THE INFLUENCE OF COLLOIDS ON THE PARTICLE SIZE AND STABILITY OF O/W EMULSIONS IN THE PRESENCE OF NONIONIC SURFACTANTS

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Abstract
The effect of the presence of colloids on formation, stability and particle size of n-undecane in water nanoemulsions prepared by the Emulsion Inversion Point (EIP) method using nonionic surfactants (Igepals) was studied. The influence of oil-to-water ratio, the HLB value and concentration of the surfactant on their physico-chemical characteristics was also investigated. The stability of emulsions was assessed from changes in the particle size and the particle size distribution as well as through visual assessment of their appearance. The results obtained show that the anticipated positive effect of the colloids on emulsion stability was not confirmed. Phase separation of these systems usually occurred soon after preparation or during a few days of their storage. The particle size of emulsions prepared in the presence of colloids was mostly bigger in comparison with emulsions where colloids were absent. Nevertheless, nanoemulsions with the particle size smaller than 200 nm were also obtained. The best long-term stability was observed in emulsions prepared at the HLB value of 11.5, oil content of 20 and 30 wt. % stored at 4 °C.

Keywords: Emulsion, Gelatin, Hydrophile-lipophile balance, Stability, Surfactant

1. INTRODUCTION
Many recent studies have focused on the preparation and stability of nanoemulsions [1,2,3] due to their outstanding properties and growing number of promising applications. As given in theory, nanoemulsions are transparent or translucent in appearance [4] because of their characteristic size, ranging typically from 20 to 200 nm [5,6], or 20 to 500 nm, as given in publications [7,8,9]. This feature together with a low viscosity, high kinetic stability against creaming or sedimentation and large interfacial area make them attractive in a variety of the practical fields. [5,10,11] However, emulsion utilization is limited by their non-straightforward preparation procedure. [11] Therefore, a good knowledge of their production processes is necessary in order to achieve the desired applications and obtain systems with long-time stability. [12]

Gelatin is well-known to be an effective agent in stabilizing disperse systems; for example, emulsions. [13] The possibility of using gelatin as emulsion stabilizer has generated wide interest in various industrial applications such as food, pharmaceutical and textile industries. [14] Gelatin is a vitreous, brittle solid and is faintly yellow in color. It is nearly tasteless and odorless. When immersed in cold water, it hydrates into separate swollen particles. After heating the swollen particles pass into solution. The important properties of these solutions are functions of pH, method of production, thermal history and concentration. [15]

Proteins, including gelatin, are surface active agents. They can be absorbed on O/W interfaces and penetrate by their hydrophobic parts into the oil phase. Besides to gelatin, surfactants play an important role in reducing of interfacial tension. [14] In particular, gelatin-surfactant interaction is considered an important parameter affecting the stability of O/W emulsions. In studies dealing with the interactions of gelatin with the surfactants it was found that due to this interaction, surface activity of
gelatin is increased. [16,17] Other important gelatin characteristics are the molecular-weight and molecular-weight distribution which also affect its properties, such as viscosity and gel strength. [14] Several studies have been reported on the stabilization of O/W emulsions by gelatin and surfactants. [18,19,20] In literature, mainly gelatin interactions with anionic surfactants, especially sodium dodecyl sulphate have been described. [20] Conversely, information about gelatin behavior in the presence of nonionic surfactants is very rare. [16]

In this study the stability of n-undecane in water emulsions and analogous emulsions prepared under the same conditions but with the addition of different colloids, such as gelatin or collagen hydrolyzate were compared. For production, nonionic surfactants and Emulsion Inversion Point method were applied. Moreover, the influence of colloid presence, HLB value and O/W ratio on particle size, particle size distribution and stability of emulsions was investigated. For this purpose, photon correlation spectroscopy and visual assessment were performed.

2. EXPERIMENTAL

2.1 Materials

The n-undecane (Sigma Aldrich) and purified water were used as oil and water phases, respectively. Two grades of nonionic Igepal (polyoxyethylene nonylphenylether, NPE) supplied by Sigma Aldrich were used as surfactants. Specification of surfactants is following: Igepal 520 (HLB 10) and Igepal 720 (HLB 14.2) with nonyl phenol ethoxylate chain length of 5 (NPE5) and 12 (NPE12). Gelatin B (molecular weight 3x10^5 g.mol^-1) with bloom value 150 (PB Gelatins), gelatin B (molecular weight 4x10^5 g.mol^-1) with bloom value 220 (SKW Biosystem) and collagen hydrolyzate Hycol with molecular weight 1.8 x10^4 g.mol^-1 (Tanex s.r.o) were used throughout the study.

2.2 Preparation of nanoemulsions

Nanoemulsions were prepared by the Emulsion Inversion Point technique (EIP). The pairs of nonionic surfactants were used to prepare mixtures with a required range of HLB values. Surfactants with a low-HLB value and a high-HLB value were pre-dissolved in the appropriate ratios in the oil and water phase, respectively. Water phase was in addition added respective colloid in the concentration of 1 wt%.

Emulsifications were carried out using an RZR 2020 stirrer (Heidolph). Using a burette, water phase containing surfactant (Igepal 720) and colloid was added drop wise at the rate of 1ml/min to the oil phase containing the second surfactant (Igepal 520). Emulsifications were carried out at room temperature (25 °C) and the stirring rate was controlled at 1050 rpm. Oil to water (O/W) ratios of 5/95, 10/90, 20/80, 30/70 and overall concentration of both Igepals of 3 wt% were used. The HLB values of 10.5, 11 and 11.5 were applied.

For comparison, emulsions prepared by the same method and under the same conditions but without addition of colloids were studied and compared with those mentioned above.

2.3 Measurement of particle size distribution

The particle size and particle size distribution were determined by photon correlation spectroscopy (PCS). Zetasizer Nano ZS (Malvern Instruments, UK) was used for this purpose. The measurements were carried out at a scattering angle of 173 ° and at the constant temperature of 25 °C.

2.4 Emulsion stability

Emulsion stability was assessed visually as well as by measuring the particle sizes as a function of time by PCS. Emulsions were stored at two different temperatures (4 °C and 25 °C) and observed daily at regular time intervals.
3. RESULTS AND DISCUSSION

3.1 Particle size

The effects of presence of different colloids, HLB value and O/W ratio on emulsion particle sizes are summarized in Fig. 1. The obtained results showed that the nature of colloid is an important parameter affecting the particle size of all studied systems. Fig. 1 a) illustrates the comparison of particle size of emulsions with O/W ratio of 5/95, produced with and without addition of colloids, determined immediately after their preparation. As it can be seen, the smallest particle size (60 nm) was observed in emulsion produced with O/W ratio of 5/95, the HLB value of 11 and in the presence of gelatin B220. In this case, the particle size was even smaller than the size of emulsions where colloids were absent. On the contrary, the largest particle sizes were determined in emulsions (O/W ratio 5/95) with the HLB value of 10.5 and at the presence of gelatin B220 or Hycol. In case of HLB value 11.5, the particle sizes of all tested systems were very similar, ranging from 150 to 180 nm. It is also obvious from the figure that emulsions with the particle size smaller than 200 nm, that implies nanoemulsions, were obtained at HLB values of 11 and 11.5, regardless of the type of used colloid.

![Fig. 1](image-url)  
Fig. 1. Particle size of emulsions with a) O/W ratio of 5/95 b) O/W ratio of 20/80 determined immediately after the preparation.

Similar results, in terms of the effect of colloid presence on the particle size of emulsions, were observed in samples with O/W ratios of 10/90 and 20/80. Both emulsion systems exhibited similar behavior after addition of colloids. As example, the relationship between the HLB value and the particle size of emulsions with O/W ratio of 20/80 is depicted in Fig. 1 b). From the figure it is clear that the addition of colloids affected particle sizes of emulsions prepared at HLB 11.5 only insignificantly, but caused an increase in particle sizes at the HLB values of 10.5 and 11 compared to systems prepared without colloids. This trend could be related to the influence of the colloid molecular weight. At HLB value of 11, it seems that emulsion droplets virtually decreased with decreasing molecular weight of colloid (gelatin B220 > gelatin B150 > Hycol). Nevertheless, true nanoemulsions (< 200 nm) were also found within the systems with oil to water (O/W) ratios of 10/90 and 20/80 and the HLB value of 10.5 in the presence of Hycol and gelatin B220. Exception here were emulsions prepared with gelatin B150 showing particles bigger than 200 nm. Corresponding findings were detected in emulsions with O/W ratio of 30/70 and the HLB value of 10.5. Also in this case, the emulsion droplets reduced with decreasing molecular weight of colloid (data not
presented). Moreover, droplets of emulsions with O/W 30/70 prepared with colloids were all relatively big in size and lay prevailingly in the size range of 470 – 760 nm, regardless of the HLB used. In addition to observed effect of HLB, the results also showed the influence of O/W ratio on particle size of emulsions. It was found that an increasing content of the oil phase from 5 to 30 wt. % resulted in growth of emulsion droplets.

3.2 Emulsion stability

Emulsion stability was evaluated by visual assessment as well as by measuring changes in their particle sizes with time of storage at the different conditions. It is obvious from the results that emulsion stability is affected by their composition, especially, by the presence of different types of colloids and by the storage temperature. Emulsions were stored at 4 °C and 25 °C which both are favorable storage temperatures. Visual observation of samples was performed daily and the height of separated phase in cm was observed and measured as a function of time. From the results it is clear, that for the preservation of stable emulsions the storage temperature of 25°C was evaluated as worse than the temperature of 4 °C. It follows, that the phase separation in emulsions stored at 25 °C occurred more often and sooner than in emulsions stored at 4 °C, irrespective of their composition. For example, in emulsion with oil-in-water ratio 5/95, HLB value of 10.5 prepared with absence of colloids, phase separation occurred after three days of storage at 25 °C. Conversely, the same emulsion stored at 4 °C was stable more than 40 days with a negligible creaming. Moreover, the influence of O/W ratio on emulsions stability was also significant. It was found that with an increasing content of oil phase, all studied emulsions (irrespective of their composition) became more stable over time, even though creaming was occasionally observed. Another variable notably affecting emulsion stability is the HLB value. The trend demonstrating improvement of stability with an increasing HLB value was detected at all studied systems, regardless of the composition. It follows, that the most stable emulsions were prepared at the HLB value of 11.5.

According to the theoretical assumption, the gelatin-surfactant interaction may have a positive impact and improve stability of emulsions. [16] For example, the formation of double W/O/W emulsions with improved stability due to the possible formation of interfacial complex between gelatin and sorbitan monooleate (Tween 80) have been published in [21]. The stability was assessed microscopically and showed no significant changes in droplet sizes of double emulsions within 2 weeks. However, this hypothesis was not fully confirmed by the present data. The stability of emulsions prepared in the presence of colloids was worse compared with emulsions prepared only with the mixture of nonionic surfactants. In the case of colloids usage, phase separation has already occurred soon after the preparation or during the first days of storage at both temperatures (25 °C and 4 °C). This effect was especially notable in emulsions prepared at HLB values of 10.5 and 11 and the lower oil content (O/W 5/95 and 10/90). On the contrary, the emulsions produced under the same conditions but without colloids were stable for a long time, especially those stored at 4 °C.

Simultaneously with the visual observation, the stability of emulsions was also evaluated from the measuring of changes in their particle sizes with time by PCS. Fig. 2 describes intensity based particle size distributions of samples containing gelatin B220, with O/W ratio 20/80, HLB value of 11.5 stored at 25 °C. From the figure it is clearly visible a gradual increase in particle size as well as the shift and broadening of entire particle size distribution during sample storage. Solid line illustrates a monomodal particle size distribution with z-average diameter of 520 nm obtained immediately after the preparation. After three-days of storage at 25 °C, the particle size rose to 900 nm (dashed line) and after fourteen-days of storage the emulsion z-average increased to 1050 nm (dotted line). As it can be also seen, the dotted line is approaching the measuring limit of the PCS instrument (6 μm) which indicated the presence another population of particles with the size in the range of micrometers.
4. CONCLUSION

In this work the Emulsions inversion point (EIP) technique was used to prepare n-undecane/surfactants/water emulsions in the presence of colloids with different molecular weight. The emulsion droplet size and stability in the presence of colloids were compared with those of emulsions containing only nonionic surfactants. Moreover, the influence of different types of colloids, the HLB value and O/W ratio on the emulsion particle size and stability was also investigated.

The overall results showed that the nature of colloid is one of the main parameters which influences the particle size and stability of emulsions. Generally, the systems prepared in the presence of colloids were bigger in their particle sizes compared to systems without colloids. However, also within these samples, the true nanoemulsion were found. For example, nanoemulsions with the particle size smaller than 200 nm were formed in the presence of all studied colloids (gelatin B220, B150, Hycol) in the case of O/W ratio of 5/95 and the HLB values of 11, 11.5. Interestingly, in emulsions without colloids the smallest particle size was found at the HLB value of 10.5 in all studied O/W ratios. In this optimal HLB, the particle size of emulsions was in the range of 50 – 200 nm and then increased with the HLB value higher (11 and 11.5) than the optimal one.

The stability of emulsions was mostly affected by the storage temperature, the presence of colloid and the HLB value. Anticipated positive effect of the presence of colloid on emulsion stability was not confirmed. Phase separation of colloid-containing emulsions occurred soon after the preparation or during the first days of their storage, especially at the lower content of oil phase (O/W 5/95 and 10/90) and at the HLB values of 10.5 and 11. On the whole, the best long-term stability was observed in emulsions produced with absence of colloids, at the HLB value of 11.5 and at the higher oil content (O/W 20/80 and 30/70) stored at 4 °C.

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