Department of Pharmacy, Ernst Moritz Arndt University, Greifswald, Germany

Influence of different test parameters on *in vitro* drug release from topical diclofenac formulations in a vertical diffusion cell setup

S. KLEIN

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Prof. Dr. Sandra Klein, Department of Pharmacy, Institute of Biopharmaceutics and Pharmaceutical Technology, Center of Drug Absorption and Transport, Felix Hausdorff Street 3, 17487 Greifswald, Germany
sandra.Klein@uni-greifswald.de

Dedicated to my co-editor Theo Dingermann, Frankfurt, on the occasion of his 65th birthday.

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In the past decades, the vertical diffusion cell has emerged as a useful device for testing drug release of topical dosage forms. However, to date neither a general USP method nor formulation-related monographs have been published in international pharmacopoeia. The purpose of the present work was to examine the influence of different test parameters in a vertical diffusion cell setup on *in vitro* drug release from semi-solid preparations for cutaneous application. Diclofenac was selected as the model compound. Release experiments were performed in a 7 ml Microette® vertical diffusion cell system. Various test parameters, including the media composition and pH, degassing, membrane material and pore size, stirring speed and stirrer type, were varied. Results obtained with different test parameter settings clearly indicate that both drug properties and instrumental details can have a huge impact on the outcome of *in vitro* diffusion/drug release studies with the vertical diffusion cell. Thus, the selection of adequate test parameters is crucial for the success of the release experiments and, as shown in the present study, optimal test parameters/conditions need to be established and validated on a case by case study.

1. Introduction

Since many years the vertical diffusion cell, also called Franz diffusion cell (Franz 1975) has been used in studying skin penetration of drug molecules. This simple *in vitro* method can be very useful in measuring the rate and extent of drug transport across the skin and/or permeation into the skin. Provided that a relevant skin model is applied, it represents a useful tool for estimating the percutaneous drug absorption, particularly when the aim is to evaluate new drug candidates or to screen new drug formulations for cutaneous application (Godin and Touitou 2007). In the past decades, the use of the vertical diffusion cell also came into the focus of product performance testing of topical and transdermal drug products. However, when the *in vitro* test is intended to assess the quality of a topical or transdermal product directly after manufacture, during shelf life or after scale up and post-approval changes (SUPAC), a skin-based diffusion model is not longer applicable. Test methods utilizing human skin or animal skin are for sure required to understand the processes, pathways and driving forces of various agents across the skin of the target species (Godin and Touitou 2007), but due to the large intra- and even more interspecies variability in skin properties, are not applicable for generating reproducible and robust test conditions that are crucial for quality control (QC). Thus, for QC purposes, where the objective is to focus on the physical attributes (solubility, microscopic viscosity, emulsion state, particle size etc.) that can affect drug release from a semi-solid formulation (Flynn et al. 1999), skin has to be replaced by an appropriate membrane. Moreover, in such experiments all test parameters relevant to drug release must be well defined to obtain a discriminating and robust QC test method.

There was an ongoing discussion on how to establish an official test method ensuring the product performance of semi-solid dosage forms. As a result, the draft of a general chapter *Topical and Transdermal Drug Products - Product Performance Tests <725>*, describing the apparatus and procedures that can be used to evaluate the *in vitro* performance of topical and transdermal products, was published in the Pharmacopeial Forum 2009 (USP 2009). Amongst other apparatus, in this chapter application of the vertical diffusion cell is described. However, this general chapter did not yet become official. Thus, to date, the FDA *SUPAC-SS Guidance for Industry: Nonsterile solid Dosage Forms "Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation* (FDA 1997) is the only official guidance discussing the use of the vertical diffusion cell for QC purposes. But, even in this guidance recommendations rather than detailed specifications of the essential test parameters can be found.

A multitude of methods using the vertical diffusion cell for examining both skin penetration of drug molecules to estimate the effectiveness of a new formulation and, drug release from semi-solid preparations for QC purposes can be found in the scientific literature. However, a detailed screen of some of these case studies (Pillai et al. 2001; van de Sandt et al. 2004; Khan et al. 2011; Chen et al. 2012) chosen in a random manner reveals that the test conditions are heterogeneous and often not presented in all details relevant for assuring the validity of the method. The studies are particularly lacking in information on the hydrodynamics in the acceptor phase. Thus, with respect to QC purposes, it is not clear, if sink conditions were provided in such experi-

ments. Moreover, even in recent case studies, the application of relatively low stirring speeds in the range of 100 rpm (Manosroi et al. 2012) to 300 rpm (Lucero et al. 2013) is reported. Depending on the cell dimensions and the stirrer type, low stirring speeds could bear the risk of insufficient mixing of the acceptor fluid which again could affect the diffusion rate. It thus is not clear, if the outcome of some of such studies is 100 % representative of the impact of formulation parameters on drug release.
Since diffusion experiments have evolved into important methods for examining cutaneous/transcutaneous drug administration, in the last decade the importance of setting adequate test parameters and validating VDC methods was highlighted by several research groups (Shah et al. 1999, 2003; Ng et al. 2005; Ueda et al. 2009; Ng et al. 2010, 2012).
The purpose of the present work was to examine the influence of different test parameters on the *in vitro* drug release profiles from semi-solid diclofenac preparations for cutaneous application obtained in a vertical diffusion cell setup.

2. Investigations results and discussion

2.1. Impact of membrane material and pore size

Voltaren® Emulgel® and Diclofenac-ratiopharm® Gel, two gel formulations with different composition and consistency, were used to study the impact of membrane material and pore size on the *in vitro* diffusion rate. Phosphate buffer pH 7.4 was used as the acceptor phase and the stirring speed was 800 rpm. According to the FDA SUPAC-SS guidance (FDA 1997), the release rates were plotted as the slope of the regression of the cumulative amount released versus the square root of time. The results are presented in Fig. 1.
The linearized diffusion profiles indicate that both material and pore size can have an impact on the *in vitro* diffusion rates of the diclofenac gel formulations. Whereas there was no big difference in the results obtained with most of the membranes tested, diffusion rate from both formulations was by far higher when using the PET membrane. However, this is most likely an artefact resulting from the handling of this membrane. As it was very difficult to fix this membrane in place and since the membrane was subject to crinkling, it was not used for subsequent studies Overall, the polysulfone (Tuffryn®) membrane was regarded as the most suitable membrane and was thus used for subsequent screening experiments.

2.2. Impact of acceptor-media pH

Diclofenac is a weak acid with the sodium salt having a pKa of 4.0 at 25 °C in water. Based on the acidic properties of the drug it was anticipated that the acceptor-medium pH would have an impact on the diffusion rate. To screen the impact of the acceptor-media pH on the diffusion rate, drug release from Voltaren® Emulgel® and Diclac® Schmerzgel was examined using three different acceptor phases of pH 7.4, 6.5 and 5.0. The Tuffryn® membrane was used to separate formulation and acceptor phase and the acceptor phase was agitated with magnetic bar and helix stirrer at 800 rpm. The results are shown in Fig. 2.
As expected, for both formulations the highest drug release rate was obtained in phosphate buffer pH 7.4. Applying acceptor media with pH values closer to the pKa of the drug resulted in a slow-down in the release rate, which can particularly be seen in the release profile of Diclac®. In the pH 5.0 buffer the drug concentration reached a plateau which reflects the poor solubility of diclofenac at this pH and also indicates that this buffer is not appropriate when the experiment is intended to examine drug release under sink conditions. To achieve sink conditions, the acceptor medium must have a high capacity to dissolve or carry away the drug, and the drug concentration in the acceptor medium should not exceed 10 % of the drug solubility in the releasing matrix (USP 2009). As sink conditions are hard to define for new drug candidates, before starting a release experiment solubility of the compound should be determined in the proposed acceptor fluids to ensure selection of the right acceptor medium. For acidic drugs like diclofenac, the acceptor media should be pKa + at least 2 pH-units when the intention is to observe diffusion that is not hindered by pH-dependent dissociation (as can be calculated with the *Hendersson-Hasselbalch* equation) and solubility of the drug compound.

2.3. Impact of degassing

Various experiments were performed with and without degassing the acceptor phase prior to use (data not shown here). Whenever the medium was not degassed and gas bubbles showed up under the membrane, this resulted in a – depending on the number and the size(s) of the gas bubble(s) – by far lower diffusion rate. Nevertheless, plotting the μg drug released over the square root of time still resulted in a linear relationship. However, these experiments clearly indicated the importance of degassing. In addition, we could observe that the degassing method described in the USP (USP 2012) is an effective method for the preparation of media to be used in diffusion cell experiments.

2.4. Impact of stirring speed and stirrer type

The impact of applying different stirring speeds and using different stirrer types, i.e. a magnetic stirring bar with and without an additional helix stirrer (see Fig. 6) was studied using Voltaren® Emulgel® as the test formulation. It could be clearly shown that both the stirring speed and the type of stirrer have an impact on the release/diffusion rate (Fig. 3).
The results shown in the left panel of Fig. 3 indicate that the use of a simple magnetic stirring bar at the bottom of the diffusion cell does not represent an optimal setup since particularly at low stirring speeds, the hydrodynamics in the cell are not sufficient for adequate mixing. The profiles shown in Fig. 3 also indicate that a critical stirring speed exists for each stirrer type. Adequate mixing with the magnetic stirring bar required very high stirring rates (> 800 rpm). Using magnetic stirring bar and helix stirrer, at a stirring speed of 200 rpm poor diffusion and high standard deviations could be observed. However, increasing the stirring speed resulted in reproducible release rates with the profiles being superimposable at stirring speeds equal or higher than 600 rpm. For this reason, the helix stirrer setup, run at 800–1000 rpm should be a good choice for QC experiments.

2.5. Drug release from marketed semi-solid diclofenac formulations under optimized conditions

Based on the results obtained in the first set of experiments, the following test parameters were regarded as being applicable for obtaining reliable and reproducible *in vitro* release profiles from semi-solid diclofenac formulations for cutaneous application: 32 °C, a phosphate buffer pH 7.4, a polysulfone membrane with 0.45 μm pore size and, a magnetic stirring bar with helix stirrer driven at 800 rpm. Release experiments with these settings should be capable of detecting changes in the finished product's drug release characteristics as requested in the USP general chapter <725> draft (USP 2009). These test parameters were thus applied to a) a freshly prepared aqueous diclofenac sodium solution (pH 7.4), b) marketed diclofenac gel formulations and c) marketed diclofenac medicated plaster formulations to exam-

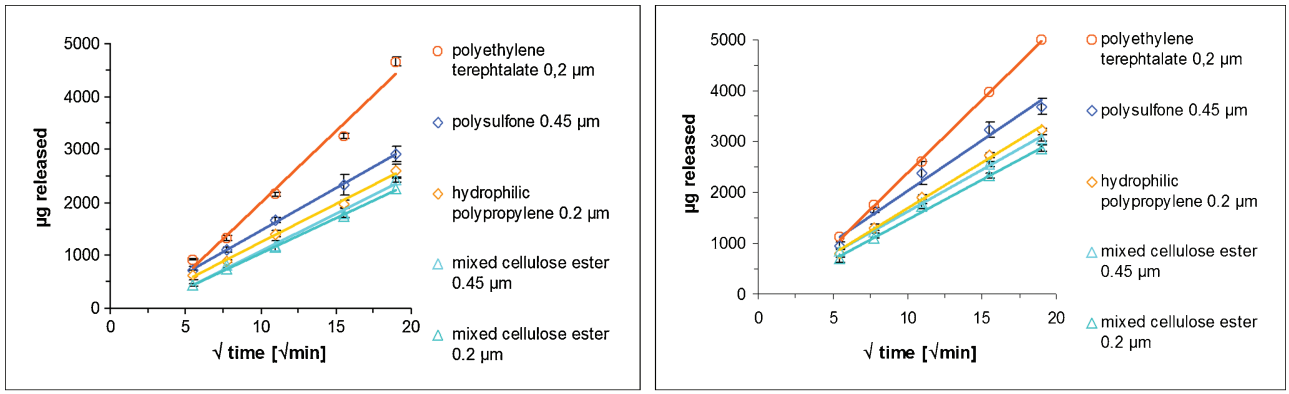


Fig. 1: Impact of membrane material and pore size on drug diffusion from Voltaren® Emulgel® (left panel) and Diclofenac-ratiopharm® Gel (right panel), pH 7.4, 800 rpm, mean (n = 3) ± S.D.

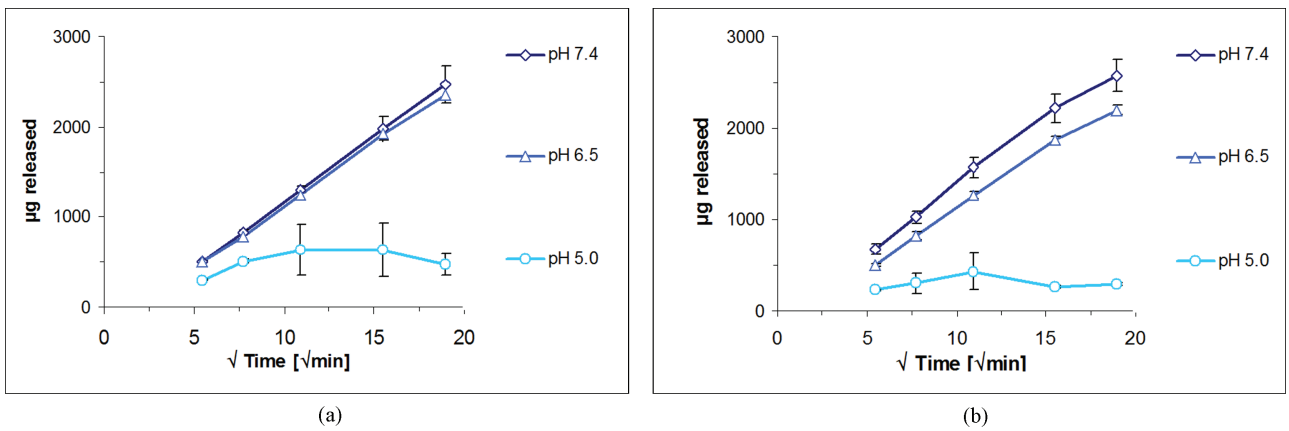


Fig. 2: Impact of acceptor-media pH on drug diffusion from Voltaren® Emulgel® (left panel) and Diclac® Schmerzgel (right panel), 800 rpm, Tuffryn® membrane, mean (n = 3) ± S.D.

ine, if different drug release characteristics can be determined. The resulting release profiles are presented in Figs. 4–5.

Figure 4 shows the release profiles obtained from the diclofenac sodium solution and the gel formulations. As can be clearly seen, the membrane had no impact on drug diffusion from the liquid formulation, indicating that an adequate membrane was chosen. Among the gel formulations, the Diclofenac-ratiopharm® gel showed the highest drug release rate. This is in good agreement with the consistency of the gel which was significantly lower than that of all other formulations tested. The remaining formulations showed similar release profiles. Voltaren® Emulgel® and Voltaren® Schmerzgel resulted in even superimposable profiles and the same observation was made for the profiles of arthrex® Schmerzgel, Diclac® Schmerzgel and Sandoz Schmerzgel. After a closer look at the composition of these formulations (see Table), this observation is not astonishing since there is no difference in the inactive ingredients of the two Voltaren® products and the same is true for the inactive ingredients of arthrex® Schmerzgel and Sandoz Schmerzgel. The similarity of the release profiles of the latter two formulations to that of Diclac® Schmerzgel cannot be explained by the composition of the product, but as the products are registered for the same indication, it is likely that they were designed to be bioequivalent which is then likely to be

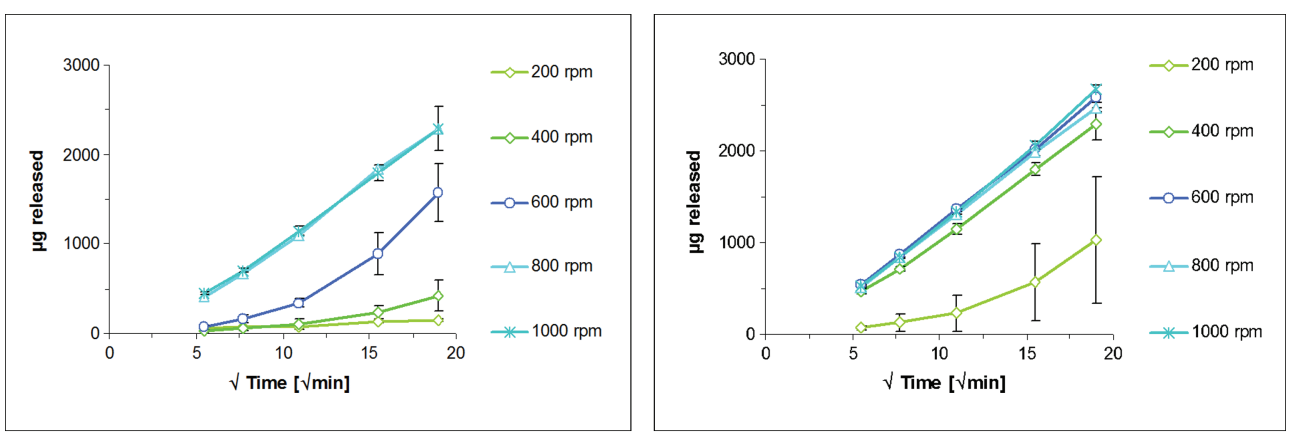
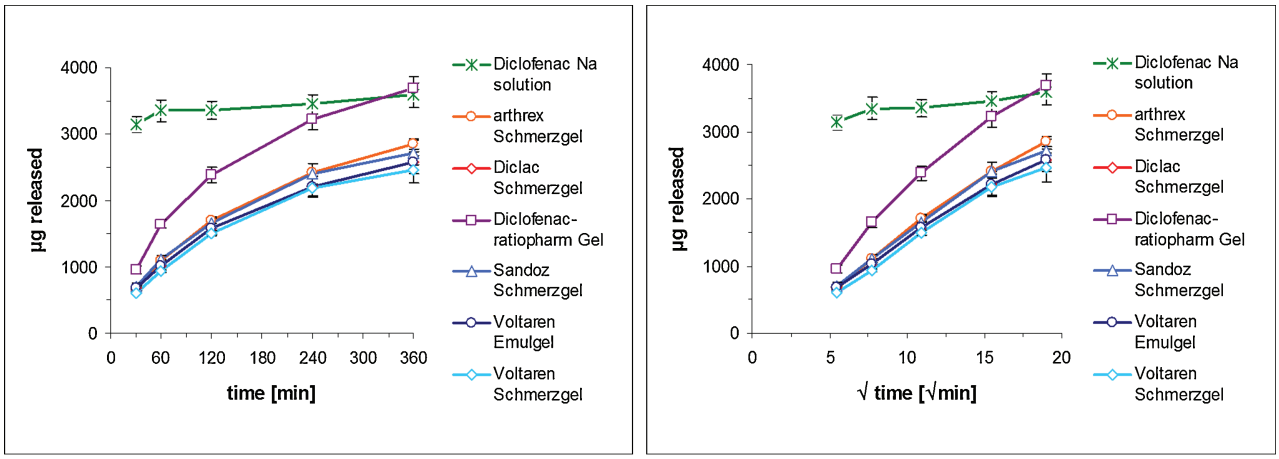
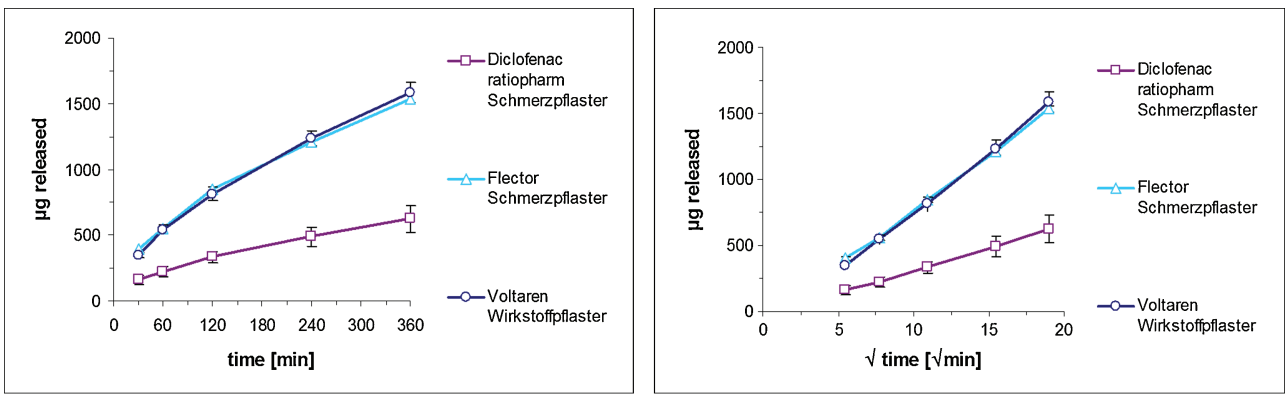


Fig. 3: Impact of stirring speed and stirrer type on drug diffusion from Voltaren® Emulgel®: stirring bar without helix stirrer (left panel) and stirring bar with helix stirrer (right panel), pH 7.4, 800 rpm, Tuffryn® membrane, mean (n = 3) ± S.D.



(a) (b)

Fig. 4: Drug diffusion/release versus time (left panel) and versus square root of time (right panel) from a diclofenac solution and several marketed semi-solid diclofenac gel formulations, 32 °C, pH 7.4, Tuffryn® membrane, stirring bar with helix stirrer, 800 rpm, mean (n = 3) ± S.D.



(a) (b)

Fig. 5: Drug release versus time (left panel) and versus square root of time (right panel) from marketed diclofenac medicated plaster formulations, 32 °C, pH 7.4, Tuffryn® membrane, stirring bar with helix stirrer, 800 rpm, mean (n = 3) ± S.D.

reflected in the *in vitro* release profiles obtained with the present setup.

In Fig. 5 the release profiles of the medicated plasters are shown. It becomes obvious that also two of the three diclofenac plaster formulations (Flector® Schmerzpflaster and Voltaren® Wirkstoffpflaster) show superimposable release profiles. However, since based on the information given in the package insert, these two formulations consist of identical diclofenac salts and identical inactive ingredients, it is most likely that the formulations are completely identical and thus also exhibit identical release behaviour. In contrast, drug release of Diclofenac-ratiopharm® Schmerzpflaster was characterized by a much lower release rate which is in good agreement with the composition of this medicated plaster formulation (different diclofenac salt and different inactive ingredients).

Overall, the release profiles obtained in the plaster diffusion experiments indicate that the plaster matrix controls drug release over the entire test period.

Comparing the release profiles of the diclofenac gel formulations with those of the medicated plaster formulations, it becomes obvious that the formulations exhibit different release kinetics. Most of the release profiles obtained from the gel formulations can be linearized by plotting the cumulative drug release versus the square root of time, indicating the momentary rate of release being proportional to $1/\sqrt{t}$. This release behavior is typical for solution-, suspension- or emulsion type semi-solid systems. In contrast, almost zero-order drug release could be observed from the medicated plasters where the release rate is controlled by the plaster matrix.

Overall, the results obtained with the optimized test parameters indicate a robust and discriminating *in vitro* method that is appli-

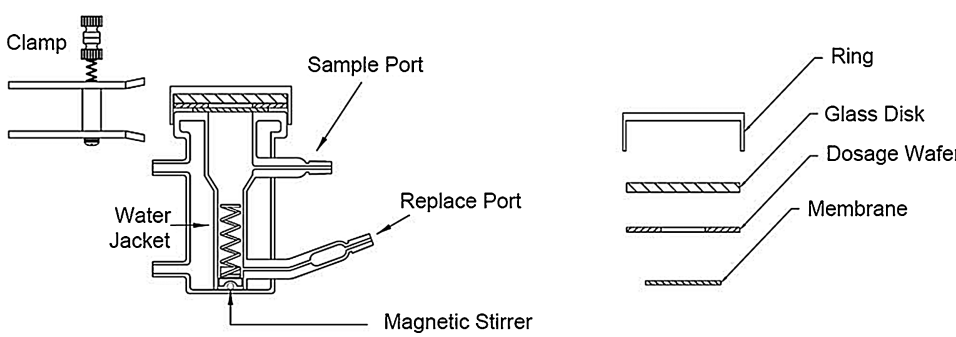


Fig. 6: Microette® vertical diffusion cell (Diagram courtesy of Hanson Research Corporation).

Table 1: Composition of the tested gel formulation as indicated by the manufacturer

Ingredients (from manufacturer's label)	arthrex Schmerzgel	Diclac Schmerzgel	Diclofenac-ratiopharm Gel	Sandoz Schmerzgel	Voltaren Emulgel	Voltaren Schmerzgel
Diclofenac diethylamine salt					x	x
Diclofenac sodium salt	x	x	x	x		
Carbomer 974 P					x	x
Octanoic acid / decanoic acid esters					x	x
Cetomacrogol					x	x
Diethylamin					x	x
Isopropanol (Propan-2-ol)	x	x	x	x	x	x
Paraffine					x	x
Perfume		x			x	x
Propylen glycole	x		x	x	x	x
Purified water	x	x	x	x	x	x
Ammonium hydroxide		x				
Carbomer 980		x				
Decyloleate		x				
Sodium edetate		x				
Octyl dodecanol		x				
3-*sn*-Phosphatidylcholine		x				
RRR-α-Tocopherole		x				
Hypromellose	x		x	x		
Poly(oxyethylen)-7-Glycerol(mono,di)alcanoat (C8-C18)*			x			
Macrogol glycerol cocoate*	x			x		
pH (± 0.5)	6.5	7.0	6.5	6.5	6.5	6.5

* most probably the same.

cable to detect different drug release mechanisms and release properties of semi-solid diclofenac formulations for cutaneous application.

3. Conclusion

The present series of experiments focused on basic aspects but was very useful to elucidate typical pitfalls in the course of developing *in vitro* release test methods for semi-solid dosage forms for cutaneous application. The results obtained with different test parameter settings indicate that the selection of adequate test methods is crucial for the success of the release experiments. Therefore, results from the present study might be very helpful for the future development of *in vitro* release methods for semi-solid formulations. However, since both drug properties and instrumental details can have a huge impact on the outcome of *in vitro* drug release studies, the optimal test parameters established for the present task should not simply be adapted to other experiments since they depend on both the dimensions of the vertical diffusion cell and the drug properties. As has been done here, optimal test parameters/conditions should be established and validated on a case by case study. Last but not least when publishing the setup and the results of a diffusion cell experiment, it is crucial to report all relevant test parameters to ensure the validity of the reported test results.

4. Experimental

Diclofenac, an acidic, poorly soluble, non-steroidal anti-inflammatory drug, was used as the model compound. Experiments were performed with marketed semi-solid dosage forms for cutaneous application. In these formulations, diclofenac is available as the sodium-, diethylamine- or epolamine salt. Diclofenac sodium, lot # 180900 / PD004133, CFM Farmaceutica Milanese S.p.A., Milano, Italy was used as the standard substance for analysis.

4.1. Products studied

4.1.1. Gel formulations and their ingredients

- arthrex® Schmerzgel (10 mg diclofenac sodium/g gel), lot # 6R0877, 1A Pharma, Oberhaching, Germany
- Diclac® Schmerzgel (10 mg diclofenac sodium/g gel), lot # 1108064, betapharm Arzneimittel GmbH, Augsburg, Germany
- Diclofenac-ratiopharm® Gel (10 mg diclofenac sodium/g gel), lot # G10998, ratiopharm GmbH, Ulm, Germany
- Sandoz Schmerzgel (10 mg diclofenac sodium/g gel), lot # 62319, Sandoz Pharmaceuticals, Ismaning, Germany
- Voltaren® Emulgel® (11.6 mg diclofenac diethylamine/g gel, corresponds to 10 mg diclofenac sodium), lot # W3388, Novartis Pharma, Nürnberg, Germany
- Voltaren® Schmerzgel (11.6 mg diclofenac diethylamine/g gel), lot # W4502, Novartis Pharma, Nürnberg, Germany

4.1.2. Medicated plasters

- Diclofenac-ratiopharm® Schmerzpflaster (140 mg diclofenac sodium/ plaster (10x14 cm), lot # 100806, ratiopharm GmbH, Ulm, Germany
- Flector Schmerzpflaster (180 mg diclofenac epolamine, corresponds to 140 mg diclofenac sodium/ plaster (10x14 cm), lot # 080705, IBSA Farmaceutici Italia Srl, Lodi, Italy
- Voltaren® Wirkstoffpflaster® (180 mg diclofenac epolamine/ plaster (10x14 cm), lot # 060525, Novartis Pharma, Nuernberg, Germany

4.2. Membranes studied

- GH Polypro, 0.45 μm, hydrophilic polypropylene, Pall Life Sciences, Ann Arbor, MI, USA
- ME 25®, cellulose acetate/nitrate (20/80), 0.45 μm, Schleicher & Schuell, Dassel, Germany
- ME 24®, cellulose acetate/nitrate (20/80), 0.20 μm, Schleicher & Schuell, Dassel, Germany
- MEMFIL PET, polyethylene terephthalate, 0.2 μm, membraPure GmbH, Bodenheim, Germany

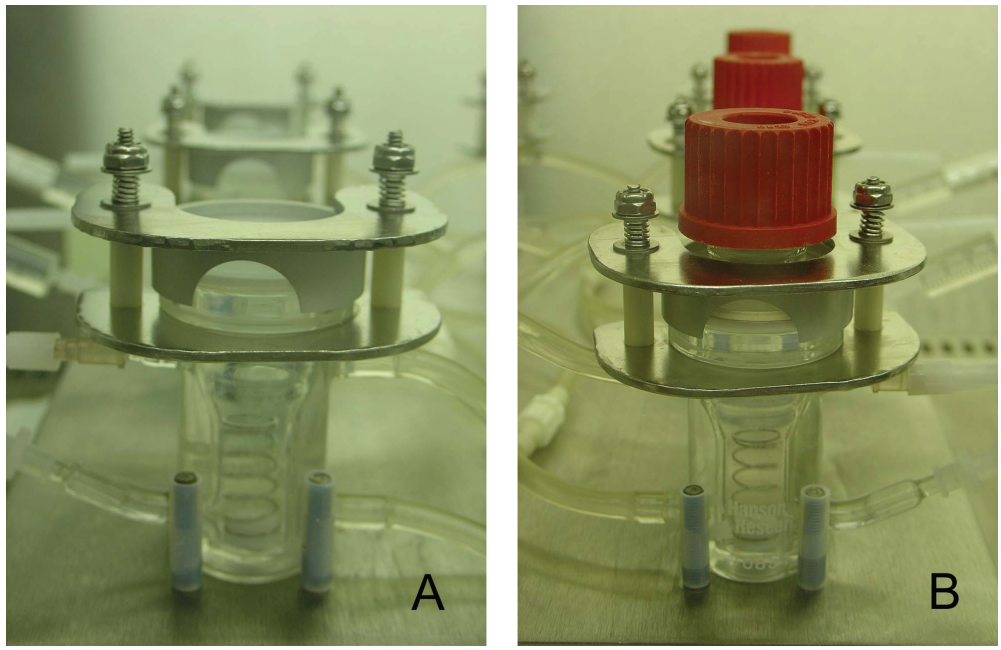


Fig. 7: Microette® vertical diffusion cell setup for semi-solid (A) and liquid (B) formulations.

- Tuffryn®, polysulfone, 0.45 μm, Pall Life Sciences, Ann Arbor, MI, USA

4.3.. Experimental setups

A 7 ml Microette® vertical diffusion cell system (Hanson Research Corporation, Chatsworth, CA, USA) with a 15 mm orifice was used for all experiments (see Figs. 6–7).

In the first set of experiments drug release of diclofenac gel formulations was tested while various test parameters were subject to changes. These included: (i) material and pore size of the membrane, (ii) pH of the acceptor medium, (iii) degassing of the medium, (iv) stirring speed, (v) type of stirrer. All experiments were performed in triplicate at a temperature of 32 °C and over a period of 6 h. The temperature was not varied but kept at 32 °C to represent the average surface temperature of the skin in all experiments. The major part of experiments was performed using phosphate buffer pH 7.4 USP 35 (USP 2012) as the acceptor medium. To examine the impact of the buffer pH on the diffusion rate, additional experiments were performed using an acetate buffer pH 5.0 and a phosphate buffer pH 6.5. All buffers had an osmolarity of ∼280 mOsmol/L.

In the second set of tests, drug release of a selection of marketed semi-solid diclofenac preparations for cutaneous application was examined. Experiments were performed at 32 °C using properly degassed phosphate buffer pH 7.4 as the receptor medium which was agitated with magnetic bar and helix stirrer at 800 rpm. Where a membrane was required, a polysulfone (Tuffryn®, 0.45 μm) membrane was used. Based on the observations made in the first part of the study, from a QC perspective these settings represent optimal, reproducible and reliable test conditions for comparing drug release of the diclofenac formulations tested.

In all experiments the cells were filled with the receptor medium and equilibrated to 32 °C. The *in vitro* experiments performed with the gel formulations required the use of a membrane to keep the product and the acceptor medium separate and distinct. Before starting the experiment, the membrane was soaked in receptor medium for approximately 30 min and then placed on a dosage wafer with a 15 mm orifice. Subsequently, membrane and wafer were placed over the cell opening. While fixing the dosage wafer with two fingers, an adequate amount of the gel formulation (∼300 mg) was placed upon the surface of the membrane. When the hollow in the wafer was completely filled and no air bubbles were visible, the sample was covered with a glass disk and a ring to hold the dosage wafer and the glass disk in position. Finally, the cell top was fixed with a clamp (see Fig. 7A) and stirring was initiated.

Gel formulations are hardly applicable to screen for an appropriate membrane since it then will be hard to distinguish whether diffusion is controlled by the gel matrix, the membrane or both. However, with the choice of an adequate membrane one can expect to observe unhindered drug diffusion from a drug solution. Thus, to prove the applicability of the selected polysulfone (Tuffryn®, 0.45 μm) membrane, an additional experiment was performed using a 300 μL of a freshly prepared solution of diclofenac sodium in phosphate buffer pH 7.4 (10 mg diclofenac sodium/g solution). The diclofenac concentration in this solution corresponded to that in the gel formulations and the sample size was chosen to approximate the diclofenac dose used in the gel experiments. While using the same membrane and wafer, a different cell top (see Fig. 7B) had to be applied to enable testing drug release from this liquid formulation.

No membranes and dosage wafers were required for determining drug release from medicated plasters. In these experiments, plasters were cut into circles with a diameter of 40 mm and directly attached to the cell top which has an outer diameter of 40 mm and an orifice of 15 mm in diameter. The plaster circles were then covered and fixed as described above, before stirring was initiated.

In all experiments, the diffusion surface was 1.77 cm^2. During a synchronized media replacement/sampling procedure samples (600 μl) were taken after 0.5, 1, 2, 4 and 6 h (FDA 1997). The first 200 μl of each sample were intended for flushing the sampling tube and therefore sent to waste. The following 400 μl were collected for subsequent quantification. After appropriate dilution, the samples were analyzed at a wavelength of 276 nm using a UV-spectrophotometer (U 2000, Hitachi Ltd, Tokyo, Japan).

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