Assessment of Risk Factors of the Container-Closure System for PAT Guidelines

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Abstract

This paper first gives an overview of the Process Analytical Techniques (PAT) guidelines and pays particular attention to product quality and analyzing a material or an inline process with regard to risk factors that could lead to producing a poor product. It will be shown that risk of a poor product can be reduced by increasing the confidence level and reducing confidence interval by increasing the number of samples. This principle is demonstrated by using a new analytical technique for determining the surface contact area of a vial that can affect the drying process. The relative surface area of two different size tubing vials were analyzed and compared with one another with regard to affecting the lyophilization process.

Frequency distributions of the relative moisture in two different lots of 20 mm elastomer closures were determined using a new analytical technique. The relative moisture in individual closure was determined for the closures as they were received from the manufacturer, after steam sterilization and after drying. Results indicate that not only how the closures are sterilized and dried is important but also the fabrication of the closure. It is shown how one can assess risk by a comparison of frequency distribution having a confidence level of 99% and a low confidence interval.

Key words: vial, surface contact area, Process Analytical Techniques (PAT), risk assessment, closures, moisture

I. Introduction

A. Process Analytical Techniques (PAT)

The Food and Drug Administration (FDA) and other regulatory agencies throughout the world are encouraging manufacturers of health care products to adopt a new set of guidelines known as Process Analytical Techniques (PAT). The FDA defines PAT in general terms as follows (1):

"A system for designing, analyzing and controlling manufacturing through timely measurements (i.e., during process) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality."

I have underlined what I consider being two key words in this definition and would now like to briefly discuss both of them.

1. Quality

The first term is quality. In accordance to the PAT guidelines, product quality should be built-in to the process by design and not tested into the final product. The concept of building quality into process offers manufacturers an opportunity of achieving real time release of their products rather than having to wait until test results show that the product is within specifications and can be released for distribution to the public. But product quality really extends beyond the duration that the product is in the manufacturing site but also at the time when the product is administered to the patient. In other words, the quality of the product should be the same when the patient receives it as when it left the manufacturing facilities.

2. Analyzing

In order to ensure product quality and to achieve real time release, it will be necessary to analyze the attributes of raw and in-process materials and manufacturing processes by using chemical, physical, microbiological, mathematical and risk analysis. Thus each raw and in-process material and manufacturing process should be analyzed to ensure it is within defined limits so as to reduce the risk of producing a poor product. Without question this is a momentous task and challenge for any manufacturer but once achieved the benefits will be greater productivity and perhaps even a reduction in regulatory control.

3. Risk

By definition risk is a measure of the uncertainty. In terms of PAT, risk will be related to the uncertainty of producing a poor product. Now the risk of producing a poor product will be inversely related to understanding, i.e., that each raw and in-process material and manufacturing processes are within specified limits. While the actual function of the relationship between the risk and understanding will vary with each material or process, nonetheless, the relationship will look similar to that shown in Figure 1.

There is yet another risk and that is the risk of spending a great deal of time and money analyzing a material or process where the actual risk of producing a poor product is low. In order to prevent such an occurrence from happening, it will be essential that some assessment be made of the risk or a management of the risk parameters associated with a material or process is made to define just what kind of analytical technique is required and the presentation of the data. It is the presentation of the data and just how it will used to assess the risk of producing a poor product that can be most perplexing. I will have more to say about risk management later in this paper.

4. Lowering Risk

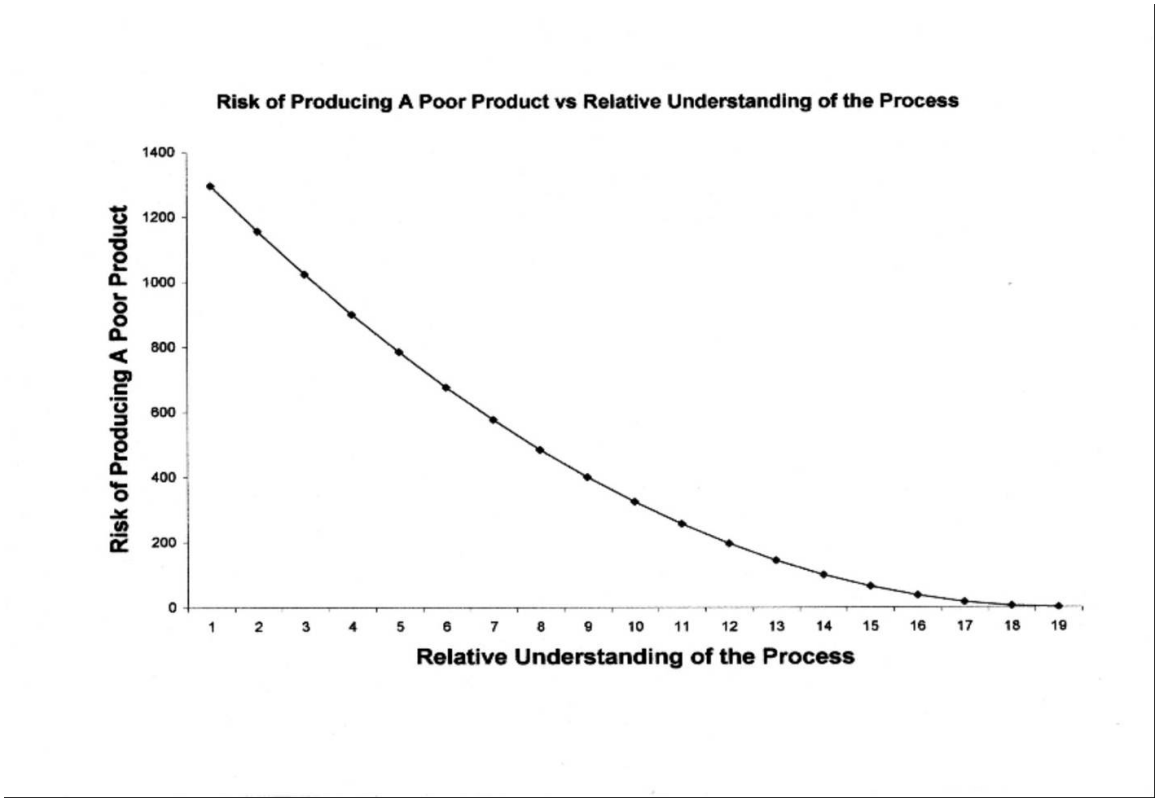

Figure 1 A hypothetical relationship between the risk of producing a poor product and understanding of the materials and processes.

We can not entirely eliminate risk of a poor lyophilized product but we can take steps to greatly reduce it. We can reduce that risk by introducing new analytical techniques that will allow us increase our confidence in the attributes of raw and in-process materials and manufacturing processes. In order to increase such confidence it is my feeling that we need to introduce statistical methods to examine the analytical data. In that case, we should be considering the confidence level.

The *confidence level* is that portion of the samples analyzed that will contain the true mean value. Typical confidence levels that are used are 90%, 95% and 99%. If the confidence level of the sample is just 90% then the remaining 10% of the samples may greatly affect of observed mean value. However, we can have a much greater confidence in the measured mean if the confidence level is 99%. Then only 1% of the measured sample can have an impact on the observed mean value. Currently some companies are considering confidence levels even closer to 1 (2) The confidence level will be dependent on the sample size to be analyzed and the *margin of error* or *confidence interval* (3). The effect of the confidence level on the required sample size of a given batch size for a confidence interval of 5% is shown by Table I. While not shown in Table 1, to decrease *confidence interval* one must increase the sample size. For example, if for a confidence level of 99% and a batch size of 100,000, we decrease the confidence interval from 5% to 2.5% if the sample size is increased from 661 to 2,594. For a more comprehensive discussion of the *confidence level*, *margin of error or confidence interval*

Table 1 Sample sizes for various batch sizes for a given confidence level with a confidence interval of 5%.

Batch Size	95% Confidence Level	99% Confidence Level
1,000	278	400
5,000	357	588
10,000	370	624
50,000	381	657
100,000	383	661
200,000	383	663
1,000,000	383	663

and *sample size*, I suggest that the reader consult the many texts that have been written on theses subjects.

B. Objectives

With the above concepts in mind, let us now consider how we can apply them with regard to the effect that (a), the surface contact area of the vial can have on the drying process and (b), the moisture content in the closures will have on the long term stability of the final product.

II. Vial surface contact area

For a number of years I was puzzled when people would tell me that they would find partial meltback in a vial that was surrounded by other vials that had completed the primary drying before commencement of the secondary drying process. This effect would be more pronounced for lyophilization processes in which the chamber pressure was equal or lower than 13 Pa (100 mTorr). I would ask if the closures on that vial may have been improperly seated and generally all the closures were properly seated. It was then I began to study the heat transfer coefficient of vials (C_o) which is defined as

$C_o = KA/d$ (energy/(unit area ^oC sec) (1)

Where,

K is the thermal conductivity of the glass
A is the contact area between the vial and the shelf
and d is the thickness of the glass

A measure of the C_o for from 25 – 30 vials showed that there was a frequency distribution of C_o for a given lot of vials (4). Thus the mean value C_{om} is best expressed as

C_{om} +/- standard deviation (SD)

As a result of the frequency distribution it was concluded that in order to ensure that all of the vials had completed the primary drying, one would have to take into account those

vials that would require a longer primary drying time, e.g. a 64 hour primary drying process may have to be extended to 84 hours. This additional 20 hours was just to make sure that a relatively few number of vials had completed the primary drying process. If we could remove these vials that were prolonging the drying process then the primary drying process could be safely reduced some 20 hours.

Recently I developed an analytical technique that can rapidly (≈ 1 sec) measure the contact area between the vial and the shelf surface and thus provide a means for removing those vials prior to the filling operation. This analytical technique will be described in a future publication. At this time, I would like to share with you the results of two experimental tests using this analytical technique.

A. Experimental Test #1

Figure 2 shows the experimental results for the measurement of the relative surface area of a given sample of tubing vials (lot A). From the results of this method, it is possible to calculate the percent contact area but that would not alter the nature of the observed frequency distribution since the analytical technique is based on first principles.

An examination of Figure 2 shows that the mean value (RA_m) of the relative surface area was 147 and the standard deviation (SD) was 8. The standard deviation in Figure 2 shows how the relative surface area values, at a given standard deviation, differ from that of the mean value. In order to assist the reader in understanding the significance of the standard deviation, let us consider Table 2. Using Table 2 in conjunction with figure 2 shows that the odds of finding a vial with $(RA_m - RA_j)/SD = 0.67$ is 1 or highly certain. However, if one were to randomly select vials the odds against one of finding one where $(RA_m - RA_j)/SD = 2$ would be 21 to 1 and would suggest it would be possible. However, to select a vial where $(RA_m - RA_j)/SD = 3$ would be 369 to 1, and although possible not likely since only one vial was found with that value.

Table 2 Relationship between $(RA_m - RA_j)/SD$ and Odds Against One

$(RA_m - RA_j)/SD$	Odds Against One
0.67	1
1	2
2	21
3	369
4	15,700
5	1.74×10^6
6	5×10^8
7	3.9×10^{11}

Since it is now possible to determine those vials that had low C_o values equal 3 SD or higher, we can put these vials through the desired primary drying process of 64 hours and ascertain if they would produce a poor product. If these vials produce a poor product, then those vials having relative surface areas lower than a given value should be removed prior to the filing process. However, Figure 2 is the frequency distribution of just 91

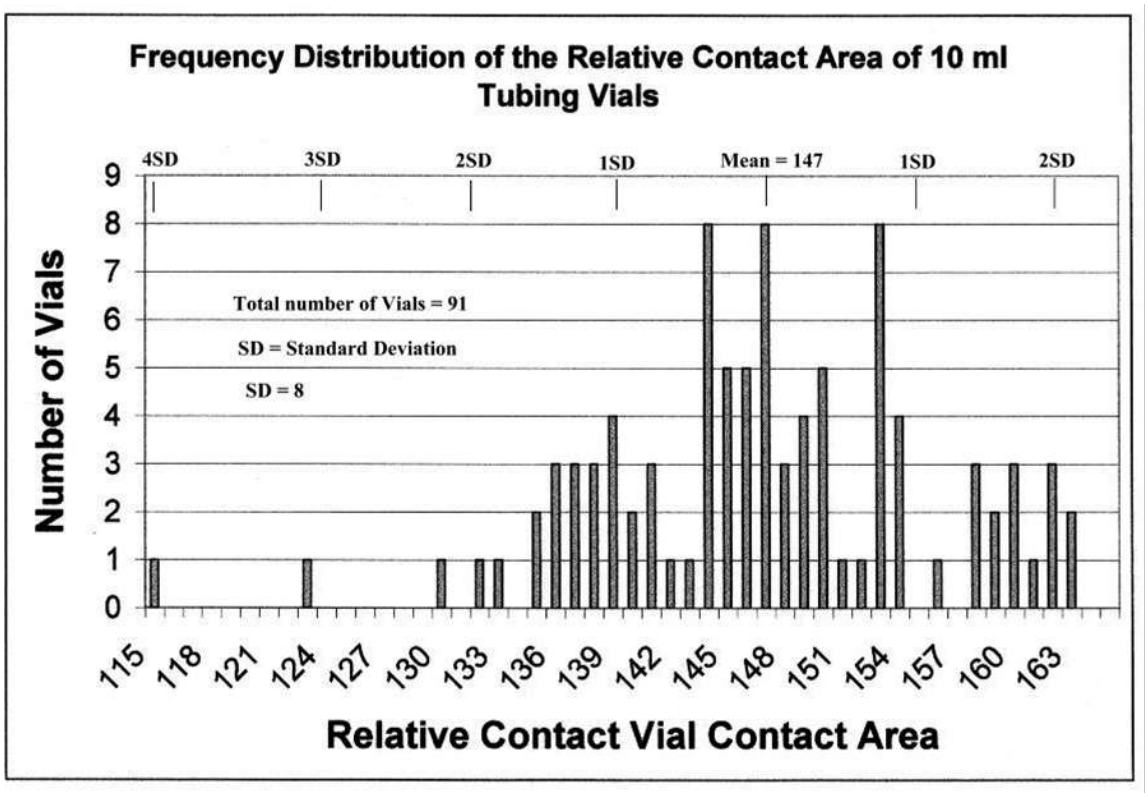

Figure 2 Frequency distribution of the relative surface area of 91 tubing vials.

samples it is now of interest to understand what is the confidence interval at a confidence level of 99%. Since vials tend to be purchased in large lots, e.g., 2,000,000 vials the confidence interval for the results shown in Figure 2 would be about 14%. This would mean one cannot have a high degree of confidence in the results since the mean relative contact surface area could range from 126 (147 - 21) to 167 (147 + 21).

To demonstrate the poor contact vials can make with a surface, one of the vials having a relative contact area of 147 was taken and the bottom was pressed on an ink pad and then onto a white sheet of paper. The print made by the vial is shown by Figure 3. The figure clearly shows areas where there is little contact between the vial and the paper. Because the ink will spread when it comes in contact with the paper, the actual contact area is smaller than that shown by the Figure 3. For that reason and others, it is not advisable to try and measure the relative contact area by making an ink print on paper.

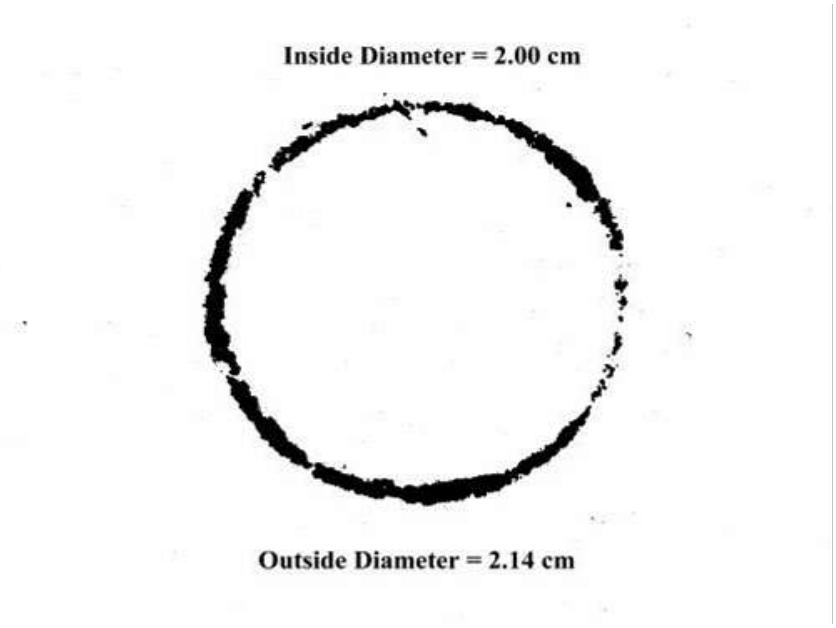

Figure 3.Print of the surface contact area of a vial

Experimental test #2

Figure 4 shows the frequency distribution of the relative contact area of tubing vials known as lot B. It is somewhat surprising that the observed mean had a value of 149 and agrees closely with the mean observed in Figure 2. Even the standard deviation of 8 is the same as that in Figure 2; however, based on a lot size to 2,000,000 vials, the confidence interval for these results is 3.9% for a confidence level of 99%. Thus the mean value could range from 143 (149 – 6) and 155 (149 + 6).

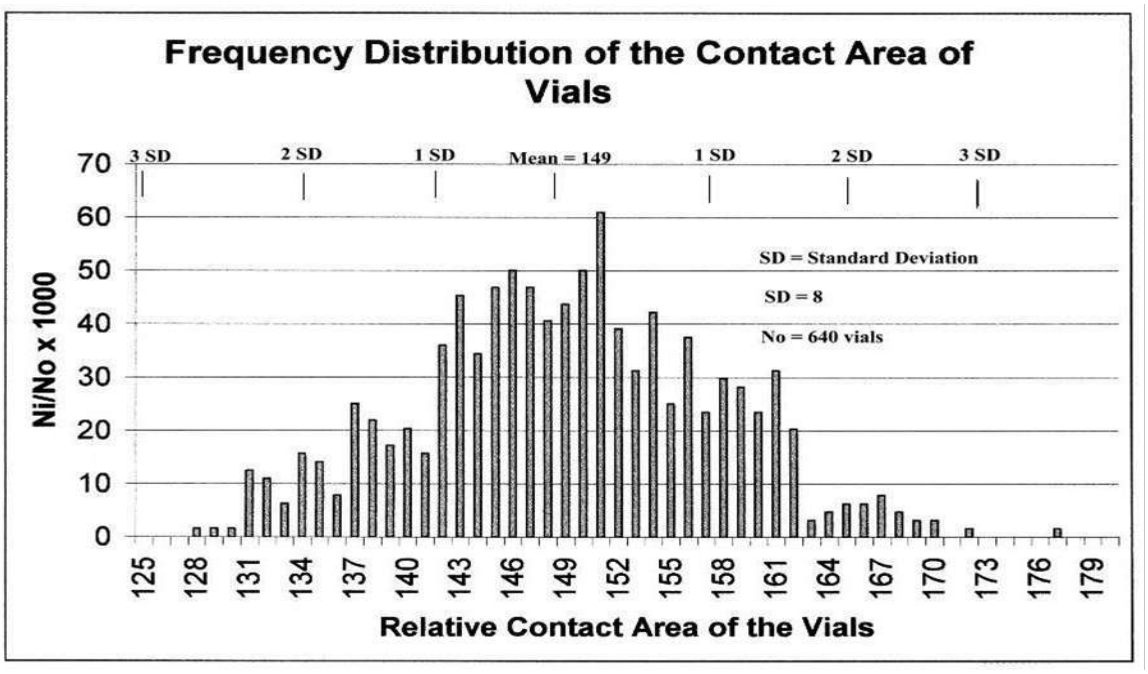

Figure 4 Frequency distribution of the relative surface area of 640 tubing vials.

A comparison of the two sets of vials.

The outside diameter of the vials in lot A was 3.02 cm and had an outside contact diameter shown in Figure 3 of 2.14 cm. The outside diameter of the vials for lot B was 2.36 cm and the outside contact diameter was 1.92 cm. Thus from expression (1) it would appear that the C_o for both vials would be quite similar and that may explain why the mean values for relative contact areas of the two sets of vials were similar in spite of the differences in their volumes. For fill volumes having the aspect ratio (width of the vial/height of the fill-volume), these results would suggest that a formulation in the larger vial (Figure 2) would require longer to dry than that of the small diameter vial (Figure 4) because the larger vial contains more formulation than the smaller vial but will have close to the same C_o.

III Moisture in closures.

A. Outgassing of water vapor

The closure plays an important part in providing a lyophilized product. When properly seated on the vial during the lyophilization process, the closure provides a path by which water vapor can be removed from the vial. After completion of the lyophilization process it provides an effective seal to protect the dried product from the environment and finally offers a means by which the dried product can be reconstituted without jeopardizing the sterility of the product prior to administrating to a patient. In spite of all these attributes the closure can affect the shelf life of the product by the outgassing of water vapor and other gases from the closure (5). Outgassing from a closure can be defined as ***"when the rate of desorption of a gas specie from a surface is greater than the rate of adsorption of that specie on the surface."***

The rate of outgassing of water vapor from a closure (Q_w) can be expressed as the following function,

$$Q_w = f(M_w, T, P_w)$$

where

M_w is the quantity of water in the closure,
T is the temperature
and
P_w is the vapor pressure water vapor in the container.

A. Effect temperature on outgassing rate

1. General description of the experimental method

The outgassing rate of water vapor from closures at various temperatures was determined by a differentially pumped mass spectrometer system attached to a freeze dryer (6). The

empty clean and dry freeze dryer at ambient temperature was first purged with dry nitrogen for more than 8 hours at a pressure of 266 Pa (2 Torr). The gas purge was turned off and the pressure in the chamber was evacuated and maintained at 13 Pa (100 mTorr) while the temperature was set for the test temperature. When the shelf temperature was within the desired range for no less than 1 hour, the chamber was isolated from the pumping system and the increase in partial pressure of the chamber gases were measured as a function of time. These results served as a blank.

The shelf temperature was then adjusted to ambient temperatures and the above was repeated with a tray containing elastomer closures. From the difference in the pressure rise rate of the water vapor of the chamber with the closures and the blank, the average outgassing rate of the closures was determined for a given temperature. The outgassing rate was expressed in terms of time required to increase the moisture content of one mg of dried product by 1 % assuming that the sticking coefficient is one, i.e., all the water vapor goes onto the dried cake.

2. Results

The results of this study are shown in Table 3. Table 3 shows that for the closures examined the average outgassing rate for temperatures equal to or lower than 25 ^{o}C, the rate of outgassing would not have a major impact on the residual moisture content of most lyophilized products. However, at temperatures approaching or exceeding 40 ^{o}C the outgassing from the closures could represent a serious threat to the stability of lyophilized products.

Table 3 Time required to increase the moisture content of 1 mg of dried cake by 1% assuming a sticking coefficient of one.

Temperature Range ^{o}C	**Time**
4 - 8	Approx. 20 years
20 - 25	18 – 20 months
=> 40	=< 8 hours

The reader should bear in mind that the above results only represent an average value and some closures will be outgassing at a higher rate than others. Thus it is possible that some closures, e.g. at 20 – 25 ^{o}C, may increase the moisture in a product such as to significantly reduce its shelf life.

<u>B. Methods for measuring the moisture content in closures.</u>

There are two basic methods for determining the actual moisture content in closures. The following will give a brief overview of each method and also introduce a new analytical method for determining the relative moisture content of a closure.

1. Loss on Drying (LOD)

In this method, the closure is first weighed and then either whole or in sections placed in an oven where it is heated to 80 to 100 ^{o}C in either a vacuum at less than 133 Pa (1 Torr) or under a constant flow of a dry gas such as nitrogen. After some period of time the closure is removed and weighed and loss in weight is recorded. The closure is placed back into the oven and the latter process is repeated until the closure attains a constant weight within some defined limits. The loss in weight is assumed to be from the removal of moisture from the closure.

The LOD method is perhaps the most reliable and accurate of all the methods used to measure the moisture in closures. Its main disadvantage is that it is very time consuming and would not be suitable as a built-in analytical method to satisfy PAT requirements.

2. Karl Fischer method.

For the Karl Fischer method the closure is weighed and then sectioned. The moisture is removed from the sections either by soaking them in a Karl Fischer reagent like methanol and then the increase in the moisture in the solvent is determined by titration using a reagent containing iodine where the end point is the presence of excess iodine. The increase in moisture in the solvent is determined from the amount of the iodine reagent that is consumed (7).

An alternative Karl Fischer method is to once again section the closure and place it in an oven where it is heated to some predetermined temperature. A constant flow of gas passes over the closure and then through a reagent were the moisture can once again be determined (8).

While this Karl Fischer method is not as accurate as that of the LOD nonetheless it is sufficient for assaying the moisture content in closures. However, the method is destructive in nature and it too would not be suitable to be used as an in-process method for monitoring the moisture in closures.

3. New analytical method

I have developed an analytical method that can be used to measure the “relative moisture” in closures from the dielectric properties. The detailed description of this analytical technique is beyond the scope of this paper and will be described later in another publication. However, the following lists some key features of this analytical method for measuring the “relative moisture” in closures.

- Measurement is rapid (about 1 second) and is nondestructive

- Suitable for measuring sample sizes that provide a frequency distribution with confidence levels that approaches or exceeds 99% with low confidence intervals.

- Closures with established excess moisture levels can be discarded or returned for additional drying thus making this method suitable for use as a process analytical technique.

- Allows for assessment of the effectiveness of the sterilization process and/or drying process.

The following are the results of measurement of the frequency distributions of the "relative moisture" in two separate batches of closures. For each batch the relative moisture was measured in closures as they came from the closures manufacturer, after steam sterilization and after drying. Just so there is no misunderstanding, different closures are represented in each test.

C. Test results for closure A

Because of a confidentially agreement I cannot disclosure the process under which the closures in the following distributions are sterilized or dried. I can only tell you that they were 20 mm closures and were not processed in bags.

1. Frequency distribution of 490 closures (A) as they were received from the manufacturer.

The frequency distribution for the closures as they were received from the manufacturer is shown in Figure 5. The frequency distribution appears to be quite broad, with a standard deviation of 10, and skewed left. The mean relative moisture for this distribution is 100.

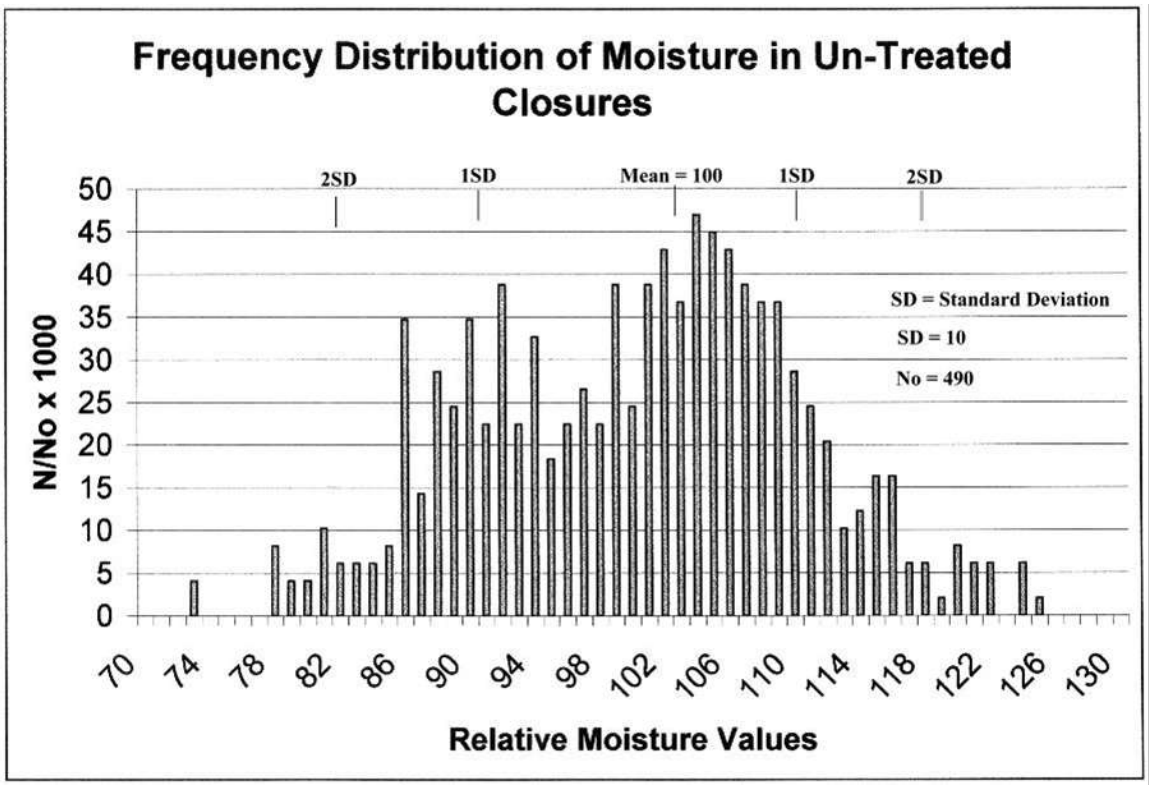

Figure 5 Frequency distribution of the relative moisture in 490 closures (20 mm) as they were received from the manufacturer and identified a closure A.

2. Frequency distribution of 498 closures (A) after steam sterilization.

After steam sterilization, the frequency distribution of the relative moisture content is shown in Figure 6. The resulting frequency distribution is more normal than that seen in Figure 5 and the mean relative moisture content has been increased to 112. However, the distribution is still rather broad having a standard deviation of 9. A comparison of the frequency distributions shown in Figures 5 and 6 indicates that moisture was adsorbed by the closures during the steam sterilization process. This is an important result for if there was an adsorption of moisture then the closures had to come into contact with the hot steam.

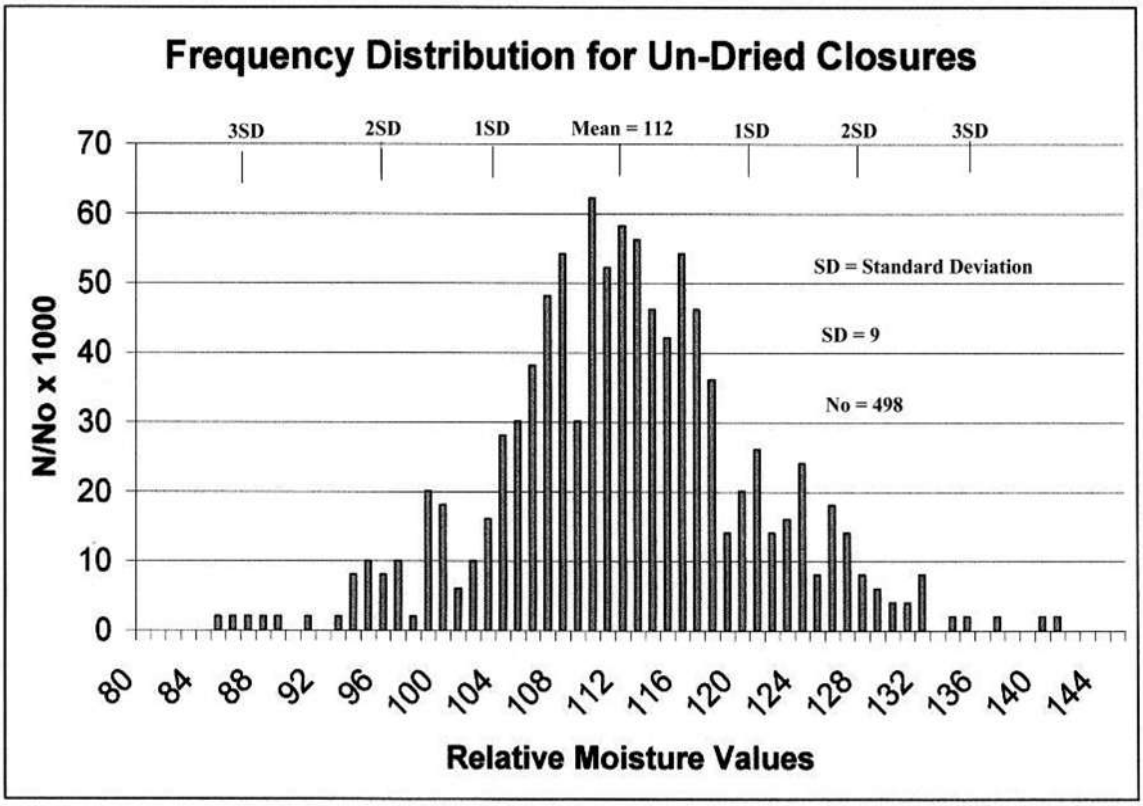

Figure 6 Frequency distribution of the relative moisture in 498 closures (20 mm) after steam sterilization.

Assuming a confidence level of 99% and the closures were purchased in lots of 2 million, the confidence interval will be 5.8 % or the true mean will exist between 106 and 118. In order to increase the confidence in the observed mean value one would have to increase the sample size.

3. Frequency distribution of 486 of steam sterilized closures (A) after drying.

Figure 7 shows a marked change in the relative moisture values of the closures after drying and shows a normal frequency distribution with a mean relative moisture of 81 and a standard deviation of 5. Assuming a confidence level of 99% and the closures were

purchased in lots of 2 million, the confidence interval will be 5.8 % or the true mean will exist between 76 and 87. Once again, in order to increase the confidence in the observed mean value one would have to increase the sample size.

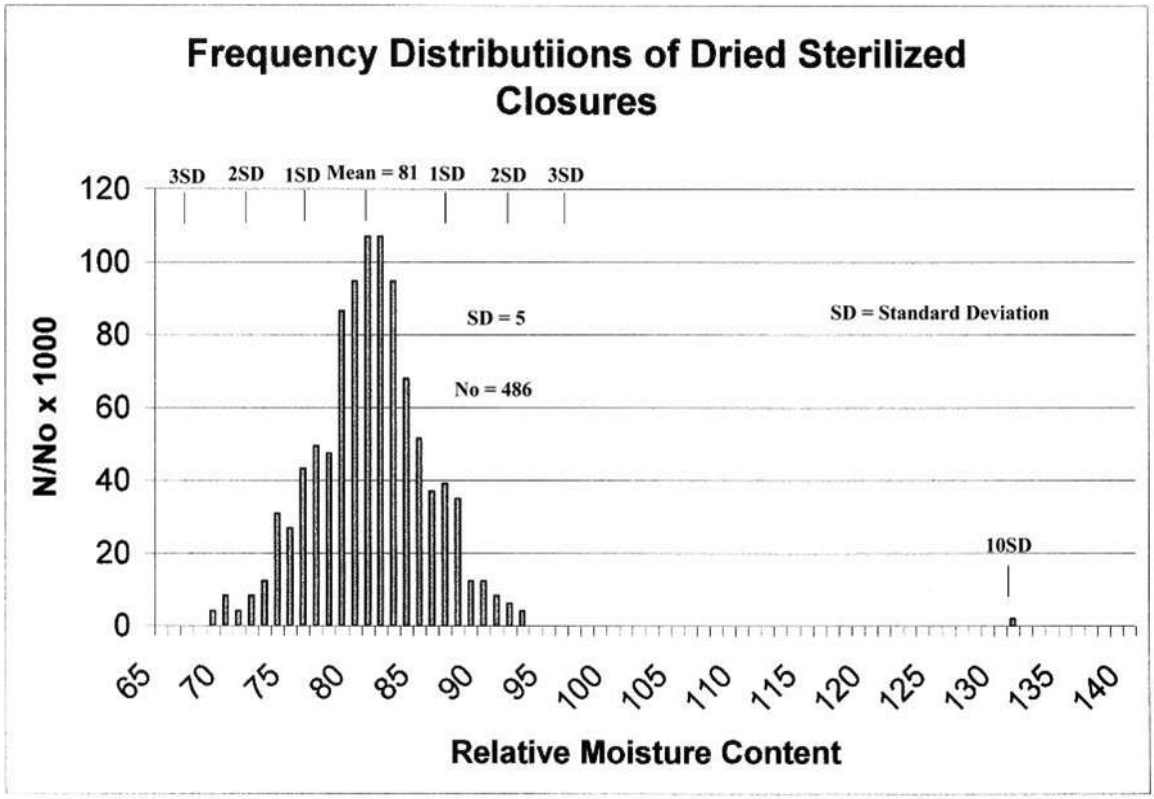

Figure 7 Frequency distribution of the relative moisture in 486 of steam sterilized closures after drying.

4. A comparison of the frequency distributions of the un-dried and dried steam sterilized closures (A).

The comparison of the frequency distribution of the un-dried and dried steam sterilized closures is shown in Figure 8. Examination of this latter figure shows that some dried closures (Figure 7) will have the same relative moisture value as that which was measured for those closures that were steam sterilized but not dried. However, the real question is whether or not those closures will affect the product stability over the shelf-life of the product. Given that this method allows for the sorting of the closures according to their relative moisture, those closures having relative moisture values in question can be identified and tested on lyophilized product to ascertain if these closures can have any impact on the product stability.

5. Actual moisture content verses relative moisture content of closures
The question that now needs to be answered is would it make any difference if the frequency distributions shown in Figures 5-8 were expressed in grams of water? In my opinion the answer would be no for it is not the units of the measurement that are

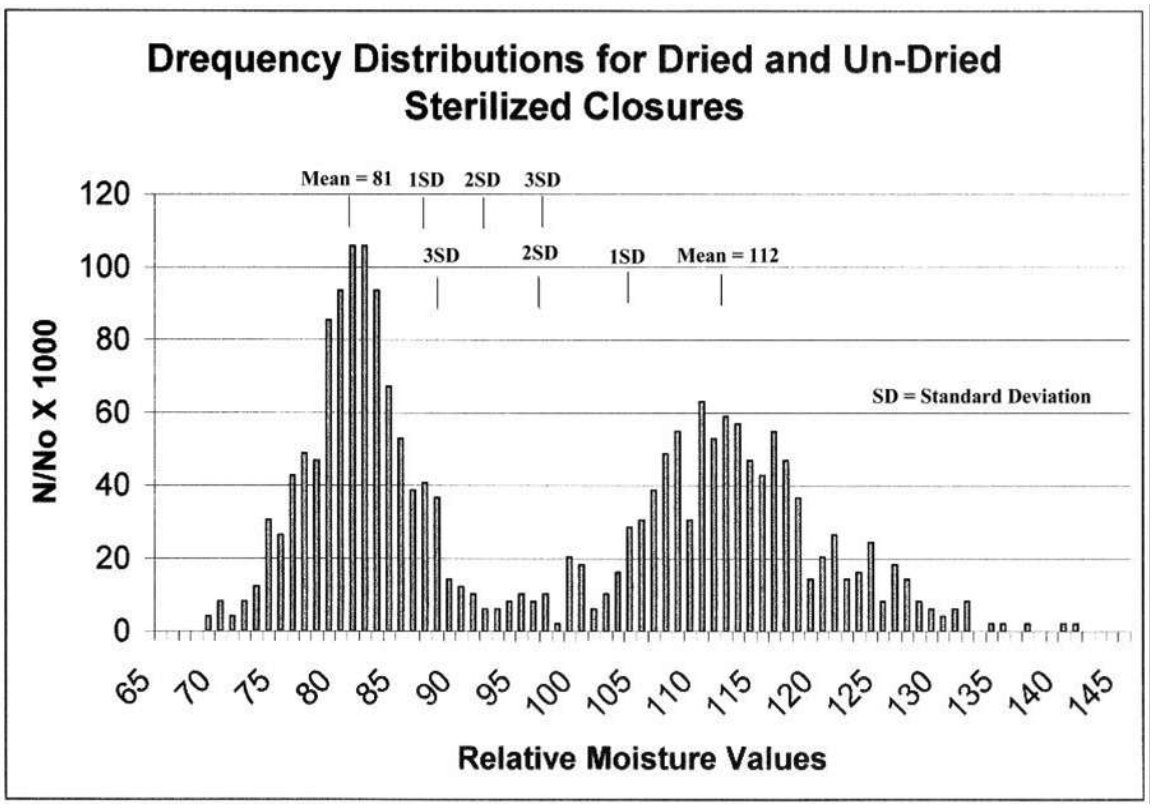

Figure 8 Comparison of the frequency distributions for steam sterilized closures A that were un-dried and dried.

important but to establish if any values of the dried sterilized closures will have an adverse affect on the stability of the product. Should any of the relative moisture values have an adverse affect on the final product then a risk management decision would have to be made whether to change the drying process or simply prevent those closures from being used in the manufacture of a lyophilized product.

C. Test results for closure B

The following data was taken from 20 mm closures that were sterilized and dried in bags. I am sorry but I cannot provide you with any further details; however, the results do give us an insight as to the possible effect that the bag may play in the sterilization or drying process.

1. Frequency distribution of 696 closures (B) as they were received from the manufacturer.

Figure 9 is the frequency distribution for 696 closures from lot B as they were received from the manufacturer. This figure shows that frequency distribution for these closures were not skewed; however, the distribution was much broader because of a larger standard deviation. The standard deviation of the untreated closures in lot A was 10 while the standard deviation for these closures was 13. In addition, the mean relative moisture

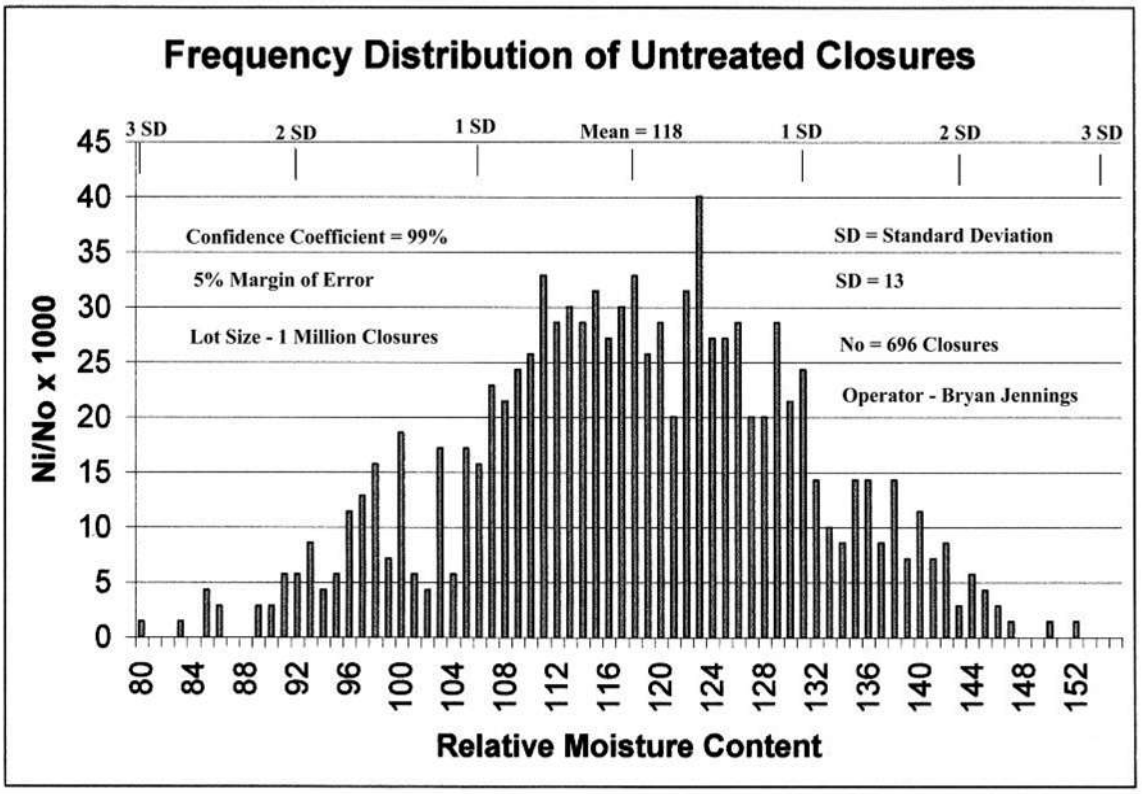

Figure 9 Frequency distribution of the relative moisture in 696 closures (20 mm) as they were received from the manufacturer and identified a closure B.

for this lot was 118 while the mean for the untreated closures of lot A was only 100. It is also interesting to note that mean relative moisture for these closures was shown to be greater than that of the steam sterilized closures (112) in lot A. Since the closures from lots A an B came directly from the manufacturer, it is not known if difference in the frequency distributions for these two lots of closures is a result of the composition or the manufacturing of the closures.

2. Frequency distribution of 1981 closures (B) steam sterilized but not dried.

The effects of steam sterilization of a bag of closures of lot B containing 1981 closures are shown in Figure 10. The effects of steam sterilization on the untreated closures was to increase the mean relative moisture from 118 to 128; however, the standard deviation also increased from 13 (from manufacturer) to 16 (steam sterilization). Assuming a confidence level of 99% and the closures were purchased in lots of 2 million, the confidence interval for this distribution was 2.2 % or the true mean will exist between 125 and 131.

3. Frequency distribution of 1958 closures (B) steam sterilized and dried.

Figure 11 shows the frequency distribution for the relative moisture in 1958 (one bag) sterilized closures (B) after drying. The mean relative moisture was 110 while the standard frequency distribution was 12. Assuming a confidence level of 99% and the

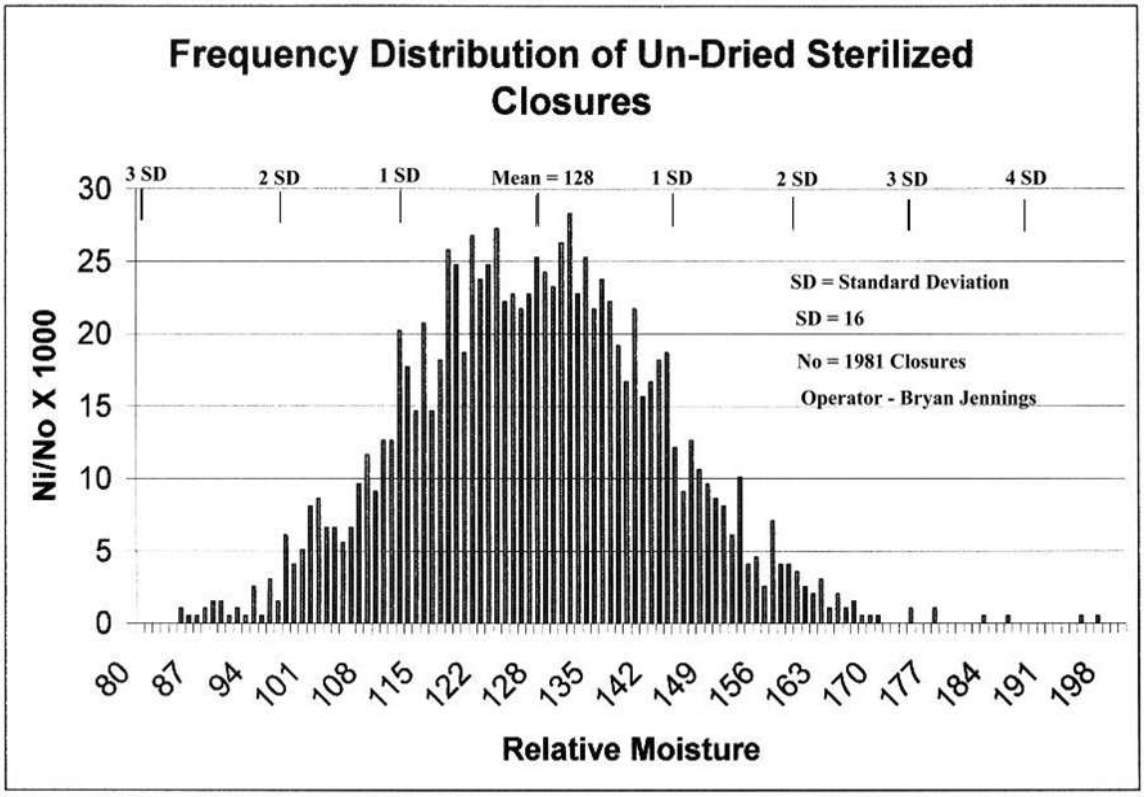

Figure 10. Frequency distribution of the relative moisture 1981 closures (B) steam sterilized but not dried.

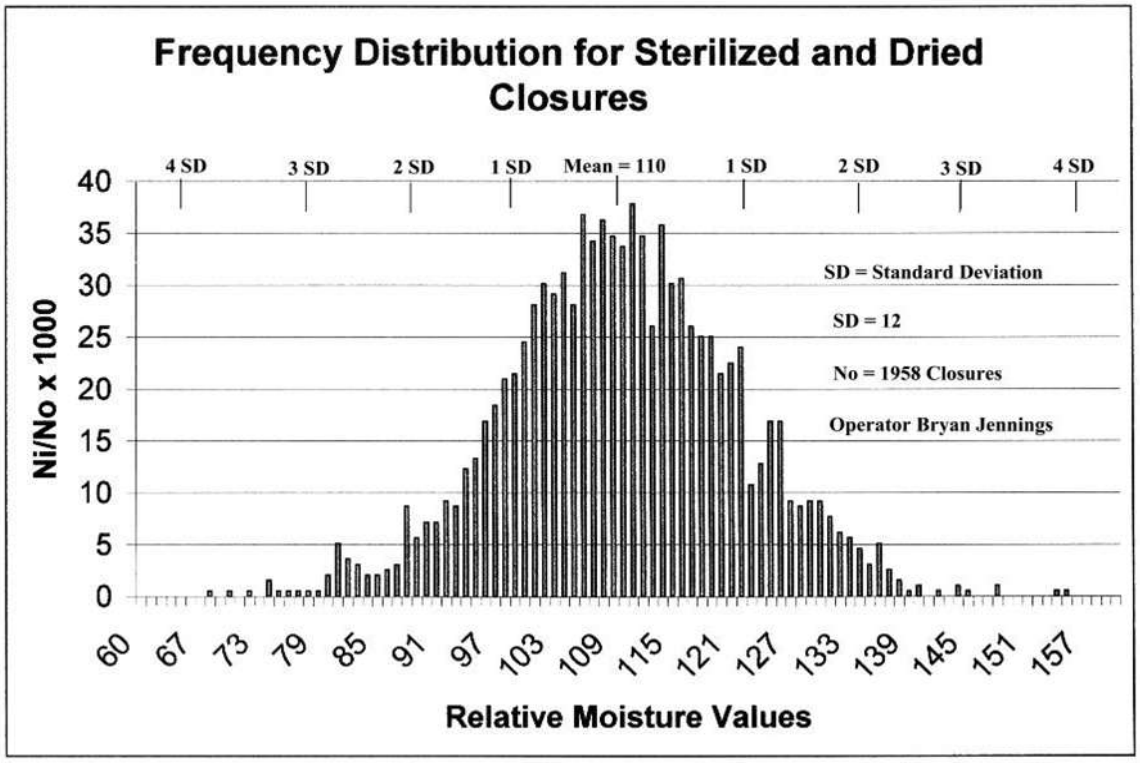

Figure 11. Frequency distribution of 1958 closures (B) steam sterilized and dried.

closures were purchased in lots of 2 million, the confidence interval for this distribution was 2.2 % or the true mean will exist between 108 and 112.

4. A comparison of the frequency distributions of the un-dried and dried steam sterilized closures (B).

An examination of Figure 12 shows that there is little separation between the two distributions and if there is any problem with outgassing of moisture affecting the stability of the final product then there would be ample justification for changing the drying process of the closures in the bag.

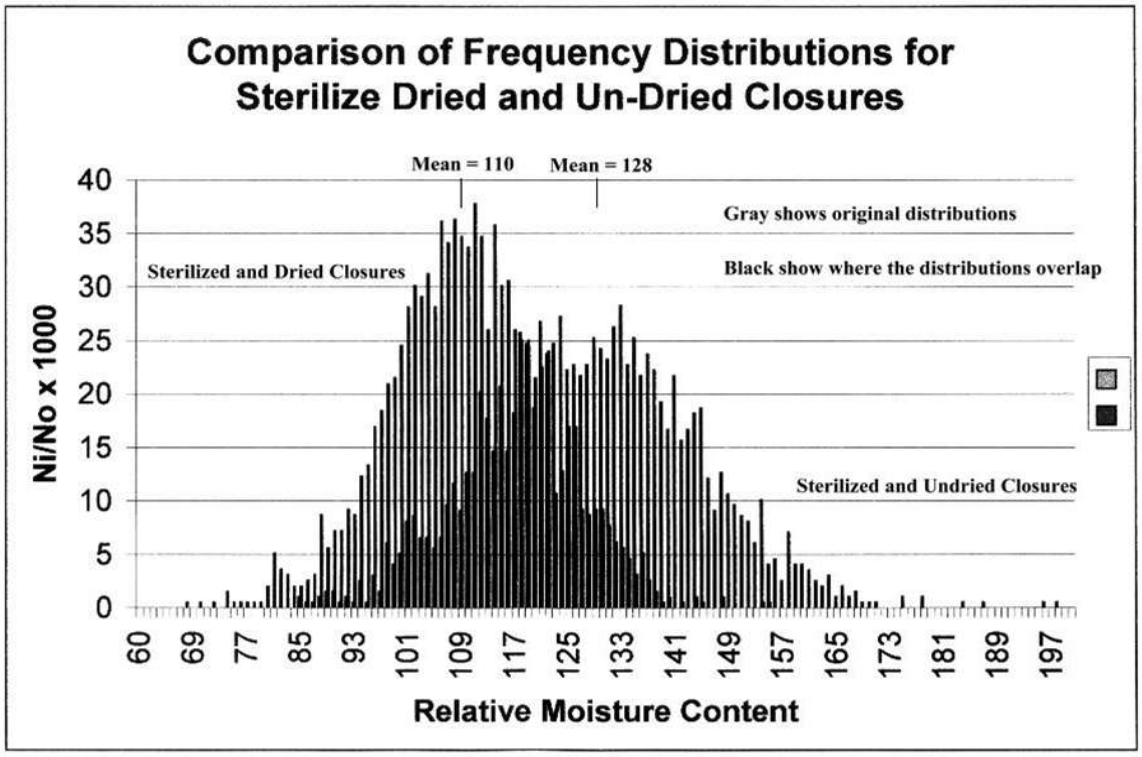

Figure 12. A comparison of the frequency distributions of the un-dried and dried steam sterilized closures (B).

IV Summary and Conclusions

In order to adequately assess the risk associated with a raw material or in-line process one must set confidence limits of 99% or higher (99.99999%) with low confidence intervals. To achieve meaningful frequency distributions having such requirements one must use large sample sizes.

By using an analytical technique that permits the measurement of the relative surface contact area of a vial, permits one to remove vials that would significantly and adversely affect the drying time. In addition, by drawing attention to the contact surface area one can select vials that will permit more rapid drying processes.

The analytical technique for determining the frequency distributions for relative moisture in closures allows us to analyze the raw materials and the effects that steam sterilization and drying of the sterilized closures.

- These results show that the closures received from the manufacturer already contain a significant amount of moisture.

- The frequency distributions for relative moisture in closures A and B where quite different and such differences may occur as a result of differences in composition or in the manufacturing process of the closure. Thus this analytical method is a rapid and non-destructive method for determining if a lot of closures are acceptable for use in the lyophilization process.

- Since the sterilization and drying of the closures A and B were performed using different methods one can assess the risk associated for a given method by comparing frequency distributions of relative moisture of sterilized closures before and after drying.

- The above results show the advantage of using frequency distribution as a means acceptance testing of components or the inline processing of closures.

Acknowledgements

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