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Review Article

Analytical Applications of Plant Extract as Natural pH Indicator: A Review

Pathan Mohd Arif Khan*, Mazahar Farooqui
Post graduate and research centre, Maulana Azad College, Aurangabad (MS) India
**Corresponding author: arif7172@rediffmail.com*

ABSTRACT
In this review importance of pH indicators in analytical chemistry alongwith the use of plant material or the extracts obtained from plant is described. In the present review some theories related with mechanism of pH indicator in analytical quantifications involving volumetric analysis is described.

Keywords: pH indicators, Flavonoids, Anthocyanin, Natural indicator, Titration error.

1. INTRODUCTION

The world has become aware of environmental issue in recent years. Synthetic compounds are highly polluting, hazardous and much more costly. Researchers are working in the field of natural products extensively as they are less hazardous, low cost, easily available, and eco-friendly.

Literature survey revealed that, many researchers have conducted studies on isolation, separation [1-3] and characterization of compounds present in plants and animals and they also studied the extractions procedures, optimization of extraction conditions to get pure and maximum yield of naturally occurring compounds from different parts of plants. Literature survey also shows that chemists are studying the medicinal [4-12] bacteriological and anti-oxidant [13] activities of the extracted compounds. Saxena [14] and coworkers studied the antifungal activity from *melilotus indica* extract while Meera Harit [15] successfully carried out the antifungal activity of unsaponifiable fraction of fixed oils of *Trischosanthes* seeds. α- Pyrone are isolated from *Aniba duckei* [16], *Aniba firmula* [17], *Aniba gardneri* [18], *Coto bark* [19], show promising antibacterial, antifungal [20], antibiotic [21] and anti-tumor activities

Many researchers adopted the suitable techniques under different conditions for the isolation of compounds from flowers and used as dye [22]. The elucidations of structure of compounds are done with the help of recent techniques viz UV, IR, NMR and Mass spectroscopy etc. Eloi Pale et al [23] have isolated triacylated and tetraglucosylated anthocyanins from *Imponoea asarifolia* flowers. Kumi Yashida [24] and coworkers isolated UV-B resistant polyacylated anthocyanin from blue petals of morning glory. R. Filippini [25] and co-workers studied the production of anthocyanins by *Cathranthus Roseus*. Use of natural dye as photosensitizers for dye sensitized solar cell is successfully done by Sancun Hao et al [26].

Colours of substances make the world a wonderful place. Because of the colours and structures; flowers, plants, animals, and minerals show their unique characters. There are various organic and inorganic compounds responsible for natural colours. Some of the organic compounds i.e. flavonoids, flavonols, acylated flavonoids, anthocyanins, glucosylated acylated anthocyanin, quinines, imines, polymethines, napthaquinones, anthraquinonoids, indigoids; dihydropyrans diarylmethanes carotene etc. imparts colours to the flower. Among them anthocyanidins and flavones are main.

2. FLAVONES

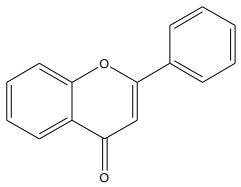
Flavones as well as flavonols and their glycosides continue to attract attention of biologist, because of their presence in many food plants, and in commonly available medicinal plants. Currently, most attention is being given to their anti-inflammatory and antioxidative activities

Flavones are soluble in water and alcohol and can be extracted by chopping and macerating the plant material, soaking it for a few minutes in hot water or rubbing with alcohol.

These are yellow pigments which occur in plant kingdom either in the free state or as glycosides or associated with

tannins. These are also known as the anthoxanthins. Chemically they are hydroxylated derivative of flavone (2-phenyl chromen-4-one).

In most of flavones positions 5 and 7 are hydoxylated and also one or more positions 3', 4', 5' are also hydroxylated. Further positions 3' and 5' are often methylated.



2-Phenyl-chromen-4-one

The main property that is recognized for flavonoids is "Venoactivity" i.e. their ability to decrease capillary permeability and fragility in animal models; they can decrease the signs of experimental vitamin C deficiency. Because of this property, they were first referred to as "vitamin P" Since they are not vitamins (flavonoid deficiency does not cause any particular syndrome)

They were later referred to as "vitamin P factor" or better yet, "P factors" Nevertheless, these terms were ambiguous and they are practically no longer used: instead these natural products and their derivatives are now referred to as "venoactive" Many Scientists have isolated and characterized flavonoid and related compounds from flowers and worked on variety of their applications. Yellow flavonoids in *Centaurea ruthenica* by Tamaki Mishio [27], from *Kalacnchoe Blossfeldiana* varieties by Allan Holmi [28] et al, from *Bassica rapa* as the UV absorbing nectar guide by Katsunori Sasaki [29], From *Cocus chrysanthus biflorus* "Eye –catcher" and "Spring Pearls" *Iridaceae* family by Rikke Norbaek [30].

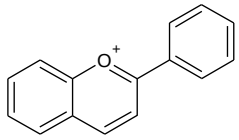
Tadigoppula Narender [31] isolated furano flavonoids and studied antidyslipidemic activity while Melanie K [32] and co worker succeeded to report yellow pink inter-specific hybridization between *dianthus Plumarius* and related with yellow flowers.

In all known flavonoid glycosides, glucose is present in the α-D-pyranose form, as has been reported in herbacetin 3 glucofuranoside from *Jungia paniculata* [33] Unfortunately, the NMR data supporting the furanose form for the sugar are not completely convincing. Researchers have reported flavonoid sulphate from gossypetin 7,8 dimethyl ether 3,3' disulphate from flowers of *Erica cinerea* [34] the other is isorhamnetin 3-(4"sulphatorutinoside) from Zygophyllum dumosum [35].

Acylated derivatives, of flavone and flavonol are also known. These can convert what are usually water soluble flavonoid glycosides into lipophilic substances.

3. ANTHOCYANINS

The other pigment present in plants is anthocyanin and substituted anthocyanin. These are the water soluble pigments and are largely responsible for attractive colours of flowers, leaves, fruits etc.



Chemically, anthocyanins are glycosides and their aglycones, i.e. the sugar free pigments are known as the anthocyanidins. The various anthocyanins were shown to possess the same carbon skeleton and differed only in the nature of substituent groups. The fundamental nucleus is 2-phenyl chromenylium chloride or (flavylium chloride).

All the anthocyanin has been considered to be derivatives of 3.5.7 trihydroxy flavylium chloride. Various anthocyanins and anthocyanidins differ in the number, and nature and position of other hydroxyl groups, methoxy groups and sugar residue. Most common sugar residues are glucose, galactose, and rhamnose. Some pigments are acylated derivatives.

The most important anthocyanidine are pelargonidin, cyanidine, peonidine, delphinidin, malvidin and petunidine. These aglycones differ in the number of hydroxyl and methoxyl groups in the B ring of the flavylium cation. Anthocyanins can also acylated organic acids, which are attached to anthocyanins glycosyl units through an ester bond, are usually either aromatic phenolic acids or aliphatic dicarboxyl acid or combination of both. The acids are most commonly linked to the 6 position of the monosaccharide, but anthocyanins with acyl substitution at the 2, 3, and 4 positions of monosaccharide have been elucidated by Cabrita [36].

The most common phenolic acids in anthocyanins are the derivatives of hydroxyl cinnamic acid i.e. p-coumaric , ferulic caffeic and sinapic acids and hydroxy benzoic acids, viz gallic acid. The most common aliphatic acids occurring in anthocyanin molecules are malonic, acetic, malic, succinic, and oxalic acids [37].

The exact functions of anthocyanins in plants are not known with certainty. However, they are assumed to carry out the following function in plants.

a) The anthocyanins may cause an increase in osmotic pressure of the sell sap
b) The beautiful colours of anthocyanins help in attracting the insect and thus assist the plants in cross pollination.

c) In respiration and photosynthesis, anthocyanins are expected to play some vital role.
d) The anthocyanin may act as light filters and thus hinder the decomposition of chlorophyll against strong light

Anthocyanins are highly unstable and easily susceptible to degradations. The stability of anthocyanins is affected by pH, storage temperature, presence of enzymes, light, oxygen, structure and concentration of the anthocyanins, and the presence of other compounds such as other flavonoids, protein, and minerals.

Researchers are taking keen interest in isolation, characterization and studying their physical, chemical, and physiological activities. Fumi Tatsuzawa [38] with his workers isolated cyanidin glycosides in flowers of Geneus *corydalis,* Toshio Honda et al [39] obtained acylated anthocyanin from violet blue flowers of *Orychophragonus violaceus,* Kosaku Takeda [40] with his researchers isolated and characterized components of protocyanin blue pigment from the blue flowers of *Centaurea cyanus* and Ana Paula et al [41] studied photo period and temperature effects on in vitro growth and flowering of *epiphytic orchid*.

Jamal uddin [42] and co worker successfully studied the seasonal variations in pigmentations and anthocyanidin phenetics in commercial *Eustoma* flowers. Kaung Liang Huang et al [43] studied flower colours and pigments in hybrid *tuberose,* Kenjiro Toki [44] and co workers isolated anthocyanin from scarlet flowers of *Anemone coronaria*.

Moshe Reuveni [45] et al studied the decrease in vacuolar pH during petunia flower opening. And Y Ben Tal [46] studied environmental factors involved in coloration of flowers of *Kangaroo Paw*. Many researchers isolated and characterized the anthocyanins from flowers. Kjell Torskangerpoll [47] and co workers isolated from tulip, Tomio Ishikawa [48] isolated from blue petals of *Salvia uliginosa,* Rikke Norbaek [49] from flowers of *Crocus*, Ben Erik et al [50] isolated from *Lobostemon* and many other researcher are working on natural dye stuff of flower.

Making dyes from common plants is not a new thing. It is, in fact, how we first got colour. Our ancestors knew they could extract certain colours from certain plants, such as yellow from goldenrod or purple from berries.

4. INDICATORS

Indicator is a substance used to locate the end point in a titration process, for example phenolphthalein, methylene blue, methyl red methyl orange etc are indicators used in acid base titrations.

Classification of indicators: Indicators are classified as an external (added externally during the titration) and internal or self indicator is one which is not added externally but one of the titrating solutions behaves as an indicator. They are further classified as redox indicator used in redox titrations, precipitating indicator/adsorption indicators used in precipitation titration, complexing indicator used in complexation titration and acid base or pH indicators.

5. pH INDICATORS

The pH indicators are substances whose solutions change color due to changes in pH. These are also called acid-base indicators or neutralization indicator. They are usually weak acids or bases, but their conjugate base or acid forms have different colors due to differences in their absorption spectra.

Indicators are complicate organic weak acids or bases with complicated structures. For simplicity, we represent a general indicator by the formula HIn for acidic indicators and InOH for basic indicator. The ionization of acidic indicator in a solution is represented by the equilibrium,

$$HIn \rightleftharpoons H^+ + In^-,$$

Some Common Indicators: There are various indicators used in acid base titration, some common indicators used along with their pH range & colour changes are given in table 1.

Table 1: pH range for colour change of some common indicators

Name	Acid Color	pH Range of Color Change	Base Color
Methyl violet	Yellow	0.0 – 1.6	Blue
Thymol blue	Red	1.2 – 2.8	Yellow
Methyl orange	Red	3.2 – 4.4	Yellow
Bromocresol green	Yellow	3.8 – 5.4	Blue
Methyl red	Red	4.8 – 6.0	Yellow
Litmus	Red	5.0 – 8.0	Blue
Bromothymol blue	Yellow	6.0 – 7.6	Blue
Thymol blue	Yellow	8.0 – 9.6	Blue
Phenolphthalein	Colorless	8.2 - 10.0	Pink
Thymolphthalein	Colorless	9.4 - 10.6	Blue
Alizarin yellow R	Yellow	10.1 - 12.0	Red

6. NATURAL INDICATORS

Natural indicators have been used for hundreds of years, long before chemists made synthetic acid-base indicators. The 17th century chemist Robert Boyle, described indicators extracted from roses and other plant materials in his book "The Experimental History of Colours" Boyle included the ability to turn plant juices red among the properties of acids. Red cabbage is not the only natural indicator that goes through a variety of colour changes but there are some other compounds from plant origin act as indicators.

It is also observed that some extracts exhibits different colour at different pH values. Cherry juice, for example, may be red (pH=2.5), orange (pH=4.5), brown (pH=7), or green (pH=10). Roses turnip skins, violets plum skins, primroses radish skins, hydrangeas rhubarb skins, cherries red grapes, beets red wine, curry powder, Geranium petals, Pansy petals , tea, tulip petals, thyme, turmeric, purple peonies, petunia petals, blue berries grape juice concentrate etc are only a few of many has different colours in acid and base. Almost any highly coloured fruit, vegetable or flower petal has the potential for use as an acid-base indicator.

Many of the natural dyes have extracted and isolated from different parts of plants. Which have different colours at different pH. Robert boyle [51] has given the method of making of indicator from juice of roses, *brazil-wood* [52], *cochineal* and litmus. Similarly red cabbage (*Brassica oleracea, capitata rosa)* [53-54] juice and tea pigments show different colors when the pH is different. The color of tea darkens in a basic solution, but the color becomes lighter when lemon juice is put into a tea. Red cabbage juice turns blue in a basic solution, but it shows a distinct red color in an acidic solution. Among the naturally occurring compounds show change in colour at different pH, are turmeric, Jack tree heartwood (*Artocarpus heerophyllus*) [55], Ratanjot (*Arnebia nobilis*) [56], yellow onion (*Allum cepa*) skins [57], beetroot (*Beta Vulgaris*) [58], Jungle flame (*Ixora coccinea*) [59-60].

7. INDICATOR PROPERTY

Nature is colourful due to the presence of flowers and other coloured material. The colour intensity depends on various factors including the composition. In plants the colour is due to the presence of naturally occurring organic substances like flaovenes, flavonol, xanthine, anthocyanins, azo compounds etc. Some of the naturally occurring flavones, flavonol, anthocyanins and other coloured substances are pH sensitive. They exhibit different colours in acids and basic medium. These substances give sharp distinct and stable colour change on a change of acid to alkaline medium. Thus they may be used as acid base indicators in volumetric analysis.

Few of them are having different colours at different pH i.e. pH 2, 4, 6, 8, 10, 12 etc. such substances make a very good educational experiment for a pH check in laboratory without using expensive pH meter and synthetic indicators.

It is reported that the aqueous extract of red cabbage shows colour variation [61-62] red (pH=2), purple (pH=3), violet (pH=5), blue (pH=7), blue green (pH=9), green (pH=12). It is thought that the change in colour is due to presence of one or more colour compounds in it. The colour components varies with the species to species, plant to plants, for example Red cabbage has only one anthocyanin [63] while blue berries has three namely delphinidin, petundin, and malvidin.

Flavones and anthocyanins are found as glycosides. On acid hydrolysis they remove carbohydrate moiety. Red cabbage contains anthocyanin which is diglycoside. Some of the flavones and anthocyanins are the ester derivatives of different organic acids. In acidic medium they exhibit different colour while has another colour in basic medium.

Hence, these can be used as indicator in titration. The necessary condition is that they should exhibit sharp, accurate and distinct colour change at equivalence point and should be stable of at least several hours. Some of the natural compounds posses these qualities and can be successfully used in titrations. The aim of the present work is also same i.e. to search a new natural indicator which can be successfully replace traditional synthetic colours. This will also help to reduce water pollution due to synthetic dye. The natural colours are biodegradable and decays aerobically or non-aerobically, hence removed easily from environment.

8. MECHANISM OF pH INDICATOR

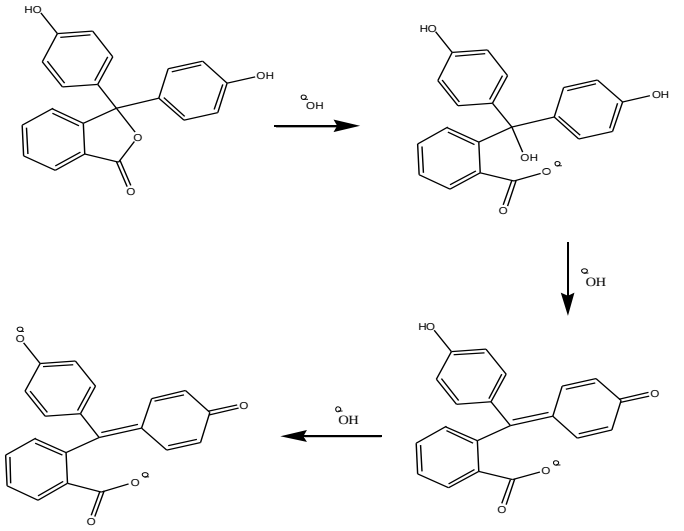
Phenolphthalein is weak organic acid. In presence of dilute alkali laclone ring of the phenolphthalein opens and triphenyl carbinol is formed [64] which undergoes loss of water to produce resonating ion which is red. But in excess alkali, the red colour disappears owing to the conversion to benzenoid structure (Scheme 1).

The pH range of phenolphthalein is 8.2 to 10.0 pH. It has PKin 9.6. Therefore phenolphthalein is not suitable for the titration of weak base with strong/weak acid

Methyl orange is weak organic base with PKin 3.7 and pH range 2.9 to 4.6. It shows red colour in acid while yellow in base due to the existence of following structures (Scheme 2).

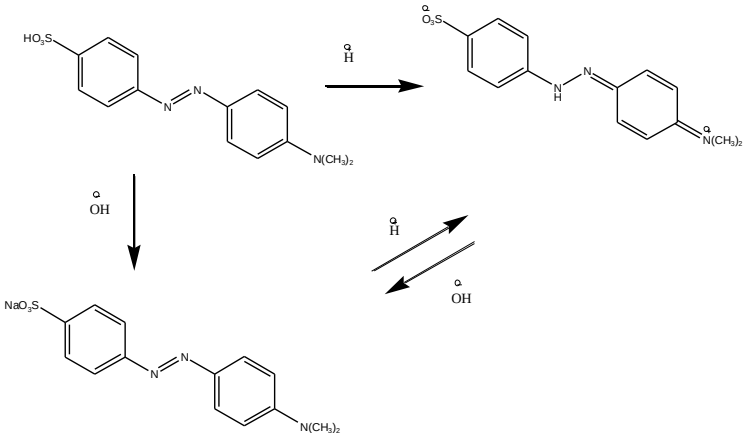
For the anthocyanin colour changes is suggested by Brouillard [65]. The anthocyanin exists in dilute acids solution as positively charged oxonium ions termed as flavylium cation. This results in extended conjugation of double bond. The

coloured flavylium cation is in equilibrium with colourless pseudo base. This conversion can be explained using Handerson-Hassel Balch equation [66-67] (Scheme 3).



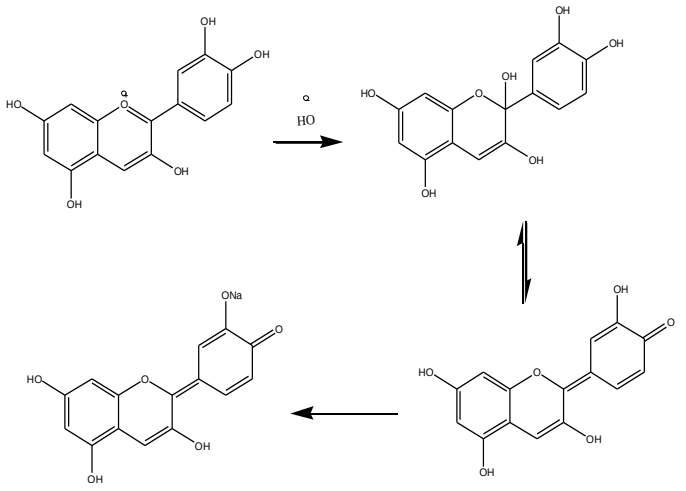
Scheme 1

Anthocyanins are more stable in acidic media at low pH values than in alkaline solutions with high pH values. In acidic aqueous solutions anthocyanin exist as four equilibrium species qinonoidal base, flavylium cation AH^+, the carbinol or pseudobase, and chalcone. Increasing the pH inflicts in decrease of both the colour intensity and the concentration of flvylium cation, as it is hydrated by nucleophilic attack of water, to the colourless carbinol form. The carbinol form has lost its conjugated double bond between A and B rings. Also a rapid proton loss of flvylium cation takes place as the pH shifts higher and the coloured quinonoidal form rises.

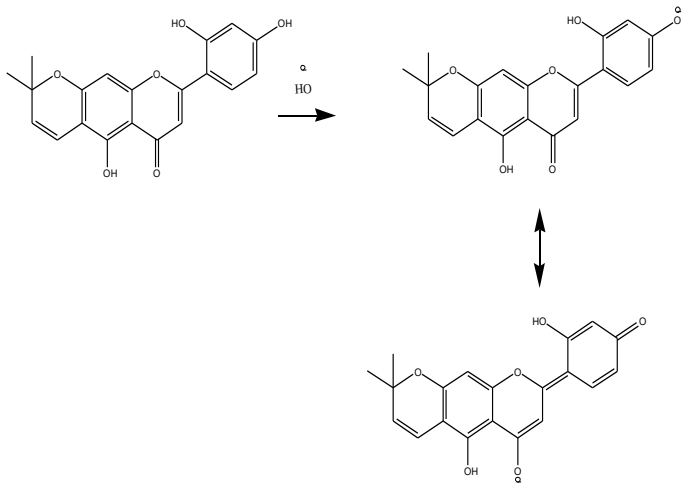


Scheme 2

A compound cycloartocarperin is isolated from the heartwood of Jack Tree [68-69] and studied for its property. In alkaline medium it has mustered yellow colour due to salt formation and quinonoid structure in dihydric phenolic ring is suggested for the change in colour. It has creamish colour in acid medium (scheme 4).



Scheme 3



Scheme 4

9. THEORY RELATED WITH TITRATION ERROR

Strong acid against strong base: Assume that a volume V_1 of HCl of Concentrated N_1 is titrated with a volume V_2 of NaOH of concentrated N_2. Then the mass balances are

$$[Cl^-] = \frac{N_1 V_1}{V_1 + V_2} \qquad ---(1)$$

$$[Na^+] = \frac{N_2 V_2}{V_1 + V_2} \qquad ---(2)$$

The Charge balance is

$$[H^+] + [Na^+] = [OH^-] + [Cl^-] \qquad ---(3)$$

Substituting equation (1) & (2) in (3), together with ionic product of water, we get

$$\frac{N_2 V_2 - N_1 V_1}{V_1 + V_2} = \frac{kw}{[H^+]} - [H^+] \qquad ---(4)$$

Since N_1, N_2 and V_1 are given; this equation gives $[H^+]$ as a function of V_2, and is the exact equation of the titration curve.

A more convenient form of equation 4 is expressed in terms of the fraction titration. The equivalent point is reached when

$$N_1V_1 = N_2V_2$$

And the fraction of the volume required to reach this point is the fraction titration

$$\phi = \frac{N_2V_2}{N_1V_1} \qquad ---(5)$$

At the equivalence point $\phi=1$

In terms of this variable, the equation of titration curve is

$$\frac{N_1V_1}{V_1+V_2}(\phi-1) = \frac{kw}{[H^+]} - [H^+] \qquad ---(6)$$

Before the equivalence point $[H^+]$ is large compared to $[OH^-]$, and hence

$$\frac{kw}{[H^+]} << [H^+]$$

Therefore equation 6 becomes

$$\frac{N_1V_1}{V_1+V_2}(\phi-1) = [H^+] \text{ (acid approximation)}$$

After the equivalence point $[OH^-] >> [H^+]$

$$\therefore \frac{kw}{[H^+]} >> [H^+]$$

Hence equation (6) becomes

$$\frac{N_1V_1}{V_1+V_2}(\phi-1) = \frac{kw}{[H^+]} \qquad \text{(base approximation)}$$

When an indicator is used to locate the end point of titration of strong acid against strong base, the change in colour is not sharp, but occurs over a range of pH and this is cause of titration error. The other cause is that the indicators may not change colour at the same pH as the equivalence point. The equivalence point of HCl vs NaOH is near to 7.00 pH.

Once the titration curve has been calculated and the pH at the end point is known. The volume of titrant required to reach the end point, V_{ep}, can be obtained from the curve. The titration error is then expressed as absolute error, relative error or percentage error

Absolute error = $V_{ep} - V_1$

$$\text{relative error} = \frac{V_{ep} - V_1}{V_1}$$

The positive value for the titration error means that the end point comes after the equivalence point. A negative value means that the end point curves before the equivalence point.

The titration error can be calculated from titration curve

Titration error = $\phi_{ep} - 1 =$

$$\frac{N_1+N_2}{N_1N_2}\left(\frac{kw}{[H^+]_{ep}} - [H^+]_{ep}\right)$$

Weak acid and strong base: If V_1 mL of weak acid HA of concentration N_1 are titrated with V_2 mL of strong base NaOH the mass balances are

$$[HA]+[A^-] = \frac{N_1V_1}{V_1+V_2} \qquad ---(7)$$

$$[Na^+] = \frac{N_2V_2}{V_1+V_2} \qquad ---(8)$$

The charge balance is

$[Na^+] = [H^+] = [OH^-] + [A^-]$ --- (9)

Substituting equation (8) in (9)

$$[A^-] = \frac{N_2V_2}{V_1+V_2} + [H^+] \qquad ---(10)$$

The degree of dissociation of weak acid is defined as

$$\alpha = \frac{[A^-]}{[HA]+[A^-]} \qquad ---(11)$$

The fraction titrated can be defined as

$$\phi = \frac{N_2V_2}{N_1V_1} \qquad ---(12)$$

Substituting equation (7) and (10) in (11) and using equation (12) we get

$$\phi = \alpha + \frac{V_2+V_1}{N_1V_1}([OH^-]-[H^+]) \qquad ---(13)$$

We can calculate the titration error by using formula

$$\text{Titration error} = \phi_{ep} - 1 = \frac{N_1+N_2}{N_1N_2}\left(\frac{kw}{[H^+]_{ep}} - [H^+]_{ep}\right) - \frac{[H^+]_{ep}}{ka}$$

Weak Base and Strong Acid: If V_1 mL of weak base BOH of concentration N_1 is titrated with strong acid (V_2 mL) of concentration N_2 mL, the mass balances are

$$[BOH]+[B^+] = \frac{N_1V_1}{V_1+V_2} \qquad ---(14)$$

$$[A\text{-}] = \frac{N_2V_2}{V_1+V_2} \qquad ---(15)$$

The charge balance is

$$[B^+] + [H^+] = [OH^-] + [A^-] \qquad --- (16)$$

Substituting (15) in (16)

$$[B^+]+[H^+] = [OH^-] + \frac{N_2V_2}{V_1+V_2}$$

$$[B^+] = [OH^-] - [H^+] + \frac{N_2V_2}{V_1+V_2} \qquad ---(17)$$

The degree of dissociation for weak base can be given as

$$\alpha = \frac{[B^+]}{[BOH]+[B^+]} \qquad ---(19)$$

The fraction titrated can be defined as

$$\phi = \frac{N_2V_2}{N_1V_1} \qquad ---(20)$$

$$\phi = \frac{([OH^-]-[H^+]) + \frac{N_2V_2}{V_1+V_2}}{\frac{N_1V_1}{V_2+V_1}}$$

$$= \frac{V_2+V_1}{N_1V_1}([OH^-]-[H^+]) + \frac{N_2V_2}{N_1+V_1}$$

$$\therefore \phi = \alpha - \frac{V_1+V_2}{N_1V_1}([OH^-]-[H^+])$$

$$\phi = \alpha + \frac{V_1 + V_2}{N_1 V_1}([H^+] - [OH^-]) \quad \ldots\ldots (21)$$

The titration error can be calculated as

$$\text{Titration error} = \phi_{ep} - 1 = \frac{N_1 + N_2}{N_1 N_2}\left([H^+]_{ep} - \frac{kw}{[H^+]_{ep}}\right) - \frac{kw}{kb[H^+]_{ep}} \ldots (22)$$

Weak Acid And Strong Base: If a volume V_1 mL of a weak acid HA of concentration N_1 is titrated with a volume V_2 mL of weak base of concentrated N_2, the mass and charge balances can be combined to give the equation for the titration curve

$$\phi\left(\frac{kb[H^+]}{kw + kb[H^+]}\right) - \left(\frac{ka}{ka + [H^+]}\right) = \left(\frac{V_1 + V_2}{N_1 V_1}\right)([OH^-] - [H^+]) \ldots\ldots\ldots (23)$$

At the equivalence point, the solution is identical with that of the salt of a weak acid and a weak base. Under the approximation that $[H_+]$ is small compared to Ka and $[H^+]$ is large compared to Kw/kb, the titration error is given by following equation

$$\phi_{ep} - 1 = \left(\frac{N_1 + N_2}{N_1 N_2}\right)\left(\frac{kw}{[H^+]_{ep}} - [H^+]_{ep}\right) + \frac{kw}{kb[H^+]_{ep}} - \frac{[H^+]_{ep}}{ka} \ldots (24)$$

Owing to these solutions is somewhat over titrated this gives rise to small error in titration known as indicator error. It is expressed in terms of excess acid or alkali remaining at the end point. If the excess alkali is strong the error are described as "$^-$OH errors", for weak bases it is called as alkali errors, these are calculated by the formulae given below

$$^-\text{OH error} = \frac{10^{-(14\text{-pT})}}{N} \times 2 \times 100 \quad percent$$

$$\text{alkali error} = 10^{pK + pT - 14}$$

10. REFERENCES

1. Mingbo Zhao, Yoichiro Ito and Pengfei Tu. *Journal of Chromatography A*, 2005; **1090(1-2):** 193-196.
2. Navindra P Seeram, Rupu Lee, H Samuel Scheuller and David Heber. *Food Chemsitry*, 2006; **97(1):** 1-11
3. Jia-Ping Lai, Yew Heng Lim, Jin Su, Han-Ming Shen and Choon Nam Ong. *Journal of Chromatography B*, 2007; **848(2)**: 215-225.
4. Zafar Iqbal, Muhammad Lateef, Abdul Jabbar, Ghulam Muhammad and Muhammad Nisar Khan. *Journal of Ethnopharmacology*, 2005; **102(2)**: 256-261.
5. Maribel Herrera-Ruiz, Yolanda García-Beltrán, Sergio Mora, Gabriela Díaz-Véliz, Glauce SB Viana, Jaime Tortoriello and Guillermo Ramírez. *Journal of Ethnopharmacology*, 2006, **107(1):** 53-58.
6. Adam J Matich, Barry J Bunn, Martin B Hunt and Daryl D Rowan. *Phytochemistry*, 2006; **67(8):** 759-763.
7. Maria Inês Soares Melecchi, Valéria Flores Péres, Cláudio Dariva, Claudia Alcaraz Zini, Fernanda Contieri Abad, Migdália Miranda Martinez and Elina Bastos Caramão. *Ultrasonics Sonochemistry*, 2006; **13(3):** 242-250.
8. Sancun Hao, Jihuai Wu, Yunfang Huang and Jianming Lin. *Solar Energy*, 2006; **80(2):** 209-214.
9. Xiang-Yu Cui, Jin-Hwa Kim, Xin Zhao, Bao-Qiong Chen, Bum-Chun Lee, Hyeong-Bae Pyo, Yeo-Pyo Yun and Yong-He Zhang. *Journal of Ethnopharmacology*, 2006; **103(2):** 223-228.
10. Evelise F Pietrovski, Kelson A Rosa, Valdir A Facundo, Katiuscia Rios, Maria Consuelo A Marques and Adair RS Santos. *Pharmacology Biochemistry and Behavior*, 2006; **83(1):** 90-99.
11. Nayak BS, Vinutha B, Geetha B and Sudha B. *Fitoterapia*, 2005; **76(7-8):** 671-675.
12. Mangathayaru K, Lakshmikant J, Shyam Sundar N, Swapna R, Fatima Grace X and Vasantha J. *Fitoterapia*, 2005; **76(7-8):** 752-754.
13. Zoran Maksimović, Silvana Petrović, Milica Pavlović, Nada Kovačević and Jelena Kukić. *Fitoterapia*, 2007; **78(3):** 265-267.
14. Saxena V K and Swati Nigam. *Asian J of Chem*, 1996; **8(2):** 337-340.
15. Meera Harit and Rathee PS. *Asian J of chem*, 1996; **8(1):** 180-182.
16. Gottlieb OR, Megathaes and Mors WB. *Ann Ass Brasil Quim*, 1957; **18:** 37.
17. Gottlieb OR, Mors WB. *J Org Chem*, 1959; **24:** 17.
18. Van Bulow MV, Gottlieb OR. *An Acad Brasil Cienc*, 1968; **40(3)**.
19. Elderfield RC. *J Hetrocylic chem*, 1950; **1:** 7.
20. Poulton GA and Cyr TD. *Can J Chem,* 1980; **58:** 2158.
21. Biskupiak JE and Ireland CM. *Tetrahedron Lett*, 1983; **24(30):** 3055.
22. Rakhi Shanker and Padma S Vankar. *Dyes and Pigments*, 2007; **74(2):** 464-469.
23. Elio Pale, Marie Koura-Bonafos, Moushoussine Nacro, Maurice Vanhaelen and Renee Vanhaelen-Fastre. *Phytochem*, 2003; **64(8):** 1395-1399.
24. Kumi Yashida, Mihoko Mori, Miki Kawachi, Reiko Okuno, Kiyoshi Kameda and Tadao Kondo. *Tetrahedron Lett*, 2003; **44(43):** 7875-7880.
25. Filippini R, Caniato R, Piovan A and Cappellletti EM. *Fitoterapia*, 2003; **74(1-2)**: 62-67.
26. Sancun Hao, Jihuai Wu, YunfangHuang and Jianming Lin.*Solar Energy*, 2006; **80(2)**: 209-214.
27. Tamaki Mishio, Tamaki Honma and Tsukasa Iwashina. *Biochemical systematics and Ecology*, 2006; **34(2)**: 180-184.
28. Allan Holm Nielsen, Carl Erik Olsen and Birger Lindberg Moller. *Phytochem*, 2005; **67(24):** 2829-2835.
29. Katsuori Sasaki and Takashi Takahashi. *Phytochem*, 2002; **61(3):** 339-343.
30. Rikke Norbaek, Jens K Nielsen and Tadao Kondo. *Phytochem*, 1999; **51(8):** 1139-1146.
31. Tadigoppula Narender, Tanvir Khaliq, Anju Puri, Ramesh Chander. *Bioorganic & Medicinal chemistry letters*, 2006; **16(13):** 3411-3414.
32. Melanie K Gatt, Keith R W Hammet, Kenneth R Markham and Brian G Murray. *Scientia Horticulturae*, 1998; **77(3-4):** 207-218.
33. Agostino MD, Senatore F, De Feo V and De Simone F. *Fitoterapia*, 1995; **66:** 283.
34. Chulia AJ, Bennini B, Kaoudadi M, DP 'Allais and Delage C. *J Nat Prod,* 1995; **58:** 560.
35. LI CJ, Elgamal MH, Sharker KH, Ahmed AA and Mabry TH. *Nat prod Lett*, 1996; **8:** 281.
36. Cabrita L, Fosson T, Andersen OM. *Food Chem*, 2000; **68:** 101-107.
37. Francis FJ. *Crit Rev Food Sci Nutr*, 1989; **28:** 273-314.

38. Fumi Tatsuzawa, Yuki Mikanagi, Norio Saito, Koichi Shinoda, Atsushi Shigihara and Toshio Honda. *Biochemical Systematics and Ecology*, 2005; **33(8):** 789-798.
39. Toshio Honda, Fumi Tatsuzawa, Nao Kohayashi, Hiroko Kasai Seiji Nagumo, Atsushi Shigihara and Norio Saito. *Phytochem*, 2005; **66(15):** 1844-1851.
40. Kosaku Takeda, Akiko Osakabe, Shinomi Saito, Daisuke Furuyama, Atsuko Tomita, Yumi Kojima, Mayumi Yamadera and Masaaki Sakuta. *Phytochem,* 2005; **66(13):** 1607-1613.
41. Ana Paula A Vaz, Rita Decassia L Figueiredo Ribeiro and Gilberto B Kerbauy. *Plant Physiology and Biochem*, 2004; **42(5):** 411-415.
42. Jamaluddin AFM, Fumio Hashimoto, Toshiki Miwa, Katsuhiro Ohbo and Yusuke Sakata. *Scientia Horticulturae*, 2004; **100(1-4):** 103-115.
43. Kuang-Liang Huang, Ikuo Miyajima, Hiroshi Okubo, Tsai Mu shen and Ta-shiung Huang. *Scientia Horticulturae*, 2001; **88(3):** 235-241.
44. Kenjiro Toki, Norio Sito, Atsushi Shigihara and Toshio Honda. *Phytochem*, 2001; **56(7):** 711-715.
45. Moshe Reuveni, Dalia Evernor, Bracha artzi, Avi Perl and Yair Erner. *J of Plant Physiology*, 2001; **258(8):** 991-998.
46. Ben Tal Y, and King RW. *Scientia Horticulturae*, 1997; **72(1):** 35-48.
47. Kjell Torskangerpoll, Torgils Fossen and Oyvind M Andersen P. *Phytochem*, 1999; **52(8):** 1687-1692.
48. Tomio Ishikawa, Tomoko Kondo, Takeshi Kinoshita Hideyuki Haruyama, Saori Inaba, Kosaku Takeda, Renee J Grayer and Nigel C Veitch. *Phytochem*, 1999; **52(3):** 517-521.
49. Rikke Norbaek and Tadao Kondo. *Phytochem,* 1998; **47(5):** 861-864.
50. Ben Erik Van wyk, Pieter JD Winter and Matt H Buys. *Biochemical System and ecology*, 1997; **25(1):** 39-42.
51. R Boyle, " Experiments upon Colours" 1663
52. Indrayan AK, and Guleria BS. *J Indian chem Soc,* 2001; **78:** 476.
53. Swain T "chemistry and biochemistry of Plant pigments" ed. T. W. Goodwin academic London 1976; 425-463.
54. Forster M. *J Chem Educt*, 1978; **55:** 107.
55. Indrayan AK, Shukla RK, and Kumar R. *Asian J Chem*, 1999; **11:** 141.
56. Indrayan AK and Yadav V. *Asian J Chem*, 1999; **11:** 611.
57. www. science.esustan.edu/persona/2000/2002-acidbase.htm.
58. "LFRA Ingredients Handbook: Food Colours" Leather head food research agency, 1997.
59. Cobb V. " Science Experiments You can eat" Harper Trophy, USA, 1994
60. Tate J. "Labs Alive" Achimoa School, Ghana, 2000.
61. Mebane RC and Rybolt TR. *J Chem Education*, 1985; **62:** 285.
62. C-Borg ford and Summerlin L. "Chemical activities." American chem. Society: Washington DC 1988; 92-94.
63. Curtright R, Rynearson JA and Markwell J, *J Chem Educt*, 1996; **73:** 306.
64. Vogel's Text Book of Quantitative Inorganic Analysis, 4th Ed. ELBS, London, 1985.
65. Brouilard R. *Phytochem*, 1983; **22:** 1311.
66. Curtright RD, Rynearson JA and Markwell J. *J Chem Educt*, 1994; **71:** 682.
67. Badgar GM. "The Chemistry of Heterocyclic Compounds", Academic, New York, 1961 (452)
68. Indrayan AK, Shukla RK and Kumar R, *Asian J Chem*, 1999; **11:** 141.
69. Indrayan AK, Kumar R and Sharma V. *J Indian Chem Soc,* 2001; **78:** 476**.**