Ph D Thesis

Optimization and evaluation of topical used pharmaceutical organogels

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TABLE OF CONTENTS

Table of contents 1

Publications related to the subject of the thesis 2

1 Objectives 3

2 Survay of literatures 4

2.1 The organogelators and the main groups of organogels 4

2.1.1 Anhydrous organogels 4

2.1.2 Microemulsion-based organogels 7

2.2 Pharmaceutical application of the organogels 8

2.2.1 Topical effect on the skin 8

2.2.2 Transdermal drug delivery 8

2.2.3 Ophtalmic vehicle 9

2.2.4 Delivery for vaccines and antigens 9

2.2.5 Rectal sustained-release preparation 9

3 Materials and methods 10

3.1 Materials 10

3.2 Methods 10

3.2.1 Organogeling ability of different surface active agents 10

3.2.2 Preparation of the organogels 10

3.2.3 Optical microscopy 10

3.2.4 Rheological measurements 12

3.2.5 Investigation of spreadability 12

3.2.6 Oil number 12

3.2.7 Determination of the wetting conditions 13

3.2.8 Sensory analysis 13

3.2.9 Determination of solubility 13

3.2.10 Determination of partition coefficient and penetration coefficient 14

3.2.11 *In vitro* penetration study 14

3.2.12 *In vivo* anti-inflammatory effect 14

4 Results and discussion 16

4.1 Development of novel organogel compositions 16

4.2 The gel formation 19

4.3 Sensory evaluation 22

4.3.1 Texture development of the GMSOs 22

4.3.2 Organogel category review 25

4.3.3 Attribute diagnostic 27

4.4 Description of the structure 28

4.4.1 Microscopic observations 28

4.4.2 Phenomenological and microstructural approach by rheology 29

4.5 Description of the stability 31

4.5.1 Thermal stability 31

4.5.2 Mechanical stability 33

4.5.3 Changes of the structure upon storage 34

4.6 Drug release and penetration profile 36

4.6.1 *In vitro* approach for prediction of skin penetration 36

4.6.2 *In vivo* anti-inflammatiory 41

4.6.3 *In vitro – in vivo* correlation 44

5 Summary 45

Acknowledgements 47

6 References 48

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

Papers

I. **T Pénzes**, F Ferrari, S Rossi, C Caramella, I Csóka, I Erős: Rheological characterization of organogels based on glyceryl monostearates. *www.rheofuture2002.de*

II. **Pénzes T**, Csóka I, Erős I: Gyógyászati és kozmetikai organogélek típusai és jellemzőik. *Olaj, szappan, kozmetika* 2003 (**52**) 45–49.

III. **T Pénzes**, I Csóka, I Erős: Rheological analysis of the structural properties effecting the percutaneous absorption and stability in pharmaceutical organogels. *Rheol Acta* (2004) 43: 457-463.

IV. **T Pénzes**, G Blazsó, Z Aigner, Gy Falkay, I Erős: Topical absorption of piroxicam from organogels - *in vitro* and *in vivo* correlations. *Int. J. Pharm.* (accepted for publication: March 2005)

Abstracts

- **Pénzes T**, Erős I: Reológiai módszerek alkalmazása az organogélek kutatásának területén, *Kedvessy György Emlékülés*, Abstract p. 114-118 (2004)
- **Pénzes T**, Blazsó G, Sipos P, Falkay Gy, Erős I: Piroxicam abszorpció organogélekből – *in vitro*/*in vivo* korreláció*, X. Farmakokinetika és Gyógyszermetabolizmus Szimpózium*, Abstract p. 91 (2004)
- **T Pénzes**, I Almeida, F Bahia, I Csóka, I Erős: Lipophilic surfactant gels as novel alternative to hydrocarbon bases, *Proc. 5th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology*, p. 419-420 (2004)
- **T Pénzes**, I Csóka, I Erős: Effect of structural properties of organogels on percutaneous absorption and stability *Proc. Annual European Rheological Conference*, p. 39 (2003)
- **Pénzes T**, Csóka I, Erős I: Lipofil emulgensgélek – új típusú organogélek a dermális terápiában, *Congressus Pharmaceuticus Hungaricus XII.*, Abstract p. 85 (2003)
- **Pénzes T**, Csóka I, Erős I: Emulgens tartalmú organogélek, *XIV. Országos Gyógyszertechnológiai Konferencia*, Abstract p. 37 (2002)
- **T Pénzes**, I Csóka, I Erős: Organogels designed for pharmaceutical use, *Proc. 4th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology*, p. 1243-1244 (2002)

1 OBJECTIVES

A great variety of surfactant self-assemblies (Fig. 1) are known [1] and several of them provide drug delivery matrices [2-6]. However there are relatively few data on nonaqueous systems inspite of their potencial advantages as lipophilic drug deliveries. The surfactant organogels are promising for the research of dermatological vehicles with multifunctionality. The number of the ingredients could be reduced since surfactants both form a delivery matrix [7] and act as penetration enhancer [8-11]. The simple compositions have the advantages of easy preparation and less skin irritancy.

The investigation of the following phenomena were the objectives of my research:

- Development of novel surfactant organogels
- Investigation of the conditions and terms of the formation
- Texture optimization according to the consumer's requirements
- Description of the structure and the stability
- Investigation of the vehicle effect on the *in vitro* drug release
- Investigation of the vehicle effect on the drug bioavailability *in vivo*

The novel organogels were compared with some traditional organogels as regards to the above-mentioned viewpoints.

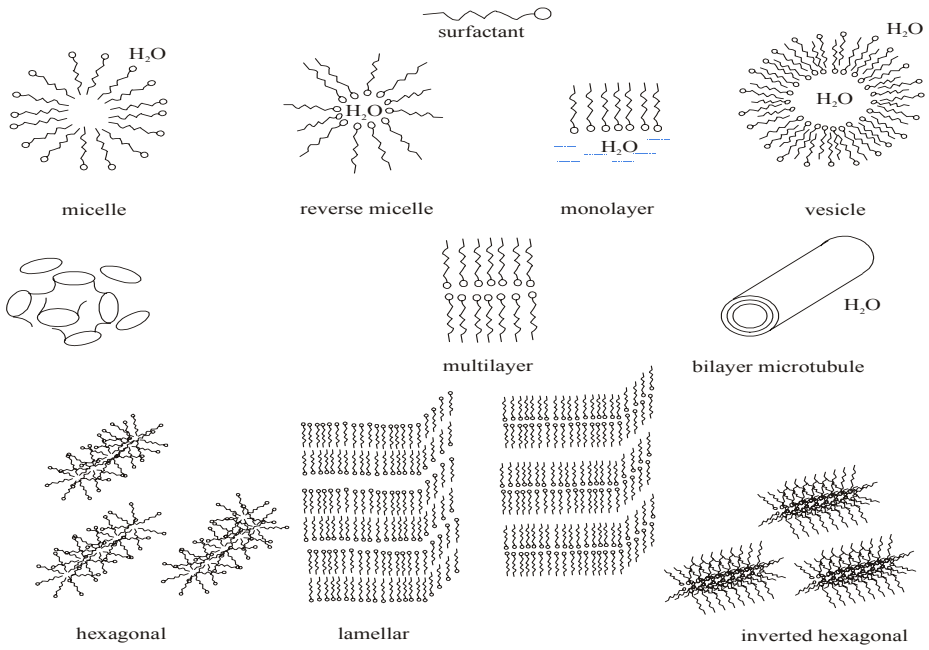


Figure 1. Schematic representation of surfactant self-assembly

2 SURVAY OF LITERATURES

Gels can be divided into two major groups of hydrogels and organogels as regards to the aqueous or nonaqueous nature of the liquid phase included [12]. Although there are certain differences between the term of organogel, oleogel and lipogel*, these names are usually used as synonyms indicating gels based on nonaqueous liquids.

2.1 Organogelators and main types of the organogels

2.1.1 Anhydrous organogels

2.1.1.1 Low molecular mass organic gelators (LMOGs)

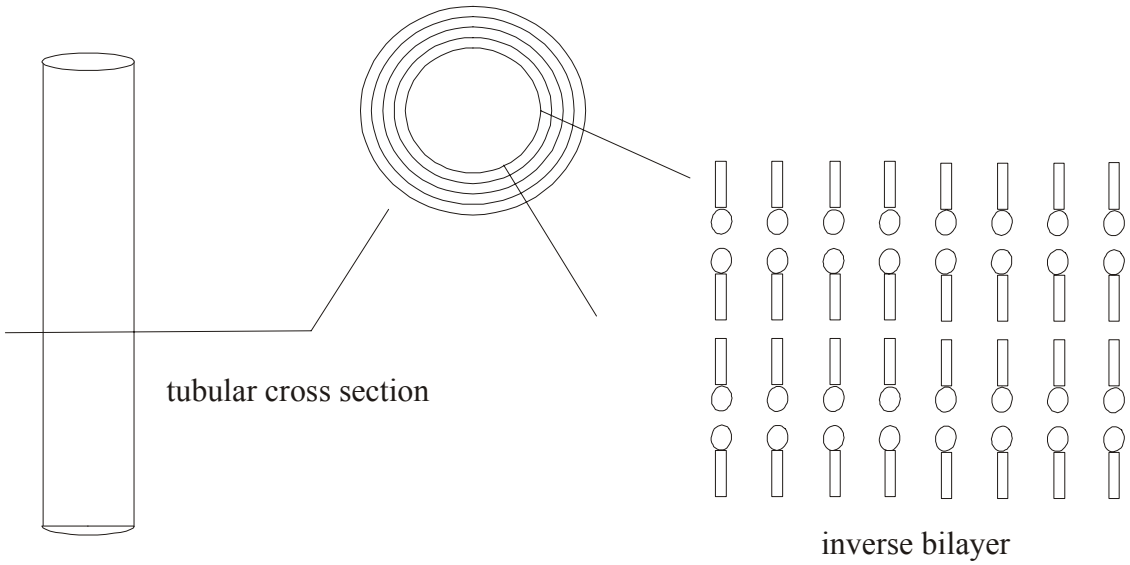
The members of this organogelator group show special variety as regards to their molecular structure (Table 1). They have the commonality of being applied usually ≤ 2% w/w. The LMOG assemblies can be stabilized by strong intermolecular forces such as hydrogen bonding (LMOGs with heteroatom), electrostatic attraction (LMOG salts), but London dispersion or van der Walls forces alone can be sufficient also (ALS). LMOG self-assemblies have forms of fibre, strand or tape, which are frequently crystalline. The LMOG organogels are thermoreversible and thixotropic [13].

The simplest members are the low molecular mass *n*-alkanes (C_n = 24 - 36) which can be found in the traditional pharmaceutical organogel bases where they build up or take part in the coherent gel structure (e.g. solid paraffin or mycrocrystalline paraffin in Paraffin Ointment, PhHgVII). In addition, branch-chain paraffins can also form coherent network in pharmaceutical organogels, e.g. the "fuzzy micelles" of White petrolatum [14].

Table 1. Low molecular mass organic gelators

Abbreviation	*Structure*
ALS	Basic components are an aromatic (A) and a steroidal group (S) with connecting atoms (L) [15, 16]
AL_2	Containing one aromatic and two linker groups
LS	Containing a "linking" chain and a steroidal group
S	Consisting of only a steroidal group
L	Alkyl chains with minimal functionalization

* **organogels**: gels based on nonaqueous dispersing phase; **oleogels**: gels containing inorganic colloidal gelators; **lipogels**: gels containing waxes, fatty alcohols and vegetable oils



Scheme 1. Schematic diagram of the amphiphil vesicules and cross section of the aggregates

2.1.1.2 Fatty acids and fatty acid esters

Gelation is achieved by dispersing the organogelator in hot nonaqueous solvent, which on cooling set to the gel state reversibly ("melt-type organogels"). Cooling the dispersion results in a reduced solubility of the gelator molecule in the dispersing phase, with a corresponding decrease in solvent–gelator affinity, causing the gelator self-assembly into vesicles. Once formed, the vesicles ("tubules") associate with others, and a three-dimensional network is formed which immobilizes the nonaqueous solvent. The tubules consist of concentric sheets of inverse bilayers (Scheme 2) [17, 18].

The above-mentioned gelation procedure was described in case of sorbitan fatty acid esters (Spans®) [19], and probably the same mechanism occurs with fatty acid esters of glycerol [20, 21] and 12-hydrostearic acid [22].

2.1.1.3 Synthetic polymers

Since the alkyl metacrylates are monomers including oleophilic group, their crosslinked polymer can interact with oils and thus result in organogels. Such organogelators are Eudragit®L and S (copolymers, anionic in character, consisting of methacrylic acid and methacrylic acid methyl ester). Eudragit® organogels based on polyhydric alcohols such as glycerol, propylene glycol and liquid polyethylene glycol were already reported [23]. They are chemical organogels and capable to absorb oil without the need for heat, and they have excellent shape holding capacity. In contrast with the hot melt-type organogelators, they are expensive.

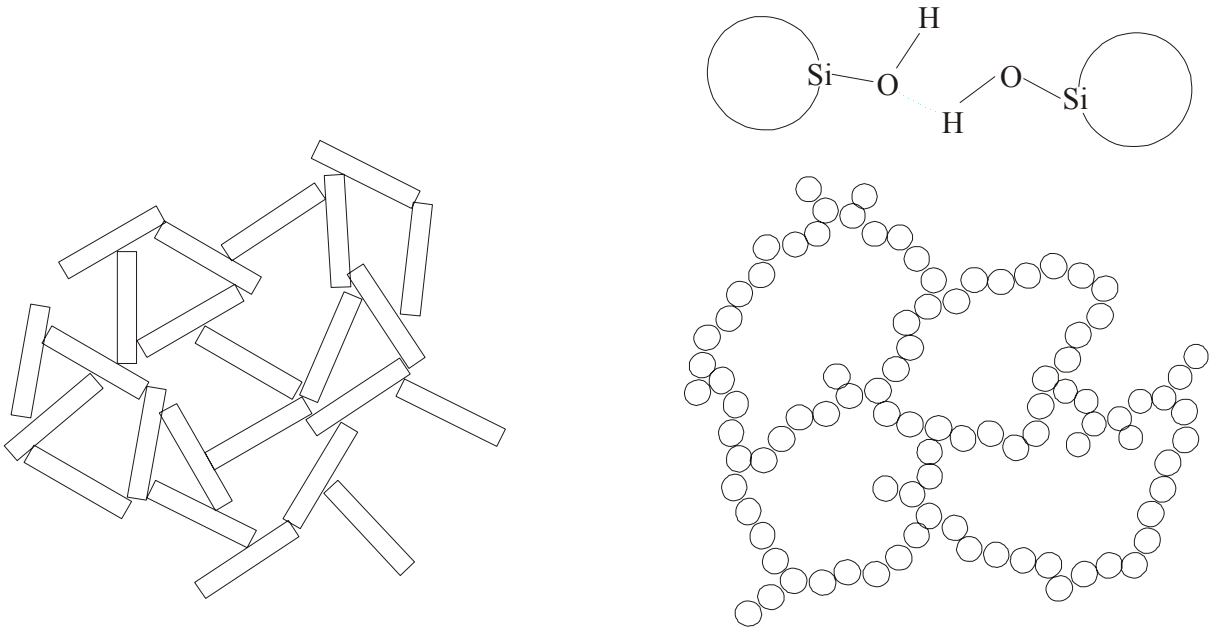
High molecular weight polyethylene glycols (MW > 20 000) (PEGs) are known also as organogelators of mineral oils (Plastibase®, Jelene®). On cooling, the polymer precipitates and

causes gelation. The mineral oil is immobilized in the network of entangled and adhering insoluble polyethylene chains, which probably even associate into small crystalline region. The organogels of high molecular PEGs are easily washable from the skin, and the water-solubility is associated with the presence of the two alcoholic -OH group in the molecule and to the hydratation of the etheric oxigen. However, solid PEGs are not soluble in liquid polyethylene glycols (lower molecular weight with MW < 400), since their mixture leads to white, pasty gels (Carbowax®) [24].

2.1.1.4 Inorganic gelators [25]

Organic clay can be obtained by reacting the hydrophilic smectites (silicate layers) with monoquaternary compounds. The fatty chains are attached to the face of the clay platelets, leaving the edges free to hydrogen bond. The fatty chains provide solubility in the organic base, whereas the edge-to-edge hydrogen bonding of the platelets provides suspension ability. The organic clays may be based on hectorite or bentonite hydrophilic clays (Benton®, Claytone®) [26].

Synthetic silica dioxide (Aerosil®) is called fumed silica because of preparing by a vapour process. The silica network forms by interparticle hydrogen bonding due to the surface silanol group. The agglomerated aggregate configuration is necessary for efficient interparticle interaction (Scheme 2).



edge-edge aggregation of organoclays silica interparticle interaction

Scheme 2. Diagrammatic representation of the interparticle interactions in inorganic organogels

2.1.2 Microemulsion-based organogels

2.1.2.1 Water-in-oil organogels of sorbitan monostearate

In order to obtain these water-in-oil organogels, first w/o emulsion (sol state) have to be prepared using sorbitan monostearate, which is both the organogelator and the principal emulsifying agent, and a secondary hydrophilic nonionic surfactant (in order to increase the gel lifetime). Cooling the emulsion results in a lowered solvent–gelator affinity. The surfactants self-assemble into tubular aggregates and these interact with one other. The tubules are composed of multiple inverted-bilayer of sorbitan monostearate, and the aqueous phase is accommodated within these inverse bilayers, bound by the polar headgroups of the surfactant at 60°C. The hydrogen bound may further stabilize the gel. The w/o sorbitan monostearate gels are thermoreversible similarly to the "dry" sorbitan monostearate gels [27].

2.1.2.2 Gelatin-microemulsion based organogels (MBG)

Gelatin is a natural polypeptide, which induces gelation in w/o microemulsions under appropriate temperature and polymer concentration. The secondary and/or tertiary structure of the gelatin solubilized in the microemulsion differs from that encountered in pure solvent. It can be considered as a polimeric cosurfactant with its hydrophilic side chains dissolved in the water pools and its hydrophobic side chains absorbing on the interface covered by surfactants. The nanodroplets have the ability to aggregate irreversibly due to interdroplet tropocollagen-like helix formation [28, 29]. MBGs are isotropic and thermoreversible organogels.

MBGs are formulated with bis(2-ethylhexyl)-sodium-sulphosuccinate (AOT) using it as principal emulsifying agent. Since AOT is an anionic surfactant, its major part was attempted to replace with the pharmaceutically more accepted nonionic surfactants in order to minimize the risk of toxicity. However, MBGs could not be formulated yet using nonionic surfactants alone [30].

2.1.2.3 Lecithin organogels

Solution of purified lecithin in nonaqueous solvents can be transformed into transparent gels by addition of a critical amount water, glycerol or formamide. Spherical inverse micelles transform into giant cylindrical micelles, which overlap, interpenetrate and entangle, thus forming a three-dimensional network. The transition to polymer-like micelles is accompanied with a formation of hydrogen bonds between the phosphate group of the lecithin molecule and the polar liquid. Lecithin organogels are in jelly-like state, isotropic and thermoreversible [31-33].

2.2 Pharmaceutical application of the organogels

2.2.1 Topical effect on the skin

When dermatological preparations are used in order to obtain local topical effect (rubefacient, adstringent, keratolytic, antikeratoid, antiparasite, topical antibacterial, -viral, -microbal, etc.), the active agent must remain on the skin surface such long-lasting as possible. In addition, when the active agent can provoke skin irritancy, it is a goal to prevent the penetration. In these cases organogels could be indicated as topical vehicle [34, 35].

A common indication of organogels is to treat eczema and dry psoriasis where they work as emollients.

Due to their oleophilic nature, organogels are slightly washable from the skin, so they are often unpopular. However, the more water-resistant the product, the longer it remains on the skin, providing more time for displaying the desired effect. On the other hand, water-resistency is especially important in those cases when skin is exposed to water permanently (e.g. sun care products, industrial protective handcreams).

2.2.2 Transdermal drug delivery

In addition to the occlusive effect obtained by oleophilic bases, the presence of fatty acids, fatty acid ethers, -esthers or lecithin might potenciate the transdermal drug delivery since these chemicals cause pertubation of the lipid bilayer of the *stratum corneum*.

Lecithin organogels have the ability to host both hydrophilic and lipophilic drugs, and the transdermal transport of several drugs (e.g. NSAIDs, capsaicin, scopolamine, ketamin, progesterone) from this matrix are reported [36-38]. Although interaction with the *stratum corneum* is confirmed, the role of the lecithin remained unclear [39].

Pluronic® lecitin organogels (PLO) unique the penetration enhancer effect of Pluronic®, lecithin and isopropyl palmitate. They have already gained acceptance as the delivery of NSAIDs, and are recommended as vehicles for delivering topical (local) analgesia [40-42] or transdermal hormon replacement. They are commercially available with the tradenames: Phojel® and PLO-Transderma®.

MBGs are electrically conducting and therefore have potential application in iontophoretic drug delivery [43-45]. Iontophoresis enhances transdermal drug transport via direct electrophoresis, electroosmosis or enhanced diffusion [46-48].

2.2.3 Ophtalmic vehicle

Since eye is especially sensitive to any physical and chemical stimulus, thus it is particularly important to select non-irritable ingredients and apply them in the smallest amount when formulating ophtalmic preparations. Another challenge with these vehicles is that the product should remain as long as possible on the cornea or on the connective tissue under eyelid inspite of intensive and permanent lacrimation.

Lecithin organogels prepared with paraffin oil, isopropyl palmitate, Miglyol® 812N (caprylic/capric triglycerides) and cyclooctane were already evaluated as potencial ophtalmic vehicles. Except of the gels based on cyclooctane, the lecithin organogels showed good ocular tolerability in rabbits during *in vivo* and *ex vivo* experiments [49-51].

2.2.4 Delivery for vaccines and antigens

Oily systems form a localised depot, unlike aqueous formulations, which spread along the muscle fibres. The Span® organogels showed slower release of the antigen as compared with the aqueous solution after i.m. administration. *In vitro* observations of these organogels revealed that fluid penetration into the organogels via the tubular network and emulsification at the surface resulted in gel breakdown and so was responsible for a relatively short-lived depo. The short depot effect may, however, be sufficient for certain applications, e.g. immunoadjuvants, where a short depot action is thought to be enhancing the immune response to antigens [52, 53]. Immunogenicity studies showed that the w/o gels as well as the vesicle-in-water-in-oil gels (v/w/o) posess immunoadjuvant property [54].

2.2.5 Rectal sustained-release preparation

Rectal administration of NSAIDs is common in order to produce fast-evolving effect, and to avoid GI side effects. When long-lasting administration is necessary, sustained-relese preparations can contribute to the patient compliance by decreasing the frequency of the inconvenience occuring with rectal administration.

The rectal delivery of various NSAIDs (salicylic acid, sodium salicylate, ketoprofen) in Eudragit® organogels was evaluated. *In vitro* esentially different release patterns from Eudragit® L and S organogels were observed. The former was a bioerosion-controlled monolithic system with zero-order release kinetics, whereas the latter provided a matrix-controlled diffussion. *In vivo* the Eudragit® L organogels showed sustained-release as compared to the traditional Witepsol® H-15 suppositories [55].

3 MATERIALS AND METHODS

3.1 Materials

All of the compositions studied are summarized in Table 2, where % means % w/w. The materials used were from the following sources:

Miglyol® 812 (glyceryl tricapryliate/caprate) was purchased from Hüls (Germany) [56]; Tegin® Pellets (glyceryl mono/distearate SE), Tegin® M Pellets (glyceryl mono/distearate), Tegin® 90 Pellets (glyceryl stearate/palmitate), Span® 60 (sorbitan monostearate) were from Franken Chemie (Germany); Imwitor® 900 (glyceryl stearate) was from Dynamit Nobel (Germany). Cetostearyl alcohol, Glyceryl monostearate, Hard paraffin, Liquid paraffin, Virgin castor oil, White beeswax, Lanolin Alcohols and White petrolatum were obtained from Hungaropharma (Hungary). Aerosil® (collidal silica dioxide), Citric acid, Sodium dihydrogen phosphate ($Na_2H_2PO_4$*2 H_2O) and Isopropyl myristate were obtained from Merck (Germany). Piroxicam (4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide) was donated by Egis (Hungary) [57].

3.2 Methods

3.2.1 Organogeling ability of different surfactants

Gelation was considered effective when 5% w/w of the surfactants solidified the oil (the sample was not pourable from the baker), and separation of the phases was not observed in one week.

3.2.2 Preparation of the organogels

The transparent dispersion of the solid components and oil was prepared at 70°C, using water bath. The melted mixture was allowed to cool down to room temperature under continous stirring (120 rpm). 1% w/w of piroxicam (Px) was then dispersed in the organogels.

3.2.3 Optical microscopy

The solid structure of the organogels was observed under light microscope (Zeiss, Germany) at 40× and 100× magnification. The microscopic pictures were taken with a photocamera using Leica Q500 MC image analysis Software.

Table 2. Composition of the organogels

	Glyceryl monostearate organogels (GMSO)	***Traditional organogels***			
		Silicoparaffin Gel, *Unguentum silicoparaffini*[1] *(SP)*	*Paraffin Ointment,* *Unguentum paraffini*[2] *(PR)*	*Simple Ointment,* *Unguentum simplex*[2] *(SX)*	*Oily Ointment* *Unguentum oleosum*[2] *(OL)*
Ointment base	Miglyol® 812	Liquid paraffin	Liquid paraffin	White petrolatum	Virgin castor oil
Solid fraction	Tegin® Pellets (*G1*) Tegin® 90 Pellets (*G2*)	10% Aerosil®	30% Hard paraffin	3% Cetostearyl alcohol	15% Cetostearyl alcohol, 10% White beeswax
Surfactant	Tegin® M Pellets (*G3*) Imwitor 900 (*G4*)*	—	—	6% Lanolin Alcohols	5% Lanolin Alcohols

* Glyceryl monostearates (GMS) were used in 5, 7, 9, 11, 13, 15, 17% w/w

[1] Hungarian National Formulary 6th Edition

[2] Hungarian Pharmacopoeia 7th Edition

3.2.4 Rheological measurements

A HAAKE RS1 (Thermo Electron, Germany) rheometer was used with a cone–plate measuring system (1/35 TI). All measurements were performed in triplicates. Data were evaluated with RheoSoft 2.84 Software.

3.2.4.1 Temperature sweep

The *G' = G"* crossover was observed while the temperature was increased/decreased in 0.1°C/min rate, at 5 Hz.

3.2.4.2 Flow curves and viscosity curves

Controlled rate-ramp ($\Delta\dot{\gamma}/\Delta t = 0.333$) was applied in up and down cycle, and the result was recorded as $\tau = f(\dot{\gamma})$ rheograms and $\eta = f(\dot{\gamma})$ viscosity curves. Thixotropy was defined as the area between the up- and down curves [Pa/s].

3.2.4.3 Linear viscoelastic region

In oscillation mode the shear stress was increased in the range of 0.5 - 50 Pa and in the range of 5 - 150 Pa.

3.2.4.4 Mechanical spectra

The oscillatory frequency sweeps were performed in the frequency range 0.1 - 10 Hz at 10 Pa. Complex viscosity ($\eta^* = G^*/\omega$) and loss factor ($tan\delta = G'/G''$) were calculated.

3.2.5 Investigation of spreadability

Using a pattern 0.3 g of organogel was placed in a 10 mm diameter circle on a glass plate. After being covered with another glass plate, the sample was loaded by 500 g weight. The diameters before and after the loading were determined on millimetre scale placed under the lower glass plate, and spreadability was defined as the difference of these values.

3.2.6 Oil number

Using a pattern, equal amounts of organogels were placed on filter-paper (595 Schleider & Schuell) (m_1), than the samples were kept at 32 ± 1ºC for 10 hours. The mass of the oil released (m_O), which increases the weight of the filter-paper (m_2), and oil number (mean value of 5 parallel tests) were calculated as:

$$m_o = m_1 - m_2 \quad (1)$$

$$oil\ number = (m_o / m_2) * 100 \quad (2)$$

3.2.7 Determination of the wetting conditions

4 µl of the oil was dispensed to the smooth surfaces prepared from the surfactant melts. The contact angle, Θ was detected by an optical contact angle measuring device (OCA 20, DataPhysics, Germany, software SCA 20 1.3), at 25 ± 1°C. The sessile drop method was applied, and ellipse fitting was used as calculation method. Results reported are mean values of 7 parallel measurements.

3.2.8 Sensory analysis

A group of 10 people of different sex and ages (23 – 55 years) was selected as panels for the sensory tests. The training of the panels and the performance of the sensory tests took place within controlled conditions (light, temperature and relative humidity) upon the ASTM E–1114 Guideline [58].

Questionnaires were prepared to evaluate appearance, texture, skin feel and overall liking [59, 60]. The test types and their statistic analysis can be summarized in Table 3. Correlation was investigated with 95% confidence interval.

Table 3. Sensory tests and their statistical evaluations

Test		*Statistical analysis*
Preference	Simple ranking	Summary statistics, Friedman's test
Acceptance	Scaling	Mean score, Friedman's test
	Just right test	Frequency distribution

Except the just right tests, which were based on the intensity of the specific attribute, categories in hedonic scales were used [61].

The scores obtained by the scaling tests were normalized by dividing each score by the sum of all scores given by the same panel to a specific attribute. In this way the individual scale interpretation could be eliminated [62].

3.2.9 Determination of solubility

An excess amount of Px was added to phosphate buffer (pH 5.4 ± 0.1) containing 0.5% w/w surfactant. The mixtures were shaken for 7 days at 25°C, the supersaturated solution was filtrated

and the Px content of the saturated solution was determined spectrophotometrically with a Unicam UV2 UV/Vis spectrometer (Unicam, England) at 356 nm.

3.2.10 Determination of partition coefficient and penetration coefficient

The mixture of 0.5% w/w surfactant and 0.5% w/w Px was added to the water saturated with *n*-octanol and to the *n*-octanol saturated with water. The mixtures were shaken for 7 days at 25°C, and c_{oct} and c_w was then determined spectrophotometrically. The permeability was estimated via the equation derived by Potts and Guy [63]:

$$\log Kp = -2.7 + 0.71 \log P - 0.0061 MW \qquad (3)$$

where Kp is the penetration coefficient, P is the *n*-octanol/water partition, and MW is the molecular weight.

3.2.11 *In vitro* penetration study

Franz type vertical diffusion cell [64] (Hanson Research, USA) was used to model the skin penetration *in vitro* [65-68]. The acceptor phase was phosphate buffer (pH 5.4 ± 0.1), thermostated at 32 ± 1°C. Cellulose acetate membrane (Sartorius, Germany, Ø=3.0 μm) was soaked in isopropyl myristate in order to mimic a lipophilic barrier such as the *stratum corneum* [69]. At predetermined intervals, 0.75 ml sample was taken from the acceptor phase and replaced with fresh buffer solution. The diffusion time was 5 hours, and Px was detected spectophotometrically (Heλios α® UV-Vis spectrophotometer, Thermo Electron, Germany).

The accumulated drug amount penetrated through the unit diffusion surface (Q) was calculated and plotted versus time. The steady-state flux (J_s) of Px was estimated from the slope of the linear portion of the penetration curves and expressed as

$$J_s = \frac{V}{A} * (dc/dt) \qquad (4)$$

where V is the acceptor volume, A is the diffusion surface area, c is the Px concentration in the acceptor phase, and t is time.

Statistical analysis was performed by Neumann-Keuls's test, at $p<0.05$ significance level.

3.2.12 *In vivo* anti-inflammatory effect

Female Sprague–Dawley rats (180-200 g) were assigned to weight-balanced groups (n = 6 in the first experiment; n = 8 in the second experiment). All measurements were performed at

24 ± 1°C, in an air-conditioned room. In the first experiment, 150 mg of organogel was applied, non-occlusively to the right hind paw (whole hairless skin of legs). The animals were anaesthetised with 400 mg/kg chloral hydrate i.p. in order to prevent the adsorption of the applied organogels by sawdust. The anaesthesia provided sufficient time (~1 hour) for the complete absorption of the organogels. In the second experiment, 300 mg of organogel was spread over the depilated (Veet®, Reckitt Benckiser, France) dorsal skin (15 cm^2 area). After 1 hour, 0.1 ml of a 0.5% carrageenan suspension (Viscarin®, Marine Colloids, USA) was injected into the subplantar area of the right hind paw. The left paw used as control was treated with physiological saline solution without carrageenan. Paw volume was measured with a plethysmometer (Hugo Sachs Elektronik, Germany) 5 hours after the carrageenan injection. The degree of paw swelling was calculated as

$$swelling\ \ (\%) = \frac{V_i - V}{V} * 100 \tag{5}$$

where V_i is the volume of the carrageenan treated, V is the volume of the non-treated paw.

On the basis of Eq. 5, the percent oedema inhibition was calculated as [70]

$$inhibition\ \ \% = 1 - \left(\frac{swelling_{treated}}{swelling_{control}} \right) * 100 \tag{6}$$

where $swelling_{treated}$ is the mean value observed in the treated group, $swelling_{tcontrol}$ is the mean value observed in the control group, which was treated with placebo organogel.

One-way ANOVA was performed followed by Neumann-Keuls's test, at a significance level of $p<0.05$.

The relative bioavailability (*RBA*) as regards the systemic effect was calculated as

$$RBA = \frac{inhibition\%1}{inhibition\%2} \tag{7}$$

where $inhibition_1$ is the percentage oedema inhibition of the different GMSOs, $inhibition_2$ is the percentage oedema inhibition of the different traditional organogels.

4 RESULTS AND DISCUSSION

4.1 Development of novel organogel compositions

Among the surfactants tested, the esters and the alcohols of stearylic acid were found to be the most effective gelators of the oils (Table 4). Their gels are melt–type, since the products solidify from the melted mixture of the ingredients. The gelation took place after the aggregation model: on cooling aggregates occurred with the decrease of the solubility of the surfactant in the oil, and the transparent sol became an opaque gel.

The various fatty acid esters had different gelation ability depending on their solubility in the test oil. Among the homologous fatty acid esters, the oil solubility increases with the increasing chain lenght, e.g. sorbitan palmitate < stearate < oleate in case of the Span® family. Since sorbitan palmitate has lower oil solubilty than that one of sorbitan stearate, thus the interaction

Table 4. Organogelation of surfactants and oils; ◙ gelified, – not gelified

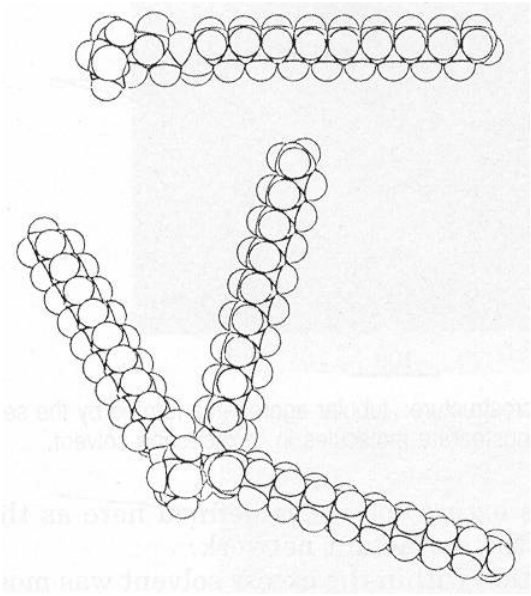
	Semipolar oils		Apolar oils	
	Isopropyl myristate	Miglyol 812	Liquid paraffin	Silicon oil
Fatty acid esters				
Sorbitan palmitate (Span® 40)	◙	◙	◙	◙
Sorbitan stearate (Span® 60)	◙	◙	◙	◙
Glyceryl monostearate, cc. 40-55% (Imwitor 900)	◙	◙	◙	◙
Glyceryl monostearate, cc. 90% (Imwitor® 940)	◙	◙	◙	◙
Glyceryl monostearate, cc. 30% + K-stearate (Imwitor® 965)	◙	◙	◙	◙
Sorbitan tristearate (Span® 65)	–	–	–	–
Glyceryl stearate/ palmitate (Tegin® 90)	◙	◙	◙	◙
Glyceryl mono-, distearate (Tegin® M)	◙	◙	◙	◙
Glyceryl mono-, distearate SE (Tegin®)	◙	◙	◙	◙
Sorbitan oleate (Span® 80)	–	–	–	–
Polygycerols				
Poliglyceryl stearate (Hostacerin® DGMS)	–	–	–	–
Poliglyceryl oleate (Hostacerin® DGO)	–	–	–	–
Poliglyceryl-2-sesquioleate (Hostacerin® WO)	–	–	–	–
Fatty alcohol				
Cetostearyl alcohol	◙	◙	◙	◙

between the aggregates might strenghten in time, resulting in the separation of the phases. In contrast, sorbitan oleate has too high solubility in the oil, and the aggregates were not able to form on cooling.

The good organogelator ability of the stearylic derivatives (sorbitan and glyceryl monostearate) is assumed to be the aggregation and the ability of these aggregates to connect with each other. About 50°C the stearate group undergoes a crystalline–amorphous transition, and on cooling recrystallize in aggregates which may serve as connecting points [71].

Stearate monoesters have cylindrical shape which allows molecular packing into bilayers such that the molecules can assemble into tubular aggregates responsible for the gelation. However, the conical shape of tristearates is not appropriate for organization in the same mode since their large molecular size and sterical configuration (Scheme 3).

As compared the organogelation process of three stearyl derivatives with different hydrophilic part (sorbitan monostearate, glyceryl monostearate, cetostearyl alcohol), different behaviours were observed. When increasing the concentration of the solid phase, the liquid dispersion became only denser in case of sorbitan monostearate, whereas the sample was solidified in the baker at certain concentrations using glyceryl monostearate and cetostearyl alcohol. For further investigations, glyceryl stearates were selected. Since commercially available glyceryl monostearates (GMS) are obtained from different sources and by different methods, they are mixtures of glyceryl esters, with the stearate and palmitate esters predominating [72]. In this



Scheme 3. Three-dimensional orientation of sorbitan mono- (a)and tristearate (b) molecule

study four GMSs and their organogels were investigated which INCI name is glyceryl monostearate but have different mono/diester contents [73, 74].

The role of the oil phase is to influence the sterical and molecular packing and also the solubility of the gelator [75]. In apolar oils, the attractive forces preveal better between the lipophilic tail end of the surfactants. This might be the reason of those findings that: (i) same surfactant concentrations resulted in greater viscosity in apolar environment, than those of in semipolar oils, (ii) and the samples were solidified into the container at lower surfactant concentration in apolar oils, than those in semipolar oils (Table 5). Since my purpose was to obtain cream-like consistency, concentrations below these values were not investigated henceforth.

Among the oil phases, Miglyol® 812 was chosen for my further investigations, because of the ability of being absorbed easily, low cost, good skin tolerability, and skin penetration enhancement [76]. In contrast, Liquid paraffin (mixture of C 8 – 10 paraffins) and Silicon oil are occlusive and they can leave residue on the skin (which property anyway makes them suitable for being involved in special formulations, e.g. water–repellent skin protective products).

Table 5. Comparison of the gelation concentration resulted in solid-like consistency

		Oil			
		Miglyol® 812		Liquid paraffin	
		c (% w/w)	*η* (Pas)[a]	*c* (% w/w)	*η* (Pas) [a]
Surfactant	Glyceryl monostearate	9	407.01	5	575.10
	Sorbitan monostearate	5*	127.98	5*	232.13
	Cetostearyl alcohol	7	540.32	5	560.32

[a] initial viscosity

* the mixture has became denser but did not be solidified

4.2 *The gel formation*

The start of the sol–gel phase transition is induced by the aggregation/crystallization of the surfactant on cooling, which is practically a competition between two phenomena: the solubility and the aggregation of the surfactant molecules. Therefore the gel point (expressed as geation temperature, T_g in Table 6) corresponds to the solubility of the surfactant in the oil.

Rheology serves a powerful method to describe the organogelation process of GMSs, since the reversible change in the elastic (*G'*) and viscous properties (*G"*) on cooling/heating can be followed during temperature sweep tests. The gel point could be determined at *G' = G"*, above which the elastic properties became dominant (Fig. 2) [77-80]. In addition, rheology is capable not only to define the gel point, but also to describe the kinetic of the gelation.

Table 6. Gelation temperature of different GMSOs

Organogels	T_g *(°C)**
G1	50.4
G2	47.2
G3	42.8
G4	31.0

* 15% w/w surfactant content

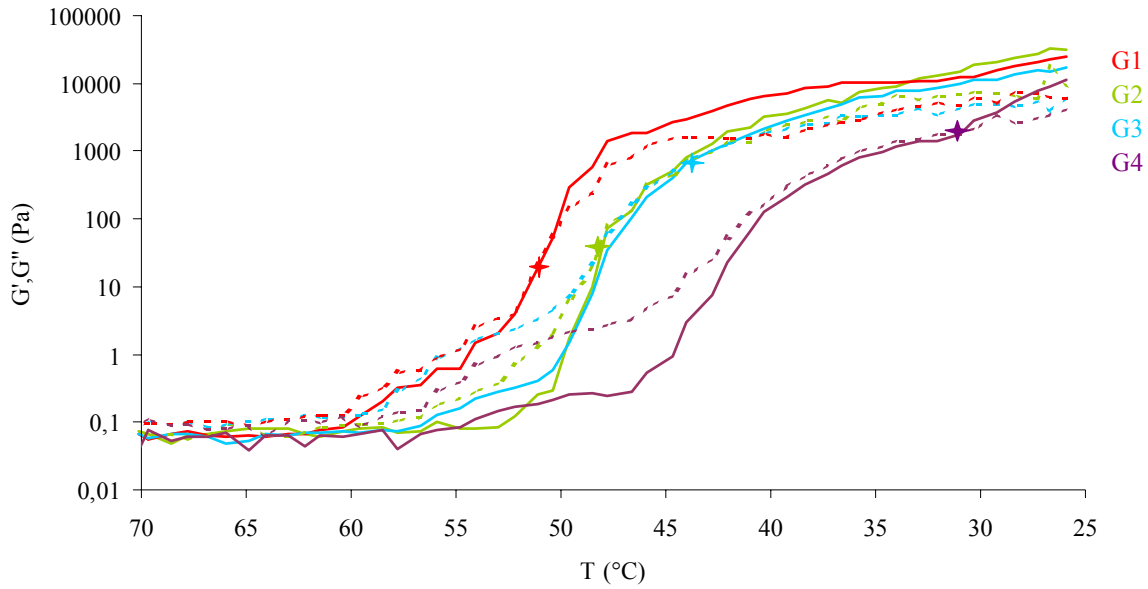


Figure 2. Comparison of the rheological behaviours of different GMS/oil mixtures on cooling (15% w/w surfactant content); G' solid line, G" dashed line; × gel point

The gel formation is usually described by the power law equation and by the Avrami's equation. The power law governs in time the relaxation function (shear modulus, G) (Eq. 8) and the elastic (G') and viscous (G'') moduli too (Eq. 9, 10):

$$G(t) = St^{-m} \tag{8}$$

$$G' = \frac{\pi}{2\Gamma(m)\sin(m\pi/2)} S\omega^m \tag{9}$$

$$G'' = \frac{\pi}{2\Gamma(m)\cos(m\pi/2)} S\omega^m \tag{10}$$

The kinetic of phase transformation can be described mathematically by a standard equation known as the Kolmogorov–Johnson–Mehl–Avrami (KJMA) equation after the individuals who derived it [81-84]:

$$\chi = 1 - \exp\left(-Kt^n\right) \tag{11}$$

where χ is the gel fraction developed at time t, K is the temperature dependent rate constant and n is the Avrami's constant.

Here the KJMA equation was used for the rheokinetic analysis of the organogel formation, assuming that the reaction advance is proportional to the increase in the storage modulus, G' due to the increase in elastic active bonds within the colloidal network, and related to the aggregation. Based on Eq. 9, the absolute value of the hill slope of the logG' = f(T) curves correspond to the gelation rate (Table 7) [85].

As the crystallization is concentration dependent, when the GMS concentration was increased, the phase transition occured earlier (at higher T), the hill slopes and the G' values reached were higher (Fig. 3). Above 13% w/w of GMS both the rate of the gelation and the energy reversible stored as elastically bounds do not change significantly, which means that the supramolecular gel network become more complex, but the basic structure does not change [86].

Table 7. Comparison of the hill slopes [Pa/°C] of logG' = f(T) curves of different GMSOs

	9% w/w	*11% w/w*	*13% w/w*	*15% w/w*
G1	4.42	6.39	9.56	9.44
G2	4.65	6.34	9.41	9.32
G3	5.15	7.55	8.72	8.11
G4	3.56	3.97	4.73	5.08

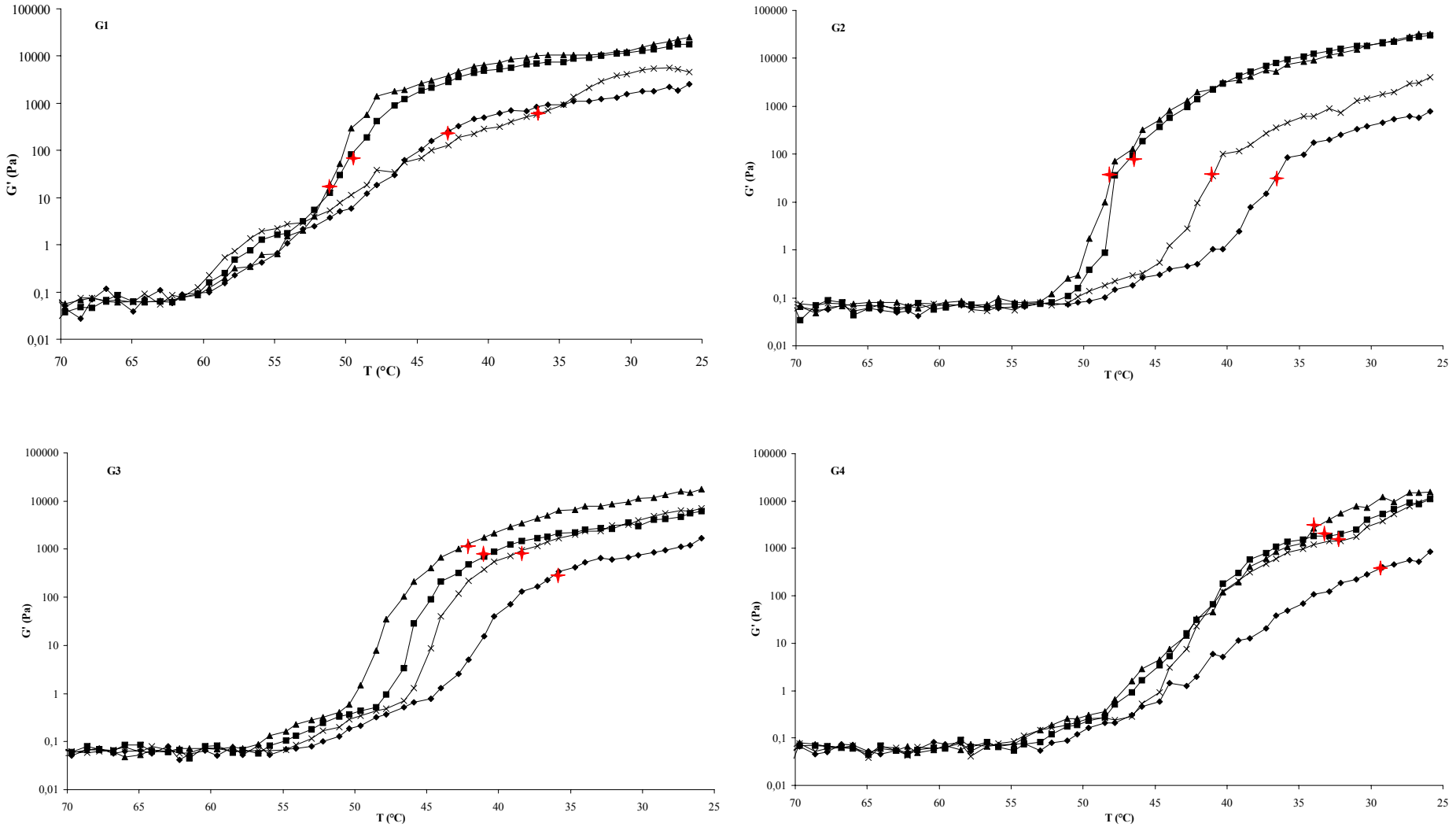


Figure 3. Comparison of the gelation kinetics of the different GMSOs, ▲: 15% w/w; ■: 13% w/w; ×: 11% w/w; ♦: 9% w/w; ✸ gel point

4.3 Sensory evaluation

In addition to the therapeutic efficacy and the commercial price, the sensory attributes influence considerably the consumers' opinion about the product. In the sensory tests the subjective opinions on the product are evaluated statistically, and the role of sensory evaluation is to provide a valid and reliable information to R&D, production and management in order to make business decisions about the perceived sensory property of the product. Furthermore, correlating the subjective opinions resulted from the sensory tests with the objective data obtained by rheological measurements, different texture properties such as firmness, spreadability and stickiness can be predicted and developed in the early research phase. This method is also called psychorheology [87, 88].

In this study, the sensory attributes of the organogel samples were evaluated by the panels in the view of they could used them as topical vehicles of analgetics.

4.3.1 Texture development of GMSOs

As some researchers reported with similar organogels, different gelator concentrations in the systems resulted in significantly different texture [89, 90]. The same findings were obtained here: Applying the different GMSs below 9% w/w liquid-like semisolids were obtained, while samples with 9 - 17% w/w of GMSs were soft creams and ointments.

The modification of the GMSO texture was reflected in the pronounced change of complex viscosity (η^*), yield value (τ_0) and spreadability (Fig. 4). In all GMSOs, η^* and τ_0 increased with the increase of the gelator concentration up to 13% w/w, but after this level did not change significantly. The spreadability decreased with the increase of the gelator content. In fact, the greater number of crystals involved in the formation of the reticulated structure, the more compact and resistant the structure was to the deformation. However, viscoelasticity ($tan\delta$) changed slightly ($p>0.05$) when GMS concentration was changed, so this feature seems to depend rather on the surfactant type than on the surfactant concentration [91].

From the variety of samples containing different surfactant amounts and so having different firmness, the concentration resulted in the most appropriate firmness for a pharmaceutical topical organogel was selected upon the subjective opinion of the panels. In the sensory tests firmness was defined as the ease of mixing the product with a glass stick, and the force to require to fully compress it between the thumb and forefinger.

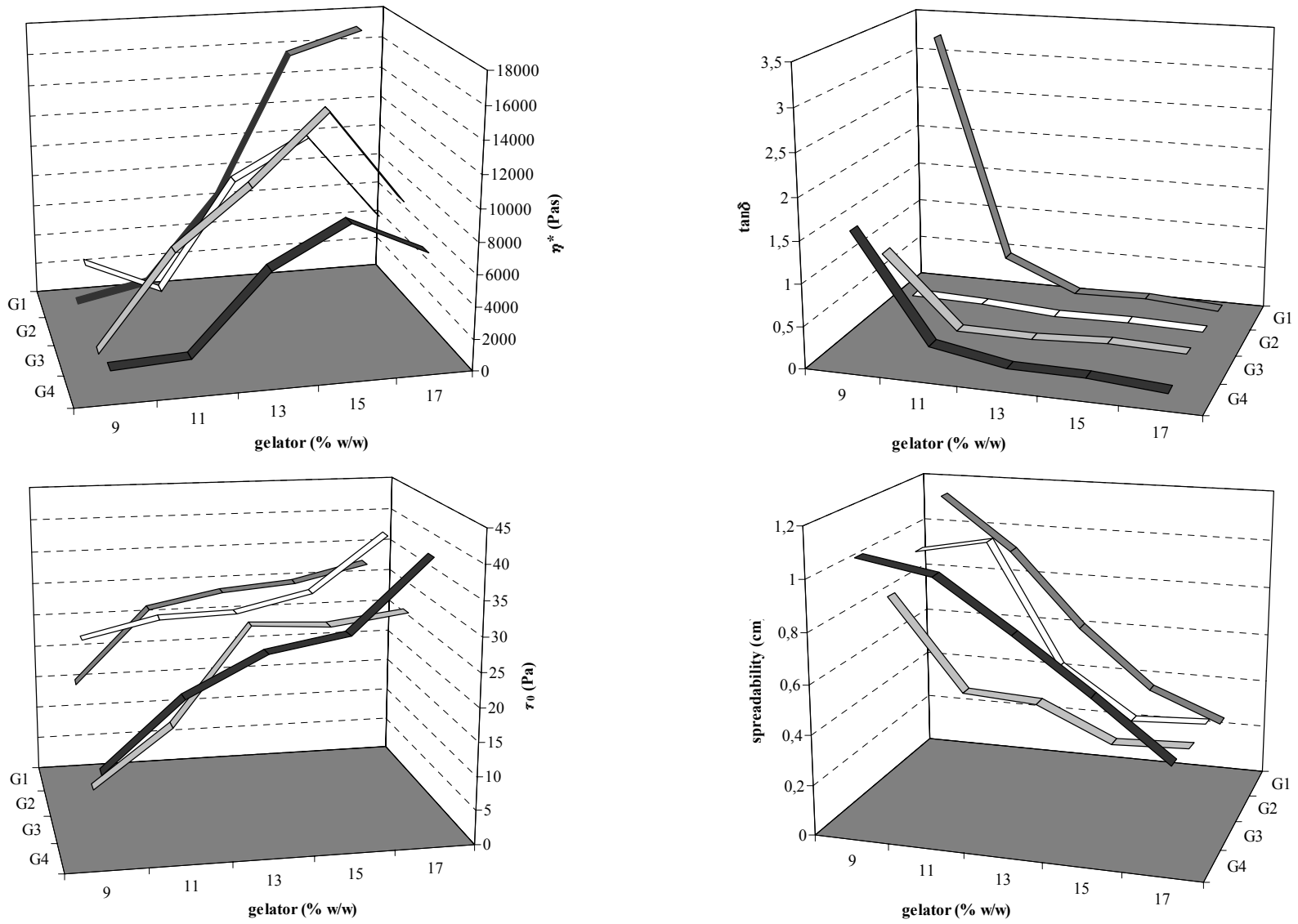


Figure 4. Effect of the gelator concentration on the rheological parameters and the spreadability of the GMSOs

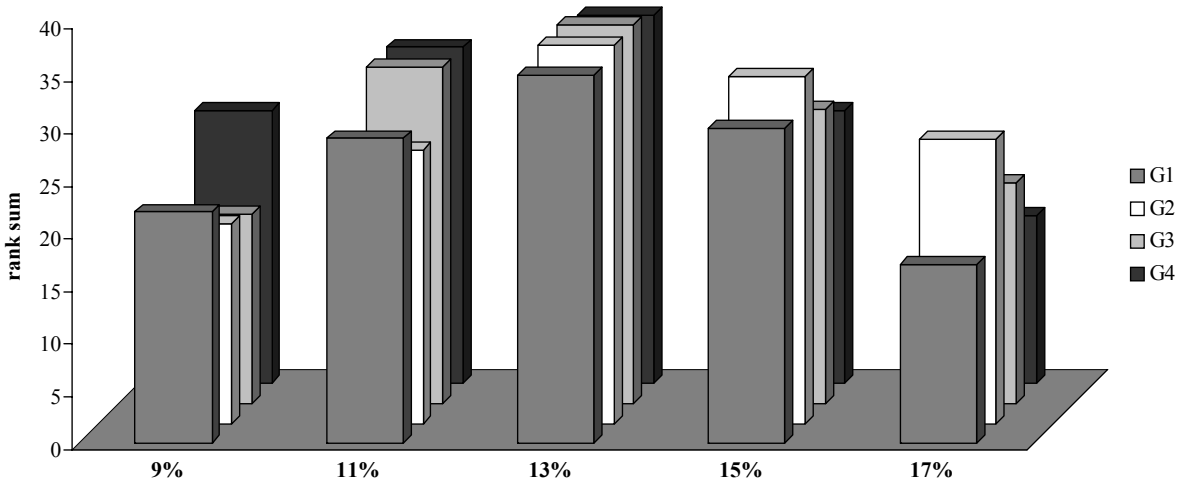


Figure 5. Sensory evaluation of the texture of organogels: simple ranking

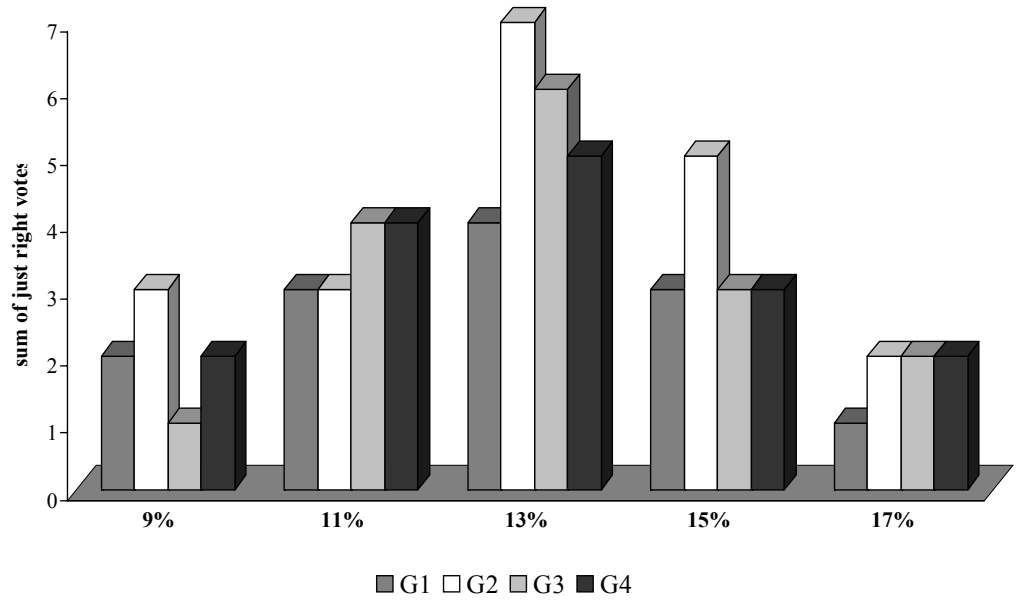


Figure 6. Sensory evaluation of the texture of organogels: just right test

First, the panels had to arrange the organogels in the order of liking their firmness. Independently of the gelator type, the greatest rank sums were reached by the organogels including 13% w/w of gelator, indicating that these samples were found optimally firm, whereas those samples including 17% w/w of gelator were preferred least of all (Fig. 5).

Then the just right test allowed the panels to assess the intensity of the firmness relative to the optimal value determined before (Fig. 6). The results confirmed that the organogels with 13% w/w of GMS possessed the optimal texture for the purpose of applying them as

pharmaceutical creams. The samples with 9% w/w of GMS were termed too soft and those with 17% w/w too firm.

As compared the rank sum of the certain samples to their rheological data, we could establish that τ_0 of approximately 20 – 30 Pa was defined as optimal. The τ_0 over or below this interval had low rank sum values. In case of the organogels with low η^* and low τ_0, the extent of the spreadability was so high to handle them without any difficulty while picking up from the petri dish and applying them to the skin. Therefore, such samples had also low rank sums.

Based on these findings which indicate that 13% w/w of GMS resulted in optimal firmness, the organogels containing this concentration of the different GMSs were investigated henceforth.

4.3.2 Organogel category review

G2 organogels, which were evaluated as the best, and G1 organogels, which were evaluated as the worst, both including 13% w/w of gelators, were selected to compare them with the traditional organogels (SX, OL, SP) regarding to the sensory attributes like overall liking, appearance, texture and skin feel. Figure 7 shows the picture of the samples.

As regards to the overall liking (Fig. 8), the statistical probe did not show any significant differences between G1, SX and OL. However, considering the mean scores, G2 proved to be better than OL ($p<0.05$) respectively to the overall liking, and the same finding was made in case of SP.

Figure 7. Samples for the organogel category review. From the left to the right: G1, SP, OL, G2, SX

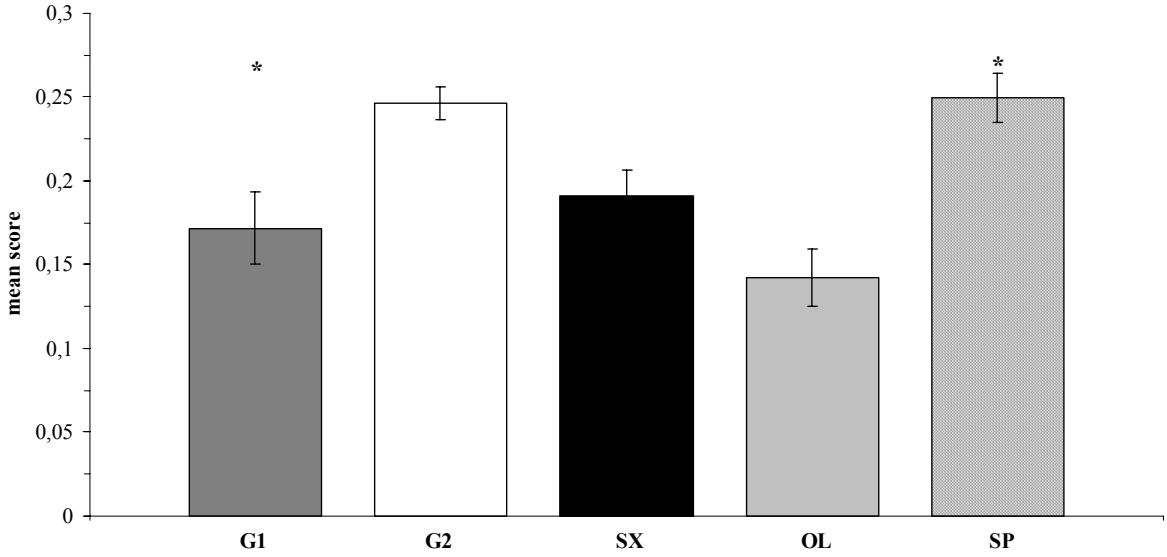


Figure 8. Evaluation of the overall liking; n=10, ± S.E.

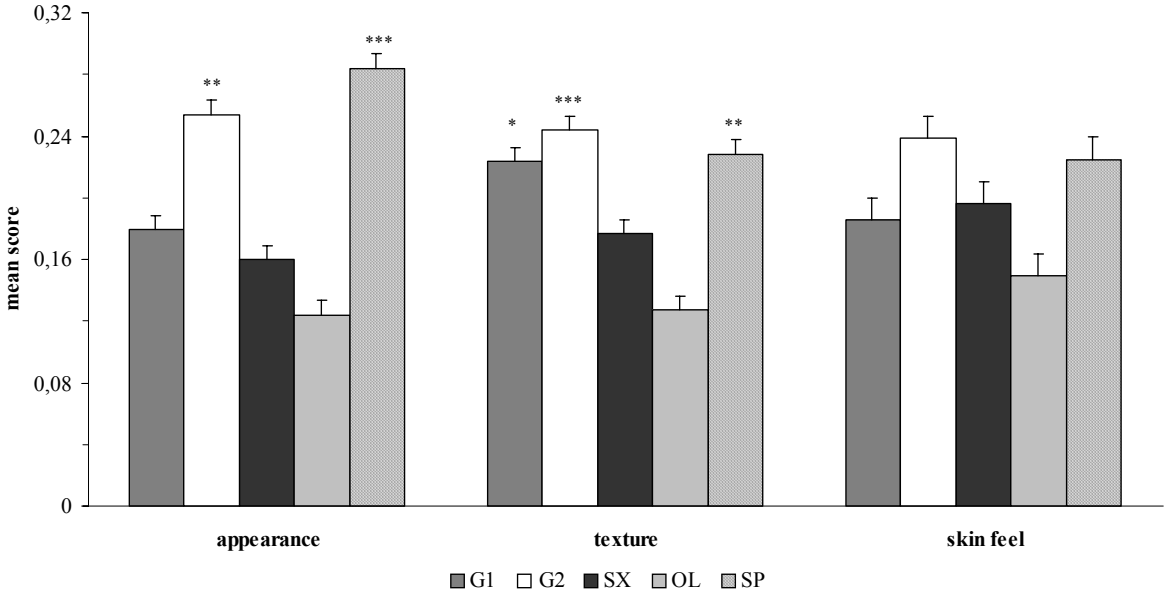


Figure 9. Evaluation of the appearance, the texture and the skin feel; n=10, ± S.E.

As regards to the appearance, G2 and SP were found to be the best again, and although there was no significant difference between them, the transparent SP could be considered slightly favourable, because it showed a more expressed difference as compared with SX and OL (Fig. 9). The appearance of OL had the smallest mean score.

As regards to the texture, OL had the smallest mean score, only SX was not better from this aspect. The next in this order was G1, which had a smaller mean value than G2. G2 and SP were

found to be the best, but while the former one's texture and the latter one's appearance could be considered the best.

Although panels perceived a slight difference regarding the skin feel of the different organogels, statistically it was not significant.

4.3.3 Attribute diagnostic

Based on the results of the category review, the relationships between the overall liking and the sensory attributes (appearance, texture, skin feel) were analysed. The correlation coefficients (r^2 values) summarized in Table 8 reveal a strong correlation between the overall liking and the appearance, as well as between the overall liking and the skin feel. Both correlations are more significant than the one exists between the overall liking and the texture.

Then, the relationships of the rheological parameters with the texture and skin feel were investigated (Table 9). The texture showed strong correlation both with τ_0 and η^*, but no relationship was found with *tanδ* and the spreadability. The same rheological data did not correlate with the subjective opinion on the skin feel.

In conclusion, attribute diagnostic revealed that the overall liking of the organogels was influenced mostly with the appearance and the skin feel of the samples, and that the subjective perception of the texture could be correlated with complex viscosity and yield value.

Table 8. Correlations between the overall liking and the sensory attributes (r^2 values)

	Appearance	Texture	Skin feel
Overall liking	0.9732	0.8013	0.9582

Table 9. Correlations between the rheological parameters and the sensory attributes (nonparametric Spearman correlation coefficients)

	η^*	tanδ	τ_0	Spreadability
Texture	-0.8481	-0.3953	-0.9602	-0.0159
Skin feel	-0.7715	-0.1721	0.5932	-0.1132

4.4 Description of the structure

4.4.1 Microscopic observations

The structure of the GMSOs in microscopic scale was investigated under light microscope. The rod-like aggregates are connected to each other and this network intermeshes throughout the oil (Fig. 10). The different glyceryl stearate assemblies possess similar shape and size and they look like the tubules described in sorbitan monostearate organogels [92].

In the microscopical pictures the heterogeneous network structure can be clearly recognized, and can be characterized like those of being formed by multiple percolation clusters [93].

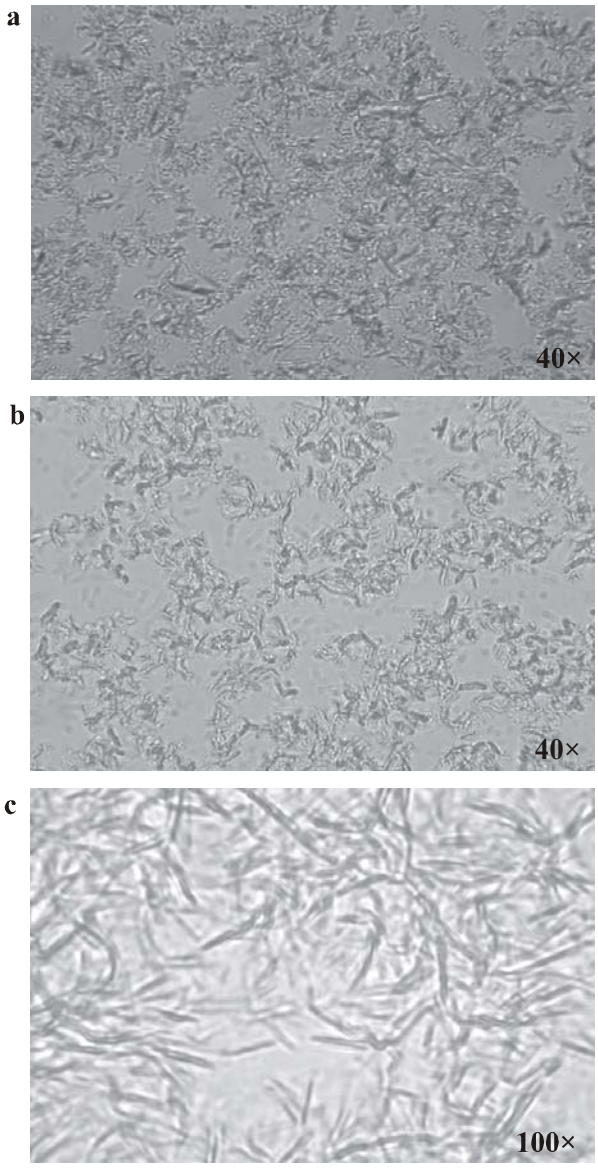


Figure 10. Microscopic picture of the organogels. a) G1; b) G2; c) G4

4.4.2 Phenomenological and microstructural approach by rheology

Figure 11 represents the dynamical rheological behaviour typical of the GMSOs. The *G'* exceeds *G"* over the whole frequency range tested, and the organogel network exhibits lifetime long enough to consider the systems as gels. Since *G'* exceeded *G"*, the smaller the *tanδ*, the more expressed the elastic nature is [94, 95].

The shear rate dependence can be described with the Cross model [96]:

$$\tau = \dot{\gamma}\left(\eta_0 - \eta_\infty\right)/\left(1 + \left(\dot{\gamma}/\dot{\gamma}_b\right)^n\right) \tag{12}$$

In the view of the viscoelasticity the relationship between the stress and strain is defined by the complex modulus, *G**:

$$G^*(\omega) = \tau^*/\gamma^* = G'(\omega) + G''(\omega) \tag{13}$$

The energy stored in the material (E^s) and the energy dissipated (E^l) correlate with the dynamic moduli, and the complex viscosity ($\eta^* = \eta' + \eta''$) represents the total resistance of the network structure against the applied strain [97, 99]:

$$E^s = G'(\omega)\gamma_0^2/4 \tag{14}$$

$$E^l = \pi G''(\omega)\gamma_0^2 \tag{15}$$

where E^s and E^l is average energy per unit volume in one cycle of the oscillation.

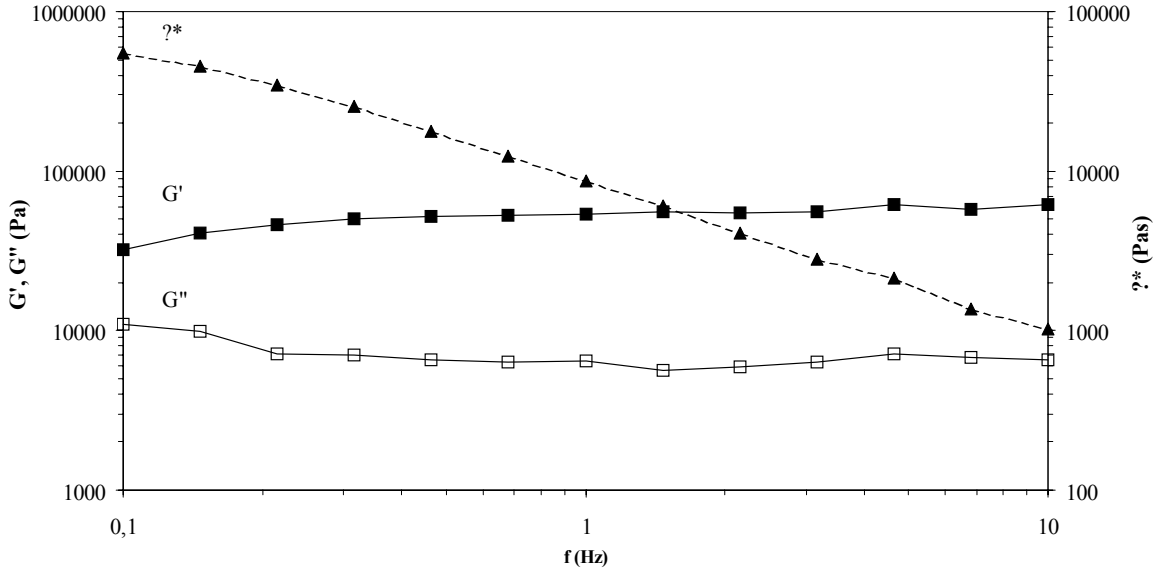


Figure 11. Typical dynamical rheological behaviour of the GMSOs

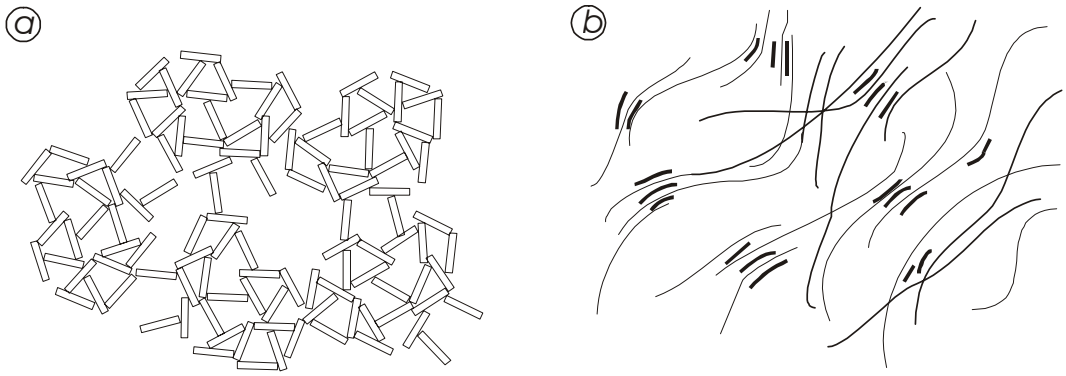
Table 10. Comparison of the rheological parameters of different organogels

	G (Pa)*	*η* (Pas)* [a]	*tanδ* [a]
OL	143 000	50 135	0.512
PR	120 000	33 115	0.168
SX	47 300	6 335	0.664
G1	44 950	10 114	0.126
G2	42 200	9 081	0.157
G3	32 247	6 559	0.132
G4	28 667	6 597	0.115
SP	26 522	5 518	0.035

G*: mean value identical to the linear range. n=3
[a] at f=1Hz

The four surfactants resulted in organogels with approximately same G^* and η^* values and the GMSOs have viscoelastic nature (Table 10). As compared to the GMSOs, PR and OL have significantly greater G^* and η^* values ($p<0.001$), but we have to consider that both traditional organogels include more solid ingredients (30 and 20% w/w) than GMSOs (13% w/w). Since 13% w/w of glyceryl monostearate resulted in approximately the same G^* value than the 81% w/w petrolatum based SX, as well as 10% w/w colloidal silica dioxide (SP), GMSs are good organogelators, and produce sufficiently stable organogels with moderate energy.

The network of glyceryl monostearate and hard paraffin (PR) consist of rod-like crystalline aggregates (Scheme 4.a). There are physical interactions between the crystals, which are in weak absorption interaction with the oil. This crystalline structure is fragile, but before a critical yield value shows elastic deformation. The three-dimensional structures of SX and OL are based on solid paraffins and Cetostearyl alcohol crystals, and their small crystallites are surrounded by fibres of Lanolin Alcohols and with amorphous wax intermeshed [100]. This arrangement is named as fuzzy micelle, and seems to be more restricted, characterized with viscous behaviour (Scheme 4.b). The silica network in SP forms by interparticle hydrogen bonding due to the surface silanol group. This interparticle interaction revealed highly elastic behaviour.



Scheme 4. Schematic diagram of the network of crystallites (a) and of fuzzy micelles (b)

4.5 Description of the stability

4.5.1 Thermal stability

After thermal load, the GMSOs has shown considerably great oil number, except of G2 organogels (Fig. 12). These oil numbers were approximately four times less than the oil numbers of the other GMSOs, which did not differ significantly. The oil number is usually used to quantitify the phenomenon of syneresis. As the syneresis results from the separation of the oil phase because of the shrinkage of the gel network, the oil number describes the interaction between the solid disperse phase and the liquid dispersing medium [101, 102]. The smaller the oil number, the stronger the interaction is between the GMS surfactant and Miglyol® 812.

Although the oil number was principally determined by gelator type, slightly depended on its quantity. The more the gelator the less the oil number was, because presumably more GMS could interact with the oil when increasing the GMS amount.

As compared with the traditional organogels, the oil numbers of the GMSOs were significantly higher ($p<0.001$), except of G2 organogels (Fig. 13).

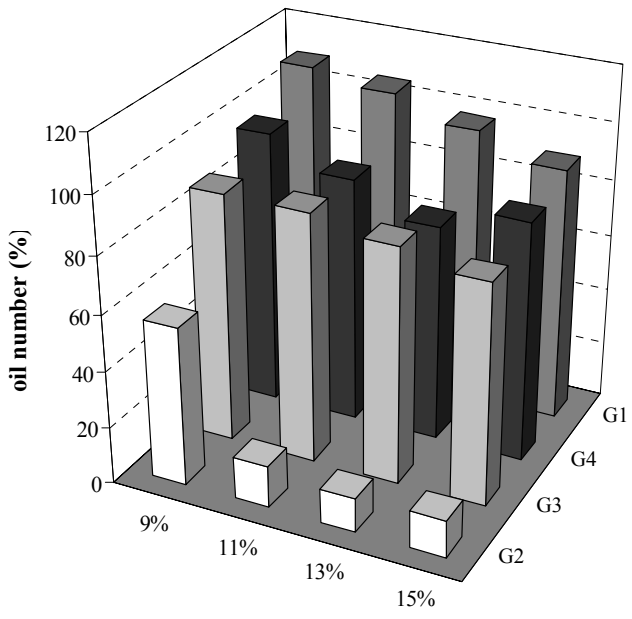


Figure 12. Oil numbers of different GMSOs

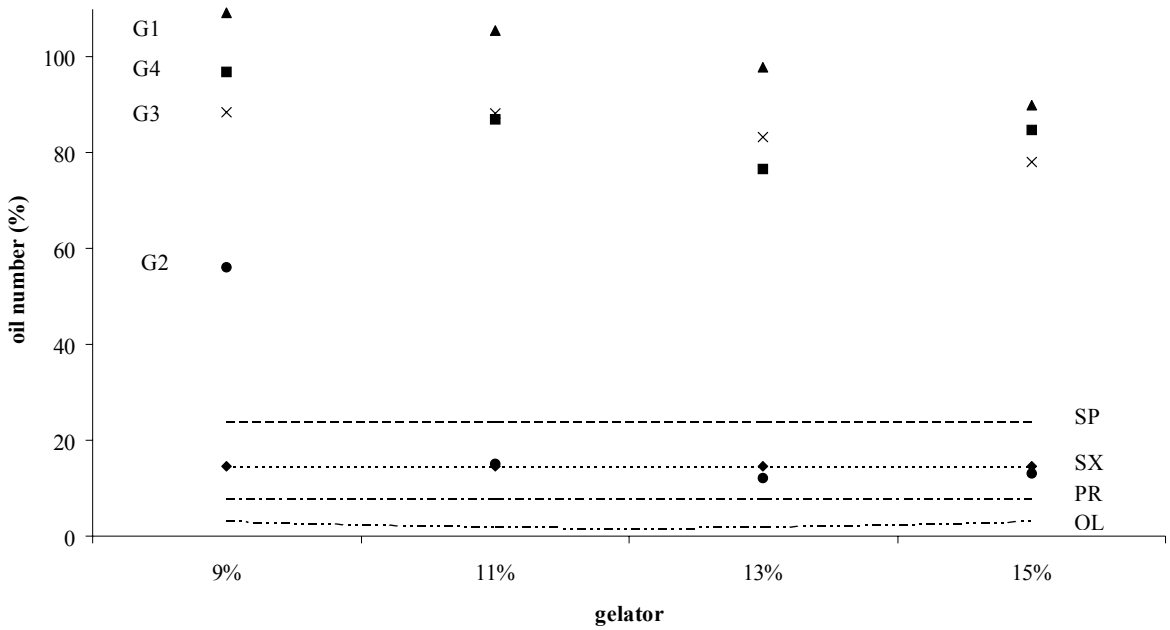


Figure 13. Comparison of the oil numbers of different organogels

The contact angle of the wetting (θ) is also a parameter which characterizes the interaction between the liquid and solid phases. The smaller the θ, the better the wetting is, so the interaction between the GMS surfactant and Miglyol® 812 is stronger (Table 11). Furthermore, the stronger the interaction, the less influenced by the temperature, consequently the less the syneresis is. The small θ value (~the good wetting) of Tegin® 90 with Miglyol® 812 corresponded to the small oil number values of G2 organogels.

Table 11. Wetting of the thermally treated surfactants with the oil phase

Organogel	*G1*	*G2*	*G3*	*G4*
Surfactant	Tegin® Pellets	Tegin® 90 Pellets	Tegin® M Pellets	Imwitor® 900
Θ	51.15 ± 0.59	44.33 ± 0.38	47.08 ± 0.39	47.91 ± 0.34

θ recorded at t=17 s. n=7; ±S.E.

4.5.2 Mechanical stability

The mechanical resistance of the dermatological products has an essential importance as regards to the application (removing from the container, spreading on or rubbing into the skin) [103]. It is usually characterized with the static yield stress value, which indicates the shear stress where the samples start to flow or with the dynamic yield stress which determines the transition from the low shear to high shear behaviour.

The yield point can be approximated from the aspect of the viscoelasticity too. The end value of the linear viscoelastic region indicates that the steady–state structure was disturbed or even destroyed. One of the most important features of a physical network is the mobility of the crosslinking sites: in the non-linear viscoelastic region the applied stress can be large enough to separate transient links, the network is destroyed and the sample readily undergoes shear thinning.

The structure of the GMSOs and that of PR were broken down at small stress values, whereas OL, SX and SP showed no changes (Fig. 14). The short linear region of the GMSOs and PR indicate that these structures are fragile. The crystalline gel nature of GMS and hard paraffin networks are responsible for the sensitivity to mechanical strain.

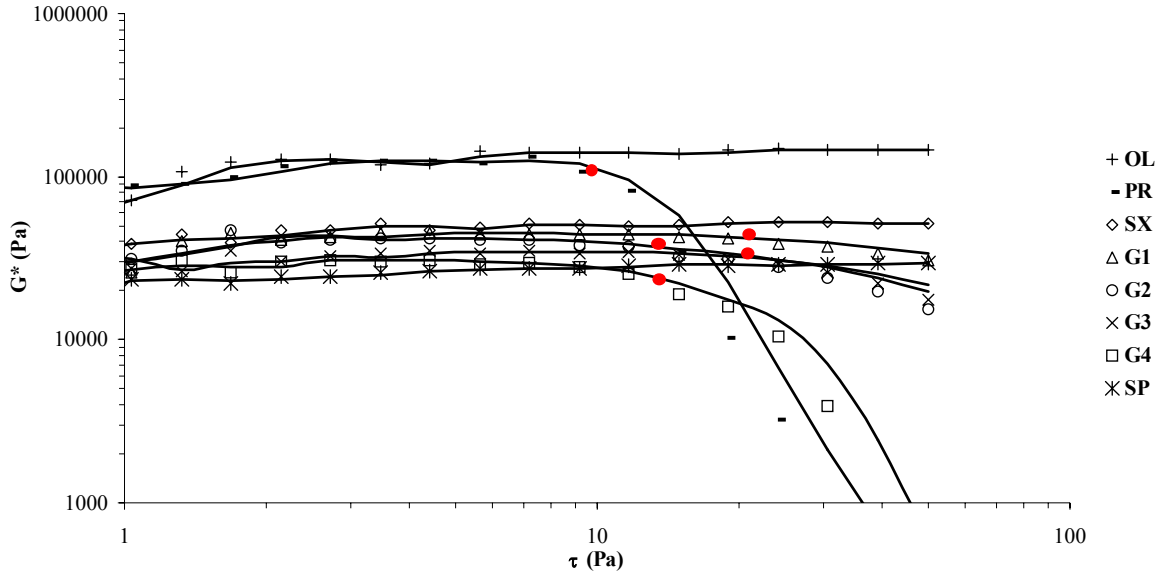


Figure 14. Oscillatory stress curves (GMSOs with 13% w/w GMS); red circles sign the end of the linear viscoelastic region

Regarding to the application, low yield value predicts easy spreadability, which has a paramount importance for a dermal product, especially when applied on inflamed, sensitive skin.

As already mentioned, the fuzzy micelles in SX and OL contain both crystalline and amorphous parts. Together with SP, these structures are highly intermeshed and have more extended linear region indicating that to be more resistant to mechanical strain.

4.5.3 Changes of the structure upon storage

During the storage time (4 weeks) the following changes were observed in the structure and were expressed in the changes of the flow curves (Fig. 16, next page):

On the rheograms of G2, G3, G4 bulges had been expressed soon after the preparation. The bulges on the rheogram relate to the degradation of the network, and this maximum can be accepted as a measure of the strength of the system in which some of the three dimensional structure must break down before the material can significantly flow [104].

The bulges became higher upon storage, which indicated further microcrystal formation and the hardening of the organogels [105]. The larger bulges were associated with greater thixotropy (Fig. 15), because this is the area determined by the up- and down curves. In cases of G2 and G3 both bulge and thixotropy were expressed considerably. On the flow curves of G1 we can not found any bulges, and the thixotropic area decrease in time, which indicate the weakening of the gel structure and syneresis at lower concentration (9% w/w).

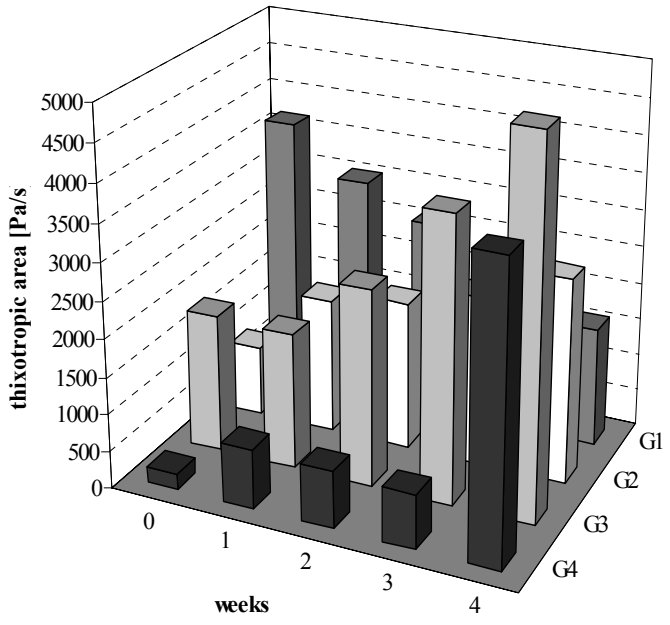


Figure 15. Change of thixotropic area upon storage (13% w/w GMS)

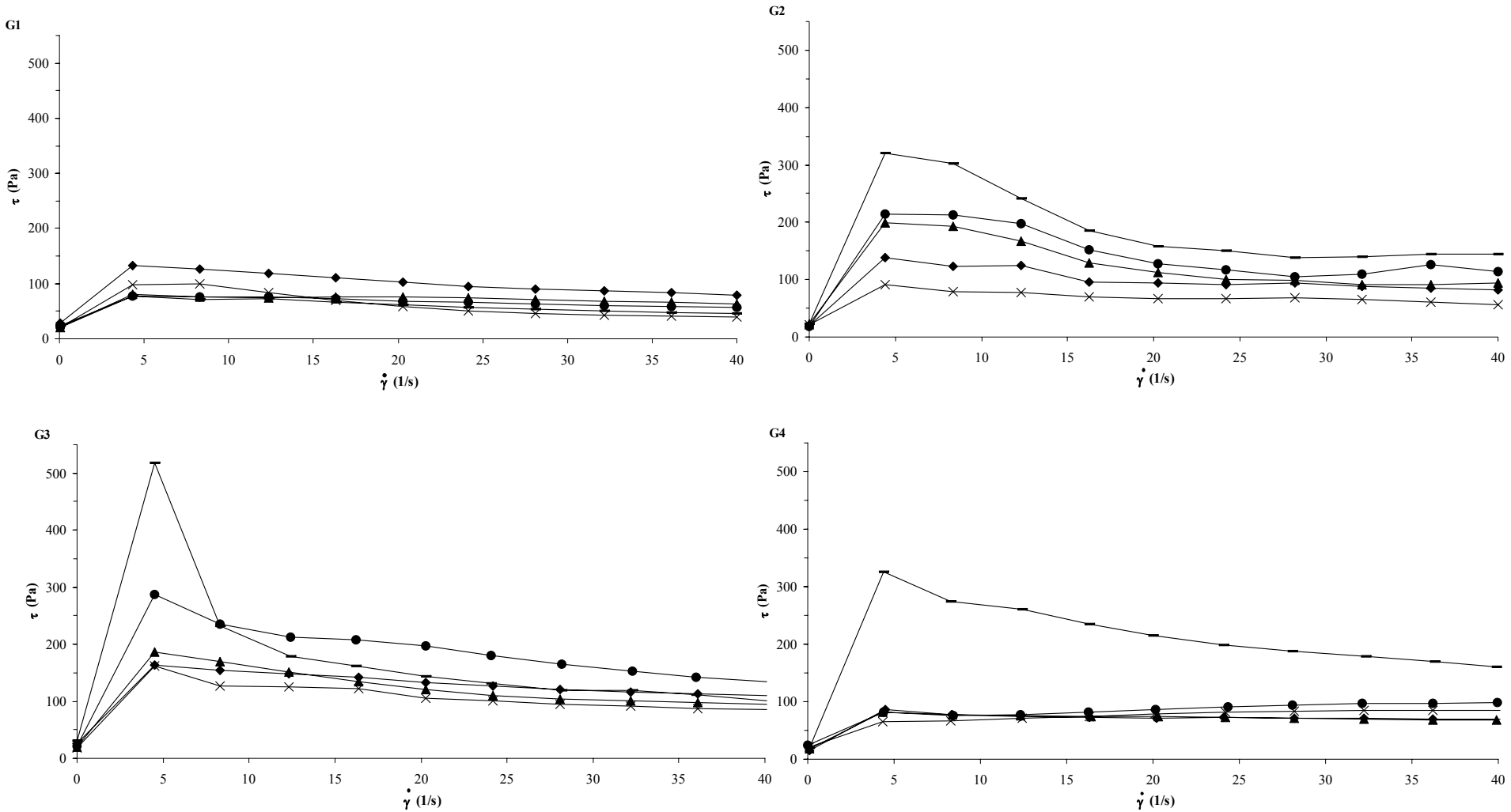


Figure 16. Change of flow curves upon storage time (13% w/w GMS);. × time 0; • 1 week, • 2 weeks; • 3 weeks; – 4 weeks

4.6 Drug release and penetration profile

Piroxicam was used as model drug, which is a NSAID, indicated in case of inflammatory diseases, articular complaints, rheumatoid arthritis and osteoarthritis [106-111]. If these are to be treated topically, a vehicle capable of ensuring the deep skin penetration has to be used. Resulting from their surface active nature, different GMSs included in the organogels can contribute to the overall penetration enhancement of Px [112, 113].

In order to predict the extent of *in vivo* penetration, the penetration coefficient and the *in vitro* penetration were measured. Then the *in vitro – in vivo* correlations was established.

4.6.1 *In vitro* approach for prediction of skin penetration

Fig. 17 shows the cumulative amounts of Px at different diffusion times during *in vitro* penetration. The process has been described by the Higuchi equation [114-117], with good regression coefficients (Fig. 18). This model describes drug release as a diffusion process based in the Fick's law, square root time dependent:

$$Q = \sqrt{D(2c - c_s)c_s t} \qquad (16)$$

where Q is the accumulated drug penetrated after time t per unit area, c is the initial drug concentration, c_s is the drug solubility in the vehicle, D is the diffusion coefficient.

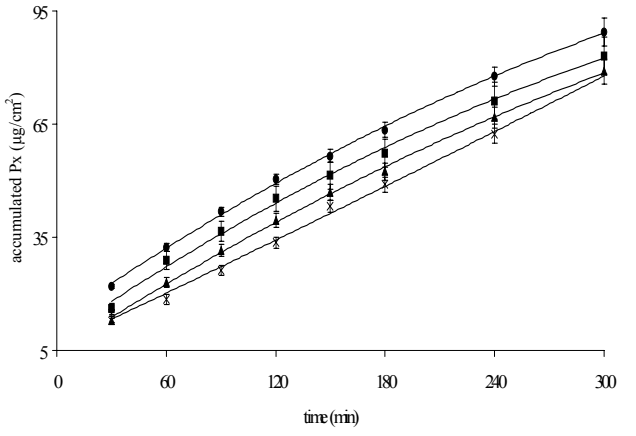


Figure 17. Accumulated amount of Px penetrated vs. time; n=5, ±S.E.

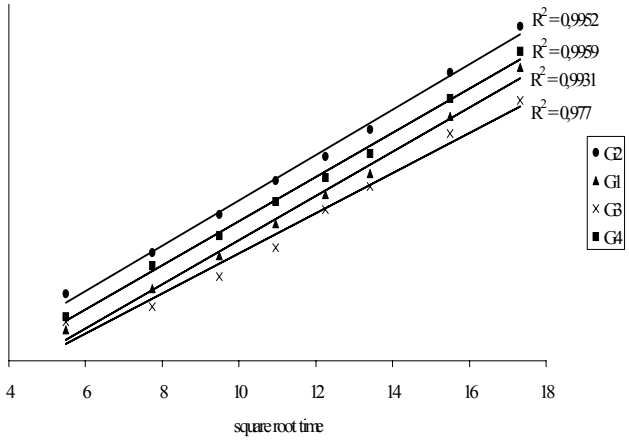


Figure 18. Accumulated amount of Px penetrated vs. square root time; n=5

Table 12. Quantitative comparison of the penetration profiles of Px from different GMSOs; n=5, ±S.E.

	K_p ($\mu g/cm^2/h$)[a]	Q ($\mu g/cm^2$)[b]	J_s ($\mu g/cm^2/h$)
G1	0.2402 ± 0.0798	78.97±3.21	1.5911
G2	0.2461 ± 0.0096	89.43±3.71	1.6302
G3	0.2201 ± 0.0092	70.64±2.88	1.4579
G4	0.2392 ± 0.0110	83.14±4.87	1.5845

[a] slope of the accumulated drug plotted against square root time, [b]Q at t = 300 min

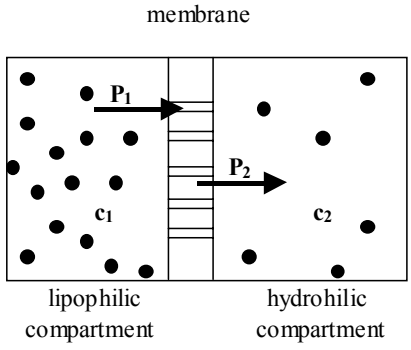
A slightly higher amount of Px penetrated from G2, and both the k_p and J_s were also higher as compared with G1, G3 and G4 (Table 12). However, statistically there were no significant differences between the *in vitro* penetration profiles, so according to this prediction, different GMSs will have similar effect on the penetration of Px *in vivo*.

There is considerable literature evidence that skin penetration can be predicted *in vitro* via log *P*, since the K_p correlates with log *P* (Eq. 3). A good potency of penetration is most probable when log *P* ~2 [118-123]. From the log *P* values (Table 13), the sequence of skin absorption should be G2>G4>G3>G1.

4.6.1.1 Effect of physicochemical properties

The GMSs might influence the *in vitro* penetration by the following mechanisms (Scheme 5):

(i) by increasing the degree of saturation by decreasing the drug solubility in the vehicle (c_1),
(ii) by increasing the partition into the lipophilic layer (P_1),
(iii) by increasing the lipophilic/hydrophilic partition between the lipophilized membrane and the acceptor phase (P_2),
(iv) by increasing the solubility of the poorly water-soluble Px (c_2) in the hydrophilic compartment (acceptor phase) by solubililization.



Scheme 5. The route of Px during the *in vitro* penetration experiment

Table 13. Effect of GMSs on the *n*–octanol/water partition and on the solubility

	c_{oct} (μg/ml)	C_w (μg/ml)	log P	log K_p	S_w (μg/ml)
Px	0.300	0.017	1.2466	-3.8364	1.420
G1	0.348	0.222	0.1953	-4.5828	6.341
G2	0.210	0.002	2.0211	-3.2856	3.762
G3	0.260	0.039	0.8239	-4.1366	1.053
G4	0.224	0.018	1.0950	-3.9441	1.751

In order to ascertain which physicochemical properties govern the *in vitro* penetration [124, 125], the influence of the GMSs on the solubility of Px in the acceptor phase (S_w) and its hydrophilic/lipophilic partition (P) was investigated (Table 13). Investigating the GMSOs under light microscope, Px crystals were detectable in all GMSOs (Fig. 19). Whereas Tegin® and Tegin® M increased the S_w (4.47- and 2.65-times), Imwitor® 900 did not changed it significantly and Tegin® 90 slightly decreased it (0.74-times). Similar changes in c_w occurred during the determination of partition, and the *n*–octanol/water partition of Px was consequently increased

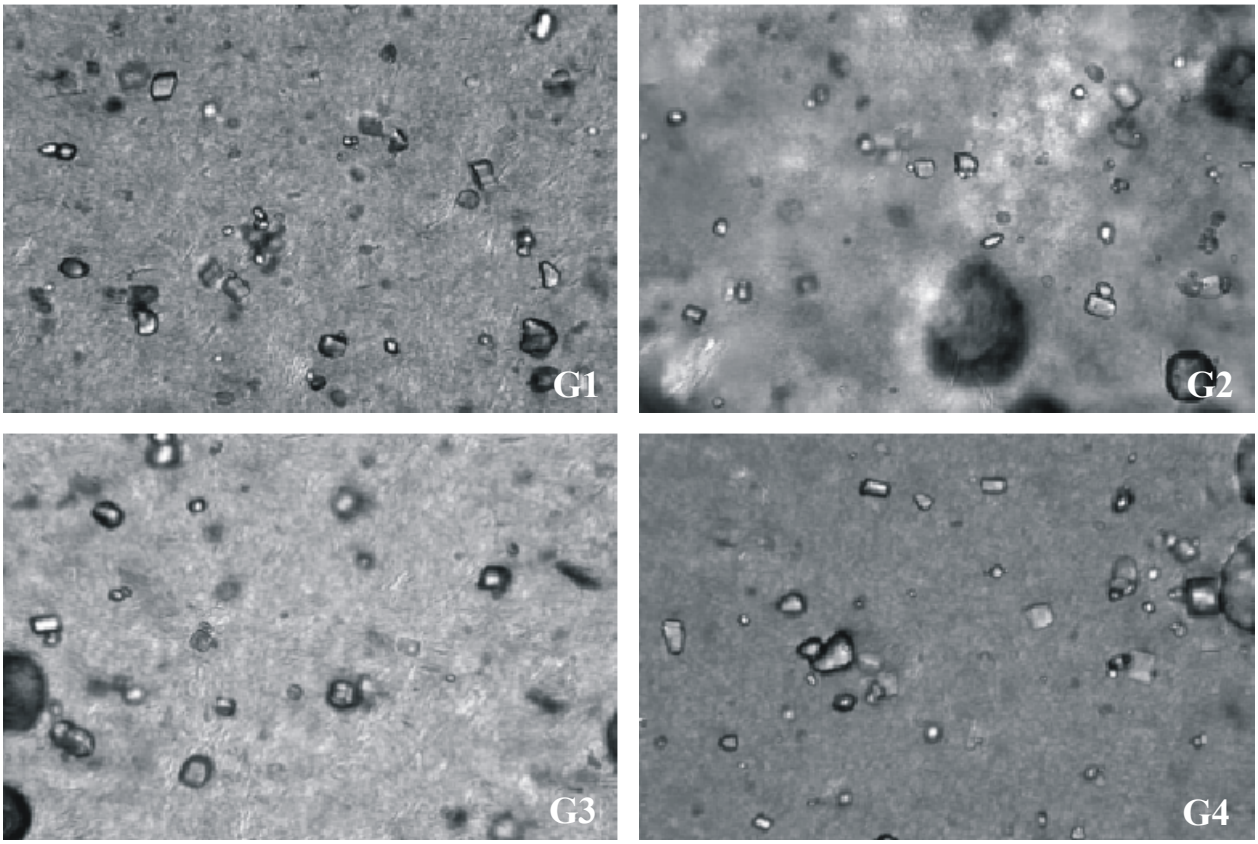


Figure 19. Suspended Px crystals in different GMSOs under light microscope (40×)

by Tegin® 90, but decreased by Tegin®and Tegin® M. Different S_w values did not result in significantly different Q values, probably because the lipophilic–hydrophilic characters of Tegin® and Tegin® M were not favourable for diffusion through a lipophilized membrane. In contrast, the more lipophilic Tegin® 90 might have favoured the penetration, but it did not increase the S_w as Tegin® and Tegin® M did.

4.6.1.2 Effect of viscosity

The diffusion coefficient is partially influenced by the friction. In semisolid matrices, the friction is usually associated with the viscosity, which affects the step when the drug reaches the diffusion interface. The following relationships present the diffusion coefficient as a function of viscosity:

$$D = kT/6\pi\eta r \tag{17}$$

where k is the Boltzmann constant and $(6\pi\eta r)$ is the Stokes' force.

$$\eta = kT/b\pi rD \tag{18}$$

where η is the microviscosity [126].

The viscosity effect on the *in vitro* penetration was estimated by comparing G2 samples with the same Px content (1%), oil phase (Miglyol®) and GMS type (Tegin® 90), but with different amount of GMS (5, 7, 13, 17% w/w), consequently with different η values (29.88, 91.14, 17940, 68090 Pas). Fig. 20 represents that the rate of the diffusion and the penetrated Px amount decreased with the increased η (initial viscosity). Nevertheless, modifying the η in wide range can even produce significant differences in the penetration profile ($p<0.001$, η = 29.88 Pas), but we have to keep in mind the consistency norms and the stability. Since the sample with 29.88 Pas of η is a pourable, pseudoplastic material, it does not have appropriate consistency for dermal use, because it is difficult to handle. Only the samples with η value about 91.14 Pas – 68090 Pas are stiff enough, but in these cases we could not find any significant difference in the Px penetration.

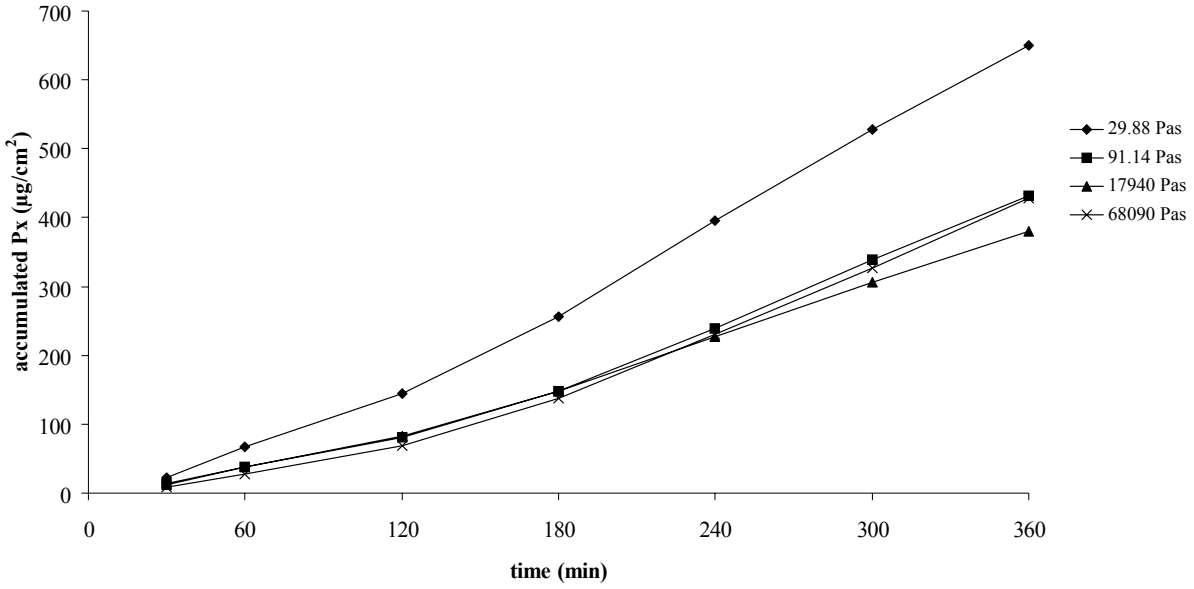


Figure 20. Effect of different viscosity on the *in vitro* Px penetration from G2 organogels

4.6.1.3 Effect of vehicle

As compared GMSOs with the traditional organogels (Table 14), significantly higher Px penetrated from G2 and G4 ($p<0.001$ vs. PR; SX; SP; $p<0.01$ vs. OL), while there was no significant difference between G1, G3 and the traditional organogels (OL, PR, SX and SP). Nevertheless it is not possible to draw general conclusions from this comparison, because the physicochemical state of Px is very different in these compositions. The solubility of Px varies in the different organogel bases (Miglyol® – GMSOs, Virgin castor oil - OL, White petrolatum- SX and Liquid petrolatum - SP), there are big differences in the viscosity values, and whereas some compositions include surface active agents (GMSOs, OL and SX), the others are lack of them (PR and SP). In addition, the surface active agents are different also (GMSs – GMSOs and Lanolin Alcohols – OL, SX). Such differences in the physicochemical state of the drug might lead to different thermodynamical potency of Px in the compositions, which resulted in the different *in vitro* penetration.

Table 14. AUC of the different *in vitro* penetration profiles of Px from deifferent organogels

	G2	G4	G1	G3	OL	PR	SX	SP
AUC (µg/cm²/h)	15965***,**	14445***,**	13160	11940	7855	5124	4882	3274

*** vs. PR, SX and SP

** vs. OL

4.6.2 *In vivo* anti-inflammatory effect

In order to test the anti-inflammatory effect *in vivo*, G1 and G2 were selected. When Px pretreatment was applied locally to the carrageenan-treated area (150 mg of 1% sample), G1 resulted in 48.6%, G2 in 59.4% oedema inhibition as compared with the control group treated with placebo (Fig 21/A). Consequently, both samples proved effective ($p<0.01$), with no significant difference between them. It was found also that Px incorporated in these GMSOs produced not only a local, but also a systemic anti-inflammatory effect. Px pretreatment of the dorsal skin (300 mg of 1% sample) inhibited the acute formation of carrageenan-induced paw oedema (Fig. 21/B), which indicated a systemic effect via transdermal absorption. As compared with the control group, the oedema volume was significantly reduced both by G1 ($p<0.05$) and G2 ($p<0.01$). However, the oedema-inhibiting ability of G1 (27.2%) was slight relative to that of G2 (50.3%). The reason for the difference between G1 and G2 is assumed to be the more lipophilic character of Tegin® 90, which promoted the diffusivity of Px into the skin, and consequently the efficacy of oedema inhibition.

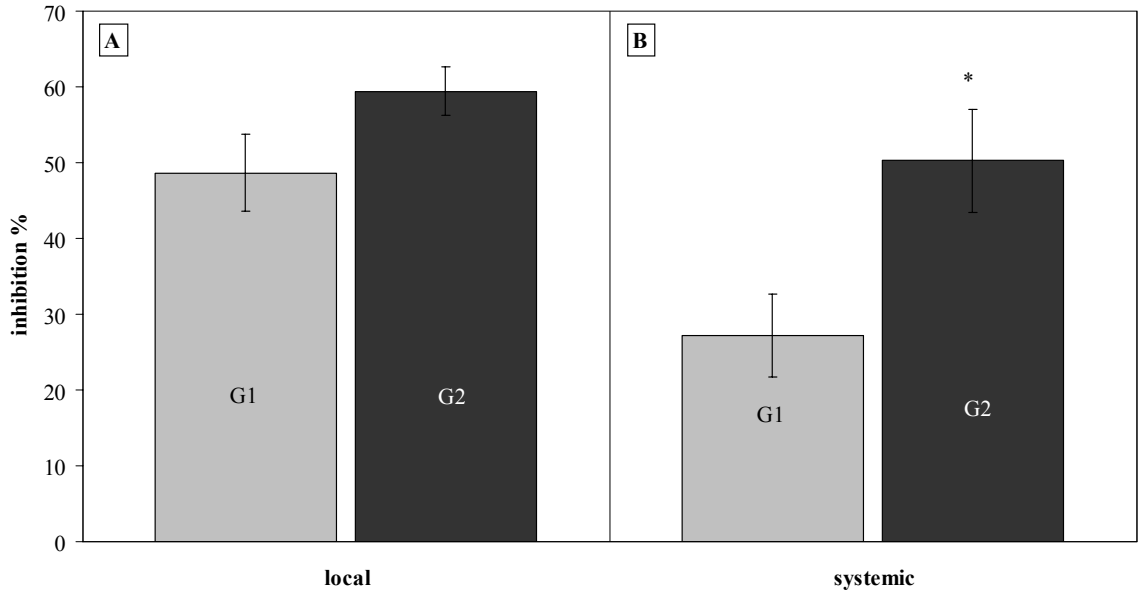


Figure 21. Comparison of extents of inhibition of paw oedema following administration of different organogels to the paw, B: administration of different organogels to the dorsal surface

4.6.2.1 Effect of Px concentration

Figure 22 depicts the systemic oedema-inhibiting effects of Px incorporated in various concentrations into G2. Within the concentration range examined, the degree of *in vivo* oedema inhibition increased with increasing Px dose in accordance with a power law ($r^2=0.8613$).

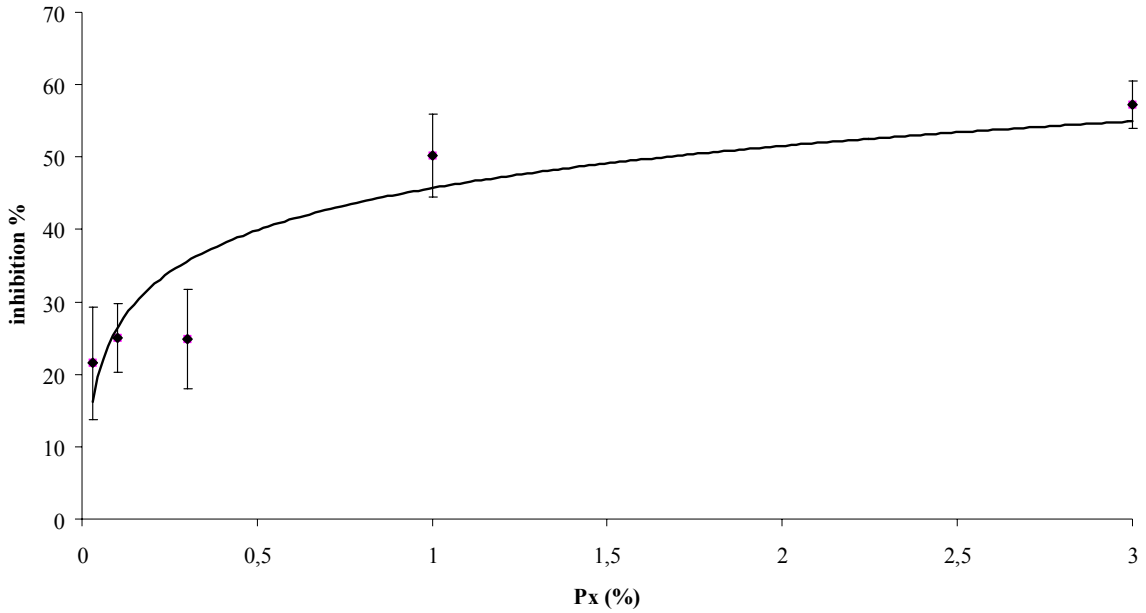


Figure 22. Dose–response relationship of the Px incorporated into G2

4.6.2.2 Effect of the GMS concentration

In order to investigate the amount of the GMSs on the *in vivo* anti-inflammatory effect via transdermal absorption, 11, 13 and 15% w/w of Tegin® 90 were applied. Figure 23 shows that - similarly to the *in vitro* penetration of Px from G2 organogels containing these GMS quantities - changing the Tegin® 90 amount in this concentration range did not produced significant difference in the systemic anti-inflammatory effect.

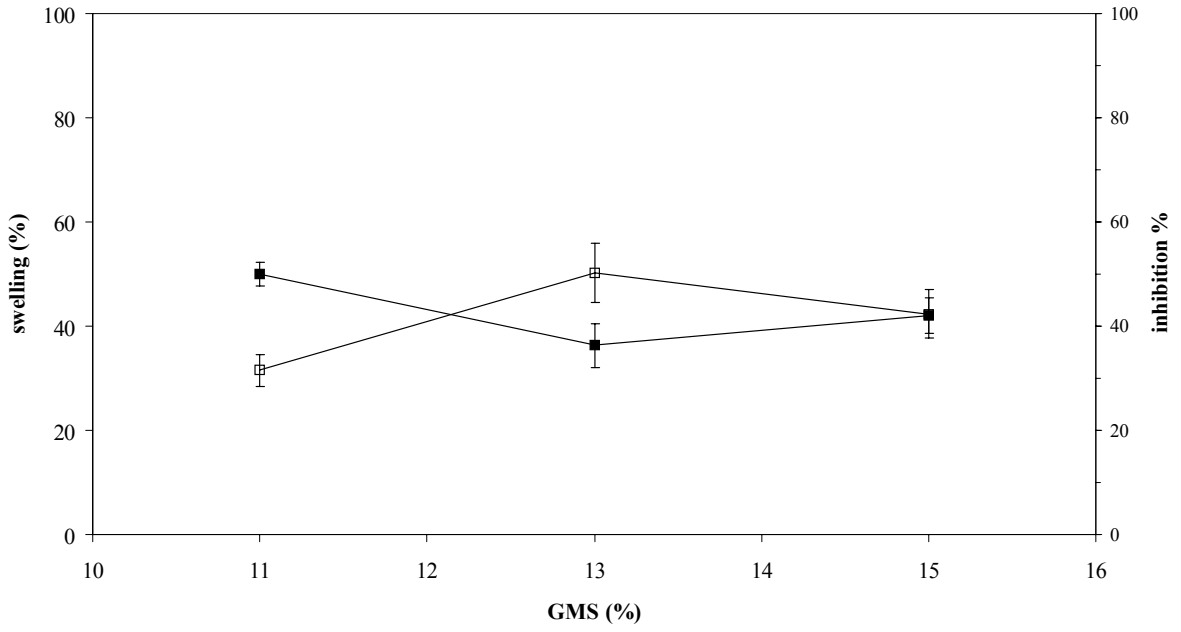


Figure 23. Effect of Tegin 90® amount on swelling and inhibition% following administration on the dorsal skin

Table 15. Comparison of extents of inhibition of paw oedema applying different organogel vehicles

	Local	*Systemic*
G1	48.64±5.1	27.17±5.5
G2	59.40±3.2	50.25±7.2
SP	12.5±7.1	14.33±5.7
SX	36.39±6.8	27.59±6.8

4.6.2.3 Effect of vehicle [127-131]

The anti-inflammatory effect of G1 and G2 was compared with those of two widely-applied lipophilic vehicles (SX, SP), which are indicated for drug delivery in order to treat rheumatic, inflamed areas. When used locally, G1 and G2 were more effective than SX ($p<0.001$), but they did not exhibit a significant difference as compared with SP (Table 15). As concerns the *in vivo* systemic effect, G2 was more effective than SX ($p<0.001$) and SP ($p<0.05$), but there was no significant difference between G1 and either SX or SP. The *in vivo* experiments revealed that the *RBA* of G2 was better than those of SP and SX (1.81; 3.51), while the *RBA* of G1 was better only than that of SX (1.89).

Generally, hydrophilic vehicles are preferred to the lipophilic ones because of the ease of washability and the fewer residues left on the skin. Regarding to these advantages hydrophilic systems as well as pretreatment techniques [132-135] were developed in order to achieve better skin absorption of Px [136-140]. However, bicomponent GMSOs are biocompatible and economic systems and their overall liking was found good in sensory evaluation. Thus they provide a notable solution for Px topical delivery. The oedema–inhibiting effect (via transdermal absorption) of G2 was compared with those of an o/w cream and a hydrogel containing carbamid as penetration enhancer (Fig. 24). The GMSO showed approximately the same oedema inhibition ability than the hydrogel and was found better than the o/w cream.

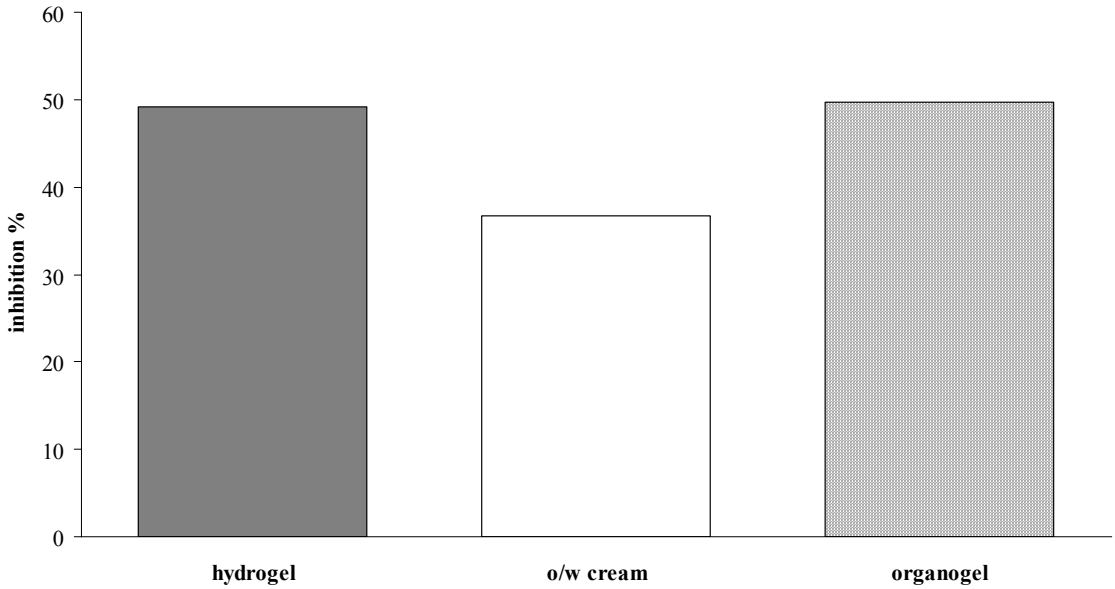


Figure 24. Comparison of extents of inhibition of paw oedema following administration of different vehicles to the dorsal skin

4.6.3 *In vitro – in vivo* correlation

As mentioned earlier, the optimum log *P* for NSAIDs is ~2. Below this value the absorption rate increases, while above it the absorption rate decreases. From the log *P* values calculated, the sequence of skin absorption should be G2 > G1. This is confirmed by the *in vivo* data, because log *P* for Px was closer to the optimum (2.0211) in the presence of Tegin® 90, and the skin penetration of Px was better from G2.

However, a different tendency was observed among the organogels as regards their *in vitro* penetration and *in vivo* oedema inhibition effects. *In vitro*, there was no significant difference between the G samples, while *in vivo* G2 proved to be better than G1 regarding to both local and systemic oedema inhibition effects.

The reason for the *in vitro* penetration and *in vivo* absorption differences could stem from the different natures of the model barriers used [141-144]. Cellulose acetate soaked in isopropyl myristate is an inert membrane *in vitro*, which interacts neither with the active agent nor with the vehicle. Thus, this method models the vehicle/*stratum corneum* and the *stratum corneum*/*dermis* partitions. However, *in vivo* absorption is more complex, because penetration enhancers such as glyceryl fatty acids can interact with the multilamellar lipid matrix of the *stratum corneum*; the skin is therefore regarded as an active barrier. Accordingly, conclusions must be drawn carefully from comparisons between the results of application of a synthetic barrier *in vitro* for the prediction of *in vivo* skin penetration.

5 SUMMARY

During my research for novel organogel compositions I have found that:

- Glyceryl monostearate (GMS) is a surfactant which gells several oils both of apolar and semipolar natures, and results in stable organogels already at low concentrations. The benefits of using GMSs are:
  - less risk of skin irritancy because of their nonionic nature,
  - multifunctionality because they act both as gelator and penetration enhancer. Reduction of the number of the ingredients contributes to the ease of formulation and less skin irritancy.
- Commercially available GMSs are obtained from different sources and by different methods, so they have different composition regarding to the mono- and diester type and ratio. Thus they have different organogelator behaviours and their organogels have different texture, stability and drug release characteristics.
  - The sensory attributes are determined by the surfactant included.
  - Oil number correlates with the surfactant wettability with the oil.
  - Upon storage the structure of G2 – 4 organogels show hardening while G1 organogel shows weakening.
  - Piroxicam penetration correlates with the log *P* changed by the surfactants.
- The gel formation of the glyceryl monostearate organogels (GMSO) is thermoreversible and the gel point and the gelation kinetcs depend on the surfactant (i.e. gelator) type and concentration.
- Investigation of structure, consistency and stability of GMSOs revealed that:
  - Under light microscope the heterogeneous network structure can be clearly recognised. The rod-like GMS aggregates are connected to each other and this network intermeshes throughout the oil.
  - Changing the ratio of the two ingredients will change of the yield value, viscoelasticy, viscosity, spreadability of the bicomponent GMSOs. From the aspect of the product optimization, this provides a quite simple way.

- 13% w/w surfactant results in optimal consistency for dermal pharmaceutical application independently from the surfactant type. As comparing to some traditional organogels, the overall liking of GMSOs is found to be better than those of SX and OL. The overall liking is correlated with the macroscopic appearance and the skin feel.
- The network energy of GMSOs are moderate. The fine crystalline structures building up the gel network resulted in fragility, thus GMSOs show low yield values.
- As compared to the traditional organogels, the thermal stability of GMSOs is small, except of those of G2.

- Px incorporated into G2 exhibits a notevole inhibition of oedema either when applied locally, or via transdermal absorption. Comparisons with traditional galenic organogels revealed that the relative biological availability of Px was better from G2 organogels. Thus Tegin® 90 could be suitable enhancers in Px formulations.
  - The extent of *in vivo* skin absorption is in accordance with the *in vitro* penetration coefficient. However, the *in vitro* penetration through a synthetic membrane did not correlate with the *in vivo* results, the reason for which might be the different natures of the model barriers.

In conclusion, different GMSOs offer pharmaceutical or skin care formulators an opportunity to develop a variety of topical applied vehicles. These GMSOs show distinct advantages over traditional organogelators when formulators are searching for new tools to formulate novel products with multifunctionality.

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