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The mechanism of antimicrobial action of Electro-Chemically Activated (ECA) water and its healthcare applications

by

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"Whoever undertakes to set himself up as judge in the field of truth and knowledge is shipwrecked by the laughter of the Gods."
Albert Einstein, October 26, 1929, The Saturday Evening Post.

The supreme goal of all theory is to make the irreducible basic elements as simple and as few as possible without having to surrender the adequate representation of a single datum of experience
"Theories should be as simple as possible, but no simpler"
Albert Einstein, 1933

In every true searcher of Nature there is a kind of religious reverence, for he finds it impossible to imagine that he is the first to have thought out the exceedingly delicate threads that connect his perceptions.
Albert Einstein, 1920.

"Everyone who is seriously involved in the pursuit of science becomes convinced that a spirit is manifest in the laws of the Universe—a spirit vastly superior to that of man.... In this way the pursuit of science leads to a religious feeling of a special sort, which is indeed quite different from the religiosity of someone more naïve".
Albert Einstein , January 24, 1936; Einstein Archive 42-601

I don't try to imagine a God; it suffices to stand in awe of the structure of the world, insofar as it allows our inadequate senses to appreciate it.
Albert Einstein , April 16, 1954; Einstein Archive 30-1154

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DECLARATION

I, the undersigned, herewith declare, that this dissertation, which I hereby submit for the degree of Ph.D. (Microbiology) is my own original work and has not previously been submitted at this, or any other University.

Signed

This..... day of....., 2009



LIST OF ABBREVIATIONS

ACC: Available Chlorine Concentration
ADP: Adenosine Diphosphate
AEW: Acidic Electrolysed Water
AFM: Atomic Force Microscopy
AIR: Airborne Infection Research
AISI: American Iron and Steel Institute
AME: Aminoglycoside Modifying Enzyme
ATCC: American Type Culture Collection
ATP: Adenosine Triphosphate
CAT: Catalase
CDC: US Centres for Disease Control and Prevention
CFU: Colony Forming Unit
CSIR: Council for Scientific and Industrial Research
DNA: Deoxyribonucleic Acid
DBP: Disinfection Bi-Product
DPD: N,N-Diethyl-p-Phenylenediamine.
EAW: Electrochemically Activated Water
ECA: ElectroChemical Activation
EDTA: Ethylenediaminetetraacetic acid
EMF: Electromotive Force
EOW: Electrolysed Oxidising Water
EPA: Environmental Protection Agency
EPS: Extracellular Polymeric Substance
ESBL: Extended Spectrum β Lactamase
EW: Electrolysed Water
FAC: Free Available Chlorine
FAD: Flavin Adenine Dinucleotide
FAO: Free Available Oxidants
FDA: Food and Drug Administration
FEM: Flow Electrochemical Module
HAA: Haloacetic Acid
HAI: Hospital Acquired Infection
HELP: High Electric Field Pulses
LPS: Lipopolysaccharide
MDR: Multi Drug Resistant

MIC: Minimum Inhibitory Concentration
MMC: Minimum Microcidal Concentration
MRSA: Methicillin Resistant *Staphylococcus aureus*
MSSA: Methicillin Sensitive *Staphylococcus aureus*
MPO: Myeloperoxidase
NAD: Nicotinamide Adenine Dinucleotide
NADH: Nicotinamide Adenine Dinucleotide (Reduced)
NB: Nutrient Broth
NICU: Neonatal Intensive Care Unit
OADC: Oleic acid Albumin Dextrose Catalase
ORP: Oxidation Reduction Potential (=REDOX)
PBDW: Phosphate Buffered Diluent Water
PBS: Phosphate Buffered Saline
PEF: Pulsed Electrical Fields
PMF: Proton Motive Force
PPD: Protein Purified Derivate
RFE: Flow Electrochemical Reactor
RNA: Ribonucleic Acid
ROS: Reactive Oxygen Species
SAMRC: South African Medical Research Council
SEM: Scanning Electron Microscopy
SOD: Superoxide Dismutase
SOW: Super-Oxidized Water
SPM: Scanning Probe Microscopy
STP: Standard Temperature and Pressure
SRB: Sulphite Reducing Bacteria
THM: Trihalomethane
TOX: Total organic halogen
TSA: Tryptone Soy Agar
TSB: Tryptic Soy Broth
TST: Tuberculin Skin Testing
USDA-FSIS: United States Department of Agriculture – Food Safety Inspection Services
USPTO: United States Patent and Trade Mark Office
VRE: Vancomycin Resistant *Enterococcus*
WIPO: World International Patent Organisation



Glossary of ElectroChemically Activated Radical species

Anolyte solution

Cl_2 - Chlorine

Cl^- - Chloride ion

$\text{Cl}\cdot$ - Chlorine radical

OCl^- - Hypochlorite anion

$\text{ClO}\cdot$ - Hypochlorite radical

ClO – Chlorine Oxide

Cl_2O – di-Chlorine oxide

ClO_2 – Chlorine Dioxide

ClO_2^- - Chlorite anion

ClO_3^- - Chlorate anion

ClO_4^- - Perchlorate anion

HClO , HOCl , ClOH – Hypochlorous acid

HOClO – Chlorous acid

HOClO_2 – Chloric acid

HOClO_3 – Perchloric acid

HCl – Hypochloric acid/hydrochloric acid

NaClO_2 – Sodium chlorite

O_2 – Oxygen

O_3 – Ozone

$\text{O}\cdot$ - Oxygen radical

$^1\text{O}_2$ – Singlet oxygen

O_2^- - Superoxide radical

$\text{O}_2\cdot^-$ - Superoxide anion

O^{2-} - Oxide ion

O_2^{2-} - Peroxide anion

ONO_2^- - Peroxynitrite

H_2O_2 – Hydrogen Peroxide

OH^- - Hydroxyl

HO_2^- - Hydrogen dioxide, hydrogen peroxide anion

$\text{HO}\cdot$ / $\text{OH}\cdot$ - Hydroxyl radical

$\text{HOO}\cdot$ - Hydroperoxyl radical / Perhydroxyl radical



Catholyte solution

NaOH – Sodium Hydroxide

Na – Sodium

H₂O₂ – Hydrogen Peroxide

H₃⁻O₂⁻ - Stable peroxide

O₂⁻ - Superoxide radical/Dioxide

O²⁻₂ – Peroxide anion

HO₂ - Hydrogen dioxide

HO₂⁻ - Hydrogen dioxide, hydrogen peroxide anion

HO₂· – Hydrogen dioxide radical

H₂ – Molecular Hydrogen

H· - Hydrogen radical

OH⁻ - Hydroxyl

OH· - Hydroxyl radical

**The mechanism of antimicrobial action of Electro-Chemically Activated (ECA) water
and its healthcare applications**

by

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Promotor : Prof. T.E. Cloete

Department : Microbiology and Plant Pathology

Degree : PhD

Summary

The Electrochemical Activation (ECA) of water is introduced as a novel refinement of conventional electrochemical processes and the unique features and attributes are evaluated against the universal principles that have described the electrolytic processes to date. While the novel and patented novel reactor design retains the capacity to generate products common to conventional electrolysis, it also manipulates the properties of the reagent solutions to achieve an anomalous Oxidation-Reduction potential (ORP or REDOX) that cannot be replicated by traditional chemical and physical interventions. As a contemporary development in the field, the technology continues to undergo rigorous assessment and while not all of its theoretical aspects have been exhaustively interrogated, its undisputed biocidal efficacy has been widely established.

Microbial vitality has been shown to be directly dependent upon the confluence of a diverse variety of physical and chemical environmental conditions. Fundamentally important in this regard is the electronic balance or REDOX potential of the microbial environment. The intricate balance of metabolic pathways that maintain cellular integrity underwrites the measures of irritability required for sustained viability. Aside from the direct effects of the conventional electrolysis products, overt electronic disruption of the immediate microbial environment initiates a cascade of secondary and largely independent autocidal molecular events which compromise the fundamental integrity of the microbe and leads to cell death.

The distinctive capacity to impart unique physicochemical attributes to the ECA derived solutions also facilitates the characterisation of the same outside of the conventional physicochemical and gravimetric measures. These adjunct measures display a substantial relationship with the predictability of antimicrobial effect, and the direct relationship between inactivation of a defined microbial bioload and the titratable measures of REDOX capacity have been shown to describe a repeatable benchmark.

The use of ultra-microscopy to investigate the impact of the ECA products on bacterial cell structures has shown this tool to have distinctive merit in the imaging and thus refined description of the consequences of exposure to biocidal solutions.

The distinctive differences of the ECA solutions relative to conventional antibacterial compounds would suggest a heightened suitability for application in conditions where the efficacy of conventional biocidal compounds had been limited. Aerosolisation of the ECA solutions for the decontamination of airspaces challenged with tuberculosis pathogens revealed that despite initial success, further refinements to the application model will be required to meet the unresolved challenges.

The health care benefits associated with the application of the ECA solutions in a medical environment substantiate the merits for the adoption of the technology as a complementary remedy for the management of nosocomial infections. The relative novelty of the technology in the commercial domain will raise questions regarding the potential for resistance development, and it has been proposed that the distinctive mechanism of biocidal action will not contribute to diminished bacterial susceptibility, as it does not reveal any cross- or co-resistance when assessed against multiple antibiotic resistant strains.

These benefits are further reinforced by the capacity to install the technology for both on-site and on-demand availability, and being derived from natural ingredients (salt and water) the ECA solutions are regarded as safe and compatible for general in-contact use. Notwithstanding the multiple benefits that the technology may provide, further assessments into materials compatibility as well as potential by-products formation following environmental exposure are imperative before the unfettered adoption of this technology as a cost-effective, safe and reliable alternative to conventional disinfection can be promoted.

Patents

Rawhani, S and Kirkpatrick, R.D. (2003). Method for the management and/or treatment of microbially contaminated environments and the use of a new class of microbicidal reagent in such management. WIPO, WO 03/04546 A1.

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Annandale, C.H., Schulman, M.L. and Kirkpatrick, R.D. (2008). The use of electrochemically activated saline as a uterine instillation in pony mares. *Journal of the South African Veterinary Association*. 79(1), 36-38.

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