

Formulation Development And In Vitro Evaluation Of A Novel Drug Delivery System For Enhanced Therapeutic Performance

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Abstract

Most therapeutically potential drugs do not perform optimally in clinical practices because of constraints related to traditional dosage formulations, such as low solubility, bioavailability, instability, and delivery to the sites. Novel drug delivery systems (NDDS) are now an effective approach to overcome such issues, as it allows controlled drug delivery, better pharmacokinetic characteristics and increased therapeutic accuracy. This review critically examines contemporary approaches to formulation development and in vitro evaluation of NDDS with an emphasis on their role in enhancing therapeutic performance. Key formulation design considerations, including drug physicochemical properties, excipient and carrier selection, and targeting or stimuli-responsive strategies, are discussed in relation to their impact on delivery efficiency and biological performance. Advances in fabrication technologies and systematic optimization approaches, particularly Quality by Design and statistical experimental designs, are highlighted as essential tools for achieving robust and reproducible formulations. The review further emphasizes the importance of comprehensive physicochemical and structural characterization in establishing stability, compatibility, and structure–performance relationships. In vitro drug release and biological performance assessment are examined as critical components for understanding delivery mechanisms and guiding formulation optimization, while acknowledging persistent challenges in achieving reliable in vitro–in vivo correlation. Regulatory, manufacturing, and scale-up matters are also taken into account to offer a translational view and extract future prospects of matching formulation innovation with clinical and industrial needs. This review highlights the importance of coordinated, foresighted, and outcome-based development measures to help the successful translation of the new system of drug delivery into effective therapies.

Keywords: novel drug delivery systems, formulation development, in vitro evaluation, drug release kinetics, translational drug delivery

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1. Introduction

Even though there have been tremendous improvements in drug discovery and pharmaceutical research, the traditional system of drug delivery still has serious limitations that limit its use in clinical settings. Most of the therapeutic agents have low aqueous solubility, low membrane permeability, rapid clearance by the body, or are unstable at physiological conditions, which in combination lead to low bioavailability and unreliable therapeutic effects¹. These issues may lead to increased

dosages or frequency of administration, which can further lead to systemic toxicity, adverse effects and diminished patient compliance. These deficiencies can be observed specifically in the management of chronic conditions, cancer, and diseases that demand a high degree of drug targeting, as these areas have continued to have unmet clinical needs due to conventional drug delivery systems². These challenges have been further increased by the increased complexity of modern drug molecules. New chemical entities and biologics have

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unfavorable physicochemical and biopharmaceutical characteristics that are not easily compatible with traditional dosage forms. This has led to the development of drug delivery systems that were initially viewed as mere carriers of drugs to become very important determinants of therapeutic outcome. The failure to achieve sufficient control of drug release, lack of selectivity, and insufficient success in overcoming biological barriers remain as obstacles to clinical translation of many promising drug candidates³. These problems have led to a necessity for new delivery strategies that can enhance drug localization, extend therapy action, and reduce off-target exposure.

The science of formulation is pivotal and facilitative in overcoming such shortcomings by combining drug characteristics with the design of delivery systems to maximize the efficacy of therapy. Formulation development can be employed to improve solubility, prevent the degradation of labile drugs, fine-tune release kinetics and pharmacodynamics and pharmacokinetics through rational selection of excipients, carrier materials and fabrication methods⁴. The recent advances in the field of formulation science have provided an opportunity to create new systems of drug delivery that respond to physiological stimuli, can be targeted to a specific site and can be adjusted to various routes of administration. Such innovations have greatly increased the potential of treating drugs that would not otherwise have been used clinically. Drug delivery research has, over the years, evolved to move beyond traditional formulations with more advanced systems to meet the intricate clinical demands. The trend here is an extension of the move in medicine towards precision and individualized therapy, in which delivery systems are designed to fulfil disease-specific and patient-specific requirements⁵. Nevertheless, it is difficult to successfully translate innovative drug delivery systems despite these developments. The problems of reproducibility of formulations, scale-up ability, regulatory compliance, and predictive evaluation still impede the advancement of most advanced systems out of lab research into clinical practice. In vitro testing has become a vital part of the formulation development process, and these tests offer the necessary information regarding drug release profile, stability and biological interactions before conducting studies in vivo. Strong in vitro testing enables early screening and optimization of formulations to shorten the development process and save on costs and enhance chances of clinical success⁶. Nevertheless, traditional in vitro systems, in most cases, simplify the conditions of the human body and cannot be used to determine performance in the field with much accuracy, which is why more standardized and relevant methods of evaluation are necessary. The discreet choice and elucidation of in vitro tests are thus vital to finding purposeful associations between formulation features as well as therapeutic results.

This review critically discusses the use of formulation development strategies and in vitro evaluation methods used in new drug delivery systems, focusing on their importance in improving the performance of the

therapeutic. Some of the critical design considerations, techniques of formulation, physicochemical characterization, and biological assessment methods are covered within the scope of the review, in addition to the contemporary challenges and future developments. This review aims to review the current amount of knowledge and the recent progress to compile a coherent model of the role of formulation science and in vitro assessment in the development of successful and translational drug delivery systems.

2. Evolution and Classification of Novel Drug Delivery Systems

The development of drug delivery systems is an indication of gradual responsiveness to the drawbacks of the traditional dosage forms and the growing complexity of therapeutic agents. The early methods of drug delivery were mainly concerned with the immediate-release formulations that aimed at reaching the systemic exposure, having little control over the drug distribution and activity. With an increase in knowledge about pharmacokinetics, disease pathophysiology, and biological barriers, the emphasis slowly changed to controlled-release and targeted delivery concepts to increase the therapeutic effect and reduce the adverse effects⁷. This shift was the springboard of the new drug delivery systems (NDDS), which combine materials and engineering with formulation science. The establishment of NDDS has been motivated by the desire to deal with problems related to low solubility, instability, low bioavailability, and non-specific drug distribution of many contemporary drug molecules. The increased variety of delivery platforms due to the late twentieth century and early twenty-first century developments in the fields of polymer science, lipid technology and nanotechnology offered a large number of novel approaches to pharmaceutical delivery⁸. These innovations made the design of carriers that could prevent drug degradation, control the release profile, and enhance the interaction with biological membranes possible. Therefore, drug delivery systems developed as passive vehicles and became multifunctional platforms that can actively affect therapeutic results.

Novel drug delivery systems can be categorically divided according to the carrier type, route of administration and targeting strategy. By the type of carrier, there are lipid-based systems, liposomes and solid lipid nanoparticles; polymeric systems, microspheres and polymeric nanoparticles; inorganic carriers; and multilateral hybrid or composite platforms that use a combination of multiple materials to generate synergistic activity⁹. Of those, nanoparticle-based systems have become of special interest as the size, surface properties, and surface functionalization allow them to control the drug release and the targeted delivery at the cellular and subcellular scales¹⁰. The second classification criterion is the route of administration, which greatly determines the formulation design and therapeutic performance. NDDS are formulated to be delivered orally, parenterally, transdermally, by lung and nose, by eye, and by local site, with each of these routes

being associated with physiological differences and formulation problems¹¹. The route-specific delivery systems are intended to increase the absorption of drugs, patient compliance, and increase the localized or systemic effects of drugs as needed. As an example, oral NDDS are designed to address poor solubility and the first-pass system, whereas parenteral systems are addressed by long circulation time and tissue target. Targeting strategy is another category of classification and functional complexity of NDDS. Passive targeting is based on physiological processes like increasing permeability and retention, and active targeting uses ligand-receptor interactions to deliver drugs directly to the site. Also, the stimuli-responsive systems have been invented which can be induced to emit drugs in reaction to internal or external stimuli like pH, temperature, enzymes or magnetic field, providing spatiotemporal control of drug activity¹². Such methods can be notably

applied in treating cancer, inflammatory diseases, and other diseases that need localized treatment.

The clinical applicability of the various NDDS platforms is that they will enhance therapeutic index, decrease dosing frequency and improve patient outcomes. A number of NDDS have already made it to the stage of clinical application, and this shows the potential of advanced delivery technologies on a translational basis. Nevertheless, safety, scalability, regulatory acceptance and reproducibility are some of the factors that indicate the clinical applicability of each platform. Learning about the historical development and systematic classification of NDDS is thus important to select the formulations rationally and lead to future innovations in drug delivery research. Table 1 summarizes the major classes of novel drug delivery systems and their defining characteristics.

Table 1. Classification of Novel Drug Delivery Systems

NDDS Type	Carrier Material	Typical Size Range	Route(s) of Administration	Key Applications
Liposomes	Phospholipids	50–1000 nm	IV, oral, topical	Cancer, vaccines
Polymeric nanoparticles	Synthetic/natural polymers	10–500 nm	Oral, IV	Oncology, CNS
Solid lipid nanoparticles	Lipids	50–1000 nm	Oral, topical	Dermal, oral
Inorganic nanoparticles	Metals/silica	5–200 nm	IV	Theranostics
Hybrid systems	Multi-material	Variable	Multiple	Precision therapy

3. Design Considerations for Formulation Development

3.1 Drug Physicochemical and Biopharmaceutical Constraints

The design of a novel drug delivery system must be done successfully, and that necessitates a detailed knowledge of the physicochemical and biopharmaceutical properties of the drug substance. The ability to dissolve, molecular size, lipophilicity, ionization constant, and chemical stability are properties that have a strong impact on the formulation feasibility and performance. Low-solubility drugs or those that are poorly permeable tend to have unpredictable absorption and low bioavailability and require formulation strategies to change drug interactions with the biological environment¹³. Moreover, biopharmaceutical limitations (specificity of absorption site, enzymatic degradation, first pass metabolism and half-life of elimination) need to be put into consideration to allow the delivery system to be matched to the desired route of delivery and therapeutic objective. These parameters can be systematically considered, and this will enable formulators to establish possible barriers at an early stage of development and pick the right carriers or technologies to overcome them. The principles of engineering-based design also underlie this process since it allows the regulation of structural and functional properties of the delivery system, including surface area

and porosity, as well as release kinetics, which directly influence the absorption and distribution of the drugs¹⁴. New analytical and biopharmaceutical methods have also enhanced the characterization of drug behavior in physiologically relevant conditions that facilitate more rational formulation decisions and minimizes uncertainty in the development process¹⁵.

3.2 Excipient Selection and Carrier–Drug Interactions

The choice of excipients is a decisive factor with regard to formulation stability, performance and safety. Modern excipients are understood to be active agents in drug delivery and not mere formulation constituents. They have the potential to have a substantial impact on the drug solubilization, degradation protection, and release characteristics due to their physicochemical characteristics and functional properties. Nevertheless, poor drug excipient interactions can lead to chemical instability, dissolution profile or bioavailability that can lead to decreased therapeutic efficacy¹⁶. This means that compatibility studies and mechanistic analysis of the interaction pathways are required during the early formulation development. The process of the selection should also take into account excipient functionality, regulatory status, toxicity, and such selection criteria as large-scale manufacturing. Recent breakthroughs in excipient science have provided multifunctional

materials which can improve the solubility, alter permeability and provide structural integrity to intricate delivery systems¹⁷. These excipients are strategically incorporated into the carrier matrices, allowing the accurate control of the formulation characteristics without affecting the patient safety and the quality of the products.

3.3 Targeting Strategies and Stimuli-Responsive Design

Targeting strategies constitute a further design of formulation with the aim of enhancing the therapeutic accuracy and reducing systemic adverse effects. Passive targeting takes advantage of physiological attributes like increased permeability of diseased tissues, whereas active targeting is based on ligand-mediated binding between the delivery system and particular cellular receptors. These methods allow concentration of drugs at the intended place of action, giving them a preferential accumulation with an increased therapeutic index. Simultaneously, the stimuli-responsive delivery systems have gained strength as a potent means of attaining

controlled and on-demand drug release. They are designed to react to internal stimuli: a pH gradient, redox state or even enzymatic activity, or external ones, such as temperature, light, or magnetic field¹⁸. Stimuli-responsive platforms have been provided to provide spatiotemporal management of drug delivery and are especially useful in cancer and other diseases that need local therapy.

The successful design development requires a systems approach that incorporates drug-specific limitations, excipient functionality, carrier engineering and targeting approach. The systematic and evidence-based approach to these design issues is likely to increase the success rate of creating strong, scalable, and clinically viable innovative drug delivery systems. Table 2 describes how the critical parameters of formulation design affect the performance of delivery and therapeutic outcome. The interdependent design issues that dictate the development of the formulations, such as the drug-related considerations, are depicted in Figure 1, and also the excipient and carrier development and targeting considerations.

Table 2. Key Formulation Design Parameters Influencing Therapeutic Performance

Design Parameter	Representative Examples	Impact on Formulation	Effect on Therapeutic Outcome	Key Challenge
Drug solubility	Poorly soluble APIs	Carrier selection	Enhanced bioavailability	Stability
Excipient choice	Polymers, lipids	Release modulation	Reduced toxicity	Compatibility
Particle size	Nano vs micro	Uptake, clearance	Targeting efficiency	Reproducibility
Surface properties	Charge, ligands	Cellular interaction	Improved efficacy	Immunogenicity
Stimuli responsiveness	pH, enzymes	Controlled release	Site-specific action	Predictability

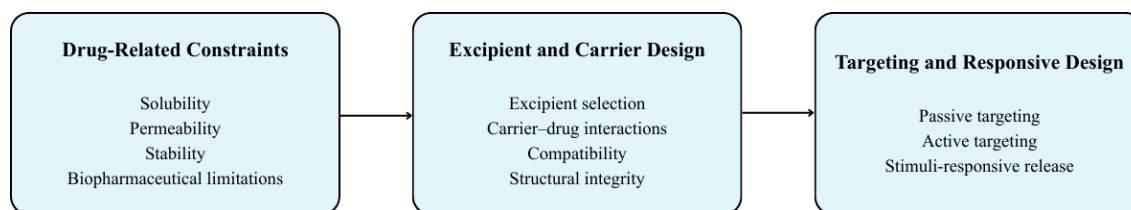


Figure 1. Key Design Considerations in Formulation Development of Novel Drug Delivery Systems

4. Advanced Formulation Techniques and Optimization Approaches

4.1 Conventional and Advanced Fabrication Techniques

Development of formulations has taken a new twist and is no longer based on the conventional methods of fabrication but rather on more sophisticated and detailed technologies. The traditional methods of wet granulation, evaporation of solvents, emulsification and straightforward coating are old and used because of their simplicity, scalability, and familiarity with regulatory agencies. Nevertheless, such approaches do not provide much control over important characteristics like particle size distribution, uniformity of drug loading and release kinetics, which may negatively affect therapeutic performance in complex drug delivery systems¹⁹. The constraints of traditional methods of fabrication have been growing more and more pronounced as drug

molecules and therapeutic objectives have become more complex. More sophisticated formulation methods have risen to meet these demands by allowing more control over the architecture and functionality of a system. Nanoprecipitation, spray drying, supercritical fluid processing, microfluidics and electrospinning technologies enable the precise control of carrier size, morphology and surface characteristics. These techniques are helpful to build up delivery systems that have better reproducibility, high stability, and customized release profiles. The shift between the traditional and the advanced methods of fabrication signifies the paradigm shift to precision-based formulation design that can address the complicated therapeutic needs²⁰.

4.2 Process Parameters Influencing Formulation Performance

Irrespective of the mode of fabrication used, parameters in a process are determinant in the ultimate performance of a drug delivery system. Critical quality attributes (mixing speed, temperature, solvent composition, flow rate, and processing time) can be heavily affected by variables such as mixing speed, temperature, solvent composition, flow rate, and processing time. Bad management of these parameters can lead to batch-to-batch variation and unreliable therapeutic response, which is a significant development and scale-up issue. This makes it necessary to conduct a systematic assessment of process variables to find out their influence on performing formulation and make it robust. The methodologies of experimental design allow the determination of important parameters and their interaction with each other, and eliminate the use of trial-and-error approaches, enhancing development efficiency²¹. Knowledge of the impact of process conditions on formulation properties can allow developers to define optimal operating regimes that can be used to maintain consistent product quality and product performance.

4.3 Quality by Design and Statistical Optimization

Quality by Design (QbD) is an emerging methodology of systematic engineering and optimization of drug delivery systems that is driven by science. QbD focuses on an in-depth knowledge of the variables of products and processes, starting with the description of a quality target product profile and the essential quality attributes. It is the method to allow proactive risk evaluation and risk control measures during the formulation process as opposed to executing a full-scale test of end products only at the end of the process²². The use of statistical optimization methods, such as factorial designs, response surface methodology, and central composite designs, is part of the delivery of successful QbD implementation. These are tools allowing the exploration of formulation and process variables efficiently and reducing the experimental burden. Using statistical models, the developers are able to create the best combinations of the formulation components and processing conditions that maximizes the performance and stability, and the reproducibility is confirmed²³. These methods are mostly useful in complicated delivery systems in which various variables respond to each other non-linearly. The combination of the latest statistical software has also contributed to the creation of intelligent and reactionary drug delivery systems. Optimization techniques are not only effective in enhancing the formulation performance, but also enable scalability and acceptance by regulators due to a clear scientific explanation of design decisions and control methods²⁴. Regulatory agencies are progressively promoting the development of QbD-based programs as it has been determined to improve the quality of products, lessen the risks of product development and lifecycle management.

The combination of sophisticated formulation strategies coupled with systematic optimization methods in drug delivery is one of the pillars of contemporary research

on drug delivery. The adoption of modern technologies through substituting the traditional fabrication practice with new technologies assisted by QbD and statistical software allows the creation of robust delivery systems with high performance that have the potential to fulfil the complex therapeutic problems and enhance the translational success.

5. Physicochemical and Structural Characterization

5.1 Size, Morphology, Surface Properties, and Solid-State Behavior

Structural characterization and physicochemical characterization are a pillar of the development of novel drug delivery systems because these two features directly govern the formulation performance, stability, and biological interaction. The size and size distribution of the particles are especially important to particulate and nanocarrier-based systems, and it affects the rate of dissolution, cellular absorption, biodistribution, and clearance. Shape and surface roughness, morphologic features that alter interactions with biological membranes and proteins, also further regulate morphologic interactions and influence circulation time and therapeutic efficacy. In-depth in vitro and in vivo characterization of nanocarriers has shown that the slight change in such parameters can result in significant changes in pharmacodynamics and pharmacokinetics, behavior²⁵.

Another critical part of physicochemical characterization is the solid-state behavior of the drug in the delivery system. The drug is physically in either crystalline, amorphous, or polymorphic form, and this affects the solubility, dissolution kinetics and stability of the drug over time immensely. Solid-state alterations in the properties of the product during the processing or storage of the formulation can lead to a decrease in therapeutic performance or shelf life. Comprehensive solid-state characterization thus offers vital information on phase behavior, molecular mobility and stability risks, which allows deliberate choice of formulation strategies that would guarantee uniform quality and performance²⁶.

5.2 Drug Encapsulation, Stability, and Compatibility Assessment

Advanced drug delivery systems are characterized by drug encapsulation efficiency and loading capacity since they define the accuracy of the dose, release behavior, and protection of the active pharmaceutical ingredient against environmental stress. Formulation composition, carrier structure, and fabrication technique are highly important in determining the outcomes of encapsulation. Such processes as nanoprecipitation have been actively used by their simplicity and the capacity to reach a high level of encapsulation and control over particle size and shape. Nonetheless, effective encapsulation should maintain the integrity of drugs and prevent unwanted phase separation or drug leakage during storage or delivery to the body during administration²⁷.

Stability testing involves stability (physical and chemical) of the formulation in conditions of storage and

physiological conditions. Physical instability can be either aggregation, sedimentation or alteration of the extent of the particle size spread, whereas chemical instability is associated with degradation or alteration of the drug substance. The compatibility testing thus plays a very important role in formulation characterization especially using complex or combination delivery systems. Analytical assessment of drug-drug interactions and drug-excipient interactions furnishes the necessary details of possible incompatibilities that may impair the stability, bioavailability or safety. Informed excipient choice and optimization of formulation. During the initial phases of product development, systematic compatibility studies can be used to support the choice of excipients and maximization of formulations²⁸.

5.3 Analytical Tools and Their Limitations

A wide variety of analytical methods is used to describe the physicochemical and structural properties of new drug delivery systems. Dynamic light scattering, electron microscopy and zeta potential analysis are standard methods employed to determine size, morphology and surface charge. Thermal, spectroscopic and diffraction-based techniques can give an understanding of the behavior of solid states, efficiency of encapsulation and stability. Combining these tools allows for formulating structure-property relations that

are used in formulation development and optimization. However, in spite of their usefulness, the analytical methods are related to the limitations that are inherent to them and should not be overlooked. There are approaches that are very demanding in terms of sample preparation and preparation that can distort the structure of the delivery system in use, whereas others produce averaged data that can mask heterogeneity between complex formulations. Moreover, the predictive quality of some methods could be constrained by differences between the results of analyses in simplified in vitro systems and in reality, in vivo²⁹. Critical interpretation of these drawbacks is thus crucial to the right choice and interpretation of characterization information to guarantee that the physicochemical assessment has a purpose in supporting formulation design and translational decision-making.

Systematic physicochemical and structural characterization provides the scientific basis for the understanding of formulation behavior, optimization of performance, and quality and reliability of novel drug delivery systems during the development and clinical translation. Table 3 lists some of the common physicochemical and in vitro characterization methods used on novel drug delivery systems. The workflow to be followed in sequential order in physicochemical and structural characterization of novel drug delivery systems is shown in Figure 2.

Table 3. Physicochemical and In Vitro Characterization Techniques for NDDS

Technique	Parameter Assessed	Information Obtained	Relevance to NDDS	Key Limitation
DLS	Particle size	Size distribution	Stability, uptake	Shape assumption
SEM/TEM	Morphology	Shape, surface	Interaction behavior	Sample preparation
Zeta potential	Surface charge	Colloidal stability	Cellular uptake	Medium dependent
DSC/XRD	Solid-state	Crystallinity	Stability, dissolution	Data complexity
Release assays	Drug release	Kinetics, mechanism	Performance prediction	Limited IVIVC

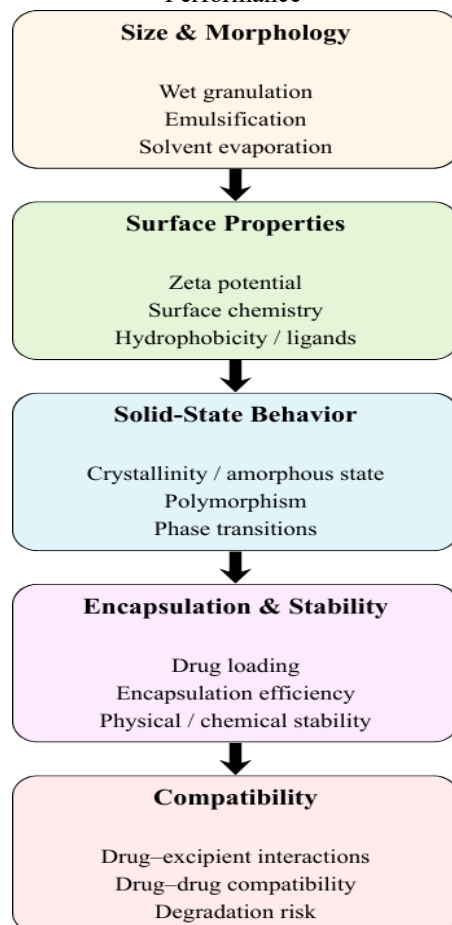


Figure 2. Sequential Physicochemical and Structural Characterization Strategy for Novel Drug Delivery Systems

6. In Vitro Drug Release

6.1 Release Models and Kinetic Interpretation

In vitro drug release is an essential part of the formulation development process that gives a crucial understanding of how the drug is liberated by the delivery system and the rate of liberation. The drug release behavior is mathematically formulated by release models and used to explain the underlying mechanisms, which may involve either diffusion, erosion, swelling or a combination of both. Some of the most extensively used kinetic models are zero-order, first-order, Higuchi, Korsmeyer-Peppas and Weibull models, which provide a particular mechanistic understanding, based on system architecture and release behavior. Proper choice of release model is necessary to do a meaningful interpretation of in vitro data, as well as to compare the performance of the formulation in different systems³⁰. Rational formulation optimization through mechanistic analysis of release profiles aims to provide the relationship between structural and compositional properties of the delivery system and experimental release behavior. Yet, the use of one kinetic model can simplify multifunctional or layered systems in release processes. Comparative analysis has also concluded that interpretation through multiple model-based mechanistic interpretation combined with experimental findings offers a stronger insight into the dynamics of drug release and is more able to predict in vivo performance³¹.

6.2 Influence of Formulation and Environmental Factors

The release behavior of drugs has a close relation to the formulation factors of drug physicochemical properties, carrier composition, matrix structure, and technique of fabrication. Parameters such as solubility, crystallinity and molecular interactions of the drug on the carrier matrix may grossly change the release rate and mechanism. Formulation design is a key determinant of the controlled and reproducible drug release profiles in the case of extended-release and modified-release systems. Even the type of excipient used, grade of polymer, or carrier structure can lead to significant changes in dissolution properties and therapeutic predictability³². The environmental conditions make the in vitro release assessment even more difficult because the testing environment might not be fully representative of the physiological environment. The solubility and diffusion of drugs may be significantly influenced by parameters like pH, temperature, ionic strength, agitation speed, and release medium composition. Environmental sensitivity is part of formulation performance in delivery systems that are to be targeted to be site-specific or stimuli-responsive. It is then crucial to comprehend the interactions between attributes of formulation and testing conditions to prevent the misleading inference, and to ensure that the release

studies are representative of clinically pertinent situations³³.

6.3 Predictive Value of Release Testing Methods

The in vitro drug release testing is a predictive test whose usefulness lies in its ability to act as a surrogate of in vivo performance and aid in formulation selection, optimization, and quality control. The well-designed release methods allow the discrimination of the formulations and give early warning of the possible bioavailability problems. Reliable correlations between in vitro release data and in vivo behavior are, however, a big challenge, especially with complex delivery systems and non-oral dosage forms. The discrepancy tends to occur as a result of physiological variables that are not easily replicated in the laboratory environment³¹. Recent progress in the area of modeling and simulation has increased the predictive power of studies of in vitro release. Supplied by statistical and hybrid techniques, mechanistic and empirical modeling methods enable the combination of the formulation variables, process

parameters, and release data in order to predict drug behavior with even more precision. These methods enhance the usefulness of in vitro testing as a tool in formulation development, as well as for regulatory decision-making and evaluation of product release, and so on³⁴. However, predictive models rely on how well the experimental data is obtained and how relevant the selected methodology of testing is.

In vitro drug release and mechanistic analysis offer crucial means of formulation behavior knowledge and the development decision-making. To improve the translational relevance of new drug delivery systems and guarantee the efficacy of these systems as therapeutic agents, it is necessary to be more critical and systematic with the release testing, which involves the use of proper models, attention to both formulations and the environment and the use of higher predictive tools. The development of the traditional methods of fabrication to modern formulation and QbD-oriented optimization is demonstrated in Figure 3.

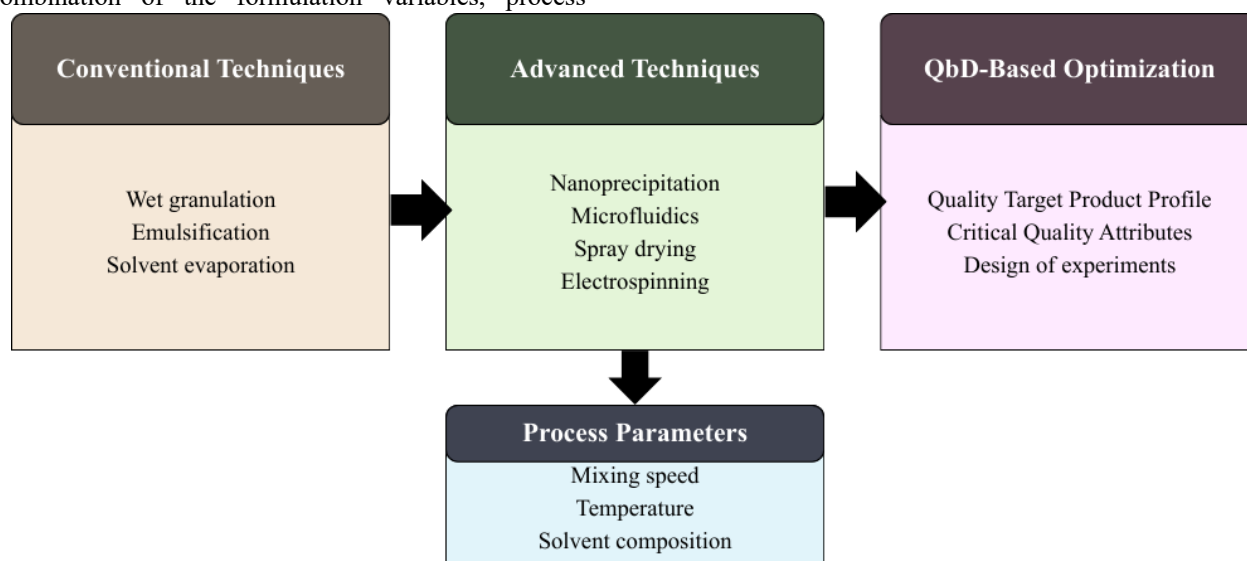


Figure 3. Advanced Formulation Techniques and Quality by Design–Based Optimization in Drug Delivery System Development

7. In Vitro Biological Performance Assessment

7.1 Cytotoxicity, Biocompatibility, and Safety Screening

In vitro biological performance testing is a major phase in the innovation of new drug delivery methods, since it determines the safe profile of formulations before the development of advanced efficacy testing. Cytotoxicity assays are frequently utilized in establishing whether carrier materials, surface alterations or degradation products cause undesirable cellular reactions regardless of the drug content. These tests give early warning of the formulation tolerability and assist in the removal of systems that have unacceptable safety risks. Such data are increasingly being analyzed within model- informed drug development contexts, which combine in vitro bio-performance data with predictive evaluations of drug exposure and behavior in a variety of routes of administration, and enhance decision-making in early

formulation development processes³⁵. Biocompatibility testing goes beyond the short-term cytotoxicity test to cellular adaptation mechanisms, and inflammatory responses, as well as prolonged interactions with the biological system. This is especially in the case of a delivery system that is programmed to be used in long-term residence or multiple dosing, in which the effects can be cumulative and change therapeutic viability. Planned protection screening validates that the promising systems for delivery progress according to their efficacy potential and acceptable biological compatibility.

7.2 Cellular Uptake, Transport, and Targeting Efficiency

Effective cellular absorption and proper intracellular delivery are critical parameters of the therapeutic performance of most sophisticated drug delivery

systems. The process of uptake is determined by the interaction of delivery carriers with cell membrane constituents, such as electrostatic interactions, lipid membrane affinity, and receptor-mediated binding. Rational surface property engineering, e.g., of charge, hydrophobicity, and ligand functionalization, can enhance internalization to a large degree and reduce nonspecific uptake and off-target effects³⁶. After internalization, subcellular drug distribution has a decisive role in efficacy, toxicity, and resistance. The effectiveness of the therapy is not necessarily determined by the therapeutic entry of the cell, which is determined by whether the drug enters the cell or not. Localization or sequestration in endosomal or lysosomal compartments can hamper efficacy, but specific delivery to a particular organelle can enhance efficacy, and also increase the risk of toxicity. The processes controlling subcellular trafficking and localization are thus of fundamental use to designing systems of drug delivery that can be compatible with the mode of action in drug and the therapeutic purpose of the drug³⁷.

7.3 Advanced In Vitro Models: 3D Cultures and Organ-on-Chip Platforms

Although traditional two-dimensional (2D) cell culture models are highly employed in screening, most of them cannot simulate the structural and physiological complexity of native tissues. Compared to 2D cell culture systems, 3D systems have an increased

biological relevance by recapitulating tissue architecture, extracellular matrix interactions, and diffusion gradients. Such models often expose the delivery barriers, penetration limitations, and heterogeneity of responses not reflected in 2D systems, and hence they are useful in a more realistic assessment of drug delivery performance and targeting efficiency³⁸. Organ-on-a-chip models are another development of in vitro models, which combine microfluidic flow, mechanical stimuli, and multicellular assembly to model dynamic physiological signals. With these systems, it is possible to investigate drug transport, accumulation and biological response under conditions more closely related to those in vivo. Their use in tumor-targeted drug delivery studies has shown a better predictive ability of both efficacy and safety, as well as justifying a stronger ability to translate in vitro results to clinical environments³⁹.

In vitro biological testing gives fundamental information on safety, uptake mechanisms and functional significance of new drug delivery systems. The combination of cytotoxicity screening, cellular transport studies, and high-level in vitro models enhances the predictive utility of preclinical testing and assists in the rational development of delivery platforms towards clinical development. Table 4 provides a comparison of popular in vitro biological models with their relevance in translation.

Table 4. In Vitro Biological Models and Translational Relevance

Model Type	Primary Endpoint	Key Strength	Major Limitation	Translational Value
2D cell culture	Cytotoxicity	High throughput	Low physiological relevance	Low-moderate
3D spheroids	Penetration, efficacy	Tissue-like structure	Limited standardization	Moderate-high
Co-culture models	Targeting, transport	Cell-cell interaction	Experimental complexity	High
Organ-on-chip	Transport, response	Dynamic physiology	Cost, expertise	High
Ex vivo models	Tissue response	Closest to in vivo	Limited availability	Very high

8. Translational Relevance and Predictive In Vitro Models

The main challenge in the development of new drug delivery systems is to establish strong translational relevance between the findings of in vitro research and in vivo/clinical performance. Despite the essential role of in vitro models to screen at early stages and understand a drug's mechanism, these models do not always reflect the physiological complexity of drug behavior in vivo. Moving blood flow, interactions of the immune system, heterogeneity of tissue, and disease-specific microenvironmental factors are some of the factors that lead to disparities between laboratory and clinical results. These restrictions are especially strong when it comes to the advanced delivery platforms, in which carrier properties and biological interactions are dominant factors in establishing therapeutic efficacy.

Thus, IVIVC of new delivery systems in vitro to in vivo is frequently poor or unpredictable, and there is a need to be cautious in the interpretation of in vitro data and employ complementary assessment systems⁴⁰.

The selection and design of experimental models are vital in improving the predictive value of in vitro evaluation. The traditional two-dimensional cell cultures, although useful in high-throughput screening, do not offer much information on drug transport, penetration, and distribution in more complex tissues. The more complex in vitro platforms, including three-dimensional cultures of cells, provide a higher level of physiological relevance by including a cell-cell interaction, components of the extracellular matrix, and concentration gradients that modify drug delivery performance. Such systems have been found to have better reflectance of the therapeutic response and

resistance mechanisms over the traditional models, and thus enhancing their potential in predicting behavior in vitro and optimization of formulation⁴¹.

The gap between formulation design and clinical outcome needs a unified framework that would involve a combination of biologically relevant in vitro models with quantitative and predictive models. Mathematical and mechanistic modelling models have become useful as supplementary tools to experimental research, to allow the analytical combination of formulation property, biological and release data to model drug behavior in the in vitro and in vivo environment. These methods work especially well with complex systems like nanoparticle-based delivery platforms, where multiscale transport effects make desk formulation of the results of an experimental study difficult. Through endpoints of clinical relevance, a juxtaposition between formulation design, in vitro testing, and predictive models can be achieved by reducing translational uncertainty and increasing the chances that promising delivery systems will exhibit significant therapeutic benefit in clinical studies⁴².

9. Regulatory, Manufacturing, and Scale-Up Considerations

Regulatory expectations and compliance requirements have a strong impact on the successful translation of novel drug delivery systems that are developed in the laboratory to clinical use. The regulatory bodies are putting a larger emphasis on the overall knowledge of material safety, biocompatibility and risk management, especially on polymer-based and advanced delivery systems. To prove safety, it is necessary to have a solid in vitro screening with the help of systematic risk assessment strategies to discuss material composition, degradation behavior, and possible toxicological issues. In numerous new delivery systems, the absence of completely standardized regulatory routes complicates the situation, and the initial correlation of the design of the formulation, its characterization, and evaluation approaches with the regulatory expectations is the key to the effective development and approval of a drug⁴³. The key issues of manufacturing feasibility and scale-up of advanced drug delivery systems are still significant challenges in the industrial translation of the latter. Recipes that perform well at the laboratory scale usually face problems at scale-up as the recipe is sensitive to processing conditions, raw material variability, and manufacturing equipment constraints. It is necessary to ensure that critical quality parameters like particle size, drug loading, and release behavior are maintained at uniform values to guarantee the therapeutic performance across batches. Moreover, the good manufacturing practice (GMP) needs validated processes, extensive documentation, and strict quality assurance systems, and all this needs to be addressed early in the development to facilitate quality commercialization⁴⁴. The focus of regulatory acceptance and long-term clinical reliability of innovative drug delivery systems is quality control and reproducibility. The high-grade formulation structures and multiphase production cycle elevate the

chances of variation from batch to batch that may affect the performance of the product and patient well-being. It is hence necessary to establish sound analytical procedures, well outlined quality requirements and repeatable manufacturing conditions to promote consistency and regulatory trust. The lack of control over the features of formulation may induce variability in clinical outcomes, which reduces the therapeutic utility of advanced delivery platforms⁴⁵.

The future opportunities of the domain of novel drug delivery systems are directly interconnected with the incorporation of regulatory science, new technologies of manufacturing, and predictive development methods. Scalability and reproducibility should be improved through the growing use of quality-by-design concepts, non-stop and continuous production, and real-time monitoring of the processes. Simultaneously, more efficient regulatory decision-making and less translation risk can be achieved through better predictive in vitro models and model-informed development strategies. Increasing the level of cooperation between the formulation scientists, manufacturing engineers and regulatory agencies will be necessary in speeding up the process of developing and clinically adopting novel drug delivery systems, which will ultimately enhance the patient outcomes and therapeutic efficacy.

10. Conclusion

It has also been shown that, as the development of novel drug delivery systems has progressed, the performance of therapeutic activity is not only a matter of the active pharmaceutical ingredient, but also reflects the complexity of formulation design and assessment measures. This is highlighted in this review, where rational formulation development, based on an appreciation of drug physicochemical constraints, carrier-excipient interactions and process control, is a key to overcoming long-standing constraints of traditional delivery methods. It is also essential that rigorous physicochemical characterization and mechanistic in vitro testing can be used to build a structure performance relationship to inform formulation optimization. Although this has been done successfully, translation of advanced delivery platforms to clinical success is still limited by imprecision in the predictive in vitro models, a lack of consistency between in vitro-in vivo correlations, and difficulties of scale-up of manufacturing and regulatory compliance. The key to solving these barriers lies in a more integrated design of the development paradigm, where formulation design, bio-performance testing, and manufacturability issues are considered at an earlier design phase. Translational predictability is likely to be enhanced by the adoption of biologically relevant in vitro models, quantitative and model-driven development strategies and quality-driven manufacturing strategies. In sum, the future role of new drug delivery systems will be based on whether they will be able to combine innovation and clinical relevance. An evidence-based, systematic and performance-oriented formulation development and in vitro assessment model will play a central role in the translation of novel

delivery technologies into safe, effective and patient-centred therapies.

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