A new simple and practical method for establish available chlorine

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ABSTRACT

Disinfection is a necessary and obligatory procedure in the framework of good professional practice. In practical terms, it means eliminating microorganisms capable of producing disease through the use of a physical or chemical agent.

The chlorine in the form of hypochlorite or bleach, is a powerful oxidant being disinfectant more important there because combines all the advantages required, including easy and cost convenient dosage. Available chlorine responsible for the bleaching and disinfecting functions, is defined as the oxidizing power of the chlorine content and is expressed in grams of available chlorine per liter (gCl_2/L). Chlorine concentration tends to decrease over time, resulting determination important because it relates to the minimum dose recommended use to fulfill their function a parameter being evaluated in the accreditation and certification standards institutions. The recommended method is the iodometric titration which is laborious and tedious. In this method the assay is performed for colorimetric automated by adaptation reagents routinely used in any clinical laboratory.   © 2013 Trade Science Inc. - INDIA

INTRODUCTION

Disinfection is a necessary and obligatory procedure in the framework of good professional practice. It aims to ensure the quality of the provision and security of the players involved. In practical terms, disinfection means eliminating the microorganisms capable of producing disease. In disinfection using a physical or chemical agent to destroy pathogens. Chlorine, in the form of hypochlorite or bleach, a powerful oxidant is, without doubt, the most important thing there disinfectant, because it brings together all the required benefits, including easy convenient dosing and

cost[1]. Some of its salts are also disinfecting power. The most used are calcium hypochlorite and sodium hypochlorite disinfectant whose efficiency is identical to that of chlorine and similar reactions in the water[2]. The hypochlorous acid (HClO) dissociates into hydrogen ions and hypochlorite (ClO⁻)[3,4]:

$$HClO \rightarrow H^+ + ClO^-$$

The hypochlorous acid (HClO) and hypochlorite ion (OCl⁻) are the so-called "free chlorine". By their nature, HClO is the ultimate disinfectant and its power is much greater than the hypochlorite ion (OCl⁻) (100 times)[2].

Available chlorine is defined as the oxidizing power

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of chlorine in the water bleaches (HClO + OCl⁻) and expressed in grams per liter of available chlorine (gCl_2 / L.), being responsible for the functions of whitening and disinfecting.

Hypochlorite solutions provide the same balance of ionization. For example[3,4]:

$$NaClO + H_2O \rightarrow Na^+ + ClO^- + OH^- \qquad (1)$$

$$H^+ + ClO^- \rightarrow HClO \qquad (2)$$

The reactions (1) and (2) depend on the pH of the medium. Thus, the first predominantly with low pH values   and the second with high values   . The quantities of hypochlorous acid and hypochlorite ions formed in the above reactions is equivalent in oxidizing ability, the amount of chlorine original. When the pH is less than 4.0 the chlorine is in the form of molecular chlorine (Cl_2). At pH 5.0 to 6.0; existing chlorine is in the form of hypochlorous acid. Above pH 6.0 there hypochlorite ions, which predominate when the pH exceeds 7.5[5].

The bleach stability is poor (3 months), whereby the concentration tends to decrease over time, decomposing slowly on contact with air. The rate of decomposition increases with increasing concentration of sodium hypochlorite in the solution and as the temperature increased[5].

Exposure to sunlight also accelerates decomposition[5,6]. When heated to decomposition, sodium hypochlorite releases chlorine (Cl_2), which is a toxic substance[5-7]. The concentration of available chlorine is important as it is related to the minimum dose recommended use to fulfill the functions of bleach or disinfectant and is a parameter evaluated in the accreditation standards and certification institutions requiring disinfection processes (food industries, dairy, poultry and pig farms, laboratories and medical institutions), plus excess chlorine in solution, corrosive effects exerted even stainless steel[2,3,6-10].

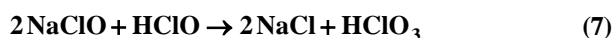The sodium hypochlorite solution are decomposed in two ways:

- to sodium chloride (NaCl) and molecular oxygen (O_2) (reaction 1)
- by disproportionation to chloride and sodium chlorate ($NaClO_3$) (reaction 2)

$$2\,NaClO \xrightarrow{\text{catalyst}} 2\,NaCl + O_2 \qquad (3)$$

$$3\,NaClO \rightarrow 2\,NaCl + NaClO_3 \qquad (4)$$
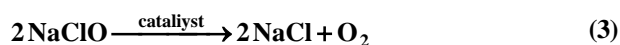
The last of these reactions occurs in two steps: an initial slow step that forms the sodium chlorite ($NaClO_2$) (reaction 3), and a fast paced disproportionation between hypochlorite and chlorite (reaction 4):

$$2\,NaClO \xrightarrow{\text{slow}} NaCl + NaClO_2 \qquad (5)$$

$$NaClO + NaClO_2 \rightarrow NaCl + NaClO_3 \qquad (6)$$
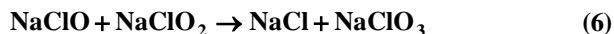
Sodium hypochlorite can also react with hypochlorous acid (reaction 5), thus:

$$2\,NaClO + HClO \rightarrow 2\,NaCl + HClO_3 \qquad (7)$$

This last equation is often used to emphasize the fact that in acid, hypochlorite decomposes more easily than in a basic medium, which is why the solutions of sodium hypochlorite at pH was maintained greater than 11[5-7,9].

According to a study on the mechanism of action of sodium hypochlorite on microorganisms, this compound acts as a solvent of organic matter, specifically fatty acids, who transformed into fatty acid salts (soap) and glycerol ($C_3H_8O_3$), reducing the surface tension of the remaining solution. Furthermore, amino acids neutralized, forming water and salts. With the decrease of hydroxyl ions (OH⁻) through the formation of water, the pH is reduced by stimulating the presence of hypochlorous acid with in contact organic components acts as a solvent, liberates chlorine combines with the amino group of the protein, forming chloramines. The hypochlorous acid and hypochlorite ions and lead to degradation of amino acid hydrolysis[2,3,8].
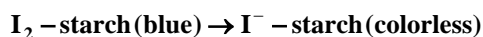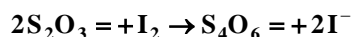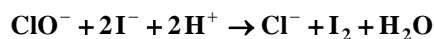
The recommended method for determining the concentration of active chlorine is iodometric titration[11,12] which is laborious and time consuming. In this new method is performed by adapting automated determination reagents routinely used in any clinical laboratory.

MATERIALS AND METHODS

Iodometric method[12]

The determination of available chlorine is a redox titration, hypochlorous acid and sodium hypochlorite present in a known volume of solution become Cl_2 in an acidic medium, which oxidizes iodide ion to I_2. The iodide ion is added to the solution in the form of potassium iodide (KI), iodine (I_2) produced was titrated with a solution of known concentration of sodium thiosulfate

$(Na_2S_2O_3)$ in the presence of starch as an indicator.

$$ClO^- + 2I^- + 2H^+ \rightarrow Cl^- + I_2 + H_2O$$

$$2S_2O_3 = +I_2 \rightarrow S_4O_6 = +2I^-$$

$$I_2 - starch\,(blue) \rightarrow I^- - starch\,(colorless)$$

Reagents and materials[12]

- Burette for titration, Iodine bottles and magnetic stirrer
- Glacial Acetic Acid
- Potassium Iodide (KI) solid
- Standardized Solution Sodium thiosulfate (Na2S2O3) 0.1 N.
- Starch Solution 1%

Assay[12]

Place the bottle for iodine with a diver on the magnetic stirrer. Dilute bleach previously shown 1:25, take 5.0 mL. Add 20 mL. distilled water, 1 mL. of glacial acetic acid, 0.5 g. of potassium iodide and 1 ml. of the starch solution. Titrate with the standardized sodium thiosulphate to turn to colorless.

Automated colorimetric method

The determination of available chlorine is a colorimetric endpoint automated method in which the hypochlorous acid and sodium hypochlorite present in a known volume of solution, produce oxidative coupling reagent (4-aminophenazone and phenol) to give a compound addition of different color (quinonimine) whose intensity is proportional to the concentration of available chlorine. As an additional advantage of the method, part of the reagent used in the Trinder reaction used to quantify glucose, cholesterol, uric acid and triacylglycerols[13]. The analytical sensitivity of the proposed reaction can be adjusted to the needs of the system and can be determined concentrations of the order of mg/L. increasing the sample / reagent and setting the parameters to that relationship (calibration curve, linearity, limit of quantification).

Reagents and materials

- Autoanalyzer or spectrophotometer
- Working Reagent: 4-aminophenazone 25 mM, 55 mM phenol. diluted 1:20 with distilled water pH 6.0. Stable for 2 months at 4 °C.
- Standard chlorine. Concentration determined by

Reference Method (3500-Cl APHA-AWWA-WPCF. - 1992)[11]. Stable at 96 hours dark sealing jar at 4°C.

Assay

The assay was performed in a clinical chemistry analyzer ROCHE COBAS 6000 ™ with the following parameters:

TABLE 1

| Temperature | 37°C. | | Wavelength | Primary | 505 nm. |
				Secondary	Cancel
Assay	1 endpoint		Calibración	2 linear points	Sense growing
Reaction Time	10 minutes		Abs. Limit	32000	Units g Cl2/L.
Sample Volume (µL.)			Volume (µL.) and Reagent Type		Calibration
Normal	16	0 0	R1	275 0 0	Cal 1. Cal.2
Decreased	8	0 0	R2	0 0 0	16 µL. 16 µL.
Increased	20	0 0	R3	0 0 0	

Statistical analysis

The data analysis was performed using the computer program InfoStat v3.35; MultiQC and Westgard.com internet site.

RESULTS

To evaluate the method developed were determined 6 (six) parameters.

√ Limit of Detection
√ Limit of Quantitation
√ Accuracy (bias)
√ Precision (intra-and Intermediate)
√ Linearity range
√ Method Comparison

Limit of detection (L_D)[14]

TABLE 2

	0,0067	0,0074	0,0069	0,0066	0,0078
	0,0074	0,0053*	0,0072	0,0073	0,0078
Abs.	0,0070	0,0069	0,0075	0,0077	0,0067
Baseline	0,0068	0,0075	0,0077	0,0078	0,0069
	0,0077	0,0066	0,0088*	0,0070	0,0074
	0,0072	0,0069	0,0078	0,0072	0,0073
Statistics	μ_B	σ_B	$L_D=$	Abs.	
	0,0072	0,00040	0,0079	0,0079	
Limit of Detection			0,50 g Cl$_2$/L.		

**Outliers*

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Limit of quantitation (L_Q)[14]

Calculated as three times the detection limit (LQ = 3xLD) 0.50 x 3 = 1.50 g Cl_2/L. As the method allows automated retest the sample test using higher fraction, our work parameters set in the analyzer contemplated increased fraction of assay of 1.25 times (20 μL./16 uL.) which improves sensitivity at low concentrations. The quantification limit was reduced to 1.20 g Cl_2/L.

Accuracy[15]

Veracity determined as expressed in terms of bias ($s = x - μ$).

TABLE 3

Concentrations (ASSIGNED = 55 g Cl_2/L.)[11]							
	57	57	57	56	59	55	56	53
Determined	53	54	54	57	52*	58	54	55
(x)	53	57	55	56	57	57	55	55
	55	58	56	56	57	57	58	57
Statistics	Average (x)		σ		(x - μ)		Bias (%)	
	55,93		1,59		+0,93		+1,69	

*Outliers

Precision (intra-and Intermediate)[16]

Determined as repeatability (intra-assay CV) and intermediate precision (interday and interassay).

TABLE 4

Concentrations g Cl_2/L.	65	66	68	69	66
	68	70	66	68	68
Repeatability High Level (Figure 1)	Average (x)		σ		CV%
	67,38		1,58		2,34
Concentrations g Cl_2/L.	12	13	14	13	13
	13	13	12	13	11
Repeatability Low Level (Figure 2)	Average (x)		σ		CV%
	12,68		0,823		6,49

To determine the intermediate precision were purchased four (4) individual sachet 250 mL commercial bleach. belonging to the same batch. Each sample was stored refrigerated at 4°C. protected from light to ensure that there is no change in concentration. Sequentially, every three days we proceeded to open a sachet, which was used to determine the content of active chlorine 3 times on successive days. Total determinations were 12 (twelve) along the same number of days.
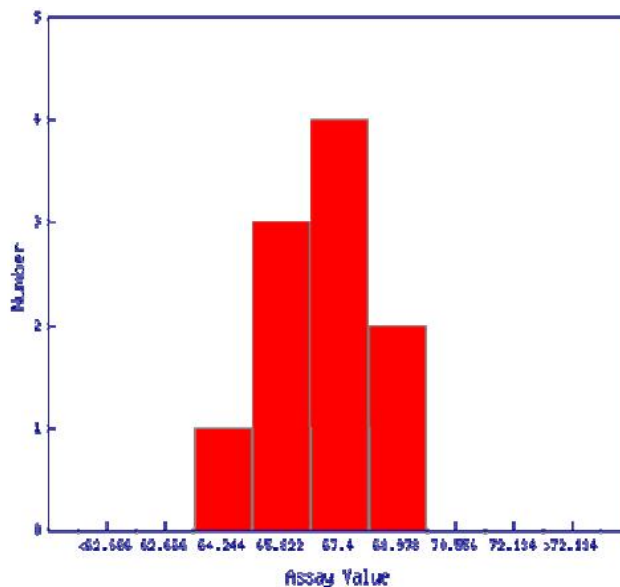

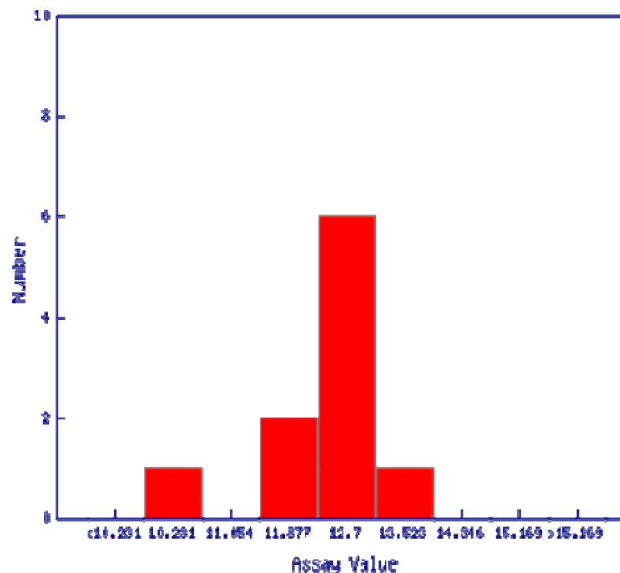**Figure 1 : Repeatability high level.**
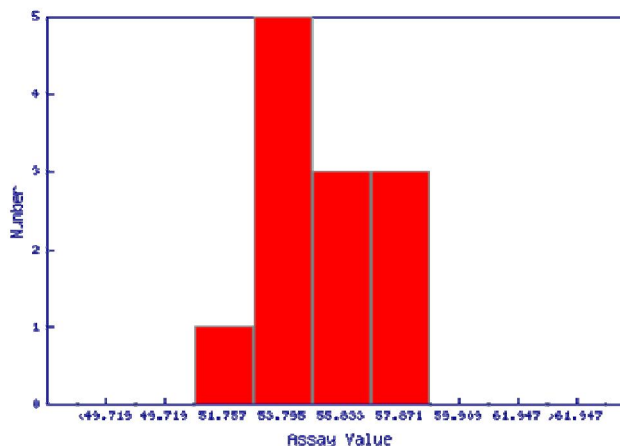

Figure 2 : Repeatability low level.

Figure 3 : Intermediate precision.
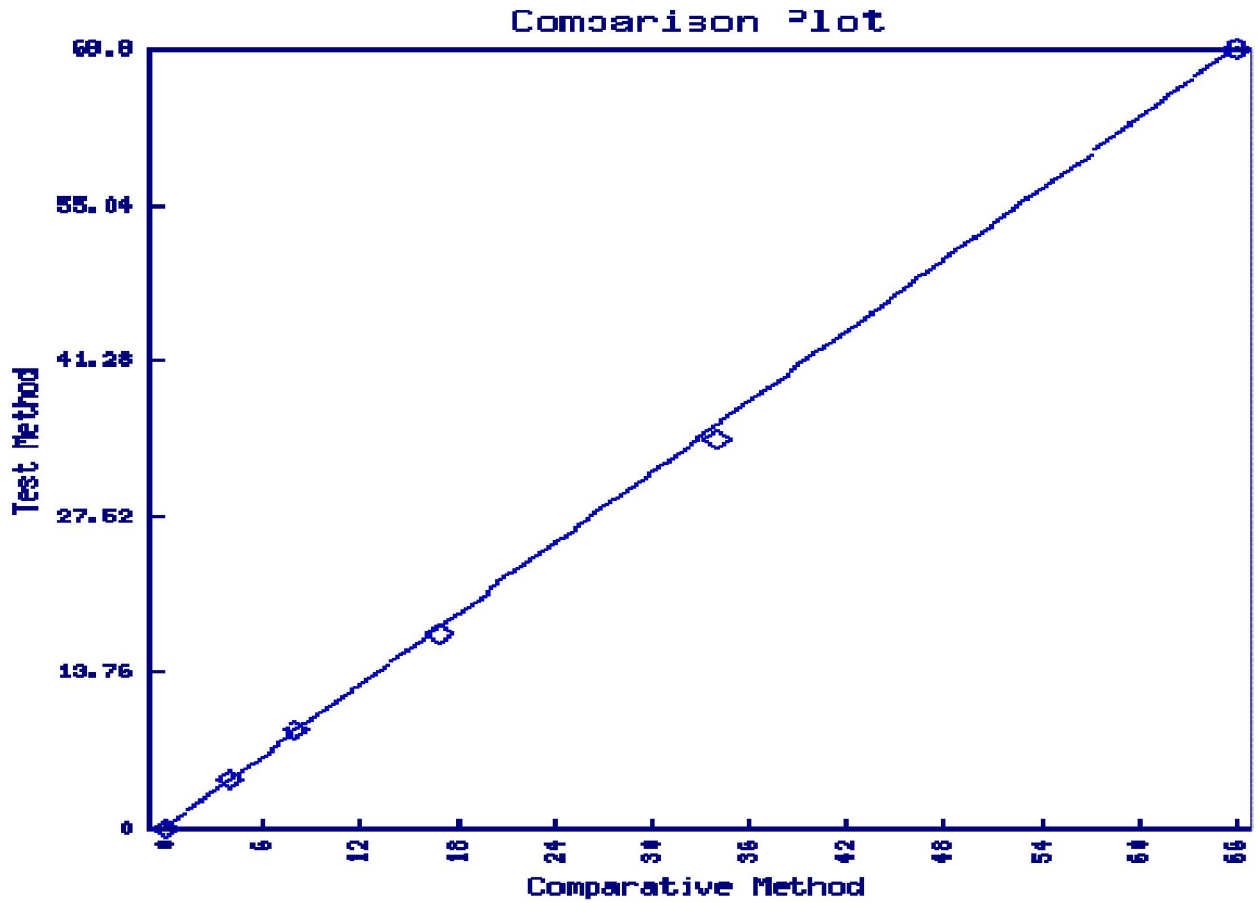
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Figure 4 : Linearity of calibration curve.
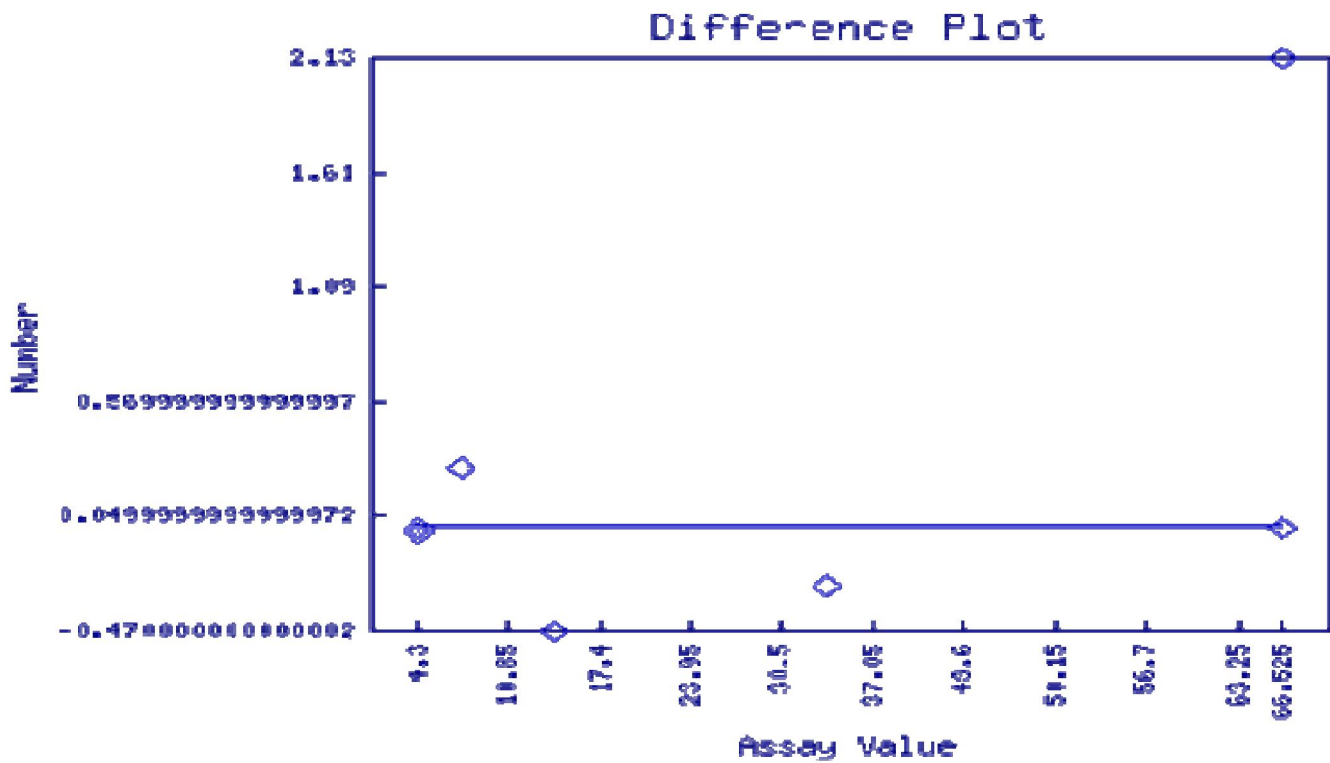
Figure 5 : Difference of linearity of calibration curve.

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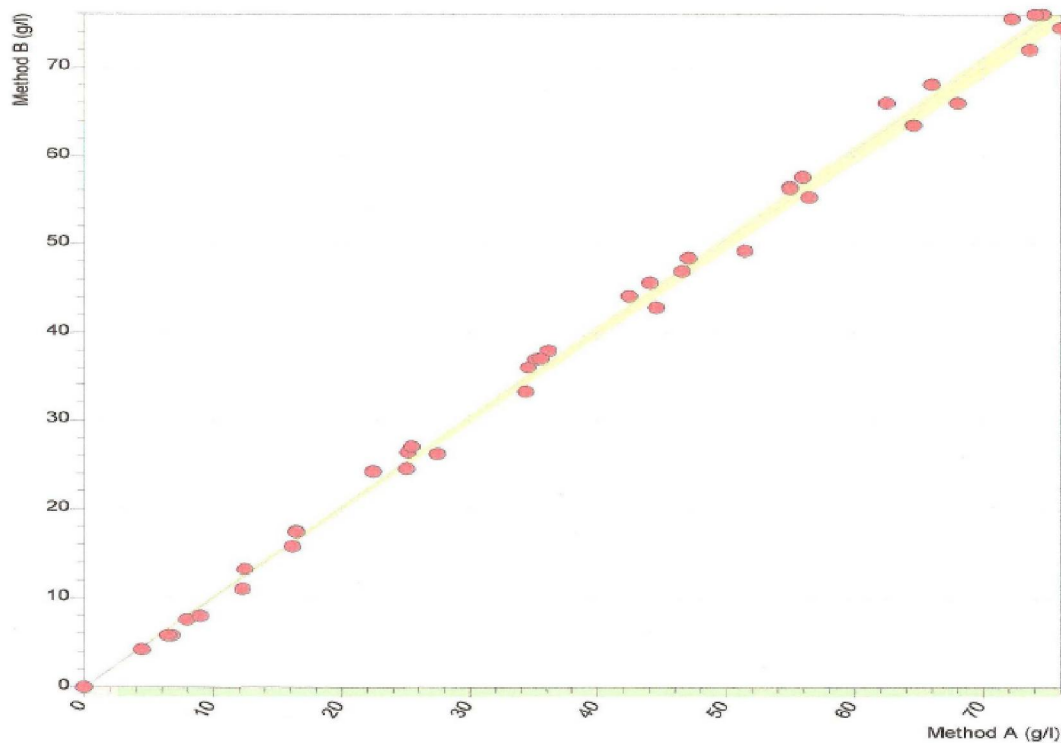
Figure 6 : Comparison of methods (Simple Linear Regression).
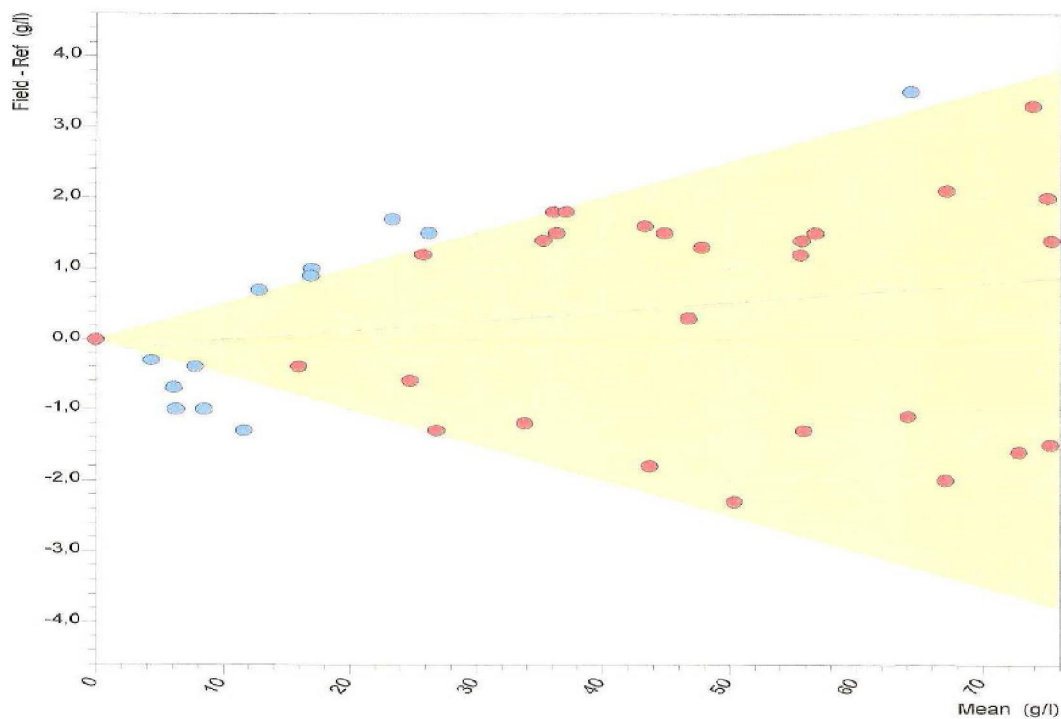
Figure 7 : Difference (30% Tolerance).

5) Linearity range[17]

From a bleach whose concentration was determined by the reference method (APHA-AWWA-WPCF. 3500-Cl - 1992)[11], were performed 4 (four) equidistant dilutions.

Method comparison[18]

Comparing the processing method was developed 40 (fourty) different samples versus the same determination by the reference method (APHA-AWWA-WPCF. 3500-Cl - 1992)[11] covering a wide range of

concentrations.

TABLE 5

Concentrations g Cl₂/L.	55	58	55	57	57	59
	56	55	54	58	54	52

Intermediate Precision (interday and interassay) (Figure 3)	Average (x)	σ	CV%
	55,80	2,04	3,65

TABLE 6

Value (μ)	Determinated			Average (x)	Estimated	Difference	Error (%)	Linearity Error < 5%
	1	2	3					
68,8	67	66	67	66,67	68,01	-0,79	-1,14	Accepted
34,4	34	35	35	34,67	34,03	-0,37	-1,08	Accepted
17,2	18	18	17	17,67	17,03	-0,17	-0,97	Accepted
8,6	8	8	9	8,33	8,54	-0,06	-0,73	Accepted
4,3	4	5	4	4,33	4,29	-0,01	-0,23	Accepted
0,0	0	0	0	0,0	0,04	+0,04	+4,0	Accepted

Concentration (g Cl₂/L.)

Deming linear equation (Figure 4 and 5)	y = 0,988x + 0,040	r = 0,9997
Confidence interval (95%)	0,930 to 1,034 -0,340 to 1,000	1,4 to 68,8 g Cl₂/L.

TABLE 7

Concentration (g Cl₂/L.)

Method (A)	Method (B)	Method (A)	Method (B)	Method (A)	Method (B)
0,0	0,0	12,3	11,0	16,5	17,5
4,5	4,2	74,6	76,0	22,5	24,2
8,0	7,6	51,5	49,2	47,1	48,4
12,5	13,2	27,5	26,2	56,0	57,5
25,2	26,4	6,8	5,8	25,1	24,5
55,0	56,4	9,0	8,0	34,4	33,2
68,0	66,0	35,2	37,0	42,5	44,1
6,5	5,8	72,2	75,5	66,0	68,1
34,6	36,0	62,5	66,0	16,5	17,4
36,2	38,0	74,0	76,0	44,6	42,8
73,6	72,0	56,5	55,2	16,2	15,8
35,6	37,1	64,6	63,5	46,6	46,9
25,5	27,0	55,0	56,2	76,0	74,5
44,1	45,6				

Line equation		Confidence interval (95%)
Simple linear regression (Figure 6 and Figure 7)	y = 1,011x – 0,07	0,991 to 1,032 -1,01 to 0,87
Deming linear equation	y = 1,013x – 0,15	0,991 to 1,036 -0,85 to 0,55
Line equation Passing - Bablok	y = 1,018x + 0,13	0,994 to 1,042 -0,85 to 0,89

CONCLUSIONS

The method developed meets standards required sensitivity and linearity for quantification of the concentration of available chlorine in disinfecting solutions. It has the advantage of using a reagent commonly used in clinical laboratories, thus avoiding, private investment in a specific methodical as would be the iodometric method, which in turn, is a manual method that requires time and is cumbersome for non-technical acquainted personnel with it also requires the use of a sodium thiosulfate solution normality exactly known, which is not stable and must be renewed frequently. The coefficient of variation of the method developed, it is just greater than the reference method (data not shown), which represents another advantage when reporting results with the same analytical grade.

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