al., 1996; Li et al., 1998; Sternberg et al., 1998), as well as minimal non-specific capture by the reticuloendothelial system (Gabizon et al., 1990; Woodle and Lasic, 1992; Litzinger et al., 1994; Seki et al., 2004b; Sonoke et al., 2008), and also show high vascular permeability via diffusion at target tissues (Nagayasu et al., 1999; Seki et al., 2004a; Fukui et al., 2003).

Lipid nanodispersions are generally prepared by means of high-energy emulsification methods (Anton et al., 2008). One such method is the use of a high-pressure homogenizer, which is frequently employed for large-scale production of liposomes (Talsma et al., 1989; Brandl et al., 1990, 1998; Bachmann et al., 1993; Lasic, 1993). Since the particle size of lipid nanodispersions produced in a high-pressure homogenizer is very sensitive to changes in the manufacturing parameters, it is important to identify and evaluate critical manufacturing parameters during the development process (Chen, 2008; Verma et al., 2009). The effects of emulsification pressure, number of passages through the high-pressure homogenizer and temperature on particle size have been reported (Washington and Davis, 1988; Arii et al., 1999; Barnadas-Rodriguez and Sabes, 2001; Wörle et al., 2007). Conventional high-pressure homogenizers (e.g., microfluidizers and Manton-Gaulin homogenizers) generate back pressure which functions as a resistance to emulsification (Fig. 1), and the effect of back pressure on particle size has been examined (Pandolfe, 1982). However, the effects of these manufacturing parameters were only examined for particles more than 100 nm in diameter in the previous reports, and in particular, the effect of back pressure was only examined for particles more than 500 nm in diameter. Currently, it is important to understand the effects of these parameters on the preparation of

Abbreviations: dw, weight average diameter; dn, number average diameter; FDA, Food and Drug Administration; CDER, Center for Drug Evaluation and Research.

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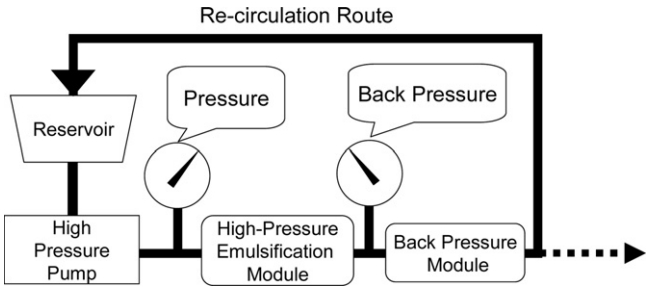

Fig. 1. Schema of emulsification with a high-pressure homogenizer. Crude emulsion or liposomes in the reservoir are forced by a high-pressure pump through the high-pressure emulsification module and back pressure module. After passing through the back pressure module, the emulsion and liposomes are re-circulated. The pressure gauge in front of the high-pressure module indicates the emulsification pressure. The pressure gauge in front of the back pressure module indicates the back pressure.

particles less than 100 nm in diameter, because such particles are easy to sterilize and show superior tissue distribution in vivo, as noted above.

In the present study, we evaluated the effect of back pressure on the preparation of lipid nanodispersions with a particle diameter of less than 100 nm with a high-pressure homogenizer.

2. Materials and methods

2.1. Materials

Purified egg lecithin and soybean oil for parenteral use were purchased from Q.P. Corporation (Tokyo, Japan) and Ajinomoto Co., Inc. (Tokyo, Japan), respectively.

2.2. Preparation of emulsion and liposomes

Purified egg lecithin (10 g) and soybean oil (10 g) were added to 100 mL of distilled water and the mixture was dispersed by a high-shear mixer to give a crude emulsion. Crude liposomes were prepared similarly, except that soybean oil was not added. Crude emulsion and crude liposomes were further diluted with distilled water to make 200 mL, and the resulting crude dispersion was emulsified with a Microfluidizer M110-E/H (Microfluidics International Corporation, Newton, MA, USA) at an emulsification pressure of 108 MPa or 135 MPa with various levels of back pressure. The back pressure was adjusted with a pressure-regulating needle valve connected to the microfluidizer instead of back pressure module. The temperature during emulsification was maintained at 30 °C. The number of passages through the microfluidizer was adjusted for each batch in accordance with the predetermined experimental design.

2.3. Determination of particle size

The particle size of emulsions and liposomes was measured with a laser dynamic light scattering particle sizer, DLS-700 (Otsuka Electronics, Inc., Osaka, Japan) equipped with a He–Ne laser source (wavelength, 632.8 nm). Samples were diluted with distilled water in order to obtain an appropriate scattering intensity (Zhang and Kirsch, 2003). All measurements were made at a scattering angle of 90° and a temperature of 25 °C. The mean diameter and particle size distribution were calculated by the cumulant method and the histogram method, respectively.

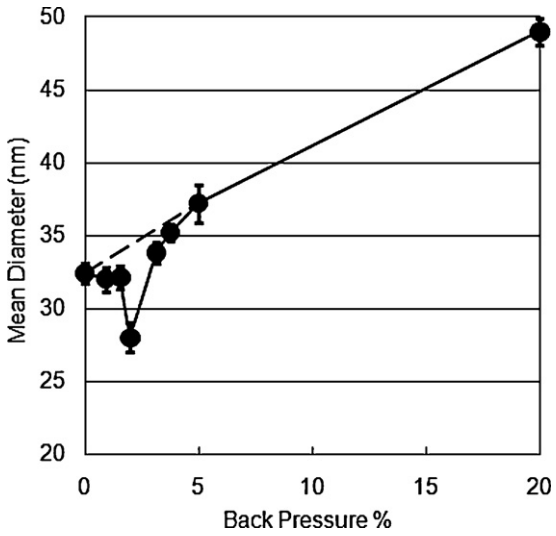

Fig. 2. Relationship of mean diameter to back pressure for emulsion prepared at 108 MPa after 90 passages. Mean diameter of emulsion was determined by dynamic light scattering. The emulsion was prepared at the emulsification pressure of 108 MPa with back pressures of 0%, 0.9%, 1.6%, 2.0%, 3.1%, 3.8%, 5.0% and 20.0%. Data are presented as the mean ± S.D. of five independent experiments.

2.4. Heat stress stability study

Prepared emulsion samples were sealed in vials and put in the stability test chamber (Tabai Espec Co., Ltd., Osaka, Japan) at 60 ± 0.5 °C and ambient relative humidity for 7 days in the dark. Their mean diameters and their d*w*/d*n* values, calculated as weight-average diameter (d*w*) divided by number-average diameter (d*n*), were determined with a DLS-700. Their zeta-potentials were determined by laser Doppler electrophoresis with a Zetasizer 2000 (Malvern Instruments, Worcestershire, UK). Statistical significance was assessed with the unpaired Student's *t*-test, and *p* values of 0.05 or less were considered to be statistically significant.

3. Results and discussion

3.1. Comparison of particle diameter in emulsions obtained with various back pressures

To examine the influence of back pressure, we used a Microfluidizer as a high-pressure homogenizer and a lipid nanodispersion composed of purified egg lecithin and soybean oil, which are commonly used as pharmacopeial excipients in peripheral parenteral nutrition.

First, it was necessary to find the optimum level of back pressure for the preparation of emulsions with the high-pressure homogenizer. Fig. 2 shows the mean diameter of emulsions obtained at the emulsification pressure of 108 MPa with back pressures ranging from 0% to 20.0% after 90 passages. As the back pressure was increased from 0% to 20.0%, the mean diameter of emulsion particles increased and a plot based on the 3 points of 0%, 5.0%, and 20.0% was essentially linear. However, the diameters at back pressures ranging from 0.9% to 3.8% were smaller than those expected from the linear relationship, and the diameter was smallest when the back pressure was 2%. Fig. 3 shows the emulsion particle size distributions at back pressures of 2.0% and 20.0%. The particle size distribution at the back pressure of 2.0% was smaller and narrower than that at 20.0% back pressure. Since the back pressure functions as a resistance to emulsification by decreasing the net emulsification pressure (actual pressure), the change of actual pressure affects the particle size. However, the result of the smaller diameters at 0.9–3.8% back pressure than expected from the linear

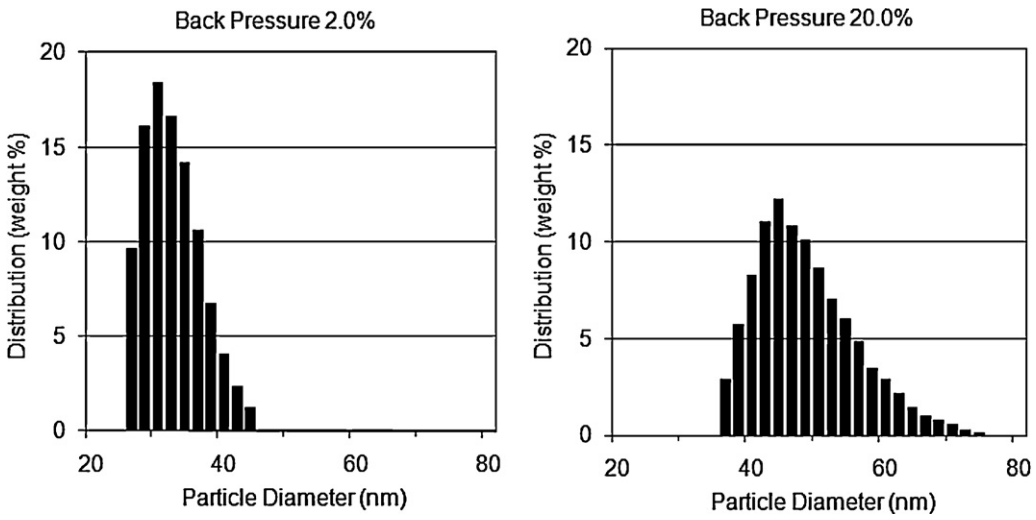

Fig. 3. Particle size distribution of emulsion prepared at 108 MPa with back pressure of 2.0% and 20.0% after 90 passages. Particle distributions of emulsion were determined by dynamic light scattering. The emulsion was prepared at the emulsification pressure of 108 MPa with back pressure of 2.0% (left) and 20.0% (right).

relationship indicates that there are certain factors in back pressure effect other than actual pressure affected the emulsification. The effective back pressure percentage found in the present study is not in agreement with reported values, 10–20% (Pandolfe, 1982). In the present study, the mean diameters at 10% back pressure and 20% back pressure after 100 passages (mean ± S.D., $n = 3$: 42.6 ± 0.4 nm and 49.5 ± 0.8 nm, respectively) were smaller than the diameter at 30% back pressure after 100 passages (62.2 ± 0.5 nm) similar to the report by Pandolfe (1982), but the mean diameter at 10% back pressure after 100 passages was above 40 nm and larger than those at 0.9–3.8% back pressure after 90 passages. Since the back pressures examined in Pandolfe's report were only 0%, 10%, 20% and 30%, the apparent discrepancy between that report and our result could be explained by the absence of back pressure measurement between 0% and 10% in that report.

3.2. Effect of actual pressure on the back pressure effect

Fig. 2 shows the relationship between mean diameter and back pressure. Since the diameter at 0.9–3.8% of back pressure was smaller than that expected from the linear relationship, it was suggested that there are certain factors in back pressure effect other than actual pressure. However, the back pressure functions as a resistance to emulsification by decreasing the net emulsification pressure, so the actual pressure in the high-pressure emulsification module at 20% back pressure (86.4 MPa = 108.0–21.6 MPa) was about 20% lower than the actual pressure at 2% back pressure (105.8 MPa = 108.0–2.2 MPa). We therefore examined the influence of actual pressure on the emulsification. Table 1 shows the mean particle diameter of emulsions obtained at various actual pressures with back pressures of 2% and 20%. The mean diameter at an actual pressure of 108.0 MPa with 20% back pressure was 61.0 nm and was close to the diameter (63.9 nm) at an actual pressure of 86.4 MPa with 20% back pressure, rather than the diameter (32.0 nm) at an actual pressure of 105.8 MPa with 2% back pressure. In contrast, the mean diameter (32.4 nm) at an actual pressure of 132.3 MPa with 2% back pressure was the same as the diameter at an actual pressure of 105.8 MPa with 2% back pressure. There was no difference of the particle diameter between actual pressures of 132.3 MPa and 105.8 MPa with 2% back pressure. This may indicate that the actual pressure of 105.8 MPa was enough for efficient emulsification in the present study. In addition, these results indicate that the back pressure effect cannot be explained in terms of the difference of actual pressure.

The mechanism through which particle size is decreased by a high-pressure homogenizer is generally considered to involve the combination of shear force generated by the jet stream from the slit of the high-pressure emulsification module, turbulent flow generated by eddies after passage through the slit of the high-pressure emulsification module and cavitation generated by gas bubbles in the liquid (Barnadas-Rodriguez and Sabes, 2001; Anton et al., 2008; Constantinides et al., 2008). Cavitation is divided into collapse cavitation with bubble implosion and non-collapse cavitation without implosion (Husseini et al., 2005). Collapse cavitation produces an acoustic pressure wave and non-collapse cavitation produces oscillation. It was reported that the emulsifying effect of non-collapse cavitation was increased by inhibition of collapse cavitation in the case of the emulsification with an ultrasound generator, because collapse cavitation interfered with non-collapse cavitation (Richardson et al., 2007). In the present study, the noise generated from the high-pressure emulsification module was decreased by imposing back pressure (data not shown). Therefore, it is possible that the mechanism of the 2% back pressure effect was enhancement of emulsification by suppression of collapse cavitation in the high-pressure emulsification module. On the other hand, it was considered that 20% back pressure suppresses not only

Table 1
Effect of emulsification pressure on particle size of emulsions prepared with various levels of back pressure.

Emulsification pressure (MPa)	108.0		135.0	
Back pressure (MPa) (back pressure as percent of emulsification pressure)	2.2 (2.0%)	21.6 (20.0%)	2.7 (2.0%)	27.0 (20.0%)
Actual pressure (MPa)	105.8	86.4	132.3	108.0
Number of passages	60	60	60	60
Mean diameter (nm)	32.0 ± 1.3	63.9 ± 1.8	32.4 ± 1.1	61.0 ± 2.1

Data are presented as the mean ± S.D. of three independent experiments.
Mean diameter was determined by dynamic light scattering.

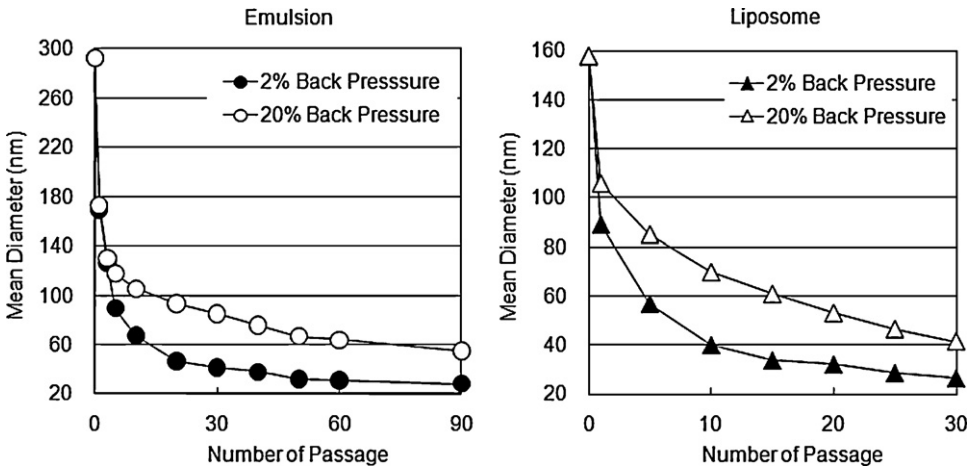

Fig. 4. Effect of back pressure on emulsification profiles of emulsion and liposomes prepared at 108 MPa. Mean diameters of emulsion and liposomes were determined by dynamic light scattering. The emulsion was prepared at the emulsification pressure of 108 MPa with back pressure of 2% (closed circle) and 20% (open circle). The liposome was prepared at the emulsification pressure of 108 MPa with back pressure of 2% (closed triangle) and 20% (open triangle).

collapse, but also non-collapse cavitation, so that emulsification at 20% back pressure was decreased as compared to emulsification without back pressure.

3.3. Effect of back pressure on the emulsification process

It has been shown that the number of passages through a high-pressure homogenizer influences emulsification (Washington and Davis, 1988; Arii et al., 1999; Barnadas-Rodriguez and Sabes, 2001). To estimate the effect of the number of passages on emulsification, we compared the relationship of mean diameter to number of passages at back pressures ranging between 2% and 20%. Fig. 4 shows mean diameter profiles of lipid nanodispersions at an emulsification pressure of 108 MPa with back pressures of 2% and 20%. The mean diameter of emulsion decreased with increasing passage number and reached final values of 28.0 nm at 2% back pressure and 55.0 nm at 20% back pressure. Moreover, we examined whether or not back pressure affects liposome preparation. The decrease of liposome particle size at 2% back pressure was greater than that at 20%. The mean diameter of liposomes at 2% back pressure after 30 passages became 26.5 nm. These results indicate the back pressure effect is not dependent on the type of lipid nanodispersion or the number of passages.

As shown in Fig. 4, all profiles of mean diameter consist of two phases, the early emulsification phase up to 10 passages and the later phase. When the slopes of the early ($Slope_{1-10}$) and the later ($Slope_{after10}$) phases were calculated by linear regression (Table 2), the difference in the slope (D_{1-10}) of the early phase between 2% and 20% back pressure was 4.2 for emulsion and 1.3 for liposomes, while the difference in the slope ($D_{after10}$) of the later phase was much smaller. This may be because the particle sizes of emulsion and liposomes were already close to the ultimate diameter in the later phase. It has been reported that the ultimate diameter of emulsion and liposomes is physicochemically determined by the composition ratio of oil to emulsifier phase (Handa et al., 1990). The mean final diameters of emulsion and liposomes in the present study were 28.0 nm and 26.5 nm, respectively. These values are close to the reported diameters for emulsion (27–29 nm) and liposomes (25 nm) of the same composition ratio as used in the present study. This result supports the idea that the back pressure effect is greater in the early emulsification phase (within 10 passages) than in the later emulsification phase because the range of diameter decrease is less in the later phase than in the early phase.

3.4. Heat stress stability

As indicated in the FDA guidance for liposome drug products, stability is an important issue for lipid dispersions (CDER/FDA, 2002), because the changes in physicochemical properties of lipid dispersions during storage influence the biopharmaceutical properties. Although the particle sizes of emulsion were less than 100 nm in diameter, as shown in Fig. 3, the particle size distri-

Table 2
Comparison of emulsification phase for emulsion and liposomes prepared at 108 MPa with back pressure of 2% and 20%.

Phase		Back pressure (%)			
		Emulsion		Liposomes	
		2	20	2	20
Early	$Slope_{1-10}$	−10.722	−6.554	−5.360	−3.961
	D_{1-10}	4.169		1.398	
Later	$Slope_{after10}$	−0.440	−0.547	−0.570	−1.160
	$D_{after10}$	0.107		0.590	

Values were calculated from the mean diameter profile.
$Slope_{1-10}$ was calculated from the mean diameter of 1–10 passages by linear-regression analysis.
D_{1-10} is the absolute difference in $Slope_{1-10}$ between back pressures of 2% and 20%.
$Slope_{after10}$ was calculated from the mean diameter at 20 passages to 60 passages for emulsion and from the mean diameter at 15 passages to 30 passages for liposomes by linear-regression analysis.
$D_{after10}$ is the absolute difference in $Slope_{after10}$ between back pressures of 2% and 20%.

Table 3
Heat stress stability of emulsion at 60 °C/ambient relative humidity.

	Back pressure (%)	Number of passages	60 °C/ambient RH	
			Initial	7 days
Zeta potential (mV)	2	15	−49.9 ± 3.8	−51.6 ± 2.6
	2	60	−52.8 ± 3.0	−49.7 ± 3.2
	20	60	−48.2 ± 2.4	−47.9 ± 4.2
Mean diameter (nm)	2	15	54.8 ± 2.2	132.3 ± 3.4*
	2	60	34.0 ± 1.2	32.2 ± 3.3
	20	60	55.2 ± 2.1	119.9 ± 5.0*
d*w*/d*n*	2	15	1.14 ± 0.37	1.39 ± 0.42
	2	60	1.02 ± 0.04	1.02 ± 0.08
	20	60	1.18 ± 0.16	1.36 ± 0.36

Data are presented as the mean ± S.D. of three independent experiments.
Zeta potential was determined by laser Doppler electrophoresis.
Mean diameter and d*w*/d*n* were determined by laser light scattering measurement.
* Statistically significantly difference from initial value (Student's *t*-test, $p < 0.01$).

butions were different between 2% and 20% back pressures. To estimate the influence of the back pressure difference on the physicochemical characteristics (particle size and zeta potential) of the emulsion, the stability of emulsions was examined under heat stress. Table 3 shows zeta-potentials, mean diameters and d*w*/d*n* values before and after the heat stress. Zeta-potential was unchanged even after storage for 7 days at 60 °C. The value of zeta potential ranged from −47.9 mV to −52.8 mV, which is within the range of −40 mV to −60 mV for stable particles (Washington, 1996).

The mean particle diameter of emulsion obtained at 108 MPa with 2% back pressure after 60 passages was not changed after the heat stress. On the other hand, the mean diameters of emulsions obtained at 2% back pressure after 15 passages and at 20% back pressure after 60 passages were significantly increased after the heat stress ($p < 0.01$) and were above 100 nm. Since particles of more than 100 nm in diameter show inferior vascular permeability compared to particles of less than 100 nm in diameter (Nagayasu et al., 1999), the tissue distribution of emulsions obtained under the latter conditions could change during storage.

The d*w*/d*n* values of emulsion obtained at 2% back pressure after 60 passages before and after heat stress were below 1.1. However, the d*w*/d*n* values of emulsions obtained at 2% back pressure after 15 passages and at 20% back pressure after 60 passages were 1.14 and 1.18 before heat stress, but were increased to 1.39 and 1.36 after the treatment, respectively. Since it was reported that stable emulsions have d*w*/d*n* values of below 1.1 (Arii et al., 1999), the emulsion at 2% back pressure after 60 passages was stable because of its narrow distribution. On the other hand, since the emulsion with broad size distribution was easy to increase the diameter by Ostwald ripening compared to the emulsion with narrow size distribution (Taylor and Ottewill, 1994), the increased diameter of heat-stressed emulsions prepared at 2% back pressure after 15 passages and at 20% back pressure after 60 passages was caused by the broader size distribution, including large particles. The results of stability study indicate that emulsification at 2% back pressure with adequate recirculation provides lipid nanodispersions with narrow size distribution and good stability.

In conclusion, we found that the particle diameter of emulsions and liposomes obtained with 2% back pressure were smaller than those of emulsions and liposomes obtained without back pressure or with greater levels of back pressure, and the physicochemically optimal diameter was attained. We found that this back pressure effect was independent of the actual pressure and was due to suppression of collapse cavitation in the high-pressure emulsification module. The present study suggests that the emulsification with a low level of back pressure is effective for production of stable lipid nanodispersions with narrow size distribution.

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