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Quantitation of perchlorate ion by electrospray ionization mass spectrometry (ESI-MS) using stable association complexes with organic cations and bases to enhance selectivity†

Edward T. Urbansky,* Matthew L. Magnuson, David Freeman and Christopher Jelks

United States Environmental Protection Agency (EPA), Office of Research and Development, National Risk Management Research Laboratory, Water Supply and Water Resources Division, Treatment Technology Evaluation Branch, Cincinnati, OH 45268, USA.
E-mail: urbansky.edward@epamail.epa.gov; magnuson.matthew@epamail.epa.gov

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Quantitation of trace levels of perchlorate ion in water has become a key issue since this species was discovered in water supplies around the United States. Although ion chromatographic methods presently offer the lowest limit of detection, ≈40 nM (4 ng ml^{-1}), chromatographic retention times are not considered to be unique identifiers and often cannot be used in legal proceedings without confirmatory testing. Mass spectrometry can provide such confirmation; however, detection capabilities can impose a practical limitation on its use. Moreover, quadrupole mass spectrometers cannot provide sufficient accuracy and precision in *m*/*z* to identify conclusively an ion as perchlorate when samples are run directly without prior chromatographic or electrophoretic separation. We report on the abilities of (1) tetralkylammonium cations and (2) minimally nucleophilic, sterically hindered organic bases to increase selectivity in the electrospray ionization mass spectrometric (ESI-MS) determination of perchlorate ion without concomitant loss of sensitivity. Selectivity arises from the formation of a stable association complex between a base molecule and a perchlorate anion. The best results were obtained using 10 μM chlorhexidine in methanolic solution; the lower limit of detection (LLOD) for $S/N \geqslant 2$ was less than or equal to 0.10 μM (10 ng ml^{-1}). This compares favorably with the LLOD determined for perchlorate in the absence of any complexing agents (≈0.05 μM = 5 ng ml^{-1}). For the other bases, which were diazabicyclo compounds (DBN, DBU, DBO), sensitivity was lower by 90% or more. The chlorhexidine–perchlorate complex (*m*/*z* = 605) can be observed even in the presence of equiformal nitrate, nitrite, hydrogensulfate, chloride, bromide, bromate, and chlorate (all together) down to approximately 1 μM; thus, the method is rugged enough to find application to systems containing multiple inorganic anions.

Introduction

Perchlorate ion was identified in ground and surface waters of the western United States in 1997, and has since been found at sites around the country. It may be found in the ground or surface waters near wherever perchlorate salts have been manufactured, stored, or used. Such sites are often associated with defense or aerospace programs (or supporting industries) since perchlorate salts find use as solid oxidants or energetics boosters in rockets and missiles. The analytical chemistry of perchlorate and the significance of quantifying this contaminant have been reviewed and described in detail elsewhere.[1,2]

Because of the low concentrations of perchlorate found at most of the contaminated sites (5–50 ng ml^{-1}), the primary technique used for water analysis has been ion chromatography (IC). However, chromatographic retention time is not considered to be a unique identifier, and additional confirmation is required to initiate legal action. Although IC may be used for routine monitoring, periodic confirmatory testing must be carried out. As a consequence, techniques such as mass spectrometry can be expected to find a role in secondary confirmation even if the instrumentation is not generally available on-site to potable water utilities, responsible parties, or regulatory governmental agencies for primary environmental monitoring purposes.

There are essentially six different ways of assaying perchlorate. Gravimetry and titrimetry only apply to standardization of fairly concentrated laboratory (≥0.01 M) solutions.[1] Ion-selective electrodes suffer from a number of anionic interferences as well as lower limits of detection (LLODs) of ≈7 μM = 70 ng ml^{-1},[1] which is at least twice what the no observable adverse effects level is predicted to be based on current research.[3] Although Hauser *et al.* report a detection limit of 10 ng ml^{-1} for an ion-selective electrode, this was in conjunction with a capillary electrophoresis (CE) separation.[4] Barnett and Horlick used electrospray ionization mass spectrometry (ESI-MS) to obtain an LLOD of 0.050 μM (5 ng ml^{-1}).[5] While spectrophotometric determinations are reported to have similar LLODs to the ESI-MS method,[6] they are not sufficiently selective, and dye purity can be an issue. Without prior analyte separation, quadrupole mass spectrometers cannot measure *m*/*z* ratios accurately and precisely enough to identify conclusively an ion with a mass of 99 u as perchlorate.

Despite the selectivity generally regarded as associated with mass spectrometric identification, a large number of small mass (< 300 u) hydrophilic inorganic or organic anions can be found in natural water sources as well as disinfected potable water supplies. Mass spectrometry offers less ambiguity than IC, but with a reduction in sensitivity. Introduction/ionization techniques for the involatile perchlorate ion are most likely constrained to either electrospray, which was used by Barnett and Horlick,[5] or thermospray, which has not been reported for perchlorate. Extraction of perchlorate salts from aqueous solution is possible, but can be difficult to take advantage of analytically. Use of large cationic organic dyes, *e.g.*, Brilliant Cresyl Blue or Brilliant Green, is reported for spectrophoto-

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metric determinations of perchlorate.[3] With a mass of 99 u, perchlorate anion has an *m*/*z* ratio in the region of many other ions commonly found in US waterways, from either natural or anthropogenic sources. Chlorinated potable water supplies contain other anions, *e.g.*, chlorate and chloride, at substantially higher levels than perchlorate can be expected to be found. Additionally, some ions can be created in the electrospray process by direct electrochemical reduction. Consequently, it is highly desirable to improve not only sensitivity, but also selectivity, for perchlorate ion determination by electrospray mass spectrometry. To this end, we have explored the ability of a number of compounds to increase the selectivity of ESI-MS as a technique for determining perchlorate ion concentration in water through the formation of stable association complexes amenable to electrospray ionization.

Experimental†

Instrumentation

A Finnigan (San Jose, CA, USA) electrospray apparatus and a Finnigan TSQ 700 quadrupole mass spectrometer were used throughout. Samples were introduced *via* a Rheodyne (Rohnert Park, CA, USA) 7725 injector with a 200 μl sample loop. The liquid carrier (Fisher Optima® (Pittsburgh, PA, USA) methanol) was supplied by a Waters (Milford, MA, USA) 600-MS pump. Mass spectra were acquired in both positive and negative ion modes. ESI is a sufficiently soft ionization technique that fragmentation is generally not observed for these analytes. Table 1 gives additional parameters for operation and data acquisition.

Reagents

Solvents. All organic solvents were pesticide residue analysis grade, such as Fisher Optima®, Aldrich (Milwaukee, WI, USA), or Mallinckrodt Nanograde® (Phillipsburg, NJ, USA).

Inorganic salts. A stock solution of 1000 μg ml^{-1} ClO_4^- was prepared by dissolving ammonium perchlorate, NH_4ClO_4 [7790-98-9] (Aldrich), in doubly de-ionized water (Barnstead Nanopure, Dubuque, IA, USA). Additional solutions were prepared by quantitative dilution of this stock solution. Stock aqueous solutions of NH_4ClO_4 (Aldrich), $NaClO_3$ (Fisher), NaCl (J. T. Baker, Phillipsburg, NJ, USA), $NaBrO_3$ (Fisher), NaBr (GFS, Columbus, OH, USA), $NaNO_2$ (GFS), NH_4NO_3 (GFS), and $NaHSO_4$ (Fluka, Buchs, Switzerland) were prepared at 0.10 M. Potassium salts were not used so as to avoid precipitation.

Cationic organic dyes. Stock solutions of Brilliant Cresyl Blue [81029-05-2], Brilliant Green [633-03-4], and Crystal Violet [548-62-9] were prepared at 0.01 M by dissolving the solids in doubly de-ionized water containing 25% v/v methanol. Dyes were obtained from Spectrum (New Brunswick, NJ, USA).

Organic bases. Stock solutions of five minimally nucleophilic (2° or 3°) sterically hindered organic bases were prepared: 1,4-diazabicyclo[2.2.2]octane (DBO, Dabco®, triethylenediamine) [280-57-9] **1**, 1,5-diazabicyclo[4.3.0]nonene (DBN) [3001-72-7] **2**, 1,8-diazabicyclo[5.4.0]undec-7-ene, (DBU, 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine) [6674-22-2] **3**, 1,1′-hexamethylenebis[5-(4-chlorophenyl)-biguanide] (chlorhexidine) [55-56-1] **4**, 4,5-dihydro-2,4-diphenyl-5-(phenylimino)-1*H*-1,2,4-triazolium hydroxide, inner salt (nitron) [2218-94-2] (structure not shown), and triethanolamine (all from Aldrich). Concentrations of 0.010 or 0.10 M were obtained by dissolving known masses of the commercial reagents in doubly de-ionized water. Ethanoic (acetic) acid (HOAc, Mallinckrodt) at several concentrations was used to solubilize the chlorhexidine and the nitron.

Table 1 ESI-MS operating conditions and data acquisition parameters

Applied ESI spray potential/kV	4.0
Baseline ESI spray current/μA	0.342
Capillary temperature/°C	200 (optimal)
Liquid carrier	Methanol
Carrier flow rate/ml min^{-1}	0.30
Scan time/s	0.50, for $\Delta(m/z) = 1.0$ u
Nebulizer gas pressure/kPa	482 = 70 psi
Number of injections	3
Injection volume/μl	50.0
Relative standard deviation of replicate injections (%)	<15

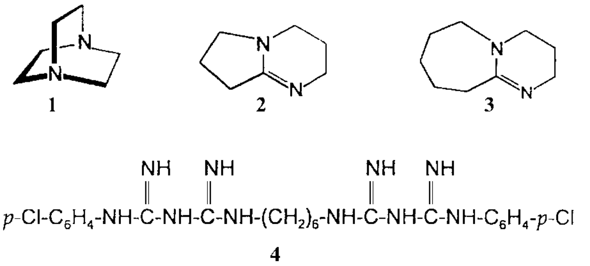
Quaternary cations. NMe_4OAc [10581-12-1], NEt_4OA-c·$4H_2O$ [67533-12-4], NPr_4OH [4499-86-9], NBu_4Br [1643-19-2], $NHex_4Br$ [4328-16-6], $NOct_4Br$ [14866-33-2], carbamyl-β-methylcholine (bethanechol) chloride [590-63-6] and $As(C_6H_5)_4Cl{\cdot}H_2O$ [507-28-8], were obtained from Aldrich or Sigma (bethanechol chloride). Stock solutions (0.10 M) were prepared by dissolving the solids in doubly de-ionized water and/or methanol. NPr_4OAc was prepared by reacting NPr_4OH with HOAc *in situ*.

Sample preparation and treatment

Test solutions. Methanolic (high-purity) solutions (containing 10–16% v/v water from dilution of stock reagents) were prepared containing perchlorate ranging from 0 to 50 μg ml^{-1} and organic complexing agents at concentrations from 1 μM to 10 mM (depending on the complexing agent). Organic bases (except chlorhexidine) were run with and without acetic acid. Organic dye concentration was 1 mM. For comparison, propan-2-ol solutions of several agents were also prepared. Methanolic solutions of ammonium perchlorate with no other reagents were used as a control group.

To evaluate ruggedness (resistance to matrix effects), methanolic solutions were prepared as described below (<5% v/v water). Each solution contained the following mix of electrolytes at equiformal conditions: NH_4ClO_4, $NaClO_3$, NaCl, $NaBrO_3$, NaBr, $NaNO_2$, $NaNO_3$, and $NaHSO_4$. In addition, each solution contained 1.0 mM DBN, DBU, or DBO or 10 μM chlorhexidine. Test solutions were prepared containing 1.0, 2.0, 5.0, 7.5, 10, 20, or 50 μM of each salt with all salts in every test solution.

Solvent extraction of cationic organic dye–perchlorate complexes. Aliquots of 100 ml of 1 mM dye and 50 μg ml^{-1} perchlorate were extracted with equal volumes of ethyl acetate (EA) or *tert*-butyl methyl ether (MTBE). Solvent was drawn off by rotary evaporation under house vacuum at 60 °C (MTBE)



$$p\text{-Cl-C}_6\text{H}_4\text{-NH-}\overset{\text{NH}}{\overset{\|}{\text{C}}}\text{-NH-}\overset{\text{NH}}{\overset{\|}{\text{C}}}\text{-NH-(CH}_2)_6\text{-NH-}\overset{\text{NH}}{\overset{\|}{\text{C}}}\text{-NH-}\overset{\text{NH}}{\overset{\|}{\text{C}}}\text{-NH-C}_6\text{H}_4\text{-}p\text{-Cl}$$

Scheme 1 Structures of organic bases.

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†Mention of specific brand names and trademarks should not be construed to reflect an endorsement of products or manufacturers by the United States Government.

or 70 °C (EA). The residue was dissolved in a minimum of methanol, transferred into a 50 ml beaker, and evaporated to dryness on a warm hot-plate. The residue was redissolved in 10 ml of methanol and subjected to ESI-MS analysis.

Results and discussion

Perchlorate complexes with organic bases

Complexation. Table 2 gives the masses of molecular ions that were found by ESI-MS or for which we monitored the corresponding *m*/*z* ratio (assuming *z* of unity); masses of significant neutral species are given for reference. As can be seen from Table 2, the protonated organic bases, DBO, DBN, and DBU, form complexes with the empirical formula $[(HB^+)(ClO_4^-)_2]$. The mass spectrum of the molecular ion for the DBU–perchlorate complex of this form is shown in Fig. 1. As Fig. 2 shows, chlorhexidine is capable of multiple protonations and up to three perchlorate complexations with molecular ions of the form $[\text{chlorhexidine}\cdot ClO_4(HClO_4)_n]^-$; $n = 1$ or 2.

Although we expected that a source of acid would be needed, we found that there was no effect on the peak areas when the acetic acid concentration was varied from 0 to 0.010 M with DBO, DBN, and DBU; at 0.10 M HOAc, the signal fell off dramatically, presumably due to a loss of electrospray efficiency induced by ionic strength. Apparently, the water present from the stock perchlorate solution (or the methanol itself) is sufficient to provide a source of protons. For chlorhexidine, varying [HOAc] from 0.01 to 10 × (molar basis) the chlorhexidine concentration had no measurable effect on the peak areas.

Selectivity and sensitivity. Of all the bases, chlorhexidine gave the greatest enhancement of selectivity as shown by Fig. 3, which includes perchlorate concentrations in the ppm range (1 ppm = 100 μM). Sensitivity must be gauged in terms of instrument response relative to a total concentration of complexing agent. It should be noted that the highest total chlorhexidine concentration in Fig. 3(a) is 10 μM while the DBN concentration in Fig 3(b) is 10 μM, 1000 times greater. Although the DBU and DBO calibration lines in Fig. 3(b) have slopes about 1.5 times that of the chlorhexidine line with highest slope, this is observed where the chlorhexidine concentration is 10 mol% that of the other two bases. Accordingly, the relative sensitivity for chlorhexidine is computed to be about 7 times greater. The chlorhexidine–perchlorate complex gave a signal equal to approximately 10% of the signal found for perchlorate alone at low concentrations. Barnett and Horlick reported a LLOD of 5 ng ml^{-1} for perchlorate ion alone.[5] Based on the data in Fig. 4, we determined LLOD $\leqslant$ 0.10 μM (10 ng ml^{-1}) for the perchlorate–chlorhexidine complex. This concentration gave a measurable signal distinct from the average noise by a factor of at least 2. With regard to capillary temperature, we eventually opted for 200 °C, which seemed consistently to give the greatest sensitivity and injection-to-injection reproducibility.

Table 2 Masses of investigated and/or identified molecular ions and reference neutral molecules[a]

Species	Mass/u	Species	Mass/u
DBO^+	112 f	$N(EtOH)_3{}^+$	149 f
$DBO(ClO_4)^-$	211 f	$N(EtOH)_3(ClO_4)^-$	248 f
$DBO(ClO_4)_2H^-$	311 f	$N(EtOH)_3(ClO_4)_2H^-$	348 f
DBN^+	124 f	Nit^+	312 f
$DBN(ClO_4)^-$	223 f	$Nit(ClO_4)^-$	411 n
$DBN(ClO_4)_2H^-$	323 f	$NMe_4(ClO_4)_2{}^-$	271 n
DBU^+	152 f	$NEt_4(ClO_4)_2{}^-$	328 f
$DBU(ClO_4)^-$	251 f	$NPr_4(ClO_4)_2{}^-$	384 f
$DBU(ClO_4)_2H^-$	351 f	$NBu_4(ClO_4)_2{}^-$	440 f
CHD^+	505 f	$NHex_4(ClO_4)_2{}^-$	552 f
$CHD(ClO_4)^-$	604 f	$NOct_4(ClO_4)_2{}^-$	664 f
$CHD(ClO_4)_2H^-$	704 f	$Beth(ClO_4)_2{}^-$	359 f
$CHD(ClO_4)_3H_2{}^-$	804 f	$HClO_4{}^0$	100 n
$ClO_4{}^-$	99 f		

[a] DBO = 1,4-Diazabicyclo[2.2.2]octane. DBN = 1,5-Diazabicyclo[4.3.0]-non-5-ene. DBU = 1,8-Diazabicyclo[5.4.0]undec-7-ene. CHD = Chlorhexidine. $N(EtOH)_3$ = Triethanolamine. Nit = Nitron. $Beth^+ = C_7H_{17}N_2O_2{}^+$, from bethanechol chloride. Identification: f = found, n = not found, based on peaks in ESI mass spectra.

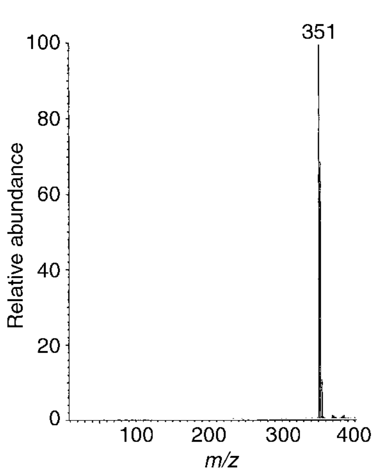

Fig. 1 Negative ion ESI mass spectrum obtained for complex of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) with perchlorate. Similar results were obtained for DBO and DBN. See Table 2 for molecular ion identification.

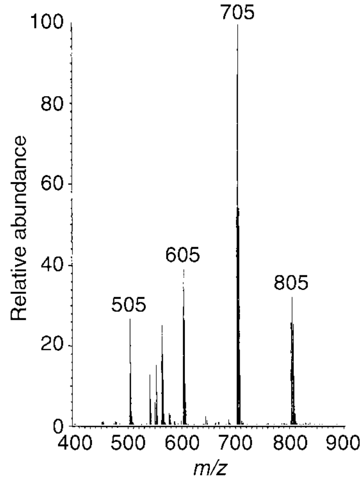

Fig. 2 Negative ion ESI mass spectrum obtained for complexes of chlorhexidine with perchlorate. Note that multiple perchlorate ions can associate with a single chlorhexidine molecule. Complexes have the following empirical formula: $[\text{chlorhexidine}\cdot ClO_4(HClO_4)_n]^-$; $n = 1$ or 2. See Table 2 for molecular ion identification.

In terms of absolute sensitivity, comparison of Fig. 3(a) and (b) suggests that 1 mM DBU would be preferable to 10 μM chlorhexidine. However, at concentrations of perchlorate below 10 μM, the instrumental response for perchlorate was indistinguishable from the blank in 1 mM DBU. Meanwhile, as noted above, Fig. 4 indicates an acceptable response for chlorhexidine complexes in this region of perchlorate concentration. Therefore, chlorhexidine outperforms DBU in terms of absolute sensitivity at the lower perchlorate concentrations and is an overall better choice.

In the presence of equiformal anions, molecular ions of the form $[(HB^+)(ClO_4^-)_2]$ were not observed for DBO, DBN, or DBU. Given that the sum of the anion concentrations is more than 8 times the chlorhexidine concentration, we had expected

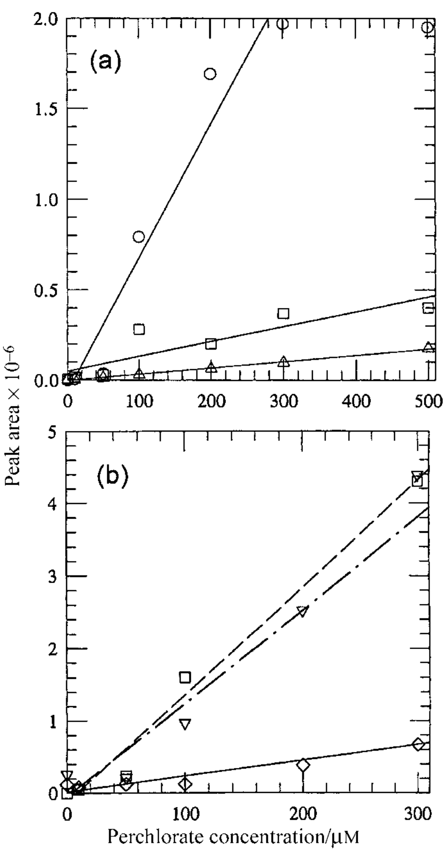

Fig. 3 Calibration graphs (peak area *versus* perchlorate concentration) for ESI-MS determination of perchlorate: (a) top to bottom, in (○) 10 μM, (□) 1 μM, (△) 0.1 μM chlorhexidine, MeOH solution; (b) (□, – –) 1 mM DBU, (◊, —) 10 mM DBN, (▽, -·-) 1 mM DBO. All lines are based on least-squares regression.

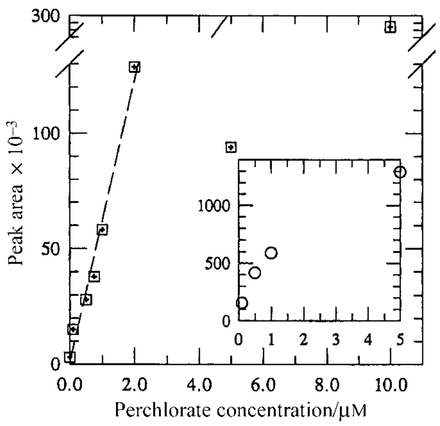

Fig. 4 Calibration graph (peak area *versus* perchlorate concentration) for ESI-MS determination of perchlorate in 10 μM chlorhexidine, MeOH solution. First six points used for least-squares regression line. Presumably, points 7 and 8 suffer from loss of electrospray efficiency due to ionic strength. Inset: Calibration graph for perchlorate anion without any complexing agents, MeOH solution, provided for comparison. Non-linear response at higher perchlorate concentration is observed with and without complexing agents.

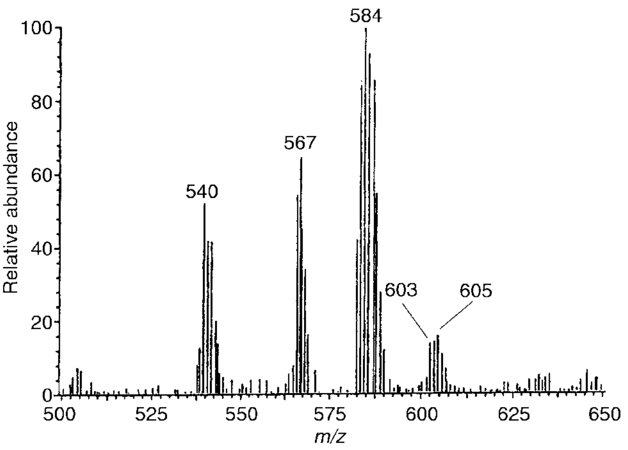

Fig. 5 Negative ion ESI mass spectrum for 1 μM (equiformal) NH_4ClO_4, $NaClO_3$, NaCl, $NaBrO_3$, NaBr, $NaNO_2$, $NaNO_3$, and $NaHSO_4$ in 10 μM chlorhexidine, MeOH solution. In this case, the only perchlorate–chlorhexidine association complex observed is that containing one chlorhexidine molecule and one perchlorate ion. See text for additional discussion.

to see an unresolvable system of mixed anion complexes in the mass spectrum or perhaps nothing at all, but this was not the case. Instead, we found molecular ions of the form [chlorhexidine·X^-] for perchlorate, chloride, nitrate, and bromide. However, there was no evidence for chlorhexidine complexes with nitrite, hydrogensulfate, acetate, bromate, or chlorate. It is worth pointing out that we have never seen a peak that corresponds to an association complex of acetate with any of the reagents. We assume that a peak at $m/z = 586$ corresponds to a chlorhexidine–hydrogensulfite complex that formed from sulfate reduced by the electrospray process, and we suspect that chlorate, bromate, and nitrite are reduced as well. Further exploration of these peaks and the fate of the other anions was beyond the scope of this work and was not pursued.

The peaks for the chlorhexidine complexes of chloride, nitrate, and bromide are larger than the peak for the perchlorate complex; nevertheless, Fig. 5 does show that the perchlorate response remains visible even under competition for the chlorhexidine. Although the sensitivity is reduced relative to perchlorate in the absence of the other inorganic anions, a non-linear response curve can be generated, as shown in Fig. 6. Attenuation of the signal at high concentration is probably due to reduced electrospray efficiency resulting from ionic strength effects.

Based on Fig. 3 and 6, we believe that this method could be applied under a variety of conditions to both analytical solutions and real water samples. Some variation may be needed for optimization, such as changing pH or chlorhexidine concentration. Preconcentration may be advised for some samples, and we expect that standard approaches would work, *e.g.*, simple evaporation, lyophilization, or on-column retention (using a strong anion-exchange resin). The suitability of these would depend on both analyte concentration and matrix constituents. Given the ionic content of the test solutions, we expect that matrix effects could readily be accounted for in drinking water by using the method of standard additions, but this would require validation and optimization on a per case basis. Fig. 5 suggests that chlorhexidine might even prove useful for selectively enhancing a number of different anions, but further exploration of the phenomenon was beyond the scope of this work.

Applicability to potable water analysis. Because of the high ionic strength of potable water, which includes sodium chloride at considerably higher concentration than the analyte, suppression of the electrospray signal is to be expected. Two different potable waters were tested: Cincinnati tap water and Tri-Township Water (TTW). The source of Cincinnati tap water is the Ohio River. TTW is supplied by a well in Dearborn County, Indiana, and probably experiences some infiltration from the Whitewater River. The TTW sample was collected from a residential faucet in Logan Township.

A 20 ml sample of TTW water was evaporated to dryness at 60 °C and reconstituted in an equal volume of 10% v/v H_2O–MeOH. Aqueous chlorhexidine was added to give a final chlorhexidine concentration of 10 μM in the reconstituted

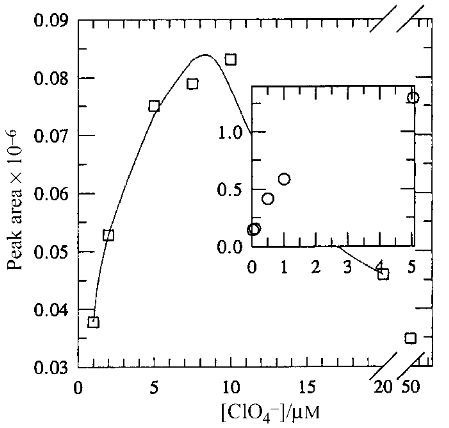

Fig. 6 Perchlorate response (peak area *versus* concentration) for ESI-MS determination in 10 μM chlorhexidine in the presence of seven other equiformal anions, MeOH solution; see Fig. 5 caption. This is not a true calibration graph, because the sample matrix (but not the chlorhexidine) varies in concentration, too. Inset: Calibration graph for perchlorate anion without any complexing agents, MeOH solution, provided for comparison. Non-linear response at higher anion concentration is observed, presumably due to ionic strength effects on electrospray efficiency.

sample. It is important to note that the mineralized residue resulting from evaporation does not redissolve in the final solution. In order to promote as much dissolution of perchlorate salts as possible, the reconstituted samples were permitted to sit for 20–40 min before decanting the solvent. A signal distinguishable from that of unspiked samples was obtained at 20 and 40 μM perchlorate with a response factor of 16 000 area units per 20 μM spiked into the sample. Much of the chlorhexidine peak ($m/z = 505$ u) is lost from the supernatant, presumably due to adsorption onto the mineralized residue. For this reason, spiking the supernatant with additional perchlorate after decanting from the evaporation vessel gives no increase in signal. Nonetheless, if the chlorhexidine concentration is returned to 10 μM (as indicated by the peak at $m/z = 505$ u), further perchlorate spikes give appropriate response.

Because of the electrospray suppression, large dilution factors are required if the waters are to be run without preconcentration. We found that a 5% v/v dilution of tap water samples gave the best results. Of course, this would mean that the perchlorate concentration in the water sample would have to be 20 times greater than the post-dilution concentration. In both TTW and Cincinnati samples, a post-dilution concentration of 250 nM perchlorate (1.25 μM pre-dilution) gave a response of about 9200–9500 area units above the unspiked blank. We must point out that the response is not linear in these matrices, and quantification of perchlorate under such conditions would require careful construction of a calibration graph in the matrix under study.

Simple dilution is adjudged not to be a generally suitable approach for quantifying perchlorate in the presence of multiple other inorganic anions. Ideally, a preconcentration technique would select for perchlorate over other anions. We speculate that preconcentration using certain highly selective resins, such as those recently developed by the Oak Ridge National Laboratory,[7] would dramatically improve the performance of this method and could reduce the LLOD by a factor of 100 or more.

Methanol *versus* propan-2-ol. We compared the signal of 50 μg ml^{-1} ClO_4^- standards with DBU and chlorhexidine in propan-2-ol *versus* methanol. No measurable difference was found. In addition, we were concerned that clusters of $(MeOH)_2(H_2O)_2$ might be responsible for units of $\Delta(m/z) = +100$ rather than $HClO_4$. Since these peaks are also observed in the propan-2-ol solutions but not in perchlorate-free methanolic blanks, we conclude that the perchlorate is in fact responsible, rather than some cluster of solvent or spectator species.

Quaternary cations and other species

Performance of the tetraalkylammonium ions as complexing agents was so much poorer than the organic bases that it suffices to say that these will be of little analytical utility. No signal was detected for the molecular ions of the tetramethylammonium, tetraethylammonium, or tetrapropylammonium cations, $[(NR_4^+)(ClO_4^-)_2]$. Triethanolammonium and bethanechol gave observable signals, but these did not compare favorably with chlorhexidine or the diazabicyclo bases. Tetrabutylammonium, tetrahexylammonium, and tetraoctylammonium gave signals whose magnitude increased with the length of the carbon chain for equiformal $[NR_4^+]$. However, these salts are limited by their solubilities in water and methanol (or combinations thereof), and did not compare favorably with the results obtained for the organic bases above. No signal was found at the m/z ratio predicted for the molecular ion of tetraphenylarsonium cation associated with a perchlorate anion and either another perchlorate anion or a chloride anion. No signal was detected for a protonated nitron associated with perchlorate anion; neutral nitron is zwitterionic, and it is usually protonated with acetic acid to solubilize it and to form the precipitant cation. In positive ion mode ESI-MS, however, all of the expected cations could be identified. Consequently, we feel confident in saying that there is a lack of the complexation behavior characteristic of the other bases rather than an inability of the cations to be carried into the vapor phase.

Cationic organic dyes

We did not observe any evidence for the presence of complexes of perchlorate with cationic dyes in the ESI mass spectra when directly injecting the methanolic dye–perchlorate solutions or the reconstituted extracts. While we are uncertain whether the extractions were successful, we suspect that the failure of the direct injection is probably due to the inability of the electrospray apparatus to nebulize and ionize these species effectively. Regardless, this particular strategy does not appear to be worth pursuing further, even though it may be suitable for the reported spectrophotometric determinations.[6]

Conclusions

Chlorhexidine gave the greatest improvement in selectivity without loss of sensitivity for perchlorate detection and quantification, and it was the only base to show resistance to matrix effects, even at much lower base concentration. Although the non-nucleophilic organic bases (*i.e.*, DBO, DBN, DBU) gave acceptable increases in selectivity, losses of sensitivity were rather high. Surprisingly, cationic precipitants used in perchlorate gravimetry, *i.e.*, tetraphenylarsonium and nitron, gave no signal at all. Because the organic bases substantially elevate the mass—by a factor of 3–8—the peak is completely separated from the low mass area where other anions are commonly found. In addition, complexes with water or methanol molecules are not seen in the mass spectrum; thus, peaks of species such as $H_2O{\cdot}^{81}Br^-$ ($m/z = 99$), which was seen by Barnett and Horlick,[5] are conveniently absent. The use of complexing reagents therefore permits ESI-MS analysis without separation on account of its increased selectivity. Nonetheless, use of IC or CE is not precluded, and hyphenated techniques (*i.e.*, LC-MS or CE-MS) may find a use for such reagents.The thoughtful use of complexing agents such as chlorhexidine will perhaps provide a valuable tool in terms of increased selectivity without diminished sensitivity in the analytical determination of the perchlorate anion.

Acknowledgements

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