AL-MUSTAQBAL UNIVERSITY

COLLEGE OF SCIENCE / DEPARTMENT OF BIOCHEMISTRY

First Experiment: **Preparation of Nucleotide Standards**
Practical Biochemistry–Third Stage

By: ABBAS HAMZA KHUDAIR

Theoretical Background:

Nucleotides are the fundamental building blocks of nucleic acids (DNA and RNA). Each nucleotide consists of a nitrogenous base (adenine, guanine, cytosine, thymine, or uracil), a sugar (ribose or deoxyribose), and phosphate groups. These molecules absorb ultraviolet (UV) light strongly at 260 nm due to the presence of aromatic nitrogenous bases.

In biochemical and molecular biology experiments, it is essential to prepare accurate standard solutions of nucleotides to:

- Quantify nucleic acid concentrations using spectrophotometry
- Calibrate analytical instruments (UV-Vis, HPLC)
- Perform enzymatic or labeling reactions with precise substrate concentrations

The most common method for determining nucleic acid concentration is spectrophotometry at 260 nm. Additionally, the A260/A280 ratio **helps assess the purity of nucleic acids by detecting protein contamination.**

Objectives:

- To prepare accurate standard solutions of nucleotides (ATP, GTP, CTP, UTP, dATP, dGTP, dCTP, dTTP).
- To practice calculations involving molarity (M = mol/L).
- To verify nucleotide concentration using UV absorbance at 260 nm.
- To assess purity based on the A260/A280 ratio.

Materials & Chemicals and Their Purpose:

Material/Chemical	Purpose
Nucleotide powders (ATP, etc.)	Main solutes to prepare standard solutions
TE buffer (Tris-EDTA)	Dissolving medium that stabilizes nucleotides; Tris maintains pH, EDTA binds metal ions (e.g., Mg^{2+}) to prevent degradation
Distilled water	Alternative solvent (less preferred due to lack of buffering capacity)
Microcentrifuge tubes (labeled)	For sample preparation, labeling, and cold storage
Gloves & Lab coat	Personal protective equipment to avoid contamination

Instruments & Equipment and Their Function:

Equipment	Function
Analytical balance	Precisely weigh small quantities of nucleotide powders (milligram scale)
Micropipettes & tips	Accurate and sterile transfer of small liquid volumes (µL range)
Vortex mixer	Mixes and ensures complete dissolution of nucleotide powders
UV-Vis spectrophotometer	Measures absorbance at 260 nm and 280 nm to quantify concentration and assess purity
Ice box or Freezer (−20°C)	Stores nucleotide standards to prevent degradation over time
Quartz cuvettes	Used in spectrophotometer (UV-transparent) for accurate absorbance measurement

Procedure:

1. Calculate the required mass of each nucleotide using:
   $M = m / (MW \times V)$
   → Rearranged: $m = M \times MW \times V$
   (Example: to prepare 0.1 M solution of ATP in 1 mL, $m = 0.1 \times MW \times 0.001$)
2. Weigh each nucleotide powder accurately using an analytical balance.
3. Transfer to a labeled microcentrifuge tube and add the required volume of TE buffer or water.
4. Vortex the solution until the powder is fully dissolved and appears clear.
5. Measure absorbance at 260 nm (and 280 nm if purity check is required) using a UV-Vis spectrophotometer.
6. Calculate concentration using Beer–Lambert Law:

C (µg/mL) = A260 × ε × dilution factor
(ε = 50 for dsDNA, 40 for RNA)

7. Adjust concentration if needed by dilution with buffer or water.
8. Store prepared standard solutions at **−20°C.**

Observations:

- Nucleotide solutions appear clear and colorless.
- Absorbance at 260 nm should be within readable range (e.g., 0.1–1.0 AU).
- **A260/A280 ratio** close to 1.8 (for DNA) or 2.0 (for RNA) indicates purity.

MCQs – Multiple Choice Questions:

1. What is the main purpose of preparing nucleotide standard solutions?
   A. To test enzyme activity
   B. To provide known concentrations for quantification
   C. To synthesize nucleotides
   D. To purify proteins

2. What is the ideal solvent for nucleotide standards?
   A. Normal saline
   B. Acetate buffer
   C. TE buffer
   D. Tris-HCl only

3. What is the expected absorbance maximum for nucleotides?
   A. 280 nm
   B. 240 nm
   C. 320 nm
   D. 260 nm

4. What does a low A260/A280 ratio (<1.8) suggest?
   A. High purity
   B. RNA contamination
   C. Protein contamination
   D. DNA fragmentation

5. Why should nucleotide standards be stored at −20°C?
   A. To prevent evaporation
   B. To increase pH
   C. To prevent degradation
   D. To improve solubility

Theoretical (Short Answer) Questions:

1. Why is TE buffer preferred over distilled water?
   → **TE buffer provides pH stability (Tris) and protects nucleotides from degradation by chelating metal ions (EDTA).**
2. How is nucleotide concentration determined using absorbance?
   → **Using Beer-Lambert Law: C = A / (ε × l), where A = absorbance, ε = extinction coefficient, and l = path length (cm).**
3. What is the ideal A260/A280 ratio for pure DNA?
   → **Around 1.8**
4. What is the role of EDTA in TE buffer?
   → **EDTA binds divalent metal ions like Mg^{2+} that could activate nucleases and degrade DNA/RNA.**
5. Why must nucleotide standards be stored in a freezer?
   → **To minimize enzymatic and chemical degradation over time.**

References:

- Nelson, D. L., & Cox, M. M. (2021). Lehninger Principles of Biochemistry (8th ed.). W.H. Freeman.
- Wilson, K., & Walker, J. (2018). Practical Biochemistry: Principles and Techniques (7th ed.). Cambridge University Press.