CENTRE UNIVERSITAIRE ABDELHAFID BOUSSOUF – MILA
Faculty of Natural and Life Sciences | Department of Biological Sciences

Course: Pharmacology-Toxicology
Level: 3rd Year Biochemistry & Microbiology
Instructor: Mr. B. Moussaoui

PRACTICAL SESSION NO. 2: ALCOHOMETRY

Preparation of External Antiseptic Solutions (Surgical Alcohol) by Dilution ("Mouillage")

I. OBJECTIVES & THEORETICAL BACKGROUND

Antiseptics are antimicrobial chemical agents designed to inhibit or destroy microorganisms on living tissues (skin, mucous membranes), whereas disinfectants are restricted to inert surfaces and medical equipment. Aliphatic alcohols—primarily ethyl alcohol (ethanol) and isopropyl alcohol (isopropanol)—are widely used antiseptic agents in clinical practice.

Pharmacological Mode of Action: Alcohols act primarily through non-specific protein denaturation and membrane disruption. Optimal bactericidal activity occurs in aqueous solutions containing **60% to 80% v/v ethanol**, with peak activity at **70% v/v**. Efficacy drops significantly in pure ethanol (>95% v/v) due to poor protein ionization in the absence of water.

II. ANTIMICROBIAL SPECTRUM & VIRUCIDAL EFFICACY

- **Bactericidal Action:** Rapidly bactericidal against vegetative bacteria (e.g., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*). Ineffective against bacterial endospores.
- **Virucidal Action:** Highly effective against **enveloped viruses** (e.g., Coronaviruses, Influenza, HSV, HIV) by solubilizing their viral lipid envelope. Variable efficacy against non-enveloped (hydrophilic) viruses (e.g., Poliovirus, Adenovirus).
- **Fungicidal Action:** Effective against most yeasts and vegetative fungi.

III. FUNDAMENTAL DEFINITIONS IN ALCOHOLOMETRY

Term	Definition	Standard Units
Alcoholometry	The set of analytical operations used to determine ethanol content in hydroalcoholic mixtures.	—
Volumetric Titer (TAV)	Number of volumes of pure ethanol at 20°C contained in 100 volumes of solution at 20°C.	% v/v, % vol, °GL
Mass Titer (TAP)	Ratio of the mass of pure ethanol to the total mass of the hydroalcoholic mixture.	% m/m, % mass
Volumetric Contraction	The decrease in total solution volume observed when mixing ethanol and water due to hydrogen bonding.	ΔV (mL)

IV. DILUTION CALCULATIONS ("MOUILLAGE") & DENSITY CONVERSIONS

Due to volumetric contraction, preparing alcoholic solutions by mixing exact measured volumes introduces significant concentration errors. Therefore, accurate pharmaceutical preparation requires weighing mass components rather than measuring volume.

Reference Densities at 20°C (from official alcoholometric tables):

- Density of pure ethanol ($\rho_{ethanol}$): ≈ 0.789 g/cm³ (or g/mL)
- Density of stock concentrated alcohol (95° v/v): $\rho_{95°} \approx 0.805$ g/cm³
- Density of target surgical alcohol (70° v/v): $\rho_{70°} \approx 0.885$ g/cm³

Volume-to-Mass Fraction Conversion Formula:

$$C_{mass} = (V_{v/v} \times \rho_{ethanol}) / \rho_{solution}$$

Fundamental Mass Dilution Formula: $m_{stock} = (C_{final} \times m_{final}) / C_{initial}$

Where m represents mass (g) and C represents concentration expressed in mass fraction (% m/m).

V. LABORATORY REAGENTS & EQUIPMENT

- **Equipment:** Precision analytical balance (±0.001 g), glass beakers (50 mL and 100 mL), glass funnel, dropping pipette, watch glass.
- **Reagents:** Stock concentrated ethanol (95% v/v), purified/distilled water, methylene blue solution (0.1% aqueous).

VI. STEP-BY-STEP EXPERIMENTAL PROCEDURE

Task: Prepare exactly 10.0 g of 70% v/v surgical alcohol using 95% v/v stock ethanol.

1. **Balance Setup:** Power on the analytical balance, ensure it is level, and verify zero reading.
2. **Taring:** Place a clean, dry 50 mL beaker onto the balance pan and press the **TARE** button (ensure the display reads 0.000 g).
3. **Stock Addition:** Using density-derived mass fractions, calculate and weigh the precise mass of 95% v/v stock ethanol into the tared beaker.
4. **Water Addition:** Add distilled water dropwise until the total target mass of 10.0 g is reached (mass of water = 10.0 g - mass of stock ethanol).
5. **Homogenization:** Gently swirl the beaker for 30 seconds to ensure complete mixing and temperature stabilization.
6. **Coloration:** Add exactly 1 drop of methylene blue solution to yield a faint blue coloration (used in clinical settings to distinguish surgical alcohol from non-antiseptic clear solvents).

> **Methodological Note for Students:** Always use the technical term "to tare" when zeroing the balance container weight. Do not confuse volumetric concentration (% v/v) with mass fraction (% m/m) during density conversion calculations.