SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal

ESTIMATION OF QUININE SULPHATE BY USING PHOTO FLUOROMETER

Rutuja Santosh Aghav[1*] Yogiraj P. Muley[2] Gokul Nagorao Rathod[3]
[1]*Student of bachelor's in pharmacy, Dr. Babasaheb Ambedkar Technological University, Raigad, Lonere.*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Chemistry, Dr. Babasaheb Ambedkar Technological University, Raigad, Lonere*
[3]*Student of bachelor's in pharmacy, Dr. Babasaheb Ambedkar Technological University, Raigad, Lonere*

[*]*Corresponding Author: Rutuja Aghav*

ABSTRACT
Estimation of Quinine Sulphate Using a Photo Fluorometer Quinine sulphate, a widely used antimalarial drug, can be accurately quantified using photo fluorometry due to its inherent fluorescence properties. This study aims to develop and validate a sensitive, precise, and reproducible method for the estimation of quinine sulphate in pharmaceutical formulations.

The procedure involves preparing standard solutions of quinine sulphate and measuring their fluorescence intensity at an excitation wavelength of 350 nm and an emission wavelength of 450 nm. The calibration curve, constructed by plotting fluorescence intensity against quinine sulphate concentration, demonstrates linearity within the range of 0.1 to 10 µg/mL, with a correlation coefficient (R^2) greater than 0.99. The method exhibits high sensitivity, with a detection limit of 0.05 µg/mL and a quantification limit of 0.1 µg/mL.

KEYWORDS : *Quinine sulphate, Photo fluorometry, Fluorescence, Pharmaceutical analysis, Method validation, ICH guidelines.*

1. INTRODUCTION

The estimation of quinine sulphate using a photo fluorometer involves a procedure that utilizes fluorescence spectroscopy to determine the concentration of quinine sulphate in a sample. This method is based on the fluorescent properties of quinine sulphate when excited by specific wavelengths of light.

Key steps in this process include preparing standard quinine solutions of known concentrations, measuring their fluorescence intensity, plotting a calibration curve, measuring the fluorescence of the unknown sample, and using the calibration curve to determine the sample's concentration. Fluorescence spectroscopy offers a fast, simple, and cost-effective approach for quantifying analyte concentration through its fluorescence behaviour under specific light excitation.

The estimation of quinine sulphate using a photo fluorometer involves utilizing the fluorescence properties of quinine sulphate to quantitatively determine its concentration in a solution. Quinine sulphate is a compound known for its fluorescent properties when exposed to ultraviolet (UV) light. This characteristic makes it suitable for analysis using a photo fluorometer, a device designed to measure the intensity of fluorescence emitted by a substance.

The introduction may also briefly describe the principle behind fluorescence spectroscopy, emphasizing how molecules like quinine sulphate absorb light energy at specific wavelengths and re-emit it at longer wavelengths, resulting in fluorescence. This phenomenon allows for sensitive and selective detection of quinine sulphate in solution.

2.RESARCH PROBLEM

2.1 Malaria

Malaria is caused by parasite transmitted from one person to another through the biting of certain species of mosquitos in parasite endemic regions.Those at creates risk of severe from of the disease the age of 5 years & pregnant women. An infection caused by a plasmodium parasite transmitted by the bite of infected mosquitoes.

Malaria mostly spreads to people through the bites of some infected female Anopheles mosquitoes. Blood transfusion and contaminated needles may also transmit malaria. Left untreated, P. falciparum malaria can progress to severe illness and death within 24 hours.

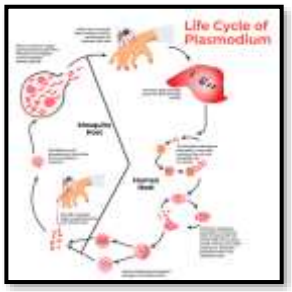
SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal

- **Life cycle of Malaria**

When a person is bite by a mosquito, sporocytes that are formed in the blood of the mosquito (from male and female hematocytes) enter the body. Then, sporocyte leaves circulation and localizes in the hepatocytes whereby they transform, multiply and develop into tissue schizonts. The primary asymptomatic tissue stage lasts for fifteen days and tissue schizonts rupture. Every tissue schizont releases thousands of merozites. Released merozites nvade more RBCs to continue the cycle.

Synchronous rupture of RBCs and release of merozites into the circulation leads to febrile patter attacks on day one and three; hence, designation is Tertian malaria'. Then the flagellation of male gametocyte is followed by male gametogenesis and fertilization of The female gametocytes in the mosquito's gut. The resulting zygote, which develops as an bocyte in the gut wall, finally gives rise to infective sporozoite, which invades the salivary glands of the mosquito. Then, by taking a blood meal, mosquito may infected another human.

Fig. 1 - Life Cycle of Malaria



- **History**

Malaria has troubled humans for thousands of years. Disease resembling malaria has been described for more than 5000 years. In 2700 BCE, tertian and quartan fever due to malaria was described in Nei Ching (The Canon of Medicine). The father of medicine Hippocrates noted the symptoms of malaria like disease in 4th century. He also linked malaria to appearance of Sirius the Dog Star. In Susruta (Sanskrit medical literature), the description of malaria like symptoms are given. Vedic literature (1500-800 BCE) called malaria the 'king of diseases'. Malaria is not seen in the books of Mayans or Aztecs. Alexander Great may have died of malaria. Romans attributed malaria disease to the swamps. [1]

- **Causative Agent**

Malaria is caused by protozoa of the genus Plasmodium.
Four species cause disease in humans:

1. Plasmoium falciparum
2. Plasmodium vivax
3. Plasmodium ovale
4. Plasmodium malariae[2]

- **Symptoms**

Requires a medical diagnoses: Chills, fever and sweating, usually occurring a few weeks after being bitten.
People may experience

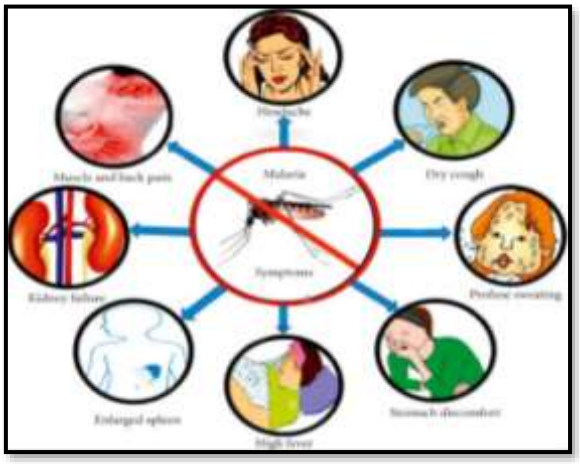
Pain areas: In the abdomen or muscles

Whole body: Chills, Fatigue, Fever, Night Sweats, Shivering, Or Sweating

Gastrointestinal: Diarrhoea, Nausea, Or Vomiting.

SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal

Fig.2 - Symptoms of Malaria



2.2 **Nocturnal Leg Cramps**

Tired muscles and nerve problems occurs nocturnal leg cramps. Kidney failure, diabetic nerve damage cause night leg cramps. The risk of night leg cramps increase with age, pregnant females are most likely the have night leg cramps.

Nocturnal leg cramps are common in older people. Such cramps are associated with many common diseases and medications. Physiological methods may be useful for preventing cramps in some people, but there have been no controlled trials of these approaches. Quinine is moderately effective in preventing nocturnal leg cramps. In patients with severe symptoms, a trial of 4±6 weeks' treatment with quinine is probably still justified, but the efficacy of treatment should be monitored, for example using a sleep and cramp diary.[3]

3.HYPOTHESIS STATEMENT

Hypothesis: The concentration of quinine sulphate in a solution can be accurately estimated using a photo fluorometer due to the direct proportionality between the fluorescence intensity emitted by the quinine sulphate and its concentration in the solution.

Explanation

Quinine sulphate is known to exhibit fluorescence when exposed to ultraviolet light. A photo fluorometer measures the intensity of this fluorescence, which is expected to be directly proportional to the concentration of quinine sulphate in the solution. Therefore, by calibrating the photo fluorometer with standard solutions of known quinine sulphate concentrations, it should be possible to estimate the concentration of unknown samples accurately.

4. METHOLOGY

4.1 Photo Fluorometer

Fluorometer is an instrument for measuring the intensity of fluorescence. A fluorometer involves using a beam of light, usually ultraviolet light. The emitted light is proportional to the concentration of the analyte being measured (up to a maximum concentration).

A fluorometer measures the fluorescence or light emitted by different fluorescing object. Fluorescence occurs when light of specific wavelength hits and excites electrons in a sample, and the electrons in that sample instantly emit or fluoresce light of a different wavelength.[4]

Common detector configurations of fluorometer include photomultiplier tubes and photodiodes. These parameters are used to identify the presence and the amount of specific molecules in a medium.

Photo Fluorometer designed for analysis of fluorophores such as vitamin, quinine, fluorescence metal complexes, etc. The Fluorescence is caused by the absorption of radiant energy and the re-emission of some of its energy in the form of light.[5]

SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal

- **History of Fluorometer**

Clair Loring Farrand (1895-1981), an inventor best known for his work with the cone loudspeaker, began his career as a Marconi radio operator. He established the Farrand Optical Company in 1941, bought a factory in the Bronx, and made optics for the U.S. Army and Navy during World War II. The firm moved to Mount Vernon, New York, in the mid-1960s.



Fig.4 – First Fluorometer

Definition of Fluorescence is a spontaneous emission of radiation from an electronically excited species (or from a vibrationally excited species) not in thermal equilibrium with its environment.

4.2. Fluorescence

When a beam of light is incident on certain substances they emit visible light or radiations. This is known as fluorescence. Fluorescence starts immediately after the absorption of light and stops as soon as the incident light is cut off. The substances showing this phenomenon are known as flourescent substances.

Fluorescence is one of two kinds of emission of light by a substance that has absorbed light or other electromagnetic radiation. Fluorescence involves no change in electron spin multiplicity and generally it immediately follows absorption; phosphorescence involves spin change and is delayed. [6]

4.3. Fluorescence Spectroscopy or Fluorometry

An analytical technique for identifying and characterizing minute amounts of a substance by excitation of the substance with a beam of ultraviolet/visible light and detection and measurement of the characteristic wavelength of fluorescent light emitted.

Fluorescence spectroscopy is a very sensitive and selective analytical technique for detecting and measuring trace amounts of organic compounds. The selective nature of this technique arises because each compound is characterized by an excitation.

- **X-ray Fluorescence Method**

In these methods, X-rays are generated within the sample and by measuring the wavelength and intensity of the generated X-rays, one can perform qualitative and patative analysis, X-ray fluorescence method is non-destructive and frequently requires very sample preparation before the analysis can be carried out.[7]

4.4. Principle

When any molecule absorbs UV/Visible radiation, its electrons transmit from singlet ground state to singlet excited state and as this excited state is not stable, it emits the radiation and returns to the stable singlet ground state. This phenomenon of emission of radiation is known as fluorescence. The fluorimeter is the measurement of this emitted radiation.

The emitted radiation (fluorescence intensity) is directly proportional to the concentration of the substance present which can be measured by fluorimeter. Quinine sulphate in 0.1 N sulphuric acid gives blue fluorescence and the fluorescence intensity can be measured by fluorimeter with the excitation wave length of 360 nm using primary filter and with the emission wave length of 485 nm using secondary filter.[8]

4.5. Instrumentation of Photo Fluorometer

- **Photo fluorometer is consist of –**
  1. Light source
  2. Filters or monocromators

SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal

3. Sample holder
4. Detector
5. Galvanometer[9]

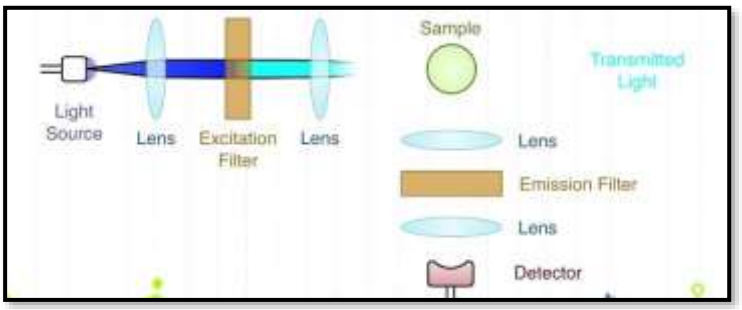


Fig.5 – Instrumentation of Photo fluorometer

A. Light Source

A mercuric vapour lamp with glass or fused silica is commonly used as source.

For more sophistication Instruments, high pressure xenon lamp used as source of radiation

I. **Deuterium and Hydrogen lamps**

A pair of electrodes is enclosed in a glass tube containing hydrogen or deuterium gas. When current is passed in electrodes electron discharge is occurring which exited the gas molecule which results in the emission of radiation (UV & Visible).

Wavelength: 160-800 am

Quartz window must be employed.





Fig. 4 Deuterium lamp **Hydrogen lamp**

II. **Xenon arc Lamp:** It consists of two tungsten electrodes form an arc at a specific distance and xenon gas is stored (under pressure) in quartz or fused silica tube. It emits radiation with a higher intensity (500 nm) than a hydrogen discharge lamp.

Wavelength: 750-1000 nm.



Fig. 2 - Xenon arc Lamp

III. Tungsten Halogen Lamp:

It is also known as a halogen lamp. It is an incandescent light source. It is consists of a filament made up of tungsten enclosed in a quartz vessel containing an inert gas and a small quantity of iodine or bromine (Halogen).

Its 85% emitted light lies in IR and near IR region, 15 % in the visible region, and less than 15% in the UV region.

SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal



Fig. 3 – Tungsten halogen lamp

IV. Mercury Vapor Lamp

These lamps are ideal light sources that provide high-intensity light in the deep UV to visible regions. It consists of 2 alloys (tungsten) electrodes which are placed together in a medium containing mercury vapor and 25-50 torr of pure argon gas. These electrodes are enclosed in an elliptically shaped in a silica glass tube. It provides clear white light, high intensity with 24000 Hrs. of life.



Fig. 4 - Mercury Vapor Lam

B. Filter And Monochromator

i. Filter

Filter is a device used to get selected wavelength. It allows the light pass through it but absorbed the light of different wavelength may partially and fully. A specific filter is used to obtain the desired wavelength for special analysis like Primary filter and Secondary filter.

Primary filter-absorbs visible radiation and transmit UV radiation.

Secondary filter-absorbs UV radiation and transmit visible radiation.

ii. Monochromator

They convert polychromatic light into monochromatic light. They can isolate a specific range of wavelength or a particular wavelength of radiation from the source.

a) **Excitation monochromators:** Provides suitable radiation for excitation of molecules.
b) **Emission monochromators:** Isolate only the radiation emitted by the fluorescent molecules.

C. Sample Holder

Cuvettes are used for the handling of samples. These are rectangular or cylindrical in shape with two rough and two smooth sides, and made up of glass, quartz or fused silica.

SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal



Fig.6. Sample Holder

D. Detector

Detector in a device which transforms light energy into electrical signals that are observed on recorder. The characteristic of ideal detector is give quantitative response, high sensitivity, low noise, shot response time, and response quantitative to wide spectrum of radiation received.Some commonly used detectors are as follows.

a) Barrier layer cell/Photovoltaic cell

It is consist of a coated silver of gold thin layer of metallic film which acts as an electrode and another metal plate acts another electrode. Both of the layers are separated by selenium layer that act semiconductor. When UV radiation falls on selenium layer, an electron become mobile and is taken up by transparent metal layer that results a potential difference between the electrodes & causes the flow of current.

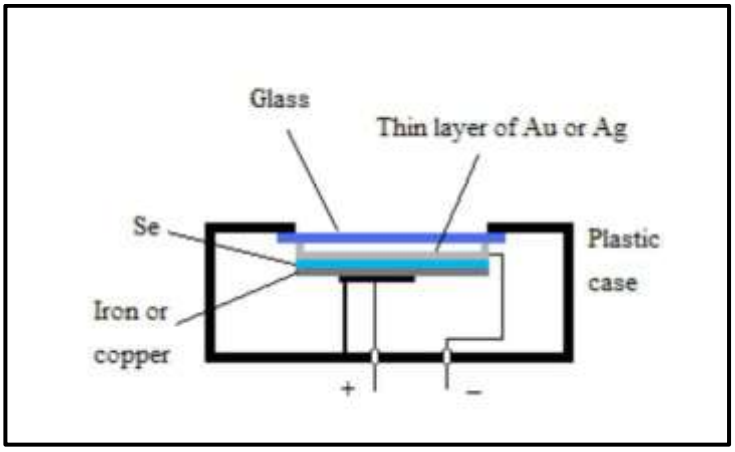


Fig. 8 - Barrier layer cell

b) Phototubes/ Photo emissive tube

It is consists of an evacuated glass tube with a photocathode and a collector anode. The surface of photocathode is coated with a layer of elements like cesium, silver oxide and its mistures. When radiant energy falls on photosensitive cathode, electrons are emitted which are attracted to anode causing flow of current. It is more sensitive than barrier layer cell.

SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal

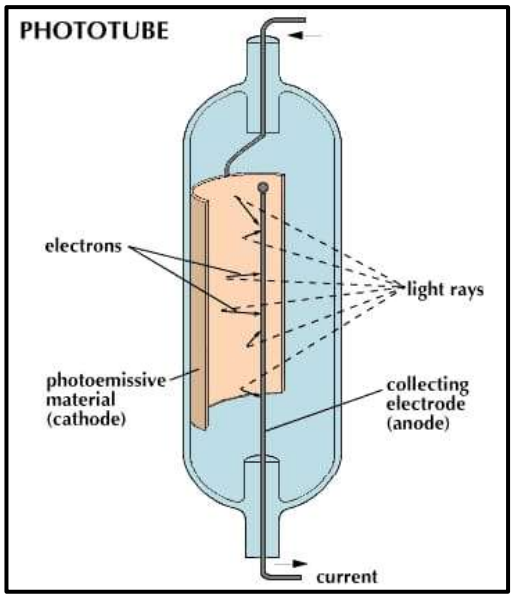


Fig. 9 - Phototubes

c) Photomultiplier Tube

Photomultiplier tube is multiply the photoelectrons by secondary emission of electrons. A primary photo-cathode is fixed in a vacuum tube which receives radiation from the sample. Some 08 to 10 dynodes are fixed each with increasing potential of 75-100V higher than preceding one. Near the last dynode electron collector electrode is fixed. It is extremely sensitive to light and detect weaker or low radiation.[10]

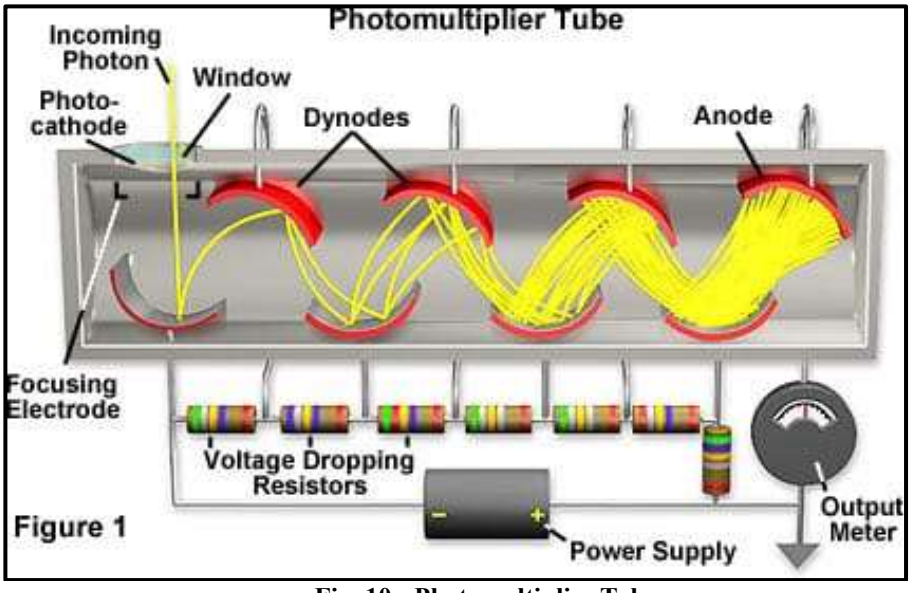


Fig. 10 - Photomultiplier Tube

4.8 Types of Photo Fluorometer:

Two main types of Photo Fluorometer –

1)Single beam photo fluorometer

2) Double beam photo fluorometer

1) Single beam Photo Fluorometer

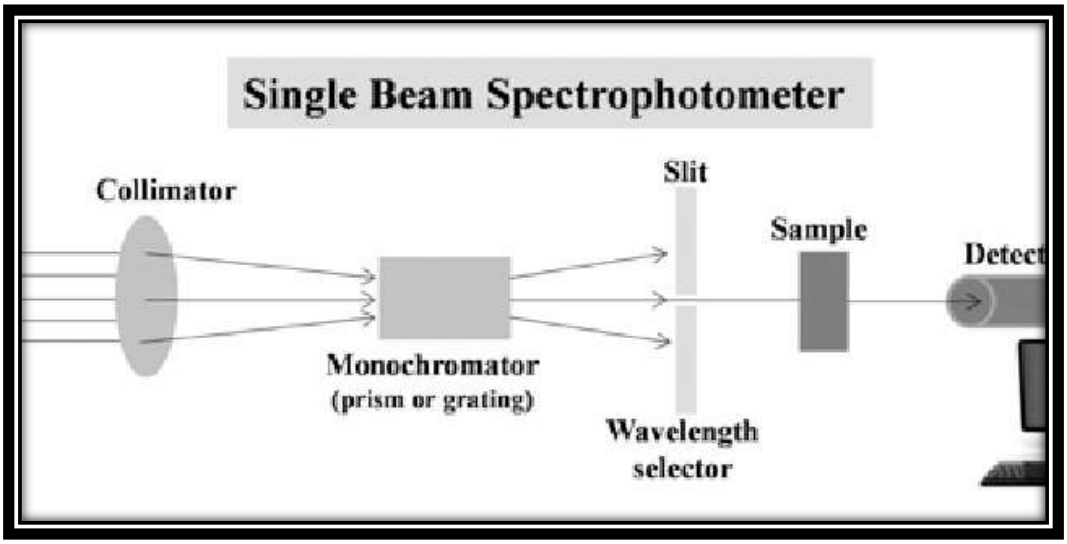
In single beam fluorimeter, all the light waves pass through the sample.

Primary filter-absorbs visible light.

Secondary filter-absorbs UV light.

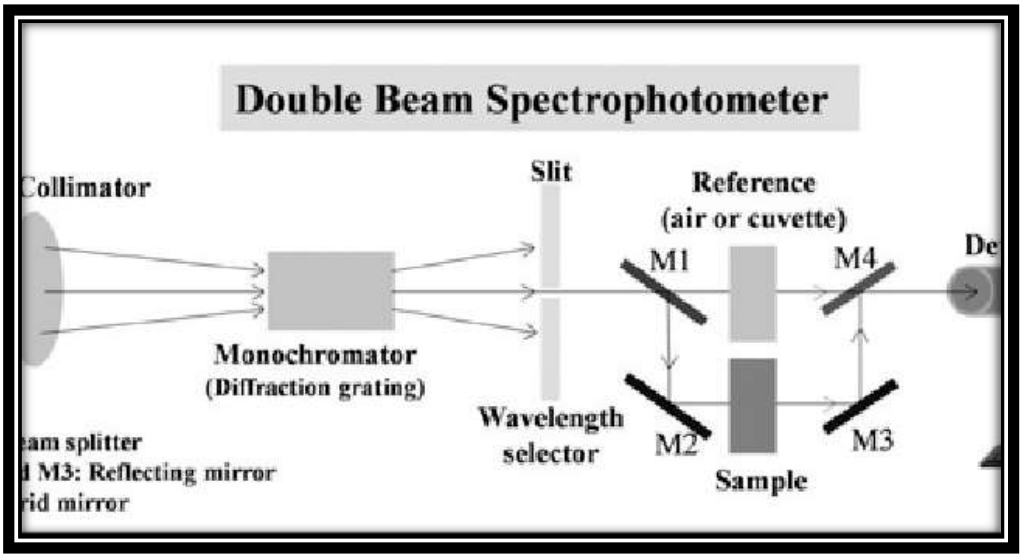
Reference and sample cant be analysed simultaneously.

SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal



2) Double Beam Photo Fluorometer

In double beam fluorimeter the light beam splits into two parts and only one part passes through the sample.
Primary filter-absorbs the light and passes to the sample.
Secondary filter - absorbs the light from the sample and sent to detector.
Reference and sample can be analyzed simultaneously.



4.6. Uses

- **Dairy Industry**

Fluorimetry is widely used by the dairy industry to verify whether pasteurization has been successful. This is done using a reagent which is hydrolysed to a fluorophore and phosphoric acid by alkaline phosphatase in milk. If pasteurization has been successful then alkaline phosphatase will be entirely denatured and the sample will not fluoresce.[11]

- **Protein aggregation and TSE Detection**

Thioflavins are dyes used for histology staining and biophysical studies of protein aggregation. For example, thioflavin T is used in the RT-QuIC technique to detect transmissible spongiform encephalopathy-causing misfolded prion

- **Oceanography**

Fluorometers are widely used in oceanography to measure chlorophyll concentrations based on chlorophyll fluorescence by phytoplankton cell pigments. Chlorophyll fluorescence is a widely-used proxy for the quantity (biomass) of microscopic algae in the water.

- **Molecular Biology**

Fluorometers can be used to determine the nucleic acid concentration in a sample.[11]

SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal

4.9 Advantages

1) **Sensitivity:** it is give accurate result even sample is present in microgram concentration in solution.
2) **Precision:** upto 1% can be achieved easily.
3) **Specificity:** This method is more specific than absorption method.

4.10. Disadvantages

1. Change in pH effect fluroscence
2. oxygen may decrease fluroscence.
3. Heavy metal presence are also decrease fluroscence.

4.11. Applications of Photo Fluorometer

1) Inorganic/organic chemistry

i. Determination of ruthenium
ii. Determination of aluminium in alloys
iii. Estimation of bismuth
iv. Determination of beryllium in Silicates
v. Determination of cadmium
vi. Assay of thiamine
vii. Estimation of quinine sulphate
viii. Investigation of chemical structures and reactions.

2) Biochemical Analysis

Fluorimetry helps in the analysis of concentration, structure, and interactions of compounds such as proteins, and nucleic acids, and their quantification.

Fluorometer is used in determination of alkaline phosphatase enzyme in milk.[12]

3) Biology & Biotechnology

Fluorometer are used to measure the chlorophyll fluorescence to investigate plant physiology.

Fluorescence spectroscopy is mainly used to determine the content of certain components in biological samples, analysis of biotechnology and immunotechnology, such as the determination of deoxyribose and deoxyribonucleic acid, DNA, antibodies, antigens, and other aspects of research. Fluorometer can be used to determine the nucleic acid concentration in sample.

4) Pharmaceutical Industry:

Fluorescence detection is used for dissolution testing of tablets and products in the pharmaceutical industry when the use of UV absorption is not appropriate.

4.12. Factor Affecting Fluorescence

- **Effect of Structure:**

The nature of the chemical structure of a molecule in terms of flexibility and rigidity is of made influence on the fluorescence and phosphorescence signal. Molecules that have a high degree of flexibility will tend to decrease fluorescence due to higher collisional probability. However, more rigid structures have a lower probability of collisions and thus have more potential.

Effect of Solvent:

Solvents affect the luminescent behaviour of molecules. There are three common effects can be recognized-

a. **The polarity of Solvent:** A polar solvent is preferred as the energy required for the P is lowered.
b. **The viscosity of Solvent:** Highly viscous solvent is perfected since collisional deactivation will be lowered at higher viscosities.
c. Heavy Atoms in Solvent: Solvents contain heavy atoms, fluorescence quantum efficiency will decrease and phosphorescence will increase.

- **Effect of Temperature**

Molecule experiences larger collisional deactivation at high temperatures due to an increase in the movement and velocity of molecules. Therefore, lower temperature are preferred for analysis.

- **Effect of Dissolved Oxygen**

Dissolved oxygen affects florescence at large scale. Molecules experience microsystem crossing due to it is paramagnetic nature.

- **Effect of Concentration**

The Fluorescence is directly proportional to the amount of absorbed radiation. When the concentration of the fluorescent molecules increases in a sample solution, the fluorescence intensity is reduced.

5. PLAN OF WORK

Estimating quinine sulphate using a photo fluorometer involves measuring the fluorescence emitted by quinine sulphate solution under specific conditions. Here's a general plan of work for this estimation:

SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal

5.1. Preparation of Solutions:

Prepare a stock solution of quinine sulphate of known concentration. Dissolve a known mass of quinine sulphate in a known volume of solvent (usually distilled water or buffer solution) to obtain a desired concentration (typically in mM or μm).

5.2. Instrument Setup:

Calibrate the photo fluorometer according to the manufacturer's instructions. This involves setting the appropriate excitation and emission wavelengths for quinine sulphate fluorescence.

6.Material Requirenment :

6.1 Apparatus: Cuvette, Primary Filter, Secondary Filter, Volumetric Flask, Beaker, Measuring Cylinder.

6.2 Chemical: Quinine Sulphate, Sulphuric Acid (H_2SO_4), Distilled Water.

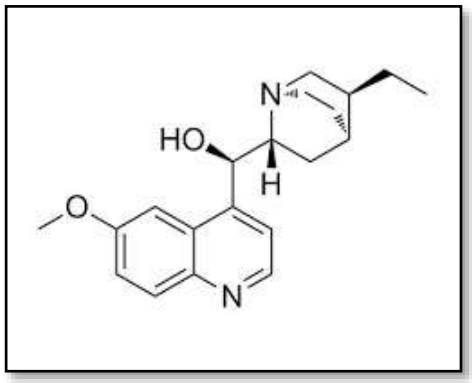
6.3 Instrument: Photo Fluorometer.

- **Definition**

The sulfate of a natural substance, quinine, obtained from the bark of various species of cinchona including Cinchona succirubra, Pavon. (red cinchona); Cinchona officinalis, Linn; Cinchona calizaya, Wenddell; Cinchona ledgeriana, Moens.

Quinine is used to treat malaria caused by Plasmodium falciparum. Plasmodium falciparum is a parasite that gets into the red blood cells in the body and causes malaria. Quinine works by killing the parasite or preventing it from growing.[13]

- **Structural formula:**



- **Molecular formula:** $C_{40}H_{50}N_4O_8S$
- **IUPAC Name:** (R)-[(2S,4S,5R)-5-ethenyl-1- azabicyclo[2.2.2]octan-2-yl]-(6-methoxyquinolin- 4-yl)methanol; sulfuric acid .
- **Formula weight:** 782.96.
- **Mechanism of Action:** It has many mechanisms of action like reduction of oxygen intake and carbohydrate metabolism, disruption of DNA replication and transcription via DNA intercalation and reduction of the excitability of muscle fibers via alteration of calcium distribution. It is toxic to malarial parasite, Plasmodium falciparum'. It interferes with the parasite's ability to break down and digest haemoglobin.[14]
- **Metabolism:** It is metabolized into 3-hydroxyquinine (major metabolite, less active than Quinine), 2-quinone, O-dimethylquinine, and 10,11-dihydroxydihydroquinine mainly by the liver oxidative cytochrome P450 (CYP) pathways. Then, these primary metabolites are further metabolized into six secondary metabolites.[15]
- **Clinical uses**: Antimalarial, Analgesic, Mild antipyretic, Non-narcotic, Flavouring agent, Muscle relaxants
- **Adverse effects:** Headache, Low blood platelets Irregular heartbeat**,** Sweeting[16].

7. EXPERIMENTAL METHODOLOGY :

7.1) Calibration of instrument:

Taken 5ml of Dis. water in cuvette and calibrate the photo fluorometer.

7.2) Preparation of 0.1N H_2SO_4:

Adding 5.4 ml of concentrated H_2SO_4 in 100ml of H_2O and dilute in 1L (for standard).

Adding 0.54 ml of conc. H_2SO_4 in 10ml and dilute with 100ml of H_2SO_4.

7.3) Preparation of standard solution:

1) Weight accurately 100mg of Quinine sulphate powdered drug.
2) Dissolve in 100ml of 0.1N H_2SO_4 (1mg/ml)
3) Taken 10ml of above solution and dilute to 100ml with 0.1 N H_2SO_4.
4) Again taken 10 ml of above solution dilute to 100ml with 0.1N H_2SO_4.

SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal

5) To get the resulting solution of 0.5, 1.0, 1.5, 2.0, and 2.5 µg/ml of above solution respectively and dilute to 10ml with 0.1N H_2SO_4.

7.4) Preparation of sample solution: Pipette 1ml of given sample solution and make up the volume to 10ml with 0.1 N H_2SO4.



Fig. 13 – Stock solutions

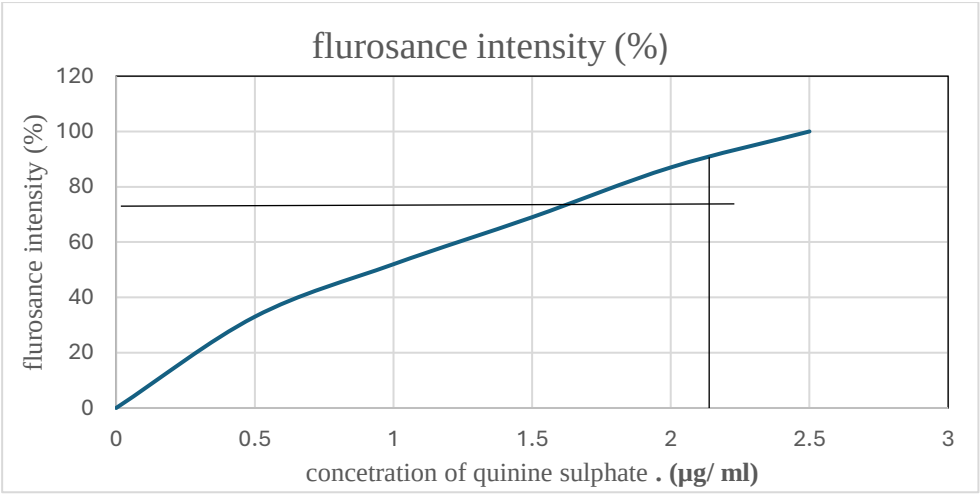
7.5) Operation of fluorimeter

1) Switch on the instrument and stabilized for 10min.
2) Keep the primary and secondary and secondary filters at the wavelength of 3486A and 4308A respectively in instrument.
3) Set the fluorescence intensity to 0% using 0.1 N sulphuric acid as blank.
4) Set the fluorescence intensity to 100% by using highest concentration of the standard solution (2.5 µg/ml)
5) Measure the percentage fluorescence intensity of different standard solution (0.5,1,1.5,2 µg/ml)
6) Measure the percentage fluorescence intensity of the sample solution.
7) Plot a graph between concentrated verses fluorescence intensity and determine concentration of sample by extrapolating the fluorescence intensity.

7.6) Observation table

Sr. no	Conc. (µg/ml)	Fluorescence intensity (%)
1	0.0	0
2	0.5	13
3	1.0	36
4	1.5	52
5	2.0	78
6	2.5	100
7	3.0	64

- **Graph**



SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 6 | June 2024 - Peer Reviewed Journal

➢ **INFERANCE**

Estimating quinine sulphate using a Photo fluorometer involves measuring the fluorescence emitted by the compound when exposed to specific wavelengths of light. By comparing this fluorescence to a standard curve of known concentrations, you can infer the concentration of quinine sulphate in your sample. This method is sensitive and precise, commonly used in pharmaceutical and research settings for quantifying various compounds, including quinine sulphate.

RESULT:

Concentration of unknown sample of quinine sulphate was found to be 2.1 (µg/ml) by using photo fluorometer.

CONCLUSION

In conclusion, the estimation of quinine sulphate using a photo fluorometer proved to be an effective method with several notable findings. Through calibration curves generated at various concentrations, we established a linear relationship between the intensity of fluorescence and the concentration of quinine sulphate. Our experimental data exhibited high precision and accuracy, as evidenced by low standard deviations and minimal deviation from theoretical values.

Furthermore, the method demonstrated good sensitivity, with the photo fluorometer capable of detecting even minute concentrations of quinine sulphate. This sensitivity is particularly advantageous in pharmaceutical analysis, where precise quantification of active ingredients is essential for quality control.

REFERANCES

1. *De, A., & Kumar, R. (Year). Life cycle of malaria. In A Textbook of Medicinal Chemistry (pp. 4.2-4.12). Rizali Publication.*
2. *Bhise, S. B., & Mis, R. (Year). Causative agent. In A Textbook of Pharmacology - III (p. 3.53). Mitali Publication.*
3. *Butler, J., Mulkecsin, E. C., & O'Keefe, S. T. (Year). Nocturnal leg cramps. Unit IV, Merlin Park Regional Hospital, Galway, Iceland.*
4. *Shinde, M. A., & Department of Chemistry, Savitribai Phule Pune University. (Year). Photo fluorometer. [Additional details if available]. Pune, India.*
5. *Wotsfold, P. J., Clinch, J. R., & Cazay, H. (Year). Spectrophotometric method for analysis. Analytica Chimica Acta, 5, [pages].*
6. *Valeur, B., & Berbern, M. (Year). [Title of the work]. Conservatoire National des Arts et Métiers, 292 Rue Saint Martin, F-75008 Paris, & Institut d'Alembert, Laboratories PPSM, ENS Cachan, 61 Avenue du Président Wilson, F-94235 Cachan Codex, France. Centro de Quimica - Física Molecular & Nanoscience, Instituto Superior Técnico, Nanotechnology, 1049-007 Lisbon, Portugal.*
7. *Chatwal, G.R., & Anand, S.K. (2014).A Textbook of Instrumental Methods of Chemical Analysis. Himalaya Publishing House.*
8. *Pal, R., & Pandey, P. (M.Pharm). Pharmaceutics, NAMS Institute of Pharmacy, MIMS University, Jaipur, Rajasthan, India.*
9. *Mahadik, K.R. (2010). A Textbook of Instrumental Methods of Analysis, (pp. 4.6-4.7). Sathiyrical Publication.*
10. *Skoog, D.A., Holler, F.J., & Crouch, S.R. (2007).Principles of Instrumental Analysis (6th ed.). Cengage Learning.*
11. *Beckett, A.H., & Stenlake, J.B. (2001).** Practical Pharmaceutical Chemistry, Volume 2 (4th ed.). CBS Publishers & Distributors.*
12. *Jolanta Geazyna Rola, Maciej Sonowsks. Animal Department of Origin, Rational Veterinary Research Institute, 24-100, Pulawy, Poland.*
13. *Kathryn Lawson Wood, Kieran Fuans. Perkin Elmer Inc., Seer Green, UK. In Journal of Applied Fluorescence Spectroscopy.*
14. *D.T. Patil, S.I. Bhatter, G.B. Kolekar, S.R. Patil. Fluorescence Spectroscopy Research Laboratory, Department of Chemistry, UniDefine, Hapur.*
15. *Martin Smilkstein, Mangluk Seiwilaijazoen, Jane Xu Kelty, Prapan Wilaitat, Michal Riscoe. Medicinal Research Service, Department of Veterans Affairs Medical Center & Oregon Health & Science University, Portland, Biochemistry Department, Mahidol University, Bangkok.*
16. *Sawyer, D. I., Heineman, W. R., & Beebe, J. M. (Year). Chemistry Instrumental Method. Biochemical Analysis, 15, 16.*