Film forming polymeric solutions as drug delivery systems for the skin

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CHAPTER 1

General introduction

1. Introduction

The skin is a very attractive organ for the application of pharmaceutically active substances due to its considerable size and easy accessibility. The aim of the drug administration via the skin can be either the local therapy of dermatological diseases or the transdermal delivery of drugs to the underlying tissues or the systemic circulation.

The transdermal delivery of drugs as alternative to oral dosage forms has been the subject of research for many decades. Due to the considerable advantages of the transdermal application route for some drugs different dosage forms have been developed for the drug delivery through the skin: polymeric patches and semisolids. Currently, the patches still represent the majority of preparations for this application route, but the semisolids (and among them especially the alcoholic hydrogels) have gained more and more acceptance in recent years. Although the number of transdermally applied drugs is limited the existing dosage forms are quite successfully marketed for various indications (the annual market volume for transdermal patches alone in the United States of America was approximately 3 billion USD in 2004 [1]). However, each of the dosage forms is associated with certain drawbacks that can negatively influence the patient compliance or limit the usage of the dosage form (for details see section 4.2.3). Hence the search for alternatives to the conventional transdermal dosage forms is reasonable to further improve the transdermal drug application for the patient.

2. Objectives of this work

The scope of the present work is to develop and investigate a novel delivery system for the skin as alternative to the existing transdermal dosage forms. The approach chosen for the new dosage form is a film forming polymeric solution. On the skin surface the solution solidifies into a film which is able to deliver the active moiety to the body. In a first step desirable properties of the novel delivery systems are to be defined and an evaluation system based on these properties is to be established to perform a formulation screening process. With the help of the developed evaluation system various polymeric materials and formulation parameters will be investigated in terms of suitability to provide the technological basis for the dosage form. The film forming formulations resulting from this process will be characterized concerning their mechanical and occlusive properties. The relevance of the utilized evaluation and characterization methods for

the film forming systems will be assessed. To evaluate their potential as drug delivery systems film forming solutions loaded with drugs of different lipophilicity are to be studied concerning their drug release and drug permeation behaviour. Crucial parameters will be investigated to control and improve the drug permeation from the potential delivery systems. The drug permeation from drug loaded film forming solutions will also be compared to registered transdermal patches in vitro and possibly in vivo to gain a realistic assessment of their drug delivering capability. Finally, the possibilities and limitations of the novel film forming systems and their advantages and disadvantages in comparison to the conventional dosage forms are to be discussed.

3. Anatomy of the skin

The skin is the largest organ of the human body with a surface area of approximately 1.5 – 2.0 m^2 [2] and an average thickness of 0.5 mm (ranging from 0.05 mm to 2 mm) [3]. As interface between the body and the outside world the skin fulfils important protective as well as sensory functions. It contains a variety of receptors to receive different impulses such as pressure, touch, temperature and pain for the communication between the body and the environment. Through its capillary system and the subcutaneous fatty tissue the body temperature is regulated. The mechanical strength of the skin protects the body against mechanical stress. Its low permeability for a broad range of substances shields the body against chemical and microbiological noxes and prevents the dehydration of the body by limiting the transepidermal water loss. Melanocytes in the skin serve as protection against harmful ultraviolet radiation. Apart from this, the skin performs endocrine functions such as the synthesis of Vitamin D and the production of pheromones.

Basically the skin can be divided into three layers: the epidermis, the dermis and the hypodermis. The different structures are displayed in Fig. 1 and will be described more detailed in the following.

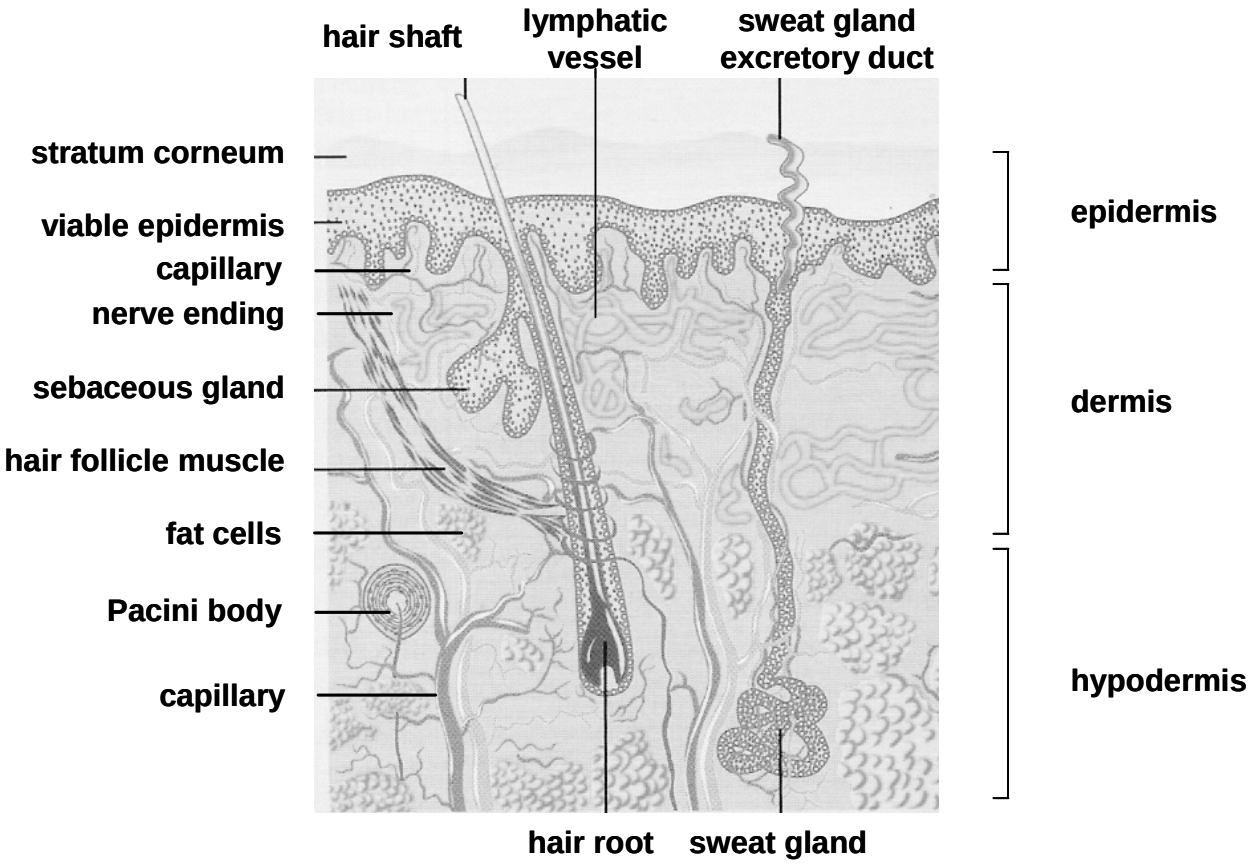

Fig. 1: Schematic cross-section of the skin (adapted from Thews [4])

3.1. The epidermis

The epidermis is the uppermost layer of the skin with an average thickness of 0.1 – 0.15 mm [5]. As it is a non-vascularized tissue, it receives its nutrition by diffusion from the capillary system of the dermis. Apart from the keratinocytes, which represent the majority of the cells in the epidermis, other cell types like melanocytes, Langerhans cells and Merkel cells are located in this skin layer. Furthermore, a number of catabolic enzymes such as esterases, phosphatases or lipases are present throughout this tissue [3]. The epidermis can be further subdivided into a viable part, consisting of the stratum basale, the stratum spinosum, the stratum granulosum and (only in certain anatomical regions) the stratum lucidum, and a non-viable part, the stratum corneum.

3.1.1. The viable epidermis

The viable part of the epidermis consists mainly of three, in certain anatomical regions of four different layers. The lowest layer, the stratum basale represents the border of the epidermis to the dermis. Above the stratum basale the stratum spinosum is located, followed by the stratum granulosum. The stratum granulosum borders on the non-viable tissue of the stratum corneum, which is the outermost layer of the skin. In the skin of the soles of the feet and the inner sides of the hands a fourth layer is found, the stratum lucidum, which is located between the stratum corneum and the stratum granulosum. The average thickness of all layers of the viable tissue is approximately 100 µm [6].

The stratum basale consists of a single layer of actively dividing cells (the only ones in the epidermis). These are connected to the basal membrane and the dermis via hemidesmosomes. Adjacent and overlying cells in the cell layer are connected via desmosomes. The main function of the stratum basale is the regeneration of the epidermal tissue. The cells that result from the mitotic activity of these epidermal stem cells, the keratinocytes, migrate in the direction of the stratum corneum where they are finally shed after approximately 30 days [7]. During the migration the keratinocytes undergo a differentiation process (loss of phospholipids, increase in sphingolipid and cholesterol content, loss of cell organelles) at the end of which they have become the dead, completely cornified corneocytes that represent the main body of the stratum corneum. Apart from the keratinocytes melanocytes and Merkel cells (pressure receptors) are also located in the stratum basale. The melanocytes, which are responsible for the protection of the basal layer against UV radiation, can also be located in the lower layers of the stratum spinosum.

The stratum spinosum, which is located next to the stratum basale, consists of 4 - 8 cell layers. The high number of desmosomes that connect adjacent cells accounts for the spiny appearance of these cells in histological sections. In this layer an increased synthesis of proteins and lipids takes place in the cells including the production of the Odland bodies. These lipid-enriched lamellar bodies are secreted by the keratinocytes later in the migration process at the border of the stratum granulosum to the stratum corneum and form the intercellular lipid matrix in the stratum corneum [5]. A first aggregation of the keratinous filaments in the keratinocytes is observed in the stratum spinosum. In the upper layers a re-orientation of the cell bodies takes place as the keratinocytes become horizontally oriented in relation to the cells in the stratum

basale. In addition to the keratinocytes the stratum spinosum contains immunologically active Langerhans cells.

The layer above the stratum spinosum, the stratum granulosom, contains only 2 – 5 layers of flattened cells with keratohyalin granules. These granules contain various proteins such as keratins, profillagrin or loricrin [5]. The profillagrin is converted into fillagrin that serves to aggregate and align the keratin filaments in the further cornification process. The stratum granulosum is also the location for the formation of the cornified envelope. This structure, consisting predominantly of the cross linked protein loricrin, is a protein layer on the inner side of the cell membrane that serves to mechanically stabilize the keratinocytes. During the migration of the keratinocyte through the stratum granulosum the transition from granular to cornified cell takes place with the cytoplasmatic degradation of the cellular organelles performed by various proteases.

3.1.2. The stratum corneum

The stratum corneum, the uppermost layer of the epidermis, consists typically of 18 - 21 cell layers and has a thickness of 10 – 20 µm [3]. On the external side it is covered by a liquid, weakly acidic film (pH 5 – 6) for antimicrobial protection. The cells in this layer are corneocytes (terminally differentiated keratinocytes) that are embedded in a lipid matrix consisting of the secreted contents of the lamellar Odland bodies. This structure has been characterized by Elias as brick-and-mortar-organization [8] where the protein rich corneocytes represent the bricks and the hydrophobic lipids organized in lamellar structures the connecting mortar. Corneodesmosomes improve the cohesiveness of this cell layer [9]. The corneocytes contain between 10% and 30% of water [5]. Their degree of hydration is related to the presence of the degradation products of fillagrin, a blend of amino acids, uric acid and further compounds termed as natural moisturizing factor [10].

The intercellular lipid matrix consists mainly of ceramides, cholesterol and fatty acids besides small amounts of triglycerides, glycosphingolipids and cholesterol sulphate [11]. Ceramides (approximately 50% of the total lipid mass) are responsible for the lipid organization, cholesterol (approx. 25%) improves the mixing of the different lipid compounds. The free fatty acids (approx. 10%) are mainly of the saturated type with a chain length of 20 carbon atoms or more, with additional smaller fractions of oleic and linoleic acids. Contrary to other biological lipid membranes polar phospholipids are not present in the lipid mixture. The intercellular lipids are

basically organized in multilamellar bilayers, containing crystalline, liquid crystalline and possibly also gel phases [5]. The presence of additional liquid phases has also been suggested [12].

3.2. The dermis

The dermis is a highly elastic tissue made of a network of protein fibres (different types of collagen and elastin) which is embedded in an amorphous ground substance consisting of glycosaminoglycan [3]. Apart from the protein network the dermis contains nerve endings and blood vessels as well as lymphatic vessels. Additionally, fibroblasts, macrophages, mast cells and leukocytes are found in this skin layer. The dermis is subdivided into the stratum papillare, that borders on the epidermis, and the stratum reticulare, which is located near the hypodermis. Both tissues differ in the strength of the collagenic fibers which are rather fine in the stratum papillare and fairly strong in the stratum reticulare.

3.3. The hypodermis

The hypodermis is located below the dermis and displays a high content of fatty tissue. Due to this the main functions that can be attributed to the hypodermis are the protection of the body against cold and the provision of energy resources.

3.4. Skin appendages

Throughout the skin different skin appendages can be found such as nails, hairs, sebaceous glands and sweat glands. Hair follicles as well as sebaceous and sweat glands can be found in the hypodermis as well as in the dermis and traverse through the epidermis to the surface. Sebaceous glands are often connected to hair follicles. For nutrition purposes the hair follicles are connected to the capillary system of the dermis.

4. Transdermal drug delivery

4.1. Delivery routes through the skin

Due to its size the skin is an attractive organ for the application of pharmaceutically active substances. However, the drug delivery via the skin is challenging due to its very efficient barrier properties. In order to reach the capillary or lymphatic system in the dermis and finally the systemic circulation a drug has to permeate several layers of the skin with different chemical properties. After the release from the formulation the drug has to partition into the uppermost layer of the skin, the stratum corneum. After diffusion through this first layer with its highly lipophilic intercellular matrix the drug has to partition from the stratum corneum into the hydrophilic viable epidermis. From the viable epidermis it diffuses into the dermis to be taken up into the local capillary network of the dermis. Once the drug has entered the capillary system perfect sink conditions and therefore a maximum concentration gradient for the drug diffusion are achieved. In this permeation process the uppermost layer of the skin, the stratum corneum, with its protein rich corneocytes and the intercellular lipid structure has been acknowledged as main barrier for many years [13].

For the permeation of substances through this first and principal barrier several different routes are discussed (Fig. 2): the intercellular route, the transcellular route and appendageal pathway [14]. Traditionally, it was postulated that hydrophilic drugs prefer to diffuse through the stratum corneum on the transcellular route, that is along the keratinous fibers through the corneocytes, while lipophilic drugs tend to diffuse through the lamellar lipid structures between the cells [6]. The transcellular route, however, requires the drug to pass not only the hydrophilic corneocytes but also the lipid intercellular matrix between adjacent corneocytes. This circumstance complicates the drug passage on this permeation route. Therefore the intercellular route is presently considered the major pathway for lipophilic and hydrophilic drugs, assuming that the hydrophilic compounds preferably diffuse along the polar head-groups of the bilayers while lipophilic drugs choose the non-polar tail-groups [15]. Apart from the transcellular and the intercellular passage way another possible route is the drug diffusion along hair follicles, sebaceous glands or sweat glands, the so-called shunt pathways. Scheuplein has proposed this diffusion route as opportunity predominantly for ionic drugs and large molecules to pass not only

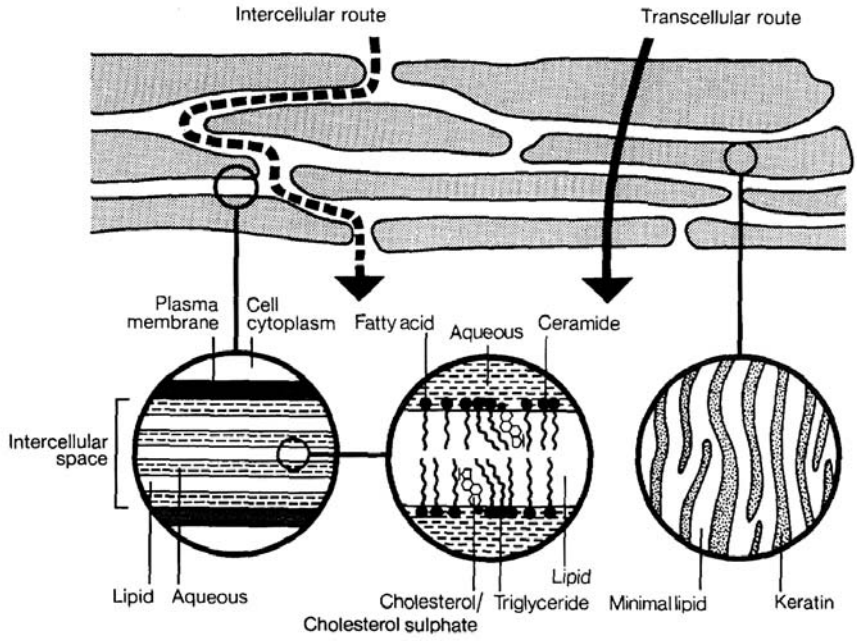

Fig. 2: Possible permeation routes through the skin according to Barry [16]

the stratum corneum but the complete epidermis [6]. Barry has shown with the help of a skin sandwich technique that hydrophilic permeants might possibly use this diffusion route contrary to lipophilic drugs [17]. As the appendages cover only 0.1% of the surface area of the skin their importance for drug absorption is limited. However, the appendageal pathway might be of relevance for the uptake of special delivery systems such as liposomes or nanoparticles [18, 19]. The existence of an additional aqueous polar pathway in the stratum corneum for hydrophilic permeants is discussed controversially in the literature [20, 21].

Mitragotri has recently suggested four permeation pathways that are used by permeants in relation to their lipophilicity and molecular size [22]. He postulated that hydrophobic molecules ($\log P_{oct} > 1$) with low molecular weight ($M_r < 400$ Da) diffuse through the stratum corneum by free-volume diffusion while larger hydrophobic solutes rather use lateral diffusion in the lipid layers. He proposed that the free-volume pathway is also preferred by low molecular weight, moderately hydrophilic molecules ($0.01 < \log P_{oct} < 1$) while small and excessively hydrophilic solutes ($\log P_{oct} < 0.01$) use a porous pathway generated by imperfections in the lipid structures. Large hydrophilic molecules, finally, are supposed to diffuse through the shunt pathway. The different pathways suggested by Mitragotri in dependence of the size and lipophilicity of the molecule are illustrated in Fig. 3. The model Mitragotri proposed on the basis of these assumptions was well supported by experimental data.

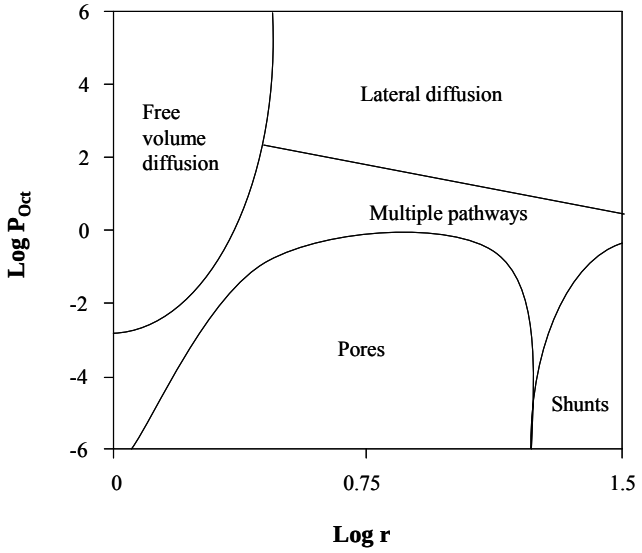

Fig. 3: Relative role of diffusion pathways in dependence of the permeant size and lipophilicity adapted from Mitragotri [22].The regions correspond to a set of parameters where the named pathway determines more than 50% of the overall skin permeability.

All described permeation options are passive diffusion processes, no active transport has been identified yet [23].

With respect to the different lipophilic and hydrophilic structures of the skin it is obvious that drugs with different chemical properties encounter different problems concerning their passage through the skin. Hydrophilic drugs for example typically show low permeabilities in the stratum corneum as they do not partition to a great extent into the lipophilic domains of the intercellular pathway. Highly lipophilic drugs on the other hand can readily partition into the stratum corneum, but cannot diffuse easily into the hydrophilic viable epidermis. This circumstance can slow down the diffusion process and can lead to an enrichment of the drug at the interface stratum corneum – stratum granulosum [24]. Drug with hydrophilic and lipophilic moieties are less problematic as they can partition into hydrophilic as well as into lipophilic domains. This is the reason for the recommendation to choose drugs for a transdermal application with a log P_{oct} between 1 and 3. Other properties that are beneficial for the transdermal absorption are a low molecular weight (< 500 Da), the absence of charges in the molecule, a low melting point and a low number of hydrogen-bonding groups [25, 26].

Due to the generally low permeability of human skin it is still a challenge to achieve sufficient delivery levels in the human body for most drugs. Therefore much research has been done to find ways to improve the drug delivery and to open the transdermal application route for a larger group of drugs, especially for larger drugs such as proteins or peptides. Examples for different approaches to overcome the skin barrier are described in various review articles [15, 27, 28] and are summarized in Table 1.

It has to be kept in mind that the transdermal absorption of a drug is not only influenced by the chemical properties of the drug and the functionalities of the vehicle but that it also underlies various inter- and intraindividual variations with respect to the application site on the body, the condition of the skin (healthy or diseased) and other factors such as age or race [29, 30].

Table 1: Examples for penetration enhancement techniques

Chemical enhancement		**Physical enhancement**	
• Pro-drugs	• Complexes	• Iontophoresis	• Magnetophoresis
• Ion pairs	• Vesicles	• Electroporation	• Heat assisted delivery
• Chemical enhancers	- Liposomes - Transfersomes	• Microneedles	• Needle-free injection
• Supersaturation	- Niosomes - Ethosomes	• Acoustical methods	• Chemical skin abrasion
• Eutectic systems	- Solid lipid nanoparticles	- Ultrasound - Short-duration shock waves	

4.2. Delivery systems

Although the transdermal delivery of drugs has been the subject of research for more than 100 years [27] the active development of transdermal products began to gain pace only in the last 40 years. The first transdermal product that was approved by the FDA in the United States of America was a scopolamine patch against motion sickness in 1979. Since then various transdermal products have entered the market. The number of drugs that are administered transdermally, however, remains small due to the abovementioned difficulties that are associated with this application route.

The predominant dosage form for the systemic administration of drugs via the skin is the transdermal patch. However, in recent yeast the conventional patches have been joined by various semisolid formulations that aim to use the advantages of the transdermal application route while avoiding some of the disadvantages of the patches.

4.2.1. Transdermal patches

The group of transdermal patches can be divided into two subgroups, the matrix-type patches and the reservoir type patches.

In matrix type patches the drug is either dissolved or suspended in a hydrophilic or lipophilic polymeric matrix. This matrix either has good adhesive properties itself (drug-in-adhesive type) or is covered completely or partly with an additional adhesive layer to provide a reliable adhesion to the skin. The outside part of the drug containing matrix is covered with an impermeable, often occlusive backing layer to protect the delivery system and to prevent diffusion of the drug to the outer surface of the patch. The opposite side of the patch, which will be brought in contact with the skin, is covered with a thin, inert release liner which can be removed easily and without residue before the application of the patch. The structure of a matrix type transdermal patch is illustrated in Fig. 4.

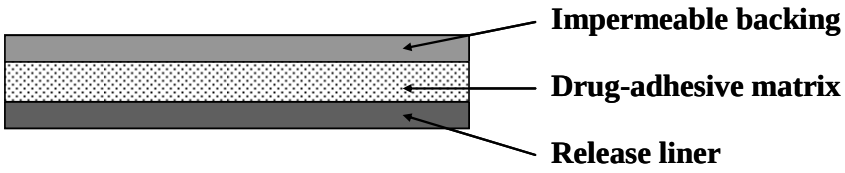

Fig. 4: Structure of a matrix, drug-in-adhesive type transdermal patch (not in original scale)

In reservoir type patches the drug is incorporated into a liquid or semisolid reservoir (for example in a hydrogel) that is located below an impermeable backing layer. The drug release from this reservoir is controlled by a non-porous or microporous membrane. If the membrane is non-adhesive an additional adhesive layer ensures a close contact between the patch system and the skin. Fig. 5 shows the structure of a reservoir-type patch.

The polymers that are used for the manufacturing of the transdermal patches are selected according to their function in the different patch layers. The backing layer can consist of natural or synthetic polymers (for example cotton tissue, polyester or polyethylene) and can contain an aluminium layer if a strong occlusive effect is desired.

The release liner can be manufactured from a thin, inert polyester foil, often covered with an additional layer (for example made of silicone) to allow a residue free removal of the transdermal system from the protective liner.

For the rate-controlling membrane in the reservoir-type systems materials such as poly(ethylenvinylacetate) or polyethylene are used [31].

The adhesive layer of the patch is a very crucial part as it is responsible for an undisturbed drug release by maintaining close contact of the drug loaded system to the skin. In case of the drug-in-adhesive systems it is also the main body of the patch and controls the drug release from the patch. The three polymer groups that are predominantly used for the manufacturing of this

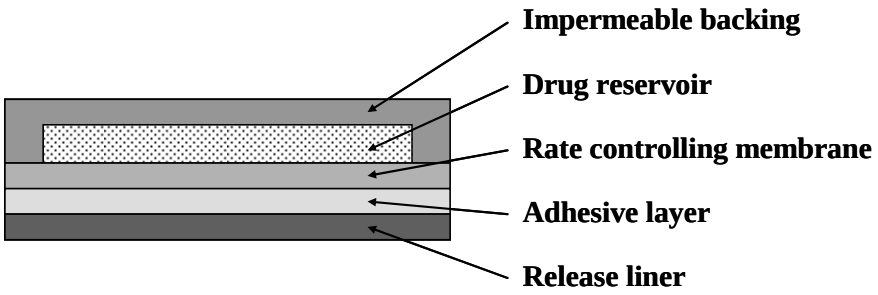

Fig. 5: Structure of a reservoir type transdermal patch (not in original scale)

layer (‘pressure sensitive adhesives’) are polyisobutylenes, polysiloxanes (silicones) and polyacrylate polymers [32]. The basic structures of these polymer groups are illustrated in Fig. 6. In addition to these basically hydrophobic polymers the usage of hydrophilic polymers such as polyvinylpyrrolidone/polyethylene glycol, aminoalkylmethacrylate copolymers or polyurethanes has been discussed [32].

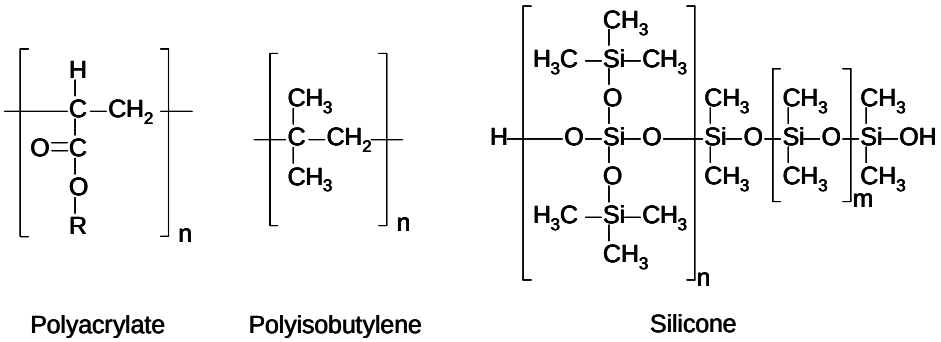

Fig. 6: Basic polymer structures of pressure sensitive adhesives according to Tan [32]

Apart from the polymers for the different patch layers further excipients can be incorporated into the patch for various purposes (for example to improve the drug solubility in the formulation, the drug permeation, the chemical or physical stability of the drug during storage or the adhesive properties of the adhesive layers).

Currently transdermal patches, either of the reservoir- or of the matrix type, are marketed only for a small number of drugs such as buprenorphine, clonidine, fentanyl, lidocaine, nicotine, nitroglycerine, estradiol, oxybutinin, scopolamine or testosterone [33, 34]. Recently patches with methylphenidate, selegiline or rotigotine have entered the market indicating that research continues to make use of this special application route. For the indications hormone replacement and contraception there are also patches available that supply a combination of two drugs such as ethinylestradiol/norelgestromin (EVRA®, Janssen-Cilag GmbH, Germany) or estradiol/levonorgestrel (Fem7® Combi, Solvay Arzneimittel GmbH, Germany). Table 2 shows some examples for commercially available transdermal patches.

Special attention in research is paid to the transdermal delivery of proteins or peptides with the help of novel enhancement techniques. Examples for drugs from this group are human growth hormone (microneedles, phase I), insulin or parathyroid hormone (thermal enhancement, phase I) [26].

Table 2: Examples for commercially available transdermal patches [33, 34]

Active ingredient	**Trade name**	**System type**	**Wearing time**	**Clinical indication**	**Patch size (cm^2)**
Buprenorphin	Transtec® PRO	Matrix	3 days	Analgesia	25 - 50
Clonidine	Catapres-TTS®	Reservoir	7 days	Hypertension	20
Estradiol	Estraderm®	Reservoir	3 - 4 days	HRT[1]	5 - 20
	Estradot®	Matrix	3 - 4 days	HRT[1]	2.5 - 10
	Fem7®	Matrix	7 days	HRT[1]	15 – 22.5
Fentanyl	Durogesic®	Reservoir	3 days	Analgesia	10.5 - 42
Lidocaine	Lidoderm®	Matrix	12 hours	Post-herpetic neuralgia	140
Methylphenidate	Daytrana®	Matrix	9 hours	ADHD[2]	12.5 – 37.5
Nicotine	Nicorette®	Matrix	1 day (16 hours)	Smoking cessation	10 - 30
	Nicotinell®	Reservoir	1 day	Smoking cessation	10 - 30
Nitroglycerin	Nitro-Dur®	Matrix	12 – 14 hours	Angina	5 - 40
Oxybutinin	KENTERA™	Matrix	3 - 4 days	Overactive bladder	39
Rotigotine	Neupro®	Matrix	1 day	Parkinsons disease	10 - 40
Selegiline	EMSAM®	Matrix	1 day	Depression	20 - 40
Scopolamin	Scopoderm® TTS	Matrix	3 days	Motion sickness	2.5
Testosterone	Androderm®	Reservoir	1 day	Hypogonadism	37 - 44
Estradiol + Norethindrone Acetate	Estragest TTS®	Reservoir	3 - 4 days	HRT[1]	10
Estradiol + Levonorgestrel	Fem7® Combi	Matrix	7 days	HRT[1]	15
Ethinylestradiol + Norelgestromin	EVRA®	Matrix	7 days	Contraception	20

[1] HRT = Hormone Replacement Therapy

[2] ADHD = Attention Deficit Hyperactivity Disorder

4.2.2. Transdermal semisolids

Traditionally, semisolid formulations were only used for the therapy of dermal diseases where the target of the drug was the skin itself and not the systemic circulation. However, due to the fact that semisolid formulations show certain advantages compared to transdermal patches and have proved that they can equally well deliver sufficient amounts of drug to the systemic circulation [29, 35, 36] these systems have been developed as an alternative to the traditional polymeric patches. Especially alcoholic hydrogels have gained acceptance in this field.
The materials used for these formulations depend on the formulation type (gels or cream). They range from different kinds of solvents (for example water, alcohols, glycols) over hydrophilic or hydrophobic polymers (acrylates, cellulose derivatives) and emulsifiers to fatty compounds (paraffins, oils, waxes).
Although considerable research is done in this field the number of commercially available products is limited. Transdermal semisolids are for example marketed with steroidal hormones for the indications hypogonadism (for example Testogel®, Jenapharm GmbH & Co. KG, Germany) or postmenopausal symptoms (Estreva® gel, Solvay Arzneimittel GmbH, Germany, or Cordes® Estriol Creme, APS GmbH, Germany).

4.2.3. Advantages and disadvantages of current systems

The transdermal administration of a sufficient dose is challenging and only feasible for a small number of drugs. Still the advantages of the transdermal application route in general are numerous [23, 25, 26]: with a transdermal application the gastrointestinal tract is avoided altogether. This is especially advantageous for drugs that are sensitive to degradation due to the pH conditions or enzymatic activity in the gastrointestinal tract. There is no impact on the reliability of the medication of the gastrointestinal activity such as gastric emptying or disorders (vomiting, diarrhoea). Mucosal irritation that can be caused by the drug or an excipient is also avoided. Moreover, the hepatic first-pass effect is omitted. In consequence the administered drug dose can be reduced and less metabolic degradation products occur in the systemic circulation which reduces the risk of side effects. The plasma levels achieved by the controlled delivery of the transdermal systems are fairly steady. Plasma level peaks or troughs, that are often observed after oral application, are minimized which also reduces the appearance of side effects. The assistance of the patient (consciousness, ability to swallow) is not necessarily required for the

application. Contrary to oral dosage forms the medication can be instantly removed in case of an emergency. Depending on the drug, however, a possible reservoir formation in the upper skin layers has to be kept in mind that might prolong the effect of the drug beyond the time of removal. Finally, the administration of the transdermal system is non-invasive, painless and convenient for the patient. These application conditions together with the often considerably reduced dosing frequency (up to once weekly) especially for drugs with a short half-life can positively influence the patient compliance.

Besides these positive aspects there are also limitations to this delivery route [25, 26]. Apart from the already discussed problem to overcome the efficient skin barrier the drugs can also suffer from degradation on their way to the systemic circulation due to the presence of metabolic enzymes in the skin. In addition to this, an inter- and intraindividual variability of the drug resorption has been described with respect to the location of the application site (differing thickness of the stratum corneum and skin hydration), the skin type (varying lipid compositions), enzymatic activity, age or health condition of the skin (healthy or diseased). Due to the relatively slow onset of the drug action the transdermal delivery is not suitable for indications where a rapid, bolus-type drug input is required. Finally, skin irritation and skin sensitisation are often responsible for the discontinuation of the treatment, although this problem occurs predominantly with transdermal patches and not with semisolids.

Except for the last point the abovementioned general advantages and disadvantages of the application route account for all transdermal delivery systems independent of their design. In addition to these, however, a few special pros and cons have to be mentioned that are associated with the different dosage forms.

Transdermal patches for example reach a high precision as to the dosing of the drug, but the dosing flexibility is usually limited for manufacturing reasons [23]. The often occlusive climate below the patches can be an advantage as it can promote the permeation of some drugs [37, 38]. On the other hand, it can also be a disadvantage as occlusion can be the source for skin irritation [39, 40]. For some drugs the low dosing and the considerable reservoir size of the polymeric patch can allow longer application intervals up to one week which can positively influence the patient compliance. However, a sufficient adhesion to the skin is not always provided by the contact layer of the patch. Cases of adhesion failure occur especially for the weekly patches [41]. The usage of strong adhesives, on the other hand, reduces this risk but can be problematic

with respect to skin irritation and residues on the skin that are difficult to remove by the patient and aesthetically unattractive. Although much work has been done to improve the appearance of the patches some are still criticized by patients for their high visibility and the resulting lack of discreetness [29]. An example for a highly visible patch is shown in Fig. 7. Finally it has to be pointed out that the production of polymeric patches requires specialized production equipment and generates considerable manufacturing costs.

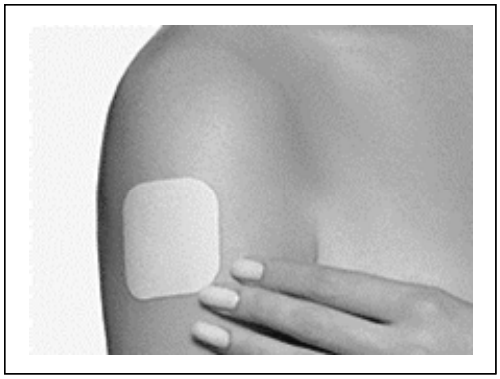

Fig. 7: Ortho-EVRA/EVRA® patch as shown on the internet [42]

In contrast to this the typical semisolid formulations can be manufactured with a fairly simple equipment. They offer a high dosing flexibility, which is an advantage in comparison to the patches, but they lack the dosing accuracy. The latter results from the fact that a correct dosing of the drug relies on the patients ability to distribute the fixed amount of formulation on a skin area of appropriate size. The application sites for the semisolids that are required to produce adequate dosing levels are considerable larger than the sizes of the transdermal patches (the Testim® gel (5 g) for example is distributed on one shoulder and arm [43]). Similar to the patch application the administration of the semisolids is simple for the patients. However, due to the fact that the administered semisolid is usually not completely absorbed a sticky or greasy residue can remain on the skin directly after application which can negatively influence the patient compliance. The incomplete absorption carries also the risk of environmental contamination [44]. Part of the drug that remains on the skin surface after application can be transferred to other persons or can contaminate the patients clothes which can raise safety concerns especially for highly potent drugs. The lack of permanence on the skin is also the reason why a sustained release is difficult to achieve with semisolid formulations in contrast to the patches.

5. Film forming pharmaceutical preparations

For this work film forming preparations are defined as non-solid dosage forms that produce a substantial film in situ after application on the skin or any other body surface. Such compositions can either be liquids or semisolids with a film forming polymer as basic material for the matrix. The formed film is sufficiently substantial to provide a sustained drug release to the skin.

In the past film forming preparations have been known predominantly from the field of surgery or wound care. Film forming solutions or gels have been used for example as tissue glues for the sealing of operative wounds [45]. The film formers mainly used in this area are fibrin as natural material and cyanoacrylates (octyl- and butylcyanoacrylate) as synthetic polymers [46-48]. Cyanoacrylates or recently acrylate polymers have also been used for the closure of superficial wounds as liquid bandages [49, 50]. Examples for commercially available products of this type are given in Table 3. While most film formers are incorporated into the formulations as already polymerised material the cyanoacrylates are often applied as monomers. The polymerisation of the monomers takes place in situ and is catalysed for example by the presence of water on the skin. The velocity of the polymerisation process has to be controlled thoroughly to avoid inconveniences for the patient as the process is exothermic [51, 52].
Wound care preparations can either be drug free or combined with antimicrobial drugs to reduce the risk of infections in the wounds [53-55].

Apart from the wound care film forming preparations are also administered in ostomy care to protect the skin surrounding the ostomy wound from the aggressive bodily fluids [56].

For dermal therapy a few liquid film forming products are approved, mainly for the therapy of warts and calluses. Examples are Verrumal® (Hermal oHG, Germany) or Clabin® Plus (Chefaro, Germany). Furthermore some film forming products for the therapy of nail mycoses are registered such as Loceryl® (Galderma GmbH, Germany) or Penlac® (Dermik Laboratories, USA).

Table 3: Examples for film forming wound care products

Trade name	**Manufacturer**	**Film forming polymer**
Dermabond®	Ethicon GmbH, Germany	Octylcyanoacrylate
EPIGLU® Gewebekleber	Meyer-Haake GmbH, Germany	Ethylcyanoacrylate, Poly(methylmethacrylate)
Flint® Sprühverband	Togal, Germany	Poly(butylmethacrylate, methylmethacrylate)
Band Aid® Sprühpflaster	Ethicon GmbH, Germany	Cellulose Acetate Butanoate
Opsite® Spray	Smith&Nephew GmbH, Austria	Poly(methylacrylate)

The film forming systems that have been described so far are used in the pharmaceutical field but are not designed for the transdermal administration of pharmaceutically active substances. Only very few preparations that aim at a sustained delivery over a longer period of time have been described in the literature.
Misra et al. have investigated a film forming solution with a mixture of polyvinyl pyrrolidone and polyvinyl alcohol as film former in isopropanol for the transdermal delivery of testosterone [57, 58]. The group reported that the film forming solution provided a sustained release of the steroidal hormone with a biphasic pharmacokinetic profile. So far, no liquid film forming preparation has been approved for transdermal drug delivery.

Bryan et al. reported on a film forming composition in the form of a cream for the local delivery of a eutectic mixture of Lidocaine and Tetracain for pre-surgical anaesthesia [59]. The cream was left to dry on the skin for at least 20 minutes, after which it was removed by peeling. During this time the formulation had delivered a sufficient amount of the local anaesthetics through the skin to provide a significant pain reduction for the patient during the following laser treatment. The product has recently been approved by the FDA (S-Caine™ Peel, Zars Inc., Salt Lake City, USA).

Another film forming semisolid preparation was described by An et al. [60]. This group investigated a transdermal hydrogel on the basis of polyvinyl alcohol and polyisobutylene that solidified into a substantial film in situ on the skin. The formed film was able to provide a

sustained release of testosterone over 24 hours. Due to its cohesive structure the formed film was removable by peeling.

The fact that the preparation investigated by An et al. produced a substantial and robust film on the skin, which is the prerequisite for a sustained drug release, distinguishes it from other transdermal gels. In these gels the main purpose of the gelling agents, that can be film forming polymers as well, is not to form films but to increase the viscosity by establishing a gel structure in the preparation. Due to this the gelling agents are not selected for their film forming ability and are often used in low concentrations so that the resulting films (if formed at all) are rather weak and show little persistence on the skin. Therefore most transdermal gels cannot provide a sustained release to the skin and can thus not be considered film forming preparations in the sense of this work.

Apart from the preparations that form a polymeric film on the skin for the transdermal drug delivery there are also other, mostly liquid formulations that are worth mentioning in this context. These are solutions of drugs, with or without enhancers, in volatile solvents that are typically sprayed onto the skin where they form liquid films. Due to the fact that the formulations do not contain a film forming polymer these films vanish shortly after application with the evaporation of the solvent, leaving a drug loaded residue or re-crystallized drug behind. Examples for solutions of this type have been investigated for example by Leichtnam et al. with testosterone [61, 62], Tucker et al. with lidocaine [63] or Morgan et al. with steroidal hormones [64-66]. Based on the findings of Morgan et al. products with a metered dose transdermal technology are being developed by Acrux Ltd., Melbourne, Australia, for several indication with the drugs estradiol, testosterone, Nestorone®, fentanyl and buspirone [67].

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CHAPTER 2

Development and characterization of film forming polymeric solutions for skin drug delivery

1. Abstract

Film forming polymeric solutions as a novel approach for skin drug delivery were developed and characterized concerning their mechanical properties and water vapor permeability. They were developed by varying type and content of the film forming polymer as well as nature and content of the plasticizer. The resulting formulations were evaluated according to five criteria: drying time, cosmetic attractiveness, outward stickiness, integrity on skin (after 18 hours) and viscosity. Among the 14 tested polymers ten film formers yielded formulations with a positive evaluation in all five test criteria. Selected formulations were then investigated for tensile strength and elongation at break in vitro and for water vapor permeability in vitro (WVP) and in vivo (TEWL). Their mechanical properties determined in vitro were found to be not predictive for the flexibility and abrasion resistance observed on living skin. Similar to this, the results derived from the WVP and the TEWL methods were not in accordance with each other. Obviously, the investigated in vitro methods do not characterize the properties of the thin films on living skin satisfactorily. Nevertheless, the identified film forming solutions are a promising approach and will provide the basis for the further development of this novel dosage form.

Keywords:
Film forming polymeric solution; Mechanical Properties; Water Vapor Permeability; Transepidermal Water Loss

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2. Introduction

The skin is a very important route for the dermal or transdermal delivery of pharmaceutically active substances. Film forming polymeric solutions are a novel approach in this area that might present an alternative to the conventional dosage forms used on the skin, such as ointments, creams, gels or patches. The polymeric solution is applied to the skin as a liquid and forms an almost invisible film in situ by solvent evaporation.

So far only very few authors have described the use of film forming systems for the delivery of drugs to the skin. Misra et al. [1, 2] reported on a liquid film forming solution for the biphasic delivery of testosterone but investigated only one formulation containing a mixture of polyvinylpyrrolidone and polyvinyl alcohol in isopropanol as film forming matrix without performing a polymer screening. Also, Misra did not investigate the mechanical or cosmetic properties of the formed film but focused mainly on the drug permeation from this system in vitro as well as in vivo. Other film forming systems described in the literature are not applied as liquids but as transdermal gels [3, 4] or cream [5]. Similar to the works of Misra these groups investigated only one individual formulation without testing a broader range of film formers and focused also mainly on the drug delivery from the film forming system [3, 4] or the clinical efficacy of the formulation [5].

Due to the fact that film forming solutions can provide many advantages over patches (higher dosing flexibility, higher patient compliance due to improved cosmetic appearance) or semisolid preparations (rub off resistance) the aim of this study was to test a wider range of materials, to select suitable excipients and to characterize the properties of the resulting formulations to provide a broader technological basis for the development of this novel dosage form.
In a first step formulation experiments were performed with 14 polymers from different chemical classes. Basically the compositions contained a film forming polymer dissolved in a volatile solvent. Further excipients such as plasticizers or crosslinkers were added if necessary. Mainly polymer content, type of plasticizer and plasticizer content were varied to find the best composition for the desired purpose.

Since no suitable evaluation method for these new application systems was available from the literature a simple score system had to be developed in order to identify suitable formulations for the intended application. The testing of the formulations was performed in vivo as pre-experiments had shown that the special properties of the skin (surface structure and movement) were very important for the differentiation between the formulation variants. The evaluation system was based on five criteria: viscosity, drying time, outward stickiness, cosmetic attractiveness and integrity after a certain wearing time (18 hours). These properties were considered key features for the practical application of the novel dosage form especially from the patients' point of view: The viscosity of the film forming solution is required to be low to enable an application of the dosage form as spray, which would ensure an accurate, but at the same time flexible dosing and would be most convenient for the patient. In order to avoid long waiting times for the patient the novel dosage form is supposed to dry quickly on the skin. The formed film is required to be non-sticky to avoid adhesion to the clothes of the patient. Considering the fact that many patients complain about the high visibility of transdermal patches which is considered cosmetically unattractive the formed film is supposed to be almost invisible. In addition to this, the delivery system is required to show a certain permanence on the skin in order to be able to provide a continuous drug supply over a prolonged period of time.

Following the polymer screening selected formulations with polymers from different chemical groups were characterized concerning their water vapour permeability and mechanical properties. Based on the observation that the developed formulations had displayed similar mechanical properties, that is flexibility and abrasion resistance, during the in vivo evaluation the assumption was that they would also show similar mechanical properties in the in vitro test method. If this was the case the in vitro method could serve as a useful instrument in further polymer screening experiments for a more objective evaluation of the developed formulations. The mechanical properties (tensile strength and elongation at break) were determined according to a standard test method [6] that has been frequently used in the literature for the characterisation of strength and flexibility of free polymeric films [7-11]. In addition to the mechanical properties, the water vapor permeability of the same four formulations was investigated in vitro according to a method from the British Pharmacopoeia [12] and in vivo by measuring the impairment of the transepidermal water loss to assess the occlusive properties of the formed films. Finally, characterization methods for the film forming solutions and important parameters for the development of this novel dosage form are discussed.

3. Materials and Methods

3.1. Materials

All polymers (Table 4, Appendix 1) were kindly provided by the manufacturers: Eudragit® RL PO, Eudragit® E 100, Eudragit® S 100 and Eudragit® NE 40D (Roehm Pharma Polymers, Darmstadt, Germany), Avalure® AC 118 (Noveon Inc., Cleveland, USA), SGM 36 and Dow Corning® Q7-9180 (Dow Corning S.A., Seneffe, Belgium), DynamX® and Dermacryl® 79 (National Starch and Chemical Company, Bridgewater, USA), Oppanol® B 100, Oppanol® 10SFN, Kollidon® 12 PF and Kollidon® VA 64 (BASF, Ludwigshafen, Germany), Hydagen® HCMF (Cognis, Düsseldorf, Germany), EC® NF1 and Klucel® LF (Hercules Inc., Wilmington, USA). Polyethylene imine 1800 was a gift from Alfa Aesar GmbH&Co KG, Karlsruhe, Germany. Polyvinyl alcohol 72000, ethanol (96 %), butyl acetate, isopropanol, aceton, polyethylene glycol 400, triethyl citrate, dibutyl phthalate, triacetin, succinic acid, lactic acid, glycerol and polysorbat 80 were purchased from Merck, Darmstadt, Germany.

3.2. Preparation of the polymeric solutions

Film forming solutions were prepared by adding the polymer to the solvent and stirring the solution overnight to ensure complete dissolution of the polymer. The solvent used was Ethanol (96 %) for all preparations except the silicone formulation. For the silicone formulation the silicon gum (SGM 36) was dissolved in a volatile silicone (Dow Corning® Q7-9180, hexamethyldisiloxane/octamethyltrisiloxane). Having obtained a clear polymeric solution other optional excipients such as crosslinker or plasticizer were added. After addition of all excipients the solution was stirred for another 24 hours before use. The formulations were stored in glass vials sealed tightly with a siliconized rubber plug and an aluminium cap.

Table 4: Polymers used in the formulation experiments

Trade name	Polymer
Avalure® AC 118	Acrylates copolymer
Dermacryl® 79	Acrylate/octylacrylamide copolymer
DynamX®	Polyurethane-14 and AMP-acrylates copolymer
Eudragit® E 100	Poly(butyl methacrylate, (2-dimethylaminoethyl)methacrylate, methyl methacrylate) 1:2:1
Eudragit® NE 40D	Poly(ethyl acrylate, methyl methacrylate) 2:1
Eudragit® RL PO	Poly(ethyl acrylate, (2-trimethylaminoethyl)methacrylate, methyl methacrylate) 1:0.2:2 chloride
Eudragit® S 100	Poly(methacrylic acid, methyl methacrylate) 1:2
Hydagen® HCMF	Chitosan
Kollidon® 12 PF	Polyvinylpyrrolidone
Kollidon® VA 64	Polyvinylpyrrolidone-vinyl acetate copolymer
Klucel® LF	Hydroxypropylcellulose
Oppanol® B100 / 10SFN	Polyisobutylene
PVA 7200	Polyvinyl alcohol
SGM 36	Silicon gum

EC® NF1 (ethylcellulose) and polyethylene imine 1800 are not listed here as they were not used as main film forming compounds in the formulations.

3.3. Evaluation of the formulations

For a first assessment of the suitability of film forming solutions, the obtained formulations were evaluated according to a rating system for five characteristics: viscosity, drying time, stickiness of the outer surface, cosmetic attractiveness and integrity on the skin after 18 hours (Table 5).

The viscosity of the solution was evaluated visually and rated as low (water-like), medium (glycerol-like) or high (syrup-like).

Table 5: Rating system for the evaluation of the film forming polymeric solutions

Rating score	1	2	3
Viscosity	low	medium	high
Drying time	< 5 min	5-7 min	> 7 min
Outward stickiness	low	medium	high
Cosmetic attractiveness	high	medium	low
Integrity on skin (after 18 hours)	complete film, no cracks, no flaking	complete film with cracks or sporadic flaking	film partly or completely missing

For the assessment of the drying time the formulation was applied to the inner sides of the forearm of a volunteer, who participated in the study on informed consent basis, with the help of a steel positioning device and a pipette. The applied volume was 10 µl/cm^2 as a pre-experiment had shown that this amount was small enough to be applicable without flowing away from the application site. A dosing range from 5 – 10 µl/cm^2 is also recommended by the OECD for the conduct of skin absorption studies [13]. After 5 minutes a glass slide was placed on the film without pressure. If no remains of liquid were visible on the glass slide after removal, the film was considered dry. If remains of liquid were visible on the glass slide the experiment was repeated with a drying time of 7 minutes instead of 5 minutes.

The stickiness of the outer surface was tested by pressing cotton wool on the dry film under low pressure. Depending on the quantity of cotton fibers that were retained by the film the stickiness was rated high (dense accumulation of fibers on the film), medium (thin fiber layer on the film) or low (occasional or no adherence of fibers).

The cosmetic attractiveness of the films was assessed by visual examination of the dry films. Transparent films with a low skin fixation had a high attractiveness as they were almost invisible. Opaque films and films with a medium skin fixation were considered less attractive as they exhibited an increased visibility and a slight wrinkling of the skin. Whitish films and films causing heavy wrinkling of the skin due to strong skin fixation displayed only a low attractiveness.

To test the integrity on skin the formulation was applied to the forearm of a volunteer as described for the assessment of the drying time. The dry film was then worn overnight by the test subject. After 18 hours the test area was examined visually with the help of a magnifying glass (magnification 10x) for completeness of the film, appearance of cracks or flaking.

Three rating scores were assigned to each criterion with 1 representing the most positive evaluation (meaning that the film characteristic closely matched the target) and 3 the most negative result. Formulations were considered successful when all five criteria were rated 1. These formulations showed a low viscosity, short drying time, low outward stickiness, high cosmetic attractiveness and stayed intact on the skin for a prolonged time. For these successful formulations the evaluation on skin was repeated on two further volunteers to support the

positive findings. Formulations with one or more criteria rated 2 were considered acceptable with limitations, formulations with one or more criteria rated 3 were not acceptable.

3.4. Determination of the mechanical properties

For the determination of the mechanical properties polymeric films were produced by solvent evaporation in a teflon mould (6 cm x 10 cm). Into this mould 15 ml of the polymeric solution were cast and left to dry at room temperature for 72 hours (24 hours ventilated in the open air for the evaporation of Ethanol, then in an exsiccator containing orange gel as desiccant). The dry films were cut into rectangular samples of 10 mm x 40 mm with the help of a scalpel. Film thickness was measured at 10 places with a digital micrometer (Mitutuyo, Kawasaki, Japan). The mechanical properties of the films were determined with a tensile tester (UPM Z010, Zwick/Roell, Ulm, Germany) based on the ASTM D882-02 [6] with a modification of the sample size. The testing device was equipped with a 20N load sensor. The films were carefully placed between the two vertical grips of the tester that were covered with a silicon gum to prevent slippage of the films during the test. The movable grip was then driven upward with a speed of 500 mm/min until the rupture of the film. From the recorded load-time profiles tensile strength (σ) and percent elongation at break (ε) were calculated representing abrasion resistance and flexibility, respectively. The tensile strength (σ) was calculated as

$$\sigma = \frac{F_{\max}}{A} \qquad \left[\frac{N}{m^2}\right] \qquad \text{(Eq. 1)}$$

where F_{max} [N] is the maximum force and A [m^2] is the crossectional area. The values for percent elongation at break were calculated with the following equation:

$$\varepsilon = \frac{L_R}{L_0} * 100 \qquad [\%] \qquad \text{(Eq. 2)}$$

where L_R [m] is the extension of the sample in the moment of rupture and L_0 [m] is the original sample length. Each experiment was repeated five times.

3.5. Investigation of the water vapor permeability

The water vapor permeability (WVP) was investigated according to a method modified from the British Pharmacopoeia [12]. Films were produced with a solvent evaporation technique by pouring 3 ml of the preparations (50 µl/cm^2) into a teflon mould (6 cm x 10 cm) on a polycarbonate filter (IsoporeTM Membrane Filters, Millipore, Billerica, USA filter; pore size 0.2 µm, thickness 11 µm,) as supporting membrane. The films were left to dry for 72 hours at room temperature (three hours ventilated in the open air to allow the evaporation of Ethanol, afterwards in an exsiccator containing orange gel as desiccant). Circular samples with a diameter of 2.0 cm were cut from the dry film sheets with the help of a scalpel. For the sample preparation 10 ml glass vials with an opening of 1.2 cm diameter (A = 1.13 cm^2) were filled with approximately 8 g of distilled water, covered with the circular film samples and a silicone ring and sealed tightly with an aluminium vial cap. To start the experiment, the top of the vial cap was opened and the weight of the vial was determined with an analytical scale (Sartorius, type MC BA 100, Göttingen, Germany). The vials (six replicates per formulation) were then placed into an exsiccator containing either a desiccant to create a climate of low relative humidity (approximately 0%) or a saturated solution of sodium bromide (Merck, Darmstadt, Germany) creating an atmosphere of 58% relative humidity [14, 15]. They were kept at a determined temperature (25°C, 32°C or 37°C) for 72 hours and weighted after predetermined intervals after having adjusted to room temperature for one hour. From the weight loss of the vials W [g] the WVP was calculated as the amount of water that had permeated through the film in relation to the surface area A [cm^2] and the time t [24 hours]:

$$WVP = \frac{W}{A * t} \quad \left[\frac{g}{cm^2 * 24h} \right] \qquad \text{(Eq. 3)}$$

The WVP ratio shows the relation of the WVP of the vials covered by the tested film to the WVP of the vials with unlimited permeability (filter only samples):

$$WVP\,ratio = \frac{WVP\left(filter + polymeric\ film\right)}{WVP\left(filter\right)} \qquad \text{(Eq. 4)}$$

For each formulation the mean value and the standard deviation were calculated. Controls for this experiment were vials with the supporting filter without polymeric film (representing 100 % WVP) and vials covered with aluminium discs (thickness 20 µm) to verify the tightness of the seal.

3.6. Transepidermal water loss measurement

Twelve healthy volunteers (seven males, five females) participated in the study on informed consent basis. The subjects were aged between 25 – 39 with a mean age of 29.7 years. None of the subjects had any dermatological diseases in their history. Before the experiment the subjects were asked not to use any skin care products for at least 12 hours before the test. Temperature and humidity in the laboratory were monitored throughout the experiment and showed little variation (temperature 21.3°C ± 0.5°C, relative humidity 54.3 % ± 4.3%).

For the determination of the TEWL the ventral sides of both forearms were chosen as test locations as they provide a fairly even surface with only little hair-growth, which might otherwise disturb the measurements, especially on male volunteers. On each arm two test areas (2 cm x 2 cm, minimum distance between the test fields 2 cm) were limited by applying a silicon paste (windowcolor, simplicol, Brauns-Heitmann GmbH&CoKG, Warburg, Germany) to the borders. The silicon paste was left to dry for 15 minutes. The area between the two test areas on each arm remained uncovered and served as a reference value for the TEWL measured on the test sites. The test subjects were allowed to acclimatize and calm down for 30 minutes before the start of the experiment. A volume of 200 µl of each formulation (50 µl/cm^2, corresponding to the amount applied for the in vitro experiment) were applied to one of the test fields. This amount was higher than the amount applied for the formulation screening experiments under the assumption that the differences in the TEWL impairment caused by the different films would be more pronounced with thicker films and therefore better detectable in spite of the considerable variations associated with this test method. The formulations were left to dry ventilated in the open air at room temperature for two hours.

The TEWL was measured according to published guidelines [16] with a Tewameter (Tewameter 300, Courage + Khazaka, Cologne, Germany) on the test sites and on the reference sites located close to the test sites. The TEWL ratio was calculated from the TEWL on the test sites after two hours drying time in relation to the TEWL measured on the uncovered reference sites:

$$TEWL\ ratio = \frac{TEWL\ on\ dry\ film}{TEWL\ without\ film} \qquad \text{(Eq. 5)}$$

From the ratios for each individual subject the overall average ratio, standard deviation and confidence intervals (P<0,05, two sided) were calculated.

4. Results

4.1. Formulation experiments

A selection of 14 polymers from different chemical groups, all described by their manufacturer or in the literature as good film formers were tested in the formulation experiments. With these polymers over 150 formulations were manufactured containing basically one of the polymers, a plasticizer and a volatile solvent. Mainly polymer content, type of plasticizer and plasticizer content were varied for every one of the chosen polymers to determine the composition with the highest scores in the evaluation system. The evaluation of these features was performed in vivo as casting the formulations on an artificial surface such as a glass slide did not offer the possibility for a realistic assessment of the film properties. The stress exerted on the formed film by the movement of the skin is one of the key challenges for the flexibility and the adhesive properties of the film and is difficult to imitate on artificial substrates. Also the cosmetic attractiveness can be judged more realistically on skin as an increased skin fixation and wrinkling often becomes more apparent with the movement of the skin. Table 6 shows the formulations that produced the best scores in the rating system as they were rated 1 in all categories.

Table 6: Composition of the positively evaluated formulations; concentrations in % [w/w]

Formulation		**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Polymer		Avalure® AC 118	Dermacryl® 79	DynamX®	Eudragit® E 100	Eudragit® NE 40D	Eudragit® RL PO	Eudragit® S 100	Kollidon® VA 64	Klucel® LF	SGM 36
		10.0	7.0	10.0	10.0	7.0	20.0	5.0	10.0	5.0	10.0
Plasticizer	Triethyl citrate			1.0	1.0		6.0	1.6		1.0	
	Triacetin		2.1								
	Dibutyl phthalate								4.0		
Solvent	Ethanol	75.0	90.9	72.2	88.1	82.5	74.0	93.4	86.0	94.0	
	Water	15.0		16.8		10.5					
	Q7-9180										90.0
Other Ingredients	Succinic acid				0.9						

One of the positively evaluated films applied on the skin of a human forearm is shown in Fig. 8. All formulations could either be removed by ethanol wipe or could be washed off with water and gentle rubbing.
The detailed results for all tested formulations are given in Appendix 2.

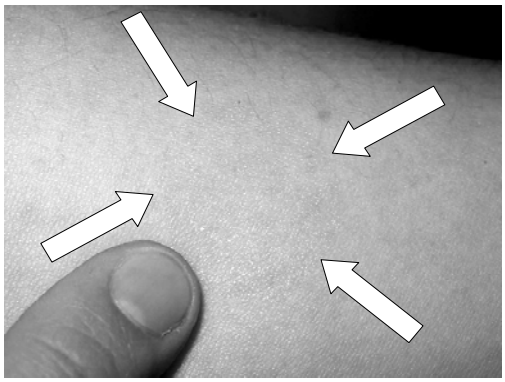

Fig. 8: Polymeric film on human forearm (Formulation F, 10mg/cm^2)

4.2. Mechanical properties

Mechanical properties such as tensile strength and percent elongation at break are determined to characterize polymeric films for their abrasion resistance and flexibility, respectively. Deducted from these two values polymeric films can be classified as shown in Table 7. This classification, however, is not based on absolute values for the two parameters tensile strength and elongation but has to be seen as a relative comparison between different polymeric films. Hard and tough films have properties suited best for the intended application as drug delivery systems for the skin: they are flexible enough to follow the movements of the skin without breaking but at the same time they show an increased strength to prevent abrasion of the film caused for example by contact with clothing.

Table 7: Classification of polymeric films according to Aulton et al. [17]

Tensile strength	Elongation at break	Film description
Low	Low	Soft and weak
Low	High	Soft and tough
High	Low	Hard and brittle
High	High	Hard and tough

To investigate if these features can be determined by an in vitro method three of the positively evaluated formulations (C, F and I, Table 6) with the polymers Eudragit® RL PO, DynamX®, Klucel® LF were tested for their mechanical properties. The SGM 36 formulation could not be tested as it does not form cohesive films. All these formulations had scored the highest rating in the evaluation criterion "integrity on the skin (after 18 hours)" indicating that they contained sufficient strength and flexibility not to crack or to be rubbed off during the wearing period. Based on this observation it was expected that all three formulations formed films with similar mechanical properties, most probably films classifiable as hard and tough.

Table 8: Mechanical properties of different polymeric films; mean values ± standard deviation
evaluation criterion: integrity on skin after 18 hours
rating 1: complete film, no cracks, no flaking
rating 2-3: film partly or completely missing, cracks, flaking

Formulation	Polymer	Tensile strength [N/mm^2]	Elongation at break [%]
Films with sufficient strength and flexibility in vivo (rating 1)			
C	DynamX®	12,2 (± 1,0)	323,4 (± 42,1)
F	Eudragit® RL PO	1,0 (± 0,1)	798,4 (± 93,9)
I	Klucel® LF	5,0 (± 0,3)	131,4 (± 5,8)
Films with non-sufficient strength and flexibility in vivo (rating 2-3)			
F (var1) (F with less plasticizer)	Eudragit® RL PO	1,3 (± 0,1)	662,0 (± 83,7)
F (var2) (F with different plasticizer)	Eudragit® RL PO	1,3 (± 0,1)	515,2 (± 19,8)
I (var1) (I without plasticizer)	Klucel® LF	10,7 (± 0,8)	107,2 (± 1,2)

The upper half of Table 8 shows the results for tensile strength and percent elongation at break for the three tested films (C, F and I). Surprisingly, the results for the three films revealed considerable differences. While the Eudragit® RL PO film showed a high elongation with a low tensile strength (rather soft and tough), the Klucel® LF film displayed a low elongation with medium tensile strength (fairly soft and weak in comparison to the Eudragit® RL PO film). Only the third film, the DynamX® formulation, could be classified as hard due to its comparatively high tensile strength. Concerning the elongation this film was weaker than the Eudragit® RL PO

film but tougher than the Klucel® LF film. The similar strength and flexibility of these three films observed on living skin was apparently not reflected in the results of the in vitro experiments.

For a better interpretation of these results the mechanical properties of three additional films were determined. The formulations F (var1), F (var2) and I (var1) were variations of the positively evaluated films F and I with changes in the plasticizer type or content. Contrary to the previously tested formulations these films had not displayed a sufficient strength or flexibility on the skin as they had cracked or flaked off during the integrity test (rating 2 or 3). The change in the plasticizer type or content resulted in harder and less flexible films as indicated by an increase in tensile strength and a decrease in the elongation values in comparison to the formerly tested films F and I with the same polymers (the results are shown in the lower half of Table 8). This might explain why F (var1), F (var2) and I (var1) had cracked up while the original formulations F and I had not displayed any cracks during the wearing period. Unexpectedly, however, the non-successful formulations F (var1) and F (var2) with the polymer Eudragit® RL PO displayed higher elongation values than the successful formulations I and C with other polymers. This implies that even though the elongation value is an indicator for the flexibility of a polymeric film it cannot serve to predict if a formulation will show the desired film properties in vivo when formulations with different polymers are concerned.

4.3. Water vapor permeability

The human body is constantly losing water to the environment by evaporation through the skin. This transepidermal water loss (TEWL) is a passive diffusion process and very important for skin functions such as body temperature control. Occlusion – meaning impairment of the TEWL – influences several properties of the skin such as hydration of the stratum corneum, skin temperature and blood flow and can therewith increase the percutaneous absorption of certain drug substances depending on the anatomic site and the drug vehicle [18-21]. Various skin parameters such as pH and bacterial flora are also influenced by an occlusive treatment resulting in an increased risk of infection and skin irritation [22, 23]. Accordingly, the degree of occlusion is an important feature of a drug delivery system that is supposed to be worn on the skin for a prolonged period of time.

Fig. 9 shows the absolute water vapor permeability (WVP) of the four tested films and the WVP for the control representing unhindered water vapor permeation. According to the British Pharmacopoeia a material can be considered permeable to water vapor when the WVP exceeds 0,05 $g*cm^{-2}*24h^{-1}$ [12]. Although the four films displayed different WVP values all of them showed a permeability above the limit set in the Pharmacopoeia and can therefore be considered non-occlusive.

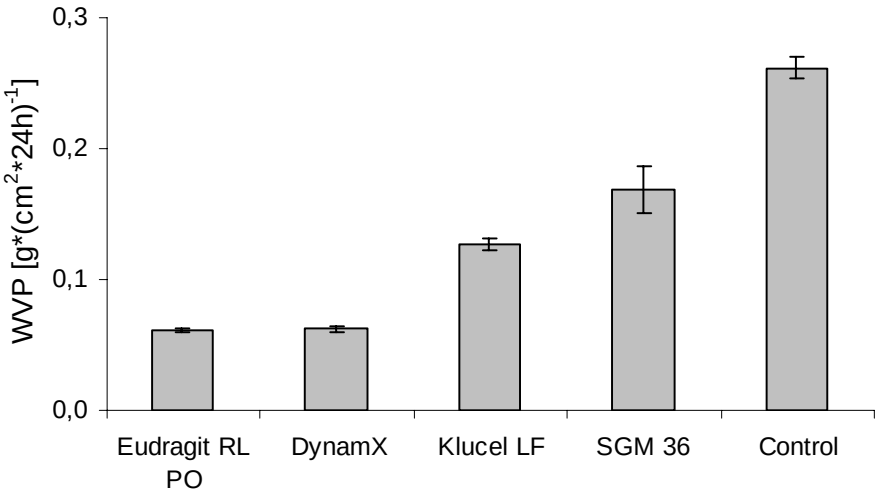

Fig. 9 WVP of the formulations F (polymer: Eudragit® RL PO), C (DynamX®), I (Klucel® LF), J (SGM 36) and control (without polymeric film); test conditions: 37°C, 0% r.h.; mean values ± standard deviation; n = 6

These in vitro results, however, could not be compared directly to the results of the TEWL measurements with the same films in vivo as the test conditions (37°C, 0% r.h.) differed from the conditions in the in vivo experiment. The skin temperature for example is considerably lower than 37°C. In the literature the skin surface temperature is reported to be between 28°C and 32°C [16], a range that our own measurements supported. As the temperature severely influences not only the TEWL measurements [24, 25] but also the WVP properties of polymeric films [26] the test conditions for the in vitro test were modified, investigating the WVP at different test temperatures. Fig. 10 shows the WVP for the four tested films at 25°C, 32°C and 37°C in a climate of low relative humidity (approximately 0% r.h.). The absolute WVP values increased with rising temperatures for all tested films. This was expected as higher temperatures increase evaporation and lead to a higher water vapor pressure driving more water through the films.

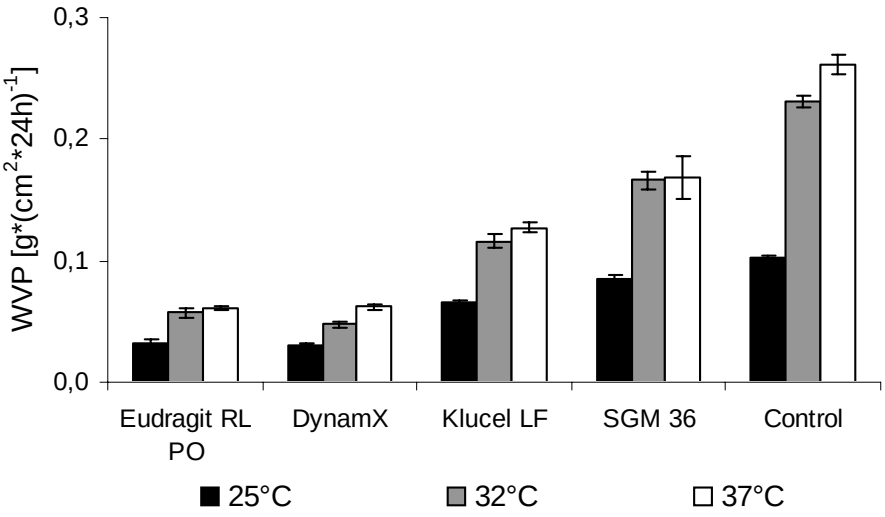

Fig. 10: WVP of the formulations F (polymer: Eudragit®RL PO), C (DynamX®), I (Klucel® LF), J (SGM 36) and control (without polymeric film); test conditions: 25°C, 32°C or 37°C, 0% r.h.; mean values ± standard deviation; n = 6

The second condition to be modified to match the test conditions of the TEWL measurements more closely was the humidity gradient. During the TEWL experiment the climatic conditions in the test chamber were measured and the relative humidity was found to be 54,3% ± 4,3%. Assuming a relative humidity of 100% on the other side of the tested film (that is inside the body of the test subject) the resulting water vapor gradient is much lower than the gradient used for the in vitro test (100% r.h. inside the vials and approximately 0% r.h. on the outside). Therefore the

in vitro experiment was repeated under modified humidity conditions by using a saturated solution of Sodium bromide instead of the desiccant to create a climate of approximately 58% relative humidity. Fig. 11 shows the WVP values for the four tested films and the control for both tested humidity degrees at 37°C. As expected the WVP values dropped with a higher ambient humidity as the humidity gradient is the strongest driving factor for water vapor permeation [27].

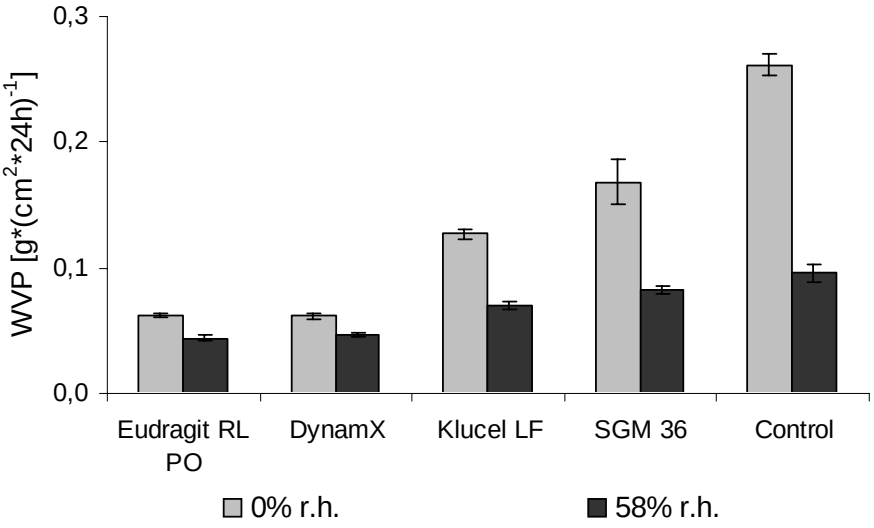

Fig. 11: WVP of the formulations F (polymer: Eudragit® RL PO), C (DynamX®), I (Klucel® LF), J (SGM 36) and control (without polymeric film); test conditions: 37°C, 0% r.h. or 58% r.h.; mean value ± standard deviation; n = 6

To compare the WVP values determined in vitro with the TEWL values in vivo the results from an experiment performed at 32°C and 58% r.h. were chosen as these test conditions came closest to the test conditions of the TEWL experiment.

4.4. Transepidermal water loss

TEWL measurements are a well-established method for characterizing the influence of chemical substances on the barrier function of the skin. An increase in TEWL usually indicates the disturbance of this protective barrier either by physical trauma, chemical treatment or occlusion which often results in skin irritation. Therefore TEWL measurements are often conducted to characterize the occlusive properties of pharmaceutical preparations such as transdermal patches [28].

To investigate if the WVP values determined in vitro are predictive for the occlusivity of the polymeric films in vivo the impairment of the natural TEWL by the films was measured. From literature many factors are known that can influence TEWL measurements. These factors are either instrument-related, environmental-related or individual-related [16]. Among these factors the high inter-individual variations seen in TEWL values on untreated skin play an important role [29]. Due to these high inter-individual variations comparisons between absolute TEWL values measured on different test subjects are highly problematic. Therefore we decided to compare TEWL ratios instead of absolute values and to compare them with the in vitro WVP results also calculated as WVP ratios (Eq. 4).

Fig. 12 shows the ratios of the in vitro (WVP) and the in vivo (TEWL) experiments. Clearly a close correlation between the results from the different methods could not be established. While both values for the Klucel® LF formulation correlated closely and the ratios for the SGM 36 formulation were fairly similar, the results for the Eudragit® RL PO and the DynamX® formulations differed widely. Nevertheless the in vivo results support the finding that all tested films are non-occlusive on the skin. This can be concluded from the high TEWL ratios (> 0,7) for all films indicating that more than 70% of the water vapor given off by the skin can permeate through the films.

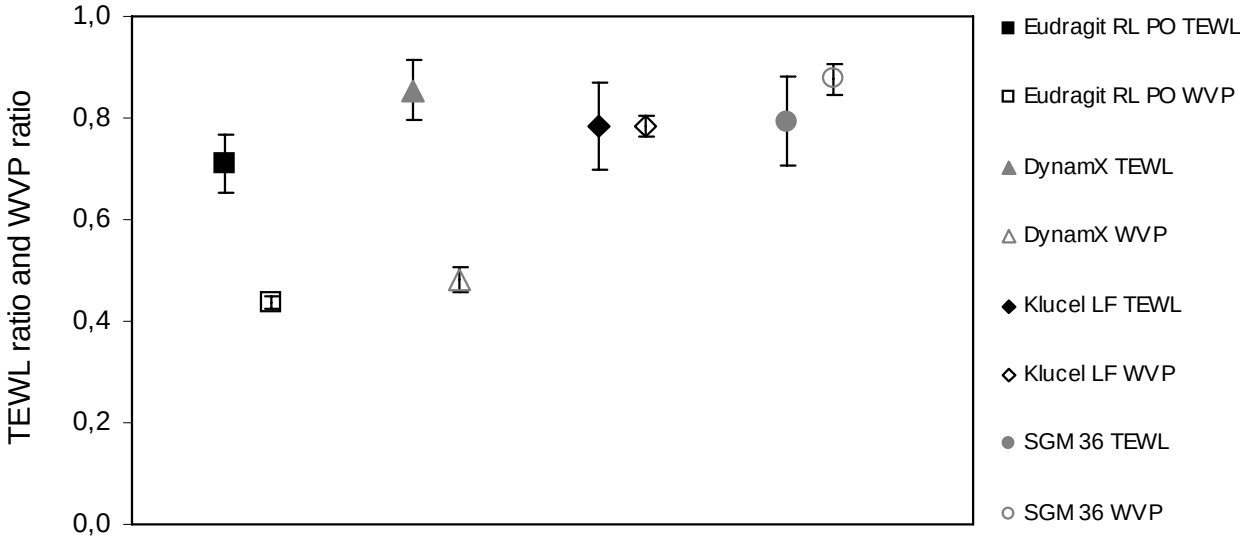

Fig. 12: Comparison of WVP ratio (open symbols) and TEWL ratio (closed symbols) of the formulations F (polymer: Eudragit® RL PO), C (DynamX®), I (Klucel® LF) and J (SGM 36); mean values with confidence intervals (P<0,05); n = 6 (WVP), n = 12 (TEWL)

5. Discussion

5.1. Important formulation parameters for film forming polymeric solutions

For the formulation experiments preparations were manufactured with 14 different polymers varying polymer content as well as nature and content of the plasticizer for every one of the chosen polymers. All these parameters have an impact on the properties of the resulting film and should therefore be considered with care. The first and most important parameter for the development of a film forming polymeric solution is the choice of polymer. Suitable excipients are polymers that form clear, flexible films at moderate temperatures. This is required as the polymeric film is formed *in situ* on the skin which has a surface temperature of approximately 28°C to 32°C. Furthermore the polymer has to be soluble in a skin-tolerant, highly volatile solvent such as ethanol, isopropanol or ethyl acetate. Film forming polymers requiring a high percentage of water in the solvent are not suitable for the formulation of film forming solutions due to the comparatively low volatility of water that results in prolonged drying times.

Besides the type of film former the polymer content is another crucial point in the formulation process. While the loading capacity for drug substances increases with rising polymer content in the solution due to the increasing thickness of the formed films the cosmetic attractiveness of the films deteriorates. Thicker films are less “invisible” and often show a stronger skin fixation than thinner films. Since solutions of different polymers with the same polymer concentration do not result in films of the same thickness and the same properties the appropriate polymer content has to be determined individually for each polymer and has to be a compromise between drug loading capacity and cosmetic attractiveness. Another limiting factor for the polymer content is the increase of viscosity of the solution caused by the polymer. To permit an application of the formulation by spraying (which would be most convenient for the patient and most exact as to dosing accuracy) a low viscosity of the polymeric solution is required. Therefore the fact that different polymers may lead to different viscosities when dissolved in a given solvent has to be taken into account as well when determining the appropriate polymer content for the formulation.

Apart from polymer and solvent other excipients such as plasticizers or crosslinkers can be incorporated into the formulation. As a general observation derived from the formulation experiments with over 150 different preparations, especially the plasticizer exerts a strong

influence on the properties of the formed film. In polymeric films plasticizer interact with the polymer chains reducing the number of active centers available for rigid polymer – polymer contacts [17]. These interactions result on the one hand in a decrease in glass transition temperature and a higher flexibility of the films, on the other hand in a changed permeability for drug substances and water vapor [30]. The plasticizer content is also decisive for the adhesive properties of the film. Films with a low plasticizer concentration in the formulation did not display a sufficient adhesion to the skin. Films with a high plasticizer concentration showed sufficient adhesion but became sticky on the outer surface. Therefore determining the right amount of plasticizer is essential for a successful formulation of this dosage form. It is important to note that the adequate plasticizer concentration is individual for every plasticizer – polymer combination as the efficiency of a plasticizer is polymer dependant.

During the formulation experiments 10 of the 14 tested polymers yielded film forming polymeric solutions with the required characteristics. Experiments with the other four polymers, however, did not result in satisfactory preparations for various reasons: For Oppanol® no skin-tolerant solvent could be found contrary to the manufacturer's specifications. Hydagen® HCMF required a high water content in the solvent resulting in unacceptably long drying times. Additionally, Hydagen® HCMF showed a strong increase of viscosity already at low polymer concentrations. PVA 72000 displayed poor skin adhesion even with high plasticizer contents and developed a profound increase of viscosity during storage. Kollidon® 12 PF produced sticky films with insufficient integrity on skin after a longer wearing period.

Taking all this into account it can be stated that a careful composition of the film forming solution with a suitable polymer in an adequate concentration and an individually adjusted plasticizer content is essential to achieve a formulation with the required properties concerning viscosity, drying time, outward stickiness, cosmetic attractiveness and integrity on skin after a longer wearing period. Minor variations might be acceptable but major changes in the composition should be avoided as they would have an unfavorable impact on the properties of the film forming system and lead to a deterioration of the mechanical or cosmetic performance of the system on the skin.

5.2. Characterisation methods for film forming polymeric solutions

The finding of suitable methods for the characterization and evaluation of film forming polymeric solutions posed a considerable problem during the development process. No screening process for film forming polymeric solutions and therefore no evaluation method of the macroscopic properties of this dosage form was available from the literature. Beneficial macroscopic properties such as a short drying time or a good cosmetic appearance, however, are prerequisites for the acceptance of a new dosage form by the patients. Therefore the development of a simple evaluation method covering several important macroscopic properties of the formulation (viscosity, drying time, outward stickiness, cosmetic attractiveness, integrity on skin after a longer wearing time) was necessary. The evaluation was performed on living skin as this allowed the assessment of the performance of the formed film under actual wearing conditions. Casting the formulations on artificial substrates such as glass slides did not provide the opportunity to distinguish between different formulations (almost all formulations yielded smooth, transparent films) and was therefore no adequate testing method.

Although the developed evaluation method was fairly simple and to a certain extent subjective it surprisingly turned out to be an efficient method for the differentiation between the various formulations. Especially the criterion “integrity on skin (after 18 hours)” provided valuable information during the screening process to eliminate those formulations that formed attractive films on the skin but that were not suitable for the practical application due to their lack of persistence on the skin.

The results of the screening process indicated that the flexibility of the film is a very important parameter for the successful formulation of this dosage form as considerable mechanical stress is exerted on the formed film by the movement of the skin. However, the attempt to replace the visual inspection concerning the appearance of cracks by an established in vitro method for the determination of the film flexibility was not successful. Although the tested formulations had displayed a similar flexibility on skin in vivo the in vitro results differed widely. In Table 8 it is shown that the non-successful formulations F (var1) and F (var2) yielded higher elongation values than the successful formulations I and C with other polymers. Even though the elongation value is a measure for the flexibility of a polymeric film it does apparently not indicate if a formulation will also show the required flexibility in vivo when formulations with different polymers are regarded. This is surprising as the formed films are supposed to display the same

properties, that is to remain intact and free of cracks during the wearing time, *independent* of the polymer that is used for the formulation. Apparently it is not possible to define a polymer-independent limit value for the elongation at break that has to be reached by a formulation in order to yield a film of sufficient flexibility on the skin. Therefore the in vitro determination of the mechanical properties cannot serve as a suitable method for a further polymer screening for this novel dosage form and cannot replace the described visual assessment of the formed films in vivo at this point.

Similar to the observations for the mechanical properties the in vitro and in vivo values for the water vapor permeability were not in good accordance either as demonstrated in Fig. 12. Based on these results we speculate that the film and its properties are considerably influenced by the contact with the skin. It is possible that a part of the plasticizer is absorbed by the skin [31]. This would lead to a plasticizer depletion in the film, resulting in harder and more brittle film. On the other hand it is also possible that the films absorb water that is evaporating from the skin [32] and that the water serves as additional plasticizer in the film [33, 34], resulting in softer and more flexible films. While these complex diffusion processes between skin and film might not lead to a noticeable change in commonly used transdermal patches they might alter the properties of the film forming system to a considerable extent due to the extreme thinness of the formed films (approximately 5 – 25 µm). Such changes in the film properties, however, that are related to the contact of the film with the skin cannot be mirrored sufficiently under the artificial conditions of an in vitro experiment. We presume that this might be one possible explanation for the different results seen in vitro and in vivo. However, due to the fact that a suitable in vitro method could not be established for an objective evaluation of developed polymeric film forming solutions an in vivo evaluation similar to the one we used in our investigations seems to be inevitable at this point if further polymers or further formulations are to be screened.

6. Conclusion

Film forming solutions were successfully formulated with polymers from different chemical groups such as acrylates (Eudragit® RL PO, Eudragit® S 100, Eudragit® NE 40D, Eudragit® E 100, Dermacryl® 79, Avalure® AC 118), polyurethane-acrylates (Dynamx®), cellulose derivatives (Klucel® LF), polyvinylpyrrolidones (Kollidon® VA 64) and silicones (SGM 36). These formulations contained one of the polymers, a volatile solvent and other

optional excipients such as plasticizers and were fixed compositions concerning the concentrations of all excipients involved. The developed rating system, even though based on simple test methods, provided a good basis for the evaluation of the developed formulations concerning the five key criteria viscosity, drying time, outward stickiness, cosmetic attractiveness or integrity on the skin (after a defined wearing time). The in vitro testing methods for the determination of the water vapor permeability and the mechanical properties of the films did not adequately describe the film properties observed in vivo. Further research will be necessary to develop adequate in vitro testing methods for this new dosage form. At this point, however, an evaluation on living skin seems inevitable if further polymers or formulation variations are to be tested. Independent of this, the positively evaluated preparations resulting from the formulation experiments provide the basis for the development of film forming polymeric solutions as a novel dosage form for the skin. This development will be pursued further with the incorporation of drug substances into the formulations and the investigation of drug release from the polymeric films to evaluate the actual potential of these formulations as dermal or transdermal drug delivery systems.

7. Acknowledgements

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CHAPTER 3

First release and permeation experiments with drug loaded film forming polymeric solutions

1. Abstract

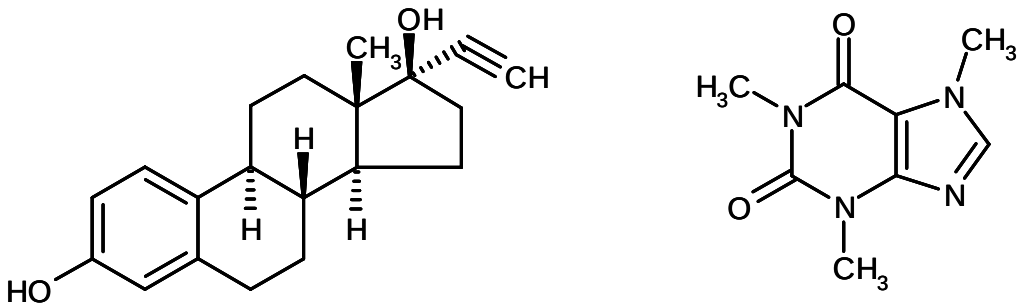
Drug loaded film forming polymeric solutions with polymers from different chemical groups were investigated concerning their potential as drug delivery systems for the skin. The formulations were tested in terms of drug release through an artificial membrane and drug permeation through heat separated human epidermis with caffeine and ethinylestradiol as drug substances. The influences of the drug loading and the solvent on the drug permeation were also investigated with ethinylestradiol as therapeutically relevant drug. A distinction between the different formulations was possible in the release as well as in the permeation experiments. The results of the two methods, however, were not in accordance with each other as different rank orders between the formulations were observed. In the permeation experiments with human epidermis the polyurethane-acrylate formulation with DynamX® as film former displayed the highest permeation results for both drugs. The results indicate a permeation enhancing effect of the polymeric formulation in comparison to the ethanolic reference solutions of the drugs. The ethinylestradiol permeation rose proportionately to the drug loading of the tested formulation. Of the investigated solvents only the mixture of ethyl acetate with ethanol (1:1) achieved an increase of the ethinylestradiol permeation in comparison to the formulation with neat ethanol, but the improvement was not substantial. The results of the permeation experiments support the idea of film forming solutions as dosage form for the skin and provide the basis for further research with this novel approach to transdermal drug delivery.

Keywords:
Film forming polymeric solution, Drug release, Drug permeation, Skin

2. Introduction

Film forming polymeric solutions are a novel approach for the application of drugs via the skin. In a previous study several drug free polymeric compositions had been screened to identify suitable formulations for the intended application. The identified formulations showed the required properties in terms of drying time, outward stickiness, cosmetic appearance and stability on the skin during wearing (chapter 2). The formulations were fixed compositions concerning polymer content as well as nature and concentration of the plasticizer since these parameters define the macroscopic properties of the formed films. Four of these formulations with polymers from different chemical groups (acrylates, polyurethane-acrylates, cellulose derivatives, silicones) were then selected to investigate drug loaded film forming solutions.

The aim of the current study was to test the drug release and permeation properties of the different drug loaded film forming systems in order to gain a first impression of their drug delivering potential. The drugs that were incorporated into the film forming solutions were firstly caffeine (M_r = 194.2, log P_{oct} ≈ - 0,07) and secondly ethinylestradiol, (M_r = 296.4, log P_{oct} ≈ 3,7). Caffeine is a frequently used hydrophilic model drug for percutaneous absorption experiments and OECD standard compound [1-4]. Ethinylestradiol is a therapeutically relevant, highly lipophilic estrogenic compound used mainly in oral or transdermal contraceptive systems [5, 6]. The structures of the drugs are illustrated in Fig. 13. The initial drug loading in the formulations was intended to be 1% (w/w) in the solution but had to be reduced to 0.2% in the case of caffeine due to the low solubility of the drug in the main solvent ethanol.



Ethinylestradiol Caffeine

Fig. 13: Chemical structures of the incorporated drugs

For the release experiments a simple silicone membrane served as inert physical barrier between donor and receptor compartment [7, 8]. For the permeation experiments heat separated human epidermis was used as biologically relevant barrier membrane [9].
Following the comparison of the different polymeric compositions in the release and permeation experiments the formulation with the best permeation performance for the therapeutically relevant ethinylestradiol was selected. It was then modified concerning drug loading and nature of the solvent to evaluate if these parameters could be utilized to control and improve the drug delivery from this novel dosage form for the skin.

3. Materials and Methods

3.1. Materials

Ethinylestradiol was kindly supplied by Schering AG, Berlin, Germany. Caffeine was purchased from Caesar & Loretz GmbH, Hilden, Germany. The polymers used were provided by Roehm Pharma Polymers, Darmstadt, Germany (ammonio methacrylate copolymer type A, Eudragit® RL PO), National Starch and Chemical Company, Bridgewater, USA (polyurethane-14 and AMP-acrylates copolymer, DynamX®), Hercules Inc., Wilmington, USA (hydroxypropylcellulose, Klucel® LF) and Dow Corning S.A., Seneffe, Belgium (Silicon gum, SGM 36). Ethanol (96%) and triethyl citrate were purchased from Merck, Darmstadt, Germany. Dow Corning® Q7-9180 (hexamethyldisiloxane/octamethyltrisiloxane) and Dow Corning® 193 Fluid (PEG-12 dimethicone) were gifts from Dow Corning Corning S.A., Seneffe, Belgium. All chemicals used for the phosphate buffered saline were of analytical grade and purchased from Merck, Darmstadt, Germany. γ-Cyclodextrins were kindly provided by Wacker, Eddyville, USA.

3.2. Tested formulations

For the preparation of the solutions the drug substance was dissolved in ethanol. In case of caffeine moderate heat (50°C for 10 minutes) was applied to facilitate the dissolution of the drug. Having obtained a clear solution the polymer was added and the preparation was stirred overnight for complete dissolution of the polymer. Finally the plasticizer was added and the solution was stirred for another 24 hours before use. An exception to this was the preparation of

the silicone formulation. Since neither of the drugs dissolved in the volatile silicone that is necessary for the dissolution of the silicone gum, an emulsion was formulated. This emulsion consisted of the polymer, the volatile silicone, a silicone emulsifier (193 Fluid) and the smallest amount of ethanol (in case of ethinylestradiol) or water (in case of caffeine) necessary to dissolve the drug. All formulations were stored in glass vials sealed tightly with a siliconized rubber plug and an aluminium vial cap. The composition of the tested formulations are given in Table 9 and Table 10.

For the comparison of the different film forming compositions the polymeric formulations were tested together with reference solutions. These references were polymer free ethanolic solutions of the drugs with the same drug concentrations as in the polymeric formulations (0.2% for caffeine and 1.0% for ethinylestradiol).

Table 9: Composition of the tested film forming solutions and the reference with caffeine

Formulation		**EUD**	**DYN**	**KLU**	**SIL**	**REF**
Polymer		Eudragit®RL PO	DynamX®	Klucel®LF	SGM 36	-
Polymer content	[%]	20.0	10.0	5.0	10.0	-
Triethyl citrate	[%]	6.0	1.0	1.0	-	-
Ethanol	[%]	73.8	72.0	93.8	-	99.8
Water	[%]	-	16.8	-	15.0	-
Q7-9180	[%]	-	-	-	69.8	-
193 Fluid	[%]	-	-	-	5.0	-
Caffeine	[%]	0.2	0.2	0.2	0.2	0.2

Table 10: Composition of the tested film forming solutions and the reference with ethinylestradiol

Formulation		**EUD**	**DYN**	**KLU**	**SIL**	**REF**
Polymer		Eudragit®RL PO	DynamX®	Klucel®LF	SGM 36	-
Polymer content	[%]	20.0	10.0	5.0	10.0	-
Triethyl citrate	[%]	6.0	1.0	1.0	-	-
Ethanol	[%]	73.0	71.2	93.0	5.0	99.0
Water	[%]	-	16.8	-	-	-
Q7-9180	[%]	-	-	-	82.0	-
193 Fluid	[%]	-	-	-	2.0	-
Ethinylestradiol	[%]	1.0	1.0	1.0	1.0	1.0

3.3. Release experiments through a silicone membrane

For the release experiments a silicone membrane (Perthese® silicone sheeting, non reinforced, thickness 125 µm, obtained from Perouse Plastie, Bornel, France) was clamped into vertical all glass `Franz` type diffusion cells (Fig. 14) with an exposed surface area of 1.76 cm^2 and a receptor volume of 12 ml. The receptor phase was phosphate buffered saline. For the experiments with the lipophilic hormone ethinylestradiol (EE) a solubilizer was added to the receptor solution as ethinylestradiol is practically insoluble in water. γ-Cyclodextrins in a concentration of 0.5% (w/v) were selected for this purpose as they solubilize lipophilic substances such as steroid hormones without changing the barrier function of the skin [10]. The receptor compartment was kept at 32°C and stirred continuously with a magnetic stirrer.
The experiment was started after the application of 300 mg of the polymeric solution to the membrane with the help of a pipette. This amount proved to be necessary to achieve full and even coverage of the membrane surface. The release experiments (four replicates per formulation) were conducted over a 24 hours period. During this period samples (200 µl) were drawn at predetermined intervals and replaced by aliquots of the receptor fluid. The concentration of the drugs in the receptor compartment was below 10% of the saturation concentration c_s of the drugs in the respective receptor fluids throughout the experiments ($c_{s\ caffeine}$ = 18,3 mg/ml, $c_{s\ ethinylestradiol}$ = 557 µg/ml).

3.4. Permeation experiments through heat separated human epidermis

The skin used for the preparation of the epidermal membrane was obtained from abdominal plastic surgery. After removing the subcutaneous fatty tissue the skin was kept frozen until further use within six months [11, 12]. For the sample preparation adequate pieces of the frozen skin were punched out, cleaned with Ringer solution and immersed in water of 60°C for 90 seconds. After this treatment the epidermis was carefully removed from the underlying tissue with the help of forceps [13]. The epidermis was soaked in the receptor fluid for 30 minutes. After this time the epidermis was placed on a supporting membrane (regenerated cellulose, MC 10000, thickness 44 µm, Medicell, London, UK, soaked in demineralised water overnight) with the stratum corneum side facing the donor compartment. Based on the results of preliminary experiments with ethanolic solutions of the drugs (data not shown) the supporting membrane was considered non-rate limiting for the drug diffusion. Both membranes were clamped

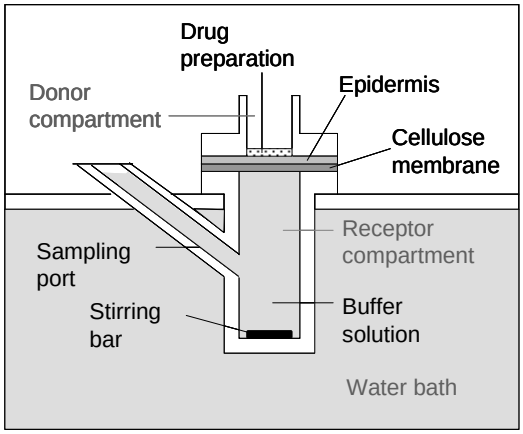


Fig. 14: Experimental setup for the permeation experiments with heat separated human epidermis

carefully into the same diffusion cells `Franz` type described for the release experiments. The experimental conditions (receptor phase, temperature, stirring velocity) were the same as in the release experiments. In contrast to the release experiment, however, only 100 mg of the formulations was applied to the epidermal membrane. This amount was sufficient for a complete coverage of the membrane due to the different surface properties of the skin and and was closer to the amount that can be applied to a patient under realistic conditions (approximately 10 mg/cm^2). The permeation experiments (four replicates per formulation) were conducted over a 24 hours period. At predetermined intervals samples of 200 µl were drawn and replaced by aliquots of the receptor fluid. Sink conditions were maintained throughout the experiment.

3.5. Chromatographic analysis

The samples were analysed for caffeine or ethinylestradiol by HPLC (autosampler model 717plus, pump model 600, all Waters, Milford, USA). No sample pre-treatment was required. The solid phase used for both drugs was a reversed phase column (Lichrospher 100 RP 18, 125 x 4mm, 5 µm) at ambient temperature. The mobile phase for the caffeine assay was acetonitril/phosphate buffered saline pH 2.6 (1:9). With a flow rate of 1.2 ml/min the retention time for caffeine was approximately 4.8 minutes. Caffeine was detected with an UV-Vis detector model 468 (Waters, Milford, USA) at 262 nm. For the ethinylestradiol assay the mobile phase was acetonitril/water (1:1) at a flow rate of 1.5 ml/min. Ethinylestradiol was detected with a fluorescence detector (SFM25, Kontron, Zurich, Switzerland) using a wavelength of 280 nm for excitation and 310 nm for emission [14]. The retention time for ethinylestradiol was approximately 2.7 minutes. The chromatography software was Millennium® (Waters, Milford, USA). Both methods provided good precision and linearity in the required concentration range (caffeine: 0.2 – 100 µg/ml, $R^2 = 0.9999$, ethinylestradiol: 0.1 – 25 µg/ml, $R^2 = 0.9999$).

4. Results

4.1. Comparison of film forming solutions with different polymers

4.1.1. Caffeine release through a silicone membrane

The caffeine release from the film forming preparations with different polymers is displayed in Fig. 15. After 24 hours the SIL and the KLU formulations had released considerably more caffeine than the reference REF, whereas the other two formulations had released a similar amount (DYN) or less (EUD). The caffeine flux from all formulations decreased during the course of the experiment.

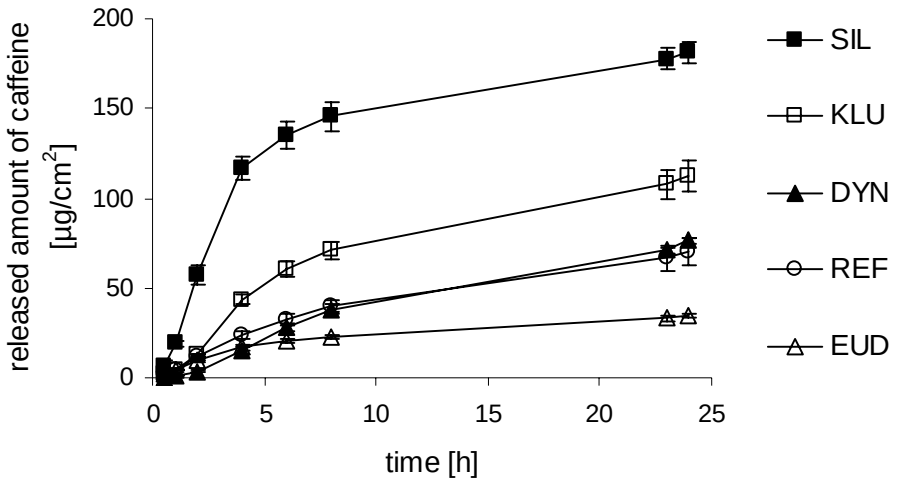


Fig. 15: Caffeine release from different polymeric solutions/films and an ethanolic reference solution through a silicone membrane; 0.2% (w/w) caffeine in the solution; mean values ± standard deviation; n=4

4.1.2. Caffeine permeation through heat separated epidermis

Fig. 16 shows the permeation of caffeine from the polymeric systems through heat separated human epidermis. Two of the formulations, DYN and SIL, delivered more caffeine through the epidermis in 24 hours than the polymer-free ethanolic reference solution (REF). EUD and KLU, on the other hand, showed permeation values that were below those of the reference, although the values for KLU were only slightly lower than those of the ethanolic caffeine solution. All formulations displayed a fairly steady caffeine permeation except for SIL. The silicone formulation showed a high drug permeation in the early stages of the experiment, but the drug flux started to decreased after the first four hours of the experiment.

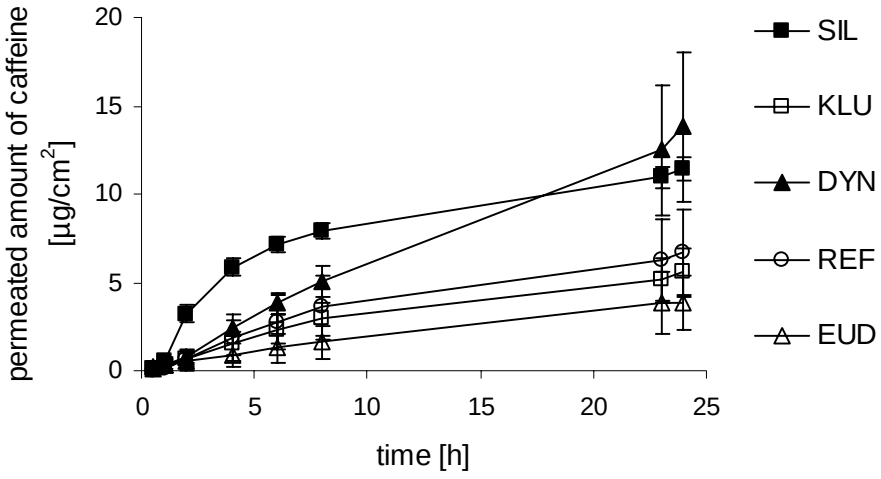


Fig. 16: Caffeine permeation from different polymeric solutions/films and an ethanolic reference solution through human epidermis; 0.2% (w/w) caffeine in the solution; mean values ± standard deviation; n=4

4.1.3. Ethinylestradiol release

The release of ethinylestradiol from the different formulations is displayed in Fig. 17. Similar to the results for caffeine the highest ethinylestradiol release was achieved by the silicone preparation SIL, followed by the cellulose formulation KLU. The DYN formulation with the polyurethane-acrylate DynamX® as film former released slightly less, the preparation with the acrylate Eudragit® RL (EUD) considerably less ethinylestradiol than the polymer-free ethanolic reference solution (REF). Contrary to the release experiment with caffeine all formulations except SIL displayed a continuous flux over 24 hours. The silicone formulation SIL showed a high immediate release followed by a decrease in flux during the later stages of the experiment.

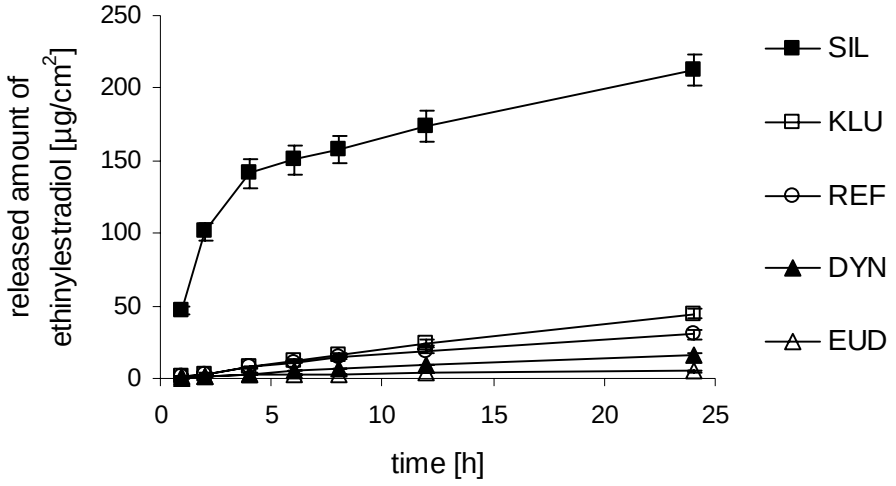


Fig 17: Ethinylestradiol release from different polymeric solutions/films and an ethanolic reference solution through a silicone membrane; 1.0% (w/w) EE in the solution; mean values ± standard deviation; n=4

4.1.4. Ethinylestradiol permeation

Only two of the preparations, DYN and KLU, showed higher permeation values in comparison to the ethanolic solution (REF) as demonstrated in Fig. 18. Among these two formulations DYN displayed not only the highest absolute values but showed also the strongest increase in flux during the early stages of the experiment. Similar to the caffeine experiment the acrylate formulation EUD delivered less drug substance through the membrane than the polymer-free reference. The formulation with silicon gum as film former, finally, did not have a strong impact on the EE permeation in either direction as it delivered a similar, only marginally lower amount of drug through the epidermis than the reference. Neither of the formulations showed a considerable decrease in flux over 24 hours.

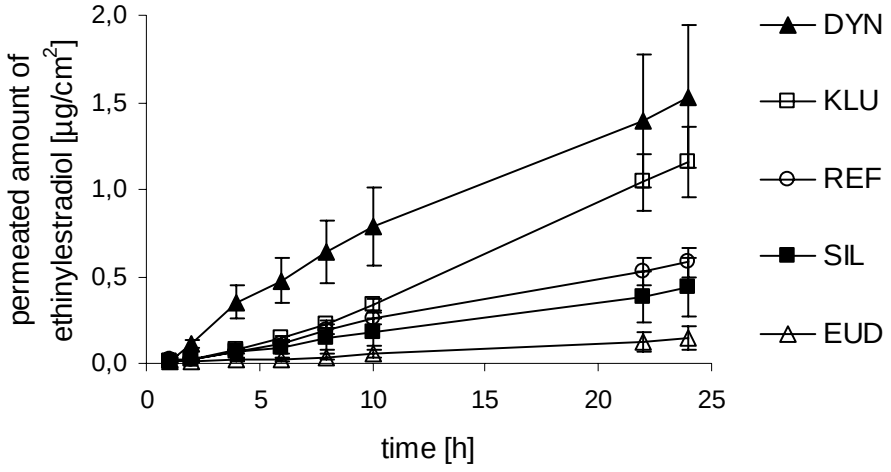


Fig. 18: Ethinylestradiol permeation from different polymeric solutions/films and an ethanolic reference solution through human epidermis; 1.0% (w/w) EE in the solution; mean values ± standard deviation; n=4

4.2. Impact of selected formulation parameters on the EE permeation

4.2.1. Drug concentration

Fig. 19 presents the relative drug permeation (percentage of the applied drug that had permeated through the epidermis at a given point of time) for the polyurethane-acrylate formulation DYN with three different ethinylestradiol concentrations (1%, 2% and 5% (w/w) in the solution). In spite of the different drug loadings all formulations showed similar permeation curves. All preparations delivered the same percentage of ethinylestradiol through the epidermis during the course of the experiment indicating that the permeation increased proportionately to the drug content in the formulation.

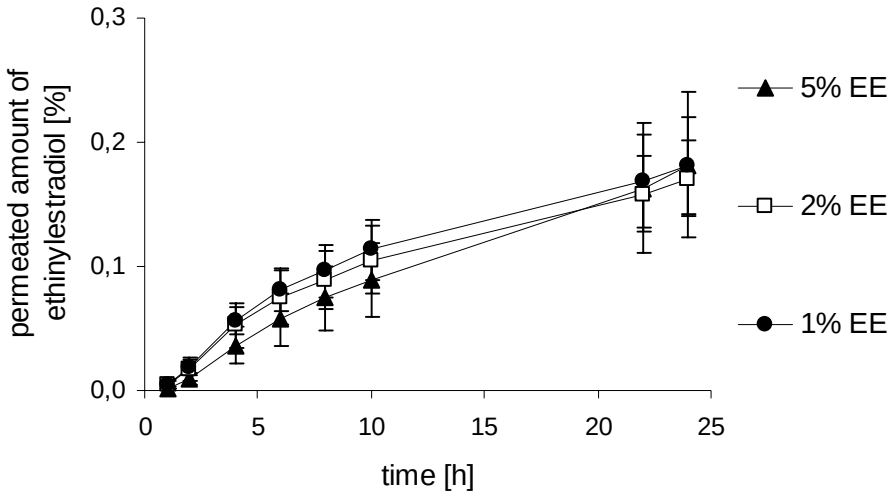


Fig. 19: Relative EE permeation from DynamX® film forming solutions (DYN) with different drug concentrations; mean values ± standard deviation, n = 4

4.2.2. Solvent

Fig. 20 shows the permeated drug amounts of the polyurethane-acrylate formulation DYN with different solvents after 24 hours in the human epidermis model. The replacement of ethanol with neat isopropanol or a binary mixture of isopropanol and ethanol did not improve the ethinylestradiol permeation but, to the contrary, reduced the ethinylestradiol amount that arrived in the receptor compartment. The usage of ethyl acetate instead of ethanol resulted also in a slight decrease of the ethinylestradiol delivery. Only the preparation with the mixture of ethanol and ethyl acetate (1:1) showed slightly, but not substantially higher permeation values than the formulation with neat ethanol.

The differences between the ethinylestradiol permeation from the formulation with neat ethanol in the two sets of experiments resulted from the fact that skin samples of two different donors were used due to capacity reasons.

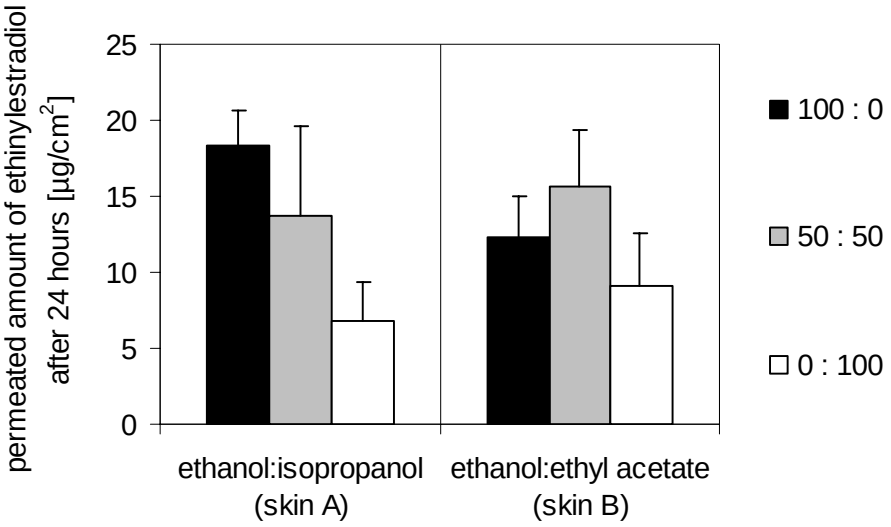


Fig. 20: EE permeation from DynamX® film forming solutions (DYN) with ethanol, isopropanol, ethyl acetate or binary mixtures of isopropanol or ethyl acetate with ethanol as solvents; ethinylestradiol: 5% (w/w) in the solution; mean values ± standard deviation, n = 4; skin samples from two different donors

5. Discussion

5.1. Drug release and permeation from different film forming solutions

In a first evaluation the four different film forming polymeric solutions were compared in terms of their release and permeation behaviour. The studies were carried out with two drugs of different polarity, caffeine and ethinylestradiol.

The release experiments through the artificial membrane with both drugs revealed noticeable differences in the release rates of the polymeric formulations and the polymer-free reference solution (Fig. 15 and Fig. 17). However, the ranking of the formulations was similar for both drugs in spite of their different physico-chemical properties.

In the caffeine experiment (Fig. 15) all formulations showed a decreasing drug flux over time while a similar effect occurred only with the SIL formulation in the ethinylestradiol study (Fig. 17). The reason for this was probably the depletion of the formed films during the course of the experiments that resulted in a lower concentration gradient between formulation and receptor compartment. A lower concentration gradient leads to a reduced drug flux according to Fick's first law of diffusion

$$J = \frac{dm}{dt \times A} = D \times K \times \frac{dc}{h} \qquad \left[\frac{\mu g}{cm^2 \times h}\right] \qquad \text{(Eq. 6)}$$

where J represents the drug flux, m is the permeated drug amount at a given point of time t, A is the permeation area, D is the diffusion coefficient of the drug in the membrane, K is the partition coefficient of the drug between formulation and membrane, c is the concentration gradient over the membrane and h is the membrane thickness.

The depletion was more pronounced for the caffeine films (5.7% - 30.2%) than for the ethinylestradiol films (0.2% - 7.1%) because the release level for caffeine was higher than for ethinylestradiol, but the drug loading in the formulations was considerably lower for caffeine. This could be the reason why all caffeine formulations displayed a decreasing drug flux, but none of the ethinylestradiol preparations except SIL, which showed a substantially higher release than the other polymeric systems with ethinylestradiol.

The extraordinary high release of the SIL formulation in both experiments might be related to the fact that both the membrane as well as the film forming solution consisted mainly of silicone. Hence it can be speculated that the drug diffusion at the interface between the formulation and the membrane was facilitated due to this similarity of the chemical structures [15]. It is also possible that part of the silicone formulation penetrated into the membrane changing its permeability for the drugs.
Although the results for the SIL formulation should therefore be interpreted with caution the silicone membrane was beneficial for the release testing of the polymeric formulations in a different respect. In account of the high membrane lipophilicity and the lack of pores [8, 16] the diffusion of water from the receptor into the donor compartment of the diffusion cell was limited. This limitation was sufficient to allow the forming of the polymeric film even with water soluble polymers. A previous experiment had shown that other frequently used membranes such as regenerated cellulose were not able to limit the water diffusion to this extent, not even after a lipophilic impregnation of the membrane with isopropyl myristate. These membranes hindered the film forming for example in case of the hydroxypropylcellulose formulation (KLU) and were therefore not suited for the release testing.

Heat separated human epidermis allowed a distinction between the different polymeric compositions and the polymer-free reference solution as well (Fig. 16 and Fig. 18). The ranking of the formulations, however, was different in the permeation experiments (caffeine: release SIL>KLU>DYN≈REF>EUD, permeation DYN>SIL>REF>KLU>EUD, ethinylestradiol: release SIL>KLU>REF>DYN>EUD, permeation DYN>KLU>REF>SIL>EUD). An exception to this was merely the acrylate formulation with the film former Eudragit® RL as this formulation produced the lowest results of all formulations in the release as well as in the permeation experiments and for either of the tested drugs. In the literature a good correlation between the drug permeation through a silicone membrane and through human skin has been described for some compounds [16, 17]. However, this correlation does not generally occur for all drugs and all drug vehicles [18-20] and was apparently not observed in our experiments. This underlines that release experiments cannot be utilized to predict the drug permeation through the epidermis [21] and that their value in a formulation selection process is limited. The decision for one of the formulation candidates should rather be based on the permeation experiments with human epidermis as relevant biological barrier.

In the relevant permeation model the formulation with the polyurethane-acrylate DynamX® as film former (DYN) reached the highest permeation values after 24 hours among the four tested polymeric formulations. This was the case for the hydrophilic caffeine as well as for the lipophilic ethinylestradiol although the delivery levels varied for the two drugs. The film forming system DYN achieved higher results than the corresponding polymer-free reference solutions which indicates an enhancing effect on the permeation of both drugs. On the basis of these positive results the polyurethane-acrylate formulation DYN was selected as core formulation for the following experiments.

5.2. Influence of drug concentration and solvent on the EE permeation

As film forming systems are a fairly novel and not yet well investigated dosage form for transdermal delivery it is useful to gain further information about possibilities to control the drug delivery from the system and to increase its efficiency. Hence drug loading and solvent influence were further investigated.

Fig. 19 demonstrates that the permeation from the tested formulation (DYN) increased proportionately to the drug loading. This was expected as the concentration gradient between polymeric film and skin increased with the rising drug loading of the formulation. This gradient is a major driving force for the drug flux according to Fick's first law of diffusion (Eq. 6). However, the drug concentration can only be utilized in a calculable way to increase the drug flux as long as no crystallization occurs in the formed film. In the course of the film formation on the skin the drug concentration in the system increases continuously with the ongoing evaporation of the volatile solvent. During this process the concentration of the drug in the forming film might reach a point where the solubility in the formulation is exceeded and drug crystals start to form. From research with conventional transdermal patches it is known that the formation of drug crystals leads to a reduction in drug flux as the thermodynamic activity of the drug in the matrix decreases [22-24]. Due to this it can be speculated that the apparent proportionality between the drug loading and the drug permeation will no longer be given as soon as crystallization occurs in the film. In our experiments with an ethinylestradiol concentration up to 5% in the solution, however, no drug crystals appeared. This was confirmed by light microscopic observations of the films. Therefore no negative impact on the drug flux was observed and the proportionality between drug loading and drug permeation was given.

Nevertheless, the risk of crystallization should be kept in mind should the ethinylestradiol loading of the polymeric solution be further increased (beyond 5%) in this film forming system.

Another parameter with possible influence on the drug permeation is the solvent of the formulation. Due to the fact that the solvent in a film forming formulation has to be highly volatile, compatible with the polymer and the drug and tolerable for the application on the skin, the choice of possible solvents is limited. Ethanol for example complies with all the requirements and was therefore chosen as basic solvent for the development of this dosage form.
Apart from its function to solubilize the other excipients and the drug, however, the solvent can also serve as permeation enhancer. Ethanol for example is known to be a good permeant for the skin and has a permeation enhancing effect for many drugs. The mechanisms that are discussed for this effect are an alteration of the partitioning of the drug into the skin, interactions with the skin lipids or solvent drag [25-28]. Other solvents such as isopropanol and ethyl acetate have been described in the literature to have an even stronger enhancing effect on the permeation of steroidal hormones than ethanol [29-33]. For this reason it was investigated if an exchange of ethanol against isopropanol, ethyl acetate or a binary mixture of these solvents with ethanol could lead to an improvement in drug permeation for a further optimisation of the delivery system.
As demonstrated in Fig. 20 the formulations with isopropanol or a mixture of isopropanol and ethanol did not achieve an increase of the ethinylestradiol permeation in comparison to the original formulation with neat ethanol but rather reduced the drug flux. Neat ethyl acetate did also fail to increase the ethinylestradiol transport through the epidermis. In consequence, these solvents cannot serve to improve the ethinylestradiol permeation from the film forming system. Only the mixture of ethyl acetate with ethanol showed a slightly higher permeation than the original formulation with neat ethanol.
This result is in accordance with the findings of Catz et al. concerning the enhancing effects of solvent mixtures on the permeation of levonorgestrel, a highly lipophilic steroidal hormone like ethinylestradiol, though hairless mouse skin [30]. However, it does not agree with other findings of the same group for a similar experiment with human cadaver skin [31]. In those experiments the solution of levonorgestrel in neat ethyl acetate produced the best results followed by the mixture of the two solvents and neat ethanol. A reason for the different findings might be that the results of Catz et al. for levonorgestrel cannot be transferred to other steroidal hormones in spite of the similar chemical properties. Another reason could be that the contact time of the solvent to the skin is of importance [34] which was different in the two experiments. In our experiments the

solvent evaporated quickly after the application to the epidermal membrane (open donor chamber). In contrast to this the contact between solvent and skin was provided throughout the whole experiment in the work of Catz et al. (closed donor chamber), offering more time for possible interactions of the solvents with the skin. The condition of a closed donor chamber, however, was not suitable for our experiments as the film forming solutions are not supposed to remain in a liquid state but to dry quickly after application to the skin.

The results of the permeation experiments with different solvents indicate that a mixture of ethyl acetate und ethanol might have a positive impact on the ethinylestradiol permeation in comparison to the usage of neat ethanol. However, since the observed improvement was only moderate a change of the solvent does not seem to be a promising measure to substantially increase the delivering efficiency of the film forming solution for ethinylestradiol.

The two experiments have shown that both parameters, drug concentration and solvent, can have an impact on the drug permeation, but that only the drug loading is a viable option to considerably improve the drug delivery from the film forming system.

6. Conclusion

The performed experiments with caffeine and ethinylestradiol revealed distinct differences between four film forming solutions concerning their drug release and permeation properties. However, the rank order observed in the release experiments with an artificial membrane was not in accordance with the results obtained with human epidermis as relevant biological barrier. For both tested drugs the formulation with the polyurethane-acrylate DynamX® showed a permeation enhancing effect in comparison to the polymer-free reference solution. It also displayed the highest permeation results among the tested formulations in the relevant human epidermis model and was therefore selected for further experiments. The ethinylestradiol amount delivered by the DYN formulation increased proportionately with rising drug loading of the film forming system but was not substantially improved by changes of the volatile solvent in the composition. These results indicate that a drug delivery from film forming solutions to the skin seems generally feasible and that further research is encouraged to prove the relevance of this novel approach for the transdermal drug delivery.

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CHAPTER 4

Delivery of ethinylestradiol from film forming polymeric solutions across human epidermis in vitro and in vivo in pigs

1. Abstract

Film forming polymeric solutions may present an alternative to the common transdermal dosage forms such as patches or gels. To evaluate the potential of these systems for transdermal drug delivery the permeation of ethinylestradiol from four formulations with different polymers was tested across heat separated human epidermis. The formulation with the best results was then modified by incorporating chemical enhancers to further increase the efficiency of the delivery system. Finally, drug delivery from the developed film forming systems was compared to a commercially available transdermal patch in vitro as well as in vivo in pigs. Among the tested preparations the formulation with polyurethane-14-AMP-acrylates copolymer (DynamX®) showed the highest ethinylestradiol permeation. The drug transport was further increased with the incorporation of oleic acid as penetration enhancer, especially when used in combination with propylene glycol. The enhancing effect of oleic acid/propylene glycol was concentration dependant and increased disproportionately with rising enhancer content. The film forming solution showed a higher ethinylestradiol permeation through heat separated human epidermis than the commercial EVRA® patch in vitro and achieved measurable plasma concentrations of ethinylestradiol in vivo in pigs. These promising results encourage the further development of film forming polymeric solutions as novel transdermal dosage form.

Keywords:
Film forming polymeric solution; Transdermal drug delivery; Human epidermis; Pigs; Ethinylestradiol

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2. Introduction

Film forming polymeric solutions have been formulated as a new approach for the transdermal delivery of drugs. In a previously performed screening process several formulations with polymers from different chemical classes had been identified that formed fast-drying, flexible films with good adhesion to the skin (chapter 2). Four film forming solutions resulting from this screening process were selected for further investigations.

The aim of this study was to assess the capability of the film forming polymeric solutions for the transdermal delivery of ethinylestradiol (EE). Ethinylestradiol is a lipophilic steroidal hormone mainly used in the indication contraception. The delivering efficiency of the film forming solutions for EE was investigated in permeation experiments through heat separated human epidermis. The polymers used in the tested formulations were hydroxypropylcellulose (Klucel® LF), ammonio methacrylate copolymer type A (Eudragit® RL PO), polyurethane-14 and AMP-acrylates copolymer (DynamX®) and silicon gum (SGM 36). Along with the four formulations a polymer free ethanolic solution of the drug was tested to evaluate the influence of the different formulations/polymers on the EE permeation. Previous experiments with an ethinylestradiol concentration of 1% had already indicated a preference for one of the formulations. However, as the drug concentration can be used to improve the permeation (chapter 3) it was decided to increase the drug loading for these experiments from 1% to 5% in the solution. The comparison of the film forming preparations with different polymers was repeated with the higher drug loading to confirm the results from the first study for the formulation selection.

Based on the results of these experiment the formulation with the best performance was selected and modified in its composition in order to further increase the drug permeation. For this purpose selected chemical penetration enhancers (laurocapram, N-methyl-pyrrolidone, propylene glycol, propylene carbonate, oleic acid or R-(+)-limonene) were incorporated into the film forming preparations. Subsequently, binary enhancer combinations were tested for a further improvement of the film forming system. Propylene glycol has been described in the literature to act synergistically with many enhancers such as oleic acid, R-(+)-limonene or isopropyl myristate [1-3]. Hence it was chosen as co-enhancer for oleic acid and R-(+)-limonene in this study.

Having determined the most efficient enhancer or enhancer combination for the film forming system the influence of the enhancer concentration was investigated.

In the last step, the film forming solution was compared to a commercial transdermal product that evidentially delivers a therapeutically sufficient amount of EE to the systemic circulation in humans (EVRA® patch). The comparison with the transdermal patch was performed in vitro in the heat separated human epidermis model as well as in vivo in pigs. The pig has been widely recognized as relevant model for dermal or transdermal investigations due to the similar structure of pig skin in comparison to human skin [4, 5]. The in vivo experiment was a single dose application study over a time period of seven days during which the EE plasma levels in the animals were determined at predetermined intervals. In addition to the plasma levels, a first impression of the local tolerance and the persistence of the developed film forming solution on living skin was gained from the in vivo experiment that could be valuable for the further development of this novel dosage form.

3. Materials and Methods

3.1. Materials

Ethinylestradiol was supplied by Schering AG, Berlin, Germany. The polymers were kindly provided by Roehm Pharma Polymers, Darmstadt, Germany (ammonio methacrylate copolymer type A, Eudragit® RL PO), National Starch and Chemical Company, Bridgewater, USA (polyurethane-14 and AMP-acrylates copolymer, DynamX®), Hercules Inc., Wilmington, USA (hydroxypropylcellulose, Klucel® LF) and Dow Corning S.A., Seneffe, Belgium (silicon gum, SGM 36). Ethanol (96%), triethyl citrate, propylene carbonate, propylene glycol and R-(+)-limonene were purchased from Merck, Darmstadt, Germany. Oleic acid (super refined) was a gift from Croda GmbH, Nettetal, Germany. Laurocapram (Azone®) was purchased from Yick-Vic Chemicals, Hong Kong, China. N-methyl-pyrrolidone (Pharmasolve®) was a gift from ISP, Cologne, Germany. Dow Corning® Q7-9180 (hexamethyldisiloxane/octamethyltrisiloxane) and Dow Corning® 193 Fluid (PEG-12 Dimethicone) were gifts from Dow Corning S.A., Seneffe, Belgium. EVRA® (Janssen-Cilag GmbH, Neuss, Germany) patches were purchased from a local pharmacy. EVRA® is a matrix type patch ($A = 20\ cm^2$) containing 0.6 mg ethinylestradiol and 6 mg norelgestromin. All chemicals used for the phosphate buffered saline

were of analytical grade and purchased from Merck, Darmstadt, Germany. γ-Cyclodextrins were kindly provided by Wacker, Eddyville, USA.

3.2. Preparation of the tested formulations

For the preparation of the solutions all ingredients (drug substance (EE), polymer, plasticizer and enhancer) were dissolved in ethanol (96%) and stirred overnight on a magnetic stirrer. For the silicone formulation an emulsion was formulated with the polymer, the volatile silicone (Dow Corning® Q7-9180), a silicone emulsifier (Dow Corning® 193 Fluid) and the smallest amount of ethanol (96%) required to dissolve the drug. This was necessary as EE did not dissolve in the volatile silicone used for the dissolution of the silicone gum. The EE concentration in all formulations was 5% (w/w) in the polymeric solution. In this concentration the drug was completely dissolvable in all preparations. The formulations (Table 11) were stored in glass vials sealed tightly with a siliconized rubber plug and an aluminium vial cap.

Table 11: Composition of the tested film forming solutions and the reference with ethinylestradiol

Formulation		**EUD**	**DYN**	**KLU**	**SIL**	**REF**
Polymer		Eudragit® RL PO	DynamX®	Klucel® LF	SGM 36	-
Polymer content	[%]	20.0	10.0	5.0	10.0	-
Triethyl citrate	[%]	6.0	1.0	1.0	-	-
Ethanol	[%]	69.0	67.2	89.0	25.0	95.0
Water	[%]	-	16.8	-	-	-
Q7-9180	[%]	-	-	-	51.5	-
193 Fluid	[%]	-	-	-	8.5	-
Ethinylestradiol	[%]	5.0	5.0	5.0	5.0	5.0

3.3. In vitro permeation experiments through human epidermis

The skin used for the preparation of the epidermal membrane was obtained from caucasian patients who had undergone abdominal plastic surgery. The patients were in good health and had no medical history of any dermatological disease. The approval from the ethics committee of the 'Caritas-Traegergesellschaft Trier e.V.' was available.

The preparation of the epidermal sheets and the experimental setup (all glass `Franz` type diffusion cells, 1.76 cm^2, 12 ml, phosphate buffered saline with 0.5% γ-cyclodextrin as receptor fluid) has been described earlier (see chapter 3, sections 3.3, 3.4 for details).

The experiments were started after the application of 100 mg of the formulation to the epidermis (2.84 mg EE/cm^2). This amount was necessary to ensure complete and even coverage of the epidermis without the need of an additional distribution device such as a brush to avoid mechanical stress for the epidermal membrane.

For the comparison with the commercial transdermal patch, however, only 20 mg of the preparations (0.57 mg EE/cm^2) were applied because this amount is small enough to be applicable to a patient under realistic conditions without flowing away from the application site and complies with the OECD Guideline No. 28 for the conduct of skin absorption studies [6]. Due to the small quantity of the sample a soft brush was required for the application in these experiments.

From the commercial transdermal matrix patches circular discs with a diameter of 1.5 cm (containing 0.03 mg EE/cm^2) were prepared with a scalpel and pressed carefully to the epidermis before mounting the membranes in the diffusion cells.

The donor compartment was kept open throughout the whole experiment. The permeation experiments (four replicates per formulation) were conducted over a 24 hours period. During this period samples (200 µl) were drawn at predetermined intervals and replaced by aliquots of the receptor fluid. The saturation concentration c_s of EE in the receptor solution was $c_{s\ ethinylestradiol}$ = 557 µg/ml, sink conditions were maintained at any time.

The samples from the permeation experiments were analysed for ethinylestradiol by HPLC (autosampler model 717plus, pump model 600, all Waters, Milford, USA). No sample pre-treatment was required. The solid phase used was a reversed phase column (Lichrospher 100 RP 18, 125 x 4mm, 5 µm) at ambient temperature. The mobile phase was acetonitril/water (1:1) at a flow rate of 1.5 ml/min. Ethinylestradiol was detected with a fluorescence detector (SFM25, Kontron, Zurich, Switzerland) using a wavelength of 280 nm for excitation and 310 nm for

emission [7]. The retention time for ethinylestradiol was approximately 2.7 minutes. The method provided good precision and linearity in the required concentration range (0.1 – 25 µg/ml, $R^2 = 0.9999$). The chromatography software used was Millennium® (Waters, Milford, USA).

The results of the permeation experiments are shown as cumulated drug amount [µg] permeated per unit surface area [cm^2] plotted as a function of time.

For the experiments with chemical enhancers an enhancement factor was calculated by dividing the absolute drug amount permeated from the formulation with enhancer after 24 hours by the absolute drug amount permeated from the enhancer free formulation after 24 hours

$$Enhancement\ factor = \frac{permeated\ drug\ amount\ with\ enhancer\left[\mu g\right]}{permeated\ drug\ amount\ without\ enhancer\left[\mu g\right]} \quad \text{(Eq. 7)}$$

Direct comparisons between different formulations within one set of experiments were always performed with skin samples from the same donor.

3.4. In vivo permeation study in pigs

The in vivo study was performed with 8 female pigs (German Landrace, age 3-4 months, Charles River Laboratories, Sulzfeld, Germany) in accordance with the German animals act [8] and under approval from the responsible authority (Niedersächsiches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Oldenburg, Germany).

The pig race selected for this study was the German Landrace pig. In this race the bristle-growth is less dense than in the commonly used Goettingen minipig [9].

The animals were divided into two groups of four animals, the patch group and the film forming solution group. The allocation to the groups was performed by body weight randomization to yield groups with approximately equal mean body weight (approximately 23.5 ± 0.5 kg). On the day before the start of the experiment the test areas on the back of the animals were shaved without damaging the skin. Prior to the application of the test medication the skin was cleaned carefully with ethanol to avoid adhesion problems caused by fatty residues on the skin. The animals of the patch group were treated with the commercially available EVRA® patch (one patch per animal, A = 20cm^2, 0.03 mg EE/cm^2). In case of a partly detachment of the patch during the course of the experiment (less than 90% skin contact) the patch was removed and a fresh patch was applied to a different location on the back of the animal. In the other group

300 µl of the prepared film forming polymeric solution (DYN, 5% (w/w) ethinylestradiol in the solution) were applied to the test area (A = 20cm^2, corresponding to the size of the commercial patch) with the help of a syringe. The applied dose per surface area (0.57 mg EE/cm^2) corresponded to the dose applied in the in vitro study with human epidermis. Following the administration the animals were restrained for at least one hour in slings. The slings allowed the free movement of the head but prevented access to the application site by the animals. The liquid formulation dried on the skin within five minutes. Both test items, the patch as well as the film forming solution, were applied once at the beginning of the experiment and were supposed to remain in contact with the skin for the recommended wearing period of the commercial patch (seven days). On a daily basis the appearance of the test items, any occurring skin reactions and necessary patch replacements were recorded.

For the determination of the ethinylestradiol plasma levels 5 ml blood samples were drawn before the application and after 6 h, 12 h, 24 h, 48 h, 72 h, 144 h and 168 h from the vena jugularis of each animal, processed for lithium-heparin plasma by centrifugation and kept frozen until sample analysis.

The ethinylestradiol plasma concentration in the samples was determined by GC-MS. For the analysis ethinylestradiol was extracted from the plasma samples, derivatized and spiked with D4-EE as internal standard. The capillary column used for the analysis was a 50%-phenyl-50%-dimethyl-polysiloxane phase (Zebron™ ZB50, 30m x 0.32mm, 0.25 µm film, Phenomenex Ltd., Aschaffenburg, Germany). The retention time for EE and D4-EE was approximately 7.6 minutes. For the detection a mass spectrometer (R10-10C, Nermag, Rueil-Malmaison, France) was used with negative ions chemical ionization (NICI) using ammonia as reagent gas. Ethinylestradiol was detected with the mass/charge ratio (m/z) of 490, D4-EE (internal standard) with m/z 494. The method assured good linearity and precision in the defined range (10 – 500 pg/ml, $R^2 = 0.998$).

The determined ethinylestradiol plasma concentrations are given in pg/ml and are plotted as a function of time. The area under the plasma concentration versus time curve over seven days ($AUC_{0-168\,h}$) was calculated by the trapezoidal rule.

4. Results

4.1. EE delivery from film forming solutions with different polymers

Of the four tested formulations the preparation with polyurethane-14 and AMP-acrylates copolymer DynamX® (DYN) as film forming polymer transported the highest amount of EE through heat separated human epidermis (Fig. 21), followed by the hydroxypropylcellulose Klucel® LF formulation (KLU). The fact that these preparations delivered a higher amount of drug substance than the ethanolic reference solution indicates that the presence of these polymers and/or the plasticizer had an enhancing effect on the drug flux. Contrary to this, the formulation with the methacrylate copolymer Eudragit® RL PO (EUD) seemed to retain the drug substance rather than promote its permeation through the epidermis. The silicone formulation with SGM 36 (SIL), finally, had neither a retarding nor an enhancing effect on the drug flux in comparison to the ethanolic reference solution. Due to the fact that the DYN formulation showed not only the highest but also the fastest drug delivery (in accordance with the earlier results with the lower drug concentration, chapter 3) it was considered the formulation with the highest potential and was therefore selected for all further investigations.

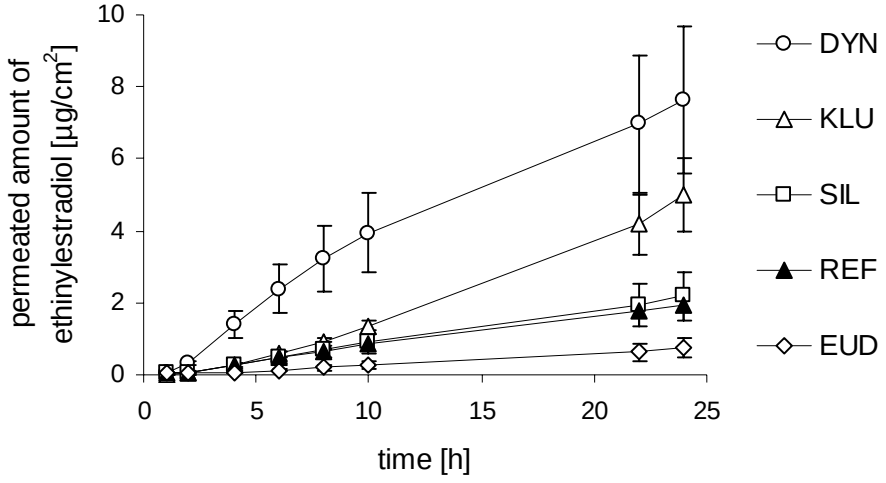

Fig. 21: Ethinylestradiol permeation from different polymeric solutions/films and an ethanolic reference solution through human epidermis; 5% (w/w) EE in the solution; mean values ± standard deviation, n = 4

4.2. Incorporation of chemical enhancers

4.2.1. Influence of different enhancers on the EE permeation

For the selection of a suitable enhancer for ethinylestradiol several DYN formulations were tested that contained one of the pre-selected enhancers in a concentration of 5% (w/w). Of the six selected substances only oleic acid (OA) and R-(+)-limonene (LIM) showed a moderately enhancing effect for ethinylestradiol in the DYN formulation (enhancement factor 1.9 and 1.4 based on the permeated drug amounts after 24 hours; Fig. 22). Propylene carbonate (PC), propylene glycol (PG) and N-methyl-pyrrolidone (NMP) achieved no increase in drug permeation. Laurocapram (Azone®, AZO) even reduced the permeated amount of drug substance (factor 0.5) compared to the enhancer free formulation.

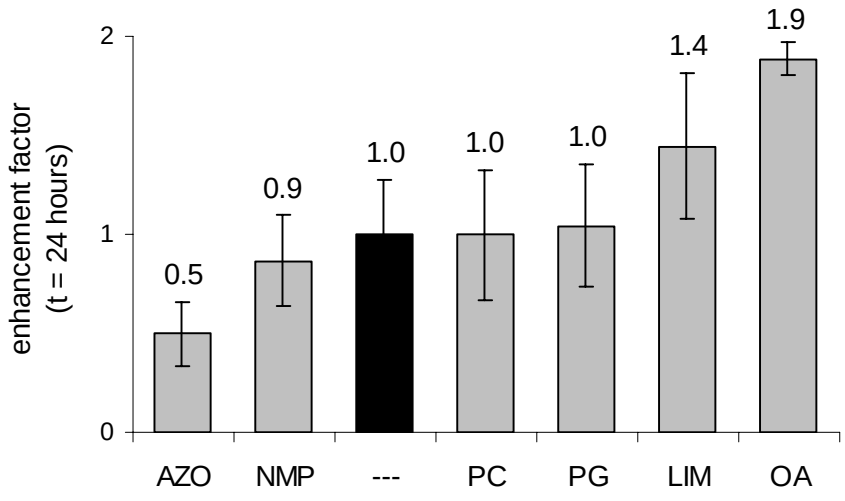

Fig. 22: Enhancement factors for the ethinylestradiol permeation from DynamX® film forming solutions (DYN) with the enhancers laurocapram (Azone®, AZO), N-methyl-pyrrolidone (NMP), propylene carbonate (PC), propylene glycol (PG), R-(+)-limonene (LIM), oleic acid (OA) and from an enhancer free film forming solution (-) based on the permeated drug amounts after 24 hours; enhancer content: 5% (w/w) in the solution; ethinylestradiol content: 5% (w/w) in the solution; mean values ± standard deviation, n = 4

4.2.2. Synergistic effects of binary enhancer combinations

To make use of possible synergistic effects DYN formulations with binary enhancer combinations were tested containing propylene glycol and one of the enhancers that had previously proved to be efficient for ethinylestradiol (oleic acid or R-(+)-limonene). Propylene glycol and the co-enhancer were each incorporated in a concentration of 2.5% (w/w) adding up to a total enhancer concentration of 5% (w/w) in the preparations. Fig. 23 shows the enhancement factors resulting from these enhancer combinations in comparison to the factors achieved by the formulations containing only one single enhancer in a concentration of 5% (w/w). Both enhancer combinations increased the ethinylestradiol permeation much more efficiently than the single enhancer compounds. Similar to the results seen with the single compounds the propylene glycol combination with oleic acid achieved a higher permeation than the combination with R-(+)-limonene (enhancement factor 4.6 for oleic acid/propylene glycol versus 3.3 for R-(+)-limonene/propylene glycol).

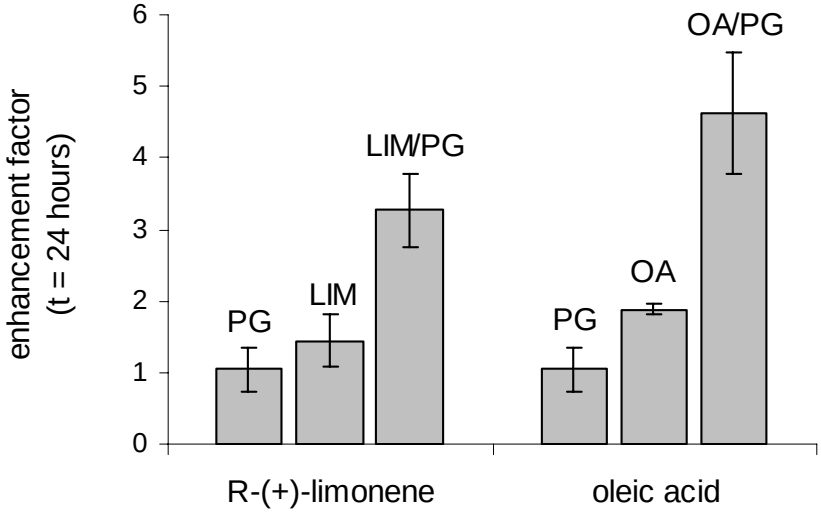

Fig. 23: Enhancement factors for the ethinylestradiol permeation from DynamX® film forming solutions (DYN) with the enhancers propylene glycol (PG), R-(+)-limonene (LIM), oleic acid (OA) and binary mixtures (1:1) of propylene glycol with R-(+)-limonene (LIM/PG) or propylene glycol with oleic acid (OA/PG) based on the permeated drug amounts after 24 hours; total enhancer content: 5% (w/w) in the solution; ethinylestradiol content: 5% (w/w) in the solution; mean values ± standard deviation, n = 4

4.2.3. Impact of the enhancer concentration on the EE permeation

In the previous experiments the chemical enhancers were incorporated into the DYN formulation in a concentration of 5% (w/w). However, permeation enhancers such as oleic acid are a possible source for skin irritation [10] and are therefore to be used with caution. For a better evaluation of the benefits of the enhancer content on the ethinylestradiol permeation DYN preparations with different enhancer concentrations (1%, 3% or 5% w/w) and an enhancer free formulation were compared. For these experiments the binary enhancer combination oleic acid/propylene glycol (1:1) was chosen as this combination had displayed the highest enhancing efficiency for EE in the previous experiment. Fig. 24 shows the permeated drug amounts after 24 hours for the formulations with different enhancer contents. As expected the preparation with the highest enhancer content achieved also the highest EE permeation. However, the ethinylestradiol permeation did not rise linearly but showed a disproportionately high increase with rising enhancer concentrations.

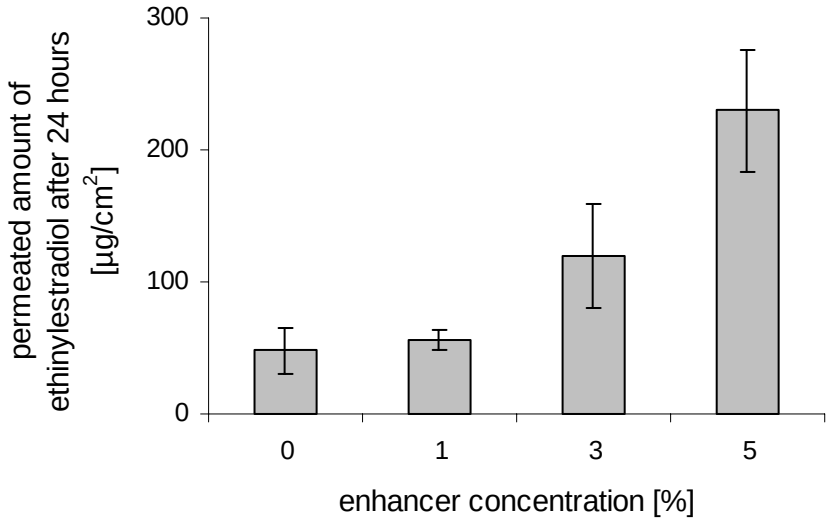

Fig. 24: Ethinylestradiol permeation from DynamX® film forming solutions (DYN) with different enhancer concentrations through heat separated human epidermis based on the permeated drug amounts after 24 hours; enhancer: oleic acid/propylene glycol (1:1); ethinylestradiol content: 5% (w/w) in the solution; mean values ± standard deviation, n = 4

4.3. Comparison of the film forming solution to a transdermal patch

4.3.1. In vitro permeation

Fig. 25 shows the ethinylestradiol permeation from the developed DYN film forming solution with enhancer (propylene glycol/oleic acid (1:1), 5% w/w) or without enhancer in comparison to the permeation from the commercially available patch (EVRA®) through human epidermis in vitro. Both film forming formulations showed a higher permeation than the commercial patch over 24 hours. While the enhancer free formulation transported more than double the ethinylestradiol amount of the marketed patch, the formulation with oleic acid and propylene glycol as permeation enhancer delivered about seven times as much ethinylestradiol than the EVRA® patch through heat separated human epidermis.

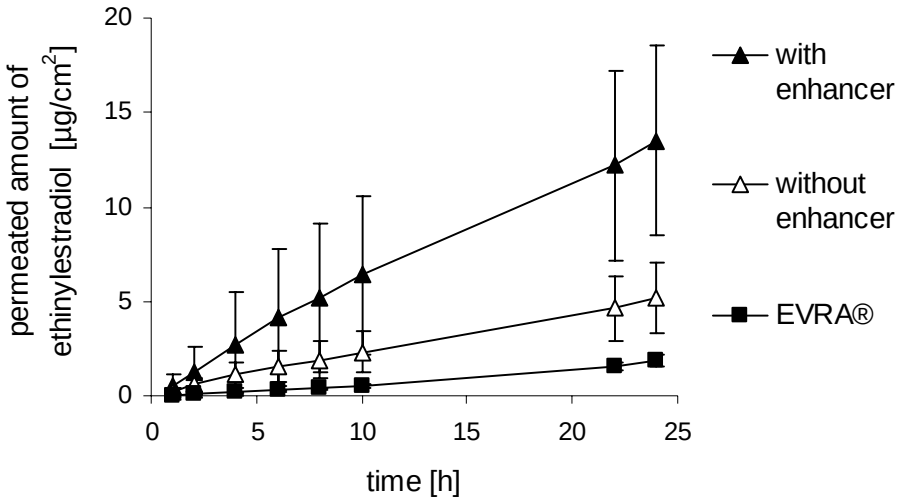

Fig. 25: Ethinylestradiol permeation from DynamX® film forming solutions (DYN) and from the EVRA® patch through human epidermis; enhancer: 5% (w/w) oleic acid/propylene glycol (1:1); ethinylestradiol dose: 0.57 mg/cm² in the solution, 0.03 mg/cm² in the patch; mean values ± standard deviation, n = 4

4.3.2. In vivo study in pigs

In the animal study both tested dosage forms, the commercial patch and the developed film forming solution showed sufficient contact to the skin. In the patch group only one patch had to be replaced on day four due to loss of contact. The developed film forming system DYN also showed good adhesion. After application the liquid formed a clear, glossy film on the skin, which started to display cracks after one day. The films ceased to be visibly detectable after three to six days. After seven days no residues were visible on the skin of the animals in this group. In the patch group brown residues were observed after removal of the patches.

Both dosage forms, the patch and the developed film forming system, were well tolerated. No erythema or other skin reactions were observed. The reddening noted in one animal of the patch group after removal of the patch was most probably related to the mechanical stress of the removal procedure. Both dosage forms showed also a good systemic tolerance as none of the animals died or displayed any item-related changes of behaviour, body weight, food consumption or external appearance.

The plasma levels of ethinylestradiol in the animals of the two test groups during the course of the experiment are shown in Fig. 26, 27 and 28. Due to the fact that the detected ethinylestradiol concentrations in the plasma were lower than expected some of the data points were below the validation range and had to be calculated by extrapolation. Therefore the given data is only

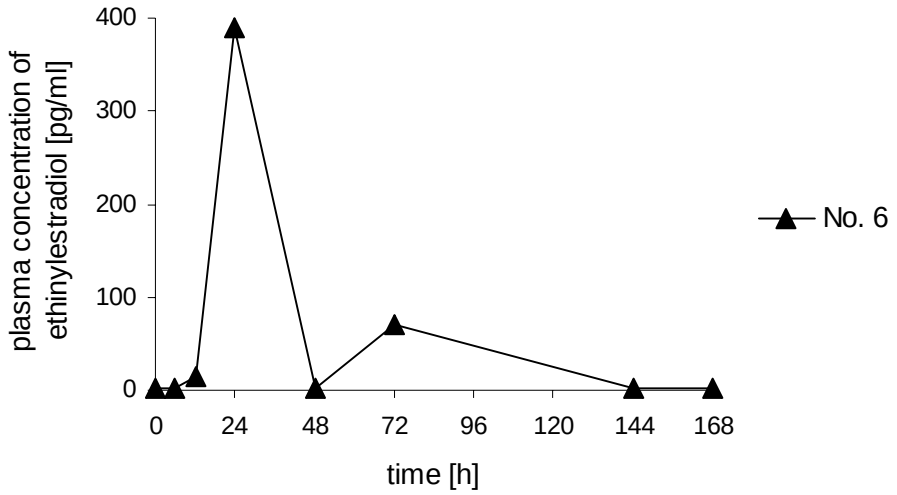

Fig. 26: Ethinylestradiol plasma concentration in pigs after single application of the DynamX® film forming solution (DYN), (0.57 mg EE/cm^2, 20 cm^2; total EE dose: 11.4 mg)

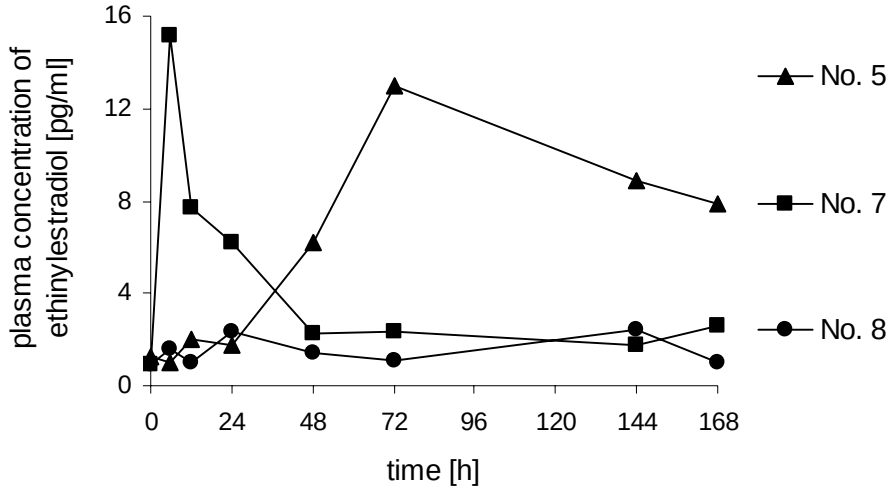

Fig. 27: Ethinylestradiol plasma concentration in pigs after single application of the DynamX® film forming solution (DYN), (0.57 mg EE/cm^2, 20 cm^2; total EE dose: 11.4 mg)

semi-quantitative and has to be interpreted with due caution. The determined plasma concentration levels showed considerable variations in both groups. While maximum plasma concentrations were reached after 48 hours (t_{max}) in three of the four animals in the patch group the animals in the film forming group displayed varying t_{max} values: 6 hours (animal no. 7), 24 hours (animal no. 6) and 72 hours (animal no. 5). In one animal of each group (animals no. 3 and 8) extremely low plasma concentrations of ethinylestradiol were detected without identifiable maximum. Although the determined ethinylestradiol plasma levels showed considerable variations in both groups the calculated area under the plasma concentration versus time curve ($AUC_{0-168\ h}$) was on a higher level for the animals with the film forming solution than for the animals with the patches (Table 12).

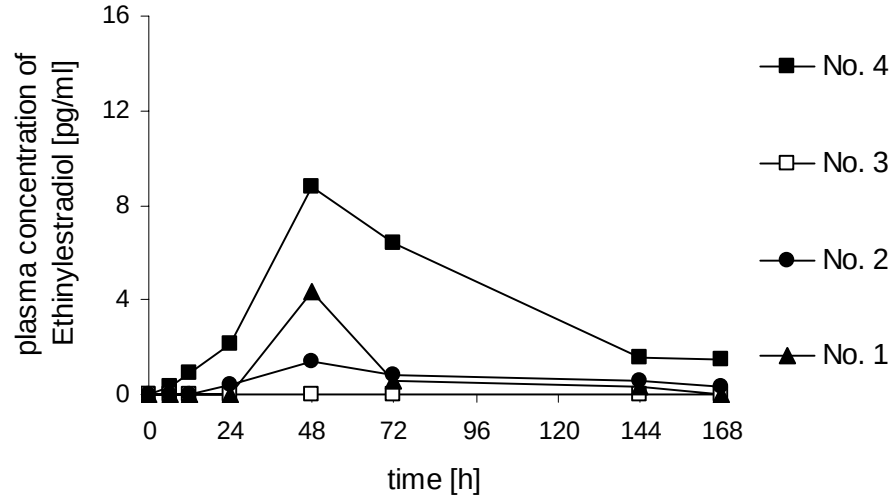

Fig. 28: Ethinylestradiol plasma concentration in pigs after single application of the EVRA® patch (0.03 mg EE/cm^2, 20 cm^2; total EE dose: 0.6 mg)

Table 12: t_{max} and AUC $_{0-168h}$ values based on the ethinylestradiol plasma concentration versus time curves after single application of the EVRA® patch or the DYN film forming solution to pigs

EVRA® patch			**Film forming solution**		
Animal No.	t_{max} [h]	AUC $_{0-168h}$ [pg*ml^{-1}*h]	Animal No.	t_{max} [h]	AUC $_{0-168h}$ [pg*ml^{-1}*h]
1	48	146	5	72	1353
2	48	111	6	24	10572
3	-	-	7	6	555
4	48	659	8	-	279

5. Discussion

5.1. Effects of the film forming polymer

For the development of film forming polymeric solutions as drug delivery systems the choice of a suitable polymer for the formulation is very important. The film former does not only influence the mechanical properties of the formed film such as flexibility or abrasion resistance, the adhesion to the skin (in cooperation with the plasticizer) or the appearance of the film (transparency, smoothness, gloss) (chapter 2) but affects also the drug permeation from the film into the skin as demonstrated in Fig. 21. Depending on their chemical properties, polymers and drugs can interact in different ways, for example by ionic forces, hydrogen bonding or through the degree of solubilization of the drug in the polymer [11, 12]. An important factor with impact on the drug permeation can be the ability of the polymer to influence the physical state of the drug in the matrix by acting as crystallization inhibitor. When the formulation is applied to the skin the drug is completely dissolved. With the evaporation of the solvent the drug concentration within the formulation rises which increases the thermodynamic activity of the drug in the formulation and with it the drug flux [13, 14]. However, when the saturation level of the formulation is exceeded crystallization might occur, which has a negative impact on the drug flux. Antinucleating polymers such as DynamX® can accordingly contribute to an increased drug delivery as they can prevent crystallization [15-17] in the formed films. In films of polymers that lack this antinucleating ability such as the silicon gum SGM 36 crystallization was observed

after the evaporation of the solvent which might contribute to the comparatively low permeation results of this formulation. However, to what extent the suggested effects contribute to the different performance of the formulations cannot be clarified without further investigations due to the diversity in the chemical structures of the utilized polymers.

5.2. Incorporation of chemical enhancers

On the basis of the positive results with the DYN formulation further measures were taken to increase the delivering efficiency of the selected formulation.

One commonly applied approach to overcome the skin as efficient absorption barrier is the incorporation of a chemical penetration enhancer. Chemical enhancers may improve the drug absorption by various mechanisms such as improving the solubility of the drug in the vehicle, promoting the partitioning of the drug into the skin or disrupting the lipid bilayer structures in the skin [18]. The enhancing efficiency of a chemical enhancer is drug specific and dependant on the chemical properties of the drug substance such as the lipophilicity [19]. Many substances have been described in the literature as penetration enhancers for estradiol [20-26], which is very similar to ethinylestradiol concerning the chemical structure. Six of these enhancers were tested in the human epidermis model to identify the most efficient enhancer for ethinylestradiol in the developed polymeric system. Of the six enhancers only oleic acid (OA) and R-(+)-limonene (LIM) achieved positive results while propylene carbonate (PC), propylene glycol (PG), N-methyl-pyrrolidone (NMP) and laurocapram (Azone®, AZO) did not increase the delivery of ethinylestradiol (Fig. 22). R-(+)-limonene and oleic acid act mainly by causing a disorder in the intercellular lipid structures of the horny layer [27, 28] which facilitates the permeation of lipophilic drugs such as ethinylestradiol through the skin. A similar mechanism of action is also described for laurocapram [29, 30] and examples can be found in the literature for its enhancing effect on the permeation of highly lipophilic steroids such as estradiol [31, 32]. Our experiments, however, a permeation enhancement for the highly lipophilic ethinylestradiol ($\log P_{oct} \approx 3.7$) by laurocapram was not observed. The results rather support the findings of Diez-Sales et al. who described that although the enhancing efficiency of laurocapram for lipophilic drugs increased with rising enhancer concentrations it showed no effect for compounds of very high lipophilicity ($\log P_{oct} > 3$) [33].

In contrast to oleic acid, R-(+)-limonene and laurocapram the three other enhancers follow a different mechanism of action. They serve mainly as co-solvents that increase the solubility of the drug in the formulation as well as in the skin [25, 34]. As ethinylestradiol was already completely dissolved in the liquid formulation as well as in the formed film (no drug crystals were observed in the film), the solubility of the drug in the vehicle could not be further improved. It is possible that the co-solvents improve the partitioning of lipophilic drugs into the skin by solubilizing them in the aqueous regions in the intercellular structures of the horny layer as suggested by Barry et al. [35]. However, this effect did either not apply to ethinylestradiol as drug molecule or was too weak to result in a measurably increased drug permeation in our experiments. Especially the efficiency of propylene glycol as sole penetration enhancer is discussed controversially in the literature. While some authors describe a permeation promoting effect of propylene glycol for lipophilic drugs [18, 23, 36] others have not seen similar effects [20, 37, 38]. The results of our experiments support the latter as the permeation of the lipophilic ethinylestradiol from the film forming system was not increased by the presence of propylene glycol alone. However, when the enhancer was used in combination with co-enhancers such as oleic acid or R-(+)-limonene a synergistic enhancing effect on the ethinylestradiol permeation was observed (Fig. 23). This is in accordance with findings in the literature where a similar effect has been reported for other, hydrophilic and lipophilic drugs [1, 23, 39-41]. The observation that the ethinylestradiol permeation did not rise linearly with the enhancer concentration but showed a disproportionately high increase (Fig. 24) might also be related to the synergistic action of the two enhancers. It can be speculated that this synergy results from an improved partitioning of the oleic acid into the stratum corneum due to the presence of the propylene glycol.

5.3. EE delivery from film forming solutions versus transdermal patch

The permeation experiments through heat separated human epidermis have shown that both developed film forming formulations, the DYN formulation with propylene glycol/oleic acid (1:1) as enhancer and even the formulation without enhancer, delivered a higher drug amount through the epidermis in 24 hours than the commercial transdermal patch (Fig. 25). At least in this in vitro model the incorporation of a chemical enhancer such as propylene glycol/oleic acid (1:1) was not required to reach the same delivery level as the commercial patch. However, the delivering efficiency of the film forming solution was lower than that of the commercial patch. Only 0.8% of the applied drug was transported through the epidermis by the film forming

formulation without chemical enhancer while the patch delivered 6.2% of the administered drug amount in 24 hours. Therefore the incorporation of enhancers might be useful to increase the delivering efficiency of the system. With this measure the required drug concentration in the system can be reduced in order to save drug substance and lower the environmental contamination caused by the amount of drug remaining in the system after usage. A prerequisite for this measure would be, however, that the enhancer containing formulation demonstrates a good local tolerance on the skin.

The positive findings from the in vitro experiment concerning the potential of film forming polymeric solutions as transdermal delivery system for ethinylestradiol were supported by the in vivo results. Although the variations were high and the delivery kinetic remained unclear it can be stated that it was possible to induce detectable plasma levels of ethinylestradiol in vivo in a relevant animal model with the film forming solutions. A trend was observed that the delivered ethinylestradiol amounts were higher than those delivered by the commercial transdermal patch (Table 12). However, additional experiments are required to profoundly investigate the delivery kinetic of the drug from the developed system.

6. Conclusion

Film forming polymeric solutions with and even without chemical permeation enhancers have demonstrated potential as transdermal drug delivery systems for ethinylestradiol in vitro and in vivo. Further research is necessary to prove the relevance of film forming solutions as transdermal dosage form but the obtained results are encouraging for the further development of this novel drug delivering technology for the skin.

7. Acknowledgements

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CHAPTER 5

Permeation of levonorgestrel from film forming polymeric solutions across human epidermis in vitro

1. Abstract

The aim of this study was to investigate film forming polymeric solutions for the delivery of steroidal hormones for hormone replacement therapy or contraception. In previous in vitro experiments a film forming solution had successfully delivered a similar amount of ethinylestradiol through human epidermis than a commercially available contraceptive patch (EVRA®). Estrogens are usually administered in combination with progestins to reduce their side effects on various hormone sensitive tissues. Therefore the aim of the current study was to test the developed film forming system concerning a possible co-administration of the progestin levonorgestrel with the estrogenic compound ethinylestradiol. The performed permeation experiments with heat separated human epidermis indicated that the film forming system was capable of simultaneously delivering ethinylestradiol and levonorgestrel. No mutual influences on the drug permeation occurred between the two drugs in the tested concentrations. The polymeric formulation as such and oleic acid/propylene glycol as additional chemical enhancers had a positive impact on the levonorgestrel permeation, similar to the effects observed for ethinylestradiol. With an enhancer content of 5% of oleic acid and 5% propylene glycol the film forming system delivered a similar amount of levonorgestrel through the epidermis than the Fem7® Combi patch, which is marketed for the indication hormone replacement therapy. These results demonstrate the potential of film forming polymeric solutions as novel dosage form for the skin and encourage their further development as transdermal delivery system for steroidal hormones such as ethinylestradiol or levonorgestrel.

Keywords:
Film forming polymeric solution, Transdermal delivery system, Drug permeation, Levonorgestrel

2. Introduction

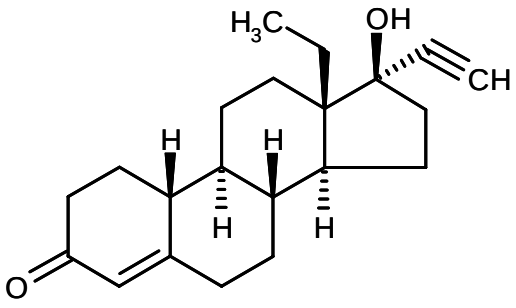
Transdermal delivery devices, mainly in the form of matrix or reservoir patches, are currently used for a number of indications, among them hormone replacement therapy (HRT) and contraception. In comparison to the widely used oral dosage forms the transdermal hormone delivery can provide several advantages: Transdermally applied ethinylestradiol for example, a frequently used synthetic estrogen for contraception, does not underlie the hepatic first pass effect. This effect is responsible for the low bioavailability of the drug after oral administration (bioavailability approximately 40-50% [1]) and various changes in the hepatic serum parameters [2]. In contrast to the oral application route plasma peaks are minimized with the transdermal application, reducing the occurrence of side effects. Moreover, the efficacy of the medication is not affected by disorders of the gastrointestinal tract like vomiting or diarrhoea.
Several polymeric patches are available for HRT with estrogens like estradiol and progestins such as levonorgestrel (Fem7® Combi, Solvay, Germany) or norethindrone acetate (Climara Pro™, Berlex, USA, CombiPatch®, Novartis, USA). For contraception the only currently marketed product is the EVRA® (EU) or Ortho-EVRA™ (US) patch. It contains ethinylestradiol as estrogenic compound and norelgestromin as progestin. The EVRA® patch is continuously worn by the patient for a period of seven days after which it is replaced by a fresh patch. After three weeks of application a patch-free week completes the menstrual cycle. This dosing scheme especially addresses women who are opposed to oral medication or who do not want to be obliged to remember taking an oral contraceptive on a daily basis [3, 4].

To benefit from the advantages of the transdermal application route without the disadvantages often associated with transdermal patches (skin irritation, high visibility) film forming polymeric solutions have been suggested for the transdermal delivery of the steroidal hormones. The basic principle of these delivery systems and their development has been described earlier (chapter 2).

The aim of this study was to investigate if film forming solutions can serve as drug delivery systems for the indications contraception or HRT. A film forming solution that had displayed encouraging results in earlier experiments was selected as core formulation for these experiments. The selected preparation contained polyurethane-14 and AMP-acrylates copolymer (DynamX®) as film former, triethyl citrate as plasticizer, water and the volatile solvent ethanol.

In a previous permeation experiment this preparation loaded with 5% (w/w) ethinylestradiol (EE) had delivered a higher amount of the estrogen through heat separated human epidermis than the contraceptive EVRA® patch (chapter 4). This result indicated that the delivery of a sufficient amount of a suitable estrogenic compound from the developed film forming system was feasible in vitro. For contraceptive purposes (and often also for HRT), however, estrogens are not administered as mono therapy as they are supposed to increase the severe risk of endometrial hyperplasia and breast cancer [5-7]. To reduce these side effects the estrogen is combined with a progestin because of the anti-estrogenic activity of these progesterone or testosterone derivatives (down regulation of the estrogen receptors, decrease of the endometrium thickness) [8]. Hence a novel film forming solution for contraception or HRT is required to supply not only a sufficient amount of ethinylestradiol but also the necessary amount of a suitable progestin to the systemic circulation.

Based on this requirement the selected core formulation was tested concerning the simultaneous delivery of ethinylestradiol and levonorgestrel as progestin. Levonorgestrel (LN), a testosterone derivative (M_r = 312.45, log P_{oct} ≈ 3.5, Fig. 29), was chosen from the group of progestins due to its high potency [8] and the consequently low required dose. Furthermore it was one of the three progestins (levonorgestrel, norelgestromin and norethindrone acetate) that were already used in commercial transdermal patches. For the first experiments the core film forming solution was loaded with 5% of EE (corresponding to the drug loading in previous experiments) and 0.3% of LN (w/w in the solution). 0.3% was the highest LN concentration that could be reached in the formulation without the additional incorporation of a solubilizer. The drug permeation from this formulation was then investigated in the biologically relevant heat separated human epidermis model.



Levonorgestrel

Fig. 29: Chemical structure of levonorgestrel

In a first step the film forming solution with EE and LN was compared to the corresponding mono formulations containing either EE or LN to measure possible interactions between the two drugs with effect on the drug permeation. In the next step the impact of the polymeric formulation was investigated. In previous experiments the polymeric formulation as such had acted as moderate permeation enhancer for EE (chapter 4). Moreover, the effect of additional chemical penetration enhancers on the permeation of the two drugs was studied. A binary mixture of propylene glycol and oleic acid (1:1) was chosen as chemical enhancer. This combination had considerably improved the EE permeation in earlier experiments (chapter 4) and was expected to have a positive effect on the LN permeation as well. The efficiency of a permeation enhancer is closely connected to the chemical properties of the drug substance [9] and both drugs, LN as well as EE, are hormones with a steroidal structure and similar lipophilicity ($\log P_{oct}$: EE ≈ 3.7, LN ≈ 3.5). Finally, the LN formulation with and without enhancer was compared to a marketed transdermal patch containing LN to evaluate if a corresponding delivery level for LN could be reached with the developed formulation. Since no contraceptive patch with LN was available, the Fem7® Combi patch ("Phase 2") for hormone replacement therapy was selected as reference. The film forming composition was further optimised to match the drug delivery of this commercial patch in vitro.

3. Materials and Methods

3.1. Materials

Ethinylestradiol (EE) and levonorgestrel (LN) were supplied by Schering AG, Berlin, Germany. polyurethane-14 and AMP-acrylates copolymer (DynamX®) was kindly provided by National Starch and Chemical Company, Bridgewater, USA. Ethanol (96%), triethyl citrate and propylene glycol were purchased from Merck, Darmstadt, Germany. Oleic acid (super refined) was a gift from Croda GmbH, Nettetal, Germany. Propylene carbonate was kindly provided by Huntsman Corp., Zaventem, Belgium. Fem7® Combi patches "Phase 2" (Solvay Arzneimittel GmbH, Hannover, Germany) were purchased from a local pharmacy. Fem7® Combi "Phase 2" is a matrix patch (A = 15 cm^2) containing 1.5 mg estradiol hemihydrate and 1.5 mg levonorgestrel. All chemicals used for the phosphate buffered saline were of analytical grade and purchased from Merck, Darmstadt, Germany. γ-Cyclodextrins were kindly provided by Wacker, Eddyville, USA.

3.2. Preparation of the tested formulations

For the preparation of the film forming solutions the drug substances (5% EE, 0.3% LN, w/w) were dissolved in ethanol (96%). Having obtained a clear solution the polyurethane-14 and AMP-acrylates copolymer (10% w/w), dissolved in a mixture of water and ethanol, was added and the preparation was stirred overnight for complete dissolution of the polymer. Finally, the remaining ingredients such as plasticizer (triethyl citrate) or enhancer (oleic acid/propylene glycol) were added and the solution was stirred for another 24 hours before use. The formulations were stored in glass vials sealed tightly with a siliconized rubber plug and an aluminium vial cap. For the ethanolic reference solution 5% EE and 0.3% LN (w/w) were dissolved in ethanol (96%).

3.3. Permeation experiments

The skin used for these experiments was obtained from Caucasian patients who had undergone abdominal plastic surgery. The patients were in good health and had no medical history of any dermatological disease. The approval from the ethics committee of the 'Caritas-Traegergesellschaft Trier e.V.' was available.

The preparation of the epidermal sheets and the experimental setup for these experiments has been previously described (chapter 3, sections 3.3, 3.4).

The experiment was started after the application of a defined amount of the formulation to the epidermis. For the experiments where the simultaneous application of EE and LN was tested 100 mg of the preparations (56.8 mg/cm^2) were applied to the membrane with a pipette in order to avoid any mechanical pressure on the epidermis. For the comparisons with the marketed patch, however, only 20 mg of the preparations (11.4 mg/cm^2) were applied due to the fact that larger amounts tend to run-off from the application site under actual exposure conditions [10]. In these experiments a soft brush was carefully used for the application to ensure complete coverage of the membrane with this comparatively small amount of preparation. The donor compartment was kept open throughout the whole experiment. For the comparison with the commercially available transdermal patch circular discs with a diameter of 1.5 cm were prepared from the patches with a scalpel and pressed carefully to the epidermis before mounting the membranes between the upper and the lower part of the diffusion cells. The permeation experiments (four replicates per formulation) were conducted over a 24 hours period. During this period samples (200 µl) were drawn at predetermined intervals and replaced by aliquots of the

receptor fluid. The saturation concentrations c_s of ethinylestradiol and levonorgestrel in the receptor solution were $c_{s\ ethinylestradiol} = 557$ µg/ml and $c_{s\ levonorgestrel} = 123$ µg/ml, sink conditions were maintained at any time.

Direct comparisons between different formulations within one set of experiments were always performed with skin samples from the same donor.

3.4. Chromatographic analysis

The samples from the permeation experiments were analysed for ethinylestradiol or levonorgestrel by HPLC (autosampler model 717plus, pump model 600, all Waters, Milford, USA). No sample pre-treatment was required. The solid phase used was a reversed phase column (Lichrospher 100 RP 18, 125 x 4mm, 5 µm) at ambient temperature. The mobile phase was acetonitril/water (1:1) at a flow rate of 2.0 ml/min. Ethinylestradiol was detected with a fluorescence detector (SFM25, Kontron, Zurich, Switzerland) using a wavelength of 280 nm for excitation and 310 nm for emission [11] after approximately 2.0 minutes. LN was detected with an UV-VIS detector model 468 (Waters, Milford, USA) at 243 nm after approximately 3.1 minutes. The method provided good precision and linearity in the required concentration range (EE: 0.1 – 25 µg/ml, $R^2 = 0.9999$; LN: 0.05 – 10 µg/ml, $R^2 = 0.9999$). The chromatography software used was Millennium® (Waters, Milford, USA).

4. Results

4.1. Simultaneous delivery of EE and LN from a film forming solution

Fig. 30 compares the simultaneous delivery of EE and LN from a film forming solution to the delivery of both hormones from film forming preparations containing only one of the drugs. The permeation curves from the formulation with the drug combination corresponded closely to the curves shown by the mono preparations with EE or LN. No mutual influences on the permeation behaviour of the two drugs was observed at the tested concentrations.

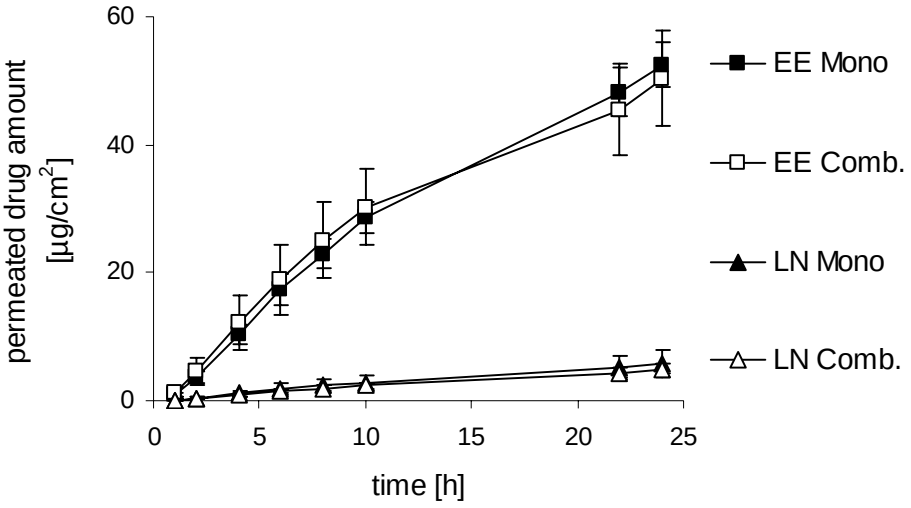


Fig. 30: Drug permeation through human epidermis from a film forming solution with two drugs (= comb.) in comparison film forming solutions with only one drug (= mono); drug content: 5.0% EE, 0.3% LN (w/w) in the solution; mean values ± standard deviation, n = 4

4.2. Permeation of EE and LN from film forming solutions with and without chemical enhancer

Fig. 31 and Fig. 32 show the delivery of EE and LN from the developed film forming solutions with and without enhancers and from a polymer-free reference solution of the drugs in ethanol. The delivery of LN was apparently increased by the presence of the polymer and/or the plasticizer as the polymeric formulation displayed higher permeation values than the polymer-free reference solution. This was similar to the effect seen for EE. The factor by which the

permeation from the polymeric solution exceeded the permeation from the polymer-free preparation was lower for LN than for EE (2.9 for LN versus 4.2 for EE). The incorporation of 5% (w/w) of oleic acid and propylene glycol in a binary mixture (1:1) increased the permeation of LN further, similarly to EE. Again the enhancement was more pronounced for EE than for LN (factor 8.9 for EE versus 5.0 for LN in comparison to the polymer-free ethanolic reference solution with the two drugs).

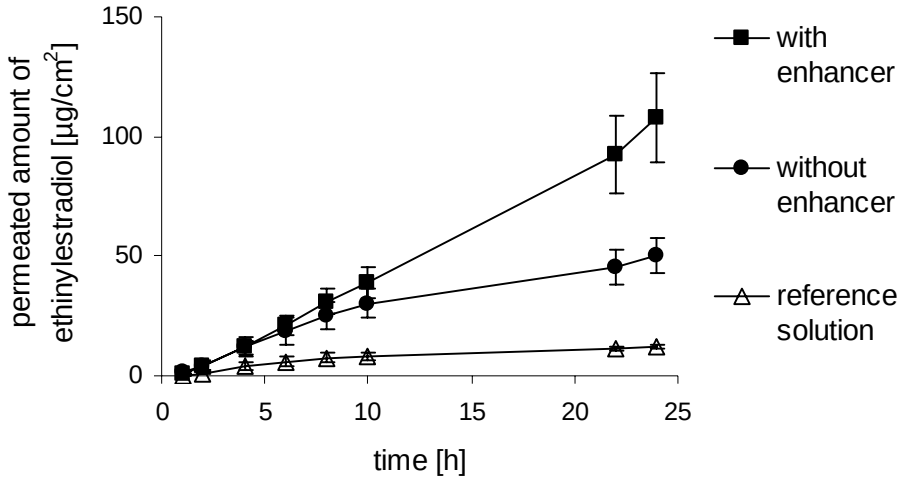


Fig. 31: EE permeation through human epidermis from film forming solutions with EE and LN with and without enhancer (oleic acid/propylene glycol 1:1, 5% w/w) and from an ethanolic reference solution; drug content: 5.0% EE, 0.3% LN (w/w) in the solution; mean values ± standard deviation, n = 4

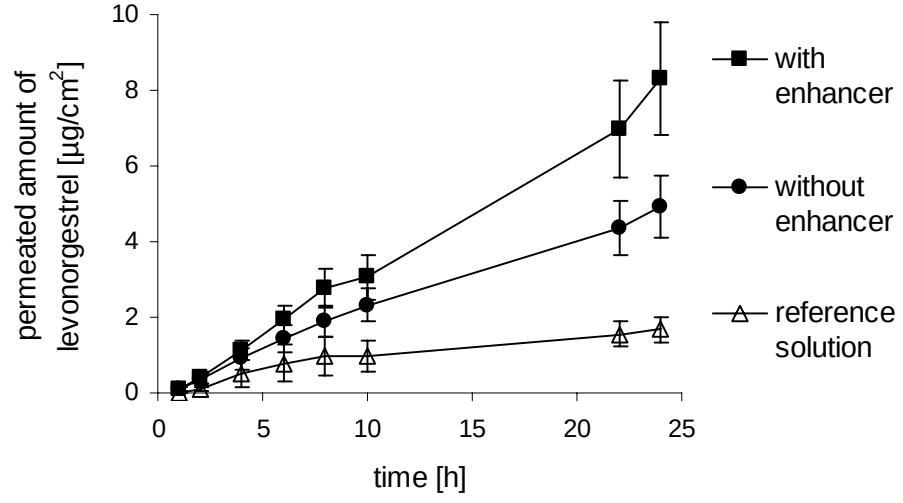


Fig. 32: LN permeation through human epidermis from film forming solutions with EE and LN with and without enhancer (oleic acid/propylene glycol 1:1, 5% w/w) and from an ethanolic reference solution; drug content: 5.0% EE, 0.3% LN (w/w) in the solution; mean values ± standard deviation, n = 4

4.3. Comparison of the film forming solution to a transdermal patch

Fig. 33 displays the LN permeation from the film forming solutions with or without 5% (w/w) of propylene glycol and oleic acid (1:1) as enhancer in comparison to the permeation from the commercially available Fem7® Combi patch. Neither the enhancer-free formulation nor the preparation with the chemical enhancer mixture was able to reach the delivery level of the commercial patch after 24 hours. Therefore an optimisation of the formulation was required. In order to increase the LN permeation from the film forming system three different approaches were selected: Firstly, the drug loading was increased from 0.3% to 0.4% LN by incorporating 5% (w/w) of propylene carbonate as solubilizer for LN. Secondly, the oleic acid content in the binary enhancer mixture was increased from 2.5% to 3.75% (w/w) while the propylene glycol content was reduced from 2.5% to 1.25% (w/w) to keep a total enhancer content of 5% (w/w). And thirdly, the total enhancer content was raised from 5% to 10% (w/w) of the binary oleic acid/propylene glycol (1:1) mixture. All three approaches had a positive effect on the LN permeation as demonstrated in Fig. 34. However, of the three variations the increase of the total enhancer content showed the highest improvement. By raising the total enhancer content from 5% to 10% a drug delivery level similar to that of the Fem7® Combi patch could be reached in vitro as displayed in Fig. 35.

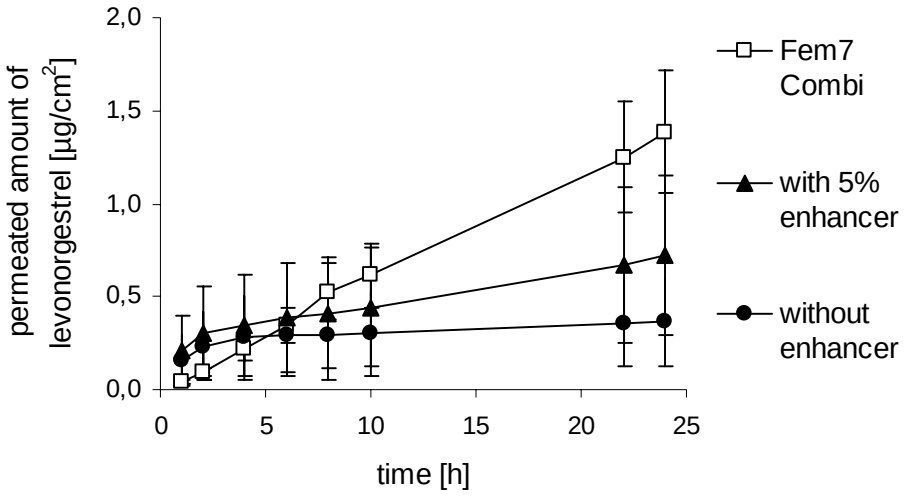


Fig. 33: LN permeation from film forming solutions with and without enhancer (oleic acid/propylene glycol 1:1, 5% w/w) and from the Fem7® Combi patch through human epidermis; LN dose: 0.034 mg/cm² in the solution, 0.100 mg/cm² in the patch; mean values ± standard deviation, n = 4

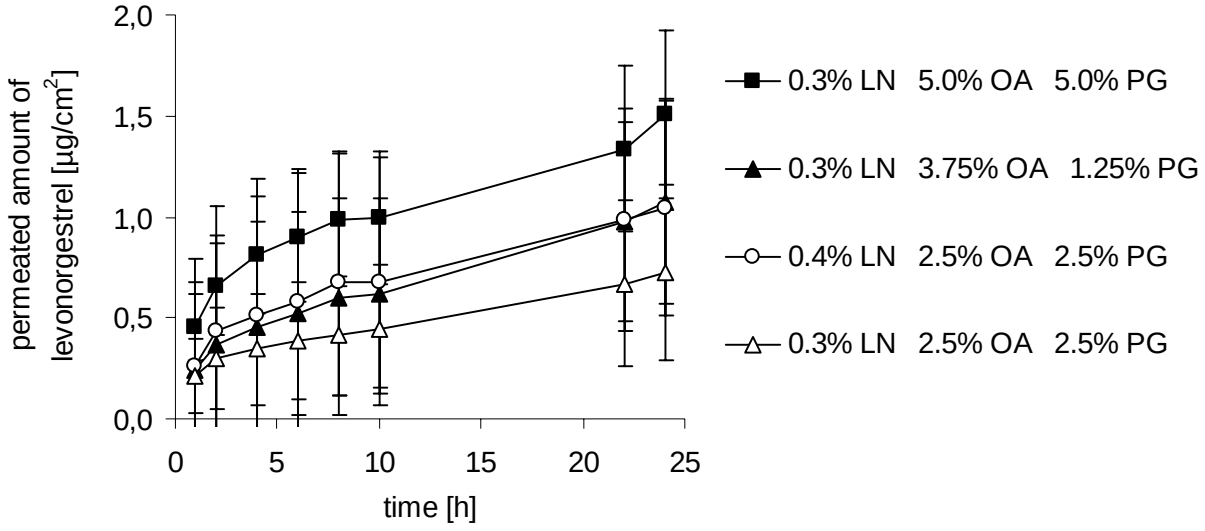


Fig. 34: LN permeation through human epidermis from film forming solutions with different contents of levonorgestrel (LN), oleic acid (OA) or propylene glycol (PG); concentrations (w/w) in the solution; mean values ± standard deviation, n = 4

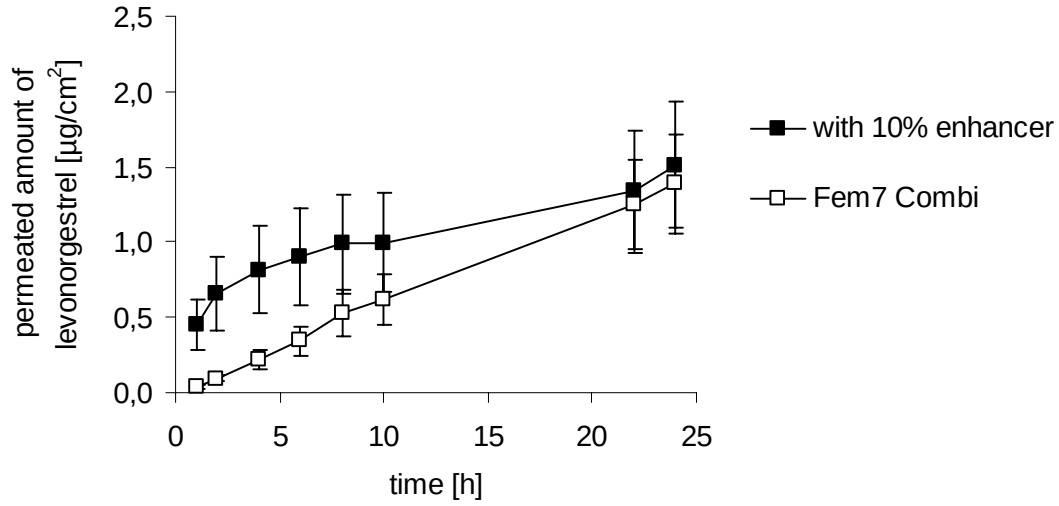


Fig. 35: LN permeation from the optimised film forming solution with enhancer (oleic acid/propylene glycol 1:1, 10% w/w) and from the Fem7® Combi patch through human epidermis; LN dose: 0.034 mg/cm² in the solution, 0.100 mg/cm² in the patch; mean values ± standard deviation, n = 4

5. Discussion

5.1. Delivery of LN from film forming solutions through human epidermis

Based on the previous positive results with EE the aim of this study was to continue the investigation on the film forming solution as delivery system for steroidal hormones for contraception or HRT. The results of the permeation experiments with the estrogen EE and the progestin LN indicate that the simultaneous delivery of both drugs from the developed film forming polymeric solution seems generally feasible. Up to 0.3% of LN could be dissolved in the polymeric formulation with EE without causing any solubility problems during manufacturing. Also no crystallization with a negative impact on the drug flux occurred in the formed film (microscopic observation) in spite of the higher total drug content. No mutual influences on the permeation of the two drugs, neither positive nor negative, were observed as demonstrated in Fig. 30.

The polymeric formulation itself and the chemical enhancers propylene glycol and oleic acid in a total concentration of 5% had a positive effect on the permeation of both drugs (Fig. 31 and Fig. 32). However, the delivering efficiency of the polymeric system was fairly low for both drugs as less than 3% of the applied drug amounts permeated through the epidermis in 24 hours. In spite of the positive impact of the enhancer mixture on the LN permeation it was not possible to reach a similar delivery level as the commercial HRT patch that was used as 'positive control' (Fig. 33). One reason for this was certainly the solubility related low drug loading of the film forming system that resulted in a lower LN dose compared to the patch (0.034 mg/cm^2 in the film forming solution versus 0.100 mg/cm^2 in the patch). Therefore a further optimization of the formulation was required.

The first attempt to improve the LN delivery through the epidermis was to increase the drug loading of the preparation with the help of a solubilizer. A higher drug loading leads to a higher concentration gradient at the membrane and consequently a higher drug flux. The concentration of the solubilizer was confined to 5% to limit the expected changes of the film properties (drying time, adhesion) due to the additional presence of the non-volatile solubilizer. In a preliminary experiment several co-solvents such as propylene glycol, polyethylene glycol and propylene

carbonate in the aforementioned concentration of 5% were tested as solubilizers for LN. Only propylene carbonate achieved an increase in the LN loading from 0.3% to 0.4%. Apart from these solubilizing qualities propylene carbonate possesses additional positive properties. It is miscible with ethanol, non-toxic for the administration on the skin and non-irritant [12] and was therefore considered a suitable solubilizer for LN in the film forming composition. The increase in drug loading from 0.3% to 0.4% LN (factor 1.3) achieved by propylene carbonate resulted in a similar increase in permeation (factor 1.4). This was in accordance with our earlier findings for EE where the permeation rose linearly with the drug content of the tested formulation (chapter 3). An additional enhancing effect of propylene carbonate itself, as described for other co-solvents in the literature [13, 14], was not observed. Although the LN permeation increased with this measure the improvement was not sufficient to reach the delivery rate of the commercial patch Fem7® Combi.

Another attempt to improve the LN delivery was to increase the oleic acid concentration in the formulation. Oleic acid is assumed to be the main enhancing principle in the utilized binary enhancer mixture as it also showed a permeation promoting effect when administered without a co-enhancer (chapter 4). In contrast to this, propylene glycol had a rather negligible enhancing effect when used as sole enhancer (chapter 4). It serves rather as co-solvent in the mixture to promote the partitioning of its co-enhancer into the skin which subsequently increases the enhancing efficiency of oleic acid [15]. One approach to increase the oleic acid content in the formulation was to simply double the concentration of the binary enhancer mixture. An increase of the total enhancer content, however, could have a non-desirable side effect. The non-volatile enhancer compounds might have a plasticizer-like effect in the formed film with a negative impact on the film properties such as drying time or adhesion/stickiness (chapter 2). Therefore another approach was to keep the total enhancer content at 5% but to shift the balance between the two enhancers in favour of the oleic acid portion in the mixture (from 50% to 75%, corresponding to a total oleic acid content of 3.75% instead of 2.5%). However, the improvement of the LN permeation that was achieved with this measure was not sufficient to reach the targeted delivery level. Only the doubling of the total enhancer content in the 1:1 mixture from 5% to 10% (5% oleic acid, 5% propylene glycol) produced a LN permeation similar to that of the marketed product after 24 hours (Fig. 35).

Although a similar drug concentration in the receptor compartment was achieved by the optimized film forming solution the permeation profiles of the two dosage forms differed. While the patch showed a steady permeation during the experiment the film forming solution displayed

a high permeation in the early stages followed by a decrease in flux (Fig. 35). However, such a permeation profile cannot be considered typical for the film forming system at this point as it was not observed in experiments with the skin of another donor.

The results of the enhancer variations supported our assumption that the oleic acid content is the dominating factor for the permeation enhancing effect. With rising oleic acid content the LN permeation rose, although not linearly probably due to the synergy of oleic acid with propylene glycol. This effect was similar to the observation made with EE in earlier studies (chapter 4). Surprisingly, the formulation with 3.75% oleic acid fit well into this picture in spite of the lower propylene glycol content in comparison to the tested formulations where both enhancers had been present in equal parts (Fig. 36). Although the presence of propylene glycol is required for a synergistic effect [15-17] it is apparently not necessary to supply the propylene glycol in the same quantity as the oleic acid. This observation might be useful in the future for a further optimization of the enhancer mixture in the film forming formulation. Possibly the propylene glycol portion and with it the total enhancer content could be reduced in order to minimize possible changes of the film properties without losing the synergistic permeation enhancing effect of the binary enhancer mixture.

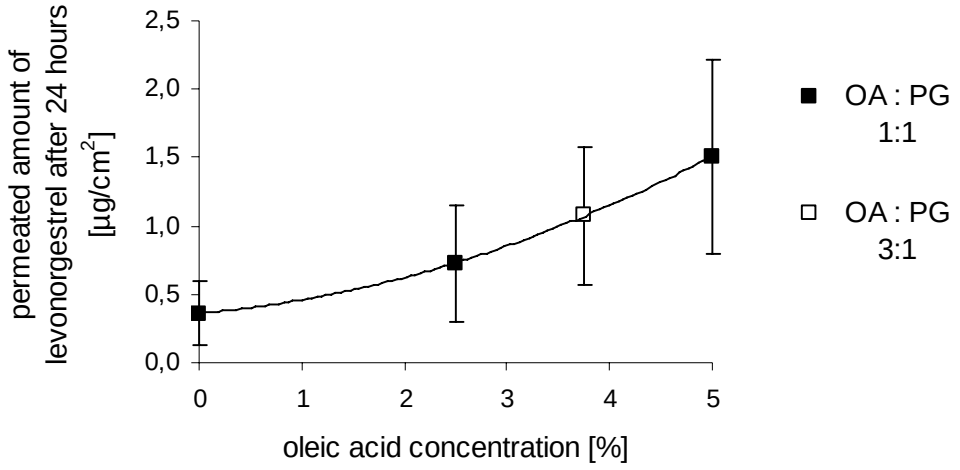


Fig. 36: LN permeation from film forming solutions with different oleic acid concentrations and varying oleic acid (OA) / propylene glycol (PG) ratios through human epidermis; LN content: 0.3% LN (w/w) in the solution; mean values ± standard deviation, n = 4

5.2. Further optimization steps for a film forming contraceptive system

The in vitro comparison of the improved film forming solution with the marketed transdermal patch (Fig. 35) indicates that the developed film forming system has a certain potential as transdermal delivery system for steroidal hormones. This is at least the case for HRT as this is the indication of the Fem7® Combi patch. It remains subject to further research, however, if the film forming system with EE and LN can also be utilized for contraceptive purposes as the required LN doses for contraception might be higher than for HRT.

Due to the lack of a contraceptive patch with LN a direct comparison of the film forming system with a registered product, that evidentially delivers a sufficient amount of LN for contraception, was not feasible in the in vitro model. In the literature LN delivery rates of 35 µg/day [18] for mono products or 5 – 50 µg/day [19] for combination products with 25 – 50 µg estradiol per day are suggested for contraception. Provided that the results of the in vitro experiments are reproducible in vivo and that the developed film forming system can indeed deliver the same LN amount as the Fem7® combi patch (10 µg/day [20]), the LN delivery would be in the lower range or even below the required daily dose of LN for a contraceptive application. It is therefore highly probable that further optimization steps are needed to further improve the progestin delivery from the film forming system.

For the above mentioned delivery rate of 10 µg/day the previously tested film forming formulation with 0.3% LN and 10% of the enhancer combination oleic acid/propylene glycol (1:1) would have to be applied with a formulation dose (D_{Form}) of approximately 11.4 mg film forming solution/cm^2 (corresponding to the dose in the in vitro study) on an area (A) of 15 cm^2 (corresponding to the size of the Fem7® combi patch). This would provide a drug dose (D_{LN}) of 34.2 µg/cm^2 and a total dose ($D_{LN\ Total}$) of 513 µg LN. A first measure to improve the LN delivery without changing the actual composition of the formulation could of course be to simply increase the total applied LN dose ($D_{LN\ Total}$). This could be achieved in two ways, either by enlarging the application area (A) without changing the formulation dose (D_{Form} = 11.4 mg/cm^2) or by increasing the formulation dose (D_{Form}) while keeping the size of the application site constant (A = 15 cm^2). However, both measures have their limitations. Application sites exceeding 20 – 30 cm^2, for example, are unlikely to be accepted by most patients. Applying a higher formulation dose per surface area, on the other hand, holds the risk that part of the

formulation flows away from the application site before drying [10] due to the low viscosity of the formulation. This would lead to an uncontrolled enlargement of the application site (A) and variations in the film thickness and thus the LN dose / surface area (D_{LN}). In addition to this, a higher formulation dose / surface area (D_{Form}) might result in thicker films with a stronger skin fixation and a more pronounced wrinkling of the skin (chapter 2), which is aesthetically unattractive and might have a negative impact on the patient compliance.

Apart from these merely physical measures another option to improve the LN delivery from the system might be a quantitative or qualitative change of the film forming composition. Quantitative changes in the composition with a positive effect on the LN delivery might include a raise of the LN loading with the help of a solubilizing agent or a further increase of the oleic acid enhancer content. However, such changes also have their limitations as they might negatively affect the film properties (as described earlier) or might be problematic concerning the tolerability of the formulation (oleic acid is known to be moderately irritant [21, 22]). Qualitative changes in the film forming composition with a possible positive effect on the delivered LN amount could for example be the exchange of the solvent ethanol against a mixture of ethanol with ethyl acetate [23], the incorporation of a different permeation enhancer for LN (the tested enhancer mixture was very efficient for EE, but might not be ideal for LN) or the usage of a pro-drug instead of LN. Friend for example reported an increase of the LN delivery by factor 32 with Levonorgestrel-Glycidol [24]. The improvement was explained with a more favorable hydrophilic lipophilic balance of the drug that led to a higher solubility in the different domains of the skin and to higher permeation values in spite of the increased size of the molecule.

Should all the discussed measures fail to increase the LN delivery to the required level, however, it might also be an option to replace LN by another progestin to develop an efficient contraceptive film forming solution. Suitable alternatives for LN might be progestins with either a higher potency than LN (resulting in a lower daily dose), a higher skin permeability (which would facilitate reaching the required daily dose) or simply a higher solubility in the volatile solvent of the formulation (to achieve a higher drug loading of the system). One example for an alternative progestin is 16-methylene-17α-acetoxy-19-norprogesterone (Nestorone®), a progesterone derivative that is soluble in ethanol, has a higher progestational activity than LN [8, 25] and is currently in clinical trial for the transdermal application in a metered dose topical

aerosol [26]. However, as the substance is still protected by intellectual property [27] it was not available for purchase at the time of our study.

In summary it can be stated that several possibilities are available for a further optimization of the progestin delivery from the film forming system should the LN delivery from the tested system be insufficient for contraceptive purposes. However, further research and especially in vivo studies are required to clarify if this is actually the case.

6. Conclusion

The developed film forming system with polyurethane-14 and AMP-acrylates copolymer (DynamX®) as film former was able to simultaneously deliver an estrogen and a progestin, ethinylestradiol and levonorgestrel, through heat separated epidermis in vitro. The polymeric formulation itself and the presence of oleic acid/propylene glycol as chemical enhancers had a positive effect on the permeation of both drugs. The developed formulation was capable of transporting a similar amount of LN through the epidermis than the commercial Fem7® Combi patch for HRT although a total enhancer content of 10% (oleic acid/propylene glycol, 1:1) was required to achieve this result. Even though a further optimization of the system should be considered for the LN delivery these results underline the potential of the film forming polymeric solutions and encourage the further development of the tested film forming formulation as transdermal delivery system for steroidal hormones such as EE or LN.

7. References

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CHAPTER 6

Final discussion: Film forming polymeric solutions – novel drug delivery systems for the skin?

1. Introduction

The aim of this work was to investigate film forming polymeric solutions as alternative dosage form for the skin. The results of the performed formulation and permeation experiments were encouraging for a further development of film forming systems for transdermal drug delivery. However, the investigations also revealed certain limitations of the novel dosage form with regard to the selection of excipients or the drug delivery. For a future use of this type of delivery system it is therefore important to discuss opportunities and limitations concerning the formulation compounds, to evaluate the drug delivering capacity of the formulations and to reflect on further aspects concerning the practical application that have not been addressed yet. Finally, the advantages and disadvantages of the film forming systems in comparison to the conventional dosage forms will be discussed.

2. Considerations for the composition of film forming polymeric solutions

2.1. Suitable drugs

The preferred application for film forming polymeric solutions is clearly the transdermal drug delivery. The usage of the developed formulations for dermatological indications is theoretically possible as well but cannot be recommended due to the nature of the solvent in the compositions. Dermatological diseases are often associated with inflamed skin where the administration of ethanolic solutions might be painful for the patient and thus not acceptable.

For a transdermal application suitable drugs have to fulfil certain requirements that are independent of the dosage form [1]: Due to the fact that the skin is a very efficient protective barrier for the body, not merely against physical or microbiological noxes but also against drugs, only potent drugs are eligible for this application route with a daily dose of less than 10 mg. The size of the molecule is required to be small in order to provide a sufficient mobility in the skin structures (molecular weight below 500 Da). As the drug has to pass lipophilic as well as hydrophilic areas in the skin on its way into the systemic circulation it is advantageous if the drug is neither very hydrophilic nor extremely lipophilic (log P_{oct} between 1 and 3). Uncharged molecules show a better permeation than charged molecules as they do not interfere with

charged moieties of the skin such as the negatively charged keratins. Therefore molecules with a pH value between 5 and 9 in aqueous solution are preferred for the transdermal application. Further parameters that are beneficial for the transdermal delivery of a drug are a small number of hydrogen bonding groups (< 2) and a low melting point [2].

Besides these general requirements for transdermal drugs some other points have to be considered for the film forming solutions in particular.
The reservoir size of the dosage form is comparatively small due to the extreme thinness of the films (approximately 5 – 25 µm). With a formulation dose of 10 mg/cm^2 and an application area with an acceptable size of for instance 30 cm^2 the total applied formulation dose would be 300 mg. Assuming a drug loading of 10% (which is higher than the drug loading that was used in this work) the applied drug dose would be approximately 30 mg. In order to reach the limit daily dose that is assumed for transdermal patches (10 mg/day) more than 30% of the applied dose would have to be absorbed. Such a high absorption was not achieved with the steroidal hormones that were investigated in this work. With these drugs the absorption was clearly below 10% of the applied dose. Taking this into account it seems obvious that the film forming solutions will be mainly attractive for drugs that have

1. a high potency (example: the progestin Nestorone®)
2. a high skin permeability (example: nicotin)
3. a high solubility in the solvent (example: ethinylestradiol).

A high potency is beneficial as it results in low required daily doses for the drug. A high skin permeability promotes a high exploitation of the thin reservoir provided a suitable polymeric matrix is given. A high solubility in the formulation, finally, allows high drug loadings and the establishment of a high gradient between formulation and skin. Ethinylestradiol for example, one of the therapeutically relevant drugs used in this work, fulfils the requirements in terms of high potency and sufficient solubility in the solvent. Levonorgestrel on the other hand, is also highly potent, but dissolved to a much lesser extent in the solvent than ethinylestradiol (0.3% versus 5%). However, drugs with less favourable properties such as levonorgestrel are not generally excluded from an application with this dosage form as additional measures can be taken to improve the performance of the delivery systems (usage of pro-drugs, enhancers etc, see chapter 5).

An efficient delivery system, meaning a high exploitation of the drug reservoir throughout the wearing time, is generally desirable for all drugs. If this cannot be achieved the film forming system might not be attractive for expensive drugs due to the considerable portion of drug that is wasted.

It remains subject to further research if the film forming solutions are suitable for drugs with a narrow therapeutic window as the kinetics of the delivery system are not yet known. With respect to the novelty of the film forming systems a thorough and individual evaluation for each new drug candidate is still inevitable until a clearer picture of the capabilities of the film forming solutions in general has been gained.

2.2. Appropriate excipients

2.2.1. Polymer

The excipient that predominantly determines the properties of the film forming system is the polymer (chapter 2). The polymer influences the viscosity of the formulation and is responsible for the visual appearance of the formed film. In co-operation with the plasticizer it determines the flexibility of the film and its adhesion to the skin. Furthermore, the polymer has an impact on the solubility and the physical stability of the drug in the film for example by acting as crystallization inhibitor (chapter 4).

The prerequisites for the polymer selection for this type of dosage form have been discussed in chapter 2. In short the polymer is required to be able to form films at the skin surface temperature (28°C-32°C) and should have a certain inherent flexibility and affinity to the skin to avoid the usage of excessive amounts of plasticizer. It has to be soluble in a highly volatile, skin-friendly solvent. Moreover, strong gelling agents should be avoided as film former as they prevent an application of the formulation by spraying (which is the preferred option, see below).

In spite of the many requirements the polymer screening experiments have demonstrated that the majority of the tested polymers could be formulated into a film forming composition with suitable macroscopic properties. Only four of the 14 tested polymers (chitosan, polyisobutylene, polyvinyl alcohol, polyvinylpyrrolidone, chapter 2) lacked some of the required properties and were therefore abandoned. The fact that the successfully utilized polymers differed widely in

their chemical structure (chapter 2, appendix 1) indicates that the formulation of this dosage form is not limited to certain polymer groups. It is highly probable that many more candidates for film forming solutions can be identified among the numerous polymers that are available on the market.

Although various polymers yielded films with suitable macroscopic properties, however, the permeation studies have shown that some polymers are superior to others with respect to the drug delivery (chapters 3 and 4). The results indicated that the polymers do not only immobilize the drugs in a matrix on the skin, but that they may also have an enhancing (in case of the DynamX® formulation) or a retarding (in case of the Eudragit® RL formulation) effect on the drug permeation. These effects can result on the one hand from complex interactions of the polymeric formulation with the skin, on the other hand also from interactions of the polymer with the drug (chapter 4). The extent of the latter is specific for each drug – polymer combination, depending on the physico-chemical properties of the two compounds such as charge or lipophilicity. This should be kept in mind for the selection of a film forming formulation for a new drug candidate. The formulation with DynamX® as film former achieved good results with two drugs of different polarity (chapter 3) in this study. The investigated drug molecules were either weakly acidic (ethinylestradiol, no charge or negative charge due to deprotonation of the phenolic ring) or a weak acid/weak base combination (caffeine, positive and negative charge at the same time at neutral pH values). However, in the enhancer free film forming solutions (pH 6 – 8) the drugs were practically non-charged. Therefore the question remains open if the formulation with the anionic film former DynamX® is also favorable for charged molecules, especially if the drugs are positively charged.

2.2.2. Solvent

The solvent is also a very important compound in the film forming solution although it is not part of the actual film on the skin due to its quick evaporation. The solvent must have a sufficient solubility for the polymer as well as for the drug. Only a high solubilizing power of the solvent for the drug allows substantial variations of the drug loading to modulate the drug delivery to the skin (chapters 3 and 5). Apart from this indirect impact on the permeation the solvent can also exert a direct influence on the drug flux. Depending on the nature of the solvent and its permeation enhancing properties it can promote the drug transport to different extents in spite of its short contact time with the skin (chapter 3). This should be kept in mind for a further formulation development.

In addition to its solubilizing properties for the polymer and the drug a suitable solvent for a film forming solution is required to be highly volatile to provide short drying times and thus a good patient compliance. Together with the polymer it is supposed to spread well on the skin after application to produce a smooth film with a uniform thickness on the application site. Both requirements are not met for example by the solvent water. During the formulation experiments an aqueous chitosan formulation displayed unacceptably long drying times (chapter 2) and an uneven spreading on the skin due to the high surface tension of the aqueous polymeric formulation. Consequently, water cannot be considered a suitable solvent for the formulation of a film forming polymeric composition. Solvents such as ethanol, isopropanol or ethyl acetate with a higher volatility and a better spreading are to be preferred.

2.2.3. Plasticizer

In polymeric applications the main purpose of a plasticizer is to facilitate the film forming and to increase the flexibility of the resulting film. Additionally, the formulation experiments have shown that the skin adhesion of the films can be modulated with the help of plasticizers (chapter 2).

The plasticizer has to be thoroughly selected with regard to the film former. It has to miscible with the polymer to produce clear films with low visibility on the skin. Since the efficiency of a plasticizer is polymer dependant no general rule can be applied as to which plasticizer content is required to produce films with the desired properties. The individual determination of the adequate plasticizer content is inevitable. An insufficient amount of the excipient leads to brittle films with low skin adhesion. An excessive amount of plasticizer on the other hand results in smooth, but sticky films (chapter 2). Both results are unacceptable for a reliable drug delivery by the film forming system and a good patient compliance.

The plasticizer should preferably have a low skin permeability to prevent leaking from the formed film. A substantial leaking would not only raise safety concerns but would also lead to a deterioration of the film properties. In case of a loss of plasticizer the film becomes brittle and loses part of its adhesive properties. Such an effect was for example observed in a pre-experiment for the animal study with rats (chapter 4). Contrary to the behaviour of the DynamX® film forming solution on human skin or pig skin (chapters 2 and 4) the films on the rat skin

became brittle after less than two hours and started to flake off. It was speculated that the higher permeability of the rat skin in comparison to human or pig skin was responsible for this different behaviour. It might have facilitated the diffusion of the plasticizer from the film into the skin, leaving the brittle, plasticizer depleted film behind. Such an effect should be avoided to ensure a reliable skin contact of the film forming system throughout the full wearing time.

It is noteworthy that not all film formers required a plasticizer in the formulation experiments (chapter 2). The acrylate polymers Eudragit® NE 40D and Avalure® AC 118 as well as the silicone gum formed adequate films without the help of a plasticizing agent.

2.2.4. Further excipients

Apart from the basic compounds of a film forming polymeric solution (polymer, solvent and plasticizer) it can be appropriate to incorporate further excipients into the preparation.
For some polymers such as the acrylate Eudragit® E 100 it is beneficial to add a crosslinker (succinic acid) to the composition to improve the film stability. For some drugs a solubilizer or co-solvent can be required (chapter 5) in order to increase the drug loading of the formulation and the with it the drug flux. For the same purpose chemical enhancers can be included (chapters 4 and 5). Further examples for supplementary excipients are antioxidants to stabilize oxidation sensitive drugs in the preparation during storage, sun screens for the protection of photosensitive drugs or dyes to facilitate the localisation of the formed film for the patient.

A precondition for the incorporation of further excipients is the compatibility of the materials with all other compounds. Furthermore it has to be kept in mind that every change in the film composition might negatively affect the macroscopic properties of the formed film such as stability, adhesion to the skin or stickiness of the outer surface of the film. Therefore it is advisable to re-evaluate the macroscopic properties of the formed film after any adjustment of the composition.

3. Evaluation of the drug delivering capacity of film forming solutions

3.1. Efficiency

The results of the permeation experiments through heat separated human epidermis have demonstrated that the delivery of caffeine, ethinylestradiol and levonorgestrel was basically feasible with the developed film forming systems (chapters 3, 4, 5). For the therapeutically relevant drugs ethinylestradiol and levonorgestrel it was also possible to achieve similar delivery levels than commercially available transdermal patches (chapters 4 and 5). In spite of this positive result, however, it became also clear during the experiments that the delivering efficiency for the two steroidal hormones was limited. Less than 5% of the drug loading permeated through the epidermis in 24 hours even in the presence of chemical enhancers. The overall efficiency of the film forming system is closely connected to the length of the wearing period (see section 3.3). If a film with a drug release of less than 5% per day is worn only for one or two days (which is highly probable with respect to the observations in the pig study, chapter 4) a high percentage of the drug substance is wasted. This can be an economic issue. In case of water soluble polymers it can additionally be an environmental concern if the film with the residual drug is showered off and deposited with the sewage water.

For film forming solutions with drugs of low skin permeability such as most steroidal hormones [3, 4] it is therefore recommended to search for further options to improve the delivering efficiency of the polymeric system. Some options for this have been discussed in chapter 5 for the example levonorgestrel. Among these are the identification of the most efficient chemical enhancer or enhancer combination for the individual drug or the usage of pro-drugs such as esters that are activated by esterases in the skin. The usage of occlusive conditions is no option to improve the drug flux from the current film forming solutions as the developed systems were all non-occlusive (chapter 2). In order to use this effect further research would be required to identify polymers that fulfil the requirements listed in section 2.2.1 and that are additionally able to induce occlusive conditions under similarly thin films (5 – 25 μm).

If the need for a further optimization of the film forming system is actually required, however, is mainly dependant on the drug itself and its skin permeability. The delivering efficiency of the

film forming system for caffeine for example was considerably higher than for the steroidal hormones in spite of the low drug loading of the films. In the caffeine experiments between 10% and 15% of the applied dose permeated through the epidermis even without chemical enhancer. This underlines that a careful choice of drug is very important for the development of an efficient film forming system.

3.2. Delivery kinetics

Apart from the delivering efficiency in terms of absolute amounts or exploitation of the reservoir the delivering kinetics are an important feature of a dosage form. The steady plasma levels that are achieved by transdermal patches are one of the major advantages in comparison to oral dosage forms. However, from the performed in vivo study with pigs no clear information about the delivery kinetics from the tested film forming solution (with ethinylestradiol and DynamX® as film former) could be derived. Further research is necessary to gain more information in this respect and to investigate if the film forming solutions can provide a similarly steady drug supply as the transdermal patches.

3.3. Wearing time and removability

Most indications where transdermal dosage forms are administered require a permanent medication. While transdermal semisolid dosage forms have to be applied at least on a daily basis the conventional transdermal patches aim at longer dosing intervals (twice weekly or weekly application). It is supposed that most patients prefer a less frequent dosing and that a weekly application serves to improve the patient compliance.

With respect to the low depletion of the drug reservoir that was observed in the permeation experiments with ethinylestradiol and levonorgestrel (chapters 4 and 5) an application frequency for the DynamX® film forming solution of once weekly seems theoretically feasible. However, the observations during the in vivo study with pigs (chapter 4) suggested rather a shorter dosing interval. After one or two days the films on the animal skin began to display cracks and started to flake off. The observation led to the assumption that a wearing period of one or maximally two days can be achieved with the tested DynamX® formulation. This estimate is supported by the fact that the polymer is basically water-soluble, being originally designed for hair care applications. In spite of a certain water resistance the film is completely removed during the

showering and drying process (observation during the formulation experiments). Although the lack of water permanence reduces the possible dosing intervals for this particular formulation it is not purely a disadvantage. A positive aspect is that it also facilitates the handling of the medication. The patient can simply shower the remaining film off without residue and apply a new dose after the shower as part of his or her daily routine.

In contrast to the water-soluble film forming preparations, non water-soluble polymeric formulations (for example with the acrylate Eudragit® RL PO) might provide longer dosing intervals. However, in the course of time these films will suffer from abrasion by the patients clothing or will begin to flake off. At that point a replacement of the film is required to ensure a reliable drug delivery. Due to their extreme thinness and low cohesion the films cannot be peeled away. The non-water-soluble films have to be removed with the help of organic solvents such as ethanol or isopropanol. This is clearly a disadvantage compared to the water-soluble films as the frequent usage of organic solvents can be harmful for the skin. However, in case of emergencies such as life threatening adverse effects or allergic reactions the organic solvents allow the instant removal of the formed films.

Based on the observations in this study with the DynamX® formulation it can be assumed that the film forming systems are suited for a daily or possibly a twice weekly application. Even for the non water-soluble formulations a weekly application does not seem to be an option due to the thinness of the formed films. It is unlikely that they will be able withstand the mechanical strain exerted by the constant movement of the skin and the contact to clothing for as long as seven days. However, further studies on human skin are required to verify this estimate.

For the practical application the resistance of the formed films to perspiration will also have to be addressed in the further development of the film forming systems. With regard to transdermal patches heavy sweating can lead to adhesion failure and loss of the patch [5]. Therefore the influence of sweating on the films formed by the polymeric solutions has to be determined to investigate the reliability of the drug delivery under different climatic conditions and bodily exercise. This is especially important for the formulations with water-solube polymers as sweat is an aqueous medium [6].

4. Further aspects for the future development process

4.1. Tolerability

The tolerability of the formulation is a very important issue and should already be considered during the selection of the formulation compounds. A safety testing of the film forming solutions was not part of this investigation but has to be addressed during the further development of the film forming solutions. For a first orientation however, it can be noted that during the polymer screening process (chapter 2) and in the preliminary in vivo study in pigs (chapter 4) no signs for skin irritation were observed with the tested DynamX® formulation. Additionally, the tested film forming systems were found to be non-occlusive (chapter 2) which also reduces the risk of skin irritation. The incorporation of chemical penetration enhancers into the film forming formulations can be problematic in this respect as many enhancers are potential skin irritants. However, it should be taken into consideration that the tolerability of a formulation always has to be seen in relation to the benefits of the medication for the patient.

For future testing of the film forming solutions on human subjects it has to be kept in mind that sufficient safety data is not only required for the film forming composition but also for the single excipients in the formulation. This might generate considerable effort for excipients that are not monographed in a pharmacopoeia. For materials that are originally cosmetic ingredients (such as the acrylate polymer Avalure® AC 118 and the polyurethane-acrylate DynamX®) a basic toxicological characterization is often provided by the manufacturer, but the data is mostly not sufficient to allow the usage of the excipient in clinical trials.

4.2. Application device

One open question for the further development of film forming solutions as dosage form for the skin is the application device. Principally, a simple application with the help of a pipette or a brush is feasible. However, the most reliable way to administer the film forming liquid would be an application by spraying. This would ensure a controlled application, that is the administration of a precise volume on a defined skin area, with only minimal involvement of the patient. The use of a spraying device would also be very convenient for the patient [7], which is a prerequisite for a good patient compliance.

Suggestions for appropriate spraying devices are found in the literature. Morgan et al. for example developed a metered dose topical aerosol for the application of ethanolic solutions of steroidal hormones [8]. The device consisted of a pressurized aerosol container with a metered valve, a nozzle with a defined spray angle and a nozzle shroud to ensure a perpendicular position of the nozzle to the skin. A cross-sectional drawing of the device is shown in Fig. 37. Leichtnam et al. have suggested similar devices, pressurized and mechanical, for the application of an ethanolic testosterone spray [9]. Mechanical spraying devices are primarily used for liquids with low viscosities and have the advantage that the filling can be performed without special equipment. Pressurized devices on the other hand are more expensive to manufacture but can also deliver liquids with higher viscosities. They usually provide a finer droplet size and thus a more uniform distribution of the formulation on the sprayed surface than the mechanical devices [9].

In the formulation experiments one of the evaluation criteria was the viscosity of the polymeric solution and the target was to formulate solutions with low to moderate viscosity in order to provide the opportunity of an application by spraying. The DynamX® formulation fulfilled this criterion by displaying a low, water-like viscosity (chapter 2). A preliminary experiment with a mechanical pump spray device (100 µl per spray valve) indicated that a distribution of this formulation with a mechanical device was principally feasible. Therefore both options, pressurized or non-pressurized aerosols, can be considered for the application of this film forming system.

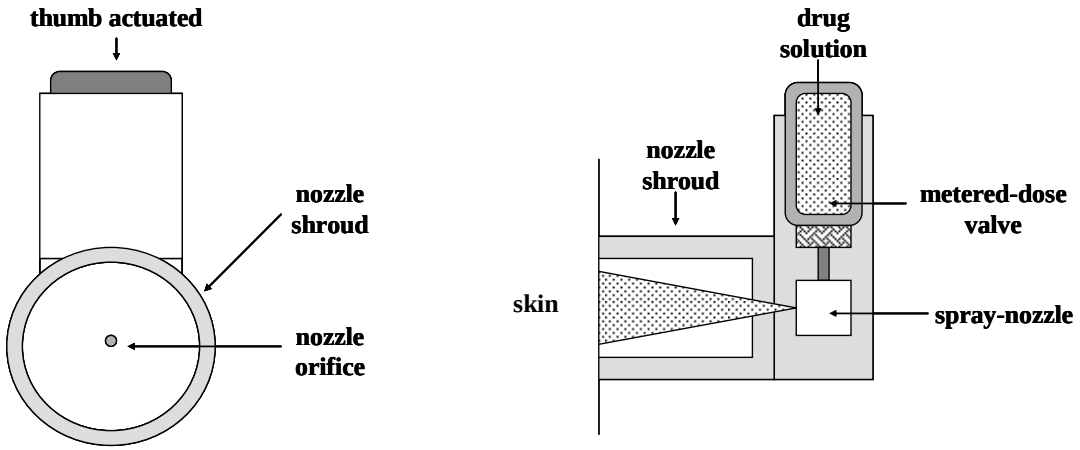

Fig. 37: Cross-sectional drawing of a metered dose spraying device according to Morgan [8] (modified)

The technical details of the application device such as valve volume, nozzle shroud length and diameter have to be adjusted to the individual formulation. In the formulation experiments (chapter 2) the evaluation of the film properties was based on an application volume of 10 mg of the polymeric formulation per cm^2. This dose was small enough not to flow away from the application site and can therefore be considered a target formulation dose for the spraying device. The size of the application area that is to be covered with the formulation depends on the delivering efficiency of the film forming system and the target daily dose of the delivered drug. It should not exceed 20 – 30 cm^2, on the one hand for reasons of patient compliance, on the other hand because a larger area would limit the locations on the body where the application device could be placed (the area should be even for a uniform distribution of the liquid). The application area together with the formulation dose then defines the valve volume, the targeted spray angle of the valve and the dimensions of the nozzle shroud.

Metered dose spraying devices have been used before with sufficient precision for the application of steroidal hormones [9, 10]. It is expected that a similar precision can be reached for the application of the film forming system. However, the dose uniformity and the even distribution of the drug loaded film on the skin will have to be confirmed once an appropriate application device has been developed.

An individualization of the dose is also imaginable. This could either be achieved by varying the number of sprays or by adjusting the valve volume (‘dial-a-dose’ principle) although the latter would require a more sophisticated valve technology.

5. Advantages and disadvantages of film forming systems in comparison to conventional transdermal delivery systems

5.1. Wearing comfort

The film formed on the skin by the polymeric solution is colourless, transparent and therefore almost invisible (chapter 2, Fig. 8). This is clearly an advantage in comparison to some commercial patches that are highly visible on the skin as illustrated in chapter 1, Fig. 7. The low visibility of the formed film could have a positive impact on the patient compliance especially in female patients who prefer a discreet medication [11].
Due to their thinness and flexibility the formed films are more comfortable to wear than the considerably thicker and more rigid transdermal patches, especially when these are of a large size.

Transdermal semisolids such as gels or creams are usually rubbed into the skin after application. They do not persist visually on the skin and are therefore as discreet as the film forming compositions. Semisolid formulations are usually comfortable to wear but may leave a sticky or greasy feel directly after application. This is avoided with the non-sticky and non-greasy film forming compositions (chapter 2).

5.2. Application

In contrast to the application of semisolid formulations the envisaged administration of the film forming solutions by spraying is convenient and non-messy for the patient, similar to the administration of conventional patches. However, for the application of the film forming system a spraying device is required that is not necessary for the other dosage forms.

With an appropriate spraying device the dosing of the film forming solution can be performed with a higher accuracy than in case of the semisolid formulations due to the precisely defined size of the application area. It remains an open question, though, if the high dosing accuracy of the transdermal patches can be matched.

Due to their liquid character the dosing flexibility of the film forming systems is higher compared to the conventional patches. For manufacturing reasons these are mostly offered in a limited number of doses only. A convenient flexibility can especially be provided for the sprayable formulations if a 'dial-a-dose' device can be realized as suggested by Thomas et al. for the Metered Dose Topical Spray [2].

5.3. Drug delivery

Concerning the application frequency the film forming solutions can presumably compete with daily patches or possibly twice weekly patches, but they are unlikely to reach the weekly applied transdermal systems in this respect. However, the pitfalls associated with weekly patches such as skin irritation, patch loss or persistent residues on the skin are also avoided.

In comparison to the semisolid formulations the application frequency of the film forming solutions is similar (daily) or possibly lower (every other day or twice weekly). Contrary to the semisolids, however, the film forming compositions can provide a sustained drug release during the full wearing period as the film represents an external drug reservoir on the skin. The polymeric matrix serves as fixation for the drug which is also an advantage because the drug cannot be wiped off contrary to the situation with semisolids. Drug loss or environmental contamination, which is especially problematic with highly potent drugs such as hormones, is therefore prevented.

Another point that can be both an advantage and a disadvantage is the non-occlusive property of the film forming system. Compared to many patches this can be an advantage as occlusion is often a source for skin irritation. On the other hand it can also be a disadvantage because occlusive conditions can have a penetration enhancing effect for some drugs and on certain areas of the body.

In the permeation studies with steroidal hormones only a low percentage of the drug loading had permeated from the film forming solutions through the epidermis in 24 hours (2.8% with ethinylestradiol, 4.4% with levonorgestrel). In the same time period 6.2% of the ethinylestradiol loading had permeated from the EVRA® patch and 1.4% of the levonorgestrel loading from the Fem7® Combi patch. Therefore the drug delivering efficiency in terms of exploitation of the reservoir was comparatively low in all tested systems. However, the transdermal patches are

designed for a wearing period of seven days. Such a wearing time will presumably not be achieved with the film forming systems. Therefore the weekly patches might have an advantage in this respect as the overall exploitation of their drug reservoir is higher.
However, the amount of comparative data gathered so far is not sufficient to draw any general conclusion concerning the delivering efficiency of one dosage form or the other. A direct comparison of the film forming solutions with semisolid formulations in this respect remains also subject to further research. Furthermore it has to be kept in mind that the efficiency of the drug permeation is predominantly dependent on the permeation properties of the drug and on the individual formulation (excipients, enhancers etc.) and less on the type of dosage form.

5.4. Costs

With regard to the manufacturing costs the film forming solutions have the advantage that the manufacturing process is fairly simple. Contrary to the production of transdermal patches expensive manufacturing equipment is not required.

In contrast to the patches or semisolids a special device is needed for the precise and patient convenient application of the film forming systems. The design and the manufacturing costs for such an application device would generate additional costs in comparison to the other dosage forms.

The costs for the formulation as such depend on the singular excipients and cannot be compared in general.

6. Conclusion

The investigations within this work have demonstrated that film forming solutions as drug delivery systems for the skin can be formulated with a variety of excipients, but that the selection of the excipients has to be performed with care. Film forming solutions take an intermediate position between the transdermal patches and semisolids and combine properties of both dosage forms. As a result the novel dosage form features a different combination of advantages and disadvantages (Table 13) distinguishes it from the existing dosage forms. Further research concerning the delivery kinetics and the suitability of this dosage form for a broader range of

drugs is necessary for a better evaluation of the opportunities and the limitations of these delivery systems. Once this knowledge has been gained the film forming polymeric solutions might indeed present a viable alternative to the conventional dosage forms for the skin in the future.

Table 13: Advantages (highlighted in grey) and disadvantages or the film forming system in comparison to the conventional transdermal dosage forms

	Patch	**Film forming system**	**Semisolid**
Wearing comfort			
Appearance	Highly visible	Almost invisible	Invisible
Flexibility	Low	High	High
Skin feel	Non-sticky, non-greasy	Non-sticky, non-greasy	Sometimes sticky, greasy
Application			
Application process	Convenient	Convenient[1]	Sometimes messy
Dosing accuracy	High	High[1]	Low
Dosing flexibility	Low	High[1]	High
Drug delivery			
Dosing interval	1 – 7 days	1 – 2 days	1 day or less
Sustained release	Yes	Yes	No
Occlusive properties	Yes	No	No
Contamination of clothing / people	No	No	Possible
Persistent residues after removal	Possible	No	No
Costs			
Manufacturing equipment	High	Low	Low
Application device	No	Yes	No

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Summary

The aim of this thesis was to develop and investigate a novel delivery system for the skin in the form of film forming polymeric solutions. For this purpose various excipients were studied and formulation experiments were performed to provide the technological basis for the new delivery system. Methods for the evaluation and characterization of the novel dosage form were developed and assessed. Finally, the drug delivery from the film forming systems was investigated. Comparative in vitro and in vivo studies with registered transdermal patches were carried out to assess the drug delivering potential of the new dosage form.

In a first step of the study 14 film forming polymers from different chemical groups were screened to identify suitable excipients for the new dosage form. Formulations with varying polymer content, plasticizer type and plasticizer concentration were manufactured and evaluated. For this purpose a score system on living skin was established. The score system based on five macroscopic key properties for the film forming system, which were viscosity, drying time, outward stickiness, cosmetic attractiveness and permanence on the skin. The experiments revealed that a careful choice of the excipients as well as the determination of their suitable concentrations in the composition is a crucial factor. Only 10 of the over 150 different compositions produced films with the desired properties on the skin.
To gather more information about the properties of the film forming systems four of the 10 formulations containing the film formers Eudragit® RL PO (an acrylate), DynamX® (a polyurethane-acrylate), Klucel® LF (a cellulose derivative) and SGM 36 (silicon gum) were further characterized. All four films were assessed to be non-occlusive in vitro as well as in vivo, although the exact results of the two methods differed. The mechanical properties of the formulations were also determined in vitro but did not match the observations made in the in vivo evaluation concerning flexibility or strength of the films on skin. Therefore the in vitro method cannot replace the in vivo evaluation at this point.

In the next step drug loaded film forming solutions were investigated with regard to their drug delivery characteristics. For this purpose two different drugs, the hydrophilic model drug caffeine and the lipophilic, therapeutically relevant ethinylestradiol, were incorporated into four selected film forming solutions. Release tests through an artificial silicone membrane as well as permeation experiments through heat separated human epidermis were performed. All

experiments showed differences among the selected film forming solutions and a polymer-free reference solution. In case of the permeation studies with human epidermis either penetration enhancing or retarding effects of the different polymeric systems were indicated. However, the results of the release and the permeation methods were not in accordance in terms of the ranking of the formulations. This underlines the importance of using a biologically relevant membrane for formulation selection processes. Based on the results of the permeation experiment with human skin the formulation with the polyurethane-acrylate film former DynamX® was selected for further studies as it had shown the best results for both tested drugs at this point.
Further permeation experiments with ethinylestradiol revealed that the solvent of the formulation can influence the drug permeation by acting as penetration enhancer. This could be measured in spite of the short presence of the solvent on the skin. Moreover, it was shown that the permeation improved linearly with an increasing drug loading within the tested range of 1% - 5% in the solution. A further improvement of the ethinylestradiol permeation was achieved by adding chemical penetration enhancers to the film forming composition. Oleic acid and R-(+)-limonene were successful in this respect, especially in combination with propylene glycol. The enhancing effect of the oleic acid/propylene glycol (1:1) combination, which was the most efficient enhancer for ethinylestradiol in these experiments, increased disproportionately with the enhancer concentration due to the synergy of the two compounds.

The comparison of the DynamX® film forming system with the commercial EVRA® patch revealed that the film forming formulations were able to deliver a higher amount of ethinylestradiol through human epidermis in vitro in 24 hours than the patch. Unexpectedly this was even measured without chemical enhancers. An in vivo study with pigs supported these positive findings. A single application of the film forming system without enhancer induced measurable plasma levels of ethinylestradiol. The plasma levels tended to be higher than those of the parallel group with the EVRA® patch. However, considerable variations were observed and the kinetics of the drug delivery from the film forming systems remained unclear.

Based on the positive results with ethinylestradiol the thought of developing a film forming delivery system for hormone replacement therapy or contraception was pursued further. For this purpose the simultaneous delivery of a progestin, levonorgestrel, together with ethinylestradiol was investigated. Permeation experiments revealed that a simultaneous delivery of both hormones was feasible without detectable mutual influences on the permeation of the two drugs. The penetration enhancing effects of the polymeric formulation and the enhancer combination

oleic acid/propylene glycol on levonorgestrel were similar to those observed for ethinylestradiol. The comparison to a commercial transdermal patch with levonorgestrel, Fem7® Combi, revealed that the delivery level of the transdermal patch through human epidermis in vitro could be reached with the film forming system, but only with an enhancer content of 10% (5% oleic acid, 5% propylene glycol).

The present work has demonstrated that film forming solutions for the application on the skin can be formulated with a variety of excipients, but that the selection of the excipients has to be performed with care. The potential of the film forming solutions as drug delivery systems has been shown in comparative permeation experiments with registered transdermal patches. However, further research concerning the delivery kinetics and the suitability of this dosage form for a broader range of drugs is necessary for a better evaluation of the opportunities and the limitations of these delivery systems. Due to the fact that the properties of the film forming solutions show similarities to the patches in some respects (for example convenient application, sustained release, no environmental contamination) and to the semisolids in other respects (such as comfortable wearing, dosing flexibility, low visibility) they feature a combination of advantages and disadvantages that distinguishes them clearly from the conventional dosage forms. In summary these results are encouraging for the further development of film forming polymeric solutions as alternative drug delivery systems for the skin.

Zusammenfassung

Das Ziel der vorliegenden Arbeit war es, eine neue Arzneiformen für die Haut in Form von filmbildenden Polymerlösungen zu entwickeln und zu untersuchen. Zu diesem Zweck wurden verschiedene Hilfsstoffe getestet und Formulierungsversuche durchgeführt, um eine technologische Basis für die neue Darreichungsform zu schaffen. Methoden zur Bewertung und Charakterisierung der Formulierungen wurden entwickelt und bewertet. Abschließend wurde die Wirkstoffverabreichung aus den filmbildenden Systemen untersucht. Es wurden vergleichende Untersuchungen mit zugelassenen transdermalen Pflastern durchgeführt, um das Potential der neuen Arzneiform hinsichtlich der Wirkstoffverabreichung einschätzen zu können.

In einem ersten Schritt der Arbeit wurden 14 filmbildende Polymere untersucht, die unterschiedlichen chemischen Gruppen angehörten, um geeignete Hilfsstoffe für die Arzneiform zu identifizieren. Durch Variation des Polymergehalts, sowie des Weichmacher-Typs und –Gehalts wurden unterschiedliche Rezepturen erzeugt und bewertet. Für die Bewertung wurde ein Punkte-Systems auf lebender Haut entwickelt. Das Punkte-System basierte auf fünf makroskopischen Bewertungskriterien, die als wichtig für die spätere Anwendung erachtet wurden: Viskosität, Trocknungszeit, äußere Klebrigkeit, kosmetische Attraktivität und Nachhaltigkeit auf der Haut. Die Formulierungsversuche machten deutlich, dass der sorgfältigen Auswahl der Hilfsstoffe (Polymer, Weichmacher und Lösungsmittel), sowie der genauen Festlegung des Gehalts der einzelnen Komponenten eine große Bedeutung zukommt. Lediglich 10 der über 150 Formulierungen waren in der Lage waren, Filme mit den gewünschten Eigenschaften auf der Haut zu erzeugen.
Um zusätzliche Information über die Eigenschaften der filmbildenden Systeme zu sammeln, wurden vier dieser 10 Formulierungen mit den Filmbildnern Eudragit® RL PO (ein Acrylat), DynamX® (ein Polyurethane-Acrylat), Klucel® LF (ein Cellulose-Derivat) und SGM 36 (Silikongummi) weitergehend charakterisiert. Alle Filme waren nicht-okklusiv, weder im in vitro-, noch im in vivo-Versuch, wobei die genauen Ergebnisse beider Methoden nicht übereinstimmten. Die mechanischen Eigenschaften der Polymerfilme wurden ebenfalls in vitro bestimmt, entsprachen jedoch ebenfalls nicht den in den Evaluierungsversuchen gemachten Beobachtungen hinsichtlich Flexibilität und Festigkeit der Filme auf der Haut. Die in vitro Methode kann daher zu diesem Zeitpunkt die in vivo Bewertung nicht ersetzen.

Im nächsten Schritt wurden wirkstoffhaltige filmbildende Lösungen im Hinblick auf ihr Freisetzungs- und Permeationsverhalten untersucht. Dazu wurden zwei verschiedene Arzneistoffe, die hydrophile Modellsubstanz Coffein sowie der therapeutisch relevante Arzneistoff Ethinylestradiol, in vier verschiedene filmbildende Formulierungen eingearbeitet. Mit den wirkstoffhaltigen Formulierungen wurden Freisetzungsversuche durch eine synthetische Silikonmembran sowie Permeationsversuche durch Humanepidermis durchgeführt. Alle Experimente zeigten Unterschiede zwischen den vier Polymerformulierungen und einer polymerfreien Referenzlösung. Im Falle der Permeationsuntersuchungen an Humanepidermis deutete dies auf permeationsfördernde oder –hindernde Effekte der Polymerformulierungen hin. Beide Ergebnisse der Freisetzungs- und Permeationsmethoden wiesen jedoch keine Übereinstimmung hinsichtlich der Rangfolge der Formulierungen auf. Dies unterstreicht, dass eine Formulierungsauswahl nur anhand von Versuchen mit einer biologisch relevanten Membran getroffen werden sollte. Auf Basis der Permeationsexperimente mit Humanepidermis wurde die Formulierung mit dem Polyurethane-Acrylat Filmbildner DynamX® für alle weiteren Versuche ausgewählt, da sie die besten Ergebnisse für beide Arzneistoffe, das hydrophile Coffein und das lipophile Ethinylestradiol, erzielt hatte.
Weitere Versuche mit Ethinylestradiol zeigten, dass das Lösungsmittel der Formulierung die Wirkstoffpermeation beeinflussen kann, indem es als Penetrationsbeschleuniger wirkt. Dies war trotz der nur kurzen Kontaktzeit des Lösungsmittels mit der Haut erkennbar. Weiterhin konnte gezeigt werden, dass sich die Wirkstoffpermeation durch Erhöhung der Arzneistoffbeladung des Systems innerhalb der getesteten Grenzen (1% - 5% in der Lösung) verbessern ließ. Eine weitere Verbesserung der Ethinylestradiol-Permeation ließ sich durch Zusatz eines chemischen Penetrationsverbesserers erzielen. Ölsäure und R-(+)-Limonen waren in dieser Hinsicht erfolgreich, vor allem in Kombination mit Propylenglykol. Der permeationsverbessernde Effekt der Kombination Ölsäure / Propylenglykol (1:1), die für Ethinylestradiol die besten Ergebnisse erzielte, stieg überproportional mit der Enhancerkonzentration durch die Synergie der beiden Komponenten.

Der Vergleich des filmbildenden DynamX® Systems mit dem kommerziell erhältlichen EVRA® Pflaster zeigte, dass die filmbildende Lösung in der Lage war, in 24 Stunden eine größere Menge an Ethinylestradiol durch die Humanepidermis diffundieren zu lassen als das Pflaster. Dies war überraschenderweise sogar ohne Penetrationsverbesserer möglich. Eine in vivo-Studie mit Schweinen unterstützte diese positiven Ergebnisse. Nach einmaliger Applikation des

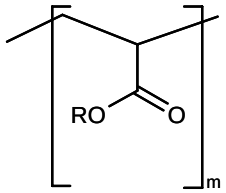
filmbildenden Systems ohne Enhancer wurden messbare Ethinylestradiol-Plasmaspiegel erreicht, die tendenziell sogar höher waren als in der Vergleichsgruppe mit dem EVRA® Pflaster. Jedoch wurden bei dem Versuch erhebliche Schwankungen beobachtet und die Kinetik der Arzneistoffabsorption blieb unklar.

Auf Basis der positiven Ergebnisse mit Ethinylestradiol wurde der Gedanke, ein filmbildendes System zur Hormonersatztherapie oder Kontrazeption zu entwickeln, weitergeführt. Dazu wurde die gleichzeitige Verabreichung eines Gestagens, Levonorgestrel, zusammen mit Ethinylestradiol untersucht. In Permeationsuntersuchungen wurde gezeigt, dass beide Hormone ohne gegenseitige Beeinflussung des Wirkstofftransports verabreicht werden konnten. Die permeationsfördernden Effekte der Polymerformulierung und der Penetrationsverbesserer Ölsäure / Propylenglykol auf Levonorgestrel waren denen auf Ethinylestradiol vergleichbar. Der Vergleich mit dem kommerziell erhältlichen transdermalen Levonorgestrel-Pflaster Fem7® Combi zeigte, dass eine vergleichbare Arzneistoffpermeation durch Humanepidermis in vitro erreicht werden konnte, jedoch nur mit einer Enhancer-Konzentration von 10% (5% Ölsäure, 5% Propylenglykol).

Die vorliegende Arbeit hat gezeigt, dass die Entwicklung einer filmbildenden Polymerlösung zur transdermalen Arzneistoffverabreichung mit einer Reihe von Hilfsstoffe möglich ist. Bei der Auswahl der Hilfsstoffe ist jedoch sorgfältig vorzugehen. Das Potential der entwickelten Systeme hinsichtlich der Wirkstoffapplikation konnte anhand vergleichender Permeationsuntersuchungen mit zugelassenen transdermalen Pflastern gezeigt werden. Weitere Untersuchungen mit Kinetik-Studien und einer breiteren Arzneistoffauswahl sind jedoch erforderlich, um die Möglichkeiten und Einschränkungen dieser Arzneiform besser bewerten zu können. Aufgrund der Tatsache, dass die filmbildenden Polymerlösungen auf der einen Seite Gemeinsamkeiten mit den Pflastern aufweisen (z.B. bequeme Applikation, verlängerte Freisetzung, keine Kontamination der Umwelt), auf der anderen Seite aber auch Übereinstimmungen mit einigen Eigenschaften der halbfesten Arzneiformen zeigen (etwa hinsichtlich Tragekomfort, Dosierungsflexibilität oder geringe Sichtbarkeit), weisen sie eine Kombination von Vor- und Nachteilen auf, die sie deutlich von den konventionellen Arzneiformen unterscheidet. Zusammenfassend sind diese Ergebnisse ermutigend für die weitere Entwicklung der filmbildenden Polymerlösungen als alternatives Wirkstoffapplikationssystem für die Haut.

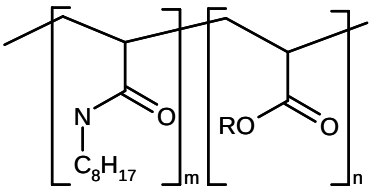
Appendix 1

Schematic structures of polymers used in the formulations experiments



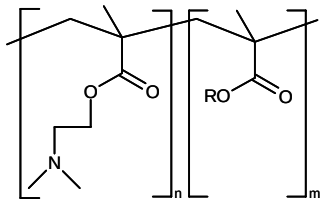
R = H, alkyl

Avalure® AC 118



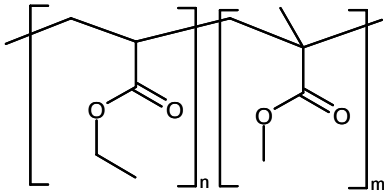
R = alkyl

Dermacryl® 79

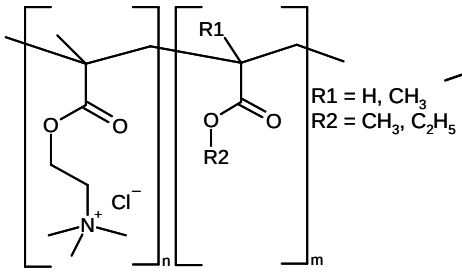


R = CH_3, C_4H_9

Eudragit® E 100

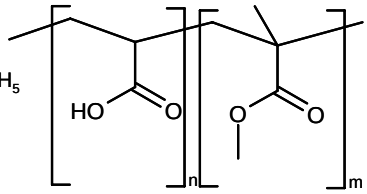


Eudragit® NE 40D

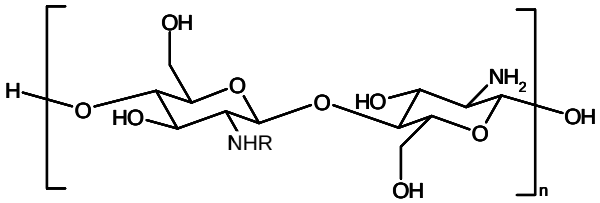


R1 = H, CH_3
R2 = CH_3, C_2H_5

Eudragit® RL PO

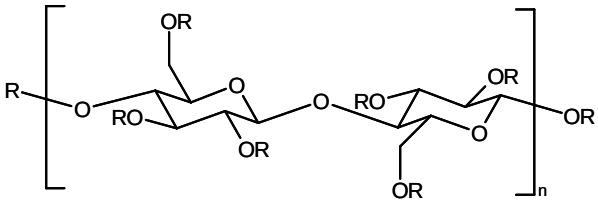


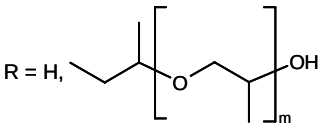
Eudragit® S 100



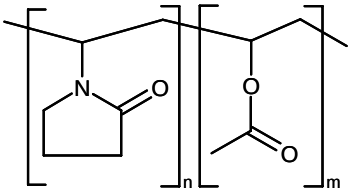
R = H, $COCH_3$

Hydagen® HCMF

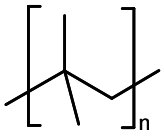




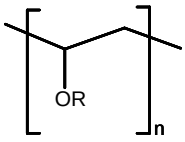
Klucel® LF



Kollidon® VA 64

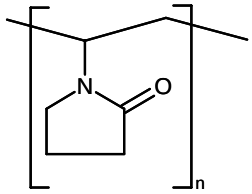


Oppanol® B100/10SFN

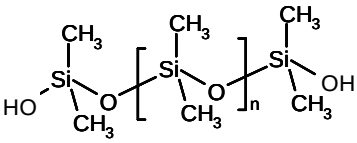


R = H, $COCH_3$

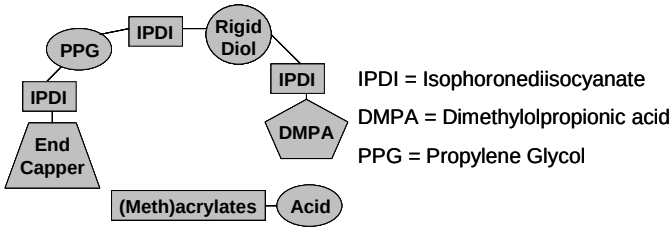
PVA 72000



Kollidon® 12 PF



SGM 36



DynamX®

Appendix 2

Results of the formulation experiments with the polymers

Avalure® AC 118, Dermacryl® 79, DynamX®, Eudragit® E 100

Excipients												Evaluation				
Polymer	Formulation number	Polymer	Triethyl citrate	Triacetin	Succinic acid	Water	Ethanol (96%)	Isopropanol	Aceton	Kollidon 12 PF	Klucel LF	Viscosity	Drying time	Outward stickiness	Cosmetic attractiveness	Integrity on skin (after 18 h)
Avalure® AC 118	A1	7.0				10.5	82.5					1	1	1	1	2
	A2	10.0				15.0	75.0					1	1	1	1	1
	A3	15.0				22.5	62.5					1	1	1	2	1
	A4	15.0	3.0			22.5	59.5					1	1	2	2	1
	A5	15.0	4.5			22.5	58.0					1	1	2	2	1
Dermacryl® 79	B1	3.0	0.9				92.3				3.0	2	1	1	2	1
	B2	7.0					93.0					1	1	1	2	1
	B3	10.0	2.0				88.0					2	1	1	2	3
	B4	15.0	3.0				82.0					2	1	1	2	3
	B5	15.0	4.5				80.5					2	1	1	2	3
	B6	20.0	6.0				74.0					2	1	1	1	3
	B7	7.0		2.1			90.9					1	1	1	1	1
	B8	7.0		2.1			87.9			3.0		1	1	1	1	3
	B9	10.0		3.0			87.0					1	1	1	2	1
	B10	10.0		4.0			86.0					1	1	1	1	3
	B11	15.0		4.5			80.5					2	1	1	2	1
	B12	15.0		6.0			79.0					2	1	1	1	2
	B13	20.0		8.0			72.0					2	1	1	1	3
DynamX®	C1	4.2	0.2			33.0	62.6					1	2	1	1	1
	C2	4.2	0.2			7.1	88.5					1	1	1	1	2
	C3	10.0	0.5			16.8	72.7					1	1	1	1	2
	C4	10.0	1.0			16.8	72.2					1	1	1	1	1
	C5	10.0	3.5			16.8	69.7					2	1	2	1	1
	C6	15.0	0.9			25.2	58.9					2	1	1	1	3
	C7	15.0	1.5			25.2	58.3					2	1	1	1	2
	C8	20.0	7.0			33.6	39.4					3	2	2	1	1
Eudragit® E 100	D1	42	18.9		3.8		11.7	2.3	21.0			3	1	3	3	2
	D2	42	18.9		3.8		11.7	23.3				3	1	3	3	2
	D3	42	18.9		3.8		35.0					3	1	3	3	2
	D4	30	13.5		2.7		53.8					2	1	3	3	1
	D5	20	9.0		1.8		67.4					1	1	2	2	1
	D6	10	1.0		0.9		88.1					1	1	1	1	1
	D7	20	2.0		1.8		76.2					1	1	1	1	2
	D8	20	3.0		3.0		74.0					1	1	1	1	3
	D9	20	4.0		0.9		75.9					1	1	2	1	1
	D10	20	4.0		1.8		74.2					2	1	2	1	3
	D11	20	4.0		3.6		72.4					2	1	2	1	3
	D12	20	4.0		3.8		72.2					1	1	2	1	1
	D13	20	4.0				76.1					1	1	1	2	1
	D14	30	6.0				64.1					2	1	3	2	3
	D15	10	3.0		1.4		75.6			10.5		1	1	2	1	1
	D16	15	1.5		1.4		77.2			5.0		1	1	1	1	2

Evaluation score system : see chapter 2
Highlighted formulations: rating 1 in all five evaluation criteria

Results of the formulation experiments with the polymers

Eudragit® NE 40D, Eudragit® RL PO, Eudragit® S 100

Excipients													Evaluation				
Polymer	Formulation number	Polymer	Triethyl citrate	Triacetin	Succinic acid	Water	Ethanol (96%)	Q7-9180 Silicone	Isopropanol	Polyethylene glycol 400	Kollidon 12 PF	Eudragit RL PO	Viscosity	Drying time	Outward stickiness	Cosmetic attractiveness	Integrity on skin (after 18 h)
Eudragit® NE 40D	E1	5.0				7.5	87.5						1	1	1	1	2
	E2	7.0				10.5	82.5						1	1	1	1	1
	E3	7.0				10.5	80.4			2.1			1	1	2	1	1
	E4	10.0				15.0	75.0						2	1	1	1	1
	E5	15.0				22.5	62.5						3	1	1	2	1
Eudragit® RL PO	F1	10.0	2.0				88.0						1	1	1	1	2
	F2	20.0	4.0				76.0						1	1	1	1	3
	F3	30.0	6.0				64.0						2	1	1	2	2
	F4	40.0	8.0				52.0						3	1	1	2	3
	F5	10.0	3.0				87.0						1	1	1	1	2
	F6	20.0	6.0				74.0						1	1	1	1	1
	F7	30.0	9.0				61.0						2	1	1	2	1
	F8	40.0	12.0				48.0						3	1	1	3	1
	F9	40.0	18.0				42.0						3	1	2	3	1
	F10	10.0	2.0				68.0						1	1	1	2	3
	F11	15.0	0.9				69.1				20.0		2	1	1	2	2
	F12	15.0					70.0				15.0		2	1	1	2	3
	F13	20.0	4.0				66.0				15.0		2	1	2	2	3
	F14	20.0	4.0		1.8		74.2				10.0		1	1	1	1	2
	F15	20.0	4.0		3.8		72.2						1	1	1	1	2
	F16	20.0		6.0			74.0						1	1	1	1	3
Eudragit® S 100	G1	0.5				1.0			91.5		7.0		insoluble				
	G2	5.0	1.6				93.0						1	1	1	1	1
	G3	7.0	2.8				90.2						1	1	1	1	2
	G4	10.0	3.0				87.0						2	1	1	1	3
	G5	10.0	4.0				86.0						2	1	1	1	3
	G6	10.0	5.0				85.0						2	1	1	1	3
	G7	10.0	6.0				84.0						2	1	1	1	3
	G8	20.0	6.0				74.0						3	1	1	2	2
	G9	10.0		3.0			87.0						2	1	1	2	2
	G10	10.0	2.0						86.0			2.0	2	1	1	1	2
	G11	10.0	5.0						83.0			2.0	2	1	1	1	3

Evaluation score system : see chapter 2
Highlighted formulations: rating 1 in all five evaluation criteria

Results of the formulation experiments with the polymers

Kollidon® VA 64, Klucel® LF, SGM 36, Oppanol®

Excipients										Evaluation				
Polymer	Formulation number	Polymer	Triethyl citrate	Dibuty phtalate	Ethanol (96%)	Q7-9180 Silicone	Butyl acetate	Polyethylene glycol 400	Ethylcellulose	Viscosity	Drying time	Outward stickiness	Cosmetic attractiveness	Integrity on skin (after 18 h)
Kollidon® VA 64	H1	5.0			95.0					1	1	2	1	3
	H2	10.0			90.0					1	1	1	2	2
	H3	15.0			85.0					1	1	1	2	2
	H4	20.0			80.0					2	1	1	2	2
	H5	10.0			89.0			1.0		2	1	1	1	3
	H6	10.0			88.0			2.0		2	1	1	1	3
	H7	10.0			87.0			3.0		2	1	1	1	3
	H8	10.0			86.0			4.0		1	1	1	1	3
	H9	5.0			88.0			2.0	5.0	2	1	1	2	3
	H10	15.0	0.75		84.3					2	1	1	1	3
	H11	15.0	1.5		83.5					2	1	1	1	3
	H12	15.0	3.0		82.0					2	1	1	1	3
	H13	15.0	4.5		80.5					1	1	2	1	3
	H14	15.0	6.0		79.0					1	1	2	1	3
	H15	15.0	7.5		77.6					1	1	3	1	o
	H16	10.0		1.0	89.0					1	1	1	1	3
	H17	10.0		2.0	88.0					1	1	1	1	3
	H18	10.0		3.0	87.0					1	1	1	1	2
	H19	10.0		4.0	86.0					1	1	1	1	1
Klucel® LF	I1	2.0			98.0					1	1	1	1	3
	I2	5.0			90.0					2	1	1	1	2
	I3	10.0			90.0					3	1	1	2	2
	I4	5.0	0.5		94.5					1	1	1	1	2
	I5	5.0	1.0		94.0					1	1	1	1	1
	I6	5.0	1.5		93.5					1	1	2	1	1
SGM 36	J1	7.0				93.0				1	1	1	1	2
	J2	10.0				90.0				1	1	1	1	1
	J3	12.0				88.0				2	1	1	1	1
	J4	15.0				85.0				3	1	1	1	1
	J5	20.0				80.0				3	1	2	1	1
Oppanol® 10SFN	M1	10.0					90.0			insoluble 20°C (24h, 500 rpm)				
Oppanol® 10SFN	M2	10.0					90.0			insoluble 50°C (24h, 500 rpm)				
Oppanol® 10SFN	M3	10.0					90.0			insoluble 90°C (24h, 500 rpm)				
Oppanol® B100	M4	10.0					90.0			insoluble 20°C (24h, 500 rpm)				
Oppanol® B100	M5	10.0					90.0			insoluble 50°C (24h, 500 rpm)				
Oppanol® B100	M6	10.0					90.0			insoluble 90°C (24h, 500 rpm)				
Oppanol® 10SFN	M7	5.0					95.0			insoluble 90°C (24h, 500 rpm)				
Oppanol® B100	M8	5.0					95.0			insoluble 90°C (24h, 500 rpm)				

Evaluation score system : see chapter 2
Highlighted formulations: rating 1 in all five evaluation criteria

Results of the formulation experiments with the polymers

Hydagen® HCMF, PVA 7200, Kollidon® 12 PF

Excipients														Evaluation				
Polymer	Formulation number	Polymer	Triethyl citrate	Triacetin	Dibuty phtalate	Water	Ethanol (96%)	Glycerol	Polysorbat 80	Propylene glycol	Polyethylene glycol 400	Lactic acid	Polyethylene imine 1800	Viscosity	Drying time	Outward stickiness	Cosmetic attractiveness	Integrity on skin (after 18 h)
Hydagen® HCMF	K1	1.0				39.8	60.0					0.6				insoluble		
	K2	1.0				45.0	50.0					0.6				insoluble		
	K3	1.0				50.0	48.4					0.6		3	3	1	2	3
	K4	1.0				50.0	48.0			0.25		0.75		3	3	1	2	3
	K5	1.5				75.0	22.5			0.1		0.9		3	3	1	2	3
	K6	1.75				87.5	9.5			0.15		1.1		3	3	1	2	3
	K7	1.0				50.0	48.2			0.2		0.6		3	3	1	2	3
	K8	1.0				50.0	48.1			0.3		0.6		3	3	2	2	3
	K9	1.0				50.0	48.0			0.25		0.75		3	3	2	2	3
	K10	2.0					96.9					1.2		3	3	1	2	3
	K11	3.0					95.3					1.8		3	3	1	2	3
	K12	4.0					93.7					2.4		3	3	1	2	3
	K13	5.0					92.0					3.0		3	3	1	2	3
	K14	6.0					90.0					3.6		3	3	1	2	3
PVA 7200	L1	10.0				90.0										insoluble		
	L2	10.0						90.0								insoluble		
	L3	10.0				73.0	4.0	13.0								insoluble		
	L4	10.0				62.0	8.0	20.0								insoluble		
	L5	10.0				40.0	50.0									insoluble		
	L6	5.0				60.0	25.0	10.0								insoluble		
	L7	5.0				65.0	20.0	10.0						2	1	1	1	3
	L8	5.0				60.0	23.0	10.0	2.0					2	1	1	1	3
	L9	5.0				60.0	28.0	5.0	2.0					2	1	1	1	3
	L10	5.0				60.0	20.0	10.0	5.0					2	1	1	1	3
	L11	5.0				59.0	28.0	5.0	2.0				1.0	2	1	1	1	3
	L12	5.0				58.0	28.0	5.0	2.0				2.0	2	1	1	1	3
	L13	5.0				55.0	28.0	5.0	2.0				5.0	2	1	1	1	2
	L14	5.0				50.0	28.0	5.0	2.0				10.0	3	1	1	1	2
	L15	5.0				53.0	28.0	5.0	2.0				7.0	2	1	1	1	2
	L16	5.0				50.0	30.0	5.0					10.0	2	1	1	1	2
Kollidon® 12 PF	N1	15.0					80.5				4.5			2	1	2	1	3
	N2	15.0					82.0				3.0			2	1	2	1	3
	N3	15.0					83.5				1.5			2	1	2	1	3
	N4	15.0					85.0							2	1	2	1	3
	N5	10.0					90.0							1	1	2	1	3
	N6	5.0					95.0							1	1	2	1	3
	N7	5.0	1.0				94.0							1	1	3	1	3
	N8	5.0		1.0			94.0							1	1	3	1	3
	N9	5.0			1.0		94.0							1	1	3	1	3

Evaluation score system : see chapter 2

Publication list

Research articles

I. Zurdo Schroeder, P. Franke, U.F. Schaefer, C.-M. Lehr, Development and characterization of film forming polymeric solutions for skin drug delivery, Eur. J. Pharm. Biopharm. 65 (2007) 111-121

I. Zurdo Schroeder, P. Franke, U.F. Schaefer, C.-M. Lehr, Delivery of ethinylestradiol from film forming polymeric solutions across human epidermis in vitro and in vivo in pigs, J. Control. Release 118 (2007) 196-203

Conference proceedings

I. Zurdo Schroeder, P. Franke, U.F. Schaefer, C.-M. Lehr, Film Forming Polymeric Solutions - Invisible Patch-like Delivery Systems for Dermal or Transdermal Application (Poster), 32nd Annual Meeting of the Controlled Release Society, Miami Beach, USA 2005

I. Zurdo Schroeder, P. Franke, U.F. Schaefer, C.-M. Lehr, Film Forming Polymeric Solutions as "Invisible" Transdermal Delivery Systems for Ethinylestradiol (Poster), 33rd Annual Meeting of the Controlled Release Society, Wien, Austria 2006

Patent applications

I. Zurdo Schroeder, Intendis GmbH, P. Franke, epinamics GmbH, S. Bracht, BayerScheringPharma AG, C.-M. Lehr, U.F. Schaefer, Universität des Saarlandes, Verwendung filmbildender Haarpflegepolymere aus der Gruppe der Polyurethane und diese Polymere enthaltende pharmazeutische Zubereitungen und Pflaster, patent application in preparation

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Abstract (English)

The aim of this thesis was to develop and investigate film forming polymeric solutions as a novel delivery system for the skin. These solutions form very thin, flexible and almost invisible films on the skin which can serve as a reservoir for the transdermal delivery of drugs. In a first step various excipients were studied and formulation experiments were performed to provide the technological basis for the new delivery system. Compositions with different polymers were identified that provided suitable properties for the intended application (short drying time, low viscosity, permanence on the skin). Methods for the evaluation and characterization of the novel dosage form were developed and assessed. The drug delivery from the film forming systems through human epidermis was investigated with caffeine as model drug and steroidal hormones as therapeutically relevant compounds. The impact of different parameters on the drug permeation from the polymeric system was tested. Among these parameters were the nature of the solvent, the drug concentration or the incorporation of chemical enhancers. Finally, comparative in vitro and in vivo studies with registered transdermal patches were carried out to assess the drug delivering potential of the new dosage form for steroidal hormones. The obtained results have demonstrated that film forming polymeric solutions are a promising approach for transdermal drug delivery that should be pursued further in the future.

Abstract (Deutsch)

Das Ziel der vorliegenden Arbeit war es, filmbildenden Polymerlösungen als neue Arzneiform für die Haut zu entwickeln und zu untersuchen. Diese Lösungen bilden auf der Haut sehr dünne, flexible und nahezu unsichtbare Filme, die als Reservoir für die transdermale Wirkstoffapplikation dienen können. In einem ersten Schritt wurden verschiedene Hilfsstoffe getestet und Formulierungsversuche durchgeführt, um eine technologische Basis für die neue Darreichungsform zu schaffen. Zubereitungen mit verschiedenen Polymeren wurden identifiziert, die geeignete Eigenschaften für die beabsichtigte Anwendung aufwiesen (kurze Trocknungsdauer, geringe Viskosität, Nachhaltigkeit auf der Haut). Methoden zur Evaluierung und Charakterisierung der Formulierungen wurden entwickelt und bewertet. Die Wirkstoffverabreichung aus den filmbildenden Systemen durch Humanepidermis wurde mit der Modellsubstanz Koffein sowie mit therapeutisch relevanten steroidalen Hormonen untersucht. Der Einfluss verschiedener Parameter auf die Wirkstoffpenetration aus den Polymersystemen wurde getestet. Zu diesen Parametern gehörten die Art des Lösungsmittels, die Wirkstoffbeladung und die Gegenwart von chemischen Penetrationsverbesserern. Schließlich wurden vergleichende Untersuchungen mit zugelassenen transdermalen Pflastern durchgeführt, um das Potential der neuen Arzneiform hinsichtlich der Wirkstoffverabreichung einschätzen zu können. Die erzielten Ergebnisse haben gezeigt, dass filmbildende Polymerlösungen ein vielversprechender Ansatz für die transdermale Wirkstoffgabe sind, der in Zukunft weiterverfolgt werden sollte.