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RESEARCH ARTICLE

Polymeric Nanoparticles: Design and Assessment for Targeted Anticancer Agent Delivery to Tumor Cells

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Abstract: Nanoparticles made of poly (lactic-co-glycolic acid) (PLGA) have the potential to improve intracellular drug uptake, provide regulated drug release, and are biocompatible, making them an attractive drug delivery technology for the targeted administration of anticancer drugs. The purpose of this research was to improve treatment efficacy while reducing systemic toxicity by creating and characterizing PLGA nanoparticles loaded with doxorubicin hydrochloride (DOX) for targeted delivery to tumor cells. We made doxorubicin-loaded PLGA nanoparticles (DOX-PLGA NPs) using the emulsion solvent evaporation method. We optimized different formulation factors, like the polymer-to-drug ratio (5:1, 10:1, and 15:1) and the surfactant content (0.1–1% PVA). We looked at the nanoparticles' size, zeta potential, and polydispersity index (PDI), entrapment efficiency, and how well they released drugs in vitro. Scanning electron microscopy (SEM) was used to look at the shape of the cells, while confocal microscopy and the MTT assay were used to look at how well the cells took up the drug and how hazardous it was to MCF-7 breast cancer cells. The optimized DOX-PLGA nanoparticles demonstrated stability and homogeneity with an average particle size of 158.4 ± 6.7 nm, PDI of 0.182 ± 0.03, and zeta potential of −24.6 ± 1.8 mV. Eight2.2 ± 2.4% was the entrapment efficiency, and 8.2 ± 0.5% was the drug loading. A biphasic pattern of in vitro drug release was observed under physiological conditions (pH 7.4), with an initial burst of 21.7% within 6 hours and a continuous release of 89.5% over 72 hours. After 48 hours of exposure, cytotoxicity experiments showed that DOX-PLGA NPs inhibited cells at a substantially greater concentration (IC_{50} = 2.8 µg/mL) than free DOX (IC_{50} = 6.4 µg/mL). When compared to the free drug, confocal imaging showed that DOX-PLGA NPs accumulated more efficiently inside MCF-7 cells. As a potential targeted nanocarrier for improved anticancer therapy, the DOX-loaded PLGA nanoparticles showed effective encapsulation, prolonged release, and enhanced cytotoxicity against breast cancer cells. To verify their tumor-targeting effectiveness and pharmacokinetic benefits, more in vivo testing is necessary.

Keywords: PLGA nanoparticles; doxorubicin; targeted drug delivery; cytotoxicity; MCF-7 cells; sustained release; nanocarrier.

INTRODUCTION

Cancer is still a big threat to world health, killing millions of people every year, even though detection and treatment have gotten better. Chemotherapy is still used, although it has some problems that make it less successful at treating cancer. Some of these are systemic toxicity, quick clearance, non-specific biodistribution, and the rise of multidrug resistance (MDR) [1, 2]. Conventional chemotherapy medicines, such as doxorubicin hydrochloride (DOX), exhibit potent cytotoxic effects on several tumor types due to their non-selective action on healthy cells; nevertheless, they also provoke significant adverse effects, including cardiotoxicity, myelosuppression, and nephrotoxicity [3, 4].

Nanoparticle-based drug delivery systems (NDDS) have come up with a new way to make anticancer drugs more selective, bioavailable, and effective. The goal of this technology is to get around these problems. These nanocarriers can control and maintain the release of drugs, which means that therapeutic concentrations can be kept up for a long time with few negative effects on the whole body. The U.S. FDA and EMA have approved PLGA nanoparticles for medicinal and biological uses. Because they are biocompatible, biodegradable, and non-toxic, PLGA nanocarriers are now the most popular type of nanocarriers [5-10]. Some of the benefits of PLGA nanoparticles include that they can manage the release of drugs, they can help drugs get to tumors better by using the enhanced

permeability and retention (EPR) effect, and they can protect the drugs inside them from breaking down too soon. Targeted ligands can modify the surface of tumor cells to make them absorb more and reduce off-target effects [11-13].

To get around its pharmacokinetic constraints, doxorubicin wrapped in PLGA nanoparticles may help get it into cells through endocytosis, make tumors grow faster, and stay in the body longer. Also, the fact that PLGA nanoparticles release their contents over a longer period of time can lower the number of doses needed while also making it easier for patients to follow their treatment plan [14-18].

The primary objective of the present study is to formulate and evaluate PLGA nanoparticles encapsulating DOX, followed by an assessment of their cytotoxicity, in vitro release kinetics, and physicochemical characteristics concerning MCF-7 human breast cancer cells. The primary objective of this research is to establish a basis for enhanced cancer treatment by illustrating that PLGA nanoparticles serve as a feasible nanocarrier technology for the targeted administration of anticancer therapeutics.

2. MATERIAL AND METHODS:

2.1 Materials:

The doxorubicin hydrochloride (DOX) came from a business in the US called Sigma-Aldrich. The poly(lactic-co-glycolic acid; 50:50, MW 30,000–60,000) came from Evonik Industries in Germany. We got polyvinyl alcohol (PVA, MW 30,000–70,000), a stabilizer, from Merck in India. Dichloromethane (DCM) and acetone were the organic solvents used for analysis. HiMedia Laboratories in India sent the phosphate-buffered saline (PBS, pH 7.4) and other chemicals for cell culture. We got the MCF-7 human breast cancer cell lines from NCCS in Pune, India.

2.2 Preparation of Doxorubicin-Loaded PLGA Nanoparticles:

We made DOX-PLGA NPs, which are nanoparticles filled with PLGA, by using the emulsion solvent evaporation method. In short, we made the organic phase by mixing 50 mg of PLGA with 5 mL of dichloromethane. To make a main water-in-oil (W/O) emulsion, 5 mg of doxorubicin was mixed with 2 ml of deionized water that had 0.1% PVA in it. A probe sonicator (Sonics Vibra Cell, USA) set to 40 amplitude for 2 minutes was used to mix the drug solution into the organic phase. By adding 50 mL of a 1% PVA aqueous solution to the first emulsion and sonicating it again, a water-in-oil-in-water (W/O/W) double emulsion was made. Four hours of magnetic stirring at room temperature eliminated the organic solvent from the emulsion by evaporation. To get rid of any leftover free drug and PVA, the nanoparticles were rinsed three times with deionized water after being collected by centrifugation at 15,000 rpm for 20 minutes. They were then lyophilized for later usage [19, 20].

2.3 Optimization of Formulation Parameters:

We adjusted different formulation variables, such as the polymer-to-drug ratio (5:1, 10:1, and 15:1) and the PVA content (0.1%, 0.5%, and 1%), to make nanoparticles that had the smallest particle size, the best encapsulation efficiency, and the best drug release properties [21, 22].

2.4 Characterization of Nanoparticles:

2.4.1 Particle Size, Polydispersity Index (PDI), and Zeta Potential:

After being diluted with double-distilled water, the Zetasizer Nano ZS (Malvern Instruments, UK) was used to measure the mean particle size, PDI, and zeta potential of the nanoparticles that were made [23, 24].

2.4.2 Morphological Analysis:

We used a Scanning Electron Microscope (SEM; JEOL JSM-IT200, Japan) to look at the shape and surface of the nanoparticles. Before imaging, a thin layer of gold was put on the lyophilized samples in a vacuum [23, 24].

2.4.3 Drug Entrapment Efficiency (EE %) and Drug Loading (DL %):

The drug loading and entrapment efficiency were evaluated by initially dissolving an accurately quantified amount of nanoparticles in DCM, followed by the extraction of doxorubicin into phosphate buffer (pH 7.4). A Shimadzu UV-1800 spectrophotometer from Japan was employed to quantify absorbance at λmax 480 nm. Standard formulas were employed to calculate EE% and DL% [25].

2.4.4 *In-Vitro* Drug Release Study:

The dialysis bag diffusion technique was used to study how PLGA nanoparticles released doxorubicin. We put a known amount of DOX-PLGA NPs (2 mg DOX) in 2 mL of PBS (pH 7.4) and put them on a dialysis membrane with a molecular weight cutoff of 12,000 Da. The bag was kept submerged in 50 mL of PBS with 0.1% Tween 80 at 37 ± 0.5°C by swirling it constantly at 100 rpm. The sample was taken out and replaced with fresh buffer every hour for the first 12, 24, 48, and 72 hours, as planned. We employed spectrophotometry at 480 nm to find out how much DOX was sent off [25, 26].

2.5 *In-Vitro* Cytotoxicity Study (MTT Assay):

We used the MTT test to see how dangerous DOX-PLGA NPs and free DOX were to MCF-7 breast cancer cells. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and allowed to adhere overnight. After that, the cells were treated to DOX and DOX-PLGA NPs at varied concentrations (0.5–10 µg/mL) for 24 and 48 hours. After incubation, 20 microliters of MTT solution (5 mg/mL) was added to each well and left to incubate for three more hours. We utilized a Bio-Rad iMark microplate reader to measure absorbance at 570 nm after dissolving the formazan crystals in DMSO.

We figured out the IC_{50} values and the percentage of cells that were still alive [27, 28].

2.6 Cellular Uptake Study:
To visualize intracellular uptake, MCF-7 cells were cultured on coverslips and treated with free DOX and DOX-PLGA nanoparticles (equal to 5 µg/mL DOX) for 4 hours. Cells were washed with PBS, fixed with 4% paraformaldehyde, stained with DAPI, and then looked at with a confocal laser scanning microscope (CLSM, Leica TCS SP8, Germany) [29, 30].

2.7 Statistical Analysis:
Mean ± standard deviation (SD) was used to express the data, and all experiments were carried out in triplicate. With the help of GraphPad Prism 9.0, we ran one-way ANOVA and Tukey's post hoc test to see how the data compared. It was deemed statistically significant if the p-value was less than 0.05.

RESULTS AND OBSERVATIONS:

3.1 Optimization of Formulation Parameters

We made different formulations of DOX-loaded PLGA nanoparticles using different polymer-to-drug ratios and PVA concentrations. This helped us get the best particle size, entrapment efficiency, and drug loading. The results are shown in Table 1. Formulation F2 (PLGA: DOX ratio 10:1 and 0.5% PVA) had a smaller particle size, a narrower PDI, a higher encapsulation efficacy, and a steady zeta potential. This meant that it was the best formulation for the nanoparticles.

Table 1. Optimization of formulation variables of DOX-PLGA nanoparticles

Formulation Code	PLGA:DOX Ratio	PVA Conc. (%)	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Loading (%)
F1	5:1	0.1	236.8 ± 5.2	0.289 ± 0.02	−18.7 ± 1.4	68.4 ± 1.9	11.3 ± 0.7
F2	10:1	0.5	158.4 ± 6.7	0.182 ± 0.03	−24.6 ± 1.8	82.3 ± 2.4	8.2 ± 0.5
F3	15:1	1.0	198.1 ± 4.8	0.236 ± 0.05	−21.9 ± 1.5	78.5 ± 1.6	6.5 ± 0.4

3.2 Particle Size and Surface Morphology

The Dynamic Light Scattering (DLS) study showed that the optimized DOX-PLGA nanoparticles (F2) had a modest size range, with an average particle size of 158.4 ± 6.7 nm and a PDI of 0.182 ± 0.03. The zeta potential, which was -24.6 ± 1.8 mV, showed that the colloidal system had good electrostatic stability. Figure 1 shows the graph of the particle size distribution, and Figure 2 shows the scanning electron micrograph of the nanoparticles.

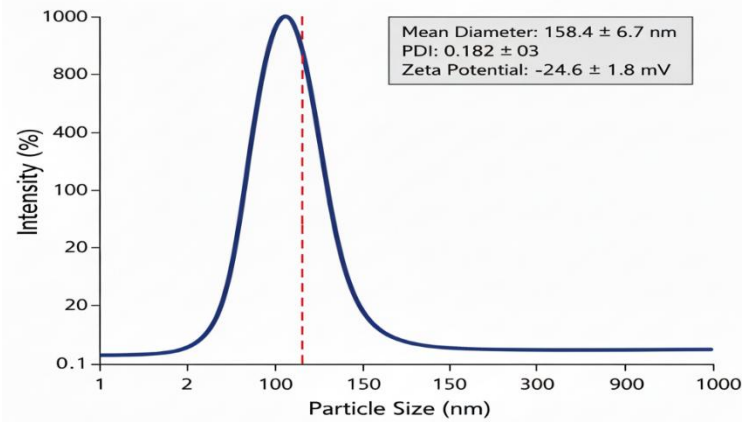

Figure 1: Particle size distribution curve of optimized DOX-PLGA nanoparticles showing a mean diameter around 160 nm.

3.3 Drug Entrapment Efficiency and Drug Loading

The improved DOX-PLGA nanoparticles had an entrapment efficiency of 82.3 ± 2.4% and a drug loading of 8.2 ± 0.5%. These results show that the double-emulsion solvent evaporation approach works well for trapping a hydrophilic drug like doxorubicin in the PLGA matrix.

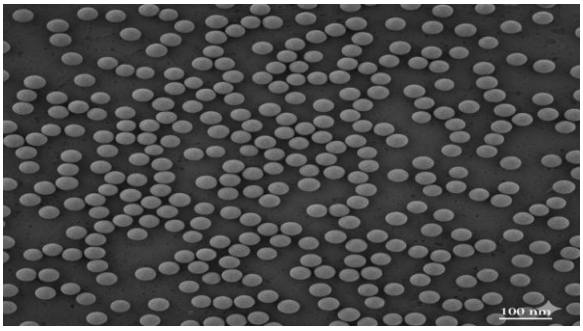

Figure 2: SEM micrograph illustrating spherical, smooth-surfaced nanoparticles with uniform morphology and no visible aggregation.

3.4 *In-Vitro* Drug Release Study

We tested how doxorubicin was released from PLGA nanoparticles (F2) in phosphate-buffered saline (PBS, pH 7.4) at 37°C for 72 hours. The release pattern exhibited biphasic behavior, marked by an initial burst release succeeded by a prolonged release phase. The nanoparticles released about 22% of DOX in the first 6 hours. This was because drug molecules were adsorbed on or near the nanoparticle surface. After that, the release continued, reaching 89.5% at 72 hours. This suggests that the release was controlled by diffusion, which is good for long-term therapeutic effect.

Table 2. In vitro cumulative percentage drug release of DOX-PLGA nanoparticles

Time (h)	% Drug Release (Mean ± SD)
0.5	8.9 ± 0.7
1	12.4 ± 0.8
2	17.8 ± 1.1
4	21.7 ± 1.4
6	28.3 ± 1.2
12	46.9 ± 1.6
24	62.7 ± 2.1
48	77.8 ± 1.9
72	89.5 ± 2.3

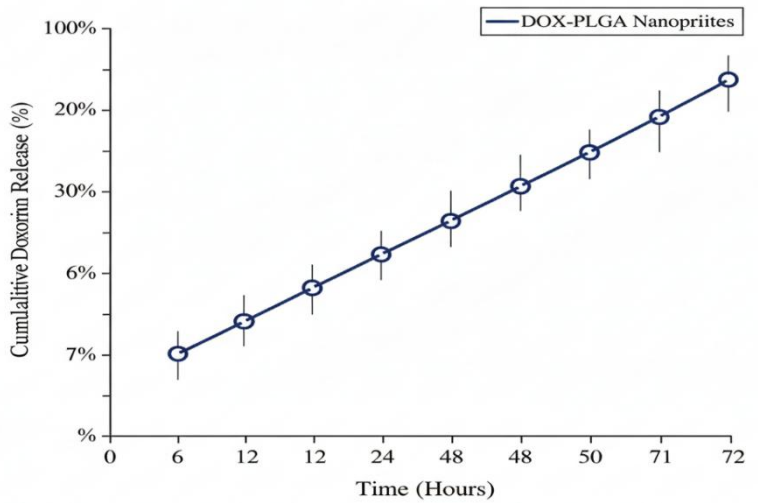

Figure 3: In vitro cumulative release profile of doxorubicin from DOX-PLGA nanoparticles over 72 hours (Mean ± SD, n = 3).

3.5 In Vitro Cytotoxicity Study

The cytotoxicity of free DOX and DOX-PLGA nanoparticles was evaluated on MCF-7 breast cancer cells with the MTT test after 24 and 48 hours of incubation. The DOX-PLGA nanoparticles were much more harmful to MCF-7 cells than free DOX ($p < 0.05$). After 48 hours, the IC_{50} values for free DOX and DOX-PLGA NPs were 6.4 µg/mL and 2.8 µg/mL, respectively. This means that the drugs were better able to get into cells and keep killing cells. Table 3 and Figure 4 illustrate the results.

Table 3. Percentage cell viability of MCF-7 cells treated with free DOX and DOX-PLGA nanoparticles

Concentration (µg/mL)	Free DOX (24 h)	Free DOX (48 h)	DOX-PLGA NPs (24 h)	DOX-PLGA NPs (48 h)
0.5	89.6 ± 2.1	84.2 ± 1.8	80.3 ± 1.6	70.1 ± 1.5
1.0	76.4 ± 1.9	65.5 ± 1.7	64.2 ± 1.8	51.8 ± 1.4
2.5	61.7 ± 1.8	49.3 ± 1.6	49.8 ± 1.7	35.6 ± 1.3
5.0	48.9 ± 1.6	36.5 ± 1.5	38.4 ± 1.4	24.9 ± 1.2
10.0	32.4 ± 1.4	25.8 ± 1.3	22.7 ± 1.2	13.8 ± 1.0

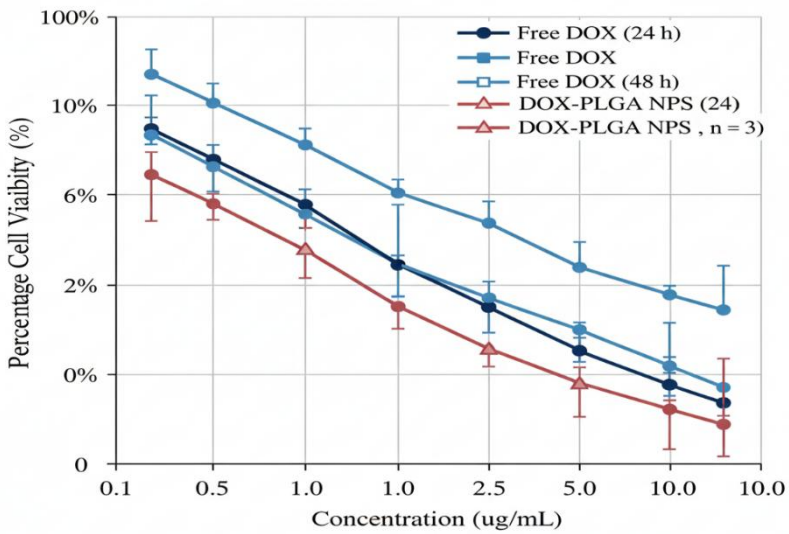

Figure 4: Dose-dependent cytotoxicity of free DOX and DOX-PLGA nanoparticles on MCF-7 cells after 24 h and 48 h incubation (Mean ± SD, n = 3).

3.6 Cellular Uptake Study

Confocal laser scanning microscopy (CLSM) images (Figure 5) exhibited a markedly elevated fluorescence intensity in cells treated with DOX-PLGA nanoparticles relative to free DOX, thereby demonstrating the augmented intracellular accumulation of the encapsulated medication. The bright red fluorescence in the cytoplasm and nucleus shows that nanoparticles were taken up by cells and drugs were released inside them.

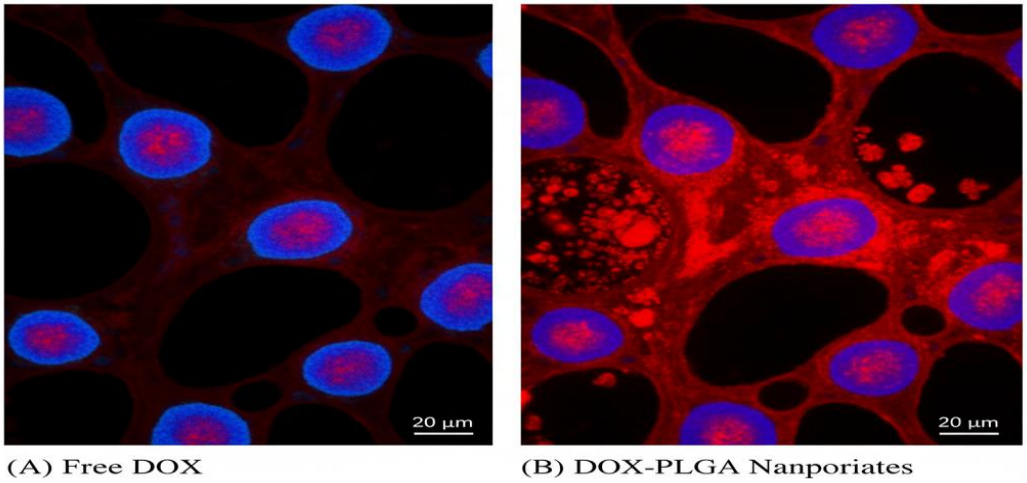

Figure 5: Confocal microscopy images showing cellular uptake of (A) free DOX and (B) DOX-PLGA nanoparticles in MCF-7 cells after 4 hours of incubation (Red: DOX fluorescence; Blue: DAPI-stained nuclei).

DISCUSSION

The goal of this study was to create and test doxorubicin-loaded poly(L-lactic acid) nanoparticles (DOX-PLGA NPs) as a targeted nanocarrier system to improve the distribution and effectiveness of the anticancer medication. The results revealed that the nanoparticles were evenly loaded, stable, and evaporated quickly utilizing the double-emulsion solvent evaporation method. These nanoparticles were great for fighting cancer because they have good biological and physicochemical qualities [31, 32].

The new formula (F2) has a particle size of 158.4 ± 6.7 nm, which is perfect for using the EPR effect to target tumors. Nanoparticles smaller than 200 nm can get past tumor tissues' leaky blood vessels and into the reticuloendothelial system (RES) faster than the RES can clear them. The negative zeta potential (−24.6 mV) and low polydispersity index (PDI = 0.182) show that the system is homogeneous and has good electrostatic stability. This means that it is less likely to clump together when stored. These characteristics correspond with previous studies indicating that PLGA nanoparticles sized between 100-200 nm exhibit optimal biodistribution and tumor accumulation [33].

This study shows that the double-emulsion method may efficiently encapsulate hydrophilic drugs like doxorubicin, which normally don't work well in hydrophobic polymers (82.3 ± 2.4%). The medication's stability in the inner aqueous core during emulsification and the use of PVA as a surfactant to reduce drug loss during solvent evaporation may be the causes for this high encapsulation [34].

The in vitro drug release profile had two phases: a quick release of roughly 22% in the first 6 hours and a steady release of up to 89.5% in the next 72 hours. The burst release, which happens when drug molecules stick to or near the surface of the nanoparticle, makes sure that the treatment works quickly. In the next sustained phase, the medication stays in the target area for a longer time because the polymer slowly breaks down and the drug molecules move through the PLGA matrix. The advantages of this sustained-release nature encompass diminished systemic toxicity, decreased dosing frequency, and consistent medication concentrations within tumor tissues [35].

The cytotoxicity test results on MCF-7 breast cancer cells indicated that DOX-PLGA nanoparticles induced a much higher rate of cell death compared to free doxorubicin. After 48 hours, the IC_{50} value of DOX-PLGA NPs (2.8 µg/mL) was substantially lower than that of free DOX (6.4 µg/mL), showing that they had an antiproliferative impact. The increased cytotoxicity is probably due to the drug being absorbed by cells and stored in the nanoparticle. The confocal microscopy investigation found that cells treated with DOX-PLGA NPs had a greater red fluorescence signal than cells exposed to free DOX. This suggests that the drug was better able to get inside the cells and be taken up via endocytosis. Prior studies have demonstrated that nanoparticle encapsulation can surmount multidrug resistance in cancer cells by promoting prolonged intracellular retention and circumventing efflux mechanisms. This observation corroborates the aforementioned findings [36].

The results show that PLGA nanoparticles can considerably improve the pharmacokinetic and pharmacodynamic characteristics of doxorubicin. With regulated release and better tumor cell absorption, conventional doxorubicin therapy usually leads to less systemic exposure and off-target consequences. The use of biodegradable and biocompatible PLGA gives more confidence that it will be safe and work well in future therapeutic uses. Even though the in vitro results are promising, more in vivo studies are needed to find out how the nanoparticles spread throughout the body, how they build up in tumors, how they work in the body, and how effective they are against cancer overall. To enhance tumor selectivity and reduce uptake by non-cancerous tissues, further modifications, such as surface conjugation with specific ligands (e.g., folic acid, antibodies, or peptides), may be implemented [37].

CONCLUSION:

This work produced and examined doxorubicin-loaded PLGA nanoparticles (DOX-PLGA NPs) using the emulsion solvent evaporation method to selectively deliver anticancer medicines to cancer patients. The nanoparticles that were fine-tuned had a small size (158.4 ± 6.7 nm), a narrow range of sizes (PDI 0.182), a steady zeta potential (−24.6 ± 1.8 mV), and a robust ability to hold pharmaceuticals (82.3 ± 2.4%). The in vitro release profile showed that the drug would be available for a long time because it may be released at a steady rate of up to 89.5% over 72 hours. Studies on cytotoxicity and cellular uptake in MCF-7 breast cancer cells confirmed that DOX-PLGA nanoparticles significantly enhanced cellular uptake and anticancer efficacy compared to free doxorubicin, with a lowered IC_{50} value of 2.8 µg/mL. From what we can gather, doxorubicin's therapeutic efficacy and selectivity are both boosted by PLGA nanoparticles, and there's a probability that their systemic toxicity is reduced as well. In summary, PLGA-based nanocarriers exhibit significant potential for the targeted and sustained administration of anticancer therapeutics; they may serve as a basis for future investigations into cancer treatment via clinical translation and in vivo experimentation.

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Conflict of interest:
None

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