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Article

A Facile Spectrophotometric Method for the Determination of Hypochlorite using Rhodamine B

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Um método espectrofotométrico simples, rápido e sensível foi desenvolvido para a determinação de hipoclorito usando Rodamina B. O método proposto considera a reação de hipoclorito com iodeto de potássio em meio ácido, com liberação de iodo. O iodo liberado descora a solução vermelho-rosada de Rodamina B que pode ser medida em 553 nm. Este decréscimo na absorbância é diretamente proporcional à concentração de hipoclorito e obedece a lei de Beer no intervalo de 0,1 a 4,0 μg mL^{-1} de hipoclorito. A absortividade molar, sensitividade de Sandell, limite de detecção e de quantificação do método são 2,57×10^5 L mol^{-1} cm^{-1}, 2,01×10^{-3} μg cm^{-2}, 0,070 μg mL^{-1} e 0,212 μg mL^{-1}, respectivamente. As condições ótimas de reação e outros parâmetros analíticos foram avaliados. O efeito dos íons interferentes na determinação é descrito. O método proposto foi aplicado com sucesso na determinação de hipoclorito em várias amostras de água de torneira, água mineral e leite.

A simple, rapid and sensitive spectrophotometric method has been developed for the determination of hypochlorite using Rhodamine B. The proposed method reports the reaction of hypochlorite with potassium iodide in an acid medium with iodine liberation. The liberated iodine bleaches the pinkish red color of the Rhodamine B and can be measured at 553 nm. This decrease in absorbance is directly proportional to the hypochlorite concentration and obeys Beer's law in the range of 0.1 – 4.0 μg mL^{-1}of hypochlorite. The molar absorptivity, Sandell's sensitivity, detection limit and quantitation limit of the method were found to be 2.57×10^5 L mol^{-1} cm^{-1}, 2.01×10^{-3} μg cm^{-2}, 0.070 μg mL^{-1} and 0.212 μg mL^{-1} respectively. The optimum reaction conditions and other analytical parameters were evaluated. The effect of interfering ions on the determination is described. The proposed method has been successfully applied to the determination of the hypochlorite in various samples of tap water, natural water and milk.

Keywords: hypochlorite determination, spectrophotometry, Rhodamine B

Introduction

Hypochlorite is very high tonnage chemical for sanitizing and bleaching purposes.[1] The determination of hypochlorite is industrially important in connection with water analysis.[2] Hypochlorite is inherently an unstable compound. Hypochlorite is widely used in textile industry as a bleaching agent[3] and disinfectant in fabrics, wood pulp food and milk[4] industries. A medical application of the compound is in the treatment of skin cancer.[5] Another application of the chemical has been a valuable tool for the narcotics department as it could be applied to track the banned drugs like cocaine.[6] Sodium hypochlorite is used as a screening agent[6] for the identification of cocaine. The lethal action of calcium hypochlorite on *Bacillus anthracoides* spores[7] appeared to result from alteration of the structural organization of the spores which led to disturbance of the normal permeability barrier with loss of life – sustaining components and unbalance of metabolic process. It is also used for the preparation of surgically active anticeptic compounds for controlling and preventing infection in wounds. Its determination in environmental and biological samples such as natural water and tap water can be of interest in biochemical research. Hence there is a need for a rapid and sensitive method for its determination. Iodometric,[8] coulometric,[9] polarographic,[10] bromination of fluorescein,[11] chemiluminescence,[12-14] colorimetric,[15] and radiolysis[16] methods are most commonly used. However, colorimetric methods are often preferred as they involve less expensive

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instrumentation and provide better sensitivity when appropriate chromogenic reagents are available.

In the present investigation a rapid, accurate, sensitive and selective method has been proposed for the determination of hypochlorite with a reagent Rhodamine B.

Experimental

Apparatus

A Secomam Anthelie NUA 002 UV–Visible spectrophotometer with 1 cm quartz cell was used for the absorbance measurements and a WTW pH 330, pH meter was used.

Reagents

All chemicals used were of analytical reagent or chemically pure grade and double distilled water was used through out the study. A standard stock solution (1000 µg mL^{-1}) of hypochlorite was prepared by dissolving 1.4442 g of sodium hypochlorite (Merck Limited, Mumbai) in 1000 mL of water and standardized by the iodometric method.[8] Hydrochloric acid (S.D. fine – Chem Ltd, Mumbai) 2 mol L^{-1}, potassium iodide (Merck Ltd, Mumbai) 2%, sodium acetate 1 mol L^{-1} were used. A 0.05% aqueous solution of Rhodamine B (Merck Limited, Mumbai) was used.

Procedure

An aliquot of a sample solution containing 0.1-4.0 µg mL^{-1} of hypochlorite was transferred into a series of 10 mL calibrated flasks, 1 mL of 2 mol L^{-1} hydrochloric acid and 1 mL of 2% potassium iodide were added and the mixture was gently shaken until the appearance of yellow color, indicating the liberation of iodine. A 0.5 mL of 0.05% Rhodamine B solution was then added to it followed by the addition of 2 mL of 1 mol L^{-1} sodium acetate and the reaction mixture was shaken for 2 min, the contents were diluted to 10 mL with distilled water and mixed well. The absorbance of the resulting solution was measured at 553 nm against distilled water. A blank was prepared by replacing the analyte(hypochlorite) solution with distilled water. The absorbance corresponding to the bleached color which in turn corresponds to the analyte(hypochlorite) concentration was obtained by subtracting the absorbance of the blank solution from that of test solution. The amount of the hypochlorite present in the volume taken was computed from the calibration graph.

Procedure for the determination of hypochlorite in natural/tap water samples

An aliquot of natural/tap water samples containing not more than 4.0 mg µL^{-1} of hypochlorite was treated with 0.5 mL of 1 mol L^{-1} NaOH and 0.5 mL of 0.2 mol L^{-1} EDTA. The solution was mixed and centrifuged to remove any precipitate formed. The centrifugate was transferred to a 10 mL calibrated flask[17] and the hypochlorite content was determined as per the method discussed above (Table 1).

Procedure for the determination of hypochlorite in milk

A known volume of milk (20 mL) was placed in a 50 mL beaker and coagulated with 8-10 mL of 1 mol L^{-1} citric acid. The precipitate was centrifuged off. The solution was transferred to a 100 mL calibrated flask[17] and the

Table 1. Determination of hypochlorite

Proposed method				Reference method[17]			
Samples	Hypochlorite added/ (µg mL^{-1})	Hypochlorite found[a]/ (µg mL^{-1})	Relative error	Hypochlorite found[a]/ (µg mL^{-1})	Relative error	*t*-test[b]	*F*-test[c]
Tap water-1	1.00	98.98 ± 0.04	-0.02	1.04 ± 0.06	+0.04	0.67	2.25
	2.00	4.04 ± 0.02	+0.02	2.08 ± 0.02	+0.02	3.01	1.00
	3.00	3.05 ± 0.02	+0.02	3.07 ± 0.03	+0.02	0.50	2.25
Tap water-2	1.00	1.02 ± 0.01	+0.02	1.06 ± 0.01	+0.06	4.00	1.00
	2.00	2.06 ± 0.03	+0.03	2.10 ± 0.05	+0.05	1.33	2.72
	3.00	3.07 ± 0.10	+0.02	3.09 ± 0.02	+0.03	0.40	4.00
Natural water	1.00	0.97 ± 0.04	-0.03	1.06 ± 0.05	+0.06	3.00	1.56
	2.00	1.98 ± 0.06	-0.01	2.01 ± 0.10	+0.01	0.60	2.72
	3.00	3.01 ± 0.02	+0.01	3.04 ± 0.04	+0.01	1.50	4.00
Milk	1.00	1.02 ± 0.03	+0.02	1.05 ± 0.04	+0.05	1.50	1.71
	2.00	4.05 ± 0.01	+0.03	2.09 ± 0.01	+0.05	4.00	1.00
	3.00	3.04 ± 0.02	+0.01	3.06 ± 0.02	+0.02	2.00	1.00

[a]Hypochlorite concentration (µg mL^{-1}) ± Standard deviation; [b] tabulated *t*-value for (4,4) degrees of freedom at 95% probability level is 2.776; [c]tabulated *F*-value for (4,4) degrees of freedom at 95% probability level is 6.39.

hypochlorite content was determined as per the method discussed above (Table 1).

The developed method has been successfully employed for the determination of hypochlorite in various samples of tap water, natural water and milk.

Results and Discussion

This method involves the liberation of iodine by the reaction of hypochlorite with potassium iodide in an acidic medium. The liberated iodine selectively bleaches the color of Rhodamine B and is measured at 553 nm. This decrease in absorbance is directly proportional to the hypochlorite concentration and obeys Beer's law in the range of 0.1 – 4.0 µg mL^{-1} of hypochlorite. The absorption spectrum of Rhodamine B is presented in Figure 1. and the reaction system is represented in Scheme 1.

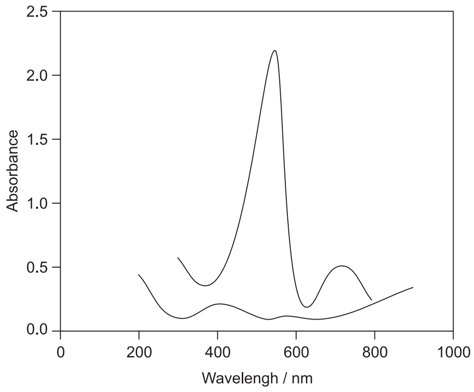
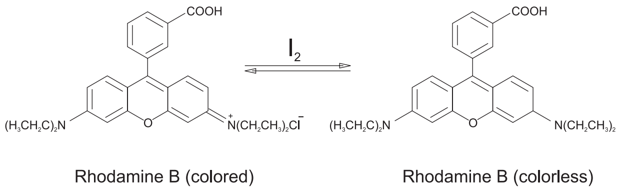


Figure 1. Absorption spectrum of colored species Rhodamine B.



$$NaOCl + HCl \rightarrow NaCl + HOCl$$
$$KI + HCl \rightarrow HI + KCl$$
$$HOCl + 2HI \rightarrow I_2 + HCl + H_2O$$

Scheme 1.

Effect of iodide concentration and acidity

The effect of iodide concentration and acidity on the decolorisation was studied with 2.0 mg mL^{-1} of hypochlorite. The oxidation of iodide to iodine by hypochlorite was effective in the pH range 1.0-1.5, which could be maintained by adding 1 mL of 2 mol L^{-1} HCl in a final volume of 10 mL. The liberation of iodine from potassium iodide in an acidic medium was quantitative. The appearance of yellow color indicates the liberation of iodine. It was found that 1 mL of 2% KI and 1 mL of 2 mol L^{-1} HCl were sufficient for the liberation of iodine from iodide by hypochlorite and 0.5 mL of 0.05% Rhodamine B was used for subsequent decolorization. Effect of concentration of iodide in reaction system is presented in Figure 2.

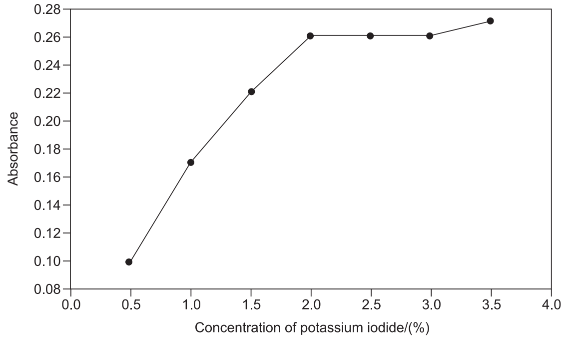
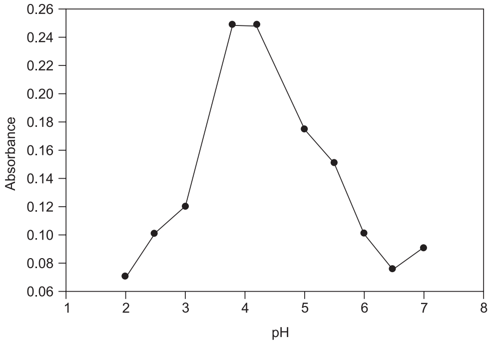


Figure 2. Effect of concentration of potassium iodide.

Constant and maximum absorbance values were obtained at the pH = 4 ± 0.2. Hence the pH of the reaction system was maintained at 4 ± 0.2 throughout the study. This could be achieved by the addition of 2 mL of 1 mol L^{-1} sodium acetate solution in a total volume of 10 mL. Effect of pH on color stability is presented in Figure 3.



Ficure 3. Effect of pH on color intensity.

Analytical data

The adherence to Beer's law was studied by measuring the absorbance values of solutions varying hypochlorite concentration. A straight line graph was obtained by plotting absorbance against concentration of hypochlorite. Beer's law obeyed in the range of 0.1-4.0 µg mL^{-1} of hypochlorite. The molar absorptivity and Sandell's sensitivity for colored system was found to be 2.57×10^{5} L mol^{-1} cm^{-1}, 2.01×10^{-3} µg cm^{-2} respectively. Correlation coefficient (n = 10) and slope of the calibration curve are 0.995 and 0.471 respectively. The detection limit (D_L = 3.3 s /s) and quantitaion limit (Q_L = 10 s /s), where s is the standard deviation of the reagent blank (n=5) and s is the slope of the calibration-curve for hypochlorite determination were found to be 0.070 µg mL^{-1} and 0.212 µg mL^{-1}, respectively. The molar absorptivity and Sandell's sensitivity of the reference method is 1.489×10^{4} L mol^{-1} cm^{-1}, 3.25×10^{-3} µg cm^{-2}.

Effect of diverse ions

The effect of various diverse ions on the determination of hypochlorite by the proposed procedure was examined. The tolerance limits of interfering species were established at the concentration required to cause not more than a ± 2% error in the recovery of hypochlorite at 1.00 µg mL^{-1}. The tolerance limits of diverse ions are summarized in Table 2. The interference of iron(III) can be masked by the addition of 1 mL of 2% sodium fluoride,[17] while cupric ions were masked by the addition of 1 mL of 1% EDTA.[17]

Table 2. Effect of diverse ions on the determination of 1.0 mg mL^{-1} of hypochlorite

Foreign ion	Tolerance limit/µg	Foreign ion	Tolerance limit/µg
Hg(II)	50	Mo(VI)	50
Cd(II)	50	W(VI)	50
Zn(II)	50	U(VI)	100
Ni(II)	75	Chloride	150
Co(II)	50	Phosphate	150
Mg(II)	75	Bromide	250
Ba(II)	25	Nitrate	250
Cu(II)[a]	25	Acetate	125
Fe(III)[a]	75	Sulphate	100
In(III)	50	Borate	100
V(IV)	100		
Ti(IV)	50		
Al(III)	50		

[a]Masked by masking agents (see text).

Application

The proposed method is capable of determining with a high degree of precision the amount of hypochlorite in samples of tap water, natural water and milk. The results, listed in the Table 1, compared favorably with those from a reference method.[15,17] Statistical analysis of the results by the use of *t* and *F* tests showed that, there was no significant difference between the accuracy and precision of the proposed and reference methods. The precision of the proposed method was evaluated by replicate analysis of samples containing hypochlorite at different concentrations.

Conclusions

For the first time, Rhodamine B has been used as a chromogenic reagent for the spectrophotometric determination of hypochlorite. The proposed method, which is simple and rapid, offers the advantages of sensitivity and wide range of determinations without the need for extraction or heating. The method does not involve any stringent reaction conditions and can be compared favorably with the other methods. The proposed method has been successfully applied to the determination of hypochlorite in various analyte samples.

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