Formulation approaches for Biologics and Peptide Stability

Nishantha JN Rao, Pooja Pratik, Pankaj Kumar Sah*

Department of Pharmacy Practice, East West College of Pharmacy, Bengaluru, Karnataka, India.

Abstract

Biologics and peptide-based therapeutics represent a rapidly expanding class of medicines with significant potential for treating cancer, autoimmune diseases, metabolic disorders, and infectious conditions. Their clinical impact arises from high specificity and potent biological activity, yet their successful application is limited by inherent instability. Structural complexity, susceptibility to chemical and physical degradation, and sensitivity to environmental factors contribute to challenges during manufacturing, storage, and administration. Formulation strategies play a decisive role in overcoming these limitations. Approaches such as lyophilization and dry powder technologies enhance stability by reducing molecular mobility, while excipients including sugars, amino acids, and surfactants protect against aggregation, oxidation, and denaturation. Advances in delivery platforms—ranging from depot formulations and microneedles to hydrogels and implantable devices—improve patient compliance and therapeutic performance. Analytical and characterization techniques such as calorimetry, spectroscopy, chromatography, and advanced imaging are essential for identifying degradation pathways and optimizing formulations. Emerging innovations, including computational modeling, artificial intelligence, nanotechnology-enabled systems, and bioprinting, are expanding opportunities for personalized and sustainable biologics development. Regulatory frameworks, particularly those guided by Quality by Design principles, provide structured pathways for ensuring safety, efficacy, and product consistency. Integration of stabilizers, delivery technologies, and predictive computational tools is anticipated to drive the next generation of biologics and peptide therapeutics. Overall, this article underscores the critical role of multidisciplinary strategies in advancing biologic and peptide formulation science, ultimately supporting the translation of fragile molecules into stable, patient-friendly therapeutics.

Keywords: Biologics, Peptides, Formulation strategies, Stability, Drug delivery systems, Quality by Design

1. Introduction

Biologics and peptide-based therapeutics have emerged as one of the fastest-growing classes of pharmaceuticals, offering highly specific mechanisms of action, reduced off-target effects, and the potential to treat complex diseases that are often refractory to conventional small-molecule drugs[1,2]. Monoclonal antibodies, recombinant proteins, therapeutic peptides, and vaccines

represent the largest segment of this category and contribute significantly to the global biopharmaceutical market[3]. Their clinical success has been particularly evident in oncology, autoimmune disorders, metabolic diseases, and infectious diseases [4].

Despite their therapeutic promise, biologics and peptides present unique stability challenges compared to small-molecule drugs. These macromolecules possess complex higher-order structures that are highly sensitive to environmental and chemical stresses[5]. Instabilities such as aggregation, oxidation, hydrolysis, and deamidation can compromise efficacy, reduce shelf life, and increase the risk of immunogenic responses in patients[6,7]. In addition, peptides are prone to enzymatic degradation and poor oral bioavailability, further limiting their formulation flexibility and clinical utility[8].

Therefore, the development of robust formulation approaches is critical to ensure the stability, efficacy, and safety of biologics and peptide therapeutics throughout manufacturing, storage, and delivery[9,10]. A variety of strategies—ranging from lyophilization and stabilizing excipients to encapsulation technologies and controlled-release systems—are being actively explored to overcome these challenges[11]. Understanding these approaches not only facilitates the design of more effective dosage forms but also accelerates the translation of biologics and peptide-based drugs into clinical practice (Figure 1).

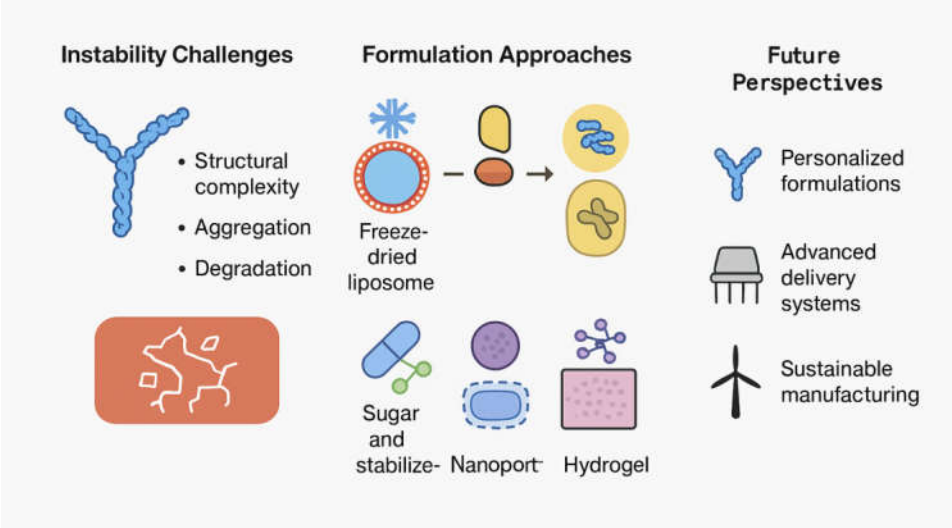

Fig 1. Overview of formulation approaches for Biologics and Peptide stability

2. Challenges in Biologics and Peptide Stability

Biologics and peptide-based therapeutics are inherently unstable due to their structural complexity and sensitivity to external conditions. Unlike small-molecule drugs, these macromolecules rely on intricate secondary, tertiary, and quaternary structures that are easily perturbed during manufacturing, storage, and administration[5]. Even subtle changes in temperature, pH, ionic strength, or agitation can induce conformational alterations, leading to reduced efficacy or immunogenicity[6,7].

2.1. Physical Instability

Proteins and peptides are prone to aggregation, denaturation, and precipitation, particularly under stress conditions such as freeze–thaw cycles or agitation during transportation. Aggregation is one of the most critical challenges, as it not only diminishes therapeutic activity but also increases the risk of immune responses in patients[7,10]. In addition, processes like adsorption to container surfaces and subvisible particle formation can further compromise product quality[9].

2.2. Chemical Instability

Biologics undergo a variety of chemical degradation pathways, including oxidation, deamidation, hydrolysis, and isomerization. Methionine and cysteine residues are especially susceptible to oxidation, while asparagine deamidation can cause structural destabilization and altered bioactivity[5,11]. For peptides, chemical instability is compounded by susceptibility to proteolytic cleavage, which severely limits their half-life and oral bioavailability[8].

2.3. Environmental Factors

Temperature fluctuations, light exposure, and moisture represent major threats to biologic and peptide stability. Many protein formulations require cold-chain storage, and deviations can accelerate degradation kinetics[1,5]. Similarly, exposure to ultraviolet or visible light can trigger photo-oxidation reactions, whereas humidity can promote hydrolysis in solid-state formulations[12].

2.4. Biological Challenges

Beyond physicochemical instability, peptides and proteins also face biological barriers, such as rapid clearance, enzymatic degradation, and immunogenicity. For example, peptide drugs administered orally are rapidly hydrolyzed by gastrointestinal proteases, resulting in poor bioavailability[8]. Additionally, the formation of aggregates or impurities in protein formulations has been linked to increased immune responses, which can compromise both safety and therapeutic effectiveness[6,7,10].

3. Formulation Approaches to Enhance Stability

3.1. Lyophilization and Dry Powder Formulations

Lyophilization, or freeze-drying, is one of the most widely employed strategies to improve the stability of biologics and peptides. In this process, the aqueous formulation is frozen, and water is removed by sublimation under reduced pressure, resulting in a dry powder with significantly improved long-term stability[13]. The removal of water minimizes hydrolytic degradation, while immobilization of the protein matrix reduces conformational mobility and aggregation[5]. Lyophilized formulations are therefore particularly advantageous for products that require extended shelf life and global distribution without dependence on stringent cold-chain conditions[1]. The success of lyophilization depends heavily on the inclusion of cryoprotectants and lyoprotectants, which safeguard the protein structure during freezing and drying stages. Commonly used excipients include sugars such as sucrose and trehalose, polyols like mannitol, and certain amino acids[14]. These molecules act by replacing water molecules through hydrogen bonding and by forming a glassy amorphous matrix that preserves protein conformation in the dry state[13,14].

However, lyophilization also presents challenges, including protein denaturation during freezing, collapse of the lyophilized cake, and potential incompatibilities with certain excipients. Process optimization, such as controlled freezing rates, annealing steps, and use of stabilizer combinations, remains essential for achieving robust formulations. Advances in analytical methods now allow deeper characterization of solid-state stability, enabling rational design of lyophilized biologics and peptides[12].

3.2. Use of Stabilizers and Excipients

Stabilizers and excipients play a pivotal role in maintaining the structural integrity of biologics and peptides during manufacturing and storage. Sugars and polyols, such as sucrose, trehalose, and mannitol, are widely used as stabilizing agents. They act by substituting for water molecules through hydrogen bonding and by forming an amorphous glassy matrix that reduces protein mobility and prevents unfolding[15]. Amino acids like glycine, arginine, and histidine can suppress aggregation and improve solubility. Surfactants, particularly polysorbates (Tween 20 and Tween 80), protect proteins against interfacial stresses encountered during agitation, freeze–thaw cycles, or filtration[16]. Buffer systems are equally important, as fluctuations in pH can trigger conformational changes and chemical degradation such as deamidation. Histidine, citrate, and phosphate buffers are commonly employed to maintain optimal stability profiles for both peptides and proteins[17]. Careful selection of stabilizers and buffers is therefore critical to ensure formulation robustness.

3.3. Encapsulation Strategies

Encapsulation in liposomes, polymeric nanoparticles, and microspheres provides physical protection of biologics and peptides from enzymatic degradation and environmental stresses. Liposomes have been successfully applied to deliver peptides such as insulin and calcitonin, improving their pharmacokinetic profiles and prolonging circulation time[18]. Poly(lactic-co-glycolic acid) (PLGA) microspheres and nanoparticles have been widely studied as biodegradable carriers for sustained release of peptide therapeutics (Figure 2)[19]. PEGylation, the covalent attachment of polyethylene glycol chains, remains one of the most effective strategies to enhance stability and half-life. This approach reduces renal clearance, shields the protein from proteolytic enzymes, and decreases immunogenicity, with several PEGylated peptides and proteins already on the market (e.g., pegfilgrastim, peginterferon). Conjugation strategies with polymers, lipids, or Fc-fragments are also being developed to further optimize stability and delivery[20].

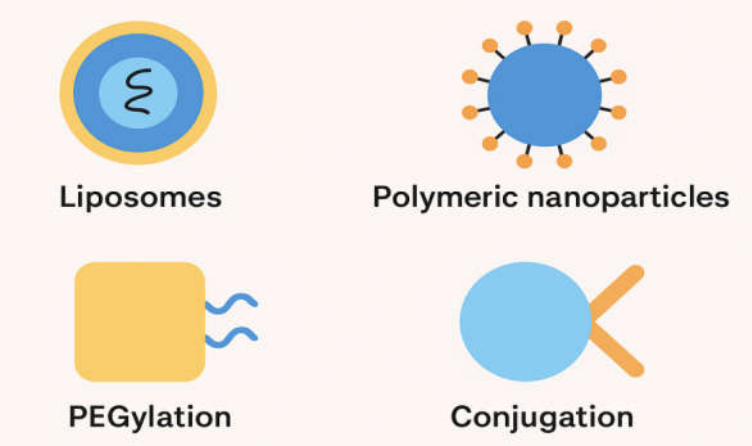

Fig 2. Encapsulation strategies

3.4. Alternative Delivery Systems

Traditional parenteral administration of biologics often suffers from poor patient compliance. Hence, alternative systems are being explored. Depot formulations, such as PLGA-based injectables, provide sustained release over weeks to months and reduce dosing frequency[21]. Transdermal systems, including microneedle patches, offer a minimally invasive route for peptide delivery with improved patient acceptability[22]. Oral delivery of peptides remains challenging due to enzymatic degradation and low permeability across the intestinal epithelium. However, approaches such as enteric coatings, enzyme inhibitors, and permeation enhancers are showing promise in clinical development. Pulmonary delivery via dry powder inhalers and nebulizers has been successfully utilized for peptides like insulin, offering rapid systemic absorption[23].

3.5. Controlled Release Systems

Controlled release platforms are designed to maintain therapeutic levels of biologics and peptides over extended periods. Hydrogels are attractive carriers due to their high water content, biocompatibility, and ability to encapsulate sensitive biomolecules without harsh processing. Similarly, biodegradable polymers such as PLGA allow sustained release by gradual degradation of the matrix[24]. Advanced osmotic pumps and implantable devices have also been investigated to achieve long-term controlled delivery of peptides, particularly for chronic conditions requiring frequent dosing. These technologies can minimize peaks and troughs in drug concentration, improving both safety and efficacy[25]. Table 1 gives various formulation approaches to enhance stability.

Table 1. Various formulation approaches to enhance stability[13-25]

Approach	Stability challenge addressed	Strategy/Mechanism	Examples
Lyophilization and Dry Powder Formulations	Instability in aqueous solutions;	Removal of water reduces hydrolytic degradation; cryo/lyoprotectants (sugars,	Freeze-dried monoclonal

	degradation during storage	polyols) preserve native conformation	antibodies; insulin powders
Use of Stabilizers and Excipients	Aggregation, oxidation, denaturation	Sugars (trehalose, sucrose), amino acids (glycine, arginine), surfactants (polysorbates) and buffer optimization to stabilize proteins	Trehalose-stabilized vaccines; polysorbate-stabilized mAbs
Encapsulation Strategies	Rapid degradation, poor bioavailability	Encapsulation in liposomes, polymeric nanoparticles, PEGylation, conjugation to carriers for protection and sustained release	Liposomal exenatide; PEGylated interferons
Alternative Delivery Systems	Limited patient compliance; instability in systemic circulation	Depot formulations, microneedles, transdermal patches, pulmonary and oral delivery systems	Octreotide LAR depot; oral semaglutide tablets
Controlled Release Systems	Short half-life, frequent dosing	Hydrogels, biodegradable polymers (PLGA), osmotic pumps, implantable devices enabling sustained release	Exenatide microspheres (Bydureon®); PLGA-based peptide implants
Spray Drying and Advanced Drying Methods	Thermal and moisture sensitivity	Mild drying techniques that produce stable amorphous powders; inclusion of stabilizers during process	Spray-dried protein inhalation powders
Nanotechnology-Enabled Delivery	Enzymatic degradation, poor permeability	Nanoparticles, nanogels, dendrimers to shield biologics and enable targeted delivery	siRNA-loaded lipid nanoparticles; polymeric nanogels
Chemical Modifications	Short plasma half-life; proteolysis	PEGylation, glycosylation, lipidation to increase solubility, stability, and circulation time	PEGylated asparaginase; lipidated GLP-1 analogs
Bioconjugation with Polymers or Carriers	Rapid clearance, immunogenicity	Conjugation with polymers, albumin, or antibodies for stabilization and long circulation	Albumin-fused peptides; antibody-drug conjugates
Protein Engineering Approaches	Intrinsic instability	Rational mutagenesis or fusion proteins to improve conformational stability	Fc-fusion proteins (etanercept); stabilized insulin analogs

4. Analytical and Characterization Techniques

Robust analytical techniques are essential for evaluating the stability and integrity of biologics and peptide formulations. A combination of thermal, spectroscopic, chromatographic, and imaging methods is often required to fully characterize degradation pathways and to ensure product quality.

Thermal analysis methods such as Differential Scanning Calorimetry (DSC) provide information about protein unfolding transitions and thermal stability profiles, allowing formulation scientists to assess the effect of excipients and buffers on protein stability[26]. Circular Dichroism (CD) spectroscopy is widely employed to monitor secondary structure content and conformational changes, while Fourier Transform Infrared (FTIR) spectroscopy complements CD by detecting alterations in protein secondary structure and hydrogen bonding[27].

Chromatographic and electrophoretic techniques are vital for detecting chemical modifications and aggregation. Size-exclusion chromatography (SEC) remains the gold standard for quantifying soluble aggregates, whereas reversed-phase and ion-exchange chromatography are frequently applied to detect oxidation, deamidation, and charge variants[28]. Capillary electrophoresis (CE) provides high-resolution separation of charge heterogeneity and peptide mapping[29].

In addition, advanced imaging and spectroscopy methods are increasingly applied for aggregation studies. Techniques such as dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) allow real-time monitoring of subvisible particles. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) provide morphological insights into aggregates and fibrils[30]. Moreover, mass spectrometry-based methods are now indispensable for detailed peptide mapping, identification of chemical modifications, and in-depth characterization of degradation pathways[31].

Together, these complementary techniques enable a comprehensive understanding of biologic and peptide stability, guiding rational formulation design and quality control strategies.

5. Emerging Approaches

5.1 Computational and AI-Based Formulation Design

The application of computational tools and artificial intelligence (AI) has become increasingly important in addressing the complexity of biologic and peptide formulations. Molecular dynamics (MD) simulations provide atomistic insights into protein folding, unfolding, and misfolding processes, as well as the influence of temperature, pH, and excipients on conformational stability[32]. For example, MD can predict aggregation-prone regions within a protein sequence, guiding excipient selection and formulation optimization. Beyond MD, AI and machine learning algorithms are being trained on large datasets of experimental stability outcomes to identify predictive markers of instability. Such models can accelerate formulation design by screening excipients virtually and suggesting optimal formulations without exhaustive experimental trials[33]. These tools reduce development timelines and cost, while also enabling personalized formulation approaches for emerging biologics such as monoclonal antibodies and peptide vaccines.

5.2 Novel Excipients and Synthetic Stabilizers

Conventional stabilizers, including sugars and surfactants, have limitations in preventing long-term degradation of biologics. Therefore, the development of novel excipients is a growing

research frontier. Modern approaches include designing synthetic polymers with zwitterionic or hydrophilic properties that mimic the natural hydration shell of proteins, thereby preventing aggregation and denaturation[34]. Ionic liquids and amino acid derivatives are also being investigated as multifunctional stabilizers that simultaneously suppress aggregation and chemical degradation pathways. Importantly, ATP and other biological hydrotropes have been shown to act as natural solubilizers, providing inspiration for the design of synthetic stabilizers with enhanced biocompatibility[35]. The introduction of such excipients requires careful toxicological evaluation and regulatory approval, but their potential to extend shelf-life and improve storage conditions of sensitive biologics could represent a paradigm shift in formulation science.

5.3 Advanced Drying Methods (Spray Drying, Supercritical Drying)

Lyophilization remains the gold standard for stabilizing protein formulations, but it is resource-intensive and has scalability limitations. Spray drying offers a scalable and cost-effective alternative, producing amorphous powders suitable for inhalation or oral peptide delivery. By carefully controlling inlet temperature, atomization, and excipient composition, spray drying can yield stable formulations with preserved activity[36]. Moreover, spray-dried powders can be tailored for controlled release or targeted deposition in the lungs. Supercritical fluid drying (SFD) is another emerging approach, utilizing supercritical CO_2 as a drying medium. This method minimizes thermal and oxidative stress, producing particles with unique morphology and stability advantages[37]. Although still underexplored compared to lyophilization, SFD holds promise for formulating sensitive biologics and peptides, particularly where mild processing conditions are essential. Both techniques highlight the trend toward alternative solid-state stabilization methods that reduce dependency on cold-chain storage.

5.4 Nanotechnology-Enabled Delivery Systems

Nanotechnology has revolutionized drug delivery by enabling the design of carriers that protect fragile biologics from degradation and improve their pharmacokinetics. Polymeric nanoparticles, nanogels, and dendrimers provide controlled microenvironments that prevent enzymatic degradation while offering sustained release profiles[38]. Lipid-based systems, including liposomes and solid lipid nanoparticles, have demonstrated success in stabilizing peptides and enabling targeted delivery across biological barriers. Importantly, stimuli-responsive nanocarriers are being developed to release drugs in response to environmental cues such as pH, redox potential, or enzymatic activity, enhancing both stability and therapeutic precision. Beyond protection, nanocarriers can also reduce immunogenicity by shielding epitopes and prolonging circulation time through surface modifications such as PEGylation. With increasing clinical translation of nanomedicines, these platforms are poised to play a pivotal role in next-generation biologic and peptide formulations[39].

6. Regulatory Considerations and Quality by Design (QbD)

6.1 ICH Guidelines for Biologics Stability

The International Council for Harmonisation (ICH) provides comprehensive regulatory guidance for the development and stability evaluation of biologics. Key documents such as ICH Q5C outline requirements for stability testing of biotechnological and biological products, including

recommended storage conditions, stress testing, and evaluation of degradation products[40]. Additionally, ICH Q6B provides specifications for testing biologic quality attributes such as purity, potency, and immunogenicity. These guidelines emphasize the need for long-term, accelerated, and stress stability studies to ensure product quality throughout its lifecycle[41]. Compliance with these standards is critical to obtaining global regulatory approval and maintaining consistency across international markets.

6.2 Risk-Based Approaches for Formulation Development

The adoption of Quality by Design (QbD) principles has transformed biologics formulation development. QbD emphasizes a risk-based framework, where critical quality attributes (CQAs), such as aggregation propensity, potency, and immunogenicity, are identified early in development[42]. Formulation and process variables are then systematically optimized using design of experiments (DoE) to build robust control strategies. Regulators encourage QbD approaches, as they provide deeper process understanding, ensure consistent product quality, and facilitate regulatory flexibility. For peptide formulations, QbD enables the identification of excipients and process parameters most likely to influence stability, thereby reducing late-stage failures and streamlining development timelines[43].

6.3 Regulatory Challenges in Peptide Formulations

Despite increasing interest in therapeutic peptides, their regulatory evaluation remains challenging due to their intermediate position between small molecules and large biologics. Issues such as chemical instability, susceptibility to enzymatic degradation, and variable bioavailability require specialized analytical and stability testing strategies. Moreover, the absence of harmonized international guidelines specifically for peptide formulations complicates regulatory submissions. Developers must often adapt requirements from small-molecule and biologic frameworks, leading to case-by-case evaluations. Additional challenges include demonstrating bioequivalence for generic peptides, addressing immunogenicity concerns, and ensuring consistency in solid-state peptide formulations. To address these gaps, regulators are increasingly promoting science- and risk-based approaches, while encouraging dialogue with sponsors during early development phases[44].

7. Case Studies

7.1 Successful Marketed Biologics with Novel Formulations

Several biologics have successfully reached the market owing to innovations in formulation science. Insulin analogs represent one of the most prominent examples, where modifications in amino acid sequence and formulation have enabled rapid-acting and long-acting profiles. For instance, insulin glargine employs pH-dependent solubility to form microprecipitates at physiological pH, providing sustained release and stable glycemic control. Similarly, insulin degludec uses multi-hexamer formation and phenol/zinc excipients to achieve ultra-long duration of action, significantly reducing dosing frequency[45]. Monoclonal antibodies (mAbs) also highlight advances in formulation approaches. High-concentration antibody formulations, essential for subcutaneous delivery, are stabilized using excipients such as sugars and amino acids to mitigate aggregation and viscosity issues. The development of adalimumab, the first fully human mAb,

underscores how formulation optimization enabled stable liquid products suitable for patient self-administration, revolutionizing therapy for autoimmune diseases[46].

7.2 Peptide Drug Formulations

Peptide therapeutics has traditionally faced challenges of short half-life, enzymatic degradation, and poor oral bioavailability. Several successful formulations illustrate strategies to overcome these barriers. Exenatide, a GLP-1 receptor agonist for type 2 diabetes, was initially developed as a twice-daily injectable but later reformulated into a long-acting release microsphere system (Bydureon®), using poly(lactic-co-glycolic acid) (PLGA) for sustained release over a week[47]. Another example is octreotide, originally requiring multiple daily injections, later advanced into a long-acting release formulation (Sandostatin LAR®) using biodegradable microspheres, significantly improving patient compliance[48]. Recent oral peptide formulations provide additional insights. Semaglutide, the first oral GLP-1 analog approved for diabetes, leverages an absorption enhancer (sodium N-[8-(2-hydroxybenzoyl) amino] caprylate, SNAC) that protects the peptide in the stomach and facilitates transcellular absorption in the small intestine[49]. These case studies demonstrate that innovative formulation strategies—including depot systems, excipient-based absorption enhancers, and solid-state stabilization—are central to transforming fragile peptides into viable therapeutic products.

8. Future Perspectives

8.1 Personalized Formulations

The increasing diversity of biologics and patient-specific needs is driving the concept of personalized formulations. Advances in pharmacogenomics and biomarker-based patient stratification are enabling tailored therapies that account for individual variability in drug response, metabolism, and immunogenicity[50]. Formulation strategies may evolve to provide dose flexibility, patient-specific excipient profiles, and adaptable delivery systems, ensuring optimal therapeutic outcomes while minimizing adverse effects. Emerging computational and AI-driven approaches could further support personalized formulation design by integrating patient data with predictive models of stability and pharmacokinetics[51].

8.2 Bioprinting and Next-Generation Delivery Systems

Bioprinting technologies are opening new avenues for localized and customizable delivery of biologics and peptides. Three-dimensional (3D) printing of hydrogel- or polymer-based scaffolds enables the incorporation of fragile proteins and peptides into precisely engineered structures, allowing controlled spatial and temporal release[52]. This approach could be transformative for regenerative medicine, vaccines, and oncology applications, where localized, sustained release is critical. Beyond bioprinting, next-generation delivery systems such as microneedle patches, implantable devices, and bioresponsive hydrogels offer patient-friendly alternatives that improve compliance and therapeutic efficiency[53]. These innovations are expected to expand the landscape of biologic and peptide drug administration in the coming decade.

8.3 Sustainability in Biologics Formulations

Sustainability is becoming a central concern in pharmaceutical development, including biologics. Traditional cold-chain logistics and energy-intensive lyophilization processes contribute

significantly to the environmental footprint of biologics manufacturing and distribution. Future strategies will likely focus on energy-efficient drying technologies, recyclable packaging, and eco-friendly excipients. Moreover, the adoption of continuous bioprocessing and single-use systems can reduce waste and resource consumption in biologics production. Incorporating sustainability principles early in formulation design will not only align with global environmental goals but also improve the long-term economic viability of biologic therapies[54].

9. Conclusion

Biologics and peptide-based therapeutics continue to transform modern medicine, yet their clinical success is tightly linked to overcoming inherent stability challenges. Advances in formulation strategies, including lyophilization, excipient optimization, encapsulation, and controlled release systems, have significantly extended product shelf life, enhanced delivery efficiency, and improved patient compliance. At the same time, novel approaches such as nanotechnology-enabled carriers, computational formulation design, and AI-based predictive modeling are reshaping the development landscape.

Future progress will depend on integrative strategies that combine stabilizing excipients with innovative delivery platforms, guided by advanced analytics and supported by regulatory frameworks such as Quality by Design (QbD). Personalized formulations, sustainable manufacturing practices, and next-generation technologies such as bioprinting hold promise to further optimize safety, efficacy, and accessibility of biologics. Ultimately, a multidisciplinary approach—bridging pharmaceutical sciences, computational biology, and regulatory science—will be essential for ensuring that biologics and peptides achieve their full therapeutic potential in diverse patient populations.

Acknowledgement: The authors are thankful to the faculty and management of East West College of Pharmacy, Bengaluru for their support in completion of this work.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Walsh G. Biopharmaceutical benchmarks 2022. *Nat Biotechnol*. 2022;40(12):1722–1735. doi:10.1038/s41587-022-01582-x.
2. Kaplon H, Reichert JM. Antibodies to watch in 2023. *mAbs*. 2023;15(1):2153410. doi:10.1080/19420862.2022.2153410.
3. Shukla AA, Wolfe LS, Mostafa SS, Norman C. Evolving trends in mAb production processes. *Bioeng Transl Med*. 2017;2(1):58–69. doi:10.1002/btm2.10061.
4. Carter PJ, Lazar GA. Next generation antibody drugs: pursuit of the 'high-hanging fruit'. *Nat Rev Drug Discov*. 2018;17(3):197–223. doi:10.1038/nrd.2017.227.
5. Manning MC, Chou DK, Murphy BM, Payne RW, Katayama DS. Stability of protein pharmaceuticals: an update. *Pharm Res*. 2010;27(4):544–575. doi:10.1007/s11095-009-0045-6.

6. Jiskoot W, Randolph TW, Volkin DB, et al. Protein instability and immunogenicity: roadblocks to clinical application of injectable protein delivery systems for sustained release. *J Pharm Sci*. 2012;101(3):946–954. doi:10.1002/jps.23018.
7. Wang W, Singh SK, Li N, Toler MR, King KR, Nema S. Immunogenicity of protein aggregates—concerns and realities. *Int J Pharm*. 2012;431(1–2):1–11. doi:10.1016/j.ijpharm.2012.04.040.
8. Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. *Drug Discov Today*. 2015;20(1):122–128. doi:10.1016/j.drudis.2014.10.003.
9. Carpenter JF, Randolph TW, Jiskoot W, et al. Overlooking subvisible particles in therapeutic protein products: gaps that may compromise product quality. *J Pharm Sci*. 2009;98(4):1201–1205. doi:10.1002/jps.21530.
10. Rosenberg AS. Effects of protein aggregates: an immunologic perspective. *AAPS J*. 2006;8(3):E501–E507. doi:10.1208/aapsj080359.
11. Chen Y, Mutukuri TT, Wilson NE, Zhou Q. Pharmaceutical protein solids: drying technology, solid-state characterization and stability. *Adv Drug Deliv Rev*. 2021;172:211–233. doi:10.1016/j.addr.2021.02.016.
12. Hawe A, Wiggenhorn M, van de Weert M, Garbe JH, Mahler HC, Jiskoot W. Forced degradation of therapeutic proteins. *J Pharm Sci*. 2012;101(3):895–913. doi:10.1002/jps.22812
13. Wang W. Lyophilization and development of solid protein pharmaceuticals. *Int J Pharm.* 2000;203(1-2):1-60. doi:10.1016/S0378-5173(00)00423-3.
14. Allison SD, Chang B, Randolph TW, Carpenter JF. Hydrogen bonding between sugar and protein is responsible for inhibition of dehydration-induced protein unfolding. *Arch Biochem Biophys.* 1999;365(2):289-298. doi:10.1006/abbi.1999.1175.
15. Pikal MJ, Rigsbee DR. The stability of insulin in crystalline and amorphous solids: observation of greater stability for the amorphous form. *Pharm Res*. 1997;14(10):1379-1387. doi:10.1023/A:1012111014923
16. Arakawa T, Ejima D, Tsumoto K, Obeyama N, Tanaka Y, Kita Y, Timasheff SN. Suppression of protein interactions by arginine: a proposed mechanism of the arginine effects. *Biophys Chem*. 2007;127(1-2):1-8.
17. Kerwin BA. Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: structure and degradation pathways. *J Pharm Sci*. 2008;97(8):2924-2935. doi:10.1002/jps.21190
18. Strickley RG. Solubilizing excipients in oral and injectable formulations. *Pharm Res*. 2004;21(2):201-230. doi:10.1023/B:PHAM.0000016235.32639.23
19. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. *Adv Drug Deliv Rev*. 2013;65(1):36-48.
20. Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev*. 2012;64:72-82.

21. Veronese FM, Pasut G. PEGylation, successful approach to drug delivery. *Drug Discov Today*. 2005;10(21):1451-1458. doi:10.1016/S1359-6446(05)03575-0
22. Prausnitz MR, Langer R. Transdermal drug delivery. *Nat Biotechnol*. 2008;26(11):1261-1268. doi:10.1038/nbt.1504
23. Drucker DJ. Advances in oral peptide therapeutics. *Nat Rev Drug Discov*. 2020;19(4):277-289. doi:10.1038/s41573-019-0053-0
24. Patton JS, Byron PR. Inhaling medicines: delivering drugs to the body through the lungs. *Nat Rev Drug Discov*. 2007;6(1):67-74. doi:10.1038/nrd2153
25. Hoffman AS. Hydrogels for biomedical applications. *Adv Drug Deliv Rev*. 2012;64:18-23. doi:10.1016/j.addr.2012.09.010
26. Santini JT, Richards AC, Scheidt RA, Cima MJ, Langer R. Microchips as controlled drug-delivery devices. *Angew Chem Int Ed Engl*. 2000;39(14):2396-2407. doi:10.1002/1521-3773(20000717)39:14.
27. Kelly SM, Jess TJ, Price NC. How to study proteins by circular dichroism. *Biochim Biophys Acta*. 2005;1751(2):119–139. doi:10.1016/j.bbapap.2005.06.005
28. Hong P, Koza S, Bouvier ES. Size-exclusion chromatography for the analysis of protein biotherapeutics and their aggregates. *J Liq Chromatogr Relat Technol*. 2012;35(20):2923–2950. doi:10.1080/10826076.2012.743724
29. He Y, Lacher NA, Hou W, Wang Q, Isele C, Starkey J, Zhang Z. Analysis of monoclonal antibody charge variants with capillary isoelectric focusing and capillary zone electrophoresis. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2010;878(15–16):1547–1555. doi:10.1016/j.jchromb.2010.03.034
30. Tyagi AK, Ruotolo BT. Characterization of protein–protein interactions using ion mobility-mass spectrometry. *Curr Opin Chem Biol*. 2021;62:46–54. doi:10.1016/j.cbpa.2020.11.002
31. Zhang Z, Pan H, Chen X. Mass spectrometry for structural characterization of therapeutic antibodies. *Mass Spectrom Rev*. 2009;28(1):147–176.
32. Daggett V. Molecular dynamics simulations of protein folding and unfolding. *Acc Chem Res*. 2002;35(6):422–429. doi:10.1021/ar010019k
33. Carpenter KA, Huang X. Machine learning-based virtual screening and its applications to Alzheimer's drug discovery: a review. *Curr Pharm Des*. 2018;24(28):3347–3358. doi:10.2174/1381612824666180911163607
34. Mensink MA, Frijlink HW, van der Voort Maarschalk K, Hinrichs WLJ. How sugars protect proteins in the solid state and during drying (review). *Eur J Pharm Biopharm*. 2017;114:288–295. doi:10.1016/j.ejpb.2017.01.024
35. Patel A, Malinovska L, Saha S, Wang J, Alberti S, Krishnan Y, Hyman AA. ATP as a biological hydrotrope. *Science*. 2017;356(6339):753–756.
36. Maa YF, Costantino HR. The effect of operating and formulation variables on the morphology of spray-dried protein particles. *Pharm Dev Technol*. 2000;5(2):213–223. doi:10.1081/PDT-100100529

37. Rantakyla M, Kallioinen H, Heikkinen A, Heinämäki J. Supercritical fluid drying in pharmaceutical applications — A review. *Int J Pharm*. 2019;567:118463. doi:10.1016/j.ijpharm.2019.118463
38. Kamaly N, Yameen B, Wu J, Farokhzad OC. Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. *Chem Rev*. 2016;116(4):2602–2663. doi:10.1021/acs.chemrev.5b00346
39. Rathore AS, Winkle H. Quality by design for biopharmaceuticals. *Nat Biotechnol*. 2009;27(1):26–34. doi:10.1038/nbt0109-26
40. ICH Q5C: Stability Testing of Biotechnological/Biological Products. International Council for Harmonisation. 1995.
41. ICH Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products. International Council for Harmonisation. 1999.
42. Yu LX, Amidon G, Khan MA, Hoag SW, Polli J, Raju GK, Woodcock J. Understanding pharmaceutical quality by design. *AAPS J*. 2014;16(4):771–783. doi:10.1208/s12248-014-9598-3
43. Lau JL, Dunn MK. Therapeutic peptides: historical perspectives, current development trends, and future directions. *Bioorg Med Chem*. 2018;26(10):2700–2707. doi:10.1016/j.bmc.2017.06.052
44. Vlieghe P, Lisowski V, Martinez J, Khrestchatisky M. Synthetic therapeutic peptides: science and market. *Drug Discov Today*. 2010;15(1–2):40–56.
45. Owens DR. Insulin preparations with prolonged effect. *Diabetes Technol Ther*. 2011;13 Suppl 1:S5–S14. doi:10.1089/dia.2011.0063
46. Heise T, Hermanski L, Nosek L, Feldman A, Rasmussen S, Haahr H. Insulin degludec: four times lower pharmacodynamic variability than insulin glargine under steady-state conditions in type 1 diabetes. *Diabetes Obes Metab*. 2012;14(9):859–864. doi:10.1111/j.1463-1326.2012.01627.x
47. Shire SJ, Shahrokh Z, Liu J. Challenges in the development of high protein concentration formulations. *J Pharm Sci*. 2004;93(6):1390–1402.
48. DeYoung MB, MacConell L, Sarin V, Trautmann M, Herbert P. Encapsulation of exenatide in poly-(D,L-lactide-co-glycolide) microspheres produced an investigational long-acting once-weekly formulation for type 2 diabetes. *Diabetes Technol Ther*. 2011;13(11):1145–1154. doi:10.1089/dia.2011.0050
49. Oberg K. The use of long-acting somatostatin analogues in the treatment of neuroendocrine tumours. *Drugs*. 2012;72(17):2235–2244. doi:10.2165/11640820-000000000-00000
50. Buckley ST, Bækdal TA, Vegge A, et al. Transcellular stomach absorption of a derivatized glucagon-like peptide-1 receptor agonist. *Sci Transl Med*. 2018;10(467):eaar7047. doi:10.1126/scitranslmed.aar7047
51. Vamathevan J, Clark D, Czodrowski P, et al. Applications of machine learning in drug discovery and development. *Nat Rev Drug Discov*. 2019;18(6):463–477. doi:10.1038/s41573-019-0024-5

52. Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol*. 2014;32(8):773–785. doi:10.1038/nbt.2958
53. Ita K. Transdermal delivery of drugs with microneedles: Strategies and outcomes. *J Drug Deliv Sci Technol*. 2015;29:16–23. doi:10.1016/j.jddst.2015.06.002
54. Konstantinov KB, Cooney CL. White paper on continuous bioprocessing. *Biotechnol Bioeng*. 2015;112(9):1654–1666. doi:10.1002/bit.25529