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Original Research Article

Combining 3D printing, dialysis adapters, and agarose hydrogels for dissolution testing of suspensions

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Abstract: Dissolution test methods described in the pharmacopeias were developed mainly for solid oral dosage forms or transdermal drug delivery systems. Dissolution studies of other dosage forms, such as suspensions, are challenging and may require special dissolution equipment that is not commercially available. 3D printing represents a way to close this gap by providing the opportunity to easily produce custom-made adapters. In this work, adapters for dialysis membranes and molds for producing hydrogels incorporating suspensions were manufactured with stereolithography. The adapters were tested in combination with different monographed dissolution apparatuses to study release from a model drug suspension. The hydrogels led to a slower dissolution than the direct injection into the dissolution medium. Agarose-based hydrogels, with their interconnected pores and potential to regulate drug release, proved to slow down the dissolution process and provide a controlled environment. Using dialysis membranes slowed down the dissolution even more. To investigate the impact of the dialysis membrane properties, different molecular weight cut-offs were used. The molecular weight cut-off did not impact the dissolution speed for the tested paracetamol suspension. The agarose concentration in the hydrogels was also no factor in dissolution speed. The described methods could be of further interest in investigating poorly soluble drugs resulting in a more extended dissolution profile.

I. Introduction

Dissolution studies are fundamental in developing, testing, and quality assurance of pharmaceutical dosage forms. These studies provide valuable insights into the release behavior of active ingredients from dosage forms, aiding in formulation optimization and ensuring consistent drug delivery to patients. Pharmacopeias typically outline monographed dissolution methods that are widely adopted in the industry. However, when it comes to certain dosage forms, such as long-acting injectable (LAI) suspensions, these conventional methods may not adequately mimic the situation and the application site. LAI suspensions are parenteral formulations designed to gradually release active ingredients over an extended period, typically from several weeks to months [1]. Conventional in vitro release methods fail to replicate this prolonged release, hindering accurate assessments of in vivo behavior. Furthermore, crucial factors like particle size, which influence dissolution and in vivo exposure time, cannot be adequately evaluated. As a result, various alternative methods have been explored in the literature to extend in vitro release. [2], [3].

The United States Pharmacopeia (USP) introduced the general chapter "<1001> in vitro release test methods for parenteral drug preparations" in 2022, recommending, for example, the basket apparatus (USP Dissolution Apparatus 1, USP1), the paddle apparatus (USP2), the flow-through cell (USP4), and the reciprocating holder (USP7) for parenteral preparations [4]. In the case of suspension, separation of the undissolved drug from the sample drawn from the surrounding release media is an additional challenge. For this purpose, a dialysis tube or a Float-A-Lyzer can be used A dialysis tube is a porous membrane that allows dissolved molecules to pass while retaining suspended solid particles. However, diffusion rates can be influenced by factors such as molecule size, solubility, and molecular weight cut-off (MWCO) of the membranes. An alternative to separate the suspended particles from the release media may be to use gel-based methods. In this case, the suspension may be placed inside a hydrogel which

separates the suspended particles from the sample and creates a controlled environment that regulates drug release from suspended solid particles, thereby reducing the dissolution rate. Commonly in research used hydrogels include agarose at varying concentrations, as well as gelatin or hyaluronic acid [5].

Innovative approaches are required to bridge the gap between these separation methods and the recommended dissolution apparatuses. While commercial adapters for dialysis membranes already exist for the USP4 [6], additional adapters need to be developed for other methods. This gap can be filled through 3D printing, a cost-effective and time-saving alternative to conventional manufacturing methods that is widely employed in pharmaceutical research. Previous studies have showcased the use of 3D-printed dissolution equipment, utilizing techniques like fused deposition modeling (FDM) and stereolithography (SLA), offering design flexibility to influence release times in experiments [7]–[9].

Therefore, this study aims to design and evaluate dialysis adapters for different compendial dissolution systems. These should be tested with a paracetamol suspension as a model drug. Additionally, suitable hydrogel molds will be 3D printed to form gels incorporating the suspension.

II. Material and methods

A suspension of paracetamol, also called acetaminophen, (Atabay, Istanbul, Turkey) with a 40 mg/mL concentration was prepared for the dissolution studies. The suspension was formulated using 2.0 g paracetamol, 3.15 g Syrspend SF pH 4 (Fagron, Glinde, Germany) and 44,85 g phosphate-buffered saline (PBS) with a pH of 7.4. Agarose from Bio-Budget Technologies (Krefeld, Germany) was used to prepare the hydrogels. For the dissolution experiments, SpectraPor (Repligen, Waltham, USA; MWCO 10 kDa and 25 kDa) dialysis tubes were combined with the printed adapters.

3D printing

The 3D models were printed with Clear Resin (Formlabs, Somerville, MA, USA) using a Form 3 SLA printer (Formlabs). The CAD files were designed using FreeCAD, sliced with Preform (v3.29.1), and printed with a layer height of 50 µm using the default settings. After printing, the SLA prints were washed in isopropanol for 20 minutes using a Form Wash (Formlabs). Once dried, they were post-cured for 30 minutes at 60 °C in the Form Cure (Formlabs).

Gel preparation

The hydrogels were formed using silicone molds, as shown in Figure 1A. These molds were created from a negative that was previously 3D printed, as depicted in Figure 1B. The paracetamol suspension was heated in a beaker and mixed with the appropriate amount of agarose (0.5%, 1%, 2%) to prepare the hydrogels. Once the mixture was cooled to approximately 50 °C, it was poured into the silicone molds. After the curing process, the resulting gels exhibited a cylindrical shape with a diameter of 6.5 mm and a height of 28 mm, as illustrated in Figure 1C.

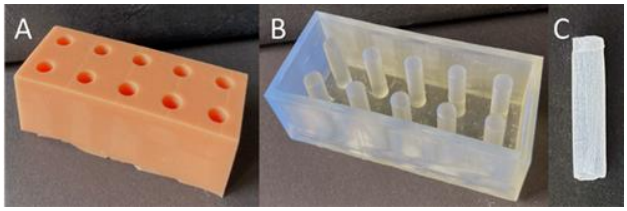

Figure 1: Silicone molds for casting of hydrogels (A), 3D printed negative for silicone molds (B), and hydrogels containing paracetamol suspension (C)

Dissolution experiments

Table 1 provides an overview of the dissolution studies performed in this work. Four different dissolution apparatuses were used with three different kinds of dissolution setup: Injection of the suspension directly into the vessel, incorporation of the suspension in agarose hydrogels, and injection of the suspension into a dialysis tube adapter.

Basket apparatus

The dissolution studies with the basket apparatus were conducted using a Sotax AT7 smart dissolution apparatus. The dissolution was performed in 1000 mL of pH 7.4

Table 1: Overview of the used dissolution apparatuses; USP = Unites States Pharmacopeia, PBS = phosphate-buffered saline

Name	USP1	USP2	USP4	USP7
Type	Basket Apparatus	Paddle apparatus	Flow-through cell	Reciprocating holder
Device	Sotax AT7 smart	Sotax AT7 smart	Sotax CE7 Smart	Agilent 400-DS
Dissolution Medium	1000 mL PBS 7.4	1000 mL PBS 7.4	1000 mL PBS 7.4	10 mL PBS 7.4
Agitation	75 rpm	75 rpm	8 mL/min	30 dips/min
Setups	Gel, Dialysis Tube	Depot, Gel, Dialysis Tube	Depot, Gel, Dialysis Tube	Gel, Dialysis Tube

phosphate-buffered saline (PBS) at a temperature of 37 °C. For the dissolution from the suspension-loaded hydrogel, the hydrogel cylinder was placed in a mesh basket. To carry out the dialysis tube experiment, the basket was removed from the holder and a 3D-printed adapter was attached to the rotating rod to carry out the experiment. The adapter was mounted with a dialysis tube which was securely sealed using two O-rings and parafilm. Both setups, the hydrogel and the dialysis tube experiment, involved rotating the adapter at 75 rpm. At predefined time intervals, 10 mL of the dissolution medium was withdrawn and replaced with fresh PBS pH 7.4.

Paddle apparatus

The same Sotax AT7 smart also served as USP2 under the same conditions. In the USP2 setup, the dissolution medium was stirred using a paddle instead of a rotating basket. The suspension was either injected directly into the vessel, incorporated into an agarose hydrogel which was placed at the bottom of the vessel, or injected into a dialysis tube adapter. In the case of the dialysis tube adapter, it was securely sealed using O-rings and parafilm, similar to the previous dialysis tube experiment setup, and also placed at the bottom of the vessel.

Flow-Through Cell

A Sotax CE7 Smart was used for the flow-through cell studies. A 6 mm ruby sphere was first placed in the flow-through cell, and then the conical part was filled with smaller glass spheres. The paracetamol suspension was subjected to three different approaches within the flow-through cell setup. Firstly, the suspension was directly injected on top of the glass spheres. Alternatively, the suspension was homogeneously incorporated into an agarose gel. Lastly, the suspension was injected into a 3D-printed dialysis adapter. The hydrogel and the dialysis adapter were placed onto the glass spheres, respectively. To maintain a constant flow, 1000 mL of pH 7.4 phosphate-buffered saline (PBS) tempered to 37 °C were continuously pumped from an external reservoir. This was achieved using an Ismatec IPC/IP peristaltic pump (Ismatec, Grevenbroich, Germany) at a rate of 8 ml/min, ensuring a closed-loop system. At predefined time points, 10 mL of the media was withdrawn and replaced with fresh PBS 7.4.

Reciprocating holder

For the reciprocating holder, the studies were performed in an Agilent 400-DS. The suspension was either incorporated into an agarose gel placed inside the commercially available PEEK holder or injected into a 3D printed holder with dialysis tubing. In each case, it was dipped at 37 °C in 10 mL of phosphate buffer 7.4 at a rate of 30 dips/min. At defined time points, 4 mL of sample was withdrawn, and the entire medium was replaced with 10 mL of fresh PBS 7.4.

Analytical Quantification

Quantification of the released amount of paracetamol was performed by UV/Vis (UV-1800, Shimadzu, Japan) at a wavelength of 246 nm. The calibrated range was between 0.6 to 25 µg/mL (R^2=0.9999).

III. Results and discussion

Due to their nature, suspensions, consisting of solid particles dispersed in a liquid medium, present unique challenges in dissolution testing. Understanding the dissolution behavior of suspensions is essential for ensuring the efficacy and bioavailability of the drug in the formulation. Selecting an appropriate dissolution apparatus and method is critical in overcoming these challenges. The choice of apparatus should consider factors such as sink conditions, hydrodynamics, and agitation mechanisms to ensure representative and reproducible dissolution testing. Furthermore, developing suitable holders or adapters that accommodate suspensions in the chosen apparatus is essential for accurate and consistent testing.

Direct injection of the paracetamol suspension was assessed in the USP2 and USP4 setups, resulting in complete dissolution within 12.5 minutes (Figure 2). These results align with expectations, as the direct injection of the suspension allows for efficient dissolution. Since the suspension is not hindered by any additional barriers or matrices, it can easily dissolve due to the presence of an adequate amount of free liquid.

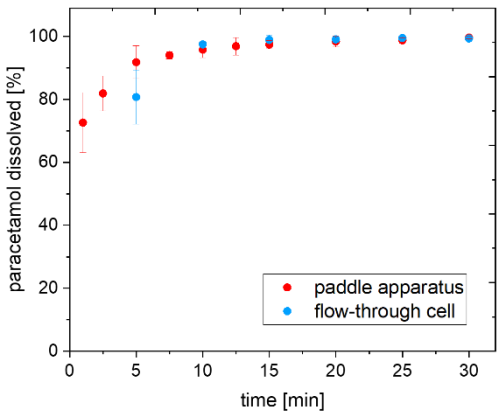

Figure 2: Dissolution curves of paracetamol suspension 40 mg/mL directly injected into the paddle apparatus or the flow-through cell. Dissolution in 1000 mL phosphate buffered saline pH 7.4 at 37 °C, mean ± SD, n = 3.

The embedding of the suspension in a hydrogel cylinder was evaluated to ensure the separation of the suspended particles from the dissolution medium. Agarose-based hydrogels are three-dimensional networks made up of agarose, a natural polysaccharide extracted from seaweed. When agarose is dissolved in hot water and cooled, it forms a gel matrix by forming physical crosslinks. The gel

structure comprises interconnected pores that can absorb and retain water, resulting in highly hydrated agarose hydrogels. The size of these pores is inversely proportional to the concentration of agarose [10]. In Figure 3, the dissolution profile in different apparatuses is depicted. After 120 minutes, dissolution was nearly complete in all setups, which is ten times slower compared to direct injection.

The preparation of hydrogels ensures that the suspension is uniformly distributed within the matrix, leading to an initial burst release of the drug near the surface. Within the aqueous environment of the gels with a water content well above 95 %, the suspension has ample opportunities to dissolve. However, the dissolved drug must diffuse through the gel matrix, resulting in a slower dissolution process compared to the direct injection. Among the various apparatuses, the USP7 setup exhibits slightly faster dissolution than the others. The gel's diffusion layer may be experiencing high shear due to the dipping mechanism of the USP7 device.

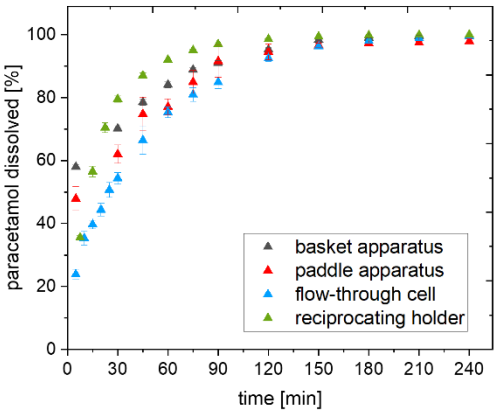

Figure 3: Dissolution curves of paracetamol suspension 40 mg/mL incorporated in 2% agarose hydrogels. Dissolution in the basket apparatus, paddle apparatus, flow-through cell, or reciprocating holder. Dissolution in 1000 mL (basket, paddle and flow-through cell) or 10 mL (reciprocating holder) phosphate buffered saline pH 7.4 at 37 °C, mean ± SD, n = 3.

The concentration of agarose for hydrogels varies in the literature. Leung et al. used 1% agarose gels for an in vitro diffusion model for subcutaneous, parenteral formulations [11]. Other researchers used a concentration of 2%. To determine the influence, dissolution experiments were performed in the USP4 with different concentrations. Figure 4 shows the different dissolution profiles. For paracetamol, there is no influence on the dissolution speed. Even though the concentration of the gelling agent has been shown to influence the pore size [10], for the diffusion of the relatively small molecule paracetamol this does not seem to impact the diffusion speed as the pores are most likely large enough. For other gel drug combinations, there have also been reports on similar diffusion coefficients in hydrogels compared to pure water [12].

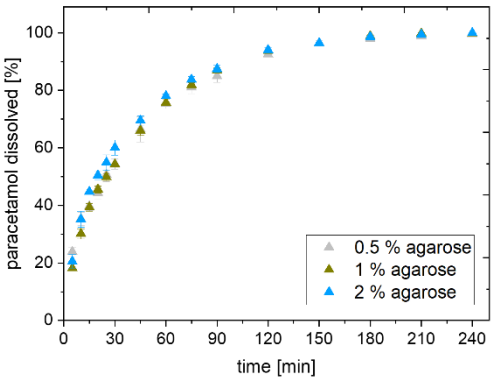

Figure 4: Dissolution curves of paracetamol suspension 40 mg/mL incorporated in 2% agarose hydrogels. Dissolution in the flow-through cell in 1000 mL phosphate buffered saline pH 7.4 at 37 °C, mean ± SD, n = 3.

Individual adapters for dissolution studies of liquid dosage forms are already described in the literature. For example, the release of dexamethasone from various liquid formulations was investigated by Bhardwaj et al. in 2010 using a dialysis adapter for the USP4. They found that release from suspensions was significantly slowed in this setup compared to solutions. In 2017, Probst et al. used self-manufactured dialysis adapters for the USP4 and USP7 [2]. They investigated the release of a suspension containing the active ingredient paracetamol or prednisolone. Both research groups concluded that the velocity of the flowing medium in the USP4 or the dip rates in the USP7 does not influence the release rate. However, a design for a dissolution adapter suitable for different dissolution apparatuses has yet to be established.

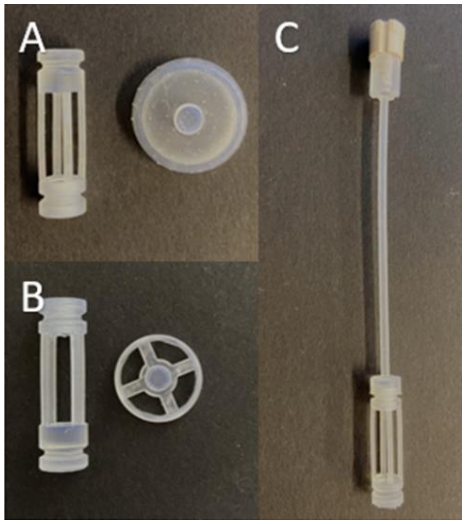

Figure 5: 3D printed dissolution adapters. Adapter for the basket apparatus (A), for the paddle apparatus and the flow-through cell (B), and the reciprocating holder (C).

The 3D printed adapters for dialysis tubes can be seen in Figure 5. Due to the SLA technique, it was possible to print fine structures. The surface for diffusion is identical for all adapters. The adapters only differ in the kind of top cover. The adapter for the rotating basket has a cap with a screw for attaching the adapter to the rotating rod, while the

adapter for the USP2 and USP4 contains a simple cover. In the case of the holder for the USP7, a rod with a thread is attached to the holder, which is used to mount a magnet. This magnet allows the holder to perform a dip movement like commercially available ones.

All printed adapters were suitable for dissolution testing of the paracetamol suspension used. There was no leakage of suspension from the adapters in any of the tests. Figure 6 shows the printed dialysis adapters in the different setups. A homemade sinker out of paper clips had to be attached to the adapter in the paddle apparatus to prevent it from floating, which would result in incomplete surface contact with the dissolution medium.

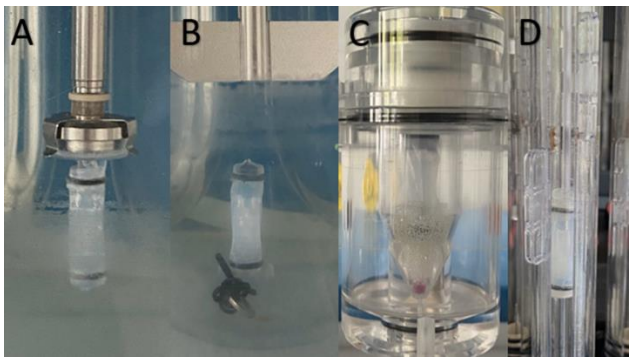

Figure 6: 3D printed dialysis adapter in the different setups. Attached to the rod in the basket apparatus (A), with a sinker in the paddle apparatus (B), in the flow-through cell (C), and in the reciprocating holder (D).

Figure 7 shows that after 12 hours, over 90% of the suspension has been released in all apparatuses. However, when comparing the setups, some differences can be seen. The dissolution rate in USP7 is slightly higher compared to the other three setups, followed by that in USP1. The different movements of the adapters in the medium could offer a possible explanation for this.

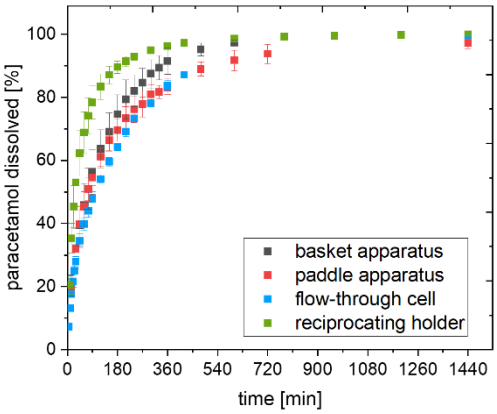

Figure 7: Dissolution curves of paracetamol suspension 40 mg/mL injected into 3D printed dialysis adapters. Dissolution in the basket apparatus, paddle apparatus, flow-through cell, or reciprocating holder. Dissolution in 1000 mL (basket, paddle and flow-through cell) or 10 mL (reciprocating holder) phosphate buffered saline pH 7.4 at 37 °C, mean ± SD, n = 3.

Due to the up and down in the dip motion in USP7, not only the adapter itself moves but also the suspension inside. This reduces the risk of sedimentation. In the USP1, the adapter rotates, which could also cause the already sedimented suspension to be slightly shaken up. In comparison, the adapters in USP2 and USP4 are somewhat fixed, and the medium around them moves. The drug sedimentation on the non-membrane bottom part of the adapter, which is expected to show a slower dissolution, seems to be more likely when using the holder with the USP2 and USP4 apparatus.

To assess the impact of the dialysis membrane, an additional release experiment was conducted in the USP4 apparatus using a dialysis membrane with a lower MWCO. Figure 8 illustrates that both dialysis membranes resulted in similar release rates. This observation aligns with expectations, considering the molecular size of paracetamol, which is 151,16 g/mol. The large pores of the dialysis membrane, more than 100 times larger than the paracetamol molecules, allowing the unhindered diffusion of dissolved particles. However, further investigations are necessary to examine the influence of MWCO on the dissolution of larger molecules.

Diffusion through the dialysis membrane is slowed down compared to the hydrogels used. Assuming that the pores of the hydrogel and the dialysis membrane are large enough, similar results could be expected. The larger surface area could explain the faster diffusion through the hydrogels. The surface area of the gels, excluding the bearing surface, is about 1400 mm^2. Only the lateral surfaces are available for diffusion through the dialysis adapters, which amount to about 848 mm^2 after subtraction of the bars. To directly assess any influence by the membrane, a comparison would have to be made with hydrogels of the same surface area in future studies. However, it has to be considered that hydrogels may swell, potentially increasing their surface area. For the duration of this experiment, there was no increase in size observed. When it comes to longer dissolution studies, the changes in size have to be investigated.

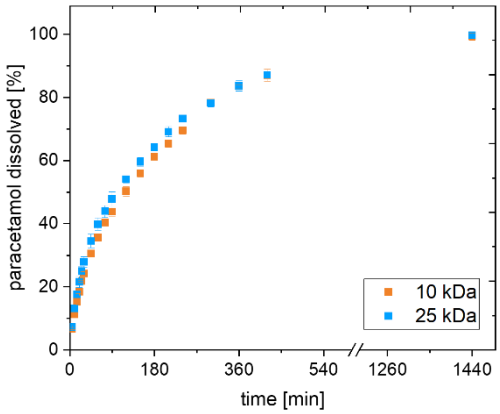

Figure 8: Dissolution curves of paracetamol suspension 40 mg/mL injected into 3D printed dialysis adapters attached with different dialysis tubes. Dissolution in the flow-through cell in 1000 mL phosphate buffered saline pH 7.4 at 37 °C, mean ± SD, n = 3.

The results demonstrated the successful design and testing of 3D printed adapters for four different dissolution apparatuses, allowing for the incorporation of dialysis membranes and agarose hydrogels. Both setups slowed down the release of the paracetamol suspension, providing a means to study the influence of factors such as agarose concentration and dialysis membrane molecular weight cutoff (MWCO) on release rates. The dissolution profiles obtained from various setups showed comparable results within the respective modifications, confirming the suitability and versatility of the proposed methods. In further studies, suspensions of lipophilic, larger, or poorly soluble drugs whereby the agarose concentration or MWCO might have a greater impact, could be investigated. 3D printing makes it possible to customize both the molds for gel production and, thus, the shape of the gels and the adapters. A combination of both methods would also be conceivable.

IV. Conclusion

Additive manufacturing represents a great opportunity to produce equipment for dissolution studies of different dosage forms. In this work, dialysis adapters for suspension release studies were designed and tested, which can be used for several USP release apparatuses. 3D printing can also be a valuable tool for the fabrication of custom-molded hydrogels for intermediate steps in the manufacturing process. With creative freedom, 3D printing can fill gaps in the feasibility of dissolution testing.

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Author's statement

The authors state no conflict of interest. Informed consent has been obtained from all individuals included in this study.

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