Analyzing some main components of MDF-500 disinfectant produced in the United States

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ABSTRACT

The demand for disinfectants has increased exponentially globally following the COVID-19 pandemic to prevent and control the risk of exposure to harmful microorganisms in humans, the environment, and infrastructure. Contaminated surfaces are places where microorganisms can survive for many days. MDF-500 disinfectant has been recommended by the Environmental Protection Agency (EPA) of the USA. MDF-500 disinfectant has a broad spectrum of antibacterial activity against chemical and biological agents. Although widely used, information on the composition of MDF-500 is minimal. Therefore, it is necessary to conduct analytical research to determine the main components of MDF-500 using qualitative and quantitative techniques, as a basis for improving and upgrading technical features. The main components identified include hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl), benzalkonium chloride, cetyltrimethylammonium bromide, and cetylpyridinium chloride. The analysis showed that MDF-500 disinfectant consisted of 1.25% H_2O_2, 9.47% NaOCl, and pH = 10.74.

Keywords: MDF-500; Disinfectant; Hydrogen peroxide.

1. INTRODUCTION

Disinfectants (liquid, gel, foam) are one of the best methods used to inactivate or kill pathogens (such as viruses, bacteria, fungi, and spores), thereby reducing the spread of infectious diseases [1, 2]. For example, SARS-CoV-2 is a virus that is more susceptible to inactivation by disinfectants and cleaning methods [3]. Currently, due to increased demand, improper and indiscriminate purchase, hoarding, storage, and use of disinfectants can lead to adverse effects on humans, animals, and the environment and ecosystems [2, 3]. Therefore, a series of guidelines on the use of disinfectants in health care and non-healthcare facilities issued by many agencies must be followed [2]. Specifically, in 2017, the United States banned the use of triclosan and triclocarban in soap, hand sanitizer, and topical antiseptic products because they hurt the aquatic environment [2]. When disinfectants are not properly washed off, they will leave residue on the surface, affecting the quality of materials [2, 3].

Among the various disinfectant formulations developed globally, such as Supertropical bleach, decontamination solution number 2 (DS2), M258 skin decontamination kit, M291 kit, are highly toxic, highly corrosive, and highly effective decontamination only against one group of biological agents or chemical agents, but not both [4]. MDF-500, produced in the United States, has attracted great attention because the product complies with the regulations on disinfectants in European Union countries, is properly evaluated before being put on the market, and is recommended for use by the EPA [2]. However, it is an increasingly popular disinfectant due to the chemical composition of MDF-500 published in US patent 6,566,574 B1 and US 7,271,137 B2 [5, 6]. However, MDF-500 is an imported product from the United States, not available in Vietnam, with complicated purchasing procedures and long shipping times. Meanwhile, disinfectants need to be used in large quantities and urgently when an epidemic outbreak occurs or in an emergency [4]. Therefore, identifying the main component of MDF-500 disinfectant is very important to elucidate its disinfection mechanism [5, 6]. Furthermore, the component of this disinfectant also shows the content of active ingredients, thereby understanding the disinfection efficiency, dilution instructions and use for each type of material surface to ensure safety and health for the person spraying the disinfectant [1]. However, identification and quantification of components in MDF-500 disinfectant can be complicated by the influence of fatty alcohols and water-soluble polymers [5, 6].

This study aimed to analyze and identify the main components, determine physical properties (such as pH, color, boiling point, and density), and evaluate the role of the components in MDF-500. By applying standard analytical techniques and based on the data published in the MDF-500 patent, this study identified 5 main components and 4 technical parameters of MDF-500.

2. EXPERIMENTAL AND RESEARCH METHODS

2.1. Chemicals

pH buffer [pH = 4, pH = 7, pH = 10], chloroform, diethyl ether, acetic acid, and sulfuric acid (H_2SO_4, trace metal grade): Fisher chemicals. Potassium permanganate ($KMnO_4$, Jiangtian, Tianjin, China), sodium thiosulfate ($Na_2S_2O_3.5H_2O$, Xilong, China), potassium iodide (KI, Thermo scientific chemicals), starch soluble (Xilong, China), bromophenol blue (Budapest, Hungary), methyl orange (Shanghai, China), sodium lauryl sulfate (Bio Basic, Markham, ON, Canada), Eosin Yellow dye (Merck-Germany), once-distilled water (Vietnam).

2.2. Equipment

Analytical balance (Shimadzu AUW220D, Shimadzu Corporation, Kyoto, Japan, precision, ± 0.01 mg), Stabitherm Prolabo water bath (Paris, France), UV-Vis spectrophotometry (Shimadzu-1900i, Kyoto, Japan with wavelength accuracy of ± 0.3 nm for the entire range from 190 to 1100 nm, photometric repeatability accuracy is 0.0002 Abs max), pH meter (Mettler Toledo Seven Compact S220, Switzerland). Inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900, USA) with accuracies of 1-3% for most elements. Other necessary glassware is available in the laboratory of Institute of New Technology.

2.3. Methods

Figure 1 shows a schematic of the experimental procedure of this study. MDF-500 consists of part A, part B, and a mixture of part A and part B in a 1:1 volume ratio (figure 2 ab).

- Determination of dry matter content by gravimetric analysis for determination of surface residue after disinfection: Evaporate 20 mL of sample on a water bath at 60 °C, evaporate to dryness, and dry the residue at 105 °C for 1 hour [7].

- Determination of pH to evaluate disinfection efficiency: Calibrate the pH meter at 3 points (pH 4, 7, 10). Put the sample to be measured into a 15 mL falcon tube and measure the pH of the sample [7].

- Determine the boiling temperature to determine the volatility of the disinfectant: Set up the experiment as shown in figure 2c, heat the flask until boiling, and read the temperature when the first drop of liquid is collected [8].

- Determine the specific gravity to determine the type of disinfectant packaging: Take each sample from 1 mL to 5 mL in turn and put it into a 15 mL falcon tube. Soak the falcon tube in a thermostatic bath at 20 °C for 30 minutes [9].

The specific gravity of the sample is determined by the formula = $\frac{m_1 + A}{m_2 + A}$.

Where m_1 is the mass of the falcon tube containing the sample at 20 ºC, g; m_2 is the mass of the falcon tube containing water at 20 ºC, g; A is the buoyancy correction = $\rho_a \times m_2$, where ρ_a is the density of air ≈ 0.0012 g/mL.

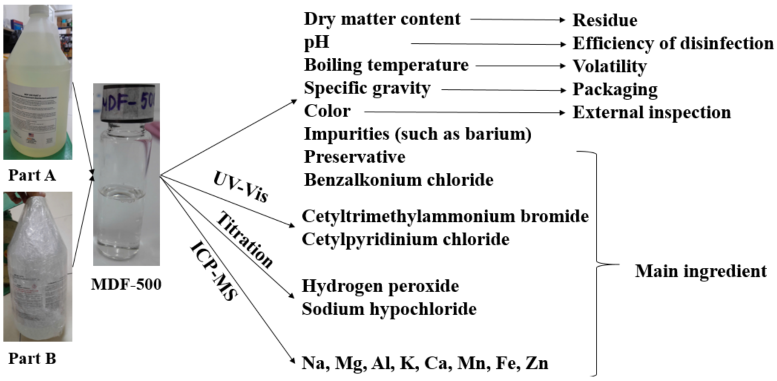


Figure 1. *Schematic of the experimental process.*

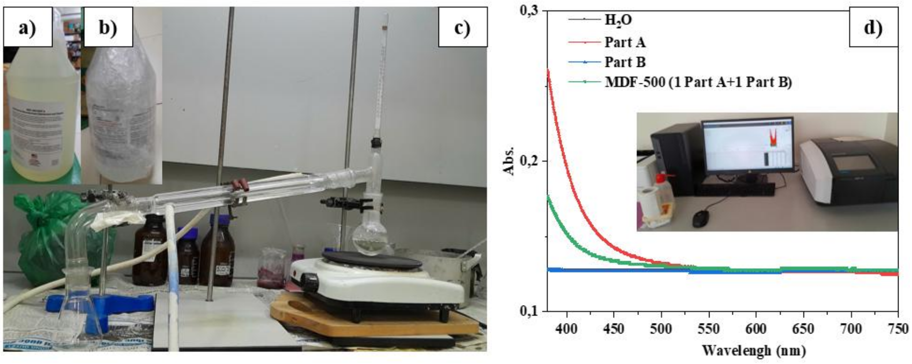


Figure 2. *Commercial MDF-500 product images: Part A (a), part B (b), and experimental design for determining boiling point (c); Color measurement results of MDF-500 (d).*

- Determination of color: The sample was placed in a glass jar, tightly closed (to avoid contact with air, which may cause a reduction reaction that changes the color). Measure the UV-Vis of the samples (figure 2d) [10].

- Determination of preservatives: 100 mL of the sample was extracted with a three-fold dilution solution, respectively 50, 25, and 25 mL. The dilution solution was a mixture of chloroform and diethyl ether in a volume ratio of 3:2. The components were evaporated to dryness at room temperature in a weighed glass dish, and then dried over silica gel for 2 hours [7].

- Determination of hydrogen peroxide (H_2O_2): Dissolve 2 mL of the sample in a conical flask containing 20 mL of distilled water. Add 20 mL of 2 N H_2SO_4, shake well, and titrate with 0.1 N $KMnO_4$. Each mL of 0.1 N $KMnO_4$ is equivalent to 1.701 mg of H_2O_2 [7].

- Check for impurities (such as barium) in the sample: Add two drops of 2 N sulfuric acid to 10 mL of the sample, and observe the color change of the solution after 10 minutes [7].

- Determination of sodium hypochlorite (NaOCl): Add 0.5 g of KI and 10 mL of 6 N acetic acid to 2 mL of the sample in a conical flask with a ground-glass stopper. Titrate the liberated iodine with 0.1 N $Na_2S_2O_3$ solution, adding 2 mL of 1% starch near the endpoint. Each mL of 0.1 N $Na_2S_2O_3$ solution is equivalent to 3.722 mg of NaOCl [7].

- Identification of benzalkonium chloride (BAC): Dissolve 1 mL of the sample in 16.9 mg of a mixture of bromophenol blue, methyl orange, and sodium lauryl sulfate (SLS) in a mass ratio of 3:1:13. If the solution is blue, the sample contains BAC [11].

- Identification of cetyltrimethylammonium bromide (CTAB): Measure the UV-Vis of a mixture of 10 mL of bromophenol blue 8.10^{-6} M and 1 mL of the sample. If the peak appears at about 410 to 600 nm, the sample contains CTAB [12].

- Identification of cetylpyridinium chloride (CPC): UV-Vis measurement of the following solutions: Solution I consists of 10 mL of eosin yellow 0.01 mM and 10 mL of the sample. Solution II consists of 10 mL of Solution I and 10 mL of SLS 8 mM solution. If the peak appears at 517 nm, the sample contains CPC [13].

- Metal cations in MDF-500 disinfectant were analyzed by ICP-MS according to US EPA Method 200.8 (dilute sample 20 times with double-distilled water).

3. RESULT AND DISCUSSION

3.1. Compounds contained in part A, part B and MDF-500 disinfectant

3.1.1. Hydrogen peroxide (H_2O_2)

The analysis results showed that part A did not contain H_2O_2. Part B and MDF-500 contained H_2O_2 at concentrations of 3.63% and 1.25%, respectively (table 1). H_2O_2 is known to reduce residual permanganate (purple) to soluble, colorless $MnSO_4$ [14]. The reaction between H_2O_2 and permanganate is as follows [14]:

$$2KMnO_4 + 5H_2O_2 + 3H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 8H_2O + 5O_2$$

Table 1. *Identification and quantification of compounds in MDF-500 disinfectant.*

Compound	Part A, %	Part B, %	MDF-500, %	Range of concentration MDF-500, % (in patent US 6,566,574 B1)
Hydrogen peroxide	-	3.63	1.25	0-4
Sodium hypochloride	9.98	-	9.47	0.1-10
Benzalkonium chloride	+	-	+	0.1-10
Cetyltrimethylammonium bromide	+	-	+	
Cetylpyridinium chloride	+	-	+	

Note: The symbol "-" means absent, "+" means present compound.

H_2O_2 is commonly used as a strong oxidant in bleaches, detergents, disinfectants, sterilants, and antiseptics [3, 14, 15]. The mechanism involved in the antibacterial effect of H_2O_2 is the formation of hydroxyl radicals that can be redox-activated with ≈ 2.8 eV. These radicals are strong oxidizing agents that can react rapidly with bacterial biomolecules, such as the thiol groups of proteins, causing irreversible structural modifications and subsequent cell death [16].

MDF-500 does not contain barium compounds, because the product does not react to form a white precipitate with sulfuric acid according to the equation:

$$BaO_2 + 3H_2SO_4 \rightarrow BaSO_4 + H_2O_2$$
$$BaCl_2 + H_2SO_4 \rightarrow BaSO_4 + 2HCl$$

In industrial bleaching, disinfectants containing H_2O_2 are more effective when pH > 10 [15]. This result is consistent with the pH of part A, part B, and MDF-500. The pH of the MDF-500 disinfectant is 10.74, which is consistent with the dissociation of H_2O_2 to form perhydroxyl anion (HO_2^-) [15]. Perhydroxyl anion plays an important role in removing stains [15]. The preservative, which may be a quaternary ammonium compound such as BAC, is present in part A, part A and MDF-500 at 0.15; 0; 0.02 g/mL, respectively [17]. This result is consistent with the study when determining the presence of BAC in MDF-500.

3.1.2. Sodium hypochloride (NaOCl)

The analysis results showed that part B did not contain NaOCl. Part A and MDF-500 contained NaOCl at a concentration of about 10%. Furthermore, the disinfectant with a pH of about 11, would cause corrosion and determine the shelf life [16]. The reaction mechanism occurs as follows:

$$NaOCl + CH_3COOH \rightarrow HOCl + CH_3COONa$$
$$HOCl + CH_3COOH + 3KI \rightarrow KI_3 + 2KCl + H_2O$$
$$KI_3 + \text{starch} \rightarrow [KI_3][\text{ starch}] \text{ (dark blue)}$$
$$[KI_3][\text{ starch}] \text{ (dark blue)} + 2\ Na_2S_2O_3 \rightarrow KI + 2NaI \text{ (colorless)} + Na_2S_4O_6 + \text{starch}$$

According to the laboratory biosafety manual recommended by the World Health Organization (WHO), it should be diluted to obtain a final concentration of 1 g/L (suitable for environmental sanitation) and 5 g/L (when dealing with high-risk situations) [16]. When NaOCl is dissolved in water, it produces a weak base and hypochlorite acid (HOCl). Since HOCl has no charge, it penetrates better into the microbial cell wall or any protective layer and kills them by oxidizing the side chains of protein amino acids [16]. The reaction mechanism occurs as follows:

$$OCl^- + H_2O \leftrightharpoons HOCl + OH^-$$
$$HOCl + H^+ + Cl^- \leftrightharpoons Cl_2 + H_2O$$
$$2HOCl + OCl^- \rightarrow ClO_3^- + 2Cl^- + H^+$$

H_2O_2 has a low solubility in all organic components, but in the presence of NaOCl solution, even at lower concentrations, all these components are dissolved [15]. Interestingly, NaOCl, also known as “bleach”, is much more effective at disinfecting hard surfaces than equivalent concentrations of hydrogen peroxide solution and exhibits a much broader spectrum of antibacterial activity at different temperatures [2, 3].

3.1.3. Benzalkonium chloride (BAC)

The results showed that part A and MDF-500 had a dark blue color, indicating the presence of BAC (figure 3ab). The reaction mechanism is due to BAC reacts to neutralize with SLS [17]. When BAC is dissolved with an SLS solution, this solution is in the form of independent molecules (called monomers) on the surface, reducing the surface tension [17]. At a certain concentration, BAC is no longer able to concentrate on the surface and is in the form of aggregated particles (micelles) [17]. These micelles form complexes with bromophenol blue to form a blue solution. This result is consistent with the study of Burel et al., when studying the presence of BAC [18]. BAC concentrations typically range from 0.01% to 15% [16]. BAC (figure 3e) is recommended by the FDA for use in soaps, hospital cleaning kits, and sanitary wipes because of its role in inhibiting bacterial growth, spore germination, and sporicidal activity [2, 3].

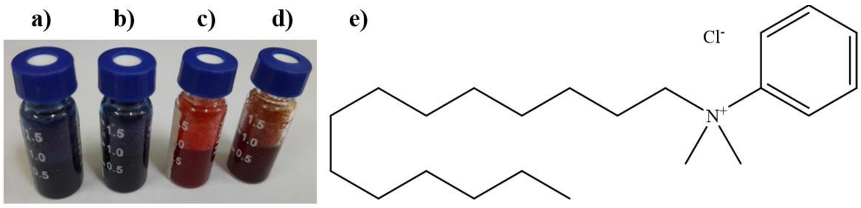


Figure 3. *Solution containing MDF-500 (a); Part A (b); part B (c); Water (d); Structure of BAC (e).*

3.1.4. Cetyltrimethylammonium bromide (CTAB)

The analytical results showed that part A and MDF-500 had CTAB due to the appearance of peaks in the range of 590-605 nm (figure 5) [19, 20]. The peak shift was attributed to the increase in CTAB concentration [19]. The color of the samples before UV-Vis measurement was consistent with the pH measurement results, indicating that part B had a yellow color in an acidic environment, while part A and MDF-500 had a blue color in an alkaline environment (figure 7). This result was explained by the fact that CTAB has a positively charged head group. Above the critical micelle concentration, CTAB forms micelles. Bromophenol blue (BPB) can be inserted into micelles, especially into the palisade layer (charged surface) (figure 5). The electrostatic attraction between $CTAB^{+}$ and BPB^{-} leads to ion pairing [19, 20]. CTAB (also known as cetrimonium bromide) contained in MDF-500 disinfectant plays an important role in maintaining surface activity and detergency properties (Figure 4). The reaction mechanism between CTAB and BPB is as follows, in which R+ is the ion of CTAB; HD^{-}, D^{2-} are the ions of BPB:

$$\left.\begin{array}{l} R^{+} + HD^{-} \rightarrow [R^{+}HD^{-}] \\ 2R^{+} + D^{2-} \rightarrow [R_2^{+}D^{-}] \end{array}\right\} \text{Mixed micelles}$$

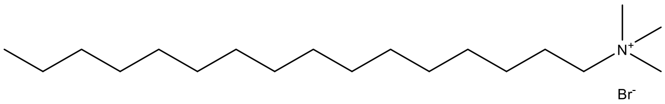
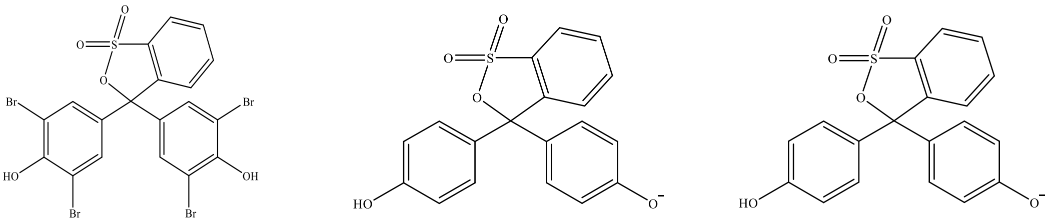


Figure 4. *Structure of CTAB.*



BPB (purple). *HD^{-} (yellow).* *D^{2-} (blue).*

Figure 5. *Structure of bromophenol blue.*

3.1.5. Cetylpyridinium chloride (CPC)

The results showed that part A and MDF-500 contained CPC due to the appearance of peaks in the range of 517-531 nm, consistent with Erdinc's publication [21]. CPC (cationic surfactant) (figure 6) reacts with eosin yellow (oppositely charged anion), through electrostatic interactions and hydrophobic interactions [21]. Figure 8 shows the maximum absorption spectrum of eosin yellow at 517 nm, while that of other samples shifts to higher wavelengths [21]. The advantages of CPC are its broad-spectrum antibacterial effect, safety, and infection prevention [22]. CPC and SLS surfactants have lower peak intensities than when SLS solution is not added, which is believed to be CPC and SLS inhibitors. These inhibitors form a protective film, reducing the corrosion potential of materials, especially on metal surfaces [23].

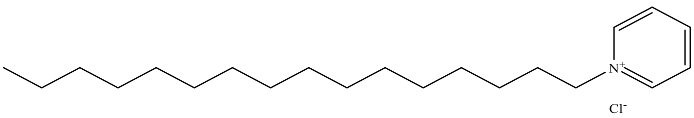


Figure 6. *Structure of CPC.*

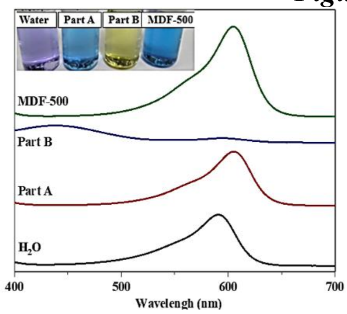


Figure 7. *UV-Vis measurement results to determine CTAB.*

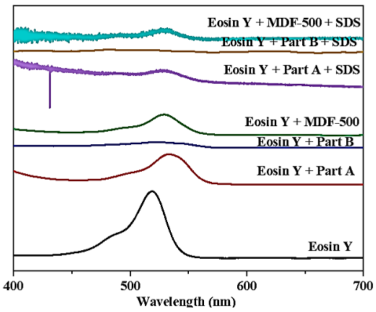


Figure 8. *UV-Vis measurement results to determine CPC.*

3.2. Dry matter, color, pH, boiling point, specific gravity of part A, part B and disinfectant MDF-500

The results of dry matter determination showed that part B, containing H_2O_2 left no residue, while part A, containing quaternary ammonium compounds, left more (table 2). H_2O_2 does not leave a residue because it decomposes into water and oxygen on the surface. The reaction mechanism occurs as follows:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Table 2. *Dry matter, pH, boiling point, density of part A, part B, and disinfectant MDF-500.*

Characteristic	Research results			Commercial product results announced		
	Part A	Part B	MDF-500	Part A	Part B	MDF-500
Dry matter, %	17.82	0.23	9.20	-	-	-
pH	11.10	2.07	10.74	10-12	2-3	8.45-9.5
Boiling point, ºC	98 - 100			101	101	< 101
Specific gravity	1.10	1.00	1.05	1.11	1.01	1.01

The MDF-500 disinfectant leaves negligible residue on the surface, thus, it is safe to use and complies with ASTM E3263-22 standard for the evaluation of equipment quality for visual detection of residue. Part A and MDF-500 are alkaline solutions, while part B is an acidic solution (table 2) [24]. The results showed that the disinfection efficiency of MDF-500 is increased due to the presence of quaternary ammonium compounds, which increase the degree of ionization of the bacterial surface functional groups, leading to increased binding [16]. Furthermore, the pH of the solution plays an important role in determining the shelf life, which is specifically stated when determining the compounds present in the solution [16]. The boiling point shows that the MDF-500 solution can evaporate quickly and leave a residue (table 2). This result is consistent with the study on dry matter. In addition, the specific gravity of the disinfectant also suggests that the recommended packaging is a transparent, hard plastic box with a tight lid made of HDPE or PET for safe storage and transportation [25]. Furthermore, the results of the determination of specific gravity also show that MDF-500 disinfectant should be used in the form of a mist spray, instead of immersion, for large-scale surface disinfection [1]. This result is consistent with the manufacturer's recommendation [24].

The color determination results showed that part A and MDF-500 were colorless to pale yellow, while part B was colorless (figure 2d). Similar to the study by Karsten et al., when studying colored and colorless disinfectants on human skin before surgery, it was shown that colored disinfectants were more effective than colorless ones in disinfection [4].

3.3. Content of elements in part A, part B, and disinfectant MDF-500

The analytical results showed that the MDF-500 disinfectant mainly contained Na^+ ions, followed by Mg^{2+}, K^+, and Ca^{2+} ions. This result was similar to the elemental composition of commercial disinfectants [2]. The MDF-500 disinfectant met the safety standards for trace metal content in chemicals used on surfaces in the United States Pharmacopoeia USP 39. In addition, table 3 shows that no heavy metals were detected in the disinfectant, demonstrating that this product is safe for humans and the environment.

Table 3. *Concentrations of elements in MDF-500 disinfectant.*

Sample	Element (mg/kg)							
	Na	Mg	K	Ca	Fe	Zn	Al	Mn
Part A	75.981	42.917	16.062	15.262	0.2967	0.2813	0.0705	0.0153
Part B	15.340	24.166	20.243	24.403	0.5056	0.3872	0.2795	0.0047
MDF-500	53.594	0.8641	15.223	15.223	0.3331	0.2642	0.0504	0.0074

Therefore, the findings of the study may lead to the improvement of disinfectant formulations from peroxide, hypochlorite, and quaternary ammonium compounds at different concentrations of the ingredients, using the concentration in MDF-500 as a reference point for future formulation optimization in terms of safety, efficacy, environmental friendliness, and low cost.

4. CONCLUSIONS

MDF-500 disinfectant is commercially produced in the United States and is recommended for use by the Environmental Protection Agency (EPA). This study analyzed the major components of MDF-500, determining the presence of hydrogen peroxide, sodium hypochlorite, benzalkonium chloride, cetyltrimethylammonium bromide, and cetylpyridinium chloride. The analytical results showed that MDF-500 consists of 1.25% H_2O_2, 9.47% NaOCl, and has a pH = 10.74. In addition, ICP-MS results indicated that the product is safe and environmentally friendly. This study suggests that the formulation of the disinfectant solution should target a broad spectrum of microorganisms, including peroxide, hypochlorite, and quaternary ammonium compounds, at the reference concentration of MDF-500 to ensure safety and efficacy. These findings contribute to a better understanding of the formulation of MDF-500 and its application for future disinfectant formulation improvements and the development of more effective public health strategies.

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TÓM TẮT

Nghiên cứu phân tích một số thành phần chính của chất khử trùng MDF-500 do Hoa Kỳ sản xuất

Nhu cầu chất khử trùng đã tăng lên theo cấp số nhân trên toàn cầu sau đại dịch COVID-19 nhằm phòng ngừa, kiểm soát nguy cơ tiếp xúc với vi sinh vật độc hại cho người, môi trường và hạ tầng. Các bề mặt bị ô nhiễm là nơi vi sinh vật có thể tồn tại và sống sót trong nhiều ngày. Chất khử trùng MDF-500 đã được Cơ quan bảo vệ môi trường (EPA) khuyến cáo sử dụng. MDF-500 có phổ hoạt tính kháng khuẩn rộng đối với tác nhân hóa học và sinh học. Mặc dù được sử dụng rộng rãi nhưng thông tin về thành phần của MDF-500 còn rất hạn chế. Do đó, việc nghiên cứu phân tích xác định thành phần chính của MDF-500 bằng các kỹ thuật định tính và định lượng, làm cơ sở cải tiến, nâng cấp tính năng kỹ thuật là cần thiết. Các thành phần chính được xác định bao gồm hydrogen peroxide (H_2O_2), natri hypochloride (NaOCl), benzalkonium chloride, cetyltrimethylammonium bromide và cetylpyridinium chloride. Kết quả phân tích cho thấy MDF-500 gồm H_2O_2 1,25%, NaOCl 9,47% và có pH = 10,74.

Từ khóa: MDF-500; Khử trùng; Hydrogen peroxide.