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Re-imagining Chemistry Laboratory Experiments by using Natural Pigments– Fostering Chemistry Students towards Sustainability

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Abstract

Green Chemistry and Sustainability are the two widely used concepts in the present-day Higher Education Chemistry curriculum. This paper issues a call for action for chemistry instructors to build stronger connections in the chemistry curriculum to sustainability. An acid-base titration is a commonly used experiment in undergraduate laboratories where synthetic indicators are generally used to locate the endpoint by observing the color change. In this paper, the ability of aqueous extracts of *Lagerstroemia speciosa* (locally known as Azar) as a natural indicator was investigated by using it instead of commonly used synthetic acid-base or neutralization indicators like Phenolphthalein, Methyl Orange, etc. The natural acid-base indicators are non-toxic, easily available, cost-effective, and environmentally benign. This inquiry-based investigation project can raise the standard of chemistry education while preserving local resources that can be used to address today's sustainability challenges. Thus, by considering several interconnected challenges to identify sustainable solutions that can be adopted in both higher secondary school and undergraduate chemistry education, such studies will enrich students' understanding of chemistry.

Keywords: Green chemistry**;** Sustainability; Acid-base titration; Synthetic indicators; Natural indicators; Chemistry education.

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1. Introduction

Sustainability is a contemporary concept that helps people understand the simultaneous harmonization of mankind's needs with the needs of the environment, and it can only be achieved through education. There is little doubt that human activity has a pervasive and growing impact on the environment and human health.

Chemistry is often considered the "Central Science," which plays a pivotal role in fulfilling the needs of human society in a more sustainable way. Therefore, there is a need to apply green and sustainable approaches and practices, more specifically in chemistry education at all levels, to reduce the impact on the environment and human health.

In 2015, the United Nations (U.N.) issued the 2030 agenda, wherein it defined seventeen Sustainable Development Goals (SDGs) to be achieved by the year 2030,

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amongst them SDG 4, "quality education," considers education for sustainable development [1], which in turn also helps in achieving other SDGs. An evaluation of the United Nations Environment Program (UNEP), particularly the Global Chemical Outlook II (GCO II), gives importance to Chemistry Education [2]. The GCO II suggests that since Chemical Science is linked either directly or indirectly to almost all the SDGs, Chemistry Education becomes more important for achieving SDGs at all educational levels, starting from school education [2,3]. Recent studies reported the importance of systems thinking in chemistry education in addressing the global challenges for sustainable development [4-7].

This results in the incorporation of Green Chemistry in the chemistry curriculum for the undergraduate level [8-10]. Though green chemistry is introduced in the undergraduate chemistry curriculum, it becomes equally important to include sustainability in chemistry education so that the students can develop a deep consciousness of the importance of sustainability strategies in chemistry research and industry. At the same time, it can also relate the learning outcome of chemistry education to the cross-disciplinary and societal interfaces. [11,12]. It therefore becomes important to teach the aims and objectives of Green Chemistry in relation to sustainability to younger students not only theoretically but also practically to meet the SDGs. However, integrating sustainability in Chemistry Education has many obstacles and challenges, which are very well documented by Jane *et al.* [13].

As it is fully recognized that experimental activities have a distinctive and crucial role in achieving positive learning outcomes, green laboratory experiments have already been introduced in undergraduate courses. However, the students did not find laboratories helpful in learning Green Chemistry completely since they followed the procedure described in the study materials recommended by the concerned universities/colleges. This indicates that it is not enough for students to practice Green Chemistry just by doing experiments in the laboratory. They must also engage themselves in the learning process of Green Chemistry through some simple inquiry-guided investigatory projects.

Acid-base titration in laboratory classes is a basic concept explained to undergraduates where indicators are used to locate the endpoint in the titration process. The acid-base indicators or neutralization indicators are organic weak acids/bases, but their conjugate bases/ acid forms have distinct colors. Many synthetic indicators, such as Methyl Red, Phenolphthalein, and Methyl Orange, have been commonly used in academic laboratories. Besides the adverse effects of these indicators on human health, these also seem to affect the bodies of water. Synthetic indicators used frequently during practical sessions in academic laboratories are washed down the sink since undergraduate college laboratories in most of the educational institutions in this part of India do not have a better disposal system, so it is directly put in the sink, which gets into the surface and underground water systems. These accumulate with time, which causes a reduction in the penetration of light and oxygen content in water, making aquatic life very difficult. Excess Phenolphthalein causes discoloration as well as elevated acidity, resulting in the death of fish [14]. Jadhav *et al.* reported that the use of Phenolphthalein deteriorates the water quality of fish tanks by increasing the Biological Oxygen Demand (BOD) and hardness and reducing the Dissolved Oxygen (D.O.), pH, and alkalinity [15]. Methyl Red was reported to have mutagenic effects besides severe consequences on Human Health, such as cancer, and has a detrimental environmental effect [16]. Methyl Orange is a toxic, mutagenic, or carcinogenic pollutant in water bodies. The presence of Methyl Orange in water (if not treated) causes hazards to living organisms, as reported by Alabbad [17]. Therefore, the treatment of such wastewater containing dyes is necessary before discharging them into the aquatic ecosystems. But instead of going for treatment after the generation of pollutants, it will be more beneficial if we use alternative ecofriendly indicators or substances which will be safer for both human beings as well as different life forms of water bodies [The First Principle of Green Chemistry: Prevention of waste]. This encourages the use of different natural indicators that originate mainly from plant sources. Amongst them, flavonoids like anthocyanin (glycosylated anthocyanidin) have been widely used due to their color-changing properties in the broad range of pH.

The name anthocyanin is derived from the Greek word *Anthos*, which means flower, and *Kyanos* (blue) [18]. Anthocyanins are ubiquitous, natural, bioactive, water-soluble phenolic plant pigments with a skeleton (Fig. 1) of 15 carbon atoms containing three rings-two phenolic (B and C) and one pyran (A) rings.

Anthocyanins produce different colors in fruits, flowers, leaves, stems, roots, and seeds ranging from orange, red, blue, and blue-violet [19-21]. Anthocyanins mainly occur as glycosides of their respective aglycone anthocyanidin chromophores with a sugar moiety.

Fig. 1. Basic structure of anthocyanidin.

There are about seventeen anthocyanidins found in nature, but only six (cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin) are the most common [22] (Table 1).

Table 1. Major anthocyanidins in plants.

Anthocyanidin	R_1	R_2	Colors produced
Delphinidin	OH	OH	Purple, Mauve and Blue
Petunidin	OH	OCH_3	Purple
Malvidin	OCH_3	OCH_3	Purple
Peonidin	OCH_3	H	Magenta
Cyanidin	OH	H	Magenta and Crimson
Pelargonidin	H	H	Orange salmon

The conjugated bonds of anthocyanins are associated with red, blue, or purple coloration in almost all plant kingdoms. The color also depends upon the number of hydroxyl (-OH) and methoxy (-OCH_3) groups. Studies reported that if there are more hydroxyl groups, the color goes towards a bluish shade, and as the number of methoxy groups increases, the color tends to become more reddish. An increase in the hydroxylation on the B-ring results in a bathochromic shift (a shift of λ_{max} toward longer wavelengths), which gives a blue color [23-25].

With this background, this simple inquiry-based investigative project is assigned to increase student's motivation toward sustainability.

The present study was assigned to the honors students as a part of their undergraduate course. The objectives of this study are:

1) Understanding the need for substitutes for environmentally and health-hazardous synthetic indicators
2) Developing effective, eco-friendly, locally available, and economic indicators for acid-base titration

The assigned study also helps the students gain insights into the way in which the chemistry conceptual knowledge can be used to find practical solutions in developing a new type of eco-friendly and locally available indicator for acid-base titration, which will also facilitate the young students in realizing the needs of SDGs.

As students have prerequisite knowledge about the acid-base concept as well as laboratory acid-base titration using indicators such as Phenolphthalein, Methyl Orange, and Methyl Red, which are synthetic so, this study was assigned to the students to motivate the students towards sustainability and Green Chemistry.

It is on this premise that this study sought to investigate the suitability of the extract of flowers of *Lagerstroemia speciosa* (locally known as Azar) as indicators in acid-base titrations. It is reported that the use of the methanolic extract of flowers of Lagerstroemia speciosa as an indicator, but our study attempts to use the aqueous extract instead of methanolic as Methanol (MeOH) is highly flammable and an acute toxicant [26].

2. Experimental

2.1. *Chemicals and apparatus*

Simple chemicals and apparatus easily available in the Undergraduate Chemistry Laboratory, like NaOH, NH_3, HCl, CH_3COOH, mortar and pestle, weighing balance, beakers, conical flask, funnel, burette, pipette, spatula, stirrer and filter paper (Whatman 40).

Fig. 2. Lagerstroemia speciosa Flower.

2.2. *Collection of flowers*

Flower petals of *Lagerstroemia speciosa* (Family Lythraceae) were collected from the college premises of Pragjyotish College, Guwahati-9, Assam, India.

2.3. *Preparation of aqueous extract of Lagerstroemia speciosa*

The fresh light violet-colored flower petals were separated from the whole flower and cleaned thoroughly by washing with water and then with distilled water to remove dirt. The petals were cut into 2-3 pieces, placed in a mortar, and then ground using the pestle.

Fig. 3. LSAE

5.0 g ground flower was then transferred into a conical flask containing 100 mL distilled water, corked tightly, and stirred for about 10 min. The violet-colored solution mixture was then filtered into a clean, labeled bottle with a stopper. The obtained Lagerstroemia Speciosa Aqueous Extract (LSAE) was then used as a natural indicator in different weak and strong Acid-Base titrations instead of synthetic indicators like Phenolphthalein and Methyl Orange.

2.4. *Experimental procedure*

The whole experimental work was done by using the same volumetric set of glassware for titrations. The calibration of apparatus like burettes, pipettes, and volumetric flasks was carried out accordingly [27].

The acid-base titrations were performed between a strong acid (0.1 M HCI) against a strong base (0.1 M NaOH), strong acid (0.1 M HCI) against weak base (0.1 M NH_3), weak acid (0.1 M CH_3COOH) against strong base (0.1 M NaOH) and weak acid (0.1 M CH_3COOH) against weak base (0.1 M NH_3) using LSAE as ecofriendly, natural indicator. Similar titrations were run with the standard synthetic indicators Phenolphthalein and Methyl Orange. The results obtained in both cases were compared.

Each titration was carried out with 10.0 mL weak or strong base solution and either by using two drops of specific synthetic indicators or 1 mL of the LSAE separately. For each acid-base titration, eight sets of titrations were conducted using LSAE (eco-friendly acid-base indicator) and synthetic indicators. Each titration was carried out three times, and the results are presented as mean ± Standard Deviation.

3. Results and Discussion

Before carrying out the titrations, the volumetric glassware was calibrated so that systematic and random errors were avoided. The outcome of this part of the work helps the students gain practical skills in measuring accurate and precise volumes, which are essential for maintaining the data quality and the data reliability.

In our study, the dried grounded flower petals were just put in distilled water and stirred for only 10 min without heating. This extraction procedure is considered to be ecofriendly as water is invariably a green solvent. We avoided the use of hazardous organic solvents like methanol and acidic conditions. This procedure may be considered as effective as well as efficient, as one of the previous studies reported that the extraction of anthocyanins from water could be done with a soaking step for several hours [28]. In some cases, heating was also necessary for extraction [29], but in our study, the extraction was carried out for 10 minutes without heating, which gives an advantage in terms of time. As extraction with higher temperatures and long extraction time result in the deterioration of anthocyanins [30,31], so heating was avoided in our extraction process.

The first steps of this study involve sample collection and extraction of anthocyanins from flowers, combining chemistry and biology to provide students with interdisciplinary learning experiences. Further, this study also introduces students to the basic principles of extraction and the various effects of different extraction parameters in plant pigment research. This type of study gives the students an opportunity to understand the relevance of basic chemistry principles like the polarity of solvents, the effect of pH in acid-base titration, and solubility, which in turn will help the students in the near future to carry out studies with different parts of the plants.

In the present study, extraction was carried out with water, so it can be concluded that the LSAE contains anthocyanin instead of anthocyanidin. Anthocyanin aglycone has higher solubility in alcohol than its glucoside, whereas glycosylated anthocyanin is highly soluble in water [32]. The polyphenolic structure of anthocyanin adds a hydrophobic characteristic to it and makes it soluble in organic solvents. The exclusive anthocyanin aglycone (Anthocyanidin) in our study may be delphinidin as the color of the extract is violet owing to the presence of three -OH groups attached to its B-ring and is also more soluble in water [32] (Table 1).

The underlying objective of the present study, designed for undergraduate students, was also to demonstrate how the substituents on an π-conjugated system can affect the color of the polyphenolic compounds. After the completion of the work, students were expected to gain a deeper understanding of the relationship between the color of an organic compound and its structure, which is also incorporated into the theory paper.

The violet-colored LSAE was tested with acid (strong and weak) and base (strong and weak) solutions, i.e., with $NaOH$, NH_3, HCl, and CH_3COOH (Fig. 4).

Since the extracts produced pronounced color changes (Fig. 4), they can be used to detect the acidity or alkalinity of acidic and basic solutions. This study also reveals the presence of Anthocyanin in LSAE extract.

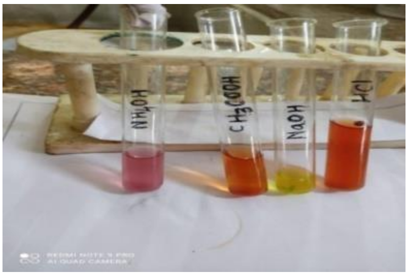

Fig. 4. LSAE in NH_3, CH_3COOH, NaOH, HCl.

During the activity, the students developed the skill of preparing the indicator from locally available colored flowers. In our study, the students prepared an aqueous extract of flowers of Lagerstroemia Speciosa, which is easily available on the institute premises and could visualize that the color changes depending on the different strengths of acidic/basic solution and hence is used as a natural acid-base indicator. Though the hazardous effect of synthetic indicators is mentioned in the introductory lab class, the health effects of synthetic indicators on the human and animal kingdom are understood more through the literature survey. Thus, by employing safer reagents and reducing the production of hazardous and toxic waste, this work may represent a novel approach to understanding chemistry from a sustainable perspective.

The volume of the acids needed to neutralize the bases, along with the color changes in both LSAE and synthetic indicators, are presented in Table 2.

Table 2. Comparison of Titrant reading with LSAE and Synthetic Indicators.

Titrand/titrant	Indicator	Volume of Titrant mean ± S.D.	Color Changes
NaOH/HCl	LSAE	9.30 ± 0.06	olive green to red
	Phenolphthalein	9.50 ± 0.05	pink to colorless
NH_3/HCl	LSAE	9.20 ± 0.03	pine green to red
	Phenolphthalein	9.30 ± 0.04	pink to colorless
NaOH /CH_3COOH	LSAE	9.50 ± 0.02	red to olive green
	Phenolphthalein	9.60 ± 0.05	pink to colorless
NH_3/CH_3COOH	LSAE	7.70 ± 0.04	pine green to red
	Methyl Orange	9.20 ± 0.03	yellow to red

The results in Table 2 show that the endpoints obtained with LSAE are very close to those obtained using synthetic indicator Phenolphthalein except in weak acid and weak base with methyl orange. This reveals that the LSAE may be considered a suitable indicator for all the titrations except for weak acid-weak bases. Since the primary component of LSAE is the aqueous extract of phytochemicals called anthocyanins, which have the potential to be used as therapeutic substances, aquatic life will not be negatively impacted if they are discharged into the water body.

Since the LSAE extract contains anthocyanin pigments that show distinctive colors in different acid and basic solutions. An earlier study reported that the anthocyanin pigment shows distinctive color changes in different pH ranges due to the change in the molecular structure of Anthocyanin [24]. The four different major forms of anthocyanin, which show colors based on pH, are depicted in Fig. 5.

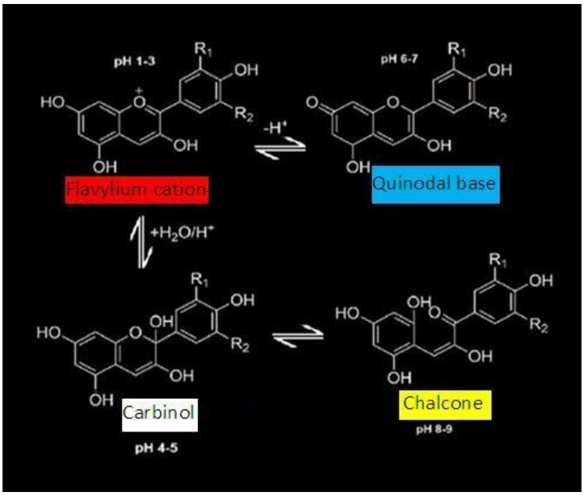

Fig. 5. Different forms of anthocyanin in different pH.

The different forms are red flavylium cation (pH range 1-3), colorless carbinol (pH range 4-5), Blue quinodal base (pH range 6- 7), and yellow chalcone (pH range 8-9). The color of LSAE is violet, which may be because of a nearly equal mixture of flavylium (red) and quinoidal (blue) forms of anthocyanin. In our study on the addition of CH_3COOH and HCl, the pH was lowered, and the color of the solution became red due to the presence of more flavylium cation. On the contrary, when the pH was raised by the addition of NaOH and NH_3 solutions, the color became green since both quinoidal (blue) and chalcone (yellow) forms were present.

Scientists in academia and industry have now been forced to "think chemistry differently" in order to create new environmentally friendly and sustainable procedures and methods for chemistry that are "benign by design"[33], so this type of approach can help the students in designing their laboratory procedure in a sustainable way which also gives students more pride and ownership of work.

4. Conclusion

Our study shows that there is a need to improve and re-imagine the current Chemistry Education to find a way to live with nature; thereby, our finding is a small example of redesigning chemistry laboratory experiments to connect the curricular content and address both the environmental and societal interfaces with Chemistry Education meaningfully. While there are numerous barriers to incorporating sustainability into chemistry education, the current study makes an effort to get over some of them by utilizing naturally occurring resources that are readily available locally. Apart from these, our study, which was designed by re-imagining the chemistry laboratory experiments, can also be viewed as a positive contribution to the knowledge base in Chemistry Education. Our study indicated an alternative way of equipping the laboratory with natural, eco-friendly acid-base indicators extracted from flowers or different parts of the plants that are around nature. Thus, it can be inferred that this kind of inquiry-based investigatory study will help students approach the challenges that have emerged for society with a more chemistry-infused mindset. Here, students are exposed to both tools and skills that can be used to experiment in a more sustainable way. The students are also encouraged to think and compare the commonly used reagents, solvents, and conditions in any kind of experiment of other courses. In the long term, it could be expected that students experiencing such experiments may use the lens of Green Chemistry in other courses or their own sustainability behaviors. Thus, it can be concluded that this type of study can be considered as a catalyst for fostering chemistry students towards sustainability.

References

1. Transforming Our World: The 2030 Agenda for Sustainable Development (United Nations, 2015).
2. United Nations Environment Program (UNEP) (Global Chemicals Outlook II, 2019).
3. V. Zuin, I. Eilks, M. Elschami, and K. Kummerer, Green. Chem, **23**, 1594 (2021). https://doi.org/10.1039/D0GC03313H
4. A. R. Szozda, P. G. Mahaffy, and A. B. Flynn, J. Chem. Educ. **100**, 1763 (2023). https://doi.org/10.1021/acs.jchemed.2c00955
5. D. J. C. Constable, iScience **24**, 103489 (2021). https://doi.org/10.1016/j.isci.2021.103489
6. L. Mammino, J. Chem. Educ. **96**, 2881 (2019). https://doi.org/10.1021/acs.jchemed.9b00302
7. P. Mahaffy, S. Matlin, M. Potgieter, B. Saha, A. Visa et al., Chem. Int. **43**(4), 6 (2021). https://doi.org/10.1515/ci-2021-0402
8. J. A. Haack, J. E. Hutchison, M. M. Kirchhoff, and I. J. Levy, J. Chem. Educ. **82**, 974 (2005). https://doi.org/10.1021/ed082p974
9. J. Andraos and A. P. Dicks, Chem. Educ. Res. Pract. **13**, 69 (2012).
10. T. J. Collins, J. Chem. Educ. **72**, 965 (1995). https://doi.org/10.1039/C1RP90065J
11. B. Braun, R. Charney, A. Clarens, J. Farrugia, C. Kitchens et al., J. Chem. Educ. **83**, 1126, (2006). https://doi.org/10.1021/ed083p1126
12. I. Eilks and F. Rauch, Chem. Educ. Res. Pract. **13**, 57 (2012). https://doi.org/10.1039/C2RP90003C
13. E. Jane, V. Aurelia, B. Saha, A. M. Stephen, G. M. Peter et al., J. Chem. Educ. **98**, 1061 (2021). https://doi.org/10.1021/acs.jchemed.1c00284
14. C. Boyd, C. Tucker, and V. Viriyatum, North Am. J. Aquacul. **73** (2011). https://doi.org/10.1080/15222055.2011.620861
15. U. Jadhav, A. Rajeshirke, and P. Kumari, Int. J. Emerg. Technol. Innov. Res. **910**, b671 (2022).
16. K. Sharma, S. Pandit, A. S. Mathuriya, P. K. Gupta, K. Pant, and D. A. Jadhav, Water **15**, 56 (2023). https://doi.org/10.3390/w15010056
17. E. A Alabbad, Open. Chem. J. **7**, 16 (2020). https://doi.org/10.2174/1874842202007010016
18. X. Sui (New York: Springer, 2016).
19. S. Gupta (India, Springer, 2006).
20. D. Pascual-Teresa, D. A. Moreno, and C. García-Viguera, Int. J. Mol. Soc. **11**, 1679 (2010). https://doi.org/10.3390/ijms11041679
21. B. Salehi, J. Sharifi-Rad, F. Cappellini, Z. Reiner, D. Zorzan et al., Front Pharmacol. **11**, 1300, (2020). https://doi.org/10.3389/fphar.2020.01300

22. T. C. Wallace and M. M. Giusti, Foods **8**, 550 (2019). https://doi.org/10.3390/foods8110550
23. D. Verma, N. Sharma, and U. Malhotra, Pharma Innov. J. **12**, 1366 (2023). https://doi.org/10.22271/tpi.2023.v12.i7p.21416
24. L. Cabrita, T. Fossen, and O. M. Andersen, Food. Chem. **68**, 101 (2000). https://doi.org/10.1016/S0308-8146(99)00170-3
25. S. Patra, P. N. Makhal, S. Jaryal, N. More, and V. R. Kaki, Int. J. Plant Based Pharm. **2**, 118 (2022). https://doi.org/10.62313/ijpbp.2022.22
26. M. Gupta and N. Yadav, World J. Pharm. Res. **9**, 2384 (2020).
27. J. Lembeck, NBSIR **74**, 461 (1974). https://doi.org/10.6028/NBS.IR.74-461
28. M. Paludo, R. Colombo, J. Teixeira, I. Hermosín-Gutiérrez, C. Ballus, and H. Godoy, J. Braz. Chem. Soc. **30**, 1506 (2019). https://doi.org/10.21577/0103-5053.20190047
29. R. Dibazar, G. B. Celli, M. S. L Brooks, and A. Ghanem, J. Berry. Res. **5**, 73 (2015). https://doi.org/10.3233/JBR-150100
30. P. Loypimai, A. Moongngarm, P. J. Chottanom, Food. Sci. Technol. **53**, 461 (2016). https://doi.org/10.1007/s13197-015-2002-1
31. I. Aprodu, S. A. Milea, E. Enachi, G. Rapeanu, G. E. Bahrim, and N. Stanciuc, Foods **9**, 1593 (2020). https://doi.org/10.3390/foods9111593
32. G. C. G. Vidana, Y. Y. Lim, and W. S. Choo, Front Plant Sci. **12**, 792303 (2021).
33. A. Satheesh, H. Usha, D. S. Priya, A. V. L. N. H. Hariharan, and M. V. V. Ramanjaneyulu, J. Sci. Res. **15**, 481 (2023). https://doi.org/10.3329/jsr.v15i2.60649