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Coulter counting and light diffraction analysis applied to characterisation of oil–water emulsions

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Abstract

Coulter counting and light diffraction techniques were successfully applied to the characterisation of the droplet concentration and size distribution in camphene–water and cyclohexane–water emulsions. Both of these techniques required a dilution of the emulsion prior to analysis, and it was found that the destabilizing effect on the droplets of such dilution could be overcome by performing the analyses at temperatures below the melting point of the oil phase. The storage stability of the camphene–water samples at 60°C was reasonably good with a 5–20% change in the investigated parameters over a 24 h period. At room temperature camphene–water samples left to stand undisturbed were unaffected after 24 h, while continuous mixing of the emulsion on a roller board brought about a rapid amalgamation of the particles into larger aggregates. This fusion process was fully described only with the light diffraction analysis due to the broader measuring range of this technique. However, analysing emulsions with a droplet size range covered by both techniques gave identical results. © 1997 Elsevier Science B.V.

Keywords: Electrical sensing zone; Coulter counting; Light diffraction; Physicochemical characterisation; Oil–water emulsions; Camphene; Cyclohexane

1. Introduction

In recent years the pharmaceutical industry has turned to colloidal and particulate systems for parenteral use. Such substances are commonly explored as drug targeting systems, but also include contrast agents for medical imaging with ultrasonography such as Echovist®, Levovist® (trademarks of Schering, Germany) and Albunex™ [1] (trademark of Molecular Biosystems, USA). These contrast agents are generally based on the effective reflection of sound from various types of physically stabilized gas envelopes in the 1–10 μm size range. Several substances belonging to this group are, or have recently been, in preclinical or early clinical phases [2–6] and the sharp focus on such disperse systems has led to a need for well documented, accurate and precise methods for their physicochemical characterisation. Coulter counting (electrical sensing zone) and light diffraction are commonly used for the

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characterisation of particle size and concentration in suspensions of solid particles, and have earlier been evaluated as assay methods for Albunex [7]. This paper presents applications of the two techniques for characterisation of the droplet size and concentration in camphene–water and cyclohexane–water emulsions. The emulsion systems investigated are intermediates in a process for production of hollow polymer microspheres intended for use as a contrast agent for ultrasonography [6].

2. Materials and methods

2.1. Materials

Camphene (Fluka Chemie, Switzerland) or cyclohexane of p.a. quality (Merck, Germany) constituted the oil phase in the investigated emulsions which were prepared with the aid of an emulsifier added to the water phase. For emulsions with camphene, human serum albumin (HSA) (5% (w/v) in water, Swiss Red Cross, Switzerland) was used as emulsifier. For the cyclohexane–water emulsions an amphiphilic polymer consisting of monomethylpoly-ethyleneglycol modified with a hydrophobic tail (Nycomed Imaging) was used as emulsifier.

2.2. Preparation of emulsions

Emulsions were prepared by rotor/stator homogenisation of the oil phase in the water–stabilizer solution at 60–75°C. The oil volume fraction in all the investigated emulsions was 0.25. The homogenisation speed and time was varied to produce samples with variable droplet size distributions. Immediately after homogenisation, 1 ml emulsion aliquots were filled in 2 ml glass vials, capped and stored undisturbed at 60°C until analysis, which was performed within 1 h of preparation. The stability of the camphene emulsion aliquots was investigated by storage of the vials for up to 24 h at both 25 and 60°C. The vials were stored either undisturbed or under slow continuous mixing on a roller board at 20 rmp (Mixer 820, Swelab Instruments, Sweden) and were carefully homogenized immediately prior to analysis by gentle manual agitation.

2.3. Droplet concentration and size distribution by Coulter counting

The concentration and size distribution of droplets in the emulsions were measured with a Coulter Multisizer Mark II E model with 'Accucomp for Windows' software, version 1.15 (Coulter Electronics, UK). A 50 μm aperture was used and the instrument was calibrated with a 5 μm calibration standard (Coulter Electronics, UK). The gain factor was set to 4 and the current was set to 1035 mA to yield a measuring range 1.0–33.1 μm. The analysis was set up with 64 logarithmically spaced size channels. Isoton II (Coulter Electronics, UK) was used as electrolyte and double filtered through a 0.22 μm filter (Millipak 40, Millipore, USA) prior to use. The camphene–water emulsions were diluted and analyzed in Isoton II electrolyte equilibrated to 27.0 ± 0.5°C prior to use. Samples of the cyclohexane–water emulsions were analyzed in Isoton II at both 27.0 ± 0.5 and 0.5 ± 0.5°C. Some cyclohexane–water emulsions were also analyzed in electrolyte to which was added 1% (w/v) of stabilizer and pre-saturated with cyclohexane prior to use. Saturation was achieved by stirring a cyclohexane–electrolyte mixture for 24 h before separation in a funnel. A sample volume 3–30 μl was diluted in 200 ml electrolyte just prior to analysis, and each dilution was analyzed three consecutive times. The analytical (siphon) volume was 50–500 μl. As response parameters the volume concentration and the volume distribution were followed together with the volume median diameter.

2.4. Droplet size distribution by light diffraction

The size distribution of droplets in the camphene–water emulsions was measured with a Malvern Mastersizer 1002 (Malvern Instruments, UK) set up with a small volume sample cell (MS 1) unit. For characterisation of freshly prepared emulsions, the 100 mm focus lens was utilized to yield a measuring range 0.5–180 μm. For investigations of emulsion stability a 300 mm lens was

used, with a measuring range 1.2–600 μm. Sample aliquots of 50–100 μl were suspended in 100 ml of Isoton II equilibrated to 27.0 ± 0.5°C and each test sample was analyzed in triplicate. Results were calculated using the independent calculation algorithm, which does not make assumptions about the shape of the resulting size distribution. The refractive index of the droplets was set to 1.45 and their absorption coefficient was set to 0.01. A medium refractive index of 1.33 was used. As response parameters the volume distribution was followed together with the volume median diameter.

3. Results and discussion

3.1. Analysis of camphene–water emulsions: Coulter counting versus light diffraction

Both the Coulter and the light diffraction analysis gave stable and reproducible results on the camphene–water emulsions. No marked changes with time were observed in the droplet size distribution after dilution of the test sample in the measuring system. The repeatability relative standard deviation (R.S.D.) of all investigated parameters was better than ± 5% R.S.D. ($N = 3$). Results for a typical sample are visualized in Fig. 1 which shows that the measured volume distributions were consistently described by the two techniques. Compared to the Coulter analysis, the light diffraction technique yielded a distribution with a downwards shift of approximately 0.5 μm and a tailing towards smaller droplets. The consistency of the two methods is also illustrated by comparison of the volume median diameter in 12 samples with varying droplet sizes, as shown in Fig. 2. The zero intercept and unit slope ($P = 0.05$) from the linear regression of the observed diameters confirmed that the two techniques yielded essentially identical results for this parameter.

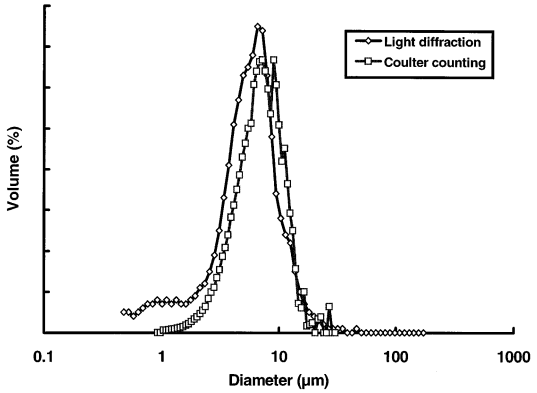

Fig. 1. Volume distribution of droplets in a camphene–water emulsion, as observed with light diffraction (◇) and Coulter counting (□).

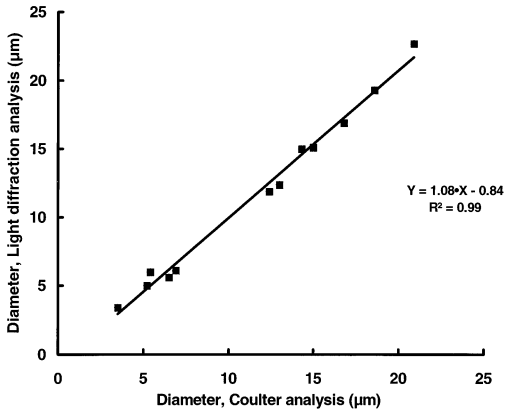

Fig. 2. Comparison of the volume median diameter (μm) of droplets in various camphene–water emulsions measured with light diffraction and Coulter counting. Results from linear regression included.

3.2. Stability of camphene–water emulsions

The stability of the camphene–water emulsions was evaluated by Coulter analysis over a 24 h period, both at room temperature and at 60°C. The emulsion aliquots were either mixed continuously on a roller board or left to stand undisturbed. It should be noted that as the melting point of camphene is 52°C [8] the oil phase solidifies at room temperature causing the emulsion to become a suspension of solid particles. A 20% reduction in droplet volume concentration and a 5–10% increase in the median diameter were observed in both continuously mixed and undisturbed emulsions at 60°C. At room temperature and without agitation the changes were almost negligible. The continuously mixed emulsions,

however, deteriorated rapidly at room temperature with a 50% reduction in droplet volume concentration in 3 h, and almost complete destruction after 24 h. Typical results are shown in Fig. 3.

To explain the apparent loss in droplet concentration, the emulsion aliquots stored on a roller board at room temperature were also investigated by light diffraction analysis. As seen from Fig. 4, the results obtained with this technique were consistent with the Coulter analyses as they confirmed a gradual decrease in the droplet concentration contained in the primary peak (2–20 μm). However, this technique also revealed a gradual build-up of larger aggregates in a secondary peak (20–300 μm). As the measuring range of the Coulter method is 1–30 μm, the secondary peak will go undetected, and the observed droplet concentration will, consequently, decrease. Hence, it's broader detection range clearly makes the light diffraction technique better suited to study the investigated process completely.

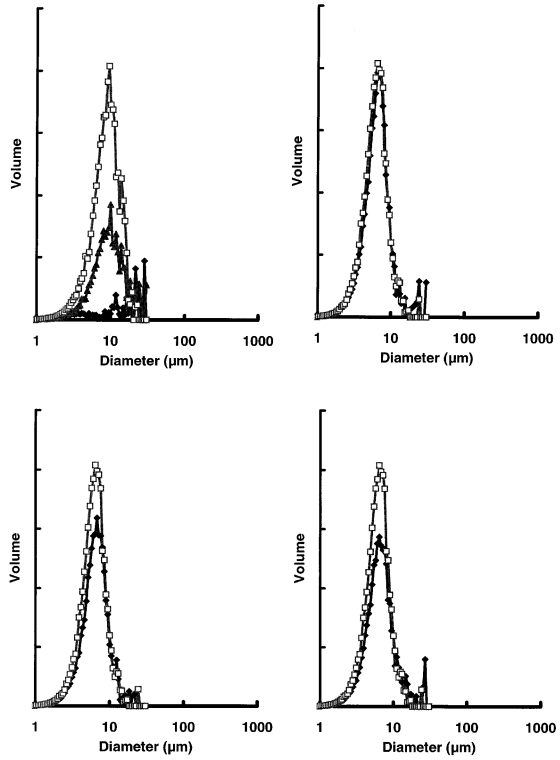

Fig. 3. Storage stability of droplet distribution in a camphene–water emulsion measured with Coulter counting. Storage conditions: room temperature/agitated (upper left); room temperature/undisturbed (upper right); 60°C/agitated (lower left); 60°C/undisturbed (lower right). Freshly prepared emulsion (□), 3 h storage (▲), 24 h storage (◆).

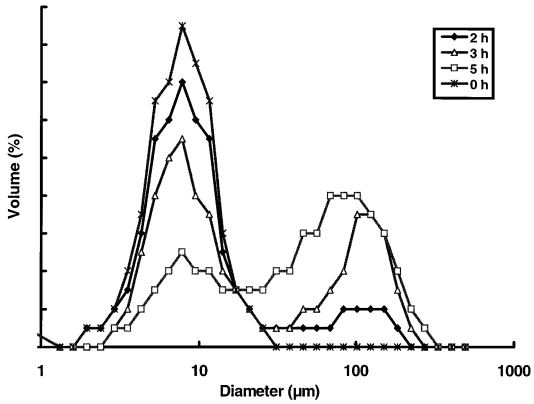

Fig. 4. Storage stability of droplet distribution in a camphene–water emulsion measured with light diffraction. Storage condition: room temperature/agitated. Freshly prepared emulsion (∗), 2 h storage (◆), 3 h storage (△) and 5 h storage (□).

The results demonstrate that the camphene–water emulsion is relatively stable at 60°C and that it deteriorates slowly, probably through a process related to Oswaldian ripening. At 25°C and under continuous mixing conditions, the solidified droplets are continuously made to collide with a relatively high kinetic energy, causing irreversible amalgamation and the rapid formation of a population of larger aggregates. It was observed that the individual droplets of the emulsion were most effectively preserved by undisturbed storage and at a temperature below the melting point of the oil phase. In this case the low frequency and energy of droplet collisions arising both from thermal motion and flotation were apparently not sufficient to cause a significant amount of irreversible fusion/aggregation over the time span investigated.

3.3. Analysis of cyclohexane–water emulsions

Whereas the camphene–water emulsion was stable during analysis at 27°C, emulsions with

cyclohexane were not. After addition of the cyclohexane–water emulsions to the electrolyte solution used as a dilution medium during the Coulter analysis, the droplet concentration decreased rapidly. Only 10–15% of the initial concentration of the oil phase remained in the test sample 1–2 min after dilution. These observations could be caused by a destabilisation of the emulsion due to the low concentration of emulsifying agent in the diluted test sample, leading to aggregation/fusion of droplets. However, results from the light diffraction analysis showed no evidence of a population of large particles outside the measuring range of the Coulter analysis, but confirmed the rapid disappearance of the droplets in the diluted test sample. The strong dilution yielded a volume concentration of the oil phase of only $6 \cdot 10^{-4}$% (v/v) during the Coulter analysis. As the solubility of cyclohexane in water is approximately $8 \cdot 10^{-3}$% (v/v) [9], the observed behaviour could thus be caused by the dissolution of the cyclohexane in the electrolyte solution. Attempts were made to prevent such dissolution by addition of emulsification agent and by saturation of the electrolyte with cyclohexane prior to dilution and analysis. These changes in analytical conditions had, however, no measurable effect on the stability of the sample and the observed instability of the cyclohexane droplets remains unexplained.

The temperature of the electrolyte solution was 25°C below the melting point of camphene and the droplets in the camphene–water samples were in fact measured as solidified particles or strongly undercooled droplets. As the solid nature of the droplets could be the reason for the stability of the camphene–water samples, the analysis of the cyclohexane–water emulsions was repeated at an electrolyte temperature of 1°C, which is below the 6°C melting point of cyclohexane [10]. The results from these investigations are shown in Fig. 5. As seen from this figure the lowering of the analytical temperature below the melting point of the oil phase stabilized the sample almost completely and yielded a detected volume concentration comparable to the initial value.

These observations indicate that oil–water emulsions may be difficult to investigate with these methods at analytical temperatures above the melting point of the oil phase. In order to avoid destabilisation of the diluted test sample, the dilution medium should have a temperature below the melting point of the oil phase.

4. Conclusions

Coulter counting and light diffraction techniques have been successfully applied to characterize the droplet size in camphene–water and cyclohexane–water emulsions. In order to avoid destabilisation of diluted test samples during analysis, observations indicate that the analytical temperature should be below the melting point of the oil phase. The storage stability of the camphene–water emulsion aliquots were reasonably good at 60°C, with a 5–20% change in investigated parameters over a 24 h period. At room temperature, where the camphene phase has solidified, continuously mixed samples deteriorated within a few hours, whereas samples left undisturbed were practically unaffected even after 24 h. Comparing results for several emulsions, which spanned a broad range of sizes within the measuring range of both techniques, the two methods yielded essentially identical results. In some cases, however, where the droplet size distribution was particularly broad, the emulsions were more fully charac-

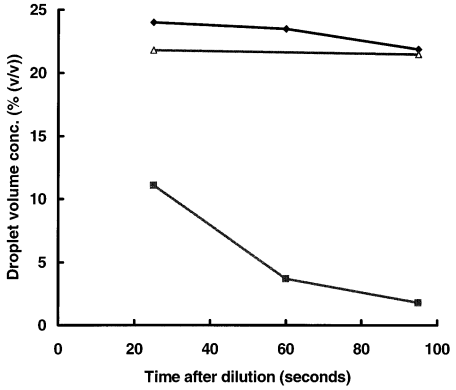

Fig. 5. Detected volume concentration of droplets as a function of time after dilution in the measuring system: camphene–water emulsion analyzed at 27°C (△); cyclohexane–water emulsion analyzed at 27°C (■) and 1°C (◆).

terized by the broader measuring range of the light diffraction technique.

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