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A stabilized TMB-based kinetic method for low-range online free chlorine monitoring in treated water: method development and preliminary prototype evaluation

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Abstract

Continuous free chlorine monitoring is important for drinking-water treatment and treated processwater control, but online implementation can be limited by reagent instability, multi-reagent fluidics, and maintenance burden. We report a stabilized tetramethylbenzidine (TMB)-based kinetic method for low-range online free chlorine monitoring in treated-water matrices. The method was implemented in prototype online analyzers using a single-reagent architecture and a fixed low-reagent operating condition. During formulation screening, lower-concentration TMB variants were attractive for nominal low-range measurement in simple standards but showed greater susceptibility to matrix-related interference in real treated-water samples. An optimized stabilized formulation was therefore adopted in the final method to improve color-development robustness and practical linearity. Prototype evaluation included a representative on-site point comparison with a handheld DPD-based comparator, a small matrix-comparison dataset covering treated-water-related samples, routine repeated testing at 24 measurements per day over approximately three months, and separate representative higher-frequency runs at a 2.5-minute measurement cycle. Under routine repeated operation, repeatability was around 1% RSD. Sealed-storage stability testing further supported longterm reagent stability under room-temperature and accelerated conditions. Together, these results support the feasibility of stabilized TMB kinetic chemistry for low-range online free chlorine monitoring in treated-water applications.

Keywords

free chlorine, online monitoring, tetramethylbenzidine, TMB, treated water, kinetic method, residual chlorine

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Abstract

Continuous free chlorine monitoring is important for drinking-water treatment and treated process-water control, but online implementation can be limited by reagent instability, multi-reagent fluidics, and maintenance burden. We report a stabilized tetramethylbenzidine (TMB)-based kinetic method for low-range online free chlorine monitoring in treated-water matrices. The method was implemented in prototype online analyzers using a single-reagent architecture and a fixed low-reagent operating condition. During formulation screening, lower-concentration TMB variants were attractive for nominal low-range measurement in simple standards but showed greater susceptibility to matrix-related interference in real treated-water samples. An optimized stabilized formulation was therefore adopted in the final method to improve color-development robustness and practical linearity. Prototype evaluation included a representative on-site point comparison with a handheld DPD-based comparator, a small matrix-comparison dataset covering treated-water-related samples, routine repeated testing at 24 measurements per day over approximately three months, and separate representative higher-frequency runs at a 2.5-minute measurement cycle. Under routine repeated operation, repeatability was around 1% RSD. Sealed-storage stability testing further supported long-term reagent stability under room-temperature and accelerated conditions. Together, these results support the feasibility of stabilized TMB kinetic chemistry for low-range online free chlorine monitoring in treated-water applications.

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1. Introduction

Residual free chlorine monitoring remains a core requirement in drinking-water treatment, finished-water quality control, and treated process-water management because a measurable disinfectant residual is needed both for operational control and for preservation of microbiological stability during downstream distribution [1–3]. In routine practice, utilities commonly maintain free chlorine residuals in the range of a few tenths of a milligram per litre, and levels below approximately 0.2 mg/L in distribution systems have long been associated with increased risk of bacterial regrowth and coliform occurrence [1–4]. Reliable low-range measurement is therefore important not only at the treatment plant, but also at points where chlorine residuals are expected to be modest and analytically more vulnerable to drift, interference, or handling delay [1–4].

Among available analytical approaches, colorimetric methods remain dominant for residual chlorine determination because they are sensitive, inexpensive, and readily adaptable to field and process monitoring [5–7]. The most widely used routine approach is based on N,N-diethyl-p-phenylenediamine

(DPD), as reflected in Standard Methods, ISO 7393-2, and U.S. EPA Method 334.0 [5–7]. DPD chemistry is also widely implemented in commercial continuous analyzers for free- or total-chlorine monitoring in drinking-water and wastewater applications [8]. Despite its broad acceptance, however, online deployment of DPD-based methods can still be constrained in practice by multi-reagent fluidics, routine reagent replacement, baseline drift, optical fouling, and general maintenance burden, particularly in decentralized or low-maintenance monitoring settings [5,8].

From a water-chemistry perspective, free chlorine in treated waters exists primarily as hypochlorous acid and hypochlorite, with speciation, decay behavior, and apparent analytical response depending on pH, temperature, and matrix composition [9–11]. These factors are especially relevant in online monitoring because real samples may differ substantially from simple laboratory standards even within the nominally narrow concentration range used for residual control [9–11]. As a result, practical online chlorine analysis requires not only sufficient analytical sensitivity, but also reagent stability, reproducible signal formation in real matrices, and compatibility with simplified automated fluidics.

Tetramethylbenzidine (TMB) is a well-established chromogenic substrate in oxidation-based analytical systems and has also been investigated for chlorine determination in water [12–15]. Earlier studies showed that TMB can provide sensitive colorimetric response to chlorine, including direct colorimetric determination and sequential-injection spectrophotometric analysis [12,13]. More recent studies further highlighted the suitability of TMB-based chlorine indication for low-level water analysis [14,15]. These reports support the analytical promise of TMB chemistry, but they do not by themselves establish that a TMB-based reagent can be translated into a practical, storage-stable, low-maintenance online monitoring platform.

An additional route to practical simplification is kinetic quantification based on early-stage signal development rather than endpoint absorbance. For online chlorine monitoring, such an approach may reduce dependence on full color development, shorten the useful acquisition window, and improve compatibility with compact automated analyzers. However, to be practical in treated-water applications, a kinetic chlorine method must be paired with a reagent formulation that remains stable during storage and produces sufficiently robust color development across real water matrices.

The present work describes the development of a stabilized TMB-based kinetic method for low-range online free chlorine monitoring in treated water. The method was not intended as a universal chlorine method for all matrices and was not developed for total chlorine in the present prototype configuration. Instead, the aim was to establish a practically relevant low-maintenance online method for residual free chlorine in treated-water systems.

The main contribution of the study is threefold. First, it shows that a stabilized TMB-based kinetic approach can provide a practically relevant route to low-range free chlorine measurement in treated water. Second, it extends the work beyond laboratory method development by implementing the chemistry in functioning prototype analyzers and evaluating operation under two separate practical modes: routine repeated testing at 24 measurements per day over approximately three months, and representative higher-frequency runs at a 2.5-minute measurement cycle. These two operating modes served different purposes and were evaluated separately. Third, it shows that the optimized formulation provided greater robustness against matrix-related interference than lower-concentration formulations, which is important for reliable operation in real treated-water samples. Stability testing also supported extended sealed-storage stability of the formulation, which is relevant to practical deployment.

2. Materials and methods

2.1 Method principle

The method is based on oxidation of a stabilized TMB reagent by free chlorine species in water, followed by kinetic optical readout in an online analyzer format. The target analyte in the present work was residual free chlorine in treated-water matrices.

2.2 Formulation screening and final formulation selection

Early development work evaluated lower-concentration TMB formulations for low-range free chlorine measurement. Although these formulations were suitable in simple standards, testing with real tap-water and treated-water samples showed greater susceptibility to matrix-related effects, including weaker or less stable color development in some samples. To improve practical robustness, an optimized stabilized formulation was adopted in the final low-range method. This decision was based on signal robustness, consistency, and practical linearity in real treated-water samples rather than on extension of the nominal upper range.

2.3 Prototype online analyzer

The method was implemented in prototype online analyzers designed for treated-water monitoring. Representative prototype units included compact cabinet-based systems with integrated fluidics, touchscreen control, optical detection, and a single-reagent delivery path.

2.4 Operating conditions and practical evaluation

The prototype method was operated under a fixed low-reagent configuration. Two practical operating modes were evaluated separately. For representative continuous-operation demonstration, the analyzer was run at a 2.5-minute measurement cycle. Separately, for routine endurance-style evaluation, approximately three months of repeated testing were carried out at a frequency of 24 measurements per day to assess practical repeatability and operational consistency under development-stage conditions.

2.5 Comparator measurements and representative matrix dataset

Comparator measurements were obtained using DPD-based chlorine measurements, including portable field meters where available. In selected same-site point comparisons, the prototype online analyzer was compared directly with a handheld chlorine meter. Representative matrix-comparison samples included tap water, finished drinking water, treated wastewater effluent, and process water. For development-stage prepared matrix samples, nominal target free-chlorine levels were assigned as prepared concentrations, while comparator DPD measurements were recorded separately where available. These values were used as practical comparison points rather than as formal certified reference values.

2.6 Stability assessment

Stability of the stabilized TMB reagent was evaluated under sealed storage using both room-temperature and accelerated conditions. Remaining activity was assessed at defined storage intervals by comparison with the initial analytical response. These studies were used to support the conclusion that the reagent maintained acceptable analytical activity during extended sealed storage.

3. Results and discussion

3.1 Final formulation selection for low-range treated-water monitoring

A central finding of development was that nominal low-range suitability in simple standards did not automatically translate to practical robustness in real water samples. Lower-concentration TMB formulations showed stronger susceptibility to matrix-related interference, particularly in tap-water and more complex treated-water samples. For this reason, the final low-range method adopted an optimized stabilized formulation that gave more reliable color development and more stable behavior in real treated-water matrices. The use of a more robust stabilized formulation in a low-range method therefore reflects engineering optimization for real-sample performance rather than a contradiction in method intent.

3.2 Prototype implementation and practical operation

Prototype implementation demonstrated that the stabilized TMB chemistry could be integrated into a cabinet-based online instrument with a simple single-reagent fluidic path. Representative higher-frequency operation at a 2.5-minute measurement cycle supported the feasibility of continuous online application. In a separate routine evaluation mode, repeated testing over approximately three months at 24 measurements per day showed repeatability of around 1% RSD, supporting stable low-range monitoring behavior under development-stage conditions.

3.3 Representative field comparison and matrix performance

A representative same-site point comparison showed close agreement between the prototype online analyzer and a handheld comparator meter. In one representative comparison, the prototype analyzer reported 0.38 mg/L free chlorine, while the handheld comparator reported 0.37 mg/L, corresponding to an absolute difference of 0.01 mg/L. Across the small representative matrix-comparison dataset, the stabilized TMB method showed encouraging practical comparability with nominal prepared concentrations and comparator DPD measurements, although differences varied by matrix. These data support the practical applicability of the method for low-range residual free chlorine monitoring in treated water, while also indicating that the present evidence base is preliminary and restricted to treated-water matrices rather than heavily contaminated wastewater or total chlorine measurement. Broader validation across larger real-water datasets would be warranted before wider performance claims are made.

Table 1. Representative development-stage comparison of the stabilized TMB method with nominal prepared concentrations and comparator DPD measurements in treated-water-related matrices.

Sample ID	**Matrix type**	Nominal prepared concentration (mg/L)	**Stabilized TMB (mg/L)**	**Comparator DPD (mg/L)**	TMB deviation from nominal (%)	DPD deviation from nominal (%)
S1	Tap water	0.15	0.16 ± 0.01	0.14 ± 0.01	+6.7	-6.7
S2	Finished drinking water	0.30	0.32 ± 0.01	0.30 ± 0.01	+6.7	0.0
S3	Treated wastewater effluent	0.50	0.48 ± 0.01	0.42 ± 0.02	-4.0	-16.0

Sample ID	Matrix type	Nominal prepared concentration (mg/L)	Stabilized TMB (mg/L)	Comparator DPD (mg/L)	TMB deviation from nominal (%)	DPD deviation from nominal (%)
S4	Process water	0.80	0.83 ± 0.01	0.90 ± 0.03	+3.8	+12.5

In the representative comparison dataset, agreement was encouraging at low residual chlorine levels, although differences between TMB and DPD responses varied somewhat by matrix. These observations are consistent with the method being positioned as a practical treated-water monitoring approach rather than a universal method across all chlorine-related matrices.

3.4 Storage stability and practical deployment implications

A practical advantage of the TMB-based method is its single-reagent architecture. Conventional online DPD systems commonly rely on separate indicator and buffer streams, whereas the present prototype used a single stabilized reagent, reducing the number of reagent lines, containers, and fluidic handling steps. Stability testing further indicated extended sealed-storage stability, which is relevant to practical online implementation because it may reduce reagent replacement pressure and simplify logistics for low-maintenance deployment.

4. Conclusions

A stabilized TMB-based kinetic method was developed for low-range online free chlorine monitoring in treated-water applications. The optimized stabilized formulation was selected because it provided more robust color development and better practical behavior in real treated-water matrices than lower-concentration formulations screened earlier in development. The method was implemented in functioning prototype online analyzers and showed practical feasibility under representative operating conditions, including routine repeated testing over approximately three months at 24 measurements per day, separate higher-frequency operation at a 2.5-minute measurement cycle, and representative point comparison against a handheld comparator. The single-reagent architecture and extended sealed-storage stability are practical features that may support low-maintenance online deployment. Taken together, the results support the feasibility and potential utility of stabilized TMB kinetic chemistry for low-range residual free chlorine monitoring in treated-water applications, while broader validation would still be needed for wider deployment claims.

Declarations

Funding

The authors declare that no external funding was received for this study.

Conflict of interest

The authors are involved in the development of stabilized TMB reagents and prototype online chlorine analyzers related to the subject of this work. This affiliation may be perceived as a potential competing interest.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Representative development-stage records may be provided where appropriate.

Figure captions

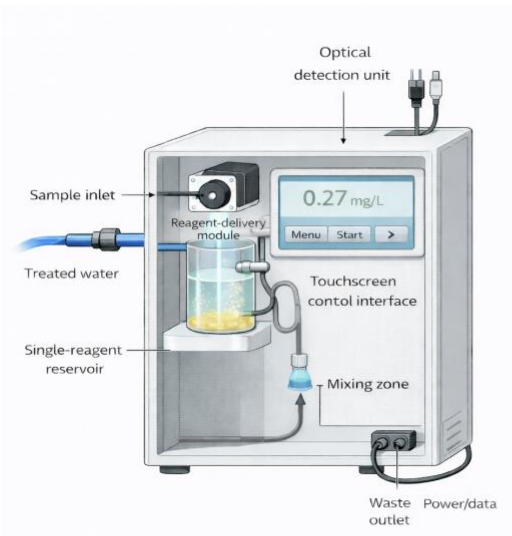

Figure 1. Schematic of the prototype online analyzer for stabilized TMB-based free chlorine monitoring. The diagram shows the overall prototype architecture, including sample inlet, single-reagent reservoir, reagent-delivery module, reaction/mixing segment, optical detection unit, control interface, and waste outlet.

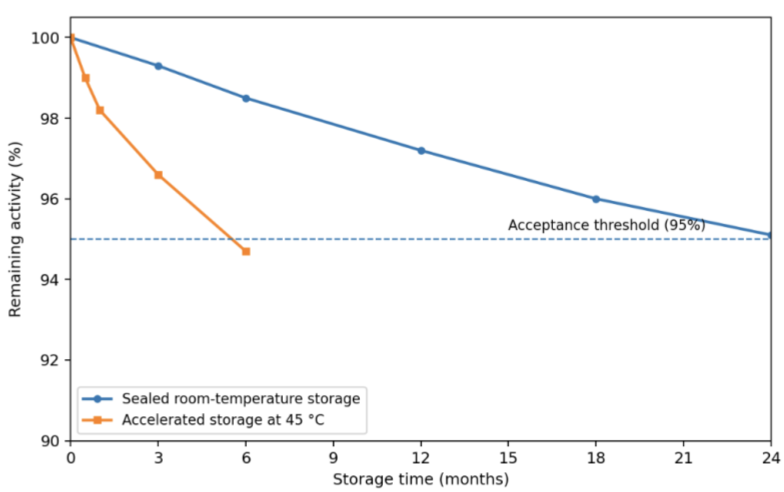

Figure 2. Storage stability of the stabilized TMB reagent under sealed room-temperature and accelerated conditions. Remaining activity is shown as a function of storage time. The dashed line indicates the 95% acceptance threshold used to support assignment of a 24-month sealed-storage shelf life.

References

[1] World Health Organization, Chlorine in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality, WHO, Geneva, 2003.

[2] Health Canada, Guidelines for Canadian Drinking Water Quality: Guideline Technical Document — Chlorine, Ottawa, 2016.

[3] P.W. LeChevallier, T.M. Babcock, R.G. Lee, Full-scale studies of factors related to coliform regrowth in drinking water, Appl. Environ. Microbiol. 62 (1996) 2201–2211.

[4] A.O. Al-Jasser, Chlorine decay in drinking-water transmission and distribution systems: pipe service age effect, Water Res. 41 (2007) 387–396.

[5] APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, 23rd ed., American Public Health Association, Washington, DC, 2017.

[6] ISO, ISO 7393-2:2017. Water quality — Determination of free chlorine and total chlorine — Part 2: Colorimetric method using N,N-diethyl-p-phenylenediamine (DPD), International Organization for Standardization, Geneva, 2017.

[7] U.S. Environmental Protection Agency, Method 334.0: Determination of Residual Chlorine in Drinking Water Using an On-Line Chlorine Analyzer, EPA, Cincinnati, OH, 2009.

[8] Hach, CL17sc Chlorine Analyzer Product Documentation, Hach Company, Loveland, CO, USA, accessed March 2026.

[9] D.G. Wahman, M.J. Pressman, C.J. Chinn, J.E. Vanderford, A drinking water relevant water chemistry model for free chlorine and cyanuric acid systems, Environ. Sci. Technol. 53 (2019) 4979–4988.

[10] D.G. Wahman, J.M. Kirisits, J.A. Rayne, Chlorinated cyanurates: review of water chemistry and implications for free chlorine analysis, Environ. Sci.: Water Res. Technol. 4 (2018) 987–1002.

[11] B. Kowalska, Chlorine decay in water distribution systems, Environ. Prot. Eng. 32 (2006) 5–16.

[12] F. Bosch Serrat, Colorimetric method for determination of chlorine with 3,3′,5,5′-tetramethylbenzidine, Talanta 41 (1994) 2091–2094.

[13] R.B.R. Mesquita, M.L.F.O.B. Noronha, A.I.L. Pereira, A.O.S.S. Rangel, Use of tetramethylbenzidine for the spectrophotometric sequential injection determination of free chlorine in waters, Talanta 72 (2007) 1186–1191.

[14] P. Palladino, M. Quijada-Garrido, F. de la Escosura-Muñiz, et al., 3,3′,5,5′-Tetramethylbenzidine as a multi-colorimetric indicator of chlorine in water in line with health guideline values, Anal. Bioanal. Chem. 412 (2020) 7241–7249.

[15] Y. Guo, J. Wang, Z. Liu, et al., Colorimetric detection of hypochlorite in tap water based on the oxidation of 3,3′,5,5′-tetramethylbenzidine, Anal. Methods 7 (2015) 6438–6442.