Monodispersed Polymers

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Synthesis of Poly(ethylene oxide) Approaching Monodispersity**

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Abstract: *Polydispersity in polymers hinders fundamental understanding of their structure–property relationships and prevents them from being used in fields like medicine, where polydispersity affects biological activity. The polydispersity of relatively short-chain poly(ethylene oxide) [$(CH_2CH_2O_2)_n$; PEO] affects its biological activity, for example, the toxicity and efficacy of PEOylated drugs. As a result, there have been intensive efforts to reduce the dispersity as much as possible (truly monodispersed materials are not possible). Here we report a synthetic procedure that leads to an unprecedented low level of dispersity. We also show for the first time that it is possible to discriminate between PEOs differing in only 1 ethylene oxide (EO) unit, essential in order to verify the exceptionally low levels of dispersity achieved here. It is anticipated that the synthesis of poly(ethylene oxide) approaching monodispersity will be of value in many fields where the applications are sensitive to the distribution of molar mass.*

Well controlled anionic polymerization of PEOs can achieve polydispersity indices approaching 1.04 or less.[1] However, this dispersity is still too high in many cases. Furthermore, synthesizing PEO approaching monodispersity is meaningless without a method capable of determining very low levels of polymer dispersity, something that established method, such as gel permeation chromatography (GPC), cannot do.

Conceptually, there are four ways in which ethylene glycol chains may be elongated starting from shorter, high purity oligo(ethylene oxide)s.[2]

A) iterative coupling of a mono-protected building block to one end, $L = n(1+g)$
B) iterative coupling of mono-protected building blocks to both ends, $L = n(1+2g)$
C) chain doubling, $L = 2^g n$
D) chain tripling, $L = 3^g n$

where: L = oligo/polymer length, g = number of generations of coupling, n = number of monomeric units in starting oligomer.

Although (C) and (D) offer the fastest methods for chain elongation they do not provide the opportunity to synthesize bespoke chain lengths (i.e. PEO chains with a specific number of EO units). Furthermore, methods (C) and (D) in practice involve a significant number of experimental steps.

As noted above, a major barrier to synthesizing PEOs of very low dispersity is how to establish the quantity of chain lengths that deviate from the target chain length, that is, the chain length purity. Conventional methods of measuring dispersity, such as GPC, do not offer the level of discrimination between different molecular weights that are necessary to distinguish two PEO chains that differ in only one EO unit (Figure 1). Long-chain molecules differing only in one monomer unit have such similarity in physical properties, size and shape that discrimination is difficult. ^{1}H NMR spectroscopy or elemental analyses are not sufficiently sensitive to distinguish between such molecules either.

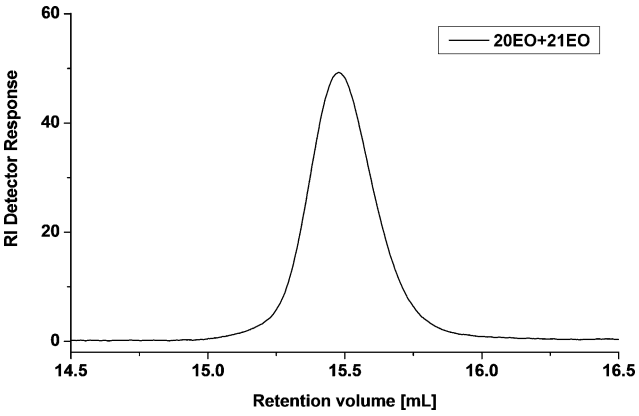

Figure 1. Size exclusion chromatographic results for an equimolar mixture of 20EO and 21EO, exhibiting only one peak and showing that GPC cannot resolve PEO differing between 20 and 21 EO units in the mixture.

Mass spectrometry (MS) is one of the few, perhaps the only, method that could, in principle, provide the means of distinguishing reliably between molecules differing by only 1 EO unit.[2,3] However, results in the literature concerning the use of different MS methods do not provide sufficient evidence that any one of the established MS based protocols can reliably do so.[4] To investigate this further, we used a 12EO synthesized by the procedure developed by Tanaka et al.[5] Electrospray ionization MS time-of-flight (ESI-MS-TOF) clearly showed one species with the main peak at 569.16 Da (and its isotopic peak 570.25 Da) corresponding exactly to $12EO + Na^+$. Since no other molecules were detected, high purity would be assumed. When the same material was subjected to MALDI-TOF-MS (MALDI = matrix-assisted laser desorption/ionization) two species were detected: the desired $12EO + Na^+$ (569.24 Da, intensity 100%) and $11EO + Na^+$ (525.21 Da, intensity 8%). From

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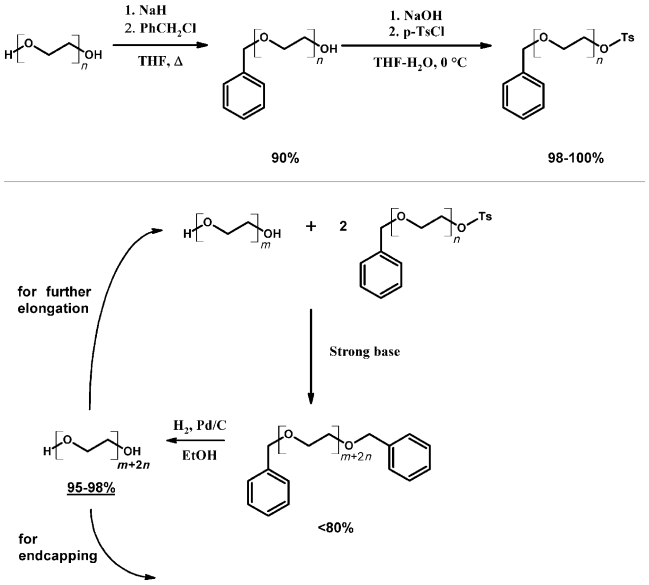
these results, MALDI appeared to be the superior method for our purposes. However, previous reports in the literature cast doubt on whether MALDI is suitable.[4b] For example, under MALDI conditions PEOs may decompose.[6] No evidence of molecules which could result from decomposition of the PEO were observed in our spectra, which led to the conclusion that under our MALDI operating conditions decomposition is not observed and the $(x-1)$EO by-products must be generated during the course of the synthesis. Another problem with MALDI reported in the literature is the reaction between the matrices used in MALDI and PEO derivatives,[4c,7] however, for OH-, CH_3O- and benzyl-terminated PEOs, as used here, such side reactions were not observed in our measurements. Shimada et al.[4b] attempted to evaluate the quantitative capability of MALDI by examining an equimolar mixture of monodispersed PEOs of $x = 6$–40. In their investigation laser power (LP) and species of adduct cations were the variables. Although their general conclusion was that MALDI is not a quantitative method, when sodium is added and LP is not higher than 5.0, the discrimination of neighbouring chain lengths was possible, making it suitable for evaluation of an impurity which is 1 EO unit smaller than the target product.

The conclusion we can draw from our studies of MALDI is that the problems reported in the literature are due to the particular protocols used, and provided the protocol described in the present work is followed, MALDI is a suitable, perhaps the only suitable, method capable of discriminating between chain lengths differing by only 1 EO unit and with sufficient sensitivity to low levels of the unwanted chain lengths that the procedure can be used to establish the level of chain length purity necessary in syntheses attempting to approach monodispersity.

Turning to the synthesis, details of the procedures are given in the Experimental Section. Here we focus on discussing the main features of the method that relate to the level of dispersity and purity of the final products. Our target was a set of monodispersed dimethoxy end-capped PEOs of specific chain lengths, in the range 19–24 EO units. The basis of the synthetic process is the iteration of well controlled Williamson's ether synthesis.[8] We selected modified mode B, also used earlier by Tanaka et al. (Scheme 1)[5] which involves one end-protection of a short (up to 6 EO units) high-purity glycol, functionalization of its other end, an elongating ether coupling reaction, deprotection, and iteration of the ether coupling either as elongation or as end-capping.

In the procedure described by Tanaka et al., all the steps, except the deprotection, are performed in a basic environment, which generates PEO-alkoxides leading to chain scission,[2,9] and hence was identified as the source of polydispersity. Davis et al. tried to address this problem[2] by replacing NaH in THF with KO*t*Bu in DMF (in presence of 18-crown-6) and changed the order of addition of the reactants to minimize the concentration of the reacting alkoxide. The monodispersity of the resultant PEO was indeed improved, however we found it difficult to separate the elongated product from 18-crown-6, which was added in substantial amounts in order to improve the solubility of KO*t*Bu in DMF. Instead we used THF rather than DMF as it



Scheme 1. Established monodispersed PEO synthesis methodology, modified mode (B).[5]

is a good solvent for KO*t*Bu and does not require any additives. It is also less toxic,[10] easier to dry and to evaporate (66–67 °C compared to 153 °C for DMF) after the reaction. Moreover, we discovered that although lowering the temperature of the ether coupling reaction should slow down its kinetics (as it follows an S_N2 mechanism), it did not affect the yield and allowed further improvement in the purity of the product. We also preserved the original order of addition of reactants, which is easier since the solution of KO*t*Bu tends to clog the injection system as the salt crystallizes.

As indicated by Tanaka et al.[5] separation of unreacted OH-terminated PEOs from benzyl or methyl end-capped product can be achieved by simple extraction, however other side products or unreacted coupling molecules have to be separated by means of flash chromatography. PEO is a very polar polymer because of its terminal (OH) groups.[11] By introducing non-polar end groups (e.g. benzyl or methyl) the polarity can be significantly decreased and becomes highly dependent on the chain length, especially for shorter chains. This opens the possibility of using normal phase silica gel chromatography instead of expensive reversed phase. However, rather polar eluents still have to be used. Several different solvent systems (such as EtOAc/acetone, EtOAc/MeOH, $CHCl_3$/MeOH, MeOH/DCM, MeOH/acetone/toluene, EtOAc/MeOH) were studied by others,[3a,5,12] but our own evaluation proves that for benzyl-terminated PEOs the highest resolution is achieved with DCM/acetone ($\Delta R_{f\ BBP8\text{–}12EO} = 0.23$, $\Delta R_{f\ BBP12\text{–}20EO} = 0.25$; BBP$x$EO = bisbenzyl-protected x-(ethylene glycol)), while DCM/MeOH ($\Delta R_{f\ BBP8\text{–}12EO} = 0.06$, $\Delta R_{f\ BBP12\text{-}20EO} = 0.09$) should only be used when mono-OH terminated molecules have to be separated from diols.

As discussed above, MALDI-TOF-MS was selected to verify the amounts of generated $(x-1)$EO side products and it shows that our procedure generates 98 % (often > 99 %) of the desired chain length on the first ether coupling chain

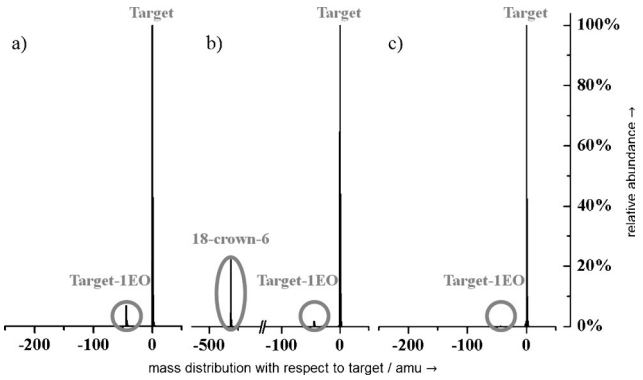


Figure 2. MALDI-MS results for the product of the first ether coupling chain extension synthesized using different procedures: a) Tanaka's, b) Davis's and c) this work. The data show that the synthesis described here achieves unprecedented low levels of dispersity (i.e. a higher level of chain length purity).

extension. This is far superior to the results which we achieved with previous methods, as they typically generated either ca. 5% or 10% of $(x-1)$EO side products (Figure 2).

It is interesting to note that single-crystal diffraction carried out on our monodispersed CH_3O- end-capped PEO with 24 EO units exhibits the same helical conformation as was observed in fiber diffraction studies of OH-terminated PEO with a molar mass of 4000000 Da in 1973.[13] The conformation is different from that of the monodispersed PEO reported by Davis et al.[2] However, the latter consisted of OH-terminated PEO with 16 EO units, that is, much shorter chains than those in the fibre diffraction study. It may be that the relatively high proportion of OH end groups in the study in Ref. [2] influenced the conformation, whereas our CH_3O- end-capped material possesses interactions more closely resembling long-chain PEO.

In conclusion, we report a procedure capable of synthesizing PEOs with the lowest dispersity ever reported (>98% chain length purity after 1 ether coupling and >95% after 3 ether couplings) in multigram scale. In addition, we demonstrate that MALDI-MS is capable of correctly assessing this level of dispersity, something established methods for measuring dispersity cannot achieve.

Experimental Section

The details of synthesis and complete characterization of products can be found in the Supporting Information.

Gradient flash chromatography was performed on a Biotage SP1 system equipped with a SNAP 100 g KP Silica cartridges.

For GPC a single solution was prepared with a volume of solvent expected to give a concentration of 10.0 mg mL^{-1}. The solutions were left for a minimum of 4 h to dissolve and were then thoroughly mixed before being filtered through a 0.2 μm membrane. The instrument was a Malvern/Viscotek Model 301 TDA with associated pump and autosampler, Columns were PLgel guard plus 2 × mixed bed-E, 30 cm, 3 mm. Tetrahydrofuran (stabilised with antioxidant) was used as a solvent, 20 μL was injected, flow-rate 1.0 mL min^{-1} (nominal), temperature 30°C (nominal). A refractive index detector (with differential pressure and light scattering) was used. The data were collected and analysed using Malvern/Viscotek "OminSec" software.

A sample, dissolved in the appropriate solvent, was introduced to the MALDI target along with a matrix and a sodium salt, and allowed to dry. MALDI-MS was acquired using a 4800 MALDI TOF/TOF Analyser (ABSciex, Foster City, CA) equipped with a Nd:YAG 355 nm laser and calibrated using a mixture of peptides. The spot was analysed in positive MS mode over the appropriate mass range, by averaging 1000 laser spots. The laser intensity was adjusted to give ≈2000 counts for the most intense peak in the spectrum.

For ESI-MS the sample was dissolved in either 50:50 acetonitrile:water or methanol at a concentration of 1 ng μL^{-1} and delivered to an electrospray ionization mass spectrometer (LCT, Micromass, Manchester) at 20 μL min^{-1} via a syringe pump and analysed in positive ionization mode, using a capillary voltage of 3200 V and a cone voltage tuned to the specific sample. The instrument was calibrated using a series of sodium formate adducts.

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