

Determination of Rate Constants for the Abstraction of Sodium Ions from Poly(ethylene glycol) by Crown Ether

Sara C. McGrath and Gary L. Glish

Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599

Introduction. MALDI has traditionally been preferred over ESI for the analysis of polymers.¹⁻³ Although MALDI produces mainly singly charged ions and relatively simple mass spectra, molecular weight information (M_n , MWD) calculated from this data does not always agree well with gel permeation chromatography. ESI has the advantage of being an easier technique to use than MALDI and may lead to a better measure of M_n and MWD for polymers. However, ESI generates ions with a distribution of charge states. Polymers already exist in a distribution of masses, so the distribution of charge states convoluted with the distribution of masses greatly complicates analysis of ESI mass spectra. We have shown that ESI mass spectra can be chemically deconvoluted by using crown ether to abstract cations from the polymer ions, reducing the polymer ions from multiple charge states to a single charge state.⁴ Analysis of unknown polymers is possible when the charge state of the ions is known. Of fundamental interest is the rate of cation abstraction, as this can provide insight into issues such as the gas phase structure of multiply charged polymers. The rate of this abstraction process has been measured for several ions over several charge states, and rate constants have been obtained for the conversion of oligomers from one charge state to another.

Experimental. Poly(ethylene glycol) (PEG) polymers in the molecular weight range of 2500 to 7100 Da were studied. All polymer solutions were 0.5 mM in methanol with sodium acetate added in equal concentration to facilitate ionization. No helium was present in the ion trap during experiments. Static pressure of 15-crown-5 was present at a partial pressure of $1-10 \times 10^{-6}$ torr (uncorrected, as measured by a Bayard-Albert ionization gauge). Experiments were performed on a Finnigan ITMS with a custom-built nanoESI source. Oligomers were reacted with the crown ether for up to 10 seconds. Ion intensity was monitored at regular intervals for the reaction of the ions of interest.

Results. Crown ether ion/molecule reactions are useful in the deconvolution of polymer spectra generated by ESI. Figure 1 shows reaction of PEG 2500 with 15-crown-5 over 9 seconds at a pressure of 1.05×10^{-6} torr. After 9 seconds, nearly the entire population of ions has been converted to the 2^+ charge state.

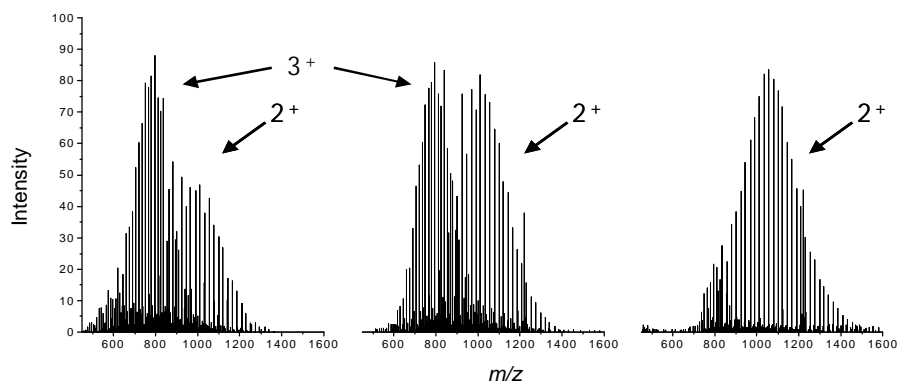


Figure 1. PEG 2500 at 0, 1, and 9 second reaction

We have measured the rate of Na^+ abstraction for several masses and charge states of ESI-generated PEG ions. Five groups of PEG oligomers with masses in the range of 2500 to 7100 Da and charge states ranging from 2^+ to 7^+ have been studied. Abstraction of Na^+ causes the oligomer to shift to a higher m/z value in the mass spectrum. The intensity of the ion of higher charge state is measured at regular intervals as it decreases over the reaction time. Because the crown ether is present in excess, the reaction should proceed as pseudo first order and follow Equation 1, where A is ion intensity, t is reaction time (seconds), m is crown ether pressure (molecules/ cm^3), and k is rate constant (sec^{-1}). Plotting $\ln(A_t/A_0)$ vs. time gives a linear slope; dividing the slope by the crown ether pressure gives the rate constant for the reaction.

$$\ln\left(\frac{A_t}{A_0}\right) = -(kmt)$$

Equation 1

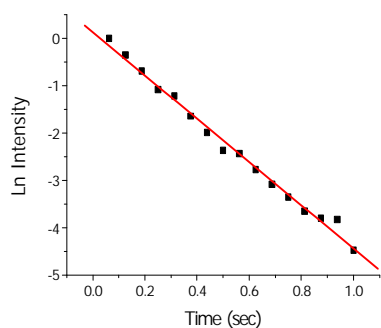


Figure 2. Linear fit of m/z 1100 reacting with 15-crown-5

Figure 2 shows a representative linear fit for the reaction of a 5^+ PEG ion of m/z 1100 (122 repeat units) with 15-crown-5. Rate constants have been obtained in the range of $3\text{--}10 \times 10^{-12} \text{ cm}^3 \text{ molecules}^{-1} \text{ sec}^{-1}$ for these reactions. Figure 3 shows the rate constants for all masses and charge states plotted vs. a) charge state, b) M_n , and c) m/z ratio.

A trend showing increased rate at higher charge states or oligomer lengths is not evident. With the exception of the lowest point on plots 3a and 3b, the trend may point in the opposite direction from what was predicted. Plot 3c shows a general trend of ions of increasing m/z ratio reacting at a faster rate, also indicating that within each M_n group, ions with fewer charges react faster.

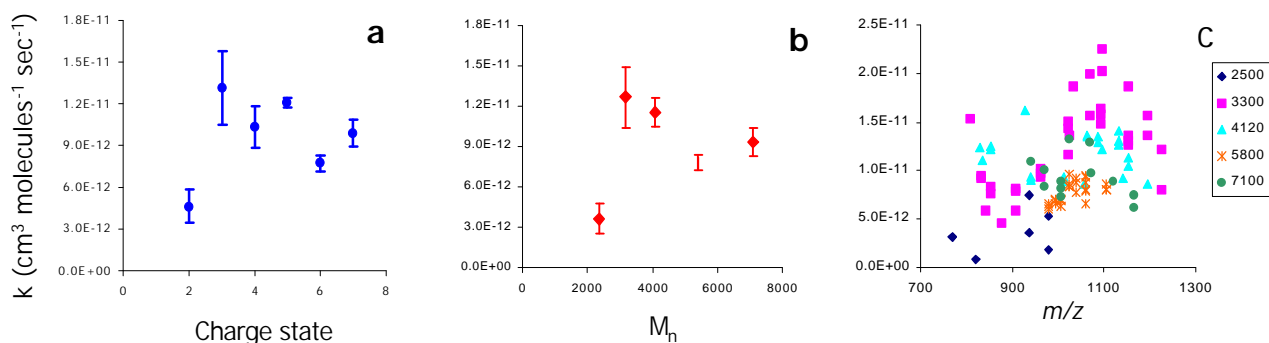


Figure 3. Compilation of PEG data showing k vs. a) charge state, b) M_n , and c) m/z ratio

Work done with H^+ abstraction from proteins has demonstrated that protein ions with more H^+ react faster in analogous ion/molecule reactions.⁵⁻⁷ The number of H^+ present on the protein affects the structure of the ion and is a function of the amino acid sequence. As the protein charge state decreases the relative basicity of neighboring residues may change in response. This would affect the rate at which subsequent H^+ were removed. Rate constants measured for the abstraction of Na^+ from PEG are 2-3 orders of magnitude slower than those measured for the above-mentioned H^+ abstraction experiments. Because polymers have a much more regular structure, the affinity for Na^+ is not likely to differ greatly from site to site or with subsequent charge removal. Even at the highest charge states seen in this study, the Na^+ ions can still be separated by 20-25 monomer units. The Na^+ are also strongly ligated to the polymer (multiple ether oxygens), making their removal by the crown ether more difficult.

Conclusion: Reactions of crown ether with polymer ions can greatly simplify electrospray data, leading to more reliable calculations of M_n and MWD. Previous work with H^+ abstraction from proteins has shown that the rate of abstraction is correlated with the charge state of the ions. We have not seen such a correlation between rate of Na^+ abstraction and charge state of the polymer ions. It is likely that the affinity of the polymer for Na^+ does not change significantly with respect to charge state due to the homogenous structure of the polymer.

References:

1. Bahr, U.; Deppe, A.; Karas, M.; Hillenkamp, F. *Anal. Chem.* **1992**, *64*, 2866
2. Montaudo, G.; Montaudo, M. S.; Puglisi, C.; Samperi, F. *Macromolecules* **1995**, *28*, 4562
3. McEwen, C.N.; Jackson, C.; Larsen, B.S. *Int. J. Mass Spectrom. Ion Proc.* **1997**, *160*, 387
4. Lennon, J.L.; Cole, S.P.; Glish, G.L. *Proceedings of the 45th ASMS Conference*, **1997**, 1271
5. McLuckey, S.A.; Van Berkel, G.J.; and Glish, G.L. *J. Am. Chem. Soc.* **1990**, *112*, 5669
6. Cassady, C.J.; Wronka, J.; Kruppa, G.H.; and Laukien, F.H. *Rapid Commun. Mass Spectrom.* **1994**, *8*, 394
7. Williams, E.R. *J. Mass Spectrom.* **1996**, *31*, 831