



VALIDATION OF AN ANALYTICAL QUANTITATIVE DETERMINATION METHOD OF CHLORIDE ANION FROM DRINKING AND SURFACE WATER, USING DIRECT POTENTIOMETRY WITH CHLORIDE-SELECTIVE ELECTRODE (I)

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abstract The purpose of this study was to elaborate, develop and validate an analytical method for quantitative determination of chloride anion from drinking and surface water using direct potentiometry with Cl⁻-selective electrode. The quantitative determinations of chloride ion realized in this way are quick and relatively cheap. It will be proved that such a method is accurate, precise, linear, sensitive and selective, presenting an adequate linear working range. In this first part will be proved that the method presents an appropriate working range and that is linear.

key words validation, analytical method, ion selective electrodes, potentiometry.

Introduction

The possibility of quantitative determination of Cl⁻ from drinking and surface water using direct potentiometry with Cl⁻-selective electrode constitutes an opportunity for routine measurements / tests.

Analytical method validation is a “necessary evil” for the quality assurance and production processes safety, but represents the first step or the first level of quality assurance within a testing and measurement analytical laboratory. Validation is realized according to national and international standards (ISO 9000, ISO 17025), but also according to BPL-OECD norms regarding good laboratory practice within testing and measurement in analytical laboratory (GLP) (HG 63/2002 modified with HG 266/2006 and HG 448/2007).

For chloride anion determination from waters are used, with good results, both gravimetric and volumetric analysis methods, as well as instrumental analysis methods. Classical methods presume gravimetric determination by insoluble chloride precipitation, or volumetric titration [1÷4]. From instrumental methods can be reminded UV-VIS molecular absorption spectrometry (mercury thiocyanate method introduced by Japanese scientists [5÷7] in 1952, mercury chloroanilate [8,9]), atomic absorption spectrometry (indirect method) [10,11] and direct and indirect potentiometry [12÷18].

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Elaboration, development and validation of a quantitative determination method for Cl^- using direct potentiometry will prove that this method is quick and cheap and shows some performance characteristics like accuracy, precision, linearity, sensibility, applicability and an adequate linear working range. It was chosen this way because the level of chloride concentration in drinking and surface waters fits relatively well with the working range of the Cl^- -selective electrode. It is known that "Quality norms that surface waters used to obtain potable water must comply with – NPTA 013" (HG 100/2002 modified with HG 662/2005 and HG 567/2006) accept a maximum chloride level of $200 \text{ mg}\cdot\text{L}^{-1}$.

In this paper it will be presented only the second part of the method validation: characterization of the performance parameters of the method. The proposed analytical method makes possible quantitative determination of chloride ion from drinking and surface waters by direct potentiometry with Cl^- -selective electrode as working electrode.

Direct potentiometry ($i = 0$) is a well known technique, that can supply a determination method of a ion concentration (cation or anion) by measuring emf (electromotive force) of a potentiometric cell with liquid junction, made of an working electrode (chloride ion-selective electrode) and a reference electrode [19,20].

Experimental

All the used reagents had analytical purity quality. A 10.000 ppm chloride stock solution (WTW Germany, NIST (National Institute of Standards and Technology) traceable) was used to obtain standard solutions. KNO_3 was used to obtain the inert electrolyte solution ($1 \text{ mol}\cdot\text{L}^{-1}$). For standard and working solutions preparation adequate volumes of stock standard solution were measured and then they were put in volumetric flasks. Tri-distilled water was used for all solutions preparation and for vessel cleaning.

The necessary measurements for quantitative determination of chloride ion from drinking and surface waters have been carried out using a pH/mV-metre WTW Inolab 740, with 6 point calibration; chloride-selective electrode as working electrode; R503/D-WTW reference electrode; 50, 100, 500, 1000 mL volumetric flasks (A class); 100 mL Berzelius glasses; 1, 2, 5, 10 mL pipettes (A class).

In order to prepare the standard solutions, in 50 mL volumetric flasks were put: fixed volumes (5 mL) of 1 M KNO_3 solution, increasing volumes of standard chloride solution (100 ppm) and tri-distilled water. Drinking or surface water samples were prepared as follows: in a 50 mL volumetric flask was added 25 mL from the water sample, 5 mL inert electrolyte solution ($1 \text{ mol}\cdot\text{L}^{-1} \text{ KNO}_3$) and tri-distilled water. The samples were decanted into the potentiometric cell and emf was measured.

Results and discussions

According to ICH (International Conference of Harmonization) recommendations [21,22] for analytical methods validation, the following performance parameters must be taken in consideration: selectivity / specificity, working range, linearity, precision, accuracy,

detection limit, quantification limit, robustness. From these, in this first part will be discussed the working range and the linearity.

Before establishing the working range, the chloride-selective electrode response is followed up on a wide concentration range ($1 \cdot 10^{-6}$ – $1 \cdot 10^{-1}$ mol·L⁻¹), in aqueous medium of 0.1 mol·L⁻¹ KNO₃. The experimental results are graphically represented in Fig. 1. They emphasize that the response curve of chloride-selective electrode presents a sigmoid form; there is a large enough concentration range for which the dependence is linear; this can be used for quantitative determination of chloride ion.

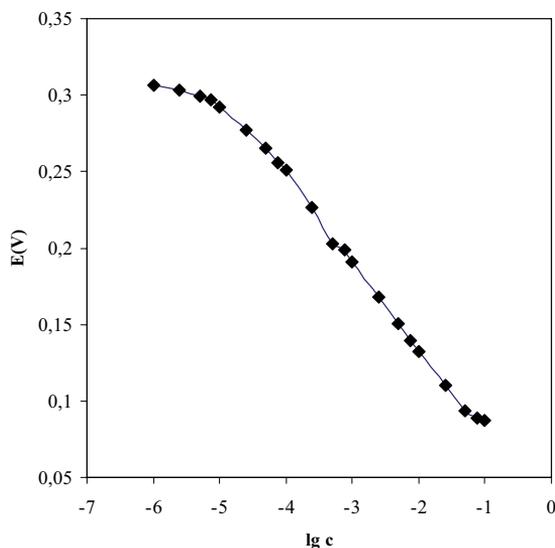


Fig. 1 The response curve $E = \lg(c_{\text{Cl}^-})$ for chloride-selective electrode
 Cl-500-WTW, in 0.1 mol L⁻¹ KNO₃

1. Working concentration range

The working concentration range, or, shortly, working range, is specific for determination of each analyte and is established to confirm the fact that the developed analytical procedure provides an acceptable linearity, accuracy and precision degree when applied to samples including the analyzed species inside or at the limits of the specified range.

In the working concentrations range, the analytical response may be or may not be linear and, in order to prove this fact, it is necessary to realize several calibration points (more than 6 different concentrations of the analyte, preferable 10-11). If the relationship between the instrument response and analyte concentration is not perfectly linear (it may be an exponential relationship), the analytical procedure can be used, but the calibration curve has to be reported on daily basis.

Establishing the working concentration range can be done in two ways: using multi-points calibration or graphically: representing $E/\lg c = f(\lg(-\lg c))$.

If the working range is established by calibration [23], then is worth mentioning that every calibration experiment begins with selection of a preliminary working range. The informative values are presented in Table 1 (for the lowest concentration were made 10 replicates and for the highest concentration were also made 10 replicates). Informative concentration values were equidistantly distributed on the entire established preliminary working range.

Table 1 Calibration data for establishing the working range used for quantitative determination of chloride from drinking and surface waters by direct potentiometry with chloride-selective electrode (preliminary range $1 \cdot 10^{-5} - 2,5 \cdot 10^{-3}$ mol L⁻¹)

i	j	x_i (mol L ⁻¹)	$y_{i,1}$	$y_{i,2}$	$y_{i,3}$	$y_{i,4}$	$y_{i,5}$	$y_{i,6}$	$y_{i,7}$	$y_{i,8}$	$y_{i,9}$	$y_{i,10}$
1		$1.0 \cdot 10^{-5}$	0.3025	0.2975	0.2973	0.2964	0.2967	0.2969	0.2970	0.2971	0.2966	0.2966
2		$2.5 \cdot 10^{-5}$	0.2868									
3		$5.0 \cdot 10^{-5}$	0.2650									
4		$7.5 \cdot 10^{-5}$	0.2557									
5		$1.0 \cdot 10^{-4}$	0.2509									
6		$2.5 \cdot 10^{-4}$	0.2269									
7		$5.0 \cdot 10^{-4}$	0.2092									
8		$7.5 \cdot 10^{-4}$	0.1989									
9		$1.0 \cdot 10^{-3}$	0.1910									
10		$2.5 \cdot 10^{-3}$	0.1676	0.1658	0.1679	0.1665	0.1685	0.1662	0.1689	0.1652	0.1661	0.1669
		$\bar{y}_1 = 0.28746$		$s_1 = 0.00180207$					$s_1^2 = 3.25156 \cdot 10^{-6}$			
		$\bar{y}_{10} = 0.16696$		$s_{10} = 0.0122$					$s_{10}^2 = 1.49 \cdot 10^{-6}$			

For establishing the dispersion homogeneity, 10 repeated determinations (replicates) were done for the lowest and for the highest concentration (x_1 and x_{10}). For each of these 2 series were obtained 10 informative values y_{ij} .

The next step consists in the *dispersion homogeneity test* using the two concentration levels x_1 and x_{10} to calculate the dispersions s_1^2 and s_{10}^2 according to the formula:

$$s_i = \frac{\sum_{j=1}^{10} (y_{ij} - \bar{y}_i)}{n_i - 1}, \quad (1)$$

with \bar{y}_i - average value of e.m.f.

For $i = 1$ and $i = 10$ there were obtained: $\bar{y}_1 = 0.28746$; $s_1 = 0.00180207$; $s_1^2 = 3.25156 \cdot 10^{-6}$; $\bar{y}_{10} = 0.16696$; $s_{10} = 0.0122$; $s_{10}^2 = 1.49 \cdot 10^{-6}$.

Obtained dispersions were tested using the F test to examine the significant differences at the working concentration range limits. The F test requires the *testing value*, PG , determination starting with the formula:

$$PG = \frac{s_1^2}{s_{10}^2} \text{ for } s_1^2 > s_{10}^2, \quad (2)$$

that is $PG = 2,17554$.

The obtained PG value was compared to the table values for the F distribution. Consulting the table containing values of F (dispersion homogeneity test for $v_1 = v_2 = n - 1 = 9$ freedom degree for dispersions s_1^2 and s_{10}^2), it was obtained $F_{9;9;0,99} = 5,35$. In this case, $PG = 2,17554$ and $F_{9;9;0,99} = 5,35$, so $PG < F_{9;9;0,99}$; the deviation between dispersions s_1^2 and s_{10}^2 wasn't significant and the working range was correctly selected: $1 \cdot 10^{-5} - 2,5 \cdot 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ ($0,5 - 50 \text{ mg} \cdot \text{L}^{-1}$) Cl^- .

Comparison of calculated value PG , with the table value didn't showed significant difference. Dispersions are homogenous and are allowing the simple (linear) regression analysis.

In the *statistic linearity test*, calibration data are used to calculate a linear calibration function and also a non-linear calibration function, both of them showing a standard residual deviation s_{y_1} and s_{y_2} . Dispersions difference DS^2 can be calculated using the formula:

$$DS^2 = (N - 2)s_{y_1}^2 - (N - 3)s_{y_2}^2, \quad (3)$$

for $v = 1$ freedom degrees.

DS^2 and the dispersions of the non-linear calibration function are subject of an F test in order to examine the significant differences. The PG value requested for F test is calculated using the formula:

$$PG = \frac{DS^2}{s_{y_2}^2} = 31,75117. \quad (4)$$

Coming back to calculation, for the linearity test was obtained: standard deviation of linear regression $s_{y_1} = 0.00147358$ and $s_{y_1}^2 = 2.171 \cdot 10^{-6}$; standard deviation of a nonlinear regression $s_{y_2} = 0.000738786$ and $s_{y_2}^2 = 5.4580 \cdot 10^{-7}$.

Dispersion difference was: $DS^2 = (N - 2)s_{y_1}^2 - (N - 3)s_{y_2}^2 = 1.7329794 \cdot 10^{-5}$.

Comparing PG calculated value with $F_{9;9;0,99} = 5$, it was ascertained that $PG > F$, so the nonlinear function doesn't supplies an improvement; the calibration function that must be used is the linear function.

2. Linearity

Linearity of a quantitative analytical method represents its ability to obtain results proportional with analyte concentration from the sample. Linearity can't be expressed, it must be demonstrated directly on analyte or spiked samples, using at least five concentrations on working range.

Besides the visual evaluation of the analytical signal as a concentration function, it is recommended to do the corresponding statistical calculations, like linear regression. Reporting statistical parameters like the slope of the calibration curve, b and its intersection to the origin, a , residual values squares amount and correlation coefficient is a mandatory condition.

Linear calibration function must result from obtained data, starting from a working range from x_1 to x_{10} , as it was established starting from uncorrelated measurements for blank samples. Usually, mustn't be included any blank sample value in calibration experiments and in least squares method.

Linear calibration function is described by the equation:

$$y = a + bx \quad (5)$$

The linear model is, no doubt, the most important one for processing dimensional data. Over sizing the equations system represents the regression analysis focussing point, meaning the determination of more coordinate pairs then the obtained ones (x_1, y_1) and (x_2, y_2), requested to calculate a and b using the classic algebra. The least squares model was selected, leading to a straight line subject (submitted) to the restriction:

$$\sum (r_i)^2 = \sum (y_i - \bar{y})^2 = \text{minim} \quad (6)$$

Here, r_i represents the residual values starting with measurement i .

The estimated parameters of regression analysis realised for quantitative determination of chloride by direct potentiometry using chloride-selective electrode are presented in table 2.

Using the calibration data, it was calculated a linear calibration function ($y = a + bx$) and a nonlinear calibration function ($y = a + bx + cx^2$). The results achieved when calculating first and second degree functions are presented in Table 2.

Table 2 Experimental results and parameters values of the calculated regression functions

i	c_{Cl^-}	$x_i = \lg c_{Cl^-}$	y_i	x_i^2	x_i^3	x_i^4	y_i^2	$x_i y_i$	$x_i^2 y_i$	$y_i - (a + bx_i)$
1	$1.0 \cdot 10^{-5}$	-5.000	0.3025	2.50E+01	-1.25E+02	6.25E+02	9.15E-02	-1.51E+00	7.56E+00	-2.00E-03
2	$2.5 \cdot 10^{-5}$	-4.602	0.2818	2.12E+01	-9.75E+01	4.49E+02	7.94E-02	-1.30E+00	5.97E+00	-2.53E-04
3	$5.0 \cdot 10^{-5}$	-4.301	0.2650	1.85E+01	-7.96E+01	3.42E+02	7.02E-02	-1.14E+00	4.90E+00	-7.64E-05
4	$7.5 \cdot 10^{-5}$	-4.124	0.2557	1.70E+01	-7.01E+01	2.89E+02	6.54E-02	-1.05E+00	4.35E+00	6.06E-04
5	$1.0 \cdot 10^{-4}$	-4.000	0.2509	1.60E+01	-6.40E+01	2.56E+02	6.30E-02	-1.00E+00	4.01E+00	2.80E-03
6	$2.5 \cdot 10^{-4}$	-3.602	0.2269	1.30E+01	-4.67E+01	1.68E+02	5.15E-02	-8.17E-01	2.94E+00	1.25E-03
7	$5.0 \cdot 10^{-4}$	-3.301	0.2092	1.09E+01	-3.60E+01	1.19E+02	4.38E-02	-6.91E-01	2.28E+00	5.24E-04
8	$7.5 \cdot 10^{-4}$	-3.124	0.1989	9.76E+00	-3.05E+01	9.52E+01	3.96E-02	-6.21E-01	1.94E+00	2.06E-04

9	1.0 10 ⁻³	-3.000	0.1910	9.00E+00	-2.70E+01	8.10E+01	3.65E-02	-5.73E-01	1.72E+00	-7.00E-04
N=10	2.5 10 ⁻³	-2.602	0.1676	6.77E+00	-1.76E+01	4.58E+01	2.81E-02	-4.36E-01	1.13E+00	-1.65E-03
$\sum_{i=1}^N$		-37.656	2.3495	1.47E+02	-5.94E+02	2.47E+03	5.69E-01	-9.15E+00	3.68E+01	7.02E-04

Linear function y = a + bx

Equation	$y = 0,0564x + 0,0225$
Slope – sensibility, <i>b</i>	$b = \frac{\sum_{i=1}^N (x_i - \bar{x})(y_i - \bar{y})}{\sum_{i=1}^N (x_i - \bar{x})} = 0,0564$
Intercept, <i>a</i>	$a = \bar{y} - b\bar{x} = 0,0225$
Residual standard deviation : $s_{y_1} = \sqrt{\frac{\sum_{i=1}^N [y_i - (a + bx)]^2}{N - 2}}$	$s_{y_1} = 0,00147358$
Standard deviation of the method: $s_{x_{01}} = s_{y_1} / b$	$s_{x_{01}} = 0,026127305$
Coefficient of variation (RSD%): $V_{01} = (s_{x_{01}} / \bar{x}) \cdot 100$	$V_{01} = 0,6938418$
Standard deviation of the slope: s_b	$s_b = 0,000639912$
Standard deviation of the intercept: s_a	$s_a = 0,002454$
Correlation coefficient: <i>R</i>	$R = 0,9994$
Determination coefficient: R^2	$R^2 = 0,9989$

Non-linear function: y = a + bx + cx²

Equation of the curve	$y = -0,009681719 - 0,0740515x - 0,0023263x^2$
<i>c</i> coefficient: $c = \frac{(\varrho_{xx} \cdot \varrho_{x^3}) - (\varrho_{x^2y} \cdot \varrho_{xx})}{(\varrho_{x^3})^2 - (\varrho_{xx} \cdot \varrho_{x^4})}$	$c = -0,0023263$
<i>b</i> coefficient: $b = (\varrho_{xy} - c \cdot \varrho_{x^3}) / \varrho_{xx}$	$b = 0,0740515$
Intercept <i>a</i> : $a = (\sum y_i - b \sum x_i - c \sum x_i^2) / N$	$a = -0,009681719$
Sensibility at the centre of the working range: $E = b + 2cx$	$E = -0,056531601$
Standard deviation: $s_{y_2} = \sqrt{\frac{\sum_{i=1}^N [y_i - (a + bx + cx^2)]^2}{N - 3}}$	$s_{y_2} = 0,000738786$
Standard deviation of the method: $s_{x_{02}} = s_{y_2} / E$	$s_{x_{02}} = -0,013068541$
Variation coefficient (RSD%): $V_{x_0} = s_{x_{02}} / \bar{x}$	$V_{x_0} = 0,347050694$

Although the correlation coefficient (*R*) and the determination coefficient (R^2) obtained during the regression analysis, are often used to prove the linearity ($R > 0.997$) [24], their values are not able to do that; they can only prove if statistical processed values are correlated ($R = |1.000|$) or uncorrelated ($R = 0.000$).

An alternative approach for using the correlation coefficient to linearity establishment consists in dividing the sample signal to the corresponding concentration ($-\lg c_{Cl^-}$) and the

graphical display of these “relative responses versus $\lg(-\lg c_{\text{Cl}^-})$ ”. It must be obtained a horizontal straight line with positive deviation at low concentrations and negative deviation at large concentrations.

Conclusion

In this first part of the validation study it was proved that the method is linear and presents an appropriate linear working range.

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