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# Gas-Phase Ion Association Provides Increased Selectivity and Sensitivity for Measuring Perchlorate by Mass Spectrometry

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## Abstract

Perchlorate ( $\text{ClO}_4^-$ ) competitively inhibits the uptake of iodide by the thyroid gland. Trace quantities of perchlorate are being increasingly detected in food and environmental samples. There is great concern that perchlorate contamination may be far more widespread than believed until now. Increasingly sensitive and unambiguous methods are needed for measuring perchlorate. We report here an ion chromatography–ion association–electrospray ionization–mass spectrometry (IC/IA-ESI-MS) method of substantially greater selectivity and sensitivity than other available single-stage MS approaches. A long chain dipositive cationic agent ( $\text{D}^{2+}$ ) is added postcolumn in low concentration. This ion associates with perchlorate, even in the gas phase. Perchlorate is, thus, detected as  $\text{DClO}_4^+$  in the positive ion mode at an  $m/z$  value between 300 and 400 (depending on the choice of  $\text{D}^{2+}$ ). This results in much better S/N and selectivity, as compared to detecting  $^{35}\text{ClO}_4^-$  at  $m/z$  99, where  $\text{HSO}_4^-$  also responds. We show results for various dicationic agents which vary in their selectivity and affinity for  $\text{ClO}_4^-$ , typically being at least 1 order of magnitude more selective for  $\text{ClO}_4^-$  over  $\text{HSO}_4^-$ . For a 100- $\mu\text{L}$  injected standard, limits of detection (LOD, S/N = 3) are as good as 25 ng/L on a single quadrupole mass spectrometer. Calibration for concentrations up to 100  $\mu\text{g/L}$  displays an  $r^2$  value of  $\geq 0.9993$ . We show applicability to various real samples. A number of the studied reagents are suitable for such applications.

## Keywords

Chromatographic analysis, Mass spectrometers, positive ions, dicationic agents, gas-phase ion association, perchlorate, thyroid glands, chlorine compounds, cation, iodine, electrospray mass spectrometry

## Disciplines

Biochemistry | Chemistry

## Comments

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# Gas-Phase Ion Association Provides Increased Selectivity and Sensitivity for Measuring Perchlorate by Mass Spectrometry

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Perchlorate ( $\text{ClO}_4^-$ ) competitively inhibits the uptake of iodide by the thyroid gland. Trace quantities of perchlorate are being increasingly detected in food and environmental samples. There is great concern that perchlorate contamination may be far more widespread than believed until now. Increasingly sensitive and unambiguous methods are needed for measuring perchlorate. We report here an ion chromatography–ion association–electrospray ionization–mass spectrometry (IC/IA-ESI-MS) method of substantially greater selectivity and sensitivity than other available single-stage MS approaches. A long chain dipositive cationic agent ( $\text{D}^{2+}$ ) is added postcolumn in low concentration. This ion associates with perchlorate, even in the gas phase. Perchlorate is, thus, detected as  $\text{DClO}_4^+$  in the positive ion mode at an  $m/z$  value between 300 and 400 (depending on the choice of  $\text{D}^{2+}$ ). This results in much better S/N and selectivity, as compared to detecting  $^{35}\text{ClO}_4^-$  at  $m/z$  99, where  $\text{H}^{34}\text{SO}_4^-$  also responds. We show results for various dicationic agents which vary in their selectivity and affinity for  $\text{ClO}_4^-$ , typically being at least 1 order of magnitude more selective for  $\text{ClO}_4^-$  over  $\text{HSO}_4^-$ . For a 100- $\mu\text{L}$  injected standard, limits of detection (LOD, S/N = 3) are as good as 25 ng/L on a single quadrupole mass spectrometer. Calibration for concentrations up to 100  $\mu\text{g/L}$  displays an  $r^2$  value of  $\geq 0.9993$ . We show applicability to various real samples. A number of the studied reagents are suitable for such applications.

Presently, there is great concern that the lower Colorado River is contaminated with significant levels of perchlorate.<sup>1</sup> The source of this contamination is believed to be ammonium perchlorate used as missile propellant. In March 2004, California became the first state to set a drinking water public health goal of 6  $\mu\text{g/L}$ ;<sup>2</sup> presently, there is intense debate on what regulatory limit should

be set.<sup>3</sup> Seven other states have advisory levels ranging from 1 to 18  $\mu\text{g/L}$ .<sup>4,5</sup>

Perchlorate competitively inhibits the uptake of iodide by the thyroid gland. Thyroid hormones are essential for metabolism and normal neurological development both in the fetus and the newborn. Perchlorate has been found in measurable quantities in milk,<sup>6–8</sup> broadleaf vegetables,<sup>9,10</sup> fruits such as cantaloupe,<sup>10</sup> and a variety of forage and edible crops.<sup>11</sup> Trace quantities of perchlorate have also been measured in rainwater.<sup>12</sup> We have recently reported that perchlorate was detectable in 36 of 36 human milk samples from 18 states, the average level being substantially higher than the average perchlorate level in dairy milk thus far reported, and equally important, the average level of iodide in the milk was much lower than that reported in the 1980s.<sup>8</sup> Perchlorate contamination might be a wider problem than previously thought. To detect low concentrations of perchlorate in environmental and biological samples, a sensitive and selective method is vital. In fact, we extensively used the presently described approach for the human milk study.

Determination of trace perchlorate in high-salinity water samples by preconcentration/preelution suppressed conductivity ion chromatography (PC/PE-IC) with the limit of detection (LOD) as low as 0.77  $\mu\text{g/L}$  has been previously reported.<sup>13</sup> However, IC

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does not provide unambiguous identification. In many real samples, we have observed high concentrations of species that elute in the same region as perchlorate, but mass spectrometry showed that no perchlorate was present in the eluate.

In principle, perchlorate can be measured without chromatography, by mass spectrometry alone.<sup>14</sup> In practice, some separation from the matrix is necessary in most real samples. This can be provided by solvent extraction after pairing with a suitable cationic reagent to form a neutral adduct. Several such ion-pairing/solvent-extraction electrospray ionization-mass spectrometry (ESI-MS) detection of perchlorate has been reported.<sup>15–17</sup> Similar extraction methods with spectrophotometric measurements have long been used<sup>18</sup> and are now being rediscovered as field screening methods.<sup>19</sup> ESI-MS/MS methods have also been developed for groundwater with an LOD of 0.5  $\mu\text{g/L}$ . However, because of matrix-dependent ionization suppression, standard addition must be used; in 12 of 16 samples analyzed, ESI-MS/MS results were higher than corresponding IC results.<sup>20</sup> The extant literature on the determination of various analytes in many environmental matrixes suggests that almost always chromatography–mass spectrometric methods are the most useful. For milk, both IC/ESI-MS<sup>8</sup> and IC/ESI-MS/MS<sup>10</sup> methods have been used. In the latter case,  $\text{ClO}_4^-$  is fragmented to  $\text{ClO}_3^-$ , albeit the LOD is not necessarily better. Others have been more successful with LC/ESI-MS/MS methods with an LOD of 0.05  $\mu\text{g/L}$  in real groundwater samples;<sup>21</sup> however, one must make extensive use of barium-loaded ion exchange cartridges to remove sulfate in high-sulfate samples. To avoid using off-line ion exchange cartridges, in-line diversion of matrix ions including chloride and sulfate to waste has been adopted for IC/ESI-MS and IC/ESI-MS/MS.<sup>22</sup> By using ion-exchange columns which selectively elute chloride, carbonate, and sulfate earlier than perchlorate, an in-line valve is used to divert the early-eluting ions to waste. The analytical stream is switched back into the mass spectrometer inlet  $\sim 2$  min before perchlorate elutes. This method is effective for matrix ion concentrations up to  $\sim 1000$  mg/L. By using  $^{18}\text{O}$ -labeled  $\text{ClO}_4^-$  as an internal standard, it is possible to confirm perchlorate elution and correct for ionization suppression. With state-of-the-art LC/MS/MS instrumentation, it is possible to achieve 10–25 ng/L LODs in samples ranging from water to urine.<sup>23,24</sup> It would be

desirable to develop comparably sensitive methods with simpler and more affordable LC/IC/ESI-MS instrumentation.

Compared to higher- $m/z$  ions, low- $m/z$  ions are often not as efficiently transferred by ESI due to mass discrimination.<sup>25</sup> Furthermore, background noise tends to be higher at low  $m/z$ . Most commercial single quadrupole mass spectrometers are designed to have their optimum performance at  $m/z$  values significantly higher than 100. One potential way to convert a low-mass analyte to a higher-mass measurand is to form an adduct with a reagent of appreciable mass. As previously stated, there are numerous methods in which perchlorate is extracted into an organic solvent as a neutral ion pair using a singly charged cationic reagent. However, this is not directly useful because an adduct that remains uncharged in the mass spectrometer will not be detectable. What is desired is an adduct that already is or produces a charged species at a low ionization/fragmentation voltage. Of potential reagents that will produce a charged adduct, it will be difficult to bind an anion by another anionic species. We know of no neutral ionophores that will bind perchlorate, although alkali metal complexes of neutral ionophores do bind perchlorate.<sup>26</sup> If a cationic reagent is used, it must be multiply charged so that one or more net positive charges remain. Any such reagent ion should ideally be dipositive to maximize the  $m/z$  value; namely, the reaction of interest should be

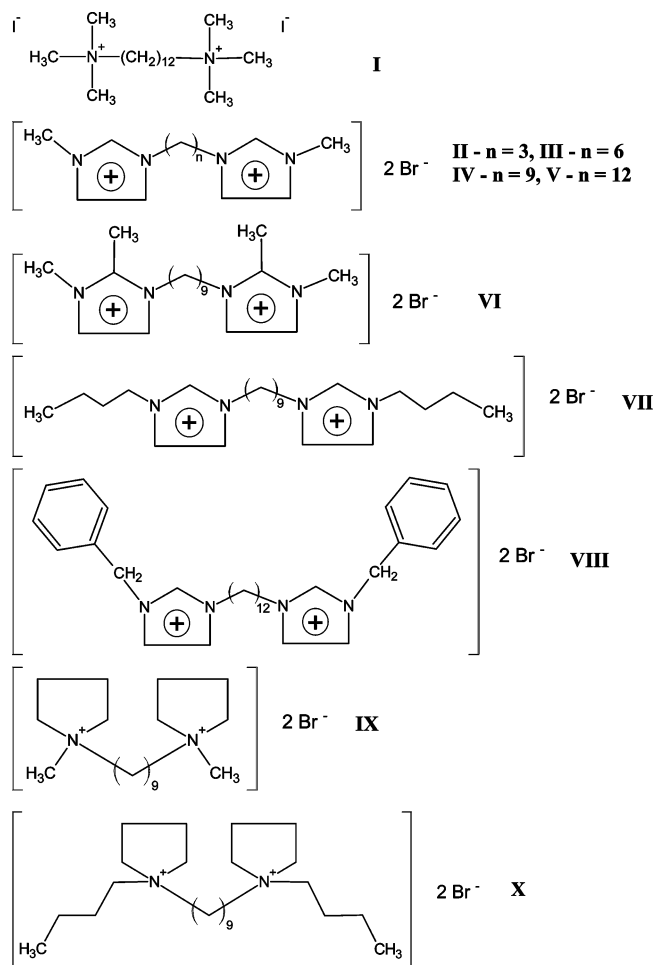


An optimum reagent ion should bind perchlorate with high affinity and selectivity. If such a positively charged perchlorate adduct can be efficiently generated, sensitivity benefits will also result: positive ions are, in general, more easily detected in ESI-MS.

ESI-MS has already proven to be a valuable tool for studying the equilibrium/association constants of host–guest complexes.<sup>27–30</sup> ESI-MS is a soft ionization technique so that the gas-phase mass spectra can be used to shed light on the nature of the species in solution.<sup>31,32</sup> Although the ion formation process might disturb the solution-phase equilibria,<sup>33</sup> in most cases, the relative ion abundances of the species observed in ESI-MS correlate well with equilibrium distribution of the corresponding species in solution.<sup>26</sup> Although from an analytical point of view in the present situation, we are concerned solely with affinities and selectivities in the gas phase, the gas-phase data can thus provide important insights into the behavior of these ions in solution. In this paper, we use ESI-MS to evaluate 10 dicationic reagents for the detection of perchlorate according to eq 1.

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**Figure 1.** Structures of different dicationic ion pair agents used in this study.

## EXPERIMENTAL SECTION

**Reagents.** The dicationic reagents tested are shown in Figure 1. Compound **I**, containing tetraalkylammonium end groups and a straight C-12 chain connecting them, 1,12-bis(trimethylammonium) dodecane diiodide, was synthesized from the  $\alpha,\omega$ -diamine and excess  $\text{CH}_3\text{I}$  according to the literature.<sup>34</sup> All the other dicationic reagents (**II–X**) consisted of substituted imidazolium or pyrrolidinium end groups, also joined by straight hydrocarbon chains (C3–C12) and synthesized as the bromide.<sup>35</sup>

To maximize the production of the dication  $\text{D}^{2+}$  in the mass spectrometer from the dihalide, the bromide or iodide salt was converted to the fluoride form  $\text{DF}_2$  by anion exchange. Four milliliters of a strongly basic anion-exchange resin (Amberlite IRA-400) in the chloride form was packed in a 10-mL disposable syringe. The resin was converted to the hydroxide form by passing 10 column volumes of 1 M NaOH and then washed thoroughly with water. The resin was next converted to the fluoride form by passing 7 column volumes of 0.5 M NaF, followed by washing with 10 column volumes of water. One milliliter of a 0.1 M solution of the dicationic compound was passed through the resin bed and collected in a 10-mL volumetric flask. The bed was washed further

with water, and the washings were collected in the flask to make a 10 mM stock solution.

**Ion Chromatography (IC).** Except as noted, ion chromatography with suppressed conductivity detection was carried out on a Dionex DX-600 ion chromatograph with a GS50 gradient pump, an ASRS-Ultra 4-mm suppressor, and a CD25 conductivity detector. Columns used were 4 mm in bore except as noted. PeakNet V6.2 was used for system control, and area-based analyte quantification (external standard mode, 5-point calibration) was used. The preelution/preconcentration (PC/PE) IC method<sup>13</sup> was used to separate the perchlorate. For PC/PE, a TAC-LP1 anion preconcentrator column was used for sample loading. Samples (1 mL) were loaded onto the preconcentrator column and preeluted with 2.0 mL of 10 mM NaOH and then switched to the main separation column (Dionex IonPac AG16/AS16, 75 mM NaOH eluent @ 1 mL/min). Note that electrogenerated eluent, which would have lowered detector background and improved overall performance, was *not* used in this configuration for purposes of economy.

**ESI-MS.** Except as stated, mass spectrometric studies were performed using a ThermoQuest Finnigan AQA single quadrupole mass spectrometer in the positive ion mode with Xcalibur (version 1.1) used for acquiring the MS data. The electrospray voltage and desolvation temperature were maintained at  $-3.0$  kV and  $350$  °C, respectively. The source (ionization) voltage used was  $+2$  V.

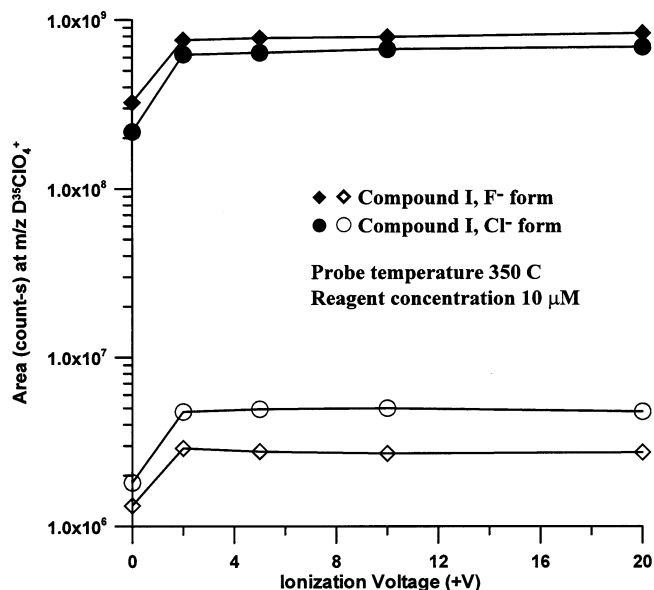
For direct introduction into the MS, solutions containing different concentrations of perchlorate and the dicationic reagent were prepared, and a  $100\text{-}\mu\text{L}$  aliquot was injected into the system using a six-port valve and air as carrier. For postcolumn reagent (PCR) addition, the conductivity detector effluent was split at a tee. Using a restrictor,  $0.4$  mL/min was allowed to go to waste. The dicationic reagent ( $100\ \mu\text{M}$  in water) was introduced at a second tee into the  $0.6$  mL/min stream at a flow rate of  $0.06$  mL/min and flowed through a  $0.8$  mm i.d.  $\times$   $1.15$  m mixing coil before entry into the MS. Direct testing of interference from sulfate and other anions was conducted in a 2-line flow injection manifold that simulated the chromatographic system in its flow scheme. Samples containing perchlorate or sulfate or other ions were injected into a water carrier ( $0.6$  mL/min) and flowed through a cation-exchange resin bed in  $\text{H}^+$  form (that acts as a suppressor) and merged with a flow stream of  $100\ \mu\text{M}$   $\text{DF}_2$  ( $0.06$  mL/min) before proceeding through a mixing coil into the mass spectrometer.

## RESULTS AND DISCUSSION

**System Optimization.** Initial system optimization was carried out with **I**, and a perchlorate concentration of  $25\ \mu\text{g/L}$  was used. A probe temperature of  $350$  °C was chosen on this basis. The thermal stability of most of the dicationic compounds (**II–X**) studied here was previously reported to be very high;<sup>35</sup> no significant thermal decomposition of any of these compounds is therefore expected within the short residence time in the desolvation zone. The best S/N was reached with a relatively low reagent concentration of  $10\ \mu\text{M}$  in the final mixture. Figure 2 shows the behavior of  $\text{DCl}_2$  and  $\text{DF}_2$  as a function of the fragmentation voltage.  $\text{DF}_2$  gives a better signal-to-blank ratio with a  $25\ \mu\text{g/L}$  perchlorate sample producing a signal 2.5 orders of magnitude greater than the blank. It will be observed that an ionization voltage of  $+2$  V is sufficient to produce the optimum

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**Figure 2.** Blank and 25  $\mu\text{g/L}$  signal response with 10  $\mu\text{M}$  I as PCR as a function of ionization voltage. Note logarithmic ordinate.

signal level, because  $\text{D}^{2+}$  is intrinsically charged. A low ionization/fragmentation voltage also reduces unwanted fragmentation of adventitious large MW species, thus reducing detector background counts at low  $m/z$  values.

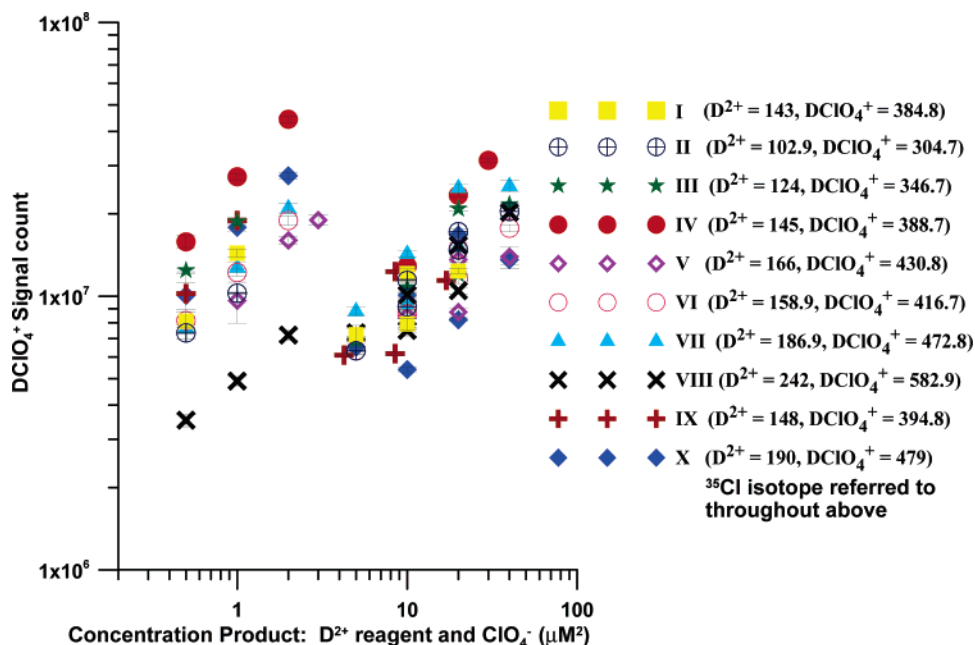
**Affinity for Perchlorate.** For all of the compounds tested, the propensity for forming  $\text{DClO}_4^+$  was very high. An exact measurement of gas-phase equilibrium constants for the process  $\text{D}^{2+} + \text{ClO}_4^- \leftrightarrow \text{DClO}_4^+$  is difficult because detector response for the different species can vary considerably. Nevertheless, let us consider the following data. The injection of 0.1 mL of 10 nM (1  $\mu\text{g/L}$ )  $\text{ClO}_4^-$  produced an area count of  $4.5 \times 10^4$  for  $^{35}\text{ClO}_4^-$ . In the presence of 7.9  $\mu\text{M}$  introduced  $\text{DF}_2$  (compound I), the  $^{35}\text{ClO}_4^-$  area count decreases to  $1.6 \times 10^4$  and the  $\text{D}^{35}\text{ClO}_4^+$  response provides an area count of  $2.25 \times 10^5$ . If we interpret this as if all

**Table 1. Relative Signal Intensities of Different Dicationic Reagents**

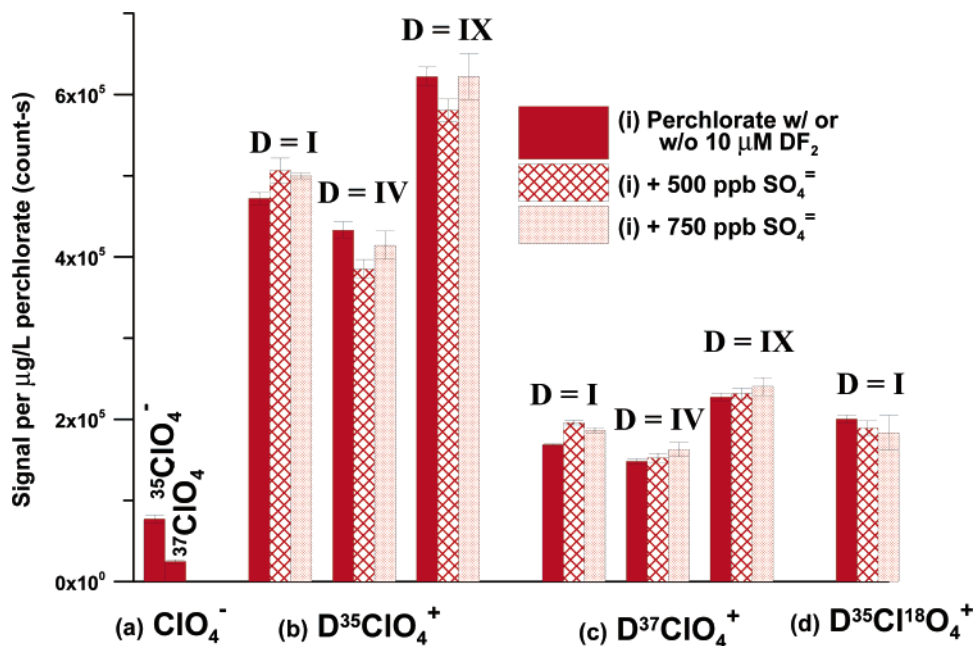
dication	$\text{D}^{2+}$ signal intensity for 1 $\mu\text{M}$ $\text{DF}_2$ ( $10^9$ count-s)	slope	
		$[\text{DF}_2][\text{ClO}_4^-]$ product, $\mu\text{M}^2$	product, $\mu\text{M}^2$
I	1.67	0.4–3	0.39
II	0.39	0.48	0.72
III	0.34 <sub>5</sub>	0.59	0.8
IV	0.89	0.74	0.82
V	0.70 <sub>5</sub>	0.63	0.69
VI	0.86 <sub>5</sub>	0.61	0.62
VII	1.1	0.72 <sub>5</sub>	0.75
VIII	1.18	0.52	0.53
IX	0.35	0.89	1.02
X	0.65	0.73	0.68

the processes are taking place in the liquid phase, on the basis of the eluate peak base width, we estimate that the average concentration of  $^{35}\text{ClO}_4^-$  prior to the introduction of I is 2.1 nM. This decreases to 0.75 nM upon introduction of the dication, and we can assume that the balance 1.35 nM (64% of the total) is converted to  $\text{D}^{35}\text{ClO}_4^+$ . This is notable given the low concentration of  $\text{DF}_2$  introduced (which almost certainly does not quantitatively produce  $\text{D}^{2+}$ ). It is also apparent that the perchlorate adduct ion produces nearly an order of magnitude greater signal than the original perchlorate; this greatly increases detection sensitivity.

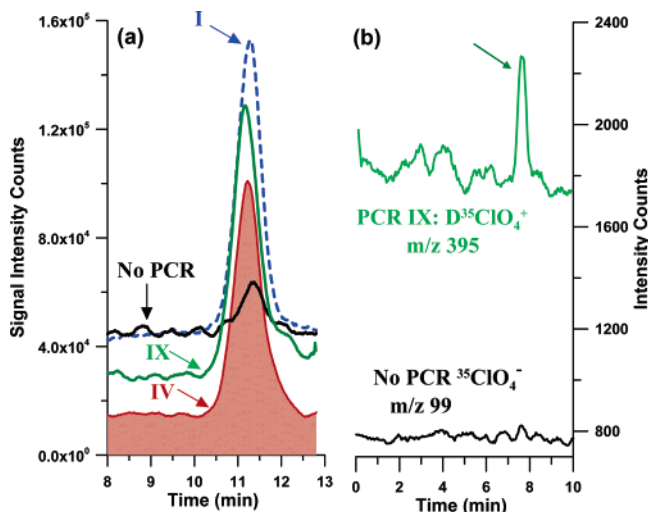
The intensities for  $\text{D}^{2+}$ ,  $^{35}\text{ClO}_4^-$ , and  $\text{D}^{35}\text{ClO}_4^+$  were simultaneously monitored for the 10 different dicationic reagents in concentrations of 1–20  $\mu\text{M}$  of the reagent and 0.5–6  $\mu\text{M}$  of  $\text{ClO}_4^-$ , with 8–12 different combinations tested for each reagent. The results are shown in Figure 3 in a log–log plot with the adduct ion intensity being plotted against the product of the concentrations of the reactants. In general, the reagents that produce the highest  $\text{DClO}_4^+$  signals, at a given  $[\text{DF}_2][\text{ClO}_4^-]$  concentration product (as introduced, especially at low values of the same),



**Figure 3.** Log–log plot of  $\text{DClO}_4^+$  signal counts and the product of concentration of various dicationic ion pair agents ( $\text{D}^{2+}$ ) and perchlorate ( $\text{ClO}_4^-$ ).



**Figure 4.** Immunity of dicationic reagents to sulfate interference.

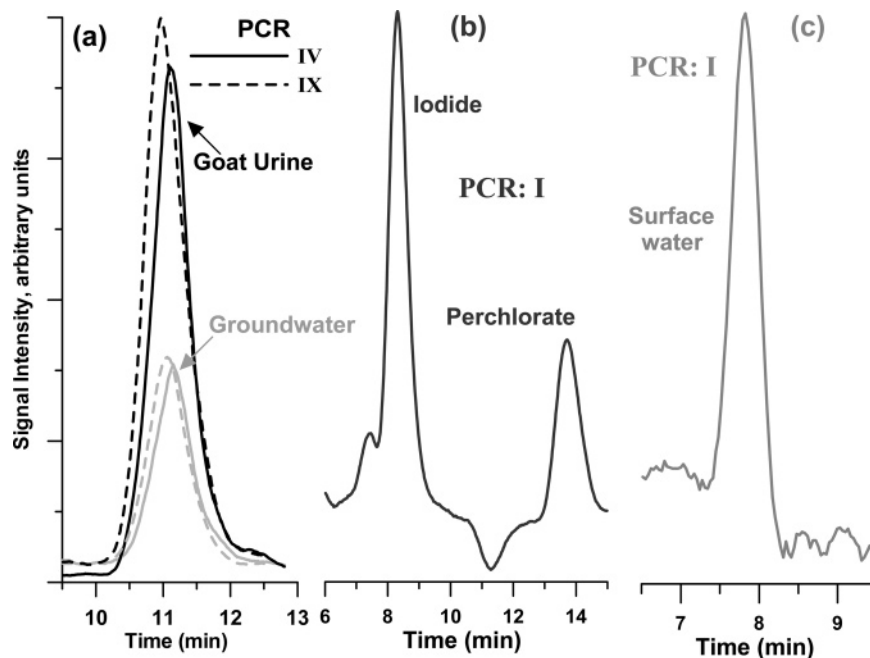


**Figure 5.** (a) Perchlorate (1 µg/L) with and without postcolumn reagent (PCR) addition. The intensity was monitored at  $m/z$  98.7 at  $-35$  V for perchlorate without PCR and at  $m/z$  384.8, 388.7, and 394.8 at  $+2$  V, corresponding to the  $\text{DClO}_4^+$  for **I**, **IV**, and **IX**, respectively. (b) Perchlorate (100 µL of 100 ng/L) injected on 2-mm AS 21 column, 21 mM KOH eluent at 0.35 mL/min, and 100 µM **IX** in 50% acetonitrile at 0.03 mL/min as PCR. Detector: ThermoQuest Finnigan MSQ.

would seem to be the better candidates. Table 1 numerically indicates the relative sensitivities. For the different dicationic reagents, we first list the intensity of the  $\text{D}^{2+}$  ion signal originating from a fixed constant concentration of  $\text{DF}_2$ . Assuming the intrinsic response of the detector to various  $\text{D}^{2+}$  ions is comparable, this value is essentially a measure of the ease with which  $\text{DF}_2$  ionizes into  $\text{D}^{2+}$ . The slope is the analytical sensitivity,  $\Delta\text{DClO}_4^+$  signal divided by the increase in the product of the input concentrations  $\Delta[\text{DF}_2][\text{ClO}_4^-]$ . For an actual use situation for which  $[\text{DF}_2]$  is invariant, this is, indeed, the observed experimental calibration slope ( $\Delta\text{DClO}_4^+$  signal/ $\Delta[\text{ClO}_4^-]$ , count-s/ $\mu\text{M}$ ). Since this may not be linear over large ranges of  $[\text{DF}_2][\text{ClO}_4^-]$ , we separately

record the slope for two different range of values of  $[\text{DF}_2][\text{ClO}_4^-]$  in two different orders of magnitude. Compounds **VI–VIII** have essentially the same sensitivity throughout, and compounds **I** and **X** have lower sensitivity at higher values of  $[\text{DF}_2][\text{ClO}_4^-]$ , whereas all the others have higher sensitivity at higher values of  $[\text{DF}_2][\text{ClO}_4^-]$ . For trace-level perchlorate measurement, the  $[\text{DF}_2][\text{ClO}_4^-]$  product will always be small. We chose, therefore, to conduct further experiments with **I**, **IV**, and **IX**, the three compounds that displayed the highest sensitivities at low values of the  $[\text{DF}_2][\text{ClO}_4^-]$  concentration product. It will be observed that these three compounds fall in the top, middle, and the bottom of the list in their ease of ionization. If overall sensitivities are comparable, it follows that compound **IX** probably has the highest affinity for forming the perchlorate adduct.

**Selectivity over Sulfate.** In trace perchlorate analysis, sulfate is most commonly the biggest problem. First, it is present in many samples in high concentrations. Second, it elutes last among common anions, closest to perchlorate, such that the profile of perchlorate response in a suppressed-conductivity-based chromatogram of a high-sulfate sample is that of a minuscule shoulder on the tail of a very large sulfate peak, resembling the topography of a shrub on the slope of a mountain.<sup>13</sup> Third, the natural abundance of  $^{34}\text{S}$  is 4.3%;  $\text{H}^{34}\text{SO}_4^-$  is only  $\sim 0.01$  amu heavier than  $^{35}\text{ClO}_4^-$ , and the required  $M/\Delta M$  resolution of  $10^4$  is well beyond quadrupole MS. A single quadrupole MS cannot distinguish between  $\text{H}^{34}\text{SO}_4^-$  and  $^{35}\text{ClO}_4^-$  or  $\text{DH}^{34}\text{SO}_4^+$  and  $\text{D}^{35}\text{ClO}_4^+$ . With reference to (a) conventional measurement at  $m/z$  98.7 and 100.7 for  $^{35}\text{ClO}_4^-$  and  $^{37}\text{ClO}_4^-$ , respectively, the performance of the dicationic reagents **I**, **IV**, and **IX** was tested using the 2-line flow injection analysis manifold mentioned in the Experimental Section. Samples containing 25 µg/L of  $\text{ClO}_4^-$  or 500 µg/L and 750 µg/L  $\text{SO}_4^{2-}$  were injected while monitoring (b)  $\text{D}^{35}\text{ClO}_4^+$ , (c)  $\text{D}^{37}\text{ClO}_4^+$ , and (d)  $\text{D}^{35}\text{Cl}^{18}\text{O}_4^+$ . In the last case, an additional (nominally 5 µg/L)  $^{35}\text{Cl}^{18}\text{O}_4^-$  was spiked into the sample. The relative intensities of  $\text{DH}^{32}\text{SO}_4^+$  and  $\text{DH}^{34}\text{SO}_4^+$  in samples containing only sulfate was



**Figure 6.** Chromatograms: (a) Groundwater sample (11.2  $\mu\text{g/L ClO}_4^-$ ) and a goat urine sample (diluted 12.5 $\times$ , originally containing  $\sim 450 \mu\text{g/L ClO}_4^-$ ) with IV and IX PCRs. (b) Human milk sample<sup>8</sup> with 22  $\mu\text{g/L I}^-$  and 2  $\mu\text{g/L ClO}_4^-$ ; both  $\text{D}^{129}\text{I}^+$  and  $\text{D}^{35}\text{ClO}_4^+$  were monitored. (c) Surface water sample (specific conductance 0.4 mS/cm) containing 0.2  $\mu\text{g/L}$  perchlorate; conditions for this chromatogram are as in Figure 5b. The ordinate scales in the three chromatograms differ.

**Table 2. Comparative Analysis of Various Samples with and without the Use of a Dicationic Agent as a Postcolumn Reagent**

sample	perchlorate ( $\mu\text{g/L}$ )	
	with I as PCR	no PCR
5 $\mu\text{g/L}$ nominal standard	5.6 $\pm$ 0.0	5.6 $\pm$ 0.6
10 $\mu\text{g/L}$ nominal standard	10.3 $\pm$ 0.4	11.0 $\pm$ 0.5
20 $\mu\text{g/L}$ nominal standard	19.7 $\pm$ 0.1	19.3 $\pm$ 0.7
deionized water (DIW)	ND	ND
groundwater	12.1 $\pm$ 0.0	13.1 $\pm$ 1.0
groundwater	12.4 $\pm$ 0.2	13.0 $\pm$ 1.8
groundwater spiked with 5 $\mu\text{g/L ClO}_4^-$	18.1 $\pm$ 0.4	17.6 $\pm$ 0.4
10 $\mu\text{g/L}$ nominal standard	10.8 $\pm$ 0.1	9.9 $\pm$ 0.4
rain 1	ND	ND
rain 1 spiked with 5 $\mu\text{g/L ClO}_4^-$	5.1 $\pm$ 0.0	4.9 $\pm$ 0.0
rain 1 spiked with 5 $\mu\text{g/L}$ nominal standard	5.4 $\pm$ 0.1	4.6 $\pm$ 0.3
DIW spiked with 5 $\mu\text{g/L ClO}_4^-$	5.0 $\pm$ 0.2	6.3 $\pm$ 0.2
milk 1	5.7 $\pm$ 0.0	5.3 $\pm$ 0.5
milk 1 spiked with 5 $\mu\text{g/L ClO}_4^-$	12.0 $\pm$ 0.1	9.2 $\pm$ 0.1
chlorine bleach (20 $\times$ dilution)	12.4 $\pm$ 0.1	9.5 $\pm$ 0.8

first measured, and the latter amounted to  $\sim 9\%$  of the former, rather than the tabulated value of 4.3% for the  $^{34}\text{S}/^{32}\text{S}$  abundance ratio. For selectivity calculations, in samples in which both perchlorate and sulfate were present, the  $\text{D}^{35}\text{ClO}_4^+$  was corrected for the  $\text{DH}^{34}\text{SO}_4^+$  contribution by subtracting from it 0.09 times the  $\text{DH}^{32}\text{SO}_4^+$  signal. The results are shown in Figure 4. It is observed that the  $\text{DClO}_4^+$  signals not only are much more intense (approximately an order of magnitude greater than the  $\text{ClO}_4^-$ ) signals, within experimental uncertainty, but also are unaffected by the presence of 500 and 750  $\mu\text{g/L}$  sulfate (up to 30 times the amount of perchlorate taken). Similar immunities were observed for interference from other ions, such as carbonate and nitrate.

Carbonate and nitrate were also well-resolved from perchlorate chromatographically and, thus, unlikely to interfere with its MS quantitation following chromatography.

**Detection Limits and Calibration Behavior.** Figure 5a shows the chromatogram for 1  $\mu\text{g/L}$  perchlorate without and with three different postcolumn reagents. At the 1  $\mu\text{g/L}$  perchlorate level, the S/N was 4 without any PCR and 27, 53, and 28 with I, IV, and IX as PCR, respectively. The improvement is obvious. Figure 5b shows 100  $\mu\text{L}$  of a 100 ng/L standard injected on a 2-mm-bore IC system coupled to a single-quadrupole MS system. The bottom chromatogram shows the signal at  $m/z$  99 for unreacted  $^{35}\text{ClO}_4^-$ , and the top chromatogram shows the signal at  $m/z$  395 for  $\text{D}^{35}\text{ClO}_4^+$ . With PCR, the S/N = 3 LOD is calculated to be 25 ng/L.

Typical calibration curves with I and IV as PCR,  $\text{D}^{35}\text{ClO}_4^+$  intensity exhibited excellent linearity for  $\text{ClO}_4^-$  concentrations up to 100  $\mu\text{g/L}$ .

$$\text{I: } \text{D}^{35}\text{ClO}_4^+ (m/z 385, \text{count-s}) = 2.64 \pm 0.02_5 \times 10^6 [\text{ClO}_4^-, \mu\text{g/L}] + 2.86 \pm 1.27 \times 10^6$$

$$r^2 = 0.9993, n = 10 \quad (1)$$

$$\text{IV: } \text{D}^{35}\text{ClO}_4^+ (m/z 389, \text{count-s}) = 1.34 \pm 0.01 \times 10^6 [\text{ClO}_4^-, \mu\text{g/L}] + 8.50 \pm 5.95 \times 10^6$$

$$r^2 = 0.9994, n = 10 \quad (2)$$

On the same instrument, calibration at  $m/z$  99 exhibited significantly poorer precision, linearity, absolute sensitivity, and sensitivity relative to background counts.



$$^{35}\text{ClO}_4^- (m/z\ 99\ \text{count-s}) = 2.35 \pm 0.09 \times 10^4 [\text{ClO}_4^-, \mu\text{g/L}] + 7.79 \pm 4.52 \times 10^4$$
$$r^2 = 0.9884, n = 10 \quad (3)$$

**Application to Real Samples.** We have found the presently described analytical method to be of great value for quantifying perchlorate with greater accuracy and certainty in samples that represent a complex matrix (typical biological samples) or with very low perchlorate content or both.<sup>8,12</sup> Figure 6 shows several examples. Panel (a) shows the perchlorate response region of the chromatogram of a high salinity (specific conductance  $\sim 3$  mS/cm) groundwater sample<sup>13</sup> containing  $11.2\ \mu\text{g/L}$  perchlorate and a urine sample from an animal that has been drinking water containing  $250\ \mu\text{g/L}$  perchlorate, with two different dicationic reagents. Such reagents also greatly enhance the sensitivity of detection for other hydrophobic ions, such as iodide, which is of considerable interest in many samples in which perchlorate quantification is sought, most notably, milk.<sup>8</sup> This is shown in panel (b). Panel (c) shows the perchlorate present in very low

concentrations ( $200\ \text{ng/L}$ ) in surface water samples can be easily detected by the present approach.

Table 2 shows comparative analytical data on various samples with and without **I** as PCR. With increasing demand for detecting perchlorate in very low concentration in increasingly complex samples, this technique can be of significant benefit in IC/MS analysis of samples containing perchlorate. We expect to report the results of adaptation of similar strategies to IC/MS/MS with these and other ion association reagents soon.

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