



METHOD DEVELOPMENT AND ASSAY OF BENZOIC ACID USING PLANT EXTRACT AS INDICATOR FOR NEUTRALIZATION TITRATION

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Abstract: This study aims to develop a method for assaying benzoic acid using the fresh leaf extract of *Vitex negundo* (Lamiaceae) as an indicator in acid-base titration. Fresh leaves of *Vitex negundo* are rich in anthocyanins, which are pH-sensitive and change color according to the pH environment, making them potential indicators for neutralization titration. A known quantity of benzoic acid was dissolved in ethanol and titrated against a 0.1M NaOH solution using 2.5 ml of 10% leaf extract as an indicator at 27°C. For comparison, the same procedure was performed using phenolphthalein as an indicator. The percentage purity of benzoic acid was calculated, and the values of the standard and test samples were compared. The titre values using phenolphthalein and leaf extract were found to be 42.6 ml and 42.4 ml, respectively. The percentage purity of benzoic acid was determined to be 95.38% using phenolphthalein and 94.8% using the leaf extract. The method was validated with parameters including precision, accuracy, standard deviation, and robustness. As anthocyanins are widely distributed in plants, they could be a feasible source for indicators. This method adheres to the standard method using phenolphthalein.

Keywords: Anthocyanins, Benzoic acid, indicator, titration.

I. INTRODUCTION

TITRATION:

- The most often used quantitative chemical analysis technique in laboratories for figuring out an analyte's concentration is titration. The majority of contemporary labs use digital automatic titrators with sensors (voltage electrode/pH sensor), some of which don't need indications. Compared to traditional titration techniques, these sophisticated titrators provide great accuracy and minimize human error. To provide great accuracy, however, kinetic parameters pertaining to the chemical reaction and the indicating system's response are essential. It is necessary to take into account elements like cell configuration, stirring, and the location of the titrant input and endpoint detector.
- Piston burettes and peristaltic pumps are frequently used in automatic titrators to transfer titrant. Peristaltic pumps are adaptable but need regular calibration because of variations in the physical characteristics of the flexible tubes they use, and they have a relatively short lifespan, whereas piston burettes are very dependable but costly. In many underdeveloped nations, where traditional titrimetric methods are still widely employed in analytical and research laboratories, the cost of automatic titrators and the problems they cause are significant barriers to their widespread usage.

INDICATORS:

- In analytical chemistry, "indicator" is a material or piece of equipment that gives the chemist information, especially during titration procedures. When an equivalent amount of titrant has been added to the analyte, it can be determined with the use of an indicator. This can be accomplished by a change in the titration system that can be measured or by an obvious or observable occurrence that emerges spontaneously at the end of the titration procedure. Indicators can be applied to qualitative analysis, quantitative applications (such colorimetry), and endpoint detection.
- Depending on the particles exchanged during the titration process, several kinds of indicators are needed. Because proton exchange is particularly significant, acid-base indicators belong to a different class. They are the oldest kind of indication in

history. Oxidation-reduction ("redox") indicators for electron exchange processes and indicators for donor-acceptor processes involving particles other than protons and electrons are examples of other types of indicators.

NATURAL INDICATORS:

- Natural indicators are more affordable, easier to get, and have less of an adverse effect on the environment than synthetic ones. Plant parts like flowers and leaves, which contain highly coloured chemicals that may change colour in response to pH changes, are the source of these indicators. Because they change colour at different pH levels, natural plant dyes and pigments including flavonoids, anthocyanins, and carotene can be utilized as pH indicators. Red cabbage juice, for instance, is a natural pH indicator because it includes anthocyanin, a pigment that responds differently to acids and bases.
- The acidity of molecules is indicated by their PKa values. *Vitex negundo* water extract was utilized in finding environmentally acceptable solutions and conducting research to ascertain their efficacy as a natural indicator are the goals of studying natural indicators.

TAXONOMY:

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Lamiales
- Family: Verbenaceae
- Genus: *Vitex*
- Species: *Vitex negundo*

VERNACULAR NAMES:

- Tamil: Nirkundi, Karu-nochi
- English: Five-leaved chaste tree
- Hindi: Shivari, Nirgundi

DESCRIPTION:

- Growth: Woody, aromatic deciduous shrub or small tree (10-20 ft tall).
- Leaves: Opposite, digitate with three to five leaflets, dark green above, greyish beneath.
- Flowers: Bisexual, fragrant, appearing from May to August.
- Fruits: Succulent, globose drupe turning black when ripe.
- Bark: Slightly rough, pale reddish-brown peeling in papery flakes.

HABITAT AND USES:

- Found in humid places, wastelands, and mixed open forests across India, Pakistan, Sri Lanka, and more. Traditional medicinal uses include anti-inflammatory, analgesic, and hepatoprotective properties. Used in Ayurveda, Unani, and Chinese medicine.

II. SYNTHETIC INDICATOR VERSUS NATURAL INDICATOR

- The five-leaved chaste tree, or *Vitex negundo*, is a woody, fragrant deciduous shrub or small tree that can reach a height of 10 to 20 feet. Dark green on top and greyish underneath, its opposing, digitate leaves have three to five leaflets. The fruit of the plant is a succulent, globose drupe that turns black when ripe, and it produces fragrant, bisexual blooms from May to August. The pale reddish-brown, somewhat scratchy bark peels off in papery flakes. In areas like India, Pakistan, and Sri Lanka, *Vitex negundo* grows well in humid climates, wastelands, and mixed open forests.
- There are several sections of the plant that are utilized for their hepatoprotective, analgesic, and anti-inflammatory qualities in Ayurvedic, Unani, and Chinese medicine. The plant goes by a number of colloquial names, such as Vaavili in Telugu, Shivari in Hindi, and Nirkundi in Tamil.

III. POTENTIAL OF HERBAL INDICATOR

- Plant-based compounds known as herbal indicators undergo colour changes in response to environmental variables such as PH. They can be used as natural textile and art dyes, environmentally friendly pH indicators (like red cabbage, beetroot, hibiscus, and turmeric), and ecological monitoring instruments (like soil and water quality testing). They are also utilized in pharmaceuticals, cosmetics, traditional medicine, and the food business (for example, freshness testing and natural colouring). Although there are many advantages to using herbal indicators, issues with stability, standardization, and extraction procedures exist.
- An herbal indicator is a naturally occurring material made from plant extracts that functions as an acid-base indicator by changing colour in response to pH variations. These indicators operate on the basis of naturally occurring pigments found in plants, such as carotenoids, flavonoids, or anthocyanins, which change colour in response to pH variations.

IV. MECHANISM OF HERBAL INDICATOR

PRESENCE OF NATURAL PIGMENTS:

- Herbal indicators contain plant pigments like anthocyanins, which are pH-sensitive compounds. Anthocyanins are commonly found in flowers, fruits, and leaves, such as in hibiscus, red cabbage, turmeric, and beetroot.
- Other pigments, such as flavonoids or carotenoids, may also contribute to colour changes under specific conditions.

PH-DEPENDENT COLOUR CHANGES:

- These pigments have a structure that is influenced by the concentration of hydrogen ions (H^+) or hydroxide ions (OH^-) in the solution. In acidic solutions (low pH): The molecular structure of the pigment stabilizes in a protonated form, which displays one colour.
- In basic solutions (high pH): Deprotonation occurs, and the molecular structure changes, resulting in a different colour.

For example:

- Red cabbage (anthocyanins)
 - Acidic: Red to pink
 - Neutral: Purple
 - Basic: Green to yellow
- Turmeric (curcumin)
 - Acidic: Yellow
 - Basic: Reddish-brown

STRUCTURAL CHANGES IN PIGMENTS:

- The structure of the active pigment in the herbal indicator shifts between different ionic forms (e.g., quinoidal base, flavylum ion, or neutral molecule) depending on the PH.
- These structural transitions alter the wavelengths of light absorbed and reflected, producing a visible colour change.

EXTRACTION AND USAGE:

- The pigments are typically extracted using solvents like water or ethanol.
- The extract is applied to a solution or used to prepare pH indicator papers.
- The resulting colour is compared to a standard pH scale to determine the solution's acidity or basicity. {19,20}

EXAMPLES OF HERBAL INDICATORS:

Hibiscus petals:

- Acidic: Pinkish-red.
- Basic: Green.

Red cabbage:

- Acidic: Red.
- Neutral: Purple.
- Basic: Green/Yellow.

Turmeric:

- Acidic: Yellow.
- Basic: Reddish-brown.

Beetroot:

- Acidic: Pinkish-red.
- Basic: Yellowish-brown.

Advantages of Herbal Indicators:

- Eco-friendly and biodegradable.
- Readily available and cost-effective.
- Non-toxic and safe to handle.

V. LIMITATION

- Some herbal indicators may lack precise transitions.
- Colour changes may be influenced by impurities or degradation over time.
- Not all are universally applicable for all pH ranges.
- By utilizing the natural chemistry of plant-based pigments, herbal indicators offer a sustainable alternative to synthetic pH indicators in various applications.

VI. PROPERTIES AND APPLICATION OF HERBAL INDICATOR

- Herbal indicators are natural, non-toxic plant-based compounds that change colour in response to PH. They are accessible, environmentally friendly, and offer unique colour changes across the pH spectrum. Applications include acid-base titrations, soil pH testing, water quality evaluation, educational purposes, and home use. While beneficial, challenges include stability, standardization, and extraction processes.

VII. MATERIALS AND METHODS

- Fresh leaves of Vitex negundo were collected, authenticated, and washed to remove dust. For extraction, 100 grams of leaves were divided into portions, placed in round bottom flasks with distilled water, and heated to 60°C for 20 minutes. After cooling, the extract was filtered and dried to obtain powder used as an indicator. Water, a polar solvent, is ideal for

extraction due to its affordability, non-toxicity, and effectiveness, though it promotes bacterial growth and requires significant heat for concentration. Key solvent selection factors include selectivity, safety, cost, reactivity, recovery, viscosity, and boiling temperature.

VIII. FACTORS CONSIDER ON EXTRACTION METHODS

- Choosing an extraction method depends on the stability of the plant material to heat, with heat-stable materials using Soxhlet or microwave-assisted extraction and heat-sensitive ones using maceration or percolation. The nature of the solvent also matters; water-based extractions use maceration, while volatile solvents suit percolation and Soxhlet extraction. Duration is another factor, with maceration suited for long exposure and microwave or ultrasound-assisted extraction for shorter times. The final volume required influences the choice, with maceration for volume products and percolation or Soxhlet for concentrated products. Intended use also plays a role, with consumable extracts typically prepared by maceration and experimental products by other methods. Chemicals used include distilled water, nitric acid, sodium hydroxide, magnesium turnings, hydrochloric acid, lead acetate, and ferric chloride. Equipment needed includes round bottom flasks, condensers, filter papers, funnels, weighing balances, test tubes, droppers, pipettes, standardization flasks, and burettes.

IX. PHYTOCHEMICAL TEST

PRELIMINARY TESTS:

- Acid Test: Extract with distilled water, treat with concentrated nitric acid (HNO₃).

Base Test: Treat with diluted sodium hydroxide (NaOH).

Flavonoids Tests:

- Alkaline Reagent Test: 1 ml leaf extract + 3 ml NaOH.
- Shinoda Test: 1 ml extract + 3 ml magnesium turnings, add concentrated HCl dropwise.
- Lead Acetate Test: 1 ml leaf extract + 3 ml lead acetate solution.

Anthocyanins Test:

- Ferric Chloride Test: 1 ml leaf extract + 3 ml ferric chloride solution.
- These tests help identify primary and secondary metabolites like flavonoids and anthocyanins in *Vitex negundo* plant leaf extract.

VALIDATION PARAMETERS

Precision:

- Determines variation in repeated analytical processes.
- Includes repeatability, intermediate precision, and reproducibility.
- Measures reproducibility within a set, often using standard deviation.

Accuracy:

- Measures closeness to the true result.
- Determined by absolute or comparative methods, reported as percentage bias.
- Involves replicate analysis and calibration using linear functions.

Linearity:

- Validates if results are proportional to analyte concentration.
- Prepare a high-concentration stock solution and dilute it into at least five concentrations (20%-120% range).
- Analyze each concentration using methods like HPLC or UV-Vis.
- Plot a calibration curve (response vs. concentration).

Perform linear regression to obtain R² and the equation of the line.

Table No: 1 Indicator and Observed PH

S.NO	Indicator	Observed pH
1.	Phenolphthalein	8.5 to 10
2.	Plant extract as indicator	8.7

X. RESULTS AND DISCUSSION

- The percentage yield of the plant extract was: **84%w/w**
- Approximately **4.2g** of plant extract in **7ml** of solvent taken



**Fig: 1 Test for Acid Base Results
(Colour Changes of the Plant Extract in Various pH Solutions)**

- The plant extract's performance as a pH indicator was compared to standard indicators.
- In acidic solutions, the extract turned a colour to **Red**.
- In basic solutions, the extract turned a colour to **Green**.

PHYTOCHEMICAL TEST:

- This test for flavonoids confirms the presence of flavonoids

Table No: 2 Test for Flavonoids

S.NO	TEST	OBSERVATION	RESULTS
1.	Ferric chloride test	Colour change to bluish green	Presence of flavonoids

Table 3: Test for Anthocyanins:

S.NO	TEST	OBSERVATION	RESULTS
1.	Alkaline reagent test	Intense Yellow colour turns to colourless on addition of few drops of HCL.	Presence of Anthocyanins
2.	Shinoda test	Pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.	Presence of Anthocyanins
3.	Lead acetate test	Appearance of yellow or green precipitate.	Presence of Anthocyanins

- This test for Anthocyanins confirms the presence of Anthocyanins



Fig: 2 Phytochemical Tests

ACID BASE TITRATION USING PHENOLPHTHALEIN

Table 4: Standardization of NaOH using Phenolphthalein Indicator

BURETTE READING	STANDARDISATION OF BENZOIC ACID	INDICATOR	ENDPOINT
26.7 ml	0.0917 N	Phenolphthalein	Appearance of pale pink colour

- Number of experiments: 1
- Titre value: 26.7
- Equivalent weight factor = 0.2042 N
- The standardization of NaOH using phenolphthalein as indicator = 0.0917 N

Table 5: Assay of Benzoic Acid Using Phenolphthalein Indicator

S.NO	BURETTE READING	Assay of benzoic acid	INDICATOR	ENDPOINT
1.	42ml	94.8% w/v	Phenolphthalein	Appearance of pale pink colour
2.	41.6ml	93.15% w/v	Phenolphthalein	Appearance of pale pink colour
3.	42.6 ml	95.38% w/v	Phenolphthalein	Appearance of pale pink colour



Fig: 3 Assay of Benzoic Acid Using Phenolphthalein Indicator
(Each ml of 0.1N NaOH is equivalent to 0.01221g of benzoic acid)

- Equivalent weight factor of benzoic acid = 0.01221 N
- The strength of NaOH was found to be: **0.0917N**
- The Assay of benzoic acid using phenolphthalein indicator Acceptance level: **0.1N**
- The Assay of benzoic acid using phenolphthalein indicator Titre value: **42.06 ml.**
- The average purity of benzoic acid by using phenolphthalein as a indicator was found to be: **94.4%w/v**

ACID BASE TITRATION USING PLANT LEAF EXTRACT AS INDICATOR**Table 6: Standardization of NaOH Using Plant Leaf Extract as Indicator:**

BURETTE READING	STRENGTH OF NAOH	INDICATOR	ENDPOINT
24.9 ml	0.0984N	Extract	Disappearance of Brown colour

Number of experiments: 1

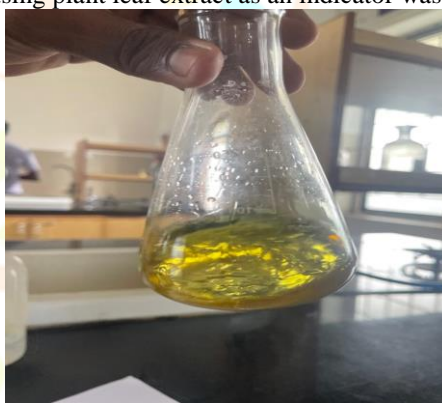
Titre value: 24.9 ml

Equivalent weight factor of NaOH= 0.204N

Table 7: Assay of Benzoic Acid Using Plant Leaf Extract as Indicator

Burette Reading	Assay of Benzoic Acid	Indicator	Endpoint
38.9ml	93.46% w/v	Extract	Appearance of yellowish green colour
38.5ml	92.5% w/v	Extract	Appearance of yellowish green colour
39.5ml	94.9% w/v	Extract	Appearance of yellowish green colour

- Equivalent weight factor of Benzoic acid = **0.01221 N**
- The strength of NaOH was found to be = **0.0984 N**
- The Assay of benzoic acid using plant leaf extract as indicator Acceptance level = **0.19N**
- The Assay of benzoic acid using plant leaf extract as indicator average Titre value = **38.96 ml.**
- The average purity of benzoic acid by using plant leaf extract as an indicator was found to be= **93.62% w/v**

**Fig: 4 Assay of Benzoic Acid Using Plant Leaf Extract as Indicator****VALIDATION PARAMETERS****Table 8: Accuracy**

Indicator	Accuracy	Accepted Critieria
Sample	- 6.88	93.62%

Assay acceptance level 99.0-100.5%

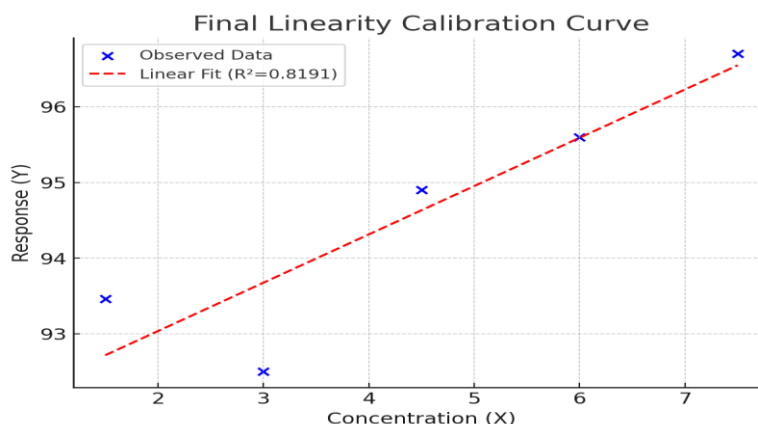
Accuracy = $\pm 0.5 \pm 1.5\%$ **Sample meets the accepted Accuracy level compared with synthetic indicator**A lower **percent error** indicates better accuracy. Since the error is above **5%**, the measurement has moderate accuracy.The **recovery percentage** is slightly below the ideal range (**98-102%**), indicating **underestimation of the true value**.**Table 9: Precision**

S.NO	Indicator	Titration value	Accepted criteria
Day 1	Vitex negundo indicator	38.9ml	93.46%
Day 2	Vitex negundo indicator	38.5ml	92.5%
Day 3	Vitex negundo indicator	39.5ml	94.9%

Precision = $\pm 1.0 \pm 1.5\%$ **Mean (Average): 93.62**

- **Standard Deviation (SD): 1.21**
- **Relative Standard Deviation (RSD %): 1.29%**
- A lower RSD% indicates higher precision. Since 1.29% is quite low, the data is **reasonably precise**.

Sample meet the accepted precision level compared with synthetic indicator

LINEARITY:**Fig 5: Linearity Calibration Curve**

The coefficient of determination (R^2) is **0.8191**, indicating a **moderate correlation** between concentration and response. While some degree of linearity is present, the method **does not meet the standard acceptance criteria ($R^2 \geq 0.990$)** required for reliable quantitative analysis.

XI. SUMMARY AND CONCLUSION

Objective: Assay of benzoic acid using Vitex negundo extract as a natural pH indicator.

Extraction: Aqueous process; yield - 84% w/w; contains anthocyanins and flavonoids.

Titration: Endpoint colours:

Phenolphthalein: pink, titre - 42.6 ml, purity - 95.38%.

Leaf extract: yellow-green, titre - 42.4 ml, purity - 94.8%.

Accuracy, precision, and linearity are among the metrics used to validate the procedure. When the calculation figures are compared to the synthetic indicator (phenolphthalein) as a standard, the natural indicator's compression passes every test that we performed in accordance with IP guidelines, suggesting that it might be a reliable and effective source of indicator

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