Baby Formula Water Activity Testing Guide

Introduction

The research and development team for the AquaLab line of instruments is conducting water activity best testing practices research. This is an ongoing study, but we have results available for baby formula and we wanted to present these results.

The objectives of this study are:

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 provide in the normal ~5 minute test time.
4. Identify the value of the custom mode testing option in Series 4 instruments and find which setting provides the best precision.
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 aw, 0.5 aw, 0.76 aw, and 1.00 aw. 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 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 aw. Once started, the instrument will then continue taking tests until 3 results are within +/- 0.003 aw 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 aw, 5 tests within +/- 0.001 aw, 3 tests within +/- 0.003 aw, and 5 tests within +/- 0.003 aw. 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 3 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 with no moisture barrier for seconds during sampling. However, the starting humidity in the testing chamber of each instrument would be at ambient humidity when each test begins.

The results in Table 1 indicate that the water activity test results for baby formula were impacted by ambient humidity, but not in a general linear way. Tests results for all instruments were equally influenced by ambient humidity. The average aw across all instruments for 10%, 30%, and 50% RH was 0.153, 0.320, and 0.388, respectively. So while changing from 30% to 10% ambient humidity resulted in a 0.167 change in aw, changing from 30% to 70% resulted in only 0.068 change in water activity. The moisture sorption isotherm shown in Figure 1 illustrates why the change in water activity was larger when exposed to dry conditions. In the range of water activities below 0.40 aw, the water activity can change from one value to another very quickly with very little change in the amount of water, illustrated as a very flat curve, due to limited availability of hydrogen binding sites in the amorphous baby formula. Consequently, the water activity of baby formula samples exposed to dry conditions during sampling were lower by an average 0.167 aw.

At water activities greater than 0.40, the formula begins to experience a phase change and the isotherm curve becomes much steeper with only small changes in water activity for large changes in the amount of water. While the phase change is time dependent and would not have time to progress appreciably during the few seconds of exposure while sampling, the sharp change in

Table 1. Average water activity, test time, and standard deviation of water activity readings for 5 different water activity instruments at 3 different ambient humidities. A comparison of the initial and final reading for the dewpoint instruments is also provided. The initial reading is the value provided by the instrument after the initial water activity reading is complete. To obtain the final reading, water activity readings are repeated for up to hour and the final value is reported.

Instrument	% Ambient Humidity	Reading	Water Activity	Time (min)	Std Dev of a_w
Series 3TE	10	Initial	0.151	6.623	0.037
		Final	0.151	66.715	0.035
	30	Initial	0.337	9.156	0.038
		Final	0.316	72.773	0.023
	70	Initial	0.413	7.961	0.009
		Final	0.396	68.292	0.010
Series 4 TEV 1	10	Initial	0.175	7.708	0.027
		Final	0.155	68.525	0.020
	30	Initial	0.316	7.018	0.027
		Final	0.320	72.270	0.021
	70	Initial	0.405	8.346	0.014
		Final	0.387	68.728	0.012
Series 4 TEV 2	10	Initial	0.156	10.226	0.040
		Final	0.155	65.936	0.038
	30	Initial	0.323	12.247	0.030
		Final	0.316	72.436	0.025
	70	Initial	0.397	13.049	0.014
		Final	0.380	71.771	0.032

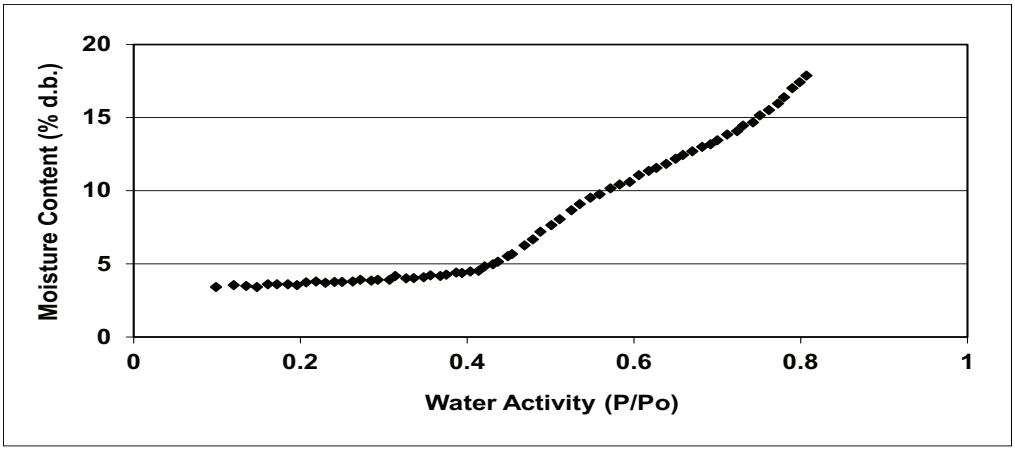

Figure 1. Moisture sorption isotherm for spray dried baby formula at 25°C showing a phase change occurring at a critical water activity of 0.43.

sorption properties at water activities close to 0.40 aw has a buffering effect on the water activity change due to the environment. Consequently, the water activity of the sample only slight increases by 0.068 aw.

T tests comparisons 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 dry powders have long vapor equilibration time constants and 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 long read times does not improve the repeatability of test results.

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

Table 2 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. However, there did appear to be a slight improvement in repeatability when using the custom mode when comparing to doing single tests as shown in Table 1. This increase in

Table 2. Average water activity values, test times, and standard deviations for an AquaLab Series 4TEV instrument at 3 different ambient humidities when using 4 different custom mode settings.

Instrument	% Ambient Humidity	Custom Settings	Water Activity	Time (min)	Std Dev of a_w
Series 4 TEV	10	Custom - 3 w/in 0.001	0.136	27.810	0.020
		Custom - 3 w/in 0.003	0.147	15.977	0.018
		Custom - 5 w/in 0.001	0.138	50.063	0.020
		Custom - 5 w/in 0.003	0.137	25.570	0.016
	30	Custom - 3 w/in 0.001	0.335	15.524	0.017
		Custom - 3 w/in 0.003	0.338	16.392	0.016
		Custom - 5 w/in 0.001	0.335	55.231	0.018
		Custom - 5 w/in 0.003	0.337	23.693	0.016
	70	Custom - 3 w/in 0.001	0.379	22.548	0.010
		Custom - 3 w/in 0.003	0.391	36.764	0.008
		Custom - 5 w/in 0.001	0.371	32.100	0.018
		Custom - 5 w/in 0.003	0.382	24.150	0.010

repeatability does require additional test time as evidenced by the long read times for the results in Table 2 and the decision to use the custom mode would depend on the willingness of the user to sacrifice some speed for a +/- 0.01 aw increase in repeatability . If the custom mode is going to be used for testing baby formula, the most practical solution would be to use the least stringent setting of 3 tests within +/- 0.003 aw 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 3

Water activity is temperature dependent, but the level and even direction of the dependency is very product specific. The results in Table 3 indicate that the temperature dependency even depends on the ambient humidity conditions where samples are taken. When ambient humidity is low, the water activity is lower, as shown in part 1, but does not change with temperature. However, at 30% and 70% RH, there is considerably change in water activity with temperature. The water activity significantly increases as temperature decreases at both humidity levels. The expected trend is for water activity to increase with temperature, so the observed trend is 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 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% and 70% RH. However, solubility does not explain why the water activity did not change with temperature at low ambient humidity. It is likely that at very dry conditions, accompanied by very low water activity, there is little to no water with high energy that can lower its energy due to increased availability of hydrogen binding sites at higher temperatures and the effect is insignificant.

Summary

Water activity test results on baby formula are not improved by long, extended test times. There are no significant differences between water activity values or the repeatability of aw 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 not necessary to run additional water activity tests on baby formula when using an AquaLab even if the test time is less than 10 minutes.

Table 3. Average water activity values and test times for 2 AquaLab Series 4TEV instrument at 3 different ambient humidities and 3 different temperatures.

Instrument	% Ambient Humidity	Temperature	Water Activity	Time (min)
4TEV 1145	10	25	0.175	7.708
		15	0.176	4.333
		45	0.179	3.317
	30	25	0.316	7.018
		15	0.414	5.512
		45	0.243	3.307
	70	25	0.405	8.346
		15	0.454	15.164
		45	0.366	1.906
4TEV 1169	10	25	0.156	10.226
		15	0.152	6.975
		45	0.164	13.243
	30	25	0.323	12.247
		15	0.39	15.397
		45	0.261	3.088
	70	25	0.397	13.049
		15	0.458	26.036
		45	0.341	4.197

Water activity testing results for baby formula can change with changes in lab ambient humidity, even if the sample is only exposed for seconds during sampling. However, this change is much more significant at lower humdities (lower than 20%) than at higher humidities (above 40%) due to the sorption properties of baby formula. The changes in water activity are not just the result of exposure to ambient humidity during sampling, but also exposure to the headspace of the instrument. The headspace of the instrument will be at ambient conditions and while the volume of headspace is usually not large enough to cause changes in water activity, for very moisture sensitive samples, such as baby formula at low water activity, a very dry headspace could contribute to lower aw readings. If room humidity is 30% RH or higher, water activity testing of baby formula shouldn't be impacted, but if humidity is very low, it may be advisable to test in an environment with higher ambient humidity.

The custom mode settings in the AquaLab Series 4 did not lessen the effects of ambient humidity or change the average water activity values. There was a slight improvement in repeatability when using the custom mode, but this improvement was not made better by making the custom mode settings more stringent. 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, but only at humidities above 30% RH.

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