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A REVIEW ON SPECTROPHOTOMETRIC DETERMINATION OF VARIOUS DRUGS BY USING IMPORTANT ANALYTICAL REAGENTS

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ABSTRACT

The main aim of this work is to develop a simple, specific colorimetric methods for determination of pharmaceutical dosage forms using the colorimetric reagents. These colorimetric reagents are highly sensitive and very easy to determine analysis of analyte by a colorimetric reagent to a derivative has various spectral properties. Hence in this present work we use different reagents, their properties, procedures and examples of drugs, applications etc. These procedures are mostly used in many quality control laboratories and also have number of applications in novel analytical techniques.

KEYWORDS: Colorimetric analysis, Spectroscopy, Colorimetric reagents, Pharmaceutical forms.

INTRODUCTION

Calorimetry is a scientific technique that is used to determine the concentration of colored compounds in solutions. A substance to be estimated calorimetrically, must be colored or it should be capable of forming chromogens through addition of reagents. Colored substance absorb light in relation to their color intensity. The color intensity will be proportional to the concentration of colored substance. The instruments used in this method are colorimeter or photometer.

The amount of light absorbed or transmitted by a colored solution is in accordance with two laws:

Beer's law

Beer's law - When a monochromatic light passes through a colored solution, amount of light transmitted decreases exponentially with increase in concentration of colored substance i.e., the amount of light absorbed by a colored solution is directly proportional to the concentration of substance in the colored solution.

Lambert's law

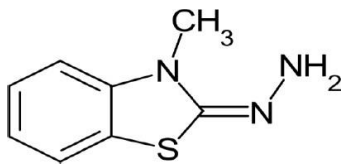
Lambert's law - The amount of light transmitted decreases exponentially with increase in path length of colored solution through which light passes i.e., the amount of light absorbed by a colored solution depends on path length of the colored solution.

List of reagents

1. MBTH reagent
2. 2,4 - DNP reagent
3. FC Reagent
4. Carr - price reagent
5. Gibb's reagent
6. 2,3,5 - Triphenyl tetrazolium salt
7. BM reagent
8. PDAB reagent

1. MBTH reagent

- 3-Methyl-2-benzothiazolinone hydrazine was first synthesized by Besthorn in 1910.
- MBTH is Introduced in analytical chemistry as a sensible
- Reagent for the estimation of carbonyl compounds.
- MBTH is a chromogenic reagent used for colorimetric estimation of drug containing phenolic, aromatic amines and active methylene groups.



Structure: -3-methyl-2- benothiazolinone hydrazine.

Reagent profile

- Synonym: MBTH, Sawicki's reagent
- IUPAC name: 3-methyl-2- benothiazolinone

hydrazone

- Molecular Formula: C8H10N3S. H2O
- Molecular Weight: 233.72
- Color: White to half white powder
- Solubility: freely soluble in distilled water

Principle of MBTH

Basic principle is oxidation followed by coupling.

- MBTH undergoes oxidative coupling reaction catalyzed by ferrous ion.
- Under reaction conditions MBTH loses two electrons and one proton forming an electrophilic intermediate which is an active coupling species.
- This intermediate undergoes electrophilic substitution with the phenols, amines, aldehydes to form colored product.

Reaction with phenol

- The reaction of MBTH with phenols is carried out in acidic, alkaline (or) neutral medium and in the presence of oxidizing agents by oxidative coupling.
- The colors obtained with phenols.
- Generally, co-substituted phenols get orange to red and Alkyl constituted phenols get violet (or) less intense color.
- As said by Hunning and Fritsch, phenol reacts in the para position to the hydroxy which is to some extent common in oxidative couplingreactions.
- Pays and Bourdon and Wanda subjected the colored reaction products to thin layer chromatography (TLC) and observed that phenol and Orto and meta cresols gave single red sports.
- The MBTH condenses blue cation the reaction of MBTH with phenols is carried out in acidic, alkaline (or) neutral medium and in the presence of oxidizing agents by oxidative coupling. The colors obtained with phenols.
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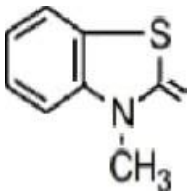


Colored product

Electrophilic intermediate of MBTH reagent

Factors affecting the nature and intensity of colour

Generally, five factors affect the nature and intensity of the resulting colored chromogen include

- Reagent (MBTH) concentration.
- pH of the medium.
- Temperature.
- Nature and concentration of oxidizing agent.
- Order of addition of reagents.

Applications of MBTH

The most widely used oxidants are ferric ammonium sulphate and ferric chloride.

- Less time is required for production of color.
- It is utilized for the determination of drugs at very small
- Concentrations, in biological fluids such as blood and urine.
- It is applicable to determination of drugs like acyclovir, ganciclovir, ceftazidine, Cefradoxil and Nicorandil.
- Utilized in the identification of aldehyde, amines, phenols, aryl amines.
- It is applicable to analysis of drinking water, domestic and industrial wastes.
- MBTH is utilized for estimation of samples which contain higher concentrations of aldehyde like disinfectants.

2.2,4-DNP REAGENT

2, 4-Dinitrophenylhydrazine (2, 4-DNPH or DNPH) is the organic compound.

- DNPH is a red to orange solid.
- It is a substituted hydrazine.
- The solid is relatively sensitive to shock and friction.
- For this reason, DNPH is usually handled as a wet powder.
- DNPH is a precursor to the drug Sivifene
- 2,4-Dinitrophenylhydrazine can be used for the qualitative identification of ketone or aldehyde functional group carbonyl functionality.
- A positive test is indicated by the formation of a precipitate known as dinitrophenyl hydrazone, yellow, orange, or red.

Synthesis of 2, 4-DNP

- It can be prepared by the reaction of hydrazine
- sulfate with 2,4-dinitrochlorobenzene:

Figure: - Synthesis of 2,4-DNP.

Reagent profile

- Synonym: Brady's reagent, DNPH, Broches' reagent
- IUPAC name :(2,4-Dinitrophenyl) hydrazine
- Molecular Formula: $C_6H_6N_4O_4$
- Molecular Weight: 198.14 g/mol
- Appearance: Red or orange powder
- Solubility: Slightly Soluble

Principle OF 2,4-DNP

2,4-DNP first attaches at the carbon-oxygen double bond to give an intermediate compound which then loses a molecule of water and results in formation of condensed chromogen.

Figure: - Reaction of 2,4-DNP with ketone.

Applications OF 2,4-DNP

Mainly used to determine Aldehydes and Ketones

- Used to determine the drugs spectroscopically like corticosteroids, flavanones, atorvastatin, ezetimibe, valproic acid.
- DNP is used for testing enzymes, especially enzymes found in living tissues and cells. Researchers use DNP to see how these enzymes work and how they are affected by different chemicals.

IMPACTS OF 2,4-DNP

DNP is a dangerous chemical that can be harmful to both humans and the environment.

- It is a skin and respiratory irritant and can cause burns, blisters, and other injuries.
- DNP is also toxic to aquatic life and can contaminate water supplies.
- Ingesting or coming into contact with DNP can cause serious health problems, including death.
- For these reasons, it is important to handle DNP carefully and take precautions when working with it.

3. Folin – Ciocalteu reagent

$$Mo(VI) + e \longrightarrow Mo(v)$$

Also known as Folin-phenol or Folin denis reagent.

It is a mixture of phosphomolybdic acid and phosphotungstic acid.

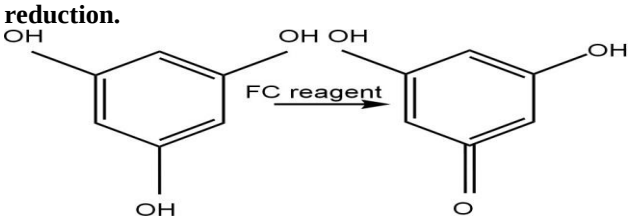
Physical properties

- Appearance: clear bright yellow solution Boiling point: 101 C
- Solubility: easily soluble in cold water, hot water.Solublein methanol.
- Used for the calorimetric assay of phenolic and polyphenolic antioxidants. Also called the Gallic acid equivalence method.

Principle

When FC reagent reacts with drug products containing phenols or non- phenol reducing agents like stannous chloride, ascorbic acid, ferrous sulphate etc., the molybdenum tungsten in FC reagent gets reduced to give rise to one or more intense blue colored reduced species. The color changes from yellow to intense blue is due to transfer of electron to reduce the phosphomolybdotungstic acid complex to basic pH conditions. The so formed reduced species or detected by spectrophotometric analysis.

Reagent oxidizes the drug and itself undergoes reduction.



Preparation of FC reagent

- Take 10g of sodium tungstate then add 25g of sodium molbdate, 70ml of water.
- Dissolve above solution and add 5ml of 85%phosphoric acid, 10mlof concentrated HCL.
- Reflux for 10 hours, then refluxed solution and add 15g of lithium sulfate, then add 5ml of water and 1drop bromine.
- Reflux for 15mins.
- Cool to room temperature.
- Makeup volume up to 100ml with water. Gives FC reagent.

Example of FC reagent Formation of Omeprazole Principle

Omeprazole effects of 1,2,3 oxygen atoms from tungsten & molybdate of FC reagent there by producing 1 or more possible reduced species which have a characteristic blue color which is estimated at742nm.

Preparation of standard solution

- To 100mg of crude drug, add 10ml of 0.1N NaOH, made up to 100ml with distilled water(SOLUTION-1)
- From above solution, pipette out 10ml and make to 100ml of distilled water (SOLUTION-2)
- From solution-2 pipette out 0.2, 0.8, 1.4, 2.0, 2.6ml of solution and add 2ml of FC reagent, 9ml of sodium carbonate make to 25ml with distilled water.
- Keep aside for 10mins.
- Measure absorbance at 742nm

Assay of Omeprazole capsules

- Weight of powder equivalent to 10mg was dissolved in 10ml of sodium hydroxide solution.
- From above solution pipette out 2ml and add 2ml of FC reagent, add 9ml of sodium carbonate make up to 25ml with water.
- Keep aside for 10mins.
- Measure absorbance at 742nm.

Applications

- Used as spray reagent in many chromatographic procedures.
- Used for determining protein concentration.
- Several drugs like aspirin, Piroxicam, acetazol amideare estimated by FC reagent.
- FC reagent id used to determine nitrogen content in the soil.

4. Carr – Price reagent
It is mainly used in determination of vitamin – A.

Principle
Anhydrous antimony trichloride in chloroform reacts with a dilute solution of vitamin A to form transient blue color. Reaction occurs between antimony chloride and unsaturated side chain of vitamin A.

The formed blue color is measured at 550nm.

Preparation of Carr-price reagent
25g of antimony trichloride dissolve in 100ml of chloroform then add 2ml of acetic anhydride. Gives Carr-price reagent.

Procedure of Carr-price reagent
- Vitamin-A (Drug solution) and add 3ml of Carr-pricereagent.
- Blue color measured at 550nm.

Example of Carr-price reagent Preparation of standard solution
- Dissolve 100mg of vitamin A in a 10ml of dry chloroform.
- Take 1ml of this solution and dilute to 5ml in dry chloroform.
- Take 0.10,0.25,0.50 and 1.0ml of this solution in four different clean and dry test tubes to make final volume 1.0ml by adding 0.90, 0.75, 0.50 of chloroform.
- Take 9.0ml of 25% antimony trichloride solution in 10 test tubes.
- Take 1.0ml of chloroform, 9.0ml of 25% antimony trichloride solution to use it as blank. The instrument is set 100% transmittance with this solution.
- Add the content 1.0ml of test tube to 9.0ml of antimony trichloride solution test tube. Immediately mix and & take the maximum absorbance at 620nm, within 15sec as the color is time sensitive and fade gradually.

Assay of vitamin a
The supplied sample is diluted to 5ml in dry chloroform and take 1.0ml of this solution. The total content of each of this solution is added to reagents of large test tube containing 9.0ml of 25% antimony tri chloride in dry chloroform. Plot the optical density in standard curve and calculate the amount of vitamin A in the supplied sample.

Applications
Carotenoids can be estimated.

Vitamin A when reacts with antimony tri chloride; in chloroform a blue color is developed which may have detected spectrometrically with wavelength of 620nm.

5. Gibb's reagent
Gibb's reagent is an organic compound used as an colorimetric indicator to detect phenolic compounds. Upon reaction with phenol itself, 2,6-dichlorophenolindophenol is formed, a chemical that is used as a redox indicator.

Gibb's reagent was used in paper chromatography in an experiment conducted to semi quantitatively estimate tyramine and octapamine. It can also be used as a chromatographic spray reagent, mainly for phenols, antioxidants, few primary and secondary amines, on silica gel chromatographic plates.

Reagent profile
IUPAC NAME: - 2,6-Dichloro-4-(chloramine)cyclohexa-2,5-diene-1-one.
Chemical formula: - C_6 H_2 Cl_3 NO Molar mass: - 210.44 g.mol Appearance:-Yellow crystalline powder

Structure
Structure of Gibb's reagent

Principle
When phenolic compounds react with Gibb's reagent, coupling reaction may takes place.

Reaction with phenols:
Phenols: Imide portion of Gibb's reagent reacts with phenolic compounds and gives corresponding products coupling with nucleophilic sites by elimination of chlorides.
Reaction with amines
Gibb's reagent couples with amine by elimination of hydrochloric acid and results in colored complex which is measured at characteristic maximum wavelength.

Preparation of reagent
A solution of 0.5-2% of 2,6-dichloroquinone-4-chloroimide in ethanol (reagent stable for 3 weeks when refrigerator)

Drugs estimated using Gibb's reagentLamotrigine
- Lamotrigine is in a class of medications called anti-convulsant used in treatment of epilepsy.
- Drug is mixed with methanol
- 1ml of 0.5% Gibb's reagent is added
- The resulting mixture is heated for 15 minutes
- It is measured spectrophotometrically at the wavelength of 403nm

Pregabalin
- Drug is mixed with ethanol
- 1.5 ml of 0.5% Gibb's reagent is added
- Mixture is heated for 10 minutes
- The drug is estimated at the wavelength of 440nm
- It also belongs to the pharmacological class of anticonvulsants used to treat neuropathic pain conditions.

Bisoprolol fumarate

- Drug is mixed with isopropanol and 5 ml of Gibb's reagent is added
- The mixture is heated and estimated at the wavelength of 532nm
- Bisoprolol is a beta-1 adrenergic blocking agent used to prevent myocardial infarction.

Applications

- It is used in spectrophotometric determination of phenolic sympathomimetic like Ritodrine Hydrochloride.
- It is a very good reagent for determination of Vit-B12.
- It is used for the identification of unsubstituted and p-alkoxy phenols.
- It is used in thin layer chromatography to produce colour spots, for example; Sulphur containing compounds show coloured spots when sprayed with gibb's reagent.

6. 2, 3, 5-Triphenyl tetrazolium SALT Synonym: - Tetrazolium Red Structure: - Structure of 2,3,5-triphenyl tetrazolium salt.

Triphenyl tetrazolium chloride, TTC, or simply tetrazolium chloride is a Redox indicator commonly used in biochemical experiments specially to indicate cellular respiration. It is a white crystalline salt, soluble in water, ethanol and acetone but insoluble in ether

Reagent profile

Chemical formula: - C_{19} H_{15} C_lN_4 Molar mass: - 334.8g/mol
Appearance: - White Crystalline Powder Solubility in water: - Soluble
Solubility in water: - ~1mg/ml Solubility in PBS: - ~1mg/ml Solubility in Ethanol: - ~1mg/mllog P: - -2.4

Synthesis of 2,3,5 tetrazolium chloride

- 15 gms of (0.05 mile) of triphenylformazan were dissolved in 100ml of Chloroform and the solution was cooled at 20 degrees.
- 30g of Lead tetra acetate was added until the red colour is disappeared. The chloroform is evaporated and the residue is taken in water.

HCL was added and the lead chloride was removed by filtration. The monobasic filtrate by three successive extractions with chloroform leaving the more acid in water. The chloroform solution was concentrated on the steam bath. Addition
of ether to this solution precipitated the tetrazolium salt is long, silky needles.

Principle

Triphenyl tetrazolium chloride (TTC) is a redox indicator commonly used in biochemical experiments to indicate cellular respiration. In presence of steroid with a α-ketol side chain group, tetrazolium salts are reduced to their colored formazan derivatives. Several formulations containing corticosteroids are assayed using TTC.

Reaction of 2,3,5-triphenyl tetrazolium salt
Applications Catecholamines

- Epinephrine and norepinephrine react with 2,3,5 triphenyl tetrazolium salt in presence of 0.1 N KOH and alcohol.
- This results in formation of blue color observed at (485nm).

Some of the other drugs spectroscopically measured with 2,3,5- triphenyl tetrazolium salt are cefepime hydrochloric acid, cefuroxime sodium, isoniazid, and rifampicin.

A Spectrophotometric Assay for Robust Viability Testing of Seed Batches Using 2,3,5-Triphenyl Tetrazolium Chloride: Using Hordeum vulgare L. As a Model

A comparative analysis was carried out of published methods to assess seed viability using 2,3,5-triphenyltetrazolium chloride (TTC) based assays of seed batches. The tests were carried out on seeds of barley (Hordeum vulgare cv. Optic) as a model. We established that 10% [w/v] trichloroacetic acid (TCA)/methanol is superior to the acetone and methanol-only based methods: allowing the highest recovery of formazan and the lowest background optical density (OD) readings, across seed lots comprising different ratios of viable and dead seeds

Radical indicator reaction for determination of 1,1-dimethylhydrazine

2,3,5-Triphenyl-2H-tetrazolium chloride (TPT) has been proposed as a redox indicator for determination of 1,1-dimethylhydrazine (DMH) which is used as a rocket propellant. It has been found that TPT is a sensitive chromogenic reagent due to its potentiometric and spectral characteristics. Products of the reaction between DMH and TPT have been characterized by mass spectrometry and EPR spectroscopy. In the EPR studies, C, N-diphenylnitrone (DPN) has been used as the radical trap and the obtained data indicate the radical nature of the reaction. The EPR spectrum of a mixture containing DPN and DMH has been recorded and calculated, whereas the spectrum of a three-component mixture containing DPN, TPT, and DMH is measured but it is complex for interpretation. A passive chemical dosimeter as a paper indicator strip with a polymer substrate impregnated with TPT has been manufactured and tested for continuous monitoring and determination of DMH in air in out-of-laboratory conditions at a concentration as low as 0.006 mg/m3 (warehouse operation area) and 0.5 mg/m3 (a warehouse box).

7. BM reagent

It is chemically n-1-naphthyl ethylene diamine dihydrochloride

Widely used for the determination of drugs and pharmaceuticals containing free primary aromatic amino group

Reagent profile
IUPAC: N-1-naphthyl ethylene diaminedihydrochloride

The primary aromatic amino group is first diazotizedwith sodium nitrite and HCL.The excess nitrous acid is neutralized by treating with ammonium sulfamate

Molecular formula: C H CL N reagent. Finally, diazonium ion can couple with BM12 16 2

Colour: Graycrystallinesolid

Principle
Reagent to produce azo dye complex measured at 550nm.

Preparation of bm reagent
Applications of BM reagent
Used for spectrophotometric determinations of phenols, Thiols and Sulfonamides.
Also used for estimation of chloramphenicol, sulphamethoxazole, dapsone.

Estimation of dapsone by BM reagent
Preparation of stock solution
Dissolve 10mg of dapsone in 10ml methanol (1000µg/ml)

Preparation of calibration curve
Pipette out 0.1,0.2,0.3,0.4,0.5 to that 1ml 5n HCL+1ml 0.1% sodium nitrate and maintain in cool condition for 10mins after that 1ml of ammonium sulphate and 1ml BM reagent and measure absorbance at 543nm

Radical indicator reaction for determination of 1,1-dimethylhydrazine.
2,3,5-Triphenyl-2H-tetrazolium chloride (TPT) has been proposed a redox indicator for determination of 1,1-dimethylhydrazine (DMH) which is used as a rocket propellant. It has been found that PT is a sensitive chromogenic reagent due to its potentiometric and spectral characteristics. Products of the reaction between DMH and TPT have been characterized by mass spectrometry and EPR spectroscopy. In the EPR studies,C,N- diphenylnitrone (DPN) hasbeenused as the radicaltrap and theobtaineddataindicatethe radicalnature of the reaction. The EPR spectrum of a mixture containing DPN and DMH has been recorded and calculated, whereas the spectrum of a three-component mixture containingDPN,TPT,andDMH is measured.

8. PDAB reagent
It is an aromatic compound and chromogenic
Used for estimation of amine group containing drugs.

Reagent profile
Synonym: Ehrlich's reagent
Iupac: 4- (dimethyl amino)-benzaldehyde
Molecular formula: C H NO 9 11
Color: white
Solubility: soluble in alcohol

Principle
The primary amine group which is present in the structure of drug reacts with carbonyl group in the PDAB reagent and forms schiff base which can be measured calorimetrically.

Preparation of PDAB reagent
Applications
PDAB reagent is used in
Micro determination of urea in urinesamples Detection of sulphonamides, amines, ergot alkaloids Used as TLC stain Used for spectrophotometric determination of ranitidine, chloramphenicol, metronidazole, sulpha methoxazole Example

Estimation of chloramphenicol: The aromatic nitro group present in the chloramphenicol is reduced to aromatic amine group by refluxing with zinc duct in presence of HCL for 10min.

Procedure
Preparation of the stock solution
Preparation: To 100mg of drug+500mg of zinc dust +2ml of 5M HCL, boil for 30mins
Filtrate was made up to 10ml with methanol

Determination of wavelength
- Pipette out 0.1ml from stock solution +2mlof PDAB reagent
- Make up to 10ml with methanol
- Scan from 400-800nm, and wavelength was found to be at 441nm

Applications
Catecholamine's
- Epinephrine and norepinephrine react with 2,3,5 triphenyl tetrazolium salt presence of 0.1 N KOH and alcohol.
- This results in formation of blue color observed at (485nm).
- Some of the other drugs spectroscopically measured with 2,3,5- triphenyl tetrazolium salt are cefepime hydrochloric acid, cefuroxime sodium, isoniazid, and rifampicin.

N-acetyl cysteine
- Drug is mixed with absolute ethanol and 1ml of gibb's reagent is added.
- The resulting mixture is heated and the drug is estimated at wavelength of 438nm.
- This compound belongs to the class of **Mucolytic agents** used to treat paracetamol overdose and to loosen thick mucus in individuals with chronic disorders like pneumonia and bronchitis

CONCLUSION

According to this review most of the reagents are collected from the best available reliability, reproducibility and accuracy.

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