

## EFFECT OF THE MOUTHWASH CONTAINING CHLORINE DIOXIDE AND CHLORHEXIDINE ON HYDROGEN SULFIDE GAS IN HALITOSIS TREATMENT

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### ABSTRACT

**Objectives:** To evaluate the effectiveness of mouthwash that contains chlorhexidine and chlorine dioxide in neutralizing hydrogen sulfide gas. **Methods:** Hydrogen sulfide was prepared from sodium sulfide and sulfuric acid, and the amount of gas was calculated by the standard curve method. Evaluate the interaction between hydrogen sulfide gas (H<sub>2</sub>S) and chlorine dioxide (ClO<sub>2</sub>) and chlorhexidine (CHX) by letting the H<sub>2</sub>S gas through the flask containing ClO<sub>2</sub> 0.05%, 0.1%, and the mixture of ClO<sub>2</sub> 0.05% + CHX 0.01%, ClO<sub>2</sub> 0.1% + CHX 0.01%, and performing the UV-vis absorption spectra change of dye solutions. The results were compared to the one when H<sub>2</sub>S gas passed through water. **Results:** When the volume of H<sub>2</sub>S was 0.262 μmol, over 90% of H<sub>2</sub>S reacted with the mouthwash containing ClO<sub>2</sub>. As the ClO<sub>2</sub> concentration increases, the amount of responding H<sub>2</sub>S also increases. The CHX + ClO<sub>2</sub> mixture did not affect the capacity of H<sub>2</sub>S neutralization. **Conclusion:** Chlorine dioxide was a potential agent to reduce the oral unpleasant breath.

**Keyword:** chlorine dioxide, halitosis, hydrogen sulfide, mouthwash, neutralization.

### I. INTRODUCTION

Halitosis, or bad breath, affects a significant portion of the global population,

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**Date of receipt:** 12/8/2024

**Date of scientific judgment:** 16/9/2024

**Reviewed date:** 14/10/2024

with prevalence ranging from 22% to over 50%. It is primarily caused by volatile sulfur compounds, such as hydrogen sulfide, produced by oral bacteria breaking down organic matter in the mouth. These compounds, including dimethyl sulfide, methyl mercaptan, and hydrogen sulfide, are produced by specific microbes in the oral cavity. Treatment for halitosis involves mechanical and chemical methods. Mechanical techniques like brushing teeth, tongue scraping, and interdental cleaning reduce bacteria in the mouth and disrupt the synthesis of volatile sulfur compounds. Chemical interventions like toothpaste, mouthwashes, and other oral hygiene products with active ingredients like chlorhexidine, cetylpyridinium chloride, essential oils, triclosan, chlorine dioxide, hydrogen peroxide, and zinc can effectively reduce bad breath. However, not all active ingredients have the same effectiveness. Researchers are exploring new products that minimize the adverse effects of chlorhexidine without compromising its efficacy or improving it. Recently, chlorine dioxide (ClO<sub>2</sub>) has become known as a possible way to treat foul breath because it can neutralize volatile sulfur compounds without the bad side effects that come with traditional mouthwashes like chlorhexidine [2].

Research has not yet been conducted on the effectiveness of mouthwash that combines chlorhexidine and chlorine dioxide in managing halitosis. However, according to

Anna H. (2019), the fact that the two agents do not interact supports the idea of using them together [1]. This paper assesses the ability of mouthwash containing chlorhexidine and chlorine dioxide to neutralize hydrogen sulfide gas, which is the main origine of halitosis.

## II. MATERIAL AND METHODS

### • Preparation of H<sub>2</sub>S gas

Based on the equation  $\text{Na}_2\text{S} + \text{H}_2\text{SO}_4 \rightarrow \text{Na}_2\text{SO}_4 + \text{H}_2\text{S}$ , calculate the amount of Na<sub>2</sub>S and put it into a 40-mL vial with a septum. Close the lid, inject 1:1 H<sub>2</sub>SO<sub>4</sub> with a volume equal to Na<sub>2</sub>S, vortex 30s.

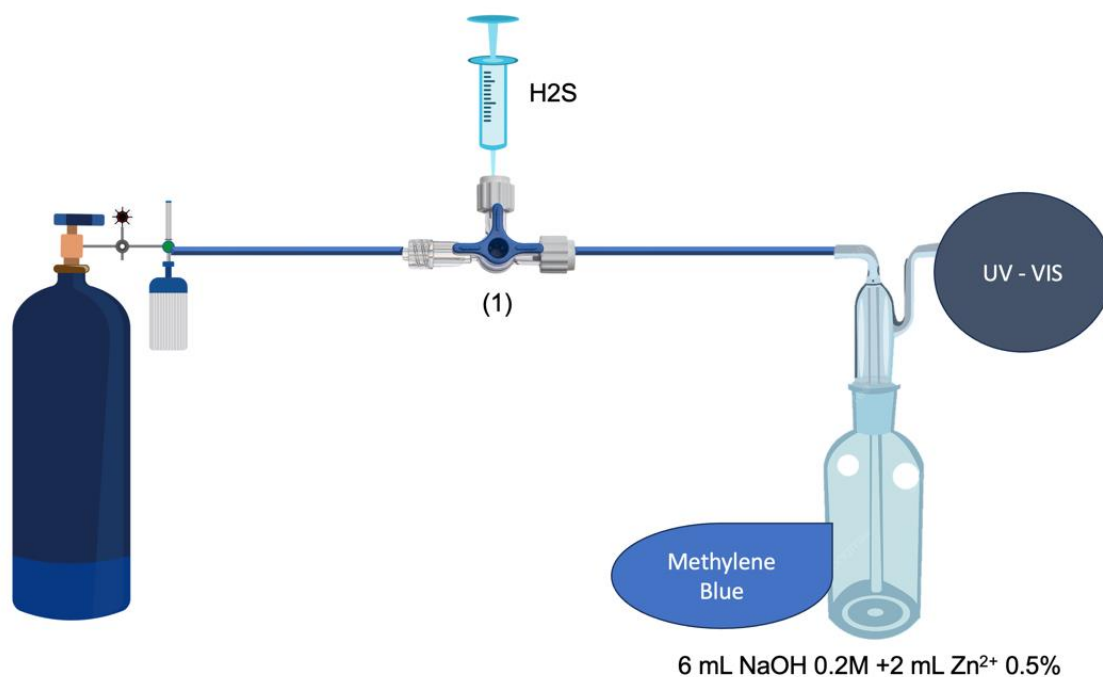
After the sample is ready, 100 $\mu$ L, 50 $\mu$ L, and 10 $\mu$ L of the H<sub>2</sub>S gas in the vial are sucked out and injected into the T-tube (1). The flow of inert Argon gas pulls the H<sub>2</sub>S into the absorption vessel, which has 6 mL of 0.2 M NaOH and 2 mL of 0.5% Zn<sup>2+</sup> in it. The reagent is added to the absorption

solution to produce a methylene blue color and photometric measurement at 665 nm wavelength.

The amount of H<sub>2</sub>S in the absorber was determined using the standard curve method. The amount of H<sub>2</sub>S in the preparation vial is calculated based on the three-proportion rule. The amount of H<sub>2</sub>S in the vial is the average of the results of different injection volumes.

### • Investigate the interaction between H<sub>2</sub>S, ClO<sub>2</sub>, and water.

H<sub>2</sub>S gas does not pass through the investigation solution. The system will not have a flask, and the gas line will lead directly from the T-tube (1) to the absorber. Inject H<sub>2</sub>S gas into (1). Argon gas blows at a 40 mL/min flow rate, attracting all the H<sub>2</sub>S into the absorption vessel. After about 30 seconds, the UV-vis (Ultraviolet visible) absorption spectra change of dye solutions is performed (Figure 1).

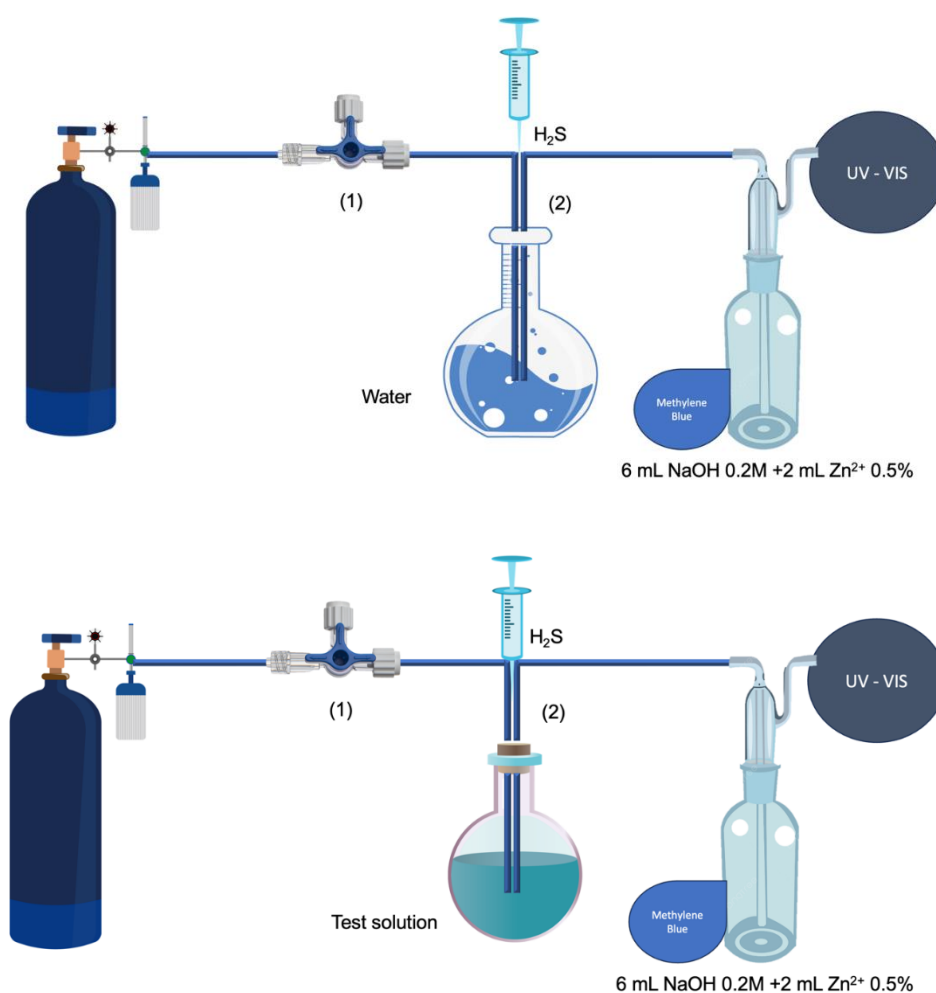


**Fig. 1:** System diagram when H<sub>2</sub>S gas does not through the investigation solution

H<sub>2</sub>S gas through the investigation solution. The system will look like Figure 2. Put 10 mL of the investigation solution into the flask. Before injecting H<sub>2</sub>S gas, adjust the flowmeter to about 5 mL/min and unplug the gas line from the flask to the absorption flask. Inject V (μL) H<sub>2</sub>S into position 2 and shake the flask for 30 seconds. After shaking, reattach the gas line leading to the absorber to the flask, reopen the gas flow to the 40 mL/min mark, and blow air for 10 minutes to ensure complete blowing of the gas in the

flask into the absorber. Perform the UV-vis absorption spectra change for dye solutions.

The amount of H<sub>2</sub>S prepared will be presented as micromol (μmol), the prortion of H<sub>2</sub>S in the gaseous phase and liquid phase will be presented as percentage (%). The calculation will be performed by Microsoft Exel ver. 16.9.2 (Microsoft, USA). The study will be conducted at Faculty of Chemistry, University of Sciences, Vietnam National University Ho Chi Minh City.



**Fig. 2: System diagram when H<sub>2</sub>S gas through the investigation solution**  
*\*The flask has a septum lid*

### III. RESULT

#### The volume of H<sub>2</sub>S in the preparation vial

**Table 1: The volume of H<sub>2</sub>S in vial**

Injected volume (μL)	A1	A2	A3	Am	n H <sub>2</sub> S (μmol)	n H <sub>2</sub> S vial (μmol)
100	0.42	0.418	0.415	0.418	0.260	109.21
50	0.215	0.219	0.213	0.216	0.129	108.47
10	0.099	0.091	0.094	0.095	0.051	106.52
					Mean	108.07

The H<sub>2</sub>S gas prepared in the vial was approximately 108 μmol (48.9 ppmv H<sub>2</sub>S), correspond to 0.26 μmol H<sub>2</sub>S in the absorber. This amount of H<sub>2</sub>S will be used for the reaction experiment with the mouthwash containing chlorine dioxide.

#### H<sub>2</sub>S with the test solution

When the amount of H<sub>2</sub>S was 0.262 μmol, over 90% of H<sub>2</sub>S reacted with tested

solutions, a significantly higher percentage than H<sub>2</sub>S dissolved in water, which accounted for about 32% (Table 2 and Figure 3). At a low concentration in the experiment, with 0.131 μmol, the presence of H<sub>2</sub>S was undetectable in the gaseous phase, indicating a complete reaction with the tested solutions (Table 3 and Figure 4).

**Table 2: The reaction of H<sub>2</sub>S with the mouthwash solution at 48.9 ppmv H<sub>2</sub>S**

		n H <sub>2</sub> S (μmol)	%RSD
	Not through flask (μmol)	<b>0.262</b>	1.1
Through the flask containing water	Gaseous phase (μmol)	0.166	1.4
	Liquid phase (μmol)	0.078	6.3
	Recovery rate (%)	93.1	
Through the flask containing ClO <sub>2</sub> 0.05% solution	Gaseous phase (μmol)	0.022	9.0
	The volume H <sub>2</sub> S reacting to ClO <sub>2</sub> (μmol)	0.240	
Through the flask containing ClO <sub>2</sub> 0.1%	Gaseous phase (μmol)	0.011	11.4
	The volume H <sub>2</sub> S reacting to ClO <sub>2</sub> (μmol)	0.251	
Through the flask containing ClO <sub>2</sub> 0.05% + 0.01% CHX	Gaseous phase (μmol)	0.015	8.8
	The volume of H <sub>2</sub> S reacting to the solution (μmol)	0.248	
Through the flask containing ClO <sub>2</sub> 0.1% + 0.01% CHX	Gaseous phase (μmol)	0.008	12.8
	The volume of H <sub>2</sub> S reacting to the solution (μmol)	0.254	

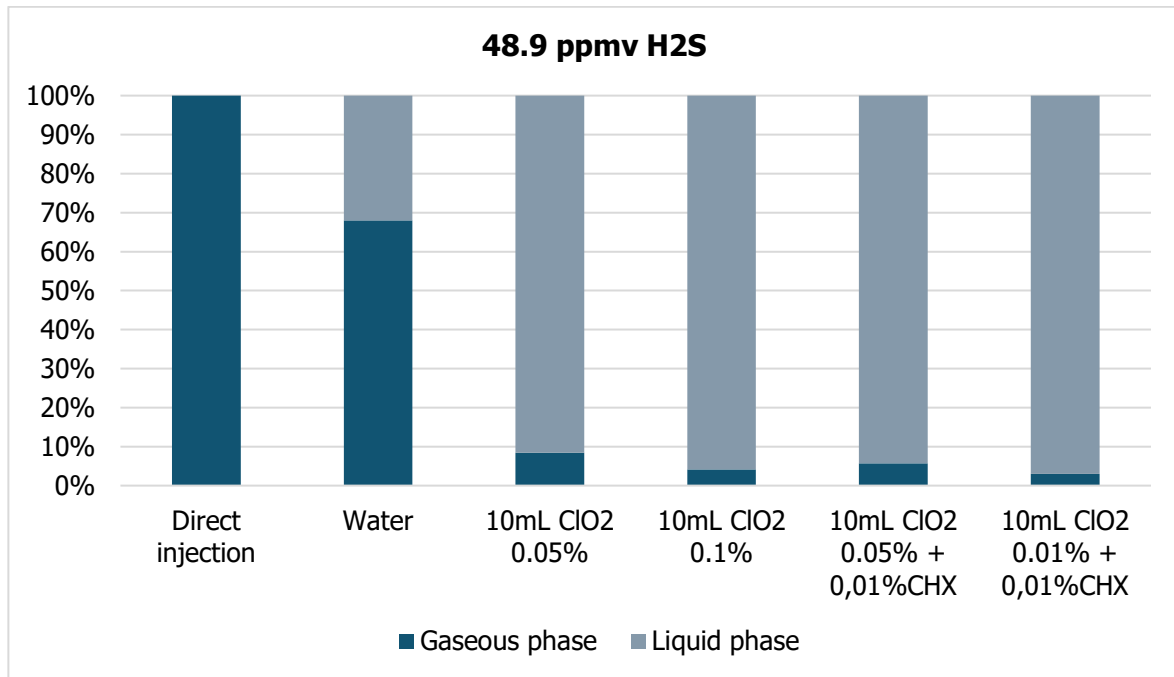
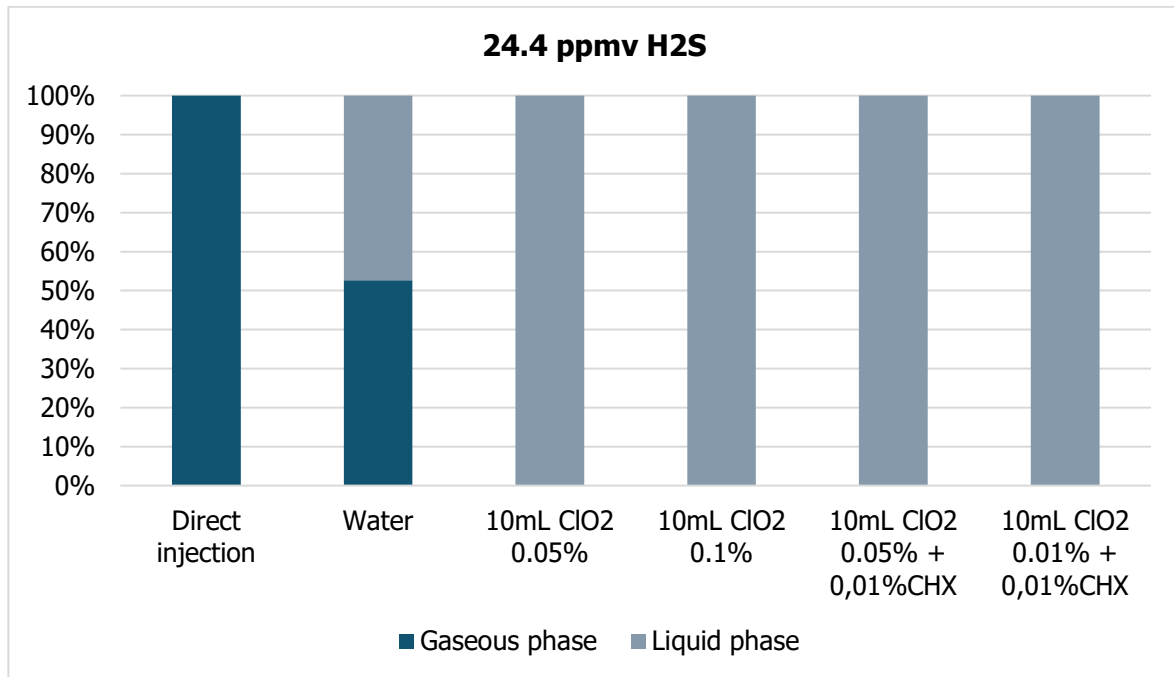


Fig 3: The proportion of H<sub>2</sub>S in the gaseous phase and liquid phase at 48.9 ppmv H<sub>2</sub>S

Table 3: The reaction of H<sub>2</sub>S with the mouthwash solution at 24.4 ppmv H<sub>2</sub>S

		n H <sub>2</sub> S (μmol)	%RSD
	Not through flask (μmol)	<b>0.131</b>	2.5
Through the flask containing water	Gaseous phase (μmol)	0.059	10.0
	Liquid phase (μmol)	0.053	11.0
	Recovery rate (%)	85.8	
Through the flask containing ClO <sub>2</sub> 0.05% solution	Gaseous phase (μmol)	Not detected	0.0
	The volume H <sub>2</sub> S reacting to ClO <sub>2</sub> (μmol)	0.131	
Through the flask containing ClO <sub>2</sub> 0.1%	Gaseous phase (μmol)	Not detected	0.0
	The volume H <sub>2</sub> S reacting to ClO <sub>2</sub> (μmol)	0.131	
Through the flask containing ClO <sub>2</sub> 0.05% + 0.01% CHX	Gaseous phase (μmol)	Not detected	0.0
	The volume of H <sub>2</sub> S reacting to the solution (μmol)	0.131	
Through the flask containing ClO <sub>2</sub> 0.1% + 0.01% CHX	Gaseous phase (μmol)	Not detected	0.0
	The volume of H <sub>2</sub> S reacting to the solution (μmol)	0.131	



**Fig 4: The proportion of H<sub>2</sub>S in the gaseous phase and liquid phase at 24.4 ppmv H<sub>2</sub>S**

#### IV. DISCUSSION

Hydrogen sulfide (H<sub>2</sub>S) is a harmful gas generated naturally or by industrial processes like oil and gas extraction, wastewater treatment, and pulp and paper production. It poses a significant risk to human health and the environment. Oral volatile sulfur compounds (VSC) are abundant in dentistry and play a role in oral diseases by controlling cell function and affecting the immune system. H<sub>2</sub>S is a neurotransmitter that controls cellular functions and has multiple effects on various disorders, including halitosis. Therefore, regulating its production and release is crucial [5]. In dentistry, anti-VSC medicines diminish oral malodor by inhibiting VSC formation and providing antibacterial action. Antimicrobials such as triclosan, zinc chloride, chlorhexidine, chlorine dioxide, cetylpyridinium chloride, essential oils, and zinc chloride get rid of bad breath by killing the bacteria that cause it [2].

Chlorhexidine is a strong denaturing agent that can stain teeth by exposing proteins to sulfhydryl groups and breaking them down. Mouthwashes containing chlorhexidine have antibacterial properties, breaking down membranes and killing cells. They are available in concentrations of 0.2% and 0.12%. Chlorhexidine is effective in curing oral malodor but can cause taste changes, increased mucosa shedding, calculus production, and tooth and tongue discoloration. Prolonged use can lead to adverse effects, so small amounts (0.05%) are used. It is recommended to mix CHX with other active medications to minimize these effects [2], [7].

Chlorine dioxide (ClO<sub>2</sub>) is a chemically stable compound with an unpaired electron and dissolves easily in water, making it a topical antiseptic for oral cavities or dentures. Besides, ClO<sub>2</sub> has many practical uses due to its quick reaction, long-lasting effect, large area coverage, ease of use, and

ability to eliminate contaminants. It is unstable in the presence of light and cannot remain in the air for long periods, making it less risky to individuals and the environment than chlorine gas. However, when comparing to CHX, CHX 0.2% inhibits biofilm more powerfully than ClO<sub>2</sub> 0.01% and works better than ClO<sub>2</sub> four hours after use [7], [8].

To our knowledge, no study has yet been conducted on the combination of chlorhexidine and chlorine dioxide. However, the lack of chlorhexidine effectively prevents the growth of bacteria that produce volatile sulfur compounds (VSCs), while chlorine dioxide acts as an oxidizing agent that can eliminate mouth odors. Nevertheless, does this combination have a synergistic effect in guaranteeing the capacity to counteract volatile sulfur gas? The inquiry pertains to the efficacy of a mouthwash solution that combines chlorhexidine and chlorine dioxide in neutralizing H<sub>2</sub>S.

In this study, pure water did not remove H<sub>2</sub>S, as evidenced by the 93% recovery rate when the H<sub>2</sub>S gas passed through the flask with water. In contrast, ClO<sub>2</sub> in 0.05% and 0.1% can react with H<sub>2</sub>S more than 94%. In addition, adding CHX 0.01% does not seem to reduce this capacity for neutralizing H<sub>2</sub>S. 98% H<sub>2</sub>S at 48.9 ppmv reacted with the mixture of CHX 0,01% and ClO<sub>2</sub> 0,1%, while 100% H<sub>2</sub>S at 24.4 ppmv participated in this reaction. In reality, to diagnose halitosis in a patient using gas chromatography, the H<sub>2</sub>S value must be greater than 122 ppb. The cut-off value of H<sub>2</sub>S in halitosis patients was  $52.72 \pm 96.23$ . H<sub>2</sub>S levels were significantly higher in the gingivitis ( $100.51 \pm 183.69$  ppb) and periodontitis ( $91.57 \pm 132.06$  ppb) groups than in healthy controls ( $14.97 \pm 31.22$  ppb) [4]. These values are low

compared to our experiments, so we cannot detect H<sub>2</sub>S reaction with the mouthwash mixture.

The effect of chlorine dioxide on hydrogen sulfide has been explained through some studies. Csekö G's study (2018) explored the kinetics and mechanism of sulfide oxidation by chlorine dioxide in a highly alkaline solution. The writers came up with a theory about a reaction that involves moving electrons and oxygen atoms around one after the other. This creates sulfur in the form of Sx<sup>2-</sup> and sulfate. The sulfide oxidation by chlorine dioxide occurs in two stages, each with its own distinct time-scale process. ClO<sub>2</sub> and ClO<sub>2</sub><sup>-</sup> are reactive oxidants that exhibit potent reducing properties on VSCs [3]. Lynch et al. discovered that mixing L-cysteine with ClO<sub>2</sub> and ClO<sub>2</sub><sup>-</sup> led to the main reaction, which was the disulfide cystine. The oxidation of thiols can take place in a consecutive, two-step reaction sequence using ClO<sub>2</sub> and/or ClO<sub>2</sub><sup>-</sup> [6].

As far as we know, many people have thought about using chlorine dioxide to control H<sub>2</sub>S in oil, gas, and water treatment. Also, mouthwash with ClO<sub>2</sub> has been shown to lower VSC mostly in clinical tests. However, our study is the first to confirm that H<sub>2</sub>S gas reacts with mouthwash with ClO<sub>2</sub> and CHX through gas absorption.

## V. CONCLUSION

Under the investigated conditions, H<sub>2</sub>S reacts well with ClO<sub>2</sub>. As the ClO<sub>2</sub> concentration increases, the amount of responding H<sub>2</sub>S also increases. The CHX + ClO<sub>2</sub> mixture did not affect the capacity of H<sub>2</sub>S neutralization.

These findings enhance our understanding of H<sub>2</sub>S reactivity and open new possibilities for its application in halitosis treatment.

**CONFLICT OF INTEREST**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**ACKNOWLEDGMENT**

This study was supported by Hong Bang International University, Ho Chi Minh City under grant number: GVTC18.11.

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