

A topical w/o/w multiple emulsions prepared with Tetronic 908 as a hydrophilic surfactant: Formulation, characterization and release study

Figen TIRNAKSIZ, Ozlem KALSIN. Gazi University Faculty of Pharmacy, Department of Pharmaceutical Technology, Etiler – 06330, Ankara, Turkey.

Received February 14, 2005, Revised May 25, 2005, Accepted June 6, 2005, Published August 5, 2005

ABSTRACT Purpose: The aim of this work was to prepare the stable water/oil/water multiple emulsions (MEs), to investigate the usage of poloxamine 908, to observe the influence of surfactant percentage on the properties of MEs. **Method:** MEs were prepared by liquid paraffin, cetyl dimethicone copolyol and poloxamine 908 by a two-step emulsification procedure. Caffeine was used as a water-soluble model. The viscosity, conductivity and globule size of MEs were followed over time. **Results:** The formulations containing 1% cetyl dimethicone copolyol and 1 or 2% poloxamine 908 were the most stable systems. The globule size of MEs ranged from 20 to 37 μm and did not change during time. The yield of MEs was between 99.6% and 98.7%. The conductivity increased and the viscosity of systems decreased during time. Increase in poloxamine 908 influenced the viscosity of the system, with the viscosity decreasing as the hydrophilic surfactant concentrations were increased. Caffeine release from the MEs was slow; the release was affected by both surfactant concentrations. **Conclusion:** Poloxamine 908 could be used as a hydrophilic surfactant for formulation of w/o/w MEs. The concentration of poloxamine 908 was a very important parameter in preparing stable MEs. It was concluded that caffeine might be transported out by molecular diffusion and through a reverse micellar mechanism controlled by the viscosity of the system

INTRODUCTION

Multiple emulsions are vesicular and complex systems [1]. They can be considered as emulsions of emulsions and have shown promise in cosmetic [2, 3], pharmaceutical and separation sciences [4].

Their potential pharmaceutical applications include uses such as taste masking, adjuvant vaccines, an immobilization of enzymes and sorbent reservoir of overdose treatments, and for enhancement of enteral or dermal absorption [5-8]. Multiple emulsions have been formulated as cosmetics, such as skin moisturizer [9]. Prolonged release can also be obtained by means of multiple structures [10-13]. These systems have some advantages, such as the protection of the entrapped substances [14, 15] and the incorporation of several actives in the different compartments [16]. Despite their potential usefulness, applications of multiple emulsions have been limited because of thermodynamic instability and their complex structure.

Water/oil/water (w/o/w) multiple emulsions consist of dispersed oil globules containing smaller aqueous droplets; each inner aqueous droplet is separated from the outer aqueous phase by an oil phase layer. The presence of at least two surfactants is required. One of them is predominantly lipophilic for stabilizing the primary water/oil (w/o) emulsion and the other is hydrophilic for the secondary oil/water emulsion. To produce a w/o/w emulsion, the lipophilic and hydrophilic surfactants are dissolved in oil and continuous aqueous phase, respectively. The most common preparations of w/o/w double emulsions are based on the two-step emulsification process. The stability and release characteristics of multiple emulsions are influenced by different factors, such as surfactant type, surfactant ratio and some physical properties of the system (globule size, viscosity, conductivity, phase volume ratio, etc) [1, 17, 18].

The Poloxamine 908 (Tetronic[®]908) used in this study as a hydrophilic surfactant is nonionic and a block copolymer [19]. We could not find any published work on the usage of the Tetronic[®]908 in the formulation of multiple emulsions. Cetyl dimethicone copolyol (Abil[®]EM90) used as a lipophilic surfactant is the siliconic polymeric

Corresponding author: Figen TIRNAKSIZ, Gazi University Faculty of Pharmacy, Department of Pharmaceutical Technology, Etiler – 06330, Ankara, Turkey. e-mail: feegosh@yahoo.com

surfactant, and shows good trapping capacity, prolongs the release of active molecules, and produces a w/o emulsion with strong interfacial film [4, 20, 21].

The purposes of this research were *i)* to investigate the usage of Tetronic[®]908 for w/o/w multiple emulsion formulation, *ii)* to evaluate the formation and stability of the multiple emulsion system, *iii)* to investigate the properties of the system and to observe the influence of hydrophilic and lipophilic surfactant percentage on the characteristic properties of multiple emulsions.

EXPERIMENTAL

Chemicals

The following substances were used for the preparation of multiple w/o/w emulsions: The oil used was liquid paraffin (η : 110-230 mPa.s, Merck). Abil[®]EM90 (cetyl dimethicone copolyol, Goldschmidt, France) was used as a lipophilic surfactant. Tetronic[®]908 (poloxamine 908, BASF-New Jersey, USA) was used as a hydrophilic nonionic surfactant. This is a poly (oxyethylene)-poly (oxypropylene) block copolymer containing approximately 80% oxyethylene.

Caffeine (Merck) frequently used in cosmetic products was selected as a hydrophilic model substance for release experiment. Sodium chloride (NaCl) was incorporated into the aqueous internal phase and used as a conductimetric tracer.

All materials were of analytical grade and used without further purification.

Preparation of w/o/w multiple emulsion

Multiple emulsions were prepared by a two-step emulsification process [2]. The first emulsification was to prepare the w/o primary emulsion and the second emulsification step provided the formation of the w/o/w multiple globules. The compositions of formulations are shown in Table 1.

Electrolyte or both electrolyte and caffeine were dissolved merely in the aqueous phase of the primary w/o emulsion. The osmotic pressure of the internal aqueous phase of multiple emulsions containing or not-containing caffeine was adjusted to 3.4 atm.

The concentrations of paraffin oil and electrolyte in primary emulsions were fixed at 30 and 0.3%, respectively. The percentage of the primary emulsion was also constant at 80%.

Table 1: Composition of the w/o/w multiple emulsions

First emulsification (for primary w/o emulsion)

Without caffeine

Oil phase

Liquid paraffin	30%
Abil [®] EM90	2 or 4%

Internal aqueous phase

Sodium chloride	0.3%
Distilled water to	100

With caffeine

Oil phase

Liquid paraffin	30%
Abil [®] EM90	2 or 4%

Internal aqueous phase

Sodium chloride	0.03%
Caffeine	1.5%
Distilled water to	100

Second emulsification (for w/o/w multiple emulsion)*

Oil phase

Primary w/o emulsion	80%
----------------------	-----

External aqueous phase

Tetronic [®] 908	1, 2 or 4%
Distilled water to	100

*The caffeine concentration of multiple emulsions = 1.2%

Water/oil primary emulsions were prepared with different concentration of a lipophilic surfactant (Abil[®]EM90). Two concentration of Abil[®]EM90 were used in the primary emulsion (2 and 4%). The oily mixture of Abil[®]EM90 and paraffin was heated to 75°C and then aqueous solution at the same temperature was added to this mixture by stirring at 1500 rpm at 75 °C for 30 minutes. The first step was carried out using the mechanical mixer to produce fine droplets. The emulsion prepared was then cooled to room temperature. The mixer used to prepare the MEs was the similar of Microvortex mechanical mixer (Granier Charvet

SA, France) and it was produced in Anadolu University (Eskişehir-Turkey).

The stability of the w/o primary emulsion prepared at the first emulsification was an important factor determining the stability of the w/o/w emulsion. Therefore, the accelerated physical stability of primary emulsions was tested under centrifugation (Mechanica Precyzynjna, Warszawa, MPW-340, Poland) during 1 hour at 3500 rpm (10060 m/s² or 1026 g), and the occurrence of phase separation was examined. The stability of w/o emulsions under centrifugation reflects the strength of the interfacial film between the aqueous and oil phases. No change was macroscopically observed in any of the primary emulsions in this study, which were quite stable. Moreover, no phase separation was observed during 6 months at room temperature.

Water/oil/water multiple emulsions containing caffeine were prepared by the same emulsification procedure under the same conditions. Both caffeine and NaCl were dissolved into the inner aqueous phase. The osmotic pressure created by adding caffeine was calculated according to Van't Hoff's equation, and NaCl concentration was decreased to equilibrate the osmotic pressure increased by the presence of caffeine in the internal droplets.

For the preparation of the w/o/w multiple emulsion, the freshly prepared w/o primary emulsion (80g) was emulsified further in an external aqueous phase (20 g) inside which the hydrophilic surfactant was dissolved at room temperature. The mixture (100 g) was stirred using mechanical mixer at 700 rpm until the formation of multiple globules. To avoid bursting the multiple globules, stirring was moderate and was stopped after their formation. During the mixing procedure, a small drop of the emulsion sample was placed on a glass slide with a small cavity in the center, the slide was mounted in an optical microscope, and the formation of w/o/w emulsion structure was confirmed.

The formation times of multiple globules were determined, and this experiment was done with multiple emulsions containing caffeine. The caffeine concentration of MEs was 1.2%.

Characteristics of multiple emulsions

After preparation of the multiple emulsions not containing caffeine, several physical tests were carried out in order to determine their

characteristics, such as viscosity, mean multiple globule size and conductivity.

In this study, the physical stability of w/o/w multiple emulsions prepared was also examined using viscometric, conductimetric and granulometric methods. In addition, formulations were evaluated by visual observation of the phase separation. Macroscopic analysis was carried out to observe the homogeneity of the systems.

The multiple emulsions were stored at 25 and 40 °C, and the characteristic properties of the systems were followed until phase separation was observed. At a definite time, the emulsions were allowed to return to room temperature before observation, and then the viscosity, mean multiple globule size and conductivity values were determined.

The data were evaluated by statistical analysis according to the Student's-t test. In addition, standard error (SE) was computed for every mean value.

Microscopic analysis, measurement of globule and droplet size

Microscopic analysis was carried out using an optical immersion microscope (Soif, Model 4GX, AN-KA, Istanbul-Turkey), and observations were made at 100x16 magnification after diluting in the appropriate external phase of emulsion. This examination provided direct information on the multiple structures. We could see the internal aqueous phases as droplets in a w/o/w emulsion structure. The existence of multiple globules was checked microscopically during all experiments until the phase separation was observed.

Granulometric analysis was carried out in order to characterize the globule size of multiple emulsions and the droplet size of w/o primary emulsion. The mean size of dispersion phase for every emulsion was determined with a laser diffraction particle sizer (Sympatec GmbH, HELOS Particle Size Analysis, Germany). As primary emulsions were diluted with liquid paraffin, the samples of multiple emulsions were diluted both in the distilled water and in iso-osmotic NaCl solution with regard to the internal aqueous phase. Fresh dilutions were made for all measurements. The dilution with water did not affect the size of multiple globules; in fact, the globule size of multiple emulsions was the same whether distilled water or iso-osmotic solution was used.

Table 2: Main properties of multiple emulsions after immediately preparation

Abil EM90	Tetronic 908	Viscosity* at 5 rpm (mPa.s)	Globule size (µm)	Conductivity (µS)
2%	1%	60000	20.6	21.3
2%	2%	58000	20.6	26.8
2%	4%	46000	34.6	28.4
4%	1%	60000	29.5	28.9
4%	2%	54000	30.2	53.9
4%	4%	44000	36.8	64.0

Measurement of viscosity

For the viscometric measurements, the samples of multiple emulsions were examined using Brookfield LV rotational viscometer (Brookfield Engineering Lab. Inc. MA, USA) at 2.5, 5, 10 and 20 rpm. Each reading was taken after equilibration of the sample, at the end of two minutes. The flow curves of all multiple emulsions were obtained by directly reading the viscosity (mPa.s) and shear stress (rpm) from the viscometer. The measurements at 2.5, 5, 10 and 20 rpm were also carried out during storage time at 25 and 40 °C, and all measurements were performed three times. The viscosity values that were only at 5 rpm were selected for tables (Tables 1 and 4) and figures (Figures 4 and 6).

Conductimetric analysis

The conductivity was measured with conductimeter (HI-9033, Hanna Inst.). It was necessary to dilute multiple emulsions with water or iso-osmotic NaCl solution to measure conductivity. The preliminary studies were performed to establish whether further dilution could influence the measurement. The measurements were made at room temperature on samples of the multiple emulsion diluted 1/20 in distilled water. The dilution with water did not affect the conductivity of the emulsion, as confirmed by using the NaCl solution that is iso-osmotic with the internal aqueous phase of multiple emulsions.

The entrapment percent or yield value ($E\%$) was calculated according to the following equation:

$$E\% = 100 \times (C_i - C_t) / C_i$$

where C_i is the conductivity of the internal aqueous phase (0.3% NaCl solution, 4800 µS) and C_t is the conductivity value of multiple emulsion at a given time t .

In vitro release studies

Release study of caffeine from multiple emulsions was investigated by a dialysis method. Before this experiment, the saturation solubility of caffeine in buffer solution (pH 5.2) at 32 °C was determined in triplicate. An excess amount of caffeine was added into 50 mL of buffer solution stirred. After 24 hours, the supernatant was filtered and caffeine was assessed spectrophotometrically.

Dialysis tubing (seamless, D-0405, lot:01H0713, Sigma) was used as a membrane for the release study and was washed several times with distilled water and left soaking in buffer solution (pH 5.2) overnight before use. Immediately after preparation, 2 g of the multiple emulsions containing 1.2% caffeine was introduced into the dialysis tubing double-tied at each end and dialyzed in 300 mL buffer solution of pH 5.2 as the release medium at 32±1 °C under constant stirring. Two milliliters were withdrawn from the dialysis medium and replenished with the same volume of fresh buffer solution at an hour interval. The concentration of the caffeine dialyzed was analyzed spectrophotometrically at 271 nm (Beckman DU-650, USA). Release studies were carried out over times up to 6 hours, in triplicate.

RESULTS and DISCUSSION

In the development of w/o/w multiple emulsions, it is necessary to estimate their physical stability experimentally. Viscosity, multiple globule size

and conductivity measurements are convenient methods for this purpose [22].

In this study, we tried to formulate a stable multiple emulsion with Tetronic[®]908 in the external phase as the hydrophilic surfactant and with Abil[®]EM90 in the inner aqueous phase as the hydrophobic surfactant. Two different concentrations of the lipophilic surfactant and three different concentrations of the hydrophilic surfactant were used in order to evaluate the influence of the surfactant percentage on the characteristics of multiple emulsions (Table 1).

It was shown that the stability of the w/o emulsion requires the presence of electrolyte in the aqueous phase; it is well known that the electrolyte concentration or osmotic pressure of the internal aqueous phase of w/o/w emulsions plays a critical role in the physical stability of the system [23]. Thus, the optimum balance should be ensured between the internal and external aqueous phase of w/o/w multiple emulsions [24, 25]. Therefore, we examined the literature regarding the formulation of w/o/w multiple emulsions, and it was decided to use NaCl at a concentration of 0.3% (0.0513 molar) as electrolyte [26].

Viscosity, mean globule size and conductivity were determined to investigate the characteristics of multiple emulsions not containing caffeine as a function of time. Measurements were made up to five months at monthly intervals following preparation of the emulsions. In addition, phase separation and phase inversion of multiple emulsions were also investigated, and the results of visual observations are summarized in Table 3. The beginning of phase separation is shown, which revealed a destruction of multiple globules of the system. During storage time, no phase inversion was observed in any multiple emulsions.

It is well known that coalescence is amplified with temperature. In this study, the beginning of phase separation at 40 °C occurred earlier than at 25 °C, except for formulations containing 4% Abil[®]EM90. For example, phase separation was observed at the fourth month for the multiple emulsion containing 2% Abil[®]EM90 at 40 °C, whereas phase separation was observed at the fifth month at 25 °C.

When the physical stability of multiple emulsions containing varying amounts of Tetronic[®]908 and a constant of Abil[®]EM90 was evaluated, it was observed that the stability decreased as Tetronic[®]908 concentration increased. At high Tetronic[®]908 concentration (4%) coalescence

occurred, resulting in phase separation within three or four months. On the contrary, no phase separation or physical stability problem occurred over five months at 25°C for multiple emulsions with prepared lower concentrations of Tetronic[®]908 (1 and 2%). A similar finding was reported in other investigations [27-31]. Matsumota et al. [27] explained the effect of an increase in the amount of hydrophilic surfactant on the physical stability of multiple emulsions as: During the second emulsification, some of the molecules of the hydrophobic surfactant from the oil layer may be solubilized in the external aqueous phase due to amount of hydrophilic surfactant that is above critical concentration. This phenomenon may be a very important cause of the rupture of the oil layer covering the internal aqueous phase droplets after preparation of the system.

Microscopic and globule size analysis

Microscopic technique is the useful direct method to assess the formation and to follow the stability of multiple globules. The various stability problems, such as globule deformation and phase inversion to simple emulsion, may be detected using this method.

All multiple emulsions were prepared successfully using the method described. The structure of w/o/w multiple emulsions was observed under the microscope during manufacturing. Many multiple globules were observed very well and many small droplets were seen in the internal phase of multiple globules. The microscopic view of a multiple emulsion is shown in Figure 1.

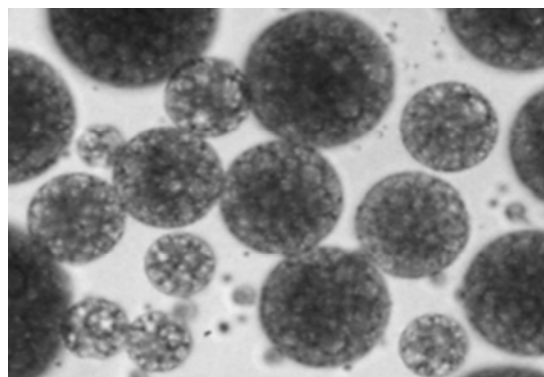


Figure 1: Microscopic view of multiple emulsion (100x16 magnification).

To evaluate the type of multiple emulsions, a drop of a water-soluble dye (methylene blue) solution was added to the system and dissolution of dye solution was observed. The colored mixture was

then examined by optical microscopy. This procedure revealed that water was the continuous phase and that the sample was a w/o/w emulsion.

The size of globules is the most important parameter in rheology and physical stability of any emulsion. The globule size of multiple emulsions as

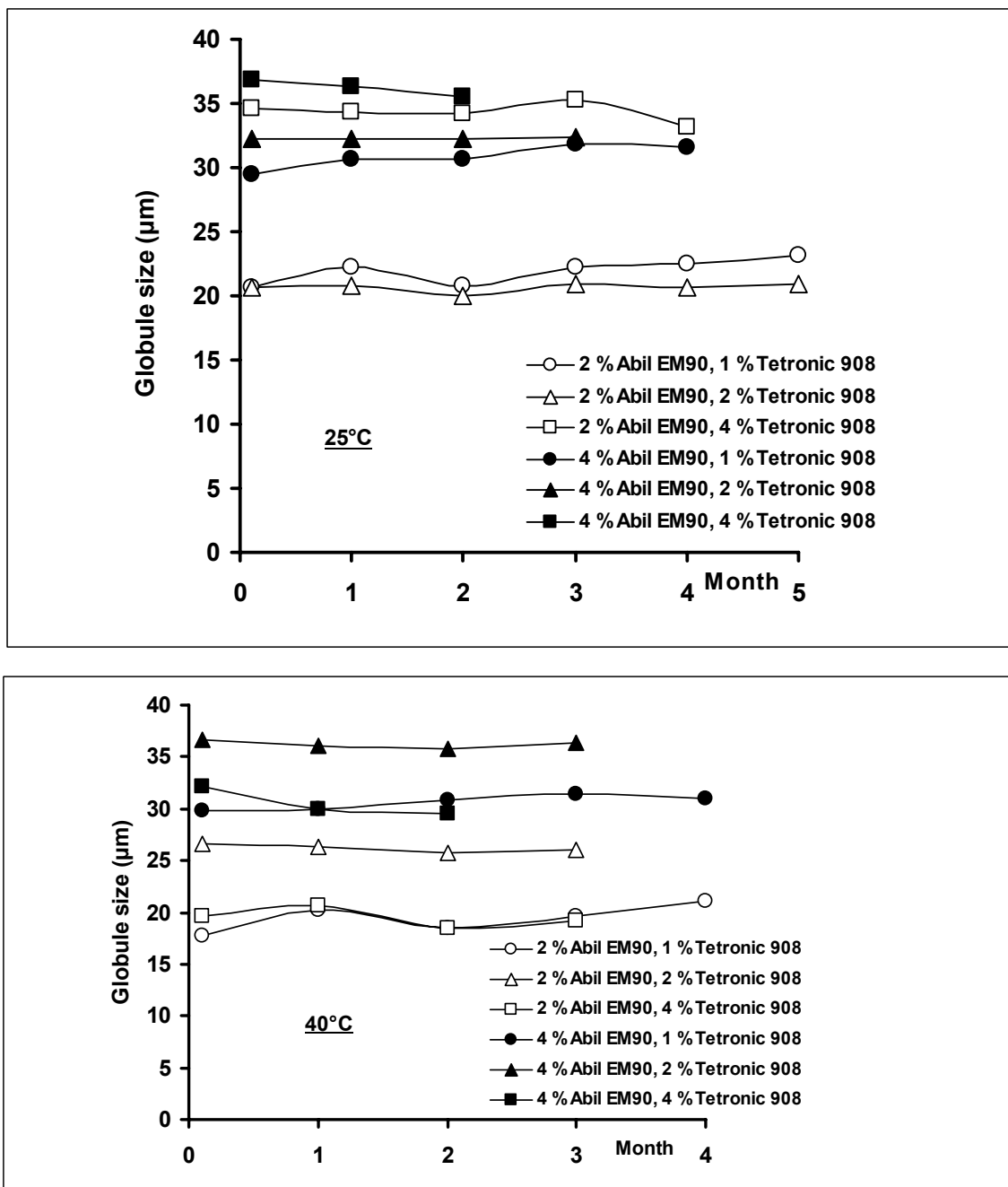


Figure 2: Globule size of multiple emulsions as a function of time

a function of time is shown in **Figure 2**. When change in multiple globule size was investigated, no significant increase or decrease was observed in the mean size during storage time at 25 and 40 °C; this may not be evidence of non-coalescence. A

similar result was also reported by **Florence and Whitehill** [32] and **Omotsho** [33]. They reported that an increase in globule size of multiple emulsions due to coalescence might be offset by a

decrease in size due to water loss from the internal to the external aqueous phase [32]. This may explain why no significant change in globule size of multiple emulsions was observed. Although the globule size of multiple emulsions was stable

during storage time, the viscosity significantly decreased and unfortunately, phase separation was observed in some of the multiple emulsions.

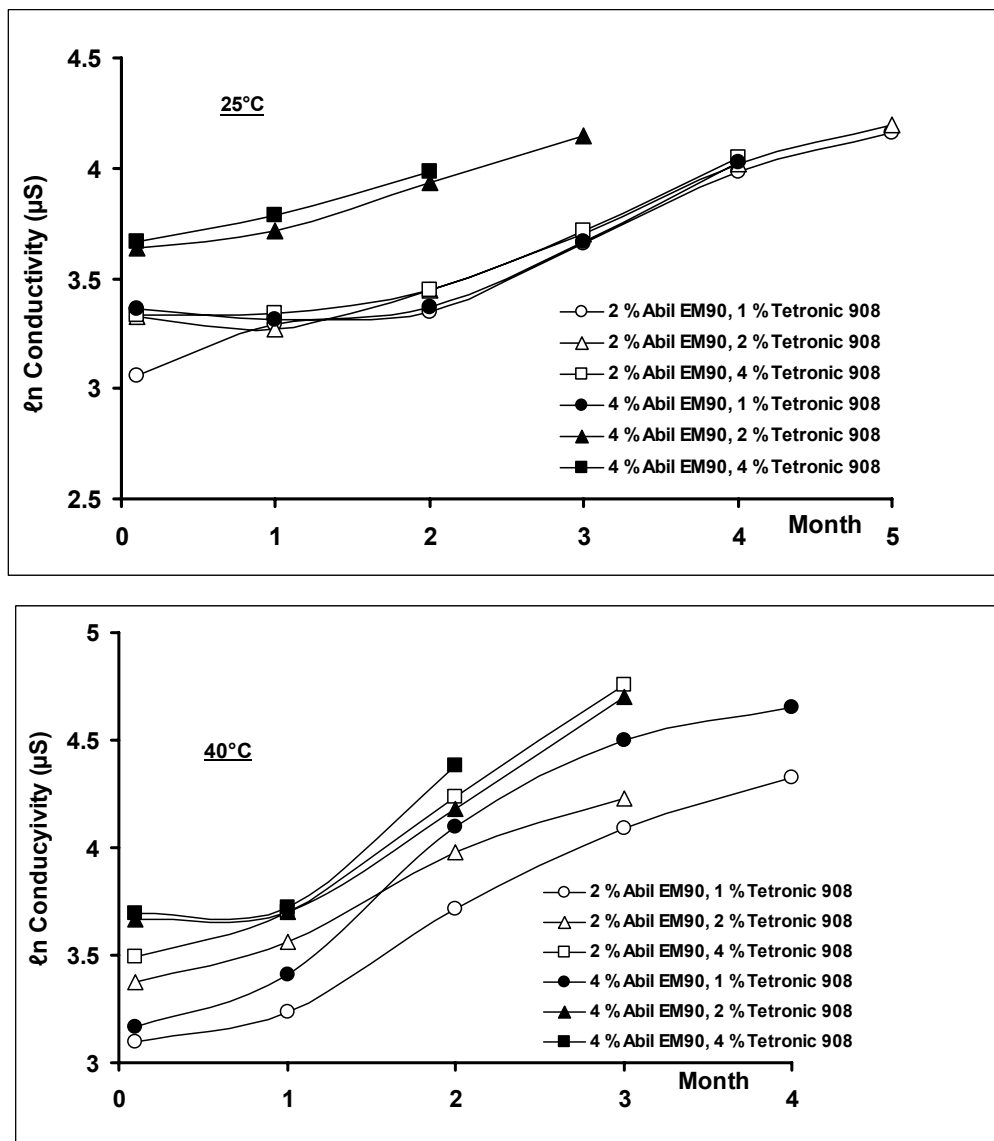


Figure 3: Effect of temperature on conductivity of w/o/w multiple emulsions during storage time Abil[®]EM90, respectively [34]. These values were statistically the same (p>0.05). This result showed

The mean multiple globule sizes of emulsions were recorded in the range of 20±0.025 µm and 37±0.031 µm (mean±confidence interval). In our previous study, the particle size measurements of w/o primary emulsions demonstrated a mean droplet size (±CI) of the aqueous phase, 3.38±0.0216 µm and 3.44±0.0541 µm for emulsions prepared with 2% Abil[®]EM90 and 4%

that there was no significant effect of high lipophilic amount (4%) on the interfacial tension between water and oil phase; the effective reduction of interfacial tension was probably ensured by 2% of Abil[®]EM90. The globule size of primary emulsions did not change, and no visual

sign of physical destabilization was observed in systems at 25 and 45 °C during storage time.

As expected, the mean globule size of multiple emulsions depended on the surfactant concentrations.

When the effect of Tetronic[®]908 concentration on globule size of multiple emulsions was investigated, a small increase with increasing surfactant amount was observed; the higher concentration of hydrophilic surfactant most likely caused the coalescence of globules due to rupture of oil layer between two aqueous phases during manufacturing [27]. However, there was no significant effect of Tetronic[®]908 concentration (for low concentration, 1 and 2%) on globule size of multiple emulsions containing the same percentage of Abil[®]EM90 (Table 2) ($p>0.05$).

When the percentage of Abil[®]EM90 was increased from 2% to 4%, the globule size logically increased. The excess of Abil[®]EM90 was located in a molecular form in the oil phase between external and internal aqueous phase, and this caused swelling of the oil globules (Table 2). On the contrary, the globule size of multiple emulsions containing caffeine decreased with increasing Abil[®]EM90 percentage and the same concentration of hydrophilic surfactant (Table 4). It was concluded that caffeine influenced the tension of the second interface during the second emulsification step.

Conductimetric analysis

This analysis was carried out in order to directly measure the entrapped electrolyte within the internal aqueous phase and to confirm the

emulsion type. Conductivity data generated good information about the emulsion type. Both dying method and low positive conductivity values demonstrated the w/o/w type emulsion.

Multiple emulsions yielded low conductivity values immediately after preparation of formulations. The yield of the emulsions (entrapment percent, $E\%$) was between 99.6% and 98.7%, meaning that a portion of the NaCl in the inner aqueous phase, approximately between 0.4% and 1.3%, leaked into the external aqueous phase during the preparation of multiple emulsions.

Although the yield values were high, unfortunately phase separation was observed in some formulations during storage time at 25 and 40 °C (Table 3).

During storage of w/o/w emulsions, the conductivity values increased with time (Figure 3). The increase in conductivity during storage time can be ascribed to the increase in NaCl in the external aqueous phase due to *i*) a diffusion of NaCl through the mineral oil film [35] or *ii*) the coalescence of internal and external aqueous phases [36], destruction of oil film because of osmotic pressure and then expulsion of internal aqueous phase. This most likely explains the phase separation observed in this study. Due to rising conductivity, declining viscosity and the approximately stable globule size of multiple emulsions during storage time, it was concluded that the solute and water molecules were transported from the oil membrane by a diffusional mechanism.

Table 3: Effect of surfactant concentration on physical stability of multiple emulsions

Abil [®] EM90	Tetronic [®] 908	1 month	2 months	3 months	4 months	5 months
2%	1%	—	—	—	—	no PS PS at 40°
2%	2%	—	—	—	no PS at 25° PS at 40°	no PS at 25° PS at 40°
2%	4%	—	—	—	no PS at 25° PS at 40°	PS at 25° PS at 40°
4%	1%	—	—	—	—	PS at 25° PS at 40°
4%	2%	—	—	—	PS at 25° PS at 40°	PS at 25° PS at 40°
4%	4%	—	—	PS at 25° PS at 40°	PS at 25° PS at 40°	PS at 25° PS at 40°

‘—’ indicates phase separation neither at 25 °C nor at 40 °C; PS, phase separation

The conductivity profiles showed that the transport rate of NaCl molecules from inner to outer aqueous phase decreased with time (first order kinetics) (Figure 3). The cause for the decreasing rate was interpreted as: The inner aqueous droplets of w/o/w emulsions probably grow with time due to coalescence of smaller droplets because of the higher percentage of hydrophilic surfactant. However, in this study, the volume of the oil phase was constant, and therefore the oil layer covering inner growing aqueous droplets thickened. Thus, the thickened layer delayed the transport of electrolyte molecules.

The temperature accelerated the diffusion or transport of NaCl molecules from inner to external aqueous phase. The calculated conductivity rate constants ($\ln \mu\text{S/month}$) were significantly different when compared with the formulations having the same composition at 25 or 40 °C ($p < 0.05$). For example, the conductivity rate constants of multiple emulsions containing 1% Tetronic[®]908 and 2% Abil[®]EM90 were calculated as $0.229 \pm 0.0183 \ln \mu\text{S/month}$ and $0.339 \pm 0.03 \ln \mu\text{S/month}$ at 25 and 40 °C, respectively.

The effect of Abil[®]EM90 concentration on conductivity of the system was investigated, and no significant difference was obtained among formulations containing 1% Tetronic[®]908. Conductivity values were similar with 2 or 4% Abil[®]EM90 concentration at 25°C. At higher concentrations of Tetronic[®]908 (2 and 4%), the two-fold increase in lipophilic surfactant did not hinder the increase in conductivity (Figure 3). Unfortunately, the increase in concentration of lipophilic surfactant could not provide the physical stability of the system (Table 3). This finding was in contrast with previous literature [27, 37, 38].

There was a direct relationship between the percentage of Tetronic[®]908 and the conductivity of multiple emulsions. As could be seen in Figure 3, the conductivity of systems containing the same amount of lipophilic surfactant increased with increasing Tetronic[®]908 concentration. This result was also observed by the other investigators [27, 31, 37, 38]. As mentioned previously, the excess amount of Tetronic[®]908 in the external aqueous phase probably caused the formation of micelles and then the solubilizing of Abil[®]EM90 molecules, thus weakening or rendering unstable the interface of the w/o system. Therefore, NaCl molecules

migrated more easily from the internal to external aqueous phase.

Viscometric analysis

Measurement of viscosity gives us useful data, especially in quality assurance of emulsions. The variation in viscosity of systems may explain some differences between formulations and at the same time provide important information about coalescence or globule size change.

The viscosity of the w/o/w multiple emulsions was measured at four different rpm to follow the time dependence of viscosity. The viscosity values only at 5 rpm were selected for tables and figures.

The viscosities of emulsions at first day after preparation are shown in Table 2.

There are many factors affecting the viscosity of multiple emulsions; one is the multiple globule size. There was a direct relationship between the viscosity and globule size of multiple emulsions. As expected, the minimum viscosity was observed with the higher globule size (Figure 4). Higher viscosity may be attributed to the small globule size of multiple emulsions prepared with the same concentration of Abil[®]EM90.

Increased hydrophilic surfactant influenced the viscosity of system, with the viscosity decreasing as the Tetronic[®]908 concentrations were increased (Table 2), whereas no significant difference in viscosity as a function of Abil[®]EM90 percentage was observed ($p > 0.05$).

All the multiple emulsions exhibited non-newtonian flow and shear thinning behavior. It means the viscosity of system decreased with increasing shear stress. The viscosity of multiple emulsions reduced with increasing shear stress from 5 rpm to 20 rpm. Only one example of the plots of viscosity versus shear stress (rpm) is shown (Figure 5). In some literatures, the bursting of multiple globules [39, 40] or phase inversion under shear stress [41] have been shown, but we did not observe any destruction of multiple globules or phase inversion after viscosity measurement using optical microscopy. It was concluded that shear stress used in this study did not induce irreversible structural changes in multiple emulsions, such as coalescence or phase inversion.

Table 4: Release rate constant of caffeine and some physical characteristics of multiple emulsions containing caffeine

Tetronic 908, %	2% Abil EM90			4% Abil EM90		
	1%	2%	4%	1%	2%	4%
k_r^o	2.31	2.89	5.66	2.56	3.82	6.95
(Released %/hour) ^a	(0.369) ^b	(0.496) ^b	(0.511) ^b	(0.198) ^b	(0.331) ^b	(0.552) ^b
Viscosity (mPa.s)	54000	32000	30000	56000	48000	32000
Globule size (µM)	25.5	34.6	36.6	19.6	20.6	35.8
Released % (±SD) ^c	14.8	15.9	18.3	17.2	19.0	30.9
(at first two hours)	(±1.17) ^c	(±1.55) ^c	(±0.673) ^c	(±0.708) ^c	(±0.154) ^c	(±1.76) ^c
Released % (±SD)	26.3	26.8	41.1	26.4	27.6	54.2
(at 6 th hours)	(±1.09)	(±1.09)	(±1.00)	(±0.462)	(±1.51)	(±0.905)

^a $r^2 \geq 0.997$, ^b SE of regression, ^c ±standard dev., n=3

Table 5: Calculated parameters of the kinetic model suggested by Magdassi and Garti (54) for release of drug from multiple droplets

Tetronic [®] 908	2% Abil [®] EM90			4% Abil [®] EM90		
	1%	2%	4%	1%	2%	4%
Slope	0.00211	0.00234	0.00714	0.00198	0.00330	0.0123
Intercept	0.000205	-0.000796	-0.00978	0.00127	-0.00297	-0.00839
r^2	0.993	0.998	0.978	0.998	0.978	0.989

$$\text{Slope} = \frac{(3 \times D \times C_s)}{(r_o^2 \times C_o)}$$

Viscosities of all the multiple emulsions decreased continuously during storage with time (Figure 6). This may be due to *i*) diffusion of water molecules from the inner to the outer aqueous phase and then the volume decrease of globules in the w/o/w emulsions, or *ii*) bursting of multiple globules due to osmotic pressure. When all multiple emulsions were compared considering phase separation at 25 °C, systems containing 2% Abil[®]EM90 and 1 or 2% Tetronic[®]908 were stable at least five months, although increasing conductivity and decreasing viscosity with time were observed. In these formulations, water molecules probably migrated through the liquid paraffin layer without affecting

the entirety of multiple globules. This has been described by *i*) micellar transport mechanism, *ii*) the hydrated surfactant mechanism or *iii*) diffusion of water molecules across the oil layer between internal and external aqueous phase [4, 17, 28, 36, 42, 43].

Formation time of multiple globules

The effect of surfactant concentration on formation time of multiple globules is shown in Figure 7. As can be seen, the required formation time of multiple emulsions prepared with 2% Abil[®]EM90 was similar with multiple emulsions prepared with 4% Abil[®]EM90 for the same concentration of Tetronic[®]908. In contrast, the formation time was

significantly affected by the percentage of Tetronic®908; the formation time of multiple globules increased with an increase in hydrophilic surfactant. It was considered that Tetronic®908 may influence the interphase between the external aqueous phase and oil layer (or oil lamella), which has been described by Garti [17]. Abil®EM90 was probably solubilized via the micelles formed by part of the Tetronic molecules. Thus, as Tetronic concentration increased, formation of multiple globules was delayed; the higher the Tetronic concentration, the longer the formation time.

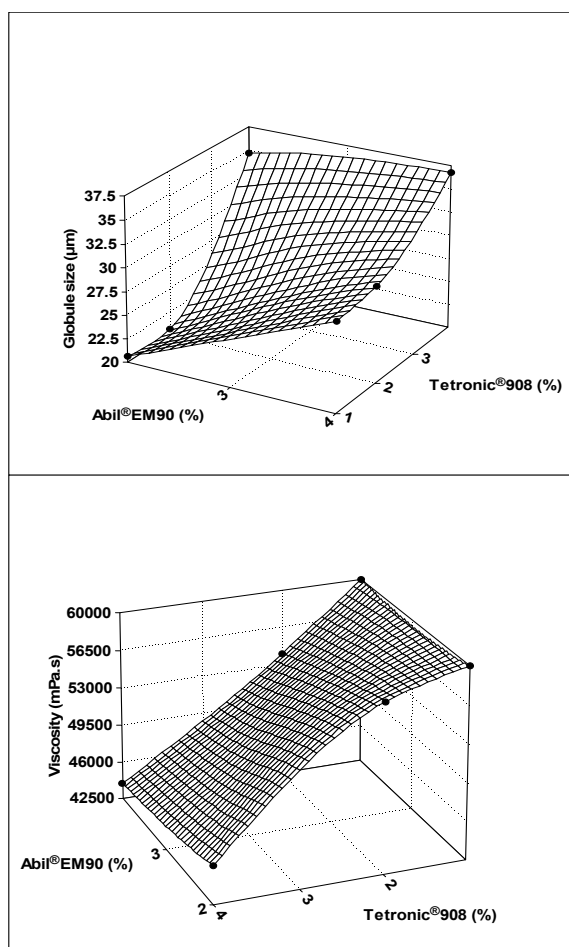


Figure 4: Effect of surfactant concentration on globule size and viscosity of multiple emulsions.

In vitro release studies

To investigate the release characteristics of multiple emulsions, caffeine was used as the model solute. When caffeine was introduced in the internal aqueous phase of multiple emulsions, the viscosity and multiple globule size of emulsions changed (Table 4).

The solubility of caffeine was found to be 20.4 ± 0.93 mg/mL (saturation value, C_s) (pH: 5.2). That result agrees with those of Clément et al. [44] who found that the solubility of caffeine was 19.64 ± 0.59 mg/mL at pH: 7.4.

During release experiments, the release rate should not be influenced by the concentration gradient between the caffeine concentration of release medium (C_{rm}) and the saturation concentration of caffeine (C_s) in release medium. If the C_{rm} is less than or equal to " $C_s \times 10/100$ " for every sampling time, it is said that a sink condition is maintained. So, when we used 300 ml of release medium, even if all of the caffeine (0.024 g) in two grams of ME released, C_{rm} could not be higher than " $C_s \times 10/100$ " value ($=20.4 \times 10/100 = 2.04$ mg/ml). Therefore, it was concluded that the volume of dialysis medium was not rate limiting in diffusion of caffeine and the release experiments were conducted under sink conditions.

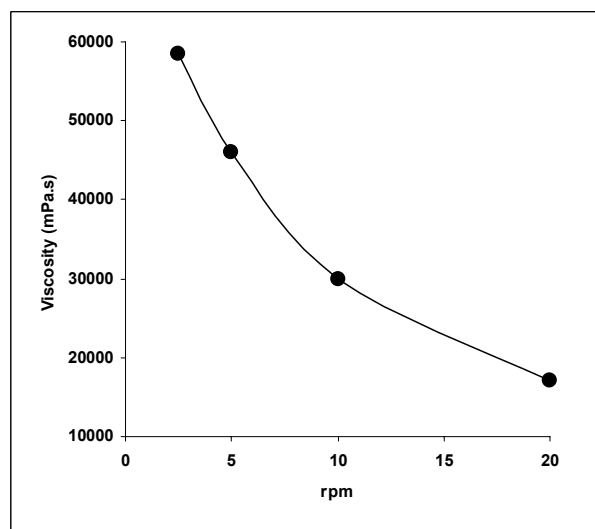


Figure 5: Viscosity as a function of shear stress – Non-Newtonian flow behavior of multiple emulsion containing 2% Abil®EM90 and 2% Tetronic®908

The cumulative percent release of caffeine from multiple emulsions is shown in Figures 8 and 9. Points and bars in each figure represent mean value \pm standard error (SE). When the SE was smaller than the size of symbol, no bar was shown.

All release profiles exhibited an initial rapid release phase and then a slower release of molecules. First phase of release profiles (the first hour of profile) might be due to caffeine existence in the external aqueous phase during the manufacturing; during the second emulsification, caffeine molecules probably leaked out of the internal aqueous phase. Therefore,

the secondary emulsification was stopped immediately after observation of multiple globules on microscope. The drug molecules at the external

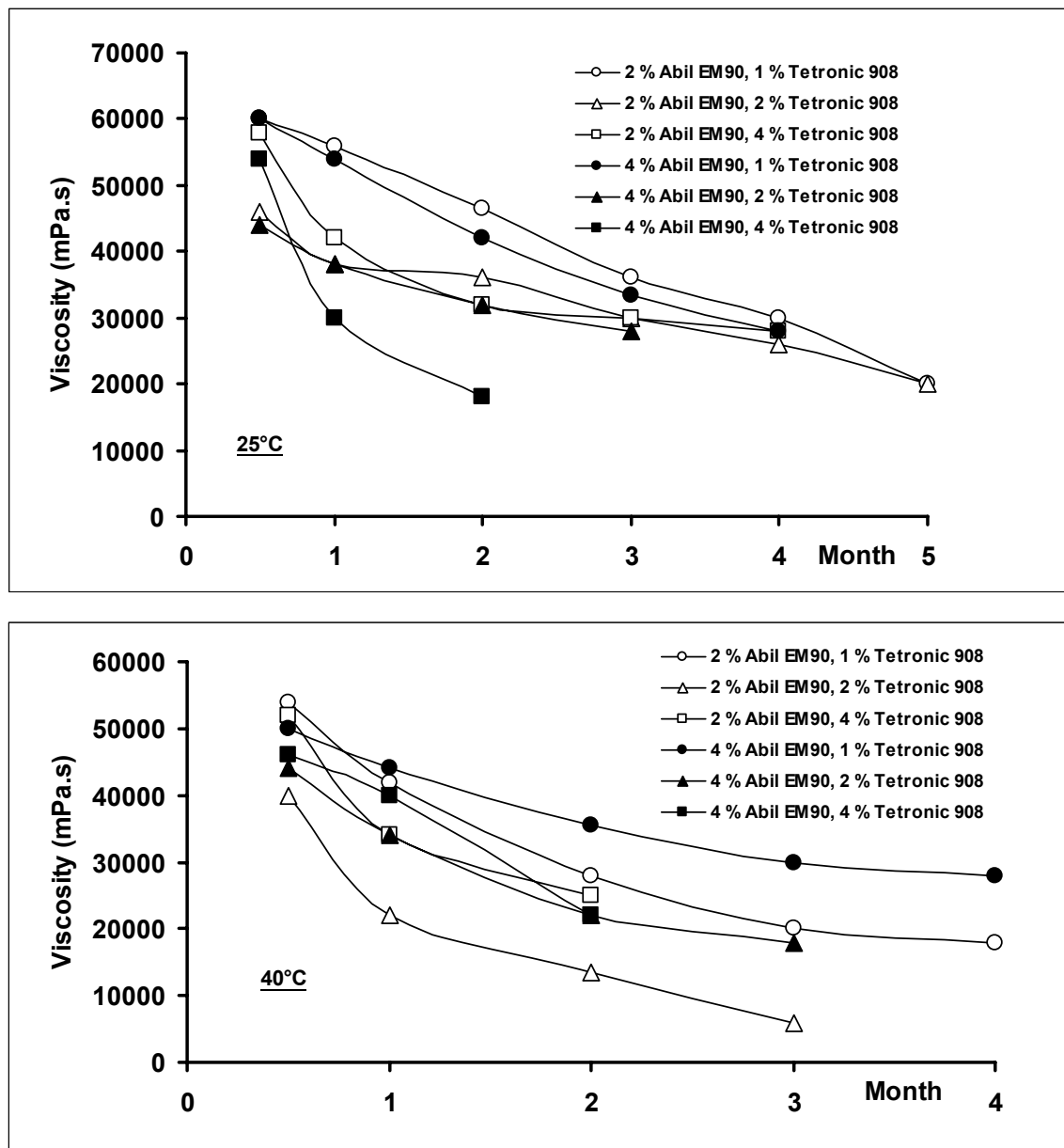


Figure 6: The decreasing of viscosity as a function of time at 25 and 40°C (shear stress: 5 rpm)

aqueous phase were free molecules that were released immediately. The release of caffeine in the external phase of w/o/w emulsions was completed within approximately 2 hours. Negligible drug release from multiple emulsions was observed between the 1st and 2nd hours. This may be attributed to the slow transport of caffeine from the internal aqueous phase; a period of time

was probably required for caffeine molecules to transport from internal droplets to the dialysis membrane.

After 2 hours, caffeine in the internal aqueous phase was released slowly at a rate governed by the interphase barrier between the inner and outer aqueous phases because the hydrophilic drug molecules could not pass freely through the oily

layer. So the second phase might be attributed to a prolonged release of caffeine from the internal aqueous phase.

The release rates presented in Table 4 were determined from slopes of the release percentage versus time between the 2nd and 6th hours. These rates were used the explanation and comparison of the effect of surfactant concentration on the release profiles.

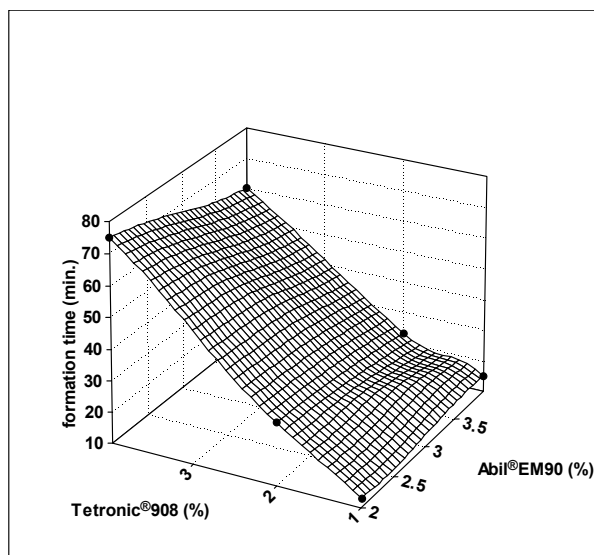


Figure 7: Effect of surfactant concentration on formation time of multiple globules.

The effect of hydrophilic surfactant concentration

Profiles shown in Figures 8 and 9 indicate that emulsions containing 4% Tetronic[®]908 enhanced the drug released in comparison with the other concentration of this surfactant. It was also observed that multiple emulsions containing 1 or 2% of Tetronic[®]908 gave almost similar patterns of caffeine levels. When the release percentages at the end of the release experiment were compared, the difference between values was not very pronounced, except for the emulsions containing 4% Tetronic[®]908. In fact, the release percentages of caffeine from formulations containing 1 or 2% Tetronic[®]908 after 6 hours were not significantly different ($p=0.849$). On the contrary, when the release percentages for multiple emulsions prepared with 4% Tetronic[®]908 were compared after 6 hours, the difference was statistically significant ($p=0.0297$), 41.1 and 54.2% of the total dose. When we used the ANOVA, it was found that there was the significant difference between the release rates of MEs containing different amount of hydrophilic surfactant ($p=0.017$).

Although the release rate or release % of caffeine for formulation containing 4% Tetronic[®]908 was relatively large, the physical stability of those emulsions was not better than the other MEs containing 1% or 2% Tetronic[®]908 as shown in Table 3; the high concentration of hydrophilic surfactant did not improve the physical stability of system. This finding agrees with those of Matsumoto et al. [27] and Jiao and Burgess [31]. These authors suggested that the usage of a high concentration of hydrophilic surfactant caused the solubilization of lipophilic surfactant and then disruption of multiple globules. In our study, the higher concentration of Tetronic[®]908 in the outer aqueous phase may have served to solubilize the molecules of the lipophilic surfactant, which probably led to the rupture of the oil layer and then the rapid release of molecules of caffeine to the external aqueous phase. In addition, this situation probably caused the phase separation.

In this study, Tetronic[®]908 was used in concentrations of 1, 2 and 4%, and these percentages are much higher than critical micelle concentration defined by Atwood et al. [45] and Dong et al. [46], 0.020% at 40 °C and 0.16% at 25 °C, respectively. Therefore, Tetronic[®]908 chains probably formed micelles in both aqueous and oil phases and this facilitated the transport of caffeine molecules through the oil phase, as described Omotosho et al [47].

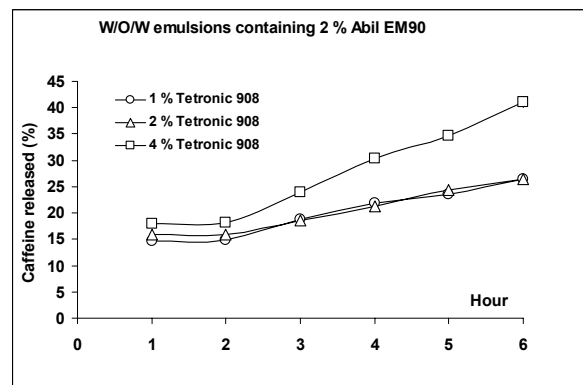


Figure 8: Release profiles of caffeine from w/o/w multiple emulsions containing different concentration of Tetronic[®]908 (mean±SE, n: 3)

The effect of lipophilic surfactant concentration

Previous literatures have shown that the percentage of the lipophilic surfactant plays an important role in the release amount of active molecule. In some of these reports, when the lipophilic surfactant concentration was increased, the release of solute

molecules decreased due to maximum viscosity of the system. The cause of increasing viscosity has been explained as the maximum swelling of multiple globules of the system [37, 48-51]. In contrast, in other reports, an increase in lipophilic surfactant amount caused an increase in the release rate of solute and water [17, 47, 52, 53]. This has been explained by the authors as follows: During the first emulsification, the reverse micelles form in the oil phase of w/o primary emulsion due to the higher concentration of lipophilic surfactant that is above critical value. In addition, during the second emulsification, the existence of hydrophilic surfactant may cause the formation of mixed inverse micelles. Thus, both water and water-soluble molecules in the micelles could be easily carried across the oil layer between the internal and external aqueous phase of w/o/w emulsion.

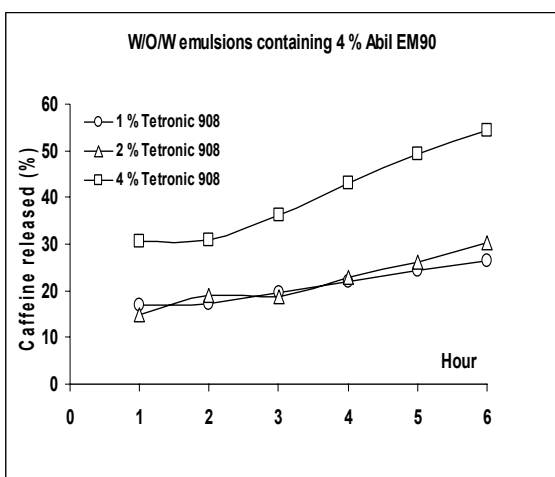


Figure 9: Release profiles of caffeine from w/o/w multiple emulsions containing different concentration of Tetronic[®]908 (mean±SE, n: 3).

In this study, the effect of Abil[®]EM90 concentration on the release rate or release amount of caffeine is shown in Table 4. It can be seen that as the concentration of lipophilic surfactant in the primary emulsion (w/o) increased, the release rate of caffeine from multiple emulsions increased. In addition, the percentage of caffeine released from multiple emulsions containing 4% Abil[®]EM90 was greater than that of the other multiple emulsions containing 2% Abil[®]EM90 prepared with same concentration of hydrophilic surfactant. The release rates of MEs containing 2% of lipophilic surfactant were compared to the release rates of MEs containing 4% of lipophilic surfactant using ANOVA test. It was found that the difference was not statistically significant (p=0.114).

In our study, the droplet size of w/o primary emulsion was approximately the same for two concentrations of Abil[®]EM90, which likely indicates that the two-fold increase of lipophilic surfactant no longer significantly decreased the interfacial tension. Thus, it is plausible to assume that a concentration of 2% Abil[®]EM90 was just sufficient to cover the inner aqueous phase of the primary emulsion; the excess amount was likely located in a molecular and micellar form (containing water, caffeine and NaCl) in the oily phase of the primary emulsion. So, when a higher concentration of Abil[®]EM90 is used in multiple emulsions, caffeine molecules in the inner aqueous phase may be transported by micelles in addition to the molecular diffusion.

The release studies showed that the release rate of caffeine was faster for those systems with lower viscosity at the same percentage of Abil[®]EM90; this also depended on increasing Tetronic[®]908 concentrations. The viscosity of systems was more effectual than the globule size of multiple emulsions, because emulsions with smaller globules were unable to generate the faster release rate. Therefore, although the globule size did not affect the rate of drug released, the viscosity of multiple emulsions did due to consistency of the system.

Based on results of release experiments, it was concluded that caffeine might be transported out by molecular diffusion and through a reverse micellar mechanism controlled by the viscosity of the system.

The possible kinetic model for release of caffeine from multiple droplets

In the previous studies, Higuchi's mechanism for release of molecule from dispersed polymeric matrix was modified and adapted for MEs [16, 53, 54]. Magdassi and Garti applied this model to w/o/w type of MEs and defined the following equation [54]:

$$\frac{3}{2} [1 - (1 - F)^{2/3}] - F = \frac{3 \times D \times C_s}{r_o^2 \times C_o} \times t$$

where F is the fraction of the drug release from globule; D is the diffusion coefficient of drug through oil membrane; C_s is the drug solubility in the oil membrane between the inner aqueous phase and the outer aqueous phase; r_o is the radius of the mean globule size of ME; C_o is the initial concentration of the drug. For simplification the

value of " $\frac{3}{2} \{1 - (1 - F)^{2/3}\} - F$ " was called B.

In our study, we used this equation and the calculated B values were plotted against time, t (Figure 10). The caffeine solubility in the paraffin film and “ D ” value were assumed to be constant. The swelling of the globules of MEs was also cancelled, because as mentioned before the mean size of multiple globules was not changed during time (Figure 2).

As shown in Figure 10, the profiles exhibited two phase for all the MEs. This was the unexpected situation; the relationship between B and t could be the linear. Sela et al [53] explained the first phase of profile as resulting from the time required for the formation of reverse micelles and solubilization of water and drug in these micelles. The second and linear phase was the straight line as expected; this confirmed the validity of the equation and the r^2 values was higher than 0.978 (Table 5). As a result, it can be concluded that the release of the caffeine molecules through the paraffin film probably was controlled by diffusion mechanism.

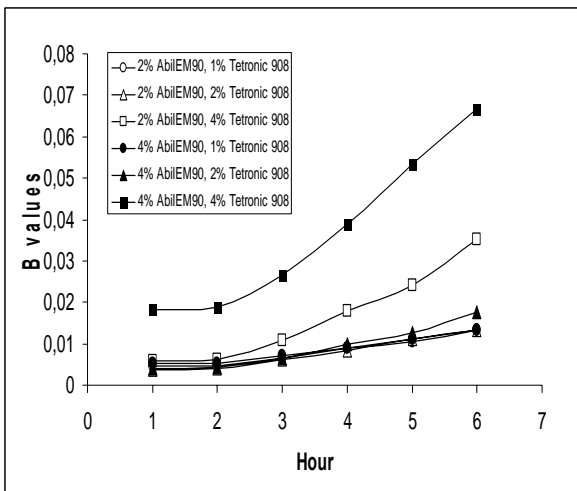


Figure 10: B versus t profiles for release of caffeine from multiple droplets

CONCLUSIONS

The investigations presented lead us to conclude that the multiple emulsions prepared with Tetronic[®]908 were highly uniform and high yields of w/o/w multiple emulsions were obtained. Relatively stable systems were formed using Tetronic[®]908 and Abil[®]EM90 surfactant pair. It is possible to formulate an optimal multiple emulsions consisting of Tetronic[®]908 (1%) in the outer aqueous phase and Abil[®]EM90 (2%) in the oil phase. The results indicated that Tetronic[®]908 as hydrophilic surfactant might be good emulsifier for the preparation of w/o/w type emulsions.

Stability measurements showed that the concentration of hydrophilic and lipophilic surfactant were very important parameter. The release of caffeine as model hydrophilic substance from system studied; an initial rapid release followed by a much slower rate of release was obtained. Multiple w/o/w emulsion systems can be utilized as potential prolonged release dosage forms. It was concluded that surfactant concentrations affected the release rate.

Further in-vitro examinations such as different type of oil phase or the different combinations of aqueous phase are still required and it is recognized that further formulation studies are needed to obtain the most stable formulations using Tetronic[®]908.

REFERENCES

- 1) De Luca, M., Vaution, C., Rabarron, A., Seiller, M., Classification et obtention des emulsions multiples. S.T.P. Pharma. Sci., 4(8): 679-687, 1988.
- 2) De Luca, M., Grossiord, J.L., Médard, J.M., Vaution, C., A stable w/o/w multiple emulsion. Cos. Toilet., 105: 65-69, 1990.
- 3) Taelman, M.C., Loll, P., Multiple emulsions in cosmetics. ICI Surfactants, Reprint, RP112/94E, 1994.
- 4) Garti, N., Aserin, A., Double emulsions stabilized by macromolecular surfactants. Adv. Coll. Interface. Sci., 65: 37-69, 1996.
- 5) Omotosho, J.A., Florence, A.T., Whateley, T.L., Absorption and lymphatic uptake of 5-fluorouracil in the rat following oral administration of w/o/w multiple emulsions. Int. J. Pharm. 61: 51-56, 1990.
- 6) Ferreira, L.A.M., Doucet, J., Seiller, M., Grossiord, J.L., Marty, J.P., Wepierre, J., In vitro percutaneous absorption of metronidazole and glucose: Comparison of o/w, w/o/w and w/o systems. Int. J. Pharm., 121: 169-179, 1995.
- 7) Silva-Cunha, A., Chéron, M., Grossiord, J.L., Puisieux, F., Seiller, M., W/O/W multiple emulsions of insulin containing a protease inhibitor and an absorption enhancer: Biological activity after oral administration to normal and diabetic rats. Int. J. Pharm., 169: 33-44, 1998.
- 8) Silva-Cunha, A., Grossiord, J.L., Seiller, M., Multiple Emulsions: Pharmaceutical Potentiality, in: Grossiord J.L., Seiller M (eds.), Multiple Emulsions: Structure, Properties and Applications, Editions de Sante, France, pp 279-312, 1999.
- 9) Jager-Lezer, N., Denine, R., Grossiord, J.L., Wepierre, J., Rault, S., Seiller, M., Formulating

- multiple emulsions with moisturizing actives. *Cosm. Toilet.*, 111: 53-58, 1996.
- 10) Omotosho, J.A., Whateley, T.L., Florence, A.T., Release of 5-fluorouracil from intramuscular w/o/w multiple emulsions. *Biopharm. Drug. Disp.*, 10: 257-268, 1989.
 - 11) Okochi, H., Nakano, M., Basic studies on formulation, method of preparation and characterization of water-in-oil-in-water type multiple emulsions containing vancomycine. *Chem. Pharm. Bull.*, 44: 180-186, 1996.
 - 12) Doucet, O., Ferrero, L., Garcia, N., Zastrow, L., O/W emulsion and w/o/w multiple emulsions: Physical characterization and skin pharmacokinetic comparison in the delivery process of caffeine. *Int. J. Cosm. Sci.*, 20: 283-295, 1998.
 - 13) Okochi, H., Nakano, M., Preparation and evaluation of w/o/w type emulsions containing vancomycin. *Adv. Drug. Del. Rev.*, 45: 5-26, 2000.
 - 14) Morishita, M., Matsuzawa, A., Takayama, K., Isowa, K., Nagai, T., Improving insulin enteral absorption using water-in-oil-in-water emulsion. *Int. J. Pharm.*, 172: 189-198, 1998.
 - 15) Gallarate, M., Carlotti, M.E., Trotta, M., Bovo, S., On the stability of ascorbic acid in emulsified systems for topical and cosmetic use. *Int. J. Pharm.*, 188: 233-241, 1999.
 - 16) Raynal, S., Grossiord, J.L., Seiller, M., Clause, D., A topical w/o/w multiple emulsion containing several active substances: formulation, characterization and study of release. *J. Cont. Rel.*, 26: 129-140, 1993.
 - 17) Garti, N., Double emulsions – Scope, limitations and new achievements. *Coll. Surf. A: Physicochem. Engineer. Asp.*, 123-124: 233-246, 1997.
 - 18) Seiller, M., Grossiord, J.L., Silva-Cunha, A., Multiple Emulsions: Pharmaceutical Potentiality, in: Grossiord JL: Seiller M (eds.), Multiple Emulsions: Structure, Properties and Applications, Editions de Sante, France, pp 55-80, 1999.
 - 19) Schmolka, I.R.,) A comparison of block copolymer surfactant gels. *J. Am. Oil. Chem. Soc. (JAOCS)*, 68: 206-209, 1991.
 - 20) Clément, P., Laugel, C., Marty, J.P., In vitro release of caffeine from concentrated w/o emulsions: Effect of formulation parameters. *Int. J. Pharm.*, 207: 7-20, 2000.
 - 21) Olivieri, L., Seiller, M., Bromberg, L., Besnard, M., Duong, T.N.L., Grossiord, J.L., Optimization of a thermally reversible w/o/w multiple emulsion for shear-induced drug release. *J. Cont. Rel.*, 88: 401-412, 2003.
 - 22) Ollivon, M., Potier-Guméry, L., Multiple Emulsions: Pharmaceutical Potentiality, in: Grossiord JL: Seiller M (eds.), Multiple Emulsions: Structure, Properties and Applications, Editions de Sante, France, pp 195-222, 1999.
 - 23) Aronson, M.P., Petko, M.F., Highly concentrated water in oil emulsions: Influence of electrolyte on their properties and stability. *J. Colloid Interface Sci.*, 159: 134-149, 1993.
 - 24) Kawashima, Y., Hino, T., Takeuchi, H., Niwa, T., Stabilization of water/oil/water multiple emulsion with hypertonic inner aqueous phase. *Chem. Pharm. Bull.* 40: 1240-1246, 1992.
 - 25) Opawale, F.O., Burgess, D.J., Influence of interfacial properties of lipophilic surfactants on water-in-oil emulsion stability. *J. Colloid. Interface. Sci.*, 197: 142-150, 1998.
 - 26) Kanouni, M., Rosano, H.L., Naouli, N., Preparation of a stable double emulsion (w₁/o/w₂): Role of the interfacial films on the stability of the system. *Adv. Coll. Interface. Sci.*, 99: 229-254, 2002.
 - 27) Matsumoto, S., Kita, Y., Yonezawa, D. J., An attempt at preparing water-in-oil-water multiple-phase emulsions. *Coll. Interface Sci.*, 57: 353-361, 1976.
 - 28) Magdassi, S., Frenkel, M., Garti, N., On the factors affecting the yield of preparation and stability of multiple emulsions. *J. Dispers. Sci. Tech.*, 5: 49-59, 1984.
 - 29) Laugel, C., Chaminade, P., Baillet, A., Seiller, M., Ferrier, D., Moisturizing substances entrapped in w/o/w emulsions: Analytical methodology for formulation, stability and release studies. *J. Cont. Rel.*, 38: 59-67, 1996.
 - 30) Opawale, F.O., Burgess, D.J., Influence of interfacial rheological properties of mixed emulsifier films on the stability of water-in-oil-in-water emulsions. *J. Pharm. Pharmacol.*, 50: 965-973, 1998.
 - 31) Jiao, J., Burgess, D.J., AAPS PharmSci., Rheology and stability of water-in-oil-in-water multiple emulsions containing Span 83 and Tween 80. 5: Article 7, 2003 (<http://www.pharmsci.org>).
 - 32) Florence, A.T., Whitehill, D.J., Some features of breakdown in water-in-oil-in-water multiple emulsions. *J. Coll. Interface. Sci.*, 79: 243-256, 1981.
 - 33) Omotosho, J.A., The effect of acacia, gelatin and polyvinylpyrrolidone on chloroquine transport

- from multiple w/o/w emulsions. *Int. J. Pharm.*, 62: 81-84, 1990.
- 34) Sever, S., Ocağ (Tırnaksız), F., A topical w/o/w multiple emulsion prepared with Pluronic F-127 or Tetronic 908 as a hydrophilic surfactant: Formulation, characterization and release Studies, in Yazan Y: Baser KHC (eds.), 4th International Cosmetics Symposium, 7-8 June 2000, Istanbul, pp 212, 2000.
 - 35) Pays, K., Giermanska-Kahn, J., Pouligny, B., Bibette, J., Leal-Calderon, F., Double emulsions: How does release occur?. *J. Cont. Rel.*, 79: 193-200, 2002.
 - 36) Wen, L., Papadopoulos, K.D., Osmotic pressure on water transport in $w_1/o/w_2$ emulsions. *J. Coll. Interface Sci.*, 235: 398-404, 2001.
 - 37) Jager-Lezer, N., Terrisse, I., Bruneau, F., Tokgoz, S., Ferreira, L., Clausse, D., Seiller, M., Grossiord, J.L., Influence of lipophilic surfactant on the release kinetics of water soluble molecules entrapped in a w/o/w multiple emulsions. *J. Cont. Rel.*, 45: 1-13, 1997.
 - 38) Csóka, I., Erős, I., Stability of multiple emulsions I. Determination of factors influencing multiple drop breakdown. *Int. J. Pharm.*, 156: 119-123, 1997.
 - 39) Terrisse, I., Seiller, M., Rabaron, A., Grossiord, J.L., Rheology: How to characterize and to predict the evolution of w/o/w multiple emulsions. *Int. J. Cosm. Sci.*, 15: 53-62, 1993.
 - 40) Muguet, V., Seiller, M., Barratt, G., Ozer, O., Marty, J.P., Grossiord, J.L., Formulation of shear rate sensitive multiple emulsions *J. Cont. Rel.*, 70: 37-49, 2001.
 - 41) Kawashima, Y., Hino, T., Takeuchi, H., Niwa, T., Horibe, K., Rheological study of w/o/w emulsion by a cone-and-plate viscometer: Negative thixotropy and shear-induced phase inversion. *Int. J. Pharm.*, 72: 65-77, 1991.
 - 42) Magdassi, S., Garti, N., Release of electrolytes in multiple emulsions: Coalescence and breakdown or diffusion through oil phase?. *Coll. Surf.*, 12: 367-373, 1984.
 - 43) Wen, L., Papadopoulos, K.D., Visualization of Water Transport in $W_1/O/W_2$ Emulsions. *Coll. Surf. A: Physicochem Eng. Aspects*, 174: 159-167, 2000.
 - 44) Clément, P., Laugel, C., Marty, J.P., Influence of three synthetic membranes on the release of caffeine from concentrated w/o emulsions. *J. Cont. Rel.*, 66: 243-254, 2000.
 - 45) Attwood, D., Collett, J.H., O'Connor, C.A., The effect of gamma irradiation on the surface, rheological and micellar behavior of the block copolymer, Tetronic 908. *Int. J. Pharm.*, 65: 201-205, 1990.
 - 46) Dong, J., Chowdhry, B.Z., Leharne, S.A., Surface activity of poloxamines at the interfaces between air-water and hexane-water. *Coll. Surf. A: Physicochem Eng. Aspects*, 212: 9-17, 2003.
 - 47) Omotosho, J.A., Whateley, T.L., Florence, A.T., Methotrexate transport from the internal phase of multiple w/o/w emulsions. *J. Microencaps.*, 6: 183-192, 1989.
 - 48) Geiger, S., Tokgoz, S., Fructus, A., Jager-Lezer, N., Seillere, M., Lacombe, C., Grossiord, J.L., Kinetics of swelling-breakdown of a w/o/w multiple emulsion: Possible mechanisms for the lipophilic surfactant effect. *J. Cont. Rel.*, 52: 99-107, 1998.
 - 49) Geiger, S., Jager-Lezer, N., Tokgoz, S., Seiller, M., Grossiord, J.L., Characterization of the mechanical properties of a water/oil/water multiple emulsion oily membrane a micropipette aspiration technique. *Coll. Surf. A: Physicochem. Engineer. Asp.*, 157: 325-332, 1999.
 - 50) Yan, J., Pal, R.J., W/O/W multiple emulsions: A review of release mechanisms by break-up of the oily membrane. *J. Memb. Sci.*, 190: 79-91, 2001.
 - 51) Grossiord, J.L., Seiller, M., W/O/W multiple emulsions: A review of release mechanisms by break-up of the oily membrane. *STP Pharma. Sci.*, 11: 331-339, 2001.
 - 52) Omotosho, J.A., Whateley, T.L., Law, T.K., Florence, A.T., The nature of the oil phase and the release of solutes from multiple (w/o/w) emulsions. *J. Pharm. Pharmacol.*, 38: 865-870, 1986.
 - 53) Sela, Y., Magdassi, S., Garti, N.J., Release of markers from the inner water phase of w/o/w emulsions stabilized by silicone based polymeric surfactants. *J. Cont. Rel.*, 33: 1-12, 1995.
 - 54) Magdassi, S., Garti, N., A kinetic model for release of electrolytes from w/o/w multiple emulsions. *J. Cont. Rel.*, 3: 273-277, 1986.