Ph.D. Thesis

Formulation and Investigation of Gel-Emulsions Containing Polymeric Emulsifiers

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ARTICLES RELATED TO THE PH.D. THESIS

I. **Szűcs Mária**, Budai Szabolcs, Erős István, Gel-emulsion systems I: Physical-chemical characterisation, *Acta Pharmaceutica Hungarica 78 (2008) 11-21* **IF: -**

II. Budai Szabolcs, **Szűcs Mária**, Erős István, Gel-emulsion systems II: Stability, *Acta Pharmaceutica Hungarica 78 (2008) 23-30* **IF: -**

III. **Mária Szűcs**, Giuseppina Sandri, M. Cristina Bonferoni, Carla M. Caramella, Patrizia Vaghi, Piroska Szabó-Révész, István Erős, Mucoadhesive behaviour of emulsion containing polymeric emulsifier, *European Journal of Pharmaceutical Science 34 (2008) 226-235* **IF: 3.127**

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OTHER PUBLICATION

I. Péter Sipos, **Mária Szűcs**, András Szabó, István Erős, Piroska Szabó-Révész, An assessment of the interaction between diclofenac sodium and ammonio methacrylate copolymer using thermal analysis and Raman spectroscopy, *Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 288-294* **IF: 2.761**

ABSTRACTS

I. **Szűcs Mária**: Polimer emulgensekkel stabilizált emulziók, Ph.D. *Tudományos Nap, Szegedi Akadémiai Bizottság Székháza, 2006. május 3.*

II. István Erős, **Mária Szűcs**, Szabolcs Budai, Erzsébet Csányi, Zsolt Makai, Péter Sipos, András Fehér and Piroska Szabó-Révész: Physico-chemical investigation of acrylate based polymeric emulsifiers, *5th World Meeting on Pharmaceutics Biopharmaceutics and Pharmaceutical Technology, Geneva, Switzerland, 27th to 30th March 2006*

III. **Szűcs Mária**, Révész Piroska, Erős István: Polimer emulgensek fizikai-kémiai vizsgálata, *Congressus Pharmaceuticus Hungaricus XIII, Budapest, 2006. május 25-27.*

IV. **Szűcs Mária**, Révész Piroska, Erős István: Polimer emulgenst tartalmazó emulziók szerkezetvizsgálata, *Gyógyszerkutatási Szimpózium, Debrecen, 2006. november 24-25.*

V. **Szűcs Mária**: Polimer emulgensek bioadhéziója, *Magyar Tudomány Ünnepe, Szegedi Akadémiai Bizottság Székháza, 2007. november 6.*

VI. Erős István, **Szűcs Mária**: Gél-emulziók tervezése és vizsgálata, *Kozmetikai Szimpózium 2007, Budapest, 2007. november 8.*

VII. **Szűcs Mária**, Giuseppina Sandri, Carla Caramella, Szabóné Révész Piroska, Erős István: Polimer emulgenseket tartalmazó rendszerek bioadhezív tulajdonságainak jellemzése, *Gyógyszerkutatási Szimpózium, Szeged, 2007. november 9-10.*

VIII. **Mária Szűcs**, Giuseppina Sandri, M. Cristina Bonferoni, Carla M. Caramella, Patrizia Vaghi, Piroska Szabó-Révész, István Erős, Bioadhesive study of gel-emulsions: effect of the components and the microstructure, *6th World Metting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Barcelona, Spain, 7th to 10th April 2008*

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1 INTRODUCTION

Emulsions have been used for centuries in various domains in the pharmaceutical, cosmetic, food, paint and road industry.

Manufacturers of pharmaceutical and cosmetic products have recently shown increasing preference for multifunctional products in which different active agents can be incorporated, and for controlled drug delivery systems which decrease the usage frequency (hence increase patient compliance) and can decrease side effects or toxicity due to a lower active agent content. Emulsions are able to ensure these terms.

In pharmacy and medicine they are formulated for virtually all the major routes of administration: dermatological, oral and parenteral. Although emulsions have several advantages over other dosage forms (often improve bioavailability and/or reduce side effect) they are not used as extensively as other dosage forms (mainly oral and parenteral) because of emulsion instability, which results in unpredictable drug release profiles and possibly toxicity. Therefore one of the most important tasks is to ensure the kinetic stability of these systems. In addition to stability, other requirements also have to be satisfied by emulsions used in cosmetic and pharmaceutical industries, e.g. appropriate consistence and safety of ingredients.

2 LITERATURE SURVEY

2.1 Emulsions, types of the emulsions

An emulsion is a heterogeneous preparation composed of two immiscible liquids (by convention described as oil and water), one of which is dispersed as fine droplets uniformly throughout the other [1].

The types of the emulsion can be grouped as follows:

I. Macroemulsions:
   - Primary emulsions: Oil-in water (o/w) and water-in-oil (w/o)
   - Secondary emulsions: w/o/w or o/w/o

II. Microemulsions: o/w, bicontinuous and w/o

III. Nanoemulsions: o/w and w/o

Macroemulsions are not stable thermodynamically. These emulsions are typically polydispersed, their droplet diameter is various, usually ranging from 1 to 100 µm.

Microemulsions, in spite of the similarity of their term “emulsion”, are absolutely different from macroemulsions in their physical and thermodynamic properties and their structure. They are thermodynamically stable, apparently homogenous dispersions of water in oil (w/o) or oil in water (o/w). These isotropic, solubilised systems can form in the presence of surfactants, sometimes the presence of a cosurfactant is also required [2]. Microemulsions are liquid and behave as a newtonian liquid, so they have low viscosity. However, recently increasing preference can be observed for microemulsion-based gels which can contain a viscosity enhancing agent [3-5]. These higher viscosity systems are more suitable for topical administration.

Microemulsion formation is spontaneous and does not require much energy. The application field of these systems is diverse. They are frequently used as intravenous drug carriers because of their low viscosity, biocompatibility and high capacity to improve the bioavailability of drugs**.** This phenomenon is more important in the case of high toxicity drugs such as antitumor drugs [6-9]. Microemulsions are well known to improve the absorption and bioavailability of many compounds [10, 11], so their topical application is widespread, especially in the case of anti-inflammatory drugs [5, 12-14] or hormones [15].

In the last two decades, nanotechnology has been developing rapidly as one of the most promising and attractive research fields. The technology offers the potential to significantly improve the solubility and bioavailability of many drugs [16]. Emulsions with nanometric droplet size (typically in the range of 20–200 nm) are transparent or translucent systems and are also frequently called mini-emulsions. They are often referred to as submicron emulsions, which are not equivalent to thermodynamically spontaneous microemulsions [17]. The cause of the confusion is their long-term physical stability without apparent flocculation and coalescence. The attraction of nano-emulsions for application in different fields is due to the following advantages: i) no creaming or sedimentation occurs on storage (because of the reduced gravity force and the Brownian motion of the small droplets); ii) small droplets also prevent any flocculation; iii) no coalescence because the droplets are non-deformable and hence surface fluctuation is prevented, and the relatively thick surfactant film prevents any thinning or disruption of the liquid film between the droplets; iv) the large surface area and the small droplets improve the penetration of the actives; v) pleasant aesthetical character and good skin feeling; vi) lower surfactant concentration than in microemulsions (5-10% w/w instead of 20% w/w).

In spite of the advantages mentioned above, nanoemulsions have been focused on only in last decades for the following reasons: i) the preparation of a nanoemulsion requires special

techniques such as high pressure homogeniser as well as ultrasonics and hereby sometimes it is very expensive; ii) lack of the understanding of the mechanism of production of submicron droplets and the role of the surfactant and cosurfactant; iii) lack of the understanding of the interfacial chemistry; iv) lack of the knowledge about Ostwald ripening, which is the most frequent stability problem of the nanoemulsions [18]. For these reasons, nanoemulsions are widely used in pharmaceutics, cosmetics and they also play an important role in the synthesis of polymer dispersions and nanoparticles [19, 20]. During the last years, nanoemulsions have been designed to deliver drugs by various administration routes such as intravenous, oral, ocular for therapeutic needs [21-22].

As a summary, research in the last decade about emulsions in pharmaceutical technology can be divided into four main groups:

1. To find new type emulsions as drug delivery systems [23-26]: Increasing interest has been shown for the design and evaluation of targeted emulsion systems in the cancer, the human gene, the macrophage targeted therapy or the intestinal insulin therapy, especially PEGylated [27, 28], mannosylated [29] or folate-tethered emulsions have been used in the first three cases [30] and w/o/w [31, 32] or s/o/w [33-34] (solid-in-oil-in-water) emulsions systems have been used in the last case.
2. Investigation of the structure and stability of emulsions with new methods [35-37]. In addition to the classical methods (conductivity, rheology, pH, centrifugation etc.) some new ones can be found in the literature, such as Fourier transform infrared spectroscopy (FTIR) [38], confocal laser scanning microscopy or thermoanalysis (differential scanning calorimetry, thermogravimetry or thermogranulometry).
3. Investigation of drug release from emulsions systems [39-43].
4. Lipid formulations for improving the bioavailability of poorly water-soluble drugs such as spray-dried redispersible emulsions [44-46].

The great number of the publications, the wide range of the application fields of emulsions and their usage in modern therapy all indicate that these systems can be regarded as effective and up-to-date drug carriers even in the 21st century.

2.2 Stability of emulsions

As the life span of most pharmaceutical dosage forms is a few (3-5) years, sufficient physical stability is required in this time, therefore the most important task is to develop long-term stable new emulsions. This can only be achieved by the adequate control of the instability processes, which is often challenging since emulsion instability is a complex process and may

involve a combination of different mechanisms (creaming or sedimentation, flocculation and coalescence) [47].

Emulsions are not stable thermodynamically; the stable state of an emulsion is the form of its phases in layers separated by interfaces. Several processes are known to lead to the destruction of the emulsion structure, such as: i) flocculation ii) creaming or sedimentation iii) coalescence iv) phase inversion v) Ostwald ripening.

Flocculation is the aggregation of droplets due to van der Waals attraction when there is not sufficient repulsion between the droplets. Generally, for flocculation to take place emulsion droplets have to pass via a stabilizing energy barrier to a position close enough to be trapped in an energy minimum. Flocculation can be reduced (or eliminated) by an energy barrier between the droplets which can be an electrical double layer (e.g. by ionic surfactant) or non-electrical layer (by non-ionic surfactants or polymers) [48, 49].

Creaming or sedimentation is separation caused by the upward or downward motion of the emulsion droplet with lower or higher density than the continuous phase. The rate of creaming or sedimentation can be described by Stoke's law. In the case of a concentrated emulsion the rate of creaming or sedimentation is lower than predicted by Stoke's law because of the limited movements of the droplets. The most common method to reduce creaming or sedimentation is to use thickeners, viscosity enhancing agents [48, 49].

Coalescence, where dispersed phase droplets merge to form larger droplets, takes place in two distinct phases: i) thinning of the liquid film between the droplets and ii) its disruption. A special case of coalescence is partial coalescence. Partial coalescence occurs between partially crystalline droplets when the crystals on one droplet penetrate a second droplet. During this state each droplet retains its individual identity (like in flocculation) but there is a molecular contact between their contents (like in coalescence). Over the melting point the crystalline network is destroyed and the partially coalesced droplets will coalesce [48, 49].

Ostwald ripening occurs when there is significant miscibility between the oil and water phase. Droplet size distribution also changes because of the molecular diffusion from small to larger droplets due to the difference in the Laplace pressure [1, 50, 51]. Kelvin was the first who related the solubility of the particle with radius r, S(r) to one of a particle with infinitive radius S(∞):

$$\mathbf{S(r)=S(\infty)\exp(2\gamma V_m/rRT)} \qquad \mathbf{(1)}$$

where γ is the interfacial tension, V_m is the molar volume of the dispersed phase, R is the gas constant, and T is the absolute temperature. For two droplets (with r_1 and r_2) the Ostwald equation can be written as:

$$(RT/V_m)\ln(S_1/S_2)=2\gamma(1/r_1-1/r_2) \qquad (2)$$

The rate of Ostwald ripening (ω) can be described by the LSW (Lifshitz, Slezov and Wagner) theory:

$$\omega=d(r_c^3)/dt =8DS_{(\infty)}V_m/9RT \qquad (3)$$

where r_c is the critical droplet radius (neither growing nor decreasing in size), D is the diffusion coefficient of the dispersed phase in the continuous phase.

The following methods can be used to reduce Ostwald ripening: i) reduction of the interfacial tension, ii) enhancement of Gibbs Elasticity (using polymeric surfactant less insoluble in the continuous phase) or iii) incorporation of a small amount of highly insoluble oil [52].

Knowing the stability problems of emulsions, it can be concluded that three basic conditions must be met to form a stable emulsion [53]: i) the two liquids must be immiscible or mutually insoluble in each other ii) sufficient agitation must be applied to disperse one liquid into the other iii) an emulsifying agent or a combination of emulsifier must be present.

2.3 Gel-emulsions

Gel-emulsions, besides microemulsion gels and creams, belong to coherent emulsions [35]. Possibilities to form a gel-emulsion are the following:

The first is gel formation of the water phase by hydrophilic polymers such as polysaccharides, carrageenan, gelatine etc. [54, 55], or gel formation of the oily phase by hydrophobic polymers such as wax [56]. These gel-emulsions can be considered as a gel matrix in which droplets are embedded. In this way emulsions can be prevented from creaming and coalescence. Some pieces of work have focused on the effect of the presence of a dispersed phase on gel properties. It was established that the rheological properties of this type of gel-emulsions depend on the volume fraction and on the interaction between the gel matrix and the droplets [55, 57]. This phenomenon can be explained by the difference between their deformation behaviour during shearing (Fig.1).

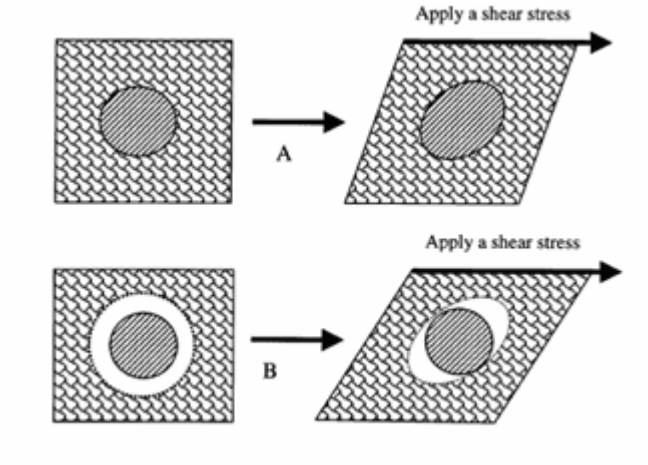

Fig.1 Difference in the rheological properties of the emulsions containing active (A) and inactive (B) fillers

Filler particles which interact with the gel matrix, they are called active fillers, are able to decrease or increase the gel strength. In the case of protein gels containing protein-covered oil droplets, gel stiffness can be improved when the adsorbed protein layer interacts with the protein gel matrix [58, 59]. Contrarily, inactive fillers have just a little chemical or physical affinity for the molecules forming the gel matrix and always decrease the gel strength [60] (Fig.1). The interaction between the oil droplets and the gel matrix depends on the surface properties of the droplets and the nature of the surfactant [61]. In addition, the particle size of the dispersed phase can also affect their properties [62].

The second possibility is in situ gelation of the polymer at the interface or in the continuous phase during/after the emulsification procedure or after the application (Fig.2) [3, 63, 64]. The latter allows the injectable formulation of gel implants. In situ gelling systems can be divided into two categories [65]: i.) systems are created upon irradiation with visible or UV-light; ii.) self-assembly systems. Photopolymerizable gels are formed in situ but they are not self-gelling. They require a photoinitiator. Self-assembling gels are formed spontaneously or after a certain trigger such as temperature, pH or electrolytes concentration.

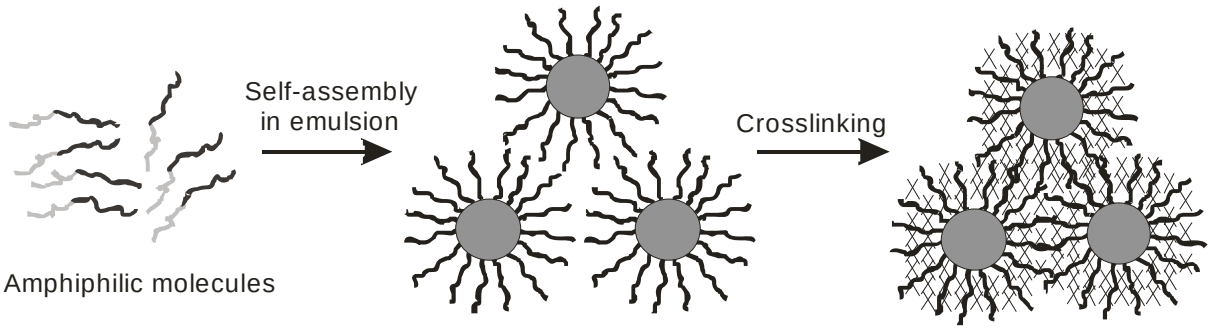

Fig.2 In situ gelation at the interface

The third possibility is using polymeric emulsifiers.

2.4 Polymeric emulsifiers

The formation of a stable emulsion requires the presence of a third component, an emulsifier, which is adsorbed at the oil-water interface and reduces interfacial tension. In the last decades the attention has been focused on two different types of emulsifiers: i.) polymeric emulsifiers, and ii.) Pickering-type emulsifiers.

Pickering-type emulsions are described as surfactant-free emulsions. Pickering-type emulsifiers are solid particles arranging between the continuous and dispersed phase, where they provide a steric hindrance [66, 67]. The solid (nano- or micro-) particles can function similarly to a surfactant molecule but have different properties, e.g. they do not assemble into an aggregate in the same way as the surfactants form micelles [68].

Polymeric emulsifiers are one of the most effective stabilizers as manifested by lower usage concentration. Three different types of polymeric emulsifiers can be found in the literature: i) linear block, ii) graft and iii) star copolymers (Fig.3). In addition to the stabilization of the emulsion, the application of a polymeric emulsifier is very different. Increasing preference has been shown for polymeric surfactants in emulsion polymerization in order to prepare microparticles, nanoparticles. As compared to low molecular weight surfactants they have a relatively low critical micelle concentration and thus their micelle formation is improved. Some authors have dealt with polymeric surfactant-based micelles as drug delivery systems. They can solubilize poorly soluble drugs and stay in the body for an extended time. On the one hand, the usage of protective polymers increases their circulation time; on the other hand it decreases their accumulation in the RES [69]. PEG-coated particles are the most common examples. Numerous scientific works verified the efficiency of “PEGylated” drug carriers: they prolong the residence time and the accumulation in the target area (e.g. tumor) [70, 71].

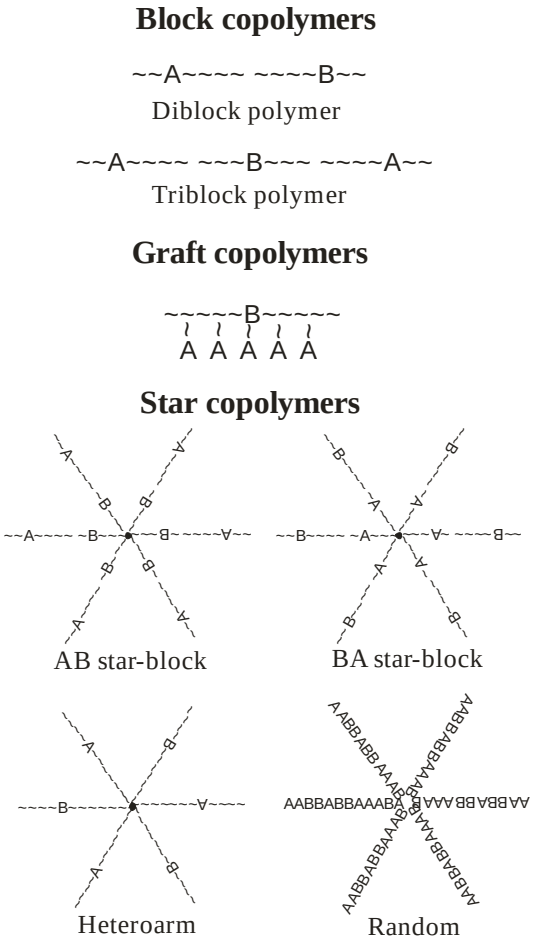

Fig.3 Grouping of the polymeric surfactants

Block copolymers have surface activity since one of the blocks is soluble in one of the phases and the other is soluble in the other phase. These amphiphilic molecules are able to form micelles. However, the aggregation number of triblock copolymers is smaller. The most widely used tri- and diblock copolymers contain polyethylene oxide (PEO) polypropylene oxide (PPO) (Pluronics, Synperonic) [72-74] and acrylic polymers [75]. In addition to the examples above, several block copolymers have been mentioned in the literature: ethoxylated aniline, 2-amino benzene thiol and benzene sulphonamide [76], hydrophobically modified ethyl(hydroxyethyl)cellulose [50], polystyrene-polyvinyl alcohol diblock, polystyrene-polyethylene oxide triblock, polyethylene glycol-oligolactide ABA block [77] or proteins, polysaccharides which are produced by a wide range of microorganisms. The latter are called “bioemulsans” [78].

Graft copolymers contain a polymeric backbone B and numerous A chains, which form a “brush” at the oil-water interface. They can also form micelles in solution, but with a small aggregation number. The most common graft polymers consist of a polystyrene or

polymethyl methacrylate backbone and polyethylene oxide chains (Atlox 4913, Hypermer CG-6), but other types can also be found such as: inulin base surfactants [79], chitosan-based surfactants [80], poly(methacrylic acid-g-ethylene glycol) [81] or grafted siliconic emulsifiers [82].

Star copolymers have attracted much less attention; there are only a few studies on their use [83, 84]. They are mainly used as polymerization stabilizers and showed better efficacy in this field than linear polymers.

2.5 Experimental aims

The aims of my research were the following:

1) To get to know the properties of the polymeric emulsifier (surface activity, wetting, swelling).
2) To determine the effect of the pH on the gels prepared with polymeric emulsifier and their oil loading capacity.
3) On the basis of the preformulation studies to define the formulation environment of the emulsions containing polymeric emulsifier.
4) To investigate the structure and properties (rheological behaviour, droplet size distribution, bounding of the water, gel structure) of emulsions and gels by: i) direct methods: image analyser, confocal laser scanning microscopy; and ii) indirect methods: rheology, thermogravimetry.
5) To study the stability of the emulsions using the methods mentioned above during 3-month storage (on 25°C).
6) To allocate the possible application fields of these systems by examining: bioadhesive behaviour and drug release profile using lipophilic and hydrophilic model drugs.
7) To determine the relationship between the formulation and the structure, the formulation and the stability, the formulation and the applicability.

The structure of the experimental work can be seen in Fig.4.

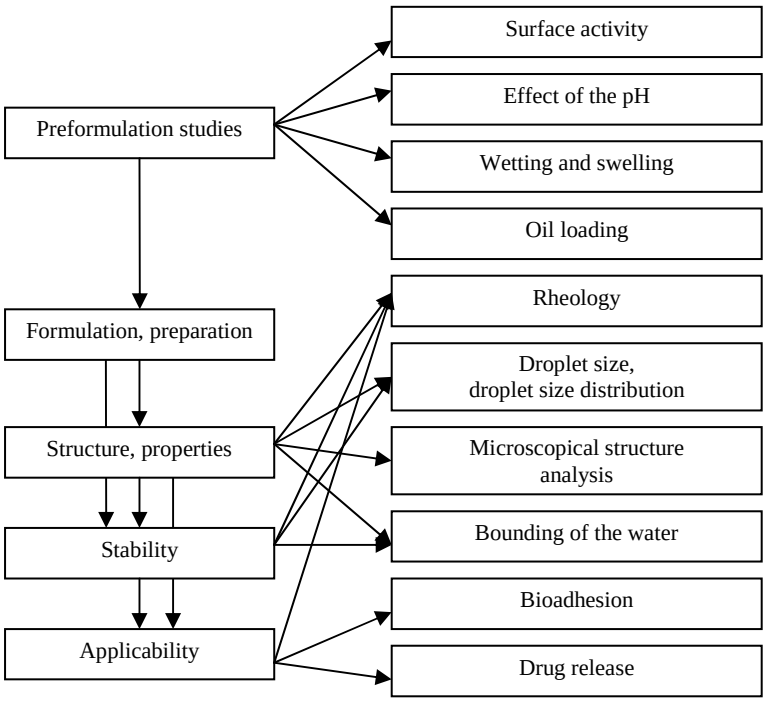

Fig.4 Structure of the examination

3 MATERIALS AND METHODS

3.1 Materials

Polymeric emulsifiers: Pemulen TR1 and TR2

Pemulen TR1 and Pemulen TR2 (PTR1 and PTR2) are cross-linked block copolymers of poly(acrylic acid) and hydrophobic long-chain methacrylates [85]. Traditional ionic or non-ionic surfactants stabilize oil-in-water emulsions principally by adsorbing and forming lamellar liquid crystalline layers at the emulsion interface requiring usage levels of 3-7% w/w of surfactant. Contrarily, emulsions created with very low levels of Pemulens are highly stable, because the hydrophobic portion of the polymer anchors in the oil phase while oil droplets are protected and held in place as a result of the viscous aqueous gel formed by the lipophilic part of the molecule around each oil droplet (Fig.5) [86]. The benefits of these polymeric emulsifiers can be summarized as follows: i) universal emulsification, the cause of which is that Pemulens do not depend on building a liquid crystalline structure, so they can be used with virtually any oil phase; ii) excellent stability; iii) low irritancy, the cause of which, on the one hand, is that Pemulens, being macromolecules, do not penetrate into the biological tissue, on the other hand they are used in a very low concentration (less than 1.0 % w/w, w/w) [87]; iv) simplifies emulsion formation procedures (at any temperature); v) potential reduction

of application frequency, since the oil phase is not readily re-emulsifiable so it remains in the surface, possibly minimizing the need for reapplication.

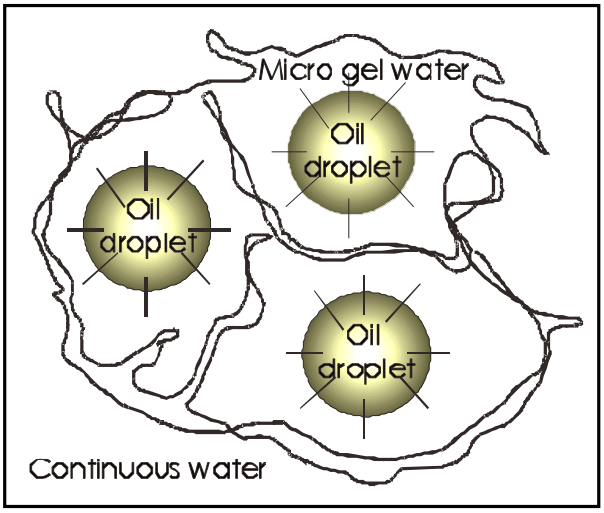

Fig.5 Structure of the gel-emulsions containing Pemulens

Other components

Poloxamers (Synperonic PE/L 31, 61, 62, 101)

Synperonic PE/Ls (S31, S61, S62 and S101) are ethylene oxide-propylene oxide block copolymers. It is used as wetter, dispersant, emulsifier (o/w and w/o), antifoam, building block [88]. The different numbers indicate different polymerization-degrees.

Miglyol 812 (Fractioned coconut oil, Triglycerida saturata media)

Miglyol 812 is a triglyceride of medium-chain saturated fatty acids, mainly of caprylic acid and capric acid. It is used as a solvent, stabiliser, base of pharmaceutical products or source of medium-chain triglycerides [89, 90].

Trolamine (Triethanolamine)

Its chemical name is 2, 2', 2"-nitrilotriethanol. Trolamine is applied mainly combined with fatty acids such as stearic and oleic acid; equimolecular proportions of base and fatty acid form a soap which can be used as an emulsifier at about pH 8 [89, 90]. It is widely used in hydrogels as a neutralizing agent.

Metronidazole

Its definition is 2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethanol. Metronidazole has antiprotozoal and antibacterial actions and is effective against Trichomonas vaginalis and other protozoa including Entamoeba histolyca and Giardia lamblia, and against anaerobic bacteria [89-91]. Metronidazole is known to be effective used in bacterial vaginosis. Conventionally, its dose is 500 mg orally twice daily for 7 days (or 250 mg three times daily

for 7 days in pregnancy) [92]. Clinical examinations have verified the efficacy of 0.75% w/w Metronidazole vaginal gel twice daily for five days in the therapy of bacterial vaginosis, which was similar to that of the standard oral Metronidazole treatment and was associated with fewer gastrointestinal side effects [93].

Lidocaine

Its chemical name is 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide. Lidocaine is a local anaesthetic. It is readily adsorbed from the gastrointestinal tract, from mucous membranes and through damaged skin. It is used for infiltration anaesthesia and regional nerve blocks. Lidocaine is also a classic antiarrhythmic used in the treatment of ventricular arrhythmias, especially after myocardial infarction [89, 90].

3.2 Methods

3.2.1 Preparation of emulsions

The Pemulens were added to purified water containing trolamine and preservative. The pH of the gel was 5-5.5. After 24 hours the oil was added to this gel while the sample was stirred with a mixer (MLW ER-10, 800 rpm) for 20 minutes. In the samples containing coemulsifiers, the mixture of the coemulsifier and oil was added to the water phase. The components of the emulsion can be seen in Table1.

Table 1 Components of the emulsions

Component	Concentration (% w/w)	Function
Pemulen TR1 or Pemulen TR2 (Noveon, USA)	0.1 – 1.2	Primary emulsifier
Synperonic PE/L 31, 61, 62, 101 (Uniqema, UK)	0.001 – 1.00	Coemulsifier
Trolamine (Ph. Hg. VIII.)	at pH 5.5 - 6	Neutralizing agent
Miglyol 812 (Sasol, Germany)	10 - 70	Oily phase
Purified water (Ph. Hg. VIII.)	30 - 90	Aqueous phase
Lidocaine base (Ph. Hg.VIII.)	1.00	Lipophilic model drug
Metronidazole (Ph. Hg.VIII.)	0.75	Hydrophilic model drug

3.2.2 Measurement of the surface tension

The measurement of the surface activity was carried out with a Krüss tensiometer. A series of the Pemulen solutions was prepared between 0.00005% w/w and 0.25% w/w. The air-liquid surface tension was detected. Each study was repeated three times.

3.2.3 Contact angle measurements

Dataphysics OCA20 was used to determine the wetting especially the contact angle between the polymer probes and the water, and between them and the oil. The contact angle was calculated from the Young-Laplace equation.

3.2.4 Measurement of the Enslin number

The measurements were performed with Enslin instrument. 0.10 g of the polymer was laid onto the filter paper (surface area = 12.57 cm^2) of the instrument. The swelling was followed for 10 min, and the loaded water was determined.

3.2.5 Droplet size analysis

The particle size and the particle size distribution were measured with the Leica Q500MC image analyser system. 500 droplets were analysed in each emulsion.

3.2.6 Thermogravimetric investigation

The measurements were carried out with a MOM Derivatograph-C (MOM GmbH, Hungary) instrument. Samples were weighed (40-50 mg) in platinum pans (No.4). The reference was a pan containing aluminium oxide. The samples were heated from 25 to 200 ^oC at 5 $^oC\ min^{-1}$ or at 10 $^oC\ min^{-1}$. TG (weight loss % vs. temperature), DTG (derivative TG) and DTA curves were plotted. Each study was repeated three times.

3.2.7 Rheological investigation

HAAKE RheoStress 1 Rheometer (HAAKE GmbH., Germany) with cone and plate geometry (diameter 35 mm, cone angle 1º and the gap 0.048 mm in the middle of the cone) was used to study the rheological profile of the samples. The flow curve and the viscosity curve of the samples were determined by rotation tests controlled shear rate. The shear rate was changed from 0.1 s^{-1} to 100 s^{-1} and then from 100 s^{-1} to 0.1 s^{-1}. The storage (G'), the loss (G") moduli and loss tangent ($\tan\delta$= G"/G') were examined as function of frequency (from 0.1 Hz to 100

Hz) at 1.0 Pa (in case of PTR1) and at 0.1 Pa (in case of PTR2). These values of the shear stress were within their linear viscoelastic range. Each examination was repeated three times.

3.2.8 Tensile test

The mucoadhesive properties of the gel-emulsions were investigated by a TA-XT2 Plus Texture Analyser (Stable Micro Systems, Enco, Italy). The samples (20 mg) were laid on a filter paper fixed with double sided adhesive tape on the bottom of the upper probe. The porcine buccal tissues were placed in the lower probe. The upper probe with the sample was lowered at a speed of 1.0 mm sec^{-1} onto the surface and a downward force of 6000 mN was applied for 1 min to ensure intimate contact between the sample and the tissue. After the preloading the upper probe was moved upwards at a speed of 4.0 mm s^{-1}. The detachment force was determined and the adhesive work was calculated from the area under the force-distance curve. Each study was repeated twelve times.

3.2.9 Confocal laser scanning microscopy

The visualization of the gel structure and the bioadhesive bond between the emulsion and the mucin was carried out with a Confocal Microscope System Leica TCS SP2 (Leica Microsystems Heidelberg GmbH., Germany) interfaced with a Leica DMIRBE inverted microscope and using a 40X oil immersion objective with 1.25 numerical aperture. The excitation source was a Green Helio-Neon (λ_{ex} = 543 nm) laser, the fluorescence emission of rhodamine B was recorded between 580 and 630 nm. Rhodamine B (0.002% w/w) was suspended in the oil phase and the oil was added to the water phase. 8.0% w/w mucin disperion was prepared from mucin and buffer solution, pH 6.4. This solution was added to the emulsions. 10:1 and 5:1 emulsion-mucin ratios were applied.

3.2.10 Drug release test

In vitro drug release tests were carried out with Hanson SR8-PlusTM Dissolution Test Station (Hanson Research Corporation, USA) using special ointment cells. 0.60 g of the sample was placed into the ointment cell as a donor phase. The membrane was a Porafil membrane filter (pore diameter was 0.45 μm). The acceptor phase was 70 ml buffer pH 4.5 in the case of Metronidazole (this pH is about the pH of the vagina in bacterial vaginosis) and 70 ml buffer pH 5.4 in the case of Lidocaine (this pH approaches the natural pH of the human skin). The quantitative determination of the drugs was performed with a UV-VIS spectrophotometer

(Unicam Heλios-α, Spectronic Unicam, UK) at a wavelength of λ= 319 (Metronidazole) and λ= 230 (Lidocaine). 7 parallel measurements were made.

4 RESULTS AND DISCUSSION

4.1 Preformulation studies

In the course of the preformulation studies, it was established that the wetting of these polymeric emulsifiers is very weak with purified water, which is indicated by the contact angle at about 90° but better with Miglyol, which is indicated by the lower value (Table 1). There is no remarkable alteration between the values of different polymerization-degree polymers.

Table 2 Contact angle of the Pemulens

Contact angle Θ (± SD, n=7)		
	PTR1	PTR2
Purified water	81.7 ± 5.67	82.64 ± 1.24
Miglyol 812	27.9 ± 0.88	26.8 ± 0.49

The swelling of the polymers takes a long time, the amount of the water taken up is quite low (low Enslin number) (Fig.6). These two properties have to be considered for the preparation of the gels or emulsions. This is the cause why the samples had been stored for 24h before the emulsification procedure.

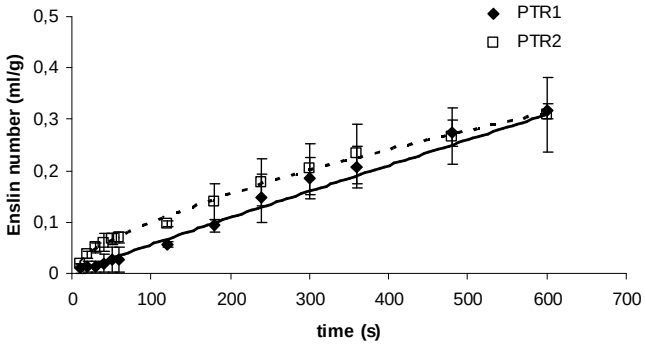

Fig.6 Swelling (Enslin number) of the polymeric emulsifiers

In the course of the surface tension measurements Pemulens like other polymeric emulsifiers showed low surface activity, which suggests that the presence of a coemulsifier is also required to facilitate the emulsification procedure [94]. The critical micelle concentration (CMC) is determined by the minimum of the concentration vs. surface tension plot. These emulsifiers have a low CMC value, which can be explained by the improved micelle

formation ability of the amphiphilic macromolecules. In the case of Pemulens this value is 0.005 % w/w (Fig.7).

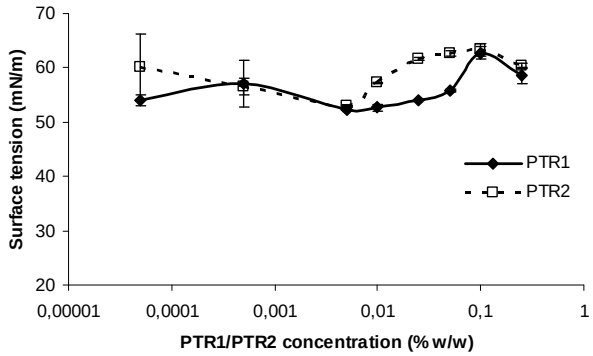

Fig.7 Surface tension of the polymeric emulsifiers

When the pH was changed at low polymer content, constant value was detected; contrarily, at high (1.00% w/w) concentration the pH affected the viscosity especially at PTR2 (Fig.8). For the further examination the pH of the samples was set at about pH 5.0-5.5 with the exception of samples containing Metronidazole.

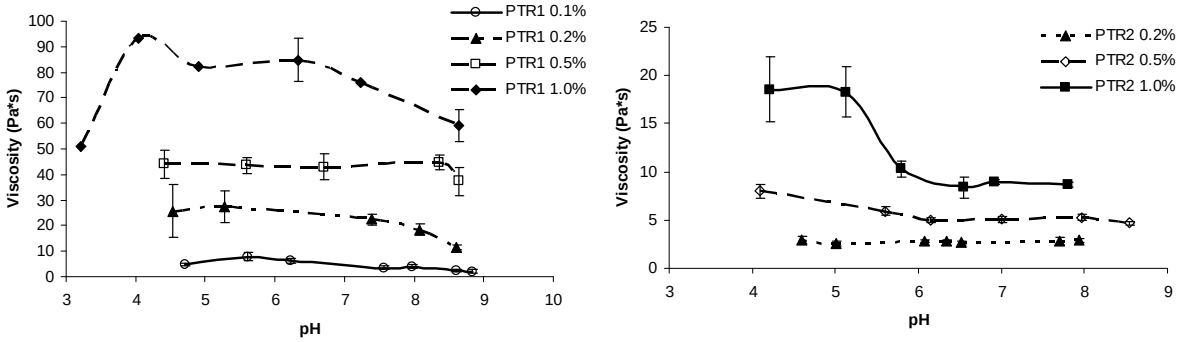

Fig.8 Effect of the pH on the viscosity of the gel containing polymeric emulsifier

Adding Miglyol to the gel drop wise, the maximum oil concentration was determined (using burette). The maximum oil concentration was the point when the two phases separated for the next oil drop. The two different polymerization-degree polymers showed alteration (Fig.9). The gels containing the lower polymerization-degree polymer could take up more oil than the higher one, which can be explained by flexibility at the interface.

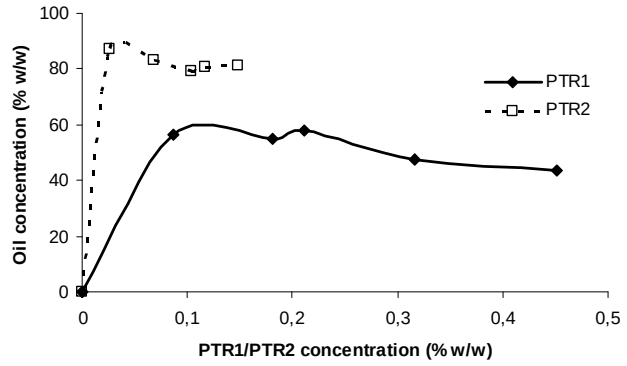

Fig.10 Oil loading ability of the gels containing polymeric emulsifier

4.2 Structure and properties of the emulsions and the simple gels

4.2.1 Rheological investigations

The knowledge of the rheological characteristics of the systems is very important for monitoring the changes of the microstructure and the bioadhesive behaviour. Few pieces of information can be found in the literature about the Pemulen's rheological characteristics. An increase in viscosity was described with the polymeric emulsifier and the concentration and the emulsions showed thixotropy or antithixotropy [95, 96]. In my studies initial viscosity (η_0), and the damping factor (tanδ) were used to characterize the rheological properties of the gels and emulsions.

Initial viscosity was determined by the power law model:

$$\eta = \eta_0 D^n \qquad (4)$$

where η is the viscosity, η_0 is the initial viscosity, D is the shear rate and n is the power (shear thinning) index. (In the further results viscosity means the initial viscosity.)

For the viscoelastic characterization of the emulsions tanδ (loss tangent or damping factor), G' (storage modulus) and G" loss modulus were used.

$$\tan\delta = G''/G' \qquad (5)$$

Where G' is the storage modulus, G" is the loss modulus. The smaller tanδ (or the greater G') is, the stronger the interaction is in the gel structure [97].

4.2.1.1 Effect of the polymer concentration

By correlating the viscosity values of the emulsions to one of the simple gels it can be concluded that there is not a pregnant difference between the gels and emulsions with the same polymer content for PTR1 samples. Contrarily, for PTR2 ones the inner phase increased the viscosity (Fig.11). The correlation between the viscosity and the polymer concentration was the following exponential equation:

$$\eta=\eta_e * \exp(m*c) \qquad (6)$$

where c is the polymeric emulsifier concentration, η_e is the viscosity extrapolated to the initial concentration and m is a structural coefficient.

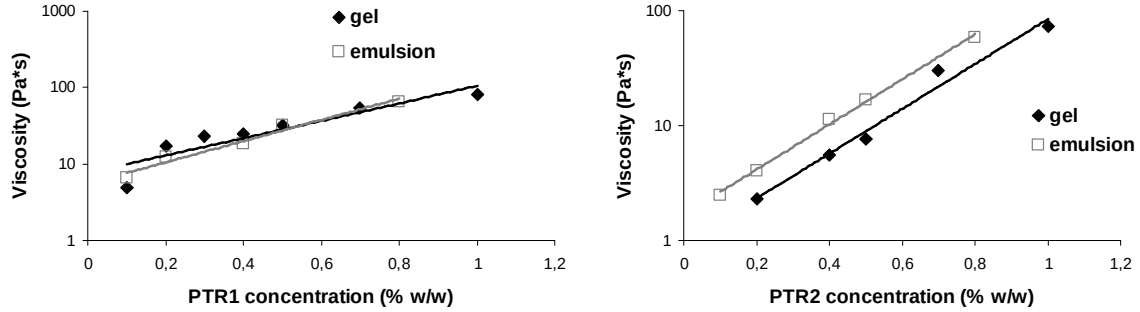

Fig.11 Viscosity as function of polymeric emulsifier concentration (oil 20% w/w)

In the course of the oscillation measurement at low polymer content the emulsions showed higher elasticity, while at high concentration the gels did (Fig.12). It can be supposed that the presence of the inner phase improves the formation of the gel structure and therefore the elasticity at low concentration, but (relatively) decreases that at a high one.

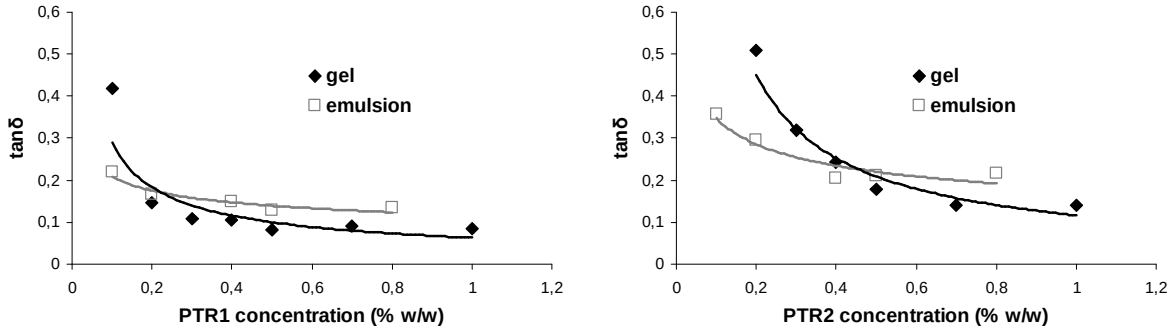

Fig.12 Damping factor as function of the polymeric emulsifier concentration (oil 20% w/w)

4.2.1.2 Effect of the oil concentration

Raising the amount of the oil increased the viscosity, which can be written by an exponential equation similarly to the changing of the polymer content. In turn, the damping factor showed an increase with the oil concentration in the PTR2 samples, which indicates that the increase of the volume fraction depresses the elasticity in these sample types (Fig.13).

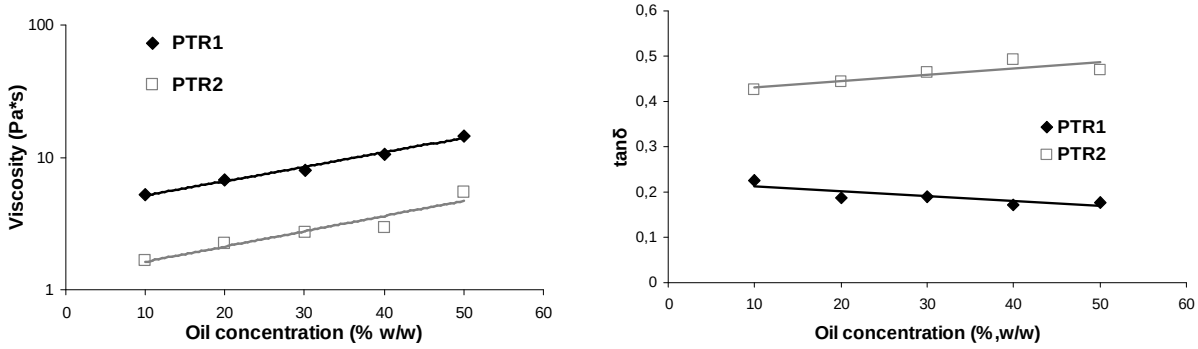

Fig.13 Viscosity and damping factor as function of the oil concentration (PTR1 or PTR2 0.10% w/w)

4.2.1.3 Effect of the coemulsifier concentration

Some authors have examined emulsions based on the combination of Pemulens and non-ionic emulsifier. They have established that the addition of a mixed emulsifier significantly modified the rheological characteristics of the emulsions [98, 99]. The viscosity of the emulsion increased with the amount of the non-ionic emulsifier. When Polysorbate 80 was used as a non-ionic emulsifier, this phenomenon was explained by the interaction between the polymeric emulsifier and the non-ionic emulsifier. In our study, when Synperonics were used, the viscosity usually increased with the cosurfactant concentration, while the damping factor decreased (Table 3). This tendency was the most remarkable in the case of S101 where those values changed extremely at high (1.00% w/w) concentration (Fig.14).

Table 3 Viscosity and damping factor values

η_o (Pa*s)

PTR1 0.20 % w/w, oil 20% w/w						
Coemulsifier	**Concentration (% w/w)**					
	0.00	**0.01**	**0.05**	**0.10**	**0.50**	**1.00**
S31	19.4	14.2	16.8	11.3	11.8	20.4
S61		18.1	11.8	14.5	25.9	25.4
S62		19.4	14.5	14.8	23.4	19.7
S101		21.0	23.6	23.7	18.0	22.3
PTR2 0.20 % w/w, oil 20% w/w						
Coemulsifier	**Concentration (% w/w)**					
	0.00	**0.01**	**0.05**	**0.10**	**0.50**	**1.00**
S31	2.5	2.4	2.8	2.5	1.9	2.0
S61		2.4	2.7	2.2	2.0	1.9
S62		2.5	2.5	2.4	2.2	2.6
S101		2.4	2.6	2.9	2.5	3.8

tanδ

PTR1 0.20 % w/w, oil 20% w/w						
Coemulsifier	**Concentration (% w/w)**					
	0.00	**0.01**	**0.05**	**0.10**	**0.50**	**1.00**
S31	0.140	0.116	0.140	0.132	0.136	0.128
S61		0.116	0.120	0.109	0.110	0.113
S62		0.125	0.117	0.117	0.108	0.108
S101		0.125	0.121	0.112	0.111	0.095
PTR2 0.20 % w/w, oil 20% w/w						
Coemulsifier	**Concentration (% w/w)**					
	0.00	**0.01**	**0.05**	**0.10**	**0.50**	**1.00**
S31	0.650	0.624	0.579	0.608	0.612	0.601
S61		0.612	0.581	0.615	0.582	0.573
S62		0.583	0.582	0.616	0.665	0.624
S101		0.624	0.544	0.529	0.489	0.358

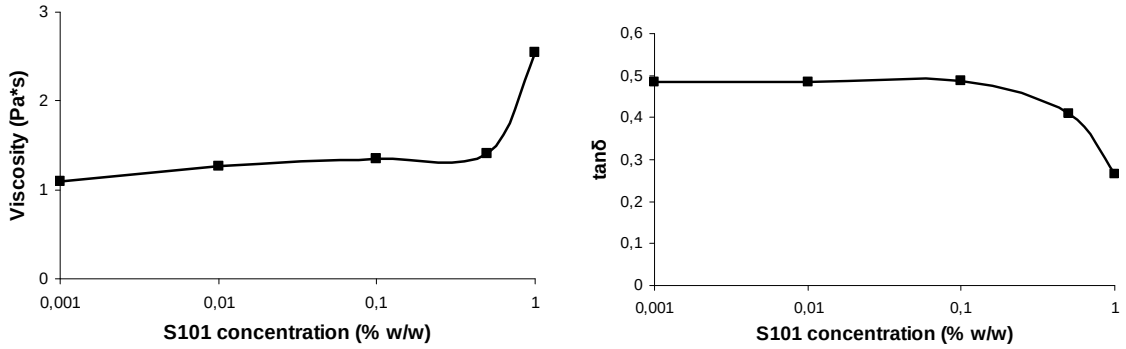

Fig.14 Variation of the viscosity and the damping factor in function of the S101 concentration (PTR2 0.10% w/w, oil 20% w/w)

4.2.2 Thermoanalytical investigations

Thermoanalytics is becoming increasingly important in the structure examination of pharmaceutical dosage forms. Recently, in addition to the research of solid dosage forms [100-102], it has also been used successfully in the investigation of liquid and semi-solid systems. Thermoanalytical measurements allow investigating the microstructure of emulsions, creams and other semi-solid systems. Several papers about the structure of various semi-solid pharmaceutical preparations and cosmetic products (e.g. creams and liquid crystals) have been published in literature [103-107]. The majority of the investigations focus the attention on the binding of water: free, bound or interlamellar types of water are distinguished [108-112]. The choice of the heating rate is a very important factor in the examination of the emulsions containing Pemulens. The shape of the TG and DTG curves can be absolutely different (Fig.15). The cause is the special gel structure in these emulsions. Our basic assumption was that the polymer, due to its surfactant nature, migrates towards the interface; consequently its concentration will decrease in regions far from the oil droplets. If this concentration difference is considerable, two aqueous phases are obtained, which can be separated well on the thermograms. The first one is the bound water in the micro gel, and the second one is relatively free water. But in these emulsion there is no barrier between the different gel phases as it is known in the case of e.g. lamellar structures. If the heating rate is lower, the water will evaporate simultaneously from the all the water phase. Contrarily, if the heating rate is higher, the free water will evaporate first and the bound water later. Figure 16 clearly shows that two peaks can be separated well in the DTG curve, one peak corresponds to free water at about 100 ºC, the other to micro gel (bound) water at about 140 ºC. Whereas a simple gel with the same polymeric emulsifier concentration has a one-peak DTG curve (Fig.16).

For the examination of the evaporation from gels or emulsions, and of their hydration state it is better to use a lower heating rate. Fitting a linear equation to the linear part of the TG curve, its slope will give the evaporation rate of the whole sample.

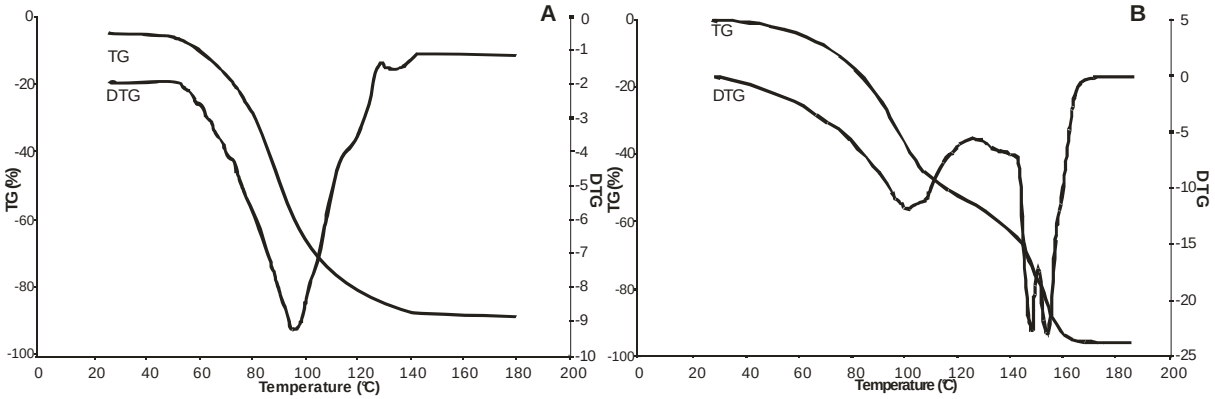

Fig. 15 Thermograms of the gel-emulsion at a heating rate 5°C/min (A) and at 10°C/min (B)

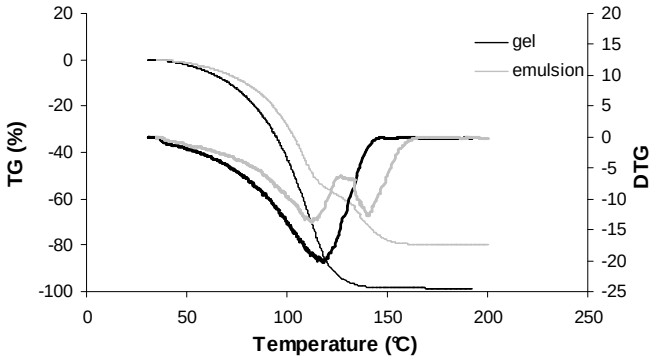

Fig.16 Difference between the thermogram of the gel emulsion and one of the simple gels

4.2.2.1 Effect of the polymer concentration

The examination of the evaporation rate showed, as it was expected, that it was slower for the emulsions than for the gels (Fig.17). Because of water evaporation and heating, an oil layer separated onto the surface, which functions as an occlusive layer decreasing the evaporation; on the other hand, the binding of water can also change, as it could be seen previously.

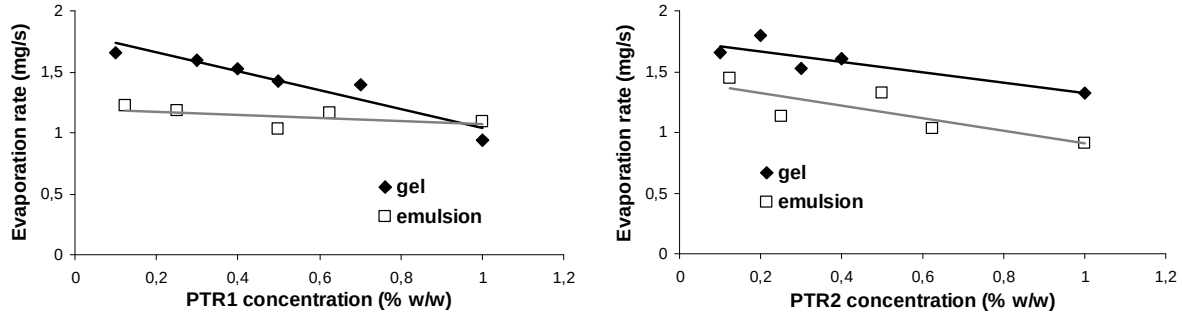

Fig.17 Evaporation rate as function of the emulsifier concentration (oil 20% w/w)

When the quantity of the polymer is increased, two processes can be expected to occur in the gel structure of the emulsions: i) the interface becomes saturated so the excess polymer will not appear in the boundary layer any more, therefore it will reduce the concentration difference between the interface and the more distant areas; ii) the increased polymer concentration will result in a greater number of interactions between the chains, which in turn over a certain concentration will inhibit the orientation of the polymers towards the interface to some extent. As a consequence, the differentiation of the gel structure can be expected to disappear with increasing polymer content. When the quantity of the polymer is increased (over 0.40% w/w) the two peaks disappear as expected, and only one peak can be observed (Fig.18).

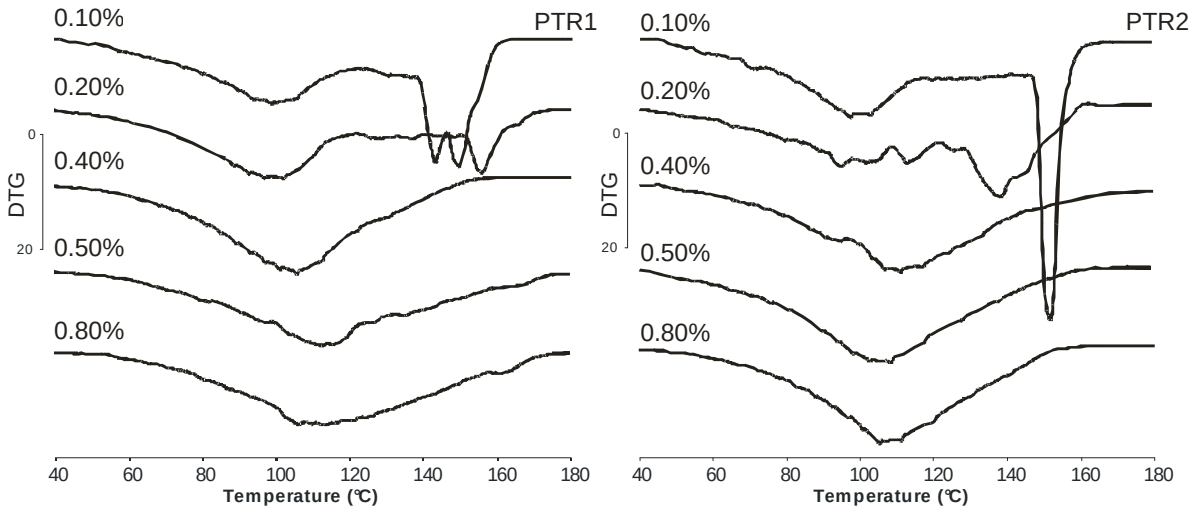

Fig.18 DTG curves of emulsions with increasing polymeric emulsifier content (oil 20% w/w)

4.2.2.2 Effect of the oil concentration

The evaporation rate linearly decreased with the amount of the oil. The higher the oil content, the larger the occlusive layer which hinders evaporation. In the samples containing different polymers where the oil content was the same, the evaporation rate was perfectly equal

(Fig.19). Consequently, the main factor in evaporation is the oil concentration or the thickness of the oil layer.

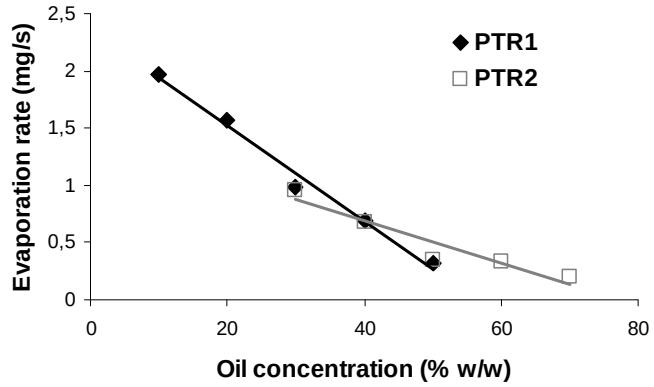

Fig.19 Evaporation rate as the function of the oil concentration (PTR1 or PTR2 0.10%)

The TG curves of the emulsions at different oil concentrations are shown in Figure 20. The higher the oil content is, the greater ratio of the bound water can be observed, which can be calculated from the height of the steps. At low (10% w/w) and high (50% w/w) oil concentrations only one step can be seen in the curves while at middle (20-40% w/w) concentration two steps can be separated. At a low concentration the surface and so the orientation of the polymer may not be significant enough to be detected. At a high oil concentration the polymer-water ratio is so high that the entire aqueous phase is bound by the polymer gel. In the case of the samples containing PTR2, in which the lower polymerization-degree-polymer was applied, the two steps on the TG curve can be detected at high oil concentration, too, because the smaller polymer chains can move easily so they can orient towards the oil droplets even at a relatively high polymer content.

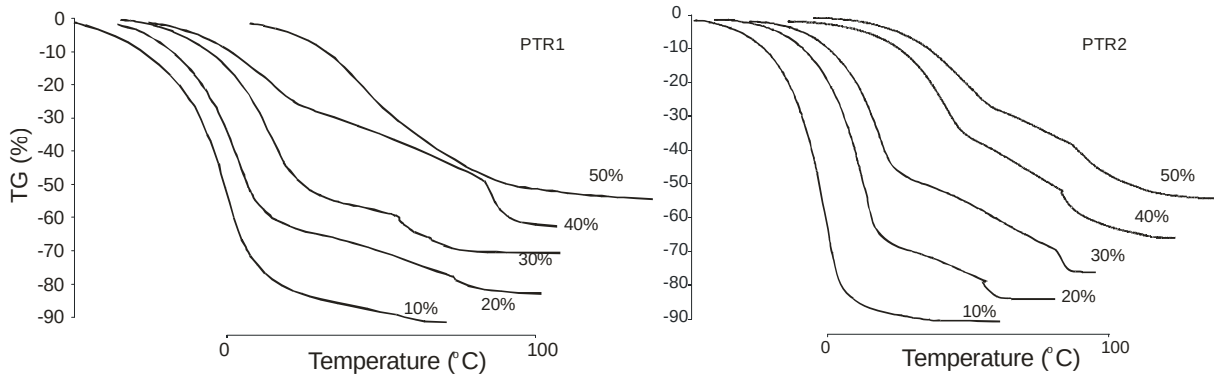

Fig.20 TG curves at different oil concentration (PTR1 or PTR2 0.10% w/w)

4.2.2.3 Effect of the coemulsifier concentration

If a coemulsifier is also used, changes in the microstructure can be assumed. The coemulsifier with its smaller molecules is also oriented on the interface; therefore in a higher concentration

it can displace the polymeric emulsifier with greater molecules. As a result, the micro gel around the droplet will disappear.

It can be said generally that the application of a coemulsifier decreases the evaporation of the gel, so the changes of the micro gel structure affected that (Table 4). In the case of S101 this change can be described with the following semi-empirical equation (Fig.21):

$$v = 0.597c^{-0.0613} \qquad (7)$$

where v is the evaporation rate and c is the S101 concentration.

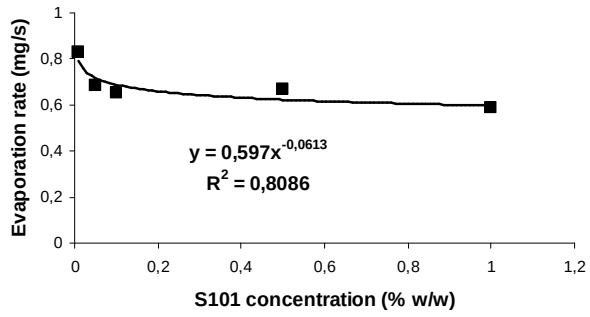

Fig.21 Evaporation rate in function of the S101 concentration (PTR1 0.20% w/w, oil 20% w/w)

Table 4 Evaporation rate

Evaporation rate (mg/s)						
PTR1 0.20% w/w						
Coemulsifier	**Concentration (% w/w)**					
	0.00	**0.01**	**0.05**	**0.10**	**0.50**	**1.00**
S31	0.773	0.752	0.593	0.657	0.566	0.613
S61		0.569	0.710	0.516	0.656	0.732
S62		0.586	0.734	0.742	0.583	0.642
S101		0.829	0.686	0.651	0.667	0.588
PTR2 0.20% w/w						
Coemulsifier	**Concentration (% w/w)**					
	0.00	**0.01**	**0.05**	**0.10**	**0.50**	**1.00**
S31	1.257	0.667	0.833	0.490	0.449	0.556
S61		0.508	0.488	0.545	0.486	0.458
S62		0.491	0.432	0.372	0.516	0.542
S101		0.529	0.408	0.466	0.989	0.676

Figure 22 shows the changes of TG curves as the function of the coemulsifier concentration. The difference between the extents of the two steps increases, the bigger the coemulsifier concentration is, the smaller ratio of bound water can be measured. At high (1.00% w/w) concentration the two steps absolutely disappear, so probably the polymer forms a homogenous gel structure and there is no micro gel around the droplets. Furthermore, the two peaks of the DTG curve are shifted with an increasing coemulsifier concentration. The polymeric emulsifier is displaced from the interface and will gelate, thus the first peak will be shifted towards a higher temperature. At the same time the water on the interface will also evaporate from the system at a higher temperature. The quantity of water bound in different ways can be calculated from the step height of the TG curves. If the quantity of the micro gel water on the interface is examined with respect to the total quantity of water with increasing coemulsifier concentration, it can be stated that the amount of the micro gel water gradually

decreases and finally disappears as a homogeneous gel is created by the polymer in the aqueous phase (Table 5).

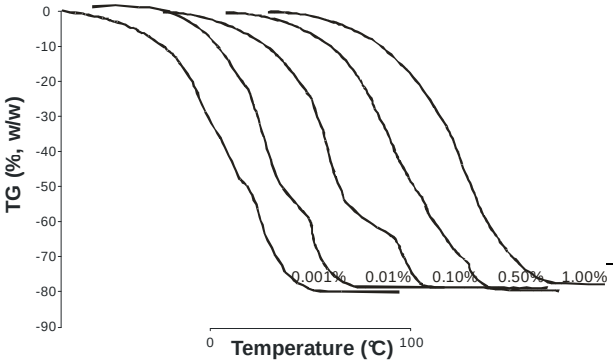

Fig.22 TG curves of the emulsions at different S101 concentration (PTR2 0.10% w/w, oil 20% w/w)

Table 5 Peaks of the DTG curves and the amount of the micro gel water (PTR2 0.10%, oil 20%)

Coemulsifier conc. (% w/w)	1st peak ºC	2nd peak ºC	Micro gel water (% w/w)
0.001	108 ± 4	131 ± 2	36.8±6.0
0.01	113 ±2	138 ± 4	24.0±3.0
0.10	113 ± 1	145 ± 4	25.4±9.5
0.50	119 ± 4	150 ± 6	16.4±7.8
1.00	133 ± 4	-	-

The relationship between microstructure and rheology is illustrated well by Figure 23, showing the relationship between the quantity of micro gel water and the rheological constants (viscosity, storage modulus), which can be described with a power function.

$$\eta = 4.45c^{-0.39} \qquad (R^2 = 0.980) \qquad \textbf{(8)}$$

$$G' = 17.24c^{-0.51} \qquad (R^2 = 0.851) \qquad \textbf{(9)}$$

where η is the viscosity, G' is the storage modulus and c is the water content in the micro gel. The small quantity of gel water detectable around the droplet indicates that the distribution of the polymer is becoming more and more homogeneous, which means that the built-up homogeneous gel structure increases the viscosity and elasticity of the systems.

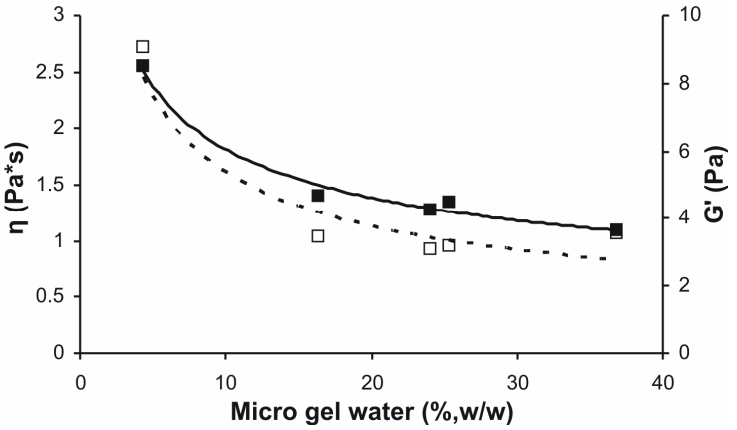

Fig.23 Correlation between the micro gel water and the viscosity/ storage modulus of the emulsions containing PTR1 and S101 at constant water oil ratio (PTR2 0.10%, oil 20%)

4.2.3 Microscopical investigations

4.2.3.1 Droplet size analysis

The average droplet size of the emulsions exponentially decreased with the emulsifier content as it was expected (Fig.24). The phenomenon would have been more remarkable unless the improved elasticity of the samples had hindered the emulsification procedure.

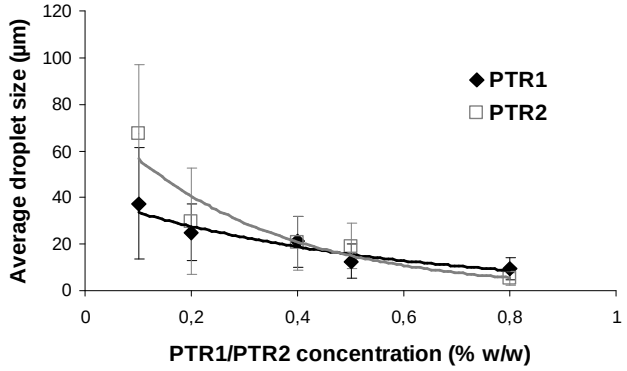

Fig.24 Average droplet size as function of the polymeric emulsifier concentration (oil 20% w/w)

By increasing the oil concentration, maximum points were on the curves (Fig.25). At both polymeric emulsifiers a maximum average droplet size was shown at 30% w/w oil content. Above this value the droplet size started decreasing. This phenomenon can be explained with the better stability of the emulsion at about the same concentration of the two phases.

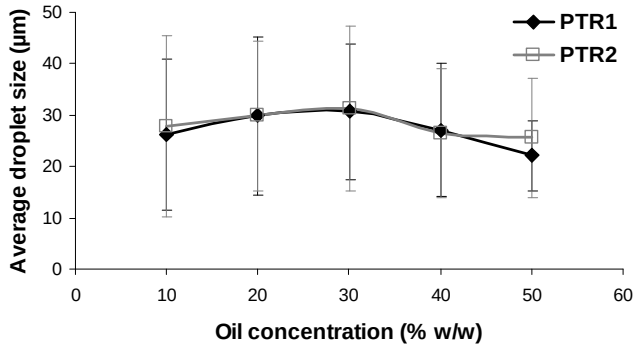

Fig.25 Average droplet size as function of the oil concentration (PTR1 or PTR2 0.10% w/w)

With the use of coemulsifier the changes of the droplet size are not definite. At a low coemulsifier content the droplet size oscillated. The interface may still be changing in this range; it is not a good ratio of the two emulsifiers to form a stable interface. Contrarily, at a high coemulsifier concentration a new interface could have been built up and formed a stable structure. The combination of the Pemulens and S101 was shown as the best one.

Table 6 Average droplet size

Average droplet size (µm)						
PTR1 0.20% w/w, oil 20% w/w						
Coemulsi-fier	**Concentration (% w/w)**					
	0.00	**0.01**	**0.05**	**0.10**	**0.50**	**1.00**
S31	14	17	15	12	16	13
S61		13	12	13	11	8
S62		16	12	13	11	7
S101		14	13	10	5	4
PTR2 0.20% w/w, oil 20% w/w						
Coemulsi-fier	**Concentration (% w/w)**					
	0.00	**0.01**	**0.05**	**0.10**	**0.50**	**1.00**
S31	16	33	18	19	17	20
S61		17	14	16	11	10
S62		21	19	17	8	9
S101		18	13	9	6	4

4.2.3.2 Confocal laser scanning microscopy

By using confocal laser scanning microscopy emulsions can be visualized either by dyeing the dispersed phase (perhaps continuous phase) or by utilizing fluorescent or fluorescent-labelled surfactant. The application fields of confocal microscopy in the case of emulsions are very different. In food industry the interaction between the surfactant and proteins was investigated [113, 114] or in a few cases the displacement of emulsifiers from the water and oil interface was studied [115, 116]. Some authors deal with the distribution of interdroplet forces in a compressed emulsion system [117, 118]. In this study the location of the polymer was detected by this method. With the use of Rhodamine B, which can be considered as a tertiary amine, hydrogen bonding or electrostatic interaction may form between the carboxyl groups of the polyacryl-acid and the fluorophore [119], so the dye concentration will be higher where the polymer concentration is higher. Fig.26 shows the difference between the dye distributions of the different types of fluorophores.

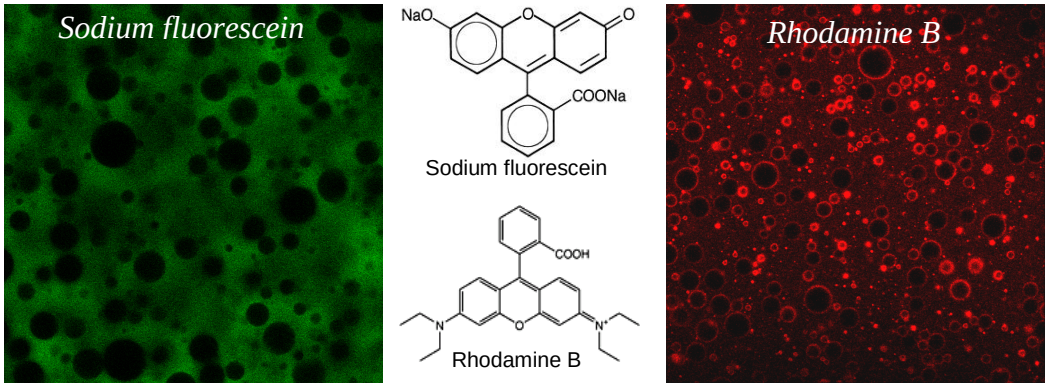

Fig.26 Difference of the dye distribution of the sodium fluorescein and rhodamine B

Pictures made with confocal microscopy are confirmed by thermogravometric results. In the case of a low concentration (Fig.27) a sharp contour is dyed by rhodamine B around the droplet, indicating a higher polymer concentration around that, while with higher concentrations the dye has homogeneous distribution.

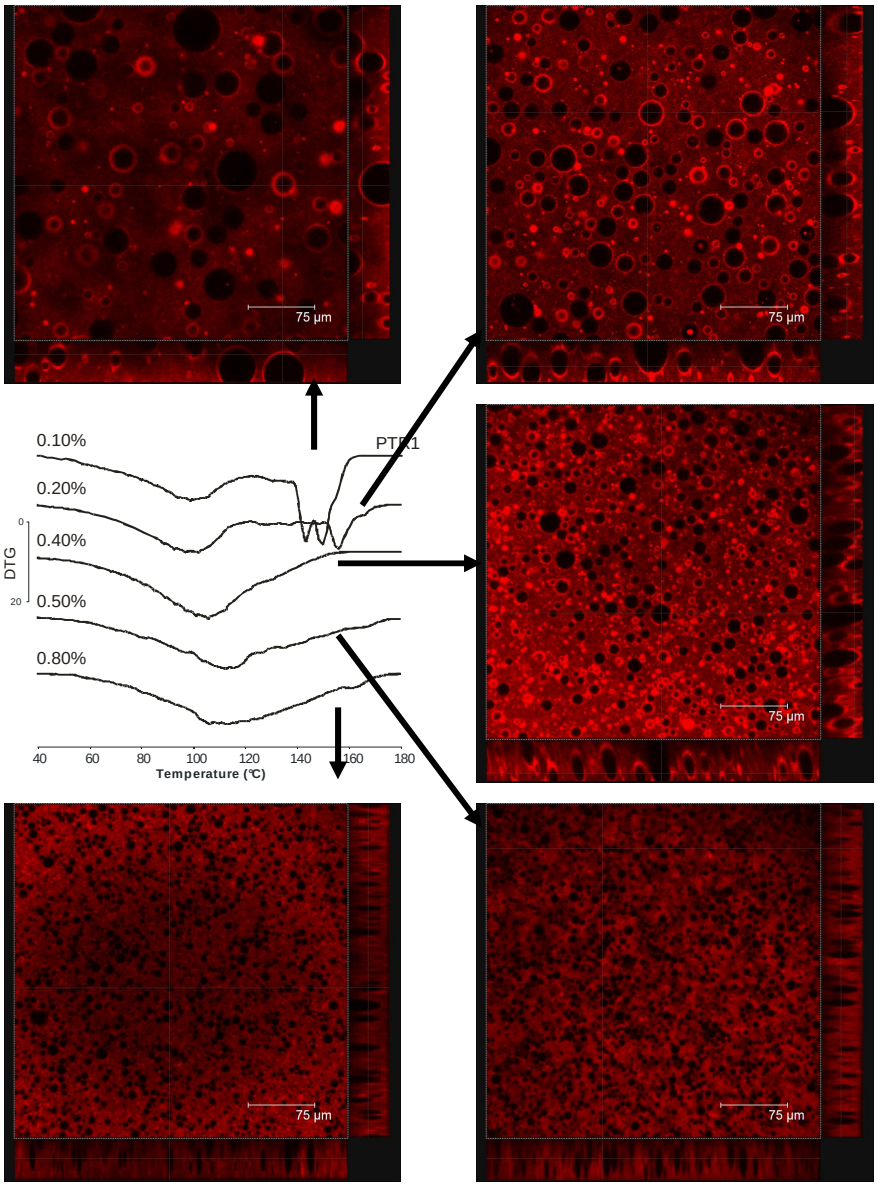

Fig. 27 Correlation between the CLSM pictures and the DTG curves (oil 20% w/w)

4.3 Stability

For studying the stability of the gel emulsion, the gel structure (hydration state, micro gel structure) was examined on the one hand, and the emulsion structure (flocculation, creaming, coalescence etc.) on the other hand.

In the case of the gel structure investigation the evaporation rate and the amount of the bound water were followed during a 3-month storage time.

Several procedures have been applied to predict the stability of emulsions such as: turbidity measurement (for predicting the flocculation, sedimentation or creaming); centrifugation (for the sedimentation or creaming), heating and cooling cycles, droplet size measurements (for coalescence) or rheology. In my work microscopical droplet size analysis and rheological methods were used and the parameters were followed during a 3-month storage time.

If flocculation occurs on storage (without Ostwald ripening and/or coalescence) the value of the initial viscosity (η_0) and the yield value (σ_0) will increase. The presence of Ostwald ripening and/or coalescence can complicate the analysis of the results because both of them can decrease those factors. If η_0 increases while σ_0 shows some decrease, it is from a flocculation occurring in an irregular way (producing strong and tight flocs). In my study η_0 was calculated from the power law as it had been described previously and σ_0 was calculated from the flow curve model on the basis of the Herschel-Bulkley equation:

$$\sigma = \sigma_0 + kD^n \qquad (10)$$

where k is the flow coefficient and n is the Herschel-Bulkley index.

The cohesive energy (E_c) is also used to measure the extent and strength of the flocculated structure:

$$E_c = \tfrac{1}{2} G'\gamma_{cr}^2 \qquad (11)$$

where G' is the storage modulus and γ_{cr} is the critical strain value, which is the minimum strain over which the structure breaks down. The higher E_c is, the more flocculated the structure is. E_c depends on the volume fraction and the droplet size distribution. In this type of gel emulsions flocculation is the most expected process during storage.

Fig.28 shows clearly that the evaporation rate (v) decreased during the storage time, which can be described with a semi-empirical equation:

$$v = A_0 t^k \qquad (12)$$

where A_0 is the evaporation rate at t=0, t is the storage time and k is the velocity constant.

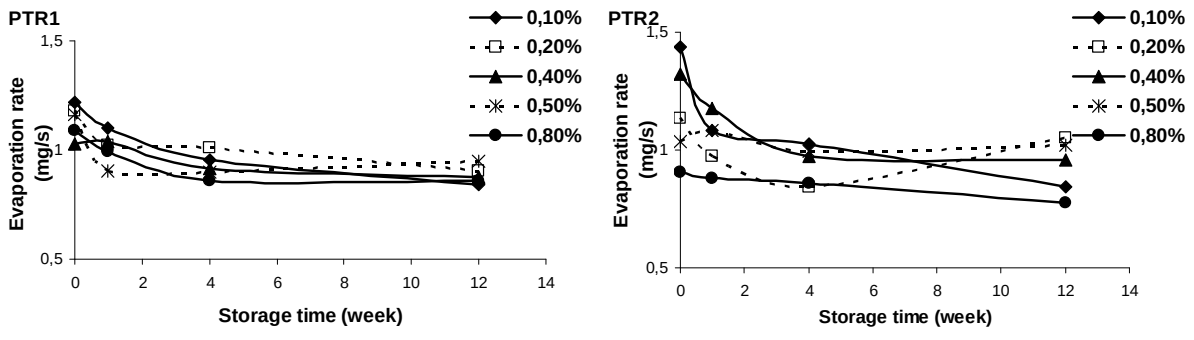

Fig.28 Changing of the evaporation rate during the storage time (oil 20% w/w)

Besides the hydration state of the emulsions, other changes occurred in the micro gel structure. In the case of PTR1 the amount of the micro gel water decreased, while it increased in the case of PTR2. So during storage the micro gel in the PTR1 emulsions expanded while in the PTR1 emulsions it sintered (Fig.29).

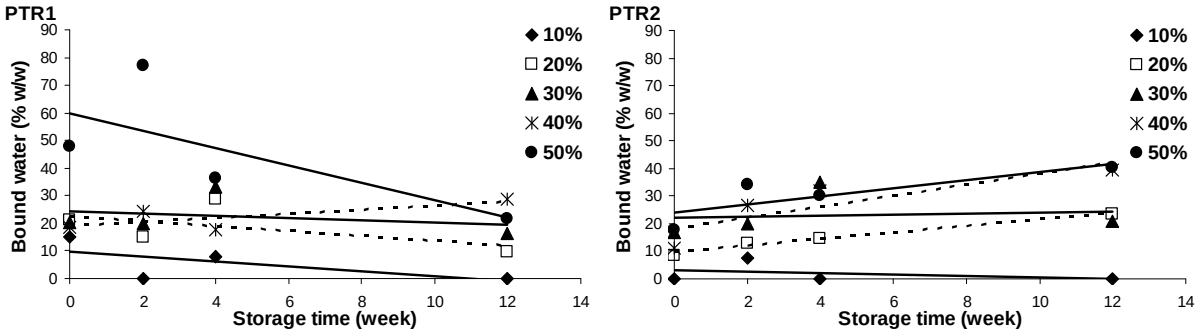

Fig.29 Changing of the amount of the bound water during the storage time (PTR1 or PTR2 0.10% w/w)

The viscosity and yield value changed parallel in the course of the rheological tests (Fig.30, Fig.31). Only at a high polymer concentration did they show some increasing, which can probably be explained by the flocculation and/or increased hydration (Fig.28). In the case of a gel-emulsion it is not possible to separate the gel structure changes and the emulsion breakdown processes from each other, so the increase in the viscosity and yield value may have happened because of one or both of them. However, at a high polymer concentration a coherent gel structure is dominant instead of the micro gel structure (as it had been shown in the thermogravimetric investigation), so the chance that the micro gel structure flocculates is quite small.

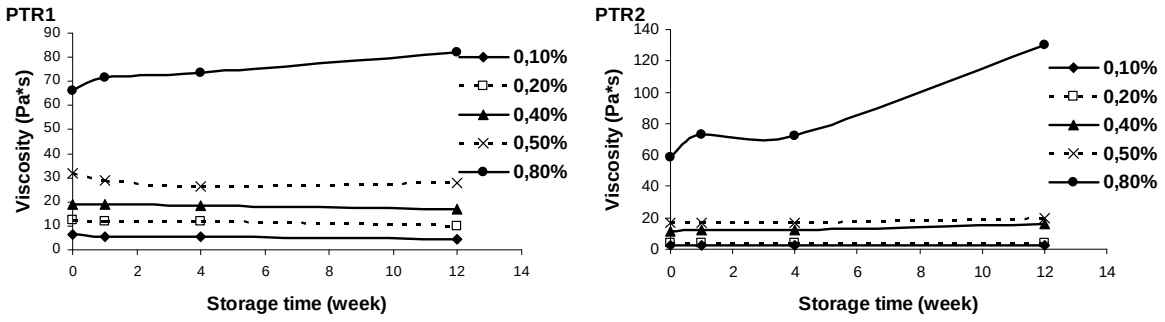

Fig.30 Changing of the viscosity during the storage time (oil 20% w/w)

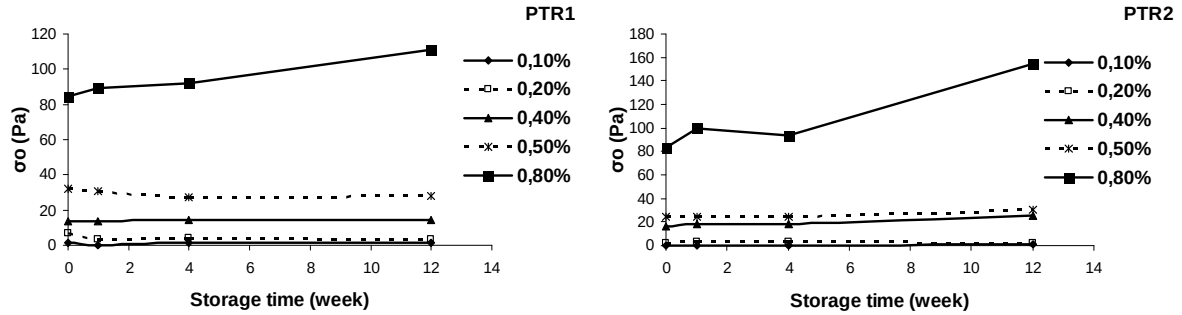

Fig.31 Changing of the yield value during the storage time (oil 20% w/w)

With the use of the dynamic oscillation test the cohesive energies were calculated (from equation 11) (Fig.32). This value can also indicate the extent of the flocculation. At higher concentrations E_c showed some increases in the first weeks but a little decrease after the 4th week. This phenomenon cannot be explained by the flocculation, but can be by the gel structure changes.

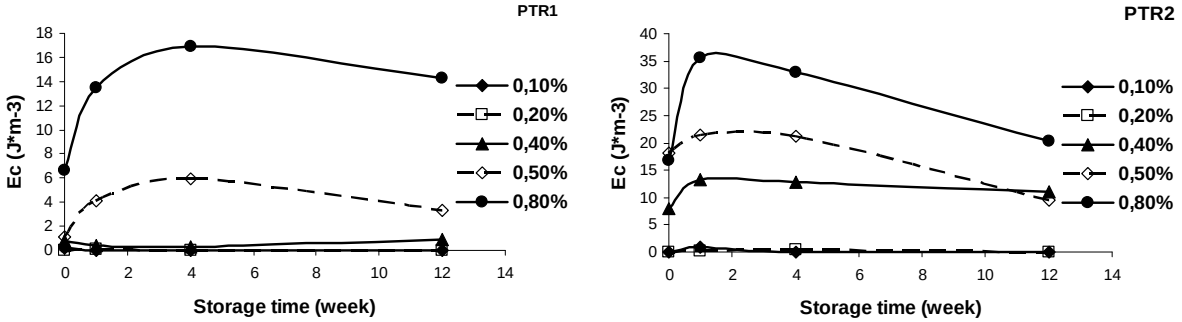

Fig.32 Changing of cohesive energy during the storage time at different polymer concentration (oil 20% w/w)

In the course of the image analysis there were no remarkable changes in the average droplet size of the emulsions during storage (Fig.33). It means that coalescence had not occurred during that time.

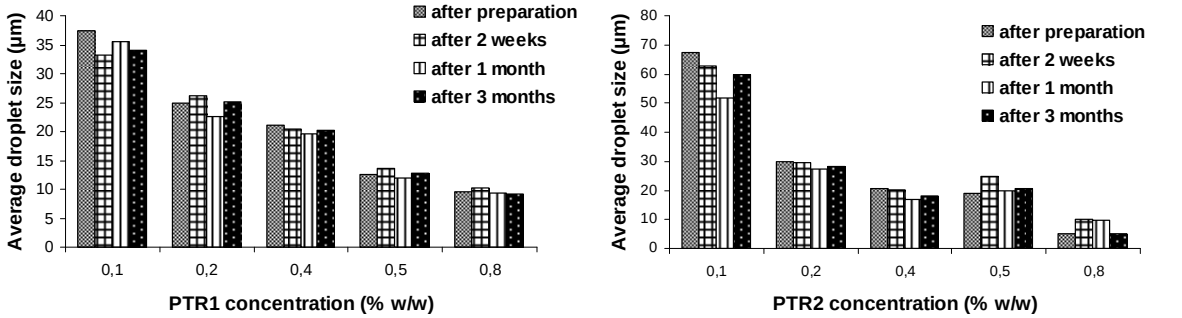

Fig.33 Changing of the average droplet size during the storage time at different polymer concentration (oil 20% w/w)

Emulsions with a very low polymeric emulsifier concentration have mainly micro gel structure (as it had been presented in the thermogravimetric results). In these cases the change of the viscosity and the yield value was different (Fig.34). It may suggest that special flocs formed or coalescence and/or Ostwald ripening occurred in the emulsions.

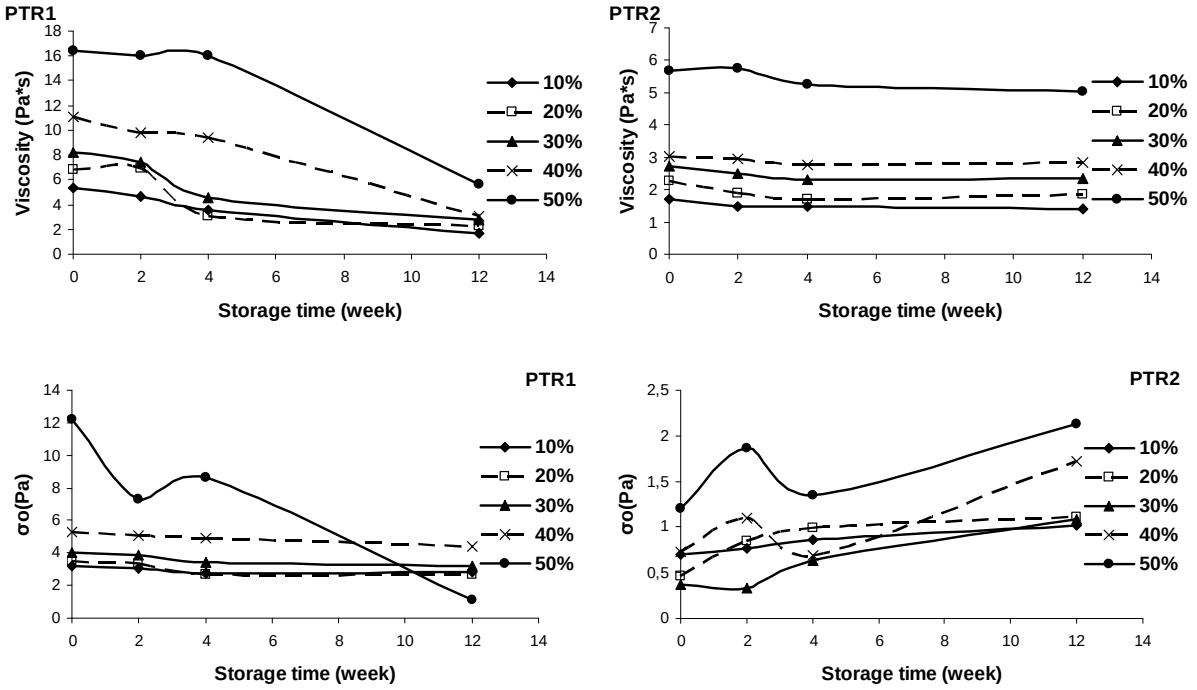

Fig.34 Changing of the viscosity and the yield value during the storage time at different oil concentration (PTR1 or PTR2 0.10% w/w)

In the emulsions where the micro gel is dominant, the integration of the polymer chain is in process during storage. After preparation a part of the molecule integrated into the oil droplets while the other part remained in the bulk water linking the micro gels around the droplets with each other. During storage the latter one also integrated abolishing the linking between the micro gels. As a result, the flocculation rate may decrease. This is why cohesive energy also decreased (Fig.35).

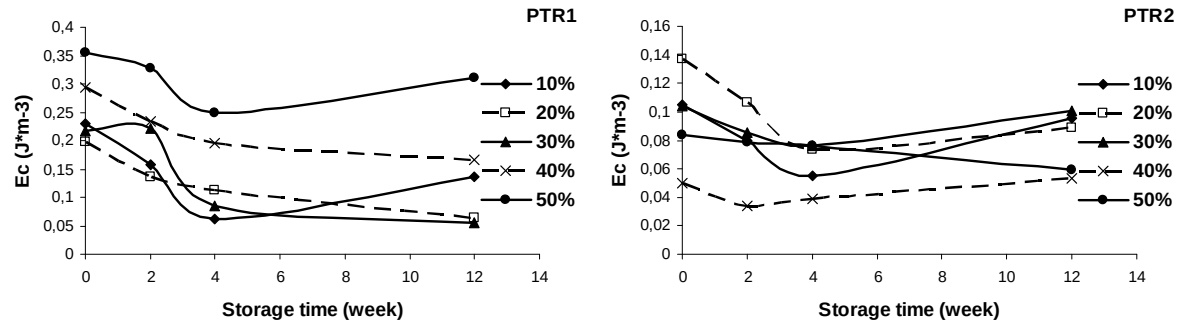

Fig.35 Changing of the cohesive energy during the storage time at different oil concentration (PTR1 or PTR2 0.10% w/w)

The droplet size analysis indicated that only little coalescence occurred in the PTR2 emulsions with higher oil concentration and in the PTR1 ones with lower oil concentration, but it is not remarkable (Fig.36).

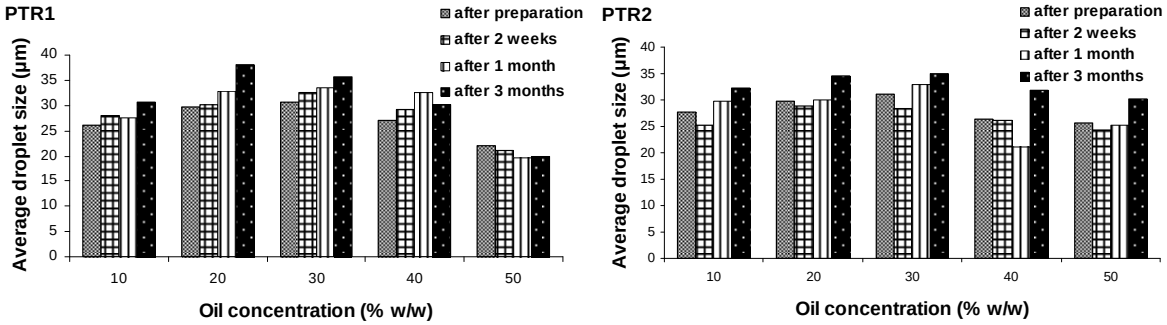

Fig.36 Changing of the average droplet size during the storage time at different oil concentration (PTR1 or PTR2 0.10% w/w)

4.4 Applicability

The further applicability of the emulsions was studied with bioadhesive measurements and drug release tests.

4.4.1 Bioadhesion

Over the last two decades attention has been focused on mucoadhesive dosage forms as a possibility to improve the residence time on a specified region of the body. One group of the most widely used polymers is constituted by poly-acrylates (and their derivatives or cross-linked modifications).

In the literature some examples can be found where the bioadhesive behaviour of different types of dosage forms containing poly(acrylic acid) type polymers has been reported, mainly as components of hydrogels [120, 121] or tablets [122, 123]. In the case of poly(acrylic acid)s, the crosslinking density of these polymers has been established to influence interpenetration, because interpenetration of a larger polymer is more difficult than that of a smaller one and the number of the functional groups which are able to form bioadhesive bonds may decrease. Another important factor in the bioadhesivity of poly(acrylic acid)s is the pH. Protonated and hydrated carboxylic groups are needed for the interaction between mucin glycoproteins and acrylates, but extreme swelling may decrease their adhesivity. Therefore an ideal pH range has been determined at around pH= 4-6 or at around the pK(a) of a certain type of poly(acrylic acid) [124]. In the last few years considerable interest has been shown in new-type polymers such as thiolated polymers, which

form covalent bonds with the mucin in contrast with the weak, non-covalent bonds of the traditionally used polymers. In addition, they are not influenced by the ionic strength or pH, and beyond the latter they also have enzyme inhibitor and permeation enhancing effects. Different type poly(acrylic acid)s-cysteine conjugates were synthesised to improve the bioadhesive property of the dosage form by covalent bond with the cysteine of the mucin glycoprotein [125-128].

By applying gel-emulsions, it is possible to incorporate a lipophilic active agent in a hydrophilic dosage form easily, thereby avoiding the behaviour of the lipophilic vehicle to adhere slightly to the hydrophilic biological surface. When Pemulens are used, the polymer chains build up a special structure instead of a continuous polymer network (Fig.5). When compared with continuous polymer texture, this special structure can modify interpenetration into the mucus (Fig.37).

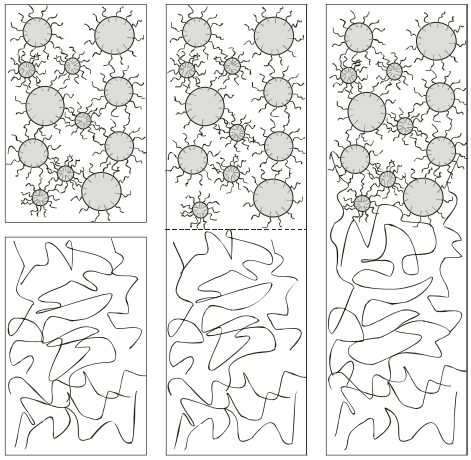

Fig.37 Interpenetration between gel-emulsion containing polymeric emulsifier and mucus

The bioadhesive behaviour of the emulsions was different depending on the different polymerization-degree polymers used in the preparation, as it can be observed in Fig 38. When increasing the polymer concentration at low values, there were changes neither in detachment force nor in adhesive work. In this range the coherent polymer network has not built up yet, as it had already been mentioned previously. Above 0.2% w/w both detachment force and adhesive work decreased with the amount of the polymer in the case of the higher polymerization-degree polymer and increased in the case of the lower polymerization-degree polymer.

PTR1 showed remarkable elasticity in the course of the rheological measurements, which suggested that these systems try to retain their integrity instead of forming chemical or physical bonds with the mucus. Contrarily, emulsions containing PTR2 with lower elasticity are more capable of forming bonds with the surface.

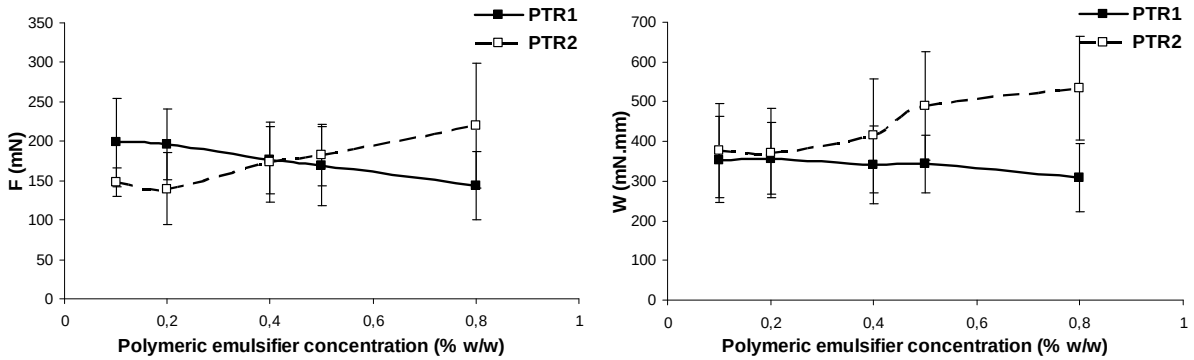

Fig.38 Detachment force and adhesive work as function of the polymeric emulsifier concentration (oil 20% w/w)

No significant change was observed in detachment force when increasing the oil concentration (our previous thermogravimetric measurements had shown the presence of micro gel in almost all these samples). There was a slight decrease in both detachment force and adhesive work between the simple gel and emulsion (Fig.39), which suggests that the added oil reduced the bioadhesivity of the samples.

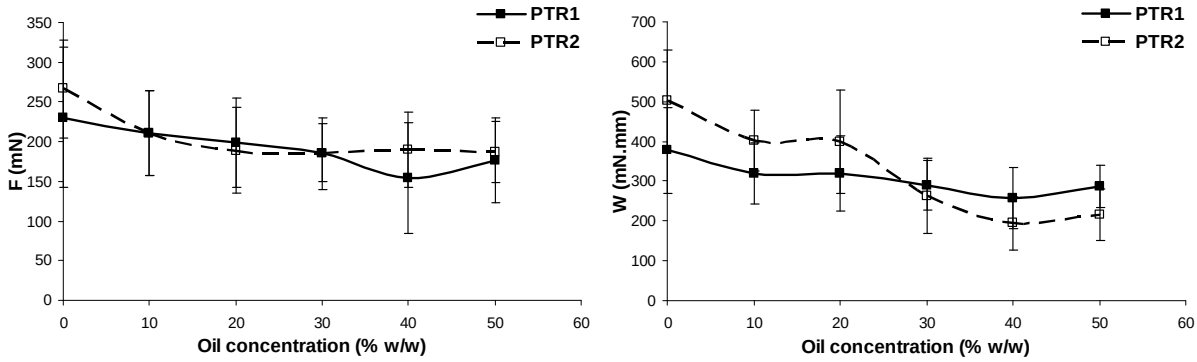

Fig.39 Detachment force and adhesive work as function of the oil concentration (PTR1 or PTR2 0.10% w/w)

The shape of the curve of adhesive work was similar at first to the one of detachment force, but at higher oil concentration, in the case of PTR2, a considerable decrease of the values was observed (Fig.39). In emulsions prepared with PTR2, the damping factor decreased with the increase of the oil concentration (Fig.13), so the deformability of these samples was stronger. Therefore the structure of the emulsion could be destroyed by the downward force. The chemical bonds could build up but physical entanglement could not develop. This explains why detachment force did not change, while adhesive work, which depends on the interpenetration of poly(acrylic acid) chains into the mucus [123], decreased at high oil concentration.

When a coemulsifier was used, a decrease in detachment force and adhesive work was observed, which is more expressed at a high S101 concentration (Fig.40). The viscosity and the elasticity of these samples were higher at a high coemulsifier content (Fig. 14). On increasing the amount of the coemulsifier, the accumulation of the polymeric emulsifier at the interface was inhibited, so the coherent polymer network was built up progressively. These changes in the microstructure influenced the rheological and bioadhesive behaviour. Based on the thermogravimetric and bioadhesive measurements, it can be concluded that the coherent polymer network can decrease the bioadhesivity of the samples as compared to the ordered micro gel system.

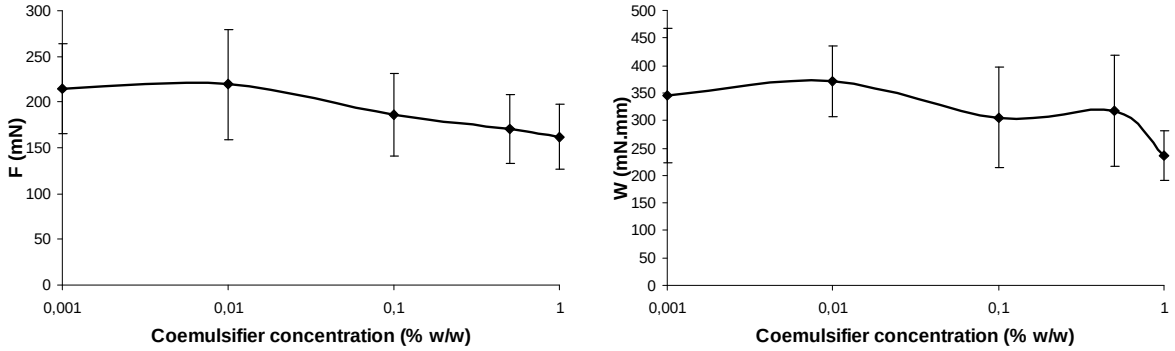

Fig.40 Detachment force and adhesive work as function of the coemulsifier concentration (PTR2 0.10% w/w, oil 20% w/w)

As the dye concentration indicates the place of the polymeric emulsifier, if the polymeric emulsifier forms a bond with the mucin, structural changes will take place in the samples, which will appear in the distribution of the dye.

At low polymer concentration, due to the interaction between mucin and poly(acrylic-acid), polymer agglomeration can be observed in the pictures. In addition, oil droplets were retained in them. It can be assumed that mucin formed bioadhesive bonds with the micro gel around the droplets and not with a network. In the course of the tensile test measurements the samples in this range did not show changes in the bioadhesive behaviour. At high polymer content (above 0.2% w/w) no agglomeration can be seen, so interaction arose with the total polymer network (Fig.41).

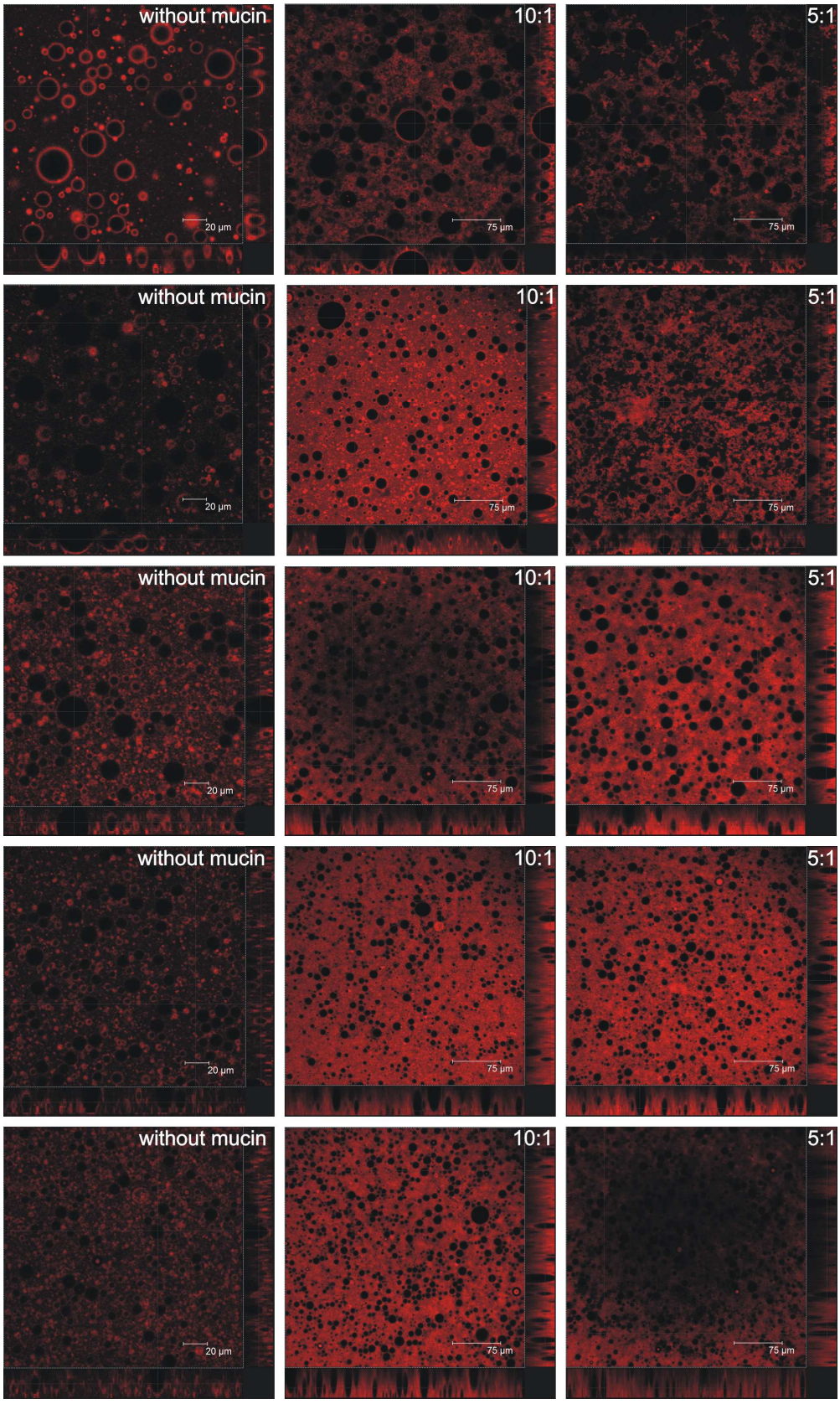

Fig.41 CLSM images of the simple emulsion (first column); and 10:1 (second column) and 5:1 (third column) emulsion-8.0% w/w mucin mixtures (from the first row to fifth row the polymer concentrations are the following: 0.10; 0.20; 0.40; 0.50 and 0.80% w/w)

4.4.2 Drug release

In vitro dissolution has been known as an important element in drug development. In the case of topical administration, it is preferred to apply a bioadhesive dosage form, but in addition to bioadhesivity, controlled drug release from the dosage form is also desirable. As it was established, the gel emulsions have a special micro gel structure. It suggests that not only the components but also the gel structure can modify drug release.

No considerable difference could be observed using water soluble Metronidazole. Only a few alterations can be seen in Fig.42 between the simple gels and the gel-emulsions. Emulsion structure lowers the dissolution rate in the first hours. The micro gel structure may have slowed drug release.

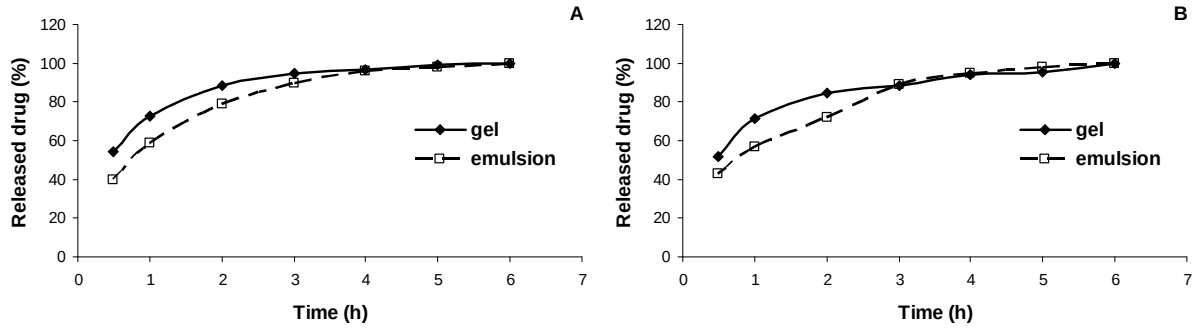

Fig.42 Comparison of the Metronidazole release from emulsions and gels with 0.10% w/w (A) 0.40% w/w (B) PTR1 concentration (oil 20% w/w)

When the components such as polymer (Fig.43A), oil (Fig.43B) or coemulsifier (Fig.43C) concentration of the emulsions were changed, no difference was found in the dissolution profile.

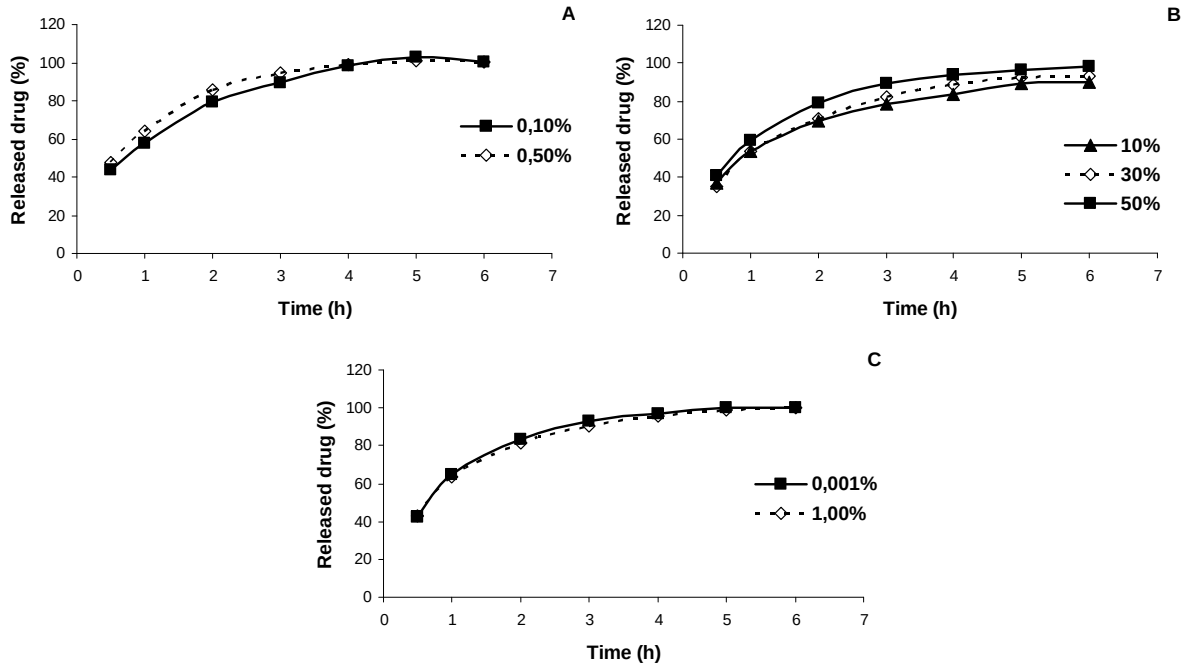

Fig.43 Effect of the components on the Metronidazole release

When a Lidocaine base was dissolved in the oil phase, the components of the emulsion affected drug release. With the increase of the polymer concentration it can be observed that the rate of drug release and the amount of the released drug increased (Fig.44). Three different factors can have an effect on drug release, such as the viscosity of the continuous phase, the interface area (due to the droplet size), and possibly the gel structure. Viscosity increases with the polymer concentration, so if it had been the main affecting agent, the decrease of the rate would have been seen on the curves. In fact, it was not, which suggests the other two factors play a role in drug release. As it had been presented previously, the average droplet size had decreased with the polymer concentration, so the interface area had increased. On the basis of Fick's law, diffusion increases with the increasing of the area, which accelerates the Lidocaine diffusion from the oil phase to the water phase and provides quicker drug release. This tendency can be observed in Fig. 44. The third factor was the gel structure. At a low emulsifier concentration (less than 0.40% w/w), a micro gel layer forms around the droplets that can hinder drug diffusion from the oil phase, so the rate and the amount of the released drug may decrease. Probably these two factors determine the drug release profile.

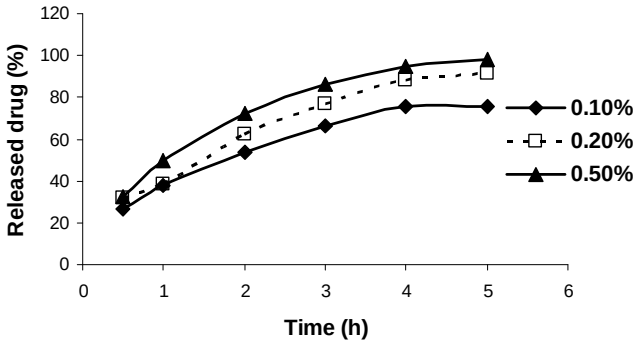

Fig.44 Effect of the emulsifier concentration on the Lidocaine release (oil 20% w/w)

With the change of the oil concentration three factors can influence the release profile, such as viscosity, the oil-water ratio and gel structure (the droplet size had not changed remarkably). If the oil concentration is higher, the concentration gradient between the aqueous and oily phase will decrease, which slows drug release (because diffusion will slow down on the basis of Fick's law). On the other hand, the amount of the micro gel increased with the oil concentration (as it had already been shown in the thermogravimetric results), so the extent of the gel layer increased, which hindered release. These two latter phenomena and the increase of viscosity (as it had been presented in the rheological results) may result in the decrease of the release with the oil concentration (Fig.45).

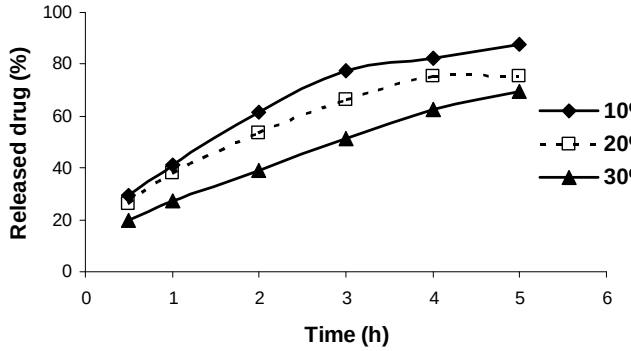

Fig.45 Effect of the oil concentration on the Lidocaine release (PTR1 0.10% w/w)

When a coemulsifier is used, a change in the interfacial layer can be expected. In addition to this change, the decrease of the droplet size and possibly the increase of viscosity (but only at 1.00% w/w!) can influence the release profile. The droplet size continuously decreased with the S101 concentration (Fig.46) so the increase of the release must happen. Contrarily, a minimum release rate can be observed at 0.10% w/w coemulsifier (Fig.46). It suggests that the change of the interfacial layer has an important role in this phenomenon. 0.10% w/w can be the best combination rate of the two emulsifiers at which the distribution of the Lidocaine between the two phases is the slowest.

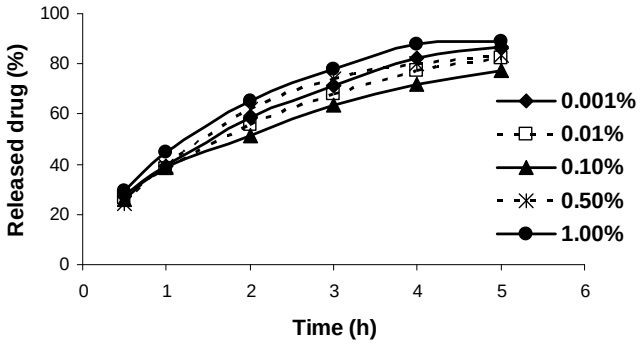

Fig.46 Effect of the coemulsifier concentration on the Lidocaine release (PTR1 0.10%, oil 20%)

5 SUMMARY

The aim of this research work was to identify and clarify the formation, structure and stability of gel-emulsions. As a summary of my experimental work, the following conclusions can be made:

- The wetting and the swelling of these polymeric emulsifiers are a prolonged procedure; Pemulens show a low surface activity, which suggests that the presence of a coemulsifier is also required to facilitate the emulsification procedure**.** These phenomena have to be considered in the course of the emulsion preparation.
- Gel-emulsions have viscoelastic properties, the polymer, the oil and the coemulsifier concentration have an influence on the rheological properties.
- Gel-emulsions containing Pemulens form a special (micro gel) structure which can be identified by thermogravimetric investigations and by confocal laser scanning microscopy. The results of the two methods can be compared and showed a good correlation.
- The stability of the emulsions can be divided into two groups: change of i) macrostructure, and ii) microstructure. Macrostructure means the droplet size while microstructure means the solvation of the polymer chain, the evaporation rate, the amount of the micro gel water, the rheological properties. The macrostructure of the emulsions can be considered quite stable while the microstructure changed continuously during storage.
- The oil added to the emulsion slightly modified their rheological and bioadhesive behaviour. With increasing polymer concentration, the two different polymerization-degree polymers showed different results. The added coemulsifier modified the structure of the emulsions, which influenced the bioadhesive characteristics. Comparing the thermogravimetric end bioadhesive measurements, it can be concluded that i) the emulsion containing mainly micro gel around the droplets shows a more remarkable

bioadhesive force than the sample with a coherent polymer network; ii) there is no difference between the bioadhesivity of the samples where the coherent gel structure had not built up.

- Drug release from the gel emulsions is influenced by the special gel structure in addition to the well known factors (e.g. viscosity, droplet size, water-oil ratio etc…). This phenomenon could be observed in the case of a lipophilic drug (Lidocaine base), but could not in the case of a hydrophilic drug (Metronidazole).

In conclusion, gel emulsions can be used well as bioadhesive topical dosage forms. Their structure is influenced by different factors. As it was presented in this study, the gel structure can modify the bioadhesive behaviour and the drug release profile of the systems. It suggests that a structure analysis is needed to predict the properties and hence the applicability.

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ANNEX

I.

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Gél-emulziós rendszerek I. rész Fizikai kémiai jellemzés

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Szegedi Tudományegyetem Gyógyszer-technológiai Intézet, Szeged, Eötvös u. 6. – 6720

Summary

Szűcs, M., Budai, Sz., Erős, I.: Gel-emulsion systems I. Physical-chemical characterisation

Emulsion gels prepared with polyacrylic acid–alkyl acrylate diblock copolymer surfactants were studied. It was supposed that the polymer surfactants surrounding the oil droplets formed a microgel structure and this structure stabilized the emulsions sterically. This assumption was verified by thermoanalytic investigation. The effect of polymer concentration and the amount of oil on the rheological characteristics, the rate of water evaporation and droplet size distribution was analysed. It was established that gel emulsions had viscoelastic the properties, the viscosity increased exponentially with increasing emulsifier concentration and amount of oil. Water was present in two forms: i) in microgel surrounding oil droplets and ii) in dispersion medium. The distribution of droplet size was generally a monodisperse one, the average droplet size decreased with polymer concentration.

Összefoglalás

A szerzők poliakrilsav-alkil-akrilát diblokk polimerekkel előállított emulziós géleket tanulmányoztak. Feltételezték, hogy a polimer emulgensek az olacseppek körül elhelyezkedve mikrogélt képeznek és e mikrogél sztérikusan stabilizálja az emulziókat. Ezt a feltételezést termoanalitikai vizsgálatokkal igazolták. A polimer mennyiségének és az emulgeált olaj töménységének hatását vizsgálták a reológiai jellemzőkre, a víz kötésmódjára, a párolgási sebességre és a cseppméret eloszlásra. Megállapították, hogy a gél emulziók viszkoelasztikus rendszerek, az emulgens koncentrációjával és az emulgeált olaj mennyiségével exponenciálisan nőtt a viszkozitás. A víz kétféle formában van jelen: az olajcseppek körüli mikrogélben és az emulziók diszperziós közegében. A cseppméret-eloszlás általában monodiszperz, az átlagos cseppméret a polimer mennyiségének növelésével csökken.

Bevezetés

Az emulziókat, mint hatóanyag-hordozó rendszereket, már a 17. század óta ismerik és alkalmazzák. Tudományos vizsgálatuk *Graham* és *Gibbs* munkásságával kezdődött, majd számos más kolloidkémikus eredményei alapján váltak széles körben elterjedté a gyógyszerészetben, a vegyiparban, az élelmiszeriparban és a mindennapi életben [1].

A 20. század második felétől kezdve a gyógyszer-technológiai kutatások egyik irányvonala, új gyógyszerhordozó rendszerek keresése, fejlesztése, programozott hatóanyag-leadás céljából [2]. Ennek eredményeképpen az emulziók ismét előtérbe helyeződtek, mivel külső fázisuk viszkozitását változtatva a hatóanyag-leadás szabályozható. Ez és még számos előnyös tulajdonságuk szól amellett, hogy még ma is korszerű gyógyszerhordozó-rendszereknek tekinthetők ezek a sajátos diszperz rendszerek.

Az emulziók gyógyszer-technológiai kutatásának napjainkban három alapvető irányvonala van:

- új emulziós gyógyszerhordozó rendszerek kidolgozása [3-6],
- az emulziók reológiai jellegének és szerkezeti stabilitásának kutatása korszerű módszerekkel és készülékekkel [7, 8],
- az emulziók gyógyszerleadásának beható és részletes tanulmányozása [9, 10].

Kísérletes munkánk az első és a második kutatási irányvonalhoz csatlakozva egy új gyógyszerleadó rendszer, a gél-emulziók szerkezetének, fizikai kémiai sajátságainak és stabilitásának megismerésére irányult.

A gél-emulziók előállítására az alábbi módszerek alkalmazhatók:

- az emulzió vízfázisának gélesítése vízoldékony polimerekkel,
- az emulzió olajfázisának gélesítése,
- a két fázis határfelületén polimer védőréteg létrehozása *in situ* polimerizációval,
- az olaj- és vízfázis határfelületén mikrogél kialakítása polimer emulgenssel.

Kísérletes munkánk során ez utóbbi módszert alkalmaztuk.

A polimer emulgenseknek alapvetően két fő csoportja van:

1. Blokk polimerek, amelyek lehetnek di- vagy triblokkok, ill.
2. Graft polimerek.

A polimerek blokkjai eltérő oldékonyságúak, ezáltal az olaj/víz határfelületen fognak feldúsulni. Stabilizáló hatásuk kisebb részben a felületi feszültség csökkentéssel, nagyobb részben a sztérikus gátlással magyarázható.

Az általunk használt polimer emulgens a Pemulen TR1 és a TR2 volt, melyek poliakrilsav-alkilakrilát diblokk polimerek. (Előállító: Lubrizol Corp., USA). A két emulgens polimerizációs fokukban különbözik egymástól, a Pemulen TR2 kisebb, a Pemulen TR1 nagyobb polimerizációs fokú. Alkalmasak o/v típusú emulziók előállítására, mivel rövid lipofil karakterű részük az olajcseppbe integrálódik, míg hosszabb hidrofil karakterű részük a vizes fázisban gélt képez közvetlenül az olajcsepp körül. A rendszerben sajátos kettős gélrendszer jön létre: a határfelületi orientáció miatt a polimer koncentrációja az olajcsepp körül nagyobb lesz, ez alkotja az un. mikrogélt, az emulzió külső vizes fázisát gélesíti a feleslegben alkalmazott polimer. (*1. és 2. ábra*)

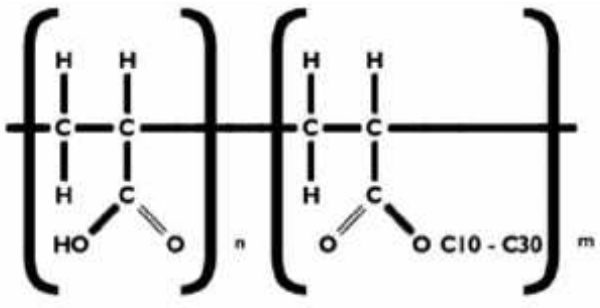
Vizsgálataink célja a következő volt: részletesen tanulmányozni kívántuk a polimer emulgensekből készített gélek és emulziók képződését,

- kutattuk a rendszerek szerkezetét, reológiai tulajdonságait,
- tanulmányoztuk a víz kötődési mechanizmusait,
- ezek alapján megfelelő hatóanyag-hordozó rendszereket szándékoztunk kidolgozni.

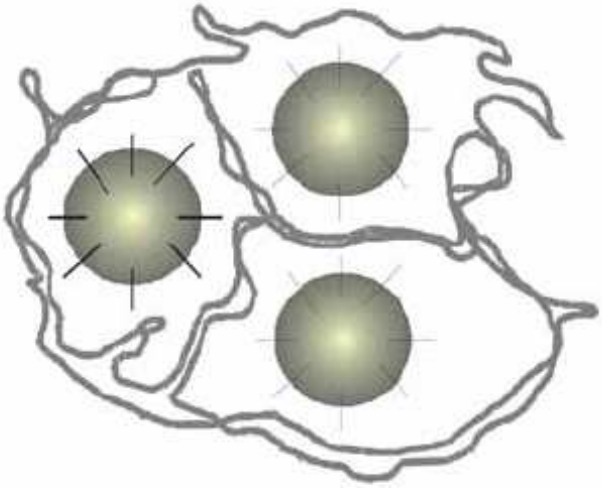
Anyagok és módszerek

Anyagok

Polimer emulgensként a Pemulen TR1 és TR2-t alkalmaztuk. Vízfázisként a gyógyszerkönyvi tisztított vizet (Aqua purificata, Ph. Hg. VIII.), olajfázisként Miglyol 812-t (Sasol GmbH, Németország) használtuk. Semlegesítő komponens trolamin (Ph. Hg. VIII.) volt.



1. ábra: A Pemulen TR1 és Pemulen TR2 szerkezeti képlete



2. ábra: A polimer emulgenseket tartalmazó emulziók teoretikus modellje

A minták előállítása

Először alapgélt készítettünk, melynek polimertartalma 1,0% volt. A víz teljes mennyiségében 24 órán keresztül duzzasztottuk a polimert, majd hozzáadtuk a semlegesítő komponenst olyan mennyiségben, hogy a pH 5,0-5,5 között legyen. A továbbiakban az alapgélből készítettünk megfelelő hígításokat és a gél-emulziós mintákat. A gél-emulziók előállítása során gélekben emulgeáltuk az olajat kis részletekben. A minták pH-ját üvegelektród segítségével határoztuk meg.

Vizsgálati módszerek

Felületi feszültség mérése

A méréseket Krüss-féle tenziométerrel végeztük. Hígítási sort készítettünk 0,00005% és 0,25% között. A levegő/polimer-oldat közötti határfelület stabilizálódása miatt 5 perc várakozás után végeztük el a mérést. Minden koncentráció esetében 3 párhuzamos meghatározást végeztünk.

Polimerek vízfelvételének mérése

A méréseket Enslin készülékkel végeztük. A készülék G2-es üvegszűrőjére szűrőpapírt helyeztünk hézagmentesen, majd 0,10 g polimert rétegeztünk

rá. A szűrővel egy szintben, vízszintesen elhelyezett pipettáról olvastuk le a polimer emulgens által adott idő alatt felszívott víz mennyiségét ml-ben. A leolvasást 10 percig végeztük és 3 párhuzamos mérést középértékeltünk.

Nedvesedési peremszög meghatározása

A vizsgálatok a cseppszétterülés mérésén alapuló Dataphysics OCA készülék segítségével történtek. A polimerekből 0,20 g-os próbatesteket préseltünk (10 másodpercig 5 tonna nyomással), melyeket 24 órára exszikkátorba helyeztünk. Ezt követően határoztuk meg a nedvesedési peremszöget desztillált vízzel és Miglyol 812-vel.

Reológiai vizsgálatok

A reológiai méréseket HAAKE RheoStress 1 (HAAKE GmbH, Németország) kúp-lap geometriájú készülékkel végeztük 25 °C-on. Szabályozott nyírási sebesség-gradiens mellett felvettük a minták folyás- és viszkozitás-görbéjét (a felszálló ágat 0,1 s^{-1}–től 100 s^{-1}-ig, a leszálló ágat 100 s^{-1}-től 0,1 s^{-1} intervallumban határoztuk meg). Az oszcillációs mérések folyamán meghatároztuk a minták lineáris viszkoelaszticitási tartományát (a nyírófeszültséget 0,1 és 100 Pa között változtattuk). E tartományon belül választottunk egy nyírófeszültség értéket és ezen értéken határoztuk meg az emulziók és gélek tárolási és veszteségi moduluszát a frekvencia függvényében (0,1 és 100 Hz között). Minden esetben 3 párhuzamos mérést középértékeltünk.

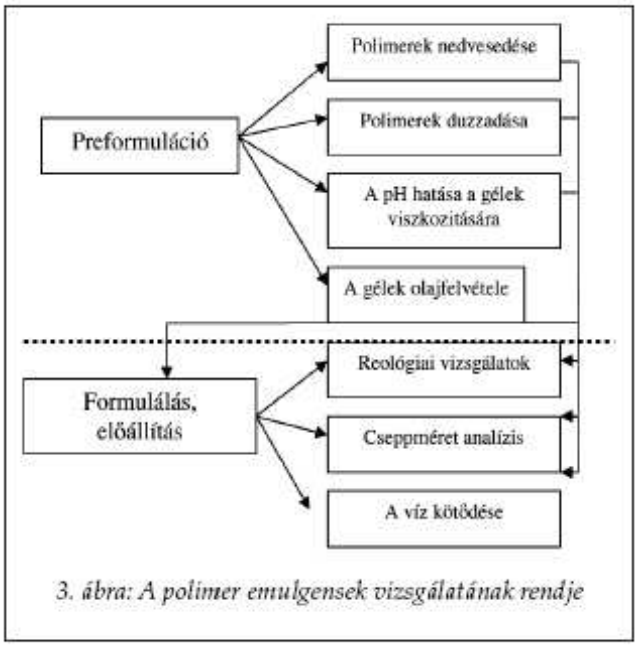
Termoanalitikai mérések

A termoanalitiakai méréseket Derivatograph C (MOM, Magyarország) készülékkel végeztük. 50 mg mintát mértünk platina tégelybe. A mintákat 25-től 200 °C-ig fűtöttük 5 °C/perc sebességgel, és felvettük a TG (tömegcsökkenés az idő függvényében) és dTG (TG időszerinti deriváltja) görbéket. A TG görbék közel lineáris szakaszára egyenest illesztettünk, melynek meredekségéből következtettünk a víz párolgási sebességére a mintákból. A víz kötődési mechanizmusának meghatározására 10 °C/perc fűtési sebességet használtunk.

Cseppméret-analízis

A cseppméret-analízist Leica képanalizátorral végeztük. 500 csepp átmérőjét mértük meg és Leica Q500MC Qwin V01.02 szoftver segítségével értékeltük az eredményt.

Kísérleteink rendjét a *3. ábrán* szemléltetjük.



3. ábra: A polimer emulgensek vizsgálatának rendje

Eredmények és értékelés

Preformulációs vizsgálatok

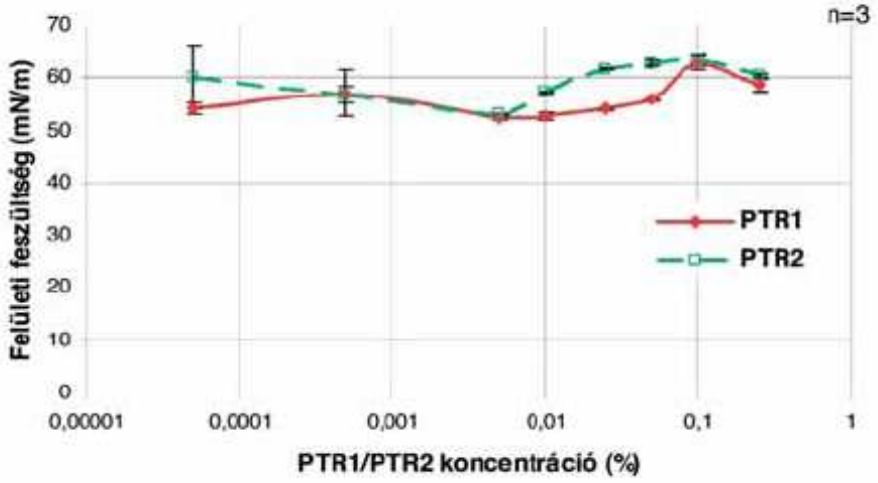
A preformulációs vizsgálatok során megállapítottuk, hogy a polimer emulgensek csekély felületi feszültség csökkentő hatással rendelkeznek. A víz 72 mN/m felületi feszültségéhez képesti 50-60 mN/m közötti érték nem mondható jelentős csökkenésnek. A felületi feszültség–koncentráció összefüggést vizsgálva mindkét emulgens esetében minimumon átmenő függvényt kaptunk, a minimum helye 0,01% koncentráció körül volt (ez tekinthető a felületaktív polimer kritikus micellaképződési koncentrációjának). A két emulgens felületaktív jellege nem tért el jelentősen egymástól, tehát a polimerizációs fok nem befolyásolta a felületi feszültség csökkentő hatást. (*4. ábra*)

A polimerek viszonylag rosszul nedvesedtek vízzel (Θ=81-82°), míg olajjal sokkal jobb nedvesedést mértünk (Θ=26-27°). A két különböző polimerizációs fokú polimer ebben az esetben is hasonló értékeket mutatott, tehát a polimerizációs fok nem befolyásolta a polimerek nedvesedését sem. (*1. táblázat*)

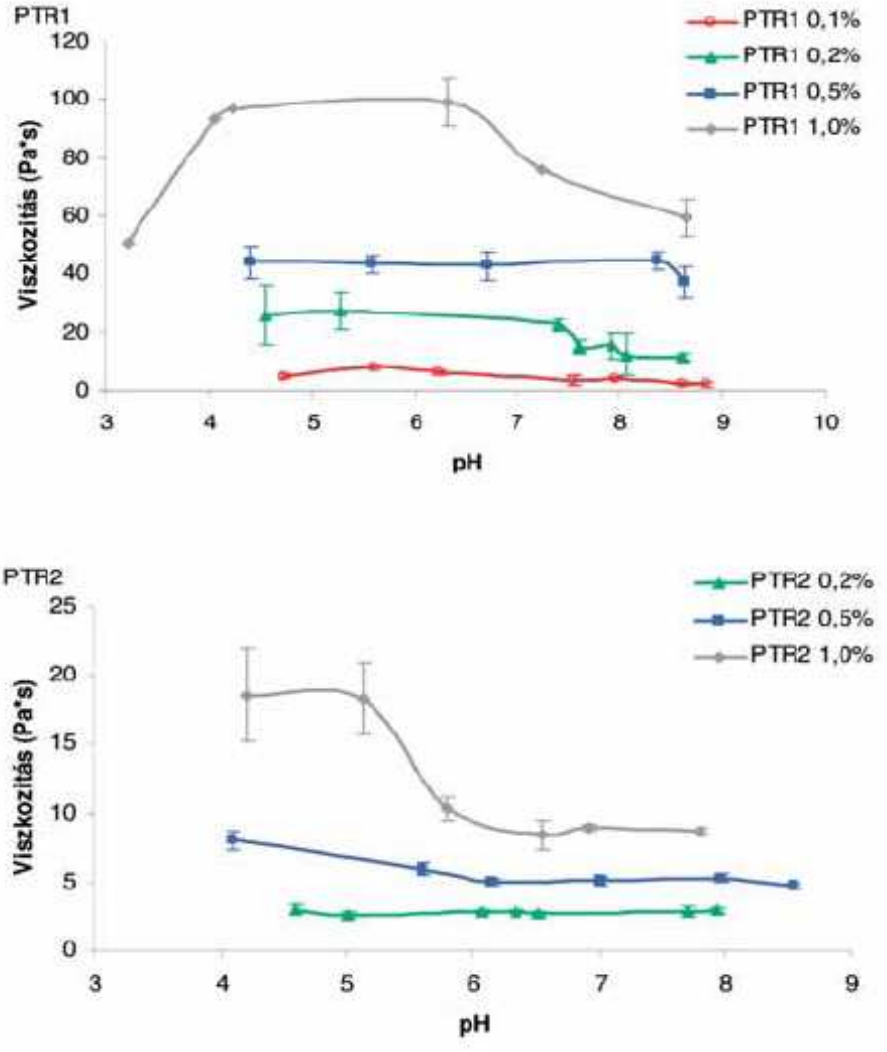
1. táblázat

A polimerek nedvesedési peremszöge vízzel és olajjal

Nedvesedési peremszög, Θ (± SD, n=7)		
	PTR1	PTR2
Tisztított víz	81,7 ± 5,67	82,64 ± 1,24
Miglyol 812	27,9 ± 0,88	26,8 ± 0,49



4. ábra: A polimer emulgensek felületi feszültségének változása a polimer-tartalommal

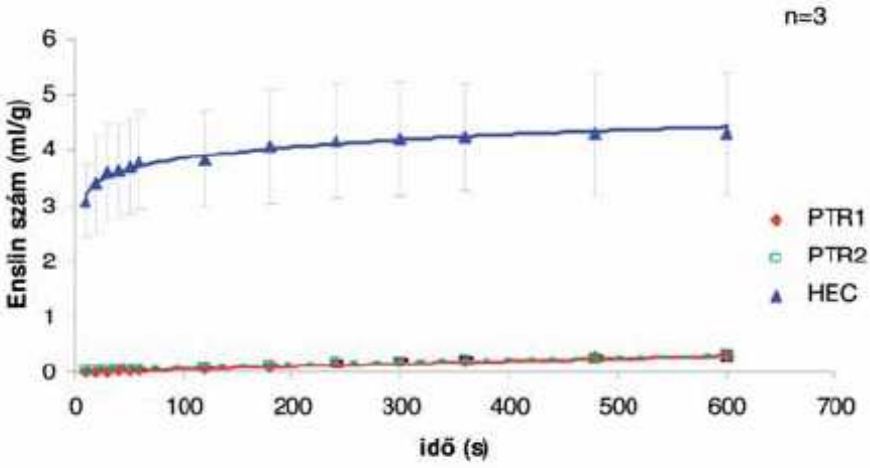


5. ábra: A polimerek vízfelvételének összehasonlítása a hidroxi-etil-cellulózzal

Enslin készülékkel vizsgálva a polimerek duzzadását, azt a következtetést vontuk le, hogy a duzzadás lassú és elhúzódó folyamat. Összehasonlítva a széles körben alkalmazott hidroxi-etil-cellulózzal (HEC), jelentősen elmaradnak ez utóbbi Enslin-értékétől (*5. ábra*).

A polimerek hidratációja és ez által a hidrogélek viszkozitása általában pH függő jelenség. Különböző koncentrációjú polimer emulgensből képződött hidrogél viszkozitását vizsgáltuk a pH függvényében. A pH-t trolamin hozzáadásával változtattuk és pH-mérő üvegelektród segítségével mértük. Az eredmények alapján elmondható, hogy a viszkozitás csak nagy polimer-koncentrációnál mutat jelentős pH-függést (1,00%) mindkét polimerizációs fokú emulgensnél. 1,0%-nál kisebb koncentrációban nem befolyásolta a kémhatás a gélek viszkozitását. A további vizsgálatokhoz 5,5 pH-jú géleket és emulziókat állítottunk elő. (*6. ábra*)

A hidrogélek olajfelvételét a következőképpen határoztuk meg. A különböző koncentrációjú hidrogélekhez büretából cseppenként adtuk hozzá a semleges olajat mindaddig, míg a rendszer szétvált. A fogyott olaj térfogatát tömegre átszámoltuk, majd kiszámoltuk az emulziók pontos százalékos összetételét. Megállapítottuk, hogy a nagyobb polimerizációs fokú emulgenssel 80%-os olajtartalmú emulziók is előállíthatóak, míg a kisebb polimerizációs fokú csak 50-60%-os olajfelvételt eredmé-



6. ábra: A polimer emulgensekből képzett gélek viszkozitásának függése a pH-tól

nyezett. Abban az esetben, ha az olajat nem cseppenként, hanem csak apró részletekben adagoltuk, 50% feletti olajtartalmú stabil rendszereket nem sikerült előállítani. (*7. ábra*)

Termoanalitikai vizsgálatok

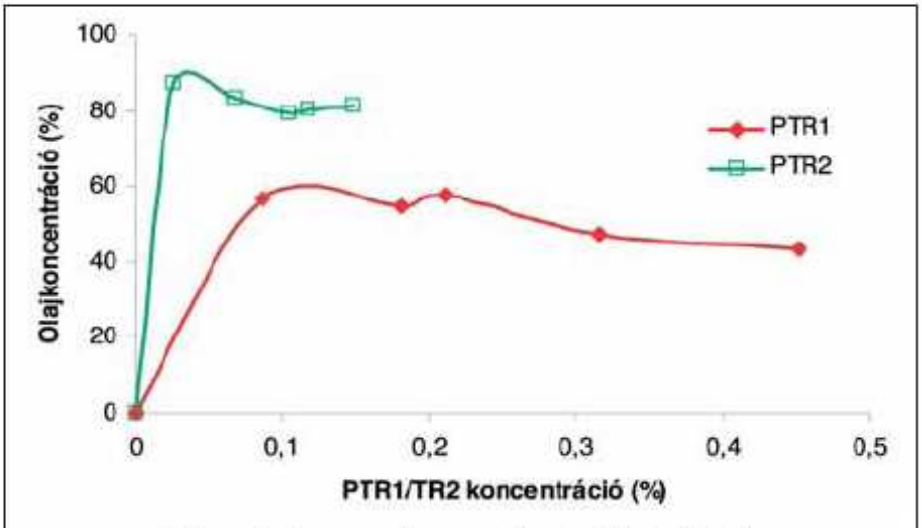
Feltételezésünk szerint a polimer emulgens amfifil sajátsága folytán a határrétegben nagyobb koncentrációban lesz jelen, mint a fázisok belsejében. Ennek következménye, hogy egy koncentráltabb „mikrogél" alakul ki közvetlenül a cseppek körül. A cseppektől távolabbi helyek polimerben szegényebbek lesznek. Ha a feltételezésünk igaz, az emulziók összefüggő külső vízfázisában kétféle módon kötött vizet kell találnunk:

1. A cseppek körüli polimerek által kötött vizet (gélvizet);
2. A cseppektől távolabbi területeken lévő, a polimerhez nem kötött vize (töltő vizet).

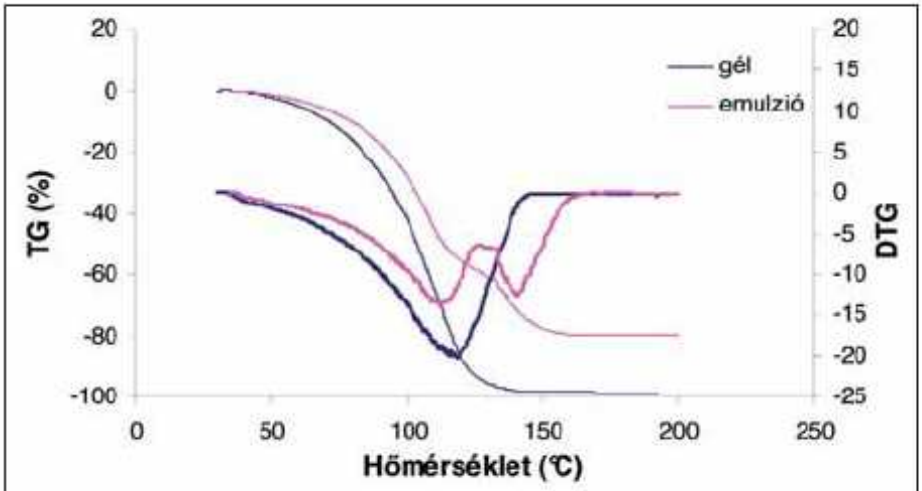
A félszilárd krémrendszerekben jelenlévő víz kötöttségével sok szerző foglalkozott [-17]. Ezek részletes tanulmányozása több szempontból is fontos, mivel dermatológiai készítményekkel kapcsolatban összefüggésbe hozható a hidratáló képességgel, valamint a rendszerekből a víz párolgási sebességével, tehát a hűtőhatással. Amennyiben vízoldékony hatóanyag hordozója a kétféle módon kötött vizet tartalmazó rendszer, módosul a hatóanyag eloszlása és ezáltal a gyógyszer felszabadulás kinetikája.

A *8. ábrán* jól látható a polimert ugyanolyan mennyiségben tartalmazó Pemulen hidrogél és a gél-emulzió vízkötése közötti különbség. A hidrogél esetében egy lépcsőben távozik a víz teljes mennyisége, egyszerű lefutású a TG görbe valamint a dTG görbe is egy csúcsot mutat. Ezzel szemben a gél-emulziók esetében két lépcső különül el a TG görbén és a dTG görbén is két éles csúcs jelentkezik. Ez alátámasztja azon feltételezésünket, miszerint a gél-emulziókban jelen van szabad vízfázis (a cseppektől távolabbi területeken), amit a dTG görbe első csúcsa jelez 110 °C körül, valamint jelen van kötött víz (a cseppek környéki mikrogélhez kötve), amit a dTG görbe második csúcsa reprezentál 140 °C-nál. Ezzel szemben a hidrogéleknél ez a differenciáltság nem figyelhető meg.

Összehasonlítva a gél-emulziók és az olajat nem tartalmazó gélek párolgási sebességét, a vártnak megfelelően a gélekből a víz párolgása sokkal erőteljesebb volt. E jelenség többek között azzal is magyarázható, hogy melegítés hatására az olaj kiválik



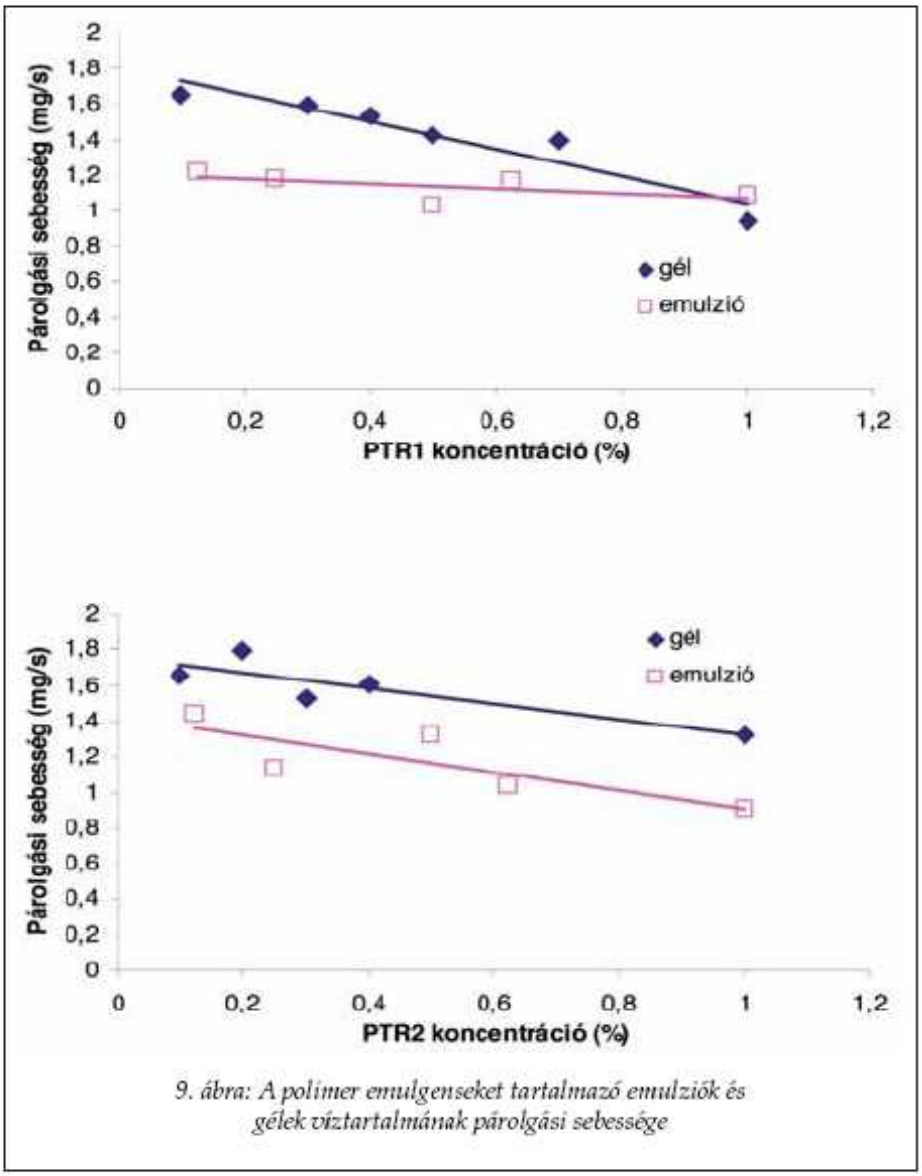
7. ábra: A polimer emulgenst tartalmazó gélek olajfelvétele



8. ábra: Az emulziók és gélek vízkötése közötti különbségek

mányban elasztikus jellegük van, tehát mind az elasztikus, mind a viszkózus jelleg megtalálható. Ezt az elasztikus jelleget kvantitatívan az oszcillációs reométerek képesek meghatározni. A készülék fejének folyamatos oszcilláló mozgásával a mintára időben szinuszosan változó nyíróáramot adunk, τ_0 amplitúdóval, és detektáljuk a rendszerben ébredő deformációs feszültséget, ennek amplitúdója γ_0. A minta által adott válasz szintén szinu-

a rendszerből és a minta tetején összefüggő olajréteget alkot, ami a későbbiek folyamán akadályozni fogja a víz párolgását. A *9. ábrán* jól látható, hogy a párolgási sebesség a polimer mennyiségével folyamatosan csökken, tehát a víz nagyobb mértékben hidrát burok formájában van jelen. Ez alól kivételnek tűnik a PTR1-et tartalmazó hidrogél-sorozat, itt a párolgási sebesség állandó, nem változik a polimer koncentráció függvényében. Ezekben a rendszerekben valószínűleg a polimer tartalmat növelve nem a víz-polimer kölcsönhatás növekszik, hanem a polimer láncok közötti kölcsönhatások lesznek intenzívebbek. Ezzel szemben, ha emulziós rendszerekben alkalmazzuk a PTR1-et, a párolgási sebesség csökkenést mutat a polimer mennyiségének növekedésével.



9. ábra: A polimer emulgenseket tartalmazó emulziók és gélek víztartalmának párolgási sebessége

Reológiai vizsgálatok

Régóta ismert, hogy a makromolekulás térhálóknak bizonyos nyírófeszültség tarto-

szos jellegű lesz, de a két hullám között általában fáziskésés (δ) lép fel. Ideálisan rugalmas testek esetében ez 0°, ideális viszkózusoknál 90°, reális rendszerek fáziskésése 0° és 90° közötti érték. A két hullám amplitúdó arányából és fáziskéséséből származtathatjuk a reológiai jellemzőket [18].

A tárolási modulusz (G') a rendszer elasztikusságát jelenti:

$$G'=\tau_0/\gamma_0 *\cos(\delta);\ [Pa] \quad (1)$$

A veszteségi modulusz (G") a rendszer viszkózus jellegét mutatja meg:

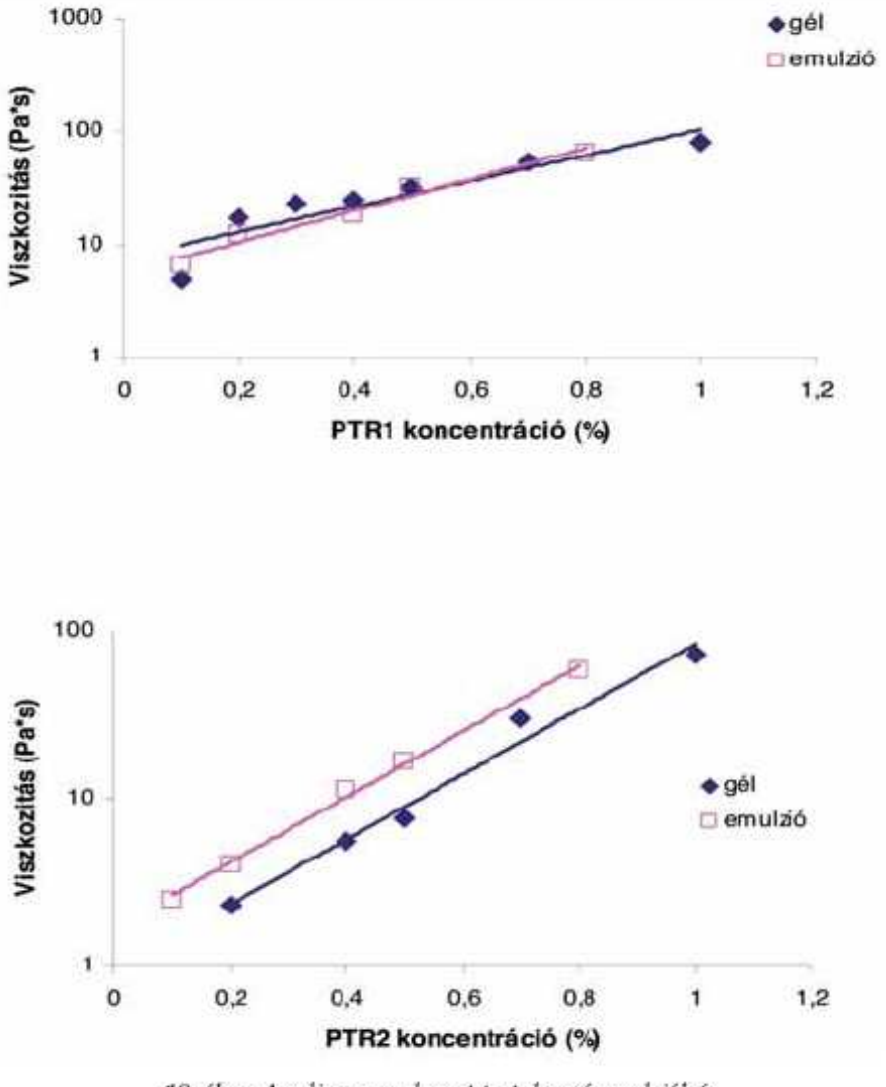
$$G''=\tau_0/\gamma_0 *\sin(\delta);\ [Pa] \quad (2)$$

A gél-emulziók és az olajat nem tartalmazó gélek reológiai tulajdonságait összevetve megállapítható, hogy az azonos polimer tartalmú emulziós és hidrogél rendszerek viszkozitás értékei között nincs jelentős különbség csak viszonylag nagy (1,00% körüli) polimer tartalomnál (*10. ábra*). A rendszerek viszkozitása és a polimer tartalom között exponenciális összefüggést tapasztalhatunk (*11. ábra*):

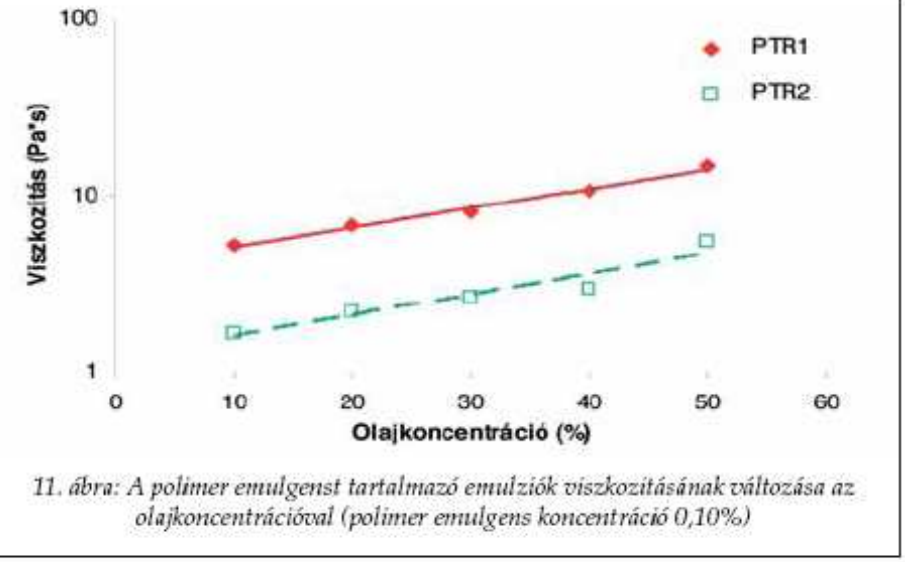
$$\eta=\eta_0 *\exp(m*c) \quad (3)$$

ahol
c = polimer koncentráció,
η_0 = 0 polimer-koncentrációra extrapolált viszkozitás
m = a függvény állandója, egységnyi koncentráció-növeléshez tartozó viszkozitás-növekedés.

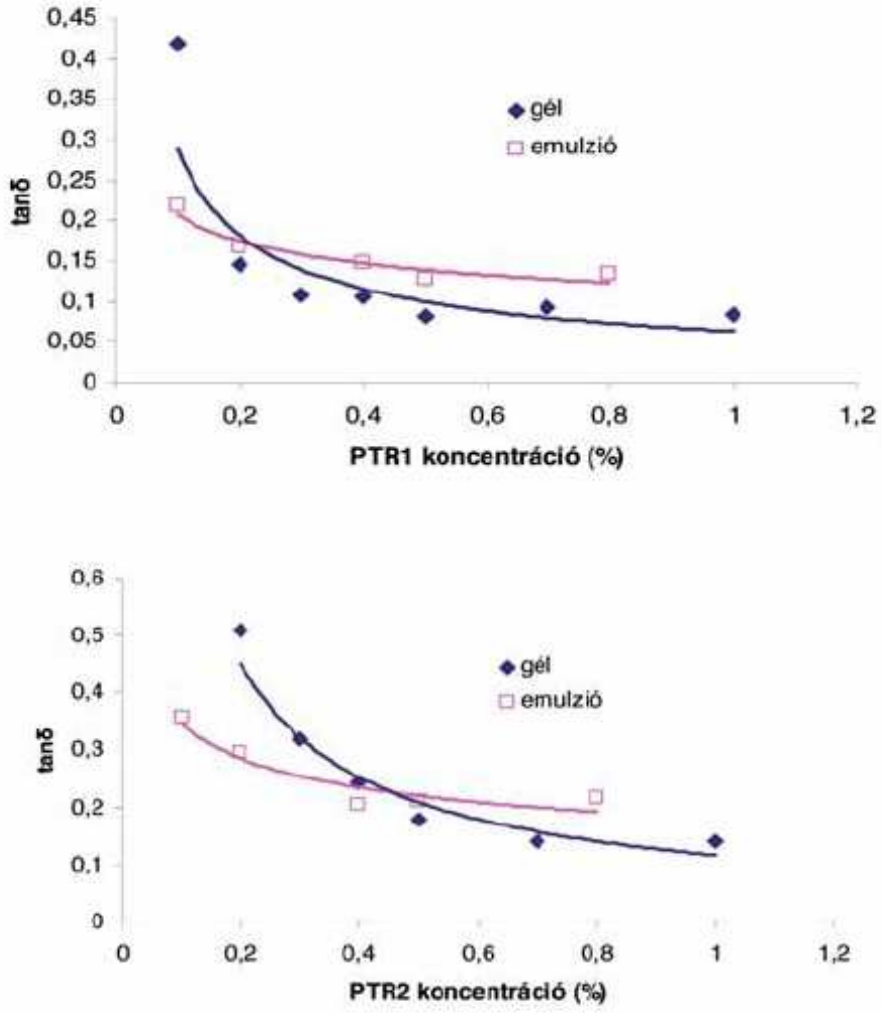
Hasonló összefüggéssel jellemezhető a viszkozitás vál-



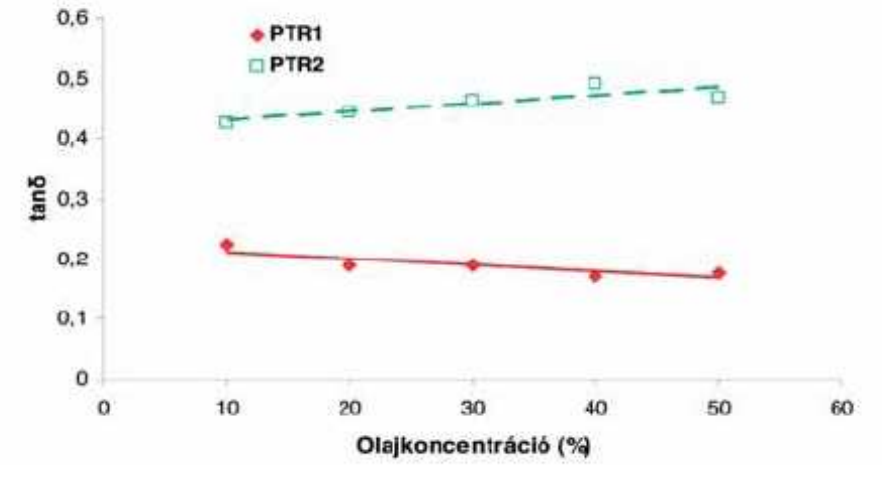
10. ábra: A polimer emulgenst tartalmazó emulziók és gélek viszkozitásának változása a polimer koncentrációjával



11. ábra: A polimer emulgenst tartalmazó emulziók viszkozitásának változása az olajkoncentrációval (polimer emulgens koncentráció 0,10%)



12. ábra: A polimer emulgenst tartalmazó emulziók és gélek veszteségi tangensének változása a polimer koncentrációjával (emulziók olajtartalma 20%)



13. ábra: A polimer emulgenst tartalmazó emulziók veszteségi tangensének változása az olajkoncentrációval (polimer tartalom 0,1%)

tozása az olajkoncentráció függvényében. Ebben az esetben η_0 a 0 olajkoncentrációra extrapolált viszkozitást jelenti.

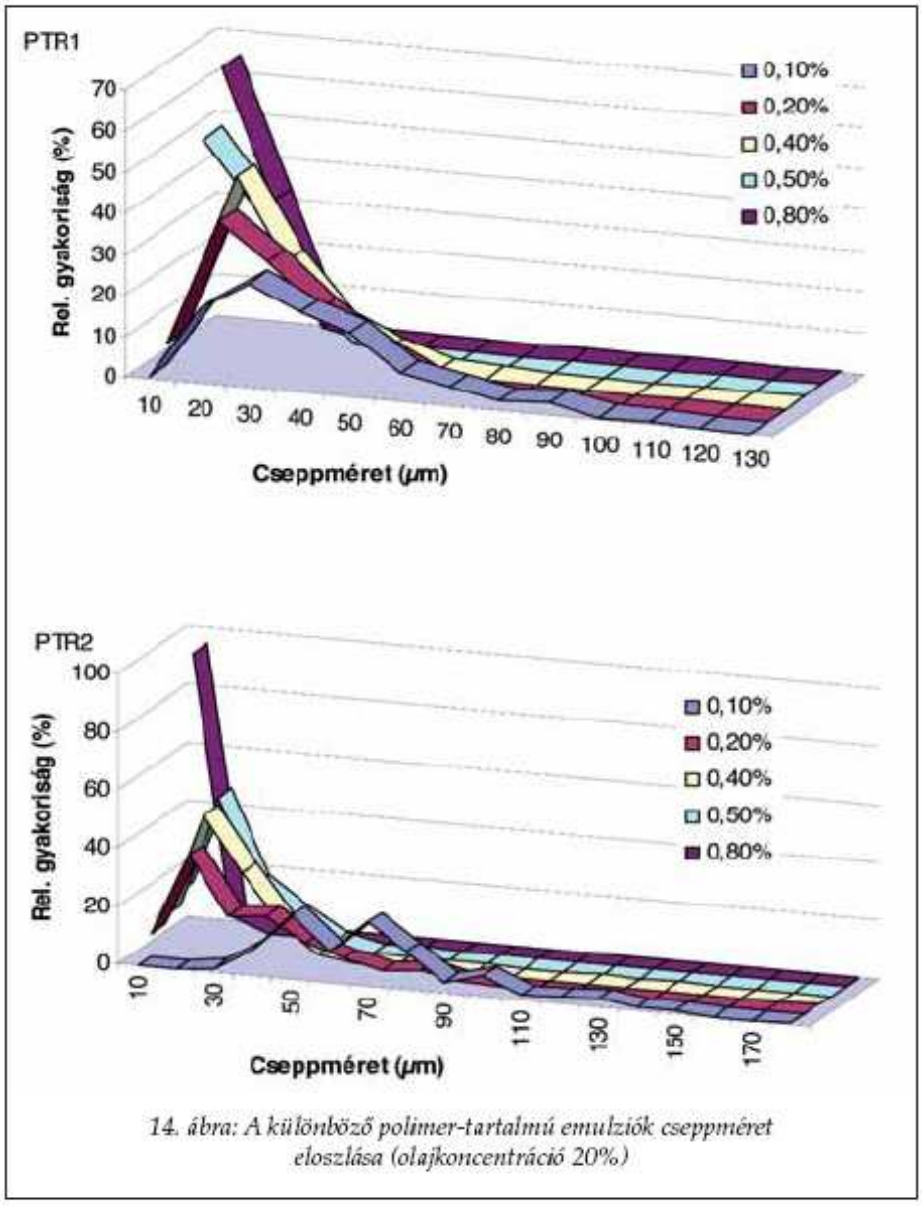
Meghatároztuk a rendszerek veszteségi tangensét (tanδ) (f=1Hz értéknél) a polimer tartalom függvényében (*12. ábra*). A veszteségi tangens az alábbi egyenlet alapján számolható ki:

$$\tan\delta = G''/G' \qquad (4)$$

ahol
G'' = veszteségi modulusz,
G' = tárolási modulusz.

Abban az esetben, ha ez az érték 1-nél kisebb, a mintában az elasztikus jelleg dominál, és minél kisebb értéket vesz fel, annál jellemzőbb az elaszticitás. Az ábrákon jól látható, hogy kis polimer tartalomnál az emulziók mutatnak erőteljesebb elasztikus jelleget, míg nagy koncentrációknál a gélek. Emulziók esetében a víz mennyisége relatíve kevesebb a diszpergált olaj miatt, ennek következtében sokkal több kölcsönhatás tud kialakulni a polimer láncok között, mint amennyit a polimer létre tud hozni relatíve nagyobb mennyiségű vizes fázisban. Nagyobb polimer tartalomnál viszont a nagyobb térfogatú vízfázis már nem okoz ilyen jellegű különbséget, viszont a diszperz fázis jelenléte miatt az emulziós rendszerek elaszticitása kisebb lesz, mint az ugyanolyan polimer-koncentrációjú gélek elaszticitása. Az a polimer-koncentráció, amely felett a gélek már nagyobb elaszticitással rendelkeznek

mint az emulziók, a PTR1 esetében kisebb polimerkoncentrációnak felel meg, mint a PTR2 esetében. Ennek oka, hogy a PTR1 nagyobb polimerizációs fokú polimer, így a polimer láncok között már kisebb koncentrációban is jelentős mennyiségű kötés tud kialakulni, szemben a kisebb polimerizációs fokú emulgenssel. Növelve az emulziók olajkoncentrációját, PTR1 esetében a veszteségi tangens csekély csökkenést, míg a PTR2-t tartalmazó mintáknál kismértékű növekedést mutat, tehát a PTR1 tartalmú minták elaszticitása fokozódik az olajkoncentrációval, míg a PTR2-t tartalmazóké csökken. (*13. ábra*)



14. ábra: A különböző polimer-tartalmú emulziók cseppméret eloszlása (olajkoncentráció 20%)

Cseppméret-analízis

Az emulziók cseppméret-eloszlása jelentősen függ a polimer emulgens koncentrációjától és az emulgeált olaj mennyiségétől. Növelve a polimer-koncentrációt, az eloszlási görbe a kisebb cseppek irányába tolódik, és egyre inkább monodiszperz jelleget mutat (*14. ábra*). Növelve az olajkoncentrációt az eloszlási görbe egyre szélesebb lesz, egyre inkább heterodiszperz eloszlású rendszereket kapunk, majd 30% feletti koncentrációnál a görbe ismét éles csúcsot ad, tehát egyre inkább homogén eloszlásúak lesznek az emulziók (*15. ábra*). Az átlagos cseppméret, mint az eloszlási görbék alapján várható volt, exponenciálisan csökken a polimer emulgens növekedésével, míg növekvő olajkoncentráció mellett a cseppméret kezdetben nő, maximum értéket vesz fel 30%-nál majd ismételten csökken (*16-17. ábra*).

Megbeszélés

Az elvégzett preformulálási vizsgálatok alapján megállapítottuk a következőket:

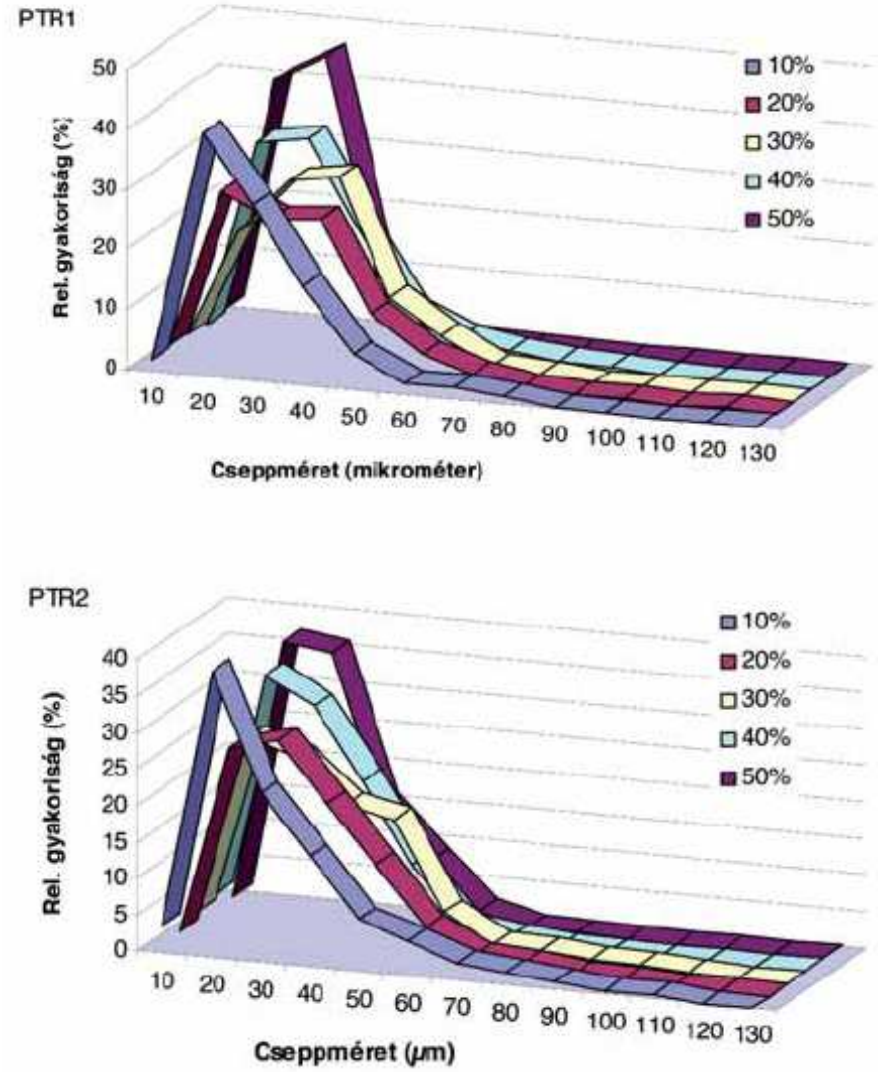
1. A Pemulen TR1 és TR2 polimer emulgensek stabilizáló hatásukat sztérikus gátlással fejtik ki, azaz az olaj fázist mechanikailag tartják zárva, felületi feszültség csökkentő hatásuk nem jelentős.
2. A polimerek vízzel rosszul nedvesednek.
3. Desztillált víz hatására mindkét polimer igen lassan duzzadt, és a megkötött víz mennyisége jellemzően kevesebb volt, mint a hidroxi-etil cellulózé.
4. A Pemulen TR1 polimer képes volt 50%-nyi olaj stabilizálására is, a Pemulen TR2 emulgens pedig alkalmasnak bizonyult 70%-os olajmennyiség emulgeálására.

A termoanalitikai vizsgálatok alapján elmondhatjuk, hogy a gél-emulziók a hagyományos gél-

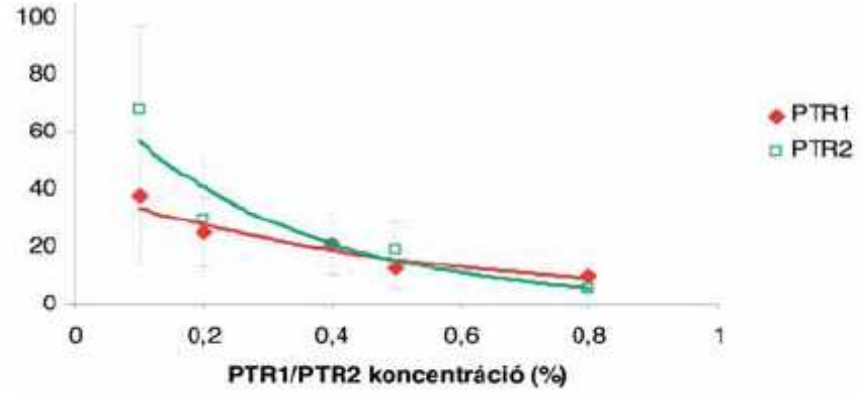
szerkezettől jelentősen eltérnek, kimutattunk mikrogélhez kötött vizet és szabad vizet. Megállapítottuk azt is, hogy a nagyobb polimer tartalom növelte a víz kötöttségét, ami a kisebb párolgási sebességben nyilvánult meg.

A reológiai mérések alapján tisztáztuk, hogy a rendszerek viszkozitása és a polimer- valamint olajtartalom között exponenciális összefüggés áll fenn. Az oszcillációs mérésekből kiderült, hogy kis polimer tartalomnál az ugyanolyan polimer tartalmú gél-emulzióknak erőteljesebb elasztikus jellegük van mint a hidrogéleknek, nagyobb koncentrációk esetén ez a különbség nem áll fenn, a hidrogélek elasztikus jellege válik nagyobbá.

A gél-emulziók cseppmérete exponenciálisan csökkent a polimer emulgens mennyiségével, az eloszlási függvény pedig egyre inkább balra, a kis cseppek tartománya felé tolódott. Az olajtartalmat változtatva, maximális volt az átlagos cseppméret 30%-os olajtartalomnál, ugyanakkor az eloszlási függvény



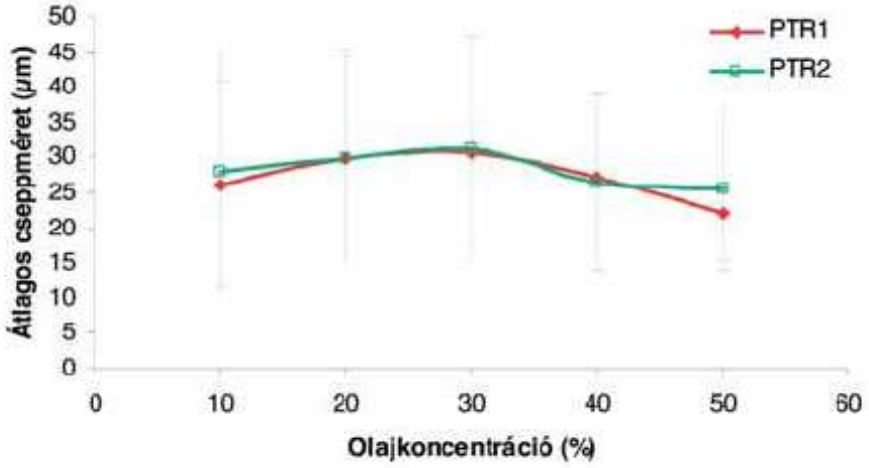
15. ábra: A különböző olajtartalmú emulziók cseppméret eloszlása (polimer emulgens koncentrációja 0,10%)



16. ábra: Az átlagos cseppméret változása a polimer emulgens koncentrációjával (olajkoncentráció 20%)

is itt mutatott leginkább polidiszperz jelleget.

A komplex fizikai kémiai vizsgálatok alapján megállapíthatjuk, hogy a polimer emulgenst tartalmazó rendszerek szerkezete összetett. Az emulgens mennyiségének változtatása jelentős mértékben kihat a rendszerek reológiai tulajdonságaira, mivel nemcsak a cseppméret és a határfelület változására kell számítanunk, hanem polimer hatására a külső fázis



17. ábra: Az átlagos cseppméret változása az olaj koncentrációjával (polimer emulgens koncentrációja 0,10%)

szerkezete is módosul: differenciálódik a gélszerkezet és a víz kötődése is változik. Mindezek ismeretében az ilyen típusú gél-emulziók szerkezetének vizsgálatakor érdemes és fontos egymás mellett vizsgálni mind az emulziók makroszerkezetét (cseppméretét, cseppméret-eloszlását), mind a mikroszerkezetét (vízkötési mechanizmusok, gélszerkezet).

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[Érkezett: 2008. 02. 22.]

II.

Acta Pharmaceutica Hungarica 78. 23-30. 2008.

Gél-emulziós rendszerek II. rész Stabilitás

BUDAI SZABOLCS, SZŰCS MÁRIA, ERŐS ISTVÁN

Szegedi Tudományegyetem Gyógyszertechnológiai Intézet, Szeged, Eötvös u. 6. – 6720

Summary

*Budai, Sz., Szűcs, M., Erős, I.: **Gel-emulsion systems. II. Stability***

Viscosity, elastic character of gel emulsions containing polymeric emulsifiers and change of droplet size distribution under storage were studied. The quantitative change of free (non-bound) water and immobilized one in microgel form, and that of the evaporation rate under storage were examined. The phenomena were divided into two groups: change of i) macrostructure, and ii) microstructure. It was determined that macrostructure (e.g. average droplet size) was stable, it did not change during the 3 month storage period. On the other hand, the microstructure (e.g. viscosity, elastic character, solvatation of polymer chains, immobilized water in microgel, rate of water eveporation) were characteristically changed during storage. These processes could be related to the sustained hydration of polymer chains.

Összefoglalás

A szerzők polimer emulgenseket tartalmazó gél emulziók viszkozitásának, elasztikus jellegének változásait, valamint a cseppméret eloszlásban bekövetkező változásokat vizsgálták az eltartás függvényében. Meghatározták a szabad és a mikrogélben kötött víz mennyiségi változását és a párolgási sebesség változást a tárolás során. A jelenségeket két csoportra osztották: a makroszerkezet és a mikroszerkezet változásaira. Megállapították, hogy a gél emulziók makroszerkezete (pl. az átlagos cseppméret) stabil, nem változik 3 hónapos tárolás alatt. Ezzel szemben a mikroszerkezet (viszkozitás, elasztikus jelleg, a polimer szolvatációja, a mikrogélben kötött víz mennyisége és a víz párolgási sebessége jellemzően változik az eltartás folyamán. E változásokat a polimer elhúzódó hidratációjával hozták összefüggésbe.

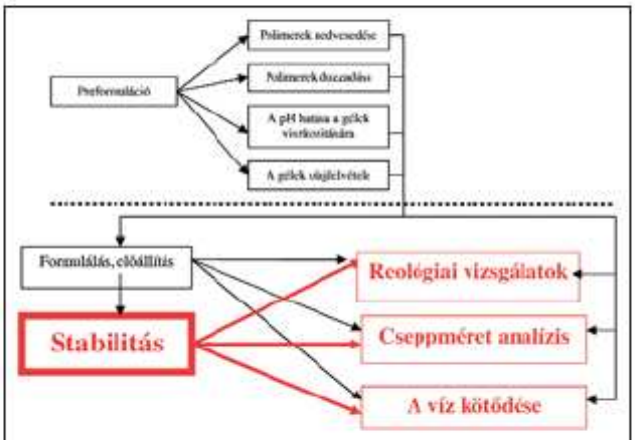
Bevezetés

A gyógyszerészetben használatos emulziók kutatásában különös figyelmet kell fordítani a rendszerek stabilitására és stabilizálására [1]. A stabilitás jelenségének értelmezésekor a képződés feltételeiből kell kiindulnunk. Hosszabb-rövidebb ideig stabilis rendszer képződésének 3 előfeltétele van [2]:

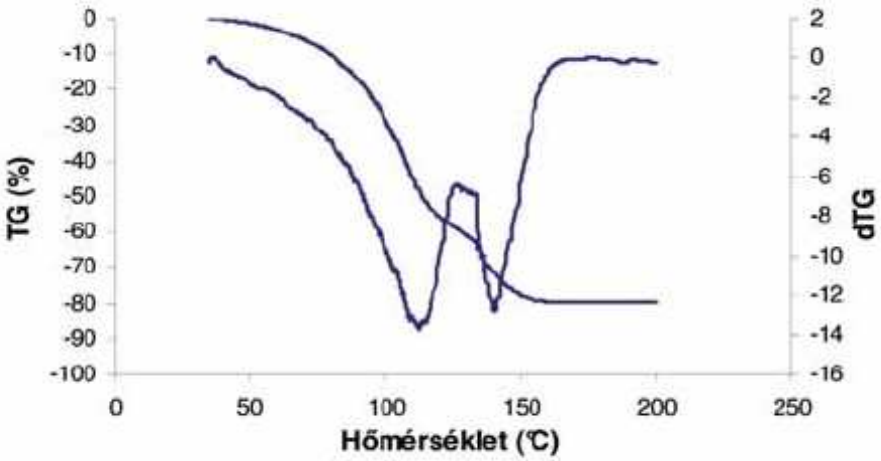
1. A két fázis nem elegyedhet egymással;
2. Elegendő nagyságú legyen az emulgeálási munka, illetve
3. Emulgensből vagy emulgens keverékből megfelelő határfelületi réteg alakuljon ki.

Az emulziók „metastabil" rendszereknek tekinthetőek, így kezdeti szerkezetük változik tárolás vagy szállítás folyamán, hőhatás, mechanikai terhelés, fizikai behatás vagy biológiai hatás következtében [3]. Számos olyan folyamat ismeretes, amely az emulziós szerkezet megtöréséhez vezet. Megkülönböztetünk reverzibilis és irreverzibilis mechanizmusokat. A reverzibilis változások közé tartozik a *fölöződés, ülepedés, flokkuláció*, az irreverzibilis jelenségek közé sorolható a *koaguláció* és az úgynevezett *„Ostwald ripening"*.

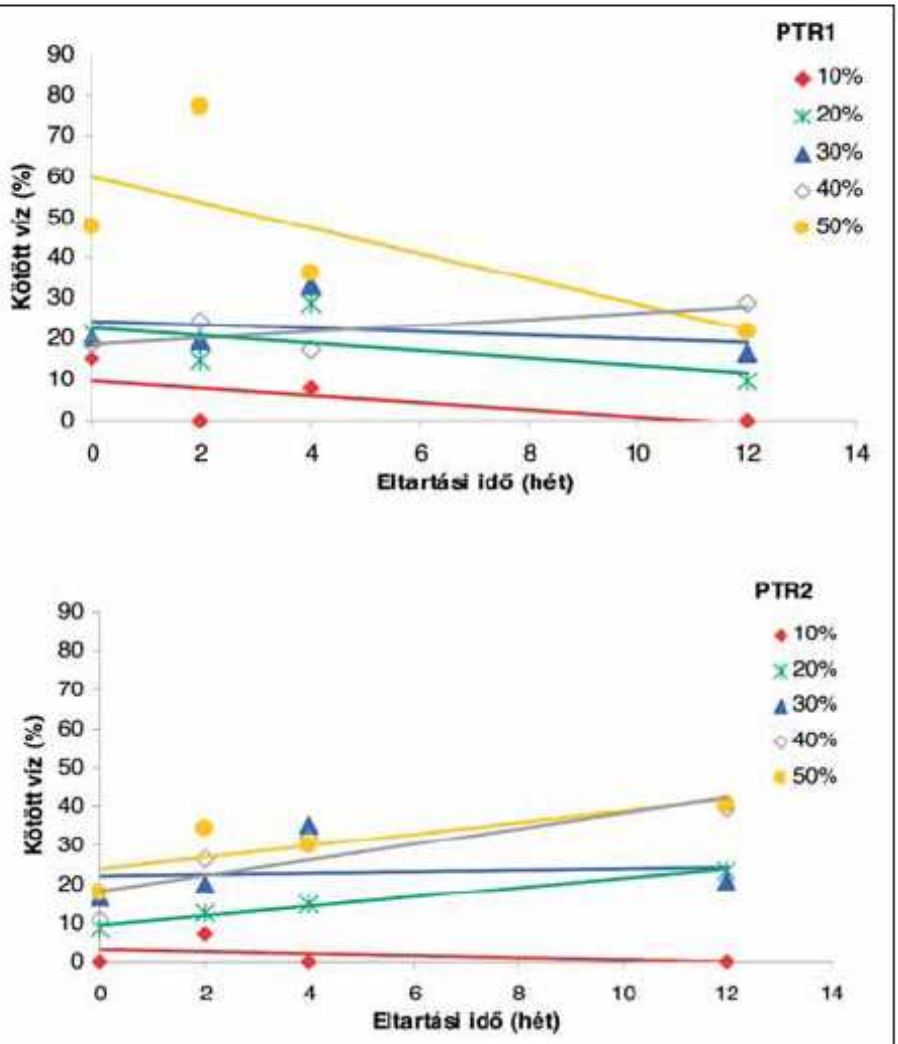
Fölöződés és *ülepedés* folyamán a gravitáció hatására a diszpergált cseppek felfelé vagy lefelé mozognak. Főként híg, kis diszperz fázist tartalmazó rendszerekben figyelhető meg a *Stokes* törvénynek megfelelően, ha jelentős sűrűségkülönbség van a két fázis között. *Flokkulációról* akkor beszélünk, ha a vonzó erők nagyobbak a cseppek közötti taszító erőknél, így a cseppek összetapadnak a gyenge van der Waals kölcsönhatások következtében. A cseppek *koagulációja* folyamán az emulgeált cseppek összefolynak a köztük lévő filmréteg elvékonyodása és megszűnése következtében. Így



1. ábra: A polimer emulgensek vizsgálatának rendje



2. ábra: A gél-emulziók TG és dTG görbéje



3. ábra: A gél-emulziók kötött vizének változása az eltartási idővel különböző olajkoncentrációk esetén

végeredményként két vagy több kisebb cseppből egy nagyobb csepp keletkezik. Az úgynevezett *„Ostwald ripening"* polidiszperz emulziókra jellemző, amikor is a nagyobb cseppek fokozatosan növekednek, a kisebb cseppek pedig egyre kisebbek lesznek. A jelenség a különböző méretű cseppek oldékonyság különbségéből adódik [1].

Két fő módszer ismeretes az emulziók stabilizálására:

1. az elektrosztatikus stabilizálás, főként kismolekulájú felületaktív anyagokkal,
2. a sztérikus stabilizálás.

Ez utóbbi esetében felületaktív polimereket alkalmaznak, melyek kötődése a határfelülethez gyengébb, mint egy kismolekulájú felületaktív anyagé, de hatásosnak bizonyulnak elektrolit tartalmú közegben, illetve magasabb hőmérsékleten is [4]. A kozmetikai és gyógyszeriparban is az utóbbi években a hagyományos kismolekulájú felületaktív anyagokkal szemben egyre inkább előnyben részesítik a polimer emulgenseket, illetve a polimerek felületaktív anyagként való alkalmazásáról is egyre több szerző tesz említést [5-7].

A polimer emulgensek két újabb képviselőjét már előző közleményünkben [8] bemutattuk. Jelen munkánkban a polimer emulgenst tartalmazó rendszerek stabilitási vizsgálatával kapott eredményeinkről számolunk be (*1. ábra*).

Az emulziók stabilitási vizsgálatára az egyik legtöbb

információt adó módszercsoport a *reológiai mérések.* Ezek a vizsgálatok érzékenyen jelzik az emulziók szerkezetében történő változásokat, így például a koagulációt, a flokkulációt, vagy akár a fölöződést, szedimentációt, mivel ezek a folyamatok mindig jellemző viszkozitás-változással járnak [9-11]. *Termoanalitikai vizsgálatok*kal lehet tanulmányozni a víz kötődésének változását, illetve a gélszerkezet változását [12], valamint *optikai módszerek* segítségével detektáltuk a cseppméret, cseppméret-eloszlás alakulását az eltartás során.

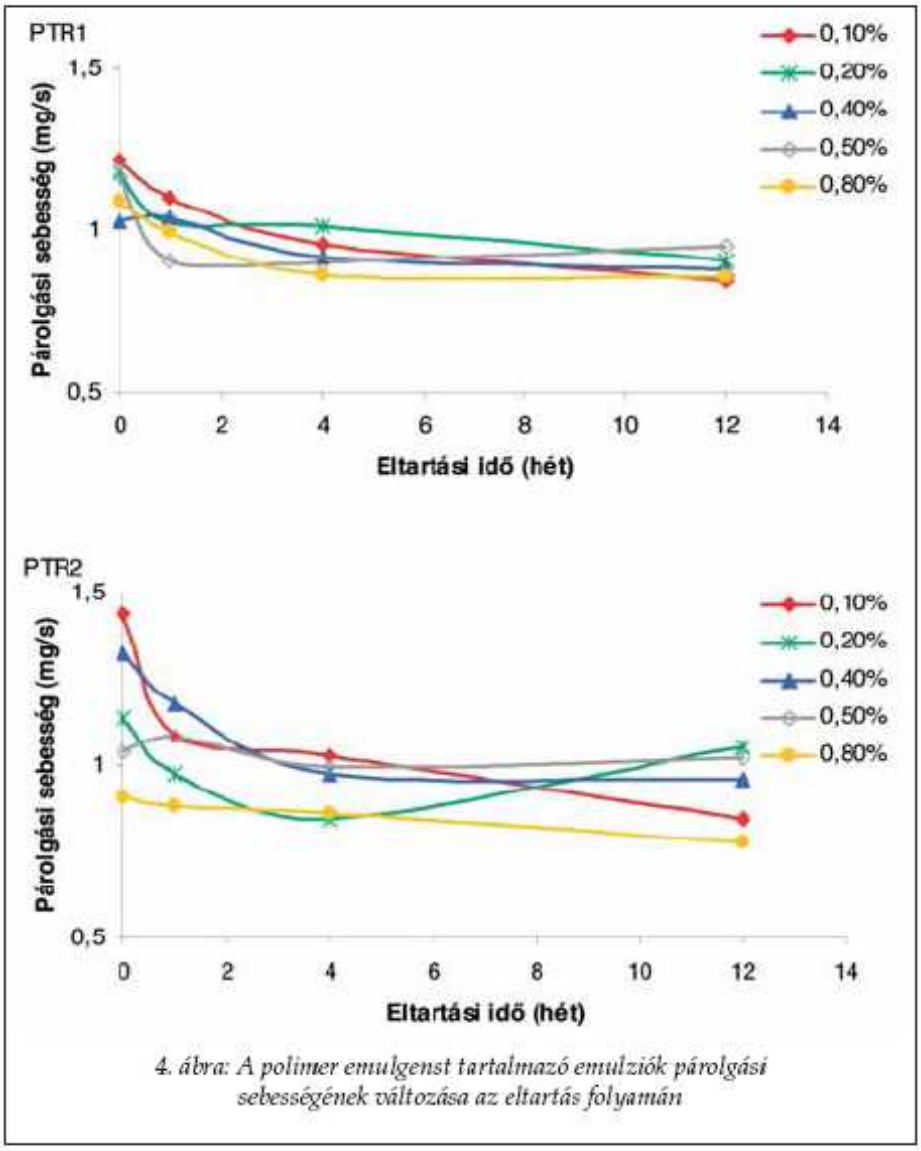
Anyagok és módszerek

Anyagok

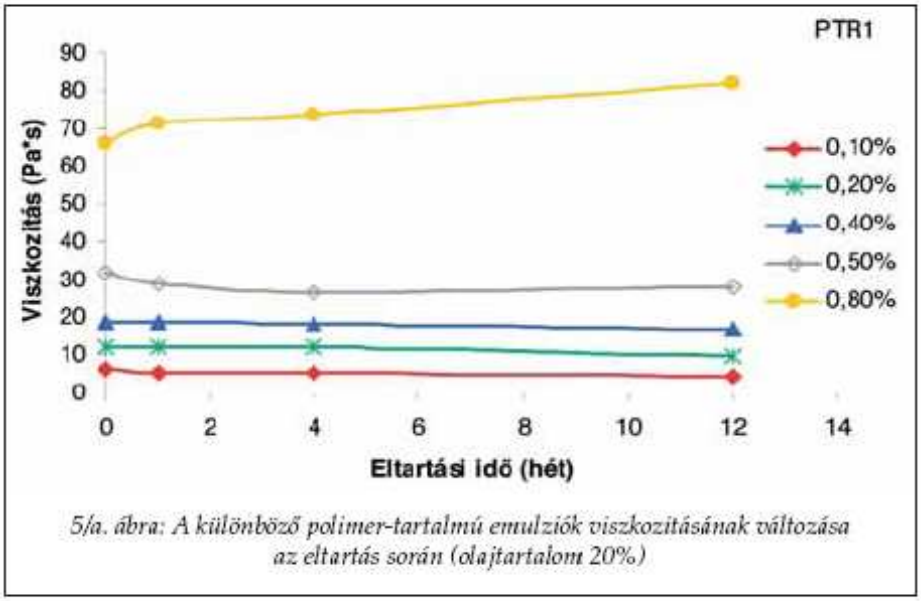
Polimer emulgensként a Pemulen TR1 és TR2-t (gyártó: Noveon AG) alkalmaztuk. A gél emulziók vízfázisa a gyógyszerkönyvi tisztított víz (Aqua purificata, Ph. Hg. VIII.), olajfázisa a Miglyol 812 (gyártó: Sasol GmbH, Németország) volt. Semlegesítő komponensként gyógyszerkönyvi trolamint (Ph. Hg. VIII.) használtunk.

Reológiai vizsgálatok

A reológiai méréseket HA AKE RheoStress 1 (HAAKE GmbH, Németország) kúplap geometriájú készülékkel végeztük szobahőmérsékleten. Szabályozott nyírási sebesség-gradiens mellett felvettük a minták folyás- és viszkozitás-görbéit (a felszálló ágat 0,1 s^{-1}-től 100 s^{-1}-ig, a leszálló ágat 100 s^{-1}-től 0,1 s^{-1}-ig).



4. ábra: A polimer emulgenst tartalmazó emulziók párolgási sebességének változása az eltartás folyamán



5/a. ábra: A különböző polimer-tartalmú emulziók viszkozitásának változása az eltartás során (olajtartalom 20%)

Meghatároztuk az emulziók és gélek tárolási és veszteségi moduluszát a frekvencia függvényében (0,1 és 100 Hz között).

Termoanalitikai mérések

A termoanalitikai méréseket Derivatograph C (MOM, Magyarország) készülékkel végeztük. A mintákat 25 °C-tól 200 °C-ig fűtöttük, a víz kötődési mechanizmusának meghatározására 10 °C/ perc fűtési sebességet használtunk. A párolgási sebesség meghatározásához 5 °C/perc sebességet alkalmaztunk. Felvettük az emulziók TG (tömegcsökkenés az idő függvényében) és dTG (TG időszerinti deriváltja) görbéit. A TG görbék egyenes szakaszára regressziós egyenest illesztettünk, melynek meredekségéből következtettünk a minták párolgási sebességére.

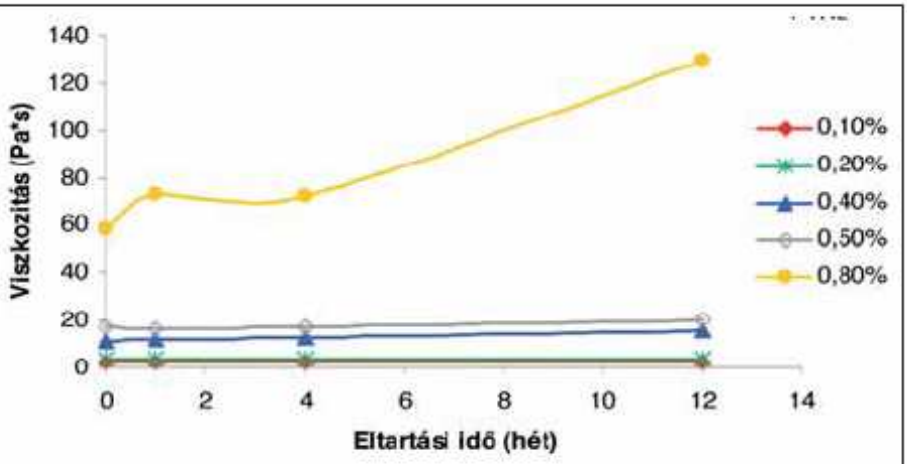
Cseppméret-analízis

A cseppméret-analízist Leica képanalizátorral végeztük. 500 csepp átmérőjét mértük meg és elemeztük Leica Q500MC Qwin V01.02 szoftver segítségével.

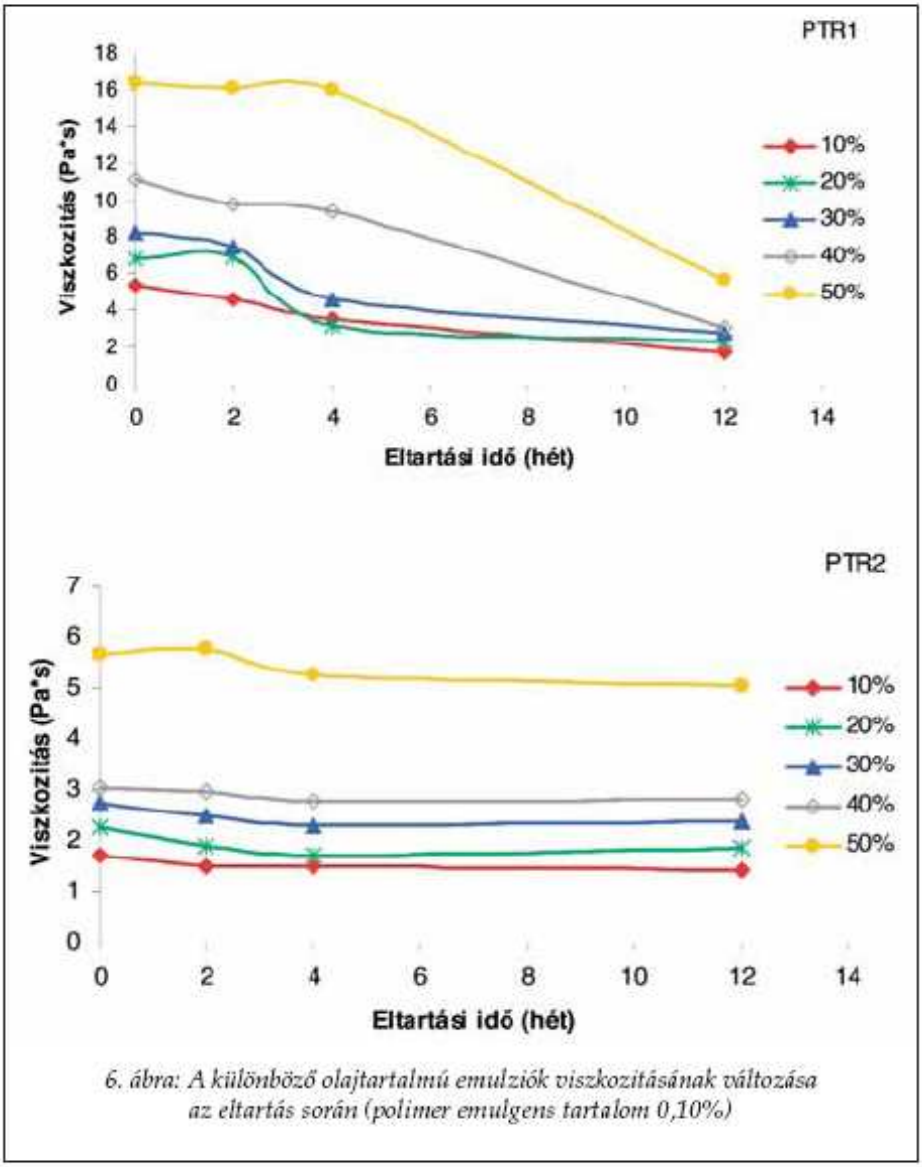
Eredmények és értékelés

Termoanalitikai vizsgálatok

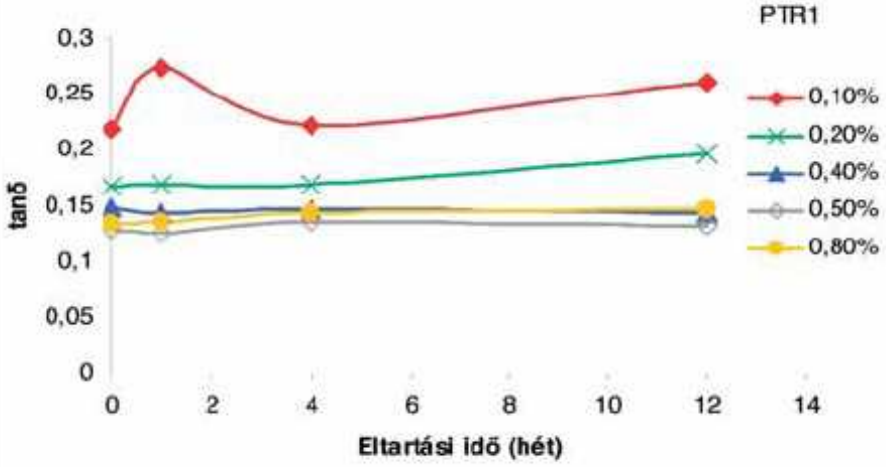
Az előző közleményünkben [8] bemutattuk az emulziók speciális szerkezetében hőközlés hatására lejátszódó változásokat (*2. ábra*). E változás lényege a különböző módon kötött víz eltávozása volt. Meghatároztunk szabad

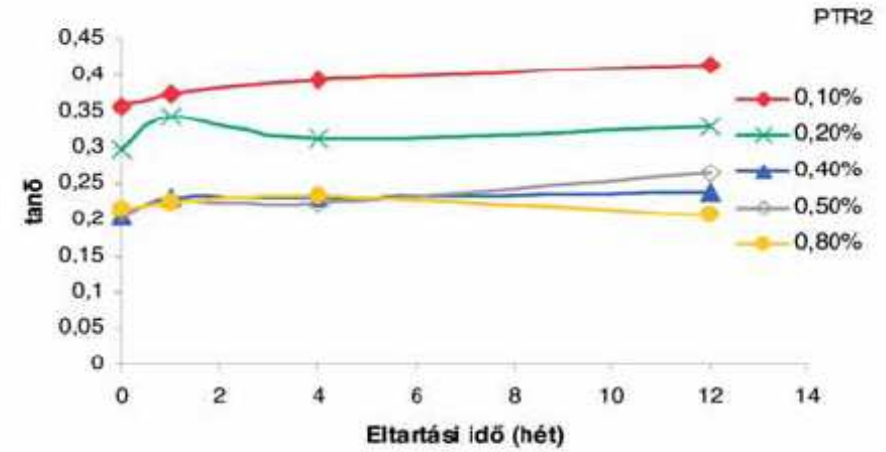


5/b. ábra: A különböző polimer-tartalmú emulziók viszkozitásának változása az eltartás során (olajtartalom 20%)

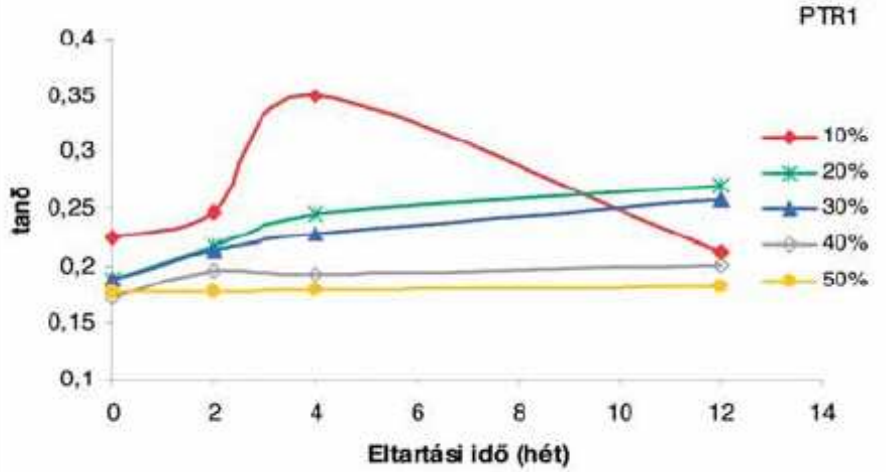


6. ábra: A különböző olajtartalmú emulziók viszkozitásának változása az eltartás során (polimer emulgens tartalom 0,10%)





7. ábra: A különböző polimer-tartalmú emulziók veszteségi tangensének változása az eltartás során (olajtartalom 20%)



8/a. ábra: A különböző olajtartalmú emulziók veszteségi tangensének változása az eltartás során (polimer emulgens tartalom 0,10%)

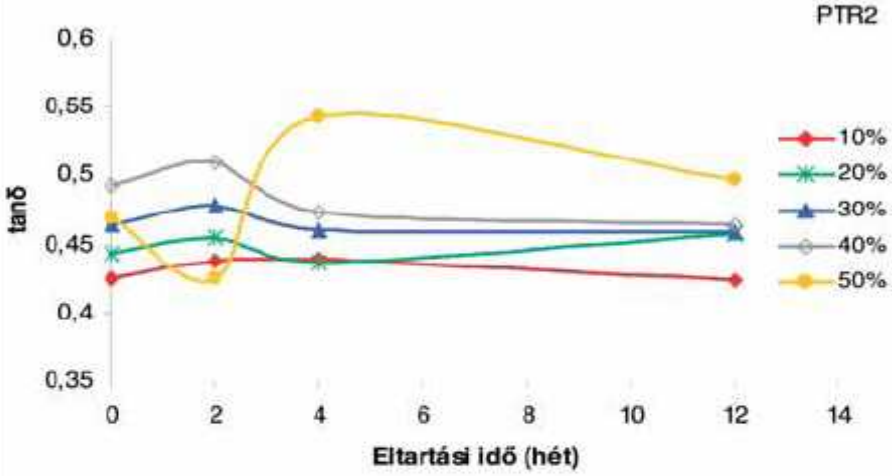
vizet (első csúcs a dTG görbén) és mikrogélhez kötött vizet (második csúcs a dTG görbén). Meghatároztuk második csúcsokhoz tartozó víz mennyiségét, azaz a kötött víz mennyiségét, és a teljes vízmennyiség százalékában kifejezve ábrázoltuk az eltartási idő függvényében (*3. ábra*). A kötött víz mennyisége csökkenést mutatott a PTR1-et tartalmazó minták esetében, míg növekedést tapasztaltunk a PTR2-t tartalmazóknál. Tehát a PTR1 emulziók mikrogél rendszere zsugorodott az eltartás során, ellentétben a PTR2-t tartalmazókéval, ahol a mikrogél duzzadása volt megfigyelhető.

Az eltartási idő alatt a polimerek hidratációja növekedett, ennek következtében a párolgási sebesség csökkent. Minél jobban hidratált egy makromolekula, annál kisebb a vízvesztés sebessége. Az adatok szerint a párolgás sebessége fokozatosan csökkent az eltartás alatt (*4. ábra*). E jelenségnek az a magyarázata, hogy a polimer fokozatosan duzzadt a vízzel való érintkezéskor és a hidrát burok nem pillanatszerűen, hanem az idő függvényében fokozatosan alakult ki. Így a hidratáció növekedésével egyre csökkent a vízvesztés sebessége. A sebességi állandókat az idő függvényében ábrázolva hatványfüggvénnyel leírható összefüggést kaptunk.

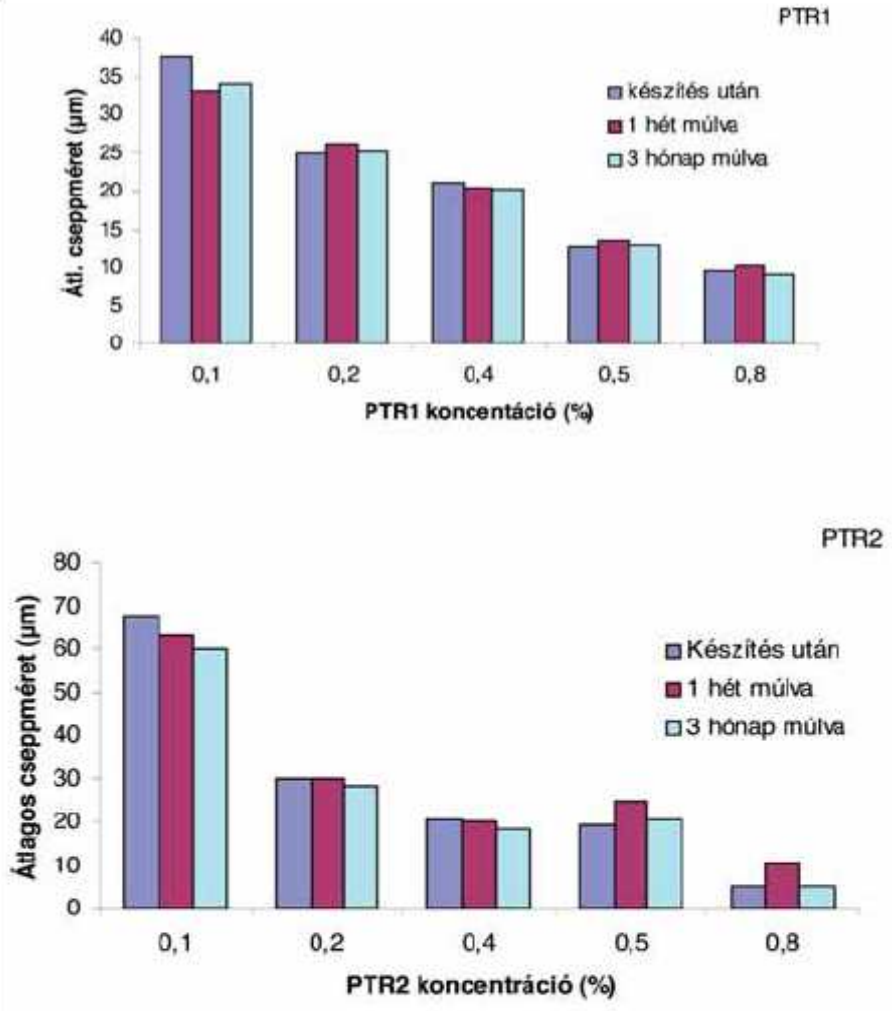
$$\Delta m/t = A_o t^k \qquad (1)$$

ahol

$\Delta m/t$ = párolgási sebesség,



8/b. ábra: A különböző olajtartalmú emulziók veszteségi tangensének változása az eltartás során (polimer emulgens tartalom 0,10%)



9. ábra: A különböző polimer-tartalmú emulziók átlagos cseppmérete az eltartás folyamán (olajkoncentráció 20%)

A_0 = kezdeti értékhez tartozó sebesség,
t = az idő,
k = sebességi állandó.

Reológiai vizsgálatok

A gél-emulziók viszkozitásának jellemzésére a 0 nyírási sebesség-gradiensre extrapolált viszkozitást alkalmaztuk és ennek változását vizsgáltuk az eltartás során. A minták elasztikusságát a veszteségi tangens (tanδ) segítségével elemeztük.

$$\tan\delta = G''/G' \qquad (2)$$

ahol
G'' = veszteségi modulusz,
G' = tárolási modulusz.

Minél kisebb értéket vesz fel tanδ, annál jellemzőbb és kifejezettebb az emulziók elaszticitása.

Azokban a mintákban, ahol kis olajkoncentráció (20%) mellett változtattuk a polimer koncentrációját, kismértékű viszkozitás növekedés volt tapasztalható főként a nagy polimer tartalmú mintáknál (*5. ábra*). Ez a jelenség jól magyarázható ugyanezen minták hidratációjának fokozódásával, amit a *4. ábra* szemléltet. Azokban az összetételekben viszont, ahol kis polimer-tartalom (0,10%) mellett növeltük az olaj koncentrációját (*6. ábra*), a PTR1-et tartalmazó minták jelentős viszkozitás-csökkenést mutattak.

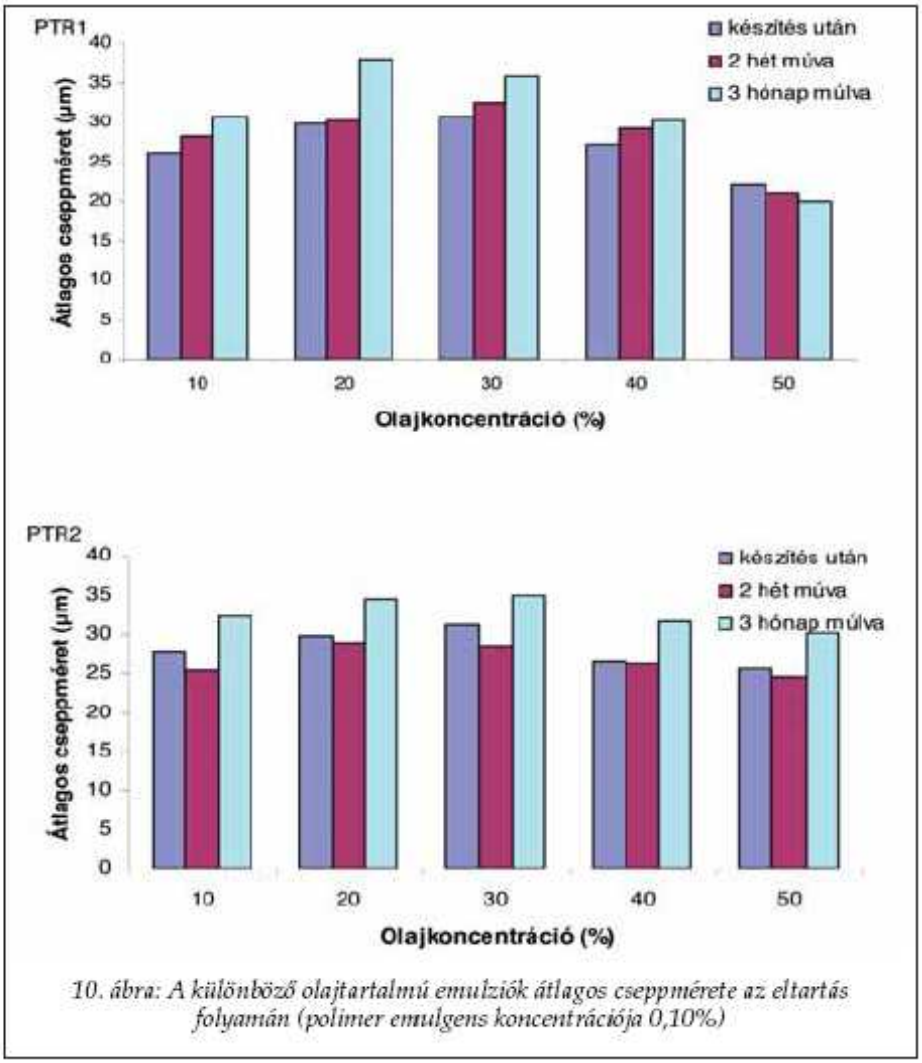
Ezzel párhuzamosan a minták veszteségi tangense folyamatosan nőtt az eltartás folyamán, ami arra utal, hogy az elasztikus jellegük csökkent. Ez utóbbi megállapítás

is főként a PTR1-et tartalmazó mintákra igaz (*7. és 8. ábra*). Ugyanezen összetételekben a mikrogél szerkezet zsugorodását állapítottuk meg a gélszerkezet vizsgálatakor (*3. ábra*).

Cseppméret-analízis

A mikroszkópos vizsgálatokkal a koaguláció és az „Ostwald ripenning" jelensége követhető nyomon, mindkettő esetén cseppméret növekedést tapasztalhatunk. Azokban a mintákban, ahol állandó olajtartalom mellett változtattuk a polimer emulgens mennyiségét, cseppméret változás nem következett be, illetve nem volt megfigyelhető.

Változás a kis polimer tartalmú minták esetében volt látható és csak a 3. hónap elteltével. Ezt főként a PTR1-et és 20-30% olajat tartalmazó rendszereknél figyeltük meg, valamint a kisebb viszkozitást mutató PTR2-t tartalmazó rendszerekben tapasztaltuk. Ez a növekedés viszont egyik esetben sem volt jelentős (9. és 10. ábra).



10. ábra: A különböző olajtartalmú emulziók átlagos cseppmérete az eltartás folyamán (polimer emulgens koncentrációja 0,10%)

Megbeszélés

A stabilitási vizsgálatok során megállapítást nyert, hogy a polimer emulgens tartalmú emulziók esetében két, egymás mellett jelenlevő szerkezeti formáról beszélhetünk. A *makroszerkezet* jelenti az emulgeált olajcseppek számát, méretét és méret szerinti eloszlását. Ez nem változott jelentősen az eltartás során. A *mikroszerkezet* jelenti a polimer láncok szolvatációját. (Pontosabb és szabatosabb lenne heterogén szerkezetről és kolloid szerkezetről beszélni, mivel az emulgeált cseppek a heterogén diszkontinuitás tartományába, a polimer láncok szolvát burka és a mikrogél pedig kolloid dimenzióba tartoznak. Mivel a heterogén fogalmat főként a diszperz rendszerek eloszlásával kapcsolatosan használják, ezért a terminológiai zavart megelőzendő, megtartjuk a makro- és mikrostruktúra fogalmát.)

A hidratáció megismeréséhez nyújtottak adatokat a TG-görbék. A TG-görbék meredeksége hatványfüggvény szerint csökkent az eltartás során, ez a hidratáció növekedésével, a polimer és a víz közötti fizikai kémiai kölcsönhatás fokozódásával magyarázható. Mindezek mellett az emulziók mikrogél szerkezete is változást szenvedett. Ezzel párhuzamosan a reológiai vizsgálatok is mikroszerkezetbeni változásokat jeleznek az eltartás folyamán. Főként viszkozitás- és elaszticitás-csökkenésről beszélhetünk, ami azért is figyelemre méltó, mivel főként azokban az összetételekben jelentkezett ez a csökkenés, ahol az átlagos cseppméret (mint egyik legfőbb befolyásolója ezen faktoroknak) egyáltalán nem változott, valamint a hidratáció fokozódásával éppen az elaszticitás és a viszkozitás növekedése lenne várható. Ezt az ellentmondást a hidratáció

makro- és mikroszerkezetben betöltött ellentétes szerepével lehet értelmezni. A hidratáció növeli a folyási egységek térfogatát, ami viszkozitás növekedésében jut kifejezésre. Viszont a hidratáció során a polimer láncokban eltávolodnak egymástól azok a funkciós csoportok, amelyek között szekunder kötőerők jönnek létre, tehát a mikroszerkezetet meghatározó kölcsönhatások spektruma ezáltal csökken. Valószínű, hogy ez a csökkenés nagyobb mértékű, mint a duzzadás által okozott viszkozitás növekedés [13].

Az eredmények alapján elmondható, hogy polimer emulgensek segítségével makroszerkezet szempontjából stabil emulziós rendszerek állíthatóak elő. A mikroszerkezetet tekintve viszont az ilyen típusú emulziók gélszerkezete nem állandó, az emulgeálást követően folyamatosan változik.

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Mucoadhesive behaviour of emulsions containing polymeric emulsifier

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ABSTRACT

Over the last two decades the attention has been focused on mucoadhesive dosage forms as a possibility to improve the residence time on a specified region of the body. In addition to bioadhesivity, controlled drug release from the dosage form is also desirable. Pemulen TR1 and Pemulen TR2 are cross-linked block copolymers of poly(acrylic acid) and hydrophobic long-chain methacrylates. They are able to stabilize o/w emulsions because their short lipophilic part integrates into the oil droplets whilst their long hydrophilic part forms a micro-gel around the droplet. In this study, correlations between the microstructure of these emulsions and the bioadhesive behaviour were found. Rheological and thermogravimetric methods were used to examine the microstructure of the emulsions. The mucoadhesive measurements were performed by tensile test and the bioadhesive bond between the polymer emulsifier and mucin was visualized by confocal laser scanning microscopy. It was established that (i) these emulsion form a special structure, which depends on the components, (ii) there were no remarkable changes in bioadhesive force and work when the oil content was increased in the emulsions, and (iii) the emulsions in which the polymeric emulsifier formed a special structure showed stronger adhesivity than the ones with simple polymer network.

1. Introduction

Bioadhesion has been defined as the attachment of synthetic or biological macromolecules to a biological tissue (Peppas and Buri, 1985). A special case of bioadhesion when the biological tissue is a mucosal epithelium is mucoadhesion (Junginger, 1991).

Over the last two decades the attention has been focused on mucoadhesive dosage forms as a possibility to improve the residence time on a specified region of the body. In addition to bioadhesivity, controlled drug release from the dosage form is also desirable. Plenty of polymers have been known as excellent bioadhesive materials. The most widely used polymers are constituted by poly-acrylates (and their derivatives or cross-linked modifications), chitosans (and their derivatives), sodium alginates and cellulose derivatives. At least one of the following polymer characteristics are required to obtain adhesion: (i) sufficient group forming hydrogen bonds (hydroxyl or carboxyl groups), (ii) anionic surface charge, (iii) high molecular weight, (iv) high chain flexibility, and (v) surface tensions

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that will induce spreading into the mucous layer (Chickering and Mathiowitz, 1999). The hydrogels as a pharmaceutical dosage form composed by the polymers mentioned above should fulfil some criteria: (i) to be loaded substantially by the active compound(s), (ii) to give no interaction with the active compound(s), (iii) to swell in the aqueous biological environment, (iv) to interact with the mucus, (v) to control release of the active agent from them, (vi) to be biocompatible, (vii) to be not absorbed from the administration site, and (viii) to be excreted unaltered or biologically degraded to inactive, non-toxic oligomers or monomers (Junginger et al., 2002).

In the literature some examples can be found where the bioadhesive behaviour of different type dosage forms containing poly(acrylic acid) type polymers has been reported, mainly as components of hydrogels (Zaki et al., 2007; Ceulemans and Ludwig, 2002) or tablets (Park and Munday, 2002; Ponchel et al., 1987). In case of poly(acrylic acid)s, the cross-linking density of these polymers has been established to influence interpenetration, because interpenetration of a larger polymer is more difficult than of a smaller one and the number of the functional groups which are able to form bioadhesive bonds may decrease (Park and Robinson, 1987). Another important factor in the bioadhesivity of poly(acrylic acid)s is the pH. Protonated and hydrated carboxylic groups are needed for the interaction between the mucin glycoproteins and acrylates, but extreme swelling may decrease their adhesivity. Therefore an ideal pH range has been determined at around pH 4–6 or at around the pK_a of the certain type poly(acrylic acid) (Mortazavi et al., 1993; Park and Robinson, 1987). In the last few years, considerable interest has been shown in new type polymers such as thiolated polymers, which form covalent bonds with the mucin in contrast with the weak, non-covalent bonds of the traditionally used polymers. In addition, they are not influenced by the ionic strength or pH, and beyond the latter they also have enzyme inhibitor and permeation enhancing effects. Different type poly(acrylic acid)s-cysteine conjugates were synthesised to improve the bioadhesive property of the dosage form by covalent bond with the cysteine of the mucin glycoprotein (Bernkop-Schnürch et al., 2000; Calceti et al., 2004; Leitner et al., 2003; Marschütz and Bernkop-Schnürch, 2002).

Pemulen TR1 and Pemulen TR2 are cross-linked block copolymers of poly(acrylic acid) and hydrophobic long-chain methacrylates (acrylate/C10–C30 alkyl-acrylate cross-polymer) (Goodrich, 1992). They can stabilize o/w emulsions because their short lipophilic part integrates into the oil droplets whilst the long hydrophilic part of the molecules forms a micro-gel around the droplet, so this micro-gel stabilizes the oil droplet. In this way, it is possible to incorporate easily a lipophilic active agent in a hydrophilic dosage form thereby avoiding the behaviour of the lipophilic vehicle to adhere slightly to the hydrophilic biological surface. One of their advantages is the low irritancy (Noveon, 2003) the cause of which is on the one hand Pemulens, being macromolecules, do not penetrate into the biological tissue, on the other hand they are used in a very low concentration (less than 1.0%, w/w). When these polymeric emulsifiers are used, the polymer chains have built up a special structure instead of a continuous polymer network (Fig. 1). When compared with continuous polymer texture, this special structure can modify the interpenetration into the mucus (Fig. 2).

Different methods have been published to show interaction between the poly(acrylic acid)s and the mucin glycoproteins. Although the most widely used methods are mechanical tests such as the rheological (Hägerström and Edsman, 2003; Hassan and Gallo, 1990; Madsen et al., 1998; Mortazavi, 1995) and tensile test (Caramella et al., 1994; Riley et al., 2001; Tamburic and Craig, 1997) measurements, numerous examples can be found in the literature where spectroscopic methods have been used to evidence the ability to form bioadhesive bond of the poly(acrylic acid), such as ATR–FTIR (Jabbari et al., 1993), ^{1}H ^{13}C nuclear magnetic resonance (Mortazavi, 1995) and X-ray photoelectron spectroscopies (Patel et al., 2003). In this study we have presented confocal laser scanning microscopy (CLSM) as a new method by means of which the bioadhesive bond can be visualized.

The emulsions containing polymeric emulsifiers can give a potential new drug delivery system, which can control drug release. The mucoadhesion of these emulsions is the physicochemical and biological precondition of drug release. The mucoadhesive behaviour of the gel-emulsion has not been encountered in the literature yet, so our research can be regarded as a novelty in this topic. Similarly, the correlations between the microstructure and mucoadhesion are presented in this paper for the first time.

The aim of our research was to examine the influence of the emulsified oil and the microstructure on bioadhesive behaviour and to find a correlation between the microstructure and bioadhesive behaviour. In case of emulsions containing polymeric emulsifiers numerous factors can influence their microstructure such as polymer concentration, oil concentration, changing of oil–water interface (due to the presence of coemulsifier) or droplet size (in this work we have disregarded the review of this last one). If the microstructure undergoes changes, the ability of the emulsions to form chemical bonds or physical entanglement with the mucus could be modified. The microstructure was investigated with rheological and thermoanalytical methods, whilst tensile test and confocal microscopy were used to examine the bioadhesive behaviour of the samples.

2. Materials and methods

2.1. *Materials*

Pemulen TR1 (PTR1) and Pemulen TR2 (PTR2) (Noveon, USA) were used as primary emulsifier, viscosity enhancing agent and bioadhesive. Pemulen TR1 (PTR1) is the higher polymerization-degree-polymer whilst Pemulen TR2 (PTR2) is the lower polymerization-degree-polymer. Coemulsifier, Synperonic PE/L 101 (S101) (Uniqema, UK) was added to certain emulsions to influence the microstructure. The oil phase was Miglyol 812 (Sasol Germany GmbH, Germany) and the aqueous phase was purified water (Ph.Eur.5). The neutralizing agent was trolamine (Ph.Eur.5).

In the fluorescence samples rhodamine B (Fluka, Milan) was used as fluorophore. Mucin (from bovine submaxillary glands, Type I) (Sigma–Aldrich, Milan) was added to the emulsions to visualize the bioadhesive bond. For the tensile tests porcine buccal tissue was obtained from freshly slaughtered

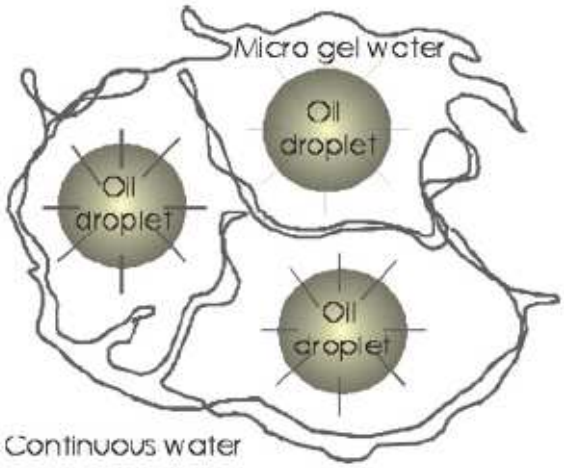

Fig. 1 – Theoretical structure of the emulsions containing polymeric emulsifier.

animals and frozen immediately after the cleaning procedure. The tissue was stored at −40 °C in a freezer until required. It was allowed to equilibrate to room temperature for 24 h before measurements.

2.2. *Emulsions preparation*

The Pemulens were added to purified water containing trolamine and preservative. The pH of the gel was 5–5.5. After 24 h the oil was added to this gel whilst the sample was stirred with mixer (MLW ER-10, 800 rpm) for 20 min. In the samples containing coemulsifiers, the mixture of the coemulsifier and oil was added to the water phase. The components of the emulsion can be seen in Table 1.

2.3. *Thermogravimetric investigation*

The measurements were carried out with MOM Derivatograph-C (MOM GmbH, Hungary) instrument. Samples were weighed (40–50 mg) in platinum pans (no. 4). The reference was a pan containing aluminium oxide. The samples were heated from 25 to 200 °C at 10 °C min^{-1}. TG (weight loss % vs. temperature), derivative TG (dTG) and DTA curves were plotted. Each study was repeated three times.

2.4. *Rheological investigation*

HAAKE RheoStress 1 Rheometer (HAAKE GmbH, Germany) with cone and plate geometry (diameter 35 mm, cone angle 1° and the gap 0.048 mm in the middle of the cone) was used to study the rheological profile of the samples. The flow curve and the viscosity curve of the samples were determined by rotation tests controlled shear rate. The shear rate was changed from 0.1 to 100 s^{-1} and then from 100 to 0.1 s^{-1}. The storage (G'), the loss (G'') moduli and loss tangent ($\tan\delta = G''/G'$) were examined as function of frequency (from 0.1 to 100 Hz) at 1.0 Pa (in case of PTR1) and at 0.1 Pa (in case of PTR2). These values of the shear stress were within their linear viscoelastic range. Each examination was repeated three times.

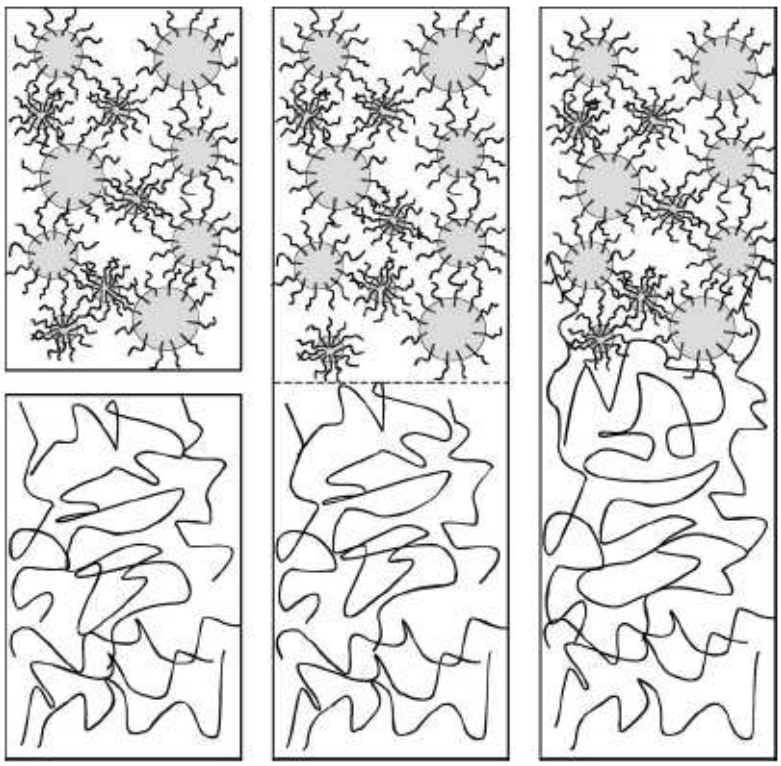

Fig. 2 – Interpenetration between gel-emulsion containing polymeric emulsifier and mucus.

Table 1 – Components of the emulsions containing polymeric emulsifier

Sample	Components (%, w/w)				
	PTR1	PTR2	S101	Oil	Water[a]
1	0.1	–	–	20	80
2	0.2	–	–	20	80
3	0.4	–	–	20	80
4	0.5	–	–	20	80
5	0.8	–	–	20	80
6	–	0.1	–	20	80
7	–	0.2	–	20	80
8	–	0.4	–	20	80
9	–	0.5	–	20	80
10	–	0.8	–	20	80
11	0.1	–	–	0	100
12	0.1	–	–	10	90
13	0.1	–	–	20	80
14	0.1	–	–	30	70
15	0.1	–	–	40	60
16	0.1	–	–	50	50
17	–	0.1	–	0	100
18	–	0.1	–	10	90
19	–	0.1	–	20	80
20	–	0.1	–	30	70
21	–	0.1	–	40	60
22	–	0.1	–	50	50
23	0.1	–	0.001	20[b]	80
24	0.1	–	0.01	20[b]	80
25	0.1	–	0.10	20[b]	80
26	0.1	–	0.50	20[b]	80
27	0.1	–	1.00	20[b]	80

[a] Together the water, the polymeric emulsifier and the trolamine.
[b] Together the oil and the S101.

2.5. *Tensile test*

The mucoadhesive properties of the gel-emulsions were investigated by TA-XT2 Plus Texture Analyser (Stable Micro Systems, Enco, Italy). The samples (20 mg) were laid on a filter paper fixed with double sided adhesive tape on the bottom of the upper probe. The tissues were placed in the lower probe. The upper probe with the sample was lowered at a speed of 1.0 mm s^{-1} onto the surface and a downward force of 6000 mN was applied for 1 min to ensure intimate contact between the sample and the tissue. After the preloading the upper probe was moved upwards at a speed of 4.0 mm s^{-1}. The detachment force was determined and the adhesive work was calculated from the area under the force–distance curve. Each study was repeated twelve times.

2.6. *Confocal laser scanning microscopy*

The visualization of the bioadhesive bond between the emulsion and the mucin was carried out with Confocal Microscope System Leica TCS SP2 (Leica Microsystems Heidelberg GmbH, Germany) interfaced with a Leica DMIRBE inverted microscope and using a 40 × 1.25 NA oil immersion objective. The excitation source was a Green Helio-Neon (λ_{ex} = 543 nm) laser, the fluorescence emission of rhodamine B was recorded between 580 and 630 nm. Rhodamine B (0.002%, w/w) was suspended in the oil phase and the oil was added to the water phase. 8.0% (w/w) mucin solution was prepared from mucin and buffer solution, pH 6.4 (USP). This solution was added to the emulsions. 10:1 and 5:1 emulsion–mucin ratios were applied.

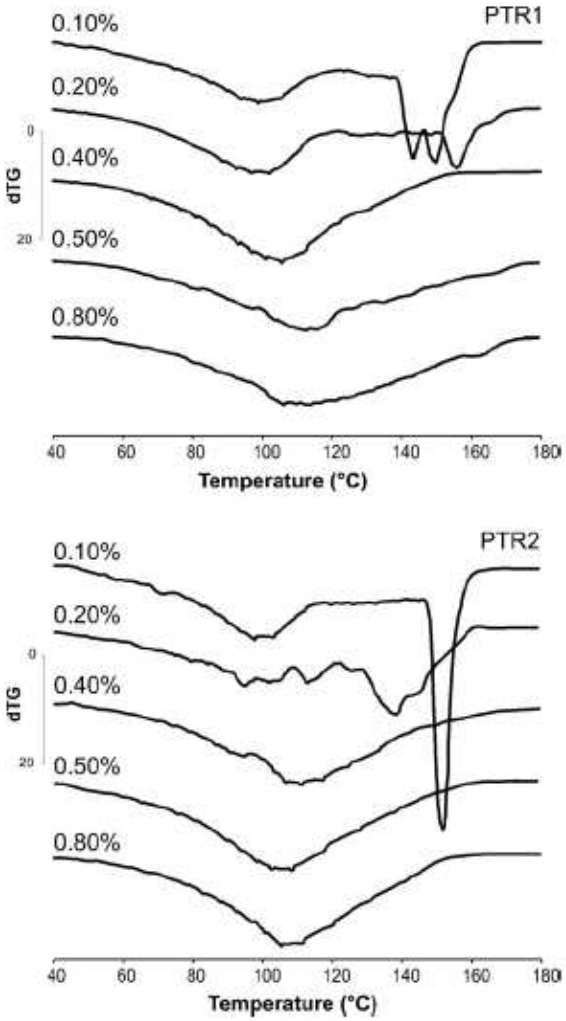

Fig. 3 – The dTG curves of the emulsions at different polymeric emulsifier concentration.

3. Results and discussion

3.1. *Thermogravimetric analysis*

Thermogravimetric measurements allow the investigation of the microstructure of emulsions, creams and other semi-solid systems, because (Junginger, 1984; Peramal et al., 1997; Kónya et al., 2003) free (or bulk) and (mechanically and chemically) bound water can similarly be identified and quantified.

It was assumed that this type of polymeric emulsifier, due to its surfactant nature, builds up a special gel structure in the emulsions instead of a continuous gel network. The polymer is enriched on the interface around the droplets and consequently its concentration will be lower in areas farther from the oil droplets. If this concentration difference is

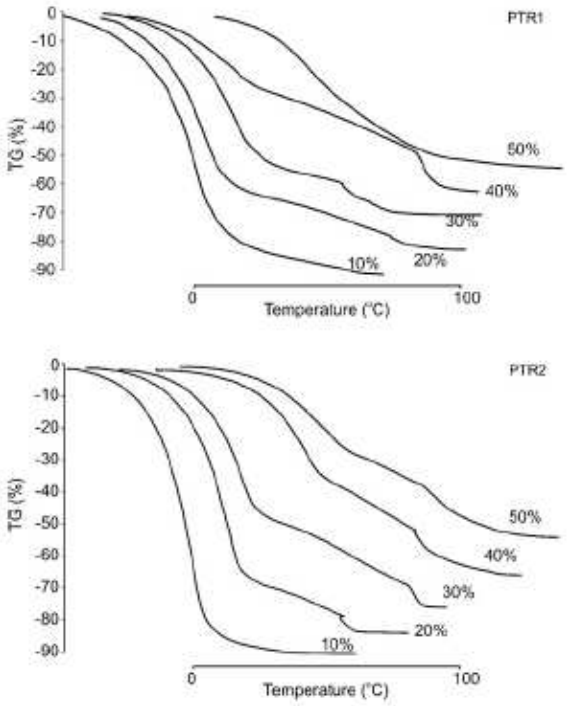

Fig. 4 – TG curves of the emulsions at different oil concentration.

considerable, two aqueous phases are obtained, which can be separated well with thermogravimetric investigations.

Fig. 3 shows the dTG curves of the emulsions at different polymeric emulsifier concentrations. Two peaks can be seen at lower concentrations (0.1–0.2%, w/w) and only one at higher ones (0.4–0.8%, w/w). The curve shows two weight loss peaks: one peak corresponds to free water at about 100 °C, the other to micro-gel (bound) water at about 140 °C. The integration of the polymers toward the oil–water interface can happen in samples with low polymer content, because the movement of the chains is not inhibited and the interface has not been saturated yet. At a high polymer content, the interaction between the chains increases so their movement may be inhibited and probably the interface is saturated, too. In this last case the different water phases are not separated.

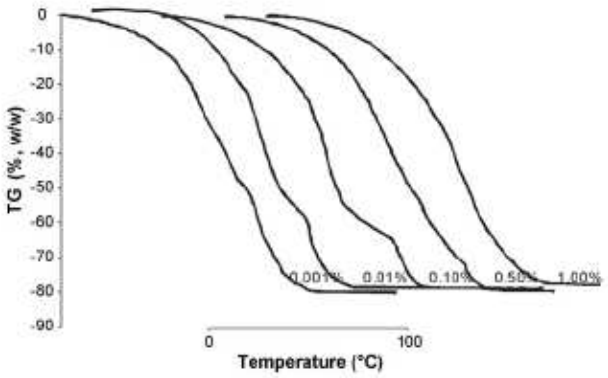

Fig. 5 – TG curves of the emulsions at different coemulsifier concentration.

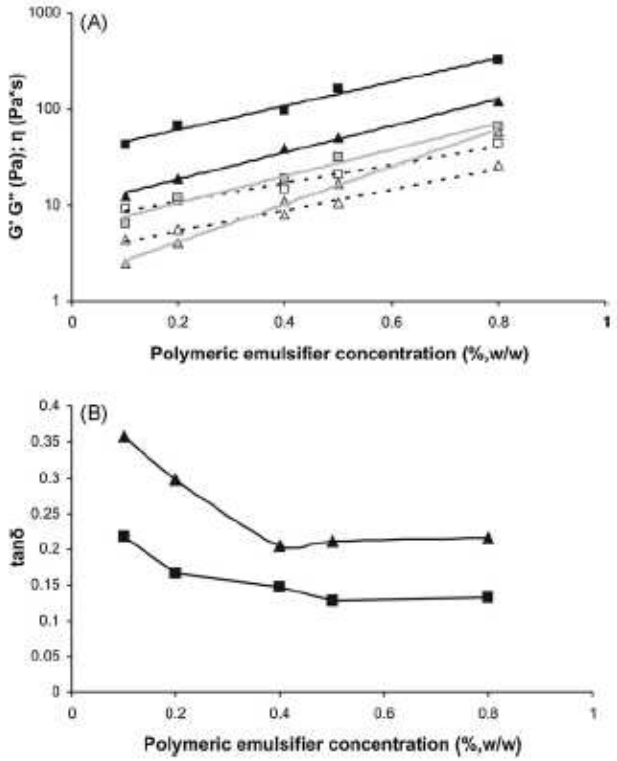

Fig. 6 – Variation of the viscosity (grey colour), the storage (black colour), loss (white colour) moduli (A) and loss tangent at 1.0 Hz (B) in function of PTR1 (square) and PTR2 (triangle) concentration.

The TG curves of the emulsions at different oil concentrations are shown in Fig. 4. The higher the oil content is, the greater ratio of the bound water can be observed, which can be calculated from the height of the steps. At low (10%, w/w) and high (50%, w/w) oil concentrations only one step can be seen in the curves whilst at middle (20–40%, w/w) concentration two steps can be separated. At a low concentration the surface and so the orientation of the polymer may not be significant enough to be detected. At a high oil concentration the polymer–water ratio is so high that the entire aqueous phase is bounded by the polymer gel. In the case of the samples containing PTR2, in which the lower polymerization-degree-polymer was applied, the two steps on the TG curve can be detected at high oil concentration, too, because the smaller polymer chains can move easily so they can orient towards the oil droplets even at relatively high polymer content.

In some samples the coemulsifier was applied in various concentrations (0.001%, 0.01%, 0.10%, 0.50%, and 1.00%, w/w). On the basis of our assumption this coemulsifier (S101) may inhibit or decrease the accumulation of the polymeric emulsifier at the water–oil interface, so the amount of the micro-gel water around the oil droplets may decrease or disappear.

Fig. 5 shows the changes of TG curves as the function of the coemulsifier concentration. The difference between the extents of the two steps increases, the bigger the coemulsifier concentration is, the smaller ratio of bound water can be measured. At high (1.00%, w/w) concentration the two steps

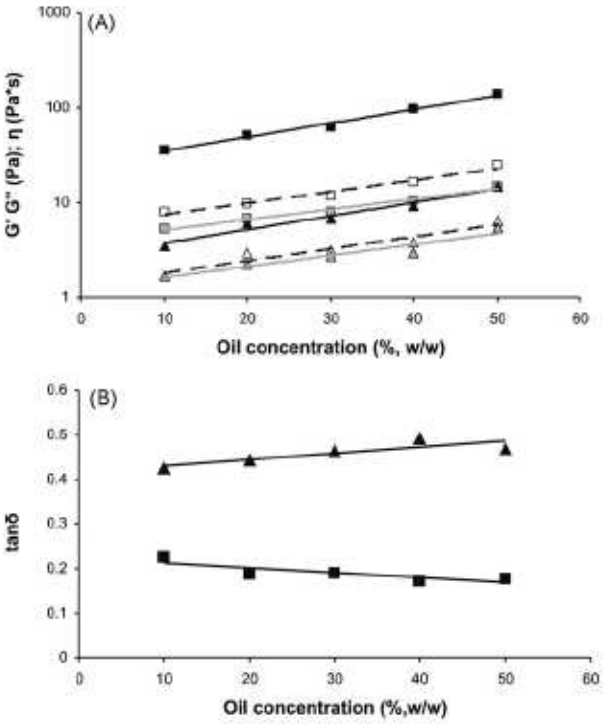

Fig. 7 – Variation of the viscosity (grey colour), the storage (black colour), loss (white colour) moduli (A) and loss tangent at 1.0 Hz (B) in function of oil concentration (PTR1 (square) and PTR2 (triangle)).

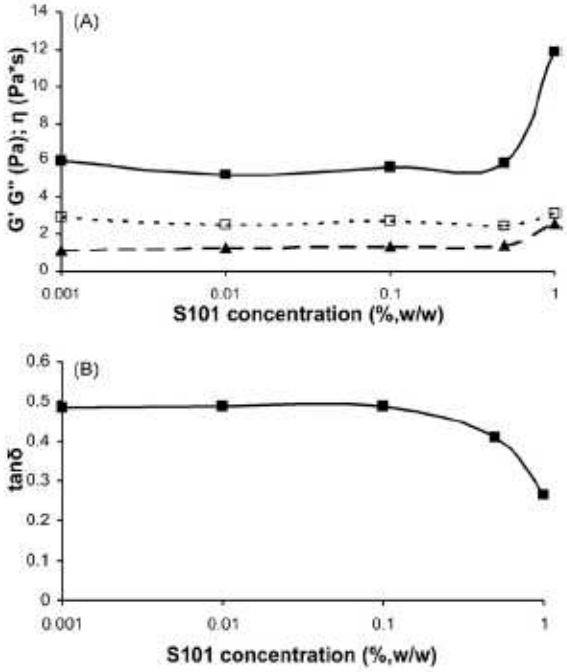

Fig. 8 – Variation of viscosity (▲), the storage (■), loss (□) moduli (A) and loss tangent at 1.0 Hz (B) in function of coemulsifier (S101) concentration.

absolutely disappeared, so probably the polymer formed a homogenous gel structure and there is no micro-gel around the droplets.

3.2. *Rheology*

The knowledge of the rheological characteristics of the systems is very important for monitoring the changes of the microstructure and the bioadhesive behaviour. Fig. 6A shows that the viscosity increases exponentially with the polymer concentration. Few pieces of information can be found in the literature about the Pemulen's rheological characteristics. Increasing of the viscosity was described with the polymeric emulsifier and the concentration and the emulsions showed thixotropy or antithixotropy (Milic-Askrabic et al., 1998; Simovic et al., 1998). On the basis of our examination it can be said that this increase of viscosity in the emulsion containing PTR2 is more pronounced; therefore, at 0.8% (w/w) both of the polymerization-degree polymers had the same viscosity value. Contrarily, the emulsion containing higher polymerization-degree-polymer showed more remarkable elasticity even if high polymer concentration was applied.

For the viscoelastic characterization of the emulsions tan δ (loss tangent or damping factor), G' (storage modulus) and G'' loss modulus were used.

$$\tan\delta = \frac{G''}{G'} \quad (1)$$

where G' is the storage modulus, G'' is the loss modulus. The smaller tan δ (or the greater G') is, the stronger the interaction is in the gel structure.

The loss tangent decreased with the amount of the polymer at low concentration whilst high polymer concentration did not alter that (Fig. 6B). This phenomenon can be explained by the changes of the microstructure. In the thermogravimetric results it was already shown that at low concentration (below 0.4%, w/w) the micro-gel structure around the droplets prevailed instead of the coherent polymer network, and this network is built up progressively with the increase of the amount of the chains. Therefore, the increase of elasticity can be more powerful than viscosity, so the loss tangent decreased. Above 0.4% (w/w) the network is completely built up, so the

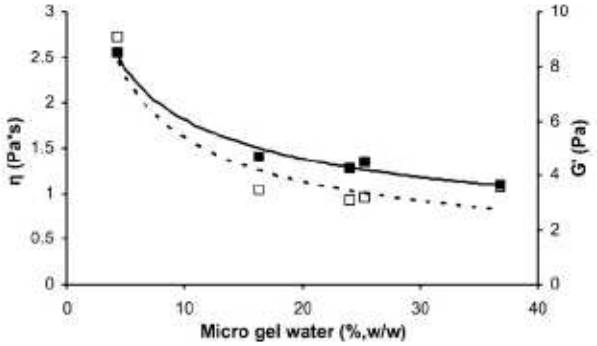

Fig. 9 – Correlation between the micro-gel water and the viscosity (■)/storage modulus (□) of the emulsions containing PTR1 and S101 at constant water oil ratio.

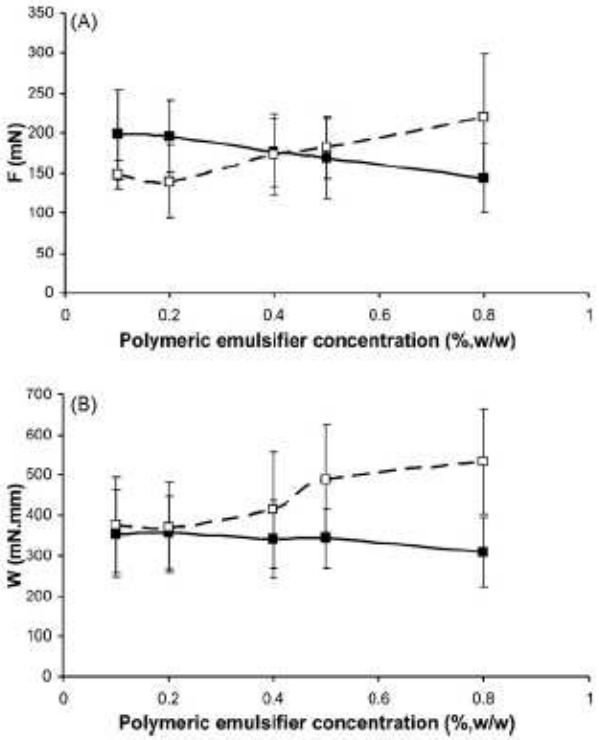

Fig. 10 – Variation of the detachment force (A) and the adhesive work (B) in function of PTR1 (■) and PTR2 (□) concentration.

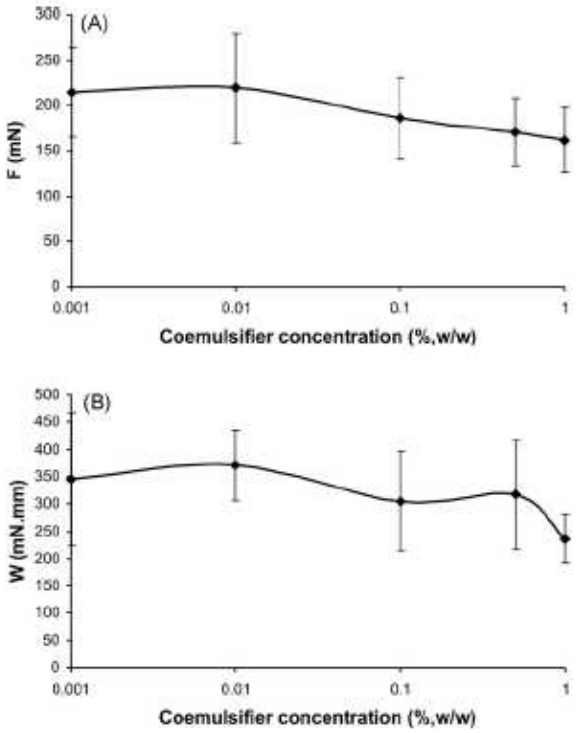

Fig. 12 – Variation of the detachment force (A) and the adhesive work (B) in function of coemulsifier concentration.

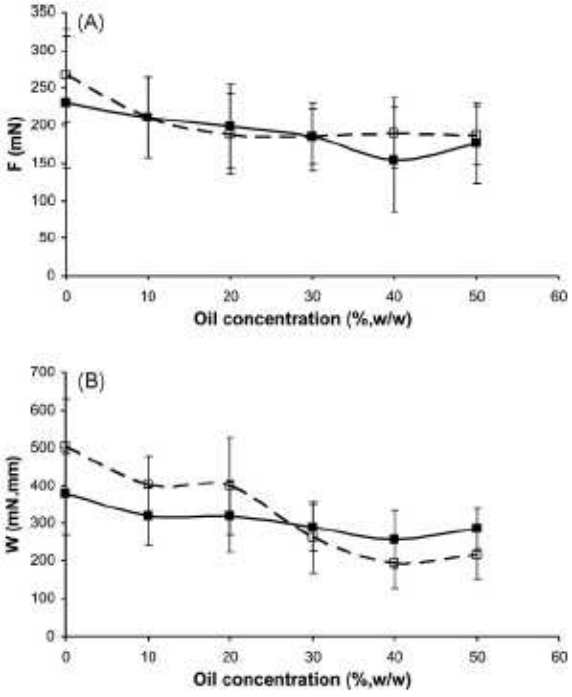

Fig. 11 – Variation of the detachment force (A) and the adhesive work (B) in function of oil concentration (PTR1 (■) and PTR2 (□)).

storage and the loss moduli changed parallel, therefore the loss tangent was constant.

Viscosity increased exponentially with the oil concentration in the case of both polymerization-degree polymers (Fig. 7A), whilst loss tangent showed a slight increase with the oil concentration in the case of PTR2 and a slight decrease in the case of PTR1 (Fig. 7B). On the basis of the loss tangent it can be concluded that the deformability of the emulsion containing PTR2 is more remarkable with the increase of the oil content.

Some authors have examined emulsions based on the combination of Pemulens and non-ionic emulsifier. They have established an addition of mixed emulsifier significantly modified the rheological characteristics of the emulsions (Savic et al., 2002; Simovic et al., 1999). The viscosity of the emulsion increased with the amount of the non-ionic emulsifier. When Polysorbate 80 was used as non-ionic emulsifier this phenomenon was explained with the interaction between the polymeric emulsifier and the non-ionic emulsifier (Simovic et al., 1999). In our study when coemulsifier (S101) was used, there were no changes below 0.5% (w/w) coemulsifier concentration, but at 1.00% (w/w) the viscosity and elasticity of the emulsion showed higher values (Fig. 8A). As already seen on the TG curves, the coemulsifier inhibited the accumulation of polymeric emulsifier at the water–oil interface and at 1.00% (w/w) the differentiated gel structure completely disappeared, so a coherent polymer network could evolve. The built network can explain the increase of the viscosity, elasticity and the decrease of the loss tangent at this concentration (Fig. 8B).

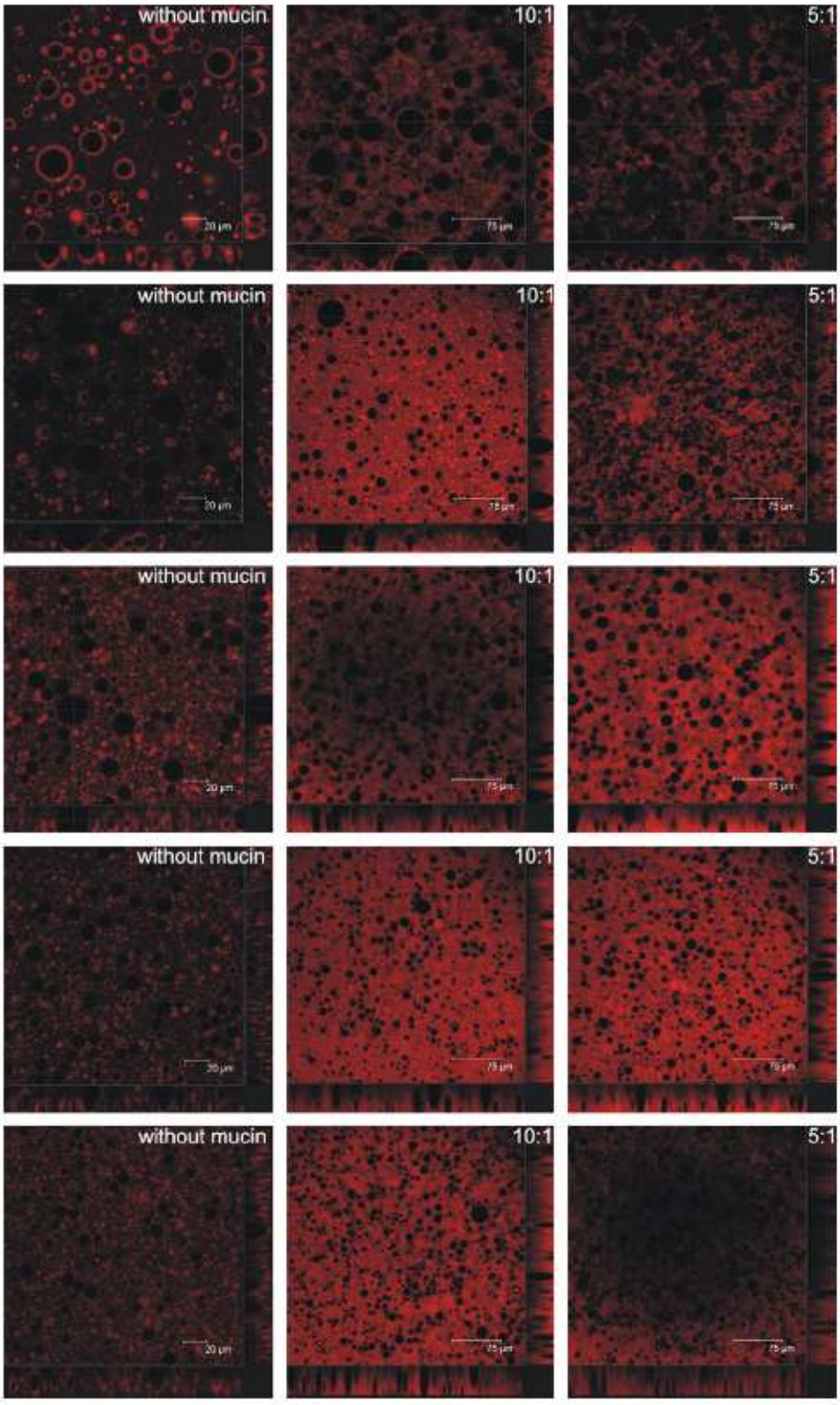

Fig. 13 – CLSM images of the simple emulsion (first column); and 10:1 (second column) and 5:1 (third column) PTR2 emulsion-8.0% (w/w) mucin mixtures (from the first row to fifth row the polymer concentrations are the following: 0.10%, 0.20%, 0.40%, 0.50% and 0.80%, w/w).

The relationship between microstructure and rheology is illustrated well in Fig. 9, showing the relationship between the quantity of micro-gel water and the rheological constants (viscosity and storage modulus), which can be described with a power function.

$$\eta = 4.45c^{-0.39} \quad (R^2 = 0.980) \tag{2}$$

$$G' = 17.24c^{-0.51} \quad (R^2 = 0.851) \tag{3}$$

where η is the viscosity, G' is the storage modulus and c is the water content in the micro-gel.

The small quantity of gel water detectable around the droplet indicates that the distribution of the polymer is becoming more and more homogeneous, which means that the built-up homogeneous gel structure increases the viscosity and elasticity of the systems.

3.3. *Tensile test*

The bioadhesive behaviour of the emulsions was different depending on the different polymerization-degree polymers used in the preparation, as it can be observed in Fig. 10. When increasing the polymer concentration at low values, there were changes neither in detachment force nor in adhesive work. In this range the coherent polymer network has not built up yet, as it had already been mentioned previously. Above 0.2% (w/w) both detachment force and adhesive work decreased with the amount of the polymer in the case of the higher polymerization-degree-polymer and increased in the case of the lower polymerization-degree-polymer.

PTR1 showed remarkable elasticity in the course of the rheological measurements, which suggested that these systems try to retain their integrity instead of forming chemical or physical bonds with the mucus. Contrarily, emulsions containing PTR2 with lower elasticity are more capable of forming bonds with the surface.

No significant change was observed in detachment force when increasing the oil concentration (our previous thermogravimetric measurements had shown the presence of micro-gel in almost all these samples). There was a slight decrease in both detachment force and adhesive work between the simple gel and emulsion (Fig. 11), which suggests that the added oil reduced the bioadhesivity of the samples.

The shape of the curve of adhesive work was similar at first to the one of detachment force, but at higher oil concentration, in the case of PTR2, a considerable decrease of the values was observed (Fig. 11B). In emulsions prepared with PTR2 loss tangent decreased with the increase of the oil concentration (Fig. 7), so deformability of these samples was stronger. Therefore the structure of the emulsion could be destroyed by the downward force. The chemical bonds could build up but physical entanglement could not develop. This explains why detachment force did not change, whilst adhesive work, which depends on the interpenetration of poly(acrylic acid) chains into the mucus (Ponchel et al., 1987), decreased at high oil concentration.

When a coemulsifier was used, a decrease in detachment force and adhesive work was observed, which is more expressed at a high S101 concentration (Fig. 12). The viscosity and the elasticity of these samples were higher at a high coemulsifier content (Fig. 8). On increasing the amount of the coemulsifier, the accumulation of the polymeric emulsifier at the interface was inhibited, so the coherent polymer network was built up progressively. These changes in the microstructure influenced the rheological and bioadhesive behaviour. Based on the thermogravimetric and bioadhesive measurements, it can be concluded that the coherent polymer network can decrease the bioadhesivity of the samples as compared to the ordered micro-gel system.

3.4. *Confocal laser scanning microscopy*

As Pemulens are modified poly(acrylic acid)s, they can be marked with cationic fluorescent dye such as rhodamine B. Using rhodamine B, which can be considered as a tertiary amine, hydrogen bonding or electrostatic interaction may form between the carboxyl groups of the polyacryl-acid and the fluorophore (Guan et al., 2006), so the dye concentration will be higher where the polymer concentration is higher. Therefore sharp fluorescence activity can be seen on the border of the droplets in the emulsion because the polymer concentration is higher around the droplet (Fig. 13, pictures on the left side). If the polymeric emulsifier forms a bond with the mucin, structural changes will take place in the samples, which will appear in the distribution of the dye.

At low polymer concentration, due to the interaction between mucin and poly(acrylic acid), polymer agglomeration can be observed in the pictures. In addition, oil droplets are retained in them. It can be assumed that mucin formed bioadhesive bonds with the micro-gel around the droplets and not with a network. In the course of the tensile test measurements the samples in this range did not show changes in the bioadhesive behaviour. At high polymer content (above 0.2%, w/w) no agglomeration can be seen, so interaction arose with the total polymer network.

4. Conclusion

On the basis of the results it can be said that the oil added into the emulsion slightly modified their rheological and bioadhesive behaviour. Under increasing polymer concentration the two different polymerization-degree polymers showed different results. In the case of the flexible low polymerization-degree-polymer, bioadhesive force and work increased with the amount of the polymer, whilst the adhesivity of the less flexible, high polymerization-degree-polymer decreased because of its increased elasticity. Contrarily, the high elasticity of the samples prepared with PTR1 meant an advantage in the case of higher oil content because these systems are able to retain their structure, and thus also their bioadhesivity, under different effects (e.g. downward force, application on the surface). The added coemulsifier modified the structure of the emulsions, which influenced the rheological and bioadhesive characteristics.

It can be concluded that (i) the emulsion containing mainly micro-gel around the droplets shows more remarkable bioadhesive force than the sample with coherent polymer network; (ii) there is no difference between the bioadhesiv-

ity of the samples where the coherent gel structure had not built up.

Acknowledgements

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IV.

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THERMOANALYTICAL AND MICROSCOPICAL INVESTIGATION OF THE MICROSTRUCTURE OF EMULSIONS CONTAINING POLYMERIC EMULSIFIER

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Polymeric emulsifiers provide exceptional stability to oil-in-water, water-in-oil or multiple emulsions by their steric stabilization. Pemulens as polymeric emulsifiers are able to stabilize *o/w* type emulsions because their short lipophilic part integrates into the oil droplets while their long hydrophilic part forms a micro gel around the droplet. In our present study the microstructure and integration of the polymeric emulsifier at the water–oil interface was investigated with thermogravimetric and microscopical methods. It was established that depending on the amount of both of the polymeric emulsifier and added coemulsifier the microstructure of the system changes.

Keywords: *confocal laser scanning microscopy, gel-emulsion, microstructure, polymeric emulsifier, thermogravimetry*

Introduction

An emulsion is a heterogeneous disperse system of two immiscible liquids (by convention described as oil and water), one of which is dispersed as fine droplets uniformly throughout the other [1]. They are not stable thermodynamically, several processes are known to lead to the destruction of their structure, such as: flocculation, creaming, sedimentation, coalescence, phase inversion and Ostwald ripening. Therefore one of the most important tasks is to ensure the kinetic stability of these systems. In addition to stability, other requirements also have to be satisfied by emulsions used in cosmetic and pharmaceutical industries, as appropriate consistence and safety of ingredients [2].

Emulsifiers are used both to advance emulsification and to ensure stability during storage and application. Polymeric emulsifiers appeared at the end of the last century. They provide exceptional stability to oil-in-water, water-in-oil or multiple emulsions by their steric stabilization. Some of these polymeric emulsifiers have been designed to act both as primary emulsifiers and viscosity enhancing agents. Pemulens (CTFA/INCI designation: Acrylate/C10-C30 alkyl-acrylate cross polymer) belong to this group. One of their most important properties resides in their affectiveness in stabilizing *o/w* type emulsions even for very low Pemulen concentrations (0.1–0.4 mass/mass%). The short lypophilic part of Pemulens is integrated into the oil droplets while the long hydrophilic part of the molecules forms a micro gel around the droplet so this micro gel stabilizes the dispersed phase [3].

Thermal analysis is becoming increasingly important in the structure examination of pharmaceutical dosage forms. Recently, in addition to the research of solid dosage forms [4–7], it has also been used successfully in the investigation of liquid and semi-solid systems. Thermoanalytical measurements allow investigating the microstructure of emulsions, creams and other semi-solid systems. Several papers about the structure of various semi-solid pharmaceutical preparations and cosmetic products (e.g. creams and liquid crystals) have been published in literature [8–12]. The majority of the investigations focus the attention on the binding of water: free, bound or interlamellar water is distinguished [13–17]. In the case of Pemulens free and bound (micro gel) water can similarly be identified and quantified with thermogravimetric measurements.

Emulsions can be visualized with confocal laser scanning microscopy either with the fluorescent dyeing of the disperse phase (or more rarely of the dispersion medium) or with the use of fluorescence-labelled surfactants [18–22]. In the present study our aim is to determine the location of the polymer with the second method. When rhodamin B is used as a fluorophore, H bonds and electrostatic interactions arise between the latter and the carboxyl group of the polymer as the structure of the rhodamine B is similar to a tertiary

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amine [23], as a consequence, the concentration of the dye will be higher where the polymer concentration is also higher.

Our aims were the following: 1) to perform thermogravimetric and microscopical examinations in order to learn about the microstructure of the gel emulsions as so far, such examinations have not been encountered in the literature of pharmaceutical technology yet; 2) to determine the binding of the water in the system; 3) to describe the changes arising in the microstructure due to the effect of the coemulsifier.

Experimental

Materials and emulsions preparation

The polymeric emulsifier was acrylate/C10-C30 alkyl-acrylate cross polymers (Noveon, Pemulen TR2). Coemulsifier was PEO-PPO-PEO triblock polymer (Synperonic PE/L 101; S101, Uniqema, UK). The oil phase was Miglyol 812 (Sasol, Germany) and the aqueous phase was purified water (Ph. Hg. VIII.) containing 0.01 mass/mass% methyl paraben (Ph. Hg. VIII.). The neutralizing agent was trolamine (Ph. Hg. VIII.). The fluorescent dye was rhodamine B (Fluka, Italy). The polymeric emulsifier was added to purified water containing trolamine and a preservative agent, than they were stored at room temperature for 24 h (pH was 5–5.5). The oil (containing suspended rhodamine B) was added to this gel by drop wise while the sample was being stirred with mixer (MLW ER-1, 800 rpk, 20 min). In the samples containing coemulsifiers, the coemulsifier was added to the oil phase. In the first series, the polymer concentration was changed under constant water oil ratio (80:20) and in the second one the secondary emulsifier was varied under constant polymer water oil ratio. The samples were made in mass/mass% concentration.

Methods

Thermogravimetric investigation

The measurements were carried out using MOM Derivatograph-C (MOM, Hungary) instrument. Samples were weighed (40–50 mg) in platinum pans (No. 4). The reference was a pan containing aluminium oxide. The samples were heated from 25 to 200°C at 10°C min^{-1}. TG (mass loss% *vs.* temperature) and DTG (derivative TG) curves were plotted. Each study was repeated three times.

Confocal laser scanning microscopy

Image acquisition was performed by Confocal Microscope System Leica TCS SP2 (Leica Microsystems Heidelberg GmbH, Germany) interfaced with a Leica DMIRBE inverted microscope and using a 40×1.25 N.A. oil immersion objective. The excitation source was a Green Helio-Neon (λ_{ex}=543 nm) laser, the fluorescence emission of rhodamine B was recorded between 580 and 630 nm.

Results and discussion

Our basic assumption was that the polymer, due to its surfactant nature migrates toward the interface; consequently its concentration will decrease in regions far from the oil droplets. If this concentration difference is considerable, two aqueous phases are obtained, which can be separated well with thermogravimetric investigations. When the quantity of the polymer is increased, two processes can be expected to occur: 1) the interface becomes saturated so the excess polymer will not appear in the boundary layer any more, therefore it will reduce the concentration difference between the interface and the more distant areas. 2) The increased polymer concentration will result in a greater number of interactions between the chains, which in turn over a certain concentration will inhibit the orientation of the polymers towards the interface to some extent. As a consequence, the differentiation of the gel structure can be expected to disappear with increasing polymer content. Figure 1 clearly shows that in the case of a low polymer content two peaks can be separated well in the DTG curve, one peak corresponds to free water at about 100°C, the other to micro gel (bound) water at about 140°C. When the quantity of the polymer is increased, the two peaks disappear as expected, and only one peak can be observed instead. This is confirmed by pictures made with confocal microscopy. In the case of a low concentration (Fig. 2) a sharp contour is dyed by rhodamin around the droplet, indicat-

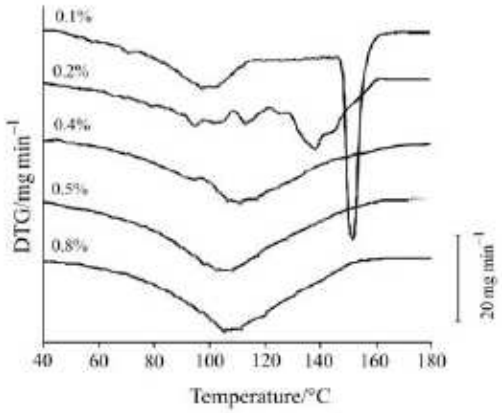

Fig. 1 DTG curves of emulsions with increasing polymeric emulsifier content

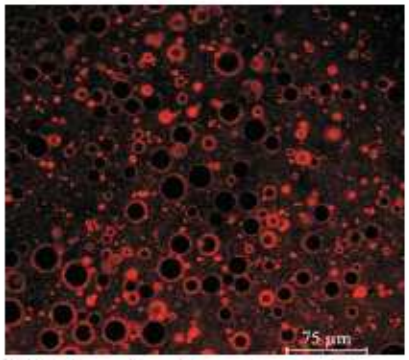

Fig. 2 CLSM picture of emulsion containing 0.1 mass/mass% polymeric emulsifier

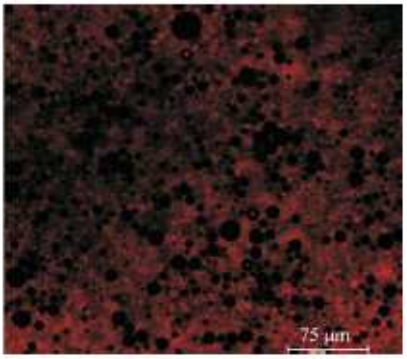

Fig. 3 CLSM picture of emulsion containing 0.8 mass/mass% polymeric emulsifier

ing a higher polymer concentration, while with higher concentrations the dye is of homogeneous distribution (Fig. 3).

If a coemulsifier is also used, changes in the microstructure can be assumed. The coemulsifier with its smaller molecules is also oriented on the interface, therefore in a higher concentration it can displace the polymeric emulsifier with greater molecules. As a result, the micro gel around the droplet will disappear. The two peaks of the DTG curve are shifted with an increasing coemulsifier concentration. The polymeric emulsifier is displaced from the interface and will gelate, thus the first peak will be shifted towards a higher temperature. At the same time the water on the interface will also evaporate from the system at a higher temperature. The quantity of water bound in different ways can be calculated from the step height of the TG curves. (In certain case large relative error can be seen in the water content determination which can be explained by the inhomogeneity of the macroemulsion systems.) If the quantity of the micro gel water on the interface is examined with respect to the total quantity of water with increasing coemulsifier concentration it can be stated that the amount of the micro gel water gradually decreases and finally disappears as a homogeneous gel is created by the polymer in the aqueous phase (Table 1).

Table 1 Peaks of the DTG curves and the amount of the micro gel water of the emulsions containing coemulsifier

Coemulsifier concentration/ mass/mass%	DTG		Micro gel water/ mass/mass%
	1st peak/ °C	2nd peak/ °C	
0.001	108±4	131±2	36.8±6.0
0.01	113±2	138±4	24.0±3.0
0.10	113±1	145±4	25.4±9.5
0.50	119±4	150±6	16.4±7.8
1.00	133±4	–	–

Conclusions

Gel-emulsions containing Pemulens form a special (micro gel) structure. It was established that the increase of the polymeric emulsifier and coemulsifier concentration leads to the disappearance of the micro gel structure. In case of the polymeric emulsifier the probable reason is the saturated surface and/or the improved polymer–polymer interaction, while in case of the coemulsifer the reason is its stronger affinity to the interface. According to the previous statement instead of two peaks only one peak can be seen in the DTG curve which corresponds to the homogenous water phase. Parallel with the latter, fluorophore does not dye a sharp contour around the droplets but is distributed homogenously in the total amount of the water.

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