PEGylation technology and biopharmaceuticals

Simple modification with polyethylene glycol (PEG) is not only capable of improving the pharmacological properties of a drug, especially for peptide and protein therapeutics, but has also to be considered with regard to its life cycle extension. Dr Frank Leenders of celares GmbH describes PEGylation technologies and how they are impacting biopharmaceutical development.

PEGylation has become a well-accepted method for the delivery of biopharmaceuticals - especially peptide and protein drugs. Simple modification with polyethylene glycol (PEG) is not only capable of improving the pharmacological properties of a drug but has also to be considered with regard to its life cycle extension. Thus, PEGylation today is mandatory to think about when developing a biopharmaceutical drug. This article gives an overview of established PEGylation technologies and new developments in the field.

celares GmbH is a drug delivery company specialising in PEGylation. The company provides proprietary drug delivery systems and licenses PEGylation technology to other users. celares also carries out contract development, with customised PEGylation and the supply of tailor-made PEG reagents.

What is PEGylation?

PEGylation is the covalent coupling of non-toxic, hydrophilic polyethylene glycol (PEG) to active pharmaceutical ingredients. The technology was developed from pioneering work carried out in the 1950s and 1960s on the coupling of polymers to proteins, and by the 1970s Frank F. Davis was using PEG for protein modifications. The first PEG-protein company was Enzon, founded in 1981, and the first approved PEG-drug product was PEG-adenosine deaminase, approved in 1990. Nowadays, PEGylated proteins represent a significant business: sales in 2005 for these products amounted to more than $5 billion.

The modification of a biopharmaceutical with polyethylene glycol (PEG) increases its hydrodynamic radius and reduces immunogenicity and antigenicity. Other benefits include decelerated renal excretion, improved stability towards proteolysis and increased solubility of the biopharmaceutical in aqueous solutions.

linear – traditional form

Y-shaped – large PEGs

hyper branched – 4 -9 PEG
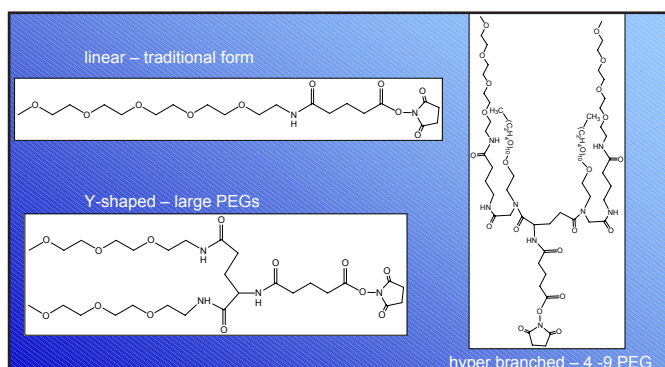
Structure of PEG.

MEET DR FRANK LEENDERS

Dr Frank Leenders is Managing Director Operations at celares GmbH, where he is responsible for both contract and preclinical development of PEGylated biopharmaceuticals. Dr Leenders was previously responsible at LIBB GmbH for the area of medicinal diagnostics and at Medical Enzymes for the preclinical and clinical development of PEGylated protein drugs. In 2003 he joined the management board of celares GmbH. Dr Leenders is co-inventor of four patents in the fields of PEGylation and protein drugs. He received his PhD degree in Biochemistry/Protein Chemistry from the Technical University of Berlin.

PEGylation is a new delivery technology that differs from traditional formulation in a number of ways. For formulated products such as tablets, liquids and capsules, the formulation process is reversible, the drug becomes active after its release from the formulation and the API remains unchanged. In PEGylated products, on the other hand, the API is chemically modified in a durable fashion, and the drug is not released from a formulation but has a permanent action and is in fact classed as a new API. Consequently, PEGylation has to be considered early in the drug development process.

The production of PEGylated proteins can be achieved through the application of a range of chemistries and other technologies, including traditional peptide chemistry; genetic engineering combined with chemical methods, for example in the introduction of unnatural amino acids; or the use of enzymatic technologies such as glycoPEGylation® or transglutaminase reactions.

An example of the utilisation of unnatural amino acids is the introduction of para-azidophenylalanine by the use of a genetically engineered yeast (E. coli tyrosyl tRNA/tRNA synthetase pair) and the reaction of an azido group specifically with a PEG-alkylene derivative (Deiters A. et al., BMCL 14 (2004) 5743-5745).

An example of an enzyme-catalysed PEGylation is the transglutaminase reaction for PEGylation of glutamine using a specific transglutaminase and requiring a specific peptide motif (Haruya Sato, ADDR 54 (2002) 487-504).

An example of a glycoPEGylation® reaction is the PEGylation of glycosylation sites using a specific sialyltransferase (Neose, Inc).

Advantages and drawbacks

Chemical and enzymatic/genetic PEGylation technologies each have their own advantages but equally they each have their own disadvantages too.

MALDI-MS of PEG.
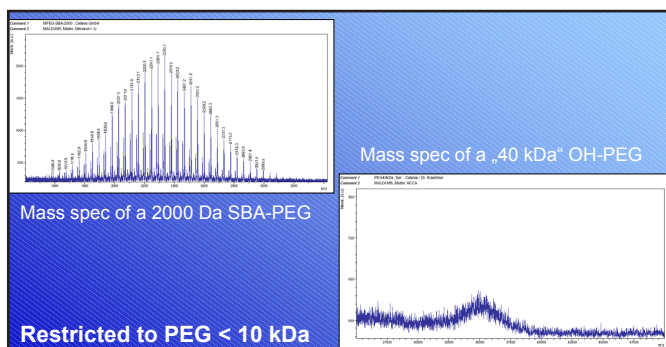
QC by biochemical testing.

The advantages of chemical technologies are that they include the use of established chemistry procedures, reactions occur in high yields and they have broad applicability. Some of the disadvantages are that reactions are not highly specific, side-reactions can occur and PEGylations can be incomplete.

Enzymatic/genetic processes, on the other hand, are highly specific and there are few side-reactions. The disadvantages are that these technologies are restricted to a limited number of applications, processes require a recognition site, and the enzyme has to be separated at the end of the process.

The choice of PEGylation strategy therefore depends on the parameter to be modified; the size of the drug molecule; the function and structure of the molecule; the availability of reactive groups; and whether the option of genetic engineering is available. The message is that PEGylation has to be tailored to each drug.
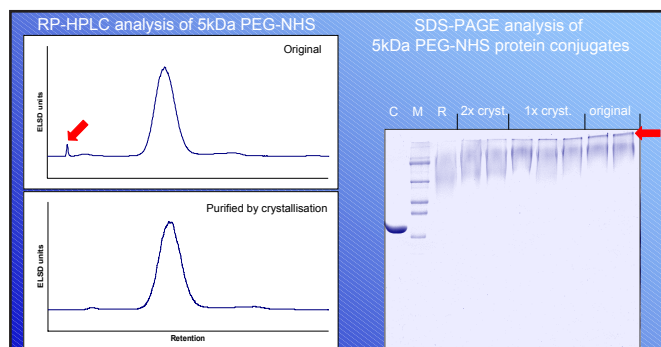
Quality control considerations

PEG quality is important in order to achieve reproducible PEGylation and reliably meet the specification of the PEGylated drug. This is illustrated, for example, by the recall of five lots of PEG-Asparaginase that occurred between 2000 and 2003 due to high deviations in activity.

Traditional PEG systems are polydispersed. Problems with polydispersed PEGs include working with heterogeneous polymer mixture; the occurrence of structurally determined fluctuations in quality; challenging analytics; and the possibility of cross-linking. Which analytical technology is best for the quality control of PEG? GPC can be used for the determination of dispersity index, and other techniques that may be applicable are MALDI-MS and HPLC. NMR can be used for qualitative determination of functional groups, and perhaps biochemical methods could have a role in the analysis of PEG-protein compounds. The answer is to find the right balance between the application of polymer analytics and small-molecule analytical techniques. One way to overcome most analytical problems is the use of monodispersed PEG. CelaSYS is the first example of a 4.2 kDa monodispersed PEG for pharmaceutical applications. CelaSYS is a multi-branched polyethylene glycol based reagent. Monodispersion produces a high-quality, reproducible reagent, and branching provides efficient protection of the drug molecule. Analytical techniques used for quality control of monodispersed PEG include RP-HPLC, NMR and MALDI-MS.

In summary, the QC of PEGs is challenging and a set of analytical methods is needed. The use of monodispersed PEGs reduces analytical efforts and makes drug specifications much more significant. Finally, only biochemical testing procedures will reveal the quality of a PEG reagent.

Increasing drug performance with PEGylation

In a recent study, a globular protein drug of molecular weight 60 kDa, an amino acid depleting enzyme that was co-factor dependent, had a very low *in vivo* half-life, and high immunogenic and allergenic potential. The aim of the study was to determine how to increase the drug's *in vivo* half-life. A PEGylation strategy was adopted that required the masking of immunogenic epitopes, and the use of high surface protection to prevent proteolysis. A multiple lysin random PEGylation procedure was used. The experimental procedures comprised the screening of PEGylation conditions, the optimisation of PEGylation, the development of the purification process, analytical procedures, and the completion of pharmacokinetic/pharmacodynamic studies in mice.

The PEGylation procedure was further developed through experiments to determine the optimal PEG/protein ratio. Initial results showed poor PEGylation efficiency and the production of an inhomogeneous conjugate. Optimisation of the PEG gave good PEGylation efficiency with no residual unmodified protein.

High reproducibility of PEGylation was achieved by performing the PEGylation for 2 hours at a pH of 9.5 and subsequently performing a one-step chromatographic purification. Using this procedure, 45% of amino groups were modified and the overall yield was > 85%. MALDI-MS was used for mass determination: the product had a molecular weight of 112 kDa with about 10 PEG per molecule. The PK/PD study in mice demonstrated favourable results.

Established technology with broad potential

In summary, PEGylation is an established drug delivery technology for biopharmaceuticals that has been marketed for more than 15 years. The process results in the formation of a new API and thus has to be considered early in the drug development process. The quality of PEG used is a crucial parameter in order to achieve reproducible PEGylation and reliably meet the specification of the PEGylated drug. In principle, PEGylation can be applied to any biopharmaceutical drug. However, the success of PEGylation is determined by the strategy adopted, the experimental set-up, and the application of experience and expertise. sp²