MICROSPHERES NANOPARTICLES BACHEM

LEADING PARTNER IN TIDES

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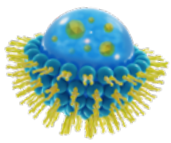
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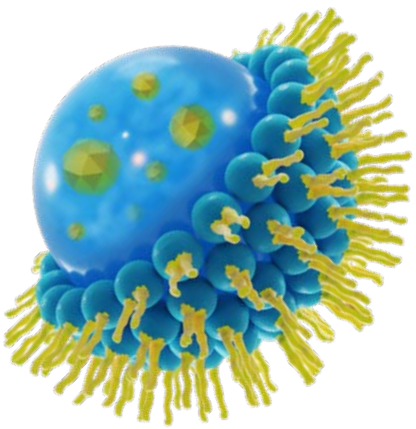
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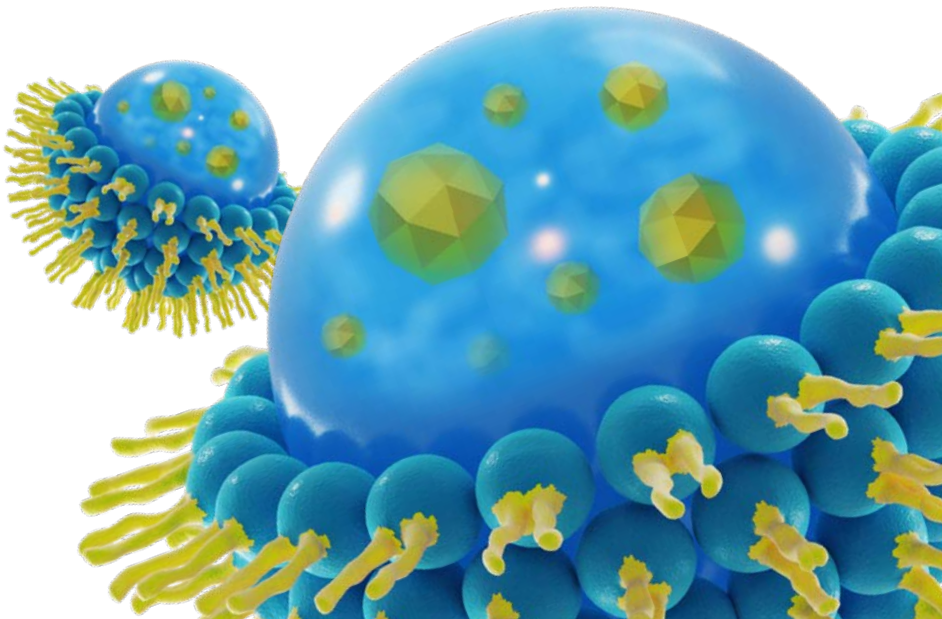
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NAVIGATING THE BENEFITS AND CHALLENGES OF MICROSPHERES AND NANOPARTICLES FOR PEPTIDE DELIVERY

Executive Summary

Proteins and peptides fulfill an irreplaceable role as medicines due to their high affinity, specificity, low toxicity, and ability to alter protein-protein interactions. They are widely recognized as therapeutic agents in the treatment of a variety of conditions, including diabetes, osteoporosis, non-Hodgkin's lymphoma, and leukemia. Clinical development is on-going as the pharmaceutical industry works to harness the potential of peptides for many other conditions. While incredible progress has been made in designing peptide- and protein-based drugs for multiple therapeutic uses, challenges remain. This paper explores challenges of peptide- and protein-based new chemical entities [NCEs] including potential chemical and physical instabilities, enzymatic degradation, and their rapid elimination from circulation.

Addressing these challenges has led to growing demand for more innovation and enhanced technological capabilities. The formulation of nanoparticles and microspheres for peptide delivery, to enhance bioavailability and therapeutic efficacy, is emerging as one innovative approach.

Bachem is dedicated to exploring the potential of discoveries in the field of life science research and development. Innovation is integral and the company strives to continuously expand its know-how in chemistries and technologies through internal research projects as well as by collaboration with external research institutions. We are currently developing a proprietary fully automated peptide synthesis system in-house which in combination with high capacity and state-of-the-art production equipment will allow us to achieve short delivery times without compromising on quality.

Bachem's support and expertise is vital in achieving successful formulation of microspheres and nanoparticles, where the production of high quality active pharmaceutical ingredients (APIs) in the first instance is critical. This includes the intrinsic properties of the peptides themselves, for example solubility, as well as the procedures of API release following production. To meet these high standards, development teams are being driven to access Bachem's specialist expertise in the production of superior quality APIs.

Introduction: the trends driving interest in nanoparticles and microspheres

The delivery of proteins and peptides to specific targets is a difficult task owing to several factors. While the parenteral route is the most common method of administration, the short half-life of these drugs necessitates frequent injections which can result in reduced patient compliance. For this reason, non-invasive drug delivery routes (nasal, transdermal, pulmonary, and oral methods) have attracted growing interest.

Oral delivery of proteins and peptides is challenging due to the presence of proteolytic enzymes in the gastrointestinal tract (GIT), as well as their inherent physiochemical and biological properties, including poor stability, large molecular size, and poor permeation across the gastrointestinal membrane. Large size and proteolytic instability can cause poor absorption of therapeutic proteins across nasal and pulmonary mucosal surfaces, while physiological barriers such as mucociliary clearance may further limit protein and peptide absorption. Hydrophilicity and large molecular size can also restrict transdermal peptide/protein delivery with peptide therapeutics usually requiring chemical or physical interventions to increase skin permeability transiently.

The encapsulation of peptides or proteins in microparticles or nanoparticles (NPs) during formulation may lead to a better drug profile, while also helping to mitigate solubility problems and addressing the needs of long-acting injectable depot formulations and specific drug targeting options. Microencapsulation comprises the coating of solid, liquid, or gaseous materials with a film of polymer to generate free-flowing micrometric particles. The products obtained by this process are called microparticles, microcapsules or microspheres, which are differentiated by morphology and internal structure. Particles smaller than 1µm are known as NPs, nanocapsules or nanospheres. Microspheres are small spherical particles with diameters in the micrometer range typically from 1 to 1000µm (1mm). Marketed drugs that are microencapsulated include aspirin, theophylline derivatives, vitamins, pancrelipase, progesterone, potassium chloride, goserelin peptide, triptorelin peptide and leuprolide peptide.

The larger size of microspheres mean they can encapsulate larger amounts of drugs. Although they cannot be easily used for intravenous or systemic delivery, as they may

agglomerate and cause clotting, they are effective for local delivery, such as subcutaneous injection, and can be used in sustained-release systems.
NPs have a high surface area to volume ratio, which promotes a high degree of surface absorption by drugs, proteins, and other molecules. This also allows for increased interaction with other particles, which leads to changes in physical properties. NPs can be made aggregation free, making them useful for intravenous or systemic delivery of drug molecules that are otherwise difficult to handle. They are also more likely to bypass the reticuloendothelial system and biological barriers such as the blood–brain barrier (BBB). Additionally, NPs can enter all cells via pinocytosis.

Microspheres for peptide delivery
Biodegradable microspheres composed of biocompatible polymers have been studied as systems for the controlled release of proteins and peptides. Generally, a drug is distributed through the polymeric matrix and released by two mechanisms: diffusion through the matrix and polymer degradation, which erodes the particles.

Advantages of microspheres
Microspheres can support the controlled release of a drug to specific target sites, offering protection of the unstable drug and the ability to manipulate the in vivo action, pharmacokinetic (PK) profile, tissue distribution and cellular interaction of the drug. The use of peptide/protein-encapsulated microspheres could potentially reduce the frequency of administration resulting in better acceptance by the patient. They can also increase therapeutic benefit, eliminate fluctuations in serum concentrations of a drug and decrease the total dosage required for successful treatment owing to higher bioavailability of the dose administered. Additionally, in enabling lower doses, they may decrease the likelihood of adverse events.

Production of microspheres
Several methods are available for the preparation of microspheres. These include single-emulsion techniques, double-emulsion techniques, polymerization techniques (normal polymerization or interfacial polymerization), coacervation phase separation techniques, spray drying, spray congealing and solvent extraction. Peptide and protein molecules are fragile under experimental conditions, accounting for the main limitations of their microencapsulation conditions. Therefore, among the different techniques available, only a few are frequently used to obtain microspheres loaded with peptide substances.
Physiochemical characterization is also an important aspect of microsphere formulation and confirms the quality and quantity of the drug (including drug loading, absence of incompatibility, stability and crystallinity) encapsulated in the microspheres, quality of the microspheres (such as particle size, shape and percentage entrapment) and quality of the microencapsulation process (including yield, percentage encapsulation and particle size distribution). Determining the percentage drug loading, entrapment/encapsulation efficiency and particle size distribution of microsphere formulations requires the use of optical microscopy, scanning electron microscopy, Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC) and X-ray diffraction (XRD).
Commercially available peptide drugs that use microsphere-based formulations include leuprolide, triptorelin, octreotide, lanreotide, human growth hormone, buserelin, abarelix and exenatide.

Challenges of microspheres
While clear advances have been made in the use of microspheres for delivering biopharmaceutical drugs, challenges remain. Disadvantages of microspheres include the difficulty of large-scale manufacturing, inactivation of the drug during fabrication and poor control of drug release rates. Specifically, further advances in controlling the burst release can increase the duration of drug release. In addition, methods that provide active control of the rate of drug release, including on-demand termination of release, could create new opportunities. A reduction in the size of the microspheres, albeit still within the micron range, could lead to subsequent reduction in the needle size required for administration while maintaining the rate of sustained release. Additional unmet needs include the requirement for a robust manufacturing process that can be achieved at a reasonable cost. In addition, it is important to consider issues such as ease of injection – that is, addressing the reconstitution of the lyophilized product and the clogging of needles with drugs. Many of these issues are not unique to protein- and peptide-based drugs but are common to microsphere-based drugs.
Proteins can also lose their structure and biological activity upon prolonged incubation with biological fluids under physiological conditions. For example, aggregation and incomplete release from the microsphere have been observed with growth hormones, while a covalent dimer formed in a microsphere formulation of darbepoetin alfa. The local degradation of polymers at the injection site can also lower the pH inside the microspheres, which can further add to the potential for protein inactivation. The addition of magnesium hydroxide and other antacids within the microspheres has been shown to negate the adverse effects of low pH.

Nanoparticles for peptide delivery

NPs made from natural and synthetic polymers (biodegradable and nonbiodegradable) have attracted attention as they can be customized for the targeted delivery of drugs, improving bioavailability, and providing controlled release of a drug from a single dose. Due to their small size and large surface area, drug NPs show increased solubility and enhanced bioavailability, with additional ability to cross the BBB, enter the pulmonary system and be absorbed through the tight junctions of endothelial cells of the skin. Different types of NPs are available, including liposomes, polymeric micelles, polymeric NPs, nanoemulsions, nanogels, dendrimers, fullerenes, carbon nanotubes, magnetic NPs, metal NPs and quantum dots.

Advantages of NPs

Micelles and liposomes offer an important avenue for the delivery of chemotherapeutic agents. Micelles also provide a useful means to make insoluble drugs soluble owing to their hydrophobic core and hydrophilic shell. If the micelle's surface is further PEGylated, it increases the ability of the nanocarriers to pass through the fenestrated vasculature of tumors and inflamed tissue through passive transport, resulting in a higher drug concentration in tumors.

Dendrimers are highly branched macromolecules with many functional groups available for the attachment of drugs. NP therapeutics based on dendrimers can improve the therapeutic index of cytotoxic drugs by employing biocompatible components and surface derivatization with PEGylation, acetylation, glycosylation, and various amino acids.

Among nanocarriers, polymeric NPs have demonstrated significant advantages over other delivery systems. They exhibit high stability in biological fluids compared with liposomes and solid lipid NPs. The properties of polymeric NPs are significantly affected by the nature of the polymers, either natural or synthetic, and the method of preparation. Commonly employed natural polymers include chitosan (CS), gelatin and alginate. Among natural polymers, CS has shown the most interesting potential, which is attributed to its better solubility at intestinal pH, improving mucoadhesivness and permeation enhancement. In the small intestine, CS NPs can adhere to and infiltrate into mucus layers and open the tight junctions between contiguous epithelial cells. Furthermore, pH-sensitive CS NPs can disintegrate and release the encapsulated drugs, which then penetrate through the opened paracellular pathway. Among synthetic polymers, polyesters, alone or in combination, are the most relevant and most frequently studied for protein delivery. In contrast to natural polymers, synthetic polymers permit adjustable controlled drug release for a period of several days to weeks.

Polymeric NPs can offer an alternative strategy to improve the bioavailability of encapsulated proteins and peptides by providing protection against degradation in the gastrointestinal (GI) environment, enhancing cellular contact with the intestinal membrane and promoting absorption in the small intestine.

Production of NPs

Proteins/peptides can be encapsulated, absorbed, or chemically linked to the surface of polymeric NPs. Protein incorporation in polymeric NPs can be achieved by various methods. Natural polymers are generally more sensitive to the processing conditions. Therefore, NPs with natural polymers are generated using mild techniques including ionic gelation, polyelectrolyte complexation and coacervation. NPs composed of synthetic polymers are normally prepared by more extensive techniques such as interfacial polymerization, emulsification–polymerization, emulsification–solvent evaporation, nanoprecipitation, salting out, supercritical fluids and emulsification–solvent diffusion.

Peptide NP formulation examples

Marketed NP-based medicines include doxorubicin liposomes for the treatment of ovarian cancer and AIDS-associated Kaposi's sarcoma, as well as paclitaxel-containing albumin NPs for second-line treatment of patients with breast cancer. Several examples of NP-mediated delivery of small-molecule chemotherapeutic agents are at the clinical and preclinical stages. There are fewer examples of the use of NPs for delivering proteins and peptides and these agents are in the early stages of development.

Limitations of NP delivery systems

NP drug delivery systems are only useful if a drug is released effectively. As the particle size becomes smaller, the surface area to volume ratio becomes larger. This implies that more of the drug is closer to the surface of the particle compared with a larger molecule, and this leads to faster drug release. It would be beneficial to create NP systems that have a large surface area-to-volume ratio; however, toxicity must always be monitored. The size of the NP determines the biological fate. The vascular and lymph systems are responsible for the filtering and clearance of foreign matter and chemicals. This is a key factor that must be engineered into the ideal NP system.

It has been shown that particles 200nm or larger tend to activate the lymphatic system and are removed from circulation faster. The optimum size for an NP is approximately 100nm. At this size, the particle can pass through the BBB with a sufficient amount of drug delivery due to the high surface area-to-volume ratio and avoiding immediate clearance by the lymphatic system. In general, systematically delivered NPs face several physiological barriers

before reaching their target. Technological advances for overcoming these hurdles are important to enable future success in this field; these include PEGylation for prolonging the circulation of NPs and enhancing tissue penetration, optimization of particle size and the use of substances to enhance deep tumor penetration of NPs.

Summary: an evolving approach to peptides

Microparticle formulations have already been successfully applied in several commercial products containing peptides as APIs. However, despite the advances made in the use of microparticles for delivering peptides and proteins, challenges remain. These include the difficulty of large-scale manufacturing, inactivation of the drug during fabrication and poor control of drug release rates. Further advances in increasing the duration of drug release and in methods that provide active control of the rate of drug release, including on-demand termination of release could establish new opportunities.

There are several examples of NP mediated delivery of small-molecule chemotherapeutic agents at the clinical and preclinical stages. Still, the use of NPs for delivering proteins and peptides is in its infancy and is expected to mature in the coming years. Recent studies have shown that for the creation of an optimum NP drug delivery system, several factors, such as appropriate targeting ligands, surface curvature and reactivity are important. This could potentially address the prevention of aggregation, stability, and receptor binding, as well as subsequent pharmacological effects of the drug.

As the development needs for these products quickly evolve, drug development teams are increasingly seeking experienced partners who can work with them to explore the full potential of microsphere and NP drug delivery for their product. At Bachem our proven track record in successful development projects makes us an ideal API manufacturing partner, bringing trusted experience in working with drug developers from early clinical phases to supply of a finished drug product.

Bachem: A leading partner in tides

Innovative strength has been a cornerstone of Bachem's business success for almost 50 years. As the leading independent supplier of peptidic active pharmaceutical ingredients (APIs), we have a proven track record in process development and large-scale cGMP manufacturing about 150 projects for new chemical entities (NCEs). To date, we have supported our customers in the delivery of 70 approved peptides for the treatment of multiple conditions.

At Bachem we offer customers a full range of services to bring breakthrough products to market, including GMP manufacturing of peptides, feasibility studies, process development, scale-up, and process validation. We have the capacity to produce peptide APIs from gram scale to annual quantities of hundreds of kilograms, all to cGMP standards. In addition to NCEs, we also support customers with generic products helping to achieve successful market authorizations and guiding them through demanding approval processes.

Bachem adopts a comprehensive approach to innovation management spanning all our business units, backed by a positive and change-orientated corporate culture. Our team is committed to continuously expanding its know-how in chemistries and technologies through internal research projects and collaboration with some of the world's most prominent research institutions.

For more information, visit:
https://www.bachem.com/api-products/



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The content of this whitepaper is based on Thundimadathil's original chapter Formulations of Microspheres and Nanoparticles for Peptide Delivery in Peptide Therapeutics: Strategy and tactics for chemistry, manufacturing and controls (2019). He has published more than 60 papers, including peer-reviewed articles, reviews, invited articles in popular scientific magazines, technical information in encyclopedia of chemical reagents, book chapters and patents.

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