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Green chemistry: Petal sap of *Delonix regia* as a substitute for hazardous internal indicators in volumetric analysis

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ABSTRACT

Suffering from the rapid depletion of the natural resources, the present world scenario calls for the need of sustainable development so as to obtain eco friendly environment. Chemicals are one among the various things that are extremely dangerous but still their use cannot be avoided. Green Chemistry thus emerges as a significant tool to mitigate the use of hazardous chemicals. It encourages innovation and promotes the creation of products that are environmentally and economically sustainable. Synthetic chemicals which are used as internal indicators in acid- base titrations being hazardous can be substituted by using petal sap extract which gives results with the same accuracy. Natural indicators are easy to prepare and are easily available. Volumetric titrations between acid and base show sharp colour change at the equivalence point. The equivalence point obtained by natural sap indicator coincide with the equivalence point obtained by standard synthetic indicators as Methyl orange, Phenolphthalein, Phenol red etc. Petals of flower like *Butea monosperma, Hibiscus rosa sinensis, Dahlia* etc. can be used for this purpose which contain anthocyanins as active component responsible for colour change. Thus petal sap indicators are found to be simple and economical in acid base titrations. Such natural indicators also satisfy the principles laid down by Green Chemistry.

KEY WORDS: NATURAL INDICATOR, ANTHOCYANINS, ACID BASE TITRATION, DELONIX REGIA

INTRODUCTION

Volumetric analysis refers to the quantitative chemical analysis carried out by determining the volume of a solution of accurately known concentration which reacts quantitatively with a measured volume of a solution of the substance to be determined. The process of adding standard solution until the reaction is complete is termed as titration. Acidimetry and alkalimetry include the titration of free bases with a standard acid and the titration of free acid with a standard base. The end point or equivalence point of the reaction is known with the help of indicators. Latter may be used internally or externally and are termed as internal indicators or external indicators respectively.

In acid base titration methyl orange, phenolphthalein, phenol red etc. are used as indicators. In such titrations the hydrogen and hydroxyl ions combine together to form neutral water. The indicators signal the completion of the reaction i.e. it gives the equivalence point. Methyl orange, phenolphthalein, phenol red etc. are hazardous chemicals, they pollute the

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environment, water and soil, thus are harmful for health (Yang, 2010; USEPA, 2011).

Methyl orange is an azo dye, as it gives sharper end points. In acidic medium it is reddish while in alkali medium it turns yellow. According to Kulkarni, (1985) and Zollinger, (1987) azo dyes are released by many industries such as paper, plastic, leather, food, cosmetics and pharmaceutical industries. Yang, (2010) reported significant environmental pollution from these effluents. Chung *et al.*, (1992) and Kusic *et al.*, (2006) have reported that azo dyes and their degradation products are recognized as potential carcinogens. As per Santa Cruz Biotechnology Methyl orange may cause eye and skin irritation. If swallowed it may cause gastrointestinal irritation with nausea and vomiting. It may cause respiratory tract irritation.

According to Hassen *et al.*, (2013) the photo degradation of aqueous solution containing azo dye methyl orange decreases the chemical oxygen demand (COD) and total organic carbon (TOC). The toxicity of soil wetted with methyl oranges solution was tested on earthworm (*Eisenia andrei*) High sensitivity was observed.Merzouk *et al.*, (2009) reported that waste water generated by textile industries contain large amounts of toxic aromatic compounds especially azo dyes. Md. Ali *et al.*, (2011) estimated that 10 to 15 % of the overall production of dyes is released into the environment mainly via waste water It has been reported that the discharge of very small amount of dyes (less than 1 ppm.) is aesthetically displeasing, impedes light penetration, affects gas solubility and may be toxic to treatment processes, food chain organisms and aquatic life.

Phenolphthalein is also used as internal indicator in acid base titration. AHFS (1995) reported that the main target organ for the toxic effects of phenolphthalein is intestine which causes chronic ulcerative colitis and involves thinning of intestinal wall and loss of normal mucosal pattern of terminal ileum. Nishikawa, (1981) reported that phenolphthalein at doses of 25 to 50 Ug/ml was cytotoxic in cultured liver cells causing decreased cell growth and increased anaerobic glyolysis.

Tice *et al.*, (1998) studied the effects of phenolphthalein at various concentrations in the diet of transgenic female mice for p53 gene, over a six month period. They found significant increase in the frequency of micro nucleated erythrocytes; these were observed at doses comparable to those to which humans are exposed. According to Sarco, (2010) over dose of phenolpht-halein have been associated with abdominal pain, diarrhoea, vomiting, electrolyte imbalance, dehydration, malabsorption, weight loss, muscle weakness and prostration. Studies have shown that it is carcinogenic in mice at high dose level and has a weak genotoxic activity *in vivo*.

Phenol red is also used in acid-base titration. It changes colour from yellow to red over pH range 6.8 (yellow) to 8.2 (red). Above pH 8.2 turns bright pink colour. Phenol red is phenolsulphonphthalein (PSP). It is a weak acid having phenolic and sulphonic groups. Warner *et al.*, (1985) reported that phenol red may cause harmful effect on the central nervous system and heart resulting in disrhythmias, seizures and coma. Budavari, (1996) reported that compounds containing phenolic group can cause eye irritation, inflammation of skin, respiratory irritation which can cause further lung damage.

Green Chemistry is the solution to these problems. According to the United State Environmental Protection Agency, (2011) it is also called as sustainable chemistry is a philosophy of chemical research and engineering that encourages the design of chemical products and processes minimizing the use and generation of hazardous waste substances. The principles underlying Green Chemistry also emphasize on the use of substances that possess little or no toxicity to human health and environment.

Flower petals are the substitute for such hazardous internal indicators. Flowers are the miracle of the nature, by God, for the world. Anthocyanins are notable plant pigments which are responsible for red violet blue colour seen in petals (Harborne, 1998). According to Vanker, (2008) the appearance of red colour is due to the presence of flavonoids, phenols and anthocyanins. Latter may serve as pH indicator in acid base titration. According to Jain *et al.*, (2012) petal extract acts as an indicator in acidimetry and alkalimetry.

Singh *et al.*, (2010) used *Nerium oleander, Tecoma stans, Calatropis gigentia, Albizia lebbeck* and *Cassia fistula* flowers as acid base indicator. Ramling *et al.*, (2010) used flower extract of *Bombax malabaricum*, Agrawal *et al.*, (2011) investigated the indicator activity of methanolic activity of *Punica granatum*. Jain *et al.* (2012) have used aqueous extract and methanolic extract of *Dahlia pinnata* and *Hibiscus rosa sinensis* petal extract as acid base indicators.

In the present study *Delonix regia* (Boj. Ex. Hook.) petal extract was selected as natural indicator for acid base titration. It is commonly known as Gulmohar and in English it is given the name Royal Poinciana or Flamboyant. This tree is commonly cultivated in tropics and subtropics including Madagascar. It belongs to family Fabaceae sub family Caesalpinioidea. It is grown as an ornamental tree and is noted for its fern like leaves and flamboyant display of flowers. *Delonix regia* is also having economic importance. Shewale *et al.*, (2012) studied the inflammatory activity of the plant. Parekh *et al.*, (2005) studied the antibacterial activity of aqueous and methanolic extract of *Delonix regia*. Rajababu *et al.*, (2011) observed antidiarrhoeal activity of *Delonix regia* in Wistar albino rats.

MATERIAL AND METHODS

Delonix regia was collected was collected for the purpose of study of natural indicator. It was identified from the flora of Botany Department of M. J. Government Girls P.G. College, Indore. Flowers were collected during April to June as it is the blooming season of this plant. The flowers possess four scarlet or orange red spoon shaped petals and a fifth upright petal called the 'standard' which is slightly larger and spotted with yellow or white.

The spoon shaped petals were plucked from the flower. The standard petal was left away. The petals were cleaned with distilled water and were kept in strong sunlight until they get completely withered. The petals were grinded into fine powder with a mechanical blender. The dried petals were soaked in two beakers one containing 40 ml methanol while the other having 40 ml distilled water for 48 hours

and then triturated in mortar and pestle. The resulting solution was filtered through muslin cloth. Thus methonilic and aqueous extracts were prepared for acid base titration. The extracts were preserved in light closed container and stored away from direct sunlight.

Analytical grade HCl, NaOH, CH_3COOH, NH_4OH, phenolphthalein, methyl orange and phenol red were made available by the Department of Chemistry, Government Holkar Science College, Indore. Reagents and volumetric solutions were prepared as per standard. The experimental work was carried out by using the same set of glassware for all types of solutions. 0.1N, 0.5N and 1.0N acid and base solutions were prepared. The titration were carried out between NaOH/HCl; HCl/NH_4OH; CH_3COOH/NaOH and CH_3COOH/NH_4OH i.e. strong acid v/s strong base; strong acid v/s weak base; weak base v/s strong base and weak acid v/s weak base. The equinormal titrations were performed using 10 ml of titrant with 5 drops of natural methanolic and aqueous indicator. A set of four experiments each for all types of acid base titration were carried out. The t- value and standard deviation for each type of titration were calculated from results obtained.

RESULTS AND DISCUSSION

In the present study, methanolic and aqueous extract of *Delonix regia* were selected as natural internal indicator for acid base titration. The strength of acids HCl and CH_3COOH and bases NaOH and NH_4OH used were of 0.1N, 0.5N and 1.0N strength. Four different types of titration performed were between strong acid strong base (HCl v/s NaOH); strong acid weak base (HCl v/s NH4OH); weak acid strong base (CH_3COOH v/s NaOH) and weak acid weak base (CH_3COOH v/s NH_4OH).

The change in colour of acid or base when titrated with synthetic indicator, methanolic floral extract and aqueous floral extract is shown in table A. It has been observed that both methanolic and aqueous natural indicators exhibit sharp cooler change like synthetic indicator.

TABLE 1: Change of Color in Titration.

Acid	Base	Change in Color with		
		Synthetic indicator	Methanolic floral extract	Aqueous floral extract
HCL	NaOH	Colorless to pink (PH)	Pink to green	Pink to greenish yellow
HCL	NH4OH	Pink to yellow (MO)	Pink to dark green	Pink to green
CH3COOH	NHOH	Colorless to pink (PH)	Pink to yellow	Pink to yellow
CH3COOH	NH4OH	Yellow to red(PR)	Pink tored	Pink to green

PH = Phenolphthalein; MO = Methyl orange; PR= Phenol red; HCl = Hydrochloric Acid
CH3COOH = Acetic Acid; NaOH = Sodium Hydroxide; NH4OH = Ammonium Hydroxide

TABLE 2: Statistical data of Methanolic and Aqueous Floral Extract of *Delonix regias* as Natural Indicator

Titration	Normality	Methanolic Floral Extract	t-Value±SD*	Aqueous Floral Extract	t-Value±SD*
	0.1 N	PH v/s MFR	**0.446±0.09**	**PH v/s AFR**	**1.3444±0.09**
NaOH/ HCL	0.5N	PH v/s MFR	0.4728±0.35	PH v/s AFR	0.8622±0.11
	1.0 N	PH v/s MFR	0.2340±0.54	PH v/s AFR	0.1682±0.23
	0.1N	MO v/s MFR	1.2723±0.25	MO v/s AFR	0.4582±0.09
NH_4OH/ HCL	0.5N	MO v/s MFR	0.4526±0.09	MO v/s AFR	1.6824±0.09
	1.0N	MO v/s MFR	0.0482±0.05	MO v/s AFR	1.9256±0.05
	0.1N	PH v/s MFR	0.2884±0.12	PH v/s AFR	0.2436±0.09
NaOH/CH_3COOH	0.05	PH v/s MFR	0.8632±0.09	PH v/s AFR	0.8544±0.06
	1.0	PH v/s MFR	0.9546±0.24	PH v/s AFR	0.4156±0.12
	0.1	PR v/s MFR	0.0482±0.09	PR v/s AFR	1.1654±0.06
NH_4OH/CH_3COOH	0.5	PR v/s MFR	0.2576±0.16	PR v/s AFR	1.1886±0.04
	1.0	PR v/s MFR	0.4238±0.20	PR v/s AFR	0.7892±0.19

All values are t- values ± SD for n = 4
PH=Phenolphthalein; MO= Methyl orange; PR= Phenol red; MFR= Methanolic Floral Extract;
AFR= Aqueous Floral Extract.

The results obtained by titration between acid and base with natural indicators were compared with the results given by synthetic indicators as phenolphthalein, methyl orange and phenol red. The t- value and standard deviation between four titrations performed are shown in table B. The standard deviation for methanolic and aqueous floral extract of *Delonix regia* was found to be in the range of ± 0.05 to ± 0.54.

CONCLUSION

The synthetic indicators like phenolphthalein, methyl orange and phenol red are not only hazardous to health but are also prominent pollutants. The fundamentals of Green Chemistry prove that these unsafe chemicals can be substituted by the petal extract as an indicator for acid base titration. The accuracy of the observed results has been examined by performing titration between different acids and bases of varying normality. The results are also supported by less variation in the value of standard deviation and t-value from that of synthetic indicators. Thus, the use of natural indicators in the acid base titrations is statistically proved. The natural indicator prepared from *Delonix regia* flower petal is neither harmful to the environment nor it causes any health hazard. This aqueous extract is equally effective as the methanolic extract or any other synthetic indicator and provides reliable and accurate results. Moreover, *Delonix regia* flowers are easily available and the petal sap can be easily prepared. Therefore, the use of natural indicator like petal sap extract is more economical, simple, harmless, pollution free and inert.

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