DETECTION OF PROTEINS ON GELS BY A PLANT DYE FROM *LAWSONIA INERMIS*

By

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CERTIFICATE

This is to certify that the work entitled, "***Detection of proteins on gels by a plant dye from lawsonia inermis***" submitted by Shweta Gupta in partial fulfillment for the award of degree of Bachelor of Technology in Bioinformatics of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Dr. Chanderdeep Tandon

ACKNOWLEDGEMENT

The project in this report is an outcome of continual work over a period of five months and intellectual support from various sources. Obligations thus occurred in completing the work have been many. It is therefore almost impossible to express adequately the debts owed to many persons who have been instrumental in imparting this work a successful status. It is however a matter of great pleasure to express my gratitude and appreciation to those who have been contributing to bring about this project.

I take this opportunity to thank Dr. Soma Marla, Head of Bioinformatics department, JUIT Waknaghat, for providing me an opportunity and the facilities for understanding this project.

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Finally, I thank all my colleagues for their constant support and encouragement. Their unobtrusive support and suggestions bolstered my confidence as usual. Their inspiring words will be a guiding force in all my endeavors to attain greater heights.

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LIST OF ABBREVIATIONS

APS-Ammonium per sulphate

TEMED-N N N' N'-Tetramethlyethylenediamine

EDTA-Ethylenediaminetetraacetic acid

SDS-Sodium Dodecyl Sulfate

PAGE-Polyacrylamide Gel Electrophoresis

BSA -Bovine serum albumin

ABSTRACT

Scientifically referred to as *Lawsonia inermis*, Henna is most commonly used to dye hair. But with the new popularity of tattoos, henna and the art of Mehndi is considered a safe, painless and non-permanent alternative form of body ornamentation. Three species of the Lawsonia plant are used as henna: Inermis, Alba and Spinoza; we know these three as red henna, neutral henna and black henna based on the characteristic color that each plant produces. These ornamental shrubs, six feet in height, are indigenous to Arabia, Persia, India, Egypt and Australia. They are also cultivated throughout North Africa, Pakistan and China.

Various stains are used to identify proteins e.g. Coomassie Brilliant Blue R-250, Amido Black, Silver stain etc. A new method is to be developed to detect proteins by plant-based dye.

Henna paste is prepared by mixing crushed dry henna leaves with a mild acidic ingredient. e.g. lemon juice. The acid releases the lawsone from the plant(the active chemical constituent , formula is C10H6O3.). At room temperature, it normally takes about a day for the acid to activate the dye and three days for the paste to lose its staining capabilities. The process is faster in hotter environments.Heat causes the hennatannic acid to cling to the proteins. Ability of henna to stain proteins from polyacrlamide gel electrophoresis is to be compared with routine staining methods.

CHAPTER ONE

LAWSONIA INERMIS

1.1 Introduction

Henna is Lawsonia Inermis, family Lythraceae.Henna is a small tree or large shrub, growing to six meters high. Three species of the Lawsonia plant are used as henna: Inermis, Alba and Spinoza; we know these three as red henna, neutral henna and black henna based on the characteristic color that each plant produces. These ornamental shrubs, six feet in height, are indigenous to Arabia, Persia, India, Egypt and Australia. They are also cultivated throughout North Africa, Pakistan and China.

It has lateral branches with leaves that grow in pairs, two to four centimeters. long.Henna is a juvenile plant for the first two years. The leaves do not have high lawsone content, and the branches do not have thorns. In mature plants, thorns develop at the leaf buds during dormant phases.Henna leaves have a red-orange dye, lawsone, and the highest dye concentration is in the petiole (the central vein). Young leaves have the highest petiole dye content. Older leaves have lower dye content. Henna will only grow where minimum temperatures stay above 50 degrees fahrenheit or 11 celsius. It will tolerate extreme heat and long droughts. It grows wild near desert oasis, and in semi-aid regions. It thrives in alluvial soils, where there is annual precipitation of 0.2 to 4.2 meters and a soil pH of 4.3 to 8.0 (4.1-31). Henna is cooling and specific for devitalized, fragile, fine hair. This ancient plant has a wonderful conditioning and smoothing effect on the hair. This is an excellent hair conditioner and tonic. The leaves of the plant are astringent and used as a prophylactic against skin diseases. Largely used as a hair dye when mixed with other natural dyes. It is dried and crushed into a fine powder that is then mixed with oils and natural ingredients and applied to the skin. The paste dries and flakes off, leaving a beautiful stain of the design of your choice in your skin that lasts a week and a half of longer depending on skin type and after care. It is harmless to body system and causes no irritation to skin.

Henna is the only natural hair color with an FDA monograph for safe and effective use as a natural hair color. as NO p-Phenylenediamine (PPD), No

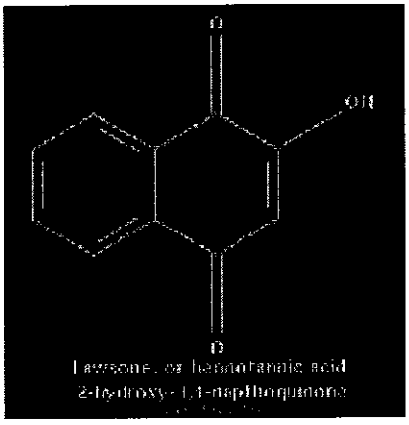
Ammonia, No Peroxide, No Chemicals are used and it has no contaminants such as heavy metals or pesticides.

Besides leaves ,roots are bitter, refrigerant, depurative, diuretic, emmenagogue, abortifacient and trichogenous, and are useful in burning sensation, dispsia, leprosy, skin disease, strangury amenorrhoea, dysmenorrhoea, and premature graying of hair. The leaves are bitter, astringent, acrid, refrigerant vulnerary, liver tonic, haematinic, styptic febriguge and trichogenous.

They are useful in wounds, ulcers strangury cough, bronchitis, vitiated conditions of kapha and pitta, burning sensation, fever, ophthalmia, amenorrhoea, dymenorrhoea, falling of hair, greyness of hair and jaundice. The flowers are intellect promoting, cardiotonic, refrigerant, soporific, febrifuge and tonic. They are useful in cephalalgia, burning sensation, sardiopathy, amentia, insomnia, and fever.

1.2 Characteristics and Constituents

The leaves of lawsonia inermis contain soluble matter, lawsone, 2-hydroxy - 1:4 - naphthoquinone resin and tannin, gallic acid, glucose, mannitol, fat, resin and mucilage are also present. The colouring matter is the quinone. The principal coloring mater is lawsone, 2-hydroxy-I; 4-naphtaquinone which is present in dried leaves in a concentration of I-O-I-4%, behenic, arachidic, stearic, plamitric, oleic.



Henna leaves (Lawsonia inermis, L. alba, L. spinosa, Lythraceae) are used for hair dye, externally for impotence, as a fungicide and as a sunscreen. The active chemical constituent is lawsone. The formula is $C_{10}H_6O_3$. When henna leaves are crushed in an acidic medium and applied to skin, the lawsone molecules migrate from the henna paste, traverse the outermost layer of the hair, the cuticle and stain the hair. Prolonged

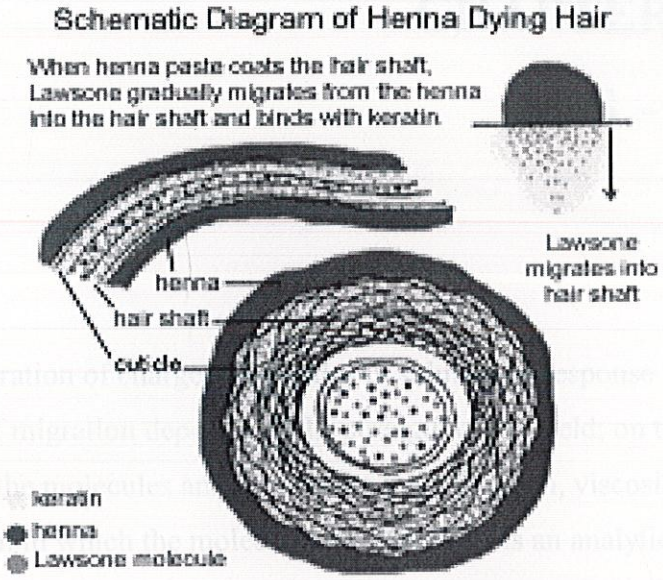
applications of henna result in diffusion of the pigment deeply into the hair. Chemically, the molecule of lawsone is 2-hydroxy-1, 4-naphthoquinone. Industrial classifications also describe lawsone as Natural Orange 6 and C.I. 75480. The name and molecular structure (see picture) of lawsone show its congeniality to naphthalene. In lawsone, two oxygen atoms are attached to the naphthalene carbons at positions 1 and 4 to form 1,4-naphoquinone and a hydroxyl (–OH) group is present at position 2. Its molecule contains 10 carbons, 6 hydrogens and 3 oxygens ($C_{10}H_6O_3$), giving a total molecular weight of 174.16 atomic units of mass. Pure lawsone is an orange powder, insoluble in water, with a melting point higher than 192°C and optical absorption maximum of 452 nm.

The size of the lawsone molecule, approximately 6.5x5x1.3 Å, compares to that of naphthalene. It is slightly bigger than the amino acids, the building blocks of proteins, and is slightly smaller than that of the sugar (sucrose) molecule. This is rather small in comparison to the size of biologically important molecules like proteins, containing typically hundreds or thousands of amino acids or even to phospholipids (8x8x40Å), which are major constituents of the membranes of cells and are also present in the top layer of skin Stratum corneum.

When dyes are used on skin, typically only the outermost layer is affected. Weak bonds are formed and it is the dye that has the colour, not the skin.

1.3 Action of Henna Dye

Henna paste is prepared by mixing crushed dry henna leaves with a mild acidic ingredient. e.g. lemon juice. The acid releases the lawsone from the plant. At room temperature, it normally takes about a day for the acid to activate the dye and three days for the paste to lose its staining capabilities. The process is faster in hotter environments. Henna coats each hair shaft with a natural, semi-permanent protein called hennatannic acid. Heat causes the hennatannic acid to cling to the proteins found in the hair (or nails and skin).



Lawsone dye infuses skin, hair, and porous surfaces but does not permanently or chemically alter them. The dye molecules, which are about the same size as amino acid molecules, migrate from the henna paste into the stratum corneum-that is, the outermost layer of the skin. The dye penetrates down through the stratum and does not spread like ink on blotting paper. As a result, the stains initially appear darkest on hands and feet because they have thicker strata cornea than other parts of the body.

The skin cells that are stained with the dye gradually in the act of skin regeneration are naturally shed, and with it, the colored cells are gradually replaced with uncolored cells, so the design fades gradually from the skin.

CHAPTER TWO

SDS – PAGE

2.1 Introduction

Electrophoresis is the migration of charged molecules in solution in response to an electric field. Their rate of migration depends on the strength of the field; on the net charge, size and shape of the molecules and also on the ionic strength, viscosity and temperature of the medium in which the molecules are moving. As an analytical tool, electrophoresis is simple, rapid and highly sensitive.

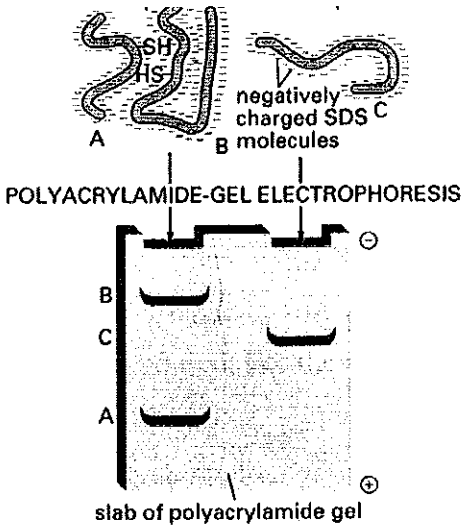
Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis is a procedure to separate proteins and determine their Molecular Weights.

2.1.1 What is so special about SDS?

- SDS is a negatively charged detergent.
- Disrupts secondary and tertiary protein structures by breaking hydrogen bonds and unfolding protein.
- 'Masks' charge on protein so that all proteins act the same as regards charge.
- Prevents protein aggregation.
- Prevents protein shape from influencing gel run.

2.1.2 Steps in SDS-PAGE

- Extract Protein
- Separate Proteins on a gel
- Stain proteins (visualization)
- Analyze and interpret results



2.1.3 Uses of SDS-PAGE

- Determine protein size
- Identify protein
- Determine sample purity
- Identify existence of disulfide bonds
- Quantify amounts of protein
- Recycle back to the PM.
- Recycle back to TGN

2.1.4 Solutions/Chemicals required For Gel Preparation

- Acrylamide (30%)
- Lower gel buffer
- Upper gel buffer
- Running buffer
- TEMED
- APS
- SDS

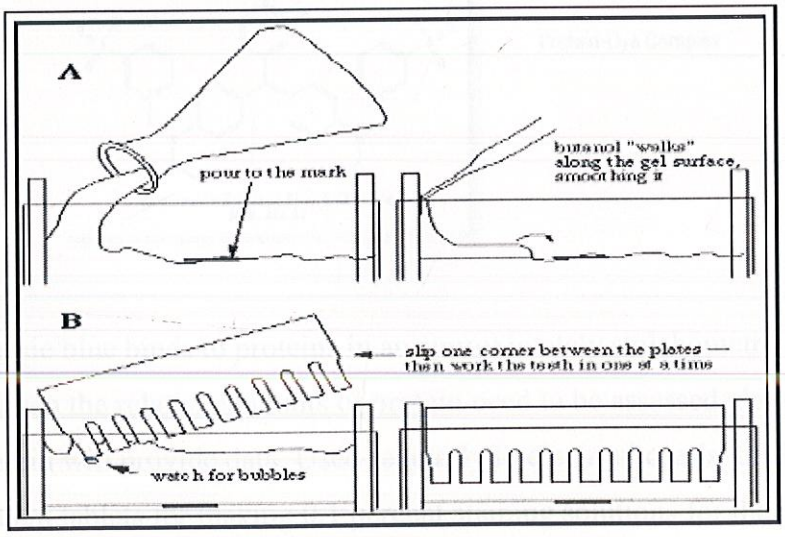
2.2 General Protocol for SDS-PAGE

Two glass plates, washed with ethanol (one notched) were placed on each other, separated by two separators known as spacers. Plates were joined by tape. Leakage was checked by pouring water in between two plates. Before pouring lower gel buffer, plug gel was prepared.

Decanted water and poured plug gel .After polymerization of plug gel, lower gel (also called as separating gel) was poured. For the various experiments performed, 10% lower gel was used.

Here we waited for the polymerization of the gel. pH of the lower gel is 8.8.After polymerization of lower gel, upper gel(also called as stacking gel) was added. The pH of stacking gel is 6.8.After pouring upper gel; a comb is placed for the formation of wells.

After polymerization, comb was removed and wells were rinsed with water. Remove the tape from bottom side so as to make the circuit complete and placed the plate on apparatus containing running buffer in upper and lower reservoirs. This was followed by loading of preheated sample buffer containing samples and selectable marker, whose molecular weight was known. After this electric field was applied. Proteins having negative charge move towards positive electrode at 100V and bands were formed. These bands were visible after staining and destaining of the gel with different dyes.

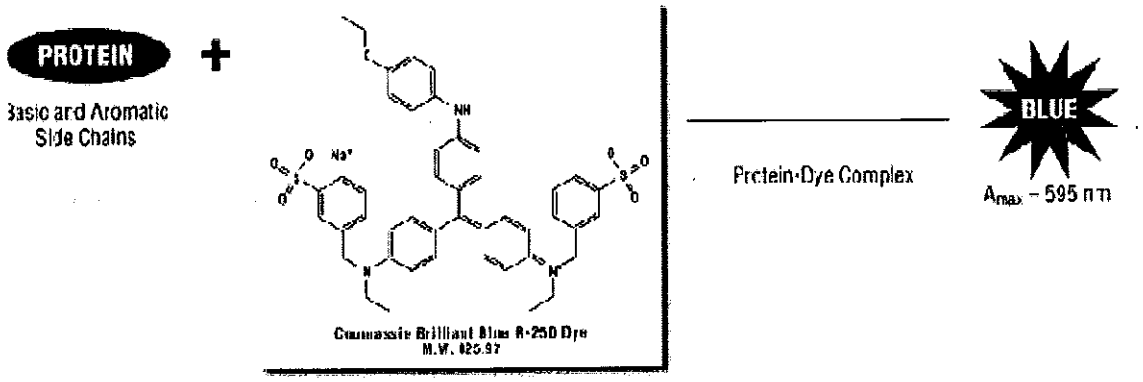


2.3 Action of Coomassie Blue

Known also as brilliant blue, coomassie blue is a synthetic heterocyclic organic stain, which binds nonspecifically to virtually all proteins. Although not as sensitive as silver staining, brilliant blue and its derivatives are widely utilized because of convenience.

2.3.1 Highlights:

- Develops intensely colored complexes with proteins
- Can determine as little as 0.5 $\mu g/cm^2$ of protein present in a gel matrix
- Anion of Coomassie Brilliant Blue formed in the acidic staining medium combines with the protonated amino groups of proteins by electrostatic interaction; resulting complex is reversible under the proper conditions
- When dissolved in 0.01 M citrate buffer at pH 3.0, has an absorption maximum at 555 nm; protein-dye complex is characterized by a peak slightly broader than that of the free dye with a maximum at 549 nm



Coomassie blue binds to proteins in an approximately stoichometric manner, meaning that when the relative amounts of protein need to be assessed, densitometry of the stained protein will provide data. Used regularly in electrophoresis, brilliant blue is sold in convenient tablets for making 0.1 percent staining solutions for the protein gels. A destaining process is often coupled to the procedure to remove excess unbound dye.

Brilliant blue appears as deep blue fine crystals with a faint reddish-blue cast. Acidified coomassie blue dye changes from reddish-brown to blue when it binds to protein molecules. Other uses for brilliant blue dyes are detection of lipids in thin layer chromatography, for detection of proteins in solution and on cellulose acetate plates, and as a traceable dye for following water uptake in the roots of plants.

2.4 Solutions

Solutions (a, d-f) are stored at 4°C, (b, c, g-h) at room temperature

(a) Stock acrylamide, 30%

73 g acrylamide

1.95 g Bis acrylamide

to 250 ml with dH_2O

(b) Sample buffer, 1x or 5x

0.72 g Tris base

6.25 g glycerol

-pH to 6.8

2.5 mg bromophenol blue

2.5 g SDS - NEVER use the pH meter with SDS

add dH_2O to 25 ml for 5x or to 125 ml for 1x

(c) Running buffer, 10x stock

3 x 202 g Tris-OH

10 x 288 g Glycine

2 x 100 g SDS

dH_2O to 20 liter mark on carboy

-do not adjust pH, should be 8.3-8.7

-if you check the pH, use pH paper not the pH meter

(d) ***Lower gel buffer, LGB***

45.43 g Tris base

5.75 ml concentrated HCl

- do not measure directly out of stock bottle, put a few ml in a beaker

-check pH - adjust to pH 8.8 if necessary

1 g SDS - NEVER use the pH meter with SDS

to 250ml with dH_2O

(e) ***Upper gel buffer, UGB***

7.58 g Tris base

~4.8 ml concentrated HCl

- do not measure directly out of stock bottle, put a few ml in a beaker

-check pH - adjust to pH 6.8 if necessary

0.5 g SDS

to 125 ml with dH_2O

(f) ***Ammonium persulfate***

100 mg per ml dH_20

-prepare fresh every two weeks

(g) ***Coomassie blue stain and fixative***

500 ml methanol

400 ml dH_2O

100 ml glacial acetic acid

-1 g Coomassie Brilliant Blue R-250

-may reuse stain multiple times; discard when staining is too light

-alternate method does not require destaining: add ~5 ml of stain to ~300 ml destain and place gel overnight in this -can dry the next morning

(h) ***Destain***

300 ml methanol

2.49 l dH_2O

210 ml glacial acetic acid

-these volumes are already marked on the destain jug

-a small piece of foam sponge added speeds up destaining and conserves destain solution

(i) ***Gel overlay spray solution***

22.7 g Tris-base, concentrated HCl to pH 8.8 (~2.9 ml)

-bring to 500 ml

-add 0.5 g SDS

-put in spray bottle

-after pouring gel, spray this onto the gel surface to assist its polymerization

(j) ***Gel overlay w/o Tris - for overlaying stacker w/o comb***

-0.5 g SDS

-500 ml dH_2O

-after pouring stacking gel, spray this onto the gel surface to assist its polymerization

2.5 Gel preparation data

Lower gel

SDS-PAGE: Use 7.5 ml per 1.5 mm minigels; run at 150V x 30-45 min					
Stock	5%	7.5%	10%	12.5%	15%
H_2O	8.75ml	7.5ml	6.25ml	5.0ml	3.75ml
LGB	3.75ml	3.75ml	3.75ml	3.75ml	3.75ml
Acrylamide (30%)	2.5ml	3.75ml	5.0ml	6.25ml	7.5ml
APS (100 mg/ml)	25µl	25µl	25µl	25µl	2 µl
TEMED	25µl	25µl	25µl	25µl	25µl

Upper gel

4.8% Stacker	
H_2O	4.4ml
UGB	1.9ml
Acrylamide (30%)	1.2ml
APS (100 mg/ml)	25µl
TEMED	25µl

Plug in gel

H_2O	700µl
UGB	700µl
Acrylamide (30%)	700µl
APS (100 mg/ml)	70µl
TEMED	7µl

CHAPTER THREE

LOWRY'S METHOD

Introduction

Lowry's assay for total protein is one of the most common colorimetric assays performed by biochemists. This procedure is particularly sensitive because it employs two color forming reactions. It uses the Biuret reaction in which Cu2+ (in the presence of base) reacts with the peptide bond to give a deep blue color. In addition Folin-Ciocalteu chemistry, in which a complex mixture of inorganic salts reacts with tyrosine and tryptophan residues to give an intense blue-green color, is also used. The combination of the two reactions gives an assay that is more sensitive that either reaction alone.

Lowry's assay is not without problems. In particular, it is sensitive to "interference" by many other compounds. Interference, the production of color by substances other that the analyte of interest, is a common problem with indirect colorimetric assays. In an attempt to overcome some of the problems of Lowry's method, many other assays for protein have been proposed.

Materials

- **Chemicals required**: Sodium Carbonate (Na_2C0_3), Sodium hydroxide (NaOH), Copper Sulfate (CuSO4.5H2O), Sodium Potassium tartrate, Folin-Ciocalteau reagent and human serum protein.

- **Apparatus Required**: Spectrophotometer, test tubes, test tube stand, micropipette.

Methodology

- **Reagents**
  - 2 % (w/v) Alkaline sodium carbonate solution (Reagent A): 2 g of sodium carbonate was dissolved in 100 ml sodium hydroxide.

- 2 % (w/v) Sodium potassium tartrate solution: 2 g of sodium potassium tartrate was dissolved in 100 ml of double distilled water.
- 1 % (w/v) copper sulphate solution: 1 g of copper sulphate was dissolved in double distilled water to make volume 100 ml.
- Copper sulfate sodium potassium tartrate solution (Reagent B): 1 ml of 1% (w/v) $CuSO_4$ mixed with 1 ml of 2 % (w/v) Na^+-K^+ Tartrate.
- Alkaline solution(Lowry's): mix 50 ml of reagent A and 1ml of reagent B.
- Folin- Ciocalteau reagent: Dilute commercial reagent with equal volume of distilled water.
- Stock standard: 1 mg of BSA was dissolved in double distilled water and made volume to 1 ml.
- Working standard: 1 ml stock was diluted to 5ml in double distilled water (conc. 0.2 mg/ml).

- **Procedure**

S. no.	Reagent	Test 1	Test 2	Test 3	Test 4	Test 5	Standard	Blank
1.	Sample	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml (H_2O)
2.	Lowry's	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
3.	Mixed and allowed to stand at 37°C for 10 minutes							
4.	Follin's	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
5.	Mixed and allowed to stand at 37°C for 30 minutes							

The optical density (OD) is taken at 650 nm.

Result

The concentration of protein in serum sample comes out to be 51.5 mg/ml

DYE PREPARATION

Different types of dyes used

1) Henna - Dried leaves dye
2) Henna - $Ca(OH)_2$ dye
3) Henna - Wet leaves dye (leaves stored at -20°C)
4) Henna - Mixed leaves dye
5) Henna - Ammonia, Petrol and Lemon juice Dye (leaves stored at -20°C)
6) Henna -Normal dye (leaves stored at -20°C)

Henna - Dried leaves dye

75g of dried henna leaves were crushed to make powder. Powder was mixed with 25 ml of double distilled water and 25 ml of ethanol. Mixture was homogenized and was kept at room temperature for one hour. Then it was filtered and used.

Henna – $Ca(OH)_2$ dye

150g of green mehndi leaves (stored at -20°C) were grinded in 20ml of 5% calcium hydroxide. After that 25ml of ethanol and same amount of water was added to the paste. It was then homogenized using a homogenizer. Homogenized paste was kept for 24 hours at room temperature before its first use.

Henna - Wet leaves dye

Green mehndi leaves (stored at -20°C) were grinded. 75g of paste was further grinded with 25ml of ethanol and 25 ml water. The paste was then homogenized along with 25ml ethanol and 75ml water. After that 50 ml ethanol was added and it was left for 5 hours. Then dye was filtered and used.

Henna - Mixed leaves dye

To the wet leaves dye (prepared previously) 50g of dried henna powder were added to make it thicker.

Henna - Ammonia, Petrol and Lemon juice Dye

120 g of wet henna leaves (stored at -20°C) were crushed in 20 ml of petrol. To the paste formed 50 ml of lemon juice and 25 ml each of ammonia and ethanol was added. 20 g of sugar was also added to the paste. The constituents were mixed thoroughly and the paste was made to stand at room temperature for 24 hours. Then the dye was filtered.

Henna - Normal dye

50 g of wet henna leaves were grinded and a paste was formed. To the paste 75 ml of ethanol and 25 ml of double distilled water was added. The constituents were further grinded and paste was heated at 40°C – 45°C for 15 - 20 minutes and kept at room temperature for 24 hours. Then the dye was filtered out and used.

EXPERIMENT NO. 1

Aim: Aim of this work is to use henna as a dye for staining proteins after doing SDS-PAGE.

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was BSA (Bovine serum albumin), chicken liver and egg.
    - BSA: 0.01g in 0.1ml distilled water. To it 40μl sample buffer was added. . 40μl sample was loaded in each well of the plate and experiment was started.

- **Staining:** Half of the gel was put in henna and half in coomassie blue. Coomassie blue staining was done for half an hour and henna staining for 1 hour.

- **Destaining:** Gel stained with henna was put into the destainer (7% acetic acid) for one and a half hours.

Result: No bands were visible in henna stained gel. Gel stained with coomassie blue showed bands.

EXPERIMENT NO. 2

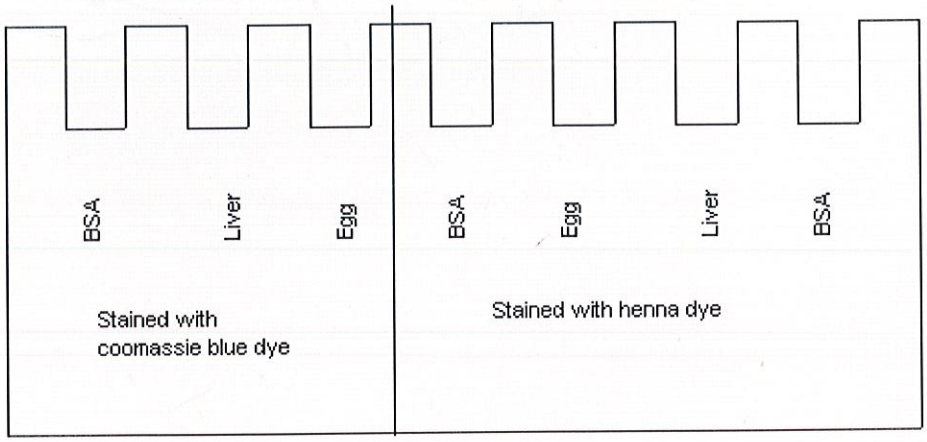
Aim: Aim of this work is to use henna as a dye for staining different proteins after doing SDS-PAGE.

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein samples used for this experiment were BSA (Bovine serum albumin), chicken liver and egg.
    - BSA: 0.01g in 0.1ml of double distilled water. 100μl sample was added to 40μl sample buffer.
    - Chicken liver: 100μl liver solution was added to 40μl of sample buffer.
    - Egg: 100μl solution was added to 40μl of sample buffer.

- **Loading:** All the samples were heated and 40μl sample was loaded in each well of the plate as shown in diagram. and experiment was started.



- **Staining:** Half of the gel was put in henna and half in Coomassie blue. Coomassie blue staining was done for half an hour and henna staining for 1 hour.

- **Destaining:** Gel stained with henna was put into the destainer (7% acetic acid) at 60-65°C in oven for one and a half hours. Overnight destaining of coomassie blue stained gel was done.

Results: Bands were present in the gel stained with henna dye. Liver protein was not visible both with coomassie and henna dye. Position of bands was same in both heena and coomassie stained gel.

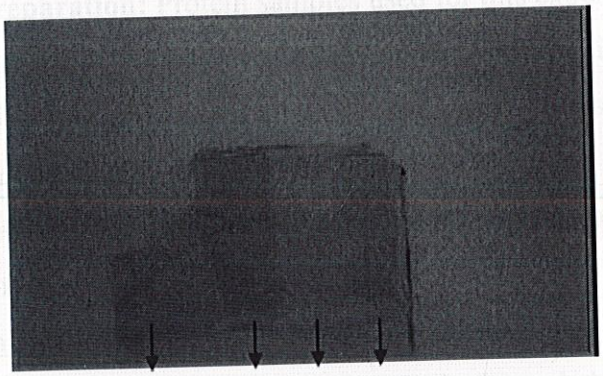


Figure1:Different proteins stained by henna dye

EXPERIMENT NO. 3

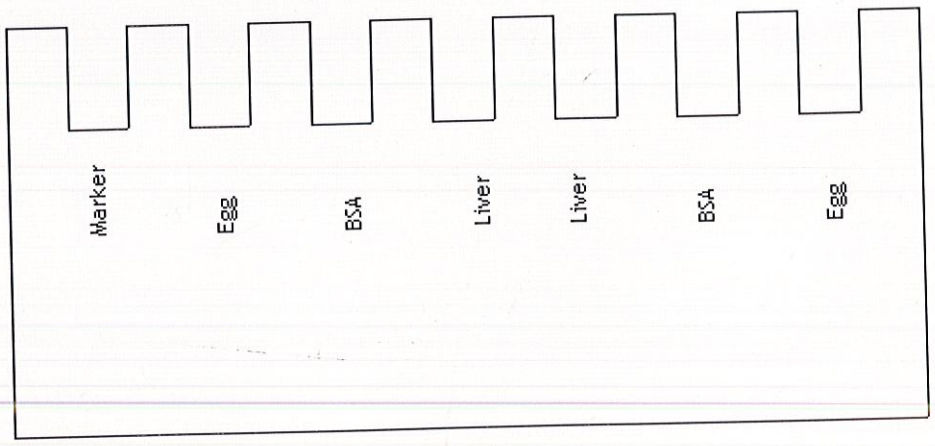
AIM: To see the effect of using 7.5% gel and use the molecular weight markers while using the henna dye.

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 7.5%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein samples used for this experiment were molecular weight markers, BSA (Bovine serum albumin), chicken liver and egg.
    - BSA: 100mg/1ml of double distilled water. 80μl sample was added to 40μl sample buffer.
    - Marker: 10μl of molecular weight markers were added to 4μl of sample buffer.
    - Chicken liver: 80μl liver solution was added to 40μl of sample buffer.
    - Egg: 80μl solution was added to 40μl of sample buffer.

- **Loading:** All the samples were heated and 30μl from each (molecular weight markers 12μl) was loaded in each well of the plate as shown in diagram and experiment was started.



- **Staining:** Half of the gel was put in henna and half in Coomassie blue. Coomassie blue staining was done for half an hour and henna staining for 1 hour.

- **Destaining:** Gel stained with henna was put into the destainer (7% acetic acid) at 60-65°C in oven for one and a half hours. Overnight destaining of coomassie blue stained gel was done.

Results: Marker did not show the henna stain. The bands were lower as we have used 7.5% gel. Rest of the bands were same as that in coomassie blue.

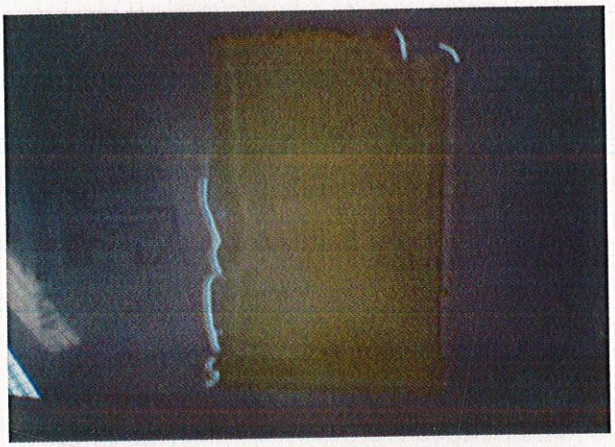


Figure 2:Henna dye on 7.5%gel

EXPERIMENT NO. 4

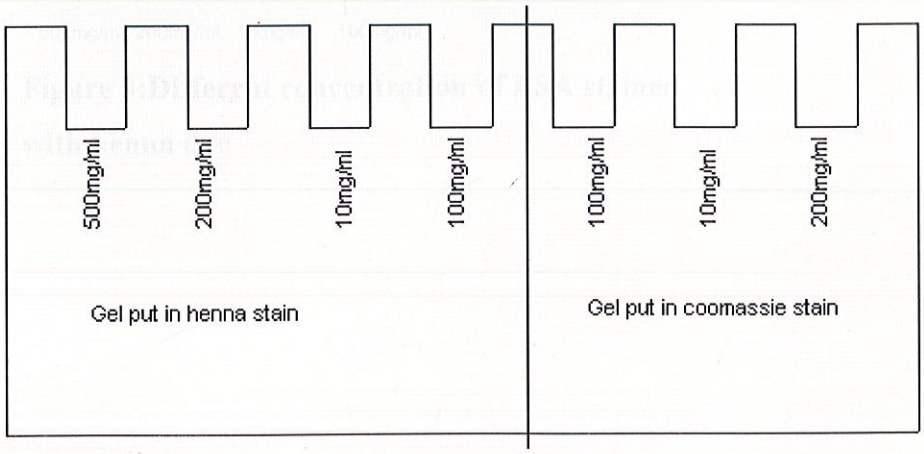
Aim: To use different concentrations of BSA protein and see the effect of henna dye.

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was BSA (Bovine serum albumin). Different concentrations of BSA used were:
  - 10mg/1ml of double distilled water.
  - 100mg/1ml of double distilled water.
  - 200mg/1ml of double distilled water.
  - 500mg/1ml of double distilled water.

- **Loading:** 20μl of sample buffer was added to 40μl sample. All the samples were heated and 20μl sample was loaded in each well of the plate as shown in diagram and experiment was started.



- **Staining:** Half of the gel was put in henna and half in Coomassie blue. Coomassie blue staining was done for half an hour and henna staining for 1 hour.

- **Destaining:** Gel stained with henna was put into the destainer (7% acetic acid) at 60-65°C in oven for one and a half hours. Overnight destaining of coomassie blue stained gel was done.

Results: Bands were visible in case of henna dye after one and a half hours of destaining. 10mg/ml concentration of BSA showed lightest colored band. Coomassie stained gel took more then 24 hours for desatining.

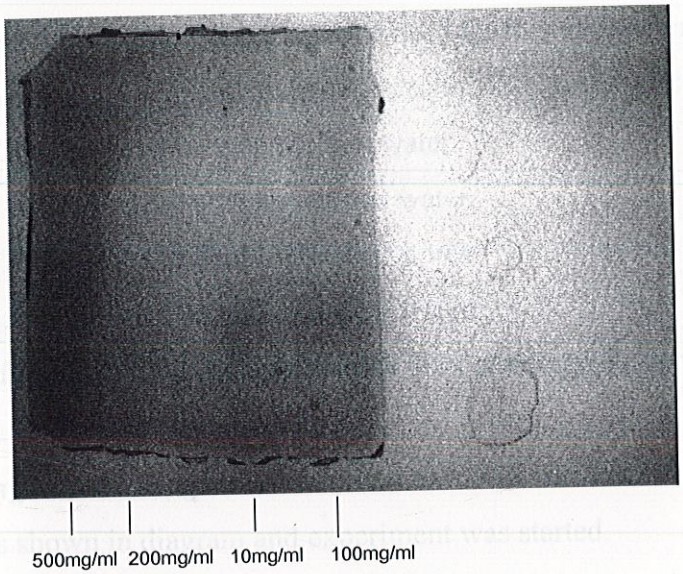


Figure 3:Different concentration of BSA stained with henna dye

EXPERIMENT NO. 5

Aim: To see the difference between the henna dye with and without calcium hydroxide.

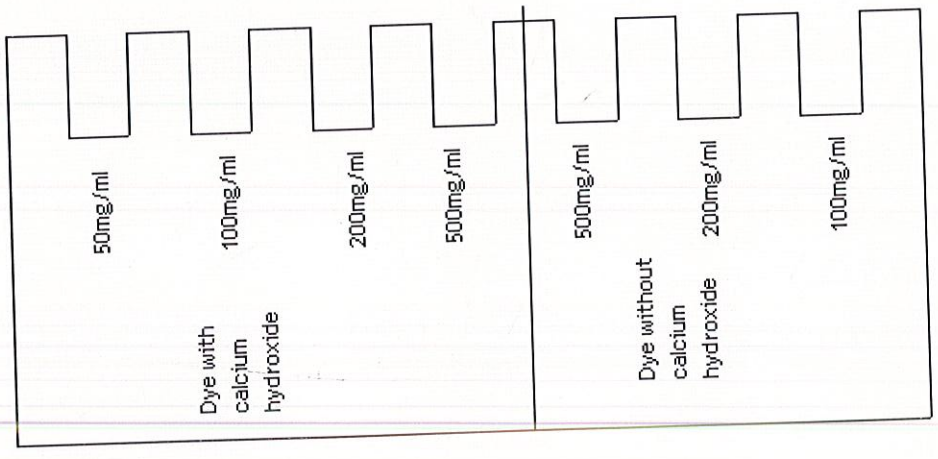
Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was BSA (Bovine serum albumin). Different concentrations of BSA used were:
  - 50mg/1ml of double distilled water.
  - 100mg/1ml of double distilled water.
  - 200mg/1ml of double distilled water.
  - 500mg/1ml of double distilled water.

  80μl of each solution was mixed with 40μl of sample buffer.

- **Loading:** All the samples were heated and 30μl was loaded in each well of the plate as shown in diagram and experiment was started.



- **Staining:** Half of the gel was put in henna dye without calcium hydroxide and rest in henna dye made by suing calcium hydroxide. Both were stained for 1 hour.

- **Destaining:** Both gels were put into the destainer (7% acetic acid) at 60-65°C in oven for one and a half hours.

Results: Stain given by henna dye (in calcium hydroxide) was not strong. Bands show white color when seen against black background. In case of normal dye bands were brown in color.

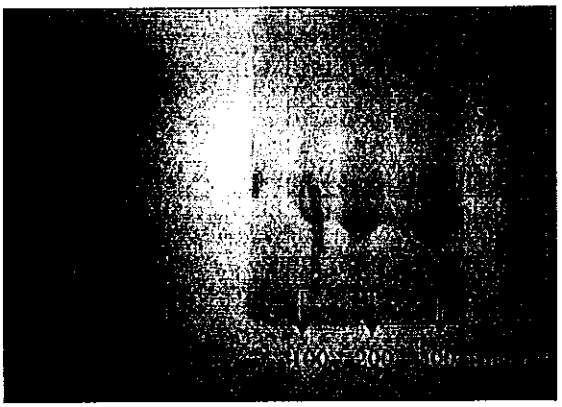


Figure 4:Gel stained with henna dye (5% Calcium hydroxide)

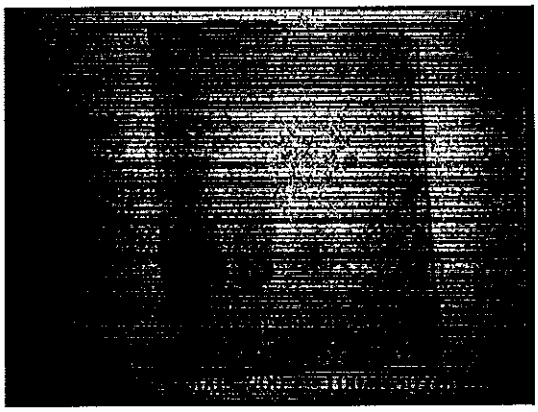


Figure 5:Gel stained with normal henna dye

EXPERIMENT NO. 6

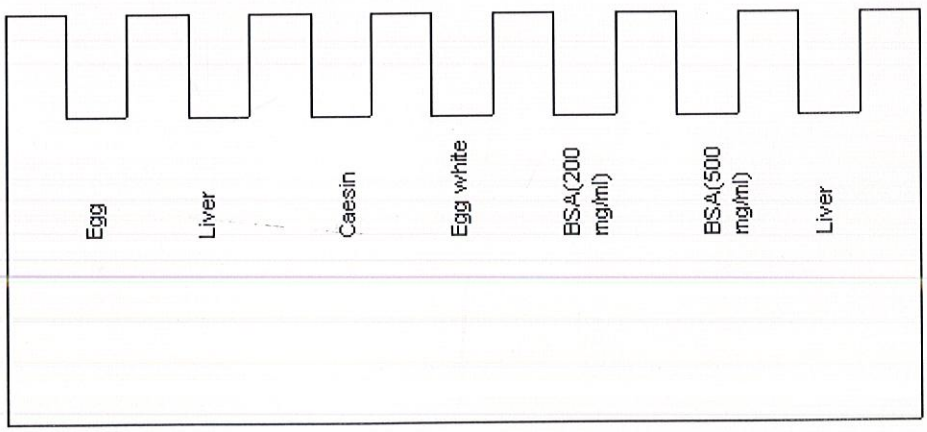
Aim: To see the effect of henna dye made with calcium hydroxide on wide range of proteins.

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was BSA (Bovine serum albumin). Different concentrations of BSA used were:
    - BSA: 200mg/ml of double distilled water. 40μl sample was added to 20μl sample buffer.
    - BSA: 500mg/ml of double distilled water. 40μl sample was added to 20μl sample buffer
    - Chicken liver: 40μl liver solution was added to 20μl of sample buffer.
    - Egg: 40μl solution was added to 20μl of sample buffer.
    - Egg white: 40μl solution was added to 20μl of sample buffer.
    - Caesin: 10mg/ml of double distilled water.

- **Loading:** All the samples were heated and 20μl sample was loaded in each well of the plate as shown in diagram.



- **Staining:** Gel was put in the henna dye made from calcium hydroxide. Bands were white in color after destaining. Then again it was put in normal henna dye for 45 minutes.

- **Destaining:** Gel stained with henna was put into the destainer (7% acetic acid) at 60-65°C in oven for one and a half hours.

Results: Brown bands were visible after staining with normal henna dye. In case of liver bands were not present.

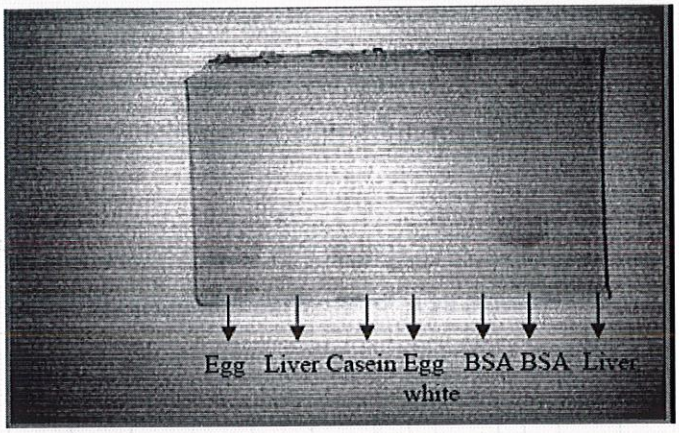


Figure 6:Different proteins stained with $Ca(OH)_2$henna dye

EXPERIMENT NO. 7

Aim: To see the effect of destainer on the protein samples.

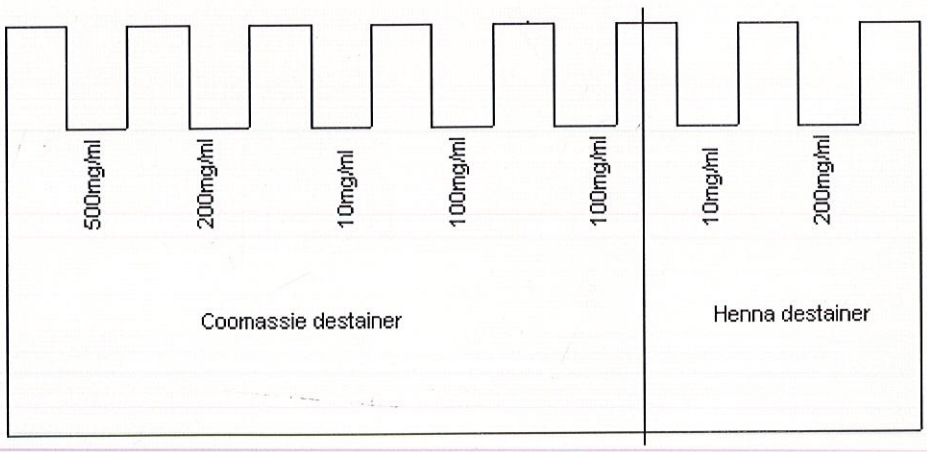
Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was BSA (Bovine serum albumin). Different concentrations of BSA used were:
    - 50mg/1ml of double distilled water.
    - 100mg/1ml of double distilled water.
    - 200mg/1ml of double distilled water.
    - 500mg/1ml of double distilled water.

  80μl of each solution was mixed with 40μl of sample buffer.

- **Loading:** All the samples were heated and 30μl was loaded in each well of the plate as shown in diagram and experiment was started.



- **Staining:** Half of the gel was put in Coomassie destainer and rest in henna destainer.

Results: White bands were visible in both the cases within 15 minutes. This was due to the fixation of proteins and this also shows that the dye with Calcium hydroxide does not give stronger stain.

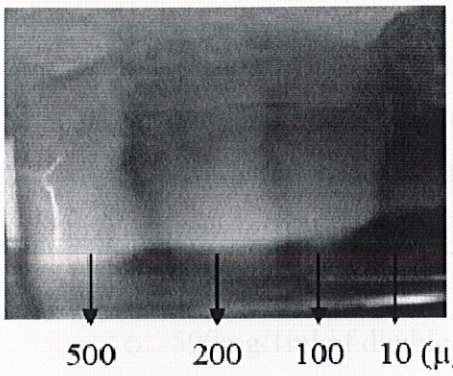


Figure 7:Gel stained with coomassie destainer

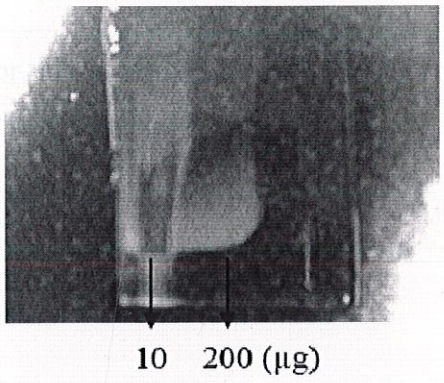


Figure 8: Gel stained with heena dye destainer

EXPERIMENT NO. 8

Aim: To see the effect of different pH while using dye with calcium hydroxide.

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was BSA (Bovine serum albumin). Different concentrations of BSA used were:
    - 50mg/1ml of double distilled water.
    - 100mg/1ml of double distilled water.
    - 200mg/1ml of double distilled water.
    - 500mg/1ml of double distilled water.

  80μl of each solution was mixed with 40μl of sample buffer.

- **Dye:** The pH of the dye was set at 5, 11 and 14.
- **Loading:** All the samples were heated and 30μl was loaded in each well of the plate as shown in diagram. Two plates were made and were put in dye with different pH.

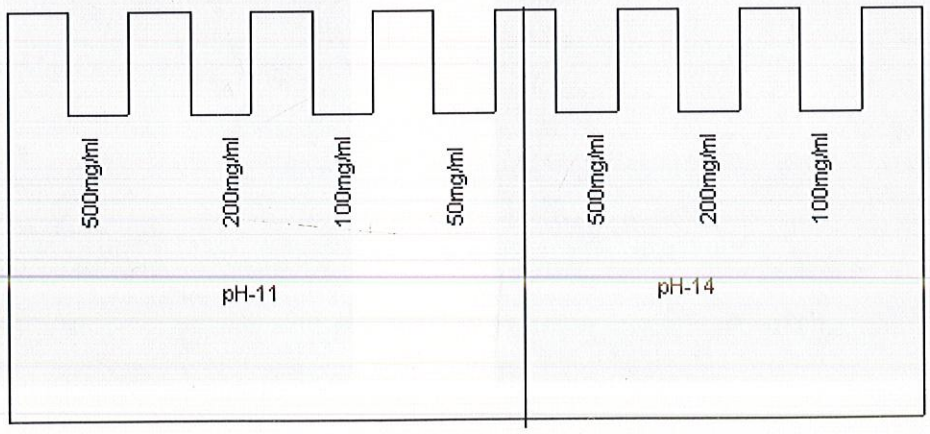


Plate – 1

Plate – 1

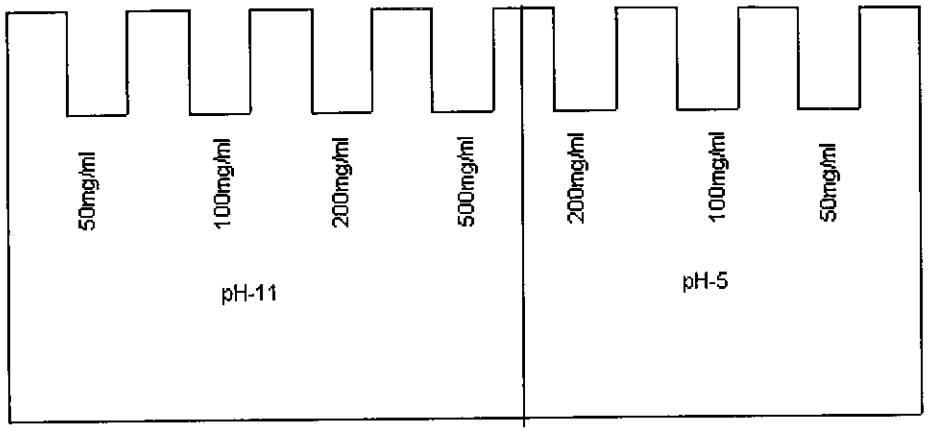


Plate - 2

- **Staining:** Gels were put in dyes with different pH as shown in the diagram.

Results: Best results were given by the dye with pH – 11. Dye with pH – 11 also took the least time to get destain, bands were visible in one and a half hours where as in case of dye with pH – 5 bands were visible only after two and a half .In case of pH – 5 bands are more or less same as that of pH – 11. Bands with pH – 14 were not good.

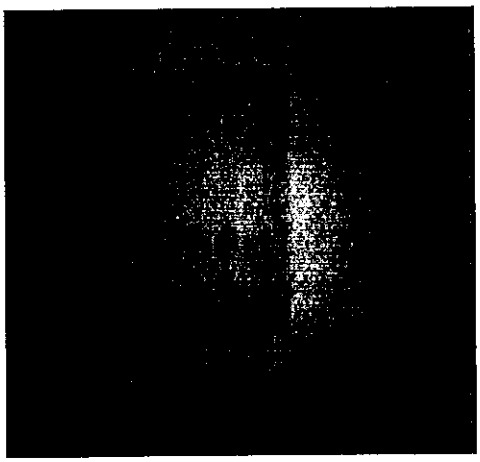


Figure 9: Plate 1 at pH-11

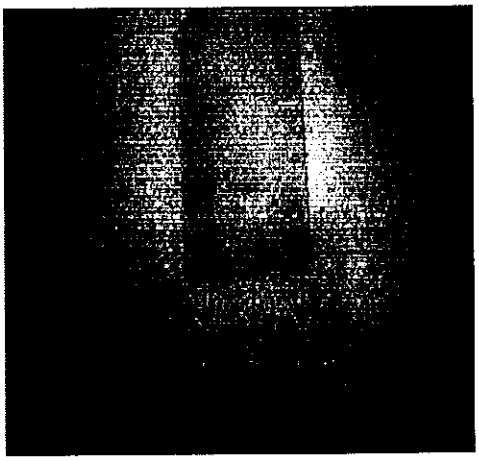


Figure 10:Plate 1 at pH-5

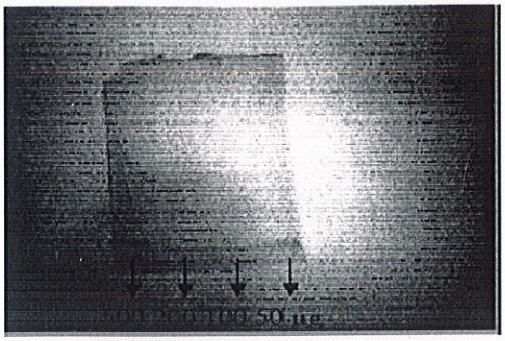


Figure 11:Plate 2 at pH-14

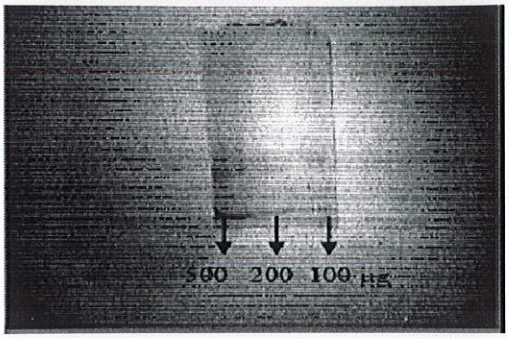


Figure 12:Plate 2 at pH-11

EXPERIMENT NO. 9

Aim: To see the use of different pH values on dye without calcium hydroxide.

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was BSA (Bovine serum albumin). Different concentrations of BSA used were:
  - 10mg/ml of double distilled water.
  - 50mg/1ml of double distilled water.
  - 100mg/1ml of double distilled water.
  - 200mg/1ml of double distilled water.
  - 500mg/1ml of double distilled water.

  80μl of each solution was mixed with 40μl of sample buffer.

- **Dye:** The pH of the dye was set at 2, 5 and 11. Darkness of the dye color increases from 2 to 11, with dye at pH - 2 being the lightest in color.

- **Loading:** All the samples were heated and 30μl was loaded in each well of the plate as shown in diagram. Different conditions of pH were used.

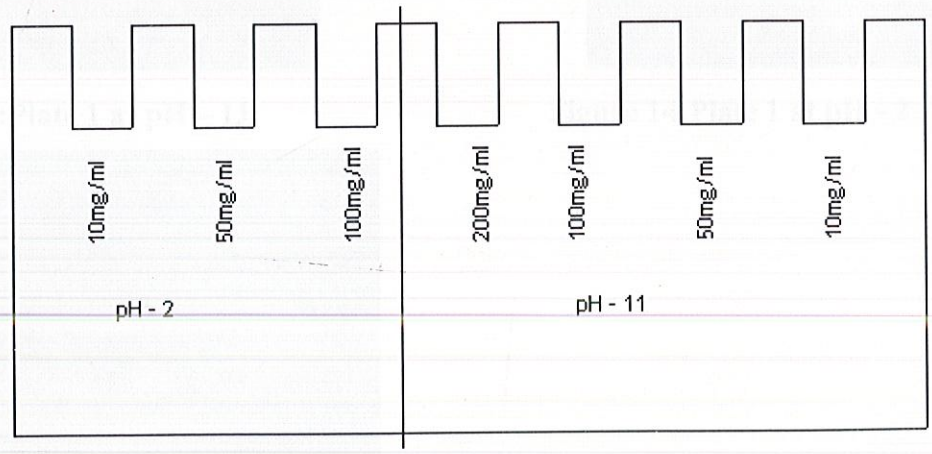


Plate – 1

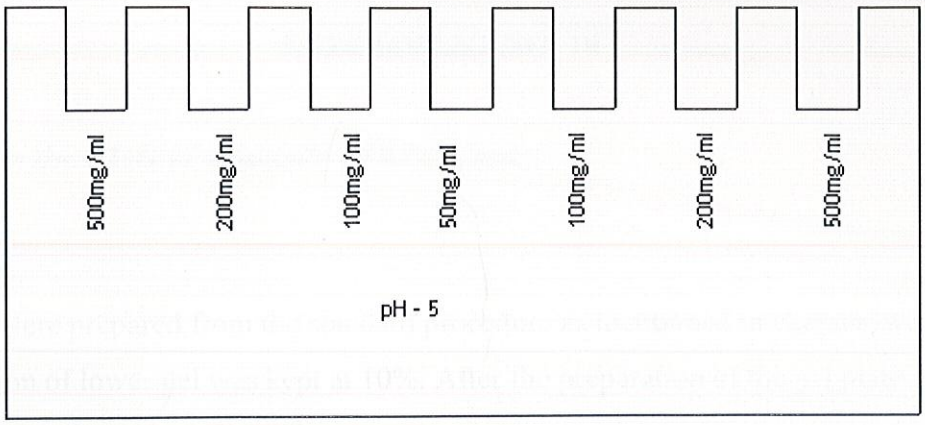


Plate - 2

- **Staining:** Gels were put in dyes with different pH as shown in the diagram.

Results: Best results were given by the dye with pH – 11 and pH – 5. Dye with pH – 11 also took the least time to get destain, bands were visible in one and a half hours. Dye with pH -2 did not give good bands.

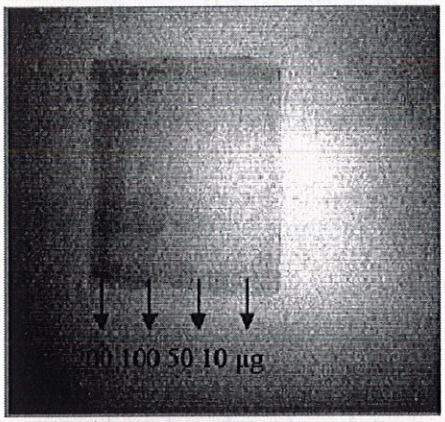


Figure 13:Plate 1 at pH – 11

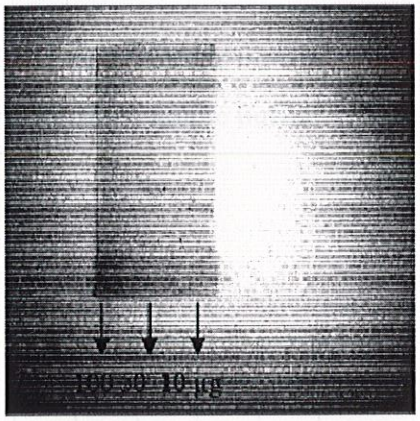


Figure 14:Plate 1 at pH - 2

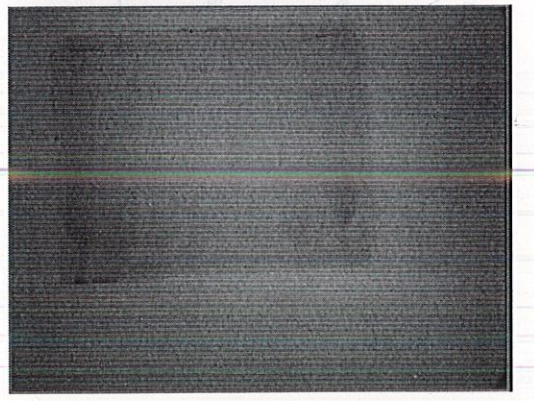


Figure 15:Plate 2 at pH - 5

EXPERIMENT NO. 10

Aim: To see the effect of ammonia on henna stain.

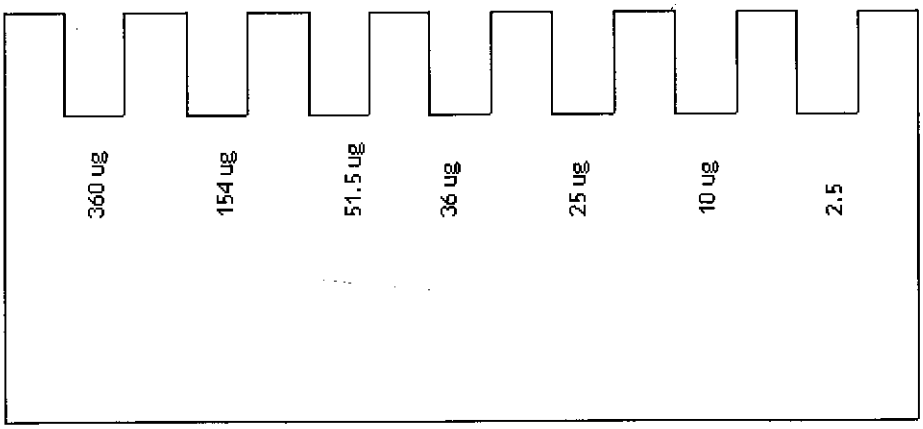
Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was serum.
  - 7 µl serum in 15 µl sample buffer.
  - 3 µl serum in 15 µl sample buffer.
  - 1 µl serum in 15 µl sample buffer.
  - 0.7 µl serum in 15 µl sample buffer.
  - 0.5 µl serum in 15 µl sample buffer.
  - 4 µl of 20 times diluted serum sample in 15 µl of sample buffer.
  - 1 µl of 20 times diluted serum sample in 15 µl of sample buffer.

- **Loading:** All the samples were heated at 60°C-70°C.

Samples were loaded in each well of the plate as shown in diagram.



- **Staining:** Gel was put in dye as shown in the diagram. Staining time for henna is 45 min – 60 min.

- **Destaining:** Henna stained gel was destained for one and a half to hours at 65°C in 7% acetic acid.

Results: After destaining the gel plate was put in ammonia for 5 minutes. The stain got darker.

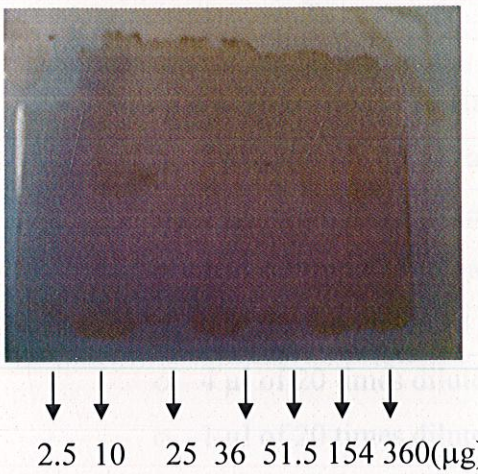


Figure 16: Serum samples stained in henna dye before applying ammonia

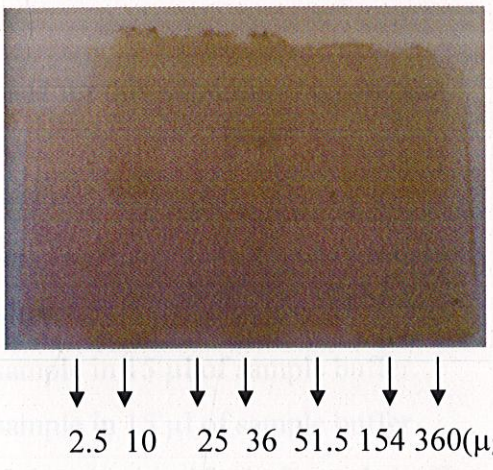


Figure 17: Serum samples stained in henna dye after applying ammonia

Conclusion: Ammonia darkens the henna stain.

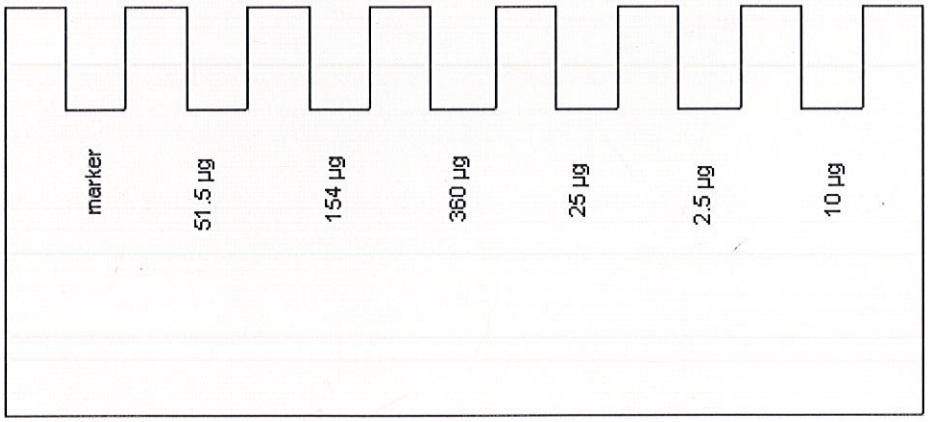
EXPERIMENT NO. 11

Aim: Compare the sensitivity of henna and coomassie blue dye along with the use of protein molecular weight markers.

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was serum.
  - 7 µl serum in 15 µl sample buffer.
  - 3 µl serum in 15 µl sample buffer.
  - 1 µl serum in 15 µl sample buffer.
  - 0.5 µl serum in 15 µl sample buffer.
  - 4 µl of 20 times diluted serum sample in 15 µl of sample buffer.
  - 1 µl of 20 times diluted serum sample in 15 µl of sample buffer.
  - 15 µl of Protein molecular weight markers in 15 µl of sample buffer.
- **Loading:** All the samples were heated at 60°C-70°C and was loaded in each well of the plate as shown in diagram and experiment was started.



- **Dye:** Henna: Previously prepared dye
- **Staining:** Staining time for henna dye is 45 – 50 minutes.
- **Destaining:** Henna stained gel was put in 7% acetic acid at 60°C – 65°C for one and a half to two hours
- **Results:** Bands with 2.5 µg protein sample are visible both in henna and in coomassie blue.

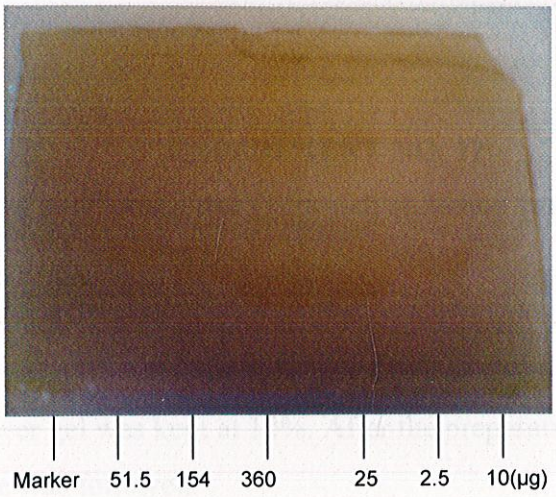


Figure 18:2.5µg protein detected by henna dye

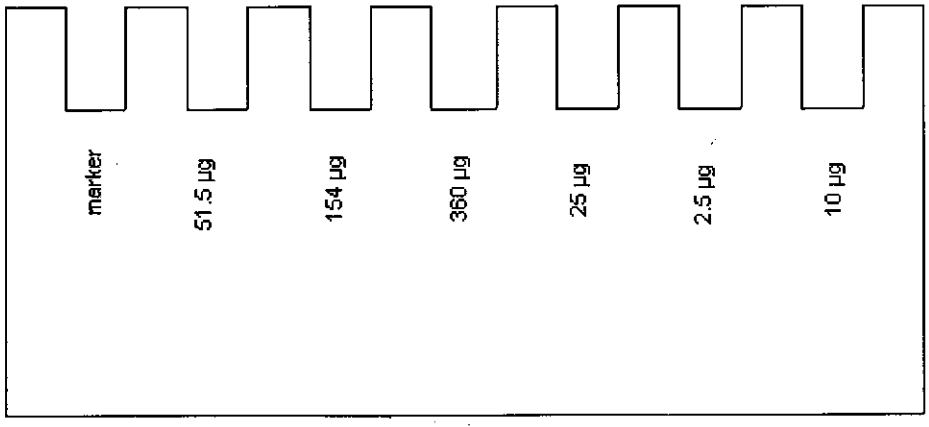
EXPERIMENT NO. 12

Aim: Comparison of new henna dye (with petrol, lemon juice and ammonia) with the previous dye.

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was serum.
  - 7 µl serum in 15 µl sample buffer.
  - 3 µl serum in 15 µl sample buffer.
  - 1 µl serum in 15 µl sample buffer.
  - 0.5 µl serum in 15 µl sample buffer.
  - 4 µl of 20 times diluted serum sample in 15 µl of sample buffer.
  - 1 µl of 20 times diluted serum sample in 15 µl of sample buffer.
  - 15 µl of Protein molecular weight markers in 15 µl of sample buffer.
- **Loading:** All the samples were heated at 60°C-70°C and was loaded in each well of the plate as shown in diagram and experiment was started.



- **Dye:** Henna: Previously prepared dye and newly prepared henna dye with petrol, lemon juice and ammonia. Color of new dye was darker then the previous one.
- **Staining:** Staining time for previous henna dye was 45 – 50 minutes at 40°C - 45°C, for new henna dye it was one hour at 40°C - 45°C.

- **Destaining:** Previous normal henna stained gel was put in 7% acetic acid at 60°C – 65°C for one and a half to two hours. New henna stained gel was also destained for the same time.

Results: In the previous normal dye bands were good and 2.5 µg of protein sample was also visible after two hours. In case of new dye the visibility of bands was poor.

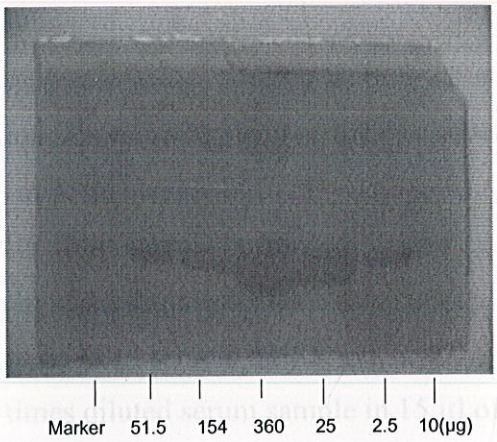


Figure 19:Gel stained with normal dye along with marker

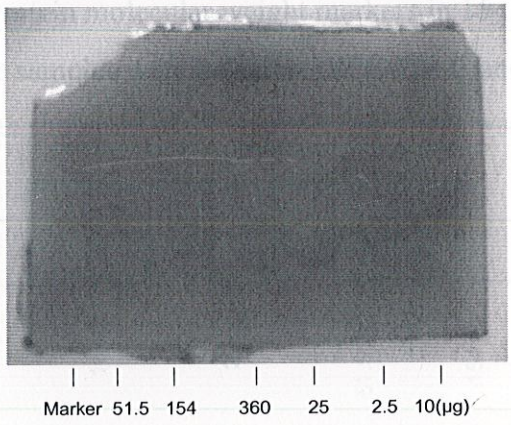


Figure 20:Gel stained with petrol dye along with marker

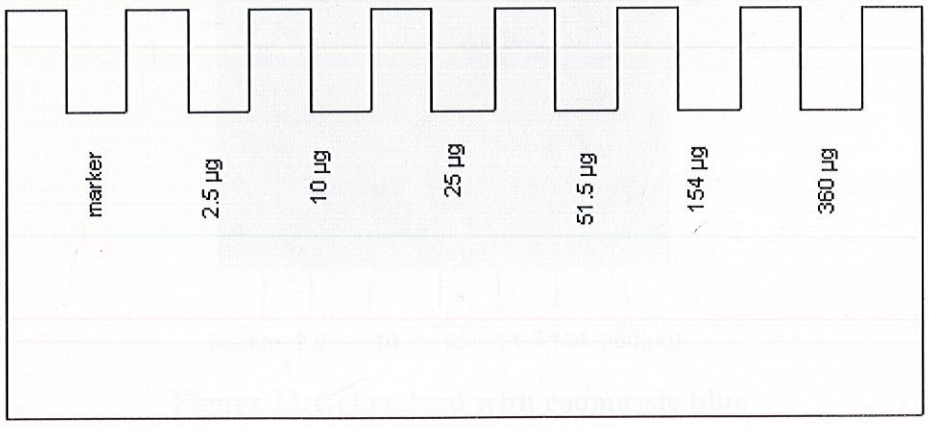
EXPERIMENT NO. 13

Aim: Comparison of new henna dye (with petrol, lemon juice and ammonia) with the previous dye and with coomassie blue dye.

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was serum.
  - 7 µl serum in 15 µl sample buffer.
  - 3 µl serum in 15 µl sample buffer.
  - 1 µl serum in 15 µl sample buffer.
  - 0.5 µl serum in 15 µl sample buffer.
  - 4 µl of 20 times diluted serum sample in 15 µl of sample buffer.
  - 1 µl of 20 times diluted serum sample in 15 µl of sample buffer.
  - 20 µl of Protein molecular weight markers in 15 µl of sample buffer.
- **Loading:** All the samples were heated at 60°C-70° C and was loaded in each well of the plate as shown in diagram and experiment was started.



- **Staining:** Gel was put in dye as shown in the diagram. Staining time for Normal henna dye was 50 – 55 minutes at 40°C - 45°C, for henna (with petrol, lemon juice and ammonia) dye was 25 – 30 minutes at 40°C - 45°C.
- **Destaining:** New normal henna stained gel was put in 7% acetic acid at 60°C – 65°C for one and a half to two hours. Henna (with petrol, lemon juice and

- ammonia) stained gel was destained with 50% acetic acid for 35 – 40 minutes and then with 7% acetic for around one hour fifteen minutes at 60°C – 65°C temperature.

Results: 2.5 µg of protein sample was stained by using all the dyes. Markers are visible in all the dyes.

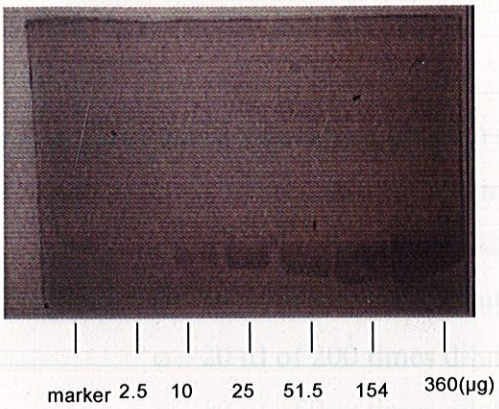


Figure 21:Gel stained with petrol dye in increasing serum concentration

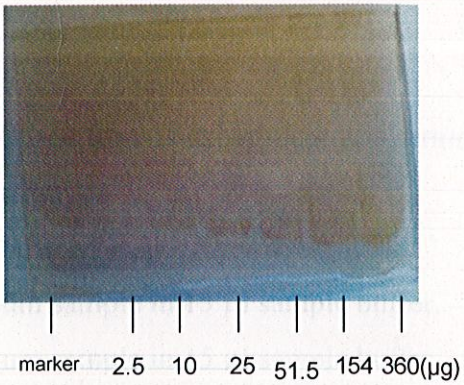


Figure 22:Gel stained with normal dye in increasing serum concentration

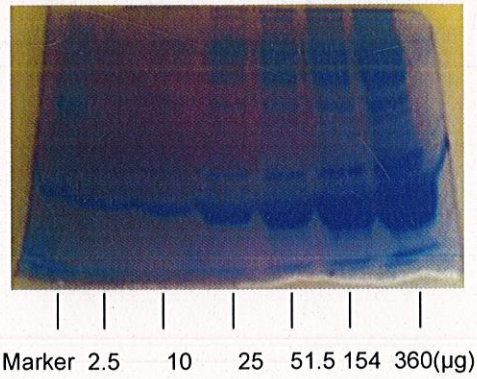


Figure 23:Gel stained with coomassie blue in increasing serum concentration

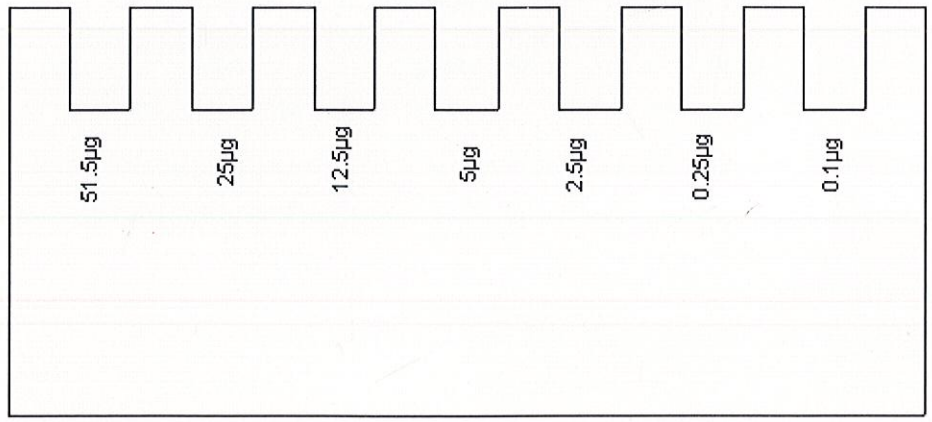
EXPERIMENT NO. 14

Aim: To check the sensitivity of normal henna and henna(with petrol, lemon juice and ammonia).

Procedure:

Gel plates were prepared from the standard procedure as mentioned in chapter two. The concentration of lower gel was kept at 10%. After the preparation of the gel plate following procedure was followed.

- **Sample preparation:** Protein sample used for this experiment was serum.
  - 1 µl serum in 15 µl sample buffer.
  - 0.5 µl serum in 15 µl sample buffer.
  - 50 µl of 200 times diluted serum sample in 15 µl sample buffer.
  - 20 µl of 200 times diluted serum sample in 15 µl sample buffer.
  - 10 µl of 200 times diluted serum sample in 15 µl of sample buffer.
  - 1 µl of 200 times diluted serum sample in 15 µl of sample buffer.
  - 0.5 µl of 200 times diluted serum sample in 15 µl of sample buffer.
- **Loading :**All the samples were heated at 60°C-70°C and was loaded in each well of the plate as shown in diagram and experiment was started.



:

- **Staining:** Gel was put in dye as shown in the diagram. Staining time for Normal henna dye was 50 – 55 minutes at 40°C - 45°C, for henna (with petrol, lemon juice and ammonia) dye was 25 – 30 minutes at 40°C - 45°C.

- **Destaining:** New normal henna stained gel was put in 7% acetic acid at 60°C – 65°C for one and a half to two hours. Henna (with petrol, lemon juice and ammonia) stained gel was destained with 50% acetic acid for 35 – 40 minutes and then with 7% acetic for around one hour fifteen minutes at 60°C – 65°C temperature.

Results: 0.1 µg of protein sample was visible with normal henna. In henna with petrol, ammonia and lemon juice it was not clearly visible.

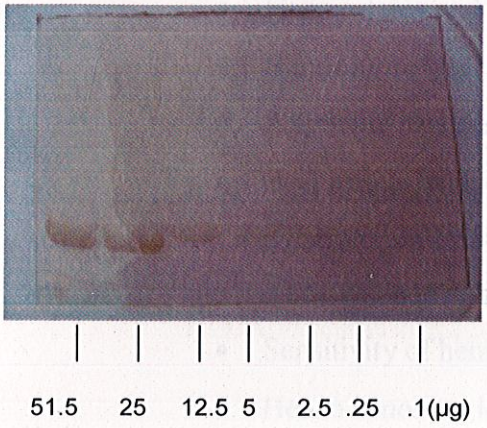


Figure 24: 0.1µg protein detected by petrol henna dye

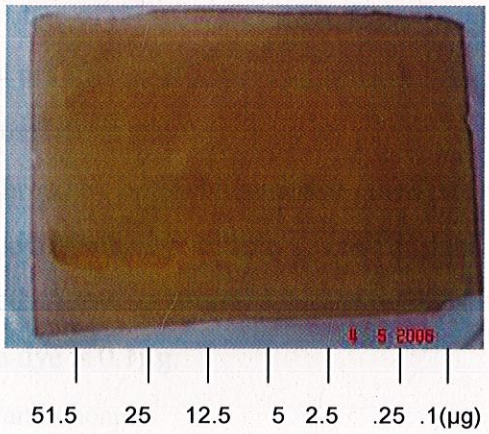


Figure 25:0.1µg protein detected by normal henna dye

Summary and Conclusion

- The active chemical constituent is lawsone. The formula is $C_{10}H_6O_3$.
- The acid releases the lawsone from the plant. The process is faster in hotter environments.
- Heat may cause the hennatannic acid to cling to the proteins.
- Henna stains wide range of proteins e.g. BSA, Egg, Serum, Caesin.
- Bands more clear at pH 11.
- Ammonia darkens the henna stain.
- Best results were shown by normal henna dye , then petrol henna dye.Calcium hydroxide henna dye didn't gave very good results as it wasn't able to stain the proteins strongly.
- Sensitivity of henna dye is 0.1µg.
- Henna is non toxic and cheap.

	Coomassie blue	Normal henna dye	Petrol henna dye
Toxicity	Cause irritation to skin and eyes	Non toxic	Non toxic
Cost	Expensive	Cheap	Cheap
Staining Time	15 min	1 to 2 hr	35 to 40 min
Destaining Time	overnight	1.5 to 2 hr	1 to 1.5 hr
Sensitivity	0.5µg	0.1µg	0.25µg

Figure 26:Comparison chart among different dyes

GLOSSARY

B

Buffer- A solution containing either a weak acid and its salt or a weak base and it's salt, which is resistant to changes in pH.

Biuret Reaction- A reaction characterized by a violet color upon the addition of copper sulfate to all compounds with two amide or peptide bonds linked directly or through an intermediate carbon atom. Used in the detection and estimation of proteins and peptides having more than two amino acids.

D

Dye- Any substance, natural or synthetic, used to color various materials, especially textiles, leather, and food.Natural dyes are so called because they are obtained from plants, animals and from certain naturally occurring minerals .They have been almost entirely replaced in modern dyeing by synthetic dyes.

Dye front - In the SDS PAGE experiment, this is the horizontal line that the blue dye in the sample buffer makes as it travels through the gel during electrophoresis. The dye front provides a visible indication of progress during electrophoresis, and helps determine if the run has gone on long enough.

E

Electrophoresis-The migration of charged colloidal particles or molecules through a solution under the influence of an applied electric field usually provided by immersed electrodes.It is also known as cataphoresis.

M

Matrix - The immediate physical environment in which a process or reaction occurs. In the SDS PAGE experiment, the matrix is the polyacrylamide gel which contains linked chains of polyacrylamide and other reagents such as buffers and Sodium Dodecyl Sulfate.

Molecular weight - The mass of a single molecule of a given compound. In the case of large molecules, it is usually expressed in KiloDaltons. In the case of significantly smaller molecules, it is expressed in atomic mass units.

P

pH -A measure of the hydrogen ion content of a substance, and thus a means of expressing the acidity or alkalinity of any substance or solution.

Protein- A class of biological molecules that are formed by connecting a long chain of amino acid molecules together.

Power supply -A source of electric current used. A power supply is used in the SDS PAGE experiment, as well as in many other types of laboratory experiments. Some power supplies are adjustable, and have different modes of operation such as constant current and constant voltage modes.

Polyacrylamide - A polymer formed by cross linking monomers of acrylamide

R

Reagent- A generic term referring to the chemical substances and solutions used during analysis, synthesis, and other processes where chemicals are consumed.

Running buffer - A buffer used in the SDS PAGE experiment which contains SDS and buffering agents.

S

Serum- The clear liquid that can be separated from clotted blood. Serum differs from plasma, the liquid portion of normal unclotted blood containing the red and white cells and platelets. It is the clot that makes the difference between serum and plasma.

Sensitivity -Degree of exposure to some sort of risk.

SDS PAGE - A technique that is used to separate proteins in a mixture for the purpose of identifying them.

Sodium dodecyl sulfate (SDS) -A compound with detergent properties. In the SDS PAGE technique, SDS associates with the protein and denatures it, and gives it an overall negative charge.

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