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Research Article

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The Photo- and Electro-Chemical Properties of Anthocyanin Extracts of Red Cabbage

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Abstract Photo- and electro-chemical properties of anthocyanin extracts of *Brassica oleracea var. capitata f. rubra*were studied. Variations in absorption spectra of anthocyanin with respect topH, chelation with various metal ions and an applied potential (electrochromic behavior) wereinvestigated. Photo- and electro-chemical properties of Fe^{3+}, Al^{3+}, $Cu^{2+}$and Ni^{2+}-anthocyanin were investigated. Anthocyanin produceschelated metal ion complexes with Fe^{3+} and Cu^{2+}. Fe^{3+}-anthocyanin produced a linear variation in absorbance at 281 nm with the Fe^{3+}-anthocyanin concentration. However,Fe^{3+}, Al^{3+}, Cu^{2+} and Ni^{2+}-anthocyanin complexes failed to produce significant electrochromic behavior under aqueous conditions at pH = 4.

Keywords anthocyanin, anthocyanin-metal ion complex,Fe^{3+} sensor

Introduction

Anthocyanins are one of the most important pigments in the plant kingdom which provide scarlet to blue coloration to plant parts [1,2]. They belong to the broad range of phytochemicals called flavonoids which is a subgroup of plant polyphenolics. Flavonoids have a basic C_6-C_3-C_6 carbon skeleton. The biosynthesis of polyphenolics involve two major metabolic pathways,[3,4]*viz.* the polyketide pathway and the shikimic acid pathway. According to the current understanding, most flavonoids (as well as anthocyanins) derive from both pathways. Ring A (Figure 1) is usually derived from the polyketide pathway, whereas the rings B and C derive from the shikimic acid pathway. There are around 17 naturally occurring anthocyanins, although, only six are prominently widespread.

Anthocyanidin	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Figure 1: Structures of common anthocyanidins. A,B, and C refer to the standard nomenclature of the rings, In anthocyanins, the sugar moieties are usually attached to the 3-, 5-, or 7- position

Anthocyanins are *O*-glycosides. They have sugar moieties substituted in the places of OH groups through glycosidic bonds. Common sugars include glucose, galactose, arabinose, rhamnose, and xylose. The free forms (aglycone forms) are termed anthocyanidins [5]. Figure 1 shows the basic structure, numbering, and the most commonly occurring anthocyanidins in nature. In aqueous phase, anthocyanins occur in a series of pH-dependent equilibria [6]

as shown in Figure 2. Depending on the position of the equilibrium, an anthocyanin solution would impart a characteristic color (acidic pH: reddish, basic pH: bluish).

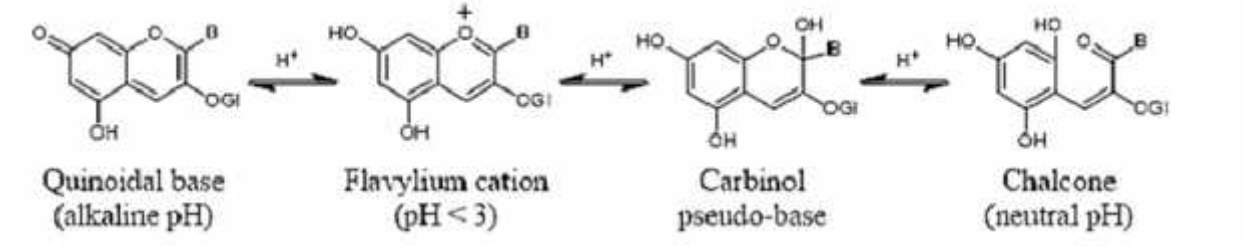

Figure 2: Aqueous equilibria of anthocyanins. Ring B do not undergo any change and "GI" is the sugar moiety.

Anthocyanins have the ability to form complexes with metal ions. Such interactions result in the changes of the absorption spectra of these compounds. Anthocyanin-metal complexes are called metalloanthocyanins. Initial studies of metalloanthocyanins were performed in 1920s due to a controversy over blue colored flowers [7]. The prevailing theory at the time argued that the blue color of flowers was due to pH variations in plant cells which resulted in the anthocyanins converting into the quinoidal base form. However, as such a basic medium was not possible in the plant cell saps, this theory was dismissed and metalloanthocyanin theory became prominent.

Although many studies have been carried out on various anthocyanin-metal pairs, there appears to be no universal consensus about the general structure of these complexes. The complexation mainly takes place using the OH groups of the B ring [8]. According to current research work, naturally occurring metalloanthocyanins have extremely complicated structures comprising of polynuclear metal centers, multiple anthocyanin molecules, and in some cases, other molecules such as flavonoids. For example, Shiono *et al.* [9] have determined the structure of commelinin, which is the pigment responsible for the color of the flower of *Commelina communis L.* According to their findings, the pigment is comprised of two Mg^{2+} ions chelated to six anthocyanin molecules and another two Mg^{2+} ions chelated to six flavone molecules, giving rise to a supramolecular assembly. Another example is that the pigment cyanosalvianin, which is responsible for the blue color of *Salvia uliginosa* petals. Cyanosalvianin is also a supramolecular metalloanthocyanin having a complicated chemical structure [10].

Molecular electronics are electronic components designed using a single molecule or a small group of molecules [11]. They may include wires, switches, memory elements, and implementation of logic gates (molecular logic gates). De Silva *et al.* [12] paved the way to the development of this field in 1993 by inventing a photoionic fluorescent system which was capable of acting as an AND gate. In 2000, De Silva and McClenaghan proved [13] that the principle of molecular level arithmetic by forming a set of molecular logic gates using a compound capable of binding Ca^{2+} and H^{+} at its two terminals and thereby changing its photophysical properties (i.e. fluorescence activity, absorption spectrum *etc.*). Kim *et al.* [14] implemented aNOR logic gate using curcumin in aqueous solution in combination with Hg^{2+}.The gate was implemented as an electrochromic cell consisting of a curcumin-Hg^{2+} complex sandwiched between two indium-tin oxide (ITO) electrodes. Electrochromism is the reversible change of absorption spectrum of a substance due to the application of the potential which results in an electrochemical (redox) reaction[15]. Most of the research related to electrochromism has been based on inorganic materials. Kim *et al.* have used a natural pigment for developing a functional electrochromic device.

The aim of this research is to investigate the electrophysical and photophysical properties of anthocyanin extracts from *Brassica oleracea var.* and *capitata f. rubra* (red cabbage). Red cabbage is a biennial, leafy plant that contains anthocyanin pigments [16]. Investigation of the effect of pH and chelation with metal ions on the absorption spectrumof anthocyanin and of theelectrochromic behavior of anthocyanin are the objectives of this research.

Methodology

All chemicals used in this research were of analytical grade. Distilled water was used for thepreparation of solutions. The organic solvents were doubly distilled.

Preparation of red cabbage extract

A red cabbage was cut into pieces (342 g) followed by equilibrating in boiling distilled methanol (600 mL). After 30 min, the solution was filtered and rotary evaporatedat 40 ºC for 8 h for a constant volume of 50 mL, followed by the preparation of diluted extracts with 10-times and 2-times dilutions. Figure 3 shows the key steps of the extraction procedure. All extract solutions were stored at 4 ºC.

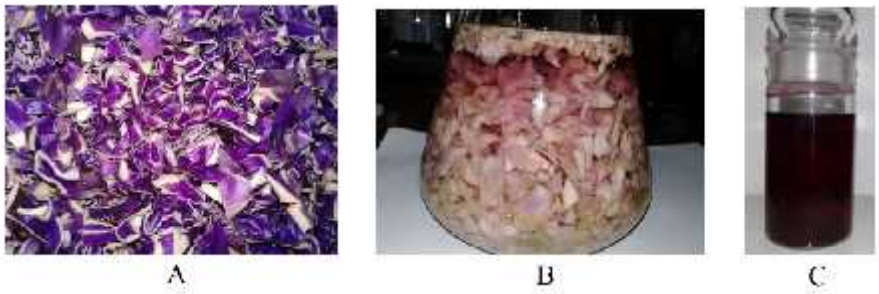

Figure 3: The major steps of the process of extraction of red cabbage, A: red cabbage pieces, B: extraction with ethanol, C: final anthocyanin extract.

Determination of effect of pH on the absorption spectra of the extracts

To determine the effect of pH on the absorption spectra of extracts, 2.00 mL of the 10-times diluted extract was diluted to 25.00 mL using buffer solutions given in Table 1. The compositions of the buffers are given in Table 1.

Table 1: Compositions of the pH buffer solutions (final volume in each case wasset as100.00 mL).

pH of the buffer	Composition
2	50.00 mL of 0.2 M KCl + 13.00 mL of 0.2 M HCl
4	15.066 g of NaOAc + 57.40 mL of AcOH
6	100.00 mL of 0.1 M KH_2PO_4 + 11.20 mL of 0.1 M NaOH
8	100.00 mL of 0.1 M KH_2PO_4 + 93.40 mL of 0.1 M NaOH
10	100.00 mL of 0.025 M $Na_2B_4O_7$ + 36.60 mL of 0.1 M NaOH
12	100.0 L of 0.05 M Na_2HPO_4 + 53.80 mL of 0.1 M NaOH

The absorption spectra were recorded against the same buffers as the reagent blanks using 1 cm quartz cuvette and a ThermoFisher Scientific GENESYS 10S UV-Vis spectrometer. The scan parameters were as follows: wavelength range: 250 – 700 nm, wavelength interval: 1.0 nm, scan speed: fast.

Determination of the effect of metal ion chelation on the absorption spectra of anthocyanin extracts

Dilute HCl (0.002 M) was prepared using conc. HCl. Solutions of 0.01 M Al^{3+}, Ni^{2+}, Cu^{2+}, and Fe^{3+} were prepared in 0.002 M HCl using $Al(NH_4)(SO_4).12H_2O$, $NiSO_4.6H_2O$, $CuSO_4.5H_2O$, and $Fe(NH_4)(SO_4)_2.12H_2O$, respectively. These solutions were diluted to prepare 0.001 M solutions of each metal ion.

For each metal ion, a series of extract-metal ion solutions (25.00 mL) were prepared with varying volumes (v mL) of metal ion solution, 2.00 mL of the 2-times diluted extract, (12.50 – v) mL of 0.0002 M HCl, and topping up with distilled water. The final pH of the ion solutions was maintained at approximately 4. The absorption spectra of these solutions were recorded. The final concentrations of metal ions are given in Table 2.

Table 2: Concentrations of metal ions in final solutions

Volume (v) of 0.001 M metal ion (mL)	Concentration of the metal ion in final solution (mol L^{-1})
1.00	4×10^{-5}
2.00	8×10^{-5}
3.00	1.2×10^{-4}
4.00	1.6×10^{-4}
5.00	2.0×10^{-4}

6.00	2.4×10^{-4}

Investigation of electrochromic properties of anthocyanin-metal ion aqueous solutions

The electrochromic effects were investigated using a cell consisting of two carbon electrodes as the working and the counter electrode and a saturated Ag/AgCl micro-reference electrode. The cell was a standard 1 cm quartz cell and the setup is shown in Figure 4.The cell was prepared in a way that allowed it to be conveniently mounted on the cell holder of the UV-Visible spectrophotometer. Pine Research WaveDriver 20 potentiostat was used to apply the necessary potentials to the working electrode.

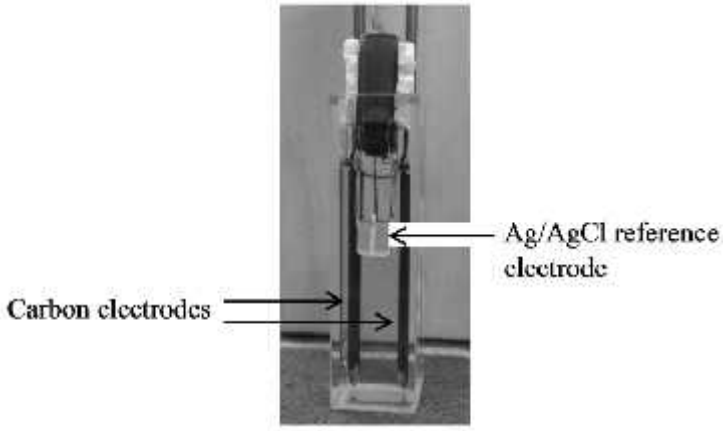

Figure 4: The cell setup used for the determination of electrochromic properties

For each metal ion, a solution of extract-metal ion (25.00 mL) was prepared using 2.00 mL of 2-times diluted extract, 6.00 mL of the 0.001 M metal ion solution (same as used in section 2.4), (25.00 – v) mL of 0.0002 M HCl, and topping up with distilled water. Bulk electrolysis technique under constant potential mode was utilized to provide the necessary potentials. Potentials from -1 V to +1 V (w.r.t. the reference electrode) were applied to the working electrode and in each case and the absorption spectrum was recorded after 40 seconds. The same procedure was followed for a set of freshly prepared metal ionsolutions, which was used as the blanks.

Results and Discussion

Effect of pH on the anthocyanin extracts

Figure 5shows the color changes obtained for the pH variation of the extract. The extract became greenish blue at pH 10, however, it is not clearly visible in Figure 5. Figure 6 illustrates the UV-Visible spectra of anthocyanin at pH = 2, 4, 6, 8, 10 and 12.

Figure 5: Color of the red cabbage anthocyanin extract at the pH = 2, 4, 6, 8, 10 and 12.

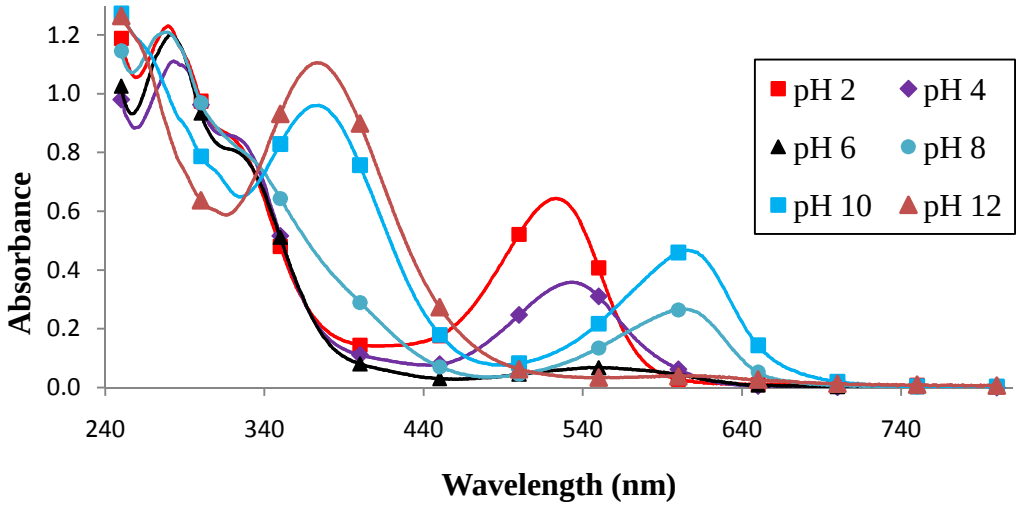

Figure 6: The UV-Visible spectra of extract at the pH = 2, 4, 6, 8, 10 and 12.

Based on Figure 6, an isosbestic point can be identified at 340 nm. The effect of metal ion chelation on the absorption spectrum of the anthocyanin extract was studied at pH = 4. This was due to the fact that most metal ions tend to either precipitate or undergo side reaction at higher pH values.

Figure 7 shows the absorption spectra of Al^{3+}-anthocyanin and Ni^{2+}-anthocyanin solutions. Both of these spectra indicate that there is no significant effect on metal ions on the extract's absorption spectrum. This may be because these two metal ions do not form complexes with the anthocyanins in the extract at this pH = 4.

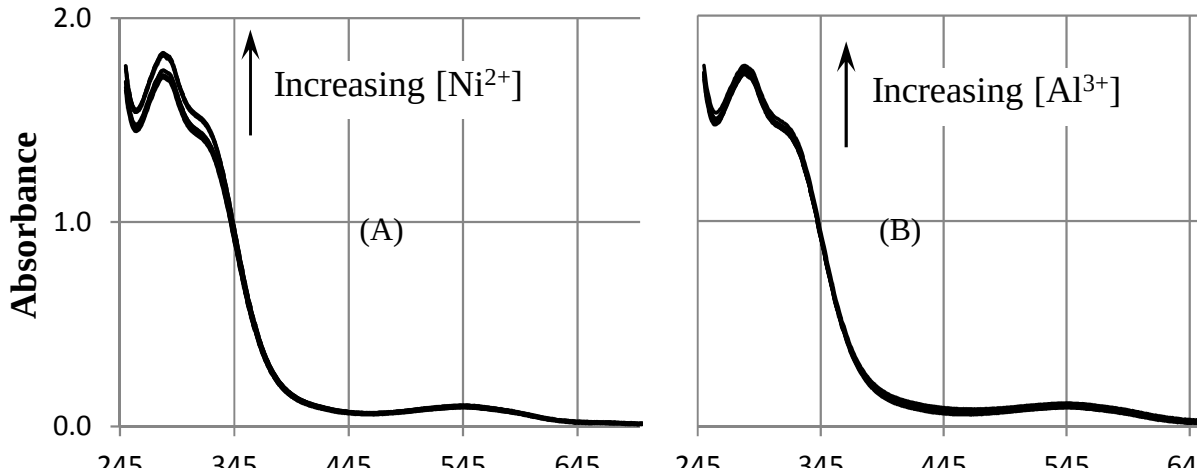

Figure 7: Absorption spectra for extract-metal ion complexes at pH 4 for (A): Ni^{2+} and (B): Al^{3+}.

Comparatively, the Fe^{3+}-anthocyanin system (Figure 8) and the Cu^{2+}-anthocyanin system (Figure 9) behave in a very different manner. Based on Figure 8, the overall absorbance has increased in the region 250 – 380 nm. The absorbance at 281 nm of the free extract gradually increased as the Fe^{3+} concentration increases. This implies that Fe^{3+}chelates with anthocyanins in the extract. There is no significant change in the spectra above 380 nm. A minor peak shift towards lower wavelengths can also be observed as the Fe^{3+} concentration increases.

A similar behavior can be observed with the Cu^{2+}-anthocyanin system. The absorbance at 283 nm gradually increased as the Cu^{2+} concentration increased. A minute peak shift toward shorter wavelengths was also noticed.

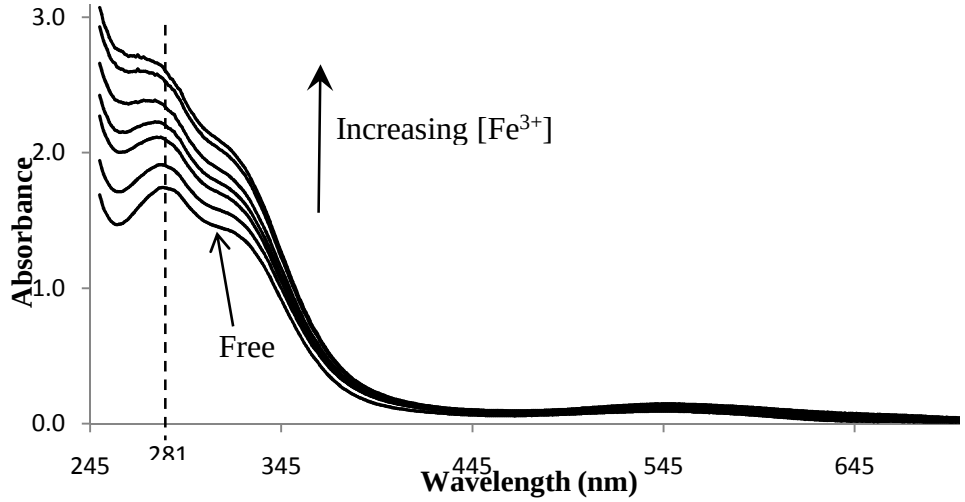

Figure 8: Absorption spectra for Fe^{3+}-anthocyanin complexes at pH 4

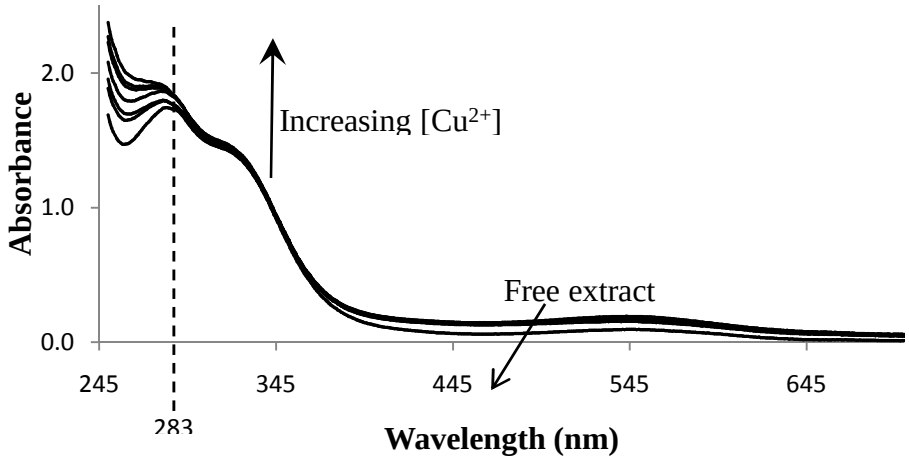

Figure 9: Absorption spectra for Cu^{2+}-anthocyanin complexes at pH 4.

Figure 10 shows the absorbance vs. concentration graph for Fe^{3+}-anthocyanin mixture at 281 nm. The graph shows a linear relationship between the absorbance at 281 nm and the concentration of Fe^{3+}.

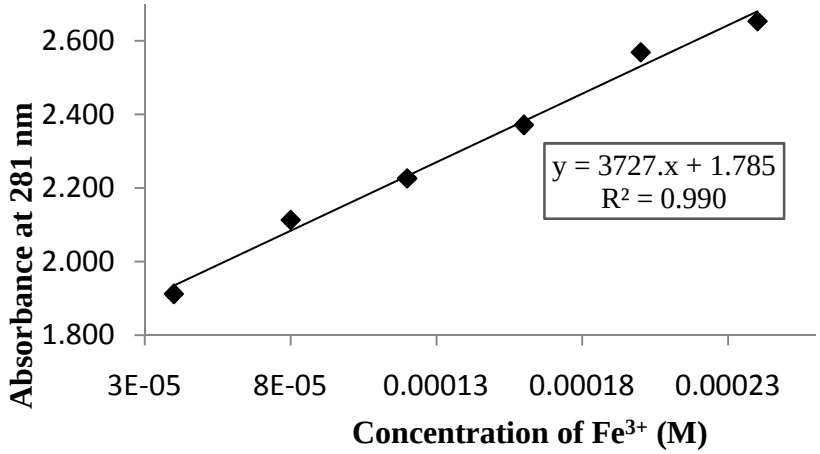

Figure 10: Absorbance vs. concentration graph for Fe^{3+}-anthocyanin at 281 nm

Based on results shown in Figure 10, the anthocyanin extract gives a linearly proportional response to the Fe^{3+} concentration and therefore, it is possible to use anthocyanins as chemical sensors for Fe^{3+}. In contrast, Cu^{2+} does not yield such a linear curve and this can be observed in Figure 11.

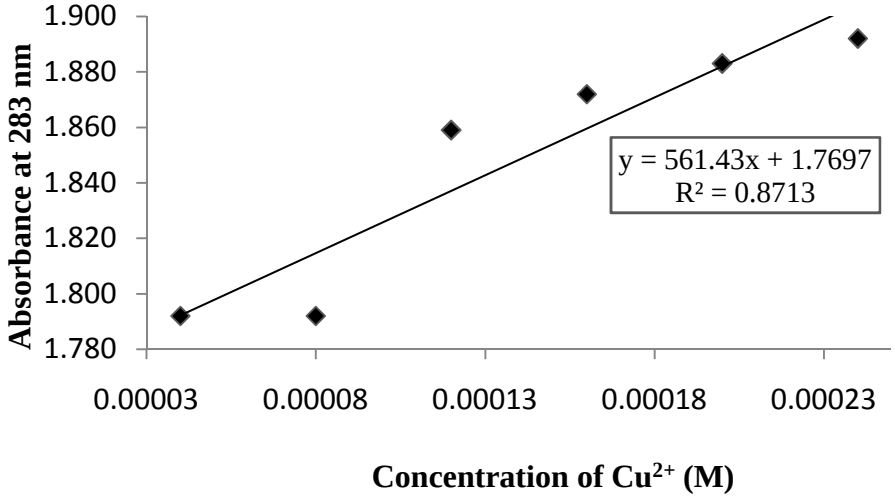

Figure 11: Absorbance vs. concentration graph for Cu^{2+}-anthocyaninat 283 nm

Investigation of the electrochromic properties of anthocyanin

None of the metal-ion-anthocyanin mixtures showed electrochromic properties under the test conditions used in these experiments (Figure 12). Absorbance spectra at all the potentials superimposed on each other and no significant change was observed with the applied potentials under the conditions used in aqueous conditions.

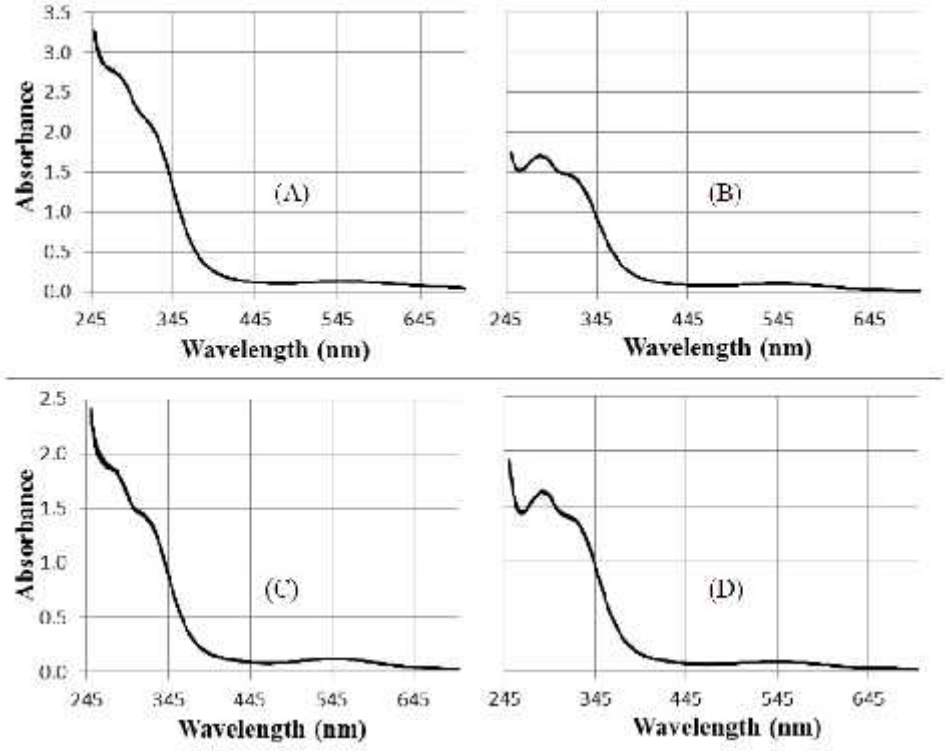

Figure 12: Absorption spectra for extract-metal ion mixtures at pH 4 under different potentials for(A): Fe^{3+}-anthocyanin, (B): Al^{3+}-anthocyanin, (C): Cu^{2+}-anthocyanin, (D): Ni^{2+}-anthocyanin.

Conclusions

The pH of the medium plays a significant role in the photophysical properties of red cabbage extracts.Fe^{3+} and Cu^{2+} have a strong affinity towards anthocyanins in red cabbage extract. Fe^{3+} concentrations produced a linear relationship with absorbance. The anthocyanins can be used to develop a chemical sensor to detect Fe^{3+} in aqueous solutions. Further experimentation is necessary to investigate whether red cabbage extracts or any of its anthocyanin components can be utilized as electrochromic materials under different conditions.

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