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## PRELIMINARY PHARMACEUTICAL CHARACTERIZATION OF FLOWERS AS NATURAL INDICATOR: ACID-BASE INDICATOR

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## ABSTRACT

Today synthetic indicators are the choice of acid-base titrations. But due to environmental pollution, availability and cost, the search for natural compounds as an acid-base indicator was started. The present vocation highlights the exploit of the acidified ethanolic extract of the flowers of few medicinal plants as an acid-base indicator in acid-base titrations. This natural indicator is easy to extract as well as easily available. Promising results were obtained when it was tested against standard synthetic indicators. Titration shows sharp color change at the equivalence point. The equivalence points obtained by the flowers extract coincide with the equivalence points obtained by standard indicators. In case of weak acid and weak base titration, the results obtained by the flowers extract matched with the results obtained by mixed indicator. This natural indicator is found to be a very useful, economical, simple and accurate for the said titrations.

**Keywords:** Acid-base indicator, Natural indicator, Anthocyanins, Titration.

## INTRODUCTION

The method of wet chemistry such as titrimetric analysis and gravimetry still has an important role in modern analytical chemistry. There are many areas in which titrimetric procedures are invaluable. The term titrimetric analysis refer to quantitative chemical analysis carried out by determining the volume of solution of accurately known concentration which is required to react quantitatively with a measured volume of a solution of the substance to be determined[1].

Indicators are dyes or pigments that can be isolated from a variety of sources, including plants, fungi, and algae. Almost any flower, for example, that is red, blue, or purple in color contains a class of organic pigments called anthocyanins that change color with pH. The use of natural dyes as acid-base indicators was first reported in 1664 by Sir Robert Boyle in his collection of essays Experimental History of Colors. Indeed, Boyle made an important contribution to the early theory of acids and bases by using indicators for the experimental classification of these substances[2]. Presence of color pigments was investigated as per IP and the tests are given in Table 1.

*Nerium oleander* L. (Apocyanaceae) is evergreen, glabrous shrub upto 6 m high, native of the Mediterranean region and extending as far as Iran. It is often grow in Indian gardens for ornament and also as fence and wind-break. Flowers of the plant are salver-shaped, pink or white, scentless, present as terminal cymes. Leaves, flowers and stem bark possess cardiotonic properties. Small amount of glycosides and alkaloids are present in flowers. Flowers yield 0.03 % of an essential oil[3].

*Tecoma stans* Juss. (Bignoniaceae) is an erect, branched, sparingly pubescent or nearly glabrous shrub, 2-4 m high distributed in tropical America, cultivated and wildly obtained in parts of India. Flowers are racemosely arranged on the few branches with green calyx, 5 mm long, 5 toothed and yellow corolla 4-4.5 cm long, tube inflated upward[4]. The leaves are used as anti-diabetic. Methanolic extract of flowers yielded the flavanone, 7, 8-dihydroxy-4', 6-dimethoxy flavones along with kaempeferol, β-carotene and G-zeaxanthin[5].

*Calotropis gigantea* R. Br. (Asclepiadaceae) is a tall shrub reaching 2.4-3 m high distributed throughout India, Ceylon, Malay Islands and China. Flowers of plant are inodorous, purplish or white, arranged in umbellate lateral cymes, peduncles from between the petioles. Pedicels are much longer than the flowers, calyx are divided to base, sepals 6 by 4 mm, ovate, acute, cottony, corolla 2 cm long or more. The flowers are used as sweet, bitter, anthelmintic, analgesics, astringent, anti-inflammatory, anti-tumor, Kapha, rat bite, good in ascites[4].

*Albizia lebbeck* Benth. (Mimosaceae) is an unarmed deciduous tree 12-21 m high obtained throughout India, tropical and subtropical Asia and Africa. Flowers are white, fragrant, in globose umbellate heads 2-3.8 cm diameter. Calyx are 4 mm long, pubescent, corolla are 1 cm long, tube glabrous, lobes 2.5 mm long, triangular, acute, pubescent outside. Stamens are much longer than corolla, filaments are connate at the base into a short tube. The flowers are given for asthma and for snake-bite. It also used as aphrodisiac, emollient, maturant, cooling medicine, externally applied in boils, eruptions and swellings. Their smell is useful in hemicrania.

*Cassia fistula* Linn. (Caesalpiniaceae) is a tree with 6-9 m high, obtained throughout India, Ceylon, Malaya and China. Flowers are in lax racemes 30-50 cm long, pedicels 3.8-5.7 cm long, slender, pubescent or glabrous. Calyx 1 cm long, divided to the base, pubescent. Corolla are 3.8 cm across, yellow in colour with 5 petals, which are subequal, obovate, shortly clawed and veined. The flowers have flavors with a bitter acrid taste, cooling, astringent, cure kapha and biliousness, cause flatulence[6].

Anthocyanins are characterized by Band- 275-280 nm (UV region). Actual color of extract is depending on number and position of hydroxyl and methoxy group. When these are fixed, the color then depends upon the pH and solvent[7].

Hence the present vocation was attempted to appraise the flowers as a natural indicator.

## MATERIAL AND METHOD

### Material

Fresh flowers were collected from the local market of Rajkot region, Gujarat, and they were authenticated from NISCAIR, New Delhi, Ref No: NISCAIR/RHMD/Consult/2010-11/1468/66. All other ingredients were of analytical grade and purchased from Loba Chemie Pvt Ltd, Mumbai.

### Method

The flowers were cleaned by distilled water and cut into small pieces and macerated for two hours in 25ml of 90% ethanol. The extract was preserved in tight closed container and stored away from direct sun light[8].

The experiment was carried by using the same set of glassware's for all types of titrations. As the same aliquots were used for both titrations i.e. titrations by using standard indicators and flowers extract, the reagents were not calibrated. The equimolar titrations were performed using 10 ml of titrant with three drops of indicator. All the parameters for experiment are given in Table 1.

A set of five experiments each for all the types of acid base titrations were carried out. The mean and standard deviation for each type of acid base titrations were calculated from results obtained. The extract was also analyses for its λmax in Ultra Voilet range on Systronics single beam spectrophotometer (Shimadzu UV 1800).

## RESULT

The extract was found to contain compound anthocyanins as it gives blue color to aqueous sodium hydroxide solution, yellow orange color to concentrated sulphuric acid while red color which fed out on standing with magnesium-hydrochloric acid solution (Table 2).

**Table 1: Standard chart for phytochemical identification**

| *Phytochemicals* | *Color with aq. NaOH* | *Color with Conc. $H_2SO_4$* | *Color with Mg-HCl* |
|---|---|---|---|
| Anthocynins | Blue violet | Yellow to orange | Red (fades to pink) |
| Flavones | Yellow | Yellow to orange | Yellow to red |
| Flavonones | Yellow to orange (cold)<br>Red to purple (hot) | Crimson Orange | Red, magenta, violet, blue |
| Isoflavones | Yellow | Yellow | Yellow |
| Leucoanthocyanins | Yellow | Crimson | Pink |

**Table 2: Technological characterization for analysis of chemical test.**

| Sample | Poly-Phenolic compound | | Flavonoid | Anthocyanins | | |
|---|---|---|---|---|---|---|
| | *Color with $FeCl_3$* | *Color with Lead acetate* | *Shinoda test* | *Color with aq. NaOH (Blue violet)* | *Color with Conc. $H_2SO_4$ (Yellow orange)* | *Color with Mg-HCl (Red)* |
| NOPI | + | + | + | + | + | + |
| TSPI | + | + | + | + | + | + |
| CGPI | + | + | + | + | + | + |
| ALPI | + | + | + | + | + | + |
| CFPI | + | + | + | + | + | + |

**+; Presence of compound**

The flowers extract of *N. oleander, T. stans, C. gigantea, A. lebbeck, C. fistula* showed λmax in Ultra Violet region (Table 3). The λmax suggested the presence of anthocyanins in the extract.

The flowers extract was screened for its use as an acid base indicator in various acid base titrations, and the results of this screening were compared with the results obtained by standard indicators methyl red, phenolphthalein and mixed indicator [methyl orange: bromocresol green (0.1:0.2) results are presented in Table 4.[9]

**Table 3: Determination of UV absorption**

| Sample code | UV λmax |
|---|---|
| NOPI | 324 |
| TSPI | 320 |
| CGPI | 304 |
| ALPI | 305 |
| CFPI | 304 |

**Table 4: Technological characterization of acid-base titration using standard indicator**

| Titration (Titrant v/s Titrate) | Strength in moles | Indicator | Mean ±S.D.* | Color | pH |
|---|---|---|---|---|---|
| **NaOH v/s HCl** | 0.1 | MR | 12.3±0.12 | Yellow to pink | 12.32-5.77 |
| | 0.5 | MR | 11.2±0.16 | Yellow to pink | 12.55-4.87 |
| | 1.0 | MR | 11.2±0.15 | Yellow to pink | 12.63-3.30 |
| **HCl v/s $NH_4OH$** | 0.1 | PT | 05.9±0.01 | Pink to colorless | 10.50-6.74 |
| | 0.5 | PT | 06.6±0.08 | Pink to colorless | 10.61-8.28 |
| | 1.0 | PT | 06.5±0.16 | Pink to colorless | 10.98-8.29 |
| **$CH_3COOH$ v/s NaOH** | 0.1 | MR | 12.0±0.11 | Yellow to light red | 12.33-6.01 |
| | 0.5 | MR | 11.9±0.14 | Yellow to light red | 12.56-5.96 |
| | 1.0 | MR | 12.0±0.09 | Yellow to light red | 12.67-5.99 |
| **$CH_3COOH$ v/s $NH_4OH$** | 0.1 | MI | 05.0±0.05 | Orange to green | 03.25-4.52 |
| | 0.5 | MI | 05.6±0.19 | Orange to green | 02.81-4.68 |
| | 1.0 | MI | 06.1±0.17 | Orange to green | 02.86-4.73 |

*All values are mean ± S.D. for n=3

HCl: Hydrochloric acid, $CH_3COOH$: Acetic Acid, NaOH: Sodium Hydroxide, $NH_4OH$: Ammonium Hydroxide, MR; Methyl Red, MI: Mixed Indicator, PT: Phenolphthalein.

The titrations of strong acid with strong base (HCl & NaOH), strong acid with weak base (HCl & $NH_4OH$), weak acid with strong base ($CH_3COOH$ & NaOH), and weak acid with weak base ($CH_3COOH$ and $NH_4OH$) were carried out using standard indicators and flowers extract. The results of these titrations are given in Table 5,6,7,8,9. It could be due to these flavonoids, the sharp end point appeared in the above mentioned titrimetric analyses. The Flowers extract of *N. oleander, T. stans, C. gigantea, A. lebbeck, C. fistula* was found to have Poly-Phenolic, flavonoids, anthocyanins and is pH sensitive. The end point determination of acid base titrations by the traditional indicators, compared with flowers extract indicator, it was observed that traditional indicators gave incorrect results due to addition of excess of titrant (base) after the neutralization reaction was completed, but flowers extract indicator has given sharp end point because solutions give sharp color change at the equivalence points. Thus natural indicator employed in the acid base titrations was found economic, safe and an efficient alternative for traditional indicators. In comparison to this, chemical indicators were found more expensive and hazardous, which proves that Flowers extract of *N. oleander, T. stans, C. gigantea, A. lebbeck, C. fistula* as a natural indicator is more worthy.

**Table 5: *Nerium oleander* L. as indicator**

| Titration (Titrant v/s Titrand ) | Strength in moles | Indicator | Mean ±S.D. | Color | pH |
|---|---|---|---|---|---|
| **NaOH v/s HCl** | 0.1 | NOPI | 11.5 ± 0.13 | Yellow to colorless | 12.78-6.85 |
| | 0.5 | NOPI | 11.1 ± 0.19 | Yellow to colorless | 12.67-4.90 |
| | 1.0 | NOPI | 4.9 ± 0.12 | Yellow to colorless | 13.03-5.19 |
| **HCl v/s $NH_4OH$** | 0.1 | NOPI | 2.0 ± 0.14 | Yellow to colorless | 10.2-2.5 |
| | 0.5 | NOPI | 0.9 ± 0.11 | Yellow to colorless | 10.30-2.0 |
| | 1.0 | NOPI | 5.7 ± 0.10 | Yellow to colorless | 11.02-2.51 |
| **$CH_3COOH$ v/s NaOH** | 0.1 | NOPI | 19.1 ± 0.13 | Yellow to colorless | 13.12-6.61 |
| | 0.5 | NOPI | 11.2 ± 0.15 | Yellow to colorless | 6.60-6.60 |
| | 1.0 | NOPI | 10.0 ± 0.16 | Yellow to colorless | 12.55-7.34 |
| **$CH_3COOH$ v/s $NH_4OH$** | 0.1 | NOPI | 4.0 ± 0.13 | Yellow to colorless | 10.65-7.14 |
| | 0.5 | NOPI | 0.9 ± 0.14 | Yellow to colorless | 6.6-6.4 |
| | 1.0 | NOPI | 5.4 ± 0.14 | Yellow to colorless | 11.27-5.75 |

*All values are mean ± S.D. for n=3

HCl: Hydrochloric acid, $CH_3COOH$: Acetic Acid, NaOH: Sodium Hydroxide, $NH_4OH$: Ammonium Hydroxide, NOPI: *Nerium oleander* L.

**Table 6: *Tecoma stans* Juss. as indicator**

| Titration (Titrant v/s Titrand ) | Strength in moles | Indicator | Mean ±S.D. | Color | pH |
|---|---|---|---|---|---|
| **NaOH v/s HCl** | 0.1 | TSPI | 5.2 ± 0.18 | Yellow to colorless | 12.82-13.35 |
| | 0.5 | TSPI | 6.2 ± 0.13 | Yellow to colorless | 13.03-12.83 |
| | 1.0 | TSPI | 4.6 ± 0.15 | Yellow to colorless | 12.92-11.80 |
| **HCl v/s $NH_4OH$** | 0.1 | TSPI | 6.2 ± 0.16 | Yellow to colorless | 11.06-1.22 |
| | 0.5 | TSPI | 3.2 ± 0.14 | Yellow to colorless | 10.22-1.18 |
| | 1.0 | TSPI | 7.0 ± 0.15 | Yellow to colorless | 11.68-6.64 |
| **$CH_3COOH$ v/s NaOH** | 0.1 | TSPI | 5.4 ± 0.16 | Pink to colorless | 6.62-6.62 |
| | 0.5 | TSPI | 10.1 ± 0.13 | Pink to colorless | 12.75-11.86 |
| | 1.0 | TSPI | 7.7 ± 0.15 | Pink to colorless | 12.73-7.41 |
| **$CH_3COOH$ v/s $NH_4OH$** | 0.1 | TSPI | 2.5 ± 0.16 | Pink to colorless | 10.62-8.7 |
| | 0.5 | TSPI | 2.1 ± 0.14 | Pink to colorless | 10.7-8.02 |
| | 1.0 | TSPI | 7.4 ± 0.13 | Pink to colorless | 11.2-5.02 |

*All values are mean ± S.D. for n=3
HCl: Hydrochloric acid, $CH_3COOH$: Acetic Acid, NaOH: Sodium Hydroxide, $NH_4OH$: Ammonium Hydroxide, TSPI: *Tecoma stans* Juss.

**Table 7: *Calotropis gigantea* R. Br. as indicator**

| Titration (Titrant v/s Titrand ) | Strength in moles | Indicator | Mean ±S.D. | Color | pH |
|---|---|---|---|---|---|
| **NaOH v/s HCl** | 0.1 | CGPI | 13.1 ± 0.14 | Yellow to colorless | 12.61-9.31 |
| | 0.5 | CGPI | 10.5 ± 0.12 | Yellow to colorless | 12.65-11.68 |
| | 1.0 | CGPI | 8.5 ± 0.11 | Yellow to colorless | 12.80-12.44 |
| **HCl v/s $NH_4OH$** | 0.1 | CGPI | 5.8 ± 0.14 | Yellow to colorless | 10.56-8.77 |
| | 0.5 | CGPI | 5.7 ± 0.13 | Yellow to colorless | 11.06-9.90 |
| | 1.0 | CGPI | 6.3 ± 0.12 | Yellow to colorless | 11.38-8.95 |
| **$CH_3COOH$ v/s NaOH** | 0.1 | CGPI | 11.0 ± 0.15 | Yellow to colorless | 12.65-9.60 |
| | 0.5 | CGPI | 10.6 ± 0.29 | Green to colorless | 12.65-9.86 |
| | 1.0 | CGPI | 10.5 ± 0.15 | Green to colorless | 12.85-12.08 |
| **$CH_3COOH$ v/s $NH_4OH$** | 0.1 | CGPI | 3.7 ± 0.16 | Green to colorless | 10.52-9.2 |
| | 0.5 | CGPI | 3.2 ± 0.14 | Green to colorless | 11.4-9.56 |
| | 1.0 | CGPI | 1.0 ±0.15 | Green to colorless | 11.23-10.37 |

*All values are mean ± S.D. for n=3
HCl: Hydrochloric acid, $CH_3COOH$: Acetic Acid, NaOH: Sodium Hydroxide, $NH_4OH$: Ammonium Hydroxide, CGPI: *Calotropis gigantea* R. Br.

**Table 8: *Albizia lebbeck* Benth. as indicator**

| Titration (Titrant v/s Titrand ) | Strength in moles | Indicator | Mean ±S.D. | Color | pH |
|---|---|---|---|---|---|
| **NaOH v/s HCl** | 0.1 | ALPI | 11.5 ± 0.13 | Orange to colorless | 12.38-6.33 |
| | 0.5 | ALPI | 10.1 ± 0.13 | Orange to colorless | 12.53-5.77 |
| | 1.0 | ALPI | 10.2 ± 0.15 | Orange to colorless | 12.66-2.61 |
| **HCl v/s $NH_4OH$** | 0.1 | ALPI | 8.9 ± 0.16 | Yellow to colorless | 10.26-2.78 |
| | 0.5 | ALPI | 10.2 ± 0.15 | Yellow to colorless | 10.82-1.58 |
| | 1.0 | ALPI | 7.0 ± 0.12 | Yellow to colorless | 11.11-1.94 |
| **$CH_3COOH$ v/s NaOH** | 0.1 | ALPI | 11.3 ± 0.16 | Orange to colorless | 12.46-7.82 |
| | 0.5 | ALPI | 10.9 ± 0.19 | Orange to colorless | 12.74-8.64 |
| | 1.0 | ALPI | 11.4 ± 0.16 | Orange to colorless | 12.81-7.08 |
| **$CH_3COOH$ v/s $NH_4OH$** | 0.1 | ALPI | 7.7 ± 0.14 | Orange to colorless | 10.49-5.68 |
| | 0.5 | ALPI | 6.3 ± 0.13 | Orange to colorless | 11.02-7.02 |
| | 1.0 | ALPI | 7.0 ± 0.16 | Orange to colorless | 11.36-6.44 |

*All values are mean ± S.D. for n=3
HCl: Hydrochloric acid, $CH_3COOH$: Acetic Acid, NaOH: Sodium Hydroxide, $NH_4OH$: Ammonium Hydroxide, ALPI: *Albizia lebbeck* Benth.

**Table 9: *Cassia fistula* Linn. as indicator**

| Titration (Titrant v/s Titrand ) | Strength in moles | Indicator | Mean ±S.D. | Color | pH |
|---|---|---|---|---|---|
| **NaOH v/s HCl** | 0.1 | CFPI | 9.5 ± 0.14 | Light yellow to colorless | 12.33-10.95 |
| | 0.5 | CFPI | 9.1 ± 0.15 | Light yellow to colorless | 12.53-11.46 |
| | 1.0 | CFPI | 7.9 ± 0.11 | Light yellow to colorless | 12.60-12.41 |
| **HCl v/s $NH_4OH$** | 0.1 | CFPI | 4.3 ± 0.16 | Light yellow to colorless | 10.34-9.15 |
| | 0.5 | CFPI | 8.0 ± 0.12 | Light yellow to colorless | 10.79-1.97 |
| | 1.0 | CFPI | 5.3 ± 0.16 | Light yellow to colorless | 11.10-8.76 |
| **$CH_3COOH$ v/s NaOH** | 0.1 | CFPI | 2.5 ± 0.18 | Light yellow to colorless | 12.52-12.33 |
| | 0.5 | CFPI | 4.6 ± 0.13 | Light yellow to colorless | 12.76-12.58 |
| | 1.0 | CFPI | 9.5 ± 0.19 | Light yellow to colorless | 12.76-12.45 |
| **$CH_3COOH$ v/s $NH_4OH$** | 0.1 | CFPI | 3.1 ± 0.11 | Light yellow to colorless | 10.42-9.36 |
| | 0.5 | CFPI | 2.6 ± 0.13 | Light yellow to colorless | 10.92-9.62 |
| | 1.0 | CFPI | 1.0 ± 0.15 | Light yellow to colorless | 11.21-10.31 |

*All values are mean ± S.D. for n=3
HCl: Hydrochloric acid, $CH_3COOH$: Acetic Acid, NaOH: Sodium Hydroxide, $NH_4OH$: Ammonium Hydroxide, CFPI: *Cassia fistula* Linn.

## CONCLUSION

The results obtained in all the types of acid-base titrations lead us to conclude that, it was due to the presence of flavonoids sharp color changes occurred at end point of the titrations. We can also conclude that, it is always beneficial to use *N. oleander, T. stans, C. gigantea, A. lebbeck, C. fistula* flowers extract as an indicator in all types of acid base titrations because of its economy, simplicity and wild availability.

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