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# A comprehensive revision on the nanocarrier drug delivery systems with special reference to artificial intelligence

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***Abstract*---**Nanocarriers outperform traditional medication dosage forms in terms of efficacy, safety, and tolerability because of their small size, large surface area, and potential for precise targeting. More and more researchers are looking to produce nanocarriers that can be used to treat a variety of illnesses. Dendrimers, liposomal nanoparticles, polymersomes, polymer–drug conjugates and peptide nanoparticles are only some of the nanocarriers that have been developed for drug delivery. Other nanocarriers include carbon nanotubes, nanoshells and carbon dioxide nanoparticles. Nanocarriers have been characterised using a variety of approaches during the past few decades, both in vitro and in vivo. Most nanocarriers are characterised using fundamental in vitro, ex vivo, ex situ, and in situ techniques, which emphasise their advantages and limitations as well as regulatory and manufacturing issues that hinder the transfer of nanocarriers from laboratory to clinical use, as described in this review. There is also a discussion of the integration of artificial intelligence with nanotechnology and the advantages and disadvantages of artificial intelligence in the creation and optimization of nanocarriers, along with future prospects.

***Keywords*---**nanocarriers characterization, challenges, artificial intelligence, future perspectives, stability, regulatory aspects, safety considerations.

## Introduction

Drug bioavailability, stability, and organ targeting have all been the focus of pharmaceutical research in recent years. Submicron-sized drug delivery vehicles, pharmaceutical nanocarriers are highly adaptable and versatile delivery systems. It includes nanoparticles of many kinds, such as polymeric and lipidic and inorganic niosomes, as well as many more. A nanocarrier surface can theoretically have ligands added to improve absorption and targeting [1–3]. There are two ways in which drugs can be included into the nanocarrier matrix. For example, nanocarriers may be tailored to a variety of different sizes, charges, surfaces, and targeting moieties in order to control their absorption, biodistribution, targeting, and removal. Parenteral [5], nasal [6], topical [7,8], or oral [9,10] methods can be used to give them. There is a growing need for nanocarriers that target many illnesses and have a wide variety of features because of the advantages listed above. Because of this, numerous methods of characterising nanocarrier behaviour in vitro and in vivo have been devised and employed during the last few decades. Nanocarriers' physical and chemical characteristics, drug loading, release rate, mechanical behaviour, stability, tissue permeability, probable toxicity, and in vivo destiny may all be evaluated using routinely used characterisation techniques. For most nanocarrier-based drug delivery systems, the most important characterisation approaches are summarised below (DDSs). There are other regulatory and scalability concerns that must be overcome in the production of nanocarriers, which are discussed in detail.

**Physicochemical Characterization**

Particle size, size distribution, surface charge, hydrophobicity, and shape are some of the physicochemical features of nanocarriers. Physical stability and entrapment efficiency may both be predicted to a large extent based on the drug nanocarrier's physicochemical qualities [11].

**Particle Size and Polydispersity**

When it comes to nanocarriers, it's all about size, shape, and dispersion (the polydispersity index, or PDI, measures particle heterogeneity). Nanocarrier biodistribution and elimination are influenced by particle size and shape [12–14]. Also, their attachment, firm adhesion, phagocytosis, circulatory half-life, cellular distribution, cellular uptake and endocytosis [18,19] are affected. This section provides an overview of the most commonly used techniques for determining particle size and PDI, as well as its advantages and disadvantages.

**Dynamic Light Scattering Spectroscopy**

Dynamic light scattering (DLS) uses Brownian motion and light scattering characteristics to calculate the particle diameter. It's possible that DLS may miss out on certain huge particles since their movement may be too sluggish. A particle sizer with particle sizing software is used to estimate the mean particle diameter and PDI [21]. Samples should be in a known viscosity solution or suspension. Using this method, you can quantify particles as small as one nanometer down to 10 micrometres in diameter. Particle size, size distribution, and particle distribution index (PDI) may all be easily analysed statistically using the results that were collected. Polydisperse and multimodal sample findings, sedimentation of big particles, and sample concentration may restrict the implementation of DLS. If you add a fractionation phase before using the DLS approach, you can circumvent these constraints. [22]. An unloaded, narrow-opened channel is used for asymmetrical flow field flow fractionation (AF4) [23]. When the channel intake is opened, a single-carrier flow enters and separates into two separate flows, as seen in Figure 1. The nanoparticles are carried to the channel outlet by a parabolic velocity profile line in the channel flow. It's a different story when it comes to nanoparticles. The cross-flow flows from top to bottom, pushing them down the accumulation wall, which is constructed of an ultra-filtration membrane and a porous frit. For this last step in size fractionation, a restriction on nanoparticle diffusion causes smaller particles to reach an equilibrium position higher up in the channel with a greater flow velocity that allows for early elution of bigger particles [22–24].

Furthermore, temperature and pH might have an effect on the accuracy of the readings. Generally speaking, the use of DLS is not recommended for the study of biological media [25]. A fresh and distinct AF4 technique must be devised for each type of measured nanoparticle sample based on its composition, average size, surface features, and size distribution [26]. This is critical. Transmission electron microscopy (TEM) or scanning electron microscopy (SEM) are frequently used to verify the accuracy of the data produced using this technique [22]. Having spherical samples isn't a guarantee, and this may not be the case in all cases. It's

also possible to have turbid or translucent samples, in which case light absorption by dispersed particles interferes with detection. Additionally, aggregated particles that can't be distinguished from individual particles may be present.

**Static Light Scattering**

It is necessary to measure the intensity of scattered light waves and then apply an acceptable mathematical model (often Mie theory) in order to translate the scattering pattern into a particle size distribution for static light scattering. All of the spherical, non-interacting particles are assumed to have a certain refractive index in this model. In reality, the vast majority of biopolymeric particles do not comply with these claims. As a result of sample preparation, the integrity or aggregation of biopolymer particles may be affected. Because of this, static light scattering measurements should be utilised with caution [28].

**Atomic Force Microscopy**

Particle size may be measured with ultra-high resolution using atomic force microscopy (AEM), which uses a probe tip of atomic scale to examine the submicron particle levels. Using a sharp probe, the appliance creates an accurate topographic map of the sample based on how hard the probe hits the sample. Imaging fragile biological and polymeric nanocarriers is made easier by this technology since it doesn't require any specific preparation for nonconducting materials. [29] Of primary importance, it does so without applying any algorithmic treatment and does it in the most accurate manner possible. In the case of complicated materials or specimens, such as biological cells, it is important to point out that proper data collection and interpretation of results need considerable skill. One of the most pressing issues is the quality of the tip and support surface chemistries, which may degrade during the data collecting process for vesicles of various shapes and sizes. Poor sample procedures and time consumption may also be a problem because of the instrument's sluggish scanning methodology, which does not have the capacity to detect individual molecules. Single-molecule force spectroscopy with AFM cantilever tip containing particular ligands or molecules that can detect certain functional groups can alleviate the latter issue, though. As a result, before conducting their first experiment, researchers must have a thorough grasp of the principles and limits of various AFM modalities [27,30].

**Centrifugal Liquid Sedimentation**

This method of fractionation is called centrifugal liquid sedimentation (CLS), and it is used to extract various monodisperse fractions within a sample by centrifugation. It is possible that CLS is more suited for polydisperse samples than DLS. However, if the size distribution is too wide, the fractionation process becomes much more difficult. CLS measurements are better suited to spherical nanocarriers with a smaller size distribution and higher density [32,33]. During sedimentation, samples must not undergo any chemical or physical changes. Particles and liquid mediums must also differ in refractive index and density in order to yield valid findings. The particle size of silica nanoparticle dispersion in

the 35–50 nm range has previously been determined using the DLS and CLS techniques.

**Surface Charge and Hydrophobicity**

Nanocarriers' bioavailability, stability, cellular absorption, and biodistribution all depend on their surface characteristics [12,34,35]. Nanocarrier units' aggregation tendencies are affected by the zeta () potential, which expresses their surface charge and may be used to pick appropriate coating materials [36]. Laser Doppler velocimetry [37] can be used to measure the mobility of nanocarriers when an electrical current is sent through the sample. Because of its precision, sensitivity, and adaptability, electrophoretic light scattering has been the most widely used approach to date. The ionic strength and pH are also important considerations for determining zeta potential. Before doing any measurement, it is common practise to dilute the sample. The trustworthiness of the interpreted data may be affected by mixtures of oppositely charged nanocarriers [20]. Adsorption probe technique, hydrophobic interaction chromatography, contact angle measurements, and biphasic partitioning may all be used to determine a nanocarrier's hydrophobicity. It is also possible to anticipate the hydrophobicity of nanocarrier surfaces using X-ray photon correlation spectroscopy [38].

**Morphology of Nanocarriers**

A variety of biological features, such as half-life, targeting effectiveness, and toxicity, are affected by the shape and aggregation behaviour of nanocarriers. Many non-spherical forms have a significant impact on biological processes, including discs, ellipses, cylinders, hemispherical spheres, cubes, cones, and other complicated structures [12]. Nanocarriers may be studied with great resolution using atomic force microscopy, which does not change the characteristics of the material before measurement. There are various advantages to using electron microscopy techniques to study morphology and particle size, but they lack information on the real population mean and size range.

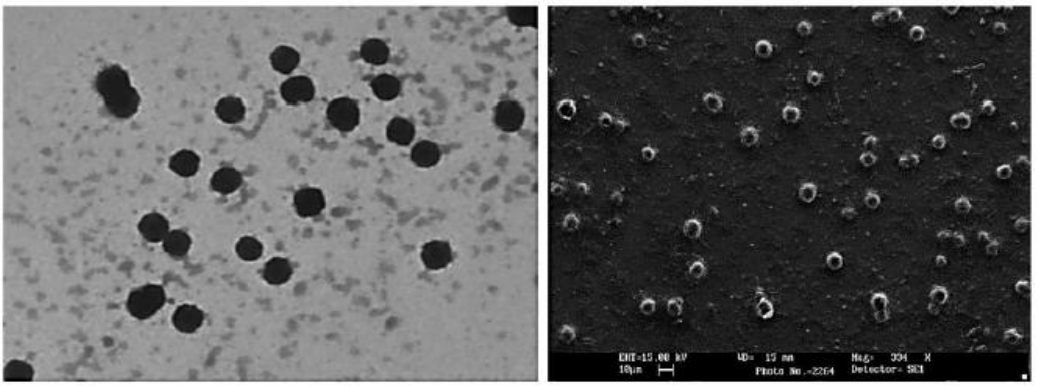
**Scanning Electron Microscopy**

Direct viewing of nanocarrier topography is possible thanks to scanning electron microscopy (SEM). Drying and sputter coating are the most common methods for coating samples, which are often coated with gold or another metal with a high electrical conductivity, such as silver. Focused electrons scan the surface of the sample and record the secondary electrons that are released from it [40]. In a perfect world, nanocarriers scanned by SEM would be impervious to the coating material, the electron beam, and the vacuum. New SEM procedures have been developed, which do not need the drying of samples. If you're looking to analyse hydrated materials, for example, wet SEM is a great option. Samples must be frozen for Cryo-SEM, another modified approach [43].

**Transmission Electron Microscopy**

The transmission electron microscope (TEM) provides information on the shape of nanocarriers, which act as conduits for electrons. As samples must be ultra-thin

to allow for electron transmission, sample preparation for TEM is time consuming and difficult [44]. There are two ways to bind the nanocarrier sample onto support films or grids: either a negative stain agent (e.g. Phosphotungstic acid) or plastic embedding (uranyl Acetate). Cryo-TEM [45], on the other hand, involves submerging the sample in vitreous ice before exposing it to liquid nitrogen. Biopolymeric materials usually require strong metal staining in order to impose adequate contrast for identification. After fixing, drying, and cutting the sample, the interior structure of particles may be examined. An illustration of the contrast between SEM and TEM images may be seen in Figure [47].



(a) (b)

Figure. TEM and SEM pictures of lyophilized DLX-NLC. In these micrographs, DLX-NLC was shown to be spherical and nanoparticulate (80.17–127.73 nm). The TEM and SEM pictures of lyophilized DLX-NLC are shown in Figure 3 (a) and (b). Images of DLX-microstructure NLC's indicated that it is a spherical nanoparticle (80.17-82.73 nm).

**Composition, Loading Efficiency, and Mechanical Properties**

Because of the small dimensions involved, determining the precise particle constitution and allocating the active components inside the particles might be a difficult task following the creation of the nanocarriers [20]. The chemical composition of the nanocarriers' surface may be examined using X-ray photoelectron spectroscopy and elemental analysis to determine if the medication has been properly encapsulated [48]. The molecules' surface characteristics may also be determined by Raman spectroscopy based on their vibrational transitions [49,50]. Differential scanning calorimetry (DSC) is widely used to study the interactions between the polymers and crystals of an encapsulated chemical. Polymeric interactions may be studied using infrared spectroscopy [53], whereas nanocarrier crystallinity can be determined using X-ray diffraction studies [54]. Nanocarrier characterisation relies heavily on entrapment efficiency (EE), a critical parameter. The direct and indirect techniques of EE measurement are frequently employed [55]. The unentrapped drug concentration in the supernatant layer after centrifugation is used to calculate EE using the indirect technique (Equation (1)).

$$\%EE = \frac{\text{Initial amount of the drug} - \text{Free unentrapped drug}}{\text{Initial amount of the drug}} \times 100 \quad (1)$$

While in the indirect technique, a suitable solvent is used to solubilize the nanoparticles, the concentration of the entrapped drug is then filtered out and analysed using the appropriate method (Equation (2)).

$$\%EE = \frac{\text{Amount of entrapped drug}}{\text{Initial amount of the drug}} \times 100 \quad (2)$$

The EE values found by Fresta et al. [55] using the two alternative equations were almost identical (3%). The main challenge in determining EE is the accuracy of drug analysis. This can be done quantitatively using UV spectrophotometry [56] or liquid chromatographic methods like high-performance liquid chromatography (HPLC) [55], depending on the drug's chemical structure. Nanoindentation, atomic force microscopy, micropipette aspiration, particle poking, and optical tweezers are used to study the mechanical characteristics, elasticity, and hardness of nanocarriers [57,58].

**Invitro Drug Release**

The in vivo behaviour of drugs released from nanocarriers is a key determinant of therapeutic efficacy and adverse consequences. In vitro release testing aids in improving the formulation and assessing batch-to-batch variance. Compendia and label claim compliance can also be ensured by using this method [59,60]. A regulatory standard for detecting drug release from nanocarriers is a major challenge, thus careful consideration was given to the development of the right method to measure in vitro release [59]. Preparation of an in vitro drug release profile often begins with preincubating nanocarriers in release media, followed by periodic sample withdrawals and analysis to quantify the amount of released drug. In vitro drug release from nanocarriers has been studied in a variety of ways, as shown in Table 1.

Table 1. In vitro drug release assessment techniques adopted for variable nanocarriers.

| In Vitro Release Model | Subtype Model | Nanocarriers System | Reference |
|---|---|---|---|
| Dialysis | Regular Dialysis | Solid Lipid Nanoparticles | [62,63] |
| | | Proniosomes | [64] |
| | | Magnetic Nanoparticles | [65] |
| | | Nanosponges | [66] |
| | Reverse Dialysis | Nanoemulsion | [67] |
| | | Niosomes | [68] |
| | | Liposomes | [69] |
| | Side-by-Side Dialysis | Nanospheres | [70] |
| | | Nanostructured Lipid Nanoparticles | [71] |
| | | Lipid Nanocapsules | [72] |
| Sample and Separation | Membrane Filters | Nanocrystals | [73] |
| | | Mesoporous Nanoparticles | [74] |
| | Centrifugation | Chitosan Nanoparticles | [75] |
| | Ultracentrifugation | Liposomes | [76] |
| | Ultrafiltration | Chitosan Nanoparticles | [77] |
| | | Liposomes | [76] |
| Continuous Flow | | Nanoparticles Incorporated in Strip-Films | [78] |
| Dynamic Dissolution Microdialysis | | Nanosuspension | [79] |
| | | Nanofibers | [80] |
| | | Nanoparticles | [81] |

## Dialysis Method

Since it's simple to set up and allows for time sampling, this in vitro drug release approach is the most used. It uses a dialysis membrane with a molecular weight cut-off (MWCO) at least 100 times greater than the drug's [69] and a two-compartment system. The amount of drug diffusion from one compartment to the next is measured. Nanospheres [82], liposomes [69] and nanoemulsion [67] are only a few of the nanocarriers to which this technology has been successfully used. In terms of the configuration and volume of the donor and recipient compartments, dialysis is classified as 'regular' [83],'reverse' [69], or 'side-by-side' [82]. Drug concentrations in the assessed drug concentrations represent both release from nanocarriers and diffusion via dialysis membrane, notwithstanding dialysis method's simplicity. There are several variables that may be taken into consideration when selecting dialysis membrane characteristics such as the MWCO and charge and binding affinity as well as accurate mathematical models for data interpretation. [84,85].

## Sample and Separation Method

Using syringe filters [73,74,86], centrifugation, ultracentrifugation, or ultra-filtration [75,87,88], the nanocarriers can be effectively separated from the release medium. Fresh medium is added to the release medium to maintain the sink state following sampling. The set-up may be customised by altering the container size, agitation method, and sample approach. Set-ups with USP I (basket), USP II

(paddle), and vials have been widely documented. Here, agitation of the release media is critical in order to prevent nanocarrier agglomeration. Magnetic stirrers and orbital shakers, as well as USP I and USP II equipment, can all be used to agitate samples. Filters might become blocked during sampling and drugs can become adsorbent on filters, both of which pose difficulties for this approach in the lab [89]. Non-sink settings have been characterised as advantageous for poorly soluble medications [90] despite sink conditions being advised.

**Continuous Flow Method**

The USP IV or a modified version of it is utilised. Small volumes of pumped release medium are passed via filters before being tested on nanocarriers [91]. Subcutaneous or intramuscular injection of nanocarriers is well-suited to this method because the nanocarriers are contained within the administration site and are only exposed to a small number of biological fluids. Complicated set-up procedures, filter blockage by drugs, and the inability to keep the flow rate steady all contribute to inconsistent outcomes [78].

**Dynamic Dissolution Method**

Because it doesn't need the separation of samples, this procedure is simple and rapid. Using a dialysis membrane and a drug-selected electrode, Moreno-Bautista and Tam [85] demonstrated that hydrophilic medicines could be quantified using this method. Mora et al. [93] used voltametric electrodes to monitor the release of chemotherapeutic compounds from liposomes. This method, however, lacks sensitivity and consistency in responsiveness. It is, thus, ineffective. These electrochemical approaches are supplemented by other non-electrochemical ones, such as calorimetry, turbidimetry, and laser diffraction [94].

**Microdialysis Method**

Probes are inserted into the dissolving containers, and a tube is utilised to continuously provide the release medium to the microdialysis probes. In between the internal tube and the dialyzing film, the medium returns to steam. The medicine that has been leaked into the public eye is then subjected to a variety of tests. A successful in vitro release study of nanocapsule-based products has been achieved using this technology [97]. Fortunately, this method does not interfere with the equilibrium between the encapsulated and free medicine. Optimum formulations can only be achieved by mathematical modelling of drug release [98]. Nanocarriers typically have a short release time. As the drug is freed from the surface in the first stage, a generalisation stage occurs in which the drug is liberated at a faster rate than in the first stage, a gradual release stage, and a final liberation stage. Drug placement, solubility, and diffusion via the nanocarrier matrix all influence the release kinetics of nanocarriers. The Weibull, reciprocal-powered time, and three-parameter models are examples of mathematical models that may be used for nanocarrier release profiling [100,101].

Because of this, IVIVC is critical to determining the link between DDS blood plasma concentrations and in vitro tests [102]. Making a reasonable IVIVC is

difficult because it necessitates the use of standardised reference materials, optimised in vitro testing, the assessment of nanocarrier biodistribution and pharmacokinetic control, as well as the investigation of nanocarrier transfer across multiple compartmental borders and the development of an appropriate risk–benefit model. Contrarily, Cao et al. [104] found for 72 hours a good IVIVC linear connection between the in vitro dissolution of the produced mesoporous silica nanoparticles encapsulating the silybin meglumine. For trans-resveratrol, Singh and Pai [105] used Eudragit RL 100 to improve the nanoparticulate (NP) DDS (t-RVT). In vitro drug release data and a good IVIVC were produced for the increased formulation of t-RVT NPs as well as for the pure drug and the commercialised formulation. This sort of correlation is usually a straight line, showing a point-to-point connection, and it is seen to be the most instructive.

**Permeability Assessment**

Nanocarrier permeability experiments are used to determine the in vivo behaviour of nanocarriers and to assist understand how the shape of the nanocarriers may impact their bioavailability [132]. Ex vivo, in vivo, in situ organ perfusion, and cell culture-based models can all be used to measure permeability.

**Ex Vivo Models**

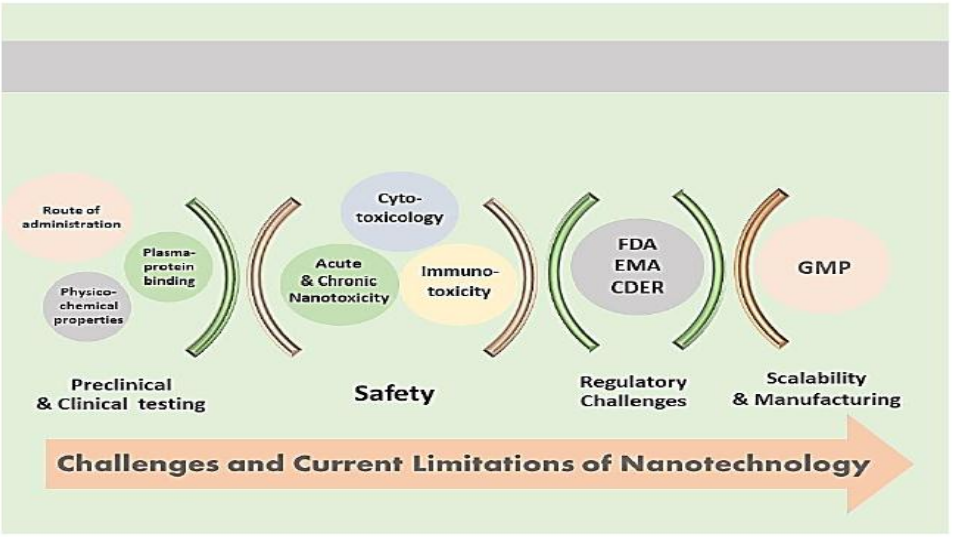
If you're looking for a way to study nanocarriers, this model is ideal. For drug-loaded oral nanocarriers with high duplicability, everted or non-everted gut sac models are commonly used [133,137]. The bioavailability of nanocarriers can also be evaluated using different excipients. Solubility, intestinal permeability, and enzyme activity may be affected by the use of these excipients. The jejunum, duodenum, or ileum is removed, divided into 5–6 cm sections, rinsed, and everted on a glass rod in the everted gut sac model. The clamped end of the intestine is filled with 37-degree Celsius Krebs solution. Nanocarriers in oxygenated media are placed in a 37 C incubation flask and the other end of the tube is tied off. Samples are then taken at different intervals over time. Factors such as the species, age, disease state, and diet of animals, as well as the gut segment factors (duodenum, jejunum, ileo and colon) all influence the permeability results (pH, aeration). Because of short-term intestinal viability, loss of enzyme activity, constrained sampling [151], eversion-induced damage, and a lack of sink conditions due to the small size of the receiver compartment [152], this model's application is limited. An alternative gut sac model uses the small intestine cut into segments, each of which is filled with the nanocarrier suspension and then tied on both ends before being submerged in Ringer's solution. Samples are removed from the sac and analysed outside of the sac, and the medium is completely replaced with new medium at regular times. Methods such as this one are easier to use because they require less suspension volume and are less likely to cause intestinal morphological damage [153]. Since only two hours of intestinal viability can be maintained, ex vivo models are generally ineffective for testing nanocarriers with sustained release profiles. Since the enzymes and bile salts used in oral digestion cannot be precisely characterised, these models cannot be used to accurately assess oral bioavailability, leading to an inadequate correlation with in vivo profiles, particularly for liposomes, which have a high degree of digestive accountability.

**In Vivo Methods**

The in vitro and physicochemical features of nanocarriers alone may not be sufficient to accurately predict their in vivo performance. Drug bioavailability can be affected by efflux transporters and metabolic enzymes because of these biological factors. The pharmacological effectiveness of nanocarriers can only be gauged by determining their oral bioavailability. After oral administration, the drug's plasma concentrations can be measured for this. Using non-human primates for bioavailability testing is the most accurate but also the most expensive. As compared to humans, rats exhibit a weaker correlation with the data [154]. In other research, rabbits were also used. By way of an oral gavage, nanocarriers are given to fasting animals. Over a period of time, blood samples are obtained and examined. To avoid reflux into the esophagus in rats, the supplied dosage volume should not exceed 350 L [159]. The released drug's biodistribution can also be tracked using in vivo imaging techniques. [160] The following are examples: Gamma scintigraphy (MRI), singlephoton computed tomography (SPECT), PET, and magnetic marker monitoring [160]. It was found that gamma scintigraphy can be used to study the distribution and pharmacokinetics of DLX-NLC, which is loaded with duloxetine for nose-to-brain delivery, in an investigation by Alam et al. [47]. The radionuclide technetium was used to identify the medication's composition and dosage form (99mTc). Researchers delivered the nanocarrier via nasal route and studied plasma samples from rats, while radioactivity in organs was measured using a shielded well-type gamma scintillation counter. The results show that the nanocarrier is safe and effective for use in humans. A single-photon emission computerised tomography (SPECT) gamma camera was employed for the whole-body gamma imaging research, as shown in Figure 4 [47]. As a result of its short half-life (6 hours) and low radiation energy (140 keV), 99mTc is an excellent radiotracer [161].

**Challenges and Limitations of Nanocarrier Characterization**

Researchers and regulatory organisations dealing with drug-loaded nanocarriers encounter several difficulties (Figure 5). These issues necessitate the use of robust characterisation methodologies, scalable optimization approaches, safety recommendations, and stability maintenance [118,171,172] in order to succeed.




Challenges and existing limitations in pharmaceutical nanotechnology are depicted in Figure 5. "FDA," "EMA," "CDER," and "GMP" all stand for the Food and Drug Administration, "European Medicines Agency," "Center for Drug Evaluation, and Research," respectively.

**Correlation of Preclinical Characterization to Clinical Testing**

At the moment, various nanocarriers are being developed to serve as instruments for a variety of purposes, including drug delivery, imaging, and therapeutics. For preclinical characterisation, their physical and chemical characteristics are strongly affected by the physiological environment. The development of in vitro and in vivo models that replicate clinical cases requires the use of reliable criteria for the quality evaluation of nanomaterials. It is the goal of regulatory agencies, such as the National Cancer Institute's Nanotechnology Characterization Laboratory (NCL), Food and Drug Administration (FDA) and the National Institute of Standards and Technology (NIST), to develop and validate standardised characterization protocols for nanocarriers that are constantly updated to include a wider range of nanotherapeutics.

The development of principles for portrayal and the subsequent therapeutic use of nanocarriers may be hampered by several challenges. Traditional techniques of characterisation are frequently hampered by surfactants used in nanocarrier formulations to aid dispersion. Citric acid-coated nanocarriers are more effective in penetrating cells' membranes than neutral or anionic nanocarriers. Adsorption of pollutants and impurities to nanocarrier surfaces is also possible [173]. Nanocarriers' physicochemical properties, such as charge and hydrodynamic diameter, are strongly influenced by physiological variables such as temperature, pH, and ionic strength. The distribution and clearance of nanocarriers may be affected by the binding to plasma proteins following delivery, and the drug release profile may be entirely altered in physiological fluids [174]. The toxicity and biocompatibility of a substance can be drastically altered if its polydispersity

changes as a result of contact with bodily fluids [175]. Methods of characterization are also greatly influenced by the route of administration. In contrast to nanocarrier formulations designed for oral, nasal, or topical delivery, intravenously injected nanocarrier systems need fewer characterization techniques [176]. That's why in vitro characterisation must account for any potential interference from in vivo variables. In vitro experiments often include analytical samples and control samples with noticeable features to assure data reliability [177].

**Safety Considerations**

Safety is a major concern when it comes to nanocarriers being used in DDSs. The basic notion of toxicity reduction is one of the nanocarriers' distinctive qualities in DDSs [178]. In part, this is due to the fact that nanocarrier DDS has a lower dosage and a superior cellular absorption and targetability. Different studies have examined the toxicity of various nanocarriers in the human body, however, to assure their safety [179–182]. Size, shape, surface charge, delivery method, and medication dosage have all been linked to nanocarrier toxicity [183, 180]. In contact with tissues and biological fluids, nanocarriers' nanoscale size might contribute to toxicity [173]. The in vivo characterisation of nanocarriers should include cytotoxicity tests, as this is an important aspect of the research. Hemorrhaging, oxidative stress and mitochondrial dysfunction are only some of the acute toxicological interactions that might occur, but chronic toxicities are far more complex [185,186].

Conventional toxicity evaluation methods established for classical medications are commonly utilised, and the results are often insufficient.... Because of their simplicity and low cost, cell culture models are commonly used in acute nanotoxicity research. Despite this, they cannot be utilised to study long-term toxicological effects because of their poor cellular viability [187]. In addition, repeated exposure of tissues to nanocarriers has not been fully explored in terms of toxicology. Immunogenic responses are possible with many nanocarrier systems, which can lead to major adverse effects and even anaphylactic shock [188]. Toxicology and effectiveness of nanocarriers have previously been reported to be affected by long-term storage stability issues. Because of the large-scale nature of nanocarrier production, it is necessary to carefully monitor exposure levels and any resulting effects. A good safety profile may be achieved with better control over the nanocarrier production process. Particokinetics and multiparametric assessment were previously described as novel toxicological techniques. To our knowledge, there is no standard method for evaluating nanocarriers' toxicological effects. The toxicity of nanocarriers may be predicted using their size, surface charge, and solubility, according to several international standard-setting agencies [188]. In vitro toxicity tests that closely mimic in vivo settings should be used instead of those that don't. Toxicological outcomes may be explained by biodistribution investigations [189].

**Regulatory Challenges in Nanomedicine Development**

As a result, nanocarriers are controlled by the FDA and the European Medicines Agency (EMA), along with other regulatory authorities, such as CDER. It has been

30 years since 21 nanocarrier compositions were authorised. Currently, liposomes are the most often used and authorised nanocarriers, whether they are given intravenously or orally. Toxicities are reduced, but effectiveness is not enhanced [191]. [191] [191]. To get nanocarriers out of the lab and into patients' hands, it appears that there are no defined and biorelevant criteria for characterisation and quality control. For example, establishing a standardised in vitro release technique for nanocarriers [192] is quite challenging. As there is no historical history of acceptance in the literature for nanocarrier safety and characterisation, there is a significant need to establish and evaluate new standard techniques. In addition, regulators should differentiate between OTC cosmetic goods based on nanocarriers, such as sunscreens, and those based on medicinal formulations [193].

**Manufacturing Considerations**

Manufacturing approaches that can be scaled to produce nanomedicines with optimal bioavailability and excretion characteristics are needed as nanocarrier technology advances. Batch-to-batch repeatability is difficult to obtain because of the polydispersity problem. Polymer to drug ratios and lipid to drug ratios, as well as other largescale process parameters, must be carefully managed [145].

**Integration of Artificial Intelligence (AI) with Nanotechnology**
**AI in Pharmaceutics and Drug Delivery**

Recently, pharmaceutics and drug delivery have taken on a greater role in pharmaceuticals since current molecular commodities have taken longer to develop, cost more, and are less productive. Even Nevertheless, the creation of new formulations is still based on time-consuming, costly, and unreliable conventional trials. 'Computational pharmaceutics,' a novel system born from the exponential rise in computer power and algorithms over the last decade, proposes a fundamental shift in the way drugs are delivered by merging big data with artificial intelligence (AI) and multiscale modelling. Pre-formulation physical and chemical qualities and predicting activity, in vitro release, physical stability, in vivo pharmacokinetic parameters and in vivo–in vitro correlation [194] are now being used to apply AI methodologies to pharmaceutical product development. Using machine learning, Run Han and colleagues predicted the physical stability of solid dispersions at 3 and 6 months [195]. By 2021, Hanlu Gao and colleagues used machine learning to study the dissolution of solid dispersions. After five-fold cross-validation, the "spring-and-parachute" and "maintain supersaturation" dissolution profiles could be distinguished using a random forest algorithm with an accuracy, sensitivity, and specificity of 85%. Using 5-fold cross-validation, a regression model with a mean absolute error of 7.78 percent was built using the random forest technique [194].

**Applications of AI in the Development and Optimization of Nanocarriers**

Multiple receptors in the body are a current problem with medication delivery, resulting in decreased performance of a specific function [196]. Because nanocarriers may be functionalized to target certain disease-specific cells, they can avoid toxicities from being activated in healthy cells [197]. The size, shape,

chemical composition, and surface qualities of nanocarriers all have a role in drug delivery. It is, nonetheless, a difficult task to prepare the ideal nanocarrier DDS. AI and computational techniques may be used to help in the optimization of nanocarrier–drug compatibility by evaluating drug loading, drug retention, and formulation stability. The methods and results of nanotechnology experiments are changing dramatically. Currently, many labs employ automated techniques; however, the expansion of nanocarriers and AI-based databases holds great potential for translation. With the goal of merging automation and AI, therapeutic nanocarriers tailored to particular cell types and patients have the potential to improve [199]. First and foremost, studies of nanocarrier DDSs have examined I nanocarrier synthesis and conformation, (ii) nanocarrier transport and interactions, and (iii) nanocarrier surface characteristics and adsorption on diverse surfaces [200]. [200?].

In vitro, in vivo, and in disease regions, nanocarriers' characteristics are being tested in a rising number of experiments. To anticipate nanocrystals in 2020, Yuan He and his colleagues employed machine learning techniques. Wet ball milling and anti-solvent sedimentation procedures are among the 910 particle size data and 310 PDI data that were included in the study. Nanocrystals generated by high-pressure homogenization and wet ball-milling processes performed well in the LightGBM models [194]. It is also possible to reduce the need for several tests with diverse medication combinations by employing cost-effective theoretical computational procedures Most commonly utilised are molecular dynamics and Monte Carlo simulations, both of which fall within the theoretical category. As a result, simulations may be used to clarify quantitative measures that are difficult to collect experimentally. Determining which nanocarrier scaffold is most appropriate for a given application is difficult [201]. In addition, each nanocarrier may be tailored to perform in a certain way. The development of a repository that aids researchers in identifying an appropriate nanocarrier scaffold and its functional groups for particular drug encapsulation and release would be a significant step forward in this respect The "Collaboratory for Structural Nanobiology" [202] has made efforts to develop a database repository of nanocarriers, where scientists may acquire 3D structures and physical and chemical characteristics. Similar to the Protein Data Bank, this resource provides an overview, organisation, and verification of these nanocarrier structures, making it possible to link their structural data to their toxicological, physical, chemical, and biological properties. and effects. The Nanomaterial Registry is another another archive for all of the accessible literature on various types of nanocarriers, such as metallic nanocarriers, polymers, and dendrimers. An extensive database devoted solely to the safety of nanomaterials is eNanoMapper.

**AI Problems in the Development and Optimization of Nanocarriers and Pharmaceuticals**

Because of the recent advancements in AI technology, medications and nanocarriers may now be rationally designed and optimised. The effective use of diverse AI algorithms has reduced development time, ensured product quality, and facilitated successful pharmaceutical research and development. When implementing machine learning algorithms, a common issue is data loss. This issue arises as a result of the high costs of pharmacological trials and the

extensive time spent on research, planning, and optimization by major pharmaceutical corporations. Furthermore, those who are satisfied with the performance of machine learning models but also want to understand how they function are no longer satisfied. The creation of pharmaceutical formulations can be better understood with the use of interpretable machine learning algorithms. Research & development in pharmaceuticals will benefit from more integration between the pharmaceutical sector and AI approaches in the future [194]. The lack of nanocarriers in the 3D atom may also present researchers with the option to conjugate nanocarriers with diverse functional groups, which might lead to new discoveries. Researchers might use such a resource to quickly choose the best scaffold for molecular simulations [204]. In addition, there is a critical shortage of data analysts and handlers [200].

## Conclusions

Nanocarriers are new platforms for minimising toxicity, increasing effectiveness, and achieving targeted medication delivery. As a result of the decades-long development of hundreds of nanocarrier formulations, multiple in vitro and in vivo characterization methods now exist. Standards for regulating regulatory clearance of these cutting-edge devices have grown increasingly difficult as a result. Nanocarrier-based formulations may be easier to distribute to global markets if certain concerns are taken into account. First, the toxicity of nanoformulations should be studied in relation to their administration, biodistribution, metabolism, and elimination. As a second step, it is necessary to improve features of the nanocarriers' scalability, characterisation, and batch-to-batch repeatability. A collaborative effort among scientists, regulatory agencies and industry is essential to the rapid development of nanocarrier DDS that is effective, safe and stable. Computer simulation and artificial intelligence will never replace laboratory tests, but they play a critical role in speeding up and enhancing target and drug discovery procedures and generating new, effective, computational methods for nanoparticle and DDS simulations. In addition, more researchers who are interested in processing and interpreting data are urgently required.

## Future Perspectives

Applicants for future work involving tailored medication delivery can now be recruited by nanocarriers thanks to improvements in nanoparticle surface technology. When it comes to the diagnosis, prevention, and treatment of sickness, nanotechnology has a significant impact. It is now possible to diagnose and/or treat several forms of cancer using nanocarriers that have been given the green light for usage in clinical trials. Various formulations are also being tested in various phases of clinical testing. Passive targeting, active targeting, solubility, and triggered release are all options for medication delivery using nanocarriers. In addition to increasing therapeutic efficacy, nanocarriers reduce the effective dose and reduce the risk of systemic, unwanted consequences. Biological challenges, large-scale manufacture, biocompatibility and protection, intellectual activity, authority rules, and overall cost efficiency in comparison to present medicines were all addressed as key obstacles related with the clinical development of nanocarriers.

They included. When using nanomedicines, it is best to design a treatment plan for each patient specifically based on their unique genetic and medical profiles. For therapeutic usage, scientists should explore lowering the complexity of nanocarriers and considering the ultimate dose form for human use. Due to new designs for nanoparticle systems, it is now possible to create vaccinations with improved efficacy by addressing issues in immunology and vaccine development using advances in nanotechnology. Toxicity difficulties arise when nanodrugs accumulate in tissues they are not supposed to be there. The bioavailability of nanoparticles after systemic injection must thus be taken into account in clinical research. The use of nanocarriers in vaccinations against SARS-CoV-2 (the virus that causes COVID-19) has recently been extensively studied, with some effective late-stage clinical tests. Companies like Moderna and BioNTech use nanocarriers to encrypt mRNA for the COVID-19 allergen with the use of nanocarriers. Phase III clinical testing conducted by Moderna and BioNTech/Pfizer since 30 November 2020 have met their primary efficacy cutoffs, and the companies are now requesting an immediate user authorisation. Solid tumor therapy is in need of more sensible combinations of chemotherapeutic medicines and nanocarriers, based on recent findings that clearly imply that nanomaterials have a bright future. Radiopharmaceuticals must also be created to target cancer cells at the nanoscale level. An essential area for future research will be the development of nanomedicine safety evaluations, as well as the possible economic ramifications of this technology.

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