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User's Manual

Hooded Combination (Macro) Kjelhdahl Systems

Models

21176 Series
21177 Series
21178 Series

To receive important product updates, complete your product registration card online at **register.labconco.com**

Please read the User's Manual before operating the equipment.

Warranty

Labconco Corporation provides a warranty to the original buyer for the repair or replacement of parts and reasonable labor as a result of normal and proper use of the equipment with compatible chemicals. Broken glassware and maintenance items, such as filters, gaskets, light bulbs, finishes and lubrication are not warranted. Excluded from warranty are products with improper installation, erratic electrical or utility supply, unauthorized repair and products used with incompatible chemicals.

The warranty for Hooded Combination Kjeldahl Systems will expire one year from date of installation or two years from date of shipment from Labconco, whichever is sooner. Warranty is non-transferable and only applies to the owner (organization) of record.

Buyer is exclusively responsible for the set-up, installation, verification, decontamination or calibration of equipment. This limited warranty covers parts and labor, but not transportation and insurance charges. If the failure is determined to be covered under this warranty, the dealer or Labconco Corporation will authorize repair or replacement of all defective parts to restore the unit to operation. Repairs may be completed by 3rd party service agents approved by Labconco Corporation. Labconco Corporation reserves the rights to limit this warranty based on a service agent's travel, working hours, the site's entry restrictions and unobstructed access to serviceable components of the product.

Under no circumstances shall Labconco Corporation be liable for indirect, consequential, or special damages of any kind. This warranty is exclusive and in lieu of all other warranties whether oral, or implied.

Returned or Damaged Goods

Do not return goods without the prior authorization from Labconco. Unauthorized returns will not be accepted. If your shipment was damaged in transit, you must file a claim directly with the freight carrier. Labconco Corporation and its dealers are not responsible for shipping damages.

The United States Interstate Commerce Commission rules require that claims be filed with the delivery carrier within fifteen (15) days of delivery.

Limitation of Liability

The disposal and/or emission of substances used in connection with this equipment may be governed by various federal, state, or local regulations. All users of this equipment are required to become familiar with any regulations that apply in the user's area concerning the dumping of waste materials in or upon water, land, or air and to comply with such regulations. Labconco Corporation is held harmless with respect to user's compliance with such regulations.

Contacting Labconco Corporation

If you have questions that are not addressed in this manual, or if you need technical assistance, contact Labconco's Customer Service Department or Labconco's Product Service Department at 1-800-821-5525 or 1-816-333-8811, between the hours of 7:30 a.m. and 5:30 p.m., Central Standard Time.

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Chapter 1: Introduction

Components Shipped

Carefully check the contents of your Hooded Combination Kjeldahl System for shipping damage while it is still on the shipping plate. Do not discard the packaging material until the contents have been checked and the equipment has been approved for installation.

The Hooded Combination Kjeldahl Nitrogen System has been shipped in one complete crate to minimize damage that may occur in transit.

The Hooded Combination Kjeldahl Nitrogen System as shipped has two components that must be assembled. The top exhaust bonnet component must be installed on the main body and the interior light must be electrically connected.

Make sure to inspect the product thoroughly **prior** to installation and report any damage that may have occurred in transit (see Warranty page for instructions).

Figure 1-1

General Description

Labconco's Hooded Combination (***Macro***) Kjeldahl Nitrogen System is designed to facilitate the determination of Total Nitrogen content within materials such as feeds, grains, soils, fertilizers, plant tissue, water, organic wastes, and food products. The expression **'*Macro*'** identifies the size of sample vessel and the sample size that can be used with this equipment. Other Kjeldahl systems are available, which use smaller sample vessels and require smaller sample sizes. The apparatus can be used for the digestion and/or distillation of all types of nitrogen containing samples. The system consists of the Lower Digestion rack and Upper Distillation rack. Both are used in standardized (***Macro***) Kjeldahl Nitrogen Determination Methods.

This Kjeldahl system is enclosed by a ventilated hood system which captures excess heat and provides for personnel safety during the digestion and distillation reactions. High temperature acid digestion and caustic distillation comprise the two-step Kjeldahl Nitrogen determination method.

2117703 Twelve Place Hooded Combination Macro-Kjeldahl System

Performance

The Hooded Combination Kjeldahl Digestion and Distillation Systems have been designed for use in the determination of nitrogen (or ammonia) concentrations. By calculation nitrogen can be converted to protein values for products such as plant tissues, meats and other food substances. Nitrogen determinations with the Hooded Combination (***Macro***) Kjeldahl Systems can accommodate sample sizes up to 5 grams due to the size of the digestion/distillation flasks. However, higher levels of organic content within a specific sample type will require smaller sample sizes due to the vigorous digestion reaction. Detailed procedures developed by professional laboratory organizations such as American Association of Analytical Chemists (AOAC) and American Association of Cereal Chemists (AACC) should always be consulted for step by step analytical procedures when operating this equipment. For methodology precision and accuracy please consult the specific published method for the type of sample or substance that will be analyzed.

Component Identification

1. **Electric Heaters**. 600-watt heaters are used in both the lower digestion and upper distillation rack of the unit. Infinite control switches regulate each of the heaters. The curved heater elements are provided and allow for the round base of the Kjeldahl Flasks, providing faster and more evenly distributed heat. The heating element assembly on the digestion rack can be moved closer to or away from the acid fume removal manifold to accommodate different size Kjeldahl flasks. All heaters and temperature controllers are wired to a common electrical box for connection to a facility electrical service.
2. **Lower Fume Removal Manifold**. Located in back of the digestion heater elements, this manifold is manufactured from chemical resistant chlorinated polyvinyl chloride and fitted with heat resistant PTFE nipples designed to prevent leakage of the hot sulfuric acid fumes created during the digestion process. The nipple design, extends in to the flask opening, and efficiently removes excess acids fumes during the digestion process. The flasks are oriented at a 45 degree angle to the fume removal manifold to promote the condensation and refluxing of the acid fumes back down into the digestion flask. This refluxing process prevents the samples from boiling dry during the 45+ minute digestion process.

3. **Acid Fume Piping Exhaust System**. Located on the left side of the unit, this exhaust piping removes excess sulfuric acid fumes from the digestion flasks. The Exhaust Piping must be connected to a remote blower to remove acid fumes. The remote blower creates suction and draws acid fumes through the CPVC digestion manifold and exhaust piping provided and pulls the fumes under negative pressure out through a duct work system connected to the remote blower and ultimately the building's exterior. The 6" exhaust stack on the left side of the unit must be connected to a leak-tight PVC ductwork system that vents 10 ft (3 meters) above the building roof.
4. **Temperature Gauge** The condensation rack behind the upper distillation heating elements requires cooling water. The temperature of the distillation cooling water can be adjusted to promote condensation and suit individual requirements with the flow control valve handle, located below the distillation heaters. Water temperature is indicated on the thermometer, which is located at the water outlet of the distillation manifold. The water connections are 3/4" NPT and the supply line should be 1/2" minimum for proper water flow through the unit. For facilities where the ground water temperature exceeds 80°F (27°C), a recirculation water chiller system will be required to achieve sufficient condensation results.
5. **Distillation Condensation Manifold** Epoxy coated steel outer columns with stainless steel inner condensation columns provide long life. Each of the six column manifold features 3/4" NPT tap water inlet and outlets for easy plumbing connections.
6. **Hood Exhaust Connection** At the top of each Hooded Combination Kjeldahl System are two duct work connection collars. The exhaust collar is positioned to the right of the make-up air collar. The exhaust collar must be connected to a dedicated duct work system with a roof mounted blower. This exhaust connection will vent hot air and small amounts of acid fumes. The Acid Fume Removal Exhaust duct work system **must not** be connected to the Hood Heat Exhaust duct work. Each duct work system must be routed to the building exterior independently. The make-up air collar can be connected to a duct work system which can supply up to 75% of the total exhaust air volume.

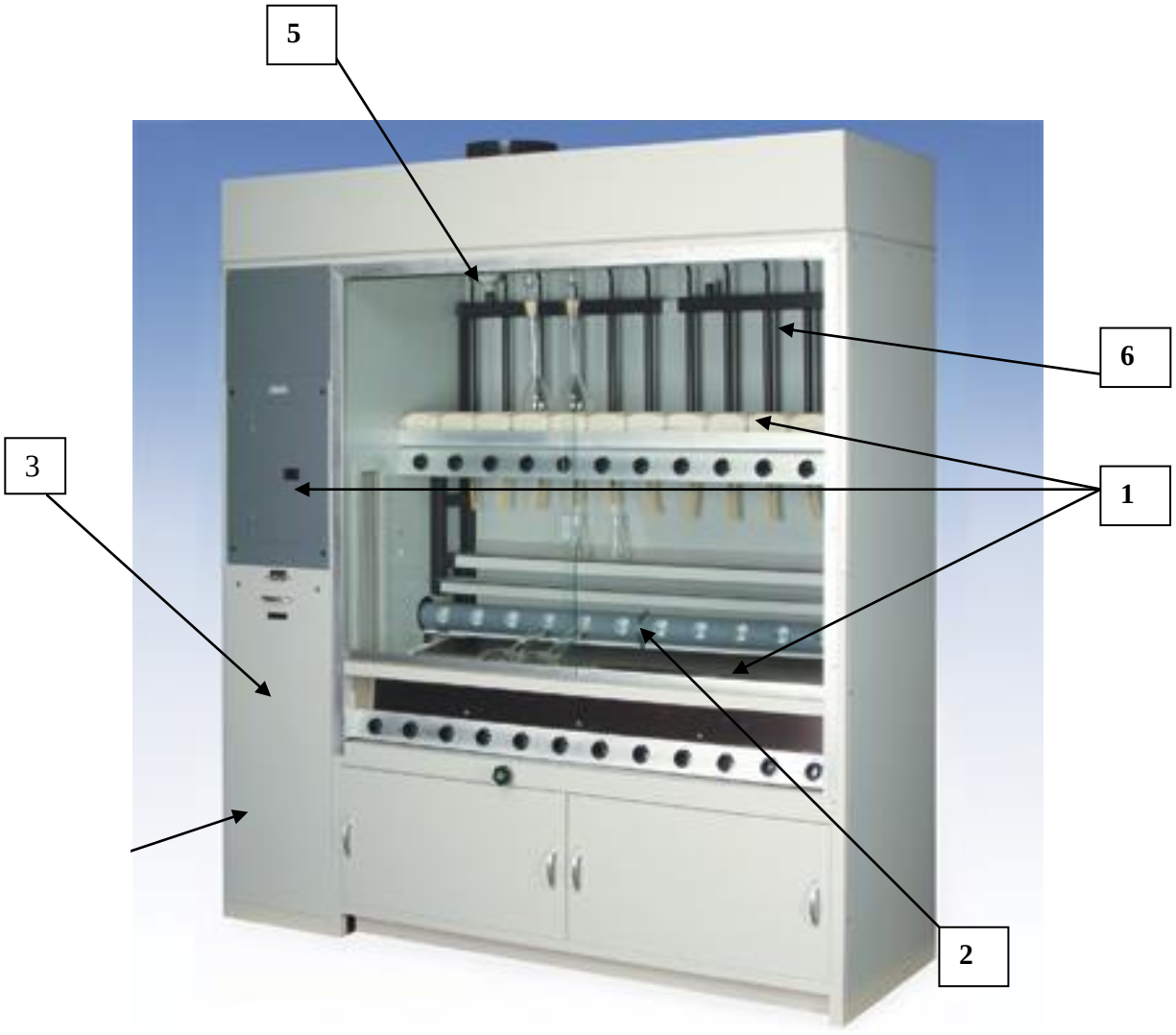


Figure 1-2

Chapter 2: Installation

General Installation Notes

The Hooded Combination Macro-Kjeldahl System is shipped in two sections. The top exhaust bonnet and glass sashes will need to be installed after the main body has been installed in its final location. Exhaust duct work, electrical service, water supply and drain connections should be completed by a licensed contractor.

The customer will need to purchase their preferred size of Kjeldahl Flasks, with one-hole-rubber-stoppers and distillate receiving flasks. The glass Connecting Bulbs and Delivery Tubes are provided with each Kjeldahl System.

Connecting Bulbs and Delivery Tubes are included with Macro Kjeldahl Systems. For installation of the Connecting Bulbs and Delivery Tubes see section on Glassware Installation and Figure 2-9.

Use caution when you remove the protective packaging material taped to the heater runways as fragile components are involved. Levelers are shipped installed, as are the distillation cooling water thermometers.

Location

If possible, the apparatus should be uncrated in the room in which it is to be placed. Hooded Combination Macro-Kjeldahl Systems are heavy and can not be disassembled to reduce their overall size. Special arrangements should be made in advance for moving the systems to their final location within a facility. Special instruction tags are attached to the apparatus; they must not be removed until the installation has been completed.

Bonnet Assembly

The hood bonnet must be installed on top of the substructure of the hood. This is accomplished by elevating the bonnet up onto the top of the substructure. It is not required to bolt the bonnet in place, as its weight on the foam gasket seal will hold it in its proper place.

Sash Assembly

The glass sashes are packaged separately and are installed into the hood assembly, by inserting the upper edge into its track first and then positioning the lower edge over the nylon runners in the lower sash track. See Figure 1-2 and Figure 2-8 for illustration of glass sashes.

Electrical Connection

The electrical line for the lights in the bonnet section of the hood assembly need to be connected to the lead wires coming off of the light switch that is located on the substructure of the hood assembly. Wire nuts have been supplied to aid in this connection.

Hood Exhaust System

The hood superstructure is fitted with a 10-3/4" I.D. duct work connection shown below. This duct work connection is used to remove the heat build up which occurs during normal operation inside the unit superstructure. Specific airflow requirements for each size of hood are shown in the air volume chart (see dimensional specification drawing in Appendix B). The duct work system should be made of an acid resistant material. It is recommended that a Laboratory HVAC technician with experience in the design and sizing of chemical fume removal systems be consulted.

Hood Auxiliary Air System

The hood superstructure is fitted with a 10-3/4" I.D. duct connection (shown below) to allow for the auxiliary air to be supplied into the hood. This air will be exhausted out through the hood exhaust connection with additional room air and the heat load that builds up inside the hood structure.

The auxiliary make up air system is used when the existing room environment does not supply enough air to the hood to adequately remove the heat build up that is experienced.

Specific airflow requirements for each size of hood are shown in the air volume chart (see dimensional specification drawing in Appendix B).

Figure 2-1

Electrical

Hooded Combination Macro-Kjeldahl Systems have been wired at the factory per the product model number ordered by the customer. Labconco model numbers ending in -11 are 115 volts. Model number ending in -12 are 230 volts. Models ending in -13 are 230 volt, three phase. Amperage requirements vary depending on the number of heaters per the digestion rack. The tables below provide the voltage and amperage electrical requirements for the Hooded Combination Macro-Kjeldahl Systems. A qualified electrician will need to connect the facility electrical supply to the main breaker box with its individual circuit breakers. The following basic steps should be followed:

1. Remove circuit breaker box cover panel.
2. The electrical service is routed through the cabinet in the front-left corner of the top and down to the electrical panel.
3. Main line lead connection terminals are identified and connections must be made accordingly.
4. Remote Blower on/off switch can be wired to control the remote blower from the unit. Remote Blower MUST be on before using the unit.
5. Line leads to the apparatus must conform to local electrical codes.
6. Provide an electrical ground to the apparatus per code.
7. Before applying power to apparatus, check the electrical panel and breakers for loose connections.
8. Reinstall box cover panel.
9. Power the breaker box and reset breakers to check circuits in the apparatus.
10. All electrical wiring and connections must conform to local codes and should be performed by qualified electricians. An earth ground must be provided.

P/N	AC Electrical Code	6-PL	12-PL	18-PL
-11	115v, single phase, 60 Hz	65 amp		
-12	230v, single phase, 60 Hz	35 amp	66 amp	96 amp
-13	208/230v, three phase, 60 Hz	20 amp	38 amp	56 amp

Figure 2-2

Tap Water Supply

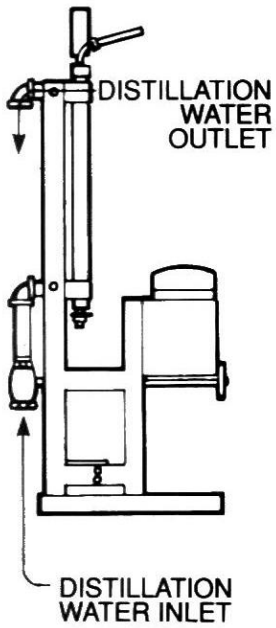
NOTE: ALL PLUMBING CONNECTIONS AND COMPONENTS MUST BE FREE OF FOREIGN MATERIAL BEFORE FINAL CONNECTIONS ARE MADE.

Distillation Manifold

The Hooded Combination Macro-Kjeldahl Systems feature an upper distillation rack with individual condensation columns, these are connected together by a common manifold (6 columns per manifold). The tap water inlet and outlet connections are 3/4" NPT and the supply line should be minimum 1/2" ID for proper water flow and heat removal capacity through the condensation columns (6-8 gallons/23-30 liters per minute). Hooded Combination Macro-Kjeldahl Systems with 12 or 18 heaters per rack will include two or three, 6 column manifolds which are connected together in series.

The plumbing line for the outlet of the distillation manifold must be free of back pressure to avoid flow restriction of the cooling water.

Water discharged from the distillation manifold is **not** acid contaminated and standard drain lines may be used for its removal.



Distillation Manifold Tap Water Connections
Figure 2-3

Acid Fume Blower Exhaust System

The exhaust duct connection on top of the blower is sized for use with 6" nominal (6-5/8" O.D.) vent duct. The exhaust piping assembly is supplied with a short piece of 6" PVC pipe and a flexible coupling with clamps to connect to the dedicated duct work exhaust. The flexible coupling must be sealed air-tight to the exhaust duct work.

All exhaust duct work must be acid-resistant and sealed air-tight. Use only acid-resistant sealant on duct work joints.

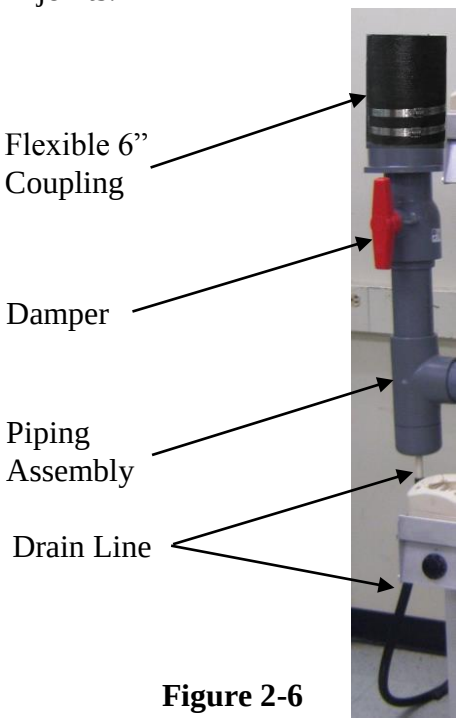


Figure 2-6

The bottom of the piping assembly is fitted with a condensate drain and corrosion resistant tubing. This condensate (identified above and on the next page) can be collected in a closed acid proof container, which should be emptied regularly or it may be plumbed to an acid resistant drain line. The condensate is concentrated acid and care must be exercised when handling it.

Blower Airflow Exhaust Volume Requirement & Velocity Test

Cubic feet per minute = 25
Average airflow velocity = 117 feet per minute

The Kjeldahl’s blower airflow volume test is based on the "Pitot Traverse in Inlet Duct" method described in the ANSI/ASHRAE 51-1999 standard. Duct velocity measurements are taken at 48" from the blower using a 6 point duct traverse pattern (Industrial Ventilation 24th Edition p. 9-10).

(Duct Area in Square Feet) x (Average Airflow Velocity in feet per minute) = (Air Volume CFM)

(Pi) x (radius^2) x (117 FPM) = 25 CFM

The exhaust stack rising from the piping assembly must be vented to the outside atmosphere via acid-resistant, sealed duct work and a remote blower (not included). If the remote blower pulls more than 25 CFM at the measurement point (48" above flexible coupling), close the damper shown in Fig. 2-6 to reduce airflow to the recommended 25 CFM.

WARNING: Acid resistant duct must be used on the exhaust connection on your Kjeldahl Apparatus.

Highly corrosive fumes produced from boiling sulfuric acid will flow through the ductwork, so acid-resistant duct must be used. Polyvinyl chloride (PVC) or fiberglass duct is recommended. The exhaust duct work **must** be supported independent of the Kjeldahl System to avoid distortion of the blower housing.

Digestion Rack Drip Shield

The digestion heaters are located in a drip shield and constructed of stainless steel which is resistant to acid attack. Liquid acid spills that occur can be drained to an acid resistant container with the drip tube located on the left side of the digestion rack.

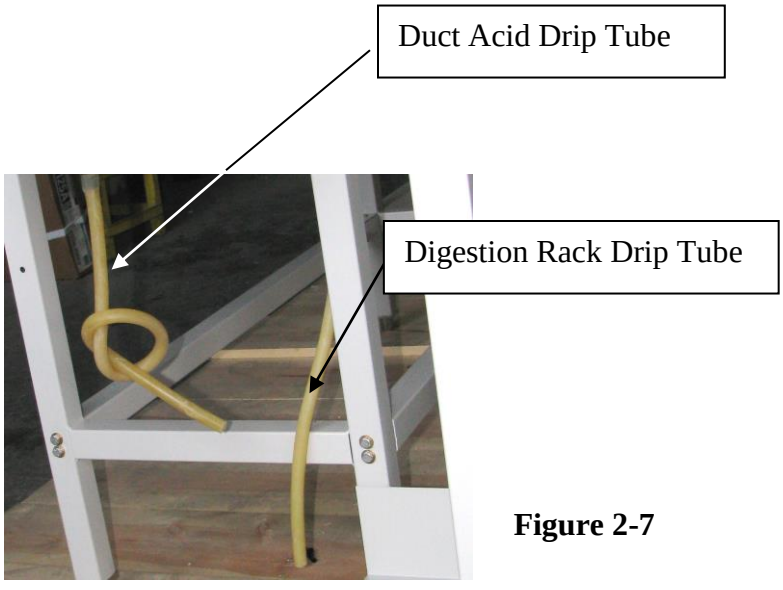


Figure 2-7

Sash Installation

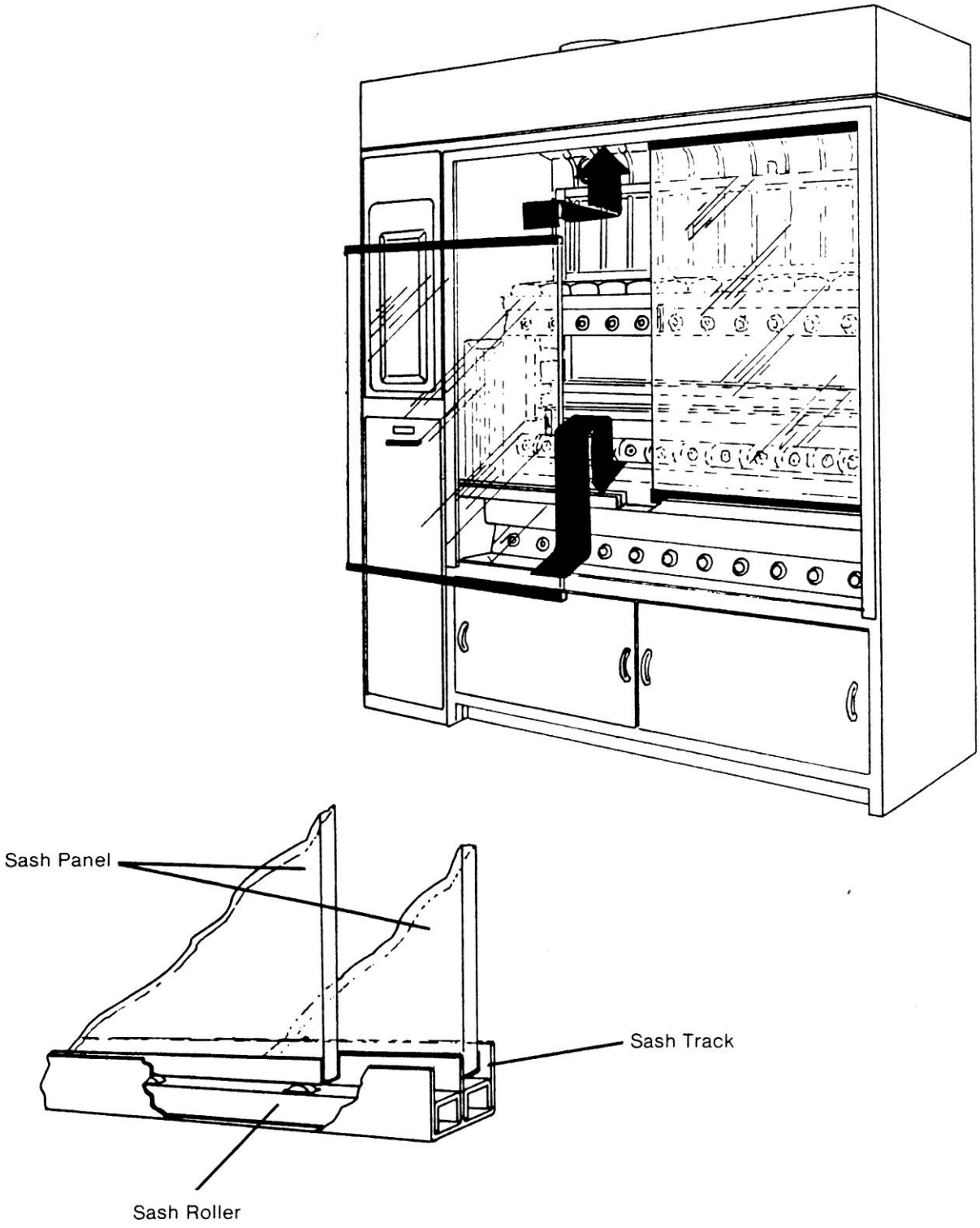


Figure 2-8

Glassware Installation

2081300 Replacement Connecting Bulbs
Connecting bulbs provide connection between Kjeldahl flask and distillation rack. The double baffle design traps caustic mist. Constructed of one-piece borosilicate glass. Six per package.
Shipping weight 4 lbs.

2128800 Replacement Delivery Tubes
Delivery tubes feature long necks, round perforated bottoms and thick walls to help eliminate pressure fluctuations in the distillation phase. Constructed of one-piece borosilicate glass. Six per package.
Shipping weight 3 lbs.

Figure 2-9

Chapter 3:
Normal Operation & Routine Maintenance

Normal Operation Start-Up

1. The KNA apparatus is designed for use with either 500 or 800-ml Kjeldahl Flasks. The digestion heaters are free to move forward or backward on the runway to accommodate either size flask.

2. Turn the heat controllers to the setting on both the Digester and Distillation racks if both racks will be used simultaneously. Wait approximately 5 to 10 minutes to insure proper preheating. Sample chemistry limits the boiling temperature of the sample, so the high heat setting may be used.

3. Start the acid fume removal blower or water aspirator (depending on model number purchased). Place a small piece of tissue paper over the nipple on the digestion manifold. Suction should be sufficient to hold 2" x 2" (51 mm x 51 mm) tissue paper in place.

4. Begin the distillation cooling water and check for leaks. For correct operation of the condensers, the temperature of the cooling water should not exceed 80°F (27°C). Watch your thermometer and regulate water flow by the control valve

5. The inner stainless steel condensing tubes on the distillation apparatus should be rinsed out by boiling nitrogen free water in Kjeldahl flasks attached to the condensation columns before commencing a distillation procedure.

A generalized Kjeldahl Total Nitrogen method is present in Appendix C. The method does not detail sample preparation or preservation procedures, which may be required depending on specific types of samples.

Clean-Up and Cosmetic Guidelines

- Keeping the Hooded Macro Kjeldahl System clean will preserve the appearance and improve life of the equipment.
- Access to the blower exhaust or water aspirator system is gained by removing the lower left front access panel, located below the electrical panel.
- The equipment can be kept clean by washing with a weak solution of sodium hydroxide and rinsing with clear water.
- Sulphate, a salt of Sulfuric Acid, may build up between the channel support and the fume removal manifold. Neutralize this with a weak solution of sodium hydroxide periodically to keep the unit clean.

Fume Removal Manifold Suction Adjustment

The suction along the fume removal manifold is adjusted as follows:

Blower Exhaust System

No adjustment is required on the suction pressure at the manifold when used. The remote blower should be inspected annually for debris, proper operation, and checked for adequate airflow as specified on pages 11-12 of this manual.

Exhaust Systems Maintenance

Sometimes the suction in the nipples is reduced due to obstruction within the exhaust system. These obstructions can usually be washed from the fume pipe by boiling water in a number of flasks placed on the digestion heaters. The remote blower should be turned on and operating to draw the steam through the fume pipe.

Drip Shield

The drip shield is constructed of stainless steel and is resistant to most acid attack. Acid spills can however cause discoloration and eventual marring of the surface unless the following proper procedures are followed in their clean up.

1. Promptly wash down all major acid spills contained by the shield.
2. Periodically clean the stainless surface with any residential stainless steel sink cleaning compound.
3. After cleaning, renew the bright shiny appearance by lightly sanding with 220 grade emery paper.

Appendix A:
Replacement Parts

Electrical Heaters and Motor/Blower Parts

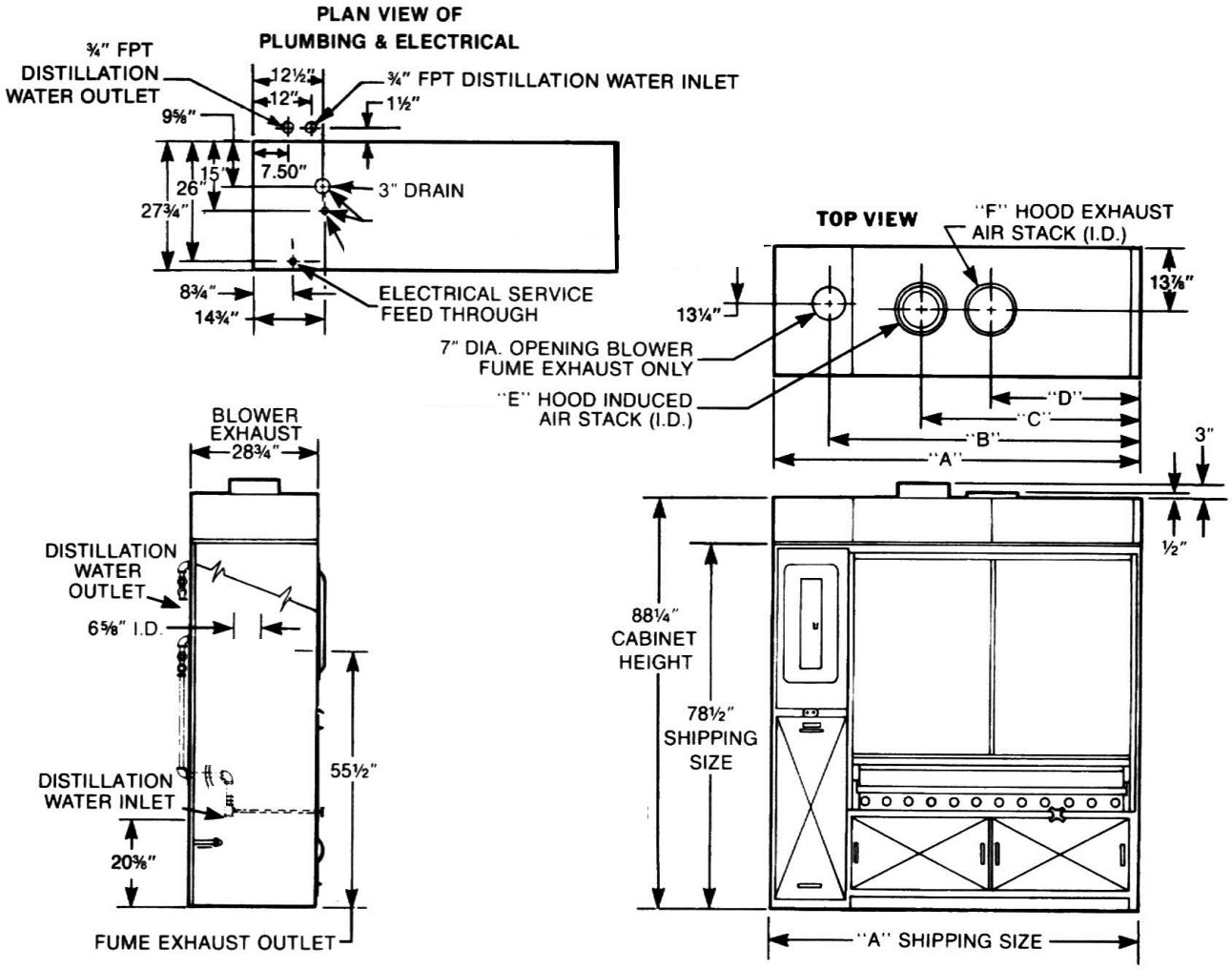
Part Number	Description
1315400	Heater Control, Infinite (115Volts)
1315500	Heater Control, Infinite (230Volts)
2011500	Core Plate Casting, (package of 6)
2023200	Heater Base Casting (electric or gas)
2033100	Heater element, 115V, 600 W, (package of 6)
2033200	Heater element, 230V, 600 W, (package of 6)
2023100	Heater Top ceramic casting (electric or gas)
2031800	Heater lead wire assembly, (package of 6 pairs)
1870200	Knob for Infinite Heater Control
1317100	Blower Motor Switch
2056600	Ceramic nipple
2053000	Water ejector nozzle
1967000	Clamp, hose
2144600	Flex sleeving
1662200	Grommet 1/2 ID x 1-1/2 OD
2165800	PTFE nipple
1880128	Plastic screws for PTFE nipple

Miscellaneous Replacement Parts

Part Number	Description
2152402	Distillation manifold – 6 place stainless steel
2038800	Wire, No 14 Black, SEWF-2, 30 ft length
2038900	Wire No. 14 White, SEWF-2, 30 ft length
2031700	Heater terminal assembly, package of 24
2146400	Assembly inlet & outlet manifold (12 unit only)
2081300	Connector bulb/caustic trap, package of 6
2128800	Delivery tube, package of 6
2078800	Kit, Replacement Fume Pipe

Appendix B: Dimensions & Airflow Requirements

B-1



SIDE VIEW

UNIT	EXHAUST CFM	AUX.-AIR CFM	MAX. KW
6	500	335	7
12	700	525	14
18	1000	750	21

FRONT VIEW

UNIT	A	B	C	D	E	F
6	49¾"	38¼"	26⅞"	17"	8⅝"	8⅝"
12	80¼"	68¾"	47½"	32¼"	10¾"	10¾"
18	110¾"	99¼"	70⅜"	47½"	10¾"	10¾"

Appendix C:
Total Kjeldahl Nitrogen Method

Titrimetric Determination

Various scientific associations offer approved Kjeldahl methods. These methods are available at their websites. The AOAC International (*Official Methods of Analysis*), Association of American Cereal Chemists (*Approved Methods*), American Oil Chemists Society, Environmental Protection Agency (*EPA Methods for Chemical Analysis of Water and Wastes*), International Standards Organization, The National Forage Testing Association, and United States Department of Agriculture.

This methodology is for reference only. It is not a citable document. It is based on data we believe to be reliable. It is offered in good faith but without guarantee.

1. **Scope and Application**
   - **1.1.** This method determines the Total Kjeldahl Nitrogen (TKN) in water, and organic substances. There are three steps within the method: 1. the digestion procedure – which converts organic nitrogen to ammonia, 2. the distillation procedure - transferring ammonia from the digested sample to an ammonia trapping solution 3. sample titration – the quantification of ammonia in the trapping solution. **NOTE 1:** Some compounds containing nitrogen may not be converted; such as amines, nitro-compounds, hydrazones, oximes, semicarbazones and some tertiary amines.
   - **1.2.** This method is described for use with Kjeldahl equipment using Macro-Size glassware (500 - 800 ml flasks).

2. **Definitions**
   - **2.1.** TKN is defined as the sum of free-ammonia and organic nitrogen compounds, converted to ammonium sulfate $(NH_4)_2SO_4$ by acid digestion.

3. **Apparatus**
   - **3.1.** Digestion Apparatus: A Kjeldahl digestion apparatus with multiple 600 watt electric burners, using 500 - 800 ml flasks and a fume removal manifold system.
   - **3.2.** Distillation Apparatus: A Kjeldahl distillation apparatus with multiple 600 watt electric burners, using 500 - 800 ml flasks. The distillation system includes connecting bulbs to prevent mechanical carryover of NaOH during distillation, water cooled condensers and

distillate receiving vessels, such as 250 ml Erlenmeyer flasks. The equipment is available as a combined system, featuring both digestion and distillation apparatus. The preheated burners should bring 250 ml of water at 25° C to a rolling boil with in 5 minutes. Condensation columns should cool distillate to 75° F (23.8°C).

3.3. Titration Class A Burets, 25-50 ml. for dispensing Standardized Acid solution.

3.4. Analytical balance, sensitive to 0.0001grams.

4. Reagents

4.1. Purified water, nitrogen free.

4.2. Sulfuric acid, H_2SO_4 concentrated, Specific Gravity of 1.84. (95-98%, nitrogen free)

4.3. Copper sulfate, $CuSO_4$, anhydrous. Nitrogen free. (catalyst)

4.4. Potassium sulfate, K_2SO_4. Nitrogen free. (boiling point elevator)

4.5. Sodium hydroxide NaOH, reagent grade Specific Gravity 1.3, nitrogen free. 45% solution, (dissolve pellets, 450g NaOH in distilled water and dilute to 1 liter).

4.6. Boiling stones, Antibumping agent, aluminum oxide stones, (Hengar granules).

4.7. Mixed color indicator: 0.75 g of Methyl Red and 0.5 g Methylene Blue in 300 ml of 95% ethanol. (Prepare fresh every 30 days)

4.8. Boric acid H_3BO_3, saturated solution, (dissolve granules, 40 g boric acid, in purified water and dilute to 1 liter). Add 3 ml of the mixed color indicator solution to the 1 liter of boric acid solution.

4.9. Sulfuric acid, H_2SO_4, standardized solution: (0.02 N) Prepare a stock solution of approximately 0.1 N sulfuric acid by diluting 3 ml of conc. H_2SO_4 (sp. Gr. 1.84) to 1 liter with CO_2-free distilled water. Dilute 200 ml of this solution to 1 liter with CO_2-free distilled water. Alternatively, premade standardized acid and base solutions with a certified specification ranges are commercially available through most Scientific Equipment Dealers. If the Sulfuric Acids standard solutions are prepared, their normality must be determined analytically.

5. Digestion

5.1. The distillation condensation columns should be cleaned before use by distilling a 1:1 mixture of distilled water and sodium hydroxide until the distillate is ammonia-free. Then repeat this cleaning procedure if the apparatus is out of service long enough to accumulate ammonia (> 4 hours).

5.2. Digestion

5.2.1. Place a homogenous measured sample (0.250 – 1.000 gram) into a 500 - 800 ml Kjeldahl flask. Weigh or measure the sample to the nearest 0.1 mg/ml.

5.2.2. Add to each flask:
20 ml sulfuric acid, 0.04 g $CuSO_4$, 15g K_2SO_4 potassium sulfate, 8-10 boiling stones. Place flask on the digestion burner in inclined position (promotes acid refluxing), with the flask neck on the fume removal system. If necessary

lower the burner heat setting to prevent sample from foaming up into neck of flask. Once, the sample comes to a smooth rolling boil return heat setting to maximum. When digestion sample clears (no black specs with pale green tint color), continue to boil for an equal length of time as required to reach the clear point, (total time approx. 1 hr). If black specs occur in the flask neck, rotate the flask 180 degrees to allow refluxing acid to rinse the internal flask surface. Allow digestion mixture to cool and **carefully** add 50 ml of distilled water to prevent K_2S0_4 salt solids from forming. All salt crystals must be dissolved before proceeding to the next step. If necessary partially reheat sample and agitate to dissolve crystals.

6. Distillation

6.1. The distillation burners should be preheated and condenser cooling water turned on. The receiving flask should be in-place with enough boric acid H_3BO_3 plus the mixed indicator solution to submerge the tip of condenser delivery tube well below the level of the boric acid receiving solution in the receiving flask. (Erlenmeyer 250 ml flasks recommended)

6.2. In a fume hood, carefully adding of 100 ml of 45% sodium hydroxide solution without mixing to make the digested sample alkaline. Tilt the flask in to the hood while adding the sodium hydroxide to the sulfuric acid digestion solution. Slowly add the heavy sodium hydroxide solution down the neck of the flask. The heavier sodium hydroxide solution will slip under the aqueous sulfuric acid solution without mixing and with out loss of free-ammonia. Do not mix until the flask has been connected to the distillation apparatus.

6.3. Immediately connect the flask to a condenser using the rubber stopper on the distillation connecting bulb attached to a condenser column.

6.4. Vigorously swirl the Kjeldahl flask to mix contents thoroughly; heat until all NH_3 has been distilled ($\geq$8-10 minutes). During the ammonia transfer the receiving solution with change color from purple to green. Lower receiving flask and let liquid drain from condenser tip. Turn off distillation burners.

6.5. For nitrogen concentrations above 1 mg/1, the ammonia can be determined titrimetrically. For concentrations below this level, colorimetric or potentiometric determination methods are recommended.

7. Titration

7.1. Depending on the samples expected nitrogen level and the sensitivity of the titration desired, select the appropriate standardized H_2SO_4 solution (0.02 or 0.10 normal). High or low nitrogen concentrations will require standardized H_2SO_4 solutions with stronger or weaker normalitys. Titrations should require at least 15 ml of titrant to be accurate. Fill a class A. buret to the zero line with the standardized H_2SO_4 solution. Titrate the H_3BO_3 receiving solution with standardized H_2SO_4 solution to first trace of the original purple color. A white stirring plate will aid color visualization of end point. Record ml H_2SO_4 titrated to the at least nearest 0.05 ml. Match the endpoint against a sample blank containing the same volume of distilled water and H_3BO_3 solution.

8. Calculation

For Dry Samples

% Nitrogen, = (ml H_2SO_4, sample - ml H_2SO_4, blank) x Normality H_2SO_4 x 1.4007/ **weight of sample in grams. (1.4007 = a single factor that takes into account the molecular weight of Nitrogen, the conversion of milli-equivalent results of V*N, and the conversion to %)**

% Nitrogen, = (ml H_2SO_4, sample - ml H_2SO_4, blank) x normality H_2SO_4 x 1400.7 x 100/ **weight of sample in milligrams.**

For Liquid Samples: calculate Total Kjeldahl Nitrogen, in mg/1, in the original sample as follows:

Milligrams Total Nitrogen per Liter = (ml H_2SO_4, sample - ml H_2SO_4, blank) x normality H_2SO_4 x 14.007x 1000/ volume of sample in milliliters.

If desired to determine % protein instead of % nitrogen, the calculated % N is multiplied by a factor, the magnitude of the factor depending on the sample matrix.

Common Protein Factors

5.7 – bread, wheat and wheat flour

6.25 – other grains

6.38 – milk

6.25 – unknown source

Total Kjeldahl Nitrogen Method Overview

Digestion is accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The end result is an ammonium sulfate solution. The general equation for the digestion of an organic sample is shown below:

Organic N + H_2SO_4 →

$(NH_4)SO_4$ + H_2O + CO_4 + other sample matrix byproducts

Distillation: Excess base is added to the digestion product to convert NH_4 to NH_3 as indicated in the following equation. The NH_3 is recovered by distilling the reaction product.

ammonium sulfate		**heat**		**ammonia gas**
$(NH_4)_2SO_4$ + 2NaOH	**→**		**$2NH_3 + Na_2SO_4 + 2H_2O$**	

Titration quantifies the amount of ammonia in the receiving solution. The amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ion in the receiving solution.

There are two types of titration—back titration and direct titration. Both methods indicate the ammonia present in the distillate with a color change.

In the direct titration, boric acid is used as the receiving solution instead of a standardized mineral acid, the chemical reaction is:

ammonia gas	**boric acid**		**ammonium-borate complex**	**excess boric acid**
NH_3 +	**H_3BO_3**	**→**	**$NH_4 + H_2BO^-_3$ +**	**H_3BO_3**

(color change occurs)

The boric acid captures the ammonia gas, forming an ammonium-borate complex. As the ammonia collects, the color of the receiving solutions changes.

ammonium-borate complex	**sulfuric acid**	**ammonium sulfate**	**boric acid**
$2NH_4$	**+ $H_2BO^-_3$**	**+ H_2SO_4 $(NH_4)_2SO_4$**	**+ $2H_3BO_3$**

(color change occurs in reverse)

The boric acid method has the advantages that only one standard solution is necessary for the determination and that the solution has a long shelf life.