Aim: Introduction to In-Vitro Pharmacology and Physiological Salt Solutions

References

1. G. L. G. G. V. B. C. L. & H. W. H. (2010). In-Vitro Pharmacology: Methods and Techniques. Springer.

2. T. G. L. & S. M. (2006). Preparation and Use of Physiological Solutions in Pharmacological Research. Academic Press.

3. Goodman & Gilman's: The Pharmacological Basis of Therapeutics (13th Edition).

Introduction

In-vitro pharmacology involves the study of drug effects on biological systems outside a living organism, typically in controlled environments like test tubes or petri dishes. This approach allows researchers to understand drug interactions, mechanisms of action, and potential side effects with high precision.

Physiological salt solutions (PSS) are crucial in maintaining the viability of biological tissues and cells during in-vitro experiments. These solutions mimic the ionic composition of bodily fluids, providing a stable environment for studying physiological processes and drug responses.

Objectives

1. Understand the Basics of In-Vitro Pharmacology: Learn about the principles and applications of in-vitro pharmacology.

2. Prepare Physiological Salt Solutions: Gain hands-on experience in preparing standard PSS used in experiments.

3. Perform Pharmacological Assays: Utilize prepared PSS to conduct basic pharmacological assays and observe drug effects.

Equipment and Materials

Equipment

- Analytical balance

- Magnetic stirrer and stir bars

- pH meter

- Water bath (if needed for heating solutions)

- Pipettes and pipette tips

- Glassware (e.g., beakers, flasks, and test tubes)

- Sterile containers (for storing solutions)

- Centrifuge (if required for specific assays)

Materials

- Distilled water

- Sodium chloride (NaCl)

- Potassium chloride (KCl)

- Calcium chloride ($CaCl_2$)

- Magnesium sulfate ($MgSO_4$)

- Sodium bicarbonate ($NaHCO_3$)

- Glucose (optional, depending on experiment)

Physiological Salt Solutions (PSS)

Common Types of PSS

1. Tyrode's Solution

2. Ringer's Solution

3. Hank's Balanced Salt Solution (HBSS)

Each solution has a specific composition tailored for different types of cells or tissues.

Tyrode's Solution Composition

Tyrode's solution is commonly used for physiological experiments and consists of:

- **NaCl:** 137 mM

- **KCl:** 2.7 mM

- **$CaCl_2$:** 1.8 mM

- **$MgCl_2$:** 1.0 mM

- **$NaHCO_3$:** 12.0 mM

- **Glucose:** 5.6 mM

- Distilled water to 1 liter

Preparation of Tyrode's Solution

1. Weigh and Dissolve Salts:

- Weigh the following amounts of each salt:

- NaCl: 8.0 g

- KCl: 0.2 g

- $CaCl_2$: 0.2 g

- $MgCl_2$: 0.1 g

- $NaHCO_3$: 1.0 g

- Glucose: 1.0 g (if using)

2. Dissolve Salts:

- Add the weighed salts into 800 mL of distilled water.

- Stir the mixture using a magnetic stirrer until all salts are completely dissolved.

3. Adjust pH:

- Check the pH of the solution using a pH meter. The pH should be around 7.4. Adjust if necessary using 1M HCl or 1M NaOH.

4. Adjust Volume:

- Add distilled water to the mixture until the final volume reaches 1 liter.

5. Sterilize:

- Sterilize the solution by filtering through a 0.22 µm filter or autoclaving.

6. Store:

- Store the prepared Tyrode's solution at 4°C if not used immediately.

In-Vitro Pharmacology Assays

Basic Assay Procedure

1. Prepare Biological Material:

- Isolate or prepare tissues/cells according to experimental needs.

2. Add PSS:

- Place the biological material into a container with Tyrode's solution or another PSS.

3. Introduce Test Compounds:

- Add the drug or compound of interest to the PSS.

4. Incubation:

- Incubate the preparation at the required temperature (often 37°C) for a specified period.

5. Observe and Record Results:

- Measure physiological responses or biochemical changes. Use appropriate techniques (e.g., spectrophotometry, microscopy) to record observations.

6. Analyze Data:

- Perform statistical analyses to interpret the effects of the drug or compound on the biological material.

Calculations

Molarity Calculation

To prepare a solution with a specific molarity, use the formula:

$$\text{Molarity (M)} = \frac{n}{V}$$

where:

- (n) = number of moles of solute

- (V) = volume of solution in liters

To convert grams to moles:

$$\text{Moles} = \frac{mass\ (g)}{molar\ mass\ (\frac{g}{mole})}$$

Example Calculation

To prepare 1 liter of a 0.1 M NaCl solution:

1. Calculate the number of moles required:

Moles = 0.1M × 1 L = 0.1 moles

2. Calculate the mass needed:

Mass = 0.1 moles × 58.44 g/mol = 5.844 g

3. Dissolve 5.844 g of NaCl in distilled water and adjust the volume to 1 liter.