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Effect of a soluble ionic polymer on the separation of anions by capillary electrophoresis

James S. Fritz[a,*], Steven A. Steiner[b]

[a]*Ames Laboratory, US Department of Energy and Department of Chemistry, Iowa State University, 332 Wilhelm Hall, Ames, IA 50011, USA*

[b]*Department of Chemistry and Engineering Physics, University of Wisconsin-Platteville, Platteville, WI 53818, USA*

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Abstract

A silica capillary for CE analysis of anions can be conditioned with NaOH, rinsed with water, coated with a cationic polymer, and equilibrated with the background electrolyte in only 2 min for each of the four steps. The coated surface has a positive charge that gives a substantial anodic electroosmotic flow (EOF) over the range of pH 2.5–12.0. The migration times of sample anions and a neutral marker (used for EOF calculations) are generally reproducible to an RSD of 1% or better, both for successive runs on a single capillary and from capillary to capillary. It was shown that the type of buffer used affects the EOF of a coated capillary. A concentration of 100 m*M* or higher sodium chloride minimizes differences in EOF with different buffers and also gives sharper peaks for sample anions.

Keywords: Coated capillaries; Inorganic anions; Organic acids

1. Introduction

In terms of theoretical plates capillary electrophoresis (CE) has at least 10 times more separation power than ion chromatography. Separation of ions by CE is fast and it is usually easy to find suitable experimental conditions for a given sample. Unfortunately, analytical laboratories have been slow to adopt CE methods for practical ion analysis. A common criticism of capillary electrophoresis has been that migration times are not sufficiently reproducible. In some cases this could lead to errors in peak identification. In an effort to obtain better reproducibility it has become commonplace to rinse the fused-silica capillary very frequently with a base such as sodium hydroxide. Such rinses are time consuming and detract from the speed and resolution of CE compared to conventional liquid chromatography.

Problems with reproducibility stem mainly from difficulties in maintaining a clean reproducible inner surface on the capillaries used in CE. In particular, variations in the surface can affect the electroosmotic flow (EOF) and hence the analyte migration times. Cohen and Grushka [1] found that EOF changed perceptibly from one run to another, especially during the first few runs.

An additional complication arises if anions are to be separated. Anion separations are apt to be very slow unless a flow-reversal reagent is added to coat

*Corresponding author. Tel.: +1-515-2945-987; fax: +1-515-2943-578.

E-mail address: kniss@ameslab.gov (J.S. Fritz).

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the capillary walls and reverse the direction of EOF. Most commonly a surfactant such as TTAB (tetradecyltrimethylammonium bromide) is added to the background electrolyte (BGE) to dynamically coat the walls to give a positive charge and thereby reverse the direction of EOF. But frequent rinsing of the silica capillary is still recommended and the reproducibility of sample ion migration times is not always satisfactory.

A very large amount of work has been published on surface modification of capillaries to be used for separation of proteins and other large biomolecules. For example, application of a capillary coating with ionic positive charges inhibits interaction of positively-charged proteins with the capillary surface and greatly improves the quality of the separation. Dynamic modification of the capillary surface by neutral or charged species has been reviewed by Corradini [2]. Dynamic coating of a silica capillary with a double-chained surfactant instead of a surfactant with a single chain gave a much more stable EOF and improved reproducibility [3]. Irreversible surface modification with neutral [4] or charged [5–7] coatings has also been used extensively. A drawback here is that multiple time-consuming steps are often required to form chemical bonds. Also, reactions at surfaces may not be as complete as they are in solution.

The coating of a fused-silica capillary with a water-soluble polymer is a simple procedure and has recently been shown to provide a positively-charged surface for separation of proteins [8–13]. Katayama et al. [11,12] and also Graul and Schlenoff [13] showed that capillaries modified with alternate surface layers of positively- and negatively-charged polymers are particularly effective for separation of proteins and other bioorganic substances. Krokhin and coworkers used capillaries dynamically coated with various polymers for CE separation of metal–cyclohexane-1,2-diamine tetraacetic acid (CDTA) complexes [14] and for inorganic anions [15].

Procedures for coating with polymers have been rather slow and involved. Wang and Dubin [10] employed an alternating high pressure (5 min) and low pressure (120 min) coating and conditioning procedure for a total of 16 h plus a 40-min equilibration with buffer. Graul and Schlenoff [13] used a conditioning and coating procedure that required 1 h for the first coating layer plus an additional 10 min for each additional coating layer. The successive-coating protocol used by Katayama et al. required ca. 2.5 h for preparation of a capillary with a negatively-charged outer layer [11] and ca. 1.5 h when the final layer had a positive charge [12].

We now describe a method for coating of a silica capillary with high-molecular-mass poly(diallyldimethylammonium chloride) (PDDAC). The PDDAC is a linear saturated positive polyelectrolyte polymer that is transparent throughout much of the UV–Vis position of the spectrum, thus it does not hinder detection in this region. It is highly water soluble but also adheres extremely well to silica surfaces due to the electrostatic attraction in between the ammonium groups of the polymer and the silanol groups on the fused-silica surface. The total time required for capillary pretreatment, coating and equilibration with buffer is only ca. 10 min. Capillaries prepared in this manner have an anodic EOF and can be used for separations between pH 2.5 and 12.0. Migration times for inorganic and organic sample ions are very reproducible and little or no treatment is required between runs.

2. Experimental

All inorganic acids, bases and salts were obtained from existing laboratory stock or purchased from Fisher Scientific (Fairlawn, NJ, USA) and were of reagent grade quality or better. The PDDAC, mesityl oxide and the organic acids were purchased from Aldrich (Milwaukee, WI, USA) and were used as received. All solutions were prepared in purified 18.2 MΩ water from a Barnsted nanopure II water purification system (Barnsted Thermolyne, Dubuque, IA, USA). Analyte solutions were typically made up in purified water in a concentration range of 1000–5000 ppm and were diluted by a factor of ten in the BGE for the injected solutions. BGEs were made in purified water by combining 25–200 m*M* NaCl as an electrolyte with acetate, borate or phosphate sodium salts added to form a buffer and the pH of the buffer is adjusted with 1.0 *M* NaOH or 1.0 *M* HCl using a Corning 440 pH meter (Corning, NY, USA).

A Waters Quanta 4000 capillary electrophoresis system (Waters, Milford, MA, USA) was used with a

negative power supply installed. Polyimide coated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were either 40 or 50 cm in length with effective lengths to the detector being 32.5 cm or 42.5 cm, respectively. All capillaries were 50 μm inside diameter and coated with a single layer of PDDAC using the conditions outlined below. Direct UV detection at 214 nm and hydrodynamic injection for 5 s at 10 cm height were used for all separations. Electropherograms were sampled at a rate of 10 points/s by the Chromperfect data acquisition system (Justice Innovations, Mountain View, CA, USA).

3. Results and discussion

3.1. Coating conditions

A wide variety of coating and conditioning procedures were investigated. The goal was to obtain reproducible EOF and migration times for sample anions using a protocol that was as fast and convenient as possible. The following procedure was selected as the most favorable:

(1) A 0.1 *M* NaOH solution was pulled through a new capillary using a −20 p.s.i. vacuum for 2 min (1 p.s.i.=6894.76 Pa). The purpose of this step was to hydrolyze siloxane groups to silanols and to ensure a clean surface on the capillary.

(2) Deionized water was pulled through the capillary under vacuum for 2 min under the same flow conditions.

(3) The capillary was coated by pulling a 0.5% aqueous solution of PDDAC through the capillary under vacuum for 2 min at the same flow-rate as in previous steps.

(4) The BGE solution was pulled through the capillary under vacuum for 2 min with an applied potential of −15 kV.

After each of the first three steps a 15–20 s air purge was applied to the capillary while switching solution vials. This effectively removed most of the previous solution. The application of −15 kV during step 4 resulted in an almost immediate flat baseline for the first electropherogram. When no voltage was applied during step 4, 10 min or more was needed to establish a stable baseline for the first electropherogram. It seems likely that the initial applied voltage induces a conditioning of the polymer that may involve a structural rearrangement and tightening of the polymer layer. If the voltage is first applied during an electropherogram rather than in step 4 of the pretreatment, there is a drift in signal (increased absorbance) as the conditioning process takes place during the run.

The quality of anion separations obtainable with a polymer coated capillary is illustrated by the electropherogram of five inorganic anions, shown on an expanded scale in Fig. 1. We calculated actual plate numbers ranging from 89 000 to 117 000 for the various peaks with an average $N=100\,500$. The peaks were remarkably symmetric as evidenced by asymmetry factors ranging from 0.96 to 1.07.

When a small cationic surfactant is used to reverse the direction of EOF, it is customary to have surfactant in the BGE to maintain equilibrium between surface bound and unbound solution surfactant on the capillary surface. This introduces the possibility of sample anion interaction with the surfactant, which could in some cases even result in precipitation. With a polymer-coated capillary the positively charged PDDAC is entirely on the surface but there is still the possibility of reaction with sample anions at the surface. Larger anions, such as organic acids in

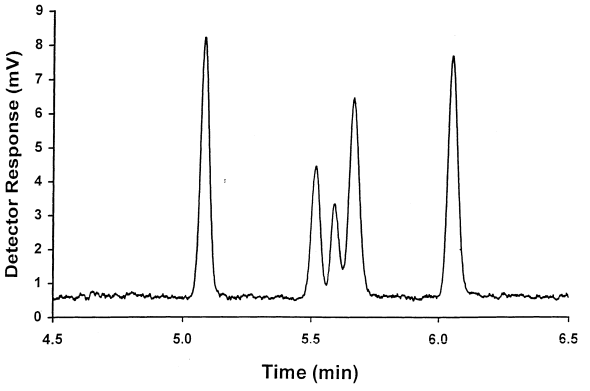

Fig. 1. Five inorganic anion mixture separated using a 50 cm PDDAC coated column with a BGE containing 200 m*M* NaCl plus 20 m*M* borate buffer at a pH of 8.5. The injection of the anion mixture was hydrodynamic for 5 s followed by separation at an applied voltage of −10 kV and detection using UV absorption at 214 nm. The injected solution concentrations ranged from 100 to 500 ppm for the five anions with the following migration order: I^-, CrO_4^{2-}, NO_3^-, SCN^-, MoO_4^{2-}.

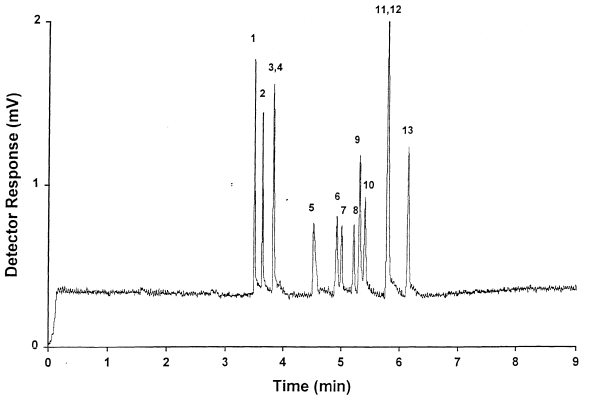

Fig. 2. Thirteen organic acid mixture separation using a 50 cm PDDAC coated column with a BGE containing 50 m*M* NaCl plus 25 m*M* borate buffer at a pH of 9.0. The injection of the acid mixture was hydrodynamic for 5 s followed by separation at an applied voltage of −15 kV and detection using UV absorption at 214 nm. The injected solution concentrations for the acids ranged from 500 to 100 ppm. Peak identification: 1=1,2,4-benzenetricarboxylic acid, 2=1,2,3-benzenetricarboxylic acid, 3=terephthalic acid, 4=isophthalic acid, 5=4-hydroxybenzoic acid, 6=benzoic acid, 7=*o*-chlorobenzoic acid, 8=*p*-anisic acid, 9=2,4-dichlorobenzoic acid, 10=3-hydroxybenzoic acid, 11=4-methoxycinnamic acid, 12=3-methoxycinnamic acid, 13=3,5-dimethoxycinnamic acid. Note: peaks 3+4 and 11+12 are merged.

Fig. 2, show a small tailing hump at very low concentrations, indicating that some interaction may occur at the coated surface. However, inorganic and smaller organic anions give symmetric peaks with little tailing. A higher concentration of a salt in the BGE, greater than 100 m*M* or 150 m*M* for example, serves to repress any surface interaction of sample anions.

The effect of several variations in the pretreatment of capillaries was studied by measuring the migration time of a neutral marker and calculating the electroosmotic mobility. Mesityl oxide was used as the neutral marker although several experiments showed that dimethylformamide gave the same results. Initial treatment of a new capillary with 0.1 *M* sodium hydroxide gave the same electroosmotic mobility (after coating and conditioning) for treatment periods of 2, 5, 10 and 20 min. The average μ_{eo} was $4.40 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} with an RSD of 0.91%. However, when there was no treatment of the capillary with sodium hydroxide before coating, the μ_{eo} decreased to $3.84 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}. The main purpose of pretreatment with sodium hydroxide would be to hydrolyze siloxane groups to silanols. It is common to treat a capillary with NaOH for 1 h or more, but excessive exposure can lead to a rough, pitted surface [16].

Insertion of a 2 min rinse with 1 *M* HCl in the treatment protocol just after the 2-min NaOH rinse gave essentially the same μ_{eo} as before; $4.39 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} with an RSD of 0.89%. This implies that the polymer adheres to the capillary surface equally well when the silanol groups are not ionized (acidic conditions) as when they are ionized (basic conditions).

Previous investigators have recommended the use of 0.5 *M* or higher salt when coating a silica capillary with an ionic polymer [9,10]. Use of a 2-min rinse with aqueous 1 *M* NaCl instead of water alone in step 2 of our protocol reduced the anodic μ_{eo} from $4.40 \cdot 10^{-4}$ to $4.17 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}. Coating with a 0.5% solution of polymer in 1 *M* NaCl instead of pure water reduced the μ_{eo} from $4.40 \cdot 10^{-4}$ to $3.79 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} with an RSD of 0.22%. Thus the need for high ionic strength polymer solutions is not evident in our work.

3.2. Reproducibility

Reproducibility is a major issue in CE. The reproducibility of PDDAC-coated capillaries was tested by repeated injections of a sample containing iodide, nitrate, molybdate and naphthylenesulfonic acid (NSA) and mesityl oxide as a marker for measurement of EOF. The capillaries were first treated according to the 8-min protocol described above. Following this initial treatment, 25 samples were run with no treatment whatsoever between runs. The results gave migration times with an RSD of 1.1% or less for each of the sample anions but the RSD of the neutral marker was 2.5% with a continuous decrease in electroosmotic mobility (longer migration times) during the succession of runs. The experiment was then repeated under the same conditions except for a 1.0-min purge with BGE between each run (Table 1). This time the RSD of the neutral marker was 0.8% with no noticeable trend during the series of runs. The migration times of the sample anions were also very reproducible with RSD averaging around 1.0%.

Table 1
Repeatability of runs using a coated capillary with a 1.0-min purge with BGE between runs

Substance	Average t_M (min)	Range (min)	RSD (%)
Iodide	1.22	1.21–1.23	0.88
Molybdate	1.31	1.29–1.33	0.99
Nitrate	1.40	1.38–1.42	1.15
NSA	2.16	2.13–2.19	0.93
Mesityl oxide	4.07	4.05–4.12	0.78

Conditions: column 40 cm×50 μm I.D. coated with high-molecular-mass PDDAC. BGE: 50 m*M* NaCl, 25 m*M* phosphate buffer at pH 6.0. Applied potential was −15 kV with direct photometric detection at 214 nm.

Capillary to capillary reproducibility was also very good. Five new capillaries conditioned according to the 8-min protocol gave average migration times for the neutral marker with a RSD of ~1.0% Dimethylformamide gave the same migration time as mesityl oxide when used as the neutral marker.

3.3. Effect of pH and buffer type

Wang and Dubin [10] found the EOF of polymer-coated capillaries to be independent of pH only when a high-M_r polymer was used and a salt concentration of ca. 0.5 *M* was used during the coating process. Graul and Schlenoff [13] observed that capillary electrophoresis with no salt added polyelectrolytes during multilayer-coating showed positive outer layers but the electroosmotic mobility was not reproducible. Liu et al. [5,7] observed a small linear decrease in the EOF of PDDAC-coated capillaries between pH 2.0 and 5.5, followed by a larger decrease between pH 5.5 and 8.5. They attributed this change to the increased dissociation of the residual silanol groups at higher pH. We initially observed a somewhat similar dependence of EOF on pH of the BGE, but further investigation indicated that changes in the type of buffer influenced the EOF.

Changes in the type of buffer ion might affect the net surface charge by interacting with the positively charged polymer coating. For example, a phosphate buffer is converted gradually from $H_2PO_4^-$ to HPO_4^{2-} as the pH is increased. In our polymer-coated capillaries with a phosphate buffer the ratio of $H_2PO_4^-$:H_3PO_4 or the ratio of HPO_4^{2-}:$H_2PO_4^-$ increases at higher pH. This could cause stronger interaction of buffer anions with the PDDAC-coated surface. This would reduce the net positive charge on the inner Helmholz layer and thus decrease the anodic EOF.

The work of Solomon et al. [17] supports this hypothesis. They showed that different cations in a buffer can have a major effect on the EOF obtained with bare silica capillaries. There is a ''wall'' effect in which the buffer cations interact with the negatively charged silanol groups on the silica surface. The authors derived and tested a model for calculating EOF as a function of buffer ion and other factors.

We made five consecutive runs of a test mixture of four anions plus a neutral marker at pH 5.0 with each of four different buffers. A new capillary coated with PDDAC was used for each series of runs. Results are summarized in Table 2. The RSD of the sample anions as well as the neutral marker was generally <0.5% except for some of the runs in which a Tris or a phosphate buffer was used. These results demonstrate that the buffer type does have a significant effect on the EOF, and hence on the migration times of sample anions. In BGE solutions containing 25 m*M* buffer and 25 m*M* NaCl the electroosmotic mobility is 9.1% lower in phosphate buffer than in acetate buffer when both are at pH 5.

When the salt concentration in the BGE is increased to 100 m*M* NaCl, the EOF is much more

Table 2
Migration times of a neutral marker (mesityl oxide) at pH 5.0 as a function of buffer type and salt content of the BGE

Buffer	25 m*M* NaCl			100 m*M* NaCl		
	t_M (min)	RSD (%)	μ_{os}	t_M (min)	RSD (%)	μ_{os}
Acetate	3.38	0.26	$4.28\cdot10^{-4}$	3.04	0	$4.75\cdot10^{-4}$
Pyridine	3.46	0.16	$4.17\cdot10^{-4}$	3.10	0.42	$4.66\cdot10^{-4}$
Tris	3.65	0.31	$3.95\cdot10^{-4}$	3.05	2.62	$4.74\cdot10^{-4}$
Phosphate	3.71	0.45	$3.89\cdot10^{-4}$	3.20	1.30	$4.56\cdot10^{-4}$

constant. Now the difference in electroosmotic mobility between acetate and phosphate buffers is only 4.0%. The higher salt concentration competes with the buffer anions for sites near the positively charged polymer surface and minimizes differences between the various buffers.

A mixed buffer containing 10 m*M* each of acetate, borate and phosphate can be adjusted to the desired pH by addition of a little strong base or acid. This buffer was found to minimize differences in migration times with respect to pH. After a series of samples has been run at a given pH and it is desired to make a substantial change in pH for the next samples, we recommend repeating the 8-min protocol used for a new capillary. This gives excellent reproducibility of migration times. Repeated recoating of the capillary surface actually seems to improve the reproducibility of the analyte migration times.

3.4. Polymer molecular mass

The PDDAC polymer used in this work is available commercially in three molecular mass ranges: low M_r is 100 000–200 000, medium M_r is 200 000–350 000, and high M_r is 400 000–500 000. The EOF and migration times of sample ions showed almost no change in the acidic and moderately basic pH range when the capillary was coated with PDDAC in the low, medium or high M_r range. Although the high-M_r polymer appears to work somewhat better than the others at pH 12, any of the three molecular mass materials are satisfactory for coating CE capillaries in the range of pH 2–12.

3.5. Scope

Capillaries coated with PDDAC provide good separation of organic as well as inorganic anions. Fig. 2 shows an electropherogram for a sample containing 13 organic anions. Good resolution of the anions was obtained except for the position isomers terephthalic and isophthalic acid, and for 3- and 4-methoxycinnamic acid.

Separations of anions are practical with the pH of the BGE as high as 12. The separation of several carboxylate anions is shown in Fig. 3. Peaks for 1,2,4-benzenetricarboxylic acid and 4-hydroxybenzoic acid are sharper and have shorter migration times at pH 12 than at a lower pH owing to more complete ionization. Migration times of all peaks in Fig. 3 gave RSDs<1.0% for five successive runs. These experiments demonstrate that separations can be carried out conveniently at higher pH values than are normally used in CE.

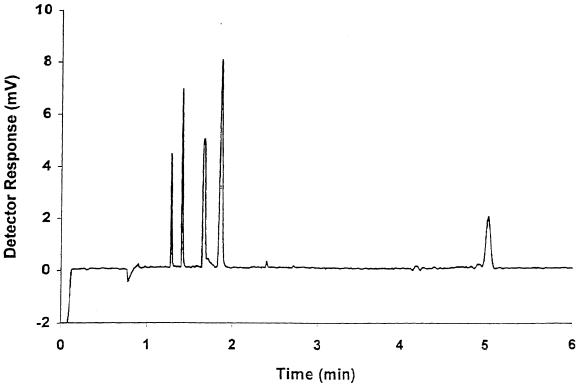

Fig. 3. Separation of inorganic, organic anions along with a neutral marker, using a 40 cm PDDAC coated column with a BGE containing 25 m*M* NaCl plus 25 m*M* phosphate buffer at a pH of 12.0. Hydrodynamic injection for 5 s, applied voltage of −15 kV, UV detection at 214 nm. The injected solution concentrations were approximately 500–1000 ppm with the following migration order: iodide, nitrate, 1,2,4-benzenetricarboxylic acid, 4-hydroxybenzoic acid and mesityl oxide.

Separation of large basic proteins has been demonstrated using CE with a PDDAC-coated column [7,9,13]. Fig. 4 shows the separation of di- and tripeptides using low-pH conditions. These conditions allow only partial protonation of the peptides and thus limit the counter migration. Even with slight counter migration the separation is achieved in under 8 min.

4. Conclusions

Anything that is inside the capillary used in CE has the possibility of affecting the inner surface by a dynamic equilibrium. This can result in changes in EOF and analyte migration times from one sample to another. Coating the capillary with a polyelectrolyte such as PDDAC is a quick and effective way to minimize changes in the capillary surface and provide excellent reproducibility in anion separations.

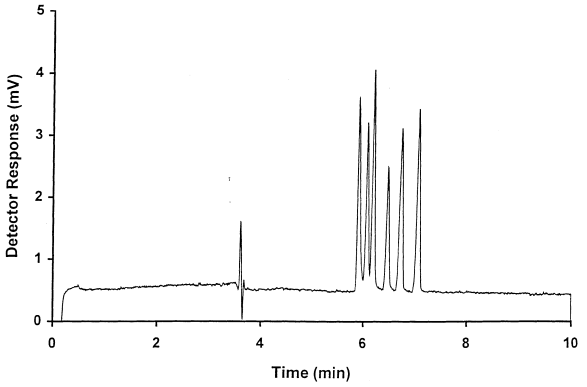

Fig. 4. Separation of a six peptide mixture using a 50 cm PDDAC coated capillary with a BGE composed of 100 m*M* NaCl plus a 25 m*M* phosphate buffer at pH of 2.5. Hydrodynamic injection for 5 s, applied voltage of −20 kV, UV detection at 214 nm. The injected solution concentrations were approximately 500 ppm for each of the following peptides in migration order: Phe–Gly, Phe–Leu, Phe–Met, Phe–Tyr, Phe–Val, Tyr–Gly–Gly.

The positive coating reverses the normal direction of electroosmotic flow and provides an almost constant EOF over the pH range of 2.5 to 12.0. Anionic analytes are generally separated with sharp, symmetric peaks.

It was shown that the type of pH buffer used can affect the analyte migration times, even on coated capillaries. The use of a relatively high salt concentration in the BGE was shown to improve both the sharpness and the reproducibility of anion peaks.

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