Implementation of Water Activity Testing to Replace Karl Fischer Water Testing for Solid Oral-Dosage Forms

Bob Snider, Peihong Liang, and Neil Pearson

For solid oral-dosage forms, water testing usually is performed to control the chemical, physical, or microbiological properties of the drug product. Measurements of total water as made with Karl Fischer (KF) techniques is not needed and water-activity often will provide a better correlation with changes in chemical, physical, or microbiological properties than KF techniques. In these cases, water activity testing can easily replace KF testing. Water-activity measurements are nondestructive, require little labor, and the equipment required is generally inexpensive. Only a few simple checks are needed to ensure the validity of measurements. Strategies for implementing water activity testing are described.

Most pharmacopeial monographs that have procedures for the measurement of water are based on the measurement of total water either by the Karl Fischer titration or by loss on drying. Karl Fischer titrations have dominated the measurement of water in pharmaceutical products for many years. Although the technique is reliable under carefully controlled conditions, it is subject to a variety of problems such as sample handling and side-reactions that cause erroneous results. Results commonly vary with changes in room relative humidity (RH). In addition, general models for describing the effect of water on physical, chemical, and microbiological characteristics recognize that different types of water may be present (1–3) and that the measurement of total water may not be the best approach for understanding the effects of water.

According to most common definitions, water can be present in at least three forms: free water, adsorbed water, and bound water. Free water is present in the void volume or in the pores. Free water can serve as a dispersing agent, as a solvent for crystalline compounds, or for microbiological growth. Adsorbed water is located on the surface of the material. Bound water is defined as the water of hydration bound to the product by strong H-bonds. Bound water relates to the monolayer of water molecules, whereas adsorbed water is present in the form of multilayers in the matrices. Water also may be present as a crystal hydrate that effectively is the same as bound water. An example of another type of water is structural water associated with hydrogen bonding between helices of polymer in a gel network (4). This explanation follows the theory of the BET isotherm describing vapor desorption or adsorption isotherms (3).

When water interacts with solutes and surfaces, it is unavailable for other hydration interactions. The term water activity (\(a_w\)) describes the (equilibrium) amount of water available for the hydration of materials. Water activity is unitless and a value of unity indicates pure water, whereas zero
indicates the total absence of water molecules. Water activity is the effective mole fraction of water, defined as:

$$a_w = \lambda_w x_w = \frac{p}{p_0}$$

in which $\lambda_w$ is the activity coefficient of water, $x_w$ is the mole fraction of water in the aqueous fraction, $p$ is the partial pressure of water above the material, and $p_0$ is the partial pressure of pure water at the same temperature (i.e., the water activity is equal to the equilibrium relative humidity [ERH]), expressed as a fraction. The relationship between water activity and weight percent water is shown in a sorption isotherm (see Figure 1). Figure 1 also shows the hysteresis observed when the sorption isotherm depends on whether the water is added to the dry material or removed from the wet material. This hysteresis is caused by nonreversible structural changes and/or kinetic effects.

The sorption isotherm for a formulated drug product is the result of the combination of the individual-component sorption isotherms. Sorption isotherms for excipients and the drug substance may be used to model the sorption isotherm of the finished dosage form (5). Figure 2 shows an example of an adsorption isotherm for a typical oral formulation. Two recently published studies present adsorption and desorption isotherms for several common excipients (5–6).

Materials of differing water activity exhibit time- and temperature-dependent water migration from areas with high to low $a_w$. For example, a gelatin capsule filled with a powder that has a different water activity will undergo water migration and, at equilibrium, the capsule shell and powder contents have the same $a_w$. For packaged products, the moisture-vapor barrier properties of the packaging become crucial in the rate of moisture loss or gain. In addition, desiccants inside a package change the initial water activity as well as the subsequent rate of water activity change. Because the humidity of the air is defined as 60% ($a_w = 0.6$) by the International Conference on Harmonization under Climatic Zones II, materials with lower $a_w$ tend to gain water and those with higher $a_w$ tend to lose water. Most solid oral-dosage forms are produced with water activities of 0.3 to 0.5 and thus gain water in stability studies.

**Use of water activity.** The food industry has long used water activity to control microbial growth and chemical stability. Consistent with the US Food and Drug Administration’s use of water activity in controlling the microbial attributes of food (7), water activity also is being used to control the microbiological properties of pharmaceutical products (8, 9). The growth of most bacteria is inhibited below approximately $a_w = 0.91$. Similarly, most yeasts cease growing below $a_w = 0.87$, and most molds stop growing below $a_w = 0.80$. The absolute limit of microbial growth is approximately $a_w = 0.6$.

The importance of water activity in controlling the microbial properties of pharmaceutical products was added to **US Pharmacopeia (USP) Chapter (2023), “Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements,” and a new USP chapter was written: (1113) “Application of Water Activity Determination to Nonsterile Pharmaceutical Products.” USP Chapter (1113) primarily describes the application of water-activity measurement for the control of a nonsterile formulation’s microbial attributes, but also mentions the control of chemical degradation. The correlation of water activity and chemical changes in food also has been investigated extensively (10). A correspondingly common practice in pharmaceutical development is to equilibrate products at different humidities (water activities) and study their degradation rates. Water activity also correlates with changes in chemical and physical stability (11, 12). The degradation rate of several pharmaceutical products is well modeled by water activity using the BET function (rate is proportional to $1/[1 - a_w]$) (11) as well as other approaches (12–15).

**Measurement of water activity.** Water activity is determined by the measurement of water in the gas phase immediately sur-

---

*Figure 1: Water sorption isotherm.*

*Figure 2: Typical adsorption isotherm for tablet formulation.*
WATER-ACTIVITY TESTING

Table I: Examples of required changes in water activity to double degradation rate.

<table>
<thead>
<tr>
<th>Example</th>
<th>Change in (a_w) to double degradation rate</th>
<th>Change in water content to double degradation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporin degradation (19)</td>
<td>0.2</td>
<td>0.3–0.5%</td>
</tr>
<tr>
<td>N-oxide formation (20)</td>
<td>0.2</td>
<td>4.1–5.7%</td>
</tr>
<tr>
<td>Waterman aspirin degradation (21)</td>
<td>0.2–0.3 (estimated)</td>
<td>Not available</td>
</tr>
<tr>
<td>Hydrolysis of nitrazepam (22)</td>
<td>0.1–0.2</td>
<td>Not available</td>
</tr>
<tr>
<td>Asparatamine degradation (23)</td>
<td>0.1–0.2</td>
<td>Not available</td>
</tr>
</tbody>
</table>

Although sample transfer can be performed rapidly to minimize changes to the sample moisture content, moisture still must be absorbed or desorbed from the sample to equilibrate the measuring chamber’s headspace. For a typical sample, the change in the sample’s moisture content during this equilibration process will not have a significant effect on the accuracy of the sample’s final \(a_w\) reading. Air at a temperature of 25 °C contains approximately 0.023 mg/mL of water at 100% RH. For a sample at 75% RH, with the external environment at 0% RH and a sample chamber headspace of 6 mL, the sample must replace only approximately 6 mL \(\times 0.023\) mg/mL \(\times (0.75 - 0.10)\) or 0.01 mg of water. With a sample weight of 2 g in the chamber and a water content of 2%, the total amount of water in the sample is 40 mg. In this case, only a very small fraction of the total water is removed from the dosage form to equilibrate the humidity in the headspace (the water content of the sample would drop by less than 0.01%).

Samples that contain very little water or have a very shallow slope for the sorption isotherm in the region of interest will be problematic with regards to bias during equilibration in the chamber. The use of laser-absorption spectroscopy (which can determine the water activity in the headspace of a sample vial without breaching the seal) would be beneficial for these types of samples. An example of a product that could be difficult is a lyophilized cake with very low water content. Nonetheless, the typical capsule or tablet product generally contains a large amount of water and is an excellent candidate for water-activity testing.

For most solid oral-dosage forms, the range of interest for water activity is 0.30–0.75. Water-activity measurements are generally precise and accurate to 0.01–0.02 units. Thus, water activity should be capable of providing 23 levels (0.75 – 0.30/0.02) of distinction over this measurement range. Sev-
eral examples of degradation as a function of water activity are shown in Table I, and water activity changes of about 0.1–0.2 are needed to double the degradation rate. In a case for which a 0.1 change in water activity is critical, tighter controls of calibration may be needed. In general, the water activity would be controlled at a level well below when significant degradation occurs. Thus, the examples in Table I represent mostly the worst cases in terms of required accuracy. Compared with Karl Fischer water testing, water activity has particular advantages when:

• the sample is hygroscopic;
• the sorption isotherm is relatively flat over the region where increased degradation rates are noted (i.e., little change in water content);
• the amount of total water is high (e.g., hydrates) and variability in the measurement makes discriminating changes in water content difficult.

Calibration. Calibration is accomplished with the use of either saturated salt solutions or solutions with well-characterized water activities. Saturated salt solutions that have reference values traceable to National Institute of Standards and Technology standards (24) are available. Figure 3 shows the effect of temperature on some typical water-activity standards.

Experiment

Over-the-counter tablets of ibuprofen, naproxen, and enteric-coated aspirin were obtained from a local retail establishment for evaluation. Two developmental capsule formulations also were evaluated. Capsule A was a starch-based formulation and Capsule B contained enteric-coated beads. The water-activity instruments used were from the same manufacturer (Novasina, Pfäffikon, Switzerland) but obtained from Omnimark Instrument Corp. (Tempe, AZ). The names of the two models are the Ms1 and the AW Lab.

Both instruments use the same measuring cell and sensor. The AW Lab model has an additional capability of determining whether the measurement has reached equilibrium by establishing a limit of the change in water activity with time. All measurements were made using the mechanical prefilter, except for those made for Capsule B. For the Capsule B measurements, all of the protective filters listed in Table II were evaluated.

Water-activity standards (saturated salt solutions) were obtained from the instrument supplier. Both Novasina instruments take into account temperature during calibration as long as the temperature is between 15 °C and 30 °C. The Novasina instruments do not allow for temperature control. To obtain temperature-effect data, the measuring chamber was placed in a plastic bag and immersed in a water bath. Measurements were only recorded after the sensor temperature reading agreed with the external water-bath temperature.

Because it is not easy to predict the effect of test samples on the instrument sensor, bracketing readings of water activity standards were performed regularly (one low and one high standard reading during each period of use, typically 24 hours).

The equilibration of dosage forms to different water activities were performed to ensure the validity of the water-activity measurement. In general, the same type of salt solutions used in sealed desiccators also were used for calibration. Intact tablets or capsules were...
equilibrated for 7–14 days. Crushed or composited dosages were equilibrated for 24 hours.

Studies requiring RH control were performed with a humidity-controlled glove box (Coy Laboratory Products, Grass Lake, MI).

Results and discussion

Key operating conditions that were investigated include the effects of sample temperature, equilibration time, and sample handling (in particular, the humidity of the environment in which samples are transferred). In addition, the factors affecting the specificity of the sensor must be considered to support the validation of the technique. Based on an earlier discussion, changes in $a_w$ of >0.01 to 0.02 were not considered significant.

Sample temperature. If the sample and the equilibration chamber are at the same temperature, then temperature has a small effect on water activity within the temperature range of most laboratories. The authors previously discussed the effects of temperature on the water activity of the saturated salt standard solutions used for calibration. Those effects are generally small and taken into account by the instrument. The effect of temperature on solid oral-dosage forms was studied for two samples: Capsule A and naproxen. The observed effects of temperature changes on water activity were 0.003 and 0.004 $a_w/°C$ and were similar to those reported by a man-
manufacturer (25). The small effect of temperature is explained by the fact that the change in the partial pressure of water vapor \( (p) \) with temperature for the sample is similar (but not equal) to the magnitude of the change of the saturation pressure \( (p_0) \) above pure water. Any change in the temperature of a hygroscopic product automatically causes the product to exchange moisture with the air (or gas) that surrounds it. Moisture is exchanged until the partial water-vapor pressure at the product’s surface and in the air is equal. For this reason, a constant temperature is important when measuring \( a_w \).

Equilibration time. To understand the time required for the headspace to come into equilibrium with the sample, water activity was measured as a function of time for the products shown in Figures 4 and 5. The results for Capsule A indicated that equilibration occurred within 10 minutes and no significant changes occurred over the subsequent 12 hours (see Figure 4). Figure 5, which includes two typical tablet formulations and an enteric-coated aspirin tablet, shows that a 30-minute equilibration time was appropriate for the tablet formulations. A longer equilibration time would be needed with the enteric-coated formulation for the same degree of accuracy. In general, the authors have used an accuracy of \( \pm 0.02 \ a_w \) as being acceptable. Another alternative would be to crush the enteric-coated tablet, but this procedure needs special precautions.

For some instruments, the equilibration time is not a concern. These instruments measure the rate of change of \( a_w \) with time and will indicate a stable reading only when the rate of change is below a selected threshold. Other instruments use a mathematical function to estimate the final equilibrium value. The advantage of measuring water activity on intact tablets is shown in Table III, in which the water activity of intact tablets is compared with that of crushed tablets under 10% and 80% RH. The total exposure time to the ambient RH was approximately 2 min (including the time to crush the tablets with a mortar and pestle). This time was used to sim-

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Water activity</th>
<th>Error compared with intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact ibuprofen tablets (different lot than in Figure 5)</td>
<td>0.345</td>
<td>—</td>
</tr>
<tr>
<td>Ibuprofen tablets crushed in a 80% relative humidity environment (2 min exposure)</td>
<td>0.530</td>
<td>+53%</td>
</tr>
<tr>
<td>Ibuprofen tablets crushed in a 10% relative humidity environment (2 min exposure)</td>
<td>0.321</td>
<td>–7%</td>
</tr>
</tbody>
</table>
ulate rapidly crushing, weighing, and transferring tablets in alternative methods such as Karl Fischer. Whereas the low humidity only created a small bias, the high humidity created a large error. There was a larger difference between the sample water activity and the ambient conditions for the high humidity (45% RH higher) versus the low humidity (22% RH lower). The rate of water uptake versus loss is not expected to be the same. A technique for reducing water changes during crushing is to use a device sold to home customers that sandwiches the sample between two holders, thus reducing contact with the ambient atmosphere.

Sample handling. The effect of ambient humidity on sample handling was assessed by transferring Capsule A in environments of 10% and 80% RH. As shown in Figure 6, the effect of high or low humidity is to increase the equilibration time with little effect on the final measured value. An equilibration time of 30 minutes was considered adequate.

A second issue with sample handling is the equilibration of water within the dosage unit. For tablets, a blended powder is compressed and there should be little inhomogeneity of water content. For capsules, the powder fill will be at a different water activity than the gelatin. A study was undertaken to measure the time required for equilibration to occur between the fill and the capsule. For dry contents ($a_w = 0.38$) within a wetter gelatin capsule shell ($a_w = 0.54$), approximately one hour was required to be within 0.01 of the final water-activity value. The same contents within a very dry shell required approximately three hours to be within 0.01 of the final water-activity value. Thus, equilibration within the dosage unit would only be a concern for in-process testing. For in-process testing, the capsule could be opened, and the capsule shell and powder could be placed within the testing chamber.

Validation. Specificity. For the Novasina instruments, the sensor responds to any polar volatile component that affects the resistance or the capacitance of the polymer used in the sensor. Most pharmaceutical tablets or capsules either will have no solvents or the solvents are removed during the processing and drying of the dosage form. Bracketing check standards (one low and one high standard check) were used during each period of use (typically 24 hours) to ensure that any changes in sensor response were readily detected. Potential interferences also were removed through the use of prefilters. Interferences from the enteric-coated beads in Capsule B caused some difficulty. The problem appeared as a failure of the check standard and subsequent poor sensor performance with respect to drift. The sensor was replaced with a new sensor, and the authors evaluated each of the chemical filters described in the experiment section for their ability to prevent sensor failure. All filters were capable of removing the interfering components, but the general chemical filter (activated carbon) greatly reduced response time and required as much as an hour for equilibration to occur. The interference arises from the degradation of the hydroxypropyl methylcellulose acetate succinate (HPMCAS) polymer to yield acetic and succinic acids. The acetic acid is a known potential interferant for this type of measurement cell (26).

Linearity. The general calibration approach ensures a linear response to within 0.01 $a_w$ over the operating range of the instrument.

Accuracy. Accuracy at equilibrium is assessed by comparing the water activity of equilibrated samples with the theoretical water activity (the RH to which the sample was equilibrated using a controlled humidity chamber). Samples were equilibrated over the same type of saturated salt solutions used to calibrate the instrument. This approach was evalu-
ated with an enteric-coated aspirin tablet and two other tablet formulations as shown in Table IV with a general accuracy of 0.01–0.02 \( w_a \). The same approach was taken by Mahajan et al., with a similar degree of accuracy (17). As previously mentioned, a 30-minute equilibration time generally has been adequate for capsules and tablets.

**Precision.** Replicate measurements were made for two tablet formulations and one capsule formulation (see Table V). The relative standard deviations for replicate measurements were 1–3%.

**Instrument qualification of maintenance (Novasina Instrument).** Using the guidelines published in a recent white paper (27), water-activity instruments would be Class B, principally requiring calibration to ensure performance. At the initial installation and every 6 months thereafter, four standards are checked (0.11, 0.33, 0.75, and 0.90). If the measured results are more than 0.02 \( w_a \) outside of the theoretical value, then the instrument calibration for that standard is changed. Note that the 0.53 standard may be used for laboratories with an expectation of standards that are dissimilar from the RH of the room.

**Water activity as part of a control strategy**

Water measurements as part of a control strategy generally must be quantitative (correcting for water content in a drug substance) or must be related to a change in a chemical or physical property. For solid oral-dosage forms, water is usually measured because of an adverse effect of water on either chemical stability or a physical attribute of the formulation such as drug release. Much of current measurement of water by Karl Fischer testing could be replaced with water-activity testing because water activity is easier to measure, less subject to handling problems, and as good or better at correlating to physical and chemical changes than total water.

Additional advantages of water activity testing are:
- One immediately knows whether the sample is likely to add or lose water relative to the external environment.
- The risk of microbial growth is assessed readily based on the earlier discussion in which \( w_a < 0.6 \) has no risk for microbial growth.
- Samples with water activities approaching the area of the sorption isotherm where “free” water is present are at a higher risk of adverse chemical or physical effects.

Where water measurements are part of a regulatory commitment, water-activity results would need to be related to Karl Fischer measurements by the sorption isotherm. The specification for water activity then could be set to correspond to the Karl Fischer specification.

The establishment of water activity as an analytical procedure would generally involve:
- determining which instruments are most suitable for the intended application (see Table VI);
- qualifying the instruments (using white paper [26], category B, calibrate);
- using daily check standards;
- using protective filters for materials with expected volatile contaminants;
- determining which instrument standards are applicable;
- determining the RH of the environment;
- determining the RH of the environment.

### Table VI: Comparison of instruments for measurement of water activity.

<table>
<thead>
<tr>
<th>Principle of measurement</th>
<th>Manufacturer</th>
<th>Temperature control</th>
<th>Equilibration detection</th>
<th>Protective filter</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement of capacitance and resistance of polymer film</td>
<td>Novasina, a division of Axair Ltd. (Pfäffikon, Switzerland)</td>
<td>some models</td>
<td>Slope only, no extrapolation</td>
<td>yes</td>
<td>0.03–1.00 (depends on model)</td>
</tr>
<tr>
<td>Measurement of capacitance and resistance of polymer film</td>
<td>Rotronic Instrument Corp. (Huntington, NY)</td>
<td>no</td>
<td>Slope and extrapolation modes</td>
<td>no</td>
<td>0–0.99</td>
</tr>
<tr>
<td>Chilled mirror</td>
<td>Decagon Devices, Inc. (Pullman, WA)</td>
<td>some models</td>
<td>Extrapolation</td>
<td>no</td>
<td>0.03–1.00</td>
</tr>
<tr>
<td>Laser absorption spectroscopy</td>
<td>Lighthouse Instruments, LLC (Charlottesville, VA)</td>
<td>yes</td>
<td>Not usually applicable</td>
<td>no</td>
<td>0–1.00</td>
</tr>
</tbody>
</table>
WATER-ACTIVITY TESTING

• validating methods once they are being used for lot-release purposes.

Table VI describes some differences in the types of instruments. Only representative manufacturers are included.

The approach used by all of the instruments is nondestructive. The instruments vary in the cutoff at low RH, the control of temperature, and the detection of the equilibration end-point. Measurement time is faster for the laser absorption and the instruments that extrapolate the detection of the end-point; but, temperature control generally lengthens the measurement time to 10–30-minutes for all the instruments. The Lighthouse instrument would be especially useful for lyophilized formulations because the measurement can be made on the headspace of the product vial. Reference 16 has more details about differences in cost and applicability.

The control strategy also must be consistent with the regulatory strategy. Table VII summarizes options relative to the product registration status.

Routine control of instrumentation. For the instruments that have sample contact with the sensor, bracketing readings of the standard solutions should be used to ensure that the sensor is providing accurate results and that the samples did not contaminate the sensor. The use of a protective filter should be tracked such that the filter is replaced at least as frequently as the manufacturer suggests. Standards that are saturated salt suspensions also require some maintenance to replace lost water from evaporation. These standards should be checked each time they are used.

Table VII: Options for implementing water-activity testing as a function of registration status.

<table>
<thead>
<tr>
<th>Product registration status</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marketed product with Karl Fischer water test</td>
<td>Establish water activity as an alternate procedure with a limit based on sorption isotherm.</td>
</tr>
<tr>
<td>Marketed product with no registered Karl Fischer water test</td>
<td>Follow normal practices for changing analytical procedures.</td>
</tr>
<tr>
<td>Product starting registration stability or earlier</td>
<td>1. Implement with the intent to use water activity as an internal GMP test (e.g., not registered). 2. Correlate Karl Fischer and water activity tests to keep options open, but use water activity as the principal test.</td>
</tr>
</tbody>
</table>
Conclusions

Water activity is a valid alternative to total water measurements (KF) in assessing the potential for water to adversely affect the formulation’s microbial or chemical quality. Water activity has been accepted in the food industry and widely discussed in the pharmaceutical industry as an appropriate measurement for water to prevent microbial growth. Likewise, water activity also can be used to evaluate the potential effect of water on chemical and physical changes in the formulation. The Novasina m1 and AW instruments in particular have proven to be sufficiently precise and accurate to measure the water activity of typical solid oral-dosage forms.

Acknowledgments

Thanks to Jim Farmer for his assistance.

References

7. U.S. Food and Drug Administration, Code of Federal Regulations, Title 21 (FDA, Rockville, MD, 2006), Part 113.3(ii) and 113.81 (f).
20. Unpublished data.
25. Rotronic Instrument Corp., “Water Activity Variations by Roughly 0.005 to 0.005 Aw (0.05 to 0.5 %RH) when Temperature Changes by 1 °C” (Rotronic Instrument Corp., Huntington, NY), http://www.rotronic-usa.com/datasheets/Ref/Aw/aw_define.htm, accessed Jan. 11, 2007.