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REGULATORY EVALUATION OF DRUG RELEASE FROM DERMAL SEMISOLID DOSAGE FORMS

PhD Thesis

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ARTICLES RELATED TO THE PHD THESIS

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VI. Anita Kovács, **Éva Petró**, István Erős, Ildikó Csóka: *The role of dissolution testing in quality control*. Per. Pol. Chem. Eng. 58 (2014) 17-20. **IF: 0.269**

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I. **Petró Éva**: *Normák és irányelvek szerepe a félszilárd gyógyszerformák hatóanyag kioldódásának validálásában*, PhD Tudományos Nap, Szegedi Akadémiai Bizottság Székháza, 2008. november 27., Szeged

II. **Petró Éva**, Csóka Ildikó, Erős István: *Félszilárd gyógyszerformák hatóanyag kioldódásának validálása*, Congressus Pharmaceuticus Hungaricus XIV., 2009. november 13-15., Budapest, Gyógyszerészet 53. 2009/11 Suppl. I., S100. (P-70.)

III. **Éva Petró**, Ildikó Csóka, István Erős: *Validation process of drug release from semisolid dosage forms.* 7th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, March 8-11 2010 Malta, Valletta (P206.)

IV. **Éva Petró**, Ildikó Csóka, Ágnes Balogh, Gábor Blazsó, István Erős: *In vitro and in vivo evaluation of drug release from semisolid dosage forms*. 8th Central European Symposium on Pharmaceutical Technology and 4th International Graz Congress for Pharmaceutical Engineering, September 16-18 2010 Graz, Austria (PDD08)

V. **Petró Éva**, Csóka Ildikó, Balogh Ágnes, Blazsó Gábor, Erős István: *Félszilárd gyógyszerformák in vitro és in vivo vizsgálata*, Magyar Gyógyszerésztudományi Társaság XVI. Gyógyszertechnológiai Konferencia és VIII. Gyógyszer az Ezredfordulón Konferencia, 2010. október 20-22., Siófok (EA-22)

VI. Baracsi Zita, **Petró Éva**, Erős István, Csóka Ildikó: *In vitro hatóanyag felszabadulási vizsgálatok validálása Franz vertikális diffúziós cella és USP 2 kenőcscella esetén*, Magyar Gyógyszerésztudományi Társaság XVI. Gyógyszertechnológiai Konferencia és VIII. Gyógyszer az Ezredfordulón Konferencia, 2010. október 20-22., Siófok (P-3)

VII. **Petró Éva**, Csóka Ildikó, Erős István: *Félszilárd készítmények hatóanyag liberációjának meghatározására szolgáló módszerek (diffúziós cellák) összehasonlító vizsgálata*, Magyar Kémikusok Egyesülete Kozmetikai Szimpózium 2010, 2010. november 18., Budapest

VIII. **Éva Petró**, Ágnes Balogh, Gábor Blazsó, István Erős, Ildikó Csóka: *In vitro and in vivo investigation of drug release from semisolid products in case of synthetic membrane and rat skin*. Skin Forum 12th Annual Meeting, March 28-29 2011 Frankfurt, Germany (P24.)

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X. **Éva Petró**, István Erős, Ildikó Csóka: *Dissolution testing of semisolid dosage forms*. 8th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, March 19-22 2012, Turkey, Istanbul (P162.)

XI. **Petró Éva**, Erős István, Csóka Ildikó: *Hatóanyag felszabadulás félszilárd gyógyszerformákból – módszerek és eszközök*, Molekulától a gyógyszerig, Szeged, 2012. május 24-25.

XII. **Petró Éva**, Csóka Ildikó, Erős István: *Ex vivo módszerek a biológiailag aktív anyagok liberációjának kutatásában*, Magyar Kémikusok Egyesülete Kozmetikai Szimpózium 2012, 2012. november 22., Budapest

XIII. **Petró Éva**, Erős István: *Biológiailag aktív anyagok szabaddá válása krémekből, gélekből és gélemulziókból*, Magyar Kémikusok Egyesülete Kozmetikai Szimpózium 2013, 2013. november 21., Budapest

XIV. **Petró Éva**, Csóka Ildikó, Erős István: *Félszilárd dermatológiai mátrixok gyógyszerleadását meghatározó tényezők,* Congressus Pharmaceuticus Hungaricus XV., 2014. április 10-12., Budapest (P-116.)

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ABBREVIATIONS

API	Active Pharmaceutical Ingredient
DS	Diclofenac sodium
ECVAM	European Centre for the Validation of Alternative Methods
FDA	Food and Drug Administration
F(V)DC	Franz (vertical) diffusion cell
GE	Gel-emulsion
GLP	Good Laboratory Practice
HG	Hydrogel
IPM	Isopropyl myristate
IVIVC	*In vitro-in vivo* correlation
IVRT	*In vitro* release testing
OG	Organogel
o/w//OW	Oil-in-water
PD	Pharmacodynamic
PDMS	Polydimethylsiloxane
Ph. Eur.	Europen Pharmacopoeia
PTFE	Polytetrafluoroethylene
PTR-2	Pemulen^{TM} TR-2
QC	Quality control
REF GE	Reference gel-emulsion
REF HG	Reference hydrogel
SC	*Stratum corneum*
SD	Standard deviation
SUPAC-SS	Scale-Up and Postapproval Changes Semisolids
USP	United States Pharmacopoeia
w/o//WO	Water-in-oil

1. INTRODUCTION

In the pharmaceutical industry *in vitro* dissolution testing - which methodology has been established for solid dosage forms - is an important tool in both drug development and quality control (QC) [1]. Dissolution testing is frequently used also in the examination of other dosage forms and it is quite widespread in case of semisolid dosage forms [2].

Properties of and drug release from semisolid preparations have recently found the interest of researchers from application [3-6] and regulatory perspectives [7] as well as regarding their effects on the skin [8, 9]. Although many *in vitro* methods are used for studying the penetration through the skin [10-14], moreover, guidelines and recommendations are also available [1, 15-17], in contrast to solid dosage form testing, there are neither generally accepted pharmacopoeial methods available to date for their *in vitro* characterization nor adequate *in vivo* sampling techniques for their investigation [18].

The goal of regulatory authorities is reducing the number of animal studies and substitute their use with *in vitro* investigations. At present, *in vitro* studies are not required by regulators. Methods for measuring percutaneous absorption are: studies with mathematical models, model membranes, *stratum corneum*, keratome slices, perfused or whole skin and *in vivo* techniques. Models for them can be: mouse, rabbit, rat, guinea pig, swine, primate and human. The confidence level is high in case of *in vivo* investigations and use of human skin. The physiological hierarchy is increasing with decrease of hairless strains. From the above mentioned list I chose the model membrane and *in vivo* study for my investigation [19]. There have been several methods to predict drug penetration in humans with human [20, 21] and animal *in vitro* models, although animal skin (mouse [22, 23], rat [24, 25], pig [26, 27], guinea pig [28, 29], rabbit [30, 31], snake skin [32, 33] tend to be more permeable than human. Rat abdominal skin has been shown to be a reasonable model based on the *in vitro* static cell experiments [34, 35]. Fundamental requirement for these studies is the establishment of the *in vitro*-*in vivo* correlation (IVIVC) [36, 37] posing a major challenge not only for solid, but also for semisolid dosage forms [13, 38-40].

The guidelines mostly recommend the use of Franz vertical diffusion cell [41] as the *in vitro* testing apparatus which is widely used by researchers as an analytical tool [42, 43, 44]. However many critical points were observed in connection with the methodology itself such as the very low amount of receiving medium, the complicated tube system and the possibility of bubble formation. They emphasize the necessity to validate the method.

Another apparatus to study *in vitro* drug release from semisolid preparations has recently been offered. Hanson Research Company has extended its SR8-Plus Test Stations for a small-volume system. This device is an adaptation of the United States Pharmacopoeia (USP) Apparatus 2. It has a special small-volume vessel (Hanson Ointment Cell [45], modified holding cell) and a mini-paddle. The former includes a donor chamber for topical drug application. After mounting a selected membrane on the mouth of the chamber it is immersed in the receiving medium before starting the test [46].

The technical differences between the Franz vertical diffusion cell (which will referred to as Franz cell) and the modified holding cell - mini-paddle system (which will be referred to as the modified USP) are as follows (Franz cell *versus* modified USP):

- cell volume: 7 ml (which is most commonly used) *versus* 70 ml,
- sample volume: 800 µl (replaced with fresh receiving medium) *versus* 2.00 ml (not replaced),
- semisolid sample amount: 0.24-1.65 g *versus* 0.40-0.70 g,
- stirring rate: 450 rpm *versus* 100 rpm,
- sampling: automated *versus* manual.

The sample surface is 1.767 cm^2 in both cases.

For quality control and product development purposes, however, simple (non-impregnated), inert, porous synthetic membranes are recommended [42]. In my study a cellulose acetate membrane of 0.45 µm average pore size was used with and without isopropyl myristate (IPM) impregnation.

Despite the increasing number of publications within this field, no standard experimental conditions are used, therefore the comparison of the data is very difficult.

2. LITERATURE SURVEY

2.1. Formulation and characteristic features of semisolid dosage forms

2.1.1. Main representatives of dosage forms for dermal drug delivery

Semisolid dosage forms including creams, ointments and gels [47] are dermatological formulations which are commonly used for local and regional skin disorders. Usually the ointments are applied to the skin and mucous membranes [10, 48-51]. Semisolids can change their shape, because they have a plastic behaviour [52-55], except gels with viscoelastic effect because of showing liquid and solid properties [56, 57].

Creams can be rubbed into the skin. They are homogenous, soft and spreadable [48]. They have two types depending on the internal phase: oil-in-water creams (o/w creams) or hydrophilic creams and water-in-oil creams (w/o creams) or hydrophobic creams [52]. The base of the o/w creams is miscible with water. They contain o/w emulsifying agents, e.g. sodium or triethanolamine soaps, sulfated fatty alcohols, and polysorbates. W/o creams are anhydrous. They contain emulsifying agents, e.g. wool fat, wool alcohols, sorbitan esters and monoglycerides [10, 50]. Because creams have a softly texture, they are often used in the cosmetic industry [51, 55].

Ointments such as creams are homogenous. The most common forms of ointments are hydrophobic, water-emulsifying and hydrophilic. The hydrophobic ointments are anhydrous. Their bases are hydrocarbons and paraffin in hard, soft and liquid form, vegetable oil, animal fats, waxes, synthetic glycerides and polyalkylsulfones. Water-emulsifying ointments consist of hydrophobic fatty bases. The w/o emulgents are wool fat, woot alcohols, sorbitan esters, monoglycerides and fatty alcohols. With use of them the cream will be hydrophilic. Consisting of an aqueous solutions they are called as w/o emulsions. The hydrophilic ointments have a base which is miscible with water [10, 48, 50]. They protect the skin and are used as emollients [51, 55].

Gels or jellies [47] consist of inorganic or organic molecules and liquid. They are a two-component system [48, 49]. The liquid phase builds a three-dimensional polymeric matrix which results a physical or chemical cross-linking [52]. The continous structure results solid like behaviour. They are usually homogenous and clear. Gels have two types: organic solvent based, hydrophobic or organogels and water based, hydrophilic or hydrogels [51, 55, 56]. The hydrophobic gels have a base consisting of liquid paraffin with polyethylene or fatty oils gelled with colloidal silica, aluminium or zinc soaps. They have a base consisting of water,

glycerol, or propylene glycol gelled with natural materials such as tragacanth, carrageen, pectin, agar, alginic acid and starch; semisynthetic agents are: cellulose derivatives such as methylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose, carboxyvinyl polymers, e.g. carboxymethylcellulose and magnesium aluminium silicates [50]; synthetic polymer is Carbopol. The cellulose derivatives form a colloidal solution. They disperse in water and because of acidic pH the solution have to neutralized by amines, e.g. triethanolamine or bases, e.g. sodium hydroxide. Depending on pH range Carbopol have different grades, e.g. Carbopol 934, 940, etc. Because of the appropriate concentration the consistency of the gel is constant and stabil [10].

2.1.2. Dosage form testing methodologies: rheology measurements

Rheology is the study of how matters deform and flow under the influence of external forces. The deformation is influenced by the inner structure that is why rheological investigations can describe different materials [58].

Rheological analysis is a useful tool to follow product parameters during the development. It is a well known methodology in characterization of semisolid dosage forms [59, 60]. The technology provides indirect information on the structure and consistency [61, 62]. The latter includes viscosity and drug release [10, 63].

It shows the reproducibility of manufacturing process, that is why in my study the reproducibility of my products was first tested with rheological measurements. It characterizes the dosage form and provides information about the drug release rate of the formulations. The use of semisolids is an important parameter from point of the patient. Because of consistency characteristics, rheological behaviour is the most predicting tool in drug development and can be used also as quality control tool, therefore they have effect not only on spreadability, but also on use of products and their biopharmaceutical parameters. (See next chapter.)

2.2. Biopharmaceutical evaluation of dermally used semisolids

2.2.1. *In vivo* methods

2.2.1.1. Animal models in general

Ethical considerations limit the use of humans [46] that is why animal studies are widely offerred. They have many advantages: they are available and act as a physiologically intact system. In animal research studies a Good Laboratory Practice (GLP) must be demonstrated.

The disadvantage of its using, that animal skin is more permeable than human skin is. Frequently rat skin is used in the research [19, 24, 25, 34, 35]. Other types of animal skins in the field of percutaneous testing are: mouse [22, 23], pig [26, 27], guinea pig [28, 29], rabbit [30, 31] and snake skin [32, 33]. However the anatomy, physiology and biochemistry of the human skin is hard to demonstrate replacing it with animal skin [10, 64].

Figure 1 represents the physiology hierarchy of models for percutaneous absorption.

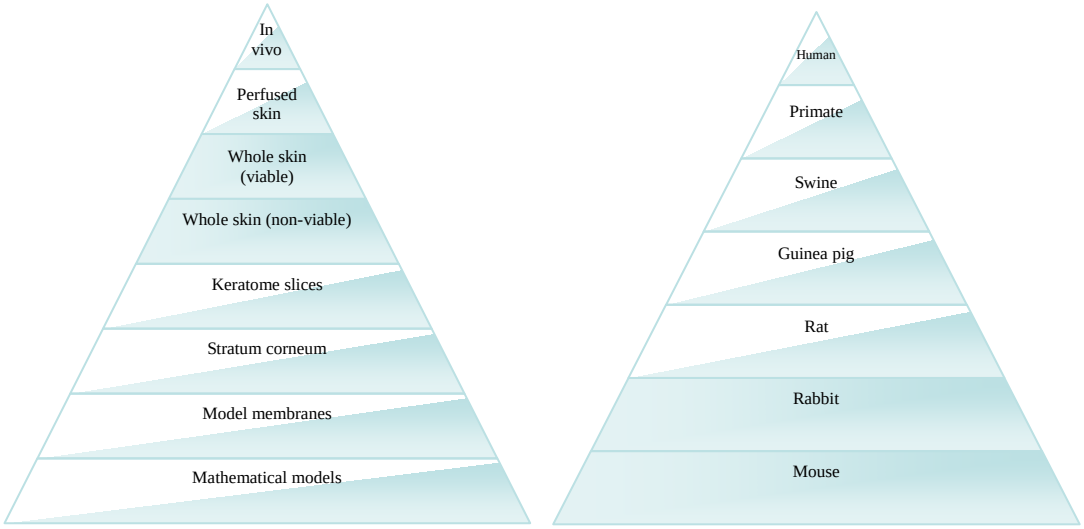

Figure 1 Physiology hierarchy of models for measuring percutaneous absorption [19]

2.2.1.2. Pharmacodynamic studies

In vivo behaviour is the consequence of both polar and apolar ways of absorption.

Many *in vivo* techniques are available to test the normal and diseased skin. *In vivo* tests are able to measure kinetic parameters of drugs, but they are nonspecific or traumatic to be used in both normal and diseased skin [65]. The cost, the technique and ethical problems limit the use of this test in humans. This method requires a lot of subjects and samples, which leads to invasiveness.

The test must be invasive and provide an adequate concentration in the target organ. The systemic circulation must be monitored and demonstrated also with clinical studies. The type of test depends on the pharmacological activity of the drug and dosage form. Some difficulties obtain during the process. If animal is used in the study, the permeation must be determined. *In vivo* tests employ physiological end point, although *in vitro* methods monitor

steady state fluxes across the intact *stratum corneum*. Drugs can penetrate the living skin through the shunt routes, but *in vitro* tests are not able to measure it accurate. The drug can be metabolized in the living organism during crossing the tissues of the skin. In spite of that fact, *in vitro* methodologies determine the absorption indirectly. When using laboratory animals, the permeation rate is often increased because of shaving them. Animals must be also prevented from ingestion or inhalation of the drug [10].

In spite of a large number of new technologies there are no adequate testing in order to demonstrate bioavailability and bioequivalence of dermal formulations. [18].

There are different factors which influence the process in the drug therapy, as seen in Table 1.

Table 1 Factors influencing percutaneous absorption [10, 19, 66]

	Parameters
Drug substance	• Molecular weight • Diffusion coefficient • Water/lipid partition coefficient • Permeability coefficient • Ionization
Vehicle	• Solubility/polariry • Volatility • Concentration • Distribution in a stratum corneum • Excipients • Penetration enhancer • PH
Skin	• Species • Age, sex, race • Anatomy • Temperature • Hydration of stratum corneum • Damage of stratum corneum • Metabolism
Site of application	• Skin area dose (film thickness, concentration) • Total skin area in contact with vehicle • Duration of exposure

2.2.2. *In vitro* models

2.2.2.1. Penetration/absorption models

The *stratum corneum* is an impermeable barrier, although many efforts have been made with penetration enhancers to reduce its resistance [67].

Figure 2 shows the absorption models which are available for evaluation of semisolid dosage forms. This model studies the percutaneous absorption of drugs. Depending on the type of the skin, with this model *in vitro* and also *in vivo* techniques can be studied [68].

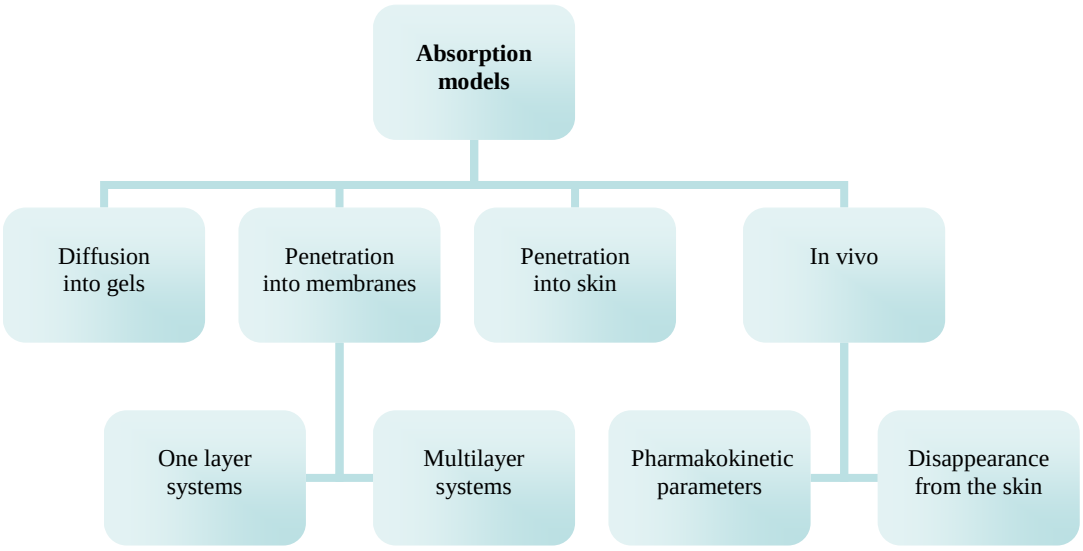

Figure 2 Absorption models [68]

In vitro penetration studies can be performed with products by measuring the diffused drug amount through synthethic membranes soaked in IPM, a penetration enhancer which can be used as a surrogate of different animal skin models. IPM is a liquid which mimics the skin lipids [64] and is an appropriate model for percutaneous absorption [69, 70]. A simple IPM model can simulate the partition phenomenon, which occurs when absorbing through the skin. That is why the absorption is higher with use of IPM impregnated membranes than with phosphate buffer soaked membranes. According to the FDA SUPAC-SS guideline, 1997 [16] and study of Siewert, 2003 [47], the Franz diffusion cell mounted with IPM impregnated membrane was found to be proper for prediction of *in vivo* results [71]. The with IPM impregnated membrane measured results will be between the *in vivo* and drug release data.

2.2.2.2. Testing of drug release

In vitro testing can be used prior to *in vivo* animal and human testing also at semisolids. Drugs, chemicals and cosmetics can be investigated with this method. Advantages of the technique are: human skin can be used, which decreases the number of living animals using in research. With *in vitro* testing the 3 R imagination can be carried out, so the use of laboratory animals can be reduced, refined and replaced. The European Centre for the Validation of Alternative Methods (ECVAM) - which was established in 1993 - promotes the regulatory acceptance and validation of alternative methods.

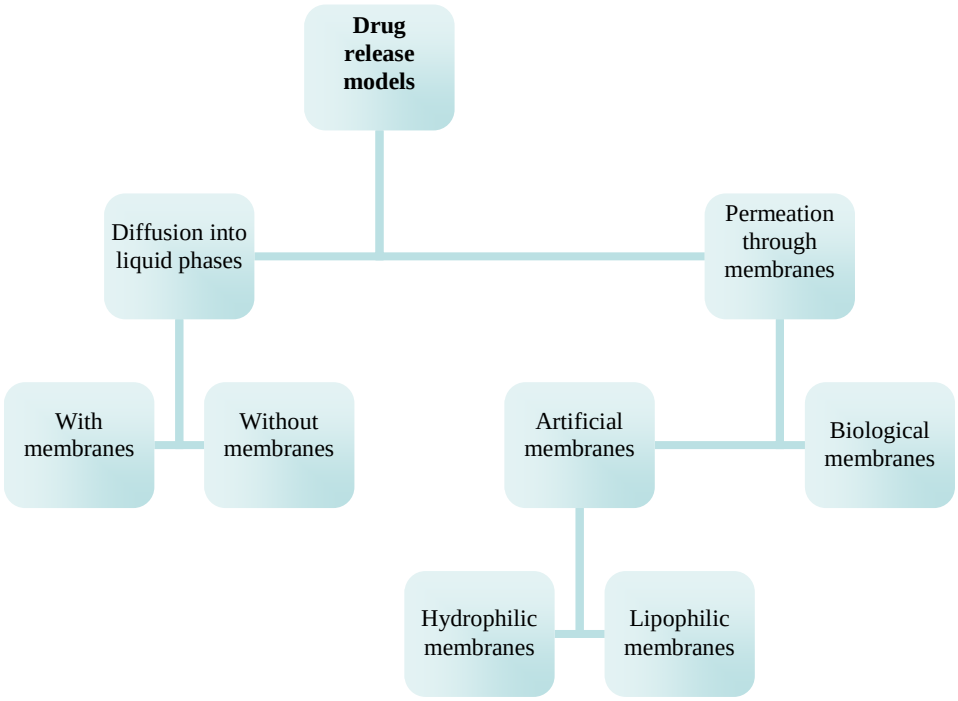

Figure 3 Release models [68]

Figure 3 shows the drug release models.

For measuring drug release a donor and acceptor compartment are necessary. The donor compartment consists of the testing formulation and the receptor medium can be found in the acceptor compartment. The model is performed with or without a membrane. In the case membrane is used, it separates the test material from the acceptor phase [68]. The schematic representation of it is illustrated in Figure 4.

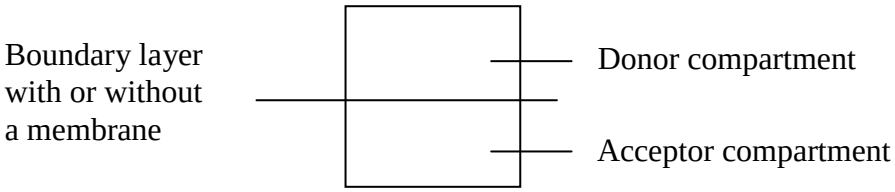

Figure 4 Schematic representation of drug release model [68]

Dissolution testing is a commonly used method in drug release studies. It was developed first for solid dosage forms and its using is widened for semisolid dosage forms nowadays. In case of semisolids dissolution is called *in vitro* release testing (IVRT), because the active ingredient is in dissolved state in the vehicle and it has to diffuse and release by the vehicle and penetrate into the skin. In the last two decades IVRT has became more important by testing semisolid dosage forms [4]. The components of an *in vitro* release test are: the assay, the apparatus, the conditions – temperature, membrane, acceptor or receiving medium, sampling time and analysis of the data [45]. After selection these elements, the drug release test has to be validated following the next parameters: repeatability, reproducibility and robustness. Repeatability means the cell-to-cell variability. Reproducibility is the variability of the measurement system in different days. Robustness is keeping the experiment against changes [72].

Many efforts have been made to test the *in vitro* drug release of semisolid preparations. The Franz vertical diffusion cell is the “gold standard” in the research [72] offered by the guidelines [73]. Another type of apparatus is the modified holding cell or Hanson Ointment Cell [45, 46]. Semisolid testing is possible in both apparatuses with using a synthetic membrane - soaked in the receiving medim, usually in phosphate buffer -, choosen according to the type of semisolid, although the cell volume is different in the two equipments (7 ml *versus* 70 ml). Representations of the cells are presented in Figure 5.

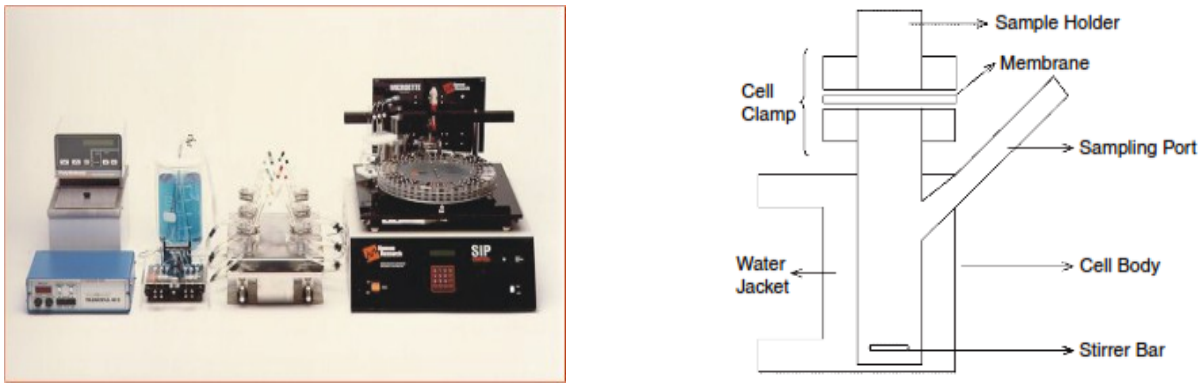

Figure 5a) Representation of Franz vertical diffusion cell [46, 73, 74]

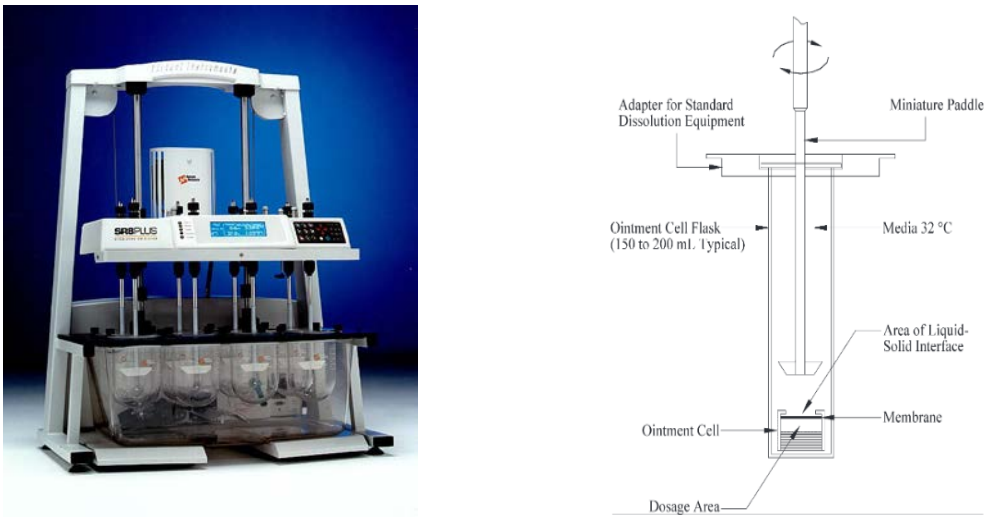

Figure 5b) Representation of modified holding cell or Hanson Ointment Cell [46, 73, 74]

Semisolid dosage forms are mainly tested by the Franz vertical diffusion cell with and without a membrane and by the enhancer cell [1, 73].

In vitro release tests are often used to assure product or batch-to-batch quality and performance. They can reflect the physical and chemical properties of the active ingredient and rheologyical behaviour of the dosage form [16, 38]. They can screen the compositions prior to *in vivo* animal testing, although there are many anatomical and physiological factors, which are not properly represented under *in vitro* conditions [10, 75].

2.2.2.3. Testing of drug release with synthetic membranes or without membranes

2.2.2.3.1. Drug release models with membranes

Membranes have two types: they can be porous or lipid membranes.

2.2.2.3.1.1. Porous membranes

Types of porous membranes are: cellophane, cellulose derivatives, nephrophane, hydrogel, fluorinated vinyl polimer and polycarbonate. Disadvantages of porous membranes are that the phase separation affects drug transport [73].

2.2.2.3.1.2. Lipid membranes

In order to avoid these disadvantages, artificial lipid membranes are used. Polydimethylsiloxane (PDMS) and polytetrafluoroethylene (PTFE) and silicone are the most commonly used membranes [73].

2.2.2.3.2. Drug release models without membranes

The model is used when the distribution of the drug between the base and the test medium will be the same as between the base and the therapeutic area. In this case drug-vehicle interactions can be investigated [64].

2.3. Possibilities and difficulties concerning the evaluation of IVIVC in case of semisolid dermal dosage forms

In vitro-in vivo correlation is a mathematical relationship between an *in vitro* parameter (drug release, rheological property, e.g. viscosity or spreadability) and *in vivo* property (pharmacodynamic [PD] measurement, e.g. time course of plasma concentration curve) [13]. The plasma concentration profiles have to be converted according to *in vivo* release or absorption data by pharmacokinetic or linear model analyses. The *in vivo* data are collected from drug concentration measurements in the blood system. The correlation can be linear or nonlinear. The calculation is based on average of *in vitro* and *in vivo* results. From regulatory point of view, the IVIVC is acceptable when 12 individual points of *in vitro* dissolution are measured and the coefficient variation at the sampling points is under 10% [39]. IVIVC is used in pharmaceutical development in order to reduce time and optimize the formulation, although because of different conditions and choosing the appropriate method the evaluation of the results is a complicated one.

3. OBJECTIVES

In order to filling the gap of regulatory requirements of semisolids, the aim of my thesis was the evaluation of drug release from dermal semisolid dosage forms with different testing methodologies.

The following purposes can be summarized:

- development of semisolid compositions,
- their comparison to reference (marketed) products,
- rheological study of semisolid dosage forms,
- biopharmaceutical evaluation of dermally used semisolids and
- verifying the results by statistical analysis.

The biopharmaceutical studies include:

- *in vivo* measurement of the anti-inflammatory effect of my compositions,
- *in vitro* drug penetration/absorption testing and
- *in vitro* drug release studies.

The *in vivo* data were compared to *in vitro* drug penetration and *in vitro* drug release ones to assess whether there is an *in vitro-in vivo* correlation or not.

The comparison of two *in vitro* dissolution methods, the Franz vertical diffusion cell and the USP method with modified holding cell was carried out. Performances of the two apparatus were studied.

The aim was:

- to investigate the release rates of diclofenac sodium (DS) from my bases and from the reference gels through synthetic membrane with the two equipments *in vitro*,
- to compare the drug release data generated by the two cells,
- to observe critical parameters and technical differences between the apparatuses and
- to evaluate the investigated pharmaceutical dosage forms with the use of similarity (f2) and difference (f1) factors.

4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Active agent - diclofenac sodium

Because of its good tolerability administration of diclofenac, a non-steroidal anti-inflammatory agent is one of the most commonly prescribed drug worldwide [76].

Its sodium salt, micronized diclofenac sodium (DS, sodium-[(dichlorophenyl)amino]-phenylacetate, Figure 6, marketed since 1973 [76], complying with the European Pharmacopoeia [Ph.Eur.], TEVA-Human Co., Debrecen, Hungary) was chosen as a model hydrophilic drug [77, 78, 79]. It inhibits the action of cylooxygenase enzyme [80]. Because of its analgesic and antipyretic effect [81] it is used in treatments of inflammatory as well as painful rheumatic and non-rheumatic diseases [82] such as osteoarthritis, rheumatoid arthritis, dysmenorrhea, migraine and gout [83].

In the present work I decided to use the DS because it is more soluble than the diclofenac acid is under the conditions of normal skin penetration (see later).

It is poorly soluble in acidic (pH 1-3), but is rapidly soluble in alkaline conditions (pH 5-8) [80, 84].

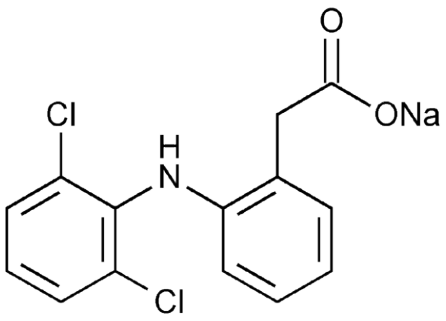

Figure 6 Chemical structure of diclofenac sodium [85]

4.1.2. Preparation of semisolid compositions

Different semisolid formulations were developed and investigated in my study, such as hydrogels, organogels, gel-emulsions, oil-in-water and water-in-oil creams. When developing these compositions, I focused on a limited number of additives that could sensitize the skin and also on their use in lower concentrations to extend the present product range. Their

rheological properties were adjusted to perform a pleasant sensation on the skin and to be easily spread over it.

Hydrogels (HG) were formulated with Carbopol 934 P (prop-2-enoic acid) obtained from BF Goodrich, Brussels, Belgium. It was added to purified water (Ph.Eur.) and dissolved at constant stirring at 450-625 rpm (Stuart Heat Stir, Sterilin Ltd., Keisen Products, Chelmsford, England) at room temperature. After complete dissolution, triethanolamine (Ph.Eur., Hungaropharma Co., Budapest**,** Hungary) was added until the three-dimensional network was built up: the pH of the sample was then adjusted to 7.0 (ISFET pH Meter, IQ Scientific Instruments Inc., San Diego, USA) to form a neutralized clear gel. The gel was stored at cold temperature (10°C) for one day before adding the active agent with vigorous stirring.

Organogel (OG) samples were produced by melting sorbitan monopalmitate (Span 40) ordered from Sigma-Aldrich, Hungary and a neutral oil, Miglyol 812 N oil (Ph.Eur., fractionated coconut oil, glyceryl tricaprylate/caprate) donated by Sasol GmbH, Hamburg, Germany, together on a water-bath at 80°C with continuous stirring and homogenization. The gel was stored in cold temperature at 10°C for one day before adding the active agent with vigorous stirring.

Gel-emulsions (GE) were prepared with the use of Pemulen TR-2 (PTR-2) which was a gift from Wickliffe, USA. It was added to distilled water followed by continuous stirring. Triethanolamine was then added to the sample for neutralization until building a gel structure. Mygliol 812 N oil and distilled water were then added to the gel with vigorous stirring (1000 rpm, Stirrer DLH, VELP Scientifica, Usmate, Italy) until the appropriate consistency was achieved for topical application.

Oil-in-water preparations (OW): cetostearyl alcohol, liquid paraffin (Hungaropharma Co., Budapest**,** Hungary) and Tagat S (PEG-30 glyceryl stearate) supplied by Evonik Ind. AG, Essen, Germany were melted together on a water-bath at 80°C and mixed in order to prepare the oil phase. The active agent was dissolved in purified water and the solution was heated up to a similar temperature at 80°C. The phases were mixed and homogenized until the cream cooled down to room temperature then allowed to stand for one day before use.

Water-in-oil preparations (WO): cetostearyl alcohol, liquid paraffin and Imwitor 780 K (Sasol GmbH, Hamburg, Germany) were melted together on a water-bath at 80°C and mixed. The active pharmaceutical ingredient DS was dissolved in purified water and the solution was

heated up to a similar temperature (about 80°C). The phases were mixed, homogenized and cooled down to 25°C then allowed to stand for one day before use.

The semisolids formulated in-house were compared to two marketed reference medicines. The **reference hydrogel (REF HG)** and the **reference gel-emulsion (REF GE)** were Diclofenac-Ratiopharm® (Ratiopharm Hungaria Ltd. Budapest, Hungary) and Voltaren Emulgel® 1% (Novartis Hungaria Consumers Healthcare Ltd., Budapest, Hungary), respectively and were purchased from Hungarian pharmacies.

4.1.3. The formulations studied

Table 2 summarizes the compositions of the dermatological bases developed for and investigated in this study. The concentration of diclofenac sodium used in the formulations was 1.0 w/w% and it was uniformly dispersed in the bases. The REF GE contained diclofenac diethyl amine salt. The numbers used in each coded sample series indicate the concentration of a particular ingredient of the formulation, as follows: hydrogel (HG) contains 0.8, 0.9 and 1.0 % Carbomer 934P; organogel (OG) contains 25, 30 and 35 % Span 40 emulsifier; gel-emulsions (GE) include 40, 45 and 50 % Pemulen TR-2 gel; oil-in-water (OW) creams consist of 65, 70 and 75 % while water-in-oil (WO) creams 40, 45, 50 % purified water.

Table 2 Compositions of the formulations (w/w%)

Components (%)	Composition character and code (%)				
	Hydrogels HG 0.8 HG 0.9 HG 1.0	Gel-emulsions GE 40 GE 45 GE50	Organogels OG 25 OG 30 OG 35	O/W* OW 65 OW 70 OW 75	W/O* WO 40 WO 45 WO 50
Liquid paraffin	—	—	—	15 10 5	45 40 35
Triethanolamine	q. s.**	—	—	—	—
Mygliol 812	—	50 50 50	75 70 65	—	—
Imwitor 780 K	—	—	—	—	5 5 5
Tagat S	—	—	—	10 10 10	—
Carbopol 934 P	0.8 0.9 1.0	—	—	—	—
PTR-2 gel***	—	40 45 50	—	—	—
Span 40	—	—	25 30 35	—	—
Cetostearyl alcohol	—	—	—	10 10 10	10 10 10
Purified water	to 100	10 5 0	—	65 70 75	40 45 50
REF GE	contains diaethylamine, Carbopol 974 P, Cetomacrogol 1000, Cetiol LC, liquid paraffin, prolylene glycol, isopropyl alcohol, purified water, “Crème 45” perfume				
REF HG	contains lactic acid, sodium disulphite, hydroxypropylcellulose, diiospropyl adipinate, isopropyl alcohol, purified water				

* Creams
** Quantum satis: add to reach pH=7.0
*** PTR-2 gel consisted of 2.0 g PTR-2 and 1.0 g triethanolamine, mixed and diluted to 200.0 g with distilled water

4.2. Methods

4.2.1. Rheology

A HAAKE RS1 (Thermo Electron, Germany) rheometer was used with cone-plate configuration (1/35 TI). All measurements were carried out in triplicates at 25 ± 0.1 °C. Experiments were run in the shear rate range 0.1 - 100 1/s.

Controlled rate-ramp ($\Delta\gamma/\Delta t = 0.333$) was applied in up and down cycle and the result was recorded as $\tau = f(\gamma)$ rheograms and $\eta = f(\gamma)$ viscosity curves. In case of flow curves shear stress was measured as the function of increasing and decreasing shear rate.Thixotropy was defined as the area between the up- and down curves [Pas/s]. Data were evaluated with RheoSoft 2.84.

4.2.2. Biopharmaceutical studies

4.2.2.1. *In vivo study*

A carrageen-induced oedema study was used to test *in vivo* efficacy of the formulations. For this technique I refer to the literature [86, 87] for the details. The experiments were approved by the Animal Ethics Committee of the University of Szeged, Hungary (IV/01758-6/2008). Male Wistar rats (150-181 g) were used. All measurements were performed at 24 ± 1°C in an air-conditioned room. The animals were kept under standard 12 h light/12 h dark conditions with food and water '*ad libitum*'. All experiments were carried out in the same period of the day (1-4 p.m.) to exclude diurnal variations in pharmacological effects. Each rat was tested only once. One day prior to the application of the preparations, the back of each rat (15 cm^2) was carefully shaven and depilated by Veet® depilatory cream (Reckitt Benckiser, Massy, France) in 5 minutes under 2.5-3.5% isoflurane anaesthesia (Forane® solution, Abbott Laboratories, Budapest, Hungary). The skin of the animals was cleaned by wiping with water containing cotton. The rats were dried under infrared lamp for 10 minutes.

On the day of the experiment, the animals were anaesthetized with Forane® solution and exposed to different test compositions. A 300 mg sample from each formulation was applied onto the depilated dorsal skin of the rat. Local inflammatory response was elicited by 0.1 ml subplantar injection of carrageen (Viscarin, Marine Colloids Inc., Springfield, USA) solution given into the right hand paw one hour after the treatment. The concentration of carrageenan solution was 0.5% which was prepared in a physiological saline solution. The left paw, used as the control, was treated without carrageenan. Paw volume was measured with a plethysmometer (Hugo Sachs Elektronik, March, Germany) 5 hours after the injection.

The volume differences between the carrageenan- and saline-injected paws were used for the evaluation of the inflammatory response. The degree of paw swelling was calculated as:

$$\text{Swelling\%} = 100 \quad \frac{(V_t - V_0)}{V_0} \qquad \text{Eq.1.}$$

where V_t and V_0 were the treated and untreated paw volumes, respectively.

4.2.2.2. *In vitro* study

4.2.2.2.1. Drug penetration/absorption study

Cellulose acetate membranes (Porafil, Macherey-Nagel GmbH, Düren, Germany and Pall Life Sciences, Batavia, USA) with an average pore size of 0.45 µm were used. The area for diffusion was 1.767 cm^2. The penetration process from different bases (n=12) and 2 reference gels were measured through synthetic cellulose acetate membrane soaked in isopropyl myristate.

The receiving medium was to mimic the penetration through the skin surface and the *stratum corneum*. According to various text books, sebaceous and eccrine secretions lay down an acid mantle on the skin surface and *stratum corneum* with a pH about 5 [64], although this value varies with the state of the body, gender, environmental conditions, season, etc. up to plus-minus half pH unit [64, 88, 89]. Thus, a phosphate buffer of pH 5.4 ± 0.1 (Orion Star pH, Thermo Electron Co., Singapore) was chosen as the receiving medium. Its temperature was maintained at 32 ± 0.5°C to reflect the usual external skin temperature [1].

The absorbances of the samples were measured by UV spectrophotometry (Unicam Helios α UV–Vis Spectrophotometer, Cambridge, England) at 275 nm and their diclofenac sodium content calculated using a calibration curve. The blank ointment bases (compositions without DS) were also tested but, for their absorbance remained below 2% of those of the diclofenac containing samples throughout the experiments; the blank values were not taken into account.

The experiments were run in triplicate. The results, because of their calculated precision, as a general rule, were rounded to three digits.

4.2.2.2.1.1. Equipment

The **Franz vertical diffusion cell** system (Hanson Research Co., Chatsworth, USA) used contained 6 cells and equipped with an autosampler (Hanson Microette Autosampling System). The receptor chamber volume was 7 ml. The membranes, soaked previously in IPM

(see above) were mounted on the top of the cells. A stirring rate of 450 rpm was set. Quantities of 0.24-1.65 g ointment samples (depending on the type and consistency of the different compositions) were placed evenly on the surfaces of the membranes. 800 µl samples were taken after 0.5, 1, 2, 3 and 6 hours and replaced with fresh receiving medium.

4.2.2.2.2. Drug release

Cellulose acetate membranes (Porafil, Macherey-Nagel GmbH, Düren, Germany and Pall Life Sciences, Batavia, USA) with an average pore size of 0.45 µm were used. The area for diffusion was 1.767 cm^2. The rates of release of the active pharmaceutical ingredient DS were studied by using non-impregnated, but soaked in phosphate buffer cellulose acetate membranes.

A phosphate buffer of pH 5.4 ± 0.1 (Orion Star pH, Thermo Electron Co., Singapore) was chosen as the receiving medium. Its temperature was maintained at 32 ± 0.5°C to reflect the usual external skin temperature [1].

The absorbances of the samples were measured by UV spectrophotometry (Unicam Helios α UV–Vis Spectrophotometer, Cambridge, England) at 275 nm and their diclofenac sodium content calculated using a calibration curve. The blank ointment bases (compositions without DS) were also tested but, for their absorbance remained below 2% of those of the diclofenac containing samples throughout the experiments; the blank values were not taken into account.

The experiments were run in triplicate. The results, because of their calculated precision, as a general rule, were rounded to three digits.

4.2.2.2.2.1. Equipments

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The **modified USP apparatus** was the SR8-Plus Dissolution Test Station (Hanson Research Co., Chatsworth, USA) with modified holding cell for semisolids. It was used for release studies exclusively. The apparatus contained 8 cells with 70 ml volumes. Quantities of 0.40-0.70 g samples were placed on the membrane surfaces of the small ointment sample

holders which were then dropped into the glass vessels containing the receiving medium. The stirring rate was set to 100 rpm. Two ml samples were taken manually after the same time intervals as described for the Franz cell.

4.2.3. Data and methods for statistical comparison

The release of diclofenac from the compositions studied was described as:

$$\frac{\mu g}{cm^2} = a\, t^{1/2} + b \qquad \text{Eq.2.}$$

where

a is the slope representing the release rate of diclofenac sodium [16];

t is the sampling time (hour) and

b is the intercept.

In my further statistical comparisons

Q is the cumulative amount ($\mu g/cm^2$) of the diclofenac released in 6 hours,

s is standard deviation expressed in relative %,

repeatability stands for the average *s* of the indicated values within the parallel runs (average intra-run *s*),

reproducibility stands for the *s* of the results of the indicated values determined in the three parallel runs (inter-run *s*) and

r is the coefficient of correlation,

CIs are confidence intervals at 95% significance level ($p<0.05$). They could be calculated by using the simple method proposed by Shah et al [15]. However, to be consistent with the swelling % results, the CIs were calculated by the common statistical method [90]:

$$CI = x_m \pm t_{95}\, s_x \qquad \text{Eq.3.}$$

where

x_m is the arithmetic mean;

t_{95} is the Student factor at 95% significance level ($p<0.05$) selected for the corresponding degree of freedom (the number of measuring points taken into account was 15 in all cases);

s_x is the standard deviation of x_m.

In order to compare not fully linear experimental curves, I applied the studies of the dissolution from solid dosage forms, where there are methods when not the apparent rate constants, but the curves itself are compared. This is the utilisation of the difference (f1) and similarity (f2) factors [91].

$$f1 = 100 \frac{\sum_{t=1}^{n} (R_t - T_t)}{\sum_{t=1}^{n} (R_t)} \qquad \text{Eq.4.}$$

$$f2 = 50 \log \left(\frac{100}{1 + \frac{\sum_{t=1}^{n} (R_t - T_t)}{n}} \right)^{½} \qquad \text{Eq.5.}$$

where

T_t is the amount (%) of the active ingredient liberated from the test preparation at t time;

R_t is the same for the “reference” product;

n is the number of the parallel experiments.

However, it has been pointed out, that f1 and f2 have shortcomings such as being sensitive to the number of time points and the absolute values of the per cent liberated [92], thus, these factors may only be used for tablet dissolution purposes together with some corresponding rules (requiring maximum coefficient of variation of mean liberation data, definition of the number of points and the maximum per cent of the liberated substance, i.e. one measuring point above 85%, etc. [91]). Moreover, in their original forms they are not suitable to study the release (up to a few per cent) of active ingredients from semisolid dosage forms. To overcome this issue, when comparing two release curves, not the percentages of the liberated active substance were used, rather

- the curve of the faster liberation was taken as ”reference”,
- its amounts liberated ($\mu g/cm^2$) was taken as 100% in every time points and
- the amounts liberated at the same time points from the other (”test”) curve were calculated as the percentages of the above values.

Thus, values calculated in such a way may be called relative difference and similarity factors $f1_{rel}$ and $f2_{rel}$. The maximum 10% difference between the curves (generally used for f1

and f2 [91]) means that if f1>10, the two curves seem to be “different”, while if the f2>50, they are ” similar”.

Statistical analysis of the *in vivo* (swelling %) data was performed by one-way ANOVA, followed by Newman-Keuls Multiple Comparison Test. At a significance level of $p<0.05$, the anti-inflammatory effect was titled “moderate” and at a significance level of $p<0.001$ it was called “significant” (GraphPad 4.0).

Linear and power trend line fitting between *in vitro* penetration data and *in vivo* absorption studies were used.

5. RESULTS AND DISCUSSION

5.1. Rheological characteristics of investigated products – hydrogels

The DS incorporation in the hydrogels did not influence the viscosity of the formulation remarkably (Table 3). It could be established, that the structure of the hydrogels could be deformed slightly easily as the structure of the reference products (Figure 7). The flow, viscosity characteristics and yield value of the hydrogel samples were similar to the reference products. With decrease of the yield value and shear stress, the spreadability of the products increased, which effected a higher anti-inflammatory effect in therapy.

Table 3 Rheological characteristics of compositions

Compositions	Flow maximum [Pa]	Viscosity				Thixotropic area [Pas/s]
		Viscosity minimum [Pas]	*a*	*b*	*r*	
Hydrogels						
DSHG 0.8	252.53	2.53	42.551	-0.558	0.9839	1204
DSHG 0.9	299.79	2.87	47.379	-0.582	0.9810	1798.66
DSHG 1.0	281.15	3.28	43.57	-0.565	0.9789	1129
Gelemulsions						
DSGE 40	159.63	1.35	15.720	-0.487	0.9987	-396.65
DSGE 45	188.65	1.65	19.649	-0.507	0.9983	-466.55
DSGE 50	214.80	1.59	28.575	-0.561	0.9980	618.9
Organogels						
DSOG 25	51.82	0.56	6.343	-0.5363	0.9053	423.33
DSOG 30	78.53	0.78	9.502	-0.528	0.9337	1289
DSOG 35	126.40	1.29	19.320	-0.558	0.9190	2552.33
Oil–in–water creams						
DSOW 65	123.5	1.34	28.950	-0.677	0.9981	1572
DSOW 70	83.61	0.87	15.611	-0.629	0.9988	1304
DSOW 75	58.96	0.67	6.9312	-0.523	0.9889	532.73
Water–in–oil creams						
DSWO 40	57.55	0.58	3.7574	-0.504	0.9127	-1611.33
DSWO 45	29.00	0.29	2.751	-0.541	0.9619	-426.23
DSWO 50	64.99	0.65	5.6929	-0.520	0.9777	-802.97
Reference preparations						
REF GE	227.7	2.18	31.257	-0.572	0.9971	-166
REF HG	168.57	1.68	24.791	-0.533	0.9100	286.07

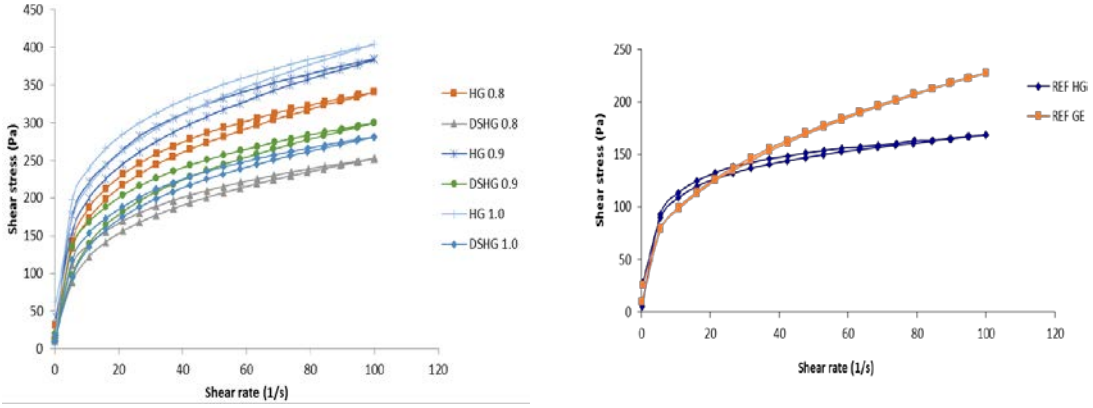


Figure 7a) Flow curves of hydrogels and **b)** reference products

5.2. Results of biopharmaceutical data

5.2.1. Evaluation of *in vivo* data

Several studies examined the paw oedema decreasing effect of different compounds [93, 94]. This carrageenan-induced oedema study was used to test *in vivo* efficacy of the formulations by me as well. Its results were then compared to the *in vitro* penetration and release rate data to evaluate IVIVC.

The data (average swelling %) are shown in Table 4, they are presented in an easy-to-compare way in Figure 8 and as in CIs in Figure 9.

Diclofenac sodium 1% (w/w%) in 40%, and 45% w/o creams ($p<0.05$, labelled with *) exerts only a moderate oedema inhibition compared to the control group. One percent (w/w%) of the active ingredient DS incorporated in 50% w/o cream, in the o/w cream basements, in hydrogel and organogel preparations and in case of both marketed reference products showed to be efficient in comparison with the non-treated group ($p<0.001$, labelled with ***). The highest oedema swelling inhibition rate was measured in case of the 35% emulsifier containing organogel, which effect was more significant than both of the registered products. The lowest effect was observed in the 45% water containing w/o formulation.

Similar to a previous study of Csóka et al. [95] – where *in vitro* and *in vivo* percutaneous absorption of ketamine hydrochloride and piroxicam were investigated – I also found that my hydrogel samples showed to be efficient in the *in vivo* investigations.

In the *in vivo* experiments the average order of the preparations is as follows: reference gel-emulsion > hydrogels > organogels > o/w creams > reference hydrogel > w/o creams.

Table 4 Swelling% data measured *in vivo*

Composition	Swelling (%)
DSHG 0.8	45.7
DSHG 0.9	47.2
DSHG 1.0	50.0
DSOG 25	29.1
DSOG 30	50.0
DSOG 35	58.2
DSOW 65	37.5
DSOW 70	41.8
DSOW 75	43.6
DSWO 40	18.1
DSWO 45	16.3
DSWO 50	28.3
REF HG	38.3
REF GE	51.8

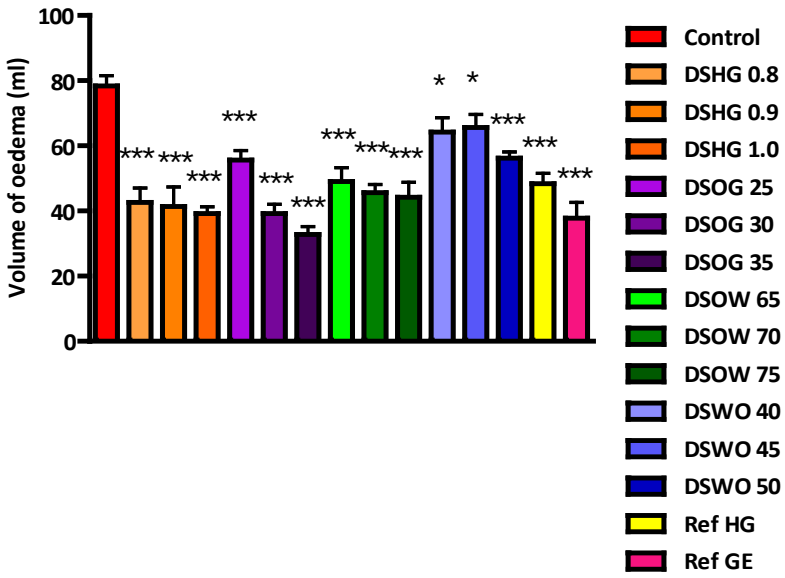


Figure 8 Anti-inflammatory effect of different preparations containing 1% diclofenac sodium on carrageenan-induced oedema in rats

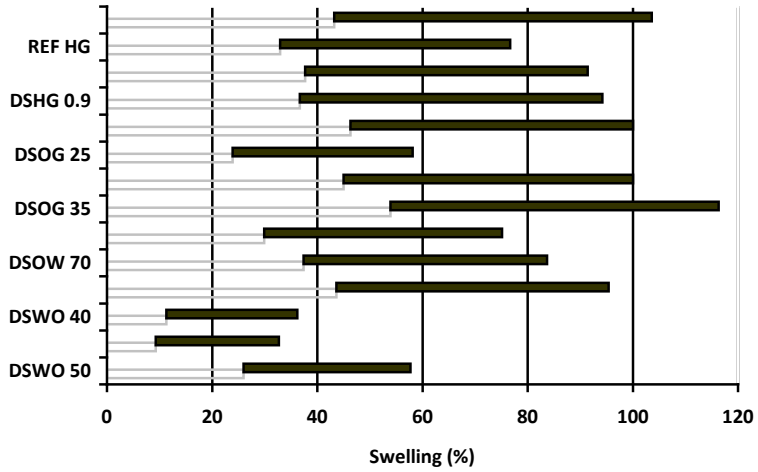


Figure 9 CIs of the swelling % results

More than half of my developed products reached and exceeded the oedema decreasing effect of the reference hydrogel and one preparation exceeded the value of reference gel-emulsion. It means that my aim to develop new products with fewer additives, that reach the oedema decreasing effect of reference gels, was successful.

5.2.2. Evaluation of *in vitro* drug penetration/absorption data

Impregnation of membranes with IPM for *in vitro* penetration studies is often used by researchers [96] for different types of the model barriers used in the experiment influenced the results**.** This was presented as the plausible reason for *in vitro* penetration and *in vivo* absorption differences. The synthetic cellulose acetate membrane is an inert barrier, in contrast, the skin is an active barrier.

Based on the *in vitro* penetration results many products could be excluded because of their low penetration rate, although they were effective in the *in vivo* studies.

The Franz diffusion cell was used.

Figure 10 shows the penetrated diclofenac sodium amounts (in percentages) against time through IPM soaked membrane. (To be consistent with my data, in all product codes the “DS” precedes the codes in Table 2).

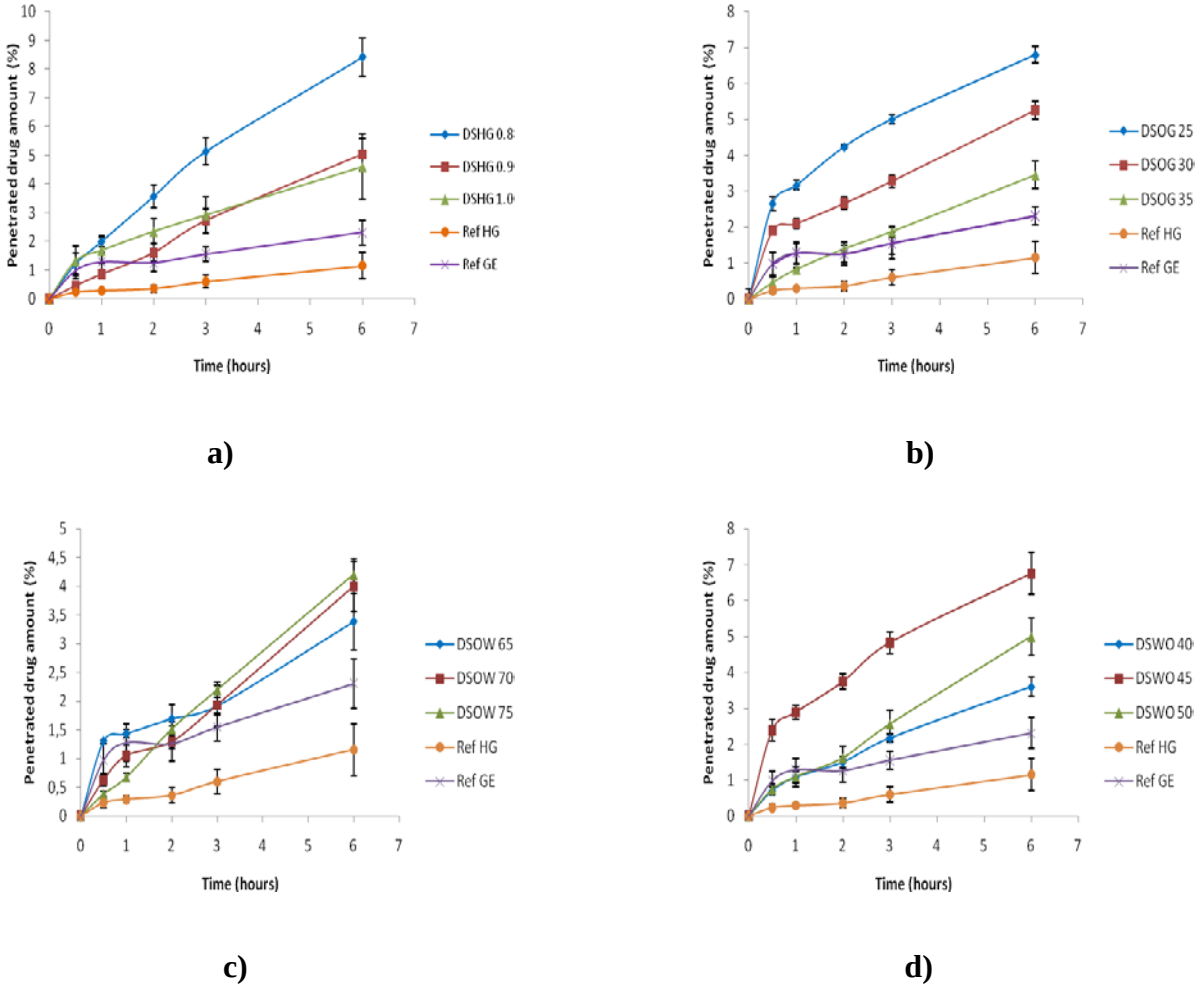


Figure 10a-d) Cumulative penetrated diclofenac sodium amounts through IPM soaked synthetic membrane plotted against time

Cumulative penetrated amounts of DS in 6 hours and their relative standard deviations are presented in Table 5.

It can be seen that all of my products reached the drug penetration level of the marketed (reference) gels (1.16% and 2.31% for reference hydrogel and reference gel-emulsion, respectively). The hydrogel samples containing the 0.8% polymer showed the highest *in vitro* penetration rate (8.41%) and the reference hydrogel was the last in the order.

The standard deviations (SD) were changing in the range from 0.46% (DSOG 25) to 2.29% (DSHG 1.0).

The penetration through the IPM soaked membrane decreased in the following order: hydrogels > organogels > w/o creams > o/w creams > reference gel-emulsion > reference hydrogel.

Table 5 Penetrated DS amounts through IPM soaked membrane in 6 hours

Composition	Penetrated drug amount ± SD (%)
DSHG 0.8	8.41 ± 1.34
DSHG 0.9	5.03 ± 1.12
DSHG 1.0	4.60 ± 2.29
DSOG 25	6.8 ± 0.46
DSOG 30	5.25 ± 0.51
DSOG 35	3.45 ± 0.77
DSOW 65	3.39 ± 0.99
DSOW 70	4.00 ± 0.86
DSOW 75	4.20 ± 0.54
DSWO 40	3.60 ± 0.54
DSWO 45	6.75 ± 1.17
DSWO 50	5.00 ± 1.05
Ref HG	1.15 ± 0.91
Ref GE	2.31 ± 0.86

5.2.2.1. *In vitro* penetration – *in vivo* correlation

Moderate (x<0.90), significant (0.90<x) or no correlation at all were measured between the *in vitro* penetration results compared to that of *in vivo* efficiency studies with difference fitting.

Best IVIVC was established in case of IPM soaked membrane in o/w creams and in organogel samples in case of linear fitting and in o/w creams in case of power trend line fitting. IVIVC rate was not remarkable in w/o samples (Table 6).

Table 6 IVIVC correlation coefficients in case of IPM soaked membrane with linear and power trend line fitting

Compositions	R^2 with linear fitting	R^2 with power trend line fitting
Hydrogels	0.6913	0.7331
Organogels	0.9176	0.8100
o/w creams	0.9732	0.9714
w/o creams	0.0403	0.0228

It can be concluded, that o/w creams had the best (0.9732 and 0.9714) and w/o creams the worst fitting (0.0403 and 0.0228) also in case of linear and power trend line fitting.

However, the *in vitro* penetration and *in vivo* results showed significant differences in the dosge form types and even within each group of dosage forms.

In contrast with the investigation of Csóka et al. [95], in my experiments correlation was found between the *in vitro* penetration and *in vivo* data of organogels, o/w creams and moderate IVIVC was found in case of hydrogels.

IVIVC with the animal tests in use was found in some cases. Evaluating the data and IVIVC results I can offer my hydrogel and organogel compositions for clinical use.

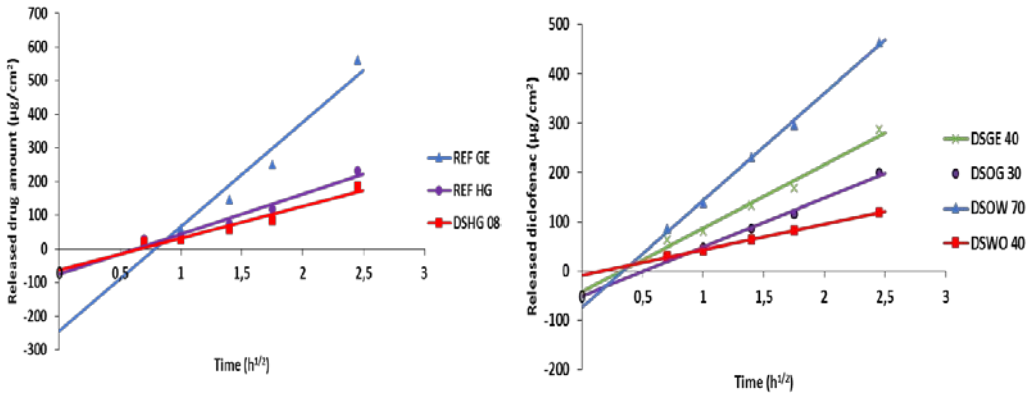
5.2.3. Evaluation of *in vitro* drug release data

5.2.3.1. Characteristics of diclofenac release from semisolid preparations and its transport through cellulose acetate membrane

Eq. 2., i.e. linear relationship between the amount released and the square root of time is valid only when sink conditions persist. If not, the release curves show saturation.

The solubility of diclofenac varies considerably with pH. According to the most reliable literature data, conditions of pH=5.5 and 23°C, which are close to my experimental conditions, give the solubility as 36 µg/ml [97]. In my experiments the highest amounts dissolved were around 1100 and 2500 µg in the Franz cell and modified USP studies, respectively. Perfect sink conditions persist if the drug concentration in the receiving medium does not exceed 10% of the solubility of the drug under the given conditions at any time point [98]. Thus, even if the 9°C temperature difference between the literature data and my experiments is taken into account, sink conditions were not expected to occur at pH 5.4 in my experiments. Researchers using sink conditions in similar studies applied higher pH values [43, 44, 99].

Characteristic release curves (one composition each) are shown on Figures 11-12 (Franz cell method), and Figure 13 (modified USP method, separated for better clarity).



a) **b)**

Figure 11a-b) Released diclofenac sodium with the Franz cell method (the best linear fit indicated)

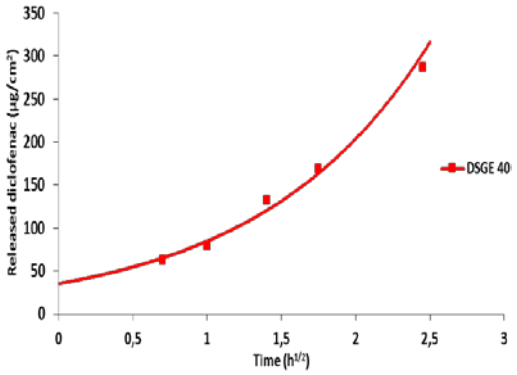
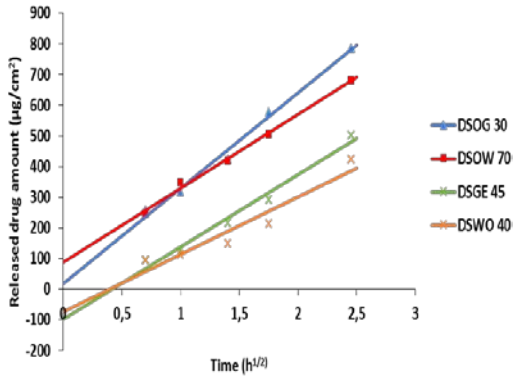
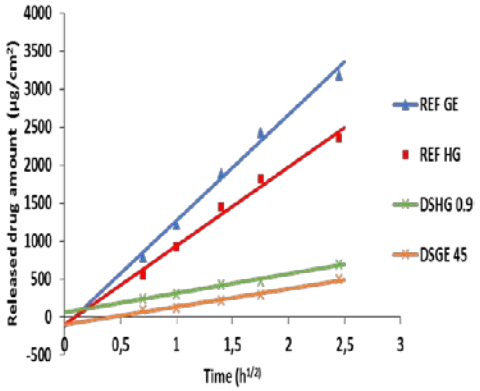


Figure 12 Release of diclofenac from DSGE 40 using the Franz cell method (a non-linear, exponential best fit)





a) **b)**

Figure 13a-b) Released diclofenac sodium with the modified USP method (the best linear fit indicated)

The release curves show no saturation. The figures indicate the best linear fit, however are also not completely linear as should follow from Eq. 2. This phenomenon can be seen in the case of the DSGE, DSOG and DSWO series of preparations using the modified USP apparatus while it occurs in almost all the cases using Franz cell experiments. Exponential rather than linear curves (see Figure 12) are seen, as if the release-rate would slightly increase in time. This phenomenon suggests that the release is not controlled by one single process (diffusion) of diclofenac alone.

The following mechanism is proposed to explain these findings. It is well known that surface active agents and lubricants (like IPM used in my former experiments) may modify both (non-impregnated) artificial membranes and the solubility of substances in the receiving medium. In my present study the dermatological bases studied contained such agents. During the dissolution not only the diclofenac was released through the membrane, but other ingredients as well. The latter modified both the membrane structure (making it more suitable for penetration) and the receiving medium dynamically. This explanation may account for the shapes of the curves and the increased solubility of the diclofenac reaching sink conditions in the receiving medium.

The idea that components of creams and ointments modify the characteristics of the membranes they penetrate into and through is not new. It has also been described for human skin [99].

It can be seen that the slower the diclofenac release the poorer the linearity of the curve (see e.g. the Franz cell experiments *versus* the modified USP experiments). Using the Franz

cell the intercepts were always negative with poor precision, thus indicating a lag-time (diffusion of the analyte through the membrane). This trend is not so obvious with the modified USP method when the release-rates were higher.

5.2.3.2. Statistical comparison of the performances of the Franz cell and the modified USP systems

The results of the *in vitro* release date are presented in Table 7. Slopes, intercepts and Q data in these tables represent average values for three parallel runs and all cells working.

Comparing the standard deviation data in Table 7, one can see that, in most of the cases, the precision (both repeatability and reproducibility) of the modified USP method was found superior to that of the Franz cell method.

As a rule, the precision of Q-values was better than that of the slopes while those of the intercepts (*b*) were the poorest. This should be a consequence of the fact that the "linear" release curves are actually not completely linear.

Standard deviations for the correlation coefficients did not provide any new information.

Table 7a-b) *In vitro* release data using the Franz cell and modified USP method

Compositions	*a*	*b*	*r*	Q µg/cm^2	Repeatability				Reproducibility			
					s_a	s_b	s_r	s_Q	s_a	s_b	s_r	s_Q
Hydrogels												
DSHG 0.8	91.4	-38.8	0.9527	202	13.8	20.3	6.81	12.9	13.4	57.7	6.32	17.0
DSHG 0.9	104	-33.3	0.9782	217	18.6	23.9	1.83	9.78	26.0	62.4	5.38	29.1
DSHG 1.0	111	-21.9	0.9750	250	15,3	18.9	4.13	12.6	27.6	68.2	3.63	20.1
Gelemulsions												
DSGE 40	130	-43.9	0,9901	287	3.80	38.4	0.93	5.99	29.3	17.7	4.20	15.9
DSGE 45	163	-49.1	0.9931	360	17.9	10.8	1.35	8.07	28.2	60.3	2.20	14.0
DSGE 50	113	-35.1	0.9929	250	24.9	44.8	5.95	7.92	36.7	80.4	3.75	25.4
Organogels												
DSOG 25	97.8	-36.9	0.9954	208	6.15	25.5	1.15	6.59	29.1	34.8	2.15	13.6
DSOG 30	93.4	-50.6	0.9957	190	7.75	24.4	1.05	13.9	22.6	47.3	3.96	18.7
DSOG 35	54.9	-26.7	0.9948	110	18.2	32.3	1.52	9.31	8.90	15.9	3.35	13.9
Oil–in–water creams												
DSOW 65	115	-30.5	0.9998	252	7.79	16.3	1.19	7.12	13.2	14.7	2.13	10.3
DSOW 70	219	-70.0	0.9989	465	5.51	18.6	1.13	7.14	10.0	14.2	3.07	8.17
DSOW 75	270	-8.61	0.9996	649	12.8	10.9	1.17	6.23	14.6	11.7	2.82	9.37
Water–in–oil creams												
DSWO 40	43.7	2.30	0.9910	109	16.7	12.9	1.92	9.32	22.3	17.3	4.31	16.9
DSWO 45	21.9	-7.03	0.9763	47.5	18.3	15.9	2.42	8.66	23.2	24.4	3.18	16.8
DSWO 50	82.4	-18.2	0.9940	186	15.5	16.3	1.60	5.72	17.6	21.5	3.74	14.2
Reference preparations												
REF GE	296	-194	0.9798	547	8.56	15.3	0.79	6.75	8.39	25.9	2.61	9.17
REF HG	120	-76.4	0.9749	225	10.4	12.7	2.42	5.10	18.0	19.3	2.78	7.02

Compositions	*a*	*b*	*r*	Q µg/cm^2	Repeatability				Reproducibility			
					s_a	s_b	s_r	s_Q	s_a	s_b	s_r	s_Q
Hydrogels												
DSHG 0.8	242	71.8	0.9895	687	9.32	26.6	1.91	4.10	12.1	39.6	2.90	9.98
DSHG 0.9	250	45.5	0.9993	651	10.0	21.1	0.57	6.78	14.1	23.4	1.89	9.03
DSHG 1.0	259	35.5	0.9804	661	10.8	18.4	3.00	8.50	7.35	23.3	3.19	9.20
Gel-emulsions												
DSGE 40	191	-30.3	0.9728	451	11.9	18.6	0.58	6.97	19.2	20.5	1.28	11.9
DSGE 45	231	-82.5	0.9824	511	11.7	24.9	9.08	5.61	11.1	29.8	7.82	10.6
DSGE 50	200	-96.9	0.9635	424	13.7	16.3	2.26	8.81	16.1	28.4	3.95	11.0
Organogels												
DSOG 25	294	131	0.9546	850	18.3	18.2	2.95	8.72	22.8	31.8	1.76	10.5
DSOG 30	322	-0.92	0.9838	832	15.6	25.3	0.80	7.46	19.4	37.9	0.79	9.03
DSOG 35	339	49.2	0.9731	899	18.3	28.9	2.53	9.29	15.1	43.0	1.20	13.8
Oil–in–water creams												
DSOW 65	146	75.2	0.9828	425	11.1	23.6	1.83	5.54	18.7	23.3	2.08	13.9
DSOW 70	243	-15.7	0.9943	688	8.85	17.6	2.13	3.31	24.3	25.8	2.00	4.41
DSOW 75	305	116	0.9982	866	7.02	17.9	1.97	4.90	29.5	23.1	2.61	6.27
Water–in–oil creams												
DSWO 40	178	-80.0	0.9682	442	14.5	23.3	4.13	7.65	19.8	42.1	5.13	11.9
DSWO 45	121	39.9	0.9678	355	19.4	37.0	3.43	6.99	23.4	44.4	4.98	12.8
DSWO 50	164	0.28	0.9598	438	14.3	24.8	1.68	8.39	19.9	38.3	2.65	14.1
Reference preparations												
REF GE	1460	-115	0.9911	3330	2.13	16.6	0.94	6.30	6.45	17.4	1.10	7.09
REF HG	1070	-166	0.9802	2340	7.05	15.2	0.64	6.66	14.3	17.7	3.24	11.1

5.2.3.3. Effect of the composition of the different dermatological bases diclofenac release using cellulose acetate membrane

5.2.3.3.1. Qualitative evaluation of the release rates

First, I have tried to describe and understand the *in vitro* results taking the average values into account exclusively.

In case of the DSOW compositions the release-rates (the slope *a* in Eq. 2, see Table 7, measured either by the modified USP or the Franz cell methods increased with increasing water (and decreasing paraffin) content. A similar, although much less pronounced, trend could be observed in the DSHG series with increasing Carbopol and consequently, the neutralizing triethanolamine content. By contrast, the "worst" and "best" compositions were found with the DSWO and DSGE series. In the modified USP method, the release-rates for the two marketed creams were much higher than those of my other compositions. This difference was not so pronounced with the Franz cell method.

The results observed for the DSOG series were surprising. The modified USP method indicated slightly, surely not significantly increasing diclofenac release rates with an increasing amount of the emulsifier Span, while this trend was just the opposite for the Franz cell experiments. The only relevant differences between the two *in vitro* systems which might account for this phenomenon were the receptor volumes and the sampling. Using the Franz cell, the 0.8 ml/7.0 ml samples taken were always replaced with fresh receiving medium at all time points. Using the modified USP method the volumes were not replaced. In my present working hypothesis the emulsifier, when penetrating into the membrane, facilitates the passage of the diclofenac, possibly forming micellar structures which helps in its solubilization in the receiving medium. The structure and composition of micelles depend on the concentration of the emulsifier. When the receiving medium is diluted after sampling, the concentration of the emulsifier decreases in the membrane as well initiating micelle changes during the passage.

Apart from this explanation, my experiments suggest that beside the fact that the release rates are naturally much faster when the modified USP method is used, the two test systems do not mimic the *in vivo* processes in the same way.

It should be emphasized that it may be true only for my selected experimental conditions, that the following conclusions may be drawn.

Comparing the modified USP and the Franz cell methods, the former seems to be more precise. Moreover, because it is a more complex piece of equipment the Franz cell needs more frequent maintenance than the USP modified apparatus method.

At the same time, the Franz cell is more discriminative.

Although the release of the active principle, namely the diffusion through the cellulose acetate membrane, seems to be a more complex process which results in not fully linear curves. This presumably involves co-penetration of excipients modifying both the membrane and the apparent solubility of the active principle in the receiving medium dynamically.

5.2.3.4. Statistical evaluation by comparing the CIs

As a second step, the CIs of the apparent release rate constants were calculated and compared to those of the *in vivo* swelling % data.

The CIs are shown on Figures 14 a-c) (for the release of the marketed medicinal products was much faster than those performed by the semisolids produced in-house, to have data more comparable with the Franz cell ones, Figure 14c) reproduces the data of the latter without those of the reference preparations).

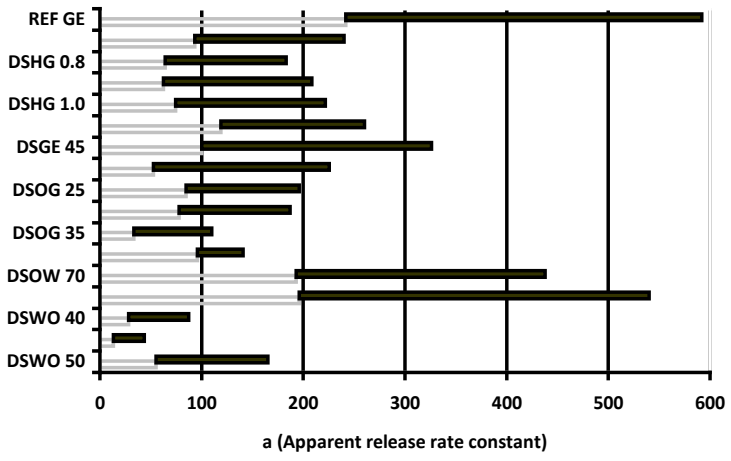


Figure 14a) CIs of the apparent release rate constants in the Franz cell experiments

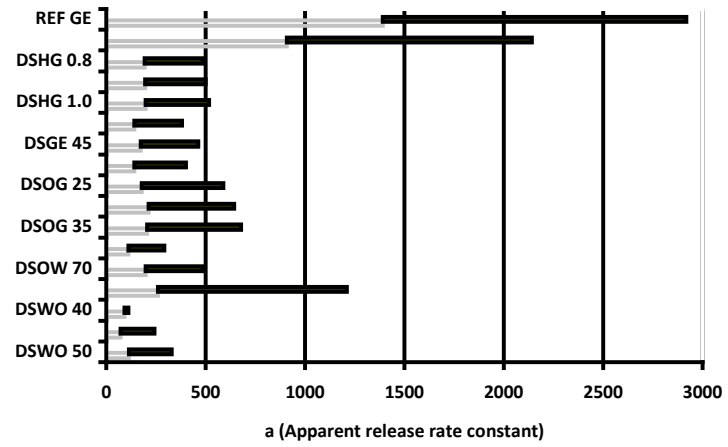


Figure 14b) CIs of the apparent release rate constants in the modified USP experiments

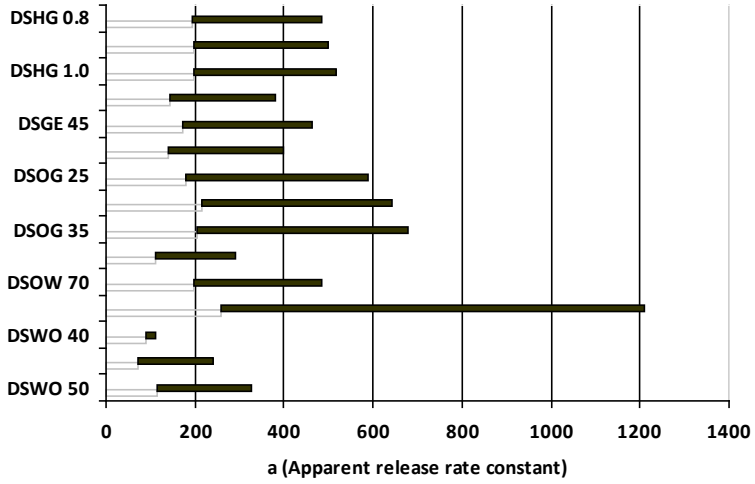


Figure 14c) CIs of the apparent release rate constants in the modified USP experiments, without the data of the reference preparations

The results are summarised in Table 8.

This evaluation of the data was done comparing the CIs looking for significant ($p<0.05$) differences. However, it resulted only in limited information, for the CIs of the *in vitro* results were quite wide. This may be explained by my findings that the liberation did not follow the pure diffusion controlled model for the membrane layer was modified by certain excipients continuously. (For this reason, the linear curve fitting to non-linear curves gave only apparent rate constants with the biases of the approximation, i.e. with wider CIs.)

The following basic conclusions can be drawn:

- Now it is proven statistically, that the Franz cell is more discriminative than the modified USP holding cell.
- Liberation from the two marketed gels (REF GE and REF HG) was much quicker than that from my formulations, when the modified USP cell was used but not with the Franz cell and the *in vivo* studies.
- Liberation from the organogel with the highest Span content (DSOG 35) gave the best results both *in vivo* and with the Franz cell but not with the modified USP holding cell.

Table 8 Summary of the results based on the comparison of CIs of the apparent release rate constants

Method	Results	WO	OW	OG	HG	GE
In vivo	Liberation rate	Slow	Medium/ Quick	Quick	Quick	
	Component content increase	Water, positive/ U- curve?	Water, negative	Span, very positive	Carbomer No effect seen	
	Significant?	No/partly	No	Partly	No	
Franz cell	Liberation rate	Slow	Better	Slow	Medium	Medium
	Component content increase	Water, u-shaped curve?	Water, positive	Span, slightly negative	Carbomer No effect seen	Pemulen No effect seen
	Significant?	No/partly	Partly	Partly	No effect seen	No effect seen
Modified USP cell	Liberation rate	Medium	Medium, quick	Quick	Medium/quick	Medium
	Component content increase	Water, u-curve?	Water, positive	Span No effect?	Carbome No effect seen	Pemulen No effect seen
	Significant?	No	Partly	No	No	No

5.2.3.4.1.Statistical comparison of release curves based on the difference and similarity factors

The use of these (particularly the f2) factors is well accepted in the comparison of the dissolution curves of solid dosage forms. In order to avoid biases caused by the linear approximation (Eq. 2) of not completely linear curves (which results inevitably in biased and less precise apparent rate constants, i.e. wide CIs) I have tried to apply these factors (in their relative form, see in *4.2.3. Data and methods for statistical comparison)* for the evaluation of the release curves. To my knowledge this is the first time when f1 and f2 are used for semisolid dosage forms.

Table 9 showes the $f1_{rel}$ and $f2_{rel}$ factors of those experiment where the apparent rate constants did not differ significantly ($p<0.05$), i.e. the CIs were overlapping.

It should be remarked that the $f1_{rel}$ and $f2_{rel}$ values indicated the same results in all cases, i.e. when two curves were found "different" ($f1_{rel}>10$) then they were also "not similar" ($f2_{rel}<50$). Thus, use of only one of them (preferably the relative f2 [91]) is advised in the future. Further on, these results are referred to as "f-results".

Studying the effect of the variation of the compositions of the various ointment bases to the active principle liberation rate using the $f1_{rel}$ and $f2_{rel}$ factors revealed the followings.

Use of these factors indicates the differences (non-similarities) of the experimental curves in a much more sensitive way than the CIs of the apparent release constants. Only the release curves of the DSHG series, when tested with the modified USP holding cell became "not different/similar".

Table 9 The $f1_{rel}$ and $f2_{rel}$ factors

DSOW series				
		DSOW 75/DSOW 70		
Franz cell	$f1_{rel}$	39.8		
	$f2_{rel}$	19.5		
USP modified holding cell	$f1_{rel}$	20.2		
	$f2_{rel}$	33.9		
DSHG series				
		DSHG 1.0/DSHG 0.9	DSHG 0.9/DSHG 0.8	DSHG 1.0/DSHG 0.8
Franz cell	$f1_{rel}$	17.6	40.0	51.9
	$f2_{rel}$	36.5	18.9	13.4
USP modified holding cell	$f1_{rel}$	5.4	3.3	5.1
	$f2_{rel}$	61.0	72.1	59.7
DSOG series				
		DSOG 25/DSOG 30	DSOG 30/DSOG 35	DSOG 25/DSOG 35
Franz cell	$f1_{rel}$	15.4	44.2	51.9
	$f2_{rel}$	36.9	17.6	14.2
		DSOG 35/DSOG 30	DSOG 30/DSOG 25	DSOG 35/DSOG 25
USP modified holding cell	$f1_{rel}$	13.6	22.5	11.1
	$f2_{rel}$	42.4	30.1	37.1
DSWO series				
		DSWO 45/DSWO 40	DSWO 50/DSWO 45	DSWO 50/DSWO 40
Franz cell	$f1_{rel}$			32,7
	$f2_{rel}$			24,1
USP modified holding cell	$f1_{rel}$	24.0	14.2	18.8
	$f2_{rel}$	33.0	37.8	31.7
DSGE series				
		DSGE 45/DSGE 50	DSGE 45/DSGE 40	DSGE 40/DSGE 50
Franz cell	$f1_{rel}$	25.2	21.5	14.5
	$f2_{rel}$	27.7	31.8	38.6
USP modified holding cell	$f1_{rel}$	24.0	12.6	28.1
	$f2_{rel}$	27.6	41.3	24.3

O/W creams: both the *in vivo* and the two *in vitro* methods indicated quicker release with increasing water content. The differences in release rate constants were not significant ($p>0.05$) *in vivo* and only partly (the DSOW 65 *versus* the other two) *in vitro*. However, the f-results indicated different experimental release curves. Thus, it can be stated the rate of release from the DSOW creams increases with the water content.

Hydrogels: increase in the Carbomer 934 P content indicates a small, but non-significant increase of the release rates in all (*in vivo* and *in vitro*) experiments. Even the f-results did not show differences in the experimental curves. This non-significant tendency can only be supported by the f-values in the Franz cell experiments.

Organogels produced the most surprising results: the increasing Span 40 content resulted in increasing release rates in the *in vivo* (the DSOG 30/DSOG 25 difference is significant) and similar tendency in the modified USP holding cell experiments (not significant). However, the f-results confirmed this tendency while the Franz cell produced opposite results: the release from the DSOG 35 was the slowest. Also this was confirmed by the f-results.

W/O creams: the increase in the water content resulted in a U-shaped context (the release rate is the lowest at 45% water content, but it is not (the modified USP holding cell experiments) or partly significant. However, the f-values clearly indicated different experimental curves. Further research is needed to explain why the 45% water content in the emulsions performs a slower release that the 50% or 40% ones.

Gel-emulsions: the *in vivo* data are lacking. *In vitro* data indicate a reversed-U-shaped context, i.e. the 45% Pemulen TR-2 content assured the highest release rate (not significant rate constant differences, the tendency can be better seen in the USP modified holding cell experiments). The f-results confirmed this tendency.

5.2.3.5. *In vitro* drug release – *in vivo* correlation

In this part of the work the correlation between *in vivo* swelling % results and the average apparent rate constants or Q released values generated in the two *in vitro* dissolution methods was studied. Table 10 summarizes the correlation coefficients.

Table 10 Correlation between *in vitro* and *in vivo* data

Compositions	Slope			Q		
	USP/ Franz	*USP/ Swelling*	*Franz/ Swelling*	*USP/ Franz*	*USP/ Swelling*	*Franz/ Swelling*
DSHG	0.9805	0.9904	0.9440	-0.8065	-0.5663	0.9440
DSGE	0.8488			0.8488		
DSOG	-0.8417	0.9943	-0.7794	-0.9373	0.5123	-0.7794
DSOW	0.9973	0.9939	0.9999	0.9965	0.9926	0.9993
DSWO	0.6047	0.4125	0.9632	0.7490	0.5830	0.9750
All data*	0.5306	0.3378	0.4233	0.3385	0.2665	0.3385

For r and not r^2 data are presented, the negative r values indicate opposite trends in the data (i.e. increasing and decreasing release rates with the concentration of a component).

5.2.3.5.1. Correlation between the slopes (apparent release rate constants) and swelling data

When all data generated either with the modified USP or with the Franz cell method were correlated with the *in vivo* swelling % results, no correlation was found. Thus, neither of these two methods was predicting IVIVC, in general. The same is valid for the correlation of the two *in vitro* methods.

However, varying the concentrations of the components within one type of composition, a good correlation was be achieved between *in vitro*, generated by the modified USP method and *in vivo* data in the DSHG, DSOG and DSOW series. Similar findings, with a somewhat poorer correlation, can be observed with the Franz cell and *in vivo* data for the DSHG, DSOW and DSWO series. The IVIVC was the best for both *in vitro* methods in the case of the DSOW compositions. Only one of the two *in vitro* methods gave somewhat acceptable correlation in the case of the DSOG and DSWO preparations using the modified USP and Franz cell methods, respectively.

When the correlation within the DSOW series was the best, one may postulate that the release of diclofenac sodium from oil-in-water bases was the closest to a single diffusion controlled process.

5.2.3.5.2. Correlation between Q and swelling data

Correlations for the cumulative amount dissolved in 6 hours (Q) data, as a rule, were much poorer that those for the slopes. It was not surprising for my proposed explanation described the dissolution of diclofenac from semisolids as a complex procedure with co-dissolution of other ointment components modifying the membrane and the solubility of diclofenac dynamically. The slope, although biased by the previously mentioned sub-processes, was determined by the whole curve characterizing the main release process. It should have been a better correlation with the *in vivo* data than the Q-values when calculated as the mean of single measuring points.

5.2.3.5.3. Conclusions

The slopes (*a*) of the release curves (Eq. 2) are the best parameter for IVIVC, although the amount dissolved at a given time (Q) can be measured with higher precision. The latter may be better for quality control purposes.

My experiments show that when I use a non-impregnated cellulose acetate membrane neither the modified USP nor the Franz cell is suitable to establish IVIVC. In general however, Csóka et al. [95] and other researchers [71] could obtain acceptable IVIVC for some

semisolid compositions using the Franz cell. Moreover, varying the composition within the same types of semisolid dermatological bases good IVIVC may be achieved. In this respect the performances of the two *in vitro* methods are not necessarily the same, depending on the type of base utilized. Thus, both methods could be suitable for optimization studies, but this is not true for all types of dermatological compositions. Consequently, to support such studies, it is advisable to establish at least qualitative IVIVC with parallel *in vivo* experiments.

6. SUMMARY

The aim of my research work was to study the drug penetration and release processes of semisolid dosage forms and to evaluate existing methods from regulatory points of view.

6.1. The experiments carried out

In the frame of this work various types of semisolid drug bases were developed and prepared, such as hydrogels, organogels, gel-emulsions, o/w and w/o creams with 1% diclofenac sodium content. The bases were tested first *in vivo* then *in vitro* in order to ascertain their drug penetration and release characteristics.

For *in vivo* data, the anti-inflammatory effect of semisolids was investigated on a carrageenan-induced oedema model in rats.

For the *in vitro* penetration studies, the Franz vertical diffusion cell was used with cellulose acetate membranes soaked in isopropyl myristate.

In the *in vitro* release studies both the Franz cell and the USP modified holding cell with mini paddle apparatuses, both with not impregnated cellulose acetate membranes were used.

Performances of the experimental methods were evaluated, on the one hand, from regulatory points of view, on the other hand from their ability to predict *in vitro-in vivo* correlation.

6.2. *In vitro* drug release from semisolid dosage forms

I have pointed out that the literature is not equivocal on the methodology. The Franz vertical diffusion cell is generally used with cellulose acetate membrane, impregated or not impregnated. In addition, data on another apparatus, the USP modified holding cell with mini paddle appeared in the literature.

Results of my work:

- I compared the performances of the Franz cell and the modified USP holding cell in studies of drug release from semisolid dosage forms. To my knowledge, no such comparison was described in the literature before.
    - Data generated by the Franz cell are less precise (presumably due to its inherent characteristics, the complicated tube system, the very low amount of

receiving medium and the possibility of bubble formation.) The much simpler USP modified holding cell gives more precise results.

- By contrast, the Franz cell is more discriminative.
- The two methods are not interchangeable. Depending on the types of semisolids studies, they may give similar or contradictory results (between types of bases and/or within the same type when the composition was varied.)

- I recognized that the release was not necessarily a fully diffusion controlled process, for the released amounts *versus* time$^{1/2}$ curves were not completely linear. To my knowledge this is a new finding in the literature. A plausible explanation comprising co-release of surface active base components, modifying both the membrane and the receiving medium dynamically was proposed. Thus, not real but "apparent" release rate constants with the biases of the linear approximation of not fully linear curves could be calculated.
- In order to overcome the above biases, first in the literature I applied the f2 similarity (and also f1 difference) factors (in their relative forms) to compare drug release curves from semisolid dosage forms.

6.3. *In vitro-in vivo* correlation

- My semisolid formulations were able to show anti-inflammatory effects and to decrease the carrageenan-induced oedema in rats. More than half of my products reached or exceeded the oedema decreasing effect of the reference (marketed) hydrogel.
- The clinical relevance of my findings might be that my hydrogel and organogel samples showed significant anti-inflammatory effects. Rheological characteristics of both formulations showed that they can be easily spread on the skin. They exceeded the value of the reference gel-emulsion and showed a significant IVIVC in the penetration studies. However, no correlation was found between the *in vitro* and *in vivo* results in case of reference gels.
- My experiments showed that when using a non-impregnated cellulose acetate membrane neither the modified USP nor the Franz cell is suitable to establish IVIVC although these *in vitro* and the *in vivo* qualitative pictures may coincide

within given types of bases. It is advisable to perform both *in vitro* release studies with *in vivo* ones.

7. REFERENCES

1. Brown C.K., Friedel H.D., Barker A.R., Buhse L.F., Keitel S., Cecil T.L., Kraemer J., Morris J.M., Reppas C., Stickelmeyer M.P., Yomota C., Shah V.P. Dissolut. Technol. 18 (2011) 51-64.
2. Sznitowska M., Pietkiewicz J., Stokrocka M., Janicki S. Pharmazie 59 (2004) 814-815.
3. Silva A.C., Santos D., Ferreira D.C., Souto E.B. Pharmazie 64 (2009) 177-182.
4. Wu L., Tang C., Yin C. Pharmazie 65 (2010) 493-499.
5. Belal T.S., Shaalan R.A., Haggag R.S. Pharmazie 94 (2011) 503-512.
6. Chen M., Pan X., Wu H., Han K., Xie X.,Wedge D.E., Repka M.A.,Wu C. Pharmazie 66 (2011) 272-277.
7. Csóka I., Bozsik E., Erős I., Paál T.L. Eur. J. Pharm. Sci. 32S (2007) S6-S21.
8. Yamaguchi Y., Nagasawa T., Kitagawa A., Nakamura N., Matsumoto K., Uchiwa H., Hirata K., Igarashi R. Pharmazie 61 (2006) 112-116.
9. Elsayed M.M.A., Abdallah O.Y., Naggar V.F., Khalafallah N.M. Pharmazie 62 (2007) 133-137.
10. Barry B.W. Dermatological Formulations - Percutaneous Absorption, Marcel Dekker, New York and Basel (1983)
11. Corbo M. Dissolut. Technol. 2 (1995) 3-6.
12. Hanson R., Gray V. Handbook of Dissolution Testing, Dissolution Technologies, Hockessin, Delaware (2004)
13. Shah V.P. Eur. J. Pharm. Biopharm. 60 (2005) 309-314.
14. Azarmi S., Roa W., Lobenberg R. Int. J. Pharm. 328 (2007) 12-21.
15. Shah V.P., Elkins J., Schuirmann D., Pelsor F., Schrivastava S., DeCamp W., Schwartz P., Williams R. Dissolut. Technol. 5 (1998) 5-11.
16. Guidance for Industry. Nonsterile Semisolid Dosage Forms. Scale-Up and Postapproval Changes: Chemistry, Manufacturing and Controls. In Vitro Release Testing and In Vivo Bioequivalence Documentation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. SUPAC-SS (1997)
17. D'Souza S.S., Lozano R., Mayock S., Gray V. Dissolut. Technol. 17 (2010) 41-45.
18. Kanfer I., Tettey-Amlalo R.N.O., Au W.L., Hughes-Formella B. Assessment of Topical Dosage Forms Intended for Local and Regional Activity. In: Generic Product Drug

Development: Specialty Drug Products, Informa Healthcare, New York and London (2010) 54-103.

19. Howes D., Guy R., Hadgraft J., Heylings J., Hoeck U., Kemper F., Maibach H., Marty J.-P., Merk H., Parra J., Rekkas D., Rondelli I., Schaefer H., Täuber U., Verbiese N. Altern. Lab. Anim. 24 (1996) 81-106.
20. Tadini K.A., Maia Campos P.M.B.G Pharmazie 64 (2009) 818-822.
21. Dragievic-Curic N., Gräfe S., Gitter B., Winter S., Fahr A. Int. J. Pharm. 384 (2010) 100-108.
22. Wasdo S.C., Juntunen J., Devarajan H., Sloan K.B. Int. J. Pharm. 373 (2009) 62-67.
23. Heo J.H., Cho Y.S., Cheon H.G. Pharmazie 65 (2010) 906-912.
24. Shakeel F., Baboota S., Ahuja A., Ali J., Shafiq S. Pharmazie 63 (2008) 580-584.
25. Melero A., Lehr C.M., Schäfer U.F., Garrigues T.M. Int. J. Pharm. 384 (2010) 137-139.
26. Songkro S., Rades T., Becket G. Pharmazie 64 (2009) 110-115.
27. Caon T., Oliveria Costa A.C., Leal de Oliviera M.A., Micke G.A., Oliveria Simoes C.M. Int. J. Pharm. 391 (2010) 1-6.
28. Barbero A.M., Frasch H.F. Toxicol. In Vitro 23 (2009) 1-13.
29. Doan K., Bronaugh R.L., Yourick J.J. Food Chem. Toxicol. 48 (2010) 18-23.
30. Nicoli S., Penna E., Padula C., Colombo P., Santi P. Int. J. Pharm. 325 (2006) 2-7.
31. Meshali M.M., Abdel-Aleem H.M., Sakr F.M., Nazzal S., El-Malah Y. Pharmazie 63 (2008) 49-53.
32. Haigh J.M., Beyssac E., Chanet L., Aiache J.M. Int. J. Pharm. 170 (1998) 151-156.
33. Ngawhirunpat T., Panomsuk S., Opanasopit P., Rojanarata T., Hatanaka T. Pharmazie 61 (2006) 331-335.
34. Farahmand S., Maibach H.I. Int. J. Pharm. 367 (2009) 1-15.
35. Kumar D., Aqil M., Rizwan M., Sultana Y., Ali M. Pharmazie 64 (2009) 80-85.
36. Wissing S.A., Müller R.H. J. Contr. Rel. 81 (2002) 225-233.
37. Buch P., Holm P., Thomassen J.Q., Scherer D., Kataoka M., Yamashita S., Langguth P. Pharmazie 65 (2010) 723-728.
38. Guidance for Industry. Topical Dermatological Drug Product NDAs and ANDAs – In Vivo Bioavailability, Bioequivalence, In Vitro Release and Asssociated Studies. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (1998)
39. Cardot J.-M., Beyssac E., Alric M. Dissolut. Technol. 14 (2007) 15-19.

40. Retting H., Mysicka J. Dissolut. Technol. 15 (2008) 6-8.
41. Franz T. Percutaneous absorption. J. Invest. Dermatol. 64 (1975) 190-195.
42. Shah V.P., Elkins J., Shaw S., Hanson R. Pharm. Dev. Technol. 8 (2003) 97-102.
43. Sanna V., Peana A.T., Moretti M.D.L. Curr. Drug Deliv. 6 (2009) 93-100.
44. Shivhare U.D., Jain K.B., Mathur V.B., Bhusari K.P., Roy A.A. Dig. J. Nanomater Biostruct. 4 (2009) 285-290.
45. Zatz J.L., Segers J.D. Dissolut. Technol. 5 (1998) 3-17.
46. Hanson R. Dissolut. Technol. 17 (2010) 33-35. (http://www.dissolutiontech.com/DTresour/201011Articles/DT201011_A04.pdf)
47. Siewert M., Dressman J., Brown C.K., Shah V.P. AAPS Pharm. Sci. Technol. 4 (2003) 1-10. and Dissolut. Technol. 10 (2003) 6-15.
48. Ueda C.T., Shah V.P., Derdzinski K., Ewing G., Flynn G., Maibach H., Marques M., Rytting H., Shaw S., Thakker K., Yacobi A. Dissolut. Technol. 17 (2010) 12-25.
49. Alexander A.E., Johnson P. Colloid Sci. Oxford Univ. Press, New York (1949)
50. http://apps.who.int/phint/en/p/docf/
51. http://www.farmavita.net/content/view/372/34/
52. http://www.pharmainfo.net/free-books/novel-semisolid-dosage-forms
53. Idson B., Lazarus J., Lachman L., Lieberman H.A., Kang J.L. Theory and Practice of Industrial Pharmacy, Verghese Publishing House, Bombay, India (1991) 534-563.
54. Swarbrick J., Boylan J.C. Encyclopedia of Pharmaceutical Technology, Vol. 14, Marcel Dekker, New York (1996) 31-59.
55. http://www.pharmtech.com/pharmtech/data/articlestandard/pharmtech/112002/12404/article.pdf
56. Anand B., Pisal S.S., Paradkar A.R., Mahadik K.R. J. Sci. Ind. Res. 60 (2001) 311-318.
57. Ramachandran S., Chen S., Etzler F. Drug Dev. Ind. Pharm. 25 (1999) 153-161.
58. Laba D. Rheological properties of cosmetics and toiletries. Marcel Decker Inc., New York (1993)
59. Welin-Berger K., Neelissen J., Bergenstahl B. Eur. J. Pharm. Sci. 13 (2001) 309-318.
60. Bonacucina G., Cespi M., Misici-Falzi M., Palmieri GF. Int. J. Pharm. 307 (2006) 129-140.
61. Davis S.S. J. Pharm. Sci. 60 (1971) 1351-1356.
62. Korhonen M., Lehtonen J., Hellen L., Hirvonen J., Yliruusi J. Int. J. Pharm. (2002) 247, 103-114.

63. Welin-Berger K., Neelissen J., Bergenstahl B. Eur. J. Pharm. Sci. 14 (2001) 229-236.
64. Katz M., Poulsen J. In: Brodie B.B., Gilette J.: Handbook of Experimental Pharmacology, 28., Springer-Verlag, New York (1971)
65. Surber C., Davies A.F. Bioavailability and bioequivalence of dermatological formulations. In: Walters K.A.: Dermatological and Transdermal Formulations, Marcel Dekker, New York, USA (2002)
66. Kydonieus A.F., Berner B.B. Transdermal Delivery of Drugs, Vol.I-III., CRC Press, Bosa Roca (1987)
67. Arellano A., Santoyo S., Martín C., Ygartua P. Eur. J. Pharm. Sci. 7 (1998) 129-135.
68. Neubert R.R.H., Wohlrab W.A. Acta Pharm. Technol. 36 (1990) 197-206.
69. Schroeter A., Engelbrecht T., Neubert R.H.H. Chem. Sci. Eng. 7 (2013) 29-36.
70. Hadgraft J., Ridout G. Int. J. Pharm. 39 (1987) 149-156.
71. Tashtoush B.M., Al-Safi S.A., Al-Fanek K.J. Pharmazie 59 (2004) 143-146.
72. http://www.admet.de/uploads/media/FCT08_SUPAC_SS_01.pdf
73. Liebenberg W., Engelbrecht E., Wessels A., Devarakonda B., Yang W., Villiers M. J. Food Drug Anal. 12 (2004) 19-28.
74. Operational Manual – Hanson Microette Autosampling System – Hanson Research Corp. (1997)
75. Naegel A., Hansen S., Neumann D., Lehr C.-M., Schaefer U.F., Wittum G., Heisig M. Eur. J. Pharm. Biopharm. 68 (2008) 368-379.
76. Zhai X.J., Yu Y., Chen F., Lu Y.N. Curr. Ther. Res. 75 (2013) 53-58.
77. Aurora Prado M.S., Steppe M., Tavares M.F.M., Kedor-Hackmann E.R.M., Santoro M.I.R.M. Pharmazie 85 (2002) 333-340.
78. Lopes P.S., Kaneko T.M., Takano C.Y., Lacerda A.C.L., Latorre L.R., Kato M.J. Pharmazie 86 (2003) 681-684.
79. United States Pharmacopoeia and National Formulary, The United States Pharmacopoeial Convention Inc., Rockville MD (1995)
80. Tripathi K.D. Essentials of Medical Pharmacology, Jaypee Brothers Medical Publishers Ltd., Delhi (1995)
81. Mourão S.C., Silva C., Bresolin T.M.B., Serra C.H.R., Porta V. Int. J. Pharm. 386 (2010) 201-207.
82. Sipos P., Szűcs M., Szabó A., Erős I., Szabó-Révész P. J. Pharm. Biomed. Anal. 46 (2008) 288-294.

83. Elzayat E.M., Ibrahim M.F., Abdel-Rahman A.A., Alanazi F.K., Habib W.A. Arab. J. Chem. In Press (2014)
84. Manjunatha K.M., Ramana M.V., Satyanarajana D. Indian J. Pharm. Sci. 69 (2007) 384-389.
85. http://www.pharmacopeia.cn/v29240/images/v29240/g-1337.gif
86. Calpena A.C., Escribano E., Martinaa H.S., Laurobaaa J., Obachb R., Domenechaa J. Arzneim-Forsch. 49 (1999) 1012-1017.
87. Uslu M., Kilincoglu V., Toker S, Kalender A.M., Dogan A., Sebik A. Acta Orthop. Traumatol. Turc. 44 (2010) 484-491.
88. Wagner H., Kostka K.H., Lehr C.M., Schaefer U.F. Eur. J. Pharm. Biopharm. 55 (2003) 57-65.
89. Klee S.K., Farwick M., Lersch P. Coll. Surf. A.: Physicochem. Eng. Aspects 338 (2009) 162–166.
90. Pharmacopoeia Hungarica Ed. VI., Vol. I. Medicina, Budapest, pp. 226-229. (1986)
91. Shah V.P., Tsong Y., Sathe P., Williams, R. Dissolut. Technol. 6 (1999) 15.
92. D. Liu, J.P., Chow, S.C. Drug Inf. J. 30 (1996) 881-889.
93. Blazsó G., Miklós G. Pharm. Res. 35 (1997) 65-71.
94. Szabados-Nacsa Á., Sipos P., Martinek T., Mándity I., Blazsó G., Balogh Á., Szabó-Révész P., Aigner Z. J. Pharm. Biomed. Anal. 55 (2011) 294-300.
95. Csóka I., Csányi E., Zapantis G., Nagy E., Fehér-Kiss A., Horváth G., Blazsó G., Erős I. Int. J. Pharm. 291 (2005) 11-19.
96. Pénzes T., Blazsó G., Aigner Z., Falkay G., Erős I. Int. J. Pharm. 298 (2005) 47-54.
97. Chuasuwan B., Binjesoh V., Polli J.E., Zhang H., Amidon G.L., Junginger H.E., Midha K.K., Shah V.P., Stavchansky S., Dressman J.B., Barends D.M. J. Pharm. Sci. 98 (2009) 1206-1219.
98. Klose D., Delplace C., Siepmann J. Intern J. Pharmaceutics 404 (2011) 75–82.
99. Escribano E., Cristina C.A., Josep Q., Rossend O., Jose D. Eur. J. Pharm. Sci. 19 (2003) 203-210.

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ANNEX

I.

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In vitro and *in vivo* evaluation of drug release from semisolid dosage forms

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This study presents the *in vitro* and *in vivo* testing of anti-inflammatory drug containing creams, hydrogels and organogels for dermal use. *In vitro* penetration studies were performed with products by measuring the diffused drug amount through synthetic membranes soaked in isopropyl myristate (IPM). Our developed preparations were investigated under *in vitro* conditions together with two marketed medicinal products used as reference preparations. *In vivo* studies were carried out on anaesthetized male Wistar rats; the carrageenan-induced paw oedema decreasing effect of twelve different formulations and the reference products were measured in comparison with a control group. All - previously *in vitro* screened - selected products reduced paw oedema in rats. Significant differences were found among the developed products both *in vitro* and *in vivo*. Correlation between the *in vitro* penetration studies and *in vivo* results were found in the case of o/w creams, organogels and hydrogels.

1. Introduction

Properties of and drug release from semisolid preparations have recently found the interest of researchers from an application (Silva et al. 2009; Wu et al. 2010; Belal et al. 2011; Chen et al. 2011) and regulatory perspective (Csóka et al. 2007) as well as regarding their effects on the skin (Yamaguchi et al. 2006; Elsayed et al. 2007). Dissolution testing - which is frequently used in the examination of other dosage forms -, is also widespread in case of semisolid dosage forms (Sznitowska et al. 2004). Although many *in vitro* and *in vivo* methods are used for studying the penetration through the skin (Schmitt et al. 2010; Azeem et al. 2010; Hoffmann and Müller-Goymann 2005; Shakeel 2008, 2009; Shams et al. 2010), there are neither validated methods for their *in vitro* characterization in any Pharmacopoeias (USP 23/NF 18 1995) nor adequate *in vivo* sampling techniques for their investigation (Kanfer et al. 2010).

The aim of regulatory authorities is reducing the number of animal studies and precede their use with *in vitro* investigations. At present, *in vitro* studies are not required by regulators.

Methods for measuring percutaneous absorption are: studies with mathematical models, model membranes, stratum corneum, keratome slices, perfused or whole skin and *in vivo* techniques. Models for them can be: mouse, rabbit, rat, guinea pig, swine, primate and human. The confidence level is high in case of *in vivo* investigations and use of human skin. The physiological hierarchy is increasing with decrease of hairless strains. From the above mentioned list we chose the model membrane and *in vivo* study for our investigation (Howes et al. 1996).

In vitro tests can be used for screening the compositions prior to *in vivo* animal testing, although there are many anatomical and physiological factors, which are not properly represented under *in vitro* conditions (Barry 1983; Naegel et al. 2008).

There have been several methods to predict drug penetration in humans with human (Tadini and Maiai Campos 2009; Dragievic-Curic et al. 2010) and animal *in vitro* models, although animal skin (mouse (Wasdo et al. 2009; Heo et al. 2010), rat (Shakeel et al. 2008; Melero et al. 2010), pig (Songkro et al. 2009; Caon et al. 2010), guinea pig (Barbero and Frasch 2009; Doan et al. 2010), rabbit (Nicoli et al. 2006; Meshali et al. 2008), snake skin (Haigh et al. 1998; Ngawhirunpat et al. 2006)) tend to be more permeable than human. Rat abdominal skin (Farahmand and Maibach 2009; Kumar et al. 2009) has been shown to be a reasonable model based on the *in vitro* static cell experiments.

Guidelines offer as methodology for these studies the Franz diffusion cell (FDC) which is frequently used (Franz 1975; Fasolo et al. 2009; Kim et al. 2009; Ogita et al. 2010).

Fundamental requirement for these studies is the *in vitro-in vivo* correlation (IVIVC) (Wissing and Müller 2002; Buch et al. 2010) posing a major challenge not only for solid, but also for semisolid dosage forms (FDA Guidance 1997 and 1998; Shah 2005; Cardot et al. 2007; Retting and Mysicka 2008).

The purpose of this study was to evaluate developed compositions both *in vitro* and *in vivo* (Medeiros et al. 2009) and to assess whether there is an *in vitro-in vivo* correlation or not.

Despite the increasing number of publications within this field, no standard experimental conditions are used, therefore the comparison of the data is very difficult.

2. Investigations and results

Because of high variability among *in vitro* skin techniques and different data with high standard deviation measured with FDC, we investigated the *in vitro* and *in vivo* characteristics and *in vitro-in vivo* correlation of our products. A simple isopropyl myristate (IPM) model was chosen to simulate the partition phenomenon, which occurs when absorbing through the outermost layer of the skin. It is a question, whether the usage of IPM under *in vitro* circumstances can help in screening prior to *in vivo* testing (Barry 1983; Thakker and Chern 2003).

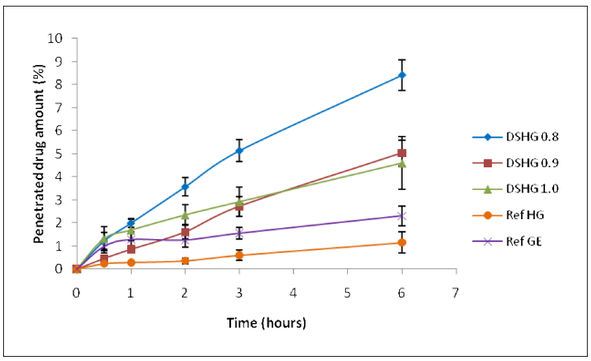

Fig. 1: Cumulative penetrated diclofenac sodium in hydrogels through IPM soaked synthetic membrane

In our study the suitability of an *in vitro* methodology for predicting *in vivo* performance was studied. Diclofenac sodium (DS), a non-steroidal anti-inflammatory agent was chosen as a model drug (Aurora-Prado et al. 2002; Lopes et al. 2003).
When developing these compositions, we focused on a less number of additives sensitizing the skin and also using them at lower concentrations to extend the present product range. These developed products were compared to the reference products in efficiency.
Our hypothesis was, that *in vitro* penetration studies carried out by means of IPM can be used as a surrogate of different animal skin models and brings us closer to the proper *in vitro* selection of products for *in vivo* studies. Results of the *in vitro* penetration were compared, and correlation between these results and the *in vivo* performance were observed.

2.1. *In vitro penetration study of diclofenac sodium*

The penetration process from different vehicles (n = 12) and two reference gels were measured through synthetic cellulose acetate membrane soaked in IPM.
Figures 1–4 and Table 1 show the penetrated diclofenac sodium amount in percentage against time through IPM soaked membrane. *In vitro* penetrated drug amount during a given time period was 6.01% from hydrogel formulations, 5.17% from organogels, 3.63% in the case of o/w creams and 5.12% from w/o creams in average. All of our products reached the drug penetration level of reference gels (penetrated drug amount of reference hydrogel was 1.16% and reference gelemulsion was 2.31%). The hydrogel samples containing 0.8% polymer showed the highest *in vitro* penetration rate (8.41%) and the reference hydrogel was the last in the order. Standard deviation (SD) were in the range from 0.46% (DSOG 25) to 2.29% (DSHG 1.0).

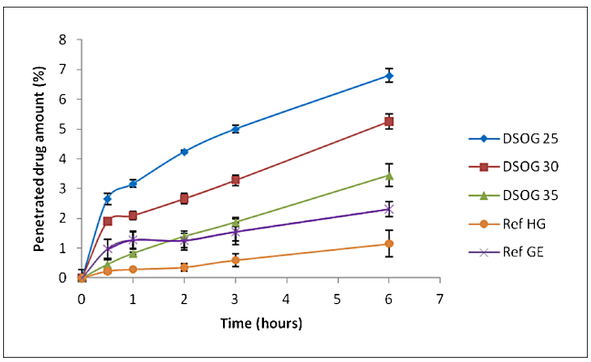

Fig. 2: Cumulative penetrated diclofenac sodium in organogels through IPM soaked synthetic membrane

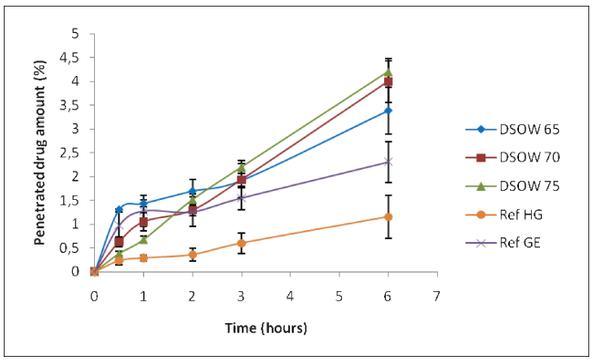

Fig. 3: Cumulative penetrated diclofenac sodium in o/w creams through IPM soaked synthetic membrane

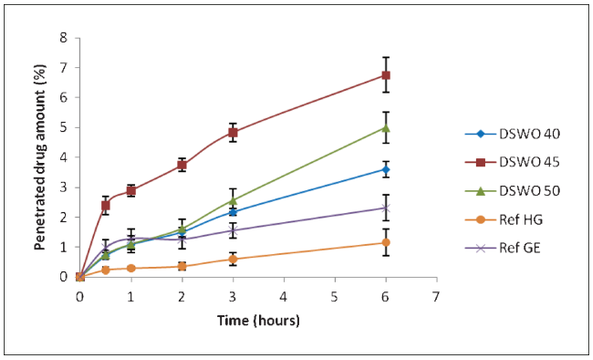

Fig. 4: Cumulative penetrated diclofenac sodium in w/o creams through IPM soaked synthetic membrane

2.2. *In vivo percutaneous testing of diclofenac sodium*

A carrageenan-induced oedema study was used to test *in vivo* efficiency of formulations Data are presented in Fig. 5. Diclofenac sodium 1% (w/w) in 40%, 45% w/o creams ($p < 0.05$) (labelled with *) exerts only a moderate oedema inhibition compared to the control group. One percent (w/w) active agent incorporated in 50% w/o cream, in the o/w cream vehicles, in hydrogel and organogel preparations and in case of both marketed reference products showed to be efficient in comparison with the non-treated group ($p < 0.001$) (labelled with ***). The highest oedema swelling inhibition rate was measured in case of

Table 1: Cumulative penetrated diclofenac sodium measured within 6 h in case of IPM soaked membrane

Composition	Penetrated drug amount ± SD (%)
DSHG 0.8	8.41 ± 1.34
DSHG 0.9	5.03 ± 1.12
DSHG 1.0	4.60 ± 2.29
DSOG 25	6.8 ± 0.46
DSOG 30	5.25 ± 0.51
DSOG 35	3.45 ± 0.77
DSOW 65	3.39 ± 0.99
DSOW 70	4.00 ± 0.86
DSOW 75	4.20 ± 0.54
DSWO 40	3.60 ± 0.54
DSWO 45	6.75 ± 1.17
DSWO 50	5.00 ± 1.05
Ref HG	1.15 ± 0.91
Ref GE	2.31 ± 0.86

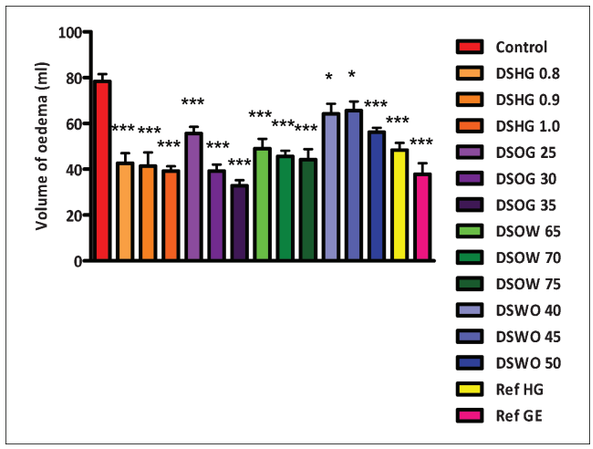

Fig. 5: Anti-inflammatory effect of different preparations containing 1% diclofenac sodium on carrageenan-induced oedema in rats

the 35% emulsifier containing organogel, which was more significant than both of the registered products. The lowest effect was observed in the 45% water containing w/o formulation.
More than 58% of our products reached or exceeded the oedema decreasing effect of the reference hydrogel, and 8% of our formulations exceeded the value of the reference gelemulsion.

2.3. In vitro-in vivo correlation (IVIVC)

Moderate ($x < 0.90$), significant ($0.90 < x$) or no correlation at all were measured between the *in vitro* penetration results compared to that of *in vivo* efficiency studies with difference fitting. Best IVIVC was established in case of IPM soaked membrane in o/w creams and in organogel samples in case of linear fitting and in o/w creams in case of power trend line fitting. IVIVC rate was not remarkable in w/o samples (Table 2). It can be concluded, that o/w creams had the best (0.9732 and 0.9714) and w/o creams the worst fitting (0.0403 and 0.0228) also in case of linear and power trend line fitting.
Based on evaluation of absolute values between *in vitro* penetration and *in vivo* efficacy studies the 0.9% hydrogel, the 30% organogel and the 40% w/o cream had the best IVIVC order numbers and the reference gel emulsion had the worst order number. IVIVC was also established in case of 0.8% and 1.0% hydrogel, 65%, 70%, 75% o/w creams and in 50% w/o cream. Correlation in reference gels was not significant. No correlation was found between the *in vitro* penetration study and *in vivo* results in case of reference gels.
The reference hydrogel was compared to the 0.9% hydrogel. *In vitro* penetration order number studies showed, that our product had better values than the reference hydrogel (Table 3).
The reference gel emulsion was not compared with gel emulsion sample, because in earlier studies their *in vitro* drug release rate (results under publication, not published here) proved to be the lowest among our developed products.

Table 2: IVIVC correlation coefficients in case of IPM soaked membrane with linear and power trend line fitting

Composition	R^2 with linear fitting	R^2 with power trend line fitting
Hydrogel	0.6913	0.7331
Organogel	0.9176	0.81
O/w cream	0.9732	0.9714
W/o cream	0.0403	0.0228

3. Discussion

Impregnation of membranes with IPM for *in vitro* penetration studies was already analysed previously (Pénzes et al. 2005). It was shown that different natures of model barriers (synthetic cellulose acetate membrane and stratum corneum) used in the experiment resulted in significant differences in the *in vitro* penetration and *in vivo* absorption. The synthetic cellulose acetate membrane is an inert barrier, while skin is an active barrier. Although the rate limiting step of diffusion through the skin is the stratum corneum which consists of dead cells. That is why we employed the reliability of diffusion through the stratum corneum with IPM soaked membrane in order to mimic a lipophilic barrier.
In vitro penetration studies were performed in order to evaluate the effect of IPM. Diffusion through the IPM soaked membrane decreased in the following order: hydrogels > organogels > w/o creams > o/w creams > reference gel emulsion > reference hydrogel. Based on the *in vitro* penetration results many products could be excluded because of their low penetration rate, although they were effective in the *in vivo* studies. This questions the applicability of IPM for screening.
Several studies examined the paw oedema decreasing effect of different compounds (Blazsó and Gábor 1997; Szabados-Nacsa et al. 2011). The paw oedema decreasing effect of our products ($n = 12$) and two reference gels were also investigated in our work. Similar to a previous study (Csóka et al. 2005) - where *in vitro* and *in vivo* percutaneous absorption of ketamine hydrochloride and piroxicam were investigated -, we also found, that our hydrogel samples showed to be efficient *in vitro* and *in vivo*. In contrast to this investigation, in our experiments *in vitro-in vivo* correlation was found among our o/w cream, organogel and hydrogel samples.
In the *in vivo* experiments the average order of the preparations was as follows: reference gel emulsion > hydrogels > organogels > o/w creams > refrence hydrogel > w/o creams. More than half of our developed products reached and exceeded the oedema decreasing effect of the reference hydrogel, and one preparation exceeded the value of reference gel emulsion. It means that our aim to develop new products with fewer additives, that reach the oedema decreasing effect of reference gels, was successful.
All the developed products with less additives than the reference gels, except the 25% organogel, 65% o/w cream and w/o compositions, reached minimum the same *in vivo* effect as the reference hydrogel. The anti-inflammatory effect of one formulation (DSOG 35) was higher than the reference gel emulsion. However, the *in vitro* penetration and *in vivo* results showed significant differences in the dosage form types and even within each group of dosage forms. Correlation was found between the *in vitro* penetration and *in vivo* data of o/w creams and organogels and moderate IVIVC was found in case of hydrogels.
In the guidelines edited by the FDA and in cooperation of International Pharmaceutical Federation (FIP) and American Association of Pharmaceutical Scientists (AAPS), the Franz vertical diffusion cell is accepted as a 'gold standard' method for semisolid investigations (FDA Guidance for Industry SUPAC-SS 1997; Siewert et al. 2003). Similar to the profile of these guidelines, the FDC was found to be proper for prediction of *in vivo* results (Tashtoush et al. 2004). However many critical points were observed in connection with the methodology itself; the very low amount of receiving medium, the complicated tube system, and the possibility of bubble formation emphasises the necessity to validate the method (results under publication, not published here).
Implementation of IPM as a "skin model" as a surrogate for different animal skin models can be used only together with release data. The release results are handled as the drug diffuses 100%

Table 3: ***In vitro-in vivo*** **number according to** ***in vitro*** **penetration and** ***in vivo*** **oedema decreasing effect**

Composition	*In vitro number*	*In vivo number*	Absolute value of *in vitro-in vivo* difference	Order number of absolute value
DSHG 0.9	5	4	1	1
DSOG 30	4	3	1	1
DSOW 75	8	6	2	2
DSWO 40	10	11	1	1
Ref HG	14	7	7	4
Ref GE	13	2	11	10

through the polar channels, partitioning from IPM which represents the 100% lypophilic way of absorption through the stratum corneum. The *in vivo* behaviour will be between these data as the consequence of both polar and apolar ways of absorption. Our conclusion is, that hydrophilic preparations diffused more readily through synthetic membranes, while an opposite order is experienced in case of penetration studies.

We strengthen the fact, that penetration studies are not acceptable without *in vivo* feedback. But what type of *in vivo* studies can be accepted? Which one gives a good prediction for clinical use? Differently permeable animal skin investigated under very different circumstances (pH, temperature, methodology, thickness) or even cadaver human skin or a simple physicochemical model (like IPM) showing no interindividual variability will give a good prediction.

In the present study IVIVC with the animal tests in use was found in some cases. Evaluating the *in vitro*, *in vivo* and IVIVC data we can offer our hydrogel and organogel compositions for clinical use.

Our developed products were able to show anti-inflammatory effects and to decrease the carrageenan-induced oedema in rats.

4. Experimental

4.1. Materials

Micronized diclofenac sodium (DS) (Ph.Eur. 6) was used as hydrophilic active ingredient (Human Co., Hungary). The following materials were used as vehicle components. Carbomer 934P (prop-2-enoic acid) was obtained from BF Goodrich, Brussels, Belgium. Sorbitan monopalmitate (Span 40) was purchased from Sigma-Aldrich, Hungary. Miglyol 812 N (fractionated coconut oil, glyceryl tricaprylate/caprate) and Imwitor 780K (isostearyl diglyceryl succinate) was donated by Sasol, Germany. Pemulen (PTR-2) was a gift from Novean, USA.Tagat S (PEG-30 glyceryl stearate) was supplied by Evonik, Germany. Isopropyl myristate 98%, disodium hydrogenphosphate and citric acid were ordered from Merck, Germany. All other additives, triethanolamine, liquid paraffin, cetostearyl alcohol and sodium hydroxide were purchased from Hungaropharma Co., Hungary. All components used were of Ph.Eur. 6 grade. The reference hydrogel (Ref HG) and the reference gel emulsion (Ref GE) are commercial medicinal products.

Table 4 summarizes the compositions selected and investigated in this study. The concentration of DS used in the formulations was 1% (w/w) and it was suspended in the vehicles. The numbers used in each coded sample series indicate the concentration of a particular ingredient of the formulation, as follows: hydrogel (HG) contains 0.8, 0.9 and 1.0% Carbomer 934P; organogel (OG) contains 25, 30 and 35% Span 40 emuslifier; gel emulsion includes 40,45 and 50% Pemulen TR-2 gel; oil in water (O/W) cream consists of 65, 70 and 75% purified water; water in oil (W/O) cream includes 40, 45 and 50% purified water.

4.2. Preparation of the formulations

In case of hydrogels, Carbopol 934 P was used as polymer and it was added first to purified water. Constant stirring at room temperature at 450 rpm - 625 rpm (Stuart heat-stir, England, Sterilin Ltd.) was continued until the complete dissolution of the powder. It was followed by adding triethanolamine until the three-dimensional network was built up and the pH of the sample was adjusted to 7.0 (ISFET pH Meter, IQ Scientific Instruments, Inc., USA) to form a neutralised clear gel. The organogel samples were produced by melting (80 °C) sorbitan monopalmitate and Miglyol 812 N oil together under continuous stirring and homogenization.

Cetostearyl alcohol, liquid paraffin and Tagat S were melted together (80 °C) and mixed in order to prepare the oil phase of the oil-in-water cream preparations. The aqueous phase containing purified water was heated up to a similar temperature. The phases were mixed and homogenized until the cream cooled down to room temperature.

In water-in-oil creams cetostearyl alcohol, liquid paraffin and Imwitor 780 K were melted together (80 °C) and mixed. Purified water was heated up to similar temperature, mixed with oil phase, homogenized and cooled down to 25 °C.

The hydrogel and organogel samples were stored at cold temperature (10 °C) for 1 day before adding the active agent. The o/w and w/o creams were stored at the same temperature written above (already containing the active agent) for 1 day before testing.

4.3. In vitro penetration study of diclofenac sodium

A franz vertical diffusion cell system (Hanson Research Co., USA) containing six cells and equipped with autosampler (Hanson Microette Autosampling System, USA) was used. The area for diffusion was 1.767 cm^2 and the receptor chamber volume was 7 ml. Cellulose acetate membranes (Porafil, Machenerey-Nagel, Germany and Pall Life Sciences, USA) with an average pore size of 0.45 μm were used. Membranes were soaked in isopropyl myristate for *in vitro* penetration studies to mimic a lypophilic barrier like the stratum corneum (Shas et al. 1991). Membrane filters were mounted on the top of the Franz diffusion cells. A stirring rate of 450 rpm was used. The dissolution medium temperature was maintained at 32 ± 0.5 °C. Phosphate buffer (pH 5.4 ± 0.1) (Orion Star pH, Thermo Electron Co., Singapore) was chosen as receiving medium as representative of the physiological values of dermis and skin surface. On one hand, our aim was to validate our *in vitro* drug release results (results under publication, not published here). In the drug release investigations the receiving medium pH was pH 5.4 ± 0.1. In order to compare the *in vitro* drug release to the penetration results, pH was set the same as in our previous study, because in the validation method experiment conditions can not be changed. Our first examinations were carried out with 5.4 pH. In the future we plan to study the penetration results with 7.4 pH, because the physiologically conductive fluid usually used is phosphate buffer pH 7.4 (Barry 1983).

The receptor medium allows sufficient amount of active ingredient released within a reasonable time period to ensure accurate analysis. Diclofenac sodium was chosen as an active agent. On the other hand, diclofenac sodium is poorly soluble in acidic (pH 1-3), but is rapidly soluble in alkaline conditions (pH 5-8) (Tripathi 1998; Manjunatha et al. 2007). That is why our first step was to maintain alkalic (pH 5.4) conditions in this study.

Samples (0.24–1.65 g, amount depended on types and consistency of the vehicles) of different compositions were placed evenly on the surface of the membrane, and 800 μl samples were taken after 0.5, 1, 2, 3 and 6 h and replaced with fresh receiving medium. The absorbance of the diclofenac sodium content was measured by UV Spectrophotometer (Unicam Helios α UV–Vis Spectrophotometer, England) at 275 nm, based on prior calibration curve. The blank vehicles without active agents served as references in the analytical measurements. No sink conditions were used.

Four parallel measurements were done with plotting the penetration amount of diclofenac sodium in percentage over a 6 hours time period. Results were expressed as the mean ± S.D.

4.4. In vivo percutaneous testing of diclofenac sodium

4.4.1. Experiments

Products for *in vivo* testing were selected based on *in vitro* results (results under publication, not published here). Experiments were approved by the Animal Ethics Committee of the University of Szeged, Hungary (IV/01758-6/2008).

Male Wistar rats (150–181 g) were studied. All measurements were performed at 24 ± 1 °C in an air-conditioned room. The animals were kept under standard 12 h light/12 h dark conditions with food and water *ad libitum*. All experiments were carried out in the same period of the day (1–4 p.m.) to

Table 4: Composition of formulations (%, w/w)

Component ↓	Hydrogel (HG)	Organogel (OG)	o/w cream (O/W)	w/o cream (W/O)
	DSHG 0.8	DSOG 25	DSOW 65	DSWO 40
	DSHG 0.9	DSOG 30	DSOW 70	DSWO 45
Marking →	DSHG 1.0	DSOG 35 (w/w %)	DSOW 75	DSWO 50
Liquid paraffin	–	–	15–10–5	45–40–35
Triethanolamine	quantum satis	–	–	–
Miglyol 812 N	–	75–70–65	–	–
Imwitor 780 K	–	–	–	5
Tagat S	–	–	10	–
Carbopol 934 P	0.8–0.9–1.0	–	–	–
Span 40	–	25–30–35	–	–
Cetostearyl alcohol	–	–	10	10
Purified water	Ad 100	–	65–70–75	40–45–50

exclude diurnal variations in pharmacological effects. Each rat was tested only once. One day prior to the application of the preparations, the back of each rat (15 cm^2) was carefully shaven and depilated by Veet® depilatory cream (Reckitt Benckiser, France) in 5 min under 2.5–3.5% isoflurane anaesthesia (Forane® solution, Abbott Laboratories, Hungary). The skin of the animals was cleaned by wiping with water containing cotton. The rats were dried under infrared lamp for 10 min.

On the day of the experiment, the animals were anaesthetized with Forane® solution. Experimental animals were exposed to different vehicles (hydrogels, organogels, o/w and w/o creams) containing 1% (w/w) diclofenac sodium and to the two reference gels. Each formulation (300 mg) was applied onto the depilated dorsal skin of the rat. One group (n = 5) served as absolute control – it was not treated at all. The 12 remaining groups (n = 60) were treated with different vehicles containing diclofenac sodium, and 2 groups (n = 10) treated with commercial preparations (reference products). Local inflammatory response was elicited by 0.1 ml subplantar injection of carrageenan (Viscarin, Marine Colloids Inc., Springfield, USA) solution given into the right hand paw one hour after the treatment. The concentration of carrageenan solution was 0.5% which was prepared in physiological saline solution. The left paw, used as control, was treated without carrageenan (Gábor 2000). Paw volume was measured with a plethysmometer (Hugo Sachs Elektronik, Germany) 5 h after the injection.

The volume difference between the carrageenan- and saline-injected paws was used for the evaluation of the inflammatory response. The degree of paw swelling was calculated as:

$$\text{Swelling}\,(\%) = \frac{Vi - V}{V} \times 100 \qquad (1)$$

where *Vi* is the volume of the carrageenan-treated paw, *V* is that of the non-treated paw.

On the basis of Eq. (1), the percentage of oedema inhibition was calculated as:

$$\text{Inhibition}\,(\%) = \left(\frac{1 - \text{swelling}_{\text{treated}}}{\text{swelling}_{\text{control}}}\right) \times 100 \qquad (2)$$

Where $\text{swelling}_{\text{treated}}$ is the mean value observed in the treated group, and $\text{swellingt}_{\text{control}}$ is the mean value observed in the control group.

4.4.2. In vivo data analysis

Statistical analysis was performed by one-way ANOVA, followed by Newman-Keuls Multiple Comparison Test. At a significance level of $p < 0.05$, the anti-inflammatory effect was titled moderate, and at a significance level of $p < 0.001$ it was called significant (GraphPad 4.0). Data are presented as means ± S.E.M.

The relative bioavailability (RBA) regarding the systemic effect was calculated as:

$$\text{RBA} = \frac{\text{inhibition}\%_{\text{D}}}{\text{inhibition}\%_{\text{T}}} \qquad (3)$$

where $\text{inhibition}_{\text{D}}$ is the percentage oedema inhibition for the different D samples and $\text{inhibition}_{\text{T}}$ is the percentage oedema inhibition for the different T samples.

4.5. In vitro-in vivo correlation

Correlation rate was calculated between the *in vitro* penetration and *in vivo* absorption data.

Although a good correlation is a tool for predicting *in vivo* results based on *in vitro* data (Cardot et al. 2007), in case of semisolid dosage forms this correlation is not well established.

Linear and power trend line fitting between *in vitro* penetration data and *in vivo* absorption studies were used.

Correlation rate was evaluated as "good" above the coefficient rate of 0.90. The correlation was "moderate" below this value. No correlation was established when numbers were close to 0. Although many studies reported about basic concept of IVIVC, different methodologies (absorption studies, plasma concentration, AUC) are available based on the type of data (Cardot et al. 2007).

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References

Aurora-Prado MS, Steppe M, Tavares MFM, Kedor-Hackmann ERM, Santoro MIRM (2002) Comparison between capillary electrophoresis and liquid chromatography for the determination of diclofenac sodium in a pharmaceutical tablet. Pharmazie 85: 333–340.

Azeem A, Ahmad FJ, Talegaonkar S (2009) Exploration of skin permeation mechanism of frusemide with proniosomes. Pharmazie 64: 735–740.

Barbero AM, Frasch HF (2009) Pig and guinea pig skin as surrogates for human *in vitro* penetration studies: A quantitative review. Toxicol *In Vitro* 23: 1–13.

Barry BW (1983) Dermatological Formulation: Percutaneous Absorption. First ed., New York and Basel.

Belal TS, Shaalan RA, Haggag RS (2011) Gradient HPLC-Diode Array Detector Stability-Indicating Determination of Lidocaine Hydrochloride and Cetylpyridinium Chloride in two combined oral gel dosage forms. Pharmazie 94: 503–512.

Blazsó G, Miklós G (1997) Evaluation of the anti-oedematous effects of some H_1-receptor antagonists and methysergide in rats. Pharm Res 35: 65–71.

Buch P, Holm P, Thomassen JQ, Scherer D, Kataoka M, Yamashita S, Langguth P (2010) IVIVR in oral absorptionfor fenofibrate immediate release tablets using dissolution and dissolution permeation methods. Pharmazie 65: 723–728.

Caon T, Oliveria Costa AC, Leal de Oliviera MA, Micke GA, Oliveria Simoes CM (2010) Evaluation of the transdermal permeation of different paraben combinations through a pig ear skin model. Int J Pharm 391: 1–6.

Cardot J-M, Beyssac E, Alric M (2007) *In Vitro-In Vivo* Correlation: Importance of Dissolution in IVIVC. Dissolut Technol 14: 15–19.

Chen M, Pan X, Wu H, Han K, Xie X, Wedge DE, Repka MA, Wu C (2011). Preparation and anti-bacterial properties of a temperature-sensitive gel containing silver nanoparticles. Pharmazie 66: 272–277.

Csóka I, Csányi E, Zapantis G, Nagy E, Fehér-Kiss A, Horváth G, Blazsó G, Erős I (2005) *In vitro* and *in vivo* percutaneous absorption of topical dosage forms: case studies. Int J Pharm 291: 11–19.

Csóka I, Bozsik E, Erős I, Paál TL (2007) Regulatory aspects of semisolid dosage form design Eur J Pharm Sci 32S: S6–S21.

Dias M, Farinha A, Faustino E, Hadgraft J, Pais J, Toscano C (1999) Topical delivery of caffeine from some commercial formulations. Int J Pharm 182: 41–47.

Doan K, Bronaugh RL, Yourick JJ (2010) *In vivo* and *in vitro* skin absorption of lipophilic compounds, dibutyl phthalate, farrnesol and geraniol in the hairless guinea pig. Food Chem Toxicol 48: 18–23.

Dragievic-Curic N, Gräfe S, Gitter B, Winter S, Fahr A (2010) Surface charged temoporfin-loaded flexible vesicles: *In vitro* skin penetration studies and stability. Int J Pharm 384: 100–108.

Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM (2007) Deformable liposomes and ethosomes as carriers for skin delivery of ketotifen. Pharmazie 62: 133–137.

Farahmand S, Maibach HI (2009) Transdermal drug pharmacokinetics in man: Interindividual variability and partial prediction. Int J Pharm 367: 1–15.

Fasolo D, Bassani VL, Teixeira HF (2009) Development of topical nanoemulsions containing quercetin and 3-O-methylquercetin. Pharmazie 64: 726–730.

FDA Guidance for Industry SUPAC-SS (1997) Nonsterile Semisolid Dosage Forms, Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls. *In Vitro* Release Testing and *In Vivo* Bioequivalence Documentation U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research.

FDA Guidance for Industry SUPAC-SS (1998) Topical Dermatological Drug Product NDAs and ANDAs – *In Vivo* Bioavailability, Bioequivalence, *In Vitro* Release and Asssociated Studies. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research.

Franz T (1975) Percutaneous absorption. On the relevance of *in vitro* data. J Invest Dermatol 64: 190–195.

Gábor M (2000) Mouse ear models for investigating of agents in hyperproliferative or in inflammatory diseases of the skin. In: Gábor M (ed.) Mouse Ear Inflammation Models and their Pharmacological Applications, First ed., Budapest, p. 282–313.

Haigh JM, Beyssac E, Chanet L, Aiache JM (1998) *In vitro* permeation of progesterone from a gel through the shed skin of three different snake species. Int J Pharm 170: 151–156.

Heo JH, Cho YS, Cheon HG (2010) Topical effects of roflumilast on 1-chloro-2,4-dinitrobenzene-induced atopic dermatitis-like skin lesions in NC/Nga mice. Pharmazie 65: 906–912.

Hoffmann C, Müller-Goymann CC (2005) Use of artificial skin constructs in permeation studies of clindamycin phosphate. Pharmazie 60: 350–353.

Howes D, Guy R, Hadgraft J (1996) Methods for assessing percutaneous absorption, in: Ecvam Workshop Report 13. Alternatives to Laboratory Animals 24: 81–106.

Kanfer I, Tettey-Amlalo RNO, Au WL, Hughes-Formella B (2010) Assessment of Topical Dosage Forms Intended for Local or Regional Activity. In: Informa Healthcare (ed.) Generic Drug Product Development: Specialty Drug Products, First ed., New York, London, p. 54–103.

Kim ST, Jang DJ, Kim JH, Park JY, Lim JS, Lee SY, Lee KM, Lim SJ, Kim CK (2009) Topical administration of cyclosporin A in a solid lipid nanoparticle formulation. Pharmazie 64: 510–514.

Kumar D, Aqil M, Rizwan M, Sultana Y, Ali M (2009) Investigation of a nanoemulsion as vehicle for transdermal delivery of amlodipine. Pharmazie 64: 80–85.

Lopes PS, Kaneko TM, Takano CY, Lacerda ACL, Latorre LR, Kato MJ (2003) Determination of diclofenac sodium in Eagle's minimum essential medium with Earle's balanced salt solution. Pharmazie 86: 681–684.

Manjunatha KM, Ramana MV, Satyanarayana D (2007) Design and evaluation of diclofenac sodium controlled drug delivery systems. Indian J Pharm Sci 69: 384–389.

Medeiros DC, Mazon-Cardoso T, Lemos-Senna E, Poli A (2009) Comparative *in vitro* and *in vivo* evaluations of oral sustained-release formulations of diclofenac sodium in beagle dogs. Pharmazie 64: 648–652.

Melero A, Lehr CM, Schäfer UF, Garrigues TM (2010) Wistar rat skin as surrogate for human skin in nortriptyline hydrochloride patch studies. Int J Pharm 384: 137–139.

Meshali MM, Abdel-Aleem HM, Sakr FM, Nazzal S, El-Malah Y (2008) *In vitro* phonophoresis: effect of ultrasound intensity and mode at high frequency on NSAIDs transport across cellulose and rabbit skin membranes. Pharmazie 63: 49–53.

Naegel A, Hansen S, Neumann D, Lehr C-M, Schaefer UF, Wittum G, Heisig M (2008) In-silico model of skin penetration based on experimentally determined input parameters. Part II: Mathematical modelling of *in-vitro* diffusion experiments. Identification of critical input parameters. Eur J Pharm Biopharm 68: 368–379.

Ngawhirunpat T, Panomsuk S, Opanasopit P, Rojanarata T, Hatanaka T (2006) Comparison of the percutaneous absorption in shed snake skin and human skin. Pharmazie 61: 331–335.

Nicoli S, Penna E, Padula C, Colombo P, Santi P (2006) New transdermal bioadhesive film containing oxybutynin: *In vitro* permeation across rabbit ear skin. Int J Pharm 325: 2–7.

Ogita Y, Takahashi Y, Iwata M, Sasatsu M, Onishi H, Hashimoto S, Machida Y (2010) Comparison of physical properties and drug-releasing characteristics of white petrolatums. Pharmazie 65: 801–804.

Pénzes, T, Blazsó G, Aigner Z, Falkay G, Erős I (2005) Topical absorption of piroxicam from organogels – *In vitro* and *in vivo* correlations. Int J Pharm 298: 47–54.

Retting H, Mysicka J (2008) IVIVC: Methods and Applications in Modified-Release Product Development. Dissolut Technol 15: 6–8.

Schmitt S, Schaefer U, Sporer F, Reichling J (2010) Comparative study on the *in vitro* human skin permeation of monoterpenes and phenylpropanoids applied in rose oil and in form of neat single compounds. Pharmazie 65: 102–105.

Shah VP (2005) IV-IVC for topically applied preparations – a critical evaluation. Eur J Pharm Biopharm 60: 309–314.

Shakeel F, Baboota S, Ahuja A, Ali J, Shafiq S (2008) Skin permeation mechanism of aceclofenac using novel nanoemulsion formulation. Pharmazie 63: 580–584.

Shakeel F, Baboota S, Ahuja A, Ali J, Shafiq S (2009) Enhanced anti-inflammatory effects of celecoxib from a transdermally applied nanoemulsion. Pharmazie 64: 258–259.

Shams MS, Alam MI, Ali A, ultana Y, Aqil M, Shakeel F (2010) Pharmacokinetics of a losartan potassium released from a transdermal therapeutic system for the treatment of hypertension. Pharmazie 65: 679–682.

Shas VP, Elkins J, Hanus J, Noorizadeh C, Skelly JP (1991) *In vitro* release of hydrocortisone from topical preparations and automated procedure. Pharm Res 8: 55–59.

Siewert M, Dressman J, Brown CK, Shah VP (2003) FIP/AAPS Guidelines to Dissolution/*In Vitro* Release Testing of Novel/Special Dosage Forms. AAPS Pharm Sci Technol 4: 1–10.

Silva AC, Santos D, Ferreira DC, Souto EB (2009) Minoxidil-loaded nanostructured lipid carriers (NLC): characterization and rheological behaviour of topical formulations. Pharmazie: 177–182.

Songkro S, Rades T, Becket G (2009) Effects of some terpenes on the *in vitro* permeation of LHRH through newborn pig skin. Pharmazie 64: 110–115.

Szabados-Nacsa Á, Sipos P, Martinek T, Mándity I, Blazsó G, Balogh Á, Szabó-Révész P, Aigner Z (2011) Physico-chemical characterization and *in vitro*/*in vivo* evaluation of loratadine: dimethyl-β-cyclodextrin inclusion complexes. J Pharm Biomed Anal 55: 294–300.

Sznitowska M, Pietkiewicz J, Stokrocka M, Janicki S (2004) Dissolution test for ivermectin in oral veterinary paste. Pharmazie 59: 814–815.

Tadini KA, Maia Campos PMBG (2009). *In vivo* skin effects of dimethylaminoethanol (DMAE) based formulation. Pharmazie 64: 818–822.

Tashtoush BM, Al-Safi SA, Al-Fanek KJ (2004) Azathioprine transport through rat skin and its immunosuppressive effect. Pharmazie 59: 143–146.

Thakker KD, Chern WH (2003) Development and Validation of *In Vitro* Release Tests for Semisolid Dosage Forms – Case study. Dissolut Technol 10: 10–15.

Tripathi KD (1998) Essential of medical pharmacology. 4th ed., Delhi.

USP 23/NF 18 (1995) United States Pharmacopoeia and National Formulary, Rockville.

Wasdo SC, Juntunen J, Devarajan H, Sloan KB (2009) A correlation of flux through a silicone membrane with flux through hairless mouse skin and human skin *in vitro*. Int J Pharm 373: 62–67.

Wissing SA, Müller RH (2002) Solid lipid nanoparticles as carrier for sunscreens: *in vitro* release and *in vivo* skin penetration. Control Release 81: 225–233.

Wu L, Tang C, Yin C (2010) Preparation and characterization of paclitaxel system based on semi-solid lipid nanoparticles coated with poly (ethylene glycol). Pharmazie 65: 493–499.

Yamaguchi Y, Nagasawa T, Kitagawa A, Nakamura N, Matsumoto K, Uchiwa H, Hirata K, Igarashi R (2006) New nanotechnology for the guided tissue regeneration of skin – potential of lyotropic liquid crystals. Pharmazie 61: 112–116.

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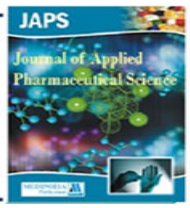

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Short Communication

Review of in vitro drug release test method's statistical evaluation to compare dissolution profile of semisolid dosage forms – Part I

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ABSTRACT

The aim of this mini-review study is to give an overview about *in vitro* drug release test methods statistical evaluation comparing dissolution profile of semisolids.

The FDA released guidance in May 1997 entitled Scale-up and Post Approval Changes for Nonsterile Semisolid Dosage Forms (SUPAC-SS). The guidance focuses on creams, gels, lotionts and ointments. The guideline desribes *in vitro* dissolution tetsing as an useful final quality control (QC) tool. The aim of it is to assure batch-to-batch quality of the product. Changes are separated in 4 categories in 3 Levels (Level 1,2,3). Level 2 recommends in vitro release (IVR) testing. Although there are several *in vitro* drug release test methods of semisolid dosage forms, their statistical evaluation is not clarified up to this day.

Our second challenge was to describe similarity and difference of these pharmaceutical dosage forms with use of similarity (f2) and difference (f1) factors. The FDA has issued these factors for solid dosage forms.

Our present work deals with calling attention on the lack of statistical validated method for semisolid dosage forms.

Keywords: semisolid dosage forms, dissolution, in vitro**.**

INTRODUCTION

In May, 1997 the FDA issued a guidance entitled Scale-up and Post Approval Changes: Chemistry, Manufacturing and Controls, In Vitro Release Testing and In Vivo Bioequivalence Documentation for Nonsterile Semisolid Dosage Forms (SUPAC-SS). The guidance focuses on creams, gels, lotions and ointments. It describes changes in 4 categories: components and composition; manufacturing equipment and process; scale (batch size); site of manufacture. Changes are categorized in 3 Levels: Level 1,2,3. Level 1 means changes that are unlikely to have any detectable impact on formulation quality and performance of the product in contrast with Level 2, which could have this impact. For Level 2 changes the guideline recommends in vitro release (IVR) testing in addition to application and compendial specifications. Level 3 is which have a significant impact on formulation quality and performance of the product. This level contains of IVR test for a site change or in vivo bioequivalence where application and compendial specifications are met.

The IVR test can characterize the performance of the product. The guideline describes several critical parameters of the method; diffusion cell system, synthetic membrane, receptor medium, number of samples, sample applications, sampling time, sample analysis, IVR rate, design of the rate comparison study. (FDA Guidance for Industry, 1997; Shah et al., 1998).

Among several mathematical methods investigated for dissolution profile, f1 and f2 by Moore and Flanner are the most common used and simplest (Moore and Flanner, 1996).

METHODS

In Vitro Release (IVR) Test Comparison

The IVR test should be carried out as a two-stage study. At the first stage, 2 runs of (six cells) *in vitro* apparatus should be carried out, yielding 6 slopes for the prechange lot (R) and 6 slopes for the postchange lot (T). IVR should be expressed in percentage. If, at the first stage, the 90% confidence interval falls within the limits of 75% to 133.33%, no further *in vitro* test is necessary. If the test is not passed at the first stage, 4 additional runs of the apparatus should be carried out, yielding 12 additional slopes or 18 in all. The first step in the statistical evaluation is to form the 36=6x6 individual T/R ratios from the post- and prechange slope data. The second step is to order the 36 rations from lowest to highest. In the third step, the eigth and twenty-ninth ordered individual ratios are the lower and upper limits. The product can pass or fail at the first stage. If the product had not passed at the first stage, an additional 4 runs would have been carried out, yielding 12 additional slopes per lot, for a total of 18 slopes. All 324=18x18 would be obtained. At the second stage, the 110th and the 215th ordered individual ratios are the lower and upper limits. The product can pass or fail at the second stage.

In case of there is only 30=5x6 individual T/R ratios, the sixth and twenty-fifth ordered T/R ratio are the limits.

Similarity (f2) and difference factors (f1)

Moore and Flanner described 2 equations – a difference factor (f1) (1) and the similarity factor (f2) (2). Both of them are acceptable methods by FDA for dissolution profile comparison, although f2 is preferred (O'Hara et al., 1998).

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \times 100 \quad \dots (1)$$

$$f_2 = 50 \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad \dots (2)$$

where n is the number of dissolution sample times, R*t* and T*t* the mean percent drug released at each time point: *t* for the reference and the test dissolution profiles.

The difference factor calculates the percent difference between the 2 curves at each time point and is a relative error between the 2 curves.

The similarity factor is a logarithmic reciprocal square root transformation of the sum of squared error and is a similarity on percentage between the 2 curves.

Curves to be considered similar, f1 values should be close to zero – between 0 and 15 and f2 values should be close to 100 – between 50 and 100 (Shah et al., 1998).

CONCLUSION

FDA has focused on a dissolution profile comparison in the pre- and post approval changes and bioequivalence. A dissolution profile can characterize the product better than a single point dissolution test. It helps to assure product performance and bioequivalence.

Our aim is to evaluate the IVR test and the similarity and difference factors with our developed products – ointments, creams and gels – in the future. We would like to discuss the deficiences of this field and validate the in vitro, in vivo and also the statistical evaluation of semisolid dosage forms.

ACKNOWLEDGEMENTS

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REFERENCE

FDA Guidance for Industry SUPAC-SS. Nonsterile Semisolid Dosage Forms, Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls. In Vitro Release Testing and In Vivo Bioequivalence Documentation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. June 1997

J.W. Moore, H.H. Flanner. Mathematical comparison of curves with an emphasis on in vitro dissolution profiles. Pharm. Tech. 1996; 20: 64-74.

T. O'Hara, A. Dunne, J. Butler, J. Devane . A review of methods used to compare dissolution profile data. Pharm. Sci. Technol. Today 1998; 1: 214-223.

V.P. Shah, J. Elkins, D. Schuirman, F. Pelsor, S. Shrivastava, W. DeCamp, P. Schwartz, R. Williams. Application of In Vitro Release Methods to Assure Product Performance of Semisolid Dosage Forms Before and After Certain Post-Approval Changes. Dissolut. Technol. 1998; 5: 5-11.

V.P. Shah, Y. Tsong, P. Sathe. In vitro dissolution profile comparison – statistics and analysis of the similarity factor, f2. Pharm. Res. 1998; 15: 889-896.

III.

TOVÁBBKÉPZŐ KÖZLEMÉNYEK

Gyógyszerészet 56. 131-137. 2012.

Félszilárd gyógyszerformák fejlesztése – a hatóanyag felszabadulás és bioekvivalencia vizsgálatok alapjai (Alkalmazott elvek, módszerek és berendezések: Irodalmi áttekintés) I. rész

Petró Éva[1], Erős István[2], Csóka Ildikó[1]

A félszilárd készítmények (kenőcsök, gélek, krémek, paszták stb.) a gyógyszerformák fontos részét alkotják, azonban biofarmáciájuk, ezen belül a hatóanyag felszabadulására vonatkozó, különböző műszerekkel végzett vizsgálataik a mai napig sem tekinthetőek megoldottnak. Míg pl. a szilárd gyógyszerformák esetében számos módszerrel rendelkezünk a hatóanyag kioldódásának vizsgálatára, addig a félszilárd készítmények kutatása során jelentős hiányosságok vannak. Nem létezik ugyanis egységes, gyógyszerkönyvi, validált módszer az ipari kutatás–fejlesztés számára. Célunk e témában összefoglaló tanulmány készítése volt, a jelenleg alkalmazott hatóanyag felszabadulási vizsgálatokról, az alkalmazott módszerekről és berendezésekről. Továbbá felhívjuk a figyelmet azokra a kritikus paraméterekre, amelyek figyelembe vételével lehetségessé válhatnának a hatósági és ipari elvárások, így egy egységes, gyógyszerkönyvi validált módszer beépítése a kutatási tevékenységbe.

Bevezetés

A hatóanyag tartalmú félszilárd gyógyszerformák (kenőcsök, krémek, gélek, paszták stb.) az összetett készítmények közé sorolhatóak, a rendszert alkotó komponensek és fázisok száma szerint [1, 2]. Bár e rendszerek hatóanyag leadása *in vitro* módszertanának területén figyelemre méltó eredmények vannak, biofarmáciájuk számos kérdése – a széleskörű vizsgálatok ellenére – még közel sem tekinthető tisztázottnak [3, 4].

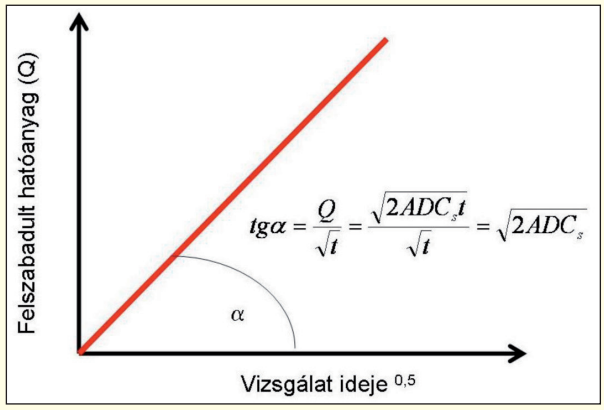
A dermatológiai gyógyszerformák gyógyszerleadásának tanulmányozására két módszercsoport terjedt el:
– a diffúziós vizsgálatok és a
– kioldódási vizsgálatok [4].

A gyakorlatban szívesen használják e két módszer kombinációját is, ami lényegében a szuszpendált hatóanyagok oldódásán és az oldott molekulák diffúzióján alapul, de a szemléletesség miatt ezt a „kombinált” módszert is kioldódási vizsgálatnak nevezik.

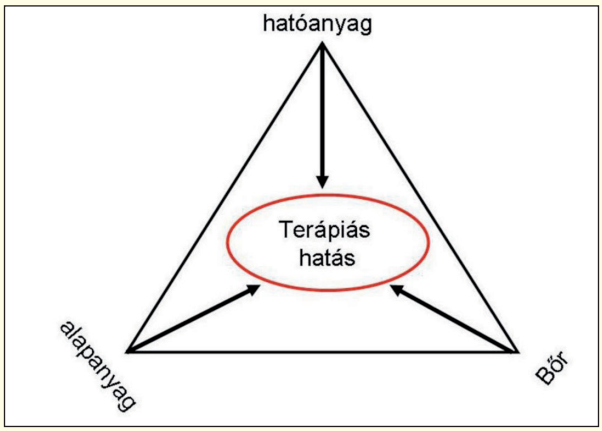
A kioldódási vizsgálatok a termék egyik legfontosabb minőségindikátorának és így a minőségellenőrzés alappillérének számítanak [1, 2]. Célszerű validált és hivatalos (gyógyszerkönyvekbe felvett, illetve az iparban alkalmazott) módszerekkel dolgozni [5]. E módszerek minőségének és robosztusságának a kutatásba történő bevezetése során figyelembe kell vennünk, hogy a vizsgált félszilárd rendszerek elsősorban emberi felhasználásra készülnek. Ezen alkalmazásukon kívül egyre fontosabb szerepet töltenek be a kioldódás vizsgálatok, mint bioekvivalencia tesztek, amelyek az *in vivo* eredmények előrevetítésére is szolgálhatnak. Ennek következtében az *in vitro–in vivo* korreláció (IVIVC) valamint a biológiai hasznosíthatóság mérésére is alkalmasak lehetnek a kioldódási vizsgálatok [1, 2].

A félszilárd készítményekre regulációs szempontból ugyanolyan szigorú elvárások vonatkoznak, mint a szilárd gyógyszerformákra [1, 2]. Míg a szilárd gyógyszerformák esetében jelentős irodalmi és kísérleti adatokkal rendelkezünk a hatóanyag kioldódás vizsgálatokra vonatkozóan, addig a félszilárd készítmények területén számos megoldatlan kérdéssel találkozunk. Ennek valószínű oka, hogy tervezésük még mindig tapasztalati alapokon és nem tudományos megfontoláson nyugszik, aminek következtében nincs egységes, validált gyógyszerkönyvi módszer az ipar számára [6].

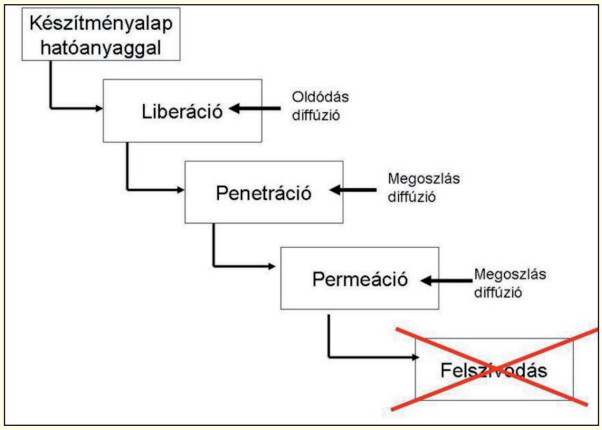
Jelen dolgozatban célunk a tervezett validáció megvalósulását elősegítő elméleti háttérismeret, módsze-



1. ábra: A Higuchi egyenlet grafikus ábrázolása. Az ábrán Q a felszabadult hatóanyag mennyiségét, A a mátrixban lévő teljes koncentrációt, D a gyógyszer diffúziós koefficiensét, C_s pedig a mátrixban oldott gyógyszer koncentrációját jelöli.



2. ábra: A bőrgyógyászati készítmények terápiás hatását meghatározó tényezők [17]



3. ábra: A terápiás hatás részfolyamatai [18]

rek, készülékek és kritikus paraméterek ismertetése és áttekinthető összefoglalása.

A hatóanyag kioldódás matematikai megközelítése

A félszilárd gyógyszerformákból a hatóanyag felszabadulását leíró matematikai modellek elsősorban a passzív diffúzió feltételezésén alapulnak. *Fick* I. törvénye a leggyakrabban alkalmazott matematikai összefüggés [7]:

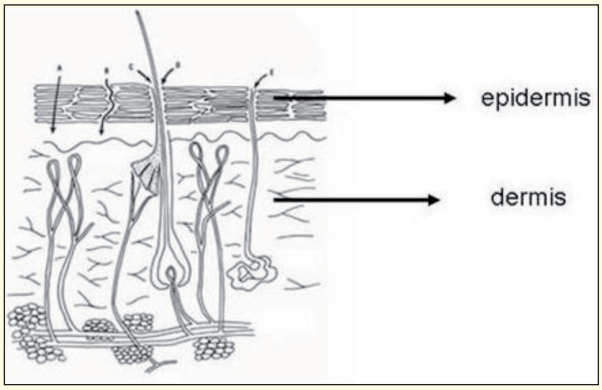
$$\frac{dm}{dt} = -DA\frac{dC}{dx} \qquad (1)$$

ahol *dm* a *dt* idő alatt *A* felületen végbemenő anyagáramlás *dC/dx* koncentráció-gradiens hatására. *D* a vizsgált molekula diffúziós állandója.

Az oldott vagy szuszpendált formában lévő hatóanyag felszabadulására vonatkozóan *Higuchi* fejlesztett ki egy geometriai megfontoláson alapuló elméletet [8]:

$$Q = \sqrt{2C_0 DC_s^0 t} \qquad (2),$$

ahol *Q* a *t* idő alatt kioldódott (felszabadult)



4. ábra: A hatóanyagok bejutási kapui [7, 11, 17]

hatóanyag mennyiség, C_S^0 a szuszpendált gyógyszer oldhatósága, *D* a gyógyszermolekula diffúziós koefficiense.

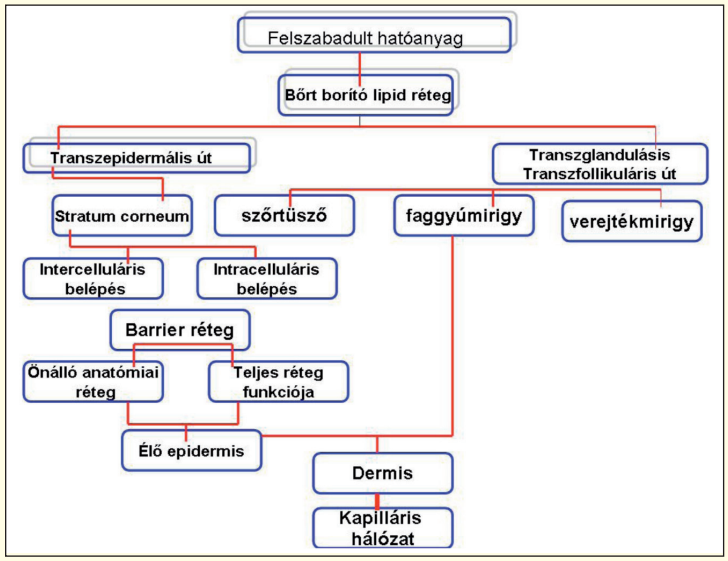
Az előbbi megállapítás során a következő feltételeknek kell teljesülniük:

– a gyógyszermolekulák átmérője sokkal kisebb legyen, mint a kenőcs- (gél-, krém-) réteg, melyen a vizsgálatot végrehajtjuk,
– a gyógyszer eredeti koncentrációja C_S^0-nál jellemzően nagyobb legyen,
– a vizsgált felület nem elegyedhet a készítményalap összetevőivel.

A modell csak azokra a rendszerekre alkalmazható, amelyekben a gyógyszer oldhatósága a hatóanyag koncentrációjához képest elhanyagolható.

A *Higuchi* egyenlet grafikus ábrázolása során (***1. ábra***) az idő függvényében a felszabadult hatóanyag mennyiségét jelenítjük meg. Ez utóbbi érték az idő gyökének függvényében egy egyenest ad [8].

Bialik [9, 10] – részben Higuchi modellje alapján, részben attól függetlenül – a gyógyszer felszabadulás kinetikájának vizsgálata során a mért adatokhoz az alábbi függvényeket illesztette:



5. ábra: A farmakon útja a bőrben [7, 15]

I. táblázat

Perkután felszívódást befolyásoló faktorok [7, 15, 16]

Hatóanyag	Molekulatömeg, diffúziós koefficiens, v/o megoszlási hányados, permeabilitási koefficiens, ionizáció
Készítményalap	Polaritás, hatóanyagot oldó képesség, illékonyság Koncentráció, a str. corneumra gyakorolt hatás Segédanyagok, penetrációt fokozó komponensek, pH
Bőr	Típus, életkor, nem, rassz, anatómia, szövettan Hőmérséklet, a str. corneum sérültsége vagy épsége Hatóanyag metabolizmusa
Alkalmazás	A bőrfelületen alkalmazott dózis (filmvastagság, koncentráció) A készítményalappal kontaktusban levő bőrfelület Az alkalmazás gyakorisága és időtartama

$$Q = A + Bt \quad (3)$$
$$Q = A \exp(Bt) \quad (4)$$
$$Q = A\, t^{B} \quad (5)$$
$$Q = A + B/t \quad (6)$$
$$Q = 1/(A + Bt) \quad (7),$$

ahol Q a felszabadult (oldódott és diffundált) hatóanyag mennyisége t idő után, A a függvény tengelymetszése és B az irányítényező. *Bialik* azt találta, hogy az (**5**) hatványfüggvény és a (**7**) reciprok összefüggés adta a legszorosabb korrelációt, ill. regressziót.

A hatóanyag kioldódás biológiai megközelítése

A bőrön alkalmazott gyógyszerkészítmények terápiás hatása bonyolult folyamat. A komplexitás oka: egyrészt a bőr többrétegű barrier felépítése és funkciója, másrészt pedig a kenőcsterápiában, a többi gyógyszerformától eltérően, számolnunk kell a készítményalap saját hatásával is. A hatás előfeltétele, hogy a hatóanyaghoz megfelelő készítményalapot válasszunk, illetve biztosítsuk, hogy a hatóanyag az alapból felszabaduljon és behatoljon a bőr külső rétegeibe.

A terápiás hatás ez utóbbi három tényező kölcsönhatásának eredményeként alakul ki (***2. ábra***) [11, 17].

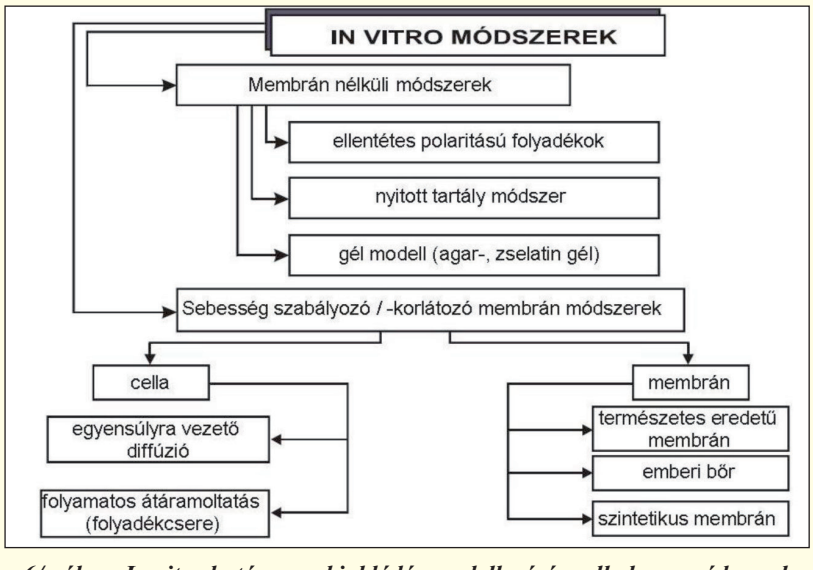
A perkután abszorpció folyamatának feltételei a következők:

– a hatóanyag készítményalapból történő felszabadulása (liberáció),
– a gyógyszermolekulák belépése a bőr külső sejtrétegeibe (penetráció),
– átjutás az elhalt és élő epidermisen (permeáció),
– a hatóanyag belépése a keringésbe (***3. ábra***) [7, 12].

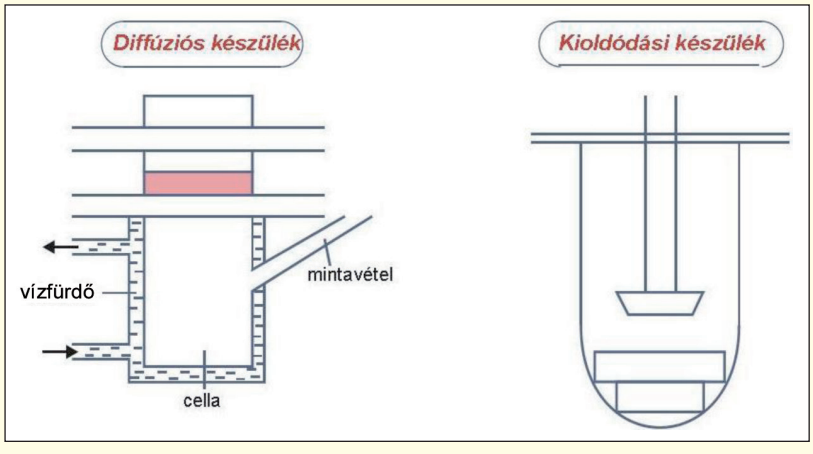
A hatóanyag felszívódása dermatológiai készítmények esetében nem kívánatos, egyes esetekben ártalmas is lehet [12, 16, 17].

A legkülső rétegen, az epidermis szarurétegén, vagyis a *stratum corneumon* keresztüli bejutás kulcsfontosságú a perkután abszorpció, valamint a dermatológiai kezelés sikere szempontjából.

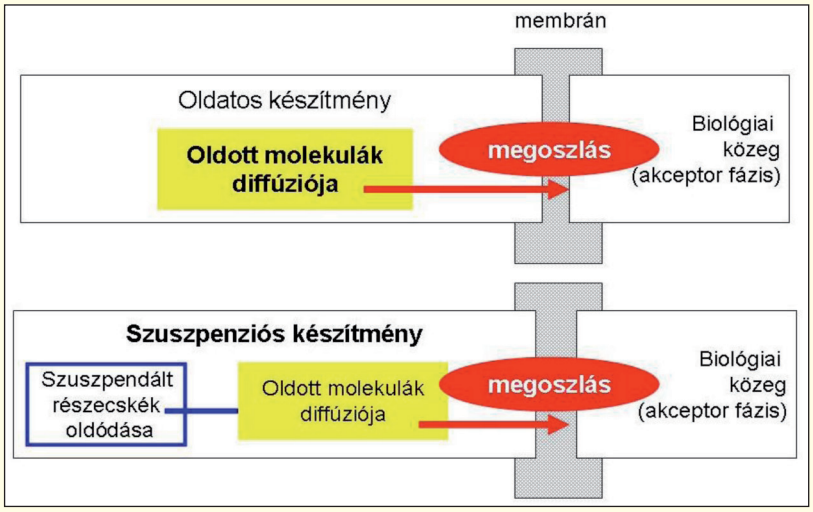
A hatóanyagok penetrációjának két módja lehetséges: a hámrétegen keresztüli és a bőrfüggelékeken keresztüli út, ezeken belül:



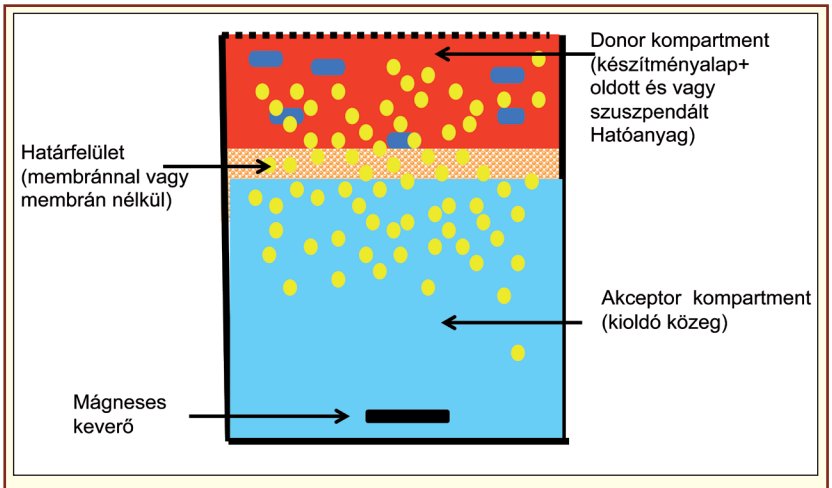
6/a ábra: In vitro hatóanyag kioldódás modellezésére alkalmas módszerek [7, 18]



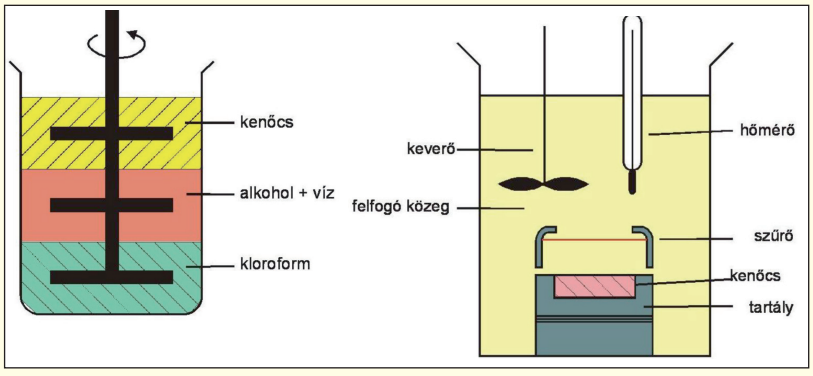
6/b. ábra: Diffúziós és kioldódási készülék vázrajza [19]



7/a ábra: Membránnal működő kioldódási készülék általános modellje



7/b ábra: Oldódás, diffúzió és megoszlás a membránnal működő készülékekben



8/a és b ábra: Membrán nélküli, a mintával nem elegyedő folyadékot tartalmazó modell (bal oldalon) [21], és nyitott tartályos, a mintával nem elegyedő folyadékot tartalmazó membrán nélküli modell [23]

– az ép hámsejteken keresztüli (intracelluláris út),
– a hámsejtek közötti (intercelluláris út),
– a szőrtüszőkön keresztüli (transzfollikuláris út) és a faggyúmirigyeken keresztüli (transzglanduláris út),
– a verejtékmirigyek által történő bejutás (szintén transzglanduláris út) (***4.*** és ***5. ábra)*** [7, 11, 12].

A megfelelő dermatológiai terápia magában foglalja a fent említett folyamatokat, a négy legfontosabb tényező, a hatóanyag, a készítményalap, a bőrstruktúra valamint az alkalmazás jellemzőinek figyelembe vételével (***I. táblázat***) [7, 15–18]. A *Tronnier*-féle hatásháromszög (***2. ábra***) szemlélteti megfelelően a komplex folyamatot.

A készítményalap és a bőr szabályozzák a hatóanyag felszabadulását (kioldódás és diffúzió), valamint a liberációt követő megoszlást, így a perkután penetráció és permeáció folyamatát a többrétegű szarurétegen (*str. corneum*) keresztül. A *stratum corneum* elhalt sejtekből áll, így a folyamatban az aktív transzport nem játszik szerepet. Az epidermisnek a *stratum corneum* alatti sejtrétegei (*str. granulosum, str. spinosum, str. basale*) élő sejtekből állnak, ezért itt már az aktív és facilitált transzport folyamatok is számításba jöhetnek [7, 11, 12, 16, 17].

Az egyes humán rasszok közötti eltérések további kérdéseket vetnek fel a kutatás során. Az afrikai bőrtípus a sűrűbb és vastagabb sejtréteg következtében rigidebb és a kaukázusinál jobban ellenálló a toxikus kémiai anyagokkal szemben, így hatékonyabb barrierként szolgál. A témával napjainkban a *geokozmetiká*nak nevezett tudományág foglalkozik [19].

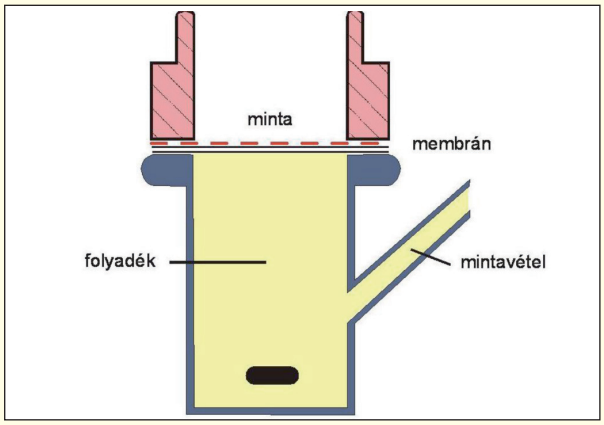
A hatóanyag kioldódás során alkalmazott vizsgálati módszerek

A bőr *in vitro* modellezése bonyolult feladat, ezért egyszerűsített modelleket használnak a gyakorlati laboratóriumi munka során ***(6/a** és **6/b ábra)*** [7, 18, 19].

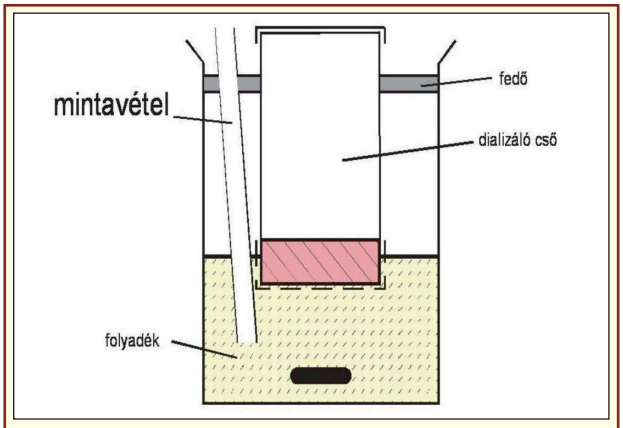
A ***6/a. ábra*** a két szerkezeti alaptípust – membrán nélküli és membránnal működő készülékeket – szemlélteti. Ez nem jelent történeti sorrendet, mivel a múlt század 20-as éveiben már alkalmaztak nagyon szellemes membrános modelleket [7, 17, 18], és a 20. század végén is találkozunk a közleményekben membrán nélküli gél-modellekkel.

A ***6/b ábrán*** a két funkcionális típus – diffúziós és kioldáson alapuló készülék – vázlata látható [19].

Az *in vitro* tesztek – amint már a *Bevezetés*ben utaltunk rá – a készítmény terápiás használhatóságá-



9/a ábra: Speciális üvegtartályú membrános készülék [23]



9/b ábra: Laboratóriumi membrános készülék (Mayer és Kedvessy [24])

nak indikátorai, valamint a bioekvivalencia tesztek is, így az *in vitro-in vivo* korreláció (IVIVC) előrejelzésére is alkalmasak [1, 2, 20].

Az *in vitro* vizsgálatok előnye, hogy a vizsgálatot végző személy laboratóriumi körülmények között a gyógyszer felszabadulását zavaró faktorokat kizárni képes. Hátránya, hogy a metódus nem képes teljesen szimulálni az *in vivo* feltételeket, különös tekintettel a vérkeringésre és metabolizmusra [7, 18, 20].

In vitro módszerek

Az *in vitro* modellek – a ***6/a ábrán*** láthatjuk – két nagy csoportra tagolhatóak: membrán nélküli, ún. *felszabadulási* és membránnal működő *diffúziós* modellekre. Ez utóbbi modelleknek két alapvető alkotórészük van: (1) a membrán, amely *természetes* (emberi, állati eredetű), illetve *mesterséges* membrán lehet, és (2) a diffúziós cella, amely a vizsgált készítményt, a bőrt szimuláló membránt, és az ún. akceptor fázist tartalmazza. A cellában a minta és az akceptor fázis között megy végbe a hatóanyag diffúziója, mely az egyensúlyi állapot eléréséig tart. Az *in vivo* körülmények szimulálását az akceptor folyamatos keringetése vagy állandó átáramoltatása biztosítja [7, 18].

A félszilárd gyógyszerformák hatóanyag felszabadulási vizsgálatára alkalmas általános berendezés felépítése látható a ***7/a és 7/b ábrákon*** [7, 16, 18].

Az *in vitro* vizsgálatok hátránya, hogy nem veszik figyelembe a bőr fiziológiai körülményeit, így az ép *stratum corneumon* keresztüli *steady state* áramlást valamint a bőrfüggelékeken történő penetrációt.

További nehézség, hogy az *in vitro* kísérletek csak a hatóanyag kioldódott, illetve átdiffundált mennyiségét mérik. Az élő szervezetben viszont számolnunk kell a gyógyszer szövetekben végbemenő metabolizációjával is. Ha a humán bőrszövet modellezésére állati eredetű bőrt használunk, figyelnünk kell a szőr eltávolítása következtében létrejött permeáció emelkedésre, valamint élő vizsgálati állat esetében a gyógyszer lenyelésének vagy inhalációjának elkerülésére.

Membrán nélküli hatóanyag kioldódási modellek

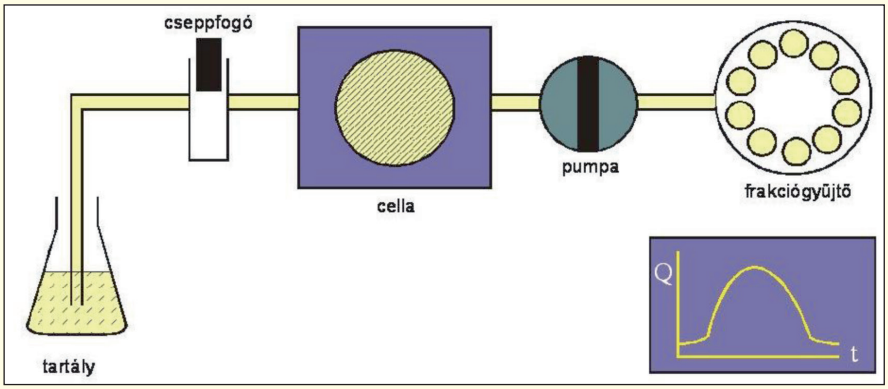
A módszer lényege a hatóanyag felszabadulás kinetikájának mérése. A feltételezés alapja, hogy a hatóanyag a készítményalap és a tesztközeg között hasonlóképpen oszlik meg, mint *in vivo* körülmények között az alap és az alkalmazott bőrfelület között. Az egyik legegyszerűbb membrán nélküli készüléket szemlélteti a **8*/a. ábra*** [21]. E módszer lényege, hogy a vizsgált készítményt két ellentétes polaritású folyadékra rétegezték. A hidrofób folyadék a szaruréteget, a hidrofil pedig az élő bőrszövetet szimulálja. Konstrukciós hiba, hogy a készítmény nem az apoláris közeggel érintkezik, hanem – fizikai kémiai megfontolások miatt – a poláris közeggel. Így nem tekinthető a fiziológiás viszonyok modelljének.

Jobban utánozza a fiziológiai történéseket a ***8/b. ábrán*** bemutatott készülék. A mintát szűrőpapír-membránnal fedik, hogy ne keveredjen az akceptor fázissal, vagy membránra, illetve alkalmas mintatartóba helyezik, annak érdekében, hogy ne mozduljon el, és ne képződjenek buborékok a határfelületen. A kioldóközeget az üvegcellába töltik. Akceptorként vizes közeg, víz, agar-gél vagy zselatin-gél használható. *Poulsen* [22] alkalmazott először akceptor közegként izopropilmirisztátot, annak érdekében, hogy megközelítse a valódi, bőrön keresztül zajló megoszlást és diffúziót.

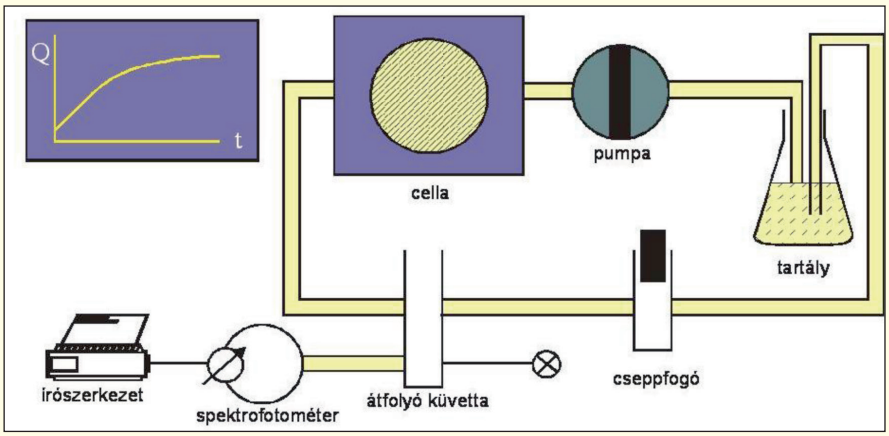
A gyógyszerformából a hatóanyag felszabadulási jellemzőinek, valamint a hatóanyag és a készítményalap közötti interakció megfigyelésére alkalmas ez az eljárás [7, 22].

Membránnal végzett hatóanyag-kioldódási modellek

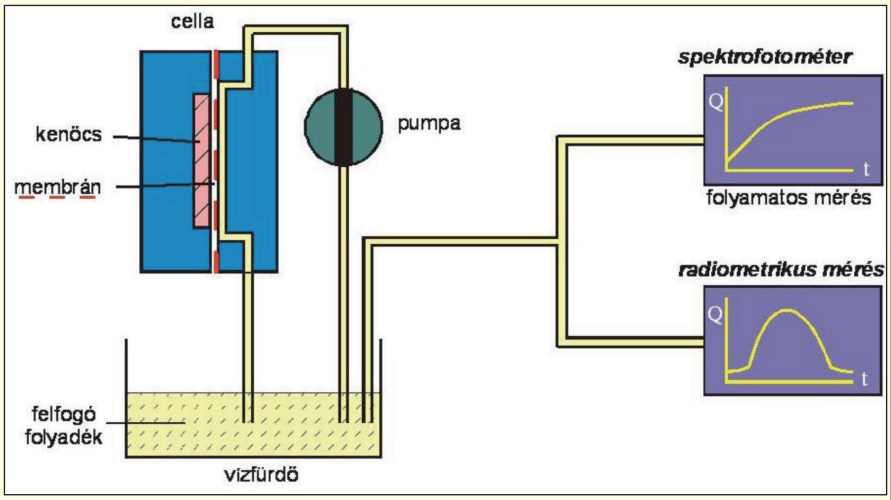
A gyakorlatban a membránon keresztüli diffúziót megvalósító *in vitro* készülékek terjedtek el legjobban [7, 17, 18]. A berendezések elve a megoszlás. A hatóanyag oldódik a készítményalapban, és az oldott molekulák a membrán és a készítmény határfelületére dif-



10. ábra: Sartorius készülék az akceptor folyadék folyamatos áramoltatásával és mintagyűjtővel [25]



11. ábra: Sartorius készülék az akceptor folyamatos áramoltatásával és a hatóanyag mennyiségének folyamatos regisztrálásával [25]



12. ábra: Diffucel készülék felépítése [26]

fundálnak. Behatolnak (penetrálódnak) a membránba, majd a membrán és az akceptor közötti megoszlás következményeként átjutnak az akceptor fázisba. A membrán a *str. corneumot*, az akceptor az élő *epidermist* szimulálja, így a történések három lényeges részfolyamatának – liberáció, penetráció, permeáció – végeredménye kvantitatív módon meghatározható. Ez a jelenség-csoport látható a ***9/a*** és ***9/b ábrákon***.

A készülék előnyei között megemlítendő a viszonylag egyszerű szerkezet, a jól reprodukálható kísérleti körülmények és az eredmények összehasonlíthatósága alapján könnyen és gyorsan kiválasztható egy összetétel sorozatból a legjobb készítményalap. Természetesen ez az eljárás sem szimulálja a fiziológiás történéseket minden részletükben. Néhány membrándiffúziós modellt mutatunk be a ***10., 11.*** és a ***12. ábrán.***

A Sartorius cég által szerkesztett készülékek (***10*** és ***11. ábrák***) a liberáció, penetráció és permeáció kinetikai viszonyairól is értékelhető képet adnak, mivel a frakciók gyűjtése és az akceptor folyadék folyamatos keringetése a vérkeringést szimulálja [25]. Hasonló célt szolgál az *Asche és mtsai* által szerkesztett készülék (***12. ábra***) [26].

A kísérletekben hidrofil vagy lipofil karakterű membránokat alkalmazhatunk. A hidrofil membrán az élő *epidermist*, a lipofil membrán pedig a *stratum corneumot* szimulálja [20].

Hidrofil típusú membránok

A celofán, különböző cellulózszármazékok, a nefrofán és fluor-vinil-polimer használatosak hidrofil membránként. Újabban a polikarbonát membránokat is megfelelőnek találták [20].

Az akceptor fázis vagy vizes közeg (víz, puffer oldat, etanol-víz elegy, agar-gél, zselatin-gél) vagy nemvizes közeg (izopropilmirisztát) lehet [7, 20].

Lipofil típusú membránok

A lipofil membránok az *in vivo* viszonyokat jobban szimulálják, mint a hidrofil membránok. Polidimetil-sziloxán (PDMS), politetraflouroetilén (PTFE) valamint szilikon membránokat alkalmaznak leggyakrabban [7, 20].

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A munkát a *TÁMOP 4.2.1/B-09/1/KONV-2010-0005 projekt* támogatásával végeztük.

IRODALOM

1. FDA Guidance for Industry: Nonsterile Semisolid Dosage Forms, Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation SUPAC-SS, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), May (*1997*). – 2. FDA Guidance for Industry: Topical Dermatological Drug Product NDAs and ANDAs – In Vivo Bioavailability, Bioequivalence, In Vitro Release, and Asssociated Studies SUPAC-SS, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), June (*1998*). – 3. *Siewert, M., Dressman, J., Brown, C.K., Shah, V.P.*: AAPS PharmSciTech *4* (1), 1-10 (*2003*). – 4. *Erős, I., Abu-Eida, Cserne, A., Csóka, I., Csányi, E.*: Acta Pharm. Hung. *70*, 29-34 (*2000*). – 5. FDA Guideline on General Principles of Process Validation, Center for Biologics Evaluation and Research, Center for Devices and Radiological Health, Food and Drug Administration, Division of Manufacturing and Product Quality, Office of Compliance, Center for Drug Evaluation and Research (CDER), Food and Drug Administration, May (*1987*). – 6. The United States Pharmacopoeia (USP) XXIII *(1995).* – 7. *Barry, B.W.*: Dermatological Formulation: Percutaneous Absorption, Marckel Decker Inc., New York, Basel *(1983).* – 8. *Higuchi, T.*: J. Pharm. Sci. *50*, 874-876 (*1961*). – 9. *Bialik, W.*:Pharmazie *39*, 763 (*1984*). – 10. *Bialik, W.*: Sci. Pharm. 93-98 (*1985*). – 11. *Thoma, K.:* Dermatika, Werbe- und Vertriebsgesellschaft Deutscher Apotheker m.b.H., München *(1983).* – 12. *Schaaf, F.:* Probleme Dermatologischer Grundlagenforschung, Dr. Alfred Hüthig Verlag Heidelberg *(1969).* – 13. www.pharmainfo.net/reviews/topical-drug-delivery-systems-review – 14. *Erős I.:* Gyógyszerészet *39*, 253-258 (*1995*). – 15. ATLA *24*, 81-106 (*1994*). – 16. *Kydonieus, A.F., Berner, B.B.* (Eds.): Transdermal Delivery of Drugs, Vol. I-III. (*1987*). – 17. *Tronnier, H.*: Über die Wirkungsweise indifferenter Salben und Emulsionssysteme an der Haut in Abhängigkeit von ihrer Zusammensetzung, Editio Cantor KG./796 Aulendorf i. Württ. *(1964).* – 18. *Erős I.*: Olaj, Szappan, Kozmetika *4*, 138-142 (*2003*). – 19. *Fares, H.M., Zatz, J.L.:* Pharm. Technology *32*, 53-59 (*1995*). – 20. *Shah, V.P., Williams, R.L.*: Roles of Dissolution Testing: Regulatory, Industry and Academic Perspectives – Role of Dissolution Testing in Regulating Pharmaceuticals, August (*1999*). – 21. *Busse, M.J., Hunt, P., Lees, K.A., Maggs, P.N., McCharty, T.M.*: British J. Dermatol, *78*, 645 (*1969*). – 22. *Poulsen, B.J., Young, E., Coquilla, V., Katz, M.*: J. Pharm. Sci. *57*, 928 (*1968*). – 23. *Turakka, L., Piepponen, T., Kahela, T.:* Labo Pharma, *32*, 540-544 (*1984*). – 24. *Mayer, A., Kedvessy, Gy.*: Pharm. Ind. *31*, 323-328 (*1969*). – 25. *Loth, H., Holla-Benninger, A.*: Pharm. Ind. *40*, 256-261 (*1978*). – 26. *Ashe, H., Botta, L., Rettig, H., Weirich, E.G.*: Pharm. Acta Helv. *60*, 232-237 (*1985*).

Petró, É., Erős, I., Csóka, I.: ***Development of semisolid dosage forms – drug release and bioequivalence investigations. Principle, methods and equipments – review. Part I.***

The semisolid preparations represent an important part of dosage forms, however their biopharmaceutics considering investigations of their drug release with different methods cannot be as good as resolved up to this day. However there are a lot of drug release methods in case of solid dosage forms, the drug release methodology in case of semisolids is still not clarified. There is no harmonised validation method for their drug release in the industrial research and development in the Pharmacopoeias. The aim of our study is to give an overview about the most used drug release experiments, methods and equipments. Furthermore we try to pay attention to these critical parameters, which can help to build a harmonised validation method into the research process. This project can fulfil the regulatory and industrial requirements.

[1]*Szegedi Tudományegyetem Gyógyszerfelügyeleti Intézet, Szeged, Eötvös u. 6. – 6720*
[2]*Szegedi Tudományegyetem Gyógyszertechnológiai Intézet, Szeged, Eötvös u. 6. – 6720*

IV.

TOVÁBBKÉPZŐ KÖZLEMÉNYEK

Gyógyszerészet 56. 195-202. 2012.

Félszilárd gyógyszerformák fejlesztése – a hatóanyag felszabadulás és bioekvivalencia vizsgálatok alapjai (Alkalmazott elvek, módszerek és berendezések: Irodalmi áttekintés) II. rész*

Petró Éva[1], Erős István[2], Csóka Ildikó[1]

In vivo módszerek

Klinikai összehasonlító vizsgálatokban (pl. glükokortikoidokkal végzett kísérletekben) és validált bioekvivalencia mérésekben (pl. dermatofarmakokinetikai vizsgálatokban) használják az *in vivo* teszteket. A félszilárd gyógyszerformák bioekvivalencia (BE) vizsgálatai a hatóanyag farmakológiai aktivitását és a gyógyszerforma (készítményalap és a benne alkalmazott egyes segédanyagok, pl. penetrációt-permeációt fokozó szerek) befolyását vizsgálják, illetve hasonlítják össze.

A helyileg alkalmazott kortikoszteroidok bizonyítottan hatásosak különböző bőrbetegségek gyógyításában, mint pl. ekcéma és psoriasis. A legelterjedtebb vizsgálatok a vazokonstriktor hatás, a kroton olajjal végzett gyulladáscsökkentő vizsgálat, valamint az adhezív *„tape stripping”* módszer. A bioekvivalencia vizsgálatok fejlesztése ezért a vizsgálati irányelvek és módszerek meghatározása szempontjából is fontos [1, 20].

Az élő állatban a perkután abszorpció folyamatának megértése érdekében először a penetrációt és permeációt kell vizsgálnunk. Azt fontos tudnunk, hogy az *in vivo* gyógyszer-permeációs vizsgálatoknál nagy a szórás mértéke, sok a hibatényező, így az *in vitro* vizsgálatokkal együtt tekinthetőek megbízható vizsgálatnak [7].

Állatkísérletes modellek

Számos kísérletet végeztek a laboratóriumi állatmodellek fejlesztésére, melyben a perkután abszorpció folyamata megközelíti az emberét. Ez sajnos csak ritkán sikerült, az emberi és állati bőr anatómiai és fiziológiai különbségei miatt. A *stratum corneum* vastagsága és felépítése, a szőrtüszők és izzadságmirigyek sűrűsége, a vérkeringés, valamint a biokémiai tényezők az ember és a kísérleti állatok esetében lényegesen különböznek [7].

További korlátot jelent, ha a kísérlet célja a gyógyszer terápiás aktivitásának vagy biohasznosíthatóságának megállapítása, ugyanis a betegségek lefolyása is eltérő az emberekben az állatokhoz képest.

A félszilárd készítmények (kenőcsök, gélek, krémek, paszták stb.) a gyógyszerformák fontos részét alkotják, azonban biofarmáciájuk, ezen belül a hatóanyag felszabadulására vonatkozó, különböző műszerekkel végzett vizsgálataik a mai napig sem tekinthetőek megoldottnak. Míg pl. a szilárd gyógyszerformák esetében számos módszerrel rendelkezünk a hatóanyag kioldódásának vizsgálatára, addig a félszilárd készítmények kutatása során jelentős hiányosságok vannak. Nem létezik ugyanis egységes, gyógyszerkönyvi, validált módszer az ipari kutatás–fejlesztés számára. Célunk e témában összefoglaló tanulmány készítése volt, a jelenleg alkalmazott hatóanyag felszabadulási vizsgálatokról, az alkalmazott módszerekről és berendezésekről. Továbbá felhívjuk a figyelmet azokra a kritikus paraméterekre, amelyek figyelembe vételével lehetségessé válhatnának a hatósági és ipari elvárások, így egy egységes, gyógyszerkönyvi validált módszer beépítése a kutatási tevékenységbe.

Az állatkísérleteket széles körben használják a lehetséges toxicitás előrejelzésére, azonban le kell szögeznünk, hogy az állatokon végzett toxicitási mérések semmi esetre sem szolgálhatnak teljesen biztonságos és alapos útmutatóként ebben a kérdéskörben.

Az állatkísérletekben a felszívódott hatóanyag mennyiségét detektálják, ami nem azonos a dermatológiai biológiai használhatósággal. Ennek alapján az állatkísérleteknek két típusa van:

– a felszívódott hatóanyag mennyiségének mérése,
– a kiváltott bőrreakció (gyulladás, ödéma stb.) megszüntetése, ill. csökkentése [7, 12, 15].

Kísérleti technológiák

A fiziológiai vagy farmakológiai válasz megfigyelése

Ha a félszilárd készítmény bármely ható- vagy segédanyaga biológiai reakciót (pl. lokális allergiát, toxicitást, izzadság- vagy faggyúmirigy szekréciót, vazo-

*Az első rész megjelent Gyógyszerészet, 56, 131-137 (2012).

II. táblázat

Az FDA és a FIP-AAPS irányelveinek összehasonlítása

FDA		FIP -AAPS
Módszer: Franz diffúziós cella		
Membrán: szintetikus poliszulfon, cellulóz-acetát, -nitrát észter, politetrafluoroetilén		
– vizes pufferrendszer – (vízoldékony hatóanyag) – víz-alkohol elegy (korlátozottan oldódó hatóanyagok)		– Alkohol-tartalmú rendszer – (és felületaktív anyag)
	kioldóközeg hőmérséklet	32 ^{0}C
300 mg	***mintamennyiség***	Jellemző dózis
5 ≤ x	***mintaszám***	
6 órás intervallum (pl. 30 perc,1, 2, 4, 6 óra) friss aliquot membrán-kioldó közeg kapcsolata	***mintavételi idő***	
Validált, specifikus, érzékeny módszer	***mintaanalízis***	

dilatációt, vaszkuláris permeabilitást, epidermális proliferációt, keratinizációt) vált ki, az erre adott válasz a penetráció kinetikájának megismerését segíti elő.

Helyi allergiás, toxikus stb. reakciók kiváltását használják fel az állatkísérletek során. Ezen kívül gyakori a bőr verejtékmirigyeinek, pigmentációjának, faggyúmirigy termelésének, és az előbb felsorolt patofiziológiás folyamatok megfigyelése, és ha lehetséges, számszerű értékelése is. Az ide tartozó legnépszerűbb technikák sorában a bőrnek a szteroidokra adott vazokonstriktor vagy fehérítő válaszát vizsgálják, valamint a bőrön alkalmazott nitroglicerinre bekövetkező vérnyomás különbségek vizsgálatát alkalmazzák [15].

A bőr változásainak vizsgálata fizikai tényezők hatására

In vivo és *in vitro* is elterjedt a bőr fizikai eltérőségeinek, ill. fizikai mérésekkel jellemezhető változásainak vizsgálata. Ide sorolhatók pl. a transzepidermális vízvesztés, hőmérsékletkülönbségek, mechanikai analízis, ultrahang, funkció és dimenzió szerinti osztályozás, spektrumanalízis, fotoakusztikus és elektronikus lehetőségek mérésén alapuló módszerek. Közülük számos kísérlet fontos információt szolgáltathat, azonban az orvosi kezelésben egyelőre nem jönnek számításba.

A hatóanyag, illetve a készítmény tömegváltozásának mérése

Az alkalmazott vivőanyag és a penetrációt fokozó anyag mennyiségének csökkenéséből lehetőség van a gyógyszer felszívódásának helyére következtetni. Mivel a bőr impermeábilis számos farmakon számára, ezért a koncentrációcsökkenés kicsi lesz, és ez pontos analízist tesz lehetővé. A koncentráció eltérések a készítményalap összetételétől, izzadságtól vagy a transzepidermális vízvesztéstől is függhetnek. A koncentráció emelkedés a készítmény bőrön történő felhalmozódásából eredhet [7].

Szövettani vizsgálatok

Ezek a vizsgálatok kevésbé pontos analízist tesznek lehetővé, mint az előző, mivel a felszívódás után a gyógyszeranyagok távol kerülhetnek az alkalmazási helyüktől.

A gyógyszer penetrációjának és lokalizációjának demonstrálására bevezették a *floureszcens mikroszkópokos vizsgálat*ot és *mikroszkópikus autoradiográfiát*. Különösen az utóbbi módszer alkalmas a radioaktív anyagok kimutatására, mivel azok elnyelik az alfa és béta sugarakat. Tríciummal jelölt izotópok alkalmazása gyenge emissziós hatásuk miatt javasolt. Erős béta-elnyelő vegyületek sötétítik a vizsgált területet.

Korábbi vizsgálatokban a kutatók a penetrációt fokozó anyagot megfestették. A fizikokémiai elmélet bizonyítja, hogy a membrántranszport molekuláris szinten, a megoszlással és diffúzióval valósul meg. A festék befolyásolja a folyamatot, így ezt a vizsgálatot ma már nem használják.

Fluoreszcens meghatározást az *A vitamin*, *tetraciklin* és *benzpirén* meghatározására használnak. A *stratum corneum* permeabilitásának meghatározására tetrakloroszalicilanilidet használnak.

A bőrpenetráció közvetlen mérését a keratolitok és emolliensek befolyásolják [7].

A hatóanyag kioldódás során alkalmazott vizsgálati berendezések

A szilárd gyógyszerformákkal ellentétben a félszilárd készítmények vizsgálatára nincs egységesen elfoga-

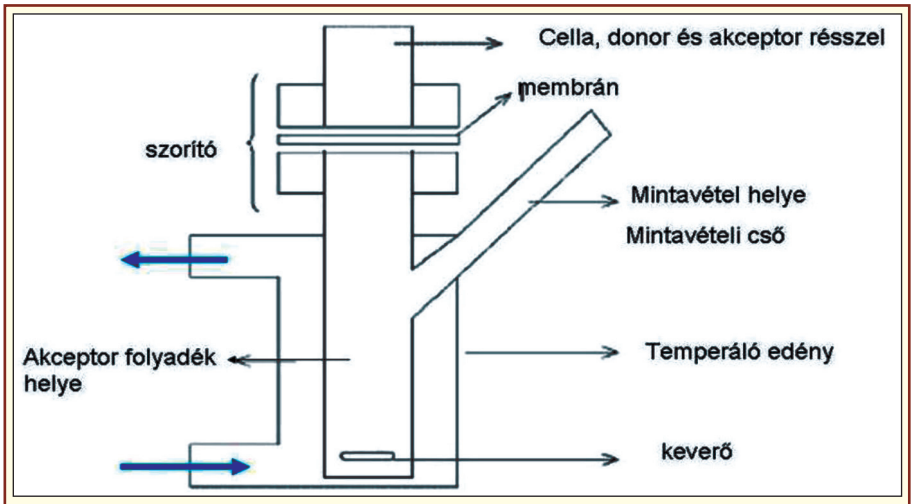
dott, gyógyszerkönyvi módszer [6]. A kioldódás vizsgálatok célja a kritikus paraméterek – a membrántípus, pórusméret, nedvesedés, felület, mintamennyiség, mintavétel valamint az akceptor közeg stb.– kioldódást befolyásoló hatásának feltérképezése, mivel a felsorolt tényezők az egyes módszerek esetében különböznek, ennek következtében nem vethetők össze az eredmények.

A gyakorlatban két berendezés-típus terjedt el:

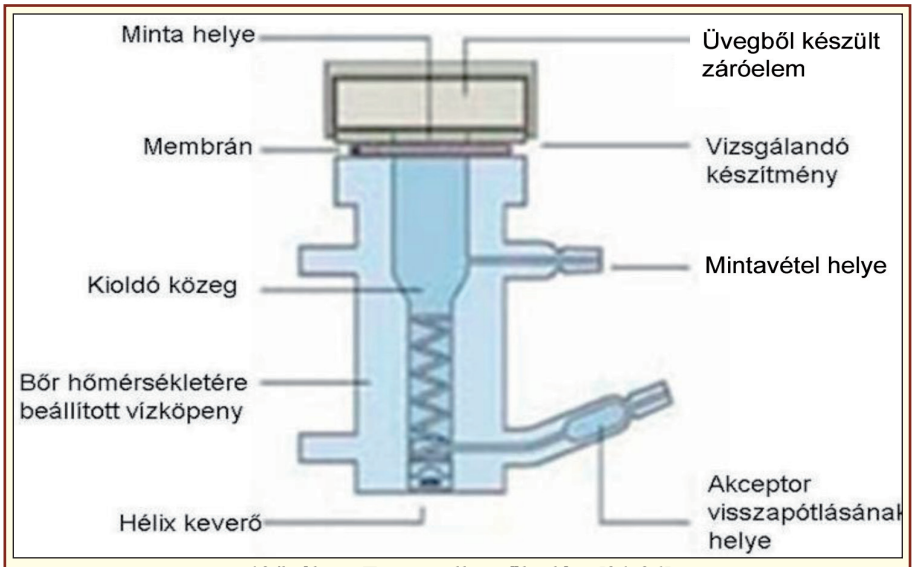
1. *Franz diffúziós cella*, amelyben a donor rész az akceptor felett helyezkedik el és a molekulák diffúzióját a gravitáció is fokozza,
2. a *forgólapátos készülékek*, melyekben a mintatartó feletti akceptor fázist a változtatható sebességgel forgó lapát állandó áramlásban tartja.

A dermatológiai készítményekben alkalmazott hatóanyagok kioldódási vizsgálatának alapmodellje a *Franz vertikális diffúziós cella*. A *Food and Drug Administration* (FDA) és az *American Associety of Pharmaceutical Scientists* (AAPS) irányelveiben legalkalmasabb készülékként – különböző típusú membránokkal ellátva – a Franz cellát jelöli meg, azonban nem határoz meg konkrét vizsgálati körülményeket a hatóanyag felszabadulás vizsgálatára vonatkozóan (***II. táblázat***) [1, 2, 3].

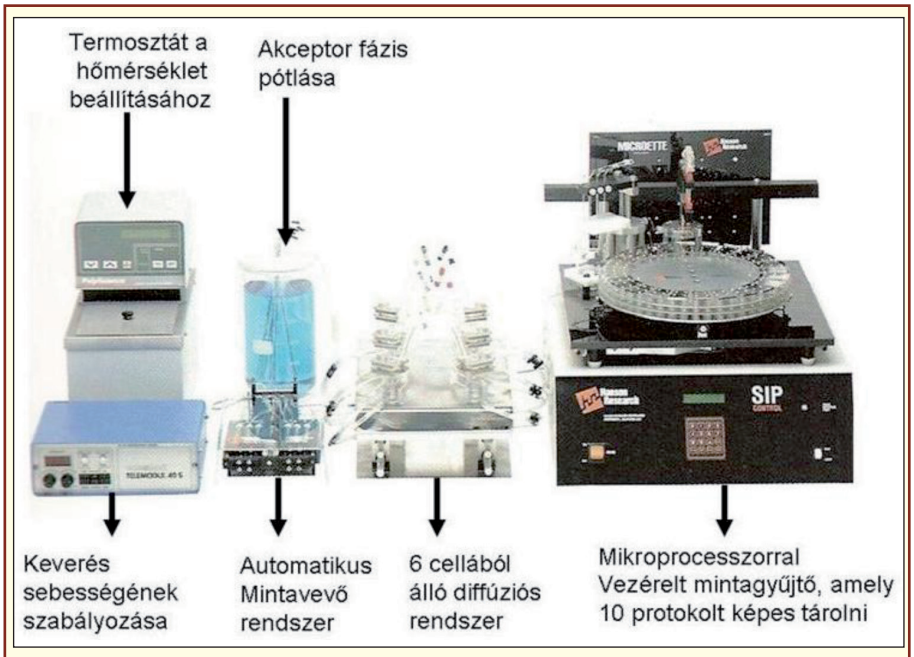
Általában a Franz cellával kapott hatóanyag felszabadulási értékek alacsonyabbak, mint a forgólapátos készülékkel kapott adatok. A két módszer közötti különbség gyakran szignifikáns. Ennek oka feltehetően az, hogy a forgólapátos készülékekben rendszerint nem alkalmaznak membránt, így a vizsgált készítmény



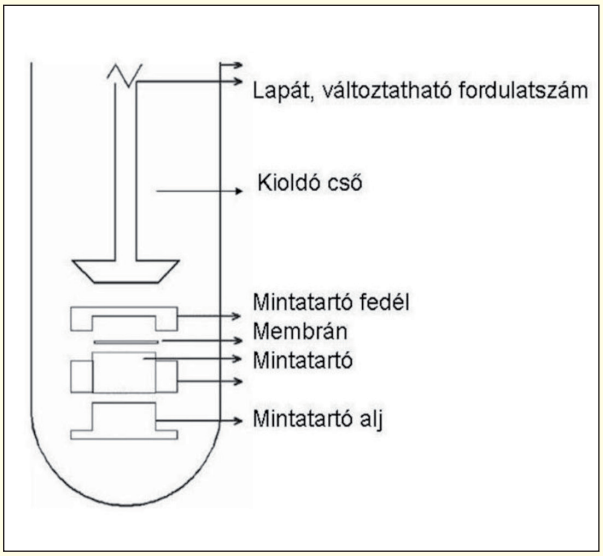
13/a ábra: Franz cella felépítése [30]



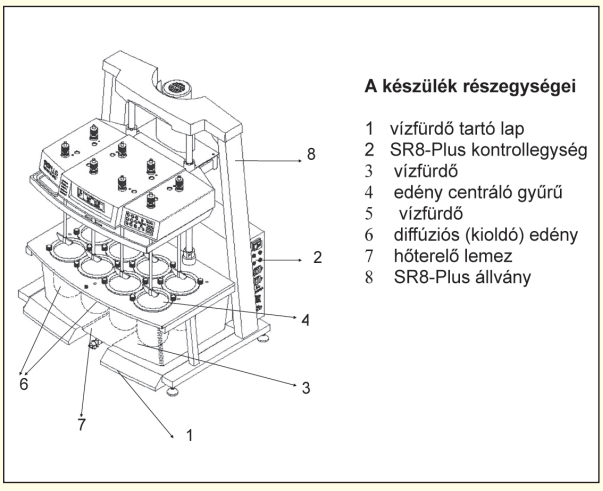
13/b ábra: Franz cella működése [31-36]



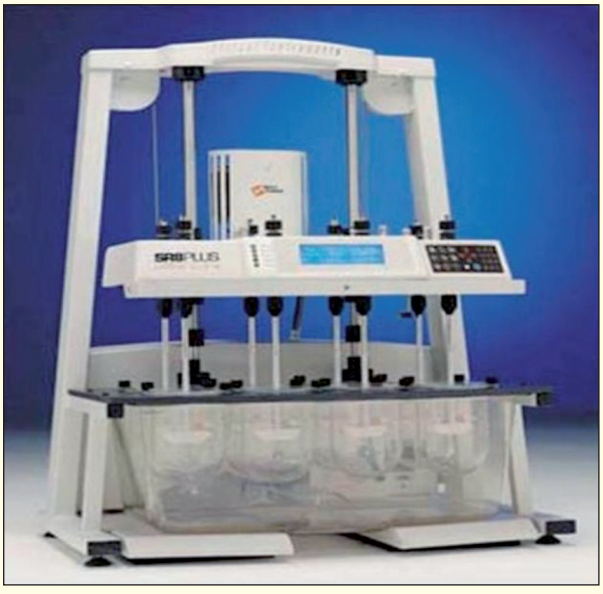
13/c ábra: Franz cellák mintavevővel, termosztáttal és mintatartóval [35]



14/a ábra: Forgólapátos készülék kioldó eleme a mintatartóval és a lapáttal [36, 37]



14/b ábra: Forgólapátos készülék szerkezete [36, 37]



14/c ábra: Hanson SR 8 PLUS készülék [37]

diszpergálódik az akceptor fázisban az állandó keverés hatására. Ekkor a készítmény és a közeg közötti érintkezési felület jelentősen megnövekszik, ez a tény a kioldódott hatóanyag mennyiségét nagymértékben megemeli [28, 29].

Az alábbiakban bemutatjuk a készülékek felépítését és működésük alapelveit.

Franz vertikális diffúziós cella

A diffúziós cella első leírása *T. J. Franz* nevéhez fűződik [30]. Bőrpermeációs kísérletekben, dermális és transzdermális rendszerek, szemészeti készítmények, bőrápolási termékek és peszticidek vizsgálatában alkalmazták elsősorban.

Ideális a félszilárd készítmények minőségellenőrzésének megvalósításához. Pontos, könnyen kezelhető, és az elmúlt 15 évben szigorú ipari és hatósági elvárásoknak vetették alá.

A Franz vertikális diffúziós cella automata mintavevő egységgel és 6 mintavevő hellyel rendelkezik. A hatóanyag felszabadulást szimuláló keverést az üvegcellákban hélix keverő biztosítja. Az akceptor fázis 4 vagy 7 ml térfogatú.

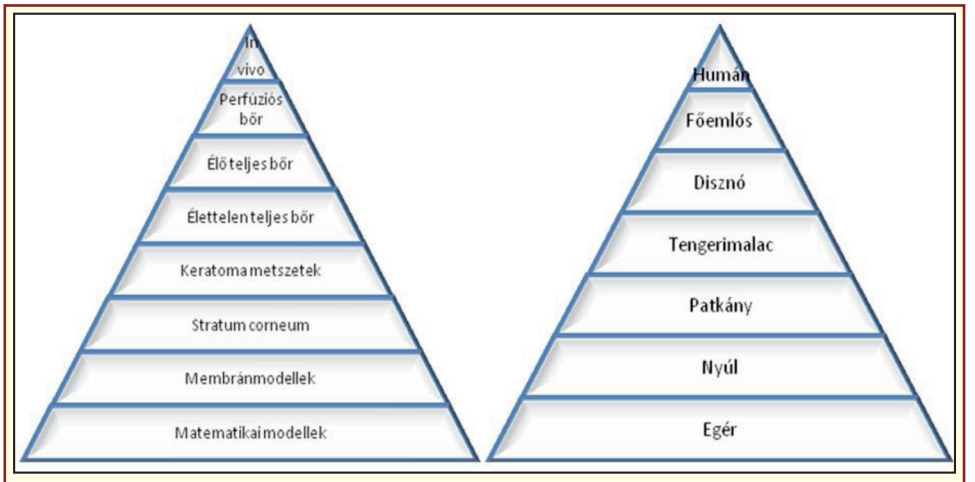
A cella donor és akceptor részből áll. A vizsgálandó rendszert (kenőcs, gél stb.) a membrán felső részére, az üvegcella nyitott részére helyezzük, itt valósul meg a hatóanyag felszabadulás. Az üvegcellákat 3 különböző méretben forgalmazzák. Az akceptor fázis az üvegcellában található. A bőr pH-ja az izzadságmirigyek, a bőrzsiradék és az *epidermisben* a Staphylococcusok által lebontott zsírsavak miatt enyhén savas (pH 4,0-6,8) jellegű, így a kioldóközeg pH-ját ennek megfelelően kell beállítani. A mintavétel a vizsgálatot végző által meghatározott időnként a 32 °C-ra termosztált kioldófolyadékból a felső csövön keresztül történik. Az alatta helyet foglaló cső a kivett minta pótlására szolgál (l. ***13/a*** és ***13/b. ábrát***). A módszer végrehajtására részletes validálási tervet szükséges készítenünk [31-36].

A kioldódott és a membránon az akceptor fázisba diffundált hatóanyag mennyiségének meghatározását HPLC készülékkel vagy egyéb analitikai módszer segítségével végezzük.

A hatóanyag kioldódás matematikai megközelítése c. fejezetben leírtak alapján általában az idő négyzetgyökének függvényében ábrázoljuk a kioldódott hatóanyag mennyiségét, mikrogramm/cm^2 (μg/cm^2) egységnyi felületre számítva. Így a lineáris regresszió segítségével legtöbbször egyenest kapunk, amelynek meredeksége jellemző a hatóanyag felszabadulás sebességére. Ez az érték készítmény-specifikus és a termék biofarmáciai minőségének jellemzésére szolgál. Az eredményeket a megfelelő nemparametrikus statisztika segítségével értékeljük ki [1, 3, 7, 28, 29].

A készülék előnyei az alábbiakban foglalhatók össze:

– tetszőleges számú minta vizsgálható és mérés végezhető egy mérési protokollon belül,
– tetszőleges, ill. előre beállítható térfogatú minta vehető az adott időpillanatban,
– a mintavételek időpontja a célnak megfelelően választható meg és előre beállítható a mérési protokoll megszerkesztésével,
– a mérési protokollok a készülék memóriájában tárolhatók,
– a mintavétel mindegyik cellából egyszerre hajtható végre a mikroprocesszoros vezérléssel,
– egyidejűleg történik a kivett akceptor fázis pótlása is,
– a mintavételt egyenesen HPLC üvegekbe végzi a készülék.



15. ábra: A perkután abszorpció meghatározására alkalmas fiziológiai hierarchia [20]

A hatóanyag felszabadulás vizsgálatának a következő területeken van kiemelt fontossága:
– a gyógyszerforma tervezésének és az összetétel optimálásának szakaszában,
– a többkomponensű dermatológiai készítmények gyártási szakaszban történő célzott monitorozásakor (pl. az alapanyag és/vagy segédanyag beszállító változása esetében, a gyártástechnológia változtatása után, az összetétel módosítását követően),
– gyártásközi ellenőrzéskor és a végtermék ellenőrzésekor,
– bioekvivalencia vizsgálatok esetében.

Azokban az esetekben, amikor az egyes gyártási tételek összehasonlító vizsgálatát végezzük a készülékkel (*„batch-to-batch”*), akkor olyan szintetikus membránt célszerű alkalmazni, amely a diffúziót nem gátolja, a vivőanyag átjutását az akceptor fázisba viszont megakadályozza. A *„Supac-SS-Scale Up and Post Approval Changed Guidelines”* [37] előírja azokat a vizsgálatokat, amelyeket az összetétel bármilyen változtatása után el kell végezni. Ezek között szerepel a készítmények hatóanyag leadásának *in vitro* vizsgálata is.

Ha a készítmény fejlesztési fázisában kívánjuk modellezni a különböző kísérleti összetételek bőrön keresztüli felszívódását, akkor az alkalmazott szintetikus membránt célszerű lipofillé tenni, és ez által lényegében a megoszlás is tanulmányozható. Ilyen előkísérletek alkalmazásával az állati bőrön végzett *in vitro* vizsgálatok és az *in vivo* vizsgálatok száma észszerűen csökkenthető, mivel az *in vitro* modell a biológiai hasznosíthatóság predikciójának tekinthető.

A vertikális cella gyártója a vizsgálati körülmények vonatkozásában a következő ajánlásokat teszi [38]:
– a vertikális cella 6 és 15 mm^2 felülettel áll a kutatók rendelkezésére,
– olyan szintetikus membránt használjunk, amely nem gátolja a hatóanyag diffúzióját, nem adszorbeál hatóanyagot, ill. nem lép kölcsönhatásba sem a készítménnyel sem az akceptorral,
– az akceptor megválasztásakor ügyeljünk arra, hogy ne lépjen fel kölcsönhatás a membrán és az akceptor, ill. az akceptor és a készítmény között,
– a vizsgált minta tömege 300 mg – 1 g között legyen,
– a mintavételek számát és a vizsgálat időtartamát úgy határozzuk meg, hogy legalább 5 mérési pont és legalább 6 óra diffúziós idő álljon rendelkezésre a kinetikai függvény megszerkesztéséhez,
– a felhasznált analitikai módszer megfelelő, specifikus és érzékeny legyen,
– az eredmények ábrázolásához a felszabadult farmakon kumulatív mennyisége–idő és a felületegységre vonatkoztatott farmakon mennyiség – diffúziós idő négyzetgyöke függvényeket javasolják.

Megjegyezzük, hogy a készülék meglehetősen drága, külön beruházás szükséges a beszerzéséhez [31-34].

Forgólapátos készülék

Széles körben használják a forgólapátos készülékeket (pl. a Hanson SR8-Plus készüléket), amely az USP 1, 2 készülékekkel azonos elven működik, és forgókosaras, ill. forgólapátos üzemmódban egyaránt alkalmazható [6, 40]). Hasonló a Magyar Gyógyszerkönyv VIII. kiadásában hivatalos tabletta kioldódást vizsgáló berendezésekhez, az akceptor fázis edényének térfogatában van csak különbség.

A forgólapátos készülékhez 8 munkahely tartozik és a mintavételezés manuálisan történik. (A mintavétel automatizált is lehet.) A mintákat mintatartóba és 70 ml kioldóközeget tartalmazó üvegcsövekbe helyezik. Az üvegcsövekben forgólapát gyorsítja az akceptor fázisban a kioldódott hatóanyag diffúzióját. A

vizsgálatok elvégzése előtt validálni kell a keverés sebességét, a forgólapát behelyezésének magasságát és annak pontos helyzetét. Ha a mintatartót nem határolja membrán, az akceptor fázis előszűrése szükséges, amely 10 mikrométeres pórusméretű szűrőn keresztül történik. A készülék lényeges elemét, a minta, a keverő és akceptor befogadását biztosító csövet a ***14/a ábra*** szemlélteti.

E készülék előnye, hogy a berendezés kiegészíthető kenőcscellával, és automatizálható. Az egyes minták eredményei közötti szórás értéke általában kisebb, mint más kioldódási módszerek eredményeinek szórása, így pontosabb, reprodukálhatóbb vizsgálatot tesz lehetővé. A műszer felépítését illetően a hibalehetőségek megegyeznek a szilárd gyógyszerformák esetében tapasztaltakkal. A kioldóközeg mennyisége meghaladja a bőr alatti szöveti folyadékmennyiséget, így humán és állati bőr *in vitro* modellezésére nem alkalmas. A gyógyszer gyakran teljesen kioldódhat a közegben, így inkább kioldódást, mint hatóanyag felszabadulást mérünk. Szuszpenziós készítmények esetében univerzális készülékként használható [28-36, 39, 40]. A teljes készülék vázrajza és a készülék fényképe a ***14/b*** és ***14/c ábrákon*** látható.

In vitro – in vivo korreláció (IVIVC)

A gyógyszer terápiás hatásossága az *in vitro* kioldódást jellemző mutatószámokból és *in vivo* biohasznosíthatóságából tevődik össze. A kioldódás mértéke – amint már rámutattunk a készülékek ismertetésekor – nagyban függ a választott vizsgálati módszertől és a készülék típusától. A műszerben, kioldóközegben, keverési sebességben stb. bekövetkező eltérések a kioldódást jelentékeny mértékben megváltoztatják [7, 41, 42].

Az *in vitro – in vivo* korrelációt gyakran alkalmazzák a termékek korai fejlesztésének stádiumában, mivel csökkenti a kutatásra szánt időt és optimálja a formulálási paramétereket, vagyis segít a leghatékonyabb összetétel kiválasztásában [41, 44].

A hatóanyagok biofarmáciai csoportosítása (*Biopharmaceutical Classification System*, BCS) a gyógyszer oldhatóságára és permeabilitására alapozva segítséget nyújt az *in vitro* kioldódás vizsgálat jellemzőinek megállapításában és a kívánt IVIVC elérésében [41].

Az IVIVC matematikai kapcsolatot teremt a gyógyszer *in vitro* felszabadulása / kioldódása és az *in vivo* eredmények között. Az *in vitro* tulajdonságokra a hatóanyag felszabadulási vizsgálatok időfüggvényeiből következtetünk, az *in vivo* eredményeket pedig az idő függvényében ábrázolt hatóanyag plazmakoncentráció értékeiből kapjuk.

A felszabadult és felszívódott hatóanyag mennyiség időfüggvényei között leggyakrabban lineáris regresszió van; nem gyakori, de előfordul nemlineáris függvénykapcsolat is.

A két módszer eredményei közötti kapcsolat (korreláció) különböző szinteken valósul meg. Az ***A*** szint a kapott eredmények teljes és tökéletes egyezését fejezi ki. Ha az *in vitro* kioldódás időfüggvényét és az *in vivo* eredmények időbeli változását egymásra fektetjük és a két függvény pontról pontra megegyezik, illetve egyezővé tehető bizonyos arányosítási tényezők alkalmazásával, akkor ***A*** szintű korrelációról beszélünk. E szint alapján az *in vitro* adatokból az *in vivo* adatok előrejelzésére lehetőségünk van.

B és ***C*** szintű korreláció kevésbé tökéletes egyezés jellemzésére szolgál. Ezekben az esetekben a két függvény nem egyezik meg pontról-pontra. A ***B*** szint kvantitatív jellemzésekor az *in vitro* kioldódási idő középértékét vetik össze az *in vivo* kioldódási idő középértékével vagy a szervezetben tartózkodási idő középértékével. A ***B*** szintű korreláció jelzőszáma nem tükrözi vissza az aktuális *in vivo* plazmaszintet, mivel a tartózkodási idő középértékéhez számos különböző *in vivo* görbe rendelhető. A ***C*** szint is egy pont kapcsolatot mutat a kioldódási paraméter (pl. a hatóanyag 50%-a kioldódásához szükséges idő) és egy farmakokinetikai paraméter (pl. vérszint görbe alatti terület, C_{max}, T_{max}) között.

A „többszörös” ***C*** szint a gyógyszer több farmakokinetikai paraméterét veti össze az *in vitro* hatóanyag felszabadulás átlagértékével [42].

Addig, amíg az *in vitro* kioldódási eredmények nem egyértelműen tükrözik az *in vivo* adatokat, (***B*** és ***C*** szintű korreláció), a módszert nem fogadhatjuk el a gyógyszer klinikai hatékonyságának megbízható előrejelzésére. Ha az adatok átfedik egymást, akkor megfelelő minőségkontrollként használhatjuk őket [20, 28, 43].

Röviden összefoglaljuk az *in vivo* vizsgálatok, illetve az ezeket szimuláló biológiai membránok nehézségeit. Bizonyított, hogy a foetus, a fiatalok valamint az idősek bőre sokkal permeábilisebb, mint a felnőtteké. Megoldatlan problémát jelent pl. az újszülött csecsemőéhez hasonló *in vivo* állati bőrmintát találni, mivel a bőrgyógyászati készítmények esetlegesen fellépő mellékhatásai a toxikus dózishatárt is elérhetik ilyen esetben.

A minta származása (abdominális, élő vagy cadaver) ugyancsak befolyásolhatja az eredményeket. A kísérletek végrehajtásához elsődlegesen humán élő szövet alkalmazása ajánlott.

Az emberi szövet beszerzésének nehézségei miatt leggyakrabban laboratóriumi állatok, így pl. rágcsálók (patkány, egér, tengerimalac), nyulak, kutyák, majmok és disznók bőrét alkalmazzák. Az állatok bőre viszont több szőrtüszőt, kevesebb izzadságmirigyet tartalmaz, valamint a *stratum corneum* vékonyabb a humánhoz képest, így jobb hatóanyag felszabadulást tesz lehetővé. E két utóbbi abszorpciós út a humán bőrön történő alkalmazásnál elhanyagolható [7]. Másik hátrányuk,

hogy az állatok szükségszerű szőreltávolítása során az esetlegesen keletkezett sérülés szintén megemeli a penetráció fokát.

A nyúlbőr a legpermeábilisebb a félszilárd gyógyszerformák számára, ezt követi a patkány bőre. Az emberi bőr struktúrájához a legközelebbi hasonlóságot a majom- és a disznóbőr mutatja, mely vizsgálatot *in vivo* kísérletek is alátámasztják.

A ***15. ábra*** [15] a perkután abszorpciót szimuláló modelleket mutatja be. A diagramok csúcsán a legmegbízhatóbb módszerek foglalnak helyet. A felsoroltakon belül előnyt élveznek a szőr nélküli fajták [7, 15, 16].

Sejtkultúrákon történő vizsgálatok

A biológiai membránok szerkezeti, kezelési és beszerzési nehézségei a humán sejtkultúrákra irányították a figyelmet, és a humán eredetű preparátumok helyettesítésére egyre több élő sejtkultúrát tartalmazó *in vitro* vizsgálati berendezés jelent meg a kutatásban. A *Living Skin Equivalent* (LSE) kollagént tartalmazó mátrixban elhelyezkedő humán dermális fibroblastok és az ezeket fedő humán keratinociták halmaza, amelyek együtt egy többrétegű *epidermist* alkotnak. Elsősorban dermatotoxikológiai és irritációs vizsgálatok elvégzésére fejlesztették ki. Hátránya az, hogy sokkal permeábilisebb a humán bőrnél [44, 45].

Az LSE sejtkultúrával az alábbi területeken végeztek vizsgálatokat:

– sejtkultúrák előállítása és tulajdonságaik vizsgálata [46-48],
– irritációs hatás, toxicitás és egyéb károsító hatás kutatása [49-56],
– a bőrön keresztüli permeáció vizsgálata [57-63]
– a hatóanyagok bőrben történő metabolizmusának vizsgálata [57, 64].

Kísérleti célokra alkalmasak továbbá a Reconstructed Human Epidermis (RHE) modellek [65-73]. Az Epiderm (MatTek, USA [74-84]), a Skinethic (Skinethik, France [84,88) és az Episkin (L'Oreal, France [89-91] a leggyakrabban alkalmazott rekonstrukturált *epidermis* típusok. Fototoxicitási, irritációs és korrozivitási kísérletek elvégzésére alkalmasak elsősorban. Míg az Epiderm és Skinethic kultúra tenyésztésének alapja mesterséges membrán, addig az Episkin kollagén alapú rétegen kifejlesztett sejthalmaz. Ezek a sejtmodellek anatómiai és előállítási szempontból jelentősen különböznek, de a dermatológiai és kozmetikai kutatásokhoz, a toxicitás, irritáció és egyéb bőrkárosító hatás vizsgálatában mára már nélkülözhetetlenné váltak [88].

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IRODALOM

1. FDA Guidance for Industry: Nonsterile Semisolid Dosage Forms, Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation SUPAC-SS, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), May (*1997*). – 2. FDA Guidance for Industry: Topical Dermatological Drug Product NDAs and ANDAs – In Vivo Bioavailability, Bioequivalence, In Vitro Release, and Asssociated Studies SUPAC-SS, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), June (*1998*). – 3. *Siewert, M., Dressman, J., Brown, C.K., Shah, V.P.*: AAPS PharmSciTech *4* (1), 1-10 (*2003*). – 4. *Erős, I., Abu-Eida, Cserne, A., Csóka, I., Csányi, E.*: Acta Pharm. Hung. *70*, 29-34 (*2000*). – 5. FDA Guideline on General Principles of Process Validation, Center for Biologics Evaluation and Research, Center for Devices and Radiological Health, Food and Drug Administration, Division of Manufacturing and Product Quality, Office of Compliance, Center for Drug Evaluation and Research (CDER), Food and Drug Administration, May (*1987*). – 6. The United States Pharmacopoeia (USP) XXIII *(1995).* – 7. *Barry, B.W.*: Dermatological Formulation: Percutaneous Absorption, Marckel Decker Inc., New York, Basel *(1983).* – 8. *Higuchi, T.*: J. Pharm. Sci. *50*, 874-876 (*1961*). – 9. *Bialik, W.*:Pharmazie *39*, 763 (*1984*). – 10. *Bialik, W.*: Sci. Pharm. 93-98 (*1985*). – 11. *Thoma, K.:* Dermatika, Werbe- und Vertriebsgesellschaft Deutscher Apotheker m.b.H., München *(1983).* – 12. *Schaaf, F.:* Probleme Dermatologischer Grundlagenforschung, Dr. Alfred Hüthig Verlag Heidelberg *(1969).* – 13. www.pharmainfo.net/reviews/topical-drug-delivery-systems-review – 14. *Erős I.:* Gyógyszerészet *39*, 253-258 (*1995*). – 15. ATLA *24*, 81-106 (*1994*). – 16. *Kydonieus, A.F., Berner, B.B.* (Eds.): Transdermal Delivery of Drugs, Vol. I-III. (*1987*). – 17. *Tronnier, H.*: Über die Wirkungsweise indifferenter Salben und Emulsionssysteme an der Haut in Abhängigkeit von ihrer Zusammensetzung, Editio Cantor KG./796 Aulendorf i. Württ. *(1964).* – 18. *Erős I.*: Olaj, Szappan, Kozmetika *4*, 138-142 (*2003*). – 19. *Fares, H.M., Zatz, J.L.:* Pharm. Technology *32*, 53-59 (*1995*). – 20. *Shah, V.P., Williams, R.L.*: Roles of Dissolution Testing: Regulatory, Industry and Academic Perspectives – Role of Dissolution Testing in Regulating Pharmaceuticals, August (*1999*). – 21. *Busse, M.J., Hunt, P., Lees, K.A., Maggs, P.N., McCharty, T.M.*: British J. Dermatol, *78*, 645 (*1969*). – 22. *Poulsen, B.J., Young, E., Coquilla, V., Katz, M.*: J. Pharm. Sci. *57*, 928 (*1968*). – 23. *Turakka, L., Piepponen, T., Kahela, T.:* Labo Pharma, *32*, 540-544 (*1984*). – 24. *Mayer, A., Kedvessy, Gy.*: Pharm. Ind. *31*, 323-328 (*1969*). – 25. *Loth, H., Holla-Benninger, A.*: Pharm. Ind. *40*, 256-261 (*1978*). – 26. *Ashe, H., Botta, L., Rettig, H., Weirich, E.G.*: Pharm. Acta Helv. *60*, 232-237 (*1985*). – 27. *Kanfer, I., Tettey-Amlalo, R.N.O., Au, W.L., Hughes-Formella, B.:* Generic Drug Product Development: Specialty Dosage Forms, 54-103, Informa Healthcare *(2010).* – 28. *Liebenberg, W., Engelbrecht, E., Wessels, A., Devarakonda, B., Jang, W., De Villiers, M.M.:* J. Food Drug Anal. *12*, 19-28 (*2004*). – 29. *Corbo, M.*: Techniques for Conducting In Vitro Release Studies On Semisolid Formulations, Dissolut. Technol., 3-6 Winter (1995); *Neubert, R., Wohlrab, W.A.*: Acta Pharm. Technol. *36* (4), 197-206 (*1990*) – 30. *Franz, T.*: J. Invest. Dermatol., *64*, 190-195 *(1975).* – 31. www.hansonresearch.com/vert-diffusion-cell.htm. – 32. www.hansonresearch.com/images/vertdiffcell-1.jpg. – 33. www.hansonresearch.com/images/

vertdiffcell-2.jpg. – 34. www.pharm.u-szeged.hu/phtech/kutatas/kutatas_muszer_felszilard_hu.html. – 35. www.hansonresearch.com/sr8-plus.htm. – 36. Able&E-Jasco Magyarország Kft.: Hanson Research SR8-Plus kioldódásvizsgáló fürdõ kezelési utasítása (Ver 3.10). – 37. Supas-SS- Quidelines. Hanson Microette Autosampling System. Hanson research Corp. Kiadványa. é.n. – 38. Hanson Model 57-6M Manual Start-Up., Diffusion test Systen. Hanson Research Corp Kiadványa. é.n. – 39. www.hansonresearch.com/images/sr8-plus.jpg – 40. www.canbev.com/hanson%201.JPG – 41. *Prior, A., Frutos, P., Correa, C.P.: C*omparison of Dissolution Profiles: Current Guidelines, Docencia, 507-509. – 42. FDA Guidance for Industry: Extended Release Oral Dosage Forms: Development, Evaluation and Application of In Vitro/In Vivo Correlations, Center for Drug Evaluation and Research (CDER), September (*1997*). – 43. *Retting, H., Mysicka, J.*: IVIVC: Methods and Applications in Modified-Release Product Development, Dissolut. Technol., 6-8 February (*2008*). – 44. *Williams, F.M.*: Toxicol. and Pharm. 814 *(2005*). – 45. *Gay, R., Swiderek, M., Nelson, D., Ernesti, A.*: Toxicol. in vitro *6*(4), 303-315 (*1992*). – 46. *Bell, E., et al*.: Toxicol in vitro, *5*, 591-596 (*1991*). – 47. *Eaglstein, W.H., Falanfa, V.*: Clin. Therap *19*, 894-905 (*1997*). – 48. *Groeber, F., Holeiter, M., Hampel, M., Hinderer, S., Schenke-layland, K.:* Adv, Drug Del. Rev. *63*, 352-366 (*2011*). – 49. *Semlin, L., Scafer-Korting, M., Borelli, C., Korting, H.C.*: Drug Discovery Today, *16*, 132-139 (*2011*). – 50. *Sugibayashi, K., Watanable, T., Hasegava, T., Tajkahashi, H., Ishibashi, T.:* Toxicology in vitro *16*, 759-763 (*2002*). – 51. *Bolesma, E., Tanajo, H., Boddé, H.E., Ponec, M.*: Toxicity in Vitro *11*, 365-376 (*1997*). – 52. *Faller, C., Bracher, M., Dami, N., Roguet, R.*: Toxicology in vitro *16*, 557-572 (*2002*). – 53. *Dykes, P.J., Edwards, M.J., O'Donoven, M.R., Morgan, H.E., Marks, R.*: Toxicity in vitro *5*, 1-8 (*1991*). – 54. *Welss, T., Basketter, D.A., Schröder, K.R.*: Toxicology in Vitro *18*, 231-243 (*2004*). – 55. *Medina, J., Fraissinette, A.B., Chibout, S-D., Kolopp, M., Kammermann, R., Burtin, P., Ebelin, M-E., Cordier, A.*: Toxicol. Appl Pharmacol. *164*, 38-45 (*2000*). – 56. *Costa, C., Pascuale, R.D., Silvari, V., Barbaro, M., Catania, S.:* Toxicology in Vitro *20*, 324-331 (*2006*). – 57. *Tai, A., Goto, S., Ishoguro, Y., Suzuki, K., Nitoda, T., Yamamoto, I.:* Bioorg. Med. Chem. Letters, *14*, 623-627 (*2004*). – 58. *Hager, D.F., Mancuso, F.A., Nasareno, J.P., Sharkey, J.W., Siverly, J.R.*: J. Controll. Release *30*, 117-123 (*1994*). – 59. *Godin, Touitou, E.*: Adv. Drug Delivery Rev. *59*, 1152-1161 (*2007*). – 60. *Andronis, V., Mesiha, M.S., Plakogiannis, F.M.:* Pharm. Acta Helv. *70*, 301-306 (*1995*). – 61. *Moss, G.P., Dearden, J.C., Patel, H., Cronin, M.T.D.:* Toxicity in Vitro *16*, 299-317 (*2002*). – 62. *Hada, N., Hasegawa, T., Takahashi, H., Ishibashi, T., Sugibayashi, K.:* J. Controll. Rel. *108*, 341-350 (*2005*). – 63. *Montenegro, L., Ademola, J.I., Bonina, F.P., Maibach, H.I.:* Int. J. Pharm. *140*, 51-60 (*1996*). – 64. *Tamura, M., Sueishi, T., Sugibayashi, K., Morimoto, Y., Juni, K, Hasegawa, T., Kawaguchi, T.:* Int. J. Pharm.*131*, 263-271 (*1996*). – 65. *Netzlaff, F., Kaca, M., Bock, U., Haltner-Ukomadu, E., Meiers, P., Lehr, C.-M., Schaefer, U.F.*: Eur. J. Pharm. Biopharm. *66,* 127-134 *(2007). – 66. Coquette, A., Berna, N., Vandenbosch, A., Rosdí, M., De Wever, B., Poumany, Y.:* Toxicology in Vitro *17*, 311-321 (*2003*). – 67. *Gabbanini, S., Matera, R., Beltramini, C., Minghetti, A., Valgimigli, L.*: J. Pharm. Biomed Anal. *52*, 461-467 (*2010*). – 68. *Rozman, B., Gasperlin, M., Tinois-Tessoneaud, E., Pirot, F., Falson, F.:* Eur. J. Pharm. Biopharm. *72*, 69-75 (*2009*). – 69. *Coquette, A., Berna, N., Vandenbosch, A., Rosdy, M., Poumay, Y.*: Toxicology in Vitro *13*, 867-877 (*1999*). – 70. *Haberland, U.F., Schafer, U., Bock, H.C., Korting, M., Scafer-Korting, M.:* Toxicology in vitro, *19*, 813-822 (*2005*). – 71. *Moretto, P., Pouthier, T., Schmith, R.W., Ynsa, M.D.*: Physics Research. Vol. *231*, 286-291 (*2005*). – 72. *Gabbanini, S., Lucci, E., Carli, M., Berlini, E., Minghetti, A., Valgimigli, L.*: J. Pharm. Biomed. Anal. *50*, 370-376 (*2009*). – 73. *Mommaas, M., Tada, J., Poneec, M.*: J. Dermatol. Sci. *2*, 97-105 (*1991*). – 74. *Hu, T., Bailey, R.E., Morrall, S.W., Aardema, M.J., Stanley, L.A., Skare, J.A.*: Toxicology Letters, *188*, 119-129 (*2009*). – 75. *Mallampati, R., Patiolla, R.R., Agarwai, S., Babu, R.J., Hayden, P., Klauser, M., Singh, M.S.*: Toxicology in Vitro *24*, 669-676 (*2010*). – 76. *Hayden, P.J., Cooney, C., Stolper, G., Klausner, M.:* Toxicology Letters Suppl.*164*, S223 (*2006*). – 77. *Hayden, P., Kubilus, J., Burnham, B., Jackson, G., Sheasgreen, J., Klauser, M.*: Toxicology Letters *144,* Suppl., 45-46 (*2003*). – 78. *Hayden, P., Bolmerchich, J., Stopler, G., Hu, T., Aaderma, M., Curren, R., Klausner, M.:* Toxicology Letters, *164,* Suppl. S225-228 (*2006*). – 79. *Kandárová, H., Hayden, P., Spiller, E., Klausner, M., Kubilus, J., Sheasgreen, J.*: Toxicology Letters, *172* Suppl. S81 (*2007*). – 80. *Hayden, P., Stopler, G., Cooney, C., Kandarova, H., Klausner, M.*: Toxicology Letters, *180* Suppl. S105 (*2008*). – 81. *Liebsch, M. et al*.: Toxicology Letters, *180* Suppl. S107 (*2008*) – 82. *Letasilova, S., Hayden, P., Stopler, G., Armento, A., Cooney, C., Klausner, M.:* Toxicology Letters, *196,* Suppl. 152 (*2010*). – 83. *Hilberer, A. et al*.: Toxicology Letters, *189* Suppl. 83 (*2009*). – 84. *Jirova, D. et al.:* Toxicology Letters, *180* Suppl. 108 (*2008*). – 85. *Alépée, N. et al.:* Toxicology in Vitro *24*, 257-266 (*2010*). – 86. *Tornier, C., Roquet, M., A de B de Frassinette*: Toxicology in Vitro *24*, 1379-1385 (*2010*). – 87. *Tornier, C. et al*.: Toxicology in Vitro, *24,* 246-256 (*2010*). – 88. *Netzlaff, F., Lehr, C.M., Wertz, P.W., Schaefer, U.F.*: Eur. J. Pharm. Biopharm. *60*, 167-178 (*2005*). – 89. *Rouget, R. et al.:* Toxicology in Vitro *12*, 295-304 (*1998*). – 90. *Netzlaff, F., Kaca, M., Bock, U., Haltner-Ukomadu, E., Meiers, P., Lehr, C.M., Scaefer, U.F.*: Eur. J. Pharm. Biopharm. *66*, 127-134 (*2007*). – 91. *Hoffmann, J. et al.:* Toxicology in Vitro, *19*, 925-929 (*2005*).

Petró, É., Erős, I., Csóka, I.: ***Development of semisolid dosage forms – drug release and bioequivalence investigations. Principle, methods and equipments – review. Part II.***

The semisolid preparations represent an important part of dosage forms, however their biopharmaceutics considering investigations of their drug release with different methods cannot be as good as resolved up to this day. However there are a lot of drug release methods in case of solid dosage forms, the drug release methodology in case of semisolids is still not clarified. There is no harmonised validation method for their drug release in the industrial research and development in the Pharmacopoeias. The aim of our study is to give an overview about the most used drug release experiments, methods and equipments. Furthermore we try to pay attention to these critical parameters, which can help to build a harmonised validation method into the research process. This project can fulfil the regulatory and industrial requirements.

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V.

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RESEARCH ARTICLE

Drug release from semisolid dosage forms: a comparison of two testing methods

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Abstract

The aim of the present work was to extend our previous *in-vitro* drug release studies using semisolid dermatological bases with non-impregnated cellulose acetate membranes. A comparison of the performances of two apparatuses, the more commonly used Franz cell and the new modified USP (mini paddle with ointment holding cell) systems were applied to this work. Five different semisolid as well as two marketed preparations containing 1% diclofenac sodium were used. Complex, slightly non-linear release curves indicating sink conditions were found. This was explained by the co-diffusion of excipients modifying the characteristics of the membrane and the receiving medium dynamically. Although our test model is, as a rule, not suitable to establish an *in-vivo–in-vitro* correlation, good qualitative as well as quantitative correlations were found within some types of dermatological bases. The correlation between the results of the two *in-vitro* methods also depends on the type of semisolids studied. The release curve characteristics and the amount of diclofenac sodium released at 6 h were measured. Their repeatability and reproducibility were calculated. The slopes and Q-values were correlated with *in-vivo* data. In general, the modified USP method provided more precise results than the Franz cell method.

Keywords

Franz cell, *in vitro*, *in vivo*, mini-paddle, modified holding cell, skin penetration

History

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Introduction

In-vitro dissolution testing is an important tool in both drug development and quality control[1]. Its methodology has been established for solid dosage forms. In the case of drug release rate testing from semisolids[2], using several *in-vitro* method variations[3–7] have led to guidelines and recommendations[1,8–10] which have been published. There is no generally accepted pharmacopoeial method available to date.

The guidelines mostly recommend the use of Franz vertical diffusion cell[11–13] as the testing apparatus. It is widely used by researchers[14–16] as an analytical tool.

In our previous work[17], the correlation between *in-vitro* release rate and *in-vivo* efficacy (IVIVC) for various semisolid formulations containing diclofenac sodium was studied. The carrageen-induced rat paw edema test represented the *in-vivo* model, while release experiments using the Franz vertical diffusion cell mounted with isopropyl myristate (IPM) soaked cellulose acetate membrane represented the *in-vitro* part of the study.

For quality control and product development purposes, however, simple (non-impregnated), inert, porous synthetic membranes are recommended[14]. Thus, our previous work should be continued with these porous synthetic membranes.

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Another apparatus with which to study *in-vitro* drug release from semisolid preparations has recently been offered. Hanson Research Company has extended its SR8-Plus Test Stations for a small-volume system. This device is an adaptation of the United States Pharmacopoeia (USP) Dissolution Apparatus 2. It has a special small-volume vessel (modified holding cell) and a mini-paddle. The former includes a donor chamber for topical drug application. After mounting a selected membrane on the mouth of the chamber it is immersed in the receiving medium before starting the test[18].

The technical differences between the Franz vertical diffusion cell (which will be referred to as the ''Franz cell'') and the modified holding cell – mini-paddle system (which will be referred to as the ''modified USP'') are as follows (Franz cell versus modified USP, resp.):

- Cell volume: 7 ml (which is most commonly used) versus 70 ml,
- Sample volume: 800 µl (replaced with fresh receiving medium) versus 2.00 ml (not replaced),
- Semisolid sample amount: 0.24–1.65 g versus 0.40–0.70 g,
- Stirring rate: 450 rpm versus 100 rpm,
- Sampling: automated versus manual.

The sample surface was 1.767 cm^2 in both cases.

To our knowledge the performances of these two systems have not yet been compared.

The primary purpose of our present work was to extend our previous *in-vitro* experiments[17], to study the release rates of diclofenac sodium using a cellulose acetate membrane of 0.45 µm average pore size without IPM impregnation from a wide range of dermatological bases.

Our secondary purpose was to compare the performances of the Franz cell and the modified USP systems.

Experimental

Materials

Micronized diclofenac sodium complying with the European Pharmacopoeia, Vol. 6 (Eur. Pharm.) was used as the hydrophilic active ingredient (TEVA-Human Co., Debrecen, Hungary). The following materials were used as base components: Carbomer 934 P (prop-2-enoic acid) obtained from BF Goodrich, Brussels, Belgium; sorbitan monopalmitate (Span 40) purchased from Sigma-Aldrich, Budapest, Hungary; Miglyol 812 N (fractionated coconut oil, glyceryl tricaprylate/caprate) and Imwitor 780 K donated by Sasol GmbH, Hamburg, Germany; Pemulen (PTR-2) was a gift from Luibrizol Co., Wickliffe, OH; Tagat S (PEG-30 glyceryl stearate) supplied by Evonik Ind. AG, Essen, Germany; and disodium hydrogen phosphate and citric acid purchased from Merck, Darmstadt, Germany. All other additives, namely triethanolamine, liquid paraffin, cetostearyl alcohol and sodium hydroxide were purchased from Hungaropharma Co., Budapest, Hungary. All components used were of Eur. Pharm. grade.

The reference hydrogel (REFHG) and the reference gel-emulsion (REFGE) were marketed medicinal products: Diclofenac-Ratiopharm® (Ratiopharm Hungaria Ltd., Budapest, Hungary) and Voltaren emulgel® 1% (Novartis Hungaria Consumers Healthcare Ltd., Budapest, Hungary), respectively, and were purchased from Hungarian pharmacies.

The formulations studied

Table 1 summarizes the compositions of the dermatological bases selected and investigated in this study. The concentration of diclofenac sodium used in the formulations was 1.0 w/w% and it was uniformly dispersed in the bases. The REFGE contained diclofenac diethyl amine salt. The numbers used in each coded sample series indicate the concentration of a particular ingredient of the formulation, as follows: hydrogel (HG) contains 0.8%, 0.9% and 1.0% Carbomer 934 P; organogel (OG) contains 25%, 30% and 35% Span 40 emulsifier; gel-emulsions (GE) include 40%, 45% and 50% Pemulen TR-2 gel; oil-in-water (OW) creams consists of 65%, 70% and 75% while water-in-oil (WO) creams 40%, 45%, 50% purified water.

Preparation of the formulations

Hydrogels (HG)

Carbopol 934 P was added to purified water and dissolved with constant stirring at room temperature at 450–625 rpm (Stuart heat-stir, Sterilin Ltd., Keisen Products, Chelmsford, England). After its complete dissolution triethanolamine was added until the three-dimensional network was built up, then the pH of the sample was adjusted to 7.0 (ISFET pH Meter, IQ Scientific

Table 1. Compositions of the formulations (w/w%).

	Composition character and code (%)				
	Hydrogels	Gel-emulsions	Organogels	O/W*	W/O*
	HG08	GE40	OG25	OW65	WO40
	HG09	GE45	OG30	OW70	WO45
Components (%)	HG1	GE50	OG35	OW75	WO50
Liquid paraffin	–	–	–	15	45
				10	40
				5	35
Triethanolamine	q. s.**	–	–	–	–
Mygliol 812	–	50	75	–	–
		50	70		
		50	65		
Imwitor 780 K	–	–	–	–	5
					5
					5
Tagat S	–	–	–	10	–
				10	
				10	
Carbopol 934 P	0.8	–	–	–	–
	0.9				
	1.0				
PTR-2 gel***	–	40	–	–	–
		45			
		50			
Span 40	–	–	25	–	–
			30		
			35		
Cetostearyl alcohol	–	–	–	10	10
				10	10
				10	10
Purified water	to 100	10	–	65	40
		5		70	45
		0		75	50
REFGE	Contains diaethylamine, Carbopol 974 P, Cetomacrogol 1000, Cetiol LC, liquid paraffin, prolylene glycol, isopropyl alcohol, purified water, ''Crème 45'' perfume				
REFHG	Contains lactic acid, sodium disulfite, hydroxypropylcellulose, diiospropyl adipinate, isopropyl alcohol, purified water				

*Creams.
**Quantum satis: add to reach pH = 7.0.
***PTR-2 (premulen TR-29) gel consisted of 2.0 g PTR-2 and 1.0 g triethanolamine, mixed and diluted to 200.0 g with distilled water.

Instruments Inc., San Diego, CA) to form a neutralized clear gel. The gel was stored at cold temperature (10 °C) for one day before adding the active agent with vigorous stirring.

Organogels (OG)

The samples were produced by melting sorbitan monopalmitate and Miglyol 812 N oil together on a water bath at 80 °C with continuous stirring and homogenization. The gel was stored under a cold temperature at 10 °C for one day before adding the active agent with vigorous stirring.

Gel-emulsions

Pemulen TR-2 (PTR-2) and diclofenac sodium were added to distilled water followed by continuous stirring. Triethanolamine was added to the sample for neutralization until building a gel consistency. Mygliol 812 N oil and distilled water were then added to the gel with vigorous stirring (1000 rpm, Stirrer DLH, VELP Scientifica, Usmate, Italy) until the appropriate consistency was achieved for topical application.

Oil-in-water preparations (OW)

Cetostearyl alcohol, liquid paraffin and Tagat S were melted together on a water bath at 80 °C and mixed in order to prepare the oil phase. Diclofenac sodium was dissolved in purified water and the solution was heated up to a similar temperature at 80 °C. The phases were mixed and homogenized until the cream cooled down to room temperature then allowed to stand for one day before use.

Water-in-oil preparations (WO)

Cetostearyl alcohol, liquid paraffin and Imwitor 780 K were melted together on a water bath at 80 °C and mixed. Diclofenac sodium was dissolved in purified water and the solution was heated up to a similar temperature at 80 °C. The phases were mixed, homogenized and cooled down to 25 °C, then allowed to stand for one day before use.

In-vitro drug release testing methods

Membrane, receiving medium and analysis

Cellulose acetate membranes (Porafil, Machenerey-Nagel GmbH, Düren, Germany and Pall Life Sciences, Batavia, NY) with an average pore size of 0.45 μm were used. The area for diffusion was 1.767 cm^2.

The receiving medium was to mimic the penetration through the skin surface and the stratum corneum. According to various text books, sebaceous and eccrine secretions lay down an acid mantle on the skin surface and stratum corneum with a pH about 5[19] although this value varies with the state of the body, gender, environmental conditions, season, etc. up to plus–minus half pH unit[19–21]. Thus, a phosphate buffer of pH 5.4 ± 0.1 (Orion Star pH, Thermo Electron Co., Singapore) was chosen as the receiving medium. Its temperature was maintained at 32 ± 0.5 °C to reflect the usual external skin temperature[1].

The absorbance of the samples was measured by UV spectrophotometry (Unicam Helios α UV–Vis Spectrophotometer, Cambridge, England) at 275 nm and their diclofenac content calculated using a calibration curve. The blank ointment bases (compositions without diclofenac sodium) were also tested but, for their absorbance remained below 2% of those of the diclofenac containing samples throughout the experiments; the blank values were not taken into account.

The experiments were run in triplicate. The results, because of their calculated precision, as a general rule, were rounded to three digits.

Diffusion cells

The Franz vertical diffusion cell system (Hanson Research Co., Chatsworth, CA) containing six cells and equipped with an autosampler (Hanson Microette Autosampling System) was used. The receptor chamber volume was 7 ml. The membranes soaked previously in phosphate buffer were mounted on the top of the cells. A stirring rate of 450 rpm was used. Quantities of 0.24–1.65 g ointment samples (depending on the type and consistency of the different compositions) were placed evenly on the surfaces of the membranes. An 800 μl sample was taken after 0.5, 1, 2, 3 and 6 h and replaced with fresh receiving medium.

The modified USP apparatus was the SR8-Plus Dissolution Test Station with modified holding cell for semisolids (Hanson Research Co.). In our study, the apparatus containing eight cells with 70 ml volumes was maintained. Quantities of 0.40–0.70 g samples were placed on the membrane surfaces of the small ointment sample holders which were then dropped into the glass vessels containing the receiving medium. The stirring rate was set to 100 rpm. Two 2 ml samples were taken manually after the same time intervals as described for the Franz cell.

In-vivo experiments

A carrageen-induced edema study was used to test *in-vivo* efficacy of the formulations[17]. As for this technique we referred to the literature[22,23] for the details. The experiments were approved by the Animal Ethics Committee of the University of Szeged, Hungary (IV/01758-6/2008). Male Wistar rats (150–181 g) were studied. All measurements were performed at 24 ± 1 °C in an air-conditioned room. The animals were kept under standard 12 h light/12 h dark conditions with food and water ''*ad libitum*''. All experiments were carried out in the same period of the day (1–4 p.m.) to exclude diurnal variations in pharmacological effects. Each rat was tested only once. One day prior to the application of the preparations, the back of each rat (15 cm^2) was carefully shaven and depilated by Veet® depilatory cream (Reckitt Benckiser, Massy, France) in 5 min under 2.5–3.5% isoflurane anaesthesia (Forane® solution, Abbott Laboratories, Budapest, Hungary). The skin of the animals was cleaned by wiping with water containing cotton. The rats were dried under infrared lamp for 10 min.

On the day of the experiment, the animals were anaesthetized with Forane solution and exposed to different test compositions. A 300 mg sample from each formulation was applied onto the depilated dorsal skin of the rat. Local inflammatory response was elicited by 0.1 ml subplantar injection of carrageen (Viscarin, Marine Colloids Inc., Springfield, IL) solution given into the right hand paw 1 h after the treatment. The concentration of carrageenan solution was 0.5% which was prepared in a physiological saline solution. The left paw, used as the control, was treated without carrageenan. Paw volume was measured with a plethysmometer (Hugo Sachs Elektronik, March, Germany) 5 h after the injection.

The volume difference between the carrageenan- and saline-injected paws was used for the evaluation of the inflammatory response. The degree of paw swelling was calculated as:

$$\text{Swelling}\,(\%) = 100\frac{(V_t - V_0)}{V_0} \qquad (1)$$

where V_t and V_0 were the treated and untreated paw volumes, respectively.

Data and methods for statistical comparisons

The release of diclofenac from the compositions studied was described as:

$$\frac{\mu g}{cm^2} = a\,t_2^1 + b \qquad (2)$$

where

a is the slope representing the release rate of diclofenac sodium (FDA Guidance 2007);

t is the sampling time (h) and

b is the intercept.

In our statistical comparisons

Q is the cumulative amount (μg/cm^2) of the diclofenac released in 6 h;

s is standard deviation expressed in relative %;

repeatability stands for the average *s* of the indicated values within the parallel runs (average intra-run *s*);

reproducibility stands for the *s* of the results of the indicated values determined in the three parallel runs (inter-run *s*) and

r is the coefficient of correlation.

Results

The results of this study are presented in Tables 2–4. Slopes, intercepts and *Q* data in these tables represent average values for three parallel runs and all cells working. Characteristic release curves (one composition each) are shown in Figures 1 and 2 (modified USP method, separated for better clarity) and Figures 3 and 4 (Franz cell method). The average swelling % data measured in our previous work[17], which were used in the present IVIVC studies, are reproduced in Table 5 (GE series of ointments were not studied *in vivo*).

Characteristics of diclofenac release from semisolid preparations and its transport through cellulose acetate membrane

Equation (2), i.e. linear relationship between the amount released and the square root of time is valid only when sink conditions persist. If not, the release curves show saturation.

The solubility of diclofenac varies considerably with pH. According to the most reliable literature data, conditions of pH = 5.5 and 23 °C, which are close to our experimental conditions, give the solubility as 36 μg/ml[24]. In our experiments, the highest amounts dissolved were around 1100 and 2500 μg in the Franz cell and modified USP studies, respectively. Perfect sink conditions persist if the drug concentration in the receiving medium does not exceed 10% of the solubility of the drug under the given conditions at any time point[25]. Thus, even if the 9 °C temperature difference between the literature data and our experiments is taken into account, sink conditions were not expected to occur at pH 5.4 in our experiments. Researchers using sink conditions in similar studies applied higher pH values[15,16,26].

The release curves seen in Figures 1–4, however, show no saturation. The figures indicate the best linear fit, however, the release curves are also not completely linear as should follow from Equation (2). This phenomenon can be seen in the case of the GE, OG and WO series of preparations using the modified USP apparatus while it occurs in almost all the cases using Franz cell experiments which demonstrated linearity. This phenomenon suggests that the release is not controlled by one single process (diffusion) of diclofenac alone.

The following mechanism is proposed to explain these findings. It is well known that surface active agents and lubricants (like IPM used in our former experiments) may modify both (non-impregnated) artificial membranes and the solubility of substances. In our present study, the dermatological bases studied contained such agents. During the dissolution not only the diclofenac was released through the membrane but other ingredients as well. The latter modified both the membrane structure (making it more suitable for penetration) and the receiving medium dynamically. This explanation may account for the shapes of the curves and the increased solubility of the diclofenac reaching sink conditions in the receiving medium.

The idea that components of creams and ointments modify the characteristics of the membranes they penetrate into and through is not new. It has also been described for human skin[20,26].

It can be seen that the slower the diclofenac release the poorer the linearity of the curve (see e.g. the Franz cell experiments versus the modified USP experiments). Using the Franz cell the

Table 2. *In vitro* release data using the modified USP method.

					Repeatability				Reproducibility			
Composition	*a*	*b*	*r*	*Q* μg/cm^2	s_a	s_b	s_r	s_Q	s_a	s_b	s_r	s_Q
Hydrogels												
HG08	242	71.8	0.9895	687	9.32	26.6	1.91	4.10	12.1	39.6	2.90	9.98
HG09	250	45.5	0.9993	651	10.0	21.1	0.57	6.78	14.1	23.4	1.89	9.03
HG1	259	35.5	0.9804	661	10.8	18.4	3.00	8.50	7.35	23.3	3.19	9.20
Gel-emulsions												
GE40	191	−30.3	0.9728	451	11.9	18.6	0.58	6.97	19.2	20.5	1.28	11.9
GE45	231	−82.5	0.9824	511	11.7	24.9	9.08	5.61	11.1	29.8	7.82	10.6
GE50	200	−96.9	0.9635	424	13.7	16.3	2.26	8.81	16.1	28.4	3.95	11.0
Organogels												
OG25	294	131	0.9546	850	18.3	18.2	2.95	8.72	22.8	31.8	1.76	10.5
OG30	322	−0.92	0.9838	832	15.6	25.3	0.80	7.46	19.4	37.9	0.79	9.03
OG35	339	49.2	0.9731	899	18.3	28.9	2.53	9.29	15.1	43.0	1.20	13.8
Oil-in-water creams												
OW65	146	75.2	0.9828	425	11.1	23.6	1.83	5.54	18.7	23.3	2.08	13.9
OW70	243	−15.7	0.9943	688	8.85	17.6	2.13	3.31	24.3	25.8	2.00	4.41
OW75	305	116	0.9982	866	7.02	17.9	1.97	4.90	29.5	23.1	2.61	6.27
Water-in-oil creams												
WO40	178	−80.0	0.9682	442	14.5	23.3	4.13	7.65	19.8	42.1	5.13	11.9
WO45	121	39.9	0.9678	355	19.4	37.0	3.43	6.99	23.4	44.4	4.98	12.8
WO50	164	0.28	0.9598	438	14.3	24.8	1.68	8.39	19.9	38.3	2.65	14.1
Reference preparations												
REFGE	1460	−115	0.9911	3330	2.13	16.6	0.94	6.30	6.45	17.4	1.10	7.09
REFHG	1070	−166	0.9802	2340	7.05	15.2	0.64	6.66	14.3	17.7	3.24	11.1

Table 3. *In-vitro* release data using the Franz cell method.

					Repeatability				Reproducibility			
Composition	*a*	*b*	*r*	*Q* µg/cm^2	s_a	s_b	s_r	s_Q	s_a	s_b	s_r	s_Q
Hydrogels												
HG08	91.4	−38.8	0.9527	202	13.8	20.3	6.81	12.9	13.4	57.7	6.32	17.0
HG09	104	v33.3	0.9782	217	18.6	23.9	1.83	9.78	26.0	62.4	5.38	29.1
HG1	111	−21.9	0.9750	250	15,3	18.9	4.13	12.6	27.6	68.2	3.63	20.1
Gel emulsions												
GE40	130	−43.9	0.9901	287	3.80	38.4	0.93	5.99	29.3	17.7	4.20	15.9
GE45	163	−49.1	0.9931	360	17.9	10.8	1.35	8.07	28.2	60.3	2.20	14.0
GE50	113	−35.1	0.9929	250	24.9	44.8	5.95	7.92	36.7	80.4	3.75	25.4
Organogels												
OG25	97.8	−36.9	0.9954	208	6.15	25.5	1.15	6.59	29.1	34.8	2.15	13.6
OG30	93.4	−50.6	0.9957	190	7.75	24.4	1.05	13.9	22.6	47.3	3.96	18.7
OG35	54.9	−26.7	0.9948	110	18.2	32.3	1.52	9.31	8.90	15.9	3.35	13.9
Oil-in-water creams												
OW65	115	−30.5	0.9998	252	7.79	16.3	1.19	7.12	13.2	14.7	2.13	10.3
OW70	219	−70.0	0.9989	465	5.51	18.6	1.13	7.14	10.0	14.2	3.07	8.17
OW75	270	−8.61	0.9996	649	12.8	10.9	1.17	6.23	14.6	11.7	2.82	9.37
Water-in-oil creams												
WO40	43.7	2.30	0.9910	109	16.7	12.9	1.92	9.32	22.3	17.3	4.31	16.9
WO45	21.9	−7.03	0.9763	47.5	18.3	15.9	2.42	8.66	23.2	24.4	3.18	16.8
WO50	82.4	−18.2	0.9940	186	15.5	16.3	1.60	5.72	17.6	21.5	3.74	14.2
Reference preparations												
REFGE	296	−194	0.9798	547	8.56	15.3	0.79	6.75	8.39	25.9	2.61	9.17
REFHG	120	−76.4	0.9749	225	10.4	12.7	2.42	5.10	18.0	19.3	2.78	7.02

Table 4. Correlation between *in-vitro* and *in-vivo* data.

	Slope			Q		
Composition	USP/ Franz	USP/ Swell	Franz/ Swell	USP/ Franz	USP/ Swell	Franz/ Swell
HG	0.9805	0.9904	0.9440	−0.8065	−0.5663	0.9440
GE	0.8488			0.8488		
OG	−0.8417	0.9943	−0.7794	−0.9373	0.5123	−0.7794
OW	0.9973	0.9939	0.9999	0.9965	0.9926	0.9993
WO	0.6047	0.4125	0.9632	0.7490	0.5830	0.9750
All data*	0.5306	0.3378	0.4233	0.3385	0.2665	0.3385

USP: modified USP method; Franz: Franz cell method; Swell: swelling %. *Including REFGE and REFHG data.

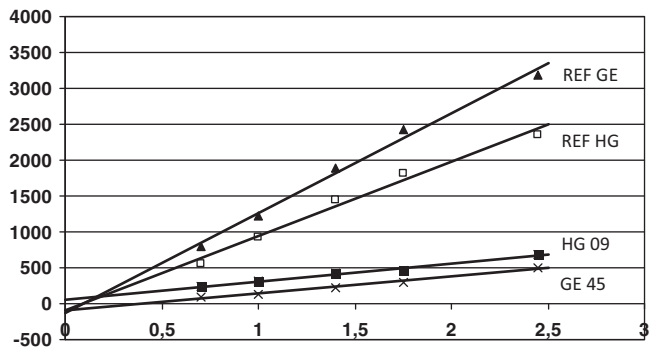

Figure 1. Release experiments with the modified USP method (the best linear fit indicated), 1/2.

intercepts were always negative with poor precision, thus indicating a lag-time diffusion of the analyte through the membrane. This trend is not so obvious with the modified USP method when the release rates were higher.

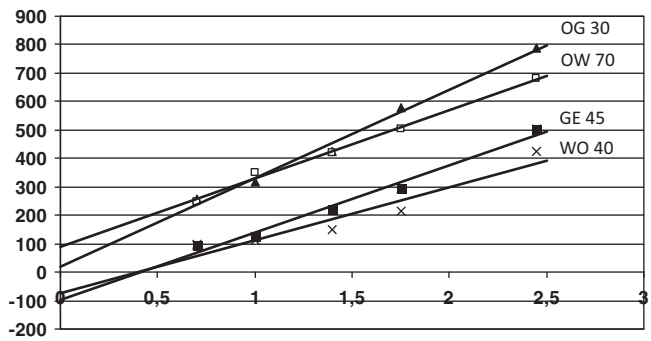

Figure 2. Release experiments with the modified USP method (the best linear fit indicated), 2/2.

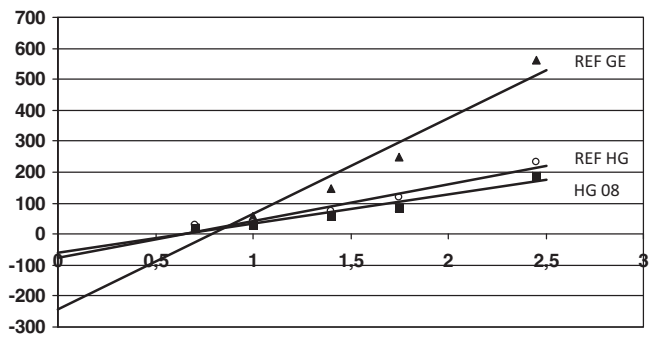

Figure 3. Release experiments with the Franz cell (the best linear fit indicated), 1/2.

Effect of the composition of the different dermatological bases on diclofenac release using cellulose acetate membrane

In case of the OW compositions the release rates (the slope *a* in Equation (2), see Tables 1 and 2) measured either by the modified

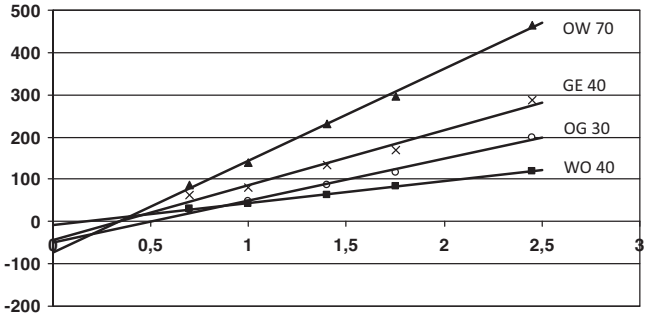

Figure 4. Release experiments with the Franz cell (the best linear fit indicated), 2/2.

Table 5. Swelling data measured *in vivo*.

Composition	Swelling (%)
HG08	45.7
HG09	47.2
HG1	50.0
OG25	29.1
OG30	50.0
OG35	58.2
OW65	37.5
OW70	41.8
OW75	43.6
WO40	18.1
WO45	16.3
WO50	28.3
REFGE	51.80
REFHG	38.30

USP or the Franz cell methods increased with increasing water (and decreasing paraffin) content. A similar, although much less pronounced, trend could be observed in the HG series with increasing Carbopol and consequently, the neutralizing triethanolamine content. By contrast, the ''worst'' and ''best'' compositions were found with the WO and GE series. In the modified USP method, the release rates for the two marketed creams were much higher than those of our other compositions. This difference was not so pronounced with the Franz cell method.

The results observed for the OG series were surprising. The modified USP method indicated slightly, not significantly increasing diclofenac release rates with an increasing amount of the emulsifier span, while this trend was just the opposite for the Franz cell experiments. Research to explain this phenomenon will be performed in the future. The only relevant differences between the two *in-vitro* systems which might account for this phenomenon were the receptor volumes and the sampling. Using the Franz cell, the 0.8 ml/7.0 ml samples taken were always replaced with fresh receiving medium at all time points. Using the modified USP method the volumes were not replaced. In our present working hypothesis, the emulsifier, when penetrating into the membrane, facilitates the passage of the diclofenac, possibly forming micellar structures which helps in its solubilization in the receiving medium. The structure and composition of micelles depend on the concentration of the emulsifier. When the receiving medium is diluted after sampling, the concentration of the emulsifier decreases as well as in the membrane initiating micelle changes during the passage.

Apart from this explanation, our experiments suggest that beside the fact that the release rates are naturally much faster when the modified USP method is used, the two test systems do not mimic the *in-vivo* processes in the same way.

In vivo–in vitro correlation

Table 5 summarizes the quantitative correlation between the results generated in the two *in-vitro* dissolution methods and the *in-vivo* swelling % (Equation (1)) data (17). For r and not r^2 data are presented, the negative r values indicate opposite trends in the data (i.e. increasing and decreasing release rates with the concentration of a component).

Correlation between the slopes (apparent release rates) and swelling data

When all data have been generated either with the modified USP or with the Franz cell method and correlated with the *in vivo* swelling % results, no correlation was found. Thus, neither method was predicting IVIVC, in general. The same is valid for the correlation of the two *in-vitro* methods.

However, varying the concentrations of the components within one type of composition, a good correlation may be achieved between *in vitro*, generated by the modified USP method, and *in-vivo* data in the HG, OG and OW series. Similar findings, with a somewhat poorer correlation, can be observed with the Franz cell and *in-vivo* data for the HG, OW and WO series. The IVIVC was the best for both *in vitro* methods in the case of the OW compositions. Only one of the two *in-vitro* methods gave somewhat acceptable correlation in the case of the OG and WO preparations using the modified USP and Franz cell methods, respectively.

When the correlation within the OW series was the best, one may postulate that the release of diclofenac sodium from oil-in-water bases was the closest to a single diffusion controlled process.

Correlation between Q and swelling data

Correlations for the cumulative amount dissolved in 6 h (Q) data, as a rule, were much poorer that those for the slopes. It was not surprising for our proposed explanation described the dissolution of diclofenac from semisolids as a complex procedure with co-dissolution of other ointment components modifying the membrane and the solubility of diclofenac dynamically. The slope, although biased by the previously mentioned sub-processes, was determined by the whole curve characterizing the main release process. It should have been a better correlation with the *in-vivo* data than the Q values when calculated as the mean of single measuring points.

Statistical comparison of the performances of the Franz cell and the modified USP systems

Comparing the standard deviation data in Tables 2 and 3, one can see that, in most of the cases, the precision (both repeatability and reproducibility) of the modified USP method was found superior to that of the Franz cell method.

As a rule, the precision of Q values was better than that of the slopes while those of the intercepts (b) were the poorest. This should be a natural consequence because the linear release correlation curves are really not completely linear. Standard deviations for the correlation coefficients did not provide any new information.

Discussion and conclusion

It should be emphasized that it may be true only for our selected experimental conditions, that the following conclusions may be drawn.

Comparing the modified USP and the Franz cell methods, the former seems to be more precise. Moreover, because it is a more

complex piece of equipment the Franz cell needs more frequent maintenance than the USP modified apparatus method.

Although the release of the active principle, namely the diffusion through the cellulose acetate membrane, seems to be a more complex process which results in not fully linear curves. This presumably involves co-penetration of excipients modifying both the membrane and the apparent solubility of the active principle in the receiving medium dynamically. The slopes (a) of the release curves (Equation (2)) are the best parameter for IVIVC, although the amount dissolved at a given time (Q) can be measured with higher precision. The latter may be better for quality control purposes.

Our experiments show that when we use a non-impregnated cellulose acetate membrane neither the modified USP nor the Franz cell is suitable to establish IVIVC. In general however, other researchers[27] and our own former studies[28] could obtain acceptable IVIVC for some semisolid compositions using the Franz cell. Moreover, varying the composition within the same types of semisolid dermatological bases good IVIVC may be achieved. In this respect the performances of the two *in-vitro* methods are different, depending on the type of base utilized. Thus, both methods could be suitable for optimization studies, but this is not true for all types of dermatological compositions. Consequently, to support such studies, it is advisable to establish at least qualitative IVIVC with parallel *in-vivo* experiments.

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Declaration of interest

The authors report no declaration of interest.

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References

1. Brown CK, Friedel HD, Barker AR, et al. FIP/AAPS Joint Workshop Report: dissolution/in vitro release testing of novel/special dosage forms. Dissolut Technol 2011;18:51–64 and Int J Pharm Sci 2011;73:338–353.
2. Ghosal K, Chandra A, Rajabalaya R, et al. Mathematical modelling of drug release profiles for modified hydrophobic HPMC based gels. Pharmazie 2012;67:147–155.
3. Barry BW. Drugs and the pharmaceutical sciences: dermatological formulation – percutaneous absorption. New York and Basel: Marcel Dekker; 1983:1–48.
4. Corbo M. Techniques for conducting in vitro release studies on semisolid formulations. Dissolut Technol 1995;2:3–6.
5. Hanson R, Gray V. Handbook of dissolution testing. 3rd ed. Hockessin (DE): Dissolution Technologies; 2004:33–71.
6. Shah VP. IV-IVC for topically applied preparations – a critical evaluation. Eur J Pharm Biopharm 2005;60:309–314.
7. Azarmi S, Roa W, Löbenberg R. Current perspectives in dissolution testing of conventional and novel dosage forms. Int J Pharm 2007;328:12–21.
8. Shah VP, Elkins J, Schuirman D, et al. Application of in vitro release methods to assure product performance of semisolid dosage forms before and after certain post-approval changes. Dissolut Technol 1998;5:5–11.
9. FDA Guidance for Industry SUPAC-SS. Nonsterile Semisolid Dosage Forms. Scale-up and postapproval changes: chemistry, manufacturing and controls. In vitro release testing and in vivo bioequivalence documentation. U.S. Department of Health and Human Services; Food and Drug Administration, Center for Drug Evaluation and Research, Washington; 2007.
10. D'Souza SS, Lozano R, Mayock S, Gray V. AAPS Workshop on the role of dissolution in QbD and drug product life cycle: a commentary. Dissolut Technol 2010;17:41–45.
11. Franz T. Percutaneous absorption. On the relevance of in vitro data. J Invest Dermatol 1975;64:190–195.
12. Chen L, Fengping T, Wang J, Liu F. Assessment of the percutaneous penetration of indomethacin from soybean oil microemulsion: effects of the HLB value mixed surfactants. Pharmazie 2012;67:31–36.
13. Chen L, Fengping T, Wang J, Liu F. Microemulsion: a novel transdermal delivery system to facilitate skin penetration of indomethacin. Pharmazie 2012;67:319–323.
14. Shah VP, Elkins J, Shaw S, Hanson R. *In vitro* release: comparative evaluation of vertical diffusion cell system and automated procedure. Pharm Dev Technol 2003;8:97–102.
15. Sanna V, Peana AT, Moretti MDL. Effect on vehicle on diclofenac sodium penetration from new topical formulations: in vitro and in vivo studies. Curr Drug Deliv 2009;6:93–100.
16. Shivhare UD, Jain KB, Mathur VB, et al. Formulation development and evaluation of diclofenac sodium gel using water soluble polyacrylamide polymer. Dig J Nanomater Biostruct 2009;4:285–290.
17. Petró É, Balogh Á, Blazsó G, et al. In vitro and in vivo evaluation of drug release from semisolid dosage forms. Pharmazie 2011;66:936–941.
18. Hanson R. A primer on release-rate testing of semisolids. Dissolut Technol 2010;17:33–35.
19. Katz M, Poulsen BJ. Absorption of drugs through the skin. In: Brodie BB, Gillette J, eds. Handbook of experimental pharmacology. Vol. 28/1. New York: Springer; 1971:103–174.
20. Wagner H, Kostka KH, Lehr CM, Schaefer UF. pH profiles in human skin. Influence of two in vitro test systems for drug delivery testing. Eur J Pharm Biopharm 2003;55:57–65.
21. Klee SK, Farwick M, Lersch P. Triggered release of sensitive active ingredients upon response to the skin's natural pH. Coll Surf A Physicochem Eng Aspects 2009;338:162–166.
22. Calpena AC, Escribanoaa E, Martinaa HS, et al. Influence of the formulation on the in vitro transdermal penetration of sodium diclofenac. Arzneim-Forsch 1999;49:1012–1017.
23. Uslu M, Kilincoglu V, Toker S, et al. Comparison of anti-edema effects of iloprost and diclofenac sodium on traumatic rat paw edema. Acta Orthop Traumatol Turc 2010;44:484–491.
24. Chuasuwan B, Binjesoh V, Polli JE, et al. Biowaiver monographs for immediate release solid oral dosage forms: diclofenac sodium and diclofenac potassium. J Pharm Sci 2009;98:1206–1219.
25. Klose D, Delplace C, Siepmann J. Unintended potential impact of perfect sink conditions on PLGA degradation in microparticles. Int J Pharmaceutics 2011;404:75–82.
26. Escribano E, Cristina CA, Josep Q, et al. Assessment of diclofenac permeation with different formulations: anti-inflammatory study of selected formula. Eur J Pharm Sci 2003;19:203–210.
27. Tashtoush BM, Al-Safi SA, Al-Fanek KJ. Azathioprine transport through rat skin and its immunosuppressive effect. Pharmazie 2004;59:143–146.
28. Csóka I, Csányi E, Zapantis G, et al. In vitro and in vivo percutaneous absorption of topical dosage forms: case studies. Int J Pharm 2005;291:11–19.

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The role of dissolution testing in quality control

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RESEARCH ARTICLE

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Abstract

Semisolid systems (creams, gels etc.) for dermal application get more and more importance in pharmaceutical and cosmetic industry. However the number of methodologies for their physico-chemical characterization have been increasing; development of methods for the measurement of the active agent's release onto the skin surface is still a challenging task.

Beside measuring the amount of the active agent reaching the skin; dissolution testing (also called release testing) can be also a good indicator of product composition changes, therefore it can be used as quality control methodology.

The purpose of this study was to investigate the in vitro drug release of active agents from hydrogels, organogels, o/w and w/o creams, emulgels and w/o/w multiple emulsions for dermal use by means of the vertical diffusion cell methodology, for quality control purposes.

Keywords

semisolid systems · dermal use · dissolution test · drug release · QC control

1 Introduction

Dissolution studies are developed and are available mainly for solid dosage forms (tablets, capsules); different methodologies validated for these forms are detailed in the main Pharmacopoeias [1,2]. There is no compulsory method in any Pharmacopoeia for semisolid dosage forms; only two guidelines are available suggesting equipment for the measurement of drug release from these preparations. According to these guidelines and also based on literature data, drug release has been extensively investigated by means of the Franz cell diffusion system with a synthetic membrane [3-6].

The experimental conditions in drug release testing such as receptor phases, membrane-types, usage of different animal skin-models etc. depend on the purpose of the experiments; whether the aim is quality control or so called bioavailability testing in order to decrease the number of animal testing or eliminating them [7,8].

Concerning cosmetic products, the product behavior on the skin and also the release of the active agent content should be tested under in vitro conditions without animals; as the EU banned cosmetic testing on animals in 2009, therefore alternatives should be found for evaluation [9].

The following figure summarizes the role of drug release studies in case of semisolid dermal preparations (Figure 1.).

Predicting the practical applicability or changes within a given system with mathematical modelling [10,11] or different

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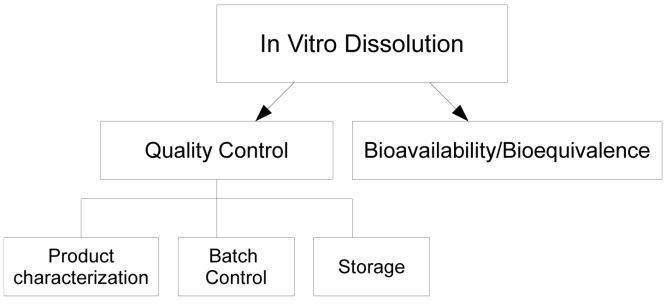

Fig. 1. Application of dissolution testing in product characterization

experimental methods – such as this drug release/dissolution measurements has increasing importance nowadays. As seen on the Figure 1.; active agent release from a cosmetic or pharmaceutical product is an important quality indicator when (1) describing a given composition; (2) it's necessary to detect the effect of changes in components and manufacturing process; and (3) we have to follow up the changes during storage (stability testing). It can be also used as bioavailability testing in order to predict the „in vivo" performance of the developed product.

2 Aim

The aim of this study is to evaluate the applicability of drug release testing in following the composition changes in case of 17 cosmetic and pharmaceutical dosage forms. The in vitro drug release data in case of two active agents were measured from different vehicles (hydrogel, organogel, w/o and o/w creams, o/w emulgels and w/o/w multiple emulsions) for dermal use. The presented compositions were developed by our research group earlier [12,13].

3 Experiments

3.1 Compositions of the investigated products

The drug release process of 1.0 w/w% diclofenac sodium from different products (n=15) and 1.0 w/w% ketamine HCl containing systems (n=2) was measured through synthetic cellulose acetate membrane soaked in the receiving medium (Table 1, Table 2).

Tab. 1. Products containing 1 % diclofenac sodium

dosage form	composition	marked as:
hydrogel	polymer (Carbomer 934 P) content (w/w%): (1) 0.8 (2) 0.9 (3) 1.0	DSHG 0.8 DSHG 0.9 DSHG 1.0
organogel	gelling agent (sorbitan monopalmitate) content (w/w%): (4) 25.0 (5) 30.0 (6) 35.0	DSOG 25 DSOG 30 DSOG 35
o/w emulgel	polymer (Pemulen TR-2) containing aqueous phase (w/w%): (7) 40.0 (8) 45.0 (9) 50.0	DSGE 40 DSGE 45 DSGE 50
o/w cream	aqueous phase (w/w%): (10) 65.0 (11) 70.0 (12) 75.0	DSOV 65 DSOV 70 DSOV 75
w/o cream	aqueous phase (w/w%): (13) 40.0 (14) 45.0 (15) 50.0	DSVO 40 DSVO 45 DSVO 50

Tab. 2. Products containing 1 w/w% ketamine HCl

dosage form	composition	marked as:
w/o emulsion	(16) aqueous phase (w/w%): 75.0	w/o
w/o/w emulsion	(17) primary ((16)w/o emulsion) phase (w/w%):40.0	w/o/w

3.2 Drug release measurements

The Franz vertical diffusion cell system (Hanson Research Co.) containing 6 cells, and equipped with autosampler (Hanson Microette Autosampling System) was used for the drug release measurements.

The products were placed on the synthetic cellulose acetate membrane (Porafil, Macherey-Nagel, Germany) with pore size of 0.45 µm. The diffusion area was 1.767 cm^2. Experiments run at 32±0.5°C and 25±0.5°C (in case of w/o/w). 800 µl samples were taken after 0.5,1,2,3,4,5,6 hours. Phosphate buffer (pH 5.4±0.1) was chosen for receptor medium. Absorbance was measured by UV spectrophotometer (Unicam Helios α UV-Vis Spectrophotometer, England) at 275 nm and 269 nm (w/o/w). The blank agents without active agents served as references in the analytical method.

4 Results and discussion

The release profiles of hydrogels and w/o creams are illustrated in Figures 2-4.

Very low amount of active agent was released even after 6 hours of experiments in case of hydrogels and w/o creams. The difference in release rates between the 0.8 and 0.9 w/w% polymers containing products wasn't significant. A slightly increased amount was released in case of 1 w/w% polymer content, which phenomena is due to the increased trietanolamine content, facilitating the diclofenac dissolution through the membrane.

Drug release from w/o creams is mainly driven by the diffusion capacity of the active agent through the compositions with slightly different viscosities (Figure 3.). No significant differences were found at this level of changes.

The following figure (Figure 4.) gives an overview about the release rates of some selected (based on the stability data) products from the different types. No significant differences in release rates were found in case of the following compositions: organogel containing 25.0 w/w% gelling agent (DSOG 25), the w/o cream with 45.0 w/w% internal water content (DSVO45) and the hydrogel product containig 1.0 w/w% polymer content (DSHG1).

The release rate from the emulgel with 45.0 w/w polymer containing water phase showed a slightly higher released acive agent amount after 6 hours, but still not evaluated as significant change. These compositions therefore, can be predicted to be equivalent in their „in vivo" performance; in spite of the fact, that they vary in composition and product type.

Significant difference from these products was found in case of the 75.0 w/w % external aqueous phase containing o/w cream (DSOV75).

Figure 5. shows the difference between the release rates of the primary w/o emulsion and a w/o/w product; after adding

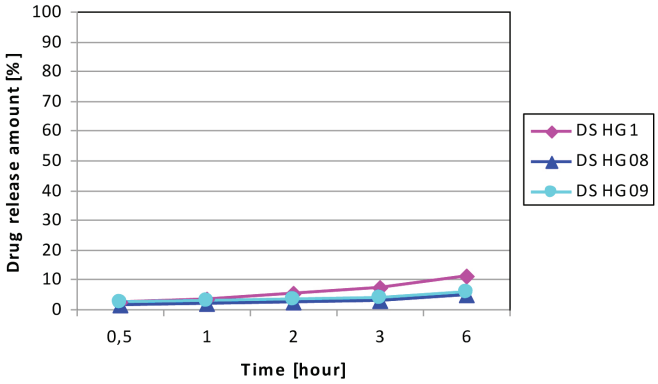

Fig. 2. Drug release from hydrogels in case of different polymer content

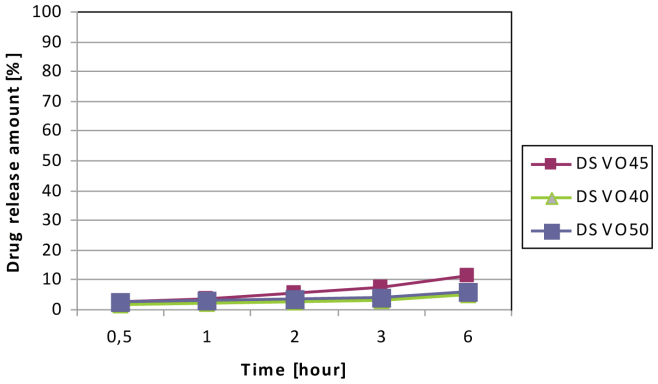

Fig. 3. Drug release from w/o creams with different aqueous phase ratio

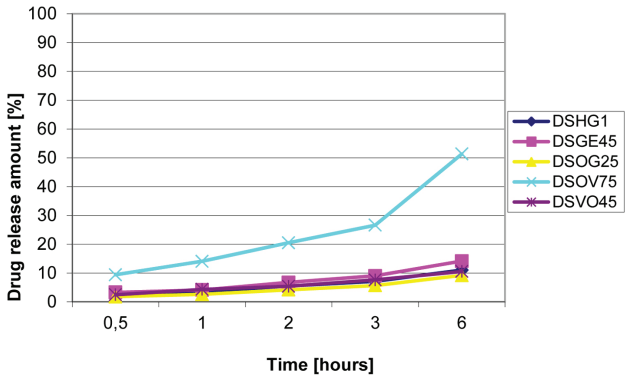

Fig. 4. Drug release from different dosage forms

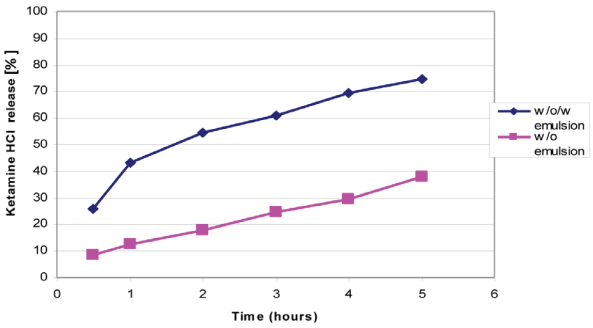

Fig. 5. Drug release from emulsions (primary w/o and the final w/o/w)

the external aqueous phase to the primary (w/o) one. The same amount of the active agent was incorporated into the aqueous phase of the primary; and in case of the inner and external water phases of the multiple emulsion. 75.0 % of the drug dissolved in the multiple emulsion released during 5 hours; while 37% from the primary simple one. The advantage of multiple emulsions for achieving faster drug release from its external phase, followed by a slower drug dissolution from the inner phases – could be detected.

5 Conclusions

Dissolution testing has an increasing role in case of all dosage forms in pharmaceutical dosage form design and development. Its applicability can be extended to cosmetic products as well; as alternative method instead of animal testing.

Different levels of product composition changes and "similarity" in case of different products can be detected only after a careful validation of the drug release measurement with Franz vertical diffusion cell system.

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References

1 **European Directorate for the Quality of Medicines & HealthCare,** *European Pharmacopoeia 7th Edition*, Council Of Europe: European Directorate for the Quality of Medicines and Healthcare, Strasbourg, France (2011).

2 **United States Pharmacopeial Convention, Usp,** *USP 35-NF 30*, United States Pharmacopeial, Rockville, MD, United States (2012).

3 **Siewert M., Dressman J., Brown C. K., Shah V. P.,** *FIP/AAPS guidelines to dissolution/in vitro release testing of novel/special dosage forms*. AAPS PharmSciTech, 4(1), 43–52 (2003). DOI: 10.1208/pt040107

4 *FDA Guidance for Industry: Nonsterile Semisolid Dosage Forms, Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation*. SUPAC-SS, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, MD, United States (1997).

5 *FDA Guidance for Industry: Topical Dermatological Drug Product NDAs and ANDAs – In Vivo Bioavailability, Bioequivalence, In Vitro Release, and Asssociated Studies*. SUPAC-SS, U.S. Department of Health and Human Services, Food and Drug Administration,Center for Drug Evaluation and Research, Rockville, MD, United States (1998).

6 **Zatz J. L., Segers J. D.,** *Techniques for Measuring In Vitro Release for Semisolids*. Dissolution Technologies, 5(1), 3-17 (1998).

7 **Shah V. P.,** *Dissolution: A Quality Control Test vs. A Bioequivalence Test*. Dissolution Technologies, 8(4), 1-2 (2001).

8 **Shah V. P., Siewert M., Dressman J., Moeller H., Brown C., K.,** *Dissolution/In Vitro Release Testing of Special Dosage Forms*. Dissolution Technologies, 9(1), 1-5 (2002).

9 *Guidance Document for the Conduct of Skin Absorption Studies*. Organisation for Economic Co-operation and Development (OECD), Paris (2004).
DOI: 10.1787/9789264078796-en

10 **Orbán-Mihálykó É., Mihálykó Cs.,** *Investigation of operation of intermediate storages applying probability density functions satisfying linear differential equation*. Periodica Polytechnica Chemical Engineering, 56(2), 77-82 (2012).
DOI: 10.3311/pp.ch.2012-2.05

11 **Bárkányi Á., Lakatos B. G., Németh S.,** *Modelling of heat exchange between drops in suspension polymerization of vinyl chloride*. Periodica Polytechnica Chemical Engineering, 56(2), 55-64 (2012).
DOI: 10.3311/pp.ch.2012-2.02

12 **Petro E., Eros I., Csoka I.,** *Rewiew of in vitro drug release test method's statistical evaluation to compare dissolution profile of semisolid dosage forms – Part I.*, Journal of Applied Pharmaceutical Science, 2(3), 180-181 (2012).
DOI: 10.7324/JAPS.2012.2329

13 **Petro E., Balogh A., Blazso G., Eros I., Csoka I.,** *In vitro and in vivo evaluation of drug release from semisolid dosage forms*.
Die Pharmazie - An International Journal of Pharmaceutical Sciences, 66(12), 936-941 (2011).
DOI: 10.1691/ph.2011.1079