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Systematic characterization of oil-in-water emulsions for formulation design

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Abstract

Oil-in-water emulsions varying in surfactant concentration and manufacturing process were prepared. About 10 experiments were performed to characterize them. The goal of this research was to find out which tests should systematically be carried out to assess efficiently the stability and the properties of an emulsified preparation. Thus, formulation design requires at least the measurement of the droplet size, the determination of the zeta potential, a TurbiScan® analysis, the investigation of the stability under centrifugation and freeze/thaw cycles. If the emulsion contains an active substance, stability under storage at 4 °C and microscopic analysis are relevant. Quality control should be improved by measurements of viscosity and pH.

Keywords: Emulsion; Characterization; Stability; Size

1. Introduction

Oil-in-water (o/w) emulsions are commonly formulated for parenteral and topical administration but also for oral and ocular routes (Tamilvanan et al., 2001; Vandamme, 2002). Each route of administration has to meet its own requirements of formulation, e.g. sterility for parenteral preparations, aesthetic attractiveness for topical products. Another interesting point for emulsions is the size of the droplets of oil dispersed in the water. The median size (Chanana and Sheth, 1995; Prinderre et al., 1998) as well as the distribution of sizes (Liedtke et al., 2000) are very important since they determine the safety of the preparation in the case of intravenous preparations (Mbela and Ludwig, 1995) or the release properties of the active ingredient in topical formulations (Friedman et al., 1995). Nevertheless, all emulsions should first be considered as dispersed systems and overall analyzed in terms of stability. Stability is often related to the zeta potential (ζ) of the system. Its value reflects the stability of the dispersed system in a chosen environment but gives poor information about the stability over time: how will the preparation age? Creaming, coalescence, flocculation, phase separation, rupture, Ostwald ripening may occur in emulsified systems (Welin-Berger and Bergenståhl, 2000), leading to numerous instability processes. In flocculation, two droplets become attached to each other but are still separated by a thin film of liquid. When more droplets are involved, an aggregate is formed, in which the individual droplets cluster together but retain the thin liquid films between them. The emulsifier molecules remain at the surface of the individual droplets during this process. When the thin liquid film between the droplets is removed, bigger droplets can be formed and coalescence can

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occur. The coalescing emulsion is characterized by a wide size distribution of the droplets, but no clusters are present. Finally, the droplets achieve such a size that they are recognizable by the naked eye: phase separation occurs and two separate layers are visible (Lieberman et al., 1988). Creaming occurs when dispersed particles either settle or float with respect to the continuous phase and when either the lower or upper portion, respectively, becomes more opaque or creamier (Lieberman et al., 1989). Ostwald ripening, or molecular diffusion, is a mechanism which causes emulsion instability, provided that the droplets are small and the disperse phase has a finite solubility in the continuous phase. In that case, many small droplets formed initially slowly disappear except for a few that grow larger, at the expense of the small droplets. The smaller droplets act as "nutrients" for the bigger droplets. As the bigger droplets grow, the area around them is depleted of smaller droplets (Welin-Berger and Bergenståhl, 2000). Rupture of an emulsion means its destruction by coalescence of droplets of the internal phase (Association Française de Normalisation (AFNOR), 1976). Predicting the in-time stability of an emulsion and predicting its degradation are topics of interest for researchers. The AFNOR has published in 1976 (AFNOR, 1976) a directive about emulsion analysis. We develop here an updated attempt of protocol, checking out which tests should be used to rationally design formulations of emulsions. The tests prescribed by the AFNOR have been carried out and completed by new techniques: particle size analysis by laser diffractometry, automatic optical characterization of the stability, and zeta potential measurement. The aim of this work was to determine which assays should be systematically performed to assess efficiently the stability and the overall characteristics of an emulsion.

2. Materials and methods

Five emulsions were analyzed. They differ by the manufacturing process and the concentration of surfactants. All the emulsions were made of 30% (w/w) soybean oil (Lesieur, France) as oily phase, polysorbate 60 and sorbitan monostearate (53:47 ratio) (free samples from Codibel, Belgium) as surfactants, and purified water containing 0.15% (w/w) of a mixture of parabens as preservatives (methyl-4-hydroxybenzoate and propyl-4-hydroxybenzoate, 8:2 ratio) (Federa, Belgium). The emulsions were prepared either by hand (emulsion H), with a Silverson L4R mixer (E.J. Payne Ltd., England) (emulsion S), or with a high-pressure homogenizer MiniDeBEE (BEEI International Ltd., Israel) (emulsion D). Emulsions H, S, and D contained 10% of surfactants (w/w of the dispersed phase). Two additional emulsions were prepared with the high-pressure homogenizer: they contained 5 and 2% of surfactants, respectively (emulsions D5 and D2). Emulsions were prepared at 65 °C: hot water was slowly added to the heated oily phase containing the melted surfactants. This technique, called the phase inversion method, is widely used in batch processing since it has been found that it can yield small droplet sizes (Lieberman et al., 1989). Emulsion S was mixed for 15 min at 6200 rpm. Emulsion D was obtained by passing emulsion S twice through the high-pressure homogenizer at 137.9×10^6 Pa (20 K psi), with the emulsification cell containing alternated reactors of 0.5 and 1.0 mm internal diameter and a 100 μm diameter nozzle. All the emulsions were prepared in triplicate and analyzed immediately after their manufacturing.

2.1. Particle size analysis

Particle size analysis can be carried out by different techniques: laser diffraction, electrical zone sensing method (or "Coulter" counter), photon correlation spectroscopy, or ultrasonic spectroscopy. For each technique previously described, we can point out disadvantages which are inherent to the method used. Laser diffraction, electrical zone sensing method, and photon correlation spectroscopy require dilution of the samples. It is not the case for ultrasonic spectroscopy but measurements with this instrument require relatively large amounts of samples (at least 100 ml). Before making a size measurement, it is important to determine which are the upper and lower limits of detection of the apparatus. As an example, photon correlation spectroscopy is a method dedicated mainly to submicron particles and is inadequate for particles larger than a few 10 μm. If the apparatus claims a broad size range capacity, the accuracy of the measurements carried out should be checked out if the results are expected to be close to the upper or the lower limit of detection (Alba et al., 1999; Washington, 1992).

Particle size analysis was performed by using a laser diffractometer Mastersizer 2000 with the Hydrosizer 2000S module (Malvern Instruments, UK). The sample was extemporaneously dispersed in purified water at 2500 rpm until an obscuration rate of 5–18% was obtained. Background and sample were measured for 12 s. Optical properties of the sample were defined as follow: refractive index 1.460 and absorption 0.00 (similarly to the particles named Intralipid in the Malvern software). Each sample was measured in triplicate.

Dilution in purified water, even if recommended by the equipment manufacturer, may induce physicochemical changes in the environment of the droplets, leading therefore to completely unreliable data. Therefore, measurements on a commercial standardized emulsion were carried out. Intralipid® 30% (Fresenius Kabi N.V., Schelle, Belgium) is an intravenous oil-in-water emulsion of soybean oil emulsified with egg phospholipids. The emulsion contains also glycerol as osmotic agent. We checked out that the extemporaneous dilution is stable and representative by concentration titrations within the ranges of the instrument. We made measurements of Intralipid® 30% (batch 1009297, expiration date July 2004) from 0.5 to 50% obscuration rate (concentration range 1–100).

2.2. Optical characterization of the stability

The TurbiScan MA2000 (Formulaction, France) is an instrument for the optical characterization of a liquid dispersion. The fresh emulsion (±6 ml) is contained in a cylindrical glass measurement cell which is completely scanned by a light source. Two synchronous detectors collect transmission and backscattering data every 40 μm. The transmission detector receives the light that goes through the sample while the backscattering detector receives the light backscattered (135°) by the sample. A pattern of the light flux as a function of the sample height is obtained, giving a macroscopic fingerprint of the sample at a given time. When the acquisition of those data is repeated over 24 h with a 12-min frequency at 21 °C ± 2, we obtain a superimposition of the product fingerprints characterizing, whether they are identical or not, the stability or instability of the product. If the emulsion is unstable, the creaming rate is calculated.

2.3. Zeta potential

If a stable emulsion is to be produced, an emulsifying agent must be added to the oil and water. It acts as a barrier to alter the rate of coalescence of droplets or creates an interfacial film which can produce repulsive electrical forces between approaching droplets. With an ionic emulsifier, a monolayer of surfactant is absorbed and a double layer is built up around the droplets. The double layer consists of the charged portion of the emulsifier at the water interface and, in the case of an anionic emulsifier, of the cations (counterions) surrounding it. If the counterion concentration is low, the thickness of the electrical double layer will be large, and long-range repulsive forces will be active, causing the droplets to repel one another when they approach. The potential produced by the double layer creates a repulsive effect between the oil droplets and thus hinders coalescence. Although this repulsive electrical potential at the emulsion interface can be calculated, it cannot be measured directly. However, a related quantity, called the zeta potential, can be determined (Lieberman et al., 1988). The zeta potential is defined as the difference in potential between the surface of the tightly bound layer of ions on the particle surface and the electroneutral region of the solution. When the zeta potential is relatively high (25 mV or more, absolute value) the repulsive forces exceed the attractive London forces. The particles are dispersed and the system is deflocculated. On the other hand, when the zeta potential is low (less than 25 mV, absolute value), the attractive forces exceed the repulsive forces, and the particles come together leading to flocculation (Lieberman et al., 1989). Zeta potential is nowadays mainly determined by measuring the electrophoretic mobility of the dispersed particles in a charged field but other instruments can be used for this purpose: electrophoretic mass transport analyzer, streaming current detector, and electrokinetic sonic amplitude device.

The zeta potential was measured by a Zetasizer 2000 (Malvern Instruments). A sample was extemporaneously diluted in Milli-Q (Millipore Corp., USA) water (1 μl/10 ml) and injected in the apparatus. The measurements were carried out in the fully automatic mode. Each sample was analyzed twice, each analysis consisting of five replicates.

2.4. Intrinsic stability

For each emulsion, three test tubes were filled with 15 ml of the emulsion and then hermetically closed. They were stored vertically at room temperature (21 °C ± 2) and were observed at time +1; +2; +4; +6, and +24 h. Any change was recorded: separation or creaming and the corresponding heights (easily transformed in volumes).

2.5. Stability under storage

Nine test tubes were filled as described in Section 2.4. Three tubes were stored vertically at room temperature (21 °C ± 2), three in a hot air oven at 45 °C ± 2, and three at 4 °C ± 2. Observations were made each week for 8 weeks.

2.6. Stability under centrifugation

Resistance of an emulsion to centrifugation depends on the difference of density between the oily and aqueous phases and also on the resistance on the interfacial film. Therefore, with quite similar formulations which exhibit small density differences, the stability under centrifugation reflects the strength of the interfacial film (Puisieux and Seiller, 1983).

Four centrifugation tubes were filled with 20 ml of emulsion and underwent a centrifugal acceleration of 15,000 m/s^2 (1529 × g) for 10 min (Heraeus Labofuge GL centrifuge, Germany). The centrifugal acceleration γ (m/s^2) is given by $\gamma = 110N^2R$, where N is the rotation speed (10^3 rpm) and R is the radius of gyration (cm), measured from the axis of the centrifuge to the bottom of the tube, in the horizontal position (AFNOR, 1976). To avoid modifications induced by possible heating, the temperature was measured in one tube at the end of the experiment. It should not exceed 30 °C.

2.7. Viscosity

Measurement of the viscosity is important since the Stokes' law asserts that the rate of phase separation (v) between liquid 1 (ρ_1) and liquid 2 (ρ_2) depends on gravity (g), on the radius of the particles (r), and on the viscosity (η) of the medium.

$$v = \frac{2r^2(\rho_1 - \rho_2)g}{9\eta}$$

Rheological measurements can be carried out by many types of viscometers with falling or rolling sphere, capillary or tube, or rotational device. Measurements should be carried out on fresh emulsions, at constant temperature since any change in the way of proceeding may induce major changes in results.

Viscosity was measured with a Brookfield DV-II+ Viscosimeter (Brookfield, USA). About 40 ml of emulsion was heated at 25 °C ± 2 in a measuring cylinder. The same spindle (spindle S61) and the same stirring rate (100 rpm) were used for each experiment except for emulsion H, which is more viscous and needed a slower stirring rate (30 rpm).

2.8. pH

An MP220 pHmeter (Mettler Toledo, UK) was used for the determination of the pH value of the emulsions at room temperature (21 °C ± 2).

2.9. Freeze/thaw cycles

Three test tubes filled with the emulsion and hermetically closed were vertically stored for 16 h in a freezer at −21 °C and then for 8 h at room temperature (21 °C ± 2). The emulsion was observed and any change was recorded. This cycle was repeated four times.

2.10. Microscopic analysis

The emulsion was diluted (1:1) in Milli-Q (Millipore Corp.) water just before a 400× magnification analysis with an Axiolab microscope equipped with an Achroplan objective (Carl Zeiss Optics, Germany). Pictures were taken with a Mitsubishi color Video Camera CCD-100 (Mitsubishi Electric, Japan).

3. Results

As expected, the high-pressure homogenizer produces the smallest droplets of oil dispersed in water. The median size of the droplets of the emulsions expressed as a percentage of the volume is reported in Table 1.

All the emulsions exhibit a tendency to creaming, except emulsion D10. The creaming kinetics calcu-

Table 1
Results of the size analysis, zeta potential measurements, stability under centrifugation, measurements of the viscosity and pH values for emulsions manufactured by hand (H), with a Silverson mixer (S), or a MiniDeBEE high-pressure homogenizer (D)

	Emulsion H	Emulsion S	Emulsion D10	Emulsion D5	Emulsion D2
Median size ± S.D. (μm)	68.876 ± 33.015	7.065 ± 1.016	0.331 ± 0.061	0.728 ± 0.024	2.312 ± 0.016
Zeta potential (peak value ± S.D. ± peak width ± S.D.) (mV)	−47.1 ± 3.0 ± 4.4 ± 1.1	−50.2 ± 2.4 ± 2.0 ± 0.4	−43.6 ± 2.3 ± 1.8 ± 0.2	−43.1 ± 0.1 ± 1.6 ± 0.0	−48.0 ± 4.1 ± 1.6 ± 0.0
Creaming volume (%) after centrifugation	47.00 ± 5.66	40.00 ± 1.41	100.00 ± 0.00	98.00 ± 1.41	74.00 ± 2.83
Viscosity (mPa s)	122 ± 17	20.4 ± 2.5	22.2 ± 1.7	12.5 ± 2.0	9.3 ± 1.7
pH	6.93 ± 0.04	6.79 ± 0.03	7.00 ± 0.23	7.00 ± 0.29	6.78 ± 0.03

Emulsions D10, D5, and D2 contain respectively 10, 5, and 2% of surfactants.

lated from the backscattering differential profiles obtained over 24 h are shown in Fig. 1.

All the zeta potential values obtained are strongly negative. They are expressed (Table 1) as the mean value of the measurements and the width of the response peak.

Stability is reflected by the value of the creaming percentage previously used to assess emulsion formulations (Krishna et al., 1998). The value of the creaming was computed for each emulsion using the following equation:

$$C = 100\frac{(V_t - V_s)}{V_t}$$

where C is the creaming volume percentage, V_t (ml) is the total volume of the sample, and V_s (ml) is the volume of the lower phase layer. According to this equation, it is worth noticing that a larger value of the

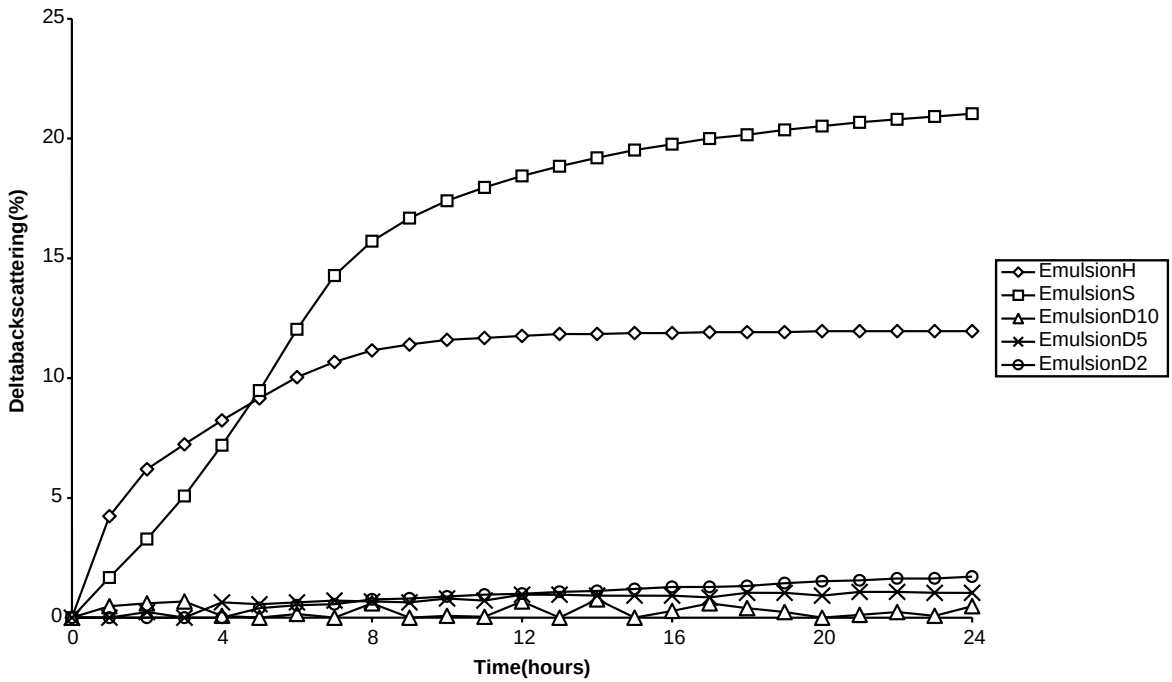


Fig. 1. Creaming kinetics of the emulsions analyzed with the TurbiScan®.

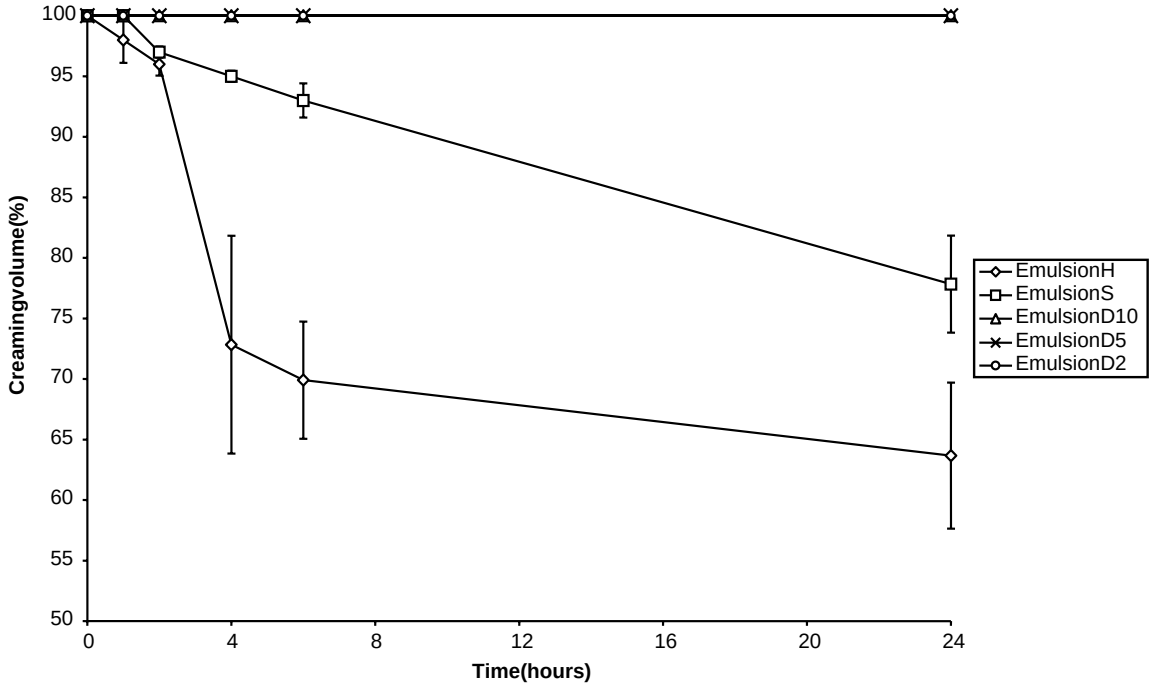
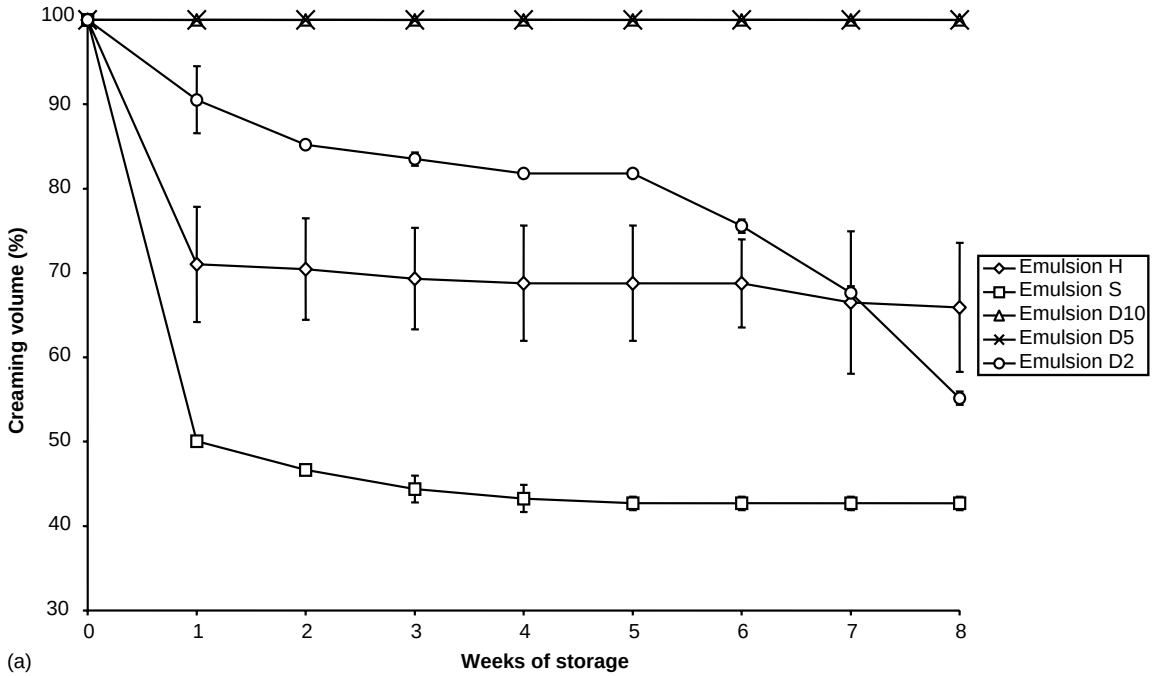
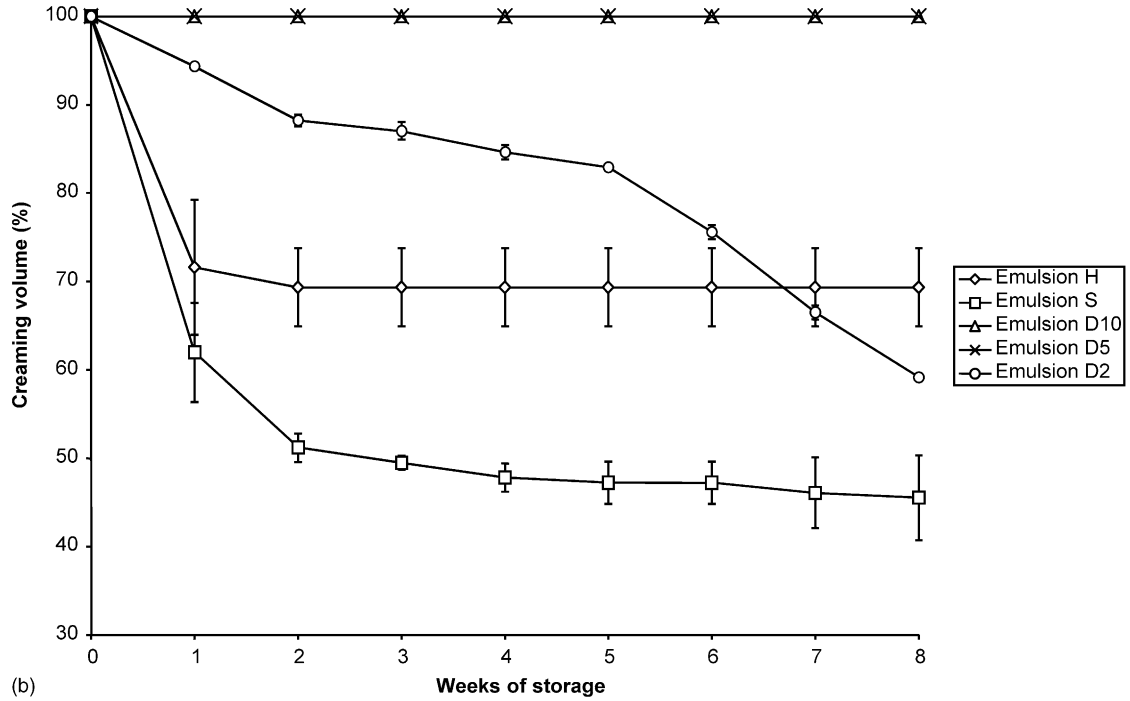


Fig. 2. Creaming volume percentage macroscopically determined over 24 h (at room temperature).

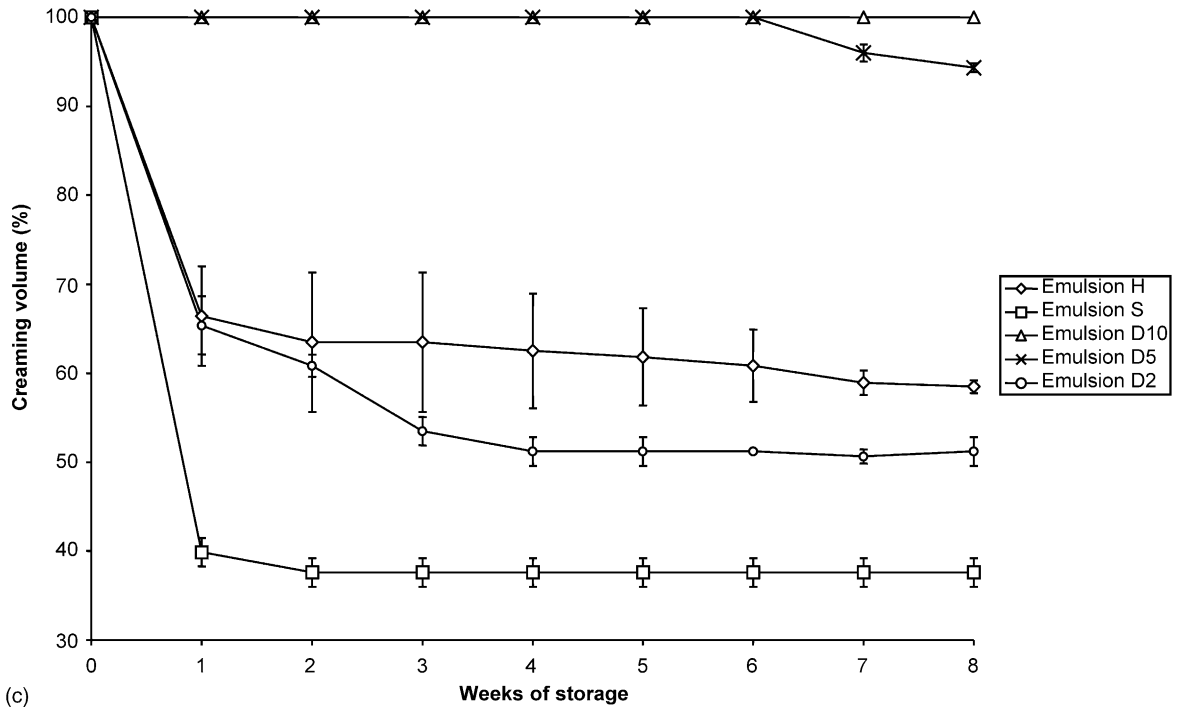


(a)

Fig. 3. (a) Stability under storage at room temperature. (b) Stability under storage at 4 °C. (c) Stability under storage at 45 °C.



(b)



(c)

Fig. 3. (*Continued*).

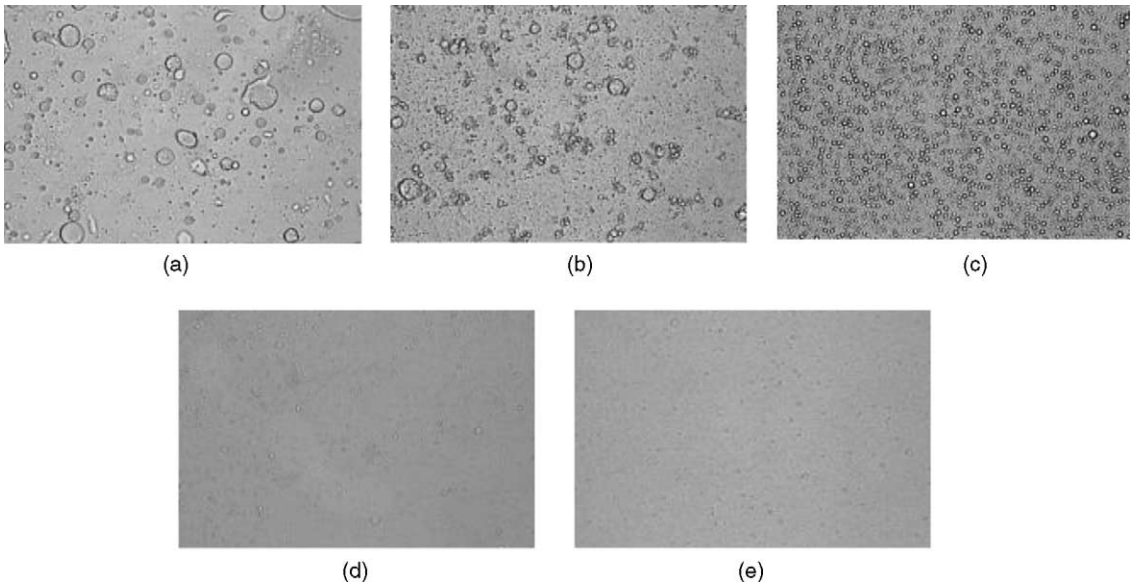


Fig. 4. Microscopic view of emulsion H (a), emulsion S (b), emulsion D2 (c), emulsion D5 (d), and emulsion D10 (e) (400× magnification).

parameter *C* is an indication of a more stable emulsion. The creaming volume percentage as a function of time is shown in Fig. 2. No change can be macroscopically observed in emulsions D10, D5, and D2 over the 24 h, therefore, the creaming volume percentage is 100.

Creaming volume percentages were compiled for each storage condition (room temperature, 4 and 45 °C) over 8 weeks; they are given in Fig. 3a–c.

No excessive heating was observed after centrifugation. All the emulsions creamed under those conditions except emulsion D10. The creaming volume percentages are given in Table 1. The viscosity data and the pH values of the emulsions are also given in Table 1.

All the emulsions underwent freeze/thaw cycles with variable damage but not a single one could withstand five cycles. Emulsions H and S creamed after one cycle and rupture occurred after three cycles. A tendency to rupture (visible droplets of oil dispersed in the emulsion) was observed in emulsion D10 after five cycles. In emulsion D5, creaming occurred after two cycles and rupture at cycle 3. Emulsion D2 creamed after one cycle and rupture was observed after two cycles.

The microscopic views of the emulsions are shown in Fig. 4.

4. Discussion and conclusion

The aim of this work was first to develop an analysis protocol for oil-in-water emulsions and then to assess its applicability in current practice. Owing to the availability of reliable instruments, former recommended tests might be omitted and replaced by a quicker and more efficient analysis. Many emulsified systems are often characterized by size analysis and zeta potential measurements (Kong et al., 2001). A lack of information available for in-time stability and aging is often noticed.

As expected, the median size of the emulsions considerably varies depending on the manufacturing process and the surfactant concentrations. Emulsions prepared with the high-pressure homogenizer and containing 10 and 5% of surfactants are submicron (median diameter (mean ± S.D.): 331 ± 61 nm and 728 ± 24 nm, respectively). When the surfactant concentration is decreased, the droplet size logically increases, as demonstrated with emulsion D2. Hand-made emulsions exhibit the largest median size with a poor reproducibility compared to the other manufacturing processes. In this case, the measurement of the zeta potential does not give any relevant information. Absolute differences in zeta potential

values should be at least 10 mV to allow prediction of distinct stability. Nevertheless, we do not observe any relevant difference despite the obvious distinction between the global stability of the systems. Moreover, the zeta potential values do no fit with the visual stability: the most visually stable emulsions (D10 and D5) exhibit the lowest zeta potential absolute values. This may be explained by the fact that size reduction is important. This would in turn result in a modification of the charge distribution as a function of surface area. On the other hand, the optical characterization of the stability by means of the TurbiScan® gives interesting information about the global stability of the emulsions. By this way, we can distinguish the behavior over 24 h of the three emulsions manufactured with the high-pressure homogenizer and predict the relative instability of emulsions D5 and D2. This sole experiment, confirmed by the stability under centrifugation, allowed us to assess that only emulsion D10 is stable. The intrinsic stability analyzed over 24 h as described is meaningless with regard to this previous powerful automatic analysis. The disadvantage of monitoring the stability under storage is the lengthy period required before any distinction can be made in the case of emulsions with small stability differences, such as emulsions D10 and D5. Indeed, creaming was observed only after 7 weeks at 45 °C. However, if the developed formulation is intended to be stored or exposed at high temperatures (storage in tropical countries, transport, etc.), this test is critical. A whole analysis should be carried out again after storage of the emulsion in the representative conditions (temperature, length of time). Storage at 4 °C may be interesting in the case of formulations containing active substances likely to crystallize. The stability study under centrifugation at 15,000 m/s^2 gives an excellent information about the stability of the system compared to the creaming volume percentages. It allowed distinction between emulsions D10 and D5 even though the 8 weeks storage period at room temperature did not. This test, easy to perform, seems as powerful as the TurbiScan® to assess the stability of emulsified systems although the TurbiScan® gives also information about coalescence and size modifications. Measurements of viscosity and pH are useful data especially in quality assurance. Their variation may explain some unexpected differences observed between emulsions. Actually, emulsions H should have been less stable than emulsions S but emulsions H were more viscous and when creaming occurred, they were thicker and the phenomenon was therefore slowed down by virtue of Stoke's law. The freeze/thaw cycle test is not powerful and may only be interesting to distinguish two formulations that are similar with regard to all other stability characteristics. However, as for storage at 45 °C, if the developed formulation is intended to be frozen, this test is critical. A whole analysis should be carried out again after thawing of the emulsion. Microscopic analysis at 400× magnification is superfluous since a particle size analysis is performed. It gives no useful information in the case of submicron emulsion. However, in the case of emulsions containing active substances, this test may reveal crystallization or other phenomena likely to disrupt the stability of the system.

In conclusion, to efficiently assess the stability of an emulsion and to characterize it in formulation design, we suggest performing a size analysis, a zeta potential measurement, and a TurbiScan® analysis, as well as a test of the stability under centrifugation and freeze/thaw cycle investigations. If the emulsion contains an active substance, stability under storage at 4 °C might be relevant. Quality control should also benefit from measurements of viscosity and pH.

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