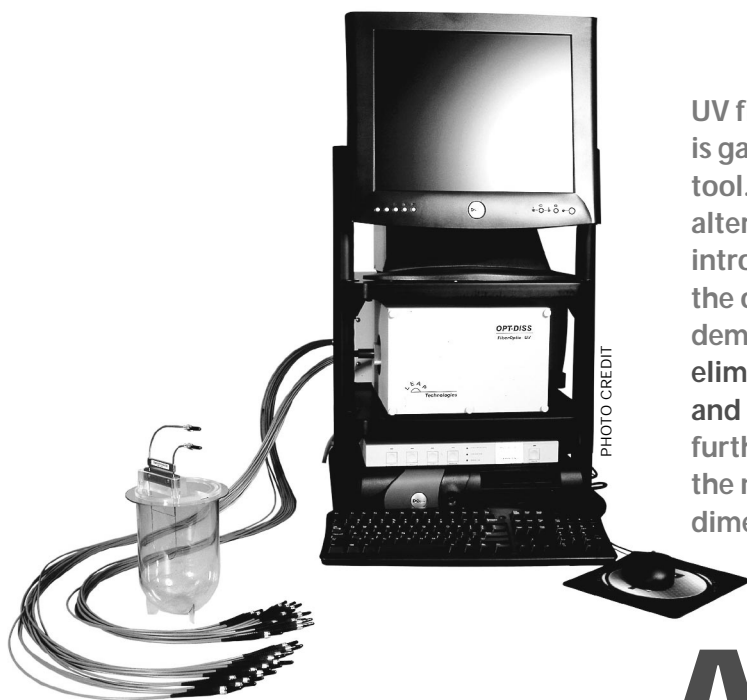


# System Optimization for In Situ Fiber-Optic Dissolution Testing

## Hydrodynamic Effects, System Performance, and Applications

Guy W. Inman,\* Eric Wethington, Kim Baughman, and Michael Horton



UV fiber-optic in situ dissolution testing is gaining acceptance as an analytical tool. However, immersion probes can alter critical solution hydrodynamics and introduce range-limiting stray light into the optical system. The authors demonstrate how a fiber-optic probe can eliminate hydrodynamic disturbances and minimize stray-light limitations. They further demonstrate the effectiveness of the new probe when coupled with a two-dimensional CCD array spectrometer.

Guy W. Inman, PhD, is principal consultant at Automation Resources, 5216 Hough Rd., Hillsborough, NC 27278, tel. 919.929.0749, fax 919.942.9232, guyinman@aol.com. Eric Wethington is products manager of Pharmtec Group, LEAP Technologies, Inc. (Carrboro, NC), Kim Baughman, PhD, is director, and Michael Horton is a chemist at Southern Testing and Research Laboratories, Inc. (Wilson, NC).

\*To whom all correspondence should be addressed.

**A** potential disadvantage of in situ fiber-optic measurement of dissolution rates is the disturbance of critical solution hydrodynamics. Probes inserted into dissolution vessels can affect dissolution rates. The effect appears to be related to the displacement volume of the insertion probe and may be more pronounced for disintegrating tablets when USP Apparatus 2 is used (1). This effect originally was documented by Wells and Savage, who showed that sipping-type probes used in automated sampling systems significantly increased dissolution rates for prednisone tablets (2,3). Rates measured with the sipping probes (tip diameters 7.2 and 8 mm) in place were 7% higher than those measured when the probes were not in place. The effect also has been observed for fiber-optic immersion probes (4).

Hydrodynamic disturbances can be minimized with the use of an automated apparatus to lower probes into the vessels only during the time required to withdraw the sample (5). However, unless the probe is withdrawn only partially after the measurement interval, bubbles might be introduced into the light path or, because of media solvent evaporation in the intervals



Figure 1: The Arch fiber-optic transmission probe.

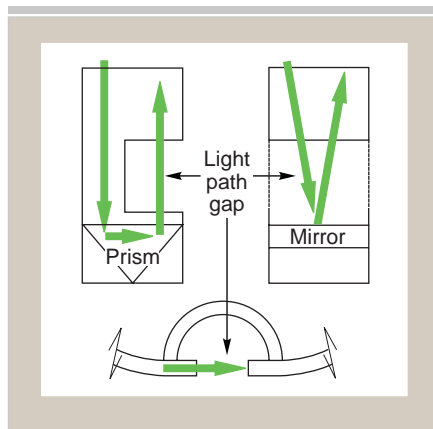


Figure 2: Schematic representations of various immersion-probe designs. The Arch design is shown at the bottom.

between measurements, solute material might be deposited onto optical surfaces. Bubbles and coated optics will reduce the amount of transmitted light, thereby producing erroneous absorbance readings. This approach also adds complexity, increases cost, and reduces the time resolution of the in situ technique.

Another approach to in situ measurement is to mount the probe inside a hollow stirring-element shaft. This method has been shown to effectively reduce hydrodynamic disturbances (6). However, both the stirring-element shaft and the dissolution bath must be modified, and the overall cost is high compared with the cost of using probes placed outside the shaft.

Immersion probes also can be a significant source of stray light in the optical system and will limit the maximum absorbance that can be measured (7). Because dilution is not an option for in situ testing, monitoring strong UV chromophores and concentrated solutions throughout a wide concentration range (0–120%) can lead to nonlinearity errors.

### Probe design

After carefully considering the specific requirements for dissolution testing and the possible advantages of coupling probes to a highly sensitive charge-coupled device (CCD) spectrometer (Optdiss, Leap Technologies, Carrboro, NC), the authors developed an in situ probe (Arch) with the following primary design goals: low displaced volume to minimize or eliminate hydrodynamic disturbances, fully transmitting light path to minimize stray light, minimal horizontal surfaces to trap bubbles or particulate matter, straightforward adaptation requiring no modifications to the bath or stirring element, and simplicity of construction (8). A photograph of the Arch probe is shown in Figure 1. Figure 2 illustrates how the design compares with those of prism-based and reflecting probes, which contain “light-bending” elements that introduce stray light.

The displaced volume of the Arch probe is less than that of a 1/16-in. o.d. sipper probe. The open structure and transverse light path minimize interference from bubbles and particulate

matter. The open structure is designed to reduce bubble and particulate entrapment as well as facilitate cleaning. The fully transmitting design, which includes no mirrors or prisms in the light path, keeps the probe contribution to stray light <0.1% (3.0 AU). The probe is constructed of passivated, spring-tempered, 316 stainless steel. The probe height adjusts to accommodate solution volumes from 350 to 1000 mL in standard 1-L vessels. Probes also can be configured to monitor 200-mL, 2-L, and 4-L vessels. The probe requires no bath or shaft modifications and can be used with waterless baths that monitor temperature by means

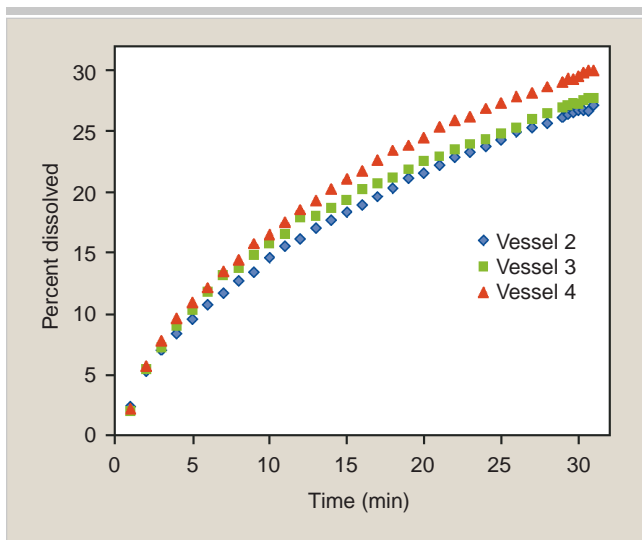
of probes inserted into the shaft. Because the Arch probe remains in place throughout a dissolution test, there is no need to add an apparatus to raise and lower the probes. Therefore, data can be collected rapidly (<5 s with the Optdiss system described in this article) to produce a dissolution curve.

### Hydrodynamic testing

To determine how effectively the Arch probe reduces hydrodynamic disturbances, the authors completed a series of tests using USP Lot M prednisone calibrator tablets. Dissolution rates for disintegrating tablets such as the prednisone calibrators are known to be highly sensitive to distortions in solution hydrodynamics (1–3). Dissolution rates for Lot M calibrators should be particularly sensitive because their 500-mL-required media volume is significantly less than the typical 900-mL media volume. Five dissolution tests were completed in ac-

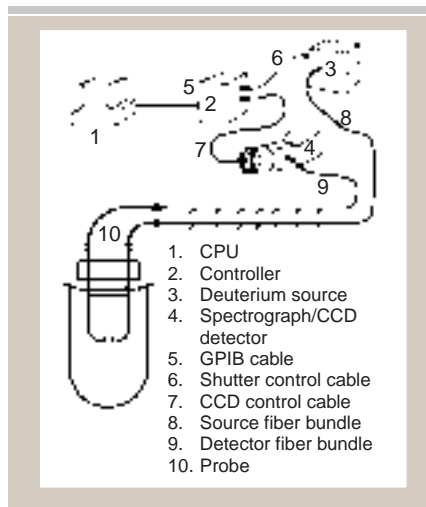
Table I: Percent-dissolved results for USP prednisone Lot M calibrator tablets.

Test	No Probes, Manual Sampling	Arch Probes, Manual Sampling	Arch Probes, Optdiss, In Situ
1	28.1	26.6	26.7
	27.4	27.4	27.2
	28.8	29.3	29.5
2	27.4	27.8	27.0
	25.9	26.0	25.4
	28.8	30.8	31.0
3	27.7	27.9	28.0
	29.7	25.9	25.0
	28.9	26.5	27.2
4	27.5	27.5	27.2
	27.3	30.3	30.0
	25.9	26.5	26.1
5	27.0	29.3	29.0
	26.5	28.8	29.9
	24.6	28.1	28.1
SD:	1.4	1.5	1.8
Mean:	27.4	27.9	27.8
Range:	24.6–29.7	25.9–30.8	25.0–31.0



**Figure 3:** In situ results from Test 1, prednisone Lot M calibrator tablets (using the Optdiss system).

cordance with USP General Chapters on Dissolution (711) and Drug Release (724) (9,10). A calibrated dissolution bath (Hanson SR-8 Plus dissolution test station, Hanson Research, Chatsworth, CA) equipped with six standard vessels and paddle shafts was used for all tests. For each test, Arch probes, mounted in specially designed vessel covers, were placed in three of the six numbered vessels. The probes were mounted such that the light-path gaps were located at the USP-prescribed sampling point midway between the top of the paddle and the solution surface. The probe mounting slot in the vessel cover placed the lateral position of the light-path gap midway between the vessel wall and the paddle shaft. The probes remained in place throughout each test. Possible vessel-to-vessel differ-



**Figure 4:** Optdiss system diagram.

ences were randomized by placing the probes in different vessels for each test.

Samples were withdrawn from the six vessels at the predetermined 30-min sampling time, filtered, and their UV absorbance measured by a calibrated, dual-beam, UV-vis spectrometer with blank media in the reference cuvette. To ensure that manual sampling times were as close to theoretical as possible, two analysts pulled samples from three vessels each. In addition, complete absorbance spectra were acquired at 1-min intervals with the Optdiss spectrometer using the Arch probes. Percent-dissolved results were calculated from the absorbance at 242 nm corrected for spectral baseline shifts by subtracting the average absorbance between 325 and 350 nm. Example dissolution curves acquired with the Optdiss system are shown in Figure 3. Percent-dissolved results from all tests are shown in Table I.

The data sets were subjected to statistical *t*-tests at the 95% confidence level ( $p = 0.05$ ) to determine if differences between the means for each possible data set pair were significant. Probability values (0.19–0.24) from the tests were much greater than 0.05 and suggest that any bias introduced by either the Arch probes or the Optdiss measurement technique was well within tablet-to-tablet variations of measured dissolution rates. This outcome indicates that no significant turbulence was introduced by leaving the Arch probes in place during a dissolution test.

### System performance

The Optdiss spectrometer (see Figure 4) uses a two-dimensional  $512 \times 512$  array CCD detector to instantaneously acquire multichannel spectra in a manner that is analogous to taking a photograph. The detector is thermoelectrically cooled and

**Table II:** Absorbance noise at various wavelengths.

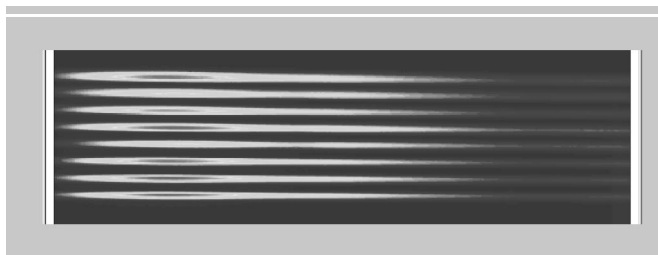
	Wavelength (nm)				
	205	250	300	350	400
1 scan	0.0011	0.0006	0.0008	0.0011	0.0012
4 scans	0.0004	0.0003	0.0004	0.0003	0.0005

**Table III:** Optdiss–Arch system stray-light results at 240 nm.

	Probe–Channel							
	1	2	3	4	5	6	7	8
%T	0.30	0.31	0.32	0.41	0.40	0.43	0.30	0.38
Absorbance	2.52	2.51	2.49	2.39	2.40	2.37	2.52	2.42

**Table IV:** Optdiss linearity ( $R^2$ ) for potassium dichromate solutions measured at various wavelengths.

	Probe–Channel					
	1	2	3	4	5	6
220 nm	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
235 nm	0.9999	0.9999	0.9998	0.9999	0.9999	0.9999
257 nm	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
350 nm	0.9998	0.9997	0.9999	0.9999	0.9999	0.9999



**Figure 5:** False-color representation of the UV spectral light bands (200–400 nm) imaged onto the CCD detector chip.

exhibits a high quantum efficiency across the UV spectrum from 200 to 400 nm. The original working prototype and first dissolution testing application for this type of system was described by Cho et al. (11). The Optdiss spectrometer can be configured to collect data from as many as 12 fiber-optic probes.

All major system functions are accessed through software that supports user compliance with 21 CFR Part 11 regulations with respect to audit trails, system access security, and file security. All raw data, method parameters, and audit trail information are stored in the same file. Thus even if a file is moved or copied, its audit trail is preserved. Additional functions are available for calibrating wavelength and response, viewing spectral and numeric data, exporting data, reporting results, and defining analytical method parameters.

Perhaps the most critical function related to data processing for in situ testing is the ability to apply various baseline corrections to measured absorbance values. Because filtration is not possible, corrections must be applied to account for baseline shifts resulting from light-scattering turbidity, source drift, and excipient absorbance. The effectiveness of various baseline-correction options has been discussed elsewhere (5,7). The Optdiss software provides four options (none, single wavelength, dual wavelength or perpendicular drop, and average-over-range) commonly used in quantitative UV spectroscopy. The single-wavelength option allows the corrected absorbance to be equal to the absorbance at the analytical wavelength ( $\lambda_{\max}$ ) minus the absorbance at a reference wavelength, which usually is selected

from a valley or flat section of the absorbance spectrum. The dual-wavelength option allows the corrected absorbance to be the difference in the absorbance at  $\lambda_{\max}$  and the absorbance at the baseline formed by two points on either side of  $\lambda_{\max}$ . The average-over-range option is similar to the single-wavelength option except that the correction is equal to the average absorbance for all values between two selected wavelengths.

The system light source is a low-noise deuterium lamp that further ensures UV spectral coverage from 200 to 400 nm, at which most active ingredients exhibit absorbance maxima. Light passing through sample solutions by way of the probes is directed through receiving fibers terminated into a vertical 12-fiber array. Light from the array passes through a slit into a fixed-grating spectrograph where it is spatially dispersed across the horizontal axis of the CCD chip onto the light-sensitive elements (pixels). The locations of the resulting multichannel spectral (200–400 nm) light bands (see Figure 5) are automatically identified during system configuration and installation qualification. Automated wavelength calibration for all configured channels is performed with a mercury lamp that is a primary National Institute of Standards and Technology (NIST) standard. The theory behind the wavelength calibration procedure is described in detail by Cho et al. (12).

The Arch probes, when coupled to the Optdiss system, do not need a lens as do most reflecting probes. The system light throughput is sufficient to rapidly saturate the CCD detector within 0.05–0.30 s, depending on the probe path length. The probe-spectrometer combination exhibits low absorbance noise (see Table II) and excellent stray-light performance (see Table III). Because the detector chip is two-dimensional, signal responses across pixel rows within each band can be averaged to produce a single absorbance spectrum for each channel. The vertical height (~11 pixels) of each light band is directly proportional to the system fiber diameter. It is also possible to average multiple spectra with respect to time to achieve further reductions in noise.

System stray-light tests were performed according to the American Society for Testing and Materials' spectrometer stray-light test at 240 nm. Arch probes with a 10-mm light-path gap were immersed in a 1% potassium iodide solution, UV spectra were measured, and the differences between absorbance values at 240 and 280 nm were used as the test results (see Table III). This is a critical test for assessing spectrometer performance for the analysis of pharmaceutical dosage forms. Typical values for the Arch probes are 2.3–2.5 AU. Stray-light performance is critical because it affects the linearity of results. If too much stray light is present, a UV method may not meet the FDA linearity requirements (0–120%) for dissolution assay methods.

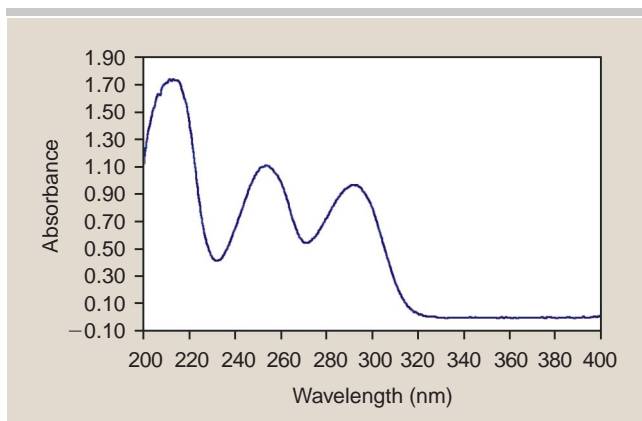
System linearity was directly assessed through potassium dichromate solutions prepared from NIST primary standard reference material 935a. Linearity results ( $R^2$ ) for baseline-corrected absorbance values

**Table V:** Optdiss linearity and reproducibility results: 0–120% API (proprietary capsule formulation).

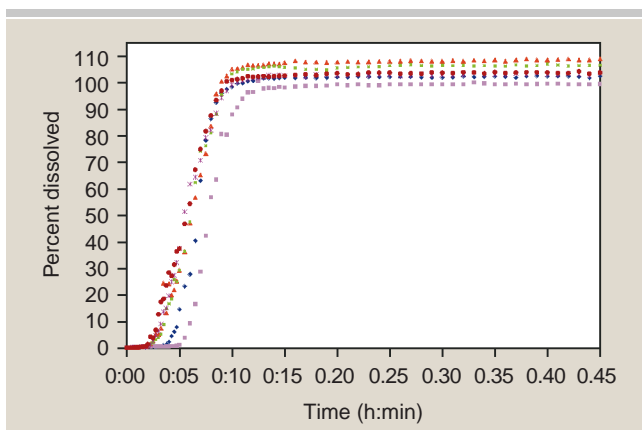
	Probe-Channel					
	1	2	3	4	5	6
$R^2$	0.9996	0.9997	0.9997	0.9995	0.9995	0.9996
Intercept	0.006	0.007	0.006	0.007	0.009	0.002
% RSD	0.14	0.13	0.19	0.24	0.14	0.32

**Table VI:** Comparison of Optdiss and manual percent-dissolved results.

		Probe-Channel					
		1	2	3	4	5	6
30 min	Optdiss	102.0	99.3	108.0	106.5	102.8	103.6
	Manual	102.5	99.4	108.9	107.4	102.9	103.8
	Difference	-0.5	-0.1	-0.9	-1.0	-0.1	-0.2
45 min	Optdiss	102.4	99.5	109.0	106.6	103.6	103.7
	Manual	103.0	99.3	109.3	107.9	103.2	103.7
	Difference	-0.6	0.2	-0.3	-1.4	0.4	0.0



**Figure 6:** Absorbance spectrum of the active pharmaceutical ingredient from the developmental capsule formulation at 100% dissolved, measured with a 2-mm Arch probe.



**Figure 7:** Dissolution curves for the developmental capsule formulation (Arch-Optdiss system).

at four wavelengths are shown in Table IV. The system exhibited excellent linearity ( $R^2 \geq 0.9997$ ) at all listed wavelengths.

### Example application

A rapidly dissolving 100-mg capsule formulation (proprietary developmental material) was selected for testing and comparison with a validated, manual UV method. Although the original method called for 1-mm cuvettes, 2-mm Arch probes were selected to deliberately stress the method by pushing the maximum absorbance above the preferred quantitation range of 0.5 AU. Table V shows the linearity and reproducibility results, and Figure 6 illustrates the spectrum of the active ingredient. Both manual and Optdiss methods used the peak at 253 nm as the basis for quantitation. To correct for baseline shifts, the Optdiss system's absorbance was corrected by subtracting the average absorbance between 340 and 350 nm from the measured absorbance at 253 nm.

A dissolution run was completed by means of a calibrated bath (Distek 2100B, Distek, North Brunswick, NJ) configured as Apparatus 2 with six vessels. The dissolution medium was 0.1 N hydrochloric acid, the solution volume was 900 mL, and the stirring speed was 50 rpm. The Optdiss method was con-

figured to collect complete UV spectra at 5-, 15-, 30-, and 60-s intervals. Both solution turbidity and particulate matter were generated during the test. Samples were withdrawn manually at 30 and 45 min, filtered through 0.45- $\mu$ m Nylon 66 membrane filters, and measured on a calibrated dual-beam UV spectrometer. Dissolution curves from all vessels are shown in Figure 7. Table VI compares the Optdiss in situ results with manual results. Results agreed within  $\pm 1.4\%$ , which is well within the typical specification for method validation comparisons. Because of the rapid dissolution of the active under these test conditions, conventional sampling methods could not characterize the early portion of the dissolution curve. Thus the Optdiss system provided previously unobtainable information.

### Conclusion

The combination of low-displacement volume fiber-optic probes and a two-dimensional CCD array spectrometer is an effective means for monitoring real-time in situ dissolution rates. Features such as rapid and simultaneous multichannel data collection are particularly useful during the early stages of formulation and method development. Probe hydrodynamic performance and spectrometer performance throughout a wide spectral and absorbance range provide tangible benefits to scientists wishing to use in situ technology for existing UV methods or to ensure quality-control acceptance of in situ methods for new dosage forms. Continuing improvements in probe design, detector technology, and software will accelerate the acceptance of in situ dissolution testing. It is anticipated that these improvements, particularly those in the area of CCD technology, will dramatically improve both the quantity and quality of dissolution data well beyond what is currently possible with manual or automated sampling techniques.

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