# A Novel and Facile Liquid Whistle Hydrodynamic Cavitation Reactor to Produce Submicron Multiple Emulsions

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*Ferrous fumarate, [$C_4H_2FeO_4$] is widely utilized in the effective treatments and prevention of iron deficiency anemia. But, its administration has been oftentimes linked with quite a few side effects than ferric products. To overcome the side effects, multiple water-in-oil-in-water (W/O/W) emulsion formulations had been proposed as a new drug delivery system for the controlled release of entrapped active iron compound. In this study, high-pressure liquid whistle hydrodynamic cavitation reactor has been developed to produce highly stable W/O/W multiple emulsions containing Ferrous Fumarate in submicron scale (∼600 nm) with the droplet-size distribution polydispersity index in the narrow regime (0.35–0.40). The microscopic observations confirmed that that the physical stability of the W/O/W emulsions was increased significantly with operating pressure and number of emulsification passes. Looking at the potential for scale-up, this could be a promising technique to produce multiple emulsions incorporated with active constituents.* © 2012 American Institute of Chemical Engineers *AIChE J,* 59: 155–167, 2013

*Keywords: hydrodynamic cavitation, ferrous fumarate, colloidal phenomena, submicron multiple emulsion, formulation, iron encapsulation*

## Introduction

Ferrous Fumarate is increasingly used in preference to the more expensive ferric sulfate for the treatment and prevention of iron deficiency anemia (IDA). This ferrous product contains iron, which is an essential component for the formation and function of red blood cells involved in oxygen transport.[1,2] In human physiology, it also plays a pivotal role in regulating cell growth and differentiation.[3,4] According to World Health Organization, iron deficiency appears as the number one nutritional disorder in the world with more than 2 billion people of the world's population may have IDA, particularly pregnant women, infants and young children.[5,6] Ferrous products are best absorbed on an empty stomach, but they are frequently associated with more gastrointestinal (GI) side effects than ferric products as it may react with superoxide ($O_2\bullet^-$) and hydrogen peroxide ($H_2O_2$) from activated neutrophils in the mucosa, leading to the production of the hydroxyl radicals (OH●). Hydroxyl radicals can attack the mucosal and cause oxidative damage, thereby result in GI inflammation. GI side effects are generally dose-related and can be associated with nausea, constipation, anorexia, heartburn, vomiting, diarrhea, dark colored stools, and/or abdominal distress. These side effects may be minimized by administering the iron supplement in divided doses or with food or can also be largely avoided by using slower or modified release tablets and capsules.[5,7] However, concurrent administration of Ferrous Fumarate with food may decrease the amount of iron absorbed whereas in modified release preparations, they are sometimes consumed with suffering from the disadvantage of poor iron absorption.

In this study, multiple water-in-oil-in-water (W/O/W) emulsion formulations had been offered as an alternative drug delivery system for controlling the release of entrapped active iron compound, thereby reducing transient drug overload and GI side effects caused by daily iron supplementation as well as masking the unpleasant taste of the iron supplements. Particularly in pharmaceutics and medicines, these complex colloidal systems have shown promise, as potential carrier in prolonged drug delivery system, in the entrapment of various hydrophilic drugs (vaccines, water-soluble vitamins, enzymes, hormones), in the protection of the entrapped active drug substances from acidic/enzymatic environment in the GI tract, in increasing the bioavailability of drugs having first pass metabolism, and in the drug targeting specially to reticuloendothelial systems for slow and controlled delivery of drugs for oral, topical, or parenteral administration.[8]

In recent years, there have been intensive studies of emulsification techniques for producing uniform and stable multiple emulsion droplets.[9–12] The preparation of multiple emulsions with controlled droplet sizes and internal structures has been a major challenge for the past few decades. Multiple emulsions prepared by single-step phase inversion are often unstable, involving a large amount of emulsifiers and producing a coarse multiple emulsion, where the host multiple droplets consist of a large number of internal aqueous droplets. Of the techniques explored to date, two-stage emulsification is the mostly used one in the practical formulation of

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multiple emulsions. This technique exploits the turbulent shear force induced by vigorous mixing so as to rupture the droplets, but the resulting internal and external droplets generally have a broad-size distribution. However, a major recent breakthrough that has been achieved is the controlled preparation of fine and monodispersed multiple emulsions by various microstructured processing techniques, but only on the smaller scale.

For these reasons, in this study, a high-pressure hydrodynamic cavitation reactor of liquid whistle type, called Sonolator was proposed as a novel yet alternative strategy for the generation of monodispersed multiple emulsions containing Ferrous Fumarate using a two-stage method of droplet formation in a closed mixing chamber. The processing technique is so unique in such a way that it employs the energy of ultrasonic vibration mechanically generated at the leading edge of the knife-like blade, when the liquid emulsion is forced through a small orifice coupled with high pressure and fluid acceleration as efficient means of breaking the emulsions into smaller droplets with desirable droplet size. In fact, the use of such liquid whistle systems in the synthesis of advanced catalysts and nanostructured materials had been recently reported.[13] The advantages of such type of ultrasound device was firstly reported in 1960 when a series of comparative studies was carried out on the efficiency of four different emulsifying equipments using mineral oil, peanut oil and safflower oil.[14] The results concluded that an ultrasonic homogenizer of Pohlman liquid whistle type[15] was superior to other three types of equipments, namely a colloidal mill, a piezoelectric quartz crystal sonicator and a barium titanate transducer, in terms of emulsion droplet size, size distribution, and stability. The whistle-type homogenizer can be used for continuous processing of flow system and is ideal for processes such as emulsification, homogenisation and dispersion. Furthermore it has immediate possibilities for scale-up since the system mainly consists of a displacement pump with no other moving parts other than tuning adjustment to the blade and to backpressure. Additionally, the system uses a less expensive pump because the pressure is operated at as much as two-thirds less than what is normally required in conventional high-pressure homogenizers.

Although hydrodynamic cavitation has been successfully employed in a variety of applications ranging from the wastewater treatment,[16] polymer chemistry,[17] synthesis of nanocrystalline materials[18,19] to in the literature, the influence of process parameters of such a unique liquid whistle hydrodynamic cavitation based reactor (LWHCR) on preparation of multiple emulsions has not yet been investigated. Furthermore, in some investigations only the mean droplet diameter was given as a parameter of distribution, although the width of the droplet size is a key emulsion property in the case of preparation of multiple emulsion systems. Thus, we investigated the influence of the inlet pressure and the number of passes of emulsification on the average size and size distribution as well as the stability of the multiple emulsion droplets generated using the hydrodynamic cavitation system.

## Materials and Methods

### *Chemicals and reagents*

Ferrous Fumarate, polyoxy 35 castor oil (Cremophor EL®) (HLB = 12–14) and sorbitan monooleate (Span 80) (HLB = 4.3) were purchased from Sigma Aldrich (Malaysia). Calbiochem® L-ascorbic acid was obtained from EMD Chemicals (NJ). Gelatin (molecular weight150,000–300,000) was purchased from Fluka Chemical. Glyceryl monolinoleate (Maisine™ 35-1), propylene glycol monocaprylate (Capryol™90) (HLB = 6) and oleoyl macrogolglycerides (Labrasol®) (HLB = 14) were kindly donated by Gattefosse (Cedex, France). D-glucose and sodium azide were received from Classic Chemicals (M) Sdn. Bhd. Deionized water was freshly obtained from Milli-Q® Plus apparatus (Millipore, Billerica) and was used throughout the experiments.

**Table 1. Composition of W/O/W Multiple Emulsion (wt %)**

| Emulsion Type | Components | wt % |
|---|---|---|
| Primary W/O emulsion (PE) | | |
| Inner $W_1$ phase | Ferrous fumarate | 0.2 |
| | Ascorbic acid | 5.0 |
| | Sodium azide | 0.1 |
| | Glucose | 0.5 |
| | Gelatin | 2.0 |
| | Deionized water | 32.2 |
| O phase | Maisine 35–1 | 25.5 |
| | Capryol 90 | 34.5 |
| | Span 80 | 0.5 |
| W/O/W multiple emulsion | | |
| Outer $W_2$ phase | Cremophor-EL | 0.02 |
| | Labrasol | 0.03 |
| | DI Water | 69.95 |
| PE | | 30.0 |

### *Preparation of ferrous fumarate colloidal dispersions*

For the preparation of multiple emulsions, Ferrous Fumarate was used as a model drug to be encapsulated into the inner aqueous phase of the formulations, which made it necessary to prepare this colloidal dispersion in advance. Firstly, L-ascorbic acid was dissolved in the deionized water containing 0.1 wt % sodium azide which was used as an antimicrobial agent. Secondly, the reddish Ferrous Fumarate powder was introduced slowly into the solution and subjected to gentle sonication using an Ultrasonic Bath (SONOREX® model, 35 kHz, peak power output: 160 W, Bandelin electronic GmbH & Co. KG) (240 × 140 × 100 mm, l × w × d) at 50 °C until a clear light brown and homogeneous colloidal dispersion is obtained. It was followed by the addition of 0.5 wt % glucose with gentle stirring. The added glucose raised the osmotic pressure of the internal aqueous phase and kept the emulsion stable against coarsening. The mixture was put to rest for 30 min to achieve a complete dissolution of the substance. Finally, 2 wt % of gelatin was added into the solution by stirring and further with bath sonication for 5 min. After 24 hr (necessary for the total gelation of the solution), a yellowish, transparent dense solution was obtained.

### *Preparation of oily and external aqueous phases*

In this work, an oily mixture of Maisine 35-1 and Capryol 90 at a weight ratio of 3:4 containing 5 wt % Span 80 with a total adjusted specific gravity of 0.9415 and viscosity of 86.9 mPa s was prepared. The aim of using these oil mixtures as an organic phase was to minimize the degree of phase separation of W/O emulsions which primarily caused by the large difference in the specific gravity between the two immiscible phases: water and oil. The external aqueous phase was constituted of 0.02 wt % Cremophor-EL and 0.03 wt % Labrasol for the formulation of multiple emulsions. The composition of W/O/W multiple emulsion has been shown in Table 1. A mixture of nonionic emulsifier,

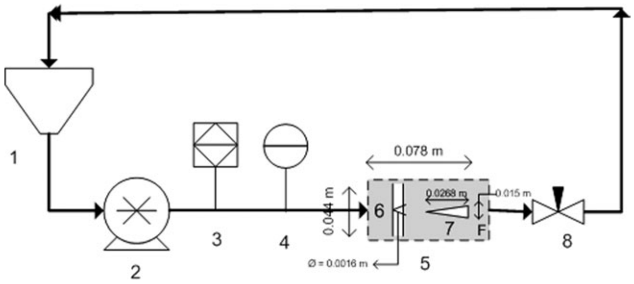

**Figure 1. Schematic representation of the experimental set-up of LWHCR (1, feed tank; 2, plunger pump; 3, PLC control board; 4, digital pressure meter; 5, cavitation chamber; 6, orifice; 7, blade; 8, back-pressure valve).**

Cremophor EL and Labrasol, has been selected due its higher HLB number as well as the nature of low toxicity and tastelessness. The addition of Cremophor-EL was reported to possess inhibitory effects on p-gp and CYP enzymes, thus, it was usually accepted for oral use.[20] As to Labrasol, it has been reported to increase the solubility of water-insoluble drugs by emulsification,[21] and it has also been demonstrated to facilitate the oral bioavailability of water-soluble drugs such as Gentamicin sulfate,[22] and amphiphilic drugs, such as Diclofenac diethylamine.[23] More importantly, it has been reported that Labrasol of low concentration (0.1 % and 1 %) enhanced remarkably the membrane permeability of D-mannitol in the chamber studies using Caco-2 cells,[24] suggesting that Labrasol has a tight-junction opening action. In addition, the clarity test shows that a homogenous translucent solution was being visually observed, indicating the good solubility of Labrasol with Cremophor EL.

### *Experimental set-up*

The emulsification device used in this work was a high-pressure hydrodynamic cavitation reactor of liquid whistle type, called Benchtop Sonolator$^{TM}$ 2000 (Sonic, CT) that employs pressure and hydrodynamic cavitational forces to deagglomerate particles and efficiently disperse and emulsify a wide range of liquids. The Model A-CIP Sonolator$^{TM}$; essentially comprises a plunger pump, a 2 HP pump motor, a Hitachi variable-frequency drive inverter and controller unit. This liquid whistle hydrodynamic cavitation reactor (LWHCR) consists of a feed vessel tank with a 5L capacity of processing volume, a plunger pump (Giant Industries, Model P220A) with a power consumption of 3.6 kW and having a speed of 1750 rpm and a small cavitation chamber (length, 0.0078 m, width, 0.044 m; height, 0.044 m) containing an orifice (area, $7.74 \times 10^{-7}$ m$^2$, diameter, 0.0016 m) and a blade (length, 0.0268 m, width, 0.0222 m; thickness, 0.0015 m) (Figure 1). The pump is capable of delivering a maximum discharge pressure of 2000 psi. The backpressure valve at the end of the cavitation chamber can be varied by adjusting the distance between the orifice and blade.

The plunger pump delivers the pre-emulsified emulsion at relatively constant flow to the cavitation chamber. The presence of the orifice creates pressure in the vessel by reducing the flow area and then intense hydrodynamic cavitation changes is envisaged to occur in a stream of process emulsion as high pressure potential energy is converted to ultrasonic acoustic energy. The coarse emulsion to be processed is forced through a special small orifice forming a flat high pressure stream. The extreme pressure generates a high velocity of fluid jet when the emulsion is projected over the edge of a stainless steel blade in which the jet of liquid emulsion sheds vortices perpendicular to the original flow. This jet impinges on the edge of the flat blade enclosed in a cavitation chamber causing it to vibrate at ultrasonic frequencies. Cavitation produces violent local pressure changes that act on the liquid, causing intense disruption of coarse emulsion consisting of two immiscible liquids. The turbulent flow conditions further imparts an enhanced hydrodynamic cavitation process in which two secondary high velocities of fluid streams is produced. The physical phenomenon which involves the conversion of high pressure potential energy into ultrasonic energy within the liquid emulsion stream is known as "jet-edge tone," as shown in Figure 2. In short, the pressure and high level of cavitation and fluid turbulence within the cavitation chamber of LWHCR are responsible for breaking the coarse emulsion into smaller uniform droplets.

### *Experimental methodology*

In this work, W/O/W multiple emulsions were prepared by means of a modified two-stage method of droplet formation in a specially shaped cavitation chamber comprising of an orifice and a blade. In the first stage, the W/O primary was generated by incorporating progressively the aqueous phase containing Ferrous Fumarate into the oily phase containing Span 80 (a lipophilic emulsifier at 80 °C ± 2 °C) at a high speed of 15,600 rpm, using Ultra-Turrax homogenizer (Ultra-Turrax, model T18 Basic, Janke Kunkel, IKA, Labortechnik, Germany). The premixing process was performed at the above speed for 20 min at room-temperature to achieve homogeneity. After premixing process, the coarse primary emulsion was then introduced into the cavitation chamber of LWHCR, where the liquid was forced through the orifice at a high dynamic pressure up to 800 psi in a single pass and then 1 ml sample was withdrawn and droplet size analysis was carried out to determine the mean droplet size and polydispersity index (PDI) of the resulting primary emulsion. This primary emulsification stage led to extremely fine droplets (312 ± 25.75 nm) with a narrow distribution as determined by Malvern Zetasizer Nano ZS. In the second stage, the primary emulsion at weigh percent of 30 % was gently mixed into a 700 ml continuous aqueous phase to form a crude W/O/W multiple emulsion at a very low speed of 3600 rpm where the external aqueous phase contains the mixtures of Cremophor EL and Labrasol as hydrophilic emulsifiers. The pre-emulsification was performed with aforementioned Ultra-Turrax T18 homogenizer for 1 min. The pre-emulsified emulsions were then passed through the

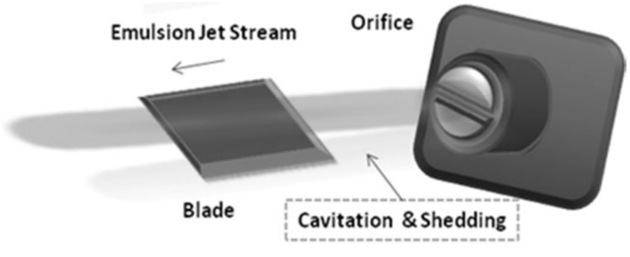

**Figure 2. "Jet-edge tone" induced by hydrodynamic cavitation in LWHCR.**

LWHCR system to achieve the formation of secondary multiple emulsions at various designated pressures.

Before the experiments, an ethanolic solution (10 % v/v) was applied for three passes at a flow rate of 2.4 l min$^{-1}$ followed by sterilized deionized water for initial clean-up of the cavitation chamber. To determine the effect of hydrodynamic cavitation produced by the LWHCR on the extent of secondary emulsification process, experiment was also carried out by passing 1 l of crude multiple emulsions containing Ferrous Fumarate through the orifice semicontinuously at various inlet pressures for a total number of 20 passes. Samples were then taken after the following number of passes: 1, 5, 10, 15, and 20; and serial characterization studies were subsequently performed to evaluate the mean globule size and the stability of the resulting multiple emulsions. It should be mentioned that unlike W/O primary emulsion, an excess or intense cavitational forces caused by high pressure and fluid acceleration can lead to the eventual rupture of the oil film separating the internal droplets and the outer continuous phase in the multiple emulsion preparations. For this reason during the secondary emulsification, the LWHCR was operated at three very mild operating inlet pressures: 100, 160, and 200 psi. In fact, fine droplets with the diameter smaller than 500 nm but with lower yield of multiple emulsion droplets were observed in any of the samples produced at an operating pressure greater than 200 psi, probably due to excessive disruptive force at higher pressures. Besides, the emulsion throughput flow rates at inlet pressures of 100, 160, and 200 psi are reported to be 0.356, 0.455, and 0.509 gallon/min respectively. Throughout the experiment, it was observed that the temperature of the cavitation chamber was maintained at room-temperature after 20 passes of fluid circulation; indicating there was virtually no heat generation in the system caused by the hydrodynamic cavitation. It is important to maintain the temperature of emulsion at an appropriate level as the temperature rise resulted from intense hydrodynamic cavitation generated at high pressure can easily lead to potential degradation of both the incorporated lipophilic and hydrophilic emulsifiers and the ascorbic acid encapsulated in the inner aqueous phase. Experimental conditions and the procedures were the same for the secondary emulsification treatment at these three different working pressures.

### *Emulsion droplet size and PDI measurement*

The mean droplet size (Z-average) and PDI of the formulated emulsions were determined by photon correlation spectroscopy with a Zetasizer Nano ZS (Malvern, UK). The value of *z*-average diameter which is referred to as the harmonic intensity-weighted average hydrodynamic diameter of the emulsions was reported as mean droplet size of multiple emulsions. All the measurements were repeated three times. The PDI was a dimensionless measure of the width of size distribution calculated from the cumulant analysis ranging from 0 to 1.0. A small value of PDI (<0.08) indicates a nearly monodispersed population, while a large PDI (>0.7) indicates a very broader distribution of droplet size. The measurements were performed using He-Ne laser at 633 nm. The short-term stability of five multiple emulsion formulations containing Ferrous Fumarate prepared after the following five designated passes: 1, 5, 10, 15, and 20 and at different working pressures (100, 160, 200 psi) was observed over a period of 15 days at room-temperature (20 °C ± 2 °C) after their preparation. The parameters evaluated were mean droplet size and PDI of the W/O/W multiple emulsions. The data were expressed as the mean ± standard deviation.

### *Intrinsic stability*

Three test tubes were filled with 10 ml of the multiple emulsions freshly generated by LWHCR at three different pressures after 20 passes respectively and sealed. They were then stored standing vertically at room-temperature (20 °C ± 2 °C) and were observed upon 10-days of storage period. Phenomenon of phase separation and creaming that bear on the instability of emulsions over the storage period was carefully studied.

### *Accelerated stability study*

Nine vials were filled with the generated multiple emulsions as described previously. Three tubes were stored vertically at room-temperature of 20 °C ± 2 °C, three in a hot air oven at 45 °C ± 2 °C, and three in a refrigerator at 4 °C ± 2 °C. Periodical observations on creaming phenomenon were performed at predetermined periods of 3, 5, 7, and 10 days.

### *Sedimentation stability test*

The resistance of an emulsion to centrifugation force depends not only on the difference in the density between the two immiscible phases: oily and the aqueous phases but also on the rigidity of interfacial film. It would, then, appear that the stability under centrifugation strongly reflects the strength of the interfacial film and the pressure balance between the inner and outer phases of the complex multiple formulations. Indeed, it has been well-documented that interfacial film strength can be used to predict emulsion stability.[25,26] According to Stokes' law,[27] the sedimentation velocity, $u_T$ can be described by

$$u_{\mathrm{T}} = \frac{d_{\mathrm{p}}^2(\rho_{\mathrm{s}} - \rho_{\mathrm{l}})g}{18\mu} \tag{1}$$

where $u_T$ is sedimentation velocity of droplet of diameter $d_p$; $\rho_l$ and $\rho_s$ are the densities of the continuous and dispersed aqueous phases respectively; $\mu$ is the viscosity of continuous phase and g is the gravitational acceleration. The retardation of the sedimentation process due to a decrease in the droplet size will increases the stability of the colloidal system.

First, nine centrifuge tubes were filled with 10 ml of the multiple emulsion and were subjected to centrifugation (centrifuge 5810R, Eppendorf AG, Hamburg) at 5000 rpm for 5 min at a constant temperature of 20 °C. After centrifugation many of plug layer of the compacted emulsions droplets were separated into a "creamed" layer at the top and a transparent "serum" layer at the bottom. The total height of the emulsions ($H_E$) and the height of the serum layer ($H_S$) were measured. The extent of creaming can be described by the following equation[28]

$$\text{Creaming index} = \frac{H_{\mathrm{s}}}{H_{\mathrm{E}}} \times 100 \tag{2}$$

### *Freeze-thaw test*

Three centrifuge tubes filled with the multiple emulsion samples and sealed. The samples were frozen in a refrigerator at −80 °C for 24 h and then thawed at room-temperature.

**Table 2. Calculated Results of Throat Velocity, Reynolds Number, and Cavitation Number for Emulsification Process at 100, 160, and at 200 psi**

| Inlet Pressure (psi) | Throat Velocity, $V_{th}$ (m/s) | Reynolds Number, *Re* | Cavitation Number, $C_v$ |
|---|---|---|---|
| 100 | 29.33 | $1.71 \times 10^7$ | 0.237 |
| 160 | 37.11 | $2.16 \times 10^7$ | 0.148 |
| 200 | 41.48 | $2.41 \times 10^7$ | 0.118 |

Evaluation of sample visual appearance and stability was performed to observe whether any destabilization or emulsion breakdown happened after the freeze-thaw test. All the samples were investigated after undergoing five cycles.

### *Chemical stability study*

Stability of W/O/W submicron multiple emulsion formulations containing Ferrous Fumarate was evaluated through mean droplet size and zeta potential measurement within the pH range 1.0–10.0. The droplet surface charge of the formulations was determined by the laser Doppler electrophoresis using a Zetasizer Nano ZS (Malvern, UK). The mean droplet diameter and zeta potential values of the formulations were determined at 25 °C. The zeta potential estimates the surface charge of droplets and, thus, gives information about interdroplet repulsive forces which generally reflect the physical stability of the emulsion.

### *Microscopic observations*

Morphology and structure of the formulated W/O/W submicron multiple emulsions were studied using field emission scanning electron microscopy (FEI Quanta 400F ESEM, OR) with the aid of xT Microscope Control imaging software. Scanning transmission electron microscopy (STEM) mode was used to reveal the form and size of the emulsions and to determine homogeneity of the colloidal systems. To perform TEM observations, the concentrated multiple emulsion was firstly diluted with deionized water (1/200) and then a sample drop (1 $\mu$l) was deposited directly onto holey formwar-coated copper grid (Agar Scientific, Essex, UK). The sample was then immediately stained with 2 $\mu$l of 2 % (w/w) phosphotungstic acid and dried for 30 s at room-temperature. The excess liquid was drawn off with a Whatman filter paper. A positive is seen in which the multiple emulsion droplets appear dark with the bright surrounding aqueous medium.

## Results and Discussion

### *Hydraulic characteristics of LWHCR*

In this LWHCR system, the flow conditions inside the cavitation chamber are analyzed based on Reynolds number while the intensity of hydrodynamic cavitation is characterized in term of cavitation number. Cavitation number, $C_v$ is the dimensionless number which can be defined as

$$C_v = \frac{2 \times (p_2 - p_v)}{\rho \times V_{th}^2} \tag{3}$$

where $p_2$ is the fully recovered downstream pressure, $p_v$ is the vapor pressure of the emulsion at the bulk liquid temperature, and $V_{th}$ is the velocity of the emulsion through the orifice holes, which was estimated with the knowledge of the emulsion throughput flow rate and the total flow area of orifice. Table 2 shows the relationship between the effect of inlet inlet pressure on the stream flow rate of multiple emulsion as well as the cavitation number. As indicated in Table 2, an increase of operating inlet pressure results in an increased turbulent flow through the cavitation chamber. This is evidently due to the higher value of $V_{th}$ generated when the liquid emulsion is forced through the extremely small orifice hole. Besides, it can be observed that an increase of inlet pressure leads to a higher degree of cavitational intensity, indicating an enhanced hydrodynamic cavitational activity. This observation follows the same trend in the results as that of the earlier studies reported with a orifice plate with multiple holes.[29]

### *Effect of hydrodynamic cavitation on the mean droplet diameter and PDI of ferrous fumarate W/O/W submicron multiple emulsions*

The influence of the operating inlet pressures and the number of passes through the cavitation chamber on the resultant mean diameter of the submicron multiple emulsion droplets as well as the droplet-size distribution using LWHCR has been shown in Figures 3a, b. Clearly, the mean droplet diameter of W/O/W multiple emulsions was greatly reduced from 1000 nm to 870 nm after a single pass as the pressure is increased from 100 to 200 psi. The substantial reduction in the mean droplet diameter at increased pressure is attributed to higher cavitational intensity. This would account for the fact that a higher cavitation intensity results in an increase in the cavity collapse event and an increased turbulence and shear.[30] The generation of this hydrodynamic cavitation in the system leads to an enhanced synergistic emulsification process. It should be mentioned here that the

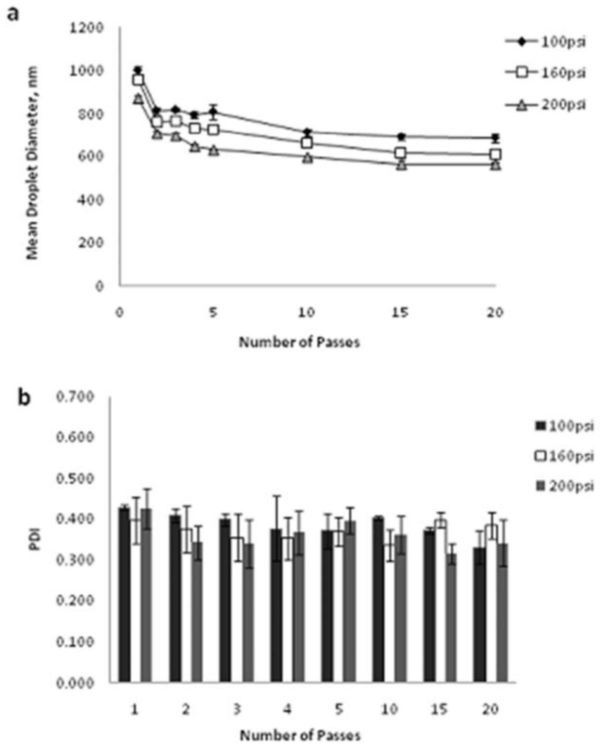

**Figure 3. Influence of the operating pressure and number of passes through the LWHCR cavitation chamber on the (a) mean droplet diameter and (b) PDI of W/O/W submicron multiple emulsions stabilized with 5 % Span 80 in the oil phase; re-emulsified with a 0.5 % solution containing Cremophor-EL and Labrasol at room-temperature (25 °C).**

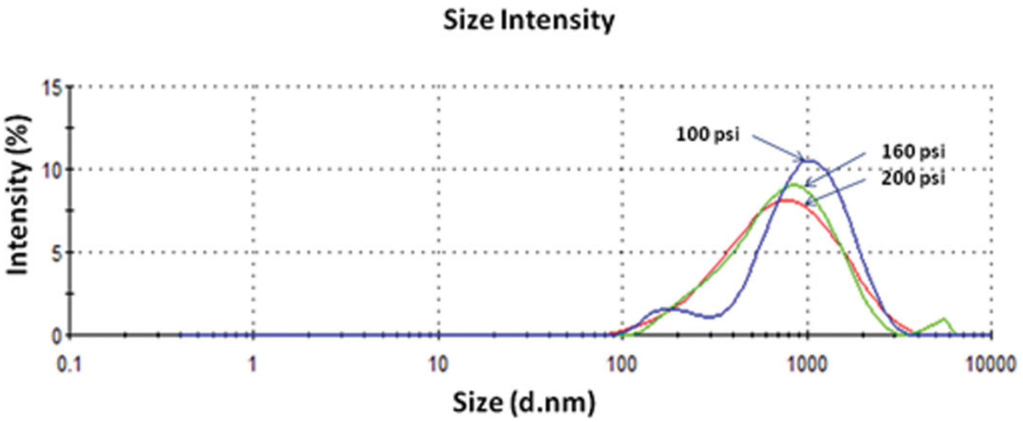

**Figure 4. Intensity-weighted particle-size distributions of W/O/W submicron multiple emulsions stabilized with 5 % Span 80 in the oil phase; re-emulsified with a 0.5 % solution containing Cremophor-EL and Labrasol at room-temperature (25 °C).**

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

operating inlet pressure, $\Delta p_s$ is used to overcome the flow resistance caused by the LCWHR blade and interfacial forces, i.e. to deform and disrupt large multiple emulsion droplets.

$$\Delta p_S = \Delta p_f + \Delta p_d \tag{4}$$

In above equation, the pressure loss for overcoming the flow resistances due to the blade, $\Delta p_f$ should be proportional to the flow velocity of liquid emulsion stream while the working pressure for droplet disruption, $\Delta p_d$ is proportional to the increase in the interfacial area of multiple emulsions. For all W/O/W multiple emulsions generated at three inlet pressures, the mean droplet diameter decreased gradually as the number of passes increased, asymptotically reaching a limiting value after 15 passes, beyond which it decreased slightly (Figure 3a). In other words, with increasing the number of passes, the major peak in droplet-size distribution tended to shift, though not appreciably, to smaller droplet diameter region. Therefore, it can be said that the reduction in the mean droplet diameter due to the cavitation progressively decreased with the number of emulsification passes until it became negligible after 15 passes. The observed phenomenon can be well explained by the reduction of availability of emulsifier, and at higher operating pressure and fixed primary emulsion content (30 wt %), the mean droplet diameter became independent of number of emulsification passes. This result is in consistent with the earlier studies on emulsification by Microfluidizer and other high-pressure homogenizers.[31–35] This indicates that all of the larger multiple emulsion droplets in the premixed coarse emulsion were almost completely disrupted to a minimum droplet size around 560–700 nm after reaching a minimum value after 10 passes. Furthermore, it has been reported by several studies that recoalescence is negligible in these experiments since the balance between the droplet break-up and the interdroplet coalescence is predominantly determined by the energy input given by the LWHCR during the emulsification process.[36,37] However, a larger deviation in the mean droplet diameter could be seen at a lower pressure of 100 psi. The size reduction of varying degrees as well as the nonuniformity of the emulsion droplets primarily stem from the different strength of cavitation intensity due to the different intensity of turbulence caused by the different magnitude of operating inlet pressure.

On the other hand, the PDI values for the W/O/W multiple emulsions prepared at 100, 160, and 200 psi after first pass were 0.428 (± 0.007); 0.397 (± 0.056), and 0.425 (± 0.048), respectively. This indicated that the increased inlet pressure in the LWHCR system cannot effectively reduce the PDI magnitude to a greater extent. Also, as shown in Figure 3b, there was obviously no significant change in the PDI values as the number of passes increased suggesting that the PDI was independent of the number of passes of emulsification through the cavitation chamber. This can be due to the fact that moderate primary emulsion content in the system (less liquidity), moderate intensity of hydrodynamic cavitation is generated leading to no beneficial effect of increased emulsification passes on the reduction in the PDI values. Despite this, the multiple emulsions prepared by LWHCR at 160 and 200 psi displayed a unimodal droplet-size distribution compared to those formulations produced at 100 psi (Figure 4). In addition, with an increase in the inlet pressure, the droplet-size distribution curve shifted gradually to the region of small droplet diameter and the size distribution became narrower. The results are, however, in contrast to some findings previously reported in the case of high pressure homogenizers, which the results showed that droplet-size distribution became broader as the homogenization pressure or speed was increased.[12,38,39] There are several possible reasons to account for this observation. Firstly, the weight fraction of dispersed W/O primary emulsion droplet phase in the W/O/W multiple emulsions containing Ferrous Fumarate was relatively high, namely 30 wt % with droplet size around 310 nm. Due to the presence of higher dispersed phase content, the emulsions cannot pass through the orifice easily and the disruption of a large number of primary emulsion droplets requires a larger amount of mechanical energy, similar to membrane emulsification conditions as demonstrated by an earlier investigation.[40]

As expected, the higher the operating pressure, the higher the uniformity of the resulting W/O/W submicron multiple emulsions generated by LWHCR since the increased pressure caused the stainless steel blade to vibrate at higher ultrasonic frequency, and thus intensified the collapse of cavitational bubbles, releasing large amount of concentrated energy into the cavitation chamber. It is hypothesized that, on projecting over the blade, the vibrational frequency of the flat blade is strongly depend on the applied inlet pressure through the cavitation chamber. Simply saying, the higher the magnitude of operating pressure, the faster the vibration motion of the blade, the higher the resulting ultrasonic frequency and thus the more intense the hydrodynamic cavitational forces generated inside the chamber, giving rise to a narrower size distribution of multiple emulsion droplets. The droplet-size distribution observed clearly proved the aforementioned hypothesis. Secondly, it should mentioned here that the role of inlet pressure is to provide a driving force for the emulsion to flow through the cavitation chamber and influence the break-up of droplets via hydrodynamic cavitation. Thus, at a lower inlet pressure of 100 psi under atmospheric back-pressure condition, it was speculated that the applied pressure was not high enough to effectively push the multiple emulsion droplets through the orifice, resulting in a low velocity of emulsion fluid jet and thus leading to subsequent poor hydrodynamic cavitational effect when the emulsion stream was projected over the edge of the blade.

### *Effect of hydrodynamic cavitation on the long-term stability of ferrous fumarate W/O/W submicron multiple emulsions*

The practical utilization of many of the W/O/W emulsions has been limited due to their inherent stability with respect to creaming, coalescence and flocculation. To inhibit the coalescence by the way of making the inner aqueous droplets into viscous-gel phase, W/O/W submicron emulsions were prepared by incorporating gelatin within the dispersed aqueous phase of the W/O primary emulsions since the usage of gelatin has been proposed as one of the alternative approaches in improving the long-term stability of entrapped inner droplets in W/O/W multiple emulsions. In this study, all the W/O/W submicron multiple emulsions containing Ferrous Fumarate produced by modified two-stage hydrodynamic cavitational emulsification process were examined for the stability of their mean droplet diameters (Figures 5a–c) and droplet-size distributions (PDI) (Figures 6a–c) at the predetermined storage period. As can be seen from Figures 6a, b, a similar trend of change in the mean diameter of the W/O/W multiple emulsions prepared after the five emulsification passes was observed at 100, 160, and at 200 psi. For the W/O/W multiple emulsions prepared after first pass at 100 and at 160 psi, the mean diameters were initially as large as nearly 1000 nm, although they all possessed nearly the same polydispersity values (~0.4), but decreased fairly slowly to ~600 nm during the storage period.

A decrease in the mean droplet diameter may be due to the gradual shrinkage of the internal droplets caused by the influx of the water from the inner aqueous phase to the outer aqueous phase during the initial 5 days of storage period.[41] It infers that the possible expulsion of the internal aqueous droplets to the external aqueous phase which leads to the formation of O/W simple emulsion with smaller mean droplet size. Comparatively, the reduction in the mean droplet size was found to be less significant with the formulations prepared at 10, 15, and at 20, indicating an improved stability with the increasing number of passes of emulsification. It could be noted that W/O/W multiple emulsions produced at 200 psi appeared to be relatively stable with no significant change in the measured droplet diameter over 15 days of storage, except the formulation prepared by using just with a single pass (Figure 5c). This result strongly suggests that the long-term stability of submicron multiple emulsions can be greatly enhanced if higher pressure is employed, preferably with the increased number of passes in the emulsification process. This can be well understood by that fact that an increase of operating pressure and number of passes results in a higher turbulent intensity, which ultimately leads to the better droplet disruption. The increase of turbulence intensity enhances the collision frequency between droplets. On the other hand, as the emulsion flow velocity increases, the mean residence time of droplet in the cavitation chamber reduces. This results in a lower coalescence probability. All these contribute to the enhanced droplet size stability of multiple emulsion.

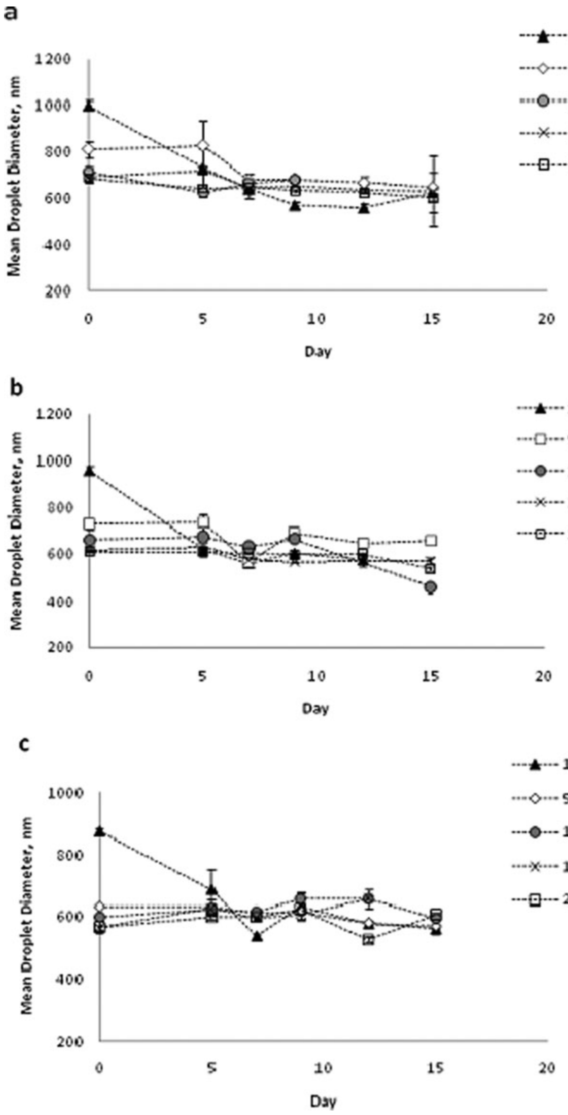

**Figure 5. Influence of the operating inlet pressure and number of passes through the cavitation chamber of LWHCR on the stability of mean droplet diameter of W/O/W submicron multiple emulsions stabilized with 5 % Span 80 in the oil phase; re-emulsified with a 0.5 % solution containing Cremophor-EL and Labrasol prepared at (a) 100 psi, (b) 160 psi, and at (c) 200 psi at room-temperature (25 °C).**

As mentioned earlier, the results from the droplet-size distribution indicated that all submicron multiple emulsions prepared after five emulsification passes at 160 and at 200 psi, respectively were almost exhibited unimodal-size distribution with their major peak shirted to smaller value region in the

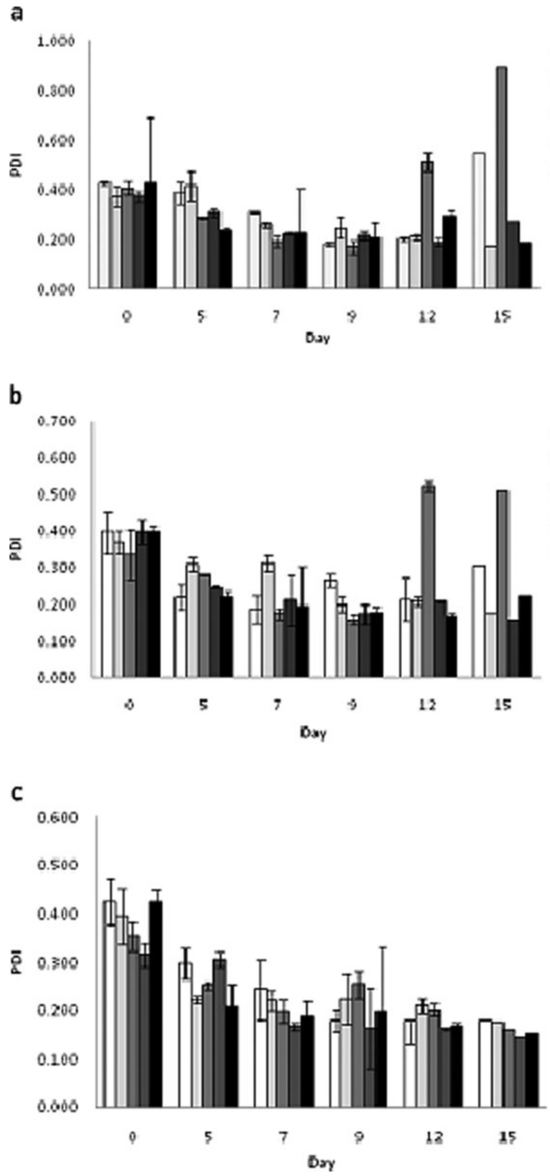

**Figure 6. Influence of the operating inlet pressure and number of passes through the cavitation chamber of LWHCR on the stability of PDI of W/O/W submicron multiple emulsions stabilized with 5 % Span 80 in the oil phase; re-emulsified with a 0.5 % solution containing Cremophor-EL and Labrasol prepared at (a) 100 psi, (b) 160 psi, and at (c) 200 psi at room-temperature (25 °C).**

droplet-size distribution with PDI value lower than that of those formulations obtained at 100 psi. Intriguingly, Figures 6a–c show that the polydispersity values for all the W/O/W submicron multiple emulsions produced by LWHCR technology decreased gradually over the storage period, until it reached a fairly constant value, indicating that the droplet-size distribution became narrower and multiple emulsion droplets appeared to be more monodispersed. This observation might be attributed to a significant prolonged cavitational effect associated with mechanical effects which favor the droplet break-up mechanism over the coalescence process within the dispersed droplets during the storage period, rendering the W/O/W multiple emulsions in the finer droplet size.

Conversely, it has been observed that there was a substantial fluctuation of PDI values for the four multiple emulsions prepared after single and 10 passes at 100 and at 160 psi, respectively (Figures 6a, b). This observation is probably attributed to the shrinkage or the swelling of internal aqueous droplets caused by the osmotic pressure gradient between these two phases, leading to the instability of PDI values. An explanation to this varying stability of the size distribution of these formulations can be offered on the basis of cavitational effects. It is entirely possible that at a lower operating pressure of 100 and at 160 psi, hydrodynamic cavitation alone provides the poor disruptive force when the first stage of hydrodynamic cavitation treatment was applied, resulting in an unstable colloidal system. Meanwhile, it is hypothesized that the droplet disruption mechanism is partly dominated by mechanical effects of fluid turbulence other than hydrodynamic cavitational effects, thereby giving rise to the instability of the size distribution of the W/O/W submicron multiple emulsions generated after 10 passes at both 100 and at 160 psi. In other way, the coarse multiple emulsion droplets have less opportunity to interact particularly at the lower pressure with the blade throughout the cavitation chamber due to the liquid emulsion stream of lower velocity. Nevertheless, a further detailed investigation is essentially needed on this area.

### *Microscopic study*

Photomicrographs for Ferrous Fumarate-loaded W/O/W submicron multiple emulsions prepared at three operating inlet pressures at 20 emulsification passes are given in Figures 7A–D. Multiple emulsion of spherical globules with a large number of inner droplets can be observed. On comparison among three W/O/W emulsions (Figures 7A–C), it is clear that W/O/W multiple emulsions generated at 100 psi displayed the largest multiple oil globules with a barely discernible oil membrane layer. This is followed on the decrease order by the W/O/W multiple emulsions made at 160 psi and 200 psi which gave relatively smaller multiple droplets size and the inner water droplets were encapsulated in a much more stable oil phase. The difference in the emulsion stability is due to the formation of less stable interfacial complex between the macromolecular compound (gelatin) and the molecules of the hydrophilic emulsifier (Cremophor EL) at the O/W interface. Therefore, the water transfers from the external to the internal phase are allowed, resulting in the thinning of the oil membrane. As in this scenario, the eventual rupture of the oil membrane would merge both internal and external aqueous phases and consequently giving rise to the release of the materials into surrounding medium. Through the ranking of the dispersion degree of multiple droplets, W/O/W submicron multiple emulsion produced at 200 psi presented the finest and homogenous vesicular multicompartment droplets with the highest swelling capacity of oil globules which may lead to a more sustained drug release. With this observation, it is suggested that hydrodynamic cavitation intensity may play a vital role in the formation of stable multiple oil globules; in this study, it could somewhat rigidify the interfaces by suppressing the diffusion process of lipophilic emulsifier. Such interaction is evidently more pronounced at the operating pressure of 200 psi, as the physical structure in the case of W/O/W

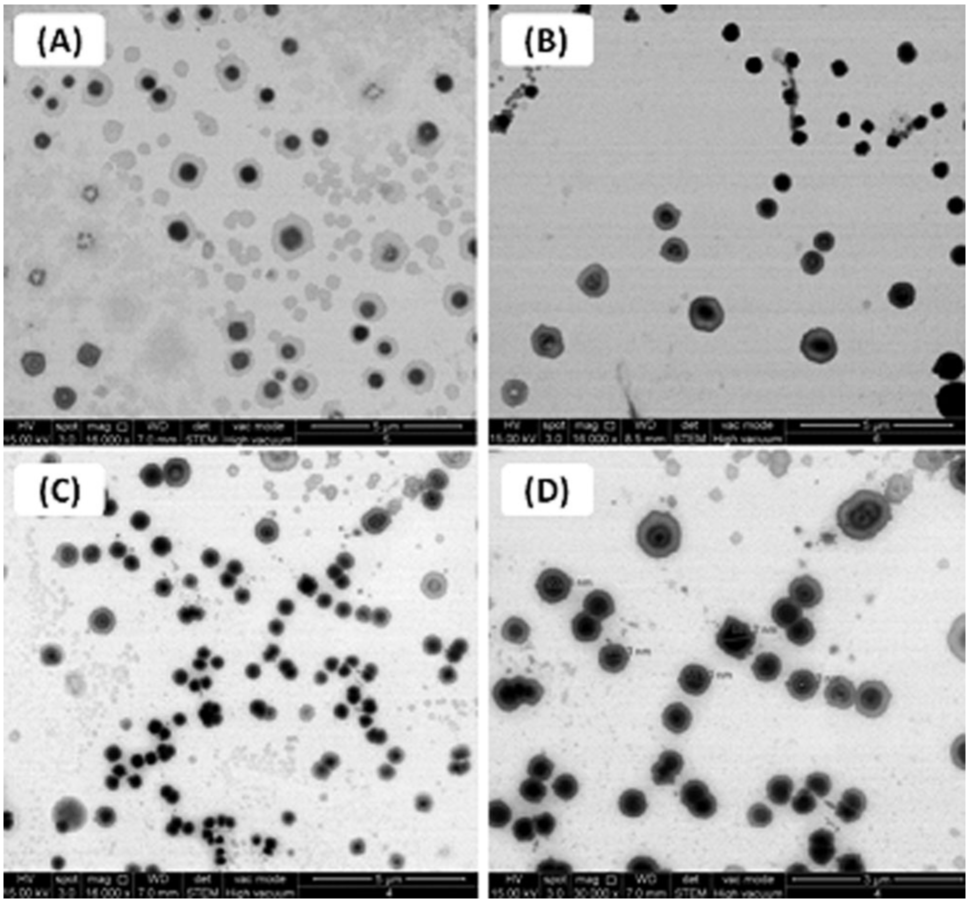

**Figure 7. Scanning transmission electron microscopy (STEM) of W/O/W submicron multiple emulsions of ferrous fumarate stabilized with 5 % Span 80 in the oil phase; re-emulsified with a 0.5 % solution containing Cremophor-EL and Labrasol prepared at (A) 100 psi, (B) 160 psi, and at (C and D) 200 psi after 20 emulsification passes through LWHCR at room-temperature (25 °C).**

submicron multiple emulsion was almost intact. These results indicated that the physical stability of submicron multiple emulsions can be substantially improved if higher pressure is employed, preferably with the increased number of passes in the emulsification process.

In the case of submicron multiple emulsions prepared at 100 psi as indicated in Figure 7A, larger internal aqueous compartment were found in the W/O/W multiple emulsion droplets as compared with the multiple emulsions produced at 160 and 200 psi. Swelling of the inner droplets eventually takes place because of the permeation of water through small oily region. In contrast, when the operating pressures is increased to 200 psi, considerable reduction of both the inner dispersed water droplets and multiple emulsion droplet size appeared. As mentioned earlier, it is recognized that smaller and more stable multiple emulsion globules was formed with a very fine inner aqueous droplets. Through droplet size measurement by SEM, the mean diameter of multiple droplets calculated was at around 600 nm and the size of inner aqueous droplets was about 300 nm, in good agreement with light scattering particle size analysis (Figure 7D). As shown in Figures 7A–C, one may observe that a variation in size distribution was considerably reduced when the operating pressures was increased from 100 to 200 psi, indicating an improved uniformity of the colloidal systems. Figure 7D also showed clearly the existence of solid phase in the core of the multiple emulsion droplets. This could be account for the fact that the drug-in-multiple emulsion core may crystallize during sample preparation before microscopic observations. These microscopic results confirmed that the morphology and physical stability behavior of the W/O/W submicron multiple emulsion containing Ferrous Fumarate are greatly governed by selection of operating pressure of LWHCR .

### *Accelerated stability study*

The stability of W/O/W submicron multiple emulsions containing Ferrous Fumarate were observed for 10 days. All freshly prepared multiple emulsions were milky white in color. As a result of the stability test, at the room-temperature, three multiple emulsion formulations individually prepared at 100, 160, and at 200 psi respectively remained intact and stable until a slight creaming layer was observed at the end of the storage period. Surprisingly, there was no change in the stability of multiple emulsions at the storage condition of −4 °C up to 10 days. Neither creaming nor sedimentation was observed. This implies that the W/O/W submicron multiple emulsions showed an excellent stability to gravitational separation at the lower-temperature.

However, it is evidently noted that the three multiple emulsion formulations stored at 45 °C showed a slower color development with a gradual creaming process throughout the observation period. The color of the multiple emulsions became yellowish white as the time went on. The change of color was detected on the first day and persisted to the last

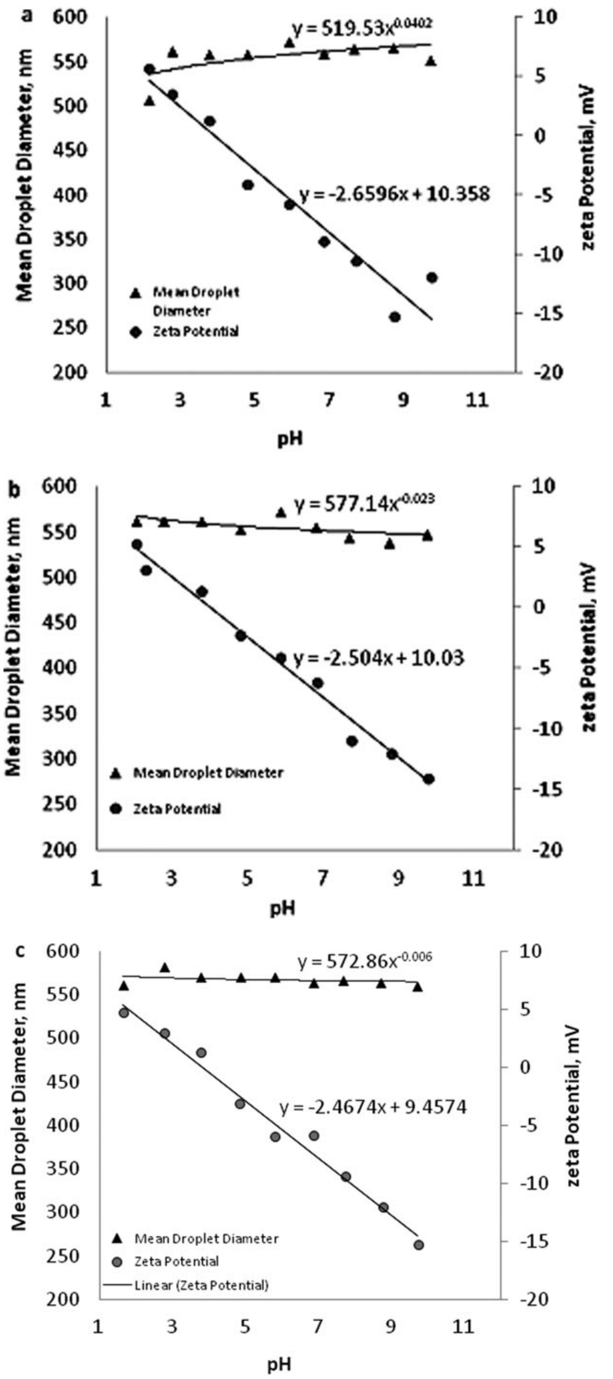

**Figure 8. Effect of pH on the mean droplet size and zeta potential of W/O/W submicron multiple emulsions containing ferrous fumarate prepared at (a) 100 psi, (b) 160 psi, and at (c) 200 psi, respectively, after 20 passes through LWHCR.**

day of the storage period. In addition, it is also observed that there was an oil-rich layer formed (~1.6 vol %) at the top of all three W/O/W multiple emulsions due to the downward movement of the water droplets after 10 days of storage at −45 °C. This result might be attributed to the change in hydrophobicity or hydrophilicity of adsorbed Span 80 and Cremophor-EL/Labrasol when the samples were stored at an elevated temperature.

Despite this, it is worth noticing that W/O/W submicron multiple emulsions containing Ferrous Fumarate prepared at 200 psi exhibited a better stability with lower creaming volume percentage of 2.8 % as compared to those two formulations produced at 160 psi (4.4 %) and at 100 psi (4.0 %). Observations with the creaming index of W/O/W submicron multiple emulsions treated by 160 and 100 psi of ultrasound seem to be quantitatively similar, and this observation may subject to some experimental errors which possibly arise from high acceleration setting of centrifugation process since the creaming velocity is highly dependent on the length of centrifugation time. This result of study proved the earlier observation that the physical stability of the W/O/W emulsion strongly depends on the applied inlet pressure and the number of emulsification passes.

### *Sedimentation stability test*

The stability of W/O/W submicron multiple emulsions containing Ferrous Fumarate as quantified by creaming index under the influence of centrifugal force was also investigated. It is well-known that creaming index provides the indirect information of the droplet aggregation or flocculation while the destabilization after centrifugation serves as an useful indicator of the degree of coalescence within the emulsion droplets.[42] In this study, all the six W/O/W multiple emulsions prepared both after 15 and 20 passes at three different pressures present the same creaming index value of 1.5 % after subjected to the centrifugation stability test (Table 3). At room-temperature, no appreciable creaming was observed after 1 week, except with a thin bulk oil layer (0.5 ml) was released in the top of these multiple emulsions. This observation might account to the fact that the use of oil mixture of Maisine 35-1 and Capryol 90 at a weight ratio of 3:4 with a total adjusted specific gravity to that of the water phase greatly retards the movement of fine emulsion droplets and thus minimizes the possibility of droplet aggregation in the W/O/W submicron multiple emulsions. The creaming instability measurements largely support the droplet size stability measurement. In this respect, Onsaard et al. reported that an increase in the creaming index would ultimately lead to an increase in the possibility of droplet aggregation due to faster movement of the emulsion droplets.[43]

### *Freeze-thaw test*

Freezing and thawing test shows that W/O/W submicron multiple emulsions generated using different operating pressures was severely damaged at the end of the experiment, though no visible creaming was detected. Neither any of the three multiple emulsion formulations individually produced after 20 passes at 100, 160 and at 200 psi could withstand five freeze-thaw cycles. All the formulations appeared to be in semitransparent continuous aqueous phase with no milky color was being observed, indicating complete rupture of the submicron multiple emulsions. In this scenario, changes in the physical stability of the multiple emulsions were observed as the number of freeze-thaw cycle was repeated. The observed destabilization might suggests that the exaggerated storage conditions (−80 °C) leads to a temperature-induced degradation of the adsorption layer of the emulsifier molecules on oil-water interface, rendering the formulations less appealing during the storage period. In fact, it is also entirely possible that exposure to temperature cycles during the emulsion is frozen and thawed significantly changes the

**Table 3. Results of the Mean Droplet Size and Polydispersity Index (PDI) Measurements, Stability Under Centrifugation, Measurements of pH Values for W/O/W Submicron Multiple Emulsions Containing Ferrous Fumarate Prepared at 100, 160, and at 200 psi, respectively, After 20 Emulsification Passes Through LWHCR**

| Operating Pressure (psi) | 100 | 160 | 200 |
|---|---|---|---|
| Mean droplet size ± S.D. (nm) | 685.6 ± 21.03 | 611.5 ± 5.941 | 564.9 ± 7.242 |
| Polydispersity index (PDI) ± S.D. | 0.330 ± 0.039 | 0.384 ± 0.033 | 0.341 ± 0.056 |
| Creaming index after centrifugation (%) | 1.5 | 1.5 | 1.5 |
| Native pH | 2.78 | 2.88 | 2.85 |

solubility of the dissolved drug and the physical properties of the whole colloidal system such as viscosity. All these consequently result in the film destabilization and poor reconstitution ability followed by an eventual loss in the emulsion stability.

### *Chemical stability study*

Additional information about the physicochemical stability of three W/O/W submicron multiple emulsions containing Ferrous Fumarate prepared at different pressures was derived from the change in the mean droplet diameter and zeta potential over a pH-range from 1 to 10. Figures 8a–c illustrate the effect of pH on the mean droplet diameter and zeta potential of Ferrous Fumarate-loaded mutiple emulsions stabilized by 20 wt % gelatin in the internal phase and 5 % Span 80 in the oil phase; re-emulsified with a solution of 0.5 % nonionic emulsifier mixture of Cremophor EL and Labrasol. It is found that alteration in the pH has a remarkable influence on the zeta potential of W/O/W submicron multiple emulsions containing Ferrous Fumarate. As indicated in Table 3, the native pH values of three freshly produced W/O/W submicron multiple emulsions after 20 passes at 100, 160, and at 200 psi were 2.78, 2.88, and 2.85, respectively, due to the drug's weak acidity with a zeta potential of around 2.9–3.5 mV. The results reveal that the formulations have a small positive droplet surface charge and these electrical positive surface charges are primarily derived from the dissociation of Span 80 molecules. The positive charges lead to electrostatic repulsion among the droplets. The absolute zeta potential decreases as ambient pH shifts from acid to alkaline and tend to be more negative as the pH approaches 10, where the negative charges stemmed from the adsoprtion of hydroxyl ions onto the droplet surface.[44,45] Admittedly, a higher absolute zeta potential values (above ±30 mV) should preferably be achieved in most of the colloidal systems to impart a good stability against coalescence of the dispersed droplets.[46] However, this suggested zeta potential cut-off point can only serve as a partial indicator for the physical stability of the emulsion obtained and cannot reliably used to predict the stability of, in this study, complex Span 80 and Cremophor-EL/Labrasol based multiple emulsions.

According to the power law relationship presented in Figures 8a–c, the results reveal that the use of higher inlet pressure appears to provide slightly better surface charge stability of submicron multiple emulsions over a pH-range from 1 to 10, as witnessed with a milder descending trend with lesser negative slope value. However, a similar stability profile has been observed for the linear relationship between zeta potential and pH for all three multiple emulsion samples produced at 100, 160, and 200 psi. These observations suggest that an increase of operating pressure and number of passes lead to an enhanced physicochemical stability of submicron multiple emulsions. Overall, the results demonstrated that submicron multiple emulsions containing Ferrous Fumarate almost retained their mean droplet size over a wider pH range, indicating an excellent droplet size stability of the entire multicompartment colloidal system. The good physicochemcial stability of the multiple emulsion formulations are mainly due to strong steric repulsion forces imparted by monolayer adsorption of the nonionic emusifiers.[47] Due to the presence of ester group of ricinoleic acid and glycerol polyoxyethylene (POE) group and the long hydrocarbon chain of Cremophor EL as well as the polyethyleneglycol esters of Labrasol, a stronger steric stabilization effect can be induced among the generated multiple emulsion droplets. On the one hand, the stability of charged droplets might be ascribed to a specific interaction due to the positive charge of the droplet in case of the multiple emulsion. In fact, the nonionic emulsifier mixture in this study is able to attain better emulsifying performance and better control of droplet size consistency as compared with those formulations stabilized by a single emulsifer.[48,49] These mixed emulsifer effects have been attributed to an increased density or closer packing of emulsifier molecules at the oil-water interface. Therefore, the droplet size of multiple emulsions containing Cremophor-EL and Labrasol were remained virtually constant showing no aggregation, creaming, and coalescence.

## Conclusions

LWHCR appears to be a novel technology and efficient yet low cost alternative to produce highly stable and uniform submicron W/O/W multiple emulsions containing Ferrous Fumarate in a semicontinuos manner. An increase in the number of passes has been proved to be capable of producing smaller and stable W/O/W multiple emulsions with the narrow droplet-size distribution. Although an increase in the inlet pressure and number of emulsification passes leads to enhanced stability of the W/O/W multiple emulsion, but, extreme pressure is not recommended for preparing W/O/W submciron multiple emulsions at the second step since the intense cavitational bubble collapse promotes the rupture of the internal aqueous droplets, which subsequently leads to the loss of the inner water phase encapsulating the active pharmaceutical or nutraceutical ingredients. The microscopic results demonstrated that a variation in multiple globule-size distribution is greatly reduced when the operating pressures is increased from 100 to 200 psi, indicating an improved homogeneity of the fine dispersion. Selection of an optimum set of operating parameters including operating pressure, number of treatment passes, temperature of the continuous phase and the distance between the blade and orifice is essential to achieve maximum benefits in the emuslification process. It can be said that LWHCR offers immediate and realistic potential for pharmaceutical industrial-scale applications. In essence, this study serve as an useful guideline to design enginner in the development and controlled manufacture of the pharmaceutical W/O/W submicron multiple emulsions in years to come.

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## Appendix

Reynolds Number, $Re = \frac{\rho V_{\mathrm{th}} D_{\mathrm{o}}}{\mu}$
$V_{\mathrm{th}}$ = velocity of emulsion through the orifice
$D_{\mathrm{o}}$ = diameter of orifice
$\rho$ = density of emulsion
$\mu$ = viscosity of emulsion
$D_{\mathrm{o}} = 0.0016$ m, $\rho = 981$ kg/m$^3$, $\mu = 0.027$ mPa s
At 100 psi, $Re = 1.71 \times 10^7$ (turbulence)
At 160 psi, $Re = 2.16 \times 10^7$ (turbulence)

At 200 psi, $Re = 2.41 \times 10^7$ (turbulence)
Cavitation Number, $C_v = \frac{2\times(p_2-p_v)}{\rho\times V_{th}^2}$
$p_2$ = fully recovered downstream pressure, Pa
$p_v$ = vapor pressure of emulsion, Pa
$\rho$ = density of emulsion, kg/m$^3$
$V_{th}$ = velocity of emulsion through the orifice, m/s

### *The relationship between orifice area, pressure, and flow (Sonolator Manual, Sonic Corporation)*

Q = 30 × A0 X
$Q$ = throughput flowrate of emulsion, gallon/min
$A_o$ = area of orifice, in$^2$
$P$ = pump-to-orifice pressure
30 or $\alpha$ = viscosity and pump slip constant
At $P = 100$ psi, $A_o = 7.74 \times 10^{-7}\text{m}^2$

$$Q = 30 \times 7.74 \times 10^{-2}\text{m}^2 \times \frac{1550\ \text{in}^2}{1\text{m}^2} \times \sqrt{100}$$
$$= 0.35591 \quad \text{gallon/ min}$$

### *Solving for throat velocity*

$$V_{th} = \frac{Q}{A_o} = \frac{0.35591\ \text{gallon}}{\text{min}} \times \frac{3.785 \times 10^{-3} m^3}{1\ \text{gallon}}$$
$$\times \frac{1}{7.74 \times 10^{-7}\text{m}^2} \times \frac{1\ \text{min}}{60\text{s}} = 29.33 \quad \text{m/s}$$

### *Calculation of cavitation number*

$$p_2 = 101,325\ \text{Pa},\ p_2 = 1680\ \text{Pa},\ \rho = 981\ \text{kg/m}^3$$
$$C_v = \frac{2 \times (10,325 - 1680)}{981 \times 29.33^2} = 0.237$$
At 160 psi, $V_{th} = 37.105$, $C_v = 0.148$
At 200 psi, $V_{th} = 41.484$, $C_v = 0.118$

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