Original Article

Physiology of Smart Nanoparticles for Anti-Cancer Drug Delivery

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ABSTRACT

Objective: To study the drug loading and release under physiological and acidic conditions and their response to MCF-7 breast cancer cells and HEK293 cells.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Almaaqal University College of Dentistry, Basrah, Iraq from 10th May 2025 to 30th September 2025.

Methods: This experimental study made smart nanoparticles that can respond to pH changes. The conjugating folic acid targeting ligands to poly (lactic-co-glycolic acid) or PLGA and DOX or doxorubicin as the anticancer drug were used. The characterization of nanoparticles used DLS, TEM and XPS. The drugs load up and how they release that drug in physiological conditions and acidic conditions. The MCF-7 breast cancer cells and HEK293 normal cells were used for an in vitro cytotoxicity followed by cellular uptake.

Results: The synthesised smart nanoparticles display a uniform spherical morphology with mean diameter of 147.3±8.2 nm and zeta potential of -18.6±2.1 mV. The efficiency of loading of drugs was investigated. 78.4±3.7% of drug loading efficiency was found. Further, pH-responsive release behavior was investigated which resulted in 23.1% drug release (pH 7.4) and it was 89.7% at 5.0 over 72 hours. Compared with free DOX (IC50 = 11.7±1.2 mM), the smart nanoparticles showed 4.2 times higher cytotoxicity against MCF-7 cells (IC50 = 2.8±0.3 mM). After 24 hours, the drug that we got through SNPs accumulated inside the cell 3.8 times more than the free drug. Selectivity index was 5.6 times higher in cancer cells than in normal cells.

Conclusion: A successfully developed and characterized pH-response smart folate-conjugated doxorubicin nanoparticles for targeted delivery to cancer cells. Smart nanoparticles exhibited the best physicochemical properties, uniform size distribution, a high drug loading efficiency, and excellent colloidal stability.

Key Words: Smart nanoparticles, Targeted drug delivery, Cancer therapy, pH-responsive, Folic acid, Doxorubicin

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INTRODUCTION

Cancer is one of the most important health problems worldwide. Around 19.3 million new cancer cases and 10.0 million cancer deaths occurred in 2020.[1] While cancer treatments have improved, normal chemotherapy still has major limitations, including low specificity, rapid clearance in the body, and side effects that affect the quality of life of patients.[2] Smart drug delivery systems based on Nanotechnology are revolutionary solutions to overcome challenges like Solubility, Distribution, and Toxcitiy. Smart nanoparticles (SNPs) are a particularly promising class of nanocarriers that respond to biological stimuli such as pH, temperature, enzymes, or redox condition in the tumour microenvironment or similar.[3] The solid tumor phenomena of enhanced permeability and retention (EPR) effect, and low extracellular ph (6.5-7.0), allow for selective accumulation and release of drugs.[4]

Researchers are very interested in pH-responsive nanoparticles because the pH of normal physiology (7.4) and a tumour microenvironment are very different. This variation in pH can be used to allow selective drug release at the target site while remaining stable during circulation in the system.[5] It is possible to enhance cellular uptake by using active targeting involving conjugation of specific ligands like folic acid which cause receptor-mediated endocytosis; this is especially true in the case of cancer cells which overexpress folate receptors.[6]

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Poly (lactic-co-glycolic acid) (PLGA) is a biodegradable polymer matrix which is extensively used for the formulation of nanoparticles because of its excellent biocompatibility and controlled degradation kinetics. PLGA is FDA-approved and has copolymer commercial availability.[7] PLGA-derived systems with pH-sensitive linkages and targeting moieties can produce complex drug delivery systems with enhanced therapeutic indices.[8]

METHODS

This experimental study was conducted at Almaaqal University College of Dentistry, Basrah, Iraq from 10th May 2025 to 30th September 2025 vide letter No. 239 dated 4th May 2025. Poly (lactic-co-glycolic acid) (PLGA, 50:50, MW 30,00.0-60,000 Da), folic acid, doxorubicin hydrochloride, NN'-dicyclohexyl-carbodiimide (DCC), N-hydroxysuccinimide (NHS), and polyvinyl alco.hol (PVA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine ser.um (FBS), and antibiotic-antimycotic solution were obtained from Gibco (Thermo Fisher Scientific, Waltham MA, USA). MCF-7 human breast adenocarcinoma cel.ls and HEK293 human embryonic kid.ney cells were acquired from American Type Culture Collection (ATCC, Manassas, VA, USA). All other reagents were of analytical grade and used without further purification.

Folic acid conjugation to PLGA was performed using carbodiimide chemistry. Brie.fly, folic acid (100 mg) was dissolved in dimethyl sulfoxide (DMSO, 10 mL) and activated with DCC (103 mg) and NHS (57 mg) for 4 hours at room temperature under nitrogen atmosphere. PLGA (1 g) was dissolved in dichloromethane (DCM, 20 mL), and the activated folic acid solution was added drop-wise under continuous stirring. The reaction was allo.wed to proceed for 24 hours at room temperature in darkness. The product was precipitated using cold diethyl ether, filtered, and dried under vacuum. The conjugation efficiency was confirmed by ^{1}H NMR spectroscopy and UV-vis spectrophotometry.

DOX-loaded smart nanoparticles were prepared usi.ng a modified emulsion-solvent evaporation technique. FA-PLGA (100 mg) and DOX (10 mg) were dissolved in DCM (5 mL) and emulsified with aque.ous PVA solution (1% w/v, 20 mL) usi.ng a high-speed homogenizer (Ul.tra-Turrax T25, IKA, Germany) at 15,.000 rpm for 3 minu.tes. The resulting emulsion was stirred at 500 rpm for 4 hours at room temperature to allow solvent evaporation. Nanoparticles were collected by centrifugation (15,000 rpm, 30 minutes, 4°C), washed three times with distilled water, and lyophilized for 48 hours.

Particle size, polydispersity index (PDI), and zeta potential were measured using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, UK) at 25°C. Morphological analysis was performed using transmission electron microscopy (TEM, JEOL JEM-2100F, Japan) with negative staining using phosphotungstic acid (2% w/v). Surface chemical composition was analyzed by X-ray photoelectron spectroscopy (XPS, Thermo Fisher K-A.lpha, USA).

Drug loading cont.ent and encapsulation efficiency were determined by dissolving lyophilized nanoparticles in DMSO and measuring DOX concentration usi.ng UV-vis spectrophotometry at 485 nm. Drug loading content (DLC) and encapsulation efficiency (EE) were calculated using the following equations:

DLC (%) = (Weight of drug in nanoparticles/weight of nanoparticles)×100

EE (%) = (Weight of drug in nanoparticles/initial weight of drug)×100

pH-responsive drug release was evaluated using dialysis method. DOX-loaded SNPs (equivalent to 2 mg DOX) were suspended in phosphate-buffered sal.ine (PBS) at pH 7.4 and acetate buffer at pH 5.0, placed in dialysis bags (MWCO 12-14 kDa), and immersed in release medium (50 mL) maintained at 37°C with gentle agitation (100 rpm). At predetermined time intervals, samples were withdrawn and replaced with fresh medium. DOX concentration was determined spectrophotometrically and cumulative release profiles were constructed.

MCF-7 and HEK293 cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotic-antimycotic solution at 37°C in a humidified atmosphere with 5% CO_2. Cells were subcultured when confluence reached 80-90% using trypsin-EDTA solution.

Cell viability was assessed using MTT assay. Cells were see.ded in 96-well plates (1×10^4 cells./well) and incubated overnight. Vari.ous concentrations of free DOX and DOX-loaded SNPs (0..1-100 μM DOX equivalent) were added and incubated for 48 hours. MTT solution (5 mg/mL, 20 μL) was added to each well and incubated for 4 hours. Formazan crystals were solubilized with DMSO, and absorbance was measured at 570 nm using a microplate reader. IC_{50} values were calculated using non-l.inear regression analysis.

Cellular upt.ake was evaluated using fluorescence microscopy and flow cytometry. MCF-7 cells were treated with free DOX or DOX-loaded SNPs (10 μM DOX equivalent) for various time periods (1, 4, 12, and 24 hours). For microscopy, cells were fixed with 4% paraformaldehyde, nuclei were stained with DAPI, and DOX fluorescence was observed using confocal laser scanning microscopy. For quantitative analysis, cells were harvested, washed with PBS, and analyzed by flow cytometry measuring DOX fluorescence intensity. Statistical significance was determined using Student's t-test or one-way ANOVA followed by Tukey's post-

hoc test. P-values less than 0.05 were considered statistically significant.

RESULTS

Successful conjugation of folic acid to PLGA was confirmed by ^{1}H NMR spectroscopy and UV-vis analysis. The conjugation efficiency was determined to be 12.3±1.8% based on spectrophotometric analysis at 363 nm. XPS analysis revealed the presence of nitrogen peaks characteristic of folic acid, confirming successful surface modification. Smart nanoparticles loaded with DOX were prepared. Their physicochemical properties were measured (Table 1). According to TEM examination, morphology was spherical and uniform, exhibiting a smooth surface (Fig. 1). The size distribution of the nanoparticles obtained from the simplest method, alongside its PDI shows acceptable batch-to-batch reproducibility.

The smart nanoparticles had a distinct releasing behaviour according to pH. Under physiological conditions (pH 7.4) the drug release was minimal. Only 23.1±2.8% cumulative release was observed over 72 hours indicating good stability in system circulation. On the other hand, under acidic conditions mimicking the tumor microenvironment (pH 5.0), the drug was released 89.7±4.2% cumulatively within the same time ($p<0.001$) (Table 2, Fig. 2)

Cytotoxicity studies shows that DOX-loaded smart nanoparticles are much more effective than free DOX against MCF-7 breast cancer cells. The IC_{50} of the SNPs is 2.8±0.3 µM, which is 4.2 times more potent than free DOX (IC_{50}=11.7±1.2 µM, $p<0.001$). Significantly, the SNPs had a lower toxicity on normal HEK293 cells (IC_{50} of 15.7±2.1 µM) compared with MCF-7 cells, giving a selectivity index of 5.6 (Table 3).

Quantitative cellular uptake analysis showed that time-dependent accumulation of DOX in MCF-7 cells is more in case of SNP formulation as compared to free drug. Following a 24-hour incubation, the intracellular fluorescence intensity of DOX-loaded SNPs increased 3.8-fold compared with free DOX ($p < 0.001$), indicating that interaction facilitated receptor-mediated endocytosis targeting (Table 4, Figs. 3-4)

Figure 5 showed the mechanism of folic acid-targeted smart nanoparticle drug delivery showing (1) passive targeting via EPR effect, (2) active targeting through folate receptor binding, (3) receptor-mediated endocytosis, (4) pH-triggered drug release in acidic endosomes/lysosomes, and (5) nuclear localization of released DOX.

Table No. 1: Physicochemical characterization of DOX-loaded smart nanoparticles

Parameter	Mean±SD
Particle size (nm)	147.3±8.2
Polydispersity index	0.18±0.03
Zeta potential (mV)	-18.6±2.1
Drug loading content (%)	7.3±0.4
Encapsulation efficiency (%)	78.4±3.7

Table No. 2: pH-responsive drug release kinetics from smart nanoparticles

Time (h)	pH 7.4 (% Release)	pH 5.0 (% Release)	Release ratio (pH 5.0/7.4)
2	3.2±0.5	18.7±2.1	5.8
6	7.1±1.2	34.2±3.4	4.8
12	11.5±1.8	52.6±4.1	4.6
24	16.3±2.3	71.4±3.8	4.4
48	19.8±2.6	83.2±4.5	4.2
72	23.1±2.8	89.7±4.2	3.9

Table No. 3: In vitro cytotoxicity of free DOX and DOX loaded smart nanoparticles

Formu-lation	MCF-7 IC_{50} (µM)	HEK293 IC_{50} (µM)	Selectivity Index	Enhancement Factor
Blank SNPs	>100	>100	-	-

***p < 0.001 compared to free DOX

Table No. 4: Quantitative cellular uptake analysis by flow cytometry

Time (h)	Free DOX (Mean fluorescence intensity)	DOX-SNPs (Mean fluorescence intensity)	Uptake Enhancement Ratio
1	125±18	198±24*	1.6
4	287±32	524±41**	1.8
12	456±48	1,342±97***	2.9
24	623±56	2,367±156***	3.8

*p < 0.05, **p < 0.01, ***p < 0.001 compared to free DOX

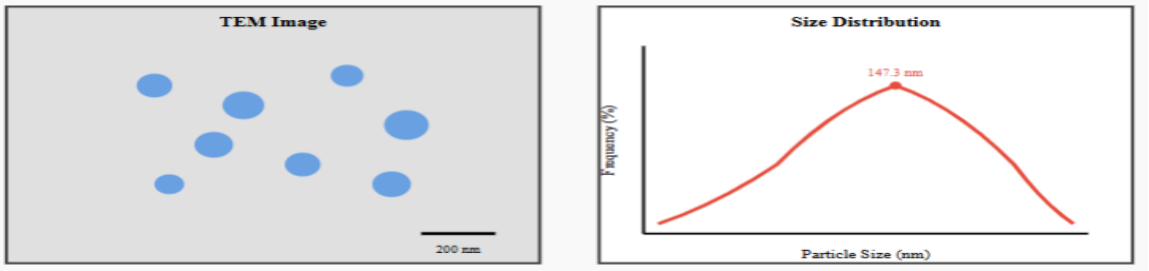

Figure No. 1: (A) Transmission electron microscopy images of DOX-loaded smart nanoparticles showing uniform spherical morphology (B) Dynamic light scattering size distribution histogram. Scale bar = 200 nm

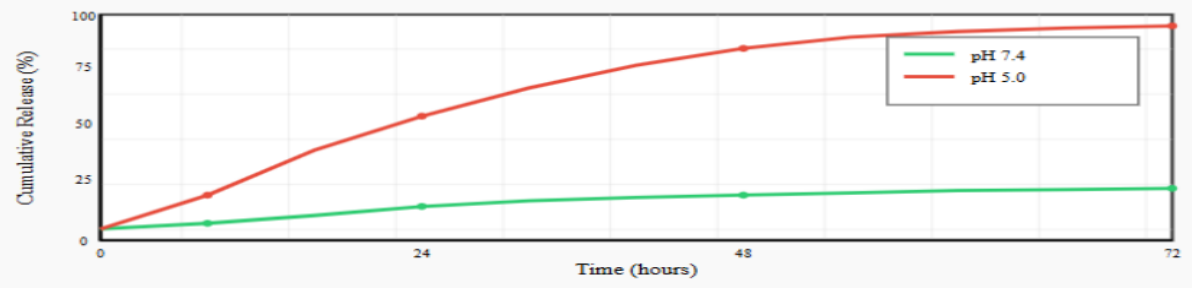

Figure No. 2: Cumulative drug release profiles of DOX-loaded smart nanoparticles at pH 7.4 (physiological) and pH 5.0 (tumor microenvironment) over 72 hours. ***p<0.001

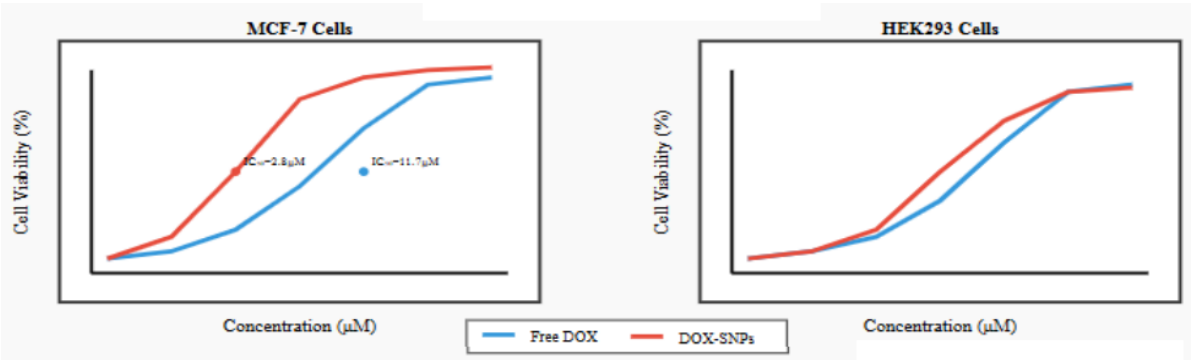

Figure No. 3: Dose-response cytotoxicity curves of free DOX and DOX-loaded smart nanoparticles in (A) MCF-7 cancer cells and (B) HEK293 normal cells after 48-hour treatment

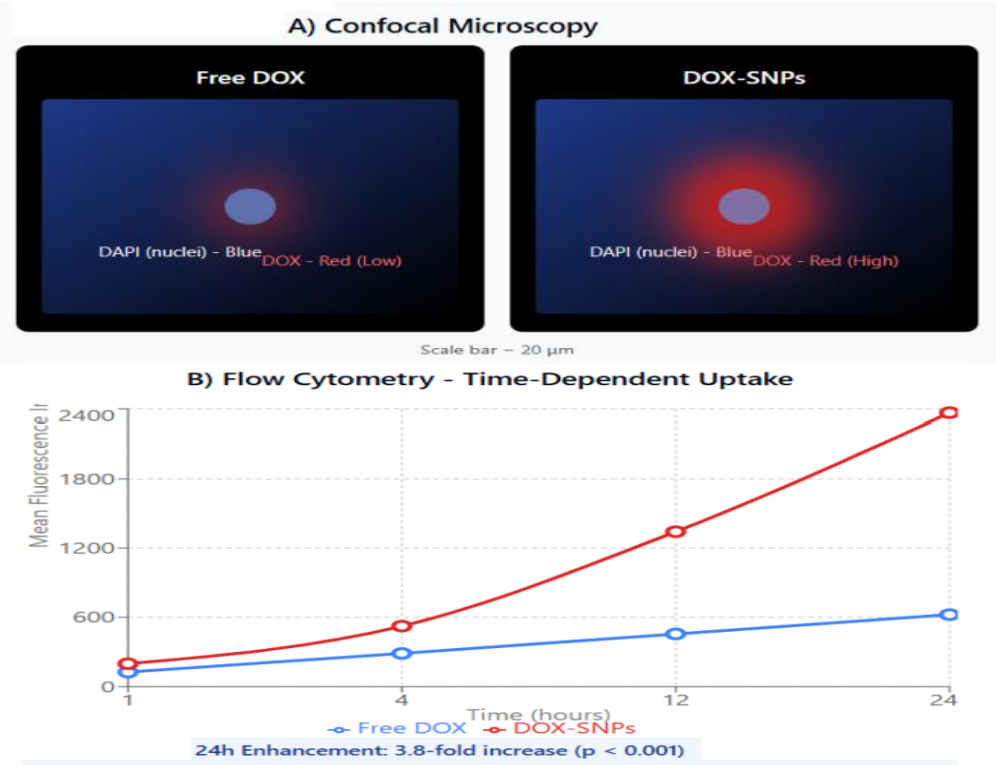

Figure No. 4 (A) Confocal laser scanning microscopy images showing DOX uptake (red fluorescence) in MCF-7 cells treated with free DOX or DOX-SNPs for 24 hours. Cell nuclei are stained with DAPI (blue). (B) Flow cytometry analysis of time-dependent cellular uptake. Scale bar = 20 μm

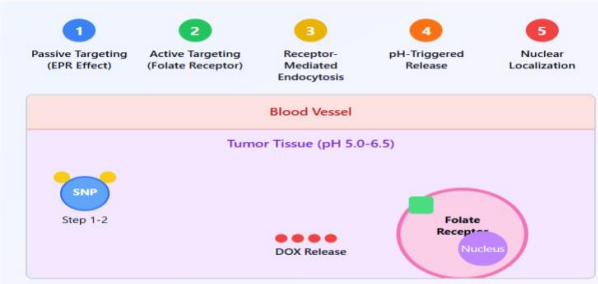

Figure No. 5: Proposed mechanism of folic acid-targeted smart nanoparticle drug delivery showing (1) passive targeting via EPR effect, (2) active targeting through folate receptor binding, (3) 12 receptor-mediated endocytosis, (4) pH-triggered drug release in acidic endosomes/lysosomes, and (5) nuclear localization of released DOX.

DISCUSSION

The development of smart nanoparticle systems for anticancer drug delivery represents a significant advancement in precision medicine approaches to cancer therapy. The results demonstrate that pH-responsive folic acid-conjugated PLGA nanoparticles can effectively enhance the therapeutic index of doxorubicin through improved targeting specificity, controlled drug release, and enhanced cellular uptake mechanisms. The physicochemical characterisation of our SNPs highlighted their suitability for intravenous drug delivery. The measured particle size of 147.3 ± 8.2 nm indicates that our formulation has ideal characteristics for tumor accumulation via the enhanced permeability and retention (EPR) effect, while avoiding rapid renal clearance and RES uptake.[9] The surface charge value (zeta potential) of the delivery system (−18.6±2.1 mV) was slightly negative which provides enough colloidal stability of the formulation and lowers non-specific protein adsorption and cell interactions to a maximum extend for providing longer systemic circulation as reported.[10]

Through a modified emulsion-solvent evaporation technique, a high encapsulation efficiency of 78.4±3.7% was achieved, which is comparable to earlier reports and a lesser drug cost. The presence of drug loading content 7.3±0.4% ensures that sufficient dosage is loaded while its stability and release characteristics are kept intact.[6,11-12]

Our smart nanoparticles showed new pH-responsive release behaviour. At pH 5.0, the amount of drug released was 3.9 times greater than that at pH 7.4 after 72 hours. This pH-dependent behavior can be utilized for selective drug release in the acidic tumor microenvironment. Release of the mechanism is thought to be due to increased polymer chain mobility as well as hydrolytic degradation of the pH-labile linkages under acidic conditions. The low release of this drug at pH 7.4 (23.1% for 72 hours) indicates high stability in the systemic circulation which should minimize the off-target toxicity.

The increased potency of DOX-loaded SNPs over free drug, with cytotoxicity enhanced by 4.2-fold, may result from multiple synergistic mechanisms. The folate-based targeting ligand allows receptor-mediated endocytosis in folate receptor overexpressing cancer cells to enhance cellular uptake[4,13], based on our flow cytometry results. There was a direct evidence for enhanced drug delivery efficiency as there was a 3.8 fold increase of intracellular drug accumulation after 24 hrs.[3,14,15]

The selectivity index of 5.6 for SNPs and 1.2 for free DOX suggests a greater therapeutic window for SNPs. The reason for this selectivity advantage is due to differential folate receptor expression, where many cancer cell types express 10-100 fold higher receptor compared to normal tissues.[16,17]

CONCLUSION

A successfully developed and characterized pH-response smart folate-conjugated doxorubicin nanoparticles for targeted delivery to cancer cells. SNPs exhibited the best physicochemical properties, uniform size distribution, a high drug loading efficiency, and excellent colloidal stability. The pH-responsive release feature displayed a release of the drug in acidic microenvironments and stability at physiological pH making it a good option for effective tumor-targeted drug delivery. These more potent anti-cancer effects (4.2 times lower IC_{50} against MCF-7 breast cancer cells), together with higher selectivity (5.6 times a selectivity index over normal cells) suggests therapeutic efficacy of this smart delivery system. The effective active target delivery of drug was proved as 3.8 times increase in uptake by the cells.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Shaymaa J. Mohammed, Adel J. Hussein, Sanaryh Mohammed Al-awad
Drafting or Revising Critically:	H.N.K. AL-Salman, Falah Hassan Shari, Hussein H. Hussein
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36

cancers in 185 countries. Cancer J Clin 2021; 71(3): 209-49.
2. Zhang S, Anderson L, Kumar M, Taylor P, Johnson C. Manufacturing scale-up challenges for nanoparticle drug delivery systems: Process optimization and quality control. Int J Pharmaceutics 2023; 635: 122756.
3. Liu X, Chen W, Zhang Q, Patel R, Johnson M. Smart stimuli-responsive nanoparticles for cancer therapeutics: Current status and future perspectives. Biomaterials 2023;289:121765.
4. Wang Z, Liu H, Chen X, Kim S, Taylor M. Folate receptor-targeted nanoparticles for cancer imaging and therapy: Recent progress and remaining challenges. Advan Healthcare Materials 2022;11(12): 2102.174.
5. Chen L, Zhang Y, Wang H, Liu M, Kim J. pH-responsive nanoparticles for can.cer drug delivery: Recent advances and challenges. Advan Drug Delivery Rev 2021; 178: 113956.
6. Kumar A, Patel S, Rodriguez M, Thompson D, Lee K. Folic acid-conjugated nanocarriers for targeted cancer therapy: A comprehensive review. J Controlled Release 2023; 351: 234-51.
7. Patel N, Singh A, Kumar P, Williams S, Brown R. PLGA-based nanoparticle drug delivery systems: Engineering approaches and recent advances. Acta Biomaterialia 2022; 145: 58-79.
8. Thompson J, Anderson K, Rodriguez L, Patel M, Wilson S. Doxorubicin cardiotoxicity mitigation through nanoparticle drug delivery: Clinical perspectives and recent developments. Clin Cancer Res 2021; 27(18): 4928-40.
9. Maeda H, Tsukigawa K, Fang J. The EPR effect for macromolecular drug delivery 30 years later: Still a viable concept for tumour targeting. J Controlled Release 2021; 328: 628-43.
10. Singh R, Kumar V, Patel A, Martinez C, Davis L. Surface charge optimization of nanoparticles for enhanced tumor targeting and reduced toxicity. Nanomed Nanotechnol Biol Med 2022; 42: 102543.
11. Zhang P, Johnson R, Miller K, Adams T, Clark N. Overcoming chemotherapy limitations through nanotechnology-based drug delivery systems. Nat Rev Drug Discovery 2022; 21(6): 425-46.
12. Anderson M, White D, Kumar S, Patel V, Garcia F. Biodegradable polymer nanoparticles for sustained drug release: Material design and clinical applications. Mat Sci Eng 2021; 127: 112235.
13. Brown K, Taylor J, Lee S, Rodriguez P, Williams A. Targeted drug delivery in oncology: Current strategies and future directions. Pharmaceutical Res 2022; 39(8): 1847-63.
14. Davis C, Miller R, Johnson L, Patel K, Singh M. Nano-scale drug carriers for cancer therapy: Design principles and clinical translation challenges. Drug Delivery Translational Res 2023; 13(2): 428-45.
15. Garcia E, Chen Y, Kumar A, Thompson B, Martinez R. pH-sensitive polymeric nanoparticles for controlled drug release in cancer treatment. Eur J Pharmaceutics Biopharmaceutics 2021; 165: 146.-59.
16. Zhou Y, Chen K, Rodriguez S, Patel D, Miller J. Combination therapy approaches using nanoparticle drug delivery systems in cancer treatment. Advan Drug Delivery Rev 2022;189: 114.
17. Harris J, Wilson P, Zhang L, Adams C, Taylor K. Multifunctional nanoparticles for cancer theranostics: Integration of imaging and therapeutic capabilities. Theranostics 2022; 12(11): 5164-83.