LEVELS OF MERCURY AND HYDROQUINONE IN SOME SKIN-LIGHTENING CREAMS AND THEIR POTENTIAL RISK TO THE HEALTH OF CONSUMERS IN GHANA.

By

Doreen Amponsah (Mrs.) BSc Chemistry

A Thesis submitted to the Department of Chemistry,

Kwame Nkrumah University of Science and Technology

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Faculty of Physical Science

College of Science

September, 2010

DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

DOREEN AMPONSAH (MRS)	……..	…….
20066614	SIGNATURE	DATE
CERTIFIED BY:		
DR R. B. VOEGBORLO	……	……
SUPERVISOR	SIGNATURE	DATE
CERTIFIED BY:		
MR G. K TUANI	……	…….....
SUPERVISOR	SIGNATURE	DATE
DR J. A. M AWUDZA	……	……..
HEAD OF DEPARTMENT	SIGNATURE	DATE

ABSTRACT

In this study, fifty (50) samples of Skin-lightening creams were analyzed for total mercury by Cold Vapour Atomic Absorption Spectrophotometry using an automatic mercury analyzer and for total hydroquinone by High Performance Liquid Chromatography. The concentration of mercury in the creams ranged from below 0.001 to 0.549 µg/g and that of hydroquinone ranged from below 0.001 to 3.45 %. All the creams sampled for mercury had concentrations less than the US Food and Drug Administration's acceptable limit of 1µg/g. The low concentrations of mercury detected in the cream samples analyzed therefore do not pose any potential risk to consumers. Eight percent (8%) of cream samples analyzed contained hydroquinone levels higher than the recommended WHO limit of two (2%). The use of such creams may lead to health hazards. Therefore, it is recommended that all skin-lightening creams should be checked for hydroquinone levels before marketing.

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DEDICATION

I dedicate this work to my mother Mrs. Elizabeth Osei for her love and care.

ACKNOWLEDGEMENT

I am indebted to God with whom all things are made possible. I also owe a debt of gratitude to my supervisors Dr R. B Voegborlo and Mr. G. K Tuani for their patience in guiding my thoughts. I thank them for taking time out of their tight schedule to direct and advise me any time I called on them. I am also indebted to Dr. W. A Jonfia-Essien and Mr. Benjamin Buckman Dove of Quality Control Company Limited (COCOBOD) for their help and support.

Special gratitude goes to Dr Henry Nagai of Family Health International and Mr. Eric Selorm Agorku of KNUST Chemistry Department. They were a real source of inspiration towards this project. I will also like to acknowledge Mr. Samuel Opoku Amponsah of Vodafone Ghana for his support and encouragement.

CHAPTER ONE

1. INTRODUCTION.

Many Ghanaian women love to keep their skin toned and beautiful but unfortunately most of them end up indulging in skin care products that bleach the skin and eventually pose potential risk to their health. Most of these bleaching products contain different kinds of chemicals that may be harmful and affect the health of women. Examples of chemicals in these products include mercury, hydroquinone, Kojic acid, Kojic acid dipalmitate, Azleic acid, Arbutin, Bearberry, Vitamin C, Magnesium ascorbyl phosphate, Calcium ascorbate, and L-ascorbic acid. The most commonly used lightening agents are known to contain mercury and hydroquinone. The skin colour is determined by the amount of melanin produced. Looking at the five hundred years of human history, humans have constantly labeled and stereotyped each other on the basis of skin colour. In most African and Asian communities, fairness is branded as beauty, grace and high social status. The darker skin is seen as being of lowest social value whereas the lighter skin is regarded as being of highest social value. This perception encourages most women to indulge in skin care products that lighten the skin.

Mercury is a very toxic element but its use has been found in many areas including use for religious, cultural and ritualistic purposes. Mercury has also been used in some traditional medicines (such as certain Traditional Asian remedies), as a preservative in some vaccines and other pharmaceuticals and in skin-lightening creams and soaps (Cole *et al*., 1930; Turk and

Baker, 1968). The application of mercurial preparations to the skin has been practiced for centuries. (Cole *et al.,* 1930; Turk and Baker, 1968). Mercury is a highly volatile element with a long atmospheric half-life. As a result of these physical properties, it is ubiquitous in the environment and exposure is not an isolated concern but rather a global threat to human health. In recent years, research has revealed that even chronic exposure to very low concentrations of exposure has the ability to cause long-lasting neurological and kidney impairment (Hutson *et al,* 1999). Mercury-based bleaching creams contain ammoniated mercury or mercrous chloride as a bleaching agent. Some of these creams may contain up to more than 2-5% mercury that will be harmful to health, thus resulting in mercury poisoning.

Mercury containing cosmetic preparations has been represented for many years as skin bleaching agents (Al-Saleh and Al-Doush, 1997). Toning creams containing mercury in the form of inorganic mercury are mainly used by dark skinned people mostly in developing countries notably in Africa and Asia to lighten their skin tone. This has been reported to be probably due to the inhibition of the production of the skin pigment melanin (Marzulli and Brown, 1972; Barr *et al.,* 1973; Bourgeois *et al.,* 1986; Katzung, 1995). Mercury containing skin bleaching preparations have resulted in the accumulation of mercury in the body after absorption through the skin especially in the kidney where it mainly accumulates in the tubular region, giving rise to the occurrence of severe reactions (Marzulli and Brown, 1972; Barr *et al*., 1973; Berlin, 1979; Bourgeois *et al*., 1986). The United States Food and Drugs Administration (US FDA) in 1992 established the maximum acceptable level of mercury in cosmetics to be 1 μg g^{-1}. A clinical investigation of Kenyan women with damaged kidneys revealed that they suffered from

a higher incidence of nephritic syndrome, which was attributed to the use of creams containing mercury (Barr *et al.,* 1973).

In Ghana little work has been undertaken to determine the levels of mercury in toning creams (Voegborlo *et al.,* 2008) even though concerns have been expressed about the wide spread of the negative effect of skin lightening creams on the skin.

Hydroquinone is potentially carcinogenic and is known to be a skin and respiratory irritant. It is also considered a primary topical ingredient for inhibiting melanin production. Hydroquinone is a strong inhibitor of melanin production, meaning that it prevents skin from making the substance responsible for skin colour. Because hydroquinone is carcinogenic it has been banned in some countries because of fears of a cancer risk. Some concerns about hydroquinone's safety on skin have been expressed, but research have shown that when it comes to topical application, it has negative reactions which are minor but major as a result of using extremely high concentrations. This is particularly true in Africa where adulterated skin lightening products are common. The Ghana Standards Board (GSB) allows a maximum of two percent in skin care products but excessive concentration can cause serious health hazards such as tinnitus, dizziness and nausea (Hutson *et al.,* 1999). Acute animal test on rats, mice and rabbits have demonstrated hydroquinone to have high acute toxicity from oral exposure (Aldrich, Chemical Co., 1990).

Hydroquinone can cause various deadly diseases such as thyroid disorder, leukemia and liver damage. Chronic occupational exposure to hydroquinone dust has resulted in eye injuries, which varied from mild irritation and staining of conjunctivae and cornea to changes in the

thickness and curvature of the cornea, loss of corneal luster and impaired vision. Prolonged exposure is required for the development of severe ocular effects. Side effects of hydroquinone are mild when used in low concentrations. Higher concentrations frequently irritate the skin and, if used for prolonged periods, cause disfiguring effects including epidermal thickening. Brown discolouration of nails has been reported occasionally when application of two percent hydroquinone is used on the back of the hand (Considine, 1987). If very low dose of hydroquinone is ingested, it seldom produces systematic toxicity. However, oral ingestion of between 5g and 15g doses has produced convulsions and hemolytic anemia (Brigs, 1982). Intraperitoneal injection of hydroquinone caused chromosomal aberrations in magnitude as in mouse bone marrow cells. Cells of intoxication have been reported after ingestion of hydroquinone alone or of photographic developing agents containing hydroquinone. Deaths have been reported after ingestion of photographic developing agents containing hydroquinone (Brigs, 1982). Hydroquinone is an important phenolic compound used in a wide variety of biological and industrial processes. Hydroquinone is used as an intermediate in the manufacturing of antioxidants for rubber, dyestuffs and food products. The major use of hydroquinone is as a reducing agent in photographic developing solution, which reduces silver halides to elemental silver in black-and-white photography and lithography. Besides its importance, it is also very much toxic and creates serious water pollution problems in many localities (The Merck Index, 1989).

Most of the skins lightening creams on the Ghanaian market are imported from Europe, USA and Cote d'voire. A few of these countries have not restricted the use of hydroquinone to 2% or less.

1.1 OBJECTIVES

The objectives of this research are;

- To determine levels of mercury in some skin lightening creams sold on the Ghanaian market.
- To determine levels of hydroquinone in some skin lightening creams sold on the Ghanaian market.
- To compare levels with standards recommended by the Ghana Standard Board and to determine if consumers in Ghana are at risk.

1.2 JUSTIFICATION

Hydroquinone and mercury have acute and chronic side effect in humans. The Ghana Standards Board allows a maximum of two percent of hydroquinone and 1µg/g of mercury in skin care

products. The Kenya Bureau of Standards banned some hydroquinone containing skin lightening creams (Aldrich, Chemical Co., 1990). Despite the side effects of mercury and hydroquinone, skin lightening creams containing these harmful chemicals are still found on the Ghanaian market and are sold to the public.

Considering the toxic effect of mercury and hydroquinone, it is important to control their exposure to humans. This can only be achieved if their levels in skin lightening creams are known.

In Ghana, little work has been undertaken to determine the levels of mercury and hydroquinone in toning creams even though concern have been expressed about the wide spread use of skin lightening creams (Voegborlo *et al*., 2008).

CHAPTER TWO

2. LITERATURE REVIEW

2.1 THE HUMAN SKIN

The skin is the body's largest organ, covering the entire outside of the body and weighing approximately six pounds (Dahl *et al.,* 2004).

Throughout the body, the skin's characteristics vary (i.e., thickness, color, texture). For instance, the head contains more hair follicles than anywhere else, while the soles of the feet contain none. In addition, the soles of the feet and the palms of the hands have much thicker layers.

The skin is made up of the following layers, with each layer performing specific functions:

- epidermis
- dermis
- Subcutis

EPIDERMIS

The epidermis is the thin outer layer of the skin. The epidermis itself is made up of three sub-layers:

- Stratum cornea (horny layer)

This sub-layer contains continually shedding, dead keratinocytes (the primary cell type of the epidermis). The keratin, a protein formed from the dead cells, protects the skin from harmful substances.

- Keratinocytes (squamous cells)

This sub- layer contains living keratinocytes (squamous cells), which help provide the skin with what it needs to protect the rest of the body.

- Basal layer

The basal layer is the inner layer of the epidermis, containing basal cells. Basal cells continually divide, forming new keratinocytes and replacing the old ones that are shed from the skin's surface.

The epidermis also contains melanocytes, which are cells that produce melanin (skin pigment).

DERMIS

The dermis is the middle layer of the skin. The dermis is made up of the following:

- blood vessels
- lymph vessels
- hair follicles
- sweat glands

The dermis is held together by a protein called collagen, made by fibroblasts (skin cells that give the skin its strength and resilience). This layer also contains pain and touch receptors.

SUBCUTIS

The subcutis is the deepest layer of the skin and is also known as the subcutaneous layer. The subcutis, consisting of a network of collagen and fat cells, helps conserve the body's heat while protecting other organs from injury by acting as a "shock absorber" (Dahl *et al.*, 2004).

In effect, the skin can be said to be made up of two layers that cover a third fatty layer. The outer layer is called the epidermis; it is a tough protective layer that contains melanin (which protects against the rays of the sun and gives the skin its colour). The second layer (located under the epidermis) is called the dermis; it contains nerve endings, sweat glands, oil glands, and hair follicles. Under these two skin layers is a fatty layer of subcutaneous tissue.

2.2 HISTORY OF SKIN BLEACHING

Skin bleaching is a phenomenon that can be traced back to the ancient times amongst nations such as Japan. Skin bleaching was also practiced in ancient and medieval times in Asia, Egypt, Europe and China; it has gained real momentum in Ghana only recently.

In ancient Japan, the Geisha were known for their painted white skin, which represented beauty, grace, and high social status. The skin colour was a psychological and social issue that persisted and still persists in their society till today. However, the skin-whitening products are not used in such a wide scale in Japan today. Geisha paint their skin white in geisha-based ceremonies to celebrate their culture and background. In ancient Persia, during the Achaemenid dynasty, farmers and civil workers used pure hydroquinone to keep their skin clear and soft. During the epoch of the middle Ages and down to the middle of the 19th century, the

white gentle skin with a matte shine was considered to be an attribute of a high origin (Dahl *et al.,* 2004). Therefore, beauties from high society preserved their skin against solar beams and wind. Skin whitening secrets were considered as family secrets and were passed down from mother to daughter. The royal family also kept skin whitening secrets because the royal person's beauty should have amazed all citizens. The modern ways of skin whitening treatments vary greatly to the extent that clients can choose a clarification technique for their skin according to their taste. Besides bleaching skin on the face and neck, there are special techniques of bleaching the skin of the lip, armpit and genital area. The more effective, but much harsher way of skin whitening treatments is bleaching the skin with the help of artificial chemical ingredients such as zinc, iron, hydroquinone, azelaic, glycolic, and citric acid. These substances remove the top layer of epidermis and interfere with the development of melanin, which is the basic painting pigment in our skin. During application with these ingredients to the skin, it is necessary to watch the time of the procedure and to concentrate on a rendered preparation carefully (Dahl *et al.,* 2004). In addition, it is recommended to use special protective creams that interface with the penetration of the ultra violet beams. The application of protective creams can improve the results of skin bleaching considerably. In both cases bleaching is a very serious procedure. Therefore, everyone who wants to pass an exam of bleaching procedures should remember that it is necessary to define the degree of skin sensitivity and to consult a doctor and a cosmetologist. Today, skin whitening products are available in the form of creams, pills, soaps or lotions. The mechanism of permanent whitening is usually by the breakdown of melanin by enzymes, such as that contained in the droppings of the Japanese bush warbler (a drab-coloured and secretive bird normally seen in spring before

there is foliage in the trees) or reducing agents such as hydroquinone. Most whitening creams also contain protective creams which prevents sun damage to the skin. It must also be said that whiles some white westerners love to tan their skin and use tanning products or solarium coffin; the opposite side of the world like Asia, Africa and Middle East also enjoy "skin whitening"(Dahl *et al.,* 2004).

2.3 MELANIN AND PIGMENTATION

Uneven pigmentation affects most people, regardless of ethnic background or skin color. Skin may either appear lighter or darker than normal; there may be blotchy, uneven areas, patches of brown to gray discoloration or freckling. Skin pigmentation disorders occur because the body produces either too much or too little melanin. Melanin is the pigment produced by melanocyte cells. It is triggered by an enzyme called tyrosinase, which creates the color of skin, eyes, and hair shades. Melanin has two major forms that combine to create varying skin tones. Eumelanin produce a range of brown skin and hair colour, while pheomelanin produces a yellow to reddish blue. Increased melanin production is known as hyper pigmentation; - is often referred to as melasma, chloasma or solar lentigens (Dyall-Smith, 1990)..

Melanin provides some amount of sun protection for the skin by absorbing ultraviolet light. Darker skin colors are less susceptible to sunburn and the overall effects of sun damage.

- Melasma is a general term describing darkening of the skin.
- Chloasma is generally used to describe skin discolorations caused by hormones. These hormonal changes are usually the result of pregnancy, birth control pills or estrogen replacement therapy.
- Solar lentigenes is the technical term for darkened spots on the skin caused by the sun. Solar refers to sunlight and lentigene describes a darkened area of skin. These spots are quite common in adults with a long history of unprotected sun exposure.

Aside from sun exposure and hormones, hyper pigmentation can be caused by skin damage, such as remnants of blemishes, wounds or rashes. This is especially true for those with darker skin tones (Yetunde *et al* 2008).

The most typical cause of darkened areas of skin, brown spots or areas of discoloration is unprotected sun exposure. Once incorrectly referred to as liver spots, these pigment problems are not connected with the liver. Skin lightening is a controversial topic as it is intertwined with the detrimental effects on the health, identity, self image, racial supremacy and colonial mentality. Specific zones of abnormally high pigmentation such as moles and birthmarks may be pigmented to match to the surrounding skin with skin lightening creams and soap containing mercury (Yetunde *et al* 2008).

Conversely, in the case of vitiligo, unaffected skin may be lightened to achieve a more uniform appearance. Vitiligo is an acquired skin disease characterized by patches of unpigmented skin (often surrounded by a heavily pigmented border). Some people treat larger areas to lighten the natural complexion. Out of aesthetic preference or to avoid social or work discrimination

and gain access to better income or higher social position. An additional application is genital and or anal bleaching, intended to reduce the typically darker pigmentation of the genital and perianal area (Yoshimura, 2001).

Asian men no longer believe that fairness is only for women. Indian men, as well as their counterparts in other Asian countries, including Korea and Japan are turning to fairness cream. According to trade analysts, men's fairness product is valued at Rs 30 million, and constitutes 35 percent of the market (Al-Saleh, I. and Al-Doush, I. 1997). All fairness cream or skin whitening creams are effective only if the pigment is in the epidermis. If the pigment is deeper, the product cannot help or make changes. Thus, fairness cream can help remove a tan or discoloration due to some pigment in the top layer of the skin. It cannot make a dark person fair (Al-Saleh, I. and Al-Doush, I. 1997).

2.4 BLEACHING AGENTS

Most skin bleaching products contain one of the two active ingredients; hydroquinone and mercury. Other agents with skin lightening properties include alpha arbutin, beta-arbutin, licorice extract, niacinamide, mulberry extract, glycolic acid, lactic acid, lemon juice extract, emblica, vitamin C, potato and Tumeric. Potato is a natural bleaching agent. When the face is massaged with a slice of raw potato as often as possible, the skin can be lightened in colour. The slice must not be washed before massaging the face as it will lose its natural properties (Yetunde *et al*., 2008).

2.5 MERCURY

Cosmetic preparations containing mercury compounds are often applied with regularities and frequency for prolonged periods. Such chronic use of mercury-containing skin bleaching preparations has resulted in the accumulation of mercury in the body after absorption through the skin; especially in the kidney where it mainly accumulates in the tubular region, giving rise to the occurrence of severe reactions (Marzulli and Brown, 1972; Barr *et al.,* 1973; Berlin, 1979; Bourgeois *et al*., 1986). Mercury from the products enters the body by penetrating the skin and also via inhalation (WHO, 1991; Glahder *et al.,* 1999; Al-Saleh *et al.,* 2004; Al-Saleh and

Shinwari, 2004). Research has shown that mercury was readily absorbed through the skin of both albino and pigmented mice as evidenced with its accumulation in the brain, kidney and liver tissues (Al-Saleh *et al.,* 2004). The most frequently observed effects of inorganic mercury on humans are connected with the central nervous system, the kidneys and the skin (WHO, 1991). Nephro-toxic effects have been attributed to the application of inorganic mercury salts (Silverberg *et al*., 1967; Barr *et al.,* 1973; Kibuhamusohe *et al.,* 1974; Lyons *et al.,* 1975; Berlin, 1979; Jeddeloh *et al.,* 1985). Studies have also indicated that permanent kidney dysfunction can be induced by exposure to nephrotoxic chemicals during prenatal periods (Neubert *et al.,* 1985; Lauwerys *et al.,* 1987). Exposure of placental cells to mercury causes accumulation of the metal in the placental membrane and lowers the membrane fluidity, which may affect membrane function and cause damage to the developing foetus (Boadi *et al.,* 1992). It has been demonstrated that low mercury levels in mothers during pregnancy affect the ability of their children to solve mental problems (Grandjean *et al.,* 1999). Studies have shown that women using creams containing mercury attain mercury concentrations from 0.03 to 0.15 mg per litre in their urine. At these concentrations, there is a major risk of negative effects on their central nervous system and kidneys (Glahder *et a*l., 1999). Research was carried out on Fair and lovely cream. It was applied on mice for a period of one month at different interval after which mercury levels were measured in the liver, kidney, and brain tissue samples of a total of 75 adult female mice. While the kidney was found to have the highest mercury content, the brain was found to have the lowest content (Al-Saleh *et al.,* 2005).

Marked histological changes were clearly noted in the kidney to a lesser extent, in the brain and liver. Studies have shown that women using soap and cream containing mercury attain mercury

concentrations from 0.03 to 0.15 mg per litre in their urine (Glahder *et al*., 1999). At these concentrations, there is a major risk of negative effects on their central nervous system and kidneys, since such effects have been demonstrated at concentrations from 0.02 to 0.5 mg per litre (Glahder *et al.,* 1999). There is therefore the justification for banning mercury-containing soaps and creams, which are manufactured and sold under the presumption of being antiseptic, although the real purpose is to bleach human skin and hair. Mercury-containing products are, however, manufactured in several European countries and these are exported and sold illegally in the Third World Countries including Ghana. English Community Health Authorities have identified several brands of skin-lightening creams containing ammoniated mercury between 1 and 5% (Marzulli and Brown, 1972) and in some cases between 5 and 10% (Barr *et al.,* 1973). It has also been shown that even under conditions of good manufacturing practice; trace amounts of mercury in cosmetics are unavoidable.

The United States Food and Drug Administration (US FDA) in 1992 established the maximum acceptable level of mercury in cosmetics to be 1µg/g.

Mercury compounds have been used with varying success to lighten skin pigment. The mercury ions are thought to inhibit the synthesis of melanin, a black pigment responsible for darkening the skin (Giunta *et al,* 1983).

The skin colour is determined by the amount and type of melanin, the pigment in the skin. Compounds of mercury were used to prevent infection before germ theory of disease was established, because of their high toxicity and severe caustic action. Such inorganic mercurials as mercuric chloride, mercuric oxycyanide and potassium mercuric iodide have

been largely replaced by certain organic mercury compounds. Organic mercurial compounds are far less toxic and are nonirritating in concentrated solution. They are highly bactereostatic and in concentrated solutions germicidal as well. They are also non specific in antimicrobial activity. Cosmetic preparations containing mercury compounds are often applied with regularity and frequency for prolonged periods. This chronic use of mercury-containing skin-bleaching preparations has resulted in the accumulation of mercury in the body after absorbing through the skin; especially in the kidney where it mainly accumulate in the tubular region, giving rise to the occurrence of severe reactions. Mercury toxicity include effects like metallic taste, increase thirst, abdominal pain, bloody diarrhea, decreased flow of urine nephritis, colitis or constipation, tremor, anemia, skin problems (Giunta *et al,* 1983).

Mercury has adverse effects on the developing brain of a foetus. Mercury applied to the skin will react with ultra violet rays and reoxidize, leading to more pigmentation and premature ageing if more of the product is applied in an attempt to correct the darker blotching appearance (Olumide et al 2008). By inhibiting the production of melanin, the skin is more susceptible to skin cancer. Mercury will slowly accumulate within the skin cells striping the skin of its natural pigment leaving behind signs of gray/blue pigmentation in the folds of the skin.

In the long term the chemical will damage vital organs and lead to liver and kidney failure and mercury poisoning. Other complications of mercurial toxicity are exogenous ochronosis, impaired wound healing and wound dehiscence, the fish odor syndrome, nephropathy,

steroid addiction syndrome, predisposition to infections, a broad spectrum of cutaneous and endocrinologic complications of corticosteroids, including suppression of hypothalamic-pituitary-adrenal axis (Giunta *et al,* 1983). The mercury content in bleaching soaps and creams work by stripping the skin of its natural pigmentation. However, in dark skinned people, the pigmentation is the skin's natural protection from the sun. Once the skin has been bleached it loses its natural protective barrier, making it susceptible to damage by the sun's rays. This is also why many bleaching products contain either sunscreen, or come with instructions advising people to use sun protection creams along with the product. The skin becomes vulnerable to damage (Giunta *et al*, 1983).

. People who use bleaching products can end up with rough and blotchy skin, and then get caught up in the "bleaching trap" by using more cream to try to correct the problem, and by doing so, find themselves causing more damage to their skin. Alternatively they may find that because of the exposure to the sun, their lightened skin gets darker (Giunta *et al*, 1983).

Black skin renews itself quickly, rapidly producing new skin cells. This ability for regeneration keeps the skin looking younger. Whenever the black skin is damaged or traumatized, it produces an excess of melanin in the area. This hyper pigmentation can result in a humble spot or cut producing a dark patch where it's healed. Minnesota is the first in the United States of America to ban intentionally-added mercury in cosmetic products. Minnesota will now have a more stringent standard than the federal government. The federal government currently allows a small amount of mercury as a preservative in eye

makeup (Al-Saleh *et al.,* 2004). The implications of this new law have already been felt on a national level. However, many leading cosmetics companies are now being forced to adapt their formulations in order to comply with Minnesota's requirements. Minnesota lawmakers say they passed the bill with hopes that other states will also do so, thereby forcing the federal government to ban it nationwide (Al-Saleh *et al*., 2004).

2.5.1 Effects of Mercury in Humans

There are emotional changes in mercury poisoning and depression slowly sets in. Victims feel fatigued, listless sand lack motivation - even for crucial tasks. They lose interest in their surroundings and in their own life. They experience constant fear of losing their job and may be very tense and feel hopeless. They have a sense of impending doom and every small problem is discouraging. Minor difficulties seem overwhelming and insurmountable (Bourgeosis *et al.,* 1986).

The altered emotional state of a mercury intoxicated person leads to impaired interpersonal relationships. They become increasingly irritable and sensitive, reacting strongly to relatively innocent remarks. They may not be able to take orders, instructions, or suggestions without losing their temper. They resent criticism and may interpret innocent remarks critically. They may have an exaggerated response to stimulation and become fearful or anxious and nervous. They may project their fears and anxieties onto others, making inappropriate criticisms or attacks. They become shy and avoid dealing with strangers. While timid, they may unexpectedly lose self control with strangers (Giunta *et al.*, 1983). Intelligence gradually

deteriorates. Previously bright persons become dull and slow in thinking. They suffer from a progressive decline specifically affecting short term memory as well as the faculties for logical reasoning. Thus their ability to do things like balance the checkbook, do math, or play chess suffers. They lose the ability to concentrate. Memory problems may be more from distractibility and inability to concentrate and pay enough attention to get things into their memory than an actual failure to remember things (thus they may complain of memory problems but do well on memory tests). They cease being motivated towards their work or other tasks. Thoughts become heavy, repetitive and pedantic (Giunta *et al*., 1983). Creative thinking becomes progressively more difficult, eventually becoming impossible. They become unable to select the right words to convey their meaning, and make stylistic and grammatical errors. Their ability to express themselves declines progressively. There is a distinctive cognitive symptom of being unable to think clearly without great effort. The best description for people who have not experienced it is of a hangover without pain. People who have experienced it will recognize the term "brain fog" as entirely descriptive. As the victim's level of intoxication waxes and wanes they go through periods of life when they do or do not dream. Dreaming may be in black and white. Early physical symptoms include dizziness, tinnitus (ringing in the ears), insomnia, daytime drowsiness, loss of appetite, a tendency towards diarrhea - often alternating with constipation, cold hands and feet, a tendency towards sweating (some people have the opposite symptom and do not sweat at all), flushing or reddening of the skin - particularly on the face and neck. Some people blush frequently, but others do not blush at all. Asthma is a symptom of chronic mercury poisoning. Digestive disturbances are also common. The skin becomes dry, athlete's foot and toenail fungus progress, and the insides of the ankles, particularly behind the ankle bone

and a bit above it become dry, itchy, flaky and peel. This often becomes painful and annoying enough to keep the victim up at night. Even after fungus and yeast infection has been eliminated hyperkeratosis, often with popular erythema and itching is common.

The hair becomes thinner, dryer, duller, less strongly colored, slower growing and more brittle. The biological clock is disturbed. Waking up late and staying up late is more common than being an "early bird." Try as they might, the mercury poisoned person simply cannot control their circadian rhythm. Victims may become photophobic and find bright light uncomfortable and unpleasant. There may be visual disturbances, including alterations in color perception leading to reduced sensitivity to the color red, or color blindness. The ability to focus on distant objects may be sporadically impaired. Peripheral vision may be reduced in the most severe cases. The hands and feet often become distinctly cold. This can occur suddenly and is most distinctive when combined with sweating. Later in more severe poisoning they may also tingle or lose feeling (Bourgeosis *et al.*,1986)

The effects of mercury on the mouth are receding, sometimes spongy gums that bleed easily and teeth that are 'loose' in their sockets and can be wiggled very slightly. It also causes excessive salivation and unusually bad breath. Mercury interferes with the sense of smell which becomes less acute, and later with hearing, in which perception of sounds does not diminish as notably as the patient's ability to understand and interpret them. Victims often experience discomfort that feels like a "tight band around their head." They may also experience sharp points of discomfort in their ear canals at bedtime. Mercury also interferes with the body's ability to regulate temperature. Victims may alternate between being hot and cold when the temperature isn't changing, or have to wear more clothes than other people,

or have more difficulty than other people in staying comfortable while the temperature changes. Temperature disregulation also leads to 'night sweats (Kahatano, et al., 1988).

Mercury has the ability to cause changes at the cellular level, which has been seen in platelets and erythrocytes. These cells have been used as surrogate markers for mercury damage of neurological tissue. The addition of methyl mercury to whole blood can cause a dramatic dissolution of microtubules in platelets and red blood cells – an effect more pronounced in erythrocytes than platelets – which is consistent with the known sequestration of methyl mercury in erythrocyte. This effect on microtubules has also been found in the brain, and results in disruption of the cell cycle. This disruption can cause apoptosis (programmed cell death) in both neuronal and non-neuronal cells (Kahatano, et al., 1988).

Mercury causes apoptosis in monocytes and decreases phagocytic activity. In one study, the percentage of cells undergoing apoptosis was dependent on the mercury content of the medium, regardless of the form of mercury. Methyl mercury chloride exposure caused a decrease in the mitochondrial transmembrane potential within one hour of exposure, leading to altered mitochondrial function. Methyl mercury can also cause increased lymphocyte apoptosis. This mechanism includes a depletion of glutathione (GSH) content, which predisposes the cell to oxidative damage, while activating death-signaling pathways. On examination of synovial tissue, it was found that mercury (as well as cadmium and lead) caused a decrease in DNA content and an increase in collagenase-resistant protein formation, leading to increased risk for reduced joint function and decreased ability to

repair joint damage (Kahatano, *et al.*, 1988).

Mercury is also implicated in Alzheimer's disease and other chronic neurological complaints. Subsequent studies have shown elevated mercury throughout the brain in individuals with Alzheimer's. Furthermore, when rats were exposed to elemental mercury vapor at the same levels as documented in the oral cavity of humans with amalgams, lesions similar to those seen in Alzheimer's disease have occurred. The same lesions have been demonstrated when rat brains were exposed to EDTA-mercury complex (Kahatano, *et al.*, 1988).

Even the cosmetic industry promotes products that are designed to help dark-skinned women look "lighter". It is also reported for instance that facial attractiveness is associated with positive evaluation by others and that many African Americans believe that their lighter skinned fellows are more competent. Still, in the USA, previous studies have shown that for the same level of education, dark-skinned people earn much less than their peers with lighter skin and thus reinforcing the perception that a lighter skin tone is associated with better economic status (Yoshimura, 2001).

Interestingly, level of education, social class, marital and employment status seem to have less influence of the skin lightening practice. The prevalence of skin lightening reported among those interviewed in Africa shows some disturbing results. In Bamako in Mali, researchers calculated 25% prevalence, while in some studies in Dakar, Senegal, up to 52% prevalence was observed. A study in Pretoria, South Africa revealed up to 35%, while the most disturbing was a study in 2002 which showed up to 77% prevalence in Lagos, Nigeria. Some countries are speaking out (Al-Saleh *et al.*, 2005). The Kenya Bureau of Standards banned several lotions, gels and soaps used for skin lightening and other African countries

are now following. This however does not necessarily stem the practice. Since some of these effects do not appear quickly, and some mimic known disease patterns, they are sometimes confused and misdiagnosed even by health care professionals since patients usually deny using any skin lighteners. It is reported that up to 69% of those who practice skin lightening may suffer from at least one complication.

Skin lightening could be regarded as a disease in the broader definition of health as a 'state of mental and social well-being' conditions (Al-Saleh *et al.,* 2005).

The multitude of adverse effects that may be experienced by those who practice skin lightening will significantly impact on how they use health care services, diverting valuable resources to care for these conditions (Al-Saleh *et al.,* 2005).

Skin lightening is not only a psychological and social problem, but also a public health issue that needs to be addressed with targeted interventions aimed at changing perceptions and educating people on its consequences.

In a study investigating the histo-pathological changes cause by skin lightening agents, changes were clearly seen in the brain, kidney, and liver sections of all treated mice. The severity of pathological changes observed in tissues increased with increasing the number of applications. It is evident that repeated application of skin-lightening creams could induce permanent damage to the kidneys, brain, and liver. This study emphasizes the potential toxicity of mercury skin-lighting creams and the importance of discontinuing their manufacture and use (Al-Saleh *et al*., 2009).

2.6 ANALYTICAL METHODS FOR MERCURY DETERMINATION.

Numerous methods have been used to determine mercury levels in biological and environmental samples. Most methods have used atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), or neutron activation analysis (NAA). In addition, methods based on mass spectrometry (MS), spectrophotometry, gas chromatography and anodic stripping voltammetry (ASV) have also been tested. Of the available methods, cold vapor Atomic Absorption Spectrophotometer is the most widely used. Atomic absorption spectrophotometry is designed to determine the amount or concentration of an element in a sample utilizing the phenomenon that the atoms in the ground state absorb the light of characteristic wavelength passing through an atomic vapour of the element.

All these methods are a considerable improvement on the original 'dithozone' method. The Dithozone method was widely used in the late 1960s. Basically it involved the formation of a coloured complex with dithizone after all the mercury in the sample had been converted to Hg^{++} compounds by oxidation in strong acids. After neutralization of excess oxidant with a reducing agent, usually hydroxylamine, the coloured complex was extracted into a non-polar solvent. After washing the extract, the colour intensity was measured on a spectrophotometer and the amount of mercury estimated from a standard curve. The dithizone procedure has an absolute sensitivity of about 0.5µg of mercury. The method is based on the principle that when natural mercury (a mixture of stable isotopes) is exposed to a high flux of thermal (slow) neutrons, it is converted to a mixture of radioactive isotopes, principally ^{197}Hg and ^{203}Hg, which have decay half-lives of 65 hours and 47 days, respectively. After the sample has been irradiated with neutrons, a precise weight of carrier mercury is added and the sample subjected to digestion and organic destruction. On completion of digestion, mercury is isolated by electrodeposition on a gold foil and the radioactivity is determined with a gamma counter. The use of carrier mercury corrects for any losses of mercury during the digestion, extraction, and isolation procedures. The limit of detection is 0.1-0.3ng of mercury. The sample size is 0.3g giving a concentration limit of 0.3-1 µg/kg in most biological samples. The neutron activation procedure is regarded as the most accurate and sensitive procedure and is usually used as the reference method. It can determine both total and inorganic mercury and, by difference, organic mercury. The apparatus is inexpensive, portable, and does not require sophisticated facilities. The technique has a sensitivity of approximately 0.5ng of mercury.

2.6.1 The Cold Vapour (CV) Technique

CVAAS is a technique based on the absorption of radiation by mercury vapour. The mercury is reduced to the elemental state and aerated from solution. The mercury vapour passes through a cell positioned in the light path. Mercury is unique among other heavy metals. Mercury has a high pressure at ambient temperature (0.61 Pa at 20ºC). This uniqueness of Hg allows its determination to be exploited. The traditional methods for the determination of Hg include flameless AAS, AFS and ICP-AES, all of which exhibit poor sensitivity. The high vapour pressure of Hg at ambient temperature enables the metal to be determined by AAS without the use of an atomizer. During the determination, Hg must be simply reduced to metallic mercury from its compounds and transferred as the vapour phase. This is achieved by a simple chemical reduction reaction used to generate the gaseous mercury species. The process is known as Cold Vapour Atomic Absorption Spectrometry (CVAAS). The CVAA process has two primary advantages. First, mercury- the analyte is removed from sample matrix, which reduces the potential for matrix interferences. Second, the detection limits are improved because the entire mercury sample is introduced into the absorption cell within a few seconds. Therefore, the density of mercury in the cell during data collection (absorption, fluorescence or emission depending on the detection technique) is greatly enhanced as compared to typical sample introduction. Two reducing agents usually employed for CV analysis are tin (II) chloride ($SnCl_2$) and sodium borohydride ($NaBH_4$).

2.7 HYDROQUINONE

Hydroquinone occurs naturally as a conjugate with beta-D-glucopyranoside in the leaves, bark and fruit of a number of plants, especially the ericaceous shrubs such as cranberry, cowberry, bearberry and blueberry. Hydroquinone and its glucose conjugate, 4-hydroxyphenyl-β-D-glucopyranoside (arbutin), are naturally present in many foods and beverages. Arbutin is reported to hydrolyze readily in dilute acidic solutions to yield D-glucose and hydroquinone. Ingested arbutin is expected to be converted to free hydroquinone in the stomach. It has been detected at low levels in coffee, tea, red wine, beer, cola soft drinks, 2% milk, orange juice, corn, wheat and rice cereals, wheat germ, and various fruits, including pears, oranges, cantaloupes, cherries, asparagus, apples, blueberries and cranberries (Yetunde *et al.,* 2008). It is also known to be present in the particulate fraction of cigarette smoke. Hydroquinone is one of the two primary reagents in the defensive glands of bombardier beetles, along with hydrogen peroxide (and perhaps other chemicals, depending on the species), which collect in a reservoir. The reservoir opens through a muscle-controlled valve onto a thick-walled reaction chamber. This chamber is lined with cells that secrete catalases and peroxidases. When the contents of the reservoir are forced into the reaction chamber, the catalases and peroxidases rapidly break down the hydrogen peroxide and catalyze the oxidation of the hydroquinones into p-quinones. These reactions release free oxygen and generate enough heat to bring the mixture to the boiling

point and vaporize about a fifth of it, producing a hot spray from the beetle's abdomen.

Synthesis

- Hydroquinone is manufactured by the oxidation of aniline to quinone and the subsequent reduction of quinone to hydroquinone.
- Other routes of synthesis include the oxidative cleavage of diisopropyl benzene and the hydroxylation of phenol.

Chemistry of Hydroquinones - Structure

Hydroquinone, also benzene-1, 4-diol or quinol, is an aromatic organic compound which is a type of phenol, having the chemical formula $C_6H_4(OH)_2$.

Structure of Benzene-1, 4-diol

Described as having two hydroxyl groups bonded to a benzene ring in a para-position.

It is a white granular solid at room temperature and pressure.

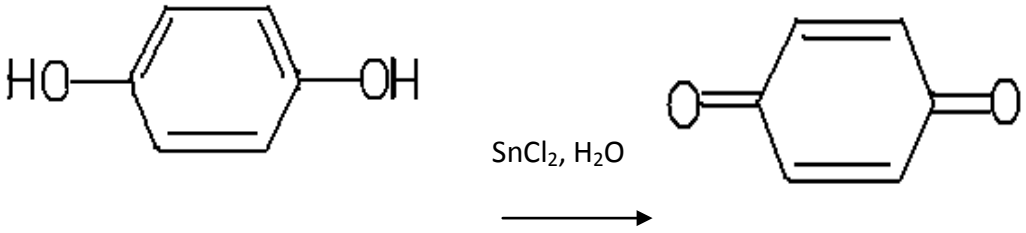

Figure 1: Oxidation in Hydroquinone

Physical Properties

- Molecular formula - $C_6H_4(OH)_2$
- Molar mass - 110.1 g/mol
- Appearance - white solid
- Density - 1.3 g/cm3, solid
- Melting point - 172 °C
- Boiling point - 287 °C
- Solubility in water - 5.9 g/100 ml (15 °C)
- Dipole moment - zero (Chambers, 1988)

Hydroquinone is a strong inhibitor of melanin production that has long been established as the most effective ingredient for reducing and potentially eliminating melasma (Yoshimura 2001), meaning that it prevents skin from making the substance responsible for skin color (Yoshimura 2001). Over-the-counter hydroquinone products can contain 0.5% to 2% concentrations of hydroquinone; 4% (and sometimes higher) concentrations are available only from physicians. The *Allium* test was used to study the cytotoxic effects of five commonly abused skin toning creams-Ikb, Tura, Top gel, Dorot and Mililo in Nigeria

(Udengwu and Chukwujekwu, 2008). These creams are commonly used by some black skinned people (especially the females) as skin lightening (bleaching) agents. The results showed that all the five bleaching creams were mito-depressive in action. They exhibited both chromatoclassic and mitoclassic effects. Their depressive effects were found to increase with duration of treatment. The induced abnormalities included chromosome contraction, spindle breakages, c-metaphase, star anaphase, chromosome stickiness and sticky bridges, precocious chromosome movement as well as endomitosis. It is suggested that since all eukaryotic cells are basically the same, these observed abnormalities could be similar to the effects these chemicals have on human skin when they are applied. (Udengwu and Chukwujekwu, 2008). Exogenous ochronosis is a paradoxical hyper-pigmentation of the skin caused by the long-term use of hydroquinone-containing bleaching creams. Ochronosis is an uncommon condition characterized by yellow-brown pigmented deposits in the dermis. Two cases of exogenous ochronosis in two female patients of the sub-Saharan African population were reported (Bongiorno and Arico, 2005). The lesions were characterized by an asymptomatic hyper-pigmentation of the face with gradually progressive blue-black macular patches, and in addition to dyschromic lesions, *striae atrophicae* were present. This phenomenon is the outcome of the use of skin care products containing high concentrations of hydroquinone- and glucocorticoid-based products, and, in addition, certain modalities in the use of bleaching products are likely to facilitate complications (Bongiorno and Arico, 2005). Available literature indicate that related studies done in this area had to do with the study of the general effects of some of the chemical compounds used in the making of some of these bleaching creams on human skin. Such studies revealed that chemicals like, hydrogen peroxide preparations, ammoniated mercury, phenols and catechols including

monobenzyl ether of hydroquinone, monomethyl ether of hydroquinone, (p-hydroxyanisole), p-tertiary butyl phenol, p-tertiary amylphenol and 4 -tertiary butyl catechol could act as demelanizing agent. Hydroquinone is one of the most popular depigmenting agents and is used extensively to treat several hyper pigmentation disorders. Depigmentation by hydroquinone is because of its ability to inhibit tyrosinase as well as its cytotoxicity to melanocytes. However, because of its carcinogenic properties, use of hydroquinone is banned or limited in cosmetic products in many countries (Engasser and Maibach 2003).

Concern about hydroquinone having carcinogenic properties is mostly related to industrial-grade materials and uses. In a study to determine the effect of the skin-depigmenting agent hydroquinone (HQ), it was found out that significant differences in its effect on DNA and RNA synthesis were observed between cell lines. HQ caused inhibition of cellular metabolism in all cells tested, but the dose that caused 50% inhibition of tritiated thymidine incorporation was approximately 30 times lower for melanotic cells. Titrated uridine incorporation was found to be 85 times more sensitive to HQ in the melanotic cells (Engasser and Maibach, 2003). These results suggested that HQ exerts its depigmenting effect by selective action on melanocyte metabolism rather than a specific effect on melanin synthesis.

Among skin-lightening agents, hydroquinone (HQ) is one of the most widely prescribed

agents in the world. However, with reports of potential mutagenicity and epidemics of ochronosis in African nations, there has been increasing impetus to find alternative herbal

and pharmaceutical depigmenting agents (Bongiorno and Arico, 2005).. A review of the literature reveals that numerous other depigmenting or skin-lightening agents are in use or in investigational stages. Some of these, such as kojic and azelaic acid, are well known to most dermatologists. Others more recently have been discovered and reported in the literature. Evidence of improvement with HQ (monotherapy) usually is observed at 4-6 weeks, with improvement appearing to plateau at about 4 months. Despite its remarkable overall safety, the physician ought to bear in mind the potential adverse effects. Contact dermatitis occurs in a small number of patients and responds promptly to topical steroids. Exogenous ochronosis is a paradoxical hyper-pigmentation of the skin caused by the long-term use of hydroquinone-containing bleaching creams (Bongiorno and Arico, 2005). Ochronosis is an uncommon condition characterized by yellow-brown pigmented deposits in the dermis. Two cases of exogenous ochronosis in two female patients of the sub-Saharan African population were reported. The lesions were characterized by an asymptomatic hyper-pigmentation of the face with gradually progressive blue-black macular patches, and in case no. 2, in addition to dyschromic lesions, *striae atrophicae* were present. This phenomenon is the outcome of the use of skin care products containing high concentrations of hydroquinone- and glucocorticoid-based products, and, in addition, certain modalities in the use of bleaching products are likely to facilitate complications (Bongiorno and Arico, 2005).

Nausea, vomiting, abdominal cramps and diarrhea occur in humans who chronically consume water contaminated with hydroquinone. No effects on people who voluntarily ate low doses of hydroquinone for less than six months (USEPA, 1987).

Side effects of hydroquinone are mild when used in low concentrations of about 1% tingling

or burning on application and subsequent erythma and inflammable were observed in eight percent of patients using a two percent concentration and thirty two percent of patients using a five percent hydroquinone concentration. Higher concentrations frequently irritate the skin and if used for prolonged periods, cause disfiguring effects including epidermal thickening (Ash and Ash, 1981).

In some cases, lesions become slightly darker before fading. Eversible brown discolouration of nails has been reported occasionally when application of two percent hydroquinone is used on the back of the hand. The discolouration is probably caused by formation of hydroquinone oxidation products. Hydroquinone is easily oxidized in the presence of light and air. Any discolouration or darkening of the cream is an indication of deterioration in the strength of available hydroquinone (Considine, 1987).

Oral ingestion of between 5g and 15g doses has produced convulsions and hemolytic anemia. Dermal applications of hydroquinone at concentrations in different bases of less than 3% caused abnormal functioning of the kidneys in males. It has also been found to increase molecular cell leukemia in females (Lewis, 1969).

There are a number of harmful effects caused by skin bleaching agent on the body. Some of these effects are on the skin whiles others affect other internal organs of the body. This is possible because at higher blood levels of mercury and hydroquinone, the effect on internal organs is prominent.

Skin bleaching destroys the black pigment found in the epidermis (top layer of the skin). Exposure of the dermis layer, underneath the epidermis layer, to the harsh weather will

increase the incidence of skin cancer. The dermis cannot compensate for the absence of the epidermis and coupled with the hot sun one will get a higher risk of cancer (Lewis, 1969).

It was found that thinning and weakening of skin, irritant dermatitis, discoloration are some of the medical problems related to regular skin bleaching. Hydroquinone caused leukemia in mice (Lewis, 1969).

2.8 ANALYTICAL METHODS FOR HYDROQUINONE ANALYSIS

Hydroquinone in cosmetics, hair products, pharmaceutical preparations, and air samples, can be determined by several analytical techniques such as flow injection analysis, kinetic spectrophotometry, gas chromatography-mass spectrometry (GC-MS), differential pulse voltammetry, capillary electrochromatography or with biosensors.

Although gas chromatography is widely used and is a powerful chromatographic method, it is limited to compounds that have a significant vapour pressure at temperatures up to about 200^{o}C. Thus, compounds of high molecular weight and high polarity cannot be separated by gas chromatography (Yang, 1989).

High performance liquid chromatography does not present this limitation. It consists of five parts; the mobile phase supply unit, the sample injection system, the column, a suitable detecting device and a recorder. HPLC has the advantage of using relatively small amounts of solvents, it is rapid and it can accomplish very difficult separations.

In HPLC, a liquid sample, or a solid sample dissolved in a suitable solvent, is carried through a chromatographic column by a liquid mobile phase. Separation is determined by solute/stationary-phase interactions, including liquid–solid adsorption, liquid–liquid partitioning, and by solute/mobile-phase interactions. In each case, however, the basic instrumentation is essentially the same. An HPLC typically includes two columns: an analytical column responsible for the separation and a guard column. The guard column is placed before the analytical column, protecting it from contamination. In liquid–liquid chromatography the stationary phase is a liquid film coated on a packing material consisting of porous silica particles. The stationary phase may be partially soluble in the mobile phase, causing it to "bleed" from the column. An important feature of HPLC instrumentation is the presence of several solvent reservoirs. Controlling the mobile phase's polarity plays an important role in improving a liquid chromatographic separation. The availability of several solvent reservoirs allows the mobile phase's composition to be quickly and easily varied. This is essential when using a gradient elution, for which the mobile-phase composition is systematically changed from a weaker solvent to a stronger solvent (Novtony, 1981).

The typical operating pressure of an HPLC is sufficiently high that it is impossible to inject the sample in the same manner as in gas chromatography. Instead, the sample is introduced using a loop injector.

Sampling loops are interchangeable and available with volumes ranging from 0.5 ml to 2 ml. As with gas chromatography, numerous detectors have been developed for use in monitoring HPLC separations. To date, the majorities of HPLC detectors are not unique to

the method, but is either stand-alone instruments or modified versions.

The most popular HPLC detectors are based on spectroscopic measurements, including UV absorption and fluorescence. These detectors range from simple designs, in which the analytical wavelength is selected using appropriate filters. When using a UV detector, the resulting chromatogram is a plot of absorbance as a function of elution time. Instruments utilizing a diode array spectrophotometer record entire spectra, giving a three-dimensional chromatogram showing absorbance as a function of wavelength and elution time. The detector suitable for determination of hydroquinone is the UV detector.

CHAPTER THREE

3. METHODOLOGY

3.1. APPARATUS AND EQUIPMENT

All glassware used were soaked in detergent solution overnight; rinsed and soaked in 10 % (v/v) HNO_3 overnight. They were rinsed with distilled water followed by 0.5 % (w/v) $KMnO_4$ and finally rinsed with distilled water before use.

Automatic Mercury Analyzer Model HG-5000 (Sanso Seisakusho Co., Ltd, Japan), equipped with mercury lamp operated at a wavelength of 253.7 nm and connected to a personal computer was used for mercury determination.

Digestion apparatus for mercury was thick walled long neck 50ml volumetric flask and a Clifton hot plate with a temperature range of 150-350 ^{0}C.

Reversed-phase High performance liquid chromatography was carried out using a High Performance liquid chromatograph model Cecil CE 4200 (England). This is equipped with an analytical column responsible for the separation. Standard solutions were injected to obtain a calibration curve. A syringe with a capacity of 50 µl was used to inject the sample into the sample loop. The mobile phase was methanol and water pumped at a flow-rate of 1.0 ml per minute. The UV detector was operated at a wavelength of 226 nm with a sensitivity of 0.50.

3.2 REAGENTS

All reagents were of analytical reagent grade (BDH Chemicals Ltd, Poole, England) unless otherwise stated. The methanol used for the hydroquinone analysis was HPLC grade.

Mercury standard solution (1000 mg/L) was prepared by dissolving 0.0677 g of $HgCl_2$ in the acid mixture HNO_3:H_2SO_4:$HClO_4$, (1:5:1) in a 50 ml digestion flask with heating on a hot plate at temperature of 200^0C for 30 minutes. The clear solution was cooled and diluted to 50 ml with water. Blank solutions were also prepared alongside. The working solutions were freshly prepared by diluting an appropriate aliquot of the stock mercury solution through intermediate solutions using blank solution. Stannous chloride solution (10% w/v) was prepared by dissolving 10 g of the salt in 100 ml of 1M HCl. The solution was aerated with nitrogen gas at 50 ml per minute for 30 minutes to expel any elemental mercury from it.

Standard solution of Hydroquinone was prepared by dissolving 1.0g hydroquinone in methanol in a 100ml volumetric flask and made up to volume using methanol. Various concentrations (0.08, 0.12, 0.16 and 0.2 g/l) were prepared by diluting aliquots of the stock hydroquinone standard solution with methanol.

3. 3 SAMPLING

Fifty samples of skin lightening creams were obtained randomly from cosmetic shops in Adum Market (Kumasi) and Makola Market (Accra).

3.4 DIGESTION OF SAMPLES FOR MERCURY

The samples were digested for total mercury determination by a modified version of an open flask procedure developed at the National Institute for Minamata Disease (NIMD) Japan (Akagi and Nishimura, 1991). Approximately 0.5 g of each cream was weighed accurately into a 50ml volumetric digestion flask and 1ml of deionised water was added. A 5 ml mixture of nitric acid and perchloric acid in the ratio of 1:1 was added and swirled. The mixture was heated at 200 ^{o}C for 30 minutes to obtain a clear solution.

The solution was allowed to cool and made up to volume with double distilled water. A blank and standard solution digest using 0, 20, 40 and 60 µl of 1 µg ml^{-1} standard Hg solution were subjected to the same treatment. The concentrations of the standard solution digests obtained were 0.4, 0.8, and 1.2 ng ml^{-1}.

The accuracy and precision of the method were determined by analysis of procedural blanks and calibrations standards, triplicate sub-samples and spiked sub-samples.

3.5 EXTRACTION OF HYDROQUINONE

About 0.10 g of each cream was weighed accurately into a 10 ml flask and 8.0 ml of methanol was added and heated at 40 ^{o}C in a water bath with occasionally shaking until it dissolved. It was allowed to cool and made up to the mark with methanol. The solution was filtered using a membrane filter before analysis.

3.6 DETERMINATION OF MERCURY

Determination of mercury in all the digests were carried out by cold vapour atomic absorption spectrophotometry using an Automatic Mercury Analyser Model HG-5000 (Sanso Seisakusho Co., Ltd, Japan) developed at National Institute for Minamata Disease (NIMD) in Japan. The analyzer consists of an air circulation pump, a reaction vessel, Tin (II) chloride dispenser, an acidic gas trap and a four way stop-cock with tygon tubes to which is attached a ball valve. The operations of the ball valve and the air circulation pump are controlled by a microprocessor. During the determination a known volume of the sample solution normally 5 ml is introduced into the reaction vessel using a micropipette (1-5 ml). The reaction vessel is immediately stoppered tightly and 0.5 ml of 10% (w/v) tin (II) chloride in 1M HCl was added from a dispenser for the reduction reaction. During this time, air is circulated through the four–way stop-cock to allow the mercury vapour to come to equilibrium and the acidic gases produced by the reaction also swept into the sodium hydroxide solution. After 30sec the four-way stop-cock is rotated through 90^{0} and the mercury vapour is swept into the absorption cell.

The schematic diagram for the analyzer is shown in chart 3.0 below.

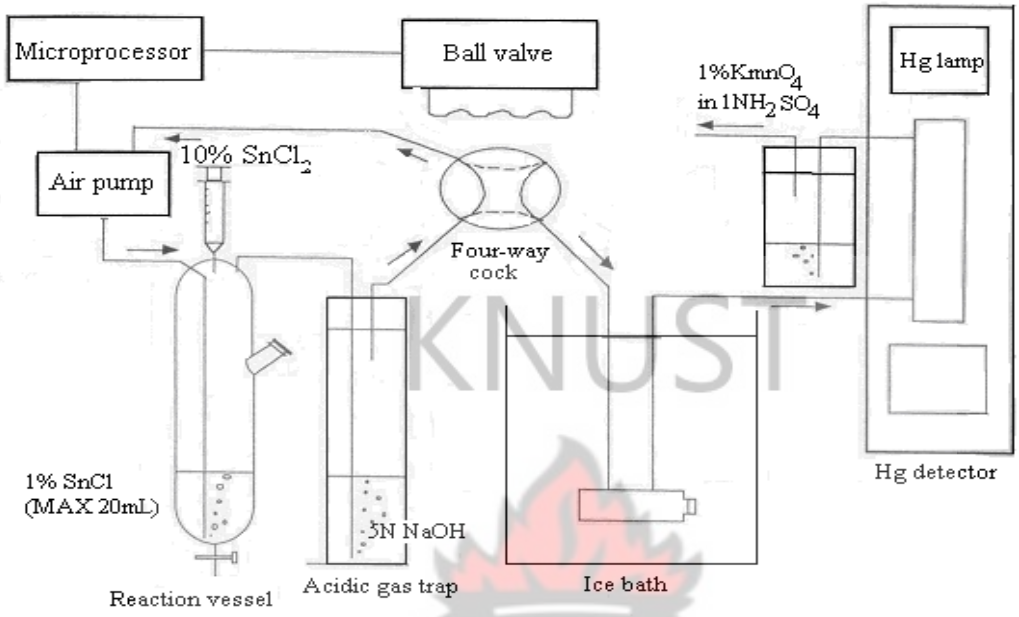

Fig 3.1 Apparatus for mercury analysis by the Cold Vapour Atomic Absorption Spectrometry.

3.7 DETERMINATION OF HYDROQUINONE.

Determination of hydroquinone was carried out by a chromatographic method using a High Performance liquid chromatograph (HPLC) model Cecil CE 4200 (England). This was equipped with an analytical column. Standard solutions were injected to obtain a calibration curve. A syringe with a capacity of 50µl was used to inject the sample into the sample loop. After loading the sample, the injector was turned to the inject position. The HPLC detector was based on spectroscopic measurements, including UV absorption. The resulting chromatogram was a plot of absorbance as a function of elution time. The signals were obtained on a computer. The schematic diagram for the analyzer is shown in Fig 3.2 below.

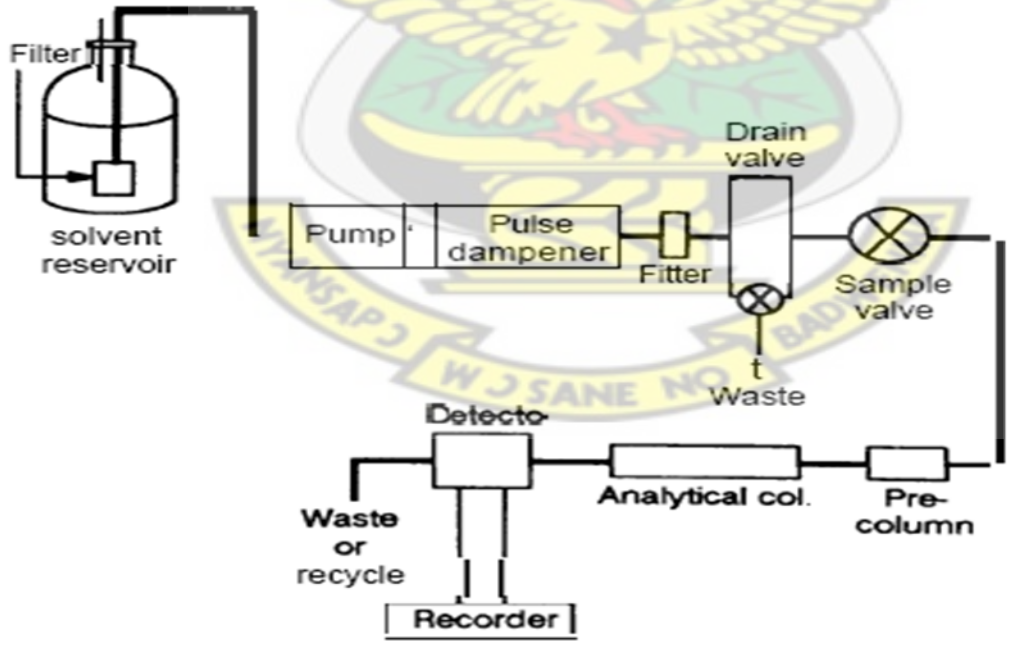
Filter
solvent
reservoir
Pump
Pulse
dampener
Fitter
Drain
valve
Sample
valve
t
Waste
Detecto
Waste
or
recycle
Analytical col.
Pre-
column
Recorder

FIG 3.2 A schematic diagram of a High Performance Liquid Chromatographic System.

3.8 Recovery of mercury and hydroquinone.

Recovery of mercury from creams was determined by adding increasing amounts of mercuric chloride solution to known weights of two different cream samples namely Zarina cream and Maprovate cream. The first flask contained only 0.5 g of each sample and the second flask contained 0.5 g of each sample and 20 ng Hg. The third flask contained 0.5 g of each sample and 40 ng Hg. They were all taken through the digestion procedure. The resulting solutions were analyzed for mercury concentrations and the results obtained are reported in table 4.1. Recovery of hydroquinone was determined by adding increasing amount of standard hydroquinone solution to known weights of two different cream samples namely Caris cream and Bioclaire cream. The first flask contained only 0.1 g of each sample and the second flask contained 0.1 g of each sample and 0.08 g of HQ. The third flask contained 0.1g of each sample and 0.12 g HQ. The resulting solutions after extraction were analyzed for hydroquinone concentrations and the results obtained are reported in table 4.1.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

Levels of mercury and hydroquinone in skin-lightening creams sold on the Ghanaian market were determined using Automatic Mercury Analyzer (Model HG-5000) for mercury and a High Performance liquid chromatograph (Model Cecil CE 4200) for hydroquinone. The accuracy of the technique used for mercury and hydroquinone determination was determined by recovery studies. Analytical and matrix recovery studies for mercury yielded results between 98% and 102% with coefficient of variation between 4% and 9%. The recovery studies for hydroquinone yielded recoveries which ranged from 97 to 102 %. The results for recovery studies are presented in Table 4.1

Table 4.1

Recovery results for hydroquinone.

Sample	HQ Present (mg)	HQ added (mg)	HQ found (mg)	HQ recovered(mg)	% Recovery
Caris cream	0.0	80	81.9	81.9	102.4
Caris cream	0.0	120	119.6	119.6	99.7

Bioclaire cream	0.2	80	78.2	78.0	97.5
Bioclaire cream	0.2	120	121.4	121.2	101.0

Recovery results for mercury

Sample	Hg Present (ng)	Hg added (ng)	Hg found (ng)	Hg recovered (ng)	% Rev

Zarina cream	26.64	25	51.64	24.8	99.2
Zarina cream	26.64	50	77.84	51.0	102.0
Maprovate cream	29.7	25	54.39	24.6	98.4
Maprovate cream	29.7	50	80.0	50.3	100.4

Mercury concentrations in the creams ranged from 0.006 to 0.549 $\mu g g^{-1}$. Hydroquinone concentrations in creams ranged from 0.01 to 3.45 %. The results for mercury and hydroquinone levels are summarized in Table 4.2.

Table 4.2 The amount of HQ and Hg in different skin lightening creams from the Ghanaian market.

NAME	C. of origin	HQ(%)	Hg(μg/g)
MOVATE CREAM	Italy	0.17	0.025
NEW AGE CREAM	Italy	ND	0.253
SWISS FORMULAR CREAM	Italy	0.02	0.045
CLAIRE DARK CREAM	Italy	ND	0.101
SKINICLES CREAM	Italy	0.20	0.155
CREAM A3 LOTION	Italy	ND	0.01
SKIN SOLUTION CREAM	Italy	0.50	0.061
CLEAR SPOT CREAM	Italy	ND	0.135

MAPROVATE CREAM	Italy	0.34	0.006
CLEAR WHITE CREAM	Italy	0.98	0.132
BIOCLAIRE CREAM	Italy	2.03	0.088
CLEAR ESSENCE CREAM	Italy	ND	0.108
LEMONVATECREAM	Italy	0.13	0.065
FADE OUT CREAM	Italy	0.15	0.011
CLEAR & SMOOTH CREAM	Italy	0.80	0.073
SKIN SUCCESS CREAM	Italy	0.27	0.031
NEO-VATE CREAM	Italy	0.43	0.007
MAXI-CLEAR CREAM	Italy	0.13	0.139
VISIBLE DIFFERENCE	Italy	0.21	0.011
CARIS CREAM	Italy	ND	0.037
CLEAR DARK SPOT CREAM	Italy	0.07	0.145
ORANVATE CREAM	Italy	0.13	0.014
ALOE VERA CREAM	Italy	0.24	0.187
CLEAN & CLEAR CREAM	Italy	0.34	0.05

TENOVATE CREAM	Italy	ND	0.011
P&C CREAM	Italy	0.50	0.127
NATUREL LEMON CREAM	Italy	0.39	0.064
WHITE MARK CREAM	Italy	0.52	0.114
SKIN MAXITONER CREAM	Italy	ND	0.271
FAIR AND BEAUTIFUL CREAM	Italy	0.21	0.09
BIOTONE TONING LOTION	Ghana	3.08	0.076
NIVEA NIGHT WHITENING MILK	Thailand	0.12	0.151
SURFAZ CREAM	India	0.01	0.186
EPIDERM CREAM	India	0.42	0.074
AMIDERM CREAM	India	0.21	0.042
CLOSOL CREAM	India	0.37	0.059
BETASOL CREAM	India	0.19	0.082
DOVE SILK CREAM	Nigeria	0.41	0.015
JERGENS SOOTHING CREAM	USA	ND	0.015
NIUMA SKIN LIGHTENING LOTION	Spain	3.45	0.079

NIVEA INTENSIVE LOTION	Spain	ND	0.047
NIVEA SMOOTH LOTION	Spain	0.83	0.08
DERMATOLOGICAL E45 LOTION	UK	ND	0.195
DOVE HYDRO FRESH CREAM	UK	0.11	0.056
DOVE SEIDIGE LOTION	UK	0.51	0.027
ZARINA CREAM	UK	1.63	0.08
LEMONVATE CREAM (SWISS)	Switzerland	0.10	0.122
DIVA MAXITONE CREAM	Cote d'voire	0.65	0.549
AKAGNI CREAM	France	3.36	0.062
FAIR AND WHITE EXCLUSIVE LOTION	France	1.94	0.185

HQ = Hydroquinone and Hg = Mercury

ND = Not detected

The range of mercury content in creams imported from Italy was 0.006-0.271 µg/g but those from Cote d'voire recorded the highest value of mercury content (0.549 µg/g). All the samples had concentration of mercury below the $1.0ugg^{-1}$ limit recommended by the United States Food and Drug Administration (US FDA). Out of the fifty samples analysed thirty two samples had levels less than $0.1\mu gg^{-1}$. Fifteen samples had levels more than 0.1µg/g but less than 0.2µg/g. New age cream and skin maxitoner cream recorded 0.253 and 0.271µg/g respectively. Diva maxitone cream recorded the highest level of 0.549µg/g but also within the recommended limit. The concentration of mercury in skin lightening creams has been the subject of study in recent years and the mercury content of some creams have been reported in Ghana (Voegborlo *et al.,* 2008). In a similar study of creams obtained in the Saudi Arabian market, mainly originating from Asia and the Middle East using Inductively Coupled-Plasma Spectrometry (ICP), mean mercury concentration was 3.76µg/g with a range from 0 to 5.65µg/g (Al-Saleh and Al-Doush, 1997) which was excessively higher than the US FDA permissible limit. Though effects of mercury poisoning on the skin itself have not been reported in relation to the use of mercury containing soap and cream, other toxic effects have been reported. Marzulli and Brown (1972) found that the use of skin lightening creams containing inorganic mercury salts result in substantial absorption and accumulation of mercury in the body. Repeated application of these skin toning creams could cause cumulative effect of prolonged low level mercury exposure, which could lead to nephritic syndrome (Giunta *et al*., 1983; Rosenman *et al.,* 1986; Emwonwe, 1987). Mercury can also be transferred from the mother to the fetus during pregnancy (Kuhnert *et al.,* 1981; Lauwerys *et al*., 1987). Mercury from soap and cream has been reported to be readily absorbed through the skin and via inhalation (WHO, 1991; Al-Saleh *et al.*, 2004). An average of 0.1 mg could develop the nephritic syndrome (renal diseases). In another study

carried out in Tanzania some women who were not active in artisanal gold mining had up to 0.1 mg mercury per liter of urine, and it was concluded that the mercury was derived from bleaching soap and cream containing mercury (Kahatano *et al*., 1998). A 46 year old woman developed membranous nephropathy following the use of a mercury-containing skin lightening cream. Results of a mercury analysis revealed that the cream contained 1% mercury by weight (Oliveira *et al*., 1987). Most of the creams analysed in this study originate mainly from Italy (30), India (5), United Kingdom (4) and Spain (3), where it is possible that manufacturing regulation concerning skin lighting creams so far as mercury is concerned is being adhered to. The wide range of skin lightening creams on the market as well as their wide use by some women in an attempt to convert their dark skin to fair skin indicate that the bleaching effect from the use of the creams may be due to other active ingredients other than mercury added to the creams. Thus as far as mercury is concerned it can be concluded that there may be no potential mercury related health risk from the use of the creams studied. One of the other active ingredients that may be added to the creams is hydroquinone.

The results of this study showed that out of the fifty samples analyzed, forty four creams contains up to 1.0% of hydroquinone. Fair & white Exclusive lotion gave 1.94 % and Zarina cream recorded 1.63% of hydroquinone. Bioclaire cream recorded 2.03% of hydroquinone. Niuma skin lightening lotion recorded the highest hydroquinone concentration of 3.45% followed by Akagni cream (3.36%) and Biotone skin lotion (3.08%). Bioclaire lightening cream contained 2.03% which is also above the recommended value. New age cream, Claire dark cream, Cream A3 lotion, Dermatological E45 lotion, Nivea Intensive lotion, Jergens Soothing cream, Skin maxitoner cream, Tenovate cream, Clear essence cream, and Clear spot cream contained non detectable levels of hydroquinone. In total, 92% of the creams analysed recorded

levels below 2% hydroquinone which is the threshold limit and 8% of the creams analysed contained more than 2% hydroquinone which are above the threshold limit. The concentrations of hydroquinone in skin lightening creams have also been the subject of study and hydroquinone content of some creams has been reported in the United Kingdom (Boyle and Kennedy, 2006). Eight out of forty one cream samples analysed were found to contain more than two (2%) percent hydroquinone which is the maximum concentration permitted by the United Kingdom cosmetic product regulations. In a similar study of creams obtained from the open market in Plateau state, Nigeria, ten cosmetic creams containing hydroquinone were subject to chromatographic test for identification. All ten creams sampled gave positive results to the test for hydroquinone. The level of hydroquinone was below two (2%) percent for seven of the creams, between 2-5 % for two and above 5 % for one (Odumoso and Ekwe, 2010).

High levels of hydroquinone detected in the cream samples analyzed poses potential hydroquinone related health risk to the consumer. Exogenous ochronosis is a paradoxical hyper-pigmentation of the skin caused by long-term use of hydroquinone-containing bleaching creams. Ochronosis is an uncommon condition characterized by yellow-brown pigmented deposits in the dermis. Two cases of exogenous ochronosis in two female patients of the sub-Saharan African population were reported (Bongiono *et al.*, 2005). In one case the lesions were characterized by an asymptomatic hyper-pigmentation of the face with gradually progressive blue-black macular patches, and in the other case in addition to dyschromic lesions, *striae atrophicae* were present. This phenomenon is the outcome of the use of skin care products containing high concentrations of hydroquinone- and glucocorticoid-based products, and, in addition, certain modalities in the use of bleaching products are likely to facilitate complications (Bongiono *et al.*, 2005). Hydroquinone has been used for decades as a skin lightening agent. As a result of concerns about

mid-term effects like leukomelanoderma, its use in cosmetics has been banned in the Netherlands since January 2001 (Kooyers and Westerhof, 2005). Until recently no attention was paid to potential long-term side-effects, despite the fact that there are indications that these may exist. It was decided that a clearer picture of these potential long term effects was needed. It appeared that since 1996 an enormous amount of articles have been published on the carcinogenicity of hydroquinone, benzene and related molecules (Kooyers and Westerhof, 2005). The literature search on hydroquinone as a skin lightening agent suggests that possible long-term effects like carcinogenesis may be expected. The risks of long-term effects (cancer) of topically applied hydroquinone may no longer be ignored. A 23-year-old left-handed white male was referred with a complaint of brown discolouration of the fingernails of his left hand. He had been applying 4% hydroquinone cream to facial melasma. The discolouration occurred within 2 months of cessation of hydroquinone (Parlak *et al.*, 2004).

CHAPTER FIVE

5. Conclusion

From the outcome of this research it can be concluded that:

- Total mercury concentration in the creams analysed ranged from 0.010 to 0.549 µg/g. Mercury concentrations in the samples are below the FAO/WHO threshold limit of 1 μgg^{-1}. This means that the general public may not be at risk through the use of toning creams as far as mercury is concerned.
- Hydroquinone concentration raged from below detection to 3.45 %.
- Eight percent of the creams analysed had hydroquinone concentration above the WHO threshold limit of 2%. This is very alarming and consumers who apply any of these creams are at risk.

5.1 Recommendations.

- Routine analysis should be conducted to ascertain the levels of mercury and hydroquinone in creams sold in Ghana.
- Manufactures should be encouraged to state the exact amount of bleaching agents in creams.

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