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Optimising the determination of nitrate and phosphate in sea water with ion chromatography using experimental design

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Abstract

A fractional factorial experimental design involving six factors is used to optimise the determination of low concentrations of nitrate and phosphate in sea water with ion chromatography. By allowing a substantial part of the interfering chloride to elute off the pre-column before the sample is injected onto the main separation column, chloride is diluted substantially more than the ions of interest. This means that nitrate and phosphate can be determined in sea water at low μM concentrations using a sample volume of only 20 μl. Detection limits for nitrate and phosphate are 0.5 and 1 μM, respectively. Repeatability measured as standard deviation is 0.15 μM for nitrate and 0.25 μM for phosphate in the 1–10 μM range.

Keywords: Water analysis; Experimental design; Nitrate; Phosphate; Inorganic anions

1. Introduction

Ion chromatography is a common method for determining anions in fresh water but not for sea water [1,2]. The high concentration of chloride in sea water overloads the column and samples have to be diluted 5–10 fold. The ions of interest will then also be diluted, making it difficult to determine low μM concentrations of nitrate and phosphate (Fig. 1).

Nutrient anions in sea water are usually determined by adding reagents and forming coloured complexes that are detected spectrophotometrically [3]. These systems normally require sample volumes of around one or a few millilitres.

When making high-resolution sediment pore-water profiles by slicing cores or using equilibration probes, only a small amount of pore water can be extracted [4,5]. The same applies for open-plug incubations of sediment [6]. With ion chromatography the sample volume is normally around 20 μl per injection, which allows for more determinations to be run when sample volume is restricted. Another

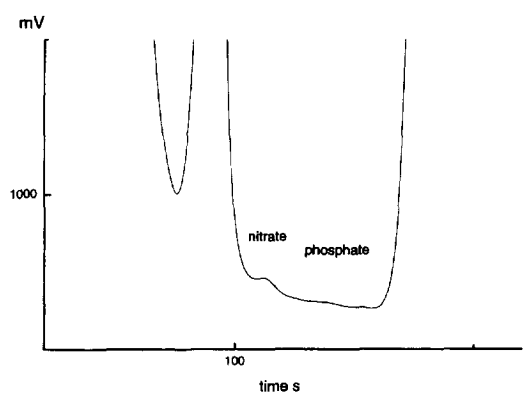

Fig. 1. Standard isocratic method for anion determination, where the sample is diluted 5 times. Concentration of both nitrate and phosphate is 5 μM in the undiluted sample.

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advantage with ion chromatography is that interferences from other anions are avoided because all anions elute at different times [3,7]. This eliminates interferences from silicate when determining phosphate and from nitrite when determining nitrate. Ion chromatography has been used for low salinity sea water, around 5, with spectrophotometric detection [8].

Naish [9] suggested that chloride could be eluted off the pre-column before injecting the remaining sample on the main column. This was done in an isocratic set-up in order to improve phosphate detection at high concentration of chloride. The chromatographic method described in this paper is a development of the method by Naish [9].

Ion strength and pH are the most common parameters that govern the separation of inorganic anions in ion chromatography [10]. Retention time for all ions is affected by ion strength, whereas pH mostly influences retention time for weak acids such as phosphate. Other factors that also can affect the separation are eluent flow-rate and as in our case, a change of eluent to achieve a gradient.

Experimental design used to optimise a separation allows for all factors to be varied in a small number of experiments, compared to if all factors had to be varied in separate experiments [11]. The advantage is that effects from a single factor, as well as combined effects from several factors, can be studied. A set of chromatograms are run where all factors involved are studied at two levels. The results from these experiments are used to construct a model for predicting an optimal separation.

In this paper we report on an improved ion chromatography method for nitrate and phosphate determination in sea water, regardless of salinity, developed using experimental design.

2. Material and methods

2.1. Chromatographic set-up

The chromatographic set-up was a Dionex 2000 and consisted of an injection valve connected to two pre-columns in line (Fig. 2). A second valve was placed between the last pre-column and the main separation column. After the separation column

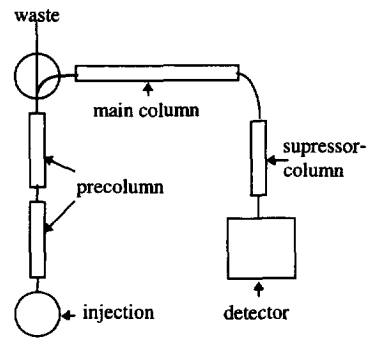

Fig. 2. Chromatographic set-up.

followed a standard suppressor system with a conductivity detector.

The separation began with a weak eluent flowing through the system at injection time, while the second injection valve was switched to waste position. This separates the chloride from the rest of the anions and chloride was eluted off into the waste line. At time 1, the weak eluent was changed to a stronger one, and at time 2, the second valve was switched towards the separation column (Fig. 3). The stronger eluent decreased the retention time and the pH in this eluent determined the resolution between nitrate and phosphate.

The pre-columns were equilibrated with the weak carbonate–hydrogencarbonate eluent for 2 min before the undiluted sample was injected, the second valve being in waste position. After the separation, when the last peak had eluted off, the valve was switched to waste position and the pre-columns were once again equilibrated with the weak eluent for 2 min. Detector output range was set to 3 μS.

2.2. Experimental design

Six factors were varied at two levels in this model; pH and ion strength in the first and second eluent, and time 1 and time 2. A full-factor model with replication of the centre point would have implied 67 separate runs. The design was therefore reduced to 19 ($2^{6-2}+3$) runs according to Table 1.

Multiple linear regression, MLR, was used for calculating the model, and analyses of residual and lack-of-fit test was used to evaluate the model. The responses used in the model were resolution, re-

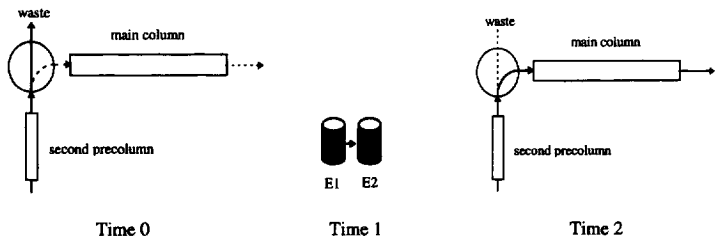

Fig. 3. At time 0, the weak eluent is directed to waste, at time 1 eluent 1 changes to eluent 2, and at time 2, the sample is switched on to the main column.

tention time, peak area, distance between peaks measured between the end of peak 1 to beginning of peak 2, and slope of the baseline.

Correct proportions of carbonate–hydrogencarbonate to achieve the desired pH were calculated using Haltafall 5.5. Ion strength was determined using the definition by Lewis and Randall [12].

2.3. Instruments and chemicals

The ion chromatographic system used was an Dionex 4000i with two guard columns, AG4A, one separator column, AS4A, and an anion micro membrane suppressor with a conductivity detector. Injection was carried out either with a Promis autoinjector, Spark Holland, or manually, in both cases using a 20-μl injection loop.

The multivariate software used in calculating, evaluating and predicting the model, was Modde 2.1, Umetri, Umeå, Sweden.

All chemicals used were from E. Merck, Darmstadt, Germany and ultra-pure water was prepared using a Milli-Q Plus 185 water purification system from Millipore, MA, USA.

Table 1
The experimental design used for optimising the separation

Run order	pH		*I* (m*M*)		Time (min)	
	Eluent 1	Eluent 2	Eluent 1	Eluent 2	*t*1	*t*2
17	8.33	9.25	1	10	0.70	1.20
4	8.33	10.50	1	10	1.10	1.20
11	10	9.25	1	10	1.10	1.60
18	10	10.50	1	10	0.70	1.60
14	8.33	9.25	1	22	1.10	1.60
3	8.33	10.50	1	22	0.70	1.60
16	10	9.25	1	22	0.70	1.20
12	10	10.50	1	22	1.10	1.20
5	8.33	9.25	1.80	10	0.70	1.60
7	8.33	10.50	1.80	10	1.10	1.60
6	10	9.25	1.80	10	1.10	1.20
9	10	10.50	1.80	10	0.70	1.20
19	8.33	9.25	1.80	22	1.10	1.20
8	8.33	10.50	1.80	22	0.70	1.20
15	10	9.25	1.80	22	0.70	1.60
1	10	10.50	1.80	22	1.10	1.60
13	9.16	9.88	1.40	16	0.90	1.40
10	9.16	9.88	1.40	16	0.90	1.40
2	9.16	9.88	1.40	16	0.90	1.40

I is ionic strength.

3. Results and discussion

3.1. Experimental design

The quality of the model calculated was evaluated as in Fig. 4. The height of the R2 bar is a measure of how well the data from the 16 runs fit the model, and Q2 shows the capability to predict the different responses. High values of both R2 and Q2 for retention time and distance between ions, show that these responses can be well predicted by the model.

To find the parameters that govern the different responses, a model for each response was made and influences were displayed as in Fig. 5 for phosphate. It can clearly be seen that it was pH and ion strength in the second eluent that were important. The other parameters, not shown here, have been taken away from the model as they had no significant influence.

The most important responses were investigated with respect to what parameters that effected them, and a prediction of chromatographic conditions for optimal runs were made from these parameters.

Three of the predictions for good separation of nitrate and phosphate were run and compared to the predictions (Table 2). There was very good agreement between prediction made from the model and experimental results. The resulting chromatogram from the best run can be seen in Fig. 6. If this chromatogram is compared to the one in Fig. 1, where the same standard is used but with dilution of the sample, it is clear that the method presented in this paper is an improvement compared with the original ion chromatographic method.

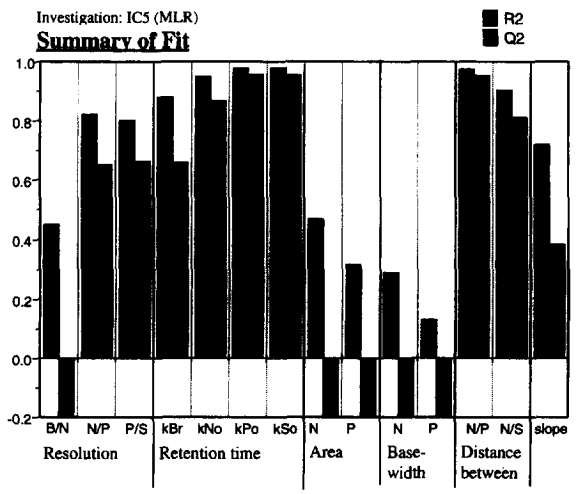

Fig. 4. R2 shows how well the data from the runs fit the model, Q2 shows how well the model can predict a certain parameter. B=bromide, N=nitrate, P=phosphate, S=sulphate.

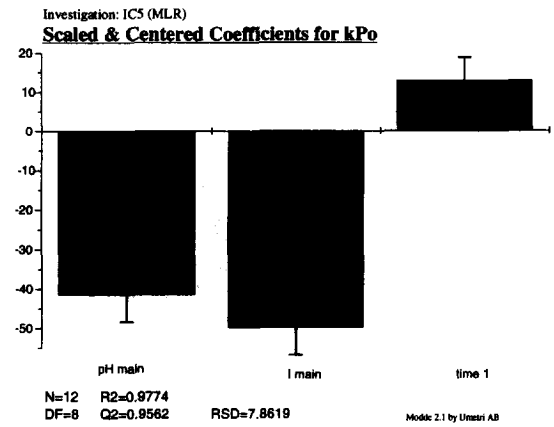

Fig. 5. Influence of different chromatographic parameters on the response factor: *k*PO: retention time for phosphate.

3.2. Determination of nitrate and phosphate

In marine sediment pore water, phosphate normally increases and nitrate decreases with depth into the sediment, making it important to design the standard curves the same way. When phosphate concentration reaches above 30 μM, it might be necessary to dilute the sample 1:1 to avoid bad resolution with nitrate, if nitrate would still be present. The calibration curves presented here are constructed so that increased nitrate concentration corresponds to decreased phosphate concentration.

The calibration curve of nitrate from this separation method is strongly dependent on salinity as can be seen in Fig. 7. The lower the salinity, the larger the response area. This would be expected as lower salinities will not overload the column as much and the initial separation on the pre-column will be more efficient. It would be possible to find chromatographic conditions for all different salinities that would produce the same chromatogram for the same concentrations of nitrate and phosphate. We chose to have the same chromatographic conditions for all salinities and rather change the salinity of the

Table 2
Calculated results from the prediction of the model, compared to the experimental results for the same input values

Input values for prediction			Predictions and experimental results					
pH main	*I* main	Time 1	k' N_p±S.D.	k' N_{exp}	k' P_p±S.D.	k' P_{exp}	k' S_p±S.D.	k' S_{exp}
9.50	19.00	0.40	225±6	224	237±7	233	286±12	284
9.50	**20.00**	**0.40**	**222±6**	**221**	**228±8**	**230**	**272±14**	**277**
9.40	20.00	0.40	225±6	221	235±8	230	285±14	279

The predictions show excellent agreement with the experimental results, proving the model to be fully functional for our purposes. The chromatogram from the highlighted run (printed in bold) is shown in Fig. 6.

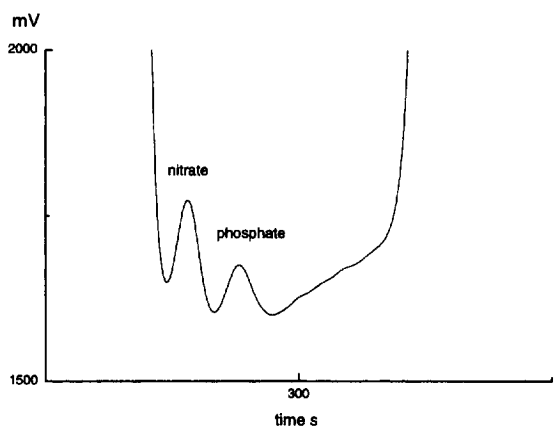

Fig. 6. Example of chromatogram for nitrate and phosphate using the new method with two hydrogencarbonate–carbonate eluents. Chromatographic conditions; eluent 1; pH 9.16, ion strength 1.4 m*M*, eluent 2; pH 9.5, ion strength 20 m*M*, *t*1=0.4 min, *t*2=1.4 min, flow=3 ml/min. Concentration of both nitrate and phosphate is 5 μ*M*.

standards. The calibration curves shown here are based on a salinity of 35, which represents high salinity seawater.

Linearity was observed for both nitrate and phosphate in concentrations valid for pore water; 1–30 μ*M* (Fig. 8). Repeatability measured as standard deviation was 0.15 μ*M* for nitrate and 0.25 μ*M* for phosphate in the 1–10 μ*M* range.

Detection limits were determined according to Miller and Miller [13], and were 0.8 and 1 μ*M* for nitrate and phosphate, respectively. A system peak appears at the same retention time as nitrate, making it difficult to further lower the detection limit. This peak is most likely caused by the change of both pH and ion strength of the eluent. The somewhat higher detection limit and repeatability for phosphate is most likely due to phosphate being a weak acid. A high level of sodium in the sample, as in a sea water sample, will cause an accumulation of hydrogen ions

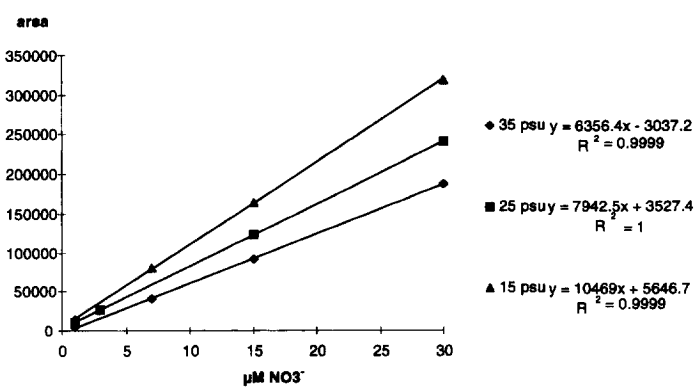

Fig. 7. Relation between peak area for nitrate and salinity.

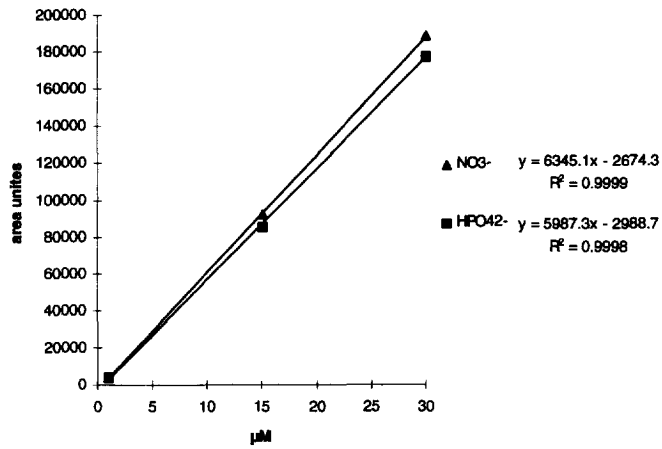

Fig. 8. Calibration curves for nitrate and phosphate at a salinity of 35.

at the surface of the suppressor membrane [14]. Phosphate can then be protonated: $HPO_4^{2-} + H^+ \rightarrow H_2PO_4^-$ causing a decrease in conductivity and thereby lowering the sensitivity.

4. Conclusions

Lower detection limits for nitrate and phosphate in sea water using ion chromatography have been shown. Using experimental design and a second switching valve as suggested by Naish, interference of a high chloride concentration can be reduced substantially and detection limits for nitrate and phosphate lowered five-fold.

Acknowledgments

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