Determining the Acid/Alkali and Color Properties of the Anthocyanin Delphinidin-3-Monoglucoside in *Hydrangea Macrophylla*

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Chemistry

3992 Words

Abstract

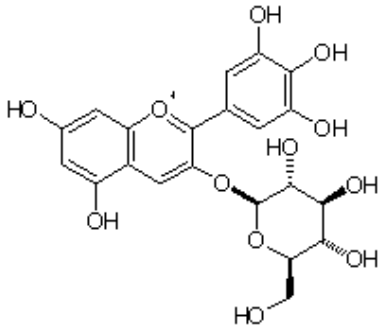
Hydrangea macrophylla, commonly known as 'big-leaf' hydrangea, is known for its two distinct colors. The same plant can exhibit brilliant pink leaves, and at the same time, another next to it can display deep blue sepals. Sometimes the plants can switch colors, depending on soil conditions. The mechanism for this change, and also the original pigmentation, is credited to anthocyanins, water-soluble pigments with three conjugated carbon rings. In this investigation, two *hydrangea macrophylla* (grown in closed soil conditions) were used to determine the acid/alkali indicator properties of the anthocyanins in red and blue *hydrangea macrophylla* sepals. Extracts were obtained by heating the sepals in ethanol, and measurements were taken. Ultimately, it was found that the red color in *hydrangea macrophylla* is the result of protonated anthocyanins—the concentration of acidic pigments was nearly twice the concentration of the blue extract. The deep blue color was determined to be the result of chelation with Al^{3+} ions, which require the presence of the alkali anhydride of the anthocyanin, which formed at a pH of 7.98. An attempt was then made to reverse the colors of the sepals, using an $Al_2(SO_4)_3$ additive to the soil of the red plants; the blue plants, because of Al^{3+} soil depletion, naturally lost their color. However, the sepal colors did not fully reverse—both plants yielded green flowers. Because the colors remained unchanging for a week, new extracts were obtained, tested, and found to have a decrease in pH difference between the red and blue extracts. This suggests that the anthocyanins in the sepals were advancing towards a color reversal, but had not fully completed changing. These findings were ultimately inconclusive and deserve further study. However, the observations made on the original extracts confirmed the sensitivity of anthocyanins in *hydrangea macrophylla* to acidity and Al^{3+} availability.

299 Words

Contents

Introduction to the Experiment

Anthocyanins are water-soluble flavonoid pigments that are found in many plants. They have been studied and used for thousands of years (see *Appendix One: History of Anthocyanins*) and are known to contribute to flower colors across the visible spectrum; the only color yet to be recorded is green. In *hydrangea macrophylla*, there is one prominent compound in the sepals that gives the plant its pigmentation. Delphinidin-3-monoglucoside, a three-ringed molecule with a sugar bonded to an sp^3 hybridized carbon atom in the second ring, is responsible for the red/blue color of the plant. One study identified 43 different types of anthocyanins in four wild forms of *garden iris*, and concluded, "There was no particular relationship between the type of pigment present, and the flower color," (Tsutomu Yabuya, 1990). This suggests that plant colors (and changes) are the result of structural changes the anthocyanins undergo.

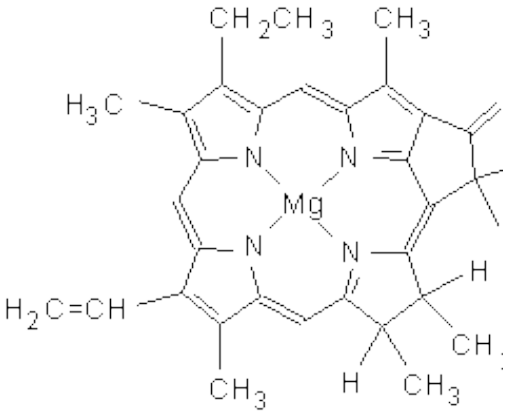


Structure of delphinidin-3-monoglucoside

However, the mechanism of this color change is widely disputed, and conflicting opinions litter the botany and gardener literature. Most agree that acidity plays a role in the color change, because soil pH has been measured around hydrangea bushes—blue is usually associated with acidic soils—but the way pH affects the plant color is not well known. The University of Georgia documents on it's agricultural website that the presence of Al^{3+} ions determines sepal color, and that differing soil pH affects the plant's ability to absorb salt compounds (see *Appendix Two: Effect of Soil pH on Hydrangea Salt Absorption*).

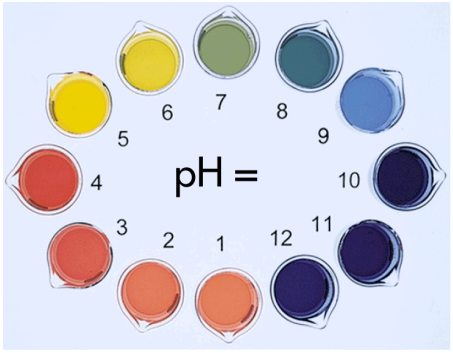
Since Al^{3+} ions do not typically display blue color properties in solutions, they could react with a compound in the flower to reflect a blue light, which would explain why adding $Al_2(SO_4)_3$ to the soil would blue the flowers. In one study, biochemists added Al^{3+} to cut Chinese Bellflowers, and observed that it rapidly made the sepals bluer. The study concluded that the

color change in Chinese Bellflowers was due to the chelation of anthocyanin with Al^{3+} (Susumu Maekawa, Noboru Inagaki and Motoichi Terabun, 1983).



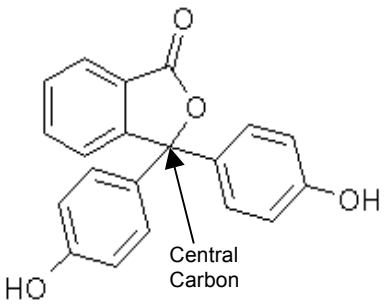
Magnesium chelated in chlorophyll, the compound responsible for photosynthesis and the green color of most plants

Chelation occurs when a metal ion is bonded with multiple sigma bonds, and this formation requires specific conditions. Primarily, metal chelation with anthocyanins requires a pH above the pK_a of the anthocyanin's phenolic group, which is the part of the pigment that bonds with the metal. Chelation occurs frequently in plants to assist in nutrient intake. Metal ions that would otherwise bind with other chemicals to make insoluble compounds are chelated with organic compounds to assist in the plant's intake of nutrients, and help prevent excess solid forming in the sepals. Additionally, because bonded ions are much more stable than non-bonded ions, chelation facilitates the plant's direction of nutrient-ions.



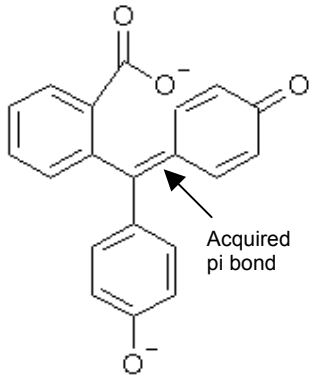
Universal Indicator (UI) in solutions of varying pH. More values are listed in Appendix Three: Common Acid/Alkali Indicators.

However, in laboratory conditions, anthocyanins can also act as pH indicators. Indicators change colors because of structural changes the compound undergoes in solutions of differing acidity. Physically, confining electrons to a smaller space makes the light they absorb bluer (reflect redder). Adding an H^+ ion to acid/alkali indicators—an acidic solution—will confine electrons to a single sigma bond with the H^+ ion, causing a bathochromic (red) shift in the indicator. Conversely, removing H^+ ion(s) from the indicator—an alkaline solution—will allow electrons to spread out in non-bonding orbitals, resulting in a hypsochromic (blue) shift.

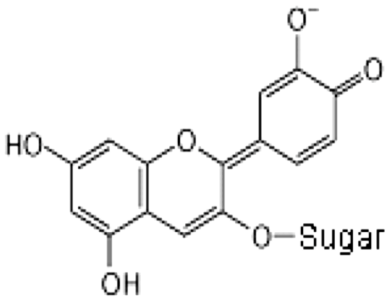


Phenolphthalein in a solution with a pH below 8.2

In organic compounds, conjugated (alternating double) bonds primarily affect the color the compound absorbs. In phenolphthalein, every carbon except for the central carbon has overlapping *p*-orbitals, which create pi bonds between these carbon atoms. The light absorbed by this structure is actually in the ultraviolet range, reflecting in the infrared range, which is why phenolphthalein in a pH below 8.2 appears clear. When phenolphthalein is in the presence of an alkali, the hydrogen atoms in phenolphthalein's hydroxide ions are removed first. In a solution with a pH higher than 8.2, the structure opens up, and the central carbon acquires a pi bond. Because electrons are less confined in pi bonds than sigma bonds, the absorption for this molecule shifts bathochromically to the blue-green range of the visible spectrum (redder than ultra-violet), which makes the light it reflects pink.



Phenolphthalein in a solution with a pH above 8.2



Cyanidin-glucoside in an alkaline solution

Anthocyanins, because of their hydroxide groups, can act much the same way as phenolphthalein and other weak-acid indicators. In an alkaline solution, H^+ ions from the anthocyanin are removed by excess hydroxide ions. This allows electrons in the anthocyanin to spread out in oxygen's *p*-orbitals, causing a hypsochromic shift, but also leaving a bonding site open. Metal ions such as Mg^{2+}, Fe^{2+}, Fe^{3+}, Ca^{2+}, and Al^{3+} are known to bond with anthocyanin compounds, and the addition of these metal ions could cause a change in color as well. Some of these metals will chelate with multiple anthocyanins, which can produce a very different color than is typically exhibited by the metal ion, or the anthocyanin itself. This also emphasizes the idea that chelation requires a pH above

the pK_a of the phenolic group, because the H^+ ions need to be removed for the metal ion(s) to have an open bonding site. Because the acidified anthocyanins are generally accepted as red in color, deprotonated anthocyanins must be present either alone, or chelated with certain metal ions to change the color.

The following experiment will investigate the acid/alkali and color properties of the anthocyanin delphinidin-3-monoglucoside, found in *hydrangea macrophylla*, and attempt to answer the following questions:

- *How do the anthocyanins in blue and red* hydrangea macrophylla *sepal extracts differ with respect to pH and concentration?*
- *Is* Al^{3+} *directly responsible for hypsochromic shifts in the anthocyanins, or does it trigger another bluing mechanism in the plant?*
- *How effective is delphinidin-3-monoglucoside as a pH indicator, and what is its* pK_a*?*
- *How are the concentrations and/or chemical properties of the anthocyanins in red or blue sepals changed when the colors are reversed?*

Qualitative tests will be performed on extracts to determine the color changing mechanism. Since anthocyanins are weak acids, titrations with NaOH will be performed to determine the concentration of acidic anthocyanins and the pK_a of delphinidin-3-monoglucoside. Using the qualitative data obtained, the sepal colors will be reversed, and the tests will then be repeated to note any changes in the properties of the anthocyanins.

Methods and Materials

Two commercially grown *hydrangea macrophylla* were obtained from a nursery. The blue and red petals were stripped from the flower stalk at the bud, so as not to include a significant amount of chlorophyll pigment, which might interfere with the anthocyanin concentration. $10 \pm .002$g of petals were added to $100 \pm .05 cm^3$ of ethyl alcohol (ethanol), which included a methanol-denaturing agent. The $250 cm^3$ beaker containing the petals and ethanol solution was then placed inside a $500 cm^3$ beaker of distilled water, and set on a hot plate at 50°C. Floating the beaker containing the sepals provided equal distribution of heat to the solution. The sepals were heated until they were translucent, indicating a substantial amount of pigment had been removed from the sepals. The extract was then collected, and the petals discarded. The ethanol left in the solution was assumed not to affect the acid/alkali color properties of the anthocyanins. The pH of the extracted solution was measured with an electronic pH meter. Qualitative tests with HCl, NaOH, $AlCl_{3(aq)}$, $FeCl_{3(aq)}$, $Ca(NO_3)_{2(aq)}$, and $Mg(NO_3)_{2(aq)}$ were then performed to determine the color change mechanism of the ethanolic extract.

A stock solution of $.01 moldm^{-3}$ NaOH was prepared by adding $.253 \pm .002$g of NaOH to $500 \pm .05 cm^3$ of distilled water in a graduated cylinder. $50 \pm .05 cm^3$ of red extract was measured in a $50 cm^3$-graduated cylinder, and titrated with the stock solution of NaOH in a plastic burette with an error of $\pm .05 cm^3$. The titrations operated on the rule that the equivalence point is reached when the moles of alkali equals the moles of acid, which would determine the concentration of weak acids if a known amount of alkali was added. The titrations were performed in triplicate on red and blue sepal extracts that had been obtained separately. The pK_a was calculated from the generated titration curve.

The colors of the red and blue *hydrangea macrophylla* were treated in the following manner: Because of Al^{3+} soil depletion, the blue flowered plants required no additive to change colors. The red plants received an $Al_2(SO_4)_3$ additive in their soil. Enough additive was added to

regulate the soil pH at 5.0. Both plants were treated as stated for one week, and sepal color was noted. When the color of the newly grown sepals had changed and remained stable for one week, the same measurements were taken.

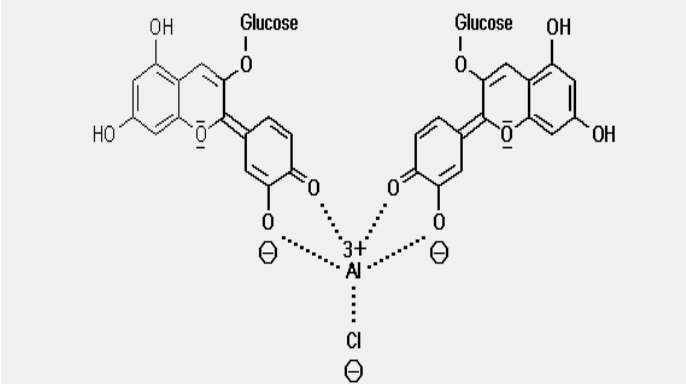
The sepals were then extracted in the same manner as before, and titrated with the same NaOH stock solution. Like the first titrations, three tests were performed on each color flower, and pK_a and concentration was calculated.

All titration points were uploaded into a calculator, and a logistics, $\frac{P}{1+Ce^{-kt}}$, or sine, $a\sin(bx)+C$, function was used to approximate the curve, depending on the amount of deviation the generated curves had around the values near the equivalence points. The inflection point on each graph (where the second derivative equaled zero) was used to determine the equivalence point. The functions generated were also used to more precisely determine other quantitative measurements taken, such as the pH values at those points and the pK_a of the system.

Results and Data Obtained

Qualitative Observations before Color Change

The unaltered extract was consistently one color, a pale pink similar to white wine (which does contain ethanol and anthocyanins). Preliminary qualitative tests suggested that the anthocyanin in an alkaline solution produced a slightly yellow color. Adding excess NaOH to the solution made a very yellow color, and adding similar amounts of HCl turned the solution clear. It was unclear at this point if the HCl actually added extra H^+ ions back to the anthocyanin, or if the excess water created from the bonding of H^+ ions from the acid and hydroxide ions from the base diluted the solution to the point where the color was not visible in small quantities. In all instances, adding any chemical to the system produced very small bubbles. Since such a small amount of bubbles were evolved, however, the produced gas was deemed negligible.



Aluminum ion chelated in a 2:1 mole ratio with anthocyanin pigments. It was assumed that iron (III) ions behaved similarly.

The alkaline anthocyanin in solution also reacted with two metal ions to produce different colors. Excess Al^{3+} ions added in the form of $AlCl_{3(aq)}$ turned the solution blue, and it appeared that the chelation of the aluminum was directly responsible for the blue tint in the flower. In this model, the Al^{3+} ion chelates with anthocyanins in a 2:1 mole ratio. Adding excess Fe^{3+} in the form of $FeCl_{3(aq)}$ produced a very dark green that had to be diluted in distilled water to accurately qualify it as green, not black. The addition of Ca^{2+} in the form of $Ca(NO_3)_{2(aq)}$ and Mg^{2+} in $Mg(NO_3)_{2(aq)}$ made no visible color change.

Quantitative Data before Color Change

The following titration data was obtained before the color of the sepals was artificially changed. All titrations were carried out with the same stock solution, and with the same burette. Titrations were stopped at 45cm^3 or if the pH of the system exceeded 12. All titration points obtained were equated to a logistic or sine function (depending on the amount of deviation), and inflection points were obtained by approximating $\frac{d^2y}{dx^2}\left[\frac{P}{1+Ce^{-kt}}\right]$ or $\frac{d^2y}{dx^2}[a\sin(bx)+C]$, depending on the parent function to which the points were equated.

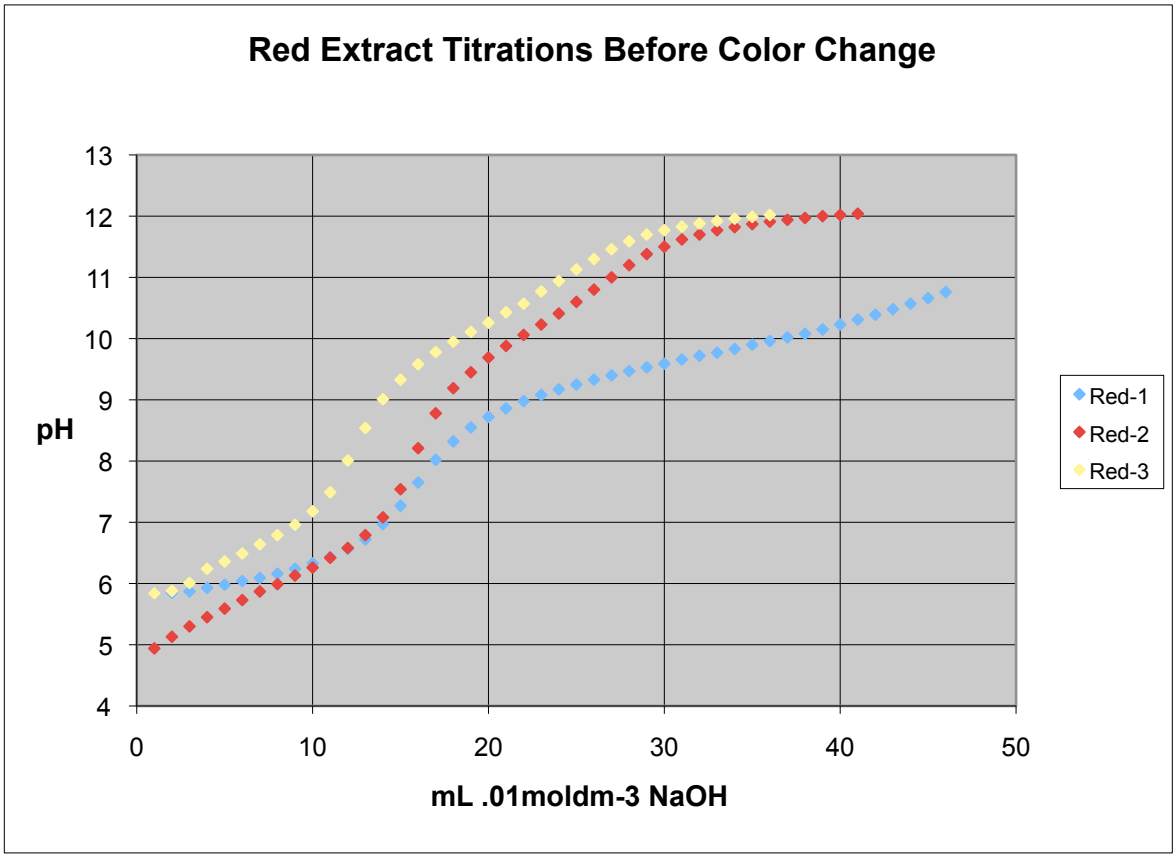
Red Extract Titrations

[.01moldm^{-3}] NaOH With 50cm^3 extract		[.01moldm^{-3}] NaOH With 50cm^3 extract		[.01moldm^{-3}] NaOH With 50cm^3 extract	
cm^3 Titrated	pH	cm^3 Titrated	pH	cm^3 Titrated	pH
0 Initial pH	5.84	0 Initial pH	4.94	0 Initial pH	5.84
1	5.85	1	5.13	1	5.88
2	5.87	2	5.30	2	6.01
3	5.93	3	5.45	3	6.24
4	5.98	4	5.59	4	6.36
5	6.04	5	5.73	5	6.49
6	6.09	6	5.87	6	6.64
7	6.16	7	5.99	7	6.79
8	6.24	8	6.13	8	6.96
9	6.33	9	6.26	9 (clear)	7.18
10	6.43	10	6.42	10	7.49
11	6.57	11	6.58	11 (mint green)	8.01
12	6.72	12	6.79	12	8.54
13	6.97	13	7.08	13	9.01
14	7.27	14 (clear)	7.54	14	9.33
15 (clear)	7.65	15 (mint green)	8.21	15	9.58
16 (mint green)	8.02	16	8.78	16	9.78
17	8.32	17	9.19	17	9.95
18	8.55	18	9.45	18	10.11
19	8.72	19	9.69	19	10.26
20	8.86	20	9.88	20	10.43
21	8.98	21	10.06	21	10.57
22	9.08	22	10.23	22	10.77
23	9.17	23	10.41	23	10.94
24	9.25	24	10.60	24	11.13
25	9.33	25	10.80	25	11.30
26	9.40	26	11.00	26	11.46
27	9.47	27	11.20	27	11.59
28	9.53	28	11.38	28	11.70
29	9.59	29	11.50	29	11.77
30	9.66	30	11.62	30	11.83
31	9.72	31	11.70	31	11.88
32	9.77	32	11.77	32	11.92
33	9.83	33	11.82	33	11.96
34	9.90	34	11.87	34	11.99
35	9.96	35	11.91	35	12.02
36	10.02	36	11.94	36	-----
37	10.08	37	11.97	37	-----
38	10.15	38	12.00	38	-----
39	10.23	39	12.02	39	-----
40	10.31	40	12.04	40	-----
41	10.39	41	-----	41	-----
42	10.48	42	-----	42	-----
43	10.57	43	-----	43	-----
44	10.66	44	-----	44	-----
45	10.76	45	-----	45	-----

Blue Extract Titrations

[.01moldm^{-3}] NaOH With 50cm^3 extract		[.01moldm^{-3}] NaOH With 50cm^3 extract		[.01moldm^{-3}] NaOH With 50cm^3 extract	
cm^3 Titrated	pH	cm^3 Titrated	pH	cm^3 Titrated	pH
0 Initial pH	6.24	0 Initial pH	6.42	0 Initial pH	6.47
1	6.32	1	6.56	1	6.54
2	6.50	2	6.70	2	6.67
3	6.64	3	6.84	3	6.77
4	6.77	4 (clear)	6.99	4	6.91
5	6.93	5	7.16	5	7.04
6	7.12	6	7.41	6	7.18
7 (clear)	7.36	7 (yellowish)	7.73	7 (clear)	7.42
8 (mint green)	7.69	8 (mint green)	8.21	8 (yellowish)	7.87
9	8.20	9	8.70	9 (mint green)	8.45
10	8.62	10	9.06	10	9.06
11	9.00	11	9.35	11	9.53
12	9.24	12	9.58	12	9.86
13	9.50	13	9.77	13	10.09
14	9.66	14	9.96	14	10.30
15	9.84	15	10.13	15	10.48
16	9.97	16	10.26	16	10.65
17	10.08	17	10.32	17	10.81
18	10.21	18	10.45	18	10.98
19	10.35	19	10.60	19	11.11
20	10.45	20	10.72	20	11.24
21	10.57	21	10.86	21	11.35
22	10.68	22	10.95	22	11.44
23	10.80	23	11.08	23	11.54
24	10.87	24	11.17	24	11.63
25	10.98	25	11.27	25	11.71
26	11.09	26	11.37	26	11.76
27	11.20	27	11.47	27	11.79
28	11.31	28	11.55	28	11.84
29	11.39	29	11.60	29	11.88
30	11.46	30	11.66	30	11.91
31	11.53	31	11.71	31	11.94
32	11.58	32	11.75	32	11.97
33	11.64	33	11.80	33	12.00
34	11.70	34	11.84	34	12.03
35	11.74	35	11.87	35	12.05
36	11.78	36	11.90	36	-----
37	11.82	37	11.92	37	-----
38	11.85	38	11.95	38	-----
39	11.87	39	11.97	39	-----
40	11.90	40	12.00	40	-----
41	-----	41	-----	41	-----
42	-----	42	-----	42	-----
43	-----	43	-----	43	-----
44	-----	44	-----	44	-----
45	-----	45	-----	45	-----

Titrations with red flower extract before changing the plant color provided the following overall data. All values obtained inherit an error of ± .01 units.

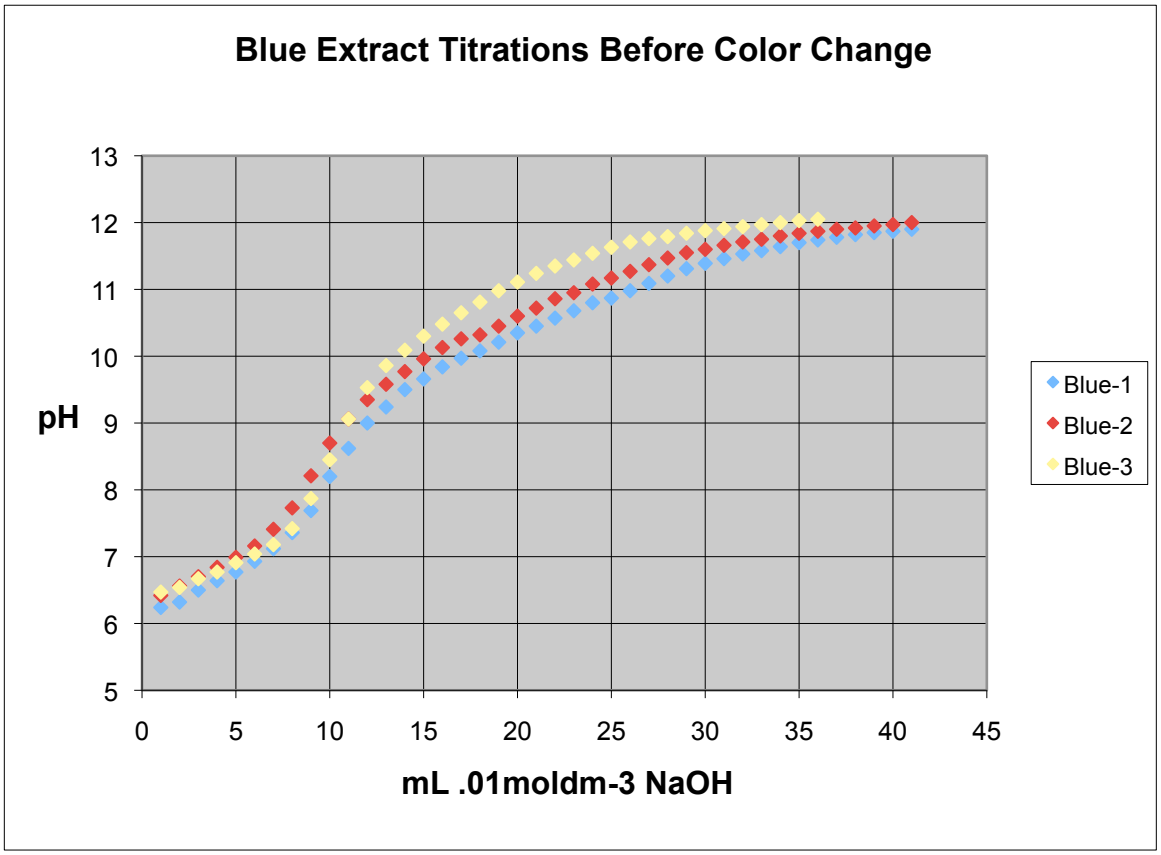


It was assumed that the discrepancies between the curves were caused by the amount of light available before the titration. This is represented by the differing color of the extracts before the titrations.

Plant Color	**Red-1**	**Red-2**	**Red-3**	**Average**
Extract Color Description	Pale Yellow	Pink	White wine	-----
Temp during Titration	Refrigerated	Refrigerated	Room temp	-----
Titrated With ($moldm^{-3}$)	[.01] NaOH	[.01] NaOH	[.01] NaOH	-----
Initial pH	5.84	4.94	5.84	5.54
Inflection Point (cm^3)	15.94	15.93	12.66	14.84
pH at Inflection Point	7.18	8.43	8.58	8.06
[Acidic anthocyanin] ($1/1000moldm^{-3}$)	3.19	3.19	2.53	2.97
pK_a of Anthocyanin	6.24	6.09	6.79	6.37

All values inherit an error of ± .01 units. Inflection points were determined by equating a line to the titrated points, with less than 4% deviation. Finding where the second derivative equaled zero provided the x-value for the inflection points.

Titrations with blue flower extract before changing the plant color provided the following overall data. All values obtained inherit an error of ± .01 units.

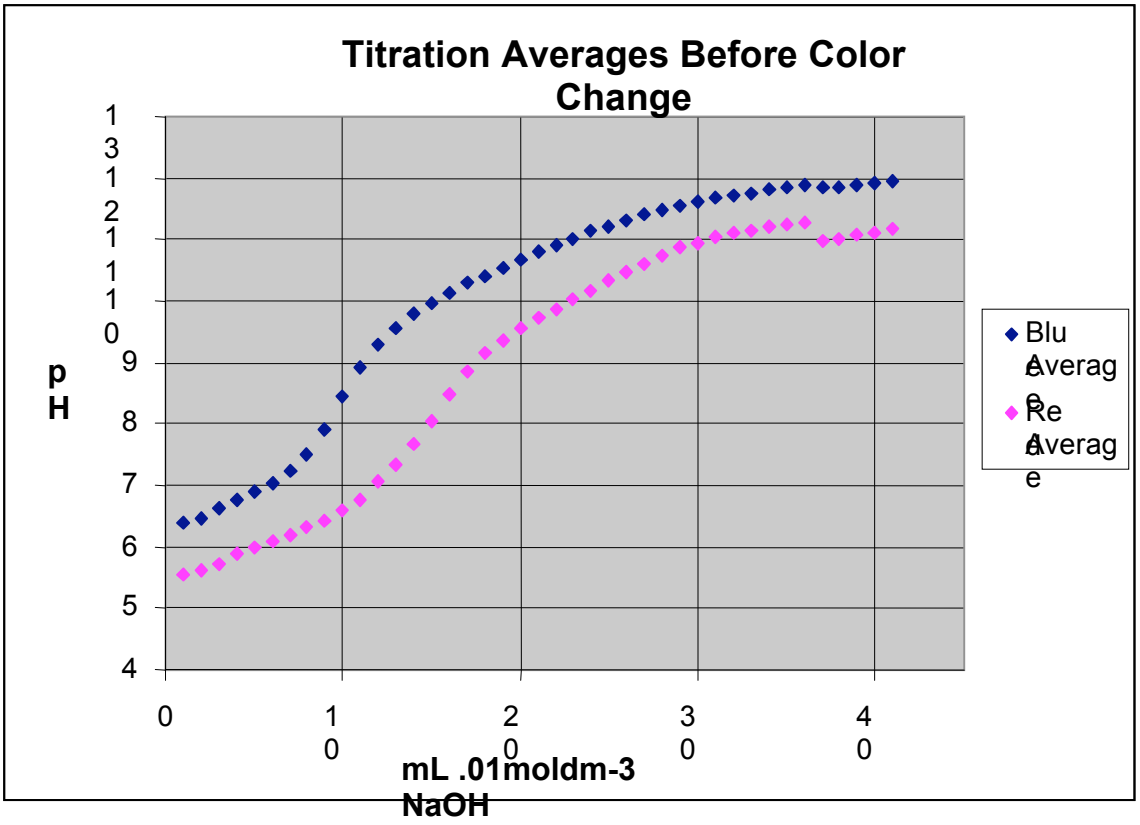


The color properties of the blue extracts were consistent with those of the red.

Plant Color	**Blue-1**	**Blue-2**	**Blue-3**	**Average**
Extract Color Description	White wine	White wine	White wine	-----
Temp during Titration	Room temp	Room temp	Room temp	-----
Titrated With ($moldm^{-3}$)	[.01] NaOH	[.01] NaOH	[.01] NaOH	-----
Initial pH	6.24	6.42	6.47	6.38
Inflection Point (cm^3)	7.45	6.53	7.82	7.27
pH at Inflection Point	7.81	7.88	8.04	7.91
[Acidic anthocyanin] ($1/1000moldm^{-3}$)	1.49	1.31	1.56	1.45
pK_a of Anthocyanin	6.85	7.01	6.93	6.93

All values inherit an error of ± .01 units. Inflection points were determined by equating a line to the titrated points, with less than 4% deviation. Finding where the second derivative equaled zero provided the x-value for the inflection points.

The average of the red and blue titration curves provided the following graph. All values obtained inherit an error of ± .01 units.



All values inherit an error of ± .01 units, and were averaged with appropriate significant digits.

Average Data Before Color Change	Red	Blue
Initial pH	5.54	6.38
Inflection Point (cm^3)	14.84	7.27
pH at Inflection Point	8.06	7.91
[Acidic anthocyanin] ($1/1000moldm^{-3}$)	2.97	1.45
pK_a of Anthocyanin	6.37	6.93

All values inherit an error of ± .01 units. Inflection points were determined by equating a line to the titrated points, with less than 4% deviation. Finding where the second derivative equaled zero provided the x-value for the inflection points.

Qualitative Data after Color Change

The red flowers were treated with $Al_2(SO_4)_3$ in an attempt to lower the soil pH and allow the plants to absorb more aluminum, which would change the sepal color to blue. The $Al_2(SO_4)_3$ was added with the intention of lowering the pH to around 5.00, which would increase the salt absorption through the roots. The red flowers had all been stripped for previous tests, and so the new buds had to develop before the blue color could set in. The sepals actually grew white at first, indicating a change in the development of the sepal pigmentation.

This cluster bears semblance to the sepal color of both plants before the second titrations

However, when the final titrations were ready to be performed, the flower colors of both the red and blue plants were the same color green. Because it seemed the color changes were not proceeding any further, the titrations were performed when the sepal color was green, but stable. The unaltered extract this time, however, did not display the color properties similar to beforehand. Instead of carrying a pale pink tint, this extract was deep green. The extracted solution did not respond to the addition of HCl, NaOH, $AlCl_{3(aq)}$, $FeCl_{3(aq)}$, $Ca(NO_3)_{2(aq)}$, or $Mg(NO_3)_{2(aq)}$ In all cases, the solution remained green, but did lighten a little in color.

Quantitative Data after Color Change

The following titration data was obtained after the color of the sepals was artificially changed. All titrations were carried out with the same stock solution, and with the same burette. Titrations were stopped at $45cm^3$ or if the pH of the system exceeded 12. In the case of the blue flowers reversed to red, not enough sepals could be collected to make 10g per $100cm^3$ of ethanol. The extraction was performed with 5g of sepal per $100cm^3$ of ethanol, and pH measurements were taken at $0.5cm^3$ intervals. All titration points were equated and analyzed in the same manner as previously done.

Red (now green) Extract Titrations

[.01moldm^{-3}] NaOH With 50cm^3 extract		[.01moldm^{-3}] NaOH With 50cm^3 extract		[.01moldm^{-3}] NaOH With 50cm^3 extract	
cm^3 Titrated	pH	cm^3 Titrated	pH	cm^3 Titrated	pH
0 Initial pH	5.99	0 Initial pH	5.89	0 Initial pH	5.72
0.5	6.32	0.5	6.16	0.5	5.98
1.0	6.53	1.0	6.39	1.0	6.14
1.5	6.63	1.5	6.76	1.5	6.26
2.0	7.04	2.0	7.32	2.0	6.58
2.5	7.05	2.5	7.60	2.5	6.23
3.0	7.45	3.0	8.10	3.0	6.98
3.5	7.62	3.5	8.62	3.5	7.08
4.0	7.88	4.0	9.05	4.0	7.35
4.5	8.33	4.5	9.60	4.5	7.63
5.0	8.47	5.0	10.13	5.0	7.75
5.5	9.13	5.5	10.68	5.5	8.25
6.0	9.52	6.0	11.11	6.0	8.44
6.5	9.97	6.5	11.45	6.5	8.92
7.0	10.51	7.0	11.88	7.0	9.29
7.5	10.77	7.5	12.00	7.5	9.59
8.0	11.21	8.0	12.25	8.0	10.06
8.5	11.45	8.5	12.35	8.5	10.21
9.0	11.78	9.0	12.45	9.0	10.56
9.5	12.00	9.5	12.51	9.5	10.76
10.0	12.12	10.0	12.57	10.0	10.97
10.5	-----	10.5	-----	10.5	11.22
11.0	-----	11.0	-----	11.0	11.37
11.5	-----	11.5	-----	11.5	11.58
12.0	-----	12.0	-----	12.0	11.71
12.5	-----	12.5	-----	12.5	11.86
13.0	-----	13.0	-----	13.0	11.96
13.5	-----	13.5	-----	13.5	12.06
14.0	-----	14.0	-----	14.0	12.15
14.5	-----	14.5	-----	14.5	12.21
15.0	-----	15.0	-----	15.0	12.27
15.5	-----	15.5	-----	15.5	-----
16.0	-----	16.0	-----	16.0	-----
16.5	-----	16.5	-----	16.5	-----
17.0	-----	17.0	-----	17.0	-----
17.5	-----	17.5	-----	17.5	-----
18.0	-----	18.0	-----	18.0	-----
18.5	-----	18.5	-----	18.5	-----
19.0	-----	19.0	-----	19.0	-----
19.5	-----	19.5	.-----	19.5	-----
20.0	-----	20.0	-----	20.0	-----
20.5	-----	20.5	-----	20.5	-----
21.0	-----	21.0	-----	21.0	-----
21.5	-----	21.5	-----	21.5	-----
22.0	-----	22.0	-----	22.0	-----
22.5	-----	22.5	-----	22.5	-----

Blue (now green) Extract Titrations

[.01moldm^{-3}] NaOH With 50cm^3 extract		[.01moldm^{-3}] NaOH With 50cm^3 extract		[.01moldm^{-3}] NaOH With 50cm^3 extract	
cm^3 Titrated	pH	cm^3 Titrated	pH	cm^3 Titrated	pH
0 Initial pH	6.46	0 Initial pH	6.21	0 Initial pH	6.10
1	6.68	1	6.58	1	6.63
2	7.01	2	6.91	2	7.10
3	7.38	3	7.21	3	7.61
4	7.79	4	7.56	4	8.55
5	8.60	5	8.08	5	9.51
6	9.59	6	8.87	6	10.27
7	10.40	7	9.76	7	10.81
8	10.86	8	10.23	8	11.22
9	11.24	9	10.74	9	11.50
10	11.50	10	11.01	10	11.76
11	11.70	11	11.29	11	11.95
12	11.89	12	11.53	12	12.10
13	12.01	13	11.71	13	12.19
14	12.14	14	11.85	14	12.28
15	12.23	15	11.98	15	12.34
16	-----	16	12.06	16	-----
17	-----	17	-----	17	-----
18	-----	18	-----	18	-----
19	-----	19	-----	19	-----
20	-----	20	-----	20	-----
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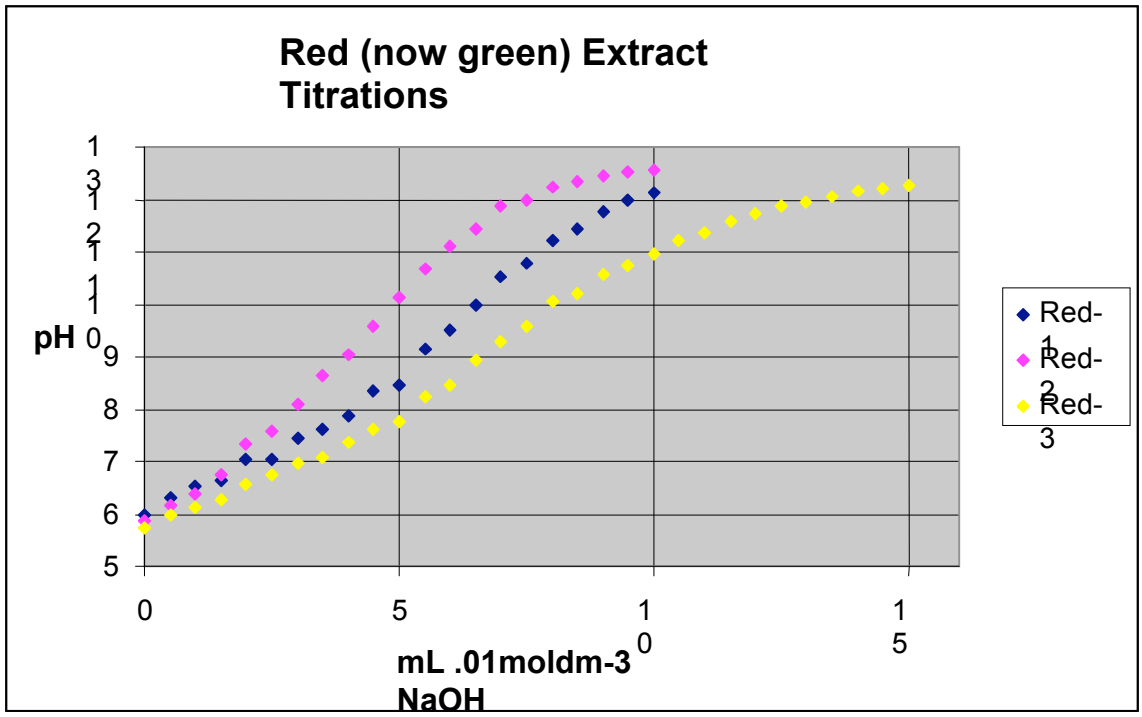
38	-----	38	-----	38	-----
39	-----	39	-----	39	-----
40	-----	40	-----	40	-----
41	-----	41	-----	41	-----
42	-----	42	-----	42	-----
43	-----	43	-----	43	-----
44	-----	44	-----	44	-----
45	-----	45	-----	45	-----

Titrations with red flower extract after changing the plant color to blue provided the following overall data. All values obtained inherit an error of ± .01 units.

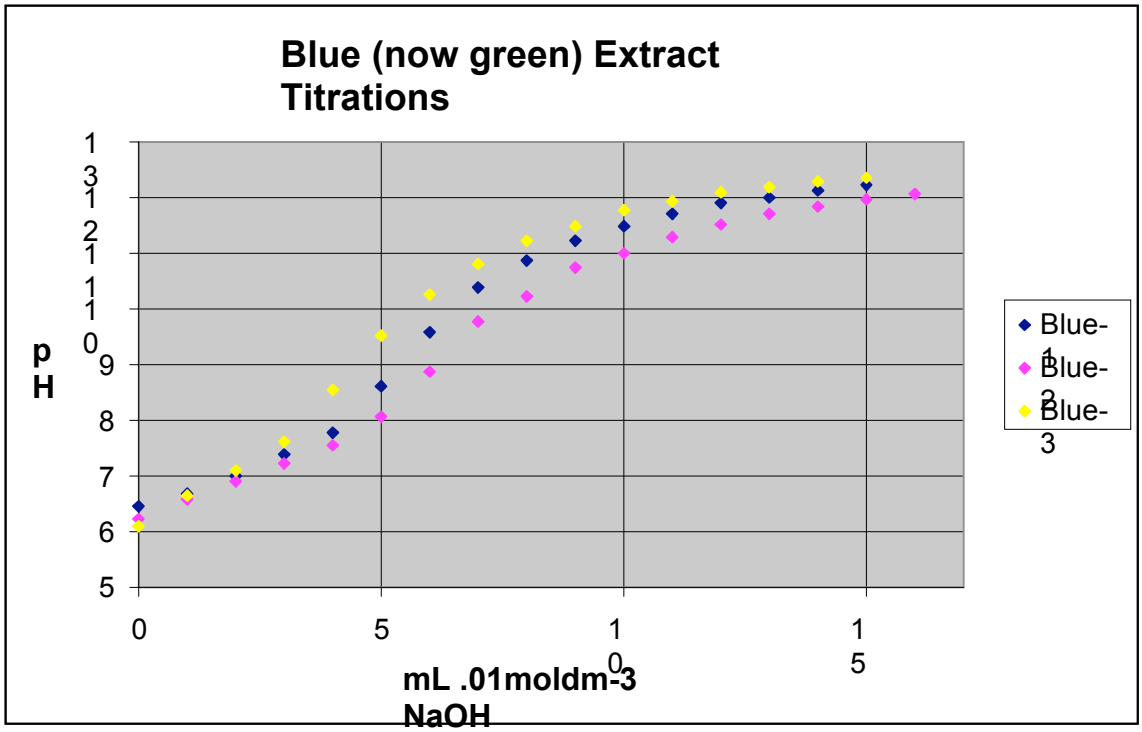


The color of the extract began as mint green and did not change during the titration.

Plant Color	Red-1	Red-2	Red-3	Average
Extract Color Description	Mint Green	Mint Green	Mint Green	-----
Temp during Titration	Room temp	Room temp	Room temp	-----
Titrated With ($moldm^{-3}$)	[.01] NaOH	[.01] NaOH	[.01] NaOH	-----
Initial pH	5.99	5.89	5.72	5.87
Inflection Point (cm^3)	5.92	3.87	6.49	5.43
pH at Inflection Point	9.49	9.02	8.92	9.14
[Acidic anthocyanin] ($1/1000moldm^{-3}$)	2.37	1.47	2.60	2.15
pK_a of Anthocyanin	7.30	7.10	6.95	7.12

All values inherit an error of ± .01 units. Inflection points were determined by equating a line to the titrated points, with less than 4% deviation. Finding where the second derivative equaled zero provided the x-value for the inflection points.

Titrations with blue flower extract after changing the plant color to red provided the following overall data. All values obtained inherit an error of ± .01 units.

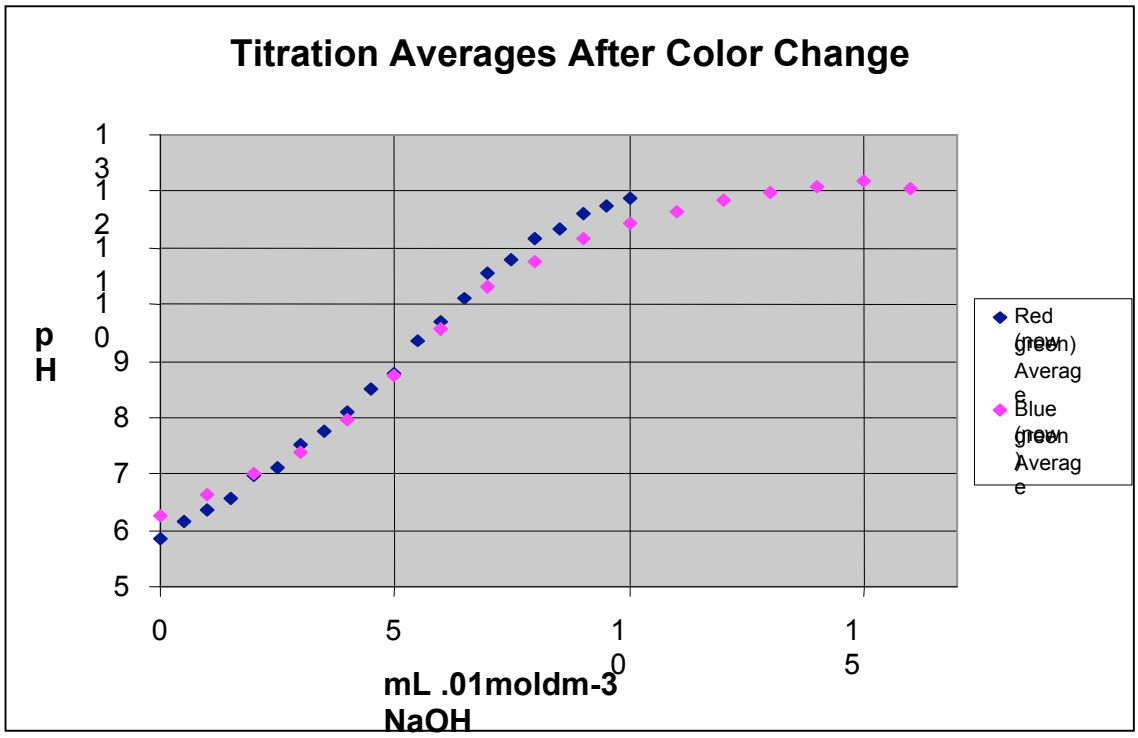


The color of the extract began as dark green and did not change during the titration.

Plant Color	Blue-1	Blue-2	Blue-3	Average
Extract Color Description	Dark Green	Dark Green	Dark Green	-----
Temp during Titration	Room temp	Room temp	Room temp	-----
Titrated With ($moldm^{-3}$)	[.01] NaOH	[.01] NaOH	[.01] NaOH	-----
Initial pH	6.46	6.21	6.10	6.26
Inflection Point (cm^3)	5.60	6.11	4.23	5.31
pH at Inflection Point	9.16	8.98	8.77	8.97
[Acidic anthocyanin] ($1/1000moldm^{-3}$)	1.12	1.22	0.85	1.06
pK_a of Anthocyanin	7.27	7.19	7.17	7.21

All values inherit an error of ± .01 units. Inflection points were determined by equating a line to the titrated points, with less than 4% deviation. Finding where the second derivative equaled zero provided the x-value for the inflection points.

The average of the red and blue titration curves after the color change provided the following graph. All values obtained inherit an error of ± .01 units.

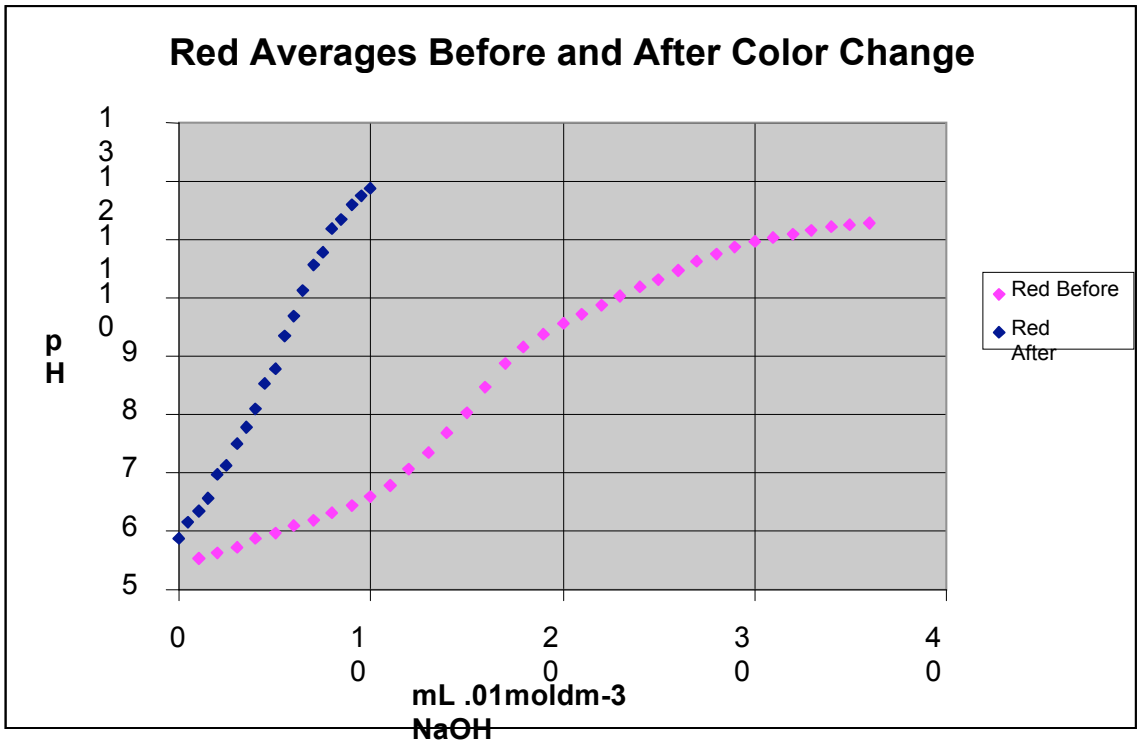


Unusual jumps in the graph are the result of the averaging of only two recorded points, after one of the earlier titrations reached the endpoint before the others.

Average Data	Red (now blue)	Blue (now red)
Initial pH	5.87	6.26
Inflection Point (cm^3)	5.43	5.31
pH at Inflection Point	9.14	8.97
[Acidic anthocyanin] ($1/1000moldm^{-3}$)	2.15	1.06
pK_a of Anthocyanin	7.12	7.21

All values inherit an error of ± .01 units, and were averaged with appropriate significant digits.

The average of the red titration curves before and after the color change provided the following graph. All values obtained inherit an error of ± .01 units.

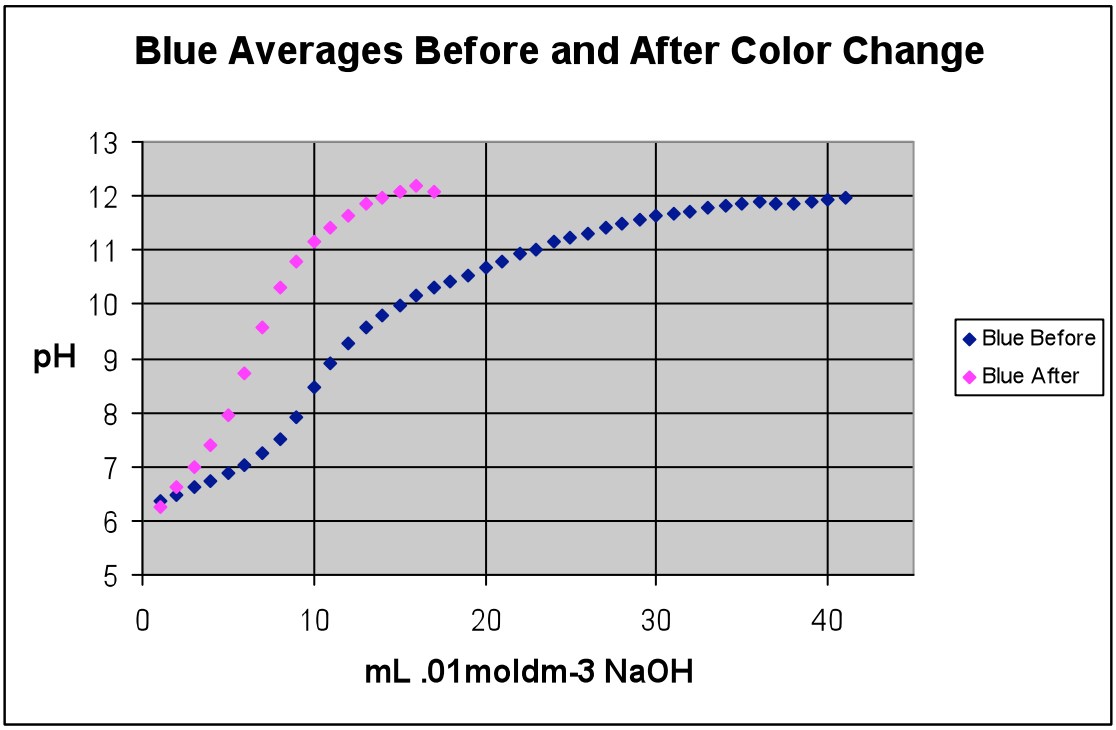


Unusual jumps in the graph are the result of the averaging of only two recorded points, after one of the earlier titrations reached the endpoint before the others.

Average Data	Red Before	Red After
Initial pH	5.54	5.87
Inflection Point (cm^3)	14.84	5.43
pH at Inflection Point	8.06	9.14
[Acidic anthocyanin] ($1/1000moldm^{-3}$)	2.97	2.15
pK_a of Anthocyanin	6.37	7.12

All values inherit an error of ± .01 units, and were averaged with appropriate significant digits.

The average of the blue titration curves before and after the color change provided the following graph. All values obtained inherit an error of ± .01 units.



Unusual jumps in the graph are the result of the averaging of only two recorded points, after one of the earlier titration data points reached the endpoint before the others.

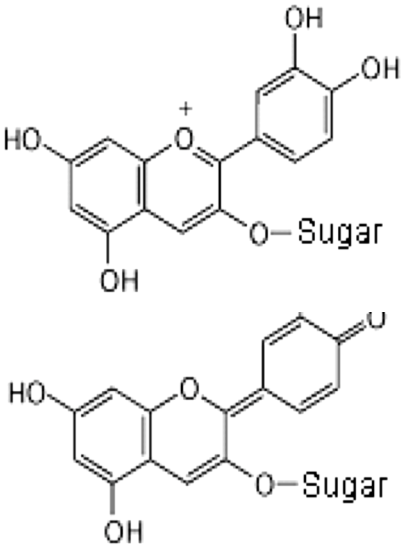
Average Data	Blue Before	Blue After
Initial pH	6.38	6.26
Inflection Point (cm^3)	7.27	5.31
pH at Inflection Point	7.91	8.97
[Acidic anthocyanin] ($1/1000moldm^{-3}$)	1.45	1.06
pK_a of Anthocyanin	6.93	7.21

All values inherit an error of ± .01 units, and were averaged with appropriate significant digits.

Data Analysis

Qualitative Data before Color Change

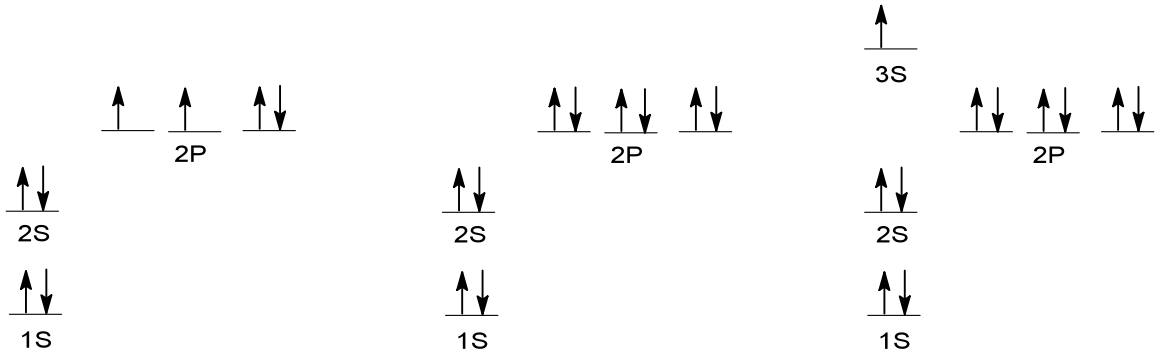
It was clear from the first qualitative test that the chelation of Al^{3+} was responsible for the blue color in the *hydrangea macrophylla* that were tested. The protonated anthocyanin produced a distinctly red color, very similar to the color of the red flowers before the extraction. When excess NaOH was added, however, the solution rapidly became very yellow. Since the anthocyanin is an organic compound, color change is heavily dependent on the number of conjugated bonds in the ring structures. Very similar to cyanidin (right), when the delphinidin molecule is protonated, there are eight conjugated bonds in the structure. However, in an alkaline solution it is deprotonated, and there are then only seven conjugated bonds between carbons. The oxygen in the middle ring also loses its positive formal charge, and the oxygen on the C-Ring loses a sigma bond and gains a pi bond, which frees electrons, contributing to the hypsochromic shift of the structure.



Cyanidin molecules protonated (top), and deprotonated (bottom)

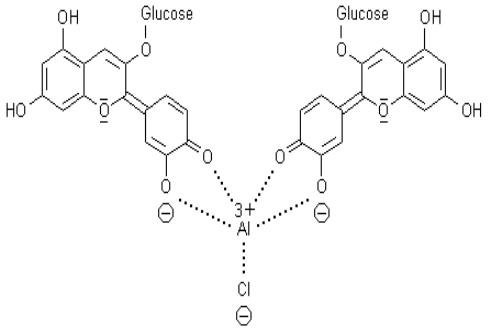
However, the molecule still underwent a hypsochromic shift despite the additional sigma bonds from the chelated aluminum. This is because the double-bonded oxygen atoms gained an electron when they bonded with the Al^{3+} ion. Since the *2p*-orbitals were already filled because of the double bonding, the electron was placed in the *3s*-orbital. The new valence electron on the oxygen atoms was unpaired, and could then spread out more than the previous electrons in the *2p*-orbitals. The valence electron was less confined, and a hypsochromic shift resulted.

lectron Configurations of the Double Bonded Oxygen Atom



Non-bonded oxygen atom *Double bonded oxygen atom* *Double bonded and chelated oxygen atom*

The Fe^{3+} produced a dark green color, which was first interpreted as a structurally different chelation with the anthocyanins. It appeared the color was darker and greener because Fe^{3+} ions have color-producing *d*-orbitals, whereas Al^{3+} ions do not. The color difference was attributed to the shorter distance that electrons would fall from the Fe^{3+} ion's *d*-orbitals to its *s*-orbitals, than the distance between Al^{3+} ion's *p*-orbitals to *s*-orbitals. Because of the decrease in distance for the electrons to fall, green light—which carries less energy than blue—would be emitted. Fe^{3+}, because it is bigger than Al^{3+}, could also have held more than two anthocyanins. There is precedent for this, because Fe^{3+} can complex with up to six ligands, whereas Al^{3+} can only complex with four, despite their identical charges.



Aluminum ion chelated with delphinidin-3-monoglucoside, which reflects a blue light.

However, it was found by adding a drop of $FeCl_{3(aq)}$ to excess ethanolic extract that Fe^{3+} chelation actually produces the same blue color as Al^{3+}, and that excess Fe^{3+} was complexing with water to produce a yellow color. The two colors combined to make an overall dark green tint. Because Fe^{3+} and Al^{3+} produced the same color when chelated with the anthocyanins, it was assumed that they chelated in the same manner, and that the color producing electrons did not land on nor fall from *d*-orbitals, because Al^{3+} does not have *d*-orbitals. This supports the argument for the 3s electron producing the blue color.

Quantitative Data before Color Change

The titrations conducted on the plants before their colors were changed were also conclusive to determine several relationships between the red and blue plants. The consistent pK_a in both red and blue extracts demonstrates that similar extracts were tested, even though the concentration and pH of the systems was significantly different. The initial pH of the red extract was almost one pH unit lower than the blue extracts', which supports the qualitative data obtained before, where the red color was demonstrated in acidic solutions. This is also reflected in the concentration of acidic anthocyanins in the ethanolic extract. Like the amount of NaOH added to reach the equivalence point, the concentration of acidic anthocyanins in red sepal extract was nearly twice that of blue extract. This supports the idea that in order for Al^{3+} ions to chelate with the anthocyanins, they have to be deprotonated first, to leave an open bonding site on the oxygen atoms.

Average Data before Color Change	Red	Blue
Initial pH	5.54	6.38
Inflection Point (cm^3)	14.84	7.27
pH at Inflection Point	8.06	7.91
[Acidic anthocyanin] ($1/1000moldm^{-3}$)	2.97	1.45
pK_a of Anthocyanin	6.37	6.93

All values inherit an error of ± .01 units, and were averaged with appropriate significant digits.

Specifically referring to the individual titrations, the pH of the solution at the endpoints varied more than would be expected. The first red titration did not exceed a pH of 11 even after $45cm^3$ of $.01moldm^{-3}$ NaOH had been added, whereas the other two reached a pH of 12 at $40cm^3$ and $35cm^3$ respectively. This is likely because of the differing amounts of ethanol remaining in the solution. Since the first titration was done on extract that had been allowed to sit overnight, and the other two were obtained the same day, the ethanol concentration was probably

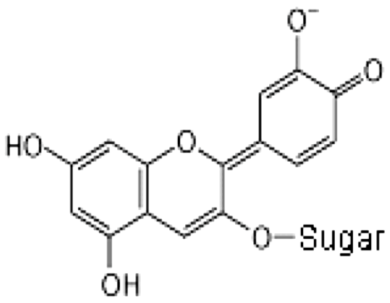
significantly different. Because ethanol is a volatile substance, allowing the first extract to sit overnight allowed the ethanol more time to evaporate out of the solution, causing a decreased amount in the extracted solution. This would have raised the concentration of acidic anthocyanins in the solution, which would have created more of a buffer solution when the NaOH was added. Because a stronger buffer was created, the pH would not have reached 12.00 as quickly, despite the consistent inflection point.

The red extract titrations also had a slight second equivalence point. However, since the color change had already occurred, and the pH was too high to take place inside the hydrangea sepals, the second inflection point was assumed to be the neutralization of another organic acid.

The blue extracts, unlike the red extracts, were tested immediately after extraction, which resulted in approximately equal endpoints for their titrations. All solutions reached a pH of 12 within $40cm^3$, with the exception of the first trial, which had a pH at 11.9. The quicker endpoints are likely the result of lesser concentration of acidic anthocyanins in the blue extract than the red. The blue extracts were tested on the same day as the extraction, but had they been allowed to refrigerate for more time, the endpoints would probably have been reached later.

Comparison to Known Values

One of the ways to check the accuracy of this experiment is to compare the results to published values, several of which were found. It was reported that deprotonization of the delphinidin occurs at a pH of 7.00. (Dragan S. Veselinovich, 1992). This experiment determined the pH at which the anthocyanins reached the inflection point before the color change to be 8.06 for the red sepals, and 7.91 for the blue. Relative to the study's findings, the data obtained differed by 15% for the red flowers, and 13% for the blue. This can be explained by the impurities in the solution while titrating with NaOH. The presence of other organic acids would mean that more NaOH would have had to been added, causing the pH of the solution to be slightly higher at the equivalence point.



Alkali anhydride of cyanidin-glucoside

The same study also found the lowest pH for Al^{3+} ions to chelate with the pigment to be at a pH of 5.85. (Dragan S. Veselinovich, 1992). Since chelation with an organic compound and a metal ion requires a pH above the pK_a of the phenolic group, then the pK_a of the C-Ring in the delphinidin compound was reported to be 5.85. Assuming that the phenolic group of the delphinidin is the only ring that undergoes structural changes in solutions of varying pH, the pK_a of the phenolic group should be equal to the pK_a of the entire compound. The error relative to the previously stated data was 9% for the red extract, and 18% for the blue. This is possibly because of the ethanol interference, because the error at the inflection point would be compounded with the calculation of the pK_a. Since pK_a was determined by using the pH value of the point halfway to the inflection point, any factors that interfered with an accurate determination of the inflection point also affected the determined pK_a for the anthocyanins.

Another study also examined hydrangea flower cells, and found the vacuolar pH in blue plants to be markedly higher than in red plants. Vacuoles inside plant cells perform many functions, but the most relevant to pH is the regulation of acidity in a cell. The study determined the vacuolar pH of blue cells to be 4.1, and the pH of red cells to be 3.3 (Kumi Yoshida, 2006). Vacuoles themselves are slightly acidic, which accounts for both pH readings being below seven. Nevertheless, these findings suggest that there is a difference of 0.8 pH units between the red and blue cells. Before the color change, the pH of the red extract was measured at 5.54 and the blue at 6.38. The difference between the two is therefore 0.84 pH units, with a 5% error relative to the known vacuolar pH values, assuming the difference remains constant after extraction.

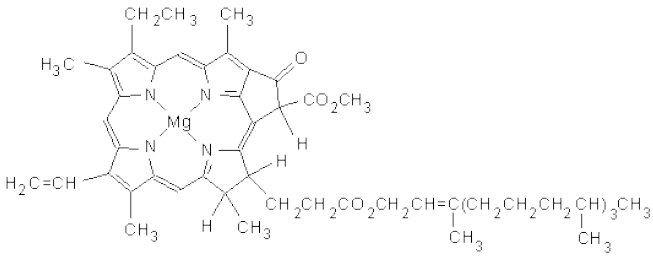
Overview of Compared Values:

Measurements and Data	Red	Blue	Published Value	Avg. % Error
pH at Formation of Alkali Anhydride	8.06	7.91	7.00	14.1%
pK_a of Acidic Anthocyanin	6.37	6.93	5.85	13.5%
pH Difference Between Red/Blue Extracts	0.84		0.80	5.0%

All values inherit an error of ± .01 units

Quantitative Data after Color Change

As the colors of the flowers were reversed, it seemed that the characteristics which previously distinguished the two colors meshed together to make the extracts indiscernible. Because the pK_a of the extract tested did remain constant between the solutions tested after the color change, it was assumed that the anthocyanins were the compound tested during the titrations. However, there were no developed patterns between the measurements before the color change and those taken after the change. The quantitative data measurements for both of the colors after the change were too similar to be definitive. Despite this, it seems that the *hydrangea macrophylla* were in the process of reversing colors, because the quantitative data changes anticipated for the change were starting to take place. A higher pH was needed in the red flowers for the anthocyanins to chelate with Al^{3+} ions, and the pH of the red extract after the color change had risen by 0.33 pH units. Conversely, the pH of the blue flowers needed to become more acidic to turn red, and the pH of the blue extract after the color change had fallen by 0.12 pH units.



Structure of chlorophyll - a

However, the calculated amount of acidic anthocyanins fell in both of the extracts after the color change. This is contrary to what would be expected with the pH differences. Because the plants and extracts were green, and because anthocyanins have never been recorded as naturally green, it would seem that chlorophyll was the most prominent pigment in the solutions titrated. Chlorophyll is not known to affect acidity (no hydrogen bonding), and so the pH differences were not affected because of the presence of chlorophyll. However, since pH is a measure of H^+ concentration, if the concentration of anthocyanins fell, then the differences in pH would be less, which would mean a smaller gap between the red and blue extract. The gap actually went from

0.84 pH units before the color change, to 0.39 pH units after. Additionally, the recorded pK_a values for the extracts after the color changed averaged 0.51 pH units higher than the values recorded before the color change. A high pK_a means that a solution is weakly acidic, and so if the concentration of the anthocyanins fell, the solution would have had a lesser H^+ concentration, and thus would be less acidic.

Another result of the decreased concentration of anthocyanins was that the endpoints of the titrations after the color change were reached much sooner. The titrations performed on the red and blue extracts before the color change reached a pH of 12.00 with an average of $39cm^3$ of $.01moldm^{-3}$ NaOH, while the titrations performed after the color change reached a pH of 12.00 after an average of $13.5cm^3$ added. Additionally, because the solution was less acidic, the pK_a was higher. The absence of a strong buffer solution suggests that there was also a marginal decrease in anthocyanin concentration.

Overall, because of the reversal of the sepal colors had not been fully completed when the titrations were performed, the concentration of anthocyanins was significantly less than the previous extracts. As a result, the measurements obtained after the attempted color change were inconclusive to determine any qualitative or quantitative changes the anthocyanins undergo when the colors of *hydrangea macrophylla* sepals are reversed. Other sources of error and uncertainty are discussed below.

Limitations and Uncertainties

With any experiment, there are certain inherent errors associated with the data obtained. Because of the limitations in precision of the equipment used, the values obtained can only be accurate to a certain degree. Additionally, such factors as differing temperatures and atmospheric conditions can affect the way similar systems behave.

The graduated cylinders used for preparing the stock solution of NaOH, the ethanol for the pigment extraction, and measuring the amount of extract before titrations carried an uncertainty of $\pm .05cm^3$. The balance used to mass the amount of NaOH had an uncertainty of $\pm$ .002g, and the burette used for the titrations had an uncertainty of $\pm .05cm^3$. Overall, the uncertainty for the graduated cylinders was 0.1%, the balance 0.2%, and the burette 0.12%. The functions to which the calculator equated the titration points also carried an error of 4%. By summing the percent uncertainties, it was determined that the total uncertainty for the experiments performed was 4.62% from the chemical equipment and the calculator.

Another source of error was the light dependency on the behavior of anthocyanins. Naturally, in plants, the amount of sunlight can affect the development of the pigment. Specifically in apples, the side that grows in the sunlight will often be redder than the rest of the apple. It was not known how this affected the anthocyanins once they were already developed, but the varying amounts of light they had absorbed before they were titrated could have affected the behavior of the system. When extracts were removed from the refrigerator, they were almost always clear. However, when the anthocyanins were allowed to sit under the room lighting, the pink tint returned, and the extract behaved similarly to the others that were titrated before refrigeration. Allowing the extract to sit in the open air could have also allowed interference with O_2, N_2, or CO_2, the latter of which would've acidified the solution before the titration.

Additionally, since only two hydrangeas were tested, the scope of the experiment is not broad enough to determine if all *hydrangea macrophylla* exhibit similar properties. These plants were grown pots, and also fed with 'Miracle Grow,' a plant supplement containing chemicals

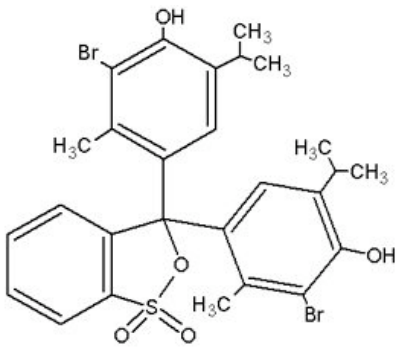
normally associated with fertilized soil. There were no nutrients added that the plant would not otherwise obtain, however with the closed soil pot, it was necessary to replenish the chemicals used by the plant. Both plants received the same additive, so the alteration was at least consistent.

The NaOH pellets used in preparing the stock solution were also very reactive hydrates, absorbing water from the atmosphere quickly. Because of this, the amount of NaOH massed on the balance might have been artificially high if a significant amount of water was absorbed before an accurate weight could be determined. This would have artificially lowered the calculated anthocyanin concentration.

The last limitation, and possibly the biggest, is that the concentration of anthocyanins obtained in the experiments cannot be related to other plants. Since no attempts were made to purify the anthocyanins from the rest of the organic compounds, the concentration obtained is not conclusive to identify the amount of pigmentation in hydrangea flowers. Also, since the ethanol that was not evaporated during the extraction was allowed to remain in the solution, the concentrations obtained with $50cm^3$ are not as consistent as they would have been if the ethanol were removed by another purification of the extract. The sepals during extraction were also weighed to about 10g per $100cm^3$ of ethanol, but the flowers were weighed while wet, and it is also possible that some of the stalks were included in the extraction, and so the mass of sepals would have been artificially high. Because of the limited extraction methods, the data can only be used in comparison with similar systems.

Conclusion

Overall, the results determined in this experiment answered three of the four research questions proposed in the introduction. Red extracts were almost one pH unit lower than the blue. The chemical additive that directly causes the blue color in *hydrangea macrophylla* was determined to be Al^{3+}, while the reddening agent was excess H^{+}, which protonated the anthocyanin to produce a pinkish color. A yellow color was also produced when the anthocyanins were deprotonated, at a pH of 7.98. The pK_a was determined to be 6.65, which is similar to bromothymol blue (see *Appendix Four: Common Acid/Alkali Indicators*). Although the delphinidin compounds in the sepals have very little similarities with the structure of bromothymol blue, it was determined that the induction properties of bromothymol blue and the phenol group of the delphinidin are about equal. This is probably because of their three benzene rings, which have very strong electron-withdrawing properties. Since the delphinidin compounds underwent color changes, it was determined that their effectiveness as an acid/alkali indicator is akin to that of bromothymol blue.



Structure of bromothymol blue

Also, the experiment determined that the ratio of acidic anthocyanins between pink and blue sepals is 2:1. Because the qualitative tests showed that excess acid made the extract red, and the pH of red extract was almost one unit lower, it was fairly conclusive that acidified anthocyanins in the sepal of *hydrangea macrophylla* made the flowers red. Triplicate titrations ensured a consistent finding, which supported the resulting ratio.

However, attempts to reverse the sepal colors were incomplete. As a result of this, the data obtained from measurements taken after the attempted color change is too similar to be definitive. The tests performed after the sepals had turned green were therefore inconclusive to

determine what, if any, structural changes the anthocyanins undergo when the flower colors are reversed.

The values obtained compared to published values ranged from a 5% error to an 18% error. Both the experimental values for the pH at the equivalence point and the pK_a were higher than the published values, which were attributed to the ethanol interference during the titrations. Overall, three out of four research questions given in the introduction were adequately answered.

Appendices

Appendix One: History of Anthocyanins

Anthocyanins have played a prominent role in the enrichment of human lives for thousands of years. Historians and scientists believe that cave paintings from as far back as 15,000 B.C.E. were colored using various plant pigments, and in Egypt and China, dyed fabrics have been found and dated back to 2,000 B.C.E. The ancient Britons used a blue plant dye to color their bodies in an attempt to frighten enemies in battle, and more recently in history, the famous "red coats," worn by British soldiers in the American Revolutionary War, were dyed with a plant called 'madder root'.

Richard Martin Willstätter was the first scientist to identify anthocyanins as the primary red/blue pigmentation in some plants and fruits. He received the Nobel Prize in Chemistry in 1915 for his work with chlorophyll in connection to anthocyanins and plant coloring. Specifically, he isolated the characteristic pigment in cornflowers, roses, pelargonias, larkspurs, and hollyhock, and showed that anthocyanidins attached to glucoses produced an anthocyanin. Willstätter also explained how the same anthocyanin can have blue or red color properties, and proposed that in roses the anthocyanin is bonded to a plant acid, which makes it red. Conversely, he claimed, in cornflower, the anthocyanin is bonded to a plant alkali, which is why it is distinctly blue.

The difference in color pattern suggests an unequal distribution of chemicals in the flower, which is not genetically determined.

The word anthocyanin is derived from two Greek words, *anthos*, which means 'flower', and *kyáneos*, which means 'purple'. Nearly three hundred different anthocyanins have been discovered, and different fruits and vegetables have their own signature mix of pigments. Red wine, for example, contains over fifteen different anthocyanin compounds, depending on the

amount and type of grapes with which it is made. The differing concentrations and types of compounds are what give wine its different color shades.

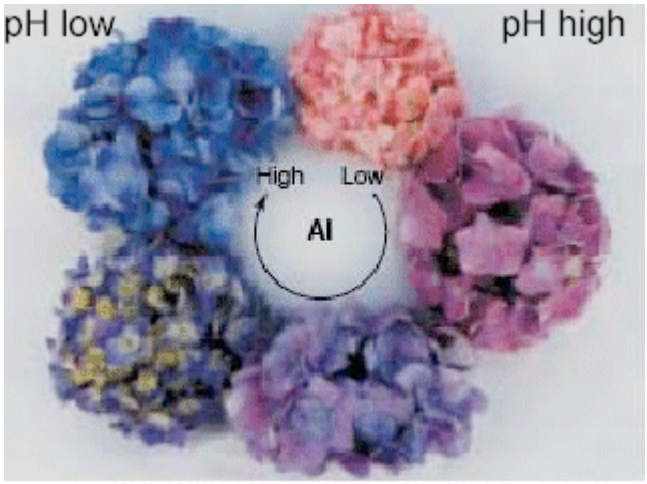
Anthocyanins are also thought to play an important role in the high antioxidant levels in fruits and vegetables. Blueberries, for example, contain a very high concentration of antioxidant compounds, which guard the cell walls of the berry from harmful free radicals existing inside the plant. When people ingest blueberries, they obtain the same protection from free radicals, which can be just as harmful to cell membranes as cell walls. Blueberries, cranberries, and cherries can contain up to 400mg of antioxidants per 100g of berry, and concord grapes—used in many red wines—can contain up to 750mg per 100g of grape (Sriram, 2004).

In the twelfth century, bilberry (Vaccinium myrtillus) was used as an herbal medicine to induce menstruation, and during World War II, British pilots took the same drug before nighttime missions to enhance their night-vision. Now, researchers know that although anthocyanins probably cannot increase nighttime awareness, nor encourage menstruation, they can prevent oxidation damage in both large and small blood vessels because of their anti-oxidant properties. Anthocyanins are also believed to inhibit degenerative nerve damage, and in laboratory conditions, delphinidin and cyanidin compounds have been found to inhibit the epidermal growth factor receptor in cancer cells, which could potentially stunt the growth of tumor cells in humans. Also under study are anthocyanins' abilities to reduce LDL (the "bad") cholesterol, and prevent blood clotting.

Appendix Two: Effect of Soil pH on Hydrangea Salt Absorption

According to the University of Georgia Agricultural Website, the development of the color of the sepals in *hydrangea macrophylla* depends on two different factors. The amount of sunlight the plant receives, and the pH of the soil in which the plant is grown. Hydrangeas of all colors cannot be left in the sunlight for more than four or five hours a day, because the leaves will dry up and wilt very quickly and easily. The pH of most garden soils ranges from 4.5 to 7.5, and most garden soils tend to be acidic, because of the salt compounds, which contain metal ions acting as Lewis acids. Because of differing conditions in which the plants are grown, variations do occur, but primarily, the color follows this pH pattern:

pH	**Flower Color**
4.5	deep, vivid blue
5.0	medium blue
5.5	lavender-purple
6.0	purplish-pink
6.5	mauve-pink
6.8	medium pink
7.0	deep, vivid pink
7.5	true red



Color of hydrangea sepals with respect to soil pH and aluminum availability in the soil

Generally, red *hydrangea macrophylla* grow best, and retain their color in a soil with a pH of 6.6 to 7.5, because at a higher pH, the roots of the pink hydrangea aren't able to absorb aluminum compounds, which turn the flowers from pink to blue. Blue hydrangeas prefer soils below 6.0, because the roots can obtain aluminum compounds from the soil much more easily than they can at a higher pH. This is due in large part to the higher amount of aluminum—which lowers pH because of its presence—and also the physiology behind the absorption of chemicals through the cell walls of the plant root.

Appendix Three: Common Acid/Alkali Indicators

Indicator Name	Effective pH Range
Alizarin Red S	4.6 - 6.0
Brilliant Green	0.0 - 2.6
Bromocresol Green	3.8 - 5.4
Bromocresol Purple	5.2 - 6.8
Bromophenol Blue	3.0 - 4.6
Bromothymol Blue	6.0 - 7.6
Chlorophenol Red	4.8 - 6.4
Congo Red	3.0 - 5.0
m-Cresol Purple	1.2 - 2.8
m-Cresol Red	2.0 - 3.0
Eosin Y	0.0 - 3.0
Methyl Orange	3.2 - 4.4
Methyl Orange - Xylene Cyanol	3.2 - 4.2
Methyl Purple	4.8 - 5.4
Methyl Red	4.2 - 6.2
Methyl Violet	0.1 - 3.2
Phenol Red	6.8 - 8.2
Phenolphthalein	8.2 —9.6
Sulfo Orange	11.0 - 12.6
Thymol Blue	8.0 - 9.2
Thymolphthalein	8.8 -10.5
Universal pH Indicator	2.0 - 11.0

All values inherit an error of ± .1 pH units.

[*] Table adapted from: <http://www.vgdusa.com/indicator_solutions.htm>

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