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High-throughput formulation screening system for self-microemulsifying drug delivery

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Abstract

Purpose: To develop a high-throughput formulation screening (HTFS) system for self-microemulsifying drug delivery system (SMEDDS) formulations. *Methods*: Formulations were prepared by dispensing surfactants and a model compound (Nilvadipine) dissolved in ethanol and oil with a robotic liquid dispenser. Screenings of emulsion particle size and phase stability were conducted for selecting SMEDDS formulations by a turbidity assay. *Results*: Formulations were prepared at 40 minute/96-formulation. Both the screenings were conducted at 1 minute/96-formulation. SMEDDS formulations and the most suitable hydrophilic surfactant (HS)/lipophilic surfactant (LS) combination, which formed the largest SMEDDS area on its corresponding phase diagram, were selected by SMEDDS–HTFS system with minimal manpower (one person) and compound consumption (0.2 mg/formulation). *Conclusions*: SMEDDS–HTFS system enabled rapid and efficient selections of SMEDDS formulations and the most suitable HS/LS combination for SMEDDS.

Key words: *Emulsion particle size; high-throughput formulation screening; microemulsion; phase stability; self-microemulsifying drug delivery system; turbidity*

Introduction

Self-microemulsifying drug delivery systems (SMEDDS) form microemulsion (ME), which is physically stable and transparent, under mild agitation in an aqueous media[1,2]. SMEDDS is employed widely to improve the oral absorption of poorly soluble drugs[3–10]. Compared with conventional formulations (tablet, capsule, etc.), the designs of SMEDDS formulations require substantial time, manpower, and amounts of candidate compounds for the following reasons: (1) many formulations (several hundred to thousands) must be screened because SMEDDS is a multicomponent system [oil, hydrophilic surfactant (HS), lipophilic surfactant (LS), etc.] and each component has many types of excipients; (2) many formulations must be prepared manually; and (3) it is difficult to dispense small amounts of viscous/semisolid surfactants or powdered compounds in preparing the formulations. These factors make it difficult to design SMEDDS formulations for drug development (particularly at the drug discovery stage) where time, manpower, and amounts of compounds are limited. We hypothesized that high-throughput formulation screening (HTFS) to efficiently and rapidly prepare and screen formulations could be an effective way to design SMEDDS formulations.

Rapid methods to prepare formulations and evaluate their emulsion properties (emulsion particle size, phase stability) were required in developing a SMEDDS-HTFS system. It was difficult to dispense small amounts of viscous/semisolid surfactants and a powdered compound in preparing formulations with a robotic liquid dispenser. Chen et al.[11] reported a rapid dispensing method for preparing intravenous formulations. In their method, water was used as a solvent for viscous or solid hydrophilic excipients. Water cannot be used in the SMEDDS-HTFS system, because LS and poorly water-soluble compounds are immiscible with water. Mansky et al.[12] reported another rapid dispensing method that adopted *n*-propanol as a solvent for HS, LS, and poorly soluble compounds. Although the formulations prepared by this

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method were dried under vacuum, some *n*-propanol could remain in the formulations. If the residual *n*-propanol is an indispensable component in the formulations, this could lead to a problem in drug development because *n*-propanol cannot be used in oral formulation[13]. Ethanol can be added to oral formulation[14]. We investigated the performance of an ethanol-based dispensing method and applied it to rapid formulation preparations.

Emulsion particle size is evaluated in the screening of ME formulations and is measured using dynamic light scattering (DLS)[3,6,15–17]. Visual inspection is used to evaluate emulsion transparency in ME screening because transparency reflects emulsion particle size[15,18,19]. The DLS and visual inspection methods are low-throughput (DLS: 5–10 minutes/formulation, visual inspection: 0.5–1 minutes/formulation), and the visual inspection method has a difficulty in comparing transparency quantitatively among many formulations. High-throughput turbidity assays can be performed by a microtiter spectrometer (1 minute/96-sample), which can quantify transparency. Therefore, we investigated the applicability of a turbidity assay to rapid ME screening by evaluating the relationships among emulsion particle size, transparency, and turbidity.

Phase stability is also evaluated to screen for physically stable emulsion formulations by visual inspection. This method is low throughput and its variation among observers is concerned. If an aqueous phase contains a physically unstable emulsion, the oily phase is separated from the aqueous phase during storage by flocculation, coalescence, and creaming. The turbidity of an unstable emulsion is expected to change during storage because the incident light in a direction perpendicular to liquid level passes through different phase states before (one phase) and after (separated phases) storage. Phase stability may be evaluated rapidly by quantitating the turbidity change. Therefore, we investigated the applicability of the turbidity assay to rapid screening for physically stable emulsion by evaluating the relationship between phase stability and turbidity changes.

The purpose of our study was to develop a SMEDDS-HTFS system that includes rapid methods of formulation preparation, ME screening, and physically stable emulsion screening. To accomplish our purpose, we investigated (1) the ethanol-dispensing performance, (2) the relationships among emulsion particle size, transparency, and turbidity, (3) the relationship between phase stability and turbidity changes, and (4) the performance of the SMEDDS-HTFS system in a pilot study.

Materials and methods

Materials

Table 1 lists oils, HS (HLB ≥ 9), and LS (HLB < 9) used in this study with their trade names, abbreviations, and HLB. MCT was purchased from Nisshin OilliO Group (Tokyo, Japan). Sefsol-218, BL2, BL4.2, BL9, HCO40, PS40, PS80, HEGML, SO10, CO3, and CO10 were supplied by Nikko Chemicals (Tokyo, Japan). Labrasol was supplied by Gattefossé SAS (Saint-Priest Cedex, France). Ethanol was purchased from Wako Pure Chemical Industries (Osaka, Japan). Nilvadipine (Nil), used as a poorly water-soluble model compound (aqueous solubility: ca. 1 μg/mL, dose: 4 mg), was purchased from Kongo Yakuhin (Toyama, Japan).

Robotic liquid dispenser

All liquids were dispensed by a robotic liquid dispenser (TECAN GENESIS Workstation, Tecan Japan, Kanagawa,

Table 1. Oils, hydrophilic surfactants (HS), and lipophilic surfactants (LS) used in this study.

Oil	Trade name	Abbreviation	
Caprylic/Capric Triglyceride	ODO-C	MCT	
Propyleneglycol monocaprylic ester	NIKKOL Sefsol-218	Sefsol-218	
HS	**Trade name**	**Abbreviation**	**HLB**
Polyoxyethylene (2) monolauric ether	NIKKOL BL-2	BL2	9.5
Polyoxyethylene (4.2) monolauric ether	NIKKOL BL-4.2	BL4.2	11.5
Polyoxyethylene (9) monolauric ether	NIKKOL BL-9EX	BL9	14.5
Glycerol caprylic/capric ester, polyoxyethylene (8) caprylic/capric ester	Labrasol	Labrasol	14.0
Polyoxyethylene (40) hydrogenated castor oil	NIKKOL HCO-40	HCO40	12.5
Polyoxyethylene (20) sorbitan palmitic ester	NIKKOL TP-10	PS40	15.6
Polyoxyethylene (20) sorbitan monooleic ester	NIKKOL TO-10MV	PS80	15.0
Hexaglyceryl monolauric ester	NIKKOL Hexaglyn 1-L	HEGML	14.5
LS	**Trade name**	**Abbreviation**	**HLB**
Sorbitan monooleic ester	NIKKOL SO-10V	SO10	4.3
Polyoxyethylene (3) castor oil	NIKKOL CO-3	CO3	3.0
Polyoxyethylene (10) castor oil	NIKKOL CO-10	CO10	6.5

Japan). The dispenser had a liquid handling arm with eight tips (200 and 1000 μL), a robotic movement arm, and a plate shaker.

Methods

Evaluation of accuracy, precision, and dispensing time for ethanol-dispensing method

Fifty %v/v surfactant ethanol solutions (BL2, BL4.2, BL9, PS40, PS80, Labrasol, HCO40, HEGML) were prepared, and 10 or 200 μL of the solutions were then dispensed into a 96-well plate. Next, a Nil ethanol solution (5 mg/mL) was prepared, and 40 μL of this solution was dispensed into a 96-well plate (this 40 μL was the dispensed volume in preparing the formulations, see *Pilot study for performance evaluation of SMEDDS-HTFS system*). After weighing the dispensed solutions, the weights were divided by their densities to calculate the dispensed volumes. Each density was preliminary determined using the weight of 10 mL of the ethanol solution (data not shown). Accuracy and precision were determined by triplicate measurements. Finally, the time of a dispensing cycle was measured, including the time to get tips at their home position, dispense solutions into one row (8-well) of a 96-well plate, eject the tips, and return to the home position.

Relationships among emulsion particle size, transparency, and turbidity

Emulsions were prepared as follows: 50 %v/v HS ethanol solutions (PS40, PS80, HCO40) and oil (MCT) were dispensed at HS : oil ratios of 9:1, 8:2, 7:3, or 6:4 into a 96-well plate (well capacity: 1.2 mL). The dispensed solutions were mixed by the plate shaker (1000 rpm, 10 minutes). The ethanol in the mixtures was removed by vacuum drying (40°C, overnight). Distilled water was added to the dried formulations (total volume of S_{mix} and oil: 50 μL) to prepare 5 and 10 %v/v solutions. The solutions were then mixed by the plate shaker to form emulsions (1000 rpm, 10 minutes). Transparency of the emulsions was evaluated by visual inspection and emulsions were classified into the following three categories: 'transparent', 'translucent', and 'opaque'. Emulsion solutions (200 μL) were dispensed into 96-well microplates, and the turbidity of each emulsion was measured at 650 nm by a microtiter spectroscopy (SPECTRAmax 190, Molecular Devices, CA, USA). Emulsion particle sizes were determined by DLS (Nicomp 380 ZLS, Particle Sizing Systems, CA, USA).

Relationship between phase stability and turbidity changes during storage

Emulsions were prepared in the same manner as described above (see *Relationships among emulsion particle size, transparency, and turbidity*). The HS used were BL2, BL4.2, BL9, PS40, PS80, Labrasol, HCO40, and HEGML and the oil was MCT. The turbidity of each emulsion (200 μL) was measured by the microtiter spectrometer at 650 nm before and after storage (25°C, 24 hours) to calculate the turbidity change ($\Delta T = |T_{Before} - T_{After}|$). Phase stability was evaluated by visual inspection, which was performed by the same observer because its variation among different observers was concerned.

Pilot study for performance evaluation of SMEDDS-HTFS system

Forty microliter of a Nil ethanol solution (5 mg/mL) was dispensed into 96-well plates (well capacity: 1.2 mL), and then 50 %v/v HS ethanol solutions (HCO40, PS40) and 50 %v/v LS ethanol solutions (SO10, CO3, CO10) were dispensed at HS : LS ratios of 10:0, 9:1, 8:2, 7:3, or 6:4. Next, oil (Sefsol-218) was dispensed into the surfactant mixtures (S_{mix}) at S_{mix} : oil ratios of 10:0, 9:1, 8:2, 7:3, or 6:4. After mixing the dispensed solutions (1000 rpm, 10 minutes), ethanol was removed by vacuum drying (40°C, overnight). Distilled water was then added to the dried formulations (total volume of S_{mix} and oil: 50 μL, Nil in formulation: 4 mg/mL) to prepare 10 %v/v formulation solutions. The solutions were mixed by the plate shaker to form emulsions (1000 rpm, 10 minutes). Emulsion solutions (200 μL) were dispensed into 96-well microplates. The turbidity of each emulsion was measured at 650 nm by the microtiter spectrometer and ME formulations were screened using a turbidity criterion, which is described in *Results*. Turbidity was measured again after storage (25°C, 24 hours), the turbidity change was calculated, and physically stable emulsion formulations were screened using a turbidity-change criterion, which is described in *Results*. Physically stable ME (SMEDDS) formulations were selected by these two screening methods and corresponding phase diagrams were plotted.

Results

Performance evaluation of the ethanol-dispensing method

Capability of direct dispensing method without ethanol

Table 2 summarizes the performance of the robotic liquid dispenser system in dispensing small amounts of surfactants. Although the dispensing of less-viscous surfactants (BL2, BL4.2, BL9, Labrasol; 15–95 mPas) was possible, this system was not capable of dispensing the more viscous surfactants (PS40, PS80, HEGML; viscosity: 289–47250 mPas) or the semisolid surfactant (HCO40). It was also not possible to dispense powdered compounds with this system.

Table 2. Capability of dispensing surfactants with a robotic liquid dispenser.

Surfactant	Viscosity (mPas)	Dispensing
BL2	15[a]	Possible
BL4	27[a]	Possible
BL9	80[a]	Possible
PS40	289[a]	Not possible
PS80	409[a]	Not possible
Labrasol	95[b]	Possible
HCO40[c]	—	Not possible
HEGML	47,250[a]	Not possible

[a]30°C. [b]20°C. [c]Semisolid.

Evaluation of accuracy, precision, and dispensing time for the ethanol-dispensing method

The accuracy, precision, and elapsed time to dispense the surfactant ethanol solutions (50 %v/v) and the Nil ethanol solution (5 mg/mL) were evaluated (Table 3). In dispensing 10-μL aliquots of surfactant solutions, the actual volumes ranged from 9.4 to 11.7 μL and the coefficient of variance (percentage CV) were less than 10.0%. In dispensing 200-μL aliquots of surfactant solutions, the actual volumes ranged from 193.2 to 201.9 μL and the percentage CV were less than 2.6%. In dispensing 40-μL aliquots of the Nil ethanol solution, the actual volume was 39.5 μL and the percentage CV was 4.7%. The elapsed time for a dispensing cycle was approximately 50 s/row (8-well).

Table 3. Accuracy and precision in dispensing ethanol solutions of surfactants and a compound (Nil).

Surfactant (50 %v/v in ethanol) Compound (5 mg/mL in ethanol)	Accuracy Mean ± SD (μL)[a]	Precision %CV
Surfactant, target volume: 10 μL		
BL2	10.6 ± 0.8	7.9
BL4.2	9.7 ± 0.4	4.5
BL9	10.8 ± 0.8	7.5
PS40	10.9 ± 1.1	10.0
PS60	9.4 ± 0.8	8.6
Labrasol	10.9 ± 0.5	4.6
HCO40	10.6 ± 0.5	5.0
HEGML	11.7 ± 0.5	4.3
Surfactant, target volume: 200 μL		
BL2	193.2 ± 0.9	0.5
BL4.2	197.5 ± 3.0	1.5
BL9	197.5 ± 5.1	2.6
PS40	195.5 ± 0.7	0.3
PS60	199.8 ± 3.8	1.9
Labrasol	200.7 ± 1.5	0.7
HCO40	201.9 ± 0.6	0.3
HEGML	194.9 ± 2.5	1.3
Compound, target volume: 40 μL		
Nil	39.5 ± 1.9	4.7

[a]$n = 3$.

Relationships among emulsion particle size, transparency, and turbidity

Relationship between emulsion particle size and transparency

Emulsion particle size and transparency were compared (Table 4). The transparency did not reflect the smallest emulsion particle size in the emulsion particle size distribution, rather it reflected the largest emulsion particle size. For example, when the largest emulsion particle size was in the range of 3012.0–4688, 173.6–1896.0, or <148.7 nm (A24), the transparency was opaque, translucent, or transparent, respectively.

Relationship between largest emulsion particle size and turbidity

The largest emulsion particle size and turbidity were compared (Figure 1). Turbidity increased with an increase of the largest emulsion particle size (Figure 1a), confirming that the turbidity reflected the largest emulsion particle size. Turbidity values for all of the transparent

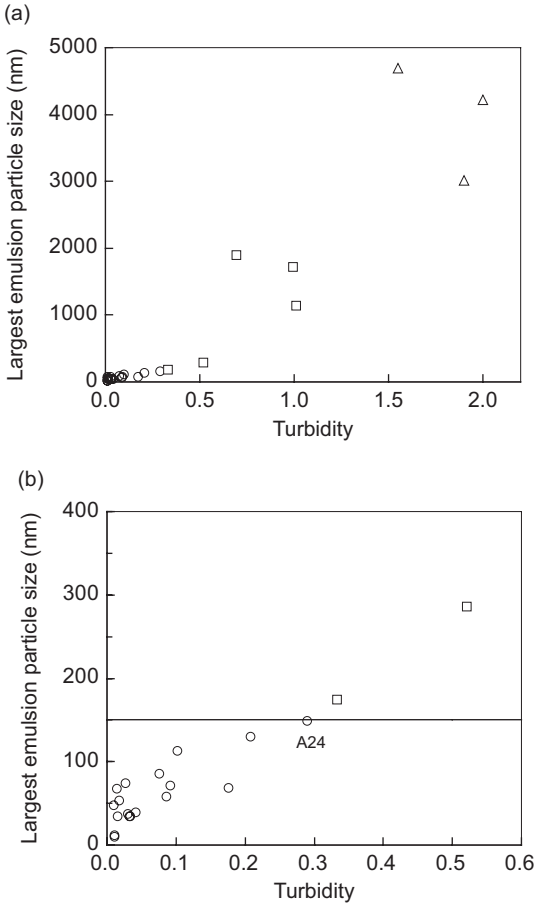

Figure 1. Comparison of emulsion turbidity with the largest emulsion particle size. The range of largest emulsion particle size: (a) full scale, (b) 0–400 nm. Symbol: (○) transparent (ME), (□) translucent, (Δ) opaque.

Table 4. Transparency, size, and turbidity of emulsions.

Formulation	Formulation				Emulsion particle size distribution								
					Peak 1		Peak 2		Peak 3		Smallest	Largest	
Formulation no.	HS	HS:Oil[a]	Water (%v/v)	Transparency of emulsion	Size (nm)	%	Size (nm)	%	Size (nm)	%	size (nm)	size (nm)	Turbidity
A1	PS40	9:1	90	Transparent (ME)	5.3	72.0	15.6	24.9	67.4	3.1	5.3	67.4	0.01
A2		9:1	95	Transparent (ME)	5.3	44.0	14.1	47.1	47.1	8.9	5.3	47.1	0.01
A3		8:2	90	Transparent (ME)	5.4	99.8	57.3	0.2			5.4	57.3	0.09
A4		8:2	95	Transparent (ME)	5.3	81.8	27.4	15.5	73.5	2.7	5.3	73.5	0.03
A5		7:3	90	Translucent	14.7	91.6	80.5	6.1	285.6	2.4	14.7	285.6	0.52
A6		7:3	95	Transparent (ME)	8.2	96.7	112.2	3.3			8.2	112.2	0.10
A7		6:4	90	Opaque	5.3	89.5	21.1	10.0	4216.0	0.5	5.3	4216.0	>2.00[b]
A8		6:4	95	Opaque	12.6	94.8	49.9	4.5	4688.0	0.7	12.6	4688.0	1.55
A9	PS80	9:1	90	Transparent (ME)	1.1	43.4	9.5	56.6			1.1	9.5	0.01
A10		9:1	95	Transparent (ME)	1.1	99.1	11.1	0.9			1.1	11.1	0.01
A11		8:2	90	Transparent (ME)	6.6	85.0	52.8	15.0			6.6	52.8	0.02
A12		8:2	95	Transparent (ME)	9.4	81.3	34.0	18.7			9.4	34.0	0.02
A13		7:3	90	Transparent (ME)	14.1	91.2	84.7	8.8			14.1	84.7	0.08
A14		7:3	95	Transparent (ME)	19.1	89.6	70.9	10.4			19.1	70.9	0.09
A15		6:4	90	Opaque	9.0	97.9	51.6	1.3	3012.0	0.8	9.0	3012.0	1.90
A16		6:4	95	Translucent	5.3	88.7	27.6	10.7	1896.0	0.6	5.3	1896.0	0.70
A17	HCO40	9:1	90	Transparent (ME)	11.1	91.5	39.1	8.5			11.1	39.1	0.04
A18		9:1	95	Transparent (ME)	16.1	82.8	36.8	17.2			16.1	36.8	0.03
A19		8:2	90	Transparent (ME)	6.2	88.3	34.1	11.7			6.2	34.1	0.03
A20		8:2	95	Transparent (ME)	5.4	89.4	34.0	10.6			5.4	34.0	0.03
A21		7:3	90	Transparent (ME)	18.3	92.1	68.0	7.9			18.3	68.0	0.18
A22		7:3	95	Transparent (ME)	7.9	63.7	26.9	34.6	129.7	1.7	7.9	129.7	0.21
A23		6.5:3.5	90	Translucent	8.8	9.3	34.3	32.9	173.6	57.8	8.8	173.6	0.33
A24		6.5:3.5	95	Transparent (ME)	30.4	34.8	148.7	65.2			30.4	148.7	0.29
A25		6:4	90	Translucent	24.7	33.0	128.8	66.2	1714.0	0.8	24.7	1714.0	0.99
A26		6:4	95	Translucent	27.7	36.6	137.2	61.5	1132.0	1.9	27.7	1132.0	1.01

[a]Oil : MCT. [b]Above the upper detection limitation of the spectrometer (2.00).

emulsions (i.e., ME) were lower than 0.29 (Figure 1b, A24). From these results, we set a criterion of turbidity '0.3', which was corresponding to approximately 150 nm, for rapid ME screening.

Relationship between phase stability and turbidity changes during storage

Phase stability and turbidity changes during storage were compared (Table 5). The physically unstable emulsions had a higher turbidity change compared with the stable emulsions. The turbidity changes of the unstable emulsions were larger than 0.1. From these results, we set a criterion of turbidity change '0.1' for rapid screening to select physically stable emulsion.

Performance evaluation of SMEDDS-HTFS system in pilot study

The performance of the SMEDDS-HTFS system was investigated in a pilot study. Small amounts of formulations (50 μL) were prepared rapidly by the ethanol-dispensing method (dispensing time: 40 minutes/96-formulation). Turbidity of the prepared emulsions was measured and the emulsions with a lower turbidity than the turbidity criterion (0.3) were selected rapidly as ME formulations (1 minute/96-formulation, Figure 2). Turbidity was measured again after storage to calculate the turbidity change, and the emulsions with changes below the turbidity-change criterion (0.1) were selected rapidly as physically stable emulsion formulations (1 minute/96-formulation, Figure 3). Physically stable ME (SMEDDS) formulations were selected by these two screenings, and the results are plotted in Figure 4. These SMEDDS formulations were confirmed by visual inspections to form transparent solutions without phase separation and drug precipitation.

The sizes of the SMEDDS areas on phase diagrams could be compared among various HS/LS combinations (Figure 4). The combination HCO40/CO3 had the largest SMEDDS area among all of the HS/LS combinations.

The HTFS was performed using minimal manpower (one person) and compound consumption (0.2 mg/formulation).

Table 5. Turbidity change and phase stability of emulsions during storage.

	Formulation			Turbidity before and after storage[a]			Phase stability[b]
Formulation no.	HS	HS:Oil[c]	Water (%v/v)	Before (T_{Before})	After (T_{After})	Turbidity change ($\lvert T_{After} - T_{Before} \rvert$)	(Visual inspection)
B1	BL2	9:1	90	0.94	0.01	0.92	Unstable
B2		9:1	95	1.48	0.90	0.58	Unstable
B3		8:2	90	1.80	0.87	0.93	Unstable
B4		8:2	95	1.99	1.71	0.28	Unstable
B5		7:3	90	1.89	1.75	0.14	Unstable
B6		7:3	95	>2.00[d]	1.93	N. C.[e]	Unstable
B7		6:4	90	>2.00[d]	>2.00[d]	N. C.[e]	Unstable
B8		6:4	95	>2.00[d]	1.34	N. C.[e]	Unstable
B9	BL4.2	9:1	90	>2.00[d]	0.13	N. C.[e]	Unstable
B10		9:1	95	1.17	0.89	0.28	Unstable
B11		8:2	90	1.76	1.60	0.16	Unstable
B12		8:2	95	1.48	0.91	0.57	Unstable
B13		7:3	90	>2.00[d]	1.70	N. C.[e]	Unstable
B14		7:3	95	1.68	0.54	1.15	Unstable
B15		6:4	90	>2.00[d]	0.96	N. C.[e]	Unstable
B16		6:4	95	1.73	0.49	1.24	Unstable
B17	BL9	9:1	90	0.00	0.00	0.00	Stable
B18		9:1	95	0.00	0.00	0.00	Stable
B19		8:2	90	0.19	0.06	0.12	Stable
B20		8:2	95	0.12	0.05	0.07	Stable
B21		7:3	90	1.83	0.29	1.54	Unstable
B22		7:3	95	1.53	0.60	0.93	Unstable
B23		6:4	90	>2.00[d]	1.75	N. C.[e]	Unstable
B24		6:4	95	>2.00[d]	1.18	N. C.[e]	Unstable
B25	PS40	9:1	90	0.00	0.00	0.00	Stable
B26		9:1	95	0.00	0.00	0.00	Stable
B27		8:2	90	0.00	0.00	0.00	Stable
B28		8:2	95	0.01	0.01	0.00	Stable
B29		7:3	90	0.59	0.51	0.08	Stable
B30		7:3	95	0.14	0.13	0.01	Stable
B31		6:4	90	>2.00[d]	>2.00[d]	N. C.[e]	Unstable
B32		6:4	95	1.65	1.43	0.22	Unstable
B33	PS80	9:1	90	0.00	0.00	0.00	Stable
B34		9:1	95	0.00	0.00	0.00	Stable
B35		8:2	90	0.00	0.00	0.00	Stable
B36		8:2	95	0.00	0.00	0.00	Stable
B37		7:3	90	0.01	0.01	0.00	Stable
B38		7:3	95	0.01	0.01	0.00	Stable
B39		6:4	90	1.81	1.70	0.12	Unstable
B40		6:4	95	0.78	0.65	0.13	Unstable
B41	Labrasol	9:1	90	0.42	0.04	0.38	Unstable
B42		9:1	95	0.53	0.03	0.50	Unstable
B43		8:2	90	0.60	0.09	0.51	Unstable
B44		8:2	95	1.11	0.02	1.08	Unstable
B45		7:3	90	1.09	0.03	1.06	Unstable
B46		7:3	95	1.54	0.14	1.40	Unstable
B47		6:4	90	1.59	0.36	1.23	Unstable
B48		6:4	95	1.35	0.27	1.09	Unstable

(*Continued*)

Table 5. (*Continued*)

	Formulation			Turbidity before and after storage[a]			Phase stability[b]
Formulation no.	HS	HS:Oil[c]	Water (%v/v)	Before (T_{Before})	After (T_{After})	Turbidity change ($\lvert T_{After} - T_{Before} \rvert$)	(Visual inspection)
B49	HCO40	9:1	90	0.01	0.01	0.00	Stable
B50		9:1	95	0.01	0.01	0.00	Stable
B51		8:2	90	0.03	0.02	0.01	Stable
B52		8:2	95	0.02	0.02	0.00	Stable
B53		7:3	90	0.23	0.21	0.02	Stable
B54		7:3	95	0.14	0.11	0.04	Stable
B55		6:4	90	0.91	0.68	0.22	Unstable
B56		6:4	95	0.85	0.71	0.14	Unstable
B57	HEGML	9:1	90	0.11	0.10	0.01	Stable
B58		9:1	95	0.12	0.11	0.01	Stable
B59		8:2	90	1.87	1.74	0.13	Unstable
B60		8:2	95	1.58	1.24	0.34	Unstable
B61		7:3	90	>2.00[d]	>2.00[d]	NC[e]	Unstable
B62		7:3	95	1.91	1.65	0.27	Unstable
B63		6:4	90	>2.00[d]	1.87	NC[e]	Unstable
B64		6:4	95	>2.00[d]	1.90	NC[e]	Unstable

[a]Storage condition: 25°C, 24 hours. [b]Phase stability of phase-separated emulsion or not-phase-separated emulsion was judged 'stable' or 'unstable', respectively. [c]Oil : MCT. [d]Above the upper detection limitation of the spectrometer (2.00). [e]NC: Not calculated.

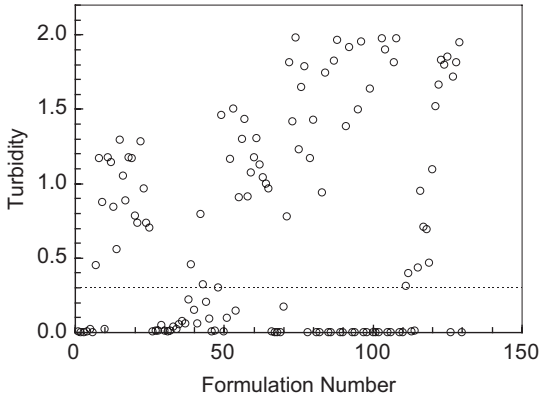

Figure 2. Turbidity of emulsion for ME screening. The dashed line shows the turbidity criterion (0.3) for selecting ME formulations.

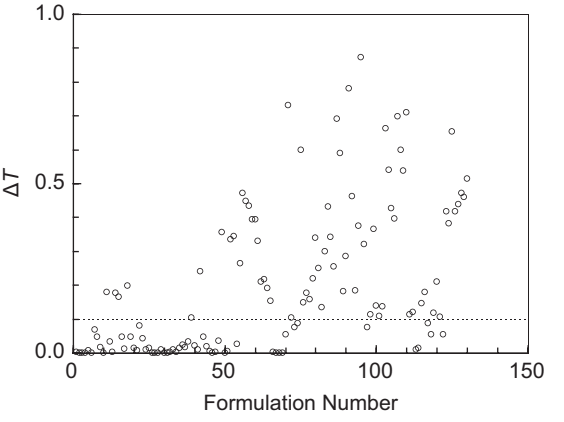

Figure 3. Turbidity change (ΔT) of emulsion between before and after storage (25°C, 24 hours) for physically stable emulsion screening. The dashed line shows the turbidity-change criterion (0.1) for selecting physically stable emulsion formulations.

Discussion

Rapid methods for formulation preparation, ME screening, and physically stable emulsion screening were required to develop a SMEDDS-HTFS system. We first focused on developing a rapid dispensing method to prepare formulations. Ethanol was used to rapidly dispense small amounts of viscous/semisolid surfactants and a powdered compound (Table 3). The effect of residual ethanol on the emulsion properties (emulsion particle size, phase stability) was a concern in developing this method. The amount of residual ethanol, which was determined by weighing, was less than 1 %w/w. This small amount of ethanol should not affect the emulsion properties[20]. Preparing these formulations by a conventional weighing method requires a significant amount of time (approximately 600–1200 minutes/96-formulation) and would consume a large amount of each compound. In contrast, the ethanol-dispensing method enabled us to prepare formulations rapidly (40 minutes/96-formulation) while consuming less amount of the compound (0.2 mg/formulation).

Second, we focused on developing a rapid ME screening method using a turbidity assay with a specific turbidity criterion. In investigating the applicability of this assay, we found that turbidity was a

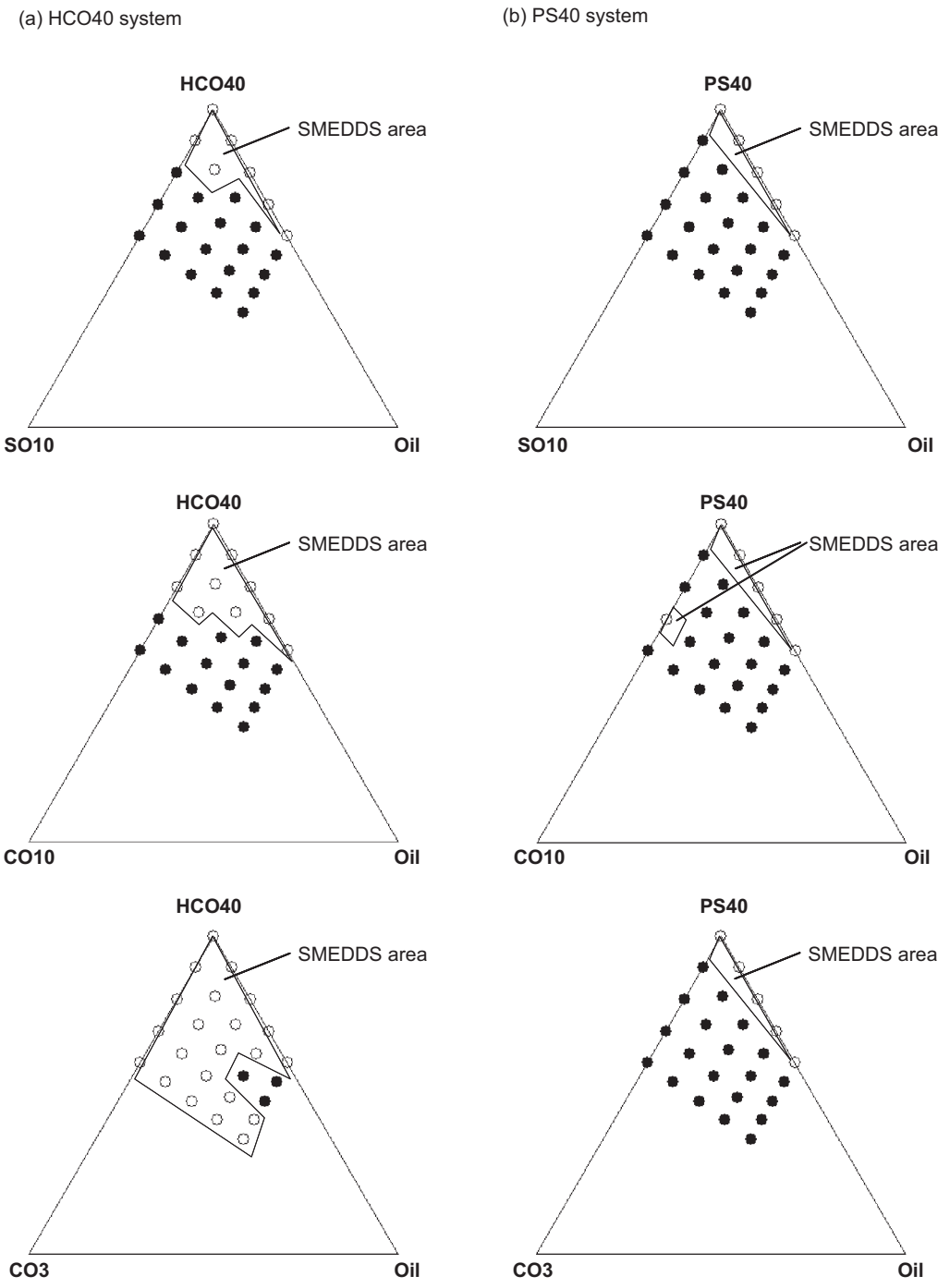

Figure 4. Phase diagrams. (a) HCO40 system, (b) PS40 system. Symbol: (❍) SMEDDS formulation, (●) non-SMEDDS formulation. The oil used was Sefsol-218. The region surrounded by a solid line shows the SMEDDS area.

function of the largest emulsion particle size in the emulsion particle size distribution (Figure 1a), consistent with Rayleigh and Mie scattering theories. The intensity of Rayleigh or Mie scattering light depends strongly on the particle size of scatterer because it is proportional to the sixth or second power of the particle size, respectively[21]. Therefore, the largest emulsion particle size would be a dominant parameter in the turbidity measurement.

Because the turbidity values for the transparent emulsions (ME) were lower than 0.29 (Figure 1b), we set the turbidity criterion '0.3' for rapid ME screening. The methods of visual inspection or DLS would require approximately 30–60 or 500–1000 minutes per 96

formulations, respectively, whereas the ME screening method using a turbidity assay required only 1 minute per 96 formulations. Thus, the turbidity assay allowed rapid ME screening (Figure 2).

Third, we focused on developing a rapid method to screen for physically stable emulsions using a turbidity assay with a specific turbidity change criterion. In investigating the applicability of this turbidity assay, we found that turbidity changes for emulsion solutions before and after storage reflected the phase stability during storage (Table 5). The reason for this result is that the incident light of a spectrometer passes through different phase states before and after storage[22]. For example, a physically unstable translucent emulsion could consist of one phase that then separates into two phases after storage (i.e., an opaque oil phase and a transparent aqueous phase).

Because the turbidity changes of unstable emulsions were larger than 0.1 (Table 5), we set the turbidity-change criterion '0.1' for rapid screening of physically stable emulsions. The method of visual inspection would require approximately 30–60 minutes per 96 formulations, whereas the physically stable emulsion-screening method using a turbidity assay required only 1 minute per 96 formulations. Thus, the turbidity assay allowed rapid screening for physically stable emulsions (Figure 3).

ME selected by turbidity criterion 0.3 has tendency to be a physically stable emulsion because its size is small. In this pilot study, all the ME selected by the turbidity criterion were physically stable. Although we have designed SMEDDS formulations using the HTFS system for some projects (data not shown), we found that some of the ME, which had lower turbidity than 0.3, were physically unstable. These formulations could be screened out by the criterion of turbidity change 0.1 for physical stability screening. Therefore, the turbidity change criterion would be important to select SMEDDS formulations.

Lastly, a pilot study was performed to evaluate the performance of the SMEDDS-HTFS system. The selected formulations (Figure 4) were confirmed by visual inspection to form transparent solutions without phase separation and drug precipitation. The SMEDDS-HTFS system enabled us to select SMEDDS formulations rapidly while requiring minimal manpower (one person) and consumption of compound (0.2 mg/formulation).

The SMEDDS-HTFS system also enabled us to select the most suitable HS/LS combination (HCO40/CO3), which had the largest SMEDDS area on its phase diagram among all of the HS/LS combinations. In the most suitable HS/LS combination, various characteristic formulations can be selected as SMEDDS formulations as follows: (1) If optimization of the HS/LS ratio for controlling the emulsification rate is required[15,23], HS (or LS)-rich SMEDDS formulations can be selected. (2) If minimizing the amount of surfactant to reduce GI irritation is required[24–28], oil-rich SMEDDS formulations can be selected.

In the HTFS system, the amount of a candidate compound dispensed is adjusted by dispense volume and compound concentration in ethanol. If the solubility of the compound in ethanol is poor, dimethyl sulfoxide (DMSO) is used instead. DMSO has a good solubility capacity and can be easily dispensed as same as water. In the case of DMSO, it is removed by vacuum drying before adding excipient ethanol solutions because DMSO in excipients is difficult to remove compared with ethanol. If the solubility of compound in these solvents is poor, other organic solvents must be sought. The maximum amount of the compound dispensed is determined by the equilibrium solubility in these solvents and the upper limitation of dispensed volume 1.2 mL, which is equal to the well capacity.

The design of SMEDDS formulations containing high compound load is desired for clinical study in some projects. Such formulations can be probably designed by performing HTFS at a target compound load.

Conclusions

In this study, we developed a SMEDDS-HTFS system that employs a rapid ethanol-dispensing method for formulation preparation and rapid screening methods of ME and physically stable emulsions using a turbidity assay. This SMEDDS-HTFS system was able to rapidly select SMEDDS formulations and the most suitable HS/LS combination, which formed the largest SMEDDS area on its corresponding phase diagram, with minimal manpower and compound consumption.

Declaration of interest: The authors report no conflicts of interest.

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