A STUDY OF WATER VAPOR TRANSMISSION RATE OF BLISTER PACKS BY USP STANDARD AND CONTINUOUS GRAVIMETRIC PROTOCOL

By

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ABSTRACT

A STUDY OF WATER VAPOR TRANSMISSION RATE OF BLISTER PACKS BY USP STANDARD AND CONTINUOUS GRAVIMETRIC PROTOCOL

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The water vapor transmission rate (WVTR) is essential in determining the shelf life of pharmaceutical products in the package. The WVTR of blister pack was determined by using USP 671 and the continuous gravimetric protocols. The WVTR values from the two protocols were compared and the accuracy was validated.

The WVTR of the blister pack determined using the USP 671 protocol was 0.0452 mg/day/blister with a standard error of 0.0010 while the WVTR using the continuous gravimetric protocol was 0.0652 mg/day/blister with a standard error of 0.0067. The WVTR were compared using t-statistic for unequal sample size with unequal variance and no significant difference was detected. The accuracy of the two protocols was compared through the pooled variance of the data points. USP 671 standard protocol had a pooled variance of 4.99×10^{-4} while for the continuous gravimetric protocol it was 2.13×10^{-5}. Therefore the continuous gravimetric protocol showed higher accuracy than the USP standard protocol.

Several recommendations have been suggested for the USP standard protocol in order to make the protocol more accurate: for the WVTR of the blister pack, the unsteady state should be considered and excluded from the calculation, Monitoring of the humidity and temperature is critical, Chamber size and the time of equilibrium for the specific relative humidity needs to be outlined.

To my dear family and friends

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Chapter 1

INTRODUCTION

1.1 Background

In early 1950's, the packaging technology took a new diversion when polymeric materials like polyethylene came into commercial use [1]. Plastics have various advantages like light weight, easy fabrication, chemical inertness and low cost which has led to the rapid increase in the consumption of these materials in different sectors. Packaging is a major market for the plastics industry, accounting for 29% of all plastic used in United States [2]. In today's market, plastic has replaced glass to a great extent.

Through different styles of packaging design, plastics have made there way in the pharmaceutical industry; many different packages like bottles, pouches, blister packs etc; made from plastics are available in the market. The primary need for the pharmaceutical industry is to insure the product safety and its efficacy, and in achieving this, barrier is an important property. The main components of pharmaceutical drugs are the active ingredient and excipients, where the active ingredient is carried in predetermined doses. The system has a specific dissolution characteristic, which once ingested will dissolve and release the active ingredient. If such a system is altered, the release of active ingredient may be compromised. Therefore it is critical to prevent the hydroscopic excipients such as starch and cellulose from being exposed to moisture [3, 4].

Recently blister packs have become one of the important packaging systems. The unique feature of the blister packs to act as a unit-dose provider, which means that the drug is dispensed one dose at a time, had attracted its attention to the pharmaceutical industry. This system overcomes the drawback of the bottle where the tablets or capsules are exposed to moisture due to repeated opening and closing of the containers [5, 6].

The problem with plastic that has always been an issue of great concern is mass transfer phenomena. A lot of industrial and academic research has been carried out to understand the mechanisms of mass transport in polymers, in order to enable design of materials with improved barrier properties. A high barrier is a very desirable property to be exhibited by polymeric materials intended to be used in the pharmaceutical industry [7]. Modern multilayer polymeric materials are able to fulfill the need of high barrier which has led to the blister packs in the pharmaceutical industry.

Blister packs are formed by thermoforming. During the process, the material is stretched, which results in decreased thickness at the walls and bottom of the cavity. This results in a situation where determining the water vapor transmission rate for the blister pack becomes a challenge [6].

1.2 Motivation

Different types of techniques based on either iso-static or quasi-isostatic methods have been developed to study the permeation process for films and packages. Mass spectrometer detector, Mocon, use of permeation cell with gas chromatography and gravimetric techniques are the commonly used techniques for measuring transmission rates.

The pharmaceutical tables and capsules are small in size; tablets varying from 6mm to 25mm in diameter and capsules varying from 11mm to 26mm in length. Due to the small size of the tablets and capsules, blister packs have small cavities which results in a challenging situation for conducting permeation studies and restricts the use of most of the techniques. The most common commercially used technique for measuring the transmission rate of blister packs is the gravimetric method.

Based on this technique, the United States Pharmacopeia (USP) has established a standard USP 671 (Container permeation) for measuring WVTR in pharmaceutical blister packages [8]. The drawbacks of this standard that need to be considered are:

1. The method can produce high noise which could be critical when high barrier materials are tested and may significantly affect the signal/noise ratio.
2. A limited number of data points are generated for the complete experiment.

The aim of this research was to compare the USP standard protocol with the continuous gravimetric protocol in order to:

1. Minimize the sample manipulation.
2. Maintain the water activity constant throughout the experiment.
3. Generate more data points to allow a thorough analysis of the mass transfer profile.

1.3 Goal and Objectives

The main goal of this study was to compare the continuous gravimetric technique with the USP 671 for measuring water vapor transmission rate in pharmaceutical blister packages.

The objectives of this study were:

1. To determine the WVTR, using the USP standard method.
2. To determine WVTR using a continuous gravimetric protocol under USP conditions.
3. To analyze and describe the differences between the two protocols.

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Chapter 2

LITERATURE REVIEW

2.1 Introduction

The use of polymer systems as pharmaceutical packaging material has increased significantly in the past few years. Polymeric packaging material serves as a barrier; its ability to minimize the exchange of moisture between the product and the outside environment is an important factor. Plastic materials have many advantages over glass containers as pharmaceutical packages. They are cheaper, lighter weight and in most cases, shatter resistant. However plastics, in contrast with more traditional packaging materials such as glass and metals, allow the exchange of low molecular weight compounds such as gases and vapors, between the inner and the outer atmosphere of the package [1].

With time, gases, light and moisture can result in the oxidation, discoloring, or degradation of the pharmaceutical products. Moisture permeation can alter the hardness or texture of a drug and it can even change the chemical formulation of the drug, making the drug inactive. Plastics offer less barrier protection against moisture; but this gap between the plastic and glass barrier properties is being narrowed by the development of high barrier plastic films and laminates [1-4].

2.2 Unit Dose Packaging

Unit dose packaging has become one of the most commonly used packaging systems in the pharmaceutical industry to allow the access to one dosage or usage at a time. It can be used for solid, liquid or powdered drugs. Most of the unit dose packages are non-reclosable. The most common unit dose packages used in the pharmaceutical industry are the pouches, blisters and strips. Along with the barrier property of the material, even the seal material and the seal integrity can affect the barrier properties of the package. An incomplete seal or a seal that is less moisture protective than the container allows moisture to invade or escape from the package. For this reason, the water vapor transmission rate (WVTR) of the package is extremely significant when considering the shelf life of a packaged pharmaceutical product [5-7].

2.3 Blister Packaging

Blister packs can be classified into four different groups: transparent blisters, opaque blisters, strip packaging, and sachets [8]. Blisters are produced from multilayer or monolayer sheet material. In a blister package each tablet or capsule is incased in a small custom-formed cavity of plastic or aluminum and sealed in place. Blister packaging provides a unit dose of product directly to consumers in a convenient easy-to-use form [9, 10].

Blister packs are manufactured by heating the plastic web and then vacuum forming it into cavity. For making a blister pack, a male die is pushed into the blister cavity, so as to improve the material distribution in the finished blister cavity. The filling of the blister and then sealing of the blister take place at the same time [11].

The common materials used for these packages are polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), cyclic olefin copolymer (COC), polyvinylidene chloride (PVDC), polychlorotrifluoroethylene (PCTFE) and chlorotrifluoroethylene (ACLAR). Polymeric films with multilayer structures are becoming popular to meet high-performance demands [3, 11, 12].

2.4 Mass Transfer in Polymer Systems

Mass transfer is a process by which the permeation of the permeant (such as oxygen, water, carbon dioxide and organic vapor) occurs in a polymeric system. It is an important phenomenon that defines the usefulness of polymeric material in the pharmaceutical industry. Mass transfer through a plastic material occurs in three stages, initially the interaction of the penetrant on the surface, followed by diffusion of the penetrant through the polymer and finally desorption of the penetrant on the other side of the polymer [13]. The permeation of vapor and simple gases through a layer of polymer film is most widely studied. This phenomenon of mass transfer is complex for structures like bottles and blister packs, where the thickness of the material is not uniform throughout [14, 15].

Sorption

Sorption is the first step in the migration process. It is defined as the uptake of the permeant molecules, such as moisture, gases and organic vapors by the polymeric material. Sorption depending upon the interaction between the polymer and permeant or between the permeant molecules, with in the polymer matrix [16].

Henry's law can be applied when both polymer/permeant and permeant/permeant interactions are weak as compare to polymer-polymer interactions. At a given temperature, the solubility coefficient is independent of concentration and is a constant value. There is a linear relationship between the concentration and vapor pressure for a sorption isotherm [17-19].

$$C = S \times p \tag{2.1}$$

where C is concentration of the permeant, S is Henry's law proportionality constant and p is the equilibrium vapor pressure of the permeant molecules.

This behavior is observed for the sorption of permeant by a rubbery polymer at low pressure and low solubility of the permeant in the polymer. The solubility coefficient, which is an equilibrium partition coefficient of the permeant between the polymer matrix and vapor phase is given by [19-21]

$$S = \frac{C_p}{C_v} \tag{2.2}$$

where, S is the mass of permeant molecules sorbed by a unit of polymer mass per unit of partial pressure, C_p is the concentration of the permeant molecules in the polymer matrix and C_V is the steady state concentration of the permeant in the vapor phase.

The solubility coefficient is temperature dependent in nature. Its temperature dependence can be explained by the Van't Hoff-Arrhenius equation.

$$S = S_0 \exp(-\Delta H_S / RT) \tag{2.3}$$

where ΔH_S is the heat of solution, R is the gas constant and T is the temperature [18, 19].

Diffusion

Diffusion can be defined as the transfer of permeant molecules across the polymer. The tendency of the permeant molecules to equilibrate across the polymer matrix, results in the molecular motion of the permeant from the high concentration to the low concentration side. The diffusion coefficient is the quantitative measurement of the rate at which a permeant diffuses. It is defined as the rate of transfer of the molecules across a unit area of the polymer, divided by the space gradient of concentration [22].

According to the Fick's law, for a unidirectional, isotropic polymeric phase, the rate of transfer of the diffusing molecule (F) can be described as [23, 24]

$$\mathrm{F} = -\mathrm{D}\frac{\partial C}{\partial x} \tag{2.4}$$

where D is the permeant diffusion coefficient, C is the concentration of the permeant molecule and x is the diffusion distance.

For the transient state, Crank described Fick's second law for a single permeant in a polymer film as

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{2.5}$$

where t is time. There are some boundary conditions that are assumed with the equation. Firstly, diffusion doesn't depend on the permeant concentration and the polymer relaxation. Secondly, It is an unidirectional process which is perpendicular to the surface of the polymeric material [20, 23].

At temperatures below and above the glass transition temperature (T_g), the diffusion mechanism varies. In rubbery polymers, diffusion can be described by Fickian behavior, whereas in case of glassy polymers the diffusion process can be more complex and may follow three different mechanisms [20, 21] .

1. Fickian diffusion: Polymer/permeant system relaxes at a faster rate than the diffusion of the permeant itself.
2. Diffusion of the permeant is faster than relaxation of the polymer/permeant system.
3. Uncharacteristic behavior where the diffusion of the permeant is comparable with the relaxation of polymer/permeant system and the presence of micro cavities in the polymer matrix, affects the diffusion process.

Permeability

Permeation is a process by which the exchange of gases, organic vapors or liquid takes place through a polymeric matrix. Permeation takes place when a concentration gradient is present across the polymeric material which results in the movement of the permeant from the high concentration to the low concentration side. It is a three step process which involves the sorption of the permeant by the polymeric material, its diffusion within the polymeric matrix and finally desorption of the permeant on the other side of the polymer. During the permeation of simple gases, a relationship between the three main permeation properties, permeability coefficient, diffusion coefficient and solubility coefficient can be observed: [20]

$$P = D \times S \tag{2.6}$$

where D is the Fickian diffusion coefficient, which represents the speed of movement of permeant molecules in the polymer and S is the solubility coefficient for distribution of the permeant between the polymer and vapor phase. The permeability coefficient (P) is the steady-state transport rate of permeant molecules through a polymer membrane of unit area per unit of thickness.

2.5 Fickian Characteristics

Diffusion of small molecular permeants in polymers is often assumed to follow Fickian characteristics at a temperature above T_g. In the case of Fickian diffusion, the sorption curve is a plot of the rate of permeant diffusion in plastic as a function of square root of time. Once solvated, these polymers assume an equilibrium state almost immediately. Rubbery polymers exhibit Fickian characteristics; the polymer chains adjust so quickly to the presence of the permeant molecules that they do not cause diffusion inconsistency [18].

In 1968, Crank indicated an important feature regarding Fickian sorption, according to which at initial stage both absorption and desorption are linear in nature. For absorption, the linear region extends over 60% or more of M_∞ and after the linear portions, the sorption curve becomes concave and approaches the final steady state condition [25].

2.6 Parameters Affecting Permeability

Permeability rate is affected by, polymer properties like chemical structure and morphology, environmental factors like temperature and relative humidity; the nature and concentration of the permeant.

Polymer Properties

Barrier properties of the polymer are affected by the polymer morphology (crystallinity, thermal history, molecular orientation, etc), polymer chemical structure (branches, molecular weight).

Polymers are not able to crystallize completely and it has been proved that the permeation of the permeant takes place mostly through the amorphous regions. Barrier properties of the polymeric material can be improved by the increasing the crystallization and orientation of the polymer. Orientation of the polymer induces crystallization by bringing the chains closer and the permeation decreases due to the decrease in the mobility of the permeant in the oriented region [26].

The free volume in the polymer is an important factor strongly associated with barrier properties. During the diffusion process, the permeant makes use of these micro-cavities; thus the transport properties of a permeant depend on the presence and the size of this free volume [21].

Temperature Dependence

Increasing in temperature can result in segmental motion. Once the energy density is sufficient, it will cause the structural transition of the polymer. Increase in temperature can also augment the micro-brownian motion of segmental units which can result in an increase in free volume. This increase in inter-chain distance causes a weakening of the molecular interactions between neighboring polymer molecules and will affect the permeation rate.

Permeation rate is affected by temperature, following the Arrhenius relationship.

$$K = Ae^{-E_a / RT} \tag{2.7}$$

where K (rate constant of chemical reactions), T (temperature), e_a (activation energy), A (pre-exponential factor), R (gas constant)

At a temperature above and below glass transition temperature the process of mass transfer works differently. Below T_g, the diffusion process is generally non-Fickian in nature. Glassy polymers usually have long relaxation time and they show an untrue steady state where as temperature above T_g, the polymer is in rubbery state and it follows the fickian kinetics. The micro-brownian motion of polymers molecules enables the polymer to reach the equilibrium rapidly and diffusion is not time dependent. An increase in temperature provides energy for a general increase in segmental motion. If energy density is sufficient, the polymer may pass through structural transitions, such as the glass and melting transitions, which further affect solution and diffusion processes [20]. The effect of increase in temperature may also be expressed in terms of the increase in free volume which is directly related to the bulk expansion of the polymer due to the increased segmental motions.

2.7 Methods for Measuring Permeability

A permeation process is a three step process, in which sorption of the permeation, diffusion and finally desorption takes place. In permeation process the permeant flow rate increases with time, until a steady state is reached in which the permeant flow rate becomes constant. P is obtained from the steady state [27]. There are two different approaches that can be used to measure permeation.

Isostatic

In this approach, a membrane acts as a separator, dividing the permeation cell into two chambers. In one chamber, the high concentration chamber (HCC), an atmosphere enriched in the permeant is generated and in second chamber, a low concentration chamber (LCC) is generated. It is a volume variable approach, where the permeated molecules are allowed to expand instead of accumulating them in the constant volume of the cell [28].

In this method, initially the permeant flow is zero. After some time, permeant molecules start to adsorb and diffuse through the polymer. At regular intervals, the low concentration chamber is flushed with a carrier gas and the quantity of permeant present is recorded. During the transition state there will be an increase in the quantity per unit time but when the steady state is attained the quantity permeated will be constant [21, 27].

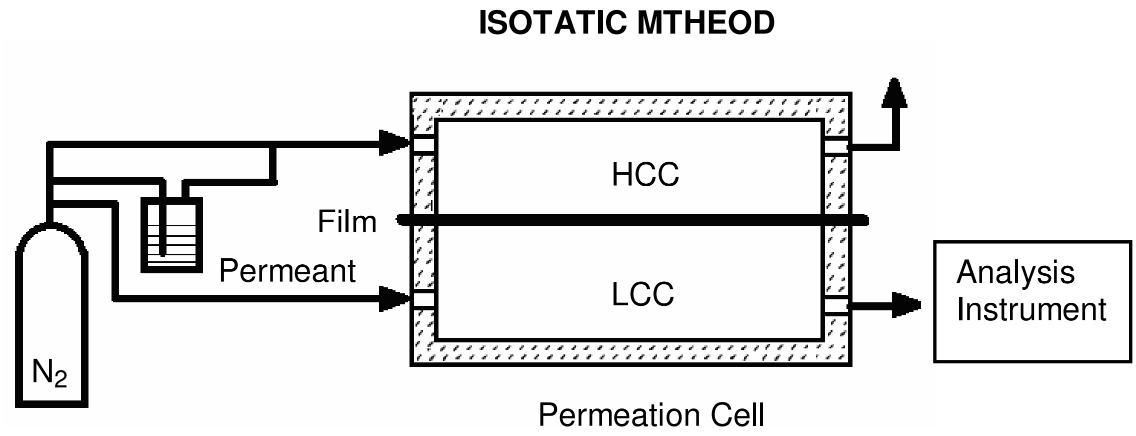

Figure 2.1: Experimental setup for the isostatic method.

By continuously purging the carrier gas, the permeant flow is recorded as function of time during the experiment [23, 27].

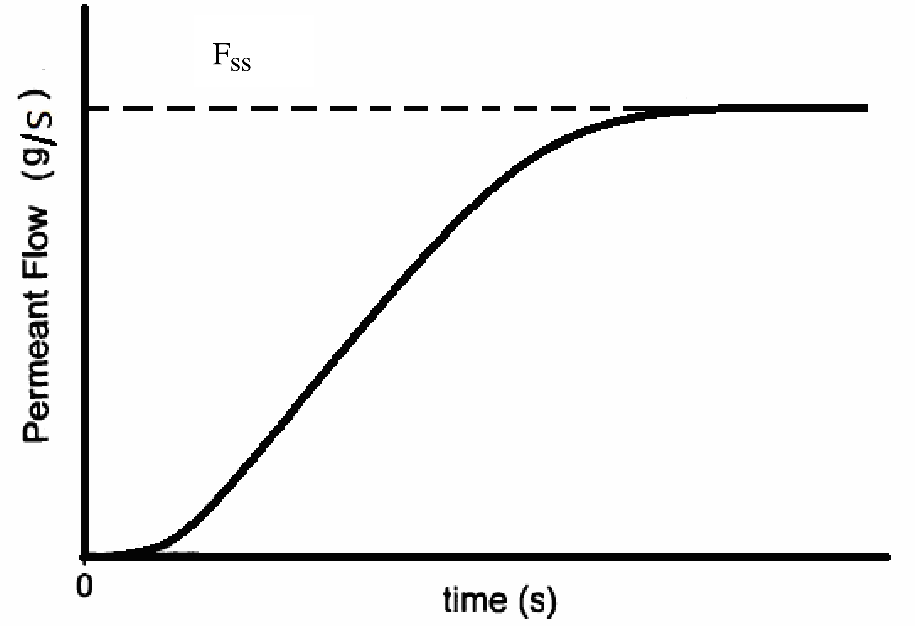

Figure 2.2: Permeant flow vs. time for an isostaic method.

From the flow at the stationary state (Fss), P is evaluated as follow:

$$P = \frac{F_{\infty} l}{A(C_{HCC} - C_{LCC})} = \frac{F_{\infty} l}{A \Delta C} \tag{2.8}$$

In this equation, l, A and Δc are film thickness, exposed area and concentration gradient (difference in permeant concentrations between the HCC and LCC atmospheres) respectively.

Quasi Isostatic

It is a pressure-variable technique, the permeant is introduced in the high concentration chamber and a concentration difference between the high and low concentration chamber is maintained. During the permeation process, the permeant increase in the low pressure chamber. This quantity increase in the low chamber side is plotted vs. time. It is necessary that the partial pressure difference between the high and the low concentration chamber is present through out the experiment [27].

QUASI-ISOSTATIC METHOD

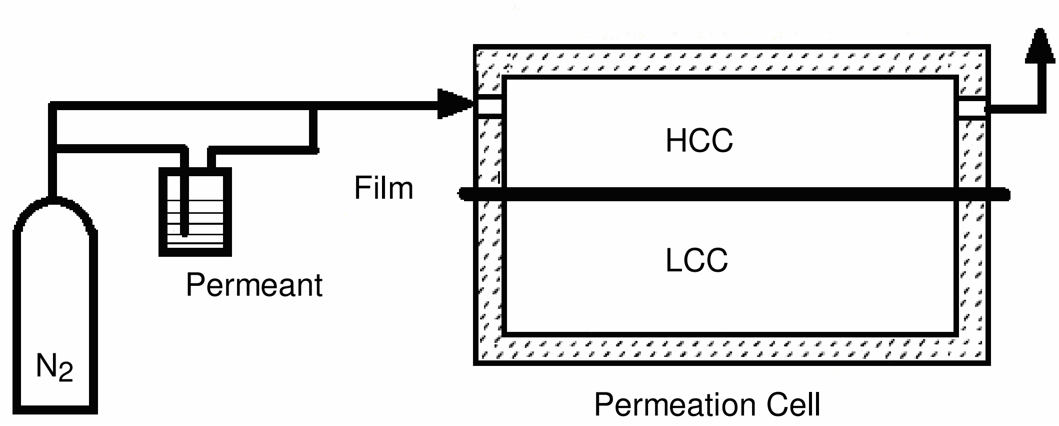

Figure 2.3: Experimental setup for the quasi isostatic approach.

Data generated through this method is permeated masses as a function of time.

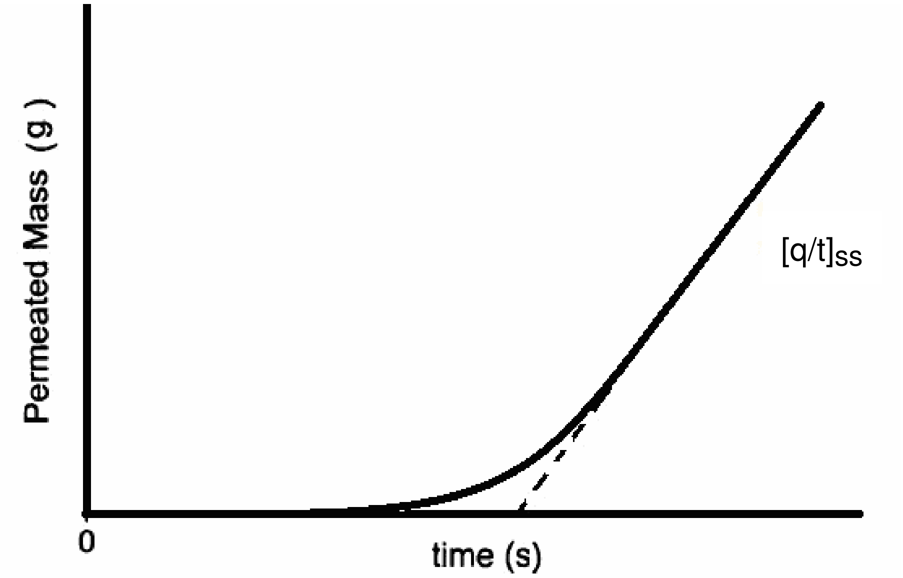

Figure 2.4: Permeated mass vs. time for a quasi isostatic method.

Mass of permeant in the LCC (initially zero) starts to increase after a period of time. The curve slope of the mass versus time plot increases with time until it reaches a constant value [27].

From the slope (q/t) at the steady state, permeability (P) is obtained through Equation

$$P = \left[\frac{q}{t}\right]_{SS} \frac{l}{A\Delta C} \qquad (2.9)$$

2.8 Standard Protocol for Study of Water Vapor Transmission Rate in Blister Packs

The United States Pharmacopoeia (USP) is a non–governmental, official public standard. It authorizes the prescription and over–the–counter medicines along with other healthcare products manufactured or sold in the United States. USP also sets widely recognized standards for food ingredients and dietary supplements. It sets the standards for the quality, purity, strength, and consistency of health care products. It is recognized and used in more than 130 countries around the globe [29-31].

In 1820, the first edition of the pharmacopoeia of the United States was published. The USP was initially published at 10-year intervals; from 1942 to 2000, at 5-year intervals; and beginning in 2002, annually.

As described earlier, blister packs are produced by the thermoforming method due to which at the edges of the cavity, thickness tend to decrease which eventually affects the permeation of the blister cavity. Hence blister packs have different permeation rate than the film itself that let to the development of USP 671 standard protocol in order to measure WVTR of the blister pack. This protocol uses a quasi-isostatic method and it is widely accepted by industry.

According to USP 671 protocol, blister packs containing multiple cavities with a desiccant placed inside the blister rather than the actual pharmaceutical product are stored at a constant temperature and humidity of 23℃ and 75%RH. The weight of the sample blister is then measured at certain intervals. From the weight gained by the individual blister packs, the WVTR is determined [29].

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Chapter 3

MATERIALS AND METHODS

3.1 Materials

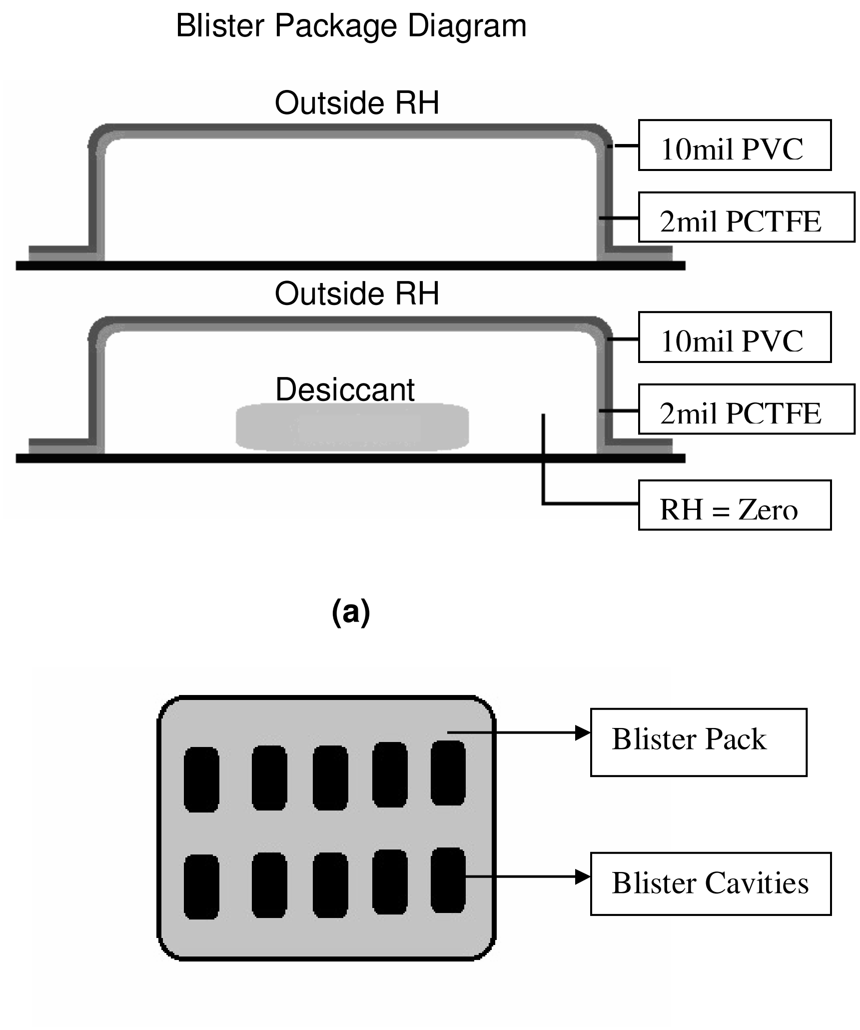
Blister Package Materials

The blister package included a blister cavity and a backing:

- The cavity was a multilayer polymeric film TEKNIFLEX® VA 10200 consisting of 10 mil polychlorotrifluoro ethylene and 2.0 mil polyvinylchloride supplied by Tekni-Plex (Somerville, NJ) [1].
- The aluminum backing TEKNILID 1250 also supplied by Tekni-Plex (Somerville, NJ) was a multilayer structure of over-lacquer/ aluminum/ heat seal-lacquer based on PVC having a thickness of 20 micron [1].

TEKNIFLEX® VA film was thermoformed in Honeywell Barrier Packaging Analytical Lab (BPAL) in Morristown, NJ. The thermoforming upper and lower platens were pre-heated to 130°C. The blister package was manufactured with a Klockner-Hansel Compacker machine and sealing was set at 170°C [2].

The blister packages were supplied in tablets that included 10 cavities as shown in Figure 3.1. Tekni-Plex provided a total of eighty blister packages: forty were empty blisters and the rest were blisters containing desiccant. The desiccant was a 83 mg moisture indicating desiccant tablet (6 mm x 2.5 mm) supplied by Medical Packaging Inc (Ringoes, NJ) having absorption capacity of 20% at 25°C and 80%RH. The blisters were packaged in hermetic high barrier aluminum pouches for transportation. The empty and filled blisters were received in two different aluminum pouches which were induction sealed. The seal was broken once the trial started.



(a)

(b)

Figure 3.1: a) Diagram of the empty blister cavity and filled blister cavity with desiccant b) A tablet with 10 cavities

3.2 USP STANDARD PROTOCOL

3.2.1 Materials

Life Latch® buckets (United State Plastic Corporation® in lima, OH) of two gallon capacity were used as chambers for storing the blisters at specific humidity of 75±3%. A saturated salt solution of sodium chloride (supplied by J.T Baker from Phillipsburg, NJ) was used to generate such RH within each bucket. Six buckets were used in this study. The buckets were placed in an environmental chamber (Enviroline™ walk-in rooms from Nor-Lake Scientific, Hudson, WI), in order to maintain the humidity buckets at 23°C. Each humidity bucket included two wire meshes having a diameter of 9 inches. The purpose of one mesh was to cover the container (with saturated salt solution) from the top and prevent the falling of the sample into the salt solution. Second mesh had hooks attached to it so that the blisters can be hung and they are surrounded by specific environment, which might not be possible if the blisters were stacked over each another. In each bucket to hold the top mesh in position, four screws fitted with a gasket were drilled.

As shown in Figure 3.3, each bucket was also equipped with a temperature and humidity recorder manufactured by LogTag Recorders (MicroDAQ.com, Ltd in Contoocook, NH). The log tag recorders were placed inside the bucket throughout the experiment in order to keep a record of the temperature and relative humidity. They can be used over a temperature range of -40℃ to 85℃ and a humidity range of 0% to 100%RH. They could store up to 16000 data points and their recording time could be set from a few minutes to days [3]. Inside the humidity buckets, these data loggers collected data at intervals of 30 minutes.

The filled and empty blisters were removed from the buckets in order to be weighed as specified by USP 671. An Ohaus Voyager V12140 analytical balance (Ohaus Corporation, Pine Brook, New Jersey) was used to weigh the samples.

Figure 3.2: Temperature and humidity recorders from LogTag.[3]

3.2.2 Method

Six humidity buckets using saturated salt solution were prepared and were stored in the environmental chamber at 23℃. Only four buckets contained a temperature and humidity sensor so as to monitor the environmental condition in real time. They collected a reading after every 30 minutes. Humidity buckets were stored in the environmental chamber for 2 days so that the environment inside the bucket stabilized to 23 ± 2℃ and 75 ± 3% RH.

Figure 3.3: Experimental Setup for the USP standard Protocol

According to the USP 671 protocol at least 4 blister packs containing 10 cavities each should be tested. Equal numbers of empty and filled (with desiccant) blister packs should be used where empty blister packs act as controls. These blisters were then placed in the chamber and exposed to a relative humidity of 75 ± 3% at 23 ± 2℃. After 24 hours and at each multiple intervals, the samples were removed from the chamber and were allowed to equilibrate in the measuring atmosphere for about 45 minutes. After 45 minutes, the control packs were weighed as one single unit and then the average was taken whereas the filled blister packs were weighed individually. If the weight increase of the filled blister pack exceeds 10%, then the test is terminated and the last reading is considered to be valid [4].

Based on the collected data, the average water vapor transmission rate in mg/day/blister in each pack was calculated using the following equation [4]

$$\frac{1}{(N \times X)} \times [(W_f - W_i) - (C_f - C_i)] \tag{3.1}$$

N = number of days

X = number of cavities per pack

$(W_f - W_i)$ = final and initial weight difference of filled blister pack

$(C_f - C_i)$ = the average final and average initial weights of control packs.

Based on water vapor transmission rate, the blister packs can be divided into four different classes.

Table 3.1: Different classes of blister packs based on USP standard

Class	Average Water Vapor Transmission Rate
A	If no pack exceeds 0.5mg
B	If no pack exceeds 5mg
C	If no pack exceeds 20mg
D	If packs test meet none of the above

According to the standard, the suitable test interval for the final weight of a class-A, should not be less than 35 days [4].

For this research, 8 blister packs with 10 cavities each were used. The samples were stored in each bucket as follow: the blisters were perforated in one of the four corners and a hook was passed through the perforation and then hung on to the wire mesh. Once blisters were all attached to the mesh using their respective hooks then the mesh with the blister was placed inside the bucket. The weighing of the samples was carried out by following the protocol outlined in USP 671. A total of 24 filled blister packs and 24 control blister packs were used. In bucket 1, 2 and 3, eight empty blister packs and in bucket 4, 5 and 6 eight filled blister packs were placed in each of the buckets.

3.2.3 Statistical Analysis

Kernel smoother

To estimate the equilibrium time for the empty blister pack, kernel smoother statistical technique was used. A kernel smoother is a statistical technique for estimating a real valued function by using its noisy observations, when no parametric model for this function is known. The estimated function is smooth in nature, and the level of smoothness is set by a single parameter. The kernel smoother represents the set of irregular data points as a smooth line or surface [5, 6].

The simplicity of the kernel nearest neighbor smoother makes it the most widely used nonparametric classification method and it is quite successful in practice in a variety of applications. In nearest neighbor smoother, each point X_0, take (n) nearest neighbors and estimate the value of $y(X_0)$ by averaging the values of these neighbors and it is represented as follow: [5, 6]

$$h_n (X_0) = \| X_0 - X_n \| \tag{3.2}$$

where X_n is the n^{th} closest to X_0 neighbor

Analysis of variance (ANOVA)

To compare the water vapor transmission rate from the three buckets, One-way ANOVA was used. It is an effective way to compare the means of three or more group with independent variables, to see if the group means are significantly different from each other. To conduct a one-way ANOVA, it is required to have a categorical variable that has at least three independent groups [7].

By using ANOVA, one can decide if the average amount of difference or variation between the scores of members of different samples is large or small compared to the average amount of variation within each sample [8]. The following assumptions are made to determine the analysis of variance:

I. Normality: The residual is normally distributed.
II. Independence of cases: The groups are independent
III. Equality of variances: The variance of data in the groups is the same.

To apply ANOVA, calculate the average amount of variation within each of the samples, known as mean square error (MS_e).

$$MS_e = \frac{SS_e}{N-K} \tag{3.3}$$

SS_e = Sum of the square error

K = Number of groups

N = number of cases combined across all groups

Now, the mean square between (MS_b) which is the average amount of variation between the groups is calculated

$$MS_b = \frac{SS_b}{K-1} \tag{3.4}$$

SS_b = Sum of squares between groups

After determining the mean square between groups and mean square error, the F value is determined as follows

$$F = \frac{MS_b}{MS_e} \tag{3.5}$$

By using the F distribution we can see if the differences between the groups are statistically significant [7, 8].

3.3 CONTINUOUS GRAVIMETRIC PROTOCOL

3.3.1 Materials

SGA-100R gravimetric analyzer was used to develop a continuous flow protocol for conducting permeation studies. The magnetic suspension electro-balance made it possible to weigh the samples contactlessly and continuously in almost all environment conditions. It had a capacity of 10 g with a resolution of 1µg. The instrument is divided into three different zones, zone one is the weighing system (microbalance), zone two is the sample chamber, and zone three is the vapor generators. The presence of temperature control systems in each zone helps in maintaining the instrument stability. The relative humidity can be accurately generated and constantly controlled.

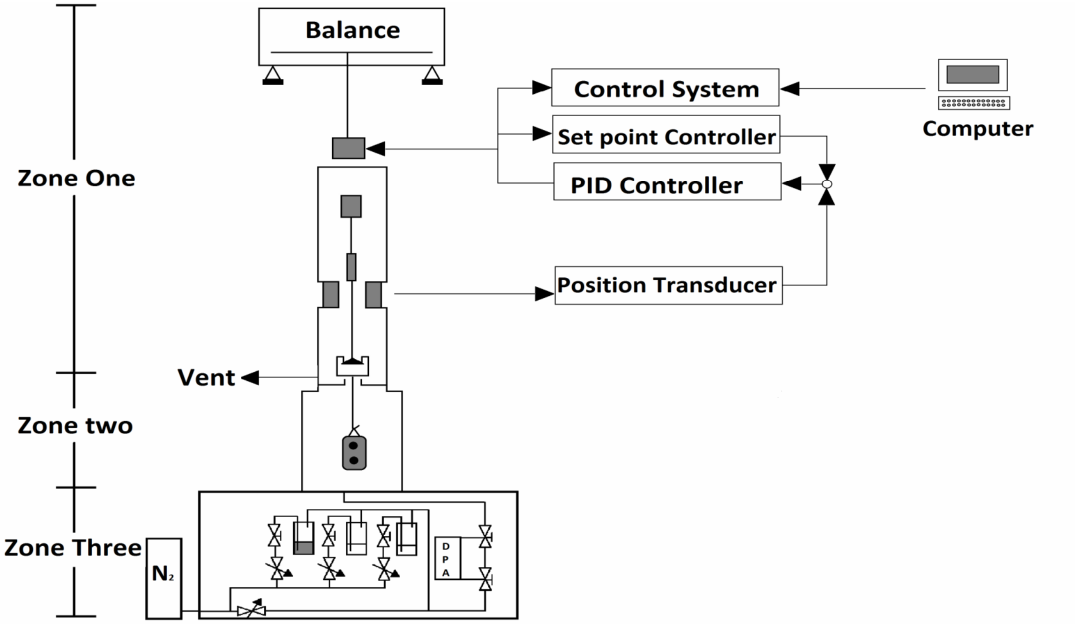


Figure 3.4: Three different zones of the magnetic suspension electro-balance with schematic of gas flow.

Zone One- Weighing Mechanism

The magnetic suspension electro-balance is the heart of the instrument. The main difficulty when using conventional gravimetric instruments is the direct connection of the measuring cell (sample atmosphere) and the weighing instrument due to which the balance can be damaged or disturbed by the measuring atmosphere Whereas by using a magnetic suspension balance, it is possible to weigh samples contactless under nearly all environments. In this device, the suspension magnet which has different parts, a permanent magnet, a sensor core and a device for coupling is linked with the sample hook [9]. The electromagnet, which is attached to the bottom of the balance, maintains a freely suspended state of the suspension magnet via an electronic control unit.

The controlled suspended state is achieved by means of a direct analogous control circle (PID controller and position transducer). The controller and transducer modulate the voltage on the electromagnet in such a way that the suspension magnet is held constantly in a vertical position. A digital set point controller allows various positions of the suspension magnet, i.e. setting the balance to zero and to the measuring position [10]. In the zero position only the weight of the suspension magnet is transmitted to the balance whereas in the measuring position the sample is weighed.

Zone Two- Sample Chamber

The sample chamber has dimensions of 7.5 cm × 7.5 cm × 14 cm (L×W×H) and it can hold a sample weighing up to 10 g. The chamber can withstand a temperature between 2℃ to 150℃. Thermostat fluid is continuously pumped into the double-walled thermostat tubes, which completely surround the sample chamber and suspension balance, so that a constant temperature can be maintained [10]. The temperature of the sample chamber and balance neck are controlled and maintained by two separated bath. To reduce lose of heat and to maintain better temperature control, ethylene glycol was used as a thermostat fluid.

The sample can be hung from the weighing hook. The chamber is tightly sealed so as to minimize the interference during measuring process. The presence of the vent in the chamber helps in maintaining constant water vapor in the chamber by discharging the excess vapors.

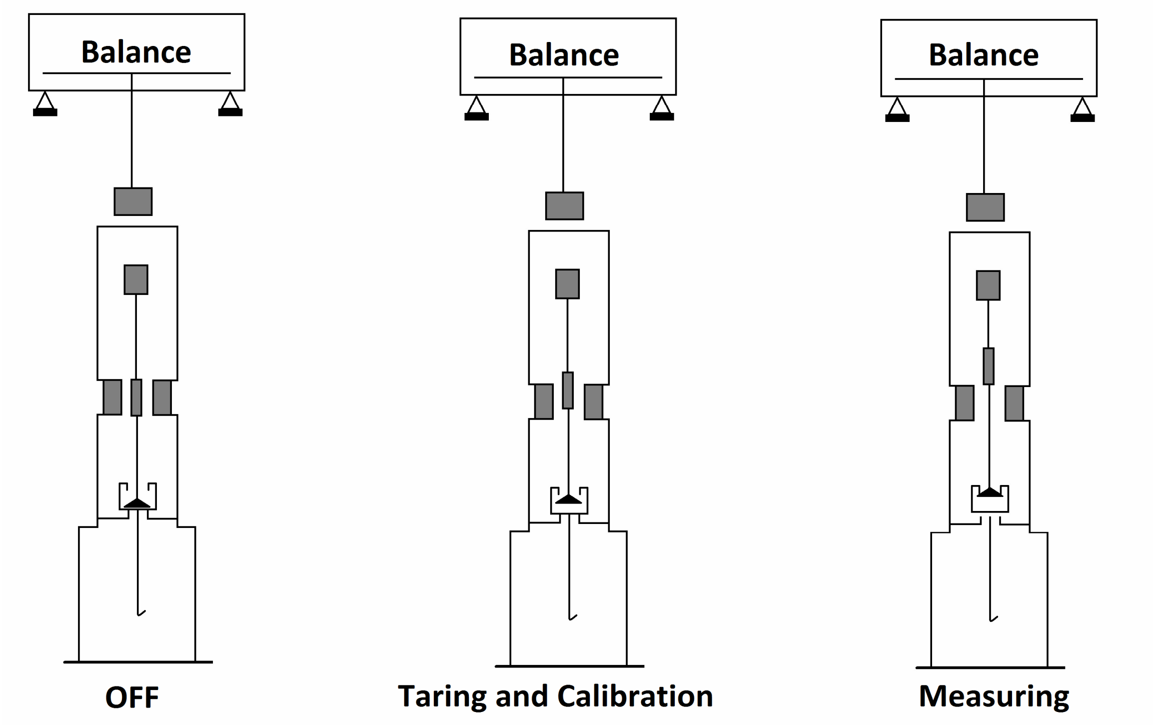


Figure 3.5: The coupling/decoupling action between the electromagnet and suspension magnet at different operating conditions.

Zone Three- Vapor Generator

This zone consists of main three components: mass flow controllers, vaporizers and chilled mirror dew point analyzer (DPA). The temperature of this zone is controlled separately and can be adjusted depending upon the permeant. For generating a particular relative humidity in the sample chamber, water vapor is generated by the vaporizer and mixed with nitrogen. DPA is a very useful device for checking the relative humidity of the water vapor. By the help of mass flow controllers and continuous analysis by the DPA, the mixing of the wet and dry stream of nitrogen is controlled, and the desired relative humidity can be attained.

3.3.2 Method

Quantification of Instrumental Noise

Calibration of the equipment is an important step for the quantification of noise. During a measurement there are two components that are present, signal and noise. Noise is the unwanted signal that can cause the information about the analyte to be undetectable. There are different types of noises that can be associated with an instrument, the first is white noise. White noise can be classified into two subcategories, thermal and shot noise. Thermal noise is generated due to the heating of the components where as shot noise is generated due to the carrying of the charge cross a junction in an electrical circuit. The second type of noise that is present with instruments is the flicker noise also known as drift. Drift is inversely proportional to the frequency of the signal [10, 11].

Quantification of the white noise and drift that were present in the magnetic suspension electro-balance was an important step. To implement this, a protocol was adopted, in which a standard stainless steel weight of 3 grams was exposed to 75% relative humidity at 23℃. Weight change of the standard stainless steel weight was recorded every 10 minutes for a period of 4,000 minutes.

Water Vapor Transmission Rate Experiment

Once the calibration was completed, the water vapor transmission rate experiment for blister packs was conducted. Due to the limitation of the size of the sample holder, for the experiment, 2 cavities were cut from the blister pack having 10 cavities. The first step was to determine the equilibrium time for the two cavity blister. Sample was suspended on the electro-balance hook and initial drying was carried out. During this process, nitrogen was purged through the chamber for 150 minutes at 27℃. Once the drying process was completed, the sample was exposed to 75% relative humidity at 23℃. The weight gain was recorded at every 10 minutes starting from time zero, untill the time there was no more weight gain by the sample for 2,000 min. Three replicates were carried out and from this, the equilibrium time for the control blister pack was determined.

The second step was to determine the WVTR of the blister pack. To measure the WVTR of the blister pack, the quasi-isostatic approach was adopted. In this approach, the blister packs containing desiccant are stored at high RH. This partial pressure differential provides a driving force for the permeation of moisture from the high concentration to the low concentration side [12]. During the steady state the desiccant absorbs the permeated moisture. Therefore the relative humidity inside the pack remains approximately zero, until the desiccant is completely saturated with moisture.

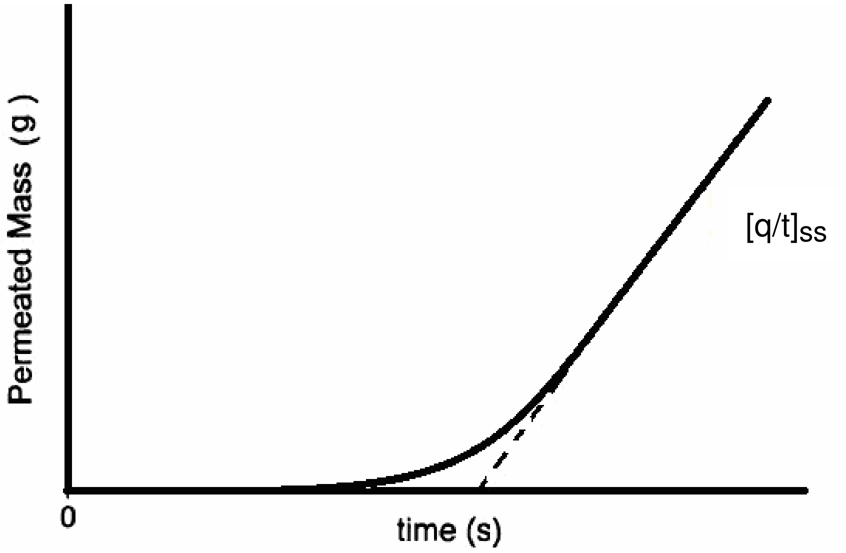


Figure 3.6: Quantity vs. time variation in a quasi-isostatic process.

As shown in the figure 3.6, the mass of the permeant in the polymer (initially zero) starts to slowly increase after some time. The permeation rate is the slope (q/t) at the steady state (figure 3.6)

$$P = \left[\frac{q}{t}\right]_{SS} \tag{3.6}$$

To measure the WVTR of the blister pack by magnetic suspension electro-balance, filled blister packs (with desiccant) having 2 cavities were used. The samples were exposed to an initial drying for 150 minutes at 27℃. Once drying was completed, the sample was exposed to 75% RH at 23℃. Based upon the time taken by the control blister to be in equilibrium, the filled blister was run for an extra 2,000 minutes or until the time it reached steady state. The slope of weight change vs. time after 11,500 minutes was used to determine the WVTR for the blister pack.

3.3.3 Statistical Analysis

Least Square Method for Regression

To evaluate the amplitude of noise and drift of the equipment, a least square method for regression was applied. The assumptions for the hypothesis testing of regression analysis are: [6]

I. There is a linear relationship between X (time) and Y (weight change).
II. The residual noise is normally distributed.
III. The variance of error is constant across different value of X (time).
IV. The errors are independent of each other.

The linear regression model for the experimental observation can be written as:

$$Y_i = B_0 + B_1X_i + e_i \qquad i = 1, 2, 3....n \tag{3.7}$$

where B_0 is the Y intercept and B_1 is the slope of the regression line.

Two Tailed T-Statistics for Unequal Variance and Unequal Sample Size

The unequal sample size and variance t-test was used to compare the water vapor transmission rate from the USP standard and the continuous gravimetric protocol.

It is applied when the two samples are not dependent and the standard deviation is not equal. The test is robust with sample size smaller than 30. It provides a test statistic for comparing the means of two independent samples for which the sample size and variance is unequal. The *t* statistic to test whether the population means are different can be calculated as follows: [13]

$$t = \frac{(\overline{X_1} - \overline{X_2}) - \mu_0}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \qquad (3.8)$$

with

$$d.f. = \frac{\left(s_1^2 / n_1 + s_2^2 / n_2 \right)^2}{\frac{\left(s_1^2 / n_1 \right)^2}{n_1 - 1} + \frac{\left(s_2^2 / n_2 \right)^2}{n_2 - 1}} \qquad (3.9)$$

where $\overline{X_s}$ is the mean of sample s, μ_0 is the hypothesized difference between sample means, n_s is the size of sample s, s_s^2 is the variance of sample s and d.f. is the degrees of freedom.

Pooled Variance

To compare the accuracy of the USP standard and the continuous gravimetric protocol, pooled variances from the two protocols were compared. Pooled variance is a method for estimating variance of several different samples where the mean may differ between samples but the true variance is assumed to remain the same. It is calculated by [14, 15]

$$s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \ldots + (n_k - 1)s_k^2}{n_1 + n_2 + \ldots + n_k - k} \tag{3.10}$$

where s_p^2 is the pooled variance, n_i is the sample size of the i^{th} sample, s_i^2 is the variance of the i^{th} sample, and k is the number of samples being combined.

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Chapter 4

RESULTS AND DISCUSSION

In order to measure the water vapor transmission rate (WVTR) of blister package commonly used in pharmaceutical products two different protocols were considered: one protocol included a continuous gravimetric method and the other is outlined by USP 671.

Commercially available blisters were used in this study. Tekni-Plex produced all the blisters from the same lot of raw materials (polymeric film for the cavity and the blister backing). All the blisters were produced the same day, in a single run closely monitoring and adjusting the environmental conditions when necessary. Since two different protocols were compared, the aim was to have minimal differences among the blisters.

In the USP 671 standard protocol it was required to test four blister samples with ten cavities each. For the experimental study, in each bucket eight samples were used. The purpose of using a higher number of samples than recommended by the USP protocol and doing six independent humidity buckets was to minimize the effect of variation among the blisters (possible changes in thickness of the polymeric cavity or variation on the sealing of the polymeric cavity with the backing etc) or chambers (hermetic seals of the buckets, etc.). Each bucket included control blisters or blisters that included desiccant. The blisters were suspended from the mesh placed on the top of the bucket to assure that each blister was surrounded by the specific environmental conditions. The relative humidity of the buckets were monitored and recorded throughout the experiment by using the data loggers in order to correlate any changes in the results with possible variations in the storage conditions of 23°C and 75% RH as required by the USP 671.

A continuous gravimetric protocol that included a magnetic suspension microbalance was used to determine the WVTR of the blister pack at USP condition. Due to the limitation of the size of the sample holder and its weight capacity, the sample size was reduced to 2 cavities per blister. The experiment was carried out three times with the empty blister and two times with the blister with desiccant. In each experimental run, the weight of the sample was recorded every 10 minutes. The gravimetric instrument automatically calibrates and re-zeros every 30 minutes in order to reduce the noise and increase the signal to noise ratio.

The WVTR from USP 671 and the continuous gravimetric protocol were determined. The outcomes from both protocols were statically analyzed and compared.

4.1 USP 671 Standard Protocol

The protocol is divided into two phases, first average weight gain by the control blister was determined and then the average weight gain profile of the blister with desiccant was obtained. From the data generated for the control blisters and filled blister with desiccant, the WVTR was determined.

Throughout both phases of the experiment it was observed that the opening and closing of the buckets was required to remove samples from the buckets for weighing. During this time, there was a drop in the relative humidity as the bucket tried to equilibrate itself with the outside atmosphere. This process was recorded by the relative humidity and temperature loggers. These loggers collected and recorded the temperature and humidity every 30 minutes for a period of 36 days. The data obtained from the logger was used to study the temperature and humidity conditions inside the bucket during the course of the experiment since this would influence the WVTR. In Figure 4.2 and 4.3, the humidity and temperature recorded during the course of the experiment is represented.

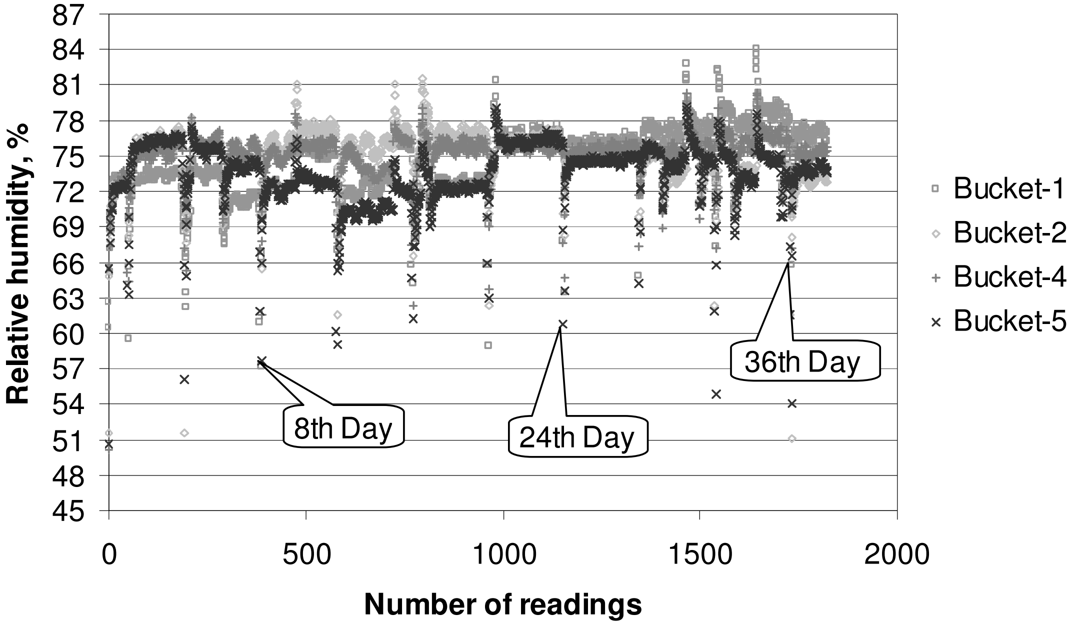

Figure 4.1: Relative humidity recorded at interval of 30 minutes for 36 days. The temperature and humidity loggers were placed in bucket 1, 2, 4 and 5.

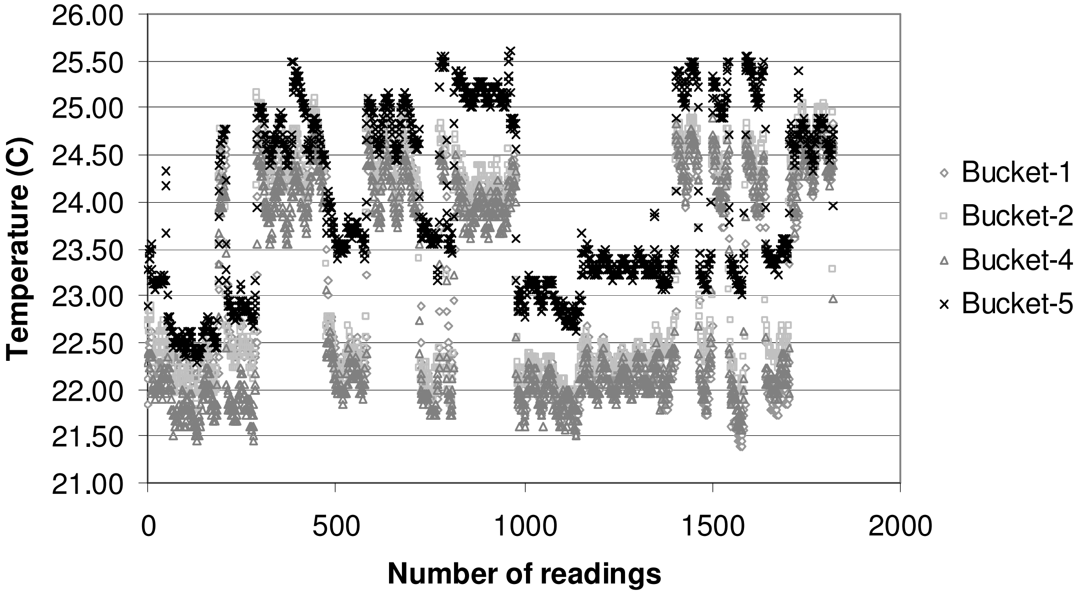

Figure 4.2: Temperature recorded at an interval of 30 minutes for 36 days. The results showed a close control over the temperature at 23 ± 2.5℃.

4.1.1 Average Weight Gain by the Control Blister Pack

To determine the moisture uptake by the blister found in the equilibrium state of it mass transfer process, the average weight gain of the control blister packs in the three buckets was determined. Eight control blister packs were stored in each bucket at 23℃ and 75% RH for 36 days. These eight blister packs in each bucket were treated as one single unit and were weighed as described by the protocol. The samples were weighed before placing them into the chamber, which was the day zero reading or the initial reading. The first reading was taken after 24 hrs, followed by consecutive readings after every four days.

To analyze the experimental data and to obtain the time of equilibrium, the kernel smoother statistical technique was adopted. This technique is used to represent irregular data points as a smooth line or surface [1]. As shown in Table 4.1, in this technique the weight of the blister pack between two consecutive readings was subtracted, resulting in a new set of data showing the weight gain between consecutive readings. If the average weight gain for three consecutive readings was ≤ 0.0001g, the blister pack was considered to be in equilibrium.

Table 4.1: Description of Kernel smoother statistical technique for bucket-1

Time (days)	Weight recorded for eight blister packs (grams)	Weight of one blister pack (grams)	Weight gain (grams)	Average weight gain for three consecutive readings (grams)
0	22.9750	2.8719	0.0000	
1	23.0126	2.8766	0.0047	0.0016
4	23.0124	2.8766	0.0000	0.0020
8	23.0220	2.8778	0.0012	0.0005
12	23.0235	2.8779	0.0002	0.0006
16	23.0261	2.8783	0.0003	0.0000
20	23.0230	2.8779	-0.0004	0.0001
24	23.0256	2.8782	0.0003	0.0000
28	23.0265	2.8783	0.0001	0.0001
32	23.0245	2.8781	-0.0002	0.0000
36	23.0247	2.8781	0.0000	

Using the kernel smoother statistical technique, the data from three humidity buckets (bucket 1, 2 and 3) were analyzed and the equilibrium time was calculated to be sixteen days.

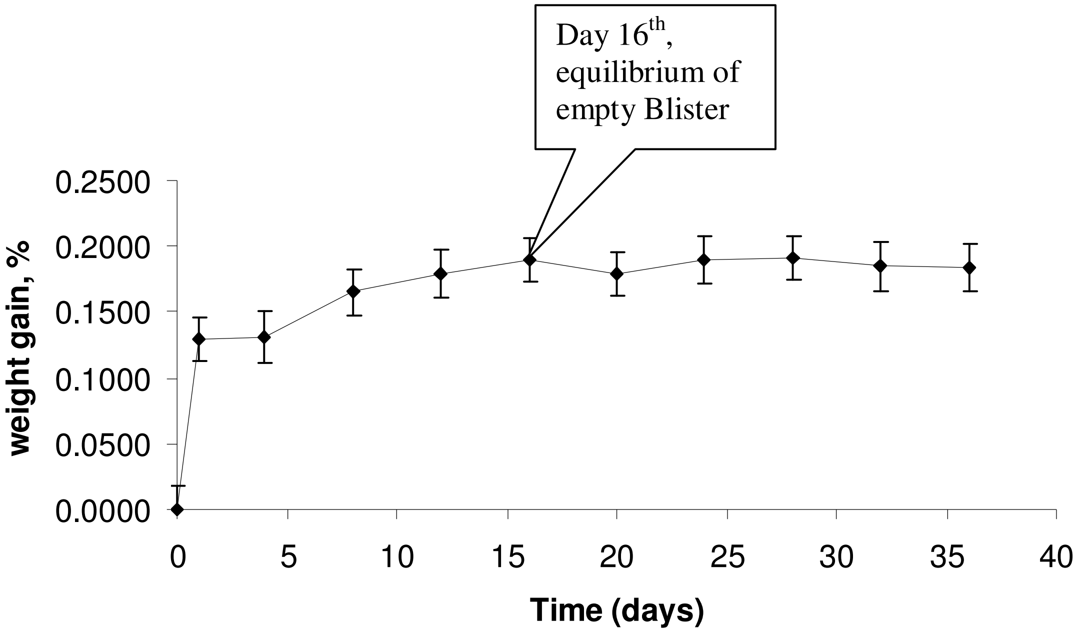

Figure 4.3: Percentage weight gain by the empty blister packs at 23℃ and 75%RH for 36 days with the standard error for three buckets.

The control blister did not gain additional moisture after 16 days as shown in Figure 4.3. The total moisture gain by the empty blister was determined by averaging the weights of the blisters in each bucket after the 16th day and this value was subtracted from the average initial weight of the blisters. This resulted in the average weight gain by an empty blister pack per bucket. The final weight gain by the control blister was determined by averaging the results of the three Buckets. The average weight gain by the blister pack with ten cavities was 5.30×10^{-3} grams with a standard error of 5.20×10^{-4}.

4.1.2 Determination of WVTR for the Blister Pack

The filled blister packs (blisters with desiccant) were exposed to 23°C and 75%RH in three buckets. The weight of each of the blister packs with desiccant was measured initially before they were placed in the buckets. The first weighing was done after 24 hrs and after that every four days, until 36 days was reached, as required by the protocol. The percentage weight gain vs. time is shown in the figure 4.4.

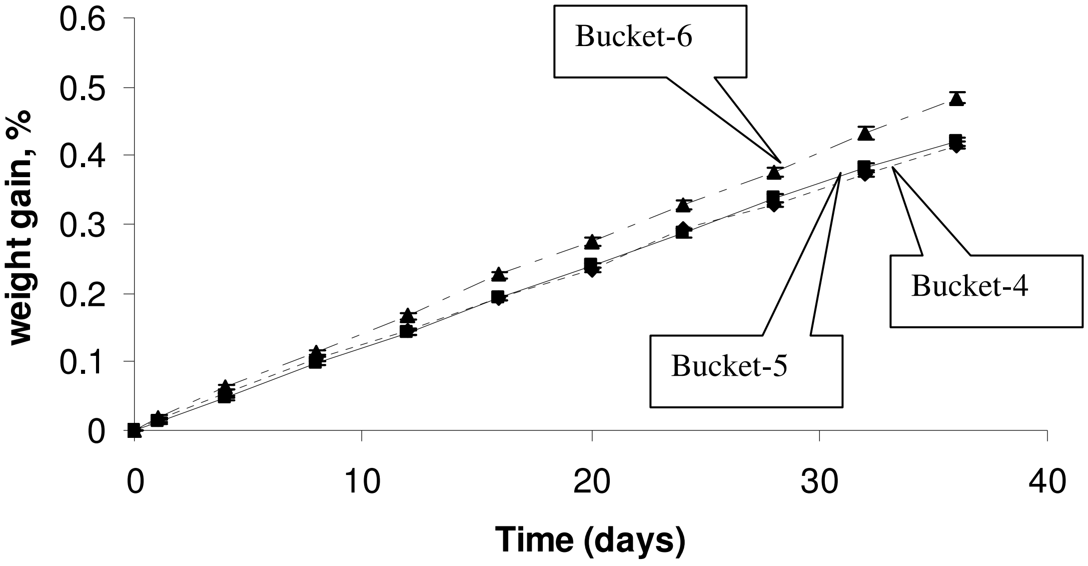

Figure 4.4: Percentage weight gain by the filled blister packs along with standard error of eight samples in three buckets at 23°C and 75%RH for 36 days.

After 36 days, the WVTR for each of the blister pack in each bucket was calculated as follow:[2]

$$\frac{1}{(N \times X)} \times [(W_f - W_i) - (C_f - C_i)] \tag{4.1}$$

N = 36 days

X = 10 cavities per blister pack

W_f = weight of filled blister on 36th day

W_i = weight of empty blister in the beginning of the experiment

$C_f – C_i$ = 0.0053 grams/blister

For each bucket average WVTR for the blister was calculated to be as follow (Table 4.2):

Table 4.2: The average WVTR and standard error for three buckets at 23°C and 75%RH with ANOVA results (means followed by the same letter are not significantly different, α=0.05)

Bucket	Average water vapor transmission rate (mg/day/blister)	Standard Error
4	4.16E-02 a	0.00050
5	4.25E-02 a	0.00093
6	5.10E-02 b	0.00091

A statistical technique, analysis of variance (ANOVA) was conducted to determine if there was a significant difference between the WVTR from three buckets (Table 4.2).

Bucket-6 shows significantly higher WVTR than other two buckets. The higher significant difference might be due to the fluctuation of the temperature and the relative humidity inside the bucket. All the eight samples in chamber-6 had higher transmission rate as compare to other two chambers.

4.2 Continuous Gravimetric Protocol

In this protocol, the water vapor transmission rate was determined under the same conditions of 23℃ and 75%RH as outlined by the USP 671. The new protocol was divided into three steps:

- Firstly the instrumental noise of the continuous gravimetric instrument was determined; this helped in setting the signal baseline
- Secondly the next step was to determine the equilibrium time of a control blister.
- Finally the WVTR was determined using filled blisters with desiccant.

4.2.1 Evaluation of Instrument Noise

There are two components that are present in an instrumental measurement, signal and noise. Noise is an unwanted signal that can originate from the fluctuation of voltage or due to the vibration from the other instruments. Theoretically when an analyte is missing, the instrument should not record any signal but in practice that's not what happens, the instrument records a random signal which can be positive or negative in nature. This makes it important to quantify the instrumental noise before starting an experiment [3].

To determine the instrumental noise for the experiment, three runs using the standard weight of 500mg were conducted. The standard weight was exposed to an atmospheric condition of 23℃ and 75%RH for 4,000 minutes. The percentage weight changes over time from three replicates were shown in Figure 4.5.

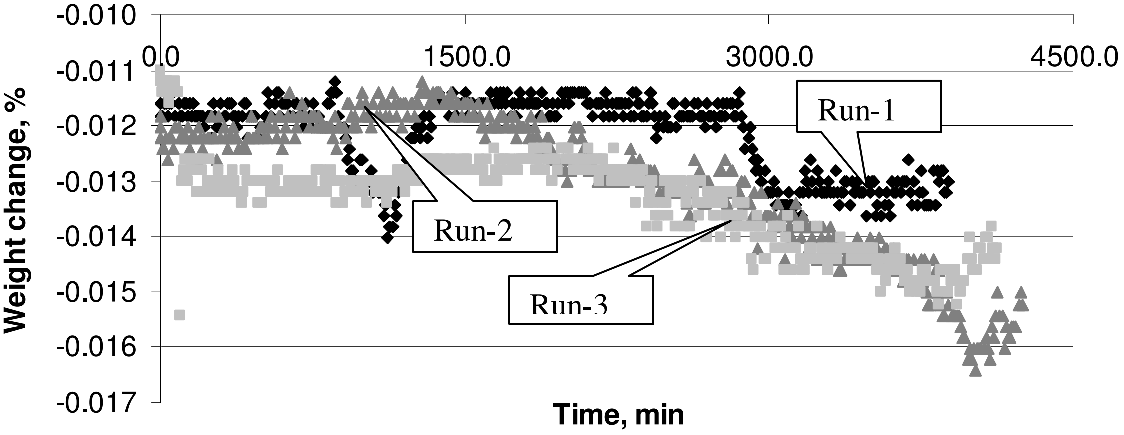

Figure 4.5: Percent weight change of standard weight (500mg) with a nitrogen flow at 23°C, 75%RH for a period of 4000 minutes for three separate runs.

The amplitude of noise and drift of the equipment was evaluated by adopting a least square method for regression. The assumptions for the hypothesis testing of regression analysis are:[4]

1. There is a linear relationship between X (time) and Y (weight change).
2. The residual noise is normally distributed.
3. The variance of error is constant across different value of X (time).
4. The errors are independent of each other.

The linear regression model for the experimental observation can be written as:

$$Y_i = B_0 + B_1X_i + e_i \qquad i = 1, 2, 3\ldots n \qquad (4.2)$$

where B_0 is the Y intercept and B_1 is the slope of the regression line.

The drift of the equipment is given by the slope of the regression line and the amplitude of the noise was obtained from the residual output as shown in the Figure 4.6.

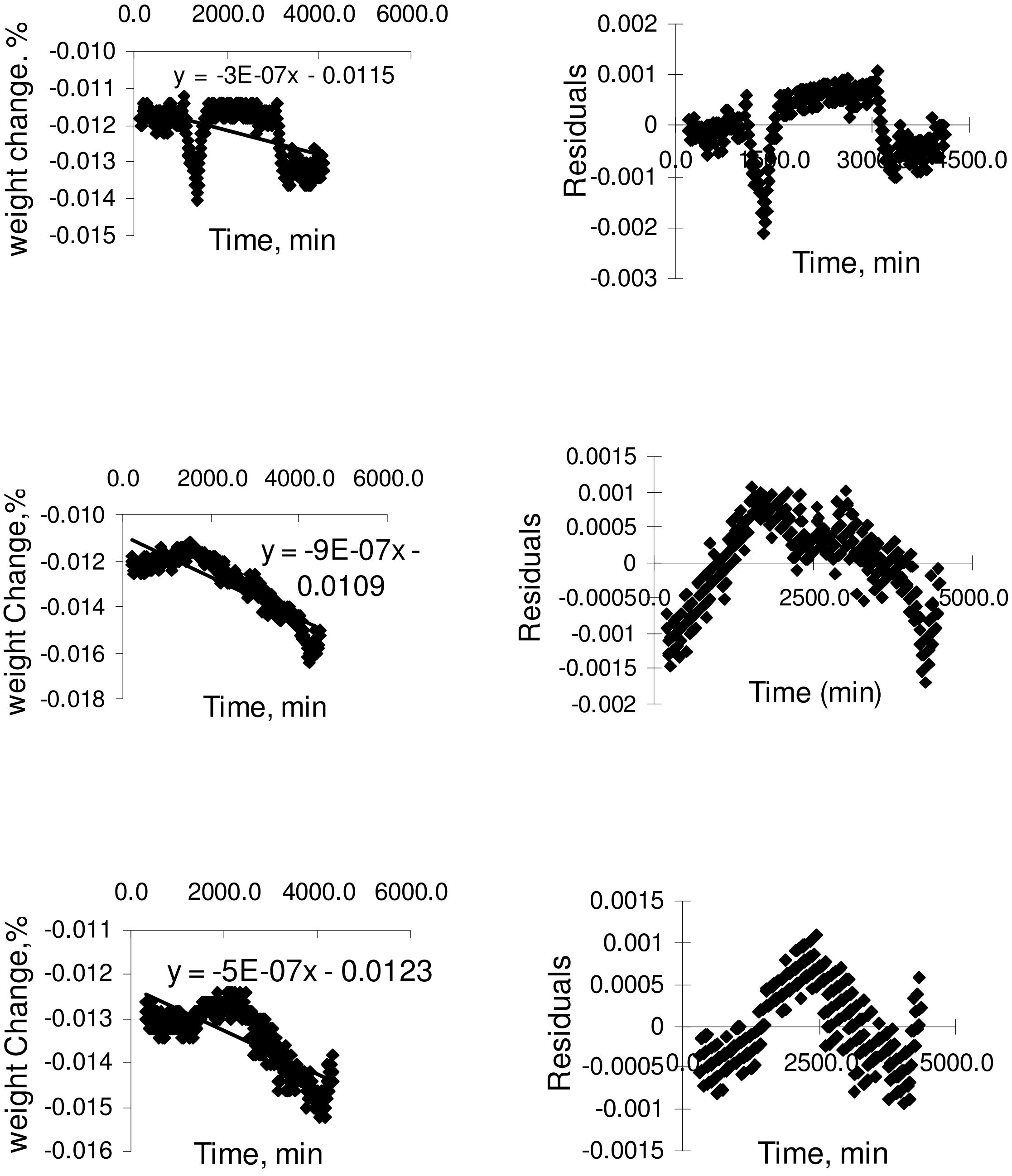

Figure 4.6: Quantification of noise for rubotherm SGA-100 gravimetric analyzer. Left column represents percentage weight change of the standard weight for 4,000 minutes. Right column represents the residual output corresponding to the experimental data.

Table 4.3 Regression analysis of the instrumental noise and drifting

Standard weight (500mg)	Regression line	ANOVA		Amplitude of Noise (3s) %	S/N
		MSE	Significant F		
Run-1	y = -3*10-7x-0.0115	3.57*10-7	3.68E-31	1.79E-03	2.79E+05
Run-2	y = -9*10-7x-0.7721	3.68*10-7	7.19E-139	1.82E-03	2.75E+05
Run-3	y = -5*10-7x-0.0123	2.17*10-7	2.68E-82	1.40E-03	3.58E+05

The residual output was obtained based on the empirical rule; y ± 3s contains 99.7% of the distribution. The amplitude of noise of the instrument was calculated as 3s. Thus the amplitude of noise lies between 0.00140% to 0.00182% for 500mg of standard weight at 23℃ and 75% RH.

The significant F values of all the repetitions were approximately zero, so we reject the hypothesis H_0: B_1= 0, thus the slope of the linear regression line is not zero and there was a drift present in the instrument.[5] In Table 4.3, high signal to noise ratio justifies that the instrument had a high sensitivity to a small change in weight.

4.2.2 Equilibrium Time for the Control Blister Pack

The aim here was to determine the equilibrium time for the control blister when exposed to 23℃ at 75%RH. The weight change of the control blister was continuously recorded for 14,000 minutes. The equilibrium condition was set to be percentage weight change of 0.0001% for 30 minutes.

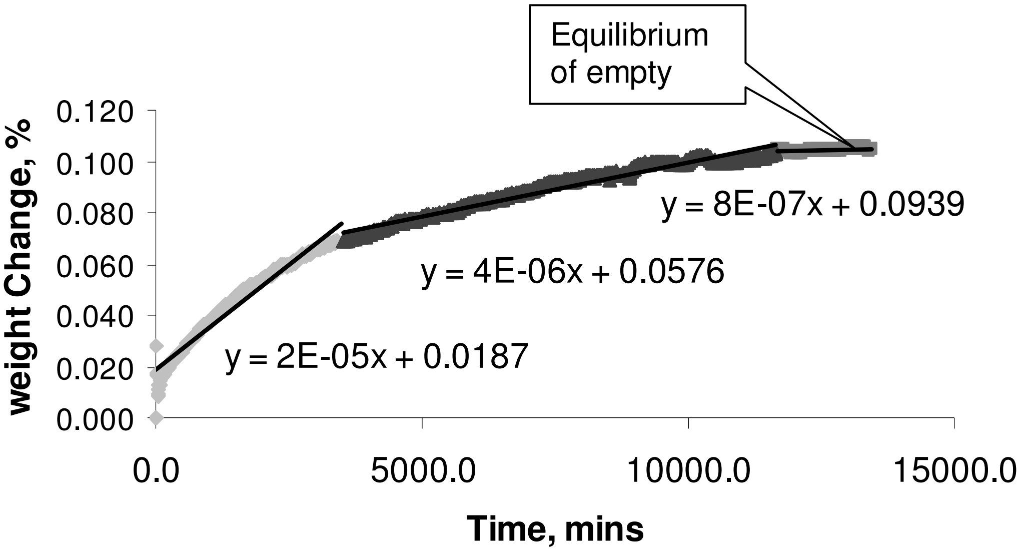

Figure 4.7: Percent weight gain of the control blisters at 23℃, 75% RH for 14,000 minutes.

In Figure 4.7, the slope of percentage weight change vs. time exhibited a linear characteristic, for first 3,500 minutes slope was 2 x 10^{-5} and then it went down to 4 x 10^{-6} for next 8,500 minutes. Final after 11,500 minutes the slope was 8 x 10^{-7} which was with in the noise level of instrument. At steady state, the slope was due to the drift of the instrument and the average equilibrium time was determined to be 11,500 minutes.

4.2.3 Determination of WVTR for the Blister Pack

The determination of WVTR for the blister packs was carried out by using blister pack samples with desiccant and the weight was continuously recorded. The control blister reached equilibrium at 11,500 minutes therefore the steady state of the blister package was beyond that time, since the desiccant absorbed the moisture permeated through the blister and kept at 0%RH. After 11,500 minutes, the slope of regression line for weight gain per unit time represents the WVTR of the blister pack with two cavities (shown in Figure B4 and B5). By dividing this transmission rate with the number of cavities, the WVTR per unit time per unit blister was obtained. The WVTR of each run of the filled blister was shown in table 4.4. The average WVTR from two blister samples was obtained to be 0.0652 mg/day/blister with a standard error of 0.0067. The percentage weight gain by the desiccant can be represented as shown in figure 4.8.

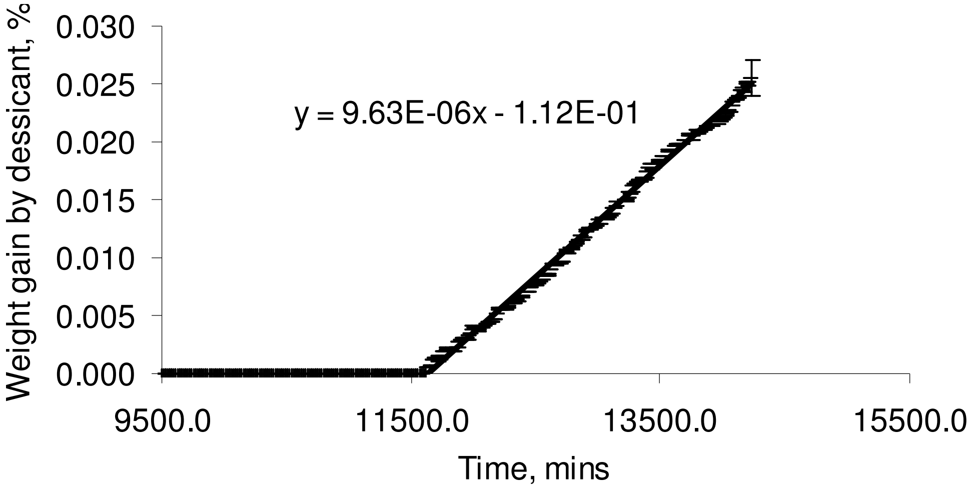

Figure 4.8: Percentage weight gain by the desiccant at 23°C and 75% RH, along with the standard error that was present for the two samples.

Table 4.4: Water vapor transmission rates in two filled blister packs

Sample	Water Vapor Transmission Rate (mg/day/blister)
Run-1	0.0719
Run-2	0.0585
Average	0.0652
Std. Error	0.0067

Temperature and Humidity Control in Continuous Gravimetric Protocol

In the Figure 4.9 and 4.10, the temperature and humidity data for the continuous gravimetric protocol was observed. During the experiment, the temperature and humidity were maintained at 23℃ and 75%RH. The temperature showed a fluctuation of ±0.5℃ and the relative humidity showed a fluctuation of ±1%RH. This pattern was observed in the temperature and humidity data collected for the standard weight, control blister pack and the filled blister packs.

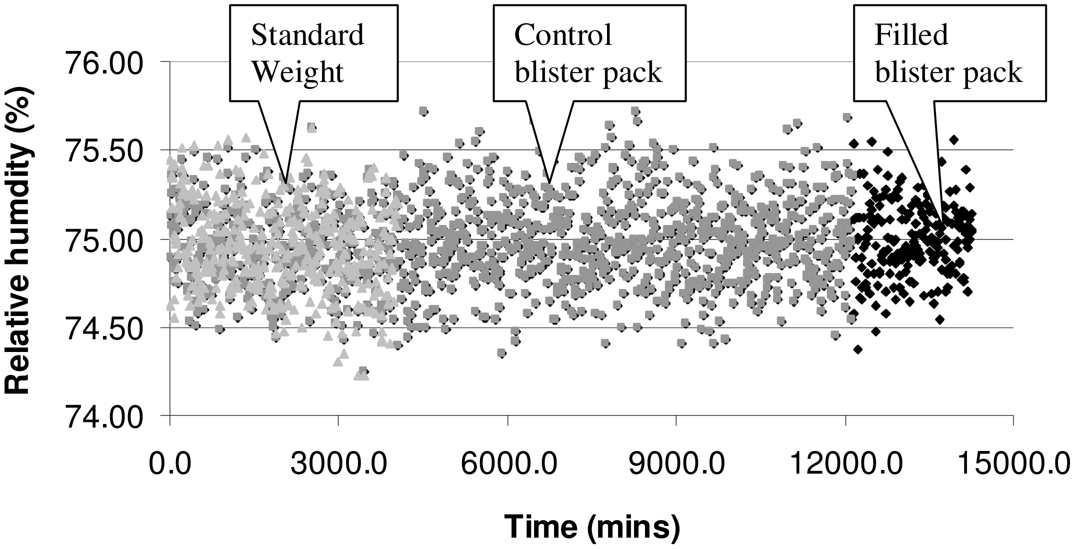

Figure 4.9: Average relative humidity recorded at an interval of 10min for a period standard weight, control pack and filled blister pack. The humidity was controlled at 75 ±1%RH.

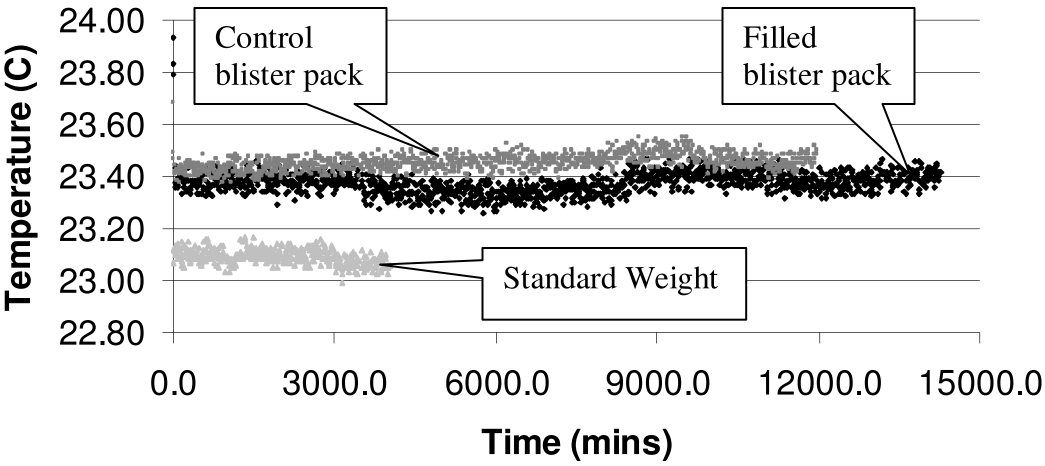

Figure 4.10: Average temperatures recorded at an interval of 10min for standard weight, control blister pack and filled blister pack. The results showed a control over the temperature at 23±0.5℃.

4.3 Comparison of the WVTR Obtained by the USP Standard and Continuous Gravimetric Protocol

To compare the WVTR, from USP standard and Continuous gravimetric protocol, two tailed t-statistic for unequal variance and unequal sample size was applied. The $|t_{calculate}|$ was smaller than $t_{(\alpha/2,\ df)}$, so it failed to reject the null hypothesis. Therefore a significant different was not observed between the WVTR by the two protocols.

4.4 Estimation of the Accuracy of USP Standard and Continuous Gravimetric Protocol

To determine the accuracy of the protocols, the pooled variance of the data points from two protocols were compared [6]. In USP, the variances between the percentage weights change on each day for 24 samples were calculated and finally an average variance was obtained by dividing the sum of the variances from each day with the number of readings. The pooled variance obtained for the USP standard was 4.99×10^{-4}.

In the same manner variance between the percentage weight changes for every 10 minutes was calculated for the two sample runs and finally a pooled variance was obtained by dividing the sum of variances with the number of data points and it was determined to be 2.13×10^{-5}.

Continuous gravimetric instrument showed a lower pooled variance than the USP standard protocol. Hence the continuous gravimetric instrument had a higher accuracy as compare to USP standard protocol.

4.5 Recommended Changes for the USP Standard Protocol

In a permeation process, the moisture will be first absorbed by the blister pack. Initially the permeation process will be in unsteady state; during this stage the permeant will diffuse and get solubilized within the polymer matrix. Once the permeant has completely solubilized and diffused in the polymer matrix, the permeation process reaches the steady state and the WVTR is determined during this state. [7]

In the case of USP standard protocol, in equation 4.1, N represents the number of days from the beginning. The suggested change to the USP standard is to use N as number of days after the control blister pack had reached equilibrium. This would result in the determination of the WVTR in the steady state and will eliminate the error that was present in the USP standard protocol which includes the unsteady state during the calculation.

Other then changing the number of days (N) for the WVTR determination, recommended changes to the USP standard protocol are as following:

- The USP protocol should outline the use of temperature and humidity sensors in the chambers throughout the experiment. This will help in monitoring of the temperature and humidity in the chambers, since these parameters have a great impact on the WVTR.
- The goal of the experiment is to expose the sample to 75%RH continuously and to achieve this it is important to maintain the chamber size so as to reduce the time necessary for chamber equilibrium to the specific RH of 75%. Longer time for the equilibrium of the bucket can impact the WVTR values.
- It is required that the samples are totally surrounded by the experimental environmental condition and they are not stacked upon each other as this can affect the experimental study. The USP 671 protocol should outline how the samples need to be placed in the chamber.

By applying suggested changes to the USP standard protocol calculation for WVTR was done. The regression analysis was applied to the weight gain by the filled blister back for the last twenty days and WVTR were determined to be as follows (Table 4.5).

Table 4.5: The average WVTR and standard error for three chambers at 23℃ and 75%RH with ANOVA results (means followed by the same letter are not significantly different, α=0.05)

Chamber	**Average water vapor transmission rate (mg/day/blister)**	**Standard error**
4	5.46E-02a	0.0006
5	5.66E-02a	0.0009
6	6.33E-02b	0.0008

By applying the changes to the USP standard protocol, average WVTR from three chambers was determined to 0.0581 mg/day/blister with a standard error of 0.0009.

In Figure 4.11, WVTR of the USP standard protocol, continuous gravimetric protocol and the USP standard protocol with changes were represented.

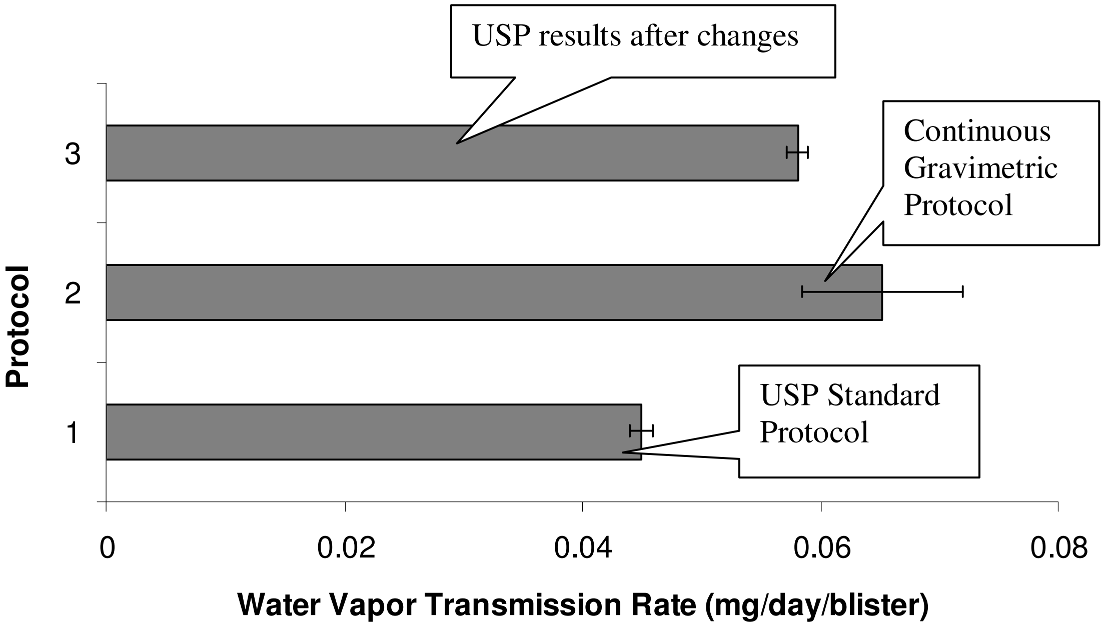

Figure 4.11: The WVTR of the blister pack by three different protocols with their standard error.

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Chapter 5

CONCLUSION

In this research, a comparative study was done between the USP standard and the continuous gravimetric protocol for the determination of the water vapor transmission rate in a blister pack. During the study, the temperature and relative humidity were 23℃ and 75%RH. Only one type of blister pack was used for this study. The main conclusions of this study can be summarized as follow:

- ✓ The temperature and humidity readings from both the protocols were analyzed and it was observed that for the USP standard protocol the temperature was controlled at 23℃ with a range of ±2.5℃. For the continuous gravimetric protocol, the temperature was controlled at 23℃ with a range of ±0.5℃. The humidity was maintained at 75% for this study. The USP standard protocol showed a drop in the relative humidity by more than 15% RH when the chamber was opened to get the sample out for measuring. The samples were exposed to a lower humidity for a certain period of time, which might had a significant impact on the WVTR of the package. Whereas, in continuous gravimetric protocol it was observed that the humidity control was well maintained at 75% RH with a range of ±1%. This proved that the continuous gravimetric instrument had a better control over temperature and humidity through out the experiment.
- ✓ In the USP standard protocol, the equilibrium moisture uptake by the control blister pack was determined to be 5.30×10^{-3} grams with a standard error of

5.20×10^{-4} and its time of equilibrium was 16 days. The WVTR for the blister pack was determined to be 0.0450 mg/day/blister with a standard error of 0.0010.

- ✓ In the continuous gravimetric protocol, the noise quantification was carried out. The drift of the instrument was identified in range of $-9*10^{-7}$ to $-3*10^{-7}$. The amplitude of noise was found to be in the range of 0.00140% to 0.00182%.
- ✓ The average equilibrium time for the control blister pack was determined to be 11,500 minutes from the continuous gravimetric protocol. The WVTR for the blister pack was found to be 0.0652 mg/day/blister with a standard error of 0.0067.
- ✓ The WVTR obtained by using USP standard and the continuous gravimetric protocol were statistically compared by:
  - t-statistic for unequal variance and unequal sample size: It was determined that the $|t_{calculate}|$ was smaller than $t_{(\alpha/2,\ df)}$, so it failed to reject the null hypothesis. Hence a significant difference was not observed between the WVTR obtained by two protocols.
  - Pooled variance: To understand the accuracy of the two protocols, the pooled variance was determined and compared. In case of USP standard protocol, the variance between the percentage weight gains on each day of reading was determined and finally a pooled variance was determined. The pooled variance for USP standard protocol was 4.99×10^{-4} and for

the continuous gravimetric protocol was determined to be 2.13×10^{-5}. Hence the continuous gravimetric protocol had a higher accuracy than the USP standard protocol, therefore making it more reliable technique when determining the WVTR in high barrier materials.

- ✓ According to the principles of the quasi-isostatic method, it is required that the WVTR for the blister pack to be calculated when the permeation process has reached steady state. For the USP 671 standard protocol, changes are recommended in the standard. It is recommended that for calculating WVTR, the numbers of days after the equilibrium of the control pack are considered. As it represents the time the permeation process was in steady state. The WVTR after applying the changes was determined to be 0.0581 mg/day/blister with a standard error of 0.0009.

Future Work

1. For this study, due to breakdown of the continuous gravimetric instrument, only two trails were completed for the filled blister pack and the average water vapor transmission rate based on these trails was compared. It is required to conduct more trails (at least three) for strong statistical analysis of the data.
2. The study was carrier for the USP standard protocol in which the atmospheric condition was set at 75%RH at 23℃. Further research needs to be done considering the environmental condition used by the other nations i.e. Asian conditions etc.

APPENDIX – A

USP STANDARD METHOD

Control Blister Pack

Table A1: Weight of eight samples in bucket 1, 2 and 3 for 36 days

Days	Recorded Weights (grams)		
	Chamber-1	Chamber-2	Chamber-3
0	22.9750	22.9478	22.7460
1	23.0126	22.9719	22.7734
4	23.0124	22.9719	22.7745
8	23.0220	22.9800	22.7803
12	23.0235	22.9828	22.7854
16	23.0261	22.9850	22.7877
20	23.0230	22.9828	22.7859
24	23.0256	22.9849	22.7887
28	23.0265	22.9848	22.7890
32	23.0245	22.9837	22.7873
36	23.0247	22.9831	22.7872

Bucket-4: Filled Blister pack

Table A2: Weight of each of the eight filled blister packs of bucket-4 for 36days

Days	Recorded Weights (grams)							
	F.1	F.2	F.3	F.4	F.5	F.6	F.7	F.8
0	4.8908	4.8780	4.8955	4.9012	4.8668	4.8656	4.9121	4.8849
1	4.8914	4.8793	4.8969	4.9012	4.8677	4.8658	4.9127	4.8859
4	4.8954	4.8804	4.8985	4.9029	4.8689	4.8673	4.9146	4.8876
8	4.8959	4.8834	4.9011	4.9057	4.8718	4.8701	4.9176	4.8899
12	4.8979	4.8852	4.9035	4.9070	4.8740	4.8724	4.9192	4.8920
16	4.9002	4.8874	4.9056	4.9095	4.8766	4.8748	4.9218	4.8943
20	4.9024	4.8888	4.9076	4.9121	4.8784	4.8769	4.9239	4.8963
24	4.9050	4.8927	4.9103	4.9147	4.8811	4.8800	4.9265	4.8989
28	4.9068	4.8935	4.9122	4.9160	4.8833	4.8817	4.9283	4.9013
32	4.9090	4.8959	4.9141	4.9183	4.8858	4.8838	4.9303	4.9036
36	4.9112	4.8986	4.9161	4.9205	4.8877	4.8854	4.9323	4.9053

Bucket-5: Filled Blister pack

Table A3: Weight of each of the eight filled blister packs of bucket-5 for 36days

Days	Recorded Weights (grams)							
	F.1	F.2	F.3	F.4	F.5	F.6	F.7	F.8
0	4.8940	4.9219	4.8743	4.9237	4.8766	4.8711	4.8802	4.8991
1	4.8940	4.9234	4.8746	4.9246	4.8768	4.8718	4.8809	4.8993
4	4.8957	4.9252	4.8765	4.9259	4.8789	4.8736	4.8825	4.9014
8	4.8987	4.9277	4.8785	4.9287	4.8812	4.8755	4.8848	4.9042
12	4.9005	4.9298	4.8808	4.9306	4.8836	4.8780	4.8873	4.9058
16	4.9033	4.9323	4.8835	4.9331	4.8859	4.8805	4.8896	4.9081
20	4.9057	4.9346	4.8855	4.9354	4.8887	4.8828	4.8917	4.9108
24	4.9074	4.9381	4.8885	4.9383	4.8901	4.8851	4.8934	4.9123
28	4.9106	4.9404	4.8905	4.9399	4.8929	4.8872	4.8968	4.9146
32	4.9127	4.9422	4.8931	4.9421	4.8954	4.8898	4.8984	4.9171
36	4.9140	4.9440	4.8940	4.9454	4.8975	4.8919	4.9003	4.9186

Bucket-6: Filled Blister Pack

Table A4: Weight of each of the eight filled blister packs of bucket-6 for 36days

Days	Recorded Weights (grams)							
	F.1	F.2	F.3	F.4	F.5	F.6	F.7	F.8
0	4.8654	4.8432	4.8539	4.9215	4.8752	4.8938	4.9098	4.9033
1	4.866	4.8442	4.8553	4.9228	4.876	4.8944	4.9109	4.9041
4	4.8686	4.8459	4.8575	4.9247	4.8784	4.8965	4.9125	4.9065
8	4.8708	4.8491	4.8600	4.9273	4.8810	4.8989	4.9145	4.9092
12	4.8736	4.8510	4.8627	4.9290	4.8836	4.9014	4.9177	4.9119
16	4.8770	4.8539	4.8655	4.9317	4.8866	4.9040	4.9206	4.9150
20	4.8794	4.8567	4.8676	4.9342	4.8892	4.9055	4.9229	4.9176
24	4.8821	4.8586	4.8706	4.9368	4.8918	4.9085	4.9253	4.9202
28	4.8849	4.8613	4.8728	4.9393	4.8936	4.9104	4.9278	4.9224
32	4.8877	4.8644	4.8762	4.9412	4.8971	4.9129	4.9303	4.9249
36	4.8896	4.8662	4.8783	4.9444	4.9001	4.9160	4.9331	4.9276

APPENDIX – B

CONTINUOUS GRAVIMETRIC METHOD

Control Blister Packs

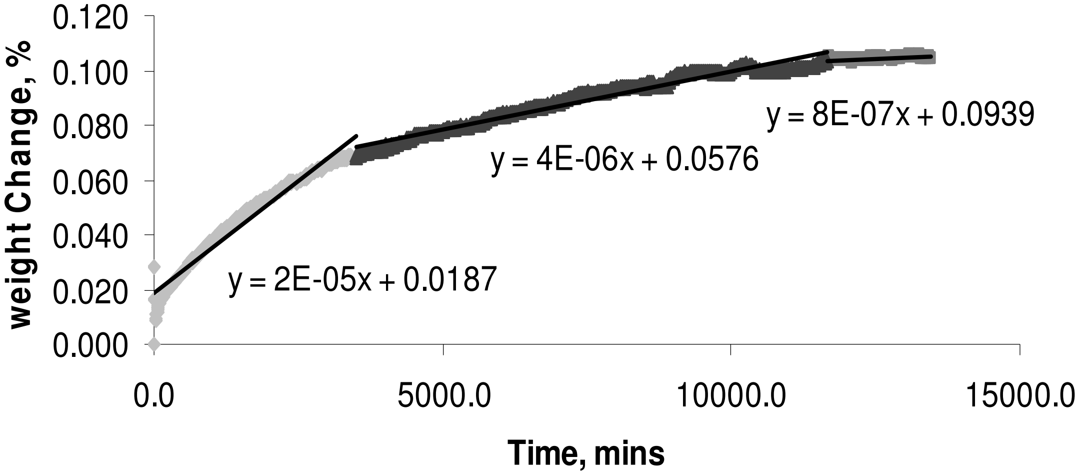

Figure B1: Percentage weight change by the 1st control blister pack with 2 cavities. The slope of the regression line after 11,500 minutes was within the noise level of the instrument and it represented the point of equilibrium for the control blister.

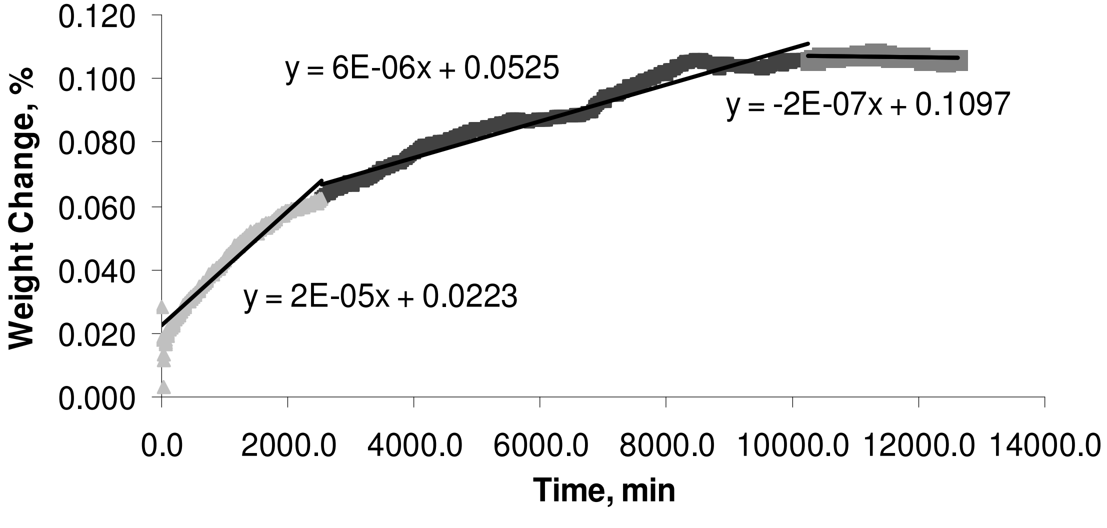

Figure B2: Percentage weight change by the 2nd control blister pack with 2 cavities. The slope of the regression line after 10,000 minutes was within the noise level of the instrument and it represented the point of equilibrium for the control blister.

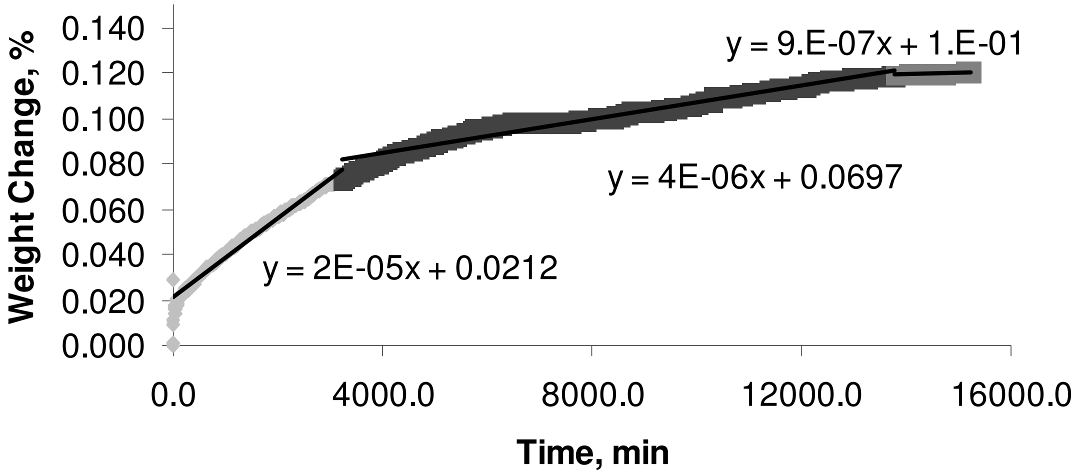

Figure B3: Percentage weight change by the 3rd control blister pack with 2 cavities. The slope of the regression line after 13,500 minutes was within the noise level of the instrument and it represented the point of equilibrium for the control blister.

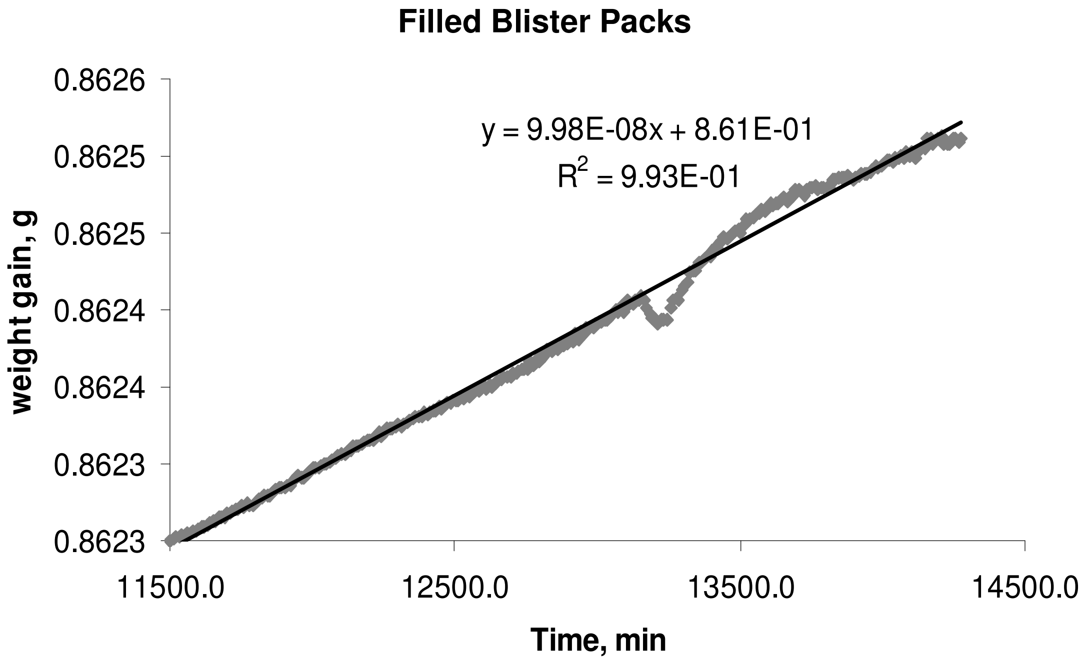

Figure B4: Weight gain of the 1st filled blister pack. The slope of the regression line after 11,500 minutes represents the WVTR of the blister pack with 2 cavities.

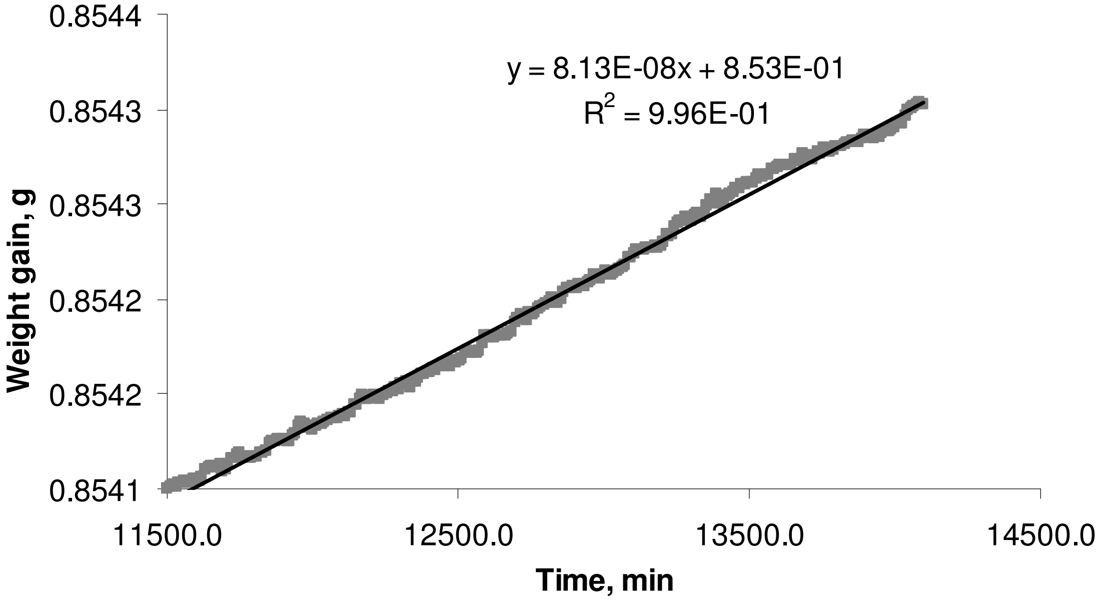

Figure B5: Weight gain of the 2nd filled blister pack. The slope of the regression line after 11,500 minutes represents the WVTR of the blister pack with 2 cavities.

Temperature and Humidity

Experimental Run of Standard Weight

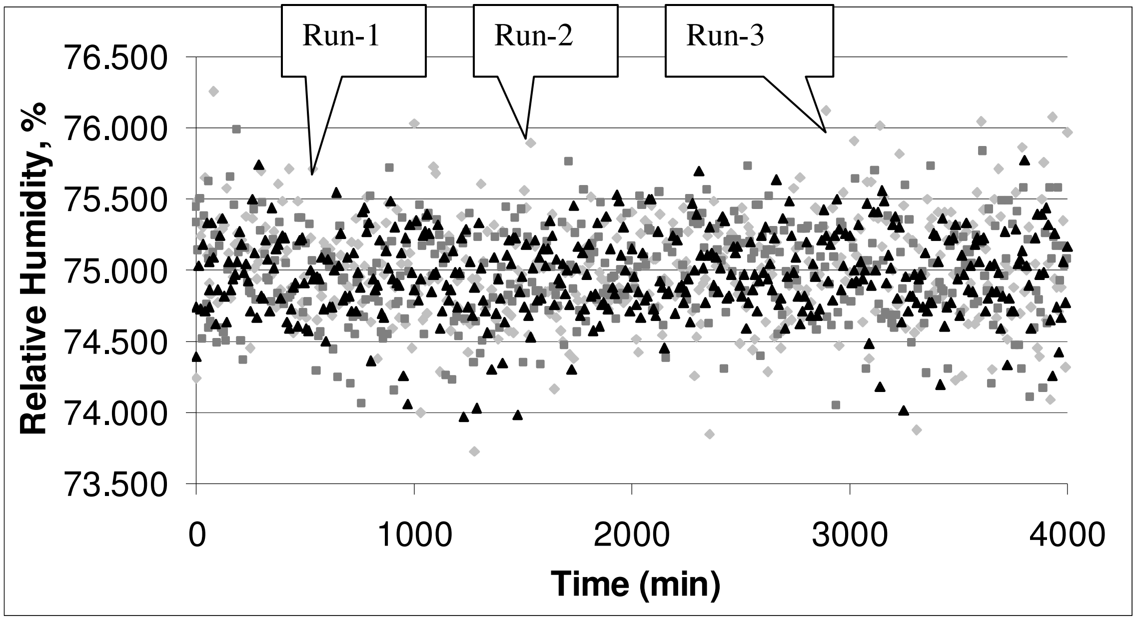

Figure B6: Relative humidity for 4,000 minutes for run-1, 2 and 3 of the standard weight.

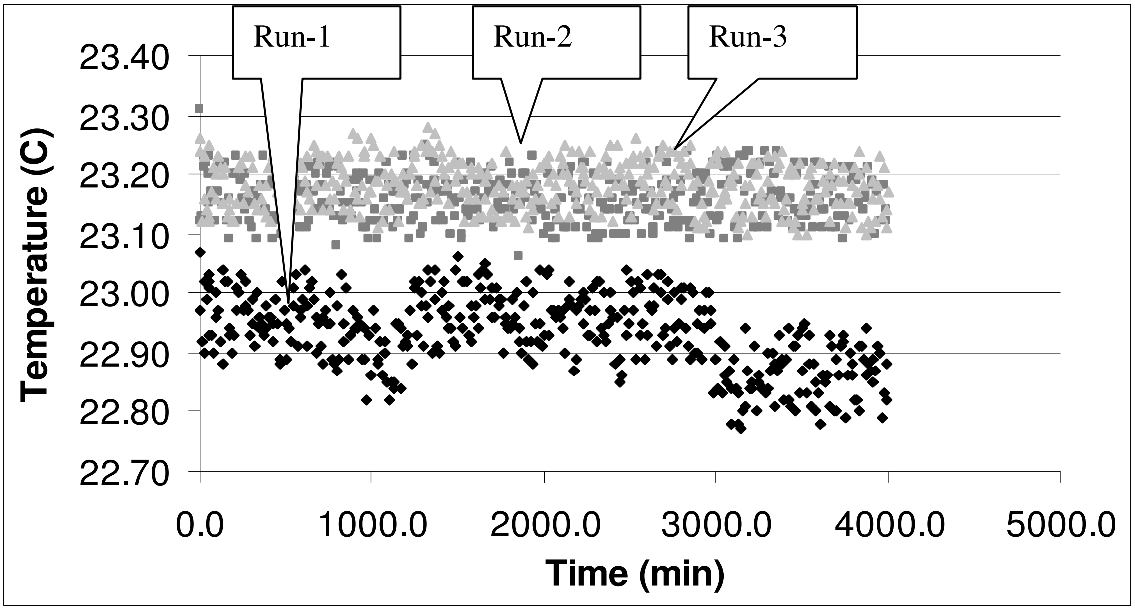

Figure B7: Temperature for 4,000 minutes for run-1, 2 and 3 of the standard weight

Experimental Run of Control Blister Pack

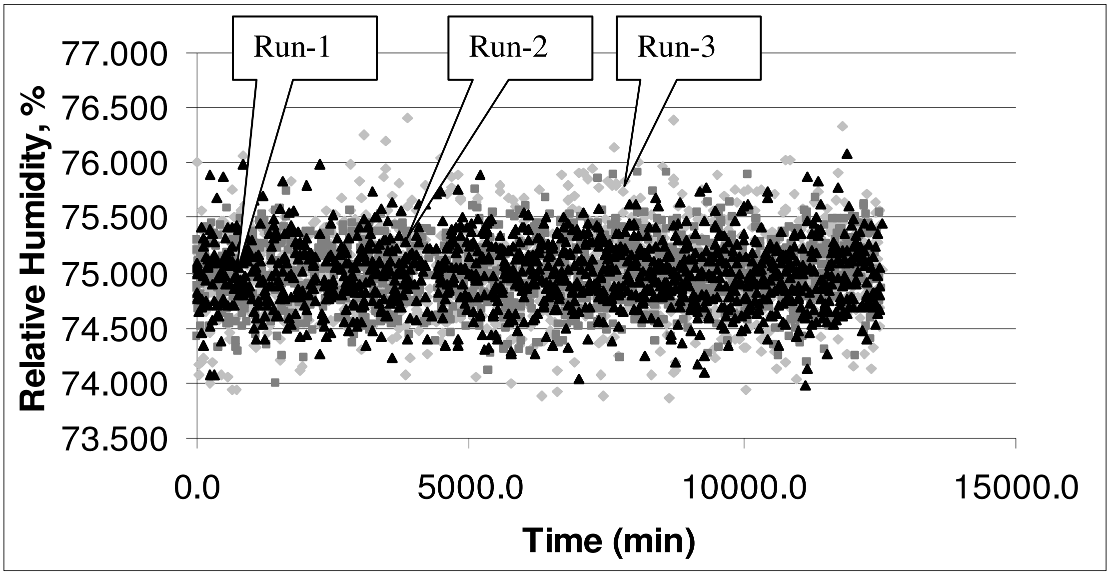

Figure B8: Relative humidity for 12,000 minutes for run-1, 2 and 3 of the control blister pack.

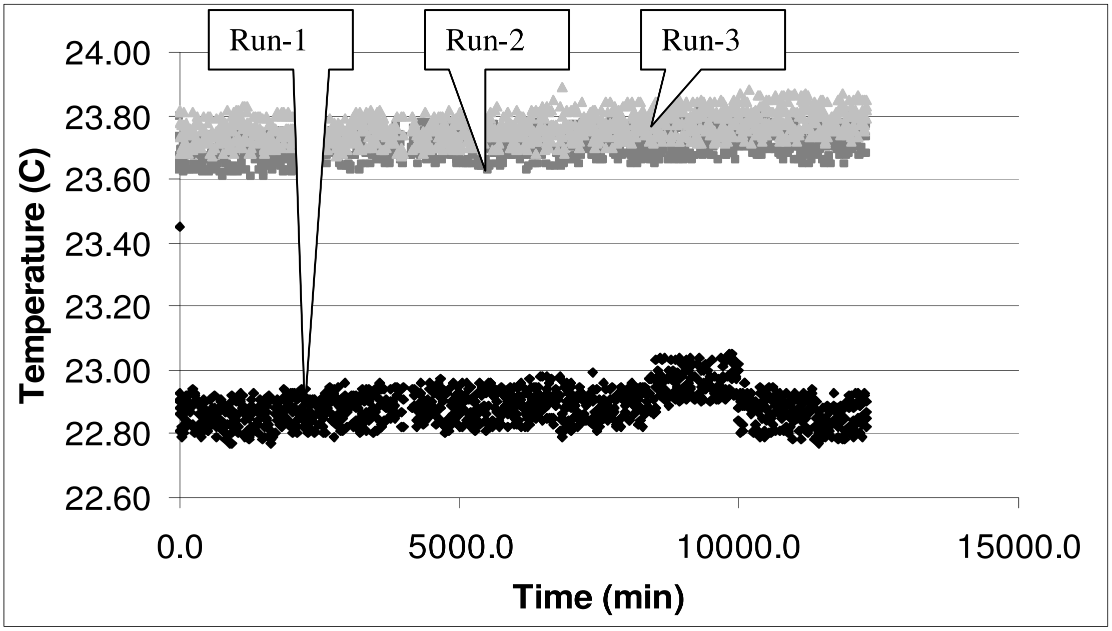

Figure B9: Temperature for 12,000 minutes for run-1, 2 and 3 of the control blister pack.

Experimental Run of Filled Blister Pack

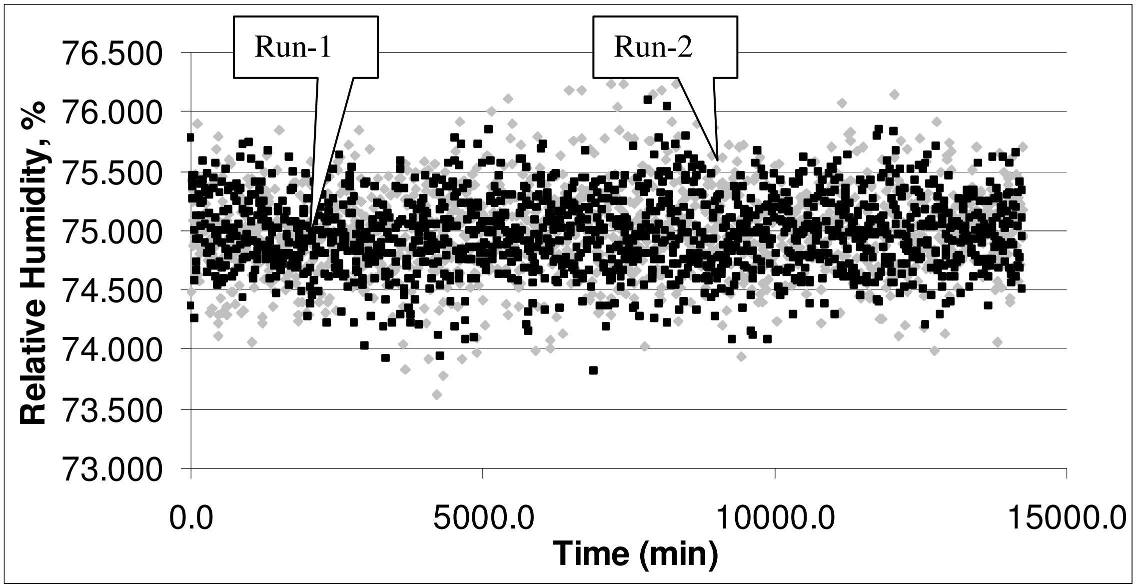

Figure B10: Relative humidity for 14,000 minutes for run-1 and 2 of the filled blister pack.

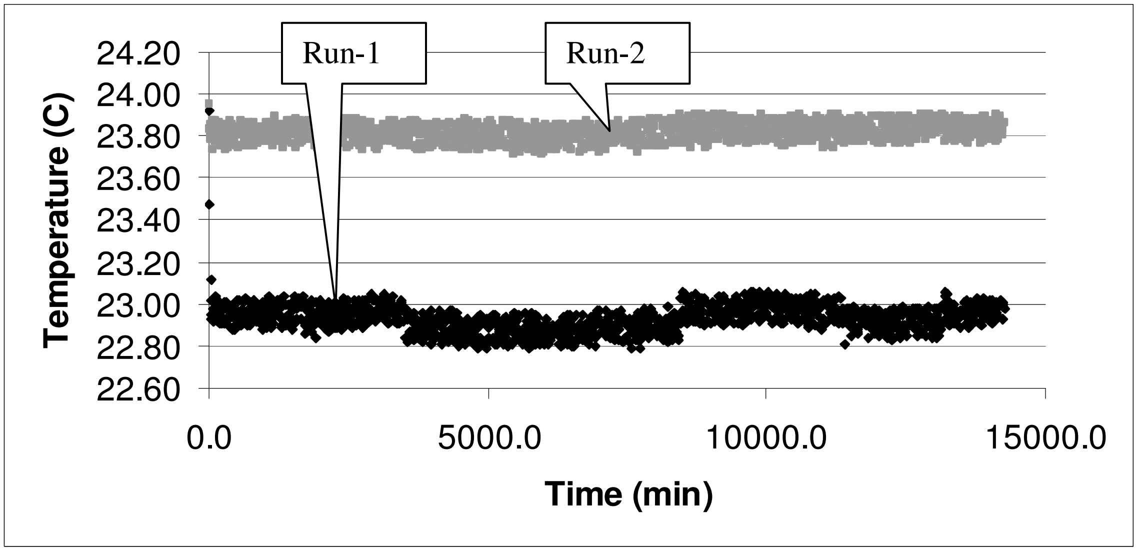

Figure B11: Temperature for 14,000 minutes for run-1 and 2 of the filled blister pack.