# Near-Monodisperse Sodium Polymethacrylates: Characterization by Linear Salt Gradient Anion-Exchange Chromatography

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## Abstract

A family of six near-monodisperse homopolymers of sodium methacrylate ($M_n$ = 1100, 3200, 5500, 7200, 14100, and 21000) is characterized by linear salt gradient anion-exchange chromatography. Although the retention times depend on the initial and final salt concentrations of the gradient, they are almost independent of the molecular weight of poly(sodium methacrylate). This suggests that anion-exchange chromatography is incapable of resolving mixtures of a given polyelectrolyte to their components of various molecular weights, and it is therefore impossible to identify the polydispersity of such a sample using this method. The independence of the retention times from molecular weight is also predicted by a theory based on stoichiometric mass-action ion-exchange. Using this theory and our experimental retention times, the equilibrium anion-exchange constant and the corresponding Gibbs free energy of anion-exchange of the monomer repeat unit are calculated to be around 2.1 and –1.8 kJ/mol, respectively.

## Introduction

Ion-exchange displacement chromatography is a powerful separation technique possessing several advantages over the conventional isocratic, linear gradient, or step gradient techniques, including higher column throughputs and concentrated products (1–3). The distinguishing feature of the process is the use of the displacer, an ionic compound whose affinity for the stationary phase must be higher than that of all components of the mixture to be separated. Polyelectrolytes have the required high affinity (4) and have been used in several instances to separate mixtures of ionic compounds, most notably proteins (5,6). Detailed characterization of the ion-exchange affinity of such polyelectrolytes is necessary to elucidate the critical structural features governing

their adsorption. To this end, the study of a series of polyelectrolytes covering a range of molecular weights but with a narrow molecular weight distribution in each sample (near-monodisperse polymers) would be most appropriate. Such samples are not readily available due to the synthetic difficulties associated with their preparation. Improvements in "living" polymerization techniques (7,8) and use of "protecting" groups (9) have enabled the preparation of such a series of polyelectrolytes, in our case poly(methacrylic acid)s, whose characterization is undertaken in this study. The chemical formula of the dissociated form of the methacrylic acid repeat unit is shown in Figure 1.

Characterization using isocratic ion-exchange chromatography techniques can be used to measure the characteristic charge ($\nu$, number of points of contact of an adsorbate with the chromatographic surface) and the Gibbs free energy of ion exchange ($\Delta G^{exchange}$) of low-molecular-weight ionic compounds (10–11). However, the same techniques are not applicable to polyelectrolytes, whose retention times have a very strong dependence upon the mobile phase salt concentration, rendering the determination of $\nu$ and $\Delta G^{exchange}$ very inaccurate. Thus, in this work, an alternative technique, linear salt gradient ion-exchange chromatography, is employed for the characterization of near-monodisperse poly(methacrylic acid)s. More specifically, the retention times of a family of six homopolymers of sodium methacrylate are determined on a strong anion-exchange column using two different linear salt gradients. The results are discussed in connection with the possibility of using ion-exchange chromatography, on the one hand, to analyze the polydispersity of a homopolyelectrolyte sample, and on the other hand, to determine the $\Delta G^{exchange}$ of such a sample.

## Experimental

The polymers, prepared by the anionic polymerization of *tertiary*-butyl methacrylate and subsequent acid hydrolysis (12), were supplied by Polymer Source Inc. (Québec, Canada). The

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narrow size distribution of the samples and completion of hydrolysis were confirmed using gel permeation chromatography (GPC) and proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy, respectively. An analytical (100 × 5 mm) Protein-Pak Q 8HR Waters (Waterford, Herts, U.K.) strong anion-exchange column packed with 8-μm quaternary methylamine-derivatized beads of 100-nm average pore size was used for the experiments. A Polymer Laboratories (Church Stretton, Shropshire, U.K.) LC1150 quaternary pump was used to deliver the solvent at a flow rate of 0.5 mL/min. A Polymer Laboratories LC1200 ultraviolet–visible (UV–vis) detector was used to monitor the column effluent. Sample volumes of 20 μL were introduced using a 7125 Rheodyne (Rohnert Park, CA) injector. The absorbance was monitored at 230 nm. Experiments were performed at pH 8.0 (Tris buffer) using two different linear salt gradients: one from 0.1 to 1.0M NaCl (to be abbreviated as 90:10) and the other from 0.25 to 1.0M NaCl (75:25).

## Results and Discussion

All experiments of this study were performed at pH 8, where the methacrylic acid repeat units in the polymers are fully dissoci-
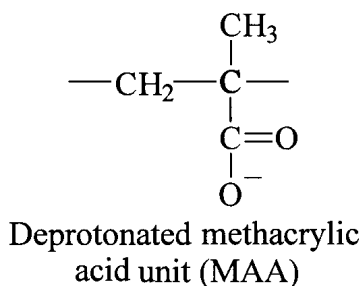


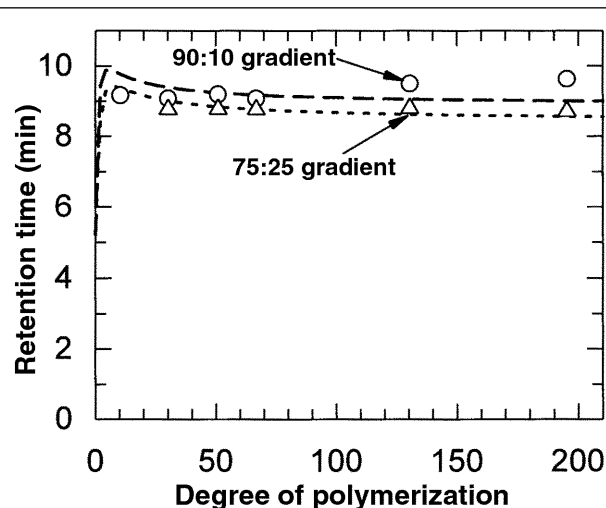**Figure 1.** Chemical formula of the dissociated methacrylic acid repeat unit.



**Figure 2.** Dependence of the linear salt gradient retention times on the degree of polymerization of PSMAs under two different gradients: 0.1–1.0M NaCl and 0.25–1.0M NaCl. The points represent the experimental data and the lines the theory fits on the data.

ated, as shown in Figure 1. Note that although the dissociation constant (p$K$) of methacrylic acid (monomer) is 4.54 (13), the effective p$K$ of the methacrylic acid repeat unit in the polymer is 5.4. The retention times ($t_R$) of the samples under the two linear salt gradients are plotted against the degree of polymerization in Figure 2. This figure shows that the $t_R$ values of the poly(sodium methacrylate)s (PSMAs) depend only on the type of the salt gradient and are independent of the polymer molecular weight. The retention times of the PSMAs under the 75:25 gradient are the same for all the homopolymers and slightly shorter than those of the 90:10 gradient. These results are in qualitative agreement with some refined (non-chromatographic) theories on polyelectrolyte adsorption (14–17) that predict the abrupt desorption of polyelectrolyte at some characteristic salt concentration (typically between 0.1 and 2M, depending on the details of the system).

The independence of the $t_R$ of the polymers upon their degree of polymerization is supported by a simple theoretical chromatographic model put forward recently by Li et al. (11). This equation was developed by considering that each surface site is neutralized either by a polyelectrolyte unit (methacrylate anion) or by a salt anion (chloride) (18,19). A mass-action formalism was also used to express the equilibrium constant of the ion-exchange process. Moreover, the small amount of analyte and the presence of a delay time ($t_d$) in the gradient development were also taken into account. Thus, the dependence of $t_R$ is given as follows:

$$t_R = t_0 + t_d + \frac{t_0}{G}\left\{\left[(\nu+1)G\left(\kappa^\nu\,\beta^\nu\,\Lambda^\nu\,\frac{t_d}{t_0}\,C_0^\nu\right) + C_0^{\nu+1}\right]^{\frac{1}{\nu+1}} - C_0\right\} \quad \text{Eq. 1}$$

where $t_0$ is the time corresponding to the column's dead volume, $\kappa$ is the equilibrium ion-exchange constant of the repeat unit, $\nu$ is the polymer's characteristic (effective) charge, $\beta$ is the column phase ratio, $\Lambda$ is the column capacity in monovalent ions, $C_0$ is the initial salt concentration in the gradient, and $G$ is the gradient's slope, given by:

$$G = \frac{C_F - C_0}{t_G/t_0} \quad \text{Eq. 2}$$

where $C_F$ is the final salt concentration in the gradient, and $t_G$ is the duration of the salt gradient.

For large values of $\nu$, Equation 1 acquires the following asymptotic form, which is independent of $\nu$:

$$t_R = t_0 + t_d + \frac{t_0}{G}(\kappa\beta\Lambda - C_0) \quad \text{Eq. 3}$$

Assuming that the characteristic charge is equal to the degree of polymerization, Equation 1 was regressed with the data of Figure 2, from which $\kappa$ was calculated for each of the two gradients. The two curves shown in Figure 2 represent Equation 1 with the best-fit values for $\kappa$. These values, along with their 95% confidence intervals, are $\kappa_{90:10} = 2.00 \pm 0.16$ and $\kappa_{75:25} = 2.16 \pm 0.06$.

The two values are similar, and their confidence intervals overlap, supporting the validity of the theory used. From these $\kappa$ values, we can calculate the corresponding Gibbs free energies of exchange of the monomer repeat unit ($\Delta g^{exchange}$) from the following equation:

$$\Delta g^{exchange} = -RT \ln \kappa \quad \text{Eq. 4}$$

where $R$ is the gas constant and $T$ the absolute temperature. At 298 K, the calculation gives $\Delta g_{90:10}^{exchange} = -1.72$ kJ/mol and $\Delta g_{75:25}^{exchange} = -1.91$ kJ/mol. These values are comparable with the value of $-1.46$ kJ/mol calculated for the ion-exchange of the carboxylic acid groups of benzene oligocarboxylic acids (11).

Our finding of the independence of polyelectrolyte ion-exchange retention time on molecular weight has two important implications. First, it suggests that the ability of linear salt gradient ion-exchange chromatography to analyze the molecular weight distribution of polyelectrolytes would be limited. For example, a homopolyelectrolyte sample with five components of different molecular weights but the same type of monomer repeat units (e.g., all sodium methacrylates) would elute as a single (narrow) peak. The same would be true for a polyelectrolyte sample with a broad molecular weight (but still unimodal) distribution. Second, it suggests that the equilibrium constant of the monomer repeat units can be readily calculated from the retention times of two or three polymers of sufficiently high molecular weight using Equation 1 (or more simply, Equation 3). This constitutes an important tool for the determination of the ion-exchange affinity parameters of large polymers for which isocratic chromatography experiments are not offered, in this case, because of the very strong dependence of the retention time upon salt concentration (18).

In agreement with our finding, Glöckner (20–22) has also observed a slight dependence of homopolymer retention time on molecular weight in reversed- and normal-phase solvent (as opposed to salt) gradient elution. Glöckner proposed the following empirical relationship for his data:

$$\phi_{NS} = A - B\ (MW)^{-1/2} \qquad \text{Eq. 5}$$

where $\phi_{NS}$ is the volume fraction of the non-solvent in the gradient at which the polymer elutes, MW is the polymer molecular weight, and $A$ and $B$ are constants. Equation 5 is qualitatively similar to our Equation 1, because it is an increasing function of the molecular weight in a "concave down" fashion. However, unlike Equation 5, Equation 1 contains parameters, each one of which has a physical significance.

## Conclusion

The retention times of a series of near-monodisperse sodium polymethacrylates have been measured on an anion-exchange column under linear salt gradient conditions, and they were found to be independent of polymer molecular weight but dependent on the initial salt concentration of the gradient. This initially unexpected result is in agreement with the results of a theoretical model that correlates solute retention to the characteristics of the column and the gradient, as well as to the affinity parameters of the solute itself. On the negative side, this finding implies that linear salt gradient ion-exchange chromatographic analysis is a poor method for identifying size heterogeneities in polyelectrolyte samples. On the positive side, the limiting (at high molecular weight) retention times of polyelectrolytes in a linear salt gradient can lend themselves to the accurate determination of the monomer repeat unit ion-exchange constant, usually inaccessible by standard isocratic elution techniques.

## Acknowledgments

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