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Ph.D. Thesis

STRUCTURAL EVALUATION OF W/O/W MULTIPLE EMULSIONS

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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* IF: 1.478

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* IF: 1.478

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)

CONTENT

1. INTRODUCTION

Emulsion systems get more and more importance in the field of both cosmetic and pharmaceutical industry. This is mainly due to the changes consumer behaviour. Nowadays there are two basic demands of customers: 1) reducing the frequency of dosage (ensuring 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 continuously 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 further 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.

2. 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)

I have carried out the following in order to achieve my aims:

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. Study the drug release under in vitro conditions
   - from w/o primary emulsion
   - from w/o/w multiple emulsion

3. LITERATURE SURVEY

3.1 Structural evaluation of w/o/w emulsions

3.1.1 Importance of multiple emulsions

Multiple emulsions are complex systems, termed „emulsions of emulsions", i.e. the droplets of the dispersed phase contain even smaller dispersed droplets themselves. The two major types of multiple emulsions are the water-oil-water (w/o/w) where the internal and external aqueous phases are separated by an oil phase and oil-water-oil (o/w/o) when an aqueous phase is between two oil phases. Because of their structure and the presence of the liquid barrier, multiple emulsions are also known as liquid-membrane systems. The existence of a liquid membrane makes multiple emulsions strong candidates for industrial applications.

Multiple w/o/w emulsions are composed of aqueous droplets, having dispersed inside oily drops. These oily drops are themselves dispersed in an external aqueous phase. There are two interfaces in these complex systems. A hydrophobic emulsifier with a low HLB value stabilizes the first one, and a hydrophilic emulsifier with a high HLB value is present on the second interface [1, 2, 3].

Due to their special structure they are widely used in pharmaceutical, cosmetic and also in food industry.

3.1.1.1 Pharmaceutical applications

O/w emulsions systems are popular dosage forms normally for oral administration of oils, and as parenteral drug delivery systems. Whereas w/o emulsions have high potential as vehicles for lipid-soluble materials and to provide a sustained release dosage systems, their high viscosity, which makes them difficult to inject, has limited their use. Multiple emulsions (w/o/w) have low viscosity and initially after injection will form diffuse depots as multiple oil drops: the external aqueous phase would be miscible with the body fluids and so dissipate leaving globules of water in oil emulsion dispersed in the body fluids. Such studies showed that w/o/w systems could combine both advantages in the formulation of antigenic material, cancer chemotherapy, insulin therapy etc. [2, 4-7].

3.1.1.2 Cosmetic applications

Using w/o/w emulsions means combining the well-known moisturising properties of w/o emulsions with pleasant skin feel of o/w emulsions. As the external phase of a w/o/w emulsion is water, it will give an immediate moisturising effect, a fresh skin feel, will be pleasant to apply and provide sustained hydration [4, 8-12].

They enable the production of sustained-release systems for additives dissolved in the internal water phase (vitamins, free radical scavengers, etc.). It is possible to use different active substances in the internal and external aqueous phases. This could allow the use of active substances interacting with each other, as they are kept apart by an oil membrane. It could also allow selection of the active substances in the external phase for immediate action, and that in the internal aqueous phase for prolonged release.

The reduction of irritation caused by some of the active substances is also possible due to their controlled release.

3.1.1.3 Applications in food industry

There are two main applications of these systems in food industry:

1) The existence of an encapsulated water (or oil) phase in w/o/w (or o/w/o) emulsions allows the protection of reactive food nutrients or volatile flavours as well as the control of their release [13, 14].
2) Since less oil phase in required to make a w/o/w emulsion compared to an o/w emulsion with the same dispersed phase volume fraction, multiple emulsions can be used in developing low calorie, reduced-fat food products e.g. low-fat salad dressings, mayonnaises, margarine spreads or butter[15, 16].

3.2 Formation and stability

Multiple emulsions belong to the thermodynamically instable macroemulsion systems. The middle oil phase (in case of w/o/w systems) acts as a ‘semipermeable’ membrane, therefore the passage of water across the oil phase can take place. This leads to either swelling or shrinkage of the internal droplet, depending on the direction of the osmotic gradient. Many studies have attempted an analysis of the possible mechanisms of instability [17-19].

Stability of multiple emulsions has two elements: 1. stability of multiple character, and alteration to a simple o/w emulsions, 2. the distributional stability, which means the continuous separation of the multiple droplets and the external water phase.

Studying the their structure, Florence and Whitehill [18] classified multiple droplets into three groups. Type A droplets contain one single inner droplet. In type B droplets there are several small inner droplets independent of each other, while type C droplets are characterized by a great number of inner droplets, which are in interaction with each other [1, 12, 20, 21]. Depending on the droplets structure swelling-breakdown kinetics of multiple systems are

different, which factor depends on numerous and various formulation parameters as follow [22]:

- manufacture of multiple emulsion/ method of preparation (the suitable agitation or share rate and time of agitation);
- the optimal concentration and type of raw materials (emulsifiers, oils, electrolytes);
- nature of entrapped materials.

3.2.1 Suitable preparation methodologies

There are several processes for the manufacture of multiple emulsions:

1. Two-step:

   This is the most widely used procedure [22-29]. The first step consists in preparing a primary emulsion. The second step entails dispersing a given amount of primary emulsion in an external phase containing secondary emulsifier (Figure 1.).

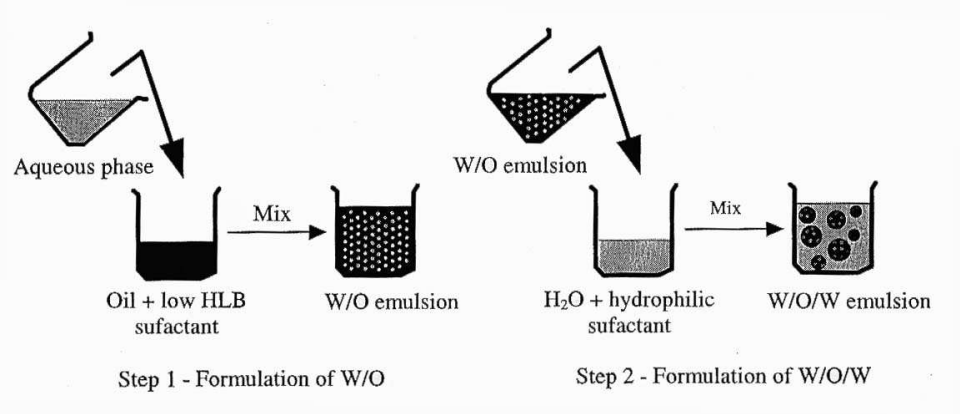


Figure 1: Elements of "two step" methods [2]

2. One-step:
   a) The phase inversion emulsification technique (Figure 2.) [1, 2, 30].
   b) Direct emulsification of oleosome-containing emulsions [4, 31].
   c) Multiple droplets born out of a lamellar crystalline gel network [2, 4].
3. Double emulsion globules prepared within a thin walled glass capillary with video-microscopy [32, 33].

4. Membrane emulsification technique, where the w/o emulsion is injected through a controlled-pore glass membrane into the emulsifying chamber, forming a w/o/w multiple emulsion [34-36].

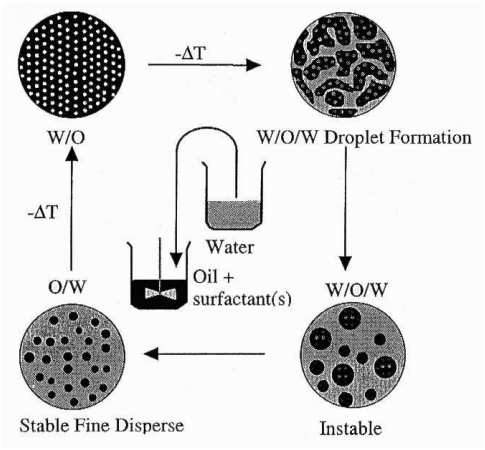


Figure 2: Production of multiple emulsions by the phase inversion technique [2]

From the industrial point of view the two step technology is the most widely used procedure, because it could be done in the simplest way and with the lowest costs.

3.2.2 Suitable composition selection

There are penal terms and requirement against the quality of raw materials both in pharmaceutical and in cosmetic industry. For example it is quite important, that the selected oil has to be natural extraction, nonirritanting to skin and biodegradable in character.

Beside the above mentioned parameters, the nature and the concentration of raw materials in multiple emulsions (oil, emulsifiers, additives) are of great importance, since they will affect the stability and the release properties as well.

Nature of the oil phase

The nature of the oil is of great importance with respect to the physical characteristics of the system[5, 22, 37]. As the oil layer acts as semi-permeable membrane between the two aqueous phases, its physicochemical properties such as a polarity (interfacial tension) [37], density and viscosity influence the behaviour of the emulsion [17].

According to Omotosho et al.[38], the nature of the oil phase in w/o/w multiple emulsion is primarily responsible for the permeability characteristics of the oil layer and, consequently, the release conditions of the drugs or markers for the internal aqueous phase, and the stability of multiple emulsion.

The effect of oil nature on the release characteristics of the multiple emulsions was studied by several research groups [39-42]. Therefore, it can be concluded that the oil nature can affect the release pattern not only through viscosity modification, but also through droplets size distribution. The most frequently used oils are hydrocarbons, esters, triglycerides and vegetable oils with different viscosity and polarity. This latter property is characterised by the oil polarity index (PI); which is defined as the interfacial tension between oil and water, determined using a tensiometer [43].

The interfacial tension $\gamma_{oil/water}$ can be deduced from the relationship (Eq. 1):

$$\gamma_{oil/water}=\gamma_{water(sat)}- \gamma_{oil(sat)} \qquad \text{(Eq. 1)}$$

in which, $\gamma_{water(sat)}$ is the surface tension of the water saturated with oil, and $\gamma_{oil(sat)}$ is the surface tension of the oil saturated with water.

Nature and concentration of the emulsifiers:

Effect of emulsifier characteristics is the most widely examined field in the course of multiple emulsion research[21, 22, 44-46].

To obtain a w/o/w multiple emulsion at least two emulsifiers needed to be introduced into the system, one lipophilic; to from the primary emulsion; and the other hydrophilic, to form the multiple emulsion. The hydrophilic/ hydrophilic (HLB) value of the primary emulsifier will be in the range from 3 to 7, while that of the secondary emulsifier from 8 to 16 [47].

The emulsifier concentration and the concentration, polarity and viscosity of the oily phase could mostly influence the size of the droplets by the amounts and accumulation of free emulsifiers on the oil/water interface [2].

Nature of additives

Increasing the viscosity of the water phase has a stabilising effect on the emulsion systems. Thickening agents, such as alginates [29, 48], xantan gum, cellulose [49],

carboxyvinyl compounds [50] and polyvinyl pyrrolidone may be added into the external aqueous phases, increase the viscosity, and consequently the stability of the emulsions. It is also possible to enhance stability by incorporating aluminium salt and fatty alcohols into the oil phase of the systems [51].

Nature of entrapped substances

Any entrapped substances within the internal or external aqueous phase will cause the disruption of the equilibrium of the system. Substances entrapped in the internal phase are active substances or markers can have an important influence on the stability of systems, meaning that the swelling-breakdown process occurs only if there is an extra concentration in the internal aqueous phase compared to the external one. Under these conditions, a resulting osmotic water flow is observed from the external to the internal phase, in order to reduce the concentration gradient. This water flow makes the inner drops swell, until a critical size is obtained. Beyond this critical size, the oily membrane breaks up [2, 22].

The presence of entrapped materials appears to be one of the most important factors in determining the stability and release of materials from multiple emulsions [52-57].

The effect of the w/o emulsion volume ratio

The fraction of primary emulsion in the final multiple emulsion has an interesting effect on the yield of preparation: bellow 40% volume, the yield of preparation increases along with the increase in volume fraction, while above 40% volume, no significant change is observed [2].

3.3 Evaluation methods for multiple emulsion characterisations

Pharmaceutical industry focuses in developing different investigation methods for mainly solid pharmaceuticals [58-61]; then again the structure analysis methods are less used in cosmetic industry. That is why it is necessary to work out easy to carry out, quick methods with good reproducibility for the examination of semisolid pharmaceuticals and cosmetic products.

The structure analysis of multiple emulsions is quite important since system characteristics have a significant influence on the swelling-breakdown kinetics of emulsions

and drug release. Furthermore, to allow for the industrial aspect, in every step of the production where the material is moving in the course of mixing, transporting and filling, the use of structure analysis methods - knowledge of structure characteristic of multiple emulsions – as well as the standardisation of the methods applied to these conditions cannot be spared.

On the base of the literature [12, 62, 63] the structure analysis methods can be devided into two basic groups: 1) direct methods and 2) indirect methods (Table 1.).

Table 1.: Methods available for multiple emulsion analysis

Direct methods	Indirect methods
Procedures: - Light microscopy - Image analysis technology - Electron microscopy	Chemical indicator compound Conductometry Centrifugation Rheological analysis Thermoanalytical methods (TG, DTG, DTA, DSC) NMR Laser Diffraction Analyser Photon Correlation Spectroscopy (PCS)

3.3.1 Direct method for structure analysis

3.3.1.1 Light microscopic and image analysis

The optical microscope is often used in qualitative evaluation of multiple emulsions. Primary characterizations provide information on microstructural aspects of a multiple emulsion: visualization methods and particle size analysis with the image analysis technology. Visualization gives direct information about the multiple structure. This is the only method, which allows the dispersed globules, as well as their internal and external aqueous phases, to be directly observed [46, 64]. Connecting with image analysis techniques, results obtained are more representative of the particle size distribution. This technique allows one to reach the two particle size populations in situ without creating disturbance. Particle size analysis is able to verify quality and reproducibility of the emulsification and changes in the particle size of multiple globules with time [23, 48, 65, 66].

3.3.2 Indirect methods for structure analysis

3.3.2.1 Thermoanalytical method

Thermoanalytical methods are becoming more and more widespreadly used in pharmacy, especially in the case of solid dosage forms [58-61]. Therefore our research group, in line with its research profile dating back to decades, performed thermogravimetric examinations to verify the microstructure of o/w creams and to detect the factors contributing to structure formation [67]. The states of water were investigated in coherent emulsions containing aqueous phases separated by a bilayer or a liquid oil membrane with thermal methods using differential scanning calorimetry (DSC) and thermogravimetry (TG) [64, 68-71].

In the case of multiple emulsion evaluation, differential scanning calorimetry is used mostly. DSC measures the temperatures and the heat flow associated with transitions in materials as a function of time and temperature. The technique provides qualitative and quantitative information about physical and chemical changes that involve endothermic or exothermic processes or changes in heat capacity using minimal amounts of sample. It has many advantages including fast analysis time, easy sample preparation, applicability to solids, semisolids and liquids, wide range of temperature applicability and excellent quantitative capability. There are many possible applications in the industry, for example: identification, characterization of active and inactive ingredients routine analysis, qualitative –and quantitative control, stability study [72-77].

Quantitative determination of volume fraction of inner aqueous phase

DSC method:

The structure of w/o/w emulsions, the presence of the inner aqueous phase can be deduced from the shape of the DSC curves. This can be indicated by the appearance of a second peak in the thermogram and the area under the peak gives the extent of the total enthalpy change. In the case of one aqueous phase, only one peak can be seen in the DSC curve. The mass fraction of the inner aqueous phase can be calculated from this value. The decrease of the area under the peak and therefore the decrease of the values of enthalpy change, and the disappearance of the second peak indicate, that the inner water loss occurs during storage and multiple droplets breakdown [68].

The evaluation of the results of the DSC measurements, the mass fraction of inner aqueous phase was calculated on the basis of the following equations:

$$\Delta h^{II}_c = [- 443.17 + 4.5026\ T^{II}_c - (1.3832 \times 10^{-2})\ (T^{II}_c)^2 + (1.5963 \times 10^{-5})\ (T^{II}_c)^3] * 4{,}1868 \quad \text{(Eq. 2)}$$

$$m^{II}_c = \Delta H^{II}_c / \Delta h^{II}_c \quad \text{(Eq. 3)}$$

$$m_f = \Delta H_f / \Delta h_f \quad \text{(Eq. 4)}$$

$$X_i^{CII}[\%] = m^{II}_c / m_f \quad \text{(Eq. 5)}$$

Where T^{II}_c [K] is the freezing temperature belonging to the 2nd peak (inner aqueous phase)

$\Delta H^{II}_{m,c}$ [J/g] is the specific freezing enthalpy of the inner aqueous phase

ΔH^{II}_c [J] is the freezing enthalpy of the inner aqueous phase

$\Delta H_{m,f}$ [J/g] is the specific melting enthalpy

ΔH_f [J] is the melting enthalpy

m^{II}_c is the mass of the frozen water of inner aqueous phase

m_f is the mass of the molten water

Δh^{II}_c [J/g] is the latent heat of freezing of inner aqueous phase

Δh_f is the latent heat of melting, it is taken from literature (79,9 cal/g(334,5253 J/g) for pure water at 0 °C).

X_i^{CII} [%] is the mass fraction of the inner frozen aqueous phase

Equation 2. describes the empiric relationship between the mass latent heat of freezing (Δh^{II}_c) and the freezing temperature of dispersed aqueous phase (T^{II}_c) in the undercooled region (223 K < T_c < 273 K) [68].

3.3.2.2 Rheological analysis

The aim and the benefit of rheological analysis [46, 78-80] are the following:

1) the changes of the structure (e.g solidification during storage, composition-change) can be properly and precisely traced by rheological measurements,
2) the rate and speed of drug release is influenced by rheological properties
3) consistency directly affects the rate of spreading, adhesion on surface of application

The rheological analysis can be achieved in case of:

- non-diluted multiple emulsions
- dilutions in pure aqueous solutions; the rate of dilution is generally 1/5 [2].

Rheological properties of multiple emulsions are affected by several factors, such as: the volume fraction, viscosity, practical size and distribution, chemical nature of the disperse phase; the chemical constitution, concentration, solubility of the emulsifiers; the physical properties of the interfacial film, viscosity, chemical constitution and polarity of the continuous phase [21].

Various types of rheological methodologies can be used in order to characterize multiple emulsions. From the two types of rheology analysis (oscillatory viscosimetry and rotary viscosimetry) [79], the most widely used rotary viscosimetry method is used in characterising the behaviour of such systems.

Rotary viscosimetry

Rotary viscosimetry can be used to record flow-curves of coherent systems, and to define structural viscosity and tixotropy. Flow-curves provide information about the structure, the forces giving cohesion to the structure and the resistance against shear rate.

The rheological changes during storage nicely reflect the structural changes of multiple emulsions. The changes are mostly expressed by viscosity and tixotropy increase.

For some multiple emulsion formulations, the shear is able to induce irreversible stuctural changes. These changes can be shown by the rheograms in the course of rheological measurement. Therefore, the shear can be considered an artificial means of inducing ageing. Consequently, it can provide predictive information on the stability of the multiple emulsion.

Instrumental rheological measurements, practical information deduced from flow and viscosity curves, numerical features of elasticity and the quantitative measurement of spreading and adhesion can be beneficially applied for planning emulsion compositions and selecting the semisolid system most appropriate for purpose [81].

Quantitative determination of volume fraction of inner aqueous phase

By means of Mooney law [1, 2, 21, 44] it is possible to obtain a quantitative relationship between the volume fraction ϕ and the relative viscosity η_r:

$$\ln \eta_r = \alpha\phi_d / 1-\lambda\phi_d \qquad \text{(Eq. 6)}$$

where α and λ are two coefficients called the form and crowding factors, respectively.

The Mooney-law is generally well adapted for dispersed systems such as multiple emulsions. The elementary measurement of the viscosity allows to quantitatively following the change in the volume fraction of the multiple globules by applying the inverted Mooney law:

$$\phi_d = \ln \eta_r / \alpha + \lambda \ln \eta_r \qquad \text{(Eq. 7)}$$

The viscosity of simple emulsions has been correlated to the volume fraction of the dispersed phase ϕ_d; for Newtonian systems which ϕ_d<0,35% (v/v) and assuming that the dispersed phase is composed of monodispersed oil globules entrapping the aqueous dispersed phase. Some of the authors [2] declared, that the Mooney equation is valid only under the above mentioned conditions.

In case of w/o/w multiple emulsions, the dispersed phase is oil and water ($\phi_d=\phi_w+\phi_o$). It is therefore possible to estimate the volume fraction of the inner aqueous phase ϕ_w from the inversed Mooney's equation:

$$\phi_w = (1-\lambda\phi_o)\ln \eta_r - \alpha\phi_d / \alpha + \lambda \ln \eta_r \qquad \text{(Eq. 8)}$$

Accordingly, from this last equation, it is possible to estimate the rate of swelling or shrinkage of the inner aqueous phase on ageing from the rate of the viscosity change.

4. EXPERIMENTAL PART

4.1 MATERIALS AND METHODS

4.1.1. Materials

Different kind of oil derivatives of various polarities give the oil phase of the investigated systems. The values of the polarity index at room temperature are given in the Table 2.

Mineral oil derivatives: liquid petrolatum /Paraffinum liquidum/ (Paraffin oil, Gustav Hess GmbH, Ph.Eur.4th), 2,2,4,4,6,6,8- heptamethylnonane /Isohexadecane/ (Arlamol HD, 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/ (Cetiol 868, Cognis, Ph.Eur.4th), (Table 2).

Table 2: *Lipophilic agents used during the preparation of w/o/w emulsions*

Lipophilic agents	Polarity Index$^{(1)}$ (mN/m)
Paraffinum liquidum	42.5
Isohexadecane	38.6
Isopropyl Myristate	24.3
Octyl Stearate	21.0
Avocado oil	11.5
Corn germ oil	9.6

(1) Polarity Index: Interfacial tension between oil and water

The surfactants used during the preparation of w/o/w emulsions were: poly(oxy-1,2ethanediyl) distearate /Steareth-2/ (Brij 72, Uniqema, Uniqema grade), poly(oxy-1,2ethanediyl) distearate /Steareth-21/ (Brij 721, Uniqema, Uniqema grade), polyoxyethylene (30) dipolyhydroxystearate /PEG-30 Dipolyhydroxystearate/ (Arlacel P135, Uniqema, Uniqema grade), block copolymer of polyethylene oxide and polypropylene oxide /Poloxamer 407/ (Synperonic PE/F 127, Uniqema, Uniqema grade) (Table 3).

Viscosity increasing agents in external aqueous phase: sodium alginate (Manugel DJX, ISP Alginates Ltd., Cosmetics grade), carbomer (Carbopol Ultrez-10, BF. Goodrich, Ph.Eur.4th) can be found in Table 4.

Six different compositions were prepared during the experiments (Table 4).

A model substance - Urea (Honeywell Co. Belgium, Ph.Eur.4th) was used in the inner aqueous phase of the compositions 2/C/a (8 m/m%) in order to simulate the osmotic pressure change induced by the active ingredient [70], and a model active agent - Ketamine hydrochloride (Calypsol, Richter Ph.Eur.4th) was used in the aqueous phases of the compositions 2/C/b (1 m/m %).

Table 3: *Surfactants used in the formulations*

Preparation of emulsion	Surfactants			
	Stabilization of the 1st interface		Stabilization of the 2nd interface	
	name	HLB value	name	HLB value
One-step technology	Steareth-2	4.9	Steareth-21	15.5
Two-step technology	PEG-30 Dipolyhydroxystearate	5.0-6.0	Poloxamer 407	high (exact value not presented by the manufacturer)

4.1.2 Methods

4.1.2.1 Emulsion preparation

Multiple w/o/w emulsions were formulated with the one-step and the two-step technology [2, 4, 9, 10]. 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 [11]. 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 [4].

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 homogenization process (5 minutes at 1000 – 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 [2].

Table 4: *Components of the multiple emulsions [m/m%]*

	Ingredients of w/o/w emulsions						
Manufacture	(1) One-step ($w_1/o/w_{1)}$			(2) Two-step ($w_1/o/w_{2)}$			
	(A)	(B)	(C)	(A)	(B)	(C)	
						C/a	C/b
<u>Oil phase</u>							
Paraffinum liquidum Isohexadecane	4.00 5.00	-	-	7.50 7.50	-	-	
Isopropyl Myristate Octyl Stearate	-	4.00 5.00	-	-	7.50 7.50	-	
Avocado oil Corn germ oil	-	-	4.00 5.00	-	-	7.50 7.50	7.50 7.50
Surfactants							
Steareth-2	3.00	3.00	3.00	-	-	-	
Steareth-21	2.00	2.00	2.00	-	-	-	
PEG-30 Dipolyhydroxystearate	0.50	0.50	0.50	4.00	4.00	4.00	4.00
Poloxamer 407	-	-	-	2.00	2.00	2.00	2.00
Viscosity increasing agents							
Sodium alginate	0.30	0.30	0.30	-	-	-	
Carbomer	-	-	-	0.30	0.30	0.30	0.75
Model active agent							
Urea	-	-	-	-	-	8.00	
Ketamine hydrochloride							1.00

4.1.2.2 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.

4.1.2.3 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.

4.1.2.4 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 [82-85] (RheoWin Pro Data Manager 1997):

$$\sqrt{\tau} = \sqrt{\tau_c} + \sqrt{(\eta_c * \gamma)} \qquad \text{(Eq. 9)}$$

Flow curve model function with the “Casson yield point” τ_c and the „Casson viscosity” η_c. Besides the flow curves, viscosity curves of the different samples were also determined. 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 (Synperonic PE127F).

4.1.2.5 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 and 4h. 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 [86-90].

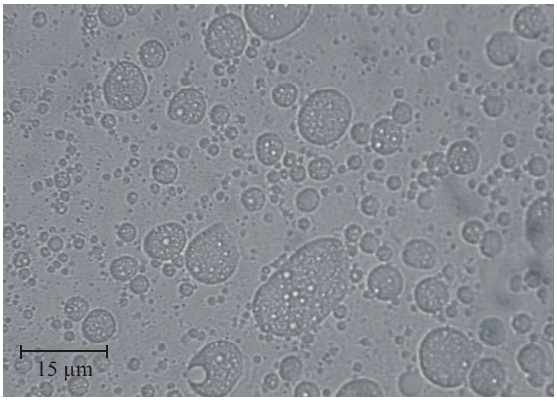
Characterization of drug release vs. time curve:

$$Q=Q_0+m\sqrt{t} \qquad \text{(Eq.10)}$$

4.2 RESULTS AND DISCUSSION

4.2.1 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 the 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 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 Figure 3.



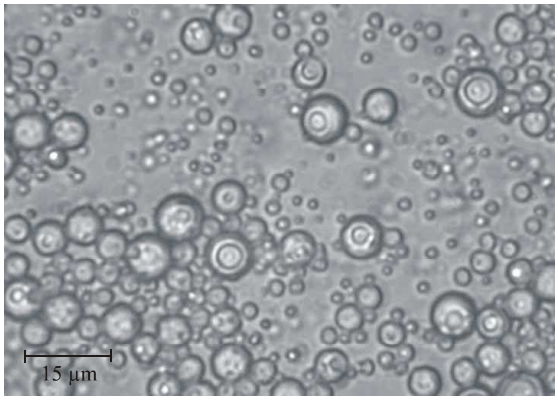


Fig. 3 a *Fig 3 b*

*Figure 3: Microscopic photograph of multiple emulsions made by one-step (**a**) and two step (**b**) procedures. Magnification 100x was used during the microscopic observation.*

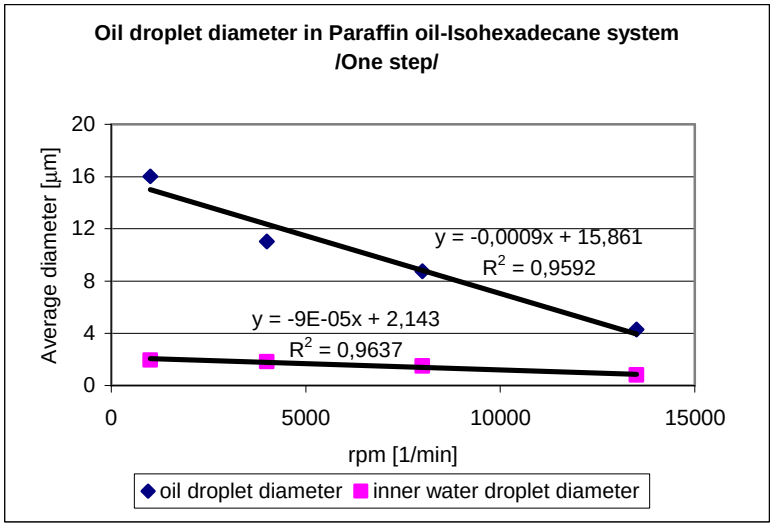
4.2.1.1 Droplet size analysis:

According to the data of droplet size analysis (Table 5a, 5b) 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.

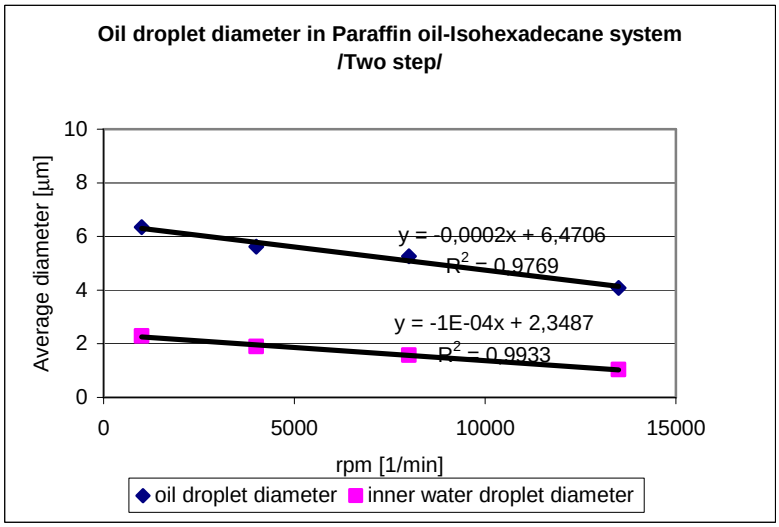
Figure 4a, 4b presenting the droplet size values showing, that with both technologies both the oil droplet size and the inner water droplet size changes, when plotted against the stirring rate.

The following diagram (Figure 5) illustrates how the investigated systems change depending on the stirring rate. It can be seen what the proper stirring rate is in case of homogeneous systems.

It can be concluded on the basis of these results (Table 5a, 5b), 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 (samples: 1A and 2A) – non-polar lipophilic substances - the number of multiple droplets (Figure 6) was considerably lower, thus these were not examined with DSC.



(a)



(b)

Figure 4: Values of the oil droplet and inner water droplet average diameter plotted against the stirring rate (a) "one step", (b) "two step"

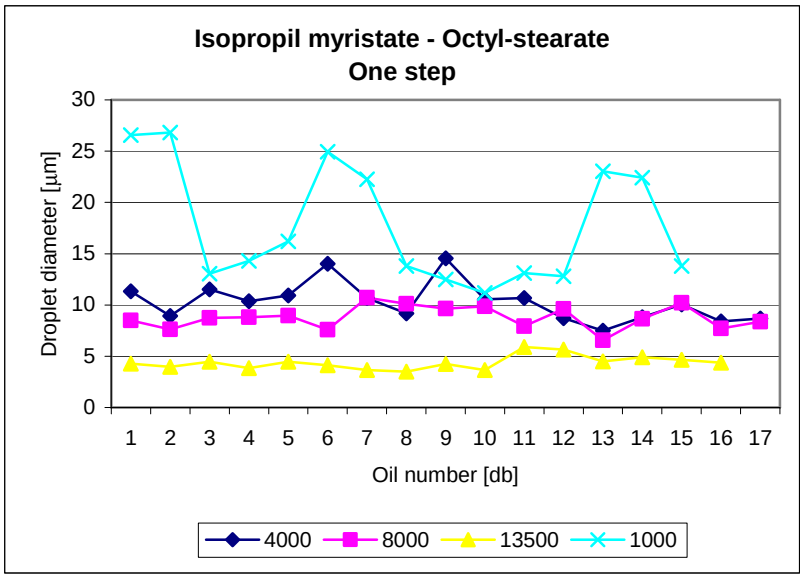


Figure 5: Change of the oil droplet diameter by increasing the stirring rate

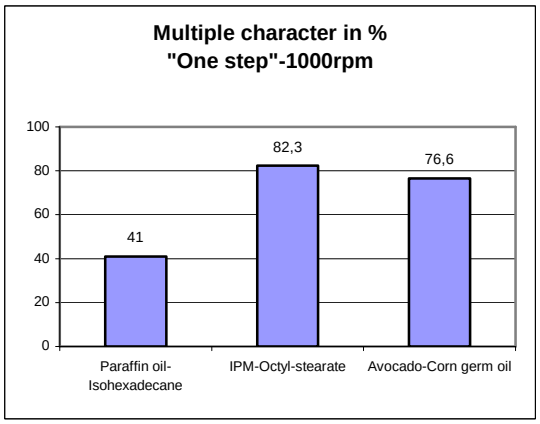


Fig.: 6 a

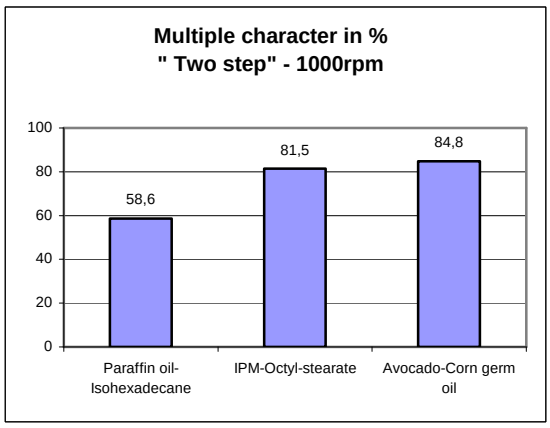


Fig.: 6 b

Figure 6: Rate of multiple droplets in multiple systems on the score of microscopic photograph

Table 5a: Data of droplet size of emulsion prepared with one-step technology

Composition	Paraffin oil-Isohexadecane		Isopropyl-myristate-Octyl-stearate		Avocado oil-Corn germ oil	
	Oil droplet	Inner water droplet	Oil droplet	Inner water droplet	Oil droplet	Inner water droplet
Average diameter of droplets /μm/ + S.D.	Average droplet∅ /μm/	Average droplet∅ /μm/	Average droplet∅ /μm/	Average droplet∅ /μm/	Average droplet∅ /μm/	Average droplet∅ /μm/
Stirring rate/rpm/						
1000	16.941 SD=±4.028	1.952 SD=±0.334	18.540 SD=±6.316	2.236 SD=±0.406	17.355 SD=±6.567	1.989 SD=±0.501
4000	11.870 SD=±2.709	1.834 SD=±0.204	11.319 SD=±1.719	1.844 SD=±0.378	14.042 SD=±2.506	1.867 SD=±0.256
8000	8.775 SD=±0.903	1.488 SD=±0.199	8.983 SD=±1.557	1.374 SD=±0.226	10.754 SD=±1.936	1.442 SD=±0.246
13500	4.308 SD=±0.633	0.795 SD=±0.178	4.384 SD=±0.169	0.941 SD=±0.136	4.226 SD=±0.401	0.866 SD=±0.149

Table 5b: Data of droplet size of emulsion prepared with two-step technology

Composition	Paraffin oil-Isohexadecane		Isopropyl-myristate-Octyl-stearate		Avocado oil-Corn germ oil	
	Oil droplet	Inner water droplet	Oil droplet	Inner water droplet	Oil droplet	Inner water droplet
Average diameter of droplets /μm/ + S.D.	Average droplet∅ /μm/	Average droplet∅ /μm/	Average droplet∅ /μm/	Average droplet∅ /μm/	Average droplet∅ /μm/	Average droplet∅ /μm/
Stirring rate/rpm/						
1000	6.343 SD=±0.713	2.294 SD=±0.751	7.862 SD=±1.411	2.158 SD=±0.414	7.114 SD=±1.131	1.988 SD=±0.274
4000	5.919 SD=±0.781	1.896 SD=±0.537	7.361 SD=±1.427	1.734 SD=±0.273	5.587 SD=±0.716	1.842 SD=±0.412
8000	5.745 SD=±0.757	1.573 SD=±0.312	5.721 SD=±1.355	1.625 SD=±0.457	5.264 SD=±0.706	1.642 SD=±0.416
13500	4.568 SD=±0.686	1.038 SD=±0.286	4.452 SD=±0.806	1.505 SD=±0.475	4.095 SD=±0.965	1.559 SD=±0.327

4.2.1.2 The effect of entrapped drugs on the structure of multiple emulsions
A) Urea

Microscopic photographs show multiple emulsions made by two-step technology (1000 rpm) with oil phase containing avocado oil and corn germ oil. Urea (8 m/m%) is only included in the inner water phase. Remained stable for half a year period at room temperature. The photos were taken of a 1/10 dilution.

It can be observed, that the presence of urea had a great impact on the droplet structure. Figure 7a shows, that 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 (Figure 7b,7c,7d), then by the gradual increase of the osmotic pressure the membrane of the oil phase broke up leading to mixture of the two aqueous phases (Figure 7e). Magnification 100x was used in the microscopic observation.

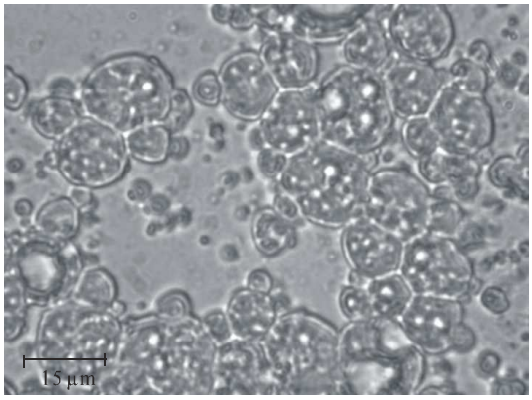


Figure 7a:Multiple emulsion containing urea at the time of preparation

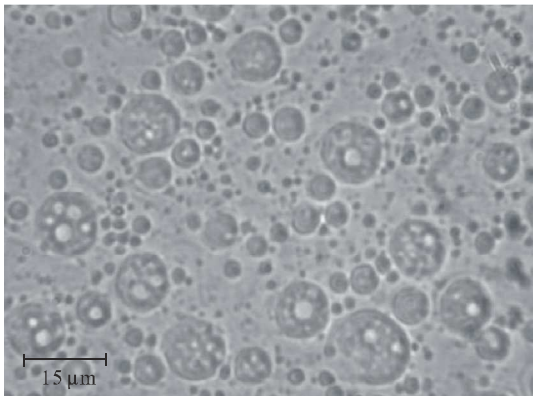


Figure 7b:Multiple emulsion containing urea (after 1 month)

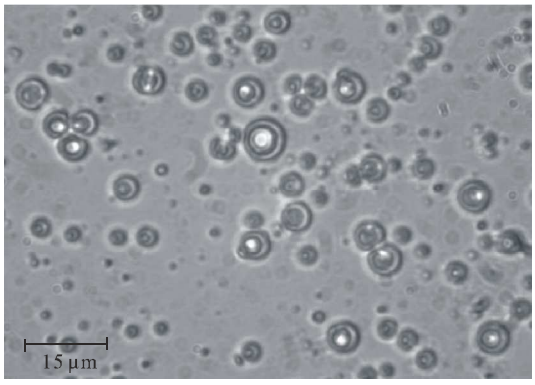


Figure 7c:Multiple emulsion containing urea (after 3 months)

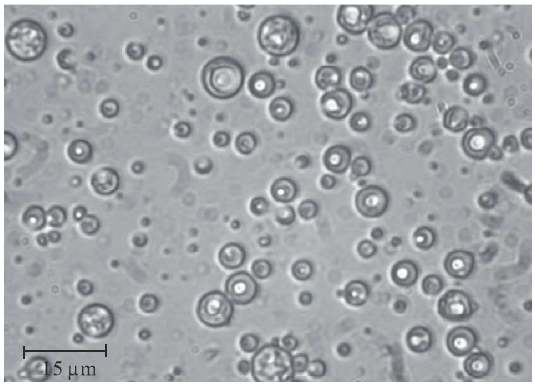


Figure 7d:Multiple emulsion containing urea (after 4 months)

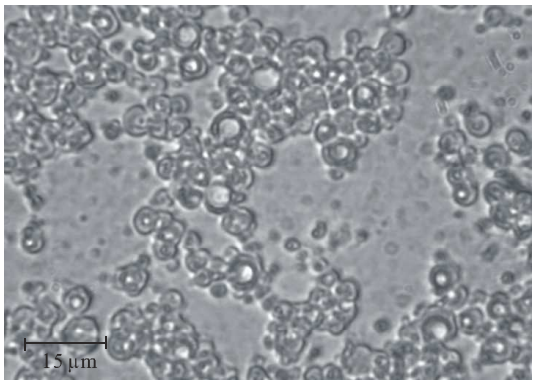


Figure 7e: Multiple emulsion containing urea (after 6 months), showing the breakdown

B) Ketamine-hydrochloride

Microscopic photographs show, the presence of the ketamine-hydrochloride in the inner aqueous phase (its ionic character) changes the osmotic pressure, consequently occurring changes in the system made by two-step technology (8000 rpm) with oil phase containing avocado oil and corn germ oil. Ketamine-hydrochloride (1 m/m%) is only included in the inner water phase. The photos were taken of a 1/10 dilution. Magnification 100x was used in the microscopic observation.

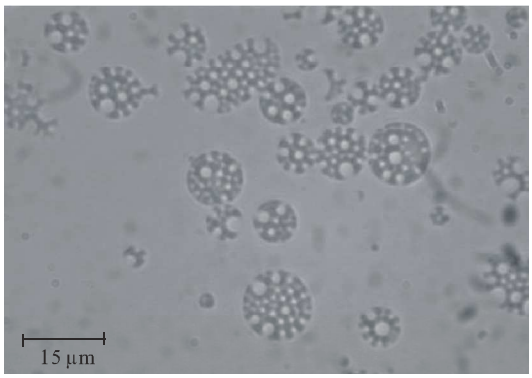


Figure 8a: 1 hour after preparation

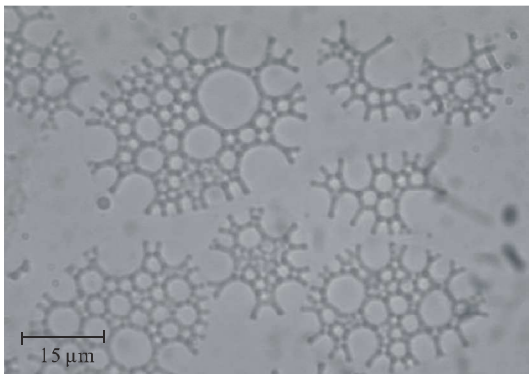


Figure 8b: 2 hours after preparation

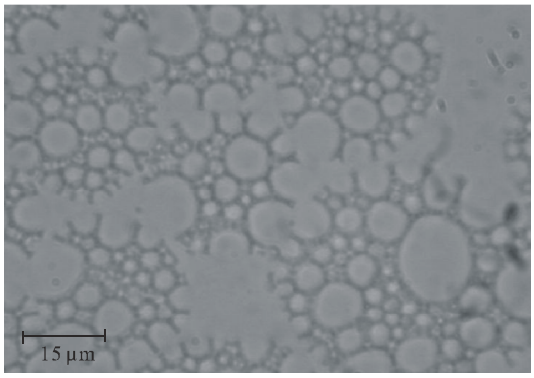


Figure 8c: 3 hours after preparation

As the microscopic photographs taken during the investigation of the multiple emulsions with ketamine hydrochloride show, that the presence of the drug in the inner aqueous phase (its ionic character) changes the osmotic pressure, consequently occurring changes in the system. 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 8a, 8b, 8c).

Modifications in the composition were needed in order to overcome this stability problem. With the aim of stabilising the multiple emulsions containing ketamine-hydrochloride 1 m/m% NaCl was added into the external aqueous phase in order to balance the external and inner aqueous phase. The experiment was unsuccessful. Then I divided the 1.0m/m% ketamine hydrochloride content, adding 0.5 m/m% drug in the external aqueous phase and 0.5 m/m% in the inner aqueous phase in order to decrease the osmotic pressure between the two aqueous phases. Furthermore, the quantity of polymer - which increases the viscosity in the external aqueous phase - was increased from 0.3 m/m% to 0.75 m/m%. This composition remained stable even after 1-year storage at 5°C (Figure 9).

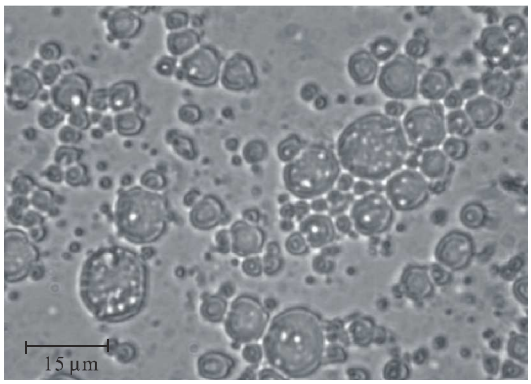


Figure 9: Multiple emulsion containing ketamine-hydrochloride (after 1 day)

In the microscopic photographs the variant behaviour of the different active agents in the same multiple system can be seen well.

4.2.2 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 10).

The size of the second exothermic peak decreases and gradually disappears, which is due to the breakdown of the multiple structure.

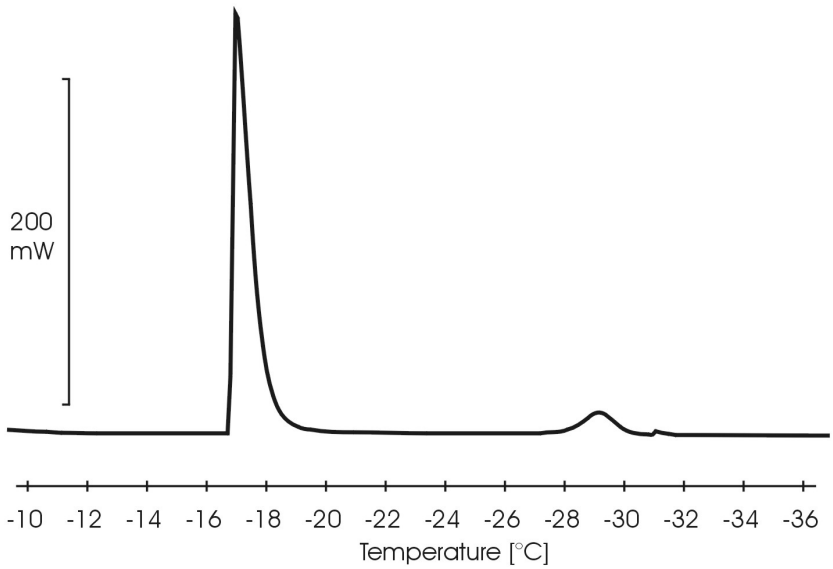


Figure 10: Typical DSC curve of w/o/w multiple emulsion. The first peak represents the external aqueous phase (-18°C) and the second peak shows the inner aqueous phase (-30°C)

b) Quantitative results

Table 6a, 6b and 7a, 7b present the values calculated with the empirical relationship presented in chapter 3. The temperatures measured in °C by the DSC equipment were converted into Kelvin (K).

Table 6: *Mass of sample (**m[mg]**), specific freezing enthalpy data of inner aqueous phase (**$\Delta H^{II}_{m,c}$[J/g]**), freezing enthalpy data of inner aqueous phase (**ΔH^{II}_{c}[J]**),freezing temperature data of inner aqueous phase (**T^{II}_{c}[°C]**), value of specific melting enthalpy (**$\Delta H_{m,f}$[J/g]**), value of melting enthalpy (**ΔH_f[J/g]**) and mass fraction of inner aqueous phase after freezing(**X_i^{CII}[%]**) in case of on- step emulsion*

Table 6a: Isopropyl Myristate(IPM), Octyl Stearate (Cetiol)

Technology	„One-step" w/o/w emulsion						
Ingredients	IPM – Cetiol						
	m [mg]	**$\Delta H^{II}_{m,c}$ [J/g]**	**ΔH^{II}_{c} [J]**	**T^{II}_{c} [°C]**	**$\Delta H_{m,f}$ [J/g]**	**ΔH_f [J]**	**X_i^{CII} [%]**
rpm							
1000	10.43	4.14	0.0432	-45.30	176.72	1.8432	3.50
4000	10.29	5.81	0.0598	-45.35	176.70	1.8182	4.92
8000	10.11	7.30	0.0738	-45.13	166.26	1.6809	6.54
13500	10.30	3.12	0.0321	-45.15	172.67	1.7785	2.69

Table 6b: Avocado oil-corn germ oil containing systems

Technology	„One-step" w/o/w emulsion						
Ingredients	Avocado oil-Corn germ oil						
	m [mg]	**$\Delta H^{II}_{m,c}$ [J/g]**	**ΔH^{II}_{c} [J]**	**T^{II}_{c} [°C]**	**$\Delta H_{m,f}$ [J/g]**	**ΔH_f [J]**	**X_i^{CII} [%]**
rpm							
1000	10.30	1.85	0.0191	-46.29	211.01	2.1734	1.33
4000	10.92	1.04	0.0114	-45.71	192.87	2.1061	0.81
8000	10.09	2.10	0.0212	-45.46	223.06	2.2507	1.41
13500	10.83	0.84	0.0091	-45.63	198.58	2.1506	0.64

Table 7: *Mass of sample (**m [mg]**), specific freezing enthalpy data of inner aqueous phase (**$\Delta H^{II}_{m,c}$ [J/g]**), freezing enthalpy data of inner aqueous phase (**ΔH^{II}_{c} [J]**), freezing temperature data of inner aqueous phase (**T^{II}_{c} [°C]**), value of specific melting enthalpy (**$\Delta H_{m,f}$ [J/g]**), value of melting enthalpy (**ΔH_f [J/g]**) and mass fraction of inner aqueous phase after freezing (**X_i^{CII}[%]**) in case of two-step emulsion*

Table 7a: Isopropyl Myristate (IPM), Octyl Stearate (Cetiol)

Technology	„Two step" w/o/w emulsion						
Ingredients	IPM – Cetiol						
	m [mg]	**$\Delta H^{II}_{m,c}$ [J/g]**	**ΔH^{II}_{c} [J]**	**T^{II}_{c} [°C]**	**$\Delta H_{m,f}$ [J/g]**	**ΔH_f [J]**	**X_i^{CII} [%]**
rpm							
1000	10.35	1.36	0.0141	-34.54	277.65	2.8737	0.65
4000	11.30	1.42	0.0160	-37.80	268.90	3.0386	0.72
8000	11.05	1.63	0.0180	-35.21	277.30	3.0642	0.78
13500	10.66	1.03	0.0110	-38.22	270.51	2.8836	0.52

Table 7b: Avocado oil-corn germ oil containing systems

Technology	„Two step" w/o/w emulsion						
Ingredients	Avocado oil-Corn germ oil						
	m [mg]	**$\Delta H^{II}_{m,c}$ [J/g]**	**ΔH^{II}_{c} [J]**	**T^{II}_{c} [°C]**	**$\Delta H_{m,f}$ [J/g]**	**ΔH_{f} [J]**	**X_i^{CII} [%]**
rpm							
1000	10.34	1.95	0.0202	-30.46	262.27	2.7119	0.94
4000	10.32	2.58	0.0266	-32.17	273.74	2.8250	1.21
8000	10.93	2.93	0.0320	-29.62	254.30	2.7795	1.45
13500	10.43	2.39	0.0249	-31.66	268.56	2.8011	1.14

The proper stirring rate can be chosen on the basis of the results presented in Tables 6a, 6b and 7a, 7b. 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 homogenity investigation (Fig.5.).

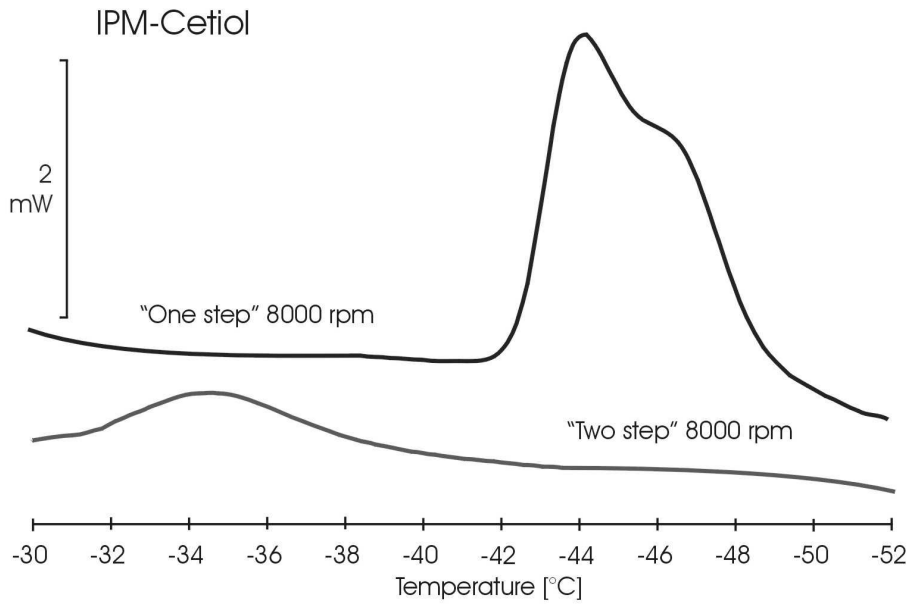


Figure 11.: *DSC curve of w/o/w emulsion containing Isopropyl Myristate(IPM) and Octyl Stearate (Cetiol) ester made by different preparation methods. (Curves indicate the crystallisation of the inner aqueous phase.)*

The shape of the thermogram (Figure 11) is in agreement with the droplet structure seen in the light microscopic images (Figure 3). While in the case of the one-step technology the second peak, indicating the presence of the inner aqueous phase, appeared at about –45°C, in the case of the two-step technology it could already been detected between –30 °C and –40 °C, which is due to the size difference of water droplets, since smaller droplets freeze at lower temperatures [72].

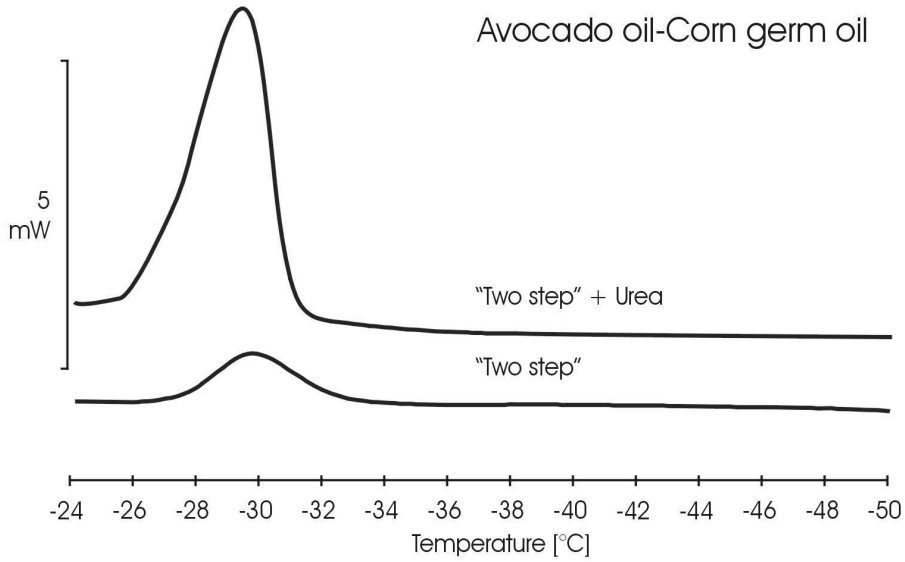


Figure 12: *DSC curve of a multiple emulsion containing avocado oil and corn germ oil with and without urea indicating the freezing of the inner aqueous phase.*

Figure 12. shows how 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 (sample: 2/C/a) 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, as it can be seen in Figure 13. 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 [73]. 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.

These result correlate with the rheological measurements (see later, chapter 4.2.3).

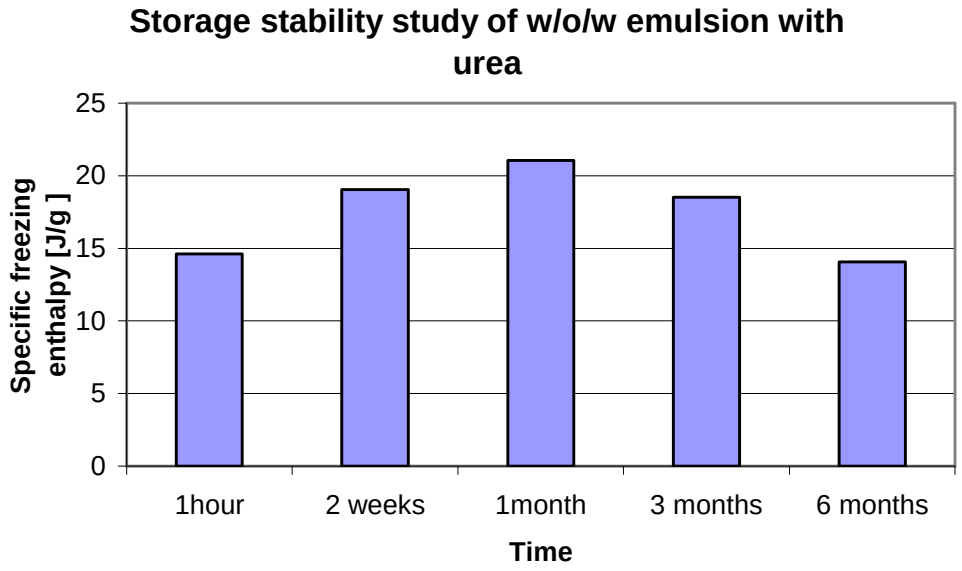


Figure 13: *Specific freezing enthalpy data (determined by DSC) of w/o/w multiple emulsion 2/C/a (urea incorporated) made by the two-step technology measured after different storage time.*

4.2.3 Rheological investigations

The rheological measurement were 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.
Most multiple emulsions are non-Newtonian and the steady state shear stress -shear rate curves show pseudoplastic flow as illustrated in Figure 14. and 16.

4.2.3.1 W/o/w emulsion with one-step technology

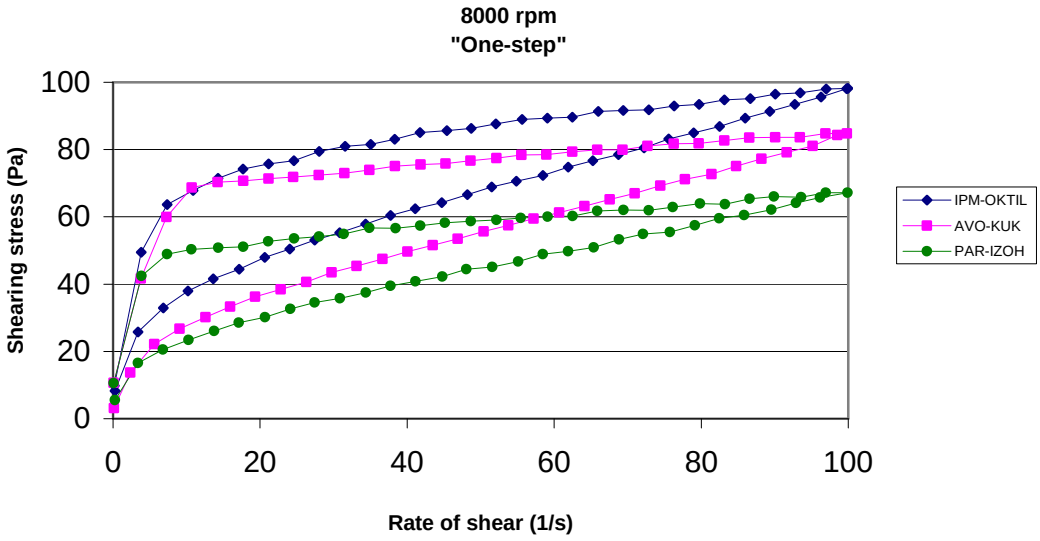


Figure 14: Flow curves of w/o/w emulsions containing different oils

It can be seen in 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 utilisation and storage better (Table 8). 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 (Figure 14).

On the basis of the data in case of one-step technology, shown in Table 8, 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 is decrease a bit, then increase at 8000rpm. The apparent/Casson yield point values can be correlated with the values in the thixotropic area in case of one-step technology.

Table 8: Apparent yield point/Casson yield point [Pa]

Casson yield point [Pa]						
	Paraffinoil-Isohexadecane.		IPM-Cetiol		Avocado oil-Corn germ oil/(Maize oil)	
r.p.m.	One step	Two step	One step	Two step	One step	Two step
1000	8.140 SD=±0.537	41.61 SD=±0.627	12.897 SD=±1.113	39.13 SD=±1.883	9.223 SD=±0.432	40.205 SD=±2.326
4000	11.670 SD=±0.877	42.825 SD=±0.262	17.337 SD=±0.592	41.550 SD=±1.011	11.235 SD=±0.573	41.005 SD=±0.995
8000	**9.859 SD=±0.567**	44.905 SD=±0.177	**8.697 SD=±295**	41.785 SD=±1.435	**10.910 SD=±0278**	42.060 SD=±1.089
13500	14.630 SD=±0.283	49.095 SD=±0.627	14.235 SD=±276	45.095 SD=±0.417	13.695 SD=±0.064	47.450 SD=±0.057

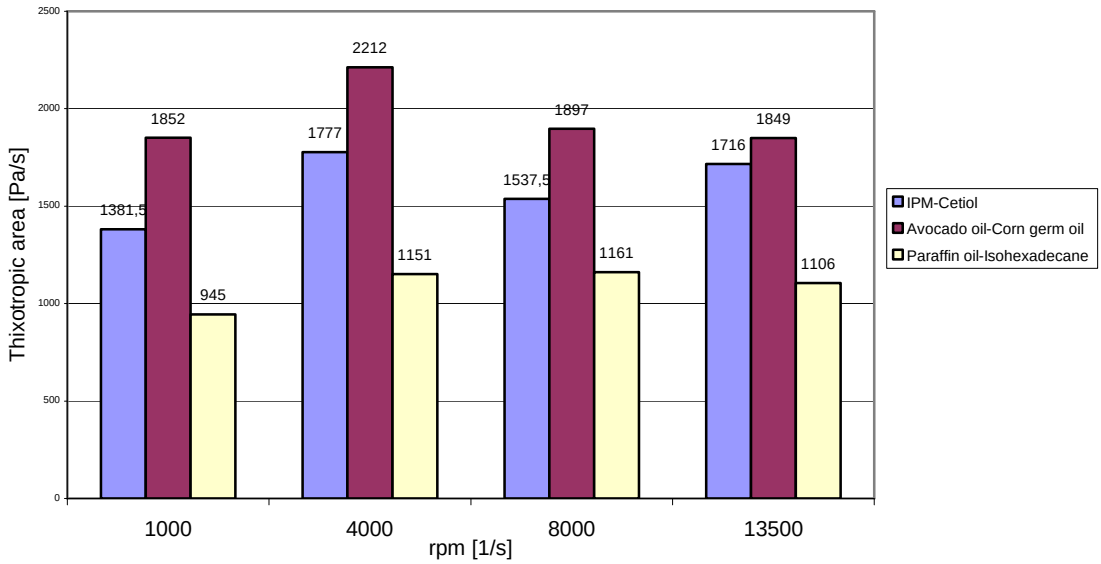


Figure 15: Thixotropic area of multiple emulsions containing different oils prepared with two-step technology

4.2.3.2 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 since the upward and downward curves were the same (Figure 16). An immediate regeneration took place in the system after deformation.

In these cases, the viscosity –the same as in the previous 4.2.3.1 chapter- decreases when the shear rate increases. This behavior is called shear-thinning.

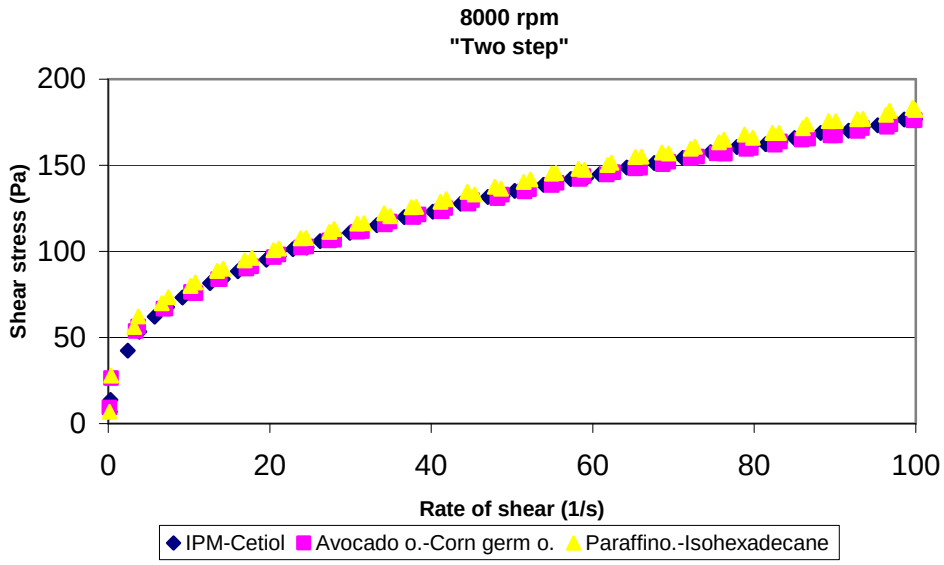


Figure 16: Flow curves of multiple emulsions containing different oils prepared with two-step technology.

Based on data presented in Table 9 the viscosity of the emulsions increase 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 data from droplet size analysis. Therefore the system is more compact, so the viscosity increases. This viscosity value also correlates with the Casson yield point (Table 8) in case of two-step technology.

The influencing effect of different active agents can be traced also with rheological investigations. 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.

Table 9: Data of viscosity of multiple emulsions containing different oils prepared with two-step technology.(shear rate is 10 1/s)

Data of viscosity [Pas]			
	Paraffinoil-Isohexadecane	IPM-Octyl-stearate	Avocado oil-Corn germ oil
r.p.m.			
1000	7.456	7.182	7.195
4000	7.793	7.541	7.445
8000	7.985	7.661	7.656
13500	8.929	8.107	8.427

4.2.3.3 Investigation of multiple emulsions containing urea

Rheograms show that viscosity is a key factor of the structure of multiple droplets. 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. The process is presented by the viscosity curves of multiple emulsion containing urea (Figure 17).

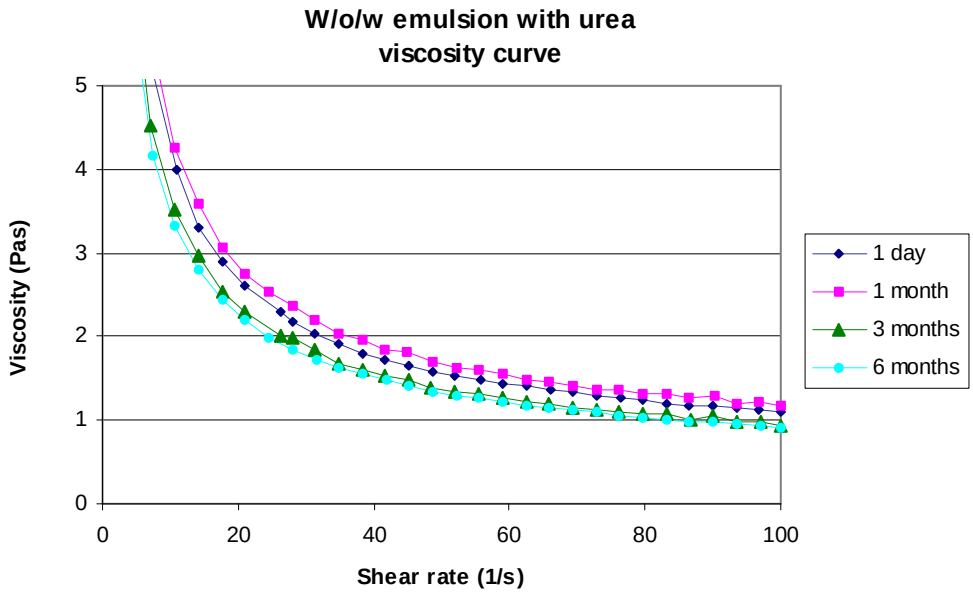


Figure 17: Change of viscosity of multiple emulsions containing urea during storage

The calculations with inversed Mooney equation (Eq.8) and its correlation with the relative viscosity and viscosity data (Table 10) also mirror the DSC measurements. The viscosity was measured at shear stress of 10 Pa (25°C). However, from inversed Mooney's equation (Eq.8), it is possible to estimate the rate of swelling or shrinkage of the inner aqueous phase on ageing from the rate of the viscosity change.

Table 10: Changes during storage: viscosity, relative viscosity and inner aqueous phase volume calculated.

	1 day	1month	3months	6months
Viscosity (mPas)	16240	17310	15095	12850
Relative viscosity	1160	1236.42	1078.21	917.85
ϕ_w (from Mooney eq.)	0.4284	0.4293	0.4270	0.4242

The change of the inner aqueous phases volume during storage can be estimated by Eq. 8. which is shown in Table 10. Since the emulsions in my studies non-Newtonian behaviour and the constants used are from the literature (based on the microscopic examination it is presumed that the given multiple emulsion consists of globules), the value derived from the inverted Mooney equation does not provide the exact volume fraction of the inner aqueous phase. It only reflects the structural changes of the w/o/w emulsions with urea during storage in absolute value.

This semiquantitative concept is suitable to follow production controls and storage. Figure 18. illustrates the viscosity changes during storage, also correlating with the DSC measurements (Figure 13).

If shear is considered an artificial means of inducing ageing, it can provide predictive information to understand the stability of the multiple emulsion.

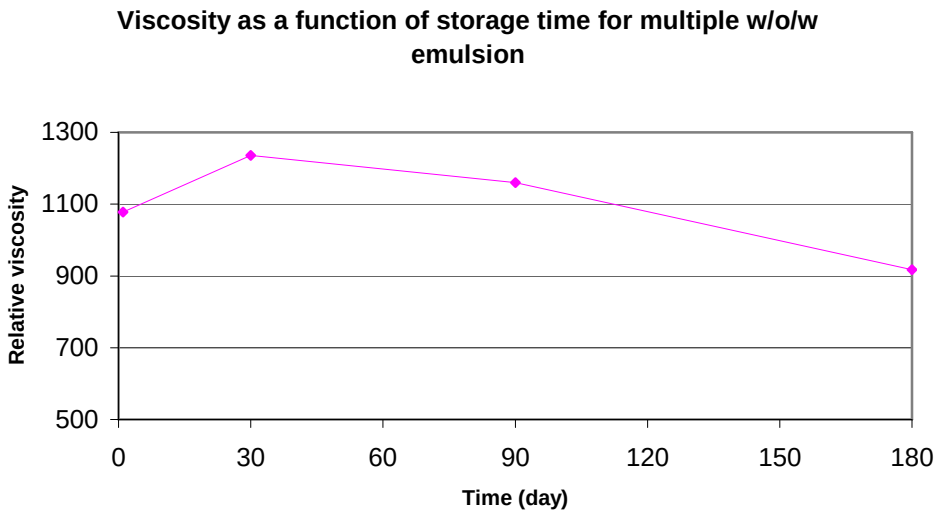


Figure 18: Viscosity as a function of storage time for multiple w/o/w emulsions containing urea in the inner aqueous phase. The viscosity was measured at shear stress of 10 Pa (25°C) and relative to the viscosity of external continuous aqueous phase.

4.2.3.4 Investigation of multiple emulsions containing ketamine hydrochloride

Figure 19 shows the changes in rheological parameters influenced by the added active agent – ketamine hydrochloride. The consistency of multiple systems in the presence ketamine hydrochloride remarkably changed.

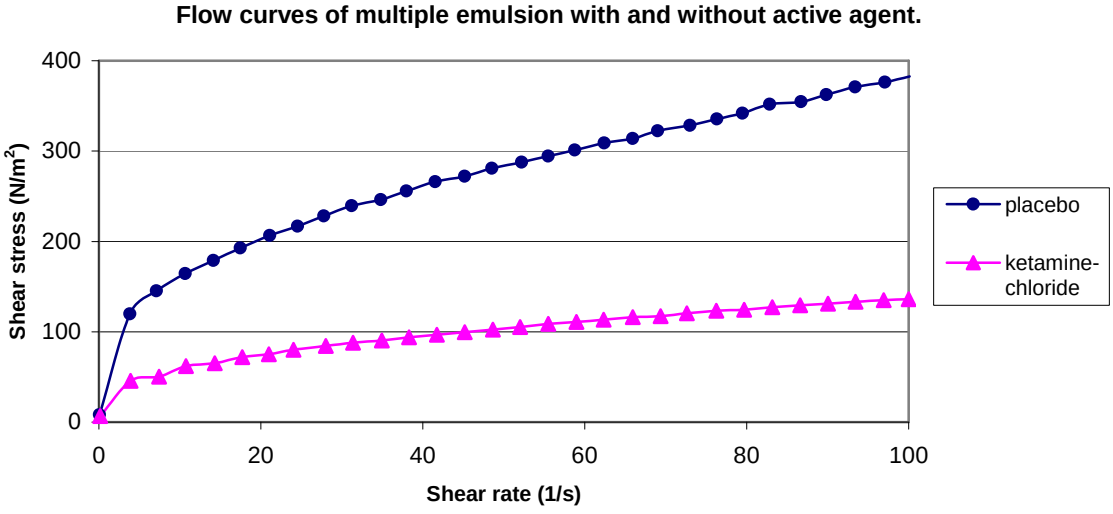


Figure 19: Comparison of the flow properties of multiple emulsion with and without the active agent.

The investigated emulsion system remained stable during storage (5°C), even after 1 year but viscosity increase was experienced that refers to the swelling of the inner aqueous phase (Figure 20.).

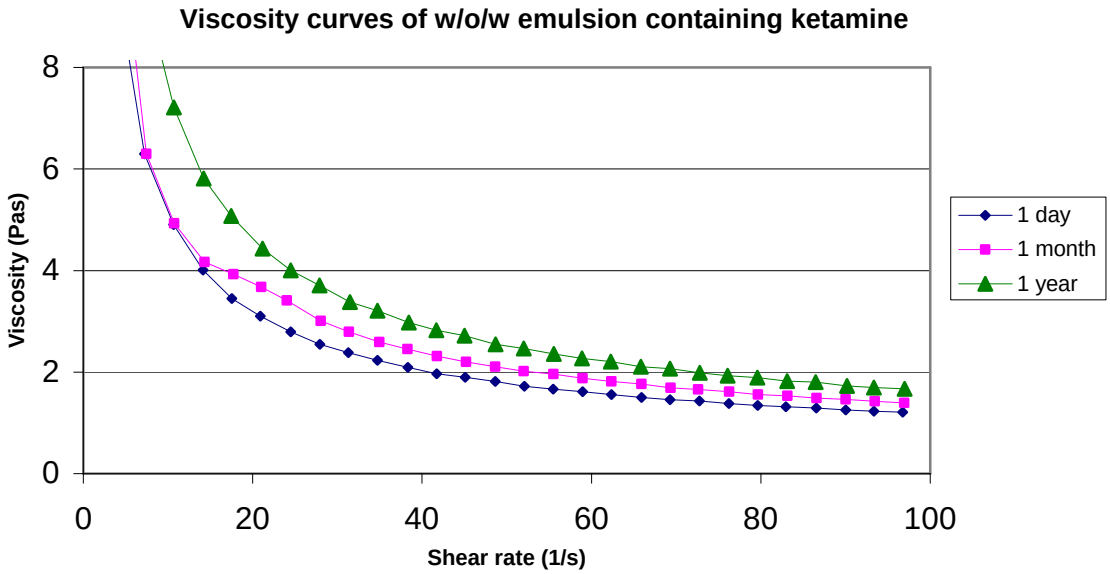


Figure 20: Viscosity changes of w/o/w multiple emulsions containing 0.5 m/m% ketamine hydrochloride in the inner aqueous phase and 0.5 m/m% ketamine hydrochloride in the external aqueous phase during storage

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, breakup) 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.

4.2.4 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.

Figure 21. shows the released drug amount in %, plotted against time.

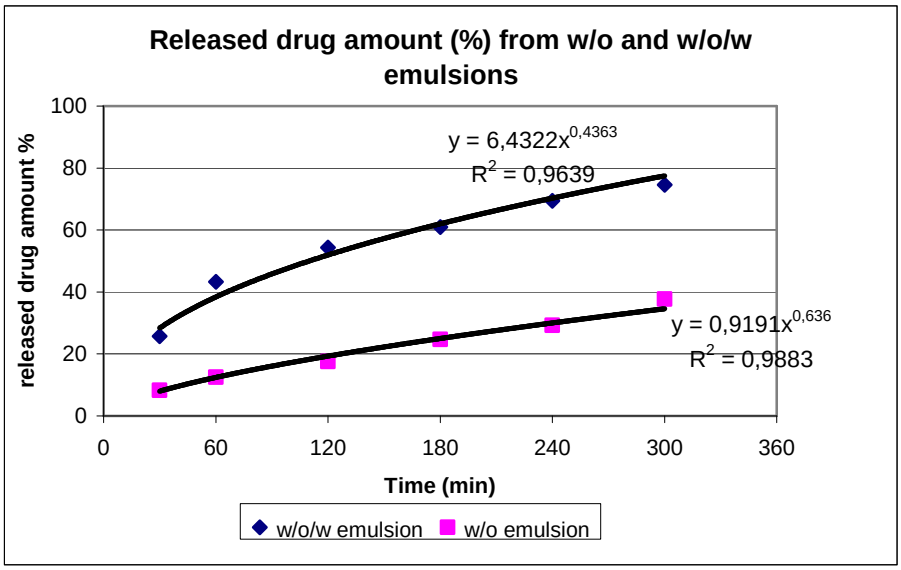


Figure 21: Released drug amount (%) from simple and multiple emulsions

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 (Figure 22.).

The next table (Table 11.) shows the differences between the two emulsions systems.

Table 11: Comparison of the diffusion rates, correlation coefficients and the cumulative released drug amounts

Compositions	**Diffusion coefficient (m)**	**Correlation coefficient (R^2)**	**Release drug amount after 5h (µg/cm^2)**
w/o/w emulsion	46,31	0,9873	1226,83
w/o emulsion	40,33	0,9799	662,56

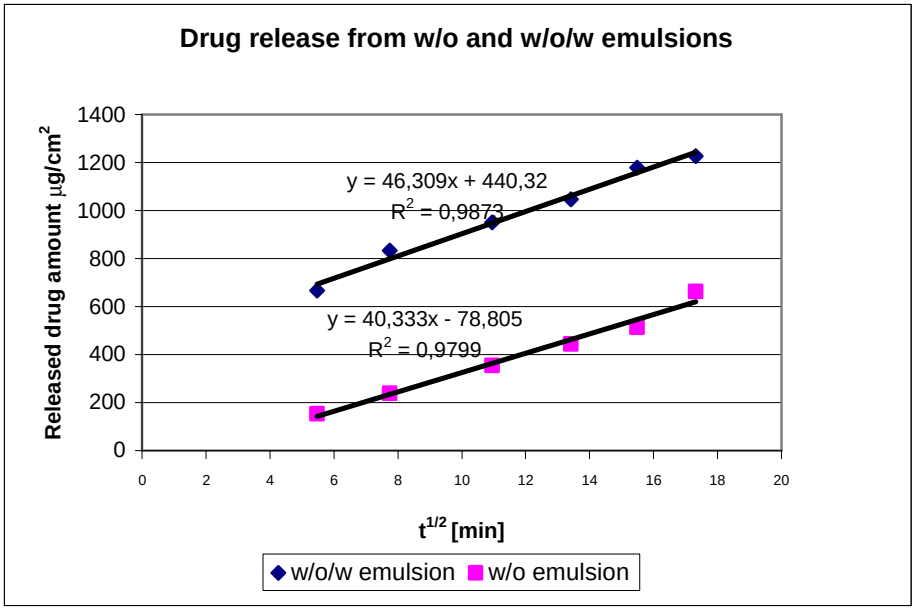


Figure 22: Cumulative released ketamine through synthetic membrane soaked in buffer solution.

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 (1) differences in the rheological parameters of the systems and (2) 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.

4.3 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 follow: 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 in the course of 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.

5. SUMMARY

The aim of this research work was to specify the role of different structural investigation methods in the 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 structure.

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:

- 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, 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 provides 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 manufacturing stage of product development and production.

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8. ANNEX

Publications related to the subject of thesis