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COLORIMETRIC METHOD FOR DETERMINATION OF CHLORINE WITH 3,3′,5,5′-TETRAMETHYLBENZIDINE

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Summary—A new method is developed for the colorimetric determination in water of chlorine (free and combined) with 3,3′,5,5′-tetramethylbenzidine. The procedure proposed achieves a detection limit of 2 ng/ml and its sensitivity is greater than that of the methods used at present.

3,3′,5,5′-Tetramethylbenzidine (TMB) is a non-carcinogenic chemical compound scarcely known as an analytical reagent outside the field of clinical chemistry. It has replaced benzidine (carcinogenic compound) for the analysis of hemoglogin, has been extensively applied, together with hydrogen peroxide, for determination of herbicides and other organic compounds by ELISA, and used for spectrophotometric determination of lipids hydroxiperoxides,[1] cyanide ion in blood,[2] chlorine in water[3] and air[4] and as a detecting reagent in the analysis of organochlorine pesticides by thin layer chromatography.[5,6]

The determination of chlorine in water can be made titrimetrically, potentiometrically and by other techniques, but molecular absorption spectrophotometry, particularly colorimetry, is the technique most frequently used because of its high sensitivity, simplicity and rapidity. Until the seventies, the most universally used method for chlorine determination was colorimetry with *o*-tolidine,[7] but this reagent has gradually decreased in use because of its carcinogenic nature. Among the reagents proposed for replacing *o*-tolidine, syringaldazine is the most outstanding.[8]

The main aim of this paper is to show the advantages of TMB as a reagent compared to syringaldazine and even *o*-tolidine for determining chlorine in water.

EXPERIMENTAL

Apparatus and reagents

Spectral measurements and absorbance readings were carried out on a Shimadzu UV-240 double beam recording spectrophotometer, using a 1 cm glass cell. A pH meter model Crison 2000 was used.

The active chlorine standard solutions were prepared from sodium hypochlorite and standardized titrimetrically with sodium thiosulfate standard solution.

A TMB solution in *N*,*N*-dimethylformamide (DMF)–water was prepared by dissolving 0.100 g of TMB in 25 ml DMF and diluting with 50 ml *o*-phosphoric acid and 25 ml distilled water.

Sodium *m*-arsenite (5 g/l.) was prepared in distilled water. All reagents were of analytical-reagent grade.

Procedure for the determination of total chlorine (free and combined)

TMB solution (2 ml) in DMF–water was placed in an Erlenmeyer flask, then 50 ml of sample was added and homogenized with the reagent. After 5 min the absorbance was measured at 450 nm against a similarly treated blank and the content of chlorine computed from a standard calibration curve containing from 0.02 to 1 mg/l. of active chlorine.

Procedure for the determination of free chlorine

TMB solution (2 ml) were placed in DMF–water in an Erlenmeyer flask, then 50 ml of sample was added at once and before ten sec elapsed 2 ml of sodium *m*-arsenite solution previously measured in a syringe was added. Then the above method was followed.

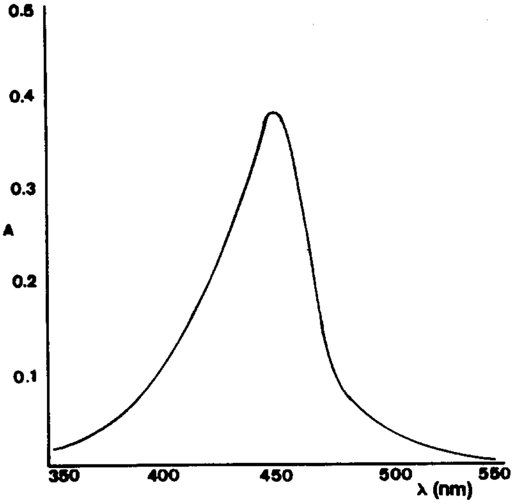

Fig. 1. Absorption spectrum (with 1 cm cell) of TMB–0.4 mg/l. of chlorine.

RESULTS AND DISCUSSION

Remarks about preparation, stability and use of TMB solutions

3,3′,5,5′-Tetramethylbenzidine is barely soluble in water. Its solubility increases in acid, being approximately 1 g/l. in 0.1*M* hydrochloric acid, but it decreases when the acid concentration increases, due to the common-ion effect. As TMB is very soluble in *N*,*N*-dimethylformamide, this solvent is more suitable than a hydrochloric acid aqueous solution, unless the presence of DMF has a negative analytical effect. The incorporation of *o*-phosphoric acid for the preparation of the reagent offers the double advantage of increasing its stability and avoiding interference of Fe(III) in the chlorine determination. The reagent prepared in this way is stable for months, if it is kept in a dark place.

In the assays with chlorine it was observed that the addition of the sample to the reagent gave better precision of the measurements, probably by minimizing the loss of analyte.

Amounts of 1 mg or more or reagent were sufficient for a quantitative reaction with 0.05 mg of chorine.

Absorption spectrum

The absorption spectrum of chlorine reaction product with TMB, presented a maximum at 450 nm at pH less than 2 (see Fig. 1).

Effect of pH *and extracting solvents*

At acid pH, the highest absorbance of TMB solutions and chlorine took place at pH 1–2. If the pH was less than 1 the colour continued to be yellow, but its intensity decreased gradually as the pH decreased. At pH higher than 2, the colour got slowly darker because the reaction product changed to another isomeric form with a bluish colour, which presented a maximum at 650 nm. At pH above 6 a yellow colour appeared again. pH 1–2 was chosen for the colorimetric determination of chlorine due to higher sensitivity and longer stability of the reaction product.

In order to increase the sensitivity of determinations, the possibility of concentration of the reaction product in an organic liquid was tested. At pH 1–2 of the solvents tested achieved a quantitative extraction, even though the reaction of volumes water/organic solvent were 1:1, being significant only in 1-pentanol or 1-hexanol and almost nonexistent in hydrocarbons, dichloromethane and barely polar solvents.

Effect of time

The reaction of chlorine with TMB was near instantaneous at room temperature, whereas nitrite ion, Fe(III) and other ions required 40 min or more to reach equilibrium. At pH 2 the spectrophotometric measurements did not change for several hours.

Table 1. Comparison of the qualities of the proposed and reference methods

	Syringaldazine method	*o*-Tolidine method	Proposed method
Equation for the calibration graph ($y=$)	$0.33x + 0.001$	$0.63x + 0.003$	$0.92x - 0.002$
SD for the slope	0.0220	0.0124	0.0095
SD for the intercept	0.0071	0.0042	0.0029
Detection limit ($y = y_B + 3s_B$)	2 *ng/ml*	3 *ng/ml*	2 *ng/ml*
RSD (0.4 *mg/l.*, $N = 6$)	1.1	1.1	1.0
Calibration range (*mg/l.*)	0.10–1	0.03–1	0.02–1
Toxicity	—	carcinogenic	noncarcinogenic

Table 2. Effect of diverse ions on determination of 0.4 *ml/l.* of chlorine

Ion added	(a)	Ion added	(a)
Sulfate, chloride	10^4	Hg(II), Se(IV), W(VI)	10
Nitrate, Mg(II), Ca(II)	$2 . 10^3$	Cr(III)	2
Al(III), Mn(II), Cu(II)	50	Fe(III)	50
Zn(II), Co(II), Ni(II)	50	Cr(VI)	1
Pb(II), Mo(VI), As(V)	50	V(V)	1

(a) Tolerance limit ion/analyte, *m/m*.

Sensitivity, precision and other qualities of the reference and proposed methods

The values recorded in Table 1 show the superiority of the proposed method over reference methods.

Effect of foreign ions

Fe(III), Cr(VI) and V(V) interfered in the determination of chlorine (see Table 2). The interference of Fe(III) decreased considerably by including *o*-phosphoric acid in the TMB reagent. Nevertheless, if the measure was not performed in less than 2 min, an iron concentration greater than 20 mg/l. slowly reduced the absorbance. Cr(IV) and V(V) did not interfere if the concentration of either of them was less than 0.2 mg/l., when spectrophotometric measurement was carried out in less than 2 min. Fortunately, these interferences are virtually irrelevant, because such high concentrations of iron, chromium or vanadium, are seldom reached in water, and in the case of drinking water this is practically impossible.

APPLICATIONS

The determination in water of free chlorine with TMB gave results which agree with those obtained by the colorimetric method of syringaldazine (see Table 3a). The total and combined amounts, the latter calculated by the difference between total and free chlorine, were determined by the procedure in this paper and also by means of the useful method of *o*-tolidine, which was deleted from the 15th edition of "Standard Methods for the Examination of Water and Wastewater" because of the toxic nature of *o*-tolidine. The results of total chlorine obtained using both procedures were practically the same, but those of combined chlorine were 1% higher with TMB than those with *o*-tolidine (see Table 3b).

CONCLUSIONS

The colorimetric determination in water of free chlorine can be carried out with 3,3′,5,5′-tetramethylbenzidine with an accuracy very similar to that obtained by means of the syringaldazine method. The sensitivity is somewhat higher with TMB, but the main advantages of the proposed method compared to the syringaldazine method are a greater stability of the reaction product, no interference by high concentrations of calcium and magnesium, and that rigorous control of pH is unnecessary.

Table 3a. Determination of free chlorine in tap water from Burjassot (Valencia) in samples of three different days ($N = 6$)

	Proposed method		Syringaldazine method	
Sample	(*mg/l.*)	RSD (%)	(*mg/l.*)	RSD (%)
1	0.62	0.7	0.59	1.1
2	0.54	1.0	0.54	0.8
3	0.52	1.0	0.50	0.9

Table 3b. Determination of total, free and combined chlorine in tap water from Burjassot ($N = 3$)

	Proposed method		*o*-Tolidine method	
Sample	Total (*mg/l.*)	Free (%)	Total (*mg/l.*)	Free (%)
1	0.45	87.1	0.44	86.0
2	0.52	86.7	0.51	85.8
3	0.41	87.3	0.43	86.4

Total and combined chlorine can also be determined with TMB, which is not possible with syringaldazine.

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