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Abstract

Genentech had a requirement for unattended robotic titration. The existing system was not robust and the manual method was too time-consuming. Per Genentech's request AB Controls developed a fully automated robotic system for conducting Karl Fischer moisture assay. AB Controls customized their "Focus" software to handle all automation and robotic tasks. The choice of measurement instruments were made by Dr. Philippe Lam, and AB Controls integrated them all on a robust and versatile platform that occupied 1/4th of the space of the previous unit. The design of the platform and robotic tooling was done such that multiple functions could be performed by the same end of arm tooling. This machine is validated for use in Genentech's manufacturing operation and has provides significant time and labor savings over the manual assay method. While some sample preparation is required from the analyst, the system fulfills moisture assay needs for all our lyophilized products.

Introduction

Karl Fischer titration is one of the few assay techniques recognized by the FDA for the determination of residual moisture in lyophilized pharmaceutical product. Because the iodine-water reaction is very specific and quantitative, this technique offers high accuracy and precision.

However, conventional Karl Fischer titration is not considered a "high throughput" assay and requires significant manual intervention from the analyst. This presents a challenge when large number of samples need to be analyzed in a very short time. Furthermore, most lyophilized products are hydroscopic and must be processed promptly after removal from the primary container. The few off-the-shelf automated systems offered by Karl Fischer titrator vendors were not suitable for our needs.

The following communication summarizes our approach at developing a reliable system intended at performing unattended Karl Fischer titration on numerous samples. We present our rationale for selecting instrumentation and discuss sample preparation strategies, both of which impact the overall design of the automated system.

Titration instrument selection

The Karl Fischer titrator is the heart of an automated moisture analysis system. There are two types of instruments available to perform titration. Both titrator types operate on the same basic principle: quantify the amount of iodine (I_2) from the titrant that reacted with the water in the sample. In both cases, the titration end point (when all the water has reacted) is determined by detecting free iodine at a double in platinum electrode. Additional details on Karl Fischer titration can be found in [1] and [2].

1) Coulometric titrators
Coulometric titrators generate iodine by *in-situ* electrochemical oxidation of iodide (I^-) contained in the reagents. The total amount of iodine generated is proportional to the total charge passed in the electrochemical cell. This allows for very sensitive detection of water, from a few ppm weight to a few weight percent.

2) Volumetric titrator
In the case of volumetric titrator, the iodine is already present in the titrant and the titrator simply measures the volume of reagent dispensed into the cell from a precision burette. Sacrificing sensitivity for range, volumetric titrators are capable of measuring water content from a few hundredth of a percent up to 100 weight percent.

For our application, the extreme sensitivity of coulometric titration is not necessary while it is very desirable to be able to analyze samples containing high moisture (> 5 wt %). Hence, we selected the Mettler-Toledo DL31 volumetric Karl Fischer titrator due to past experience with this type of instrument from that company.

Sample preparation techniques

All of Genentech's lyophilized products are packaged in glass vials. However, there are a variety of different configurations spanning various compositions, fill volumes, as well as various vial sizes. We have considered the following techniques for preparing samples:

a) *Extraction in the product vial*
This is the technique that is recommended by Mettler-Toledo for handling freeze-dried substances. The procedure calls for injecting a know amount of anhydrous solvent (methanol) directly into the sample vial and allowing some time for water to be extracted into the solvent. Agitation or sonication can be used to expedite the extraction. An aliquot of this extract is then withdrawn from the sample vial and introduced into the titration cell for analysis.

b) *Use of a drying oven*
The sample vial is placed into a small oven and heated to desorb the moisture. An inert, dry carrier gas is feed into the vial, the effluent is feed into the titration cell for analysis.

c) *Direct extraction of crushed sample in titration cell*

This is the technique that has been traditionally employed by our analysts. Here, the product vial is uncapped and the contents briskly crushed inside the vial using a smooth metal rod. A know amount of powdered sample is transferred directly into the titration vessel for analysis.

All our historical data had been collected using method (c). The following study was undertaken to determine the comparability between techniques (a) and (c) only; the experimental setup for technique (b) was not available.

A Mettler-Toledo DL31 volumetric Karl Fischer titrator with a one component Hydranal Composite 2 titrant was used for all cases. Sodium tartrate dihydrate was used as the standard to determine reagent titer.

Active product configured as a 0.88 mL fill volume in 5 cc vials was used in the study. Two set of samples, spanning the typical range of moisture normally observe for this product, were tested.

Two extraction schemes for method (a) were investigated:

I) introduction of anhydrous methanol into the product vial, 5 minute sonication, withdraw an aliquot and analyze. This is the instrument manufacturer recommended procedure.

II) introduction of anhydrous methanol into the product vial, 5 minute sonication,, 5 minutes standing, repeat 5 minutes sonication, then withdraw an aliquot and analyze.

Figure 1 summarizes the results for these experiments. For consistency with historical data, water content is reported as weight percent moisture of the lyophilized product cake. To obtain weight percent moisture values for method (a), the sample vials had to be weighed prior to extraction, emptied post extraction, cleaned, dried and re-weighed in order to obtain the actual freeze dried cake mass. These extra steps added complexity and lengthened the overall analysis time. Method (b) would also require following the same procedure.

Comparing results from the two methods, it is clear that scheme II of method (a) extracted more water than the recommended scheme I, suggesting that scheme I was not able to fully extract all the water from the sample. It is questionable whether even scheme II achieved complete extraction. Neither schemes of method (a) extracted as much water from the samples as method (c). However, it should be recognized that method (c) may be influence by the level of relative humidity in the room since the crushed lyophilized cake is exposed briefly to ambient conditions. Hence, the true moisture content of a sample may lie between the values given by method (a) and (c).

While methods (a) and (b) offer the advantage that the sample is never directly handled and exposed to ambient conditions, the additional steps required post extraction are undesirable. Furthermore, products contained in larger containers such as 50 cc or 100 cc vials, will require yet longer extraction time due to the longer diffusion path for the water. Method (a) will also require large volumes of solvent for the extraction, compounding waste disposal problems. For these reasons, it was decided to implement method (c) in the automated system.

We considered the possibility of a system where sample preparation, uncapping, crushing and dispensing of the lyophilized cake would be fully automated. However, such system would need to handle vials dimensions from 5 cc to 100 cc, be able to consistently pulverize lyophilized product cake of consistency ranging from very fluffy to very hard and of a few millimeters to a few centimeters in height. This would entail a very complex and cost prohibitive setup, just for sample preparation. Hence, we simply elected to prepare samples manually, as it is currently done. A trained analyst can perform these operations in less than two minutes per sample.

Automated system design and construction

1) Sequence of operation
Given the requirements discussed above, the automated system should operate according to the following sequence.

1) The analyst prepares the sample as per method (c) described previously. Some amount (50 to 150 mg) of crushed lyophilized is dispensed into a standard centrifuge tube and capped. The tube is placed into a sample rack. A number of samples can be prepared and loaded into the system.
2) The automated system retrieves a sample tube from the rack and transfers it to a balance where the weight is recorded.
3) The sample tube is placed in a de-caping station and the tube cap is removed.
4) The sample is dispensed into the titration cell. Titration is initiated.
5) The sample tube is re-caped and re-weighed. The actual sample mass is determined and the sample tube is discarded.
6) Titration result is returned and the titration cell is cleaned if necessary (after some predetermined number of samples have been analyzed).
7) The cycle repeats from step (2).

This sequence is basically the same as would be carried out manually. The automated system serves to shuttle the sample between the various stations (rack, balance, titrator) and record and automatically get the analysis results. Titration of the sample remains the rate limiting step so the total analysis time is comparable to that of a fully manual operation. We typically observe titration times ranging from a few minutes to over 10 minutes, depending on the composition and moisture level of the sample. However, the benefit from such automated setup stems from the ability to perform unattended analysis once the samples have been loaded into the system.

2) System construction
To minimize costs, we have incorporated as many components from off-the-shelf sources as possible.

A Mettler-Toledo XP 204 balance was selected as this model offers RS-232 serial connectivity, motorized draft doors and a removable front panel controls. The later feature is important as removing the front panel avoids accidental activation of any balance operation during a run.

The DL31 titrator includes a small vacuum pump for removing and dispensing solvent from the titration cell. However, that setup is not sufficiently robust to ensure full removal of the cell contents and accurate dispensing of solvent. Hence, on the automated system, the fluid handling, waste removal and solvent dispensing, is performed by a pair of Masterflex L/S ComputerCompatible peristaltic pumps, which also offer RS-232 serial connectivity.

The samples are shuttled between the various stations by a Mitsubishi RV-2AJ-511 five axis industrial robot arm. This component has a proven track record in industrial settings, is robust, widely used and also controlled via RS-232 serial communication. Furthermore, the robot arm controller provides several digital I/O lines used to power solenoid valves permitting the actuation of pneumatic cylinders for the decaping station and the titration cell cap.

The components are placed aboard a compact custom fabricated aluminum frame/deck and are interfaced to a standard computer running Windows OS and equipped with a multi-port serial communication card. "Focus", AB Controls automation and robotics software, was customized to control all operations.

Figure 2 shows the entire system, including the sample rack, capable of holding a total of 105 samples contained in standard 15 cc screw cap polypropylene centrifuge tubes. The system was designed primarily for robustness and ease of use, which meant that the software was customized to limit the user's options. There are only two user input parameters that directly affect system operation: the total number of samples to be analyzed and the number of titrations to be performed before the titration cell is washed. There are no product specific "methods", all samples are handled and analyzed in the same manner. After each moisture determination the result is sent to the computer and immediately printed on the attached line printer.

System testing

The system was subjected to comprehensive tests, including hardware error checking and recovery; no major difficulties were encountered. Operation testing was simplified thanks to the limited number of user input parameters. This section presents results from the most relevant studies, moisture determination of active hydroscopic freeze dried product from a fully loaded sample rack.

For these studies, the sample queue times (the time the samples were awaiting analysis while on the rack) were determined. Figure 3 summarizes the results for two sets of samples consisting of two different batches of active material lyophilized in 50 cc vials. For the second set (circle), in order to increase the queue time, analysis was paused after 4.5 hour and resumed 17 hours thereafter.

The data shows that for this product, the sample moisture increases linearly as a function of queue time, suggesting that the samples uptake moisture from the environment, presumably from

the overhead air space or through the polypropylene centrifuge tube wall. Typical laboratory ambient conditions are maintained at 21 °C and 50±15 % RH. The moisture uptake rate for the two set of samples are 0.018 wt %/h and 0.0095 wt %/h, respectively. This behavior is not unexpected and is a consequence of the sample preparation methodology we have selected. While this seems to be a shortfall of the current system, in practice, the analysts prepare 10-20 samples at a time, with samples added only after most of the initial ones have been processed. Hence, the queue time is typically only a few hours and this limitation was deemed an acceptable trade off for the convenience afforded by such a system.

Conclusion

We have demonstrated a system that is capable of performing Karl Fischer titration unattended. This instrument is currently fully validated for use in activities such as lyophilization cycle development, tech transfer between various manufacturing sites and manufacturing discrepancy investigations. Genetech decided to limit the options in favor of robustness, which because of the flexibility of the "Focus" software it was possible to do so. As a result, this system is very user friendly, low maintenance and has seen heavy usage. This setup has been a very cost effective tool, saving both labor and time.

Figure 1: Comparison between extraction methods.

Figure 2: Automated Karl Fischer titration system components
a) Side view, b) top view of titration deck with foot print of approximately 95 x 110 cm
1) Result line printer, 2) UPS for entire robotic system, 3) Windows XP Computer, 4) Mitsubishi robot controller, 5) Sample tube rack, 105 positions, 6) Titration cell pneumatic sealing mechanism assembly, 7) Mitsubishi robot arm, 8) Mettler-Toledo XP analytical balance, 9) Mettler-Toledo DL 31 volumetric Karl Fischer titrator, 10) and 11) Solvent delivery and waste removal peristaltic pumps, 12) Waste bottle, 13) Solvent bottle, 14) Pneumatic decapping station, 15) Cap holder, 16) Spent sample tube chute to trash bin, 17) Hydranal reagent bottle and holder, 18) Ionizer for static charge control

Figure 3: Effect of queue time on sample moisture.

Sample set 1 (1, dashed line), sample set 2 (#, solid line).

Figure 1

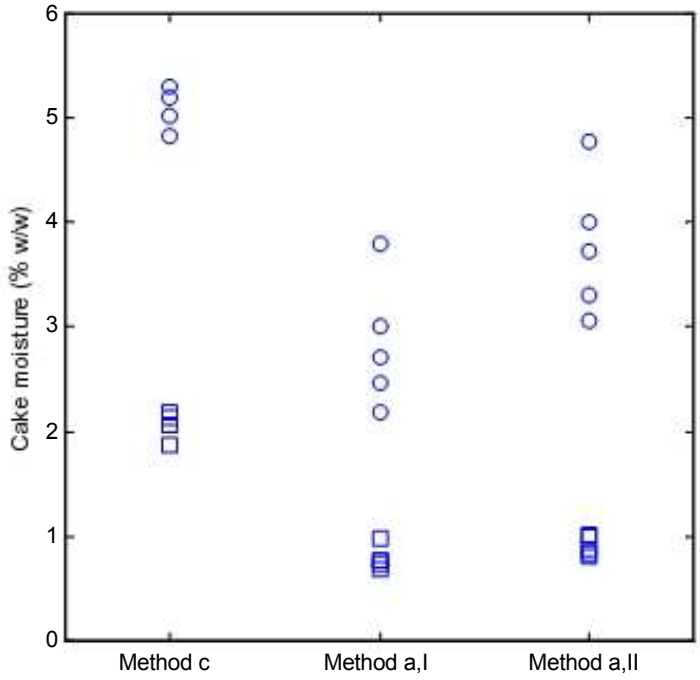


Figure 2

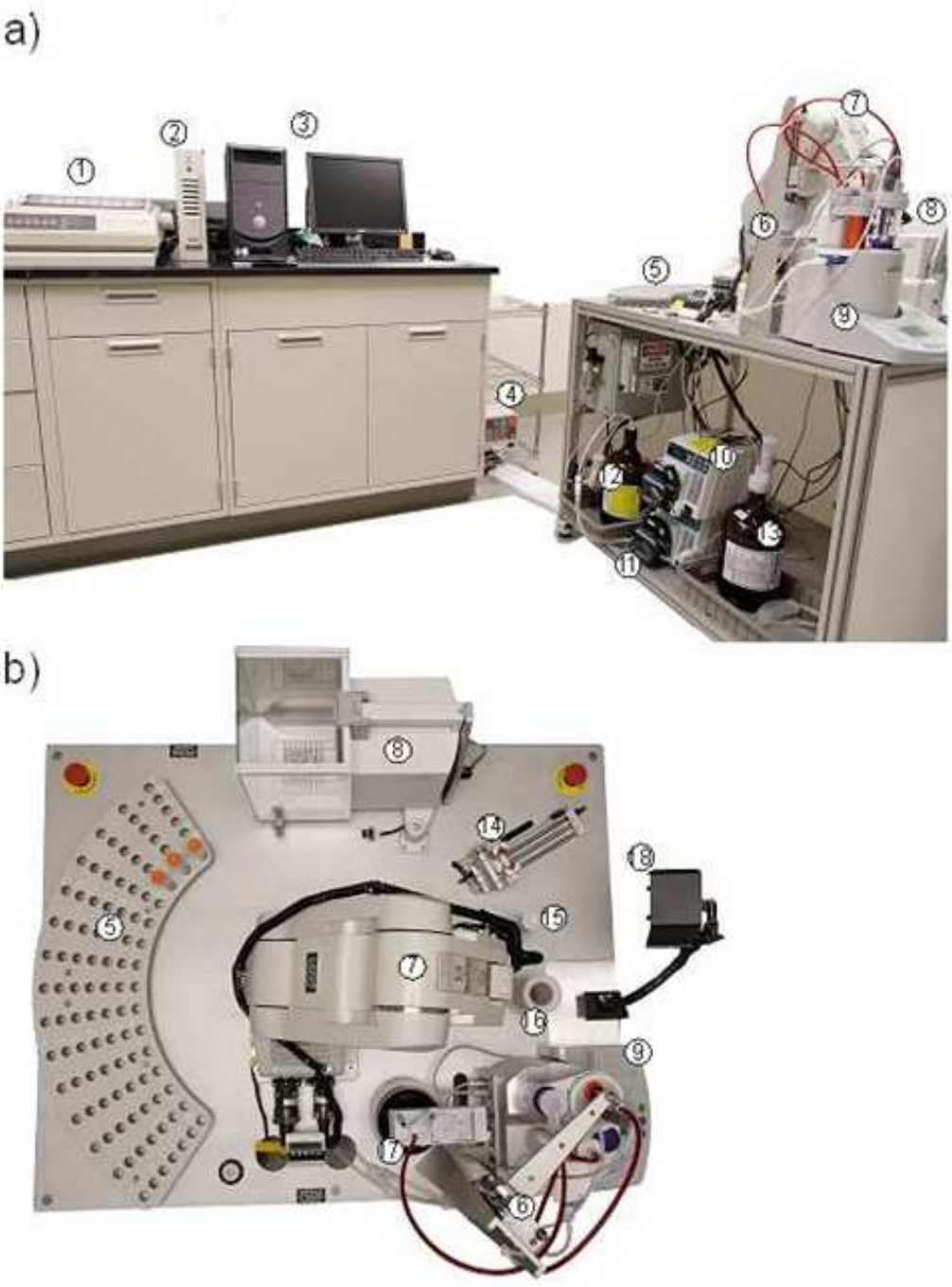
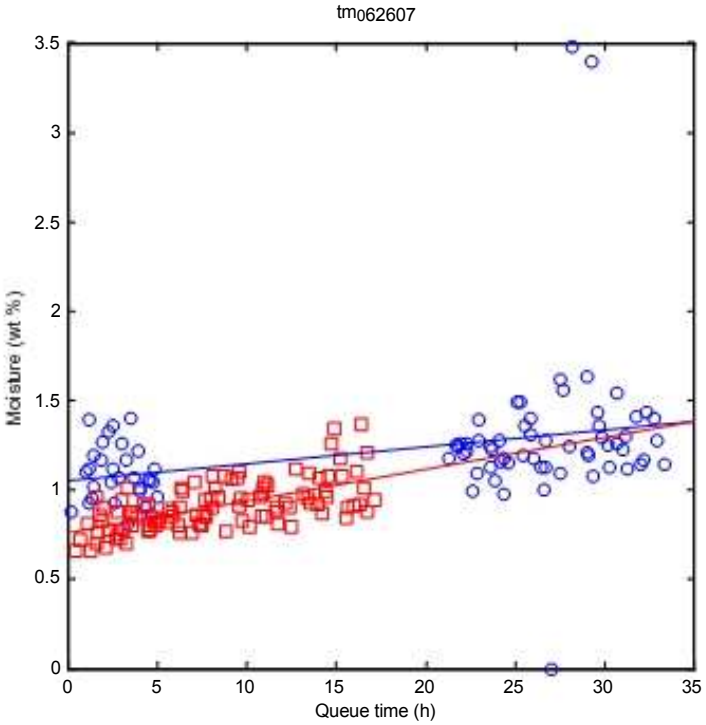


Figure 3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Moisture (wt %)
Queue time (h)
0
0.5
1
1.5
2
2.5
3
3.5
0
5
10
15
20
25
30
35