Bactericidal efficacy of electrochemically activated solutions and of commercially available hypochlorite

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ABSTRACT

Electrochemical activation (ECA) has been developed as a quick and efficient method of hypochlorite production, and many claim increased efficacy when compared to conventional disinfectant solutions. Numerous potential applications, including hospital disinfection, waste-water treatment, routine drinking water disinfection and biological decontamination have been suggested. In this study, three solutions were produced by electrochemical activation of 0.5% NaCl and compared to commercially available NaOCl. The NaOCl concentration and pH of each solution was measured, and the minimum bactericidal concentration of each was determined using seven common microbial pathogens. All solutions were effective, the most significant of which was the ECA anolyte solution. This is notable due to its neutral pH and antimicrobial efficacy that is four times that of commercially available NaOCl. This process may lead to production of a highly effective yet non-caustic disinfectant that would have countless scientific, medical, military and public health applications.

KEY WORDS: Anti-bacterial agents.
Disinfection.
Electrochemistry.
Sodium hypochlorite.

Introduction

The efficient production of effective disinfectant solutions is critical in efforts to minimise the occurrence of water-borne, food-borne, bioterrorism-related and nosocomial infections.[1] Sodium hypochlorite solution (bleach) is the most commonly used method for disinfection,[1] and its use has led to a significant reduction in worldwide mortality rates.[2] Owing to the often toxic nature of hypochlorite, the production, transport, storage and disposal of these chemicals adds significant expense and environmental impact,[2] and they can be corrosive to steel and other surfaces at relatively low concentrations.[3]

Electrochemical activation (ECA) has been developed recently as a quick and efficient method of on-site hypochlorite production that limits costs, reduces or eliminates environmental impact, and produces safer and more desirable products.[4,5] Claims have been made of increased efficacy when compared to conventional disinfectant solutions,[6,7] and several flow-through devices have been developed that use ECA-produced hypochlorite for significant reduction in bacterial numbers.[4,8] These ECA systems have been shown to be inexpensive to operate, increasingly portable and highly effective against a wide variety of organisms.[4,9,10] Many potential applications, including hospital disinfection, waste-water treatment, industrial decontamination, routine drinking water disinfection and biological decontamination have been suggested for this technique.

Electrochemical activation of water involves the exposure of water and added salts to an electrical potential difference. When NaCl is the added salt, the primary product will be hypochlorite. The complex reactions that occur within the electrochemical reactor produce a meta-stable solution containing several reactive ions and free radicals, including ozone, hydrogen peroxide, chlorine, hypochlorite, hypochlorous acid, hydrochloric acid, and hydroxide ions.[4–6,9,10] The primary method of hypochlorite production is as follows:[11]

$$2\,Cl^- \leftrightarrow Cl_2 + 2e^-$$
$$Cl_2\,(aq) + H_2O \leftrightarrow HClO + Cl^- + H^+$$
$$HClO \leftrightarrow ClO^- + H^+$$

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There is little debate over the ability of these solutions to kill bacteria because the main product of ECA is hypochlorite, a well-known and widely studied disinfectant. Studies performed using ECA water have demonstrated increased efficacy,[6,7] which many attribute to the presence of reactive oxygen species such as hydroxyl radical (·OH), ozone and hydrogen peroxide.[4–6,9,10] As the presence of these compounds is transient and difficult to measure, their exact involvement in the disinfection process has been

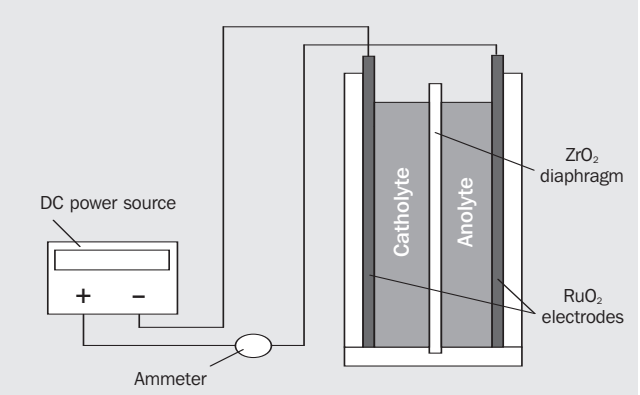

Fig. 1. Electrochemical reactor.

difficult to ascertain, especially in the presence of other active species.[5,7]

This study demonstrates the activity of these reactive oxygen species by comparing the efficacy of ECA solutions to that of commercial bleach with equivalent hypochlorite concentration. To ascertain the solution's potential usefulness as a medical- or laboratory-grade disinfectant, seven common microbial pathogens are used for this comparison. These were chosen to best represent the various morphological types and biochemical properties, such as oxidase and catalase production, that might affect the action of a given disinfectant.

Materials and methods

Electrochemical reactor

The reactor (Fig. 1) consists of an acrylic chamber containing two ruthenium oxide-coated titanium electrodes. A zirconium dioxide diaphragm (approximate pore size: 5–10 µm) separates the two electrodes. The diaphragm allows passage of ions but significantly impedes passage of the solution between the two chambers. Voltage is applied to the reactor using a DC power supply.

Hypochlorite concentration

Initial hypochlorite concentration of commercial bleach (Clorox household bleach) was determined by titration with sodium thiosulphate (Sigma-Aldrich) using an American Scientific Porta pH 4 to measure oxidation-reduction potential (Fig. 2). A wavelength scan was performed to determine the optimal wavelength for spectrophotometric measurement (Fig. 3). A series of standards was then made and measured at optimal wavelength using a Perkin-Elmer Lambda EZ201 spectrophotometer. Linear regression (SPSS) established the mathematical relationship between absorbance and concentration of hypochlorite (Beer-Lambert Law). This standard curve was then used for all subsequent hypochlorite determination.

Saline activation

The reactor was used to activate solutions at various concentrations, times and voltages to characterise the effects of electrochemical activation. Absorbance was measured using a Hach DR 4000 spectrophotometer, pH was measured using a Fisher Scientific Accumet Basic AB15, and current was measured with a TENMA 72-7720 voltmeter. For microbiological assay, 0.5% NaCl solution (5 g/L) was activated at 20 V for 30 min. The anolyte and catholyte solutions were analysed for hypochlorite concentration and pH. A second activation was performed using the same parameters without the zirconium oxide diaphragm in place. In this case, only one solution, referred to as ECA-ND solution, was produced.

Microorganisms

Seven different microorganisms, *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (ATCC 11778), *Candida albicans* (ATCC 10231), *Enterococcus faecalis* (ATCC 51299) and *Klebsiella oxytoca* (ATCC 49131) were used to test the various solutions produced. *Escherichia coli, P. aeruginosa* and *K. oxytoca* were incubated at 35°C for 24 h on MacConkey agar (MAC); *B. cereus, Enterococcus faecalis* and *S. aureus* were incubated at 35°C for 24 h on trypticase soy agar with 5% sheep blood; and *C. albicans* was incubated at 20°C for 48 h on trypticase soy agar with 5% sheep blood. Organism suspensions in sterile water were standardised to 1.0–1.5 x 10^8 organisms/mL using a Perkin-Elmer Lambda EZ201 spectrophotometer (0.09 absorbance at 625 nm equivalent to a 0.5 MacFarland standard).[12] All microbial growth media were purchased from Becton-Dickinson.

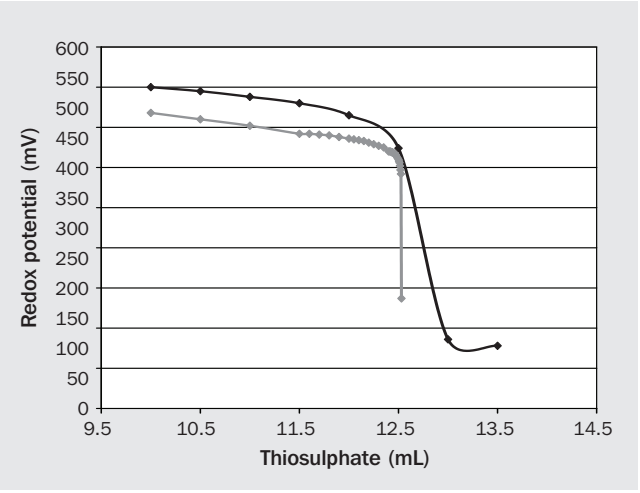

Fig. 2. Hypochlorite titration. Sodium thiosulphate ($Na_2S_2O_8$) is added until a significant drop in redox potential is observed (black curve). Repeated titration using smaller and more precise increments (grey curve) determines commercial hypochlorite concentration to be 2.33%, or approximately 40% of labelled concentration.

Minimum bactericidal concentration

Two-fold serial dilutions (1 in 2, 1 in 4, 1 in 8, 1 in 16 and 1 in 32) of each solution were prepared in trypticase soy broth using 0.5 mL of the solution to be tested. Diluted solutions

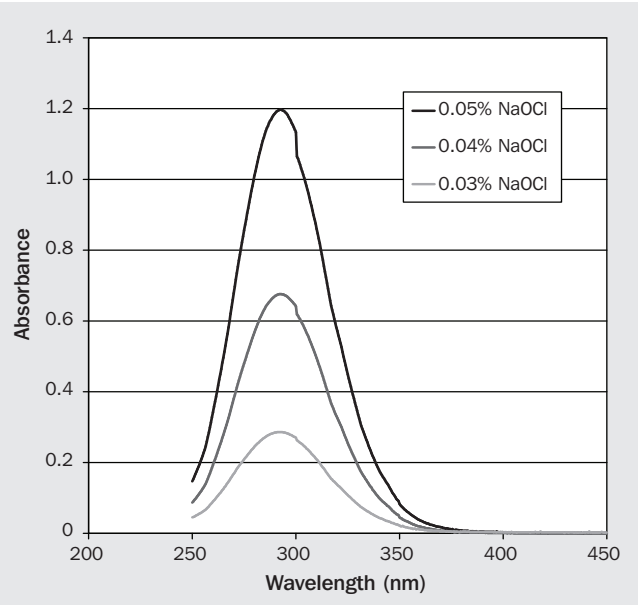

Fig. 3. Wavelength scan. Absorbance of commercially available hypochlorite is recorded every 0.5 nm from 250 nm to 400 nm to determine the optimal wavelength for quantitative measurement. 292.5 nm was used for all hypochlorite determinations in this study.

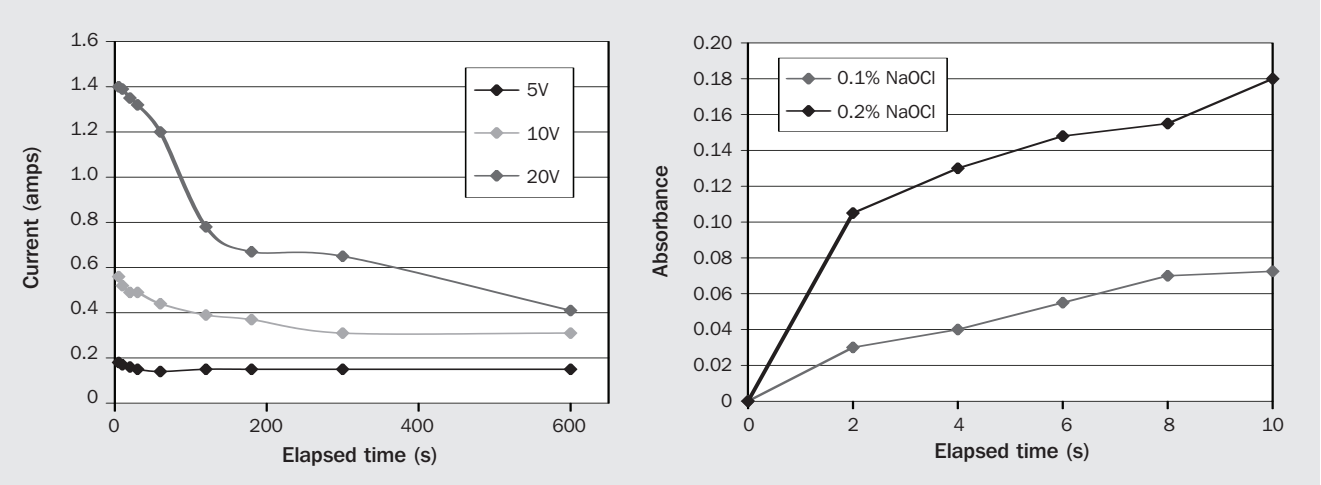

Fig. 4. Time *vs.* current and time *vs.* absorbance – ECA-ND (single) chamber, 292.5 nm.

were then inoculated with 100-µL standardised bacterial or yeast suspension. After 24-h incubation at 35°C, cloudiness in the medium indicated positive growth. Serially diluted unactivated saline served as a positive control. The absence of growth in uninoculated solutions (both pre- and post-activation) served as a sterility control. Clear tubes were plated on trypticase soy agar with 5% sheep blood to verify sterility. The lowest concentration of the solution that produced a sterile medium was determined to be the minimum bactericidal concentration (MBC).

Results

As voltage was applied to the electrochemical reactor, current decreased over time. Absorbance increased over time as hypochlorite and other constituents were produced (Fig. 4).

Repeated titration of commercial bleach confirmed that the hypochlorite concentration of commercial bleach is 2.33%, which is considerably less than the 6.15% indicated on the label (Fig. 2). Wavelength scans determined 292.5 nm to be the optimal wavelength for hypochlorite measurement (Fig. 3), and a series of dilutions was measured to produce a standard curve. Linear regression analysis provided the relationship: absorbance = –1.12 + 138.75 * concentration (R-square = 0.99).

Commercial bleach (10%; 0.233% NaOCl [pH 12.2]) was serially diluted through five tubes for MBC determination. After incubation and subculture of clear tubes, sterility was confirmed in tube 1 for *E. faecalis, P. aeruginosa* and *S. aureus*; tube 2 for *K. oxytoca* and *Escherichia coli*; and tube 3 for *B. cereus* and *C. albicans*.

Electrochemically activated saline, ECA-ND solution (0.077% NaOCl [pH 9.4]), was serially diluted through five tubes for MBC determination. After incubation and subculture of clear tubes, sterility was confirmed in tube 2 for *C. albicans* and in tube 1 for all other organisms.

The ECA anolyte solution (0.026% NaOCl [pH 6.8]) was serially diluted through five tubes for MBC determination. After incubation and subculture of clear tubes, sterility was confirmed in tube 2 for *B. cereus, Enterococcus faecalis, K. oxytoca, P. aeruginosa* and *S. aureus. C. albicans* and *Escherichia coli* showed sterility in tube 3.

The ECA catholyte solution (0.179% NaOCl [pH 12.9]) was serially diluted through five tubes for MBC determination. After incubation and subculture of clear tubes, all organisms showed sterility at tube 1.

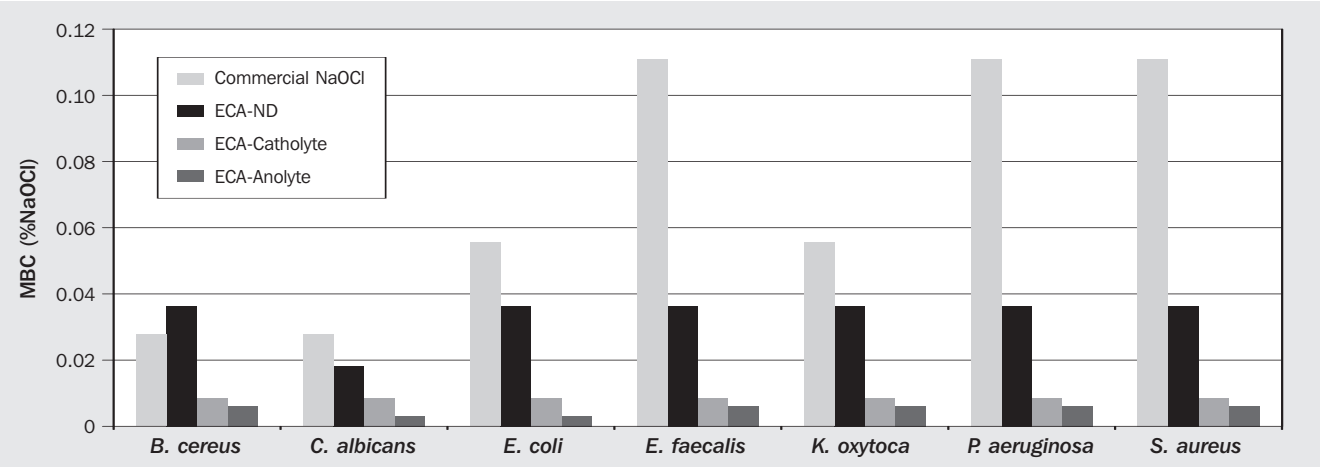

Fig. 5. Minimum bactericidal concentration. The MBCs determined by this study for the various solutions and organisms tested are compared. The lowest MBC being the most efficient, the anolyte and catholyte solutions are significantly more efficient disinfectants than the ECA-ND solution or the standard hypochlorite solution of equal concentration.

Discussion

The electrochemical reactor used in this study is able to produce several different solutions, all of which possess antimicrobial properties. All contain small quantities of hypochlorite, hydrochloric acid and hypochlorous acid.[7] The three ECA solutions produced have varying concentrations of these constituents, which accounts for their notable differences in pH.

In the caustic solutions with pH>7, ECA catholyte and ECA-ND, the predominant species produced is hypochlorite. With the membrane present, mixing of the two sides of the reactor is minimised to produce two distinct solutions. In the slightly acidic ECA anolyte solution, hypochlorous and hydrochloric acid are more prominent. Commercially available hypochlorite, which was 100% effective against all species at 0.111% NaOCl, proved to be the least effective of the solutions studied (Fig. 5).

Of particular interest is the anolyte solution, which was 100% effective against all species at only 0.006% NaOCl. This is remarkable bearing in mind its essentially neutral pH. The increased antimicrobial activity seen by the ECA solutions further demonstrates the presence of these reactive oxygen species and confirms the numerous claims of increased antimicrobial effectiveness.

The ability to produce disinfectants on-site and in low quantities emphasises the advantages of decreased shipping, packaging and storage costs, and the absence of chemical waste products suggests minimal environmental impact. The successful production of a neutral yet highly effective antimicrobial agent has numerous potential applications, including possible skin and mucous membrane disinfection. Areas of further study include antiviral, antituberculosis and antiparasitic studies, as well as composition, decomposition and biocompatibility analyses. □

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