Characterization of Seawaters by Ion Chromatography – Comparison of ionic patterns and balances

Bárbara Anes[1], Cristina Oliveira[1], Ricardo J. N. Bettencourt da Silva[1], M. Filomena Camões[1]

[1] *Centro de Química Estrutural, Faculdade de Ciências da Universidade de Lisboa, Ed.C8 Campo Grande, 1749-016 Lisboa, Portugal, bvanes@fc.ul.pt*

Abstract **– Accurate characterization of seawater composition is required to understand chemical changes occurring in the ocean and their impact on marine ecosystems.**

In this work, three different seawater samples collected from the Portuguese Coast were analyzed by Ion Chromatography. For matrix comparison purposes, the same procedure was performed in two samples collected in two different locations in the Baltic Sea, known to be composed essentially by brackish water with a salinity gradient typical of estuaries. Special focus on calcium and magnesium ions is presented since both ions are essential for biological processes in the ocean's environment.

The uncertainty associated with the results of ionic composition were evaluated to further use in the ionic balance and to assess the constancy of the proportion of major ionic components in the analysed seawaters. The collected information is of key usefulness for characterizing major components and for determining long-term trends or spatial variations of seawater composition.

I. INTRODUCTION

The distribution of chemical species in natural seawater is strongly influenced by chemical and biological processes that occur in the ocean. According to the Forchhammer's principle or Principle of Constant Proportions, the total amount of dissolved solids (salinity) in open seawater varies, but the proportion of the major ions in oceanwater remains practically constant. The observation of this principle is assumed to be valid for the tested coastal waters.

Eleven components (Na^+; K^+; Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, Br^-, F^-, NO_2^-, NO_3^-, PO_4^{3-}) are acknowledged to be present in seawater [1], although the major ions, sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), chloride (Cl^-) and sulfate (SO_4^{2-}), are the ones that are generally considered, Table 1.

Table 1. Major ions of surface seawater for salinity, S = 35 ‰ [1] *(information provided without uncertainty).*

Ion	g kg^{-1}	mol L^{-1}
Cl^-	19.35	0.5452
Na^+	10.77	0.4685
SO_4^{2-}	2.712	0.0282
Mg^{2+}	1.290	0.0531
Ca^{2+}	0.4121	0.0103
K^+	0.399	0.0102
Br^-	0.0673	8.42×10^{-4}
F^-	0.0013	6.84×10^{-5}

Monitoring the ionic composition of seawater is essential for oceanographic studies. Ion chromatography (IC) is the most widely applied analytical method for the determination of ionic species [2], [3].

The high chloride content is challenging due to its possible interference on the IC quantification of other anions. In order to overcome this interference, two detection systems were used, conductimetric for major cations and anions and UV-VIS detection for minor anions.

Through the analysis of the ionic composition of seawater it is also possible to assess some other parameters, important for seawater characterization.

Calcium and magnesium ions deserve special attention since both ions are essential for biological processes in the ocean's environment. Marine organisms use Ca^{2+} and carbonate ions (CO_3^{2-}) present in ocean waters, to build skeletons, scales, shells and teeth. Also, Mg^{2+}, the third most abundant ion in seawater, behind sodium and chloride, is an important component of chlorophyll, essential for photosynthesis. Seawater hardness is a measure of the amount of calcium and magnesium in solution, Eq. (1), hence it is an important quantity for the development and reproduction of living marine animals and plants in seawater.

$$\text{Hardness/mg eq. CaCO}_3\ \text{L}^{-1} = (2.497\ [\text{Ca}^{2+}] + 4.118\ [\text{Mg}^{2+}])/\text{mg L}^{-1} \qquad (1)$$

The $[Mg^{2+}]/[Ca^{2+}]$ ratio is relevant for both the calcification process and the final shell composition. This ratio has a fundamental importance in the biological processes of aquatic animals and it is particularly important in aquaculture processes when natural environment must be recreated. For open ocean waters, the expected ratio is close to 5, while in freshwaters it tends to be less than 1 [4].

In this work, the determination of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, F^-, NO_2^-, Br^-, NO_3^- and PO_4^{3-} was conducted by IC in three seawater samples from the Portuguese Coast. For matrix comparison purposes, the same type of analysis was performed in two samples collected in two different locations in the Baltic Sea, known to be composed essentially by brackish water with a salinity gradient typical of estuaries.

Prior to the analysis of the samples, four equidistant calibrators (standard solutions) were prepared for cations and anions determination. Calibrators were prepared from a multi-ion solution previously prepared from stock solutions of each ion.

The calibration intervals were validated by checking the assumptions of the used least-squares regression model (LSRM) from detailed information on calibrators preparation and replicate injections of each calibrator. The assumptions of the LRSM are: a) Linearity of the variation of the signal (indication according to the International Vocabulary of Metrology [5]); b) Homogeneity of the variance of the signal (homoscedasticity); c) Negligible uncertainty of the ratio of the value of any pair of calibrators given the precision of the ratio of respective signals [6].

Homoscedasticity was verified through Levene's test [7] performed on the variance estimated from 4 to 8 replicate values of the calibrators obtained under intermediate precision conditions.

Linearity was tested by ANOVA-Lack of Fit test based on the same replicate readings.

The quality of calibrators was also checked as described by Bettencourt da Silva and Camões [7].

After proving that the assumptions of the LSRM are valid, the uncertainty from quantifications performed by interpolating the sample signal in the calibration curve is evaluated by combining the standard deviation of the interpolation, estimated by the regression model, with the uncertainty from the calibrators values.

The ionic composition of the tested samples was evaluated by a *bottom-up* approach based on LSRM using an MS-Excel Spreadsheet for designing valid least-squares calibrations [7], [8]. The values reported with expanded uncertainty (k = 2, for 95% confidence level) allow an objective characterization of the seawater.

II. EXPERIMENTAL

A. Sample Collection

Seawater samples were collected at three sampling sites in the Portuguese coast: Port of Lisbon (A1), Cabo Espichel (A2) and Sesimbra (A3). In the Baltic Sea, samples were collected in two different locations (B1 and B2). The sampling sites are represented in Fig.1.

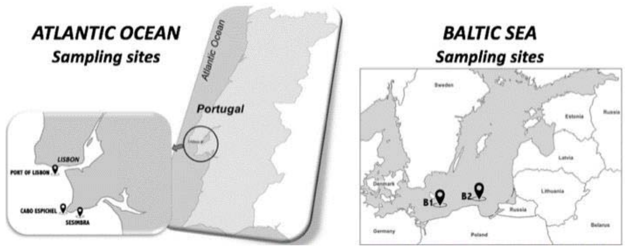

Fig. 1. Location of sampling sites in the Portuguese Coast and in Baltic Sea
(Adapted from GinkgoMaps - http://www.ginkgomaps.com and from d-Maps.com - http://d-maps.com/carte.php?num_car=5038&lang=en)

Samples were collected at 30 cm depth into clean plastic bottles fitted with tight-fitting caps at temperatures ranging from 15 °C to 18 °C.

B. Solutions preparation

All chemicals used for the preparation of both calibrators and eluents were of analytical reagent grade and the water used in all steps was obtained from a Milli-Q Academic system from Millipore® (18.2 MΩ cm).

For IC analysis, four equidistant calibrators of cations and anions were prepared from multi-ion solutions (of cations and anions, respectively) previously prepared from known concentration stock solutions of each ion. Table 2 presents the concentration (mg L^{-1}) of the calibrators used to build the calibration curves.

Table 2 - Concentrations (mg L^{-1}) of the calibration standard solutions, SS, for the cations and anions analysis

Ion	SS 1	SS 2	SS 3	SS 4
Cl^-	50	100	150	200
Na^+	25.112	50.139	75.254	100.231
SO_4^{2-}	6.29	12.59	18.88	25.17
Mg^{2+}	2.469	4.930	7.399	9.855
Ca^{2+}	0.882	1.761	2.643	3.520
K^+	0.805	1.608	2.413	3.215
Br^-	0.350	0.700	1.050	1.400
PO_4^{3-}	0.2075	0.415	0.6225	0.830
NO_2^-	0.038	0.075	0.113	0.150
NO_3^-	0.038	0.075	0.113	0.150
F^-	0.005	0.010	0.015	0.020

The eluent for the analysis of cations was a 20 mmol L^{-1} MSA (methanosulfonic acid) solution and for the analysis of anions it was a 9 mmol L^{-1} Na_2CO_3 solution. Due to their high content in chloride ions, samples were conveniently diluted. For the analyses performed with conductivity detection for both cations and anions, samples from the Portuguese Coast were diluted 1:200 and those from the Baltic Sea 1:50. For the experiments using UV-VIS detector, the sample dilution factors were 1:50 for the Portuguese Coast and 1:20 for the Baltic Sea.

C. Equipment and analytical conditions

Cationic IC measurements (Na^+, K^+, Mg^{2+}, Ca^{2+}) were performed in a Dionex® DX500 system with conductivity detection (CD20), equipped with Peaknet® software. The chromatograph was equipped with an isocratic pump IP20, a cation pre-column IonPack CG12A 4x50 mm, an analytical column IonPack CS12A 4x250 mm, a suppressor CSRS 300 - 4 mm and a loop of 25 µL. The instrumental conditions, a flow rate of 1.2 mL min^{-1}, the detector current of 50 mA and an output range of 1 mS, were chosen to get an adequate resolution of peaks and sensitivity.

Anions were analysed in a 881 Compact IC pro system from Metrohm®, equipped with a conductivity detector (used to analyze Cl^-, SO_4^{2-}, and F^-), with chemical suppression (H_2O/H_2SO_4) or an UV-VIS detector (for NO_2^-, Br^-, NO_3^- and PO_4^{3-}), an IonPacAS9-HC separation column with an AG9-HC guard-column and a loop of 100 µL. The flow rate was of 1.0 mL min^{-1}. MagIC Net 2.3 software was used for instrument control, data acquisition and processing.

The presence of anions was previously tested in the three analysed seawater samples. Through the analysis of the chromatograms, F^-, NO_2^-, NO_3^- and PO_4^{3-} were not detected in the diluted seawater samples. Hence, the working range and the LSRM assumptions were not studied for any of these anions.

III. RESULTS AND DISCUSSION

The working ranges for each type of ions were validated by collecting 4 to 8 replicate signal values for each standard solution.

The areas of the chromatogram, expressed in μS (for conductivity detectors) and mAU (for UV-VIS detector), were tested for the homogeneity of the variances and the linearity of their variation with ion concentration. Homoscedasticity, tested through Levene's test, showed that the measured areas are homoscedastic with a confidence level of 95 %; i.e., have statistically equivalent variances. Since this condition was verified, the linearity of the variation of the areas with the ion concentration was tested by the ANOVA lack of fit test that showed that the linear LSRM fits the instrumental response for all the studied ions with a confidence level of 95 %.

Analytical limits, LoD (Limit of Detection) and LoQ (Limit of Quantification), were determined based on the residual standard deviation [7]. Table 3 shows the values of the analytical limits as well as the detector used in the determination. For K^+ and Br^-, the calculated LoD is larger than the lower calibration point (SS1). However, this fact did not affect the uncertainty modelling that allows assessing if reported results have a fit-for-purpose uncertainty.

Table 3 - LoD, LoQ (mg L^{-1}) and used IC detector

Ion	LoD	LoQ	IC detector
Cl^-	12.6	38.2	Conductimetric
Na^+	1.163	3.523	Conductimetric
SO_4^{2-}	0.932	2.825	Conductimetric
Mg^{2+}	0.873	2.644	Conductimetric
Ca^{2+}	0.429	1.301	Conductimetric
K^+	0.800	2.425	Conductimetric
Br^-	0.608	1.843	UV-VIS

The uncertainty associated to each concentration value was evaluated by combining the standard deviation of the interpolation of the sample signal in the calibration curve estimated by the regression model, with the uncertainty from calibrators values [7], [9], [10].

Table 4 presents the estimated ion concentrations of seawater samples with the respective expanded uncertainty estimated for a confidence level of 95 % with a coverage factor k = 4.302653. The coverage factor corresponds to the Student's t for 2 degrees of freedom (n-2) and a confidence level of 95 % since the calibration curve was built from 4 signals (n).

Table 4 - Results of the mass concentration, c (g L^{-1}), of cations and anions in the analysed seawater samples

Ion	$(c \pm U)$ / g L^{-1}				
	Atlantic Ocean			Baltic Sea	
	A1	A2	A3	B1	B2
Cl^-	19.7 ± 1.9	19.5 ± 2.2	21.4 ± 2.2	4.06 ± 0.51	4.09 ± 0.64
Na^+	11.04 ± 0.33	11.01 ± 0.35	11.99 ± 0.36	2.282 ± 0.076	2.273 ± 0.075
SO_4^{2-}	2.73 ± 0.15	2.65 ± 0.15	3.11 ± 0.28	0.565 ± 0.087	0.585 ± 0.110
Mg^{2+}	1.28 ± 0.13	1.31 ± 0.17	1.43 ± 0.13	0.261 ± 0.033	0.248 ± 0.033
Ca^{2+}	0.433 ± 0.063	0.416 ± 0.081	0.449 ± 0.064	0.107 ± 0.016	0.101 ± 0.016
K^+	0.41 ± 0.12	0.42 ± 0.15	0.45 ± 0.12	0.084 ± 0.029	0.085 ± 0.029
Br^-	0.066 ± 0.026	0.067 ± 0.026	0.067 ± 0.032	0.0140 ± 0.0090	0.0140 ± 0.0090
Hardness	6.33 ± 0.56	6.42 ± 0.73	7.00 ± 0.56	1.34 ± 0.14	1.27 ± 0.14
Mg^{2+}/ Ca^{2+}	2.94 ± 0.52	3.14 ± 0.74	3.18 ± 0.54	2.44 ± 0.48	2.46 ± 0.51
Salinity	35.6 ‰	35.4 ‰	38.6 ‰	7.4 ‰	7.3 ‰

Total hardness of each sample was calculated by Eq. (1) and reported with uncertainty. The uncertainty of calcium and magnesium measurements were combined using the law of propagation of uncertainties assuming atomic weights are associated with negligible uncertainty [9], [10].

Based on the UNESCO publication [11], the salinity, S, was estimated by its relationship with chlorinity, Cl ($S = 1.80655$ Cl).

Seawater samples from the Portuguese Coast have the characteristics of very hard water. The obtained results match the expected values for seawater hardness (≥ 6 000 mg/L). For the Baltic Sea, total hardness lower value is explained by the relevant influence of river discharges responsible for different contents of Mg^{2+} and Ca^{2+} ions.

The values of the Mg^{2+}/ Ca^{2+} ratio, Table 4, found in all the Portuguese Coast samples are very similar and slightly bigger than those of Baltic Sea revealing the influence of brackish waters.

In order to test the principle of constant proportions of major components of seawater, it was estimated the proportions of quantified ions given the mass concentration of sodium. Table 5 presents that results reported with expanded uncertainty to allow the objective comparison of data.

The principle of constant proportions was observed for the tested samples since the proportions of each ion referenced to the mass concentration of sodium are metrologically compatible (i.e. metrologically equivalent) for a confidence level of 99 %.

Table 5. Composition ratio, expressed in %, between each tested ion and Na^+ concentration for each tested seawater sample.

Ion ratio	Atlantic Ocean A1	Atlantic Ocean A2	Atlantic Ocean A3	Baltic Sea B1	Baltic Sea B2
K^+/Na^+	2.17 ± 0.64	2.23 ± 0.80	2.22 ± 0.59	2.17 ± 0.75	2.21 ± 0.75
Mg^{2+}/Na^+	10.9 ± 1.2	11.2 ± 1.5	11.3 ± 1.1	10.8 ± 1.4	10.3 ± 1.4
Ca^{2+}/Na^+	2.25 ± 0.33	2.17 ± 0.43	2.15 ± 0.31	2.68 ± 0.41	2.54 ± 0.41
Cl^-/Na^+	116 ± 12	115 ± 13	115 ± 12	115 ± 19	117 ± 15
Br^-/Na^+	0.17 ± 0.07	0.17 ± 0.07	0.16 ± 0.08	0.18 ± 0.11	0.18 ± 0.11
SO_4^{2-}/Na^+	5.91 ± 0.37	5.76 ± 0.37	6.21 ± 0.59	5.9 ± 1.2	6.16 ± 0.94

Considering that the sum of the number of positive charges of cations should be equal to the sum of the number of negative charges of anions, a charge balance, in meq L^{-1}, was performed using Eq. (2) for all the analysed samples. The denominators are the molar masses of the ions associated with a negligible uncertainty given the uncertainty of the determined mass concentrations. If the quantified ions are the major ionic components of the seawater, the difference between the number of positive and negative changes should be metrologically equivalent to zero.

$$\frac{\frac{[Na^+]}{mg\,L^{-1}}}{22.99} + \frac{\frac{[K^+]}{mg\,L^{-1}}}{39.10} + \frac{2\times\frac{[Mg^{2+}]}{mg\,L^{-1}}}{24.31} + \frac{2\times\frac{[Ca^{2+}]}{mg\,L^{-1}}}{40.08} - \frac{\frac{[Cl^-]}{mg\,L^{-1}}}{35.45} - \frac{\frac{[Br^-]}{mg\,L^{-1}}}{79.90} - \frac{2\times\frac{[SO_4{}^{2-}]}{mg\,L^{-1}}}{96.07} = 0 \quad (2)$$

Table 6. Ionic balance parameters calculated by Eq.(2)

Ionic balance/ meq L^{-1}	Atlantic Ocean A1	Atlantic Ocean A2	Atlantic Ocean A3	Baltic Sea B1	Baltic Sea B2
∑ Cations	617 ± 18	618 ± 21	673 ± 19	128 ± 4	127 ± 4
∑ Anions	613 ± 54	607 ± 62	668 ± 62	126 ± 18	128 ± 15
Charge balance	4.2 ± 57	10.5 ± 66	5.2 ± 65	1.8 ± 19	-1.2 ± 15

The fact that the confidence intervals estimated from Eq.(2), Table 6, include the zero value (i.e. $d-U < 0$ and $d+U > 0$ or $|d| < U$; where d is the difference between the number of moles of positive and negative charges and U is the respective expanded uncertainty for 95 % confidence level ($k = 4.30$), demonstrates that the seawaters' major ions have been quantified.

The small but consistent excess of positive charges in the performed ionic balance can be explained by the not quantified presence of HCO_3^- and CO_3^{2-} (about 2.4 mmol L^{-1} for Atlantic Ocean waters and 1.7 mmol L^{-1} for the Baltic Sea, according to published values for TA [13]).

IV. CONCLUSIONS

Natural waters are dynamic systems with variable inputs and outputs of mass and energy. The analysis of the composition of natural seawaters is essential for a great variety of chemical and biological studies.

The different ranges of concentration of major and minor species present in seawater were a challenge for the optimization of the methodology used to assess the composition of seawater samples by ion chromatography. In this work, three runs were performed, one for cations and two for anions using different dilution ratios and different detectors (Condutimetric and UV-VIS).

After validating the measurement procedure, three seawater samples from the Portuguese Coast were analysed, allowing their characterization in terms of cations and anions concentration, salinity, total hardness and ions proportions, bringing additional and important information about the composition of Atlantic Ocean Coastal waters.

The results are in a good agreement with the reference values for Atlantic Ocean seawaters and the Forchhammer's principle was confirmed.

The tested Atlantic Ocean waters have a small but consistent "excess" of positive charges between its cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (Cl^-, SO_4^{2-}, Br^-). The difference found is explained by measurements uncertainty, nevertheless, it should involve the balance of the dissolved CO_2 (CO_2(aq), HCO_3^-, CO_3^{2-}) and by not quantified anions eventually present in the tested samples (F^-, NO_3^-, NO_2^-).

The carbonate and hydrogen carbonate ions cannot be determined by the used chromatographic system, since the IC eluent is itself a carbonate solution.

Two samples collected in two different locations in the Baltic Sea were also tested for composition comparison purposes. Due to its special hydrographical and climatic conditions, the Baltic Sea is considered to be composed by brackish water, as verified by the results presented in this work.

The successful ionic balance of seawater components, highlights ion chromatography as an important tool for the determination of seawater ionic composition.

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