phosphorex

Derisk Therapeutic Nanoparticle and Microsphere Programs with Stage-Appropriate Analytical Method Development

Theresa Logan, Head of Laboratory Operations, Phosphorex

Formulation, process development, and analytics are interconnected. Advancing analytical method development in a stage-appropriate manner is critical to ensure the success of therapeutic nanoparticle and microsphere programs from feasibility to clinical manufacture. Whether programs involve lipid nanoparticles, polymeric nanoparticles, or polymeric microspheres encapsulating nucleic acids, small molecules, peptides, or biologics, including proteins, developing appropriate methods during the preclinical, early, and late clinical phases can accelerate development while reducing risk.

General Analytical Considerations for Therapeutic Nanoparticles and Microspheres

Therapeutic nanoparticles encapsulate active pharmaceutical ingredients (APIs), providing cargo protection, improved pharmacokinetics and bioavailability, and enabling targeted delivery, enhanced tissue penetration, and intracellular delivery. Therapeutic microspheres also encapsulate APIs, improving drug stability and bioavailability while enabling controlled and sustained release, which can reduce side effects and toxicity and improve patient compliance.

Lipidic and polymeric materials are the most commonly used compositions, including both synthetic and biomimetic lipids and polymers. Nanoparticles can encapsulate small molecules, proteins, peptides, and nucleic acid cargos, and their small size enables nonviral intracellular delivery. In contrast, microspheres are generally too large for intracellular delivery and therefore are unsuitable for nucleic acid

delivery. However, their larger size and depot-like properties extend the effective duration of drug exposure, increasing the apparent half-life and dosing interval and making microspheres well-suited for sustained-release drug delivery.

During development including in-process testing and clinical product release, analytical methods are required to assess both the loaded particles and their payloads. The physical and chemical properties of the particles must be characterized, along with endotoxin and bioburden levels. For loaded particles, attributes such as particle size, encapsulation efficiency, cargo content and integrity, and release kinetics must also be evaluated. However, a key challenge in characterizing cargos encapsulated within particulate-based drug delivery systems is extracting the cargo from the particles without degrading or compromising their integrity. This challenge is particularly significant for sensitive cargos such as proteins and nucleic acids.

In addition to gathering data to support regulatory submissions and meet regulatory requirements at each development phase, analytical methods should be developed from the outset to ensure that physicochemical properties, stability, and biological performance are well understood. This approach helps streamline program advancement and minimizes the need for rework. A risk-based approach to method development ensures that appropriate methods are implemented at each stage, progressively increasing understanding of physicochemical properties, stability, and biological performance as the program advances from discovery to the clinic. It also ensures that methods remain fit for purpose, with method qualification and validation activities expanding as programs progress toward late-stage clinical development and commercialization.

Benefits of Stage-Appropriate Analytical Method Development

Cost- and time-effective drug development requires selecting and implementing the right set of analytical methods at each stage. Developing stage-appropriate methods minimizes analytical costs while generating the data needed to support informed, data-driven decisions throughout formulation and process development. This approach ultimately enables preparation of an investigational new drug (IND) application and the collection of first-in-human proof-of-concept data.

Working with an experienced contract development and manufacturing organization (CDMO) can be critical to making informed method development decisions, particularly when formulating specialized products such as therapeutic nanoparticles and microspheres. A CDMO can leverage prior experience from similar projects to recommend which methods are needed early in formulation and/or process development to support the assessment of the formulation's critical quality attributes (CQAs) (e.g., particle size), and which can be delayed until development progresses towards the clinic.

Understanding Physicochemical Properties Paramount at the Proof-of-Concept Stage

Several attributes of therapeutic particles must be thoroughly understood at the proof-of-concept stage. First, the physicochemical properties of the particles include particle size distribution, surface charge, cargo content, encapsulation efficiency, integrity, and release kinetics.

Particle size strongly influences particle behavior, particularly biodistribution and cellular uptake. As such, it plays an important role in determining the organs and tissues in which the particles will accumulate. Particle size also dictates the sterilization strategy that must be employed. Nanoparticles can typically be filtered through 0.2-micron sterilizing filters, while microspheres, which are generally formulated for subcutaneous (SC) or intramuscular (IM) delivery, are typically manufactured via fully aseptic processes in a controlled, sterile environment or terminally sterilized via gamma irradiation or e-beam.

Surface charge affects protein adsorption, cellular interactions, and uptake, which are particularly important in the development of therapeutic lipid nanoparticles (LNPs).

Cargo content and stability are other important properties that must be well understood early in development. For instance, it is important to confirm that protein cargos are not denatured during the encapsulation process, and that sensitive nucleic acids remain stable once encapsulated and released from the delivery system. Significant project delays can result if instability is noted during process development and scale-up rather than early in the program.

In addition to gathering data to support regulatory submissions and/or meet regulatory requirements at each development phase, analytical methods should be developed from the outset to ensure that physicochemical properties, stability, and biological performance are well understood.

Additional Expectations for Polymeric Microspheres

Polymeric microspheres require analysis using several additional, non-standard methods. Understanding the release rate is critical for extended-release formulations, as it directly impacts product safety. These delivery systems are designed to release their payloads gradually over time, and an initial burst or rapid release of a large portion of the cargo can lead to toxicity.

Process and formulation parameters can significantly influence the physicochemical properties of microspheres. Scaling up production from lab-scale equipment, such as probe-type sonicators or rotor-stator homogenizers, to clinically relevant unit operations, such as an inline homogenizer, can, for example, produce particles with altered API distributions and morphologies, particularly in porosity. The choice of solvent evaporation technique and the evaporation rate can also markedly affect API distribution, particle morphology, and release kinetics. Because these structural differences directly impact release behavior, scanning electron microscopy (SEM) is frequently used for detailed structural analysis, while Raman spectroscopy provides

information on the spatial distribution of the API within the microspheres.

Residual solvent content is another critical attribute, as it can affect the performance of polymeric microspheres. Monitoring solvent levels from the outset of process development, rather than waiting until clinical operations, is essential. Developing stage-appropriate analytical methods ensures a thorough understanding of solvent content at each process step and confirms consistency as the process is scaled.

Syringability and injectability are other important considerations for polymeric microspheres. Because these systems are used for sustained-release applications, the total amount of cargo that must be delivered over the intended release period should be evaluated early in development. This assessment informs key parameters, including required drug loading, injection volume, needle gauge selection, and helps determine whether the target dose can be delivered within the desired injection volume and needle specifications. Injectability and syringability can be studied cost-effectively using an Instron device, providing a preliminary understanding of these parameters before *in vivo* studies.

Phosphorex has the necessary experience and expertise to develop phase-appropriate analytics that assess the right attributes at the right time, with the sensitivity and robustness needed to support informed decision-making, meet or exceed regulatory expectations across all program phases, and enable rapid program advancement.

Confirming PK and Biodistribution is Crucial when Advancing to the Lead Formulation Stage

As programs advance toward lead formulation selection, understanding the pharmacokinetics (PK) and biodistribution of therapeutic nanoparticles becomes essential. Effective methods are needed to measure circulation half-life, cellular uptake, and organ accumulation, particularly when the nanoparticles are designed to target specific biological sites. Early characterization of these attributes informs formulation optimization and guides decision-making as the program progresses towards the clinic.

While *in vitro* studies provide valuable mechanistic information, it is critical to evaluate PK and biodistribution in relevant *in vivo* models as early as possible, starting with rodents and progressing to nonhuman primates (NHPs). For lipid nanoparticle (LNP) formulations, a major challenge is the limited translatability of results from *in vitro* systems to animal studies, including those in rats and NHPs. Strategies to improve this translatability are essential to increase the proportion of formulations that successfully reach clinical evaluation.

Targeted LNPs (tLNPs) present additional translational challenges because the cell-surface receptors used for targeting specific human cell- and/or tissue-types are often absent, expressed at different levels, or structurally different in small animal models. Humanized mice engrafted with human immune cells provide more predictive data than standard mice for immunogenicity, PK, efficacy, and toxicity, though the degree of humanization can significantly influence data interpretation. NHPs offer further human-relevant insights, particularly for systemic PK and immune responses. Despite these models, translatability limitations remain, making complementary data from *in vitro* studies, rodents, and humanized mice, combined with careful study design, critical for robust clinical translation.

Increasing Robustness and Confirmation of Identity, Safety, and Tolerability Required as Programs Move toward GMP

While at the preclinical stage, the focus is on determining whether a therapeutic nanoparticle or microsphere formulation solves a real biological problem and if that behavior translates into *in vitro* and then *in vivo* performance. Once that is successfully demonstrated, it is necessary to show that the biological effect is robust and reproducible. As programs get closer to the clinic, confirming the safety and tolerability of the formulation and the translatability of animal data into humans becomes paramount.

Assays in general remain similar as programs move from the proof-of-concept stage to early clinical studies. During the earliest development phases, ideal methods provide quick readouts to support evaluation of multiple formulations in parallel, yet are sufficiently robust and reproducible to ensure confidence in the ability to rank formulations. As programs advance into early clinical development and beyond, the robustness of the method becomes increasingly important and must reach a level that supports validation.

For all types of therapeutic nanoparticles and microspheres, identity determination also becomes increasingly important as programs move toward clinical manufacturing. It is essential to have robust methods to confirm the identity of both the cargo and the components of the particle delivery system (lipids, polymers, etc.). Typically, fluorescence-based assays or liquid chromatography (LC) methods are used to determine the cargo composition and encapsulation efficiency. The integrity of small-molecule, peptides, and protein cargos is typically assessed by LC, whereas a combination of capillary electrophoresis (CE) and LC is used for nucleic acid payloads.

For LNPs, it is also important to accurately establish lipid identity and determine the lipid composition. Because ionizable lipids may pose toxicity risks, regulators require confirmation of their safety. For polymeric systems such as poly(lactic-co-glycolic acid) (PLGA), despite being GRAS (generally regarded as safe) and widely used as pharmaceutical excipients, it is important to have appropriate polymer identity and content methods in place during early-phase clinical trials to ensure quality control, product performance, and batch-to-batch consistency.

Other assays that gain importance as programs advance towards the clinic include purity methods, which are typically based

Putting the Strategy into Practice at Phosphorex

Phase-appropriate method development at Phosphorex is achieved using several different approaches. Two good examples include a modified RiboGreen assay for analyzing messenger RNA (mRNA) payloads in LNPs and an *in vitro* release method for polymeric microspheres.

The RiboGreen assay is a sensitive, fluorescence-based method for quantification of mRNA in solution and encapsulation efficiency in LNPs. It is attractive for mRNA-LNP analysis because it is essentially a plug-and-play method that can be used across all LNP formulations. It is, in fact, the gold standard method used at the proof-of-concept/preclinical stage.

Because the RiboGreen method is a plate-based assay, it suffers from variability, which can reach 10%—a level not typically acceptable for API cargo methods. Phosphorex has overcome this variability issue by automating the conventional RiboGreen assay using robotic liquid handlers. This solution eliminates the variability associated with human operations, such as pipetting. It also enables the parallel screening of multiple formulations at higher throughput.

For analysis of polymeric microspheres, which are typically used in sustained-release formulations, the gold standard method for *in vitro* release assessment is the USP Apparatus 4 (Flow-Through Cell) method. This method is relatively complex in terms of development and parameter definition, and very limited in the number of samples it can test at once.

To simplify and accelerate analysis of the *in vitro* release properties of polymeric microspheres, Phosphorex adapted the USP Apparatus 2-like protocol for pharmaceutical dissolution testing. The sample is shaken in the *in vitro* release media within a vessel placed in a water bath, and the resulting data provide relative release rates (fast, medium, slow). This method is more straightforward and higher-throughput and thus provides sufficient information more quickly to appropriately rank formulations and identify the top candidates.

on size-exclusion chromatography (SEC) or ion-pair reverse-phase high-performance LC (RP-HPLC). The development of robust methods for detecting endotoxins and bacterial contaminants (e.g., sterility), residual solvents, and particulates also become essential as programs move toward the clinic.

Finally, as a program moves towards late-stage clinical studies and commercialization, scalability must be demonstrated. Appropriate analytics are needed to clearly demonstrate that any changes in process conditions that occur upon scaling do not impact the particle properties and *in vivo* performance of the therapeutic.

Deep Expertise in Particulate-Based Delivery Solutions

The inherent complexity of lipid and polymeric nanoparticles often creates significant translational gaps between preclinical and clinical development, particularly in achieving cost-effective, scalable, clinically relevant production operations. Access to expertise in therapeutic nanoparticle and microsphere technologies and the ability to develop phase-appropriate analytical methods are essential to addressing those gaps.

Phosphorex has focused solely on particulate-based drug delivery systems since its founding. Technologies covered include polymeric nanoparticles and microspheres, lipid nanoparticles and liposomes containing nucleic acids (mRNA, small interfering RNA, plasmid DNA, and other oligonucleotides), small-molecule, peptide, and protein payloads.

Over more than two decades of applying the standard methods required by regulatory agencies for particulate-based systems, the company has established a deep toolbox of techniques applicable across all three platforms. Standard and specialized methods are applied that ensure thorough characterization and accurate quantitation of both particles and their cargos. That includes the use of technologies that enable careful extraction of cargos from particles without impacting their critical quality attributes.

Similarly, for targeted LNPs and polymeric particles, Phosphorex has extensive expertise in the conjugation and characterization of targeting ligands presented on the particle surface, enabling accurate characterization and quantitation of their targeting ability.

As importantly, Phosphorex has the necessary experience and expertise to develop phase-appropriate analytics that assess the right attributes at the right time, with the sensitivity and robustness needed to support informed decision-making, meet or exceed regulatory expectations across all program phases, and enable rapid program advancement. Contact us to discuss how we can help to advance your program.

Theresa Logan
Head of Laboratory Operations

LinkedIn: www.linkedin.com/in/theresa-j-logan