

EBOOK

Particle Science in Practice:

Formulation, Process, and Scale-Up Insights for Lipid and Polymeric Drug Delivery Systems



Introduction

Lipid nanoparticles (LNPs), polymeric nanoparticles (PNPs), and polymeric microspheres offer versatile approaches for delivering a wide range of therapeutic payloads. Their applications span nucleic acid delivery, small-molecule therapeutics, targeted delivery, and sustained-release formulations. However, advancing particle-based drug delivery systems from early feasibility toward the clinic requires careful coordination of formulation design, particle characteristics, process development, and scale-up.

This eBook assembles insights from the Phosphorex team and examines several important considerations in particle-based drug delivery. Topics include the development of polymeric nanoparticles and microspheres, approaches to sustained-release formulation, the impact of formulation buffer on LNP particle size, and downstream process optimization for clinically relevant LNP scale-up.

Grounded in practical insights and experience, this eBook offers a look at how thoughtful particle engineering and process development can help advance complex drug delivery programs toward clinical readiness.

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Benefits of Formulating Advanced Therapeutics as Polymeric Nanoparticles and Microspheres

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Polymeric nanoparticles (PNPs) are a game-changing solution for delivering therapeutic agents, including small molecules, peptides, proteins, and nucleic acids. By encapsulating active pharmaceutical ingredients in biodegradable polymer nanoparticles, it is possible to protect them from degradation, thereby extending their time in the bloodstream; improving their bioavailability; enhancing their cellular uptake; and, in many cases, achieving targeted delivery through careful particle design and surface modification. Fluorescent labeling techniques also enable the use of polymeric particles in diagnostic applications.

Polymeric microsphere (PMP) formulations, when delivered subcutaneously (SC) or intramuscularly (IM), can dramatically reduce dosing frequency, increase patient convenience, and improve medication adherence.

Successful development of therapeutic and diagnostic PNPs or PMPs requires optimal particle design, which in turn necessitates selection of the most appropriate biodegradable polymers for a given drug substance and target product profile, choosing the best surface ligands and conjugation methods for active targeting, development of consistent, scalable manufacturing processes, and establishment of phase-appropriate analytical methods that ensure comprehensive analysis of both encapsulated payloads and the particulate vehicle.

Distinguishing Polymeric Nanoparticles and Microspheres

Polymeric nanoparticles and microspheres are typically distinguished by their sizes. There can be some confusion, however, as different organizations define nanoparticles differently. Literature publications often consider polymeric nanoparticles to have dimensions ranging from 1 to 1000 nm.^{1,2} The US Food and Drug Administration (FDA) defines nanomaterials as materials that have been engineered to have one or more dimensions within the nanoscale range, which, although most commonly pertains to average diameters around 100 nm, is considered to include diameters up to 1000 nm by regulatory definition.³

In 2017, an FDA study found that of more than 350 drugs containing nanomaterials submitted to the agency, the majority contained engineered nanomaterials with dimensions under 300 nm. Phosphorex considers polymeric particles with diameters < 500 nm to justify the polymeric nanoparticle (PNP) title, and those that are larger to be characterized as polymeric microspheres (PMPs).

There are three other important distinctions between PNPs and PMPs. First, PNPs can be leveraged to facilitate nonviral intracellular delivery of nucleic acid cargoes, whereas microspheres are not well suited for nucleic acid cargoes due to a considerably limited potential for intracellular uptake. Second, PNPs and polymeric microspheres exhibit very different pharmacokinetics. The *in vivo* half-lives of active pharmaceutical ingredients (APIs) delivered by PNPs and PMPs are influenced by particle size, with larger particles providing longer durations of API release.

Finally, the manufacturing operations applied for polymeric nanoparticle and microsphere production are quite different. Nanoparticle vehicles are typically filtered through commonly used 0.2-micron sterile filters prior to fill-and-finish; whereas microspheres, are too large to be filtered in this manner, and thus require terminal sterilization or fully aseptic processing within a controlled, sterile environment, depending on the cargo.

Many Benefits of Polymeric Delivery Vehicles

Polymeric nanoparticles and microspheres represent alternative methods for delivering diagnostic and therapeutic agents. Incorporation of APIs into biodegradable polymer

particles offers numerous benefits, including protection against degradation, extended drug release, and improved bioavailability.

Polymeric particles can also enable conversion of some injectables to oral formulations, enhance cellular uptake, and facilitate targeted delivery. Compared to lipid nanoparticles (LNPs) in particular, PNPs offer extended half-lives because they do not exhibit the burst-release profile common to liposomes or LNPs. Furthermore, polymeric nanoparticles can be better suited for alternative administration route strategies that liposomes or LNPs may struggle to support, provided there is sufficient formulation, process development, and chemistry, manufacturing, and controls (CMC) expertise to manage and scale these intricate systems.

Polymeric microspheres, meanwhile, provide a means to achieve true long-acting release for molecules with sufficient potency to support long-term doses in a single injection volume. Formulations requiring administration only twice per year are possible and can often be self-injected at home, dramatically increasing patient convenience and resulting in greater medication adherence. Other important benefits of formulating sustained-release products such as polymeric microspheres includes

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the potential to reduce systemic exposure, resulting in decreased side effects and the ability to enhance the therapeutic index.

Developing complex polymeric delivery systems, however, requires specialized expertise in formulation development and optimization, as the relationships among API and polymer chemistries, the target product profile, and the processing techniques required must be carefully considered. With the right expertise and capabilities, though, polymeric systems can be leveraged to improve patient convenience and compliance, enhance tolerability, capture new IP space, and even harness safer materials and more economical distribution logistics.

Choosing Nanoparticles vs. Microspheres: The What and the Why

When deciding whether to formulate a novel drug substance as a polymeric nanoparticle or microsphere, it is important to consider the delivery problem statement, as both technologies can be used to incorporate a wide variety of API formats. The particle class and API class are not typically mutually exclusive. Each approach has its own strengths, and thus, the delivery problem statement should drive the drug product selection.

The key is to determine whether a micro- or a nanoparticle is the better option given the desired physiological target and dose. It is then important to empirically determine the chemistry and particle characteristics that provide optimal performance.

For instance, peptides are often more difficult to encapsulate in polymeric particles than small molecules, which are typically more hydrophobic. Clever strategies may be needed to achieve sufficient loading of a nominated peptide or hydrophilic small-molecule API into the desired type of polymeric particle, while achieving the target product profile. Such strategies must leverage the physicochemical properties of API and marry them to the polymers and processing techniques. For polymeric microspheres and nanoparticles to be formulated as suspensions for subcutaneous (SC) or intramuscular (IM) injection, specialty formulation expertise is often required to achieve a loading capacity sufficient to meet dose requirements while maintaining injection volume limits that support safe administration.

The potential application of PNPs or even PMPs for oral delivery is an important

Phosphorex has focused on polymeric delivery vehicles since its founding over 20 years ago.

area of research. The focus is on developing PNPs that can induce relevant uptake mechanisms within the gastrointestinal (GI) tract. Even for biologics, PNPs have been shown to protect the payload and facilitate targeted delivery by providing sufficient stability in the GI tract. However, transport across the gut or intestinal wall remains challenging for many applications and will continue to be modeled and empirically investigated to realize fuller potential.

Polymeric microspheres have already been proven in commercial sustained-release products. ZILRETTA® (triamcinolone acetonide extended-release injectable suspension, Pacira Biosciences) for the treatment of osteoarthritis knee pain is formulated in biodegradable poly(lactic-co-glycolic acid) (PLGA) microparticles and is widely considered the gold standard in the field.

Polymer Chemistry Matters

The materials used to produce polymeric nanoparticles and microspheres for drug delivery consist primarily of biocompatible polymers containing hydrolysable esters for biodegradation. The fine-tuning of these polymer structures by expert formulators can be employed to improve drug loading, drug release, or the manufacturability of the target product profile. Polymer selection can also facilitate the conjugation of targeting ligands to the surface of polymeric particles.

Key attributes for selecting a specific polymer or polymer mix for any given application include the chemical structure and molecular weight of the polymer(s). These properties are tuned to facilitate critical quality attributes such as particle size, drug loading, encapsulation efficiency, and stability, as well as favorable process con-

ditions such as solvent selection, and required solvent volumes, which can be critical for scalable manufacturing.

Generally, for encapsulation of small molecules and peptides, PLGA and polylactic acid (PLA)-derived polymers are preferred because they often provide the necessary balance of API loading, tunable release rate, and manufacturability (including favorable organic solvent solubility). Tuning the specific properties is achieved, for instance, by adjusting the polymer MW and/or the ratio of lactide to glycolide (LA/GA) in the polymer.

Polymers used in PNP formulations are often modified with polyethylene glycol (PEG), which lead to smaller particles and provide sites for the attachment of surface ligands, such as antibodies and other molecules that support active targeted delivery.

Polymeric microspheres can be produced using a diverse range of biodegradable polymers, including not only PLGA and PLA but also polycaprolactone (PCL), polyanhydrides, and poly(ortho esters), with the specific choice based on the desired therapeutic effects, the desired sustained-release time frame, and the ability to reduce side effects.

In addition to these polymers, which have been used in numerous food and pharmaceutical applications with some having “Generally Recognized as Safe”, or GRAS, status, several novel cationic polymer excipients with attractive properties for encapsulation of nucleic acids into PNPs for nonviral intracellular delivery are currently being developed.

These polymers have not only hydrolysable ester groups but also accompanying cationic moieties, typically via tertiary amine groups. The cationic charges enable them to bind to negatively charged nucleic acids, pass through cell membranes, and disrupt endosomes. The most promising examples are poly(beta-amino esters) (PBAEs), which have been shown to condense and deliver nucleic acid cargoes intracellularly with minimal systemic toxicity risk (e.g., lower than widely explored polyethyleneimine).

Research is now focused on identifying PBAEs and other polyamino-based block polymers with optimized transfection efficiencies versus prior generations. Interest in this approach to nonviral gene therapies is rising in response to updates to the FDA's guidance and acceptance framework to promote the rapid development of per-

sonalized medicines and gene therapies⁴, which opens the door for PNPs designed to excel at nucleic acid delivery.

Achieving Targeted Delivery with Multiple Approaches

The ability to modify the surfaces of polymer nanoparticles enables targeted delivery beyond lipid-based approaches, and their extended half-life can be advantageous for improving target uptake. Additional phenomena, such as the enhanced permeability and retention (EPR) effect, can be leveraged with polymeric nanoparticles at solid tumor target sites. With this approach, particles within the appropriate size range preferentially accumulate (passive targeting) in solid tumors due to the presence of leaky, abnormal vasculature and poor lymphatic drainage. The key to success in this approach is determining the optimal size, duration of drug release, and surface chemistry for the tumor environment of interest.

With active targeting, ligands (e.g., antibody fragments, nanobodies, peptides, small molecules, etc.) are chemically conjugated to the surfaces of drug-loaded PNPs.

By designing delivery systems with scalability, reproducibility, and regulatory expectations in mind from the start, Phosphorex addresses the process translation gap and enables partners to maintain delivery performance while de-risking the development path from candidate selection to IND filing.

Ligand conjugation to the nanoparticle surface is typically achieved using either maleimide bioconjugation or copper-free click chemistry approaches. The specific ligand is chosen based on its ability to selectively bind to the target cell or tissue. This approach enhances the drug's potential to reach its intended destination while shielding it from degradation until release. Fluorescent dyes and imaging agents can also be conjugated to and/or encapsulated within PNPs for *in vivo* imaging applications.

Investing in Optimal Analytics Early in Development Pays Dividends

Polymeric nanoparticles and microspheres are complex systems. It is important to perform intentional, well-structured experiments from the outset of a development project to establish a deep understanding of the critical design characteristics that impact loading capacity, release rate, targeting ability, and manufacturability.

Powerful analytical methods that utilize high-performance liquid chromatography (HPLC) provide better characterization of discovery prototypes than simpler plate-based methods, leading to better candidate nomination. These techniques can, once a development candidate demonstrates full scalability potential, be further developed as stability-indicating methods for IND-enabling studies and IND filings. Additionally, with the expertise described above, it is possible to identify manufacturability issues before candidates are nominated, allowing selected candidates to move forward through development without the risk of redesign or repeated development.

Overcoming Translational Gaps

In fact, the inherent complexity of PNPs and polymeric microspheres often leads to large translational gaps when moving from preclinical to clinical development, particularly in achieving effective, economical, and scalable, clinically relevant production operations. Use of polymeric particles as drug delivery vehicles adds significant manufacturing complexity, and many manufacturers lack the expertise required for GMP production of these complex systems. In some cases, novel vehicles are developed on the benchtop only to reach a moment of truth: the technology transfer of this science to scalable, clinically relevant unit operations is not trivial. Even large contract

development and manufacturing organizations (CDMOs) with experience in the production of traditional four-component lipid nanoparticle (LNP) formulations lack expertise to address critical process development and formulation optimization challenges associated with polymeric particles.

Phosphorex has focused on polymeric delivery vehicles since its founding over 20 years ago. Today, we help clients evaluate the potential of both polymeric and lipidic targeted nanoparticle delivery systems in parallel. As a specialized partner, we guide innovators through small-scale feasibility testing and formulation optimization to ensure a balance of physical and chemical properties in the target product profile, resulting in reproducible *in vivo* performance and successful scale-up. We also know how to minimize process volumes and hold times to develop an economical process that can be efficiently tech-transferred for clinical drug product manufacturing.

By designing delivery systems with scalability, reproducibility, and regulatory expectations in mind from the start, Phosphorex addresses the process translation gap and enables partners to maintain delivery performance *while* de-risking the development path from candidate selection to IND filing.

Solving Delivery Challenges for Highly Potent APIs and Nucleic Acids

The unique properties of polymeric nanoparticles make them attractive as delivery vehicles for two challenging applications – highly potent compounds (HPAPIs) and nonviral delivery of nucleic acids. For HPAPIs, PNPs, by encapsulating the drug substance, protect the body from systemic toxicity, while the active targeting capability of ligand-modified PNPs promotes selective delivery to the target site (such as solid tumors) to mitigate off-target effects.

For nucleic acids, while LNPs will remain the predominant solution for nonviral delivery, there are certain instances where

lipidic vehicles fall short. In cases where extended half-life is advantageous for targeted gene therapy or there are tolerability challenges LNPs can't avoid, PNPs can emerge as an important part of the delivery toolkit. PNPs, for example, also hold the potential to unlock improved stability characteristics for more reliable targeting and streamlined distribution logistics. Finally, PNPs also have the potential to improve nuclear transport inefficiencies in non-viral gene-editing therapies.

Combined, the safety, tolerability, manufacturing stability, less saturated IP, and some differentiated performance properties of polymeric nanoparticles make them an exciting technology worth further investigation and investment. Indeed, the few examples presented herein highlight only some of the ways in which PNPs may help drug developers harness the full potential of non-lipidic nanoparticles and microparticles to treat highly sought-after indications with open demand for solutions.

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Engineering Polymeric Particles for Sustained Release of Small-Molecule Therapeutics

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Small-molecule drugs too often stumble in development or fail to reach their full potential, not because a compound lacks therapeutic benefit, but because of ineffective drug delivery and biodistribution. Polymeric drug delivery platforms, including nanoparticles and microspheres made from biodegradable polymers such as poly (lactic-co-glycolic acid) (PLGA), add powerful tools to the drug developers' toolbox. By encapsulating the drug within a polymer matrix, these systems control how quickly the drug is released and how long it actively circulates in the bloodstream.

Polymeric nanoparticles are well-suited to solve three distinct delivery challenges. First, they can be engineered to overcome various biological barriers, such as penetration of the mucosal surfaces of the eye and the lining of the GI tract. This physiology is cleverly designed to prevent unwanted viruses, bacteria, and other contaminants from penetrating these surfaces. However, this protection also complicates drug delivery across these surfaces. A nanoparticle that can reside on the ocular surface longer than a standard eyedrop or survive transit through the GI tract long enough to reach its absorption site, can deliver more drug to its target.

The second benefit is the ability to control the release rate to eliminate dose-limiting side effects. Many small molecules are therapeutically effective but suffer dose-limiting toxicities due to lack of target specificity, rapid systemic clearance, and off-target tissue accumulation. However, encapsulation slows release, reduces peak exposure, and keeps drug levels within a window the body can tolerate. The third advantage polymeric nanoparticles provide is improving drug biodistribution to target tissues and cell types. Nanoparticles with appropriate size and surface chemistry can circulate in the bloodstream long enough to accumulate at designated targets, such as solid tumors, via the enhanced permeability and retention effect, without requiring active targeting mechanisms.

Polymeric microspheres solve a different problem. Delivered subcutaneously or intramuscularly, they enable true long-acting release. A single injection can maintain therapeutic drug levels for weeks or months. This reduces dosing frequency, improves pharmacokinetic consistency, and meaningfully increases patient adherence, particularly for chronic conditions where daily medication is a compliance burden.

Commercial and Clinical Success of Polymeric Drug Delivery Systems

The successful commercial track record of polymeric drug delivery belongs to microspheres. There are currently 15 FDA-approved polymeric microsphere-based therapeutics,¹ including Risperdal Consta (risperidone), which delivers a sustained-release antipsychotic via intramuscular injection, eliminating the need for daily dosing in schizophrenia patients, a significant compliance advantage for a patient population where medication adherence is a persistent clinical problem. Other approved PLGA microsphere products follow the same logic: leuprolide acetate (Lupron Depot) for prostate cancer and endometriosis, naltrexone (Vivitrol) for alcohol and opioid dependence, octreotide (Sandostatin LAR) for acromegaly, and triptorelin (Trelstar) for prostate cancer. Each encapsulates a small molecule or peptide in PLGA, injects subcutaneously or intramuscularly, and releases the drug over weeks to months.

The story of polymeric nanoparticles is more complex, with several innovative platforms in late stages of clinical development. The first targeted polymeric nanomedicine to enter clinical studies,

BIND-014, was a prostate-specific membrane antigen (PSMA) targeted polymeric nanoparticle encapsulating docetaxel, which produced meaningful clinical responses across multiple tumor types in Phase I and a promising response rate in metastatic castration-resistant prostate cancer in Phase II, with manageable tolerability. Unfortunately, additional clinical testing of BIND-014 was ceased. However, BIND Therapeutic's second-generation formulations (e.g., AZD2811) demonstrated even greater clinical promise, benefiting from hydrophobic ion pairing to achieve slower drug release rates and improved encapsulation efficiencies. Based on this work, an ongoing Phase II study is evaluating AZD2811 (an Aurora B kinase inhibitor) in combination with the immunotherapy drug durvalumab as maintenance therapy after induction with platinum-based chemotherapy plus durvalumab, for the first-line treatment of patients with extensive-stage small-cell lung cancer.²

Why Polymeric Nanoparticle Formulations Are Attractive

The appeal of polymeric nanoparticles starts with compounds that display therapeutic benefits but are not tolerable when administered to patients. For example, kinase inhibitors are often highly effective at shutting down the cellular pathways that drive cancer, but these on-target effects also negatively impact healthy cells and tissues. Encapsulating kinase inhibitors in a polymeric nanoparticle slows release, reduces peak concentrations, and brings toxicity down to a level the body can tolerate without compromising therapeutic effect. In animal studies, slow-releasing formulations consistently produced the best tolerability and the strongest long-term efficacy.³

The benefits extend well beyond toxicity management. PLGA nanoparticles protect drug payloads from enzymatic degradation and from physiological conditions that would otherwise diminish potency, which is particularly relevant for oral delivery, where the GI tract presents a hostile environment for many therapeutic compounds. For pediatric formulations, encapsulation solves a different problem entirely: a bitter drug sealed inside a polymer particle never reaches the taste receptors, with direct implications for compliance in a population where palatability determines whether a medication is taken at all.

Surface modification with PEG extends circulation time by reducing opsonization and immune recognition, thereby giving particles more time to reach their target. That extended half-life creates a meaningful advantage over lipid nanoparticles, which tend to clear more rapidly from the bloodstream, opening delivery opportunities to extrahepatic tissues.

Broad Applications for Polymeric Nanoparticles

When applied to mucosal surfaces such as the ocular surface, nanoparticles can remain longer than conventional eye drops, delivering more drug before blinking clears them away. For solid tumor applications, appropriately sized nanoparticles exploit the enhanced permeability and retention effect, passively accumulating within the tumor without requiring active targeting mechanisms. Surfaces can also be functionalized with ligands, antibodies, or peptides for receptor-mediated targeting when passive accumulation isn't sufficient.

Finally, there is a commercial logic to this platform that drug developers are beginning to recognize. Reformulating a known molecule into a polymeric nanoparticle system opens new patent space, a meaningful incentive when the underlying active ingredient is mature and the IP landscape around the molecule itself is crowded. The platform doesn't change the drug. It changes what the drug can do, who can tolerate it, and in many cases, whether it reaches patients at all.

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Challenges Commercializing Polymeric Nanoparticles

The science of polymeric nanoparticles is well ahead of the commercial reality, and while there are several polymeric microsphere products on the market, there is no approved therapeutic utilizing a polymeric nanoparticle delivery system. The primary barriers are formulation and manufacturing. From a manufacturing perspective, translating a nanoparticle formulation from lab-scale production to an industrially viable, reproducible GMP process while maintaining proper characterization and quality is complex and challenging.

The formulation complexity compounds the problem. Solvent selection, co-solubilization of polymer, drug, and excipients, emulsion stability over longer processing times at larger volumes, purification, and burst-release control each require specialized expertise that few innovators or CDMOs possess. The result is a field with strong preclinical data but no approved products. Closing that translational gap requires close collaboration between manufacturing science and formulation science.

Polymer Selection: Starting with What Works

For most small-molecule applications, polymer selection begins and often ends with PLGA. The reasons are practical. PLGA is biodegradable, breaking down into lactic acid and glycolic acid, both naturally occur-

ring metabolites the body handles without difficulty. It has an extensive history of use in FDA-approved drug products, which means its behavior is well characterized and its regulatory standing is established, reducing regulatory risks.

Importantly, PLGA isn't a single material. The ratio of lactic acid to glycolic acid in the polymer chain can be adjusted to tune degradation rates and, by extension, the drug release profile. For example, higher glycolic acid content accelerates degradation, whereas higher lactic acid content slows it. Molecular weight is another lever: low-molecular-weight polymers degrade faster than high-molecular-weight versions. Polycaprolactone and polyanhydrides are available for applications requiring different release windows or degradation characteristics, but PLGA/PLA remains the default starting point for sustained release of small molecules precisely because so much is already known about its behavior.

Drug Encapsulation Strategies: Nanoemulsion and Nanoprecipitation

Once the polymer is selected, the next decision is how to incorporate the drug. The two primary manufacturing pathways are nanoemulsion and nanoprecipitation. The choice between these two approaches comes down to one practical question: which solvent(s) effectively solubilize the drug and polymer?

Nanoemulsions use water-immiscible solvents; ethyl acetate and benzyl alcohol are common examples. The polymer and drug are co-dissolved in the organic phase, then dispersed into water, essentially a controlled version of salad dressing, with small oil droplets suspended in water, each containing polymer and drug. High-pressure homogenization reduces the droplets to the 100-nanometer range, the solvent is extracted, and the drug becomes encapsulated in the solidified particle. With straightforward formulations, drug loading typically lands in the 5–9% range by weight.

Nanoprecipitation uses water-miscible solvents instead; acetonitrile and tetrahydrofuran (THF) are two examples compatible with PLGA. There is no emulsion step; as the organic and aqueous phases are mixed, the solvent diffuses out, leaving behind precipitates in the form of polymeric nanoparticles.

Both processes require the drug and polymer to be co-soluble in the chosen solvent system, a requirement that becomes

significantly more complex when additional excipients are added to the formulation.

Hydrophobic Ion Pairing: The Advanced Tool

Many small molecules (e.g., kinase inhibitors) have tertiary amine groups built into their chemical structure that create pH-dependent solubility. At high pH, the drug behaves as its most hydrophobic form (free base); upon shifting to acidic conditions, those amines become protonated, resulting in more polar, more hydrophilic behavior. Since PLGA is itself hydrophobic, encapsulation works best when the drug shares that character. By formulating at a high pH, a kinase inhibitor can be encapsulated efficiently in its freebase form, but hydrophobic ion pairing takes this further.

The strategy pairs the drug with a hydrophobic counterion (an acid in the case of a basic drug), forming an electrostatic complex that co-encapsulates within the PLGA particle far more efficiently than the drug alone. Counterion selection drives two outcomes: encapsulation efficiency and release rate.

A 1:1 molar ratio is typically optimal, but an excess of counterion can drive drug encapsulation toward 100% efficiency. Importantly, the hydrophobic ion pairing approach often reduces the extent of drug burst release, a reduction that must be avoided to maintain tolerability. Furthermore, the properties of the hydrophobic counterion (e.g., acidity, molecular weight, and lipophilicity) significantly affect the drug release rate, with formulations prepared using oleic, cholic, and pamoic acids achieving progressively longer release durations, ranging from three to over ten days, based on *in vitro* analysis.³

Hydrophobic ion pairing can also be used to encapsulate water-soluble payloads, such as nucleic acids, peptides and proteins, into hydrophobic polymeric matrices. The approach is similar to the use of ionizable/cationic lipids in lipid nanoparticles (LNPs), where nucleic acid-based cargo (e.g., mRNA, DNA) is encapsulated within the LNP during particle fabrication via electrostatic interactions between positively charged lipid species and negatively charged nucleic acids under acidic mixing conditions. This approach vastly expands the range of active pharmaceutical ingredients (APIs) that can be encapsulated within polymeric systems, a capability that was historically limited to hydrophobic APIs.

Phosphorex guides drug developers through the full development pathway for both polymeric nanoparticles and microspheres, with the specialized expertise to de-risk the science, close the translational gap, and move programs forward with confidence.

Overcoming Polymeric Nanoparticle Manufacturing Challenges

The formulation science behind polymeric nanoparticles is well understood. Getting that science to behave reliably at manufacturing scale is a different problem, and it has been the barrier to commercializing polymeric nanoparticle therapeutics.

Controlling Burst Release

Burst release, the rapid spike in drug concentration at the start of a release profile, results from drug adhering to the particle surface or from free drug that precipitated during manufacturing and wasn't fully removed during purification. Either way, that drug dissolves immediately upon administration, producing the peak concentrations the formulation was designed to prevent.

Tangential flow filtration (TFF) removes free drug from the nanoparticle suspension, but residual drug must be fully dissolved in solution to pass through the membrane. Any precipitated drug re-circulates with the drug product instead of passing through the TFF membrane. Where surface-bound drug remains a problem after standard purification, a 'conditioning' step can help: temporarily heating the formulation above PLGA's glass transition temperature of approximately 37°C dislodges the drug from the surface, and then a subsequent

TFF step removes it from the drug product suspension. Phosphorex has extensive experience in employing the appropriate scale-able downstream processing as well as hydrophobic ion pairing strategies for minimizing API burst release.

Solving Co-Solubilization

Typical formulations using hydrophobic ion pairing require that the polymer, drug, and counterion be dissolved into a single solvent system before particle formation can begin. The physicochemical properties of the API and counterion can pose challenges during this step; aromatic structures and highly hydrophobic species are prone to precipitating out of solution, and the resulting precipitates are difficult to remove during downstream purification and also present potential in-process stability concerns that worsen at larger volumes and longer processing times. Phosphorex's solution is co-solvent development: identifying a solvent blend in which all components remain stably dissolved throughout the hold times required for manufacturing. There is no universal answer, so the right system must be found empirically for each drug and counterion combination.

Maintaining Stability at Scale

At the bench scale, an emulsion can be processed before instability develops. At manufacturing scale, the same formulation must remain stable over longer hold times and larger volumes. Therefore, emulsion stability should be considered from the start of development to avoid costly delays at later stages, when the stakes are higher. Through careful excipient selection (e.g., surfactants), co-solvent systems, and solid content, scalable emulsion-based formulations can be identified and considered for selection as lead formulation candidates.

Address Therapeutic Index Challenges & Extend Intellectual Property

Polymeric nanoparticles and microspheres represent one of the most technically de-

manding areas of pharmaceutical development, but there is a tremendous commercial opportunity. For small-molecule drugs that have stalled due to tolerability problems, the right polymeric formulation can be the difference between a shelved candidate and a viable commercial product. Additionally, polymeric microsphere or nanoparticle delivery systems might be ideal for extending therapeutic patent life and creating new areas of intellectual property.

Also, programs that reformulate utilizing a polymeric strategy may offer meaningful regulatory advantages as existing safety data on the active pharmaceutical ingredient can often be leveraged, de-risking and potentially accelerating the development timeline compared to a novel chemical entity.

However, the expertise required to navigate the complexities of polymeric therapeutic development is rare. Phosphorex has spent over two decades building this capability. From initial feasibility through formulation optimization, process development, and IND-enabling studies, Phosphorex guides drug developers through the full development pathway for both polymeric nanoparticles and microspheres, with the specialized expertise to de-risk the science, close the translational gap, and move programs forward with confidence.

For developers exploring polymeric delivery for the first time, or revisiting candidates that deserve a second look, Phosphorex is the partner built for this work.

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TECHNICAL BULLETIN

Effect of Formulation Buffer Type on LNP Size

Introduction

Lipid nanoparticle (LNP) fabrication typically starts with rapid mixing of lipids in ethanol and nucleic acid-based cargos prepared in an acidic aqueous buffer solution (buffer pH < pKa of the ionizable lipid). The acidic aqueous buffer is of importance to (1) protonate the ionizable lipids so that they may bind electrostatically with the negatively charged nucleic acid-based cargos and (2) increase the solvent polarity to drive the particle formation and cargo encapsulation. Aqueous buffer solution parameters such as type of buffering species, pH and salt concentration (ionic strength) not only impact cargo stability but also LNP size and size distribution [1,2].

Many studies have reported the importance of LNP size on the *in vitro/in vivo* efficacy. The optimal LNP size largely depends on the intended indication, route of administration and animal species. For example, the ideal particle size for hepatic delivery of intravenously administered LNPs in nonhuman primates (NHPs) is between 50 to 60 nm, while 70 to 80 nm works best in rodents [3]. When LNPs are used as a vaccine and administered by intramuscular injection, different particles sizes ranging from 60-150 nm produced similar immune response in NHPs, but in mice particles > 80 nm performed the best [4]. Therefore, precise size control of LNPs during fabrication is an essential step towards maximizing the desired therapeutic effects.

The LNP size can be tuned in many ways. Increasing the molar ratio of polyethylene glycol (PEG) lipid is commonly employed to reduce particle size, stabilize LNPs and increase their circulation time. However, too much PEG on the particle surface will inhibit the cellular uptake of LNPs. In addition, PEG associated immunogenicity may result in reduced treatment efficacy or unwanted side effects. LNP size can also be adjusted by changing the mixing conditions such as the total flow rate, cargo/lipid solution flow rate ratio, and inline dilution ratio. In this study, we aim to tune the LNP size using different cargo buffer solutions while maintaining the same lipid composition and mixing conditions across all formulations.

Methods

To study the effect of aqueous buffer pH and buffer type on LNPs, polyA (model cargo with ~6,000 nucleotides) was dissolved in 10 mM citrate buffer at pH=3, 4 or 5, 10 mM malate buffer at pH=5, or 10 mM acetate buffer at pH=5. The aqueous buffer solution was then mixed with lipid excipients including Dlin-MC3-DMA (MC3), Cholesterol, DSPC, DMG-PEG2K at molar ratio of 50:38.5:10:1.5 in ethanol using Unchained Labs' Sunshine system and 190 µm Sunny XT cross-junction chip (Fig. 1). N/P ratio was 6. The flow rate ratio of aqueous solution and lipid solution was 3:1, and total flow rate was 12 mL/min. Post LNP mixing, all formulations were processed using the same downstream process parameters: LNP solutions were dialyzed against 20 mM Tris-HCl buffer (pH 7.4) using a Slide-A-Lyzer™ dialysis cassette (MWCO: 20 kDa) overnight to remove the ethanol, followed by Amicon® ultra centrifugal filter (MWCO: 100 kDa) concentration to ~200 µg/ml, and 0.2 µm syringe filtration. The mean particle diameter and polydispersity index (PDI) were determined via dynamic light scattering (DLS). The encapsulation efficiency (EE%) and total polyA concentration in prepared LNPs were determined using a Quant-iT™ RiboGreen® RNA Assay.



Figure 1: Unchained Labs' Sunshine System with Sunny XT cross-junction chip (insert)

Results

As shown in Fig. 2, particle size increases with the increase of citrate buffer pH post LNP fabrication via microfluidic mixing and size is well maintained during subsequent downstream processing steps including dialysis, amicon concentration and filtration. Although the impact of cargo buffer pH might be formulation dependent, the particle size dependency on formulation pH reported here is in good agreement with the literature [1, 5].

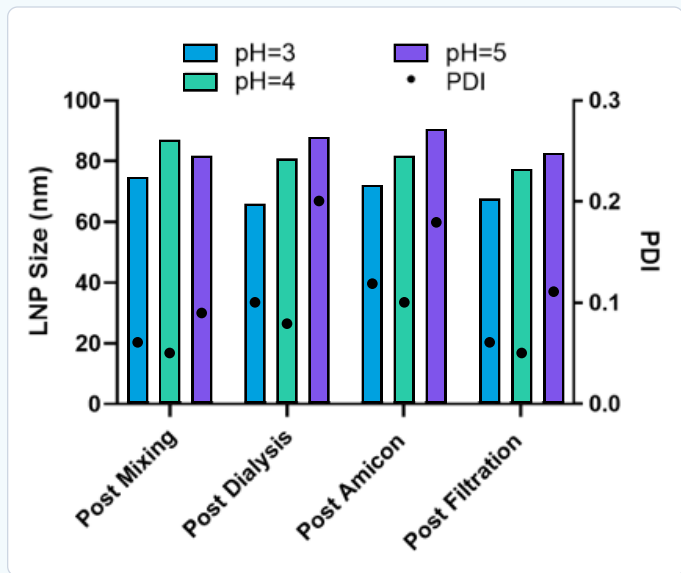


Figure 2: Effect of citrate buffer pH on LNP size and PDI

To study the effect of buffer type, 10 mM citrate (tricarboxylic acid) buffer, 10 mM malate (dicarboxylic acid) buffer and 10 mM acetate (monocarboxylic acid) buffer at pH 5.0 were used as cargo buffers for LNP preparation. In Fig. 3, LNPs fabricated using acetate buffer generated the smallest particles while citrate buffer generated the largest particles. A similar trend was observed after subsequent downstream processing steps. In addition to effects on particle size, it's important to note that the chelating ability of citric acid can reduce the hydrolysis rate of nucleic acids, therefore citrate buffers may be preferable when encapsulating less stable cargos [1, 2].

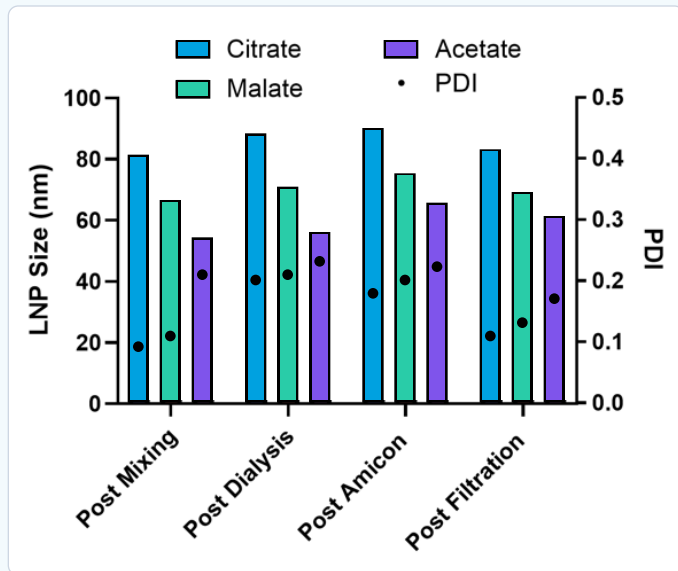


Figure 3: Effect of buffer type on LNP size and PDI

The polyA encapsulation efficiencies (EE%) for all LNPs were above 94% with no significant differences observed among the conditions tested (Fig. 4).

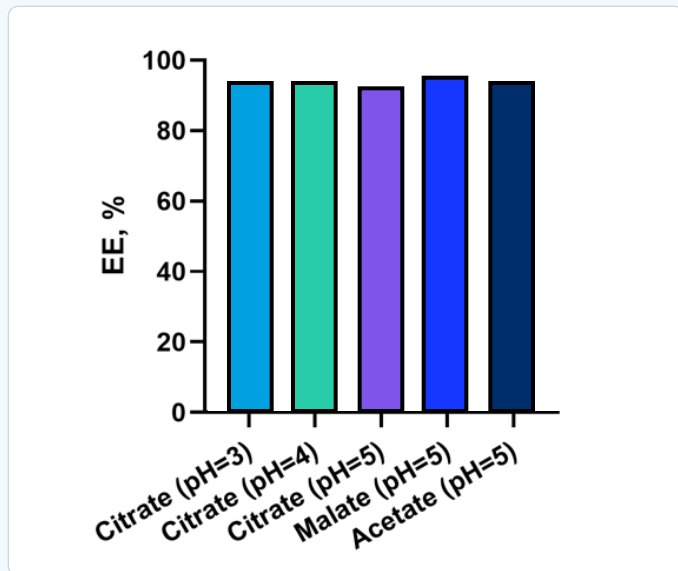
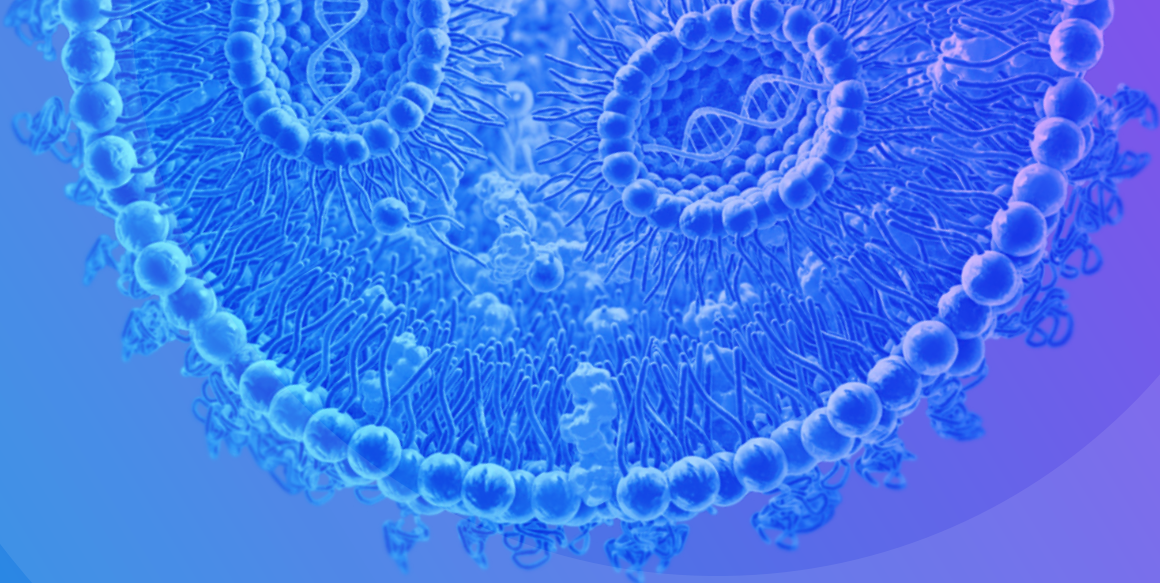


Figure 4: EE% of LNPs prepared using different cargo buffer types.



Conclusion

Here we demonstrate that LNP size can be fine-tuned through the selection of the appropriate cargo buffer. The choice of buffering species type, pH, and ionic strength must be carefully considered. Importantly, this approach enables fabrication of LNPs of various size while maintaining the same lipid composition and microfluidic mixing conditions; thereby, facilitating the investigation of LNP size effects on *in vivo* therapeutic efficacy during pre-clinical testing. Phosphorex has further expertise in LNP formulation design including optimization of the lipid components and compositions (e.g., ionizable lipid type, PEG-lipid type/content, etc.), formulation storage buffer, and preparation of actively targeted formulations through surface conjugation of targeting ligands. At Phosphorex, we pride ourselves in our ability to offer customized LNP solutions to best meet our client's project requirements.

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SUCCESS STORY

Clinically Relevant Downstream Process Optimization

Problem

An ionizable lipid chemistry innovator company developing novel LNP systems was interested in developing an effective, scalable downstream process to reliably maintain LNP critical quality attributes. Due to the unique formulation composition, the LNP demonstrated shear sensitivity in standard tangential flow filtration (TFF) protocols.

Solution

Phosphorex optimized the quality of the novel, shear-sensitive LNP formulation after mixing process scale-up and transfer of batch downstream purification from dialysis to tangential flow filtration (TFF) by:

- Determining the optimal TFF membrane chemistry, molecular weight cut-off, and surface area
- Determining the optimal concentration factor before diafiltration
- Optimizing the transmembrane pressure, feed pump flow rate, and column loading
- Optimizing the downstream buffer pH and ionic strength

Outcome

The LNPs resulting from the optimized TFF method demonstrated >90% cargo yield, a mean particle size of 96 nm, a low PDI of 0.11, and an encapsulation efficiency of 92% following successful concentration to the target range. The final TFF method was qualified for batch fabrication to support an NHP study.

At Phosphorex, we are committed to helping our partners navigate the complexities of precision drug delivery and advance their programs with confidence. We adapt to your modality, delivery target, and mission, bringing together comprehensive expertise through our Drug Delivery Engine to support every stage of development.

With payload-agnostic, non-viral delivery capabilities, deep experience, and an adaptive approach, we move at the pace of your program; iterating, refining, and aligning as needed. Through close collaboration and clear communication, we serve as a true extension of your team, focused on delivering meaningful progress.

Let's Solve Your Delivery Challenge >

Behind the Solution

Our partner required a robust tangential flow filtration (TFF) process to support the scale-up of their proprietary lipid nanoparticle (LNP) formulation for rat and non-human primate (NHP) toxicology studies. Following successful demonstration of scalability for the upstream microfluidic mixing process, Phosphorex transitioned the downstream purification workflow from dialysis/Amicon-based processing to a TFF-based approach. This process was subsequently optimized using Phosphorex's established TFF development and scale-up methodology, resulting in a greater than twofold reduction in processing time.

Importantly, the optimized TFF process maintained the critical quality attributes (CQAs) of the LNP formulation while achieving the target final LNP concentration following sucrose addition. In addition, a high overall process yield (>90%) was maintained, which was essential given the limited availability of RNA for producing the toxicology study batches.

Using the optimized TFF process, Phosphorex successfully manufactured the LNP toxicology batches and, through the LNP Alliance, supported the execution of rat and NHP toxicology studies at Neosome and Envov, respectively. Completion of these studies represented a critical milestone, enabling our partner to make informed strategic decisions regarding the further development and advancement of their proprietary LNP formulations.



Matthew Freeman, PhD

Senior Scientist

Matthew Freeman holds a PhD in Chemical Engineering from the University of Rhode Island. He specializes in nanoparticle and microparticle drug delivery systems, with publications and presentations spanning synthesis and physicochemical characterization work.



This project came down to time and material. Our partner had a limited supply of RNA, so we couldn't afford a process that wasted product, and we were on a tight timeline. Moving from dialysis and Amicon-based processing to a TFF approach, then optimizing it with our scale-up methodology, cut processing time by more than half while keeping yield above 90%. The formulation reached its target concentration after the addition of sucrose and retained the quality attributes that mattered for the studies. That gave our partner the data they needed to make decisions about the next phase of their program.



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