Summary of Ph.D. Thesis

STRUCTURAL EVALUATION OF W/O/W MULTIPLE EMULSIONS

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INTRODUCTION

Emulsion systems get more and more importance in the field of both cosmetic and pharmaceutical industry. This is mainly due to the changes in consumer behaviour. Nowadays there are two basic demands of customers: 1) reducing the frequency of dosage (ensure and control drug release); 2) applying products that have several effects in one ("multifunctional product").

Multiple emulsions are corresponding with modern consumer challenges and the different demands of consumers can be satisfied by altering formulations. Their significance lies in the separation characteristics and controlled drug release because the active agents that have not been suitable for applications because of their instability, short biological half-life or side effects can now become therapeutic tools.

Changes in the regulations concerning marketing authorization of cosmetic products, a continuous increase in the expectations concerning the quality of the preparations, and also the necessity of more modern testing methods are also challenging tasks. Since there are only few methods to qualify the products that meet the requirements of the professional expectations mentioned above, it is necessary to study the already existing ones and more new structure analysis techniques.

The extent use of multiple emulsions in industrial fields is hindered by the fact that the stability of these systems cannot be maintained for longer time.

As multiple emulsions are very sensitive systems, investigation and control of their structural changes is a very important task for researchers. In order to achieve this goal, a number of evaluation methods need to be developed, or the existing ones should be adapted to the precise examination of multiple emulsion systems.

AIMS

The aim of my research work was to look for and work out structure analysis methods and adapt methods approved in other fields with which the classical emulsion analysis methods can be supplemented and the coherences between the structure and different influencing factors can be specified.

By means of these methods, the composition, the applicable tools and instruments and the manufacturing technology can become purposable, which are emphasized in both pharmaceutical and cosmetic industry.

As a chemist working in research&development with in the cosmetic industry;

I would like to apply the chosen structure analysis methods in the following areas:

- for proper selection of the product composition and manufacturing technology,
- to use as in-process control methods in manufacturing,
- to use as controlling test during storage,
- to certify the multiple character (patent).

In order to achieve my aims I have carried out the followings:

1. Preparation of w/o/w multiple emulsions with different methods
   - with different manufacturing technology
   - with changing different parameters in the compositions
2. Application of several structure analysis methods to account for the multiple character and to characterise the important properties of multiple emulsions in qualitative and quantitative terms:
   - structure and droplet size analysis
     - in case of multiple emulsions prepared with oils of different polarities
     - in case of multiple emulsions in the presence and lack of active agents at the time of preparation
   - DSC method

     The microstructure of w/o/w emulsions, the effects of the composition (polarity of oil), the effects of method of preparation and quantitative proportions of different types of aqueous phase (internal or external) investigated with thermoanalytical methods.
     - in case of multiple emulsion without active agent at the time of preparation
     - in case of multiple emulsion containing active agent during storage
   - rheological investigations
     - in case of multiple emulsion without active agent at the time of preparation
     - in case of multiple emulsion containing active agent in the course of storage
3. Studying drug release under in vitro conditions
   - from w/o primary emulsion
   - from w/o/w multiple emulsion.

MATERIALS AND METHODS

Materials

I investigated systems including oil phases of various polarities. Three different oil compounds were used. Mineral oil derivatives: liquid petrolatum /Paraffinum liquidum/ (Gustav Hess GmbH, Ph.Eur.4^{th}), 2,2,4,4,6,6,8- heptamethylnonane /Isohexadecane/

(Uniqema, Uniqema grade) and vegetable oil derivatives: avocado oil /Persea Gratissima/ (Symrise, Cosmetics grade), corn germ oil /Zea Mays/ (Naturol, Cosmetics grade) and esters: isopropyl myristate /Isopropyl Myristate/ (Oleon NV, Ph.Eur.4th), 2-ethylhexyl stearate /Octyl Stearate/ (Cognis, Ph.Eur.4th).

The surfactants used during the preparation of w/o/w emulsions were: poly (oxy-1, 2ethanediyl) distearate /Steareth-2/ (Uniqema, Uniqema grade), poly (oxy-1, 2ethanediyl) distearate /Steareth-21/ (Uniqema, Uniqema grade), polyoxyethylene (30) dipolyhydroxystearate /PEG-30 Dipolyhydroxystearate/ (Uniqema, Uniqema grade), block copolymer of polyethylene oxide and polypropylene oxide /Poloxamer 407/ (Uniqema, Uniqema grade).

Viscosity increasing agents in external aqueous phase: sodium alginate (ISP Alginates Ltd., Cosmetics grade), carbomer (BF. Goodrich, Ph.Eur.4th).

Six different compositions were prepared during the experiments. A model substance - Urea (Honeywell Co. Belgium, Ph.Eur.4th) was used in the inner aqueous phase of the compositions (8 m/m%) in order to simulate the osmotic pressure change induced by the active ingredient, and a model active agent - Ketamine hydrochloride (Calypsol, Richter Ph.Eur.4th) was used in the aqueous phases of the compositions (1m/m%).

Methods

Emulsion preparation

Multiple w/o/w emulsions were formulated with one-step and two-step technology. The oil phase containing the surfactant and the aqueous phase were heated separately to 75 °C, in case of the one step technology. The oil phase was then added to the aqueous phase. The emulsion was homogenized for 5 minutes and cooled down to 25°C while gentle stirring, and thus a multiple $w_1/o/w_1$ emulsion was obtained which was stabilized by the liquid crystal phase. The stirring rates used were: 1000, 4000, 8000, 13500 rpm (BIOMIX LE-402/LABORMIM, Hungary, DI 25 IKA-VERKE GmbH. Germany). The result is the direct emulsification of oleosome-containing emulsions.

The two-step technology started with the preparation of a simple w_1/o emulsion, by adding the w_1 aqueous phase to the oil phase containing the hydrophobic surfactant. Both phases were heated separately to 75 °C and then mixed. After the homogenisation process (1000, 4000, 8000,13500 rpm), the emulsion was cooled down to room temperature with gentle stirring. This w_1/o emulsion was dispersed – at a low stirring rate (500 rpm) – in the w_2 aqueous phase at room temperature.

Optical observations

A computerized image analysing device connected to a light microscope was used for the microscopic observations (LEICA Q500MC Image Processing and Analysis System, Leica Cambridge Ltd, U.K.). The type and size distribution of the multiple emulsion droplets were examined at 100x magnification. The oil droplets and the inner water droplets were counted and the diameter of the droplets was determined in all cases. The number of simple and multiple droplets were counted per slide, and the multiple character was calculated in %. The homogeneity was characterized by means of the droplet diameters.

Thermoanalytical measurements

The emulsions were studied without dilution and performed with a DSC821^{e} (Mettler-Toledo GmbH, Switzerland) DSC (heat flux) instrument. The samples were first cooled down from 25 °C to –60 °C, and then they were heated steadily up to 25 °C in hermetically sealed aluminium pan. The heating/cooling rate was 5 °C/min. The weight of the samples was 10±1 mg, the measurements were performed in a nitrogen medium. An empty pan was used as a reference. The calorimeter measured and recorded the heat flow rate of the sample as a function of temperature, while the sample underwent the aforementioned cooling and heating procedure. The instrument also determined the total heat transferred in the observed thermal processes. The enthalpy changes associated with thermal transitions were evaluated by integrating the area of each pertinent DSC peak. The peak areas were evaluated with using the STAR^{e} Software.

Rheological investigations

Rheological measurements were carried out with a RheoStress 1 HAAKE rheometer (ThermoElectron, Germany). The rheological analyses were carried out with non-diluted multiple emulsions. The shear rate was increased from 0 to 100 1/s (up curve) and decreased from 100 to 0 1/s (down curve) in the CR mode; the duration of the experiment was 60s. The temperature of the sample was 25 +0,1°C. Shear stress was recorded as a function of the shear rate (flow curves). The shear rate dependence was described with the Casson mathematical model.

The investigations during the storage time were performed 1 day, 1 month and 1 year after preparation. The relative viscosity was calculated by means of the viscosity of the external aqueous phase (14mPas), which was determined with Brookfield viscosimeter (2spindle, 50 1/s, 25°C; water+ionic surfactant (Poloxamer 407).

In vitro release testing

Franz vertical diffusion cell system (Hanson Research Co., USA) containing six glass cells, and equipped with an autosampler (Hanson Microette Autosampling System) was used during the in vitro release testing process. The area for diffusion was 1,767 cm2, and the receptor chamber volume was 7 ml. Cellulose acetate membranes (Porafil, Machenerey-Nagel, Germany) with an average pore size of 0,45 μm were used. Pre-treatment of the membrane by soaking in the receiving medium was performed. The experiments run at 25±0.5 °C and the receptor medium was phosphate buffer (pH 5.4). 800μl samples were taken after 0,5, 1, 2, 3 4 and 5h. The absorbance was measured by UV spectrophotometer (Unicam Helios α UV-vis Spectrophotometer, England) at 269nm based on prior calibration curves. The blank vehicles without active agents served as references in the analytical measurements.

RESULTS AND DISCUSSION

Light microscopic image analysis

The light microscopic images revealed that the type of the multiple systems and the size of the droplets depended on the preparation method. The microscopic images showed two types of multiple emulsions: in case of the emulsions made by the one-step process several small inner drops of water were seen in the oil droplets (type B), while in the two-step emulsions contained mainly one inner water droplet within the oil droplets (type A). Droplet size decreased with the increase of the stirring rate used during preparation. The average diameter of oil droplets in the one-step emulsions varied between 5-20μm; while the diameter of inner drops of water ranged between 0.5-2,0μm. The diameter of the oil droplets in two-step emulsions ranged between 5-8 μm – depending on the stirring rate – and an inner drop of water with a greater diameter of about 0.8-2.5 μm could be seen in Figure 1.

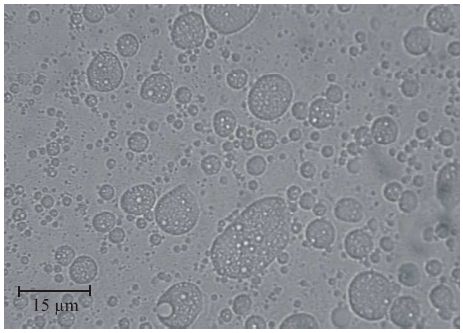

Fig. 1 a

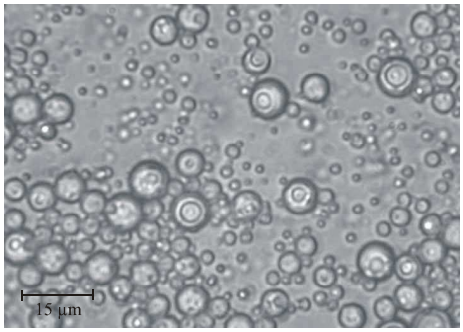

Fig 1 b

*Figure 1: Microscopic photograph of multiple emulsions made by one-step (**a**) and two step (**b**) procedures. Magnification 100x was used during the microscopic observation.*

Droplet size analysis:

According to the data of droplet size analysis; it was found, that the average diameter of oil droplets in one-step emulsions was larger than in the two-step technology system. Although the data alone – without microscopic photographs – do not show that the droplet structure is different.

It can be concluded on the basis of results, that there was no difference seen in these systems with different polarity oils and the droplet structure. Moreover, the microscopic examination revealed that in compositions prepared with Paraffinum liquidum and Isohexadecane – non-polar lipophilic substances - the number of multiple droplets was considerably lower, thus these were not examined with DSC.

The effect of entrapped drugs on the structure of multiple emulsions

A) Urea

Multiple emulsions made by two-step technology (1000 rpm) with oil phase containing avocado oil and corn germ oil and containing urea (8m/m%) in the inner water phase remained stable for half a year period at room temperature. The presence of urea had a great impact on the droplet structure at the time of preparation numerous small inner water droplets arose instead of one bigger droplet. During storage the water droplets flow into a larger droplet, then by the gradual increase of the osmotic pressure the membrane of the oil phase breaks up leading to mixture of the two aqueous phases.

B) Ketamine-hydrochloride

The presence of the ketamine-hydrochloride in the inner aqueous phase (its ionic character) changes the osmotic pressure, consequently the structure changes in the system made by two-step technology (8000 rpm) with oil phase containing avocado oil and corn germ oil. Ketamine-hydrochloride (1m/m%) is only included in the inner water phase. The breakage of the oil phase and flow out of the inner aqueous phase began immediately after preparation, which could be followed both microscopically and macroscopically (Figure 2.).

Modifications in the composition were needed in order to overcome this stability problem. The osmotic pressure between the two aqueous phases was balanced and the quantity of polymer - which increases the viscosity in the external aqueous phase - was increased from 0.3m/m% to 0.75m/m%. This composition remained stable even after 1-year storage at 5°C.

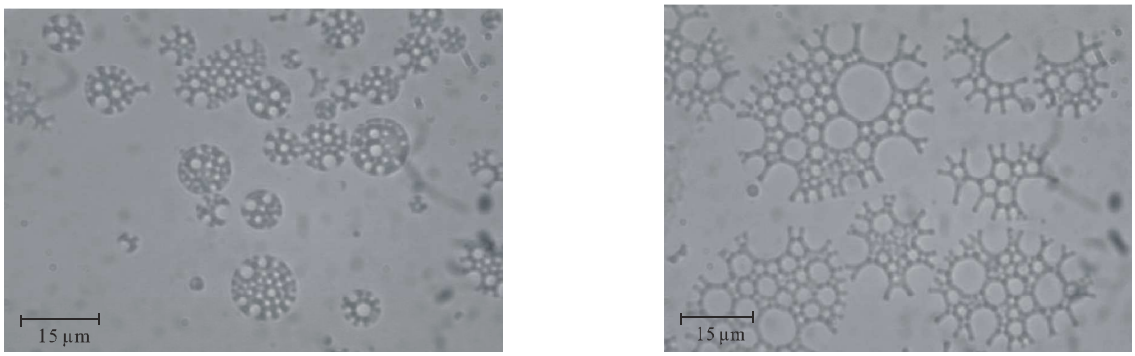

Figure 2: Swelling and breakdown of multiple emulsions

Figure 2 shows the influence active agent presence in the inner aqueous phase of multiple emulsions; the oil droplets break up, after swelling.

Thermoanalytical method

DSC method

DSC methodology is a useful tool in order to get qualitative and quantitative information about the structure of multiple emulsions.

a) qualitative results

The DSC measurement carried out in w/o/w emulsion samples with steady cooling demonstrated the presence of the two types of water, as the solidification of the external aqueous phase and that of the inner aqueous phase took place at different temperatures (Figure 3). The size, changes in the size and the disappearance of the second exothermic peak represent the breakdown process of the multiple structure.

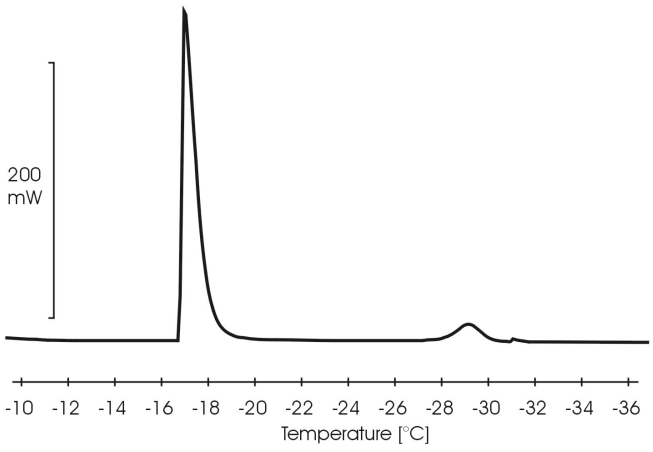

Figure 3: Typical DSC curve of w/o/w multiple emulsion. The first peak represents the external aqueous phase (-18°C) and the second peak shows the inner aqueous phase (-30°C)

The shape of the thermogram is in agreement with the droplet structure seen in the light microscopic images. While in case of the one-step technology the second peak,

indicating the presence of the inner aqueous phase, appeared at about – 45°C, in case of the two-step technology it could already been detected between –30 °C and –40 °C.

b) quantitative results

The proper stirring rate can be chosen on the basis of the results. The greatest enthalpy change (ΔH^{II}_c) was measured at the stirring rate of 8000 rpm both with the one-step and the two-step technologies, so the mass fraction of the inner aqueous phase (X_i^{CII}) was the greatest in this case. This finding mirrors the results of microscopic homogeneity investigation.

The presence of the dissolved urea influenced the dynamic equilibrium of the w/o/w multiple emulsions. Upon the effect of the dissolved material in the inner aqueous phase, the equilibrium between the inner and external aqueous phases changed as an osmotic pressure difference arose between them. The increase of the second peak indicated the migration of the external aqueous phase into the inner aqueous phase.

The DSC method was used also for studying the stability of the formed systems. The enthalpy data of the inner aqueous phase was determined in urea containing emulsions 1 hour, 2 weeks, and then 1, 3, 6 months after preparation. The value of $\Delta H^{II}_{c,norn}$ obtained during the DSC measurement increased with time for 1 month, and then it decreased. The reason for this is, that after 1 month, the water migration from the external water due to the dissolved material in the inner aqueous phase, resulted in such a great increase in the diameter of the inner droplets that the swelling droplets bursted the oil membrane. The breakage of the oil film led to the mixture of the two aqueous phases, thus the number of multiple droplets decreased, which was indicated by the decrease in enthalpy change (Figure 4). These results correlate with the rheological measurements.

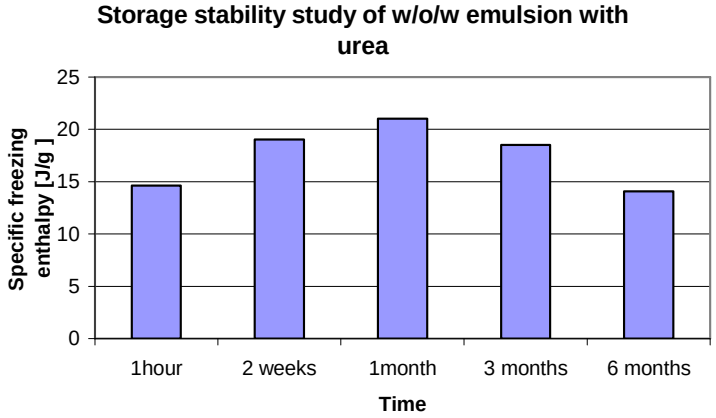

Figure 4: Specific freezing enthalpy data (determined by DSC) of w/o/w multiple emulsions (urea incorporated) made by two-step technology measured after different storage time.

Rheological investigations

The rheological measurement was done partly as this is a valuable tool to characterise pharmaceutical and cosmetic emulsion systems; and also in order to compare the resulting data with other methodologies also referring to the structural properties of these systems.

W/o/w emulsion with one-step technology

The flow curves in case of w/o/w emulsion with the given composition made with one-step technology show pseudoplastic – thixotropic behavior. The yield point was determined from the downward curves because it reflects the circumstances during the utilization and storage better. The Casson's mathematical model was fitted to these flow curves. The flow curve shows a hysteresis loop. The thixotropic area provides qualitative information about its time dependence and it is proportional to the investigated work necessary to change the structure. On the basis of the data in case of one-step technology, it can be declared that in emulsion with non-polar oils the tixotropic area is the smallest, so the shortest time is needed to rebuild the structure in these systems. Furthermore, in case of all three systems the tixotropic area decreases a bit, then increases at 8000rpm. The apparent/Casson yield point values can be correlated with the values in the tixotropic area in case of one-step technology.

W/o/w emulsion with two-step technology

The investigated multiple emulsions produced by the two-step technology show structural viscous flow behaviour and were not tixotropic systems. In these cases, the viscosity decreases when the shear rate increases. This behaviour is called shear-thinning.

The viscosity of the emulsions increases with increasing the stirring rate. This can be explained by the fact that the higher the stirring rate, the smaller the oil droplet size, which is also confirmed by the data from droplet size analysis. Therefore the system is more compact, so the viscosity increases.

The influencing effect of different active agents can be traced also with rheological investigations. I studied two active agents were: urea with hydrating function, often used in cosmetic industry, and ketamine hydrochloride used in pharmaceutical preparations. Both agents were incorporated in emulsions containing avocado oil and corn germ oil prepared with two-step technology.

Investigation of multiple emulsions containing urea

The viscosity of systems increases gradually after preparation, and after achieving a maximum value, it decreases. The background of this is the hydrostatic and osmotic pressure difference between two sides of the oil membrane resulting increase in the volume of the inner aqueous phase. This growth lasts till the multiple droplets breaks up. This process is followed by the viscosity decrease. Figure 5 illustrates the viscosity changes during storage, also correlating with the DSC measurements (Figure 4).

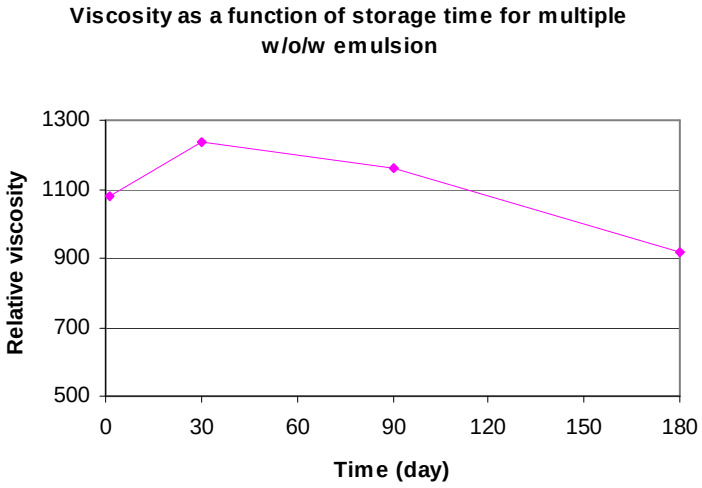

Figure 5: Viscosity as a function of storage time for multiple w/o/w emulsions containing urea in the inner aqueous phase. The viscosity was measured at shear stress of 10 Pa (25°C) and relative to the viscosity of external continuous aqueous phase.

Investigation of multiple emulsions containing ketamine hydrochloride

The consistency of multiple systems in the presence ketamine hydrochloride remarkably changed.

Based on the results of the rheological investigations, it can be concluded – in conformity with the results from the former two structural analysis methods – that the flow and viscosity curves were observed in any case, in accordance with the structure changes mechanism (swelling, break up) during storage. Both DSC method and the rheological analysis show that the inner aqueous phase reaches the largest size after 1-month storage. In case of DSC method the greatest enthalpy change (ΔH^{II}_c) can be observed, while the viscosity is the highest at this point. These phenomena can be seen in microscopic photographs. As a result, the optimum process conditions for various multiple emulsions were obtained.

In vitro release test

Based on the structural evaluation studies, a stable $w_1/o/w_2$ emulsion prepared by the „two step" technology was chosed for in vitro release experiments.

The freely water soluble active agent was incorporated into the aqueous phases (inner, external) of the multiple emulsion in order to compare the release profile of the simple and multiple systems containing same amount of the active agent.

75 % of the drug dissolved in the multiple emulsion released during the 5 hours experimental period, while only 37% from the simple one.

As these emulsion systems are mainly used as topical preparations used on the skin, our results are shown also as the released drug amount per a unit area.

Significantly more drug released from the w/o/w emulsion that from the w/o emulsion after 5 hours period. Differences between these rates are mainly due to the differences in the rheological parameters of the systems and due to the fact, that the drug is present in the external aqueous phase. This drug amount releases rapidly, achieving the function of the „loading dose", and this is followed by the continuous drug release from the inner aqueous phase. In case of simple emulsion the drug is dissolved in the inner phase – therefore both the release rate and the released drug amounts are lower within the investigated time period.

CONCLUSION

A properly documented laboratory development with reproducible data measured by validated methods is of great importance in both the pharmaceutical and the cosmetic industry. Each product gets its own file where the parameters under investigation are recorded (standard value determination), which is followed during production and quality control.

According to a general formula, the steps of product development are as follows: 1) idea, 2) preparation in laboratory, 3) pilot production and 4) manufacturing

Since multiple emulsions are very complex and sensitive systems, it is essential to choose and develop proper evaluation methods and to standardize the testing parameters.

The using of structure analysis methods in the course of product development are the follows:

I. Preparation of w/o/w emulsion with an active agent

II. Examination the structure and stability of multiple emulsions

A) Investigation with light microscope connected with image analysis

The microscopic examination follows the realization of a product from the production to stability testing during storage.

Testing parameters:

a) formation of multiple emulsions
b) type and number of multiple droplets
c) droplet size analysis
d) change occur during storage (stability test)
e) the effect of entrapped active agents on multiple emulsion

B) Rheological investigation

Consistency investigation:

a) plot and evaluation of the flow and viscosity curve
b) set up correct viscosity
c) follow changes during storage (consistency increase or decrease)

C) DSC investigation

Testing parameters:

a) check formation of multiple emulsion (2 peaks in the thermogram)
b) determination of volume fraction of inner aqueous phase
c) check quality (disappearance of the second peak) and quantity (decrease of the volume fraction of the inner aqueous) changes

Using the three structure analysing methods together the formation of a stable multiple emulsion can be investigated, in which system the changes during preparation, storage and transport can be traced properly. According to my practical knowledge, these investigation methods meet the demands of the cosmetic industry – fast, exact, low cost production and control – and provide precise description in case of studying semisolid systems.

SUMMARY

The aim of this research work was to specify the role of different structural investigation methods in case of w/o/w multiple emulsions.

It can be concluded by the results that the **microscopic observations** provide several direct and well-detectable information about the microstructures.

According to the data of droplet size analysis I found that the average diameter of oil droplets in one-step emulsions is larger than in the two-step technology system. The data alone – without microscopic photographs – do not show that the droplet structure is different. Therefore droplet size analysis with microscopic evaluation can be accepted to describe the multiple structures.

There was no significant difference shown in the droplet structure using different polarity oils; however the % of the multiple droplets was lower in compositions prepared with non-polar lipophilic agents (at constant surfactant concentration).

DSC method was found to be an adequate tool in case of multiple emulsions:

- to quantitatively measure the mass fraction of different aqueous phases,
- to detect the relationship between the stirring rate and the mass fraction of inner aqueous droplets,
- to give a feedback for the formulation technology by characterizing the structure of multiple emulsions,
- to follow the microstructural changes occurring during storage.

Consistency changes in the structure were followed with the aid of the rheology. **Rheological investigations** mirrored the results from the above mentioned two analytical methods. The character of the flow and viscosity curves were in accordance with the structure changes mechanism (swelling, break up) during storage. Both DSC method and the rheological analysis show, that the inner aqueous phase reaches the largest size after 1-month storage. In case of DSC method the greatest enthalpy change (ΔH^{II}_c) can be observed, while the viscosity is the highest at this point. These phenomena can be seen also in microscopic results.

Drug release process was also measured in case of a selected composition in order to evaluate the structural changes caused by the incorporated active agent. The differences between release rates represent the differences in the rheological features and different structures within the systems.

The combination of the above mentioned microscopic, thermoanalytical, rheological and drug release methodologies are needed in order to completely characterise w/o/w emulsions. The preparation method, the composition and the nature of the drug greatly influence the internal and external aqueous ratios, therefore modifying the drug releases process.

The joint use of the above mentioned methods provide a rapid and efficient procedure for the formulating expert, and then the required composition and production technology can be chosen. Using these techniques, the optimization of the investigated systems with the desired properties becomes feasible.

I suggest the involvement of these methodologies for both pharmaceutical and cosmetic industry in research&development phase as well as in the method of manufacture development.

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ANNEX

Publications related to the subject of thesis

Papers:

1. **A. Kovács**, I. Csóka, M. Kónya, E. Csányi, A. Fehér, I. Erős: Structural analysis of w/o/w multiple emulsions by means of DSC, *J. Term. Anal. Cal., Vol. 82 (2005) 491-497*

2. **Kovács Anita**, Csóka Ildikó, Sipos Péter, Erős István: Összetett emulziók előállítása, tulajdonságai, stabilitása és alkalmazhatósági területei a kozmetikában, *Olaj, szappan, kozmetika 54. évf.(2005)*

3. A. Fehér, E. Csányi, I. Csóka, **A. Kovács** and I. Erős: Thermoanalytical investigation of lyotropic liquid crystals and microemulsions for pharmaceutical use, *J. Term. Anal. Cal. Vol. 82 (2005) 507-512*

4. **A. Kovács**, M. Kónya, N. Zajc, I. Csóka, S. Srcic, I. Erős: Use of thermal methods in the optimisation of w/o/w coherent emulsions; *5th Central European Symp. On Pharm. Techn. And Biotechnology,* Ljubljana, Farm. Vestn. 54 465-466 (2003)

Abstracts:

1. M. Kónya, **A. Kovács**, I. Csóka, I. Erős: Influence of the microstructure on drug release from o/w and w/o/w emulsion systems; *International Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology*, Nurnberg, (2004)

2. **A. Kovács**, I. Csóka, M. Kónya, E. Csányi, A. Fehér, I. Erős: Structural analysis of w/o/w multiple emulsions by means of DSC *6th Central European Symp. On Pharm. Techn. And Biotechnology,* Siófok (2005)