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Review Article

NANO-ENABLED THERAPEUTICS: EXPLORING BIOEQUIVALENCE AND BIOAVAILABILITY FRONTIERS

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ABSTRACT

The advancement of nanotechnology has significantly transformed pharmaceutical sciences, especially in enhancing the bioavailability and bioequivalence (BE) of drugs with poor solubility and permeability. Nanoparticulate drug delivery systems including nanoparticles, nanocrystals, nanoemulsions, and liposomes exhibit unique physicochemical properties such as increased surface area, improved dissolution rates, and enhanced permeability. These characteristics collectively contribute to superior therapeutic efficacy. For example, a 25-fold increase in the bioavailability of poorly soluble drugs such as retinoic acid has been reported by the use of solid lipid nanoparticles (SLNs), which act as protective lipid matrices, augmenting drug stability and allowing sustained release. This comprehensive review explores the fundamental concepts of bioavailability and BE as they pertain to nanoformulations, focusing on key factors that influence the absorption, distribution, metabolism, and excretion of nanosized drugs. It emphasizes the mechanistic pathways by which nanocarriers overcome biological barriers, including the gastrointestinal (GI) tract and first-pass metabolism, thereby improving pharmacokinetic (PK) profiles. Furthermore, the review discusses analytical methodologies and regulatory considerations critical to evaluating the BE of nanoformulated drugs. It highlights challenges in establishing equivalency arising from altered pharmacokinetic and pharmacodynamic (PD) behaviors unique to these formulations. A comparative analysis of conventional versus nanoformulated drugs illustrates the clinical implications of nanotechnology in drug delivery, such as dose reduction, improved patient compliance, and minimized adverse effects. Additionally, recent advancements in formulation strategies and characterization techniques are synthesized, showcasing how surface modifications, size optimization, and targeting moieties enhance oral bioavailability. By evaluating preclinical and clinical studies, this article provides valuable insights into the translational potential of nanoformulations. It also identifies existing gaps and future directions in regulatory frameworks and standardized BE testing. In summary, this review offers a holistic understanding of nanotechnology's role in improving drug bioavailability and BE, serving as an essential resource for pharmaceutical scientists, clinicians, and regulatory authorities aiming to leverage nanoformulations for enhanced therapeutic outcomes. Despite notable progress, significant challenges remain especially in achieving scalable manufacturing, ensuring reproducibility of formulations and navigating complex regulatory landscapes. These challenges stress on the urgent need for internationally harmonized standards and robust quality control systems to support clinical translation.

Keywords: Nanoformulations, Bioequivalence, Bioavailability, Pharmacokinetics, Regulations

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INTRODUCTION

Creating efficient drug delivery systems remains a cornerstone of pharmaceutical research, aimed at enhancing therapeutic efficacy and improving patient adherence. For this review, a systematic literature search was conducted across PubMed, Science Direct, Web of Science, Scopus and Google Scholar to identify relevant studies on nanoparticle-based drug delivery. Keywords such as nanoformulations, bioequivalence, bioavailability enhancement, physiologically based pharmacokinetic (PBPK) modeling, regulations and bioequivalence harmonization were made use of. Publications from 2013–2024 were prioritized, with only about 10% of earlier works included before that. Peer-reviewed articles, systematic reviews, meta-analyses and regulatory guidelines with experimental or clinical data were majorly taken into consideration. Non-English, non-peer-reviewed sources, inaccessible full texts and unrelated studies were excluded. This structured approach ensured inclusion of high-quality, relevant literature for a comprehensive review.

A major challenge in drug development is the poor aqueous solubility and limited bioavailability of many biopharmaceutics classification system (BCS) class II and IV compounds, often leading to inadequate GI tract dissolution and reduced absorption. Traditional methods such as salt formation, micronization, and surfactant use provide marginal improvements and fail to comprehensively address these issues [1, 2]. Over 40% of newly developed drug candidates demonstrate inadequate water solubility, which presents a major obstacle to achieving optimal oral bioavailability and therapeutic efficacy. Conventional approaches like salt formation, micronization, and the use of surfactants have been widely utilized to enhance the solubility and bioavailability of poorly water-soluble drugs, but their effectiveness is often modest. For instance, salt formation typically improves solubility by only 2- to 3-fold, and it is unsuitable for drugs lacking ionizable groups or those unstable as salts. Likewise, micronization, which reduces particle size to the micrometer range, typically results in a 1.5-to 2-fold increase in oral bioavailability; however, this improvement is often limited by challenges such as poor wettability and particle aggregation.

Nanotechnology offers a promising solution. By manipulating drug particles at the nanoscale, it enhances drug physicochemical properties and PK performance. For example, SLNs serve as protective lipid matrices that stabilize drugs and enable sustained release, with studies reporting up to 25-fold increases in bioavailability for poorly soluble molecules like retinoic acid [3]. Mucoadhesive polymeric nanoparticles, especially those incorporating thiolated chitosan, enhance drug residence time at the intestinal epithelium and significantly improve the uptake of macromolecules by promoting close contact with the mucosal surface and facilitating paracellular transport [4]. A study conducted investigated the use of polyethylene glycol (PEG) coated zein nanoparticles for oral insulin delivery. The researchers found that these mucus-permeating nanoparticles significantly improved insulin absorption compared to their mucoadhesive counterparts. The PEGylation of nanoparticles facilitated their diffusion through

the mucus layer, allowing them to reach the intestinal epithelium more effectively, thereby enhancing the bioavailability of insulin [5]. The selection of nanocarriers is based on their ability to overcome specific physiological barriers. PEGylated zein nanoparticles use a hydrophilic surface to reduce mucus interaction, enhancing their ability to penetrate the mucus layer and reach epithelial cells. In contrast, thiolated chitosan facilitates drug transport by temporarily opening tight junctions through disulfide bond formation with mucin. These mechanisms improve drug absorption and bioavailability, especially for orally delivered therapeutics. Another effective nanotechnological strategy is the development of nanocrystals, which, due to their increased surface area, significantly improve dissolution rates and oral absorption of drugs such as curcumin [6]. Comprehensive strategies integrating particle size, surface properties (charge, hydrophobicity) and the use of ligands or stimuli-responsive coatings can further optimize oral bioavailability and enable targeted delivery [7]. Passive targeting via the enhanced permeability and retention effect remains a foundational aspect of nanoparticle mediated drug delivery, however recent reviews emphasize its heterogeneity and advocate for integrating active transport mechanisms such as endothelial transcytosis to improve efficacy and clinical translation [8]. However, challenges remain, including overcoming the mucus and enzymatic barriers of the GI tract, ensuring nanoparticle stability during transit, and managing transport variability across patient populations [9]. Collectively, the application of these nanotechnological approaches marks a significant advancement in oral drug delivery, offering robust solutions to multifaceted pharmaceutical challenges.

Bioavailability serves as a vital PK indicator reflecting how efficiently and rapidly an active pharmaceutical ingredients (APIs) is absorbed into the systemic circulation. In the context of oral administration, optimizing bioavailability is particularly challenging due to multiple physiological barriers, including poor aqueous solubility, degradation by GI enzymes, extensive first-pass metabolism in the liver and restricted permeability across the intestinal epithelium [10, 11]. These limitations necessitate advanced drug formulation approaches to enhance systemic absorption and therapeutic impact. Conventional PK measures such as the total drug exposure over time, the highest concentration of the drug observed in the blood and the time taken to reach this peak concentration are commonly used to assess how much and how quickly a drug becomes available in the systemic circulation. These parameters provide essential insights into the rate and extent of systemic drug absorption [12]. Liquid chromatography coupled with tandem mass spectrometry is a highly sensitive analytical technique widely employed for the quantification of drugs in biological matrices. Its precision and sensitivity are particularly beneficial when assessing nanoformulated drugs, where low doses and targeted delivery necessitate accurate measurement [13]. BE is the demonstration that two drug formulations provide comparable levels of systemic drug exposure, ensuring similar therapeutic effects and safety profiles. Regulatory agencies such as the U. S. food and drug administration (FDA) and the european medicines agency (EMA) require that key PK measures, including the total drug exposure over time and the peak concentration, fall within an acceptable confidence range to confirm BE [14, 15]. Nanotechnology has added new dimensions to traditional bioavailability and BE assessment. Nanocarriers such as liposomes, micelles and polymeric nanoparticles can enhance drug absorption by protecting drugs from degradation in the GI tract, increasing permeability across mucosal barriers, and promoting lymphatic uptake, thereby potentially bypassing first-pass metabolism [16, 17]. Nanoformulated drug products frequently display ADME profiles compared to traditional formulations. These differences complicate the application of conventional BE criteria. As a result, regulatory bodies increasingly recommend integrating advanced methods such as PBPK modeling, *in vitro–in vivo* correlation (IVIVC), and non-invasive imaging techniques, to better evaluate therapeutic equivalence for nanomedicines [18, 19]. Comprehensive physicochemical characterization of nanoparticle systems including assessments of particle size, surface charge (zeta potential), drug loading efficiency, and stability is essential to ensure consistent performance, reproducibility and therapeutic efficacy. These parameters critically influence nanoparticle behavior in biological environments, affecting biodistribution, cellular uptake, and overall treatment outcomes [20, 21]. Table 1 provides an overview of nanocarrier system with mechanism of bioavailability enhancement and limitations.

Table 1: Overview of nanocarrier systems and their role in enhancing bioavailability

Nanocarrier type	Mechanism of bioavailability enhancement	Example drug/formulation	Key limitations	Reference
Polymeric nanoparticles	Provide sustained release and enhance intestinal permeability	Paclitaxel-loaded PLGA nanoparticles	Potential polymer-associated toxicity; challenges in large-scale manufacturing	[22]
Solid lipid nanoparticles (SLNs)	Bypass hepatic first-pass metabolism; improve drug solubility	Simvastatin SLNs (in Phase II clinical trials)	Risk of drug expulsion during storage; polymorphic transitions affecting stability	[23]
Liposomes	Improve drug stability; encapsulate both hydrophilic and lipophilic drugs	Liposomal doxorubicin	Low encapsulation efficiency for hydrophilic compounds; susceptibility to degradation	[24]
Nanoemulsions	Increase surface area for absorption; enhance solubility	Ibuprofen nanoemulsion	Physical instability; high surfactant load; limited suitability for oral delivery	[25]
Nanocrystals	Enhance dissolution rate and saturation solubility	Fenofibrate nanocrystals	Risk of aggregation; burst release; limited control over drug release profiles	[26]
Micelles	Improve solubility of poorly water-soluble drugs	Cyclosporin A micellar formulation	Dilution instability; premature release in gastrointestinal fluids	[27]

Nanocarrier systems for enhancing oral drug bioavailability

By overcoming inherent limits including low solubility, poor permeability, and enzymatic degradation in the GI tract, the development of nanocarrier based drug delivery systems has changed the field of oral therapeutics [28, 29]. Unique physicochemical properties of these nanoscale platforms like polymeric nanoparticles, solid lipid nanoparticles, liposomes, nanoemulsions and nanocrystals increases drug absorption and general bioavailability [30].

Polymeric nanoparticles protect drugs from severe GI conditions by means of biodegradable polymers, thus enabling controlled and continuous drug release. Their mucoadhesive characteristics increase drug residence time at absorption sites, thus promoting improved absorption [31]. Combining the advantages of lipid-based carriers with nanoscale size, SLNs increase drug solubility and stimulate lymphatic flow through chylomicron-mimicking uptake of the lipid matrix. SLNs improve systemic drug exposure and offer higher formulation stability by avoiding first-pass metabolism [32]. Comprising one or more phospholipid bilayers, liposomes are spherical vesicles that can encapsulate both hydrophilic and lipophilic drug molecules. Their dual-loading capacity makes them quite flexible for uses in drug delivery. Liposomes have several benefits in the framework of oral drug delivery.

They maintain the integrity of drugs as they pass through the GI tract by shielding (APIs) from being broken down by intestinal enzymes and stomach acids. Because their phospholipid bilayers are compatible with cellular membranes, liposomes can fuse directly with epithelial cell membranes, enhancing drug uptake. Additionally, because of their

structural resemblance to biological membranes, they can fuse with intestinal epithelial cells to improve drug absorption. Targeted delivery and increased stability in the GI environment can be achieved through functional modifications like ligand attachment or PEGylation [33]. By increasing the interfacial surface area, nanoemulsions—fine oil-in-water or water-in-oil dispersions with nanosized droplets improve drug solubilisation [34]. Additionally, they temporarily open tight junctions, which makes paracellular drug transport easier. For drugs that are poorly soluble in water, nanocrystals like carrier-free nanosized drug particles significantly increase dissolution rates and saturation solubility [35].

By boosting the drug's effective surface area for dissolution, shielding it from enzymatic breakdown, enhancing intestinal epithelium penetration, and modifying efflux transporters like P-glycoprotein (P-gp), nanocarriers mechanistically improve bioavailability [36]. By avoiding the mononuclear phagocyte system (MPS), surface functionalization of nanoparticles with hydrophilic polymers like PEG not only lengthens the systemic circulation time but also increases colloidal stability in physiological settings. Furthermore, receptor-mediated endocytosis in particular tissues or cells is made possible by the conjugation of targeting ligands, such as folic acid, peptides or monoclonal antibodies. This enhances drug accumulation at the target site and reduces off-target effects. The therapeutic efficacy of formulations based on oral and systemic nanocarriers is greatly increased by this dual approach of PEGylation and active targeting [37]. Nanocarrier-based drug delivery systems have been shown in preclinical and clinical studies to significantly improve PK parameters, such as increased systemic drug exposure over time and higher maximum plasma concentration. When poorly soluble medications are reformulated with nanosystems such as lipid nanoparticles, polymeric micelles and nanoemulsions, these improvements are particularly noticeable [38]. Better patient compliance and less frequent dosing may result from these enhancements. Notwithstanding these benefits, obstacles like formulation stability, scalable manufacturing, and strict regulatory requirements still prevent broad clinical adoption. Long-standing obstacles in the development of nanomedicines are being addressed by recent advancements in nanomanufacturing methods and changing regulatory frameworks [39]. These developments are ultimately broadening the use of nanotechnology in therapeutic applications by facilitating more reliable quality control, scalable production and more transparent regulatory pathways.

Mechanisms enhancing drug availability via nanotechnology

Nanotechnology has greatly improved oral drug delivery by removing several physiological and biochemical obstacles that restrict drug bioavailability. Nanocarriers improve drug solubility, permeability, protection and systemic delivery by adjusting particle size, surface chemistry and formulation techniques. This is especially true for substances with subpar biopharmaceutical qualities. These advancements are substantiated by recent research.

Increased surface area and accelerated dissolution

According to the Noyes–Whitney equation, reducing drug particles to the nanoscale greatly increases the rate of dissolution because of an increased surface area [40-42]. This increase in dissolution makes passive diffusion across the intestinal epithelium easier, creating a greater concentration gradient.

Elevated saturation solubility

Drug formulations based on organic nanoparticles such as lipid-based carriers (e. g., SLNs, nanostructured lipid carriers) and polymeric matrices (e. g., PLGA, chitosan or PEG-based nanoparticles) and nanocrystals change the surface energy of the APIs, improving their bioavailability, wettability and rate of dissolution. These formulations improve the drug's interaction with biological fluids by decreasing interfacial tension and increasing the surface area-to-volume ratio by reducing particle size to the nanometer scale [35, 43].

Prolonged mucoadhesion and retention

Mucoadhesive nanocarriers that contain polymers like carbopol and chitosan are made to work closely with the mucosal layer of the GI tract. This mucoadhesion promotes sustained, site-specific drug release, decreases rapid drug clearance and extends the formulation's residence time. All of these processes work together to increase the oral bioavailability of different medicinal substances [44-46].

Facilitated transcellular and paracellular transport

By utilising endocytic pathways such as clathrin-mediated, caveolin-mediated, macropinocytosis and receptor-mediated endocytosis, nanoparticles dramatically improve transcellular transport. This makes it possible for therapeutic agents that are enclosed in or affixed to nanoparticles to get around conventional paracellular restrictions and efflux processes, like those that are mediated by P-gp and other ATP-binding cassette transporters. Targeting particular cell-surface receptors (such as transferrin, folate, insulin or low-density lipoprotein receptors), ligand-functionalized nanoparticles specifically encourage receptor-mediated endocytosis, which enhances the cellular uptake of the drug payload in a site-specific and energy-dependent manner. These nanoparticles aid in achieving increased intracellular drug concentrations and enhanced bioavailability by evading recognition and removal by efflux pumps, especially in tissues with tight epithelial barriers or drug-resistant cells [47-49]. However, a critical limitation of receptor-mediated transcellular transport is the potential for endosomal and lysosomal degradation of the internalized nanoparticles, which may lead to reduced therapeutic efficacy or increased intracellular toxicity if the payload is not released efficiently before enzymatic breakdown. Overcoming endosomal entrapment is crucial for effective intracellular delivery of nanocarrier-based therapeutics. One approach involves using pH-responsive materials like poly (histidine) or which destabilize endosomal membranes under acidic conditions to promote drug release. The proton sponge effect**,** commonly achieved with polymers such as PEI, leads to endosomal swelling and rupture by buffering acidic pH. Additionally, fusogenic peptides and light-activated systems generate structural or chemical disruptions to aid escape. These mechanisms significantly improve cytosolic delivery and enhance the overall effectiveness of nanoparticle-based treatments.

Lymphatic uptake and first-pass metabolism avoidance

SLNs, nanoemulsions, and self-emulsifying drug delivery systems (SEDDS) are examples of lipid-based nanocarriers that have shown great promise in improving the systemic bioavailability of medications that are poorly soluble in water. These formulations successfully avoid hepatic first-pass metabolism by facilitating the lymphatic transport of lipophilic compounds through the intestinal lymphatic system. Longer systemic circulation and increased plasma drug concentrations are the outcomes of this mechanism. However, particle size plays a crucial role in determining lymphatic uptake efficiency; nanoparticles larger than 100 nm may evade rapid clearance by the MPS but tend to show diminished uptake into the lymphatic system. Lipid composition, droplet size, polarity and the degree of association with chylomicrons after absorption all affect lymphatic uptake efficiency. Notably, SEDDS support the lymphatic transport of highly lipophilic drugs by spontaneously emulsifying in GI fluids and promoting preferential uptake via lacteals [50, 51].

Protection against gastrointestinal degradation

The GI tract's acidic pH and enzymatic activity make oral administration of therapeutic peptides and proteins difficult, resulting in degradation and decreased bioavailability. Nanocarrier systems, such as SEDDS, polymeric nanoparticles, and lipid-based nanocarriers, have been developed to get around these obstacles. By encasing therapeutic agents, these nanocarriers successfully protect them from proteolytic enzymes and acidic stomach environments. For instance, hydrophobic ion pairs of proteins and peptides can be incorporated into lipid-based nanocarriers to lessen their susceptibility to enzymatic degradation. Furthermore, some nanocarriers have mucoadhesive or mucus-penetrating qualities that lengthen their time in the GI tract and promote better absorption [52].

Modulation of efflux transporters and enzymatic barriers

It has been demonstrated that polymeric nanoparticles minimise drug efflux and enzymatic degradation by reducing metabolic

enzyme activity and modulating efflux transporters like P-gp. This dual action enhances the absorption and bioavailability of drugs that are substrates for efflux pumps and metabolizing enzymes in the GI tract [53, 54].

Improved stability and controlled release

By stabilising labile medications against oxidation, hydrolysis and enzymatic breakdown, nanoparticles can maintain their therapeutic potential. Additionally, site-specific and sustained drug release is made possible by the design of controlled or stimuli-responsive nanocarriers, which maximises therapeutic efficacy while reducing systemic side effects.

For instance, polymeric and lipid-based nanoparticles have been shown to protect drugs from aqueous and oxidative environments, while pH-sensitive and redox-responsive systems ensure triggered release in response to intracellular cues. These systems help to enhance bioavailability and PK [55, 56]. Summary of nanotechnology based mechanisms that could enhance drug bioavailability is depicted in table 2.

Table 2: Summary of nanotechnology based mechanisms enhancing drug bioavailability

Mechanism	Benefit	References
Increased surface area and dissolution	Enhances dissolution rate and promotes better drug absorption	[57]
Elevated saturation solubility	Improves solubility in gastrointestinal fluids due to nanoscale size; explained by the Ostwald–Freundlich equation, where smaller particles exhibit greater saturation solubility owing to increased surface curvature and energy	[58, 59]
Mucoadhesion and retention	Prolongs gastrointestinal residence time and improves localized absorption; polymers like chitosan bind to mucosa via electrostatic interactions	[60]
Transcellular and paracellular transport	Enhances permeability by facilitating both transcellular and paracellular pathways and overcoming efflux mechanisms	[61, 62]
Lymphatic uptake	Bypasses hepatic first-pass metabolism, resulting in increased systemic bioavailability	[63]
Protection from GI degradation	Shields the drug from degradation in acidic and enzymatic gastrointestinal environments	[64, 65]
Efflux and enzyme modulation	Minimizes drug efflux and metabolic breakdown by modulating efflux transporters and metabolic enzymes	[66, 67]
Stability and controlled release	Enhances physicochemical stability and allows for sustained and targeted drug release	[68, 69]

Analytical and regulatory challenges in nano bioequivalence testing

Comparing BE to traditional pharmaceutical formulations has become much more difficult with the introduction of nanotechnology-based drug delivery systems. Traditionally, BE is assessed using PK parameters such as maximum plasma concentration and area under the curve (AUC) to determine the rate and extent of drug absorption. However, the unique physicochemical characteristics of nanoformulations, such as liposomes, polymeric nanoparticles and lipid-based carriers, such as particle size, surface charge, and encapsulation efficiency, have a significant impact on their behaviour *in vivo*. These characteristics can affect targeted delivery, biodistribution, controlled release and absorption pathways (like lymphatic uptake), all of which conventional PK measurements might miss. Furthermore, nanomedicines are frequently categorised as Non-Biological Complex Drugs (NBCDs), meaning that minor formulation modifications can have a big impact on clinical outcomes. Regulatory bodies like the FDA and EMA understand that the traditional BE paradigm is frequently inadequate for these kinds of products. To guarantee therapeutic equivalency, a totality-of-evidence approach is thus becoming more and more necessary, encompassing physicochemical characterisation, Critical Quality Attributes (CQAs) evaluation, biodistribution profiling and occasionally clinical endpoint studies [70, 71].

Analytical challenges

Comprehensive nanoparticle characterization

To guarantee safety, efficacy, and reproducibility, drug delivery nanoparticles need to be precisely physicochemically characterised. Key factors include morphology, which influences release profiles and biological interactions, drug loading capacity that determines therapeutic payload, zeta potential which indicates surface charge and stability and particle size that influences cellular uptake and biodistribution. Common analytical methods include transmission electron microscopy (TEM) for high-resolution visualisation of shape and surface structure, dynamic light scattering (DLS) for hydrodynamic size and zeta potential and nanoparticle tracking analysis (NTA), which tracks individual nanoparticles to provide size distribution and concentration metrics. Comprehensive characterisation, which is essential for formulation optimisation, is made possible by these complementary approaches [72, 73]. Additionally, spatial techniques like Mass Spectrometry Imaging (MSI) can be employed to differentiate between nano-encapsulated and free drug forms within tissues, enabling precise assessment of drug distribution at the cellular and subcellular levels.

Drug quantification in complex biological matrices

Differentiating between free and nanoparticle-bound drug fractions in biological samples is crucial because nanoparticles significantly change a drug's PK and tissue distribution. One reliable method is ultrafiltration, which separates the drug's low molecular weight free form from forms that are attached to nanoparticles or proteins. For extremely sensitive and specific quantification, liquid chromatography tandem mass spectrometry (LC MS/MS) is then used. For example, this combined approach has been used to reliably identify unbound docetaxel or paclitaxel in plasma, showing consistent precision and reproducibility. Researchers also developed solid phase extraction with LC MS/MS in liposomal formulations like pegylated doxorubicin, and compared the results to ultrafiltration, to quantitatively separate and measure free versus encapsulated drug, which is important for assessing PK parameters and safety profiles [74, 75].

IVIVC challenges

Nanoformulations have complex release mechanisms, including burst, diffusion, and degradation phases, which are frequently formulation-specific, making it particularly difficult to establish IVIVC. First, there is a lot of batch-to-batch variability in *in vitro* release profiles because they are very sensitive to factors like temperature, media composition and sampling techniques. Second, method development is made more difficult by the lack of standardised regulatory guidance for parenteral nanoparticles. Third, these formulations often go through multi-step physiological processes that are not captured by straightforward *in vitro* assays, such as tissue-specific uptake, enzymatic degradation, and protein corona formation. As a result, real-world PK profiles are not captured by standard IVIVC models used to predict *in vivo* behaviour. Fit-for-purpose, multistage release models and sophisticated analytical tools are frequently needed to address this and produce data that is clinically relevant [76]. To enhance the predictability of IVIVC models, the use of biorelevant dissolution media such as fasted-state simulated gastric fluid (FaSSGF) and fed-state simulated intestinal fluid (FeSSIF) is strongly recommended, as they better simulate human GI conditions.

Regulatory challenges

Defining appropriate reference standards

Proprietary nanostructures are frequently present in innovator products, which makes equivalency evaluation more difficult. Biodistribution, release kinetics, and therapeutic results can be

greatly impacted by their particular particle architectures, surface coatings, or internal design elements, which are usually not revealed. Consequently, without access to the original design, generic or follow-on versions (nanosimilars) find it difficult to reproduce these CQAs. Instead of depending only on conventional BE metrics, regulatory agencies such as the FDA and EMA now demand a totality-of-evidence approach that includes advanced physicochemical characterisation, non-clinical biodistribution data, and occasionally clinical studies. This intricacy is similar to the difficulties encountered with NBCDs, where subtleties in manufacturing have a direct impact on clinical equivalency [77-79]. To improve reproducibility and comparability of evaluations, the development and adoption of standard nanocarrier references, such as those proposed by national institute of standards and technology (NIST), are crucial.

Altered PK and BE metrics

Because of their size, surface characteristics, and interactions with biological systems, nanoformulations often show different PK profiles from traditional medications. ADME processes are frequently changed as a result of these distinctive traits. Therefore, it might not be possible to completely describe their *in vivo* behaviour by depending only on conventional PK endpoints like Cmax and AUC. To thoroughly assess the therapeutic efficacy and safety of medications made with nanotechnology, longer endpoints like tissue distribution, cellular uptake and extended circulation times are becoming more and more important [80].

Lack of harmonized guidelines

Globally, regulatory guidelines for evaluating the BE of nanomedicines are still being developed. Agencies are aware that traditional BE evaluation techniques are challenged by the special complexity of nanoformulations, including their varied structures and interactions with biological systems. Although there has been progress, there are still no standardised, globally recognised standards for nano-BE, which causes differences in regulatory expectations and approval processes across the globe. This demonstrates the continued necessity of teamwork in creating thorough and uniform regulatory frameworks specifically designed for medications based on nanotechnology [81].

Table 3: Key challenges and strategies

Challenges	Strategic approaches	Validated analytical approaches	References
Variable nanoparticle attributes	Use of advanced characterization tools	DLS, Zeta potential analysis, SEM/TEM, FTIR, XRD	[84, 85]
Quantification complexity	Adoption of sensitive and selective quantification techniques	LC-MS/MS, HPLC, ELISA	[86, 87]
Difficult IVIVC	Integration of biorelevant dissolution methods and PBPK modeling	PBPK modeling, IVIVE, IVIVC computational tools	[88-90]
Lack of global BE standards	Promotion of harmonized regulatory initiatives	Simulation-based BE studies, FDA/EMA draft guidance implementation	[91]
Immunogenicity/toxicity risk	Comprehensive preclinical safety evaluation	Cytotoxicity assays, immunogenicity screening, *in vivo* animal studies	[92, 93]

Safety and immunogenicity

Because of their small size and large surface area, nanoparticles can interact with the immune system in a variety of ways. Depending on their makeup, surface characteristics, and biodistribution, these interactions may result in immunosuppression, hypersensitivity reactions or even unintentional immune activation. Furthermore, some nanomaterials can build up in organs like the lungs, liver or spleen, which can cause oxidative stress or dose-dependent toxicity. The significance of thorough preclinical assessment of immunotoxicity and biocompatibility in drug delivery systems based on nanoparticles is highlighted by these possible hazards [82, 83]. To proactively identify and manage potential safety concerns, regulatory evaluations should incorporate validated immunotoxicity assays, such as complement activation tests and cytokine release profiling, especially during the early stages of drug development. Table 3 represents the key challenges, strategies and analytical approaches adopted.

Clinical significance of nanoformulated drug bioequivalence

Nanotechnology has transformed pharmaceutical development by addressing solubility challenges, enhancing PK properties, and enabling targeted drug delivery, thereby revolutionizing treatment approaches. Polymeric, liposomal, and smart nanoparticles are examples of nano-enabled drug delivery platforms that provide controlled and sustained release profiles that have important therapeutic applications. These include better therapeutic results due to better targeting, decreased systemic toxicity because medications are precisely routed to diseased tissue and decreased dosing frequency, which improves patient adherence. Recent studies in oncology, for instance, have demonstrated that smart, extended-release nanoparticles allow for multistage targeting and prolonged release, which can support less frequent dosing while preserving efficacy and minimizing side effects. By optimising drug release, increasing solubility, and improving cellular permeability, nanoformulations improve drug ADME. By avoiding quick clearance processes, nanocarriers such as liposomes, SLNs and polymeric micelles are made to allow for controlled or prolonged drug release and to extend systemic circulation. A significant progress in polymeric nanoparticle-based drug delivery systems, highlights their capacity to enhance bioavailability and achieve targeted therapeutic action. Extensive recent advances in polymeric nanoparticle based drug delivery systems have demonstrated their profound capacity to significantly enhance bioavailability and enable targeted therapeutic action [94]. These results help us to understand on the decreased dosing frequency, more stable plasma drug concentrations, and possibly better therapeutic results. This results in increased therapeutic efficacy and possibly lower dosages and intervals. Additionally, stabilizing plasma concentrations facilitates improved disease control. [95-99]. However, this enhanced bioavailability may also pose clinical risks. For instance, nanoformulations like nano-paclitaxel have demonstrated cases of neurotoxicity due to excessive systemic exposure. In such scenarios, therapeutic drug monitoring (TDM) is strongly recommended, particularly for drugs with a narrow therapeutic index, to avoid toxicity while ensuring therapeutic effectiveness.

Selective delivery to diseased tissues is made easier by functionalizing nanoparticles with targeting moieties like aptamers, peptides or antibodies. By preventing drug buildup in healthy organs, this selective targeting lowers side effects and enhances safety profiles [100]. For example, chemotherapeutics based on targeted nanoparticles can selectively deliver cytotoxic agents to tumor sites, thereby reducing off-target toxicity to noncancerous cells. By reducing side effects linked to traditional chemotherapy, this selective action improves treatment adherence and tolerance while also improving patient quality of life [101]. Because nanoformulations increase bioavailability, dosages must frequently be adjusted to account for increased absorption, decreased clearance, and prolonged circulation, which can otherwise result in toxicity or subtherapeutic exposure [102]. This is especially true for medications with limited therapeutic windows. Therapeutic drug monitoring is crucial to ensuring safe and effective therapy because increased systemic exposure can

increase the risk of toxicity. Accurately translating BE data to patient-specific therapeutic needs is essential to its clinical utility. It is also important to critically evaluate the enhanced permeability and retention effect in humans. Although preclinical models suggest significant tumor accumulation of nanoparticles via EPR, clinical studies reveal that typically less than 5% of the administered dose reaches human tumors. This discrepancy highlights the need for caution when designing EPR-reliant targeted delivery systems and underscores the necessity of exploring alternative or complementary targeting strategies.

The complex *in vivo* fate of drugs that are nanoformulated may not be adequately captured by traditional PK parameters such as AUC and Cmax. Integrated evaluation techniques are necessary for characteristics like tissue retention, lymphatic uptake and controlled release. To properly evaluate nano-BE, regulatory bodies are increasingly utilising PD endpoints, sophisticated modelling, and specialised clinical studies. Nanoparticle-based drug delivery systems, significantly enhance drug loading, penetration, and controlled release [103]. The clinical advantages and considerations of nanoformulated bioequivalence is shown in table 4.

Table 4: Clinical advantages and considerations of nanoformulated drug bioequivalence

Clinical aspect	Description	References
Enhanced Pharmacokinetics and Dynamics	Nanocarriers optimize drug release profiles, enhance solubility, and maintain sustained plasma concentrations	[104, 105]
Targeted Delivery and Toxicity Reduction	Ligand-mediated targeting enables site-specific delivery, reducing off-target toxicity and adverse effects	[106, 107]
Dose Adjustment and Therapeutic Monitoring	Improved bioavailability may necessitate dose recalibration and close therapeutic monitoring to ensure safety	[108]
Regulatory and Clinical Challenges	Conventional pharmacokinetic metrics may be inadequate; highlights the need for integrated, model-based tools	[109, 110]

CONCLUSION

Drug delivery has been completely transformed by nanotechnology, which has made it possible to create nanoformulations that greatly increase the bioavailability and therapeutic efficacy of medications, particularly those with low solubility and restricted permeability. Nanocarriers get past biological barriers and produce superior PK profiles, which improve clinical outcomes, by utilising special nanoscale properties like targeted delivery, increased surface area, and improved drug stability. However, evaluating BE and obtaining regulatory approval are extremely difficult due to the complexity of nanomedicines. To guarantee therapeutic equivalency and patient safety, comprehensive physicochemical characterisation, sophisticated analytical techniques, and creative PK and PD study designs are crucial. Harmonised guidelines and advancements in regulatory science will be essential to expediting the approval process for these intricate formulations. In the future, the use of multifunctional nanocarriers that combine therapeutic and diagnostic properties, personalised nanomedicine techniques, and there are encouraging prospects to improve drug delivery even more and increase clinical applications with sustainable manufacturing techniques. The broad use of medications made with nanotechnology will be made possible by overcoming obstacles pertaining to cost-effectiveness, long-term safety assessment, and regulatory standardisation, which will ultimately improve patient care and public health globally. Looking forward, the evolution of nanomedicine is expected to focus on advanced multifunctional nanocarriers that not only deliver drugs but also enable real-time tracking of their distribution and therapeutic effects, a concept referred to as theranostics. Furthermore, personalized treatment strategies that incorporate individual biological differences, such as the influence of the gut microbiota on nanoparticle degradation and drug absorption, are anticipated to enhance therapeutic precision. Overcoming current barriers—such as high production costs, scalability issues, the need for long-term safety evaluation, and the lack of unified regulatory standards—will be essential for integrating nanotechnology more broadly into clinical practice. These advancements will ultimately support the development of safer, more effective, and patient-tailored drug delivery systems, significantly improving global healthcare outcomes.

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Nil

LIST OF ABBREVIATIONS

bioequivalence (BE), gastrointestinal (GI), pharmacokinetic (PK), pharmacodynamic (PD), biopharmaceutics classification system (BCS), solid lipid nanoparticles (SLNs), polyethylene glycol (PEG), food and drug administration (FDA), european medicines agency (EMA), physiologically based pharmacokinetic (PBPK), *in vitro–in vivo* correlation (IVIVC), active pharmaceutical ingredients (APIs), poly lactic-co-glycolic acid (PLGA), p-glycoprotein (P-gp), liquid chromatography tandem mass spectrometry (LC MS/MS), mononuclear phagocyte system (MPS), self emulsifying drug delivery systems (SEDDS), area under the curve (AUC), non-biological complex drugs (NBCDs), transmission electron microscopy (TEM), dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), mass spectrometry imaging (MSI), fasted-state simulated gastric fluid (FaSSGF), fed-state simulated intestinal fluid (FeSSIF), critical quality attributes (CQAs), national institute of standards and technology (NIST), therapeutic drug monitoring (TDM).

AUTHORS CONTRIBUTIONS

Aravinda Pai: Writing-original draft, writing-review and editing, Chandrashekar K S: Review and editing, Vidhi Ansul Saxena: Data curation and editing, Bhavana Bhat B: Review and Editing, Venkatesh Kamath B: Conceptualization, Editing, formal analysis, project administration, validation and visualization.

CONFLICT OF INTERESTS

Authors do not have any competing interests.

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