Estimation of dextrose by Colorimetry

AIM- Aim of the experiment is to estimate sucrose by colorimeter.

BACKGROUND-

Colorimetry is the measurement of concentration of analyte in a solution by determining absorption of a particular wavelength. The technique is based upon Beer-Lambert's law. Overall the process is accomplished with the measurement of analyte in a colored solution or development of color that appears in the solution following reaction with specific reagent.

In alkaline medium p-hydroxybenzoic acid hydrazide (PHBAH) reacts with dextrose that give products which can be assayed by colorimetry. Dextrose reacts with p-hydroxybenzoic acid hydrazide (PHBAH) reagent to give coloured product; the absorbance noted is proportional to concentration of sugar.

REQUIREMENTS-

Dextrose: 5mg
Sodium hydroxide: 3-5 pellets
p- hydroxybenzoic acid hydrazide (PHBAH) : 50 ml
Test tubes
Burette
Colorimeter
Water bath

PROCEDURE-

Alkaline solution

Put 3-5 pellets of sodium hydroxide in about 50 ml of water, stir to mix well.

Diluent

Add 10.0 ml of p-hydroxybenzoic acid hydrazide (PHBAH) solution with 30.0ml of water along with few drops of Sodium hydroxide.

Standard stock solution preparation

Weigh 5.0 mg of dextrose; add in a test tube containing 15.0 ml of water, 2-3 drops of sodium hydroxide and 5.0 ml of p-hydroxybenzoic acid hydrazide (PHBAH) solution, and mix well.

Working standard solution preparation

Dilute 5.0ml of stock solution with 15.0 ml of diluent.

Sample solution preparation

Take a test tube, add 1.0 ml of sample with burette, add 4.0 ml of water to it, and shake gently to mix. Keep the above sample solution for 30 -35 minutes at normal temperature. Again shake gently, then add 5.0 ml of p-hydroxybenzoic acid hydrazide (PHBAH) coloured solution and few drops of sodium hydroxide,

mix well and keep the test tube in water bath in boiling mode. After about 5 minutes withdraw test tube from water bath, allow to cool, then add 10 ml of water and mix well.

Instrumentation

Before measuring absorbance by colorimeter clean the glass tube with water, and ensure zero absorbance with diluent.

To finalize the light source, take sample solution in glass tube and keep first in blue light source and repeat with the available light source simultaneously recording absorbance. Use the light source with highest absorbance and record the absorbance for the followings.

1. Standard stock
2. Working standard solution
3. Sample of unknown concentration.

Note the above three different absorbance. Now plot the graph of two known solution (1 is standard stock, 2 is working standard) i.e. absorbance (Y-axis) against concentration (X-axis), plot a straight line in between two points and find the concentration of unknown sample with the sample absorbance.

CONCLUSION-

The chelate formed from the reaction of sucrose and p-hydroxybenzoic acid hydrazide (PHBAH) in alkaline medium is measured by colorimeter which is the basis of quantifying the amount of sucrose present in sample.

REFERENCES-

1. https://www.vernier.com/products/sensors/colorimeters/col-bta. Accessed on 5 August 2017.

2. Lever M. Colorimetric and Fluorometric Carbohydrate Determination with p-Hydroxybenzoic Acid Hydrazide. Biochem Med. 1973;7: 274-81

Estimation of Sodium by flame Photometry

AIM- The aim of the experiment is to estimate sodium in the given sample by flame photometry.

BACKGROUND-

Flame photometry, more precisely called *flame atomic emission spectrometry* or "*flame photometry*" is a traditional instrumental analysis method. It is originated a long back to Bunsen's flame-colour tests for the qualitative identification of some selected metallic elements. Most probably a very simple example of the atomic emission effect is fireworks for 4th of July celebrations and other events. As an analytical method, atomic emission is a fast, simple, and sensitive method for the determination of trace metal ions in solution. Because of the very narrow (*ca*. 0.01 nm) and characteristic emission lines from the gas-phase atoms in the flame plasma, the method is appreciably free of interferences from other elements. Typical precision and accuracy for analysis of dilute aqueous solutions with no major interferences present are about ±1-5% relative. Detection limits can be quite low. "Good" elements are having detection limits lying between about 1 ng/ml and 1 μg/ml. The method is appropriate for many metallic elements, especially for those metals those are easily excited to higher energy levels at the relatively cool temperatures of some flames – Li, Na, K, Rb, Cs, Ca, Cu, Sr, and Ba. Metalloids and non metals generally do not produce isolated neutral atoms in a flame, but mostly as polyatomic radicals and ions. Therefore, non-metallic elements are not preferred for determination by flame emission spectroscopic studies, except for a very few and under very specialized conditions.

Flame photometry is a highly empirical, in comparison to an absolute, method of analysis such as gravimetry. So the method must be calibrated carefully and frequently. Many different experimental variables affect the intensity of light emitted from the flame and that finding its way to the detector.

REQUIREMENTS-

1. The bottle(s) was/were washed and rinsed several times and then filled with deionized water
2. One 500 ml volumetric flask
3. Assorted volumetric and/or graduated transfer pipettes
4. Five 100 ml volumetric flasks for the standards (experiment locker)
5. Eight to ten small plastic containers for aspirating solutions (experiment locker)

PROCEDURE-

Standard sodium stock solution, 100.0 ppm

Accurately (to 0.1 mg) weighed out by difference 0.1271 g of reagent grade NaCl into a small plastic weighing boat. The exact mass was recorded, and corrected the concentrations accordingly.
(Remember: NEVER transfer chemicals inside an analytical balance.)
Carefully the salt was transferred quantitatively into a 500-mL volumetric flask. Then few squirts of deionized water was used from the wash bottle on the weighing boat and the sides of the flask to wash all of it down into the flask. [0.100 g Na/L = 100 mg/L = 100 μg/mL = 100 ppm Na). About 100 ml of deionized water was added to the flask, swirled several times, and dissolved all of the salt before diluting to volume with deionized water. This is critical.

Sodium standard calibration solutions

Deionized water is used for the "blank". A series of volume icluding 1.00, 2.00, 3.00, 4.00, and 5.00 mL of the standard 100-ppm sodium solution were taken into the first, second, third, fourth, and fifth 100-mL volumetric flasks, respectively and separately. Diluted carefully to the mark with deionized water and mixed thoroughly.

Unknown solution

The unknown was obtained from the instructor and carefully diluted to the 100-mL mark with deionized water and mixed thoroughly. Carefully followed the instructions provided for the use of the instrument and measured the emission intensity for the blank (deionized water), each standard, and the unknown(s).
When approaching to begin taking emission readings, utmost steps are followed to light the flame, stabilize the flame photometer, and for its proper and safe use. The instrument should have been turned on and the flame lit for 15 minutes [aspirating deionized water] to ensure stability. Thoroughly rinsed all the equipments used in this experiment, first with lots of distilled water, secondly with deionized water from a rinse bottle. Then filled the tall, 25 ml, capped polyethylene vials with the Blank (deionized water), the five standards (1, 2, 3, 4, and 5 ppm Na) and the unknown solution(s) – in that order – and placed in the plastic holder designed for them. Because water droplets cling to the vials, their insides will need to be pre-rinsed with small amounts of their solutions first. Then put a mL or two into a vial, caped it, shaken the contents into the sink.
Repeat at least 3 times for each vial. Aspirate deionized water until the meter reading stabilizes, this may take 30-90 sec. Then the blank knob was used to set the meter reading to 0.00. Then the highest standard (5 ppm) was aspirated until the meter reading has been stabilized. Then the fine sensitivity knob was used to set the meter reading to 5.00. The coarse sensitivity switch should be in the correct setting and not have to be switched.
Repeated the two-step calibration procedures with deionized water and the 5 ppm standard as many times as it takes to get them both stabilized at 0.00 and 5.00, respectively. The blank, the 5 standards, and the unknown(s) were aspirated in that order. Three replicate readings were done for each solution

once the meter reading has been stabilized. There may be some "bounce" (noise) in the readings, especially at the higher concentrations. For the second calibration run, place the unknown solution(s) between the two standards whose readings bracket that of unknown(s), so that the concentrations of the solutions aspirated now all increase monotonically.

Atomic emission instruments may work best when going from lower to higher concentrations.

The whole process of calibration repeated by taking triplicate readings as before at least 1 or 2 more times. The more data we have to review, the better the analysts will be able to detect and eliminate determinate error – inaccuracy in the final reported value.

CONCLUSION-

The calibration curve was done by plotting the emission intensities as a function of Na concentration. Determine the concentration of sodium in the unknown sample by reading the concentration of the sample which corresponds to its emission intensity from the calibration curve. Depending on the drift in the instrument and other factors, it may be better to average all three values for each solution and obtain one final value for the unknown, or to get three separate values for the unknown, each using its "own" calibration curve, and average the three values. If the plot appears to be reasonably linear, or at least that portion of it that includes your unknown, use the Excel LINEST function to do a linear-least-squares fit to the data, which will also be provided for some quality parameters for the fit. The "best estimate" was reported for the average concentration of sodium in ppm (μg/mL) and the associated standard deviation of the value.

REFERENCES-

1. Skoog DA, West DM, Holler FJ, Crouch SR. Analytical Chemistry: An Introduction, 7th ed., Chapter 23: 594-631.

Determination of Potassium by flame photometry

AIM- The aim of the experiment is to determine potassium by flame photometry

BACKGROUND-

Flame photometry, more precisely called *flame atomic emission spectrometry* or "*flame photometry*" is a traditional instrumental analysis method. It is originated a long back to Bunsen's flame-color tests for the qualitative identification of some selected metallic elements. Most probably a very simple example of the atomic emission effect is fireworks for 4th of July celebrations and other events. As an analytical method, atomic emission is a fast, simple, and sensitive method for the determination of trace metal ions in solution. Because of the very *narrow* (*ca*. 0.01 nm) *and characteristic* emission lines from the gas-phase atoms in the flame plasma, the method is appreciably free of interferences from other elements. Typical precision and accuracy for analysis of dilute aqueous solutions with no major interferences present are about ±1-5% relative. Detection limits can be quite low. "Good" elements are having detection limits lying between about 1 ng/mL and 1 μg/mL. The method is appropriate for many metallic elements, especially for those metals those are easily excited to higher energy levels at the relatively cool temperatures of some flames – Li, Na, K, Rb, Cs, Ca, Cu, Sr, and Ba. Metalloids and nonmetals generally do not produce isolated neutral atoms in a flame, but mostly as polyatomic radicals and ions. Therefore, non-metallic elements are not preferred for determination by flame emission spectroscopic studies, except for a very few and under very specialized conditions.

Flame photometry is a highly *empirical*, in comparision to an *absolute*, method of analysis such as gravimetry. So the method must be *calibrated* carefully and frequently. Many different experimental variables affect the intensity of light emitted from the flame and that finding its way to the detector.

REQUIREMENTS-

1. The bottle(s) was/were washed and rinsed several times and then filled with *deionized* water
2. One 500 ml volumetric flask
3. Assorted volumetric and/or graduated transfer pipettes
4. Five 100 ml volumetric flasks for the standards (experiment locker)
5. Eight to ten small plastic containers for aspirating solutions (experiment locker)

PROCEDURE-

Standard Potassium Stock Solution, 100.0 ppm

Accurately (to 0.1 mg) weighed out by difference 0.1253 g of reagent grade KCl into a small plastic weighing boat. The exact mass was recorded, and corrected the concentrations accordingly.

(Remember: NEVER transfer chemicals inside an analytical balance.)
Carefully transfer the salt *quantitatively* into a 500-mL volumetric flask. Then few squirts of deionized water were used from the wash bottle on the weighing boat and the sides of the flask to wash all of it down into the flask. [0.100 g K/L = 100 mg/L = 100 μg/mL = 100 ppm K).
About 100 ml of deionized water was added to the flask, swirled several times, and dissolved all of the salt before diluting to volume with deionized water.

Potassium Standard Calibration Solutions

Deionized water is used for the "blank". A series of volume including 1.00, 2.00, 3.00, 4.00, and 5.00 mL of the standard 100-ppm potassium solutions were taken into the first, second, third, fourth, and fifth 100-mL volumetric flasks, respectively and separately. Diluted carefully to the mark with deionized water and mixed thoroughly.

Unknown solution

The unknown solution was obtained from the instructor and carefully diluted to the 100-mL mark with deionized water and mixed thoroughly. Carefully followed the instructions provided for the use of the instrument and measured the emission intensity for the blank (deionized water), each standard, and the unknown(s).
When approaching to begin taking emission readings, utmost steps are followed to light the flame, stabilize the flame photometer, and for its proper and safe use. The instrument should have been turned on and the flame lit for 15 minutes [aspirating deionized water] to ensure stability. Thoroughly rinsed all the equipments used in this experiment, first with lots of distilled water, secondly with deionized water from a rinse bottle. Then filled the tall, 25 ml, capped polyethylene vials with the Blank (deionized water), the five standards (1, 2, 3, 4, and 5 ppm K) and the unknown solution(s) – in that order – and placed in the plastic holders designed for them. Because water droplets cling to the vials, their insides will need to be pre-rinsed with small amounts of their solutions first. Then put a mL or two into a vial, caped it, shaken the contents into the sink. Repeat at least 3 times for each vial. Aspirated deionized water until the meter reading stabilizes; this may take 30-90 sec. Then the blank knob was used to set the meter reading to 0.00.
Then the highest standard (5 ppm) was aspirated until the meter reading has been stabilized. Then the fine sensitivity knob was used to set the meter reading to 5.00. [The coarse sensitivity switch should be in the correct setting and not have to be switched.]
Repeated the two-step calibration procedures with deionized water and the 5 ppm standard as many times as it takes to get them both stabilized at 0.00 and 5.00, respectively. The blank, the 5 standards, and the unknown(s) were aspirated in that order. Three replicate readings were done for each solution once the meter reading has been stabilized. There may be some "bounce" (noise) in the readings, especially at the higher concentrations. For the second

calibration run, place the unknown solution(s) between the two standards whose readings bracket that of unknown(s), so that the concentrations of the solutions aspirated now all increase monotonically. Atomic emission instruments may work best when going from lower to higher concentrations.
The whole process of calibration repeated by taking triplicate readings as before at least 1 or 2 more times. The more data we have to review, the better the analysts will be able to detect and eliminate determinate error – inaccuracy in the final reported value.

CONCLUSION-

The calibration curve was done by plotting the emission intensities as a function of K concentration. The concentration of potassium in the unknown sample was determined by reading the concentration of the sample which corresponds to its emission intensity from the calibration curve. Depending on the drift in the instrument and other factors, it may be better to average all three values for each solution and obtain one final value for the unknown, or to get three separate values for the unknown, each using its "own" calibration curve, and average the three values. If the plot appears to be reasonably linear, or at least that portion of it that includes your unknown, use the Excel LINEST function to do a linear-least-squares fit to the data, which will also be provided for some quality parameters for the fit. The "best estimate" was reported for the average concentration of potassium in ppm (μg/mL) and the associated standard deviation of the value.

REFERENCES-

1. Skoog DA, West DM, Holler FJ, Crouch SR. Analytical Chemistry: An Introduction, 7th ed., Chapter 23: 594-631.

Determination of Sulphate by Nephelo turbid metric Method

AIM- The aim of the experiment is to determine sulphate in given sample by using turbid metric method.

BACKGROUND-

Sulphates are found in appreciable quantity in all natural waters, particularly high in arid and semi arid regions where natural waters in general have high salt content. Sulphate salts are mostly soluble in water and impart hardness. Water with high concentrations has a bitter test. Sulphate may cause intestinal disorders.

A group of environmental scientists are doing research on the effects of mining on human water supplies and the efficiency of several water treatment techniques.

When testing the water it should be kept in mind that an acceptable concentration for drinking water is 250 ppm OD sulphate. Although sulphate is not particularly toxic if the concentration is high then it may cause other problems, such as build up in high-pressure boilers, the taste of the water being bitter, and at concentrations above 1000 ppm, the water may cause diarrhoea. The turbid metric method for the determination of sulphate concentration is based on the fact that light is scattered by particulate matter in aqueous solution. When barium and sulphate react in water, they make the solution turbid, which means the concentration of the Sulphate can be measured by using a spectrophotometer. The equation for the reaction of barium and sulphate is shown below.

SO_4^{2-} (aq) + Ba_2^{+} (aq) ——> $BaSO_4$ (s)

REQUIREMENTS-

Chemicals: Concentrated HCl
Glycerol
Isopropyl alcohol
NaCl

Apparatus: Beakers
Flasks

PROCEDURE-

First a conditioning reagent was prepared by mixing 50 ml glycerol with a solution containing 30 ml concentrated HCl, 300ml distilled water, 100 ml isopropyl alcohol, and 75 g NaCl. Then, using a stock solution of 1000 ppm sulphate, create four 100 ml standards with the concentrations of 10.0 ppm, 20.0 ppm, 40.0 ppm, and 80.0 ppm.

For each of the standards the following procedure was used:

(I) Ten ml of standard and 10ml of distilled water added to a 250 ml Erlenmeyer flask. Then added with 5.0 ml of the conditioning reagent and stirred gently.

(II) Measured about 0.1-0.2 g of 20-30 mesh BaCl and added to the flask. Stirr the contents of the flask for one minute. After the completion of the stir time, quickly and carefully poured the contents into a spectrophotometer cell, and let stand for five minutes. While waiting, the spectrophotometer was auto zero at 420 nm with distilled water and then taken the absorbance of the standard.

(III) After taking the absorbance for the remaining three standards an absorbance vs. concentration curve was created, using the original concentrations of the standards. To find the concentration of sulfate in the samples, the same procedure was used for the standards.

CONCLUSION-

The value of sample in standard curve was intrapolated and the exact amount with percentage purity was calculated.

The amount of sulfate in the sample was found to be…….. mg.

REFERENCES-

1. AWWA, WEF, APHA, Standard Methods for the Examination of Water and Wastewater. 1998.

2. Sawyer CN, McCarty PL, Parkin GF. Chemistry for Environmental Engineering. Fourth Edition, McGraw-Hill, Inc., New York. 2000.

Turbid metric determination of chloride

AIM- The aim of the experiment is to do the turbid metric determination of chloride.

BACKGROUND-

Chloride is an aesthetic contaminant as it imparts a salty taste to water. The concentration of chloride in water is variable and dependent on the chemical composition of water. Normally, ground water has a lower concentration of chloride than surface water. With regard to wastewater, the C concentration can be quite elevated due to industrial processes and the high levels of sodium chloride in the diet, which pass unchanged through the digestive system.

Automated systems have already been developed for chloride determination using flow injection analysis (FIA),a methodology which presents significant advantages in comparison to conventional methods, in terms of sampling rate and reagent consumption. Some of these systems involve the reaction between chloride and mercury thiocyanate, with subsequent colorimetric measurement of iron thiocyanate, and therefore require the use of this toxic reagent (mercury thiocyanate), as well as iron nitrate. A less environmentally harmful system has been proposed, based on a turbid metric estimation involving silver nitrate with the formation of silver chloride.

REQUIREMENTS-

1. P^H Meter
2. Burette
3. All reagents must be ACS reagent-grade:

Barium chloride, anhydrous, Hydrochloric acid, 37%

Nitric acid, 70%

4. Potassium chromate indicator
5. Silver nitrate
6. Sodium chloride
7. Sodium hydroxide,1 N

PROCEDURE-

Barium Chloride, 10%

In a 500 ml beaker, 50 g of barium chloride was transferred. Then it was added with the deionized water to the barium chloride until reaching a total of 500 ml of solution. After that it was added with a stirring magnet to the beaker and stirred on a magnetic stirrer.Once all the barium chloride has been dissolved, transferred the solution to an airtight container.

Dilute Nitric Acid

Two mL of nitric acid was measured in a 10 ml graduated cylinder, and measured 38 mL of deionized water in a 50 ml graduated cylinder. Then the

deionized water and nitric acid were combined in a 200-mL tall-form beaker. Then it was stirred on a magnetic stirrer. Once the solution is thoroughly mixed, it was transferred into a drop-dispensing bottle.

Potassium Chromate Indicator

Accurately weighed 50 g of potassium chromate powder was taken in a 250-mL beaker. Hundred mL of deionized water was taken in a 100-mL graduated cylinder. Then it was added with the deionized water to the beaker with the potassium chromate. Then it was stirred on a magnetic stirrer for a while. Once all of the potassium chromate has been dissolved, the solution was transferred into a drop dispensing bottle.

Silver Nitrate, 0.1 N

On a balance, accurately weighed 17 g of silver nitrate powder was measured. In a graduated cylinder, 1 L of deionized water was measured. Then the silver nitrate and deionized water were mixed in a 1 L beaker. And then the solution was stirred on a magnetic stirrer. Once all of the silver nitrate has been dissolved, the solution was transferred into a 1 L light protective storage bottle.

Approximately 5 g of sodium chloride was dried at 212°F (100°C) for at least 1 hr. Using an analytical balance, weighed quantity of 0.2 g of sodium chloride to the nearest 0.0005 g into a tared 200-mL tall-form beaker. In a graduated cylinder, 100 mL of deionized water was measured. Then deionized water was added with to the beaker with the sodium chloride. Again the solution was stirred on a magnetic stirrer. Once the solution is thoroughly mixed, 10 drops of potassium chromate indicator added to the solution. Then a 50 ml burette was filled with the silver nitrate solution and titrated the sodium chloride solution with the silver nitrate to the first color change.

$$N = \frac{W}{(0.05844)\,V}$$

Where:

N = normality of the silver nitrate solution

W = mass of sodium chloride used for the titration, g

V = volume of silver nitrate used for the titration, mL.

The result from the titration was used to calculate the normality to at least to 3 significant digits:

Chloride ion content

50 ml of the filtered sample was pipetted out into a 200-mL tall-form beaker. Using the pH meter or pH paper, added either dilute nitric acid or dilute sodium hydroxide to adjust the sample pH to between 8–9. Then 11 drops of potassium chromate indicator was added to the sample. Then Stirred the solution until a solid yellow color persists throughout the sample. Then a 50-mL burette was filled with the 0.1 N silver nitrate solutions. It was stirred with a magnetic stirrer. Added the silver nitrate drop by drop until a brick red color persists

throughout the sample. Recorded the amount of silver nitrate used. Then the chloride ion concentration was estimated.

Calculations-

$$ppm\ Chloride = \frac{3.5433\ NVA\ (10,000)}{S}$$

Where:

N = normality of silver nitrate ($AgNO_3$)

V = volume of $AgNO_3$, mL

A = aliquot factor

S = sample weight, g.

CONCLUSION-

The amount of sulfate in the sample was found to be…….. mg.

REFERENCES-

1. Mesquita RB. Fernandes SM (https://www.ncbi.nlm.nih.gov/pubmed/?term=Fernandes%20SM%5BAuthor%5D&cauthor=true&cauthor_uid=12094945),Rangel AO (https://www.ncbi.nlm.nih.gov/pubmed/?term=Rangel%20AO%5BAuthor%5D&cauthor=true&cauthor_uid=1209 945). Turbidimetric determination of chloride in different types of water using a single sequential injection analysis system, J Environ Monit. (https://www.ncbi.nlm.nih.gov/pubmed/12094945) 2002;4(3):458-61.

Assay of Azithromycin by HPLC in tablet dosage form

BACKGROUND-

Azithromycin is administered in di_erent dosage forms to as antibiotic for the treatment bacterial infections. Few of the symptoms includes intestinal infections, middle ear infections, throat infection, pneumonia, traveler's diarrhea, and overall for respiratory tract infections. The Molecular weight and formula are 748.996g/mol and C_{38} H_{72} N_2 $O_{12.}$

AIM- Aim of the experiment is to determine the content of Azithromycin in tablet dosage form is elaborated which is simple by using HPLC with ultraviolet detector.

REQUIREMENTS-

Azithromycin Active Pharmaceutical Ingredient
Azithromycin tablets
Dipotassium hydrogen phosphate
Phosphoric acid
Acetonitrile
Volumetric flask
Mortar pestle
Weighing balance
0.45 μm Filter paper
Water
High Performance Liquid Chromatography
Column [C18 column (15 mc x 4.6 mm, 5 μm)]

PROCEDURE-

Buffer solution

Dissolve 1.16 gm of dipotassium hydrogen phosphate in water and dilute to 100 ml with water. Adjust pH to 6.5 with phosphoric acid.

Mobile phase

Mix buffer: acetonitrile : water in the ratio of 10:35:55

Diluent

Mix acetonitrile : water in the ratio of 40:60

Standard solution

Weight 20 mg of Azithromycin (API) in 100 ml volumetric flask containing about 50 ml of diluent, mix well and make up the volume up to 100 ml by diluent.

Sample solution

Triturate 20 no of azithromycin tablets in a mortar pestle. Weight equivalent to 20 mg of powdered sample in a 100 ml volumetric flask containing about 50 ml of diluent, shake vigorously to dissolve complete for about 10 -15 minutes, make up the volume up to 100 ml by diluent. Filter sample solution by 0.45 µm filter paper.

Chromatographic conditions

C18 column (15 mc x 4.6 mm, 5 µm)
Column temperature 70°C
Mobile phase flow rate 1 ml/min
Detection wavelength 215 nm
Injection volume 100 µl
Run the HPLC only with mobile phase at least for 10 minutes for conditioning of column.
Now inject standard solution six times by taking solution from volumetric flask each time and record the response/area.
Similarly inject filtered sample solution six times by taking solution from volumetric flask each time and record the response/area.

Sl. No.	Standard Area	Sample Area
1		
2		
3		
4		
5		
6		
Avg.		

Calculation

$$\text{Content (mg/tab)} = \frac{\text{Avg.sample area x 20 mg (standard weight) x 100 ml (sample dilution) x 99.8(potency of API) x Avg.wt of Tab}}{\text{Avg.Standard area x 100 ml (standard dilution) x wt.of sample powder equivalent to 20 mg x 100}}$$

CONCLUSION-

The content (mg/tab) of Azithromycin (market brand) tablet found to be ____.

REFERENCES-

1. https://medlineplus.gov/druginfo/meds/a697037.html
2. Indian Pharmacopoeia.

Separation of plant pigments by column chromatography

AIM- The aim of the experiment is to separate plant pigments by using column chromatography.

BACKGROUND-

The leaves of plants contain a number of colored pigments generally falling into two categories, chlorophylls and carotenoids. Chlorophylls a and b are the pigments that make plants look green. Carotenoids are part of a larger collection of plant-derived compounds called terpenes. Carotenoids are tetraterpenes (eight isoprene units). Lycopene, the compound responsible for the red coloring of tomatoes and watermelon, and β -carotene, the compound that causes carrots and apricots to be orange, are examples of carotenoids. Spinach leaves contain chlorophyll a and b and β -carotene as major pigments as well as smaller amounts of other pigments. Chlorophyll a and chlorophyll b are similar in structure and may not be able to be resolved in this procedure.

REQUIREMENTS-

Chemicals: Petroleum ether (hexane) and acetone
Apparatus: Round bottom flask
Chromatography column
Pipettes

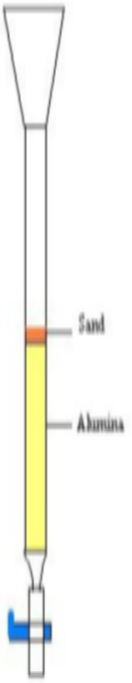
PROCEDURE-

Extraction of the pigments

About 5 grams of leaves is dried and placed in a mortar. Then the pigments are extracted by grinding the leaves with a pestle with about 5-10 ml of an 80:20 mixture (v/v), petroleum ether (hexane) and acetone. Then the liquid is decanted into a 50 ml round bottom flask. A quick filtration is done if necessary.

Preparation of the column

Wet pack method is used to prepare the column. The chromatography column is made with plastic tip with frit, the one-way stopcock, and the plastic funnel. The column is filled with enough alumina to get the required height. The dry alumina is poured into a beaker and hexane (pet ether) is added. The mixture is swirled and then poured into the column. The column is tapped gently, so air is not trapped as the alumina settles. Then it is added with a small amount of sand after the alumina has been settled. The column should not contain air bubbles and should be homogeneous. Then the solvent level is allowed to drop to the level of the alumina sand intersection.



Running the column

Using a long pipette, some of the pigment mixture is added directly onto the sand. Then it is added enough to fill the sand layer with color. Then the stopcock is opened and let the liquid level falls to the top of the alumina. Gently add petroleum ether to fill the sand layer. Then the stopcock is opened and let the liquid level fall to the top of the alumina. These steps are repeated at least three times or until all the colored compounds are in the alumina. Now the column is filled with petroleum ether.

Do not ever let the column run dry. Never let the solvent level fall below the level of the alumina!

Then the stopcock is opened to allow a drip rate of approximately 1 drop per second. First the yellow-orange β-carotene is eluted. As the yellow-orange colored product is eluted, it was collected in a test tube. 3. When the β-carotene has been eluted, the elutions of the chlorophylls are eluted by using a more polar solvent. Let the solvent level fall to the the top of the alumina. Gently the column was filled with either pure acetone or a petroleum ether acetone combination. By using 70% petroleum ether: 30 % acetone combination, it might be able to separate chlorophylls a and b. Then the chlorophylls are collected in a separate test tube.

CONCLUSION-

The R values of the above plant pigments are _____.

REFERENCES-

1. www.*web.fscj.edu/milczanowski/ten/chloro.pdf.*