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Review Article

Hypochlorous Acid - Analytical Methods and Antimicrobial Activity

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Abstract

Hypochlorous acid (HOCl) is produced by the human body's immune cells to fight infections. It is effective against a broad range of microorganisms. It is non-toxic, non-irritant and non-corrosive at proper usage concentrations. There are some available commercial products that contain HOCl. However, its low storage stability constitutes a major challenge. This review considers the antimicrobial activity of HOCl and its methods of analysis.

Keywords: *Antimicrobial activity, Hypochlorous acid, Analytical methods*

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INTRODUCTION

Hypoclorous acid (HOCl), a powerful oxidizer and deproteinizer produced by neutrophils, has a good microbicidal activity within these cells. It reacts with many biological molecules, especially thiol, thiolether, heme proteins, amino groups and carbonhydrates, as well as overcomes pathogens and fights infection [1-3]. HOCl has advantages over sodium hypochlorite (NaOCl) and hydrogen peroxide (H_2O_2) in that within its effective antimicrobial concentration range, it is non-irritating, non-sensitizing and cytotoxicity to mammalian cells is lower [1].

It can be synthesized by one of the three methods [3]:

(a) Hydrolysis of chlorine gas
$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$

(b) Electrolysis of salt solution
$2Cl^- + 2e^- \rightarrow Cl_2$ (I)

$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$ (II)

(c) Acidification of hypochlorite
$OCl^- + H^+ \rightarrow HOCl$

The proportion of HOCl and hypochlorite ion (OCl^-) in a solution depends on its pH. The predominant species is HOCl between pH 3 and 6. Within this pH range, the concentration of HOCl is optimal and its dissociation is minimal. At higher pH, OCl^- is formed, whereas at lower pH, the solution exists as a mixture of chlorine (Cl_2) and HOCl in solution [4]. Due to the challenge of maintaining storage stability, a commercial pharmaceutical formulation containing pure HOCl has not been developed [1]. Studies have shown that stabilized HOCl displays rapid and concentration-dependent activity against clinically relevant microorganisms, as long as the effective pH range is maintained [1, 3, 5-7].

COMMERCIAL PRODUCTS THAT CONTAIN HOCl

Mild acidic HOCl solutions, developed by acidifying NaOCl with HCl or electrolyzing NaOCl solutions, have been widely used as disinfectants [1]. NVC-101 is one of the commercially available product containing acidified and unbuffered solution of HOCl in saline and its concentration is low. The active ingredient of this product is primarily HOCl in equilibrium with a small amount of dissolved Cl_2. NVC-101 containing 0.01 % HOCl with a pH of 3.5 to 4.0 was demonstrated to be an effective topical antimicrobial agent when used for a brief period (15-30 min) and followed with another application. This was because of its rapid neutralization in the wound bed environment. If its effective pH range can be maintained in the clinical situation, this stabilized form of HOCl (NVC-101) could have potential application as an antimicrobial wound irrigation and treatment solution [8]. Wang *et al* [3] indicated that NVC-101 had rapid and broad spectrum antimicrobial activity against clinically relevant microorganisms *in vitro* and *in vivo*. In another study, Robson *et al* [8] reported that as opposed to other antimicrobials investigated in their study, NVC-101 controlled the tissue bacterial bioburden without inhibiting the wound healing process.

One of the commercial disinfectants containing HOCl is Medilox®, and is prepared by electrolysis of sodium chloride solution. Electrolysis yields super-oxidized water with pH of 5.0 - 6.5 and an oxidation-reduction potential of > 950 mV and containing about 30 - 50 ppm of HOCl [5] . Choi and Kim [5] evaluated the antimicrobial activity of Medilox against several clinical isolates of bacteria (methicillin-susceptible *Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus* (MRSA)*, Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus pneumoniae, Salmonella typhi, Salmonella paratyphi A, Salmonella enteritidis, Shigella flexneri, Pseudomonas aeruginosa, Acinetobacter baumanii, Escherichia coli, Serratia marcescens, Klebsiella pneumoniae, Enterobacter cloacae, Citrobacter freundii, Stenotrophomonas maltophilia, Proteus mirabilis, Citrobacter freundii, Stenotrophomonas maltophilia, Proteus mirabilis, Chryseobacterium meningosepticum)* and yeast *(Candida albicans)*. They observed that all strains of bacteria and yeast were killed within 30 seconds after exposure to 30 ppm of Medilox. Moreover, *Bacillus subtilis* was killed within 4 min after exposure to 30 ppm of Medilox, but killed within 30 s in 50 ppm of Medilox. This study showed that Medilox is effective against commonly isolated bacteria and yeast from hospital but was less effective against spore-forming bacteria.

Huang *et al* [9] evaluated the stability of Medilox and its disinfection effect on hands and on article surfaces. They stored Medilox for 90 days under room temperature and at the end of the period they determined its effective chlorine content, pH value and disinfection effect on hands and article surface. As a result, they found that its chlorine content decreased by 36 %, pH value by 17 % and the average disinfection effect of the Medilox on hands and on article surface was over 90 %. Shi *et al* [4] evaluated the bactericidal effects of Medilox at neutral pH. They found that MRSA, *Acinetobacter baumannii* and *Streptococcus pneumoniae* strains were killed within 1 min by Medilox. They indicated that Medilox has a quick and highly effective bactericidal action and it can be used for the effective disinfection of skin, instruments and surfaces.

Another HOCl containing disinfectant is Sterilox. It contains HOCl at a concentration of approximately 144 mg/L and free chlorine radicals. Its pH is 5.0-6.5 and has an oxidation-reduction potential of > 950 mV [10]. It has been shown to be non-toxic to biological tissues and is claimed to be non-corrosive and non-damaging to endoscopes [11]. The antimicrobial activity of Sterilox has been tested against *Clostridium difficile* spores*, Helicobacter pylori,* vancomycin resistant *Enterococcus species, Candida albicans* and several *Mycobacterium spp.* According to the results, it was effective (< 2 min) in achieving a 5-log 10 reduction of pathogenic microorganisms (*H. pylori*, vancomycin resistant *Enterococcus spp*, *C. albicans*, *M. avium, M. chelonei, M. xenopi* and *M. smegmatis*) in the absence of organic loading. However, its biocidal activity was reduced in the presence of organic material (5 % horse serum) [12].

ANALYTICAL METHODS FOR DETERMINATION OF HOCl

Due to its low storage stability, determination of HOCl by different analytical methods has has assumed greater importance. They include the following.

Spectroscopy

HOCl and (OCl^-) were determined by addition of bromine and fluorescein to natural (rea and fresh) waters. The resulting pink color was measured spectrophotometrically. Beside this, decrease in fluorescence intensity was evaluated in this study [13]. Isotopically, HOCl was

determined by using the microwave spectrum for the determination of its molecular structure [14]. HOCl was also determined using spectrophotometry [15]. First, the samples were treated with tris(2-carboxyethyl)phosphine (TCEP), and then the, residual amount of TCEP was measured after reaction with 5,5´-dithiobis(2-nitrobenzoic acid) via the final product, 2-nitro-5-thiobenzoate. The concentration of HOCl was calculated based on the oxidation of TCEP by HOCl in a 1:1 ratio.

In another technique, a specific ferrocene-based florescent probe was developed for HOCl [16]. This is based on the formation of a double bond between HOCl and ferrocene selectively in pH 7.4, a condition that was achieved by a 100–fold fluorescence enhancement. The developed probe was applied to HeLa cells for fluorimetric imaging of HOCl. Simultaneous determination of chlorine dioxide and HOCl in the bleaching process has also been achieved by a spectroscopic method [17]. Spectrophotometric measurement of HOCl and chlorine dioxide were carried out at 295 nm but the method was not successful for the determination of low levels of HOCl. A specific and sensitive fluorescence method was developed for imaging of HOCl produced by a microbe [18]. Beside this, a method has also been developed using three water-soluble dihydrofluorescein-ether probes for the detection of HOCl via oxidation; these probes were applied to determine accumulated hypochlorous acid in organelles in a zebra fish model [19]. HOCl has also been detected by a HOCl-promoted cyclization reaction based on fluorescence resonance energy transfer (FRET) signaling mechanism; the authors claimed that their study shed light on the development of new fluorescent HOCl probes [20]. Kim *et al* [21] developed a boron-dipyrromethene (BODIPY)-based probe for the selective detection of HOCl in living cells.

Electrochemistry

HOCl concentration has been evaluated by deposition of copper on a gold-film electrode using potentiometric stripping analysis prior to being chemically oxidized by chlorine species [22]. In another study, HOCl was determined by electrochemically establishing an anode via coating a ferrite film on a substrate [23]. Limit of detection was 0.005 mg of Cl/L for HOCl. Sournia-Saquet *et al* [24] developed an amperometric method for the determination of HOCl from drinking water and swimming pools. The reaction was evaluated via a reduction path using cylic voltammetry. A suitable potential, i.e., 400 mV, was determined by chronoamperometry and the linear response range was determined as 1 - 50 ppm. This method was compared with an iodometric method. In a similar study, HOCl was determined using cylic voltammetry for measurement of residual levels in tap water; the reaction was monitored via a reduction path at + 0.3 V versus SCE [25]. In this study, gold electrode was applied to the flow injection analysis device and the linear calibration concentration range was found to be 0.05 – 2.5 mg L^{-1} while the relative standard deviation was 2.1 %. These results were compared with a photometric method using o-toluidine. In another work, Takeshi and Yaegashi [26] developed an electrochemical sensor for the determination of HOCl in electrolyzed water. The working electrode was B-doped diamond electrode and the reference electrode an SCE. Gobet and Rychen [27] took a patent for an electrochemical sensor for the determination of HOCl in water.

Titrimetric and Thermochemical Methods

Klimenko [28] and Salzer [29] developed a titrimetric method based on the reaction with potassium iodide. HOCl has also been determined by titration with aqueous methyl orange of a minimum of 0.5 mg Cl/l) [30] while Stojkovic *et al* [31] developed a method for the determination of dissociation constant based on the measurement of pH in 5M NaCl. The values were measured from the intercept and slope of a straight line and from thermodynamic measurement. Denis [32] carried out thermochemical studies on hypobromous and HOCl in which Delta H degrees were determined.

CONCLUSION

Although HOCl is a potent antimicrobial agent and has advantages such as non-toxicity in biological tissues and environmentally friendly, it has limited applications due to its decreasing antimicrobial efficacy in the presence of organic matter and low storage stability. HOCl is present in multiply used containers as commercial products and following to each using, amount of HOCl is decreased. Hence, the determination of HOCl with various analytical methods are necessary.

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