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# Particle size determination of phase-inverted water-in-oil microemulsions under different dilution and storage conditions

Panayiotis P. Constantinides [a,*], Seang H. Yiv [b]

[a] *Department of Drug Delivery, Pharmaceutical Technologies, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406, USA*
[b] *IBAH Inc., Bio-Pharm Drug Delivery Services, Aston, PA 19014, USA*

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**Abstract**

Laser light scattering was used to monitor particle size and polydispersity of several water-in-oil (w/o) microemulsion formulations upon dilution with excess of the dispersed (aqueous) phase to induce phase inversion and generate oil-in-water (o/w) and/or water-in-oil-in-water (w/o/w) emulsions. Factors affecting particle size, such as, the extent and temperature of dilution, as well as, sample storage conditions were investigated. In addition, the particle size of diluted formulations incorporating a peptide (SK&F 110679) was determined and compared to that of the peptide-free microemulsions. The extent of dilution had a pronounced effect on particle size. Dilution at ambient temperature or 37°C produced particles with similar size unless the microemulsion was solid at ambient temperature where significant effect on particle size upon dilution was observed. As expected from the non-ionic nature of the investigated microemulsions, using different physiologically relevant diluent solutions the particle size of the diluted microemulsion was found to be unaffected by pH and/or ionic strength. Dilution with a micellar sodium deoxycholate doubled the particle size and polydispersity of the diluted microemulsion, presumably as a result of physical interactions. The presence of a small peptide SK&F 110679 (Mol. Wt = 850) in different microemulsions prior to dilution, at levels varied from 0.8 to 3.0 mg/ml of formulation, had no major effect on the size of the inverted particle. Microemulsions which have been stored at various temperatures for up to 70 days and then diluted, showed no significant changes in particle size whereas the polydispersity was increased upon storage.

*Keywords:* Water-in-oil microemulsion; Dilution; Phase inversion; Particle size; Light scattering

## 1. Introduction

Microemulsions have attracted much interest in recent years in terms of their drug delivery potential. Water-in-oil and oil-in-water microemulsions have been shown to enhance the oral bioavailability of drugs, including peptides

Abbreviations: BHT, butylated hydroxytoluene; GHRP, growth hormone releasing peptide; HLB, hydrophilic-lipophilic balance; MCM, medium-chain monoglycerides; ME, microemulsion; o/w, oil-in-water; RGD, arginine-glycine-D-aspartic acid; **w/o**, water-in-oil.

* Corresponding author. Drug Delivery Department, UW 2111, SmithKline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, PA 19406–0939, USA. Tel. 215/270-5691.


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(Ritschel, 1991). Although the mechanism of absorption enhancement is still largely unknown, drug delivery advantages offered by microemulsions include: improved drug solubilization and protection against enzymatic hydrolysis, as well as the potential for enhanced absorption largely due to the inclusion of absorption enhancers/surfactants in the formulation (Swenson and Curatolo, 1992).

We have recently developed several water-in-oil (w/o) microemulsion systems that contain an oil or a mixture of oils, a blend of surfactants having a low and high HLB (hydrophilic-lipophilic balance), and an aqueous phase incorporating a water-soluble molecule/peptide (Constantinides and Yiv, 1994; Constantinides et al., 1994). Water-soluble molecules are assumed to be located within very small spherical droplets of water surrounded by surfactant molecules in a continuous oil medium. The aqueous phase is thus the internal or dispersed phase whereas the oil makes up the external or continuous phase. These systems can solubilize up to 50% (w/w) aqueous phase incorporating peptides of different physicochemical and pharmacological characterists, such as, RGD peptides that share the amino acid acid sequence of arginine-glycine-d-aspartic acid, GHRPs (growth hormone releasing peptides), vasopressins and calcitonins. With two water-soluble molecules, calcein and an RGD peptide (SK&F 106760), we have observed significant absorption enhancement upon intraduode-

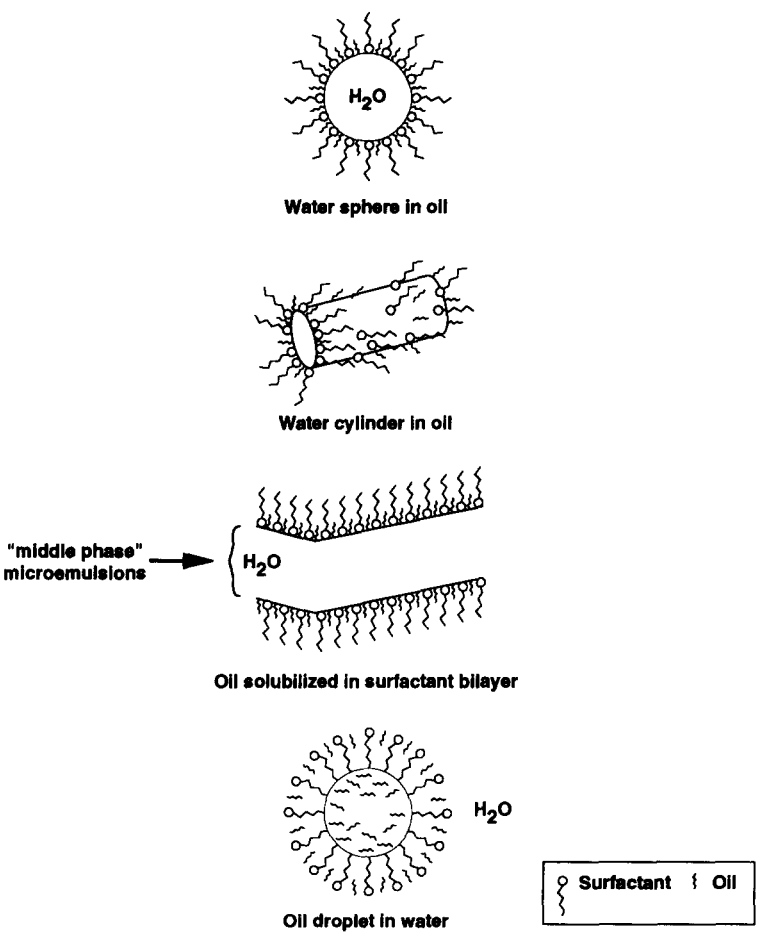


Fig. 1. Mechanism of phase inversion of a water-in-oil microemulsion (adapted from Attwood and Florence, 1983).

nal administration to rats from w/o microemulsions incorporating medium-chain glycerides (Constantinides et al., 1994).

Phase inversion of microemulsions (Leung and Shah, 1989) upon addition of excess of the dispersed phase or in response to temperature is an interesting property of these systems that can affect drug release both in vivo and in vitro. Upon dilution with excess aqueous phase w/o microemulsions are inverted into o/w emulsions/microemulsions and/or multiple w/o/w emulsions with a number of liquid crystalline phases being proposed as possible intermediates during this phase inversion process (see Fig. 1 and Attwood and Florence, 1983). During phase inversion drastic physical changes occur, including changes in particle size.

Most of the microemulsion particle sizing methods have centered around the use of scattering techniques including, photon correlation spectroscopy (Muller and Muller, 1984), time-average light scattering (Cazabat et al., 1980) and small angle neutron scattering (Caponetti and Magid, 1987). The objective of this study was to determine the particle size of several w/o microemulsions by photon correlation spectroscopy under different physiologically relevant dilution/inversion and storage conditions. The particle size upon dilution of w/o microemulsions incorporating the growth hormone releasing peptide SK&F 110679 was also measured and compared to that of the peptide-free formulations. SK&F 110679 is a water-soluble hexapeptide (His-D-Trp-Ala-Trp-D-Phe-Lys-$NH_2$) with very low octanol/buffer partitioning and at pH 5.7 the aqueous solubility exceeds 100 mg/ml (unpublished data).

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Microemulsion excipients

Captex 355 ($C_8/C_{10}$ triglycerides), Captex 200 (propylene glycol $C_8/C_{10}$ diesters) and Capmul MCM ($C_8/C_{10}$ mono-/diglycerides) were supplied by Karlshamns Lipid Specialties (Columbus, OH). Witepsol H-15 (90:10, %, w/w of $C_{12}$ glycerol triesters:diesters), with less than 2% $C_{12}$ monoester, and Imwitor 308 (80–90% wt of $C_8$ monoglycerides), were both provided by Huls America, Inc. (Piscataway, NJ). Myverol 18-92 (distilled sunflower monoglycerides) was purchased from Eastman Chemical Products Inc. (Kingsport, TN). Centrophase 31, a liquid lecithin (Mol. Wt 800) was supplied by Central Soya (Fort Wayne, IN). Tween 80 (polyoxyethylene sorbitan monooleate) was purchased from Sigma Corp. (St. Louis, MO) and Cremophor EL (polyoxyethylene glycerol triricinoleate 35 DAC) was a product of BASF Inc. (Parsippany, NJ). The HLB

Table 1
Composition (% w/w) of the investigated water-in-oil microemulsions [a]

| Component | ME1 | ME2 | ME3 | ME4 | ME5 |
|---|---|---|---|---|---|
| Aqueous [b] | 3.0 | 5.3 | 10.9 | 10.2 | 10.0 |
| Captex 355 (oil) | 65.0 | – | – | – | – |
| Captex 200 (oil) | – | 68.3 | – | 59.9 | 59.4 |
| Capmul MCM (HLB = 5.0– 6.0) | 22.0 | 8.3 | 7.9 | 13.7 | – |
| Centrophase 31 (HLB = 4.0) | – | 1.6 | – | 3.2 | – |
| Cremophor EL (HLB = 13.5) | – | 16.5 | – | – | – |
| Inwitor 308 (HLB = 6.0) | – | – | – | – | 15.3 |
| Myverol 18– 92 (HLB = 3.7) | – | – | 4.3 | – | – |
| Tween 80 (HLB = 15.0) | 10.0 | – | 38.6 | 13.0 | 15.3 |
| Witepsol H-15 [c] (oil) | – | – | 38.3 | – | – |

[a] The concentration of the peptide in ME1, ME2, ME3, ME4 and ME5 was 0.80, 1.34, 2.76, 2.50 and 2.50 mg/ml of ME, respectively.
[b] Isotonic acetate at pH 5.0 and osmorality of 300 mOsm.
[c] High melting point oil (m.p. 33–36°C).

values of the aforementioned emulsifiers are listed in Table 1.

*2.1.2. Drug supply and stock solutions*

The acetate salt of SK&F 110679 was supplied by Pharmaceutical Technologies, SB Pharmaceuticals (King of Prussia, PA), with specified chemical purity at 99.8% and peptide content at 81.6% based on an HPLC assay. A stock solution of SK&F 110679 was prepared at 27 mg/ml by dissolving the compound in sterile water for injection, (USP), and then adjusting the pH and osmolarity to 5.0 and 300 mOsm, respectively. The peptide content of the resulting isotonic solution of the drug was verified by HPLC and then aliquoted into sealed ampules (0.6 ml/ampule) and stored at 4°C in the dark. A placebo (peptide-free) isotonic acetate solution at pH 5.0 and osmolarity of 302 mOsm was prepared and used as a diluent unless otherwise indicated.

*2.1.3. Light scattering standards*

Polystyrene (latex) beads of different particle size in the sub-micron range were employed upon dilution as particle size standards.

*2.2. Methods*

*2.2.1. Microemulsion preparation*

The w/o microemulsions employed in this study were prepared as previously described (Constantinides and Yiv, 1994; Constantinides et al., 1994) by admixing appropriate quantities of the various components with gentle hand mixing, vortexing or stirring if necessary to ensure thorough mixing. For the preparation of the ME1 microemulsion (Constantinides et al., 1994) the drug (SK&F 110679) was first dissolved in the hydrophilic phase by dilution of a stock solution, then the high HLB surfactant (Tween 80) was added followed by a pre-mixed combination of the oil (Captex 355) and the low HLB surfactant (Capmul MCM). The same procedure was also used to prepare drug-free microemulsions. Microemulsions ME2–ME5 were prepared by admixing the oil (Captex 200), the blend of the low and high HLB surfactants (Capmul MCM, Centrophase 31, Cremophor EL or Tween 80) and the hydrophilic phase containing the peptide. The w/o microemulsion that is solid at room temperature (ME3), was prepared by admixing the high melting oil (Witepsol H15) with the other components as described above. The solution of components was heated to a slightly elevated temperature (30–50°C) during mixing and then cooled to a solid at ambient temperature (Constantinides and Yiv, 1994). Whilst higher temperatures (30–60°C) may be needed to solubilize all components during the preparation of microemulsion, the microemulsions which are liquid at ambient temperature can be formulated at this temperature. This is particularly advantageous for thermolabile compounds/peptides. The compositions of the investigated w/o microemulsions are shown in Table 1. The microemulsion ME3 is solid at room temperature but liquid at 37°C whereas all other microemulsions are liquid at room temperature.

*2.2.2. Physical characterization of microemulsions*

*2.2.2.1. Viscosity and refractive index measurements.* The viscosity of the microemulsions was monitored by a Cannon-Manning Semi-micro viscometer (Baxter/Scientific Products, McGaw Park, IL) size 200 with a viscometer constant of 0.0984 $mm^2/S^2$ or (cSt/s). The kinematic viscosity in centistokes (cSt) of the sample is calculated by multiplying the efflux time in seconds (s) by the viscometer constant. Multiplying this value by the density of the sample gives the viscosity in centipoise (cP) units. The instrument was calibrated with liquids of known viscosity, including oleic acid. For measuring refractive index, a Milton Roy refractometer (Thomas Scientific, Swedesboro, NJ) was used. Deionized water and oleic acid were used to calibrate the instrument.

*2.2.2.2. Particle size determination.* Photon correlation spectroscopy (PCS) using laser light scattering is frequently used to determine particle size of emulsions (Cazabat et al., 1980; Muller and Muller, 1984). A Malvern Photon Correlation Spectrometer model 4700 equipped with an argon laser model 2000 from Spectra Physics (Mount View, CA) was employed to monitor particle size of the various microemulsion systems. A

Table 2
Effect of the extent of dilution and mixing mechanism on the particle size and polydispersity of the inverted ME1 and ME2 microemulsions

| Extent of dilution and mixing mechanism [a] | Mean diameter ± SD (nm) | Mean polydispersity ± SD |
|---|---|---|
| ME1, 100× HM | 191.3 ± 108 | 0.352 ± 0.012 |
| ME1,1000× HM | 1277 ± 408 | 0.305 ± 0.024 |
| ME1, 100× SP | 61.2 ± 27.0 | 0.360 ± 0.007 |
| ME1,1000× SP | 2060 ± 523 | 0.792 ± 0.009 |
| ME2, 1000×, HM | 76.3 ± 42.6 | 0.390 ± 0.002 |
| ME2, 1000×, SP | 92.6 ± 42.9 | 0.324 ± 0.002 |
| ME2, 1000×, VX | 79.8 ± 44.7 | 0.355 ± 0.004 |
| ME2, 100×, VX | 3.5 ± 1.4 | 0.188 ± 0.036 |
| Latex beads standard, 59 nm | 57.3 ± 15.1 | 0.061 ± 0.029 |

[a] HM , SP and VX denote dilution by hand mixing, on a stirring plate and by vortexing, respectively.

120-channel controlled digital correlator was used to receive and count the photons. Samples were loaded into 1-cm$^2$ cylindrical cuvettes and placed in a thermostated scattering chamber. The aperture of the photomultiplier tube was set at 50 nm. The viscosity and refractive index of microemulsions were incorporated into the computer software which calculates the mean particle size and polydispersity from intensity, mass and number bimodal distributions assuming spherical particles. Polydispersity is a measure of particle homogeneity and it varies from 0.0 to 1.0. The closer to zero the polydispersity value the more homogeneous are the particles. Light scattering was monitored at 90° scattering angle and temperature of 25 or 37°C.

*2.2.2.3. Temperature stability samples.* Shelf-life stability of microemulsions both as a function of time and storage temperature was evaluated by both visual inspection of the intact w/o microemulsions and by UV/VIS spectroscopy (unpublished data). Stability was monitored at 4°C (refrigerator), 30, 40 and 60°C. By visual inspection criteria, stable systems were identified as those free of any physical changes, such as phase separation, flocculation and/or precipitation.

## 3. Results and discussion

The mean particle diameter and polydispersity were calculated from intensity, mass and number

Table 3
Effect of the extent and temperature of dilution on the particle size and polydispersity of the inverted ME3 and ME4 microemulsions

| Extent and temperature of dilution [a] | Mean diameter ± SD (nm) | Mean polydispersity ± SD |
|---|---|---|
| ME3, 100× RT, LST 25°C | 115.1 ± 116.9 | 0.474 ± 0.004 |
| ME3, 1000× RT, LST 25°C | 379.0 ± 71.8 | 0.438 ± 0.004 |
| ME3, 100× 37°C, LST 25°C | 46.9 ± 14.8 | 0.243 ± 0.006 |
| ME3, 1000× 37°C, LST 25°C | 50.0 ± 20.0 | 0.339 ± 0.006 |
| ME3, 100× 37° C, LST 37°C | 45.7 ± 14.1 | 0.246 ± 0.006 |
| ME3, 1000× 37°C, LST 37°C | 49.6 ± 19.8 | 0.331 ± 0.004 |
| ME4, 1000×, RT | 97.8 ± 30.2 | 0.124 ± 0.005 |
| ME4, 1000×, 37°C | 103.9 ± 30.3 | 0.120 ± 0.009 |
| ME4, 100×, RT unfiltered | 9.2 ± 4.6 | 0.402 ± 0.002 |
| ME4, 100×, RT, filtered through a 0.2 μm filter | 8.8 ± 4.4 | 0.417 ± 0.003 |

[a] RT, room temperature; LST, light scattering temperature; dilution was performed on a stirring plate.

bimodal distributions. For clarity, however, the results are presented only as mass distributions. The effect of the extent and temperature of dilution, as well as the mechanics of mixing during dilution, on the particle size of the inverted microemulsion were monitored. The results from each of these parameters are presented below.

### *3.1. Effect of the extent of dilution on the particle size of the inverted microemulsion*

The extent of dilution had a more dramatic effect on the particle size upon dilution of ME1, ME2 (Table 2) and ME4 (Table 3) microemulsions, but no significant effect on that of the ME3 microemulsion (Table 3). Specifically, at 100-fold dilution, ME2 and ME4 produced particles with an extremely and consistently small mean particle diameter ($< 20$ nm), most likely indicative of liquid crystalline/micellar intermediates, such as, hexagonal phases proposed to be formed during phase inversion of w/o microemulsions (Attwood and Florence, 1983). In the case of ME4, the presence of an extremely small particle at 100-fold dilution was verified by passing the diluted microemulsion through a 0.2 $\mu$m filter (Acrodisk). Essentially the same mean particle diameter was obtained when non-filtered and filtered samples were compared (Table 3). At 100-fold dilution, ME1 produced particles with a mean diameter between 30 and 200 nm (Table 2). At higher degree of dilution (1000-fold), ME2 converted to an o/w emulsion with a mean particle diameter of about 100 nm (Table 2). The mean diameter of the emulsions upon a 1000-fold dilution of ME1 was at least 1 $\mu$m, with polydispersity between 0.3 and 0.8, suggesting the formation of heterogeneous coarse o/w microemulsions and/or multiple (w/o/w) emulsions. The presence of these large particles was also observed at 500-fold and 10000-fold dilution (data not shown). For ME1 and ME2 microemulsions and at 1000-fold dilution, similar particle size was observed when the microemulsion was either directly diluted to 1000-fold or indirectly to 100-fold dilution first, followed by a 10-fold dilution (data not shown). At either 100- or 1000-fold dilution, ME3 (Table 3) produced very fine and transparent o/w microemulsions, with a mean particle diameter of about 50 nm, provided the dilution was performed at 37°C.

### *3.2. Effect of the dilution temperature on the particle size of the inverted microemulsion*

The temperature during dilution, as expected, had significant effect on the microemulsion ME3 which is solid at room temperature but liquid at 37°C. When ME3 was premelted at 37°C and then diluted (1000-fold) at room temperature, large and heterogeneous emulsions were produced with mean particle diameter $> 0.1$ $\mu$m and polydispersity of about 0.5 (Table 3). Fine and transparent microemulsions with a mean particle diameter and polydispersity of about 50 nm and 0.25, respectively, were obtained when the dilution took place at 37°C (Table 3). Similar particle size was obtained when light scattering was measured at 37°C as Table 3 indicates, suggesting that microemulsion dilution at 37°C generated emulsions that remained stable at room temperature, at least within few hours postdilution. In the case of the ME4 microemulsion which is liquid at room temperature, at 1000-fold dilution either at room temperature or 37°C, produced particles with essentially the same mean diameter and polydispersity (Table 3).

### *3.3. Effect of the mixing mechanism during dilution on the particle size of the inverted microemulsion*

Mixing during dilution was accomplished either by hand-mixing, on a stirring plate via a magnetic bar or by vortexing. In the last two methods the stirring/vortexing speed and time were carefully monitored. In general, at a given degree of dilution, there was no appreciable effect of the mixing mechanism on the particle size of the diluted microemulsions (Table 2). However, in order to reproduce the mixing conditions during dilution, it is recommended that stirring on a plate (Fisher Thermix Stirring Hot Plate, Model 210T, at position 3–4 for 1 min) is employed.

Spontaneous emulsification mechanism has been proposed recently (Greiner and Evans, 1990) in the formation of a water-continuous emulsion

Table 4
Diluent effect on the particle size and polydispersity of the inverted ME4 microemulsions

| Diluent [a] | Mean diameter ± SD (nm) | Mean polydispersity ± SD |
|---|---|---|
| Isotonic acetate pH 5 | 103.9 ± 30.3 | 0.120 ± 0.009 |
| Deionized water | 107.9 ± 32.1 | 0.146 ± 0.006 |
| 0.1 N HCl | 107.8 ± 31.3 | 0.148 ± 0.002 |
| Saline (USP) | 108.3 ± 31.4 | 0.138 ± 0.010 |
| PBS [b] | 103.7 ± 31.3 | 0.155 ± 0.010 |
| 0.5% SDOC [c] in PBS | 307.4 ± 100.6 | 0.308 ± 0.002 |

[a] Dilution was performed at 37°C on a stirring plate; dilution factor: 1000 ×.
[b] Phosphate-buffered saline ($Ca^{2+}$- and $Mg^{2+}$-free).
[c] Sodium deoxycholate.

by inversion of a highly viscous w/o microemulsion containing less than 10 wt% of water. It has been found that inversion led to the formation of a stable, rather homogeneous o/w emulsions containing oil droplets, as small as 150 nm (Greiner and Evans, 1990). The w/o microemulsions we have investigated in the present study are also viscous and incorporate up to 10% aqueous phase (Table 1). Therefore, a similar inversion process may take place that involves diffusion of water into inverted micelles which grow, interconnect and then invert (Greiner and Evans, 1990).

### *3.4. Diluent effect on the particle size of the inverted microemulsion*

Table 4 summarizes the light scattering data obtained upon dilution of ME4 using physiologically relevant aqueous solutions (diluents) of different pH and/or ionic strength. The w/o microemulsions employed in the present study (Table 1), incorporate non-ionic components (oils and surfactants) and should therefore be insensitive to pH and/or ionic strength changes during dilution. Indeed, as can be seen from the data in Table 4, at least with ME4, neither the particle size nor polydispersity were affected by the pH and/or the ionic strength of the diluent. Dilution, however, with a micellar solution of sodium deoxycholate in phosphate-buffered saline at 0.5% w/v or 12 mM, doubled the particle size and polydispersity of the inverted microemulsion (Table 4). These results suggest that physical changes in microemulsions/emulsions as a result of interactions with other micellar systems can be detected by laser light scattering. It is known that in vivo emulsification of the microemulsion by bile salts can lead to an increase in the particle size of the resulting emulsion (Ritschel, 1991).

Table 5
Effect of temperature and length of storage on the particle size and polydispersity upon dilution of ME1 and ME2 peptide-free and peptide-incorporating microemulsions

| Storage conditions [a] | Mean diameter ± SD (nm) | Mean polydispersity ± SD |
|---|---|---|
| ME1-C, 30°C, 2 weeks | 61.2 ± 27.0 | 0.360 ± 0.007 |
| ME1-P [b], 30°C, 2 weeks | 77.7 ± 38.7 | 0.374 ± 0.015 |
| ME1-C, 40°C, 70 days | 71.9 ± 34.1 | 0.359 ± 0.011 |
| ME1-P [b], 40°C, 70 days | 209.1 ± 114.7 | 0.380 ± 0.010 |
| ME2-C, 30°C, 2 weeks | 109.2 ± 40.8 | 0.266 ± 0.006 |
| ME2-P [c], 30°C, 2 weeks | 104.7 ± 44.2 | 0.275 ± 0.002 |
| ME2-C, 40°C, 70 days | 42.9 ± 26.9 | 0.442 ± 0.007 |
| ME2-P [c], 40°C, 70 days | 49.8 ± 31.5 | 0.425 ± 0.002 |

[a] ME1 and ME2 microemulsions were diluted 100 × and 1000 ×, respectively; C and P denote peptide-free (control) and peptide-incorporating microemulsion, respectively.
[b] Incorporating peptide (SK&F 110679) at 0.80 mg/ml of ME.
[c] Incorporating peptide (SK&F 110679) at 1.34 mg/ml of ME.

Table 6
Effect of temperature and length of storage on the particle size and polydispersity upon dilution of ME4 peptide-free and peptide-incorporating microemulsions

| Storage conditions | Mean diameter ± SD (nm) | Mean polydispersity ± SD |
|---|---|---|
| C [a], 4°C, 7 days | 97.3 ± 30.2 | 0.124 ± 0.005 |
| P [b], 4°C, 7 days | 98.7 ± 30.4 | 0.127 ± 0.003 |
| C [a], 4°C, 70 days | 84.0 ± 25.2 | 0.211 ± 0.002 |
| P [b], 4°C, 70 days | 76.2 ± 26.3 | 0.237 ± 0.006 |
| C [a], 30°C, 70 days | 74.7 ± 25.5 | 0.254 ± 0.010 |
| P [b], 30°C, 70 days | 75.4 ± 26.7 | 0.260 ± 0.012 |
| C [a], 40°C, 70 days | 74.5 ± 27.8 | 0.276 ± 0.010 |
| P [b], 40°C, 70 days | 74.5 ± 27.5 | 0.266 ± 0.009 |
| Latex beads standard, 59 nm | 54.5 ± 14.5 | 0.054 ± 0.035 |

[a] Control (peptide-free) microemulsion; dilution conditions: 1000 ×, RT.
[b] Incorporating peptide (SK&F 110679) at 2.50 mg/ml of ME; dilution conditions: 1000 ×, RT.

### 3.5. Particle size upon dilution of fresh versus stored microemulsions

Representative microemulsion formulations (ME1, ME2 and ME4) were stored at 4, 30, and 40°C up to 70 days and their mean particle diameter and polydispersity upon dilution were determined and compared to those obtained with freshly prepared formulations. The results are shown in Tables 5 and 6. Storage of ME1 microemulsions before dilution, at either 30 or 40°C had no major effect on either the mean particle diameter or polydispersity of the diluted microemulsion (Table 5). Diluted ME2 microemulsions showed a similar trend in particle size between these two temperatures, with the polydispersity being doubled, however, upon a 70-day incubation at 40°C (Table 5). A 70-day incubation of ME4 at 4, 30, and 40°C prior to dilution had no effect on the mean particle diameter or polydispersity upon a 1000-fold dilution (Table 6). When the 7- and 70-day 4°C incubated samples were compared, a 2-fold increase in polydispersity of diluted ME4 microemulsions was observed upon dilution of the 70-day stored sample (Table 6).

### 3.6. Particle size and polydispersity upon dilution of peptide-free versus peptide (SK&F 110679)-incorporating microemulsions

By comparing the mean particle diameter and polydispersity of the peptide-free and peptide-incorporating ME1, ME2 and ME4 microemulsions (Tables 5 and 6), it appears that the peptide, present at different levels in the investigated w/o microemulsions prior to dilution, had no major effect on the particle size or polydispersity of the inverted microemulsions. Similar effects were aslo observed with peptide-incorporating ME3 and

Table 7
Particle size and polydispersity upon dilution of fresh and thermally stressed ME5 microemulsions

| Storage | Mean diameter ± SD (nm) | Mean polydispersity ± SD |
|---|---|---|
| RT, 2 days | 64.8 ± 58.7 | 0.449 ± 0.001 |
| RT, 2 days with 0.2% BHT [a] | 46.4 ± 33.9 | 0.460 ± 0.004 |
| 60°C, 6 days | 69.9 ± 62.9 | 0.444 ± 0.001 |
| 60°C, 6 days with 0.2% BHT | 86.5 ± 38.1 | 0.322 ± 0.005 |
| C.P. ME [b] | 830.5 ± 582.5 | 0.987 ± 0.018 |
| Diluted/inverted C.P. ME | 316.3 ± 146.3 | 0.483 ± 0.002 |
| Latex beads standard, 220 nm | 320.8 ± 84.2 | 0.100 ± 0.046 |

[a] Butylated hydroxytoluene, a lipid-soluble antioxidant; 1000 × dilution was applied to all samples at RT.
[b] Cloud point (borderline) microemulsion.

ME5 microemulsions (data not shown). Ultraviolet/visible (UV/Vis) spectroscopy and high-performance liquid chromatography (HPLC) have demonstrated chemical changes in microemulsion-formulated SK&F 110679 upon storage at different temperatures (data not shown). These chemical changes, however, seem not to be sufficiently significant to affect the particle size and polydispersity of the inverted microemulsion as measured by light scattering.

### *3.7. Particle size upon dilution of fresh versus thermally stressed microemulsions*

One particular aspect of the temperature stability studies was to compare particle size and polydispersity between freshly prepared and thermally stressed formulations in the absence and presence of 0.2% BHT (butylated hydroxy toluene), a lipid-soluble antioxidant. The light scattering data obtained with diluted (1000-fold) ME5 microemulsion are shown in Table 7. This particular microemulsion, freshly prepared, upon a 1000-fold dilution produced o/w particles similar in size to those produced by other microemulsion formulations but with a significantly higher polydispersity (about 0.4). Neither the mean particle diameter nor polydispersity of the inverted microemulsion was found to be affected by the 6-day incubation at 60°C, although the presence of the antioxidant had some small but significant effect on the polydispersity of the inverted microemulsion (about 25% decrease).

The data obtained with ME5 microemulsion that was brought close to cloud point (borderline w/o microemulsion) before dilution were of interest and serve as a reference for microemulsion stability evaluation. As can be seen in Table 7, when ME5 was brought close to the cloud point by dropwise addition of aqueous phase, both the mean particle diameter and polydispersity were increased. An approx. 10-fold increase in the mean particle diameter and a 2-fold increase in polydispersity were obtained (Table 7). This was also evident by the presence of multi-peak light scattering histograms (data not shown). Upon further dilution with excess of the aqueous phase (1000-fold), the borderline microemulsion inverts to a larger particle compared to that produced by microemulsion away from the cloud point (Table 7). Since the structure of the borderline microemulsion is largely unknown, however, it is difficult to directly compare its particle size to that of the diluted microemulsion away from the cloud point.

## 4. Conclusions

The w/o microemulsions evaluated in this study, freshly prepared or upon storage, with ot without an incorporated peptide (SK&F 110679), when diluted with excess of the dispersed phase to induce phase inversion, produced fine microemulsions having mean particle diameter and polydispersity between 5 and 200 nm and 0.1 and 0.4, respectively. Only under extreme conditions of storage (temperatures above 60°C), or with cloud point microemulsions, can large increases in mean particle diameter and polydispersity be detected. The present work provides a useful background in determining the design of future light scattering experiments for monitoring particle size changes of microemulsions upon dilution and phase inversion.

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## References

Attwood, D. and Florence, A.T., *Surfactant Systems: Their Chemistry, Pharmacy and Biology*, Chapman and Hall, London, 1983, pp. 519–524.

Caponetti, E. and Magid, L.J., Static SANS measurements on concentrated water-in-oil microemulsions. In Rosano, H.L. and Clausse, M. (Eds), *Microemulsion Systems, Surfactant Science Series*, Vol. 24, Dekker, New York, 1987, pp. 227–298.

Cazabat, A.M., Langevin, D. and Pouchelon, A., Light scattering study of water-in-oil microemulsions. *J. Colloid Interface Sci.*, 73 (1980) 1–12.

Constantinides, P.P. and Yiv, S.H., Particle size determination of phase inverted water-in-oil microemulsions under different dilution and storage conditions. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 21 (1994) 766–767.

Constantinides, P.P, Scalart, J.-P, Lancaster, C., Marcello, J., Marks, G., Ellens, H. and Smith, P.L., Formulation and intestinal absorption enhancement evaluation of water-in-oil microemulsions incorporating medium-chain glycerides. *Pharm. Res.*, 11 (1994) 1385–1390.

Greiner, R.W. and Evans, D.F., Spontaneous formation of a water-continuous emulsion from a w/o microemulsion. *Langmuir*, 6 (1990) 1793–1796.

Leung, R. and Shah, D.O., Microemulsions: an evolving technology for pharmaceutical applications. In Rosoff, M. (Ed.), *Controlled Release of Drugs: Polymers and Aggregate Systems*, VCH, New York, 1989, pp. 185–215.

Muller, B.W. and Muller, R.H., Particle size distributions and particle size alterations in microemulsions. *J. Pharm. Sci.*, 73 (1984) 919–922.

Ritschel, W., Microemulsions for improved peptide absorption from the gastrointestinal tract. *Methods Find. Exp. Clin. Pharmacol.*, 13 (1991) 205–220.

Swenson, E.C. and Curatolo W.J., Intestinal permeability enhancement for proteins, peptides and other polar drugs: mechanisms and potential toxicity. *Adv. Drug Del. Rev.*, 8 (1992) 39–92.