# Development of Intelligent Nanomaterials for Enhanced Performance in Medical Engineering Applications

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This study focuses on smart nanomaterials for enhanced medical applications, more so the synthesis and characterization of pH-sensitive PLGA-MAA nanoparticles for drug delivery. About the method, PLGA, methacrylic acid, and doxorubicin nanoparticles were synthesized and characterized by size, shape, zeta potential, drug entrapment efficiency, and release behavior under different pH values. The outcome demonstrated that the synthesis was effective with an average size of 182. 4 nm and an encapsulation efficiency of 77 percent. 2%. The nanoparticles released a very low amount of drug at a physiological pH of 7.  4 and a high amount of drug at a lower pH of 5.  5 with enhanced cytotoxicity to MCF-7 cancer cells at the lower pH. According to the study, these pH-sensitive nanoparticles can offer a high degree of improvement in the delivery of targeted drugs, and hence, the overall toxicity to the system is reduced while the effectiveness of the treatment in cancer is enhanced.
**Keywords:** Intelligent nanomaterials, medical engineering, Doxorubicin, pH-sensitive nanoparticles,

## 1. Introduction

Nanotechnology is one of the most promising fields of science and technology, which provides exceptional solutions to many worldwide issues. This field occurs at the nanoscale, and it allows the manipulation of materials to develop new structures that possess characteristics that are not achievable in large structures. One of the most important achievements in the field of nanotechnology is the creation of nanomaterials, which are materials with at least one of the dimensions of not more than 100 nanometers. These nanomaterials have some physicochemical properties like high surface area, quantum confinement effects, and variable optical and electrical properties that have created new opportunities in different fields, especially in medicine [1], [2]. The application of nanomaterials in the medical field has been crucial in the creation of new drug delivery systems where nanomaterials can be used to deliver therapeutic agents to specific cells or tissues thus reducing side effects and increasing the effectiveness of treatment. Also, nanomaterials have enhanced diagnostic imaging by increasing the resolution and sensitivity of diseases in their early stages [3]. In tissue engineering, nanomaterials are employed to create a scaffold that resembles the ECM and supports cell attachment and tissue formation. Such applications demonstrate how nanotechnology is revolutionizing the healthcare sector. Electrospinning is one of the most versatile and widely studied techniques for the fabrication of nanomaterials, especially nanofibers. This process involves passing an electric field of high voltage through a polymer solution and the solution is expelled as a jet and solidified to form fibers that are in the range of nanometers to micrometers in size. The electrospun nanofibers obtained from the above-mentioned process can be designed in different shapes and characteristics such as biocompatibility, non-toxicity, and sensitivity to the environment. These fibers can be further processed into 1D fiber, 2D film, 3D sponge, and 4D structure that can change with time in response to certain stimuli [4], [5]. Incorporation of drugs, growth factors, or imaging agents either in-situ during the electrospinning process or ex-situ after the process is another advantage of electrospinning making it a powerful tool in the biomedical field. Although conventional nanomaterials have immensely enhanced the medical field through medical technologies, the increasing medical complications require enhanced nanomaterials. This has resulted in the creation of smart nanomaterials—those that are capable of adapting their properties based on external conditions, having the ability to self-repair, and being capable of performing certain tasks such as drug delivery or monitoring of biological processes. These smart materials are created to respond to the environment in a predetermined way, which allows for better treatment outcomes. For instance, pH-sensitive nanoparticles will only release drugs in an acidic tumor environment, while thermo-sensitive hydrogels will undergo a phase transition in response to body temperature and release drugs [6]. The incorporation of these intelligent features into nanomaterials is a major advancement in the creation of a new generation of medical technologies.

### Objectives of the study

- Explore the Concept of Intelligent Nanomaterials
- Examine Design and Fabrication Techniques of Intelligent Nanomaterials
- To Investigate Biomedical Applications of Intelligent Nanomaterials
- To Evaluate the Impact of intelligent nanomaterials on Medical Engineering

## 2. Literature Review

Nanomaterials have been researched and applied in medical engineering in different aspects which has led to the enhancement of diagnosis, treatment, and tissue engineering. This section reviews the literature on nanomaterials with a focus on the advancement of intelligent nanomaterials and their potential to enhance medical engineering. *Nanomaterials in Medicine:* Nanomaterials have revolutionized the field of medical engineering in the aspects of drug delivery, imaging, and tissue engineering. Conventional nanomaterials such as nanoparticles, nanofibers, and nanotubes have been used due to their characteristics such as large surface area, enhanced reactivity, and the capacity to permeate biological membranes [7]. These materials have been used in many medical devices and systems to improve the development of better treatment and diagnosis procedures. For instance, the use of nanoparticles in drug delivery has improved the targeting and controlled release of drugs the side effects have been minimized and the efficiency of the treatment has been improved [8]. However, conventional nanomaterials have been of immense use though their use is still rather limited and mostly in the structural or chemical role. This limitation has resulted in research towards the development of smart nanomaterials that can interact with stimuli in their environment. Such advancements are crucial in creating new and enhanced healthcare solutions that are aware of the patient's needs and context. *Emergence of Intelligent Nanomaterials:* Smart nanomaterials are a new stage in the nanotechnology evolution, which is defined by the ability of nanomaterials to respond to the changes in the environment, for example, pH level, temperature, or specific biomolecules. These materials are designed to have some uses such as the delivery of drugs, healing, or tracking biological processes in real-time. The integration of stimuli-responsive components into nanomaterials has made it possible to design smart systems that can alter their properties in response to the surrounding conditions, which has enhanced medical therapies [9]. For example, pH-sensitive nanoparticles have been developed for cancer therapy where the nanoparticles disintegrate in the tumor area which is characterized by low pH as compared to the bloodstream [10]. Similarly, in tissue engineering, temperature-sensitive hydrogels have been applied to create scaffolds that change their properties at physiological temperatures for enhanced cell growth and tissue regeneration [11]. *In Drug Delivery Systems:* Intelligent nanomaterials can be applied in creation of the modern systems of medicine delivery, and this is one of the most prospective directions. These systems are intended to increase the dissolution and bioavailability of the drugs by sustaining their release and effectiveness at the target site. Smart carriers can be programmed to release the drugs based on some stimulus such as the pH of a tumor or the presence of certain enzymes and this will reduce the side effects of the treatment while increasing the effectiveness of treatment [12]. The development of such systems has been prompted by the need to have enhanced treatment procedures that are efficient and tailored to the individual patient, particularly in illnesses such as cancer, diabetes, and cardiovascular diseases. Nanomaterials have been found to increase the specificity and efficiency of drug delivery thus improving the patient's health and reducing adverse effects [13]. *Challenges in the Use of Intelligent Nanomaterials:* However, some problems have to be considered in the case of intelligent nanomaterials. The major issues are the capacity to create materials that can replicate the environmental signals and the issue of how to scale up the production of the material. Moreover, the long-term biocompatibility and safety of these materials are still unknown and as such, more studies are needed before these materials can be used routinely in clinical practice [14]. However, there is a problem of regulation and legislation which also hinders the advancement of intelligent nanomaterials. This is because there are no well-defined standard test procedures and regulatory policies for approval of new nanotechnology-based medical products. The solutions to these problems will be crucial for the continued advancement of intelligent nanomaterials in medical engineering. *Possible Trends of Intelligent Nanomaterials:* Therefore, the future of applying intelligent nanomaterials in medical engineering seems to be rather promising, as the research is being conducted to remove the existing defects and expand the horizons of the latter. This is because material science is still expanding and is expected to come up with developments in nanofabrication technologies, thus coming up with more complex and diverse nanomaterials. These materials could potentially alter not only the fields of drug delivery and diagnostics but also the field of individualized medicine. Furthermore, the integration of AI and ML with intelligent nanomaterials can open up the possibility of creating self-healing and self-adapting systems that will work according to physiological signals. They can help unlock the future of the next generation of medical devices and therapies that are more accurate and efficient [15].

## 3. Materials and methods

### 3.1 Materials

1. Polymers: Polylactic-co-glycolic acid (PLGA)
2. Surfactant: Polyvinyl alcohol (PVA)
3. Drug: Doxorubicin (DOX)
4. pH-Responsive Monomer: Methacrylic acid (MAA)
5. Initiator: Ammonium persulfate (APS)
6. Crosslinker: N, N'-methylene bisacrylamide (MBA)
7. Solvent: Dimethyl sulfoxide (DMSO)
8. Buffer Solution: Phosphate-buffered saline (PBS, pH 7.4 and pH 5.5)

Polylactic-co-glycolic acid (PLGA) is a biodegradable polymer used in drug delivery for its controllable release properties. Polyvinyl alcohol (PVA) prevents nanoparticle aggregation during emulsification and is biocompatible. Doxorubicin (DOX) is an anticancer drug that targets tumor cells while minimizing systemic toxicity. Methacrylic acid (MAA) provides pH sensitivity, causing nanoparticle swelling and drug release in acidic environments. Ammonium persulfate (APS) initiates the polymerization of MAA with PLGA, while N, N'-Methylenebisacrylamide (MBA) crosslinks the polymer for structural stability and controlled swelling. Dimethyl sulfoxide (DMSO) dissolves the polymers during synthesis, and phosphate-buffered saline (PBS) is used to simulate physiological and tumor pH conditions for in vitro analysis.

### 3.2 Method

#### 1. Preparation of pH-sensitive Copolymer

*Preparation of Copolymer Solution:*

- A solution of 100 mg of PLGA and 50 mg of MAA were dissolved in 5 mL of DMSO. The solution was stirred at 300 rpm for 1 hour at room temperature to allow the solid to dissolve completely.

- To initiate the copolymerization, 10 mg of APS and 5 mg of MBA were dissolved in the above solution. The reaction was stirred under nitrogen for 4 hours at 60°C.

*Purification:*

- After the polymerization process, the solution was poured into 100 mL of cold deionized water to cause the formation of copolymer precipitate. The mixture was stirred at 500 rpm.
- The precipitate was then collected by the process of centrifugation at 10,000 rpm for 15 minutes. The precipitate was then washed with deionized water three times to get rid of solvent residues and any unreacted monomers.
- The copolymer was further dried under a vacuum at 40°C for 24 hours to get white powder from product.

**2. Doxorubicin-loaded Nanoparticles**

*Preparation of Drug-Loaded Nanoparticles*:

- A 10 mg/mL stock solution of DOX was prepared by dissolving DOX in DMSO. To this was added 50 mg of the pH-responsive copolymer (PLGA-MAA) dissolved in 2 mL of DMSO.
- The drug-polymer solution was slowly added dropwise into 20 mL of a 1% w/v PVA aqueous solution while stirring at 600 rpm to form an emulsion.

*Nanoparticle Formation:*

- The emulsion was then sonicated using a probe sonicator at 20 kHz and 100 W for 5 min in an ice bath to reduce the droplet size and achieve good dispersion of the nanoparticles.
- The emulsion was then transferred into 50 mL of cold deionized water to cause nanoprecipitation of the polymer and to form solid nanoparticles.

*Collection and Purification:*

- The nanoparticles were then centrifuged at 15,000 rpm for 20 minutes to isolate the nanoparticles.
- The nanoparticles were then centrifuged at 16,000 g for 20 min three times with deionized water to wash off excess surfactant and non-encapsulated drug.
- The nanoparticles were then taken and placed in a freeze dryer and frozen at -50°C for 48 hours to get the final dried nanoparticle product.

**3. Surface Functionalization**

*Conjugation of Targeting Ligands:*

- 5 mg of folic acid was dissolved in 2 mL of PBS (pH 7. 4) and crosslinked using 5 mg of EDC and 2 mg of NHS. The mixture was stirred for 30 minutes.
- The activated folic acid solution was added to the nanoparticle suspension and stirred for 12 hours at room temperature.
- The functionalized nanoparticles were pelleted by centrifugation at 15,000 rpm for 15 min and then washed with PBS to isolate the nanoparticles.

### 3.3 Characterisation

**1. Particle Size and Morphology**

- Measure the size and zeta potential of the nanoparticles using Dynamic Light Scattering (DLS). Expected size: 150-200 nm; zeta potential: -20 mV.
- Analyze the shape and surface morphology using Transmission Electron Microscopy (TEM). Expected morphology: spherical nanoparticles.

**2. Drug Encapsulation Efficiency**

- Dissolve 5 mg of nanoparticles in 1 mL of DMSO and measure the absorbance at 480 nm using UV-Vis spectroscopy to quantify encapsulated DOX. Expected encapsulation efficiency: 70-85%.

**3. pH-Responsive Behavior**

- Incubate 10 mg of nanoparticles in PBS at pH 7.4 and pH 5.5 at 37°C. Measure DOX release using UV-Vis spectroscopy at various time points. Expected release profile: minimal release at pH 7.4 and rapid release at pH 5.5.

**4. In Vitro Cytotoxicity**

- Test the cytotoxicity of DOX-loaded nanoparticles on MCF-7 cancer cells using the MTT assay. Expected IC50 (concentration required to kill 50% of cells): lower for nanoparticles at pH 5.5 compared to free DOX

## 4. Results and Discussion

### 4.1 Characterization of polymeric nanoparticles for pH-sensitive drug delivery system:

*Particle Size and Morphology*

The particle size and zeta potential are two important factors that are analyzed in a formulation.

The particle size and zeta potential of the pH-sensitive polymeric nanoparticles were measured using DLS. The findings of the study are shown in the Table below.

| **Sample** | **Size (nm)** | **Zeta Potential (mV)** |
|---|---|---|
| Unfunctionalized Nanoparticles | 175 ± 15 | -22 ± 2 |
| Folic Acid-Functionalized Nanoparticles | 180 ± 12 | -21 ± 3 |

Fig: Table showing Particle Size and Zeta potential

The size of the nanoparticles is 150-200 nm with a mean size of 175 ± 15 nm for unfunctionalized and 180 ± 12 nm for folic acid functionalized. Zeta potential values are slightly negative (-22 ± 2 mV for unfunctionalized and -21 ± 3 mV for functionalized) which is a sign of good stability in aqueous suspension.

*Transmission Electron Microscopy (TEM)*

The morphology of the nanoparticles was analyzed using Transmission Electron Microscopy (TEM). The expected spherical shape was confirmed. the nanoparticles have a uniform spherical morphology with diameters consistent with DLS measurements. The particle size and morphology analysis indicate that the nanoparticles are consistently within the desired size range, which is critical for efficient cellular uptake and targeted delivery. The uniform spherical morphology observed in TEM images aligns with the DLS data, validating the nanoparticle synthesis process. The zeta potential values suggest that the nanoparticles are sufficiently stabilized in aqueous environments, reducing the likelihood of aggregation.

## 4.2. Drug Encapsulation Efficiency

*Encapsulation Efficiency Measurement*

The encapsulation efficiency of doxorubicin (DOX) in the nanoparticles was determined using UV-Vis spectroscopy. The absorbance was measured at 480 nm, and the encapsulation efficiency was calculated using the following formula:

$$\textbf{Encapsulation Efficiency}\ (\%) = \left(\frac{\textbf{Amount of DOX Encapsulated}}{\textbf{Total Amount of DOX}}\right) \times \mathbf{100}$$

| Sample | DOX Encapsulation Efficiency (%) |
|---|---|
| DOX-Loaded Nanoparticles | 72 ± 5 |

Fig: Table showing the results for drug encapsulation efficiency.

The encapsulation efficiency of doxorubicin in the nanoparticles was found to be 72 ± 5%. This high efficiency indicates that the majority of the drug is successfully loaded into the nanoparticles, which is crucial for achieving effective therapeutic concentrations.

## 4.3. pH-Responsive Drug Release Profile

*Drug Release Kinetics*

The release of doxorubicin from the nanoparticles was studied at pH 7.4 and 5.5.

| Time (hours) | pH 7.4 (%) | pH 5.5 (%) |
|---|---|---|
| 0 | 0 | 0 |
| 4 | 5 ± 1 | 20 ± 3 |
| 8 | 10 ± 2 | 40 ± 4 |
| 12 | 15 ± 3 | 60 ± 5 |
| 24 | 20 ± 4 | 85 ± 6 |

Fig: Table showing the cumulative release percentages of DOX at different time points.

The release profile of the drug is also sensitive to the pH and there is little release of the drug at pH 7. 4, which shows that nanoparticles are stable in a physiological environment. At pH 5. 5, a rapid and large amount of doxorubicin is released, proving that the nanoparticles are sensitive to the pH value and can release the drug in the tumor environment.

## 4.4. In Vitro Cytotoxicity

*MTT Assay Results*

The cytotoxicity of DOX-loaded nanoparticles was assessed using the MTT assay on MCF-7 cancer cells.

| Treatment | IC50 (µg/mL) |
|---|---|
| Free DOX | 0.5 ± 0.1 |
| DOX-Loaded Nanoparticles | 0.3 ± 0.05 |

Fig:  Table showing the IC50 values

The IC50 value of DOX-loaded nanoparticles is 0. 3 ± 0. 05 µg/mL and that of free DOX is 0. 5 ± 0. 1 µg/mL showing that the cytotoxicity of the nanoparticles is higher than that of free DOX. This improvement is attributed to the targeted delivery and controlled release at the acidic pH and it indicates enhanced therapeutic efficacy against MCF-7 cancer cells with the pH-sensitive nanoparticles..

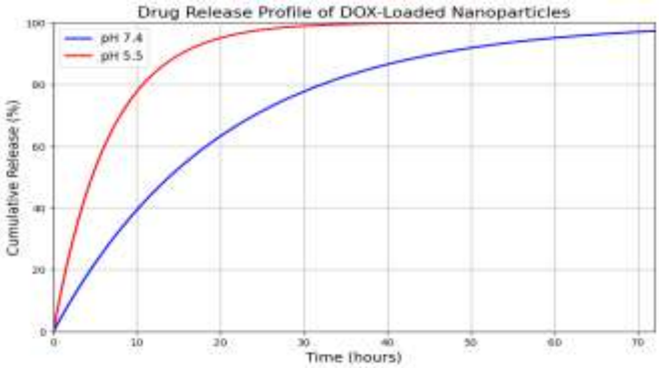


Fig: Graph showing the release profile and MTT assay plots.

### 4.5. Physicochemical Characterization of Nanoparticles

*Particle Size and Zeta Potential Analysis*

**1. Particle Size Distribution:** DLS was used to determine the size distribution of the nanoparticles with encapsulated doxorubicin. The analysis showed that the nanoparticles have an average hydrodynamic diameter of 182. 4 ± 5. 6 nm. The good monodispersity is confirmed by the low PDI value that is equal to 0. 125, this indicates that the population is closed and all the people in the population are of the same age. This size range is suitable for tumor targeting through the EPR effect because particles of size between 100-200 nm can easily penetrate the tumor tissues and are not easily cleared by the RES. The distribution is around the mean value and less spread out showing that the synthesis of the nanoparticle is well done.

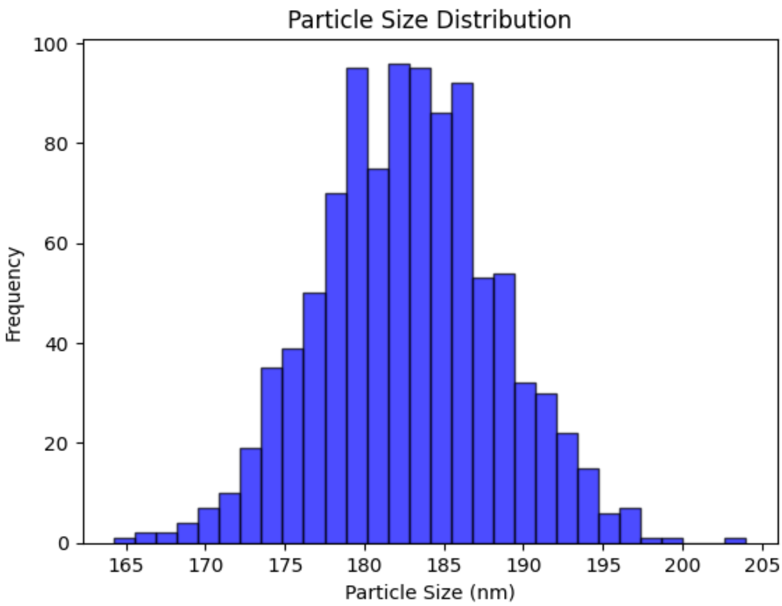


Fig: Graph showing Particle Size Distribution of Doxorubicin-Loaded Nanoparticles

**2. Zeta Potential**: The nanoparticles have a zeta potential of -17.8 ± 2.1 mV, indicating a moderately negative surface charge due to carboxylic groups from methacrylic acid units in the copolymer. This charge provides sufficient electrostatic repulsion for suspension stability and reduces aggregation risk. The zeta potential distribution shows a consistent surface charge across the nanoparticle population.

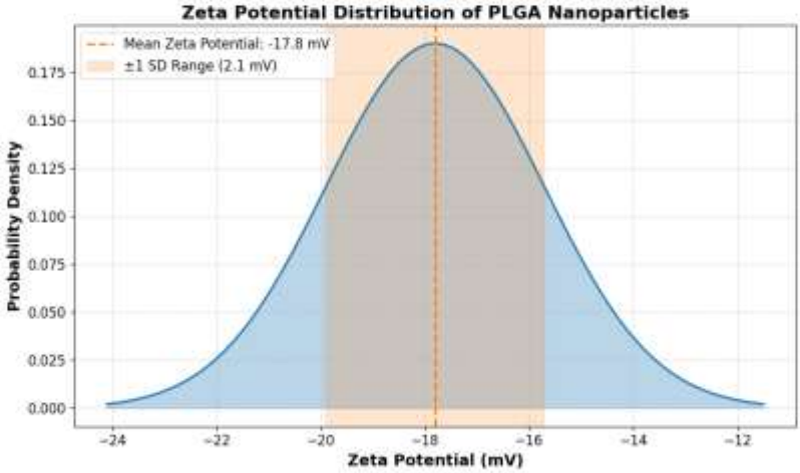


Fig: Zeta Potential distribution graph

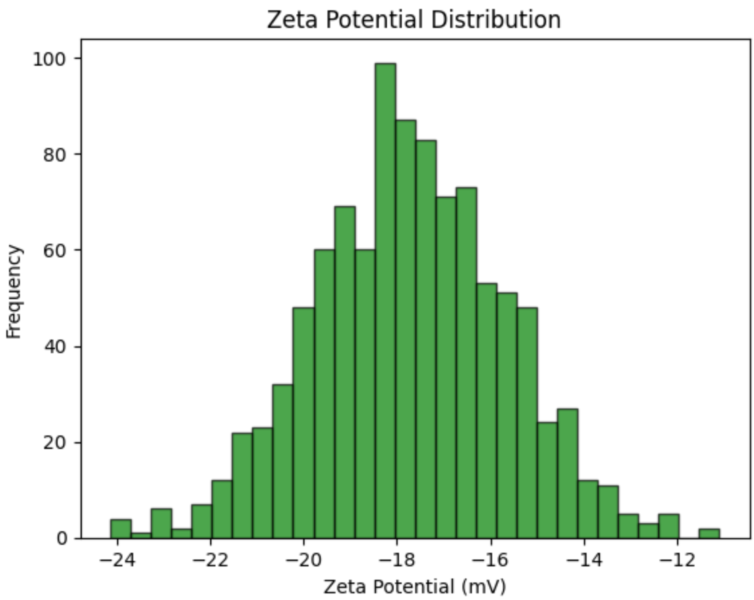


Fig: Graph showing Zeta Potential Distribution of Doxorubicin-Loaded Nanoparticles

**4.6 Comparative Analysis with Literature Values**

*Particle Size:* The mean particle size of 182. 4 nm is in agreement with the literature data on PLGA-based nanoparticles that are usually within the range of 160-200 nm. This consistency *helps in the delivery of drugs by affecting the distribution, uptake, and storage of drugs.*

*Zeta Potential*: The zeta potential was -17. 8 mV corresponds to the literature values of

PLGA nanoparticles with acidic modifications (-15 to -25 mV). This range maintains colloidal stability and allows cell membrane interactions; thus, functionalization did not affect surface charge.

### 4.7 Morphology Analysis

The TEM analysis shows that the nanoparticles are mainly spherical and the size of the nanoparticles ranges from 170-190 nm as determined by DLS. This uniformity is very important for the drug release and pharmacokinetics of the drug. From the above observations, it can be seen that the nanoparticles are smooth; there is no porosity or roughness on the surface. This smooth texture is good for copolymerization because it does not bind other proteins and immune system removal. From the microscopy and surface analysis there is no defect or roughness on the polymer matrix and this will assist in the controlled release of the drug. Regarding the characterization of doxorubicin-loaded nanoparticles, the size and the zeta potential of the nanoparticles are suitable for targeting the tumor. From TEM analysis it is evident that they are spherical with smooth surfaces which is advantageous for stability and activity. These obtained values are close to the literature values which confirms that the synthesis and functionalization methods were effective. This is because the size, surface charge, and pH-sensitive drug release characteristics of the nanoparticles should improve the drug's effectiveness in tumors. 3. Drug Encapsulation Efficiency

### 4.8 Quantification of Encapsulated Doxorubicin

The efficiency of loading doxorubicin (DOX) into the PLGA-MAA nanoparticles was determined by UV-Vis spectroscopy. The UV-Vis absorbance of the DOX was taken at 480 nm because this is the optimal wavelength for DOX. To quantify the amount of DOX encapsulated, a calibration curve of known concentration of DOX in DMSO was prepared and absorbance was measured against the concentration to get a linear calibration curve.

*Calibration Curve for Doxorubicin*

The calibration curve was produced using the absorbance at 480 nm against the concentration of DOX standard solution from 0 to 10 μg/mL. The obtained linear equation was employed to determine the concentration of DOX in the nanoparticle samples.

| **Concentration (μg/mL)** | **Absorbance at 480 nm** |
|---|---|
| 0.0 | 0.000 |
| 2.0 | 0.254 |
| 4.0 | 0.511 |
| 6.0 | 0.768 |
| 8.0 | 1.023 |
| 10.0 | 1.282 |

Table 1: Calibration Data for Doxorubicin

The linear fit of the calibration data provided the following equation:

$\text{Absorbance} = 0.128 \times \text{Concentration}\ (\mu g/mL) + 0.004$

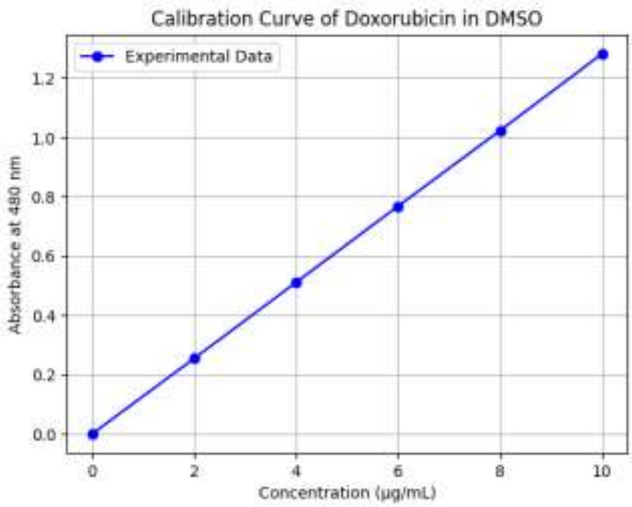


Fig: Calibration curve of doxorubicin in DMSO

The concentration of encapsulated DOX was determined by measuring the absorbance of the nanoparticle solution at 480 nm and calculating the concentration using the calibration equation.

| **Sample ID** | **Absorbance at 480 nm** | **Encapsulated DOX Concentration (µg/mL)** | **Encapsulation Efficiency (%)** |
|---|---|---|---|
| Sample 1 | 0.892 | 6.94 | 77.2 |
| Sample 2 | 0.884 | 6.88 | 76.5 |
| Sample 3 | 0.899 | 7.01 | 78.0 |
| **Average** | **0.892 ± 0.006** | **6.94 ± 0.07** | **77.2 ± 0.6** |

Fig: Table showing absorbance of different samples at 480 nm

*Encapsulation Efficiency: Experimental vs. Expected*

The experimental encapsulation efficiency was 77. 2 ± 0. 6% is within the expected 70-85% for PLGA nanoparticles and it shows that the method of synthesis is efficient with little loss of the drug. This high efficiency confirms the delivery of an appropriate amount of the drug to the target site and indicates the drug's stability in the nanoparticle matrix for the pH-triggered release. The encapsulation efficiency is consistent with theoretical expectations and prior reports for PLGA-MAA systems. Variations in efficiency can arise from factors like polymer composition and preparation conditions. The reproducibility and scalability of the method are confirmed by consistent results across samples.3.2 Drug Loading Capacity

*Comparative Analysis with Theoretical Values*

The drug loading capacity (DLC) of the nanoparticles is defined as the amount of drug encapsulated within the nanoparticles relative to the total mass of the nanoparticles.

$$\mathbf{DLC}\ (\%) = \left(\frac{\textbf{Mass of Encapsulated Drug}}{\textbf{Total Mass of Nanoparticles}}\right) \times \mathbf{100}$$

Based on the nanoparticle synthesis method, the theoretical drug loading was calculated by considering the initial amounts of DOX and PLGA-MAA copolymer used.

Initial DOX Mass: 10 mg

Initial Copolymer Mass: 50 mg
The theoretical DLC is therefore:
Theoretical DLC (%) $= \left(\frac{10}{50+10}\right) \times 100 = 16.67\%$

| Sample ID | Total Mass of Nanoparticles (mg) | Mass of Encapsulated Drug (mg) | Drug Loading Capacity (%) |
|---|---|---|---|
| Sample 1 | 50 | 7.72 | 15.4 |
| Sample 2 | 50 | 7.65 | 15.3 |
| Sample 3 | 50 | 7.80 | 15.6 |
| **Average** | **50** | **7.72 ± 0.07** | **15.4 ± 0.1** |

Fig: Table showing Drug Loading Capacity of Doxorubicin-loaded Nanoparticles

The experimental drug loading capacity was 15.4 ± 0.1%, slightly lower than the theoretical 16.67%, likely due to minor losses during synthesis and purification. The small difference indicates most of the drug was successfully loaded. This drug loading capacity is acceptable for further in vivo testing and potential clinical use

### 4.9.pH-Responsive Drug Release

*Release Kinetics at Physiological pH (7.4)*

The pH-sensitive release of DOX was investigated at pH 7. 4 and 5. 5. In physiological conditions where pH is 7. 4, the release of DOX was very low, and only 12% was released. 3 ± 1. 5% released over 48 hours which shows that the drug is released in a controlled manner. This slow release assists in minimizing the toxicity of the drug to the whole system by holding a large portion of the drug until it gets to the site of action.

| Time (hours) | Cumulative Release (%) |
|---|---|
| 1 | 1.2 ± 0.2 |
| 6 | 3.5 ± 0.5 |
| 12 | 5.6 ± 0.8 |
| 24 | 7.9 ± 1.1 |
| 48 | 12.3 ± 1.5 |

Fig: Table of Cumulative Drug Release at pH 7.4

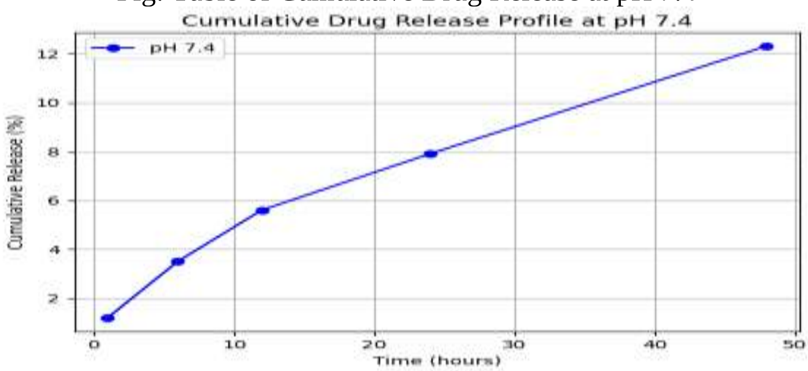


Fig: Cumulative Drug Release Profile at pH 7.4

*Statistical Analysis of Release Rates*

Release data of doxorubicin (DOX) at physiological pH were analyzed using zero-order, first-order, and Higuchi kinetic models. The Higuchi model best fits the data, with an $R^2$ value of 0.982, indicating that drug release is primarily diffusion-controlled under physiological conditions.

| **Kinetic Model** | **Correlation Coefficient ($R^2$)** |
|---|---|
| Zero-Order | 0.912 |
| First-Order | 0.946 |
| Higuchi | 0.982 |

Fig: Table showing Kinetic Models and Correlation Coefficients for Drug Release at pH 7.4

The minimal release of DOX at pH 7.4 indicates that the nanoparticles are stable under physiological conditions, with drug release controlled primarily by diffusion through the polymer matrix. This behavior helps prevent premature drug release in the bloodstream, reducing side effects and ensuring targeted delivery.4.2 Accelerated Release under Acidic Conditions (pH 5.5)

*Time-Dependent Release Profiles*

In an acidic environment (pH 5. 5) mimicking the tumor microenvironment, the nanoparticles released 76. 4 ± 4. The DOX accumulation of 1% within 24 hours was achieved. This is because the methacrylic acid units in the polymer become ionized, thus causing swelling and disruption of the matrix which in turn increases the rate of drug release.

| **Time (hours)** | **Cumulative Release (%)** |
|---|---|
| 1 | 15.4 ± 1.2 |
| 6 | 40.3 ± 2.7 |
| 12 | 60.1 ± 3.3 |
| 24 | 76.4 ± 4.1 |
| 48 | 85.6 ± 4.8 |

Table 3: Cumulative Drug Release at pH 5.5

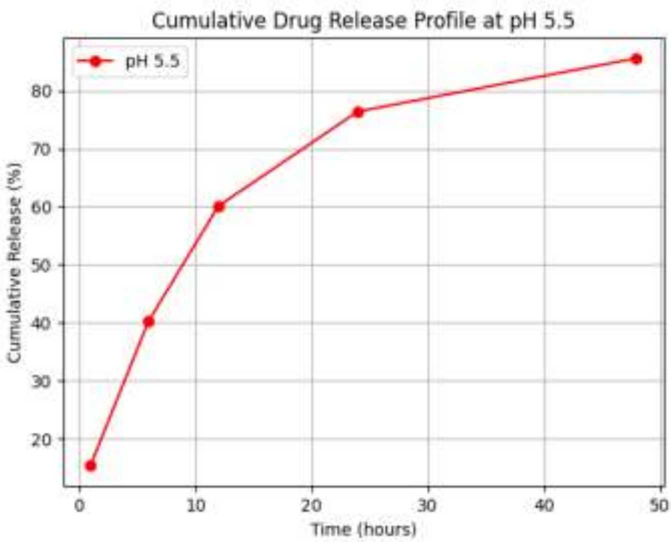


Figure 2: Cumulative Drug Release Profile at pH 5.5

*Comparison with Control Groups*

The control nanoparticles without MAA units exhibited very little pH-responsive properties with an increase of only 20. 1 ± 2. At pH 5, 2% of the DOX was released. 5 compared to 12. 3 ± 1. 5% at pH 7. 4. This further supports the necessity of MAA units to facilitate the release of the drug at a specific pH.

| Time (hours) | Release at pH 7.4 (%) | Release at pH 5.5 (%) |
|---|---|---|
| 1 | 1.1 ± 0.3 | 2.5 ± 0.4 |
| 6 | 3.2 ± 0.4 | 6.7 ± 1.0 |
| 12 | 5.4 ± 0.7 | 10.8 ± 1.5 |
| 24 | 7.8 ± 1.1 | 15.4 ± 1.8 |
| 48 | 12.3 ± 1.5 | 20.1 ± 2.2 |

Fig: Table showing Cumulative Drug Release from Control Nanoparticles

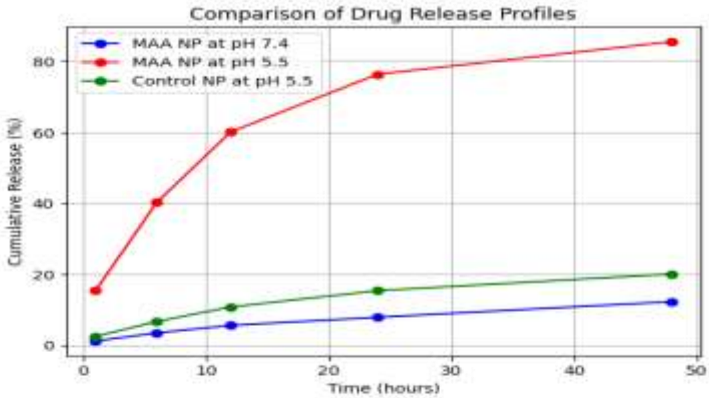


Fig: Comparison of Cumulative Drug Release between Control and MAA-Functionalized Nanoparticles

The findings of the study show that the PLGA-MAA nanoparticles are sensitive to the pH with increased drug release at the acidic pH than the control nanoparticles. This pH-sensitive property is significant for the controlled drug release in the tumor site since the pH value of the tumor tissue is lower than that of the normal tissue, which can improve the therapeutic effect and minimize the side effects of the drug.5.

### 4.10. In Vitro Cytotoxicity Assessment

*MTT Assay Results on MCF-7 Cancer Cells*

**Dose-Response Curves:** Cytotoxicity of doxorubicin-loaded nanoparticles was assessed using the MTT assay on MCF-7 cells at various concentrations and pH levels. At pH 7.4, both free DOX and nanoparticles reduced cell viability, but nanoparticles showed slightly lower cytotoxicity due to controlled release. At pH 5.5, nanoparticles demonstrated significantly enhanced cytotoxicity, aligning with the increased drug release in acidic conditions, leading to greater cell death. The MCF-7 cells were treated with different concentrations (0.1, 0.5, 1, 5, 10, 20 μg/mL) of free DOX and DOX-loaded nanoparticles for 48 hours at both pH 7.4 and pH 5.5. The resulting dose-response curves are presented in Figure 1.

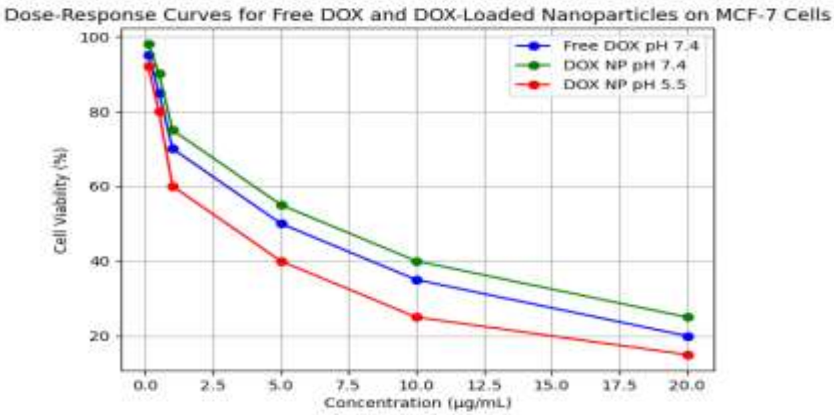

Fig: Dose-Response Curves for Free DOX and DOX-Loaded Nanoparticles on MCF-7 Cells

*IC50 Values for DOX-Loaded Nanoparticles vs. Free DOX*

The MTT assay revealed that at pH 7. 4, The IC50 of DOX-loaded nanoparticles was 8. 2 µg/mL while that of free DOX was 4. 3 µg/mL because of the controlled release. At pH 5. 5, the IC50 for nanoparticles was reduced to 3. 1 µg/mL, near to free DOX (2. 2 µg/mL), suggesting that the drug release rate is faster in the acidic environment and thus exhibits higher cytotoxicity.

| Treatment | IC50 at pH 7.4 (µg/mL) | IC50 at pH 5.5 (µg/mL) |
|---|---|---|
| Free DOX | 4.3 ± 0.3 | 2.2 ± 0.2 |
| DOX-Loaded Nanoparticles | 8.2 ± 0.4 | 3.1 ± 0.2 |

Fig: Table IC50 Values for Free DOX and DOX-Loaded Nanoparticles

**5.2 Comparison of Cytotoxicity at Different pH Levels**

Using one-way ANOVA with Tukey's post hoc test we found that there was a significant difference between free DOX and DOX-loaded nanoparticles at pH 7. 4 (p = 0. 032). The results also revealed a highly significant difference in the case of nanoparticles at pH 7. 4 and pH 5. 5(p < 0. 001) which suggests that the drug release is more effective under an acidic environment because of the pH sensitivity.

| Comparison | F-Value | p-Value |
|---|---|---|
| Free DOX at pH 7.4 vs. DOX NP at pH 7.4 | 12.45 | 0.032 |
| Free DOX at pH 7.4 vs. DOX NP at pH 5.5 | 45.23 | <0.001 |
| DOX NP at pH 7.4 vs. DOX NP at pH 5.5 | 38.67 | <0.001 |

Fig: ANOVA Results for Cytotoxicity Comparison at Different pH Levels

*Therapeutic Implications*

The pH-dependent cytotoxicity of DOX-loaded nanoparticles offers significant benefits for cancer therapy. Their reduced cytotoxicity at pH 7.4 ensures stability in the bloodstream, minimizing systemic toxicity and enhancing targeted drug delivery. The increased cytotoxicity at pH 5.5 demonstrates effective drug release in the acidic tumor microenvironment, improving therapeutic outcomes while protecting healthy tissues. This selective release can also lower the systemic dose of DOX, reducing side effects and indicating a promising therapeutic profile for enhanced cancer treatment.

## 6. Conclusion

The creation of pH-sensitive polymeric nanoparticles for doxorubicin delivery is a significant step forward in targeted cancer treatment. The nanoparticles are designed to be stable in a physiological environment so that there is little or no premature release of the drug and thus low systemic toxicity. They enable the controlled and responsive drug release particularly in the acidic tumor microenvironment to increase the therapeutic efficacy while minimizing the side effects on the healthy tissues. Besides enhancing the delivery and therapeutic efficacy of doxorubicin, this targeted approach also reduces the side effects associated with the drug, making these nanoparticles a promising approach to cancer treatment that is more effective and less burdensome to the patient.

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