

A New Discriminative Criterion for the Development of Franz Diffusion Tests for Transdermal Pharmaceuticals

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ABSTRACT – PURPOSE. *In vitro* skin/membrane permeation profiling of topical pharmaceuticals is an important overall quality attribute in the evaluation of product consistency and it is also used for IVIVR (*in vitro* - *in vivo* relationship) purposes in product development and change control. Franz diffusion cell (FDC) experiments are emerging as a generally accepted methodology in this field, where the choice of operational conditions requires a data-supported justification towards the discriminating power of the test. A response function is therefore proposed to objectively quantify the discriminating power. **METHODS.** We evaluated the usefulness of the proposed response function by studying one of the operational conditions, *i.e.* the influence of receptor medium composition, on the FDC *in vitro* penetration behaviour of the model compound testosterone formulated in four different topical preparations, using both artificial membranes and dermatomed human skin. A second application is a FDC test system for spilanthal. **RESULTS.** From the obtained cumulative amount of the active (testosterone or spilanthal) in the receptor fluid versus time curves, the permeability coefficient K_p of testosterone from each formulation was calculated. The evaluation of the discriminating power of the different media was performed using our new objective response function based upon an equal spread criterion of normalised K_p values. **CONCLUSION.** The proposed new criterion was found to be useful for the rational design of an *in vitro* diffusion test for transdermal pharmaceuticals. We demonstrated significant differences in discriminating power between the different media used: (a) for testosterone-containing formulations, it was shown that HPBCD-containing media are more discriminative compared to ethanol- or BSA-containing media; (b) for spilanthal-containing formulations, PBS containing formulations also gave better discriminating results than ethanol-based receptor media.

INTRODUCTION

The commercialization of transdermal drug delivery systems (TDS) like patches and gels has resulted in diverse products (1) such as nitroglycerin for angina pectoris, scopolamine for motion sickness, fentanyl for pain control, nicotine for smoking cessation, estrogen for hormone replacement therapy, testosterone for male hypogonadism, clonidine for hypertension, lidocaine for topical anaesthesia, and ethynylestradiol for contraception. Over the last decades, transdermal drug delivery has become increasingly popular for many reasons (2): the availability of well-characterized formulation technology (3), the development of a biopharmaceutical, pharmacokinetic and pharmacodynamic science base to allow definition

and study of important safety and efficacy issues that are related to TDS, the opportunities afforded by TDS for better patient compliance and more consistent and reliable drug concentrations over protracted dosing periods, a high degree of acceptance by both patients and health care professionals and the development or extension of market exclusivity for off-patent drugs or drugs that are losing patent or exclusivity protection.

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As a result, the pharmaceutical industry is investing increasing amounts of money and resources on the development of new products that reversibly overcome the skin barrier. Although a new transdermal product is only of value if the clinical pharmacokinetic profile delivers the appropriate pharmacodynamic response needed for the treatment of the patient, preclinical assessments strongly guide the product development. These include as well *in vitro* experiments for determining the flux of the compound across skin or membranes during feasibility studies, as *in vivo* evaluation in animal models (4). The aim of the *in vitro* experiments in transdermal delivery is to evaluate the penetration of a molecule through the skin. Typically, this is achieved by using a variety of skin diffusion cells and different experimental protocols. There have been numerous recommendations, and some of them have been selected as guidelines by both regulatory entities and committees of interested parties. The most widely known of these guidelines are "Guideline Number 428 on the Conduct of Skin Absorption Studies" by the Organization for Economic Cooperation and Development (OECD) (5), the European Centre for Ecotoxicology and Toxicology of Chemicals (EXETOC) workshop on "Methods for assessing percutaneous penetration" (6), the "Guidelines for Percutaneous Penetration / Absorption" by the European Cosmetic Toiletry and Perfumery Association (COLIPA) (7), the American Association of Pharmaceutical Scientists/Food and Drug Administration (AAPS/FDA) workshop on the performance of *in vitro* skin penetration studies (8) and the FDA "Guidance for the Industry on Non-sterile Semisolid Dosage Forms" (9). Irrespective of the guidance followed, the techniques used must be carefully considered as several variables such as the type of cell system, the skin source, skin integrity and viability, skin preparation, composition of receptor fluid and even the way as results are calculated can influence the outcome and final conclusions (10).

Determination of the release rate of the active pharmaceutical ingredient (API) and/or specific excipients from the formulation, *i.e.* its diffusion in and through a biological or artificial membrane contacting a receptor fluid, is not only an important parameter for characterizing its transdermal behaviour, but it can also be considered as a global overall quality attribute which is valuable in the

development of a suitable formulation or in the evaluation of possible changes in formulation composition (11), production parameters and shelf-life stability. Therefore, the regulatory health authorities are generally requesting these diffusion-release tests in the pharmaceutical dossier submitted to obtain the marketing authorisation. The following points should be considered when developing the *in vitro* release procedure for dermatological products (12): (a) the method(s) developed should assure batch-to-batch quality for the product, (b) the method should be general enough and should be applicable to all products that are (and will be) marketed, (c) the method should be discriminative enough to detect manufacturing changes that (may) influence product performance and (d) the method should be simple, reliable, reproducible and capable of automation. However, the development of such a global functionality quality test is currently often a matter of discussion due to a certain lack of standardisation, missing generally accepted criteria, incomplete understanding of fundamental theory and its applications, regulatory immaturity and the different objectives which are sometimes mixed up (quality-consistency versus *in-vitro-in-vivo* correlations). Whatever the context, however, a key aspect is the discriminating power of the test (13), *i.e.* the ability to distinguish different preparations as separate items. Therefore, we propose a new criterion assessing the discriminating power between different FDC-tests, based on experimentally obtained K_p values. The applicability of this criterion is demonstrated by the systematic evaluation of different FDC receptor media on the transdermal and transmembrane behaviour of the test compound testosterone, previously defined as a suitable model compound for skin penetration studies (14). A second application given here is the skin FDC test system for spilanthal containing formulations.

METHODS

Products examined

Three testosterone-containing topical products were purchased from the Ghent university hospital pharmacy: Testim[®] 50 mg gel (Ipsen, Merelbeke, Belgium), Androgel[®] 50 mg gel (Laboratoires Besins International, Paris, France) and Tostrex[®] 2% gel (Strakan, Tredegar, UK). Androtiv[®] 2% gel

(Anastore, Geneva, Switzerland) was obtained from the internet.

Reagents

0.01 M phosphate buffered saline (PBS) and $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ were purchased from Sigma (St. Louis, MO, USA). Bovine serum albumin (BSA), Na_2SO_4 , KH_2PO_4 , K_2HPO_4 , MgSO_4 and NaOH were bought from Merck (Darmstadt, Germany). NaCl, KCl, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and NaHCO_3 were obtained from UCB (Leuven, Belgium). HPLC gradient grade acetonitrile and methanol came from Fisher Scientific (Leicestershire, UK). LC-MS grade formic acid and HPLC-grade testosterone were purchased from Fluka (Buchs, Switzerland). Hydroxypropyl- β -cyclodextrine (HPBCD) and HCl were obtained from Acros Organics (Geel, Belgium). Ethanol, denatured with up to 5% ether, was bought from Chem Lab (Zedelgem, Belgium). Water was purified using an Arium 611 purification system (Sartorius, Göttingen, Germany) resulting in ultrapure water of 18.2 M Ω .cm quality.

Liquid chromatography

The testosterone content in the receptor fluid was assayed using a validated HPLC-UV method (15). Briefly, the HPLC apparatus consisted of a Waters Alliance 2695 separation module and a Waters 2996 photodiode array detector (DAD) controlled by Empower 2 software (all Waters, Millford, USA). 20 μL of each sample was injected onto a Lichrospher 100 RP C_{18} (125 mm x 4 mm, 5 μm particle size) column (Merck, Darmstadt, Germany) maintained at 30°C. Testosterone was eluted by a degassed isocratic mobile phase consisting of a mixture of 50% (A) 0.1% m/V formic acid in water, and 50% (B) 0.1% m/V formic acid in acetonitrile at a flow rate of 1 ml/min. UV detection was done at 254 nm.

Preparation of receptor media

Ethanol/ H_2O (30:70, V/V) was used as a reference receptor medium. Phosphate buffered saline was prepared according to the suppliers (Sigma) instructions, resulting in a 0.01 M solution at pH 7.4. Artificial human sweat (SS) was prepared according to Shimamura (16). Simulated body fluid (SBF) was prepared as described by Dorozhkin (17). The exact composition is given in Table 1. To assure a good solubility of testosterone, a

solubilizer was added. This was either 5% m/V BSA or 0.5% m/V HPBCD.

Table 1. Composition of the physiological receptor media used (in mM).

Component	PBS	SS	SBF
NaCl	138.00	49.96	136.80
KCl	2.70	-	3.00
CaCl_2	-	0.15	2.50
MgSO_4	-	1.00	-
MgCl_2	-	-	1.50
Na_2SO_4	-	-	0.50
NaHCO_3	-	-	4.20
KH_2PO_4	1.43	7.50	1.00
Na_2HPO_4	8.57	-	-

Determination of testosterone solubility in the receptor media

Excess testosterone was added to 2 ml of each receptor medium used and the mixture was incubated for 48 hours at 32°C with continuous shaking. The undissolved testosterone was removed by temperature-controlled centrifugation at 20 000 g. 500 μL of the clear supernatant was transferred to a new tube and an equal volume of acetonitrile was added. After 10 minutes of shaking at room temperature, the samples were centrifuged for 10 min at 20 000 g and the clear supernatant was transferred into glass HPLC vials. All tubes and pipettes used were preheated to 32°C in an oven. All experiments were performed in six-fold, except for EtOH/ H_2O as a solvent, where only three measurements were made.

Permeation study

The permeation of testosterone through both human skin and the artificial membrane was determined using static Franz diffusion cells (Logan Instruments Corp., New Jersey, USA) with a calibrated receptor compartment of 5 mL equipped with a sampling port. All conditions were performed in triplicate using a randomized blocked design. For the skin experiments, excised human skin from three different female patients (52 ± 7 years old, mean \pm standard deviation), who had undergone an abdominoplasty procedure, was used in such a way that each block of experiments was performed using skin from one donor. After

cleaning the skin with 0.01 M PBS pH 7.4 and removal of the subcutaneous fat, the skin samples were wrapped in aluminium foil and stored at -20°C for not longer than 3 months. Just before the start of the experiments, the skin samples were thawed and dermatomed to a pre-set thickness of 400 µm. The experimentally obtained thickness was determined using a micrometer (Mitutoyo, Tokyo, Japan). Skin samples or hydrophilic mixed cellulose esters filter sheets with 0.05 µm pore size (VMWP; Millipore, Schwalbach, Germany) were sandwiched between the donor (upper) and the receptor (lower) chambers of the diffusion cell. The receptor compartment was filled with receptor medium, making sure all air under the skin/membrane was removed. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor compartment was continuously stirred using a Teflon coated magnetic stirring bar (400 rpm). Before starting the skin experiments, skin impedance with PBS in the donor chamber was measured using an automatic micro-processor controlled LCR Impedance Bridge (Tinsley, Croydon, U.K.) to ensure that there was no skin damage. Skin pieces with an impedance value below 20 kΩ were discarded and replaced. A quantity of the formulation corresponding to 3 mg of testosterone was topically applied to the surface of the skin/membrane and carefully spread to achieve complete coverage. Smaller quantities of the formulations did not guarantee the full skin coverage. The donor compartment was then covered with parafilm (American National Can™, Chicago, USA) and the temperature of the receptor compartment was kept at 32 ± 2°C by a water jacket. The available diffusion area was 0.64 cm². Samples (200 µL) were drawn at regular time intervals from the sampling port (0, 0.5, 1, 1.5, 2, 4, 8, 12, 18, 21 and 24h) and were immediately replaced by 200 µL fresh solution. The analytically determined testosterone assay values were correspondingly corrected for the replenishments.

Data analysis

The skin permeation parameters were calculated from the plot of cumulative amount testosterone permeated as a function of time. Steady-state flux (J) was obtained from the slope of the linear portion of the curve. The permeability coefficient Kp was calculated using the following equation:

$$Kp = \frac{J}{C_d} \quad (1)$$

where C_d is the concentration of the drug in the dose formulation.

In case of the artificial membranes, a bending point was observed, *i.e.* two different slopes (J-values). Hence, an initial Kp from the first part of the curve and a final Kp from the second part of the curve was calculated.

All Kp values thus obtained (*i* from 1 to 20 for each of the 3 membrane systems: skin, initial- and final artificial membrane) were rescaled within each membrane system using the following normalization equation, resulting in a distribution between 0 and 1:

$$K'p_i = \text{normalised } Kp_i = \frac{Kp_i - Kp_{\min}}{Kp_{\max} - Kp_{\min}} \quad (2)$$

where K'p_{*i*} is the normalised Kp value for experiment *i*, Kp_{min} and Kp_{max} are the minimal and maximal values for Kp that were found over the total number of experiments (*n*). An overall response function F(Kp) reflecting the formulation-discriminating power of the system was defined as the percentage equal-spread of the normalised Kp-values obtained for the different formulations (in our model study: n=4), using the following formula:

$$F(Kp) = \frac{\prod_{i=1}^{n-1} (K'p_{i+1} - K'p_i)}{\left(\frac{1}{n-1}\right)^{n-1}} \times 100\% \quad (3)$$

where the denominator (in our study: 0.33³) represents the ideal situation where spreading of the normalised Kp values is maximal, and hence reflecting the maximal discriminating power between the different formulations used in the study.

There is usually an interval between the start of the experiment and the steady state being reached. This lag-time is derived from the graph of cumulative absorbed dose versus time: it is the intercept (on the time axis) of the tangent to the linear part of the absorption profile.

Spilanthol application

A recent study has evaluated the transdermal behaviour of the N-alkylamide spilanthol. Three

different formulations were compared on three different FDC test systems, differing in the receptor fluid (PBS, PBS + 0.5% m/V HPBCD and EtOH/H₂O (30/70, V/V)). Full experimental details and the transdermal results obtained are given in references (18 - 19).

RESULTS

We used our criterion in a study evaluating the receptor phase composition. The solubility of the test compound should be sufficiently high to prevent slowing down the flux. Typically, the receptor fluids used are physiological saline or phosphate buffered solutions. If necessary, these solutions are complemented with a solubilizer. However, due to interactions with the skin, the receptor fluid itself can change the transdermal behaviour of the test compound. Hence, this operational variable in FDC tests can influence the discriminative power of the test system.

Testosterone solubility in the receptor media

Before starting the skin penetration studies, the solubility of testosterone at 32°C (*i.e.* the physiological skin temperature (5)) was determined for each receptor medium. Results are given in Table 2. Without solubilising agent added, testosterone solubility was very low, ranging between 67 and 87 µg/mL. Therefore, these media without solubilising agents were not used as a receptor fluid. Ethanol-based receptor media are historically quite often used in this kind of studies, and testosterone was highly soluble in the EtOH/H₂O (30/70, V/V) receptor medium. However, because the use of organic solvents is considered not well-suited as a biocompatible receptor medium in product development and establishing IVIVR (20) resulting in a regulatory reluctance to accept these organic solvent based receptor media, different other physiological buffers were evaluated as receptor media as well. However, due to the very low solubility of testosterone in purely aqueous systems, the addition of a solubilising additive was required. In compliance with the OECD guideline 428 and considering the fact that a concentration of 50 g/L is generally accepted to be the mean albumin concentration in human serum (21), 5% m/V BSA was used. As an alternative, addition of HPBCD as a solubilizer was evaluated as well. The solubility of testosterone at

32°C in PBS was determined at different HPBCD concentrations ranging from 0 till 5% m/V. A linear relationship between the testosterone solubility and the percentage HPBCD added was found ($R^2 = 0.9991$). A working concentration of 0.5% m/V HPBCD was chosen, as this gave solubility results in the range of the physiological 5% m/V BSA. The ratio of testosterone solubility at 32°C to the maximum amount of testosterone found in the receptor fluids of the skin and of the initial flux through the artificial membrane was minimally five (*i.e.* for SS + 0.5% HPBCD): this value is sufficiently high to assure that solubility of the active ingredient in the receptor phase was not the rate limiting step for transdermal and initial transmembrane penetration, which was experimentally confirmed by the linear kinetics.

Franz diffusion cell experiments using human skin

In our development of a regulatory acceptable, *i.e.* a data-supported, justified and discriminating test method, we used four model formulations from different origin/supplier/composition which are thus expected to show differences in their diffusion behaviour. Our experimental data are given in Table 3. For all experimental conditions, a steady state linear part was reached (R^2 values ranging from 0.9566 to 0.9971) and therefore transdermal parameters for each condition could be calculated using the previously given formulae (Figure 1). As two formulations have a label claim of 2 % m/m (Androtiv[®] and Tostrex[®]) and two formulations are 1 % m/m (AndroGel[®] and Testim[®]), the permeability coefficient K_p is a more appropriate evaluation tool compared to an absolute permeated quantity, as K_p takes the concentration effect into account. Moreover, K_p reduces all the different experimental points into one concentration-standardised flux-value, contrasting a one-time-point like *e.g.* the cumulative amount penetrated after 24 hours. When the acceptor medium contained BSA, the rank order of transdermal permeation was as follows (from high to low): Tostrex[®], AndroGel[®], Androtiv[®] and Testim[®]. When 0.5% m/V HPBCD was present in the receptor chamber, Tostrex[®] and AndroGel[®] still showed the highest permeation, but testosterone penetration from Androtiv[®] was lower than from Testim[®].

Table 2. Solubility of testosterone in the different receptor media used at 32°C.	
Receptor medium	Average solubility ± S.D. (µg/mL) (n = 3-6)
EtOH/H ₂ O (30:70, V/V)	26381 ± 403
PBS	69 ± 16
PBS + 5% BSA	345 ± 10
PBS + 0.5% HPBCD	527 ± 23
SS	67 ± 43
SS + 0.5% HPBCD	534 ± 23
SBF	87 ± 33
SBF + 5% BSA	314 ± 13

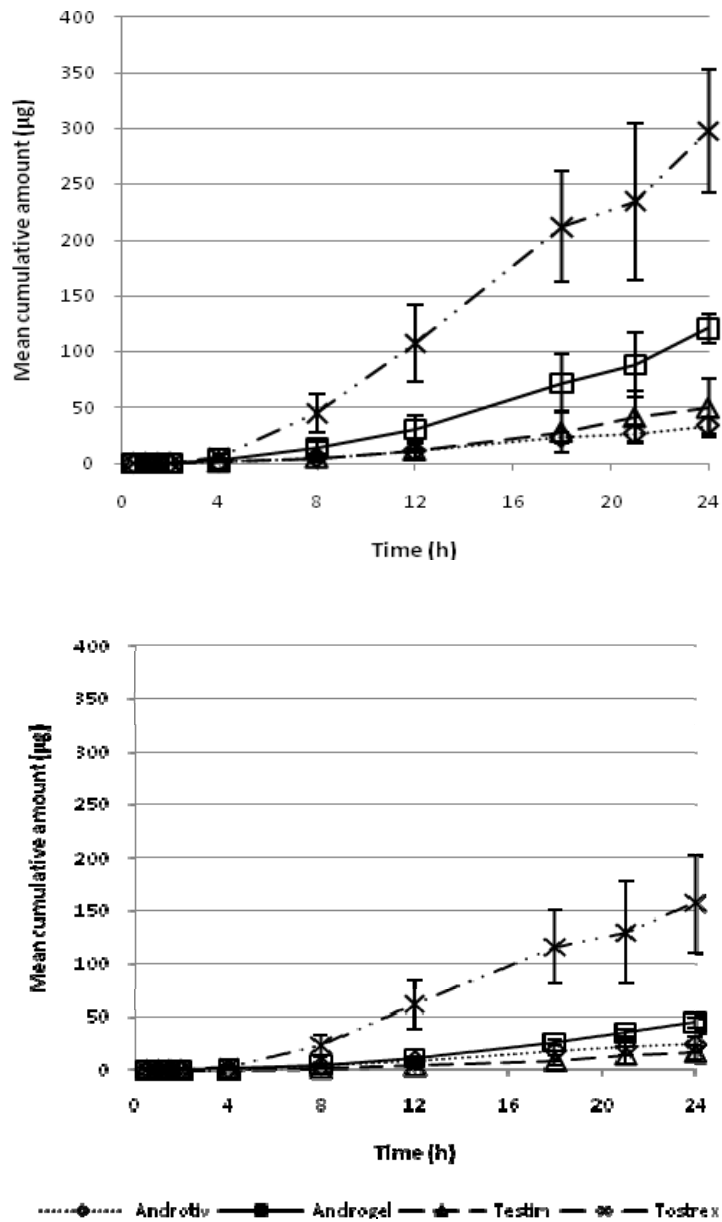


Figure 1. Cumulative amount of testosterone in the receptor fluid (µg) versus time (h) curves using human skin and PBS + HPBCD (upper panel) or PBS + BSA (lower panel) as a receptor fluid.

Table 3. Calculated permeation parameters (\pm S.D.) using different receptor media.

Parameter	Gel	EtOH/H ₂ O	PBS + 5% BSA	PBS + 0.5% HPBCD	SBF + 5% BSA	SS + 0.5% HPBCD
Skin K _p (x 10 ⁻⁴ cm/h)	Androtiv [®]	2.75 \pm 0.10	1.07 \pm 0.05	1.40 \pm 0.07	1.29 \pm 0.04	2.05 \pm 0.07
	AndroGel [®]	4.08 \pm 0.27	3.98 \pm 0.26	10.29 \pm 0.81	4.14 \pm 0.44	8.03 \pm 0.66
	Testim [®]	0.50 \pm 0.06	1.50 \pm 0.13	4.53 \pm 0.35	1.16 \pm 0.13	5.89 \pm 0.38
	Tostrex [®]	16.91 \pm 0.76	6.20 \pm 0.24	11.53 \pm 0.50	6.73 \pm 0.41	22.41 \pm 0.99
Skin T _{lag} (h)	Androtiv [®]	3.13	5.44	5.48	3.64	2.77
	AndroGel [®]	5.87	7.07	6.72	7.04	7.34
	Testim [®]	5.43	7.66	7.26	10.75	7.50
	Tostrex [®]	3.58	4.23	4.30	4.97	4.11
Membrane Initial K _p (x 10 ⁻⁴ cm/h)	Androtiv [®]	27.93 \pm 0.47	2.15 \pm 0.18	7.91 \pm 0.69	1.42 \pm 0.15	9.77 \pm 1.22
	AndroGel [®]	90.16 \pm 1.29	5.30 \pm 0.47	5.86 \pm 0.49	5.61 \pm 0.61	5.95 \pm 0.54
	Testim [®]	45.05 \pm 1.02	2.62 \pm 0.28	3.41 \pm 0.19	4.18 \pm 0.47	2.48 \pm 0.15
	Tostrex [®]	26.81 \pm 0.82	30.07 \pm 0.44	45.41 \pm 0.16	28.33 \pm 0.20	57.10 \pm 0.20
Membrane T _{lag} (h)	Androtiv [®]	0.20	0.51	0.56	0.37	0.53
	AndroGel [®]	0.17	0.48	0.54	0.53	0.50
	Testim [®]	0.11	0.63	0.42	0.32	3.63
	Tostrex [®]	0.09	0.21	0.26	0.31	0.23
Membrane Final K _p (x 10 ⁻⁴ cm/h)	Androtiv [®]	8.89 \pm 0.55	7.28 \pm 0.40	24.50 \pm 2.71	6.93 \pm 0.13	29.72 \pm 0.68
	AndroGel [®]	39.27 \pm 1.31	26.55 \pm 0.95	39.91 \pm 2.99	27.11 \pm 0.77	37.60 \pm 3.87
	Testim [®]	28.59 \pm 0.68	7.89 \pm 0.33	64.04 \pm 3.71	8.49 \pm 0.44	56.08 \pm 1.24
	Tostrex [®]	6.90 \pm 0.49	14.81 \pm 1.06	20.32 \pm 0.40	21.15 \pm 1.50	24.26 \pm 0.76

Franz diffusion cell experiments using artificial membranes

In addition to the dermatomed human skin approach, the four formulations were tested in triplicate on Franz diffusion cells using an artificial membrane as diffusion barrier and again, the cumulative amount of testosterone in the receptor fluid was plotted against time (Figure 2).

In contrast to the skin experiments, the data obtained could not be modelled directly over the complete 24 hours experimental domain by the standard steady-state linear regression due to the non-linear behaviour, with an initial linear part, a bending point region (22) and a final linear part. However, both selected linear time-regions were suitable for separate linear regression analysis: the initial 2 hours and from 8 till 24 (final) hours.

Moderate to good linear coefficients of determination (R^2 ranging from 0.9296 to 0.9999) were obtained for both selected time regions and therefore, initial and final K_p-values were calculated from both experimentally obtained J values as before (Table 3). As expected, lag times

for the initial linear part of the membrane studies were much lower compared to the human skin penetration behaviour.

Values of the discriminating power criterion

The calculated values for the response function F(K_p) are given in Table 4. Values are ranging from 0.12%, indicating that this FDC system is not capable of discriminating the different formulations, to 28% where a different behaviour of the formulations could be observed. For quality control (QC) purposes, the systems with the highest F(K_p) values are preferred, as these will be the most discriminating.

DISCUSSION

Being able to consistently observe differences in transdermal/membrane behaviour of similar transdermal products is useful in quality control (QC) environment, where the concept of clinically overdiscriminating *in vitro* tests is superfluous: whenever significant differences are observed,

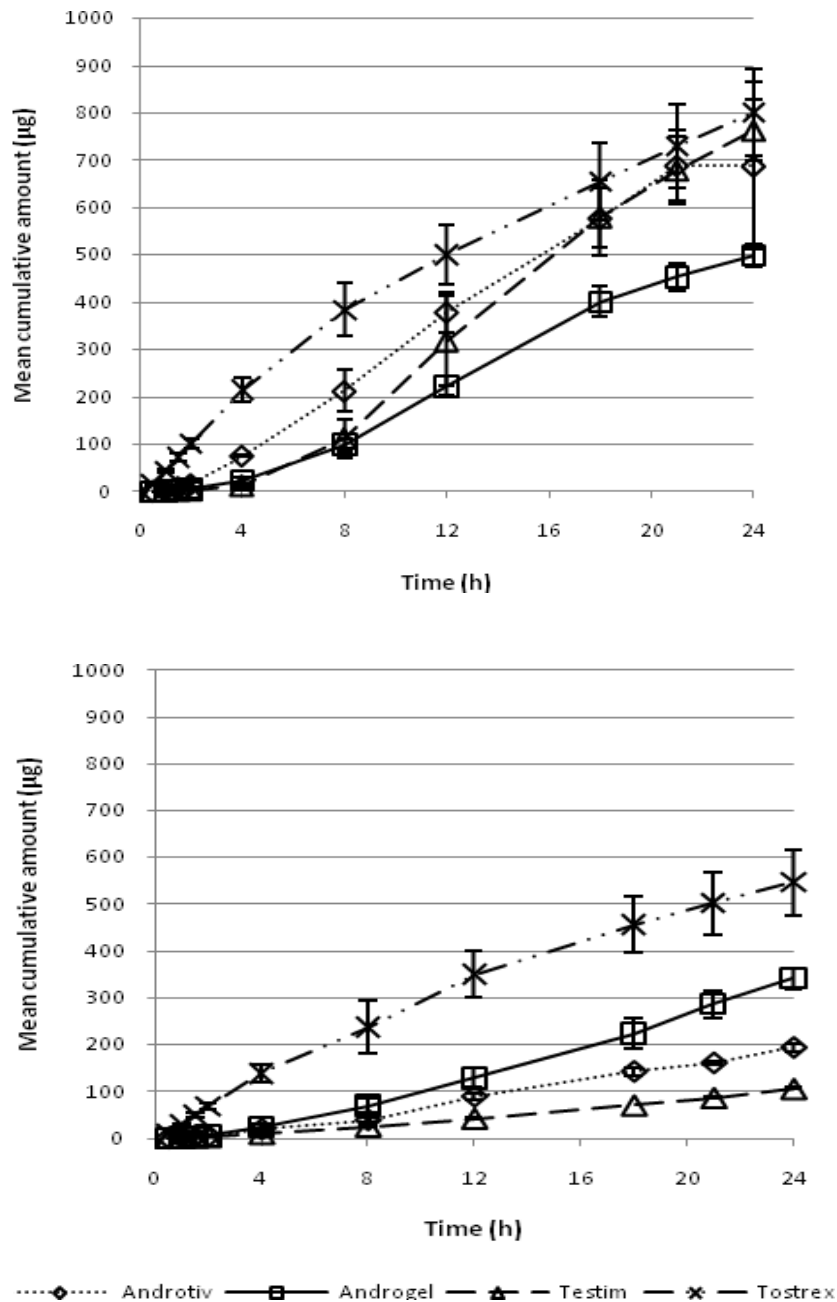


Figure 2. Cumulative amount of testosterone in the receptor fluid (µg) versus time (h) curves using artificial membranes and PBS + HPBCD (upper panel) or PBS + BSA (lower panel) as a receptor fluid.

pharmaceutical non-equivalence should be concluded. For this QC-objective, we were especially looking at the discriminating power of the Franz diffusion cell methods, which are recommended as global quality tests for transdermal products, similar to dissolution for oral solid dosage forms. To evaluate this discriminating aspect in an

objective and quantitative way, a new criterion $F(K_p)$ is proposed: the percentage equal-spread of the rescaled K_p values obtained in the different conditions investigated. We have evaluated the practical usefulness of this criterion in a study evaluating the choice of the receptor fluid.

Table 4. F(Kp) values (percentage spreading) on normalized Kp values for the testosterone preparations investigated (n = 4).

Membrane System	EtOH/H ₂ O	PBS + 5% BSA	PBS + 0.5% HPBCD	SBF + 5% BSA	SS + 0.5% HPBCD
Skin Kp	9.42	0.58	7.13	0.47	28.04
Membrane initial Kp	3.46	0.12	0.75	0.36	2.50
Membrane final Kp	6.23	0.74	23.18	1.75	11.85

Table 5. F(Kp) values for different spilanthal formulations (n = 3).

Formulation	Receptor fluid	Kp (10 ⁻⁴ cm/h) ± S.D.	F(Kp)
65% EtOH	PBS	4.38 ± 2.82	14.08
10% PG		15.34 ± 1.50	
Commercial extract	PBS + HPBCD	3.56 ± 2.17	10.42
65% EtOH		4.38 ± 2.84	
10% PG	EtOH/H ₂ O	16.62 ± 1.85	0.45
Commercial extract		4.95 ± 3.31	
65% EtOH		0.70 ± 0.25	
10% PG	EtOH/H ₂ O	5.46 ± 1.60	0.45
Commercial extract		0.64 ± 0.31	

Spilanthal application

Based upon the Kp values as experimentally obtained (18), the F(Kp) values were calculated for each of the three FDC test systems (Table 5).

The choice of the receptor solution is indeed an important determinant in the design of an *in vitro* model for percutaneous penetration, as the receptor phase interacts directly with the membrane (artificial or skin) and eventually with the formulation in the donor phase, as well as collects the drug penetrated through the skin/membrane (23). Depending upon the test conditions chosen, different conclusions about the release/permeation characteristics of the formulation can be made (24). The generally accepted OECD Guideline Number 428 on the Conduct of Skin Absorption Studies states that the selection of the receptor fluid must be compatible with the skin preparation and must meet the solubility requirements of the test substance (5). No further details on how this should be translated in practice are given. Therefore, researchers are free to choose the receptor medium they prefer for their selected application and intended objective. An overview of the most commonly used receptor media in recent (2007-2008) *in vitro* transdermal penetration literature studies (n=104) is given in Figure 3. The base solvent (left graph) is often supplemented by additives (right graph), mostly to increase the solubility of the test substance to obtain

sink conditions or to increase the stability of the system. Phosphate buffers, often resembling physiological conditions, are the most often used base solvents (60%), followed by non-buffered water (15%) or normal saline (sodium chloride) solutions (15%). Often, these phosphate buffers are derived from and are similar to those used in dissolution tests for oral dosage forms (25). To prevent bacterial degradation of the drug under investigation (26), antimicrobial preservative agents are used as an additive in 15% of the cases. Besides the maintenance of skin viability and integrity, a major requirement of the receptor fluid is to assure sufficient solubility of the test substance in order for the solubility not to limit the penetration (27). When the transdermal behaviour of compounds with low aqueous solubility is to be assessed, different solubilizers can be used to increase the solubility of the drug in the aqueous receptor medium while limiting skin interaction. Solubilization of poorly water soluble drugs is a frequently encountered challenge in the pharmaceutical industry and a number of solubilisation techniques have been developed (28). The solubility of poorly water soluble drugs can be increased by the addition of a water miscible solvent in which the drug has a good solubility. In transdermal penetration studies, solubility-increasing additives are used in 36% (n=37) of the literature reports. In these studies,

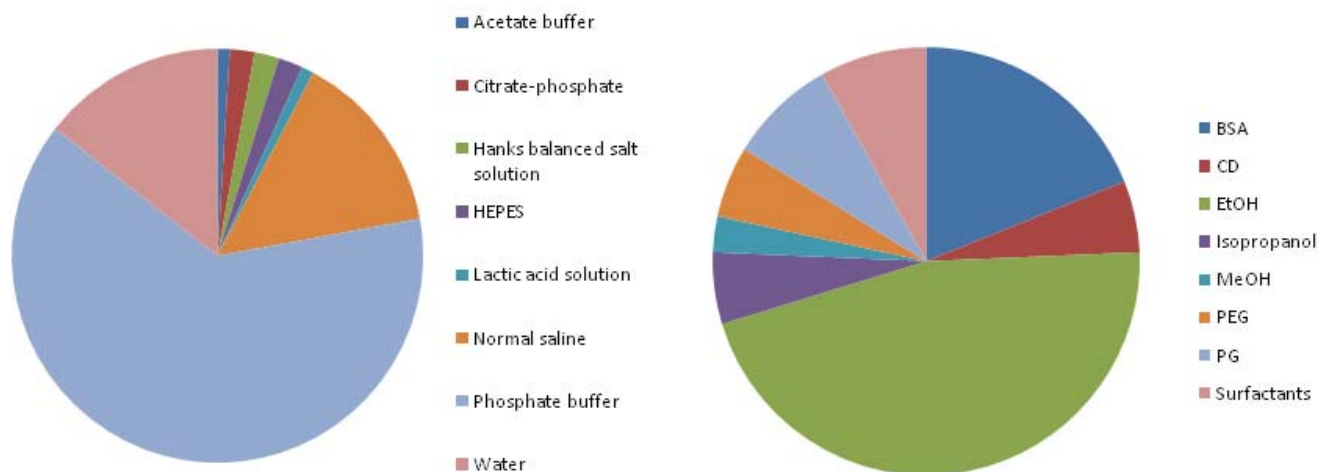


Figure 3. Overview of receptor media used in transdermal penetration studies. Left: base solvents. Right: solubility-increasing additives.

EtOH is most frequently used as a co-solvent (46%), but other solvents such as propylene glycol (PG) (8%), polyethylene glycol (PEG) (5%), isopropanol (5%), and even methanol (3%) are being employed. In 8% of the recent studies reported in the literature (period 2007-2008), surfactants like Tween20[®] or Brij98[®] have been used as a modifier to ensure adequate solubility of the test compound. Albumin binds a wide variety of hydrophobic ligands, including steroids, fatty acids, retinoids, thyroid hormone, prostaglandins and antibiotics (29). Because serum contains around 3 to 5% m/V albumin (21), addition of similar quantities BSA to the receptor medium contributes to its *in vivo* relevance. It is also recommended by the OECD as a solubilising agent and was used in almost 20% of the recent studies to increase solubility of lipophilic compounds. Alternatively, cyclodextrins can be used as they possess a lipophilic cavity and a hydrophilic exterior, thus making them ideal to form inclusion complexes with non-polar molecules in relatively polar solvents like water (30). This complexation has been applied as one of the most useful ways to improve the dissolution of poorly water-soluble drugs in intravenous and oral formulations (31). Up till now, they are only scarcely used in Franz diffusion cell experiments (approximately 5% of the literature studies), but are promising as solubilizing

agents in the field. HPBCD is a chemically modified cyclodextrin with high aqueous solubility (32). It is already successfully used in transdermal formulations (33), as well as in dissolution media for the evaluation of tablets for oral use (34).

When using human/animal skin, interactions of the receptor medium with the contacting lower skin layers can modify these layers (*e.g.* precipitation of proteins or dissolution of specific endogenous skin compounds) and consequently, the apparent diffusion characteristics of the skin for the test compound can change. This is not the case when using artificial membranes, which remain inert towards the receptor medium composition. In this case, a more direct interaction of the receptor medium with the formulation itself is expected. The homogeneous artificial membrane is thus more a passive diffusion barrier only, while the heterogeneous skin can additionally have an active role. While the above considerations are important in IVIVR, assuring product consistency during development and marketing is the key concern in pharmaceutical quality, with no obligatory link to the *in vivo* relevance. Therefore, in the QC-field, tests are expected to be maximally discriminating. The criterion $F(K_p)$ is a measure for the percentage spread of the normalised K_p values obtained in the different conditions investigated. It can be used to evaluate the discriminating effect of the FDC

method, i.e. the ability to distinguish different preparations as separate items. The maximal F(Kp) value that can be reached theoretically is 100%, meaning that the Kp-values obtained are maximally different. On the other hand, if the Kp-values found for two different preparations are identical, the test is not discriminating and the F(Kp) value obtained in this situation will be zero. In other words, the F(Kp) value indicates how the operational conditions employed during FDC experiments affect the ability of the test to discriminate different preparations.

Using our criterion F(Kp), the lowest responses were obtained with the membrane initial Kp values. Therefore, irrespective of the receptor medium used, only drastic changes in the diffusion behaviour can be seen on initial membrane Kp values, while less pronounced effects are masked and not differentiated. Despite the fact that the evaluation of the initial kinetics has the advantage of not being influenced by solubility constraints and of a relatively short time period which is well suited for QC purposes, our criterion quantitatively shows this approach is not useful. When considering skin Kp or membrane final Kp values, a better spreading and hence discriminating power was observed. Depending upon the type of receptor medium used, differences in the discriminating power of the testosterone FDC experiments were observed (Table 4). The highest discriminating response was obtained using 0.5% HPBCD containing media (up to 28.04%), while the discriminative power of the test was relatively small when using BSA-containing media (< 1.75%). For EtOH/H₂O, the F(Kp) value was intermediate (up to 9.42%). A different situation was observed for the spilanthal samples (Table 5): best discriminating results were obtained using PBS without solubilizers as a receptor medium (14.08%), followed by PBS containing 0.5% m/V HPBCD (10.42%). When using EtOH/H₂O (30/70, V/V), the test was not discriminative (0.45%).

CONCLUSIONS

For quality purposes of transdermal products, a quantitative and objective discriminative power criterion is proposed based upon the observed spreading of Kp-values. This criterion is a simple, reliable and reproducible characteristic that is experimentally obtained from the generally

accepted Franz diffusion cell test for the assessment of the transdermal behaviour of molecules. The practical applicability was demonstrated by evaluating the influence of the receptor medium composition on the discriminative power of the test system for both testosterone and spilanthal containing formulations. The best results were obtained with PBS supplemented with HPBCD for testosterone and without HPBCD for spilanthal.

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ABBREVIATIONS

AAPS	American Association of Pharmaceutical Scientists
API	Active Pharmaceutical Ingredient
BSA	Bovine Serum Albumin
CD	Cyclodextrine
COLIPA	European Cosmetic Toiletry and Perfumery Association
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EtOH	Ethanol
FDA	Food and Drug Administration
FDC	Franz Diffusion Cell
HPBCD	Hydroxypropyl- β -cyclodextrine
IVIVR	<i>In Vitro</i> – <i>In Vivo</i> Relationship
Kp	Permeability Coefficient
MeOH	Methanol
OECD	Organisation for Economic Cooperation and Development
PEG	Polyethylene Glycol
PG	Propylene Glycol
QC	Quality Control
S.D.	Standard deviation
TDS	Transdermal Delivery System

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