Annexure I: Methodology and Experimental Protocols for Nanoparticle Enhanced Radiotherapy

1. Introduction

This annexure outlines the experimental methods used in the study of nanoparticle-enhanced radiotherapy. The focus is on the synthesis of nanoparticles, their characterization, and their application in radiotherapy to enhance tumor treatment outcomes. The following sections provide detailed descriptions of the materials, procedures, and equipment involved in the experiments.

2. Materials

- **Nanoparticles:**
    - Iron oxide nanoparticles (IONPs)
- **Chemicals:**
    - Solvents (e.g., dimethyl sulfoxide, ethanol)
    - Stabilizing agents (e.g., polyvinyl alcohol, citrate)
    - Radiation sensitizers (e.g., cisplatin or other chemotherapeutic agents)
- **Biological Materials:**
    - Cell lines (e.g., DNA assay or other tumor cells)

3. Nanoparticle Synthesis

- **Iron Oxide Nanoparticle Synthesis:**
    - **Method:** Co-precipitation method
    - **Procedure:**
        1. A mixture of iron salts ($FeCl_3$ and $FeCl_2$) is dissolved in water.
        2. The solution is made alkaline by adding sodium hydroxide.
        3. The solution is heated under constant stirring to form iron oxide nanoparticles.
        4. The particles are then separated, washed, and dried.
- **Characterization:**
    - **Size and Shape:** Transmission electron microscopy (TEM), Dynamic light scattering (DLS)
    - **Surface Charge:** Zeta potential measurements
    - **Surface Area and Porosity:** BET surface area analysis

4. Nanoparticle-Enhanced Radiotherapy Protocol

1. **Nanoparticle Administration:**
    - Nanoparticles are administered via intravenous (IV) injection or intratumoral injection.
    - The concentration and dosage are optimized based on pre-study trials.
2. **Radiotherapy:**
    - Radiotherapy is delivered using a linear accelerator (LINAC)
    - The radiation dose is typically in the range of 2–10 Gy, depending on the

    Radiation Sensitization Assessment:

    - Cell survival is assessed using colony-forming assays or DNA assays.
    - The sensitization enhancement ratio (SER) is calculated by comparing the radiation dose required to reduce cell survival by 50% (D_{50}) in nanoparticle-treated versus untreated controls.

5. Analytical Methods

- **In Vitro Assays:**
    - **DNA Assay** to evaluate cell viability post-treatment.

6. Statistical Analysis

- Data will be analyzed using statistical software (e.g., GraphPad Prism).
- **Tests:** One-way or two-way ANOVA for comparing different radiation dosage