An easy 3D printing approach to manufacture Vertical Diffusion Cells for *in vitro* release and permeation studies

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ABSTRACT

Vertical diffusion cells are utilized in the pharmaceutical and cosmetic fields to study the release and permeation of active ingredients through polymeric or biological membranes. Nevertheless, the commercially available glass-based systems are expensive and need to be carefully handled due to their fragility. Fusion deposition modeling 3D printing is an additive manufacturing technique that allows producing objects by printing layer over layer different thermoplastic materials. Among them, polypropylene is a robust, flexible, and chemically inert polymer that can resist to many organic solvents and to heating. In this work, we designed and printed a vertical diffusion cell following pharmacopeia requirements by using polypropylene in a fused deposition modeling 3D printer. The model was developed to fit in a heating block to avoid the use of warm water recirculating system. The vertical diffusion cells were leak-free and presented chemical resistance and no interaction with the tested molecules (*i.e.*, caffeine, diclofenac sodium, and glycyrrhetinic acid). The 3D printed cells were compared to commercially available glass cells and then two different types of synthetic membranes (*i.e.*, PDMS and Strat-M®) were used to evaluate the permeation of a caffeine hydrogel. The developed 3D printed testing system could represent an efficient alternative to the glass-based equipment.

Keywords: 3DP; fusion deposition modeling (FDM); Franz cells; VDCs; polypropylene (PP); polymeric membranes.

1. INTRODUCTION

During the last few years, 3D printing (3DP) continued to grow as an innovative additive manufacturing (AM) technology with applications in many different areas including pharma (Melocchi et al., 2020; Ngo et al., 2018). Recently, many pharmaceutical applications have been published in the literature reporting the production of dosage forms (i.e., tablets, capsules, suppositories, and vaginal rings), testing systems (i.e., ocular, nasal, and respiratory models), and manufacturing devices (i.e., microfluidic chips) (Lim et al., 2018; Mathew et al., 2020; Tiboni et al., 2020; Trenfield et al., 2020). Among the 3D printing techniques, fused deposition modeling (FDM) presents several advantages including relatively inexpensive printers and materials, low maintenance costs, a large selection of commercially available materials, the ease of initial use, and the ability to start, stop, and integrate complexity on the fly (Romanov et al., 2018).

Taking advantage of this technology, the acronyms DIY (Do-It-Yourself) is gaining attention over research laboratories. The additive manufacturing techniques allow researchers to develop and produce almost any kind of object needed in the laboratory from the simplest to even more complex ones with a real decrease in costs (Boparai et al., 2016; Capel et al., 2018).

In the pharmaceutical and cosmetic fields, vertical diffusion cells (VDCs or Franz cells) are routinely used for the study and analysis of both release and permeation of active molecules from different formulations through the use of polymeric and biological membrane (Marques et al., 2009). These kinds of studies are important since they can determine the feasibility of delivering the cargo to and through the skin (Johal et al., 2016).

Conventional VDCs are typically manufactured from glass and they can be found in the market in many shapes, sizes and may be modified depending on the required experimental conditions. According to United States Pharmacopeia (USP, www.uspnf.com, Topical and transdermal drug products), the VDC assembly consists of two chambers (donor and receptor), separated by a membrane. Commonly, this system is used for testing the *in vitro* release rate of topical drug products such as creams, gels, and ointments. Even alternative diffusion cells that conform to the same general design can be used and can be made from any materials that do not react with or absorb the test product or samples. Commercial VDCs are commonly made from borosilicate glass that results fragile and require careful handling during their utilization.

Only one 3D printing approach was considered in the literature to produce VDCs using stereolithography (SLA) (Sil et al., 2020). This additive manufacturing technique requires the utilization of acrylate-based resins which are photopolymerized during the printing procedure and then they need to be post-cured to obtain the final object. Moreover, the type of resin utilized

presented physical and chemical interactions with the tested drug, requiring a plastic coating to avoid these problems.

FDM, in comparison to SLA, is easier to use, it has lower overall production costs and it does not need post-curing after printing. Another important aspect to consider is that the selection of thermoplastic FDM printing materials is very wide and the most appropriate one can be chosen depending on the needs.

In this work, we developed an alternative 3DP vertical diffusion cell using polypropylene (PP) as manufacturing material in a FDM printer. This material was selected since it is a robust, flexible, and chemically inert polymer that can resist to many organic solvents and to heating (Price et al., 2020). The 3DP VDCs were tested for leaking and then were compared to glass VDCs to evaluate the potential applicability.

In the *in vitro* permeability studies, different membranes can be used including human skin, animal skin as well as polymeric membranes. However, biological membranes have limitations such as cost and availability of human skin and ethical consideration for the use of animal skins. Besides, compared to synthetic membranes, biological models exhibit high variability that complicates the experimental design, statistical significance, and number of replicates required (Haq et al., 2018a). Moreover, biological models possess a short half-life, special storage requirements, higher costs, and safety issues (Haq et al., 2018b).

For our work, we selected skin-mimicking membranes (Strat-M®) which comprise two layers of polyethersulfone on top of one layer of polyolefine. These membranes possess a porous structure that imparts additional skin-like properties by creating a gradient across the entire thickness (Uchida et al., 2015).

Finally, Strat-M® membranes were compared with polydimethylsiloxane (PDMS) membranes (Ng et al., 2012), using a caffeine hydrogel as model formulation since this active is a hydrophilic molecule widely used in topical applications (Herman and Herman, 2012).

2. MATERIALS AND METHODS

2.1 Materials

Neutral polypropylene 3D printing filament was kindly provided from Verbatim (Italy). Caffeine was kindly provided by BASF (Germany), diclofenac sodium was obtained from Farmalabor (Italy), glycyrrhetinic acid, and xanthan gum were purchased from A.C.E.F. (Italy). Strat-M® membranes were obtained from Merck (Germany), 250 µm thick polydimethylsiloxane (PDMS) membranes were kindly provided by Shielding Solutions Limited (Essex, UK), Spectra/Por™ 7 Standard RC dialysis

membranes (6-8 kDa cut-off) were purchased by Spectrum Labs (USA). All the other solvents used were HPLC grade.

2.2 Design and development of the VDCs

The original 3D model project was designed using the free online computer aided design (CAD) tool Tinkercad® (Autodesk, USA). The cell was designed to fit in a heating block (IKA, Germany) used to control the temperature during experiments. The cell is composed of a receptor part in which is present a withdrawal window with its cap, two donor compartments depending on the origin of the formulation, liquid or semisolid, and a stirring block useful to adjust the receptor volume. The 3D printed stirring block presents a slot to insert a magnetic stirring bar. The 3DP VDCs present a receptor compartment volume of 9 or 11.5 mL (with or without stirring block respectively) and an effective diffusion area of 1.583 cm^2. The files were exported from the online CAD software as STL (Stereolithography interface format) to be then converted into machine language with a computer aided manufacturing (CAM) software (STL files provided in the supplementary material).

2.3 Manufacturing process of the 3D printed VDCs

3D-printed PP VDCs were produced via fused deposition modeling (FDM) using an Ultimaker 3 printer (Ultimaker, The Netherlands). The VDCs were printed at a print speed of 25 mm/s with a nozzle temperature of 205 °C. The infill density was set at 100 % and the build plate was preheated at 85 °C after the application of a polypropylene adhesion sheet (Ultimaker, The Netherland). The original STL file was converted to a print pattern using Ultimaker Cura 4.7 software (Ultimaker, The Netherlands). Layer thickness was set to 150 µm enabling the production of leak-free PP VDCs. The 3DP VDCs were tested for leaks by filling both compartments with water. The receptor compartment and the donor compartment were ulteriorly sealed with the application of laboratory sealing film. The system was examined for leaks over a minimum of 24 hours and it was considered good if no water was present on the outer wall after this period.

2.4 Compatibility studies of the 3D printed VDCs

To assess the compatibility of the VDCs with active compounds, three different model drugs were evaluated, *i.e.*, caffeine (2 mg/mL water solution), diclofenac sodium (2 mg/mL water solution), glycyrrhetinic acid (0.02 mg/mL 50% ethanolic solution). The solutions were prepared and used to fill the receptor compartment of the cell that was then closed using laboratory sealing film and warmed up at 32 °C together with 400 rpm magnetic stirring. After 24 hours, the concentration in the

receptor compartment was compared with the initial concentration to confirm that any amount of drug was retained or adsorbed from the cell wall.

The amounts of the model drugs were measured by HPLC (1260 Infinity II, Agilent, USA) using a mixture of 0.5% formic acid in water and methanol (ratio 60:40 for caffeine, 30:70 for diclofenac sodium, and 5:95 for glycyrrhetinic acid) as mobile phase, with a flow rate of 1 mL/min in an Agilent Zorbax Eclipse Plus C18, 150 x 4.6 mm, 5 µm column (Agilent, USA). The injection volume was 20 µL and the detection signals were recorded at 275 nm (caffeine and diclofenac sodium) and 276 nm (glycyrrhetinic acid) keeping the analysis system at room temperature.

2.5 *In vitro* release comparison: glass vs polypropylene 3D printed VDCs using a caffeine hydrogel

A comparison between commercial glass VDCs and 3DP VDCs was performed using a cellulose-based dialysis membrane (6-8 kDa cut-off, Spectra/Por 7 Standard RC Dry Dialysis Tubing, Spectrum Labs, USA). The selected model formulation was a caffeine hydrogel composed of caffeine (5 mg/mL), xanthan gum (0.5% w/v), and water.

The glass VDCs (Teledyne Hanson Research, USA) presented a receptor compartment volume of 7 mL and an effective diffusion area of 1.766 cm^2 meanwhile the 3DP VDCs were utilized with the stirring block presenting a receptor volume of 9 mL and an effective diffusion area of 1.583 cm^2. Water was used as receptor medium in both cell types. The receptor medium was continuously stirred at 400 rpm. The glass system was thermostated at 32 ± 1 °C with a circulating jacket meanwhile the 3DP system was thermostated at 32 ± 1 °C with a heating block positioned over a heating plate. The efficacy of heat transfer and temperature control between the heating plate and the receptor medium inside the 3DP cell was previously assessed by measuring the temperature with a thermometer. At predetermined sampling intervals (0.5, 1, 2, 3, 4, 5, 6, and 24 h), samples were withdrawn from the receptor compartment and replaced with an equal volume (0.2 mL) of fresh buffer. The content of the active compound in each sample was then determined by HPLC as reported above. A calibration curve of caffeine was performed with a concentration ranging from 0.01 to 0.5 mg/mL obtaining a correlation coefficient (R2) of 0.9997.

The amounts of the active compound released at each time point (AR_{tn}) were obtained using the eq. (1) for the first time point and eq. (2) for the subsequent time points:

$$AR_{t_1} = \frac{C_{t_1} * 1000 * V_c}{A_o} \qquad (1)$$

$$AR_{t_n} = \frac{C_{t_n} * 1000 * V_c}{A_o} + (AR_{t_{n-1}} * \frac{V_s}{V_c}) \qquad (2)$$

where AR (μg/cm^2) is the amount released at t_n sampling interval, the C_t (mg/mL) is the concentration of caffeine determined at t_n sampling interval, V_c (mL) is the volume of diffusion cell receptor compartment, A_0 (cm^2) is the cell diffusion area and V_s (0.2 mL) is the sampling aliquot volume.

2.6 *In vitro* permeation studies using 3D printed VDCs with different membrane models

Permeation studies using the PP 3DP VDCs were conducted using a caffeine hydrogel (5 mg/mL, 0.5% xanthan gum) applied to two different membranes, *i.e.*, skin mimicking Strat-M® membranes and 250 μm thick PDMS membranes. The skin-mimicking Strat-M® membranes are composed of two layers of polyethersulfone on top of one layer of polyolefine. These polymeric layers create a porous structure with a gradient across the membrane in terms of pore size and diffusivity. The porous structure is impregnated with a proprietary blend of synthetic lipids, imparting additional skin-like properties to the synthetic membrane (Kaur et al., 2018). PDMS membranes were selected as model membranes, already used in other studies, with a lower permeation compared to dialysis membranes (Ilbasmiş Tamer and Değim, 2007; Jung et al., 2012; Sil et al., 2020).

The receptor chambers were filled with water kept continuously stirred at 400 rpm. The system was thermostated at 32 ± 1 °C with a heating block positioned over a heating plate. At predetermined sampling intervals (0.5, 1, 2, 3, 4, 5, 6, 24 h), samples were withdrawn from the receptor compartment and replaced with an equal volume (0.2 mL) of water. The content of caffeine in each sample was determined by HPLC with the method reported above. Equations 1 and 2 were utilized to calculate the amount of active compound released at each time point.

2.7 Statistics

The data presented are the mean ± standard deviation of triplicate measurements and are representative of at least three independent experiments.

3. RESULTS AND DISCUSSION

3.1 Design, 3D printing, and compatibility studies of the vertical diffusion cells (VDCs)

The CAD design of the 3DP VDCs was developed following the USP guidelines presenting a receptor and a donor compartment (Figure 1).

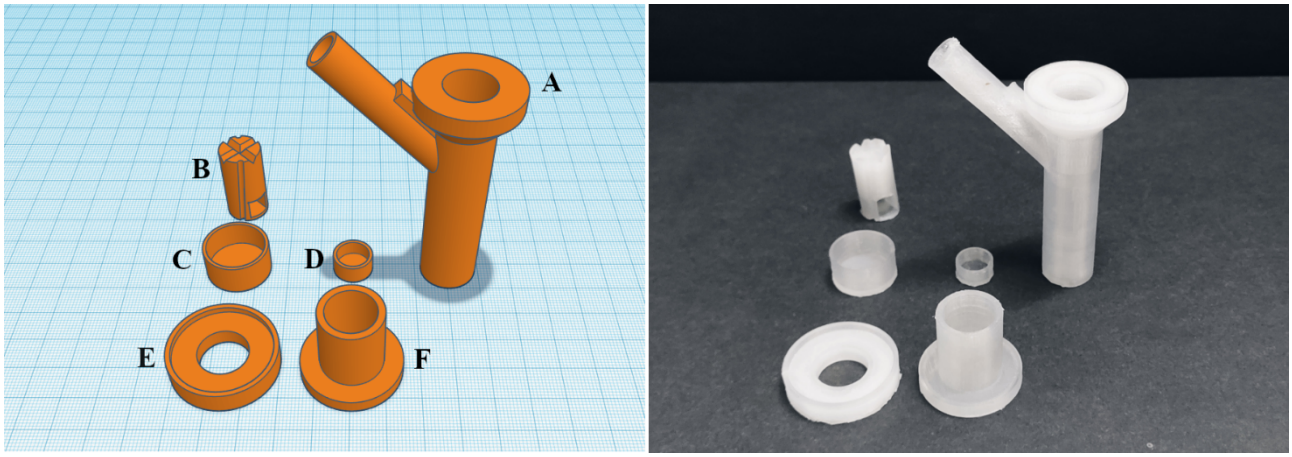

Figure 1. CAD design of the VDC parts and polypropylene 3D printed parts. A) Receptor compartment with withdrawal window; B) Stirring block; C) Cap for donor compartment for liquid formulations; D) Cap for withdrawal window; E) Donor compartment for semisolid formulations; F) Donor compartment for liquid formulations.

These two sections are separated by a membrane (*e.g.*, synthetic or biological) that allows the permeation of the tested molecule. The material selected to print out the entire system was polypropylene since it is a robust, flexible, and chemically inert polymer.

This last property is the most important to meet the pharmacopeia requirements since the cell material does not have to interact chemically and/or physically with the compound analyzed. This is also the reason because VDCs are traditionally made from glass that is a material known for its lack of interaction with active ingredients (Skelly et al., 1987). The drawbacks of this material are mainly its fragility and the high production costs. Taking advantage of FDM 3D printing, it was possible to print a VDC with a low cost and without fragility since PP results robust and flexible. The printed cell resulted semitransparent with the possibility to examine the receptor medium for the presence of air bubbles. The printed layers fusion was evaluated to prevent eventual leakage. The receptor and the donor compartments were filled to the top with water and sealed with laboratory film. After 24 hours no leakages were detected from the VDCs confirming the effective fusion of the layers produced with the FDM technique.

Then, another important step was to evaluate the compatibility of the material with active molecules even if PP is already known for its chemical resistance. Authors were more worried about eventual physical absorption into spaces between layers. We tested three different molecules varying their

chemical nature: caffeine was selected as amphiphilic molecule, diclofenac sodium as salt, and glycyrrhetinic acid as hydrophobic molecule. These molecules in their respective solutions were used to fill the receptor compartment for 24 hours and the analysis of concentration after this period showed no differences with the initial concentration confirming the compatibility with these active molecules. As it is impossible to test every type of molecule, we choose these three as models, but we suggest assessing the compatibility of each specific active compounds before utilizing it in an *in vitro* permeation test with the 3D printed VDCs.

3.2 *In vitro* release and permeation studies

In vitro release studies were performed first in both glass and 3DP VDCs to evaluate effective comparability between the two systems. A commonly used cellulose dialysis membrane was applied to divide the receptor from the donor compartment and a caffeine hydrogel was utilized. This comparison showed no significant differences in the release of the active molecule with the 3DP VDCs when compared with the glass VDCs as shown in figure 2 confirming the suitability of the developed 3DP system. Release studies with the 3DP VDCs resulted in 1164 ± 36 µg/cm^2 of caffein permeated in the receptor compartment after 24 h meanwhile the release was 1123 ± 41 µg/cm^2 for the glass homologues.

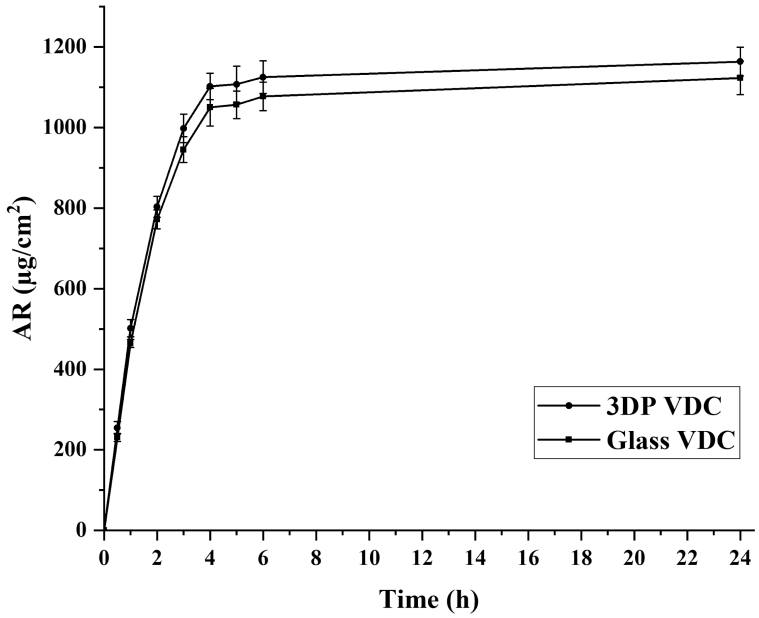

Figure 2. Comparison between glass VDCs and the 3DP VDCs using a cellulose dialysis membrane (6-8 kDa cut-off) and a 5 mg/mL caffeine hydrogel.

After the assessed suitability of the 3DP VDCs, two different membranes were employed for a permeation study using a caffeine hydrogel. Excised human and animal skins are often utilized to study skin permeation profiles of topical formulations, however, they are expensive and possess several drawbacks. Among them, there are variations of skin thickness, diseased skin states, preparation complexity, age of the donor, the density of hair follicles, and skin storage conditions that can hinder reproducibility data (Haq et al., 2018b, 2018a).

In this study, we decided to compare standardized synthetic Strat-M® membranes as a reproducible alternative to excised human skin (Haq et al., 2018a) and 250 µm thick PDMS membranes as a low permeability model membranes (Figure 3) (Ng et al., 2010).

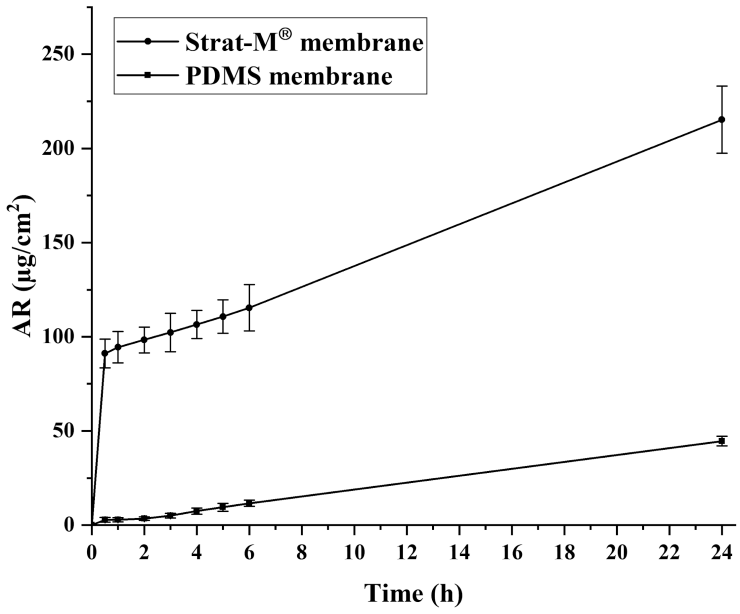

Figure 3. Comparison between Strat-M® and PDMS membranes using the 3DP VDCs and a 5 mg/mL caffeine hydrogel.

The drug permeation resulted higher with the Strat-M® membranes with an amount permeated after 24 h of 215 ± 18 µg/cm^2 meanwhile for the PDMS membranes, the drug permeated was more than 4 times lower (44.6 ± 2.6 µg/cm^2). Since this membrane is made with a hydrophobic material, the permeation through it is influenced by the nature of the tested molecule. Since caffeine result hydrophilic, its passage through this type of membrane resulted very low even after 24 hours.

In release studies, mathematical models play a crucial role in evaluating the drug release mechanism (Siepmann and Peppas, 2011). In these studies, the release profile of the drug from the xanthan gum hydrogel resulted linear with the time for the utilized membranes confirming zero-order kinetics (Strat-M® R^2 0.9972; PDMS R^2 0. 9974) (César dos Santos Nogueira et al., 2003).

4. CONCLUSIONS

In this work, we successfully developed a 3D printed VDCs model useful for the evaluation of *in vitro* drug release and permeation. The design was in accordance with the pharmacopeia requirement and the dimensions were studied to perfectly fit in a heating block to control the temperature avoiding warm water recirculatory system. As the system has been developed for 3D printing it is possible to continue the personalization based on the needs for example changing or reinventing the donor compartment. The material employed for the manufacturing of the cell (*i.e.,* polypropylene) confirmed its chemical resistance and the possibility to be used to produce leak-free FDM printed objects. Moreover, compared to commercially available VDCs (usually made with glass), the 3D printed VDCs require really low costs of production (less than 2 US $ of material) and only a few typical lab equipments such as a heating and stirring plate, a heating block and a magnetic stirring bar.

VDC *in vitro* testing results fundamental to predict results from *ex vivo* or *in vivo* studies and the possibility to have this testing system readily available in a research lab with a really low cost could increase its diffusion and utilization.

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