Pharmaceutical Development and Technology

http://informahealthcare.com/phd
ISSN: 1083-7450 (print), 1097-9867 (electronic)

Pharm Dev Technol, Early Online: 1–7
 DOI: 10.3109/10837450.2014.908308

informa
healthcare

RESEARCH ARTICLE

Equipment and method for *in vitro* release measurements on topical dosage forms

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Abstract

Context: In countries where research budgets are meager as Argentina, the tendency to innovation and improvements in the designs prototypes ''made in Argentina'' marks a growing trend adopted by researchers. This article presents a diffusion cell of original design, for release studies of Active Pharmaceutical Ingredient (API) from classical topical dosage forms, also includes the methodology for its optimization and validation. The objective was to evaluate and validate a system designed and to compare it to the Franz cells system.

Methods: Parameters, reproducibility and robustness were performed included factors as, stirring conditions, membrane stabilization treatment and temperature variation. Release and retention on membrane assay were performed using two different API and formulations.

Results: The method is reproducible and robust for the parameters tested. Release assays show that no significative difference with the Franz Cells system. Our system allows the simultaneous measurement of different parameters, representing an innovation on these methodologies. The LMC was used for assays of *in vitro* retention on membrane and the values obtained were reproducible and coincident whit values obtained for other authors.

Conclusions: The system designed and the methodology employed, are acceptable for *in vitro* release studies. The device and method has the characteristics required.

Keywords

In vitro vertical diffusion cell, retention on membrane assay, residence time

History

Received 5 February 2014
Revised 21 March 2014
Accepted 22 March 2014
Published online 15 October 2014

Introduction

The skin is a membrane selectively permeable to chemicals that can be absorbed by this route to reach the systemic circulation. The use of *in vitro* static diffusion cells to assess skin permeability has evolved into an important research methodology, providing key insights relationships between skin, active pharmaceutical ingredient (API) and formulation[1,2]. Such testing is highly useful not only for the design and development of novel formulations but also for toxicity screening and quality-control purposes[3–6]. The control of substances diffusion for which pharmacological effect is only topical such as sunscreen agents or escabicides agents is possible by the use of *in vitro* release systems. It is especially important that the measurement of diffusion through membranes of substances by their chemical structure and metabolic residues they produce, resulting in some manner toxic to humans[7,8]. Within this context, *in vitro* Franz diffusion experiments has become into one of the most important methods for researching transdermal drug administration. However, many authors sustain that in general this type of testing, often yields permeation data that has poor reproducibility, even when synthetic membranes are used as barriers, thereby eliminating the variability associated to biological tissue[9–11]. Chien and Valia[12] have compared the hydrodynamics of this cell to their own design. They report that the architecture of the Franz cell does not provide adequate solution to hydrodynamics, mixing efficiency and temperature control that is required for quantitative permeation evaluations. As there are no general directives regarding which cells should be employed, researchers have designed their own systems[13–18], although there does exist a general series of considerations which must be taken into account to guarantee the reliability of the release tests. The speed of liberation depends, among other factors, on the temperature at which the test is performed, on the type of membrane employed its integrity over time and thickness. A very important and useful parameter is the residence time on the skin of APIs and this cannot be measured during diffusion experiments using Franz cells, due to their design, which does not allow the disassembling of the semi permeable membrane until the end of the experiment. This is a determining factor in the assessment figure formulation design, toxicity and quality control APIs require a long residence time on the skin and where the risk of systemic distribution[19].

The aim of this study was to evaluate and validate a system designed in our laboratory for research purposes and compared with the traditional diffusion system: Franz Cells. Parameters reproducibility and robustness were analyzed, included factors as,

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stirring conditions, membrane stabilization treatment and temperature variation. Synthetic membranes were used, in order to eliminate variability due to biological tissue. Synthetic membranes exhibit superior permeation data reproducibility as *in vivo* variables such as skin, age, race, sex and anatomical site are eliminated[20]. Furthermore, the commercial availability, stability, interbatch uniformity and ease of usage make the use of synthetic media highly desirable[21]. As model drugs, we used benzophenone-3 and permethrin which had been previously reported as useful tools in studies of this type of evaluation[22–25]. Benzophenone-3, a common organic sunscreen in cosmetics that can be adsorbed and it accumulates in the body after passing through the kidneys, where it is metabolized[26,27]. Skin permeation and penetration of the organic and inorganic sunscreens; have been deeply studied in order to determine their safety[28,29]. Several studies have confirmed the presence of benzophenone-3 and its metabolites in human urine 48 h after administration skin in cosmetic formulations[30–34]. Permethrin is an escabicides with low toxicity associated, used to treat head lice, scabies and spread in recent years for use in the treatment of malaria[35–37]. The control of substances diffusion for which pharmacological effect is only topical such as sunscreen agents or escabicides agents is possible by the use of *in vitro* release systems. It is especially important the measurement of diffusion through membranes of substances by their chemical structure and metabolic residues they produce, resulting in some manner toxic to humans.

This article presents an original diffusion system designed to perform release studies, membrane retention studies and residence time determination of API, it also includes the methodology used for optimization and validation. To support the validation of the method and equipment, parallel assays were performed using a USP Franz Diffusion cell set, previously validated. In countries where research budgets are meager as Argentina, the tendency to innovation and improvements in the designs prototypes made in Argentina marks a growing trend adopted by researchers. In this sense, the design and validation of CML, is the subject of a patent application (UNT-CONICET), Application Record in INPI No: 20130102901. The device designed, has the three characteristics required for direct transfer of technologies (Law 24.481), novelty, inventive step and industrial applicability.

Materials and methods

Tested formulation

The selected formulations were: (1) a commercial sunscreen cream containing benzophenone-3 (BZ-3: CAS No. 119-61-9) as API[19]; (2) a commercial gel for scabies treatment containing permethrin (Pm: CAS No. 52635-53-1) as single API. BZ-3 is a hydrophobic molecule while Pm is a hydrophilic one.

Laboratory made cells

This system was comprised of a group of static vertical diffusion cells ($n = 16$ for this work) made entirely of glass. Each cell comprised a donor vessel and an acceptor vessel with a horizontal membrane as interface between them. The acceptor vessel having a capacity of 10 mL was filled with an acceptor solution. A cellulose membrane acted as interface mounted above the acceptor vessel in contact with the acceptor fluid. Then donor and acceptor vessels were assembled using stainless steel tongs as support. At this time, a calculated amount of sample could be placed on the membrane. The entire equipment was placed in a plastic container as support and then was inserted in an orbital shaker. Constant temperature is achieved by using a stove. For this work, four samplings were made (2, 4, 6 and 8 h), at each time, four cells were withdrawn for measurements. Then a volume of 2 mL of acceptor solution of each of the cells were removed and absorbance measurement were made ($n = 4$). Each cell was removed from the experiment after the readings. Figure 1 shows an individual laboratory made cell (LMC). In addition to their supportive function stainless steel tongs, also made it possible to obtain a perfect match of the membrane with the equipment (Figure 1). The vertical position provided a means to eliminate air bubbles that might remain in the chamber receptor liquid interface and distort the results, which was the main disadvantage of this type of cell[14]. A cellulose acetate membrane was used (D9527 avg. flat width 43 mm (1.7 in; Sigma-Aldrich Chemical Co., St. Louis, MO) previously soaked for 24 h in the receptor solution. This membrane was chosen for its compatibility with the components of the semisolid formulation and of the receptor phase. In our trial, diffusion cells with identical characteristics were used, within a stove at constant temperature (FAL, Dique Chico, Cordoba, Argentina).

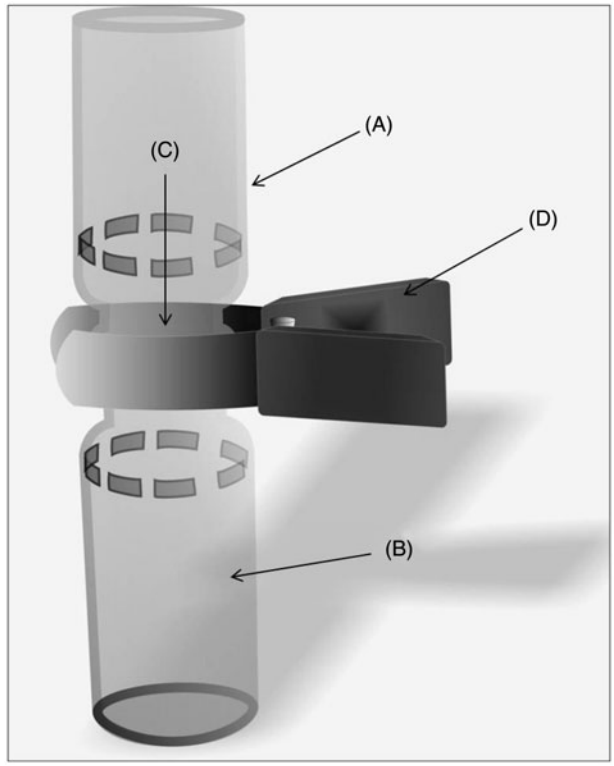

Figure 1. Individual Laboratory made cell. Donor vessel (A), acceptor vessel (B), interface membrane (C) and stainless steel tongs (D).

Acceptor solutions

For BZ-3 assays, we used propylene glycol:water, (3:2) as acceptor solutions. BZ-3 solubility in propylene glycol:water mixture was found to be about 30 times that in water[24].

For Pm assays, we used solution alcohol:propylene glycol:water (6:3:1) as acceptor solutions[38].

Validation of equipment and methods

Laboratory made cell dimension evaluations

Visual observations were used to classify the receptor cell bases as either flat or convex. Distilled water was filled carefully to the top of the compartment and the exact volume was recorded ($n = 6$). For measuring the internal diameters of the donor and receptor cells, a pair of calipers was placed on the inside lip. External diameter measurements of the receptor cells were

Table 1. Robustness of the method.

T (°C)	EH (cm)	AV (rpm) 80	100	120	140
29	0	107.34 ± 6.2	108.21 ± 9.2	108.43 ± 8.7	110.13 ± 4.7
	4	106.49 ± 8.9	105.21 ± 6.5	107.64 ± 3.7	108.43 ± 2.7
32	0	114.45 ± 5.6	116.52 ± 6.4	116.31 ± 5.8	114.87 ± 5.3
	4	115.45 ± 3.8	115.24 ± 4.4	116.42 ± 4.8	113.24 ± 4.5
35	0	143.78 ± 9.6*	143.49 ± 10.3*	142.67 ± 7.2*	145.78 ± 10.2*
	4	140.78 ± 7.9*	144.49 ± 9.8*	145.87 ± 10.2*	145.81 ± 9.6*

*Significantly different from control $p < 0.01$.

performed by laying the caliper tips on the outside of the lip. The effective diffusion area (EDA) was calculated for both donor and receptor cells using the formula πr^2, where r was the mean of the internal radius of cells, donor and receptor. The repeatability of measurements was calculated by using one-way ANOVA.

Validation tests

All parameters on the validation were measured by BZ-3 as API to diminish variation due to the change of drugs. Tests for reproducibility and robustness were performed. These are all included in the U.S.P. XXII directives for the validation of analytical methods used to characterize dosage forms (Category III; United States Pharmacopoeia, 1990). Once we conclude the LMC validation, we used a group of 4 Franz Cells for diffusion assays performed to compare our method with a validated known method in this case we used BZ-3 and Pm.

Robustness. This parameter was verified by the study of BZ-3 diffusion in presence of small variations in the main factors that would directly affect the results. These factors were: temperature (29–35 °C), agitation velocity (80–140 rpm), quantity of sample (0.15–0.25 g; data not shown), sample extraction height from the base of the apparatus (0 and 4 cm) and operator ($n = 2$). The quantitative variation of each of the factors was based on those alterations that might inadvertently occur during the test. The trial was carried out during four consecutive days (Table 1).

Reproducibility. Once we determinate the optimal conditions for the diffusion assay, the degree of reproducibility of the method was tested for simultaneously set up four diffusion cells for each sampling interval (16 for the entire assay), with an identical sample quantity (0.2 ± 0.01 g), shaking speed (120 rpm) and temperature (32 °C). The procedure was repeated six times. By comparing the results of the four diffusion cells for each interval, the degree of reproducibility was determined by calculate the mean of six assays for each time and using an statistical determination variation coefficient (VC%).

$$\text{VC\% was calculated according to: } \frac{\text{Standard deviation}}{\text{mean}} \times 100 \quad [1]$$

Diffusion assay. This assay was conducted using LMC. Cellulose membrane was mounted between the half-cells (donor and acceptor vessels) in contact with receptor fluid (propylene glycol:water, 3:2) and equilibrated for 1 h. The area available for diffusion was 1.8 ± 0.1 cm^2. Temperature fluid in the receptor compartment was 32 ± 1 °C. The amount of sample was placed in the donor compartment 0.2 ± 0.01 g. The entire assembly was kept in an orbital shaker (120 rpm), each test had a total duration of 8 h. At each time, four cells were removed from the system, aliquots (2 mL) of the receptor phase at specific time intervals (2, 4, 6 and 8 h), and was measured spectrophotometrically the diffusion of API through cellulose membrane at a wavelength (290 nm for BZ-3 and 219 nm for Pm), that was unaffected by other components of the formulations that might be released together with the API. Cumulative amounts of API (μg) permeated the unit diffusion surface (cm^2) was plotted against time (h; Figure 2 for BZ-3 and Figure 3 for Pm). In parallel, the same conditions were reproduced on a USP Franz Diffusion cell set and the data obtained were compared. All experiments were performed in triplicate ($n = 12$) and the results were expressed as mean ± SD. The flux (J_D) was determined from: the amount of API diffused divided by effective diffusion area per hour, according to literature[39] and the resulted expressed as: μg/cm^2/h.

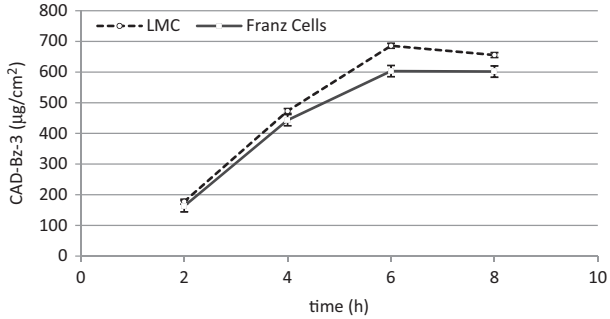

Figure 2. Comparative assay between Franz cells and Laboratory made cells (LMC). Values of total amount of Benzofenone-3 diffused (μg/cm^2; CA Bz-3) during experiment time 8 h.

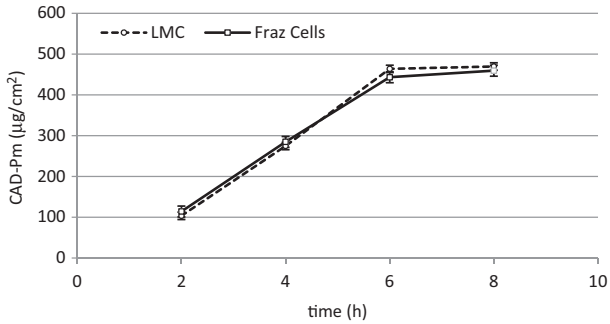

Figure 3. Comparative assay between Franz cells and Laboratory made cells (LMC). Values of total amount of Permethrin diffused (μg/cm^2; CA-Pm) during experiment time 8 h.

Retention on membrane assay

One of the most interesting advantages of the use of LMC was the possibility of simultaneous tests, as mentioned above Franz cells cannot be removed at each interval of measurement.

On these bases, we determined at each sampling interval, retention on membrane of API (μg/cm^2) assay.

For these propose, the membranes ($n = 4$) retired at each sampling interval were cut in pieces and submerged on 2 mL fresh acceptor solution. Then, all the systems were agitated for 30 min at 120 rpm at 32 °C, and spectrophotometrically evaluated at a wavelength (290 and 219 nm for BZ-3 and Pm, respectively). Each test had a total duration of 8 h at each time (2, 4, 6 and 8 h), four membranes were retreated of the system. Cumulative amounts of API (μg) retained, by unit diffusion surface (cm^2) was plotted (Figure 3 for BZ-4 and Figure 5 for Pm). All experiments were performed in triplicate ($n = 12$) and the results were expressed as mean ± SD.

Residence time

The residence time of API, on the membrane was calculated as the test time at which API concentration (μg/cm^2) decreases significantly on membrane retained values.

Statistics

One way ANOVA was used to determine differences between systems by means of the computer program Origin 4.0 (Microcal Inc., 1995; F test). To evaluate the statistical significance of the results *t*-test was performed ($*p < 0.01$).

Results

Laboratory made cell dimension evaluations

Visual observations of LMC showed that some of the bases of cell receptors were convex as a result of manufacturing variations. The dimensions of the LMC Donor recipient determinate were Volume 1.5 ± 0.01 mL and EDA was 1.8 ± 0.1 mm^2 neither of the measurements presents significant variability. The acceptor recipient volume dimension was 10 ± 0.01 mL and EDA 1.8 ± 0.1 mm^2, this values measured with calipers, showed no significant differences between them.

Validation tests

Robustness

The diffusion process was evaluated in presence of small variations in different parameters (agitation velocity, sample extraction height from the base of the apparatus and operator). The values of diffused BZ-3 (μg/cm^2/h) were always compared to the values obtained at 32 °C. The different parameters did not significantly affect the diffusion values (Table 1). However, there was a significant difference in the values of BZ-3 diffused per unit of time when the temperature was 35 °C. When the same experiment was performed with different sample quantities (0.15 and 0.25 g) and operators ($n = 2$), no significant difference in the values of diffused BZ-3 was found (data not shown).

Reproducibility

When the reproducibility of the method was determined by CV% values ranged between 0.47 and 1.00, with a uniform and reproducible behavior of all the cells employed. These coefficients are within the range considered acceptable for situations reproducibility of the analytical methods.

Diffusion assay

Figure 2 shows the evaluation of BZ-3 diffused (μg/cm^2) using the laboratory made cells (LMC) compared to a commercial Franz cell set ($n = 4$). No significant differences were found on the values of CA-BZ-3 for the sampling at 2 and 4 h for the LMC against Franz Cells. The total amount of BZ-3 diffused obtained from LMC and Franz Cells was statistically different, being 665.7 ± 7.6 (μg/cm^2) and 601.9 ± 16.2 (μg/cm^2), respectively. The values of diffusion Flux (J_D) of BZ-3 were calculated as the amount of API diffused divided by effective diffusion area per hour (μg/cm^2/h) for LMC and Franz Cells. The values obtained were: 0.0888 and 0.1181 μg/cm^2/h for LMC and 0.0813 and 0.1108 μg/cm^2/h, respectively for Franz Cells. A lineal correlation was obtained on the linear regression analysis giving the equation: $J_D = 0.059 + 0.015\,t$ ($R = 0.998$; $p = 0.001$).

Figure 3 shows the evaluation of Pm diffused (μg/cm^2) using the laboratory made cells (LMC) compared to a commercial Franz cell set ($n = 4$). No significant differences were found on the values of CA-Pm for all samples for the LMC against Franz Cells. The total amount of BZ-3 diffused obtained from LMC and Franz Cells was not statistically different, being 469.66 ± 10.5 (μg/cm^2) and 459.41 ± 8.57 (μg/cm^2), respectively. The values of diffusion Flux (J_D) of Pm were calculated as the amount of API diffused divided by effective diffusion area per hour (μg/cm^2/h) for LMC and Franz Cells. The values obtained were: 0.0728 and 0.0781 μg/cm^2/h for LMC and 0.0713 and 0.0732 μg/cm^2/h for Franz Cells, respectively.

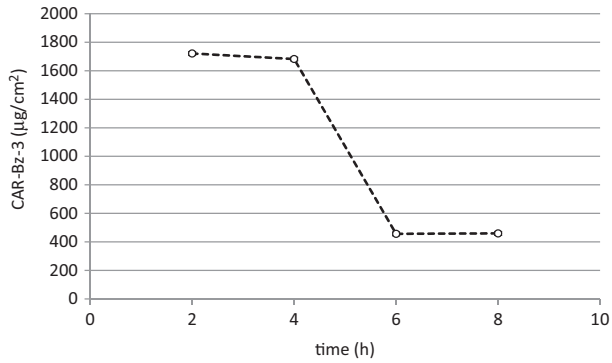

Figure 4. Benzophenone-3 retained on membrane (CAR-Bz-3;μg/cm^2) versus time (h).

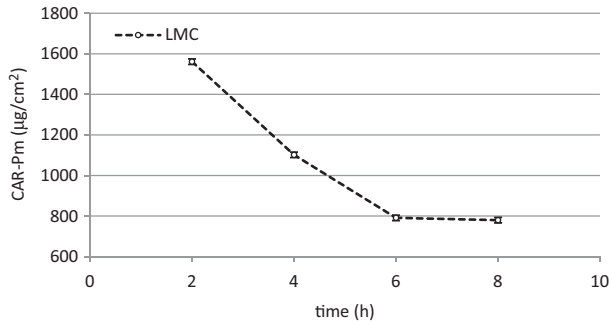

Figure 5. Benzophenone-3 retained on membrane (CAR-Pm; μg/cm^2) versus time (h).

Retention on membrane assay

Figure 4 shows the values of BZ-3 retained on membrane (μg/cm^2). The values obtained were: 1721.31 ± 12.87 and 1682.5 ± 11.60 for the first 4 h of assay then the retention decreases significantly to 456.7 ± 11.43 and 459.9 ± 7.56 for 6 and 8 h of assay, respectively.

Figure 5 shows the values of Pm retained on membrane (μg/cm^2). The values obtained were: 1561.22 ± 14.26 and 1101 ± 17.86 for the first 4 h of assay then the retention decreases significantly to 791.22 ± 7.89 and 779.89 ± 7.55 for 6 and 8 h of assay, respectively.

Residence time

The residence time of BZ-3, on the membrane was 4 h, before these time, the concentration retained decreases at 50% or less (Figure 4). The residence time of Pm on the membrane was also 2 h but the decrease of API concentrations starts at 4 h with a 37.5% decrease of Pm retained on membrane (Figure 5).

Discussion

Visual observations of LMC showed that some of the bases of cell receptors were convex this feature did not prevent a good mix because the system has external agitation. The dimensions of the

LMC, presents non significant variability. Furthermore, the values of effective diffusion area (EDA) measured and showed no significant differences between them. This allows us to state that the recipients (donor and acceptor) of different pairs of cells can be exchanged, without risk of introducing variations in measurements.

The robustness of the process was verified by the study of BZ-3 diffusion in presence of small changes in the main factors that can directly affect the results. Within the parameters analyzed to standardize the robustness of the method, the stirring speed is considered in determining the validation. Stirring of the receptor fluid is critical for the maintenance of both uniform drug distribution and temperature equilibrium[31,32]. The time taken to achieve such uniformity determines the minimum time for the first sampling interval as well as times between subsequent samples. It is noteworthy that inefficient stirring can occur when there is fast stirring near the base of the receptor chamber but negligible stirring in the upper parts of the chamber. This occurs with Franz cells due to the agitation by devices immersed in the acceptor solution can generate vortex effect, which is undesirable due to its potential to disrupt the static fluid layer adjacent to the membrane. On the other hand the presence of the side tube in Franz cells for sampling liquid modifies the behavior of local deviation causing sink conditions, the API is not evenly distributed within the fluid receiver. This generates errors in the interpretation of results when samples are taken at different heights. LMC will not require a side tube to the sampling, as the cells are removed at the time of measurement represents another advantage because it does not need to enter calculations for corrections due to dilution during data processing. In addition, the external orbital agitation system, allows the flow to remain uniform at all points acceptor system. Table 1 shows results obtained during the validation process of cell robustness of the equipment designed in our laboratory. When determined the amount of BZ-3 diffused ($\mu g/cm^2/h$) to different agitation speeds, the tabulated values showed no significant difference between them. Also, external agitation can correct errors due to sampling inefficient agitation that modifies the flow rate within the acceptor liquid as it moves away from the container base. This assertion is demonstrated in Table 1 where at different stirring speeds and sampling at two different heights no significant differences were observed between values. Furthermore, LMC also solves the problem of the vortex effect observed in Franz cells, as it does not require the side tube for sampling. Another factor commonly induce errors during the measurements is temperature. Is a known fact that the healthy skin temperature is 32 °C, which is why the *in vitro* release tests are carried out at this temperature in general, attempting to simulate *in vivo* conditions. Table 1 shows that there are significant differences in the values of BZ-3 diffused ($\mu g/cm^2/h$) when the test temperature was 35 °C, showing that the release rate is affected by changes in temperature of 3 °C or more above of the optimal T work. This is explained by the increased fluidity of the semisolid with increasing temperature, facilitating the release of the liquid within the API to acceptor and the increase in solubility with temperature. In this regard, an advantage of the system designed in the laboratory LMC, is the strict control of temperature, because the culture oven has a variability of ±1 °C, so that in this case, does not represent a limiting factor for release assays. In parallel, we monitored the influence of variation of temperature on the Franz diffusion cells where the control is done by circulating water by a double outer jacket connected to a thermostatic bath. Systematic measurements of bath temperature variations during the experiment showed at least 4 ± 1 °C, this variation increases when the ambient temperature is 10 °C or more degrees below the assay temperature. The sample amount of 0.15 and 0.25 g, and the change in the operators during the test did not significantly affect the measurements (data not shown). Also determined the height parameter extraction, samples were taken from the base unit (0 cm) and a height of 4 cm from the base. In this case, no significant differences were found in concentrations of BZ-3. When the reproducibility of the method was determined by CV% values obtained were within the range considered acceptable for situations reproducibility of the analytical methods, with a uniform and reproducible behavior of all the cells employed.

Figure 2 shows the evaluation of BZ-3 diffused using LMC compared to a commercial Franz cell set ($n = 4$). All parameters were carefully controlled in parallel experiments. No significant differences were found on the values of CA-BZ-3 for the sampling at 2 and 4 h for the LMC against Franz Cells. However, at the same conditions, our system shows less variability on the measurements CV 0.6% and CV 2.3%, respectively, even though both are considered acceptable values, we can say that our measurement system induces less variability in samples. The total amount of BZ-3 diffused on both experiments was statistically different, being higher on LMC assay. This difference is due to the above factors, external agitation, controlled temperature and its independence from sample quantity, sampling height and operator. A number of reports have been published indicating that sunscreens are absorbed through the skin. Transdermal absorbed amounts of various sunscreens were tested and calculated the J_D, which ranged from 0.5 to 130 $\mu g/cm^2/h$[40–43]. In this article, we calculate a J_D of 0.1181 and a 0.1108 $\mu g/cm^2/h$ for diffusion through membrane on LMC and Franz Cells, respectively. These are below the range considered as acute toxicity. These lower results may be due to the vehicle of the commercial sunscreen cream, as many authors report that the penetration can be affected by the vehicle[41,42]. This poor permeation represents an advantage over other previously studied sunscreens containing BZ-3 as API. Figure 3 shows the evaluation of Pm diffused ($\mu g/cm^2$) using the laboratory made cells (LMC) compared to a commercial Franz cell set ($n = 4$). In this case, we have chosen a gel formulation in order to extend the validation of the method. No significant differences were found on the values of CA-Pm for all samples for the LMC against Franz Cells. The total amount of Pm diffused obtained from LMC and Franz Cells was not statistically different, being 469.66 ± 10.5 ($\mu g/cm^2$) and 459.41 ± 8.57 ($\mu g/cm^2$), respectively. Although not found a linear correlation between the J_D values, the values of CV% were 1.8 and 2.2 for LMC and Franz cells, respectively, showed that in this case LMC induces similar variability in their measurements to Franz system.

A disadvantage of the evaluation *in vivo* measurement using urinary excretion to estimate dermal and skin penetration is underestimated, as it cannot account for sunscreen that is distributed in the tissues, metabolized to unknown metabolites or excreted through other routes. Therefore, monitoring the *in vitro* release and retention on membrane of API reduces measurement errors due to metabolization. The possibility of performing this measurement at each of the times of the assay is an advantage in itself. Figure 4 shows the values of BZ-3 retained on membrane ($\mu g/cm^2$). The values obtained were: 1721.31 ± 12.87 and 1682.5 ± 11.60 $\mu g/cm^2$ for the first 4 h of assay then, the retention decreases significantly to 456.7 ± 11.43 and 459.9 ± 7.56 $\mu g/cm^2$ for 6 and 8 h of assay, respectively. The values of BZ-3 were considerably higher than diffusion assays right through cellulose membrane. This is a result that is consistent with the statements made for other authors[37].

Figure 5 shows the values of Pm retained on membrane ($\mu g/cm^2$). The values obtained were: 1561.22 ± 14.26 and 1101 ± 17.86 for the first 4 h of assay then the retention decreases

significantly to 791.22 ± 7.89 and 779.89 ± 7.55 for 6 and 8 h of assay, respectively. In this sense, the values obtained at 6 and 8 h are steel on the range of escabicides action of Pm. The use of LMC on these kinds of assays presents a double advantage: the possibility of continuous monitoring of the amount of API retained in membrane and the realization of other parallel *in vitro* assays to support the claims made.

One primary requirement in pharmaceutical or cosmetic topic use product is that an effective concentration of the particular API is to be maintained at the site of application for a sufficient period of time such that can act locally. The residence time is an important factor to consider on design of new formulations process. The residence time of BZ-3 on the membrane was 4 h, before these time, the concentration retained decreases at 50% or less (Figure 4). For Pm, the residence time was 2 h after that the concentration of API retained on membrane diminished to 65% and less. However, Pm concentration at 8 h steel reaches active insecticide action. The parameter could be use also, for determination of time of repetition of application of the product.

Conclusions

The reproducibility of the results obtained on LMC provides initial validation of this *in vitro* method. There are three weaknesses in the use of Franz cells for quantitative assays *in vitro* release: temperature, sampling and agitation (speed and uniformity). The LMC solves them with external agitation, constant temperature in oven and no side tubes for sampling. For reproducibility test CV ranged from 0.47 to 1.00, with regular, reproducible behavior of all the cells employed. These coefficients are within the interval considered to be acceptable for situations of reproducibility for analytical methods. Robustness was determined by introducing small variations on some parameters. External agitation can correct errors due to sampling inefficient agitation. At 1 h or more (in the stable state), the method is robust for all the experimental factors analyzed except for temperature; a difference of 3 °C is sufficient to increase the quantity of the API released. No corrections are needed for sample dilution due to the absence of side tube in LMC.

Two different API and semisolids formulations were tested using LMC and Franz Cells. When we compared the values of API ($\mu g/cm^2$) obtained not showed significantly differences between methods.

By its design, our system allows the simultaneous measurement of different parameters, representing an innovation on this type of methodologies. The LMC was used for assays of *in vitro* retention on membrane and the values obtained were reproducible and coincident whit values obtained for other authors. From this study, we conclude that the system designed and the methodology employed is acceptable for *in vitro* release and toxicity studies with classical topical dosage forms. The device designed, has the three characteristics required for direct transfer of technologies, novelty, inventive step and industrial applicability. The transfer of technologies in developing countries is key pathways for technological and industrial development. Our system *made in Argentina*, responds to an economic necessity and a defined government policy.

Declaration of interest

The authors declare no competing financial interest. This work was supported by grant Consejo de Investigación de la Universidad Nacional de Tucumán Programa CIUNT N° 26/D453. The authors thank to Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, for providing funds for the Pos Doctoral grants of PhD M.E. Sesto Cabral and PhD A.N. Ramos.

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