Water Activity Best Testing Practices
Product 2. Solid Dosage Tablets

Introduction
The AquaLab research and development team now has water activity best testing practices available for solid dosage tablets. The objectives of this study were to:

1. Observe the impact of ambient humidity on water activity testing results when using normal testing practices.
2. Compare the water activity average values, test time and repeatability across several water activity instruments.
3. Determine if an advantage in precision is gained by extending read times up to an hour when using dew point instruments instead of using the initial value provided in the normal ~5 minute test time.
4. Identify the optimal settings for the custom mode testing option in Series 4 instruments.
5. Observe the impact of test temperature on water activity test results.

Materials and Methods
The water activity instruments used for testing included 1 AquaLab Series 3TE Chilled Mirror Water Activity Instrument and 2 AquaLab Series 4TEV Chilled Mirror Water Activity Instruments. Each instrument was verified daily using unsaturated salt solutions at 0.25 a_w, 0.5 a_w, 0.76 a_w, and 1.00 a_w. All testing was conducted on 3 replicates taken from 3 independent samples. Humidity was controlled using a glove box and all sampling and testing was conducted in the glove box. Humidity in the glove box was constantly monitored. Sampling was done as quickly as possible with the sample exposed for no more than 5 seconds during sampling. The ambient humidities included in the study were 10% RH, 30% RH, and 70% RH. At each humidity, testing was conducted in 3 parts. A description of each part follows.

Part 1 consisted of tests accomplished using just the AquaLab instruments. An initial water activity reading was recorded when the first test ended as indicated by the instrument, but then the instrument was set to continue taking measurements up to approximately 1 hour. The initial and final mean water activity and standard deviation across the 3 samples were compared using ANOVA to see if a significant advantage is gained in the AquaLab instruments by extending the test time. All tests in Part 1 were done at 25°C.

Part 2 consisted of comparing the results in Part 1 for tablets tested whole and tablets crushed before testing. The tablets were crushed using mortar and pestle inside the glove box at each humidity. Tablets were crushed, but not pulverized to a powder. Sample preparation was investigated as a significant source of variation again using ANOVA.

Part 3 consisted of utilizing the custom feature in the AquaLab Series 4 instruments. This mode allows setting stability specifications for ending a test, which consists of identifying a water activity range that must be met by a specified number of tests. For example, the custom setting could be 3 tests and 0.003 a_w. Once started, the instrument will then continue taking tests until 3 results are within +/- 0.003 a_w of each other. To determine the preferred custom mode to achieve the highest combination of repeatability and speed, 4 custom mode settings were compared including: 3 tests within +/- 0.001 a_w, 5 tests within +/- 0.001 a_w, 3 tests within +/- 0.003 a_w, and 5 tests within +/- 0.003 a_w. Testing was conducted using 1 Series 4TEV instrument on 3 replicates from 3 samples. The mean water activity and standard deviation across all 3 samples was then compared using ANOVA to determine if one custom mode setting provides significantly better performance than another setting. All tests in this second set were done at 25°C.

Part 4 consisted of observing the effect of temperature on the water activity readings of the product of interest. Water activity is temperature dependent, but the level of sensitivity depends on the product. To investigate the effects of temperature, each replicate from each of 3 samples was evaluated for water activity at 15°C, 25°C, and 45°C. Mean water activity and test time were compared for each temperature at each humidity level to determine if temperature resulted in significant differences in water activity.

Results

Part 1

Keep in mind that the samples were only exposed to ambient humidity without moisture barrier for seconds during sampling. However, the starting humidity in the testing chamber of each instrument was at ambient humidity when each test began.

The results in Figure 1 indicate that the water activity test results for Solid Dosage Tablets were impacted by ambient humidity, with a general increase in water activity as ambient humidity increased. Tests results for all instruments were equally influenced by ambient humidity. The average a_w across all instruments for 10%, 30%, 50% RH, and 70% RH was 0.171, 0.292, 0.397, and 0.495 respectively. The data suggests that great care must be taken to limit exposure time of tablets to ambient humidity. The data also suggests that obtaining true readings for tablets as opposed to just reading ambient humidity may be difficult. However, additional testing was done on a sampling of tablets from different manufacturers at 30% RH to see if any differences could be seen or if all samples just read at ambient and readings ranged from 0.168 to 0.410, indicating that real differences in tablets are observable. The standard deviation of repeated readings did trend higher (poorer) as ambient humidity increased for all instruments (Figure 1). This is likely the result of slight differences in the sensitivity of each sample to changes in humidity.

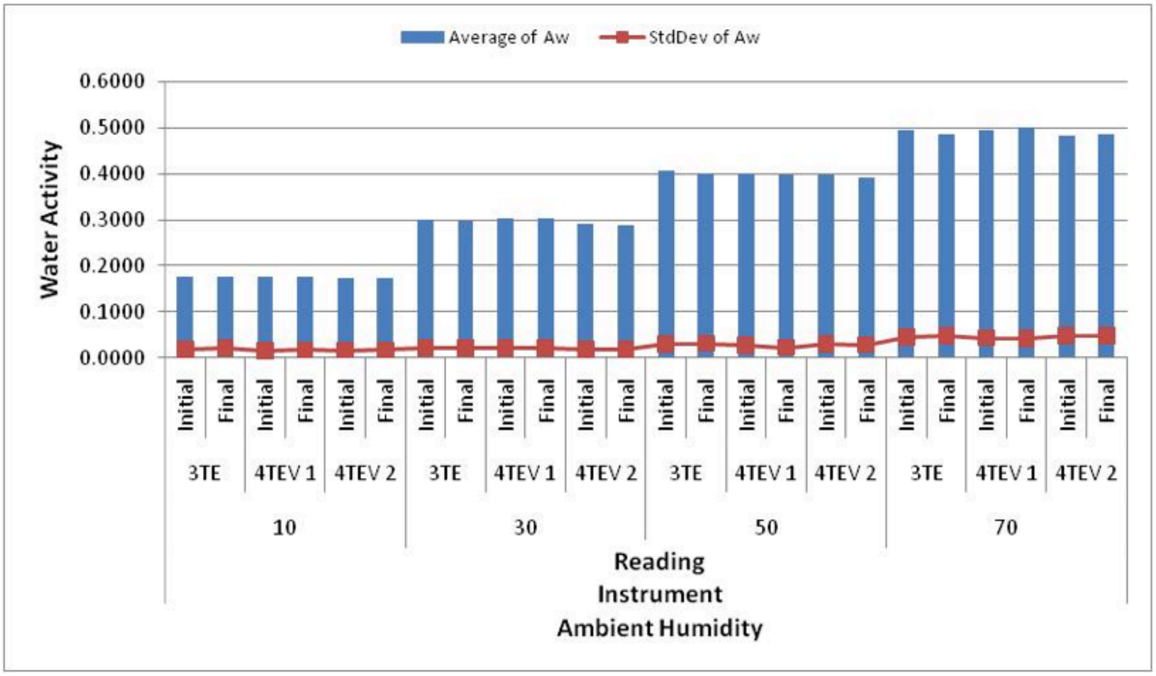

Figure 1. Average water activities and standard deviations for 5 different water activity instruments at 4 different ambient humidities. A comparison of the initial and final reading for the dewpoint instruments is also provided.

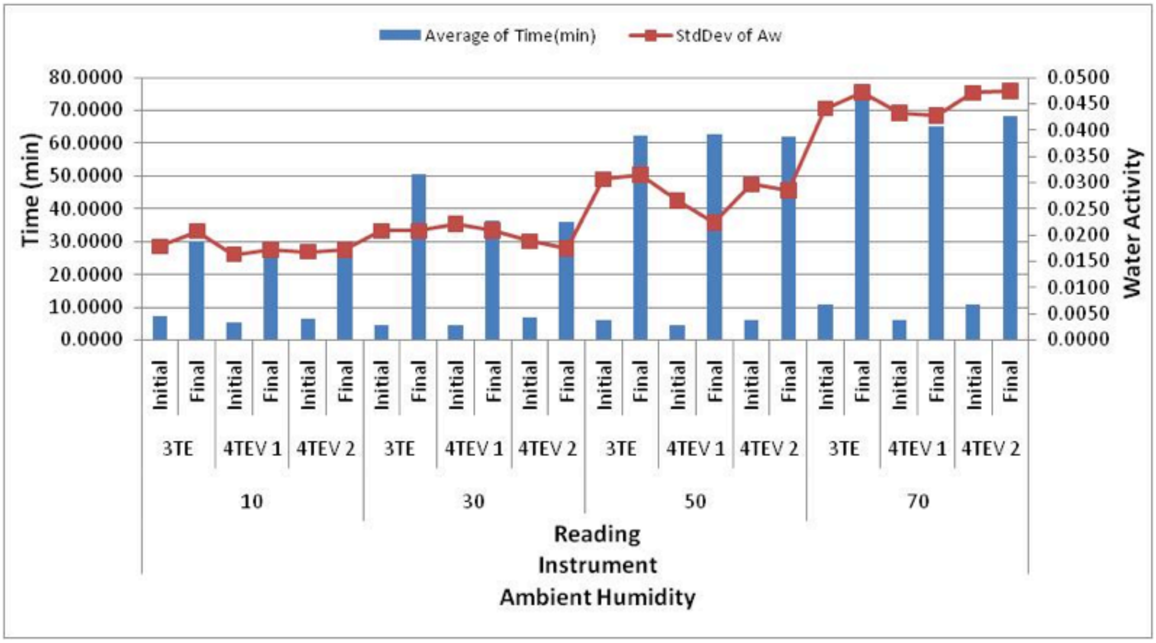

Figure 2. Average test times and standard deviations of final and initial readings of 5 different water activity instruments at 10%, 30%, 50%, and 70% relative humidity.

Tukey's multiple mean test of initial and final values for each AquaLab instrument at each humidity level showed no difference, indicating that the results do not significantly change if the test is run for 5-8 minutes vs. running for 1 hour, debunking the idea that correct readings can only be obtained from continuous reads or long read times. In addition, standard deviations were not significantly better for long read times, indicating that letting the instrument take readings for extended test times does not improve the repeatability of test results. Finally, neither water activity values nor standard deviations for initial and final readings were significantly different.

Standard deviation across multiple readings for each instrument in comparison to test time is shown for each instrument at each humidity in Figure 2. The general increase in standard deviation as humidity increases can be observed, but no trend of better precision with increased testing time can be seen, indicating that the normal read time of the AquaLab is sufficient to equal and even outperform competitor instruments.

As a side note, putting desiccant in the instrument before reading did not prove to be helpful in buffering the effects of ambient humidity. In fact, in almost all cases, the reading was higher after desiccant. There was no consistent trend in terms of read time either.

Part 2

In general, water activity results at each humidity level were different for crushed samples than whole samples (Figure 3). The trend was true for all instruments, so the data was combined. The crushed samples appear to be more susceptible to changes in ambient humidity than whole tablets, which makes sense since there would be much more surface area for sorption of water by crushed samples whereas, whole tablets would have much slower sorption rates. Standard deviations were again higher at high humidities, but were not consistently higher for one sample preparation method. However, test times were significantly lower for crushed samples. This is again likely due to the increases surface area allowing for faster equilibration. Therefore, crushed samples have the advantage of faster test times, but are more susceptible to changes due to ambient humidity.

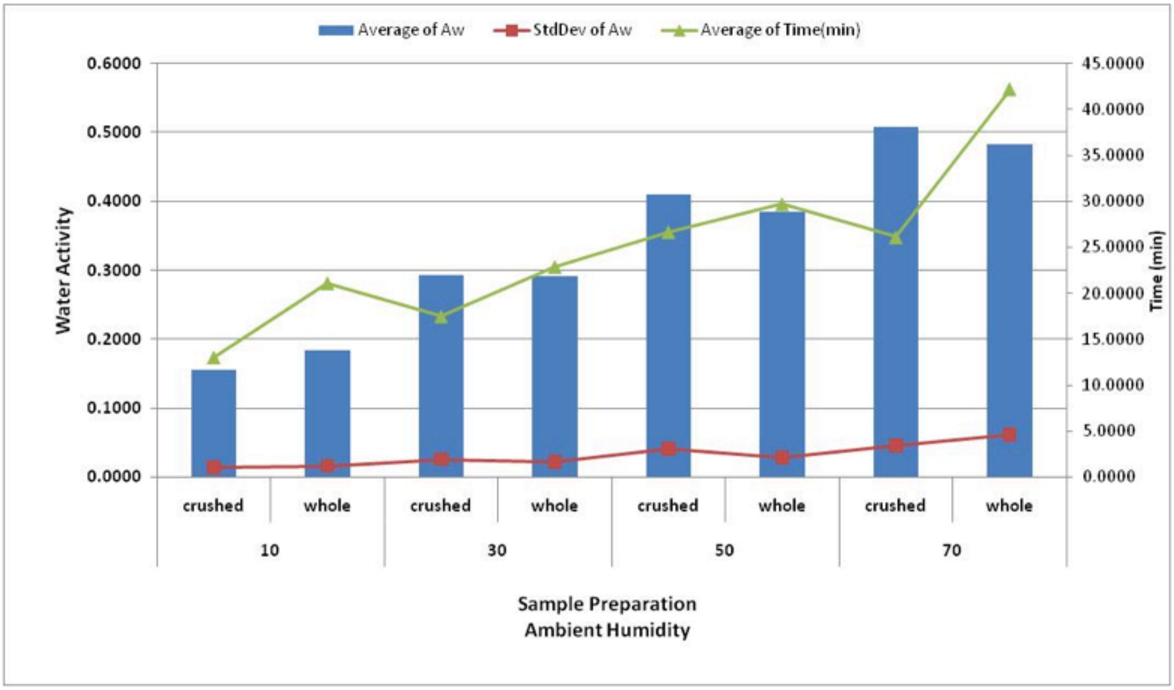

Figure 3. Average water activities, standard deviations, and test times for crushed and whole tablets.

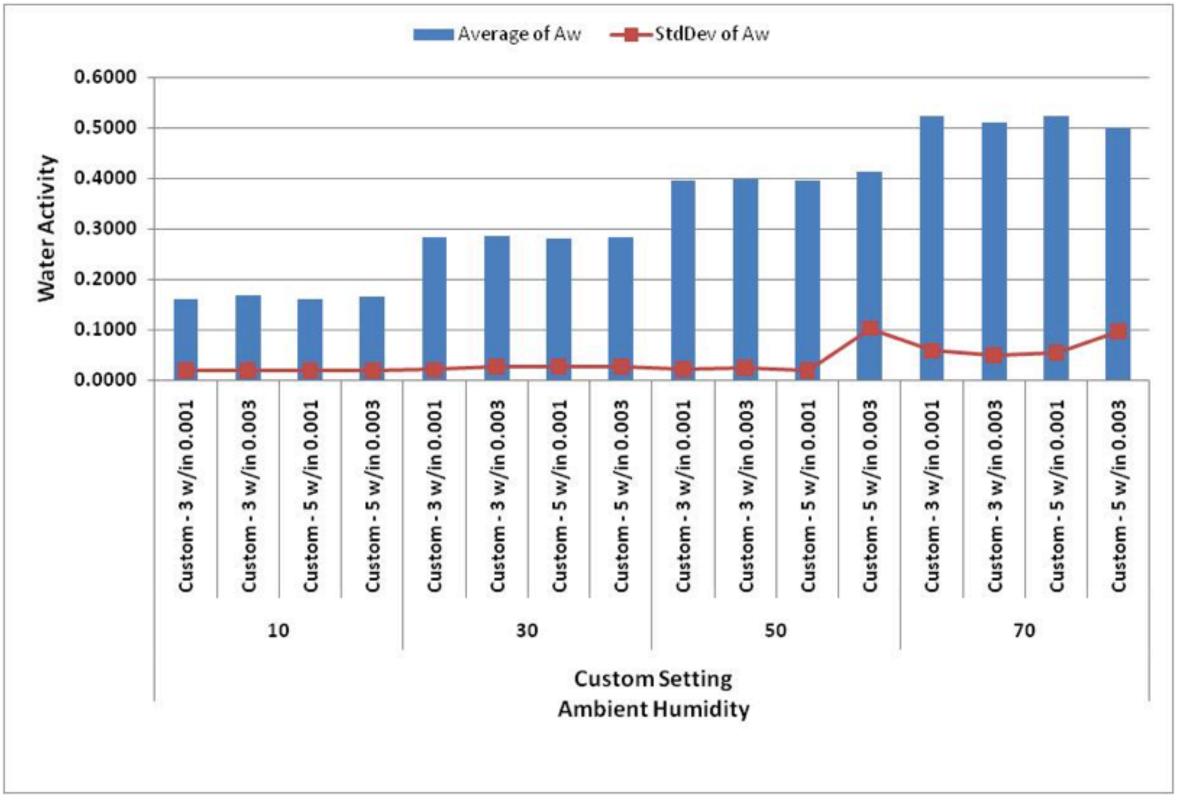

Figure 4. Average water activity values and standard deviations for an AquaLab Series 4TEV instrument at 3 different ambient humidities when using 4 different custom mode settings.

Part 3

Figure 4 shows the results for testing different custom mode settings. At a given humidity level, the average water activities were not significantly different across custom settings, nor was there significant improvement in the repeatability. In addition, the no custom setting was any less susceptible to the effects of ambient humidity (Figure 4). The data suggests that the custom mode doesn't really provide any advantage, so if the custom mode is going to be used for testing Solid Dosage Tablets, the most practical solution would be to use the least stringent setting of 3 tests within +/- 0.003 a_w since the test time was only 15 minutes (at 30% RH) and the performance was not significantly different from the other custom mode settings.

Part 4

Water activity is temperature dependent, but the level and even direction of the dependency is very product specific. The results in Figure 5 indicate that the temperature dependency even depends on the ambient humidity conditions where samples are taken, but not on the instrument used. When ambient humidity was low, the water activity was only slightly lower at both high and low temperatures. However, at 30%, 50% and 70% RH, there was a considerable change in water activity with temperature. The water activity significantly increased as temperature decreased at all 3 humidity levels. The expected trend is for water activity to increase with temperature, so the observed trend was not typical, but explainable. The interaction of temperature and water activity, while multifaceted, can be caused by changes in solubility. An increase in temperature can result in an increase in solubility, making more hydrogen binding sites available for binding water and

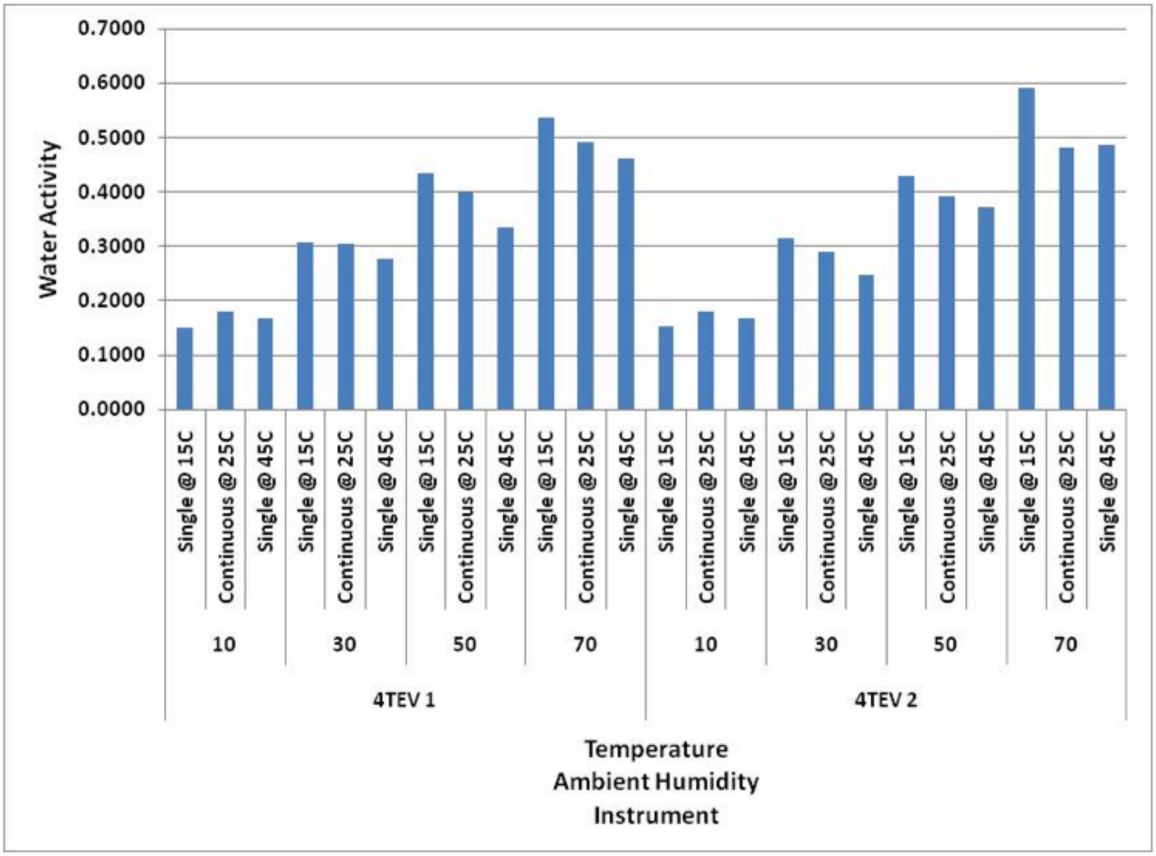

Figure 5. Average water activity values for 2 AquaLab Series 4TEV instrument at 4 different ambient humidities and 3 different temperatures.

causing water activity to decrease even though moisture content hasn't changed. This change in solubility is the likely explanation for results observed for 30%, 50% and 70% RH. However, solubility does not explain why the water activity decreased at both high and low temperatures at low ambient humidity. It is likely that at very dry conditions, accompanied by very low water activity, there is a combination of effects occurring.

Summary

Water activity test results on Solid Dosage Tablets were not improved by long, extended test times. There were no significant differences between water activity values or the repeatability of a_w tests when using the initial 5-10 minute reading from an AquaLab instrument vs. using the readings taken after a 1 hour test time. It is therefore not necessary to run additional water activity tests on Solid Dosage Tablets when using an AquaLab even if the test time is less than 10 minutes.

Water activity testing results for Solid Dosage Tablets did change with changes in lab ambient humidity, even if the sample was only exposed for seconds during sampling. The changes in water activity are not just the result of exposure to ambient humidity during sampling, but also exposure to the head space of the instrument. The head space of the instrument will be at ambient conditions and while the volume of head space is usually not large enough to cause changes in water activity, for very moisture sensitive samples, such as Solid Dosage Tablets at low water activity, a very dry head space could contribute to lower a_w readings. Care should be taken to minimize the time samples are exposed to ambient conditions.

The custom mode settings in the AquaLab Series 4 did not lessen the effects of ambient humidity, improve precision, or change the average water activity values. If the custom mode is to be used, it is not necessary for the settings to be any more stringent than 3 tests within +/- 0.003. Finally, water activity values decreased with increasing temperature at all humidities except 10% RH, where water activity decreased at both high and low temperatures.

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