ISSN: 1999-1630

SEU Journal of Science and Engineering, Vol. 12, No. 2, December 2018

An Investigation of the Analytical Potentials of Flower Extracts as Bio-Indicators in Acid Base Titration

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Abstract
This study investigates the potentials of flower extracts namely, *Hibiscus Rosa-sinensis* (red hibiscus flower), *Plumeria Alba* (White Frangipani flower), *Bombax Buonopozense* (Red flower silk cotton flower) *Tabebuia Rosea* (Pink trumpet flower) and *Tecoma Stans* (Yellow bells flower) as bio-indicators and substitutes for commonly used synthetic indicators such as methyl orange and phenolphthalein. The bio-indicators were prepared through sun-drying, pulverisation and solubilisation of the flower petals in solvents (acetone, ethanol, water, acetone/ethanol mixture and ethanol/water mixture) of various solvatrochromic parameters followed by strong agitation at room temperature.Titration of strong acid against strong base was carried out using the prepared five different indicators and the synthetic indicators (methyl orange and phenolphthalein) to serve as control. *Plumeria Alba* (White Frangipani flower) petals dissolved in ethanol was found to be a highly promising bio- indicator with a competing average titre value of 29.50 cm^3 against methyl orange with average titre value of 29.30 cm^3.The Fourier transform infra red study shows that the presence of the C=C stretch and vibration band at 1605 cm^{-1} found in the synthetic indicators used was also present in PLA flower extract. A regression model in terms of solvent polarity, Hasen dispersion bond solubility parameter and and bio-indicator pH was developed for the prediction of average titre values.The model was found to give excellent prediction only for bio-indicators obtained from solvent of low (<25) polarity index.

Keywords: *Flower extracts, Bio-Indicators, Regression model, Hasen solubility parameter, Solvatrochromic parameters*

I. Introduction

Acid-Base interactions in the presence of an indicator are reactions that are often and widely encountered in analytical Chemistry and Chemical Engineering for the standardization of solution and subsequent quantitative analysis. Details of such analysis can be found in Levine, 2002; Atkins and De Paula, 2014; Chang, 2007; Sharma, 2000; Viswanathan, 2008. In other to indicate the end and extent of the acid - base interactions, few drops of indicators are commonly introduced into the base before such interactions are monitored. An indicator is a substance that changes color as the pH of a solution changes (Tilekar *et al*., 2015). Frequently used indicators are synthetic products that are not only scarce and costly (Pathade *et al*., 2009) but also with negative toxicological records (Dunnick and Hailey, 1996).Table 1 shows some negative health implications of over-exposure to some of the frequently used indicators.

Table 1: Toxicity of some frequently used Acid-Base Indicators

	Acid-Base Indicators	**Toxicity**	Reference
1.	Methyl Orange	Skin and Eye Irritant	CHEMWATCH, 2010
2.	Methyl Red	Skin and Eye Irritant	CHEMTREC, 2013
3.	Phenolphthalein	Carcinogenic	Dunnick and Hailey, 1996

Aside the exposure to the synthetic indicators as shown in Table 1, the process technology for their synthesis involves the use of several non-green materials (Dijk *et al*., 2013), is hazardous (Dunnick and Hailey, 1996) coupled with severe

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pollution effect (Vadivel *et al*., 2016). To overcome the aforementioned negative threats and potentials of synthetic indicators, this study therefore aims to synthesize indicators from natural or bio-source that are both readily available and easily accessible. Indicators from bio source selected for use in this study also involves various solvents media before its final application for the acid-base interactions. Though, solvents present numerous environmental, health and safety challenges including human and eco-toxicity, possess safety hazards and waste management issues (Gani *et al*., 2005), they have however been carefully selected in this study to meet the challenges of green and sustainable chemistry (Table 2). Bio-indicators selected for this study are extracts of flowers which chemists believe to be a chemical entity containing several other chemical compounds. Numerous previous researches have proved the adequacies of some extracts of the brightly color flowers as a replacement for synthetic acid- base indicators. Some of the flavonoid family noted for this function are the flavones, anthocyanidins, flavonones, isoflavone, flavonols, etc For instance, Tilekar *et al*., 2015 concluded that almost any flower such as blue, purple or red in color contains a class of organic pigment called anthocyanins that changes color according to pH. Pimpodkar *et al*., 2014; Igidi *et al*., 2012; and Venugopalar *et al*., 2011 further ascertain that some of the naturally flavones, flavonol, anthocyanins and others found in plants are pH sensitive, flavonoids that can be obtained from various parts of a plant such as flowers are color pigments that are also pH sensitive (Sandeep, 2009) and finally (Oloyede, 1997) posited that any highly color fruit, vegetable, or flower petal has the potential for use as acid- base indicator.

Table 2: Solvent Selection Guide

Preferred	Usable	Undesirable
Water	Cyclohexane	Pentane
Acetone	Toluene	Hexane
Ethanol	Methylcyclohexane	Di-isopropylether
2-Propanol	tertiary Butyl methyl ether	Di-ethylether
1-Propanol	Isooctane	Dichloromethane
Heptane	Acetonitrile	Dichloroethane
Ethyl acetate	2-Methyltetrahydrofuran	Chloroform
Isopropyl acetate	Tetrahydrofuran	N-Methyl-2-pyrrolidone
Methanol	Xylenes	Dimethylformamide
Methyl ethyl ketone	Dimethylsulfoxide	Pyridine
1-Butanol	Acetic Acid	Dimethylacetamide
t-Butanol	Ethylene Glycol	Dioxane
		Dimethyloxtethane
		Benzene
		CarbonTetrachloride

Sheldon (2012) and Dunn (2011)

Some other studies have also reported the effectiveness of extract of flowers as acid-base indicators, for instance *Morus alba* (Pathade *et al*., 2009), *Nerium odorum* (Patil and Ukpumwan, 2009), *ixora corcinea* (Nwosu *et al*., 2004) and *Jacaranda acutifolia* (Patrakar *et al*., 2010), *Argyreia cuneata* (Pimpodkar *et al*., 2014). Results obtained from these previous studies though confirmed that indicators from some of the flower extracts could replace some of the synthetic indicators but they all failed to establish regression or statistical relationships in terms of models among parameters of the indicators obtained from flower extract. Moreso, in this study , five other varieties of flowers [*Hibiscus Rosa-sinensis* (red hibiscus flower, (RSN) Fig. 1a); *Plumeria Alba* (White Frangipani flower, (PLA) Fig. 1b); *Bombax Buonopozense* (Red flower silk cotton flower, (BBP) Fig. 1c), *Tabebuia Rosea* (Pink trumpet flower,(TBR) Fig. 1d) and *Tecoma Stans* (Yellow bells flower, (TCS) Fig. 1e)] were randomly selected around University of Lagos Natural environment,longitude and latitude (6.5190° N, 3.3972° E) to analyse their analytical potentials as acid base indicators in a bid to find a bio source indicator, create values for often and indiscriminately discarded natural materials and to deepen the chemistry knowledge of acid-base reactions in bio-indicators.

Figures 1: Verities of flowers (a-e)

II. Materials and Method

II.A. Materials

Brightly color flowers of *Hibiscus Rosa-sinensis* ; *Plumeria Alba*; *Bombax Buonopozense*, *Tabebuia Rosea*, and *Tecoma Stans* were obtained along Oduduwa way, Ransome Kuti road, Botanical garden and Tafawa Balewa way, respectively around University of Lagos, Longitude and Latitude 6.5190° N, 3.3972° E. The flowers were identified by a member of staff at the Herbarium, Botany department, Faculty of sciences, University of Lagos.

II.B. Methods

All reagents used herein were of analytical grades and used without further purification. The description below with respect to flowers shows the summary of procedure used in this study:

Collection → Identification → Drying → Communiting → Extraction → Characterization → Titration

After collection and identification of the flowers, they were sundried for three weeks, pulverized and communited using manual grinders. About 5g each of the pulverized flowers (BBP,TBR and TCS) and 2g of (PLA and RSN) were separately dissolved in 100 and 40ml respectively, of acetone, ethanol, distilled and de-ionized water (DDW), acetone/ethanol equal mixture and ethanol (67%)/distilled and de-ionized (33%) mixture (Edewor, 2001) for 48 hours. They were all simultaneously subjected to rigorous agitation using a laboratory shaker at 500 rpm for 30 minutes per day and subsequently left for a week.The resulting mixtures were filtered and filtrate with noted colours were kept in sample bottles for pH determination and further analysis (Fig 2). Few drops of each of the filtrates , methyl orange and phenolphthalein in hydrochloric acid, sodium hydroxide and ethanoic acid, respectively were carried out and color changes were noted. Acid-base titrations in triplicates using 25 cm^3 pippete size were then carried out for each of the indicators and average volume of acid used were calculated in each case. The bio indicators were concentrated by placing them in water bath till complete removal of the corresponding solvents (Fig. 3). Fourier Transform Infra Red (FTIR) analysis of concentrated bio-indicators which responded excellently and poorly were carried out. The overall results obtained were further analysed using statistical package for social scientists (SPSS) .

Figure 2: Bio Indicators

Figure 3: Concentrated Bio Indicators

III. Results and Discussion

The colors of the five different flower species in various solvents are tabulated in Table 3. The pH of all the bio- indicators, their corresponding colours in all acids and bases used and their end point color changes were also tabulated and compared with those of standard indicators such as methyl orange and phenolphthalein in Table 4. These aspects are important in acid-base titrations as color changes are pH dependant, (Tilekar *et al*., 2015) and to further scientifically theorise the reliability and accuracy of the bio indicators.

Table 3: Colors of flower extracts in some solvents media

S/N	Flower Species	Extract Colour in Different Solvents				
		Acetone	DDW	Acetone/ Ethanol	Ethanol/ DDW	Ethanol
1	BBP	Light Yellow	Dark Brown	Light Yellow	Dark Brown	Light Yellow
2	TBR	Light Green	Dark Brown	Light Yellow	Dark Brown	Light Yellow
3	TCS	Light Yellow	Light Yellow	Orange	Orange	Dark yellow
4	PLA	Dark Yellow	Dark Brown	Pale Yellow	Dark Brown	Light Yellow
5	RSN	Pale Yellow	Dark Brown	Pink	Pink	Light yellow

It is evidenced from Table 4 that some of the selected flower extracts are competitive candidates for acid base indicators since they changed colours in acidic and basic medium.This is a reflection of the work of Oloyede,1997 who posited as earlier said that any highly color fruit, vegetable, or flower petal has the potential for use as acid- base indicator.

RSN in ethanol, ethanol/DDW and DDW show resemblance with methyl orange with pink colouration in acidic medium while BBP in acetone, acetone/ethanol, DDW; TBR in all the five solvents; TCS in all the five solvents except acetone; PLA in all the solvents except acetone and acetone/ethanol also show some level of yellowish resemblace with methyl orange in basic medium. On the other hand, BBP in all solvents, TBR in all solvents except DDW,TCS in ethanol, PLA in all solvents except DDW and RSN in all solvents except ethanol/DDW and DDW show a color less resemblance with phenolphthalein in acidic medium and no color resemblance in basic medium.

Table 4: Colours of Bio indicators in Acid and Base media

S/N	Flower species	Solvents	Colours		
			Acid	**Base**	**End Point**
1	BBP	Acetone	color less	Light yellow	color less
2		Acetone/ Ethanol	color less	Light yellow	color less
3		Ethanol	color less	Clear solution	Nil
4		Ethanol/ DDW	color less	Light green	color less
5		DDW	color less	Pale yellow	color less
6	TBR	Acetone	color less	Light yellow	color less
7		Acetone/ Ethanol	color less	Light yellow	color less
8		Ethanol	color less	Light yellow	color less
9		Ethanol/ DDW	color less	Light yellow	color less
10		DDW	Yellow	Light yellow	color less
11	TCS	Acetone	Yellow	Pale green	color less
12		Acetone/ Ethanol	Yellow	Light yellow	color less
13		Ethanol	color less	Pale yellow	color less
14		Ethanol/ DDW	Pale Yellow	Light yellow	color less
15		DDW	Pale Yellow	Pale yellow	color less
16	PLA	Acetone	color less	Pale green	color less
17		Acetone/ Ethanol	color less	Pale green	color less
18		Ethanol	color less	Pale yellow	color less
19		Ethanol/ DDW	color less	Light yellow	color less
20		DDW	Pale yellow	Pale yellow	color less
21	RSN	Acetone	color less	Clear solution	Nil
22		Acetone/ Ethanol	color less	Pale green	color less
23		Ethanol	color less	Clear solution	Nil
24		Ethanol/ DDW	Pink	Pale green	color less
25		DDW	Pink	Pale green	color less
26	Methyl orange	DDW	Pink	Orange	pink
27	Phenolphthalein	Ethanol/ DDW	color less	Pink	color less

The 25 bio- indicators except BBP in ethanol, RSN in acetone and ethanol deviated strongly from the color less end point colouration of phenolphthalein. This typical colouless behaviour of phenolphthalein in acidic medum was also reported by Garba, 2012 where all the six flower extracts used such as Bougainvillea, Oleandar, Flamboyant, Chinese rose, Pumpkin and Dutchmann's pipe maintained colourlessness in acidic medium. However, no end point colouration was found to be similar with the yellow color end point using methyl orange.The color results further indicate dissimilarities in the strength and the likely variation in the pH of the bio- indicators. Changes in temperature, ionic strength, colloidal particles and organic solvents could be responsible for the observed variation (Skoog, 1994).

Parameters such as concentration of acid, base, volume of base pipetted and even basicity of acid were constant throughout the titration. However, the solvents used for the preparation of the bio-indicators are of varied solvents parameters,so also, the bio-indicators are of different pH. Table 5 shows the details of the solvent parameters and the values of the volume of acid used correspondingly. Polarity index or dielectric constant of solvent is a rough measurement of the solvent polarity (Owolabi, *et al*., 2016). The Hasen solubility parameter values are based on dispersion bonds(δD), polar bonds (δP) and hydrogen bonds (δH) (Abbott *et al*.,2008

and Hanse *et al*., 2007). They contain information about the intermolecular interactions with other solvents. These intermolecular interactions have been found to affect a number of chemical reactions and extraction (Horn and Matyjaszewski, 2013; Bini *et al*., 2008; Schleicher and Scurto, 2009) which are the cases in this study. For the blend of solvent (acetone/ ethanol and ethanol/water), the solvent parameters values have been taken as the weighted average of the corespnding neat solvent parameters.

A critical observation of the titre values obtained (Table 5) shows that with respect to the two synthethic indicators used, the titre values from the bio indicators are relatively competitive except for BPP in ethanol, RSN in acetone and RSN in ethanol which were complete aberration from others. PLA in ethanol, DDW and RSN in DDW responded closely to the titre values obtained from the synthethic indicators.

The parameters contained in Table 5 were statistically used to develop a regression model using SPSS statistical software at a confidence level of 95 %. Equation 1 presents the average titre value as functions of solvatrochromic and bio-indicator parameters. The polar and hydrogen components of the Hasen solubility parameters were excluded from the model due to redundancy.

$$y = 70.345 + 2.070x_1 - 5.207x_2 + 1.205x_3 \quad (1)$$

where y, x_1, x_2 and x_3 are average titre value, pH of indicator, dispersion and polarity index of solvents respectively.

Table 5: Solvents and Indicator Parameters of the bio indicator and their titre values

S/N	Flowers	Solvents	P.I[a]	HSP			pH of indicator	Titre Value (cm^3)
				δD	δP	δH		
1	BBP	Acetone	20.7	15.50	10.40	7.00	6.31	28.70
2		Acetone /Ethanol	22.63	15.65	9.60	13.20	5.38	27.20
3		Ethanol	24.55	15.80	8.80	19.40	4.96	-----
4		Ethanol/ DDW	42.85	15.70	11.18	26.96	5.63	28.30
5		DDW	80.00	15.50	16.00	42.30	5.02	29.00
6	TBR	Acetone	20.7	15.50	10.40	7.00	5.83	25.80
7		Acetone/ Ethanol	22.63	15.65	9.60	13.20	5.74	24.40
8		Ethanol	24.55	15.80	8.80	19.40	5.94	28.00
9		Ethanol/ DDW	42.85	15.70	11.18	26.96	6.20	28.60
		DDW						
10		DDW	80	15.5	16.00	42.30	5.35	29.30
11	TCS	Acetone	20.7	15.50	10.40	7.00	6.15	28.70
12		Acetone /Ethanol	22.63	15.65	9.60	13.20	5.97	30.80
13		Ethanol	24.55	15.80	8.80	19.40	5.94	28.00
14		Ethanol/ DDW	42.85	15.70	11.18	26.96	6.20	28.60
15		DDW	80	15.5	16.00	42.30	5.35	29.30
16	PLA	Acetone	20.7	15.50	10.40	7.00	6.35	28.90
17		Acetone /Ethanol	22.63	15.65	9.60	13.20	6.38	29.20
18		Ethanol	24.55	15.80	8.80	19.40	6.09	29.40
19		Ethanol /DDW	42.85	15.70	11.18	26.96	6.46	28.90
20		DDW	80	15.5	16.00	42.30	5.42	29.40
21	RSN	Acetone	20.7	15.50	10.40	7.00	6.65	-------
22		Acetone/ Ethanol	22.63	15.65	9.60	13.20	6.32	28.90
23		Ethanol	24.55	15.80	8.80	19.40	6.31	-------
24		Ethanol/ DDW	42.85	15.70	11.18	26.96	6.66	29.20
25		DDW	80	15.5	16.00	42.30	6.48	29.55
26	Me.O	DDW	80	15.5	16.00	42.30	7.89	29.50
27	Phen	Ethanol/ DDW	42.85	15.70	11.18	26.96	7.54	29.70

a = Pedro *et al*., 2007, P.I = Polarity Index , HSP = Hasen *Solubility Parameters*
δD = Dispersion, δP = Polar, δH = Hydrogen bonding, Me.O = methyl orange, Phen = Phenolphthalein

The positive signs in the models signify synergetic effects of factor while the negative sign indicates antagonistic effect (Owolabi, *et al*., 2017). It is observed from the model that the pH of bio-indicators followed by the polarity index of solvents have the largest positive effect on the average titre values while the dispersion component of the Hasen solubilty parameters showed the largest negative influence on the average titre value.Though its strong negative effects is reported herein for the first time, the positive influence of pH of indicators had already studded both stale and recent literature, for instance, Tilekar *et al*., 2015; Pimpodkar *et al*., 2014; Igidi *et al*., 2012; Venugopalar *et al*., 2011; Sandeep, 2009 and Oloyede, 1997. Similarly, for the case of polarity, Schleicher and Scurto (2009) reported, while studying the solvent effects in the synthesis of ionic liquid, that methanol, the most polar of the solvents used was found to be the slowest in terms of reaction rate. Owolabi *et al*., 2016 in their study of solution polymerization of styrene reported that solvent polarity enhances styrene conversion but though not the only

contributory factor. The present study also reveals a trend that requires further insightful analysis in our subsequent studies.

Table 6: Analysis of variance (ANOVA) for the regression model

Model	Sum of squares	DF	Mean square	F-value	Sig
Regression	287.945	3	95.982	1.059	0.387
Residual	1902.752	21	90.607		
Total	2190.697	24			

R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson	Cronbach's Alpha
.363[a]	.131	.007	9.51878	1.906	0.633

The analysis of variance (ANOVA) of the regression model (Table 6) showed value of R^2 = 0.363, indicating that the model can explain only 36.3 % of the data variation. Though, Le Man *et al*., 2010 reported that for a model to be adequate, value of R^2 should not be less than 0.75, the statistical analysis of the present study however shows some deviation. The deviation is reflected in the high predicted values of average titre value compared to the experimental when the polarity index of the solvent is high (ethanol/DDW and DDW). The model predicted 51.88 and 96.43 cm^3 average titre values instead of 28.30 and 29.00 cm^3 for the cases of ethanol/DDW and DDW, respectively. Interestingly, the same model predicted 27.64, 27.26 and 27.92 cm^3 instead of 28.70, 27.20 and 28.00 cm^3 for the cases of acetone, acetone/ethanol and ethanol, respectively. Therefore, the model is limited to solvents of relative low polarity index (ethanol, acetone, acetone/ethanol). Koocheki *et al*., (2009) however claimed that a large value of R^2 does not always imply that the regression model is a good one. Similarly, according to Rai *et al*., (2016), the difference between adjusted R^2 and predicted R^2 should be within 20 % to be in good agreement. This requirement is howerver satisfied in this study. The Durbin - Watson number which tests for autocorrelation in the residuals from the regression analysis shows a slightly positive correlation.The relative reliability of the model was also confirmed by the slightly high value of the Cronbach's alpha value.

Spectral Analysis: The infra red as depicted in Fig 4a-4b and Table 7 were used in this study to characterise the bio indicators that responded excellently and poorly to the acid base titration as compared to the synthetic indicators. The carboxylic acid (C= O) with strong intensity was detected in bio indicators with poor response (RSN in ethanol and acetone, respectively) but not in PLA in ethanol that responded excellently.The esters (C=O) with strong intensity was also detected in RSN in ethanol but not in RSN in acetone and PLA in ethanol.The N-H bend and OH carboxylic acid with strong-moderate and strong-moderate/broad intensity respectively, were detected in RSN in acetone, PLA in ethanol but not in RSN in ethanol.The detection of C=C benzene with variable intensity was observed in PLA in ethanol but not in RSN in ethanol and acetone, respectively.The C= C benzene stretching may depicts the excellent response of the PLA in ethanol bio indicator. This is evidenced in the report of Shen *et al*.,2015 where the C=C bond band of spectra was found in the neat methyl orange but absent in the modified methyl orange.Similarly, Chattopadhyay *et al*., 1989 report confirmed the presence of band at 1605 which is attributed to C = C vibration.

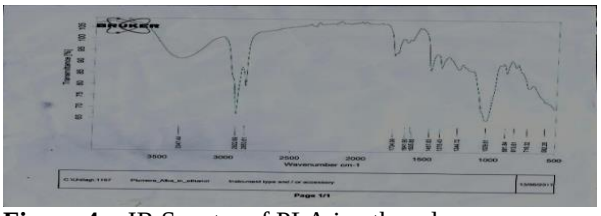

Figure 4a: IR Spectra of PLA in ethanol

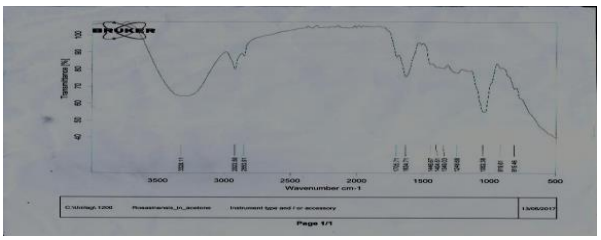

Figure 4b: IR Spectra of RSN in acetone

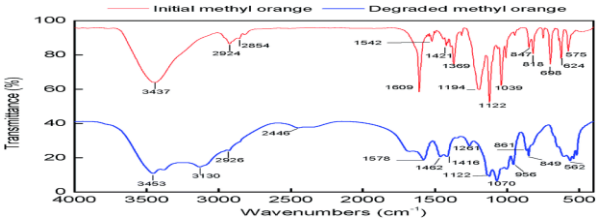

Figure 4c: IR Spectra of Neat and Degraded Me.O (Shen *et al*., 2015)

Table 7: Characteristics Bond Stretching Frequencies of Selected Bio-Indicators

S/N	Bio-Indicators	Solvents	Bond	Frequency (cm^{-1})	Intensity
1	RSN	Acetone/ Ethanol	C= C Benzene, **N-H Bend**, *Alkene C= C*	1623.52	v,**s-m,***v*
			sp^3 C-H, **OH Caeboxylic acid**	2929.98	v, **s-m,b**
2	TCS	Ethanol	N-H Bend, **Alkene C=C**, *C=O Amide*	1631.81	s-m,**v**,*s*
			sp^3 C-H, **OH Carboxylic acid**	2928.49	v, **s-m,b**
3	RSN	Ethanol	Alkene C=C, **C=O Amide**	1641.29	v,**s**
			C=O Carboxylic acid, C=O Ketone	1713.29	s
			C=O Aldehyde,C = O Ketone, C = O Ester	1736.66	s
			C-H Aldehyde, **sp^3 C-H**	2853.09	m,**v**
			sp^3 C-H	2921.96	v
4	RSN	Acetone	N-H Bend, **Alkene C=C**, *C=O Amide*	1634.71	s-m,**v**,*s*
			C=O Carboxylic acid, C=O Ketone	1705.71	s
			C-H Aldehyde, **sp^3 C-H**	2853.91	m,**v**
			sp^3 C-H, OH Carboxylic acid	2923.558	**v**,s-m,b
5	BBP	Ethanol/ DDW	N-H Bend	1601.08	s-m
			C=O Carboxylic acid	1709.26	s
			C-H Aldehyde, **sp^3 C-H,** OH Carboxylic acid	2853.85	**m**,**v**,s-m,b
			sp^3 C-H, OH Carboxylic acid	2923.5	**v**, s-m,b
6	PLA	Ethanol	C= C Benzene, **N-H Bend**	1605.85	v,**s-m**
			Alkene C=C, **C=O Amide**	1641.90	v,**s**
			C=O Aldehyde,C = O Ketone,	1734.99	s
			C-H Aldehyde, **sp^3 C-H,** OH Carboxylic acid	2853.61	**m**,**v**,s-m,b
			sp^3 C-H, OH Carboxylic acid	2922.69	**v**, s-m,b

s = strong, m = moderate, v = variable, b = broad

IV. Conclusion

A regression model limited to bio-indicators obtained from solvents of low polarity index (<25) to predict the average titre values of acid- base titration (strong acid and strong base) has been generated.PLA in ethanol bio indicator among others has also been found as an excellent replacement for either methyl orange or phenolphthalein synthetic indicators.This is as a result of the good agreement between their average titre values when they were used for the acid- base titration. The presence of the spctra band at 1605 which is attributed to C = C vibration was also attributed to the excellent response of PLA in ethanol during the titration.

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