Ph.D. Thesis

Formulation and Investigation of Gel-Emulsions Containing Polymeric Emulsifiers

Mária Budai-Szűcs

Szeged
2008
Formulation and Investigation of Gel-Emulsions Containing Polymeric Emulsifiers

Mária Budai-Szűcs

Supervisor:
Prof. Dr. Habil. István Erős Ph.D., D.Sc.

Szeged
2008
ARTICLES RELATED TO THE PH.D. THESIS


OTHER PUBLICATION


ABSTRACTS


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
# TABLE OF CONTENTS

Table of contents ......................................................................................................................................... 1

1 Introduction ............................................................................................................................................... 3

2 Literature survey ........................................................................................................................................ 3
   2.1 Emulsions, types of the emulsions ......................................................................................................... 3
   2.2 Stability of emulsions ............................................................................................................................... 5
   2.3 Gel-emulsions ......................................................................................................................................... 7
   2.4 Polymeric emulsifiers .............................................................................................................................. 9
   2.5 Experimental aims .................................................................................................................................. 11

3 Materials and Methods ............................................................................................................................ 12
   3.1 Materials .............................................................................................................................................. 12
   3.2 Methods ............................................................................................................................................... 14
      3.2.1 Preparation of emulsions .................................................................................................................... 14
      3.2.2 Measurement of the surface tension ................................................................................................. 15
      3.2.3 Contact angle measurements ............................................................................................................ 15
      3.2.4 Measurement of the Enslin number .................................................................................................... 15
      3.2.5 Droplet size analysis .......................................................................................................................... 15
      3.2.6 Thermogravimetric investigation ...................................................................................................... 15
      3.2.7 Rheological investigation .................................................................................................................. 15
      3.2.8 Tensile test ....................................................................................................................................... 16
      3.2.9 Confocal laser scanning microscopy .............................................................................................. 16
      3.2.10 Drug release test ............................................................................................................................. 16

4 Results and discussion ............................................................................................................................... 17
   4.1 Preformulation studies ............................................................................................................................ 17
   4.2 Structure and properties of the emulsions and the simple gels ......................................................... 19
      4.2.1 Rheological investigations .................................................................................................................. 19
         4.2.1.1 Effect of the polymer concentration .......................................................................................... 19
         4.2.1.2 Effect of the oil concentration .................................................................................................... 20
         4.2.1.3 Effect of the coemulsifier concentration .................................................................................... 21
      4.2.2 Thermoanalytical investigations ......................................................................................................... 22
         4.2.2.1 Effect of the polymer concentration .......................................................................................... 23
         4.2.2.2 Effect of the oil concentration .................................................................................................... 24
4.2.2.3 Effect of the coemulsifier concentration ................................................................. 25
4.2.3 Microscopical investigations ..................................................................................... 28
4.2.3.1 Droplet size analysis .............................................................................................. 28
4.2.3.2 Confocal laser scanning microscopy ...................................................................... 29
4.3 Stability ....................................................................................................................... 30
4.4 Applicability ................................................................................................................ 35
  4.4.1 Bioadhesion ............................................................................................................ 35
  4.4.2 Drug release ............................................................................................................ 40
5 Summary ....................................................................................................................... 43
6 References ..................................................................................................................... 45
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 thermogranelometry).

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(∞):

\[ S(r) = S(\infty) \exp\left(\frac{2\gamma V_m}{rRT}\right) \]  

(1)
where $\gamma$ 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:

$$(RT/V_m)\ln(S_1/S_2)=2\gamma(1/r_1-1/r_2)$$  \hspace{1cm} (2)$$

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

$$\omega=d(r_c)^3/dt = 8DS_\infty V_m/9RT$$ \hspace{1cm} (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).
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.

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].
Block copolymers

~~A~~~~ ~~~~B~~
Diblock polymer

~~A~~~~ ~~~B~~~~~~A~~
Triblock polymer

Graft copolymers

~~~~~B~~~~~~

A A A A A

Star copolymers


AB star-block

BA star-block

Heteroarm

Random

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.
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](image)

**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 Table 1.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (% w/w)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemulen TR1 or Pemulen TR2 (Noveon, USA)</td>
<td>0.1 – 1.2</td>
<td>Primary emulsifier</td>
</tr>
<tr>
<td>Synperonic PE/L 31, 61, 62, 101 (Uniqema, UK)</td>
<td>0.001 – 1.00</td>
<td>Coemulsifier</td>
</tr>
<tr>
<td>Trolamine (Ph. Hg. VIII.)</td>
<td>at pH 5.5 - 6</td>
<td>Neutralizing agent</td>
</tr>
<tr>
<td>Miglyol 812 (Sasol, Germany)</td>
<td>10 - 70</td>
<td>Oily phase</td>
</tr>
<tr>
<td>Purified water (Ph. Hg. VIII.)</td>
<td>30 - 90</td>
<td>Aqueous phase</td>
</tr>
<tr>
<td>Lidocaine base (Ph. Hg.VIII.)</td>
<td>1.00</td>
<td>Lipophilic model drug</td>
</tr>
<tr>
<td>Metronidazole (Ph. Hg.VIII.)</td>
<td>0.75</td>
<td>Hydrophilic model drug</td>
</tr>
</tbody>
</table>
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 °C at 5 °C min$^{-1}$ or at 10 °C 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 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 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 ($\lambda_{\text{ex}} = 543$ 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 $\mu$m). 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 Helios-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>
<thead>
<tr>
<th>Contact angle Θ (± SD, n=7)</th>
<th>PTR1</th>
<th>PTR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified water</td>
<td>81.7 ± 5.67</td>
<td>82.64 ± 1.24</td>
</tr>
<tr>
<td>Miglyol 812</td>
<td>27.9 ± 0.88</td>
<td>26.8 ± 0.49</td>
</tr>
</tbody>
</table>

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](image)

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](image)

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](image)

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.
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 ($\eta_0$), and the damping factor ($\tan \delta$) were used to characterize the rheological properties of the gels and emulsions.

Initial viscosity was determined by the power law model:

$$\eta = \eta_0 D^n$$

(4)

where $\eta$ is the viscosity, $\eta_0$ 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 $\tan \delta$ (loss tangent or damping factor), $G'$ (storage modulus) and $G''$ (loss modulus) were used.

$$\tan \delta = G''/G'$$

(5)

Where $G'$ is the storage modulus, $G''$ is the loss modulus. The smaller $\tan \delta$ (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:
\[ \eta = \eta_e \exp(m \cdot c) \]  

where \( c \) is the polymeric emulsifier concentration, \( \eta_e \) 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)](image)

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)](image)

### 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

<table>
<thead>
<tr>
<th>Coemulsifier</th>
<th>Concentration (% w/w)</th>
<th>ηo (Pa*s)</th>
<th>tanδ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTR1 0.20 % w/w, oil 20% w/w</td>
<td>0.00</td>
<td>14.2</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>16.8</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>11.3</td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>11.8</td>
<td>0.109</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>20.4</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>25.4</td>
<td>0.113</td>
</tr>
<tr>
<td>PTR2 0.20 % w/w, oil 20% w/w</td>
<td>0.00</td>
<td>19.4</td>
<td>0.624</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>21.0</td>
<td>0.579</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>23.6</td>
<td>0.608</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>23.7</td>
<td>0.608</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>23.4</td>
<td>0.615</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>23.4</td>
<td>0.615</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coemulsifier</th>
<th>Concentration (% w/w)</th>
<th>tanδ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTR1 0.20 % w/w, oil 20% w/w</td>
<td>0.00</td>
<td>1.016</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>1.012</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>1.011</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>1.011</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>1.011</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.011</td>
</tr>
<tr>
<td>PTR2 0.20 % w/w, oil 20% w/w</td>
<td>0.00</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>2.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coemulsifier</th>
<th>Concentration (% w/w)</th>
<th>ηo (Pa*s)</th>
<th>tanδ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTR1 0.20 % w/w, oil 20% w/w</td>
<td>0.00</td>
<td>2.5</td>
<td>0.624</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>2.4</td>
<td>0.544</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>2.4</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>2.4</td>
<td>0.489</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>2.4</td>
<td>0.358</td>
</tr>
</tbody>
</table>

Table 3 Viscosity and damping factor values
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 °C, the other to micro gel (bound) water at about 140 °C. 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.
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.17 Evaporation rate as function of the emulsifier concentration (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.18 DTG curves of emulsions with increasing polymeric emulsifier content (oil 20% w/w)**
(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%)](image)

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)](image)

### 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):

\[ v = 0.597c^{-0.0613} \]  

(7)

where \( v \) is the evaporation rate and \( c \) is the S101 concentration.

Table 4 Evaporation rate

<table>
<thead>
<tr>
<th>Coemulsifier</th>
<th>PTR1 0.20% w/w</th>
<th>Concentration (% w/w)</th>
<th>0.00</th>
<th>0.01</th>
<th>0.05</th>
<th>0.10</th>
<th>0.50</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>S31</td>
<td>0.752</td>
<td>0.593 0.657 0.666 0.613</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S61</td>
<td>0.569</td>
<td>0.710 0.516 0.656 0.732</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S62</td>
<td>0.586</td>
<td>0.734 0.742 0.583 0.642</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S101</td>
<td>0.829</td>
<td>0.686 0.651 0.667 0.588</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coemulsifier</th>
<th>PTR2 0.20% w/w</th>
<th>Concentration (% w/w)</th>
<th>0.00</th>
<th>0.05</th>
<th>0.10</th>
<th>0.50</th>
<th>0.676</th>
</tr>
</thead>
<tbody>
<tr>
<td>S31</td>
<td>0.667</td>
<td>0.833 0.490 0.449 0.556</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S61</td>
<td>0.508</td>
<td>0.488 0.545 0.486 0.458</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S62</td>
<td>1.257</td>
<td>0.491 0.432 0.372 0.516 0.542</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S101</td>
<td>0.529</td>
<td>0.408 0.466 0.989 0.676</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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).

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

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

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

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.

\[ \eta = 4.45c^{-0.39} \quad (R^2 = 0.980) \quad (8) \]

\[ G' = 17.24c^{-0.51} \quad (R^2 = 0.851) \quad (9) \]

where \( \eta \) 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)](image)

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)](image)

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

<table>
<thead>
<tr>
<th>Coemulsifier</th>
<th>PTR1 0.20% w/w, oil 20% w/w</th>
<th>Concentration (% w/w)</th>
<th>0.00</th>
<th>0.01</th>
<th>0.05</th>
<th>0.10</th>
<th>0.50</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>S31</td>
<td>17</td>
<td>15</td>
<td>12</td>
<td>16</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S61</td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S62</td>
<td>16</td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S101</td>
<td>14</td>
<td>13</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coemulsifier</th>
<th>PTR2 0.20% w/w, oil 20% w/w</th>
<th>Concentration (% w/w)</th>
<th>0.00</th>
<th>0.01</th>
<th>0.05</th>
<th>0.10</th>
<th>0.50</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>S31</td>
<td>33</td>
<td>18</td>
<td>19</td>
<td>17</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S61</td>
<td>17</td>
<td>14</td>
<td>16</td>
<td>11</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S62</td>
<td>21</td>
<td>19</td>
<td>17</td>
<td>8</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S101</td>
<td>18</td>
<td>13</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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](image)
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.

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 ($\eta_0$) and the yield value ($\sigma_0$) 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 $\eta_0$ increases while $\sigma_0$ shows some decrease, it is from a flocculation occurring in an irregular way (producing strong and tight flocs). In my study $\eta_0$ was calculated from the power law as it had been described previously and $\sigma_0$ was calculated from the flow curve model on the basis of the Herschel-Bulkley equation:

$$\sigma = \sigma_0 + kD^n$$

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:

$$E_c = \frac{1}{2} G' \gamma_{cr}^2$$

where $G'$ is the storage modulus and $\gamma_{cr}$ 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:

$$v = A_0 t^k$$

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)**
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.

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.
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.

![Graph](image1)

![Graph](image2)

*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).

![Graph](image3)

![Graph](image4)

*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](image)

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.
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.

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.

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.

![Graph](image)

*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.
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.
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).

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.
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

![Fig.46 Effect of the coemulsifier concentration on the Lidocaine release (PTR1 0.10%, oil 20%)](image-url)
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.
6 REFERENCES

35. I. Erös, M. Kónya, I. Csóka, Int. J. Pharm 256 (2003) 75-84
44. T. Hansen, P. Holm, M. Rohde, K. Schultz, Int. J. Pharm. 293 (2005) 203–211
49. J. Palanuwech, J.N. Coupland, Colloid Surface A 223 (2003) 251-262
60. G. Sala, F. van der Velde, M.A. Cohen Stuart, G.A. van Aken, Food Hydrocolloid. 21 (2007) 977-985
63. S. Liu, Steven P. Armes, Curr. Opin. Colloid Interfac. 6 (2001) 249-256
64. Z. Zhang, G. Liu, S. Bell, Macromolecules 33 (2000) 7877-7883
81. B. Drescher, A.B. Scranton, J. Klier, Polymer 42 (2001) 49-58
86. B.F. Goodrich, Technical Data Sheet 114
87. Noveon, TOX-005, Pemulen® Polymeric Emulsifiers Toxicology Studies, 2003
89. European Pharmacopoeia 4th edition, Directorate for the Quality of Medicines of the Council of Europe, Strasbourg, 2002;
97. G. Scharamm: Practical Approach to Rheology and Rheometry HAAKE GmbH, Karlsruhe, Germany, 1994
100. J.L. Ford, Int. J. Pharm. 179 (1999) 209-228


124. S.A. Mortazavi, B.G. Carpenter, J.D. Smart, Int. J. Pharm. 94 (1993) 195-201


ACKNOWLEDGEMENTS

I am very grateful to **Professor István Erős** as my supervisor for his support. I owe my warm gratitude for his encouragement and numerous advice during my Ph.D. work.

I would like to thank **Professor Piroska Szabó-Révész** for providing me with the opportunity to work in her department.

I am very grateful to **Associate Professor Dr. Erzsébet Csányi, Dr. András Fehér, Zsolt Makai, Dr. Péter Sipos** for their help and friendship.

I am greatly indebted to **Professor Carla Caramella** and **Professor Cristina Bonferoni** for providing me with the possibility to work in their department.

My warmest thanks to **Dr. Giuseppina Sandri** and **Dr. Patrizia Vaghi** for their help and useful advice.

I would like to thank to my **family** and my **husband** for their encouragement, support and for giving me a peaceful background.
ANNEX
I.
Gél-emulziós rendszerek I. rész
Fizikai kémiai jellemzés

SZŰCS MÁRIA, BUDAI SZABOLCS, ERŐS ISTVÁN

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

Summary

Szűcs, M., Buda i, Sz., Eros, I. Gel-emulsion systems I. Physical-chemical characterisation

Emulsion gels prepared with polycrylic 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 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 monodispersive one, the average droplet size decreased with polymer concentration.

Összefoglalás


Bevezetés

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

A 20. század második felétől kezdve a gyógyszer-technológiai kutatások egyik irányvonalát, új gyógyszerhordozó rendszerek keresése, fejlesztése, programozott hatóanyag-leadás céljából [2]. Ennek eredményeképpen az emulziók ismét előterbe 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 tekinthető ezek a sajátos diszperz rendszerek.

Az emulziók gyógyszer-technológiai kutatásának napjainkban három alapvető irányvonal van:
- új emulziós gyógyszerhordozó rendszerek ki-dolgozása [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 gyógyszer-leadó rendszer, a gél-emulziók szerkezeténak, fizikai kémiai sajátosságainak és stabilitásának megismerésére irányult.

A gél-emulziók előállítására az alábbi módszerek alkalmazhatók:
- az emulzió vizítáziának gélelődése vizoldékony polimerekkel,
- az emulzió olajfázisának gélelődése,
- a két fázis határfelületén polymer vécőréteg létrehozása in situ polimerizációval,
- az olaj- és vizítási határfelületén mikrogél kialakítása polymer emulgenessel.

Kísérletes munkánk során ez utóbbi módszert alkalmaztunk.

A polimer emulgiségeknek alapvetően két fő csoportja van:
1. Blockk polimerek, amelyek lehetnek di- vagy tri-blokkok, ill.
2. Graft polimerek.
A polimerek blokkjai eltérő oldékonyságúak, ezáltal az olaj/víz határfelületen fognak feldúsulni. Stabilizáló hatásuk kisebb részben a felületi feszültség csökkenetéssel, nagyobb részben a sztérikus gátással magyarázható.

Az általunk használt polimer emulgens a Pemulen TR1 és a TR2 volt, melyek poliakrilát-alkilakrilát diblokk polimerek (Előállító: Lubrizol Corp., USA). A kétféle polimergyorsító fokukban különbözik egymástól, a Pemulen TR2 kisebb, a Pemulen TR1 nagyobb polimerizációs fokú. Alkalmazás a/v típusú emulziók előállítására, mivel rövid lipofil karakterű részük az olajcseppebe integálódik, míg hosszabb hidrofil karakterű részük a vízes fázisban gelt képez közvetlenül az olajcseppek körül. A rendszerben sajátos kettős gélrendszer jön létre: a határfelületi orientáció miatt a polimer koncentrációja az olajcseppek körül nagyobb lesz, ez alakítja az un. mikrogélet, az emulzió külső vízes fázisát géllesíti a feleslegben alkalmazott polimer.

(1. és 2. ábra)

Vizsgálataink célja a következő volt: részletesen tanulmányozni kívántuk a polimer emulgesekből készített géleket és emulziók képzetőségét,
- kutattuk a rendszerek szerkezeti, reológiai tulajdonságait,
- tanulmányoztuk a víz kötődési mechanizmust,
- ezek alapján megállapítottuk a vezetők szerkezetét, s szándékoztunk kidolgozni.

Anyagok és módszerek

Anyagok

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

A minták előállítása

Először alapgélt készíttettünk, melynek polimertartalma 1,0% volt. A víz teljes mennyisébén 24 órán keresztül duzzaszattottuk 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 géleket emulgsáltuk az olajat kis részletekben. A minták pH-ját üvegelektród segítségével határoztuk meg.

Vizsgálati módszerek

Felületi feszültség mérése

A méréseket Krüss-fele tenziométerrel végeztük. Hígítási sort készítettünk 0,00005%, és 0,25% között. A levegő/polimertartalomból készítettük stabilizálódása miatt 5 perc várakozás után végeztük el a mérést. Minden koncentráció esetében 3 párhuzamos mérést végeztünk.

Polimerek vézelvételének mérése

A méréseket Ensín készülékkel végeztük. A kétszűlő G2-es üvegszűrőjére szűrőpapír helyeztünk hézamentesen, majd 0,10 g polimert rétegeztünk.
ről. A szűrővel egy szintben, vízszintesen elhelyezett pipettároló olvasztuk le a polímer emulgense által adott idő alatt felszívott víz mennyiségét ml-ben. A leolvasást 10 percig végeztük és 3 párhuzamos mérést középértékeltünk.

Nedvesedési peremcsövek meghatározása

A vizsgálatok a cseppszétertésű mérősen alapuló Dataphysics OCA készülék segítségével történtek. A polímerekből 0,20 g-os próbatesteket prélisztünk (10 másodpercg 5 torna nyomással), melyeket 24 órára összékerületbe helyeztünk. Ezt követően határoztuk meg a nedvesedési peremcsövet desztilált vízzel és Miglyol 812-vel.

Reológiai vizsgálatok

A reológiai méréseket HAAKE RheoStress 1 (HAAKE GmbH, Németország) kúp-lap geometriaú j készülékkel végeztük 25 °C-on. Szabályozott nyírói sebesség-gradiens mellett felvettük a minták folyás- és viszkozitás-görbét (a felszáló ágot 0,1 s⁻¹-től 100 s⁻¹-ig, a leszáló ágot 100 s⁻¹-től 0,1 s⁻¹ intervallumban határoztuk meg). Az oszcillációs mérések folyamán meghatároztuk a minták lineáris viszkoelaszicitási tartományát (a nyírófeszültséget 0,1 és 100 Pa között változtattuk). E tartományon belül választottunk egy nyírófeszültséget értéket és ezen értéken határoztuk meg az emulziók és gélekh tárolási és vesztegeségi moduluszát a frekvencia függvényében (0,1 és 100 Hz között). Minden esetben 3 párhuzamos mérést középértékeltünk.

Termoanalitikai mérések

A termoanalitikai méréseket Derivatograph C (MOM, Magyarország) készülékkel végeztük. 50 mg mintát mérünk platina tégelybe. A mintákat 25-től 200 °C-ig főzzük 5 °C/perc sebességgel, és felvettük a TG (tömegcsökkenés az idő függvényében) és dTG (tömegszenvedés derívtjá) görbét. A TG görbék közélineáris szakaszára egyenest illesztettünk, melynek meredekségéből következtettünk a víz párolgási sebességére a mintából. A víz kötődési mechanizmusának meghatározására 10 °C/perc fűtési sebességet használtunk.

Cseppméret-analízis

A cseppméret-analízist Leica képanalizátórral végeztük. 500 csepp átmérőjét mértük meg és Leica Q500MC Qwin V10.02 szoftver segítségével értékeltek az eredményt.

Kísérleteink rendjét a 3. ábrán szemléltetjük.

Erdemények és értékelés

Preformulációs vizsgálatok

A preformulációs vizsgálatok során megállapítottuk, hogy a polímer emulgensek csekély felületi feszültség csökkentő hatással rendelkeznek. A víz 72 mN/m felületi feszültséghéz képest 50-60 mN/m közötti értéke nem mondható jelentős csökkentésnek. A felületi feszültség-konzentráció összefüggést vizsgálva mindkét emulgense esetében minimum átmérő függvényt kapunk, a minimum helye 0,01% koncentráció körül volt (ez tekintetében a felületaktív polímer kritikus micellaképződési koncentrációjának). A két emulgense felületaktív jellege nem tér el jelentősen egymástól, tehát a polimerizációs fok nem befolyásolta a felületi feszültség csökkentő hatást (4. ábra).

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

<table>
<thead>
<tr>
<th>Nedvesedési peremcsöv, θ (± SD, n=7)</th>
<th>PTP1</th>
<th>PTP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiszta víz</td>
<td>81,7 ± 5,67</td>
<td>82,64 ± 1,24</td>
</tr>
<tr>
<td>Miglyol 812</td>
<td>27,9 ± 0,88</td>
<td>26,8 ± 0,49</td>
</tr>
</tbody>
</table>

1. táblázat

A polímerek nedvesedési peremcsöve vízzel és olajjal
Ensln készülékekkel vizsgálva a polimerek duzzadását, azt a következtetést vontuk le, hogy a duzzadás lassú és elhúzódó folyamat. Összehasonlítva a széles körben alkalmazott hidroxi-etil-cellulózal (HEC), jelentősen elmaradnak ez utóbbi Ensln-értékekétől (5. ábra).

A polimerek hidratációja és ez által a hidrogélek viszkozitása aláalában pH függő jelenség. Különböző koncentrációjú polimer emulgensekből képződött hidrogélek viszkositását vizsgáltuk a pH függvényében. A pH-t trolamin hozzáfogásával változtattuk és pH-mérő üvegelektrosis segítségével mértük. Az eredmények alapján elmondható, hogy a viszkositás csak nagy polimer-konzentrációjánál mutat jelentősen pH-függést (1,00%) mindkét polimerizációs fokú emulgenseknél, 1,0%-nál kisebb koncentrációban nem befolyásolta a kémhatás a gélek viszkositását. A további vizsgálatokhoz 5,5 pH-jú géleket és emulziókat állítottunk elő. (6. ábra)

A hidrogélek alajfelvételelét a következőképpen határozottuk meg. A különböző koncentrációjú hidrogélek-hez üregtelkeből cseppenként adtuk hozzá a semleges olajat mindaddig, míg a rendszer szétvált. A fogyott olaj térképtetést tömögre átszámoltuk, majd kiszámoltuk az emulziók pontos százalékos összetételét. Megállapítottuk, hogy a nagyobb polimerizációs fokú emulgensek 80%-os olajtartalmú emulziók is előállíthatók, míg a kisebb polimerizációs fokú csak 50-60%-os alajfelvételeit eredményezett.
nyezett. Abban az esetben, ha az olajat nem cseppenként, hanem csak apró részletekben adagoltuk, 50%-os felett olajtartalmú stabil rendszereket nem sikerült előállítani. (7. ábra)

**Termoanalitikai vizsgálatak**

Feltételezésünk szerint a polímer emulgens amifil sajátsága folytán a határértégek nagyobb koncentrációban lesz jelen, mint a fázisok belsejében. Ennek következménye, hogy egy koncentráltabb „mikrogél” alakul ki közvetlenül a cseppen körül. A cseppektől távolabbi helyek polímerben szegényebbek lesznek. Ha a feltételezésünk igaz, az emulziók összefüggő külső vízfázisában kétféle módon kötött vizet kell találnunk:

1. A cseppek körüli polímeric cseppen által kötött vizet (gőzvízet);
2. A cseppektől távolabbi területeken lévő, a polímerhez nem kötött víz (tölö vízet).

A felszínlárd krémrendszerekben jelenlévő víz kötött-ségével sok szerző foglalkozott [-17]. Ezek részletes tanulmányozása több szempontból is fontos, mivel dermatológiai készítményekkel kapcsolatban összetűzése hozható a hidratáló képességével, valamint a rendszerekből a víz párolgási sebes-ségével, tehát a hűtőhatással. Amennyiben vízeldekony 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 polímer 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 egyszerű csúcsot mutat. Ezzel szemben a gél-emulziók esetében két lépcső különül el a TG görbéből és a dTG görbéből is két éles csúcs jelentkezik. Ez alátámasztja azon feltételezésünket, miszerint a gél-emulziók 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 környékének mikrogélhez kötve), amit a dTG görbe második csúcsa reprezentál 140 °C-nál. Ezzel szemben a hidrogélekkel ez a differenciáltság nem figyelhető meg.

Összehasonlíthatja a gél-emulziók és az olajat nem tartalmazó gélek párolgási sebességét, a várfok megfelelően a gélek ből a víz párolgása sokkal erőteljesebb volt. E jelenlős többek között azzal is magyarázható, hogy melegítés hatására az olaj kíválék

![Diagram](image-url)
a rendszerből és a minta te-
tején összefüggő olajréteget
alkot, ami a későbbiek folya-
mán akadályozni fogja a víz
párolgását. A 9. ábrán jól látható,
hogy a párolgási sebességgel
polímer mennyiségével
folyamatosan csökken, tehát
a víz nagyobb mértékben
hidrát burok formájában van jelen. Ez alól kivételének tű-
nik a PTR1-et tartalmazó hid-
rogél-sorozat, itt a párolgási
sebesség állandó, nem válto-
zik a polímer koncentráció
függvényében. Ezekben a
rendszerben valószínűleg
a polímer tartalma növeve
nincs a víz-polímer kölcsön-
hatás növekszik, hanem a
polímer láncok közötti köl-
csönhatások lesznek intenzí-
vebbek. Ezzel szemben, ha
emulziós rendszerben alk-
lámpázzuk a PTR1-et, a pá-
rolgási sebesség csökkenést
mutat a polímer mennyiség-
gének növekedésével.

Reológiai vizsgálatok

Régóta ismert, hogy a mak-
romolekulás térhálóknak bi-
zonyos nyírös feszültség tar-
mányban elasztikus jellegük
van, tehát mind az elasztikus,
mind a viszkózus jelleg
megtalálható. Ezt az elasztikus
jelleget kvantitatívan az
oszcillációs reométerek ké-
pesk meghatározni. A készülekh
fejének folyamatos
oszcilláló mozgásával a minta-
tára időben szinuszosan vál-
tozó nyíróáramot adunk, V_0
amplitúdóval, és detektáljuk
a rendszerben ébredő deformációs
feszültséget, ennek
amplitudóját V_0. A minta által
adott válasz szintén szinu-

9. ábra: A polímer emulziók tartalmazó emulziók és
gélek víztartalmának párolgási sebessége.
szos jellegű lesz, de a két hullám között általában fáziskésés (δ) lép fel. Ideálisnak rugalmas testek esetében ez 0°, ideális viszkózusoknál 90°, réális rendszerek fáziskésése 0° és 90° közötti érték. A két hullám amplitudójára arányból és fáziskéséséből származtatjuk a reológiai jellemzőket [18].

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

\[ G' = \tau_0 / \eta_0 \cdot \cos(\delta); \text{[Pa]} \] (1)

A vesztési modulusz (G'') a rendszer viszközus jellegét mutatja meg:

\[ G'' = \tau_0 / \eta_0 \cdot \sin(\delta); \text{[Pa]} \] (2)

A gél-emulziók és az oltakat nem tartalmazó gélk reológiai tulajdonságait összevetve megállapítható, hogy az azonos polímer tartalmú emulziók és hidrogé rendszerek viszkozitás értékei között nincs jelentős különbség csak viszonylag nagy (1,00% körüli) polímer tartalomnál (10. ábra). A rendszerek viszkozitása és a polímer tartalom között exponenciális összefüggést tapasztalhátunk (11. ábra):

\[ \eta = \eta_0 \cdot \exp(m \cdot c) \] (3)

ahol:
- c = polímer koncentráció,
- \( \eta_0 = 0 \) polímer-konzentrációra extrapolált viszkozitás
- \( m = a \) függvény állandója, egy-ségyi koncentráció-növekedéshoz tartozó viszkozitás-növekedés.

Hasonló összefüggéssel jellemezhető a viszkozitás vál-
tozása az olajkonzentráció függvényében. Ebben az esetben \( \eta_0 \) a 0 olajkonzentrációnál extrapoliált viszkozitást jelenti.

Meghatároztuk a rendszerök veszteségi tangensét \((\tan \delta)\) \((1=1\text{Hz értéknél})\) a polímer tartalom függvényében \((12. \text{ ábra})\). A veszteségi tangens az alábbi egyenlet alapján számolható ki:

\[
\tan \delta = \frac{G''}{G'}
\]  

ahol \(G''\) = veszteségi modulusz, \(G'\) = tárolási modulusz.

Abban az esetben, ha ez az érték 1-nél kisebb, a minatban az elasztikus jelleg dominál, és miről kisebb értéket vesz fel, annál jellemzőbb az elaszticitás. Az ábrák on jól látható, hogy kis polímer tartalommal az emulziók mutatnak erőteljesebb elasztikus jellegét, míg nagy koncentrációknál a gélek.

Emulziók esetében a víz mennyisége relatív kevesebb a díszpergált olaj miatt, ennek következtében sokkal több kölcsönhatás tud ki alakulni a polímer láncok között, mint amennyit a polímer létre tud hozni relatív nagyobb mennyiségű vízes fázisban. Nagyobb polímer tartalommal viszont a nagyobb térfogati vízfázis már nem okoz ilyen jellegű különbséget, viszont a díszperz fázis jelenléte miatt az emulziós rendszerek elaszticitása kisebb lesz, mint az ugyanolyan polímer-konzentrációjú gélek elaszticitása. Az a polímer-konzentráció, amely felett a gélek már nagyobb elaszticitással rendelkeznek.
mint az emulziók, a PTR1 esetében kisebb polimer-koncentrációknak felel meg, mint a PTR2 esetében. Ennek őka, hogy a PTR1 nagyobb polimerizációs fokú polimer, így a polimer lánkok között már kisebb koncentrációban is jelentős mennyiséggel kötődik kialakulni, szemben a kisebb polimerizációs fokú emulgenssel. Növelve az emulziók olajkoncentrációját, PTR1 esetében a veszteségi tangens csekély csökkentést, míg a PTR2-t tartalmazó mintánál kismértékű növekedést mutat, tehát a PTR1 tartalmú minták elaszticitása fokozódik az olajkoncentrációval, míg a PTR2-t tartalmazóké csökken. (13. ábra)

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 monodiszperse jelleget mutat (14. ábra). Növelve az olajkoncentrációt az eloszlási görbe egyre szélesebb lesz, egyre inkább heterodiszperesz eloszlású rendszereket kapunk, majd 30% feletti koncentrációról a görbe ismét eles csúcsot ad, tehát egyre inkább homogén eloszlásuk lesznek az emulziók (15. ábra). Az átlagos cseppméret, mint az eloszlási görbék alapján várható volt, expozenciálisan csökkent a polimer emulgens növekedésével, míg növekvő olajkoncentráció mellett a cseppméret kezdetben rövid, maximum értéket vesz fel 30%-nál majd ismételten csökkent (16-17. ábra).

Megbeszélés

Az elvégzett preformulálási vizsgálatok alapján megállapítottuk a következőket:

1. A Pemulen TR1 és TR2 polimer emulgerések stabilizáló hatásukat sztérikus gátlással fejtik ki, azaz az olaj fázis mechanikailag tartják zárra, felületi feszültség csökkentő hatásuk nem jelentős.
2. A polímerek vízzel rosszul nedvesednek.
3. Desztilált víz hatására mindkét polimer igen lassan duzzadt és megkőtött víz mennyisége jellemzően kevesebb volt, mint a hidroksi-etil celulózé.
4. A Pemulen TR1 polimer képes volt 50%-nyi olaj stabilizálására is, a Pemulen TR2 emulgens pedig alkalmazásban bizonyult 70%-os olajmennyiség emulgeálására.

A termoanalitikai vizsgálatok alapján elmondhatjuk, hogy a gél-emulziók a hagyományos gél-
szerkezettől jelentősen eltérnek, kimutattunk mikrogéle
hez kötött vizet és szabad vizet. Megállapítottuk át is, hogy a nagyobb polímer tartalom növelte a víz kötött
séget, ami a kisebb párolgási sebességben nyilvánult meg.

A reológiai mérések alapján tisztázottuk, hogy a rend-
szerkezeti viszkozitása és a polímer valamint olaj tartalom között exponenciális össze-
függés áll fenn. Az oszcillációs mérések mértéktől kiderült,
hogy kis polímer tartalommal az ugyanolyan polímer tar-
talmú gél-emulzióknak erőteljesebb elasztikus jellegűek
van mint a hidrogéleknél. Nagyobb koncentrációk esetén ez a különbség nem áll fenn, a hidrogélek elasztikus
jelleg vállik nagyobbá.

A gél-emulziók csepmpé-
rete exponenciálisan csökkent
a polímer emulgens mennyi-
ségével, az eloszlási függy-
vény pedig egyre inkább balra, a kis cseppek tartomá-
nya felé tolódott. Az olajtar-
talma változata, maximális volt az átlagos csepmpéret
30%-os olajtartalomnál, ugyan-
akkor az eloszlási függvény

is itt mutatott leginkább po-
lidiszperz jellegét.

A komplex fizikai kémiai
vizsgálatok alapján megálla-
pítjuk, hogy a polímer
emulgenst tartalmazó rendszer
szerkezete összetett.
Az emulgens mennyiségének változtatása jelentős mértékben kihat a rendszerek
reológiai tulajdonságaira, mivel nemcsak a csepmpéret és a határfeletti változására kell számítanunk, hanem polímer hatására a külső fázis

![Diagram](image1)

15. ábra: A különrből olajtartalmú emulziók csepmpéret eloszlása
(polímer emulgens koncentrációja 0,10%)

![Diagram](image2)

16. ábra: Az átlagos csepmpéret változása a polímer emulgens koncentrációjának
(olajkonzentráció 20%)
szerkezet is módosul: differenciálódik a gélszerkezet és a víz kötődése is változik. Mindezek ismételten az ilyen típusú gőz-emulziók szerkezetének vizsgálatakor érdemes és fontos egy másik méltott vizsgálati érdekel minden emulzió makroszerkezetét (szeppméret, cseppméret-eloszlását), mind a mikroszerkezetét (vízkötői mechanizmusok, gélszerkezet).

IRODALOM


[Erkezett 2008. 02. 22.]
II.
Gél-emulziós rendszerek II. rész
Stabilitás

BUDAI SZABOLCS, SZÚCS MÁRIA, ERŐS ISTVÁN

Summary

Budai, Sz., Szucs, M., Eros, 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, solvation of polymer chains, immobilized water in microgel, rate of water evaporation) were characteristically changed during storage. These processes could be related to the sustained hydration of polymer chains.

Bevezetés

A gyögyírászetben használatos emulziók kutatásaiban különös figyelmet kell fordítani a rendszerek stabilitására és stabilizálására [1]. A stabilitás jelenségeken értelmezéskor a képződés feltételeiből kell kiindulnunk. Hosszabb-rövidebb ideig stabilis rendszer képződésének 3 előfeltétele van [2]:
1. A két fázis nem elégedhető egymással;
2. Elegendő nagyságú legyen az emulgésiállás munka, illetve
3. Emulgensből vagy emulgens keverékből megfelelő határfületű réteg alakuljon ki.

Az emulziók „meta-stabil” rendszerekn ek tekintetben, így kezdetei szerkezetük változik tárolás vagy szállítás folyamán, hőmérséklet, mechanikai terhelés, fizikai behatás vagy biológiai hatás következetében [3]. Számos olyan folyamat ismeretes, amely az emulzió szerkezet megtételehez vezet. Megkülönböztetünk reverzibilis és irreverzibilis mechanizmusokat. A reverzibilis változások közé tartozik a föléződés, ülepedés, fokkaludás, az irreverzibilis jelenségek közé sorolható a koaguláció és az úgynevezett „Osmwald ripening”.

Föléződés és ülepedés folyamán a gravitáció hatására a dízsgergált csepp felfelé vagy lefelé mozognak. Főként hig, kis diszperz fázist tartalmazó rendszeren kifinomítva meg a Stokes törvénynek megfelelően, ha jelentős státiszkiségülnéss van a két fázis között. Fluktatációval akkor beszélünk, ha a vonzó erők nagyrésben a csepp közötti taszító erőknél, így a csepp ősszetapadnak a gyenge van der Waals kölcsönhatások következtében. A cseppkoaguláció folyamán az emulgilált cseppként összeolynak a köztük lévő filmréteg elvékonyodása és megszűnése következtében. Így
végeredményként két vagy több kisebb cseppből egy nagyobb csepp keletkezik. Az úgynevezett „Östwald ripening” polidispersz emulziókra jellemző, amikor is a nagyobb cseppek fokozatosan növekednek, a kisebb cseppek pedig egyre kisebbek lesznek. A jelenség a különböző méretű cseppek oldókonyság különbségéből adódik [1].

Két i. módszer ismeretes az emulziók stabilizálására:
1. az elektrostatikus stabilizálás, főként kis molekulájú felületaktív anyagokkal,
2. a szterikus stabilizálás.

Ez utóbbi esetében felületaktív polimereket alkalmaznak, melyek kötődése a határ felülethez gyengébb, mint egy kis molekulájú felületaktív anyagé, de hatásosnak bizonyulnak elektrolit tartalmú közegben, illetve magasabb hőmérsékleten is [4]. A kozmetikai és gyógyszeriparban is az utóbbi években a hagyományos kis molekulájú 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 emulgonek 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 stabilizációs vizsgálatára az egyik legtöbb
információit adó módszer-csoport a reológiai mérések. Ezek a vizsgálatok örzséke- nyen jelzik az emulziók szerkezetében történő változásokat, így például a koaguláció, a flokkuláció, vagy akár a főlöződést, sedimentációt, mivel ezek a folyamatok mindig jellemző viszkozitás-változásai járnak [9-11]. Termoanalitikai vizsgálatokkal lehet tanulmányozni a víz kötődésének változását, illetve a gélszerkezet változását [12], valamint optikai módsze- rek segítségével detektáltuk a csepplméret, csepplméret-eloszlás alakulását az eltartás során.

Anyagok és módszerek

Anyagok

Polimer emulgenschként a Pemulen TR1 és TR2-4 (gyár- tó: Noveon AG) alkalmaz- tuk. A gél emulziók vizsgálása a gyógyszerkőnyvi tisztított víz (Aqua purificata, Ph. Hg. VIII.), olajzáVisz a Miglyol 812 (gyártó: Sasol GmbH, Németország) volt. Semlegesítő komponensként gyógyszerkőnyvi trolamint (Ph. Hg. VIII.) használtunk.

Reológiai vizsgálatok

A reológiai méréseket HA AKE RheoStress 1 (HAAKE GmbH, Németország) kúp- lap geometriájú készülékként végeztük szobahőmérsékleten. Szabályozott nyúlást sebesség-gradiens mellett felvettük a minták folyás- és viszkozitás-görbét a felszalló ágt 0,1 s⁻¹-től 100 s⁻¹-ig, a le- szálló ágt 100 s⁻¹-tól 0,1 s⁻¹-ig.

4. ábra: A poliorm emulenst tartalmazó emulziók párolgási sebességek változása az eltartás folyamán

5/a. ábra: A különböző poliorm-tartalmú emulziók viszkozitásának változása az eltartás során (duftartalom 20%)
Meghatároztuk az emulziók és gélek tárolási és veszteségi modulszát a frekvencia függvényében (0,1 és 100 Hz között).

**Termoanálitikai mérések**

A termoanálitikai méréseket Derivatograph C (MOM, Magyarország) készülékel végzettük. 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 (tőmegcsökkenés az idő függvényében) és dTG (TG idő szerinti deriválja) görbét. A TG görbének egyenes szakaszára regressziós egyenest illesztettünk, melynek meredekségből következtettünk a minták párolgási sebességre.

**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**

**Termoanálitikai vizsgálatok**

Vizet (első csúcscs a dTG görbén) és mikrogélehez kötött vizet (második csúcscs a dTG görbén). Meghatározuk második csúcscsokhoz tartozó viz mennyiségét, azaz a kötött viz mennyiségét, és a teljes vizmennyiség 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 tartalmazókról. Tehát a PTR1 emulziók mikrogél rendszere zsugorodott az eltartás során, ellentétben a PTR2-t tartalmazók, ahol a mikrogél duzzadása volt megfigyelhető.

Az eltartási idő alatt a polímer parólgási sebessége fokozatosan csökkent az eltartás során (4. ábra). Ez jelenségnek az a magyarázata, hogy a polimert fokozatosan duzzadt a vízzel való érintkezéskor és a hidrát burok nem pillanatszerűen, hanem az idő függvényében fokozatosan alakult ki. Így a hidrátció növekedésével egyre csökken a vízvesztés sebessége. A sebességi állandókat az idő függvényében ábrázolva hatványfüggvényen leírható összefüggést kaptunk.

\[ \Delta m/t = A_0 e^{-kt} \]  
(1)

ahol 
\[ \Delta m/t \] = parólgási sebesség.
A
\( A_0 \) = kezdeti értékehez tartozó sebesség,
\( t \) = az idő,
\( k \) = sebességi állandó.

Reológiai vizsgálatok

A gél-emulziók viszkozitásának jellemzésére a 0 nyírású sebesség-gradientre extra-polált viszkozitást alkalmaztuk és ennek változását vizsgáltuk az eltartás során. A minták elasztikusságát a veszteségi tangens (tan\( \delta \)) segítségével elemeztük.

\[
\text{tan}\delta = \frac{G''}{G'}
\]

ahol
\( G'' \) = veszteségi modulusz,
\( G' \) = tárolási modulusz.

Minél kisebb értékkel vesz fel tan\( \delta \), annál jellemzőbb és kifejezetten az emulziók elaszticitása.

Azokban a mintákból, ahol kis olajkoncentráció (20%) mellett változtattuk a polímer koncentrációját, kismértékű viszkozitás növekedés volt tapasztalható főként a nagy polímer tartalmú minták (5. ábra). Ez a jelenség jól magyarázható ugyanazon minták hidratációnak fokozódásával, amit a 4. ábra szemlélt. Azokban az összetételekben viszont, ahol kis polímer-tartalom (0,10%) mellett növeltük az olaj koncentrációját (6. ábra), a P12-k 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 gél szerkezet vizsgálatkor (3. ábra).

Cseppméret-analízis

A mikroszkópos vizsgálatokkal a koaguláció és az „Ostwald ripenning” jelen sége követethető nyomon, mindkettő esetén cseppmé ret növekedést tapasztaltunk. Azokban a mintákból, ahol állandó olajzáralom mellett változott a polimer emulgencsennyiségét, 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% olaj tartalmazó rendszerekből 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).

Megbeszélés

A stabilitási vizsgálatok során megállapítást nyert, hogy a polimer emulgenc tartalmú emulziók esetében két, egymás mellett jelenlevő szerkezeti formáról beszélhetünk. A mikroszerkezet jelenti az emulziót olajcsomag számát, méretét és méret szerinti eloszlását. Ez nem változott jelentősen az eltartás során. A mikroszerkezet jelenti a polimer láncok szolvatációját. (Pontosabban és szabályosabban lenne heterogén szerkezetről és kolloid szerkezetről beszélni, mivel az emulziót cseppeknak 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 megelő zendi, megtartjuk a makro- és mikrostruktúra fogalmát.)

A hidratáció megismeréséhez nyújtottak adatokat a TG-görcbék. A TG-görcbék megeredése hatványfüggvény szerint csökken 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 reolológiai vizsgálatok is mikroszerkezetben változásokat jeleznek az eltartás folyamán. Főként viszkozitás- és elaszticitás-csökkentésről beszélhetünk, ami azért is figyelemre méltó, mivel főként azokban az összetételekben jelentkezett ez a csökkenés, ahol az átlagos cseppméret (mint egyik legjöbb befolyásolója ezen faktoroknak) egyáltalán nem változott, valamint a hidratáció fokozódásával éppen az elaszticitás és a viszkozitás növekedése lenne várattható. Ezt az ellentmondást a hidratáció
makro- és mikroszerkezetben beültött ellentétes szerepével lehet értelmezni. A hidratáció növeli a folyási egységek törzsgatálati, ami viszkozitás növekedésében jut kifejezésre. Viszont a hidratáció során a polimer láncokban eltávolodnak egy mástól 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.

**IRODALOM**


[Érkezett: 2008. 02. 22.]
III.
Mucoadhesive behaviour of emulsions containing polymeric emulsifier

Mária Szűcsa, Giuseppina Sandrib, M. Cristina Bonferonib, Carla M. Caramella, Patrizia Vaghi, Piroska Szabó-Revézs, István Erősa,*

a University of Szeged, Faculty of Pharmacy, Department of Pharmaceutical Technology, Eötvös u. 6, Szeged H-6720, Hungary
b University of Pavia, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Valerio Taramelli 12, 27100 Pavia, Italy
c University of Pavia, Centro Studi Farmaceutici, Via Bassi 21, 27100 Pavia, Italy

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 bioadhesiveness, controlled drug release from the dosage form is also desirable. Penugel TTN and Penugel TTR are cross-linked block copolymers of poly(acrylic acid) and hydrophilic long chain methacrylates. They are able to stabilize oil emulsions because of their short lipophilic part integrates into the oil droplets whilst their long hydrophilic part forms a microgel 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 mucoadhesion measurements were performed by tensile test and the bioadhesive bond between the polymer emulsifier and mucin was visualised 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.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Bioadhesion has been defined as the attachment of synthetic or biological macromolecules to a biological tissue (Peppas and Bini, 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 bioadhesiveness, 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 is required to obtain adhesion: (i) sufficient group forming hydrogen bonds (hydroxy or carboxyl groups), (ii) anionic surface charge, (iii) high molecular weight, (iv) high chain flexibility, and (v) surface tensions...
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 fulfill 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 mucous, (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, nontoxic oligomers or monomers (Hunziger 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; Coulemaire and Ludwig, 2002) or tablets (Park and Munday, 2002; Ponchet et al., 1997). In case of poly(acrylic acid), 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) is the pH. Protonated and hydrated carboxylic groups are needed for the interaction between the mucin glycoproteins and acrylics, but extreme swelling may decrease their adhesivity. Therefore an ideal pH range for bioadhesive devices is around pH 4–6 or around the pKa 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 dialylated 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 polyacrylic acids–cysteine conjugates were synthetized to improve the bioadhesive property of the dosage form by covalent bond with the cysteine of the mucin glycoprotein (Remkop-Schröck et al., 2006; Calori et al., 2004; Leitner et al., 2002; Marschitz and Remkop-Schröck, 2002).

Femulen TR1 and Femulen TR2 are cross-linked block (copolymers of poly(acrylic acid) and hydrophobic long-chain methacrylates acrylate/C10–C30 alkyl-acrylate crosspolymer (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 (Noven, 2003) the cause of which is on the one hand Femulenes, being micromolecules, do not penetrate into the biological tissue, on the other hand they are used in a very low concentration (less than 1%, 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) and the mucin glycoproteins. Although the most widely used methods are mechanical tests such as the rheological (Hagerström and Edsman, 2003; Hassan and Galle, 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), 1H 13C 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 bioadhesive 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 polymeric 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 mucous 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

Femulen TR1 (PTF1) and Femulen TR2 (PTF2) (Noven, USA) were used as primary emulsifier, viscosity enhancing agent and bioadhesive. Femulen TR1 (PTF1) is the higher polymerization degree polymer whilst Femulen TR2 (PTF2) is the lower polymerization degree polymer. Coemulsifiers, Symperonic PE10 (Solvay, Germany) and unigem (UK) was added to certain emulsions to influence the microstructure. The oil phase was Miglyol812 (Sasol Germany GmbH, Germany) and the aqueous phase was purified water (Ph.Eur.5). The neutralizing agent was triolein (Ph.Eur.5).

In the fluorescence samples rhodamine B (Fluka, Milan) was used as fluorophore. Mucin (from bovine submaxillary glands, Type II (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
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 measurement.

2.2 Emulsions preparation

The Fernulensa were added to purified water containing trolamine and preservative. The pH of the gel was 5.5-5.5. After 24 h the oil was added to this gel whilst the sample was stirred with mixer (MLW ER 20, 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 MCM Derivatograph-C (MCM 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 DTG 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 25 mm, cone angle 1° and 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 δ = 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>PTR1 (%)</th>
<th>PTR2 (%)</th>
<th>ST01 (%)</th>
<th>Oil (%)</th>
<th>Water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>0.4</td>
<td>-</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>0.8</td>
<td>-</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>11</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>13</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>14</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>15</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>16</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>18</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>19</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>22</td>
<td>-</td>
<td>0.1</td>
<td>0.001</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>23</td>
<td>0.1</td>
<td>-</td>
<td>0.01</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>24</td>
<td>0.1</td>
<td>-</td>
<td>0.10</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>25</td>
<td>0.1</td>
<td>-</td>
<td>0.50</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>26</td>
<td>0.1</td>
<td>-</td>
<td>1.00</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>27</td>
<td>0.1</td>
<td>-</td>
<td>1.01</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

* Together the water, the polymeric emulsifier and the triolamine.
+ Together the oil and the ST01.

2.5. Tensile test

The mucroadhesive properties of the gel-emulsions were investigated by TA-XT2 Plus Texture Analyser (Stable Micro Systems, Inco, 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 500 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 DMIRE inverted microscope and using a 40× 1.25 NA oil immersion objective. The excitation source was a Green HeNe-Neon (λex = 543 nm) laser, the fluorescence emission of rhodamine B was recorded between 580 and 600 nm. Rhodamine B (0.002%, w/w) was suspended in the oil phase and the oil was added to the water phase. 8.0%

![Fig. 3 - The dTG curves of the emulsions at different polymeric emulsifier concentration.](image)

(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.

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; Paranal et al., 1997; Körny et al., 2003) free (or built) 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 further from the oil droplets. If this concentration difference is
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 (0.4-0.6%, 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 to 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. In this last case the different water phases are not separated.

Fig. 4 – TG curves of the emulsions at different oil 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%, 0.1%, 0.5%, and 1.0%, w/w). On the basis of our assumption this coemulsifier (SO1) 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 (FTR1 (square) and FTR2 (triangle)).

Fig. 8 – Variation of viscosity (A), the storage (B), loss (C) moduli (A) and loss tangent at 1.0 Hz (B) in function of co-emulsifier (S101) concentration.

Fig. 9 – Correlation between the micro-gel water and the viscosity (B)/storage modulus (C) of the emulsions containing FTR1 and S101 at constant water oil ratio.

where $G'$ is the storage modulus, $G''$ is the loss modulus. The smaller $\tan \delta$ (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

\[
\tan \delta = \frac{G''}{G'}
\]
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.

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 Fumulina and non-ionic emulsifier. They have established an addition of mixed emulsifier significantly modified the rheological characteristics of the emulsions (Saric 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 polymer 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 TE curves, the coemulsifier inhibited the accumulation of polymer 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) PTE2 emulsion-8.0% (w/w) mucin mixtures (from the first row to fifth row the polymer concentrations are the following: 0.1%, 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.

\[ \eta = 4.45c^{-2.29} \quad (R^2 = 0.980) \quad (2) \]

\[ G' = 17.24c^{-2.51} \quad (R^2 = 0.851) \quad (3) \]

where \( \eta \) 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 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.

PFR1 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 mucosa. Contrarily, emulsions containing PFR2 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 PFR2, a considerable decrease of the values was observed (Fig. 11B). In emulsions prepared with PFR2 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 mucosa (Foncet 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 Pemulen is modified polyacrylic acids, 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 carbonyl groups of the polyacrylic 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. 12, 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 PFR1 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 bioadhesi-
ity of the samples where the coherent gel structure had not built up.

Acknowledgements

We thank Noveon, Inc. for Penulen TR1 and TR2 and the Sasso Germany GmbH for the Miglycol 812.

REFERENCES


IV.
Thermoanalytical and Microscopical Investigation of the Microstructure of Emulsions Containing Polymeric Emulsifier

Mária Szűcs¹, Patrizia Vaghi², Giuseppina Sandri³, M. Cristina Bonferroni³, Carla M. Caramella³, Piroška Szabó-Révész¹ and I. Eőrő¹*

¹University of Szeged, Faculty of Pharmacy, Department of Pharmaceutical Technology, Eötvös u. 6, 6720 Szeged, Hungary
²University of Pavia, Centro Grandi Strumenti, Via Bassi 21, 27100 Pavia, Italy
³University of Pavia, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Viale Taramelli 12, 27100 Pavia, Italy

Polymeric emulsifiers provide exceptional stability to oil-in-water, water-in-oil or multiple emulsions by their steric stabilization. Polymers 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 microgel 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 co-emulsifier 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. Penamuls (CTFA/INCI designation: Acrylate/C10-C30 alkyl-acrylate cross polymer) belong to this group. One of their most important properties resides in their effectiveness in stabilizing o/w type emulsions even for very low Penamul concentrations (0.1–0.4 mass/mass%). The short lipophilic part of Penamuls is integrated into the oil droplets while the long hydrophilic part of the molecules forms a microgel around the droplet so this microgel 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 Penamuls 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

* Author for correspondence: eros@pharm.u-szeged.hu

1388-4150/20/09
© 2008 Akadémiai Kiadó, Budapest
Springer, Dordrecht, The Netherlands
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 (Novone, Pemulen TR2). Coemulsifier was PEO-PPG-PEO triblock polymer (Synperonic PEI 101; SI101, 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). 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 rph, 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 DMRBE inverted microscope and using a 40×/1.25 N.A. oil immersion objective. The excitation source was a Green Helio-Neon (λex=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, indicating...
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 macro-emulsion system.) 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).

Conclusions

Gel-emulsions containing Permulen 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 coemulsifier 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.

Acknowledgements

We thank Noveon, Inc. for the sample (Permulen TR2).

References

10. S. A. Vanapalli, J. Palanuwech and J. N. Coupland, 
11. J. I. Urguen, L. Bremer, V. Mathot and G. Groeninckx, 
14. S. Kalonimou, K. Helenius and J. Ylinu, Pharmazie, 
15. A. Feher, E. Csanyi, L. Csoka, A. Kovacs and I. Erös, 
16. V. L. Peramal, S. Tamburic and D. Q. M. Craig, 
17. M. Konya, M. Sorrenti, F. Ferrari, S. Rossi, I. Csoka, 
18. S. Kersten, C. Mugnier, B. S. Murray and E. Dickinson, 
19. S. Kersten, B. S. Murray and E. Dickinson, 
20. I. Heertje, J. Nederlof, H. A. C. M. Hendrickx and 
and E. H. Lucassen-Reynders, Food Sci. Technol., 
22. J. Bruje, S. F. Edwars, I. Hopkinson and H. A. Makse, 
23. Z. S. Guan, Y. Zhang, Q. Zhang and D. X. Li, 

Received: December 3, 2007
Accepted: March 10, 2008
OnlineFirst: June 25, 2008

DOI: 10.1007/s10973-007-8907-9