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A Study comparing the moisture barrier characteristics of flat liners versus stoppers in continuous thread closures on glass viles

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A STUDY COMPARING THE MOISTURE BARRIER CHARACTERISTICS OF FLAT LINERS VERSUS STOPPERS IN CONTINUOUS THREAD CLOSURES ON GLASS VIALS

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A STUDY COMPARING THE MOISTURE BARRIER CHARACTERISTICS OF FLAT LINERS VERSUS STOPPERS IN CONTINUOUS THREAD CLOSURES ON GLASS VIALS.

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William L. White, Author

DEDICATION

To my wife Donna

for her encouragement

and support.

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A particular acknowledgment is directed to The West Co., Phoenixville, Pennsylvania for their patience and the professionalism exhibited by their staff during the component development and production phases for both vials and closures for this project.

ABSTRACT OF THE THESIS

The combination of glass vial, elastomeric stopper, and aluminum crimp seal represents one of the principal packaging systems used to package diagnostic reagents, ophthalmic and veterinary medicines, and pharmaceutical products. This work states that elastomeric flat liners are an alternative to traditional stoppers. To confirm this position, several comparative moisture transmission evaluations are conducted to contrast the barrier performance of glued flat liners against stoppers in continuous thread closures on glass vials.

After a review of several standard packaging integrity testing methods, this thesis demonstrates the use of coulometric titration as an alternative package system testing methodology. Coulometric titrators are commercially available, and coulometric titration is well established as a precise moisture determination method for determining the micro-moisture contents in both solid and liquid products. The accuracy of coulometric titration technique is well suited for discerning the slight changes in moisture content of hygroscopic products after being packaged in candidate high moisture barrier packaging systems.

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CHAPTER 1

INTRODUCTION

Obtaining reliable measurements of sealing performance differences between various closure systems for products that contain hydrophilic pharmaceutical reagents is a difficult task. In the pharmaceutical reagent packaging industry sealed glass vials, heat seat sealed flexible high barrier thermoforms, and lidded high barrier thermoforms are the generally accepted packaging systems for protecting moisture sensitive products. Within these three broad packaging system categories, the glass vial sealed with either a fluted lypho or plug-style elastomer stopper has generally been accepted as the traditional gasketing material and/or sealing method of choice for hygroscopic products.

In addition to the traditional packaging gasketing options, there are now commercially available innovations, such as high moisture barrier flat liner materials, that can reduce the level of end-user involvement in pouring out or reconstituting granulated products. A major impediment to qualify new gasketing materials is the difficulty in accurately measuring total moisture transmission differences between candidate packaging systems.

The purpose of this paper is to examine and evaluate the feasibility of a fixed flat liner in a continuous thread (CT) polypropylene closure shell versus the use of elastomeric plugs and CT shells. The elimination of the traditional elastomeric plug in favor of a CT polypropylene closure shell presents two end-user advantages. First, a lined CT closure can reduce risk of product contamination or end-user contact when manually extracting the stopper. Second, most elastomeric plugs are held in place by an aluminum crimp closure on biological finish vials. There is the possibility of injury to end-users on the sharp edges created during the process of removing crimped seals. The overall safety of the packaging system is improved by replacing the crimped seals with one-piece lined CT closures.

The convenience and safety improvements contributed by the implementation of a flat liner closure system could be moderated by the potential negative effect on sealing performance. The primary function for most closures is to prevent product leakage out of the container. In this study, the closure's primary function is to minimize the amount of moisture vapor permeating into the package and reaching moisture sensitive "dry granulated" powder reagents. Moisture will adversely affect the pharmaceutical reagent's performance and can negatively affect the

perception of an end-user due to powder clumping and associated reconstitution problems.

This study was designed and conducted to demonstrate the performance differences between glass vials sealed with molded plugs versus vials sealed with high moisture barrier flat liner materials. As expected, the moisture transmission rates were difficult to distinguish between sealing systems using the "conventional" moisture transmission methods. To this problem, coulometric titration techniques were employed to exaggerate the amount of overall water vapor transmitted into the package over a period of time in both normal and stressed environments.

This study introduces and demonstrates the use of a micro-computerized coulometric titration instrument and commercially prepared coulometric Karl Fischer reagents as an additional packaging evaluation tool to sense micro-moisture changes in the candidate packaging systems. The coulometric titrator is designed to only measure extremely small amounts of moisture. This makes the coulometer ideal for measuring slight moisture changes in hygroscopic pharmaceutical reagent products with extremely low initial moisture contents. Surveying the published coulometric instrument specifications, many commercial titrators list moisture detection sensitivities as low as 0.1 μg H_2O (0.0000001 g/H_2O) with accuracy of ± 1 μg H_2O or ± 0.3% depending on water content ratio and sample size. The

accuracy of moisture determination results is enhanced by the composition of the coulometric Karl Fischer reagents utilized in the coulometric titrator.

CHAPTER 2

MATERIALS TESTED

Reagent Products

This paper will make continued references to diagnostic pharmaceutical reagent products. Pharmaceutical reagents are used by clinical chemistry laboratories to perform *in vitro* clinical analyses on human body fluids such as blood, urine, and other materials.[1] The body fluids or materials are combined with specific reagents designed to provide information for the diagnosis of disease or the general assessment of health. For example, some diagnostic reagents are designed to quantitatively measure a patient's level of cholesterol, glucose, urea, etc. The results are then interpreted by healthcare professionals to assist in the maintenance and treatment of medical problems.

The suitability and performance of the packaging components that contain reagent products are the critical elements in protecting product performance and indirectly insuring accurate patient results. Depending on the sensitivities of the packaged pharmaceutical reagents, products may be adversely affected by gases, light, and by

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[1]Anonymous, "Draft FDA Guidance to Manufacturer of IN VITRO Analytical Test Systems for Preparation of Premarket Submissions Implementing CLIA" U. S. Dept. of Health & Human Services, Food and Drug Adm., Drafted Dec. 17, 1992.

moisture vapor permeation.[2] This study focuses on the amount of atmospheric water vapor entering a sealed package, a process that can cause decreases in product performance related to oxidization (pH shifts), changes in the product's physical properties, discoloration, and most importantly, reductions in the active ingredients through a series of inadvertent, premature reactions among the components.[3]

The U. S. Pharmacopoeia XXI has defined the moisture permeation of containers. The lowest gas transmission category listed and optimal packaging level for the "dry" reagents is the hermetically sealed container. The hermetic container, by definition, is impervious to all gases under normal conditions. Using the hermetic container as an ideal, there are instances when it is necessary to abridge the packaging barrier performance for additional end-user features. The most frequently requested packaging modifications are to decrease the effort required in the opening of the container, to minimize the possibility of product to end-user contact, to facilitate pouring of the reagent, or to aid in the reconstitution of the product in

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[2]Ann M. Brennan, "Moisture barrier requirements for dry pharmaceutical products," Tappi Journal, (March 1992), 145-148.

[3]William D. Lakin, Ph.D., "Computer Aided Hermetic Package Design and Shelf Life Prediction," Journal of Packaging Technology 1, no. 3, (June 1987), 82-85.

the package. The general strategy is to introduce end-user packaging enhancements that will contribute to the product's differentiation within the diagnostic marketplace. Occasionally such packaging enhancements will diminish the performance of the "ideal" hermetically sealed package by a few tenths of a percentage (H_2O) but still not adversely affect overall product expectations or performance.

When developing or modifying dry reagent primary packaging, the manufacturers of pharmaceutical reagent products have a variety of commercially available packaging options that include molded glass, plastic containers, foil and composite material pouches, and thermoformed blisters. The reagent product's acceptable moisture tolerance levels are usually established during the product development phase. On the basis of stated moisture tolerances, potential candidate packaging materials and components can be selected or designed concurrently with the establishment of component evaluation protocols. Packaging selection criteria also balances the economics of in-house manufacturing capabilities and equipment along with material procurement costs, product compatibility, environmental concerns, end-user interactions, and the requirements of the diagnostic instrument, or, how the diagnostic instrument uses the pharmaceutical reagents.

For those reagents manufactured through freeze drying (lyophilization) or for blended dry powder reagents, the

traditional packaging system frequently selected is a glass (tubing or molded) container, an elastomeric gasketing stopper, and a crimped aluminum seal or continuous thread shell. When these components are correctly assembled, the reagent manufacturer can anticipate, with a high degree of confidence, that the stability of hygroscopic reagent products can be sustained. The extremely low moisture transmission rates at molecular levels will cause only slight increases in the reagent's moisture content over time, and this generally does not alter the "customary" shelf-life expectation, which normally ranges between 12 to 24 months.

Departures away from the conventional glass container, plug, and crimp seal for dry reagents has been restrained for good reasons. The pharmaceutical reagent industry has confidence in glass containers sealed with plugs. This is demonstrated by the multitude of successful product performance applications on diagnostic instruments that are now required by various Federal Food and Drug Administration applications and the CLIA[4] regulations. Excluding in-process manufacturing techniques, such as freeze drying that requires glass containers and plugs, the other primary motivation for using traditional packaging is rooted in the

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[4]Clinical Laboratory Improvement Amendments of 1988 (CLIA) (Public Law No. 100-578).

stringent institutionalized "in-house" or "organizational" reagent packaging performance evaluation specifications. The reagent manufacturer's extensive packaging experiences using glass containers sealed with stoppers has generated large volumes of satisfactory and comfortable barrier packaging performance data. The result is a narrow selection of commercially available, highly standardized catalog of glass "acceptable" barrier packaging systems that invite innovations by the industry.

When considering departures away from the traditional "cataloged" barrier glass components and sealing systems, the principal design consideration is to determine if the new features on glass containers can enhance the existing granulated reagent characteristics.

To successfully implement the "new" components that were developed from realistic design expectations, a general assessment of the capabilities and limitations of the proposed glass containers and closure manufacturing processes was completed for this study. Only packaging component manufacturers demonstrating capability and process controls were selected to participate in this project.

Engineered Sealing Surface

Before describing the specifications and details of candidate packaging components in this study, it is important to acknowledge the extensive research and

contributions of Dana Morton-Guazzo, Ph.D. regarding the sealing performance and mechanical characterizations of various elastomer gasketing materials on glass containers. Described in detail was the critical nature of compositional and dimensional component specifications along with the absence of critical defects in a parenteral packaging system. All of Dr. Guazzo's research was conducted using glass vials with serum finishes, elastomeric stoppers (plugs), and crimp seals. After examining all the potential sealing points, Dr. Guazzo concluded that at the contact points between the vial's land surface (flat horizontal plane) to the elastomer's flange under vertical compression was the only series of points (plane) that could be relied upon to "consistently" maintain product integrity.[5] This study builds upon these sealing observations to demonstrate the equivalency in sealing performance of flat barrier liners to elastomer stoppers thus reinforcing Dr. Guazzo's point of seal observations.

The "process control" on the part of the suppliers manufacturing pilot packaging components used in this study has been undocumented in this chapter. There were numerous trial runs to adjust and understand all manufacturing

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[5]Dana K. Morton-Guazzo, Ph.D., "Container/Closure Integrity of Parenteral Vials," Journal of Parenteral Science & Technology 41, no. 5, (September-October, 1987), 145-157.

processes before the final evaluation components were produced. The candidate components used in this study were manufactured to tighter tolerances than the current industry" accepted specifications as outlined by the Glass Container Manufacturers Institute (GCMI) or The Society of the Plastics Industry, Inc. (S.P.I.). Without the engineering design cooperation and excellent process controls exhibited by the West Company and Comar, Inc., the manufacturers of the glass vials and closure shells, this study would not have been possible.

Continuous Thread Shells & Materials

For evaluation uniformity all gasketing candidates (plugs or flat liners) were compressed and held on the vials using continuous thread shells. CT closures were selected based on the assumption that end-users in laboratories prefer the convenience of a CT closure versus the more traditional crimped aluminum seals. The CT closure provides a variety of downward vertical pressures on the gasketing material to effect the seal, however, the applied cap torque must be maintained (6 in. lbs. ± 1 in. lb.) and closely monitored during the filling and sealing stage of production.

All 18 mm closure shells in this study are made of white polypropylene and are produced by injection molding. The shells used in conjunction with the elastomeric plugs were

manufactured by Menshen in Germany (Figure 1); the shells with glued liners were manufactured by the West Company, Phoenixville, PA (Figure 2). A key dimension on the West print is the "E" 0.708" ± 0.003". Sample shells were measured and the "E" was within ± 0.708" ± 0.001". In a follow-up consultation with the West organization, it was acknowledged that the current process control of the injected molded shells routinely fell within the observed 0.001" tolerance.[6] This knowledge permitted tighter specifications to the vial's "T" dimensions.

Glass Vials

There are two USP[7] classified types of glass containers considered acceptable for a pharmaceutical reagent and liner project. USP Type I Borosilicate glass is customarily used for parenteral (injectable) pharmaceutical products, and USP Type II glass can be used to package large volume parenterals and sterile solids.[8] Both types of glass are commercially available with or without amber tints. American equivalent Type II amber glass in tubing is

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[6]Peter S. Sinnott, Manufacturing Engineering Manager, The West Company, Williamsport, PA, personal communication, March 19, 1993.

[7]"Containers," United States Pharmacopoeia, 21th rev., U.S. Pharmacopoeia Convention Inc., (1984), 1233-1235.

[8]Allen I. Kay, "The Selection and Evaluation of Package Components for Parenteral Products," Pharmaceutical Technology, (May 1982), 54-61.

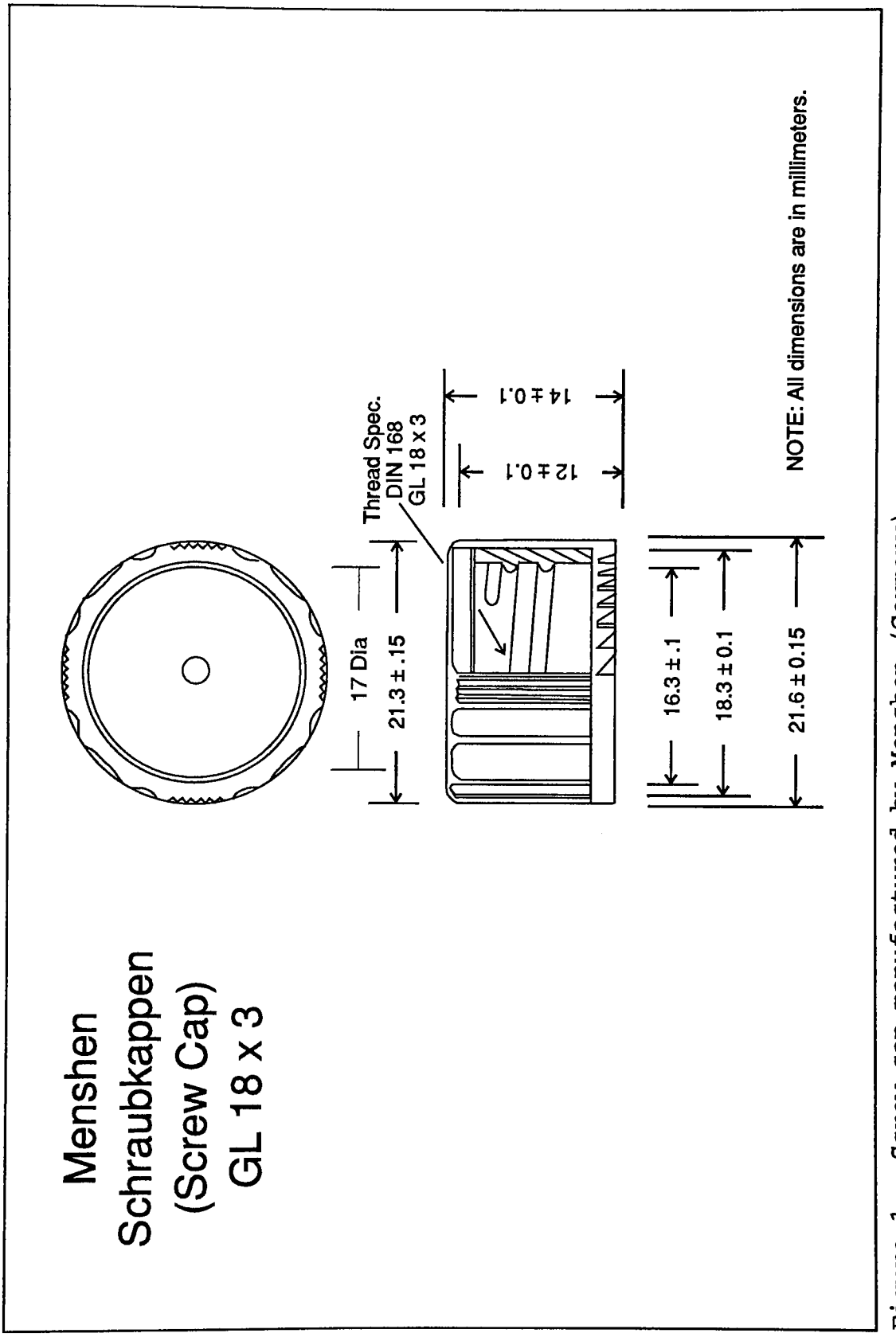

Figure 1. Screw cap manufactured by Menshen (Germany)

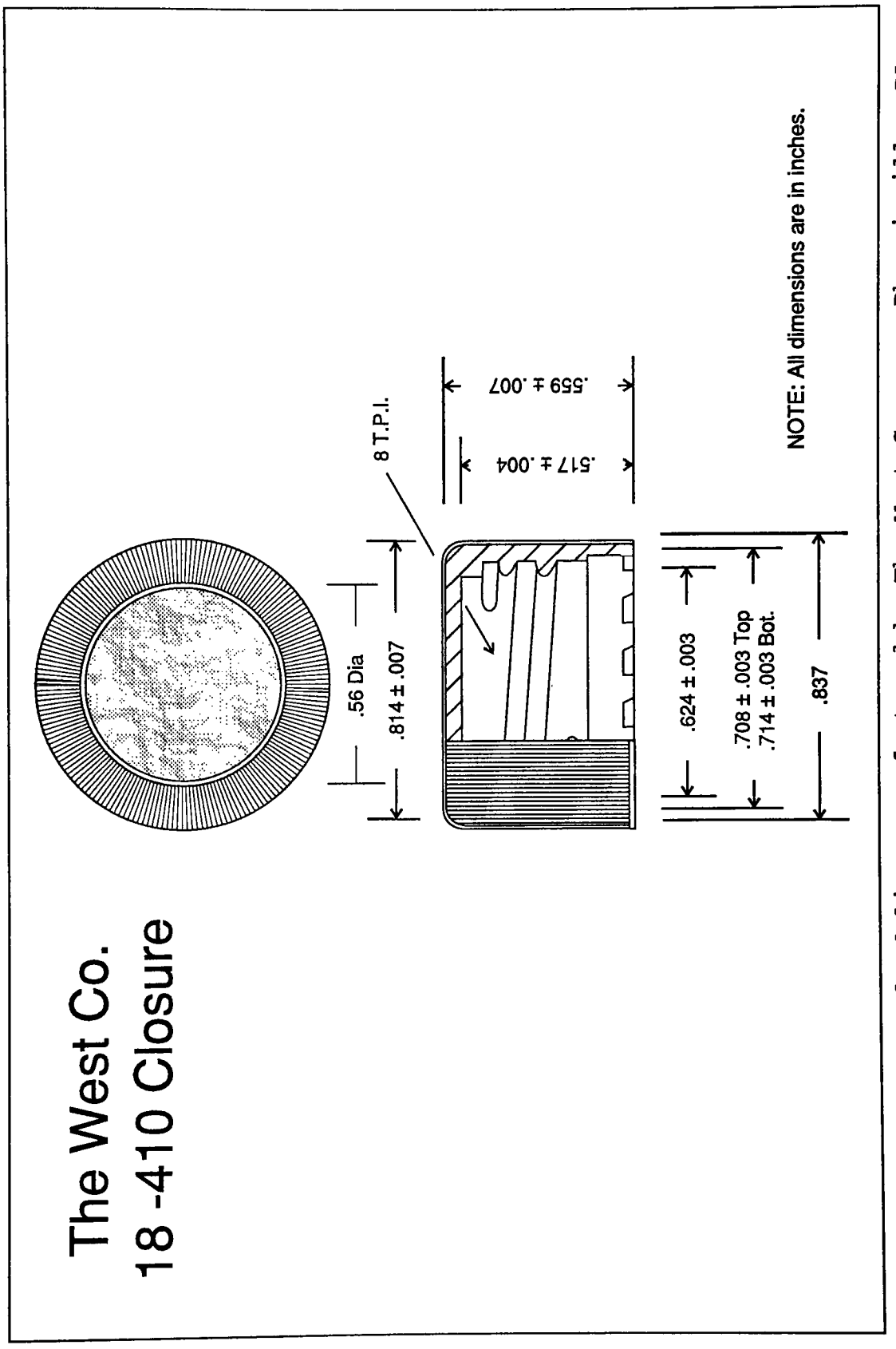

Figure 2. Shell and glued liner manufactured by The West Company, Phoenixville, PA.

commercially available, and in this case, imported from Schott Glass, Germany.

Generally, Type I glass is usually specified since it has a higher thermal resistance, low alkali content, and is free of heavy metals and zinc group elements.[9] The final selection of glass type used to manufacture the vials for "dry" chemistries is also contingent on the type of manufacturing process that is used. Due to the highly resistant nature of Borosilicate Type I glass, it usually passes the USP Powdered Glass Test[10] for alkali release. Type II glass can be qualified after passing the USP Water Attack Test,[11] but the Type II glass is not normally specified for dry reagents or controls manufactured employing lyophilization technologies. The freeze drying process facilitates and hastens the extraction of undesirable elements, such as alkali, from the glass. Alkali would cause an increase in the pH of the reagents. Critical to most diagnostic tests are reaction sensitivities to various levels or changes in pH[12] that would adversely

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[9]Norbert W. Tietz, Textbook of Clinical Chemistry, (Philadelphia, PA: W.B. Saunders Company 1986), 10.

[10]"Containers," United States Pharmacopeia, 21th rev., U.S. Pharmacopeia Convention Inc., (1984), 1233-1235.

[11]Ibid

[12]John B. Henry, Clinical Diagnosis and Management, 17th ed. (Philadelphia, PA: W. B. Saunders Co., 1984), 255.

effect optimal reagent performance, patient data, or results.

When the dry reagents are manufactured through compounding (mixing oven dried powder components) or fluid bed granulation, either USP Type I or Type II glass becomes more interchangeable. Glass to reagent contamination is slight since the dry reagents are conveyed in and then poured out of the glass container, and the reagents are rehydrated in an instrument or another container. If the reagents are reconstituted in the vial, the manufacturer's validation of working solutions is usually limited from two to eight days when stored at 2° to 8°C.[13] The contact between the reconstituted solution to glass stored under refrigeration is short enough that glass extractables do not influence overall reagent performance.

For this study the commercially available Münnerstädter Glaswarenfabrik GmbH (Germany) light amber threaded neck finish tubing vials presented in Figure 3 are to be compared to the experimental threaded vials manufactured from Type I dark amber glass tubing and manufactured by the West Company and Comar, Inc. (Figures 4 and 5). Molded vials were the first choice because of the increased dimensional stability control advantage. However, due to high mold capitalization

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[13]Norbert W. Tietz, Textbook of Clinical Chemistry, (Philadelphia, PA: W.B. Saunders Company, 1986), 10.

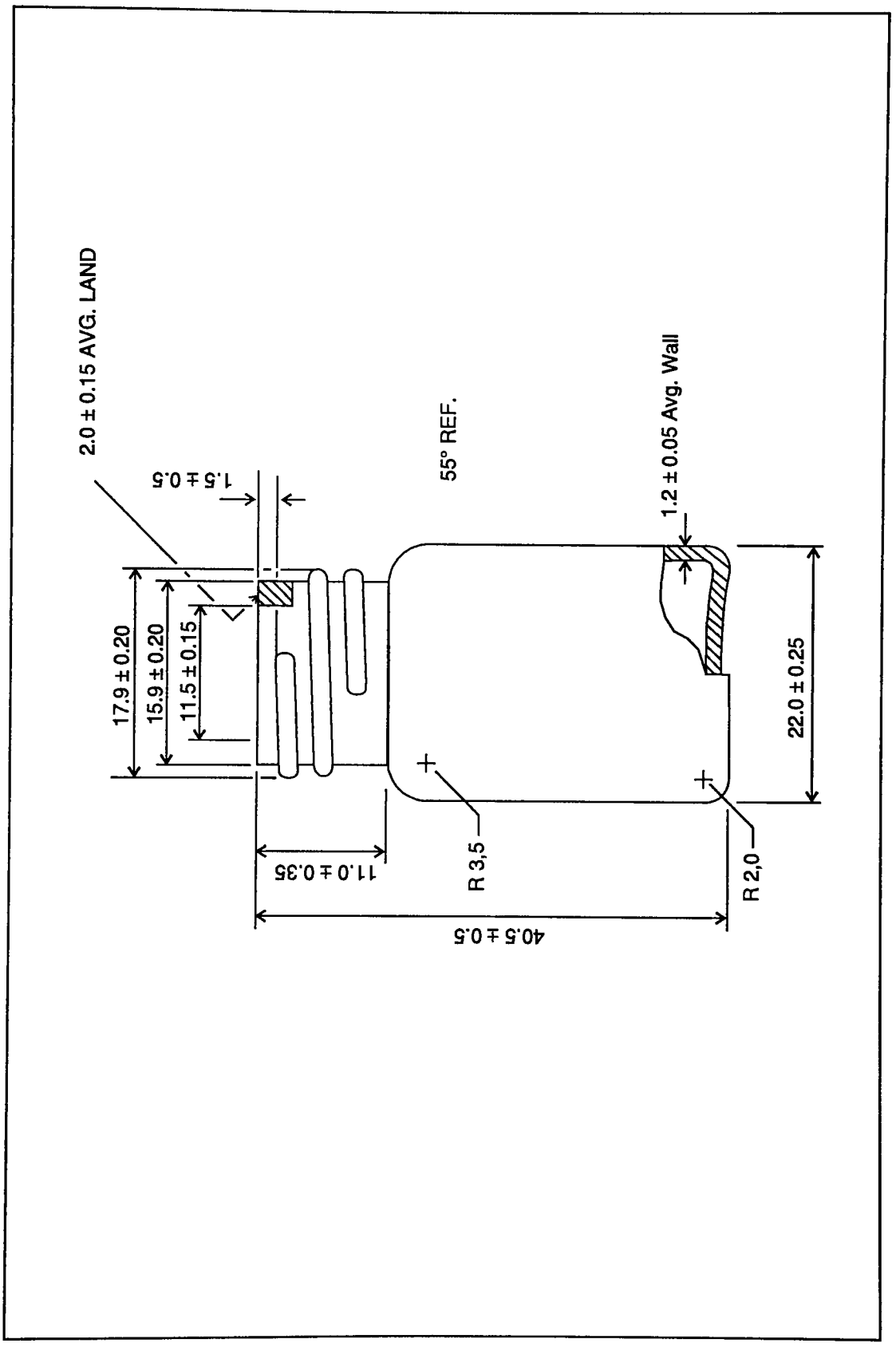

Figure 3. Threaded finish tubing vial manufactured by Münnerstädter Glaswarenfabrik GmbH.

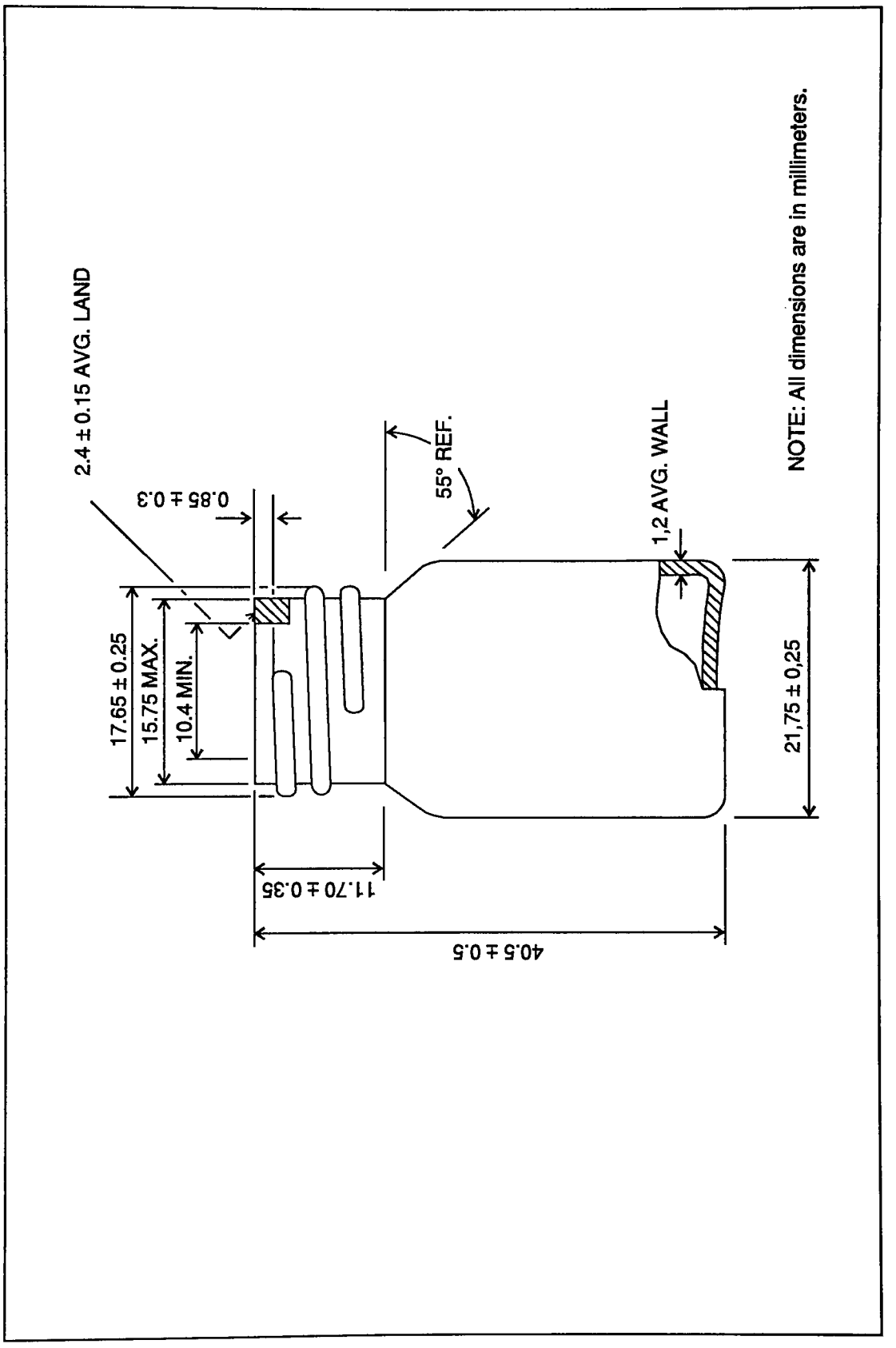

Figure 4. Experimental threaded vial manufactured by the The West Company.

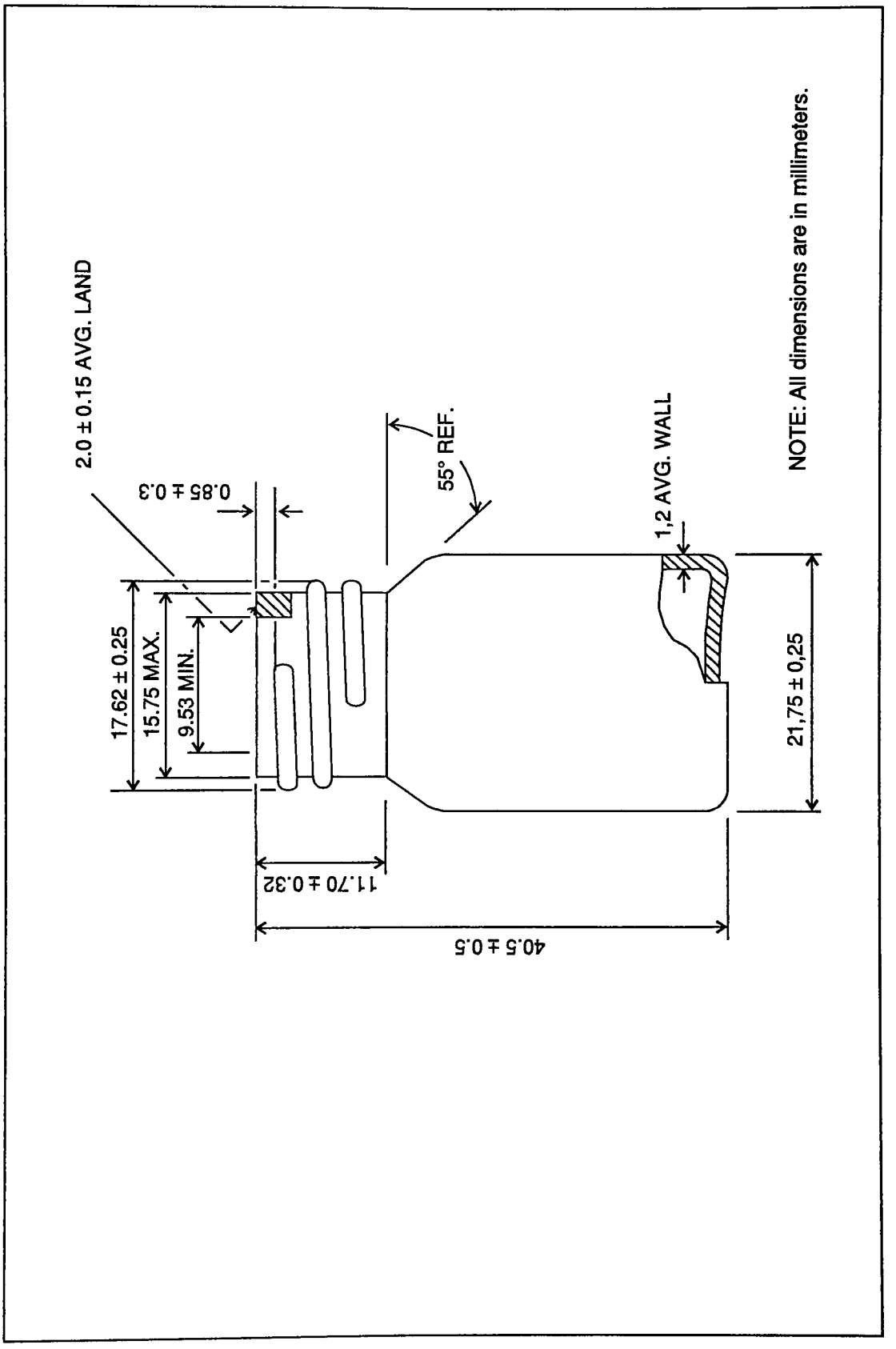

Figure 5. Experimental threaded vial manufactured by Comar, Inc.

costs and the experimental nature of this project, molding the glass vials would have been impracticable with the subsequent modifications to the neck finish tooling and land area that would be required during the initial stages of the vial's development.

Plug and Liner Materials

The elastomeric plugs (Figure 6) are formulated from a halobutyl elastomer and were molded by Pharma Gummi (The West Company) in Germany. The "base" candidate liner materials for this study were calendered 0.070" chlorobutyl isoprene blend elastomer manufactured by the West Company, Phoenixville, PA. Table 1 lists several formulation characteristics for both materials. Halogenated butyl is a preferred sealing material for vial closures in the pharmaceutical field due to low water and gas permeability.[14] One of the base candidate liners was specified with the 0.002" polytetrafluoroethylene (PTFE) film material. In the chemical industry, a Teflon® facing is specified for high purity or corrosive chemical applications.[15] For some pharmaceutical applications and

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[14]Y. J. Wang and Y. W. Chien, "Sterile Pharmaceutical Packaging: Compatibility and Stability," Parenteral Drug Association Tech. Report 5, (1984), 123.

[15]E. B. Hubbard, "New Choices in Cap Liner Materials for Demanding Packaging Applications," Journal of Packaging Technology 2, no. 4, (August 1988), 145-146.

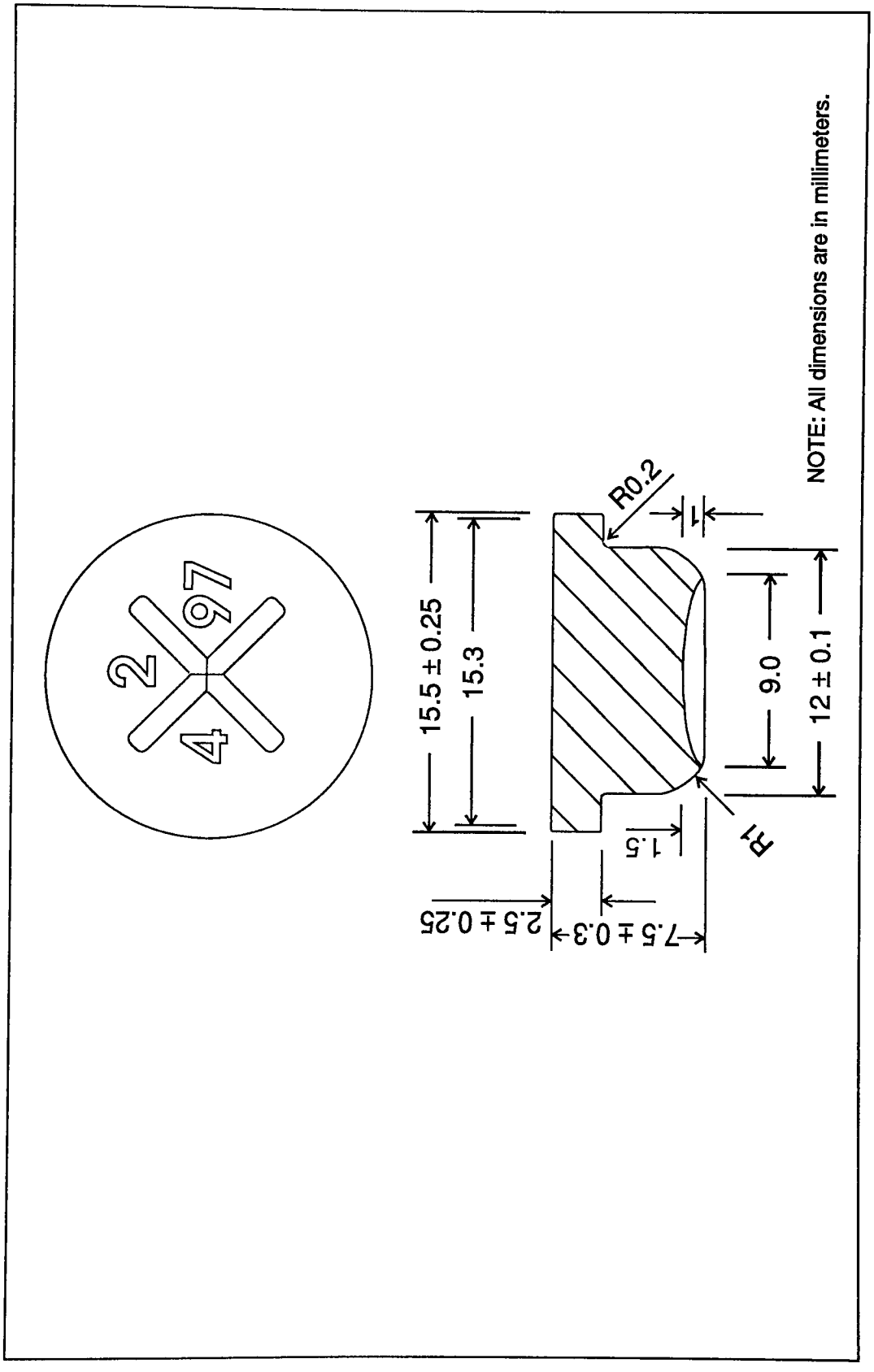

Figure 6. Plug molded from halobutyl elastomer. Manufactured by Pharma Gummi, Germany (The West Company).

TABLE 1

Formulation Characteristics - Elastomeric Plugs & Liners
Halobutyl / Chlorobutyl

Gasketing Materials	Plug	Flat Liner (only)
Formulation code The West Co.	PH 701/50 Pink	1888 Gray
Elastomer	Halobutyl	Chlorobutyl Isoprene Blend
Durometer (nominal Shore A)	50 ± 5	40 ± 5
Tensile (psi)	580	621
Compression Set (Method B) (% of orginal deflection)	18.0%	18.0%
Moisture Vapor Transmission $gm/m^2/day$	not reported	0.75

chromatography sample vials, PTFE is also specified to reduce the level of extractables exiting from the butyl or halogenated butyl rubber liner materials into the containers head space or product.[16]

The primary reason for the specification of PTFE was to emulate the lubrication characteristics of a silicone-coated elastomer and to reduce the coefficient between the liner and the container. Initial sealing evaluations of lining materials glued into a CT shell demonstrated the possibility of premature griping on the vial's land area during high speed torqueing conditions. The plain (no Teflon®) halobutyl liner materials exhibited a high degree of surface friction that inhibited the more desired liner sliding action across the vial's sealing surface. When liner stresses occurred, their appearance was spiral. Within the spiral a series of small channels were observed that would permit water vapor to ultimately enter the container.

The second reason for using PTFE was to reduce possible adherence of particulate matter (including product) to the surface of the stopper. Before sealing, silicone-coated stoppers can trap particulates on "clean" stoppers. Particulates are usually introduced through handling or from

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[16]Richard W. O. Jaehnke et al., "Interaction of Rubber Closures with Powders for Parenteral Administration," Journal of Parenteral Science & Technology 44, no. 5, (September-October 1990), 282-288.

the vibratory feeder bowls feeding stoppers to production line stopper plungers,[17] but for study, pharmaceutical reagent clinging to the silicone-coated stoppers after sealing is more of a problem than particulate contamination. The surface of the oiled stoppers trapped and became coated with reagent powders. There was the concern that product with low fill weights (100 mg.) and not reconstituted in the container would exhibit undesired inconsistencies and reductions in the final working strengths due to the powder sticking to the stopper. Those containers sealed with PTFE faced liners only required a slight tap to the container before opening to dislodge all granulations that clung to the liner's surface.

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[17]J. Z. Knapp and H. F. Dull et al., "A Wide pH Range Stopper for Improved Particulate Quality in Parenteral Solutions" Journal of Parenteral Science & Technology 38, no. 4, (July-August, 1984), 128-137.

CHAPTER 3

LEAKING AND PERMEATION

The dry pharmaceutical reagents in this study are adversely affected by the effects of inadvertent atmospheric moisture entering the candidate package systems. The barrier performance of the "proposed" flat liner elastomer is directly compared to the traditional elastomer stopper. To be successful, the performance of the flat liner system must be commensurate or surpass the moisture barrier provided by the stopper closure system. Evaluation data was generated from the change in a reagent's initial moisture content to the final moisture content after being environmentally stressed over a fixed time period. Results are reported as a percentage of moisture to reagent ratio, and the moisture content is measured and calculated by a commercial microprocessor controlled coulometer.

This study concentrates on real or total water vapor leaks occurring in sealed glass containers with candidate gasketing systems. Contributing to the changes in the reagent's water content are the localized leaks characterized as the unexpected discontinuity between two

sealing surfaces in physical contact and under compression[18] and the distributed leaks due to the permeation of water vapor through the closure and gasketing material. For this study the definition of a container leak has been limited to the liner's gasketing ability to create an effective moisture barrier. As discussed in Chapter 2, the packaging components have been selected, engineered, and manufactured to minimize gross leakage due to defects in packaging components. The only water vapor "leaks" anticipated in this study are limited microscopic transmission levels. This study does not discount the possibility of gross leaks due to unforeseen channels or passages caused by material defects, debris, or closure misapplications that would cause liner distortions or wrinkles caused by over-torqueing. Gases, other than water vapor, and other airborne contaminates were not evaluated or measured in this study.

After "physical" leaking effects, the balance of moisture transmission entering the sealed containers will be dependent upon the water vapor's permeation rate through the closure. Permeation is adsorption and desorption consisting of gases and other materials passing *through* the packaging materials via "activated diffusion".[19] In activated

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[18]Mary A. Amini and Darrell R. Morrow, "Leakage and Permeation: Theory and Practical Applications", Package Development & Systems, (May/June, 1979) 20-27.

[19]Christine M. Samaniego-Esguerra and Gordon L. Robertson, "Development of a Mathematical Model for the

diffusion the gas or vapor from the highest gas concentration side dissolves into the surface of the material and exits or evaporates at the opposite surface adjacent to the area of least concentration. The rate of diffusion and the permeation process is dependent on the concentration gradients on both sides of the packaging material or system.[20] The rates of permeation in this study are based on three factors. The first factor is the inherent packaging material barrier characteristics of the materials employed. Since the containers are made of glass, permeation in this study will be limited to an amount of water vapor passing through polypropylene closure shells and elastomer gasketing lining materials. The second permeability factor is the nature of the product and the head gas density concentrations above it. To evaluate transmission a dry powder reagent product has been packaged to sorb or absorb moisture that enters the package. The third and most significant factor effecting permeation is the differentials between internal and external packaging environments. Most sealing studies will use a combination

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Effect of Temperature and Relative Humidity on the Water Vapor Permeability of Plastic Films," Packaging Technology and Science 4, (1991) 61-68.

[20]Mary A. Amini, "Testing Permeation and Leakage Rates of Pharmaceutical Containers," Pharmaceutical Technology, 39-43, (1981).

of moderately elevated temperatures and higher than ambient humidity to accelerate stresses on the packaging systems. This study also compresses time frames by stressing normally refrigerated packaging at room temperature in an attempt to compare moisture transmission results after stressing candidate vials and flat liner systems along side commercially successful vials with plugs that have passed long-term time evaluations.

Theory

Several leaking and permeation theories have become the standard formulas in the packaging field and provide a framework of reference to assist in the understanding of gas or particle migration around or through sealed container systems. Moisture vapor leaking across the sealing surface is anticipated to be the primary cause affecting total moisture transmission into the container, and permeation through the closure is anticipated as a secondary cause.

The effects of total moisture leaking is described as the sum of convection and diffusion. The containers in the main study were filled with products having low initial moisture contents when combined with production and sealing in a low humidity area ($\leq$ 2% RH). Once the containers left the dry room any outside environment established an immediate water vapor pressure concentration gradient and resulting diffusion to equalize the pressure. Since product

was sealed under ambient atmospheric pressures and room temperatures, the slight convection or pressure gradients anticipated would be limited due to thermal fluctuations created as the candidate packages equilibrate to the stressing storage temperatures and conditions.

Beginning with the permeation component in the total leak rate, the gas (water vapor) rate of diffusion through the closure and liner at constant temperature and differential pressures can be described by Fick's first law:[21]

(3.1) $J = P \cdot A \cdot \Delta p/L$

where J = diffusion or transport rate ($g/m^2 \cdot s$)

P = permeability coefficient (m^2/s)

A = barrier area (m^2)

p = partial pressure gradient (g/m^3)

L = barrier material thickness (m)

When Henry's law is applied, Equation 3.2 is used for evaluating the permeability constant through one material (e.g., liner material):

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[21]Axelson, Lena, Cavlin, Sören, "Aseptic Integrity and Microhole Determination of Packages by Gas Leak Detection", Packaging Technology and Science 4, (1991), 9-20.

(3.2) $P = D \cdot S$

where P = permeability constant ($g/m^2 \cdot s$)

D = diffusion constant (m^2/s)

S = solubility constant (m^3(STP) $/m^3$ Pa)

$S = \Delta c / \Delta p$

c = concentration (m^3 gas/m^3 plastic)

p = partial pressure gradient (g/m^3)

For composite materials, such as the closure's liner and liner facing (e.g., Teflon), the permeability coefficient is outlined in Equation 3.3:

(3.3) $$1/P = (x_1/L)\cdot(1/P_1)+(x_2/L)\cdot(1/P_2) + \ldots$$

where P = permeability of composite ($g/m^2 \cdot s$)

L = total film thickness

$P_{1,2}$ = permeability of each layer

$x_{1,2}$ = barrier film thickness of each layer

The diffusion or transport rate can be further defined as in Equation 3.4:

(3.4) $J = \delta W / \delta t$

where J = diffusion or transport rate ($g/m^2 \cdot s$)

δW = water vapor (g)

δt = time (s)

Total leakage is the total effects of both convection (pressure differentials) and diffusion (gas concentrations) of water vapor across the sealing surface. The gradient is mathematically expressed in Equation 3.5.[22]

(3.5) $$J_A = X_A\,(j_A + j_B) - (p)(D_A)(X_A)(1/x)$$

where A = total leakage flux of species A ($g/m^2 \cdot s$)

X_A = fractional amount of species A ($g/m^2 \cdot s$)

j = convective flux if each species ($g/m^2 \cdot s$)

p = density of mixture (g/m^3)

D_A = diffusion coefficient of species A ($m^2 \cdot s$)

x = barrier material thickness (m)

As previously indicated, there are gradient driven internal processes versus external processes describing moisture transmissions in (or out) of packaging systems. It is Labuza[23] who described that a product's water sensitivity in conjunction with the container's head space combined to establish a moisture equilibrium. That internal level of equilibrium is critical in establishing the final gradient potential between the inner and outer environments.

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[22]Mary A. Amini and Darrell R. Morrow, "Leakage and Permeation: Theory and Practical Applications," Package Development & Systems, (May/June 1979), 20-27.

[23]T. P. Lubuza, "The Effects of Water Activity on Reaction Kinetics of Food Deterioration," Food Technology, (January 1980).

However, it is the product's sensitivity to physical-chemical moisture binding activity that ultimately effects the total internal moisture equilibrium. The activity is expressed in Equation 3.6.

(3.6) $A_W = p/p_o = RHE/100$

where A_W = water activity

p = vapor pressure of water exerted by product

p_o = vapor pressure of water

RHE = product's relative humidity equilibrium

In order to select a "dry" reagent's barrier packaging, barrier components, and materials these product elements must be stated or least estimated. The reagent's initial moisture content, the moisture content at which the reagent fails performance evaluations, and the moisture activity of the reagent itself under various temperature and external relative humidity must be considered.

CHAPTER 4

GENERALIZED CONTAINER LEAK TESTING METHODS

It is the responsibility of the pharmaceutical reagent manufacturer, under the current guidelines of Good Manufacturing Practices (GMP) incorporated into the Code of Federal Regulations Section 211.84, to test both closures and components.[24] The evaluation should validate the performance of the combined packaging system for safety and product performance.

During the preliminary evaluation process for dry reagent packaging the candidate must pass the evaluation in three categories. First, as discussed in Chapter 2, the composition of the packaging and the materials must be compatible and clean so not to interfere with the reagents or performance of the diagnostic instrument. The second issue is for leak testing to address package systems and product safety. Containers and closures must withstand conventional processing and handling on automated filling and capping equipment. For example, the breaking of a glass vial filled with pharmaceutical reagents that contains stabilizers (such as azides), can be potentially hazardous

[24]Code of Federal Regulations, Title 21, U.S. Government Printing Office, Washington, D.C., (April 1988).

if ingested. The package engineering of the components and the validation of the production line, inspection, filling, and sealing equipment should minimize the potentially hazardous gross component defects. The third issue centers around seal effectiveness and the component material's barrier performance. The package's moisture barrier performance and total transmission ultimately determines the reagent's published shelf-life and the reconstituted reagent performance levels that will be experienced by the laboratory instrument or the end-user.

Discussed are common leak testing methods that have been developed and generally accepted to affirm the performance of a sealed package system. These basic uncomplicated and rapid tests, such as immersion testing, are used to identify gross defects in the materials and sealing process. Upon completion of elementary leak testing, the higher levels of leak tests are utilized. These more complicated test methods will yield the data used to determine the candidate packaging system's barrier performance and final component specification. The challenge is to select practical final leak testing methods, with or without instrumentation, that realistically assess the packaging systems performance. The determination of final leak testing methods should balance the expense and the time required to generate the quality of packaging performance data needed.

Since the causes of gross defects are detected early, the primary focus shifts to the faint leaking defects that can cause a reduction in reagent performance ranging from insignificant to consequential in nature. Fortunately, most automated diagnostic instrumentation employ a system of standards and calibrators that are used periodically to measure the performance of reagent blanks (reagents not yet mixed with patient samples). When performance of a reagent blank falls outside the instrument's calibrated parameters, the instrument will shut down or alert the technician. It is the inconsistent package barrier performances that can cause minor changes in reagent performance. Reagents that remain within calibration limits but drift over the permissible range of product performance may result in less than accurate patient results. In the diagnostic reagent industry, the negative impact of variations in patient results will increase the cost of testing due to patient retesting or the verification of diagnostic inaccuracies that are reported to physicians.

The commonly accepted leak test methods described in the following pages are presented from the less to the more sensitive methodologies.

Immersion Tests

The traditional bubble tests are the among the simplest package integrity tests. The added advantage of immersion

testing is the lack of capitalization required to perform this testing procedure. This method does not require much technical expertise to perform, and the test results are immediate and easily interpreted.

Bubble tests are based on the creation of a pressure differential induced by either vacuum, thermal, or pressure means across the sealing or contact surface. A bubble test will start to detect holes of 0.005" in diameter or larger.[25] For large, cumbersome packages the seals are coated with a soapy film, whereas smaller items can be immersed in fluids. A sealing failure is indicated when gas escaping through the leak forms bubbles on the low pressure side that are noticeable. The size, number, and rate of bubbles appearing often indicates the size and location of system breaches.

An evaluation for system defects can be made more sensitive by increasing pressure differentials. This can be accomplished by using a surfactant to reduce surface tension or through the introduction of a suitable tracer agent, i.e., Helium (-10^{-6} Pa-m^3 /s) instead of air. The Helium gas flow is mainly molecular, and the nature of leak flows can be both molecular and laminar.

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[25]Robert L. Demorest, Journal of Packaging Technology 2, (October 1988), 182-190.

The downside of bubble testing includes unreproducible evaluations, undefined leak rates, wet packages, and difficulty isolating defects due to undetectable leak pathways. The immersion procedure is primarily limited to use as a quick "in-process" quality control evaluation in some food, pharmaceutical, and aerosol manufacturing facilities.

Package Weight Changes

Determining a change in package weight is a widely used method to qualify proposed packaging systems. The method presumes that changes in the total weight of a candidate packaging system are related to an amount of leaking or permeation that has occurred.[26]

Candidate containers filled either with liquids, desiccants, actual product, or controls (i.e., glass beads) are sealed and initial weights recorded. The containers are stored or stressed under defined levels of relative humidity and/or temperature over time. When package weights are recorded periodically during the evaluation, it is possible to calculate both the total transmission rate in addition to the permeation rate via periodic weighing.

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[26]Mary A. Amini, "Permeation And Leakage in Closures," Packaging Technology, (April 1986), 10-14.

The USP,[27] ASTM,[28] and PDA[29] have published several weight gain methods for the evaluation of sealed containers. Generally, the package weight gains (or losses) are observed and plotted over prescribed time intervals. Several methods are listed:

ASTM D 3199-79	Standard Test Method for Water Vapor Transmission through Screw Cap Closure Liners.
USP Containers	Permeation
PDA	Moisture Vapor Transmission (characteristics of pharmaceutical stoppers and crimp aluminum seals).

The technical level to perform weight change testing is minimal. The quality of data depends on the resolution and accuracy of the balances engaged to make weight determinations. Disadvantages include demonstrated defects but undefined causes. This method has the inability of achieving rapid determinations since some test methodologies

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[27]Containers," United States Pharmacopeia, 21th rev., U.S. Pharmacopeia Convention Inc., (1984), 1233-1235.

[28]ASTM D 3199-79, "Standard Test Method for Water Vapor Transmission Through Screw-Cap Closure Liners," American Society for Testing and Materials, Philadelphia, PA.

[29]"Elastomeric Closures: Evaluation of Significant Performance and Identity Characteristics", Technical Methods Bulletin No. 2, Parenteral Drug Association, Inc., Philadelphia, PA (1981).

stipulate at least five weeks of evaluation studies. Weight gain data does not readily distinguish between weight changes due to leaking and various permeation stages of water vapor. Weight gain testing requires critical preparation of samples. This testing is open to potential errors in measurement, calculations, and plotting errors.

Biotesting

A microbiological challenge method using bacteria or mold spores is used to detect "pin hole" leaks in sealed packaging systems. This method exploits the micro holes or seal defects in the package. If the capillary diameter of the defect is greater than the bacteria's diameter, bacteria penetration may take place. After a given incubation time, further evaluations are conducted to draw out evidence of bacterial transmissions through the package. Organizations such as the FDA, the National Food Processors Association (NFPA), and the ASTM have proposed biotest methods.[30] McEldowney and Fletcher, using microbiological challenge methods, studied the effects of physical and microbial factors to assess the integrity of food containers.[31]

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[30]D. Bernard, "Evaluating Container Integrity through Biotesting," Proceedings of National Food Processors Conference 'Packaging Alternatives for Food Processors 'The Food Processors Institute, (1984), 83-84.

[31]S. McEldowney and M. Fletcher, "A Model System for the Study of Food Container Leakage," Journal Appl. Bact. 69, (1990), 206-210.

Microbial immersion is the most common biotest method. Containers are filled with a culture media and then sterilized either by steam or gas (i.e., ethylene oxide).[32] The containers are then suspended in a microorganism bath containing, for example, Pseudomonas aeruginosa or Escherichia coli Salmonella typhimurium.[33] After exposure to the microorganism media, samples are taken from inside the package. Those samples are transferred to pour plates containing agar and incubated from one week to several weeks. In the last step the pour plates are inspected for microbial growth colonies identical to the original genus microorganisms.[34]

Microbial aerobiology is similar to the microbial immersion process described above except the sealed containers are placed in an empty chamber and microorganisms are introduced via an aerosol. The exposure to airborne bacteria is not as intensive as immersion but simulates the

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[32]Dave J. Murray, "Test Methods for Detection of Leaks and Seal Defects in Medical and Other Packaging," Tappi Journal, (April, 1991), 193-195.

[33]Nicole Richard, "Monitoring the Quality of Selected Liquid Media Used in the Official French Dilution Technique for the Bacteriological Examination of Food," Proceedings of the Symposium 'Quality Assurance and Quality Control of Microbiological Culture Media', (1982), 51-57.

[34]R. Ahvenainen and T. Mattila-Sandholm et al., "The Effect of Microhole Size and Foodstuff on the Microbial Integrity of Aseptic Plastic Cups," Packaging Technology and Science 5, (1992), 101-107.

effects of atmospheric microbial and pressure conditions on closure/container integrity.

Microbial testing evaluations are conducted under either static or dynamic conditions. During dynamic or stress phases, evaluations are designed to elevate strains exerted on seal strengths and seal creeping by exposing the subject packaging to challenge bacterial organisms under increased levels of atmospheric pressures and/or vacuums.[35]

Morton, et al, expanded on the test to generate leakage rates through a series of container closure evaluations in a microbial environment. Morton, using parenteral glass vials with crimped elastomeric stoppers, noted that microbial leakage was eliminated when the closures were compressed with a closure compression force permitting no more than 10^{-5} to 10^{-6} Pa-m^3 /s. air flow to escape under previous internal pressure evaluation.[36]

Due to the wide margin of errors generated by this methodology as compared to more mechanical tests, the microbial test is not widely used outside of the pharmaceutical and food industries. The other limitation of

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[35]"Aspects of Container/Closure Integrity," Technical Information Bulletin No. 4, Parenteral Drug Association, Inc., Philadelphia, PA (1983).

[36]D. K. Morton, N. G. Lordi, L. H. Troutman, and T. J. Ambrosio, "Quantitative and Mechanistic Measurements of Container/Closure Integrity. Bubble, Liquid and Microbial Leakage Tests," Journal of Parenteral Science & Technology 43, (1989), 104-108.

the microbial integrity method is the inability to reproduce the same results for the same package defects.

Quartz Crystal

A direct micro determination technique for determining the amount of water absorbed on a coated oscillating quartz crystal exists. The piezoelectric quartz crystal sensor is coated with a selected hydrophilic polymer (i.e., povidone). The coating causes a reduction in the resonating natural frequency of the crystal, and adsorption of water vapor further reduces the crystal's initial frequency. The changes in frequencies before and after exposure to various levels of water vapor makes the quartz crystal react similar to a micro balance.[37] Some measurements are carried out under vacuum so that the relative vapor pressure can be controlled. The advantages of this method are that the results of moisture changes are rapid and reproducible. Major disadvantages depend on the particular nature of the coated material's adsorption isotherm. The way in which the coated material accepts moisture over time, temperature, and pressure may yield a moisture isotherm that may be non-linear.

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[37]Hani M. Sadek and James L. Olsen, "Determination of Water Adsorption Isotherms of Hydrophilic Polymers," Pharmaceutical Technology, (February 1981), 40-48.

Sonic & Ultrasound

A gross qualitative leak monitoring system is demonstrated utilizing sonic and ultrasound technology. Leaks that are audible (sonic) are obviously limited to the listeners physical capacities for detection, and presumably, this could have a corresponding ratio for accurate or inaccurate determinations. In the case of small leaks (ultrasonic), the amplification of a barely perceptible acoustic signal using a heterodyned circuit to reproduce the ultrasound back into the audible range is required. A pressure or vacuum pressure differential is created, and if there is a leak of sufficient size, the expelled fluid or gas creates air turbulence on the low pressure side. Acoustical detection is dependent on variables such as the viscosity and velocity of the fluid or gas, pressure differentials, and the size of the leak. The turbulence exiting from small activate leaks generates the ultrasonic "white noise" that is detected by the ultrasonic leak detector.

Infrared Detection

The ability to quantify water vapor using infrared detection has produced a series of commercially available instruments manufactured by Modern Controls, Inc. (MOCON®). These instruments utilize a patented pressure-modulated detector that can measure diffused water vapor transmission through flat materials and packages. The diffused water

vapor molecules are transported in a closed loop stream of "pre-dried" carrier air to a sensor. The sensor measures the infrared energy that was absorbed by the water vapor, and the instrument converts the electrical signal information into a recognizable "grams per" readout.[38] The preeminent position of the MOCON® instrument in the field of moisture vapor transmission evaluations is reinforced by ASTM F 1249-90[39] and TAPPI CA900207.01 standards.

The major strength of an infrared instrument is the capacity to determine a package's steady state or moisture permeation rate through packaging materials or packaging systems. The instrument's limitation is its insensitivity to accurately transmit extremely low moisture transmission rates.[40] An infrared sensor is by design very sensitive to water vapor, but the instrument's accuracy at low transmission rates is diminished due to a calibration requirement using a known reference material. The reference

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[38]"Water Vapor Transmission Rate Measuring Systems" Promotion Literature for PERMATRAN-W® Series, Modern Controls, Inc., Minneapolis, MN. (1992).

[39]ASTM F1249-90, "The Standard Test Method for Water Vapor Transmission Rate Through Plastic Film and Sheeting Using a Modulated Infrared Sensor," American Society for Testing and Materials, Philadelphia, PA.

[40]Ray W. Wood, Michael J. Mulski, and Kuu Wei-Youh, "Prediction of Water Vapor Transport Rates Across Polyvinylchloride Packaging Systems Using a Novel Radiotracer Method," Journal of Parenteral Science & Technology 44, no. 5, (1990), 278.

standard should ideally closely approximate the anticipated transmission rate.[41]

Presently the lowest commercially manufactured reference material from Modern Control equates to approximately 4.5 gm/m^2/day. The capitalization justification for the instrument, which starts at $40,000[42] (with dual sampling stations for calibration and sampling), will depend on the confidence of packaging information being generated and the amount of instrument utilization per year.

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[41]Bradley J. Henry, Territory Account Mgr. Permeation Systems, Modern Controls, Inc., Minneapolis, MN, personal communication, October 7, 1992.

[42]Bradley J. Henry, Territory Account Manager Permeation Systems, Modern Controls, Inc., Minneapolis, MN, personal communication, October 7, 1992.

CHAPTER 5

TOTAL MOISTURE DETERMINATION METHODS

Accurate determination of the actual reagent's moisture content can serve several useful packaging purposes. Referring to Chapter 4, all of the described leak test methods indirectly measured or observed the changes in moisture or substitute materials entering or exiting the packaging system.

Most pharmaceutical organizations will design and manufacture dry reagents with an initial moisture content specification. The pharmaceutical reagent organization's materials processing, stabilities, or technical service groups will determine the permissible upper limit moisture gain specification before the "dry" reagent's performance falls below design specification.

The origin of water or the type of water originally outlined by Hillebrand[43] that might be included in final moisture tabulations should be understood. For some chemical compositions one component could be *essential* water, or the water which is an integral part of the

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[43]W. F. Hillebrand and H. A. Lundell et al., Applied Inorganic Analysis, 2 ed., (New York, NY: John Wiley & Sons, Inc. 1953), 815.

molecular structure. Within the classification of essential water there are two forms. First is the *water of crystallization* that is very stable, solid by nature, and is part of a crystal structure. The second form is *water of constitution*, which is not present in the structure but is formed when the solid undergoes decomposition, i. e., as a result of heating or chemical reaction during oven drying. The negative effects of water of constitution will become evident when Karl Fischer moisture determination methods are discussed in Chapter 6.

Nonessential water is not part of the chemical constitution of a molecular structure, and it is retained by the solid through physical forces. It is the changes in the levels of nonessential water on "dry" product that causes the product to clump and causes the total package weights to change. The classification of nonessential water is further subdivided into *absorbed water*, which is retained on the surface of the solids. The second form of nonessential water is *sorbed water*, which is held in the condensed phase in the amorphous microporous capillaries of partially dehydrated forms of polymeric colloidal silicic acid or more commonly known as silica gel.[44] Sorb water can represent up to 40% or more of the solids total weight versus absorption

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[44]Rodney L. Dobson, "Protection of Pharmaceutical and Diagnostic Products Through Desiccant Technology," Journal of Packaging Technology 1, no. 4, (1987), 127-131.

which involves only a few tenths of a percent. Physically, nonessential water on many structures has a very weak bond. The amount of absorbed and sorbed water measured at a given time is very dependent upon the temperature and humidity present.

For clarity the term "water content" measurement will be applied to describe the direct determination of the total unknown quantity of water bound to the sample material as determined by a scientifically analytical method (i.e., Karl Fischer titration). Conversely, when decribing indirect moisture determination methods (e.g., Loss on Drying) the designation total "moisture content" will be applied.

There are many methods and techniques outlined in texts and periodical literature describing methods for the determination of unknown water and moisture contents in a product sample. Listed below are several of the commonly accepted determination methods currently being used in the pharmaceutical reagent industry or mentioned in the literature. Several methods with broad water determination applications will be described in the following pages:

- Gravimetric Methods
  - Oven Drying
  - Vacuum Drying
  - Microwave

- Separation Methods
  - Distillation
  - Absorption
- Infrared Spectroscopy
- Nuclear Magnetic Resonance Spectroscopy
- Gas Chromatography
- Mass Spectroscopy
- Chemical Methods
  - Karl Fischer Titration
- Electrical Methods
  - Coulometric Titration

Many of moisture determination methods listed above are very accurate and precise, however, with the exception of infrared (MOCON®) technologies, these methods are rarely used to measure the total package system's resistance to moisture transmission in or out of the package.

Total Weight Changes Using Uncontrolled Heat

As a widely accepted reference method for moisture determination, the gravimetric method requires two stages.[45] During the first stage the weighed sample is dried in a boiling water bath. The second stage heats the same sample

[45]J. Mitchell and D. M. Smith, Aquametry: Part III "The Karl Fischer Reagent", (New York, NY: John Wiley and Sons, 1980), 5.

to a constant weight in an oven at 102° ± 1°. The sample is then cooled in a desiccator, and the final weight is noted. The weight loss is calculated, and as demonstrated in Equation 5.1, the water content is expressed as a percentage.

$$\text{percent A} = \frac{\text{weight of A}}{\text{weight of sample}} \times 100 \qquad (5.1)$$

In the above equation the "weight of sample" is defined as the initial weight of the sample. The "weight of A" is the difference between the initial and final or constant weight. Percent A is the ratio of water to the initial sample weight.

Depending on the sample that is heated, the traditional gravimetric procedures do have limitations. Bypassing the bias introduced at the weighing device the water content could be overstated on occasions when water, together with other components in the sample, is decomposed when heated at near boiling temperatures.

Total Weight Changes Using Controlled Heat

Widely used and generally accepted are several oven drying techniques for moisture determination that have become recognized water determination standards by professional organizations such as the USP and the AOAC.

Oven drying is a modification of the traditional gravimetric procedures, and the major difference is the use of controlled heat generated by an electrical element, infrared, or microwave. The premise for the controlled and lowered heating temperatures is not to "boil" off water in the sample. The principal intention is to reduce the vapor pressure around the sample to facilitate the release of bound water only on the surface of the sample and to minimize the decomposition of other volatile components due to high temperatures. The optimal heating conditions for each sample must be predetermined for each sample to insure reproducible moisture content data for each evaluation.

Sources of moisture level errors include inappropriate selection of heating temperatures, and errors can be introduced by weighing balances.

CHAPTER 6

MOISTURE DETERMINATION USING KARL FISHER METHODS

For pharmaceutical products the Karl Fischer method is widely accepted as a methodology for the determination of water content in products.[46] Since Karl Fischer (macro) and Coulometric (micro) water determination methods and reagents are interrelated, an overview of the Karl Fischer reaction and reagents provides the basis for Chapter 7, Coulometry.

The Karl Fischer (KF) method is a quantitative volumetric method for determining water content based on a titrand (solid, liquid, or gas) containing an unknown moisture amount. Various Karl Fischer application methods and reagent modifications have been extensively described. Mitchell's & Smith's monograph presents a comprehensive review on the subject of Karl Fischer methods.[47] There have been various modifications to the orginal KF method presented and patented since Karl Fischer first published the water determination method in 1935.[48] However, there

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[46]Kenneth A. Conners, A Textbook of Pharmaceutical Analysis, 3 ed. (New York, NY: John Wiley & Sons, 1982).

[47]J. Mitchell, and D. M. Smith, Aquametry: Part III "The Karl Fischer Reagent", (New York, NY: John Wiley and Sons, 1980).

are still publications and accepted standards that continue to present the "original" Karl Fischer reagent ingredients (iodine, pyridine, sulfur dioxide, and methanol) and methodologies for many moisture determinations. The Karl Fischer standards as published in the U. S. Pharmacopoeia, ASTM E 203, European Pharmacopoeia, British Pharmacopoeia, ACS "Reagent Chemical", and ISO 760 outline the basic Karl Fischer methods along with updated references to commercially produced modified Karl Fischer reagents for both manual apparatus and automatic volumetric instruments that utilize the Karl Fischer method.

The condensed procedure for water determination using KF starts with a sample material containing the unknown water content. The sample can be dissolved directly in the Karl Fischer reagent or the common alternative method is to use solvent extraction. During solvent extraction the sample with the unknown water content is dissolved into a "dried" solvent such as methanol. After the sample's water has been released into the solvent, the increase or change in the solvent's water content is determined by titration using Karl Fischer reagents and processes. Since most Karl Fischer reactions take place in the protic media ("reactive"

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[48]Karl Fischer, "New Method for the Volumetric Determination of Water Content in Liquids and Solids," Angew. Chem. 48, (1935), 394.

in excess alcohol), and using commercially available Karl Fischer reagents, the chemical reaction can be expressed by Dr. Eugen Scholz's Karl Fischer equation which follows.[49]

(6.1) $CH_3OH + SO_2 + RN \rightarrow [RHN]SO_3CH_3$

$H_2O + I_2 + [RHN]SO_3CH_3 + 2RH \rightarrow [RNH]SO_4CH_3 + 2[RNH]I$

(RN = BASE)

To summarize Karl Fischer's chemistry equation, during the first stages of the reaction sulfur dioxide reacts with the alcohol to produced a monoalkyl ester. The ester is then neutralized by the base. The anion of methyl sulfurous acid is the reactive component and is already present in the Karl Fischer reagent. The water is consumed during the oxidation of the methyl-sulfite to methyl-sulfate by the iodine. The neutralizing (RN) base component is critical in maintaining optimal reaction pH ranges (~5-8).[50] A base solution that is too alkaline will adversely affect the titration times or affect the reactions stoichiometry and change the final water content results.[51]

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[49]Eugen Scholz, Karl Fisher Titration Determination of Water, (Berlin, Germany, Springer-Verlag), (1984), 13.

[50]J. C. Verhoef and E. Barendrecht, "Mechanism and Reaction Rate of Karl Fischer Titration Reaction", Part I Potentiometric Measurements, J. Electroanal. Chemistry 71, (1976), 305-315.

The stoichiometry for water consumption during Karl Fischer titration involves one mole of iodine, one mole of sulfur dioxide, and three moles of base (or pyridine) for each mole of water consumed. Both the sulfur dioxide and base are employed in excess so that the chemical combining capacity of the reagent for water is entirely determined by its iodine content.

A first distinction between the Karl Fischer method and the Coulometry method that uses coulometric reagents is in the way iodine is added to the titration. To be further discussed in Chapter 7, the iodine in coulometric titration is electrically generated from iodide. In Karl Fischer reactions the total quantity of iodine in a "standardized" KF reagent introduced to consume the water present during titration is measured volumetrically. The water content can be calculated using the following equation using the sample's total water content as a weight or the percentage of the sample:[52]

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[51]Steven K. MacLeod, "Moisture Determinations Using Karl Fischer Titrations," Analytical Chemistry 63, no. 10, (1991), 557-566.

[52]HYDRANAL® Manual, Eugen Scholz Reagents for Karl Fischer Titration, Riedel-de Haën Aktiengesellschaft, Germany (1987).

(6.2) $\text{mg } H_2O = a \cdot WE$

$\% H_2O = a \cdot WE / 10 \cdot e$

where: a = Karl Fischer reagent consumed

WE = water equivalent (std.) of reagent mg H_2O/ml

e = weight of sample

A fundamental requirement when using Karl Fischer reagents is the reagent's periodic water standardizations. The "original" Karl Fischer reagent, when prepared to many published standards, is chemically less stable and requires "new" water standardizations every few hours. Many commercially prepared Karl Fischer reagents are improved to be chemically more stable, however, the requirement for at least one water standardization per day is not eliminated. Chapter 7 discusses how the coulometric titration instrument eliminates the need for Karl Fischer reagent water standardizations.

Both Karl Fischer and coulometric Karl Fischer reagents cannot be used to determine moisture for all substances directly due to interfering side reactions. There are instances when the sample will react with the chemical ingredients of the Karl Fischer reagents. These undesirable chemical side reactions can skew the results by under or

over-estimation of the sample's total water content. The literature identifies some of the interfering chemicals that oxidize iodine or create water resulting in an over-estimation of water content results. Conversely, the chemical side reactions between Karl Fischer reagents and samples facilitate the creation of Bisulfate complexes formed from free water, sulfur dioxide, base and carbonyl functions on aldehydes and ketones in the sample. Water in the sample becomes bound in this type of complex.

Riedel-deHaën[53] (HYDRANAL®) and EM SCIENCE (AQUASTAR®) have published extensive Karl Fischer reagent applications manuals for their respective commercial lines of Karl Fischer reagents. EM SCIENCE includes descriptions of their AQUASTAR® volumetric and coulometric automated instruments and accessories in addition to AQUASTAR® Karl Fischer reagents. Both manuals present detailed guidelines for extraction solvent selection, sample preparations, and Karl Fischer water determination methodologies.

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[53]HYDRANAL® Manual, Eugen Scholz Reagents for Karl Fischer Titration, Riedel-de Haën Aktiengesellschaft, Germany (1987).

CHAPTER 7

COULOMETRY

Coulometric Analysis

Coulometry encompasses a group of electroanalytical chemistry techniques that can determine the amounts of an unknown substance by measuring the quantity of electricity in coulombs needed to convert the analyte passing between two electrodes in an electrochemical cell.[54] Depending on the material analysis required, there are several variations of commercial coulometers commercially available, such as chemical coulometers. For example, ASTM D 3985-81[55] standard outlines a test method for gauging oxygen using a coulometric sensor. This study evaluates the barrier performance of candidate packaging systems by measuring moisture changes in the packaged hygroscopic reagents using an automatic coulometric titrator in conjunction with coulometric Karl Fischer reagents. Figure 7 is an

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[54]Douglas A. Skoog and Donald M. West, Principles of Instrumental Analysis, 2 ed., (Philadelphia, PA: Saunders College), (1980), 590.

[55]ASTM D3985-81 (1988), "The Standard Test Method for Oxygen Gas Transmission Rate Through Plastic Film and Sheeting Using a Coulometric Sensor," American Society for Testing and Materials, Philadelphia, PA.

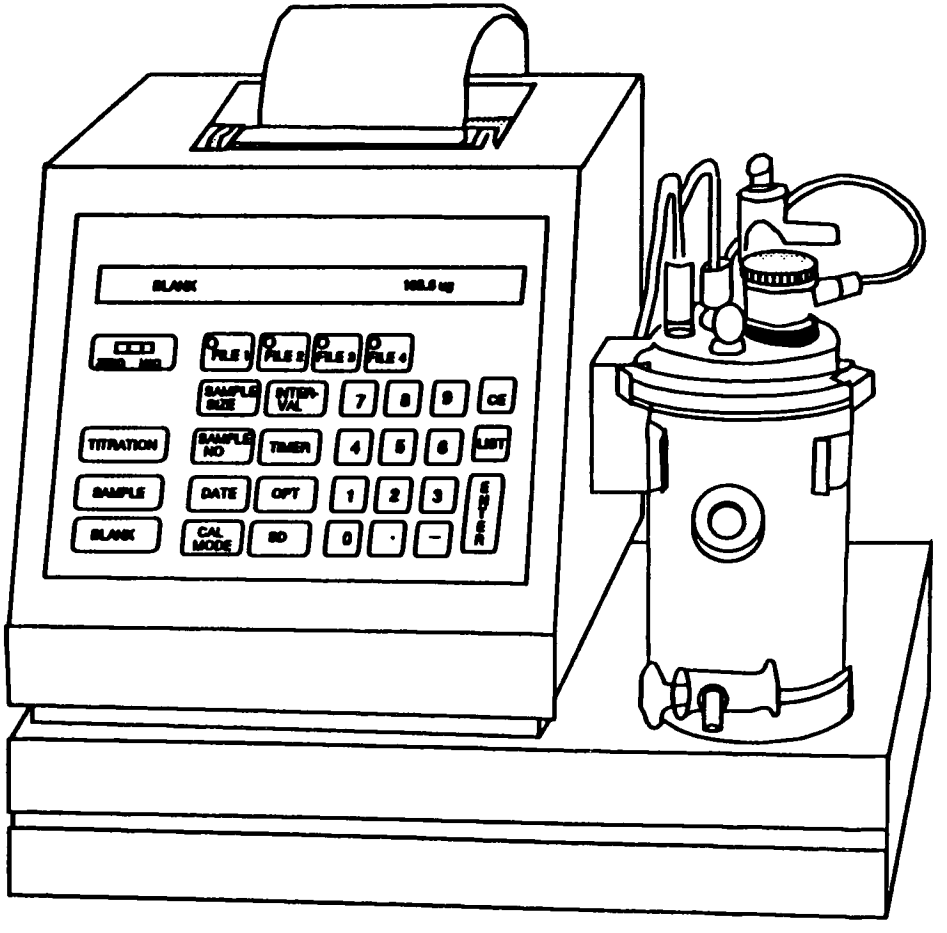

Figure 7. AQUASTAR C2000 instrument.

illustration of an AQUASTAR® C2000 one of several coulometric titration commercially available systems.

Most coulometers measure the amount of electricity expended to convert the analyte to a different substance via a reaction at the electrode in a proportional relationship. This process is represented by Faraday's first law and is expressed as demonstrated in Equation 7.1:

(7.1) $$z \cdot n \cdot F = Q$$

Where z is the numerical stoichiometric amount of electrons involved in the reduction or oxidation reaction, n the amount of substance reduced or oxidized, F the Faraday constant (= 96487 C $\cdot$ mol^{-1}), and Q is the amount of electricity (unit: C = 96500 coulombs = 96500 amperes $\cdot$ 1 second) passing through the cell.[56]

On the contrary, many coulometric titration instruments measuring moisture maintain the electrical current at a constant level, and it is the amount of time the device requires to reach the reaction's equilibrium that is recorded.[57] The equation is expressed as:

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[56]Norbert W. Tietz, Textbook of Clinical Chemistry, (Philadelphia, PA: W.B. Saunders Company, 1986), 127.

[57]G. Charlot and J. Badoz-Lambling et al., Electrochemical Reactions, (New York, NY: Elsevier Publishing Co., 1962), 247.

(7.2) $$Q = I \cdot t$$

Where Q is the amount of electricity (coulombs), I is the electrical current (amperes), and t is the time (seconds).

The coulometric titrations using modified Karl Fischer reagents are stoichiometrically equivalent to the Karl Fischer volumetric titrations described in Chapter 6. In both volumetric and coulometric reactions, when taking place in a protic medium, there is a reduction of one mole of iodine (to iodide) for each mole of water consumed during the titration. In the volumetric Karl Fischer reaction the iodine is regenerated by the interjection of incremental amounts of Karl Fischer reagent containing iodine into the titration vessel during the titration. Similar to the volumetric reaction the iodine consumes water, but during a coulometric titration the iodine I_2 is generated via electrolysis (anodic oxidation) from iodide (I^-) that is contained in the Karl Fischer coulometric reagent. Iodine is generated at the instrument's anode until all the water has been consumed according to the following reaction:[58]

(7.3) $$I_2 + SO_2 + H_2O + 3RN + CH_3OH \rightarrow 2RN \cdot HI + RN \cdot HSO_4CH_3.$$

[58]Eugen Scholz, "Karl Fisher Reagents without Pyridine", Fresenius Z Anal Chem, 314, (1983), 567-571.

Summarizing the equations, it is the quantity of the controlled electricity consumed combined with the amount of time taken within the coulometric analysis that is classified as *coulometric titration*. Meyer and Boyd[59] were among the first investigators to describe an automatic coulometric titrator and the reagent electrolytically regenerated iodine from depleted Karl Fischer reagent.

Coulometric titrators are designed to rapidly and accurately assess micro-moisture determination water quantities contained in or on a variety of substances. There are several studies where coulometric methods using Karl Fischer reagents are used for the determination of moisture in drugs and vaccines.[60],[61],[62],[63] The coulometric

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[59]A. S. Meyer and C. M. Boyd, "Determination of Water by Titration with Coulometrically Generated Karl Fischer Reagent," Analytical Chemistry 31, (1959), 557-566.

[60]Joan C. May and Elizabeth Grim et al., "Determination of Residual Moisture in Freeze-Dried Viral Vaccines: Karl Fischer, Gravimetric and Thermogravimetric Methodologies," Journal of Biological Standardization 10, (1982), 249-259.

[61]Joan C. Mary, Roscoe M. Wheeler, and Elizabeth Grim, "The Determination of Residual Moisture in Several Freeze-Dried Vaccines and a Honey Bee Venom Allergenic Extract by TG/MS," Journal of Thermal Analysis 31, (1986), 643-651.

[62]Joan C. May, Alfred Del Grosso, and Roscoe Wheeler, "TG/MS Interface: Applications to the Determination of Moisture in Polysaccharides and Freeze-Dried Biological Products," Thermochimica Acta 115, (1987), 289-295.

[63]Joan C. May, Roscoe M. Wheeler, and Elizabeth Grim, "The Gravimetric Method for the Determination of Residual Moisture in Freeze-Dried Biological Products," Cryobiology 26, (1989), 277-284.

moisture results are generally better when compared to other moisture determination techniques such as gravimetric and thermogravimetric methods, but thermogravimetric in combination with mass spectrometry analysis has demonstrated performance advantages over coulometric titration. The major drawback usually cited for coulometric titration is the possibility of a "specific" reagent or vaccine chemical incompatibility (discussed in Chapter 6) that might interfere with the reported water data. Knowledge of or the testing for possible reagent incompatibilities eliminates these problems.

The periodic literature (English language) does not reference coulometric titration as a sealed package assessment procedure for gauging moisture barrier integrity. The closest "in package" study cited in the literature was conducted by J. P. H. Wekx[64], et al. Wekx attached a micro device to a volumetric Karl Fischer titrator. The device provided the capability for performing Karl Fischer titrations inside primary containers (ampules or vials) containing lyophilized pharmaceutical products. By not removing the samples, Wekx reduced the risk of moisture contamination of the pharmaceutical samples. Wekx's group

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[64]J. P. H. Wekx and J.P. de Kleijn, "The Determination of Water in Freeze Dried Pharmaceutical Products by Performing the Karl Fischer Titration in the Glass Container Itself," Drug Development and Industrial Pharmacy 16, no. 9, (1990), 1465-1472.

successfully generated water determinations that ranged from 66 µg to 410 µg.

A coulometric titrator can accurately measure micro quantities of water in sample sizes as small as 5 mg. Referring to Table 2 the moisture determination data illustrates the exceptional repeatability of a coulometric titrator (data generated on the AQUASTAR® C2000) with small sample sizes of various powders and lyophilized reagents. Conversely, coulometric titration is not a replacement for all volumetric moisture determinations. The ideal coulometric determination should contain approximately 100 - 200 µg of water for greater reproducibility of results and decreased titration duration times.[65] The coulometric instruments do not tolerate levels of moisture above 30 mg H_2O per titration. A coulometric titrator would require a least 25 minutes to generate a final moisture determination. The extended titration times introduce the possibility of "other" non-stoichiometric chemical side reactions, as discussed in Chapter 6, that may modify the final water content results.

The second advantage of coulometric titration over the volumetric Karl Fischer method is the elimination of water standardizations. The coulometric Karl Fischer reagent

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[65]AQUASTAR® Titrators, Applications Manual, EM SCIENCE, Gibbstown, NJ.

TABLE 2

Moisture Content Determination Data for Reagents & Vaccines
Generated by the AQUASTAR® C2000

Sample	Amount of Sample (g)	Measure-ment Value (μgH_2O)	Moisture Content (%)	Titration Time (min)	Mean	Standard Deviation	Coeffient of Variation
Haemophilus b Polysaccharide Vaccine	0.0044 0.0043 0.0035	62.0 59.9 46.7	1.40 1.39 1.33	1 1 1	1.37	0.038	2.77
Meningococcal Polysaccharide Vaccine Group A,C,Y and W-135 combined	0.0239 0.0274 0.0379	385.4 432.0 563.4	1.613 1.577 1.487	1 1 2	1.559	0.0649	4.16
Antivenin Crotalidae Polyvalent	0.0647 0.1169 0.1363	170.2 283.3 326.3	0.2631 0.2423 0.2394	2 2 2	0.2483	0.0129	5.21
Lyophilized Reagent for Glycosylated Hemoglobin	0.0441 0.0441 0.0389	528.2 523.1 502.9	1.198 1.186 1.293	1 1 1	1.226	0.0586	4.78
Nicotinamide Adenine Dinucleotide	0.0253 0.0317 0.0224	1988.6 2459.9 1774.1	7.86 7.76 7.92	5 5 4	7.85	0.081	1.029

Source: EM Science

(electrolyte) is "blanked" electronically for background water by the instrument. The reagent blanking also occurs after and between each sample titration. When the titration cell is "blanked", the ambient background moisture in the electrolyte cell is continuously consumed.

Most commercial coulometric instruments are microprocessor controlled analyzers with the electrolytic titration cells. Figure 8 graphically depicts the inner connections of components found on many coulometric titrators. The critical component on the instrument is the central processor (CPU) that virtually eliminates control errors and automatically conducts these functions:

- Electrolysis Control
- End-point Detection
- End-Point Auto Stop
- Background Moisture Detection & Correction
- Background Information on the Electrolyte
- Total Moisture Consumed
- Reagent and component Installation Date

Table 3 emphasizes accuracy and precision similarities between many commercially available coulometric titrators. The moisture sensitivity for most instruments is about 0.1 µg H_2O, but accurate water determinations begin at 10 µg H_2O ± 1 µg (1 x 10^{-6} grams) H_2O. The purchase price for commercially available titrators averages $7,000 ± $2,000 each.

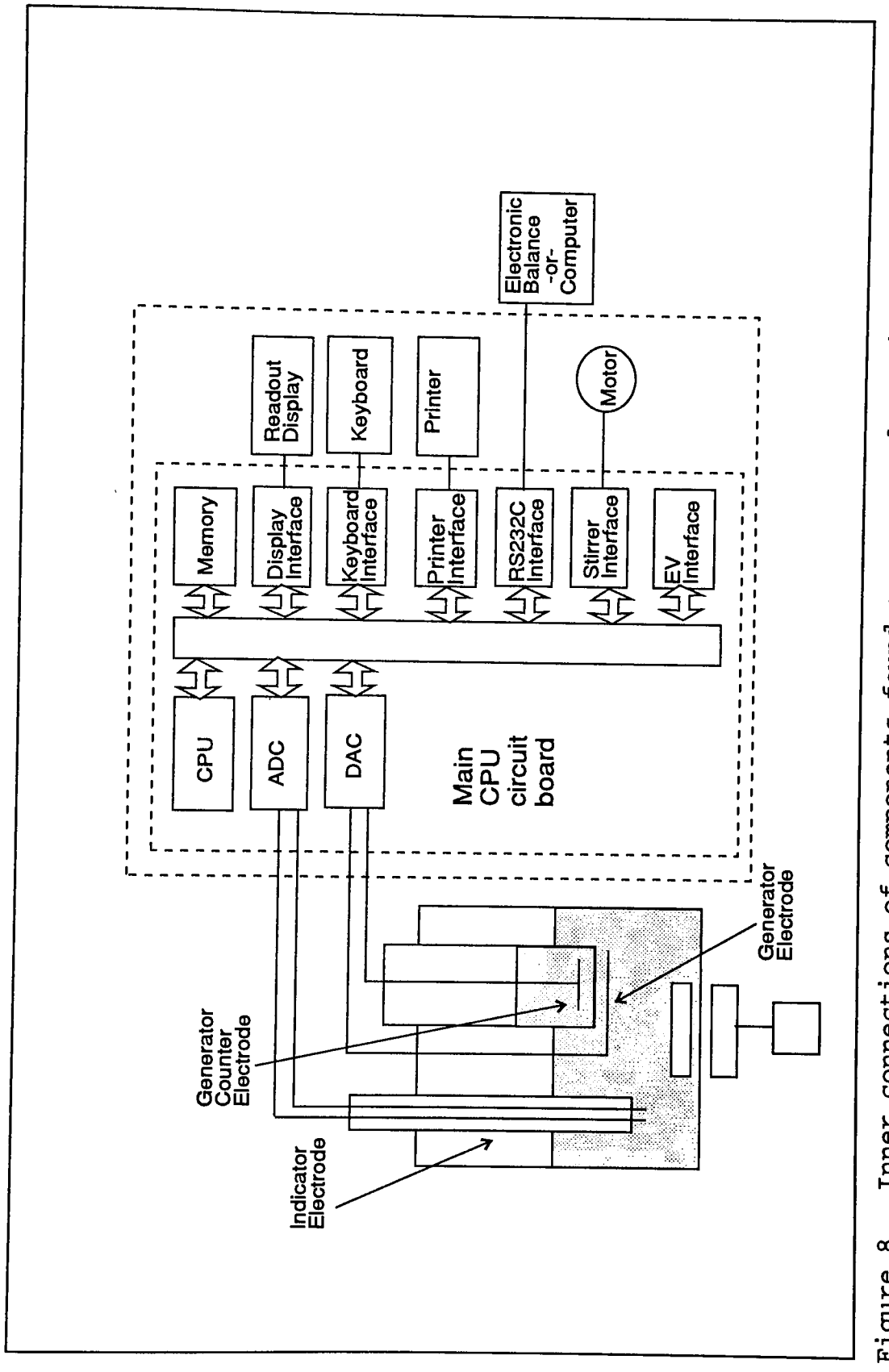


Figure 8. Inner connections of components found on many coulometric titrator instruments.

TABLE 3

Moisture Content Determination Data for Reagents & Vaccines
Data Generated by an AQUASTAR® C2000

Coulometric Titrators	Measuring Range	Sensitivity	Accuracy	Titration Speed H_2O / min.
C-2000 EM Science	10 µg to 100 mg	0.1 µg	± 1 µg (10 µg - 1 mg H_2O	1.2 mg
AQUATEST cma Photovolt	10 µg to 100 mg	0.1 µg	0.5% (C.V.) > 1 mg H_2O	2.4 mg
Model 684 KF Metrohm	10 µg to 100 mg	na	< 5 µg H_2O	2 mg
Model CA-06 Mitsubishi Kasei	10 µg to 100 mg	0.1 µg	± 3 µg for 10 µg -1 mg H_2O	2.4 mg
DL37 Mettler	10 µg to 100 mg	0.1 µg	± 0.3% > 1 mg H_2O	not available

Sources: Specifications and information as published on promotional brochures

Most coulometric titrators include LCD displays (in ppm, %, µg), built-in result printers, computer/balance interface, data/results memory, statistics, and convenient calculation modes. Sample weights are entered via a keypad, or in some cases, the balance weight data is sent directly into the CPU via a RS-232 interface. After the reaction is completed moisture results are displayed, printed, then filed in the instrument's memory. Most instruments feature automated calculation features that further reduce the possibility of moisture determination errors. Several of the available calibration modes are illustrated in Table 4.

As previously stated, the hardware on a coulometric instrument consists of the electrolytic cell that interfaces with and is controlled by a central processing unit (CPU). Figure 9 depicts the assembly of an electrolytic titration cell containing the anode, cathode, indicating electrodes, desiccant filled venting tube, and counter-electrode tube. Customarily, the electrolytic cell is filled with titrand (anode), and the counter-electrode tube is filled with titrand (cathode) solutions. For the flat liner versus stopper experiments a newer commercially available "universal" single solution pyridine free AQUASTAR® Coulomat reagent replaced the more traditional two-part (A & C) reagents.

In the start-up phase a coulometric titrator is self-contained and does not require any operator participation.

TABLE 4

Calibration Mode Comparison

CALCULATING FORMULA MODES	APPLICATIONS
$X = \frac{\text{FOUND}}{\text{SIZE (Sample weight)}} \quad (\mu g\ H_2O)$	Sampling by weight (g) Solid, Liquids
$X = \frac{\text{FOUND}}{SIZE_1 - SIZE_2}$	Weight by difference (g) Solid, Liquids SIZE is determined by the difference between the values before and after sample injection.
$X = \frac{\text{FOUND}}{\text{Density x SIZE (Volume)}}$	Volume by density (ml) Liquids The sample of known density is sampled by the volume
$X = \frac{\text{FOUND x } 1.244}{\text{SIZE}/\ (1+t/273)}$	Volume (l) Gas Calculated by measuring the water content V/V% (ppm) in a gas sample.
$X = \frac{\text{FOUND}}{\text{SIZE}}\left(\frac{B}{C} + \frac{X}{10}\right) - \frac{AB}{C}$	Solvent extraction method (g) Solid The water content in a solid sample is extracted into a solvent when the sample is insoluble in the solvent.
$X = \frac{\text{FOUND}}{\text{SIZE}}\left(\frac{B}{C} + 1\right) - \frac{AB}{C}$	Solvent dilution method (g) Solid, Liquid Measurement is made by diluting a liquid sample with a solvent or by dissolving a solid sample in a solvent.

Notes: DENS: Density of liquid sample
TEMP: Temperature of gas in degrees centigrade
A: Water content in the extracting solvent (ppm)
B: Volume of extracting solvent used (g)
C: Weight of sample used for extraction (g)

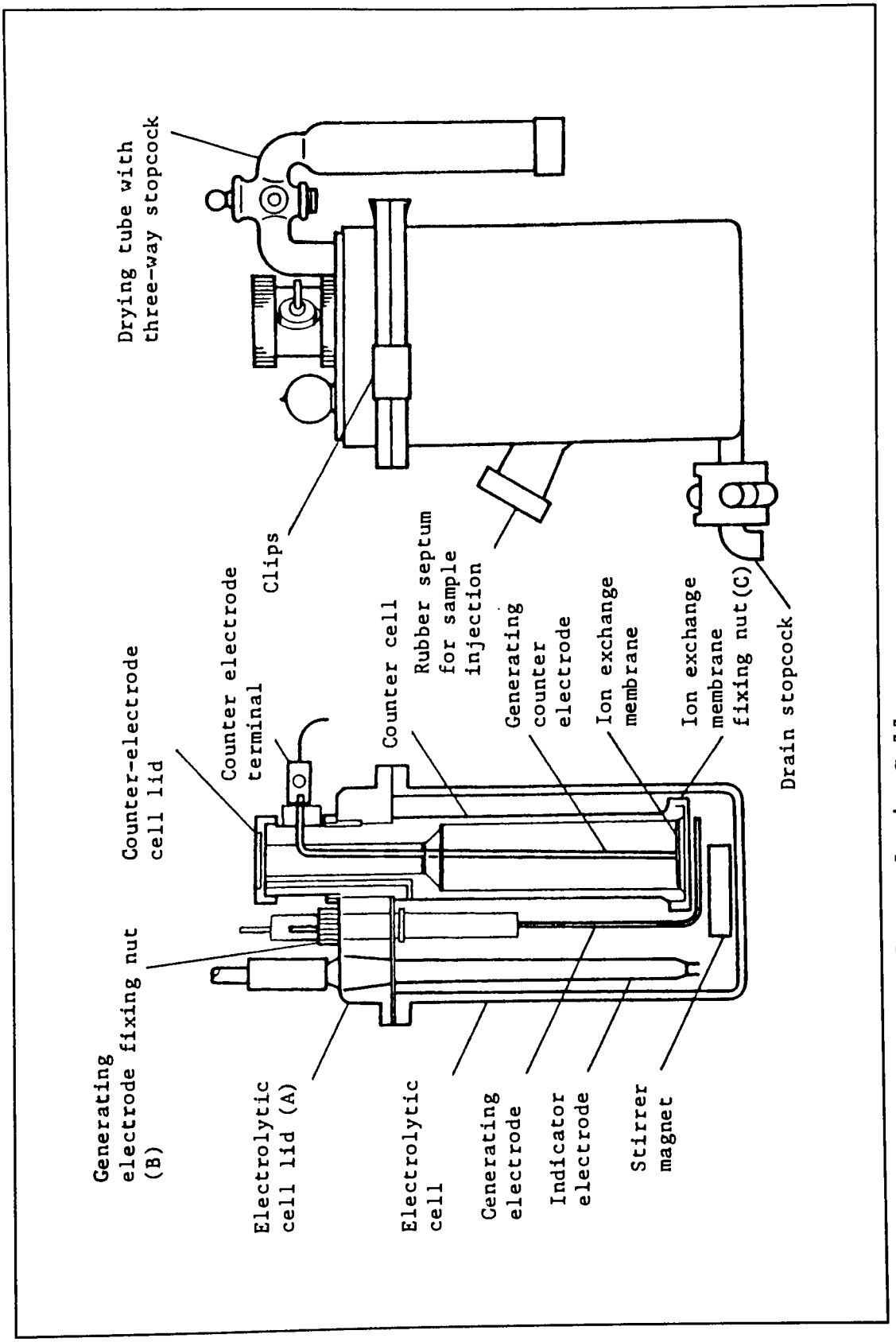


Figure 9. Assembly of Electrolytic Cell

the instrument's first function after being turned on is to remove all residual moistures absorbed into the coulometric reagents through preliminary electrolysis or "blank elimination". Once blanked a "sample" with an undetermined water content can be added directly to the titration cell or dissolved in a solvent then injected via a septum into the titration cell. Once the sample is added the indicator electrodes detect the change in the Karl Fischer solution's level of polarization and signals the CPU to start the reaction. The CPU permits electricity to reach the anode which regenerates the iodide (I^-) and produces iodine (I_2).

Figure 10 is a graphic representation of the electrolysis control sequence for both initial blanking (removing background water) and sample titration (moisture determination). Calling attention to the depiction a sample containing water being introduced into the coulometric cell on the right side of the graph. The titrator senses a change in conductivity of the coulometric reagent, which then activates the high electrical current level EL1 to start water elimination reaction. After the most of water is consumed the reagent's coductivity or potential dips below control points. The EL1 electrical level is then disconnected and the lower background current EL3 is then activated. When the reactive enters the B region between control point and end point: t1 represents wait times and t2 represents the activation of "just above" background current

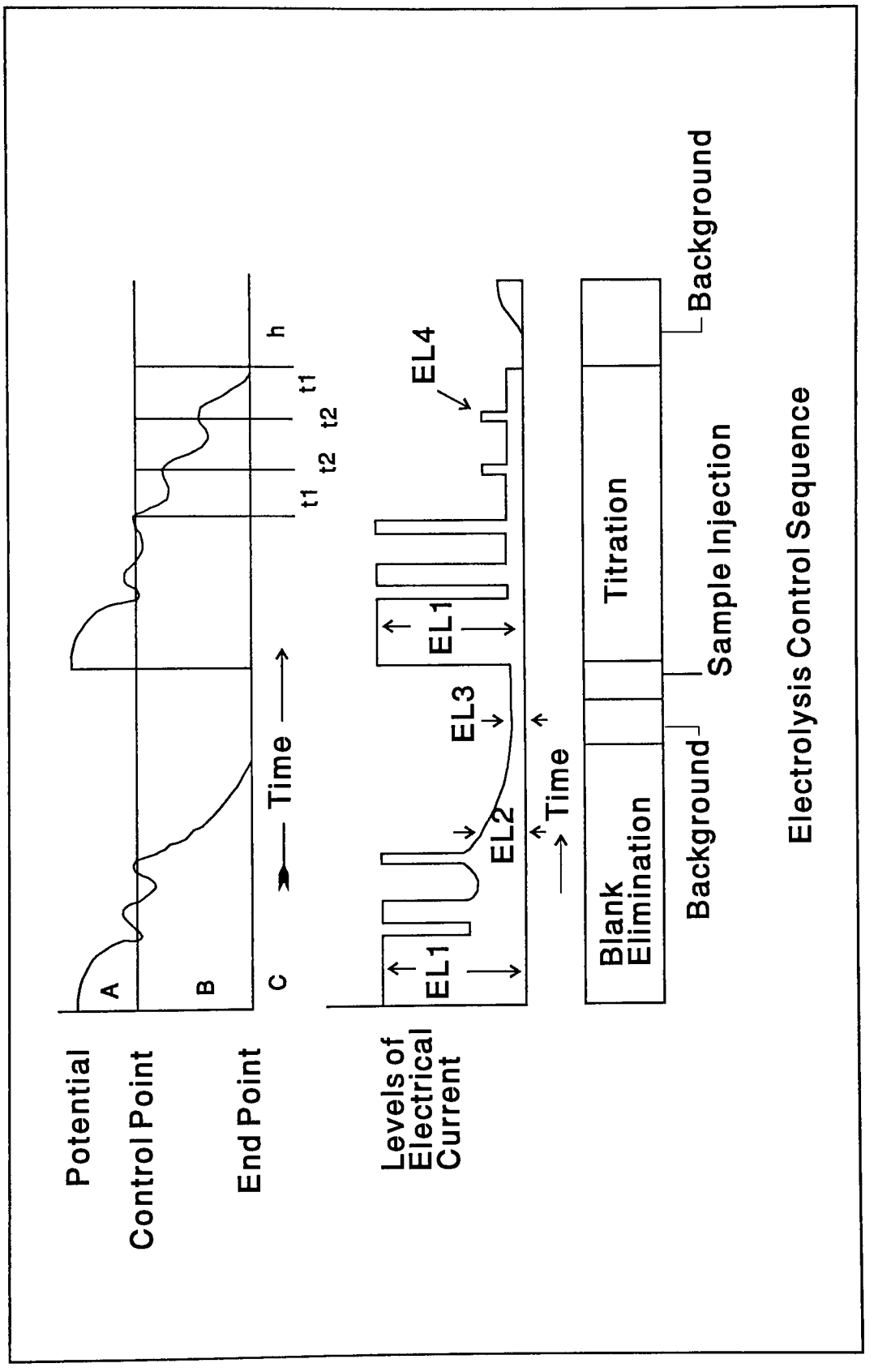


Figure 10. Electrolysis control sequence for initial blanking and sample titration.

EL4 being activated. These t levels are repeated over several cycles until the instrument's indicator electrode signals the CPU that all residual water in the cell has become undetectable by the indicator electrode. The CPU then interrupts the electrolytic flow to the anode, and the reaction ends. The quantity of electricity consumed as previously stated during the titration is based on Faraday's law and the stoichiometric amount of electrons involved in the iodine regeneration phase of the reaction are factored together. If no calculation mode was selected, the absolute volume of water (µg H_2O) or the concentration of water (%, ppm) is either displayed on the LED, printed out, or transmitted out to a personal computer.

An EM SCIENCE AQUASTAR® C2000 coulometric titrator manufactured by Hiranuma (Japan) was used for all water determinations. For addition information on the instrument the first several sections of a AQUASTAR® C2000 instruction manual has been included in Appendix 2. The manual contains detailed procedures for instrument set-up, reagent filling, moisture determination procedures. AQUASTAR® Coulomat Single Solution (universal reagent), AQUASTAR® Anhydrous Methanol (0.01% H_2O) (solvent extraction), t.h.e.® desiccants (drying tube), AQUASTAR® solid water standards, and AQUASTAR® water check solution (2-methoxyethanol) were also used to in conjunction with the packaging evaluations described in the following chapter.

CHAPTER 8

EVALUATIONS AND METHODOLOGIES

The main purpose for vial and closure evaluations is to evaluate the effectiveness of the proposed replacement flat liner versus the traditional stopper. Two sets of evaluations (#1 & #2) were conducted with materials supplied by two different North American glass vial manufacturers, the West Co. (Evaluation #1) and Comar, Inc. (Evaluation #2). Full descriptions of the packaging materials used, such as the vials, liners and closure shells are described in Chapter 2.

Within each evaluation segment the effects of minor variations in the candidate vials are explored. The filling equipment, storage conditions, and final moisture determination procedures are identical for both evaluations.

The primary purpose of Evaluation #1 is a direct moisture performance comparison between the candidate flat liners versus the conventional stopper sealing system. The candidate flat liners are seated on West, Co. vials and the barrier results are then contrasted to the control "traditional" package system consisting of the Münnerstädter screw thread vials sealed with Pharma Gummi stoppers. The packaging components are shown in Figure 11.

Figure 11. Evaluation #1 packaging components.

Within Evaluation #1 an additional moisture barrier comparison was conducted between the laminated Teflon® (0.002" PTFE/0.070" butyl) liners to the unlaminated (0.070") butyl liners. The issue is the barrier performance between the Teflon® faced butyl versus the same but unfaced butyl liner materials. There was the possibility that the Teflon® layer on the butyl could adversely affect sealing performance. The less ductile nature of Teflon® might not compress sufficiently and therefore not facilitate an effective seal into the imperfections on the vial's land or sealing surface.

The primary purpose of Evaluation #2 is to investigate barrier performance repeatability of flat liners, but this time using an alternative vendor, Comar, Inc. The packaging components are shown in Figure 12. In this evaluation only one liner is used (0.002" PTFE/0.070" butyl), and the vials produced by Comar are manufactured 1.2 or 1.4 mm glass tubing. Two different wall thicknesses are used to explore the effects of gathering more glass around the neck finish on the 1.4 mm vials. The intent is to improve the sealing surface and to permit Comar, Inc. greater control of neck characteristics during the vial manufacturing process. There are no specification changes from Münnerstädter (1.2 mm) vials and the Pharma Gummi plugs used in Evaluation #1. Also, in Evaluation #2 the moisture sensitive reagent cholinesterase blend (absorbent) is replaced with sodium

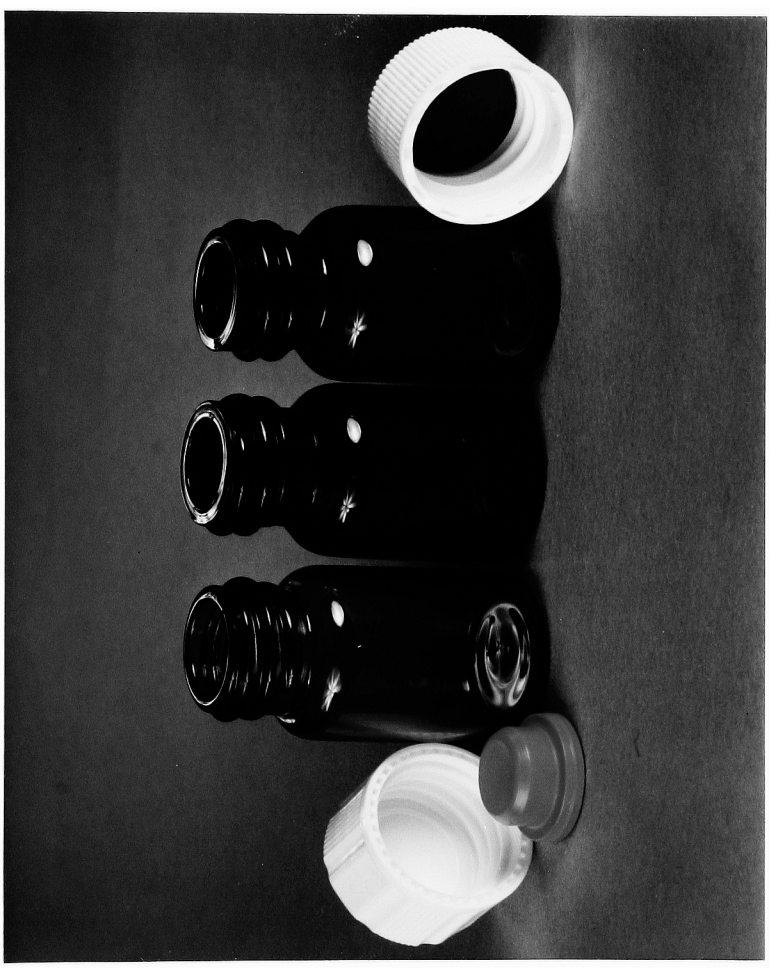


Figure 12. Evaluation #2 packaging components.

phosphate buffer to investigate the possibility of chemical side reactions affecting water content data between the absorbent reagents and the coulometric reagents.

The low humidity controlled environment for filling remained constant for both evaluations. The RH in the area is monitored by a dew point temperature and calculated using a dew point to RH conversion chart. The dew point temperatures during the filling sessions and later during solvent extraction sessions were -27°C ± 2°C (Honeywell Hygrometer) @ 70°F or approximately 2% RH @ 70°F referencing Table #B-1 in Appendix III.

Reagent Filling

In Evaluations #1 and #2 all vials are filled with 200 mg of absorbent granulations using a vacuum assisted Perry Accufil Lab Gun (Mod. FC). From a coulometric analysis standpoint, the more accurate methodology would include recording the weight of each vial's reagent; however, due to the hundreds of vials in the evaluations a semi-production Perry Accufil power filler is used to fill granulations. A statistical fill weight monitoring system was utilized during the initial set up and semi-production filling of the reagents. All representative fill weights are determined on a Sartorius four place analytic balance (type AC120S). Referencing Tables #A-1 and #A-8 in Appendix I, during the filling of each sub-group the vials were statistically evaluated for filling precision and possible bias employing

the Student's t-test at the 95% confidence interval. Immediately after each powder filling the vials are sealed. The closure shells are hand applied with a uniform 6 in. lbs. of applied torque as measured by an O-I torque meter (0 - 10 lb.).

Testing Environments

The sealed vials are divided into three subgroups and subjected to the stress or storage condition listed below for 21 days. The commercial pharmaceutical reagents packaged in both the control (stopper) and proposed (flat liner) packaging are intended by the reagent manufacturer to be stored and transported at temperatures between 2°-8°C.

- Refrigerated Chamber 2°-8°C @ 92% RH (avg.)
- Room Temperature 22°C @ 50% RH (±5% RH)
- Elevated Temperature 37°C at 80% RH (±5% RH)

Solvent Extraction of the Moisture

The sealed vials are returned to the "dry" room at the end of 21 days. The first step when preparing reagents in containers for moisture assay is to fill each vial with five milliliters of anhydrous methanol (AQUASTAR® AX 1699 M-1 ≤ 0.01% H_2O) from a butyl stoppered bottle. The procedure involved quickly uncapping and filling evaluation vials with CH_3OH. The five milliliters of methanol was transferred by a Hamilton GASTIGHT® #1010 (10 ml) syringe. In both evaluations the vials are quickly resealed and incubated at

room temperature for a minimum of 15 hours to insure complete release of all nonessential water from the reagent surfaces.

Just as in reagent powder filling, the more accurate method for coulometric analysis of filling methanol would include recording the solvent's fill weight in each vial. Due to the large number vials to be filled with solvent the gravimetric approach would be too time consuming. Referring to Table #B-2 in Appendix III compares the Hamilton GASTIGHT® syringes volumetric displacement to the actual weights of the liquids. Due to the precision and small standard deviations an overall mean weight was imputed for calculation purposes.

The initial methanol "blank" (H_2O content of CH_3OH) and water content assays after the solvent extraction process for all moisture determination is performed on the AQUASTAR® C-2000 coulometric titrator. The titrator is filled with AQUASTAR® (9255) Coulomat Single Solution universal reagent. All determinations are based on one milliliter of sample transferred from the vial using the Hamilton GASTIGHT® #1001 (1 ml) syringe.

The moisture data is randomly collected in such a manner so as to preclude instrument drift or bias. The water content data, as a percentage for each sample, is applied to the Student's t-test to evaluate if any group of data (closure type or condition) is statistically significantly different than any other group of data in the study.

Positive Controls

To monitor the total possible water absorption characteristics of the reagents, positive controls were created for Evaluations #1 and #2 by removing the closures and exposing the reagent in the test storage environments for 24 hours. The control vials were then recapped and handled similarly to the other test samples. Referring to Table 5 the only positive controls in the first evaluation were created under refrigerated storage conditions. The water contents shown in Table 6 demonstrate how much water the reagent can absorb in the event of a complete packaging failure. In the most extreme example, the 37°C/80% RH conditions causes a water content shift from 2.7% to 56% in the sodium phosphate buffer. The positive controls demonstrate the level of moisture differential existing between the sealed vial's interior atmosphere versus the external environmental conditions.

Evaluation #1 Results

The full set of data generated under the first evaluation (#1) is recorded in Tables #A-1 through #A-4 in the Appendix I. The raw data summaries are condensed in Table 7.

The final water content data generated by the various closure and storage conditions are analyzed using a Student's t-test with a 95% confidence interval. Table 8 is a summary of the results after the application of the Student's t-test. The upper portion of the t-test table contains the results in

TABLE 5

EVALUATION #1

PERCENT MOISTURE / POSITIVE CONTROLS
24 HOURS W/ NO CLOSURES

STORAGE ENVIRONMENT	1.2mm VIALS MÜNNERSTÄDTER (PLUGGED) (% H_2O)
REFRIGERATED	12.26

REAGENT: CHOLINESTERASE BLEND
AVERAGE MOISTURE CONTENT OF REAGENT: 2.7 ± 0.1% H_2O)

TABLE 6

EVALUATION #2

PERCENT MOISTURE / POSITIVE CONTROLS
24 HOURS W/ NO CLOSURES

STORAGE ENVIRONMENT	1.2mm VIALS MÜNNERSTÄDTER (PLUGGED) (% H_2O)	1.2mm VIALS COMAR (TEFLON/BUTYL) (% H_2O)	1.45mm VIALS COMAR (TEFLON/BUTYL) (% H_2O)
REFRIGERATED	14.3470	12.2605	14.4355
ROOM TEMP.	30.6595	26.1880	27.0735
37°C TEMP.	54.4355	61.4045	53.5925

REAGENT: SODIUM PHOSPHATE BUFFER
AVERAGE MOISTURE CONTENT OF REAGENT: 2.8 ± 0.1% H_2O)

TABLE 7

EVALUATION #1
MOISTURE CONTENT OF REAGENT IN PERCENT
21 DAYS

STORAGE ENVIRONMENT	1.2mm VIALS MÜNNERSTÄDTER (PLUGGED) (% H_2O)	1.2mm VIALS WEST CO. (BUTYL ONLY) (% H_2O)	1.2mm VIALS WEST CO. (TEFLON/BUTYL) (% H_2O)
REFRIGERATED			
n	23	23	23
mean	2.8753	2.7991	2.7848
sd	0.0656	0.0610	0.0895
max	2.9840	2.9780	2.9675
min	2.7445	2.7090	2.6620
range	0.2395	0.2690	0.3055
ROOM TEMP.			
n	23	23	23
mean	2.8906	2.7735	2.7857
sd	0.0775	0.0870	0.0804
max	3.0190	2.9520	3.0425
min	2.7130	2.6150	2.6680
range	0.3060	0.3370	0.3745
37°C TEMP.			
n	23	23	23
mean	2.8393	2.7365	2.7254
sd	0.0773	0.0703	0.0751
max	2.9710	2.8395	2.8745
min	2.6245	2.5745	2.6005
range	0.3465	0.2650	0.2740

REAGENT: CHOLINESTERASE BLEND

TABLE 8

STATISTICAL COMPARISONS

Sample 1	Sample 2	Null Hyp.	If null is rejected which mean is higher?	Difference 1 vs 2 (% H_2O)
Refrigerated Plug & Vial	Refrigerated Butyl	Reject	Refrigerated Plug & Vial	0.0762%
Room Temp. Plug & Vial	Room Temp. Butyl	Reject	Room Temp. Plug & Vial	0.1171%
37°C Humid Plug & Vial	37°C Humid Butyl	Reject	37°C Humid Plug & Vial	0.1028%
Refrigerated Plug & Vial	Refrigerated Teflon/Butyl	Reject	Refrigerated Plug & Vial	0.0905%
Room Temp. Plug & Vial	Room Temp. Teflon/butyl	Reject	Room Temp. Plug & Vial	0.1049%
37°C Humid Plug & Vial	37°C Humid Teflon/butyl	Reject	37°C Humid Plug & Vial	0.1139%
Refrigerated Butyl	Refrigerated Teflon/butyl	Accept	N/A	N/A
Room Temp. Butyl	Room Temp. Teflon/butyl	Accept	N/A	N/A
37°C Humid Butyl	37°C Humid Teflon/butyl	Accept	N/A	N/A
Refrigerated Plug & Vial	Room Temp. Plug & Vial	Accept	N/A	N/A
Refrigerated Plug & Vial	37°C Humid Plug & Vial	Accept	N/A	N/A
Room Temp. Plug & Vial	37°C Humid Plug & Vial	Reject	Room Temp. Plug & Vial	0.0513%

TABLE 8 - Continued

Sample 1	Sample 2	Null Hyp.	If null is rejected which mean is higher?	Difference 1 vs 2 (% H_2O)
Refrigerated Butyl	Room Temp. Butyl	Accept	N/A	N/A
Refrigerated Butyl	37°C Humid Butyl	Reject	Refrigerated Butyl	0.0626%
Room Temp. Butyl	37°C Humid Butyl	Accept	N/A	N/A
Refrigerated Teflon/butyl	Room Temp. Teflon/butyl	Accept	N/A	N/A
Refrigerated Teflon/butyl	37°C Humid Teflon/butyl	Reject	Refrigerated Teflon/butyl	0.0594%
Room Temp. Teflon/butyl	37°C Humid Teflon/butyl	Reject	Room Temp. Teflon/butyl	0.0603%

Statistic: Student's t-test, 95% Confidence Interval

Null Hypothesis: Mean of population (sample 1) is equal to the Mean of population (sample 2).

which the test environmental conditions are constant and the various liners are compared (i.e., refrigerated Münnerstädter plugged vials versus refrigerated West, Co. vials with and without Teflon® facing on butyl). The lower half of the t-test table contains results in which the closure's liners are constant and the test conditions are compared (i.e., refrigerated Teflon® versus room temperature humid Teflon®). If the statement in the null hypothesis (mean sample #1 is equal to mean sample #2) is accepted, the two samples are not significantly different. If, however, the null hypothesis is rejected, then the two sample means are considered to be significantly different. Columns 1 and 2 are the sample groups that are to be compared. Column 3 is the result of the t-test calculation. Column 4 indicates in cases where the null hypothesis was rejected, which sample mean was higher, and Column 5 shows, in percent water, how much higher the sample mean was.

Application of the Student's t-test in Table 8 to the different groups of data indicated statistically significant differences between the means of various different groups. However, the largest difference between any two statistically different means was only 0.1171%, These differences, although statistically significant, do not represent meaningful differences in terms of product performance.

It is interesting to note that where statistically significant differences existed between different closures,

it was the traditional packaging system consisting of plugged vials that generated slightly higher water contents in the reagent than either the butyl or the Teflon® test group. This would imply that the test flat liners, butyl or Teflon® faced butyl, performed as well if not better than the plugged vials within the parameters of this study.

Evaluation #2 Results

The full set of data generated under the second evaluation (#2) is recorded in Tables #A-5 through #A-8 in the Appendix I. The raw data summaries are condensed in Table 9.

The final water content data generated in Evaluation #2 by the various closure and storage conditions is again analyzed using a Student's t-test with a 95% confidence interval. Referring to Table 10 the data generated by the packaging variation for water barrier performance is analyzed for significant differences. After the application of the Student's t-test to the different groups of data indications are that within a closure system there is no difference in the mean percent moisture between the three test environments. For example, the mean percentage moisture for the Comar 1.2 mm vials with PTFE/butyl liners tested at room temperature is not statistically different from the mean for the same Comar 1.2 mm vials stored in 37°C humid or the refrigerated storage conditions. Similarly, the means for the 1.45 mm vials with PTFE/butyl liners and Münnerstädter

TABLE 9

EVALUATION #2

MOISTURE CONTENT OF REAGENT IN PERCENT
21 DAYS

STORAGE ENVIRONMENT	1.2mm VIALS MÜNNERSTÄDTER (PLUGGED) (%)	1.2mm VIALS COMAR (TEFLON/BUTYL) (%)	1.45mm VIALS COMAR (TEFLON/BUTYL) (%)
REFRIGERATED			
n	15	15	15
mean	2.8253	2.7325	2.8743
sd	0.0658	0.0742	0.1250
max	2.9310	2.8355	3.1835
min	2.6850	2.6165	2.7330
range	0.2460	0.2190	0.4505
ROOM TEMP.			
n	15	15	15
mean	2.8286	2.7412	2.8427
sd	0.0550	0.0711	0.0964
max	2.9010	2.8730	3.0410
min	2.7135	2.6165	2.6425
range	0.1775	0.2565	0.3985
37°C TEMP.			
n	15	15	15
mean	2.8364	2.6730	2.9309
sd	0.0676	0.1000	0.1930
max	2.9420	2.8775	3.3325
min	2.7170	2.5640	2.7120
range	0.2250	0.3135	0.6205

REAGENT: SODIUM PHOSPHATE BUFFER

TABLE 10

STATISTICAL COMPARISONS

Sample 1	Sample 2	Null Hyp.	If null is rejected which mean is higher?	Difference 1 vs 2 (% H_2O)
Refrigerated Plug & Vial	Refrigerated 1.2 mm Comar	Reject	Refrigerated Plug & Vial	0.0928%
Refrigerated Plug & Vial	Refrigerated 1.45 mm Comar	Accept	N/A	N/A
Refrigerated 1.2 mm Comar	Refrigerated 1.45 mm Comar	Reject	Refrigerated 1.45 mm Comar	0.1418%
Room Temp. Plug & Vial	Room Temp. 1.45 mm Comar	Accept	N/A	N/A
Room Temp. Plug & Vial	Room Temp. 1.2 mm Comar	Reject	Room Temp. Plug & Vial	0.0874%
Room Temp. 1.45 mm Comar	Room Temp. 1.2 mm Comar	Reject	Room Temp. 1.45 mm Comar	0.1015%
37°C Humid Plug & Vial	37°C Humid 1.2 mm Comar	Reject	37°C Humid Plug & Vial	0.1491%
37°C Humid Plug & Vial	37°C Humid 1.45 mm Comar	Accept	N/A	N/A
37°C Humid 1.2 mm Comar	37°C Humid 1.45 mm Comar	Reject	37C 1.45 mm Comar	0.2436%
Refrigerated Plug & Vial	Room Temp. Plug & Vial	Accept	N/A	N/A
Refrigerated Plug & Vial	37°C Humid Plug & Vial	Accept	N/A	N/A
Room Temp. Plug & Vial	37°C Humid Plug & Vial	Accept	N/A	N/A

TABLE 10 - Continued

Sample 1	Sample 2	Null Hyp.	If null is rejected which mean is higher?	Difference 1 vs 2 (% H_2O)
Refrigerated 1.2 mm Comar	Room Temp. 1.2 mm Comar	Accept	N/A	N/A
Refrigerated 1.45 mm Comar	Room Temp. 1.45 mm Comar	Accept	N/A	N/A
Refrigerated 1.2 mm Comar	37°C Humid 1.2 mm Comar	Accept	N/A	N/A
Refrigerated 1.45 mm Comar	37°C Humid 1.45 mm Comar	Accept	N/A	N/A
Room Temp. 1.2 mm Comar	37°C Humid 1.2 mm Comar	Accept	N/A	N/A
Room Temp. 1.45 mm Comar	37°C Humid 1.45 mm Comar	Accept	N/A	N/A

Statistic: Student's t-test, 95% Confidence Interval.

Null Hypothesis: Mean of population (sample 1) is equal to the Mean of population (sample 2).

plugged vials are not different when comparing water content from the three environments. Statistical comparisons of the different closures within a test environment show that means for the percent moisture in the control Münnerstädter vials are not statistically different from the Comar 1.45 mm vials.

A statistically significant difference in the water content mean did occur in Evaluation 2 when 1.2 mm Comar vials were compared to either the 1.45 mm Comar vials or the Münnerstädter vials within the same storage environment. The mean percentage moisture for the reagent inside the Comar 1.2 mm vials was lower. This was true for all three test environments. Conversely, the largest difference between any two statistically different means was only 0.243% and generally does not represent a meaningful difference in terms of package system integrity for a pharmaceutical reagent product. Although a statistical difference was demonstrable between the Comar 1.2 mm closure and the other two closures, none of the three closure systems exhibited any significant increase in moisture when exposed to increasing moisture challenges from refrigerated to room temperature humid, to 37 degrees humid. If there was a difference in the efficacy of the closure, the expectation would be an increase in the moisture adsorbed proportional to the increase of the challenge to the barrier.

CHAPTER 9

CONCLUSION

The integrity of the elastomeric flat liners versus stoppers has been verified using coulometric titration. The flat liners were used in place of the stoppers on a special line of commercial pharmaceutical reagents manufactured by EM Diagnostic Systems, Gibbstown, New Jersey. Since the commencement of commercial production using the flat liners, over two million closures have been applied to small glass vials containing moisture sensitive reagents with no reports of negative product performances related to package integrity issues. For commercial purposes the flat liners, as described on correctly engineered glass vials, have similar moisture barrier characteristics to the traditional elastomer stoppers.

The secondary goal of this research work was to introduce the coulometric titration as an additional packaging comparative evaluation tool for packaging professionals. This research does not promote coulometric titration to the exclusion of other integrity evaluations currently accepted by organizations such as ASTM or USP. Additional research should be conducted to establish correlation between coulometric titration and more traditional test methods, such as gravimetric test methods. There may be a repeatable

relationship to be defined between the total weight change methods using traditional absorbents such as silica gel in a candidate packaging system versus the actual water content determined on the coulometric titration instrument.

CHAPTER 10

FUTURE CONSIDERATIONS

The package system evaluations conducted in Chapter 8 describe the use of coulometric titration to evaluate water permeation between a traditional "acceptable" water vapor barrier vial packaging system versus an "unknown" packaging system. There is the possibility, with further investigations, that coulometric titration could have broader applications as a standard test method for water vapor permeability for a variety of packaging systems.

The ASTM standards currently list several test methods for permeation or leak evaluations for finished packages and heat-sealed packages containing dry products along with screw closure integrity evaluations. Most of the ASTM methods utilize either weight changes, bubble testing, or infrared leak detection which may not accurately assess an extremely low water vapor permeation transmission rate through or around candidate packaging systems.

Further studies involving coulometric titration techniques could demonstrate how the methodology could be used as a verification tool to supplement the weight change data generated by the traditional gravimetric methods.

With continued investigations and confidence in coulometric titration analysis, the coulometric titration

process could qualify as a stand-alone alternative to existing leak determination methods in high moisture barrier packaging systems.

It is recommended, however, that before coulometric titration is fully considered as a method of moisture determination, that the weight gain versus water content relationship between common commercially available absorbents be identified and established. Absorbents, such as silica gel or molecular sieve, are suggested as initial materials to investigate. Silica gel shows promise since it is chemically inert and should not affect the coulometric reagents. Molecular sieve is also promising since it will reach moisture equilibrium in a low (1% - 15%) relative humidity environment and has a fast water absorption rate. The candidate absorbents should be openly exposed to a series of controlled (relative humidity with temperature) environments. The absorbent's weight changes are compared to absorbent's coulometrically determined water content for correlation studies.

If there is a demonstrated repeatable relationship between the absorbents and coulometric titration, the next series of investigations could be conducted using ASTM D 3199-84 (Standard Test Method for Water Vapor Transmission through Screw-Cap Closure Liners) as an outline for further evaluations. Glass containers are suggested in order to exaggerate and expedite the evaluation process as is the

suggestion to select commercially available liner material capable of permitting a predictable level water permeation through the closure. Liners such as PVLF (pulp & vinyl) or PE solid/foam coextrusions (Tri-Seal's F-217), and shells of polypropylene are recommended.

It also advocated that several packaging system sub-populations be created to incorporate the thermal pressure differential effects created by the weekly weight checks when the packages are taken in and out of the environmental stress chambers. The other sub-populations that are stressed should remain undisturbed for the duration of the study.

APPENDIX I

TABLE #A-1

PERRY GUN FILLING PRECISION DATA IN mg

SETUP	AFTER 90 FILLS (mg)	AFTER 180 FILLS (mg)	AFTER 270 FILLS (mg)
	202.8	195.4	195.8	195.4
	204.6	198.8	199.6	200.5
	205.0	198.0	200.5	195.5
	200.7	203.2	197.8	203.4
	199.6	205.7	204.5	204.8
	203.9	204.3	203.1	200.1
	203.4	202.4	206.3	195.2
	205.8	199.4	201.2	204.3
	198.0	200.8	203.7	198.4
	197.9	199.1	209.3	199.0
n	10	10	10	10
mean	202.2	200.7	202.2	199.7
sd	2.913	3.1684	4.0351	3.6546
max	205.8	205.7	209.3	204.8
min	197.9	195.4	195.8	195.2
range	7.9	10.3	13.5	9.6

NOTE: Application of the Student's t-test at the 95 percent Confidence Interval indicated that no mean of any group of data contained in this table is statistic significantly different from any other mean. Therefore all the samples were drawn from the same population and there was no bias introduced in the filling process.

TABLE #A-2

PERCENT MOISTURE / STORED REFRIGERATED

SAMPLE NUMBER	1.2mm VIALS MÜNNERSTÄDTER (PLUGGED) (% H_2O)	1.2mm VIALS WEST CO. (BUTYL ONLY) (% H_2O)	1.2mm VIALS WEST CO. (TEFLON/BUTYL) (% H_2O)
1	2.9240	2.7535	2.6965
2	2.9220	2.8620	2.8015
3	2.7445	2.7470	2.7295
4	2.9540	2.8055	2.7155
5	2.8305	2.7600	2.6620
6	2.8815	2.7610	2.8955
7	2.9345	2.9780	2.9675
8	2.9270	2.8745	2.9085
9	2.9735	2.8485	2.9055
10	2.9120	2.8435	2.8245
11	2.9180	2.7805	2.6810
12	2.8950	2.7420	2.7570
13	2.7995	2.7090	2.7035
14	2.8430	2.8385	2.6635
15	2.8765	2.8270	2.7210
16	2.8655	2.7720	2.7460
17	2.9840	2.8010	2.8015
18	2.8060	2.8230	2.8165
19	2.9075	2.7800	2.9240
20	2.8520	2.7745	2.8610
21	2.7840	2.7425	2.7645
22	2.7940	2.7160	2.7325
23	2.8030	2.8395	2.7710
n	23	23	23
mean	2.8753	2.7991	2.7848
sd	0.0656	0.0610	0.0895
max	2.9840	2.9780	2.9675
min	2.7445	2.7090	2.6620
range	0.2395	0.2690	0.3055

TABLE #A-3

PERCENT MOISTURE / STORED ROOM TEMPERATURE

SAMPLE NUMBER	1.2mm VIALS MÜNNERSTÄDTER (PLUGGED) (% H_2O)	1.2mm VIALS WEST CO. (BUTYL ONLY) (% H_2O)	1.2mm VIALS WEST CO. (TEFLON/BUTYL) (% H_2O)
1	2.7745	2.9290	2.7400
2	2.9235	2.7770	2.7100
3	2.8155	2.7205	2.8330
4	2.9215	2.7440	2.7255
5	2.9645	2.8435	2.7445
6	2.8760	2.7025	2.7915
7	2.9010	2.6850	2.7965
8	3.0170	2.8725	2.8935
9	2.8785	2.8805	2.7220
10	2.8470	2.6970	2.7730
11	2.7130	2.7895	2.7670
12	2.8895	2.7985	2.7070
13	2.8850	2.7035	2.6680
14	2.8180	2.7860	2.8805
15	2.8690	2.6150	2.7875
16	3.0190	2.7890	2.7815
17	2.9290	2.9520	2.8370
18	2.9690	2.7175	2.8455
19	2.9915	2.7635	2.7185
20	2.8850	2.7855	3.0425
21	2.7840	2.6175	2.8160
22	2.9485	2.8070	2.7305
23	2.8645	2.8150	2.7605
n	23	23	23
mean	2.8906	2.7735	2.7857
sd	0.0775	0.0870	0.0804
max	3.0190	2.9520	3.0425
min	2.7130	2.6150	2.6680
range	0.3060	0.3370	0.3745

TABLE #A-4

STORED AT 37°C HUMID

SAMPLE NUMBER	1.2mm VIALS MÜNNERSTÄDTER (PLUGGED) (% H_2O)	1.2mm VIALS WEST CO. (BUTYL ONLY) (% H_2O)	1.2mm VIALS WEST CO. (TEFLON/BUTYL) (% H_2O)
1	2.7940	2.7565	2.7610
2	2.6245	2.6785	2.7440
3	2.7965	2.8165	2.8745
4	2.8505	2.7430	2.7455
5	2.8440	2.7190	2.7015
6	2.8620	2.7935	2.7210
7	2.9355	2.7900	2.7895
8	2.9585	2.7185	2.8100
9	2.8820	2.7325	2.6435
10	2.8690	2.7790	2.7330
11	2.7880	2.7545	2.6990
12	2.8760	2.6765	2.8735
13	2.8655	2.7185	2.6135
14	2.7965	2.7885	2.6640
15	2.7350	2.5760	2.6675
16	2.7460	2.5745	2.6370
17	2.8030	2.8395	2.6585
18	2.9280	2.7755	2.7940
19	2.8145	2.7440	2.7535
20	2.9710	2.7780	2.6005
21	2.8505	2.6310	2.6910
22	2.8740	2.7415	2.7165
23	2.8400	2.8140	2.7915
n	23	23	23
mean	2.8393	2.7365	2.7254
sd	0.0773	0.0703	0.0751
max	2.9710	2.8395	2.8745
min	2.6245	2.5745	2.6005
range	0.3465	0.2650	0.2740

TABLE #A-5

PERRY GUN FILLING PRECISION DATA IN mg

SETUP	DURING FILLS OF 1.45mm COMAR VIALS (mg)	DURING FILLS OF VIALS (TO BE PLUGGED) (mg)	DURING FILLS OF 1.2mm COMAR VIALS (mg)
	200	194	194	196
	196	201	195	197
	203	196	230	196
	200	197	198	194
	207	197	196	203
	202	196	204	189
	199	198	196	195
	197	196	200	189
	195	192	195	193
	203	199	203	198
		198	202	199
		197	202	198
			189	195
			198	193
n	10	12	14	14
mean	200.2	196.8	198.2	195.4
sd	3.68	2.30	4.34	3.75
%CV	1.84	1.17	2.19	1.92
max	207	201	204	203
min	195	192	189	189
range	12	9	15	14

NOTE: Application of the Student's t-test at the 95% Confidence Interval indicated that no mean of any group of data collected during the filling of the vials is statistically significantly different from any other mean during filling. However the mean for the Perry gun adjustment before filling is slightly (and statistically significantly) higher than the other means. Therefore it can be concluded that the technique during filling of the vials was consistant and reproducable and introduced no bias to the moisture evaluation.

TABLE #A-6

PERCENT MOISTURE / STORED REFRIGERATED

SAMPLE	1.2mm VIALS MÜNNERSTÄDTER (PLUGGED) (%)	1.2mm VIALS COMAR (TEFLON/BUTYL) (%)	1.45mm VIALS COMAR (TEFLON/BUTYL) (%)
1	2.7855	2.7285	2.9945
2	2.7610	2.7660	2.9380
3	2.6850	2.7785	2.9390
4	2.8505	2.6165	2.9915
5	2.7950	2.7525	2.8170
6	2.9310	2.7175	2.8690
7	2.8575	2.7195	2.8545
8	2.9265	2.8070	3.1835
9	2.8310	2.7320	2.8315
10	2.8615	2.7965	2.6780
11	2.7580	2.5580	2.8975
12	2.8340	2.7945	2.7570
13	2.8910	2.7225	2.7905
14	2.7965	2.6630	2.7330
15	2.8150	2.8355	2.8395
mean	2.8253	2.7325	2.8743
sd	0.0658	0.0742	0.1250
%cv	2.33	2.71	4.35
max	2.9310	2.8355	3.1835
min	2.6850	2.6165	2.7330
range	0.2460	0.2190	0.4505

TABLE #A-7

PERCENT MOISTURE / STORED ROOM TEMPERATURE

SAMPLE	1.2mm VIALS MÜNNERSTÄDTER (PLUGGED) (%)	1.2mm VIALS COMAR (TEFLON/BUTYL) (%)	1.45mm VIALS COMAR (TEFLON/BUTYL) (%)
1	2.76500	2.6560	3.0410
2	2.74550	2.8730	2.8520
3	2.72350	2.7195	2.7775
4	2.86800	2.7415	2.8590
5	2.87500	2.6165	2.8560
6	2.82100	2.7085	2.8710
7	2.86700	2.8520	2.8825
8	2.87800	2.7335	2.8865
9	2.90100	2.7570	2.7610
10	2.87250	2.7410	3.0050
11	2.80000	2.7690	2.7885
12	2.78050	2.7970	2.8315
13	2.89150	2.7370	2.7995
14	2.86465	2.6360	2.7820
15	2.82400	2.7805	2.6425
mean	2.8286	2.7412	2.8427
sd	0.0550	0.0711	0.0964
%cv	1.95	2.59	3.39
max	2.9010	2.8730	3.0410
min	2.7135	2.6165	2.6425
range	0.1775	0.2565	0.3985

TABLE #A-8

PERCENT MOISTURE / STORED 37°C

SAMPLE	1.2mm VIALS MÜNNERSTÄDTER (PLUGGED) (%)	1.2mm VIALS COMAR (TEFLON/BUTYL) (%)	1.45mm VIALS COMAR (TEFLON/BUTYL) (%)
1	2.8400	2.5815	2.9065
2	2.8105	2.5640	3.0950
3	2.8355	2.6240	2.8775
4	2.8490	2.8170	2.8285
5	2.8690	2.7910	2.9315
6	2.8355	2.6680	3.0035
7	2.8630	2.8015	2.9385
8	2.8270	2.6210	3.1720
9	2.9270	2.6760	3.3325
10	2.8960	2.5715	2.8435
11	2.7300	2.6640	2.7515
12	2.7170	2.5875	3.1700
13	2.7285	2.8775	2.7120
14	2.8465	2.7390	2.6505
15	2.9420	2.7255	2.7510
mean	2.8364	2.6730	2.9309
sd	0.0676	0.1000	0.1930
%cv	2.38	3.72	6.59
max	2.9420	2.8775	3.3325
min	2.7170	2.5640	2.7120
range	0.2250	0.3135	0.6205

APPENDIX II

AQUASTAR® C2000 INSTRUCTION MANUAL

AQUASTAR ®C2000 COULOMETRIC TITRATOR

INSTRUCTION MANUAL

Table of Contents

1. General

The AQUASTAR ®C2000 is an automatic titrator designed to measure trace quantities of water by the coulometric titration process. The AQUASTAR C2000 Titrator features rapid measurement, high efficiency and high accuracy. All operations, such as titration control, end point detection, concentration calculation and statistics are carried out by a microcomputer housed in the instrument; all necessary data is printed out on a thermal dot printer. Samples can be weighed rapidly when the instrument is connected to an elelctronic balance.

The measuring capabilities of the AQUASTAR C2000 Titrator ranges from 10 µg H_2O, with an accuracy of ±1 µg H_2O to 30 mg H_2O, with an accuracy of ±0.3%. The detection sensitivity is 0.1 µg H_2O. The instrument automatically compensates for any background moisture and sounds an alarm at the end of titration.

The AQUASTAR C2000 Titrator has six data handling modes for calculating the ideal sample size, as well as the mean, standard deviation and coefficient of variation for up to 20 repetitive analyses. It has 7 option modes with memory functions for tabulating reagent consumption and date reagents were changed. The instrument can provide a graphical display of titration vloume vs. time which can be used in selecting the optimum heating temperature when C2000 is used in conjunction with special accessories. The AQUASTAR C2000 Titrator gives the operator options for current control and titration time for each determination.

The versatility and effisiency of measurement is enhanced with the file function capabilities of the instrument. All operating parameters and titrating conditions can be stored in advance in the instrument memory and recalled when needed.

Samples can be measured in the form of liquid, solid or gas. Special accessories are available for samples that cannot be measured by direct addition. These accessories are the Solid and Oil Evaporators. Combined with the Solid Evaporator accessory the AQUASTAR C2000 Titrator permits measurement of water content in solids and powders without interference from humidity. The Oil Evaporator permits measurement of water content in oils that normally interfers with moisture determinations.

The instruction manual contains operating, maintenance and troubleshooting information for the efficient operation and handling of the AQUASTAR C2000 Titrator. We highly recommend AQUASTAR PYRIDINE FREE reagents be used in conjunction with the AQUASTAR Titrator. AQUASTAR Reagents are optimized for use with the C2000, are virtually odorless and replace conventional pyridine type reagents. It will be necessary to select the composition of the generator solution and the sampling mehtod, which may vary, depending on the properties of each smaple. To assist you we have prepared a "Selection Table"; it is in the Application Manual for the AQUASTAR instruments. AQUASTAR Reagents are available through EM SCIENCE distributors.

2. Standard Specifications and Components

2-1 Standard Specifications

Titration process	:	Coulometric titration process
Electrolytic control method	:	Constant current electrolysis, intermittent electrolysis in the vicinity of the end point only.
End point detection method	:	AC polarization potential difference detection method.
Automatic end point stop system	:	(1) Automatic stop at end point by wait time (Wait time: Max. 99 sec.) (2) Titration time setting system (Set by timer: Max. 99 min.)
Automatic compensation range for background	:	0 - 50 μg H_2O/min.
Display	:	20-digit liquid crystal display
Display unit	:	μg H_2O, %, ppm
Detection sensitivity	:	0.1 μg H_2O
Accuracy	:	Within $\pm$1 μgH_2O during 10 - 1000 μgH_2O measurement (when sampling more than 1 g of water-methanol) Within $\pm$0.3% during 1 - 30 mgH_2O measurement
Measuring range	:	10 μg H_2O - 30 mg H_2O
Required measuring time	:	1 mg H_2O/50 sec.
Titration cell capacity	:	150 ml
Printer	:	Thermal dot printer
Chart width	:	80 mm
Printing contents	:	Measuring date, sample No., measuring value (μgH_2O), sampling quantity (g, mℓ, ℓ), moisture concentration (%, ppm), average value, standard deviation, coefficient of variation, moisture quantity-time curve recording, generator reagent total electrolytic quantity and counter reagent total electrolytic quantity, Global titrating condition list.

Optional functions	:	1. Generator, Anode reagent total electrolytic quantity (mgH_2O), filling date (G TOTAL)
		2. Counter, Cathode reagent total electrolytic quantity (mgH_2O), filling date (C TOTAL)
		3. Total electrolytic quantity (mgH_2O) of ion exchange membrane, mounting date (MEMBRANE)
		4. Moisture quantity-time curve (T-CURVE)
		5. Optimum sample size (SIZE)
		6. Blank value setting (BLANK-V)
		7. Electrolytic current setting and display (CURRENT)
Timer functions	:	1. Titration timer 0-99 min. (T-TIMER)
		2. Titration start delay timer 0-99 min. (S-TIMER)
File function	:	4 files for storing titrating conditions
Alarm and display		
Key input buzzer	:	2-second alarm in case of an input error during keying entry operation
Titration end buzzer	:	5-second alarm when titration ends
Background	:	Normal display in the unit of $\mu gH_2O/min.$
Electrolytic current	:	Display by keying operation
Detection potential	:	Normal display by LED
External input/output	:	Balance interface or RS232C data output
Power supply	:	AC 110V, 50/60Hz, 100VA Rechargeable battery is included for memory storage
Dimensions	:	320mm wide x 310mm deep x 270mm high
Weight	:	9.8 kg

2-2 Standard Components

	Quantity
Basic unit (Cat #AX1696D/1)	1 unit
Electrolytic cell (Cat #3200181)	1 pc.
Indicator electrode, TPT-72 (Cat #A7108061)	1 pc.
Syringes; 5 ml with a long needle (Cat #A1000006) and 2 ml (Cat #A1000007) with a short needle	1 pc. each
Microsyringe (with a stopper) (Cat #A3202801)	1 pc.
Silicone rubber block (Cat #A7202361)	10 pcs.
Fluorine grease (Cat #A3202111)	1 bottle
Ion exchange membrane (10 sheets) (Cat #A7806481)	1 set
Rubber septums for sample injection (Cat #A1000047)	10 pcs.
Stirrer magnet (8mm dia. x 30mm) (Cat #A1000014)	1 pc.
Glass-enclosed fuse (4A) (Cat #A0000035)	2 pc.
Power cord with AC plug (Cat #A7803181)	1 pc.
Vinyl instrument cover (Cat #A7704211)	1 pc.
Ground wire 3 m (Cat #A7803201)	1 pc.
Washing bottle (with a Teflon tube) (Cat #A7806621)	1 pc.
Funnel (Cat #A1000364)	1 pc.
Chart paper (TR-80) (Cat #A2232101)	3 rolls
Instruction manual	1 copy

2-3 Reagent Kit

Generator Solution:	AQUASTAR Coulomat A Reagent for general use (Cat #AX 1697A/1)	500 mℓ
Counter Solution :	AQUASTAR Coulomat C Reagent (Cat #AX1697C/1)	25 mℓ
Silica Gel :	T.H.E.™ Desiccant (Cat #DX0017/1)	500 g
Applications Manual		1 copy

3. Installation

3-1 Location

The instrument should be located out of sunlight, in an area with minimum temperature change, avoid locating near areas of magnetic force, vibration, corrosive gas and water vapor.

3-2 Power

The power requirements is 110V A.C., single phase, 50 Hz or 60 Hz, with consumption of approximately 100 VA.

3-3 Connection of Power Cord

Caution: Insure the electrical switch, located lower left is off (downward position). Connect cord to the AC IN connector located on rear panel. Plug cord into 110V AC outlet.

3-4 Grounding

When a 3-pin outlet is used, grounding is automatic; connection of the ground wire is not required.

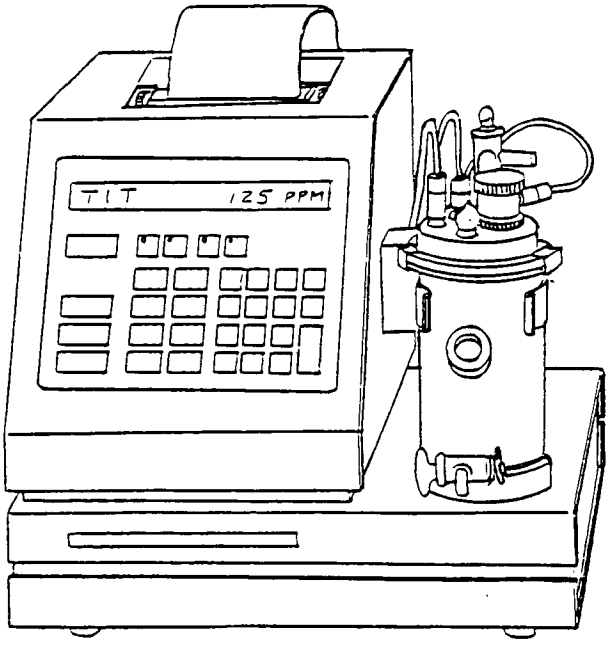


Fig. 3-1 Overall View of AQUASTAR C2000 coulometric Titrator

3-5 Loading of Chart Paper

To load chart paper, follow the procedure given below. Fig. 3-2 shows the Chart paper loading drawing.

(1) Raise the top cover of the unit.

(2) Remove the chart bobbin.

(3) Mount the chart paper to the chart bobbin, and insert into the chart holder.

(4) Insert the chart paper into the printer by feeding.

(5) The chart paper with the chart paper thumb wheel (DO NOT FORCE).
CAUTION: Use only AQUASTAR replacement paper (Cat#A2232101).
Any other type of paper may cause damage to the printer head.

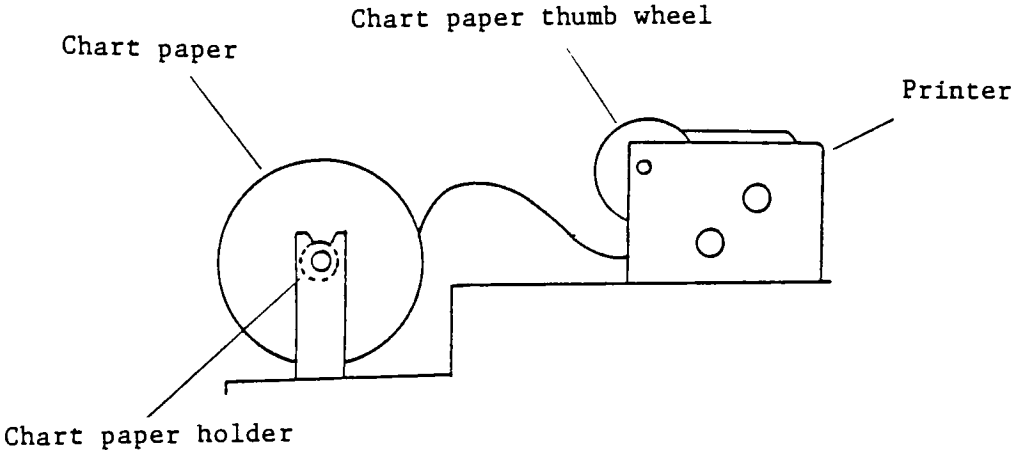


Fig. 3-2 Chart Paper Loading

3-6 Assembly of Electrolytic Cell (Fig. 3-3)

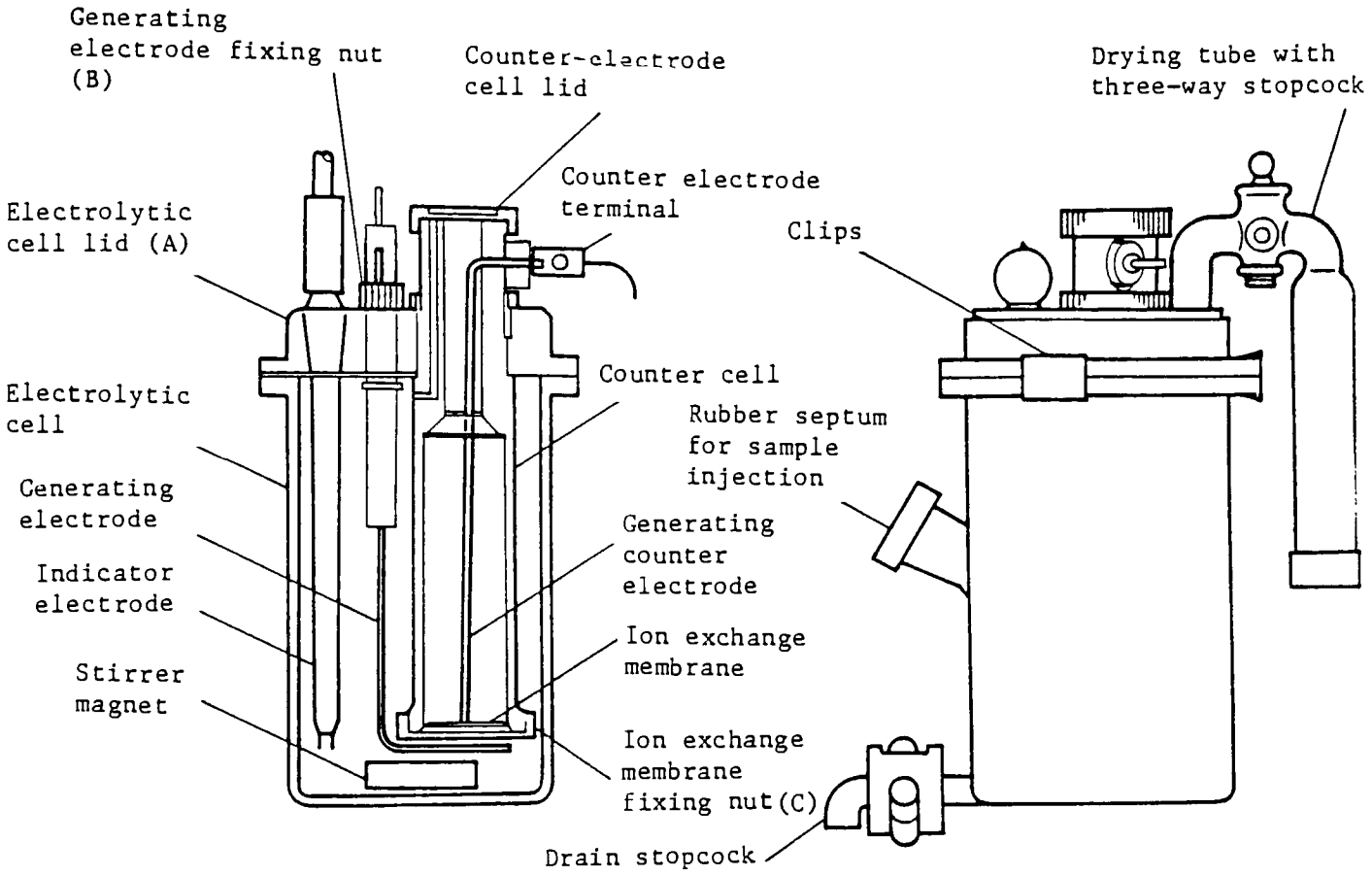


Fig. 3-3

① Preparation for the ion exchange membrane.
Remove ion exchange membrane from the container using tweezers. Be careful not to damage or bend the membrane; hold it at its end. Blot off excess liquid and crystals on membrane with filter paper. Do not dry for prolonged period or at a high temperature.
To wash the membrane, use dehydrated methanol (Cat #AX1699M-1)
Store the ion exchange membrane in a well of dehydrated generator solution (Coulomat A, item AX1697A-1).

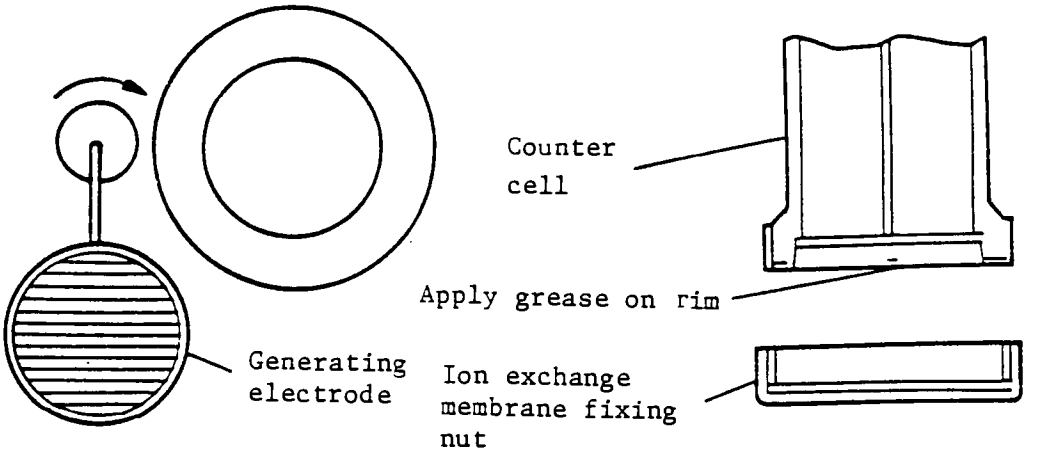
② Mounting of the ion exchange membrane
To mount the ion exchange membrane, remove the electrolytic cell lid (A) from the cell and proceed in the order of i), ii), iii), and iv) as shown in Fig. 3-4.

③ Attaching the electrolytic cell lid.
Apply fluorine grease to the fitting surface of the electrolytic cell lid, and fasten electrolytic cell lid to the electrolytic cell with three clips. (See Fig. 3-3)

④ Putting in the stirrer magnet.
Lower the stirrer magnet (supplied) into the cell through the injection port.

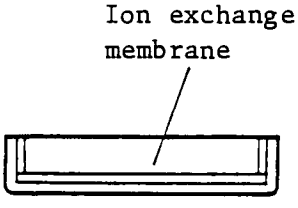
⑤ Installation of the indicator electrode.
Remove the protective cover from the tip of the electrode and carefully remove the protective sleeve from the shaft.
Apply fluorine grease to the fitting surface of indicator electrode and insert it through the electrolytic cell lid.

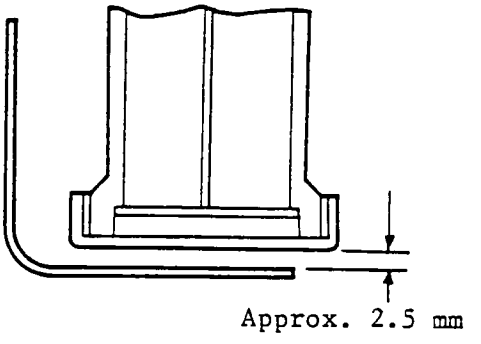
⑥ Installation of the cell.
Insert assembled electrolytic cell into the cell holder on basic unit. Place drying tube with Desiccant in back right hole after applying grease. Place small glass stopper in front hole after applying grease. Place septum over injection port.
(See Fig. 3-3.)



(i) Slightly loosen the fixing nut (B) shown in Fig. 3-3 turn the generating electrode Clockwise as shown above.

(ii) Apply thin, uniform coat of fluorine grease to the bottom rim of counter cell.





(iii) Install membrane inside the membrane fixing nut (C).

(iv) Firmly tighten fixing nut to counter cell by screwing it tight. Return generating electrode to original position under counter cell and tighten the electrode fixing nut.

Fig. 3-4 Installation of Ion Exchange Membrane

3-7 Preparation for Measurement

① For optimum results use EM SCIENCE. AQUASTAR Reagents; Coulomat A (Cat #AX1697A-1) for the generator solutions and Coulomat C (Cat #AX1697C-1) for the counter solution. See the replacement part list for other available reagents.

② Filling the generator solution

NOTE: Quickly fill generator solution to ensure the reagent does not absorb moisture.

(i) Make sure that the electrolytic cell stopcock is closed.

(ii) Open the bottle containing AQUASTAR Coulomat A Reagent.

(iii) Remove the stopper on the electrolytic cell lid, attach supplied funnel, pour the generator solution up to the 100 ml marker of the electrolytic cell.

③ Filling the counter solution

(i) Open the bottle containing AQUASTAR Coulomat C Reagent.

(ii) Remove the counter cell lid and add 5 ml of the solution in the bottle through the funnel to remove residual moisture.

(iii) Use washing bottle to remove initial counter solution. Be careful not to damage the generating electrode membrane. Note: This step is only nesessary during the initial set up.

(iv) Pour the remaining counter solution into the counter cell to near the 150 ml marker on the electrolytic cell.

(v) Put on the counter cell lid.

④ Connection of electrodes (Fig. 3-3)

(i) Insert the indicator electrode banana plug into the indicating electrode jack behind the cell holder.

(ii) Connect the red lead wire of the generating electrode plug to the generating electrode above the red fiting nut and the black lead wire to the counter electrode below the black cap.

Note: Be careful not to tighten the screw excessively.

4. Measuring Operation

The following describes operations for measuring liquid samples. For measurement of solid, gaseous or viscous samples, refer to the AQUASTAR applications manual.

4-1 Turning on Power

Turn on the POWER switch located on the left side. The stirrer rotates and a blank deleting starts immediately. The display shows [N 99 BLANK 0 µg]. The background is 99 and blinking. The detector Pilot Light (PL) is green.

4-2 Confirmation of Blank Elimination End Point

First, electrolysis is carried out continuously, and most of the water content is eliminated, when the pilot lamp of the DETECTOR changes from green PL to yellow PL. The time required for elimination of the blank is nearly in proportion to the volume of water content in the generator solution. The background value gradually becomes smaller. It will generally take about 30 minutes before the water content in the air in the titration cell and water content on the cell wall are eliminated. To shorten this time proceed as follows:

① Wait until anode reagent is a slight yellow color.
② Turn power OFF.
③ Disconnect all three electrode leads.
④ Insure three way stopcock on drying tube is closed to cell so no reagent will enter into the desiccant.
⑤ Remove entire cell from holder and gently tilt and rotate so reagent touches cell lid and septum entrance.
⑥ Return cell to holder and reconnect the three electrode leads. Turn power ON.

Note: Occasionally, the DETECTOR red PL. may light and the background of zero may be indicated from the beginning because excess iodine may be contained in the anode regent. In such a case, add 2-3 µℓ of pure water by a micro syringe or water/methanol standard (item #AX1697D-1)until the detector green PL. comes on and the electrolysis starts.

4-3 Operating Parameters

The sample weight is measured by weighing the syringe before and after sample injection using an analytical balance (CAL MODE 1).
Input by FILE Key and Function Key
Set the [FILE] key and Function key according to the sample moisture content and sampling method. The following shows an example of a general sample measurement.

Sample : methanol (water content approx. 500 ppm)
Sampler: 5 mℓ syringe
Balance: chemical balance (sensitivity 0.1 mg)

Weigh the sampled amount by measuring the weight of the syringe before and after injecting the sample.

(1) Setting FILE key

FILE → [FILE 1] selected

(2) STIRRER SW -- OFF

(3) Setting function keys

[DATE] 11/19/1987 → [ENTER]

[SAMPLE NO] 1 → [ENTER]

[CAL MODE] 1 → [ENTER] $X = \frac{\text{FOUND}}{\text{SIZE 1-SIZE 2}}$

[INTERVAL] 10 → [ENTER]

[TIMER] (T-TIMER, S-TIMER) 0

[OPT]

1	[CE] → [ENTER]	Initialization of the electrolytic volume of the generator solution, counter solution, and the date of exchange of ion exchange membrane.
2	[CE] → [ENTER]	
3	[CE] → [ENTER]	
4	[0] → [ENTER]	(T-TIMER = OFF)
6	[0] → [ENTER]	(BLANK = 0)
7	[0] → [ENTER]	(SLOW)

OPT 1, 2, 3 are not necessary unless exchange data and amount titrated need to be checked.

4-4 Sampling

① Apply furnished grease to the fitted surfaces and top end of the syringe.

② Put the needle on the syringe.

③ Aspirate a sample into the syringe and wash the interior 1-3 times with the sample. Collect about 2 mℓ of sample, aspirate air until no sample remains in the needle, and immediately put a silicone rubber block on the syringe needle end (take care not to pierce) to avoid sample leakage and moisture absorption (obtain the sampling amount with OPT 5 beforehand).

④ Quickly weigh the above syringe accurately. Assume the weighed value is 35.1251 g.

⑤ Make sure "S" mark blinks (status where background is stable). Press the [SAMPLE] key. The stirrer stops and the background value is held. The display indicates SAMPLE NO 1.
An underline is printed on the chart.

⑥ Remove the silicone rubber block from the syringe, insert the syringe into the rubber septum. Inject the sample gently taking care not to allow the needle tip to contact the generator solution.

⑦ While the syringe needle is still inside the cell draw the remaining sample in the tip of the needle into the syringe. Remove the needle and insert the needle into the silicone rubber block immediately.

4-5 Start of Titration

① Press the [TITRATION] key.
The stirrer rotates and titration starts in 5-6 seconds.
Counting starts and the result is indicated on the display in μgH_2O.

② Weigh the syringe accurately after sampling injection.
Assume the weighed value is 33.5041 g.

③ End of titration
As titration approaches the end point, electrolysis becomes intermittent. So even if counting stops, it does not mean the end of titration. The titration ends when the result of determination is printed out and the buzzer sounds.
The printer prints out the following data.

DATA	1982/1/19
SAMPLE NO	1
H_2O	965.2 µg
BLANK	0 µg
H_2O	965.2 µg

Note: Immediately before the end of titration, the indicated value may increase or decrease, this is not an erroneous operation, but a correction of over titration.

④ Input of sample size
When titration ends, BLANK eliminating operation starts automatically.
Press the SAMPLE SIZE key to input the sample amount.

[SAMPLE SIZE] [3] [5] [·] [1] [2] [5] [1] [-] [3] [3] [·] [5] [0]
[4] [1] [ENTER]

DATE	1982/11/19
SAMPLE	1
H_2O	965.2 µg
BLANK	0 µg
SIZE	35.1251 g
-	33.5041 g
	1.6210 g
H_2O	595.4 PPM

Note: The sample size can be entered any time after the end of titration until the SAMPLE key is pressed again.

⑤ Repetitive measurements
In the following measurement, be sure to follow the procedure given above after verifying "S" mark is blinking and that the background is stable.
Repetitive operation is as follows:

a) Weight syringe with sample.
b) Press [SAMPLE].
c) Carefully add sample to cell.
d) Press [TITRATION]
e) Reweight syringe
f) Input sample size by pressing [SAMPLE SIZE], enter weights, [ENTER].

Note: When copies of the data are needed, press the [SAMPLE SIZE] key and then the [ENTER] key. The same data is printed out.

4-6 Instrument Shutdown

Turn off the POWER switch. The operating parameters set in the FILE keys and function keys are stored in the battery backup memory, so it is not necessary to set them again if measurment is made under the same conditions after turning power back on. However, check the data by pressing the LIST key before measurement.

4-7 Other Sample Measurement

For other sample measurment, only the setting of the CAL MODE, TIMER, INTERVAL, etc. may differ depending on the method of sampling, method of weighing, etc.
Create files appropriate for particular samples before measurement.

4-8 Measurement Using OPT 4

When determination is made by the Solid or Oil Evaporator Accessory, record the processes of evaporation during heating graphically by using the graph to determine optimum heating temperature.

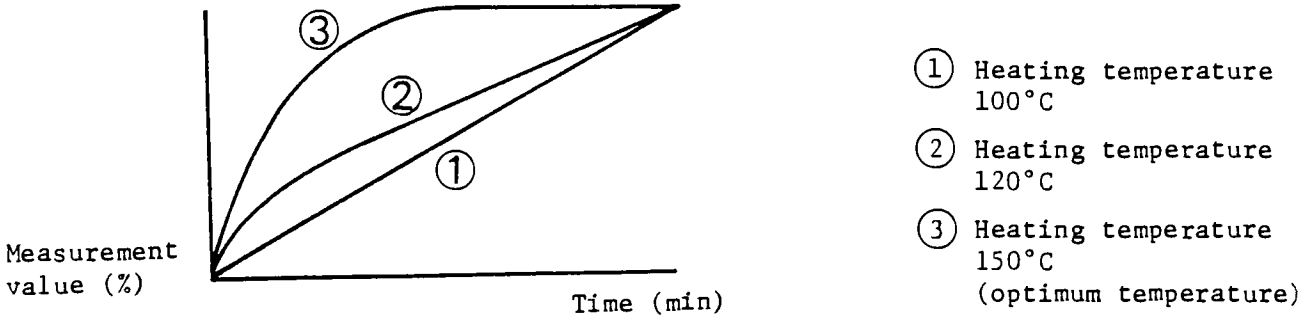


Fig. 4-1 Example of Application of OPT 4

OPT 4 is also very useful in measuring water content in certain liquids and solids after either direct injection or by using the micro solid sampler, powder sampler or viscous solid sampler (see applications manual). This graphical analysis of time vs. water content (µg, %, ppm) allows one to preset the titration time to optimize the recovery of moisture from the sample.

5. Names and Functions of Each Part

5-1 Front Panel

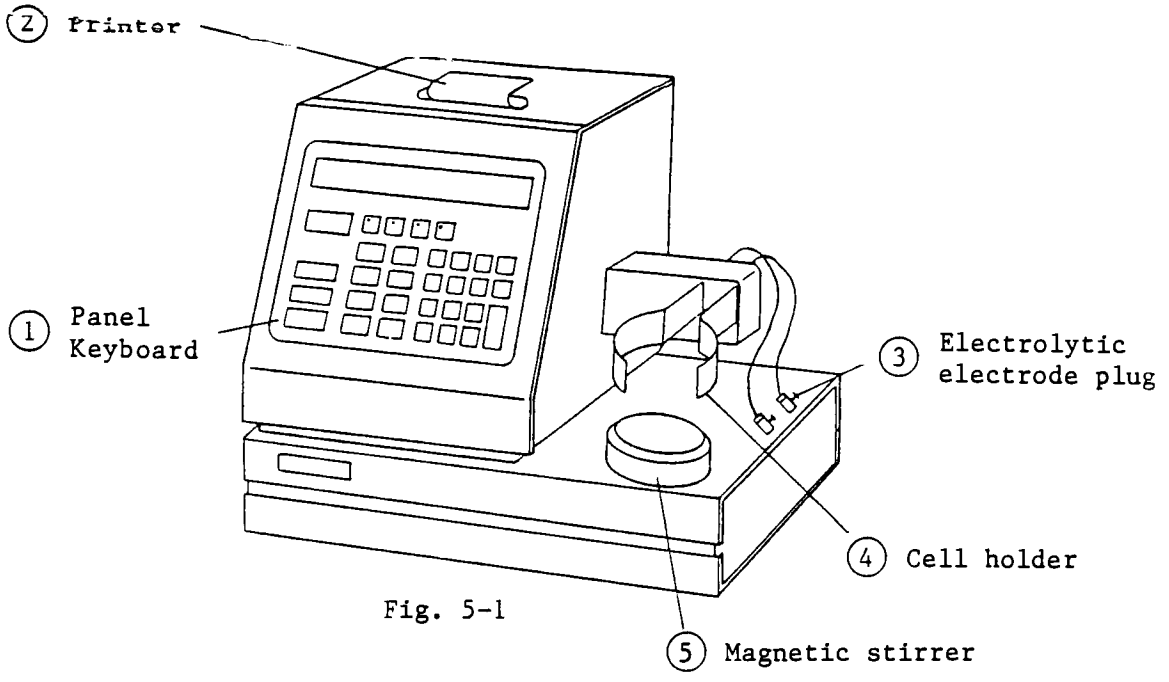


Fig. 5-1

① Keyboard panel
The chemically resistant keyboard panel includes the control and display section. Do not push it with a sharp point like a ballpoint pen tip. Press it only with fingertips.

② Printer
Data is printed out on this thermal dot graphic printer. Chart paper is fed manually by means of a thumb wheel on the left end of the chart paper.

③ Electrolytic electrode plugs
Plugs are to be connected to the electrodes, red cord to the generating electrode and the black one to the counter electrode.

④ Cell holder
The holder attaches the electrolytic cell to the basic unit.

⑤ Magnetic stirrer
This is for stirring the generator solution.

5-2 Side Panel of AQUASTAR C2000 coulometric Titrator

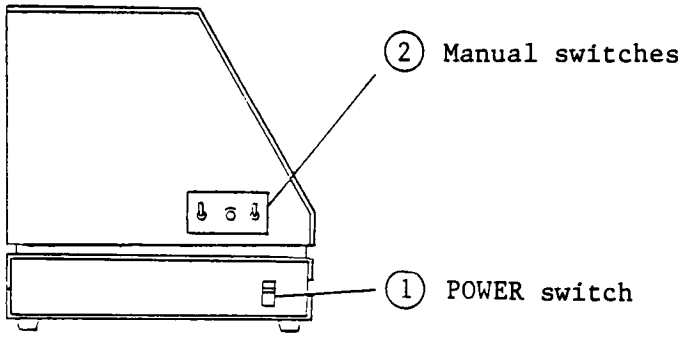


Fig. 5-2 Side View of AQUASTAR C2000 coulometric Titrator

① POWER switch
Used to turn power on and off.

② Manual switches

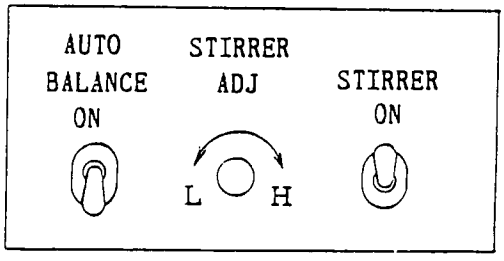


Fig. 5-3 Manual Switches

- AUTO BALANCE switch
  Used when an automatic electronic balance is connected. Moisture concentration is calculated with the data entered from the balance. Titrator must be set on MODE 0 for this function. This switch should be set to ON when the balance is used and for RS232C otuput.

- STIRRER ADJ knob
  A knob to adjust the speed of rotation of the stirrer.
  Normally, set this knob to the center position, where the speed of rotation of the stirrer is approx. 550 rpm.
  When set to L or H position, the speed of rotation can be changed by approx. 20%.

- STIRRER ON-OFF switch
  Switch for selecting whether the stirrer is to be on or off at [SAMPLE]. Normally, set it at OFF, and the stirrer will stop so sample can be added to the cell. Stirring will resume after the [TITRATION] button is activated. The ON switch is used when the water extraction rate from the sample to the generator solution is slow such as with a solid sample then the stirrer stays on so the sample can be stirred before titration or when using an evaporation accessary.

5-3 Rear Panel of AQUASTAR C 2000 coulometric Titrator

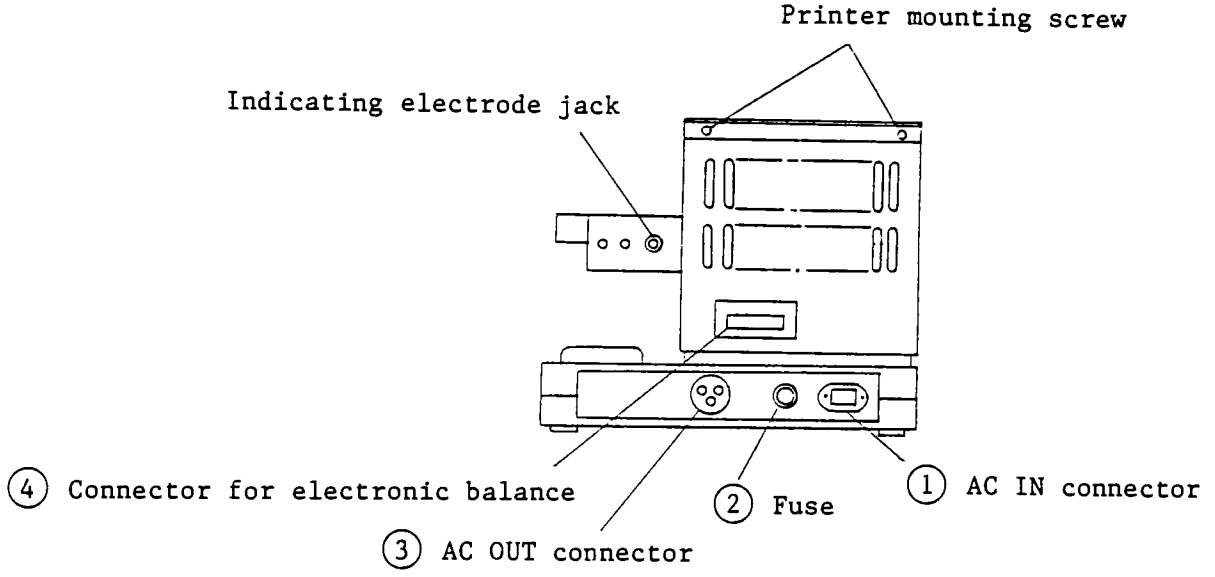


Fig. 5-4 Rear Panel

① AC IN connector
A connector to connect the power cord (accessory) with AC plug.

② Fuse holder
A holder for a glass-tube fuse (4 A).

③ AC OUT connector (1 A)
A connector for the power cord of the electronic balance. The current capacity is 1 A. Never use this connector to connect any thing other than the electronic balance.

④ Connector for electronic balance
A connector to connect the electronic balance or RS232C output cable. For the connection methods for the electronic balance and RS232C output, refer to Section 10.

6. Keyboard Panel

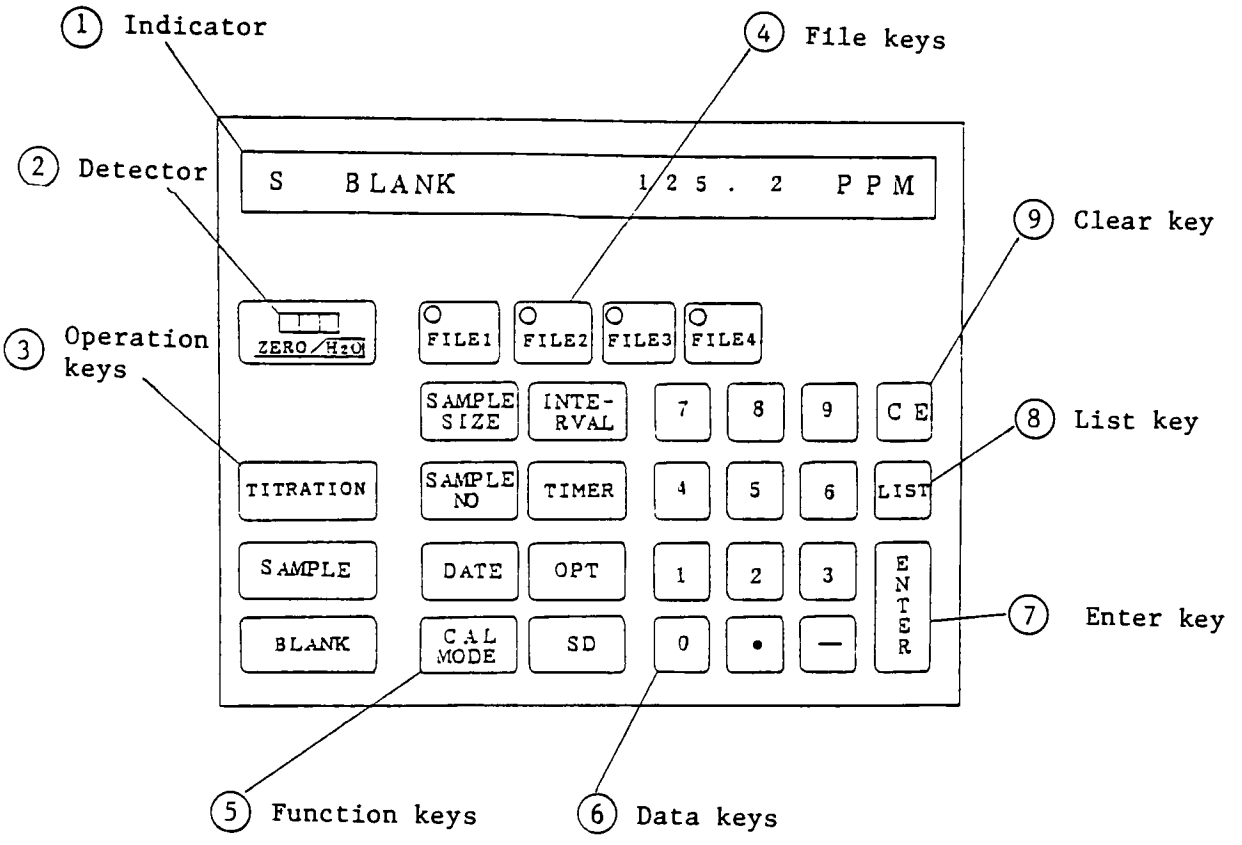
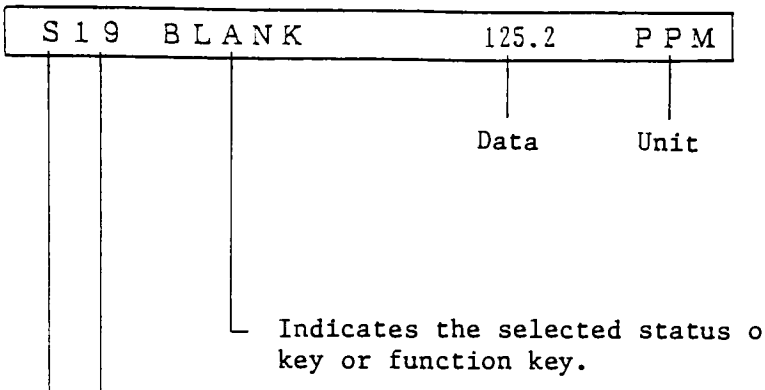


Fig. 6-1 Keyboard Panel

6-1 Indicator Display

20 digit liquid crystal display. A measured result is displayed in µg, % or ppm according to the calculation mode or sample size setting. When keying in, the particular key status and input data are displayed. The concentration is displayed in % for 0.1 % or more, or in ppm for below 0.1 %.



Indicates the selected status of operation key or function key.

The background is indicated in μgH_2O/min (0-50).

"--" indicates the background is 0 or excess iodine is present.

N: The background is not stable or is beyond the range of 0-50 μgH_2O/min.

S: The background is stable (blinks). Sample can be measured.

6-2 Detector

Indicates the status of the detecting electrode, representing increase or decrease in the water content of the generator solution.

(1) Green PL (PL = Pilot Lamp)
This lamp indicates that the water content in the generator solution is 50 to 100 μgH_2O or more. The lamp is on in the initial stage of titration. If the lamp blinks, it indicates abnormality of the indicator electrode or the generator solution.

(2) Yellow PL
This lamp indicates that the water content in the generator solution is 50 μgH_2O or less. During titration, it indicates that the water content is approaching the end point and intermittent electrolysis begins. In the [BLANK] mode, the lamp is always on if the background is within the range of 0 to 50 μgH_2O/min.

(3) Red PL

This lamp indicates that there is almost no water content in the generator solution and titration is nearly complete. At the end of a normal titration, the lamp always comes on. If the indicator electrode and the generator solution are abnormal the lamp blinks. If the indicator electrode is not connected or if excessive iodine is generated in the solution, the lamp blinks.

6-3 Operation Keys

These keys are for the titration operation and include [BLANK], [SAMPLE], and [TITRATION] keys. An operation key is activated by pressing. The operating sequence is shown in Fig. 6-2.

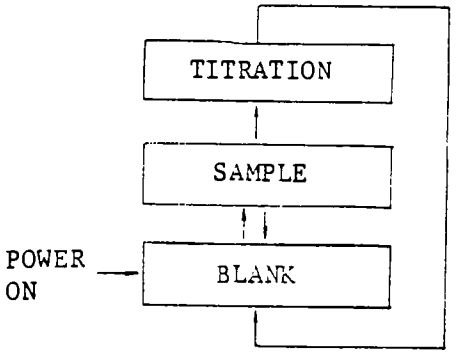


Fig. 6-2 Operation Keys

① [BLANK]

When power is turned on, the instrument is always initialized at [BLANK] and continually titrates background moisture.

② [SAMPLE]

Before injecting a sample, press this key. Make sure "S" BLANK appears on the display and that "S" blinks. Blinking "S" means the background is stable. Pressing this key indicates on the display the sample number to be measured and an underline on the chart. Pressing the [SAMPLE] key increases the sample number. When the [SAMPLE] key is depressed, the STIRRER switch turns the stirrer on or off. Before engaging or disengaging the electrolytic cell, stop the stirrer.

③ [TITRATION]

Key for starting titration. Press the [TITRATION] key after sample injection.
When the titration reaches the end point, the end of titration alarm sounds and the key is automatically reset to the [BLANK] operation.

6-4 [FILE] Keys

There are [FILE 1] to [FILE 4] keys. By pressing a [FILE] key, the corresponding pilot lamp indicates a selected status.

The instrument is provided with 4 files for independently registering the titrating conditions and operating parameters.

First select a [FILE] key, and then key in the titrating conditions, such as, [SAMPLE SIZE], [SAMPLE NO], ... [OPT] to create a file. Once the file is created it is stored in the memory backed up by a battery and protected from power shortage.

Before altering file contents, select a [FILE] key.

6-5 Function Keys

Used when setting titrating conditions or when setting data for calculating the concentration. These keys can be set independently of the operation key status.

The basic input operation by function keys is as follows.

① [SAMPLE SIZE]
Set the sample volume to be collected in CAL. MODE in terms of g, mℓ or ℓ.
[SAMPLE SIZE] [1] [.] [5] [ENTER]: SIZE 1.5 g is printed
The method of keying in the sample size depends on CAL. MODE. Refer to CAL. MODE.

② [SAMPLE NO]
Used to set a sample number in 5 digits.
[SAMPLE NO] [1] [5] [ENTER] : SAMPLE No 15 is printed
When it is desired to increase the sample No. at every measurement, hold down the [SAMPLE] key for more than 1 second and the sample number will be automatically incremented.

③ [DATE]
The date of measurement can be set (year, month, date or month, date, year).
[DATE] [1] [9] [8] [7] [.] [1] [2] [.] [0] [9] [ENTER] :
DATE . 1987/12/9 is printed

④ CAL MODE

Selects formula for calculating the concentration.
There are 2 methods of collecting a sample for moisture measurement: (1) weighing the sample by a balance in each measurement (the sample volume changes each time) and (2) sampling by volume (the sample volume is the same in each measurement). In the first method, the sample size has to be entered in each measurement while in the second, all that is needed is to set the sample size once and it is not necessary to modify the setting for each measurement. This instrument is so designed that the same calculating formula can be used in any of the 2 modes above. In the CAL. MODE 0-5, a new size is entered every time the SAMPLE key is pressed (clear mode). In the CAL. MODE 10-15, all you do is enter the size once regardless of the SAMPLE key operation.

Table 6-1 CAL. MODE and Calculating Expressions

CAL. MODE		Calculating formula	Use	SIZE unit	INDICATOR DISPLAY
Clear Mode	Set Mode				
0	10	$X = \frac{FOUND \quad (\ \mu gH_2O\ )}{SIZE\,(Sample\ weight)}$	Sampling by weight Note: When the balance is connected, automatically set in the 0 mode.	g	
1	11	$X = \frac{FOUND}{SIZE_1 - SIZE_2}$	Sampling by weight SIZE is determined by the difference between the values before and after sample injection.	g	
2	12	$X = \frac{FOUND}{Density \times SIZE(Volume)}$	Sampling by volume The sample of known density is sampled by the volume.	ml	DENS
3	13	$X = \frac{FOUND \times 1.244}{SIZE/(1+t/273)}$	Calculated by mesuring the water content V/V% (ppm) in a gas sample.	l	TEMP
4	14	$X = \frac{FOUND}{SIZE}(\frac{B}{C} + \frac{X}{10}) - \frac{AB}{C}$	Solvent extraction method. The water content in a solid sample is extracted into a solvent when the sample is insoluble in the solvent.	g	A B C
5	15	$X = \frac{FOUND}{SIZE}(\frac{B}{C} + 1) - \frac{AB}{C}$	Measurement is made by diluting a liquid sample with a solvent or by dissolving a solid sample in a solvent.	g	A B C

Note: DENS: Density of liquid sample
TEMP: Temperature of gas in degrees centigrade
A: Water content in the extracting solvent (ppm)
B: Volume of extracting solvent used (g)
C: Weight of sample (g) used for extration

(i) CAL. MODE (0, clear mode; 10, set mode) Direct weighing

. [CAL MODE] [0] [ENTER]: CAL. MODE 0 is printed

. [SAMPLE SIZE] [1] [.] [2] [ENTER]: SIZE 1.2 g is printed

Typical print at end of titration

DATE	1987/12/15	
SAMPLE NO	112	
H_2O	1121.5	μg
BLANK	0	μg
SIZE	1.365	g
H_2O	821.6	PPM [(H_2Oμg-BLANK)/SIZE]

(ii) CAL. MODE (1, clear mode; 11, set mode) Differense in weight

. [CAL MODE] [1] [ENTER] CAL. MODE 1 is printed

. [SAMPLE SIZE] [5] [.] [2] [-] [4] [.] [3] [ENTER]

SIZE 5.2 g
- 4.3 g
0.9 g

is printed

Typical print at end of titration

DATE	1987/1/15	
SAMPLE NO	115	
H_2O	891.5	μg
BLANK	11.9	μg
SIZE	5.20	g
	-4.30	g
	0.90	g
H_2O	977.3	PPM (H_2Oμg-BLANK)/SIZE

(iii) CAL. MODE (2. Clear mode; 12, set mode) Sampling by volume

. [CAL MODE] [2] [ENTER]: CAL. MODE 2 is printed

. DENS appears on the display. Key in the density.
[0] [.] [8] [2] [1] [ENTER]: DENS 0.821 is printed

. [SAMPLE SIZE] [2] [ENTER]: SIZE 2 ml is printed

Typical print at end of titration

DATE	1987/2/10	
SAMPLE NO	15	
H_2O	1289.1	μg
BLANK	0	μg
SIZE	5.00	g
DENS	0.821	
	4.105	g
H_2O	314.0	PPM

(iv) CAL. MODE (3, clear mode; 10, set mode) Measuring gas sample

. [CAL MODE] [3] [ENTER] : CAL. MODE 3 is printed

. TEMP appears on the display. Key in the temperature.
[1] [5] [ENTER] : TEMP 15°C is printed

. SAMPLE SIZE [3] [0] [ENTER] : SIZE 30 ℓ is printed

Typical print at end of titration

DATE	1983/12/10
SAMPLE NO	19
H_2O	256.1 μg
BLANK	0 μg
SIZE	30 ℓ
TEMP	25 °C
H_2O	11.59 PPM

(v) CAL. MODE (4, clear mode; 14, set mode) Solvent extraction

. [CAL MODE] [4] [ENTER] : CAL. MODE 4 is printed

. A appears on the display. Key in A (= moisture of extracting solvent in ppm).
[1] [2] [0] [ENTER] A 120 PPM is printed

. B appears on the display. Key in B (=weight of extracting solvent in grams).
[1] [0] [0] [ENTER] B 120 g is printed

. C appears on the display. Key in C (=sample quantity used for extration in grams)
[2] [5] [ENTER] C 25 g is printed

Note: Keying in SAMPLE SIZE (weight of supernatant in grams, injected into cell) during a titration changes the contents on the display from μgH_2O to % (ppm).

Typical print at end of titration

DATE	1987/1/21
SAMPLE NO	15
H_2O	259.1 μg
BLANK	0 μg
SIZE	2.5 g
A	12.0 PPM
B	100 g
C	25 g
H_2O	0.1579 %

(vi) CAL. MODE (5, clear mode; 15 set mode) Solvent dilution

- [CAL MODE] [5] [ENTER] : CAL.MODE 5 is printed
- A appears on the display. Key in A (= moisture of extracting solvent in ppm).
  [1] [2] [0] [ENTER] A 120 PPM is printed
- B appears on the display. Key in B (=weight of extracting solvent in grams).
  [1] [0] [0] [ENTER] B 100 g is printed
- C appears on the display. Key in C (=sample quantity in grams used for dilution).
  [2] [5] [ENTER] C 25 g is printed
  Sample size = weight of supernatant in grams injected into cell

Typical print at end of titration

DATE	1987/1/25	
SAMPLE NO	10	
H_2O	259.1	μg
BLANK	0	μg
SIZE	2.5	g
A	120	PPM
B	100	g
C	25	g
H_2O	998.2	PPM

⑤ [INTERVAL]

Used when setting the wait time at the end point. As the titration approaches the end point, electrolysis is changed over from continuous to intermittent, and gradually proceeds to the end point. The instrument alternates between the wait time for reaction and the electrolysis of a certain volume. The interval ends by holding the end point for the wait time (interval time) set by the [INTERVAL] key past the end point. The interval time determines the end conditions for titration. The interval is set normally at 10-15 seconds. The settable range is 1-99 seconds.

⑥ [TIMER]

There are T-TIMER (TITRATION TIMER) and S-TIMER (START TIMER).

(i) T-TIMER
The titration timer is used to set the overall time of titration. The set time constitutes a time required for total titration. It is used when it is desired to continue a titration for a certain time period after the end point is reached. The settable time is 0-99 minutes.

[TIMER]

[TIMER T=1 S=0 0] : displayed

[1] [ENTER]

[T-TIMER 1 MIN] : T-TIMER data appears on the display

[5] [ENTER] T-TIMER 5 MIN is printed

Key in [0] [ENTER] when T-TIMER is not used.

(ii) S-TIMER

Delays the start of the titration. Pressing the [TITRATION] key operates, the start timer and the display indicates the time remaining on the timer in seconds. The titration begins after the set time. The S-TIMER can be used when measuring difficult to dissolve solid samples or viscus liquid samples. When using the evaporator accessories the S-TIMER allows the water vapor to transfer to the electrolytic solution.

[TIMER]

[TIMER T=1 S=0 0] : displayed

[0] [ENTER]

[S-TIMER 2 MIN] : S-TIMER data appears on the display

[5] ENTER : S-TIMER 5 MIN is printed

Key in 0 ENTER when S-TIMER is not used.

⑦ [OPT]

Table 6-2 shows the contents of OPT keys.

Table 6-2 OPT Functions

OPT mode	Function
1	The total electrolytic volume of the generator (anode) solution and the date are stored in the memory and printed
2	The total electrolytic volume of the counter (cathode) solution and the date are stored in the memory and printed out
3	The date of exchange of the ion exchange membrane is stored in the memory and printed
4	Interim progress of titration is graphically displayed on a titration volume-time curve. This can be used in selecting optimum heating temperature by measuring the evaporation rate when using the evaporator accessories.
5	Used to calculate the sample weight in grams when the appropriate water content of sample is known.
6	The blank vlaue (BLANK V) is set
7	The electrolyzing current is set (FAST = 214 mA, SLOW = 107 mA) and checked

Input of [OPT] Keys (See Table 6-2)

(1) [OPT] 1 thru 3 are especially useful when several analysis utilize the instrument on an intermittent basis. These options initialize and store in memory the dates and total electrolytic volume of the reagents since their last exchange. Namely, the date the generator solution was changed and how much water was consumed since that exchange (OPT Mode 1), the date the counter solution was changed and how much water was consumed since that exchange (OPT Mode 2), and the date the ion exchange membrane was changed (OPT Mode 3).

(2) If an erroneous value is obtained these [OPT] Modes can be recalled by any operator to determine if the instrument is operating satisfactorily or if the reagents or ion exchange membrane need changing.

(i) Initialization of the electrolytic volume and the date of exchange OPT 1 (generator solution), OTP 2 (counter solution), and OPT 3 (ion exchange membrane).

[OPT] [1] [CE] [ENTER] Print-out G TOTAL 0 mg

[DATE 9/5/87] : display

The date of exchange can be set

[7] [.] [1] [.] [8] [7] [ENTER] print-out DATE 7/1/87

This clears memory and sets the changing date of the generator solution.

(ii) Recalling the electrolytic volume and the date of exchnage of the generator solution.

[OPT] [1] [ENTER]

[G-TOTAL 99 mg] : displayed

[ENTER]

[DATE 7/1/87] : displayed

(iii) Operate OPT 2 and OPT 3 as explained for OPT 1

(iv) [OPT] [4] prints out a graphical display of the total amount of water titrated (µg, %, ppm) vs. time (min.). This is helpful during preliminary studies to determine conditions necessary for quantitative measurements of new samples or mixtures by recording the water extracting rate. When using the solid evaporator accessory this function can help in selecting an optimum heating temperature by recording the water evaporation rate.

. Setting OPT 4

[OPT] [4] [ENTER] : OPT 4 T-CURVE is printed

[1=SET 0=CLR] : displayed

[1] [ENTER] → Keying 1 ENTER sets OPT 4.
OPT 4 SET is printed.

[0] [ENTER] → Keying 0 ENTER clears OPT 4.
OPT 4 CLEAR is printed.

Or key in [OPT] [4] [CE] [ENTER] and a graphic display will not print.

Fig. 6-1 shows a typical graphic data by OPT 4.

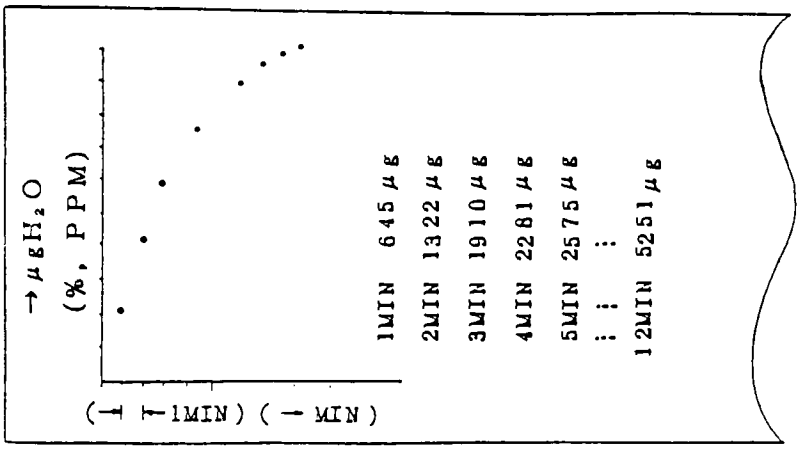


Fig. 6-1 Typical Graphic Data for OPT 4

a) The abscissa shows the integral values of the units of measurement (μgH_2O, % ppm), which is divided into the equal sections.

b) The ordinate shows the measuring time (time from the pressing of [TITRATION] key to end of titration), where one graduation corresponds to one minute.

(v) [OPT] [5] (calculates the sample amount)

[OPT] [5]

[OPT 5 SAMPLE SIZE] : displayed

[ENTER]

[CONC %] : displayed

Key in the sample moisture concentration in %.
From a guessed moisture concentration, an optimum sampling quantity is displayed and printed.
OPT 5 SAMPLE SIZE is printed
The judging criteria are based on Table 6-3.

Table 6-3 Sampling Quantity

Water content in sample	CURRENT	
	FAST	SLOW
100%	0.01 g	
10 g	0.05 g	
1%	0.5 g	
0.5%	0.7 g	
1000PPM		1 g
100PPM		2 g
50PPM		3 g
10PPM		5 g
1PPM		10 g or more

(vi) [OPT] [6] (blank value = μgH O)

Results for a blank test are measured beforehand and entered into the instruments memory. Then the results for a sample measurement are corrected automatically by subtracting the blank value. This function can be used when a certain amount of moisture is introduced when injecting samples into the electrolytic cell or evaporation accessory.

· [OPT] [6] [ENYER] : OPT 6 BLANK is printed

Data for the blank appears on the display.
Then key in [5] [.] [1] [ENTER]. BLANK 5.1 μg is printed.
When the blank value is not used, key in [0] [ENTER]. BLANK 0 μg is printed.

(vii) [OPT] [7] (Electrolytic current is set and checked refer to table 6-3)

[OPT] [7]

[OPT 7 CURRENT] : displayed

[ENTER]

[CURRENT 106.23 mA] : displayed
(it is necessary to light green detector PL in this cheak of current)

Electrolyzing current flowing currently

[ENTER]

[FAST=1 SLOW=0 1] : displayed

Set the electrolyzing current intensity.

[0] [ENTER]

(to set to SLOW)

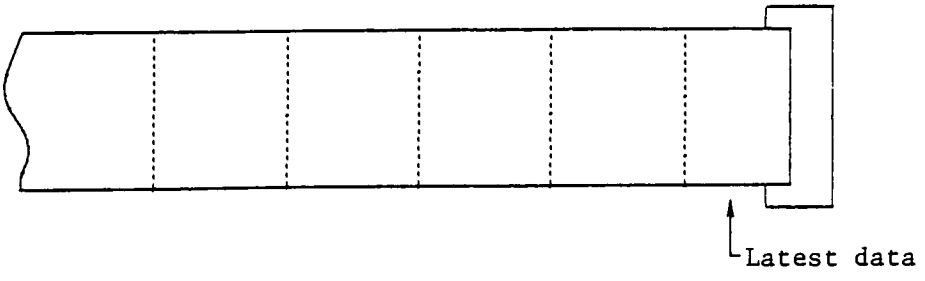
CURRENT SLOW SET is printed.

⑧ [SD] STANDARD DEVIATION

Used in statistical calculation of the measurement result. The calculation is made of the mean value, standard deviation, and coefficient of variation, and the memory capacity is up to N = 20. If this capacity is exceeded, previous data is replaced by most recent data and new data is written.

· [SD] key setting

[SD] [5] (n = number of data) [ENTER]



In the above calculation example, data of DATA 1 .. DATA 5 are statistically calculated.

Typical print

N	5
MEAN	1105%
SD	0.12%
CV	0.15%
DATA	1.12%
⋮	⋮
DATA	1.01%

} N = 5 data

Notes:

(1) When statistical calculation is performed among data with different units of concentration, only the data with the same unit as in the last data are calculated.

Data 1	0.25%
Data 2	0.21%
Data 3	2958 ppm
Data 4	2023 ppm
Data 5	0.24%

Depress [SD] [5] and [ENTER] keys, and only DATA 1, 2 and 5 (%) are calculated.

(2) Deletion of erroneous data
When an abnormal value of data is obtained and the data should not be used in the statistical calculation, change the units (μgH_2O, %, ppm) of this data, and conduct re-calculation at the time when data is obtained, (that is, before the [SAMPLE] key is activated again) by depressing [SAMPLE SIZE] [ENTER]. The re-written data is not added to the statistical calculation, however, N becomes N-1.

(3) Clearing of all measurement data is memory
Depress [SD], [0] and [ENTER] keys, and all the data stored in the memory is cleared.

⑨ [CE]

Data is cleared before the ENTER key is pressed.

⑩ [ENTER]

Data is inputted or printed.

⑪ DATA key

- [0]-[9] [.] : numeric keys
- [-] : subtract key.

⑫ [LIST]

The titrating conditions are listed out.

[FILE 1]: selected

[LIST] (typical print of file)

```
FILE1
DATE                          1987/4/7
SAMPLE NO                            1
CAL MODE                             0
SIZE                               0 g
INTERVAL                        10 SEC
T-TIMER                         0 MIN
S-TIMER                         0 MIN
OPT4 T-CURVE CLEAR
OPT6 BLANK V                       0 µg
OPT7 CURRENT SLOW SET
```

6-6 Special Functions

① Recalculating function

If the sample size inputted is found erroneous or otherwise abnormal, a recalculation is available by keying in the sample size again provided the [SAMPLE] key has not been operated yet. After the [SAMPLE] key is operated, the measured result is cleared and, therefore, a recalculation is impossible. To execute a recalculation, key in [SAMPLE SIZE] data [ENTER].

② Data copy function

The same measured results are copied. The conditions for data copy are the same as for a recalculation, that is [SAMPLE] key must not be reactivated.
To execute data copy, key in [SAMPLE SIZE] [ENTER]. Printing is made as ofter as the [ENTER] key is pressed.

③ All clear

Depress [OPT], [9], [9], and [ENTER] keys.
All set conditions are cleared.
The following items are set as given below.

- SAMPLE NO 1
- CAL MODE mode 0 (calculation mode)
- INTERVAL 10 SEC (wait time)
- TIMER all OFF OPT 4 OFF
  OPT 6 BLANK=0
  OPT 7 CURRENT SLOW

7. Cautions in Operation

7-1 Cautions in Operation

① If the fitting surface of the cell is left for a long time without applying grease, it may become unmovable. If it adheres to the electrolytic cell lid (made of Teflon), warm the lid with a hair drier, and it will come off.

② The generator solution may turn reddish, with the red lamp of the DETECTOR blinking. This is because excess iodine has been produced due to the effect of sunlight. In that event, add about 1 μℓ of water using a microsyringe to eliminate excessive iodine and stabilize the background before measurement.

7-2 Cautions in Determination

① Volume of sample to be collected
Select the sample volume and electrolytic current (CURRENT), referring to Table 7-1. (Table 7-1 is contained in OPT 5.)

Table 7-1 Volume of Sample to be Collected

Water content in sample	CURRENT	
	FAST	SLOW
100%	0.01 g	
10%	0.05 g	
1%	0.5 g	
0.5%	0.7 g	
1000 ppm	-	1 g
100 ppm		2 g
50 ppm		3 g
10 ppm		5 g
1 ppm		10 g or more

② Decrease in electrolytic current
As the sample is added, the resistance of the generator solution may become higher and the electrolytic current may decrease, resulting in somewhat long measurement time; this does not affect the measurement result. This can occur when the water content of insulating oils, hydrocarbon compounds and other nonpolar solvents are measured. Use of the [SLOW] range of CURRENT is recommended in electrolysis.

③ High background
If the background is high when a fresh generator solution and the counter solution are used, check the following:

a) Water deposits on the electrolytic cell wall. (Tilt the cell and wash the inside wall with the generator solution.)
b) Aged rubber septum; change septum, if several holes are evident.
c) Hole in the ion exchange membrane. (The counter solution and the generator solution are at the same level.)
d) Foreign matter in the generator solution.

④ The BLANK elimination is not completed. (The light blinks when the background is over 50.)

a) Too Much water in the generator solution.
b) Failure to connect the electrolytic electrode plug.
(Check the electrolytic current by pressing the [OPT] 7 key to see if electrolytic current is displayed.
c) Foreign matter on the surface of the generating electrode.
(Wash the electrode with water.)
d) Distance between anode electrode (platinum screen) cathode electrode is too far see Fig. 3-4.
e) Silica gel or cotton in three-way drying tube contain moisture.

⑤ Checking of the abnormal value
This method should be used to check the instrument for abnormality, rather than to obtain an accurate absolute value. Take recording of measurement values by normal operation using a syringe, and judge the instrument condition from the deviation. Collect 1 mℓ of Water/Methanol Standard (Cat #AX1697D/1) (1.00 mg/mℓ) using the syringe, and inject it into the generator. solution slowly. The display should read approximately 1000 µg H_2O. If the deviation is large, refer to Chapter 11 Trouble-shooting.

8. Inspection and Maintenance

8-1 Reagent usage

① One bottle of generator solution (anode reagent, volume 500 mℓ) is sufficient for 5 chargings at 100 mℓ per charge.
② One bottle of counter solution (cathode reagent, volume 25 mℓ) is sufficient for 5 chargings at 5 mℓ per charge.

8-2 Life of the Generator Solution

① The water content measuring capacity of the generator solution is 400 mgH_2O per 100 mℓ. Check the total electrolytic volume by printing by OPT 1.

② The limit of dilution of the generator solution per 100 mℓ occurs is when the solution diluted with the sample has reached 150 mℓ. Further dilution will decrease the electrolytic efficiency, resulting in high measurement values. With some samples, the electrolytic efficiency may be lower even at below 150 mℓ. It may be necessary to determine the measurable range empirically.

8-3 Life of the Counter Solution

Depending on the frequency of use and the sealing, change the counter solution once a week, especially in high humidity conditions.
If the background does not become smaller than 15 to 20 μg H_2O/min after changing the generator solution change the counter solution.
Change the counter solution when the total volume of electrolysis has reached 100 to 250 mg. To be more accurate, use the background value as a criterion for changing the solution.

8-4 Life of the Ion Exchange Membrane

The ion exchange membrane can be used for several months, if it is not mechanically damaged. It is not necessary to change it unless a leak is found.

8-5 Assembly and Disassembly of the Electrolytic Cell (Fig. 3-3)

For assembling, disassembling or washing the cell, see Fig. 3-3. To remove sample build up use appropriate solvent to dissolve residue.

Note: Do not use Acetone. Rinse with water followed by methanol dry in oven the drying temperature should be 70°C.

8-6 Chart Paper (TR-80)

One roll of chart paper is 25 meters long. When the chart paper length reaches 50 cm, a red mark will appear on the paper end.

8-7 Battery

The instrument uses a rechargeable battery. If the instrument is not used for more than six months, reset the keys before use. Charge the battery for about eight hours by connecting it to a power line once every six months.

APPENDIX III

TABLE #B-1

DEW POINT TEMPERATURE / % RH

Dewpoint in Degrees °C	H_2O Grains per Pound	% RH @ 70°F
-25.00	2.732	2.5
-25.55	2.584	2.3
-26.17	2.444	2.2
-26.67	2.311	2.1
-27.22	2.184	1.98
-27.78	2.064	1.88
-28.33	1.950	1.77
-28.89	1.841	1.67

TABLE #B-2

SYRINGE EVALUATION w/METHANOL
VOLUMETRIC FILL vs. ACTUAL WEIGHT

SAMPLE	Hamilton GASTIGHT® #1001 (1 ml) FILLED 1 ml (grams)	Hamilton GASTIGHT® #1010 (10 mL) FILLED 5 ml (grams)
1	0.7849	3.9539
2	0.7839	3.9492
3	0.7818	3.9468
4	0.7831	3.9862
5	0.7821	3.9732
6	0.7835	3.9539
7	0.7821	3.9572
8	0.7846	3.9492
9	0.7836	3.9808
10	0.7821	3.9792
n	10	10
mean	0.7832	3.9630
sd	0.0011	0.0151
max	0.7849	3.9468
min	0.7818	3.9862
range	0.0031	0.0394

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