Add the sodium chloride 70.0 g for (ii) Fluid Soybean-Casein Digest Medium (containing 5 g of sodium chloride), mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 - 7.5.

(xix) Vogel-Johnson Agar Medium

Casein peptone	10.0 g
Yeast extract	5.0 g
D-Mannitol	10.0 g
Dipotassium hydrogenphosphate	5.0 g
Lithium chloride	5.0 g
Glycine	10.0 g
Phenol red	0.025 g
Agar	16.0 g
Water	1000 mL

Mix all the components, and boil for 1 minute to effect solution. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 7.0 - 7.4. To this solution add 20 mL of sterile potassium tellurite solution (1 in 100), and mix.

(xx) Baird-Parker Agar Medium

Casein peptone	10.0 g
Meat extract	5.0 g
Yeast extract	1.0 g
Lithium chloride	5.0 g
Glycine	12.0 g
Sodium pyruvate	10.0 g
Agar	20.0 g
Water	950 mL

Mix all the components. Heat the mixture with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 6.6 - 7.0. To this solution add 10 mL of sterile potassium tellurite solution (1 in 100) and 50 mL of egg-yolk emulsion. Mix gently, and pour into petri dishes. Prepare the egg-yolk emulsion by mixing egg-yolk and sterile saline with the ratio of about 30% to 70%.

(xxi) Mannitol-Salt Agar Medium

Casein peptone	5.0 g
Animal tissue peptone	5.0 g
Meat extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Phenol red	0.025 g
Agar	15.0 g
Water	1000 mL

Mix all the components. Heat with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.2 - 7.6.

(3) Reagent·Test solution

Amphotericin B powder Amphotericin B added sodium deoxycholic acid, sterilized by γ-ray.

Amphotericin B TS Dissolve 22.5 mg of amphotericin B powder in 9 mL of sterile purified water.

Bile salts Yellow-brown powder made from dried bile of animal, consist of sodium taurocholic acid and sodium glycocholic acid, and containing not less than 45% of cholic acid. pH of 5% solution: 5.5 - 7.5.

Rose bengal $C_{20}H_2Cl_4I_4Na_2O_5$ [Special class] Red-brown powder, purple-red solution in water.

Rose bengal TS Dissolve 1 g of rose bengal in water to make 100 mL.

2,3,5-Triphenyl-2*H*-tetrazolium chloride TS (TTC TS) Prepare a 0.8% TTC solution (dissolve 0.8 g of TTC in water to make 100 mL), distribute in small tubes and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. Store in light-resistant containers.

(4) Preparation

Preparation of agar medium with TTC

Just prior to use, add 2.5 to 5 mL of TTC TS per liter of sterile medium and mix.

Preparation of agar medium with amphotericin B

Dissolve 22.5 mg of amphotericin B sterilized by γ-ray in 9 mL of sterile purified water. Just prior to use, add 2 mL of this solution in a liter of sterile medium and mix.

Preparation of agar medium with rose bengal

Add 5 mL of rose bengal TS in a liter of medium and mix. Sterilize in an autoclave at 121°C for 15 to 20 minutes.

37. Mineral Oil Test

The Mineral Oil Test is a method to test mineral oil in nonaqueous solvents for injections and for eye drops.

Procedure

Pour 10 mL of the sample into a 100-mL flask, and add 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of ethanol (95). Put a short-stemmed, small funnel on the neck of the flask, and heat on a water bath to make clear, with frequent shaking. Then transfer the solution to a shallow porcelain dish, evaporate the ethanol on a water bath, add 100 mL of water to the residue, and heat on a water bath: no turbidity is produced in the solution.

38. Nitrogen Determination (Semimicro-Kjeldahl Method)

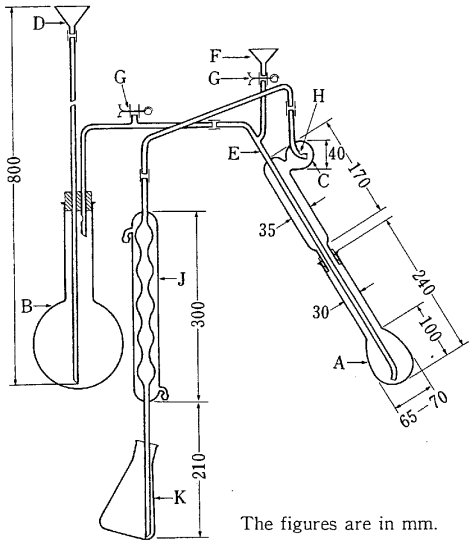
The Nitrogen Determination is a method to determine ammonia in ammonium sulfate obtained by decomposition of organic substances containing nitrogen with sulfuric acid.

Apparatus

Use the apparatus illustrated in the figure. It is thoroughly constructed of hard glass, and ground glass surfaces may be used for joints. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

Procedure

Unless otherwise specified, proceed by the following method. Weigh accurately or pipet a quantity of the sample corresponding to 2 to 3 mg of nitrogen (N:14.01), and place in the Kjeldahl flask A. Add 1 g of a powdered mixture of 10 g of potassium sulfate and 1 g of cupper (II) sulfate pentahydrate. Wash down any adhering sample from the neck of the flask with a small quantity of water. Add 7 mL of sulfuric acid, allowing it to flow down the inside wall of the flask.



The figures are in mm.

A: Kjeldahl flask
B: Steam generator, containing water, to which 2 to 3 drops of sulfuric acid and fragments of boiling tips for preventing bumping have been added
C: Spray trap
D: Water supply funnel
E: Steam tube
F: Funnel for addition of alkali solution to flask A
G: Rubber tubing with a clamp
H: A small hole having a diameter approximately equal to that of the delivery tube
J: Condenser, the lower end of which is beveled
K: Absorption flask

Then, while shaking the flask, add cautiously 1 mL of hydrogen peroxide (30) drop by drop along the inside wall of the flask. Heat the flask gradually, then heat so strong that the vapor of sulfuric acid is condensed at the neck of the flask, until the solution changes through a blue and clear to a vivid green and clear, and the inside wall of the flask is free from a carbonaceous material. If necessary, add a small quantity of hydrogen peroxide (30) after cooling, and heat again. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus washed beforehand by passing steam through it. To the absorption flask K add 15 mL of boric acid solution (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube J. Add 30 mL of sodium hydroxide solution (2 in 5) through the funnel F, rinse cautiously the funnel with 10 mL of water, immediately close the clamp attached to the rubber tubing G, then begin the distillation with steam, and continue until the distillate measures 80 to 100 mL. Remove the absorption flask from the lower end of the condenser tube J, rinsing the end part with a small quantity of water, and titrate the distillate with 0.005 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.14007 mg of N

39. Nuclear Magnetic Resonance Spectroscopy (^{1}H)

Nuclear magnetic resonance (NMR) spectroscopy is based on the phenomenon that specific radio frequency radiations are absorbed by magnetic nuclei in a sample placed in a magnetic field. These nuclei have intrinsic spin angular momentum, of which magnitude is given by $I(I+1)/h/2\pi$, where I is the spin quantum number and is integral or half-integral ($I = 1/2$ for ^{1}H). When the magnetic nuclei are placed in a magnetic field, they are oriented similarly to a bar magnet in $2I+1$ possible orientations corresponding to $2I+1$ energy levels equally spaced (two energy levels for ^{1}H). The transition between two successive quantized energy levels corresponding to the adjacent orientations can be induced by electromagnetic radiation with a suitable frequency. The precise relation between the field strength and the resonant frequency υ is given by

$$\upsilon = \gamma \cdot \frac{H_0}{2\pi}$$

where H_0 is the strength of the applied external magnetic field and γ is the gyromagnetic ratio, a constant characterizing a particular isotope. The absorption of radiation (NMR signal) can occur only when the irradiating radio frequency satisfies the resonance condition. Since this absorption coefficient (the transition probability) does not depend on the environments where the nuclei are located, the intensity of an absorption line is proportional to the number of nuclei involving in the absorption. The excess spins shifted to the higher energy levels by the transition process return to the thermal equilibrium state at various rates determined by characteristic time constants (known as relaxation time).

A nucleus is shielded from the applied magnetic field by the electrons belonging to its own atom and to the molecule. Therefore nuclei in different environments are shielded to different extents and resonate at different frequencies. The difference in resonance frequencies is defined as chemical shift (δ), which is given by

$$\delta \text{ ppm} = \frac{\upsilon_S - \upsilon_R}{\omega} \times 10^6,$$

where,

ω: The oscillator frequency in MHz (60 MHz, 100 MHz, etc.),
υ_S: The resonance frequency of the observed signal,
υ_R: The resonance frequency of the reference signal.

The chemical shifts are normally expressed in ppm, a dimensionless unit, by assuming the chemical shift of the reference compound as 0 ppm.

In addition to the shielding due to electrons, the nucleus is subjected to effects due to the spin orientations of other magnetic nuclei, resulting in an additional splitting of the signal. The spacing between two adjacent components of the signal is known as the spin-spin coupling constant (J). Coupling constants are measured in Herz and independent of the strength of the external magnetic field. The increased number of interacting nuclei will make the multiplet pattern more complex.

From the NMR spectrum the following four parameters