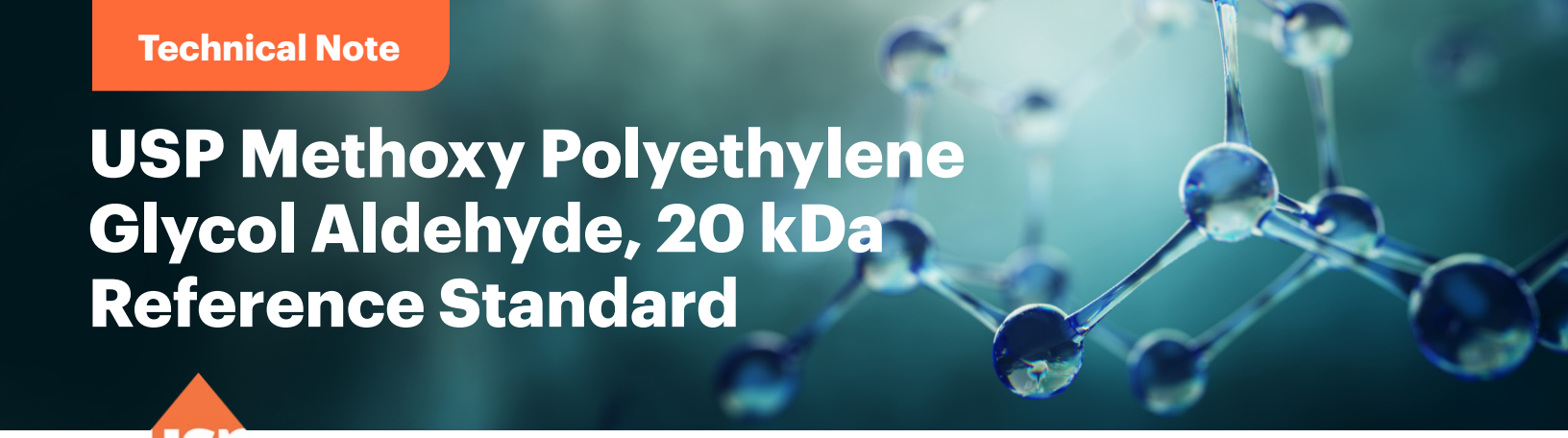
Technical Note

USP Methoxy Polyethylene Glycol Aldehyde, 20 kDa Reference Standard



Polyethylene glycol (PEG) is a linear synthetic polyether that can be produced in a range of molecular sizes. PEG is non-toxic and soluble in both water and organic solvents which makes it highly biocompatible and hence used in a wide range of pharmaceutical applications. Linking PEG to biomolecules through covalent conjugation, called PEGylation, leads to improvement in pharmacokinetic properties like increasing the circulatory life of the drug, reducing renal filtration immunogenicity and susceptibility to proteolytic cleavage. The first PEGylated biotherapeutic was ADAGEN® (Pegademase bovine), introduced in 1990 to treat severe combined immunodeficiency disease. Currently there are several PEGylated products approved in the market and many more in clinical trials[1]. Through PEGylation the PEG moiety becomes part of the Active Pharmaceutical Ingredient (API) when it is linked to the protein or peptide molecule to form the conjugated product. Hence it is a critical raw material of the manufacturing process. The quality of the PEGylation reagent has a direct impact on the conjugated product. Properties like molecular weight distribution (polydispersity), functionality of the derivatized group and presence of impurities in the PEG affects the conjugation process in terms of efficiency, yield and quality.

Impurities in PEG raw materials can result from improper manufacturing, handling, packaging, and storage. These impurities can be inadvertently introduced during the PEGylation process, resulting in reduction of PEGylation efficiency, yield and product quality. Control strategies such as qualification of the incoming raw material becomes important to ensure consistent process performance and product quality throughout a biological product lifecycle. Testing the incoming PEG raw material to ensure the purity, polydispersity and other critical attributes is important, not only for each lot but also for each vendor.

The monomethoxylated form of PEG (mPEG) has been used in most PEGylated therapeutics[1]. Monomethoxy poly(ethylene glycol) aldehyde (mPEG-ALD) is an alkylating PEG reagent that reacts with the N-terminal amino acid of the protein to form a stable bond. One end of the PEG is capped with a methoxy group to avoid unwanted crosslinking and the functional aldehyde at the other end reacts with the protein to form the conjugate. Methoxy polyethylene glycol aldehyde, 20 kDa (mPEG-ALD, 20 kDa) is one such raw material used in the conjugation of granulocyte colony stimulating factor to manufacture the approved therapeutic Pegfilgrastim. Here, we describe analytical procedures that were used to evaluate the quality of the mPEG-ALD, 20 kDa raw material and develop a mPEG-ALD, 20kDa reference standard (USP Methoxy Polyethylene Glycol Aldehyde, 20kDa RS, catalog #1419109) that can be used to qualify incoming raw material as well as to validate and demonstrate system suitability of analytical methods.

Materials and Methods

The chemical identity and structure of mPEG-ALD, 20kDa was confirmed by 1-D NMR (^{1}H and ^{13}C) and 2-D NMR (^{1}H-^{1}H COSY and HSQC) at 500 MHz. NMR experiments were performed at 300° K with deuterated chloroform ($CDCl_3$) solvent. The ^{1}H and ^{13}C NMR chemical shift values were referenced to the tetramethylsilane peak at 0.00 ppm and to the $CDCl_3$ peak at 77.00 ppm, respectively.

Suggested NMR test conditions:	
Sample	About 50 mg of Methoxy Polyethylene Glycol Aldehyde, 20kDa. Add 0.6 mL of diluent and mix well to dissolve the sample.
Diluent:	$CDCl_3$ + 0.03 vol % TMS
Sample Volume	about 0.6 mL
Pulse Program	zg
Temperature	300° Kelvin
Number of Scans	NLT 64
Field strength	300-600 MHz
Recycle delay (D1)	NLT 25 sec
Size of fid, TD	NLT 64 K
Spectral width, SW	20.0 ppm
Transmitter frequency offset, 01	6.0 ppm
LIne boardening	0.3 Hz

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Materials and Methods (continued)

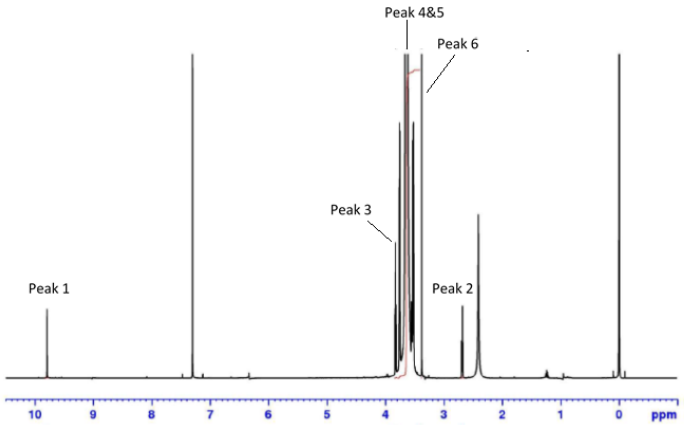
The molecular weight (Mw) and polydispersity index (PDI) of mPEG-ALD, 20kDa were determined using size exclusion chromatography with multi-angle light scattering detection (SEC-MALS). Chromatography was performed on an Alliance HPLC system equipped with an autosampler, UV detector, and Empower™ software (Waters, USA). The PL aquagel-OH size exclusion chromatography (SEC) columns (300 x 7.5 mm), 8 µm (Agilent, USA) were used at 35°C. Two columns were connected in series. The mobile phase used was 0.1 N Sodium Nitrate. A sample of 100 µL of mPEG-ALD, 20kDa (10 mg/mL) was injected and separated using an isocratic gradient. MALS was performed using the DAWN8+ (Wyatt, USA) equipped with a Wyatt QELS DLS module and an Optilab TrEX refractive index detector. Light scattering was monitored at 663.5 nm and the refractive index was monitored at 658 nm at 35°C.

Suggested SEC-MALS test conditions:	
Mobile Phase:	0.1 N Sodium Nitrate
Sample:	10 mg/mL in mobile phase
Injection Volume:	100 µL
Flow rate:	1 mL/min
Column:	PL aquagel-OH 40 & PL aquagel OH 30 (connected in series)
Column dimension and particle size:	7.5 mm x 30 cm, 8 µm
Column temperature:	35°C
Autosampler temperature:	25°C
System:	Isocratic
Run time:	40 mins
Refractive Index detector temperature:	35°C
Normalization Standard: Methoxy Polyethylene Glycol Aldehyde, 20kDa Note:	Expected dn/dc for Methoxy Polyethylene Glycol Aldehyde, 20kDa is approximately 0.13 mL/g when used as normalization standard.

Results and Discussion

Through a multi-lab collaborative study, USP has established a reference standard for mPEG-ALD, 20kDa. The chemical identity of a material is an important criterion especially when it is part of the API. The identity of mPEG-ALD, 20kDa material was established by confirming the presence of the aldehyde group and the overall structure of the methoxy polyethylene glycol moiety. A typical NMR profile for the mPEG-ALD, 20kDa is shown in Fig 1. The methoxy end group (CH_3O-) is the peak at 3.38 ppm and the aldehyde (CHO) peak is at 9.80 ppm. A comparison with a non-functional PEG control (Fig 2) shows that the above two peaks were absent in the NMR profile of the control. The peaks at 2.68 and 3.64 are assigned to protons of methylene groups (CH_2-) in the PEG blocks.

Fig 1: NMR profile of mPEG-ALD, 20kDa



Approximate ^1H chemical shifts (δ in ppm) for mPEG-ALD, 20kDa

Position	Peak	Approximate δ_H (ppm)
1	Aldehyde	9.80 (1H)
2	PEG group	2.68 (2H)
3	PEG group	3.83 (2H)
4 & 5	PEG group	3.64 $(4H)_n$
6	Methoxy group	3.38 (3H)

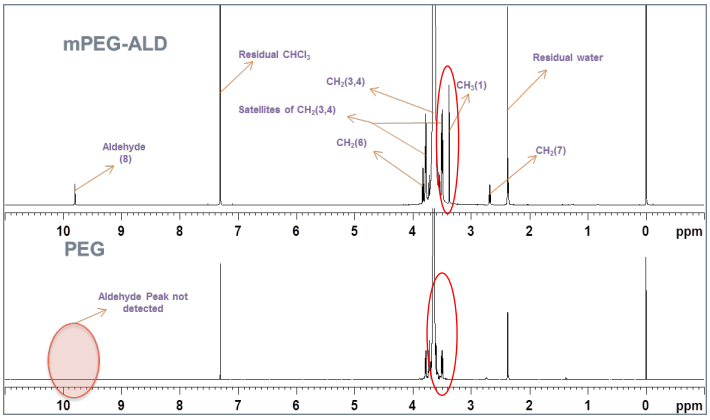


Fig 2: Comparison of NMR results for mPEG-ALD, 20kDa to a PEG control

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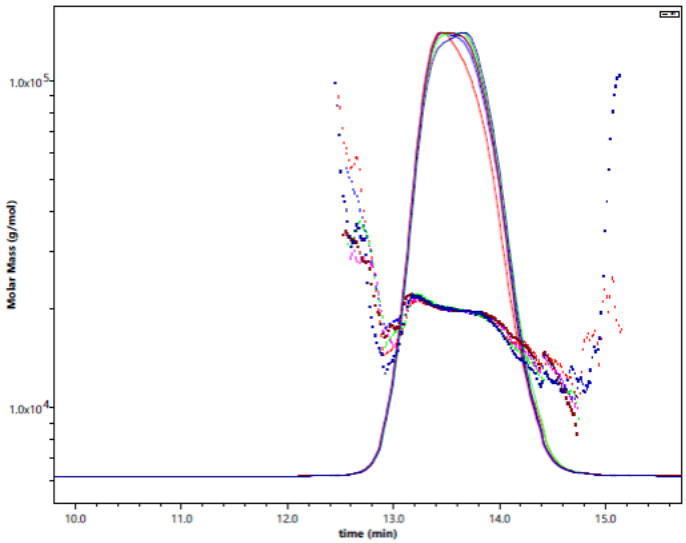
Results and Discussion (continued)

PEG is known to be a polydisperse molecule. The broadness of molecular weight distribution of a polymer or its polydispersity index (PDI) is a ratio of the weight average molecular weight to the number average molecular weight. If all the molecules in a sample have the same degree of polymerization, then the sample is monodisperse and has a PDI equal to 1.0. High molecular weight PEGs have a PDI of ~1.10. SEC-MALS is an analytical technique that directly measures the absolute molar mass and the average size of molecules based on how the sample scatters light. SEC-MALS permits accurate mass determination independent of a calibrant. The mPEG-ALD, 20kDa sample eluted as a single peak in the SEC-MALS chromatography as seen in Figure 3. The MW of mPEG-ALD, 20kDa was determined as 20.02 ± 1.5 kDa and the PDI as 1.01 indicating that the material was of good quality with low polydispersity. Note that the values of molecular weight and PDI as well as representative NMR data are provided in the USP Certificate of the Reference Standard.

Fig 3: Peak profile with distribution curve of SEC-MALS for mPEG-ALD, 20kDa



Conclusions

Quality control of PEG raw materials is essential to achieve reproducible PEGylation and consistent, high quality products that meet desired specifications. The quality control of activated PEG is challenging due to the complexity of the molecule. The molecular weight distribution and polydispersity index of PEG material used in biopharmaceuticals has been traditionally carried out using gel permeation chromatography (GPC). However, GPC relies on molecular weight estimation using a calibration curve which can introduce variations based on the calibrants used. The SEC-MALS method reported in this technical note provides a convenient, quick and powerful tool for directly measuring the MW and PDI of mPEG-ALD, 20kDa. Combined with NMR for determining critical functional groups to confirm the identity, these tests provide a suite of analytical tools to ensure the quality of mPEG-ALD, 20kDa raw materials.

USP Methoxy Polyethylene Glycol Aldehyde, 20kDa RS can be used as a control material to help biologic manufacturers confirm the quality of their PEG raw material lots and identify suitable alternative suppliers. The RS can also be used to validate analytical methods and to ensure system performance.

References:

1. Peter L. Turecek, Mary J. Bossard, Freddy Schoetens, Inge A. Ivens. PEGylation of Biopharmaceuticals: A Review of Chemistry and Nonclinical Safety Information of Approved Drugs. Journal of Pharmaceutical Sciences 105, 460-475 (2016).

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