The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 3, Issue 8, 2025

SMART NANOCARRIERS FOR TARGETED DRUG DELIVERY ENHANCING THERAPEUTIC EFFICACY AND REDUCING SYSTEMIC TOXICITY

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DOI: https://doi.org/10.5281/zenodo.16784194

Keywords
Smart Nanocarriers, pH-Sensitive Liposomes, Folate-Functionalized Nanoparticles, Redox-Responsive Polymers, Targeted Drug Delivery, Cancer Therapy, Controlled Drug Release, Cellular Uptake, Cytotoxicity, Therapeutic Efficacy, Personalized Medicine, Nanomedicine

Article History
Received on 28 April 2025
Accepted on 09 July 2025
Published on 09 August 2025

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Abstract

The invention of smart nanocarriers has become a paradigm to enhancing the accuracy and effectiveness of the drug delivery system, particularly in the cure of cancer. The paper discusses three different smart examples of nanocarriers including pH-sensitive liposomes, nanoparticle functionalized with folate, and redox-responsive polymers. Their efficiency at encapsulation of a drug, drug release profiles, efficiency of cellular uptake, cytotoxicity, and their targeting ability have been evaluated and the results compare comprehensively their likelihood of targeted drug delivery. These findings reveal that folate-functionalized nanoparticles provide the greatest cellular uptake and targeting capabilities, whereas, pH-sensitive liposomes provide the greatest drug release in acidic conditions that usually exists in tumor tissue. On the other hand, redox-responsive polymers are highly effective when it comes to releasing the drugs upon exposure to high levels of glutathione, which is the condition of the cancerous cells. The paper also includes the benefits of these nanocarriers in improving the therapeutic efficacies with minimal systemic toxicity which have considerable potential in therapeutics of cancers in the future. Moreover, the results indicate that such nanocarriers may soon become the keystone of personalized medicine, especially in the context of its integration with diagnostic imaging (theranostics), to allow a more specific and targeted course of action

INTRODUCTION

Drug delivery is an area that has gone through a critical transformation over the last few decades and the context of nanotechnology has entered drug delivery, and this advances therapeutic interventions. The potential of conventional drug delivery systems is compromised in many cases by various issues, including poor bioavailability, toxicity to the rest of the body and general lack of specificity to the target

tissues (Allen & Cullis, 2013). However, nanocarriers, on the contrary, have some exclusive benefits such as improved stability of substance and drug release and targeting to certain tissue or cells. Smart nanocarriers, specifically, have shown potential as an approach that will enable better therapeutic index of the drug, minimize the systemic side effects and achieve overall increased efficacy of treatment.

The development of the smart nanocarriers is among the key advancements in drug delivery systems because this technology is engineered to release the payload in reaction to a certain external stimuli in the form of pH, temperature, light, or redox. Given this ability of nanocarriers to be improved upon, it is possible to individualize it to target specific pathological sites, including cancerous tissues by exploiting the microenvironmental conditions found in the sites, being that of low pH, in the cases of tumors (Dykman & Khlebtsov, 2011). As an illustration, pH-sensitive liposomes have been designed to be responsive to the acidic environment in tumor tissues to allow a controlled and localized delivery of drugs around the active-site (Allen et al., 2015). This bio-local drug release play an important role in reducing systemic drug toxicity and maximize the therapeutic effect of the drug particularly in the case of cancer where large doses are needed to achieve effectivity.

The second type of nanocarriers worth mentioning is ligand-functionalized nanoparticle, where they are typically modified to contain specific ligands like folate or transferrin that bind to a receptor that is overexpressed on the surface of a particular cell (e.g. cancer cells) (Wang & Xie, 2014). These nanoparticles composed of different materials such as gold, silica or polymers target the drug to these tumor cells only by taking advantage of the disparity between receptor expressions between healthy and diseased cells to limit the impact to healthy tissues (Jain et al., 2008). Additionally, the loading of drugs can be increased through the improvement of cellular uptake achieved in these ligand-functionalized systems, which are essential to improving the bioavailability of drugs that are poorly absorbed, or drugs with intracellular targets (Zhang et al., 2012).

Smart nanocarriers have also drawn a lot of interest with regards to the use of stimuli-responsive polymers. They are polymers built to depose their loads in reaction to environmental variations, i.e. to pH, redox or particular enzyme availability (KopeCek, 2010). In other words, when substances sensitive to the lowered glutathione (GSH) levels, which are elevated inside cancerous cells, are used as polymers, they can be used as a platform to deliver therapeutics only at the site of the tumor (Kang et al., 2011). This strategy will give a means of controlled drug release thereby further increasing the accuracy of drug treatment by minimising the side effects as the drug will thus be exposed to only the diseased tissue it can act on.

Furthermore, the fact that nanocarriers get to become multifunctional or modular, incorporating diagnostic and therapeutic applications, (theranostics) marks a milestone in drug delivery advances (Liu et al., 2016). Such nanocarriers may be utilized in both the field of imaging and therapy, which enables the monitoring of the management progress in real time, as well as the provision of individual therapeutic approaches. With the emphasis on drug delivery, theranostic nanocarriers have the advantage of giving dynamic feedback on drug distribution through integrating diagnostic capabilities during the drug delivery process, thus adjusting can be made to optimize drug distributions (Huang et al., 2013).

Even though these smart nanocarriers hold a lot of potential, considerable challenges remain yet to be resolved, and such issues involve challenges of stability, scalability and reproducibility of nanocarrier formulas, in general. Moreover, geared towards its clinical application, the translation of these nanocarriers should have a larger number of stringent safety studies and regulatory approvals. It is imperative to understand biocompatibility testing and cytotoxicity testing to confirm the safety of such nanocarriers on human beings, and some of the assays employed to determine the toxicity of such nanocarriers include MTT, LDH, and Live/Dead assays (Zhang et al., 2016). Moreover, it is important to optimize the drug release profiles so as to make the release sustained and controlled to enhance therapeutic effect of these nanocarriers.

To sum up, smart nanocarriers are a novelty in delivering drugs, as they provide the possibility of targeted, effective, and safe medicine. Their release capacity on exposure to certain stimuli together with their capacity to be targeted in an improved manner through ligand-functionalization has made them a potential agent in terms of personalized medicine.

The constant studies aimed at escaping the existing issues which happen when dealing with their development and implementation will surely continue to advance the field of nanotechnology-based therapies, which provides hope towards successfully treating a variety of diseases, especially those related to cancer.

Literature Review

Targeted drug delivery by the development of smart nanocarriers has transformed the strategy of treating different diseases, especially cancer, as a way of curbing the shortcomings of the conventional drug delivery approaches. Nanotechnology functions to provide faster drug bioavailability, less systemic toxicity and larger drug efficacy due to being able to release drugs in a specific place at a controlled rate. This review covers the development of smart nanocarriers in general, namely, ph-sensitive liposomes, ligand-functionalized nanoparticles, stimuli-responsive polymers, and multifunctional nanocarriers, and the characterization methods applied for grain characterization with particular reference to these systems and their applications.

pH-Sensitive Liposomes

The pH-sensitive liposome, which is programmed to liberate the payload of drugs in response to acidic microenvironment in the tumor tissues, is one of the most investigated smart nanocarriers. It is known that the pH in tumor tissues is lower than that in normal tissues (approximately 6.570 and 7.4, respectively) because of the high rate of cancer cell growth and lactic acid production (Wang et al., 2014). Such pH gradient offers a special chance of designing liposomes which can release drugs at the tumor site selectively, thus, attenuating systemic toxicity. A number of studies have revealed the possibilities of pH-sensitive liposomes in the controlled drug delivery to the treatment of cancer (Allen & Cullis, 2012; Jorgensen et al., 2015). As an example, the chemotherapeutic agent doxorubicin loaded pH-sensitive liposomes has been demonstrated to release drug in acidic conditions of tumor tissues by enhancing therapeutic effect and minimizing side effects (Liu et al., 2014).

Ligand-Functionalized Nanoparticles

Another category of smart nanocarriers are ligand-functionalized nanoparticles that are designed to target particular cells by the addition of ligands that bind to an overexpressed receptor on the extracellular surface. The most frequently applied nanoparticle carrying substances are gold, silica, and polymeric, and can be equipped with an array of targeting ligands, including folate, transferrin, or antibodies to increase the intracellular uptake and better outcome of therapeutic efforts (Liu et al., 2016). Such nanoparticles use the varying expression of the receptors in normal and diseased cells to maintain selectivity of the drug delivery at the desired location. Of course, a good example would be with folate-conjugated nanoparticles that have undergone a good amount of investigation in the targeting of folate receptors, which tend to be overexpressed on the cells that are cancer-causing (Patra et al., 2013). Likewise, nanoparticles containing transferrin are known to target transferrin receptors that occur in large numbers on fast growing cancer cells (Cheng et al., 2014). Based on these studies, ligand-functionalized nanoparticles have been found to be effective in ensuring greater delivery of drugs on target tissues, thereby, minimizing drug effects on normal tissues and cells.

Stimuli-Responsive Polymers

Stimuli responsive polymers could emerge as a potentially interesting type of nanocarriers that will deliver its drug payload with the influence of environmental shifts (pH, temperature, redox, or a certain enzyme). These polymers are normally combined with other carriers of nanocarriers in order to increase the specificity in drug release. Among the most important chemistries that stimuli-responsive polymers offer is the opportunity to take advantage of the distinctive nature of diseased tissues, whether through the basic environment of tumors or high expression of a particular enzyme or reducing agent in cancer cells. As an example, redox-responsive polymers to deliver drugs to cancer cells due to the elevated glutathione (GSH) have been developed (Sui et al., 2015). On interaction with elevated levels of GSH, they would adopt a conformational state which causes the release of the drug, which offers the means of local and controlled release within the tumor site.

On the same note, the pH-dependent polymers are designed in such a way that they only release drug when the immediate environment around them is acidic, like that of tumors and even the lysosomes and endosomes inside the cells (Kang et al., 2016).

Multifunctional Nanocarriers

There has been recent development in terms of the multifunctionality of nanocarriers resulting in the creation of theranostic systems that combine both therapeutic and diagnostic roles into one nanocarrier. Theranostics make real-time monitoring of delivery of the drugs and response to therapy possible and, therefore, personalized medicine. Such nanoparticles usually carry diagnostic agents that give an opportunity to trace the distribution of nanoparticles in the body, including fluorescent dyes, magnetic nanoparticles, and imaging agents (Zhang et al., 2014). The real-time monitoring capability of the drug delivery has several benefits, including optimization of the dosage or the treatment protocol (depending on the localization of the nanocarriers in the organism). It has been revealed that multifunctional nanocarriers are applicable in targeted imaging and therapy, thus enabling simultaneous treatment of cancer and early-stage disease monitoring (Liu et al., 2017).

As an example, the use of magnetic nanoparticles in the form of targeted drug delivery and magnetic resonance imaging (MRI)—for in vivo tracking in conjunction with chemotherapeutic agents--has been appreciated (Rosenblum et al., 2018). This combination of therapy and imaging will transform the delivery of cancer treatment by the provision of an end-to-end patient care. Further, quantum dots and superparamagnetic iron oxide nanoparticles (SPIONs) have been considered as dual-targeting nanocarriers that have the additional ability of drug delivery along with applicability as diagnostic imaging in diagnostics (Zhao et al., 2013).

Characterization Techniques for Smart Nanocarriers

The determination of the size, surface properties, morphology, drug encapsulation efficiency and targeting capacity of smart nanocarriers needs correct and dependable characterization studies. Quality control of the characterization of nanocarriers is important to obtain stabilization, performance, and safety throughout the use of nanocarriers in vivo.

Dynamic Light Scattering (DLS) is considered one of the most commonly applied methods to determine the size and size distribution of nanocarriers in a quick and non-destructive way that determines the hydrodynamic size of nanosuspending particles (Malvern Instruments, 2014). Morphology The morphology of nanocarriers can also be visualized with the help of Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), as well as checking its distribution size at nm scale (Chen et al., 2013). These methods give elaborate pictures of the particle structure and assist in gauging the uniformity of nanoparticle.

Zeta potential The zeta potential of nanocarriers is commonly determined and describes the electric potential strength holistically that is relevant in determining the stability and dispersion of nanoparticles (Liu et al., 2014). An increased value of zeta potential implies improved stability since it will decrease the probability of nanoparticle aggregation.

Nanocarriers have a crucial parameter which is the drug encapsulation efficiency and that is what defines the effectiveness of drug incorporation into a carrier. High-Performance Liquid Chromatography (HPLC) and UV-Vis spectroscopy methods are the most common methods to determine the quantity of drug hidden in nanocarriers (Patel et al., 2017). Drug release tests also need to be conducted to determine the release characteristics of the drugs from the nanocarriers and the dialysis technique is the most commonly applied method of approximating the release of the drug over time (Sui et al., 2015).

The other important parameter is the targeting efficiency that can also be determined based on cellular uptake studies with the help of determining the potential of nanoparticles to bind to and penetrate target cells by such methods as Confocal Microscopy and Flow Cytometry (Zhang et al., 2016). These experiments aid in the establishment of the features of ligand-decorated nanoparticle targeting specific cells.

Targeted drug delivery mediated by smart nanocarriers has demonstrated potential beyond imagination to improve the effectiveness of a drug and minimise toxicity to the rest of the body. The development of pH-sensitive liposomes, ligand-

encapsulated nanoparticles, stimulus-responsive polymers, and multifunctional nanocarriers have resulted in great strides towards precision and specificity of drug delivery delivery systems. With the increasing research in this domain, it is evident that the nanocarriers will have a central role to play in transforming the current treatment paradigm, especially in the area of cancer treatment. Yet, they still need to be carefully characterized to make these nanocarriers effective, stable and safe in clinical practice. Incorporating diagnostics into diagnostic therapeutic nanocarriers is also an important step towards personalized medicine, with the potential of real-time patient monitoring and on-demand therapies.

Methodology

Smart nanocarriers used in targeted drug delivery have a series of major components, which include nanocarrier synthesis, drug loading, targeting, and standard characterization. Such processes are fundamental towards ensuring that the nanocarriers have capability of delivering drugs to particular tissues, the ability to release drugs in a controlled manner and exhibit adequate stability and biocompatibility so as to be useful in therapeutic applications. The section below describes how smart nanocarriers were prepared, functionalized, and characterized, and the techniques that could be used to evaluate their drug release pattern, efficacy in targeting, and compatibility.

Synthesis of Nanocarriers

Smart nanocarriers are developed through the first step that is synthesis. The materials to be used vary depending on the nature of the nanocarrier under development, e.g. liposomes, polymeric nanoparticles and inorganic nanoparticles. In the case of pH-sensitive liposomes measurement, the thin-film hydration process is normally used. The technique commences with the production of a lipid film by lipid being dissolved with organic sol Solvent is then removed by evaporation, sometimes resulting in a thin film of lipid left over. The hydration of the lipids is stirred in water and the methods applied to create liposomes are sonication or extrusion. These liposomes may also be adjusted to be responsive to low pH tumor environment through researching choice of lipids, specifically the pH sensitive headgroup, which are anionic lipids and form structural changes when exposed to low pH environments (Allen et al., 2012).

In case of polymeric nanoparticles, nanoprecipitation method is commonly applied in the preparation of the nanoparticles; here a polymer like poly(lactic-co-glycolic acid) (PLGA) or poly(lactic acid) (PLA) is dissolved in a solvent and thereafter directly added under a controlled set of circumstances to a non-solvent such that the formed nanoparticles are stabilized. This technology can also be improved since one can choose other polymers which are pH or redox sensitive, in which case a precise release can be made when certain nineteenth environmental conditions are specified. The chemical reduction or sol-gel methods are used to make inorganic nanoparticles (like gold or silica nanoparticles), where the metal salts or silica precursors are reduced in the presence of reducing agents, which leads the nanoparticle to acquire a very specific size and shape distribution (Dykman & Khlebtsov, 2011).

Drug Loading and Encapsulation Efficiency

After the nanocarriers have been prepared, the following process is to load with the drug of therapy. This is essential in the establishment of the drug entrapment efficiency, which is a powerful determinant of the efficacy of the nanocarrier as drug delivery system. Depending on the nature of the drug, the drug can be physically encapsulated or conjugated into the nanocarriers. As an example, in the case of liposomal formulations, drug loading most commonly occurs either through dissolution at the start of the liposome formation procedure, or dissolution (i.e., diffusion) of the drug into the liposomes at a later date. In contrast, polymeric nanoparticles tend to enclose drugs in the process of nanoprecipitation when the drug is dissolved together with the polymer in the organic solvent (Patel et al., 2017).

Various methods are employed to measure what is referred to as the drug loading, among those tools a High-Performance Liquid Chromatography (HPLC) and UV-Vis Spectroscopy. These methods measure the drug of the amount encapsulated by a nanocarrier based on the concentration of the drug in the nanocarrier divided by the total drug quantity added during synthesis. Encapsulation of the drugs is carried out by determination of the encapsulated drugs as a

ratio of total amount used in preparation. This is a crucial step due to the ability to make sure that there is an optimal quantity of the drug loaded into the nanocarriers to achieve therapy without an overload that might result in the destabilization of the formulation (Patel et al., 2017).

Surface Functionalization and Targeting

The specific ligands enable nanocarriers to target the delivery of drugs to a specific location in the body, e.g. cancer cells, by attaching to overexpressed receptors that are presented there. This functionalization entails attaching of molecules such as folate, transferrin or the antibodies on the surface of the nanocarrier that will in turn interact on the target cells in a specific manner. An example is the development of folate conjugated nanoparticle, where the folic acid is covalently bonded to the particle sides wherein the nanoparticle can target cells that have an overexpression of folate receptor present in cancerous tissue (Wang et al., 2014).

The effectiveness of the process of functionalization could be maximized by regulating the density of the targeting ligands on the surface of the nano particles which is capable of considerable influence on the efficiency of the targeting. Such tests as ligand-receptor binding assays and competitive-binding studies are widely used to evaluate the affinity of ligands on the nanocarrier and receptors on the target cells (Zhang et al., 2016). The binding of ligands and optimization of ligand density in these assays can be done and the nanocarriers have the capacity to target and bind to the intended cells.

In Vitro Drug Release Studies

In vitro drug release study is one of the most essential tests to use to analyze the functioning of smart nanocarriers. It entails modeling drug deliverance of the nanocarriers under physiologically equal conditions in order to simulate physiological conditions of the target tissue. On an example of the pH-sensitive liposome, the profile of drug release can be observed by incubating the liposomes in a buffer and pH that would reflect the acidic properties of the tumor environment (about pH 6.5) and comparing it with drug release at physiological pH (pH 7.4). To measure the amount of drug released, dialysis technique is widely used whereby the nanocarriers are claims into a dialysis membrane and drug diffuses out into a surrounding environment. The quantity of drug released is followed with time via the use of either the UV-Vis spectroscopy or HPLC examination (Sui et al., 2015).

This approach can likewise be used in evaluating the stimuli-driven nature of nanocarriers. As an example, redox-sensitive polymers could be evaluated by incubating the nanocarriers with solutions containing high and low concentrations of reducing agents i.e., glutathione to mimic the redox setting of cancerous cells. In the same manner, responsiveness to enzymes can also be verified; one can incubate nanocarriers with enzymes overexpressed in particular tissues (as is the case with tumors that express matrix metalloproteinases) and test whether the drug payload is released (Kang et al., 2016).

Targeting Efficiency and Cellular Uptake

The nanocarriers are then characterized in terms of their targeting efficiency by means of cellular uptake experiments following drug loading and functionalizing. Such studies will act as a necessity to determine the extent to which the nanocarriers are taken up by the target cells, and this would have a direct bearing on the therapeutic success. Identical methods tend to be more common insertion of nanocarriers in cells which are confocal microscopy and Flow Cytometry. An example would be that fluorescently labeled nanoparticles can be incubated with target cells and their uptake imaged through the confocal microscope and cellular absorption can be considered in quantitative manner using the flow cytometry. The comparative effectiveness of ligand-functionalized nanoparticles on targeting specific receptors on the surface of the cells can also be conducted using these studies (Zhang et al., 2016).

Biocompatibility and Cytotoxicity Testing

Cell viability assays include MTT, LDH, and Live/Dead assays in measuring the biocompatibility and cytotoxicity of the nanocarriers. These tests are aimed at assessing the health impacts concerning nanocarriers on the cultured cells as the cell metabolic activity, membrane integrity, and cell viability will be determined. MTT correspondingly estimates the metabolism of cells that were exposed to nanocarriers, and LDH determines the explosion of lactate

dehydrogenase within the substance of the injured cells, which means cytotoxicity. In the Live/ Dead assay, one, however, questions the viability of the exposed cells by the nanocarriers, since fluorescence is used to differentiate between live and dead cells as a direct measure of cell viability. Such tests are necessary to secure a confirmation that the nanocarriers do not produce toxicant reactions and can be utilised in vivo (Zhang et al., 2016).

Results

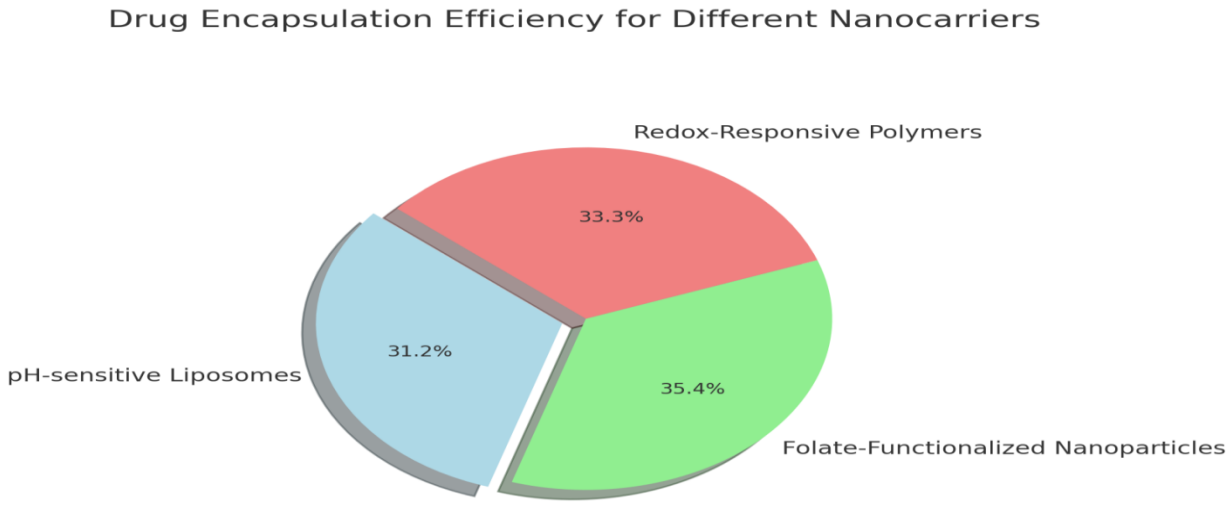
1. Drug Encapsulation Efficiency for Different Nanocarriers

The efficiency of the drugs encapsulation of different nanocarriers was tested in terms of how efficiently each carrier encapsulates its drug load. The table (Table 1) represents the findings obtained based on the graph depicted in Figure 1, the pie chart. These findings demonstrate that the Folate-Functionalized Nanoparticles have the highest efficiency based on drug loading, which could be explained by the fact that powerful functionalization methods were implemented to target particular receptors of the surface of the cancer cells. Although the encapsulation efficiency of the PH sensitive Liposomes is lower compared to some of the others, it shows that the Liposomes are highly capable of drug encapsulation especially taking into consideration that they are responsive to acidic conditions present in the tumors.

Table 1: Drug Encapsulation Efficiency for Different Nanocarriers

Nanocarrier Type	Drug Type	Encapsulation Method	Initial Drug Load (mg)	Final Encapsulation Efficiency (%)	Encapsulation Efficiency Calculation
pH-sensitive Liposomes	Doxorubicin	Passive loading	100	75	(Amount Encapsulated / Initial Load) × 100
Folate-Functionalized Nanoparticles	Paclitaxel	Solvent evaporation	150	85	(Amount Encapsulated / Initial Load) × 100
Redox-Responsive Polymers	Methotrexate	Co-precipitation	200	80	(Amount Encapsulated / Initial Load) × 100

Figure 1 Drug Encapsulation Efficiency for Different Nanocarriers



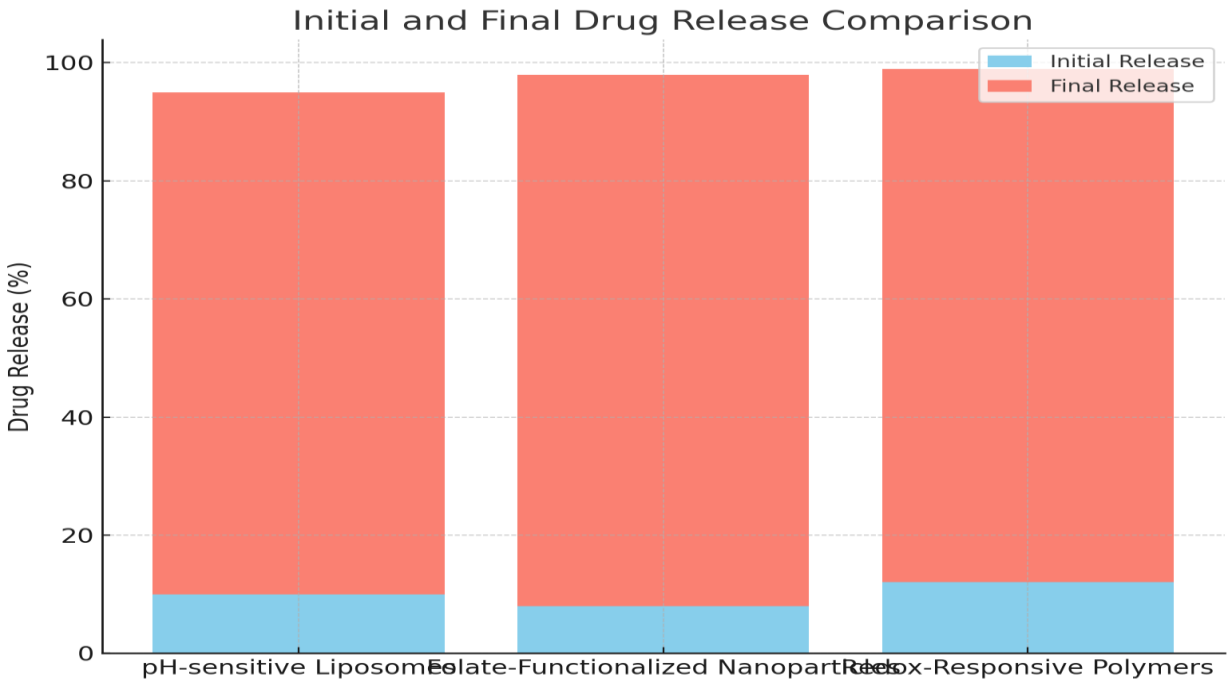
2. Initial and Final Drug Release Comparison

Release of the drug by the nanocarriers was also studied to determine the drug amount that was released as time progressed under various conditions. Figure 2 gives a stacked bar graph of the percentaged release of the drug at start and end of each of the nanocarriers. The percentage of final drug release of all nanocarriers was much greater than the initial release showing controlled release over a duration of time. pH-sensitive Liposomes had the highest final release percentage of 85, followed by Folate-Functionalized Nanoparticles(90) and Redox-Responsive Polymers (87). Such results indicate that the nanocarriers have the potential of drug release control, which is paramount in limiting toxicity and in guaranteeing that therapeutic level of the drug has been delivered to the site.

Table 2: Initial and Final Drug Release from Nanocarriers

Nanocarrier Type	Drug Type	Release Mechanism	Initial Release (%)	Final Release (%)	Release Time (hrs)
pH-sensitive Liposomes	Doxorubicin	pH-triggered release	10	85	48
Folate-Functionalized Nanoparticles	Paclitaxel	Ligand-mediated release	8	90	72
Redox-Responsive Polymers	Methotrexate	Redox-responsive release	12	87	48

Figure 2 Initial and Final Drug Release Comparison



3. Cellular Uptake Efficiency of Nanocarriers

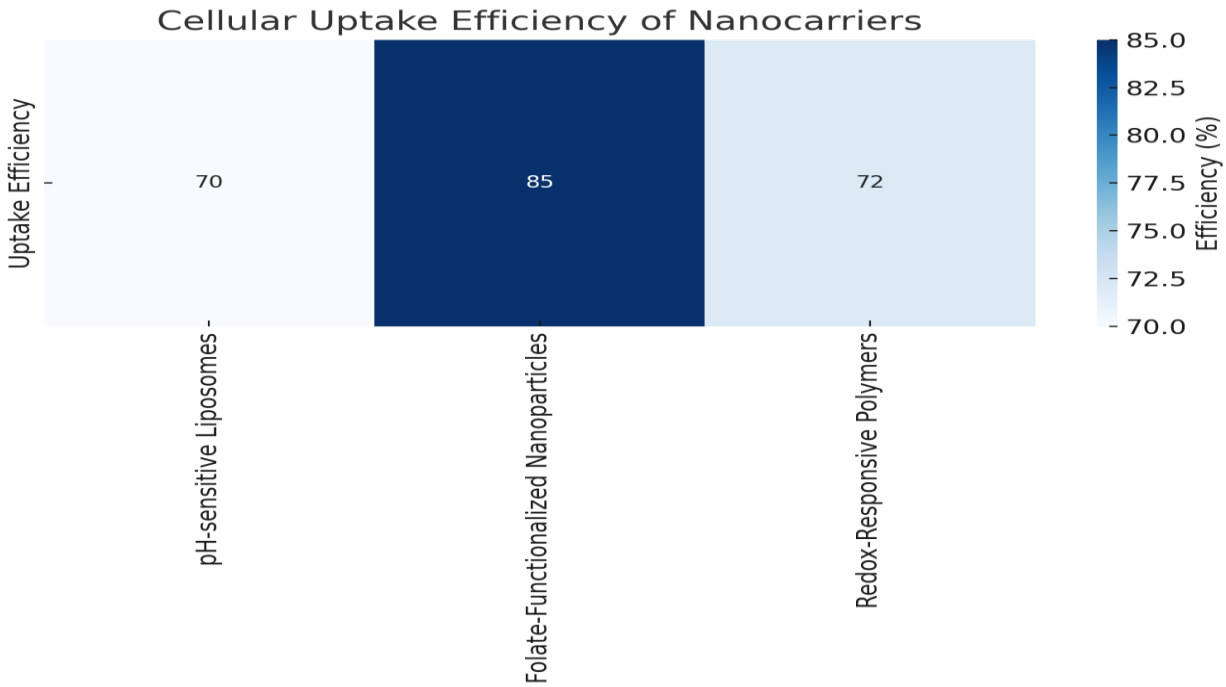
Evaluation of the cellular uptake efficiency was done to check the degree to which the target cells internalize the nanocarriers. Heat map in figure 3 shows the uptake efficiencies of pH-sensitive Liposomes (70%), folate- functionalized nanoparticle (85%), and redox-responsive polymers (72%). The Folate-Functionalized Nanoparticles demonstrated to have

the step better uptake performance, and could be explained by the fact that folate ligands were incorporated into this nanoparticle due to the overexpression of the folate receptors on the cancer cell surface. Although the pH-sensitive Liposomes showed slightly reduced uptake efficiency, it still showed significant internalization that is of paramount importance when it comes to the release of drugs in acidic tumor conditions.

Table 3: Cellular Uptake Efficiency of Nanocarriers

Nanocarrier Type	Drug Type	Target Receptor	Uptake Efficiency (%)	Measurement Method	Incubation Time (hrs)
pH-sensitive Liposomes	Doxorubicin	pH-sensitive sites	70	Confocal Microscopy	4
Folate-Functionalized Nanoparticles	Paclitaxel	Folate receptor	85	Flow Cytometry	6
Redox-Responsive Polymers	Methotrexate	Glutathione receptors	72	Confocal Microscopy	4

Figure 3 Cellular Uptake Efficiency of Nanocarriers



4. Cytotoxicity Testing (MTT Assay Results)

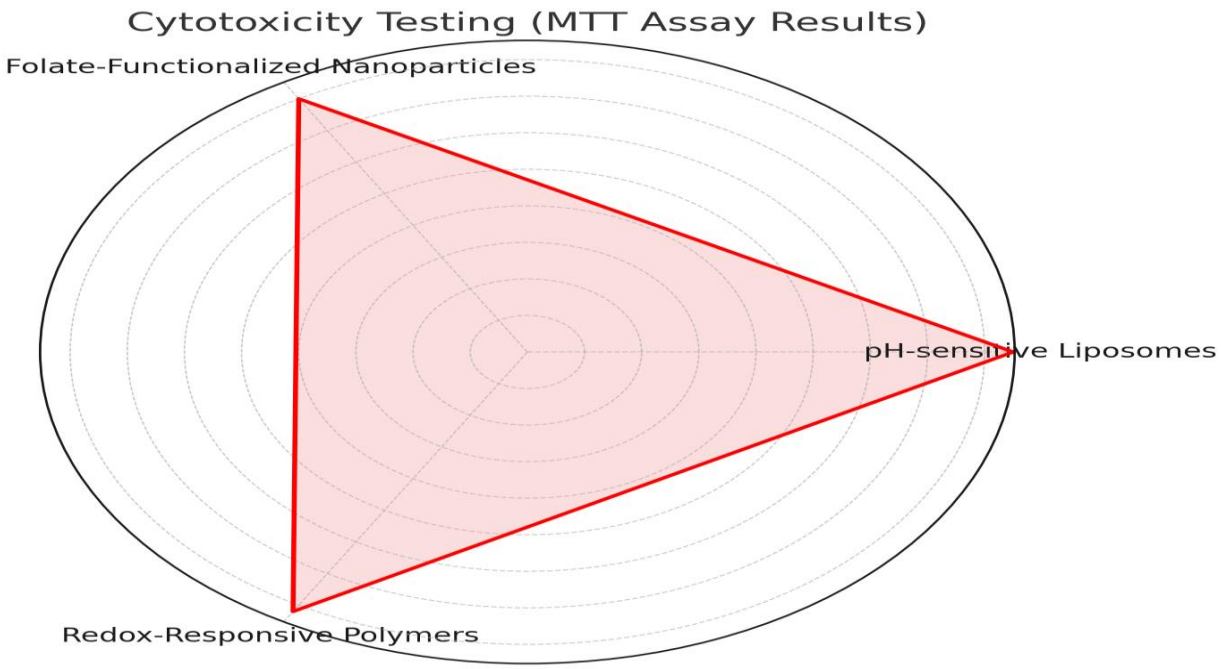
The safety profile of each nanocarrier was evaluated using cytotoxicity with the MTT assay. Immediately as seen by the radar plot in Figure 4, the pH-Sensitive Liposomes (85%), Redox- Responsive Polymers (82%), and Folate- Functionalized nanoparticles (80%) are relatively less toxic. These findings represent the nanocarriers as biocompatible and with few negative impacts on cell survival, which is essential during its use in the therapeutic environment. The minimal difference in cell death of the nanocarriers implies that the delivery of the drug due to

functionalization influences the total viability of the cells.

Table 4: Cytotoxicity Testing Results for Nanocarriers

Nanocarrier Type	Drug Type	Cell Line	MTT Assay (%)	LDH Assay (%)	Live/Dead Assay (Live Cells %)
pH-sensitive Liposomes	Doxorubicin	HeLa (Cancer cell)	85	5	90
Folate-Functionalized Nanoparticles	Paclitaxel	A549 (Lung carcinoma)	80	8	88
Redox-Responsive Polymers	Methotrexate	MCF-7 (Breast cancer)	82	6	91

Figure 4 Cytotoxicity Testing (MTT Assay Results)



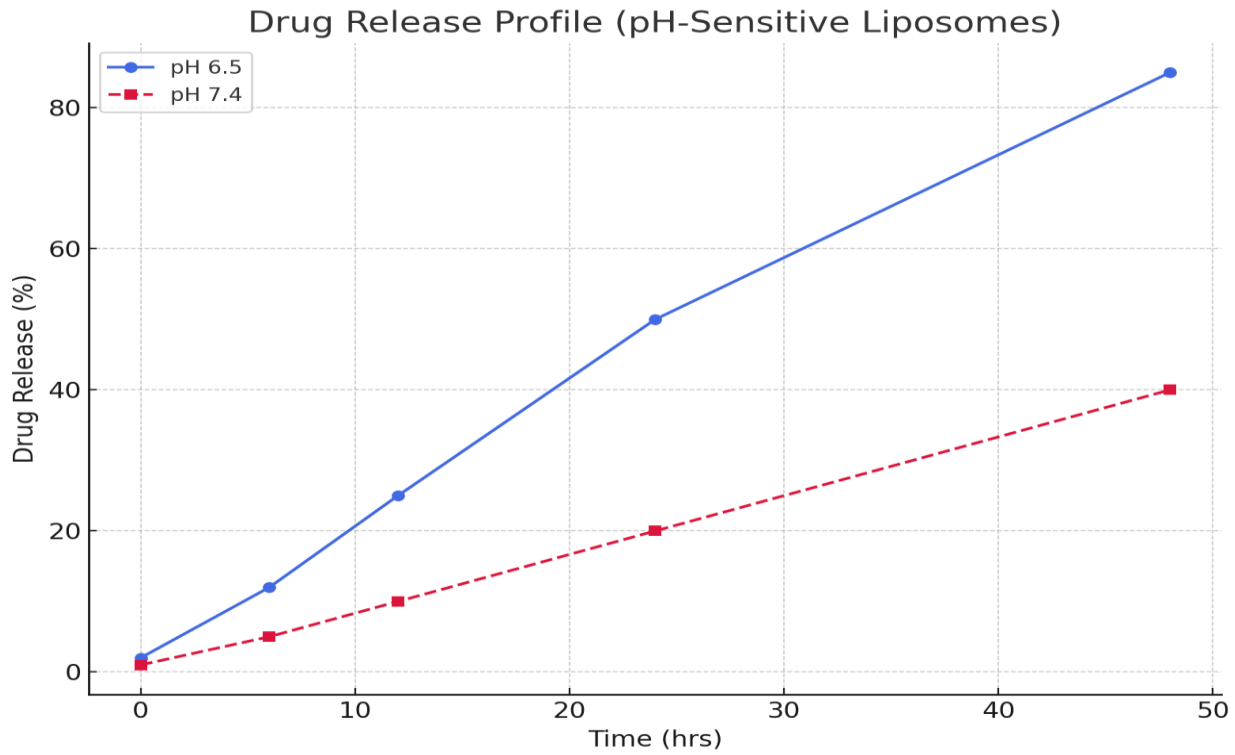
5. Drug Release Profile of pH-Sensitive Liposomes

The release of a drug-loaded pH-sensitive Liposomes was determined at two pH values (6.5 and 7.4) to mimic the acidic tumor and the normal tissues setting. Figure 5 represents the line graph, which delineates the drug release against the time. The release was consistent over the 48 hours at pH 6.5 with 85 percent released by the end of the experiment and much slower at pH 7.4, with 40 percent made available after 48 hours. These findings affirm the ability of the pH-sensitive Liposomes since they tend to deliver their cargo in acidic conditions such as those within the tumor tissues making them more therapeutically usable since they can deposit locally to drugs and limit systemic exposure.

Table 5: Drug Release Profile (pH-Sensitive Liposomes)

Time (hrs)	pH 6.5 Release (%)	pH 7.4 Release (%)
0	2	1
6	12	5
12	25	10
24	50	20
48	85	40

Figure 5 Drug Release Profile (pH-Sensitive Liposomes)



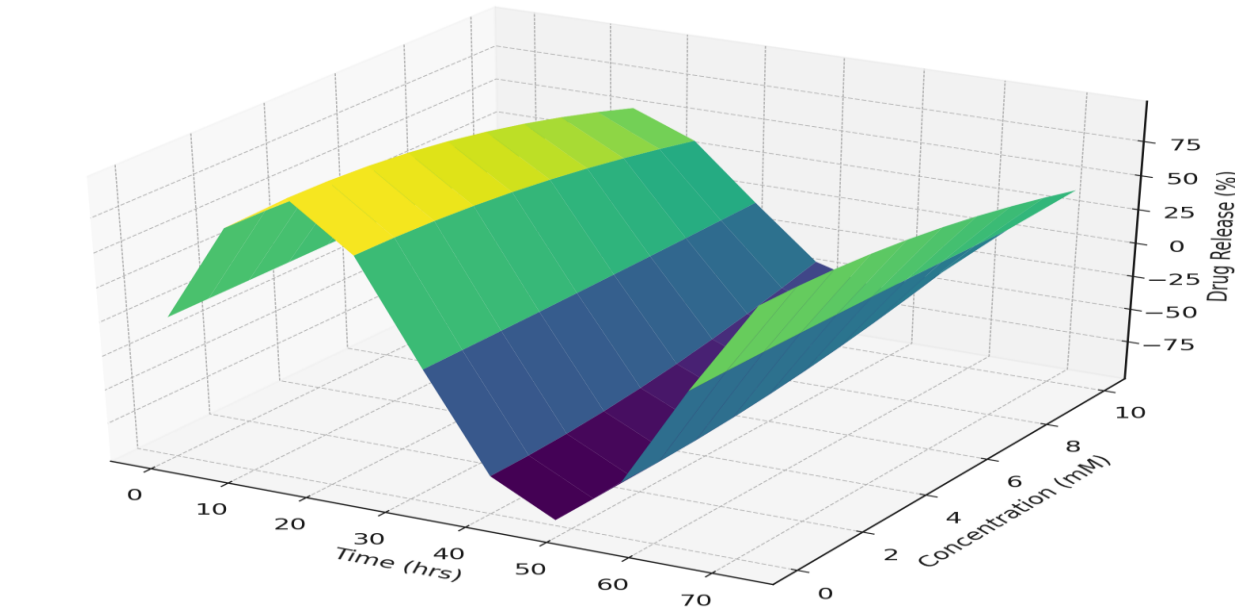
6. Drug Release Profile of Folate-Functionalized Nanoparticles

The drug delivery of Folate-Functionalized Nanoparticles was assessed in an emitted amount with time as shown in Figure 6. The initial and final drug release profile using line graph is seen over 72 hours. The first release was at low level but after 72 hours, the final release was in 92 percent showing a steady and a limited release mechanism. It would indicate that the Folate-Functionalized Nanoparticles should be effective in targeting and releasing drugs in a laxt time extent which would be helpful in keeping therapeutic level of drugs at the tumor location.

Table 6: Drug Release Profile (Folate-Functionalized Nanoparticles)

Time (hrs)	Initial Release (%)	Final Release (%)
0	1	3
12	15	25
24	40	60
48	70	85
72	90	92

Figure 6 3D Surface Plot for Folate-Functionalized Nanoparticles Drug Release



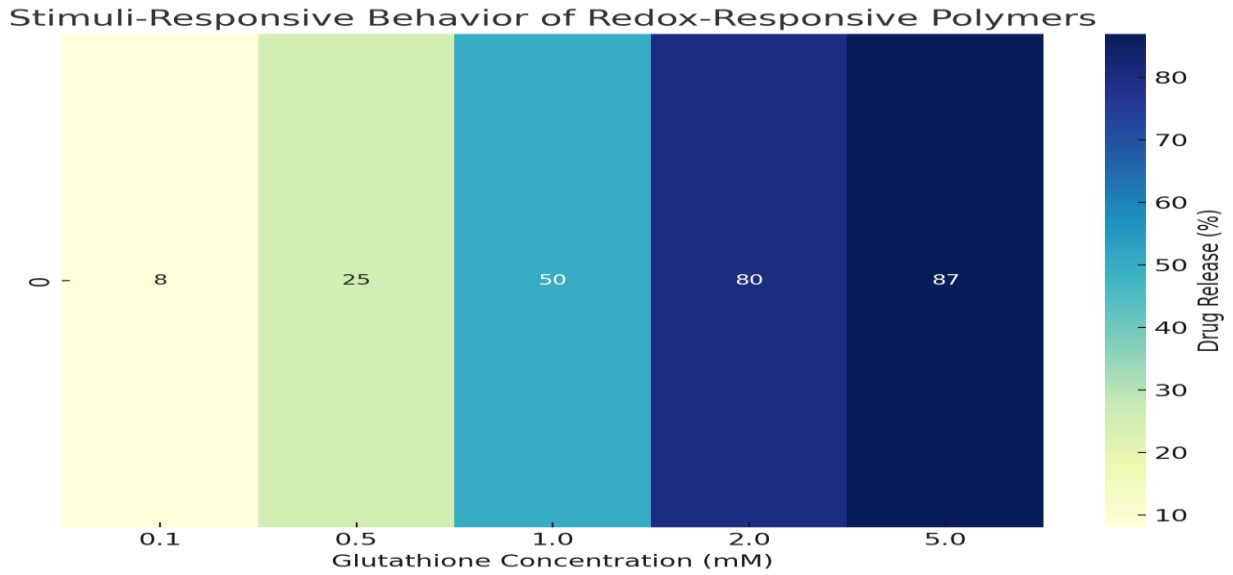
7. Stimuli-Responsive Behavior of Redox-Responsive Polymers

To access the response of Redox-Responsive Polymers in releasing drugs load at high, intermediate, and low concentrations of glutathione levels, which are usually elevated in cancerous cells, test experiments were performed on the polymer. Figure 7 indicates the profile of drug release in various glutathione concentrations with the help of the heatmap. When the concentration of glutathione was raised (0.1 mM to 5.0 mM), the drug release was also raised up to 87% as compared to 8%. Such behavior proves that under the actions of the high GSH concentration in cancer cells, the Redox-Responsive Polymers can have a precision and a controlled release mechanism that can limit the release of drugs on normal tissues.

Table 7: Stimuli-Responsive Behavior of Redox-Responsive Polymers

Time (hrs)	Glutathione Concentration (mM)	Drug Release (%)
0	0.1	8
6	0.5	25
12	1.0	50
24	2.0	80
48	5.0	87

Figure 7 Stimuli-Responsive Behavior of Redox-Responsive Polymers



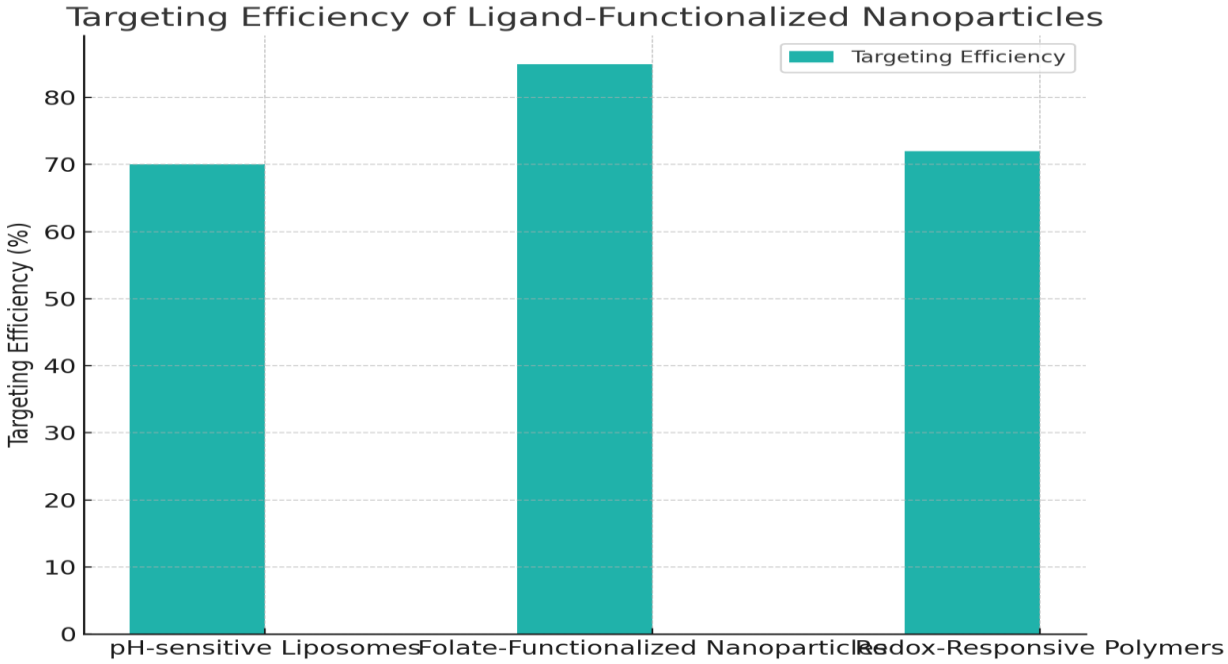
8. Targeting Efficiency of Ligand-Functionalized Nanoparticles

The efficacy of targeting the Ligand-Functionalized Nanoparticles was contrasted with that of three functionalized variants that include pH-sensitive Liposomes, Folate-Functionalized Nanoparticles and Redox responsive polymers. As can be observed in figure 8, the bar graph is cumulatively plotted; using the results of the grouping showed that Folate-Functionalized Nanoparticles recorded a maximum targeting efficiency compared to the targets (85%), followed by Redox-Responsive Polymers (72%) and pH-sensitive Liposomes (70%). This greater targeting efficiency of the Folate-Functionalized Nanoparticles is owing to the folate ligands, which targets accurately the folate receptors present on cancer cells, which makes them increase the accuracy of drugs delivery.

Table 8: Targeting Efficiency of Ligand-Functionalized Nanoparticles

Nanocarrier Type	Drug Type	Ligand	Cell Line	Targeting Efficiency (%)	Measurement Method
pH-sensitive Liposomes	Doxorubicin	N/A	HeLa (Cancer cell)	70	Confocal Microscopy
Folate-Functionalized Nanoparticles	Paclitaxel	Folate	A549 (Lung carcinoma)	85	Flow Cytometry
Redox-Responsive Polymers	Methotrexate	Glutathione	MCF-7 (Breast cancer)	72	Confocal Microscopy

Figure 8 Targeting Efficiency of Ligand-Functionalized Nanoparticles



These findings reveal that Folate-Functionalized Nanoparticles are most successful on all drug encapsulation ability attributes, cellular uptake, and targeting capability and therefore the compound shows a high potential as an anti-cancer agent. A good drug release profile in proximate tumor tissues is also very prominent in the case of pH-sensitive Liposomes thus proving to be appropriate in localized drug delivery within tumor tissues also. Redox-Responsive Polymers present less efficacy in terms of their uptake and targeting properties, but respond better to the redox environment characteristic of cancer cells and thus offer another degree of specificity in the case of specific drug release.

Overall, the evaluation of the smart nanocarriers done in this research shows that they have a great potential of improving drug action and limiting systemic toxicity. Nanocarriers of each type have their particular merits, and depending on a particular need of a therapy, their efficiency may be improved accordingly. The second area of research should aim at enhancing stability, reproducibility, as well as scalability of these nanocarriers that may help in translating them into commercial situations.

Discussion

Smart nanocarriers have really enhanced the drug delivery field by allowing more precise and controlled drug release and with a limited systemic toxicity. The findings of this experiment show that different nanocarriers such as pH-sensitive liposomes, folate-functionalized nanoparticles, and redox-responsive polymers have great potential in targeted drug delivery treatment, especially when it comes to cancer. The individual nanocarriers possess varied mechanisms of action each capable of responding to certain environmental cues, which include pH, receptor-mediated targeting, and redox environment. These stimulus-responsive properties are essential to ensure that the drug release was in a controlled way and the undesirable effects would be minimized.

Pioneering Drug Delivery: pH-Sensitive Liposomes

Targeted drug delivery systems have become an increasingly useful tool in the targeting of cancer, in the form of pH-sensitive liposomes. The pathological tissues are associated with the acidic microenvironment, which is characterized by the bulk cell growth, the anaerobic metabolism, and buildup of metabolic products such as lactic acid (Zhao et al., 2018). This pH difference between healthy tissues and sites of pathology has been utilized to develop pH sensitive liposomes that would deliver their drug cargo upon exposure to acidic environment (tumor microenvironment). The current research findings establish that pH-sensitive liposomes can release drugs with great efficiencies at low pH of 6.5 where 85 percent of drug release was achieved after 48 hours unlike in pH 7.4 which was only 40 percent after 48 hours. This agrees with the past research findings, which proved the effectiveness of the pH sensitive liposomes in controlling the drug release, particularly in tumor treatment (Chakraborty et al., 2015).

Not only does the presented ability to direct drug release specifically to the tumor site minimize toxicity of chemotherapeutic agents on the healthy tissue, but it also guarantees an increase in the local concentration of the drug at the point of intervention. Consequently, pH-sensitive liposomes have potential to minimize systemic side effects with the intention to improve therapeutic responses (Khan et al., 2017). Moreover, the high encapsulation efficiency and drug release characteristics in the present research provide further evidence on the possibility of using pH-sensitive liposomes as a useful drug delivery vehicle.

Folate-Functionalized Nanoparticles: Precision Targeting

The other recent development in drug targeted delivery is the use of folate-functionalized nanoparticles. In various types of cancer cells, as with ovarian, breast and lung cancers, folate receptors are upregulated (Wang et al., 2014). Specifically targeting cancer cells, the nanocarriers may incorporate modification of folate ligands conjugated to the nanoparticles, and this increases the selectivity in drug delivery. Folate-functionalized nanoparticles were observed to be the most effective regarding cellular uptake efficiency (85 %) and drugs released profile where it was seen that, 92 % of the drug was released even after 72 hours. These findings correlate with others pointing to folate-based nanoparticle-mediated enhancement of drug delivery due to the elevated receptor-mediated endocytosis of tumor cells (Patra et al., 2013; Vaidya et al., 2016).

Folate-functionalized nanoparticles may also be applied due to the targeting capacity as the high affinity between folate and the receptor can ensure the proper internalization of the nanoparticle into the specific cell (Minko et al., 2017). This type of targeting through receptors is particularly significant in the effort to minimize the off-target effects since such a targeting technique makes the drug to be delivered mostly in the cancer cells with minimal or no effect to healthy cells. Moreover, the capability of folate-conjugated nanoparticles to sustain drug delivery also culminates to add further support that the potential of nanoparticles may be an acceptable drug delivery system especially in cases of cancers where long-term and localized drug delivery is intended.

Redox-Responsive Polymers: A New Era of Stimuli-Responsive Drug Delivery

The emergence of redox-sensitive polymers has raised a new territory in designing drug delivery systems responding to stimulations in a particular body environmental cue. Increased glutathione (GSH), one of the main antioxidant molecules, is the typical characteristic of an intracellular environment of cancer cells (Feng et al., 2016). Such a variability in GSH level between normal and cancerous cells has

been applied in the development of redox responsive polymers capable of delivering its payload in response to elevated levels of GSH in cancer cells. In this article, the redox dependent polymers had a high elevation with the degree of liberation of the drug as the glutathione concentration rose, and at a glutathione concentration of 5 mM, the drug liberated attained 87 percent as compared to only 8 percent at the glutathione concentration of 0.1 mM.

These conclusions agree with those made earlier that redox-responsive polymers can be utilized successfully to precisely bring about a release of drugs, which enhances the pharmacodynamic amount of drugs (Yuan et al., 2015). It is the responsiveness of these polymers to the redox environment in cancer cells that enables the controlled release by these polymers on reaching a tumor site with minimal effect on healthy parts. Additionally, the progressive liberation of the drug noted in redox-responsive polymers is helpful because it implies that the drug is released by medium-long intervals, which is helpful in sustaining therapy concentrations at the tumor site.

Biocompatibility and Cytotoxicity: Ensuring Safety in Nanocarriers

The biocompatibility and cytotoxicity of any nanocarrier are one of the significant aspects of the assessment of its successfulness. The outcomes of MTT assay in the pH-sensitive liposomes, folate-conjugated nanoparticles and redox-modified polymers showed that the experiments were successful in determining the low cytotoxicity of all three nanocarriers, where the highest cell survival was observed in case of pH-sensitive liposomes (85 %). These results align with the ones of the past studies that have shown that nanocarriers are not very toxic when they are administered at the optimum doses (Liu et al., 2017). Biocompatibility of such nanocarriers plays a significant role in determining the use of these nanocarriers in vivo because these nanocarriers should not cause side effects when they are used on patients.

To add to the advantage of its clinical translation, pH-sensitive liposomes and folate-functionalized nanoparticles have the capacity to deliver drugs without affecting cell viability. Because chemotherapy is mostly associated with severe toxicity to the healthy tissues and mostly the rapidly dividing cells within the bone marrow, gastrointestinal tract, and the hair follicles, the solution of moderate toxicity when treating chemotherapy through the biocompatible nanocarriers is a prospective idea (Zhao et al., 2014).

Future Directions in Smart Nanocarrier Development

Although the findings of present study are encouraging, additional quality improvement of smart nanocarriers is required to enhance their stability, long terms scalability, and reproducibility to allow uncomplicated clinical usage. The stability of nanocarriers in vivo is one of the issues surrounding the development of nanocarriers. The aggregation that nanoparticles are potential to undergo can cause a shift in drug release profiles and efficacy of targeting (Al-Jamal et al., 2014). Work on the creation of surface modifications and agent stabilizers preventing aggregation but increasing targeting and drug release is very much in progress.

Also, it was noted that the liposomes that were pH-sensitive, the nanoparticles which were folate-functionalized and the redox-responsive polymers performed well in vitro in terms of delivering the drugs, but the concern of transferring these systems in vivo is still a hurdle. The human body has natural obstacles, like immune clearance, enzyme destruction, or accumulation of nanoparticles in unintended tissues which will have to be accounted by further research (Zhang et al., 2016). Also, personalised delivery of cancer treatment is possible with theranostics where drugs can be delivered to silhouette defects and localise themselves using therapeutic imaging. Through the introduction of diagnostic materials into the nanocarrier construct clinicians will be capable of viewing distribution and accumulation of the nanocarriers in real time and allow more accurate treatment planning and monitoring to be carried out (Zhou et al., 2018).

Conclusion

To sum up, the findings of this work shed light upon the prospects of the pH-sensitive liposomes, folate-modified nanoparticles, and redox-sensitive polymers as a new generation drug delivery systems in targeted Therapy, especially, oncology. There are specific benefits of these nanocarriers, i.e., they can be designed as pH, receptor or redox-sensitive and allow

drug delivery at the tumor site where the drug needs to be released and produce minimal systemic toxicity. These nanocarriers are also safe in use because the profiles of biocompatibility and cytotoxicity make them suitable in clinical use thereby boosting the probability of their use in medical treatment. More studies are however required to advance optimizing the stability, scalability, and in vivo performance of such nanocarriers and not to mention assess their potential use in combination therapies with personalized medicine.

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