

# Particle size analysis of some water/oil/water multiple emulsions

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## Abstract

Particle size analysis gives useful information about the structure and stability of multiple emulsions, which are important characteristics of these systems. It also enables the observation of the growth process of particles dispersed in multiple emulsions, accordingly, the evolution of their dimension in time.

The size of multiple particles in the seven water/oil/water (W/O/W) emulsions was determined by measuring the particles size observed during the microscopic examination. In order to describe the distribution of the size of multiple particles, the value of two parameters that define the particle size was calculated: the arithmetical mean diameter and the median diameter.

The results of the particle size analysis in the seven multiple emulsions W/O/W studied are presented as histograms of the distribution density immediately, 1 and 3 months after the preparation of each emulsion, as well as by establishing the mean and the median diameter of particles.

The comparative study of the distribution histograms and of the mean and median diameters of W/O/W multiple particles indicates that the prepared emulsions are fine and very fine dispersions, stable, and presenting a growth of the abovementioned diameters during the study.

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*Keywords:* Multiple emulsions water/oil/water; Particle size analysis; Medium diameter and median diameter

## 1. Introduction

Multiple emulsions are complex systems, named “emulsions of emulsions” in which the particles of the dispersed phase include smaller dispersed particles that belong to the continuous phase and which have two interfaces. In multiple emulsions, each dispersed particle forms a vesicular structure with one or more aqueous compartments separated from the aqueous phase by a film of the oily phase [1–8]. W/O/W multiple emulsions are composed of aqueous microparticles contained in oily particles, which in turn are dispersed in an aqueous phase [5–7,9,19].

Because of their structure, multiple emulsions may have numerous applications in the cosmetics, pharmaceutical and food industry, and in different separation techniques [2,3,7,9–16]. Still, the applicability of multiple emulsions in the above mentioned domains is limited by their thermodynamic instability which is due to their complex structure and, therefore, by the difficulty of their long-term stabilization.

The instability of W/O/W multiple emulsions can manifest itself through numerous mechanisms [8,10,17], which may be delayed or even avoided using different methods to stabilisation of these systems [4,7,18]. Among the numerous methods used in order to control the quality and to evaluate the stability of W/O/W multiple emulsions, the particle size analysis and the microscopic analysis (as complementary method) are presented in this paper.

The aim of this paper was the use of size analysis of multiple particles in some W/O/W multiple emulsions observed by microscopic examination to their quality and stability evaluation.

## 2. Experimental

### 2.1. Materials

The materials used are the following: lipophilic emulsifier sorbitan monooleate (Span 80, HLB = 4.3 from Fluka, Buchs, Switzerland); hydrophilic emulsifier: polyoxyethylene sorbitan monolaurate (Tween 20, HLB = 16,

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Merck, Darmstadt, Germany), glyceryl polyoxyethylene monostearate (Tagat S<sub>2</sub>, HLB = 15, T.H. Goldschmidt, A.G. Essen, Germany); paraffin oil (Ph. Eur.) (Merck Vaseline); cetostearyl alcohol (Ph. Eur.) (Merck); hydroxyethylcellulose (Merck); gelatin (Ph. Eur.) (Rousselot SA); polyacrylic acid (Carbopol 940, BF Goodrich); triethanolamine (Fluka); buffer solution pH = 6; magnesium sulphate hydrate (MgSO<sub>4</sub>·7H<sub>2</sub>O, Ph. Eur.); methyl-*p*-hydroxybenzoate, propyl-*p*-hydroxybenzoate (Ph. Eur.) (Merck); distilled water (Ph. Eur.).

## 2.2. Composition of multiple emulsions

Stable W/O/W multiple systems were obtained using two general formulas (I and II) in which the variables were the following: the concentration of the lipophilic emulsifier, the composition and concentration of the oily phase; the composition and concentration of the internal aqueous phase, the nature of the hydrophilic emulsifier, the concentration of the primary W/O emulsion in the multiple W/O/W emulsion as well as the content of the external aqueous phase. The composition (% , m/m) of the two formulations used for the preparation of the W/O/W multiple emulsions studied is presented in Table 1.

## 2.3. Procedure

### 2.3.1. Preparation of colloidal dispersions

For the preparation of the multiple emulsions, the macromolecular compounds in the table mentioned above were used as colloidal dispersions in a preservative solution, which made it necessary to prepare these colloidal dispersions in advance.

Preparation of the gelatin colloidal solution 3%: the gelatin is dissolved in the preservative solution, maintain-

ing it in the water bath at 70–80 °C under stirring until a clear and homogenous colloidal dispersion is obtained.

Preparation of the hydroxyethylcellulose colloidal solution 3%: the hydroxyethylcellulose powder was added slowly to the preservative solution heated at 60–70 °C, stirring until the macromolecular compound is completely dispersed. The mixture is put to rest for 30–40 min for a complete dissolution of the substance. Finally, in order to obtain a homogenous colloidal solution, a slow stirring is necessary.

Preparation of the Carbopol 9400.3% colloidal dispersion: the Carbopol 940 powder is introduced by stirring it into the preservative solution and after a 24 h rest (necessary for the total hydration of the powder) a diluted aqueous solution of triethanolamine is added. Through the neutralisation of the dispersion with this alkaline substance, a transparent dense mass is obtained.

### 2.3.2. Preparation of W/O/W multiple emulsions

The W/O/W multiple emulsions were obtained by using the two-step emulsification procedure.

In the first step, the primary W/O emulsion was prepared by incorporating progressively the aqueous phase (80 ± 1 °C) into the oily phase containing the lipophilic emulsifier (80 ± 1 °C) at high speed (2000 rpm), using a Eurostar digital agitator (Ika-Werke, Germany). The agitation was kept constant for 30 min until the temperature had fallen to 25 ± 1 °C.

In the second step, the primary emulsion was incorporated slowly and progressively into an aqueous phase containing the hydrophilic (secondary) emulsifier at room temperature (25 ± 1 °C). The system was stirred at low speed (800 and 500 rpm) until formation of the W/O/W multiple emulsion.

Table 1  
Composition of W/O/W multiple emulsions (% , m/m)

|                                      | W/O/W multiple emulsion no. |       |       |       |       |       |       |
|--------------------------------------|-----------------------------|-------|-------|-------|-------|-------|-------|
|                                      | 1                           | 2     | 3     | 4     | 5     | 6     | 7     |
| <b>Primary W/O emulsion</b>          |                             |       |       |       |       |       |       |
| Span 80                              | 10.00                       | 10.00 | 10.00 | 10.00 | 4.00  | 4.00  | 4.00  |
| Paraffin oil                         | 35.00                       | 35.00 | 35.00 | 35.00 | 20.00 | 20.00 | 20.00 |
| Cetostearyl alcohol                  | 10.00                       | 10.00 | 10.00 | 10.00 | –     | –     | –     |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O | –                           | –     | –     | –     | 0.70  | 0.70  | 0.70  |
| Hydroxyethylcellulose                | 1.35                        | 1.35  | –     | –     | –     | –     | 1.13  |
| Gelatin                              | –                           | –     | 1.35  | 1.35  | –     | –     | –     |
| Buffer solution (pH = 6)             | –                           | 43.65 | –     | 43.65 | –     | –     | –     |
| Preservative solution                | 43.65                       | –     | 43.65 | –     | 75.3  | 75.3  | 74.17 |
| <b>Multiple W/O/W emulsion</b>       |                             |       |       |       |       |       |       |
| Primary W/O emulsion                 | 40.00                       | 40.00 | 40.00 | 40.00 | 80.00 | 80.00 | 80.00 |
| Tween 20                             | 1.00                        | 1.00  | 1.00  | 1.00  | –     | –     | –     |
| Tagat S <sub>2</sub>                 | –                           | –     | –     | –     | 1.00  | 1.00  | 1.00  |
| Hydroxyethylcellulose                | 1.77                        | 1.77  | 1.77  | 1.77  | –     | 0.57  | –     |
| Carbopol 940                         | –                           | –     | –     | –     | 0.06  | –     | 0.06  |
| Triethanolamine                      | –                           | –     | –     | –     | 0.04  | –     | 0.04  |
| Preservative solution                | 58.23                       | 58.23 | 58.23 | 58.23 | 18.90 | 18.43 | 18.90 |

### 2.3.3. Evaluation methods of the quality and stability of the W/O/W multiple emulsions

The microscopic analysis was carried out in order to visualize and notice the dimensions changes of the multiple particles over time. This analysis was performed using an optical microscope with a polarimeter (Cole Parmer compound microscope), connected to a computer in order to obtain images and to measure the particles. The microscopic examination was made immediately, 1 and 3 months after the preparation of the W/O/W multiple emulsions.

0.50 g of sample emulsion was diluted with 4.50 g external aqueous phase of multiple emulsion (dilution 1:10). A drop from the sample of the diluted emulsion was put on a cover slip to be observed under the microscope. After all the sample fields had been examined, 10 more representative fields were selected for the respective sample and their image was kept.

The size analysis of multiple particles of W/O/W emulsions (the granulometric analysis) was performed measuring the size of multiple particles observed during the microscopic examination. For each of the seven multiple emulsions studied, the size of each 30 multiple particles belonging to each field from the 10 selected was measured. The determinations were carried out immediately, 1 and 3 months after the W/O/W multiple emulsions preparation, in order to observe the changes in the particle size over time. In order to describe the multiple particle size distribution, the value of two parameters (which define the particle size) was established beforehand: the arithmetic mean diameter and the median diameter [7,14,20].

The arithmetic mean diameter is the sum of the each particle diameter divided by the number of particles.

$$d = \frac{n_1d_1 + n_2d_2 + \dots + n_nd_n}{n_1 + n_2 + \dots + n_n} = \frac{\sum_{i=1}^n n_i d_i}{\sum_{i=1}^n n_i}$$

The median diameter is defined as the diameter that corresponds to a cumulative frequency of 50%; 50% of the particle population has a diameter below this value, 50% above.

## 3. Results and discussions

The macroscopic aspect of the W/O/W multiple emulsions formulated immediately after preparation: all the multiple emulsions obtained, no matter the formulation used in preparation, were homogenous, white, fine and creamy, with an aired, glossy aspect, proving thus the favourable influence of the target parameters on their stability.

### 3.1. The microscopic aspect of the W/O/W multiple emulsions

The aspect of the multiple emulsions studied through the optical microscopy proved the multiple feature and the spher-

ical form of the dispersed drops. Also, the homogenous distribution of the multiple emulsions particles was observed. Some of the microscopic images of this multiple emulsions are presented in Figs. 1–3.

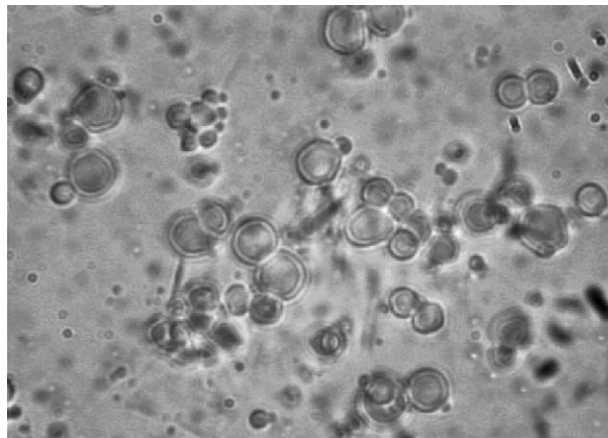


Fig. 1. Microscopic image of W/O/W multiple emulsion no. 1.

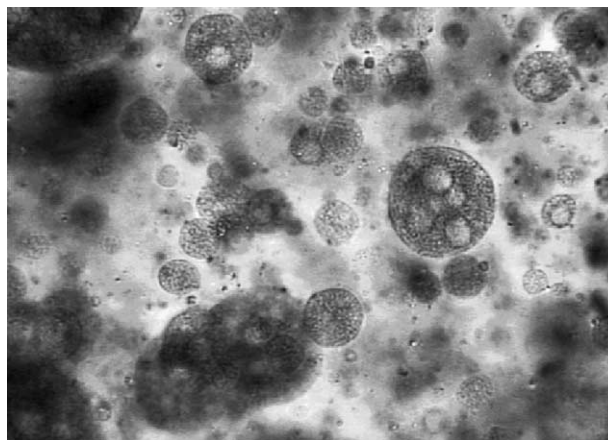


Fig. 2. Microscopic image of W/O/W multiple emulsion no. 5.

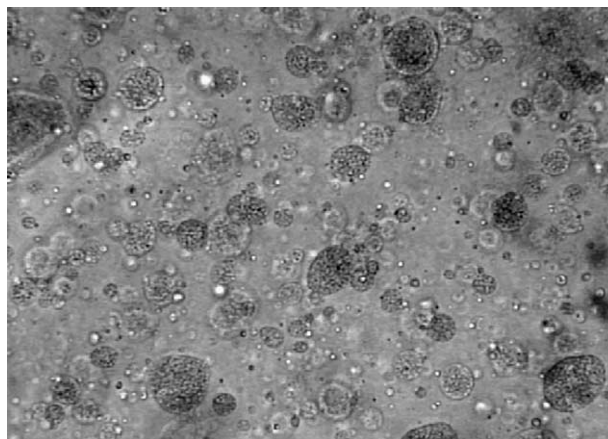


Fig. 3. Microscopic image of W/O/W multiple emulsion no. 7.

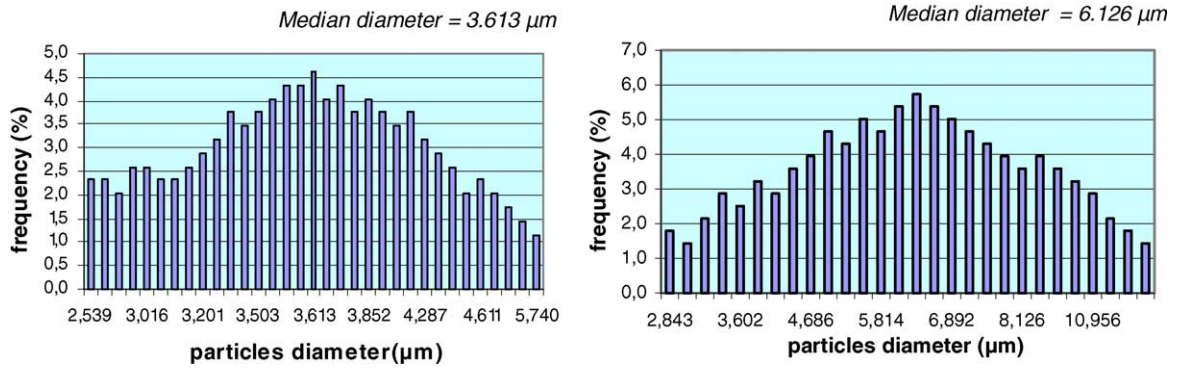


Fig. 4. The distribution histograms of multiple particles for emulsion no. 1, immediately and at 3 months after preparation.

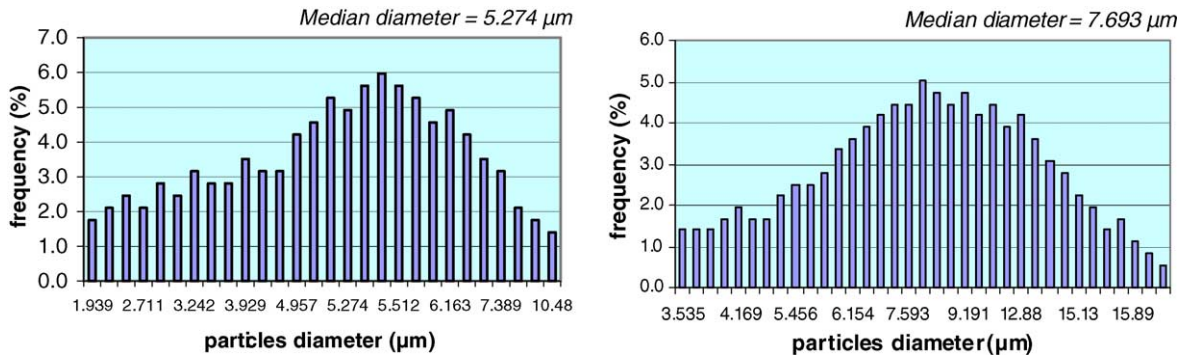


Fig. 5. The distribution histograms of multiple particles for emulsion no. 2, immediately and at 3 months after preparation.

3.2. The granulometric analysis of the W/O/W multiple emulsions

Relying on the data obtained in the experimental measurements, the distribution histograms of the multiple particle size for the seven multiple emulsions were obtained through the graphic representation of the particle diameter according to their frequency. Some of these histograms are presented in Figs. 4–7.

The comparative study of the distribution and evolution of the multiple particles dimensions of the W/O/W emulsions studied for 3 months was achieved through the val-

ues of the mean and median diameter, presented in Table 2 and Fig. 8.

The analysis of the distribution histograms of the multiple emulsions particle size done immediately after preparation shows the following:

- all histograms present a monomodal form, the exception from this being the distribution histogram corresponding to multiple particles of the emulsion no. 5 which has a bimodal form;
- the multiple emulsions no. 1 and 3 are homogenous and very fine dispersions, because 93 and 70.3%, respectively

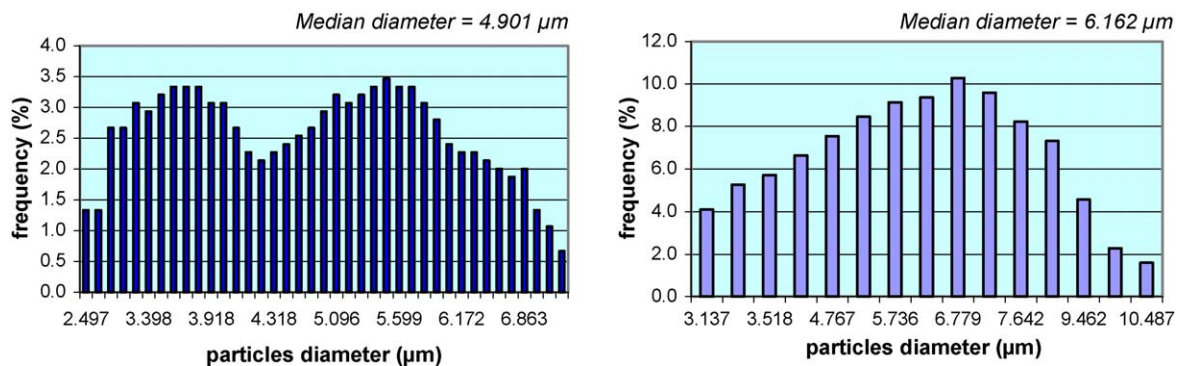


Fig. 6. The distribution histograms of multiple particles for emulsion no. 5, immediately and at 3 months after preparation.



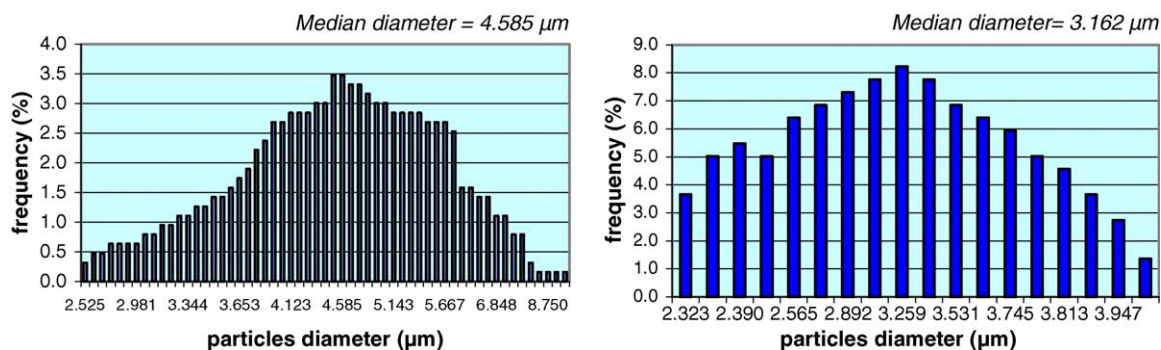


Fig. 7. The distribution histograms of multiple particles for emulsion no. 7, immediately and at 3 months after preparation.

Table 2

Evolution of the mean diameter of the W/O/W multiple emulsions in time

| Mean diameter ( $\mu\text{m}$ ) | W/O/W multiple emulsion no. |       |       |       |       |       |       |
|---------------------------------|-----------------------------|-------|-------|-------|-------|-------|-------|
|                                 | 1                           | 2     | 3     | 4     | 5     | 6     | 7     |
| Immediately after preparation   | 3.701                       | 5.219 | 4.234 | 5.153 | 4.828 | 4.914 | 4.690 |
| 1 month after preparation       | 4.694                       | 6.702 | 4.466 | 6.888 | 5.990 | 5.247 | 2.928 |
| 3 months after preparation      | 6.592                       | 9.108 | 5.873 | 8.818 | 6.181 | 2.801 | 3.189 |

from the particles have a diameter of between 2.5 and 5  $\mu\text{m}$ , while the median diameter is 3.613 and 4.265  $\mu\text{m}$ , respectively;

- the multiple emulsions no. 2 and 4 are homogenous but less fine dispersions than the preceding emulsions because 56.6 and 59.4%, respectively from the particles have a diameter of between 3.3 and 7.389  $\mu\text{m}$ , while the median diameter is 5.274 and 4.439  $\mu\text{m}$ , respectively;
- the multiple emulsion no. 5 is a heterogeneous dispersion because the maximum frequency presents the particles belonging to two different granulometric classes (31.5% from the particles have a diameter of between 3 and 4  $\mu\text{m}$  and 41.8% have a diameter of between 5 and 6.3  $\mu\text{m}$ ). The median diameter value is 4.901  $\mu\text{m}$ , corresponding to a fine dispersion;
- the multiple emulsions no. 6 and 7 are homogenous and very fine dispersions, because 83.1 and 82.7%, respectively from the particles have a diameter of between 2.6 and 6  $\mu\text{m}$ ,

while the median diameter is 4.15 and 4.585  $\mu\text{m}$ , respectively.

Through the ranking of the dispersion degree of the multiple particles, it is noticed that the finest is emulsion no. 1, followed on the decrease order by emulsions no. 6, 3, 4, 7, 5 and 2.

The comparative study of the distribution of the size of the multiple emulsions particles no. 1, 2, 3 and 4, indicated the fact that the particles proportion having a diameter of between 8 and 12  $\mu\text{m}$  is higher in emulsions no. 2 and 4 which contain in their aqueous internal phase the buffer system which enables water transport from the exterior to the interior, a phenomenon accompanied by a growth in the dimension of the particles.

Comparing the distribution histograms corresponding to the multiple emulsions no. 5, 6 and 7, one may observe the presence in higher proportion of the multiple particles with a

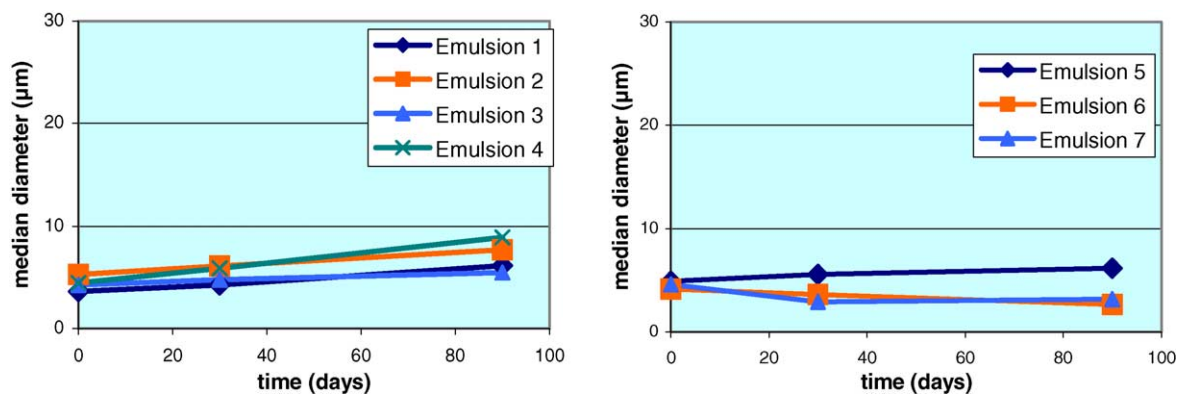


Fig. 8. The evolution of the median diameter in time, for multiple emulsions no. 1, 2, 3, 4, 5, 6 and 7.

diameter of between 8 and 18  $\mu\text{m}$  in case of the emulsion no. 6 which contains hydroxyethylcellulose in aqueous external phase. This fact is due to the formation of a less stable interfacial complex between the macromolecular compound and the molecules of the hydrophilic emulsifier at the O/W interface. Therefore, the water transfer from the external phase to the internal phase is allowed.

Following the evolution in time of the median diameter of the multiple emulsion particles no. 1, 2, 3 and 4, it was observed that its more obvious increase was after a month and especially, after 3 months from the preparation of the emulsions no. 1, 2, 4 comparatively to emulsion no. 3. The median diameter increase of the multiple particles of emulsions no. 2 and 4 over time is due to the presence of the buffer system in the internal aqueous phase, while in the case of multiple emulsion no. 1, it is favoured by the break-up of the W/O interface membrane less stabilized by the hydroxyethylcellulose—Span 80 interfacial complex.

In case of the multiple emulsions prepared according to general formula II, it was noticed that a variation in the median diameter over time is reduced, especially for emulsion no. 5, containing in its external aqueous phase Carbopol 940, a macromolecular compound which forms with hydrophilic emulsifier a stable complex at the O/W interface. The multiple emulsions no. 6 and 7 display a decrease in the median diameter a month after the preparation, its value being very close to the preceding one, after 3 months. As compared to the Carbopol 940, hydroxyethylcellulose, present in the external (emulsion no. 6) or internal aqueous compartment (emulsion no. 7), does not sufficiently stabilize O/W interface, W/O interface, respectively, allowing the water transfer through the oily membrane.

The granulometric stability hierarchy of the analysed multiple emulsions should be as follows: 2, 4, 1, 3, 5, 6, 7.

#### 4. Conclusions

In this paper the particle size analysis was presented as an evaluation method of the quality and stability of seven stable W/O/W multiple emulsions. For the W/O/W multiple emulsions formulation and preparation the factors were taken into consideration that favourably affect the stability of these systems.

The particle size analysis was accomplished by the microscopic images of the samples. The optical microscopy method confirmed the multiple structure of W/O/W multiple emulsions.

The results of the granulometric analysis of the W/O/W multiple emulsions, showed that these systems contain some

fine and very fine dispersions, stable during the whole period of study. It was also demonstrated, as is mentioned in the literature, that the use of this method in the control of the multiple emulsions stability is necessary for the following reasons:

- indirectly it offers informations about the multiple structure of these systems;
- it allows the quality and reproductibility control of emulsification;
- it quantifies the modifications of the multiple particle dimensions in time, permitting the monitoring of the mean dimensions and/or the particles numbers present in the unite volume, as well as of the instability phenomena characteristic for these systems (the coalescence of the internal aqueous particles and their expulsion in the external aqueous phase, water transfer from the external to the internal aqueous compartment, the coalescence of multiple particles).

#### References

- [1] J. Balázs, I. Erös, M. Tacsí, I. Peter, *Acta Phys. Chem. Szeged* 34 (1988) 121–133.
- [2] M.L. Cole, T. Whateley, *J. Control. Release* 49 (1997) 51–58.
- [3] I. Csóka, I. Erös, *Int. J. Pharm.* 156 (1997) 119–123.
- [4] E. Fredro-Kumbaradzi, A. Simov, *Pharmazie* 47 (1992) 388–389.
- [5] N. Garti, A. Aserin, *Adv. Colloid Interf. Sci.* 65 (1996) 37–69.
- [6] N. Garti, *Colloids Surfaces A: Physicochem. Eng. Aspects* 123–124 (1997) 233–246.
- [7] J.L. Grossiord, M. Seiller, *Multiple emulsions, in: Structure, Properties and Applications*, Editions De Sante, Paris, 1998.
- [8] F.O. Opawale, D.J. Burgees, *J. Pharm. Pharmacol.* 50 (1998) 965–973.
- [9] L. Olivieri, M. Seiller, L. Bromberg, M. Besnard, T.N.L. Duong, J.L. Grossiord, *J. Control. Release* 88 (2003) 401–412.
- [10] A. Abd-Elbary, S.A. Nor, F.F. Mansour, *Pharm. Ind.* 46 (1984) 964–969.
- [11] S. Geiger, S. Tokgoz, A. Fructus, N. Jager-Lezer, M. Seiller, C. Lacombe, J.L. Grossiord, *J. Control. Release* 52 (1998) 99–107.
- [12] T. Hino, A. Yamamoto, S. Shimabayashi, M. Tanaka, D. Tsujii, *J. Control. Release* 69 (2000) 413–419.
- [13] W. Hou, K.D. Papadopoulos, *Colloids Surf. A: Physicochem. Eng. Aspects* 125 (1997) 181–187.
- [14] Y. Kawashima, T. Hino, H. Takeuchi, T. Niwa, K. Horibe, *J. Colloid Interf. Sci.* 145 (1991) 512–523.
- [15] S.Y. Lin, W.H. Wu, *Pharm. Acta Helv.* 66 (1991) 342–347.
- [16] H. Okochi, M. Nakano, *Adv. Drug Del. Rev.* 45 (2000) 5–26.
- [17] T. Miyakawa, W. Zhang, T. Uchida, N.S. Kim, S. Goto, *Biol. Pharm. Bull.* 16 (1993) 268–272.
- [18] A.J. Khopade, N.K. Jain, *Pharmazie* 52 (1997) 562–563.
- [19] J.L. Grossiord, M. Seiller, S. T. P. *Pharma Sci.* 11 (2001) 331–339.
- [20] N. Jager-Lezer, I. Terrisse, F. Bruneau, S. Tokgoz, L. Ferreira, D. Clause, M. Seiller, J.L. Grossiord, *J. Control. Release* 45 (1997) 1–13.