INVESTIGATION OF CONSTITUENTS AND EFFECTS OF THE DIFFERENT CONCENTRATIONS OF HYDROQUINONE IN BLEACHING CREAMS

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BU/UP/2019/1699

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE AND EDUCATION DEGREE IN BUSITEMA UNIVERSITY.

APRIL 2023

DECLARATION

I ALOO PATIENCE declare that the information here is my own piece of work unless where reference is cited. The work has never ever been submitted to any other institution for any award or publication.

Signature.. Date..27TH FEB 2024

This project report has been submitted for examination with approval ofthe following supervisor:

....

Mr. Musagala Peter

ACKNOWLEDGEMENT

I thank the Almighty God who has enabled me do the project and the course at large. And glory goes back to him.

I would like to extend my sincere appreciation to my project supervisor Mr. Musagala Peter for his parental efforts, guidance and words of encouragement that made me successfully complete this project. I really do not take your help and guidance for granted, May the Almighty God bless you abundantly and continue blessing the work of your hands.

I extend great thanks to the chemistry technician Madam Nakijoba Lydia for her assistance during the process of lab work and may the Almighty God bless you abundantly.

I whole heartedly thank my parents for always sacrificing their finances to support me throughout my studies and I pray that the Almighty God blesses you.

I finally appreciate my friend Kyotalye Brian, Watila Joshua, Omuroke David N for their cooperation and team work during the research.

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ABSTRACT

Hydroquinone has been used for decades as a skin lightening agent. The use of skin lightening agents has its drawback in terms of causing harmful effects such as skin disorders like depigmentation, rashes, pimples, discolorations, kidney damage, and cancer, neurological and psychiatric disorders depending on how the agents for skin lightening are used.

A total of 3 samples of different skin whitening cosmetics were collected from local market.

They were analyzed by using thin layer chromatography and spectroscopy for qualitative and quantitative determination of their hydroquinone contents.

The hydroquinone was extracted from samples by using 96% ethanol and was subjected to TLC analysis and the exact concentrations. 3 were found to contain hydroquinone.

ABBREVIATIONS

SLC: Skin Lightening Creams.

WHO: World Health Organization

DDW: Double Distilled Water

HQ: Hydroquinone

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Generally, across the globe including Uganda, white color is regarded as a feature of beauty, superiority and prettiness. In Uganda today, one cannot ignore the sight of some light skinned women with dark feet and elbows, commonly nicknamed as Fanta face and coca cola legs. Both females and males in Uganda today use skin lightening creams (SLC) with no idea about the side effects of their constituents. The different bleaching products that they apply contain a number of chemicals for example hydroquinone, steroids, vitamin C, mercury, lead, arsenic and Magnesium ascorbic phosphate which may be harmful to human health

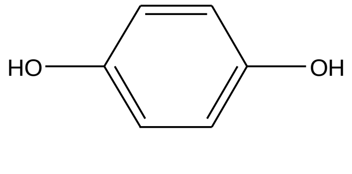
However according to the World Health Organization (WHO),the use of skin lightening products has its drawbacks in terms of causing harmful effects to human health such as rashes,pimples,discolorations,kidney damage and skin cancer depending on how the skin lightening agents are used.

Hydroquinone is one of the chemicals used in skin lightening cosmetics, which is a strong inhibitor of the melanin production process and hence lightening the skin.

Primary constituents of hydroquinone(Organization 1994)

Chemical formula: **$C_6H_4(OH)_2$**

Chemical structure:



Relative molecular mass: **110.11**

Common name: **Hydroquinone**

Synonyms: **1,4-dihydroxybenzene**

1,4-benzenediol

Hydroquinone works in a way that it decreases the production and increases the degradation of melanin pigments of the skin. This therefore increases the risk of skin exposure to the ultraviolet A ray which has a longer wave length and is associated with skin aging and risks of exposure to ultraviolet B with shorter wavelength and is associated with skin burning, which therefore increases the risk of acquiring skin related diseases like skin cancer.

According to the hydroquinone health and safety guide book that was drawn by the world health organization, WHO, in 1996, it was recommended that the concentration of Hydroquinone in skin lightening creams should be between 1.5% and 2%, but however reports show that in some bleaching creams the concentration of hydroquinone is higher than that recommended by the world health organization.(Amponsah, Voegborlo et al. 2014)

Ugandans using these creams are ignorant about the concentrations of hydroquinone in the bleaching creams they use and therefore ignorant of its effects on their skin. Therefore, this research will aid enlighten Ugandans on the constituents and effects of hydroquinone in bleaching creams that are used by Ugandans.

1.2 PROBLEM STATEMENT

People globally especially Ugandans use bleaching creams ignorant about the quantities of hydroquinone (chemical which prevents production of melanin) in the cream.

The significance of this research is to quantify the concentration of hydroquinone in bleaching creams and enlighten the users on the effect of the concentrations on the skin.

The concentrations will be comparison to the recommended concentration by the WORLD HEALTH ORGANIZATION (WHO).

1.3 OBJECTIVE OF THE RESEARCH STUDY

General objective

The research study is to investigate the concentration and the effects of the concentrations of hydroquinone on the skin lightening creams (bleaching creams) used by Ugandans

Specific objectives

1) To determine the concentration of hydroquinone in different bleaching creams used by Ugandans
2) To enlighten Ugandans on the effect of the concentrations on their skins

1.3.1 Scope of study

The study is to determine concentration of hydroquinone in bleaching creams in Uganda and the sample will be got from different cosmetic shops in tororo and mbale city.

The research is to take two months from October 13^{th} to December 13^{th}.

1.4 JUSTIFICATION

For purposes of beauty and attraction, women and some men in Uganda use different cosmetics and some which may include skin lightening creams. Therefore, in the process their skins get bleached.

1.5 RESEARCH QUESTIONS

a) What are the constituents of hydroquinone in selected SLC

b) What are the effects of the different concentrations of hydroquinone in the bleaching creams

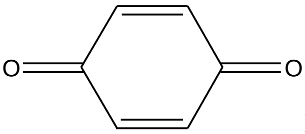
c) How does hydroquinone in the bleaching creams cause the negative effects on the skin of the consumers

1.6 LITERATURE REVIEW

1.6.1 INTRODUCTION

Hydroquinone is a substance which occurs naturally as hydroquinone β-D-glucopyranose in the leaves of several plants for example bearberry, cowberry, and cranberry, usually accompanied by its methyl arbutin.

Hydroquinone was first described in 1844 by woehler, who obtained it by addition of hydrogen to 1, 4-benzoquinone.



1, 4-benzoquinone

Hydroquinone is also produced from hydrolysis of arbutin forming hydroquinone and glucose in hot dilute aqueous acid.

1.6.2 SOURCES OF HYDROQUINONE

Hydroquinone occurs in a variety of forms as a natural product from plants and animals. It has been found in non-volatile extracts of coffee beans and other foods, and as Arbutin that is to say a glucoside of hydroquinone in the leaves of blueberry, cranberry, and cowberry and bearberry plants. Hydroquinone is considered to be the most important component of the allelopathic interaction between the perennial weed leafy spurge and the small everlasting.

Hydroquinone is also found in the bombardier beetle where it is involved in defensive biochemistry, the beetle can shoot a hot cloud of Quinone, formed by the action of hydrogen peroxide, hydroquinone and catalase-peroxidase in the explosion chamber of the beetle, towards an oncoming enemy.

Hydroquinone can also occur as a metabolite in the biodegradation of substituted phenols.

1.6.3 PHYSICAL PROPERTIES OF HYDROQUINONE

In pure form hydroquinone is a colorless crystalline solid

In stable α-form, three crystalline modifications exist, at melting point between173.8 – 174.8, is obtained as hexagonal needles by crystallization in water.

a) Commercial grades of hydroquinone are typically white to off white crystalline material solution of hydroquinone are discolored by oxidation with air.
b) The dipole moment, for hydroquinone is 1.40
c) Enthalpy of formation of HQ is -261.71kjmol^{-1}, the enthalpy of combustion of hydroquinone is -2.7×10^{3}kjmol^{-1} and heat of fusion at melting point of 27.1kjmol^{-1}
d) Hydroquinone has a boiling point of 285^{o}C at pressure 101.3KPa, critical temperature of 549^{o}C, critical volume of 3.0×10^{-4}m^{3}mol^{-1} and a critical pressure of 7.45 MPa.
e) Hydroquinone has a Gibbs free energy of formation of -176.13kjmol^{-1}, absolute entropy changes of 344.17jmol^{-1}K^{-1} and flammability limits of volume 1.6 % to 15.3%
f) It has Vander Waals volume of 5.998×10^{-5}m^{3}mol^{-1} and Vander Waals area of 8.46×10^{5} m^{2}mol^{-1}.
g) The solubility of hydroquinone per 100g of solvent at 30^{o}C is as below
    1. Ethanol is 46.4g
    2. Acetone is 28.4g
    3. Water is 8.3g

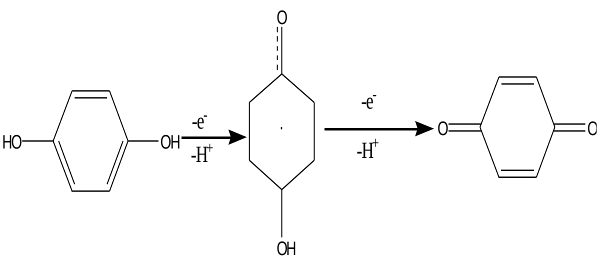
4. Benzene is 0.06g

5. Tetra chloromethane is 0.01g

2.4 CHEMICAL PROPERTIES OF HYDROQUINONE

The rate of oxidation of hydroquinone by air is accelerated in alkaline solution. The oxidation product can be added to hydrogen peroxide to give 1, 2, 4-benzenetriol, subsequent oxidation may result in formation of humic acids.

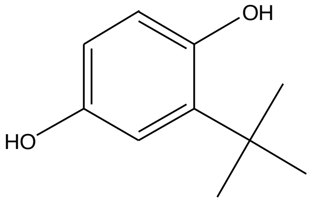
Oxidation of hydroquinone forms P-benzoquinone. Oxidation of hydroquinone involves loss of an electron and elimination of a proton to give the relatively stable semi benzoquinone radical. A second one-electron transfer and proton elimination results in formation of P-benzoquinone.

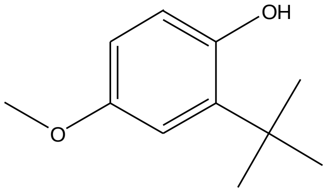


Neutral aqueous solutions of hydroquinone darken on exposure to air.

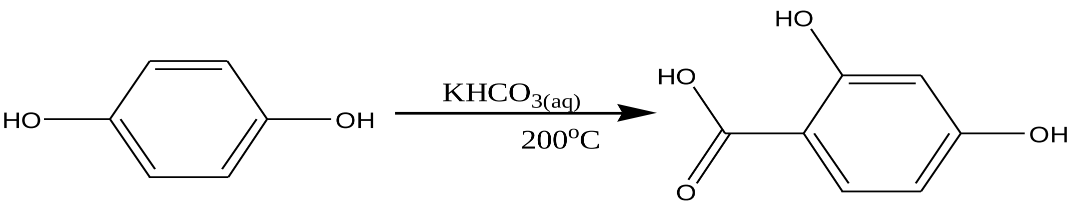
The redox potential E_0 of hydroquinone is 699mV, the half-wave potential of hydroquinone at pH 0, $E_{1/2}$, is 560mV and at pH 5, $E_{1/2}$, is 234mV.

Hydroquinone and its esters undergo Friedel crafts conditions to produce a variety of mono-substituted and di-substituted products. Among the commercially important alkylated derivatives are 2-tert-butylhydroquinone [1] and 2-tertbutyl-4-methoxyphenol [2].

1 

2 

Hydroquinone undergoes Kolbe-Schmitt carboxylation under $200^{o}C$ forming 2,4-dihydroxybenzoic acid using Potassium hydrogen carbonate as the carboxylating agent.

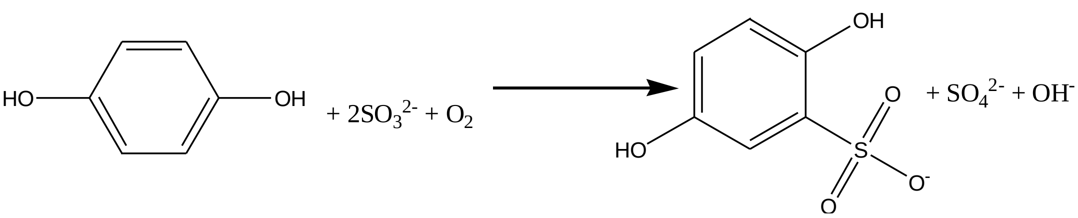


Above $200^{o}C$ yields a decrease notably due to known decarboxylated route, which is the back reaction to the reagents. Here hydroquinone forms 2,5-dihydroxybenzoic acid.

Hydroquinone was selected to test for different reactivity and decarboxylation in the Kolbe-Schmitt carboxylation due to varied electron core structure, since hydroquinone has a positional isomer of resorcinol.

Anodic treatment of aqueous hydroquinone in presence of sodium sulphite forms hydroquinone sulphonates for solution which is not strongly acidic.

The auto-oxidation of hydroquinone in aqueous media is inhibited by presence of sulphite thus forming hydroquinone sulphonates preventing the oxidation of hydroxyl groups.



Hydroquinone reacts with alkyl amine to form substituted aryl amines for example P-N-methyl aminophenol commercially known as sulphate salt.

2.5 THE INDUSTRIAL PRODUCTION OF HYDROQUINONE

There are various process used in industrial production of hydroquinone as below

I. Hydroxylation of phenol
II. Oxidation of aniline
III. Hydro peroxidation of P-diisopropylbenzene

Hydroxylation of phenol is catalyzed with 70% aqueous hydrogen peroxide producing a mixture of hydroquinone and catechol. This done at 80^{o}C.

Other catalyst can also be used for example strong mineral acid, iron (ii) or cobalt (II) salt. The ratio of the products i.e. the hydroquinone and the catechol depend on the catalyst selected.

For example, catechol is the major product, if the reaction proceeds by an ionic mechanism in which hydrogen peroxide is polarized by the strong acid catalyst and phenol is subsequently hydroxylated and resulting isomers separated be series of extractions.

The use of sharp-selective zeolite as catalyst for example use of vanadium-modified nafion per fluoro sulphate polymer gives the ratio of hydroquinone to catechol of 13:1, and thus yielding hydroquinone with 99% selectivity.

OXIDATION OF ANILINE

This is one of the oldest process used for production of hydroquinone. The production process suffers from the stoichiometric co-production of mineral salts which is the cause of serious environmental problems.

Aniline is oxidized with manganese dioxide in aqueous sulphuric acid at a temperature of (0-5)^oC to produce P-benzoquinone which is an intermediate. Hydroquinone is obtained from the formed intermediate P-benzoquinone by reduction with iron at 55-65 ^oC .

The product formed then crystallized, isolated from the typically aqueous stream by centrifugation and dried in a vacuum dryer. The overall yield of hydroquinone is 85%

HYDROPEROXIDATION OF P-DIISOPROPYLBENZENE

This process is a Friedel craft alkylation of benzene with propene. This process has been studied in more depth than other process for production of hydroquinone. P-diisopropylbenzene is converted to the dihydroperoxide by air oxidation under slightly alkaline conditions at 80-90 ^oC.

The Dihydroperoxide id separated from the reaction mixture by either extraction of by crystallization and then cleaved to hydroquinone.

The hydroquinone formed is crystallized and isolated and the yield is about 80% hydroquinone.

2.6 REACTION OF PHENOL AND BENZOPHENONE

Heating of a mixture of 2M phenol, 1M benzophenone and 0.5M CF_3SO_3H at 70^oC for about 19hours forms fuchsone. The formed fuchsone is the isolated.

The sample of fuchsone is synthesized from Ar_2CCl_2 or phenol, hydroquinone and benzophenone is got by reacting the fuchsone with hydrogen peroxide and traces strong acid.

This production process shows that is possible to produce hydroquinone from phenol and hydrogen peroxide via fuchsone in two stages.

2.7 USES OF HYDROQUINONE

One of the largest consumers of hydroquinone is the rubber industry, which requires hydroquinone for the production of antioxidants and antiozonants. Hydroquinone derivatives used in this area include N, N0 -diaryl-p-phenylenediamines for example N, N0 -diphenyl-phenylenediamine, dialkylated hydroquinone, N-alkyl-p-amino phenols, dialkyl-p-phenylenediamines, and aralkyl-p-phenylenediamines.

Hydroquinone derivative that is to say Hydroquinone dimethyl ether is used as a starting material for a family of dyes and pigments based on the 2-amino- and 2-amino 5-chloro derivatives. Quinzaine is an intermediate for textile dyes that is to say Anthraquinone Dyes and Intermediates.

The fungicide Chloroneb is produced from hydroquinone dimethyl ether as raw material. The herbicide ethofumesate is produced from p-benzoquinone. Fluazifop-butyl is representative of a new family of herbicides based on the o-alkylation of hydroquinone with 2-halopropionic acid derivative

Hydroquinone and several derivatives are used in formulation of skin bleaching and skin lightening agents for example Caro light, cocoderm,

The oxygen scavenging properties of hydroquinone are being exploited for use in boiler water treatment.

Hydroquinone and certain C-alkylated or C-arylated derivatives are useful monomers for the preparation of a variety of polymers, including liquid crystal polyesters for high-performance plastics, composites, and fibers. In addition to high tensile and impact strengths, these materials exhibit good weather ability, solvent resistance, flame retardance, transparency to microwave radiation, and retention of strength at elevated temperature.

Effects and restriction on the use of hydroquinone

Hydroquinone is restricted for use in cosmetics to 2% or less by the World Health Organization (WHO) and The US Food and Drug Authority.

Cases of intoxication have been reported after oral ingestion of hydroquinone alone or of photographic developing agents containing hydroquinone. The major signs of poisoning included dark urine, vomiting, abdominal pain, tachycardia, tremors, convulsions and coma. Deaths have been reported after ingestion of photographic developing agents containing hydroquinone. In a controlled oral study on human volunteers, ingestion of 300-500 mg hydroquinone daily for 3-5 months did not produce any observable pathological changes in the blood and urine.

Combined exposure to hydroquinone concentrations causes eye irritation, sensitivity to light, injury of the corneal epithelium, corneal ulcers and visual disturbances.

There have been cases of appreciable loss of vision. One report described cases of corneal damage occurring several years after the exposure to hydroquinone had stopped. There are no adequate epidemiological data to assess the carcinogenicity of hydroquinone in humans.

CHAPTER TWO

EXPERIMENTAL

In this chapter, I used Thin Layer chromatography for qualitative analysis and highly sensitive spectrometric method for quantitative determination of hydroquinone the skin lightening creams.

2.1 Qualitative analysis.

I used TLC to find if hydroquinone present in the skin lightening creams.

2.2.1 Apparatus

Weighing was done using ohaus corporeation7 campus drive weighing balance, 310 Parsippany NJ 07054, USA.

Measuring cylinder was used for measuring volumes of solutions, beaker were used for holding and mixing solutions, (J.V chemical, Bengaluru) TLC plates were used for analyzing the mixtures of the SLCs.

Water bath was used to maintain constant temperature, boiling Tubes were used for boiling solutions which required boiling,

Spectrum bands, NC, OP hair dryer was used to dry the TLC plate,

Global finishing solutions oven Osseo Wisconsin, USA was used to dry glassware used,

(VICI valvo instruments) developing tank was used to run TLC plates for separation.

Filter papers were used for separating a mixture of solid and liquid flow throw a semi-permeable barrier

2.2 Materials

All chemicals that were used are of analytical grade, they include the following. Ethanol, Water, Caro light cream, Coco Dem cream, Betasol beauty cream.

2.3 Sample preparation

Skin lightening cream (2 g) was weighed into a beaker, Ethanol (15 mL) was added, the mixture was transferred into a water bath of temperature 60^{o} C for 10 minutes, and then cooled in an iced bath until the separation fats occurs. The mixture was then filtered and the filtrate used for TLC analysis.

The procedure for sample preparation was repeated for all the 3 skin lightening creams.

2.4 TLC procedure.

The TLC plates were prepared using silica gel and water. The plates are then dried using a dryer.

A small drop of about diameter 2 mm of each sample is deposited on the plates. The plates then placed on a developing tank containing an appropriate mobile phase for about an hour.

The solvent front was then marked and the plates dried. Visualization was then performed and distance moved by the component marked. The Rf values was calculated and tabulated.

Quantitative analysis

I used highly sensitive spectrometric method to determine the quantity of hydroquinone present in the different skin lightening creams

2.5 Apparatus

Absorption spectra were recorded on a UV-VIS cole-parmer double beam spectrometer (cole-parmer Ltd, 2008, England.

Volumetric flask was used for measuring accurate volumes of liquids used, Oven was used for drying glassware used, and Glass rod was used for stirring solutions,

A black wall PTFE ltd microcell Quartz cuvette was used to hold samples for spectroscopic analysis.

2.6 Materials

All the chemicals used were analytical grade and they include the following, hydroquinone, Caro light cream, Coco Dem cream, Betasol beauty cream, The creams, Sulphuric acid.

2.6 Procedure

2.6.1 Preparation of standard solution

A Standard solution of Hydroquinone (1.00 g) was dissolved in 100 mL sulphuric acid (0.05M), and the solution was used as the standard solution.

2.6.2 Preparation of stock solution

Each sample (1 g) was dissolved in 20 cm3 of Sulphuric acid (0.05M) in a water bath. This solution was then transferred into volumetric flask ($25cm^3$) and Made to volume with the sulphuric acid. The solution was then filtered with a filter paper then discarded the first 5 times.

The filter paper was then rinsed with additional 5 cm3 of sulphuric acid to remove any retained sample. The Concentration of hydroquinone for each sample of the SLC was determined using a highly sensitive UV spectrophotometer at a wavelength of 290 nm (determined maximum absorbance) using quartz cuvette.

CHAPTER THREE

3.1 RESULTS AND DISCUSSION OF RESULTS

Table 1

Sample no	Sample name	Rf value
1	Caro light cream	0.51
2	Cocoderm cream	0.50
3	Betasol cream	0.80

3.2 Estimation of maximum absorption of hydroquinone

To determine the maximum absorption, standard solutions of hydroquinone in concentration of 40 ppm were prepared.

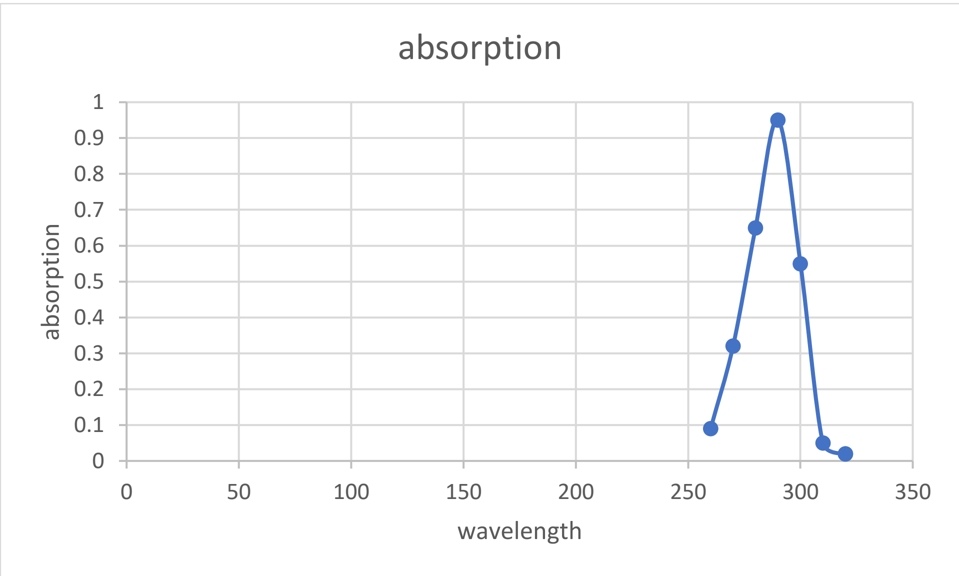
Wavelength range of HQ from 260 nm to 320 nm showed a maximum absorbance (ƛmax) at 290 nm as shown in table 2

Table 2

Wavelength (nm)	Absorption
260	0.090
270	0.320

280	0.650
290	0.950
300	0.550
310	0.050
320	0.020

Figure 1



3.3 DISCUSSION OF RESULTS

3.3.1 Calibration curve

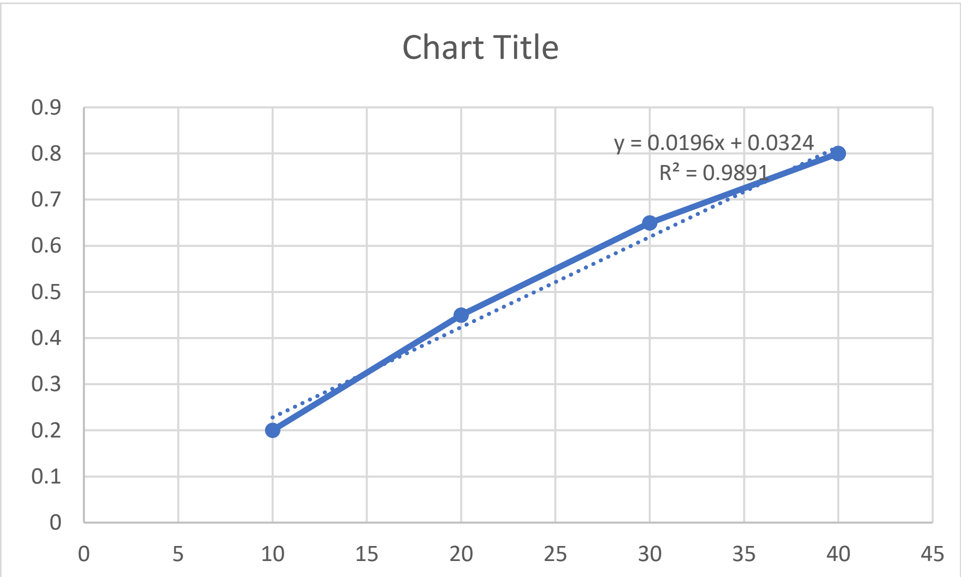
After determination of the maximum absorption of hydroquinone (290 nm) using spectrophotometer, The absorbance was then taken at a wavelength of 290 nm and traced on the calibration curve to give the concentration of hydroquinone in each samples. The calibration curve was obtained from Hydroquinone standard by serial dilutions of concentrations 10, 20, 30, and 40 ppm. Under the optimum experimental conditions, a good linear correlation was obtained between the absorbance and hydroquinone concentrations in the range 10,20,30,40 µg|mL.

For carolight cream

Table 3

HQ conc.(µg|mL)	Absorbance
10	0.2
20	0.45
30	0.65
40	0.80

Figure 2



For cocoderm cream

Table 4

HQ conc.(µg|mL)	Absorbance
10	0.15
20	0.30
30	0.60
40	0.85

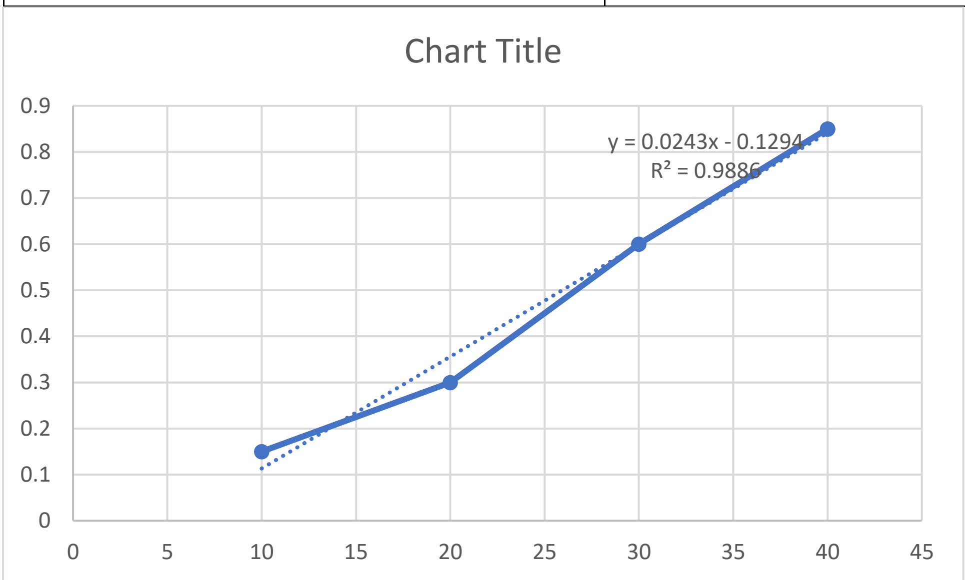


Figure 3

For Betasol cream

Table 5

HQ conc.(µg|mL)	Absorbance

10	-0.05
20	0.23
30	0.43
40	0.70

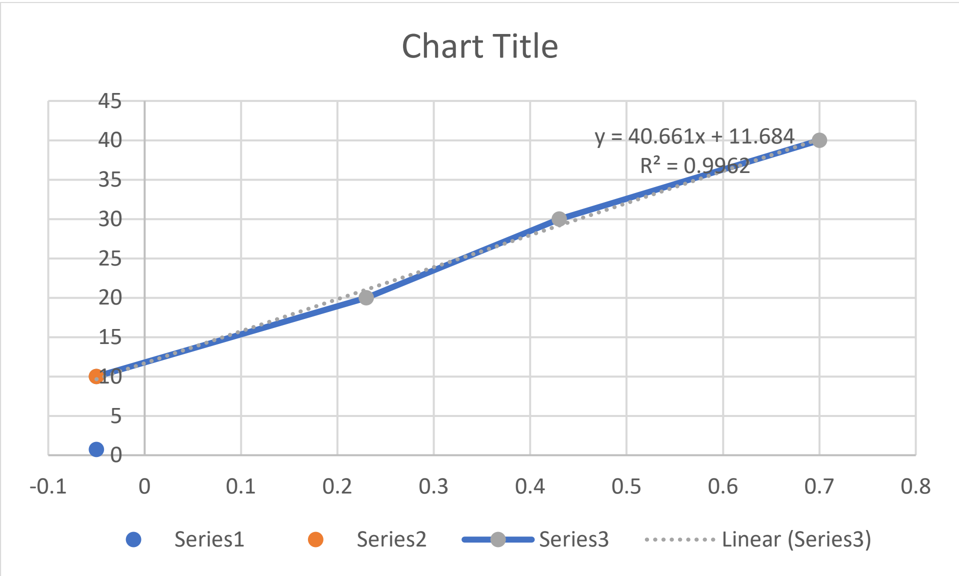


Figure 4

For carolight

Table 6

y=0.0196x+0.0324

R^2= 0.9891

Y is the absorbance, x is the concentration extinction constant (ϵm) =0.0196

absorbance	Concentration value of HQ(µg|mL)
0.2	8.551
0.45	21.306
0.65	31.51
0.8	39.163

Mean concentration=25.1325µg|mL

For cocoderm

Y=0.0243x+-0.1294

Table 7

Absorbance	Conc
0.15	11.4979
0.30	17.6708
0.60	30.0164
0.85	40.30

Mean concentration=24.87µg|mL

For betasol

Table 8

Y=40.661x+11.684

absorbance	Conc
-0.05	18.85
0.23	21.55
0.43	31.43
0.7	33.21

Mean concentration= 26.26µg|mL

26.26 microgram/mL = 0.002626x1000percentage=2.626g%

25.1325 microgram/mL = 0.00251325x 1000g percentage=2.513g%

24.87 microgram/mL = 0.002487 percentagex1000g= 2.487g%

Table 9

Cream	%concentration of hydroquinone
Carolight cream	2.626
Coco derm cream	2.487
Betasol cream	2.513

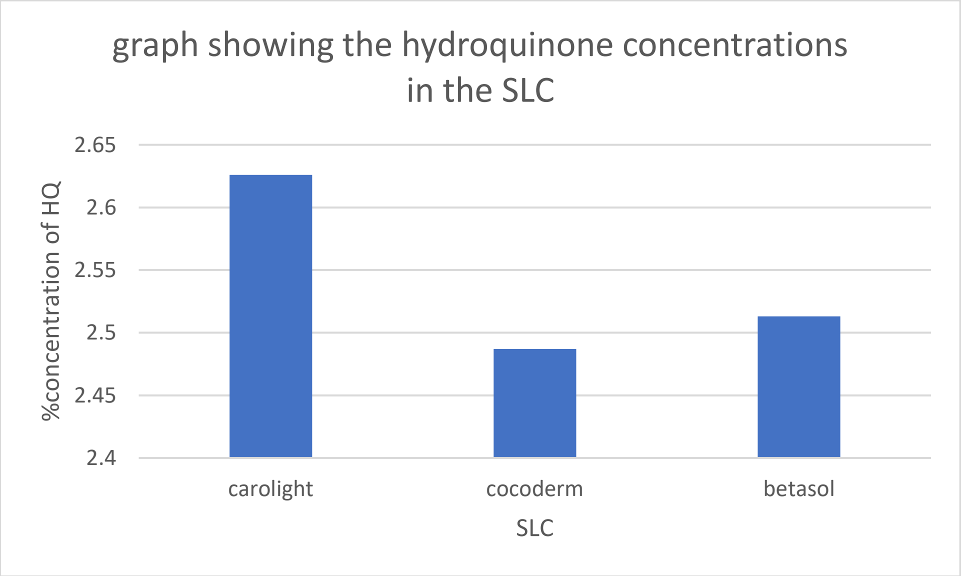


Figure 5

CHAPTER FOUR

Conclusion

The above SLC contains high concentrations of hydroquinone which is above the 2% as recommended by the WHO. From the investigation, it is evident that most of creams contain amounts of hydroquinone that are within the unacceptable range. Therefore these levels may cause permanent skin damage, disfigurement or are carcinogenic.

Recommendation

First, I highly recommend the research since it is for the wellbeing of peoples health.

For researchers on the same topic of hydroquinone in bleaching creams, I would like to recommend the use of HPLC for quantitative analysis that is to say determine the concentration of HQ rather than spectrometric method in order to minimise errors in the process.

I would also advice women out there and some men who would like use SLC to first make thorough research on the effects of SLC before using any them

Further studies should focus on affordable means on how to determine the concentration of HQ in SLC in order for the people to easily select on the creams to use and those to avoid, instead of HPLC and spectrometric method which is expensive.

Lastly I would like to encourage women and men to be comfortable in their skin color to avoid the bad health side effects of skin lightning.

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