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

by

Robin Duncan Kirkpatrick

Submitted in partial fulfilment of the requirements for the degree

Philosophae Doctor

Faculty of Natural and Agricultural Sciences

Department of Microbiology and Plant Pathology
University of Pretoria
Pretoria
South Africa

April 2009

"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

Table of Contents

Chapter 2: Microbial energetics

Chapter 3: Biocides and mechanisms of action

Chapter 4: Review of general ECA solution applications

Chapter 5: Non-gravimetric measurement of Electro-Chemically Activated water as a biocidal assessment tool

Chapter 6: Evaluation of the biocidal effects of ECA solutions using Atomic Force Microscopy (AFM)

Chapter 7: The efficacy of Electro-chemically Activated (ECA) water against aerosolised *Bacillus subtilis*, *Serratia marcescens*, *Mycobacterium parafortuitum* and *M. tuberculosis* in a controlled environment.

Chapter 8: Application of ECA solutions to control nosocomial infections in a Neonatal Intensive Care Unit

Chapter 9: Antimicrobial efficacy of Actsol®, an Electro-Chemically Activated (ECA) oxidant solution against multi-drug resistant bacteria.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to the following people and institutions who contributed towards the realisation of this dissertation.

- Professor T.E. Cloete, my supervisor, for his guidance, insight, support and encouragement during the evolution of this study

- My colleagues, both past and present at Radical Waters, without whose vision, tenacity and perseverance in the face of all adversities, would not have resulted in the global prominence with which the ECA technology and its diverse applications are now accorded.

- Dr N van den Berg of the Physics Department, University of Pretoria, for his selfless sacrifices of time and whose patient assistance helped realise the unique microscopy studies.

- The staff of the library, University of Pretoria, for their unstinting support and guidance over the years.

- My partner, family and friends for their belief, love and coercion in helping me achieve this goal

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 ß 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

Cl· - Chlorine radical

OCl^- - Hypochlorite anion

ClO· - Hypochorite 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

O· - Oxygen radical

$^1O_2$ – Singlet oxygen

O_2^- - Superoxide radical

$O_2\cdot^-$ - 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

HO· / OH· - Hydroxyl radical

HOO· - Hydroperoxyl radical / Perhydroxyl radical

Catholyte solution

NaOH – Sodium Hydroxide

Na – Sodium

H_2O_2 – Hydrogen Peroxide

$H_3^-O_2^-$ - Stable peroxide

O_2^- - Superoxide radical/Dioxide

$O^{2-}{}_2$ – Peroxide anion

HO_2 - Hydrogen dioxide

HO_2^- - Hydrogen dioxide, hydrogen peroxide anion

$HO\cdot_2$ – Hydrogen dioxide radical

H_2 – Molecular Hydrogen

H· - Hydrogen radial

OH^- - Hydroxyl

OH· - Hydroxyl radical

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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. Aeroslisation 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

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