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Review Article

AN OVERVIEW OF THE USE OF NATURAL INDICATORS IN ACID-BASE TITRATIONS

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Article History	Abstract
Received: 25-02-2024 Revised: 1-03-2024 Accepted: 30-03-2024	While titrimetric and gravimetric analysis were the most concerning analytical techniques, they are now the most commonly used techniques for identifying chemicals. The endpoint in the titrimetric analysis method is identified by colour changes from one medium to another caused by the addition of chemicals referred to as indicators. There are a lot of synthetic indicators on the market these days, which are expensive and pollute the environment. Human toxicity is produced by a number of synthetic markers. As demonstrated by their use in acid-base titrimetric analysis, floral extracts have a very promising analytical potential. Seven plant extracts were found to function well in strong acid-strong base titrations: violet cabbage, beetroot, red hibiscus flower (shoe flower), turmeric powder, red rose flower, henna leaves, and pink Mirabilis Jalapa flower (4 o'clock flower). For the violet cabbage, there was a noticeable and distinct colour shift from pale pink to violet; for the beetroot, it was dark brown to red; and for the red hibiscus flower extract, it was pale yellow to pink.
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Keywords: Natural sources, titrations, synthetic indicator, plant extract.	

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Introduction

The titrimetric analysis is a quantitative chemical analysis that involves finding the volume of a known concentration solution that reacts quantitatively with a known volume of a solution to the substance to be determined. The equivalency point in titrimetric analysis is usually defined by the detection of the endpoint. The endpoint can be detected by the color changes from one medium to another medium (either acidic or basic) with the addition of substances which is an indicator. (1-3) at a specific stage of the chemical reaction, indicators (either weak acid or weak base) change color. The identification of an acid-base indicator can be represented by a color change depending on the concentration of Hydrogen (H⁺) or Hydroxide (OH⁻) ions. (4) Methyl red, methyl orange, phenolphthalein, phenol red, methyl yellow, Penta methoxy red, bromophenol blue, and thymol blue were among the laboratory- based markers. (5)

Currently, the most usual analytical methods are established to identify compounds, though analytical methods like gravimetry and titrimetric analysis were the most concern. In the titrimetric analysis method, the endpoint is detected by the color changes from one medium to another medium (either acidic medium or basic

medium) with the addition of substances are known as indicators. Nowadays, many synthetic indicators are available, which produce environmental pollution and are costly. Several synthetic indicators produce toxicity in humans. Therefore, the search for alternative indicators from natural sources is required for cost-effectiveness and to minimize the toxicity and pollutant from the environment. Animals, fungus, and algae, can be used to isolate pigments or dye-based indicators. (6-8) Sir Robert Boyle first recorded the use of natural dyes in an acid-base indicator in his collection of assays "Experimental History of Colors" in 1664. (9,10) The various parts of the plant impart color

due to the presence of their extensive distinct character. The number of phytoconstituents including anthocyanins, glycosylated acylated anthocyanin, quinines, anthraquinonoids, naphthoquinones, flavonoids, acylated flavonoids, flavanols, imines, indigoids, polymethines, diarylmethanes, dihydropyrans, and carotene is responsible for the color property. Among them, flavones are water and alcohol soluble yellow pigments present in plant sources either in a free state or as glycosides or conjugated with tannins. In general, sometimes it is also identified as canthaxanthins (a chemically hydroxylated derivative of flavone). The water- soluble pigment is

anthocyanin which is mostly found in flowers, leaves, fruits of the plants. Anthocyanins belong to the class of glycosides, and their aglycones moieties are known as anthocyanidins.(11) Out of these, some compounds illustrate different colors at different pH levels. As a result, these features in natural substances can be used as an acid-base titration indicator. Nowadays, many synthetic indicators are available that produce pollution to the environment and are not cost-effective. (12,1).

Although there is automated attraction apparatus that determine the equivalent points between reacting species, indicators are still needed for teaching and research laboratories for simple titration (14). Natural indicators have been extracted from Hibiscus (red species), Bougainvillea and rose flowers (15). Several authors have reported on the effectiveness of natural indicators in acid-base titrations e.g. Nerium odor, Thespesia popular extract used as indicators ; Morus alba linn fruit extract indicator (17)and Ixora coccinea, Datura stramonium, Sun flower (Helianthus annus), pride of Barbados (Caesalpinia pulcherrima) and rail creeper (Ipomoea palmate) flower petal extracts . The natural indicator sources investigated in these papers have been extracted and prepared using ethanol, water, or methanol. Waakye leaves are obtained from Guinea corn which is cultivated mostly in African countries and other parts of the world(12). The plant is listed as the fourth major world cereal crop base on its production and is also considered as staple food in semi-arid tropics. The leaves are considered as good dye sources for dying hats, hand bags and wallets in Ghana (Azido 2006 unpublished Data

Celosia cristata is local plant of South America and now is distributed throughout Asia, especially, Malaysia. It is now considered as ornamental plant and frequently used for landscaping and roadside plants



due to the presence of various attractive colors of the flowers.

Figure :1 Celosia cristata

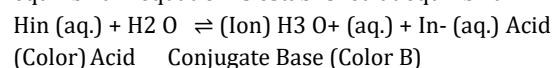
Celosia cristata produces purplish or reddish pigment due to the presence of cyanidin, a kind of anthocyanin (4). The tightly clustered blooms are said to appear as a rooster's comb, thus, the common name "Cockscomb". Flowers are in red, yellow, orange, gold and pink (16).

Some of the organic compounds. In spite of the numerous instrumental techniques currently available for the chemical analyses of various samples, conventional methods of analyses are still relevant and find application in many situations. In titrimetric, the equivalence point is determined by the end point in the titration is usually indicated by some substances added into

the analytic solution, which change color immediately after the equivalence point has been attained. Several types of indicators are available for different types of titrimetric analyses.

Acid base indicator and mechanism:

Acid-base (pH) indicators are either a weak acid or a weak base that is introduced to a solution in small amounts to visually determine the pH and change color when the pH changes. In the Arrhenius model,(15) a pH indicator is a chemical detector for Hydronium Ions (H_3O^+) or Hydrogen Ions (H^+) as shown in the Eq. no. 1. Weak acids or bases that dissociate somewhat when dissolved in water usually serve as indicators. To acquire understanding into the example of a weak acid with the formula Hin as an indicator. With its conjugate base, the following equilibrium equation is established at equilibrium.:



The colors of the acid and its conjugate base are distinct. Because the concentration of H_3O^+ is high at low pH levels, the equilibrium position shifts to the left, becoming color A. Similarly, the concentration of H_3O^+ is low at high pH levels, and the equilibrium position changes to the right, becoming color B. A universal indication is a mixture of indicators that gradually change color over a large pH range; when a few drops of the universal indicator are combined with the solution, the pH of the solution can be approximated. In most titration solutions, indicators are used to mark the conclusion of the acid-based reaction.[28].

Factors influencing the color of the indicator:

1.Effect of temperature on the color of the indicator.

The temperature has an impact on the color-based chemicals' stability. Natural pigments like Curcuma and tulip petals show no color change at 98°C and 92°C, respectively while borage at 60°C changes red-purple color. Studies have revealed that the pH of a solution shows an inversely proportional relationship with temperature except for water. A solution is considered acidic if the excess of hydrogen ions is present over the hydroxide ions. In the case of pure water, the hydrogen and hydroxide ions concentrations are always the same because of neutral characteristics (even if their pH changes)(9).

Natural Indicator:

Natural-based indicator plays a vital role during titration. Currently, various plants were used as a natural indicator, and their color changes in a different medium (acidic medium or basic medium) at different pH has listed Compounds used as a natural indicator:

Anthocyanin:

Anthocyanin The flavonoid anthocyanin has a positive charge oxygen atom on its C-ring. Anthocyanin's stability is affected by pH, light, temperature, and its chemical structure.(15) On the anthocyanin structure (Figure 1a) at the 7th position, the R group can be incorporated. Various groups such as a methoxy group, sugar, and other specific substitutions could influence the coloring behavior of anthocyanin.(11) Anthocyanin preparation derived from grape juice tanks has been allowed for use in human food, beverage production, and soft drinks, according to the Food Drug and Administration (FDA).(12)



Figure :2 Anthocyanin

At low pH, anthocyanins are stable. When subjected to heat, however, it loses its stability, resulting in color loss and browning. Anthocyanin molecules are present in the equilibrium of a solution between the colored cationic form and the colorless pseudo base form. pH has a direct impact on this equilibrium, which is critical for the color of anthocyanins. In acidic solutions, anthocyanins create red, violet or purple in neutral solutions and blue in alkaline solutions. Because anthocyanins have a flavylium cation in their structure, the cyanidin molecule is protonated and produces a cation at low pH. When the pH rises, the molecules deprotonate, and a reaction occurs. (14) The effect of changes in anthocyanin structure based on the surrounding solution and is depicted in. Therefore, most of the anthocyanin's colorant's can only be used at a pH below four. Additionally, most of the anthocyanin molecules can act as pH indicators in acid-base titration.(15) Anthocyanins are primarily found in plants' flowers, fruits, and tubers. The basic colors of anthocyanins are blue, purple, red, and orange, and are determined by the number of hydroxyl groups in the molecule, as well as an indirect relationship with the number of methoxy groups. Red clover, red pineapple sage, red rose, red hibiscus, and pink blossom are examples of red flowers that contain anthocyanin molecules. Anthocyanin is found in blue flowers including cornflower, blue chicory, and blue rosemary, as well as purple flowers like purple mint, purple passionflower, purple sage, common violet, and lavender. Apart from flowers, anthocyanin can be found in fruits such as apples. Tradescantia pallida leaves contain rich sources of anthocyanin, which are used for the prevention

of diseases. (19) Numerous anthocyanins from plants revealed different absorption spectra in the range between 465-550 nm, (11) and due to presence of these anthocyanins it produces different colors like red, pink, blue, purple, violet, and orange. Only a few aglycone anthocyanidins are much smaller (about 17). Six of the 17 anthocyanidins found in nature are cyanidin, delphinidin, pelargonidin, pelargonidin, malvidin, peonidin, and petunidin.

Cyanidin:

Cyanidin is an anthocyanidin-like natural plant pigment found in berries such as grapes, bilberry, blackberry, blueberry, cherry, cranberry, elderberry, hawthorn, loganberry, açai berry, raspberry, and other red-colored vegetables like red sweet potato, purple corn, red cabbage, and red onion. Other fruits that contain cyanidin include apples and plums. The color of this natural chemical is a distinctive reddish-purple. The cyanidin molecule has a red color when pH is less than 3, a violet color when pH is between 7-8, and a blue color when pH is greater than 11. The highest concentrations of cyanidin can be found in the seeds and skin of some fruits.

Delphinidin:

Delphinidin is a natural plant pigment that shows in the plant as a blue-reddish or purple color. Delphinidin is the blue pigment found in the flowers of the Viola and Delphinium genera. Apart from that, delphinidin, which may be found in cranberries, concord grapes, pomegranates, and bilberries, is responsible for the grape's blue-red color.

pelargonidin:

Pelargonidin is mainly a red-colored pigment, but it provides an orange color for a few flowers and red color in some fruits and berries. Pelargonidin can be found in red geraniums, spathes of philodendron Pelargonidin is found in mature raspberries, strawberries, blueberries, blackberries

Malvidin:

Malvidin is a glycosylated anthocyanidin with the sugar moiety connected at position 3 on the c-ring, resulting in malvidin-3-glucoside and malvidin-3-galactoside. Malvidin is found in the blue petal of the polyanthus group's primula (Anagallis monellin). Malvidin is also responsible for red wine's color. Blueberries (Vaccinium corymbosum) and saskatoon berries (Amelanchier alnifolia) also contain it. Malvidin solutions that are slightly acidic and neutral are red, while basic malvidin solutions are blue.

Peonidin:

Peonidin is an O-methylated anthocyanidin derived from cyanidin. Some flowers, such as peonies and roses, have a purplish-red colour due to peonidin. Some blue flowers, such as the morning glory, contain peonidin. At pH 2, peonidin is cherry red; at pH 3, it is a strong yellowish pink colour; at pH 5, a red-purple grape colour; and at pH 8 it becomes deep blue colour. It is stable at higher pH and has been isolated as a blue colorant from the brilliant

“Heavenly Blue” morning glory (*Ipomoea tricolor*).

petunidin:

Petunidin is a dark-red or purple water-soluble pigment found in many red berries, including choke berries (*Aronia* sp.), saskatoon berries (*Amelanchier* *antifolia*), and other grape species. It's also responsible for the colours of many flowers' petals. When the fruits are exposed to sunshine, it produces the deep purple colour of indigo rose tomatoes. The molecule's name is derived from the word *Petunia*.

Alizarin:

Alizarin is an orange dye that is present in the form of a glycoside in the root of the madder plant, *Rubia cordifolia* L., *Leylandii umbellata* L. (Indian Madder), *Rubia tinctorum* L. (European Madder). At pH 5.5 in 0.5%, the alcoholic solution of alizarin provides a yellow colour, and at pH 6.8, it appears to be in red.

curcumin:

Curcumin is a yellow pigment derived from the *Curcuma longa* (turmeric) plant. Turmeric contains 2 percent to 9 percent curcuminoids, depending on its origin and soil characteristics. Curcumin, desmethoxycurcumin, bis-desmethoxycurcumin, and cyclic curcumin are examples of curcuminoid chemicals. Curcumin is the primary component, while cyclic curcumin is the secondary component.

Esculin:

The esculin (7-hydroxycoumarin-6-glucoside) is a fluorescent dye obtained from *Aesculus* spp., including *A. glabra*, *A. californica*, *A. octandra*, *A. Pavia* and *A. hippocastanum*. Esculin changes the colourless to fluorescent blue at pH 1.5 – 2.

Logwood:

Logwood is a dye present in the yellow heartwood of *Haematoxylin campechianum*. The dyestuff contains the substance haematoxylin, but when exposed to air, it is oxidized and produce the purple compound haematoxylin or hematein. In an acidic medium, the colour logwood produces reddish colour, and in the alkaline medium, it produces blue shades.

pyrogallol:

Pyrogallol is derived from the aquatic plant *Microphyll spicatum*. Pyrogallol gives colourless to golden yellow in the variation of pH range 7.4 - 10.0.

The chemicals juglone [5-hydroxy-1, 4-naphthoquinone] and lawsone [2-hydroxy-1, 4-naphthoquinone] were isolated from *Juglandaceae* and *Lythraceae* plants, respectively. In an acidic media, juglone (Figure 1h) and lawsone (Figure 1i) have minor pale-yellow colours. But in the alkaline medium, they reveal pink and red colours.

Juglone and lawsone:

Plants in the *Bignoniaceae* and *Verbenaceae* families produce lapachol [2-hydroxy-3-(2-methyl-3-butenyl)-1, 4-naphthoquinone]. The compound is present mainly in the heartwood of *Tremella undulate*, *Tabebuia rosea*, and *Phylloerythrin* *Comorans*. The lapachol is also present in the stem bark of *Pteridosperm suave lens*.(13) Because protonation of the quinonoid oxygen atom suppresses its

quinonoid character, it produces colourless acidic media. However, because of its resonant structures, it has a red colour in the alkaline medium. Lapachol transition range is found to (Figure 1k) is an acid-base indicator obtained from the bodies of dried female insects *Dactylis's coccus* Costa

Cochineal:

Cochineal extract is obtained by using an aqueous-alcoholic or by alcoholic solution. But now a day's cochineal solutions are not used as indicators in acid-base titration.

Litmus:

Litmus is a dye derived from lichens of diverse types. Litmus is most commonly used to determine if a solution is acidic or basic(12). When exposed to acidic conditions, blue litmus paper turns red, and when exposed to alkaline conditions, red litmus paper turns blue. Purple is the colour of neutral litmus paper.

Materials and methods:

The chemicals and materials used for the study include; High Performance Liquid Chromatography (HPLC) grade ethanol, sodium hydroxide (NaOH), hydrochloric acid (HCl), acetic acid (CH₃COOH), sodium bicarbonate (NaHCO₃), methyl red, phenolphthalein, methyl orange of the American Chemical Society (ACS) grade, waakye leaves extract and Whatman No. 4 filter papers. The following apparatus such as; volumetric flasks (100 mL, 500 mL and 1000 mL), conical flask with volume size of 125 mL, burette of 50 mL, and graduated measuring cylinders of volume size 10, 20, 25 and 100 mL were used to carried out the experiment.

- Sample Collection and Preparation of Indicator Solution The plant materials (leaves) were purchased from an African market in Columbus, Georgia, USA and then ground into powdered form. The powder was sieved into an amber bottle and stored away from direct sun-light to prevent photolysis and decomposition. The natural indicator extract was prepared by weighing approximately 1.01 g of a powdered sample leaves into a Pyrex culture test tube (20 × 250 mm) and 25.0 mL of ethanol (99.9%) added. The mixture was vortexed for 5 minutes at ambient temperature (25°C) and then filtered using Whatman No. 4 filter paper into a new culture test tube of the same size, capped with a Teflon cap and store for use on the same day.
- Experiment with Natural Indicators Approximately 15.0 mL of 0.1 M HCl or 0.1 M CH₃COOH was titrated with 2.2 M NaOH using the natural indicator extracted from the waakye leaves in the order of strong acid versus strong base and weak acid versus strong base respectively, and then 5.0 mL of 0.1 M HCl or 0.1 M CH₃COOH was also titrated against the weak base 0.1 M NaHCO₃ in the order of (HCl v/s NaHCO₃, CH₃COOH v/s NaHCO₃). Three drops of the extracted indicator were added to each volume of acid used for the titration. The experiment was conducted in triplicate at some cases and replicates of five (5) as

- indicated The acidbase titration was carried out at room temperature.
- Experiment with Commercial Indicators For comparison, the procedure used for the commercial indicators (standard indicators) was the same as described above for the natural indicators. The experiment was conducted in triplicate and replication of five (5) and the results were analysed with simple Microsoft excel 2010 and SPSS statistical software. The statistics generated were used to discuss the results
- Determination of Total Flavonoids (TF) TF was determined by a colorimetric method as described in [6-8] with slight modification. Briefly, 250 μ L of sample was mixed with 1.25 mL of deionized water and 75 μ L of a 5% NaNO₂ solution. After 6 min, 150 μ L of a 10% AlCl₃·6H₂O solution was added to the mixture. The mixture was incubated at room temperature for 5 minutes, and then 0.5 mL of 1 M NaOH and 2.5 mL of deionized water were added. The mixture was then thoroughly vortexed and the absorbance of the light pink colour was measured at 510 nm against the blank using Perkier Elmer 8454 UV-Visible spectrophotometer. A blank solution prepared with ethanol replacing the guinea corn leaves extract. Results were expressed as absorbance per 1.0 g amount of extract dry weight (DW). All experiments were carried out using eight (8) replicates.
- Determination of Condensed Tannin (CT) CT content in sorghum bicolor crude extract was determined using a colorimetric method as described in [9, 10], with slight modification. A 1.0 mL of sample solution, 5.0 mL of vanillin/HCl reagent (0.5 g villain in 4% methanol plus 1.5 mL HCl solution (v/v)) was added. After mixing well, the mixture was allowed to stand for 20 minutes at room temperature in darkness. Absorbance was measured against the blank reagent at 500 nm using Perkier Elmer 8454 UV-Visible spectrophotometer. A blank solution prepared with ethanol replacing the guinea corn leaves extract. Results were expressed as absorbance per 1.0 g amount of extract dry weight (DW). All experiments were carried out in replication of eight.

pH of the plant extracts:

After the preparation of extracts, pH of these pure extracts was measured using a pH meter. The pH values indicate that except henna leaf extracts all other extracts are almost neutral. Henna leaf extract shows lowest pH value and indicates that it is acidic in nature. Violet cabbage extract recorded maximum pH value and shows some alkaline nature.

Sl.no	EXTRACT	PH
1	Violet cabbage	7.38
2	Beetroot	6.54
3	Turmeric power	5.19
4	Red rose flower	5.06
5	Mirabilis Jalapa flower	5.30
6	Henna leaves	3.96
7	Red hibiscus flower	6.60

Table 1, pH of pants extracts Investigation of indicator colour change with pH:

In order to study the colour, change of the plant pigment with pH, six solutions of different pH were taken and 0.1ml of the extract was added and the colour change was recorded.

NO	PLANT MATERIAL	PH	1.3	2.4	7.0	8.4	10.1	1.3
1	Violet cabbage	Colour	Crimson red	Light pink	violet	Pale green	Pale green	Golden yellow
2	beetroot	Colour	Blood red	Red	red	Dull green	Dull green	Pale green
3	turmeric	Colour	violet	Fluorescent yellow	Fluorescent yellow	Dark orange	Dark orange	Dark orange

4	henna	Colour	Light green	Dull green	orange	light brown	Light brown	brown
5	Hibiscus flower	Colour	Dark pink	light pink	violet	Pale yellow	Pale yellow	Pale yellow
6	Rose flower	Colour	orange	Pink	colourless	yellow	yellow	Light yellow
7	Four O'clock flower	Colour	violet	Violet	violet	Pale green	Pale green	Pale green

Table 2. TITRATIONS:

In order to evaluate the potential for the use of the dyes as indicators in acid-based titrimetric, a number of demonstrated titrations were conducted. The end points of the demonstrated titrations using 2 to 3 drops of the dyes are reported. The end points of the demonstrated acid-base titrations using commercially available indicators are also reported in the table. The results of the experiments for strong acid strong base (HCl and NaOH) are tabulated below: Titration of a strong acid (HCl) against strong base (NaOH)

Table. 3 Indicator end points Volume of HCl using phenolphthalein indicator: 10.7ml

No	Plant extract	Colour of the extract in base	Colour of the extract at the end point	Volume of HCl(ml)
1	Violet cabbage	light pink	violet	10.8
2	beetroot	Dark brown	red	10.2
3	hibiscus	Pale yellow	pink	11
4	henna	Brown	Light green	13.1
5	Four O'clock flower	Pale yellow	Dull pink	7.1
6	turmeric	Golden yellow	Pale yellow	9
7	rose	Dark brown	Dull brown	18.3

The violet cabbage, Hibiscus and Beet root extract had similar titre value with phenolphthalein and therefore can as well replace the commercial indicators. The colour changed from light pink to violet in the case of aqueous extract of violet cabbage, pale yellow to pink phenolphthalein for all the titrations. The results obtained showed that the routinely used chemical indicator can be replaced successfully by violet cabbage, Hibiscus and Beet root extracts. Henna extract, turmeric yellow, four o'clock flower, Rose extracts were not agreement with the end points obtained with other. This observation suggests that these extracts cannot serve as a suitable indicator in acid/base titrations. Indicators should be chosen in such a way that the pH at equivalence point lies within the pH range of indicator. For a titration between a strong alkali and a weak acid, the pH at equivalence point is below 7, so the pH ranges of the indicator should be below 7 to give a sharp colour change at end point.

Conclusion

In the present study, the results showed that the dye extracts have excellent analytical potential, as

in the case of alcoholic floral extract of Hibiscus Rosa sinensis and dark brown to red in the case of beet root. Titrations using the plant extract almost reached close to the equivalence point using the standard indicator,

demonstrated by its application in acid-based titrimetric in which it performed best in strong acid-strong base titration with a sharp and clear colour change.(20) The sharp contrast between their colours in acid and base made the pigment suitable for use as acid-base indicators. Out of the seven plant extracts prepared, violet cabbage, Hibiscus, Beet root extracts can serve as suitable indicators in acid-based titrimetric involving a strong acid and a strong base. These plant materials are readily available and the extraction procedure is simple, with excellent performance, precise and accurate results, making them an ideal replacement for presently available synthetic indicators. Thus, the use of natural indicator in acid base titration is more beneficial because of its economy, easy to prepare, simplicity, easy availability, pollution free, inert and accurate results.

Author contributions

All authors are contributed equally.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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