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In line monitoring of the preparation of water-in-oil-in-water (W/O/W) type multiple emulsions via dielectric spectroscopy

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ABSTRACT

Multiple emulsions offer various applications in a wide range of fields such as pharmaceutical, cosmetics and food technology. Two features are known to yield a great influence on multiple emulsion quality and utility as encapsulation efficiency and prolonged stability. To achieve a prolonged stability, the production of the emulsions has to be observed and controlled, preferably in line. In line measurements provide available parameters in a short time frame without the need for the sample to be removed from the process stream, thereby enabling continuous process control. In this study, information about the physical state of multiple emulsions obtained from dielectric spectroscopy (DS) is evaluated for this purpose. Results from dielectric measurements performed in line during the production cycle are compared to theoretically expected results and to well established off line measurements. Thus, a first step to include the production of multiple emulsions into the process analytical technology (PAT) guidelines of the Food and Drug Administration (FDA) is achieved. DS proved to be beneficial in determining the crucial stopping criterion, which is essential in the production of multiple emulsions. The stopping of the process at a less-than-ideal point can severely lower the encapsulation efficiency and the stability, thereby lowering the quality of the emulsion. DS is also expected to provide further information about the multiple emulsion like encapsulation efficiency.

1. Introduction

Multiple emulsions of the W/O/W type provide several promising applications in pharmaceutical (Ferreira et al., 1995; Fukushima et al., 1987), cosmetics (Tadros, 1992; Vasudevan and Naser, 2002) and food technology (Friberg et al., 2004). However, multiple emulsions are thermodynamically instable; therefore care has to be taken during the production of these emulsions to ensure a prolonged stability, to enable commercial use of the emulsions. Aside from the chemical composition of multiple emulsions, in which a sophisticated combination of hydrophilic and hydrophobic emulsifiers is used to achieve a stable emulsion, the physical production process has a big influence on the prolonged stability of multiple emulsions (Garti and Aserin, 1996). The production method used in this study, as reported by Matsumoto et al. (1976), is a 2-step procedure. In the first step, a primary water-in-oil (W/O) emulsion is prepared under high shear forces. The primary emulsion then is emulsified in the second step with the outer water phase under low shear forces to produce the multiple emulsion. The second step has great influence on the stability and the overall quality of the emulsion. Too high shear forces or too long emulsification times cause the inner water phase to come into contact with the outer water phase, thereby reducing the multiple component of the emulsion and ultimately leaving a simple oil-in-water (O/W) emulsion. On the other hand, too low shear forces and too short stirring times might leave the emulsion inhomogeneous with very large multiple emulsion droplets, thereby lowering its stability and its efficiency as a multiple emulsion. Therefore, a method to observe and control the second production step of multiple emulsions to determine the end point of the emulsification process is required. The method must fit well into the FDA-PAT guidance. The PAT guidance is a framework to encourage innovations in development, manufacturing and quality assurance of pharmaceutical products. Through real-time process

Abbreviations: FDA, Food and Drug Administration; PAT, process analytical technology; W/O/W, water-in-oil-in-water; O/W, oil-in-water; DS, dielectric spectroscopy; ε^*, complex permittivity of the emulsion; ε_c^*, complex permittivity of the continuous phase; ε_d^*, complex permittivity of the dispersed phase; Φ, volume fraction of the dispersed phase; Φ_m, volume fraction at the maximum packing limit.

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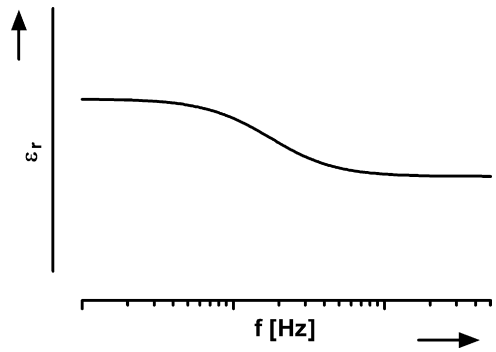

Fig. 1. Schematic β-dispersion. ε_r is the relative permittivity; f is the frequency.

control and quality assurance an efficient production cycle is to be enabled while avoiding the production of poor quality products (FDA, 2004).

Well established analytical methods of multiple emulsions include the analysis of viscosity and the observation of the evolution of droplet sizes via light scattering. However, these measurements have to be performed off line, which only gives information about the production after it is finished and increases the effort as high temporal resolution is desired. As a solution to the problems with the established methods, DS is proposed as an analysis tool. It offers an almost non-invasive, in line measurement of multiple emulsions during the second emulsification step and it yields information about the conductivity of the different water phases and about the physical structure of the emulsion.

2. Theoretical background

Dielectric spectroscopy is a measurement technique, where alternating current fields at various frequencies are applied to the sample and the resulting sample response is observed. The sample response is the ability of the material to polarize, namely its permittivity. The permittivity is measured via capacitance measurements. When a dielectric material is placed between the plates of a capacitor, the induced polarization in the material weakens the electric field between the plates, thereby increasing the capacity of the capacitor. The relative permittivity is a dimensionless constant indicating for a given material at a given frequency the factor by which the capacity of a capacitor is increased when the material is placed between the plates instead of a vacuum (Nolting, 2007). As the permittivity depends on the reorientation of charge, which is a dynamic process, it shows a frequency dependent behavior.

Depending on the frequency range, in which the permittivity is observed, it offers information about the molecular or macroscopic composition of the sample (Asami, 2002). When electric fields in the radio frequency (RF) range are applied, the dominant mechanism determining the permittivity of heterogeneous materials is interfacial polarization. If two insoluble materials of different conductivities build up an interface, charge can be accumulated across the interface, increasing the permittivity of the heterogeneous sample. At high frequencies, above ca. 100 MHz, the response of the heterogeneous medium is limited; the electrical field changes its polarity too fast to allow charge accumulation across the involved interfaces. At lower frequencies, the interfaces become fully polarized, thereby reaching a low frequency plateau. This behavior is called the β-dispersion, an example is indicated in Fig. 1.

The dielectric behavior of W/O/W emulsions in the RF range can be described as following: The oil phase acts as an insulator between the inner and the outer water phase, thereby enabling the accumulation of charge across this interface (Pal, 2008). Thus, the permittivity of W/O/W emulsions holds information about the structural composition and the conductivities of the water phases of the emulsion.

3. Materials and methods

3.1. Preparation of emulsions

The multiple emulsions were prepared according to a 2-step procedure, as reported by Matsumoto et al. (1976). The batch volume was 2500 g. Table 1 shows the composition of the multiple emulsion. The aqueous and the oil phase were heated to 60 °C; then the inner water phase was added to the oil phase and the emulsion was homogenized for 120 s using a rotor/stator homogenizer (Diax600, Heidolph, Germany) at 9500 rpm. In the second step, the outer water phase was slowly added to the chilled primary emulsion under slow stirring at 10–50 rpm (Unimix, Hagen & Rinau, Germany), until a homogeneous emulsion was obtained.

3.2. Droplet size measurement

The multiple emulsion droplet size and the size distribution of the multiple W/O/W emulsions were determined using a laser diffraction particle size analyzer (Mastersizer S, Malvern Instruments, England). The particle size distribution was calculated according to the Mie theory (Malvern-Instruments, 1997). Measurements were performed directly after sample dilution in glucose solution.

3.3. Viscosity measurement

Viscosity measurements were performed at 25 °C using a RheoStress 300 Rheometer (Thermo Haake, France) with a cone and plate geometry, a diameter of two centimeters and an angle of 2°. The apparent viscosity was measured over a shear rate of 0.1–100 s^{-1}. Applying shear to multiple emulsion might cause fragmentation of the emulsion. Several studies have shown, that for W/O/W emulsions with a non-gelified external water phase the critical shear rate before fragmentation is above 2000 s^{-1} (Muget et al., 1999). In this study, microscopic observation after the viscosity measurement confirmed the absence of severe fragmentation due to the viscosity measurement.

3.4. Dielectric spectroscopy

As a dielectric spectrometer, the iBiomass system (i-Biomass 465, Fogale, France), commonly used for biomass determination in bioreactors was used. The system measures the permittivity via a pencil electrode in the range from 180 kHz to 10 MHz. A complete frequency spectrum is obtained in 15 s. An offset was added to the measurement system to compensate for the natural occurring permittivity of the outer water phase to observe only relevant changes of the permittivity during the formation of the multiple emulsion.

4. Results

4.1. Theoretical expectations

The permittivity of emulsions and dispersions has been subject of several studies. Different models have been proposed for calculating the complex permittivity of simple as well as for double emulsions of core–shell droplet morphology (Pal, 2008; Pauly and Schwan, 1959). As the multiple emulsions used in this study have a morphology of multiple emulsion droplets including many droplets rather than a core–shell morphology, a different approach was taken. Therefore, the model originally proposed by Nielsen