ASSESSMENT OF THE QUALITY OF SODIUM HYPOCHLORITE AND HYDROGEN PEROXIDE PRODUCTS IN NAIROBI CITY COUNTY

A thesis submitted in partial fulfillment of the requirements for the award of

the degree of Master of Pharmacy in Pharmaceutical Analysis

BY

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November 2019

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This thesis is my original work and has not been presented elsewhere for examination purpose.

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DEDICATION

This work is dedicated to my guardian angel, my late sister Christine Wanjiru (Shiru), for pushing me to be myself and achieve my best. I also want to dedicate it to my wife, Jemimah and daughter, Tamara for their support, love and endurance during my absence.

My dad, Francis Wachira, mum, Mary Ndegwa and siblings, Gladys Wamuyu, Dr Eric Chomba and James Gichaga for their unconditional support during scholarly undertaking.

ACKNOWLEDGEMENTS

I wish to express my warm heartfelt gratitude and unreserved acknowledgement to my project supervisors, Dr. S.N. Ndwigah, Dr. D.S.B. Ongarora and Dr. A.O. Okaru who made this project possible through their support, excellent guidance and wisdom throughout the process.

I would also want to express my gratitude to the staff of DARU (Drug Analysis and Research Unit) and Dr. K.O Abuga, the head of DARU, for his forbearance and support throughout the practical aspects of the project. I would also want to thank Ms Joyce Syombua for her efforts in helping me throughout the whole practical aspects.

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LIST OF SYMBOLS AND ABBREVIATIONS

℃	Degree centigrade
BP	British pharmacopoeia
Co	Corporation
DARU	Drug Analysis and Research Unit
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
g	Grams
KEBS	Kenya Bureau Standards
L	Litre
LOD	Limit of detection
Ltd	Limited
M	Molar (concentration)
mA	Milliamperes
MFD	Manufacture date
mg	Milligrams
mL	Millilitres
mV	Millivolts
NMR	Nuclear Magnetic Resonance
RSD	Relative standard deviation
V	Voltage

ABSTRACT

Introduction

The growing resistance of microorganisms to antimicrobial agents due to antimicrobial resistance calls for strategies geared on prevention of infection rather than treatment. It is for these reasons that antiseptics and disinfectants continue to play an important role in prevention, against pathogenic microbes. However, they are also central to assuring on their effectiveness. Locally, no quality surveillance has been reported in literature.

Study objective

The general objective of this study was to assess the quality of sodium hypochlorite and hydrogen peroxide products available in Nairobi City County, Kenya using physico-chemical methods.

Methodology

Thirty six samples of sodium hypochlorite were evaluated for general characteristics such as adequacy of label information and pH and assay of active chlorine. Similarly, thirty three samples of hydrogen peroxide were analyzed for identity, adequacy of labeling, acidity and content of hydrogen peroxide. Analytical methods prescribed by the British Pharmacopoeia and the Kenya Bureau of Standards were used. Triplicate analyses were done.

Results

Samples of sodium hypochlorite used as bleaching agents (26), for treatment of water (5) and as hospital disinfectants (5) were collected from Nairobi County. The content of active chlorine was as follows bleaching agent (2-4 w/v %), treatment of water (0.9-1.1 w/v %) and disinfectant (4-6 w/v %) respectively. Twenty out of the twenty six samples (77 %) analyzed complied with the KEBS requirement of a minimum $\leq$ 2 % w/v) of active chlorine for bleaching agents. All 5 (100 %) samples of sodium

hypochlorite used for water treatment met label claim for BP 2017 of 0.9 % w/v and 1.1 % w/v of active chlorine. The values ranged between 1.0 % w/v and 1.3 % w/v corresponding to 92.5 % and 105 .8 % of the label claim (1.2 % w/v). All the samples (n=5) used as disinfectants did not comply with specifications for content (4-6 % w/v). One sample out of the five samples (20 %) did not comply with KEBS requirement (minimum $\leq$ 2 % w/v) for content of active chlorine.

Fifty five percent (n=11) of detergent hydrogen peroxide (3 % w/v H_2O_2) samples met BP (2017) specification of 2.5-3.5 % w/v of H_2O_2. All the samples (n=21) antiseptic hydrogen peroxide (6 % w/v H_2O_2) did not meet the BP (2017) specification of 5 %-7 % w/v of H_2O_2. The content of hydrogen peroxide samples ranged between 3.0 % w/v and 4.7 % w/v. The hydrogen peroxide laboratory reagent was found to contain 13.6 % w/v and this was out of BP specification of 29-31 % w/v of H_2O_2.

Conclusion

About 45 % of detergent hydrogen peroxide samples, all the samples (n=21) of antiseptic hydrogen peroxide and laboratory reagent 30 % w/v hydrogen peroxide did not comply with BP 2017 specifications for assay. While 23 % of the bleaching agent analyzed did not meet KEBS requirements of active chlorine. Five samples of sodium hypochlorite used for treatment of water complied with BP 2017 specifications for the assay while all the samples (n=5) of sodium hypochlorite used as disinfectants did not comply with BP 2017 specifications for content of active chlorine. There is need for continued post market surveillance and enforcement of labeling and packaging specifications by manufacturers for compliance with pharmacopoeial and Kenya bureau of standards specification.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The term Microorganism generally refers to a diverse group of unicellular or multicellular organisms that have both beneficial and non-beneficial effects (Gabor and Tibor, 1999). Some microorganisms have industrial applications such as in the production of cheese, wine, yoghurt and penicillin while others have been implicated with causing infectious diseases like cholera, dysentery and typhoid (Mihriban *et al*., 2006). There are various approaches targeting the destruction of pathogenic microorganisms. These include the use of antibiotics, sterilization and disinfection, among other approaches (Michael *et al*., 2005).

Disinfectants are chemicals that are harmful on the vegetative forms of microorganisms such as fungi and bacteria especially on surfaces and equipment but are less effective against spores (Luanne *et al*., 2004). On the other hand, antiseptics are substances that act by preventing growth of microorganisms or inhibition of microorganism activity. In living tissues, antiseptics prevent infections (USP, 2011). Many antiseptics in the market target Gram-negative and Gram-positive bacteria (Hani and Adnan, 2009).

Disinfectants and antiseptics are generally categorized based on the functional groups into alcohols, aldehydes, phenolics, quaternary ammonium compounds and oxidizing agents (Lachenmeier, 2016). Alcohols are further classified into monohydric and polyhydric alcohols (USP, 2011; William *et al*., 2008). Examples of monohydric alcohols include ethanol and isopropanol while glycerol is an example of a polyhydric alcohol (Gerald a nd Denver, 1999). The

mechanism of action of alcohols as antimicrobial agents is attributable to denaturing action of alcohol. Alcohols affect the production of metabolites essentially involved in rapid cell division (Ali *et al*., 2001). Alcohols are routinely used to disinfect oral and rectal thermometers (Jane, 2002; Judith, 2017) and fiber optics endoscopes, where 70 % alcohol is used (Arthur *et al*., 2002).

Formaldehyde, glutaraldehyde and *ortho*-phthalaldehyde are examples of aldehydes used as disinfectants (Victor, 2005). Mechanistically, they inactivate microorganisms' amino and thiol groups of proteins and the ring nitrogen of the purine bases by alkalization (Gurusamy, 2008). Reusable hemodialyzers are commonly disinfected using formaldehyde although a decline has been being witnessed (Drukker *et al*., 1996) while glutaraldehyde is used to disinfect anesthesia and respiratory therapy equipment (Nancymarie, 2017). Human exposure to glutaraldehyde may lead to skin irritation and dermatitis hence care has to be taken when handling this agent (Gloria and Nick, 2004).

Phenolics used as disinfectants include *ortho*-phenylphenol, chloroxylenol, hexachlorophene and thymol (Semyours, 2001). At high concentrations, the phenolic compounds act as protoplasmic poisons, they act through transfixion and disruption of the cell wall and precipitation of cell proteins while at low concentrations, they also cause inactivation of essential enzyme systems and leakage of essential metabolites from the cell walls leading to bacterial cell death (Vasanthakumari, 2007). Phenolic compounds are commonly used in the decontamination of non-critical medical items and lab surfaces (Semyours, 2001). However, they are not advocated for use in infant's rooms due to their potential to cause hyperbilirubinemia (Carls *et al*., 2012).

Quaternary ammonium compounds can be categorized into cationic, non-ionic, anionic and amphoteric compounds (Reyhaneh and Ali, 2015). Surfactants that are commonly used as disinfectants are cationic and are either single-chain or twin-chain compounds (Russell *et al*., 1998). Single-chain compounds include benzalkonium chloride, alkyl dimethyl benzyl ammonium chloride, alkyl dodecyl dimethyl ammonium chloride and dialkyl dimethyl ammonium chloride. Didodecyl dimethyl ammonium bromide and dioctyl dimethyl ammonium bromide are examples of twin-chain quaternary ammonium compounds (Megan *et al*., 2015). The mode of action of quaternary ammonium compounds involves inactivation of energy-producing enzymes, this causes severe deterioration of cell proteins that are essential and disarranging cell membranes (Merianos, 2001; Mansel, 2005).

Similarly to disinfectants, antiseptics are also classified based on the functional groups. The classes of antiseptics include alcohols, anilides, biguanides, bisphenols, diamidines, heavy metal derivatives and quaternary ammonium compounds among others (Russell *et al*., 1999; Denny and Marsik, 1997). The mechanism of action of alcohols is believed to involve membrane damage, denaturing of proteins, interference with the metabolism and cell lysis (Gerald and Denver, 1999). Isopropyl alcohol swabs are used before and after administration of an injection to disinfect the skin (Gittens and Bunnell, 2009).

Anilides are believed to act by absorbing and destroying the semi-permeable cytoplasmic membrane (Gerald and Denver, 1999). This, in turn, leads to cell death. Triclocarban is an anilide commonly used in soaps and deodorants (Johanna *et al*., 2017).

There are two minor classes of biguanides namely monobiguanides and polymeric biguanides used as antiseptics (Gerald and Denver, 1999). Monobiguanides such as chlorohexidine are thought to act by inhibiting membrane-bound and soluble adenosine triphosphatase as well as net potassium ion uptake in the *Enterococcus faecalis*. Chlorohexidine is used in the manufacture of hand-wash detergents and oral products (Shane and Elizabeth, 2014). On the other hand, polymeric biguanides, such as vantocil, impair the outer membrane of Gram-negative bacteria by acting on the permeability barrier. Vantocil is used to disinfect swimming pools and as a bactericide to sanitize surfaces in food processing industries (Gerald and Denver, 1999).

Triclosan is an example of a bisphenol that is commonly used in the production of hand soaps and hand rinses and toothpaste product (Damia and Andrey, 2015; Ole and Edwina, 2008). The mode of action of bisphenols as antiseptics has not been elucidated but they are postulated to affect the cytoplasmic membrane (Ole and Edwina, 2008). Triclosan has been shown to inhibit the uptake of essential nutrients in *Escherichia coli*. Triclosan (5μg/ml) leads to a rapid release of cellular components and in turn cell death occurs (Larson *et al*., 2003).

Chloroxylenol, a halophenol antiseptic, is commercially marketed as 1.2 % w/w dichloroxylenol antiseptic formulation. Chloroxylenol is believed to affect the cell wall of microorganisms such as *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* (Grillo and Ojo, 2013). Chloroxylenol commonly used as antibacterial soap (Mary, 1996).

Diamidines are commonly used as topical treatments for wounds. Diamidines such as propamidine act by inhibiting oxygen uptake and induces leakage of amino acids of *Pseudomonas aeruginosa* and *Enterobacter cloacae*. Sodium isethionate forming a salt with propamidine (4,4-diamino phenoxy propane) and dibromo (2,2-dibromo-4,4- diamidine phenoxy propane) are other good example of diamidines (Gerald and Denver, 1999).

Silver compounds that are used as antiseptics include silver nitrate and silver sulphadiazine (Abdulkareem and Wiley, 2005). The mechanism of action of silver compounds involves the interaction with the thiol groups in the amino acids of target microorganisms (Woo *et al*., 2008). Silver nitrate also interacts the cysteine and sodium thioglycolate present in *Pseudomonas aeruginosa* (Liaus *et al*., 1997).

Quaternary ammonium compounds used as antiseptics include cetrimide, chlorhexidine and salvon (combination of cetrimide and chlorhexidine). The mechanism of action of cetrimide involves membrane disorganization which leads to cell wall lysis caused by autolytic enzymes and damaging bacterial cell wall (Denyer, 1995). Figure 1.1 shows the structures representatives of compounds of various classes of disinfectants and antiseptics.

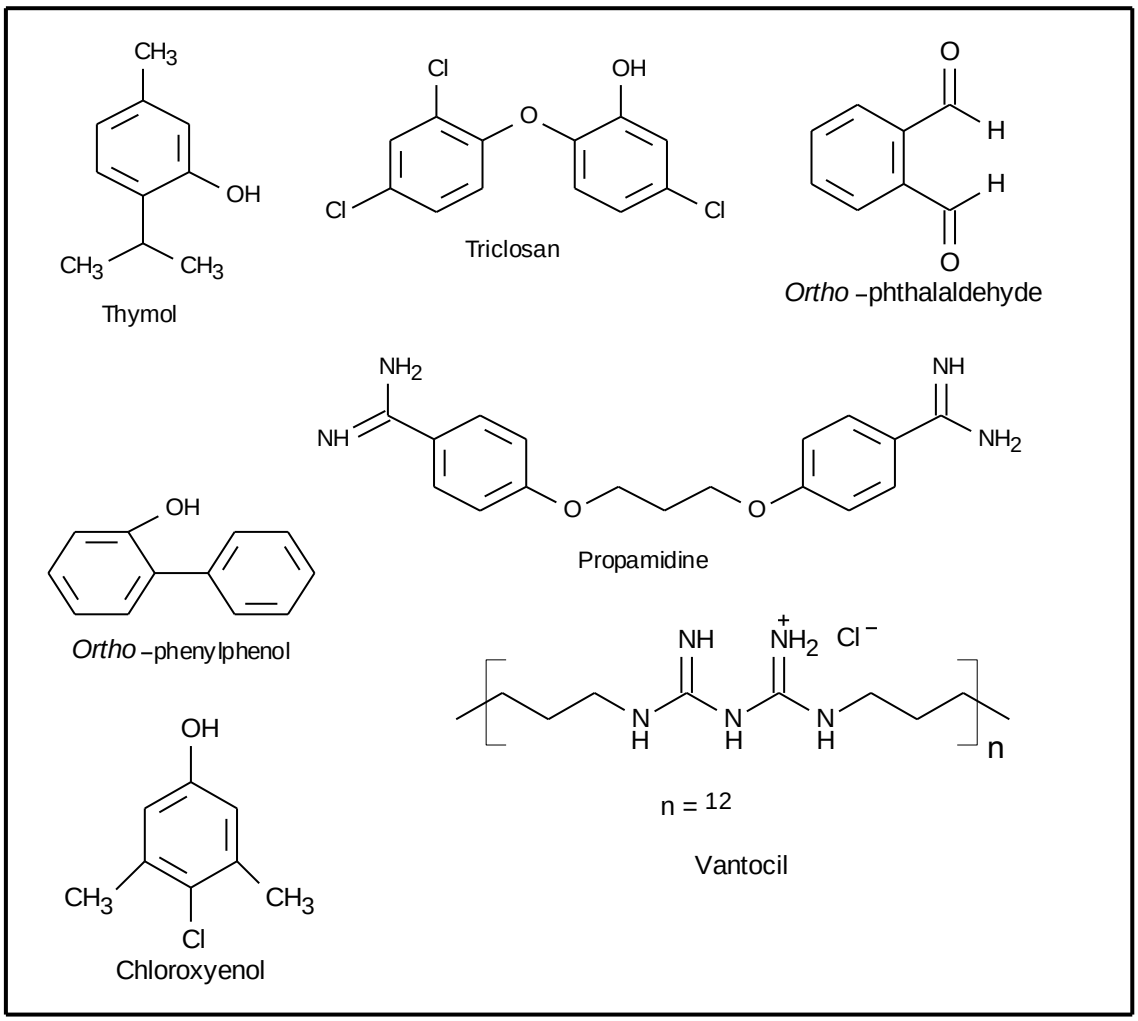


Figure 1.1: Chemical structures of some disinfectants and antiseptics

Oxidizing agents used as disinfectants and/or antiseptics are either chlorine based or oxygen based (Russell *et al*., 1999). Chlorine-based disinfectants include sodium hypochlorite, calcium hypochlorite, chloramine and chlorine dioxide. The mode of action of chlorine-based compounds has not been enumerated in existing research. Presumably, chlorine oxidizes the sulfhydryl enzymes and amino acids leading to intercellular contents loss, depleting intake of nutrients, proteins synthesis inhibition and reduction in production of adenosine triphosphate (Gerba and Rusin, 2001).

Oxygen-based disinfectants and/or antiseptics include potassium permanganate, performic acid, peracetic acid, potassium peroxymonosulfate and hydrogen peroxide (Russell *et al*.,1999). Peroxygen compounds act by producing free hydroxyl radicals. The free hydroxyl radical targets the double bonds and sulfhydryl groups present in lipids, proteins and DNA of microorganism (Alugoju *et al*., 2015).

1.2 Sodium hypochlorite

1.2.1 Properties of sodium hypochlorite

Sodium hypochlorite (NaOCl), a sodium salt of hypochlorous acid, exists either as solid sodium hypochlorite pentahydrate ($NaOCl.5H_2O$), solid sodium hypochlorite dihydrate ($NaOCl.2H_2O$) or sodium hypochlorite solution. Sodium hypochlorite pentahydrate is a colourless crystalline solid with a melting point of 18 °C and a solubility of 293 g in a liter of water at 0 °C and 942 g in a liter at 23 °C (Dale, 1995). Sodium hypochlorite pentahydrate is a strong oxidizer of organic and inorganic compounds (Masayuki *et al*., 2017). On the other hand, sodium hypochlorite dihydrate is a colourless hygroscopic crystalline solid with a melting point of 57.5 °C.

Sodium hypochlorite dihydrate is very stable in cold water and at high concentration hypochlorite ions rapidly dissociate to form chlorate and chloride ions (equation 1) (Aieta and Robert, 1985).

$$3OCl^-_{(aq)} \longrightarrow ClO^-_{3(aq)} + Cl_{2(aq)} + 2\,e^- \quad \text{.....(equation 1)}$$

Sodium hypochlorite solution is a clear to a slightly yellow solution with strong oxidizing properties. Sodium hypochlorite solution is amenable to decomposition depending on its concentration, ionic strength, pH, temperature, light and impurities. In acidic media, sodium

hypochlorite solution produces chlorine gas (Uri, 2009).

1.2.2 Production of sodium hypochlorite

Industrially, sodium hypochlorite solution is synthesized using the Hooker process (Adamson *et al*., 1963). The process involves combination of sodium hydroxide and chlorine gas produced by electrolysis of brine (sodium chloride solution). Through non-diaphragm process sodium hypochlorite is isolated (equation 2) (Fukuzaki, 2006).

$$2NaOH_{(aq)} + Cl_{2(aq)} \longrightarrow NaOCl_{(aq)} + NaCl_{(aq)} + H_2O_{(l)} \quad \text{.....(equation 2)}$$

Commercially, sodium hypochlorite solution is marketed as dilute sodium hypochlorite solution (contains between 0.90 % and 1.10 % w/w of active available chlorine in the solution) and strong sodium hypochlorite solution (contains ≥ 8 % w/w of active available chlorine) (BP, 2017). The available chlorine (active chlorine) is in three forms namely hypochlorous acid (HOCl), hypochlorite ions (OCl^-) and chlorine gas (Cl_2), where the ratio is dependent on pH and temperature. In the variation of pH, hypochlorite anion is predominant at a pH 8 followed by hypochlorous acid at pH 5-7 and chlorine at a pH 1-3 (Bjerregaard, 2014).

1.2.3 Uses of sodium hypochlorite

Sodium hypochlorite is used in many ways in diverse industries. For example in textile and paper pulp industries, sodium hypochlorite is the preferred bleaching agent (Jame *et al*., 2003). In food industries, it is used as a preservative to prevent the formation of moulds in canned foods (Mihriban *et al*., 2006). In hospitals, sodium hypochlorite (4-6 % w/w) is used to sterilize various surfaces and equipment (Sweetman, 2011). Cleaning floors in hospitals is the most notable use of sodium hypochlorite. However, sodium hypochlorite has to be diluted before use to facilitate

optimal effectiveness depending on the area of application. Table 1.1 illustrates the dilutions of 4-6 % w/w sodium hypochlorite for typical hospital application.

Table 1.1: Typical dilutions of sodium hypochlorite for hospital application

Disinfection use	**Dilution to make up to 5 L with water**
Hands and floor	100 mL
Operation theater, Stretcher, emergency wards and laboratory surfaces	25 mL
Sputum disinfection and surface contaminated	500 mL
Highly contaminated surfaces, HIV, Hepatitis B virus	1 L
Patient clothes, linen	50 mL

Sodium hypochlorite is used to disinfect swimming pools where it targets *Legionella* bacteria and biofilm which transmit water-borne diseases. Biofilm is a matrix of microorganisms that form a layer on the surface of water thus providing a safe haven for the growth and multiplication of *Listeria, Escherichia coli* and *Legionella* (Luanne *et al*., 2004). Water obtained from different sources is treated with 1 % w/w sodium hypochlorite solution to prevent the spread of water-borne diseases such as cholera, dysentery and typhoid (Alix and Michael, 2007; Save *et al*., 2014).

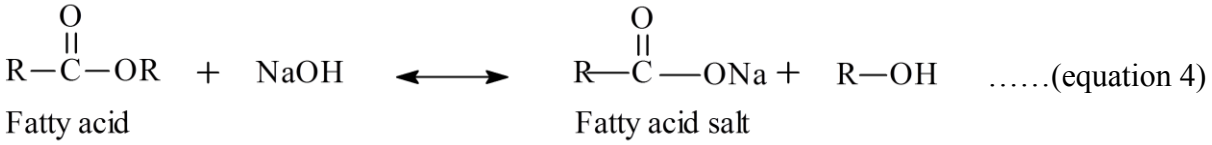
During dental root canal procedures 2.5 % w/w sodium hypochlorite solution prevents the growth of *Enterococcus faecalis* and helps dissolve dead tissues. Similarly, sodium hypochlorite exhibits activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* (Fukuzaki, 2006; Carlos *et al*., 2002). However, some precautions are advised on its use. For example, it was noted that 5 % w/w sodium hypochlorite irritates soft tissues around the tooth. Therefore, its appropriate concentration for use in root canal procedures is between 0.5 and 4.5 % w/w of NaOCl. The mechanism of action of sodium hypochlorite involves alteration of

the nucleus, mitochondria energy balance and destruction of phospholipid in microorganisms (Carlos *et al*., 2002).

Sodium hypochlorite has found use in the treatment of eczema at a concentration of 0.005 % w/w (Tanya and Kerryn, 2013) and to neutralize chemical weapons (Demetrius *et al*., 2002). Sodium hypochlorite as a saponification agent for cleaning cloths entails a series of reactions. Foremost, sodium hypochlorite dissociates to produce sodium hydroxide and hypochlorous acid as shown in equation 3 (Fukuzaki, 2006; Carlos *et al*., 2002)

$$NaOCl + H_2O \longleftrightarrow NaOH + HOCl \longleftrightarrow Na^+ OH^- + H^+ + OCl^- \quad \text{.(equation 3)}$$

Salts of fatty acid and alcohol are produced after the reaction between sodium hydroxide and fatty acid esters (equation 4). This decreases the surface tension caused by the fatty acids esters particles forming the basis for the use of sodium hypochlorite as a detergent.



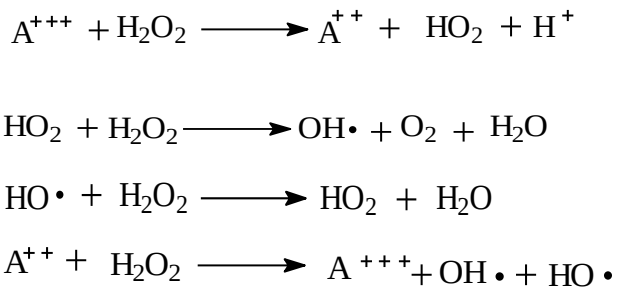
……(equation 4)

1.3 Hydrogen peroxide

1.3.1 Properties of hydrogen peroxide

Hydrogen peroxide is a clear liquid that is colourless, odourless and decomposes in air to form oxygen and water once exposed to light. A French scientist, Louis Jacques Thernard (1777 -1857) identified hydrogen peroxide (H_2O_2), as the product formed when barium peroxide was added to water in 1818 (Scott and Andrea, 1995). However, separation of the product from water was difficult due to its miscibility in water. In 1894, Richard Wolffenstein (1864 - 1926) managed to

completely separate hydrogen peroxide from the mixture using vacuum distillation (Maass and Hatcher, 1920). The decomposition of hydrogen peroxide occurs in presence of light at a wavelength of 365 nm or less. This decomposition follows a chain reaction based on Haber-Willstatter reactions in the presence of catalysts (scheme1) (Willem, 2001).

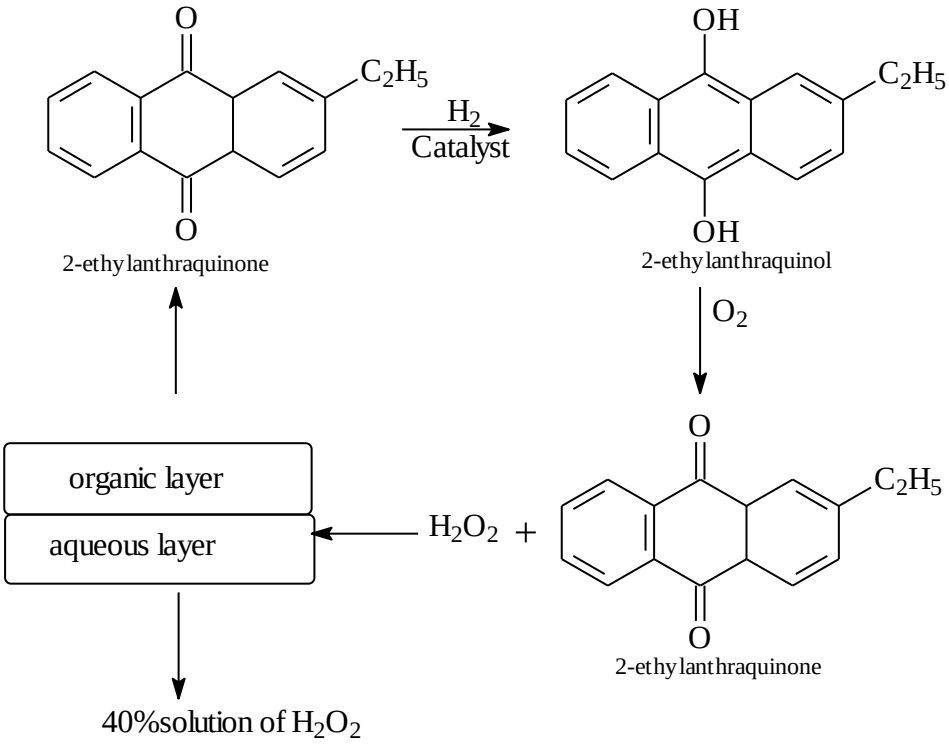


Where A is the catalyst

Scheme 1: Haber-Willstatter reaction

1.3.2 Production of hydrogen peroxide

Hydrogen peroxide can be synthesized using various methods such as the reaction between hydrogen and oxygen gas over palladium as a catalyst (Maehara *et al*, 2008). In the commercial production of hydrogen peroxide, 2-ethylanthraquinone is used in the presence of hydrogen gas. After catalytic reduction of 2-ethylanthraquinone, 2-ethylanthraquinol is obtained which is then oxidized by oxygen to produce anthraquinone and hydrogen peroxide. The catalysts used in the process include palladium and aluminum oxide (Qunlai, 2008). The synthesis is as shown in scheme 2.



Scheme 2: Preparation of hydrogen peroxide from 2-ethylanthraquinone

Hydrogen peroxide is commonly marketed either as 3 %, 6 % or 30 % w/v solution in water.

1.3.3 Uses of hydrogen peroxide

Hydrogen peroxide is an oxidizing agent that is used as antiseptic, disinfectant and deodorant. The antibacterial and antiviral properties of hydrogen peroxide are dependent on the concentration used. In the food industry, 35 % w/v hydrogen peroxide is used to sterilize plant food before packaging (Ezra *et al*., 2012). Hydrogen peroxide (1.5 % w/v) is used as a mouthwash in the treatment of acute stomatitis and as a deodorant gargle (Sweetman, 2011). Hydrogen peroxide has also been used for aesthetic purposes in whitening teeth and together with other components to make a bleaching toothpaste (Mohammet and Omer, 2015). The other aesthetic use of hydrogen peroxide is in bleaching of industrial hair and delicate fabrics which is done using 6 % w/v hydrogen peroxide diluted with equal portions of water. The dilution is done to avoid irritation in

the case of hair dye and prevent damage to the delicate fabrics (Sweetman, 2011). It is important to note that some detergents contain 3 % hydrogen peroxide. These are commonly known as color cleaning agents. The detergents are commonly used to clean coloured clothes that would be bleached if sodium hypochlorite is used (Zaharia and Suteu, 2012).

In the removal of earwax, 6 % w/v hydrogen peroxide solution diluted in three parts of water is used. Hydrogen peroxide makes the wax bubble and easily removable from the ear (Sweetman, 2011). Hydrogen peroxide is also useful for disinfecting soft contact lenses where it decomposes deposits on the contact lenses. Lenses have to be properly rinsed to avoid acute irritation of the eyes caused by hydrogen peroxide (Robin, 2001).

The use of hydrogen peroxide an antiseptic arose from the observation that honey, which contains hydrogen peroxide was employed to diabetic wounds (Fahmida *et al*., 2014). A 6 % w/v hydrogen peroxide cream is used to clean wounds and ulcers (Sweetman, 2011). Hydrogen peroxide has been shown to prevent the growth of microorganisms like *Pseudomonas species, Escherichia coli* and *Bacillus subtilis* (Fukuzaki, 2006). Hydrogen peroxide vapour system and hydrogen aerosol system are used to disinfect theaters in hospitals and have shown effects against *Clostridium difficile,* Vancomycin resistant *Enterococci,* Methicilin resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* (Holmdahl *et al*., 2011).

1.4 Adverse effects of sodium hypochlorite and hydrogen peroxide

Some adverse effects have been noted when sodium hypochlorite is used to clean dialysis machine such as hemolysis, hyperkalemia, cyanosis and cardiopulmonary arrest (Jeffrey, 2010). An

accidental 1 % sodium hypochlorite infusion leads to slow heart rate and mild hypotension (Jeffrey, 2010). It also adversely affects pulmonary functions as evident in a study done on the effect of sodium hypochlorite on the pulmonary function, when used as a cleaning agent. In the study it was found that, even at low concentrations, sodium hypochlorite resulted in clinical and functional defects arising from irritation in the airway (Demiralay, 2001).

In root canal irrigation, sodium hypochlorite (3 % w/w) led to an accidental life threatening airway obstruction. Facial swelling, bruising and pain 3 h after a root canal procedure has been reported. The major drawback in this research was the concentration of sodium hypochlorite used was not reported. The swelling and bruising persisted up to four weeks after the incident (Lachenmeier, 2016). Sodium hypochlorite has been found to affect the body length and cranial circumference of infants born from mothers who drink water decontaminated with chlorine compounds (Kanitz *et al*., 1996). An increased number of malignant and benign tumors were found in male rats exposed to sodium hypochlorite. The aforementioned tumors were a squamous cell of the fore stomach and two leiomyosarcomas in the stomach (Morando *et al*., 1997).

The major adverse effects related to the use of hydrogen peroxide as a disinfectant is irritation of gastrointestinal and respiratory tracts. it is also reported that in high concentration, hydrogen peroxide may lead to laryngospasm and hemorrhagic gastritis (Barbara *et al*., 2004). Care has to be taken while using hydrogen peroxide cream due to cytotoxicity once in contact with venous bloodstream, causing catalase tissue deposits (Robert, 2005). Further, it has been observed that high concentration of hydrogen peroxide and heat seems to lead to tooth cervical root resorption (Nikhil, 2013).

The tumorigenicity of hydrogen peroxide has been examined by administering hydrogen peroxide (0.4 %) solution in water availed for drinking to mice of various strains. It was found to cause the formation of gastric and duodenal lesions (James *et al*., 2011; EFSA, 2012). Hydrogen peroxide also affects the DNA and motility of spermatozoa stored and incubated in it. A research to investigate plasma membrane and DNA integration of human spermatozoa by hydrogen peroxide found that *in vitro* incubation of spermatozoa in hydrogen peroxide not only induces DNA fragmentation but also affects the motility of spermatozoa (Namik *et al*., 2000).

1.5 Literature review

1.5.1 Quality of sodium hypochlorite products

A study conducted on the quality of 38 proprietary and 62 of non-proprietary sodium hypochlorite products obtained from 116 pharmacies in the UK indicated that there was a significant variation in the content of sodium hypochlorite with storage temperature ($P < 0.01$). No significant ($P < 0.05$) change occurred in the quality of the products stored at ambient temperature. Nevertheless, there was a predictable reduction of available chlorine if the sodium hypochlorite was stored at 37 °C after a period of 6 months but this was dependent on concentration of the preparation. It was observed that when 5 % w/w active chlorine in sodium hypochlorite was heated at 65-80 °C for 4 h, the active chlorine increased to 6 % w/w in a covered solution and 9 % w/w in the uncovered solutions (Gulabivala *et al*., 2001).

In another study designed to probe the effect of concentration, storage condition and pH on the sturdiness of various sodium hypochlorite (5 % w/w available chlorine) solutions, it was observed

that products stored at 24 °C showed a higher rate of degradation than those stored at 4 °C. Also, it was observed that there was a slow decline in the pH of the solutions over time, correlating to the dropping amount of active chlorine present (Murat and Beyser, 1995).

A study done in Brazil to evaluate the active chlorine released from 6 % w/w sodium hypochlorite solution during a seven-day period and stored at different temperatures found that 49 samples (n=80) lost up to 58.3 % of active chlorine depending on the storage condition, time of storage and effect of prevailing temperatures. The free active chlorine concentration of sodium hypochlorite was affected by temperature. Higher temperature lowered lifetime of free residual chlorine while samples stored in refrigerated environment lost the least amount of active chlorine (Braitt *et al*., 2013).

Similarly, the stability of sodium hypochlorite (1 %, 2.62 % and 5.25 % w/w) has been analyzed and found to be affected by high temperature, air, exposure to light as well as the presence of inorganic matter and organic matter. The free residual chlorine in the solution is affected which in turn affects the disinfecting ability of sodium hypochlorite (Sirtes *et al*., 2005).

In Kenya, quality sodium hypochlorite solutions (used as detergents and for disinfection) should have the following limits maintained as shown in Table 1.2 (KEBS, 1999).

Table 1.2: Kenya Bureau of Standards requirements for sodium hypochlorite solutions

Characteristic	Requirement
Available chlorine % m/v min	≤ 2.0
Free alkali as NaOH, % m/v	0.1-1.0
Fe ppm max	0.4
Sodium Chlorate g/L (m/v)	Traces
pH min	9

1.5.2 Quality of hydrogen peroxide

Research conducted on the quality of hydrogen peroxide products (n=165) obtained from 140 medical stores and pharmacies in Tanzania which included wound cleansing agents and eardrops, reported the presence of poor quality products in the Tanzanian market. Out of the 70 samples obtained from the medical stores, 26 % of samples failed to comply with the BP (2005) specification for assay and labeling. Also, 29 % (n=70) of the samples obtained from the pharmacies failed to comply with BP (2005) specification for assay and labeling. All the twenty five samples of eardrops obtained from the pharmacies failed to comply with BP (2005) specification for assay and labeling (Kaale *et al*., 2007).

1.6 Methods used to analyze

1.6.1 Hydrogen peroxide

There are various methods for the analysis of the amount of hydrogen peroxide in different matrices these include fluorescence quenching (Zhiliang *et al*., 2011), near-infrared spectroscopy (Adriana *et al*., 2008), ^{1}H nuclear magnetic resonance (NMR) spectroscopy (Monakhora and Diehl, 2016; Siegfried *et al*., 2010), Raman spectroscopy (Stewart *et al*., 2012), ultraviolet-visible spectroscopy (Takamura and Matsumoto, 2009), chemiluminescence spectroscopy (Yamashiro *et al*., 2004), electrochemical methods (Ohura *et al*., 1996) and redox titration (BP, 2017).

Fluorescence quenching is one method of analyzing presence of hydrogen peroxide. Sodium acetate-hydrochloric buffer solution (pH of 1.99) and temperature of 60 °C together with rhodamine B for excitation in the presence of iron (II, III) oxide nano particles as catalyst are used in fluorescence quenching. Rhodamine B exhibits a strong fluorescence with an excitement peak at 584 nm. The fluorescence quenching occurs when iron (II, III) oxide nano particles catalyze the

hydrogen peroxide oxidation of rhodamine B with attendant or decrease in intensity of the fluorescence peak intensity at 584 nm. The fluorescence quenching forms a straight line (linear) against concentration of hydrogen peroxide in the region of 10-200 nmol/L and therefore this forms the basis for its use for assay of hydrogen peroxide (Zhiliang *et al*., 2011).

Near-infrared spectroscopy is used for the analysis of hydrogen peroxide in a wavelength ranging between 850-1800 nm using (1.0 mm) optical path, transflectance probe. Pure liquids, absorb the bands of near-infrared are registered at 1010, 1200, 1500, 1600, 2100, 2300 and 2930 cm^{-1}. The gaseous mixture containing water and hydrogen peroxide report an absorption band at 1420 cm^{-1} while region of 1400 to 1550 cm^{-1} absorption band forms water overtone of O-H is predominant. A reduction in the absorption is observed in the presence of hydrogen peroxide in this region while an increase in the absorption is noted at 1550 to 1750 cm^{-1}band region (Adriana *et al*., 2008).

A method utilizing ^{1}H NMR spectroscopy has been advanced for analysis of hydrogen peroxide in hairspray, hydrogen peroxide disinfectant, nail polish and chemical reagent. Dimethyl sulfoxide (DMSO)-d_6 is used to prevent coelution of OH peaks. A unique peak at δ 10.2 ppm of peroxide is then quantified counter to the signal of the CH_3 group of dimethyl formamide (δ 2.9 ppm). The LOD varied between 10^{-4} % w/v and 10^{-2} % w/v for chemical reagents and hairspray respectively. The linearity of ^{1}H NMR for 10-100 μL (R^2=0.9958) sample volume was demonstrated (Monakhora and Diehl, 2016).

Incorporation of NMR and pH measurement has also been developed to analyze hydrogen peroxide. The NMR transverse relaxation time T_2, analyzed by Carr-Purcell-Meiboom-Gill

(CPMG) multi-pulse series of aqueous hydrogen peroxide solution strongly depends on the spin of hydrogen peroxide (H_2O_2) and water (H_2O) molecules. The hydrogen peroxide concentration and pH allow for the inversion of the data as is used for the non-invasive determination of hydrogen peroxide concentration (Siegfried *et al*., 2010).

A handheld Raman spectrometer has been used for the analysis of hydrogen peroxide amounts in aqueous solution which occurs in seconds. To allow for analysis of amount, the aqueous peroxide sample was mixed 50/50 (v/v) with 4 mol/dm^3 sodium perchlorate solution (internal standard). Relative peak height (932 cm^{-1}) for the strongest perchlorate and peroxide bands (876 cm^{-1}) produced an error of 1.43 % for samples analyzed in the range 5-30 % w/v peroxide. The peaks were then compared against standard spectra library prepared using an identical range of peroxide concentration at 5 % increment. Determination of hydrogen peroxide concentration in fields for explosive manufacturing site is done using this method (Stewart *et al*., 2012).

Ultraviolet-visible spectral used to analyze the amount of hydrogen peroxide is also available. For the detection of hydrogen peroxide, two highly sensitive and selective aqueous acidic solutions were developed of Oxo [5, 10, 15, 20-tetra (4 pyridyl) porphyrinato titanium (IV) complex and Oxo [5, 10, 15, 20-tetra (4 pyridyl) porphyrinato] titanium oxide. A unique peak at 432 nm is produced by the reagents and when hydrogen peroxide is added to the reagents, a new peak at 450 nm is produced. Proportional to the concentration of hydrogen peroxide a peak is produced. Although, the peak at 450 nm was H_2O-adducted structure, modifications were done with an aim of eliminating hydrogen peroxide (Takamura and Matsumoto, 2009).

Chemiluminescence has also been used for the analysis of hydrogen peroxide. An injected flow of hydrogen peroxide identification system has been used with a part per billion identification limit to analyze the amount of hydrogen peroxide in water sample. The detection system uses luminol as chemiluminescence agent. Water sample of (20 μL) is mixed with aqueous luminol solution and cobalt ion as a catalyst in a cell and streamed to the detection cell. Photocurrent that is detected by the luminol in the identification system produced a straight line (linear) against the concentration of hydrogen peroxide. The luminous intensity of luminol chemiluminescence is at its highest at pH 11 and 0.3 parts per billion which is the lowest detectable limit (Yamashiro *et al*., 2004).

Electrochemical techniques such as potentiometric and amperometric assays have been considered for analysis of hydrogen peroxide content. A pontentiometric flow injection method, that is rapid and sensitive, is used for checking traces of hydrogen peroxide has made headway using Fe (III)-Fe (II). A buffer solution that contains bromide and Mo (VI) is used for detection. The analysis is based on a relationship that is linear between concentration of hydrogen peroxide and the potential change on the oxidation–reduction potential electrode. This is assignable to the reaction between hydrogen peroxide and the buffer solution that leads to production of bromine (Ohura *et al*., 1996).

A simple amperometric biosensor coupled with injection flow analysis system that detects hydrogen peroxide in solvents that are organic has been developed. Various modified electrode are used, catalases entangled in polyacrylamide gel and deposited on the exterior of platinum (working electrode) fixed in a Teflon coverers with silver wire (auxiliary electrode). This is followed by insertion of filter paper immersed in potassium chloride (Varma and Mattiasson, 2005).

An alternative amperometric method, for the analysis of hydrogen peroxide involves enhanced electrodes containing (PDDA) poly (diallydimethylammonium chloride) functionalized graphene-Fe_3O_4 (PDDA-G/Fe_3O_4)$_n$ overlap of films were forged with a sheet by sheet of negatively charged Fe_3O_4 nanoparticles (Fe_3O_4 NPs) as the cathode. The anode electrode contained (PDDA-G) with an electrostatic interaction, constructed with a hydrogen peroxide chemical sensor. The PDDA-G enhanced the catalytic capability of Fe_3O_4 NPs due to the increased area of contact, perfect for electric conductivity. Allowing for the hydrogen peroxide chemical sensor to exhibit an outstanding electro catalytic activity for the identification of hydrogen peroxide with a linear range of between 20 μM to 6.25 mM and with a detection limit that is low allowing for up to 2.5 μM to be measured and an (S/n) of 3 (Liu *et al*., 2011).

1.6.2 Sodium hypochlorite

Various techniques have been used for the analysis of the amount hypochlorite and chlorine in some assorted products. They include ultraviolet-visible spectroscopy (Chand and Narayana, 2007; Gengan and Jonnalagadda, 2005), chemiluminescence spectrometry (March and Simonet, 2007; Catala *et al*., 2011; Sivanildo and Boaventura, 2007), voltammetry method colourimetry *(*Kodera *et al*., 2005; Thomas, 2005). A sensitive facile spectrophotometric method has been developed to determine the amount of hypochlorite present using rhodamine B. When potassium iodide reacts with hypochlorite in acid medium, iodine is liberated. The liberated iodine decolorizes the pinkish red color of rhodamine B. The absorbance is measured at 553 nm. It was found to be directly proportional to the hypochlorite concentration (Pasha and Narayana, 2007).

Similarly, a second spectrometric method using potassium permanganate to determine the amount of hypochlorite present in commercial bleaches has also been developed. The method was based

on the reaction of hypochlorite with arsenious oxide (As_2O_3), at acidic pH 6.5 allowed for the analysis. The absorbance of the residual potassium permanganate is used to form the four ranges of the calibration curve. The curve is used to determine the hypochlorite ranging (2.5×10^{-4} to 2.4×10^{-2}M) (Gengan and Jonnalagadda, 2005).

Hypochlorite in bleaching products can be determined based on an injection flow system. This involves measuring the absorbance of hypochlorite at 292 nm and allowing for the analysis of hypochlorite in the range of 0.07-0.42 $gL^{-1}$of chlorine gas. To allow the analysis to be done, a highly sensitivity cobalt oxide containing mini-column is used to catalyze hypochlorite (allowing for decomposition to form oxygen and chloride) was put into the injection flow system. The absorbance difference was then measured after the sample was allowed to pass through the mini-column (March and Simonet, 2007).

A spectrometric method using a flow injection analysis to determine the amount of active chlorine in industrial products and water samples has been developed. The color change of the *O*-dianisidine was observed at 445 nm. The technique is linear over a range of 0.04-1.00 mgL^{-1} of chlorine gas and with a limit of detection 0.04 mgL^{-1}. The reproducibility had a RSD slope of 3.7 % for a series of 4 independent calibrations (Catala *et al*., 2011).

In an alternative photometric method, a falling drop system of the analyte (Cl_2) was collected together with *N, N′*-diethyl-*p*- phenylenediamine (50 μL), a chromogenic reagent. A green light emitting diode (515 nm) and a phototransistor were used. From the analysis, a linear response

range was 15-100mg L^{-1} (R^2=0.999) and an of LOD of 4.5 mgL^{-1}(Sivanildo and Boaventura, 2007).

The reaction of hypochlorite with luminol, using stopped-flow chemiluminescence spectrometry has also been used to determine the amount of hypochlorite in water. A conventional fluorescence detector set at 425 nm with its shutter off is used for observing the emission. The method gives a linear response over 3 orders of magnitude. This technique is sensitive and fast (Thomas, 2005).

Voltammetrically, low active chlorine concentration are determined using a reduction wave founded on anodic cyclic. The detection is observed at 600 mV using silver/ silver chloride electrode, this depends on the active chlorine concentration and switching potential. A maximum value for a switching potential at the peak wave was approximately 1350 mV showing a relationship that is linear between the peak current and concentration range of 0.2-6.0 mg/dm^{-3}. It was observed that each concentration had less than 2 % relative standard deviation (Kodera *et al*., 2005).

Colourimetric determination of chlorine uses reagents such as benzidine and 3,3'- dimethyl-derivative (*O*- toluidine). A yellow colour is produced when the reagents are treated with an oxidizing agent (chlorine). However, due to the instability of the benzidine and rapid fading of the colour, it was difficult to use the reagent. The colour obtained from using 3, 3'-dimethylnaphthidine lasted for at least 20 minutes allowing for analysis, using EEL photo-electric colorimeter. The colour produced obeyed Beer's law and it was sufficiently reproducible to enable

accurate results. This allowed for as a little as 0.05 ppm of chlorine to be measured (Geza and Livia, 2000; Ronald *et al*., 1954).

A method has been developed that enables simultaneous determination of chloride, hypochlorite, hydroxide, and chlorate ions in a sample. A flow injection method that exits in chlor-alkali cell is used to determine sub millimolar of the elements. Colorimetric iodometry method is used for the determination of chlorate and hypochlorite. While for the hydroxide heat of neutralization is determined after sample has been injected and the chloride is calculated from conductance data. Iodine is obtained after oxidization of iodide by hypochlorate and chlorate in acid solution (Tian and Dasgupta, 2000).

As oxidizing agents, sodium hypochlorite and hydrogen peroxide are therefore amenable to redox titration, the endpoint is determined either by use of starch-iodide indicator, potentiometrically or visual observation of the change in color of the reagents. Three most commonly used titrants are potassium dichromate, potassium permanganate and sodium thiosulphate. Potassium dichromate and potassium permanganate act as oxidants in acidic solutions. The half-reaction involving manganate (vii) ion is as shown equation 5 (Michael and Rosalind, 2000).

$$MnO_4^-{}_{(aq)} + 8H^+_{(aq)} + 5e^- \longrightarrow Mn^{2+}_{(aq)} + H_2O_{(l)} \quad \text{.....(equation 5)}$$

Sodium thiosulphate acts as a reducing reagent in acid media. The half-cell (equation 6) involved is as shown.

$$2S_2O_3^{2-}{}_{(aq)} \longrightarrow S_4O_6^{2-}{}_{(aq)} + 2e^- \quad \text{.....(equation 6)}$$

1.7 Standardization and assay

1.7.1 Standardization of sodium thiosulphate and assay of sodium hypochlorite

Standardization is a necessity before using a volumetric reagent as a titrant. The reason for standardization is to be assured of the concentration of prepared reagents. The standardization of sodium thiosulphate involves a series of reactions as shown below (BP, 2017).

Potassium bromate and potassium iodide in the presence of an acid react as shown in equation 7

$$KBrO_{3(s)} + 6KI_{(s)} + 6HCl_{(aq)} \longrightarrow KBr_{(aq)} + 6KCl_{(aq)} + 3H_2O_{(l)} + 3I_{2(g)} \quad \text{....(equation 7)}$$

The iodine gas liberated is then titrated with sodium thiosulphate as shown in equation 8

$$I_{2\,(g)} + 2Na_2S_2O_{3(aq)} \longrightarrow 2NaI_{(aq)} + Na_2S_4O_{6\,(aq)} \quad \text{.....(equation 8)}$$

Sodium thiosulphate is then used as a titrant in the assay of sodium hypochlorite. Initially, sodium hypochlorite, acetic acid and potassium iodide react to generate iodine gas as shown (equation 9).

$$NaOCl_{(aq)} + 2CH_3COOH_{(l)} + 2KI_{(s)} \longrightarrow NaCl_{(aq)} + 2CH_3COOK_{(aq)} + H_2O_{(l)} + I_2 \quad \text{...(equation 9)}$$

The iodine generated reacts with sodium thiosulphate as shown in equation (10)

$$I_{2\,(g)} + 2Na_2S_2O_{3(aq)} \longrightarrow 2NaI_{(aq)} + Na_2S_4O_{6\,(aq)} \quad \text{.....(equation 10)}$$

The end point is determined potentiometrically for accuracy, 2.784 mg of potassium bromate is equivalent to1 mL of 0.1 M sodium thiosulphate (BP, 2017).

1.7.2 Standardization of potassium permanganate and assay of hydrogen peroxide

Standardization of potassium permanganate involves titration with potassium iodide in the presence of sulphuric acid to produce iodine (equation 11). The iodine liberated is titrated with sodium thiosulphate to a potentiometric endpoint (equation 12).

$$2KMno_{4(aq)} + 10KI_{(aq)} + 8H_2SO_{4(aq)} \longrightarrow 6K_2SO_{4(aq)} + 2MnSO_{4(aq)} + 8H_2O_{(aq)} + 5I_{2(g)} \quad \text{..(equation 11)}$$

$$I_{2(g)} + 2Na_2S_2O_{3(aq)} \longrightarrow 2NaI_{(aq)} + Na_2S_4O_{6(aq)} \quad \text{…….(equation 12)}$$

The standardized potassium permanganate serves as titrant in the assay of hydrogen peroxide. Although in the titration is self-indicating, for accurate determination a potentiometric device is used. The ionic reaction involved is as shown (equation 13).

$$H_2O_{2(aq)} \longrightarrow 2H^+_{(aq)} + O_{2(g)} + 2e^- \quad \text{…….(equation 13)}$$

The ionic reaction for potassium permanganate can be represented as shown in equation 14.

$$MnO_4^-{}_{(aq)} + 8H^+_{(aq)} + 5e^- \longrightarrow Mn^{2+}_{(aq)} + 4H_2O_{(l)} \quad \text{…….(equation 14)}$$

The net reaction between potassium permanganate and hydrogen peroxide in the presence of an acidic media is as shown in equation 15.

$$2KMnO_{4(aq)} + 5H_2O_{2(aq)} + 3H_2SO_{4(aq)} \longrightarrow K_2SO_{4(aq)} + 2MnSO_{4(aq)} + 8H_2O_{(aq)} + 5O_{2(g)} \quad \text{…(equation 15)}$$

1.8 Justification

Sodium hypochlorite and hydrogen peroxide are common disinfectants and antiseptics in hospitals, they are used to disinfect equipment, in water treatment and wound cleansing. The use of the

correct concentration of sodium hypochlorite is critical to achieving disinfection. The concentration of sodium hypochlorite was 8000-9000 mg/L with pH 9 was effective against gram negative strains of *Escherichia coli* and *Acinetobacter calcoaceticus*. Inaccurate concentrations may lead to sub-optimal disinfection of equipment and/or subsequently lead to infections while carrying out water treatment. For example, compromises in water treatment may lead to water-borne and food-borne diseases such as cholera, dysentery and typhoid (Save *et al*., 2014). Hence, it is important to assure that the sodium hypochlorite being used is of the required quality standards.

Hospital acquired infection are a major cause of prolonged hospitalization and a factor in the spread of antimicrobial resistance. Proper disinfection and sterilization are an essential part in managing the spread of such infections. The availability of quality disinfectants and antiseptics is essential in the prevention and control of these infections. Substandard medical products in circulation can only be identified through routine post market surveillance.

Factors such as temperature, concentration and time have been shown to affect the amount of available active chlorine in sodium hypochlorite solution. Compromise during the preparation of sodium hypochlorite and storage may thus affect the quality of products (Murat and Beyser, 1995). Sodium hypochlorite is also commonly used as a household bleaching agent. It has been reported that even low concentration of sodium hypochlorite can lead to pulmonary irritation. Therefore to minimize unnecessary exposure assessment of quality is required (Demiralay, 2001).

Care is particularly needed because hydrogen peroxide is commonly used as a mouthwash and wound cleansing agent. High concentrations of hydrogen peroxide have been associated with

gastrointestinal pulmonary irritation (Barbara *et al*., 2004). Hydrogen peroxide easily decomposes to oxygen and water on exposure to air and this requires stabilizing agents such as butylated hydroxyl toluene and butylated hydroxyl anisole. Sometimes, the stabilizers may not be incorporated in the right quantities and as such hydrogen peroxide products may degrade on storage.

Consequently, market surveillance is necessary to ensure that products in the market are suitable for use. According to a review of existing literature, no study has been conducted in Kenya on the quality of hydrogen peroxide and sodium hypochlorite containing products. Therefore, this baseline study will be used to create awareness on the possibility of finding poor quality products and expose the potential risk they might pose to public health.

1.9 Problem statement

The growing resistance of microorganisms is due to microorganism's resistance. A better way of ensuring that the microorganisms are prevent from entering the host is by use of disinfectants and antiseptics in the right concentration and used in the right way. A study by Hani and Adnan in a Jordan hospital indicated that a specific concentration of sodium hypochlorite of 8000-9000 mg/L with pH 9 was effective against gram negative strains of *Escherichia coli* and *Acinetobacter calcoaceticus* with MIC of 850-1000 mg/L for the microorganisms and 4500 mg/L for the spore. Showing how paramount the concentration of the sodium hypochlorite is. Yet no study has been carried out in our country of the quality of sodium hypochlorite in the market.

A study in our neighboring county Tanzania by Kaale *et al* indicated that there was low quality hydrogen peroxide in the market. There is however no data of the same in our county.

2.0 Study questions

1. Do the samples of sodium hypochlorite in Nairobi City County comply with KEBS 1999 requirement for content?
2. Do the samples of hydrogen peroxide in Nairobi City County comply with BP 2017 specification for content?
3. Does the label information, caution, container and closure comply with KEBS and BP 2017 specification?
4. Does the pH of sodium hypochlorite samples comply with KEBS requirement?

2.1 Objectives

2.1.1 General objective

The general objective of this study was to assess the quality of sodium hypochlorite and hydrogen peroxide products available in Nairobi City County using physico-chemical methods.

2.1.2 Specific objectives

The specific objectives of the study were to:

1. Determine the amount of active chlorine in samples of sodium hypochlorite.
2. Determine the amount of hydrogen peroxide in samples of hydrogen peroxide.
3. Assess the label information, cautions, container and closure for compliance with KEBS and BP 2017 specification.
4. Determine the pH of the samples of sodium hypochlorite.
5. Determine the acidity of hydrogen peroxide samples.

CHAPTER TWO

EXPERIMENTAL

2.1 Study area

The study area for the research was the eight administrative sub-counties in Nairobi City County namely; Kasarani, Pumwani, Kibera, Dagoretti, Makadara, Embakasi, Parklands and Westlands. Additionally, the Central Business District was separately included due to the fact that it is centrally placed and is easily accessible. To ensure a representative sampling, samples were collected from retail outlets, pharmacies, wholesalers, supermarkets and pharmaceutical distributors from the eight sub-counties.

2.2 Sampling

2.2.1 Sodium hypochlorite

Samples of sodium hypochlorite detergents were obtained from major supermarkets (26samples) in the sub-counties. Two product categories namely regular bleaches and lemon bleaches were collected. Additionally, samples of sodium hypochlorite used in hospitals as a disinfectant and for water treatment were collected from pharmaceutical distributors (5 samples) and pharmacies (5 samples) respectively. Convenience random sampling was used to collect the samples. Samples were obtained from the eight sub-counties of Nairobi City County and the central business district. Table 2.1 below shows how the samples of sodium hypochlorite were picked from the various sub-counties.

Table 2.1: Distribution of sodium hypochlorite product samples collected from the sub-counties.

Sub counties	Detergents	Sodium hypochlorite used for water treatment	Hospital disinfectant
Kasarani	4	1	1
Parklands and west lands	3	1	
Pumwani	3	1	1
Kibera	3	1	
Nairobi Central business district	4	1	3
Makadara	3		
Embakasi	3		
Dagoretti	3		
Total number of samples	**26**	**5**	**5**

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2.2.2 Hydrogen peroxide

Samples of hydrogen peroxide were obtained from various pharmacies (11 samples), supermarkets (21 samples) and distributor for the laboratory reagent in Nairobi City County. Three different concentrations of hydrogen peroxide products were collected. The samples were picked from the various shopping centers with the sub counties. Table 2.2 below shows how the samples of hydrogen peroxide were picked from the various sub-counties.

Table 2.2: Distribution of hydrogen peroxide product samples collected for the various sub-counties.

Sub-Counties	Detergent	Antiseptic	Laboratory
Kasarani	1	3	
Parklands and West lands	2	2	
Pumwani	1	3	
Kibera	2	3	
Nairobi Central Business District	2	4	1
Makadara	1	2	
Embakasi	1	2	
Dagoretti	1	2	
Total number of samples	**11**	**21**	**1**

2.3 Physico-chemical analysis

2.3.1 Sodium hypochlorite

The appearance of the sodium hypochlorite solution was evaluated. Labeling information required after observation included product name, manufacture, instructions on dilution, batch number, dates of manufacture and expiry if present, volume of sample, concentration of the preparation and caution “use in well ventilated room” as well as “store below 30 °C and in cool dry place”. Clarity of the solution was to be done by observation and pH meter was to be used to determine the pH of the preparation. A titrimetric titration was used for assay and the end point was determine potentiometrically using platinum electrode

2.3.2 Hydrogen peroxide

The appearance of the solution was evaluated and test for identity was to be carried out. Label information required after observation included product name, manufacturer, instructions on dilutions, batch number, dates of manufacture and expiry, volume of sample, concentration of the preparation and cautions "to be stored away from light and in a cool place". Acidity of the test on the sample was also done. Assay was done using titrimetric method with the end-point being determined potentiometrically using a platinum electrode.

2.4 Equipment

2.4.1 Titroprocessor

TitroLine® 6000 (Si Analytics Corp, Mainz city, Germany), with a 3.5 inch-1/4 VGA TFT display with 320×240 pixels equipped with a stirrer connection was employed. A platinum electrode was used for the determination of both sodium hypochlorite and hydrogen peroxide.

2.4.2 Weighing balance

A Shimadzu AUW220 semi–micro analytical electronic weighing balance (S/N: D450012073) (Shimadzu Corp., Kyoto, Japan) unibloc P.A.T 1987 with a sensitivity of ±0.1 mg was used for weighing the various reagents and samples.

2.5 Reagents

2.5.1 Reagents for analysis of sodium hypochlorite

The analytical grade reagents that were used for these experiments include potassium iodide from Oxford Lab Chem. (Thane, India). Glacial acetic acid, sodium thiosulphate, hydrochloric acid,

sodium hydroxide all were from Loba Chemie Pvt. Ltd. (Mumbai, India). The primary standard potassium bromate was from Sigma-Aldrich, Co (Darmstadt, Germany).

2.5.2 Reagents for analysis of hydrogen peroxide

The analytical grade reagents used for assay of hydrogen peroxide namely sulphuric acid, potassium permanganate, sodium thiosulphate were from Loba Chemie Pvt. Ltd. (Mumbai, India). Potassium bromate used as primary standard was from Sigma-Aldrich, Co (Darmstadt, Germany). Sodium hydroxide and 96 % ethanol were from Loba Chemie Pvt. Ltd. (Mumbai, India). While methyl orange solution used as indicator was from Oxford Lab Chem, (Thane, India).

2.5.3 Preparation of reagents

2.5.3.1 For analysis of sodium hypochlorite

Standard 0.1 M sodium thiosulphate was prepared by dissolving 15.4470 g of anhydrous $Na_2S_2O_3$ crystals, and 0.4 g sodium carbonate in freshly prepared distilled water. The resulting solution was made up to 1000 mL of solution with distilled water. Standard 7 M hydrochloric acid was prepared by dissolving 595 mL of concentrated hydrochloric acid and made up to 1000 mL of solution with distilled water.

On the other hand, 0.1 M sodium hydroxide solution was prepared by dissolving 4 g of sodium hydroxide pellets in freshly distilled water to form 1 L of solution. Potassium iodine solution 0.01 M was prepared by dissolving 16.6 g of potassium iodide to make up 100 mL of solution. Standard 2 M glacial acetic acid was prepared by dissolving 114 ml of concentrated glacial acetic acid to make up 1000 mL solution with distilled water. The details of the preparation of reagents are described in the British pharmacopoeia, 2017 (BP, 2017).

2.5.3.2 For the analysis of hydrogen peroxide

Standard 0.02 M potassium permanganate was prepared by dissolving 6.4044 g of the crystals in 2000 mL of distilled water was heated in a water bath for 1 h cooled and filtered. Standard 0.1 M sulphuric acid was prepared by dissolving 54 ml concentrated sulphuric acid to make up 1000 mL of solution with distilled water. Methyl red solution used as indicator was prepared by adding 50 mg methyl red to a mixture of 0.1 mL of 1 M sodium hydroxide and 50 mL of 96 % ethanol and diluted to100 mL with distilled water. Standard 0.1 M sodium thiosulphate was prepared using procedure summarized in section 2.4.3.1

2.5.4 Standardization of 0.1M sodium thiosulphate

To 20 mL of 0.0167 M potassium bromate solution in a 100 ml beaker, 40 mL of distilled water, 10 mL of 0.01 M potassium iodide and 5 mL of 7 M hydrochloric acid were added. The solution was titrated with 0.1 M sodium thiosulphate solution to a potentiometric endpoint. A triplicate titration using a titroprocessor was done. A relative standard deviation of less than 2 % of the triple titration values was used for the analysis. Appendix 1 gives the samples number and titres volumes of potassium bromate used. The factor of 0.1 M sodium thiosulphate versus standardization was calculated using equation I.

$$\text{Factor}=\frac{\text{Amount of Potassium Bromate in 20mL}}{\text{volume of Sodium Thiosulphate}\times\text{Equivalence}}\qquad\text{.....(equation I)}$$

Standardization of titrant was also carried before analysis of sodium hypochlorite and hydrogen peroxide. For analysis of hydrogen peroxide appendix 2 gives the sample number and titres volume of potassium bromate used.

2.5.5 Standardization of 0.02 M potassium permanganate

Standardization of potassium permanganate 0.02 M was carried out as per BP 2017. Briefly, the procedure used was as follows, to 20 ml of 0.02 M potassium permanganate was pipetted into a 50 ml beaker, 2 g of KI and 10 mL of 1 M sulphuric acid was then added and titrated with 0.1 M sodium thiosulphate. The endpoint was determined potentiometrically. Triplicate titrations were conducted. A relative standard deviation of ≥ 2 % of the titration values was considered adequate for this analysis. Results are given in appendix 3.The factor (factor 2) for the standardization of the potassium permanganate was calculated using the formula.

$$\text{Factor 2}=\frac{\text{titre volume of } Na_2S_2O_3 \times \text{Factor 1}}{\text{vol of } KMnO_4} \quad \text{.....(equation II)}$$

2.6 Assay

2.6.1 Assay of active chlorine in sodium hypochlorite

A ten (10) mL aliquot of the sample was pipetted into a 100 mL beaker and mixed with 50 ml distilled water, 1 g of KI and 12.5 mL of 2 M glacial acetic acid. The resultant solution was titrated with 0.1 M $Na_2S_2O_3$. The endpoint was determined potentiometrically using a platinum electrode. Appendix 4 shows an ideal titration curve of the assay of active chlorine in samples of sodium hypochlorite collected.

2.6.2 Assay of Hydrogen Peroxide Preparations

2.6.2.1 Three percent (3%) w/v hydrogen peroxide (BP, 2017)

Ten (10) mL of the sample was diluted with 100 mL of distilled water. To 10 mL of the resulting solution, 20 mL of sulphuric acid R was added. The solution was then titrated with 0.02 M potassium permanganate to a potentiometric endpoint.

2.6.2.2 Six Percent (6 %) w/v hydrogen peroxide (BP, 2017)

Ten (10) mL of the sample was diluted with 100 mL distilled water. To 10 mL of the resulting solution, 20 mL of 1 M sulphuric acid was added. The solution was then titrated with 0.02 M potassium permanganate. The end-point was determined potentiometrically.

2.6.2.3 Thirty Percent (30 %) w/v hydrogen peroxide (BP, 2017)

One (1 mL) of 30 % hydrogen peroxide sample was dissolved in 100 mL distilled water. To 10 mL of the aliquot, 20 mL of sulphuric acid R was added. The solution was then titrated with 0.02 M potassium permanganate. The end-point was determined potentiometrically. Appendix 5 shows an ideal titration curve for the assay of hydrogen peroxide.

2.7 Other physico-chemical parameters of the samples

2.7.1 Determination of pH for sodium hypochlorite samples

The pH was determined by placing the pH meter sensor into a vial containing the sample. A pH value of not less than 9 was specified.

2.7.2 Test for identity of hydrogen peroxide samples

Identification test involved pipetting 0.05 mL of sample into a test tube, followed by addition of 2 mL of 1 M sulphuric acid, 2 mL of ether and 0.05 mL of 0.0257 M potassium chromate solution and color change was observed.

2.7.3 Determination of acidity of hydrogen peroxide

Ten (10) mL of the sample was dissolved in 20 mL of distilled water and 0.25 mL of methyl red solution was added. Not less than 0.05 mL and not more than 1.0 mL of 0.1 M sodium hydroxide was required to change the color of the solution from red to colorless (BP, 2017).

2.8 Calculations and data analysis

2.8.1 Determination of active/available chlorine in sodium hypochlorite samples

The content of active/ available chlorine in the samples was calculated using the formula.

$$\text{Concentration of active/available chlorine} = \frac{Titre\ vol\ of\ sodium\ thiosulphate \times Factor \times equivalence}{sample\ volume} \text{x} 100$$

The equivalence used was 1 mL of 0.1 M sodium thiosulphate was equivalent to 2.784 mg of potassium bromate.

2.8.2 Determination of hydrogen peroxide content

The content of hydrogen peroxide in the samples was calculated using the formula.

$$\%\ \text{content} = \frac{titre\ vol\ of\ KMnO_4 \times Factor \times Equivalence\ x\ Dilution\ factor}{sample\ volume} \text{x} 100$$

Where equivalence of 1 mL 0.02 M potassium permanganate is equivalent to 1.701 mg of hydrogen peroxide or 0.56 mL of oxygen (BP, 2017)

Data analysis was done using Microsoft Excel 2010 (Microsoft Corporation, Washington DC, USA) where the various concentrations, volume and weights were expressed in the tables and calculations were done.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Results

3.1.1 General characteristics of sodium hypochlorite

General characteristics of sodium hypochlorite samples assessed were, the colour of solution, odour of chlorine, miscibility with water, presence of foreign matter and adequacy of labeling (KEBS, 1999). All the samples were slightly yellow in colour, produced characteristic odour of chlorine and were miscible in water. The material used was plastic in nature for both the container and closures. All the samples had the caution to be store below 30 °C in a cool dry place. Additionally, all samples had a pH ≤ 9 as required by KEBS. Table 3.1 shows the results of the samples that did not comply with general characteristics of sodium hypochlorite and packing recommendations

Table 3.1: Non-complying sodium hypochlorite samples on general characteristics and packaging recommendations

Code No	Batch No	Clarity of solution	No foreign matter	Label Details	Caution	MFD	Expiry date
SH5a	6164003150156	x	x	x	✓	11/2017	11/2020
SH5b	6164003150163	✓	✓	x	✓	9/2016	9/2018
SH14	6489	✓	✓	✓	x	12/2017	12/2019
SH18	1611070	✓	✓	✓	x	10/2016	10/2019
SH19a	00580SH	✓	✓	✓	x	6/2017	6/2019
SH19b	005865SH	✓	✓	✓	x	3/2018	2/2020
SH22	1521	✓	✓	✓	x	8/2016	8/2018

Key ✓ complied x did not comply

NB

Caution- Use in a well-ventilated room
Label- concentration of the sample

Sample SH5a failed the clarity test as it had particulate matter. Samples SH5a and SH5b lacked label information on concentration of active chlorine. The absence of dilution would affect the way the solutions were used. Five samples namely (SH14, SH18, SH19a, SH19b and SH22) didn't have the caution to be used "in a well-ventilated area". This has dire consequence because prolonged exposure to chlorine has many untoward effects such as, affecting the pulmonary functions (Demiralay, 2001). Appendix 6 shows the general characteristics of all the sample of sodium hypochlorite.

3.1.2 General characteristics of hydrogen peroxide

All the thirty three samples of hydrogen peroxide analyzed were colourless solution and did not contain foreign particles. All samples of detergent hydrogen peroxide (3 % w/v H_2O_2) and antiseptic hydrogen peroxide (6 % w/v H_2O_2) were found to be labeled with information on the product name, batch number, net volume, manufacturing and expiry dates, name and address of the manufacturer. Additionally, the container and closures were all plastics in nature. Table 3.2 shows the samples that failed the various test carried out and also lacked full label information.

Table 3.2: Non-complying hydrogen peroxide samples on identity test, label information, caution and acidity test.

No	Batch no	Identity	Label details	Caution	Acidity	MFD and Exipry date
No label claim						
HP3a	6164003150156	x	x	x	x	9/2016-9/2019
HP3b	6164003150163	✓	x	x	✓	5/2017-5/2020
3 % w/v Hydrogen peroxide samples						
HP5a	0032	x	✓	✓	✓	7/2017 8/2020
HP5b	0035	✓	✓	✓	x	4/2017-14/2020
6 % w/v Hydrogen peroxide samples						
HP8a	23662A	✓	✓	x	✓	6/2016-6/2020
HP8b	36322A	✓	✓	x	✓	9/2017-9/2020
HP9a	878	✓	✓	x	✓	5/2017-10/2018
HP9b	990	✓	✓	x	✓	6/2017-11/2019
HP10a	331170DHP	✓	✓	x	x	5/2017-5/2019
HP10b	24517DHP	✓	✓	x	x	11/2017-11/2019
HP11a	24517FHP	✓	✓	✓	x	11/2017-11/2019
HP11b	32317FHP	✓	✓	✓	x	7/2017-7/2019
HP11c	32317FHP	✓	✓	✓	x	6/2017-6/2019
HP11d	51317FHP	✓	✓	✓	x	3/2017-3/2019
HP12a	410717	✓	✓	✓	x	7/2017-7/2020
HP12b	410117	✓	✓	✓	x	2/2017-2/2020
HP13a	H0308	✓	✓	x	x	5/2017-4/2020
HP13b	D0244	✓	✓	x	x	3/2017-2/2020
HP13c	D0248	✓	✓	x	x	3/2017-2/2020
HP14	01303H	✓	✓	x	x	3/2017-2/2020
HP15	170835	✓	✓	✓	x	4/2017-4/2020
30 % w/v hydrogen peroxide samples						
HP18	HP8801	✓	x	x	✓	2/2016-2//2021

Key ✓ complied x did not comply

NB
Label details -concentration and manufacturers name
Caution- to be stored way from light and in a cool place

Samples HP3a and HP5a were found not to meet specifications for identity. Samples HP3a and HP3b did not contain label information on the concentration of hydrogen peroxide.

Twelve samples (HP3a, HP3b, HP8a, HP8b, HP9a, HP9b, HP10a, HP10b, HP13a, HP13b, HP13c and HP14) did not contain a caution on the effects of heat and sunlight on the quality of hydrogen peroxide that usually states "to be stored away from light and in a cool place" (BP, 2017).

Sample HP18 (30 % w/v hydrogen peroxide), lacked details of the manufacturer and was packed in a transparent container against the recommendation of British Pharmacopeia 2017 which say light protected container with no stabilizer should not be stored at temperature above 15 °C. Hence, the sample would be affected by sunlight. Fifteen samples (HP3a, HP5b, HP10a, HP10b, HP11a, HP11b, HP11c, HP11d, HP12a, HP12b, HP13a, HP13b, HP13c, HP14 and HP15) failed the acidity test. Low acidity affects the concentration of hydrogen peroxide in the solution (BP, 2017). Appendix 7 shows the results of the general characteristics of all the samples of hydrogen peroxide.

3.2.3 Active chlorine in sodium hypochlorite samples

Analysis of content of samples of sodium hypochlorite was evaluated for the products. Table 3.3 shows the results of amount of active chlorine in sample of sodium hypochlorite.

Table 3.3: Content of active chlorine in samples of sodium hypochlorite used as bleaching detergents

Code No	Batch No	Percent active chlorine(% w/v) Average n=3 ± SD	% RSD	Label claim	% label claim
SH1a	BR 137	2.64±0.01	0.42	3.5	75.4
SH1b	BR 048	3.18±0.02	0.72	3.5	90.9
SH2a	CP185H1208DS/7F	2.79±0.04	1.36	4.06	68.7
SH2b	CR090N0820D8/8F	2.78±0.00	0.03	4.06	68.5
SH3a	JR2597Z0423DS/7F	3.22±0.04	1.28	3.85	83.6
SH3b	JR2650Z0627DS/2F	2.89±0.03	1.10	3.85	75.1
SH4a	0039	2.79±0.02	0.64	3.5	79.7
SH4b	0010	1.97±0.01	0.65	3.5	56.3
SH4c	0009	2.17±0.00	0.18	3.5	62.0
SH5a	6164003150156	1.18±0.01	0.47	N/A	N/A
SH5b	6164003150163	0.87±0.01	1.21	N/A	N/A
SH6a	IL2556/832Z0315	2.96±0.01	0.39	3.85	76.9
SH6b	IL26595/870208R5 DS/2F	3.33±0.04	1.17	3.85	86.5
SH7a	BLE 124	2.50±0.02	0.85	3.5	71.4
SH7b	BLE 229	2.34±0.03	1.27	3.5	66.9
SH8a	Cl084No803DS/9D	2.74±0.03	1.02	4.06	67.5
SH8b	Cl098Y0921DS/9F	3.01±0.04	1.32	4.06	74.1
SH9a	0031	2.25±0.01	0.47	3.5	64.3
SH9b	903010305063	3.10±0.02	0.76	3.5	88.6
SH10a	CF089N0817DS/9F	2.87±0.05	1.70	4.06	70.7
SH10b	CF102N10257DS/8F	2.98±0.01	0.30	4.06	73.4
SH11	2909171	2.22±0.03	1.29	3.8	58.4
SH12	2909177	1.68±0.01	0.67	3.8	44.2
SH13	1311171407	2.47±0.05	1.93	3.5	70.6
SH14	6489	1.41±0.01	0.67	3.5	40.3
SH15	BR 00018/U	0.40±0.00	0.06	3.85	10.4

Twenty out of the twenty six samples (77 %) analyzed met the requirement of a bleaching agent according to KEBS specification of minimum ≤ 2 % w/v active chlorine. Samples SH5a, SH5b, SH12, SH14 and SH15 had less than 2 % w/v of active chlorine with the values being 1.2 % w/v,

0.9 % w/v, 1.7 % w/v, 1.4 % w/v and 0.4 % w/v respectively. All the 26 samples of sodium hypochlorite detergent failed on label claim content. The percentage label claim of the samples ranged between 56.3 % and 90.9 % against the recommend range of 95 % - 110 %. However, two samples, SH5a and SH5b were not calculated since manufacture did not provide the label claim. A similar research to evaluate amount of active chlorine released and pH of sodium hypochlorite of two bleaching agents, Brilux® and Qboa® indicate that the two samples had concentrations of 2.43 % w/v and 2.45 % w/v against a label claim of 2.0 - 2.5 % w/v of chlorine (Cristhiany *et al*., 2014). The analysis is in agreement with the current research, with values being within the range of KEBS apart from the five samples. Table 3.4 below shows results of percentage of active chlorine in samples of sodium hypochlorite used in water treatment.

Table 3.4: Percentage content of active chlorine in samples of sodium hypochlorite used in water treatment with a label claim of 1.2 % w/v

Code No	Batch No	Percent active chlorine(% w/v) Average n=3 ± SD	% RSD	% Label Claim
SH16a	31	1.26±0.01	0.79	105.0
SH16b	23	1.27±0.00	0.35	105.8
SH17a	A028	1.13±0.01	1.02	94.2
SH17b	A037	1.11±0.01	0.60	92.5
SH18	1611070	1.00±0.00	0.25	83.3

All samples (n=5) of sodium hypochlorite used for water treatment met label claim requirements of 0.9 % w/v -1.1 % w/v. The values ranging between 1.0 and 1.3 % w/v corresponding to 83.3 % and 108.3 % of the label claim. All 5 samples met BP 2017 specification of content of active chlorine (0.9-1.1 % w/v). Research carried out in Malawi 330 (n=349) households used water Guard (sodium hypochlorite) in water treatment. Indicated that concentration is paramount for effectiveness against *Escherichia coli* contamination of water (Save *et al*., 2014). Research carried

out on viability of commercially available bleach for water treatment in developing countries, Kenya included Indicated that all the five samples used in the study for the case of Kenya had a different amounts of sodium hypochlorite with the one advertised in the container. The analyzed concentration were 1.5 %, 2.7 %, 3.4 %, 3.6 % and 4.4 % w/v of active chlorine while those advertised on the container were 1.0 %, 3.0 %, 3.5 %, 3.5 % and 5.2 % w/v of active chlorine respectively (Danieles, 2009). The results are in agreement with minimal difference in amount of active chlorine with the current study. Table 3.5 shows the percentage of active chlorine in samples of sodium hypochlorite used for hospital disinfection.

Table 3.5: Percentage content of active chlorine in samples of sodium hypochlorite used for hospital disinfection with a label claim of 5 % w/v

Code No	Batch No	Percent active chlorine(% w/v) Average n=3 ± SD	% RSD	% label claim
SH19a	00580SH	2.76±0.04	1.77	55.2
SH19b	005865SH	3.39±0.06	1.90	67.8
SH20	5107	2.31±0.05	1.96	46.2
SH21	44017FSH	1.03±0.05	1.40	20.6
SH22	1521	2.50±0.07	0.75	50.0

All five samples used as hospitals disinfectants failed the test on active chlorine present. The content of active chlorine ranged between 1.0 % w/v and 3.4 % w/v against a specification of between 5 % w/v -7 % w/v of active chlorine. The values corresponding to 20.6 % and 67.8 % of the label claim. Research carried out reported that a significant amount of bacteria were removed by sodium hypochlorite in hospital in North Jordan. The concentration of sodium hypochlorite was 8000-9000 mg/L with pH 9 was effective against gram negative strains of *Escherichia coli* and *Acinetobacter calcoaceticus* with MIC of 850-1000 mg/L for the microorganisms and 4500 mg/L for the spore (Hani and Adnan, 2009). Hence, if the concentration is low then some

microorganisms would not be affected by sodium hypochlorite. Research to probe the effect of concentration, storage conditions and pH illustrated that a higher rate of degradation occurred to samples stored at 24 °C than those at 4 °C over a period of time. The results are in agreement with the high degradation of the samples reason being the samples were storage at room temperature in this case between 15 °C and 25 °C.

3.2.2 Assay of hydrogen peroxide present

Table 3.6 below shows the hydrogen peroxide content in samples of detergents.

Table 3 6: Percentage content of hydrogen peroxide in detergent samples

Code No	Batch no	Percent active H_2O_2 (% w/v) Average n=3 ± SD	% RSD	% label claim
HP1a	CO39	2.61±0.01	0.23	87.1
HP1b	CO52	2.96±0.06	1.97	98.7
HP2a	JC15282z0803ds/1f	3.38±0.07	1.96	116.3
HP2b	JC15612z1311d9/1f	3.30±0.07	1.97	112.7
HP2c	JC1561z0511d8/9f	3.26±0.01	0.43	108.8
HP3a	6164003150156	0.89±0.00	0.44	N/A
HP3b	6164003150163	1.24±0.00	0.09	N/A
HP4	2909171	3.44±0.02	0.45	114.7
HP5a	0032	14.96±0.00	0.01	491.6
HP5b	0035	14.17±0.19	1.32	481.4
HP6	DC003	3.87±0.07	1.71	127.5

Out of the eleven samples of hydrogen peroxide detergents analyzed 6 (55 %) of the samples met percentage content according to BP 2017 specification of 2.5- 3.5 % w/v of hydrogen peroxide. Three samples (HP5a, HP5b and HP6) had very high content of hydrogen peroxide corresponding to 3.9 % w/v 14.2 % w/v and 15.0 % w/v hydrogen peroxide and with label claim of 127.5 %, 481.4 % and 491.6 % respectively. Samples HP3a and HP3b had very low concentration of 0.9 % w/v and 1.2 % w/v respectively of the required hydrogen peroxide content. Manufacturer did not

state the content of hydrogen peroxide for samples HP3a and HP3b hence percentage label claim content would not be calculated.

Table 3.7 below shows the assay results for samples antiseptic hydrogen peroxide

Table 3.7: Percentage content of hydrogen peroxide in antiseptics hydrogen peroxide

Code No	Batch no	Percent active H_2O_2 (% w/v) Average n=3 ± SD	% RSD	% label claim
HP7b	48706	3.51±0.03	0.73	58.6
HP8a	23662A	4.52±0.02	0.45	75.4
HP8b	36322A	2.39±0.01	0.23	39.8
HP9a	0878	3.41±0.02	0.65	56.9
HP9b	0990	3.42±0.00	0.02	57.1
HP10a	331170 HP	3.23±0.00	0.10	53.9
HP10b	24517DHP	3.23±0.01	0.31	53.8
HP11a	24517FHP	3.48±0.00	0.08	58.0
HP11b	32317FHP	3.47±0.03	0.88	57.8
HP11c	32317FHP	3.37±0.03	1.03	56.2
HP11d	51317 FHP	3.02±0.01	0.19	50.3
HP12a	410717	3.75±0.04	0.96	62.5
HP12b	410117	3.77±0.04	1.06	62.8
HP13a	H0308	3.43±0.02	0.67	57.1
HP13b	D0244	3.53±0.03	0.86	58.9
HP13c	D0248	3.57±0.02	0.43	59.5
HP14	01303H	3.44±0.01	0.17	57.4
HP15	170835	2.64±0.01	0.22	44.0
HP16	170835KAM	3.78±0.01	0.15	63.0
HP17	8456	4.69±0.03	0.69	78.1

None of the twenty one samples antiseptic hydrogen peroxide met BP 2017 specifications for content (5-7 % w/v of hydrogen peroxide). The content of hydrogen peroxide ranged between 2.39 ± 0.01% w/v and 4.69 ±0.03 % w/v of hydrogen peroxide corresponding to 39.8 % and 78.1 % label claim content. Two samples (HP8b and HP15) had less than 50 % of the label claim content with 39.8 % and 44.0 % respectively. Sample HP17 was the highest percentage label claim of 78.1 %. The beneficial effect of hydrogen peroxide on sub gingival flora on patient with periodontitis

would significantly reduce if the concentration was low (Wayne *et al*., 1990). A similar quality assessment carried out in Tanzania on hydrogen peroxide antiseptics obtained from the medical stores, 26 % of samples (n=70) failed to comply with the BP (2005) specification for assay and labeling (Kaale *et al*., 2007). The results are not agreement with the current study where all the samples failed to pass the test.

Sample HP18 (which was a laboratory sample) had hydrogen peroxide content of 13.6 % w/v out of the required 30 % w/v percentage content according to the label claim. This was just 45.2 % of the label claim. From the observation carried out in the general test, the sample was packed in a transparent container against the recommendations of BP 2017 which specifies that the sample should be protected from light. This would be the reason the sample failed

CHAPTER FOUR

CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

4.1.1 Hydrogen peroxide

The study evaluated the quality of sodium hypochlorite and hydrogen peroxide products in Nairobi County. Assay results indicated that five samples (45 %) of detergent hydrogen peroxide did not met percentage content specification of BP 2017. All the samples (n=21) of antiseptic hydrogen peroxide did not comply with BP 2017. The laboratory reagent 30 % w/v hydrogen peroxide failed to comply with BP 2017. Two samples (0.06 %) did not comply with BP 2017 specifications on identity of hydrogen peroxide. Three samples (0.09 %) did not comply with BP 2017 specification label information. Fifteen samples (45 %) did not comply with BP 2017 specifications on acidity. Thirteen samples (0.4 %) did not comply with KEBS on label information. All the thirty three samples met KEBS requirement for containers and closures.

The overall analysis and assessments indicated that 11 samples (33 %) met all the KEB and BP 2017 specifications, identity, label information, acidity, container and closures.

4.1.2 Sodium hypochlorite

Assay results indicated that six samples (23 %) of the bleaching agent analyzed did not meet the requirement for KEBS (minimum $\leq$ 2 % w/v) of active chlorine. The entire sample (n=5) sodium hypochlorite used for treatment of water met label claim requirements and assay content. All

samples (n=5) of sodium hypochlorite used as disinfectants failed on assay for content of active chlorine. Although they met the KEBS requirement, only one sample was outside the limit of a minimum ≤ 2 % w/v of active chlorine required with the value being 1.03 % w/v of active chlorine. One sample (3 %) failed the clarity test of solution and presents of presents of foreign matter, two samples (6 %) failed on the label details while five samples (13 %) did not comply with KEBS requirements on the caution.

4.2. Recommendations

- There is need for manufacturers to adhere to labeling and packing specifications. This will ultimately ensure sodium hypochlorite and/or hydrogen peroxide quality is maintained.
- There is need for post market surveillance on quality of the products continuously by both Pharmacy and Poisons Board and KEBS.
- There is need for further investigations by increasing the sample size for both sodium hypochlorite and hydrogen peroxides to certain the true position in the market.

4.3 Study limitations

The storage temperature of the samples at the point of distribution/ sale was not recorded hence this would be a limiting factor on the quality. Some tests, for example characteristic odour of chlorine would not be ascertained because the necessary olformeter equipment was not available. The sample size in this case was limited due to time and budgetary constraints.

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APPENDICES

Appendix 1**:** Standardization of 0.1M sodium thiosulphate for analysis of sodium hypochlorite

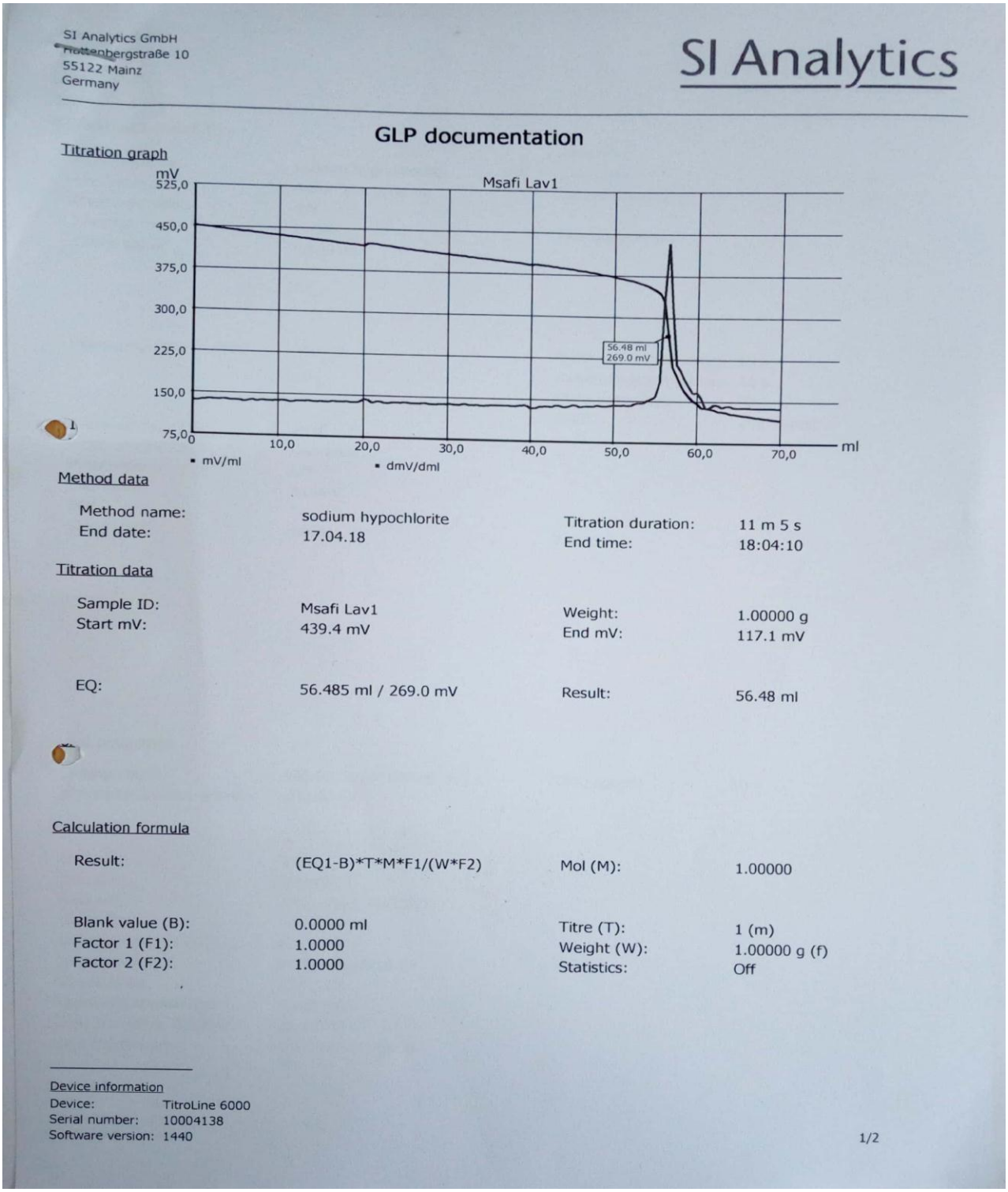
Sample No	**Titre volume of $Na_2S_2O_3$**	**Average**
Sample I	21.39	
	20.73	
	20.54	20.89
Sample II	19.89	
	19.81	
	19.73	19.81
Sample III	19.75	
	19.93	
	19.82	19.83
Sample IV	19.62	
	19.63	
	19.50	19.58
Sample V	20.14	
	19.86	
	20.07	20.02
Sample VI	18.68	
	18.22	
	18.20	18.37
Sample VII	20.24	
	20.26	
	20.25	20.25
Sample VIII	19.29	
	19.24	
	19.56	19.36

Appendix 2: Standardization of 0.1 sodium thiosulphate for the analysis of hydrogen peroxide

Sample No	Titre volume of $Na_2S_2O_3$	Average
Sample I	19.29	19.36
	19.24	
	19.56	
Sample II	21.58	21.40
	21.23	
	21.39	
Sample III	21.36	21.48
	21.27	
	21.80	
Sample IV	20.19	20.03
	19.98	
	19.91	

Appendix 3: **Standardization of 0.02 M potassium permanganate**

Sample No	Titre volume of $Na_2S_2O_3$	Average
Sample I	20.74	20.90
	21.19	
	20.77	
Sample II	21.18	21.06
	20.77	
	21.23	
Sample III	23.29	23.26
	23.18	
	23.30	
Sample IV	21.71	21.40
	21.32	
	21.16	



Appendix 4

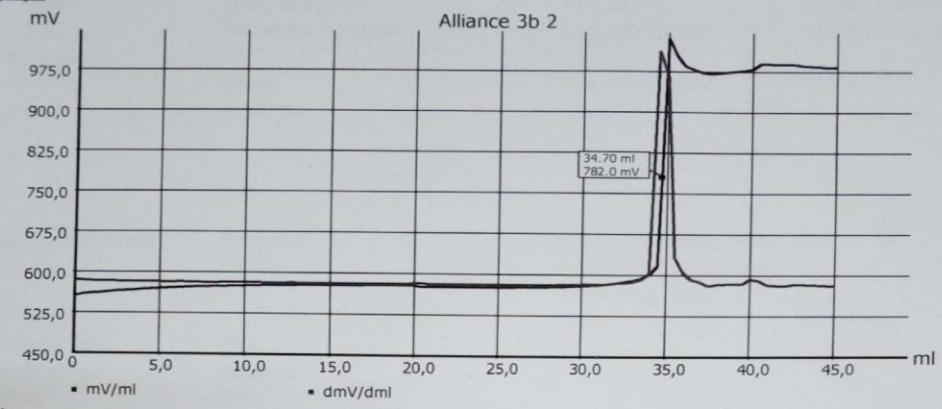
Appendix 4: Typical titration curve for the assay of active chlorine in sodium hypochlorite samples

SI Analytics GmbH
Hattenbergstraße 10
55122 Mainz
Germany

SI Analytics

GLP documentation

Titration graph



Method data

Method name:	H2O2 sample	Titration duration:	6 m 39 s
End date:	30.04.18	End time:	20:47:49

Titration data

Sample ID:	Alliance 3b 2	Weight:	1.00000 g
Start mV:	536.9 mV	End mV:	983.6 mV
EQ:	34.698 ml / 782.0 mV	Titer:	34.6980 ml
Mean value:	---	RSD:	---

Calculation formula

Titer:	(EQ1-B)*T*M*F1/(W*F2) -> WA	Mol (M):	1.00000
Blank value (B):	0.0000 ml	Titre (T):	1 (m)
Factor 1 (F1):	1.0000	Weight (W):	1.00000 g (f)
Factor 2 (F2):	1.0000	Statistics:	1 from 3

Device information

Device:	TitroLine 6000
Serial number:	10004138
Software version:	1440

1/2

Appendix 5: Typical titration curve for assay of hydrogen peroxide

Appendix 6: General characteristics, label, caution, pH, manufacturing and expire date of bleaching sodium hypochlorite samples

Code No	Batch No	Chlorine oduor	Clarity of solution	Colour Pale yellow/ White	Miscible to water	Label Details	Caution	Container and closure (plastic)	pH	MFD and Expiry date
SH1a.	BR 137	✓	✓	✓	✓	✓	✓	✓	12.54	7/2017-7/2019
SH1b.	BR 048	✓	✓	✓	✓	✓	✓	✓	12.52	2/2018-2/2020
SH2a.	CP185H1208 DS/7F	✓	✓	✓	✓	✓	✓	✓	12.77	12/2016-12/2018
SH2b.	CR090N0820 D8/8F	✓	✓	✓	✓	✓	✓	✓	12.67	8/2016-8/2018
SH3a.	JR2597Z042 3 DS/7F	✓	✓	✓	✓	✓	✓	✓	12.78	4/2017-4/2019
SH3b.	JR2650Z062 7 DS/2F	✓	✓	✓	✓	✓	✓	✓	12.77	6/2017-6/2019
SH4a.	0039	✓	✓	✓	✓	✓	✓	✓	12.03	11/2017-12/2019

Key ✓ complied x did not comply

General characteristics, label, caution, pH, manufacturing and expire date of bleaching sodium hypochlorite samples

Code No	Batch No	Chlorine oduor	Clear solution Pale yellow	No foreign matter	Miscible to water	Label details	Caution	Container and closure (plastic)	pH	MFD and Expiry date
SH4b.	0010	✓	✓	✓	✓	✓	✓	✓	12.49	6/2016-6/2018
SH4c.	0009	✓	✓	✓	✓	✓	✓	✓	12.71	8/2016-8/2018
SH5a.	6164003150156	✓	x	x	✓	x	x	✓	12.74	11/2017-11/2020
SH5b.	6164003150163	✓	✓	✓	✓	x	x	✓	12.32	9/2016-9/2018
SH6a.	IL2556/832Z0315	✓	✓	✓	✓	✓	✓	✓	12.74	3/2017-3/2019
SH6b.	IL26595/870208R5 DS/2F	✓	✓	✓	✓	✓	✓	✓	12.67	8/2017-8/2019
SH7a.	BLE 124	✓	✓	✓	✓	✓	✓	✓	12.62	6/2017-6/2019
SH7b.	BLE 229	✓	✓	✓	✓	✓	✓	✓	12.63	11/2017-11/2019
SH8a.	Cl084NO803 DS/9D	✓	✓	✓	✓	✓	✓	✓	12.72	8/2016-8/2018

Key ✓ complied x did not comply

General characteristics, label, caution, pH, manufacturing and expire date of bleaching sodium hypochlorite samples

Code No	Batch No	Chlorine oduor	Clear solution and Pale yellow	No foreign matter	Miscible to water	Label Details	caution	Container and closure (plastic)	pH	MFD and Expiry date
SH8b.	Cl098Y0921 DS/9F	✓	✓	✓	✓	✓	✓	✓	12.59	9/2016-9/2019
SH9a.	0031	✓	✓	✓	✓	✓	✓	✓	9.45	4/2017-4/2020
SH9b.	903010305063	✓	✓	✓	✓	✓	✓	✓	11.58	6/2018-3/2021
SH10a.	CF089N0817 DS/F	✓	✓	✓	✓	✓	✓	✓	12.90	8/2016-8/2018
SH10b.	CF089N0877 DS/F	✓	✓	✓	✓	✓	✓	✓	12.76	10/2016-10/2018
SH11.	2909171	✓	✓	✓	✓	✓	✓	✓	12.71	1/2018-1/2020
SH12.	2909177	✓	✓	✓	✓	✓	✓	✓	12.71	10/2018-10/2020
SH13.	1311171407	✓	✓	✓	✓	✓	✓	✓	12.57	11/2017-11/2019
SH14.	6489	✓	✓	✓	✓	✓	x	✓	12.93	12/2017-12/2019
SH15.	BR 00018/U	✓	✓	✓	✓	✓	✓	✓	12.71	9/2017-9/2020

Key ✓ complied x did not comply

General characteristics, label, caution, pH, manufacturing and expire date of water treatment sodium hypochlorite samples

Code No	Batch No	Chlorine oduor	Clear solution and Pale yellow	No foreign matter	Miscible to water	Label Details	Caution	Container and closure (plastic)	pH	MFD and Expiry date
SH16a	31	✓	✓	✓	✓	✓	✓	✓	12.32	10/2017-10/2019
SH16b	23	✓	✓	✓	✓	✓	✓	✓	12.32	9/2017-9/2019
SH17a	A028	✓	✓	✓	✓	✓	✓	✓	12.36	9/2017-9/2019
SH17b	A037	✓	✓	✓	✓	✓	✓	✓	12.37	11/2017-11/2019
SH18	1611070	✓	✓	✓	✓	✓	x	✓	12.07	10/2016-10/2019

Key ✓ complied x did not comply

General characteristics, label, caution, pH, manufacturing and expire date of disinfectant sodium hypochlorite samples

Code No	Batch No	Chlorine oduor	Clear solution Pale yellow	No foreign matter	Miscible to water	Label Details	Caution	Container and closure (plastic)	pH	MFD and Expiry date
SH19a.	00580SH	✓	✓	✓	✓	✓	x	✓	12.82	12/2017-11/2019
SH19b.	005865SH	✓	✓	✓	✓	✓	x	✓	12.89	3/2018-2/2020
SH20.	05107	✓	✓	✓	✓	✓	✓	✓	11.66	8/2017-1/2019
SH21.	44017FSH	✓	✓	✓	✓	✓	✓	✓	10.65	10/2017-10/2019
SH22.	1521	✓	✓	✓	✓	✓	x	✓	13.26	12/2017-5/2019

Key ✓ complied x did not comply

Appendix 7: Characteristics in general, label, caution, acidity, manufacturing and expire date of 3% 6 % and 30 % hydrogen peroxide

No	Batch no	Identity	Clear solution	No foreign matter	Label Details	Caution	Acidity	Container and Closure (plastic)	MFD and Expiry date
HP1a	CO39	✓	✓	✓	✓	✓	✓	✓	8/2017-8/2019
HP1b	CO52	✓	✓	✓	✓	✓	✓	✓	11/2017-11/2019
HP2a	JC15282z0803ds/1f	✓	✓	✓	✓	✓	✓	✓	8/2017-8/2019
HP2b	JC15612z1311d9/1f	✓	✓	✓	✓	✓	✓	✓	5/2017-5/2019
HP2c	JC1561z0511d8/9f	✓	✓	✓	✓	✓	✓	✓	5/2017-5/2019
HP3a	6164003150156	x	✓	✓	x	x	x	✓	9/2016-9/2019
HP3b	616400150163	✓	✓	✓	x	x	✓	✓	5/2017-5/2020
HP4	2909171	✓	✓	✓	✓	✓	✓	✓	9/2017-9/2019
HP5a	0032	x	✓	✓	✓	✓	✓	✓	7/2017 8/2020
HP5b	0035	✓	✓	✓	✓	✓	x	✓	4/2017-14/2020
HP6	DC003	✓	✓	✓	✓	✓	✓	✓	9/2017-7/2020

Key ✓ complied x did not comply

Characteristics in general, label, caution, acidity, manufacturing and expire date of 6% hydrogen peroxide

No	Batch no	Identity	Clear solution	No foreign matter	Label Details	Caution	Acidity	Containers and closure (plastic)	MFD and Expiry date
HP7a	21306	✓	✓	✓	✓	✓	✓	✓	11/2016-4/2018
HP7b	48706	✓	✓	✓	✓	✓	✓	✓	3/2017-7/2018
HP8a	23662A	✓	✓	✓	✓	x	✓	✓	6/2016-6/2020
HP8b	36322A	✓	✓	✓	✓	x	✓	✓	9/2017-9/2020
HP9a	0878	✓	✓	✓	✓	x	✓	✓	5/2017-10/2018
HP9b	0990	✓	✓	✓	✓	x	✓	✓	6/2017-11/2019
HP10a	331170 HP	✓	✓	✓	✓	x	x	✓	5/2017-5/2019
HP10b	24517DHP	✓	✓	✓	✓	x	x	✓	11/2017-11/2019
HP11a	24517FHP	✓	✓	✓	✓	✓	x	✓	11/2017-11/2019
HP11b	32317FHP	✓	✓	✓	✓	✓	x	✓	7/2017-7/2019
HP11c	22817FHP	✓	✓	✓	✓	✓	x	✓	6/2017-6/2019
HP11d	51317 FHP	✓	✓	✓	✓	✓	x	✓	3/2017-3/2019

Characteristics in general, label, caution, acidity, manufacturing and expire date of 6 % and 30 % hydrogen peroxide

No	Batch no	Identity	Clear solution	No foreign matter	Label	Caution	Acidity	Containers and closure (plastic)	MFD and Expiry date
HP12a	410717	✓	✓	✓	✓	✓	x	✓	7/2017-7/2020
HP12b	410117	✓	✓	✓	✓	✓	x	✓	2/2017-2/2020
HP13a	H0308	✓	✓	✓	✓	x	x	✓	5/2017-4/2020
HP13b	D0244	✓	✓	✓	✓	x	x	✓	3/2017-2/2020
HP13c	D0248	✓	✓	✓	✓	x	x	✓	3/2017-2/2020
HP14	01303H	✓	✓	✓	✓	x	x	✓	3/2017-2/2020
HP15	170835	✓	✓	✓	✓	✓	x	✓	4/2017-4/2020
HP16	170835K AM	✓	✓	✓	✓	✓	✓	✓	10/2017-10/2019
HP17	8456	✓	✓	✓	✓	✓	✓	✓	1/2017-1/2019
HP18	30% H_2O_2	✓	✓	✓	x	x	✓	✓	2/2016-2//2021

Key ✓ complied x did not comply