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Innovations In Nanocarrier Technology For Targeted Therapeutics: A Comprehensive Review

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Abstract
Recent advancements in medication delivery systems have brought nanocarriers to the forefront as a groundbreaking technology. Nanocarriers, ranging from 1 to 100 nanometers in size, have revolutionized the targeted delivery of therapeutic agents, such as drugs, genes, and proteins, to specific cells or tissues. This precision addresses one of modern medicine's most significant challenges: optimizing therapeutic efficacy while minimizing adverse effects. Traditional drug delivery methods often struggle with issues like low solubility, rapid degradation, and non-specific distribution, leading to suboptimal treatment outcomes and undesirable side effects. In contrast, nanocarriers offer unparalleled advantages due to their small size and customizable surface properties, enabling them to navigate complex biological environments and selectively interact with target cells. This targeted approach is particularly beneficial in cancer treatment, where precise delivery to tumor cells can significantly enhance treatment effectiveness and reduce harm to healthy tissues. Additionally, nanocarriers can be engineered to release their therapeutic payloads in response to specific biological triggers, further improving their therapeutic potential. As science and technology continue to evolve, the role of nanocarriers in targeted drug delivery is increasingly vital. Their ability to enhance drug solubility, regulate release patterns, and improve targeting precision marks a significant advancement in the pursuit of safer and more effective treatments. Nanocarriers, including nanoparticles, liposomes, carbon nanotubes, niosomes, dendrimers, and polymeric nanoparticles, offer enhanced bioavailability, stability, and organ-specific targeting, making them a superior alternative to conventional therapies, particularly for poorly soluble drugs.

Keywords:- Nanocarriers, Transdermals, Targeting, Stability, Treatment.

INTRODUCTION:

In recent years, the science of medication delivery has made amazing advances, with nanocarriers emerging as a crucial breakthrough. A nanocarrier is a tiny vehicle that transports therapeutic agents—such as medications, genes, or proteins—to specific cells or tissues [1]. These small carriers, which generally range in size from 1 to 100 nanometers, provide unmatched accuracy in drug administration, solving one of medicine's most pressing challenges: successfully targeting therapy while reducing adverse effects [2].The value of nanocarriers in modern medicine cannot be emphasized. Traditional drug delivery methods frequently encounter difficulties such as low solubility, fast degradation, and non-specific distribution, which can result in inferior treatment results and undesired side effects [3]. Nanocarriers, with their distinct features and adaptable architecture, provide a solution to these challenges. Their tiny size helps them to negotiate the body's diverse biological surroundings, and their surface may be tailored to connect with specific cells or tissues.

This precise targeting is especially important for treating illnesses such as cancer, where delivering medications to tumor cells while preserving healthy tissues can significantly improve treatment efficacy and decrease side effects. Nanocarriers can be programmed to release their payload in response to certain biological signals or environmental circumstances, therefore increasing their therapeutic efficacy. [4]

The significance of nanocarriers in targeted medication delivery is becoming more and more important as science and technology develop. Their primary significance in modern medicine is highlighted by their capacity to increase drug solubility, regulate release patterns, and improve targeting precision—all of which represent a major advancement in the search for safer and more effective therapies. [5]

The major desirability of any pharmaceutical fabrications is that they offer better drug bioavailability, enhanced stability and improved organ targeting. In this context, nanocarriers stands out because it is of submicron size and offers tremendous versatility, when it comes to medicament delivery system. Nanocarriers includes nanoparticles, liposomes, carbon nanotubes, niosomes, dendrimers, nanocomplexes, nanotubes, and polymeric nanoparticles [6]. The basic idea behind drug binding to nanocarrier is that, either the drug binds to ligand, or it eventually binds to nanocarrier which facilitates the uptake by specific organ specific drug delivery.

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Nanocarriers have an edge over conventional therapies due to its enhanced targeting and specific binding. Moreover it is a popular choice of delivery when the medicament is poorly soluble [7].

Reasons to prefer nanocarriers over conventional drug delivery systems

Better Targeting Accuracy: By focusing on certain cells or tissues, nanocarriers may be tailored to reduce the risk of harming healthy cells and increase the effectiveness of therapy.

Improved Bioavailability and Solubility: Nanocarriers boost the solubility of medications that dissolve slowly, which promotes improved absorption and higher efficacy at lower dosages [8].

Controlled and Extended Release: They can be designed to release their medicinal ingredients gradually, resulting in a long-lasting impact that requires fewer doses to achieve.

Reduced adverse Effects: Nanocarriers assist to minimize potential adverse effects by more accurately targeting medications and preventing needless exposure to healthy tissues.

Facilitated Cellular Entry: Drugs can be absorbed by cells more effectively, even those that are hard to reach with conventional techniques, thanks to the tiny size and unique surface characteristics of nanocarriers [9].

Adaptable and Customizable: Nanocarriers may be made to carry a range of medicinal substances, and their efficacy can be increased by adding targeting characteristics.

Protecting Sensitive medications: They prevent environmental variables from causing sensitive medications to deteriorate, extending their stability and guaranteeing their efficacy [10].

Optimized Pharmacokinetics: By changing the drug's absorption and distribution inside the body, nanocarriers can lower the frequency of delivery while increasing therapeutic potential.

Ability to deliver multiple Drugs at Once: Certain nanocarriers have the ability to transport and administer many therapeutic chemicals at once, allowing combination treatments for more potent therapy [11].

Categories of Nano carriers

Polymeric Micelles

Micelles are nano-sized colloidal particles with spherical shapes, characterized by a polar outer surface and a non-polar interior. These nanoparticles can carry bioactive agents either within their hydrophobic core or covalently bound to their surface. Constructed from amphiphilic block copolymers, micelles form a nano-sized core/shell structure in hydrophilic-buffered media.[12,13] The hydrophobic cavity of the micelles serves as a reservoir for lipophilic drug molecules, while the hydrophilic outer shell ensures stability in aqueous environments, making micelles suitable for intravenous administration. [14, 15]

Drug enrichment within micelles can be achieved through two methods: chemical covalent attachment or physical encapsulation. A key advantage of micelles is their ability to be engineered to quickly carry fat-soluble medications.[16,17] Micelles form spontaneously at concentrations above their critical micelle concentration (CMC) due to the self-aggregation of amphiphiles in aqueous conditions, thereby encapsulating fat-soluble bioactive compounds within their hydrophobic core. However, if the concentration falls below the CMC, micelles disassemble, releasing the encapsulated drug. [18, 19]

The properties of micelles can be influenced by their surrounding environment. For example, blood contains specific compounds that can alter the chemical gradient between the monomeric fraction in micelles and the surrounding aqueous phase, potentially increasing the CMC. [20, 21] As a result, micelles that are stable in saline solutions may lose stability in the bloodstream, causing premature drug release. A notable development in micelle technology is the creation of multifunctional polymeric micelles, which are equipped with targeting ligands, imaging, and therapeutic agents. These advanced micelles are expected to become standard in drug delivery systems. Recently, a research group developed a novel drug delivery system using ursolic acid-loaded polymeric micelles. [22, 23]

Carbon Nanotubes

Carbon nanotubes (CNTs) are carbon allotropes with a hollow, cylindrical structure. They are classified based on their layers: single-walled nanotubes (SWNTs) with a diameter of approximately 1 nm, and multi-walled nanotubes (MWNTs) with outer diameters ranging from 5 to 20 nm and inner diameters between 2 to 6 nm. [24, 25]Raw CNTs are inherently hydrophobic, which necessitates functionalization to make them water-soluble and biocompatible for biomedical

applications. Functionalization techniques, such as PEGylation, can significantly enhance the drug-loading capacity of CNTs. [26]

CNTs have found significant applications in biological fields, particularly as sensors for diagnostic purposes, detecting DNA and proteins, discriminating between different types of proteins in serum samples, and delivering vaccines and proteins. Despite their potential, the insolubility of CNTs in most solvents poses health risks.[27] However, chemical modifications and the attachment of functional groups can render them water-soluble, enabling their use in the delivery of various therapeutics, including proteins, peptides, nucleic acids, and other active compounds.[28,29]

Notable advancements in CNT-based delivery systems include the incorporation of antifungal agents like amphotericin B and the combination of antitumor agents such as methotrexate with fluorescent markers. Research has shown that drugs attached to CNTs exhibit more effective internalization compared to free drug molecules. Aromatic drugs like Doxorubicin can bind to the CNT surface through supramolecular π–π stacking. Functionalized CNTs can serve as carriers for a variety of therapeutics, from small molecules to peptides and nucleic acids, delivering active agents to different organs based on their functionalization, which may also respond to specific stimuli. [30]

Beyond their prominent role in cancer therapy, CNTs are being explored for their potential in treating other diseases. For instance, Leeper's group recently utilized PEG-functionalized SWNTs loaded with a fluorescent probe and a small-molecule inhibitor targeting the anti-phagocytic CD47-SIRPα signaling axis to prevent atherosclerosis. Additionally, due to their ability to easily penetrate cells, CNTs show promise in crossing the blood-brain barrier (BBB) to treat neurological diseases. [31, 32]

Carbon Dots

In recent years, carbon dots (Cdots) have emerged as a significant area of research in biomedical applications, providing a safer alternative to quantum dots, which are known for their toxic effects. Cdots were first synthesized and reported in 2004.[33] They are typically produced through the pyrolysis of organic compounds, often using a microwave or autoclave, which results in the formation of nanoscale pure carbon sheets with surface functional groups that depend on the initial organic compounds used.[34]

Cdots have become a promising substitute for quantum dots due to their nontoxic nature, potential for generation from natural sources, and high colloidal stability in water. For example, Cdots have been synthesized from synthetic sources like glycerol and polyethyleneimine (PEI). Additionally, they can be fabricated from natural sources, such as agave nectar, by heating it at 300°C for one hour. The resulting Cdots are then functionalized with polymers terminated with amine, carboxyl, or hydroxyl groups, dendrimers, or PEG tails. This versatility in functionalizing carbon dot surfaces opens the door to a wide range of theranostic applications. [35,36]

In another study, a one-step green synthesis of fluorescent Cdots was achieved by pyrolyzing freshly extracted bovine serum albumin (BSA), which contains hydroxyl and carboxylic functional groups, as confirmed by FTIR analysis.[37] These Cdots exhibited high colloidal stability with an average diameter of approximately 2 nm, and their stability was maintained even in varying pH and salt solutions. The Cdots achieved a relative fluorescence of around 38%, with maximum excitation at 405 nm. This unique synthesis method not only provides fluorescence but also ensures water solubility, making it highly suitable for rapid theranostic applications. [38]

A distinctive type of zwitterionic Cdots was synthesized through the microwave pyrolysis of citric acid, which provided carboxylate groups, and β-alanine, which provided amine groups. Drugs can be conjugated to these Cdots through non-covalent bonding via the carboxyl groups or through electrostatic interactions with other functional groups. [39, 40, 41] Cdots enter cells through endocytosis and passive diffusion, where the conjugated drugs are then passively released inside the cells. With a size of less than 10 nm, Cdots show promise in overcoming the challenge of delivering drugs across the blood-brain barrier (BBB) for treating neurological diseases. [42]

In one notable study, Leblanc's group developed carbon dots conjugated with targeting ligands and therapeutic drugs to treat glioblastoma brain tumors. They later created carbon nitride dots for potential use in treating pediatric glioblastoma, highlighting the evolving applications of Cdots in addressing challenging medical conditions. [43]

Polymeric nanoparticles

Polymeric nanoparticles (NPs) are versatile particles ranging in size from 1 to 1000 nm and can be formulated in various dosage forms. Based on their structural organization or preparation method, polymeric NPs are classified as either nanocapsules or nanospheres. These NPs can serve as carriers for a wide range of medications tailored to specific conditions or treatment types. The drug may be dispersed in a liquid core of oil or water, encapsulated by a solid polymeric membrane, or embedded within the polymer matrix. [44, 45]

Among the various types of NPs, polymeric NPs are known for their enhanced stability and improved encapsulation efficiency, which can be controlled through the manufacturing techniques and the characteristics of the materials used in the formulation. [46] Polymeric NPs can be prepared using either natural or synthetic polymers, and their structure can vary depending on the components of the formulation, leading to the development of nanocapsules. Nanocapsules feature a core surrounded by a polymeric wall, where the drug can be either enclosed within the particle's walls or adsorbed onto the surface. These components can be functionalized to facilitate targeted delivery. [47]

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Several polymers are utilized in the preparation of polymeric NPs, with biocompatibility, stability, appropriate biodegradation kinetics, easy processing, and retention of their properties for a specific duration in vivo being essential criteria. [48]Polymeric NPs can encapsulate a broad spectrum of drugs, releasing them based on the solubility of the polymeric matrix. One mechanism of drug release involves the swelling of the polymer due to hydration, followed by drug diffusion. Another mechanism occurs through chemical and enzymatic reactions that degrade the polymer at the release site, freeing the drug from the NP's core.[49]
Polymeric nanocarriers offer potential in various treatments by enhancing the diffusion of bioactive compounds, providing excellent stability, and increasing absorption at the target site. They can deliver higher therapeutic efficiency and reduce the adverse effects associated with conventional therapies. [50] Additionally, polymer nanoparticles can cross the blood-brain barrier (BBB) through endocytosis and evade phagocytosis by the reticuloendothelial system, thereby increasing drug concentrations in the brain. In vitro studies have demonstrated that polymer nanoparticles enhance drug delivery to the brain. For instance, the improved delivery of curcumin in the treatment of Alzheimer's disease has been shown to be more effective in reducing oxidative stress, inflammation, and plaque load. [51, 52]

Magnetic Nanoparticles
Magnetic nanoparticles (MNPs) are nanostructures ranging in size from 1 to 100 nm. Typically, MNPs consist of a central magnetic core surrounded by a surface coating that may include a functional layer. [53] These particles are often composed of pure metals such as iron, cobalt, nickel, and manganese. Among these, iron oxide nanoparticles are the most commonly used in biomedical applications. Depending on their magnetic properties, nanoparticles can be classified as paramagnetic, ferromagnetic, diamagnetic, ferrimagnetic, antiferromagnetic, or superparamagnetic materials. [54, 55]
Diamagnetic materials are repelled by an external magnetic field, resulting in zero magnetic moment in the absence of the field. Paramagnetic materials exhibit weak magnetic moments in the presence of a magnetic field but do not retain magnetic properties when the field is removed. [56, 57] Ferromagnetic materials, on the other hand, remain magnetized even without an external magnetic field due to the presence of unpaired electrons. Ferrimagnetic materials have a net spontaneous magnetic moment because they contain two different ions with unequal opposing moments. Superparamagnetic materials behave like paramagnets in the presence of a magnetic field. [58, 59]
Various features of magnetic nanoparticles, such as their coating, size, and core material, influence their biocompatibility and toxicity. [60] MNPs are widely used in biomedical and biotechnological applications. For instance, Lubbe and colleagues successfully loaded epirubicin onto magnetic nanoparticles and found them to be benign to normal tissues at concentrations below 50 mg/m² of the drug. Since then, extensive research has been conducted on MNPs as drug delivery systems (DDS). Although numerous magnetic nanomaterials are under investigation, iron oxide nanoparticles are the most widely used due to their superparamagnetic nature, biocompatibility, and relatively low toxicity? [61, 62, 63]
MNPs are effective as drug delivery devices because they reduce the in vivo distribution of cytotoxic materials and increase cellular uptake at targeted sites.[64] However, the hydrophobic surface of MNPs, combined with their high surface-to-volume ratio, can lead to the formation of clusters, which increases particle size and may cause embolization within the bloodstream, restricting blood flow. The clustering of particles also affects the superparamagnetic properties of MNPs. Additionally, these nanoparticles can cause cytotoxicity when they accumulate in the liver. [65, 66]
To address these challenges, surface modification of MNPs is necessary to prevent aggregation and reopen the potential for theranostic delivery across the blood-brain barrier and to various intracellular targets. As a result, MNPs are often functionalized with a variety of inorganic and organic substances, including polymers. These surface modifications help establish strong interactions between biomolecules and nanoparticles, enhancing the efficacy and safety of MNPs in biomedical applications. [67, 68]

Liposomes
Liposomes are phospholipid vesicles composed of lipid bilayers that enclose distinct aqueous spaces. Several attributes make liposomes excellent candidates for drug delivery systems. [69] These self-assembled nanocarriers are biocompatible, easily modifiable, and capable of carrying large drug payloads. Liposomes can encapsulate both lipophilic and hydrophilic compounds—drugs and imaging agents—within their lipid membrane and aqueous core, respectively. [70, 71]They are generally considered to have a good safety profile.
Conventional liposomal nanocarriers consist of simple self-assembled lipid bilayers that encapsulate therapeutics within their aqueous cores. [72] The lipid bilayers can be further stabilized by adding polyethylene glycol (PEG) to the surface, a process known as PEGylation. Additionally, liposomes can be functionalized by modifying their surface with specific ligands. Phospholipids are preferred for their bivalent structure, which allows the bilayer to easily adjust its fluidity and influence the release rate of the encapsulated drug.[73] Cholesterol is another key component in liposome formulations, enhancing their stability by interacting with the carbon chains of the phospholipids, thereby altering the release profile of the encapsulated bioactive agent.[74]
Liposomes are characterized by their distinctive bilayer structure, which offers increased biocompatibility and makes them valuable drug delivery vehicles. These vesicles can become reactive when exposed to extrinsic or intrinsic stimuli, exhibiting changes in their arrangement in response to factors such as temperature, pH, or electrostatic charge. [75, 76]

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For example, pH-sensitive liposomes remain stable in healthy tissues but destabilize in tissues with a pH below 7.4, leading to the release of the encapsulated bioactive substance. This property is particularly useful for mediating intracellular drug release in tumor tissues. [77] Researchers have also developed functionalized liposomes capable of targeting specific molecules on the surface of various cells, facilitating the internalization of these nanocarriers into the targeted cells. Liposomal carriers have been widely used in drug delivery, significantly improving therapeutic efficacy by stabilizing the payload and aiding targeted tissue uptake.[78,79] The FDA's approval of Doxil, a liposomal drug carrying the anti-cancer agent doxorubicin hydrochloride, marked a milestone in the clinical translation of nanocarriers. Liposomes continue to be the leading nanocarrier among all nanocarrier-assisted drug submissions to the FDA, highlighting their safety and effectiveness [80, 81].

In addition to drug delivery, ongoing research is exploring the potential of liposomal nanocarriers for nucleic acid delivery. However, one major challenge in targeted therapy using these carriers is that systemic administration often results in predominant hepatic uptake [82, 83].

There are two primary methods for integrating medications into liposomes: passive and active drug loading techniques. In passive encapsulation, bioactive molecules are entrapped within the nanoparticles during their assembly. [84, 85] In active loading, therapeutic agents are packed into already-formed liposomes. The efficiency of drug entrapment via passive methods largely depends on the liposome's ability to capture a specific volume of drug-containing solutions or solutes during vesicle formation. For water-soluble drugs, the encapsulation efficiency is directly related to the volume of aqueous solution entrapped by the nanoparticles, which is influenced by the liposome's morphology, phospholipid concentration, and the number of lamellae. For lipophilic drugs, direct interaction with the phospholipid bilayer occurs, with encapsulation efficiency depending on the type and concentration of phospholipids used. [86, 87]

Dendrimers

Dendrimers are nanoscale, three-dimensional, branched polymeric structures. Drugs can be either physically entrapped within dendrimers through non-covalent interactions or covalently attached to them. The entrapment and release of drugs can be regulated by modifying the surface of the dendrimers. [88, 89] Surface functionalization, such as attaching targeting ligands, can also enhance the targeting efficiency of dendrimers. These structures hold significant potential for biomedical applications due to their ability to encapsulate high molecular weight hydrophilic or hydrophobic substances and their high surface-to-volume ratio, making them suitable carriers for gene therapy. [90, 91]

Dendrimers also improve the solubility, stability, and oral bioavailability of many drugs. However, their translational applications have been somewhat limited due to the tendency of cationic dendrimers to interact with cell membranes, potentially causing cell lysis. [92, 93] Despite this, dendrimer-based drug delivery systems possess several unique properties, such as modifiable surfaces, monodisperse size, hydrophilic internal cavities, and multivalency. For example, polyamidoamine dendrimers conjugated with cisplatin are well-known for their use as scaffold systems. [94]

The customizable external characteristics of dendrimers allow them to be conjugated with multiple molecules simultaneously, making them multifunctional drug delivery systems. With their high density of functional surface molecules, dendrimers can be easily conjugated with various targeting agents to specifically deliver chemotherapeutics to tumor tissues. Additionally, the voids present in their core allow for the encapsulation of lipophilic macromolecules, enabling the transport of highly hydrophobic drugs. [95, 96]

Characterization of nanocarriers

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) provides precise information on the surface structure of nanocarriers through direct visualization. Typically, the samples are dried, mounted on a sample holder, and coated with a metal that has high electrical conductivity, such as gold, using a sputter coating process. The sample surface is then scanned with a focused electron beam, and the secondary electrons emitted from the surface are recorded [97]. Ideally, nanocarriers analyzed by SEM should be able to withstand the coating material, electron beam, and vacuum without undergoing significant changes. Modified SEM techniques, where sample drying is not required, have also been developed. For instance, wet SEM allows the examination of hydrated samples without the need for fixation, coating, or drying [98, 99].

The emission of electrons from the sample can involve both elastic and inelastic scattering events. Electron backscattering occurs when high-energy electrons are ejected after an elastic collision between incident electrons and the nuclei of the sample atoms. In contrast, secondary electrons are lower-energy electrons emitted as a result of inelastic scattering. These are produced when substantial energy is lost due to the ejection of loosely bound electrons from the sample atoms during collisions with the nuclei. It is crucial to consider the type of electron emitted when adjusting SEM measurements to optimize the analysis [100,101].

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is one of the most powerful techniques for characterizing nanomaterials at spatial scales ranging from the atomic level (less than 1 nm to 100 nm) to the micrometer level, enabling innovative applications. TEM utilizes more intense electron beams than SEM, offering higher resolution and greater detail, such as

insights into a particle's crystalline structure and granularity [102]. An image is generated by electrons transmitted through the sample and focused by an objective lens, which is then detected by a camera and displayed on a screen. The nanocarrier sample is typically mounted onto support films or grids and fixed using a negative stain material, such as uranyl acetate or phosphotungstic acid, or through plastic embedding. Alternatively, the sample can be exposed to liquid nitrogen after being embedded in vitreous ice, a technique known as cryo-TEM [103]. Bio-polymeric samples often require staining with heavy metals to achieve sufficient contrast for identification. The internal structure of particles can also be examined after fixation, drying, and sectioning of the sample. [104]

Atomic Force Microscopy

AFM (Atomic Force Microscopy) force spectroscopy enables the examination of the nanomechanical properties of individual molecules and particles under conditions closer to those found in physiological environments. This technique achieves extremely high resolution in particle size measurement by physically scanning particles at the sub-micron level using a probe tip at the atomic scale. The instrument generates a topographic map of the sample based on the interaction force between a sharp probe and the sample surface. AFM is particularly advantageous for imaging non-conducting samples without any special preparation, making it ideal for observing delicate biological and polymeric nanocarriers [105].

Most importantly, AFM provides the most accurate representation of size and size distribution without requiring any algorithmic corrections. However, accurate data collection and result interpretation require substantial expertise, especially when dealing with complex samples like biological cells. Challenges include ensuring the quality of the probe tip and support surface chemistries, as well as considering the potential for these factors to alter the shape and size of the measured vesicles during data collection. [106]

AFM is highly suitable for characterizing pharmaceutical nanocarriers, offering the ability to visualize them in 3D while providing both qualitative and quantitative information on physical properties such as size, surface texture, morphology, and roughness.[107 Additionally, it can characterize a broad range of particle sizes, from 1 nm to 8 µm, within the same scan. Moreover, AFM can analyze nanomaterials in various environments, including ambient air, controlled conditions, and even liquid dispersions. This versatility in analyzing samples in both liquid and gas mediums makes AFM especially beneficial for nanoparticle characterization. [108]

Dynamic Light Scattering Spectroscopy

Dynamic Light Scattering (DLS) is the most commonly used method for determining particle size in suspensions, often referred to as photon correlation spectroscopy (PCS). When the particle size is less than one-tenth of the wavelength of incident light (λ/10), the scattered light has similar energy to the incident light (elastic scattering) and is independent of the scattering angle (Rayleigh scattering) [109]. However, when the particle diameter exceeds this limit, Rayleigh scattering transitions to Mie scattering, resulting in scattered light with different energy (inelastic scattering) that is angle-dependent [110].

DLS is typically employed to analyze the size of nanocarriers within the range of 1–500 nm, though some instruments claim a working range of 0.3 nm–10 µm. Despite its widespread use, DLS faces challenges in analyzing polydisperse nanoparticle suspensions. This is because the signal strength is heavily influenced by the presence of larger particles, as it is proportional to the diameter of spherical particles raised to the power of six. Consequently, DLS is best suited for determining the size of unimodal nanoparticles. The DLS instrument detects the scattered laser light with a photon detector, and the intensity of the scattered light is proportional to the size of the nanoparticles being measured. [111,112]

For accurate DLS measurements, samples must be in a liquid state, solution, or dilute suspension with a known viscosity. The technique is sensitive to impurities and can measure particles ranging from 1 nm to 10 µm in diameter. The resulting data on particle size, size distribution, and polydispersity index (PDI) is useful for statistical analysis. However, DLS has limitations, such as producing unreliable results for polydisperse and multimodal samples, the sedimentation of large particles, and issues related to sample concentration. These challenges can be mitigated by incorporating a fractionation step to separate particles of different sizes before DLS measurement. [113]

One such method is asymmetrical flow field-flow fractionation (AF4), which involves separating samples in a narrow, open channel without packing material [114]. In this process, a single carrier flow enters the channel and splits into channel flow and crossflow. The channel flow follows a parabolic velocity profile that transports nanoparticles to the outlet for detection, while the crossflow moves from the top to the bottom of the channel, forcing nanoparticles toward an accumulation wall made of an ultrafiltration membrane supported by a porous frit. The nanoparticles' diffusion restricts the crossflow field, enabling size fractionation: smaller particles reach equilibrium higher in the channel, where faster flow velocities allow for earlier elution compared to larger particles [115].

Additionally, factors such as temperature and pH can impact the reliability of DLS measurements. As a result, DLS is generally considered unsuitable for measurements in biological media.

Differential centrifugal sedimentation (DCS)

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The principle behind differential centrifugal sedimentation (DCS) is that larger particles sediment faster than smaller ones, assuming they have the same density. Since most nanocarriers do not naturally settle due to their small size, sedimentation is facilitated by centrifugation. In this process, a hollow disc, placed inside a centrifuge tube that is optically clear and has a central opening, spins at speeds ranging from 600 to 24,000 rpm. The disc is partially filled with fluid, allowing liquid layers to separate based on density differences. The sample is injected through the central opening for measurement. DCS offers exceptionally high resolution, enabling the complete resolution of nanocarriers with size variations of less than 5%. [116] Recently, DCS has been used to study size changes in gold nanoparticles following surface modification or functionalization. For example, a shift of 0.5 nm in nanoparticle size was observed after modification, and a shift of 2.1 nm was noted when the nanoparticles were modified with high molecular weight entities such as single-stranded DNA. [117] Centrifugal liquid sedimentation (CLS) is a fractionation method in which different monodisperse fractions within a sample are separated by centrifugation before particle size measurement. Theoretically, CLS is more suitable for polydisperse samples. However, the fractionation process becomes increasingly complex if the size distribution is too broad. Spherical nanocarriers with narrower size distributions and uniform densities are better suited for CLS measurements [118].
To obtain reliable results, it is essential that the samples remain chemically and physically stable in suspension during sedimentation, and that there is a significant difference in refractive index and density between the particles and the liquid medium. Previous studies have demonstrated that both DLS and CLS methods are suitable and robust for determining the particle size of silica nanoparticle suspensions in the 35–50 nm range. [119]

Particle Size and Polydispersity
The most critical characteristics of nanocarriers are their particle size, shape, and dispersity, which is often expressed as the polydispersity index (PDI), indicating the heterogeneity of particle sizes. Particle size and shape play a significant role in determining the bio distribution and elimination of nanocarriers. [120] these factors also influence the nanocarriers' ability to attach and adhere firmly, their susceptibility to phagocytosis, circulation half-life, cellular distribution, and processes such as cellular uptake and endocytosis [121].

Challenges of advanced nanocarriers system for targeted drug delivery system
Complex design and fabrication: Nanocarriers are designing for target the site specific like specific cell or tissue that's why designing of nanocarriers are very difficult and not cost effective. In similar way scaling up the production of nanocarriers with maintaining the uniformity is very complicated.[122,123]
Biocompatibility: For construction of nanocarriers it is always important to use biocompatible to avoid adverse immune reaction. This include important materials are Polymers, lipids, metals or inorganic materials.[124,125]

Degradability: The nanocarriers materials are needed to degrade in a specific controlled manner after delivering the drug to minimize accumulation in the body balancing the release kinetics is a big challenge.[126]

Biocompatibility and toxicity: The immune system may identify nanocarriers as foreign body, which could trigger an immunological response and cause clearance of the carrier before it reaches the intended location. Certain nanomaterials may interact with biological systems in a way that causes unanticipated side effects or inherent toxicity.[127,128]
A Nanocarriers can be utilized in therapeutics in two different ways: either as a drug by itself or as a carrier for another material. These Nanocarriers interact with different biological processes and biomolecules present in living things since they are entering the biological system.[129] Because of their high surface to area ratio, Nanocarriers can be advantageous for effective medication loading, however, when they are present in a living body, they can also enhance the likelihood of interacting with other biomolecules that's some time cause of biotoxicity. [130]The great advantages that Nanocarriers provide are largely well known, but very little is known about their toxicity, non-specific protein interactions, translocation to secondary target organs, etc. The harmful effects of titanium, gold, silver, and silica nanoparticles have already been brought to light by a few in vitro and in vivo investigations.[131,132]
Like some studies had been shown that in case of Protein based Nanocarriers shows Hepatotoxicity: The inherent abundance of albumin in the body, its biocompatibility, and its ability to bind to a variety of medicines make it a popular protein for use in drug delivery.[133] Improved solubility and distribution of hydrophobic medicines, especially in cancer therapy, are the goals of albumin-based nanocarriers, like the ones found in the medication Abraxane (a nanoparticle albumin-bound formulation of paclitaxel). The liver absorbs a large amount of the albumin-based nanocarriers notwithstanding the targeting mechanisms. The albumin is recognized as a natural substrate by the liver, which is the principal location for protein metabolism and elimination.[134,135]
The hepatocytes (liver cells) and Kupffer cells (specialized macrophages) in the liver are where the nanocarriers gather. These cells are in charge of eliminating foreign materials from the circulation. The medicine that has been encapsulated is released locally as the albumin-based nanocarriers break down in the liver. A chemotherapeutic agent at high local concentrations may directly harm hepatocytes.[136,137].

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Furthermore, non-harmful byproducts from the breakdown of albumin may cause oxidative stress, inflammation, and liver cell apoptosis (programmed cell death).[138]
Nephrotoxicity, cardio toxicity. Metal nanocarriers show Anxiogenic and depression effects .Reproductive and development toxicity, genotoxicity. Lipid based Nanocarriers show cardiopulmonary distress, anaphylactic reaction, hypersensitivity reaction.[139]
Targeting efficiency: Nanocarriers are effective transport agents because of their small size and capacity to change their physical properties, such charge and shape, to deliver therapeutic chemicals to tissues. Targeted tissues, including tumors, can be heterogeneous, it can be challenging for nanocarriers to evenly reach and enter the whole area.[140,141] Nanocarriers some time may bind with non-targeted cell which cause reducing the amount of total drug delivery. The ability of nanocarriers to target specific targets, such cancers, with minimal exposure to healthy tissues is a crucial component in increasing the therapeutic efficacy of medications. The size, shape, surface characteristics, and targeting tactics used—passive targeting via the Enhanced Permeability and Retention (EPR) effect and active targeting with ligands that bind to particular receptors on target cells all affect this efficiency. [142,143]Targeting precision is further improved by stimuli-responsive designs, which release the medicine in response to internal or external events. Nevertheless, obstacles like immune system clearance, off-target accumulation, and target tissue heterogeneity can affect overall efficacy. Sustaining these challenges and enhancing targeted performance in medication delivery systems requires ongoing improvements in tailored methods and nanocarrier design.[144]

Stability and Shelf Life: The in vitro and in vivo impacts of nanocarriers (NCs) and their physicochemical qualities. The shape, size, surface chemistry, ligands, and other physicochemical characteristics are what give nanocarriers their biological identity. For this nanocarriers must stable in various physiological condition to ensure the effective drug delivery.[145]
On the same side sometime nanocarriers reducing the effectiveness after premature degradation or aggregation. Controlled Release: Nanocarriers huge surface area to volume ratio and short diffusion distance present particular difficulties in regulating medication release kinetics. Understanding the mechanisms by which a carrier retains and releases a drug, the effects of the carrier composition and morphology on the drug release kinetics, and the most recent methods for nanocarrier preparation and modification are crucial for developing nanocarriers with desirable release kinetics for target applications[146,147].

Regulatory and Clinical Challenges: The complex nature of nanocarrier systems makes it difficult to navigate regulatory pathways, as there is often a lack of standardized guidelines.[148]

Cost and Accessibility: Due to sophisticated technology is required for preparing nanocarriers it is costly than other providing method.[149]

Future Scope
According to the recent research and studies in the development of nanocarriers, achieving clinical relevance necessitates a comprehensive evaluation of formulation characteristics, pharmacokinetic behavior, and the regulatory approval pathway. [150]Due to the advancement of nanocarrier drug delivery system it has established a new paradigm in pharmaceutical field. Convergence of science and engineering leads to a new era of hope where medicines will act with increase efficacy, high bioavailability and less toxicity. Several nanoscale drug delivery systems are currently in clinical trials and few of them are already available commercially. [151]The field of cancer nanomedicine has progressed in recent years. Spatial and temporal release of nanoparticle-encapsulated drugs (as well as other biomolecules) in a regulated fashion at the site of action is one avenue that calls for attention to impart further improvement in treatment modalities. Nanoparticles with built-in tunable triggering properties platforms coupled with localized drug delivery technology will have significant impact on cancer therapy and other related diseases.[152,153] Their structural properties have been studied widely and have found to be responsible for their unique characteristics like high payload and tissue accumulation. Further, they are found to have greater emphasis on gene delivery, boron neutron capture therapy, and magnetic resonance imaging contrast agents. They allow bulk drug loading, structural flexibility, intrinsic stability, improved circulation time, and bioavailability.[154] This system provides higher drug loading and allows the conjugation of biological molecules without triggering an immune response. The ideal adjustment to the delivery conditions, such as transportation to the site of action, specific targeting or adequate delivery profile, among others, for each type of disease, requires the development of new polymers that can fit these requisites.[155]
Over the last few years, there has been a global transformation in the field of nanomedicine, which has led to a multidisciplinary and collaborative approach with promising results and success. For heat performances, whenever it is not possible to test the material intratumorally, studies can be conducted under clinical AFM conditions heat response of TR- nanosystems in a matrix or viscous media, both of which can mimic an in vivo viscous environment. [156,157]The polymer shrinkage and nanocarriers performances could be significantly different if particles were to be blocked in subcellular compartment or tumor stroma. The recently renewed interest in stimuli- responsive nanosystems has coincided

with the parallel boost in a new generation of Nanocarriers. Moving from aqueous coprecipitation methods to nonhydrolytic thermal decomposition methods, which allow better control over nanocarriers parameters, has drastically affected the heat efficiency.[158,159,160]

Conflict of Interest: None

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