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(RESEARCH ARTICLE)

Use of *Ipomoea involucrata B.* flower as metallochromic indicator for complexometric titrations

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Abstract

The present work highlights the use of *Ipomoea involucrate B.* flower extract as a complexometric indicator in complex formation between the EDTA and the following metal solutions (Cu^{2+}, Ba^{2+}, Hg^{2+}, Fe^{3+}, and Bi^{3+}). *Ipomoea involucrate* is a species of the *Ipomoea* genus, belonging to the family Convolvulaceae. This natural indicator was extracted in 2M HCl at 100 ^{0}C for 30-40 minutes, cooled and the extract filtered and extracted with ethyl acetate and the pigment extracted with amyl alcohol which was separated and concentrated over a water bath. The flower extract was screened for its use as an metallochromic indicator at different pHs in metal solutions of Cu^{2+}, Ba^{2+}, Hg^{2+}, Fe^{3+}, and Bi^{3+}.This natural indicator from the results obtained gave sharp intense color change at the equivalence point of complex formation between the metals and the EDTA at both acidic and alkaline pHs in the metal solution of Cu^{2+} and Fe^{3+} and that with different amount of EDTA used in the titration, which increased as the pH value increased, while the *ipomoea* indicator gave sharp intense color change at the equivalence point of complex formation between the metals and the EDTA at alkaline pHs in the metal solution of Ba^{2+}, Hg^{2+} and Bi^{3+} at pH > 10. Natural flower indicators could take the place of the synthetic metallochromic indicators currently used in conventional laboratories because these flower extracts have excellent performance with sharp and intense color change in end points during the complexometric titrations and simple, cost-effective and environmentally friendly extraction methods.

Keywords: *Ipomoea*; Natural pigments; Anthocyanins; Synthetic indicators substitutes; Metallochromic

1. Introduction

The reproductive component of plants, the flower, is unique in both color and form. Flowers display an apparently limitless diversity of combinations in their spectrum of color, size, form, and anatomical arrangement. From tiny blossoms to enormous blooms, they come in all sizes [18]. While certain plants, like the poppy, magnolia, tulip, and petunia, produce their flowers singularly and are generally rather large and showy, other plants, like asters, snapdragons, and lilacs, produce their blooms in characteristic clusters called inflorescences that are often quite modest. The reproduction of the species through the formation of seed is the same function served by all flowers, regardless of variety [18].

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With stems up to 4 meters long, *Ipomoea involucrata B.* is an annual or perennial twining herb a strong twines that occasionally covers the surrounding plants. Hairy peduncles, leaves, stems, and big bracts. Up to 13 cm long, generally heart-shaped with a deeply cordate base, and hairy on both surfaces, frequently more so below. Flowers emerge from unique boat-shaped bracts and have funnel-shaped heads that range in color from white to pink [9]. The capsules are spherical and hairless. Involucrate: With an involucres, referring to the bract that encircles their inflorescence and has the shape of a boat. Along the edges of forests, frequently in secondary vegetation, on the sides of roads, and as a cultivating weed found throughout northern South Africa and all of tropical Africa.

The existence of pigments, which have a variety of crucial functions to perform wherever they are located, whether in plant or animal structures, is what gives nature its various colors. The origin of a pigment determines whether it is natural, synthetic, or inorganic. Living things like plants, animals, fungus, and bacteria produce natural pigments. Laboratories are where synthetic pigments are obtained. Organic molecules make up both natural and artificial pigments. Inorganic pigments can be obtained naturally or created artificially [11]. Anthocyanin exhibits color shifts from red to blue, changing from red in acidic settings to violet in neutral conditions to blue in alkaline ones [5]. The coexistence of several anthocyanins is another element that affects how colored flowers turn out. The six kinds of anthocyanins found in the flowers of Sarracenia L. were identified by Sheridian [16]. Numerous anthocyanins and co-pigments were found in both the fresh and preserved flowers in Puckhaber's [12] investigations on the flowers of 29 species of Hibiscus. According to Soltan [17], the use of plant blossoms as natural indicators exhibits good accuracy in determining the endpoint in neutralization and complexation titrations. Flowers and fruits frequently contain anthocyanins with catechol or pyrogallol groups in their structural makeup. These chromophores typically appear as glycosylated derivatives of cyaniding, delphinidin, and petunidin.

For a very long time, qualitative tests demonstrating the existence of a catechol group in plant anthocyanins have been conducted using reactions of metal ions like iron (III) and aluminum (III) with such flavylium complexes. The test relies on the observation of a color shift or bathochronic shift of the visible maximum wavelength upon addition of an aluminum ion, often in the form of AlCl3. According to Dangles [3], the complexation of anthocyanins with aluminum, which is highly common in plants, may have biological significance in the production of blue color in flowers. Later research [4] demonstrated that anthocyanins, both natural and synthetic, can form stable complexes with tiny, highly charged metal ions like gallium (III) and aluminum (III). They demonstrated how the anthocyanins complexation produces stunning changes, including significant hyperchromic and bathchromic shifts in the pigment's visible absorption, which could be used in quantitative research on the kinetics and thermodynamics of complex formation.

According to Flaschka [2], complexometric titrations are those in which a simple ion is converted into a complex ion, and the equivalence point is established either electrometrically or using metal indicators. In analytical chemistry, complexometric indicators are used in complexometric titration to show the precise moment when all the metal ions in the solution are sequestered by a chelating agent (most commonly EDTA. According to Scarpa [13], these indications are also known as metallochromic indicators.

The pH of the vacuole where anthocyanins and its derivatives are stored affects their color, which can range from red under very acidic conditions to purple-blue in intermediate pH conditions, until yellow-green in alkaline conditions. In addition to the pH, these flavonoids' color can also be influenced by the degree of hydroxylation or methylation pattern of the A and B rings, as well as by glycosylation pattern. Anthocyanins, flavones, and metal ions form complexes to give some plant pigments their hue. It should be noted that because of the variations in chemical structure that result from pH changes, anthocyanins are frequently utilized as pH monitors. According to Shah [14], blooms of the *Ipomoea aquatica* species, which are pink or pale lilac in color, exhibit a range of colors depending on the pH, including pink between 0 and 8.0 pH, green between 8.0 and 11.0 pH, and greenish yellow above 11.0 pH.

The purpose of this study was to determine whether the extract of *Ipomoea involucrata* might serve as a good natural metallochromic indicator replacement for synthetic metallochromic indicators and to introduce the practice of employing flower pigments as indicators in complexometric titrations.

2. Materials and method

2.1. Plant flower collection and identification

The flower samples were picked from the *Ipomoea involucrate* B. plant located at Nnamdi Azikiwe University Awka campus main gate along Awka – Enugu express way Anambra state. The flower was identified and taxonomically classified at Botany Department in Nnamdi Azikiwe university Awka by Dr. Achugbu Adaeze. The flowers were washed

severally with distilled water in the laboratory and then dried for 48 hours at 70 0C. The dried flowers were powdered in a grinder, put in polythene bottles wrapped in aluminum foil, and stored in a dark cupboard.

2.2. Extraction of pigment from ipomoea flower

A small amount (10g) of the dried flowers was heated in 2M HCl in conical flask for 30-40 minutes at 1000C. The cooled extract was then filtered and extracted with ethyl acetate. Then, the aqueous extract was heated at 80 0C for 3 min to remove the last traces of ethyl acetate. The pigment was then extracted with a small volume of amyl alcohol, which was pipette off and concentrated over boiling water bath.

2.3. Complexometric Titration

The partially purified anthocyanin mixture was used to test for the colour reaction with metals, using the prepared metal solutions of these metal ions $Cu^{2,+}$ $Ba^{2,+}$ Hg^{2+}, Fe^{3+}, and Bi^{3+} and their complex formation determined by direct titration with EDTA [8]. 20 ml each of 0.01 M $CuSO_2$, $BaCl_2$, $HgCl_2$, $FeCl_3$, Bismuth solutions were buffered to different pH of 2.0, 6.0, 7.0, 8.5 and 10.91and titrated with 0.01 N EDTA. Changes in colour were observed using the flower pigment as the indicator and noted. Also, the colour changes of the different metal solutions were monitored during the titration at different pH using the flower pigment indicator.

3. Results

Table1 The complexometric titration between Cu^{2+} with EDTA at different pHs

pH	Colour of M^+solution	M^+ + In	Colour of M-EDTA	Titre value
2.0	Light blue	Pale-yellow	Light greenish	9.4±0.06
6.0	Bluish	Pale-yellow	Greenish	12.1±0.06
7.0	Bluish	Pale-yellow	Greenish	14.4±0.06
8.5	Bluish	Pale-yellow	Greenish	17.0±.06
10.91	Bluish	Amber	Greenish	19.5±0.06

M^+ = Metal ion solution; M^+ + In = Metal ion solution plus indicator; M^+ - EDTA = Metal ion complex with EDTA

Table 2 The complexometric titration between Ba^{2+} with EDTA at different pHs

pH	Colour of M^+solution	M^+ + In	Colour of M-EDTA	Titre value
2.0	Colourless	Amber	No clear colour change	-
6.0	Whitish ppt	Amber	No clear colour change	-
7.0	Whitish ppt	Amber	No clear colour change	-
8.5	Dark brown	Amber	No clear colour change	-
10.91	Dark brown	Greenish yellow	Pale-yellow	18.3±0.06

M^+ = Metal ion solution; M^+ + In = Metal ion solution plus indicator; M^+ - EDTA = Metal ion complex with EDTA

Table 3 The complexometric titration between Hg^{2+} with EDTA at different pHs

pH	Colour of M^+solution	M^+ + In	Colour of M-EDTA	Titre value
2.0	Colourless	Amber	No clear colour change	-
6.0	Whitish (milky)	Amber	No clear colour change	-
7.0	Whitish (milky)	Amber	No clear colour change	-
8.5	Dark brown	Amber	No clear colour change	-
10.91	Dark brown	Greenish yellow	Pale-yellow	27.5±0.06

M^+ = Metal ion solution; M^+ + In = Metal ion solution plus indicator; M^+ - EDTA = Metal ion complex with EDTA

Table 4 The complexometric titration between Fe^{3+} with EDTA at different pHs

pH	Colour of M^+solution	M^+ + In	Colour of M-EDTA	Titre value
2.0	Golden	Light brown	Pale yellow	7.4±0.06
6.0	Golden	Light brown	Pale yellow	12.6±0.06
7.0	Golden	Light brown	Pale yellow	19.7±0.06
8.5	Golden	Light brown	Pale yellow	21.1±0.06
10.91	Golden	Light brown	Pale yellow	27.9±0.06

M^+ = Metal ion solution; M^+ + In = Metal ion solution plus indicator; M^+ - EDTA = Metal ion complex with EDTA

Table 5 The complexometric titration between Bi^{3+} with EDTA at different pHs

pH	Colour of M^+solution	M^+ + In	Colour of M-EDTA	Titre value
2.0	Colourless	Amber	No clear colour change	-
6.0	Colourless	Amber	No clear colour change	-
7.0	Colourless	Amber	No clear colour change	-
8.5	Colourless	Amber	No clear colour change	-
10.91	Colourless	Greenish yellow	Pale-yellow	13.6±0.06

M^+ = Metal ion solution; M^+ + In = Metal ion solution plus indicator; M^+ - EDTA = Metal ion complex with EDTA

4. Discussion

The complexometric titration of $CuSO_4$ solution (Table 1) changed pale-yellow colour on addition of *Ipomoea* indicator at the different pHs of 2.0, 6.0, 7.0 and 8.5 and amber at pH10.91. When titrated with EDTA solution there was colour change to greenish colour at the different pHs as the endpoint colour which was stable as metal-ligand complex of Cu-EDTA was formed, giving a single, easily identified end point coinciding with the report by Libretexts [8]. From the titre value it was observed that the higher the pH value the higher the titre value which support the reason why high pH is maintained or employed in EDTA complexometric titration because Y^{4-} is prevalent in EDTA and Y^{4-} is needed to react with the metal ions present in the titration solution and higher pH can be used to achieve this [7]. The accuracy of an indicator's endpoint depends on the strength of the metal-indicator complex relative to that of the metal-EDTA complex. Complexometric indicators indicate the exact moment when all the metal ions in the solution are sequestered by chelating agent (EDTA). Thus, the higher the metal-EDTA complex strength the easier the formation of the Metal-EDTA complex and the amount of EDTA consumed to sequester all the free metal ion in the metal-indicator solution by the chelating agent (EDTA) to form the stable complex also will increase, but the higher the metal-indicator complex strength the less the free metal ions to react with the EDTA to form Metal-EDTA complex thus, less amount of EDTA will be consumed to sequester all the metal ion in the metal-indicator solution as the concentration of the free ions of the metal in the titrand solution available to form complex with EDTA is directly proportion to the volume of EDTA required for the complexation reaction [10]. Therefore from the titre values at the different pHs, Cu^{2+} is sequestered with less amount of Chelating agent (EDTA) at acidic medium as the metal-indicator bond tends to be strong and complex less stable, majorly caused by break down of the bond formation by the acidic medium to give light or faint green endpoint which could disappear with time compared to basic pHs which gave dark green endpoint and this report coincided with the report on Online Chemistry Dictionary [6], on complexometric quantification of Cu^{2+} using Murexide indicator on direct titration with EDTA in ammonical solution.

Table 2 for $BaSO_4$, followed similar trend like $HgCl_2$ since endpoint colour change was observed only at pH 10.91 even though there was clear colour change from the metal solution colour when the *Ipomoea* indicator was added to the metal solution at different pHs of 2.0, 6.0, 7.0, 8.5 and 10.91. Only at pH 10.91 complexation endpoint colour change was observed from greenish yellow to a stable pale yellow endpoint colour change, suggesting that pH 10.91 is the working pH for Ba^{2+} complex coordination with EDTA and this is in line with the principle and report stated on Online Chemistry Dictionary [6], on determination of Ba^{2+} ion by direct titration with methyl thymol blue indicator using EDTA.

For $HgCl_2$ solution (Table 3), the pHs 6.0, 7.0 and 8.5 gave amber colour when *Ipomoea* indicator was added to the solution while pHs 2.0 and 10.91 gave light brown and greenish yellow respectively. After titration with EDTA, clear and distinct pale yellow colour change was observed at pH 10.91 with titre value of 27.5cm^3. From the colour change observation it shows that at pH 10.91 all the Hg^{2+} in the solution was sequestered by the EDTA to form Hg-EDTA complex by giving an endpoint colour change of pale yellow as reported by Shah [14], but at other pHs there was no complexation between Hg^{2+} and the Chelating agent (EDTA) therefore, no colour change was observed as the endpoint equivalence.

For the trivalent metals like $FeCl_3$ (Table 4), at all working pHs of 2.0, 6.0, 7.0, 8.5 and 10.91 metal-indicator colour was light brown which later changed to pale yellow colour on addition of EDTA as complexation endpoint. But the report of Sharma [15] on the micro-determination of Iron (III) by complexometric titration using Solochrome Azurine B.S as indicator coincided with the result of this research by reporting that Fe^{3+} formed stable complex with EDTA at acidic pHs of range 2 to 4 but differed from the result of this research as end point colour change was noticed between F^{3+} and EDTA at pHs above 4 to alkaline mediums. Also it was observed that the amount of EDTA required in complex formation between the metal and the ligand (EDTA) increased with increase in pH value indicating less metal-indicator complex strength at higher pH thus, requiring more amount of chelating agent (EDTA) to sequester all the free metal ions in the solution to form metal-ETDA complex.

For another trivalent metal, Bismuth Bi^{3+} (Table 5), there was colour change from colourless metal solution to amber colour when indicator was added at pHs of 2.0, 6.0, 7.0 and 8.5 but greenish yellow at pH 10.91. When EDTA was added to the metal-indicator mixture only at pH 10.91 there was a clear visible and stable colour change from greenish yellow to pale yellow, the rest of the pHs did not show colour change from the metal-indicator colour, suggesting that Bi-EDTA complex formation is at increased pH from 10 and above. Similar report was also reported by Cheng [1] on using 3-Hydroxy-1,2-Benzoquinone indicator to directly titrate Bi^{3+} with EDTA which resulted also to yellow colour equivalent endpoint like could be seen in this research with *Ipomoea* indicator.

5. Conclusion

As an indicator for complexometric titration, the *Ipomoea involucrate B.* pigment was very effective in bivalent metals like Cu^{2+}, Hg^{2+}, Ba^{2+} at different pHs as there was stable colour change observed in the metal-indicator mixture and after metal-EDTA complex formation. Also using trivalent metals like Fe^{3+} and Bi^{3+}, stable complex colour change was observed at the different pHs after metal-EDTA complex formation in $FeCl_3$ solution but only in Bismuth, stable complexometric colour change was observed at high pH of 10.91 similar endpoint as reported by Shah [14].

A good chemical indicator is any substance that shows by change in its colour, the presence, absence or concentration of some other substances, or the degree of reaction between two or more other substances. Hence, *Ipomoea involucrate B.* flower indicator can serve as a good indicator for metallochromic indicator for the following bivalent and trivalent metals (Cu^{2+}, Hg^{2+}, Ba^{2+}, Fe^{3+}, Bi^{3+}) tested for complex formation with EDTA in this research work.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The flower samples were carefully collected and selected from the plant without mutilation or any further damage before drying and extraction of the pigment with high standard grade solvent.

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