dx.doi.org/10.14227/DT280221P16

A Data Set to Verify Volume and Sample Removal Correction Calculations for Dissolution Testing

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ABSTRACT

Software in the form of a spreadsheet, computer program, or web-based application is typically used to perform the appropriate mathematical corrections to dissolution profile data to correct for the amount of sample solution removed from the dissolution vessels at each time interval. Because the sample solutions contain analyte and because removal changes the volume of dissolution medium remaining in the vessels, such corrections are sometimes necessary to determine accurate values. This article presents results from a mental exercise where a theoretical dissolution profile was constructed. These data can be used to verify the accuracy of such calculations.

KEYWORDS: Dissolution sample removal correction, dissolution profile data, dissolution test calculations

INTRODUCTION

In vitro release and dissolution tests are routinely used to assess the performance of pharmaceutical formulations. In some cases a single data point provides an acceptable level of information for the test. In other cases several data points are needed to yield a profile that shows the amount of drug released from the formulation over time. The shape of the profile and the amount of drug released at one or more intervals may be important indicators of the formulation's performance.

In a typical dissolution test, sample solutions are collected from the vessels, filtered, put into test tubes or suitable vials, then taken to an analytical instrument to measure and determine the amounts of drug released. The cumulative amount of drug and medium withdrawn and removed from the dissolution vessel at each sample interval may be significant enough to affect results in subsequent sample intervals. The effect is more significant when larger volumes are removed from the dissolution vessel. Likewise, the effect is more pronounced for lower initial media volumes. Also, the number of sample collection intervals contributes to this cumulative effect. Mathematical corrections must be applied to determine accurate values, i.e., the values that would have been measured had very little or no sample solution been removed from the vessels (*1*).

The computations can be done manually with pen and paper but software (whether in the form of a spreadsheet, a stand-alone computer program, or a web-based application) is typically used for this purpose. Presented here are data and results that can be used to verify the accuracy of the numeric values produced by such software. Note that regulations defined in 21CFR11 and EU Annex 11 stipulate other aspects to consider to realize a compliant software application of this nature such as security, version control, and data integrity (*2*, *3*).

THE CALCULATIONS

Consider two different scenarios – one where the amount of solution withdrawn at each sample interval is replaced and the vessel is replenished with an equal volume of fresh medium. (This might be important, for example, if the solubility of the drug is such that without replenishment the remaining media volume would be less than that required for sink conditions.) In this case, the vessel volume remains constant throughout the dissolution test and the correction accounts for the amount of drug removed at each sample interval. Equation 1 can be applied when fresh media replaces the amount of sample removed at each interval.

$$C_{n,corr} = C_n + \frac{V_s}{V_m}\int_{i=1}^{n-1} C_i \qquad (1)$$

where $C_{n,corr}$ is the corrected concentration at sample interval n; C_n is the measured or uncorrected concentration at sample interval n; V_m is the original media volume in the dissolution vessel; V_s is the volume of sample removed at each time interval; and C_i is the uncorrected concentration at each previous sample interval i.

In the other scenario, which perhaps is more common, samples are removed and the vessels are not replenished with media. In this case the correction must account for the amount of drug removed at each interval and for the volume of sample withdrawn because the subsequent volume left in the vessel has been reduced by that amount. Equation 2 can be applied when media replacement is not done.

$$C_{n,corr} = \frac{V_m - V_s(n\text{-}1)}{V_m} C_n + \frac{V_s}{V_m} \int_{i=1}^{n-1} C_i \qquad (2)$$

Equation 3 combines Equations 1 and 2 to represent both scenarios.

$$C_{n,corr} = \frac{V_m - (V_s - V_r)(n-1)}{V_m} C_n + \frac{V_s}{V_m} \int_{i=1}^{n-1} C_i \qquad (3)$$

where V_r is the volume of media replaced at each sample interval.

If media is not replaced, then replacement volume V_r is set to zero and Equation 3 reduces to Equation 2. If media is replaced by a volume equal to the sample volume, then V_r equals V_s and Equation 3 reduces to Equation 1. Note that Equation 3 could be used if there is ever a case in which the replacement volume was different from the sample volume.

ASSUMPTIONS

For the purpose of this thought experiment, the following assumptions are made:

- The initial volume of medium in the dissolution vessel is 500 mL.
- The volume of sample removed at each collection interval is 10 mL.
- Six samples are removed from the vessels at equally spaced intervals of 15 minutes for 90 minutes total.
- Withdrawing sample and replenishing with medium has no effect on the hydrodynamics.
- The test formulation contains 60 mg of a drug.
- The drug is very highly soluble in the dissolution medium.
- The formulation releases drug in a linear fashion (zero-order) at a rate of 1 mg per minute. (A thin wafer might exhibit this pattern of release because the two surface areas exposed to the medium would remain constant throughout the dissolution test.) Drug is released from the formulation until all of it has been converted from the solid state into the liquid state, at which time the profile plateaus to the level representing 100% released. The theoretical dissolution profile is shown graphically in Figure 1.

Note that the sample volume listed above is the same as that dispensed into collection container, which is typically a vial or test tube. Many dissolution testers incorporate automated sampling, and typically a volume of sample solution equivalent to two to three times the tubing volume is delivered to waste prior to collection. This is done to ensure that the lines and filters are flushed with sample solution to minimize carryover from what previously filled those lines. In this case the entire volume, the amount collected plus the rinse volume, would be counted as the volume removed from the vessel.

DISSOLUTION TEST WITH NO MEDIA REPLACEMENT

We begin this mental experiment using the scenario where each sample withdrawal is not accompanied by media replacement. Note that for the purpose of this exercise all intermediate results are rounded and expressed to 5 places after the decimal. Any and all rounding errors are propagated through all subsequent intervals. Rounding to five places ensures that the final percent dissolved values are correctly displayed to two places beyond the decimal. The results are summarized in Table 1.

At 15 minutes, 15 mg of the drug has been released and 45 mg of drug in the solid state now remains in the formulation. The vessel contains 500 mL, so the concentration of drug in solution is 15 mg / 500 mL = 0.03000 mg/mL. A 10-mL aliquot of sample solution is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.03000 mg/mL = 0.30000 mg. The amount of drug in solution is 15 mg – 0.30000 mg = 14.70000 mg. The vessel volume is now 490 mL.

At 30 minutes, another 15 mg of drug has been released and now 30 mg of drug in the solid state remains in the formulation. The 14.70000 mg that remained in the vessel from the previous interval means that the total amount of drug in the vessel is now 15 mg + 14.70000 mg = 29.7 mg. The vessel contains 490 mL, so the drug concentration is 29.70000 mg / 490 mL = 0.06061 mg/mL. A 10-mL aliquot of sample solution is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.06061 mg/mL = 0.60610 mg. The amount of drug in solution is 29.70000 mg – 0.60610 mg = 29.09390 mg. The vessel volume is now 480 mL.

At 45 minutes, another 15 mg of drug has been released and now 15 mg of drug in the solid state remains in the

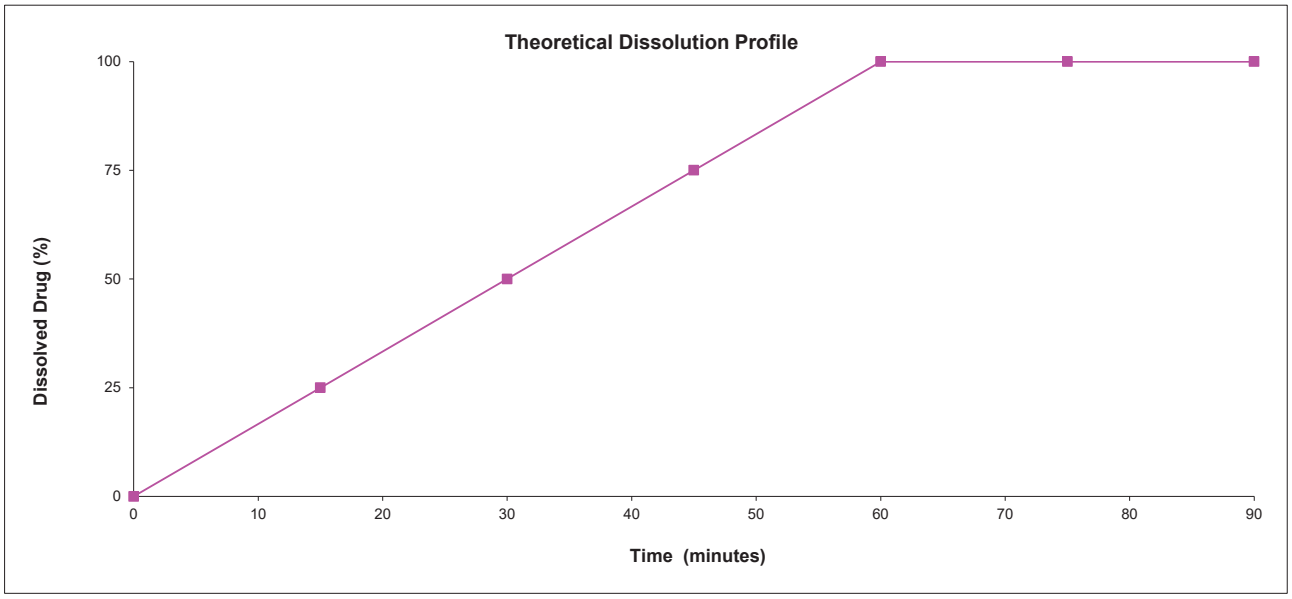

Figure 1. Theoretical dissolution profile.

Table 1. Example Dissolution Test with No Media Replacement

Time (min)	Solid State (mg)	Amount Released (mg)	From Prev Interval (mg)	Total for this Interval (mg)	Vessel Vol (mL)	Actual Conc. (mg/mL)	Amount Removed (mg)	Amount Remaining (mg)	% Released (Uncorrected)
15	45	15	0.00000	15.00000	500	0.03000	0.30000	14.70000	25.00000
30	30	15	14.70000	29.70000	490	0.06061	0.60610	29.09390	50.50833
45	15	15	29.09390	44.09390	480	0.09186	0.91860	43.17530	76.55000
60	0	15	43.17530	58.17530	470	0.12378	1.23780	56.93750	103.15000
75	0	0	56.93750	56.93750	460	0.12378	1.23780	55.69970	103.15000
90	0	0	55.69970	55.69970	450	0.12378	1.23780	54.46190	103.15000

Prev, previous; Vol, volume; Conc., concentration

formulation. The 29.09390 mg that remained in the vessel from the previous interval means that the total amount of drug in the vessel is now 15 mg + 29.09390 mg = 44.09390 mg. The vessel contains 480 mL, so the drug concentration is 44.09390 mg / 480 mL = 0.09186 mg/mL. A 10-mL aliquot of sample solution is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.09186 mg/mL = 0.91860 mg. The amount of drug in solution is 44.09390 mg – 0.91860 mg = 43.17530 mg. The vessel volume is now 470 mL.

At 60 minutes, another 15 mg of drug has been released and now no drug remains in the solid state. The 43.17530 mg that remained in the vessel from the previous interval means that the total amount of drug in the vessel is now 15 mg + 43.17530 mg = 58.17530 mg. The vessel contains 470 mL, so the drug concentration is 58.17530 mg / 470 mL = 0.12378 mg/mL. A 10-mL aliquot of sample solution is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.12378 mg/mL = 1.23780 mg. The amount of drug in solution is 58.17530 mg – 1.23777 mg = 56.93750 mg. The vessel volume is now 460 mL.

At 75 minutes, no additional drug has been released so the concentration of drug in solution is 56.93750 mg / 460 mL = 0.12378 mg/mL. A 10-mL aliquot of sample is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.12378 = 1.23780 mg. The amount of drug in solution is 56.93750 mg. The vessel volume is now 450 mL.

At 90 minutes, no additional drug has been released so the concentration of drug in solution will remain unchanged at 0.12378 mg/mL. A 10-mL aliquot of sample is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.12378 = 1.23780 mg.. The amount of drug in solution is 55.69970 mg. The final vessel volume is now 440 mL.

The actual drug concentrations at each interval were converted to uncorrected values (percent of drug release), which are presented in the last column of Table 1. These are the concentrations that would be determined from analytical measurements. With each interval up until 60 minutes the sample removed reduces the amount of drug remaining in solution while the volume in the vessel is also reduced, which effectively has the opposite effect of raising the concentration at each interval. The effects are mathematically represented in the two terms of Equation 2; the first term is the effect of reducing the vessel volume at each interval and the second term represents the effect of drug removed per interval. The corrected concentrations are obtained by applying Equation 2 (or Equation 3) to the measured concentrations at each interval. Note that the value for percent released at the 60-minute interval is 103.15% as opposed to the corrected value of 100.00%.

DISSOLUTION TEST WITH MEDIA REPLACEMENT

Now consider the case in which media is replaced at each interval. The vessels are replenished with fresh medium after each sample is withdrawn to maintain the vessel volume at 500 mL. The results are summarized in Table 2.

At 15 minutes, 15 mg of drug has been released and 45 mg of drug in the solid state now remains in the formulation. The concentration of drug in solution is 15 mg / 500 mL = 0.03000 mg/mL. A 10-mL aliquot of sample solution is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.03000 mg/mL = 0.30000 mg. The amount of drug in solution is 15 mg – 0.30000 mg = 14.70000 mg. The vessel is replenished with 10 mL of fresh medium.

At 30 minutes, another 15 mg of drug has been released and now 30 mg of drug in the solid state remains in the formulation. The 14.70000 mg that remained in the vessel from the previous interval means that the total amount of drug in the vessel is now 15 mg + 14.70000 mg = 29.70000 mg. The drug concentration is 29.70000 mg / 500 mL = 0.05940 mg/mL. A 10-mL aliquot of sample solution is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.05940 mg/mL = 0.59400 mg. The amount of drug in solution is 29.70000 mg – 0.59400 mg = 29.10600 mg. The vessel is replenished with 10 mL of fresh medium.

At 45 minutes, another 15 mg of drug has been released and now 15 mg of drug in the solid state remains in the formulation. The 29.10600 mg that remained in the vessel from the previous interval means that the total amount of drug in the vessel is now 15 mg + 29.10600 mg = 44.10600 mg. The drug concentration is 44.10600 mg / 500 mL = 0.08821 mg/mL. A 10-mL aliquot of sample solution is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.08821 g/mL = 0.88210 mg. The amount of drug in solution is 44.10600 mg – 0.88210 mg = 43.22390 mg. The vessel is replenished with 10 mL of fresh medium.

At 60 minutes, another 15 mg of drug has been released and now no drug remains in the solid state. The 43.22390 mg that remained in the vessel from the previous interval means that the total amount of drug in the vessel is now 15 + 43.22390 = 58.22390 mg. The drug concentration is 58.22390 mg / 500 mL = 0.11645 mg/mL. A 10-mL aliquot of sample solution is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.11645 mg/mL = 1.16450 mg. The amount of drug in solution is 58.22390 mg – 1.16450 mg = 57.05940 mg. The vessel is replenished with 10 mL of fresh medium.

At 75 minutes, all of the drug in the solid state has been depleted so no more has been released. The 57.05940 mg that remained in the vessel from the previous interval means that the concentration is 57.05940 mg / 500 mL = 0.11412 mg/mL. A 10-mL aliquot of sample solution is withdrawn from the vessel, so the amount of drug

Table 2. Example Dissolution Test with Media Replacement

Time (min)	Solid State (mg)	Amount Released (mg)	From Prev Interval (mg)	Total for this Interval (mg)	Vessel Vol (mL)	Actual Conc. (mg/mL)	Amount Removed (mg)	Amount Remaining (mg)	% Released (Uncorrected)
15	45	15	0.00000	15.00000	500	0.03000	0.30000	14.70000	25.00000
30	30	15	14.70000	29.70000	500	0.05940	0.59400	29.10600	49.50000
45	15	15	29.10600	44.10600	500	0.08821	0.88210	43.22390	73.50833
60	0	15	43.22390	58.22390	500	0.11645	1.16450	57.05940	97.04167
75	0	0	57.05940	57.05940	500	0.11412	1.14120	55.91820	95.10000
90	0	0	55.91820	55.91820	500	0.11184	1.11840	54.79980	93.20000

Prev, previous; Vol, volume; Conc., concentration

removed at this interval is 10 mL × 0.11412 mg/mL = 1.14120 mg. The amount of drug in solution is 57.05940 mg – 1.14120 mg = 55.91820 mg. The vessel is replenished with 10 mL of fresh medium.

At 90 minutes, all of drug in the solid state has been depleted and no more has been released. The 55.91820 mg that remained in the vessel from the previous interval means concentration is 55.91820 mg / 500 mL = 0.11184 mg/mL. A 10-mL aliquot of sample solution is withdrawn from the vessel, so the amount of drug removed at this interval is 10 mL × 0.11184 mg/mL = 1.11840 mg. The amount of drug in solution is 55.91821 mg – 1.11836 mg = 54.79980 mg. The vessel is replenished with 10 mL of fresh medium.

The actual drug concentrations at each interval are expressed as uncorrected values (percent of drug released) in the last column of Table 2. Note that the percent released at the 60-minute interval is just over 97% as opposed to the corrected value of 100%. With each interval the sample solution undergoes successive and cumulative dilutions, so the measured concentrations are lower relative to those that would have been measured without sample removal and media replacement. This effect is represented in the second term of Equations 1–3. The corrected concentrations are obtained by applying Equation 1 (or Eq. 3) to the measured concentrations at each interval.

RESULTS AND DISCUSSION

Table 3 presents results from a spreadsheet developed to perform the appropriate corrections for dissolution profiles as per Eq. 3. Results from both cases (with and without media replacement) are listed using the data set presented here. As expected, the corrected results exactly match the data in the theoretical profile presented in Figure 1.

Table 3. Measured (Uncorrected) and Corrected Results of Example Dissolution Tests

	No Media Replacement		With Media Replacement	
Time (min)	Measured % Released	Corrected % Released	Measured % Released	Corrected % Released
0	0.0000	0.00	0.0000	0.00
15	25.0000	25.00	25.0000	25.00
30	50.5102	50.00	49.5000	50.00
45	76.5519	75.00	73.5100	75.00
60	103.1476	100.00	97.0398	100.00
75	103.1476	100.00	95.0990	100.00
90	103.1476	100.00	93.1970	100.00

CONCLUSION

The data set presented here may be used by those who need to verify the accuracy of calculations used to correct dissolution profiles for sample aliquot removal. This can be part of a planned software validation effort that would also consider things like security, data integrity, version control, and other aspects decreed by appropriate regulations.

CONFLICTS OF INTEREST

The author disclosed no conflicts of interest related to this article.

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