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Optimization of disposable paper-based test strips for hypochlorous acid detection

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Abstract. The Covid-19 pandemic has increased demand for chlorine-based sanitizing solutions, most of which contain hypochlorous acid (HOCl) as an active agent. Free chlorine (HOCl) in these sanitizers is crucial for their efficacy. Disposable test strips are affordable and convenient tools for determining various qualitative and quantitative parameters. In this study, disposable opto-chemical test strips were developed by physically immobilizing 3,3’,5,5’-tetramethylbenzidine (TMB) and o-dianisidine (o-D) reagents on chromatography and filter paper-based test strips for the visualization and detection of free chlorine in the form of HOCl. The reagents undergo a rapid color change upon reaction with chlorine through a redox reaction. The paper-based test strips showed rapid color change within a minute and a low sample volume requirement (1 ml). This portable, disposable paper-based test strip is a simple and cost-effective way to rapidly detect the presence of HOCl sanitizers for home and field applications. Both TMB and o-D successfully detected chlorine. Chromatography paper proved to be the more efficient option among the two papers used as substrates for the reagents (TMB and o-D). It exhibited high retention capacity and high performance in terms of color transformation when reacting with HOCl, even after two months of storage.

Keywords: chromogenic reagents; chlorine; disinfectant; hypochlorous acid; paper test strip; sanitizer

1. Introduction

For many years, specifically during the Covid-19 pandemic, many disinfection and cleaning routines involved the use of chlorine-based solutions as sanitizers for the oxidative inactivation of pathogens, including SARS-CoV-2 (Palladino *et al*. 2020a). The water pollution caused by microcystin also increases the importance of disinfectants (Rita *et al*. 2019), and the formation of harmful disinfection by-products creates severe health problems (Wang *et al*. 2023). In the form of hypochlorous acid (HOCl), free chlorine is an effective disinfectant (Ding *et al*. 2019). Colorimetry is one of the fastest and most convenient methods to detect and quantify the presence of free chlorine in water. Other techniques, such as spectrophotometry (Fobar *et al*. 2018), high-performance liquid chromatography (Klencsár *et al*. 2016), and fluorescence (Shang *et al*. 2019, Xiong *et al*. 2015), used for chlorine determination, can similarly provide accurate and precise results but require expensive instrumentation and technical skills (Phoonsawat *et al*. 2018). Furthermore, free chlorine detection kits are available commercially and provide satisfactory results. However, these kits are manufactured from relatively complex assays and require specialized gadgets like photometers and cell phones (Arsawiset and Teepoo, 2020). Most chlorine detection and visualization tools are sophisticated, but limited studies are available on manufacturing simple kits for detecting HOCl.

Test strips are simple and convenient tools for the qualitative and semi-quantitative analysis of organic and inorganic compounds (Schwenke *et al*. 2019). The sensitivity of the test strips is defined by the reactivity of the reagent in the test zone, indicated by a color change. The intensity of the color change mainly varies with the concentration of the analyte solution and contact time. In this study, 3,3’,5,5’-tetramethylbenzidine (TMB), 3,3′-dimethoxy benzidine, and *o*-dianisidine (*o*-D) were used as chromogenic reagents for the visualization of chlorine on paper-based test strips made from cellulose chromatography and glass fiber filter paper. TMB is one of the most commonly used chromatic reagents in chlorine detection and clinical applications as a safer substitute for benzidine, which was reported to be carcinogenic (Wu *et al*. 2019). TMB-based assays have been used as colorimetric reagents based on the formation of yellow or blue diamine or diimine colors (Guo *et al*. 2015, Mesquita *et al*. 2007, Palladino *et al*. 2020a, Serrat 1994, Yamaoka *et al*. 2016, Zhang and Yang 2013). TMB, a colorless molecule in the reduced state, transforms into a blue diamine when in contact with an oxidant. Further oxidation produces a green diimine intermediate, a blend of blue diamine, and the diimine yellow endpoint (Palladino *et al*. 2020a). The observed multichromatic reaction of TMB has been documented to exhibit remarkable sensitivity to pH, as it determines the color transformations that occur during the TMB reaction (Drozd *et al*. 2016).

o-D is a colorimetric peroxidase, commonly used as a substrate in enzyme-linked immunoassay (ELISA) procedures. It has also been used to measure exposure to chlorine gas from bleach (Ha *et al*. 2021), glucose, uric

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acid, and lactate (Dungchai *et al*. 2010). *o*-D produces a vivid orange color upon reaction with an oxidizing product. Although several studies have been conducted on the oxidation of TMB for chlorine detection, studies based on *o*-D as a chlorine colorimetric reagent are scarce. TMB and *o*-D have numerous benefits compared to other chromatic reagents, such as syringaldazine, *o*-tolidine, dopamine, N,N-diphenyl-1,4-phenylenediamine (DPD), and N,N-diphenyl-benzidine (DPB). These benefits include greater sensitivity, selectivity, environmental safety, and human health (Dou *et al*. 2019, Holland *et al*. 1974, Palladino *et al*. 2020b, Schwenke *et al*. 2019). These two reagents change color simultaneously upon exposure to oxidants and are relatively environmentally friendly compared to other dyes that undergo oxidation.

This study demonstrates the application of TMB and *o*-D as colorimetric reagents for the detection of chlorine by immobilizing the reagents onto paper-based substrates. The objective is to conclude the efficiency of these reagents in detecting HOCl by evaluating their reaction times and color intensities. Furthermore, this study aims to assess the capacity of the papers used as substrates to retain the reagents.

2. Materials and methodology

2.1 Chemicals and materials

The 3,3′-dimethoxybenzidine (o-dianisidine) and 3,3’, 5,5’-tetramethylbenzidine (TMB) were purchased from Merck (Germany). Sodium hypochlorite (NaOCl), calcium chloride (CaCl2), sodium chloride (NaCl), sodium hydrogen carbonate ($NaHCO_3$), sodium nitrate ($NaNO_3$), potassium iodide (KI), potassium bromide (KBr), sodium dihydrogen phosphate (NaH_2PO_4), and magnesium sulfate ($MgSO_4$) were procured from Daejung Chemicals & Metals Co. Ltd. (Gyeonggi-do, South Korea). All chemicals purchased were of analytical grade and were used as received. Whatman No. 1 glass fiber filter and cellulose chromatography paper (3 MM CHR) were purchased from GE Healthcare, Life Sciences (Buckinghamshire, UK).

2.2 Analytical measurement

Ultraviolet-visible (UV-vis) absorption spectra were recorded at room temperature using a Hach UV-vis spectrophotometer (DR 6000^{TM}, Hach Co., USA) with 1.0 cm path length quartz cuvettes from Hellma GmbH & Co. KG (Germany).

2.3 Colorimetric detection of HOCl

A stock solution of TMB was prepared in a mixture of 1:1 v/v ethanol and water and was left to dissolve overnight at room temperature. The solution was stored in a dark bottle and refrigerated at 4 °C. The stock solution of *o*-D was also prepared by mixing a calculated quantity of *o*-D in distilled water and storing it in a dark bottle. Different concentrations of TMB (0.5, 1, and 1.5 mM) and *o*-D (0.1, 0.5, and 1 mM) were subsequently prepared from the stock solution for the optimization tests. Acetic acid–sodium acetate buffer (200 mM concentration) was used to maintain the pH of the TMB solution at 4.5. A stock solution of HOCl was prepared from commercial NaOCl with an initial active chlorine concentration of 120 g/L. To detect HOCl, 2 ml of the reagent (TMB or *o*-D) solution was mixed with 2 ml of variable concentrations of the prepared NaOCl solution. After a minute of incubation, the color intensity of the resulting solutions and the corresponding UV-vis absorption spectra were recorded. NaOCl solutions were prepared using distilled water with a pH of approximately 5.8 to ensure that the free chlorine present in the solution is HOCl (Feng *et al*. 2007), knowing that the relative concentrations of HOCl and OCl^- are pH-sensitive, with HOCl dominating the equilibrium at $4 < pH < 6$.

2.4 Optimization of HOCl detection conditions

Four parameters were investigated to establish the optimum conditions for HOCl detection: reagent concentration, pH, reaction time, and the influence of competing ions. To determine the effect of reagent concentration, 2 ml of variable concentrations of TMB (0.5, 1, and 1.5 mM) and *o*-D (0.1, 0.5, and 1 mM) were mixed with 2 ml of 15 ppm NaOCl concentration in cuvettes. The colorimetric response of the resulting solutions was analyzed, and their corresponding UV-vis absorption spectra were recorded. The color changes, A_{655} of TMB and A_{482} of *o*-D, were used to study the influence of pH. To analyze the impact of pH, 2 ml of one of the reagents (1 mM TMB or 0.5 mM *o*-D) was mixed with 2 ml of HOCl solutions of various pH values. After the reaction, the color transformations and corresponding UV-vis absorption spectra (A_{655} of TMB or A_{482} of *o*-D) were recorded. Additionally, the impact of reaction time was determined by mixing 2 ml of the reagent with 2 ml of a 15-ppm HOCl solution, at this point, the color formation and the respective UV-vis values were recorded over a 10-minute period. Furthermore, to determine the assay selectivity in the presence of competing ions, 2 ml of a 1 mM concentration of Na^+, K^+, Ca^{2+}, Mg^{2+}, Br^-, I^-, PO_4^{3-}, NO_3^-, CO_3^{2-}, and SO_4^{2-} were mixed with 2 ml of either TMB or *o*-D and allowed to react for a minute. The resultant color and UV-vis values were recorded. The experiment was repeated by adding 2 ml of a 15-ppm HOCl solution to the reagent-ion solution.

2.5 Preparation of paper-based test strips

Cellulose chromatography and glass fiber filter papers were used as substrates for the test strip preparation. TMB and *o*-D solutions of 1 mM and 0.5 mM concentrations were prepared and poured into a beaker. The chromategraphy and filter papers were immersed in the TMB solution and left to stand for 5 minutes at room temperature. The same procedure was repeated for the *o*-D solution. The papers were then dried in a fume chamber for 3 hours and cut into 1 cm × 1 cm strips. The strips were then stored in labeled, airtight plastic containers for later use.

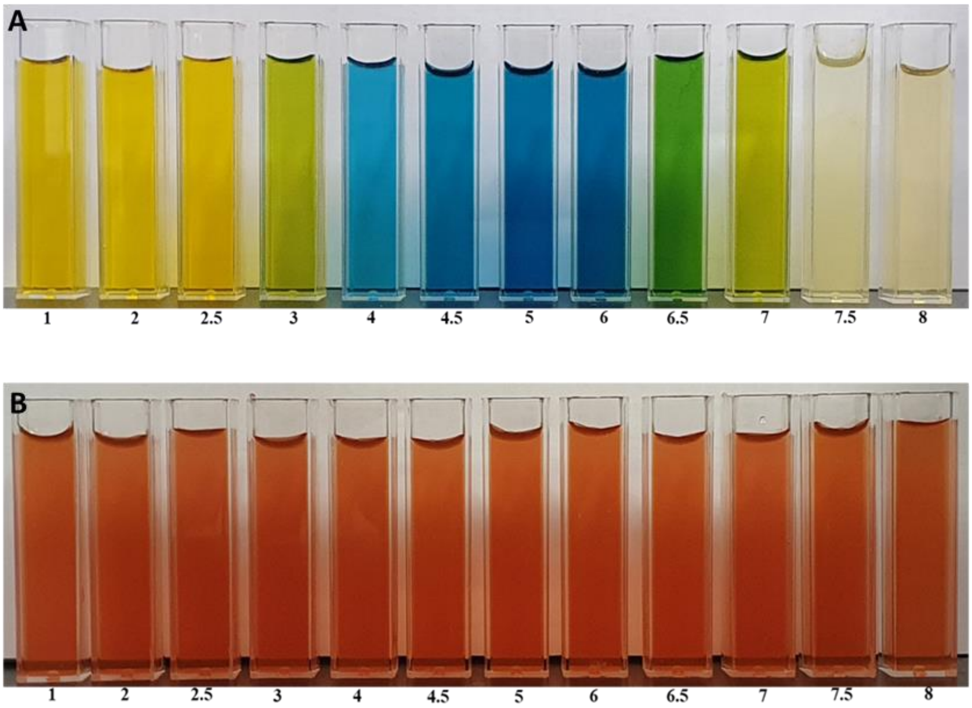


Fig. 1 Coloration of (A) TMB and (B) o-D after reacting with HOCl at various pHs

3. Results and discussion

3.1 Principle of detection (UV-vis spectroscopy)

TMB and *o*-D are colorimetric reagents that can detect chlorine due to their ability to change color instantly. The color transformation is due to the oxidation of TMB and *o*-D (reagents) in the presence of chlorine. The oxidation mechanism for TMB entails the formation of a semi-quinone imine radical cation, which reversibly forms a blue charge transfer complex with the parent diamine (Palladino *et al*. 2020a). The maximum UV-vis absorption peak of the oxidized blue TMB was observed at 655 nm. The yellow oxidized form of TMB, produced at a pH lower than 3, was observed at 450 nm. The oxidation of *o*-D upon exposure to Cl_2 and HOCl involves a redox reaction between *o*-D and chlorine, forming an orange-colored bis-azo-biphenyl product (Ha *et al*. 2021). The maximum UV-vis absorption peak of oxidized *o*-D (orange) was observed at 482 nm.

3.2 Optimization of detection conditions

3.2.1 Effect of reagent concentration

Optimal conditions for HOCl detection were established by investigating the effects of reagent concentration, pH, reaction time, and competing ions. The concentrations of TMB and *o*-D were studied methodically and optimized. Fig. S1(A) shows that the colorimetric response for the three TMB concentrations (0.5, 1, and 1.5 mM) is indistinguishable. Moreover, the UV-vis spectra measured for the three TMB concentrations are also analogous. The concentration of TMB was found to have little significance on the degree of HOCl detection as the TMB-HOCl reaction color outcome did not intensify with increasing TMB concentrations. As a result, a TMB concentration of 1 mM was employed for subsequent HOCl detection tests. On the contrary, a positive correlation was observed between the concentration of *o*-D and color intensity, simultaneously showing increased UV-vis values. The *o*-D began to exhibit crystallization (cloudy white spots) in the 1 mM concentration solution because of the reaction with HOCl (shown in Fig. S1(B)). An *o*-D concentration of 0.5 mM was selected as the optimal concentration for detecting HOCl.

3.2.2 Effect of pH

In principle, molecular chlorine (Cl_2) mainly exists at pH values < 2, with hypochlorous acid (HOCl) dominating the equilibrium at $4 < \mathrm{pH} < 6$, and hypochlorite (ClO^-) ions at pH > 8 (Kettle *et al*. 2014). Therefore, varying the pH of the sample solution within these pH ranges can influence the presence of a particular chlorine form, ultimately affecting its detection by the reagent. Variation in the pH of the TMB solution enables tracking of all non-catalytic oxidative forms of HOCl (Palladino *et al*. 2020a). Fig. 1(A) illustrates the color transformation of the TMB-HOCl reactions at variable pH values. The color of the product of the TMB-HOCl reaction was observed to be significantly influenced by pH. At lower pH values, the formation of yellow TMB-diimine was favored. At a pH range of 1–2.5, the colorless reduced-state TMB (diamine) changes to a yellow two-electron oxidized product (diimine). At a pH of 3, the yellow diimine changes to a green color, a mixture of the yellow endpoint and blue complex (charge-transfer complex). The absolute blue diamine/ diamine complex was found to be optimal in the pH range of 4 to 6, after which it began to change again at pH > 6, where charge transfer

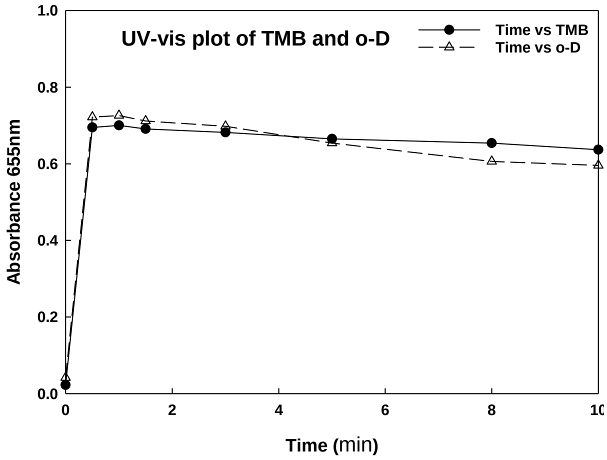


Fig. 2 Absorbance at 655 nm of TMB and o-D after reacting with HOCl at varous incubation durations

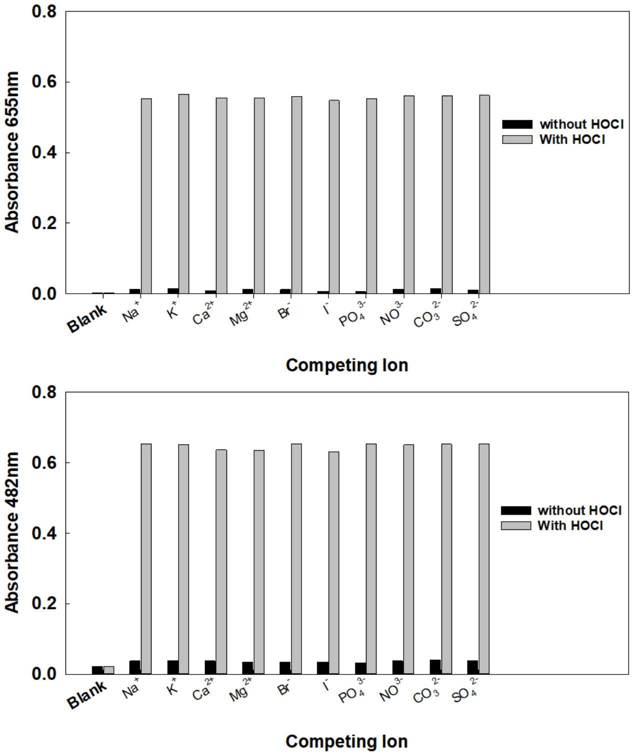


Fig. 3 Influence of competing ions on reaction of (a) TMB and (b) o-D with and without HOCl

complex formation began to decrease significantly. At a pH range of 7–8, a yellow color begins to form again, exhibiting the appearance of the solution at a pH of 1–2.5. However, the stability and intensity of this diimine is lower than that formed at a pH of 1–2.5. This multicolor (yellow-green-blue) attribute of TMB makes it a very suitable indicator for chlorine colorimetry. The A_{655} of the TMB solution changed significantly from a pH of 1.0 to 7.5. However, it stabilized at a pH of 4.5, displaying a vivid blue color with a maximum A_{655}. Thus, the optimum pH value of TMB was 4.5, and this value was maintained throughout this study. In contrast, no color deviation was observed when altering the pH values of *o*-D and chlorine solutions (Fig. 1(B)). In addition, the recorded A_{482} of *o*-D-HOCl reactions showed slight variations in the UV-vis spectra values.

3.2.3 Effect of incubation time

The oxidation of TMB and *o*-D was influenced by incubation time. HOCl rapidly oxidized both TMB and *o*-D, and the oxidation was complete within a minute (Fig. 2). This indicates the ability of these two reagents to rapidly-produce a vivid color change via a redox reaction in the presence of HOCl. All experiments were performed consecutively using the optimal conditions of 1 mM TMB and 0.5 mM *o*-D, maintained at a pH of 4.5, with a 1 min incubation time for the prime detection of HOCl.

3.2.4 Effect of competing ions/selectivity of the assay

To determine the selectivity of the assays for HOCl detection in the presence of interfering ions, series of experiments were performed with Na^+, K^+, Ca^{2+}, Mg^{2+}, Br^-, I^-, PO_4^{3-}, NO_3^-, CO_3^{2-}, and SO_4^{2-} at concentrations of 1 mM each. No color deviation was observed for the reactions of the interfering ions with TMB, as illustrated in Fig. 3(a), but a visible blue color was observed in the presence of HOCl. The TMB-HOCl-ion reaction showed higher A_{655} values, indicating that these cations and anions have no effect on the HOCl detection, as confirmed by the colorimetric response shown in Fig. 3(a). The high affinity of HOCl to oxidize TMB is attributed to this high selectivity, even in the presence of the ions reflected by the blue product.

Equivalent results were observed for the reactions of the interfering ions with *o*-D. No coloration was detected for the *o*-D-ion mix, however, an orange coloration appeared in the *o*-D-ion-HOCl reaction, indicating the presence of HOCl (Fig. 3(b)). Additionally, A_{482} indicated no effect of interfering ions on *o*-D-HOCl oxidation, as shown in Fig. 3(b).

Natural water contains variable concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+}, therefore, additional interference tests were carried out to see the effect of these four primary ions on HOCl detection when tested with both reagents. Even at a high ion concentration of 10 mM (Na^+, K^+, Ca^{2+}, and Mg^2), no ion interference was observed in the effectiveness of HOCl detection with TMB and *o*-D, where color transformation was detected for both reagents.

3.3 Chlorine (HOCl) detection (Calibration curve)

To determine the minimum concentration of HOCl detectable by the assays, the UV-vis absorption spectra of TMB and *o*-D were observed using different concentrations of HOCl solution. Color transformations indicating reagent oxidation of TMB (blue) and *o*-D (orange) were observed as the HOCl concentration was increased from 0 to 25 ppm (as shown in Fig. S2 (A) and (B)). The variation in the absorbance values at 655 nm (A_{655}) for TMB and 482 nm for *o*-D also showed the oxidation of TMB and *o*-D by HOCl. As the HOCl concentration increased, the UV-vis absorbance at 655 nm and 482 nm gradually increased for TMB and o-D, respectively. The oxidation of TMB was

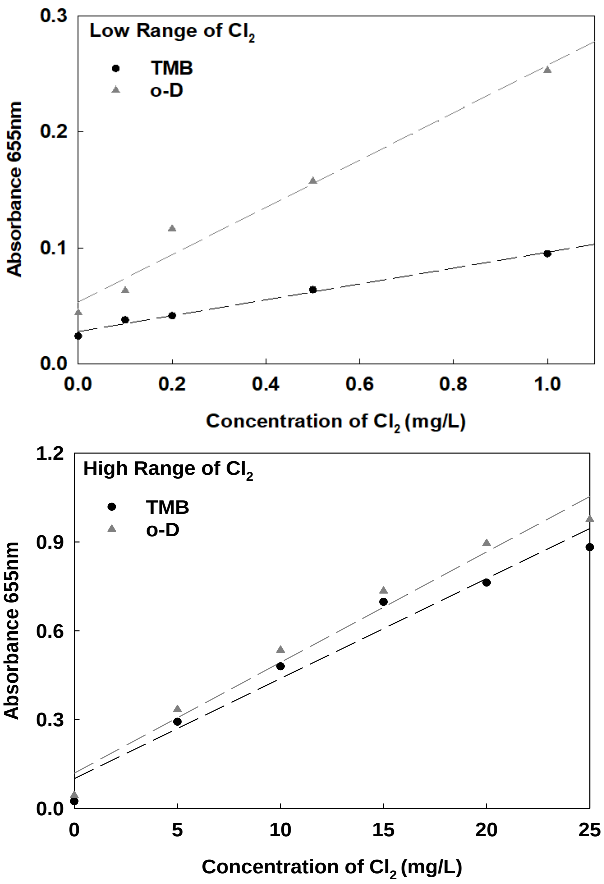
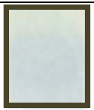
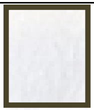


Fig. 4 Linear relationship between A655 of TMB and o-D at different concentrations of Cl

Table 1 Coloration of paper-based test strips with TMB and *o*-D after reacting with HOCl

Concentraiton (ppm)	TMB$_C$	TMB$_F$	o-D$_C$	o-D$_F$
0.5				
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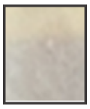
5				
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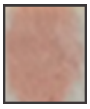
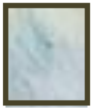
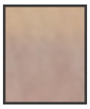
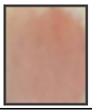
15				
20				
25				

evident at HOCl concentrations as low as 0.1 ppm. The fitting curve showed an excellent linear relationship between A_{655} and a concentration of HOCl as low as 0 ppm and up to 25 ppm, with a correlation coefficient of 0.9899 for the lower range (0–1 ppm) and 0.9608 for the higher range of 5–25 ppm HOCl concentration (Fig. 4). The oxidation of *o*-D, however, started to occur at a HOCl concentration of 0.5 ppm. The A_{482} of *o*-D also showed a good relationship with a correlation coefficient of 0.9740 for the lower range (0–1 ppm) and 0.9715 for the higher range of 5–25 ppm HOCl concentration (Table 1).

3.4 Appearance of prepared TMB and o-D paper-based test strips

Numerous capillary pores, high absorbability, and a large specific surface area make chromatography and filter papers suitable substrates for organic and inorganic reagents (Wang *et al*. 2018). With this knowledge, TMB and o-D chromogenic reagents were immobilized onto chromategraphy and filter papers as portable, user-friendly HOCl detection kits. Fig. S3 shows the TMB chromatography and filter paper strips before the reaction with HOCl. After immobilizing the papers with TMB, the chromatography paper strip transitioned to a light grey color upon drying, while the filter paper strip remained white.

When the *o*-D reagent was immobilized on the paper, the chromatography paper strip showed a slight discoloration upon drying, similarly, a slight discoloration appeared on the filter paper strips, but not as much as on the chromatography paper. The slight discoloration in the papers was due to the retention of the reagents by the papers.

3.5 Performance of test strips in HOCl detection

The prepared paper-based test strips were assessed based on the reaction time and capability of HOCl detection according to the intensity of the color produced upon the reaction with HOCl. To achieve this, paper-based test strips were tested with different concentrations of free chlorine to ascertain the response of the papers when in contact with HOCl, and the intensity of the color produced by the papers was observed. First, TMB chromatography and filter papers were tested. The TMB chromatography paper showed a rapid color change within 17 seconds when in contact with the free chlorine solution. The blue color, which indicates the oxidation of TMB, was observed (Table 1). However, the blue color was very light at the lower HOCl concentrations of 0.5 ppm and 1 ppm, while a distinct blue color was evident for HOCl concentrations above 5 ppm. Table 1 illustrates the color transformation of the TMB filter paper upon reaction with free HOCl. The color of the TMB filter paper took longer to transform than that of the TMB chromatography paper strips. Color formation started 30 seconds after being in contact with HOCl, while at the 1-minute mark, only a faint light blue color was observed.

Next, *o*-D chromatography and filter papers were tested. The coloration of the *o*-D chromatography and filter paper-based test strips upon reaction with HOCl is shown in

Table 1. The *o*-D chromatography paper showed orange color transformation slightly more rapidly than the TMB chromatography strip. The oxidation of *o*-D was observed within 12 seconds of the reaction with the free chlorine solution. However, the orange color was distinct only when the HOCl concentration reached 10 ppm. At concentrations lower than 10 ppm, the orange color was hardly observed. The *o*-D filter paper showed results equivalent to those of TMB filter paper during the testing of the reaction of *o*-D filter paper in HOCl solutions. The color transformation took approximately 1 minute and 18 seconds to appear. Although the o-D filter paper was faint orange, it was more visible than the blue color observed in the TMB filter paper strip.

Although the color development in TMB and *o*-D paper-based kits increased with increasing HOCl concentration, chromatography papers showed a more vivid color for both reagents than filter papers. In addition, the reaction time for the chromatography test strips for both reagents was much faster than that for the filter paper-based strips. The observed differences in color intensity development and reaction time can be attributed to the unique properties of the two papers. In practice, filter paper, with its relatively small pores, operates more as a sieve than a liquid retainer and is more suited for mechanical filtration. This feature of the filter paper must have been why it remained white after being immobilized with the reagents and exhibited only a faint coloration for the reagent-HOCl reaction. On the other hand, chromatography paper is functionalized with cellulose fiber as an inert support to retain the liquid required in the stationary phase during paper chromatography procedures. The retention property of chromatography paper makes it a suitable substrate for reagent test strips (Miller *et al.* 2018). Therefore, the chromatography paper test strips effectively retained the reagents, resulting in a more evident coloration upon reaction with HOCl. Among the two papers used as a substrate for the reagents, chromatography paper proved more effective for reagent retention, enhancing HOCl oxidation and providing a more accurate visualization.

3.6 Effect of storage on the performance of paper-based test strips

The prepared test strips were stored in amber-colored glass bottles in a dark cabinet at 10 °C for two months. TMB and *o*-D reagents are reported to be light-sensitive, therefore, direct and prolonged exposure to light (especially sunlight) should be avoided to prevent the degradation of the reagents (Miller, 2018) (Lehmann *et al.* 2019). The paper-based test strips were further assessed based on reaction time and intensity of color formation upon reaction with HOCl after 2 months of storage. The TMB chromatography paper showed a rapid color change within 17 seconds when in contact with chlorine. The blue color was more visible at 1 ppm chlorine concentration (Fig. S4). On the contrary, the TMB filter paper showed color transformation 35 seconds after contact with HOCl, and a pale blue color was observed after a minute. The oxidation of *o*-D was observed within 17 seconds of the reaction with the free chlorine solution. The orange color transformation was slightly more visible than when the papers were developed two months earlier. The *o*-D filter paper again took a long time to color transform (1 min and 43s). However, the faint orange color observed for the *o*-D filter paper was more visible than the blue color observed for the TMB filter paper strip (Fig. S5) provided in the supplementary data file.

Thus, the duration of the storage of the reagent paper test strips had an insignificant effect on the performance of the prepared test strips, especially the chromatography paper-based strips, which still showed the expected color appearance due to their ability to retain the reagents. Thus, no sophisticated conditions were required for storage. Storing the paper strips in dark bottles away from direct light at 10 °C was sufficient to prevent the degradation of the reagents.

4. Conclusions

This study contributes to the field of knowledge on the colorimetric determination of free chlorine using TMB and o-D as safer alternatives to carcinogenic benzidine. The chromogenic properties of TMB and o-D were utilized for the easy detection and visualization of HOCl. The two reagents (TMB and o-D) showed excellent oxidation of HOCl, as indicated by color development, and exhibited high selectivity for HOCl in the presence of other competing ions. Simple paper-based test strips were developed to visualize the HOCl in slightly acidic electrolyzed water (SAEW). The TMB and o-D paper-based test strips showed rapid coloration with a low HOCl concentration within a minute. Chromatography paper proved to be a better substrate among the two papers used for the reagents due to its high retention capability. The chromatography paper-based test strips still exhibited the required color change after two months of storage in dark conditions. These chromatography paper-based test strips can serve as a simple tool to test and visualize HOCl in SAEW without sophisticated gadgets. In conclusion, unlike TMB, this study highlights the underutilized potential of o-D as a colorimetric reagent for detecting chlorine and recommends further research in this area.

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Appendix

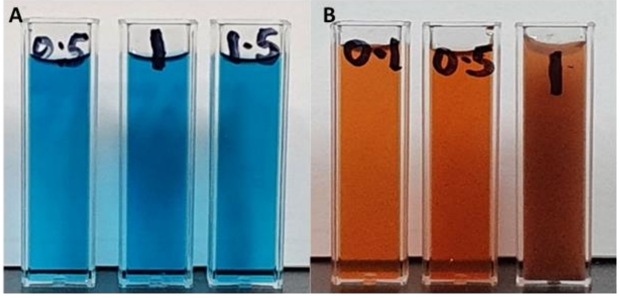

Fig. S1 Color intensity illustration of the effect of different concentrations of (A) TMB and (B) o-D solutions reacting with HOCl

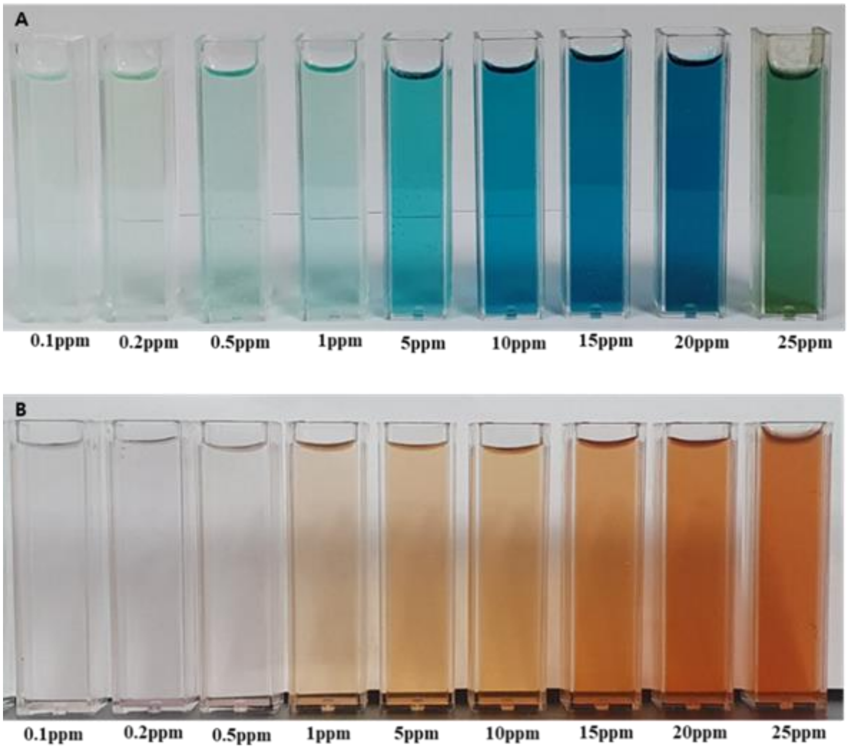

Fig. S2 Oxidation of (A) TMB (blue) and (B) o-D (orange) by HOCl

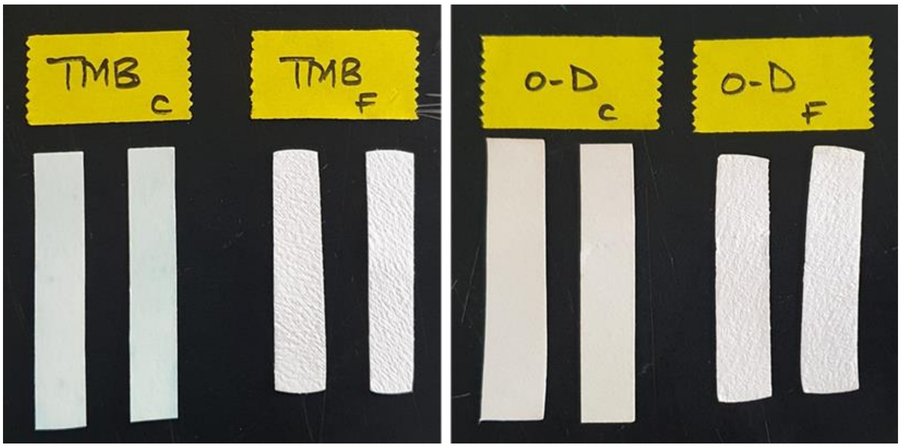

Fig. S3 Appearance of paper-based test strips after immobilizing with TMB and o-D reagent, C- Chromatography paper, F- Filter paper

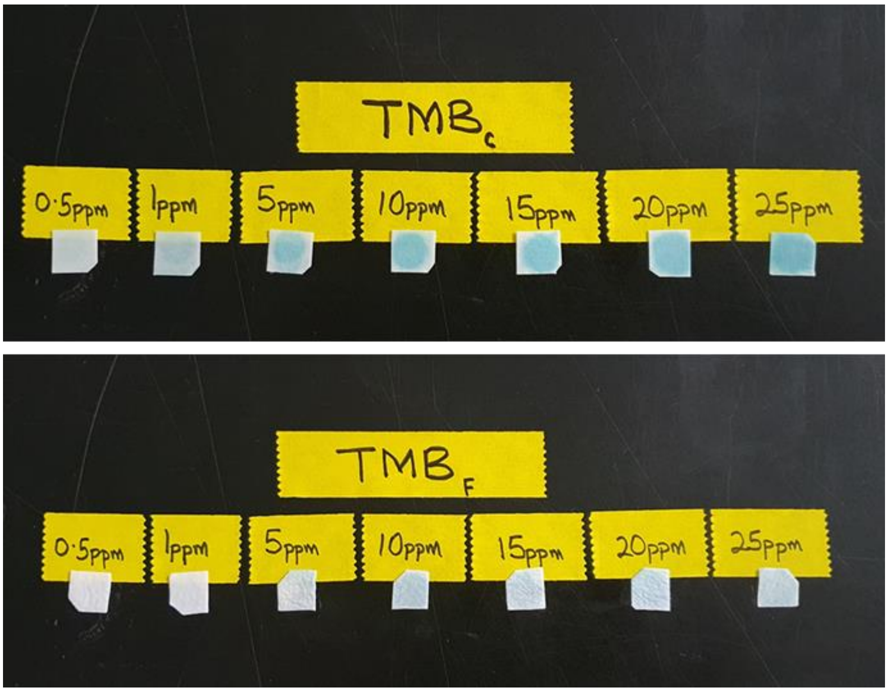

Fig. S4 TMB paper-based test strips coloration after 2 months of storage

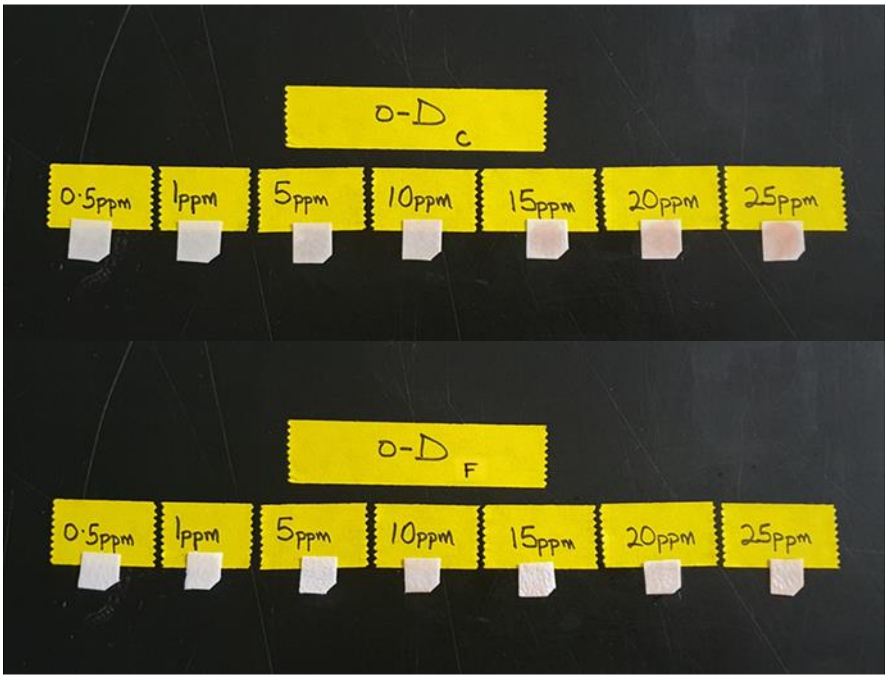

Fig. S5. *o*-D paper-based test strips coloration after 2 months of storage