JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

DEVELOPMENT AND EVALUATION OF AN ANTHOCYANIN–FLAVONOID CO-PIGMENTED COMPLEX INCORPORATED IN ACETATE BUFFER FOR PH MONITORING IN ACID – BASE TITRATION

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Abstract
In this research , a new acid – base indicator system utilizing the enhancement of anthocyanin colors from rose [Rosa indica] through the intermolecular co-pigmentation with flavonoids from Green tea is designed and its properties were studied. In this approach , anthocyanin extracted from rose is combined with flavonoid - rich source like green tea . The system's chemical reaction is brought about by the red flavylium cation , colorless hemi- ketal and pale chalcones inter conversion in a pH-controlled manner. Through non-covalent interactions like $\pi - \pi$ stacking and hydrogen bonding, flavonoid co-pigments form associations with anthocyanin-coloured structures which in turn increases and stabilizes the degree of visible color. An acetate buffer is used because first, it helps maintain an acidic condition that is well-controlled, and second, it assists in making the indicator more homogeneous so that the results of titration become more reproducible. Anthocyanins are naturally occurring water soluble pigments and are responsible for red, purple or blue colour in fruits , flowers and vegetables. The performance parameters of the color change range, the clarity of the transition from one colors to the other colors, the ease of recognition of the end point and the titration capabilities in direct comparison to classic color indicators are all ways in which the composition is measured and tested. This co-pigmentation system is aimed at offering a safer and naturally sourced substitute that can be used for pH change observation visually at the same time providing good enough colour resolution for acid-basic titration identification. The study will involve preparation of acetate buffers of different pH values and evaluation of colour changes using visual observation and UV-Visible spectrophotometer .

Index terms : Anthocyanin , Flavonoid , Co-pigmentation , UV-Visible spectrophotometer , Absorbance , Acid – base titration , pH meter

1. Introduction

The accurate monitoring of pH in acid-base titrations is one of the major requirements for getting reliable analytical results. Yet, since these experiments depend mostly on synthetic indicator compounds, it is a priority to find and develop less hazardous, natural substitutes**[1,2].** The demand for such substitutes is more relevant to educational and small-scale laboratories where the indicators needed should be cheap and easily available besides being simple to make**[1,2]**. Anthocyanin pigments are good candidates as the different structures like flavylium cations, quinonoidal bases, hemiketals and chalcones can exist in a certain equilibrium based on pH that results in a visible change of the solution's color**[3].** The buffer acetate has been selected, since the micro environment around anthocyanins will remain quite similar about pH and other variables as well as anthocyanins will be protected against degradation **[4,5]**.

The buffer also helps to preserve the red color from the natural anthocyanin, making the indicator much clearer at the endpoint**[4,5]**.Besides keeping it safe from degradation, the complex is stabilized against environmental degradation because of this overcoming the usual stability issues that prevent these natural indicators with pH sensitivity from being used reliably**[6].** In addition, this study also looks into how varying ionic strength and the buffer capacity of acetate media would affect the anthocyanin equilibrium, so changing the visible transition intervals. This is a good method as not only the composition of the pigments of a natural indicator affects the endpoint response, but also the environment in which the proton is transferred. Anthocyanins have been found to be in particular good in this way since, through the structural changes, they give rise to different red purple blue, and yellow colour states per the pH conditions, one from another only slightly**[1]**.Whereas flavonoid co-pigments can modify the intensity and stability of these states through π–π stacking, electrostatic interactions, hydrophobic effects, and hydrogen bonding **[3].** Same thing, earlier researches have shown the use of natural plant pigments as effective and affordable substitutes to commercially chemical dyes in titration experiments**[7]**. And that extracts high in anthocyanins will give a visible endpoint change of similar nature to both the other indicators and the existing generic indicator**.** The acetate buffer is this way applied as a performance-enhancing agent (beyond the solvents mode of expression) that would have bridged the gaps of the index between established reproducible conditions by imposing a reproducible acidic microenvironment, temperature buffering, and a more uniform co-pigment area during titration. The empirically optimized formulation would then work as a colourimetric standard against which the transition between acid – base performance indicators of sharpness, contrasting origin of pigmentation, repeated reliability, and endpoint visibility could be gauged within a controlled experiment. This structure also gave the ability to investigate the aggregate effects of acetate concentration, flavonoid/anthocyanin ratio temperature illumination while separating such influences of the acetate medium from potential cofactors of co-pigmentation. Such a stabilizing structure allowed a true transition from attempted empirical pigment trial-and-error to a standardised indicator system of analytical quality **[8].**

2. Literature review

Anthocyanins have a natural advantage for use in color indicator development because their color is a result of a reversible multistate equilibrium instead of just the one structural form of a compound. In a highly acidic environment, the red flavylium cation is the prevalent form but with the gradual increase of pH, we get more hydrated and deprotonated species, which are isomerized to blue, purple quinoid bases, colourless hemiketals or yellow chalcones **[9].** The relative concentrations and relative rates of interconversion of these different species will be affected by anthocyanin substitution, the solvent composition , the presence or absence of illumination, and the chemical history of the solution; so an extract may have a wide or overlapping transition interval rather than the distinct colour change observed with a synthetic, purified indicator. While this multistate behaviour has practical advantages for a visual indicator, allowing detection of from a range of pH, it does throw up problems of defining a unique endpoint and maintaining colour intensity homogeneity. Flavonoid co-pigmentation can makes better this behaviour through chemical modification. Phenolic flavonoids can associate with the planar, polarizable chromophore of the anthocyanin via various dispersion and electrostatic components of stacking, hydrophobic interactions, hydrogen-bonding, and in some systems charge-transfer effects**[10].**

Such interactions can result in shifts to longer wavelengths (barthochromic) and higher intensity (hyperchromic), which makes the observed color more pronounced and shields the anthocyanin flavylium chromophore from nucleophilic attack by the solvent**[11]** . This effectively suppresses the formation of colorless hemiketal and chalcone forms**[11]**. Previous study using spectroscopic methods revealed that flavonoid glycosides as well as other phenolic compounds are capable of interacting with anthocyanins to create detectable complexes, and such observation substantiated that co-pigmentation is more than a mere colour - enhancing occurrence, but rather it represents an actual molecular interaction that can be detected and measured quantitatively**[12].** Co-pigmentation is a powerful means of controlling the color transition of a dye but in many ways it cannot override the effects due to the surroundings. That is to say, environmental conditions like the pH, ionic strength, pigment-to-co-pigment ratio, buffer composition temperature oxygen and light can also change association equilibria and so either narrow or broaden the visible transition. So an acetate buffer is useful not only for the regulation of acidity but also for fixing a reproducible medium which enables the comparison of the anthocyanin proton-transfer equilibria and pigment, co-pigment associates in a systematic way. Naturally occurring indicators such as methyl orange and phenolphthalein are usually picked from their known transition ranges and reliable endpoint responses, whereas natural plant extracts are mixtures of anthocyanins flavonoids acids, sugars, and other compounds that can cause variability from one source to another. But, plant-based indicator investigations still document visible changes of color with end-point potential agreements of synthetic ones and highlight their advantages like low toxicity, easy availability, inexpensive, and environmentally

friendly**[7].** That is why, the major literature gap is not showing that anthocyanin extracts change in colour with varying pH levels, but rather a development of a standardization protocol that turns this natural variability into a clear, stable, and reproducible analytical signal. To fill this literature gap, anthocyanin - flavonoid co-pigmented acetate buffer has been proposed as a means to integrate the buffer, the pigment, and co-pigment to form a one indicator system whose range of transition, colour contrast, storage behaviour, and titration accuracy may be compared with the standard reference indicators in identical experimental conditions. The synergy here is that instead of working with the chemical components separately, we are treating them as an integrative indicator system whose response characteristics such as range of transition and colour change (and in this context how many degrees apart the hues can be before the human eye cannot distinguish them anymore (like the saturation), storage stability, and titration accuracy (which for a good indicator is defined as how close to the actual value (measured in pH) one can get without the use of any instrumentation other than a burette and a color scale would become the evaluation criteria for an effective standardized indicator**[12].** And, the reliability of these naturally obtained colors indicators is determined to great extent by the chemical properties of the pigments. In other words, the vividness and lastingness of the color produced largely depend on the structure of and charge of anthocyanins derivative molecules present**[13].** Because of this, tackling the problem of these natural pigments being vulnerable to environmental stress factors still appears to be one of the important conditions for achieving indicator films stable performances when used in actual analysis works. Anthocyanins can be destroyed through processes like heat light presence of oxygen and adverse pH which all factors can decrease the intensity of color and change the observed transition range**[14].** Even though co-pigmentation leads to stable color hues, it is impossible to completely eliminate media- and storage-related degradation; so, the evaluation of indicator films should cover color fastness, uniform response of color change, and clear endpoint visualization under controlled conditions. Actually, since the wider colorimetric range of anthocyanin-based systems could benefit from pH monitoring, such calibration is very helpful in fact. But, the same characteristic, if used as a criterion of titration endpoint assessment via the eye, can result in a reduction in the level of accuracy of visual determination**[15]**.The previous researches show that natural anthocyanin pigments show considerable color change with pH , but their practical use as indicators can be limited by instability and relatively weak color intensity under some condition . Therefore there is need to investigate whether co-pigmentation with flavonoids can improve color response of anthocyanin and produce a more useful natural pH indicator system. This research is mainly focusing on the hypothesis of combination of anthocyanin from rose with flavonoid from green tea for co-pigmentation that will increase colour intensity rather than using anthocyanin pigment alone. It is further hypothesized that developed co-pigmented system will exhibit reproducible colour change when exposed to acetate buffer solution of different pH value.

3. **Methodology**

In this research , we describe a methodology for the anthocyanin-flavonoid co-pigmented acetate buffer preparation. The method entails diluting concentrated plant extracts into an isotonic solution of sodium acetate to provide an environment of constant ionic strength and high chemical durability for the anthocyanin compounds as given in the figure 1**[16]** . Introducing the flavonoid co-factors at precisely controlled molar concentrations can stabilize the flavylium chromophore via intermolecular , $\pi - \pi$ stacking interaction**[17]**.

Aim : To develop and evaluate natural Anthocyanin-Flavonoid Co-pigmented buffer – indicator system using rose Rose extract and Green tea extract for approximate pH monitoring during acid – base titration.

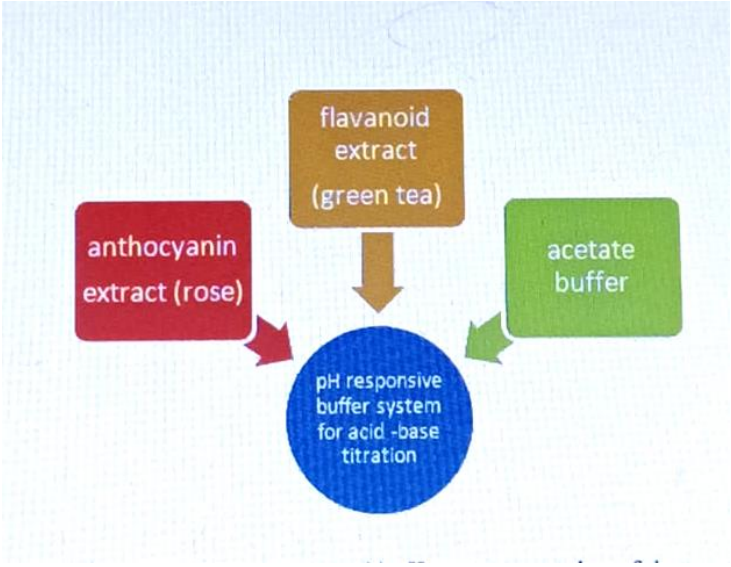

Figure-1: the basic principle of proposed buffer system consists of three components

3.1 Materials required :

i. Plant materials like fresh or strongly coloured rose petals and Green Tea .

ii. Chemicals : Distilled water , glacial acetic acid(CH_3COOH) , sodium acetate (CH_3COONa) , concentrated HCl , dilute HCl , dilute NaOH , 0.5M NaOH (sodium hydroxide), xM (CH_3COOH) acetic acid

iii. Apparatus : Beaker , Test tube , measuring cylinders ,conical flasks ,burette , pipettes / dropper , funnel and filter paper , mortar and pestle , water bath , glass rod , pH-meter , amber bottles , UV-Visible spectrophotometer.

3.2 Objectives :

3.2.1 To prepare Anthocyanin – rich extract from rose petals .

3.2.2 To prepare Flavonoid / polyphenol –rich extract from green tea .

3. 2.3 To perform preliminary qualitative test .

3.2.4 To prepare acetate buffer of different pH.

3.2.5 To prepare Anthocyanin-Flavonoid co-pigmented buffer solution and to study colour response of co-pigmented system at different pH values

3.2.6 To do acid – base titration with newly developed co-pigmented buffer to see change in color at different pH

3.2.7 To use UV-Visible Spectrophotometer for finding absorbance (A) and maximum wavelength.

Procedure to follow :

3.2.1 To prepare Anthocyanin – rich extract from rose petals

Select fresh, strongly coloured rose petals and wash gently with distilled water. Remove excess water and cut petals into fine pieces. Than weigh approximately to 7-8 grams and grind it using mortar and pestle. Transfer to 100ml beaker or conical flask. Add distilled water and markup to 100ml and let it stand still by mixing with glass rod for 1 hour so that maximum pigments get extracted. Than filter it using filter paper and funnel and store it in amber bottle so there is no direct contact with sunlight.

3.2.2 To prepare Flavonoid / polyphenol –rich extract from green tea

Weigh about 7-8grams of Green tea and then grind into finer powder so as to increase surface area for releasing flavonoid pigment. Then put it into 100ml flask and add distilled water to markup to mark and let it stand still by mixing with glass rod for 1 hour so that maximum pigments get extracted. Than filter it using filter paper and funnel and store it in amber bottle so there is no direct contact with sunlight.

3. 2.3 To perform preliminary qualitative test

For Anthocyanins :

Take 2 mL of the red rose filtrate in a separate test tube. Add a few drops of concentrated Hydrochloric Acid **(**HCl).

Observation**:** The red color will persist, stabilize, or intensify into a vibrant magenta or deep cherry red.

Inference**:** The retention or enhancement of the deep red pigment at low pH (pH 1–2) indicates the stable formation of the flavylium cation, a hallmark characteristic of anthocyanins.

For Flavonoids :

Take 2 mL of the green tea filtrate.

Add a few drops of a dilute Sodium Hydroxide (NaOH) solution to the extract.

Observation: An intense yellow color appears instantly.

Inference: When a few drops of dilute acid (HCl) are added subsequently and the yellow color turns colorless or disappears, it confirms the presence of flavonoids.

3.2.4 To prepare acetate buffer of different pH

- First we need to prepare 0.1 M anhydrous sodium acetate solution in distilled water for 200ml in beaker and 0.1M glacial acetic acid solution in distilled water for 200ml in beaker .
- For 0.1M anhydrous sodium acetate (anhydrous 0.1M CH_3COONa) : weigh 1.64 grams of anhydrous sodium acetate and add in beaker and add distilled water to make it of 200ml.
- For 0.1M glacial acetic acid (CH_3COOH) : take 1.2ml glacial acetic acid and add it in beaker and add distilled water for mark of 200ml .
- Now using this 0.1M solutions of anhydrous sodium acetate and glacial acetic acid for making acetate buffer of different pH values as given in the below table 1 :

Table 1 : volumes of 0.1M sodium acetate and 0.1M glacial acetic acid for making buffer of different pH

Target pH	**0.1M Sodium acetate**	**0.1M Glacial acetic acid**	**Final volume**
3.6	13ml	187ml	200ml
4.5	84ml	116ml	200ml
5.5	194ml	6ml	200ml

After making this buffer of different pH,check by pH meter that pH are in proper range if not than add sodium acetate in appropriate concentration to make the target range.

3.2.5 To prepare Anthocyanin-Flavonoid co-pigmented buffer solution and to study colour response of co-pigmented system at different pH values

Table 2 : use the given table to form co-pigmented buffer solution and see colour response at different pH

Conical flask	Buffer of pH	Anthocyanin	Flavonoid	Buffer solution	Total Volume	Color response
A	3.6	12ml	8ml	10ml	30ml	light yellow
B	4.5	12ml	8ml	10ml	30ml	light yellow
C	5.5	12ml	8ml	10ml	30ml	reddish/brown

Figure-2: co-pigmented buffer solution and see colour response at different pH of 3.6, 4.5 and 5.5 respectively.

As we can see colour intensity in conical flask [C] is more than compared to others that means our co-pigmented buffer solution is more effective to use as an acid-base indicator. So acetate buffer of pH 5.5 is suitable along with co-pigmented extract to use for titration .

3.2.6 To do acid – base titration with newly developed Co-pigmented buffer to see change in color at different pH

For doing acid base titration we need 0.5M NaOH (sodium hydroxide) and xM (CH_3COOH) weak acetic acid. Take 0.5M NaOH in burette and in beaker take 50ml weak acetic acid.

Then add 7-8 drops of prepared anthocyanin-flavonoid copigmented acetate buffer solution in beaker that will work similar to indicator that will change colour of overall solution when pH of solution increases. Light yellow colour initially without adding NaOH will change to dark reddish/brown colour.

Also keep pH meter calibrated and adjust temperature nob at room temperature. Standardized the pH meter using known buffer solution of pH 4 and 7.

Preparing all this , firstly measure the pH of solution when concentration of NaOH is 0ml in beaker .

Then start titrating by adding 0.5 ml NaOH into beaker drop-wise. After each 0.5 addition of NaOH in solution also measure it's pH at each successive addition of NaOH which will be easy to identify colour change in our solution. And note down the intensity and pH of solution in beaker at each step.

3.2.7 To use UV-Visible Spectrophotometer for finding absorbance (A) and maximum wavelength.

Firstly switch on the instrument and then clean the quartz cuvettes. Then fill 2/3rd or 3/4th with blank solution or zero solution that is our acetate buffer of pH 5.5 . First scan anthocyanin extract from range 400-700nm. Also repeat this for flavonoid extract , anthocyanin – flavonoid extract with buffer and at last for co-pigmented mixture with buffer of pH and also find its maximum wavelength and maximum absorbance and record the readings. Also change wavelengths of co-pigmented buffer solution so as to get maximum absorbance of it and note down the reading. This means that higher the absorbance of solution there will be more light absorbed by compound.

4. Results

[A] For doing acid – base titration with newly developed co-pigmented buffer to see change in color at different pH there will be different intensity of colors seen as we move from acidic condition to basic condition. Now we can see the result and analysis of our buffer solution with increase in pH and intensity of colour change .

Table 3: to do acid – base titration with newly developed co-pigmented buffer to see change in color at different pH the observation table is as follows :

Sr. No.	Volume of NaOH in ml	pH in beaker on adding NaOH (average)	Color of indicator	Intensity
1.	0ml	2.34	light yellow	very weak
2.	0.5ml	2.56	light yellow	very weak
3.	1ml	2.66	light yellow	very weak
4.	1.5ml	3.18	light yellow	very weak
5.	2ml	3.19	light yellow	very weak
6.	2.5ml	3.25	light yellow	weak
7.	3ml	3.33	light yellow	weak
8.	3.5ml	3.60	light brown/yellow	moderate
9.	4ml	3.64	light brown/yellow	moderate
10.	4.5ml	3.81	light brown/yellow	moderate
11.	5ml	3.91	light brown/yellow	moderate
12.	5.5ml	4.19	yellow	moderate
13.	6ml	4.56	yellow	moderate
14.	6.5ml	4.73	yellow	light
15.	7ml	5.34	reddish /brown	light
16.	7.5ml	5.58	reddish /brown	high
17.	8ml	8.7	reddish /brown	high
18.	8.5ml	10.1	reddish /brown	high
19.	9ml	10.5	reddish /brown	very high

20.	9.5ml	11.3	reddish /brown	very high
21.	10ml	11.64	reddish /brown	very high

This means that when we add drop-wise NaOH in beaker the basic nature of solution increases and thus the pH also increases , thus giving change in colour .

During this there is shift in colour intensity from light yellow to dark reddish –brown of solution in beaker that contains weak acetic acid and our newly developed buffer solution .

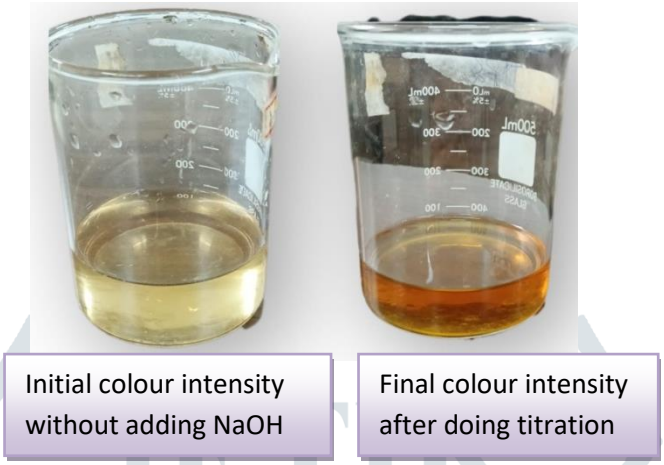

Figure -3: this image shows difference in intensity of colour of beaker solution having weak acetic acid and our newly developed buffer solution when titrated with dil.NaOH .

- We can also plot the graph for volume of NaOH Vs pH change from the experiment :

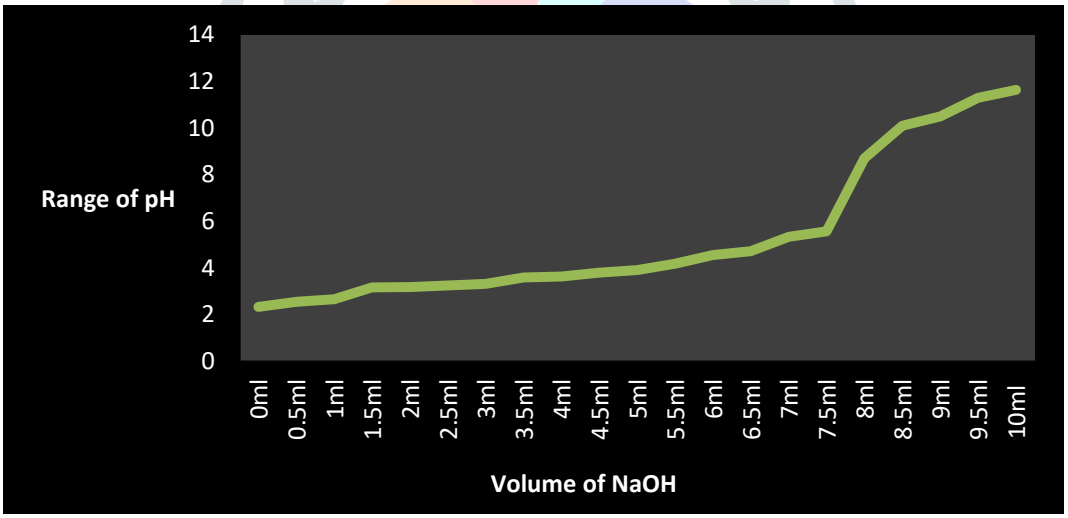

Figure-4: graph of volume of NaOH vs range of pH change with respect of titration

[B] To use UV-Visible Spectrophotometer for finding absorbance (A) and maximum wavelength

Using UV – Visible spectrophotometer we get absorbance of anthocyanin extract , flavonoid extract , anthocyanin + flavonoid mixture , anthocyanin +buffer and anthocyanin – flavonoid co-pigment complex with buffer with wavelength of approximately 400-700nm. Changes in absorption after mixing can provide additional evidence for an interaction.

Table 4: we have used UV-Visible Spectrophotometer for finding absorbance of different samples at different wavelength and the data analysis is shown in the table :

Sr. No	Samples	Extracts	Volume of each accordingly	Final volume	Absorption	Wavelength (nm)
1.	Blank	buffer of pH 5.5	5ml	5ml	0.165	400
2.	Anthocyanin extract	anthocyanin+ buffer of pH 5.5	1.5ml+3.5ml	5ml	0.447	400
3.	Flavonoid extract	flavonoid +buffer of pH 5.5	1.5ml+3.5ml	5ml	0.692	400
4.	Anthocyanin+flavonoid+ buffer	Anthocyanin+ flavonoid+ buffer of pH 5.5	1ml+1ml+3ml	5ml	0.696	400
5. [A]	Co-pigmented mixture + buffer of pH 5.5	co-pigmented mixture + buffer of pH 5.5	2ml + 3ml	5ml	1.674	400
[B]	Co-pigmented mixture + buffer of pH 5.5	co-pigmented mixture + buffer of pH 5.5	2ml + 3ml	5ml	2.631	360
[C]	Co-pigmented mixture + buffer of pH 5.5	co-pigmented mixture + buffer of pH 5.5	2ml + 3ml	5ml	2.784	380
[D]	Co-pigmented mixture + buffer of pH 5.5	co-pigmented mixture + buffer of pH 5.5	2ml + 3ml	5ml	2.349	385
[E]	Co-pigmented mixture + buffer of pH 5.5	co-pigmented mixture + buffer of pH 5.5	2ml + 3ml	5ml	2.279	390
[F]	Co-pigmented mixture + buffer of pH 5.5	co-pigmented mixture + buffer of pH 5.5	2ml + 3ml	5ml	0.904	430
[G]	Co-pigmented mixture + buffer of pH 5.5	co-pigmented mixture + buffer of pH 5.5	2ml + 3ml	5ml	1.223	500

[Note: In the table the Co-pigmented mixture is anthocyanin 12ml and flavonoid 8ml]

[C] The graphical representation for our analysis of data found during experiment :

i.We can also plot graph of Absorbance value Vs Wavelength , as shown in the below graphical data :

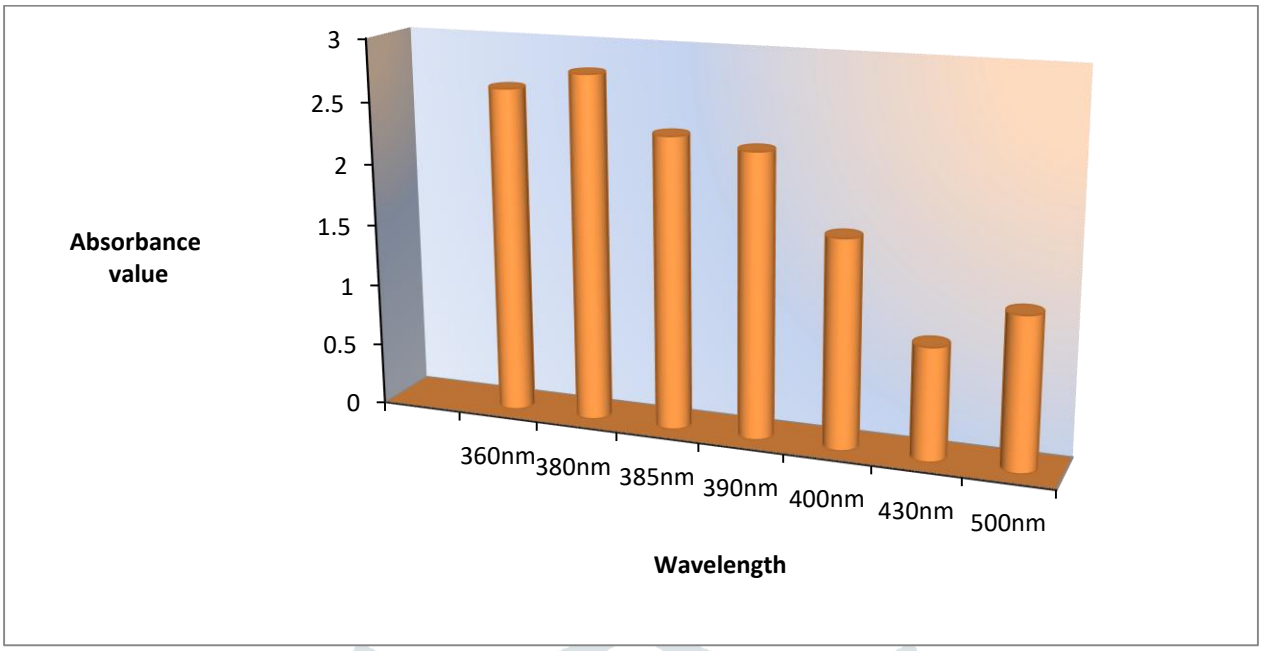

Figure-5: graphical data analysis of absorbance value vs wavelength

ii.We can also plot graph for different samples taken Vs its Absorbance value :

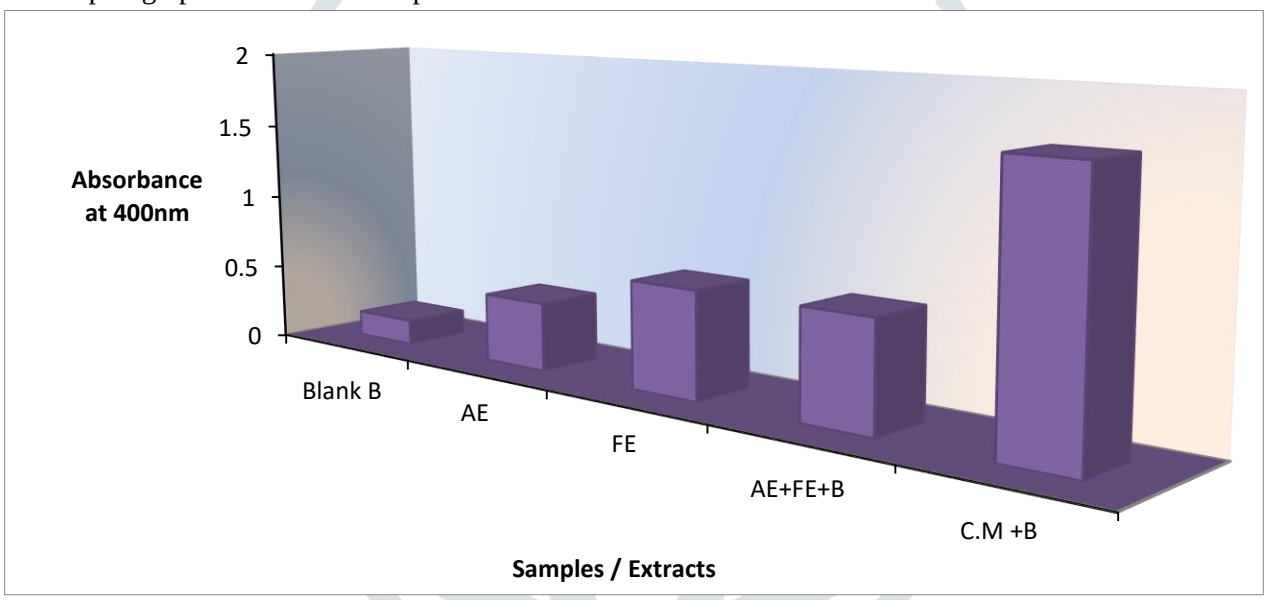

Figure-6: graphical representation of absorbance at 400nm vs different samples/extracts and from here we can find that the maximum wavelength for our developed co-pigmented buffer solution is 380nm of UV range.

Note : Here in the graphical representation the terms are known as follows :

B – buffer , AE- anthocyanin extract , FE – flavonoid extract , C.M- co-pigmented mixture

5. Discussion

This improved spectral clarity enables greater distinctiveness of the color shift at the equivalence point and so it reduces the visual uncertainties of crude anthocyanin extracts. The definite acetate medium also helps in preserving the constant ionic and pH condition, enabling the color change to be read reliably from one titration to another. Regardless, the indicator has to be selected as per the acid- base system and titrant strength because precision of the endpoint largely depends on the shape of titration curve, the relative strengths of the two reactants as well as the difference between the indicator change and the point of equivalence[**18**]. On top of that, the copigmented formulation may best be interpreted as a ready-to-use, application-specific substitute, rather than a comprehensive replacement for standard indicators, mostly in situations where the utmost precision or instrument-independent identification of endpoint is necessitated. Further fine-tuning the ratio of pigments to copigments, together with the modulation of polymer-based delivery systems, will provide a route of modifying the response characteristics and sensitivity of that sensors to perfectly align with the specific analytical goals. With modular approaches at their fingertips, the scientific community could be the ones who would be able to adjust the pH-sensing mechanisms to the various methods of titration could be accommodated, and thereby the functionalities of the natural pigments will increase a lot**[19]**.

In summary, combining both co-pigmentation and encapsulation methods gives an effective strategy for resolving the longstanding trade-off between vivid indicator colors and chemical stability over time**[20]**. Next steps would involve

researching ways to minimize the threat of oxidation, like preventing the formation of quinones, which are not only able to reduce pigment hues but can also cause pigments to turn colourless . This line of research would lead to the longer functional shelf life of such bio-based analytical reagents**[21]**. This kind of objective might be achieved through preparation and packaging in an environment with limited oxygen, storing the products in containers that block light out, and using methods to prevent exposure of colored dyes with the agents that can break them down. In addition, long-term investigation should also be carried out where scientists could determine colour retention, transition-range shifts, and endpoint reproducibility in different levels of temperatures, oxygen exposure, and time of storage**[22].**

6. Conclusion

Anthocyanin-flavonoid co-pigmented acetate buffer represents a viable environmentally safe substitute for pH measurement tools by showcasing that standardized natural substances can give analytical results similar to synthetic reagents when suitable titration systems are chosen . Its homogeneous nature together with it gives very strong color response and really makes easier the viewing of end points without losing the advantages of the use of environmentally, low-toxicity, and locally available raw materials .From figure 6 , we can also conclude that co-pigmented mixture along with buffer solution is having higher absorbance value than any other samples which also indicates that this co-pigmented buffer system is applicable for acid-base titration. Still, wider application demands its validation in different acid-base systems, various batches of pigments, storage periods, and observer conditions to confirm its accuracy precision reproducibility, and shelf life. As a result, future optimization should mainly concern itself with pigment-to-co-pigment proportion, buffer solution type, methods of guarding from oxidation and light, and encapsulation or immobilization techniques since these methods could together increase the intensity of color and also prolong the color's stability to varying degrees. Based on pH measurements made through visual inspection using colour indicators, this work shows that natural anthocyanins can be used not only to produce pigments for the dyeing of materials, but also to monitor the progress and endpoint of such reactions. An inexpensive natural indicator system has that's why been suggested. The combination of anthocyanin extract from rose petals and flavonoid extract from green tea was prepared as one solution. These were after that mixed with an acetate-buffered medium to formulate the pH indicator with a combination of the two pigments. When the pH level rose, the formulation's colour quite a bit changed, proving that anthocyanin as a colorant was quite sensitive to pH level.For an acetate buffer system, pH level of 5.5 was the lowest pH at which the system showed more red-brown colour. As per UV-Vis spectroscopic results, the co-pigmented formulation was found to absorb a larger number of photon particles than the individual extraction systems. The results obtained show that when two types of pigments get mixed, they may result in a more noticeable change in colour. And, the co-pigmented system underwent successive shifts in hue during the gradual introduction of base solution, a characteristic which will be of great value for visual tracking of pH variations.The developed systems have been suggested as being very cost-effective, naturally-derived, and potentially eco-friendly. Still, with the practical utility of the developed systems as indicators, several experimental investigations and quantification studies need to be carried out. Among others, these include, the conduct of replication of experiments, the determination of transition range accurately, the study of anthocyanin-flavonoid ratio effects systematically, detailed spectroscopic studies, stability investigations as well as the comparison with synthetic indicators to check the validity of the developed system as a reliable analytical indicator.

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