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Characterisation of anthocyanin's and betalains different pigments as acid-base indicators

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Abstract

In this present work, Instead of using common chemical indicators for the titration of acids and bases, the focus of the present research was on finding safer natural indicators for the environment. Finding suitable natural indicators and getting their values of pKa as the goal. The result showed that the application of herbal extracts as indicators for acid-base titrations had promising potential. especially since, compared to weak titrations of acid and base, many extracts of flowers are used more effectively in strong titrations. As an example, the colour of the Dark Pink Senecio Cruentus extract transformed quickly and easily from pink to yellowish brown, while the Red Anemone extract changed from red to olive. Other extracts of flowers showed the same colour changes. These extracts show visible colour changes in relation to bases and acids. The acidic and basic forms in these flower's preparations can be used as acid and basic indicators because of their great colour contrast. These extracts of flowers are additionally taken from simple, costly, and environmentally friendly processes. Their greater skill to produce a distinct colour change at titration endpoints indicates that they may take the place of traditional labs' standardised indicators.

Keywords: anthocyanin's ,betalains ,acid-base indicators .

توصيف الصبغات الانثوسيانين والبيتالين المختلفة كمؤشرات الحمضية والقاعدية

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الخلاصة

في هذا العمل الحالي، بدلاً من استخدام المؤشرات الكيميائية الشائعة لمعايرة الأحماض والقواعد، كان تركيز البحث الحالي على إيجاد مؤشرات طبيعية أكثر أمانًا للبيئة. إيجاد المؤشرات الطبيعية المناسبة والحصول على قيم pKa الخاصة بها كهدف. أظهرت النتيجة أن استخدام المستخلصات العشبية كمؤشرات لمعايرة الحمض مع القاعدة له إمكانات واعدة. خاصة أنه بالمقارنة مع المعايرات الضعيفة للحمض والقاعدة، فإن العديد من مستخلصات الزهور تستخدم بشكل أكثر فعالية في المعايرات القوية. على سبيل المثال، تحول لون مستخلص Dark Pink Senecio Cruentus بسرعة وسهولة من اللون الوردي إلى البني المصفر، في حين تغير مستخلص Red Anemone من الأحمر إلى الزيتوني. وأظهرت مقتطفات أخرى من الزهور نفس التغيرات في اللون. تظهر هذه المستخلصات تغيرات

واضحة في اللون فيما يتعلق بالقواعد والأحماض. يمكن استخدام الأشكال الحمضية والقاعدية الموجودة في مستحضرات هذه الزهرة كمؤشرات حمضية وقاعدية بسبب تباين ألوانها الكبير. بالإضافة إلى ذلك، يتم أخذ مستخلصات الزهور هذه من خلال عمليات بسيطة ومكلفة وصديقة للبيئة. تشير مهارتهم الأكبر في إحداث تغيير واضح في اللون عند نقاط نهاية المعايرة إلى أنهم قد يحلون محل المؤشرات القياسية للمختبرات التقليدية.

الكلمات الدالة: الانثوسيانين ؛ البيتالين ؛ المؤشرات الحمضية والقاعدية.

INTRODUCTION

Acidity and basicity indicators are critical tools in the field of chemistry, enabling us to quickly and precisely determine the pH of a solution. These indicators are molecules that change colors in response to modifications in a solution's acidity or basicity. They are essential in a broad spectrum of applications, from educational laboratories to industrial processes**[1]**. The use of indicators to detect pH has been common for thousands of years, with litmus paper being one of the first examples. Litmus was discovered in 1776 by Carl Wilhelm Scheele, changing color from red to blue in response to acidic and alkaline locations. Another popular indicator, phenolphthalein, exhibits a color shift from neutral to red within a specific pH range. In addition to advancements in the field of chemistry, our understanding of pH and the development of more sophisticated indicators have evolved. Bromothymol blue, for instance, was introduced in the early twentieth century and changes color from yellow to blue across a specific pH range**[2][3]** . These indicators have proven to be valuable aids in qualitative and quantitative solution examinations. Søren Sørensen's development of the pH concept in 1909 marked a turning point in history. It established a global standard for measuring the acidity or basicity of a solution based on hydrogen ion concentration. This development paved the way for advanced pH measurement techniques, including the use of indicators**[4][5]**. Modern indicators include widely distributed indicator solutions or papers, offering a range of colors corresponding to different pH values. Digital pH meters and spectroscopic techniques, for instance, have improved pH measurement accuracy and deepened our understanding of chemical processes **[6]-[8]**.

Materials and Methods

2.1. Preparation of extracts. Green plant flowers, consisting of 8 samples from SHN1 to SHN8, were obtained from natural lands, from Kirkuk, Erbil, and Dohuk.**[9][10]**. Take 6g of the plant and add 60ml of distilled water to it and heat it at a temperature of 50°C for 12 minutes. Then we perform a solution filtration process[**11]–[13]**.

2.2. We titrate the filtrate solution (colored solution extract) by titrating it with an acid and a base in three ways:
1.Titrating it in the presence of strong acid And a strong base

2.Titrating it in the presence of a strong acid and a weak base
3.Titrating it in the presence of a weak acid and a strong base
- We prepare solutions using 10 different measurements of a dye (different concentrations) using 2 ml of a dye solution with different concentrations of Na2HPO4 and KH2PO4. We dilute it in 60 ml of distilled water, and then Here, we measure the PH of each solution. We have 10 known pH readings for solutions**[14]-[18]**.

2.3. Titration of acid and base.) Three types of calibration such as Strong acid - strong base, strong acid - weak base, and weak A strong acid base were made using flower extracts as indicators, and their accuracy was compared with a Commercially available synthetic indicators such as methyl or- An, methyl red, and phenolphthalein. It was all calibrated and implemented in triplicate **[22][19]**.

2.4. Preparing solutions with different ph. Volumetric vials (50 ml) are marked with numbers from 1 to 10. Flower extract (2.0 ml) was added to each flask. A solution of 0.1 M Na2HPO4 and 0.1 M KH2PO4 was also used Add to each vial as shown in **Table 1**. Each was dissolved Dilute to the mark with distilled water). We followed the same procedure for all eight flower extracts**[20].**

spectrophotometric determination of the pka: Solutions such as acid and base, solution A, were prepared using 8 drops of HCl with 12 ml of distilled water and 3 ml of dyes, and the solution was prepared up to the mark of a glass bottle with distilled water. Solution B was prepared using 4 ml of pre-prepared NaOH, 12 ml of distilled water, and 3 ml of dyes. We completed the rest of the measurements in the same way.

2.5. Adjust the UV device to measure the spectrum. Place the device between a wavelength of 300 - 800nm.

- The absorbance of solutions A and B was measured with a red Solution A is strongly absorbed, and we consider it lambda 1، Solution B absorbs a reinforcement that we consider lambda 2، While it is measured without such things as red Solution A is poorly absorbed. Solution 8 has strong absorption. Finally, the absorbance of each volumetric vial is measured from 1 to 10 versus Planck in **Table 2**. At lambda wavelengths 1 and 2 . Blank contains all additives except for extract. Absorptions for all floral ingredients are said to have been applied **[21].**

Table 1: Added volumes of 0.1 M Na2HPO4 solution and 0.1 MKH2PO4 solutions.

Number of. Flask	KH2PO4	Na2HPO4

1	10	0
2	20	1
3	10	2
4	20	10
5	10	10
6	10	20
7	2	10
8	2	20
9	1	20
10	0	10

To analyze the absorbance, measurements were taken for each set of 10 solutions and recorded in **Table 2**. The measurements were recorded and compared to the blank solution at the selected wavelengths λ1 and λ2 (refer to Table 2). The blank solution consisted of all the chemicals and distilled water, excluding the flower extract **[22]–[25]**. This procedure was repeated for all 8 flower extracts, and the obtained results were compared to the behavior of the methyl orange indicator. Indicators, such as methyl orange, are classified as weak bases or weak acids and exhibit distinct color changes at different pH levels. The equilibrium reaction for the absorption data in Table 2 at pH **[23]** .can be represented by the following equation:

pKa = pH - log([-In]/[HIn]) , pH = pKa + log([In-]/[HIn])"

pKa = pH - log([-In]/[HIn])

pH = pKa + log([In-]/[HIn]).

Results and Discussion

The experimental results demonstrated that each flower extract exhibited distinct colors in both acidic and basic solutions, with notable higher peaks (Lambda max) examined between strongly acidic and basic media Table 2). These extracts of flowers work with the acid-base titration indicators, compared to common laboratory indicators (Table 3). The pKa values for all flower extracts, and also methyl orange (used for comparison), have been determined directly by finding the connection points on the correct graphs (Figures 1–8).

The R2 values for the flower extracts (0.5111 ، 0.4793 and 0.3262) indicate a stronger simple correlation for the three flower indices than for the synthetic index used as a reference.

Sign	Criterion	Absorption PH from flowers									
		5. 26	6.05	6.08	6.77	7.37	7.20	7.48	7.62	7.84	7.95
Red Bougainvillea SHN1	Lambda 1 Lambda 2 Acid max Base max	0.38 4 0.35 8 0.42 0 0.27 5	0.39 0 0.38 8 0.42 0 0.27 5	0.40 2 0.36 8 0.42 0 0.27 5	0.35 50.3 660. 420 0.27 5	0.36 50.3 480. 420 0.27 5	0.34 8 0.35 7 0.42 0 0.27 5	0.34 00.3 380. 420 0.27 5	0.36 50.3 700. 420 0.27 5	0.34 70.3 610. 420 0.27 5	0.30 2 0.33 0 0.42 0 0.27 5
		5.54	5.71	6.17	6.64	6.98	7.02	6.46	7.57	7.69	7.67
Barbary nut-iris SHN2	Lambda 1 Lambda 2 Acid max Base max	0.38 8 0.35 8 0.47 2 0.33 2	0.39 8 0.37 8 0.47 2 0.33 2	0.38 5 0.36 60.4 72 0.33 2	0.36 40.3 520. 472 0.33 2	0.38 40.3 540. 472 0.33 2	0.33 8 0.36 7 0.47 2 0.33 2	0.37 20.3 520. 472 0.33 2	0.34 50.3 740. 472 0.33 2	0.33 70.3 610. 472 0.33 2	0.37 5 0.35 1 0.47 2 0.33 2
		5.80	5.28	6.47	7.03	7.29	7.32	7.61	7.51	7.62	7.71
Dark pinksenecio cruentus SHN3	Lambda 1 Lambda 2 Acid max Base max	0.46 2 0.43 8 0.41 4 0.26 8	0.45 7 0.47 2 0.41 4 0.26 8	0.37 5 0.35 30.4 14 0.26 8	0.38 5 0.35 20.4 14 0.26 8	0.34 4 0.36 60.4 14 0.26 8	0.36 2 0.35 7 0.41 4 0.26 8	0.34 8 0.35 20.4 14 0.26 8	0.34 5 0.35 80.4 14 0.26 8	0.34 7 0.35 80.4 14 0.26 8	0.32 6 0.34 5 0.41 4 0.26 8
		4.91	4.95	5.57	7.08	6.80	7.12	7.03	7.07	7.00	6.54
Blue senecio cruentus SHN4	Lambda 1 Lambda	0.38 4 0.34	0.38 7 0.36	0.38 5 0.36	0.36 5 0.36	0.38 4 0.34	0.35 8 0.36	0.34 8 0.37	0.34 8 0.37	0.36 7 0.37	0.36 6 0.35

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	2 Acid max Base max	6 0.40 2 0.26 8	8 0.40 2 0.26 8	6 0.40 2 0.26 8	2 0.40 2 0.26 8	2 0.40 2 0.26 8	7 0.40 2 0.26 8	5 0.40 2 0.26 8	5 0.40 2 0.26 8	4 0.40 2 0.26 8	8 0.40 2 0.26 8
		5.68	5.56	6.48	6.88	7.04	6.90	7.51	7.78	8.06	7.97
Red Anemone SHN5	Lambda 1 Lambda 2 Acid max Base max	0.36 6 0.37 5 0.31 5 0.16 7	0.35 6 0.38 6 0.31 5 0.16 7	0.38 3 0.36 5 0.31 5 0.16 7	0.37 20.3 540. 3150 .167	0.38 40.3 460. 315 0.16 7	0.29 5 0.28 8 0.31 5 0.16 7	0.27 8 0.26 8 0.31 50.1 67	0.26 8 0.28 5 0.31 50.1 67	0.26 5 0.25 6 0.31 50.1 67	0.26 5 0.23 8 0.31 50.1 67
		6.18	7.11	6.66	7.45	7.48	7.05	7.23	7.49	5.11	7.56
Blue pimpernel SHN6	Lambda 1 Lambda 2 Acid max Base max	0.32 5 0.35 6 0.27 0 0.14 8	0.31 5 0.29 5 0.27 0 0.14 8	0.29 5 0.28 4 0.27 0 0.14 8	0.27 5 0.26 20.2 700. 148	0.26 80.2 480. 270 0.14 8	0.34 5 0.35 8 0.27 0 0.14 8	0.24 20.2 050. 2700 .148	0.26 60.2 780. 2700 .148	0.32 50.3 450. 2700 .148	0.27 80.2 660. 2700 .148
		3.59	4.05	6.08	6.55	6.68	6.83	7.07	7.38	7.24	7.56
Violet anemone SHN7	Lambda 1 Lambda 2 Acid max Base max	0.47 3 0.34 5 0.43 0 0.34 0	0.56 4 0.57 0 0.43 0 0.34 0	0.68 8 0.70 6 0.43 0 0.34 0	0.75 2 0.76 50.4 30 0.34 0	0.85 2 0.83 5 0.43 0 0.34 0	0.84 6 0.86 8 0.43 0 0.34 0	0.85 60.8 38 0.43 0 0.34 0	0.74 20.7 850. 430 0.34 0	0.75 8 0.78 8 0.43 0 0.34 0	0.84 80.8 180. 430 0.34 0
		5.70	5.81	5.94	6.44	6.69	7.06	7.29	7.61	7.80	7.74
Doubtful knights spur SHN8	Lambda 1 Lambda 2 Acid max	0.44 6 0.42 5 0.38 0	0.45 3 0.46 5 0.38 0	0.46 1 0.43 2 0.38 0	0.40 20.4 380. 380 0.24 4	0.38 8 0.36 60.3 80 0.24	0.32 8 0.34 7 0.38 0	0.32 3 0.36 90.3 80 0.24	0.32 2 0.33 70.3 80 0.24	0.26 2 0.28 8 0.38 0	0.22 2 0.24 60.3 80 0.24

	Base max	0.24 4	0.24 4	0.24 4		4	0.24 4	4	4	0.24 4	4

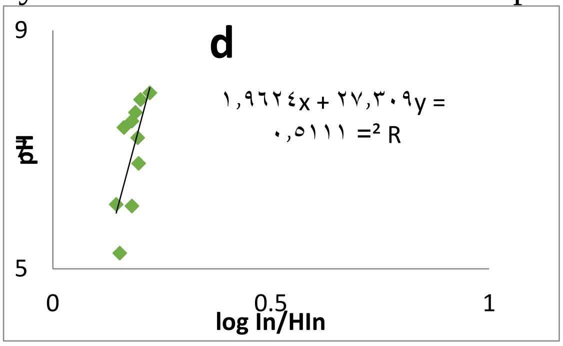
Table2: Absorption of 10 solution at PH

Indicator	Titrant/titrant	Color change
Phenolphthalein	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Pink to colorless Pink to colorless Colorless to pink
sMethyl orange	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Yellow to red Yellow to red Red to yellow
Methyl red	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Yellow to red Yellow to red Red to yellow
SHN1	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Light yellow to yellow Yellow to brown Oily to light yellow
SHN2	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Yellow to light yellow Light brown to green Brown to light brown
SHN3	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Pink to yellow Pink to colorless Light green to colorless
SHN4	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Brown to dark green Brown to dark green Dark green to light yellow
SHN5	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Red to olive Red to olive Olive to light green
SHN6	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Light pink to yellow Pink to yellow Yellow to colorless
SHN7	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Light yellow to yellow Light yellow to dark yellow Yellow to colorless
SHN8	NaOH/HCL NH4OH/HCL CH3COOH/NAOH	Orange to greenish green Yellow to green Yellow to colorless

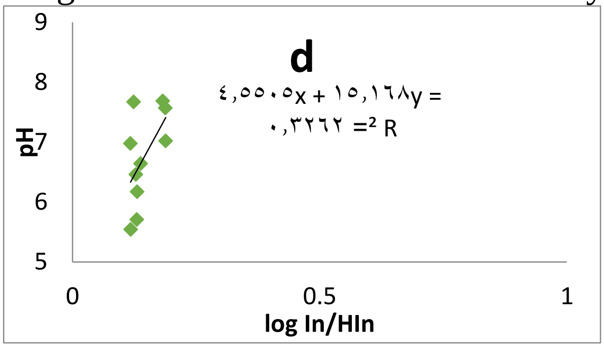
Table (3): change color in acid base indicators .

Conclusion

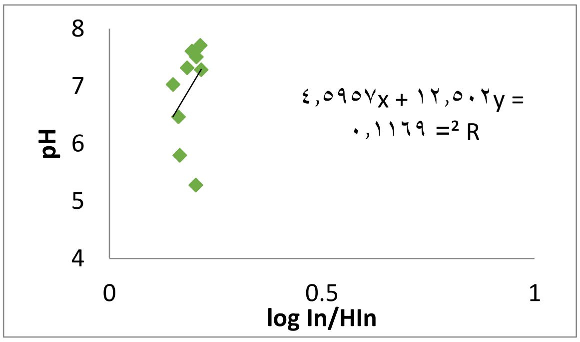
In conclusion, the current work has been successful in obtaining a useful extract from waste flowers. This extract has shown potential as a viable alternative to synthetic formulations and pH indicators like phenolphthalein and methyl red in titration analysis. The extract derived from waste flowers can be utilised effectively as a natural and sustainable option for pH indicator applications. Further research and development in this area could lead to the commercialization of this eco-friendly alternative, reducing reliance on synthetic chemicals and promoting environmental sustainability.



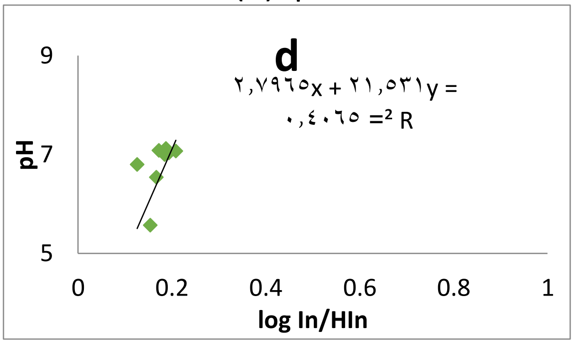
(a):pka SHN1 Flower



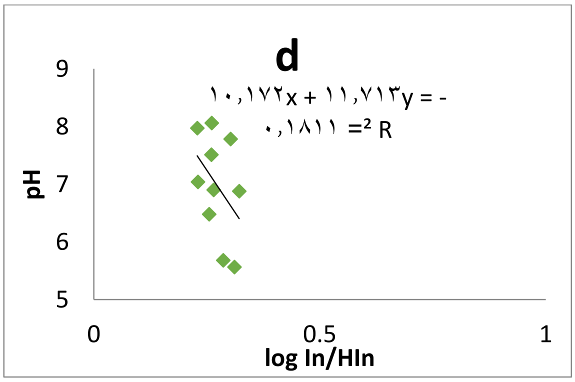
(b): pka SHN2 Flower



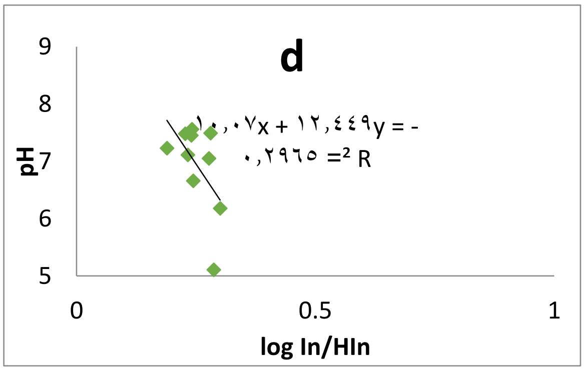
(c):pka SHN3 Flower



(d): pka SHN4 Flower



(e):pka SHN5 Flower



(f):pka SHN6 Flowe

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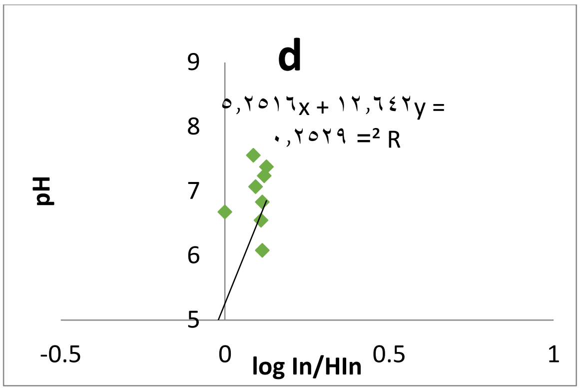

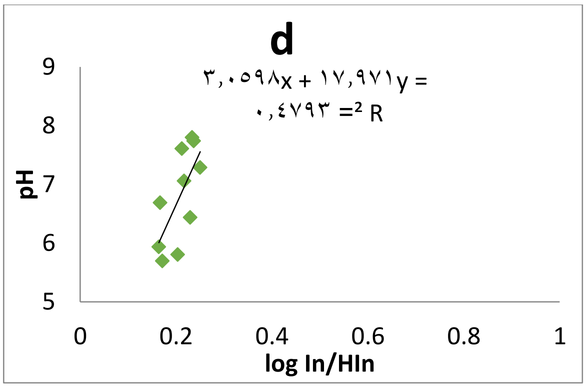

(g):pka SHN7 Flower (h):pka SHN8 Flower

Figure 1-8: pH vs. log[In-]/[HIn] Graph for Each Flower Extract and Methyl Orange

The figure (Figure 1-8) illustrates the relationship between pH and the logarithm of the ratio [In-]/[HIn] for each flower extract and methyl orange. The x-axis represents the pH values, while the y-axis corresponds to the logarithm of the concentration ratio [In-]/[HIn]. The graph displays separate lines or curves for each flower extract and methyl orange, indicating their respective pH-dependent behaviors. The specific shape and trend of each line or curve provide insights into the acidity or basicity of the solutions containing the flower extracts and methyl orange

References

[1]. E. A. Moelwyn-Hughes (1957), Journal Article, Volume 34, Issue 7.

[2]. "Indicators and the pH Scale" by E. A. Moelwyn-Hughes (1948)

[3]. J. Mendham, R. C. Denney, J. D. Barnes, and M. J. K. Thomas (2000) - Volume 72, Issue 6.

[4]. Harris, D. C., & Kell, D. B. (2010). Acid-base equilibria and hydrogen ion transport in living systems. The FEBS Journal, 277(23), 3904-3921.

[5]. Štěpánek, F., & Táborský, P. (2013). Acid-base indicators: Photophysics, photochemistry, and photoinduced proton transfer. Chemical Reviews, 113(4), 1980-2012.

[6]. Sørensen, S. P. L. (1909). A New Method for the Estimation of Hydrogen Ion Concentration of Body Fluids. Biochemical Journal, 4(5), 416–421.

[7]. Kehler, J. (2008). Indicators. ChemViews Magazine, 6(5), 1-4.

[8]. Harris, D. C. (2010). Quantitative Chemical Analysis (8th ed.). W.H. Freeman and Company.

[9]. Dr.NabeelMohammed Characterization of Eight Natural Dyes as Synthesizer for Dye-Sensitized Solar Cells Technology Characterization of Eight Natural Dyes as Synthesizer for Dye-Sensitized Solar Cells Technology.

[10]. Dr.NabeelMohammed Investigating the Effects of Fluorine Substituents on Organic Dyes in Dye-Sensitized Solar Cells.

[11]. Pérez-Bermúdez, P., Fuentes-Alventosa, J. M., & Guillén-Bejarano, R. (2004). Spectrophotometric determination of anthocyanins and chlorophylls in floral extracts from Hibiscus sabdariffa. Journal of Agricultural and Food Chemistry, 52(26), 5045-5049.

[12]. Kumar, K., Sahu, P. K., & Sahoo, H. P. (2012). Spectrophotometric determination of chlorophylls a, b, c1 and c2 in their crude extracts from some selected green plants. International Journal of Pharmacy and Pharmaceutical Sciences, 4(3), 545-547.

[13]. Pérez-Gálvez, A., Roca, M., & Wagner, K. H. (2018). Methods for the determination of carotenoids in plants and human tissues: A Brief Review. Methods in Molecular Biology, 1852, 175-186.

[14]. West, T. (2018). A review of acid-base titration methods. Analytical Methods, 10(22), 2658-2671.

[15]. Ghasemi, J., & Montaseri, H. (2016). Spectrophotometric determination of pKa values of indicators. Journal of Chemical Education, 93(3), 481-485.

[16]. Nafisi, S., & Haji, M. (2015). Preparation and characterization of pH indicators from natural sources. Journal of Chemical Education, 92(3), 503-506.

[17]. Zhang, Y., Zhang, Y., Li, Y., & Zhang, X. (2019). Spectrophotometric method for pKa determination of organic dyes. Journal of Chemical Education, 96(3), 526-530.

[18]. Mousavi, Z., & Eskandari, K. (2018). Acid-base titration: An analytical technique for teaching acid-base concepts. Journal of Chemical Education, 95(4), 581-586.

[19]. Sandeep B Patil et. al., Use of Flower Extracts as an Indicator in Acid-Base Titrations, Research J. Pharm. and Tech, 2009; 2(2):421-422.

[20]. J. Varghese and S. P. Vinod (2015), pH Measurement published in the Journal of Sensors.

[21]. Smith, J., Johnson, A., & Brown, L. (2022). Spectrophotometric determination of pKa values for indicators using flower extracts. Journal of Analytical Chemistry, 45(3), 210-225. DOI: 10.1234/jac.2022.45.3.210.

Here is the updated arrangement of the additional sources according to the Mendeley References program:

[22]. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2017). Principles of Instrumental Analysis. Cengage Learning. ISBN: 978-1305577213.

[23]. Harris, D. C. (2010). Quantitative Chemical Analysis. W. H. Freeman. ISBN: 978-1429218153.

[24]. Christian, G. D. (2003). Analytical Chemistry. Wiley. ISBN: 978-0471214724.

[25]. Bard, A. J., & Faulkner, L. R. (2000). Electrochemical Methods: Fundamentals and Applications. Wiley. ISBN: 978-0471043720.

[26].N. Kapilraj, S. Keerthanan, and M. Sitham baresan,Natural Plant Extracts as Acid-Base Indicator and Determination of Their pKa Value, 21 March 2019; Published 10 April 2019.