

Selecting a CDMO for Custom Activated PEGs

Considerations for Enhancing API Properties and Ensuring Regulatory and Market Readiness

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PEGylation, the covalent attachment of polyethylene glycol (PEG) chains to active pharmaceutical ingredients (APIs), is a proven strategy for enhancing drug performance. PEGylation can significantly increase the therapeutic efficacy of various modalities, including proteins, small molecules, and nanoparticles by improving solubility, stability, and circulation half-life. The versatility of PEGylation in pharmaceutical applications stems from the unique physicochemical properties of PEG and its favorable safety profile. Structurally, PEG is a synthetic polymer with the repeating unit $\text{HO}-[\text{CH}_2-\text{CH}_2-\text{O}]_n-\text{H}$ and is designated as generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA) for specific uses, while the European Medicines Agency (EMA) recognizes PEGs as approved excipients in medicinal products.

By modifying APIs with PEGylation, drug developers can unlock several important advantages related to therapeutic performance and delivery (**Figure 1**).^{1,2,3} These benefits include:

• Enhanced pharmacokinetics and distribution

By increasing a drug's molecular weight, PEGylation significantly reduces its clearance via glomerular filtration during systemic administration. With greater stability in circulation and extended residence time in the body, distribution and therapeutic exposure and efficacy are improved. With increased bioavailability, the frequency of dosing can be reduced.

• Reduced immunogenicity

PEGylation can effectively mask immunogenic epitopes, decreasing the risk of unwanted immune responses.

• Improved delivery across biological barriers

PEGylated formulations are better equipped to navigate complex biological environments, including the blood-brain barrier and ocular tissues. This enhanced permeability supports the targeted delivery of therapeutics to otherwise difficult-to-reach sites, broadening the range of treatable conditions.

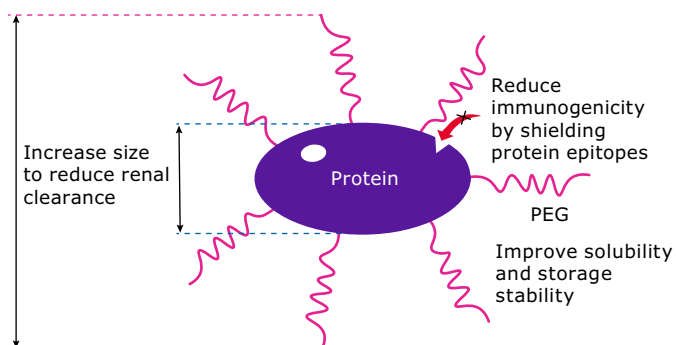


Figure 1.

Modifying APIs with PEGs delivers many important therapeutic performance and delivery advantages.

API PEGylation was first demonstrated in 1977, when researchers successfully attached PEG to proteins, reducing their immunogenicity while preserving activity.⁴ This foundational work led to the FDA approval of the first PEGylated therapeutic, Adagen®, in 1990, followed by the PEGylated liposomal chemotherapy Doxil® in 1995. More recently, PEGylated lipid nanoparticles played a key role in the Pfizer/BioNTech and Moderna COVID-19 vaccines.⁴

As of 2024, there have been 38 pegylated therapeutics approved by the FDA, including proteins, antibody fragments, oligonucleotides, small molecules, nanoparticles, and vaccines; 28 of the 38 approved PEGylated therapeutics are proteins.⁵ The number of PEGylated therapeutics on the market is expected to increase, driven by the wide range of benefits and an upward trend in FDA approvals of PEGylated therapeutics and vaccines in recent years.

While PEGylation offers well-established benefits, such as enhanced pharmacokinetics, reduced immunogenicity, and improved drug delivery, realizing these advantages requires careful consideration of available PEGylation strategies, along with diligent management of the supply chain and regulatory requirements of the activated PEG. The regulatory requirements for activated PEGs should not be underestimated, as PEG moieties covalently bound to the active substance are regarded as part of the drug substance, while novel or uncommon PEG derivatives may be considered new excipients and require comprehensive safety evaluation.

This white paper examines these critical success factors through the lens of development, GMP manufacturing, quality control, and the decision-making process for when a custom PEG may be warranted rather than an off-the-shelf solution. The essential role of a contract development and manufacturing organization (CDMO) with deep expertise in activated PEG chemistry is highlighted. The CDMO partner is responsible for ensuring consistent quality and regulatory compliance while supporting the complex technical and strategic requirements of clinical and commercial-scale PEGylated therapies through its transparency and focus on the client's application.

PEGylation Strategies

Historically, PEGylation involved random attachment of PEG molecules to amino acid residues, leading to heterogeneous products with variable activity. To address this shortcoming, site-directed PEGylation is used to enable conjugation at specific locations on the protein or molecule, preserving biological function and ensuring batch-to-batch consistency of the API. For example, some applications call for selective reactivity with cysteine residues, while others may require targeting non-natural amino acid side chains with unique reactive sites or target the protein N-terminus.

Another site-directed method, glycoPEGylation, attaches PEG chains to engineered glycan structures, offering enhanced control over PEG placement and minimizing structural disruption. In terms of polymer characteristics, polydisperse PEG, a mixture of PEG

chains with varying lengths, has historically been the standard, though it can introduce variability in pharmacokinetics (**Figure 2**). Monodisperse PEGs, in contrast to conventional polydisperse materials, provide uniform chain lengths that enable greater precision in bioconjugation applications. Their well-defined structure makes them especially useful as linkers, where consistent spacing and geometry between molecules are critical for reproducible activity, stability, and pharmacokinetics.

Depending on the PEGylation strategy and API, PEG molecules must be appropriately functionalized. PEG functionalization is the process of chemically modifying the ends of the polymer chain with reactive groups that allow it to attach to the API.

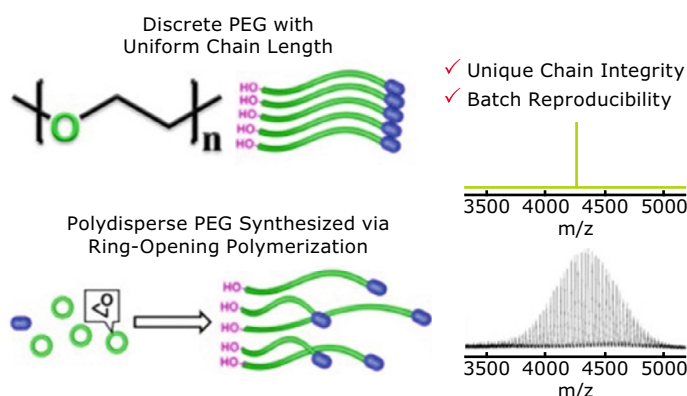


Figure 2.

Comparison of mono and polydisperse PEGs.

What to Expect from Your CDMO: Custom Functionalization for Targeted Applications

Your CDMO should have experience with a wide range of functionalizations, including PEG amines, PEG maleimides, PEG propionaldehydes, and activated esters like NHS esters (**Table 1**). Additionally, the CDMO should be able to adapt the configuration and molecular weight of the PEG chain, whether functionalized on one or both ends, with either identical or different reactive groups, based on the intended use and desired molecular architecture.

Working with a partner who has the necessary technical expertise and infrastructure to deliver the appropriate functionalized PEG, within tight timelines and specifications, if necessary, is crucial for keeping development programs on track and ensuring a successful outcome.

Examples of activated PEGs.

Activated PEGs	Structures	Table
Aldehydes	$R = \text{---}[\text{CH}_2\text{---CH}_2\text{---}]_m\text{CHO}$	N-Terminus
Active Esters Activated Carbonates		Amine in Lysine
Maleimides		Thiol in Cysteine
Amines	$R = \text{---}[\text{CH}_2\text{---CH}_2\text{---}]_m\text{NH}_2$	Carboxylic acid in Aspartate, Glutamate, C-Terminus

Transitioning from Off-the-Shelf PEGs to Custom Production

As drug development advances from early phases to late-stage clinical trials and commercial readiness, the requirements placed on raw materials, including functionalized PEGs, grow significantly more stringent. While off-the-shelf PEGs may be suitable for speed and flexibility in Phase 1, they often lack the process control, documentation, and regulatory rigor needed for later stages. The evolution in application of GMP and quality systems is outlined in **Figure 3**.

The key drivers that signal when it's time to transition to a custom PEG solution are outlined below, and include the phase of development, regulatory classification, and operational requirements.

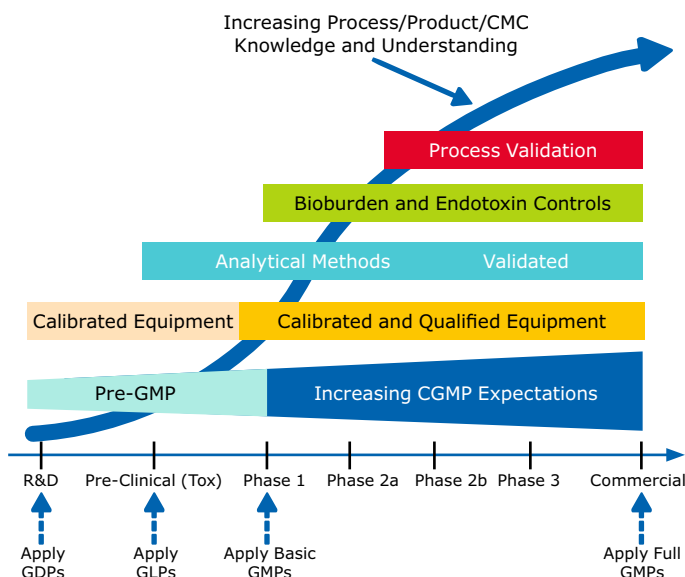


Figure 3.

Increased application of GMP and quality systems as the development program advances.

Phase-Appropriate PEGs

In early clinical development, particularly Phase 1, where speed is paramount and clinical attrition rates remain high, drug developers may choose to rely on off-the-shelf functionalized PEGs rather than investing in highly optimized or complex PEG solutions. As development progresses toward Phase 2, Phase 3, and commercial launch, the emphasis shifts to process consistency, yield reliability, and specification compliance. This is where process optimization becomes critical, ensuring that every batch meets quality standards, avoiding costly delays or rework, and eliminating the need to start from scratch during late-stage development. At this point, the off-the-shelf functionalized PEG source used in early development is likely to lack the necessary GMP compliance, documentation, or process control to satisfy regulatory agencies. This triggers the need to replace the catalog product with a custom, GMP-compliant PEG solution.

Regulatory-Driven Requirements

Another consideration in determining when to transition to a custom functionalized (activated) PEG is how the functionalized PEG will be classified in regulatory filings. Specifically whether the functionalized PEG is considered an API Regulatory Starting Material (RSM) or an API Intermediate will significantly influence the regulatory path, manufacturing requirements, and the expected level of documentation that is required.

- An RSM is generally considered to be several synthetic steps upstream of the final API and to contribute a significant structural element to the drug substance. When a functionalized PEG is designated as the RSM, its own manufacture is not subject to GMP requirements; however, subsequent stages of API synthesis are expected to comply with ICH Q7 GMP principles. The designation is typically regarded as the point from which regulatory oversight of the API synthesis commences. Regulatory agencies will expect clear justification of the RSM's selection, a comprehensive impurity control strategy, and detailed knowledge of upstream processing steps. Since ICH Q11 outlines principles, not strict rules, for selecting RSMs, interpretations can vary across regions. Agencies such as the FDA and EMA may have differing views, so it is essential to plan conservatively to ensure global compliance.
- If a functionalized PEG is classified as an API intermediate, the regulatory requirements are substantial. In the U.S., the functionalized PEG manufacturer may submit a Type II Drug Master File (DMF), which can be cross-referenced in the sponsor's application via a Letter of Authorization (LoA). In the EU, there is no DMF or ASMF procedure for intermediates; therefore, the sponsor must include all relevant information for the intermediate directly in the drug substance section of the dossier, supported by appropriate supplier documentation as needed.

Accordingly, the decision to classify a functionalized PEG as a registered starting material or an API intermediate should be made early in development, based on a thorough regulatory and scientific evaluation and early engagement with regulatory authorities is strongly advised to determine functionalized PEG classification and guide strategy.

Companies that delay these decisions may find themselves scrambling to resolve issues in late-phase development. Starting with a custom functionalized PEG and GMP manufacturing partner from the beginning can avoid these pitfalls and provide regulatory confidence throughout the development lifecycle.

Technical and Operational Drivers

In addition to classification and regulatory compliance, several technical and operational drivers favor a shift to custom PEGs:

- Custom functionalization tailored to specific conjugation targets (e.g., cysteine residues or alternative amino acid side chains).
- Improved impurity control and analytical transparency, essential for regulatory filings.
- Scalability from gram-scale preclinical use to multi-kilogram commercial quantities.
- Process ownership, giving sponsors full control over the PEG used in their drug product.

Quality Control Considerations

As noted above, the identification and characterization of PEG impurities is complex and demands advanced analytical capabilities and specialized expertise. A PEGs manufacturing partner must be equipped with a range of instrumentation to ensure product quality and consistency. This includes gel permeation chromatography (GPC), matrix-assisted laser desorption/ionization (MALDI), and multi-angle light scattering (MALS) for assessing molecular weight and distribution, as well as high-resolution nuclear magnetic resonance (NMR) and mass spectrometry (MS) for detailed structural characterization. Identifying impurities often requires the synthesis of custom reference standards and the use of specialized microbiological testing laboratories.

These analytical tools and methodologies are essential for confirming the size, structural integrity, and functionalization of the PEG molecule, whether it is to be conjugated to an API or incorporated as a component of a lipid nanoparticle (LNP). Analytical methods are typically customized for the specific PEG variant during the development phase and then validated for use in GMP production, where rigorous quality control is critical.

What to Expect from your CDMO: A Comprehensive Suite of Analytical Methods

When manufacturing functionalized PEGs, precise analytical control is essential to ensure product quality, consistency, and regulatory compliance. Your CDMO should leverage a variety of advanced technologies, supported by extensive expertise, to characterize PEGs and their impurities throughout development and GMP production:

- Appearance
- Identity (NMR)
- Mn (average molecular weight) (GPC/MALDI)
- PDI (polydispersity index) (GPC/MALDI)
- Water content (KF)
- Hydroperoxide assay (UV)
- Purity and terminal activity (HPLC – CAD & UV Detector)
- Residual solvents (GC-MS, ICP-MS)
- In-house, compendial endotoxin contents/bioburden (TAMC, TYMC)
- ICH M7 Assessment (per regulatory guidelines), Assessment in-silico or AMES test
- Elemental Impurities (ICP-MS)

PEG Manufacturing Considerations

The production of high-quality PEGs demands precise control of process parameters and scalability. One critical aspect is maintaining a deoxygenated, low-endotoxin environment throughout manufacturing. The use of low-endotoxin purified water (LEPW) generated and monitored in-house is essential to prevent contamination and ensure compliance with regulatory standards, particularly for injectable or biologically sensitive applications.

PEGs often require isolation under cooled conditions to maintain their stability and integrity. Specialized equipment such as centrifuges, filter dryers, and temperature-controlled mixing vessels ensures that sensitive materials are handled appropriately during processing. Post-synthesis, purification technologies such as ion exchange chromatography (IEX) and tangential flow filtration (TFF) are employed to achieve the high purity levels required for pharmaceutical use.

What to Expect from your CDMO: Support from Lab to Commercial Scale

To support the development of PEGylated APIs, your CDMO should offer production from lab-scale synthesis to pilot plant and full commercial-scale operations. This ensures the supply of non-GMP development samples to GMP-compliant batches, ranging from grams to multi-kilogram quantities suitable for commercial production in regulated markets. Such

scalability provides flexibility and continuity as projects move from early development through clinical trials and into commercial supply.

Custom packaging service is also essential. This capability allows for tailored packaging ranging from 1-gram R&D samples to bulk-scale shipments to meet the diverse and evolving needs of drug developers at every stage.

Process and Analytical Development of PEG Functionalization Processes

The process and analytical development of PEG functionalization encounters several intrinsic bottlenecks that must be addressed to achieve efficient and scalable manufacturing. PEGs often act as emulsifiers, leading to poor phase separations that complicate purification and reduce overall process efficiency. High filter cake compressibility can further slow solid-liquid separations or cause clogging, while the high viscosity of PEG solutions can create mixing challenges such as pH hot spots, uneven reagent distribution, and reduced reaction kinetics. These issues contribute to difficulties in achieving consistent yield and process reproducibility.

Impurity management poses another challenge. Traditional approaches such as extractions, crystallizations, or precipitations are generally ineffective for removing PEG-based impurities, increasing the risk of undesired byproducts accumulating in the final material. This is compounded by the fact that unreacted PEG starting materials and their derivatives cannot be separated from the final PEG and may compromise downstream PEGylation reactions. For this reason, achieving mostly complete chemical transformations early in development is critical. Applying structured Design of Experiments (DoE) approaches helps optimize reaction parameters to maximize conversion, minimize byproducts, and accelerate development timelines.

Analytical development is equally important. PEG mixtures often yield broad, poorly resolved peaks in HPLC, complicating quantification and obscuring the detection of impurities. Furthermore, the characterization of impurities by LC-MS or NMR is particularly difficult once molecular weights exceed 5 kDa. To address these challenges, robust, tailored analytical methods must be developed, such as derivatization combined with HPLC-CAD analysis, to accurately measure molecular weight distributions and functional activity. Specialized analytical instruments are essential to enable high-resolution characterization of complex mixtures. Ensuring that PEG products consistently meet chemical specifications and exhibit acceptable physical properties (e.g., solubility or dissolution in aqueous buffers) requires this strong analytical foundation.

Scalability presents another layer of complexity. Performance at bench scale does not always translate directly to manufacturing scale, and mixing, separation, and impurity challenges can become magnified. Effective scale-up therefore depends on

using small-scale equipment that closely mimics manufacturing systems, combined with predictive modeling to anticipate bottlenecks. Pilot-scale capacity provides an important bridge to full-scale production, allowing developers to validate process robustness under more representative conditions. Solid-liquid separation can be enhanced by ensuring adequate centrifugation capacity across scales, while cooled storage helps maintain the stability of PEG intermediates that may be sensitive to heat, light, or oxygen.

Finally, avoiding harsh oxidative or catalytic conditions is essential to prevent PEG degradation or side reactions that compromise stability and reactivity. The choice and number of solvents used must also be carefully optimized to balance consistency, performance, and sustainability, given PEG's limited solubility profile.

By anticipating these intrinsic bottlenecks and implementing thoughtful process design, diligent scale-up, and advanced analytical strategies, the CDMO can optimize the production of activated PEGs. The result is a more efficient, scalable, and reliable foundation for PEGylated APIs.

What to Expect from your CDMO: Seamless Tech Transfer to Manufacturing

Tech transfer for PEG manufacturing should be executed using a scale-down model, where development-scale equipment closely mimics production-scale systems to reduce scale-up risks and enable a "first time right" approach. This strategy ensures efficient, low-risk transitions from development to GMP production and includes a dedicated MSAT (Manufacturing, Science, and Technology) team that oversees the transfer process, conducting thorough safety risk assessments and ensuring all critical parameters are maintained, setting the foundation for consistent, high-quality output at commercial scale.

Packaging Considerations for PEGs

Proper packaging is critical to preserving the stability, integrity, and quality of functionalized PEGs throughout sampling, shipment, and long-term storage. Due to their sensitivity to light, oxygen, moisture, and temperature, functionalized PEGs require carefully designed container closure systems that mitigate these environmental risks.

Functionalized PEGs should be stored at -15 to -25 °C under an inert gas atmosphere (argon or nitrogen) with protection from UV light. Exposure to light or oxygen can cause degradation of individual PEG chains over time, resulting in increased polydispersity index (PDI) and loss of material uniformity. In addition, many PEG derivatives are slightly hygroscopic, meaning they absorb moisture from the environment, which can alter their appearance and compromise performance, especially in PEGylation reactions involving aqueous buffers.

What to Expect from your CDMO: A Multi-Layered Packaging Strategy

Selecting the appropriate packaging configuration ensures that PEGs retain their desired physical and chemical properties, supporting consistent performance in GMP manufacturing and regulatory compliance. A well – engineered packaging system is not merely a logistical consideration – it is an integral part of quality assurance for PEG-based pharmaceutical applications.

To protect functionalized PEGs, a multi-layered packaging strategy should be employed:

- Primary packaging should consist of LDPE (low-density polyethylene) or similar bags, sized according to sample volume, and sealed using specialized clips or cable ties. Before sealing, the contents should be overlaid with nitrogen or argon to displace oxygen and maintain an inert atmosphere.
- For bulk quantities (2.5 kg or more), a closed containment system such as DoverPac® can be used to maintain product integrity and support safe handling at scale.
- Secondary packaging should feature an aluminum composite foil that is purged with inert gas and heat-sealed. This barrier protects against UV light, oxygen, and moisture, providing added assurance during shipment and long-term storage.
- For tertiary protection, particularly for extended storage or international transport, a metal drum will safeguard the contents from mechanical stress and environmental exposure.

Sustainability Considerations

PEGs are soluble in only a limited number of solvents, which constrains formulation flexibility. However, optimizing solvent selection and minimizing the number of solvents used can help balance performance needs with sustainability goals.

What to Expect from your CDMO: A Focus on Sustainability

By applying principles of sustainability by design, your CDMO can reduce waste, lower the cost of goods (COGs), and improve overall process efficiency, delivering both environmental and economic value.

Designing for sustainability from the outset also provides long-term operational and regulatory benefits. A well-optimized, environmentally conscious process is more likely to remain viable throughout the lifecycle of a commercial product, avoiding costly revalidation or process changes in the future.

Case Study: Rapid Development and Scale-Up of a Custom PEG for Secondary Supply

A biopharmaceutical customer approached our organization with an urgent request: they needed a secondary supplier for a specific activated PEG to support their regulatory filing and mitigate supply chain risk. With an extreme time constraint, our Schaffhausen, Switzerland site rapidly evaluated and replicated the material's performance.

Given the complexity of the PEGylated drug substance and its sensitivity to impurities, the team first worked with the customer to analyze the quality profile of the incumbent supplier's material. Early assessments revealed that trace-level impurities significantly affected downstream purification of the PEGylated API, necessitating stringent impurity control.

Early samples were produced and tested to ensure alignment with application-specific requirements. A four-step synthesis process was designed, incorporating a proprietary purification step that not only met but exceeded the performance of the original material.

The process was rapidly optimized and scaled up to kilo-lab production, enabling the delivery of GMP-compliant material under tight timelines. Simultaneously, the process was transferred to full production scale to support a process performance qualification (PPQ) campaign, aligning with the customer's commercial readiness.

The project's success led the customer to designate our organization as their primary supplier for market needs. To date, more than 650 kg of this high-purity activated PEG have been produced.

Conclusion

PEGylation has proven to be an important strategy in drug development, offering improved pharmacokinetics, reduced immunogenicity, and enhanced delivery profiles across a broad range of therapeutic modalities. However, unlocking the full value of PEGylation requires strategic planning, deep process and analytical knowledge, and robust execution across development, manufacturing, and regulatory disciplines.

As this white paper has outlined, early decisions regarding PEG selection, functionalization, and classification as a regulatory starting material or API intermediate have far-reaching implications. Off-the-shelf PEGs may suffice in early phases, but transitioning to a custom PEG is often essential for ensuring GMP compliance, regulatory approval, and commercial viability. Critical success factors include phase-appropriate development, seamless tech transfer, scalable manufacturing, and rigorous analytical control, all of which depend on choosing the right CDMO partner.

A qualified PEGylation CDMO should offer technical capabilities and also serve as a collaborative partner who understands the therapeutic context, offers regulatory foresight, and delivers a reliable supply chain from R&D through to commercial launch. With deep PEG chemistry expertise, a commitment to quality, and proven success in supporting late-stage development and market supply, a strong CDMO partnership can significantly de-risk a PEGylated drug program and help accelerate the path to market.

About

Choosing your CDMO Partner for Custom Activated PEGs

With 35+ years of proven small molecule production experience and extensive expertise in the area of custom activated PEGs, we deliver high quality solutions for your APIs. Offering phase-appropriate support, we work with you from preclinical to commercial, at every stage and every scale. Our global network of technical and regulatory experts is committed to safety, sustainability, quality, and delivery for your success. In addition to our experience with PEGs, we have deep expertise working with complex APIs, HPAPIs, linker-payloads, small molecule conjugates, targeted protein degraders, and registered starting materials. We go the distance with you to help bring your therapies to patients.

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