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Study of the formation of micro and nano-droplets containing immiscible solutions

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ABSTRACT

There is currently significant interest in the multiple resistance to treatment using drugs (MDR), developed by bacteria and malignant tumors. One of the alternatives to the existing medicines and treatment procedures in fighting MDR is strengthening the effects of cytostatics by improving their delivery methods. Such a method is represented by the generation, transport and use of micro-/nano-droplets which contain medicines. This approach can reduce the medicines consumption by generating micro-droplets which contain drugs incorporated in solvents substances; the micro-/nano-droplets can favour a faster delivery to the targets and a higher drug concentration in them. This paper reports first, results concerning the generation of single micro-droplets containing an inner core (medicine solution in water) and a thin layer of oily liquid covering it. This generation is made one by one and it is, at the moment, not conceived as a method for mass production, or in other words for high population of such droplets. We have generated and measured stratified micro-droplets, each of them containing a solution of Vancomycin in ultrapure water as a core and a surrounding layer of Vitamin A in sunflower oil; the micro-droplets generation was made using a double capillary system. Secondly, micro-/nano-droplets were produced by mixing two immiscible solutions in particular conditions (high rotating speed and/or high pressure difference). For this we have studied the generation of emulsions of Vitamin A diluted in sunflower oil and a solution of Tween 80 surfactant in distilled water. The concentration of surfactant in water was, typically, 4×10^{-5} M. We have studied the dependence of the droplets dimensions in emulsion on the mixing rotation speed, agitation time and components ratio. The droplets diameters were measured using a light scattering method. It is found that at high enough energy input (high rotation speed, large pressure drop) and relatively small oil/water ratio, droplets diameters smaller than 100 nm were obtained.

1. Introduction

Interest in nano/submicron droplets dimensions in emulsions recently increased in the pharmaceutical, cosmetic and food domains, in parallel with the development of better performing emulsification technologies. Droplets with diameters lower than 1 μm can be used as transport vectors of medicines in parenteral, oral, ophthalmic or transdermal delivery systems which are related to therapeutic applications. The systems using droplets allow substances poorly soluble in water, but oil-soluble, such as vitamins or drugs to be incorporated in a lipophilic phase, so that the increase of their local bioavailability takes place; this also stabilizes components sensitive to enzymatic degradation, allows a slower, controlled release of components to the targets over a prolonged period of time and reduces the side effects of drugs. Based on small particles use in systemic treatments, one predicts that micro-/nano-emulsions uptake improves efficiency of lipophilic substances. Emulsions are the dispersions of an immiscible or partially miscible liquid (dispersed phase) in another liquid (continuous phase). These liquids are immiscible or are mutually only slightly soluble. The dispersed phase is present in the form of droplets in the continuous phase. Usually, in order to stabilize the dispersed phase against coalescence, the presence of a surfactant is essential as the droplets are thermodynamically metastable. An emulsion is characterized by the mean size and the size distribution of the droplets; these characteristics can be controlled by a proper choice of the dispersing apparatus and the process conditions [1–3].

Compound droplets of millimeter or sub-millimeter diameters have been reported to be related to a wide and disperse range of applications. For instance, droplets (~2.7 mm) containing emulsions of oil in water sprayed on a moving steel strip or on a rotating roll have been used for cooling the strip/roll by the water whereas efficient lubrication of the sprayed areas was achieved by the oil [4]. Stratified capsules, including immiscible liquids, were produced

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by electrohydrodynamic forces using coaxial electrified jets in the micrometer and submicrometer ranges [5]. Capsules of 0.150 μm diameter corresponded to a sphere of water of 0.1 μm in the core surrounded by a 0.025 μm thick layer of olive oil. Compound drops composed of a core water sphere encased to a diesel oil shell of diameters between 740 μm and 1 mm were generated by a double capillary coaxial system, as reported recently [6]. One of the most recent reports deals with a double emulsion (oil in water in oil) obtained from a compound drop of diameter of about 2 mm permanently bouncing onto a vibrated liquid surface [7].

Micro-/nano-droplets may be produced by mixing two immiscible solutions at high rotating speed and/or high pressure difference. It is generally accepted that the emulsification is based on two opposite processes: drop breakup resulting in the production of several smaller droplets from a larger drop, and droplet–droplet coalescence leading to the formation of a larger drop from two smaller ones [8,9]. Generally, the evolution of the drop-size distribution during emulsification is determined by the competition between these two processes. At high surfactant concentrations, the contribution of the droplet–droplet coalescence is negligible so that the process of drop breakup determines the evolution of the drop-size distribution in the formed emulsion [9,10]. After a long enough emulsification time, a "steady-state" is reached, which is characterized by a relatively slow change of the drop-size distribution in the formed emulsions [11,12].

This paper reports first, results concerning the generation of single micro-droplets containing an inner core (medicine solution in water) and a thin layer of oily layer surrounding it. These droplets were produced by using a double dosing system (coaxial capillaries). These experiments allowed assessment of the adsorption phenomena between the liquid layers of the examined droplets. The above evidence combined with information from literature on how water/oil viscosity, interfacial tension, and rate of energy dissipation affects the maximum droplet diameter, e.g., [13–22], have been used to select emulsification parameters.

Next, this work reports on the massive production of micro-/nano-droplets realized by mixing relatively large quantities of two immiscible solutions at particular conditions (high rotating speed and/or high pressure difference). We have generated emulsions of sunflower oil droplets containing Vitamin A dispersed in a solution of a surfactant in distilled water. Using a batch stirred tank system we examined the dependence of oil droplets dimensions on the rotation speed of the impeller, and components ratio. The diameter of stable droplets is measured after a sufficiently long period of emulsification, when the steady-state drop size distribution is reached. The droplet diameters were measured by light scattering. The experiments were performed using a surfactant concentration above the CMC of the surfactant in water in order to avoid droplet–droplet coalescence during emulsification.

The scope of this work is dual: first to check the interfacial stability of individual compound droplets related to drug delivery systems and second to check whether intense bulk mixing devices can create high populations of nano-sized droplets with a narrow size distribution. Whether it is possible to use intense bulk mixing of two immiscible liquids in order to create high populations of compound nano-droplets will be the topic of a subsequent study. This is of paramount significance since conventional devices (e.g., coaxial capillaries) for making individual compound droplets are not suitable to create nano-sized droplets.

2. Materials and methods

The first part of the work was devoted to the measurement of the dynamic interfacial tension of Vitamin A solutions in sunflower oil and to the generation of a droplet of Vancomycin in ultrapure water covered with a layer of Vitamin A. These experiments were performed using the Drop Profile Analysis Tensiometer (PAT1, SINTERFACE) which generates pendant droplets and allows surface or interfacial tension and viscoelasticity (oscillation) measurements over a period of several hours [23,24]. For the generation and measurement of interfacial tension of the obtained compound pendant droplets we have employed a specific double dosing module of PAT1 furnished with a double capillary with which we have generated first the exterior droplet and then the core through the inner capillary. The total volume of the droplet (10 μL) was kept constant by a built-in control loop based on streaming video images analyzed by a dedicated software.

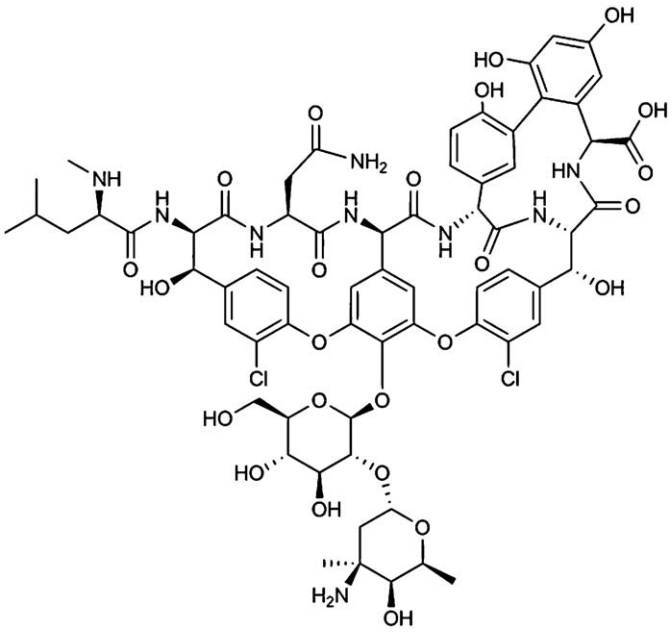

Fig. 1. The chemical structure of Vancomycin.

Vancomycin is a glycopeptide antibiotic used in the prophylaxis and treatment of infections caused by Gram-positive bacteria; the chemical structure of Vancomycin is given in Fig. 1. The experiment was conducted to check whether the adsorption of the Vancomycin at the water/oil interface is strong enough to modify the properties of a layered droplet [23].

The Vancomycin solutions were made in high purity grade water and the concentration range used was 10^{-4} M to 10^{-5} M. The oily Vitamin A (retinol diluted in sunflower oil) used for these measurements is a commercial product found in pharmacies; the chemical formula/structure of it is given in Fig. 2 [23].

In the second part, the micro-/nano-droplets were produced by mixing two immiscible solutions at particular conditions (high rotating speed and/or high pressure difference). The measurements were meant for studying the droplet dimensions dependence on different conditions. First, we have studied the variation of droplets diameter as a function of rotating speed for the same oil/water volume ratio. This allowed us to choose the proper rotating speed for further measurements. Afterwards, in order to study the dependence of droplets dimensions on the oil/water ratio, we kept the

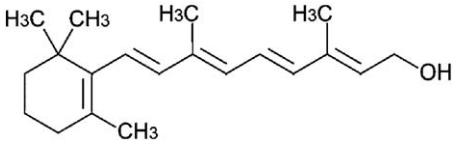

Fig. 2. The chemical structure of Vitamin A.

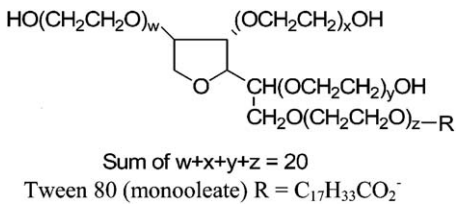

Fig. 3. The chemical structure of Tween 80.

rotating speed constant and we varied, respectively, the ratio of the two components (from 10/90% to 50/50% oil/water). The dispersed phase droplet diameters were measured using a Malvern light scattering instrument type Mastersizer Hydro 2000 M.

For these measurements we have used as emulsifiers the nonionic surfactant Polysorbate 80 (commercially known as Tween 80) derived from polyethoxylated sorbitan and oleic acid; the Tween 80 structural formula is shown in Fig. 3 [25]. Tween 80 is a nonionic surfactant and according to literature [25] its partition coefficients are low for the less polar solvents regardless of conditions. Therefore, in the present experiments the surfactant is expected to be chiefly distributed in the water phase with only a negligible amount dissolved in the oily phase. The working conditions for all the reported experiments were: 20 °C room temperature and 101.3 kPa (normal atmospheric pressure).

As dispersed phase, we used Vitamin A diluted in sunflower oil with a viscosity η_D = 48.98 mPa s at 298 K [26]. Only one surfactant concentration was employed since our objective was to examine other than the surfactant important emulsification parameters (type of mixer, rotation speed, oil/water ratio). For this, Tween 80 (FLUKA) was dissolved at a relatively high concentration of 4×10^{-5} M (50 ppm) in distilled water. This concentration is above the CMC of Tween 80 in water at ambient conditions (0.012 mM [27]) and was employed in order to suppress droplet–droplet coalescence during emulsification.

Mixing of the two immiscible liquids (total volume of the mixture: 270 ml) was performed in a stirred tank (H = 19.5 cm, Inner diameter = 7 cm) equipped with a variable speed Rushton turbine (d = 4.6 cm) placed along the central axis of the vessel. The impeller was placed at 1.7 cm above the bottom of the vessel. Immediately after switching-off the impeller, a 0.5 ml sample was withdrawn from the vessel using a 5 mm inner diameter tube (wide enough to prevent droplets jamming) and was added to a 20% w/v Tween 80 solution to prevent droplets coalescence [28].

In some runs, emulsions were also produced by high speed homogenizers (Ultra Turax T18 operated at 10,000 rpm and Ultra Turax T25 operated at 20,000 rpm). Finally, in some runs we have also employed an APV high pressure homogenizer with an exhaust valve operating at ΔP = 800 bar.

3. Results

Fig. 4 presents the values of surface tension (ST) at the oil/gas interface for different types of compound pendant droplets (the concentration of Vitamin A in sunflower oil is $\sim 8 \times 10^{-2}$ M), respectively: a drop of Vancomycin solution in water surrounded by a layer of Vitamin A; a drop containing a water inner core surrounded by a layer of Vitamin A; a simple drop containing only Vitamin A. Apparently, the value of surface tension is constant with time which indicates that there are no adsorption phenomena at the water/oil interface (in the interior of the examined droplet).

The total volume of the droplet (water solution of Vancomycin and oily Vitamin A) was 10 μl and the droplet total diameter was 2.66 mm; the water solution of Vancomycin in the droplet's core was 2 μl and the respective core diameter was 1.56 mm.

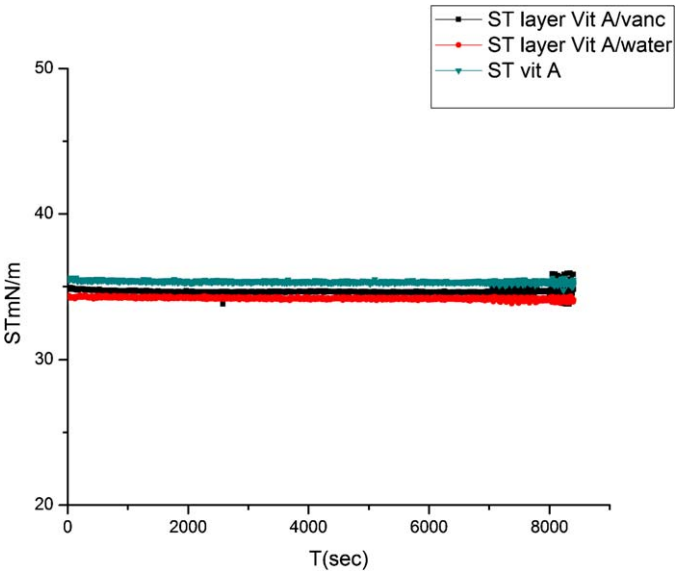

Fig. 4. Surface tension measurement for oil covered water droplets.

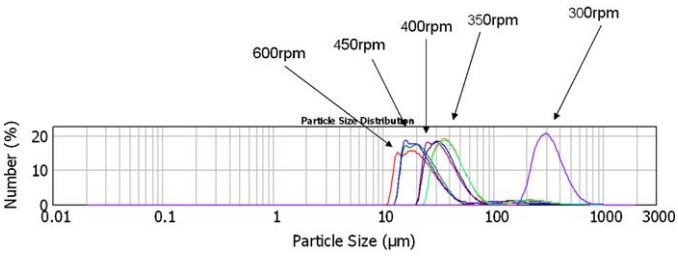

Fig. 5. Droplet dimensions as a function of rotation speed. Oil/water ratio 10/90%.

The following experiments aimed at identifying the conditions to generate droplets with nanometer dimensions in an emulsion. The dependence of droplets diameters on rotation speed, oil/water ratio and agitation time interval is studied. To do this we used a 10/90% volume ratio of oil/water and we measured the dimensions of droplets for different rotation speeds: 300 rpm, 350 rpm, 400 rpm, 450 rpm, and 600 rpm.

In Fig. 5 one can see the dependence of the produced droplets dimensions on rotation speed; by increasing the speed, droplets with smaller dimensions are obtained. For each experiment two samples were measured in order to check for reproducibility.

Next, the dependence of droplets dimensions on the oil/water volume ratio was examined. For these tests, a constant rotation speed of 450 rpm was chosen while varying the oil/water volume ratio. As it can be seen in Fig. 6, the droplets became smaller when the oil/water ratio decreased.

The above measurements have shown that in order to obtain droplets with smaller dimensions one should increase the rotation

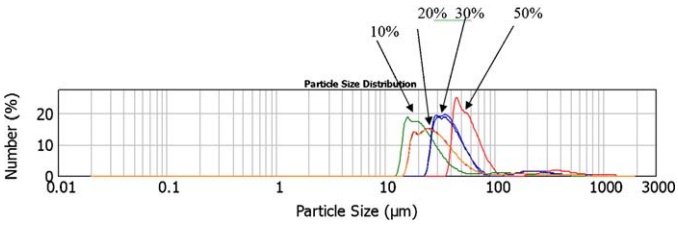

Fig. 6. Droplet dimensions as a function of the oil/water volume ratio. Rotation speed: 450 rpm.

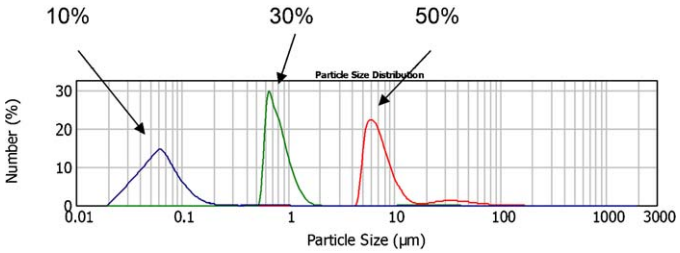

Fig. 7. Droplets dimensions obtained at 10,000 rpm for three different oil/water ratios.

speed or/and decrease the oil/water volume ratio. Because the maximum speed without sucking air (due to excessive vortexing) in the employed stirred tank is 600 rpm, a high speed homogenizer—Ultra Turax is used next. It is recognized that apart from the higher rotation speed Ultra Turax differs from the employed stirred tank also in the shape and size of the mixing section, geometry of rotor and total volume of working liquids. However, among all these parameters the higher energy input provided by the higher rotation speed is expected to play a dominant role in creating smaller droplets.

First, we have used an Ultra Turax T18 homogenizer with three different oil/water ratios – 10%, 30%, 50% of oil – at a rotating speed of 10,000 rpm (Fig. 7).

Fig. 7 shows that the droplets are classified with respect to their sizes in three distinct groups: a first one obtained for a 10% oil/water ratio for which the diameters are below 100 nm, a second one obtained for 30% oil/water ratio for which the dimensions are grouped around 800 nm and a third one for the 50% oil/water ratio where the most frequent (modal) diameter is around 6 μm.

In Fig. 8 two graphs are shown for the case of 10% oil/water ratio. Fig. 8a displays the percentage distribution of droplets number as a function of their size which shows that the mean size of the obtained droplets is approximately 65 nm. Nevertheless, there is a second significant family of droplets with diameters larger than 65 nm as it is displayed in Fig. 8b where the number of droplets larger than 0.3 μm is represented. In this family of droplets the diameters are centred on 0.6 μm. It is possible that a relatively small number out of the remaining droplets may have diameters even larger than 1 μm. The measurements presented in Fig. 8 have been performed on two different samples in order to prove their reproducibility and the results obtained were identical which diminishes the possibility of measurement errors or artefacts induced by the light scattering measuring equipment. In Fig. 8b, the two curves correspond, respectively, to the two mentioned samples.

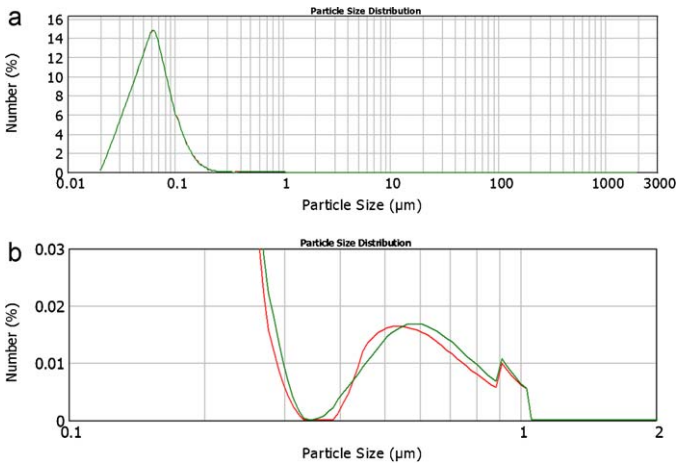

Fig. 8. Droplet size distributions for 10% oil/water ratio as a function of number of particles.

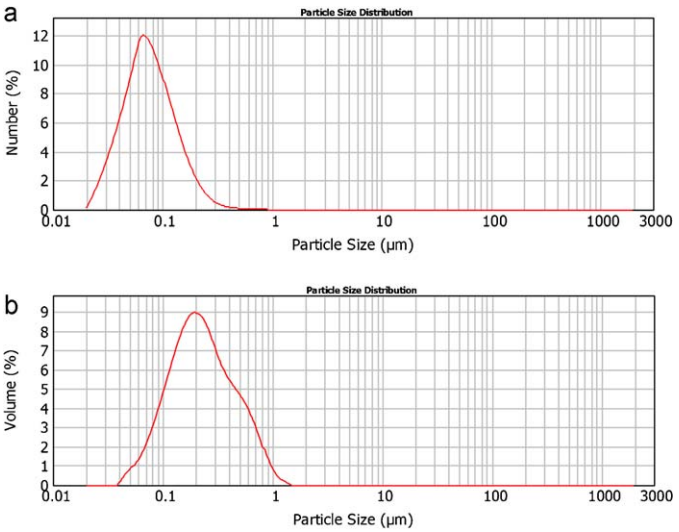

Fig. 9. Droplets dimension by number and by volume after using both homogenizers.

Fig. 8 shows that at the employed rotation speed although the nanometer droplets are dominant in number, still a non-negligible family of droplets is distributed at larger diameters; this is why we investigated further the conditions in which one may obtain only one family of droplets with nanometer sizes only.

To do this we have used an Ultra Turax T25 homogenizer operated at 20,000 and a high pressure homogenizer with an exhaust valve operated at $\Delta P = 800$ bar. The surfactant concentration in water, for this measurement only, was increased to 2.5×10^{-3} M (3200 ppm).

Fig. 9a shows that in these conditions nano-droplets with diameters centred at 65 nm are obtained; Fig. 9b confirms that there is only one family of nano-droplets, the same as the one shown in Fig. 9a. The emulsion characterized by the data shown in Fig. 9 was monitored with respect to time showing a very good time stability of, at least, two weeks (no visual phase separation).

4. Conclusions

We generated, first, compound pendant droplets consisting either of an inner core of water or of a solution of Vancomycin in water surrounded by a thin layer of Vitamin A diluted in sunflower oil. Each of these two kinds of compound droplets are stable for more than one hour. This is inferred from the measurement of surface tension at the interface between the external oily layer and air which remains constant for more than one hour after droplets production (Fig. 4) implying that there is no adsorption of material (particularly of Vancomycin) from the inner core liquid to the external oily layer. The dimensions/diameters of this kind of compound pendant droplets may be lowered by using capillaries of lower inner diameters, so that total cross section diameters of the compound droplets of the order of hundreds of micrometers could be obtained.

Second, a systematic set of experiments were conducted to generate nano-scale droplets of oil in water and to obtain emulsions by modifying parameters such as: impeller rotation speed and oil/water ratio. The present work focus on the performance of each employed technology (which is relatively simple) for the generation of nano-droplets leaving for the future the physical explanation of the underlying phenomena. Steady-state (long time) drop-size distributions were monitored showing that:

As expected, by increasing the rotation speed droplets with smaller dimensions are obtained; for the same rotation speed the

higher the oil/water ratio the bigger the droplets dimensions are. By using the high speed homogenizer with speeds around 10,000 rpm two families of droplets were obtained: small droplets less than 100 nm and large droplets in the range 800 nm–100 μm. By using an even higher speed (20,000 rpm) and a high pressure homogenizer only a single family of droplets is obtained with dimensions around 65 nm.

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