Downscaling of Semisolid Characterization: Establishment of a Systematic and Integrated Approach to Accelerate the Development of Topical Products

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Este trabalho é dedicado à minha família.

No matter how many barriers you have ahead;

Talent, perseverance and God will always prevail.

(Unknown author)

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Prof. Dr. Rolf Daniels

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Table of content

List of abbreviations

BC	base cream DAC
BM17V	betamethasone-17-valerate
CAP	Capryol® 90
CC	cold cream DAB
CG	carbomer gel DAB
CMC	critical micelle concentration
DAB	German Pharmacopeia (Deutsches Arzneibuch)
DAC	German Drug Codex (Deutscher Arzneimittelcodex)
DEGEE	diethylene glycol monoethyl ether
DMSO	dimethylsulfoxide
DSL	deeper skin layers
FDA	Food and Drug Administration
G`	storage modulus
G``	loss modulus
G*	complex modulus
HBC	hydrophobic base cream DAC
HBSS	Hank`s buffered salt solution
HLB	hydrophilic-lipophilic balance
HPLC	high pressure liquid chromatography
HS	hydrophilic ointment DAB
KRB	Krebs-Ringer bicarbonate
LAB	Labrasol®
MC	macrogol ointment DAC
MeOH	methanol
n	number of determinations
NHC	nonionic hydrophilic cream DAB
o/w	oil-in-water
PBS	phosphate buffered saline
PC	prednicarbate
PEG	polyethylene glycol
Ph. Eur	European Pharmacopoeia
PG	propylene glycol

r^2	correlation coefficient
RH	relative humidity
rpm	rotation per minute
RT	room temperature
RSD	relative standard deviation
SC	stratum corneum
SD	standard deviation
SDS	sodium dodecyl sulfate
SLC	silicone cream SR
SDS	sodium dodecyl sulfate
SLC	silicone cream SR
SUPAC-SS	scale-up and postapproval changes for semisolid dosage forms
Tan δ	loss tangent
USP	United States Pharmacopoeia
UV	ultraviolet
w/o	water-in-oil
WS	wool fat ointment DAB

As certain terms may convey different meanings in other contexts, a glossary of terminology is presented in Appendix 1 to enhance the understanding of the concepts discussed in the present work.

Abstract

The main purpose of the present doctoral thesis was to propose a systematic and integrated approach to accelerate the development of topical products. It was intended to establish an integrative characterization program with fewest logical steps based on scientific foundation to minimize development costs and time. Three topical corticosteroids (triamcinolone acetonide, clobetasol propionate and prednicarbate) were employed as model drugs. Drug solubility with cosolvents, stability in solution, pH-stability investigation, profiling of degradation products and drug compatibility with semisolid vehicles were performed employing approximately 1 mg of compound per test condition. The established alternative compatibility test could give within 1 week predictive estimates of formal stability studies. Eight semisolid base formulations with different physicochemical properties comprising the group of hydrogels, o/w creams, w/o creams and anhydrous ointments were selected as candidate vehicles and characterized with regard their rheological properties, pH and physical stability. An *in vitro* release test using Franz diffusion cells and synthetic membrane was developed and validated. The method demonstrated to be useful for quality control and for regulatory purpose detecting changes in products after postapproval modifications. Moreover, the method was used to investigate drug transport properties in several semisolid vehicles. Drug bioavailability was evaluated *in vitro* with excised human skin mounted with diffusion cells and sampled with the tape-stripping technique. Release data did not correlate with skin absorption data when formulations of different physicochemical properties were compared, demonstrating that the extrapolation of release data to skin absorption results is still complex to be obtained. The effect of five chemicals on enhancement of skin permeation and retention was examined and the results suggested that the enhancement effect is drug specific and the selection of the suitable enhancer should be according to the therapeutic target. In conclusion, employing a minimal amount of compound (<20 mg), test formulation (<30 g) and combining eight test procedures (scaled-down solubility, scaled-down stability in solution, predictive compatibility test, pH determination, flow and oscillatory measurements, centrifugation test, *in vitro* release test and skin experiments with tape-stripping technique) an effective development program could be established minimizing trial-and-error attempts by providing scientific foundation in the approaches currently used in pharmaceutical development of topical products.

Zusammenfassung

Das zentrale Ziel der vorliegenden Dissertation war, einen systematischen und integrierten Ansatz zur beschleunigten pharmazeutischen Entwicklung von topischen Produkten zu etablieren. Das im Rahmen dieser Arbeit vorgeschlagene Charakterisierungsprogramm sollte auf wissenschaftlicher Basis mit geringsten logischen Entwicklungsvorgängen und mit reduzierten Entwicklungskosten gestaltet werden. Drei topische Corticosteroide (Triamcinolonacetonid, Clobetasolpropionat und Prednicarbat) wurden als Modellsubstanzen verwendet. Die Wirkstofflöslichkeit mit Cosolvent, Wirkstoffstabilität in Lösung, pH-Stabilitätsprofil, Charakterisierung von Abbauprodukten und Wirkstoffskompatibilität mit halbfesten Vehikeln wurden anhand von etwa 1 mg Substanzmenge pro Versuchsbedingung untersucht. Eine qualitative Vorhersage über die klassische Stabilitätsprüfung konnte durch den etablierten Kompatibilitätstest innerhalb einer Woche getroffen werden. Acht halbfeste Grundlagen wurden aus der Gruppe von Hydrogelen, O/W-Emulsionen, W/O-Emulsionen und wasserfreien Salben ausgewählt und hinsichtlich ihrer rheologischen Eigenschaften, ihres pH-Werts und ihrer physikalischer Stabilität charakterisiert. Eine Wirkstofffreisetzungsmethode mittels Franz Diffusionszellen und synthetischer Membran wurde entwickelt und validiert. Die hilfreiche Anwendung dieses Prüfverfahrens zur Qualitätskontrolle und zur Zulassung halbfester Produkte wurde dargelegt. Die Methode wurde zusätzlich für die Untersuchung der Wirkstofftransporteigenschaften in den halbfesten Grundlagen angewendet. Die biopharmazeutische Charakterisierung der Formulierungen wurde mit humaner Haut und mit der Tape-Stripping-Methode untersucht. Eine direkte Korrelation der Freisetzungsdaten mit den Ergebnissen der Hautexperimente wurde nicht beobachtet. Fünf Permeationsbeschleuniger und deren Einfluss auf die Wirkstoffspermeation und -retention in der Haut wurden untersucht. Die Ergebnisse weisen darauf hin, dass die Auswahl der geeigneten chemischen Beschleuniger wirkstoff- und therapiespezifisch erfolgen soll. Abschließend konnte anhand geringer Menge von Wirkstoffen (<20 mg) und Formulierungen (<30 g) und mittels acht kombinierten Prüfverfahren (Löslichkeitbestimmung, Stabilität in Lösung, Kompatibilitätstest, pH-Bestimmung, oszillatorische Messungen, Zentrifugationstest, Freisetzungsmethode und Hautexperimente mit der Tape-Stripping-Methode) ein effektives Entwicklungsprogramm mit fundierten wissenschaftlichen Grundlagen etabliert werden.

1. Introduction and objectives

The skin is the largest organ of the body and an important entry for chemicals offering an number of opportunities as route of administration either for local treatment of skin disorders or for transdermal application aiming systemic effect and avoidance of disadvantages of oral administration (Barry, 1983; Langer, 2004). There has been a tendency among the researchers to confuse principles of topical and transdermal delivery. Drug products topically administered via the skin are classified into two general categories: those applied to achieve local action (dermal delivery) and those to achieve systemic effects (transdermal delivery). Transdermal products can be related to patches or semisolid vehicles which provide necessary drug permeation through the skin and exert systemic effect. An optimal drug flux across the skin without considerable retention in this tissue is desired for transdermal products (Shah *et al.*, 1993; Willians, 2003). On the other hand, topical or dermatological products aim the skin itself as target site and the maximum drug retention in the skin with minimum systemic exposure is favourable (Shah *et al.,* 1993).

The transdermal route present many advantages: it is a non invasive route of administration, avoid first-pass metabolism in liver and chemical degradation in gastrointestinal environment (Willians, 2003). The transdermal delivery represents one of the most rapidly advancing areas of novel drug delivery (Samad *et al.,* 2009). The market value in 2005 corresponded to $ 12.7 billion and it is expected to grow to $ 31.5 billion until the year 2015 (Samad *et al.,* 2009). However, pharmaceutical industries have been increasingly encountering barriers during the drug product development process. The average time of taking a new compound from the discovery stage to market is typically 12-15 years with an overall cost that is approaching more than $ 800 millions (DiMasi *et al.,* 2003; Wermuth, 2008; Qiu *et al.*, 2009). Given these alarming statistics, pharmaceutical companies are continually looking for strategies to speed-up development processes so that costs can be reduced.

Efficient development strategies involve a balance between rapid screening and commercially viable utilization of characterization tests (DiMasi *et al.,* 2003, Alsenz and Kansy, 2007; Dai *et al.,* 2008). During the preclinical stage of product development, several steps are involved which take place either sequentially or concurrently: the relevant physicochemical properties of the active ingredients have to be profiled; suitable excipients and/or vehicles have to be selected taking into consideration desired therapeutic effects, stability and toxicity issues;

physical and chemical stability of the final formulation have to be assured; adequate drug release and satisfactory (trans)dermal delivery according to the target site are key elements to obtain successful product. Current development strategies are still semi-empirical and mainly based on trial-and-error principles, in-house databases and formulator experience. Quite often, preformulation studies do not yield to relevant information taking into consideration the particularities of semisolid systems; formulations for clinical testing are selected without further optimization; practices and techniques during optimization phases often neglect maximization of drug in the local site (Shah *et al.,*1993); investigations of drug and excipient compatibility are frequently based on protocols used for solid dosage forms and very often provide unrealistic prognostics (Serajuddin *et al.,* 1999; Kopelman and Augsburger 2002; Sims *et al.,* 2003). An enormous list of test procedures can be utilized for running a characterization program and the selection of the most adequate procedures becomes a difficult task. The challenge during preclinical stage is to obtain an integrated and systematic development strategy with fewest logical steps which yield to relevant and supportive information, so that one can proceed with more reliance to further clinical phases.

Speeding-up development strategies has been proposed by many researchers. The utilization of high-throughput screening methods for early evaluation of drug candidate in the discovery phase has been frequently the base of the current development platforms (Bevan and Lloyd, 2000; Kerns, 2001; Gardner *et al.,* 2004; Alsenz and Kansy, 2007; Dai *et al.,* 2008). The research focus have been often on strategies to improve biopharmaceutical properties of poorly soluble drugs (Lee *et al.*, 2003; Chaubal, 2004; Strickley, 2004; Dai *et al*., 2007; Stegemann *et al*., 2007). *In silico* models have been aiding to obtain predictive estimates of pharmacokinetic properties of drugs (Agoran *et al*., 2001; Stenberg *et al.*, 2001; Bergstrom *et al.,* 2002; Boobis *et al.,* 2002). Miniaturized and automated systems have been helping to provide a faster sample preparation and higher analysis throughput during the screening phase (Shah *et al.*, 1994; Sims *et al*., 2002; Shanbhag *et al.,* 2008). The small amount of substance available for carrying out a full characterization program during the preclinical phase has been the concern of many researchers (Hariharan *et al.,* 2003; Ballbach and Korn, 2004; Dai *et al.,* 2007). Notwithstanding, downscaling the characterization is usually achieved only with aid of automation, which oftentimes is not affordable for small pharmaceutical companies. Most of the described development strategies focus on the evaluation of drug candidates during the discovery phase, which are intended for oral administration and very few of the current approaches give a particular focus on the complex semisolid dosage systems. Currently, there

is very limited published knowledge concerning systematic approaches based on scientific judgment to accelerate the development of semisolids. A screening method to rapidly identify adequate semisolid formulation for low-solubility compounds considering the limited compound supply have been recently proposed (Mansky *et al.,* 2007). An integrative approach involving prototype design taking as example a skin care cream was reported by Cheng *et al*. (2009). A systematical evaluation by selecting relevant test procedures to assess properties of emulsified preparations was documented by Roland *et al,* (2003). However, it still lacked in these approaches the evaluation of important biopharmaceutical parameters on drug percutaneous absorption. Karande and Mitragotri (2002) could achieve a high throughput screening of permeation enhancers for development optimization. Kaca (2007) documented a comprehensive work-flow for the development of semisolids using topical corticosteroids as example. A critical evaluation of the test procedures commonly employed is fundamental to achieve a rational and science-based development program. By critically directing the choice and design of test procedures and, thus, avoiding unnecessary experimental efforts, a time-saving program can be established. It is desired is to obtain a effective development strategy integrating simple, rapid and cost-effective tests, which deliver relevant information with high data quality and scientific rigor.

The central purpose of the present work was to establish a systematic and integrated approach to accelerate the development of topical drug products. It was intended to propose an integrative development platform with fewest logical stages supported by scientific foundation. A critical evaluation of the test procedures employed and appraisal of their usefulness in an integrated program were the basis to reach the research aim. It was expected with the present thesis to build scientific knowledge on semisolid development and to gain a more clear insight into the drawbacks of currently employed test procedures to develop and optimize topical products so that more reliance can be placed on laboratory studies before undertaking costly clinical and animals trials. Moreover, the present work has as secondary specific objectives:

- Utilize and evaluate *in silico* tools within preformulation studies for early assessment of relevant properties which are most likely to affect the feasibility of development of topical formulation and propose scaled-down approaches to investigate the stability and solubility of the model drugs.

- Select and characterize the base formulations to be employed in the proposed development program with emphasis on the evaluation of rheological properties and accelerated assessment of physical stability.
- Establish a new approach to compatibility test of semisolid dosage forms with high potential of rapidly identify failure formulations.
- Develop an *in vitro* release test with focus on the influence of test parameters (membrane and acceptor medium) on drug release and propose a validation protocol for *in vitro* release tests intended to be used in industrial routine.
- Investigate the effect of semisolid vehicles with different physicochemical properties, structure, macro and microviscosity on the release of topical corticosteroids.
- Characterize the biopharmaceutical properties of formulations with focus on the influence of vehicles on the drug permeation, distribution and retention in *ex vivo* human skin.
- Investigate the effect of different chemical enhancers on the drug permeation and retention in *ex vivo* human skin.
- Evaluate the utilization and integration of all employed test procedures into a development program for topical products.

2. Theoretical background

2.1. Topical corticosteroids

Topical glucocorticoids are the most frequently prescribed drugs by dermatologists and have been frequently considered as first-line drugs for inflammatory diseases of the skin (Wiedersberg *et al.,* 2008). The clinical effectiveness in the treatment of psoriasis and atopic dermatitis is related to their vasoconstrictive, anti-inflammatory, immunosuppressive, and anti-proliferative effects (Reitamo, 2003; Wiedersberg *et al.,* 2008). In humans, the naturally occurring corticosteroid is cortisol (hydrocortisone) which is produced primarily in the adrenal gland. Since demonstrations of the effectiveness of cortisol and cortisol-21-acetate in treating dermatological disorders, massive research efforts have aimed at providing more effective topical corticosteroid therapy. In earnest in 1960 with the introduction of the fluorinated corticosteroids triamcinolone, flurandrenolone and flumethasone, drugs four to six times as potent as hydrocortisone were obtained (Kuntscher *et al.,* 1961). These drugs were so-called second generation and characterized by single halogenisation (fluoridation increasing both glucocorticoid and mineralocorticoid effects by means of increased glucocorticoid receptor affinity (Hughes and Rustin, 1997). The synthesis of more potent corticosteroids lead also to more extended side effects. More recently, the efforts have been placed on the synthesis of topical corticosteroids with improved benefit/risk ratio. The introduction of double esters in the non-halogenated corticosteroids, yielding derivatives of hydrocortisone and prednisolone with significant anti-inflammatory activity, but with the least capacity to induce skin atrophy (Korting, and Schäfer-Korting, 1998; Haberland *et al.*, 2006).

2.1.1. Model drugs

Three representative corticosteroids were chosen for the investigations in the present work: triamcinolone acetonide (TAA), clobetasol propionate (CP) and prednicarbate (PC). The molecular structures of the model drugs are presented in Fig. 2-1. TAA was the first halogenated corticosteroid, which sparked a revolution in topical corticosteroid development (Reitamo *et al.*, 2003). CP is a class I or super-potent synthetic dihalogenated analogue of prednisolone. This drug is 1800 times more potent than hydrocortisone when potency is measured using the human skin blanching assay (Haigh and Kanfer, 1984). CP has been used for the short-term treatment of patients with inflammatory and pruritic manifestations of moderate-to-severe glucocorticoid-responsive dermatoses (Gordon, 1998; Brazzini and

Pimpinelli, 2002). PC is a non-halogenated, double-ester derivative of prednisolone and functionally effective while possessing low skin atrophy potential. This drug is ranked as medium range of potency compared with other topical corticosteroids and it can be effective in treating cutaneous disorders including atopic dermatitis and psoriasis (Korting and Schäfer-Korting, 1998).

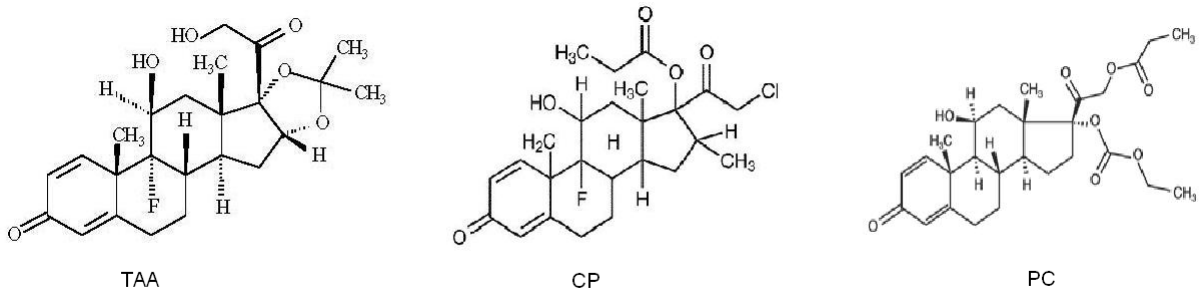


Fig. 2-1: Molecular structure of the model drugs.

2.2. Drug delivery across the skin

The terminology "topical delivery" or "dermal delivery" usually refers to the topical application targeting the skin itself, whereas "transdermal delivery" refer to cross skin barriers and reach systemic circulation (Shah *et al.*, 1993). Development strategies have to consider the therapeutic targets (local or systemic). Generally, the process of percutaneous absorption involves several consecutive steps (Kalia and Guy, 2001).

- drug in suspended state dissolves in the vehicle;
- drug in solution state diffuses in the vehicle;
- release of drug from vehicle;
- partitioning of the compound into the *stratum corneum* (SC);
- transport through the SC;
- partitioning from the lipophilic SC into the more aqueous viable epidermis;
- transport across the epidermis;
- uptake by the cutaneous microvasculature with subsequent systemic distribution.

The governing factors which determine the rate and extent in the overall process can be grouped as: vehicle-drug interaction, drug-skin interaction and vehicle-skin interaction.

2.2.1. Drug-vehicle interactions

The underlying factors that contribute to vehicle-drug interactions and their influence on the drug transport can be summarized as: (a) solubility of the drug in the vehicle; (b) diffusivity of the drug in the vehicle; (c) release properties of the drug from the vehicle into the skin. The extent and velocity of drug release are dependent on partition coefficient, solubility of the drug in the vehicle and activity of the active ingredient. By using vehicles which contain microstructures functioning as diffusional barriers, the drug release can be theoretically controlled at a desired level (Kalia and Guy, 2001). Understanding the key factors which determine drug transport is fundamental for a rational formulation design.

2.2.2. Drug-skin interactions

Physicochemical properties of the drug are important elements which determine the flux through skin. It has been suggested that an inverse relationship exists between transdermal flux and molecular weight of the molecule (Magnusson *et al*., 2004). Large molecules will tend to diffuse slowly (Hadgraft, 2004). The lipophilicity strongly influences drug permeability and binding to proteins (Cartensen, 1998; Liu *et al*., 2008). The octanol–water partition coefficient is the measure of the lipophilicity of a drug and frequently utilized as indication of its ability to cross the cell membrane. There is often a parabolic relationship between the octanol–water partition coefficient as expressed by log P and the penetration rate (Kim *et al.,* 2000). The partition coefficient is crucially important in establishing a high initial-penetrant concentration in the outermost SC layers (Barry, 2001). Interactions between drug substances and the tissue can vary from hydrogen bonding to weak van der Waals forces, and the effect of drug binding to specific proteins varies depending on the permeant. For very lipophilic drugs, the viable epidermis can act as the rate-limiting factor and clearance rate will govern its percutaneous absorption. Cutaneous biotransformation is also an important issue to be considered. The skin is comprised of a complex cellular network and it is capable of many metabolic functions common to visceral organs, including the biotransformation of drugs that penetrate through its most external layer. The biotransformation in the skin affects directly drug flux and bioavailability in target sites (Hadgraft, 2004).

2.2.3. Vehicle-skin interactions

Pharmaceutical vehicles strongly influence on drug uptake, distribution and retention in the skin. Several mechanisms are involved, as hydration effect or promotion of permeation by excipients. Penetration enhancers are pharmaceutical excipients that facilitate the absorption

of penetrant through the skin by temporarily diminishing the impermeability of the skin (Sinha and Kaur, 2000). Chemical enhancers are assumed to operate in the intercellular spaces of the SC, which is the major diffusion route for lipophilic moieties. Although exact mechanisms have not been clearly elucidated, it is believed that they have multiple effects once absorbed into the SC (Sinha and Kaur, 2000; Karande and Mitragotri, 2009).

2.3. Kinetic of percutaneous absorption

The transport of drug through human skin is a complex phenomenon involving several diffusional processes inhomogeneous in the layered skin structure.

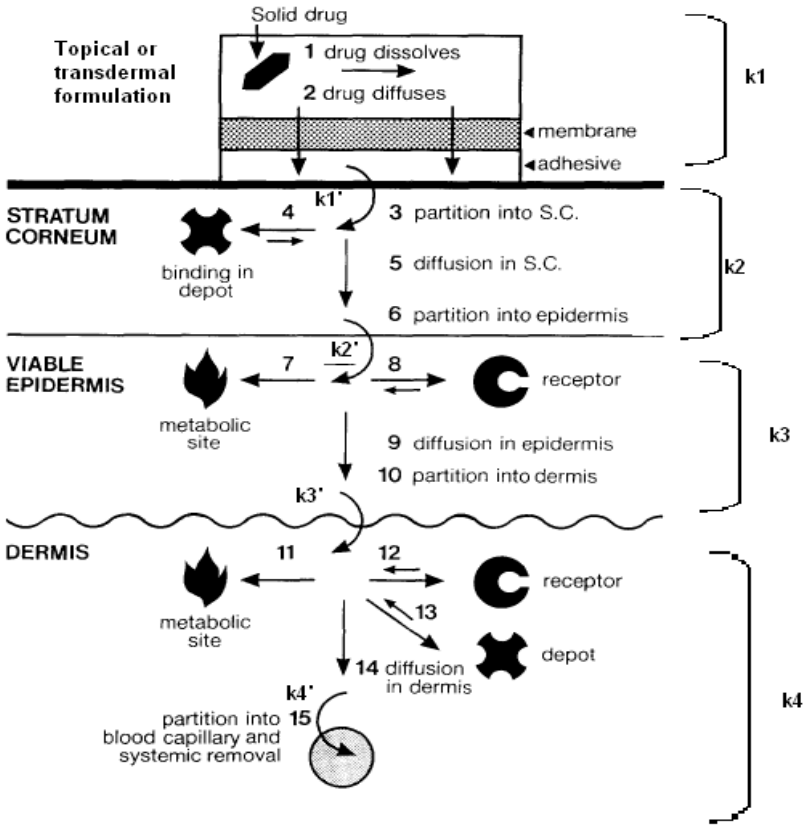


Fig. 2-2: Schematic illustration of the drug transport in the different skin layers and the determining constants which influences flux rate (adapted after Barry, 1987).

- k1 and k1'are determined by the factors affecting drug-vehicle interactions and vehicle SC partition.
- k2 and k2' are in most cases thought to be the rate-limiting step of drug transport. They represent the transepidermal transport route which can be divided into are into

the transcellular and the intercellular route (Trommer and Neubert, 2006). The more direct route is the transcellular; however the substances encounter signifcant resistance to permeation because they have to cross both lipophilic and hydrophilic structures (Trommer and Neubert, 2006). Through the intercellular route the permeant overcomes the SC by passing between the corneocytes. The governing factors are dictated by the determinant pathway (transcellular or intercellular) and factors affecting drug diffusion in the complex structure of the SC (Guy and Hadgraft, 1984). k2' describes the partition coefficient between the SC and the viable epidermis (Guy and Hadgraft, 1984).

- k3 and k3` describe the diffusion of the penetrant through the viable epidermis. Rate and extent of transport can be also determined by cutaneous metabolism and formation of a reservoir (Hadgraft, 2001). If the drug is very lipophilic**,** the viable epidermis acts as potential rate-limiting step.
- k4 and k4' are related to concurrent metabolism, formation of depot, binding to receptor and partition into capillaries.

The basic assumption supporting the mathematical theory for drug diffusion in the skin is that the rate of transfer of diffusing substance per unit area of a section is proportional to the concentration gradient (Barry, 1987). This is expressed as Fick's first law of diffusion, where (dQ/dt) is the rate of transfer per unit area of surface (known as the flux), c is the concentration of diffusing substance, x is the space coordinate measured perpendicular to the section, and D is the diffusion coefficient:

Eq. 1

$$\frac{dQ}{dt} = -DA\frac{dc}{dx}$$

The skin is a heterogeneous multilayer tissue and in percutaneous absorption the concentration gradient develops over several stracta. In terms of a laminate, each skin layer contributes a diffusional resistance (Barry, 1991). Therefore, the final rate of transport is a measure of the contribution of each rate depending of the resistance to diffusivity in each particular skin layer. The amount of drug permeated through the skin per unit time and unit area is defined as the drug-flux (J). In the case of perfect sink the flux is calculated according to Eq. 1. If perfect sink (concentration in the acceptor compartment close to zero) and infinite

dose conditions (concentration in the donor compartment constant) are maintained changes of the concentration gradient should be negligible. Then the following equation can be used:

Eq. 2

$$J = \frac{dQ}{dt \cdot A} = \frac{Ds \cdot C_0 \cdot K}{h}$$

Where:

J = Drug-flux ($g \cdot m^{-2} \cdot s^{-1}$)

$\frac{dQ}{dt}$ = Mass transfer

A = Permeation area (m^2)

Ds = Apparent diffusion coefficient in the skin ($m^2 \cdot s^{-1}$)

C_0 = Concentration of the drug in the donor ($g \cdot m^{-3}$)

K = Partition coefficient skin/donor

h = Effective skin thickness (m)

The partition coefficient as well as the effective skin thickness is difficult to be determined. They are, therefore, summarized together with the apparent diffusion coefficient to the apparent permeability coefficient ($m \cdot s^{-1}$) in equation. The thickness of the SC is not equivalent to the actual path length a molecule has to cross on its way through the SC due to its tortuosity. Therefore, permeation coefficient (P) is often labeled as Papp – the apparent permeability coefficient:

Eq. 3

$$Papp = \frac{Ds \cdot K}{h}$$

or alternatively

Eq. 4

$$Papp = \frac{DQ}{Dt} \cdot \frac{1}{AC_0} (m \cdot s^{-1})$$

simplified to

Eq. 5

$$J = Papp \cdot C_0$$

Considering saturated conditions, J is then represented by the saturated concentration of the drug in the donor.

2.4. Semisolid vehicles

In dermatology, the drug is rarely applied to the skin in the form of a pure chemical but instead is incorporated into a carrier system, a so-called vehicle. Topical dermatological bases have not only the function of "carrying" the active ingredient but they also influence directly on the therapeutic efficiency. In some cases they are also useful for providing protection from the environment on the injured areas and facilitate healing and wetting effect on dry the skin (Shah and Maibach, 1993). Transdermal permeation can be regulated by the vehicle design of the drug product. In this regulation not only physicochemical principles such as diffusion and partitioning of the active ingredient play an important role, but also the interaction with the absorptive epithelium affects the permeability of the drug (Waters, 2002). A correct interpretation of how drug transport depends on vehicle is complicated to elucidate. A profound knowledge of the formulation composition, including its physicochemical properties, rheological properties and present microstructures is therefore crucial in terms of achieving optimal topical delivery (Osborbe and Amann, 1990).

2.4.1. Type of semisolid formulations

Ointments

Ointments are water-free semisolid preparations and can be classified as hydrocarbon bases, absorption bases, water removable base and water soluble bases (Troy, 2005; Daniels and Knie, 2007). Hydrocarbon bases are lipophilic in nature containing non-polar components. Aqueous preparations may be only mechanically incorporated with difficulty in small amounts. Absorption bases consist of bases that permit the incorporation of aqueous solutions with the formation of water-in-oil (w/o) cream. Absorption bases contain in addition to the lipophilic components also emulsifiers, which could be either w/o-emulsifiers e.g. in wool fat ointment or oil-in-water (o/w) emulsifiers e.g. in hydrophilic ointment permitting, thereby, the incorporation of water resulting in the formation of w/o creams or o/w creams, respectively (Walters, 2002). Water removable bases are also described as "water-washable," since they may be readily washed from the skin or clothing with water, an attribute that makes them more acceptable for cosmetic reasons. Other advantages of the water-removable bases are that they may be diluted with water and they favour the absorption of serous discharges in dermatological conditions (Troy, 2005). The water soluble bases are also called "greaseless ointment bases" and comprise water-soluble constituents. Macrogol ointment DAC (MC) is the only pharmacopeial preparation in this group (DAC, 2009).

Creams

Creams are multiphase preparations consisting of a lipophilic phase and an aqueous phase (Daniels and Knie, 2007). Where oil is the dispersed phase and an aqueous phase is the continuous phase, the system is designated as an o/w cream. Hydrophilic cream preparations contain o/w emulsifiers such as sodium soaps or trolamine soaps, fatty alcohol sulfates, polysorbates, oresters of polyoxyl fatty acids and fatty alcohols (Daniels and Knie, 2007). A typical structure of a hydrophilic cream is presented in Fig. 2-3. Conversely, where water or an aqueous solution is the dispersed phase and oil or oleaginous material is the continuous phase, the system is designated as a w/o cream (Ph. Eur., 2008; USP, 2009a).The w/o creams can efficiently reduce the evaporative water loss from the skin by forming an occlusive layer on the skin, promoting in some cases more percutaneous absorption (Gloor *et al.*, 2004). Amphiphilic ointments are considered to be a transition between o/w and w/o creams. Sometimes amphiphilic creams are termed "supersaturated" o/w emulsion ointments, although this does not accurately describe their colloidal-chemical structure (Daniels and Knie, 2007). Creams are generally thermodynamically unstable unless emulsifying agents are added. Emulsifying agents (surfactants) concentrate in the interface between the droplet and external phase and by providing a physical barrier around the particle to coalescence (Walters, 2002; Bortnowska, 2009).

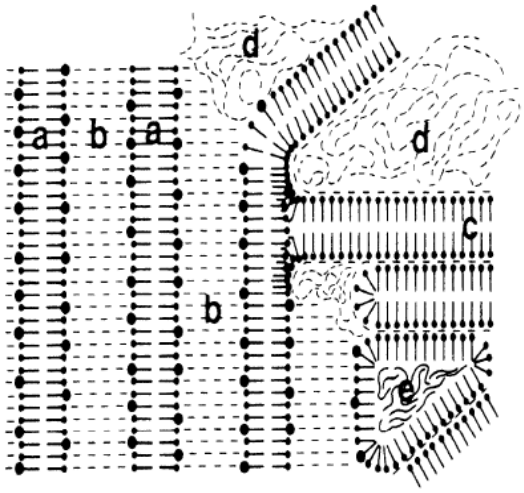


Fig. 2-3: Representative illustration of hydrophilic cream, water-containing hydrophilic ointment DAB: (a) mixed crystal bilayer of cetostearyl alcohol and cetostearyl alcohol sulfate sodium; b: interlamellarly fixed water layer; (a + b) hydrophilic gel phase; (c) cetostearyl alcohol semihydrate (lipophilic gel phase); (d) bulk water phase; e: lipophilic components (Junginger, 1984).

Gels

Gels are semisolid systems consisting of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Where the gel mass consists of a network of small discrete particles, the gel is classified as a two-phase system. In general, one component, being in the solid state, builds up a coherent three-dimensional network - often called matrix or texture - in which a second liquid component is immobilized by capillary attraction forces, lyosorption or mechanical forces (Ratner, 2009). Generally hydrogels are characterized for their morphology, swelling property and elasticity (Amin *et al.*, 2009). Swelling determines the release mechanism of the drug from the swollen polymeric mass while elasticity affects the mechanical strength of the network and determines the stability and release properties of these drug carriers (Ranjha *et al.,* 1999; Bonacucina *et al.*, 2006). The carbomer gel (CG) was introduced in the mid- to late-1950s as a group of hydrophilic, colloidal semisolid with improved thickening properties (Barry and Meyer, 1979). The hydrogen bonding in aqueous conditions must also occur but because the water molecules are small this has only a minor effect on the flexible nature of the polymer. A mechanism that takes the cross-linked nature of the polymer into account involves the absorption of water by the polymer to form a gel consisting of macroscopic, swollen particles (Bonacucina *et al.*, 2006).

2.5. Development of drug products

The process of taking a new drug from concept to commercialization involves several steps that occur in series. The successful development of new drugs is a difficult and expensive undertaking. The average cost of taking a compound from discovery stage to market for a new compound is typically 12-15 years with an overall cost approaching almost $ 1 billion until the new drug that obtains approval to go on the market (DiMasi *et al.*, 2003; Wermuth, 2008; Qiu *et al.*, 2009). For every 5000 potential new medicines developed, only very few of new chemical entities survive chemistry and preclinical studies (Gallin and Ognibene, 2007).

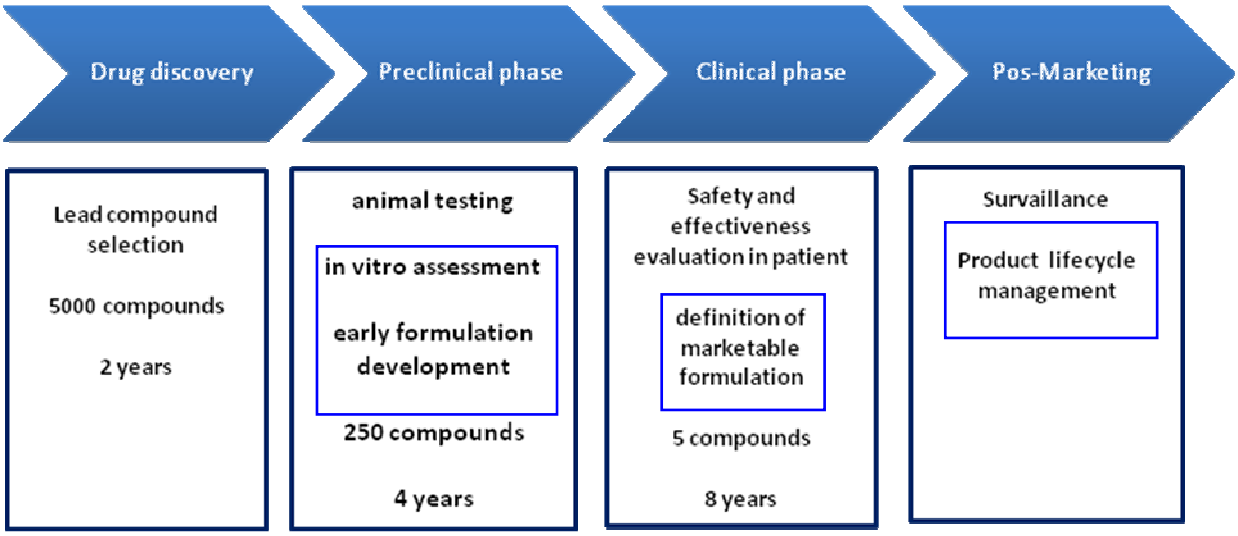


Fig. 2-4: Key development steps required to bring a new chemical entity to market (adapted after FDA, 1998a). The marked boxes represent the stages, wherein development strategies proposed in the in the present work can be applied.

In the discovery phase, a preliminary evaluation of the optimized compounds is generally conducted by predicting certain key physicochemical properties via computational chemistry (Qiu, 2009). The compound judged to have the most suitable *in vitro* properties is recommended for entry into preclinical development by a team of scientists. During the preclinical stage, only few milligrams of the lead compound is synthesized and purified for running characterization tests. In this stage occurs the physicochemical characterization of the lead compound; development of preliminary analytical methods and identification of its major synthetic impurities, and/or products of chemical degradation (Balbach and Korn, 2004; Qiu, 2009). Parallel to the physicochemical characterization of the compound, the first efforts on the early formulation development take place. Before stepping forward to the clinical phase, the potential new drugs undergo toxicology studies in animals and *in vitro.*

The clinical stage represents the longest portion of the development chain. The costs associated with the development increase during clinical research, peaking in phase 3 in which multiple trials that are conducted involving thousands of patients (Gallin and Ognibene, 2007). Therefore, it is rational to have already delineated the most suitable formulation prototypes in the preclinical phase. The formulation design steps to optimization phases and long-term stability studies in the clinical phases I and II and in this phase the marketable product is defined.

During the post-marketing phase, development strategies still can be applied. The term product lifecycle management or, alternatively, life cycle management has been a feature of the pharmaceutical industry for several decades. Reformulation of a product can provide market share protection via new patent coverage and market exclusivity, usually involving a change in dose form, dosing regimen, route of administration, and/or drug chemistry (Kvesic, 2008). Many drugs administered orally can be reformulated to transdermal semisolids and applied via skin avoiding the disadvantages of the oral route. An effective preclinical development avoids further disappointments during clinical phases. A typical workflow of preclinical development of topical semisolids is presented in Fig. 2-5.

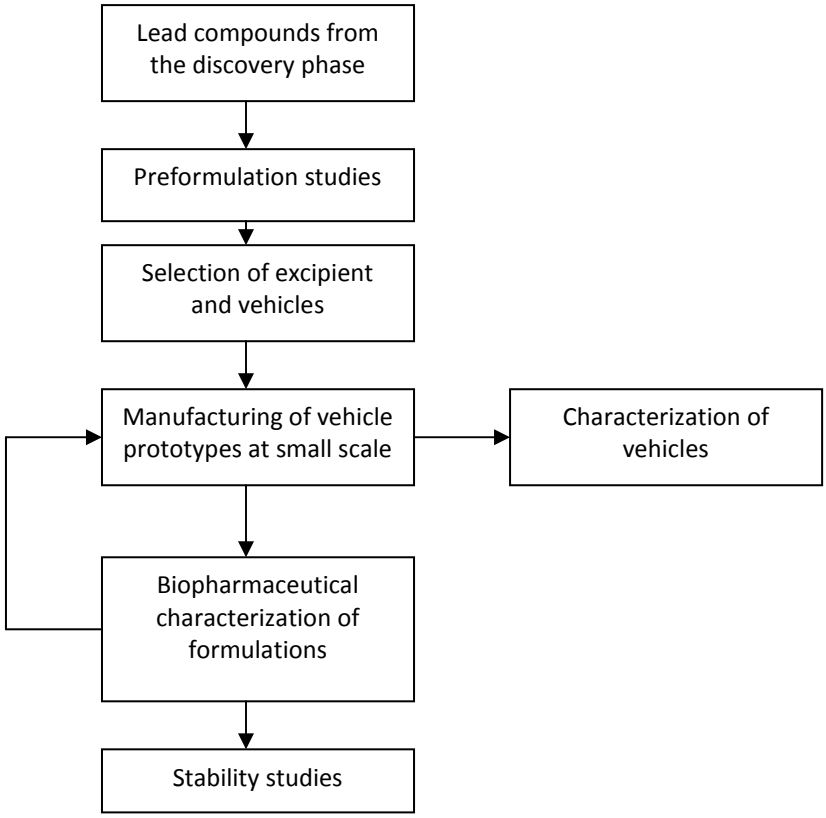


Fig. 2-5: Proposed work-flow for the preclinical development of topical and transdermal drug products. Adapted after Kaca (2007).

2.5.1. Preformulation studies

Preformulation is the study of the chemical and physical properties of the drug components prior to the design of the dosage form. The purpose is to understand the nature and

characteristics of each component and to permit the rational development of a stable, safe and efficiency dosage form (Cartensen, 1998). The focus on the preformulation studies enables:

- selection of drug substance candidates;
- orientation of choice of dosage form, excipients, composition, physical structure and method of manufacture of drug product during the formulation design;
- adjustment of pharmacokinetic and biopharmaceutical properties of the compound;
- development of first analytical methods.

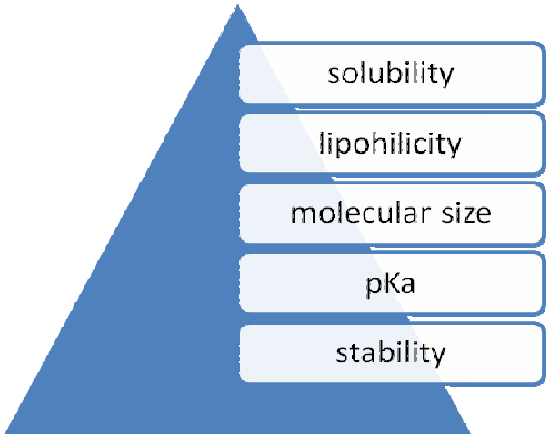


Fig. 2-6: Illustration of important physicochemical factors which affect the feasibility in developing topical and transdermal drug products.

Lipophilicity is a fundamental property of compounds and is a major contributor to solubility, permeability and protein binding (Cartensen, 1998; Liu, 2008). It relates to a compound's preference for van der Waals interactions with other organic molecules versus hydrogen bonds or dipolar interactions with water (Gibson, 2001). The compound`s melting point is a very useful parameter for identification tests and quality control of raw material, since it represents a unique characteristic of a pure substance (Gibson, 2001). The observation of the melting point data of different combinations of the drug/excipient is commonly used to investigate the compatibility before developing the dosage form (Balestrieri *et al.,* 1996; Ceschel *et al.,* 2003; Lira *et al.,* 2007). The melting point is also regarded as input parameter for the initial *in silico* assessment of solubility (Yalkowsky and Valvani 1977; Yalkowsky and Valvani, 1980; Yalkowsky *et al.,* 1998) and used for elucidation of crystal properties and polymorphism (Banker and Rhodes, 2002). Drug aqueous solubility is one of the most critical physicochemical properties for drug absorption and bioavailability (Kerns, 2001). The pKa determine the ionisation state of the molecule, affecting directly the diffusion process in the semisolid vehicle and permeation into the skin. The molecular size is related to the capacity of

percutaneous permeation. The determination of the intrinsic stability of the drug substance has an essential importance during the preformulation stage. Forced degradation studies are often designed to determine the inherent stability of the drug molecule by establishing the degradation pathways and identifying the most likely degradation products which may arise in further development phases (ICH, 2003).

2.5.2. Selection and characterization of semisolid vehicles

The selected base vehicle should provide adequate drug bioavailability on the target; be cosmetically acceptable to the patient; demonstrate safety and low irritancy properties; be stable and compatible with active ingredient (Fig. 2-7). Lipophilic bases are suitable for chronic cases, but they are more resistant to spreading than other vehicles and difficult to remove from the skin (Vender *et al.*, 2008). Patients may find w/o creams more acceptable than the oleaginous or absorption bases because they are less greasy and they spread more easily. Creams are often chosen to topically deliver a dermatological drug and may be used on moist skin areas and they are preferred for acute and subacute dermatoses (Lee *et al*., 1998). They are easily accepted by patients due to the lower oil content (Vender *et al.*, 2008). Gels are popular because of their high degree of clarity and ease of application and removal. They are indicated to acute disease states in hairy areas, particularly the scalp.

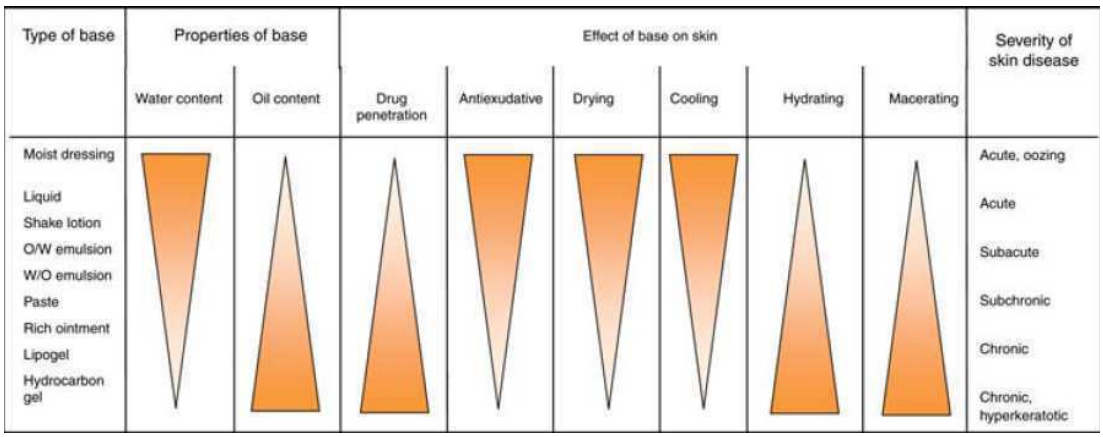


Fig. 2-7: Schematic illustration of the type of base and their respective properties (Daniels and Knie, 2007).

Aesthetic properties and pH determination

Appearance, texture and skin feeling of formulations are becoming fundamental parameters that strongly influence the physicians' choice and compliance of patients to therapeutic treatment (Draelos *et al.*, 1995; Vender *et al.*, 2008). The determination of pH of the base

formulation is an important factor for the product stability and patience compliance as well. The background information regarding the substance pH-stability profiles gathered during the preformulation studies orient the selection of the proper base and/or adjustment of the vehicle pH resulting in a stable-product. Furthermore, the pH of topical formulations plays an important role on the microbial activity of the skin and on enzymatic processes in the cornal layer (Schmid-Wendtner and Korting, 2007).

Rheological properties

The investigation of the rheological properties of semisolid vehicles provides useful information regarding the manufacturing procedures and alteration of microstructure during stability studies. Various methods may be applied such as assessment of flow properties and oscillatory techniques (Tadros *et al.,* 2004). The most common expression of non-Newtonian behavior refers to the apparent viscosity (η_{app}) which is dependent on shear rate (τ) and shear stress (γ):

Eq. 6

$$\tau = \eta_{app} \cdot \gamma$$

The dynamic mechanical "strain sweep" test examines the microstructural properties of the material under increased strain. It measures the storage modulus (G´) which is an indicator of elastic behaviour and reveals the ability of the system to store elastic energy associated with recoverable elastic deformation (Rao, 2007). The greater the G´, the more elastic are the characteristics of the material. The loss modulus (G´´) is a measure of the dynamic viscous behaviour that relates to the dissipation of energy associated with unrecoverable viscous loss. The greater the G´´, the more viscous are the characteristics of the material (Binks, 1998). The loss tangent (tan δ) is defined as the ratio of G´´to G´. It is a measure of the ratio of energy lost to energy stored in a cycle of deformation and provides a comparative parameter that combines both the elastic and the viscous contribution to the system (Petsev, 2004). The smaller tan δ, the more elastic is the material:

Eq. 7:

$$\tan\delta = \frac{G''}{G'}$$

The complex modulus (G*) relates to the complex viscosity (η*) of the material and is a measure of the material's overall resistance to deformation.

Eq. 8:

$$G^* = G' + iG''$$

The dynamic viscosity (η') is a function of the complex viscosity (η*) and is related to the steady shear or apparent viscosity. It measures the rate of energy dissipation in a viscoelastic material (Petsev, 2004).

Cream stability

Formulation stability is one of the most important factors governing the shelf-life of topical applied products. The physical stability can be defined as the ability to resist changes in spatial distribution of ingredients over the time whereas the chemical stability is the ability to resist changes in chemical structure of ingredients (Swarbrick, 2006). The information generated from physical stability studies can also be used for reformulation purposes considering properties such as the type of emulsifier and its concentration, the use of preservatives, and the selection of the primary packaging material (Santoro *et al.,* 2005). Creams are thermodynamically unstable systems and they tend to breakdown over time due to a number of different physicochemical mechanisms, which may occur concurrently such as creaming, flocculation, coalescence, phase inversion and/or Ostwald ripening.

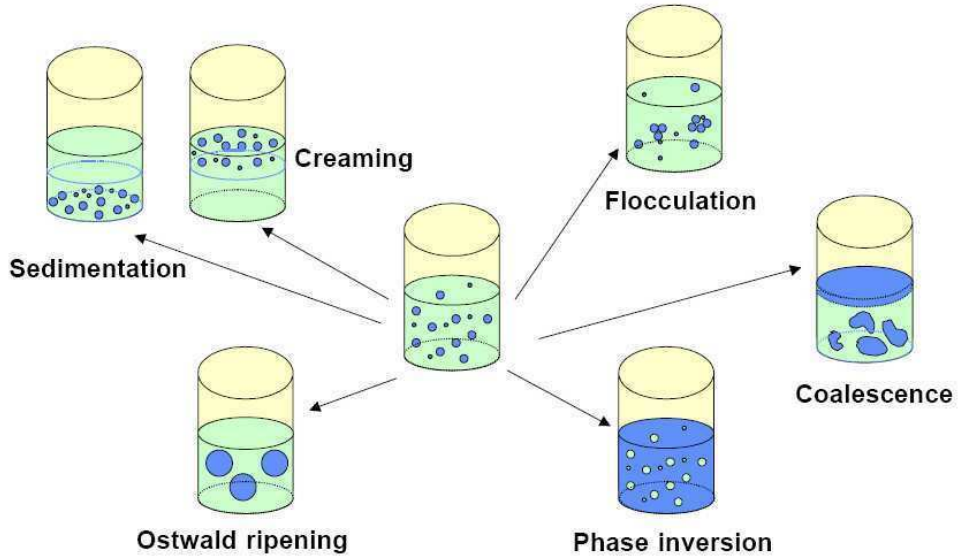


Fig. 2-8: Main breakdown processes of creams (adapted after Tadros *et al.,* 2004).

Sedimentation and creaming processes are dependent on the specific density of each single vehicle component and can be accelerated by applying centrifugal forces (Idson, 1993). An (ultra)centrifuge is an instrument designed to apply a rotational force to a mass (particle) and

if the mass is unrestricted, it will move away from the centre of rotation. Hence, acceleration of gravidity (*g)* is replaced by acceleration of centrifugation:

Eq. 9

$$g = \omega^2 x$$

Where ω is the angular velocity and x is the distance of the centrifuge tube from the axis of rotation. The force at which a centrifuge is operated is often expressed in terms of the number of times that the force of gravity is exceeded.

2.5.3. Compatibility and stability testing

Not only physical, but also chemical stability of the drug is a fundamental aspect which determines feasibility of product. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommend storage conditions (Banker and Rhodes, 2002; Huynh-Ba, 2008). Stability studies should include testing of those attributes of the drug substance that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The investigation of drug compatibility between drug and excipient is one of the strategies to try to delineate at a sooner phase possible stability problems. Traditional excipient compatibility testing usually involves the employment of binary systems mixing drug and excipients at determined ratio and submitting to accelerated temperature conditions (e.g. 40 ºC) over a period of time, usually no longer than 1 month (Morris *et al.* 1994; Balestrieri *et al.*, 1996; Kopelman and Augsburger, 2002; Ceschel *et al.* 2003). Formal stability studies encompass long-term, intermediate and accelerated tests. Long-term testing should cover a minimum of 12 months (ICH, 2003). Data from the accelerated storage condition can be used to evaluate the effect of short term excursions outside the label storage conditions (ICH, 2003). The general testing conditions of formal stability studies are summarized in Tab. 2-1.

Tab. 2-1: Types of stability studies and storage conditions (ICH, 2003).

Types of study	Storage condition	Minimum time period covered
Long term	25 °C ± 2 °C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH ± 5% RH	12 months
Intermediate	30 °C ± 2 °C/65% RH ± 5% RH	6 months
Accelerated	40 °C ± 2 °C/75% RH ± 5% RH	6 months

2.5.4. *In vitro* release test

The application field of *in vitro* release tests includes different frameworks in the field of pharmaceutical semisolid dosage forms. For quality control, the purpose of the test procedure is to demonstrate that critical manufacturing steps result in a consistent product assuring batch-to batch uniformity (Shah *et al.,* 1991; Flynn *et al.,* 1999; Azarmi *et al.,* 2007). In the pharmaceutical development, the test is assumed to be useful in the early screening of candidate vehicles or in the final optimization of the lead formulation, providing some predictive estimates with respect to the *in vivo* performance of a drug product (Shah *et al.,* 1992; Shah *et al*., 1999; Azarmi *et al.,* 2007). The composition of the semisolid vehicle strongly influence on the product performance and how rapid the drug is released into the skin (Ostrenga *et al.,* 1971; Kundu *et al.*, 1993). In this context the test procedure is often referred to performance test rather than a quality test. In the regulatory field, the test serves as a "waiver" to maintain the certification of the products which undergo alterations in the composition; manufacturing procedure or site (FDA, 1997).

Franz diffusion cells (Fig. 2-9) are usually used as test system (Franz, 1975; Flynn *et al.,* 1999; Clement *et al*., 2000; Siewert *et al.*, 2003; Borgia *et al*., 2008). A diffusion cell consists of a donor chamber and a receptor chamber between which a supporting-membrane is positioned. The formulation or substance in study is placed in the donor compartment. The cell should provide a good seal around the membrane; enable easy sampling and good mixing of the receptor solution in contact with the membrane and good temperature control of the cell and its contents.

Fig. 2-9: Picture of a Franz diffusion cell.

The theoretical model which describes the release of drugs from semisolid dosage forms was first postulated by Higuchi (Higuchi, 1961). The model derives from Fick´s law of diffusion and describes the drug release from "solution systems" in which the drug is completely dissolved in the semisolid system and from "suspension systems", in which the drug is rather suspended in the base

Eq. 10

$$Q = A \cdot \sqrt{D \cdot c_s \cdot (2 \cdot c_0 - c_S)} \cdot \sqrt{t}$$

Where

Q= Cumulative amount of the released drug (g)

A= Diffusion area (m^2)

c_0= Starting concentration of the drug in the donor ($g \cdot m^{-3}$)

c_S= Saturation concentration of the drug in the donor ($g \cdot cm^{-3}$)

t= Time (s)

Ds= Diffusion coefficient of the drug ($m^2 \cdot s^{-1}$)

Assuming that (a) the suspended drug is in a line state such that the particles are much smaller in diameter than the thickness of the applied layer; (b) $c_0 >>> c_S$; (c) the surface to which the formulation is applied is immiscible with respect to the ointment and constitutes a perfect sink for the released drug. The simplified equation (Eq. 11) was obtained for drugs in suspended state.

Eq. 11

$$Q = \sqrt{2 \cdot A \cdot D \cdot c_s} \cdot t$$

2.5.5. Skin experiments

The employment of excised skin mounted in diffusion cells is frequently used for the characterization of biopharmaceutical properties of topical semisolids (OECD, 2004a). Skin from human or animal sources can be used. Three types of skin membranes can be prepared for *in vitro* experiments: epidermal membranes (thickness of approximately 0.1 mm, prepared by heat separation, chemical or enzymatic separation), split-thickness skin (thickness of 0.2-0.5 mm prepared using a dermatome) and full-thickness skin (thickness of 0.5-1.0 mm) (SCCP, 2006). Since the main barrier function of the skin is located in the SC, all three membrane types have been used for permeation studies. The use of a physiologically conducive acceptor fluid is preferred (OECD, 2004b). The sampling is performed either in a continuous form or at pre-determined time intervals.

Assessment of *in vitro* bioavailability and toxicity with tape-stripping technique

The tape-stripping technique is a simple and efficient method for *in vitro* characterization of cosmetical and dermatological formulations (Bashir *et al.,* 2001; Herkenne *et al.,* 2007). The technique is used to assess the rate and extent of topical drug in the SC. After topical application of formulation and drug penetration, the cell layers of the SC are successively removed from the same skin area using adhesive films and the drug penetrated in the skin compartments is measured (Fig. 2-10). The removed tape strips contain the amount of corneocytes and the corresponding amount of the penetrated drug. The method assumes that topical bioavailability can be estimated from the drug concentration within the SC, which is expected to be related to the drug concentration at the target site (i.e. usually viable epidermis or dermis) since the SC is the rate limiting barrier for percutaneous absorption. Due to its mildly invasive nature, it is ideally suited to performing studies *in vivo*, but these are often expensive to perform and obtaining ethical approval can be difficult. Therefore, *in vitro* tape-stripping can be viewed as an alternative option and is particularly appropriate for preliminary investigations of formulation effects before conducting clinical studies.

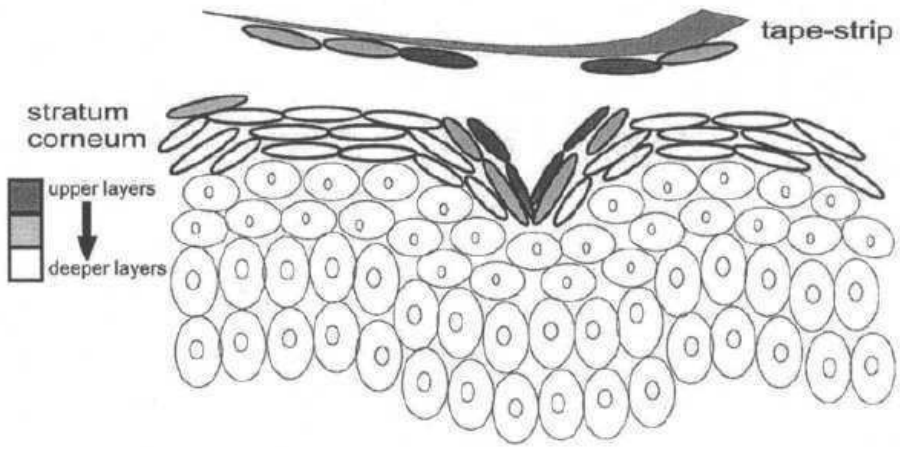


Fig. 2-10: Schematic illustration of the removal of the SC layers by tape-stripping (van der Molen, 1997).

Besides the application of the tape-stripping technique for assessing topical bioavailability, the method has been widely used for risk and safety evaluation associated to data from *in vitro* percutaneous absorption (Sartorelli *et al.,* 2000; Lademann *et al.,* 2008). In the European Union, risk assessment of dermal contact with pesticides is generally based on *in vivo* animal skin penetration data combined with an *in vitro* comparison of animal and human skin penetration results (EPA, 1999; EU, 2004). Thus the results of *in vitro* skin absorption studies are essential for evaluating whether substances are considered to be safe.

3. Preformulation studies

3.1. Introduction

Preformulation is an important phase during the drug product development. In this stage, the investigation of physicochemical properties of the drugs is the basis for a rational formulation design. This also provides valuable information for assessing the pharmacokinetic properties of potential lead candidates (Cartensen, 1998; Gibson 2001). Currently, the increasing pressure applied on the pharmaceutical industry to improve operational efficiency drives the pharmaceutical research toward to the extensive use of new technologies to the early assessment of pharmacokinetic parameters and among them the i*n silico* tools. These *in silico* methods include databases, quantitative structure-activity relationships, similarity searching, pharmacophores, homology models and other molecular modelling, machine learning, data mining, network analysis tools and data analysis tools that use a computer (Testa and Tursky, 2006). The employment of these models in the early phases of the development has been widely described (Bergstrom *et al.*, 2002; Riviere and Brooks, 2005; Hansen *et al*., 2008). The challenge placed on this evaluation is to find the proper models which deliver relevant information to support further development steps. As well as *in silico* assessment, the determination of the intrinsic stability of the drug substance has an essential importance during the preformulation stage. Forced degradation studies are often designed to determine the inherent stability of the drug molecules by establishing the degradation pathways and identifying the most likely degradation products which may arise in further development phases (ICH, 2003). The difficulty found in these tests is to limit the amount of substance to be used and to track the significant degradation products with more simple analytical techniques.

In this part of the work, the employment of *in silico* methods for evaluation of physicochemical parameters which are most likely to affect the feasibility of development of the model drugs CP, PC and TAA was described. The investigation of drug stability in solution at different forced conditions and drug solubility with colsolvents were performed with scaled-down methods. Finally, a compact preformulation program has been proposed.

3.2. Materials and methods

3.2.1. Chemicals and reagents

The model drugs (TAA, CP and PC) were purchased from Fagron GmbH Co. KG (Barsbüttel, Germany). Acetonitrile, sodium dihydrogen phosphate, phosphoric acid solution 85%, sodium hydroxide, chloric acid and hydrogen peroxide solution 30% were obtained from Merck KGaA (Darmstadt, Germany). Hydrogen peroxide solution 30% was further diluted employing ultra-pure water system (Ultra Clear®). Propylene glycol (PG) and diethylene glycol monoethyl ether (DEGEE) were purchased from Merck KGaA (Darmstadt, Germany) and used as cosolvents in the solubility experiments.

3.2.2. *In silico* models

Evaluation of drug-likeness properties

Lipinski's 'Rule of Five' (Lipinski *et al*., 1997) is a model used to evaluate the drug-likeness properties with respect to bioavailability. Initially conceptualized for oral dosage forms, the rule combines the assessment of important physicochemical properties as molecular weight, partition coefficient, hydrogen acceptor and donor bonds that are determinant for the biopharmaceutical properties. The approach was based on the experience from the author at Pfizer Central Research to guide the decisions during the drug discovery and development combining experimental and computational tools to assess drug solubility, absorption and permeability. This rapidly became known as "Lipinski's Rule of Five" because all the cut-off points are multiples of five. The model drugs were firstly evaluated with aid of the Lipinski's 'Rule of Five' in order to qualitatively verify if they raise permeability concerns. Compounds that fall in the following category are likely to have permeability issues:

(a) Molecular weight ≥500
(b) Log *P* ≥5
(c) H-bond donor ≥5
(d) H-bond acceptors ≥10

Prediction of skin permeability

Potts and Guy (1992) have analyzed the extent of skin permeability data from a variety of sources using a simple model, which depends on the size of the permeant and its octanol/water partition coefficient. The equation correlates skin permeability coefficient (K_P)

to a drug in aqueous solution with solute molecular weight (*MW*) and octanol–water partition coefficient (P). This approach has been extensively employed for estimating the permeability coefficient of compounds and it has been adopted by guidelines for dermal risk assessment (EPA, 1999). The equation follows:

Eq. 12

$$\log Kp = -6.3 + 0.71 \cdot \log P - 0.0061 \cdot MW$$

Prediction of aqueous solubility

The general solubility equation, proposed by Yalkowsky and co-workers (Yalkowsky and Valvani, 1977; Yalkowsky and Valvani, 1980; Yalkowsky *et al.,* 1998) can predict aqueous solubility (*S)* with knowledge of melting point (*Tm*) and octanol–water partition coefficient (log *P)* as shown below:

Eq. 13

$$\log[S] = -0.01(Tm - 25) - \log P + 0.5$$

Parallel to the calculation of theoretical aqueous solubility, a literature survey was performed to attain reference values of solubility.

3.2.3. Determination of drug solubility with cosolvents

The solubility of CP was determined in binary mixtures of water/cosolvent using a scaled-down method. Based on information of water solubility, an excess of substance was weighed into Eppendorf cups and filled with 1.5 mL of medium. The media consisted of binary mixtures of water and cosolvent at different proportion (10, 20, 30 and 50%). PG and DEGEE were employed as cosolvents. The equilibration followed over 24 hours at RT at 200 rpm of agitation. Samples were filtrated (Millex-HV, Millipore, 0,45 μm) before HPLC analysis.

3.2.4. Investigation of intrinsic stability and analytical profiling

The design of the study protocol has followed the recommendations reported elsewhere (Bakshi and Singh, 2002; Alsante *et al.,* 2003; Alsante *et al.,* 2007). The model drugs were treated under acid (HCl 0.1N), basic (NaOH 0.1N), water pH and oxidation (H_20_2 solution 0.3% and 15%) conditions. Samples were prepared in presence and absence of a cosolvent (acetonitrile) in order to investigate the inherent stability in suspension and solution state.

Approximately 1 mg of drug was weighed into transparent HPLC vials following the addition of reagent (20 µL) and 80 µL of cosolvent (when applicable) and vortexed (Eppendorf AG, Hamburg, Germany). The vials were closed and stored in thermostatically controlled dry air oven (Thermo Fisher Scientific, Bonn, Germany) over 48 hours at 25 ºC and at 60 ºC, respectively. After storage, a visual evaluation of the samples was done. Before analysis by HPLC, the samples were diluted into amber vials to the concentration of 0.1 mg·mL. The peak area of the degradation products were compared to the peak area of the native drug and reported in percentage.

3.2.5. Analytical method

The HPLC system used was Waters Alliance 2695 with quaternary pump. The output signal was monitored and processed using Empower2® software. The chromatographic column employed was Waters Symmetry® C18, 150 mm x 4.6 mm, 3.5 µm using as mobile phase a gradient mixture of 0.02 M sodium dihydrogen phosphate buffer pH 3.5 (mobile phase A) and acetonitrile (mobile phase B). The gradient for PC and CP followed: 6:4 (A/B) increasing linearly the concentration of acetonitrile to 3:7 (A/B) until 7 minutes, keeping this level for 2 more minutes and finally returning to 6:4 (A/B) until 11 minutes. The gradient for TAA followed the same procedure with a modification at the initial concentration of mobile phases to 7:3 (A/B). The retention times of PC, CP and TAA were 6.4, 5.8 and 5.9 minutes, respectively. The flow rate was 1 ml/min. The column temperature was maintained at 40 ºC, the auto sampler at 20 ºC and the injection volume amounted at 20 µL. The wavelength was monitored at 239 nm (TAA and CP) and 243 nm (PC). Spectral data was obtained using a Waters 996 Photo Diode Array detector in order to extract UV spectra of degradants and check peak purity by library spectra matching, confirming the absence of coeluting species.

3.3. Results and discussion

3.3.1. Evaluation with Rule of Five

The table below presents the results of parameters investigated with Lipinski model (1997):

Tab. 3-1: Summary of results of evaluation of drug-likeness properties.

Model drug	Molecular weight*	Log P*	H-bond donor*	H-bond acceptor*
TAA	434.49	2.5	2	6
CP	466.97	3.98	1	5
PC	488.57	3.83	1	8

* Obtained from ChemSpider chemical databank (http://www.chemspider.com, retrieved on 08/09/08).

None of the model drugs has violated the Lipinski`s Rule of Five. A qualitative evaluation indicated that PC is most likely to be more critical concerning development aspects due to the higher molecular weight and number of hydrogen-bond acceptors. It has been suggested that an inverse relationship exists between transdermal flux and molecular weight of the molecule (Hadgraft, 2004; Magnusson *et al*., 2004). Taking into consideration intracellular transport pathway, an excessive number of hydrogen bond donor groups impair permeability across a membrane bilayer (Abrahan *et al.,* 1994; Paterson *et al.,* 1994). Too many hydrogen bonds acceptor (nitrogen and oxygen atoms) also hinder permeability across a membrane bilayer. The sum of nitrogen and oxygen atoms is a rough measure of hydrogen bond accepting ability (Pugh *et al.,* 1996). The partition coefficient is the measure of the lipophilicity of a drug and the lipophilic-hydrophilic balance of a drug influences it interaction with extracellular lipids in the SC (Kim *et al.,* 2000). There is often a parabolic relationship between the octanol–water partition coefficient as expressed by log P and the penetration rate (Kim *et al.,* 2000). The Lipinski`s Rule of Five was useful for an initial assessment of new drugs, providing an early background concerning the physicochemical properties.

3.3.2. Prediction of skin permeability

The calculation of the permeability coefficient of the model drugs followed in parallel to the calculation of coefficients of one low permeable marker (caffeine) and one high permeable marker, propranolol, (OECD 2004a, OECD 2004b).

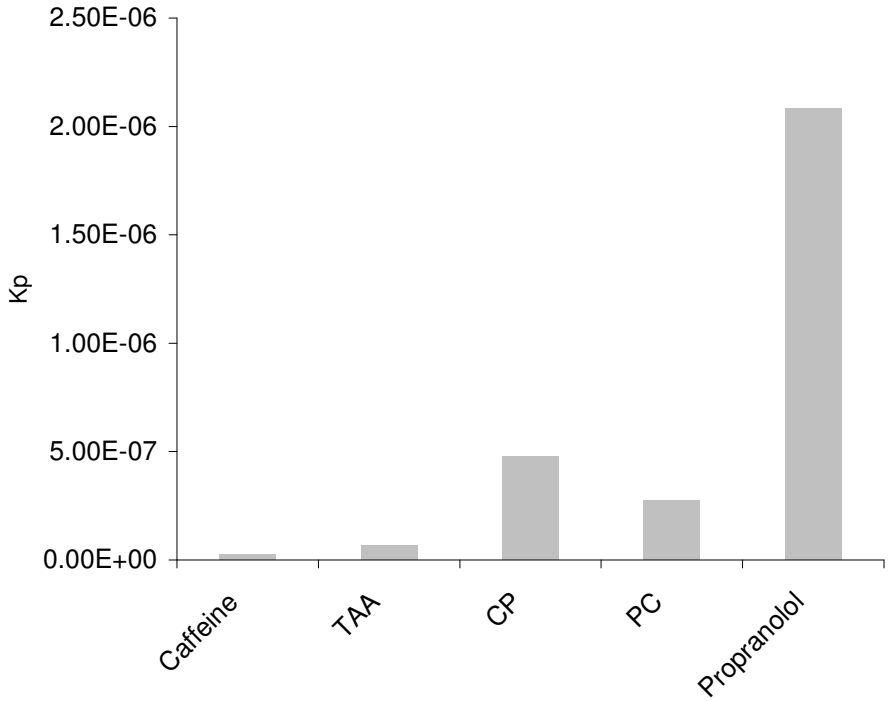


Fig. 3-1: Calculated permeability coefficients of caffeine, propranolol, TAA, CP and PC according to the model postulated by Potts and Guy (1992). The input parameters (MW and Log P) were obtained from ChemSpider chemical databank (http://www.chemspider.com).

The skin permeability followed the rank: propranolol > CP > PC > TAA > caffeine. Among the model drugs, CP has presented the highest coefficient ($4.76 \cdot 10^{-7}$), followed by PC ($2.75 \cdot 10^{-7}$) and TAA ($6.68 \cdot 10^{-8}$). This can be explained by the higher lipophilicity value of CP. The values obtained are closer to the coefficient calculated for the low-permeable marker caffeine ($Kp = 2.64 \cdot 10^{-8}$), suggesting that TAA, CP and PC are most likely to present low skin permeability.

3.3.3. Prediction of aqueous solubility

In a broad sense, solubility may be defined as the amount of a substance that dissolves in a given volume of solvent at a specified temperature (Alsenz and Kansky, 2007). In the lead compound identification during the discovery phase, solubility assays are routinely used to

guide chemists to overcome potential compound liabilities, to develop appropriate chemistry strategies, and to make decisions on potential development candidates (Alsenz and Kansky, 2007). In the drug product development, the solubility information is crucial for the choice of appropriate formulations for animal and human studies involving the selection of suitable cosolvents and excipients. Lee *et al*. (2003) investigated many Pfizer's discovery drugs and found that out of 300 compounds, 85% were formulated using pH adjustment, cosolvents or the combination of both in the preclinical phase. The calculated aqueous solubility of the model drugs and data found in the literature are presented:

Tab. 3-2: Calculated solubility results of the model drugs.

Test compound	**Melting point (ºC)**	**Log P**	**Calculated solubility (µg/mL)**	**Reported solubility (µg/mL)**
TAA	292	2.50	21.38	21.00 (1)
CP	197	3.98	6.31	3.96 (2)
PC	202	3.83	7.94	5.09 (2)

(1) Block and Patel, (2006).

(2) Wishart *et al.*, (2006).

In general, the values calculated with the model postulated by Yalkowsky and co-workers are in good agreement with the data found in the literature. The results demonstrated that all model drugs are considered to be practically insoluble (Ph. Eur., 2008). TAA was slightly more soluble in comparison to the other model drugs. Insoluble compounds are considered to be critical in the product development. In topical products, only the drug in solution state in the vehicle is assumed to diffuse, to be released and to become available to exert its action. The rate of drug soluble in the topical vehicle may directly affect the biopharmaceutical properties of the product. Hence, solubility data can also orientate the selection of an appropriate vehicle and mix of excipients toward the maximization of the drug solubility. Nevertheless, the adjustment of biopharmaceutical properties can be done with more accuracy after results of posterior pharmacokinetic characterization. All integrated information gathered in the *in silico* assessment together with the data from biopharmaceutical characterization provides a more conclusive understanding during the optimisation phase.

3.3.4. Investigation of solubility with cosolvents

Topical corticosteroids are frequently formulated into hydrogels, since this type of bases present more pleasant application, transparent, appearance (Draelos *et al.*, 1995). Low aqueous solubility in hydrogels may be surmounted by addition of water-miscible cosolvents. PG and DEGEE (Transcutol®) are commonly used cosolvents and also aid enhancing penetration of drug into the skin. In this part of the study, the solubility of CP in binary mixtures of water/cosolvent was investigated.

Tab. 3-3: Solubility values of CP in binary mixture of water with PG and DEGEE (n=3).

Concentration of cosolvent	**Solubility (µg/mL)**	
(%)	**PG**	**DEGEE**
0	*	*
10	0.87 ± 0.03	3.19 ± 0.14
20	5.30 ± 0.03	19.48 ± 0.04
30	17.55 ± 0.41	78.47 ± 0.66
50	172.53 ± 0.73	925.55 ± 3.64

* Values under the analytical lowest limit of quantification.

Varying the concentration of cosolvent, a noticeable increase of the drug solubility was observed. The limited solubility of CP in water calculated with the model of Yalkowsky and co-workers is consistent with the experimental results. DEGEE provided higher drug solubility with increasing concentration. The solubility values in PG are in good agreement with the literature (Kasongo, 2007). Most cosolvents have hydrogen bond donor and/or acceptor groups as well as small hydrocarbon regions. Their hydrophilic hydrogen bonding groups ensure water miscibility while their hydrophobic hydrocarbon regions interfere with water's hydrogen bonding network, reducing the overall intermolecular attraction of water (Wypych, 2000). By disrupting water's self-association, cosolvents reduce water's ability to squeeze out non-polar, hydrophobic compounds, thus increasing solubility (Millard *et al.*, 2002). A different perspective is that by simply making the polar water environment more non-polar like the solute, cosolvents facilitate solubilization. This is supported by the observation that cosolvents reduce the solubility of polar compounds such as amino acids, ostensibly by reducing the polarity of the aqueous environment and thereby reducing the favorable interactions between solute and solvent (Millard *et al.*, 2002). Tab. 3-3 shows an increase in drug solubility with the increase of cosolvent fraction. The log-linear model describes an exponential increase of solubility values of non-polar drugs in function of a

linear increase of cosolvent fraction (Li and Yalkowsky, 1994; Millard *et al.*, 2002). This relationship is described as:

Eq. 14

$$\log S_{mix} = \log S_w + \sigma F_c$$

Where S_{mix} and S_w are the total solute solubility in the cosolvent/water mixture and in water, respectively, σ is the cosolvent solubilization power for the particular cosolvent/solute system, and F_c is the volume fraction of the cosolvent in the aqueous mixture. Plotting the slope of log (S_{mix}/S_w) in function of cosolvent fraction, a straight line was obtained with values of regression coefficient higher than 0.9 (Fig. 3-2). This indicates that the solubilization behaviour of CP in the mixtures of water/cosolvents followed the log-linear model for the investigated concentrations of cosolvent (10-50%). The slope of the plot represents the solubilization power (σ) of PG and DEGEE.

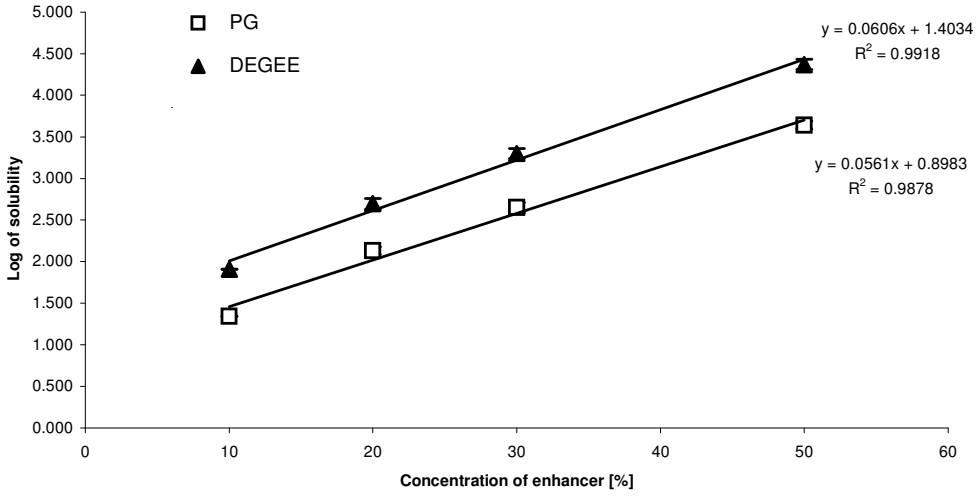


Fig. 3-2: Plot of log of drug solubility in function of fraction of PG and DEGEE. The graph displays the mean value of three determinatons (n=3) and SD.

3.3.5. Investigation of stability in solution and analytical profiling

The examination of the stability of the drug substance and the possible degradation products are key elements in the initial preformulation stage. The characterization of the impurities and/or degradation products is very important in order to protect patients from unwanted side effects. Degradation leading to sub-potency of fluocinolone in Fluocinolone Acetonide Topical Solution USP, 0.05% has lead to recall of the product in the United States (FDA 1998b). Besides the implications on the safety and toxicity of the drug product, this initial characterization also provides useful information to orientate the formulation design. Since the studies are frequently performed under different pH conditions, it provides a first insight into the optimal vehicle pH. In addition, it can deliver discerning information to the degradation products which are originated from the drug molecule and the ones related to the excipients (Bakshi and Singh, 2002). Stress testing or forced degradation studies are frequently used to determine the intrinsic stability of the drug molecule by establishing the degradation pathways in order to identify the likely degradation products and to develop and validate the stability-indicating analytical methods (ICH, 2003). These are defined as validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference (Snyder *et al.*, 2009). Analytical techniques are fundamental to new drug product development. Without them, no evaluation of the quality of materials, product precursors, final product, biological or pharmacologic responses in the preclinical or clinical stages can be measured. Analytical methods are developed before the product development stage and the optimization of the method follow the requirements of the development stages.

Degradation profile of the model drugs

An overview of the degradation profile of TAA, CP and PC under oxidative, alkaline, acidic conditions with and without cosolvent at RT and 60 ºC over 48 hours is presented (Fig. 3-3):

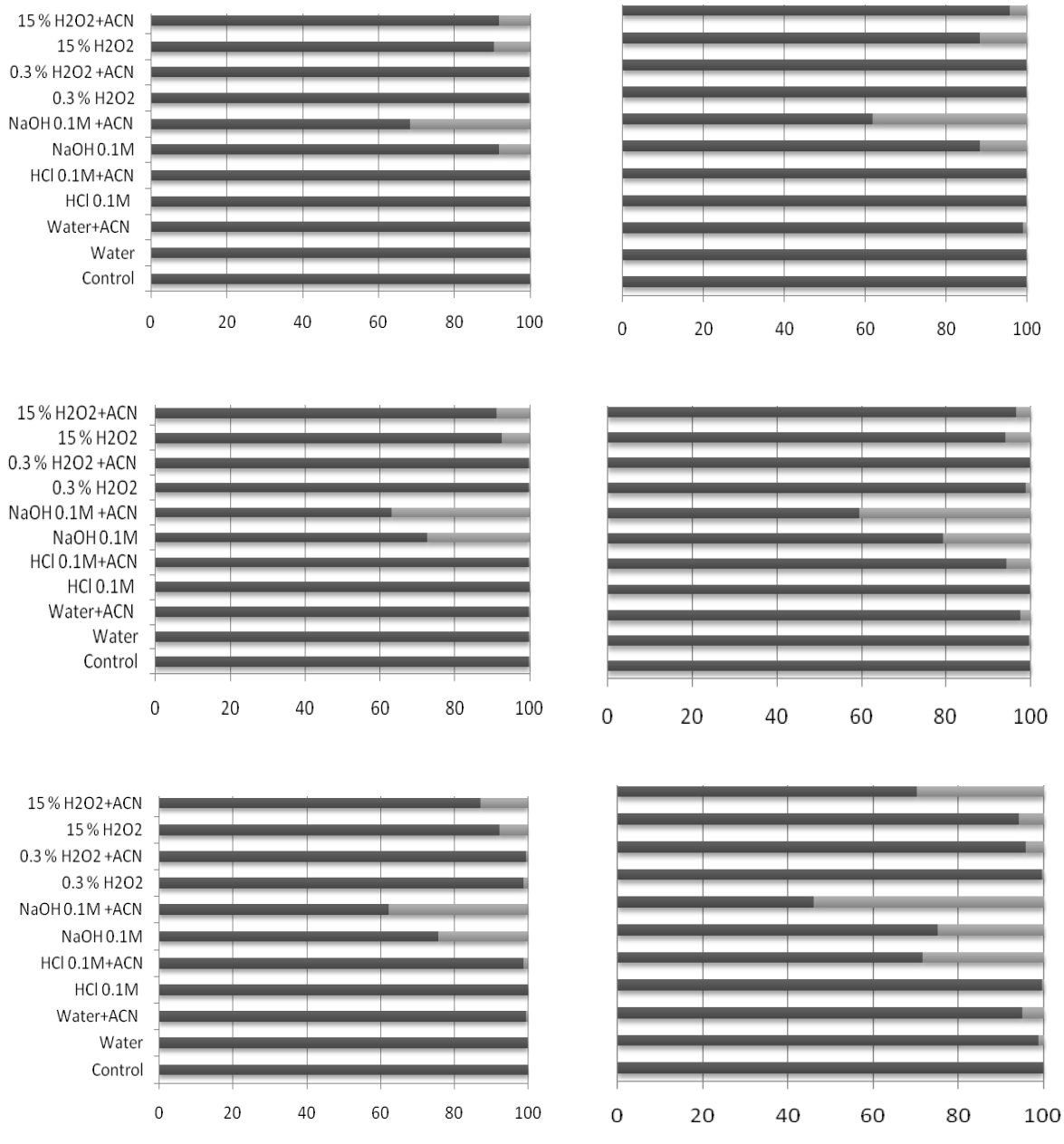


Fig. 3-3: Overall evaluation of degradation profiles of CP, TAA and PC under alkaline (0.1 N NaOH), acidic (0.1 N HCl), oxidative (0.3% and 15% H_2O_2). All the samples were prepared with and without addition of cosolvent (acetonitrile) and stored in closed HPLC vials at RT and 60 ºC over 48 hours. In the graphs, the peak area in percentage corresponding to the model drug (dark bars) and sum of degradants (light bars) are presented.

The model drugs have mostly undergone significant degradation (over 5%; ICH, 2003) under alkaline (0.1N NaOH) and oxidative conditions with solution at 15% H_20_2. A slightly yellow solution was observed for the samples stressed under basic conditions with cosolvent. The susceptibility of corticosteroids to undergo degradation catalyzed by basis was already described for bethametasone-17-valerate (Bundgard and Hansen, 1981), hydrocortisone butyrate (Yip *et al.,* 1983) and hydrocortisone-21-lysinate (Johson *at al.,* 1985). Gupta e*t al.,* (1983) have reported the general corticosteroid decomposition following to hydrolytic attack of ring A or attack on the C-17 side chain in aqueous and alcoholic solutions.

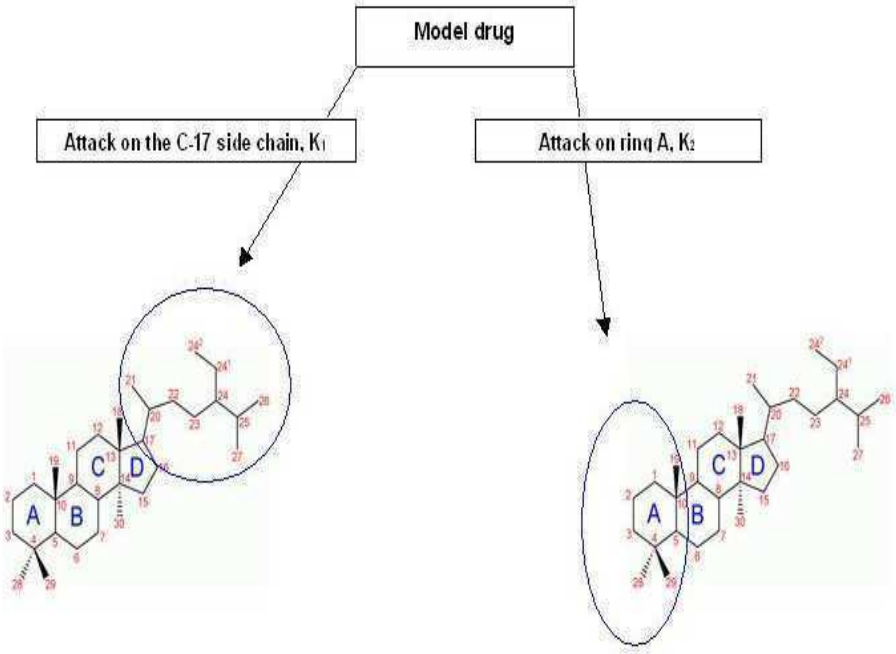


Fig. 3-4: General degradation of corticosteroids. Modified after Gupta e*t al*., (1983).

Degradation following attack on the ring-A has been particularly observed with some steroids of 1-ene-3-keto or 1,4-diene-3-keto structure, such as hydrocortisone (Allen and Gupta, 1974). In general, the degradation reactions at C-17 side chain are catalyzed by proton, hydroxide, and trace metal ions (Teng *et al.,* 2003). The clinical relevance of this degradation route for corticosteroids C-17 ester derivatives is well illustrated with bethamethasone-17-valerate, which has 15 times higher activity of the 21-isomer upon application (McKenzie and Athinson, 1964). The attack on the C-17 side chain is frequently associated with reversible ester group migration between C-21 and C-17-hydroxy groups in aqueous media. Bundgard

and Hansen (1981) have pointed out the overall decomposition of bethametasone-17-valerate reactions were found to consist of a reaction sequence involving an initial rearrangement of betamethasone-17-valerate to betamethasone-21-valerate followed by hydrolysis of the later to betamethasone (Fig. 3-5).

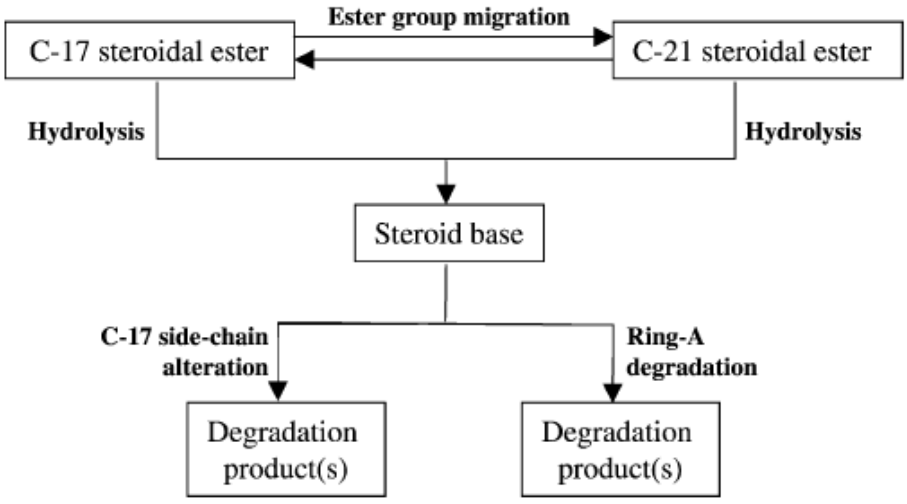


Fig. 3-5: Schematic graph showing general the degradation pathways of synthetic steroidal C-17 esters (Teng a*t al.,* 2003).

Allen and Gupta (1974) found for hydrocortisone formulated in polyethylene glycol ointment that the decomposition of the C-17 side chain was much faster (average half-life of 186 days at 60 ^{o}C) as compared with ring A (average half-life of 505 days at 60 ^{o}C). TAA, PC and CP have 1,4-diene-3-keto structure, which are susceptible to attack on ring A as previously described. For the theoretical evaluation of the possible degradation routes on C-17 side chain, the structure of the compounds has to be particularly examined. CP bears on C-17 two propionate ester groups, one of these is halogenated on C-21. TAA bears an acetonide group and a 2-one-4-hydroxy moiety on C-17. PC has on C-17 two ethoxycarbonyl groups. The two esterifications at positions C-17 of PC increase the molecule's lipophilicity, and its uptake by and affinity for the glucocorticoid receptor (Borgia e*t al*., 2008). This has brought the concept and applications of the prodrugs to the topical corticosteroids. The decomposition of PC seems to follow the description in Fig. 3-5. The hydrolysis followed by ester migration results the formation of monoesters prednisolone-17-ethylcarbonate and prednisolone-21-ethylcarbonate. The hydrolysis of monoesters leads to the steroid base (prednisolone) as previously pointed out (Gysler *et al.*, 1997). All PC metabolites differ in receptor binding affinity, which can strongly influence its therapeutics and local toxicity. The metabolite

prednisolone-17-ethylcarbonate demonstrates higher adrenocorticoid receptor affinity in comparison to PC. The higher proportion of the metabolite in comparison to the PC improves, therefore, its benefit/risk ratio (Schäfer-Korting 1998).

It can be observed that a complex chromatogram was generated with seven degradants identified from A to G (Fig. 3-6). The evaluation of the UV spectra of the decomposition components demonstrated that all are structurally similar to the native drug. The UV spectra extracted with photodiode array detector varied demonstrated that the maximum of absorption from 244 nm to 248 nm, which suggests that the cromophore group (cyclohexadienone A-ring) kept intact for all decomposition products. Thus, the present data give strong evidence that the decomposition route has most likely undergone attack on side C-17 rather than attack on ring A.

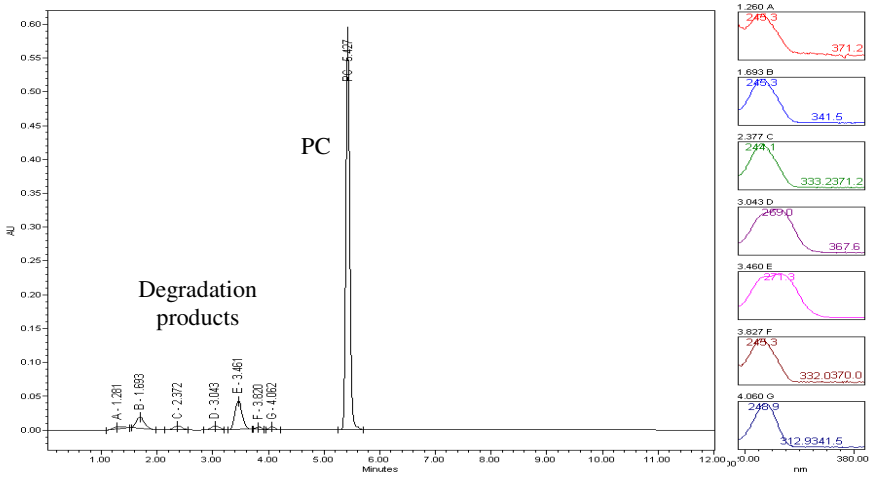


Fig. 3-6: Representative chromatogram from stressed sample of PC under alkaline conditions.

Apparently, the degradation route of TAA seems not to follow ester migration as previously described. The compound bears an acetonide group and a 2-one-4-hydroxy moiety on C-17. TAA bears two spatially separated cromophores: cyclohexadienone moiety in ring A and carbonyl group in C-20. The maximum of absorption of the predominant component C (retention time of 5.133 minutes) is identical to the native drug (Fig. 3-7). This indicates that the cromophores in the ring-A and C-20 were not affected and, therefore, decomposition of the 2-one-4-hydroxy moiety is assumed to not have occurred.

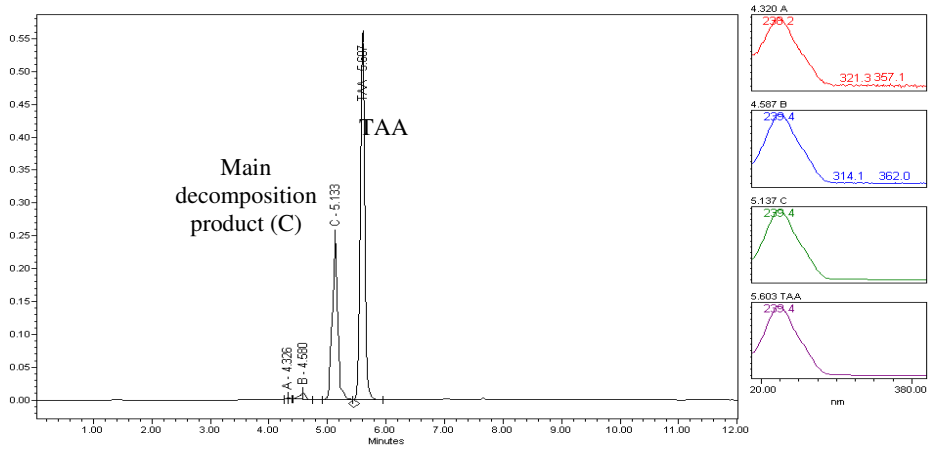


Fig. 3-7: Representative chromatogram from stressed sample of TAA under alkaline conditions.

The present results are in good agreement with the literature. Matysova *et al.,* (2003) have found that triamcinolone was the main decomposition product of triamcinolone acetonide in topical creams after long-term stability studies. Sudsakorn *et al.,* (2006) have also identified triamcinolone as main degradation product eluting earlier than the native drug in stability studies for nasal sprays. The component C is assumed to be originated from the decomposition of the acetonide group leading to triamcinolone (Fig. 3-8).

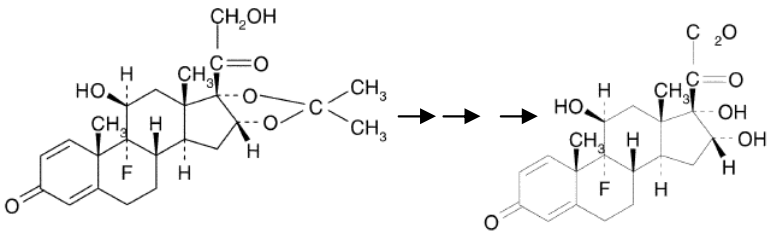


Fig. 3-8: Decomposition of triamcinolone acetonide yielding triamcinolone.

CP bears a chloride in the C-21. This halogenisation is assumed to hinder the reactivity of the carbon C-21. Therefore, the degradation consisting of ester migration was not the most likely route. It has been previously demonstrated for mometasone that the substitution of the 21-acyl by chlorine increases the resistance of this drug to degradation by esterases in the epidermis (Mori *et al*., 1994; Prakash and Benfield, 1998). Thus, the decomposition of CP is rather to undergo hydrolysis of the non-halogenated propionate group on C-17.

Evaluation of testing conditions

Different stress conditions were employed during the performance of the forced degradation studies: presence and absence of cosolvent; storage at RT and 60 ºC; two oxidative levels (solution 0.3% and 15% H_2O_2); alkaline and acidic conditions. Acetonitrile was employed in order to investigate the inherent stability of the substance in solution state, since the corticosteroids are assumed to be dissolved in the topical vehicle. Generally, the addition of the cosolvent has increased the susceptibility of the molecules to undergo hydrolytic or oxidative reactions. The solutions employed for acidic and alkaline conditions represented a drastic panorama. Indeed, the employment of forced conditions fulfilled the purpose of the forced degradation study but extrapolations to realistic conditions should be avoided. The same rationale is valid for the investigation under oxidative conditions. Two solutions with different concentrations of H_2O_2 were employed (0.3% and 15%) but significant decomposition (>5%) was observed only for the solution at 15%. Nevertheless, this concentration is unlikely to represent realistic conditions. More conclusive statements to the relevant degradants can be done only after completion of formal stability studies (long-term and accelerated). One of the critical points during the protocol design is the exact determination of the testing conditions and timing. Although the regulatory guidance documents state and define concepts of forced degradation studies, they do not provide strategies or best practices for the amount of stress which has to be applied. Especially due to the variety of the nature of drug substance and drug products, particular approaches should be considered. The amount of stress applied to a sample should be selected according to what is necessary to achieve a "purposeful degradation." An optimal degradation pattern generated during stress testing would show only those degradation products observed at the end of shelf-life in regulatory stability studies and those that might appear if the drug substance or drug product is not handled or packed properly (Alsante *et al.,* 2007). The experiments should focus on the development of a protocol to induce at least 5-20% degradation and parallel attempt to elucidate degradation pathways (Alsante *et al.,* 2003). Overall, among all approaches employed, the samples treated without cosolvent at 25 ºC during 48 hours have generated results fulfilling the assumed "adequate degradation". Then, the chromatograms generated from this protocol were employed for the posterior evaluation of the degradants.

After establishing the most suitable testing conditions, the key degradants have been identified. The analysis and judgment of significance of degradants can be very project specific. Usually individual degradants exceeding 10% of total degradation are considered

primary degradation products (Alsante *et al.,* 2007). The identification has followed by the retention time and UV spectra extracted by photodiode array detector. This analytical tool represented a fast and simple strategy for an initial elucidation and identification of degradants. The complete elucidation of molecular structure of the degradants is generally feasible only using a combination of analytical techniques as mass spectrometry and magnetic resonance microscopy. Fully elucidation is, however, required only at the final phase of clinical trials after completion of formal stability testing in further clinical phases (Alsante *et al.,* 2007). Thus, attempts to fully characterization of degradants in the preclinical phase are rather to be pointless. Photodiode array detectors allows a relatively simple and fast analysis of UV absorption spectrum and permits to drawn some conclusions with regard to the nature of the degradant based on modifications of the cromophores. Hence, the initial identification in the present investigation provided an important basis for further structure elucidation in the late development stages.

3.4. Summary and conclusion

In silico models are useful prior to actual experimentation, in particular when sample amount and timeline are restricting factors. They allow some properties to be predicted rapidly and inexpensively, permitting resources to be used to screen for other properties (Ekins and Wang, 2006). The assessment of drug-likeness properties with aid of Rule of Five postulated by Lipinski (1997) demonstrated that none of the model drugs is expected to be critical concerning developability issues. The calculated permeability data with aid of the model proposed by Potts and Guy (1992) suggested that PC, TAA and CP are most likely to demonstrate low skin permeability. Thus, the utilization of excipients or mixture of excipients has to be appropriate in order to surmount permeability issues and to provide transdermal delivery. The results also provided important information for the design of testing procedures at further development stages. The obtained aqueous solubility data have demonstrated that all model drugs are practically insoluble. Since many topical vehicles are o/w emulsion, low water solubility might become critical. Thus, the formulator becomes already aware at this early stage that the selection of cosolvents and excipients for enhancing drug solubility might be necessary for dissolving the desired amount of drug. In conclusion, the *in silico* assessment of permeability and solubility of the model drugs provided an important understanding for the excipients selection and design of testing procedures to be employed in further phases.

The determination of solubility of CP with cosolvents was performed with a scaled-down method employing approximately 1 mg of compound per test conditions. The solubility

values plotted against cosolvent fraction yielded to a straight line and the cosolvency power was calculated from the slope. The performance of forced degradation studies gave a first insight into the degradation profile of the model drugs under acidic, alkaline, water own pH and oxidative conditions. TAA, PC and CP have demonstrated to be prone to decompose under alkaline conditions. The results obtained correlated with the findings from the literature for other corticosteroids. The evaluation of UV spectra from the degradants in comparison to the native drug strongly suggests that the degradation pathway is most likely to follow attack on the side chain of the carbon 17 as described by Gupta *et al*. (1983). The decomposition on the side chain on C-17 followed presumably different routes according to the particular molecular structures of the three compounds. The degradation route of PC is most likely to undergo ester group migration and hydrolysis as previously described for metabolism studies with reconstructed human epidermis (Borgia *et al.,* 2008) and keratinocytes (Gysler *et al.,* 1997, Haberland *et al.*, 2006). The most predominant decomposition product of TAA was found to possess identical UV spectra eluting slightly earlier than the native drug. The results of stability studies in topical creams (Matysova *et al.,* 20003) and in nasal sprays (Sudasakorn *et al.,* 2006) supported the evidence that the mentioned degradant corresponds to triamcinolone. CP was less extensively decomposed in comparison to TAA and PC. The chloride on C-21 presumably lowers the reactivity on this carbon. Thus, ester migrations and hydrolysis are less probably to occur as reported for studies with esterases (Mori *et al.,* 1994). The identification of the relevant degradants has followed by retention time and UV spectra as presented in the chromatograms. This represented the first initiative to the further fully structure elucidation, required at advanced development stages (Alsante *et al.,* 2007).

In conclusion, a compact and effective preformulation program could be obtained with the utilization of *in silico* assessment, few experimental tools and a minimal amount of test substance permitting to achieve important background information with respect to the decomposition products and their degradation pathway; solubility with cosolvent; and pH-stability profile, serving to guide the design of the topical vehicles.

4. Selection and characterization of base formulations

4.1. Introduction

The choice of excipients and vehicles depends on many factors, such as the action desired, nature of the active substance to be incorporated and its bioavailability and stability. During the galenic development, several tools can be utilized to characterize the prototypes. Rheological characterization is particularly important for in manufacturing operations, changes upon transportation or behavior during administration/utilization of pharmaceutical products (Barnes, 1994; Derkach, 2009). During the industrial operations, the knowledge of the rheological properties is required for the design, selection and operation of the equipment involved for mixing, storing, and pumping of emulsions (Pal, 2000). Moreover, investigation of rheological properties is important to monitor alterations of formulation microstructure during stability studies (Tadros *et al.,* 2004). Centrifugation tests can be used for a rapid assessment of physical stability of thermodynamic unstable formulations. The utilization of this test for accelerated estimation of physical stability of emulsions has been widely documented (Tcholakova *et al.,* 2004; Villalobos-Hernandez and Müller-Goymann, 2006; Formiga *et al.,* 2007; Hayatia *et al.,* 2009). However, one of the major concerns of the employment of this test in an integrated development program is the delivery of irrelevant stability information. The magnitude of the stress should not exceed the critical force that causes deformation of the emulsion droplets and oil separation (Idson *et al*., 1993). Excessive force may yield to results which might not be observed in long-term studies representing an exaggerated stress condition. The selection of the test conditions has to be done with scientific sound to yield representative results.

This part of the study aimed to characterize the standard base formulations employed in the proposed development platform. For scientific purposes, representative vehicles widely utilized in German pharmacies with different physicochemical properties, structure, macro and microviscosities were selected from the Deutsches Arzneibuch (DAB) and the Deutscher Arzneimittelcodex (DAC) covering the group of hydrogels, o/w creams, w/o creams and anhydrous ointments. Flow and oscillation measurements were utilized for the rheological characterization. A centrifugation method with adjusted centrifugation forces to rapidly determine physical stability with realistic estimations has been proposed.

4.2. Materials and methods

4.2.1. Base formulations

The base formulations were prepared according to DAB (2010) and DAC (2009) monographs. The pharmaceutical ingredients are described below:

Base cream DAC (BC)

Glycerol monostearate 60 (4%), cetyl alcohol (6%), medium chain triglyceride (7.5%), white soft paraffin (25.5%), macrogol-20-glycerol monostearate (7%), propylene glycol (PG) (10%), water (40%).

Nonionic hydrophilic cream DAB (NHC)

Polysorbate 60 (5%), cetostearyl alcohol (10%), glycerol 85% (10%), white soft paraffin (25%), water (50%).

Silicone cream FN 5% (SLC)

Dimeticone 350 (5%), cetomacrogol 1000 (15%), decyl oleate (5%), hard paraffin (10%), sorbitol solution 70% (4%), PG (2%), water (59%).

Hydrophobic base cream DAC (HBC)

Triglycerol diisostearate (3%), isopropyl palmitate (2.4%), hydrophobic base gel DAC (24.26%), potassium sorbate (0.1%), citric acid (0.07%), magnesium sulphate (0.5%), glycerol, 85% (5%), water (64.8%).

Cold cream DAB (CC)

Yellow beeswax (7%), cetyl palmitate (8%), arachic oil (60%), water (25%).

Wool fat ointment DAB (WS)

Cetostearyl alcohol (0.5%), wool fat alcohol (6%), white soft paraffin (93.5%).

Hydrophilic ointment DAB (HS)

Emulsifying cetostearyl alcohol (type A) (30%), hard paraffin (35%), white soft paraffin (35%).

Carbomer gel DAB (CG)

Carbomer (carbopol 980®) (0.5%), NaOH sol (50 g/L) (3%), water (96.5%).

All the excipients used for the preparation of the formulations were acquired from Caesar & Lorenz GmbH (Bonn, Germany).

4.2.2. Reference formulation

Dermatop® cream was purchased from Sanofi-Aventis Deutschland GmbH (Frankfurt, Germany).

4.2.3. Visual examination

Appearance, colour and odour of the bases were examined at the same temperature, lighting and packaging conditions.

4.2.4. Determination of pH

Approximately 1 g of formulation was weighed into tubes and dispersed in 10 mL of water. After homogenized, the pH measurement of the samples was carried out with pH-meter Orion model 330 (Thermo Fisher Scientific Germany Ltd. & Co. KG, Braunschweig, Germany).

4.2.5. Rheological characterization

Flow curves were measured with samples of approximately 2 g at controlled stress (0-80 Pa) and constant temperature (20 ºC) employing the rheometer with parallel-plate geometry (Physica MCR501, Anton Paar, Graz, Austria). Oscillation stress sweep tests were performed in triplicate applying increasing strain at different ranges for hydrogel (0.1-100 Pa), creams (0.1-500 Pa) and ointments (0.1-1000 Pa) at constant frequency (1 Hz) and temperature (20 ºC).

4.2.6. Characterization of physical stability with centrifugation test

The investigation of the physical stability was performed only for the base formulations susceptible to phase separation (emulsion systems). Dermatop® cream was employed as positive reference. The comparison to an established stable registered product represented a yardstick for obtaining meaningful stability information from untested vehicles. Adequate centrifugation forces were established after preliminary experiments and oriented by previous published stability studies with Dermatop® cream (Borgia *et al.,* 2008). Two centrifugation

procedures were employed: centrifugation force of 5000 g during 10 minutes (test A) and centrifugation force of 13200 g during 20 minutes (test B). Approximately 2 g of the vehicles were placed on Eppendorf® cups and stored at 40 ºC over 3 months. Centrifugation was performed with mini-centrifuge (Eppendorf, Berzdorf, Germany) after 1 and 3 months. After the test, two fractions were identified as supernatant and sediment. The supernatant was removed and the tubes were weighed to calculate by subtraction the amount of separated phase which is reported in % of total mass.

4.3. Results and discussion

Eight standard base formulations (BC, NHC, SLC, HBC, CC, CG, WS and HS) were selected from the DAB (2010) and the DAC (2009). The rationale for this selection followed: (a) employment of vehicles with different microstructures and physicochemical properties comprising a wide range of semisolid groups (hydrogel, o/w creams, w/o creams, anhydrous ointments); (b) different clinical applications according to the severity of the skin disease; (c) utilization of standardized formulations with relatively known toxicological profile. BC is an amphiphilic cream considered as universal base due to its amphiphilic properties. The base is adequate for incorporation of cationic and anionic compounds (Garbe and Reinmann, 2005). NHC is a white hydrophilic cream adequate for incorporation of cationic and nonionic active ingredients and the treatment of acute and subacute skin dermatitis (Garbe and Reinmann, 2005). SLC is a white hydrophilic cream containing dimeticone that together with the other hydrophilic vehicles promotes prominent hydration of the skin producing a plastic film occlusion (Suetake *et al.*, 2000). BC, NHC and SLC represent the group of o/w creams with particular differences in water content and mixture of emulsifiers. HBC is a w/o cream with high water content. HBC stands between the strong occlusive bases composed by paraffin and the hydrophilic bases less adequate for dry skin (Garbe and Reinmann, 2005). CC is a yellowish cream indicated for acute and subacute dermatitis. The cooling effect is explained by the liberation of water from the cream. HBC and CC represent the group of w/o creams with more occlusive properties than o/w creams but less greasy then water-free ointments. WS is a yellowish anhydrous base with slight characteristic odour. Usually WS is indicated for the treatment of chronic disease state due its occlusion properties (NRF, 1999). HS is classified as an absorption base (DAB, 2010) constituting of hydrocarbon bases and an o/w emulsifier. The mix of the ester and his respective salt (cetostearyl alcohol and sodium cetostearyl alcohol) provides the capacity of incorporating water forming o/w creams. CG is a clear hydrophilic gel which contains 0.5% of Carbomer 980®. This base represents the group

of hydrogels which are adequate for the treatment of sensitive skin, acute dermatoses and application in hairy areas.

4.3.1. Assessment of visual properties and determination of pH

A qualitative description of the dosage form should be provided as initial quality control and identification procedure (Phar. Eur. 2008; USP, 2009). The assessment of appearance and odour was performed after manufacturing of the formulations. The pH was determined for o/w emulsions and hydrogel (CG).

Tab. 4-1: Results of determination of pH (n=3).

Base formulation	**pH value**
	Mean and SD
BC	6.15 ± 0.13
NHC	5.92 ± 0.08
SC	5.62 ± 0.15
CG	6.25 ± 0.17

The appearance and odour of the manufactured bases complied with the description in the specified monographs (DAC 2009; DAB 2010). The pH values of the bases varied from 5.6 to 6.3. The preformulation studies with TAA, CP and PC revealed that the drugs are relatively stable in neutro and slightly acid pH. Timmis and Gray (1983) reported that the optimum pH of TAA in aqueous solutions is between pH 3-6. The pH of the vehicles can be further adjusted to the pH range of the skin (pH 4.6-5.8) before the definition of the final formulation (Rodrigues *et al.,* 1995; Pinto *et al.,* 1997).

4.3.2. Flow measurements

Flow properties of topical vehicles are a subject of considerable importance and a direct manifestation of the various interaction forces that occur in the system. Rheology controls the application of semisolid systems, e.g., the spreadability, consistency and helps to obtain information on the structural properties (Rao, 2007). Understanding the relation between structure and rheology helps in designing products with specific rheological properties. The flow curves of BC, SLC, NHC, HBC and CC are shown in Fig. 4-1.

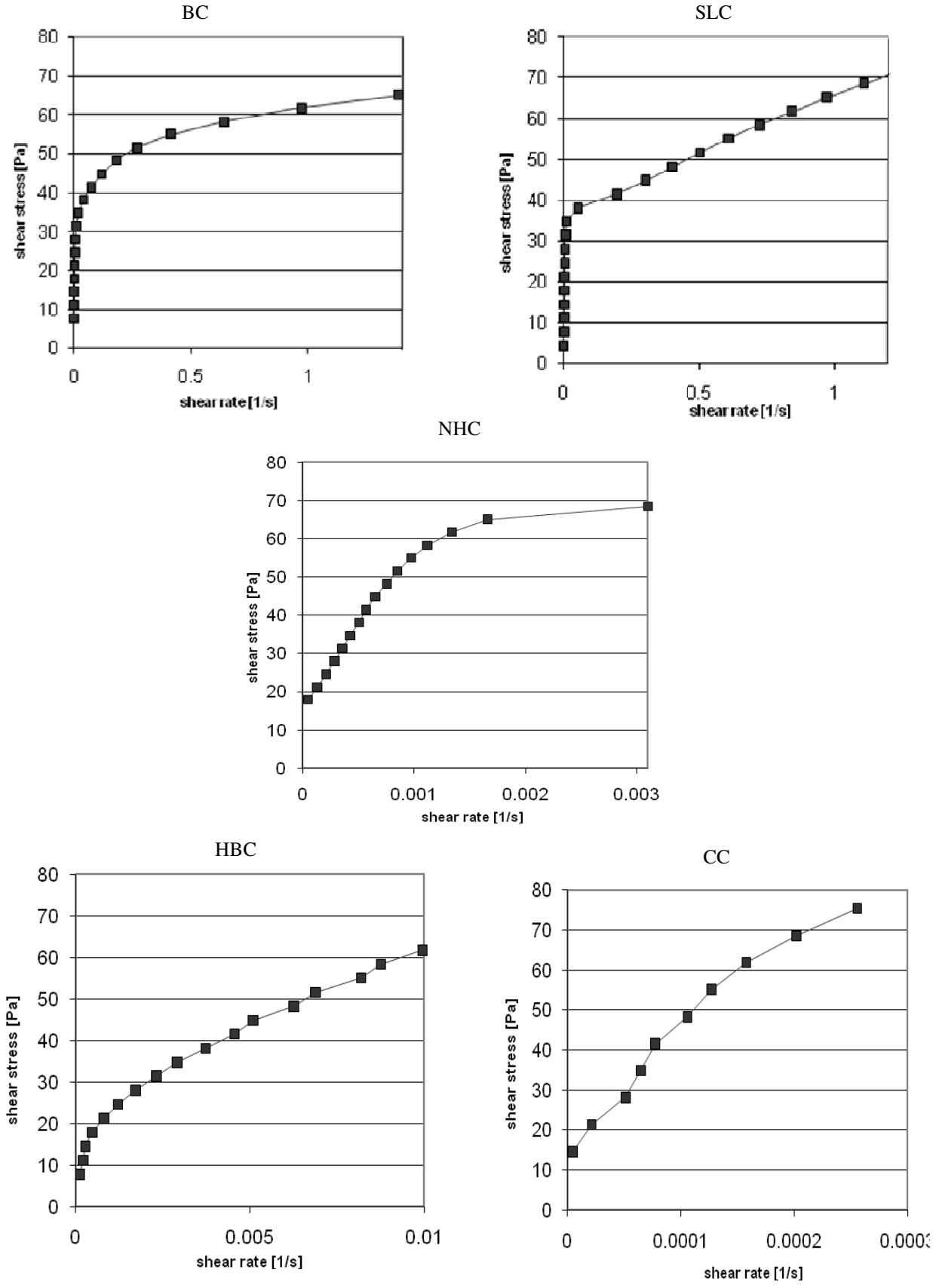


Fig. 4-1: Flow curves of BC, SLC, HNC, HBC and CC at the same strain range (0-80 Pa). The black squares represent the shear stress (n=1).

The rank of resistance to strain followed by CC > NHC > HBC > SLC > BC. For all examined creams, an increase in the shear stress resulted to a non-linear decrease of the apparent viscosity, indicating shear thinning properties typical from pseudoplastic materials. The curved rheograms results from a shearing action of long chain molecules or other arranged structures. As the shearing stress is increased, the normally disarranged molecules start to align their long axes in the direction of flow and the orientation reduces the internal resistance of the material (Robins *et al*., 2002). The apparent or shear viscosity is a relative value depending on the shear rate. Several factors can influence the viscosity of semisolids as continuous phase volume and nature; volume, concentration, nature, distribution and deformability of dispersed droplets; temperature; nature and the concentration of the emulsifying agent (Pal, 2000). Typically w/o creams presents higher apparent viscosity due to the nature of the external oily phase. This was confirmed for CC but not for HBC. This formulation presented lower resistance to strain in comparison to an o/w cream (NHC). Below the critical stress, the viscosity reaches a limiting value, namely the residual (or zero shear) viscosity (Fig. 4-2). When shear or strain increases, the system is broken down and this may be denoted as the 'true yield stress'. Above the yield stress, the apparent viscosity decreases rapidly with further increase of the shear stress (the shear thinning regime). It reaches another Newtonian plateu, which is the high shear limiting viscosity (Tadros, 2004).

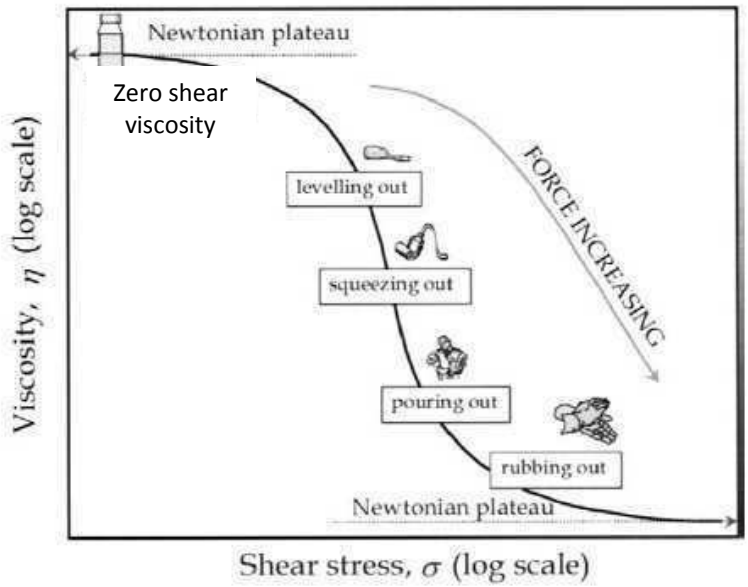


Fig. 4-2: Representation of the various parts of the flow curve of a typical cream categorized in terms of the various end-use operations. Modified after Petsev (2004).

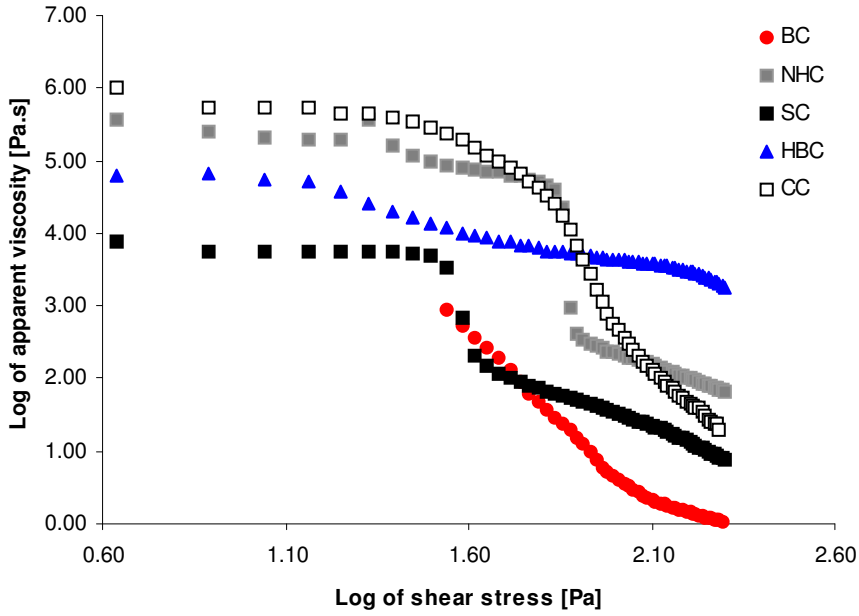


Fig. 4-3: Dependence of apparent viscosity on shear stress for BC, NHC, SLC, HBC and CC.

Taking as reference the values of zero shear rate viscosity, the following rank was obtained: CC > NHC > HBC > SLC > BC. The viscosity data indicates that BC, SLC and HBC are the creams which allow better spreadability and rubbing in the skin. Good spreadability is an important factor to ensure drug uptake in the skin and patience compliance (Draelos, 1995; Vender *et al.*, 2008). Based on the results, a greater strain is needed to cause effective spreading and rubbing of CC and NHC. The measure of the viscosity at very low stresses (or shear rates) can also be used for monitoring stability over storage (Tadros, 2004). Changes in rheological characteristics represent important early warnings of impending failure of the product. A decrease in consistency, changes in time-dependent viscous behaviour and changes in the ratio between viscous and elastic properties can concluded to be signs of instability (Petsev, 2004).

4.3.3. Oscillatory measurements

Oscillatory measurements determine the viscoelastic properties of semisolids, which means the characterization of both elastic response (G´) and viscous behaviour (G´´). In these tests, the system is exposed to oscillating (swinging) constant stress. At low stress values, the structure of the system remains intact i.e. all parameters which characterize the system are constant. If the system is overstressed, the structure will be disturbed and the parameters drastically change. The range of stress values in which the structure of the system is undisturbed is called viscoelastic range. In this region, the values of G*, G´ and G´´ remain

constant up to a critical strain value. Exemplary, the result of oscillatory measurements of CG is presented (Fig. 4-4):

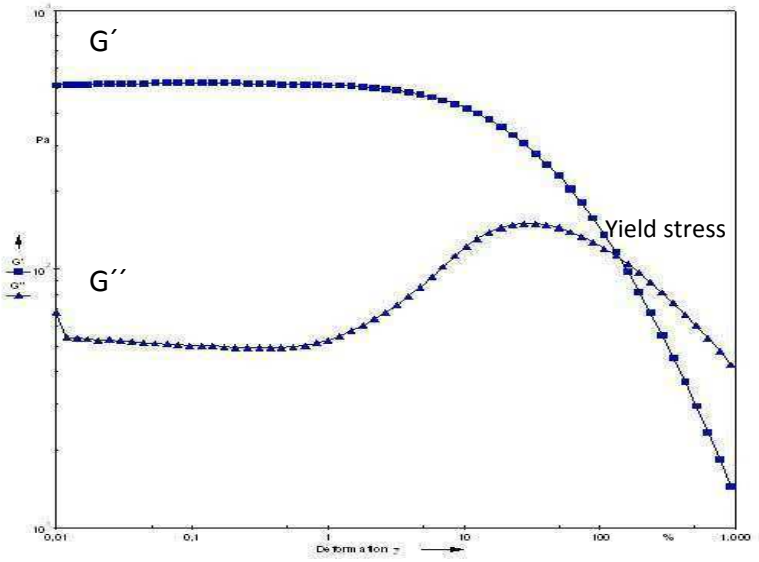


Fig. 4-4: Representative result of oscillatory measurements for CG.

G´ is the measure of how well-structured is a material and its elastic (solid-like) properties. G´´ gives information about the viscous (fluid-like) features. The loss tangent (tan δ) is the ratio between viscous and elastic properties, showing which one is the dominant one. With a tan δ value of 1, the elastic and viscous properties of the material are equal (Fig. 4-4). The smaller the loss tangent is, the more elastic is the material. The values of G´ and G´´ determined in the viscoelastic regions are presented (Fig. 4-5):

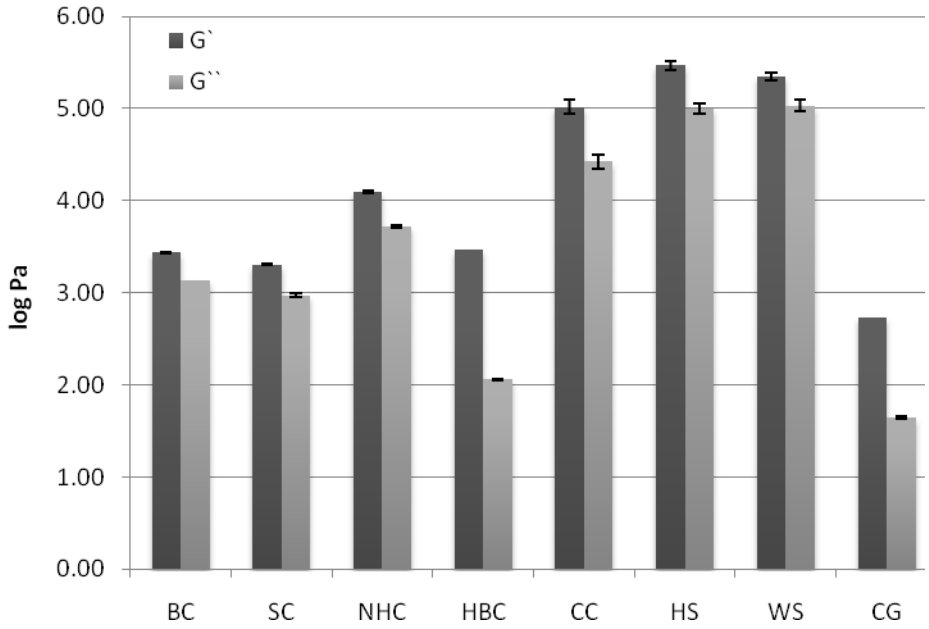


Fig. 4-5: Results of oscillatory measurements of BC, SLC, NHC, HBC, CC, HS, WS and CG. The values are plotted in logarithmic scale. The graph shows the mean of triplicate determinations (n=3) and SD.

As expected, the rank order of complex viscosity followed: ointments > creams > gel. Complex viscosity takes into account both elastic and viscous properties of the system. For all examined base formulations, the values of G´ maintained higher than G´´, indicating that the structure of the formulations were intact during the experiments. In the group of ointments, HS demonstrated higher complex viscosity than WS, which is in good agreement with previous reports (Refai, 2001).

4.3.4. Assessment of physical stability under accelerated conditions

The research focus of this part of the study was addressed to the establishment of a method to rapidly evaluate physical stability of semisolid vehicles employing rational centrifugation conditions. A known registered cream was employed in the investigation as reference to adjust centrifugations forces during the establishment of the test. Two test procedures varying in centrifugation force and time were finally used to assess physical stability. The phase separation was evaluated after storage in closed system at 40 ºC over 1 and 3 months.

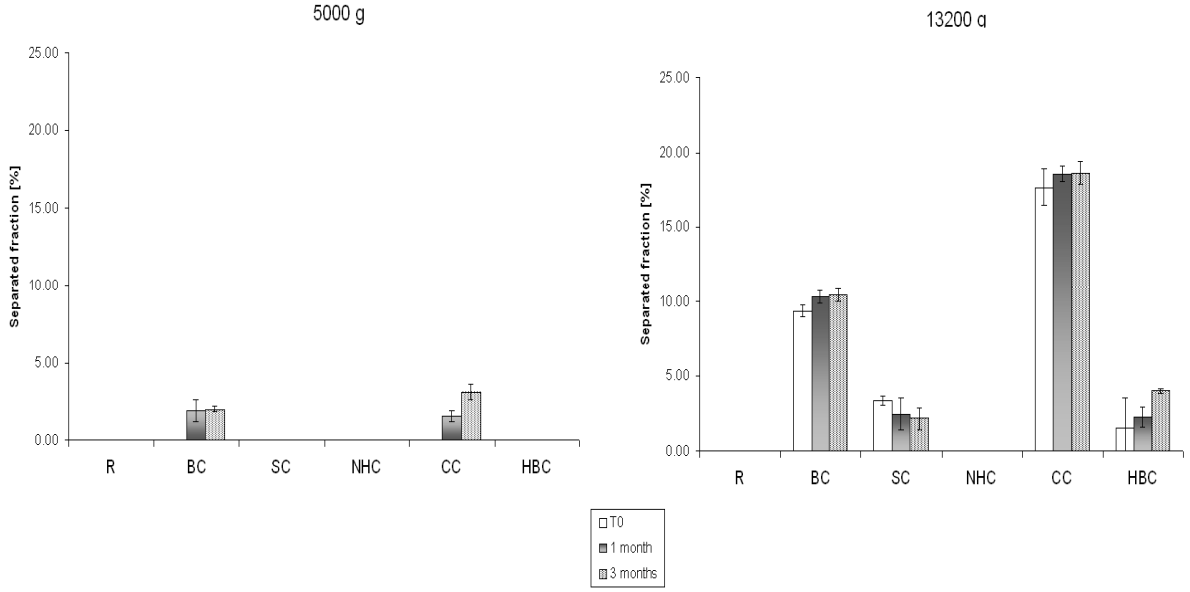


Fig. 4-6: Results of physical stability of the reference product Dermatop® cream (R), BC, SLC, NHC, CC and HBC employing two test procedures: centrifugation force of 5.000 *g* over 10 minutes (test A) and centrifugation force of 13.200 g over 20 minutes (test B). White, dark and gray bars represent results of time point zero, one month and three months, respectively. In the graph the mean value of three determinations (n=3) and SD are shown.

Phase separation profiles were clearly different applying different test conditions. Limited phase separation after 1 month (1.90 ± 0.73%) and 3 months (2.01 ± 0.15%) was observed for BC with procedure A (centrifugation force of 5000 g during 10 minutes). Similar results were obtained for CC after 1 month (1.56 ± 0.36%) and after 3 months (3.11 ± 0.51%). Differently, the application of procedure B (centrifugation force 13200 g over 20 minutes) detected phase separation of four vehicles: BC, CC, SLC and HBC. The fractioning of phases was more extensive and observed at all time points with this procedure. Assuming data obtained from the last measurements (gray bar), the rank of phase separation followed: CC > BC > HBC > SLC. After 3 months, fractioning of CC was 18.60 ± 0.77%, 6 fold higher than the separation obtained with procedure A. At the same time point, 5 fold higher separation was observed for BC (10.44 ± 0.17%). Furthermore, the test B revealed a limited phase separation for SLC and HBC, which was not revealed with method A. For all stressed vehicles, a highly viscous semi-transparent liquid phase was observed in the supernatant. The separated supernatant is most likely to be constituted by lipid components of the vehicles with lower density than aqueous phase.

The resistance of an emulsion to centrifugation usually depends on the difference of density between the oily and aqueous phases and also on lamellar structure (Petsev, 2004; Eros *et al*., 2003). The importance of emulsifier mixture and concentration on the stability of emulsions has been also well illustrated (Engels *et al.,* 1995; Kanouni *et al*., 2002; Bortnowska *et al.,* 2009). Both procedures A and B revealed phase separation for BC and CC. The critical stability of CC is already recognized in the literature (Garbe and Reinmann, 2005). The higher phase separation observed for CC in comparison to other bases presumably lies on the lack of emulsifying agent in this cream. BC, NHC and SLC possess a mixture of nonionic surfactants and fatty alcohols, which functions as coemulsifying agents. These secondary emulsifiers accumulate at the o/w interface forming condensed films that are more mechanically stable and resistant to coalescence (Barnes, 1994; Robins, 2002). The mixture of emulsifiers and their lamellar arrangements strongly influence on the lamellar structures of these creams and consequently their physical stability.

The selection of an adequate stress condition is the key factor during performance of centrifugation studies. Phase separation was not observed for the positive reference Dermatop® cream neither applying test A nor B, indicating that the conditions employed were not exaggerated. Based on the present results, one can assume that both methods (A and B) are suitable in terms of amount of stress. However, the "suitability" of the test will depend on the questions addressed in the investigation. Due to the milder conditions, the procedure A is more likely to provide a more realistic prognostic revealing the vehicles with high probability to demonstrate phase separation during long-term studies. Nevertheless, the procedure A lacks the sensitiveness fulfilled by the procedure B. Only with the aid of method B, a clear rank could be obtained between the bases yielding to a more evident distinction in the separation profiles. This becomes particularly important during the development of new vehicles, wherein comparative results are valuable information (Roland *et al.,* 2003).

4.4. Summary and conclusion

In the present chapter the rationale for the selection of base formulations for a development platform of topical glucocorticoids was presented. Eight representative standard semisolid bases have been chosen from the DAB (2010) and DAC (2009). A qualitative identification was carried out by assessing appearance and odour. The determination of pH assured the optimal pH for development of stable products. Evaluation of flow properties of the bases was valuable to understand their spreading and rubbing properties; response to external forces on application on the skin; and manufacturing characteristics. BC, HBC and SLC were the bases

with less resistance to shear, which indicates a better spreability. Oscillatory measurements provided important information on structural properties of the formulations which can be used to monitor the stability of the tested creams.

A method to accelerated assessment of physical stability has been proposed. The essential feature of the approach was the application of the centrifugation force speeding-up the process which goes on more slowly under 'normal' storage conditions. The test procedure revealed two possible critical formulations (CC and BC). The strategy of employing a commercial product as positive reference represented a rational approach with sound judgment for a stability program. The utilization of mild centrifugation force was useful to provide a more realistic prognostic, raising concern only for those products which are most likely to be grossly unstable and not those which eventually will yield to adequate stability. In addition, the strategy of utilizing a more forced stress conditions resulted to achievement of a clear discrimination between the bases and relevant information to orient the galenical development. In summary, the balanced combination of a “mild” and “forced” centrifugation protocols leads to the achievement of relevant information for a stability program and for the orientation in the development phase.

5. Establishment of a new approach to compatibility testing of semisolids

5.1. Introduction

Formal stability studies (long-term and accelerated) are very costly, laborious and usually take place in the later phases of the development during clinical trials (Alsante *et al*., 2007). It becomes crucial to have already eliminated unsuitable candidates at earlier stages to avoid more disappointment and financial disaster. Some of the common ways by which excipients may affect drug stability in the dosage form are by altering moisture content in the dosage form, changing microenvironmental pH in the dosage form, acting as general acid–base catalysts or directly reacting with drug (Cartensen and Rhodes, 2000; Banker and Rhodes, 2002). Prediction of the stability of drugs and excipients has occupied the attention of pharmaceutical scientists since the 1950s (Shah *et al.*, 1993). Traditional excipient compatibility testing usually involves the employment of binary systems mixing drug and excipients at determined ratio (e.g. 1:1, 1:10) and submitting to accelerated temperature conditions (e.g. 40 ºC) over a period of time, usually no longer than 1 month (van Dooren, 1983; Morris *et al.,* 1994; Balestrieri *et al.*, 1996; Kopelman and Augsburger, 2002; Ceschel *et al.,* 2003). However, one of the main preoccupations on performing such tests is the lack of relevance of the results generating overestimated predictions. The current practice has been criticized by many authors (Serajuddin *et al.,* 1999; Sims *et al.,* 2003; Kopelman and Augsburger, 2002; Waterman and Adami, 2005). For semisolid dosage forms the test design becomes even more critical assuming the complexicity of these multicomponent systems. The results with mixed ingredients may differ completely from results obtained with traditional compatibility studies using binary mixtures with individual ingredients. Currently, there is a lack of knowledge on the specific and complex design of compatibility tests for semisolid dosage forms. The published data reveal that most of the documented compatibility tests are focused on the development of solid dosage forms and very few of them for semisolid topical formulations.

The present investigation aimed to establish an alternative compatibility test for semisolid dosage forms with high potential of identifying possible failure formulations at an early phase. The principle of the method relied on the preparation of compatibility samples by blending drug with the vehicle prototype or base formulation producing the so-called “mini-formulation”. The prognostic capacity of this approach relied on the tailored investigation with formal stability studies and the premises that blending drug with base formulation

generating mini-formulations yields more representative results concerning the long-term stability compared to traditional approaches. Furthermore, the "back-diagnosis" approach was also presented. This represented a focused search tracking back the "guilty" ingredient based upon the results from failure mini-formulations. Ultimately, the downscaling potential of the method was evaluated.

5.2. Materials and methods

5.2.1. Materials

Betamethasone-17-valerate (BM17V) was purchased from Fagron GmbH Co. KG (Barsbüttel, Germany). This test substance did not belong to the model drugs in the development program, but it was employed for this part of the study since its susceptibility to degradation is already known (Bundgard Hansen, 1981; Cornarakis-Lentkos and Cowin, 1987). The preparation of the bases NHC and BC was described in section 4.2.1. Macrogol ointment DAC (MC) was employed as negative vehicle due to the documented instability with topical corticosteroids (Allen and Gupta, 1974). MC consists of macrogol 300 (50%) and macrogol 1500 (50%). The preparation of this base followed as described in the monograph (DAC, 2009).

5.2.2. Preparation of test formulations

The base formulations were prepared according to DAB and DAC monographs. For the stability study, test formulations containing 0.1% of BM17V were compounded in MC and NHC using a porcelain recipient. Geometrical incorporation of active ingredient was assured as recommended (USP, 2009a). The test formulation were assayed in triplicate with regard their drug content in order to assure uniform drug distribution.

5.2.3. Short-term stability study

The performance of the classical stability study has followed for the test formulations MC and NHC containing 0.1% of BM17V. The formulations were packed in recipients of 20 g and stored at 40 ºC over 60 days. The test temperature was the same used for short-term stability studies (ICH, 2003). Drug substance was determined before storage and after 15, 30 and 60 days. The test frequency has followed at least 3 data points as recommended (ICH, 2003). Prior to analysis, 100 mg of formulation was weighed into tubes (Corning Incorporated Life Sciences, NY, USA) and diluted with 5 mL of MeOH/water (50:50, v/ v). Samples were

heated at 40 ºC during 30 minutes, strongly shaked with aid of vortex and filtrated (Millex-HV, Millipore, 0,45 μm) into vials before HPLC analysis.

5.2.4. Compatibility with mini-formulations

Mini-formulations were obtained by blending drug and base in HPLC glass vials at 1:10 and 1:50 (w/w) mixtures. Blends were prepared by vortexing (Eppendorf AG, Hamburg, Germany). Samples were stored in thermostatically controlled dry air oven (Thermo Fisher Scientific, Bonn, Germany) with closed vials at 60 ºC over 1 week. The determination of degradation kinetics followed the predetermined time intervals (24, 48, 72 and 168 hours).

5.2.5. Compatibility with propylene glycol

Binary mixtures of drug and excipient were obtained at 1:10 and 1:50 (w/w). Additional samples were prepared with addition of cosolvent (acetonitrile) at 80% (v/v). Mixtures were obtained vortexing with minishaker. The samples were stored at RT over 48 hours in accordance with the protocol for determination of stability in solution described in chapter 3.

5.2.6. Analytical method

The parameters of the HPLC analytical method are described in section 3.2.5. The specificity of the method for BM17V was confirmed by library spectra matching in order to check peak purity and confirm the absence of co-eluting species. Retention time of BM17V and the main degradation product were 5.7 and 6.5 minutes, respectively. Spectral data were obtained using Waters 996 Photo Diode Array detector.

5.2.7. Data processing

The proposed reaction models (zero-order, first-order and second-order) were tested by fitting the experimental data to the appropriate equations. The correlation coefficients were used as an indicator of goodness-of-fit of the equations to the experimental data. The observed contants of degradation rate were obtained estimated from the slope of the declining concentrations versus time plots. An arbitrary semi-quantitative classification was provided based on the results of the recovery values. The limit of 10% of degradation was set to distinguish “failure” and “stable formulations” in classical stability study. The total of 5% degradation was considered significant (ICH, 2003).

5.3. Results and discussion

5.3.1. Short-term stability study

This part of the study served as reference for the establishment of the compatibility method, since the susceptibility to degradation of BM17V is already well documented (Bundgard and Hansen, 1981). Initially, the efforts were placed on the performance and evaluation of the stability of BM17V in base formulations stored at 40 ºC over 60 days. Formal short-term stability studies usually requires the period of 6 months (ICH, 2003). However, an arbitrary testing period of 60 days was set considering the preventive nature of the compatibility test. The stability results of BM17V in MC and NHC is presented in Fig. 5-1.

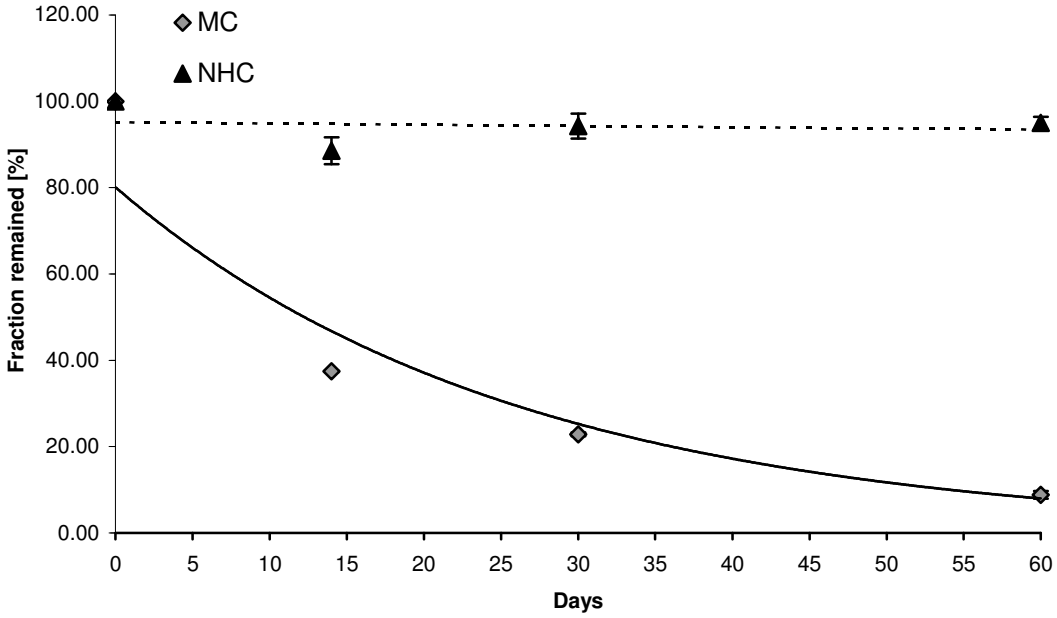


Fig. 5-1: Stability results of BM17V formulated in NHC and MC at 40 ºC over 60 days. In the graph the mean value of recovery in comparison to initial concentration from three determinations (n=3) and SD are displayed.

BM17V undergone rapid degradation in MC. Apparently an exponential decay followed over 2 months. Only 8.84 ± 0.87% of the drug was recovered from MC whereas 94.98 ± 1.41% of drug was recovered from NHC, confirming that the utilization of these vehicles as negative and positive references were appropriate. Fitting data for zero, first and second order the best-fit was found for the logarithmic plot of remaining drug in function of time, suggesting that the reaction in MC followed first-order (Fig. 5-2). Assuming that the rate of disappearance of

BM17V equals the rate of the major degradant, the observed rate constant (k_{obs}) was obtained from the slope of the logarithmic plot of drug remaining against time (Tab. 5-1). The degradation of BM17V in NHC was insufficient to determine the reaction order.

The decomposition of BM17V in creams and ointments is reported to yield the monoester betamethasone-21-valerate (Cornarakis-Lentkos and Cowin, 1987). The mechanism follows ester migration as illustrated in 3.3.5. The drug instability in MC was in good agreement with the literature (Allen and Gupta, 1974). Assuming that the degradation products are not toxic, the shelf-life of the final product is generally defined as the time for 10% degradation of the active component (Shah *et al.,* 1993). In the present work, 10% of degradation over 60 days was arbitrary set as the limit for declaring a "failure" formulation. This served as reference for the establishment of a tiered compatibility method. Moreover, published stability data of BM17V incorporated in BC supported the utilization of this base as a second negative reference (Mitryaykina, 2007). The employment of a second negative reference aided to substantiate the findings of the alternative test.

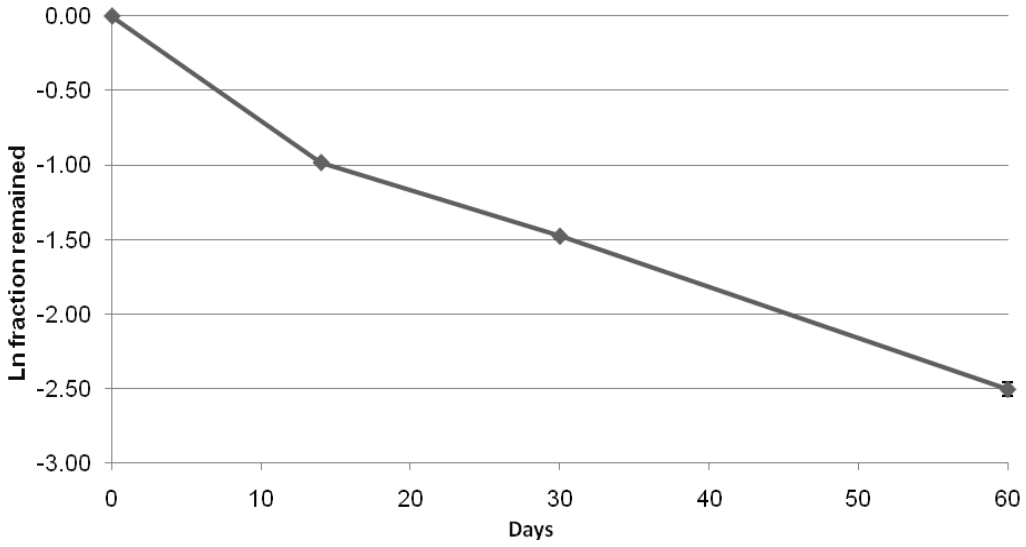


Fig. 5-2: Degradation kinetics of BM17V in MC at 40 ºC over 60 days. In the graph, the logarithmic plot of drug remained against time and SD are displayed.

5.3.2. Compatibility of drug with bases employing with the alternative method

The second part of the study aimed to qualitatively recreate the results of the classical studies in a shorter period of time employing blends of drug/base and submitting to accelerated

temperature conditions (60 ºC). The degradation profiles of BM17V in mixtures with BC, MC and NHC are presented in Fig. 5-3.

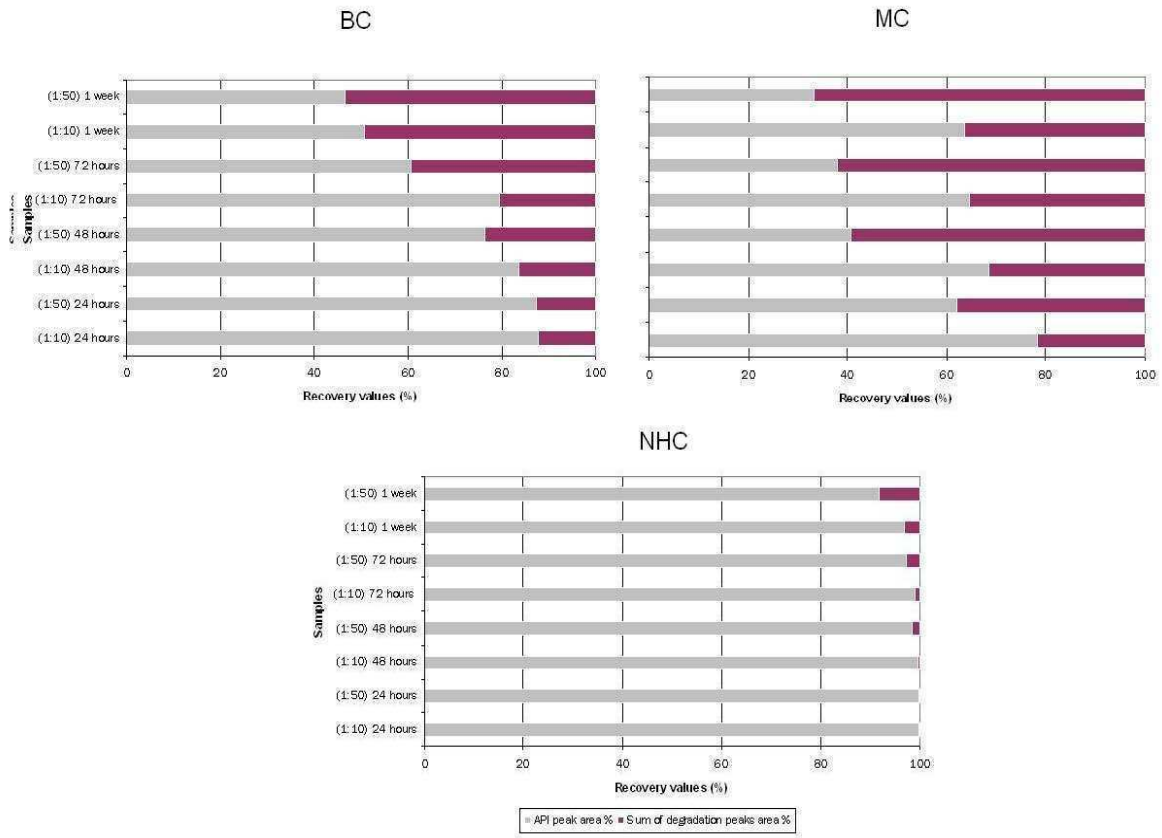


Fig. 5-3: Results of compatibility test of BM17V with the negative references (BC and MC) and positive reference vehicle (NHC) using mixtures of drug and excipient at two different ratio (1:10 and 1:50, w/w) stored at 60 ºC over 1 week. In the graph are displayed the recovery (%) of drug remaining (gray bar) and sum of degradation (red bars) obtained after 24, 48, 72 and 168 hours.

The method could clearly distinguish the degradation of BM17V in the blends with the negative references (MC and BC) and the positive reference (NHC) in a short period of time, recreating the prognostic given by the classical stability investigation. Recovery values below 90% were obtained for the drug in BC and MC at all investigated time points whereas higher drug degradation (6.15%) was observed for blends with NHC after 1 week. Apparently, the mixture 1:50 (w/w) resulted to more extent decomposition. The employment of blends at 1:10 and 1:50 (w/w) was discussed in details in section 5.3.4. The selection of higher storage temperature was intended to accelerate reaction rates, so that even relatively slow reactions become evident in a short period of time. This is accepted in practice and well founded for chemical predictions based on the classical Arrhenius theory (Cartensen and Rhodes, 2000).

The reaction constants obtained from the classical study and compatibility investigation demonstrated the acceleration potential of the test (Tab. 5-1).

Tab. 5-1: Comparison of study conditions and observed reaction constants (K_{obs}) of classical and alternative tests.

Study design	Test temperature (ºC)	Test formulation	K_{obs}
Classical	40	BM17V/MC (0.1%)	0.0385
Alternative	60	Mini-formulation BM17V/MC (1:10 w/w)	0.1444
Alternative	60	Mini-formulation BM17V/MC (1:50 w/w)	0.3336

Blends of drug/MC at 1:50 (w/w) provided 11-fold faster reaction whereas at 1:10 (w/w) the reaction was approximately 5 times higher in comparison to the classical study design. Extrapolation of accelerated results at 60 ºC to RT was avoided, since beside the temperature other variants might contribute to the rapid reaction. The ratio of drug/vehicle in the samples is one of the determinant factors, which may affect the fraction of drug soluble susceptible to reactions. In addition, at higher temperatures, the solubility of the drug can be enhanced, increasing the liability to chemical reactions.

5.3.3. Evaluation of the prognostic potential of the alternative test

In order to provide a more quantitative analysis of the present data, the magnitude of degradation was arbitrary classified. Dürug and Fassihi (1993) achieved a semi-quantitative estimation of the destabilizing effects of a range of excipients with pyridoxal hydrochloride using a classification of extent of degradation based on recovery results. The ICH Guidance on Stability Testing of New Drug Substances and Products determines as significant 5% of degradation in comparison to initial values (ICH, 2003). In the present study, the classification was established as presented in Tab. 5-2.

Tab. 5-2:Arbitrary classification of the magnitude of degradation.

Rank order	Recovery values	Classification	Abbreviation
1	> 95%	Low potential of failure	+ +
2	Between 90 and 95%	Raises concern	+
3	Between 85 and 90%	Potential of failure	-
4	< 85 %	High potential of failure	--

According to the classification, loss of drug content higher than 5% is considered to raise concern. Values beyond the limits of 90% are considered to present a potential or high potential of failure. Tab. 5-3 assigned the recovery results of alternative and classical tests according to the suggested classification.

Tab. 5-3: Assigned results of alternative and classical tests abbreviated with symbols according to the classification of extent of degradation.

Alternative				
Storage time	**vehicle**			**Blend ratio**
(days)	NHC	BC	MC	
1	+ +	-	- -	1:10
	+ +	-	- -	1:50
2	+ +	- -	- -	1:10
	+ +	- -	- -	1:50
3	+ +	- -	- -	1:10
	+ +	- -	- -	1:50
7	+ +	- -	- -	1:10
	+	- -	- -	1:50
Classical				
Storage time	**vehicle**			
(days)	NHC	BC (*)	MC	
60	+	--	--	

* Classification based on results from Mitryaykina (2007).

The results of the compatibility test provided a semi-quantitative discrimination between “failure” and “non failure” formulations. The findings are in congruence with the results of the classical studies, demonstrating the predictive capacity of the test. For all investigated time points, blends of BMV17 with negative reference vehicles yielded to results falling

below 90% of recovery, whereas for the positive reference, recovery was found to be higher than 90%. At 168 h (7 days), recovery results for drug mixture with NHC at 1:10 (w/w) have indicated "low potential of failure" whereas mixtures at 1:50 (w/w) indicated "raises concern". In fact, the prognostic for NHC gave a detailed insight into the short-term drug stability. BM17V decays to 94.14% after 60 days, which represents a significant change, but not a failure formulation yet. Therefore, the obtained prognostic applies to this base. The time point 168 hours has demonstrated to be more representative and it was set as reading endpoint. The prognostic potential of the method was also evaluated in terms of capacity to recreate results in terms of major degradation product, confirming the selectivity of a stability-indicating analytical method employed (Fig. 5-4).

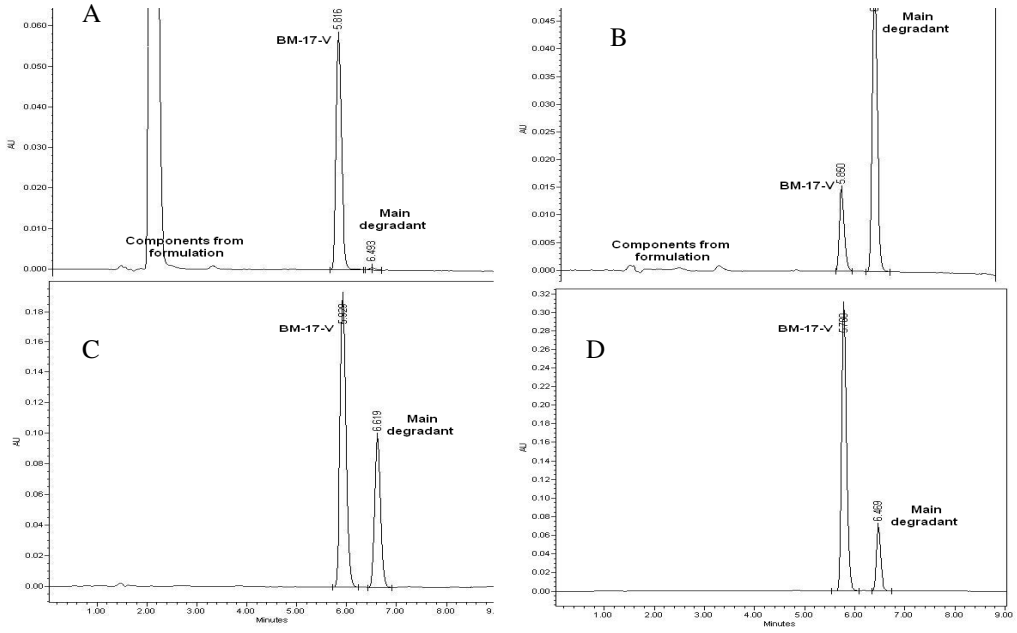


Fig. 5-4: Representative chromatograms from samples: (A) and (B) stability of BM17V in NHC and MC (at 40 ºC after 60 days), respectively. (C) and (D) compatibility of BM17V in MC and BC (at 60 ºC after 7 days), respectively.

The chromatograms demonstrated that the main degradation product obtained from the alternative test matched with the major decomposition product from classical studies. This is particularly important to assure that the system did not exceed the activation energies and did not generate alternative degradation pathways.

5.3.4. Degradation kinetics of drug in mixtures of base vehicle/drug

The degradation kinetics of BM17V at 60 ºC over 7 days using two drug/excipient blend ratio (1:10 and 1:50, w/w) is shown (Fig. 5-5).

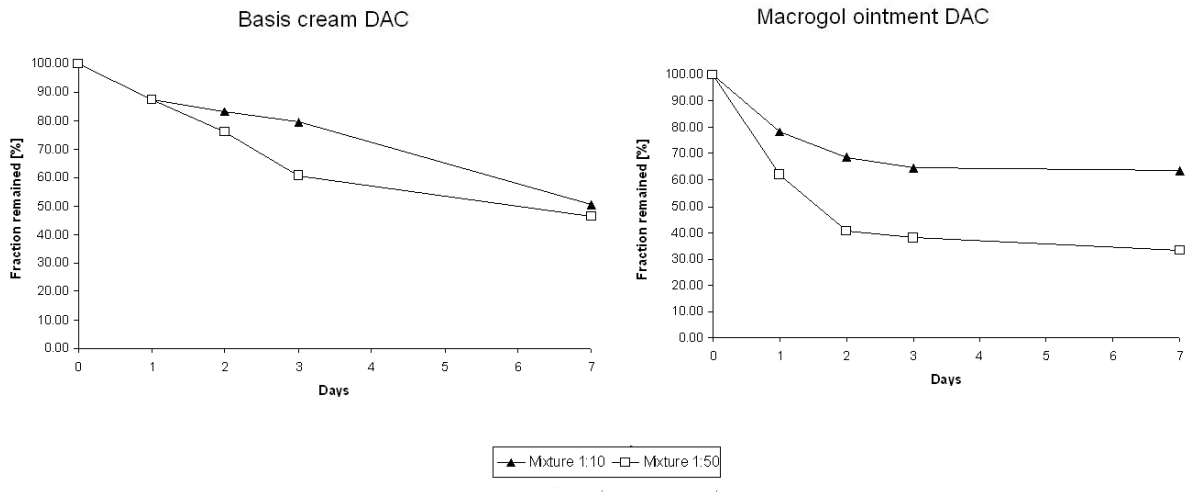


Fig. 5-5: Degradation kinetics of BM17V with mixtures (1:10 and 1:50, w/w) of BC and MC mini-formulations over 7 days at 60 ºC (n=1).

Drug degradation in MC followed initially a rapid exponential decay over 3 days and an apparent levelling to a pseudo-equilibrium level. The plot of Ln of remaining drug against the time (over 3 days) yielded correlation coefficients higher than 0.9, which supports the evidence that the reaction followed via first-order. The formation of equilibrium in drug/excipient kinetic studies can be observed when the reaction is dependent upon a depleting constituent of the system, such as water or small quantities of reactive excipients. Exponential decay followed by an equilibrium phase has been previously documented in kinetic studies with ascorbic acid with several excipients (Tardif, 1965). The results also demonstrated differences in the kinetic profile employing 1:10 and 1:50 (w/w) mixtures, suggesting that the degradation is related to the ratio of the mixture, which could have influenced on the fraction of drug dissolved in the semisolid base and consequently its degradation. The drug blends with BC have shown less evident differences in the drug/excipient ratio. The degradation in the base has followed at a reduced magnitude and the equilibrium was not evidently formed. Either the more abundant presence of water sourced

from the formulation itself or the higher drug solubility of the drug in BC in comparison to the hydrophilic MC base can explain this difference.

One of the particularities of the described method is the storage with closed-system without addition of water. In conventional drug/excipient studies for solid dosage forms, mixtures are stored in open containers at different temperature and humidity conditions. Thus, the presence of water determines its potential role in drug/excipient interactions due to the water sorption and desorption properties of the ingredient (Kontny, 1988). Excipients that strongly sorb water may prevent drug degradation by scavenging water in a closed system (Gore *et al.,* 1979; De Ritter *et al.,* 1970). Excipients with higher adsorption energy can decrease the reactivity of water in the system, compared with those with lower adsorption energy (Perrier and Kesselring, 1983). Serajuddin *et al.* (1999) argued that with exposure of the samples to high humidity, the interaction between excipient and drug depends upon the free moisture present and relative hygroscopicities. However, in the case of semisolid dosage forms the source of water is less likely to be originated from environmental variants but from the semisolid formulation itself.

5.3.5. Back-diagnosis approach: focused drug-excipient compatibility investigation

Traditional drug/excipient compatibility test are usually laborious and time consuming. At an initial phase, many formulation prototypes are tested generating an enormous list of individual excipients to be investigated. The sample preparation with individual ingredients usually requires manual operation, which makes the procedure to be extremely time-consuming. In many cases, the results may not yield to conclusive statements. The implementation of new effective strategies with focused compatibility testing becomes necessary. The “back-diagnosis approach” was based on the assumption that the diagnosis of any observed incompatibility from the detected “failure formulations” can be tracked back identifying the “culprit” excipients. The scheme (Fig. 5-6) illustrates a comparison between traditional and alternative compatibility tests.

Traditional drug excipient compatibility **Alternative drug excipient compatibility**

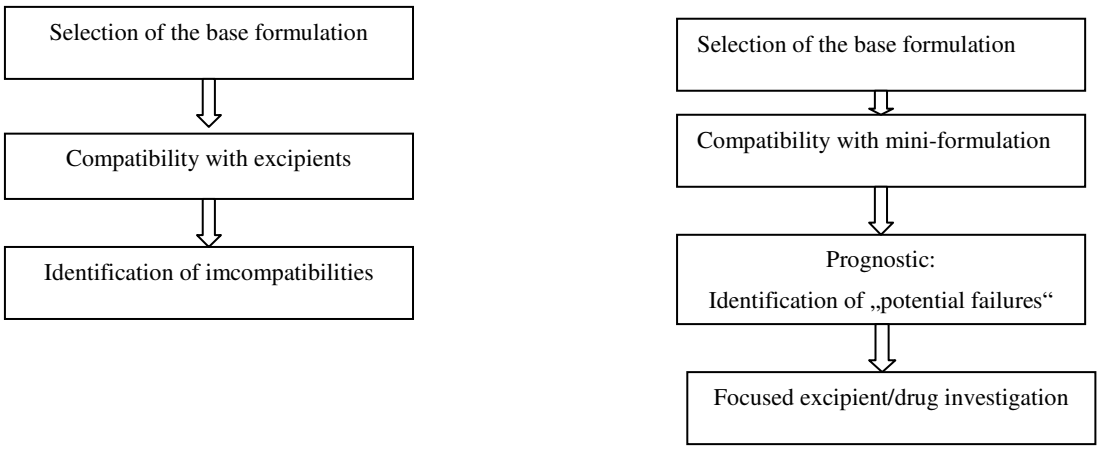


Fig. 5-6: Schematic comparison of traditional and alternative excipient compatibility test. The workflow for traditional test was adapted after Kaca (2007).

It was previously demonstrated that MC and BC were critical bases for the design of BM17V formulations. MC consists of a 1:1 mixture of macrogol 300 and 1500. The application of the back-diagnosis to this vehicle is simple. The verification of the critical component in BC is a more difficult task. BC is a more complex multicomponent system containing 6 different ingredients. Kaca (2007) has investigated the compatibility of excipients of BC with a series of corticosteroids. Apparently, PG has yielded to lowest drug recovery values in comparison to the other components (Kaca, 2007). The incompatibility of BMV17 with PG was further reported by Simonsen *et al.,* (2004). Oriented by the results of the literature, individual drug/excipient studies were performed for PG, representing the search for the "guilty" component in the formulation. Additionally, in order to substantiate the findings, mini-formulations of HBC containing different amounts of PG (5, 10, 15 and 20%) were submitted to 60 ºC over 48 hours. The original HBC base is absent from PG. The destabilizing effect of the excipient at different concentrations was the driving force in this study question. The Fig. 5-7 illustrates the results of individual compatibility studies with PG utilizing drug/excipient blends (1:10 and 1:50, w/w) and cosolvent (acetonitrile). The compatibility results with mini-formulations of HBC containing different concentrations of PG are also presented.

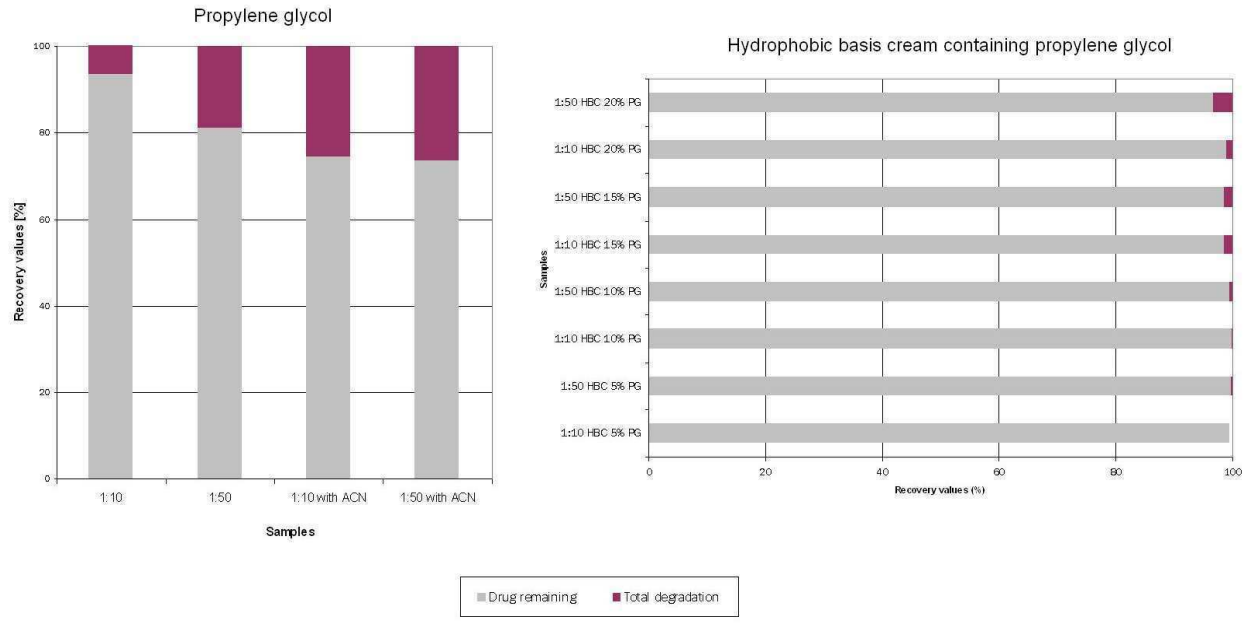


Fig. 5-7: Results of compatibility test with PG and with HBC formulated with PG at 5, 10, 15 and 20%. The graph on the left illustrates the results of compatibility test with PG employing binary mixtures of drug/excipient at two different ratios (1:10 and 1:50, w/w) with and without acetonitrile. The graph on the right shows the recovery values of BM17V after storage with HBC formulated with different concentrations of PG.

The individual studies with PG confirmed the destabilizing effect of this excipient. The higher liability of the drug in presence of acetonitrile is congruent with results of stability in solution described in chapter 3. In the studies with modified HBC, 96.63% of BM17V was recovered from the formulation containing 20% PG whereas 99.41% was recovered from the one containing 5% PG. This confirmed the destabilizing effect of the excipient when incorporated into vehicles. In addition, the higher degradation values obtained with 1:50 (w/w) blends are in accordance with previous compatibility results. These findings confirmed the sensitiveness of the method in tracing back the critical components.

5.3.6. Compatibility of model drugs with selected bases

In this part of the study the compatibility of the model drugs with the bases was investigated with the established alternative method. The results are presented in Fig. 5-8.

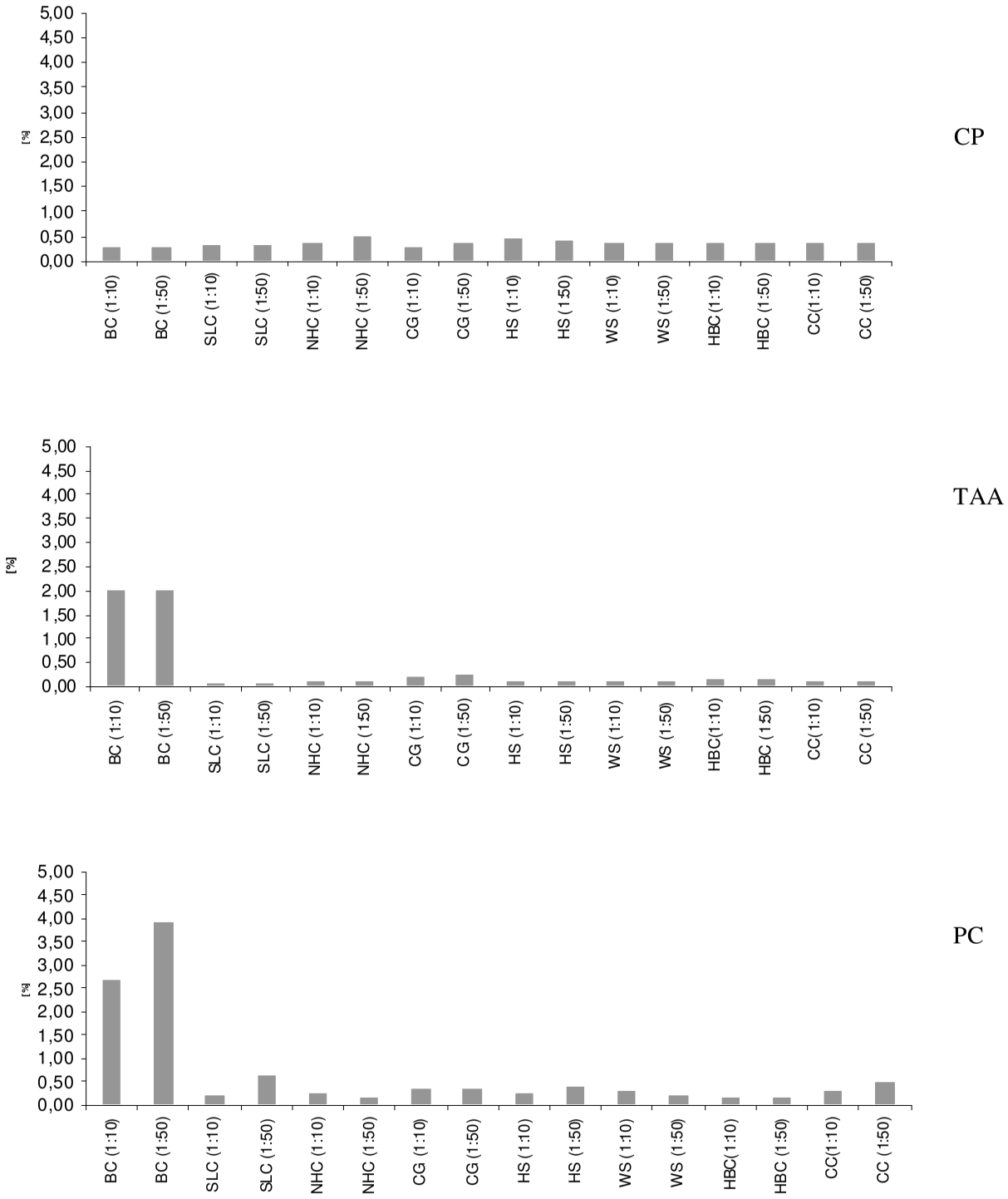


Fig. 5-8: Compatibility results of CP, PC and TAA with BC, SLC, NHC, HBC, CG, WS, HS and CC at 1:10 (w/w) and 1:50 (w/w) over 168 hours at 60 °C. The bars displayed are the sum of degradation products (%).

None of the model drugs demonstrated significant degradation (>5%) with the selected bases. The highest degradation was observed when the drugs were tested with BC, which is in good agreement with the results of BM17V. Higher degradation was found for PC, followed by TAA and CP. These results are consistent with the determination of stability in solution described in chapter 3.

5.3.7. Evaluation of the scaling-down potential of the method

One of the most important economic factors of compatibility tests is the capability of handling many samples in a short period of time. The outsourcing of such studies is estimated to cost on average 40,000 US dollars for one compound (Wakasawa *et al.,* 2008). Therefore, it is crucial to increase intern capacity and productivity in the testing procedure through new development strategies. Furthermore, it is desirable to carry out such in studies with lowest sample amount possible because of the constraints of synthesis of new substances in the early development stages. The establishment of scaled-down compatibility tests were mostly attempted with aid of automation (Carlson *et al.* 2005; Wakasawa *et al.,* 2008; Tomas and Naath, 2008). However, automation is a highly costly alternative usually not affordable for small pharmaceutical companies. Taking into account the described development program, three test substances, eight base formulations (summing up 28 different excipients) and two testing variants (blend mixtures 1:10 and 1:50, w/w) a list of 168 samples is necessary if the traditional compatibility approach is employed requiring approximately 56 hours of manual work by preparing samples. Applying the proposed test, the number of samples reduces to 48 with an overall time expenditure of 16 hours (taking into account the previously documented time for sample handling by Wakasawa *et al.,* 2008), resulting to 350% of savings in the time during sample handling (Fig. 5-9).

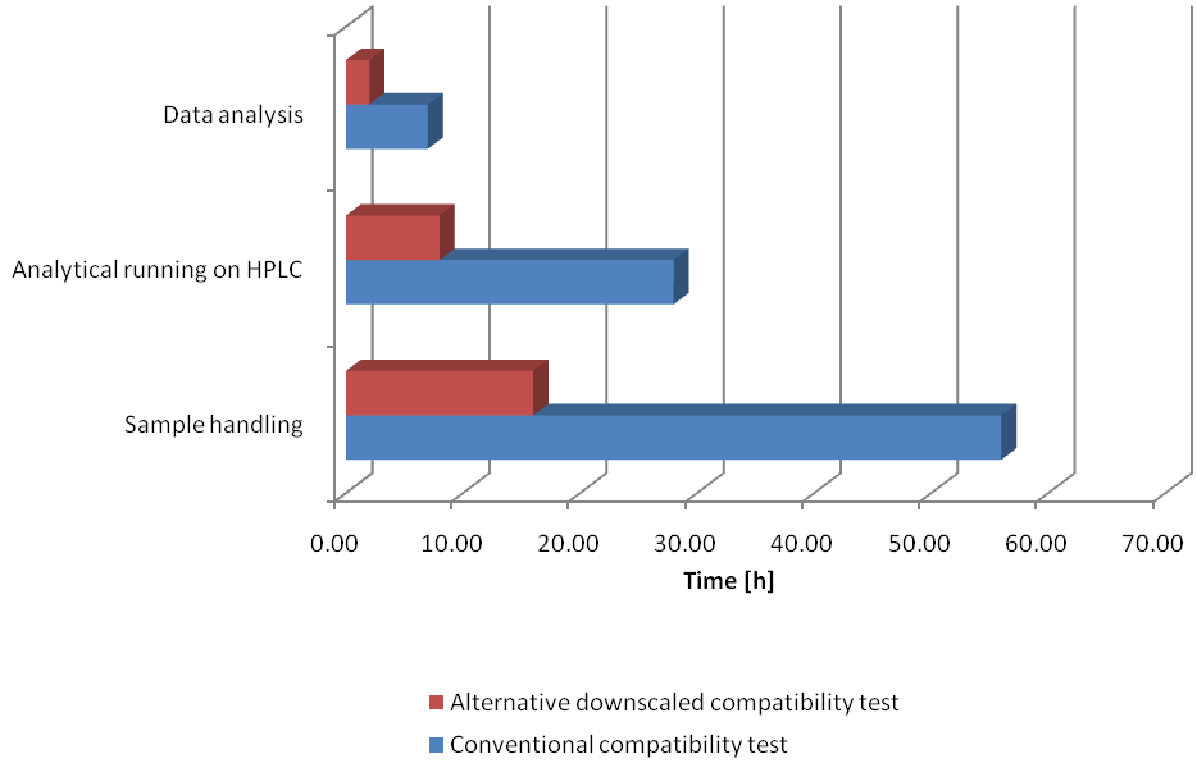


Fig. 5-9. Comparison of the time spent on experimental procedures applying conventional and the alternative compatibility test. Estimatives of sample handling and data analysis were obtained from Wakasawa *et al*. (2008). A run time of 10 minutes was considered for the calculation of time spent on samples measurement.

The designed approach was a resource and time sparing alternative for a primary screening of formulations reducing time number of samples, providing fast and more conclusive statements on formulation stability. The early identification of critical formulations permits the reallocation of resources and time for the other important development activities.

5.4. Summary and conclusion

An alternative compatibility test for semisolid development with high predictive potential has been proposed. The utilization of mixtures of drug with the base formulation generating the so-called “mini-formulations” has lead to representative results taking as reference the results of classical studies. The utilization of multicomponent systems in the sample preparation of compatibility test with the aim of enhance the predictability capacity was already attempted by some authors (Wytternbach *et al.*, 2005: Dürig and Fassihi, 1993; Sims *et al.*, 2003). However, these reports were intended to design of solid dosage forms and did not give the

particular focus on development of semisolids. The present study was the first description of the application of such strategy for semisolid development with pharmacopeial bases. The usefulness of the described method relied on the discriminating capacity demonstrated for identification of potential failure formulations allowing the scientist to proceed with more reliability to further phases. This strategy is especially useful to identify possible real interactions between formulation excipients and the drug aiding in the identification of an optimal formulation and the potential of an early discovery of alternatives for patent protection and life cycle management. Furthermore, the back-diagnosis approach was presented. The principle of this strategy was based on a focused drug/excipient investigation upon results from failure mini-formulations. This represented a rational attempt to locate the critical component, reducing material and time. The results of individual drug/excipient investigations could strongly support the hypothesis that PG was one of the critical components in BC. Furthermore, the destabilizing effect of this excipient was confirmed in studies with modified HBC manufactured at different amounts of PG. Finally, the established method was utilized to investigate the compatibility of the model drugs with the selected vehicles integrated in the development program. The results suggested that none of the bases is expected to raise instability issues. The proposed method is an innovative approach and can be regarded as efficient strategy to delineate possible failure formulation at a sooner phase, representing an important contribution for the pharmaceutical development of topical products.

6. Development and validation of an *in vitro* release test

6.1. Introduction

In vitro release testing of topical dosage forms is an emerging technology, which has been applied analogous to the dissolution tests for oral dosage forms. For the particular semisolid systems it is referred preferably as release test since the drug in suspended state has to dissolve, diffuse through the vehicle and finally be released on the skin, so that it can further permeate skin layers. The test has important application in the regulatory field. The FDA Guidance for Industry on Scale-up and Post Approval Changes for Nonsterile Semisolid Dosage Forms (SUPAC-SS) outlines the employment of the method to maintain certification of semisolid dermatological products after quantitative changes have been made in their compositions and/or after changes have been made in the sourcing of key ingredients, in their processing, batch sizes and/or site of manufacturing (FDA, 1997). This is a type of waiver to lower the regulatory burden placed on the industry while assuring the continued safety and effectiveness of drug product (Flynn *et al.,* 1999; Siewert *et al.*, 2003). Although the test does not serve as a measure of bioavailability, it has to be capable of detecting changes in the product which have potential to alter biological performance (FDA, 1997). Recently, two USP general chapters were published for public review addressing the quality and performance aspects of topical and transdermal products covering apparatus and procedures to be used to evaluate drug release (USP. 2009b). Although the chapters state the test must be reproducible and reliable, no further guidance has been given to evaluate the reproducibility and reliability of the method. Considering the increasing relevance in the quality control, the validation of the method intended to be used in a regular basis for formulation design or monitoring of batch-to-batch uniformity will be in a soon future a mandatory requirement. Currently, there is poor understanding how test parameters as synthetic membrane and acceptor medium influence on the capacity of the method of distinguishing products which undergone relevant modifications.

The aim in this part of the work was to develop and validate an *in vitro* release method with focus on the influence of test parameters (membrane and acceptor medium) on the discriminating power of the release method. Secondly, it was aimed to propose a validation protocol for *in vitro* release tests intended to be employed in quality control, regulatory field and pharmaceutical development of topical products.

6.2. Materials and methods

6.2.1. Materials

The following marketed formulations were used: Kortikoid-Ratiopharm® cream 0.1%, Kortikoid-Ratiopharm® ointment 0.1% (Ratiopharm GmbH, Ulm, Germany) and Triamgalen Cream® 0.1% (Galenpharma GmbH, Kiel, Germany). For the release experiments, Franz diffusion cells (PermeGear Inc, Bethlehem, PA, USA) made of flat ground glass, 15 mm diameter orifice, 1.76 cm² diffusion area and 12 mL volume were employed. MeOH and chemicals of HBSS buffer (Glucose, HEPES, $NaH_2PO_4 \cdot 2H_2O$, KCl, NaCl, $CaCl_2 \cdot 6H_2O$ and $MgCl_2$) were obtained from Merck KGaA (Darmstadt, Germany). The membranes used were made of regenerated cellulose, thickness of 8 µm and 25 µm (Diachema AG, Zürich, Switzerland), polycarbonate (thickness of 10 µm) and polyester (thickness of 10 µm) acquired from Whatman GmbH (Dassel, Germany).

6.2.2. Investigation of drug binding to test membranes

Standard solutions containing TAA at two concentrations (1 µg/mL and 25 µg/mL) were prepared. The test membranes were placed inside opened 15mL-syringe, which were vertically placed into a 300 mL glass beaker and incubated with the analytical solutions inside the syringe sealed with Parafilm®. After 6 hours of incubation, Parafilm® was removed. The plunger was placed in the syringe and the solutions were filtrated (Millex-HV, Millipore, 0,45 µm) into glass vials. The experiment was determined in three-fold and the samples were analyzed on HPLC (analytical method described under the section 3.2.5).

6.2.3. Drug solubility in different acceptor media

The solubility of TAA in different acceptor media was determined aligned with the experimental conditions of the *in vitro* experiments. An excess of drug was weighed into 10 mL-volumetric flasks, filled with HBSS buffer and HBSS/MeOH binary mixtures with 20, 30 and 50% MeOH. Formation of suspensions was observed. The samples were stored in thermostatically controlled dry air oven (Thermo Fisher Scientific, Bonn, Germany) at 32 ºC, withdrawn after 6 hours and filtrated into glass vials before HPLC analysis. Stability of TAA in the investigated media was previously ensured over 24 hours.

6.2.4. Determination of drug interaction between vehicle and acceptor medium

Approximately 300 mg of Korticosteroid-Ratiopharm® cream and ointment were weighed into the Franz cells. The cells were filled with 10 mL of respective media, sealed with Parafilm® and to mix at 400 rpm over 6 hours. Samples were collected from acceptor medium. Turbid samples were filtrated. Drug in the media was determined after 6 hours.

6.2.5. *In vitro* release experiments

The membranes were prior to the experiments soaked with the tested acceptor media over 1 h. The receptor phases employed were degassed to avoid bubbles formation and equilibrated at 32 ± 2 ºC prior to the start of the experiments. The diffusion cells were kept at the same temperature (32 ± 2 ºC) in thermostated water-bath along the experiments. The acceptor medium was continuously stirred at 400 rpm. Approximately 300 mg of test formulation were uniformly placed on the upper side of the membrane in the open donor chamber of the diffusion cell. The entrapment of air bubbles between formulation and membrane during application was avoided. The applied test formulations were kept occluded to prevent solvent evaporation and compositional changes using Parafilm®. Release of drug was monitored collecting aliquots (200 µL) after 0.5, 1, 2, 3, 4 and 6 hours and analysed by HPLC. After withdrawing the samples, the acceptor medium was refilled with fresh receptor medium conditioned at 32 ± 2 ºC. The dilution introduced by sampling was corrected and considered in the data processing.

6.2.6. Validation of the test

The proposed validation program aimed to examine the reproducibility and reliability of the test. Regenerated cellulose membrane (thickness of 8 µm) was employed as holding material and HBSS buffer/MeOH mixture (70:30 v/v, pH 7.4) as receptor fluid. Korticosteroid-Ratiopharm® cream was employed as test formulation during the validation phase so that it was ensured that the obtained experimental variations in the results would be accounted into the method and not into possible formulation deficiency as non-uniform drug distribution or content. The reproducibility of the results was assessed between experiments performed in different days within the same week (inter-day reproducibility); different days and different weeks (inter-week reproducibility) and between products from different batches (batch-to-batch reproducibility). Since different batches have met the classical compendial quality control requirements, no significant difference should be obtained in the release rate. The release results of 30 Franz-cells employed in 5 different validation experiments were

accounted to the evaluation of the cell-to-cell variability. The robustness of the method was assessed by determining the release rate of the same product with modifications in the experimental conditions as receptor medium (pH 7.4 and pH 6.4) and stirring rate (300 rpm and 400 rpm). All validation experiments were performed in six-fold.

6.2.7. Data processing and statistics

The plot of the cumulative amount of drug released per unit area ($\mu g/cm^{-2}$) against the square root of time ($h^{0.5}$) yields a linear curve and it was obtained from the mean of six Franz-cells for each test formulation. The slope of the regression line represents the release rate of the drug and can be employed as a parameter to monitor product quality. Because outliers are expected to occur on occasion with this test (for example, due to an air bubble between the product sample and the membrane), the nonparametric Man-Whitney U test was employed for verifying significant differences during the method validation as recommended by the FDA Guidance (FDA, 1997). The 90% confidence interval for the ratio of the median *in vitro* release rate for the first test formulation over the median of *in vitro* release rate for the second test formulation should be computed and expressed in percentage terms. If this 90% confidence interval falls within the limits of 75.00% to 133.33% (expressed as 0.75-1.33) the difference between the release rates is not significant. During the development phase, statistical differences between different membranes and acceptor media employed were assessed with two-paired t-test and one-way ANOVA with 95% confidence interval. An example of data processing with U-Test is given in Appendix 2.

6.3. Results and discussion

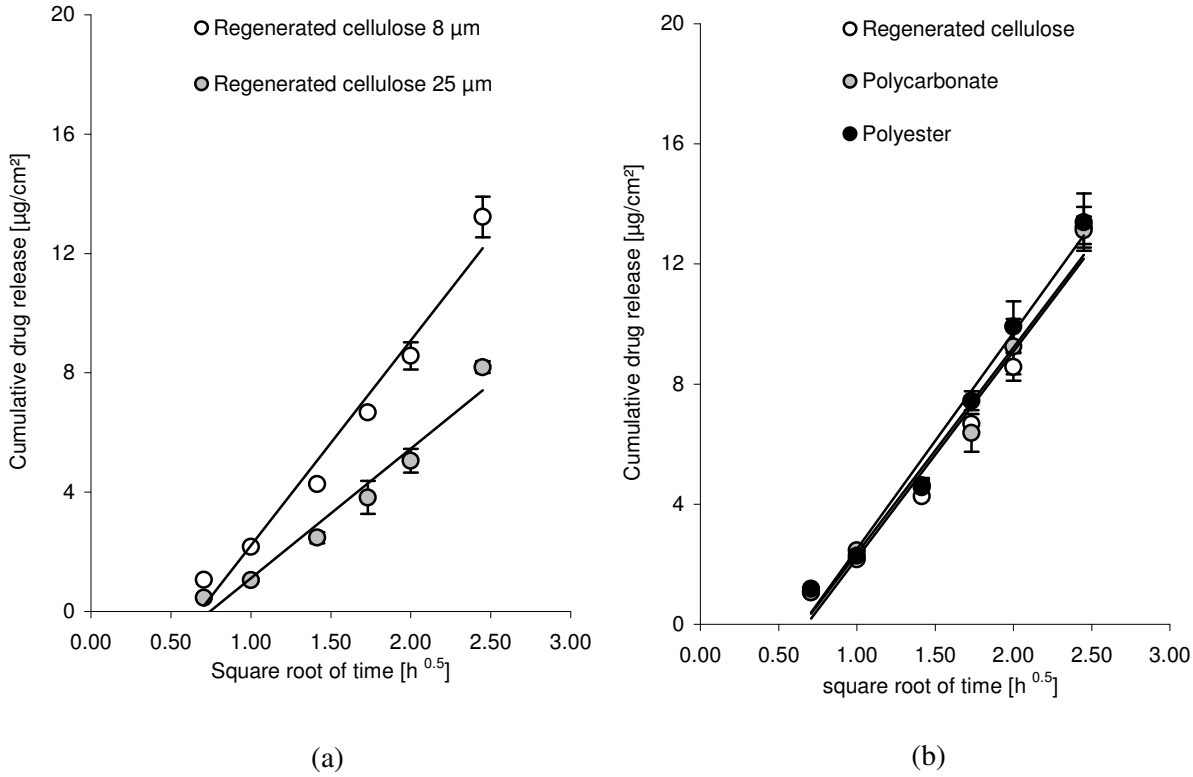
During development of *in vitro* release tests, selection of a proper membrane and acceptor medium is fundamental. According to suggestions of the FDA, membranes are selected for use which: (a) are commercially available (the practical way to assure reproducible membrane properties over time); (b) have little capacity to bind a drug; (c) have little tendency to interact with the releasing medium and; (d) offer the least possible diffusional resistance (FDA, 1997). The acceptor medium should ensure adequate drug solubility, so that the transport of drug from the vehicle into the medium is not limited. The method must be able to distinguish changes in the product which falls into the categories 2 and 3 (see Appendix 2). Firstly, the potential of drug binding to synthetic membranes was investigated. The recovery results of TAA from test solutions after incubation with regenerated cellulose, polyester and polycarbonate membranes are presented (Tab. 6-1).

Tab. 6-1: Recovery values of TAA in the test solutions at two different concentrations (1µg/mL and 25 µg/mL) after incubation with test membranes (n=3). Only mean values are presented.

Membrane type	**Drug concentration (µg/mL)**	**Recovery (%)**
Regenerated cellulose (thickness 8 µm)	1	99.00
	25	101.70
Regenerated cellulose (thickness 25 µm)	1	99.00
	25	101.50
Polyester (thickness 10 µm)	1	98.80
	25	101.90
Polycarbonate (thickness 10 µm)	1	100.00
	25	102.20

The recovery values indicated no significant drug binding to the synthetic membranes. The membrane of choice should provide an inert holding surface for the test formulation, but not a barrier, so that the drug release would reflect the vehicle properties and not the membrane rate-limiting properties (Kundu *et al.*, 1993; Clement *et al.,* 2000). Resistance to drug transport caused by the membrane directly influences on the method sensitiveness (Zatz *et al.,* 1995).

6.3.1. Influence of membrane thickness and material on drug release



(a) (b)

Fig. 6-1: (a) Release profile of TAA from Kortikoid-Ratiopharm® cream using regenerated cellulose membrane with different thickness (8 µm and 25 µm). (b) Release profile of TAA from Kortikoid-Ratiopharm® cream employing regenerated cellulose (8 µm), polycarbonate and polyester membranes. For both experiments HBSS buffer was employed as acceptor medium. The results presented in the graph are mean of linear regression from three Franz-cells (n=3) and error bars.

The release rate of TAA was significantly lower using the cellulose membrane with thickness of 25 µm (4.35 ± 0.17 µg/cm^2/h$^{0.5}$) in comparison to the cellulose membrane with thickness of 8 µm (6.88 ± 0.304 µg/cm^2/h$^{0.5}$) (t-test, $p<0.05$), indicating a considerable increase in the resistance to drug diffusion with increase of the membrane thickness. Apparently, the material (cellulose, polyester and polycarbonate) did not influence on drug transport. No statistical difference could be observed in the drug release using these three membranes with similar thickness (ANOVA, p=0.493).

The correlation coefficients obtained from the plot of the cumulative released drug in function of the square root of time for cellulosic membranes with different thickness were higher than

0.99, which indicates that the drug release follows the theoretical diffusion model postulated by Higuchi (1961). However, a clear difference could be observed in their release profiles. Not only chemical interactions, but also physical features of the membrane as porosity, tortuosity and thickness can play an important role in the drug diffusion (Zatz *et al.,* 1995).

The material of the synthetic membrane can also influence on drug release (Shah *et al.,* 1999). Clement *et al.* (2000) have investigated the release characteristics of caffeine from concentrated emulsions using three different sources of synthetic membranes. Silicone membrane has represented a rate-limiting barrier for the diffusion of caffeine whereas cellulose membrane has permitted to differentiate the release from caffeine from two formulations with different emulsifiers. For regulatory purposes, the membrane should also permit the discrimination between prechanged and postchanged semisolid products. Cellulose membranes have been extensively employed in other studies, yielding acceptable differentiation between dermatological products (Babar *et al.,* 1991; Shah *et al.,* 1999). Thus, cellulose membrane was the membrane selected for the validation experiments.

6.3.2. Drug solubility in different acceptor media

The results of solubility of TAA in HBSS buffer and binary mixtures of HBSS buffer with MeOH are presented in Tab. 6-2.

Tab. 6-2: Saturated solubility of TAA in HBSS buffer and HBSS/MeOH binary mixtures with different fractions of MeOH (n=3).

Medium	Solubility (µg/mL)
HBSS buffer	20.89 ± 0.15
HBSS buffer/MeOH (80:20, v/v)	42.92 ± 1.17
HBSS buffer/MeOH (70:30, v/v)	89.23 ± 2.33
HBSS buffer/MeOH (50:50, v/v)	892.65 ± 0.15

The solubility of TAA increased with the increase of MeOH fraction in the binary mixtures with HBSS buffer. This organic solvent functions as a cosolvent by changing the solubility parameter of the solution and helping to solvate more lipophilic compounds. The results clearly show that drug solubility is not a limiting-factor for the drug transport from the vehicle into the acceptor media.

6.3.3. Determination of drug interaction between vehicle and acceptor medium

The formulations were allowed to equilibrate with respective acceptor media over 6 hours without the support membrane (Fig. 6-2).

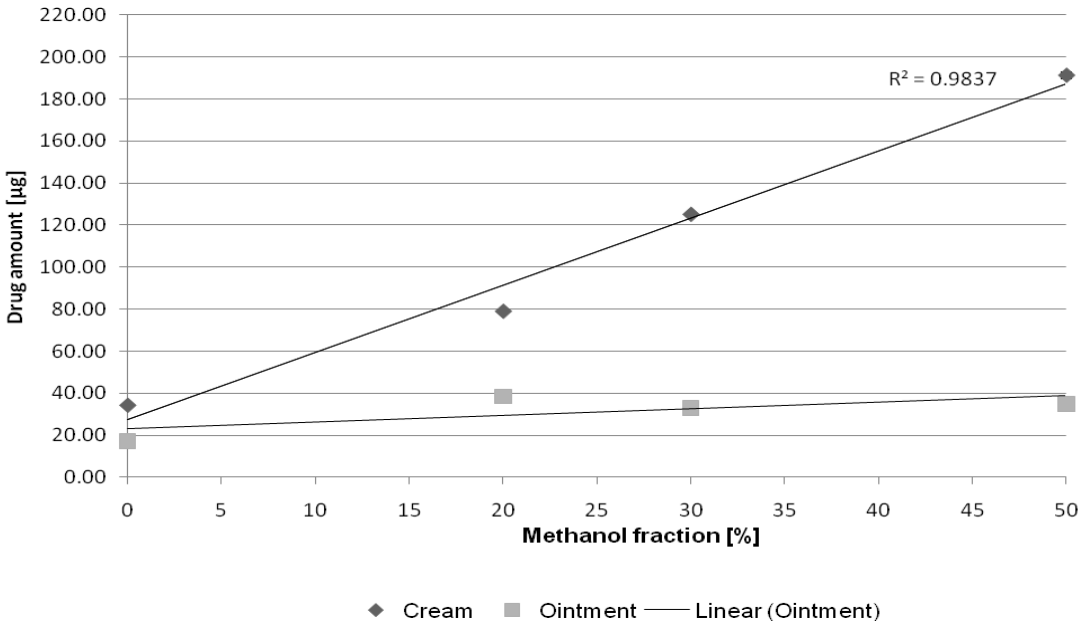


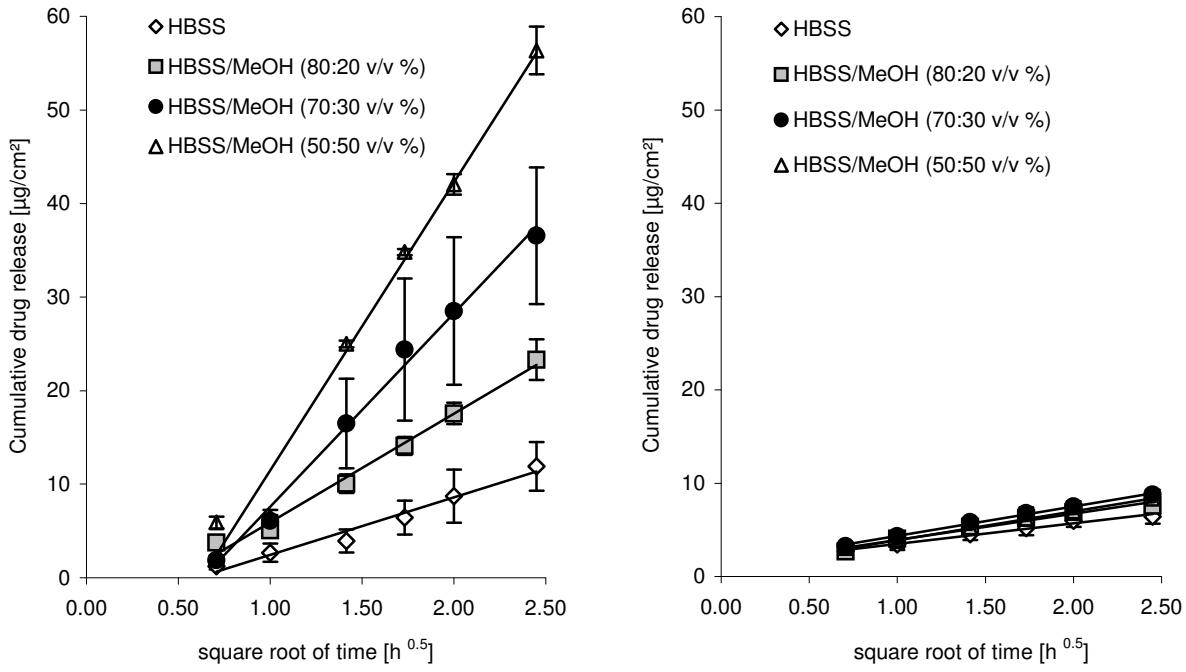
Fig. 6-2: Drug partitioned from Kortikoid-Ratiopharm® cream and Kortikoid-Ratiopharm® ointment into acceptor medium with increased fraction of MeOH after 6 hours at 32 ºC. The different acceptor media are represented by data plotted in the X axis at 0, 20, 30 and 50 (%). The graph shows the mean value of drug amount (n=3). Error bars of results of ointment were small and omitted because they overlapped with circle symbol in the graph. The dashed line represents the regression analysis of drug amount/MeOH fraction for hydrophilic cream.

After the equilibration time, an increase in the drug amount found in the acceptor medium related to an increase in the MeOH fraction was observed for the cream. The regression analysis of the partitioned drug in function of MeOH fraction demonstrated a linear behaviour (Fig. 6-2). However, the same was not observed for the ointment. Regardless the concentration of methanol in the acceptor medium, the partitioned drug was relatively constant.

Kortikosteroid-Ratiopharm® cream is an o/w cream. The partition of the lipophilic TAA from this hydrophilic vehicle into the medium is more favourable than the partition from the ointment. The drug leaves more easily the cream with the increase of concentration of organic

solvent in the medium whereas the drug "leaving potential" from ointments is unfavourable due to the oily nature of this base.

6.3.4. Influence of acceptor medium on drug release



(a) cream (b) ointment

Fig. 6-3: *In vitro* release profile of TAA from Kortikoid-Ratiopharm® cream and ointment using as acceptor medium HBSS and HBSS/MeOH binary mixtures with 20, 30 and 50% MeOH over 6 hours at 32 ± 2ºC. In the graph the mean value of three Franz-cells (n=3) and SD are displayed.

The release profile of TAA from the cream was notably different whereas no statistical difference (ANOVA, p=0.4) was observed for the release rate from ointments employing different acceptor media varying in MeOH concentration. These findings are consistent with the results of drug partition, which give strong evidence that release from the investigated vehicles was mainly governed by vehicle/acceptor medium interaction.

Plotting the release rate from the cream and TAA solubility in medium in function of MeOH fraction, a linear and exponential behaviour (Fig. 6-4) were obtained, respectively.

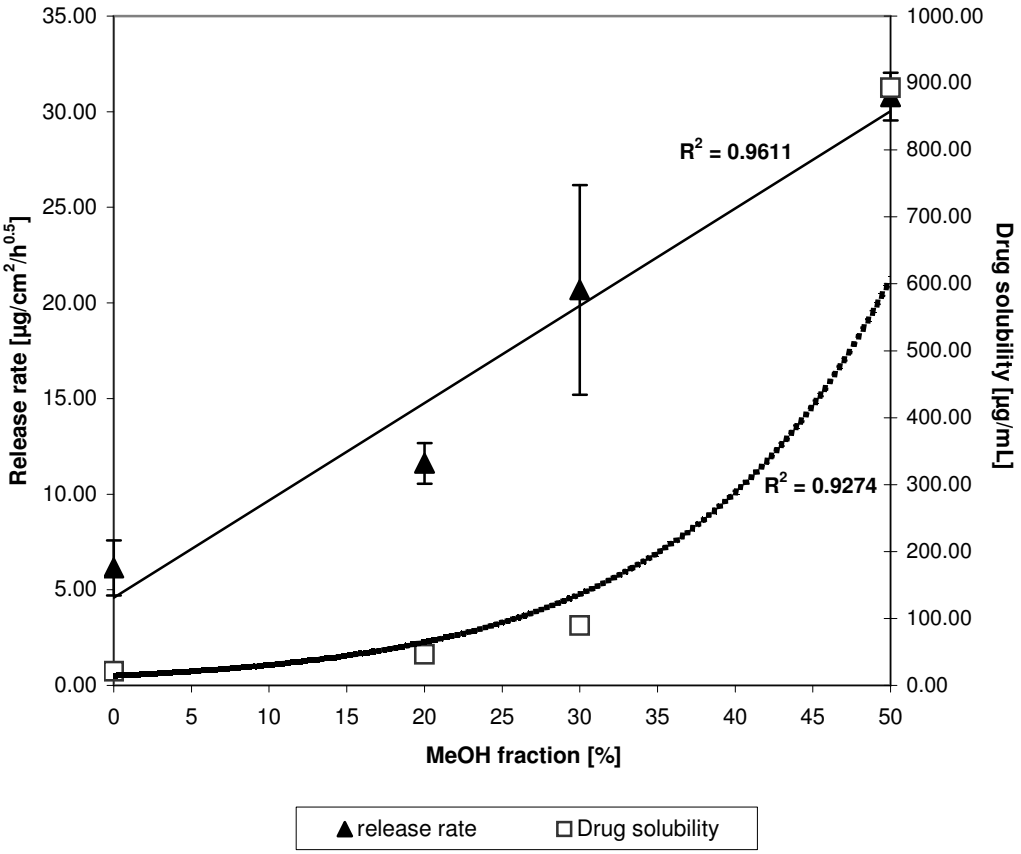


Fig. 6-4: Relationship between drug release rate (primary Y axis) and drug solubility in acceptor medium (secondary Y axis) in function of MeOH concentration in the acceptor medium (X axis). The graph displays the coefficients of regression analysis, the mean of three determinations (n=3) and SDs.

Drug release increased linearly and drug solubility increased exponentially in function of fraction of organic medium. The results indicated that solubility of TAA in binary mixtures of HBSS/MeOH follows the model linear logC-cosolvent relationship proposed by Yalkowsky and co-workers (Yalkowsky and Krzyzaniak, 1998; Millard *et al.*, 2002). This model postulates an increase in aqueous solubility for nonpolar organic compounds as the concentration of organic cosolvent is increased. Since HBSS buffer is an aqueous system, the present data closely fit to this model.

An overall evaluation of solubility, partition and release results demonstrated a very close correlation between these factors. Since drug partition is regulated by the differential solubility between two immiscible phases, release rate is dependent on drug solubility in the acceptor medium. The release process can be schematically represented as in Fig Fig 6-5:

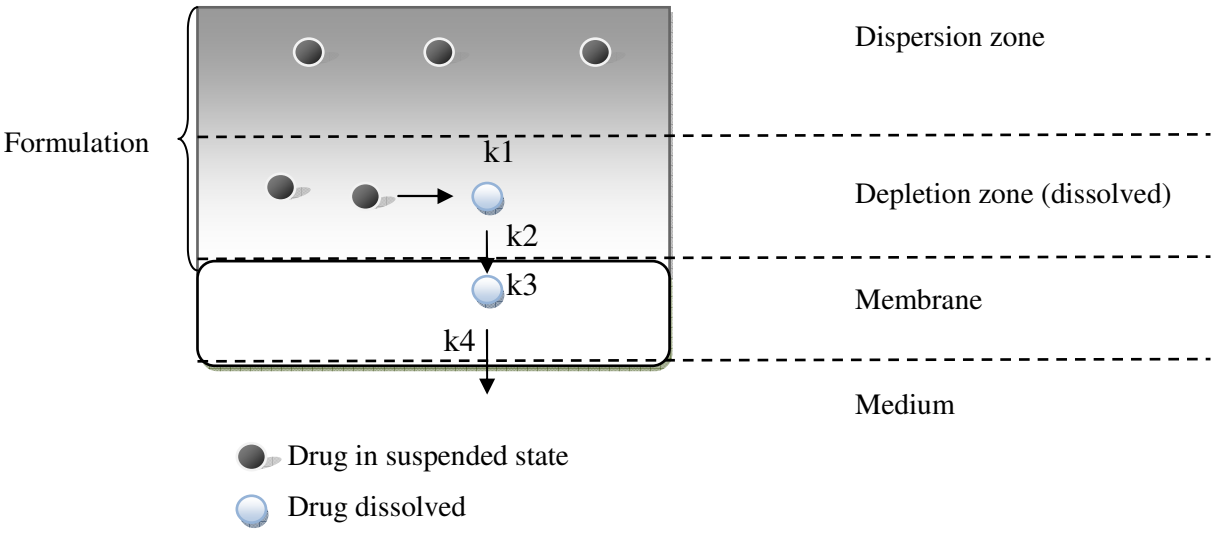


Fig 6-5: Schematic illustration of the kinetic factors influencing on drug release.

Topical corticosteroids are assumed to be mostly in suspended state in the semisolid bases. In the boundary between formulation and the membrane, a depletion area is expected to be formed (Fig 6-5). Assuming a constant temperature and negligible influence of the back diffusion of acceptor medium, the dissolution rate (k1) of the drug in suspended phase is governed mainly by the physicochemical nature of the semisolid base. The drug in solution state diffuses through the base at the rate k2. Considering that the pores of the membrane are previously soaked with acceptor medium, the drug partition from the formulation into the membrane is assumed to be mainly governed by the drug/acceptor medium partition, dictating k3. The transport of drug in the membrane, represented by k4, is governed by the properties of the membrane as tortuosity and physicochemical features.

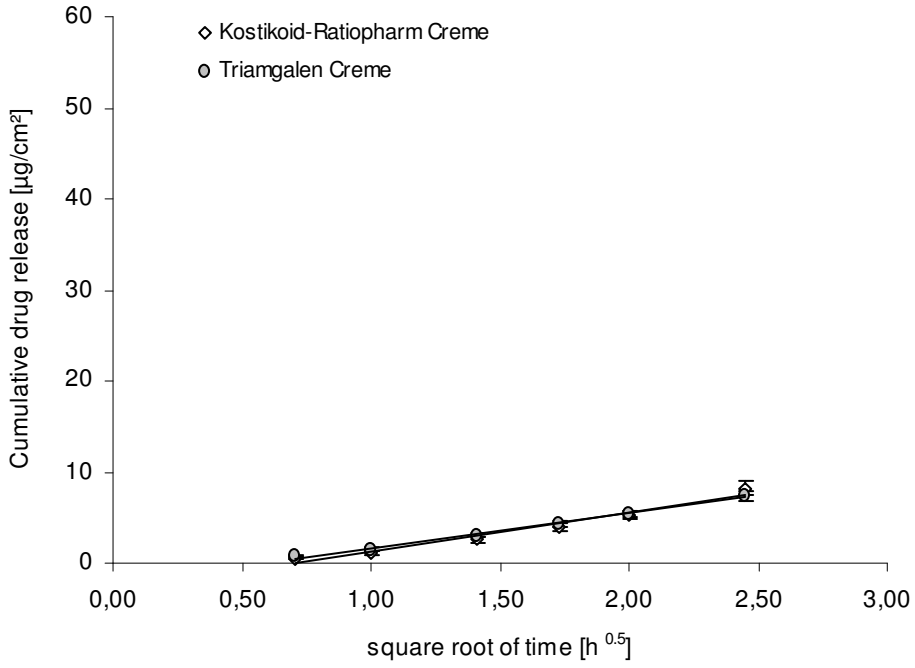
In the framework of regulatory applications, the capacity of predicting drug release from solubility data open perspectives in the current *in vitro* release practice. The discriminating power of the method may be improved with the alteration of the medium composition, enhancing or retarding the drug release, revealing more clearly relevant differences between formulations. Although the currently published USP monograph drafts state that the test must be reproducible and reliable, no further guidance has been given to evaluate the reproducibility and reliability. If the method is intended to be employed in a regular basis, the influence of the medium can be a critical point. Variations in its preparation in different days

may result in variation in the results, compromising the reproducibility. Moreover, concerning the utilization for SUPAC-SS purposes, the influence of test components as synthetic membrane and acceptor medium on the capacity of the method of distinguishing products which undergone relevant modifications has to be investigated.

6.3.5. Influence of acceptor medium on discriminating power

Drug release from generic products

Two marketed generic products with similar physicochemical properties (o/w cream systems) were used to investigate the discriminating power of the method. The assessment of equivalence of generic topical corticosteroids is a subject to increasing research interest. Jackson *et al*. (1989) reported that generic substitution would be made for the prescribed brand of TAA in 75% of the cases in local pharmacies in United States. Kortikoid-Ratiopharm® and Triamgalen® creams vary quantitatively and qualitatively in their excipients and according to these variations they fall into the level 3 of alteration (FDA, 1997). The release equivalence experiments were performed with two different acceptor media: HBSS (method 1) and HBSS supplemented with 30% MeOH (method 2).



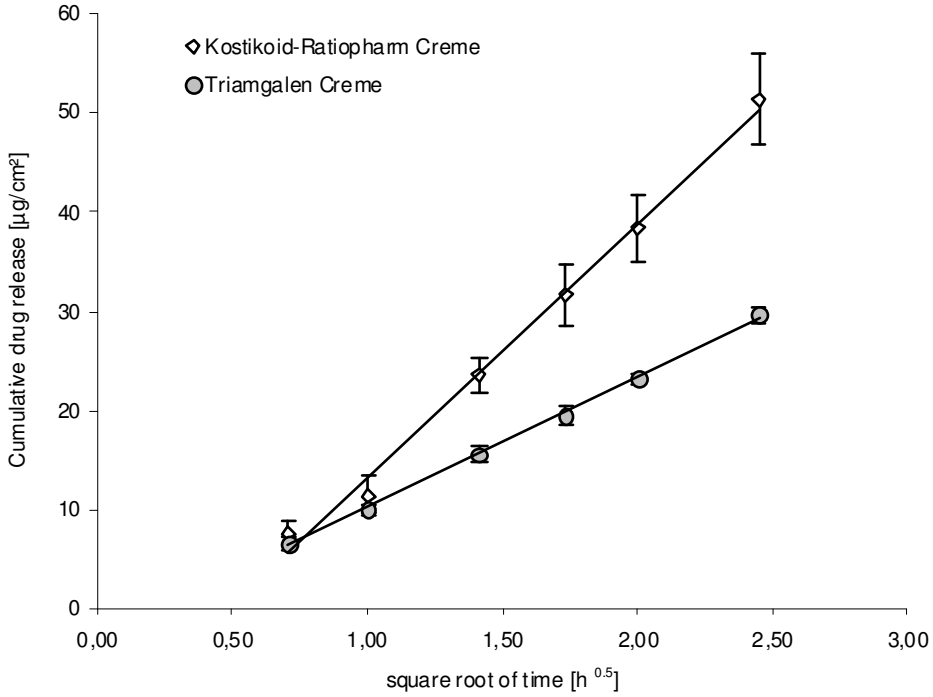
Method 1

Fig. 6-6: *In vitro* release profile of TAA from Kortikoid-Ratiopharm® cream and Triamgalen® cream using HBSS buffer as acceptor medium. The graph displays the mean value of six Franz-cells (n=6) and error bars.

Tab. 6-3: Summary of release results of two generic TAA creams using HBSS buffer as acceptor medium (n=6).

Product	**Cumulative transport per area*** **(µg/cm²)**	**Release rate (µg/cm²/h$^{0.5}$)**	**r²**	**Limits of the 90% confidence interval**		
				Lower limit	**Upper limit**	**Significant different**
Kortikoid-Ratiopharm® cream	8.21 ± 0.81	4.36 ± 0.41	0.993	1.060	1.226	no
Triamgalen cream®	7.38 ± 0.53	3.81 ± 0.30	0.966			

* Cumulative amount released per area represents the concentration of TAA in the receptor compartment at the end of the experiment after 6 hours.



Method 2

Fig. 6-7: *In vitro* release profile of TAA from Kortikoid-Ratiopharm® cream and Triamgalen® cream employing HBSS/MeOH (70:30 v/v, %) as acceptor medium. The graph displays the mean value of six Franz-cells (n=6) and error bars.

Tab. 6-4: Summary of release results of two generic TAA creams using HBSS/MeOH (70:30 v/ v, %) as acceptor medium.

Product	**Cumulative transport per area*** **(µg/cm²)**	**Release rate (µg/cm²/h$^{0.5}$)**	**r²**	**Limits of the 90% confidence interval**		
				Lower limit	**Upper limit**	**Significant different**
Kortikoid-Ratiopharm® cream	51.30 ± 4.59	25.68 ± 2.64	0.994	1.768	2.115	yes
Triamgalen® cream	29.60 ± 0.77	13.16 ± 0.35	0.963			

* Cumulative amount released per area represents the concentration of TAA in the receptor compartment at the end of the experiment after 6 hours.

The results of U-test in the first experiment demonstrated that the method was not capable to distinguish the products, whereas the method 2 could clearly detect the formulation

differences, falling out of the interval of 0.75-1.33 (U-test, lower limit of 1.768 and upper limit of 2.115).

The "release equivalence" of topical generical products is topic of intensive discussion. Following the thalidomide tragedy after 1962, evidences of efficacy and safety of drugs prior to marketing started to be strongly required (Shah and Maibach, 1993). However, some of generic topical products approved before 1962 were allowed to remain in the marked only with *in vitro* evidences of comparability, waving from *in vivo* requirements (Shah and Maibach, 1993). TAA is a pre-1962 drug and prior to this year, the FDA did not proclaimed *in vivo* bioavailability/bioequivalence requirements for topical products (Shah *et al.*, 1999). Drug release from semisolids combine different characteristics of the formulation that are not demonstrated in traditional quality control tests as viscosity, pH uniformity of content. Although all formulations have met classical quality control tests, the different composition reflects on their release properties. Similar results have been recently found for generic transdermal products containing testosterone (Baert *et al.,* 2009). The consequence of these results for the *in vivo* bioavailability and therapeutic action is still unclear.

One has to be very careful during the development of *in vitro* test for regulatory applications. The straightforward choice of the medium without considering its possible influence on the drug release may result to an unsuitable method with low discriminating power. During the performance of the test, the developer might obtain adequate fits to the theoretical model of release postulated by Higuchi and acceptable correlation coefficients and reproducibility. However, the main purpose of the method may be not fulfilled as it could be observed for the method 1. Hence, during the development of the method not only drug binding to membrane, drug solubility and compliance to theoretical models have to be verified, but it is also important to investigate the main purpose of the method to provide clear discrimination of prechanged from postchanged products avoiding in the same time an overdiscrimination.

6.3.6. Influence of methanol in the vehicle on drug release

Back diffusion of the acceptor medium into applied formulation is a critical aspect pointed out by some authors (Rolland *et al.* 1992; Zatzt, 1995). This becomes more critical for media containing organic solvents, which may enhance the solubility of lipophilic compounds in the vehicle modifying the release rate. The degree to which the back diffusion affects release is still unclear. This part of the study aimed to investigate the influence of MeOH on the release of TAA. The drug was formulated into the pharmacopeial base (BC) at same experimental

concentration (0.1%) used in the development experiments. BC was the base suitable for this investigation due to its amphiphilic character miscible either with aqueous or organic solvents (DAC, 2009). MeOH was incorporated into the vehicle at different concentrations (1, 3 and 5%). This experiment design represented the worst case scenario for the investigation of the influence of MeOH on drug release.

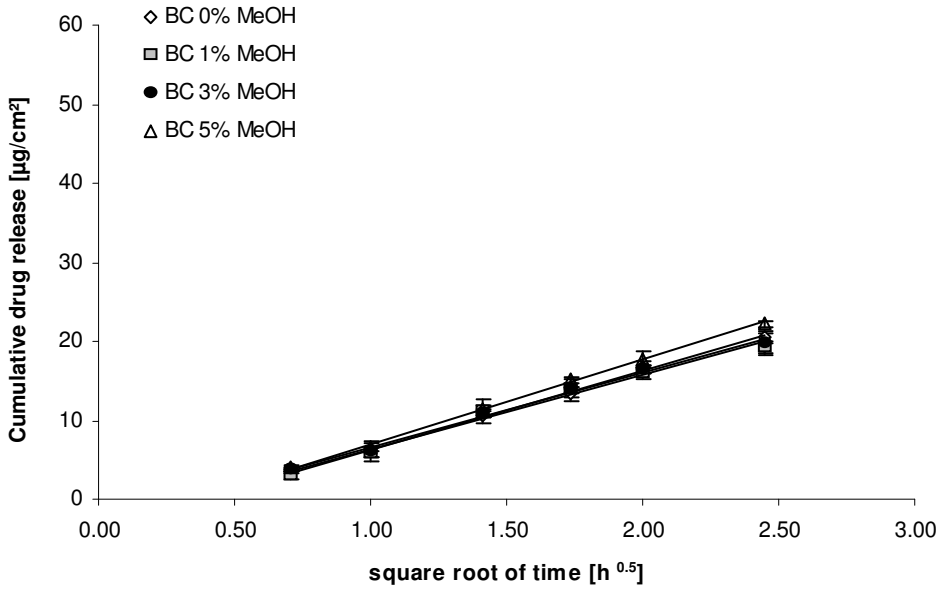
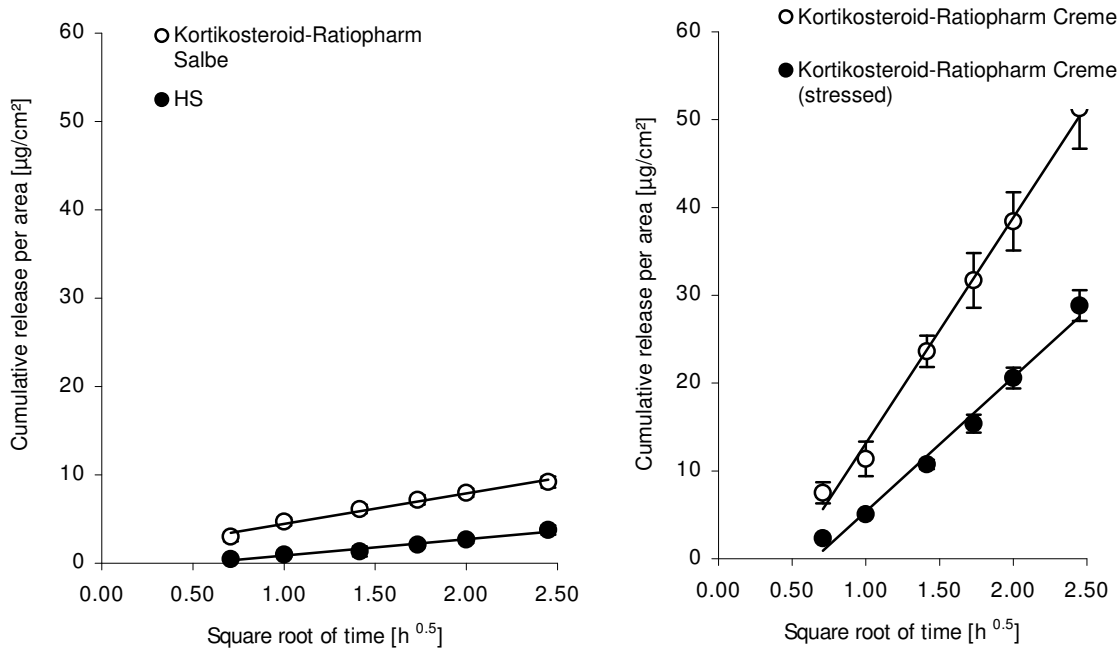


Fig. 6-8: *In vitro* release profile of TAA from BC (control) and BC modified with 1, 3 and 5% (w/w) of MeOH. In the graph, the mean of three Franz-cells (n=3) and error bars are presented.

No significant difference in the release rate of TAA was observed between control and modified test formulations (t-test, $p>0.05$). Although the diffusion of MeOH into the applied formulations could not be experimentally detected, the results indicate that at low concentrations MeOH does not significantly influence on the release of TAA from creams.

Release from water-free formulations and stressed cream

Despite the method has demonstrated satisfactory discriminating capacity for cream preparations, the discriminating power for anhydrous oily bases was still unclear. Moreover, the test should be also capable to detect small differences in the same product in order to be utilized to monitor batch-to-batch uniformity during quality control tests. Therefore, the release of TAA from water-free ointments and from a cream submitted to accelerated conditions (storage with opened recipient at 40 ºC over 1 month) were investigated.



(a) Water-free ointments (b) Stressed and untreated cream

Fig. 6-9: (a) *In vitro* release profile of TAA from water-free ointments (Kortikoid-Ratiopharm® ointment and HS) varying in composition and; (b) release profile of TAA from untreated and stressed cream. In the graphs the mean value of six Franz-cells (n=6) and error bars are displayed.

Slower drug release was observed either for Kortikoid-Ratiopharm® ointment or for HS in comparison to creams. Despite the low release rate, a significant difference was observed for both formulation (t-test, $p<0.05$). After 6 hours, the marketed ointment demonstrated approximately 3-fold higher drug released (9.19 ± 2.64 µg/cm^2) in comparison to HS (3.76 ± 2.64 µg/cm^2). These results confirmed the discriminating power of the method not only for hydrophilic bases but also for anhydrous viscous bases, which usually are critical due to lower release of drug. An evident difference was also observed in the release profile of treated and untreated formulations. After 6 hours, 51.30 ± 4.59 µg/cm^2 of TAA were released from the untreated cream whereas only 28.84 ± 1.75µg/cm^2 were released from the cream submitted to storage conditions at 40 ºC. Since the product was submitted to opened-system stress conditions, the higher temperature could have caused evaporation of water in the external phase of the formulation reflecting on its viscosity, drug concentration and mobility of drug in

the cream. The results could confirm the usefulness of the method for detecting small variation in the same product.

6.3.7. Validation of *in vitro* release method

The validation experiments aimed to investigate the reproducibility and reliability of the method. Based on the results of the development phase, cellulose membrane (8 µm) and HBSS/buffer (70:30 v/v) were chosen as adequate membrane and acceptor medium. Reproducibility was tested in different days (inter-day and inter-week) and between different lots. The robustness of the method against small changes in the test conditions (medium and stirring rate) was investigated. The reproducibility between the cells was assessed taking into account the release results of 30 cells within a series of 5 experiments. The summary of the validation results are presented in Tab. 6-5.

Tab. 6-5: Summary of validation results.

Validation parameters		**Release rate (µg/cm²/h$^{0.5}$)**	**Limits of the 90% confidence interval***		
			Lower limit value	Upper limit value	Significant different
Reproducibility (inter-day)	Day 1	25.68 ± 2.64	0.971	1.116	no
	Day 2	23.42 ± 1.51			
Reproducibility (inter-week)	Day3	22.49 ± 1.06	1.036	1.250	no
Reproducibility (batch-to-batch)	Lot A	22.49 ± 1.06	0.894	1.013	no
	Lot B	23.60 ± 1.18			
Robustness (stirring rate)	400 rpm	23.42 ± 1.51	1.016	1.163	no
	300 rpm	21.66 ± 1.07			
Robustness (medium pH)	pH 7.4	21.92 ± 1.23	0.931	1.055	no
	pH 6.4	22.03 ± 0.71			

* Ratio of *in vitro* release mean value of the formulation tested in the day 1 over the *in vitro* release mean value of the formulation tested in the day 2 with 90% of confidence expressed in percentage. If values fall within 0.75-1.33 (eighth and twenty-ninth ordered individual ratios are the lower and upper limits) the release rates are not significantly different. The experiments reproducibility day 3/reproducibility lot A and reproducibility day 2/robustness 400 rpm are the same.

Employing the U-Test, no statistical difference could be found in the release rate of experiments performed in different days within and in different weeks. Experiments using the same product from different batches also yielded reproducible results. During the investigation of the method robustness, the medium pH did not influence significantly the release of TAA. Because TAA does not dissociate in ion forms, the change of the pH apparently does not affect its partition. Performing two experiments at different stirring rates, no statistical difference could be found in the release of TAA. The stirring rate of the acceptor medium influences particularly the concentration gradient present in the diffusion boundary layer between donor and acceptor as previously described (Horsch, 1974).

The release rate of 30 cells (within 5 experiments) was plotted in a flow chat (Fig. 6-10). The process analysis using control charts was firstly postulated by Water Shewhart (Shewhart, 1931) for evaluation of statistical control of manufacturing processes. These charts can help users to develop an understanding of the performance of a process and to evaluate benefits or consequences of process interventions.

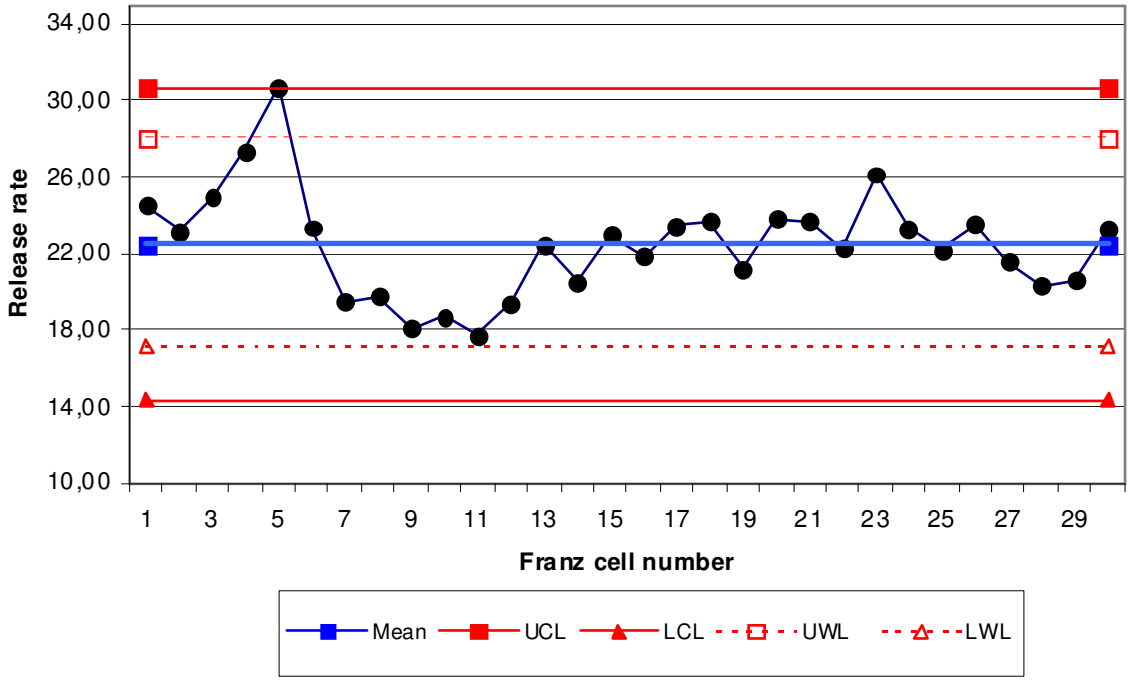


Fig. 6-10: Flow chart of the release results of 30 Franz-cells. Each plotted point represents chronologically the release of a single cell within 5 validation experiments. The central line represents the mean value of release of all investigated cells. The dashed lines represent the up-warning limit (UWL) and low-warning limit (LWL). The superior and inferior lines represent the up-control limit (UCL) and low-control limit (LCL).

Tab. 6-6: Summary of overall statistical results from 30 Franz-diffusion cells.

Parameters	Results
Number of replicates	30.00
Mean (µg/cm^2/h$^{0.5}$)	22.48
SD	2.72
RSD (%)	12.09
Maximum (µg/cm^2/h$^{0.5}$)	30.73
Minimum (µg/cm^2/h$^{0.5}$)	17.76
Up-warning limit (µg/cm^2/h$^{0.5}$)	27.92
Low-warning limit (µg/cm^2/h$^{0.5}$)	17.05
Up-control limit (µg/cm^2/h$^{0.5}$)	30.63
Low-control limit (µg/cm^2/h$^{0.5}$)	14.33

The RSD values of each single experiment varied from 3.22% to 10.28% and the mean of 30 cells yielded to 12.09%. Chilliot *et al.* (2005) determined the inter- and intra-laboratory variations in diffusion cell measurements from 18 participating laboratories. The coefficient of variation between laboratories was approximately 35%. Intra-laboratory variation was lower, averaging 10% within a single laboratory. Thus, the present results are in good agreement with the previously reported variations and they are considered to be acceptable.

In the presented control chart, the up and low control limits were initially established based on the principles of Shewhart. The empirical investigation of sundry probability distributions reveals that at least 99% of observations occurred within three SDs. Furthermore, warning limits were set follows that approximately 95% of the sample means will fall within the limits of ± 2 SDs. One of the main objectives of analysis from control charts is the identification and verification of changes and variations in the process. Most processes exhibit some variability, which can be classified into one of two categories: 'natural' or 'unnatural' (Wheeler and Chambers, 1992). The natural variability of a process is the systemic variation inherent as a regular part of the process. Because they are caused by regular sources within the process or its environment, data exhibiting natural variation occur in predictable and relatively common frequencies. Conversely, outcomes or in-process observations that have very small probabilities of occurrence, represents deviations from the regular process. Such events suggest that the process fundamentally has changed due to atypical unnatural variability that should be traced to root assignable causes for further decisions with regard its performance and improvement (Wheeler and Chambers, 1992).

According to Benneyan (1998), if one of the results falls in one of the following criteria, the process is considered to be out-of-state of statistical control:

- any single unit value outside of control limits (UCL and/or LCL);
- eight consecutive unit values on one particular side of the center line (mean value);
- three consecutive units beyond the limits of 2 SDs on a particular site of the center line;
- six consecutive groups with either an increasing or decreasing trend.

The first evaluation of the present chart reveals an outlier (cell 5), which falls outside the up control line (UCL). This cell belongs to the first experiment from a series out of 5. It can be also observed that results corresponding to the first two experiments (cell 1 to cell 12) presented higher deviations from the centre line in comparison to the experiments 3 to 5 (cells 13 to 30). Up to cell 12, the individual results start to be centrally located around the mean value, suggesting that the procedure is entering in statistical control. From the second experiment, no individual cell falls in the above mentioned criteria, demonstrating that for the evaluated population, the process is under statistical control. The guidance on *in vitro* release (FDA, 1997) outlines the expectation of outliers during the run of *in vitro* experiments, therefore a non parametric statistical method (U-test) is provided for assessment of the results. With the exception of the cell 5, no other cell falls in the tighter warning limits (LWL and UWL). After overall evaluation of the process, it can be concluded that it delivers stable results over the time exhibiting only natural variability. The process evaluation represents the first efforts toward the establishment of acceptance criteria for *in vitro* release tests.

Selection of suitable medium and further method validation are the key elements for the dissolution tests for oral solid dosage. The method has to demonstrate discriminating capacity, robustness, transferable, reproducible and be able to monitor batch-to-batch sameness (Azarmi *et al.,* 2007). *In vitro* release tests follow the same principles of dissolution test, with the difference that no specifications with regard the amount of drug to be liberated are usually required. These tests have to be individually developed taking into consideration the particularities of the dosage form and to be validated to deliver reliable results and fit its final purpose. The idea of a universal method for all semisolid formulation types is unrealistic.

6.4. Summary and conclusion

The effect of semisolid vehicle on the drug release of topical corticosteroids was investigated. Three generic products containing TAA have demonstrated different release profiles. These results raised the issue of the therapeutic equivalence of particular drugs approved prior to 1962. A different release profile may not result in a different percutaneous absorption and therapeutic action. It is recognized the test may demonstrate an overdiscriminating potential, however, this is desired for quality control purposes from a regulatory point of view, where alarming evidences are necessary prior to *in vivo* testing. The test also confirmed the potential of discriminating anhydrous ointments varying in composition, which are considered to be critical due to the low amounts of drug liberated. Moreover, small changes in the same product provoked by storage at forced conditions could be detected, confirming the usefulness of the test for quality control purposes.

Numerous apparatus and techniques have been used for investigate *in vitro* release (Rege *et al.,* 1998; Chattaraj *et al.,* 1995; Amerongen *et al.,* 1992; Rolland et *al.,* 1992, Tuomi *et al.,* 1989). The establishment of a validation protocol is an important prerequisite for ensuring the reliability of the results. During the validation, reproducibility was investigated in terms of inter-day precision, inter-batches precision and overall variability of Franz-cells within a series of experiments. During the performance of the test procedure, artifacts inherent to the operation might influence on the reproducibility of the results. The influence of MeOH on the drug release was discussed. Then, the preparation of the acceptor medium prior to the start of the experiment becomes a critical step. A marketed product was utilized in the experiments ensuring that variations in the experimental results would be originated from the method and not from the formulation, as non uniformity in the drug distribution and content. Employing the U-Test, no statistical difference could be found in the release rate of experiments performed in different days within and in different weeks. A statistical process control was employed for the evaluation of the variability in the results obtained for individual Franz-cells within several experiments. This represents also the initial efforts toward to the establishment of acceptance criteria for a quality control method. The verification of the variation using control chart permitted to monitoring an in-control process for changes in process and outcome quality; identifying, testing, and verifying process improvement opportunities and in long-term objectives, to tighten the control limits by reducing process variation.

7. Effect of vehicles on *in vitro* release

7.1. Introduction

When a drug is applied to the skin, several events may become rate-limiting steps in cutaneous permeation kinetics. The transport of drugs in the semisolid vehicles is one of the key factors which determine overall kinetics. The influence of pharmaceutical vehicles on drug release has been extensively the focus of research on semisolids (Ostrenga *et al.,* 1971; Niemi *et al*., 1989; Lu *et al*., 1998; Clement *et al*., 2000; Borgia *et al*., 2008). Several authors have reported the success in demonstrating a direct relationship between drug release and pharmacodynamic responses (Pershing *et al.*, 2002). Kazmi *et al.* (1984) reported a correlation between *in vitro* release and *in vivo* percutaneous absorption of indomethacin in rabbit skin at several concentrations in ointments. A good correlation between *in vitro* release, percent of solubilized drug in vehicle and vasoconstrictor response was also found for topical creams containing fluocinolone (Ostrenga *et al.,* 1971). A clear difference in response from skin blanching assays was observed between two brands of betamethasone valerate correlating with *in vitro* release data (Shah *et al*., 1992). However, interpretation of the data must be taken with care to avoid excessive extrapolation, as there are many anatomical and physiological factors, which are not represented under these circumstances of synthetic membranes. More understanding of physical and chemical factors which affect drug transport in semisolid vehicles is necessary to elucidate the relationship between drug release rate and percutaneous absorption. Particularly for these dosage forms, the mechanism of drug diffusion can be complex and involves properties of drug, vehicle microstructure and composition. This knowledge is essential for the selection of the most therapeutically effective base and for the determination of the most effective drug concentration in a given base.

In this chapter, the research purpose was to investigate the effect of several representative semisolid vehicles with different consistence; physicochemical properties; micro and macroviscosity on *in vitro* release of the model drugs. It was expected to obtain a better insight into the underlying mechanistic events which determine the rate of transport of topical corticosteroids in semisolid formulations.

7.2. Materials and methods

7.3. Materials

Dermatop® cream, ointment and fatty ointment were purchased from Sanofi-Aventis Deutschland GmbH (Frankfurt, Germany), Betnesol® cream and ointment; Dermoxin® cream and ointment were purchased from GlaxoSmithKline GmbH & Co. KG (Munich, Germany). Kortikoid-Ratiopharm® cream and ointment were obtained from Ratiopharm GmbH (Ulm, Germany) Triamgalen® cream was purchased from Galenpharma GmbH (Kiel, Germany). Triamcream Lichtenstein® was acquired from Winthtrop Arzneinmittel GmbH (Berlin, Germany). Test formulations were prepared as described in 4.2.1. The synthetic membrane and Franz-cells were used as described in 6.2.1.

7.4. Methods

7.4.1. Preparation of test formulations

All base formulations were prepared according to monographs in the DAB (2010) and DAC (2009) as described in 4.2.1. The model drugs were incorporated in the bases employing a mixer (Unguator, GAKO Konietzko GmbH, Bamberg, Germany) at the same strength as commercial formulations: CP at 0.05%, BM17V at 0.1% and PC at 0.25%, respectively.

7.4.2. Performance of release experiments

This experimental part was described in 6.2.5.

7.4.3. Data processing and statistics

The data processing was performed as described in 6.2.7.

7.5. Results and discussion

This part of this study involved the investigation of the release properties of the model drugs from representative base formulations covering the group of o/w creams, w/o creams, water-free ointments and hydrogels. A better insight into the mechanistic process of drug transport in vehicles varying in consistence; macro and microviscosity; and physicochemical properties was one of the foreground aims of the investigation. Considering the complex structure of the bases and possible different pathways of drug transport, more scientific rigor was applied on the evaluation of the results by comparing the release rates of test bases with marketed

products belonging to the same formulations type. This strategy represented a more level-headed evaluation avoiding misinterpretations caused by comparison between formulations with different microstructures (i.e. ointment and gels).

7.5.1. Vehicle effect on release of triamcinolone acetonide

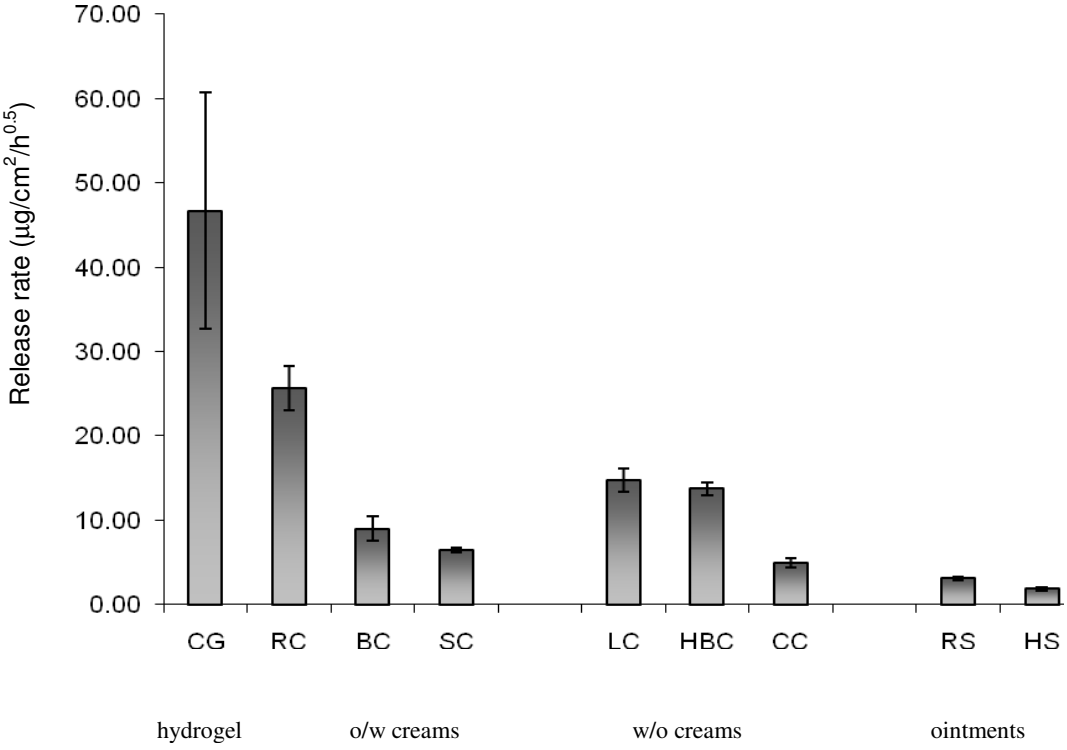


Fig. 7-1: *In vitro* release rate of TAA from CG, Kortikoid-Ratiopharm® cream (RC), BC, SLC, Triamcream Lichtenstein® (LC), HB, CC, Kortikoid-Ratiopharm® ointment (RS) and HS. The bases are grouped with the respective marketed product in function of formulation type (hydrogel, o/w creams, w/o creams, anhydrous ointment). The graph shows the mean of release rate from six Franz-cells (n=6) obtained from the plot of cumulative released drug in function of square root of time. Error bars are also displayed.

The drug release from CG was clearly faster in comparison to the other bases. After six hours, 91.31 ± 22.77 µg/cm^2 of drug were released from this hydrogel. The rank of release has followed CG > w/o creams > o/w creams > anhydrous ointment. In the group of o/w creams, the marketed cream (Kortikoid-Ratiopharm® cream) demonstrated the highest release rate (25.68 µg/cm^2/h$^{0.5}$), followed by BC (8.99 ±1.43 µg/cm^2/h$^{0.5}$) and SLC (6.48 ± 0.24 µg/cm^2/h$^{0.5}$). No significant difference was observed between the release rate of the w/o cream Triamcream Lichtenstein® (15.12 ± 0.99 µg/cm^2/h$^{0.5}$) and HBC

13.41 ± 0.41 µg/cm^2/h$^{0.5}$ (t-test, p=0.20). In the group of water-free formulations, Kortikoid-Ratiopharm® ointment has shown slightly higher release than HS. These results are a strong indicative of the significant effect of the vehicle on the diffusion and release of drugs from different semisolid vehicles, which possibly influence the percutaneous absorption and duration of efficacy of drugs in topical formulations.

The higher drug release observed for hydrogel in comparison to other vehicles is consistent with other published data. Babar *et al*. (1991) have investigated the release of chlorpheniramine maleate from topical bases using cellulose membrane and faster drug release was observed for the hydrogel in comparison to hydrophilic ointments. Lu and Jun (1998) also found higher release of metrotrexate from gels in comparison to ointments. The release of piroxicam was also demonstrated to be faster from hydrogels in comparison to creams (Rafiee-Tehfani, 2000). Carbomers® are one of the most traditional base of topical preparations (Amin *et al*., 2009). The explanation of faster drug release from hydrogels in comparison to other vehicles is possibly based on the hydrophobic nature of TAA and higher thermodynamic activity in this base in comparison to creams and ointments. Thermodynamic activity is influenced by the drug concentration, solubility and level of saturation in the vehicle. This associated to a favourable vehicle/acceptor medium partition yields to a more rapid release with increase of the so-called “drug leaving potential”.

7.5.2. Vehicle effect on release of prednicarbate

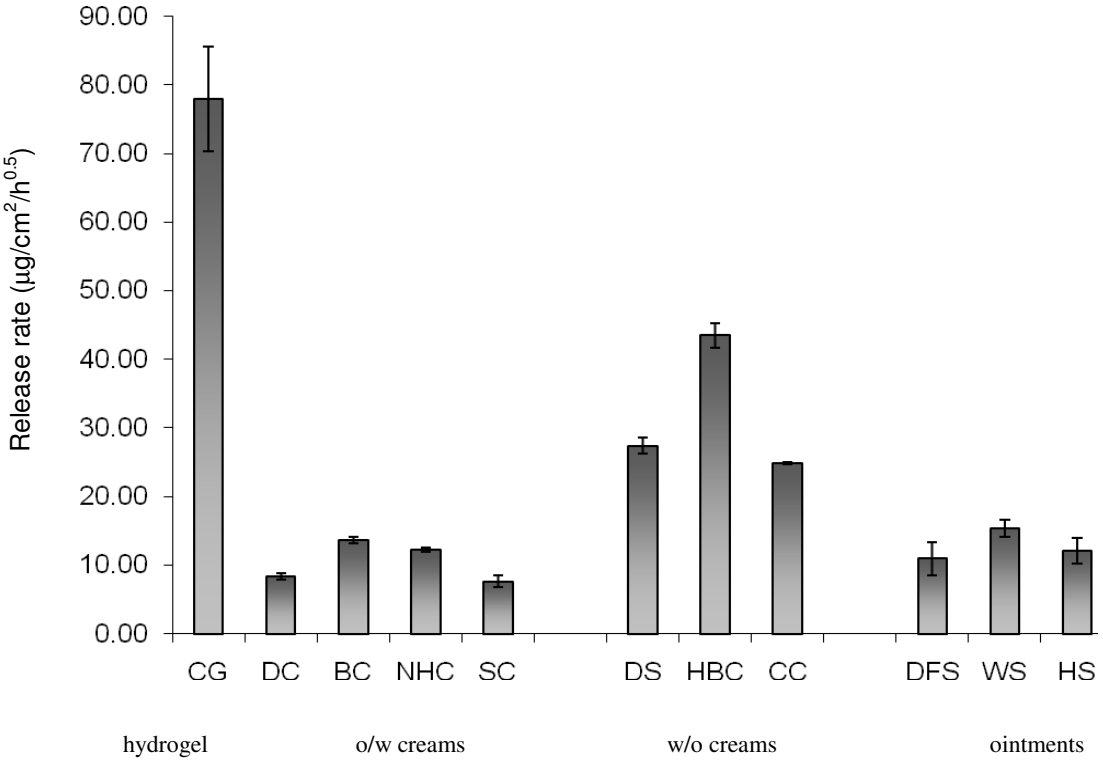


Fig. 7-2: *In vitro* release rate of PC from CG, Dermatop® cream (DC), BC, NHC, SLC, Dermatop® ointment (DS) HBC, CC, Dermatop® fatty ointment (DFS), WS and HS. The bases are grouped with the respective marketed product in function of formulation type (hydrogel, o/w cream, w/o cream, anhydrous ointment, respectively). The graph shows the mean of release rate from three Franz-cells (n=3) obtained from the plot of cumulative released drug in function of square root of time. Error bars are also displayed.

As observed for TAA, CG demonstrated the faster release of PC in comparison to all tested vehicles. After 6 hours 140.43 ± 13.10 µg/cm^2 of drug were found in the acceptor medium. Higher release rates were also found for w/o creams in comparison to o/w creams. In the group of o/w creams, the highest release rate was found for BC (13.62 ± 0.48 µg/cm^2/h$^{0.5}$). Statistically significant differences were found comparing Dermatop Cream ®, BC and NHC (t-test, $p<0.05$). However, no statistic difference was found for SLC (t-test, $p=0.37$). In the group of w/o creams, HBC has demonstrated faster release than Dermatop® ointment (t test, $p<0.05$). No significant difference was observed between Dermatop fettsalbe® and HS (t-test, $p=0.62$) and WS (t-test, $p=0.08$). The results are in agreement with the results from Borgia *et al.* (2008), who found that the release of PC through cellulose nitrate membrane ranked Dermatop® ointment > Dermatop® fatty ointment > Dermatop® cream.

7.5.3. Vehicle effect on release of clobetasol propionate

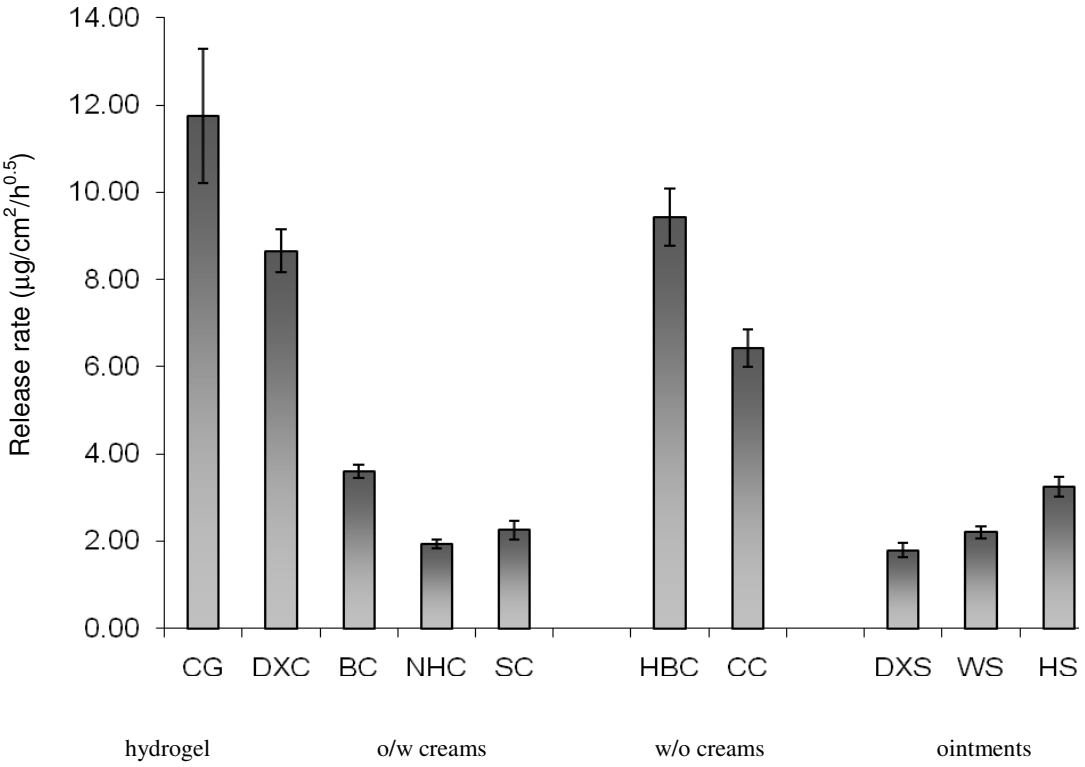


Fig. 7-3: *In vitro* release rate of CP from CG, Dermoxin® cream (DXC), BC, NHC, SLC, HBC, CC, Dermoxin® ointment (DXS), WS and HS. The bases are grouped with the respective marketed product in function of formulation type (hydrogel, o/w creams, w/o creams, anhydrous ointments, respectively). A marketed product belonging to w/o creams group was not available at the local supplier. The graph shows the mean of release rate from three Franz-cells (n=3) obtained from the plot of cumulative released drug in function of square root of time. Error bars are also displayed.

In congruence with the results of TAA and PC, the highest drug release of CP was observed for CG followed by w/o creams. However, differently from PC, the faster release was less evident. In the group of o/w creams, Dermoxin® cream demonstrated the highest release rate (11.76 ± 0.48 µg/cm^2/h$^{0.5}$), followed by BC, SLC and NHC. All three bases demonstrated statistically significant differences in comparison to reference product (t-test, $p<0.05$). Drug release from HBC was higher than CC (t-test, $p<0.05$). In the group of anhydrous ointment, the rank HS > WS > Dermoxin® ointment was obtained.

7.5.4. Vehicle effect on release of betamethasone-17-valerate

BM17V is not included in the development platform of the present work. The employment of BM17V was justified due to the similar lipophilicity to CP and PC, allowing comparison to these two drugs.

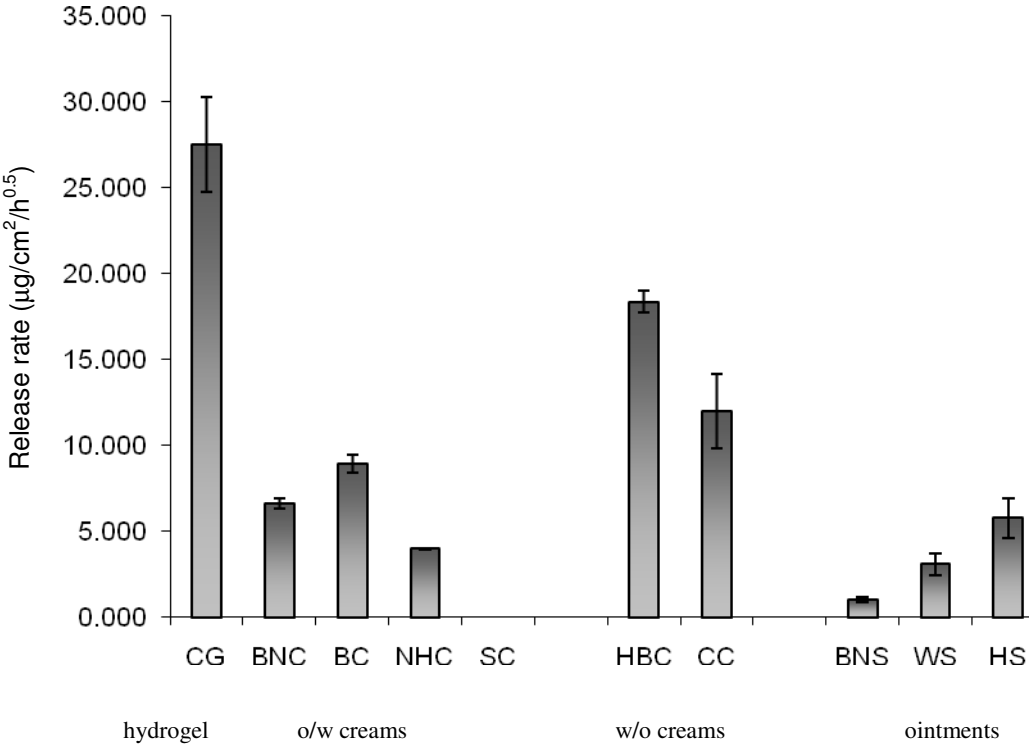


Fig. 7-4: *In vitro* release rate of BM17V from CG, Betnesol® cream (BNC), BC, NHC, SLC, HBC, CC, Betnesol® ointment (BNS), WS and HS. The bases are grouped with the respective marketed product in function of formulation type (hydrogel, o/w creams, w/o creams, anhydrous ointments, respectively). A marketed product belonging to w/o creams group was not available at the local supplier. The graph shows the mean of release rate from three Franz-cells (n=3) obtained from the plot of cumulative released drug in function of square root of time. Error bars are also displayed.

Again, CG provided the highest drug release rate followed by w/o creams. As observed for PC, BC provided higher release of BM17V in comparison to the marketed o/w cream (t-test, $P<0.05$). In the group of anhydrous ointments, the release from WS and HS were higher than the marketed formulation (t test, $p<0.05$). The table below summarizes the release results of all tested drugs.

Tab. 7-1: Summarized release results.

	Formulation type	Drug	Strength [%]	Q6h [µg/cm²]	Release rate [µg/cm²/h^0.5]	r^2	P value**
	CG	CP	0.05	20.86 ± 2.70	11.76 ± 1.54	0.984	$p<0.05^+$
		TAA	0.1	91.31 ± 22.77	46.68 ± 14.02	0.9688	$p<0.05^+$
		PC	0.25	140.43 ± 13.10	77.93 ± 7.61	0.975	$p<0.05^+$
w/o creams	R	CP	0.05	*	*	*	*
		TAA	0.1	28.51 ± 1.65	15.12 ± 0.99	0.9984	-
		BM17V	0.1	*	*	*	*
		PC	0.25	58.25 ± 3.25	27.37 ± 1.15	0.9918	-
	HBC	CP	0.05	18.72 ± 1.20	9.44 ± 0.65	0.9964	p<0.05
		TAA	0.1	28.69 ± 0.41	13.41 ± 0.41	0.9912	p=0.20
		BM17V	0.1	33.27 ± 0.43	18.35 ± 0.65	0.9929	p<0.05
		PC	0.25	88.20 ± 1.11	43.49 ± 1.76	0.9988	p<0.05
	CC	CP	0.05	12.18 ± 0.60	6.43 ± 0.44	0.9978	-
		TAA	0.1	11.20 ± 1.15	4.93 ± 0.53	0.9977	p<0.05
		BM17V	0.1	23.70 ± 3.94	11.98 ± 2.17	0.9994	p<0.05
		PC	0.25	47.25 ± 0.77	24.86 ± 0.20	0.9951	p<0.05
o/w creams	R	CP	0.05	20.53 ± 1.08	8.66 ± 0.49	0.9999	-
		TAA	0.1	51.30 ± 4.59	25.68 ± 2.64	0.9943	-
		BM17V	0.1	11.31 ± 0.45	6.63 ± 0.29	0.9509	-
		PC	0.25	17.44 ± 0.90	8.35 ± 0.44	0.9983	-
	BC	CP	0.05	7.55 ± 0.37	3.60 ± 0.14	0.9888	p<0.05
		TAA	0.1	20.53 ± 2.35	8.99 ± 1.43	0.9981	p<0.05
		BM17V	0.1	18.07 ± 1.20	8.93 ± 0.52	0.9914	p<0.05
		PC	0.25	27.73 ± 1.33	13.62 ± 0.48	0.9957	p<0.05
	NHC	CP	0.05	4.03 ± 0.17	1.95 ± 0.10	0.9954	p<0.05

(Continued).

Formulation type		Compound	Concentration (%)	Q6h ($\mu g/cm^2$)	Release rate ($\mu g/cm^2/h^{1/2}$)	Linear coefficient (r^2)	P value**
o/w creams	NHC	TAA	0.1	*	*	*	*
		BM17V	0.1	8.04 ± 0.08	3.97 ± 0.02	0.9959	p<0.05
		PC	0.25	27.39 ± 0.80	12.22 ± 0.35	0.9969	p<0.05
	SC	CP	0.05	4.96 ± 0.66	2.26 ± 0.22	0.9957	p<0.05
		TAA	0.1	14.00 ± 0.51	6.48 ± 0.24	0.9973	p<0.05
		BM17V	0.1	7.92 ± 0.54	3.72 ± 0.48	0.9947	p<0.05
		PC	0.25	15.86 ± 1.86	7.6 5 ± 0.87	0.9979	p=0.37
anhydrous ointments	R	CP	0.05	4.96 ± 0.66	2.26 ± 0.22	0.9957	-
		TAA	0.1	9.19 ± 0.62	3.46 ± 0.45	0.9829	-
		BM17V	0.1	2.54 ± 0.45	1.02 ± 0.16	0.7288	-
		PC	0.25	22.31± 4.70	10.98 ± 2.43	0.9896	-
	HS	CP	0.05	6.72 ± 0.38	3.25 ± 0.23	0.9972	p<0.05
		TAA	0.1	3.76 ± 0.53	1.85 ± 0.23	0.9921	p<0.05
		BM17V	0.1	10.80 ± 0.61	5.72 ± 0.68	0.861	p<0.05
		PC	0.25	26.41±2.57	12.10 ± 1.83	0.9809	p=0.62
	WS	CP	0.05	4.96 ± 0.29	2.21 ± 0.14	0.9758	p<0.05
		TAA	0.1	*	*	*	*
		BM17V	0.1	7.26 ± 0.40	3.10 ± 0.63	0.7863	p<0.05
		PC	0.25	31.78 ± 2.71	15.35 ± 1.19	0.9942	p=0.08

(R) Denotes reference formulation (commercial equivalent formulation).

* Product not available at the local supplier.

** Two-tailed t-test (95% confidence interval) in comparison to commercial equivalent formulation.

+ Two-tailed t-test (95% confidence interval) in comparison to hydrophilic w/o marketed cream.

In general, the correlation coefficient of the plot of cumulative drug released per area in function of square root of time yielded values higher than 0.99, strongly indicating that the

drug release followed Higuchi model (Higuchi, 1961). The high coefficients obtained also a indicator of good reproducibility and reliability of the results. The experiments with anhydrous ointment yield to poorer correlation coefficients in comparison to the other vehicles. This can be explained by the very low amounts of drug released and detected in the acceptor medium, which lead to higher variability of the results.

Considering all test runs, two potential bases for drug delivery of topical corticosteroids arose: CG and HBC. They demonstrated the highest release rates for all investigated drugs. The mechanism of drug transport was discussed in details in section 7.5.5. Based on the present results, an important question was raised: would the higher drug release from these vehicles reflect on higher percutaneous absorption? This was one of the study questions further investigated in chapter 8.

7.5.5. Influence of base type on *in vitro* release of the model drugs

Among all tested vehicles, CG was imperatively the base which provided the fastest drug release for all investigated drugs. As previously discussed, the central mechanistic assumption for the faster release of bases was the favourable partition into acceptor medium due to higher drug thermodynamic activity and leaving potential in this vehicle.

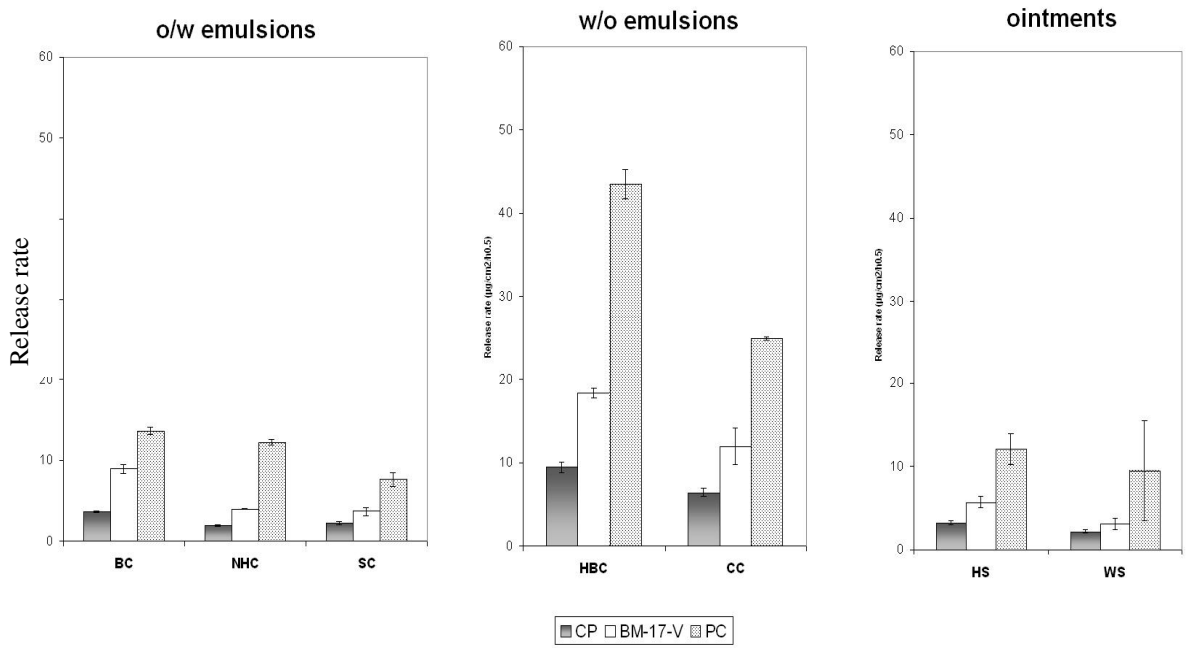


Fig. 7-5: Release rate of CP (dark bar), BM17V (white bar) and PC (grey bar) from o/w creams, w/o creams and ointments. The graph displays the mean of release rate from three Franz-cells (n=3) obtained from the plot of cumulative released drug in function of square root of time and SD.

Faster drug release was generally observed for w/o creams in comparison to o/w creams. The drug release rank followed CG (not presented in Fig. 7-5) > w/o creams > o/w creams/anhydrous ointment. Apparently, similar release rates were found for o/w creams and anhydrous ointments. In the group of o/w creams, BC was found to provide faster drug release whereas in the group of w/o creams HBC demonstrated the fastest release of drug. In order to understand the drug transport in these complex vehicles, a more detailed examination of the microstructure is necessary.

Several authors attempted to elucidate the governing factors which determine the rate of drug transport and release from creams. The simplest theory postulates that for systems where the oil phase is the intern phase, the drug transfer from the oily dispersed droplets into the external aqueous phase is the rate determining step (Madan, 1985; Friedman and Benita, 1987; Trotta *et al*., 1989). Thus, the drug diffusion through these creams involves the following events: a) partition of drug from dispersed oil phase in solution state into continuous aqueous phase; b) diffusion in the external aqueous phase; c) partition from external phase into acceptor medium. This is a simplistic model, which does not consider the effect of the surfactant film formed in the interface of dispersed droplets with external phase; influence of micelles in external phase on drug solubilization and transport; formation of gel phase and interlamellar fixed water. It has been already demonstrated that the liquid crystalline structure of the vehicle, particularly the interlamellar fixed water can act as a diffusional barrier and consequently controls the rate of drug transport (Tiemessen *et al.*, 1988). Depending on the nature and concentration of the surface-active agent and solvent, different types of aggregate structures can form different mixed crystals. Cubic, hexagonal lamellar and micellar phases can be observed in most cases, which differ from each other in their mechanical properties (Farkas *et al.*, 2000). The micelles formation and structures can strongly influence on drug solubilization state in the external phase. Based on first principles of diffusion, Goldberg *et al.* (1967) have derived equations to predict transport rates of micelle-solubilized drug from its aqueous environment to an oil phase. This physical model of drug transport included the effects of the free drug-solubilized drug equilibrium, the diffusion coefficients of both free and solubilized drug, ionic strength of the aqueous medium, and the micelle size. Ghanem *et al.* (1969), Ghanem *et al.* (1970) and Lostritto *et al*. (1987) were the first to consider the effect of the micellar phase on drug transport in emulsion systems. Yoon and Burgess (1998) postulated two mathematical models for the prediction of drug transport in triphasic (oil, water and micellar) emulsion systems as a function of micellar concentration.

Drug transport was later found to be dependent from concentration of cationic surfactants according to the drug lipophilicity (Yoon and Burgess, 1998). Chidambaram and Burgess (2000) extended this model to surface-active model drugs where the drug may compete with the surfactant for the interface and consequently can affect emulsion stability and the transport phenomenon of the model drug. In the present work, the effective diffusion coefficient of model drugs in emulsion systems seems to be controlled by several mechanisms acting sequentially and/or concurrently. The challenge, therefore, is not only to understand which factors influence on drug transport, but also among all these factors, which are dominating phenomena determinant for the rate of drug transport. Typically, an o/w cream consist of 4 phases, a hydrophilic gel phase, a lipophilic gel phase, an aqueous bulk phase and an internal dispersed lipophilic phase (Fig. 7-6). Therefore, the transport of corticosteroids in o/w creams can be represented as shown:

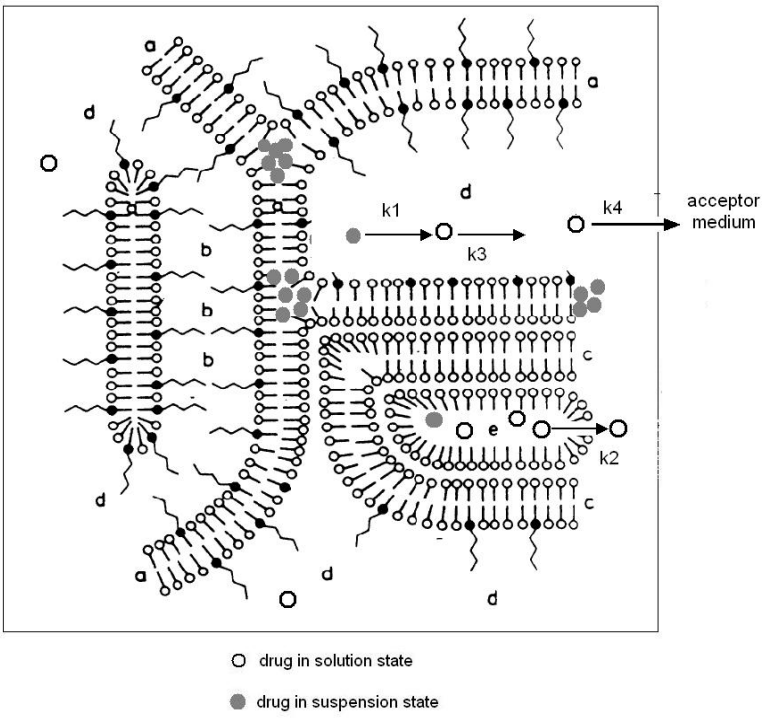


Fig. 7-6: Schematic illustration of drug transport in o/w creams and the possible determining factors which influence on transport rate. Adapted from Junginger (1984).

a) mixed crystals of non-ionic o/w.
b) interlamellar fixed water (hydrophilic gel phase).
c) lipophilic gel phase.
d) bulk water.
e) lipophilic dispersed phase.

- k1 represents the dissolution rate of drug in suspended form. This rate is mainly controlled by the nature of the external phase.
- k2 represents the transport from oily internal droplets to aqueous external phase. The rate is governed by drug partition between these phases and nature of interfacial film formed (Friedman and Benita, 1987; Chidambaram and Burgess, 2000). Since the partition from internal phase is favoured by drug solubility in the external phase, the components in this aqueous external phase are assumed to play an important role on drug transport.
- k3 depicts the drug transport in the external aqueous phase. As described above, this rate is assumed to be controlled by diffusion and solubility in this phase, which is directly related to nature of phase and interlamellar fixed water.
- k4 represents the drug release from vehicle to acceptor medium. It is assumed to be controlled by the partition from the external phase into the acceptor medium as discussed in chapter 6.

In the group of o/w creams, the release rank order followed the same for all investigated drugs: BC > SLC > NHC. As pointed out by other authors, the amount of water in the external phase is one of the governing factors on drug release (Refai, 2001). For lipophilic drugs, a lower water volume is thought to increase the release rate by decreasing the diffusion pathway. The three investigated o/w creams differ in their aqueous phase: NHC (50%), SLC (59%) and BC (50%). Indeed, the results corresponded to this theoretical expectation. The drug release was found to be higher for BC with less water content in the external phase. However, the regression analysis of the plot of release rate in function of water content demonstrates a poor correlation (r^2= 0.8745), suggesting that water concentration in the external phase is not exclusively the determining factor for the drug release. As previously discussed, the nature of oil and aqueous phase is possibly one of the variables influencing on drug transport. Omotosho *et al.* (1989) found that release rate of methotrexate was related to hydrophobic component of o/w creams. The nature of the oil phase reflected on the increasing internal droplet size of the creams, influencing on drug transport. BC, SLC and NHC differ quantitatively and qualitatively in their oil phase: BC (medium chain triglycerides 7.5%, white soft paraffin 25.5%, cetyl alcohol 6%); NHC (white soft paraffin 25%, cetostearyl alcohol 10%), SLC (dimeticone 350 5%, decyl olate 5%, hard parafin 10%). The solubility in these two phases (aqueous and oily) determine the partition of the drug. The drug solubility in the oily phases is experimentally too complex to be assessed and it is beyond the scope of the

present study questions. However, a noticeable difference can be observed in the content of aqueous phase of the bases. BC contain 10% of PG, whereas SLC 2% and in NHC this excipient is absent. The enhancement of the solubility in water with aid of PG has been previously documented for corticosteroids (Kasongo *et al.*, 2007). The higher concentration of PG in the aqueous phase of BC may enhance drug solubility which consequently favours the drug partition and diffusion. The effect of micelles formation was also previously outlined. The lowest release rate observed for SLC can be partially influenced by the concentration and nature of the surfactants in this vehicle.

The mechanism of the drug transport from w/o creams is apparently determined by the following (Fig. 7-7):

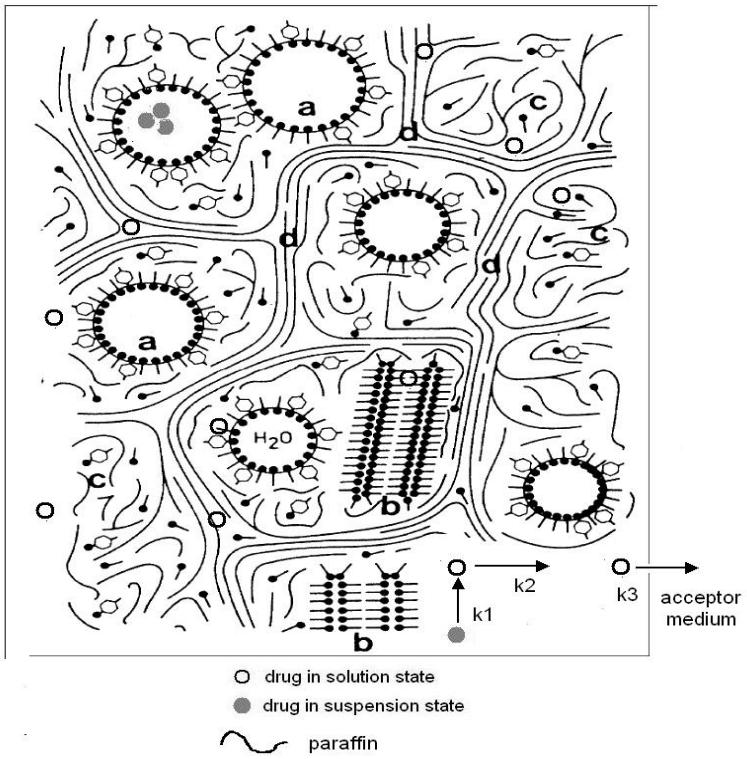


Fig. 7-7: Schematic illustration of drug transport in w/o creams and the possible determining factors which influence on transport rate. Adapted after Junginger (1984).

In these systems, lipophilic drugs are expected to be found in the external oily phase and freely diffuse. The drug partition into internal aqueous droplets and micelles considered to be thermodynamically unfavourable. Therefore, the rate of transport can be expressed as:

- k1 represent the dissolution of the drug in suspended state and it is dependent on the nature of the oily external phase.
- k2 shows the rate of diffusion in the continuous phase, which is mainly governed by interaction of drug with components of this phase influencing on drug solubility and diffusion.
- k3 represents the release into acceptor as previously illustrated for o/w creams.

The drug release from HBC was found to be higher in comparison to CC for all investigated drugs. Both w/o bases present qualitative and quantitative differences in the external phase and the drug is assumed to be mainly in suspended phase. The most noticeable difference is the volume of external phase. The oily components in the continuous phase represent only 30% in HBC whereas 75% in CC. This presumably implies to a higher concentration of drug in the external phase of HBC. The assumed higher drug concentration in the continuous phase of HBC leads to a higher thermodynamic potential. As previously discussed, thermodynamic potential is one of the key factors which dictate drug release, explaining the higher release rates.

7.5.6. Relationship between formulation viscosity and drug release

Conclusions on drug release have been often drawn based on viscosity data. Less viscous bases are expected to provide less resistance to drug transport and diffusion and consequently higher release rates. However, this relationship is more complex since other factors also play an important role in the drug diffusion. According to Einstein-Stokes equation, the diffusion coefficient of a particle becomes inversely dependent from viscosity:

Eq. 15

$$D = \frac{KT}{6\pi\eta r}$$

Where D is the diffusion constant, K is the Bolzman constant, T absolute temperature, η viscosity and r radius of molecule.

The viscosity of the gel matrix may play an important role in controlling the release of the drug into the receptor compartment when the drug diffusion through the gel matrix is a rate determining step (Arellano *et al*., 1998). Several papers have attributed faster drug release to lower macroviscosity (Al-Khamis *et al.,* 1986; Chi and Jun, 1991; Rafiee-Tehfani and

Mehramizi, 2000). However, considering current published data this relationship still remains contradictory. Refai (2001) found despite the significant difference in their viscosity, similar release rates of hydrocortisone from NHC and water-containing ointment. Sanna *et al*. (2009) found that the permeation of ketoprofen across two different gels with identical macro viscosity/ rheological properties provided despite their rheological parity, almost double the amount ketoprofen permeated through the cellulose gel in comparison to the carbomer gel.

In the present work, in the group of ointments, HS demonstrated a slightly higher release rate in comparison to WS, although this base has higher complex viscosity. WS is a lipophilic base consisting mainly of paraffin (93.5%), in which the wool fatty alcohols are partly suspended and partly dissolved. HS contains beside paraffin, cetostearyl alcohol together with sodium cetostearyl alcohol sulfate. This builds a mixed crystal forming a three-dimensional network in which paraffin is immobilized (Junginger, 1984; Niedner and Ziegenmeyer, 1992). The solubility of the model drugs is thought to be higher in HS, as already observed for hydrocortisone (Refai, 2001), which explain the higher release. In the group of o/w creams, NHC demonstrated similar drug release in comparison to SLC, despite its clearly higher values of viscosity. A possible explanation is the lower water content in this base in comparison to SLC, which facilitate the drug transport compensating the viscosity. Presumably, the pharmaceutical ingredients present in NHC may also enhance the solubility of the drugs and consequently the diffusion. These results strongly suggest that bulk viscosity does not dictate drug release. The viscosity does not account for interactions between active and excipient. Macroviscosity is an aesthetic property which does not consider the microstructure of the vehicles, particularly physicochemical interactions of drug and vehicle determinants to solubility and diffusion. The influence of microviscosity (microstructure) on drug release stills remains to be elucidated.

7.6. Summary and conclusion

The effect of representative dermatological vehicles covering the group of hydrogels, o/w creams, w/o creams and anhydrous ointments on the release of the model drugs was investigated. It was aimed to provide a better insight into the mechanistic processes involved in the drug transport from vehicles with different physicochemical features, macro and microviscosity. CG has demonstrated the fastest release for all investigated drugs, followed by w/o creams. In general, similar release rates were observed for o/w creams and water-free ointments. Different drug transport processes are involved for each particular group of vehicle. It is thought that hydrogels provide higher thermodynamic activity for lipophilic

drugs and favourable partition into acceptor medium. The transport of lipophilic molecules in o/w creams seems to be more complex process and comprises several steps sequentially or concurrently. The rates of drug partition from internal oily phase into external aqueous phase and diffusion therein, are presumably the key kinetic rates determining overall transport. They are affected mainly by the nature and volume of components in the external phase as water and/or cosolvents dictating diffusion coefficient and pathway. The transport in w/o creams is assumed to be mainly determined by the nature and volume of the external phase as well. These creams provided higher release due to the free transport of lipophilic drugs in the external oily phase. In the group of anhydrous formulations, drug diffuses freely in the homogeneous lipid phase. The solubility of lipophilic drugs in these vehicles are expected to be higher in comparison to o/w creams, however, an unfavourable partition from these vehicles into acceptor medium is thought to be the limiting factor for drug release.

Commercial products belonging to the same formulation class were employed for statistical comparisons and evaluation of drug release. Since the drug transport in different vehicles is mechanistically complex, the comparison of release data from test vehicle to those from marketed products belonging to the same formulation type represents a more rigorous and level-headed evaluation. Consistent results demonstrated that CG and HBC were the vehicles which presented fastest drug release. One may empirically assume that a faster release determine a higher percutaneous absorption. However, conclusions to drug bioavailability based on release data were until this part of the study avoided. The relationship between release rate assessed by a synthetic membrane and *in vitro* drug bioavailability using *ex vivo* human skin was one of the study questions of chapter 8.

In conclusion, the present *in vitro* release test can be seen as a useful tool to investigate the different events involved in the drug transport in semisolid vehicles aiding to step with more rigour and less empirical approaches to costly bioequivalence tests.

8. *In vitro* biopharmaceutical characterization

8.1. Introduction

In vitro biopharmaceutical characterization of topical and transdermal semisolids involves the investigation of factors governing drug uptake, distribution, retention and permeation through the skin. Conclusions of bioavailable drug delivered topically have been frequently drawn based on drug quantified in urinary excretion and/or blood (Wester *et al.,* 1977, Melendres *et al.,* 1992; Shah *et al.*, 1993). Data yielded from urinary excretion or blood are only means for bioavailability evaluation when the pharmacological response is correlated to measure of systemic bioavailability (of the topical application) and not to local bioavailability (Pellanda *et al.,* 2006). Systemic drug concentrations after topical application do not represent drug concentrations at the target site in the skin but drug concentrations after permeation through the target site, giving unrealistic estimates of topical treatment. A similar topical bioavailability within the SC does not necessarily imply a similar systemic bioavailability (Pellanda *et al.,* 2006). Therefore, *in vitro* tests to characterize biopharmaceutical properties of formulations have to be critically designed considering the therapeutic purpose of the product. If the transdermal assessment is desired, the focus is on the rate of transport across the skin without looking at processes inside the skin barrier. It is equally important for evaluating potential side effects that may result from systemic absorption of topically applied drugs. In contrast, dermal delivery experiments should be focused on analysis of drug in the target site in the SC and deeper skin layers (DSL).

The first reseach question of the present chapter was addressed to the examination of the effect of three different vehicles on the extent of drug absorption, distribution and retention in the skin. The relationship between drug release assessed by synthetic membranes (described in chapter 7) and percutaneous absorption assessed with *ex vivo* human skin was evaluated and discussed. Ultimately, the influence of a novel of pharmaceutical excipients on drug transdermal permeation and skin retention was examined. The uselfulness of experiments sampled with tape-stripping technique into an integrated development program for semisolids was discussed.

8.2. Materials and methods

8.2.1. Test formulations and chemicals

Dermoxin® ointment and Kortikoid-Ratipharm® ointment were employed as reference products. TAA and CP were formulated into CG and HBC according to the strength of the reference formulations. Diethylene glycol monoethyl ether (DEGEE) or Transcutol® and the chemicals used for the preparation of buffers were purchased from Merck KGaA (Darmstadt, Germany). Dodecylamine (DDA), Polysorbate 80, DMSO and Igepal® were acquired from Sigma-Aldrich (Hannover, Germany).

8.2.2. Skin preparation

Excised human skin was obtained from Caucasian patients, who had undergone abdominal plastic surgery. Skin was collected as soon as possible after surgery and kept at 4 ºC during the transport. The preparation of skin followed the recommendations of current guidelines (OECD, 2004a). Care was taken to ensure that the subcutaneous fatty tissue did not get into contact with the surface of the skin. Once in the laboratory, the skin used in the experiment was separated from the subcutaneous fatty layer which was removed within two hours after reception of the abdominal skin. Once separated from the subcutaneous fatty tissue, the residual full-thickness skin was stored at -20 °C. Skin from the same donor was used for each experimental setup in order to avoid inter-individual variations.

8.2.3. Determination of drug solubility in acceptor media

Saturated solubility of TAA and CP in PBS, KRB, HBSS was determined using the scaled-down method described in 3.2.3. The equilibration followed over 24 hours at RT at 200 rpm of stirring rate. Additionally, the effect of several pharmaceutical solubilizers was investigated.

8.2.4. Assessment of drug absorption, distribution and retention

On the day of the experiment, skin samples were thawed at RT, and split-thickness skin membranes were prepared with an electric dermatome (Aesculap GA 630, Tuttlingen, Germany). Skin samples were fixed on a dissection board, epidermal side up, and sections were cut at 400–500 µm depth, including the epidermis and the upper part of dermal tissue. The dermatome was applied to the skin surface with intact SC uppermost. The dermatomized skin was punched out (1.33 cm^2) and the thickness was measured with a Heidenhain thickness gage at five different places on each skin membrane. The skin membranes were mounted with

static vertical diffusion Franz-cells (PermeGear, USA) in such a manner that the dermal side of the skin was exposed to the receptor fluid and the SC was exposed to upper part where the formulation is applied. The cells have diffusion area of 2.7 cm^2 and receptor compartment volume of 20 mL. The advantage of using a static system is the increase in sensitivity for test substances, which poorly penetrate the skin (SSCP, 2006). Due to poor water solubility of the drugs, receptor fluid (HBSS buffer) was supplemented with 0.1% of polysorbate 80. The test formulations were applied (80 mg, infinite dose) and uniformly spread with spatula. The donor compartment was occluded with Parafilm®. The diffusion cells were placed in water bath with horizontal agitation (magnetic stirrers at 400 rpm) assuring the homogeneity of receptor fluid and thermostated at 32 ± 2 ºC. Four replicates were used for the study. After 24 hours of incubation time, samples (200 µL) from the receiving medium were collected in order to assess the cumulative permeated drug amount representing the drug systemic exposure. The residual formulation on skin surface was rinsed twice with 100 µL of extraction medium (acetonitrile/water 60:40 v/v, %) and the diffusion cells were carefully dismantled. The skin was pinned on a piece of isopor solid surface for sampling with tape-strip technique.

8.2.5. Sampling with tape-stripping technique

Adhesive tapes (Type 4129, width 25 mm, Beiersdorf, Hamburg, Germany) were used for the removal of the SC. For removing in standardized manner, a solid Teflon weight (500 g) was placed on the tape-strip/skin membrane for supplying a constant pressure during 10 seconds and then removed rapidly. The first two strips were discarded because it may be contaminated with residual drug from the formulations on the skin surface and they were not taken into account for the assessment of drug local bioavailability. Ten further tape-strips were used to remove the drug located in the SC as described elsewhere (Tsai *et al.,* 1999; Müller, 2003; Stelutia *et al.,* 2005). The tapes were combined in 2 pools of 5 strips for analytical purposes. After stripping, the residual skin was punched with 1.33 cm^2 area. This residual skin was sliced with scissors and the drug was extracted with acetonitrile/water (60:40, v/v) by stirring at 200 rpm over 24 hours. This represented the drug located in the DSL. Prior the performance of the experiments, the method to extract drug from skin tissues was tested in duplicate in preliminary studies using a lipophilic base (HBC) containing 0.5% of drug as donor following incubation of 6 hours with skin mounted with Franz-cell.

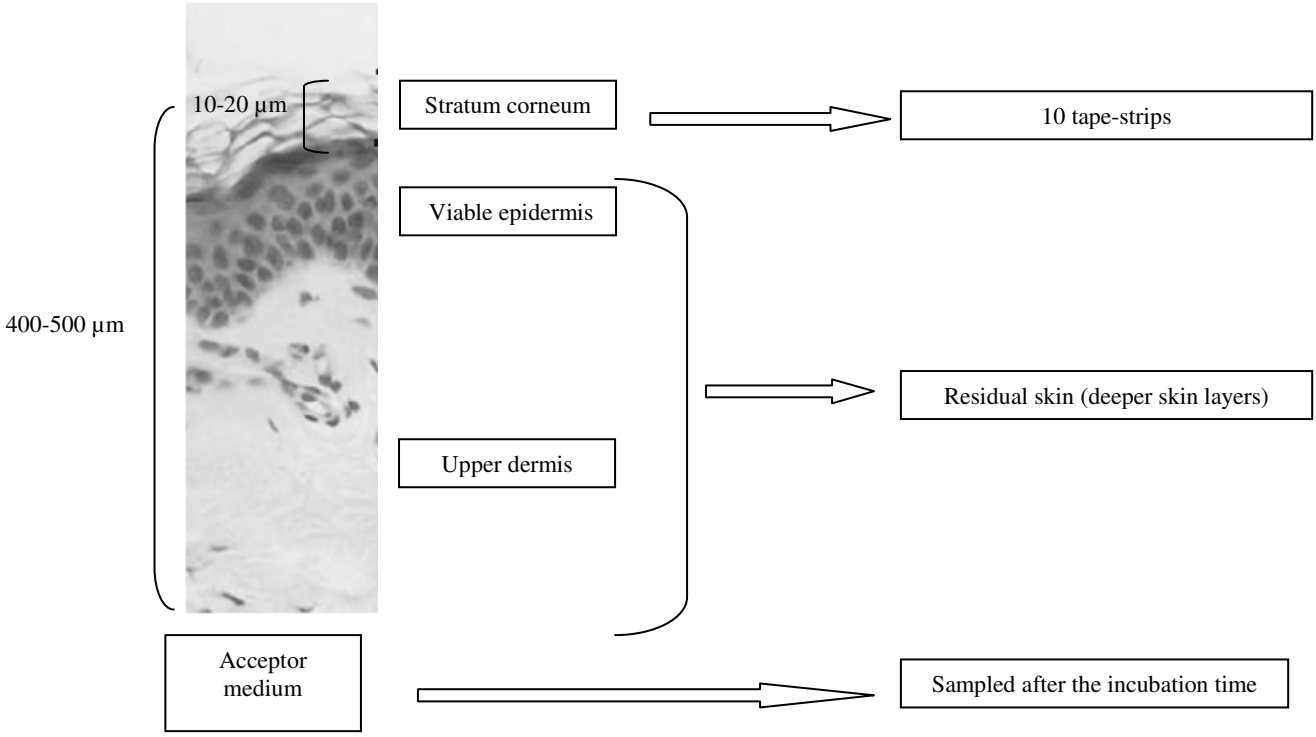


Fig. 8-1: Schematic illustration of sampling during performance of assessment of drug absorption, distribution and retention.

8.2.6. Skin quality control

As the barrier function of human skin is influenced by many factors such as age, thickness of the SC or the content and composition of the dermal lipids, skin specimens from different donors naturally exhibit variable permeability properties. To verify the skin's barrier properties of the specimens, they were tested by measuring the transport of the low-permeability marker compound caffeine (SCCP, 2006). The marker was applied as an aqueous solution (donor volume: 1 mL, donor concentration: 10.00 $mg \cdot mL^{-1}$, infinite dose) and remained on the skin throughout the whole study period. The apparent permeability coefficient determined in the present study was compared to the caffeine P_{app} measured using dermatomized skins from different donors (Bock *et al.*, 2002).

8.2.7. Effect of permeation enhancers

This experimental part followed similarly as described in 8.2.4. Differently, skin samples were mounted with Franz diffusion cells and pre-treated by adding 20 µL of each permeation enhancer on the skin surface (n=3). After the incubation of 2 hours at 32 ± 2 °C, excess of substance was gently removed with cotton swabs from skin surface. Solution saturated with

CP in MeOH/water (50:50, v/v) was applied (400 µL) and incubated over 23 hours. The application of a saturated solution with the drug ensured the same thermodynamic activity for all the samples. In order to evaluate the permeation kinetics of the drug, samples were collected (200 µL) from acceptor chamber at determined time points (after 0.5, 2, 4, 15, 18, 21 and 23 hours). Skin pieces were sampled as described in 8.2.5.

8.2.8. Data processing and statistics

The assessed drug amount in the SC and DSL (viable epidermis and upper dermis) were interpreted as local drug bioavailable as suggested by other authors (Borsardia *et al.,* 1992; Wagner *et al.,* 2002). The cumulative drug amount in the receptor fluid after the incubation time was interpreted as systemic bioavailable drug. The results were expressed in percentage related to the total drug amount applied and expressed as absolute drug amount per skin area (µg/cm^2) as recommended (OECD, 2004a). Statistical differences were assessed by two-paired, Student's t-test with 95% of confidence interval and by one-way ANOVA. Transdermal permeation enhancement was expressed as the ratio of the flux value with enhancer to that obtained without enhancer:

Eq. 16

$$ER = \frac{Flux_{enhancer}}{Flux_{control}}$$

8.3. Results and discussion

8.3.1. Effect of solubilizers on solubility of drugs in acceptor medium

One of the major concerns during the performance of *in vitro* experiments is the limited solubility of the tested drug in the employed acceptor medium. The utilization of static-vertical Franz-cells provide on one hand more sensitivity for poorly permeable substances, but on the other hand the need of ensuring adequate drug solubility to not inhibit absorption (SCCP, 2006). In the present study, all model drugs are practically insoluble. Although some guidances point out the utilization of ethanol/water as acceptor medium (OECD, 2004a) for poorly soluble drugs, ethanol may compromise the skin integrity barriers in experiments with long duration. Strategies to improve drug solubility in acceptor medium without compromising skin barrier are, therefore, necessary. In this part of the study, the solubility of two model drugs (TAA and CP) and the influence of several solubilizers on solubility in

aqueous buffer was investigated. Since considerable cutaneous degradation of PC was demonstrated in preliminary experiments, this model drug was withdrawn in this phase of the development program. Skin permeation experiments with topical drugs with high susceptibility to skin metabolism require a particular approach for quantification and evaluation of all metabolites. Therefore, the confirmation of cutaneous degradation of PC leading to several metabolites represented the endpoint for a no-go decision on further development. The solubility results of TAA and CP in common utilized acceptor media are presented in Tab. 8-1.

Tab. 8-1:Results of the determination of the saturated solubility of TAA and CP in commonly employed acceptor media for *in vitro* skin experiments (n=3).

Buffer system	**Solubility (µg/mL)**	
	TAA	**CP**
Phosphate	13.78 ± 0.36	*
HBSS	16.11 ± 0.24	*
PBS	14.69 ± 0.20	*
KRB	14.85 ± 012	*

* Values under the analytical lowest limit of quantification.

The solubility of CP was below the lowest limit of quantification of the analytical method. The limited solubility of CP in buffer systems is already recognized (Kaca, 2007). The absolute solubility values found for TAA are in congruence with the assessed solubility in water using the *in silico* model in the section 3.3.3. Limited solubility in medium can yield to underestimated results to systemic exposure. In order to overcome this problem, the effect of commonly used pharmaceutical solubilizers on the solubility of CP and TAA in HBSS buffer was investigated (Fig. 8-2).

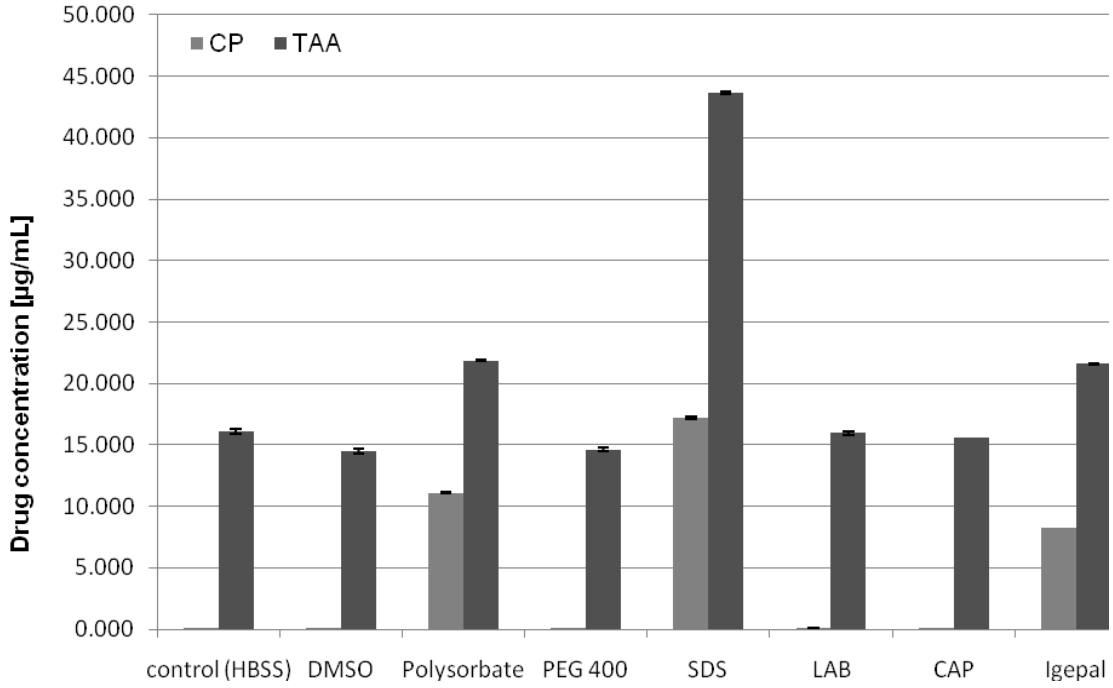


Fig. 8-2: Saturated solubility of TAA and CP in HBSS buffer (control) with DMSO, polysorbate 80, polyethylene glycol (PEG 400), sodium docecyl sulfate (SDS), Labrasol® (LAB), capryol (CAP) and Igepal® at 0.1%. In the graph the mean of three determinations (n=3) and SDs are presented.

The pharmaceutical surfactants have clearly demonstrated higher solubilization power in comparison to the cosolvents employed. Polysorbate 80, SDS and Igepal® increased significantly the solubility of TAA and CP in HBSS buffer (t-test, $p<0.05$). The rank of solubilization followed: SDS > polysorbate 80 > Igepal®. Quantifiable amounts of CP were obtained with the employment of SDS (17.20 ± 0.08 µg/mL),polysorbate 80 (11.10 ± 0.03 µg/mL) and Igepal® (8.25 ± 0.02 µg/mL). These three ingredients also significantly enhanced the solubility of TAA in HBSS buffer. At the concentration of 0.1%, all three surfactants are above of their critical micelle concentration (CMC) (Bailey and Dorsey, 2001; Hillgren *et al.,* 2002). The group of cosolvents (DMSO, PEG 400, Labrasol® and Capryol®) did not contribute significantly to the increase of solubility of both drugs due to the low concentration employed.

In the design of skin experiments, the limited solubility of the tested drug in the employed acceptor medium can be critical. A low drug amount detected in the receptor medium due to

limited solubility (and not due to limited permeation) can lead to false conclusions and underestimated results. Strategies to rapidly identify suitable solubility enhancers without compromising the skin barrier are necessary. Guidelines for experimental studies of percutaneous penetration prescribe the employment of optimal barrier integrity of the skin and the utilization of solvents at high concentration should be avoided (SCCP, 2006). In this experiments a low concentrations of solubilizers was preferred to avoid possible effect of the chemicals on the skin structure. With a rapid and simple screening of chemicals, the suitable solubilizers could be identified.

8.3.2. Influence of vehicles on drug absorption, distribution and retention in the skin

In this part of the study, the influence of topical vehicles on percutaneous absorption and distribution in skin compartments was investigated. Three representative vehicles possessing different physicochemical properties have been chosen: CG, HBC and commercial water-free ointments. CG and HBC arose in the experiments described in chapter 7 as the bases which presented fastest drug release. Commercial ointments containing TAA (0.1%) and CP (0.05%) were utilized as reference formulations, since their pharmacodynamic properties were already characterized. One of the important questions addressed in this part of the study was not only the investigation of the influence of different vehicle on *in vitro* drug bioavailability, but also the relationship between drug delivery assessed by synthetic membranes and drug (trans)dermal delivery assessed by *in vitro* experiments using excised human skin. Fig. 8-3 shows the results of drug absorption, distribution and retention.

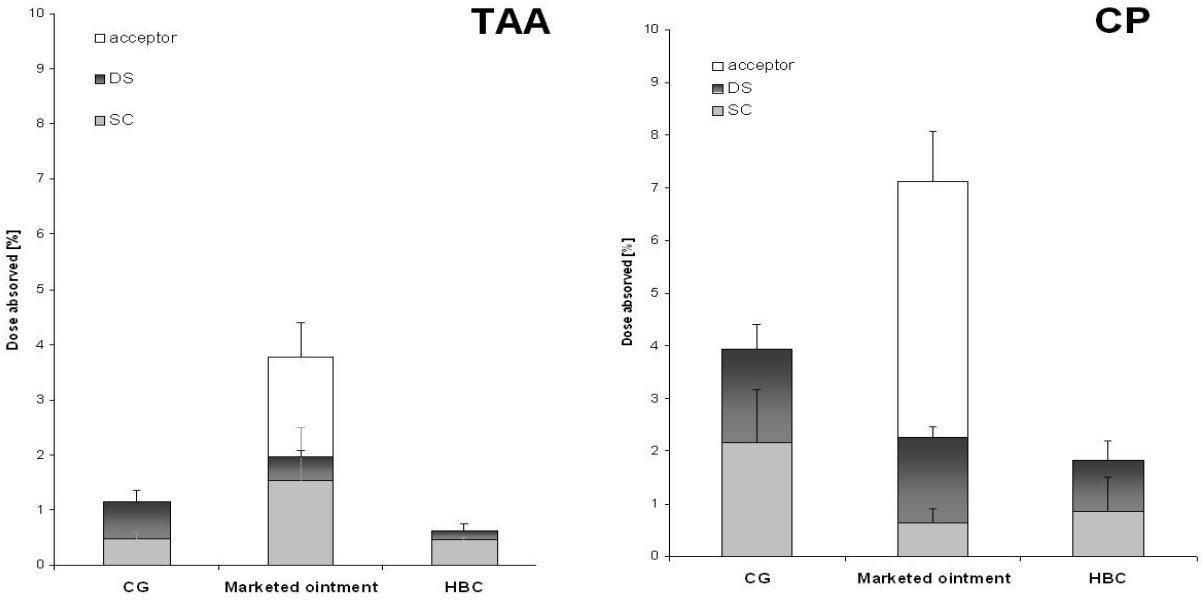


Fig. 8-3: Results of assessment of drug absorption and distribution in the SC (gray bars), DSL or residual skin (dark bars) and acceptor medium (white bars) after topical application of bases formulated with TAA (0.1%) and CP (0.5%); and marketed ointments (Dermoxin® and Ratiopharm® ointments). The graph displays the mean of four determinations (n=4) of dose absorbed and error bars

The total drug absorbed taking into account SC, DSL and acceptor medium were statistically different (ANOVA, $p<0.05$), which strongly indicates the influence of the vehicle on drug uptake in the skin. The rank has followed Kortikoid-Ratiopharm® ointment > CG > HBC. After application of ointment, 3.78% of TAA from the applied dose was found in the SC, DSL and in acceptor medium after 24 hours, whereas 1.15% of drug was found in the SC and DSL after application of CG and 0.63% for HBC. No quantifiable drug was found in the acceptor medium after application of CG and HBC. Similar results were obtained for the formulations containing CP. In agreement with results of TAA, the absorption rank Dermoxin® ointment > CG > HBC was observed. After 24 hours, 7.12% of drug was found distributed between the SC, DSL and in acceptor medium, whereas 4.18% and 1.86% were found after application of CG and HBC, respectively. Quantifiable amount of drug in the acceptor medium indicating systemic exposure could be found only for the marketed ointment, which is also in good agreement the experiments with TAA.

The influence of vehicles on drug penetration and retention in the skin is already acknowledged (Coderch *et al.,* 1996; Tsai *et al.,* 1999; Wagner *et al.,* 2001; Jacobi *et al.,*

2003; Jiménez *et al.,* 2004). Ratiopharm® and Demoxin® ointments noticeably demonstrated the greater drug absorbed in the skin layers. It has been reported that oily components present in ointments exert their effect on skin function by forming an inert, epicutaneous, occlusive membrane (Ghadially *et al.,* 1992). The occlusive film reduces transcutaneous water loss, trapping water under the skin's surface enhancing the permeation of drugs (Cornell and Stoughton, 1985). Hydration of SC can lead to profound changes to its barrier properties. The effect of water on the skin is due to the combination of the swelling of hydrated corneocytes and the water-induced expansion of the intercellular lipid lamellae. The dead cells that make up the SC are dry and desquamated. In addition, Elias *et al.,* (2002) consider the presence of an aqueous pore pathway in the SC, consisting of lacunar domains (sites of corneodesmosome degradation) embedded within the lipid bilayers. Although scattered and discontinuous under normal physiological conditions, they suggest that under high stress conditions (such as extensive hydration) the lacunae expand, interconnect and form a continuous ‘‘pore pathway’’ (Elias *et al.,* 2002). The formation of such a route would markedly enhance drug penetration. Other important factor to be considered is the uptake of excipients from the ointment into the SC in sufficient quantities to alter the solubility and diffusivity of drugs in the skin barriers. The accumulation of paraffin from ointment formulation in the intercellular lipid domains of the SC may favors the solubility and diffusivity of TAA and CP in these structures. Therefore, retention for a longer time or facilitation of drug permeation can take place. This behavior functioning as “diffusivity enhancer” was already observed for Transcutol® (Godwin *et al.,* 2002) and propylene glycol (Bendas *et al.,* 1995; Herkenne *et al.,* 2008).

8.3.3. Influence of vehicle on local drug bioavailability

The assessed drug retention/accumulation taking into account the drug amount in the SC and DSL was also found to be significantly different between the test formulations containing either TAA or CP (ANOVA, $p < 0.05$), indicating the influence of the vehicle not only on the drug permeation but also on the drug retention in the skin. For all tested vehicles, a considerable drug amount could be found retained in the SC and deeper skin layers, indicating the formation of a drug depot or “reservoir” in these skin compartments (Tab. 8-2).

Tab. 8-2: Summary of results of vehicle influence on distribution and retention of CP and TAA in the skin (n=4).

	CP		
Compartment	**Drug amount (μg/cm²)**		
	CG	ointment	HBC
SC	0.25 ± 0.15	0.10 ± 0.06	0.14 ± 0.09
DSL	0.08 ± 0.06	0.19 ± 0.08	0.10 ± 0.07
receptor	*	0.67 ± 0.20	*
SC + DSL	0.39 ± 0.14	0.29 ± 0.02	0.10 ± 0.06

	TAA		
Compartment	**Drug amount (μg/cm²)**		
	CG	ointment	HBC
SC	0.15 ± 0.01	0.32 ± 0.11	0.11 ± 0.03
DSL	0.19 ± 0.05	0.12 ± 0.03	0.05 ± 0.03
receptor	*	0.51 ± 0.19	*
SC + DSL	0.34 ± 0.05	0.55 ± 0.28	0.16 ± 0.04

* Values under the analytical lowest limit of quantification.

SC: *stratum corneum*.

DSL: deeper skin layers.

SC + DS: represents the local bioavailable drug.

The term “reservoir” referred to the skin, can be defined as an accumulation of a topically applied compound within the skin or within a particular skin layer for a longer time period (Hadgraft, 1979). The reservoir can be formed not only in the SC, but also in the viable avascular tissue in the DSL (viable epidermis and supracapillary dermis) and in the dermis (Hadgraft, 1979). This phenomenon is frequently desired and it is an important determinant of the duration of action of a topical drug (Roberts *et al.,* 2004). After stripping the SC, the drug in the remaining skin residue was quantified. This samples represented the drug in the viable dermis and supracapillary (avascular) dermis, which are the target local of action of topical corticosteroids. The active drug permeates epidermal and dermal cells membrane reacting with receptor proteins in the cytoplasm to form a steroid-receptor complex, producing the anti-inflammatory, immunosuppressive and anti-mitogenic effects participating in the treatment of proliferative and inflammatory skin diseases (Kragballe, 1989). *In vivo* situations, the drug accumulation can be mainly determined by a slow removal rate by the

capillary network. Vasoconstriction produced by corticosteroids slows down this removal rate and it is this effect causing the maintenance of a depot in the SC (Handgraft, 1979). In the context of *in vitro* experiments, the depot formation is mainly explained by the partition of the compound into a specific skin compartment (e.g., into the intercellular lipids of the SC) and subsequent slow release into deeper compartments (Roberts *et al.,* 2004). Secondly, a temporary binding to specific skin structures (e.g., to keratin, proteins, amino acids, collagen) may occur, discontinuing temporally the diffusion along deeper compartments (Walter *et al.,* 1988, Rougier *et al.,* 1983)

8.3.4. Evaluation of permeability information obtained from *in silico* assessment

The evaluation of drug permeability in the skin with *in silico* model was described in chapter 3. The results revealed a higher permeability coefficient of CP in comparison to TAA. Experimentally, higher absorbed doses were found for formulations containing CP in comparison to the ones containing TAA. The early *in silico* assessment was useful to give a first insight into the skin permeability of the model drugs, however, it was not intended to accurately predict its kinetic results. The model postulated by Potts and Guy (1992) was based on the data available from drugs in aqueous solution, which does not consider the influence of the semisolid vehicle on the skin permeation. It can be concluded that the usefulness of application of *in silico* tools to assess drug permeation lies only on the achievement of a qualitative permeation rank enabling an early classification of the candidate drugs into “low” and “high” permeable, but not accurate assess the kinetic permeation, which depend on more complex estimations.

8.3.5. Relationship between drug *in vitro* release rate and percutaneous absorption

The results presented in chapter 7 revealed that CG and HBC were the vehicles which presented the fastest drug release. It seems to be logical that vehicles that provide faster drug release provide higher skin permeation. However, this relationship is not so straightforward. The current published data are still controversial. One of the underlying research questions of this chapter was the investigation of the relationship between *in vitro* release rate employing synthetic membrane and the assessed drug bioavailability using human skin. The rank of release and skin experiments is presented in Tab. 8-3.

Tab. 8-3: Rank of results from *in vitro* release and skin experiments.

Model drug	Rank of release experiments	Rank of skin experiments*
TAA	CG > HBC > ointment	ointment > CG > HBC
CP	CG > HBC > ointment	ointment > CG > HBC

*The total dose absorbed (%) was taken into consideration.

The highest drug release rate was observed for CG followed by HBC and the marketed ointment. The mechanistic factors for this faster drug release were in detail discussed in chapter 7. However, the release rank did not directly correlate to the absorption rank obtained from the skin experiments. Taking into consideration the total drug bioavailable (SC, DSL and acceptor medium), the marketed ointments presented higher absorption rates, followed by CG and HBC. It is clear that synthetic membrane can not represent the complex physiological structure of the skin. The occlusive effect exerted by oily ointments seems to surpass the release rate and be the dominating mechanism which determines overall transport of lipophilic drugs from semisolid vehicles. Therefore, *in vitro* release test can be not adequately applied to assess drug absorption.

8.3.6. Relationship between *in vitro* bioavailability and *in vivo* response

Potency of corticosteroids is determined by complex factors of both the drug and its vehicle. The dependence of the pharmacological response relies on the drug chemical structure, concentration in the vehicle, type of vehicle (Cornell and Stoughton, 1985). Potency rating was proposed to evaluate topically applied corticosteroids based primarily on the pharmacodynamic response determined by the ability to produce vasoconstriction of the microvasculature of the skin, leading to skin blanching (whitening) at the site of application (Cornell and Stoughton, 1985). The vasoconstrictor assay involves applying topical corticosteroids to the normal skin surface of healthy volunteers and observing the ability to induce blanching and the length of their action at the application site anti-inflammatory effect of the topical corticosteroid and the results. Vasoconstrictor test is recognized as good predictors of efficacy in clinical trials (Cornell, 1992). By assessing skin blanching, the delivery of the active agent through the skin barrier, intrinsic activity at the receptor and the rate of clearance from the site of application can be evaluated (Kirkland *et al.*, 2006).

Tab. 8-4: Potency classification of topical corticosteroids (adapted after the National Psoriasis Foundation, http://www.psoriasis.org, retrieved on 03/24/10).

Classification	Product	Drug
Potent	Cyclocort Ointment, 0.1%	Amcinonide
	Diprosone Ointment, 0.05%	Betamethasone dipropionate
	Florone Ointment, 0.05%	Diflorasone diacetate
Upper Mid-Strength	Cyclocort Cream/Lotion, 0.1%	Amcinonide
	Diprosone Cream, 0.05%	Betamethasone dipropionate
	Florone Cream, 0.05%	Diflorasone diacetate
	Aristocort A Ointment, 0.1%	**TAA**
Mid-Strength	Cutivate Ointment, 0.005%	Fluticasone propionate
	Aristocort Cream, 0.1%	**TAA**
Lower Mid-Strength	Cutivate Cream, 0.05%	Fluticasone propionate
	DesOwen Ointment, 0.05%	Desonide
Mild	DesOwen Cream, 0.05%	Desonide

Topical corticosteroids at the same strength formulated in different bases may show different skin blanching results and fall into different potency classification (Wiedersberg *et al.*, 2008). Different formulations of the same steroid can shift the position of the 'dose–response' curve by either enhancing or retarding the permeation of drug into the skin (Lippold and Schneemann, 1984). The results of *in vitro* evaluation of different test formulations have shown higher dose absorbed from ointment in comparison to the other investigated vehicles. These findings are in good agreement with the potency classification of topical corticosteroids demonstrating the usefulness of the test for giving predictive estimates of vasoconstriction activity and, thereby, estimations of clinical efficacy.

8.3.7. Effect of pharmaceutical excipients on drug permeation

Over several decades, investigators have been focusing the research on overcoming the skin barrier, mainly for treating patients topically but occasionally also for systemic therapy (Langer, 2004; Karande and Mitragotri, 2009). Penetration enhancers are the pharmaceutical ingredients that facilitate the absorption of drug through the skin by temporarily diminishing the impermeability of the skin (Sinha and Kaur, 2000). More than 300 chemical enhancers including surfactants, fatty acids, fatty alcohols, and organic solvents have been used to increase transdermal drug transport, however; only few of them are actually effective in practice (Karande *et al.,* 2004). An universal chemical enhancers does not exist and the

enhancement provided by a pharmaceutical excipient can be drug specific. As example, one of the most common chemical enhancer PG, still demonstrates controversial effects. The permeation enhancement was already observed for several drugs as aciclovir (Diez-Sales *et al.*, 2005), diclofenac sodium (Arellano *et al.*, 1998) and testosterone (Leichtnam *et al.*, 2006) but this enhancer appeared inefficacious for benzodiazepines (Puglia *et al.*, 2001). Permeation enhancement should be individually investigated for each drug substance and a particular approach to identify suitable chemicals should be given. In the present chapter, CP was employed as model drug. The aim of this part of the study was to investigate the influence of chemical enhancers belonging to different chemical classes on the drug permeation and retention in the skin.

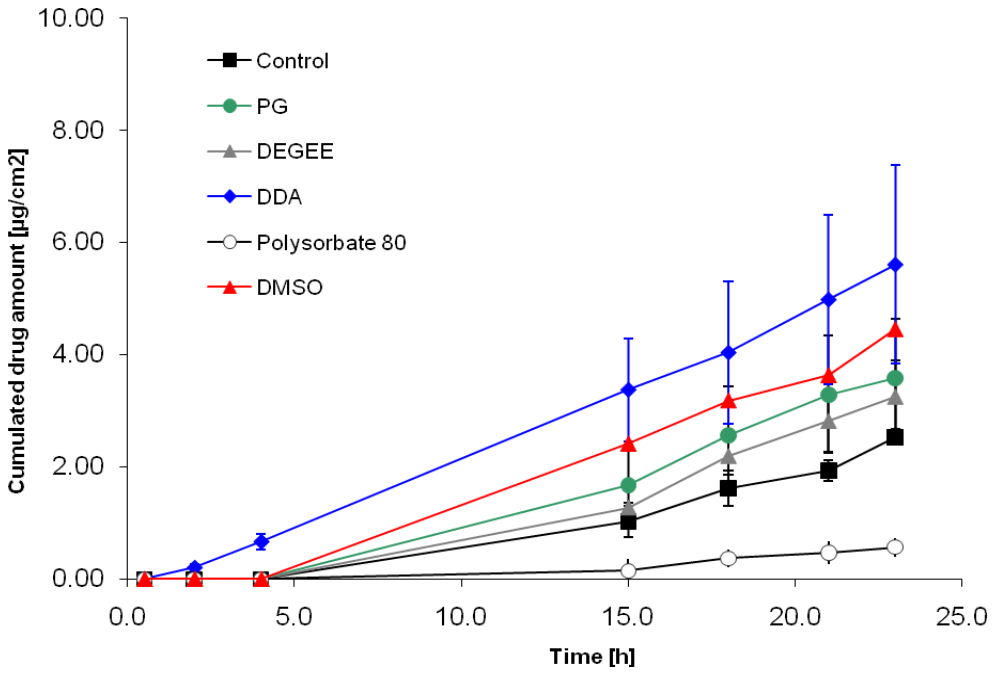


Fig. 8-4: *In vitro* skin permeation profile of CP after pretreatment with PG, DEGEE, DDA, polysorbate 80 and DMSO. The graph displays the mean of cumulated drug amount per area (µg/cm^2) over 23 hours of three Franz-cells (n=3) and SD.

Tab. 8-5: Permeation results of control (no enhancer), PG, DEGEE, DDA, polysorbate 80 and DMSO over 23 hours at 32 ºC (n=3).

Permeation enhancer	**Permeability coefficient (10^{-8} cm/s)**	**Cumulative drug amount (μg/cm²)**	**Flux (μg/cm²/h)**	**Enhancement ratio**
Control	2.46 ± 0.39	2.53 ± 0.14	0.177 ± 0.028	-
PG	3.35 ± 0.64	3.58 ± 1.06	0.242 ± 0.046	1.36
DEGEE	3.39 ± 0.97	3.26 ± 0.64	0.244 ± 0.070	1.38
DDA	4.36 ± 1.30	5.61 ± 1.77	0.253 ± 0.080	1.42
Tween 80®	0.45 ± 0.10	0.57 ± 0.17	0.051 ± 0.009	0.28
DMSO	3.42 ± 0.69	4.45 ± 1.16	0.240 ± 0.240	1.35

Different permeation profiles were obtained after the pre-treatment with the investigated enhancers, suggesting that the chemicals strongly influenced on the permeation of CP. Among all chemicals, DDA presented the higher flux, however statistically differences in comparison to control were found only for polysorbate 80. The enhancement ratio values suggest only slight contribution of the chemicals on the transdermal permeation of CP. The flux of CP after treatment with polysorbate 80 was significantly lower (t-test, $p<0.05$), indicating an inhibitory effect of this enhancer.

Several mechanisms of enhancement of drug permeation can act sequentially, and/or concurrently. For example, the enhancer can disrupt the intercellular bilayer lipid and increase the diffusivity of the drug in the skin (Benson, 2005; Willians and Barry, 2004). In some cases the enhancers penetrate into and mix homogeneously with the lipids into the bilayer, rotating, vibrating and forming microcavities which increase the free volume available for drug diffusion (Barry, 2001). A second important mechanism is the interaction with the intracellular proteins of the SC. The chemical can interact with keratin in corneocytes, opening up the dense protein structure and making it more permeable (Barry, 2001; Benson, 2005; Trommer and Neubert, 2006). The cosolvent effect is another fundamental mechanism. Enhancers functioning as cosolvents can increase drug solubility and diffusion in the vehicle. Furthermore, cosolvents can enhance partitioning into and solubility within the SC (Willians and Barry, 2004).

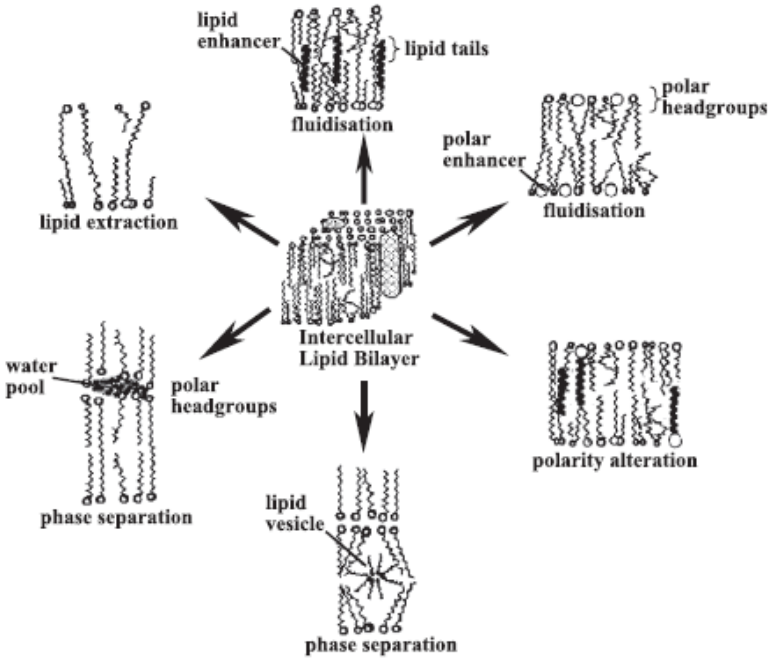


Fig. 8-5: Sites of action for accelerants in the intercellular space of the horny layer. After Williams and Barry (2004).

DDA is a saturated fatty amine cationic at low pH and non ionic at a high pH (Smith and Irwin, 2000). The enhancement effect of this chemical was already reported for testosterone (Kim *et al.*, 2001; Zhao *et al.*, 2005) and gentistic acid (Bian and Doh, 2003). PG is one of the most frequently used cosolvents in dermatology. The action as a real penetration enhancer is debated and still controversial in the literature. One of the postulated mechanisms is the solvation of keratin in the SC by competition with water for the hydrogen bond binding sites and the intercalation in the polar head groups, thus reducing drug/tissue binding (Barry, 1987). DEGEE (Transcutol®) is freely miscible with both polar and non-polar solvents. DEGEE has been recognized as a potential transdermal permeation enhancer due to its nontoxicity, biocompatibility with skin, and excellent solubilizing properties. The enhancement effect has been already reported for ivermectin (Yazdanian and Chen, 1995), clonazepam (Mura *et al.*, 2000), nimesulide (Gungor and Bergisadi, 2004), prostaglandins (Watkinson *et al.*, 1991) and theophylline (Touitou *et al.*, 1991). DMSO is one of the earliest and most widely studied penetration enhancers. DMSO is a powerful aprotic solvent with a high dielectricity constant because of the S-O-bond polarity (Trommer and Neubert, 2006). The mechanisms of the sulphoxide penetration enhancers are complex. One of the assumptions is the change of intercellular keratin conformation, from a helical to a ß-sheet (Anigbogu *et al.*, 1995; Oertel, 1997). Moreover, DMSO within skin membranes may

facilitate drug partitioning from a formulation into this solvent within the tissue. Because of its outstanding dissolving properties, DMSO is able to generate solvent-filled spaces in the SC where the solubility of the drug substances is increased. Surfactants have been widely employed as permeation enhancers (Shin *et al.*, 2005; Mukherjee *et al.*, 2005). However, the present findings indicated an inhibitory effect of polysorbate 80 on transdermal permeation of CP. The possible explanation is the entrapment of drug into micelles formed by the surfactant in the skin surface, hindering the transport into deeper SC layers. The residual surfactant which remained after the rinsing procedure could have led the formation of micelle structures, entrapping the drug hindering the flux into the skin.

8.3.8. Effect of pharmaceutical excipients on drug retention

The examination of drug retention in the skin is especially important for the development of topical semisolid, improving the local effect and minimizing systemic exposure. By analyzing only transdermal permeation and neglecting possible retention, the current practice ignores a very important aspect in the development, selecting or rejecting product candidates based on transdermal permeation data. Limited reports have described the influence of chemical enhancers on the retention of CP in the skin layers. Fig. 8-6 shows the drug retention and distribution profile after pre-treatment with the investigated enhancers.

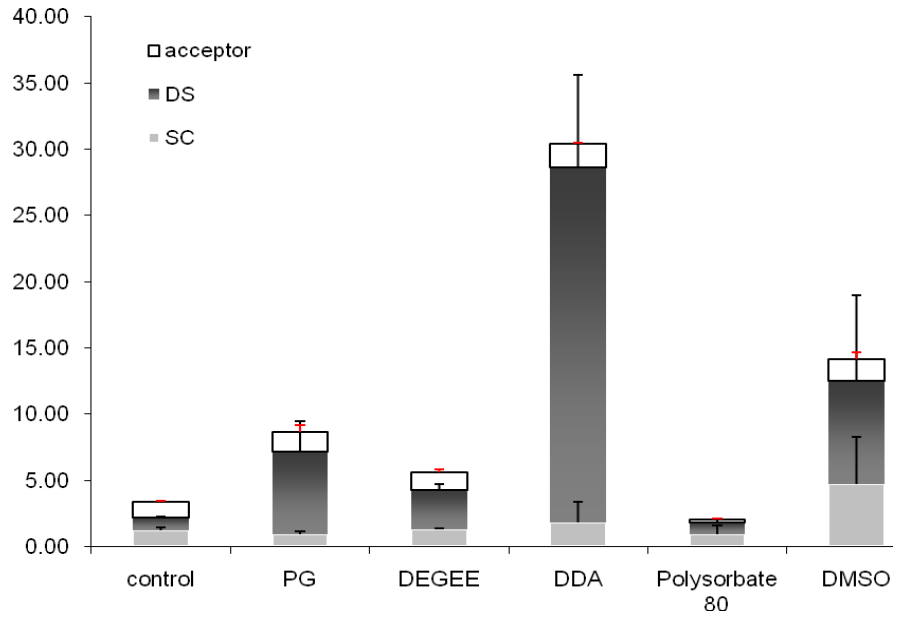


Fig. 8-6: *In vitro* skin distribution and retention profile after pre-treatment with permeation enhancers. The graph displayed the mean of cumulated drug amount per area (µg/cm^2) of three Franz-cells (n=3) and SD. Error bars represent the standard deviations of triplicate measurements. The overlapped red bars indicate the deviation of samples from acceptor medium.

Drug distribution and retention profiles were notably different observed after the treatment of chemicals. DDA has clearly demonstrated the highest dose absorption in the DSL in comparison to the other chemicals. Apparently, DMSO provided higher drug retention in the SC, however no statistical difference was obtained in comparison to control ($p>0.05$). PG and DEGEE have shown six-fold and three-fold higher retention in the DSL in comparison to control, respectively. The data suggest that the retention effect provided by the enhancers is drug specific altering drug partition in the skin layers. The explanation for the clearly higher retention effect of DDA appear to involve the disruption of lipid bilayer that are filling the extracellular spaces in the SC improving drug partitioning and diffusion in the skin (Willians and Barry, 2004). This may occur due to their resemblance in structure to the lipids in intercellular spaces. DDA is also assumed to increase solubility of lipophilic drugs in the hydrophilic environment of epidermis and supracappilary dermis, hindering the partition into aqueous medium, enhancing, therefore, the retention of the drug in these layers. This effect has been previously observed for other chemicals as Transcutol® (Mura et *al.*, 2000). It has been reported that the chain length of saturated fatty acid or amine of about 12 carbons brings about an optimal balance between partition coefficient of solubility parameter and affinity skin. It appears that saturated alkyl chain lengths of around C10–C12 attach to a polar head group as potent enhancer (Goodman and Barry, 1989; Tanojo *et al.*, 1997). Other mechanism have been postulated by Panachagnula and Ritschel, (1991) termed an ‘intracutaneous depot the increased accumulation of corticosteroid due to the swelling effect of SC intercellular lipids without alteration of their multiple bilayer structure. These swollen lipids then retain drugs (especially lipophilic compounds) to form the depot. The end result of this depot formation is an increased skin accumulation of drug with a simultaneous decrease in transdermal permeation.

8.4. Summary and conclusion

The effect of common employed pharmaceutical solubilizers on the solubility of TAA and CP in aqueous buffers was investigated. The solubility of the test compound in acceptor medium is one of the most critical issues to be considered during the performance of *in vitro* permeation experiments with human skin. The limited drug solubility in acceptor medium may lead to false conclusions that test chemical has been not permeating through the skin, demonstrating low systemic and/or toxicological potential. The solubilizers belonging to the group of surfactants have considerably enhanced the solubility of both model drugs in HBSS buffer. The cosolvents employed did not contribute significantly to the enhancement of drug solubility due to the low concentrations employed (0.1%). The utilization of polysorbate 80, Igepal® and SDS at 0.1% in aqueous buffer is, therefore, a more rational strategy to increase drug solubility in comparison to the employment of ethanol at 50% as commonly practiced.

Drug absorption, distribution and retention in the skin after application of three representative formulations (hydrogel, cream and ointment) were investigated. The effect of a commercial ointment, CG and HBC on the local and systemic *in vitro* bioavailability of TAA and CP was examined with excised human skin and the local amount of drug was evaluated by sampling with the aid of the tape-stripping technique. Dermoxin® ointment demonstrated the highest drug absorption in comparison to CG and HBC taking into account the local targets (SC and DSL) and systemic delivery (acceptor medium). Drug in the acceptor medium was detected only for the investigated ointments, indicating systemic exposure. The occlusive properties of the ointments enhanced drug permeation which is on one hand favorable for the local therapeutic treatment, but on the other hand hazardous considering the potential of systemic exposure and side-effects of topical glucocorticoids. The results of the drug release in chapter 7 were cross compared with the results of skin experiments. As expected, release data did not directly correlate with skin permeation and retention results. Dermoxin® and Kortikoid-Ratiopham® ointments presented lower release rates in comparison to CG and HBC, but highest amount of drug absorbed in the skin. This indicates that the occlusive effect of ointments surpassed other transport mechanism and determined the overall kinetic rate through the skin. Although some success has been achieved correlating release and skin absorption data, more research has to be done toward to this issue.

The effect of five chemical enhancers on the drug permeation and retention in the skin was examined. The values of enhancement ratio suggested that the chemicals slightly contributed

to the transdermal permeation of CP. However, when the local drug retention was examined, DDA, PG and DEGEE have shown a noticeable retention enhancement effect. The data indicates that permeation and retention effects are drug specific and should be separately investigated considering the desired therapeutic action. By analyzing only transdermal permeation and neglecting possible drug retention, the investigator ignores a very important aspect, selecting or rejecting product candidates based on transdermal permeation data. In conclusion, *in vitro* percutaneous sampled with the tape-tripping technique can be viewed as an appropriate approach for preliminary assessment of efficacy of dermally applied corticosteroids and their safety avoiding unnecessary *in vivo* bioavailability and toxicity studies, reducing risks to the human volunteers, contributing to speed-up the development of new drug formulations.

9. Final discussion and conclusion

Speeding-up the development of drug products is one of the most pursued aims in the pharmaceutical research. The high pressure to stretch resources forces the industries to seek strategies to rapidly and efficiently develop drug products. A large number of research papers have been devoted to propose strategies to speed-up pharmaceutical development. However, the current published data demonstrate that most of the approaches are still focused on the design of oral dosage forms and very few of them propose specific development strategies for semisolid dosage forms. An enormous list of characterization methods is available to generate data which support go/no-go decisions during projects in the overall development chain. There have been few reports, however, reporting the integrated use of these techniques in a compact development program. The key question in the present work was to define the most relevant methods and how to carry out these unit tests in a systematic and integrated manner to minimize time and expenses. It was expected with this research to reduce trial-and-error in the empirical models by providing scientific sound on testing of topical products.

The described approach combined eight experimental tests which were systematically integrated into a development platform as presented in Fig. 9-1. The starting point was the assessment of the model drugs with the aid of *in silico* tools. The drug-likeness properties were assessed with Lipinski Rule of Five (Lipinski *et al.*, 1997); drug solubility with models proposed by Yalkowsky and co-workers (Yalkowskyand Valvani, 1977; Yalkowsky and Valvani, 1980; Yalkowsky and Krzyzaniak, 1998) and skin permeability with the model postulated by Potts and Guy (1992). Drug stability in solution was profiled with minimal amount of test substance (ca. 1 mg) and speeded-up by applying forced conditions. A first insight into the pH-stability profile and the evaluation of the degradation products could be obtained. Using HPLC coupled with photodiode array detector, valuable information on mechanistic pathways of the decomposition products could be achieved. The data generated was further cross compared with data from compatibility and stability studies to confirm the relevant degradation products.

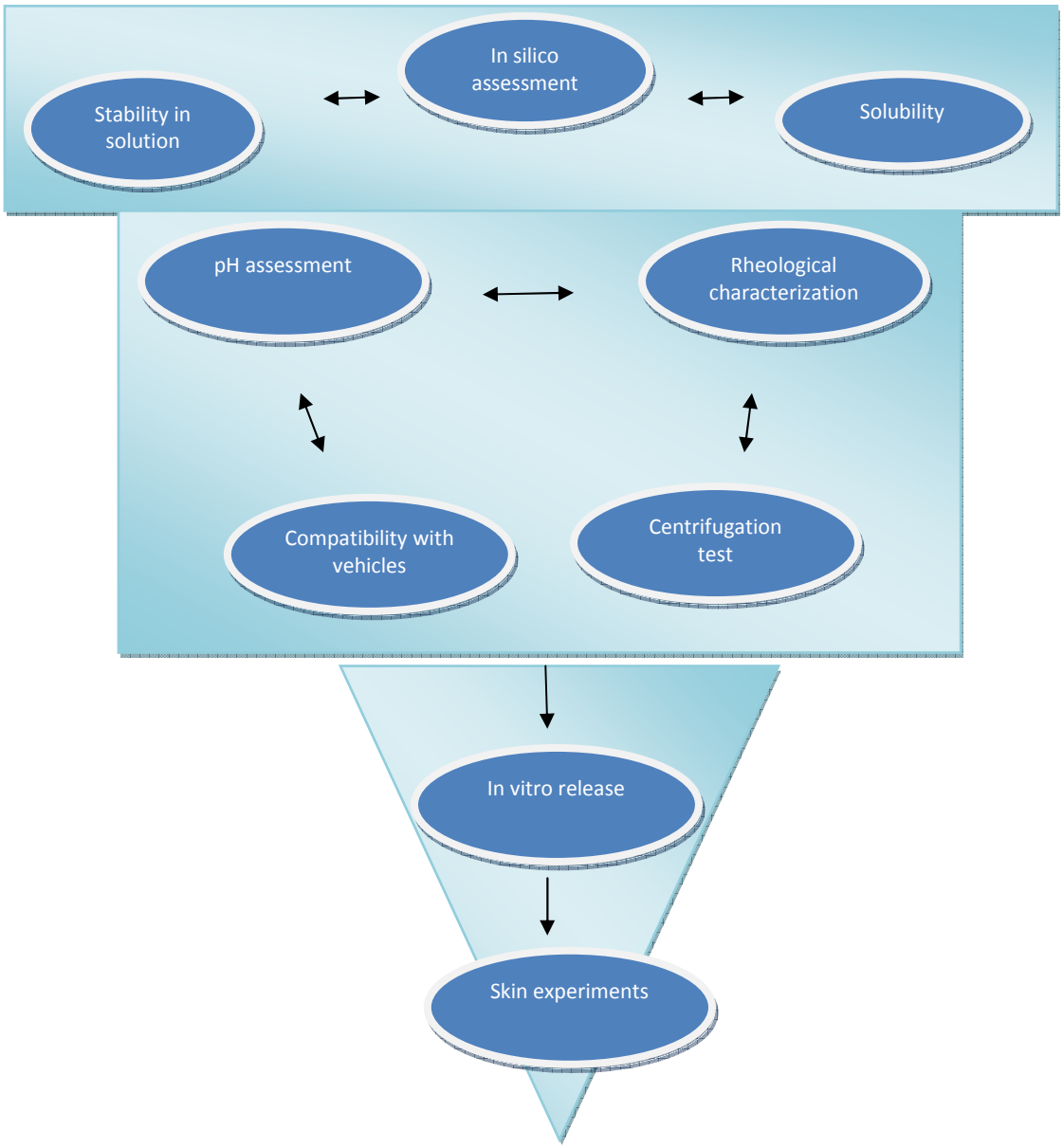


Fig. 9-1: Schematic illustration of the integration of the proposed characterization methods into a development program.

The advance of discovery research had lead to a high number of poorly soluble drug molecules which may require high-throughput methods and automated setups (Carlson *et al.*, 2005). Moreover, the classical shake-flask method can be time consuming and require large amount of substance (Alsenz, 2007). Fully automation may not fit the demand and the budget of small companies. To overcome this problem, a scaled-down method was proposed to determine drug solubility with cosolvents. The proposed test consisted of a simple setup employing only ca. 1 mg of compound and 2 mL of medium/cosolvent. The solubilization

power of the cosolvents was determined by equations obtained with the curve solubility-cosolvent fraction. Through this method, the adequate solubilizers for enhancing solubility in the acceptor medium in skin absorption studies were also identified. The established compatibility test employing mixtures of compound/base formulation generating the so-called mini-formulations demonstrated high potential to identify failure formulations at early phase, giving predictive estimates of classical stability studies within seven days. Furthermore, with the aid of a back-diagnosis approach, the critical components could be identified with individual investigations. The test design took into consideration the particularities of this complex dosage form to properly evaluate compatibility with pharmaceutical excipients. This experimental setup was a simplistic alternative with minimal utilization of test material and demonstrated to considerably save time during sample preparation, generating high quality of data.

In summary, the preformulation phase consisted of *in silico* evaluation and the performance of three tests: scaled-down stability in solution, scaled-down solubility determination and alternative compatibility test (Fig. 9-2).

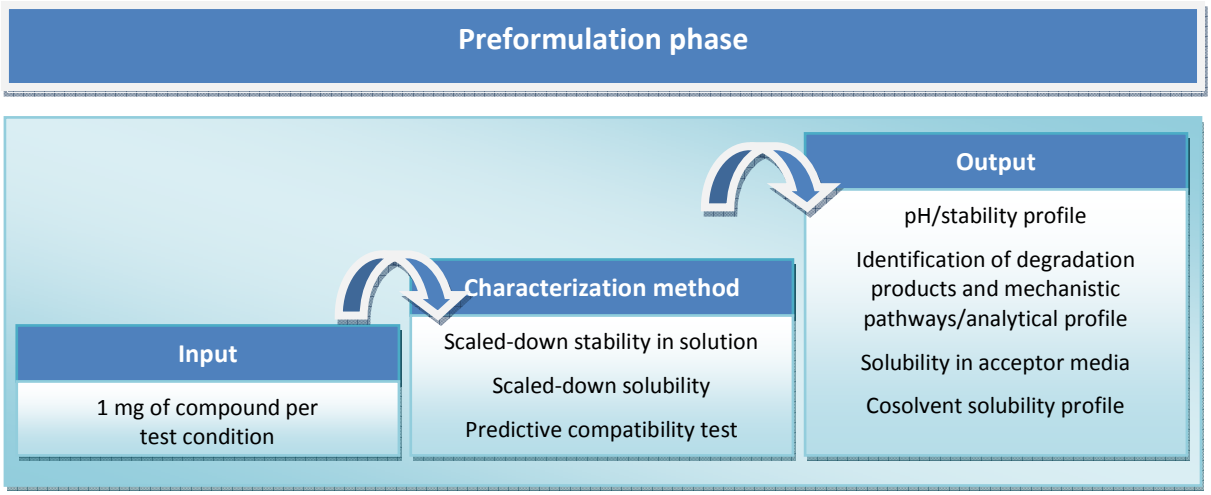


Fig. 9-2: Illustration of proposed preformulation program and the input, characterization method and outputs necessary in this development phase.

The characterization methods to be employed are very project specific and the choice of the adequate test procedures depends on several factors. Drug discovery companies usually need a full profiling program for new synthesized drugs to shorten the number of candidates which proceed to further phases (Balbach and Korn, 2004). Generic companies concentrate

the research focus on the development aspects which might affect product stability and performance, since the compound passed through the pipeline of the discovery phase and have been, thereby, partially profiled. After the described preformulation studies, the next step consisted of the selection of appropriate semisolid vehicles. Currently, approximately 15,000 pharmaceutical ingredients are available in the market (Cheng, 2009) and choosing the most adequate excipients among them can be an exhaustive task. Unknown excipients have to be evaluated with regard to cumulative irritancy, cutaneous contact sensitization, potential photoallergic contact sensitization, and phototoxicity (Pifferi and Restani, 2003), which increase research costs and prolong the development time. In the present work, the utilization of standard base formulations with already known and authorized ingredients was proposed as a simple and straightforward strategy to avoid the utilization of unprofiled excipients and unnecessary expenditures by undertaking toxicological studies. Eight base vehicles widely prescribed in German pharmacies covering the group of hydrogels, o/w creams, w/o creams and anhydrous ointments were employed. The decision of incorporating a new excipient, in case of identification of benefit (e.g. stability or permeation enhancement) can be taken in the latter optimization phases. The vehicles were manufactured at small scale for an early characterization. It stills remains a critical point to ensure robust manufacturing processes at small scales and after scaling-up to larger batches. However, development and validation of manufacturing processes were out of the scope of the present work. With a minimal amount of test formulation (<30 g) a series of tests could be carried out. Assessment of pH helps to ensure a stable product and it was used as a quality control parameter. Oscillation and flow measurements yielded important information on formulation structure, stability and manufacturing properties. The data generated from the rheological characterization were, however, not interpreted as key criteria to go/no-go decisions. The results reflected the specific properties of a product designed according to therapeutic target and patience acceptance. The data should be, therefore, used to establish specifications of the product, to monitor quality and to design the manufacturing process. The proposed centrifugation test demonstrated high capacity of detecting critical vehicles. It was intended to eliminate exaggerate conditions by adjusting centrifugation forces with known commercial product, providing more realistic estimates. The data generated from the evaluation of physical stability combined with results of compatibility test support go/no-go decisions reducing the number of candidates which proceed to further phases. Prototypes are expected to be eliminated based on failures identified at an early development phase (Fig. 9-3).

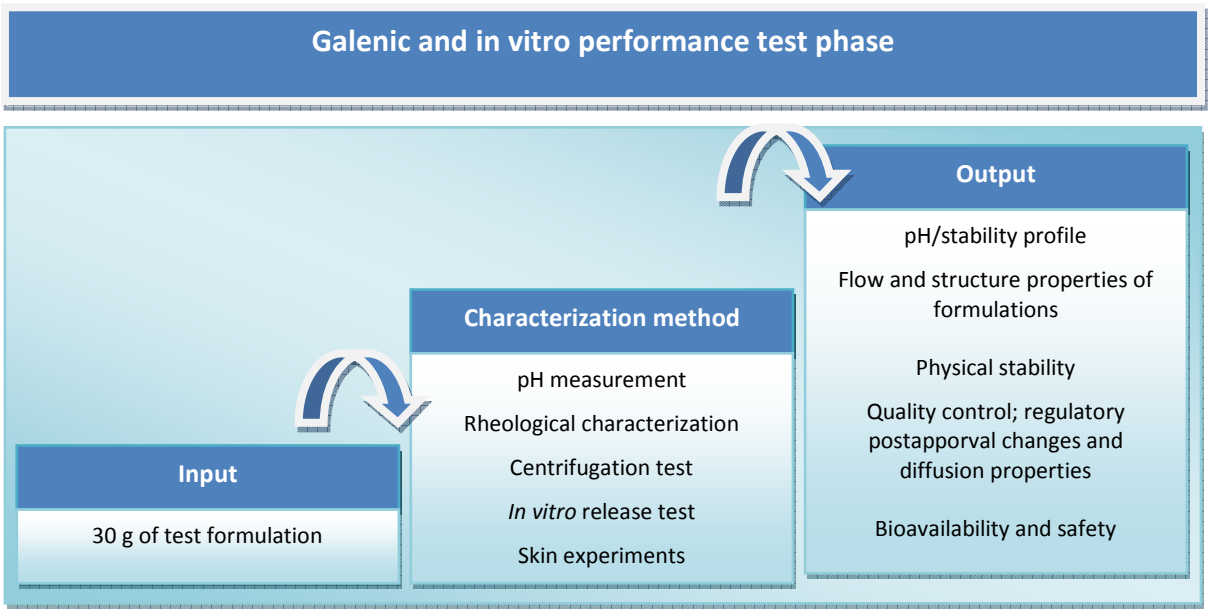


Fig. 9-3: Schematic illustration of the inputs, characterization methods and outputs during the formulation performance testing.

A method utilizing Franz diffusion cells and synthetic membranes was developed and validated to assess the drug release from semisolid vehicles. The application framework of the test poses at the interface with regulatory, quality control and performance assessment. From the regulatory point of view, the method was useful as a waiver for bioequivalence studies after postapproval changes in the product. Exemplary, the test demonstrated sufficient discriminating power when different generic products with same drug concentration and different composition were compared. From the point of view of quality control, the test is fundamental to more rigorously monitor batch-to-batch uniformity, since it reveals a combination of properties (e.g. drug solubility and diffusion) that classical quality control methods do not evaluate. The diffusion and transport properties of the investigated drug were deeply discussed and evaluated with several semisolid vehicles possessing different physicochemical properties and structures. A validation program was proposed and carried out to ensure that the results were delivered with reliability and to assure that the method is suitable to be used in industrial routine. The problems associated with the restricted availability of human skin lead many scientists to choose synthetic membranes in attempt to obtain estimations of clinical performance. Although several authors reported certain optimism in utilizing synthetic membrane as indicator of bioavailability, it was demonstrated in the present work that the extrapolation of release data

assessed by synthetic membranes to percutaneous absorption in human is not so simple. The mechanistic aspects involving the transport into the skin (e.g. hydration provided by oleaginous bases, drug binding to skin proteins and cutaneous metabolism) cannot be fully simulated with synthetic membranes generating misleading results. Skin experiments were performed with excised human skin and sampled with tape-strip technique to examine the amount of drug retained in the SC and in the upper dermis. The advantages of i*n vitro* skin experiments is that they generally afford the investigator the ability to control the experimental conditions and the possibility to monitor the rate and extent of percutaneous absorption and distribution in skin tissues, removed and analyzed separately from the rest of the body. The effect of five chemical enhancers on the permeability and retention of the drug in the skin was investigated. In the current practice, investigations of effect of chemical enhancers are concentrated on the permeation enhancement of the drug and the "retention enhancement" has been often ignored. It is, however, important to emphasize that for topical drugs the desired effect is the retention in the skin providing optimized local effect and not the permeation into blood vessels exerting systemic effects. The local-acting formulation should be able to release the drug to permeate the SC and reach adequate concentrations in the skin without reaching high serum concentrations. The results demonstrated that, for the same chemical enhancer, a noticeable difference between the permeation and retention enhancement effects, indicating that the selection of suitable enhancers has to be drug specific taking into account the desired therapeutic action. I*n vitro* percutaneous absorption experiments sampled with the tape-stripping technique can be viewed as an appropriate approach for preliminary assessment of efficacy of dermally applied corticosteroids and their safety avoiding unnecessary *in vivo* bioavailability and toxicity studies reducing risks to the human volunteers, costs and simplifies the development of new drug formulations.

Apart from the confirmation of the usefulness of several tests, the present work could also disclose some drawbacks of the described methods in the current practice. It was demonstrated that *in silico* models intended to assess drug permeability still provide qualitative information and cannot be seen as permeability predictors, since the database used to establish the model were originated from permeability information of drugs in solution, which present different properties of complex semisolid mixture of several excipients. The model yields, therefore, qualitative predictions distinguishing high and low permeable compounds. It could be confirmed that the design of compatibility studies for semisolid vehicles still lacks scientific sound when conclusions of incompatible ingredients

are drawn from investigations which are carried out only with individual excipients, disregarding the complexity of semisolid mixtures. A simple approach could demonstrate that when properly designed, the test can give useful predictions of formal stability studies. Similarly to compatibility test, a special emphasis was given to the examination of physical stability with a centrifugation test. It was shown that the adjustment of centrifugation forces is the key factor to obtain relevant stability information and small modifications in the centrifugation forces generate different prognosis. The limitations of *in vitro* release tests were also deeply discussed. It could be revealed the importance influence of acceptor medium on the discriminating power of release tests in quality control and SUPAC-SS applications. This factor is frequently ignored in the current practice, when the choice of acceptor medium usually are based on drug solubility information and neglect its influence on drug partition and consequently release extent. A second important drawback of the test was disclosed by demonstrating that release rate do not necessary indicate higher drug absorption. Synthetic membranes cannot represent the complexicity of drug transport into the skin and the extrapolation to *in vivo* results still has to be with caution. Finally, it was demonstrated that care has to be taken during the selection of suitable permeation enhancers. Very often the selection is very often based on on transdermal permeation neglecting the skin retention effect of the drug. *in vitro* skin experiments. The results exhibited although the permeation profiles of the chemicals were similar, the retention profile was considerably different, indicating a drug specific enhancement effect.

The research focus of the present work was in congruence with current discussions and principles of Quality-by-Design (ICH, 2009), which emphasize that the level of knowledge gained during pharmaceutical development, and not the volume of data, provides the basis for science-based submissions and their regulatory evaluation. The combination of the test procedures presented were designed considering development costs, time and scientific judgment enabling to establish an effective development program. It was intended with this work not to limit the characterization methods to be employed, but to provide a baseline expectation of the tests that should be carried out generating high quality data ensuring decisions based on scientific foundation.

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Appendix 1

GLOSSARY

Absorption: uptake of a substance into skin compartments or systemic circulation.

Acceptor medium: fluid placed into the receptor compartment Franz diffusion cells.

Back-diagnosis: approach to track back the pharmaceutical ingredient which originates chemical instability during compatibility investigations.

Dermal delivery: delivery of drug at local site (viable epidermis or dermis).

Deeper skin layers: upper dermal tissue assumed to be avascular.

Developability: general development factors which influence the success of turning a drug molecule into a product.

Discriminating power: capacity of method to discriminate products through their release profile with changes level 2 and 3 according to SUPAC-SS Guindance.

Dose absorbed: percentage of drug retained in the skin and permeated into acceptor medium.

Flux: The amount of drug passing through a unit area of skin in a unit time.

Permeation: penetration through one layer into a second layer that is both functionally and structurally different from the first layer.

Penetration: entry of a substance into a particular layer or structure, such as the entrance of a compound into the stratum corneum.

Prognostic potential: term used in the chapter 5 to define the capacity of compatibility tests to give predictive results and estimations of formal stability studies.

Reservoir: Depot of drug formed in stratum corneum or deeper skin layers.

Transdermal delivery: delivery of drug across the skin into systemic circulation.

Mini-formulation: blend of drug and base formulation utilized during compatibility studies **.**

Skin retention: accumulation of drug in the skin layers forming a depot.

Quality by design: A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound.

Appendix 2

Classification of postapproval changes according to SUPAC-SS

(Modified after FDA, 1997)

Level 1 Changes

Level 1 changes are those that are unlikely to have any detectable impact on formulation quality and performance.

Type of change	Examples
Components and composition	Deletion or partial deletion of an ingredient intended to affect the color, fragrance, or flavor of the drug product. Any change in an excipient up to 5% of approved amount of that excipient. The total additive effect of all excipient changes should not be more than 5%. Changes in the composition should be based on the approved target composition and not on previous level 1 changes in the composition. A change in diluent (q.s. excipient) due to component and composition changes in excipient may be made and is excluded from the 5% change limit. Change in a supplier of a structure forming excipient that is primarily a single chemical entity (purity 95%) or change in a supplier or technical grade of any other excipient.
Manufacturing	Change from nonautomated or nonmechanical equipment to automated ormechanical equipment to transfer ingredients. Change to alternative equipment of the same design and operating principles. Process changes, including changes such as rate of mixing, mixing times, operating speeds, and holding times within approved application ranges.Also, order of addition of components (excluding actives) to either oil or water phase.
Batch size	Change in batch size, up to and including a factor of ten times the size of the pivotal clinical trial/biobatch, where: (1) the equipment used to produce the test batch(es) are of the same design and operating principles; (2) the batch(es) is manufactured in full compliance with cGMPs; and (3) the same standard operating procedures (SOPs) and controls, as well as the same formulation and manufacturing procedures, are used on the test batch(es) and on the full-scale production batch(es).
Manufacturing site	Level 1 changes consist of site changes within a single facility where the same equipment, standard operating procedures (SOPs), environmental conditions (e.g., temperature and humidity) and controls, and personnel common to both manufacturing sites are used.

Level 2 Changes

Level 2 changes are those that could have a significant impact on formulation quality and performance.

Type of change	Examples
Components and composition	Changes of >5% and <10% of approved amount of an individual excipient. The total additive effect of all excipient changes should not be more than 10%. Changes in the composition should be based on the approved target composition and not on previous level 1 or level 2 changes in the composition. Changes in diluent (q.s. excipient) due to component and composition changes in excipients are acceptable and are excluded from the 10% change limit. Change in supplier of a structure forming excipient not covered under level 1. Change in the technical grade of structure forming excipient. Change in particle size distribution of the drug substance, if the drug is in suspension.
Manufacturing	Change in equipment to a different design or different operating principles. Change in type of mixing equipment, such as high shear to low shear and vice versa. Process changes, including changes such as rate of mixing, mixing times, rate of cooling, operating speeds, and holding times outside approved application ranges for all dosage forms. Also, any changes in the process of combining the phases.
Batch size	Changes in batch size from beyond a factor of ten times the size of the pivotal clinical trial/biobatch, where: (1) the equipment used to produce the test batch(es) are of the same design and operating principles; (2) the batch(es) is manufactured in full compliance with cGMPs; and (3) the same standard operating procedures (SOPs) and controls, as well as the same formulation and manufacturing procedures, are used on the test batch(es) and on the full-scale production batch(es).
Manufacturing site	Level 2 changes consist of site changes within a contiguous campus, or between facilities in adjacent city blocks, where similar equipment, standard operating procedures, (SOPs), environmental conditions (e.g., temperature and humidity) and controls, and personnel common to both manufacturing sites are used, and where no changes are made to the manufacturing batch records, except for administrative information and the location of the facility.

Level 3 Change

Level 3 changes are those that are likely to have a significant impact on ormulation quality and performance.

Type of change	Examples
Components and composition	Any qualitative and quantitative changes in an excipient beyond the ranges noted in level 2 change. Change in crystalline form of the drug substance, if the drug is in suspension.
Manufacturing	No level 3 changes are anticipated in this category.
Batch size	No level 3 changes are anticipated in this category.
Manufacturing site	Level 3 changes consist of a site change in manufacturing site to a different campus. A different campus is defined as one that is not on the same original contiguous site or where the facilities are not in adjacent city blocks. To qualify as a Level 3 change, similar equipment, SOPs, environmental conditions, and controls should be used in the manufacturing process at the new site. Changes should not be made to the manufacturing batch records except when consistent with other level 1 changes. Administrative information, location, and language translation may be revised as needed.

Data processing according to U-Test

(modified after FDA, 1997)

Suppose that the slope data obtained at the first stage are as follows:

Postchange Lot (T)	Prechange Lot (R)
1.3390	1.1331
1.3496	1.1842
1.4946	1.0824
1.4668	1.3049
1.1911	1.0410
1.2210	1.2419

The first step in the computation of the confidence interval is to form the 36 (=6x6) individual T/R ratios. This is illustrated in the following table, where the prechange lot slopes (R) are listed across the top of the table, the postchange lot slopes (T) are listed down the left margin of the table, and the individual T/R ratios are the entries in the body of the table:

	1.1331	1.1842	1.0824	1.3049	1.0410	1.2419
1.3390	1.1817	1.1307	1.2371	1.0261	1.2863	1.0782
1.3496	1.1911	1.1397	1.2469	1.0343	1.2964	1.0867
1.4946	1.3190	1.2621	1.3808	1.1454	1.4357	1.2035
1.4668	1.2945	1.2386	1.3551	1.1241	1.4090	1.1811
1.1911	1.0512	1.0058	1.1004	0.9128	1.1442	0.9591
1.2210	1.0776	1.0311	1.1280	0.9357	1.1729	0.9832

The second step in the computation of the confidence interval is to order these 36 individual T/R ratios from lowest to highest:

In the third step, the ***eighth*** and ***twenty-ninth*** ordered individual ratios are the lower and upper limits, respectively, of the 90% confidence interval for the ratio of the median in vitro release rate (slope) for T over the median in vitro release rate for R. In the example, this confidence interval is 1.0343 to 1.2863, or in percentage terms, 103.43% to 128.63% falling within the limits of 75% to 133.33%, the product, therefore, passes.

Publications

Marangon, A., Bock, U., Haltner, E., Daniels, R., Development and validation of an in vitro release test for triamcinolone acetonide from semisolid formulations. Poster presentation. PharmSciFair, Nice, France, 2009

Marangon, A., Bock, U., Haltner, E., Daniels, R., Stress testing of corticosteroids for stability-indicating assay method development. Poster presentation. HPLC 2009, Dresden, Germany.

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Prof. Dr. Arminda Lucia Siqueira	Biostatistik
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