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Evaluation of red cabbage dye as a potential natural color for pharmaceutical use

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Abstract

Red cabbage dye is a natural pigment used mainly as a food color. A class of compounds called anthocyanins attributes to this color. The pH of the red cabbage solution can also affect both its color and intensity. The objective of this study was to determine the ionization constant (pK_a) of red cabbage color, the effect of pH and temperature on its stability in solution and evaluation of this natural color as a pH indicator in pharmaceutical system. Spectrophotometric method was used to determine its pK_a. The λ_{max} and absorbencies of the red cabbage color at different concentrations and pH were determined. The analytical wavelength (AW) is the wavelength at which the greatest difference in absorbencies between ionized and molecular species occurs was determined. The absorbencies of red cabbage solution (0.12% w/v) at different pH values ranging from 5.0 to 8.0 (with increments of 0.2), was measured at the AW of 612 nm. The resulted absorbencies ranged from 0.31 to 1.91 and were used to determine its pK_a. The pK_a determined by this method was within a range of 6.8–7.2. Results from this study demonstrated that red cabbage dye could be used as a pH indicator in pharmaceutical formulations. In acidic condition, it has its original red color but at a basic pH its color changes to deep blue. This color is more stable at a low temperature and pH. Its ability to act as a pH indicator was further tested using chlorbutol solution as a model system.

Keywords: Cabbage red; pH indicator; Natural color; Chlorbutol; pK_a; Stability

1. Introduction

Red cabbage dye is a natural pigment used mainly as a food color. A colorful class of compounds called anthocyanins attributes to this color. Most naturally occurring anthocyanins occur as a glycoside and contain one of the several aglycone cores. The aglycone portion of red cabbage has been identified to be cyanidine (Fig. 1) and is attached to carbohydrate moiety at both 3 and 5 position (Curtright et al., 1996). This aglycone core can exist as a positively charged oxonium ion and termed as a flavylium cation in acidic solution as shown in Fig. 2. The flavylium

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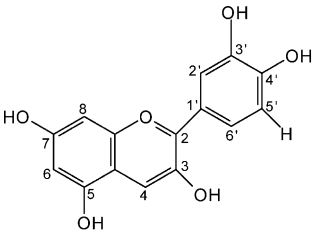

Fig. 1. Molecular structure of cyanidin the common anthocyanin aglycone core present in cabbage red.

cation can exist in equilibrium with a colorless pseudo-base form in basic pH.

The ability of anthocyanin to act as a natural pH indicator can be explained as follows. In an acidic solution, the oxonium ion results in an extended conjugation of double bonds through three rings of the aglycone moiety, which helps in the absorption of photons in the visible spectra. Addition of a base disrupts the conjugation of double bonds between the second and third rings and results in absorption of photons in the UV range rather than in the visible range. The effect of pH change on increasing the number of conjugated double bonds in the molecule lowers the energy level of the electronic transition between the ground state and the excited states, and in turn results in the absorption of photons at greater wavelength. The red cabbage dye, is a dark reddish-purple powder with a faint characteristic odor. It is soluble in water and sensitive to pH changes (Sapers et al., 1981). Stability of this pigment during extraction from natural sources has also been reported (Mazza, 1990; Baublis et al., 1994; Malien-Aubert et al., 2001). Currently, cabbage red dye is being used in beverages, candies, dry mixes, chewing gum, a variety of sauces, and yogurt (Product Information Bulletin, 2000). The use of red cabbage dye in these foods has predisposed the possible use of this natural color as a pH indicator in pharmaceutical preparations. Changes of pH during degradation of some pharmaceuticals in liquid dosage forms (Accodino et al., 1996) and leaching of alkali from soda or alkali glass containers (type III glass) are some of the examples where one may need an indicator for quick identification for such a change. Most of the FDC colors used in pharmaceutical practices are synthetic in nature and do not have any such indicator properties. Therefore, we hypothesized that cabbage red color can be a potential pH indicator for such systems. In order to test this hypothesis, we tested this indicator in an aqueous solution of chlorbutol as a model system.

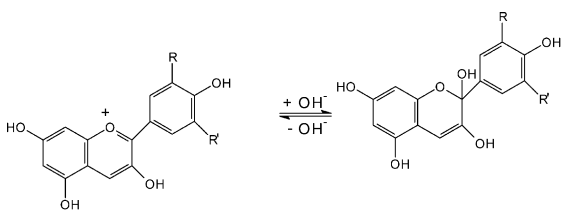

Fig. 2. Chemical structures of the two forms of anthocyanin aglycone cores responsible for pH indicator in cabbage red color.

Knowledge of ionization constants for a pH indicator is essential for its use in practice. Ionization constant can be determined using different methods such as potentiometry, spectrophotometry, solubility, thermometry, as well as other methods (Albert and Serjeant, 1971; Zimmermann, 1983). Objectives of this study were: to determine the ionization constant (pK_a) of red cabbage color using a spectrophotometric method, to evaluate the effect of temperature and pH on the stability of this color in aqueous solution, and to investigate the effectiveness of this coloring agent as a pH indicator in a pharmaceutical system.

2. Materials and methods

2.1. Materials

Powdered cabbage red color WS-5 (Chr.Hansen Company, Mahwah, NJ., Lot #: 18.104.71), potassium (KH_2PO_4) disodium phosphate ($Na_2HPO_4 \cdot 2H_2O$), sodium hydroxide, hydrochloric acid, and chlorbutol (Fisher Scientific, Fair Lawn, NJ), were used as received. The color strength of the dye expressed as $E^{1\,cm}_{1\%}$ was used to compare lot-to-lot variability. $E^{1\,cm}_{1\%}$ of the dye was 30 ± 2.0 at pH 3.0 and 520 nm.

2.2. Spectrophotometric method

A Shimadzu spectrophotometer (model: UV-160) was used to measure the λ_{max} and absorbencies of various cabbage red solutions at pH values of 5.0–8.0. Samples (0.06 g each) of cabbage red powder were dissolved in 50 ml of appropriate buffer solutions at various pH values. The λ_{max} and absorbencies of the solutions were measured using the spectrophotometer in the visible light spectra (400–800 nm). The λ_{max} is defined as the wavelength at which the greatest absorbance in the spectrum is observed.

2.3. Buffer solutions

Sorensen's phosphate buffer solutions with different pH values were prepared. Monopotassium phosphate (KH_2PO_4) 9.073 g was dissolved and qs to 1L with distilled water (Solution A). Disodium phosphate (11.87 g) was dissolved and qs to 1L with distilled water (Solution B). Different volume fractions of both the above solutions were mixed to prepare buffers with appropriate pH values.

2.4. Stability of cabbage red color

The effect of temperature and pH on the stability of cabbage red color was studied at three different temperatures. Cabbage red solutions at three different concentrations (0.1, 0.4 and 1.0 mg/ml) were prepared in buffer solutions. Samples were kept in screw-capped bottles and shaken continuously (at 80 rpm) in controlled temperature reciprocating shaker cum water bath over a period of 10 days. The absorbance of the solution at 536 nm was monitored. The degradation of cabbage red color at a particular time was determined by calculating the change in its concentration from the original concentration in percentage.

2.5. Application in pharmaceutical system

The effect of pH and color changes in a pharmaceutical system was tested using aqueous solution of chlorbutol. Three different concentrations (0.015, 0.025, and 0.05% w/v) of cabbage red color in water containing 0.5% (w/v) of chlorbutol were prepared. The pH of each solution was determined at time 0 and the spectral scans (λ-scans) were obtained immediately. These solutions were kept in tightly closed scintillation vials and exposed to 60 °C in an oven for 24 h. Spectral scans, pH and color change if any was observed visually at 12 and 24 h.

Table 1
Absorbance and λ_{max} of 0.12% (w/v) solutions of cabbage red dye at different pH values

pH	λ_{max} (nm)	Absorbance
5.0	549.1 ± 0.2	0.487 ± 0.02
6.0	553.6 ± 0.3	0.696 ± 0.03
7.0	590.4 ± 0.7	1.079 ± 0.2
8.0	612.3 ± 0.9	2.511 ± 0.1

The data provided represent mean ± S.D. ($n = 3$).

3. Results and discussion

3.1. Effect of pH on the color and intensity of cabbage red solution

A pH range of 5.0–8.0 was selected for this study, because at pH 5.0 the compound exists as a molecular species (unionized form) and at pH 8.0 in the ionized form (Sapers et al., 1981). Cabbage red solutions have shown to change their color on exposure to various pH values (Sapers et al., 1981). This was confirmed using the spectrophotometer, which showed different λ_{max} values at different pH. At pH 5.0, the sample (0.12% w/v) was red in color, whereas at pH 8.0 the sample had a bluish color. Specific λ_{max} and the corresponding absorbance values for cabbage red solutions are depicted in Table 1. Furthermore, spectrophotometric scans (Fig. 3) shows the corresponding λ_{max} at different pH values of the cabbage red solutions. At a pH of 8 the λ_{max} was around 612 nm. However, at pH 5 this was found to be around 550 nm. The effect of pH of the solution on the λ_{max} values was visually confirmed by differences in the color of the solution.

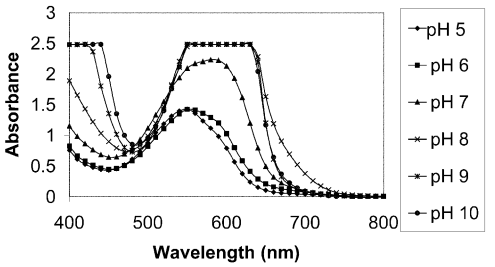

Fig. 3. λ-Scans of various cabbage red color solutions (0.12% w/v) at various pH values.

3.2. Determination of the analytical wavelength (AW)

In order to determine the pK_a of a substance by spectrophotometer, identification of the analytical wavelength (AW) is essential. The AW is the wavelength at which the greatest difference in absorbencies between ionized and molecular species occurs. At different λ_{max} values, as indicated in Table 2, the absorbencies of the solutions were measured. The AW was then determined. The AW was determined to be 612 nm as shown in Table 2. At this wavelength, a highest absorbance difference of 1.41 between the ionized and molecular species was observed.

3.3. Determination of pK_a

The absorbance of red cabbage solution (0.12% w/v) at different pH values ranging from 5.0 to 8.0 (with increments of 0.2) was then measured at the AW (612 nm). The resulting absorbances ranged from 0.31 to 1.91. The data are depicted in Table 3. The ionic strength for each buffer used in this study was calculated and also provided in Table 3 (Martin et al., 1993a). pH indicators may be considered as weak acids and weak bases. They act like buffers and exhibit color changes as a consequence of their change in degree of dissociation at different pH. The relationship between the pK_a of an indicator (pK_{in}) and pH can be described by the buffer equation or the Hendersen–Hasselbalch equation (Martin et al., 1993b) as follows:

$$\mathrm{pH} = \mathrm{p}K_{\mathrm{in}} + \log\frac{[\mathrm{base}]}{[\mathrm{acid}]}$$

The pKa of the red cabbage color was then determined using the following equation (Albert and Serjeant, 1971):

$$\mathrm{p}K_{\mathrm{a}} = \mathrm{pH} + \log\frac{d_{\mathrm{i}} - d}{d - d_{\mathrm{m}}}$$

Table 3
Absorbance of red cabbage solution at the AW (612.3 nm)

pH of the solution	Calculated ionic strength of the buffer (μ)	Absorbance (nm)
5.0	0.0676	0.3050
5.2	0.0689	0.3463
5.4	0.0703	0.3724
5.6	0.0727	0.3871
5.8	0.0763	0.4127
6.0	0.0815	0.4493
6.2	0.0892	0.5127
6.4	0.0995	0.5584
6.6	0.1128	0.6772
6.8	0.1289	0.7900
7.0	0.1448	0.9635
7.2	0.1604	1.1751
7.4	0.1736	1.4434
7.6	0.1828	1.6570
7.8	0.1900	1.7924
8.0	0.1951	1.9132

Table 2
Determination of the AW

Wavelength (nm)	Absorbance at pH 5.0 (X_1)	Absorbance at pH 8.0 (X_2)	Absorbance differences (X_2-X_1)
549.1	0.793	0.828	0.035
553.6	0.785	0.895	0.110
590.4	0.549	1.479	0.930
612.3	0.315	1.721	1.406

Table 4
Calculation of pK_a for cabbage red color

pH of the solution	d (observed absorbance at the AW)	Calculated pK_a
5.2	0.3463	6.8
5.4	0.3724	6.8
5.6	0.3871	6.9
5.8	0.4127	6.9
6.0	0.4493	7.0
6.2	0.5127	7.0
6.4	0.5584	7.2
6.6	0.6772	7.1
6.8	0.7900	7.2
7.0	0.9635	7.2
7.2	1.1751	7.1
7.4	1.4434	7.0
7.6	1.6570	6.9
7.8	1.7924	6.7

Absorbencies of the ionized and molecular species at the AW are: $d_i = 1.9132$ and $d_m = 0.3050$.

where d is the observed absorbance at the AW; d_i and d_m, the absorbances of the ionized and molecular species, respectively. The calculated pK_a values of red cabbage powder utilizing the above equation are presented in Table 4. The mean pK_a of the red cabbage dye determined by this spectrophotometric method was calculated to be 6.99 ± 0.16 (mean $\pm$ S.D.; $n = 14$). Eight different anthocyanine have been isolated from red cabbage dye (Nakatani et al., 1987). Therefore, the pK_a reported here will be considered as a macroscopic pK_a for a closely related group present in this natural color.

3.4. Stability of the red cabbage solutions in water

The stability of red cabbage color at three different temperatures, three different pH conditions and three different concentration levels were then investigated. The results were summarized in Table 5. Stability data were expressed, as percentage of degradation observed over a period of 10 days. Stability of this color was dependent on temperature, pH as well as concentration. The dye was most stable at room temperature and pH 3 (percentage of degradation was only 1–5% over a period of 10 days). However, it was least stable at 50 °C and pH 8 (percentage of degradation was 79–98% over a period of 10 days). Reversibility of the color change was also tested for this natural color. The solution was red at pH 3.0. Drop wise addition of 0.1 N NaOH turned the solution blue around pH 7. When 0.1 N HCl was added to this blue color slowly, the solution changed back to its original color red around pH 4.0. This test also confirms the reversibility of this color change with pH at room temperature.

Table 5
Effect of temperature and pH on the degradation of cabbage red color in aqueous solution

pH	Concentration (mg/ml)	Degradation of cabbage red color (%) over a period of 10 days at three different temperatures[a]		
		23 ± 1.0 °C	37 ± 0.5 °C	50 ± 0.5 °C
3.0	0.1	1.2 ± 0.1[b]	3.4 ± 0.1	9.0 ± 0.9
	0.4	1.4 ± 0.2	8.3 ± 0.9	17.5 ± 0.04
	1.0	4.5 ± 0.1	11.4 ± 0.1	18.0 ± 0.01
7.4	0.1	10.5 ± 2.5	16.3 ± 1.7	48.3 ± 4.0
	0.4	47.0 ± 0.1	67.5 ± 1.7	78.2 ± 0.6
	1.0	60.5 ± 0.2	74.7 ± 0.7	83.0 ± 2.6
8.0	0.1	66.1 ± 6.6	76.0 ± 0.8	79.1 ± 1.1
	0.4	68.0 ± 1.4	79.0 ± 2.4	81.8 ± 1.8
	1.0	75.0 ± 0.2	82.2 ± 0.6	98.1 ± 0.7

[a] Degradation (%) = (original concentration − determined concentration) × 100.
[b] Mean $\pm$ S.D.; $n = 3$.

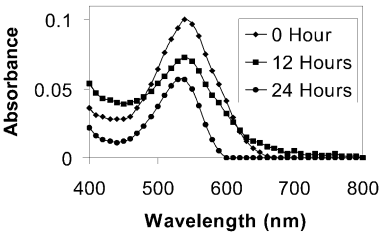

Fig. 4. λ-Scans of 0.5% (w/v) chorbutol solution containing 0.015% (w/v) of cabbage red color. The scans were obtained after exposing these solutions to a temperature of 60 °C for: (◆) 0 h, (■) 12 h, and (●) 24 h.

3.5. Application of this natural color as a pH indicator in pharmaceutical system

Effectiveness of this natural color as a pH indicator was tested in a pharmaceutical system. Aqueous solution of chlorbutol was used for this purpose. Chlorbutol is also called chlorobutanol, is a chlorinated alcohol used as a preservative at a concentration of 0.5% (w/v) in pharmaceutical systems. This compound is stable in acidic condition and hydrolyzes in basic or neutral conditions to form hydrochloric acid with a resultant decrease in the pH of the solution. This pH change has also been shown to be dependent of temperature (Accodino et al., 1996). When exposed to 60 °C, the pH changes in 0.5% (w/v) chlorbutol solution were found to be more than 1 pH unit. Therefore, this system was thought to be ideal for testing this pH indicator. The initial pH of 0.5% (w/v) chlorbutol solution in water prior to heating was 4.4 ± 0.15 (Mean $\pm$ S.D.; $n = 3$). After exposing these solutions to 60 °C for 24 h the pH was changed to 3.2 ± 0.08 (Mean $\pm$ S.D.; $n = 3$). This change in pH was visually confirmed by noticing a change in the color of the solution. Initially the color of the solution was purple and changed to red when exposed to 60 °C for even 12 h. This change in color was also confirmed by the λ-scans. The λ_{max} as well as the absorbance values of the solutions were changed during the pH shifts in the solution as shown in Fig. 4.

The color change was distinct and clearly visualized even at the lowest concentration of the cabbage red (0.015% w/v) used.

4. Conclusion

1. A spectrophotometric method was used to determine the ionization constant (pK_a) of red cabbage color and the pK_a was determined to be 6.99 ± 0.16 (mean $\pm$ S.D.; $n = 14$). Since eight different anthocyanins have been isolated from cabbage red dye, the pK_a reported represents a macroscopic pK_a for a closely related group present in this natural color.
2. Results from this study demonstrated that red cabbage dye could be used as a pH indicator in pharmaceutical formulations. At a low (acidic) pH it has its original red color, but at a basic pH its color changes to deep blue. This color change is reversible with pH at room temperature.
3. This natural color in solution was more stable at room temperature and at a low pH (pH 3). However, it was found to be least stable at 50 °C and at pH 8.
4. The color change in a pharmaceutical solution due to a change in the pH of the solution caused due to an in situ degradation can visually be confirmed even at a low concentration (0.015% w/v) of the cabbage red color used as a pH indicator.

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