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Brief/Technical Note

Dissolution Testing of Acetaminophen Suspension Using Dialysis Adapter in Flow-Through Apparatus: A Technical Note

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KEY WORDS: dialysis adapter; dissolution testing; flow through method; USP 4.

INTRODUCTION

Dissolution testing is an important tool during drug development, characterization, and quality control of both immediate- and controlled-release formulations. It has been used for evaluating candidate formulations and for understanding possible risks related to specific gastrointestinal factors, potential for dose dumping, food effects on bioavailability, and interaction with excipients (1,2).

Recently, dissolution testing has been increasingly used to test dosage forms such as suspensions, soft gelatin capsules, creams and transdermal systems. To test these special dosage forms, special accessories are sometimes required. SOTAX Inc. recently introduced a dialysis adapter for release testing of solution and semisolid formulations. This device, as shown in Fig. 1, facilitates the use of dialysis tubing to sequester a sample in the center of the USP 4 standard 22.6 mm flow cell. The applications with this dialysis adapter are mainly for *in vitro* release testing of dispersed dosage forms. One publication by Burgess' group using this method compared dexamethasone release from extruded and non-extruded liposomes (3). Results showed that the USP apparatus 4 method with this dialysis adapter could discriminate between solution, suspension, and liposome formulations of dexamethasone.

The objective of this study was to evaluate this new dialysis adapter. An acetaminophen suspension drug product and various concentration acetaminophen solution samples were used to compare the flow-through method with and without the dialysis adapter, as well as to compare two different sizes of dialysis membranes. Other parameters such as flow rate, dissolution media, and medium ionic strengths were also studied.

EXPERIMENTAL

Materials

- Acetaminophen: USP (Lot J-1) was used for UV–vis standard; Sigma (Lot# 064K0096) was dissolved in dissolution medium to prepare sample solutions with different concentrations.
- Suspension samples: Children's Tylenol (McNeil, 160 mg/5 ml, Lot# ALM404, Exp: 08/11)
- Dialysis membrane: Molecular weight cut off (MWCO) 3,500–5,000 Da (Spectrum Laboratories, Inc., cellulose ester (CE), Lot# 3247947); MWCO 50 kDa (Spectrum Laboratories, Inc., CE, Lot# 3247951)
- Dissolution medium: 0.05 M, pH 7.2 potassium phosphate buffer and simulated gastric fluid (SGF, pH 1.2, without enzyme and NaCl). For different ionic strength SGF, NaCl was added accordingly.

Instrument and Accessory

- A USP apparatus 4 (CE 7 Smart with CP7 piston pump, Sotax AG, Switzerland) with 22.6 mm (Sotax part #8820) flow-through cells was used during the study. Each cell was prepared by placing a 5 mm ruby bead in the apex of the cone to protect the inlet tube. Of the glass beads, 8.0 g of 1 mm were added to the cone area to form a glass bead bed when tested with glass beads. A 25 mm, 0.7 μm filter paper (Whatman GF/F) was placed in the head of the flow-through cell. The flow rate through each cell was verified. The temperature of the flow cell unit was 37.0±0.5 °C. The closed loop configuration was applied and 200 ml dissolution solution was used in the closed loop.

The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any agency determination or policy.

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- Dialysis adapters: Three dialysis adapters from Sotax Inc (Fig. 1) were used. The specifications of dialysis adapter reported in the previous paper are: 33 mm in height, 9 mm diameter, 1.7 ml in total volume, and ~832 mm^2 exposed surface area (3).

Procedures

- Membrane preparation: The membrane was soaked in DI water for at least 30 min at room temperature to remove the preservative agent (sodium azide). It was then rinsed thoroughly in DI water, and soaked in dissolution medium for at least 30 min before use.
- Suspension samples: About 1 ml suspension sample was taken with 5 ml disposable syringe, the total weight of sample and syringe was weighed. After injecting the suspension sample, the syringe was weighed again. The difference of the two weights was the amount of suspension sample used in the test.
- Dissolution testing: dissolution was conducted in the closed loop using 200 ml dissolution medium, with studied flow rate, at 37°C. Six flow-through channels were used, with three channels assembled with dialysis adapters, and the other three channels used as normal flow-through cells. The test for Tylenol suspension sample was run for 24 h with on-line UV–vis taking samples at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240 min, and every hour for 24 h. The test for acetaminophen solution was run for at least 4 h with online UV–vis taking samples at 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 75, 90, 105, 120, 150 min, and every 30 min for 4 h.
- All of the dissolution results (except data in sample amount study) were the average of three samples. The data for suspension sample amount study were from one dissolution test with different sample amounts in each flow cell.

RESULTS AND DISCUSSIONS

Comparison of Results with and Without Using Dialysis Adapters

The newly developed dialysis adapter is designed to be used as an accessory for the flow-through method for release testing of solutions and semisolid formulations. This study compared the test results with and without using the dialysis adapter in the flow-through method. The tests were conducted with 1 ml acetaminophen solution (0.899 mg/ml), as well as acetaminophen suspensions in SGF (without enzyme, without NaCl) dissolution medium with the flow rate of 4 ml/min at 37°C. Membranes with 50 KD MWCO were used with the dialysis adapters.

Figure 2 shows the dissolution results of samples with and without using dialysis adapters. For solution samples (labeled with triangle), since the drug is already in solution, immediate release of the drug is seen in the dissolution medium when the test is done without using dialysis adapters (without membrane). Acetaminophen releases slowly when dialysis adapters are used. With the dialysis adapter in the dissolution apparatus, the sample must diffuse through the membrane to the dissolution medium.

For suspension acetaminophen samples (labeled with square), the results show the same trend as solution samples with and without using dialysis adapters. However, suspension samples did show slower release compared with solution samples when tested at the same conditions.

Comparison of Dissolution Results with Various MWCO Dialysis Membranes

In this study, two different MWCO dialysis membranes were used, and the tests were conducted in SGF (without enzyme, without NaCl) dissolution medium with the flow rate of 4 ml/min at 37°C. Figure 3 shows four dissolution profiles obtained by using either solution or suspension samples, and two different MWCO membranes. For the acetaminophen solution sample, the dissolution profiles (solid and empty square labels) were the same indicating that the pore size of the membranes (50 and 3.5 K MWCO) does not affect the rate of acetaminophen released from the dialysis adapter for acetaminophen solution. Based on the manufacturer's definition, the molecular weight cut off is the molecular weight solute that is 90% retained by the membrane during a 17-h period (4). Since the acetaminophen molecule (API in the drug) is small with a molecular weight of 151 Da, it can freely pass through either membrane when in solution.

For Tylenol suspensions, a dramatic drug release delay was observed in Fig. 3 (solid and empty triangle labels)

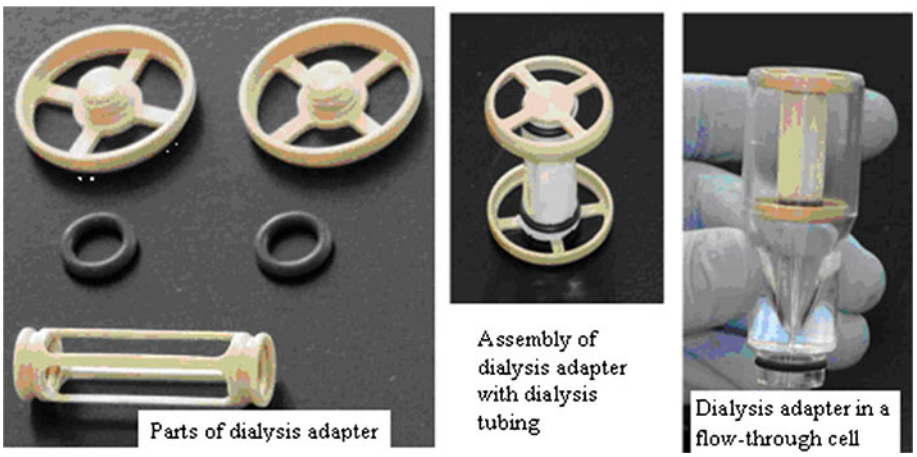

Fig. 1. Dialysis adapter from Sotax Inc

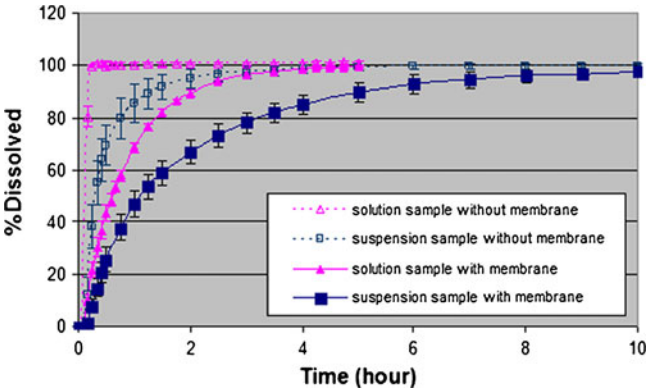

Fig. 2. Comparison of dissolution profiles with and without using dialysis adapters in dissolution testing

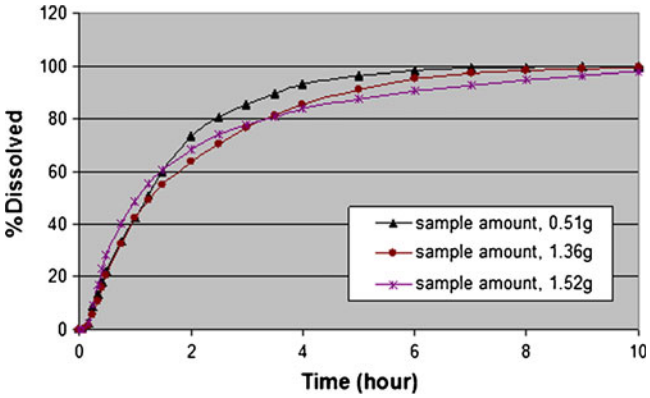

Fig. 4. Dissolution results with various amounts of Tylenol suspension samples in dialysis adapter

compared to the solution samples. This observation shows the difference between solution and suspension samples, where suspension samples still have to dissolve the drug whereas the solution samples already have drug dissolved. Additionally, the release delay may be due to the different sample physical property. The Tylenol suspension is a viscous liquid with density about 1.23 g/ml. The migration of drug substance from inside the suspension sample to the membrane interface may be controlled by the viscosity, and the migration rate could be much slower than that in SGF solution with lower viscosity. Comparing Tylenol dissolution results from the two different MWCO membranes shows a slightly higher dissolution rate for the larger MWCO membrane.

Comparison of Dissolution Results with Various Suspension Sample Amounts and Solution Concentrations in Dialysis Adapters

The specifications of the dialysis adapter reported in published paper are: 33 mm in height, 9 mm in diameter, 1.7 ml in total volume, and ~832 mm^2 exposed surface area (3). The less of the suspension sample used, the smaller volume of sample in the dialysis bag. This volume difference causes a contact surface area difference with the membrane, which could potentially cause variation for drug permeation through the membrane since the rate of drug permeation partially depends on the contact area of sample and membrane. About 1.5 g of sample was typically used in the study, but to compare sample size effect, smaller amounts were used. The dialysis membrane used in the test was 50 K MWCO, and the test was conducted once with various sample amounts in each flow-through cell at 4 ml/min flow rate and 37°C.

Figure 4 shows the normalized dissolution release profiles with various suspension sample amounts. The results showed that the amount of suspension in the dialysis adapter did not have an effect on the drug release rate. This result is in agreement with a previous published report (3).

Although different sample amounts have been studied and showed no impact on drug dissolution, sample concentration is a variable that still needs to be examined. For this reason, a sample with constant volume but different drug concentrations was explored. Acetaminophen from Sigma (Lot# 064K0096) was used to prepare various concentration solutions. A 1-ml sample was used to inject into the dialysis adapter in each test. The results (Fig. 5) show a slight trend of increasing permeation rate with higher drug concentration. This result indicates that the drug permeation through the dialysis membrane is a passive diffusion process and follows Fick's first law where the driving force of the permeation is the concentration gradient across the membrane.

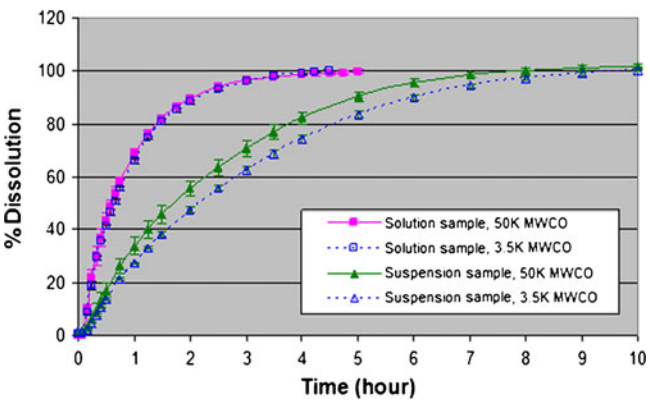

Fig. 3. Comparison of dialysis membrane effects on dissolution results with different acetaminophen samples

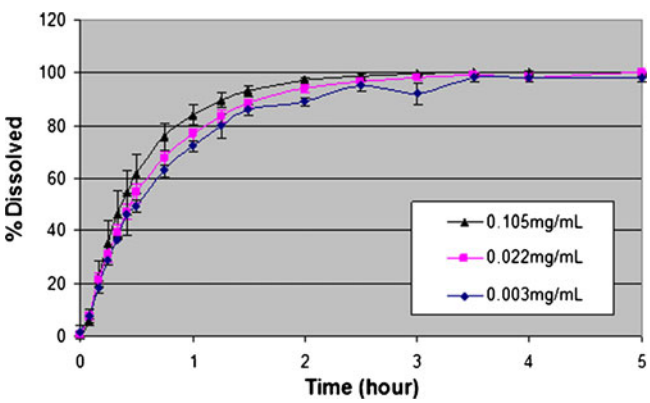

Fig. 5. Drug concentrations effects on dissolution profiles (with 1 ml solution samples in dialysis adapter)

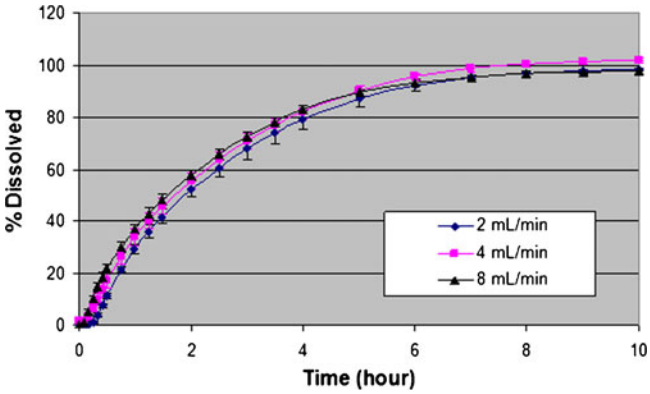

Fig. 6. Comparison of dissolution results using various flow rates

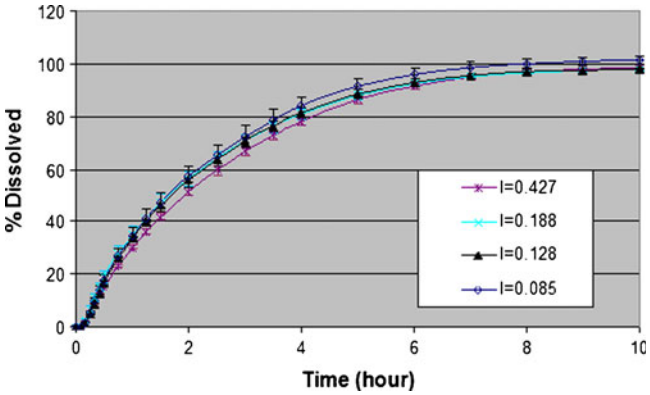

Fig. 8. Effect of ionic strength of dissolution medium on dissolution results of Tylenol suspensions

Comparison of Suspension Dissolution Results with Various Flow Rates

Flow rate of dissolution medium is one of the most important variables when developing a flow-through method. By changing the flow rate, the optimal discriminative power of the method for studied drug products can be obtained. With this dialysis adapter in place, dissolution medium is separated from drug sample by the membrane, and the flow rate may not affect dissolution as it does with the traditional flow-through cell.

To compare the flow rate effect on drug dissolution with the dialysis adapter, three different flow rates were applied. The tests were conducted at 2, 4, and 8 ml/min flow rates while keeping the other parameters the same (*i.e.*, using 50 K MWCO membrane, SGF dissolution medium, same amount of Tylenol suspension samples). The results (Fig. 6) show that the studied flow rates have no effect on Tylenol dissolution. This result is in agreement with that reported in the reference which used 8, 16, and 20 ml/min flow rates for their study (3).

Comparison of Suspension Dissolution Results with Different pH of Dissolution Medium

Figure 7 shows dissolution results using dissolution medium with different pHs. The dialysis membrane used was 50 K MWCO, and the tests were conducted using Tylenol suspension samples at 4 ml/min flow rate and 37°C.

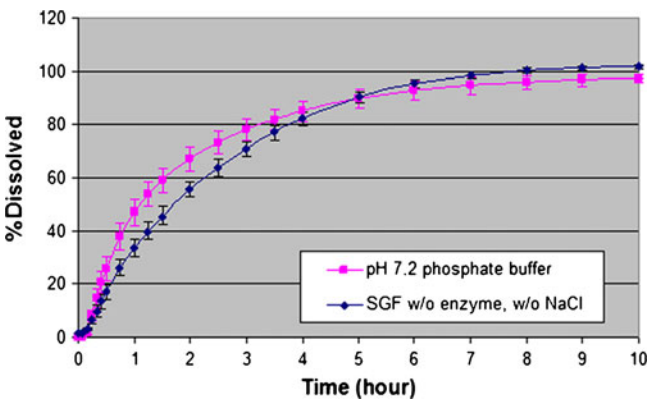

Fig. 7. Dissolution results for suspension sample in different pH medium

The dissolution rate at the early stage in pH 7.2 buffer was a little higher than that conducted in pH 1.2 SGF medium. The Student's *t* tests were checked on dissolution results at 0.5, 1.0, 1.5, and 2.0 h, and the *P* values were all less than 0.05 showing a significant difference of dissolution rate in these two different pH dissolution media.

Based on a FDA report (5), acetaminophen is a weak organic acid in a class of drugs called analgesics and is considered a borderline compound between BCS classes I and III. At 25°C the pK_a of acetaminophen is 9.6, and the solubility in water is about 13 mg/ml. Since the drug is a weak acid, it could be easier dissolved in the pH 7.2 buffer than in the simulated gastric fluid.

Comparison of Dissolution Results with Various Ionic Strength of Dissolution Medium

Ionic strength is an important factor in biochemical reactions and plays an essential role in the function of all living things. When the dissolution medium is simulating a biological fluid such as that in the GI tract, the ionic strength needs to be controlled. Since this study focused on the dissolution and permeation of suspension sample through a dialysis membrane, the medium ionic strength could potentially have an impact on the results. To focus on the medium ionic strength effect, other operational parameters were kept the same: all tests used 50 K membrane, 4 ml/min flow, and the same amount of Tylenol suspension sample. Four different ionic strength media with a fivefold difference in ionic strength were

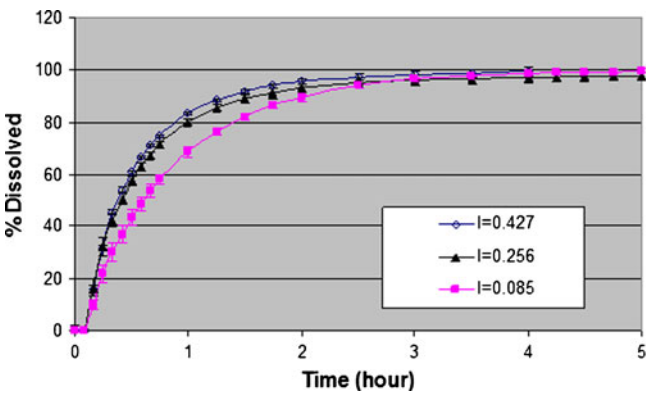

Fig. 9. Effect of ionic strength of dissolution medium on dissolution results of acetaminophen solution samples

used, and the results are shown in Fig. 8. The results indicated that the ionic strength did not have an impact on the dissolution profile for the studied suspension samples.

When drug suspension is placed in the dialysis adapter, the drug substance must dissolve in the dialysis adapter, then migrate to the interface of membrane and permeate through the membrane into the dissolution medium. In this process, dissolution, migration, and permeation could be rate-limiting steps. A published paper showed that ionic strength may affect diffusive permeability of an inorganic phosphate ion through dialysis membrane (6). The report showed that diffusive permeability to an inorganic phosphate ion increased with ionic strength. However, our studies showed no effect of ionic strength on the dissolution profile. This may be because of the physical properties of the suspension sample. As mentioned above, the Tylenol suspension is a viscous liquid, and the migration of drug substance could be controlled by the viscosity. In this case, ionic strength differences in the dissolution medium showed no impact on dissolution results.

To test this hypothesis, several more tests were conducted by using acetaminophen solution samples (0.899 mg/ml) with various ionic strength SGF dissolution media. The tests were conducted using 50 K MWCO membrane at 4 ml/min flow rate and 37°C. Figure 9 shows the dissolution profiles with solution samples at three different ionic strength dissolution media. There is a slight trend of increase in dissolution with increasing ionic strength in dissolution medium which is in agreement with the previous report (reference 6).

CONCLUSIONS

The results indicated that flow rate did not show an impact on dissolution profiles when using a dialysis adapter. This implies a fundamental change of dissolution mechanism since the hydrodynamics around the sample is relatively stationary. Additionally, the surface area between sample and dissolution medium was limited by the membrane area which was used to separate sample from dissolution medium. When drug sample is placed in the dialysis adapter, to get detected in the dissolution medium, the drug substance must dissolve in the dialysis adapter, then migrate to the interface of membrane and permeate through the membrane into dissolution medium. In this process, dissolution, migration, and permeation could all be rate-limiting steps. Since Tylenol suspension is a viscous sample, the results showed that drug dissolution and migration to the membrane surface were slow and were rate-limiting steps.

The medium pH, medium ionic strength, and membrane MWCO, examined in this study, did show some effects on the dissolution results. But these effects were sometimes dependent on sample physical properties like viscosity.

For dissolution testing of special dosage forms, there is a potential to use this newly developed dialysis adapter with the flow-through method in drug development and quality control. There is a need to more fully understand this dialysis adapter. It is recommended that more drug products and different membranes be investigated.

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