The Growing Potential of Monodisperse PEG 2000 and PEG 5000 Applications

Quality and defined chemical composition are essential for pharmaceutical formulations. Purity requirements dictated by regulatory agencies and pharmacopoeias ensure the safety and reproducibility of all ingredients in marketed formulations. Poly(ethylene glycol) (PEG) derivatives are one of the most widely used polymeric excipients, with over 50 years of applications in medicine. Initially employed to "hide" proteins from the immune system, its use has expanded to liposomal formulations, copolymers and drug conjugates. The rise of antibody drug-conjugates has demonstrated the benefit of single-length, also known as monodisperse, PEG derivatives. In this article, we summarise the current applications of monodisperse PEG derivatives in both academic and commercial settings. With the newly available monodisperse PEG 2000 and new research on PEG 5000, we also highlight potential areas where polydisperse PEG could be replaced with its monodisperse equivalent.

Uniformity and Defined Composition of Monodisperse PEG

Polymers are inherently polydisperse, consisting of a mixture of different molecule lengths, which is a consequence of the industrial polymerisation methods.[1] PEG is typically obtained via anionic ring-opening polymerisation (ROP) of ethylene oxide (EO). This method yields a mixture of PEG molecules with a Gaussian distribution of chain lengths (Figure 1). Although modern polymerisation methods have been improved with new catalysts and post-synthetic treatments to narrow the distribution, heterogeneous materials are still generated. For instance, PEG 2000 with a narrow polydispersity index (PDI) of 1.02 still contains over 25 different PEG chain lengths.[2]

For most applications, PEG is further modified with reactive groups such as amines or thiols to enable covalent attachment to proteins or nanoparticle surfaces.[3] These additional synthetic steps, when performed on polydisperse PEG, amplify its heterogeneity by generating side products and impurities that are difficult to remove efficiently.

One example of a modified polydisperse PEG is methoxy-PEG 2000 amine (mPEG 2000 amine, Figure 2), widely used for PEGylation of nanoparticles or as a hydrophilic block in biodegradable copolymers.[4,5,6] mPEG 2000 is first obtained via ROP of EO using ethylene glycol monomethyl ether as an initiator.[1] Even at this step, impurities such as dimethoxy PEG or unprotected PEG diol can be formed. Subsequent derivatisation steps to the activated intermediate, like mPEG tosylate or mPEG bromide, and finally to mPEG amine,[7] will lead to the generation of even more impurities like amine dimers or hydrolysed mPEG. Both impurities were identified in the sample of a commercial mPEG2000 amine.

Another example is 1,2-DiMyristoyl-sn-Glycero-3-methoxy PEG 2000 (DMG-PEG 2000), which is used as an ingredient in

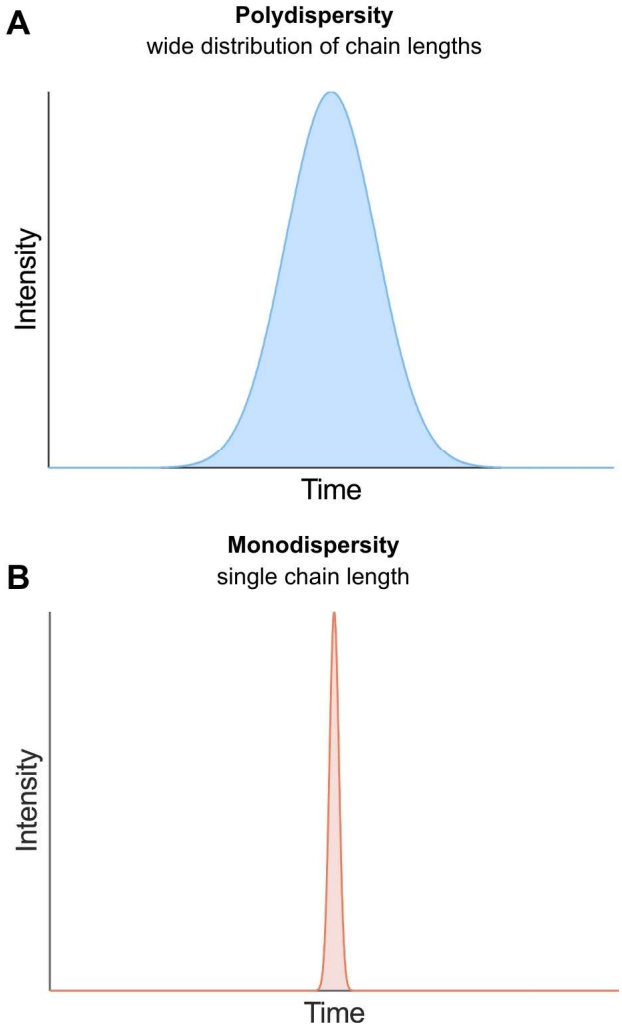


Figure 1. Cartoon of: A) polydisperse PEG and B) monodisperse PEG

lipid nanoparticles, widely known from the SARS-CoV-2 vaccine Spikevax[8] from Moderna. A recent study of commercial DMG-PEG 2000 samples revealed minor presence of impurities, less than 1.5%, generated under synthesis and hydrolysis, and a PDI of 1.01–1.02, corresponding to roughly 30 different PEG lengths.[9] Our analysis of the PEG component after cleavage of the lipid moiety (Figure 3A-C) confirms variations in the maximum PEG length across products from different suppliers. This batch-to-batch heterogeneity, even at low PDI, can be carried over into the final formulations, possibly hindering reproducibility and purity of the final product.

Monodisperse PEG, also referred to as single-length or discrete PEG, contains only one specific chain.[10,11] Contrary to the polydisperse material, monodisperse PEG cannot be obtained by conventional polymerisation methods. Rather, it is synthesised by reiterations of Williamson's ether synthesis combined with chromatographic purification.[10,11] This multi-step process allows precise control of chain length and efficient removal of

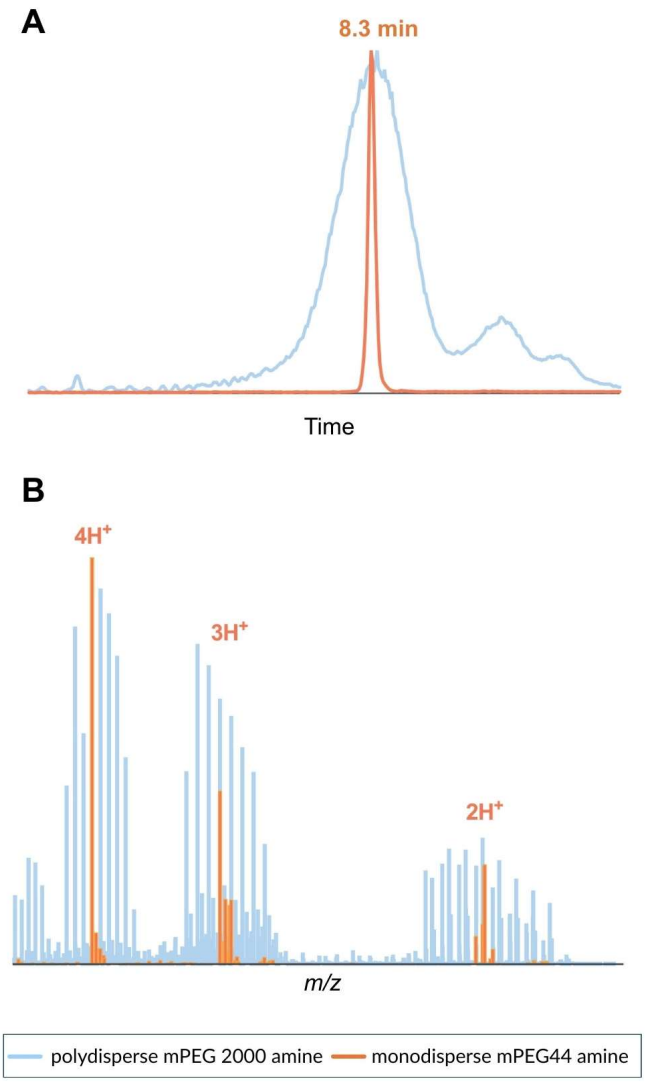


Figure 2. Analysis of polydisperse mPEG 2000 amine (blue) and monodisperse mPEG-44 amine (orange): A) HPLC chromatogram and B) MS spectra.

impurities formed during the process. The resulting high-purity PEG diol with minor impurities can then be easily derivatised into heterobifunctional species.

The synthesis of monodisperse PEG dates back to 1970, when the first PEG of n = 9 and 15 were synthesised by Bomer *et al.*[12] The first attempts lacked control of chain elongation and led to the generation of side products.[11] Later, improved methods were introduced with the use of orthogonal protecting groups and longer PEG could be generated. However, most of the described approaches to generate PEG with n = 45 were still limited to milligram quantities. Only recently, monodisperse PEG 2000 (corresponding to n = 45) has become commercially available on a multigram scale.[13]

Single-length, high-purity PEG oligomers provide an optimal starting point for further derivatisation and biomolecule attachment.[14,15] Defined chemical composition is also valued in single molecule imaging and modelling, where known chain length simplifies calculation and simulations.[16] In high-volume manufacturing, monodisperse PEG yields high reproducibility, precise control over the impurity profile and streamlined purification of the final formulation.

Heterobifunctional PEG in Diagnostics and Imaging

Before monodisperse PEG made it to approved therapeutics, its first uses were focused on surface functionalisation,[14,15] linker for imaging,[16] and conjugation with peptides.[17,18,19]

Surface functionalisation with PEG is often employed when performing biotin-streptavidin-based immunoassays.[14,15] These immunoassays are commonly used in clinical diagnostics to quantify levels of various proteins, e.g. hormones. In a typical assay, gold particles are functionalised with mPEG-thiol (typically n = 7) and biotin-PEG-thiol (typically n = 10) via the covalent thiol-gold bonds. The mPEG-thiol forms an antifouling monolayer that minimises nonspecific protein adsorption, while biotin-PEG-thiol introduces biotin groups for high-affinity binding of streptavidin. The streptavidin molecules immobilised on the surface then capture biotinylated antibodies specific to the analyte, enabling selective binding and subsequent signal generation.

In atomic force microscopy (AFM), a longer heterobifunctional PEG is employed as a flexible spacer, namely maleimido-PEG-

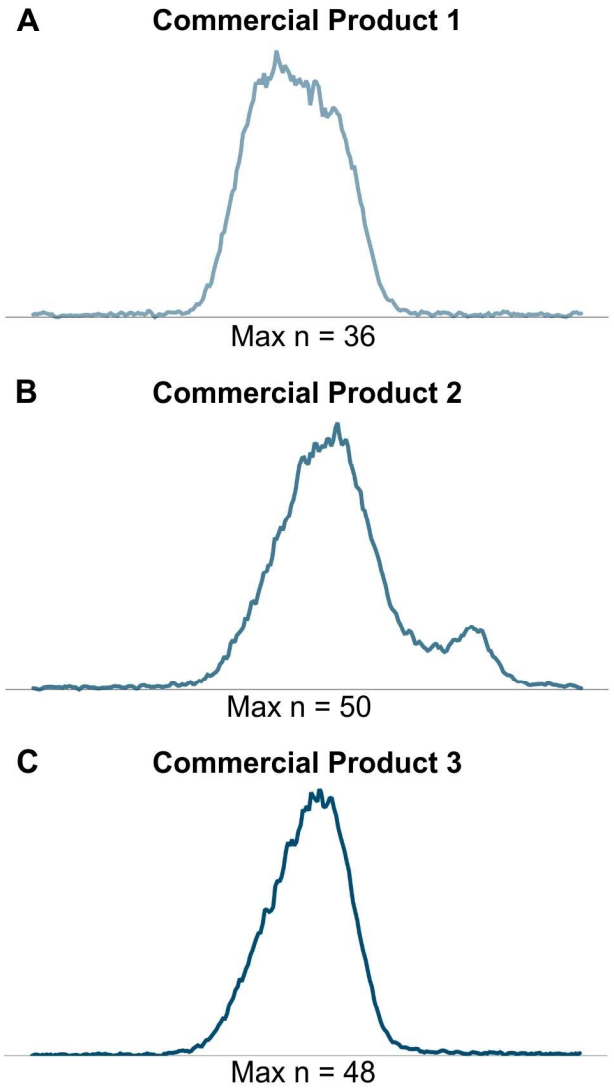
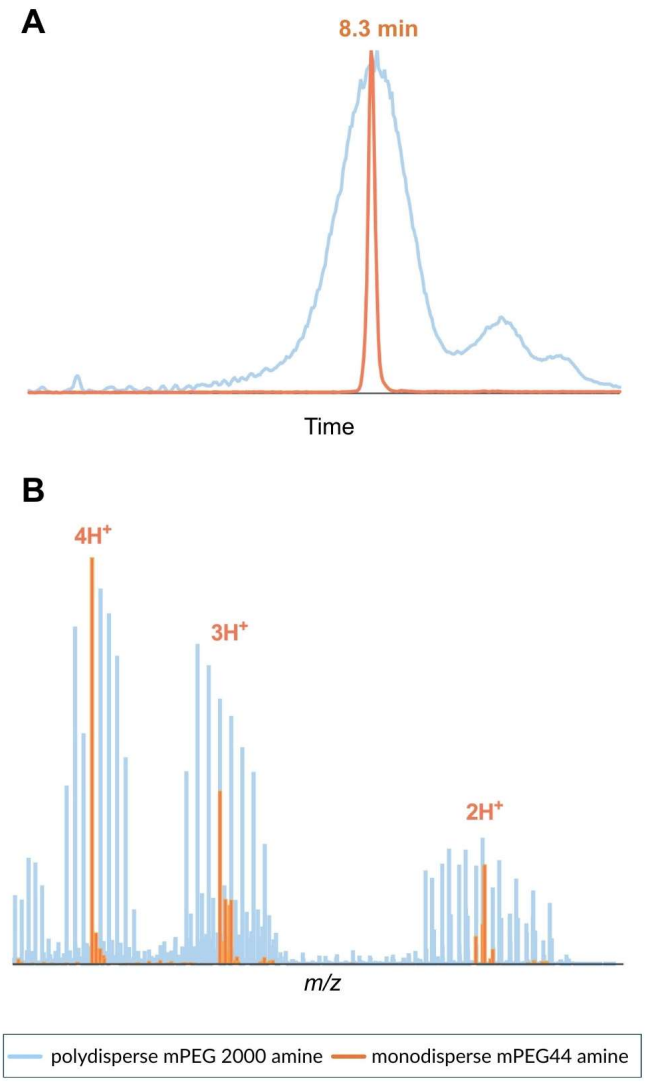


Figure 3. HPLC analysis of hydrolysed polydisperse DMG-PEG 2000 samples from different suppliers with identified variations in the maximum length of the PEG chain (Max n).



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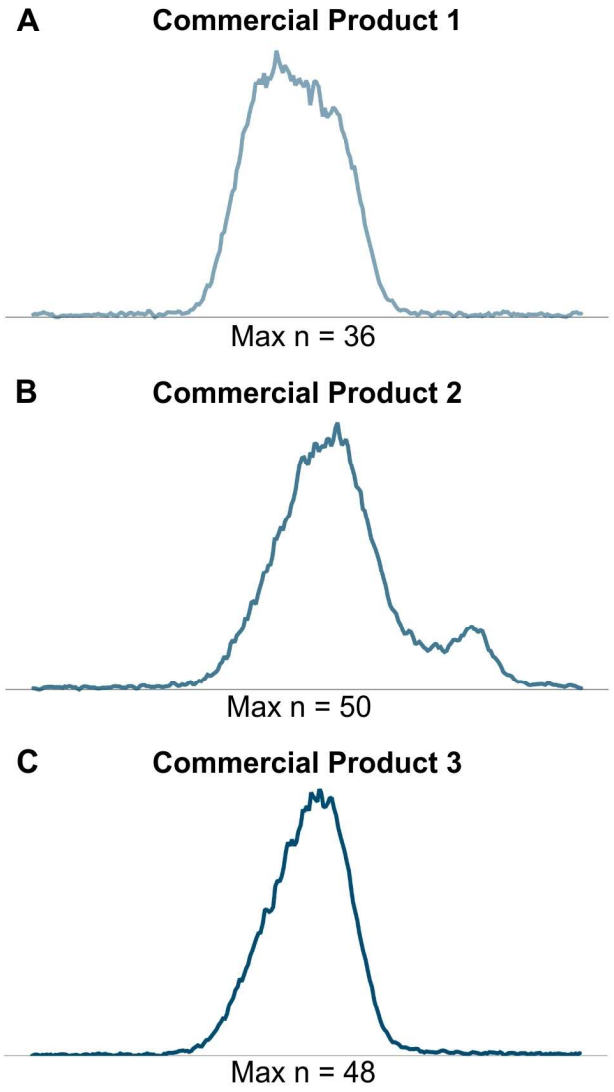

Figure 3. HPLC analysis of hydrolysed polydisperse DMG-PEG 2000 samples from different suppliers with identified variations in the maximum length of the PEG chain (Max n).

Drug Name (active substance)	PEG length
Doxil, Definity, Onivyde, Onpattro, Comirnaty, Spikevax, Elfabrio	PEG 2000
Rolvedon	PEG 3000
Adagen, Oncaspar, Somavert, Asparlas, Revcovi	PEG 5000
Krystexxa, Skytrofa, Pegintron, Sylatron	PEG 10,000
Neulasta, Plegridy, Adynovate, Udenyca, Fulphila, Palynziq, Ziextenzo, Nyvepria, Stimufend, Flynetra, Besremi, Esperoct, Izervay, Omontys, Macugen	PEG 20,000

Table 2: Examples of FDA-approved medicines with polydisperse PEG[22,23]

The number of PEGylated therapeutics on the market continues to grow. The advantages of monodisperse PEG have already been demonstrated in commercialised ADCs. With monodisperse PEG 2000 derivatives available on the market, such as mPEG-44 amine and mPEG-44 thiol (Figure 4), there is a new potential area for applications in nanoparticle and liposomal formulations.[13] However, there is still an unmet need for even longer monodisperse PEGs for protein PEGylation, particularly PEG 5000 corresponding to 114 repeating units.

The benefit of using single-length PEG 5000 for protein PEGylation was recently demonstrated by Burggraef *et al.*[25] The group showed that proteins PEGylated with conventional polydisperse PEG produce multiple PEGylated peptide fragments when analysed by mass spectrometry. The multiple signals make the evaluation and quantification of the PEGylation efficiency challenging. Contrastingly, PEGylation with single-length PEG 5000 enabled straightforward and precise analysis due to the presence of a single PEG molecular weight. This clear advantage has pushed current industrial R&D efforts to develop heterobifunctional PEG 5000 on a multigram scale to make it readily available for the pharmaceutical sector.

There is little doubt that medicines based on PEGylated proteins and enzymes would benefit from the simplified analytical determination of PEGylation efficiency and improved yields granted by monodisperse PEG. With monodisperse PEG 2000 already available and the development of monodisperse PEG 5000 currently ongoing, the more consistent and well-characterised PEGylated therapeutics are well underway and might soon enter the clinic.

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