

Use of tetramethylbenzidine for the spectrophotometric sequential injection determination of free chlorine in waters

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Abstract

A sequential injection (SI) method was developed for the spectrophotometric determination of chlorine based on the reaction between tetramethylbenzidine (TMB) and free chlorine. The advantages resulting from the use of TMB are considerable: TMB is highly selective for chlorine, it enables a fairly low quantification limit and represents a less toxic alternative to reagents such as toluidine. The use of this reaction in SI adds other advantages as it enhances the degree of automation, minimisation of reagent consumption (6.8 μg TMB/assay) and low effluent production (2.5 mL/determination). The developed method allowed a quantification limit of 90 $\mu\text{g/L}$ with a working range of 0.09–1.30 mg OCl^-/L and a determination rate of 60 det./h. Based on these features, the system was applied to tap-water and surface water samples with no previous treatment required. The results obtained with the developed system were compared to the reference method, diethyl-*p*-phenylenediamine (DPD) colorimetric method, and proved not to be statistically different.

Introduction

The public concern for water safety has led to an increase on the use of water disinfectants, which in turn has become a problem itself. This problem is emphasised if the carcinogenic by-products generated by the disinfectants are considered. The most widely used disinfectant is chlorine both in water supply networks and water treatment plants. The excess of chlorine can induce health problems of its own – stomach discomfort, eye irritation, etc. – and through its by-products (e.g. trihalomethanes) – cancer – therefore a close monitoring of its value is important. The characteristics of the water in each situation are significantly different as well as the tolerated limits for the presence of free chlorine. Considering a water treatment plant, it involves waste water that may present suspended solids and/or intrinsic colour. The possible interference of those characteristics was efficiently avoided in a recent work [1] for the determination of free chlorine

with matrix separation. The reagent used in that determination was a non-selective, mildly pollutant reagent (*o*-dianisidine) whereas the matrix separation prevented any possible interference of other species. The sample was mixed with hydrochloric acid to ensure that chlorine was in the molecular form and then diffused through the gas separation unit. Hydroxide was used in the acceptor channel to convert chlorine to hypochlorite. As effective as it was for application of any coloured water and bleaches, the quantification limit, 0.6 mg OCl^-/L , could not be as low as aimed due to the gas diffusion unit poor efficiency.

When tap-water is concerned the problems of intrinsic colour and suspended solids may not be so significant. On the other hand, the tolerated limits for the free chlorine are lower than in waste water due to its direct impact in human diet. So a low quantification limit and high reagent selectivity becomes a priority over matrix separation. Aiming for these features, a new method for free chlorine spectrophotometric determination was developed. The most commonly used reagents for chlorine determination are *o*-toluidine, methyl orange and *N,N*-diethyl-*p*-phenylenediamine (DPD) both in batch and in flow systems [2–6]. Their selectivity is high but so is their toxicity.

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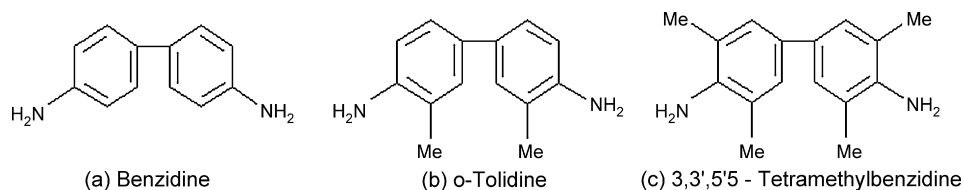


Fig. 1. Chemical structures of benzidine and its analogues.

The search for an equally sensitive and specific reaction without using those excessive pollutant reagents led to the use of 3,3',5,5'-tetramethylbenzidine (TMB).

Reagents with a benzidine structure (Fig. 1) have been recognised as human carcinogens by U.S. Environmental Protection Agency, and that carcinogenic profile results from the possible introduction of hydroxyl groups in its ortho positions. In the TMB chemical structure (Fig. 1) those positions are occupied with methyl groups, which abolishes the mutagenic activity completely [7].

Therefore the chosen reagent, TMB, is not only highly sensitive but also less harmful. Joining these features of the reagent with the known advantages of flow methodologies [8], namely sequential injection analysis, an implementation of the reaction TMB/chlorine in a SI system was aimed.

Preliminary studies of reagent solution preparation and composition as well as a fairly extensive interference study were carried out. A flow injection manifold was used for some of these studies. After all the preliminary studies, a step forward in automation was achieved by designing a sequential injection manifold. Sequential injection techniques present some advantages over flow injection techniques, namely higher degree of automation, robustness, versatility and in some situations, like the present one, lower reagent consumption. The result, after optimisation of the system parameters, was a sequential injection system for the colorimetric determination of free chlorine in waters without any previous treatment of the sample and using a selective, mildly pollutant reagent.

Experimental

Reagents and solutions

All solutions were prepared with analytical grade chemicals and boiled Milli-Q water (resistivity > 18 MΩ cm).

A sodium hypochlorite stock solution of about 200 mg OCl⁻/L was weekly prepared from the concentrated solution (6–14% active chlorine, Merck, Darmstadt, Germany) and titrated for accurate concentration. Working standards were daily prepared from this stock solution in the dynamic range of (0.1–2.3 mg/L).

A stock solution of ortho-phosphoric acid 5 mol/L was obtained from the concentrated acid ($d = 1.71$; 85%, Merck, Darmstadt, Germany).

A stock solution of hydrochloric acid 1 mol/L was prepared from the concentrated acid ($d = 1.19$; 37%, Merck, Darmstadt, Germany).

Tetramethylbenzidine solution was prepared by dissolving 2.5 mg of the solid 3,3',5,5'-tetramethylbenzidine (Fluka, Buchs, Switzerland) in 250 μL of concentrated *N,N*-dimethylformamide, DMF ($d = 0.948$; 99.8%, Sigma–Aldrich, St. Louis, USA), in a final volume of 100 mL with a proper dilution from the acids stock solutions to a final concentration of 0.05 M of H₃PO₄ and HCl.

Sample preparation

The water samples were directly introduced in the system without any previous treatment.

Flow injection manifold and procedure

The flow injection manifold used in preliminary studies depicted in Fig. 2 was composed of a Gilson Minipuls 3 peristaltic pump, a Rheodyne 6 port rotary valve and a Unicam 8625 UV/VIS spectrophotometer equipped with a Hellma 178.710—QS flow-cell (10 mm light path, 80 μL inner volume) as detection system connected to a Metrohm E 586 Labograph strip chart recorder. The flow channels were constructed using Teflon from Omnifit with 0.8 mm i.d.

The standard (S) was injected in a water carrier stream (H₂O) that merged at confluence c with the colour reagent stream (TMB). The streams were mixed at reaction coil L on the way to the detector. The following parameters were set: standard volume, 375 μL; flow rate for both streams, 2.2 mL/min; reaction coil, 50 cm.

Sequential injection manifold and procedure

The sequential injection manifold for the colorimetric determination of chlorine in waters is depicted in Fig. 3. Solutions were propelled by a Gilson Minipuls 3 peristaltic pump with

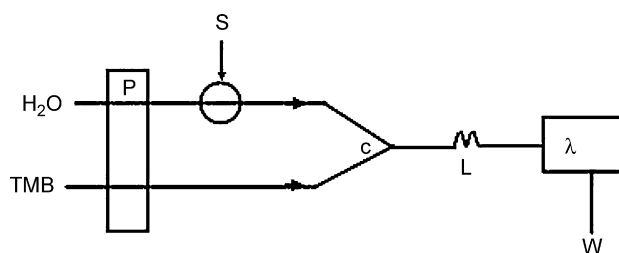


Fig. 2. FIA manifold for TMB solution study: TMB, TMB solution; S, sample/standard; W, waste; c, confluence; P, peristaltic pump; L, reaction coil 50 cm; λ, spectrophotometer at 452 nm.

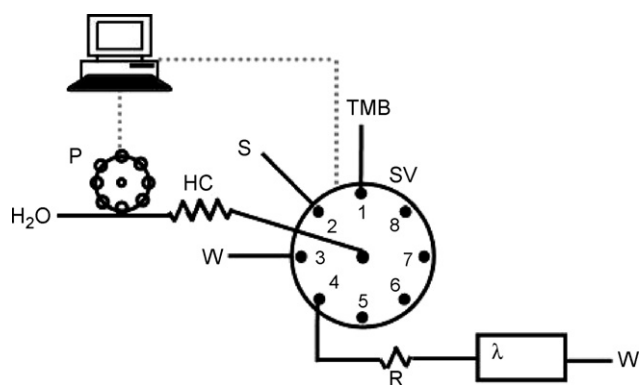


Fig. 3. Manifold for the spectrophotometric free chlorine determination in waters: TMB, TMB solution: tetramethylbenzidine 25 mg/L (0.104 mM) in 3 M DMF and in 0.05 M of both H_3PO_4 and HCl; S, sample or standard; W, waste; P, peristaltic pump; SV, 8 port selection valve; HC, holding coil 300 cm; R, reaction coil 100 cm; λ , spectrophotometer at 452 nm.

PVC pumping tubes. The pump was connected to the central channel of an eight port electrically actuated selection valve (Valco VICI 51652-E8). All tubing connecting the different components of the flow system was made of Teflon from Omnifit with 0.8 mm i.d.

A Hitachi 100-40 UV-vis spectrophotometer with a Hellma 178.711-QS flow-cell (10 mm light path, 30 μL inner volume) was used as detection system. The wavelength was set to 452 nm. Analytical signals were recorded in a Metrohm E 586 Labograph strip chart recorder.

A personal computer (Samsung SD 700) equipped with a PCL818L interface card, running with homemade software written in Quick-Basic 4.5, controlled the selection valve (SV) position and the pump sense and speed.

The sequence of the steps as well as the respective time and volume for the chlorine determination is shown in Table 1. The colour reagent, TMB solution, is aspirated into the holding coil followed by aspiration of sample/standard (S). Then the flow is reversed, promoting the mixture on the way to the detector where the absorbance of the coloured product is measured.

Table 1
Protocol sequence for the determination of free chlorine in waters

Step	Selection valve position	Operation time (s)	Pump speed/direction	Volume (μL)	Description
A	1	4.8	40/a	275	Aspirate TMB
B	2	6.2	40/a	350	Aspirate sample; starts reaction with TMB
C	4	45	40/b	2560	Propel the colour product to the detector and registration of the signal

Table 2
Summary of results with different solvents tested for TMB dissolution

Solvent	Acid conditions	Absorption maximum (nm)	Linear range (mg/L)	Calibration curve	Dissolution observations
Ethanol 30%	$[\text{H}_3\text{PO}_4] = 0.5 \text{ M}$ $[\text{HCl}] = 0.5 \text{ M}$	451	0.0131–1.31	$A = 0.5286[\text{OCl}^-] - 0.0046$; $R^2 = 0.9982$	Poor dissolution
HCl 0.1%	–	652	0.3–2.13	$A = 0.5409[\text{OCl}^-] - 0.1179$; $R^2 = 0.9921$	Very poor dissolution
DMF 25%	$[\text{H}_3\text{PO}_4] = 7.4 \text{ M}$	452	0.0524–1.31	$A = 0.6496[\text{OCl}^-] - 0.0101$; $R^2 = 0.9996$	Very good dissolution

The values concern the preparation of TMB 1 g/L.

Results and discussion

Several aspects of reagent preparation were studied. These studies included testing different solvents for dissolution of TMB and evaluation of the impact of the solvent in the method sensitivity.

The composition of the reagent solution was also studied in terms of the acidic conditions required, followed by a fairly extensive interference study. Both these studies were carried out with a flow injection system. Afterwards the sequential injection system designed and optimised was used for the determination of free chlorine in water samples.

TMB dissolution optimisation -batch

Based on a previous work [9], different solvents were tested for dissolving TMB: ethanol, hydrochloric acid and DMF. A TMB solution of 1 g/L was prepared in the mentioned solvents and afterwards spectra were traced with a chlorine standard of 0.7 mg OCl^-/L . Calibration curves were elaborated based on the procedure described by Serrat et al. [9], the results and observations of these studies are summarised in Table 2.

The choice of DMF as TMB solvent was based on the much better dissolution over the others solvents. In addition, it also presented higher sensitivity proving to be the best choice regardless of its high toxicity. The colorimetric product obtained was yellow, it was formed instantaneously and was stable.

TMB solution optimisation -FIA

After choosing the solvent, and consequently the working wavelength, further studies involving the optimisation of TMB solution continued, using the flow injection manifold presented in Fig. 2.

As above-mentioned DMF is highly toxic so a reduction on the used amount was aimed without compromising the sensitivity of the method. For this study, a concentration of 0.1 g TMB/L solution was set. This means that all the components of the solu-

Table 3
Optimisation of the TMB solution composition by FIA

TMB solution	DMF (%)	[H ₃ PO ₄] (M)	[HCl] (M)	pH	Calibration curves
1	2.5	0.74	–	≈1	$A = 0.3175 [\text{OCI}^-] - 0.1156; R^2 = 0.998$
2	2.5	0.05	0.05	≈2	$A = 0.2994 [\text{OCI}^-] - 0.0946; R^2 = 0.998$
3	1	0.05	0.05	≈2	$A = 0.3048 [\text{OCI}^-] - 0.1115; R^2 = 0.9995$
4	1	0.05	–	3 < pH < 4	$A = 0.2533 [\text{OCI}^-] - 0.098; R^2 = 0.9995$

The values correspond to the preparation of 0.1 g/L TMB in a working range of 0.7–2.2 mg OCI[–]/L.

tion will have a 10-fold dilution when compared to the previous study (Table 2).

In order to assess the minimal quantity of DMF required for dissolution of TMB the study started with 5 mg of TMB (for a final volume of 50 mL) and 100 μL of DMF. There was no complete dissolution, so increments of 100 μL of DMF were made until complete dissolution. In the end, 500 μL of DMF were added for completed dissolution, resulting in a DMF concentration of 1% (v/v). This represented a significant reduction, as it corresponds to a minimisation of 2.5× when compared to the initial percentage of 2.5% DMF (v/v).

The role of *o*-phosphoric acid is to stabilise the TMB solution and avoid interference of Fe(III) [9]. Nevertheless, it contributes to the increase of the solution viscosity so a lower final concentration was aimed.

Observing Table 2, the acidic conditions when the solvent was ethanol were different than those when the solvent was DMF, namely much lower H₃PO₄ concentration. Based on this observation, the hypothesis tested was having 0.05 M of both H₃PO₄ and HCl with the DMF as solvent. For testing this hypothesis, four TMB solutions were prepared and compared (Table 3) by flow injection analysis (Fig. 1) in the working range of 0.7–2.2 mg OCI[–]/L. Solution 1 is the TMB solution in DMF previously described (Table 2) diluted 10 times; solution 2 maintains the same quantity of DMF as in solution 1 using the acidic conditions of the hypothesis; solution 3 uses 1% in DMF (result of the minimisation study) with the acidic condition of the hypothesis; solution 4 uses same conditions of solution 3 but with no HCl.

Observing the results in Table 3, several conclusions can be drawn: from the comparison between solutions 1 and 2 the change in the acidic conditions (hypothesis tested) proves to be valid as it did not interfere with method sensitivity; comparing solution 2 with solution 3 confirms the previous study for the minimisation of DMF quantity; comparing solutions 3 and 4, it proves the need for HCl presence for the minimisation of H₃PO₄ concentration.

Therefore, the TMB solution used in further studies was prepared accordingly with the composition described for solution 3 (Table 3).

Interference studies -FIA

Having optimised the composition of TMB solution, possible interference of other species was evaluated. The search of possible interfering agents was based in previous work [9] and probable inorganic ions to be present in water samples [10]. The

values tested for each species were based on the reference values for waste water as those would be representative of the worst case scenario. For this study, a standard of 0.7 mg OCI[–]/L was used as reference and then compared, by peak height (absorbance), to another standard with the same amount of OCI[–] and the chosen quantity of the interfering agent to be tested. A summary of all the species, values tested and percentage of interference is shown in Table 4.

The results show that, within the tested values there is no significant interference, as percentage of interference <5%. This led to conclude the good selectivity of the reagent, proving to be an appropriate choice for chlorine determination.

Development of SI manifold and optimisation of the system parameters

Having concluded the preliminary studies, a sequential injection system was assembled and optimised. The SI manifold is depicted in Fig. 3. The aspiration order was set in advance being first aspirated the colour reagent, TMB solution, followed by sample/standard. Also set was the reaction coil length to 1 m.

The first SI parameter to be studied was then the aspirated volumes (Fig. 4). The volume of TMB solution was studied, ranging from 225 to 425 μL; because the sensitivity increased slightly up to 275 μL, this was the volume chosen. As for the sample volume, the studied range was from 225 to 350 μL.

Table 4
Summary of the tested possible interfering agents, with the tested values added to a standard of 0.7 mg OCI[–]/L and respective percentage of signal interference when compared to the same standard without interfering agent

Interfering agent	Concentration (mg/L)	% Interference
SO ₄ ^{2–}	1500	+0.2
Al ³⁺	11	–3.3
Al(SO ₄) ₂ [–]	92	
NO ₃ [–]	50	–2.9
MnO ₄ [–]	2	–1.7
Cu ²⁺	1	–3.7
Ni ²⁺	2	+2.3
Hg ²⁺	0.05	–2.9
Pb ²⁺	1	+2.9
Cr (VI)	0.1	+4.9
Fe(NO ₃) ₃	1.2	+0.4
Fe(SO ₄) ₂ ^{2–}	2	+4.9
Cl [–]	25	–0.2
Mg(NO ₃) ₂	50	–4.5
Ca ²⁺	100	+0.5
HCO ₃ [–]	50	–2.3

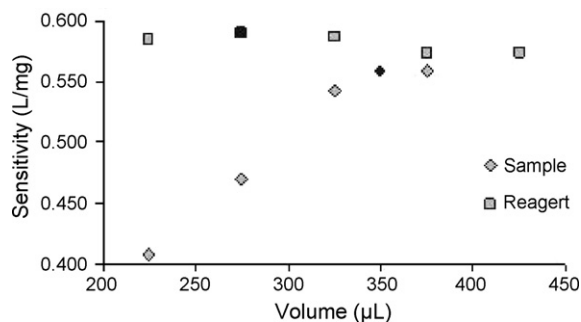


Fig. 4. Optimisation of the volumes of reagent, TMB and the sample/standard. The points in black represent the chosen values.

The results show that there was no further increase in the sensitivity for volumes above 350 µL, so this was the volume chosen.

Then, with the optimised volumes, a study of the TMB concentration was performed, maintaining the proportions of the other components (DMF, H₃PO₄, HCl). A concentration of 25 mg/L (0.104 mM) TMB was chosen within the range 10–200 mg/L (0.04–0.8 mM) as it corresponded to a maximum sensitivity with a lower reagent consumption.

Features of the system

For the dynamic concentration range 0.09–1.30 mg OCl⁻/L a typical calibration curve was calculated as a mean of four calibration curves of four consecutive weeks and was as follow:

$$A = 0.5959(\pm 0.0461) \text{ mg OCl}^{-}/\text{L} - 0.0558(\pm 0.0168);$$

$$R^2 = 0.994(\pm 0.003),$$

where the values in brackets represent the standard deviation values.

The detection and quantification limits, calculated according to IUPAC recommendations [11], were 0.08 and 0.09 mg OCl⁻/L, respectively.

A complete analytical cycle took about 1 min for water sample. An analytical cycle is the sum of the time needed for each step plus the time necessary for the port selection in the selection valve. Thus, based on the time spend per cycle, the sampling frequency was 60 determinations per hour. This corresponds to a sample consumption per determination of 0.350 mL of water sample or standard.

The overall reagent consumption per determination was: 6.8 µg TMB, 30 µg DMF, 376 µg hydrochloric acid and 140 µg *o*-phosphoric acid. The total volume of effluent produced per determination is only around 2.5 mL.

Application to water samples

The developed system was applied to chlorine determination in some water samples, tap-water (samples 1, 3, 4, 6, 9), surface water (samples 2 and 5) and spiked surface water (samples 7, 8 and 10–12) also analysed by the reference colorimetric method of DPD [12], results are presented in Table 5.

Table 5

Application of the developed system (SIA) to water samples, and comparison with the reference method (colorimetric with DPD)

Sample	DPD (mg OCl ⁻ /L)	SIA (mg OCl ⁻ /L)	%RD ^a
1	0.654 ± 0.006	0.709 ± 0.012	8.4
2	0.640 ± 0.008	0.712 ± 0.007	11.2
3	0.209 ± 0.019	0.202 ± 0.003	-3.5
4	0.318 ± 0.007	0.297 ± 0.002	-6.7
5	0.318 ± 0.014	0.337 ± 0.006	5.9
6	0.675 ± 0.021	0.705 ± 0.004	4.4
7	0.917 ± 0.039	0.921 ± 0.002	0.4
8	1.360 ± 0.052	1.287 ± 0.007	-5.4
9	0.593 ± 0.011	0.593 ± 0.008	-0.1
10	2.073 ± 0.009	2.159 ± 0.007	4.1
11	2.352 ± 0.030	2.281 ± 0.003	-3.0
12	1.356 ± 0.016	1.419 ± 0.007	4.7

^a RD, relative deviation.

To evaluate accuracy, a linear relationship between C_{SIA} (mg OCl⁻/L) and C_{DPD} (mg OCl⁻/L) was established; the equation found was:

$$C_{\text{SIA}} = 0.991(\pm 0.0767) \times C_{\text{DPD}} + 0.0216(\pm 0.0561)$$

where the values in parenthesis are 95% confidence limits. These figures show that the estimated slope and intercept do not differ statistically from the values 1 and 0, respectively. Therefore, there is no evidence for systematic differences between the two sets of results [13].

Conclusions

The use of the TMB/chlorine reaction in a sequential injection system was successful. TMB proved to be a highly selective reagent, yielding a very sensitive methodology resulting in fairly low quantification limit (90 µg OCl⁻/L) if compared to the previously described [1] (600 µg OCl⁻/L). When compared to other reported spectrophotometric flow methods, it presents a similar [2] or better sensitivity [4,5], but involving a non-carcinogenic reagent.

The choice of sequential injection technique effectively added advantages resulting in high degree of automation, low consumption values, small volume of effluent production and a quite good determination rate.

Taking advantage of the sequential injection versatility, it would be possible to accommodate, within the same manifold, both the presented system and the previously developed [1], which included the matrix separation; this would meet the requirements for both low detection limits of tap waters and need for preventing interference of waste waters.

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