KINETICS OF DEGRADATION OF DYE EXTRACTED FROM RED CABBAGE

A DISSERTATION SUBMITTED

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On the recommendation and done under the supervision of **Prof. Dr. Raja Ram Pradhananga**, this dissertation work of **Mr. Nirmal Phuyal** entitled "**Kinetics of Degradation of Dye Extracted from Red Cabbage**" has been approved hereby for the examination and is submitted to the controller of examinations, Tribhuvan University for the partial fulfillment of the degree of Masters of Science (M.Sc.) in Chemistry.

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This is to certify that Mr. Nirmal Phuyal has completed this dissertation work entitled **"Kinetics of Degradation of Dye Extracted from Red Cabbage"** as a partial fulfillment for the requirement of M.Sc. Degree in Chemistry under my supervision. To the best of my knowledge, this is his original work and has not been submitted to pursue any other degree.

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I, Nirmal Phuyal, hereby declare that the work presented herein is genuine work done originally by me and has not been published or submitted elsewhere for the requirement of a degree program. Any literature, data or works done by others and cited in this dissertation has been given due acknowledgement and listed in the reference section.

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ABSTRACT

The pH-dependent color showing property of anthocyanins as natural dye has many applications in food colouring and packaging, pH monitoring, and textiles industry. Due to the potent use of anthocyanins as natural dye, it is desirable to study its kinetic stability against oxidizing agent like sodium hypochlorite and understand the mode of reaction between them. The natural dye is extracted from red cabbage (*Brassica oleracea L. var. capitata f. rubra*) in aqueous medium. The amount of anthocyanin in the dye extracted from 100 grams fresh leaves was estimated to be about 7.8 mg. The extracted dye was buffered at pH of 2, 4, 6, 8 and 10 which showed distinct colors at different pH. The absorption spectrum of the buffered dye of each pH was recorded from 400 nm to 700 nm. The λ_{max} of the dye ranged from 525 nm to 602 nm resulting in bathochromic shift with the increase in pH of the dye with the lowest specific absorptivity at pH range of 4-6. The study of the colour and spectra showed that the simple aqueous extract of the red cabbage can be used as an excellent pH indicator.

The kinetics of dye degradation on treatment with sodium hypochlorite at different pH was studied spectrophotometrically. The rate of oxidative degradation of dyes increases significantly with the increase in acid concentration. The order of the reaction with respect to dye concentration was two as determined by graphical and half-life method. The reaction follows a complex kinetics and the decolorization of dye is very fast when the concentration of hypochlorite is appreciable. Hence, it requires an advance technique for the detailed kinetics study to establish the reaction mechanism. In the present investigation kinetic stability of the dye against hypochlorite was investigated in detail.

Keywords: Anthocyanins, natural dye, Absorption spectrum, λ_{max}, Aqueous extract Order of reaction, Specific absorptivity, Bathochromic shift

ABBREVIATIONS AND ACRONYMS

% v/v : Percentage volume by volume

% v/w : Percentage volume by weight

DF : Dilution factor

EtOAc : Ethyl acetate

EtOH : Ethanol

g/mol : Gram per mole

L/cm mol : Litre per centimetre mole

MeOH : Methanol

mg/L : Milligram per litre

mg/mL : Milligram per millilitre

Mol. Wt. : Molecular weight

MS/MS : Mass spectroscopy – mass spectroscopy (Hyphenated)

nm : Nanometre

sec : Second

TLC : Thin layer chromatography

UPLC : Ultra performance liquid chromatography

UV-VIS : Ultraviolet-visible

ε : Molar extinction coefficient

λ_{max} : Wavelength of maximum absorption

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CHAPTER I
INTRODUCTION

1.1 Background

Red cabbage is used as a leafy vegetable and found in almost every region of the world. It is scientifically known as *Brassica oleracea var. capitata f. rubra*, that belongs to the Brassicaceae family. It is closely related to other cabbage varieties, such as green cabbage, broccoli, and Brussels sprouts. It is recognized for its striking purplish-red color. The colour of this plant gives a noticeable contrast to it as well as it adds value of immense importance to this plant. The colour of this plant is due to the presence of a group of pigments called anthocyanins (Haddar et al., 2018).

Figure 1: Red cabbage and its sliced view

Red cabbage is a cool-season crop that thrives in moderate climates with temperatures ranging from 45 °F to 75 °F (7°C to 24°C). It requires well-drained soil enriched with organic matter and prefers a pH level between 6.0 and 7.5 (Denis et al., 2016). It is cultivated and produced locally in Nepal too.

This vegetable is largely consumed as fresh salads worldwide. It has numerous health benefits which helps in antiaging, boosting immunity and promoting digestive and heart health. The health benefit of this plant is largely contributed by the colour pigment, anthocyanins, in it and its chemistry has been studied extensively (Ghareaghajlou et al., 2021).

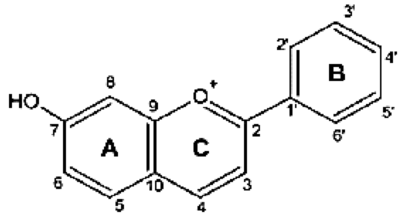
1.2 pH dependent chemistry of anthocyanins

Anthocyanins (in Greek anthos means flower, and kyanos means blue) are a group of plant pigments that are widely distributed in nature, among flowers, fruits and vegetables, and are responsible for red, purple and blue colors (Kong et al., 2003).

A variety of fruits and vegetables, including raspberries, blueberries, concord grapes, blackberries, strawberries, peaches, egg-plant, red cabbage, and red onions, contain anthocyanins that are responsible for the blue-red color and the astringent taste associated with such foods. In addition, anthocyanins exhibit a wide range of chemical properties, such as radical scavenging, metal chelation, pH-dependent color changes, and intramolecular stabilization (Galloway et al., 2015).

Chemically, the anthocyanins are polyphenols and member of flavonoids group. They are derivatives of the 7-hydroxyflavylium cation (flavylium nucleus) with additional hydroxyl group as shown in the structure scheme in figure 2(a). Several thousand known anthocyanins contain the same flavylium nucleus and are derived from one of the six basic anthocyanidin (or deglycosylated anthocyanin) structures shown in figure 2(b). They differ only in the number of hydroxyl and/or methoxy substituents in the phenyl or B-ring (Silva et al., 2016).

a)



b)

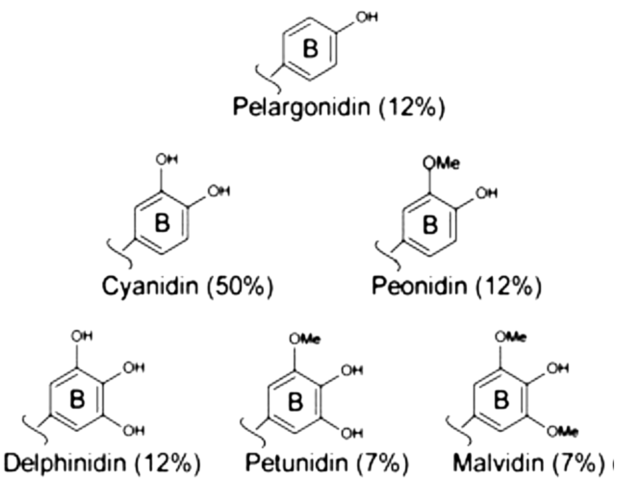


Figure 2: a) Structure of 7-hydroxyflavylium cation with standard numbering b) The six natural anthocyanidin structures with the corresponding names and relative abundances. (Silva et al., 2016)

It is well established fact that anthocyanins are water-soluble colored pigments whose color and stability depends on pH, light, temperature, solvent, and structure. The pH-dependent color showing property of anthocyanins has been extensively studied (Roy & Rhim, 2021).

There exists a series of pH-dependent chemical equilibria between four different structures: flavylium cation (AH^+), quinoidal base (A), carbinol base (hemiketal, B), and chalcone (C_E & C_Z). Each of these structures possess their characteristic colour. When pH is less than 2.5 the flavylium cation is predominant. The positive charge is delocalized through all the pyrylium moiety. This allows for the electron delocalization through the ring and give it a dark red colour (Escribano-Bailón et al., 2019).

When pH is increased, with the removal of hydrogen ions from the medium competition occurs between two reactions. One of them is the hydration of the flavylium cation to give colorless hemiketal forms (B) and the other is the deprotonation of the acidic hydroxyl groups to yield quinonoidal bases (A) at slightly higher pH. In the pH range of 3 to 6, the former reaction takes place predominantly. The flavylium cation suffers the nucleophilic addition of H_2O on C-2 of anthocyanin, leading to the formation of colorless carbinol base (hemiketal form). This species leads by a ring-opening tautomeric process to the pale yellow or colourless chalcones as shown in the reaction scheme in figure 2. At pH values close to 7, pre-existing quinonoidal bases undergo a second deprotonation leading to more bluish resonance-stabilized quinonoidal anions (Santos-Buelga & González-Paramás, 2019).

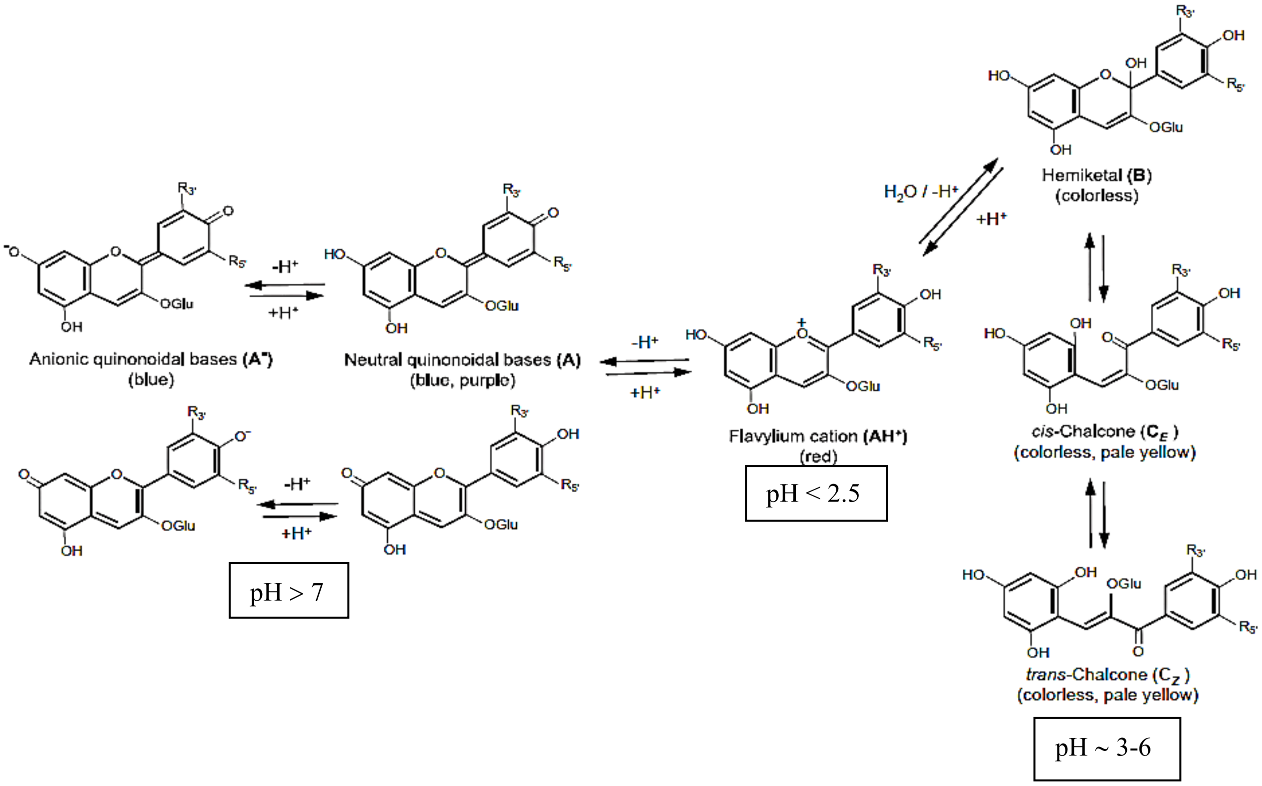


Figure 3: pH-dependent equilibria between different structures of anthocyanins

Hence, colour spectrum of anthocyanins at different pH is wide and the colour differs from each other at different pH. It is red at pH less than 3. At the pH range of 3-6 the mixed colour of quinonoidal and hemiketal form is observed, i.e, mixture of purple, blue and yellow. At pH more than 7, blue colour is vividly observed.

1.3 Stability of anthocyanins

Generally, anthocyanins are quite unstable and undergo degradation easily. Owing to this reason the usability of anthocyanins in different applications is limited. Structurally it is seen that both aromatic and aliphatic acylation and glycosylation in ring C, primarily at position 3 and 5, improves the stability. By this it becomes more resistant to hydration at slightly acidic or neutral medium. In the same way, stability also increases with the increase substitution of the hydroxyl groups in ring B by methoxy groups. The degradation of anthocyanins become easier in the presence of nucleophiles like bisulfite ion (HSO_3^-). In addition, the oxygen-rich environment and light exposure has bleaching effect on anthocyanins (Santos-Buelga & González-Paramás, 2019).

The degradation of anthocyanins is observed at medium-high temperature. High temperature affects stability prominently at high pH. Hence, keeping the temperature low enhances the stability and color is also preserved (Escribano-Bailón et al., 2019).

The use of natural and bio-degradable dye as food additives and in textiles too has obtained great attention in recent years. The researchers have been studying to replace the use of inorganic dyes and in this context, anthocyanins from natural products are seen as a potential candidate. However, the applications of anthocyanins as food colorant are seriously limited due to its stability. They easily undergo thermal degradation and suffer colour change at different pH conditions.

This research is focused to investigate the stability of natural dye obtained from red cabbage under chemical influence. Thus, it is desired to study the degradation of anthocyanins dye by sodium hypochlorite kinetically using UV-VIS spectroscopy.

1.4 Chemistry of sodium hypochlorite

Sodium hypochlorite (NaClO) is a pale greenish-yellow solution that is commonly known as bleach. It is a strong oxidizing agent and is widely used in a variety of applications, including as a disinfectant, a bleaching agent, and a water treatment chemical.

Sodium hypochlorite is a sodium salt of hypochlorous acid that comprises sodium ion (Na^+) and hypochlorite ion (ClO^-). The stability and effectiveness of sodium hypochlorite can be greatly affected by changes in pH. The normal aqueous solution of sodium hypochlorite is alkaline with pH 11 or more. It is because hypochlorite ion causes the hydrolysis in normal aqueous solution to give hydroxide ion as:

$OCl^{-} + H_2O \rightleftharpoons HOCl + OH^{-}$

The chemistry of sodium hypochlorite involves its ability to release hypochlorous acid (HClO) upon reaction with water. At the pH of 11 or higher, the hypochlorite ion (OCl^{-}) is the predominant species but as the pH decreases below 10, the hypochlorite ion gradually reacts with hydrogen ions (H^{+}) to form hypochlorous acid (HOCl) and chloride ions (Cl^{-}). As pH goes below 7, hypochlorous acid is the predominant species and its amount becomes the highest at pH around 9. When solution is increasingly acidic, at around pH less than 2, due to the addition of strong acid like HCl, elemental chorine is produced which remains either dissolved or escape from the solution (Wang et al., 2007).

$HOCl\ (aq) + Cl^{-} + H^{+} \rightleftharpoons Cl_2\ (aq) + H_2O$

$Cl_2\ (aq) \rightleftharpoons Cl_2\ (g)$

The equilibrium can be greatly shifted by the addition of strong acids or bases to favor the formation of elemental chlorine and hypochlorite ion respectively (Oxychem, 2014).

$ClO^{-}\ (aq) + 2\ HCl\ (aq) \rightarrow Cl_2\ (g) + H_2O\ (aq) + Cl^{-}\ (aq)$

$Cl_2\ (g) + 2\ OH^{-} \rightarrow ClO^{-}\ (aq) + Cl^{-}\ (aq) + H_2O\ (aq)$

With variation in pH sodium hypochlorite avails different oxidizing agents like hypochlorite ion, hypochlorous acid and elemental chlorine. These different species obviously have varying oxidizing ability and hence alter the rate of their oxidative reaction.

The stability of the sodium hypochlorite is also affected by temperature as it decomposes to produce chlorate and chlorides at high temperature. The decomposition ends up with the formation of oxygen gas (Sandin et al., 2015). The reaction is presented below.

$3\ NaOCl\ (aq) \rightarrow 2\ NaCl\ (aq) + NaClO_3\ (aq)$

$2\ NaOCl\ (aq) \rightarrow 2\ NaCl\ (aq) + O_2\ (g)$

1.5 Use of UV-VIS spectroscopy in kinetic study

Ultra Violet-visible (UV-VIS) spectroscopy is a powerful analytical technique that investigates the interaction of matter with light in the ultraviolet (UV) and visible (visible) regions of the electromagnetic spectrum. It provides valuable insights into the electronic structure and properties of molecules and materials. By measuring the absorption or transmission of light at different wavelengths, UV-VIS spectroscopy enables the identification of chemical compounds, quantification of their concentrations, and characterization of their molecular environments. It is a non-destructive technique which finds wide applications in fields such as chemistry, biochemistry, materials science, pharmaceuticals, environmental science, and forensic analysis.

UV-VIS spectroscopy is an invaluable tool for studying the kinetics of chemical reactions. By monitoring changes in the absorbance or transmittance of light at specific wavelengths, it allows to track the progress of a reaction in real-time. In a kinetic study, UV-VIS spectroscopy can provide insights into reaction rates, reaction mechanisms, and the influence of various factors on the reaction kinetics. The technique is particularly useful when the reaction involves species that exhibit distinct absorption bands in the UV- VIS range, such as chromophores or transition metal complexes. The dye component, anthocyanins, from red cabbage absorbs in the visible range and its kinetic degradation can be studied by spectroscopic method.

To perform a kinetic study using UV- VIS spectroscopy, the reaction mixture is continuously subjected to spectroscopic measurements at regular intervals. By analyzing the time-dependent changes in absorbance or transmittance, the concentration of reactants or products can be determined as a function of time. The rate of the reaction can then be calculated by analyzing the concentration-time data using appropriate kinetic models.

1.6 Determination of order of the reaction

The order of the reaction with respect to a reactant is the power to the concentration of that reactant in the rate law. Sum of all the powers to which concentration terms are raised in the rate law is the overall order of the reaction. Order of the reaction needs to be determined by performing series of experiments for further investigation of kinetics of the reaction to establish the mechanism of the reaction.

Order of the reaction for simple reaction can be determined by graphical method using integrated rate equation or half-life method. In recent methods, suitable mathematical modelling is developed and orders are determined.

1.6.1 Graphical method

In graphical method, the order of the reaction with respect to a certain reactant is verified by plotting the appropriate function of concentration against time. Concentration vs time, natural log of concentration vs time and inverse of concentration vs time is plotted to ascertain whether the reaction is following the zeroth, first or second order kinetics. Whichever plot among these gives the best straight line, corresponding order is followed by the reaction. The best fit can be found out by regression analysis of the plot and finding out the correlation coefficient (Pradhananga & Sthapit, 2008).

1.6.2 Half-life method

Half-life is the time required to complete half of the reaction. In this method, half-lives for at least two different initial concentrations are determined. If [A] represents the initial concentration, $t_{1/2}$ represents the half-life and n is the order of the reaction, they are related as:

$$t_{1/2} \propto \frac{1}{[A]^{n-1}}$$

If t_1 and t_2 are half-lives when initial concentrations are $[A]_1$ and $[A]_2$ respectively, then order of the reaction can be expressed as:

$$n = 1 + \frac{\log \frac{t_1}{t_2}}{\log \frac{[A]_2}{[A]_1}}$$

After determining half-lives for at least two different initial concentration, order can be calculated by this relation (Pradhananga & Sthapit, 2008).

CHAPTER II

LITERATURE REVIEW

Red cabbage is a suitable candidate for the extraction of anthocyanins dye. Charron et al. (2007) has reported the detection of six nonacylated and 30 acylated anthocyanins in red cabbage. Wiczkowski et al. (2013) stated that red cabbage is a rich source of acylated anthocyanins possessing strong antioxidant activity and acylated anthocyanins display marked stability. Giusti & Wrolstad (2003) concluded that the acylated anthocyanins, as those present in some vegetables like colored potatoes, black carrot, red radish, or red cabbage, usually possess greater heat and light stability, so that they are better candidates for their use as colorants. Since the degradation of anthocyanins present in red cabbage occurs comparatively slower it offers greater color stability too.

Anthocyanins from the red cabbage has been extracted using different type of extracting samples, extraction media and methods. Chandrasekhar et al. (2012) extracted anthocyanins simply from fresh leaves pieces by solid-liquid extraction method. They studied the efficiency of extracting media like water, pure and different volume mixture of acidified water, ethanol, methanol, and acetone. The highest anthocyanin content (390.6 mg/L) was obtained with the mixture of 50% (v/v) ethanol and acidified water. They also reported the demerit of the such conventional extraction methods. It was reported that such extraction methods yield pigment solutions with large amounts of byproducts such as sugars, organic acids, and proteins. Some of these impurities may accelerate anthocyanin degradation.

Wiczkowski et al. (2013) employed a very sophisticated extraction method. They used pulverized dried leaves which was obtained after lyophilization with liquid nitrogen. The extraction mixture was sonicated, vortexed and centrifuged alternately. The optimal extraction was achieved with the solvent mixture of ethanol/water/trifluoroacetic acid mixed in the ration of 0.58/0.38/0.04 (v/v/v).

Since, anthocyanins are water soluble pigments, a simple extraction procedure employing fresh red cabbage leaves and suitable extracting media at slightly elevated temperature can give good results.

The concentration of the anthocyanins extracted has been estimated by pH differential method in many researches. pH differential method is a rapid and simple spectrophotometric method based on the anthocyanin structural transformation that occurs with a change in pH (colored at pH 1.0 and colorless at pH 4.5).

Fuleki & Francis (1968) originally developed the pH differential method. Lee et al. (2005) along with eleven collaborators conducted a study to determine the efficacy of pH differential method. They employed pH differential method to determine the total monomeric anthocyanin concentration from fruit juices, beverages, natural colorants, and wines. The repeatability relative standard deviation varied from 1.06 to 4.16%. The reproducibility relative standard deviation ranged from 2.69 to 10.12%. The study director recommended the method be adopted officially for the determination of anthocyanin concentration.

The equation used by Chandrasekhar et al. (2012) for pH differential method was:

$$\text{Anthocyanin pigment concentration (mg/mL)} = \frac{\text{A} \times \text{Mw} \times \text{DF}}{\varepsilon} \times \text{L}$$

Where, $A = (A_{530} - A_{700})_{\text{pH } 1.0} - (A_{530} - A_{700})_{\text{pH } 4.5}$, Mw is the molecular weight of anthocyanin (449.2 g/mol), DF is the dilution factor, ε is the extinction coefficient (26,900 L/cm mol) and L is the path length (1 cm).

Maccarone et al. (1985) indicated that the degradation rate doubles for every 10 °C rise. Furthermore, thermal degradation of anthocyanins is influenced by pH, being more pronounced at high pH. Marҫo et al. (2011) has reported that seven different species of anthocyanins were detected in the absence of UV radiation but with the UV radiation exposure nine different species were detected. This indicated the degradation caused by the UV radiation.

This study focuses on the degradation and color changing property of the anthocyanins extracted from red cabbage due to treatment with hypochlorite. The proper mechanistic understanding of the bleaching reaction between anthocyanins and hypochlorite has not yet been fully established but thought of as being similar to the oxidative reaction with peroxide (Ramos et al., 2005; Resque et al., 2019). Resque et al. (2019) stated that the hypochlorite attacks the chromophore group of the anthocyanins, causing attenuation of the color of the anthocyanins while the hypochlorite is reduced to chloride.

Satake & Yanase (2018) studied the oxidation of cyanidin-3-O-glucoside by hydrogen peroxide and proposed a reaction mechanism. They also studied the effect of solvent on the reaction rate and the distribution of product. The reaction mechanism proposed was validated by UPLC-MS/MS using ^{18}O-labeled reagents.

Anthocyanins of red cabbage display color change over a larger range of pH-values than the majority of anthocyanins from other natural sources, being pink at pH 3, violet at pH 5 and blue at pH 7 (Dyrby et al., 2001). Consequently, in UV spectral analysis, the peak of maximum absorption also changes with the change in pH value.

The pH of the aqueous sodium hypochlorite solution is around 11-12. The introduction of hypochlorite solution changes the pH significantly due to which the color change of the anthocyanins cannot be attributed to the reaction occurred between them alone. The use of buffer is needed for the constant pH restoration throughout the proceedings of the reaction. The buffer system to be employed can be a universal buffer advised by Carmody (1963) which can produce a series of buffer solution from pH 2 to 12 or any other common buffer system. Furthermore, for the use in UV spectrophotometry, the buffer system can be adopted in accordance with the one advised by Davies (1959).

Ahmadiani et al. (2016) did the spectral analysis of the anthocyanins from red cabbage using UV spectrophotometry. In the study, pigments were dissolved in MeOH and diluted with either MeOH (0.1% HCl) or buffers to obtain final concentrations between 5×10^{-5} and 1×10^{-3} mol L^{-1}. The study has shown that the molar absorptivity (ε) in acidified MeOH and buffer pH 1 ranged between ~16,000–30,000 and~13,000–26,000 L mol^{-1} cm^{-1} respectively. Most pigments showed higher ε in pH 2 than pH 8, and lowest ε between pH 4 and 6. There were bathochromic shifts (81–105 nm) from pH 1 to 8 and hypsochromic shifts from pH 8 to 9 (2–19 nm). The solvent media was important variables which greatly influenced their ε and spectral behaviors.

CHAPTER III
OBJECTIVES

3.1 Statement of the problem

Applications of natural dyes obtained from plant and animal sources have been investigated in recent researches worldwide as colorants in textiles, food, medicine etc. and in advance technologies like dye sensitized solar cells. The instability of color component, i.e., anthocyanins, is the major problem. The instability is caused due to oxidation of the flavylium nucleus of anthocyanins primarily. The reaction mechanism of oxidation by peroxide has been proposed but the mode of oxidation and discoloration caused by hypochlorite has not been investigated. In textiles and so many other places, the bleaching is extensively used for cleaning purposes. If anthocyanin-developed natural dyes are to be used as colorants, it is worth studying the effect of bleaching caused by hypochlorite beforehand. The kinetic study of degradation of anthocyanin-dyes by hypochlorite can give insight to the mode of reaction between them and give idea about the instability of such dyes when exposed to oxidants.

3.2 General objectives

The general objective of this research is to study the degradation of natural dyes from red cabbage by hypochlorite kinetically.

3.2 Specific objectives

i. To extract the natural dyes from red cabbage in aqueous medium.
ii. To record UV-VIS absorption spectra of aqueous extract of the dye from red cabbage at different pH to explore the possibility of using the dye for pH monitoring.
iii. To study the variation of specific absorptivity of the dye with pH.
iv. To study the kinetics of degradation of the dye-color by spectrophotometric method in treatment with hypochlorite at different pH.

CHAPTER IV

MATERIALS AND METHODS

4.1 Extraction of the dye from red cabbage

Fresh red cabbage was purchased from local market in Kathmandu. The leaves of red cabbage were separated and were washed thoroughly by tap water followed by distilled water. After that, thick veinlets including midrib from the leaves was cut off and removed to obtain lamina with color-rich parts. The color-rich parts were finely chopped into small pieces as much as possible using a clean knife and scissors.

100 g of the finely cut pieces were soaked in 100 mL of distilled water at 40 °C for 3 hours. Then the extract was cooled at room temperature and filtered using Whatman filter paper no.1. The filtrate was collected for further examinations.

4.2 TLC of dye extract

Thin layer chromatography (TLC) of the dye extract was done to investigate the presence of multiple components of anthocyanins pigment. At first, aqueous extract was used to run TLC. But, because of the water content in the aqueous extract, it became difficult to concentrate the extract using rotary evaporator. Moreover, no separation could be achieved in any of the solvent system.

Aqueous extract was discarded and the methanol extract was obtained by soaking the cut pieces of red cabbage in methanol as explained above. 20-25 mL of the methanol extract was introduced into the rotary evaporator and run for half an hour at 45 °C and pressure of 200 psi to concentrate the extract.

The silica paper was used as thin layer stationary phase. The concentrated extract was introduced into the silica paper with the help of a pointed capillary tube and let to dry. The TLC was run on different combination of solvents like hexane, methanol, and ethyl acetate.

The combination ethyl acetate (EtOAc) and Methanol (MeOH) at the ratio of 5:3 and 7:3 served as the best solvent system to be used as mobile phase. The TLC was run and retention factor for each case was calculated.

4.3 Determination of available chlorine of sodium hypochlorite solution

The available chlorine of the sodium hypochlorite solution was determined iodometrically. Firstly, the sodium thiosulphate was standardized iodometrically by titrating against standard potassium bromate solution. Then, standardized sodium thiosulphate solution was used to determine the available chlorine of the sodium hypochlorite solution.

4.4 Observation of colour of the red cabbage extract at different pH

The colour of dye extract from red cabbage was observed at different pH from 2 to 12. 2 mL of dye extract each was taken in 6 test tubes. 0.1 M HCl and 0.1 M NaOH was added into each test tube from the burette dropwise as per requirement to adjust pH. pH was adjusted to get the value of 2, 4, 6, 8, 10 and 12. The pH was measured using a pH meter (HANNA instruments HI 8314) in all cases wherever discussed hereafter as well. Finally, the volume was made same for each test tube by adding distilled water manually and photograph was taken.

4.5 Preparation of buffer reagents

All the necessary buffer reagents were prepared from L.R. grade chemicals without further purification in distilled water.

a) 100 mL 0.2 M HCl solution

1.72 mL of available conc. HCl (11.66N) was transferred into 100 mL volumetric flask and distilled water was added up to the mark.

b) 100 mL 0.1 M HCl solution

50 mL of above prepared 0.2M HCl was taken and diluted to 100 mL in a 100mL volumetric flask using distilled water.

c) 100 mL 0.1 M potassium hydrogen phthalate solution

2.042 g of the potassium hydrogen phthalate (mol wt. 204.22 g/mol) was weighed and transferred into 100 mL volumetric flask and distilled water was added up to the mark.

d) 100 mL 0.1 M Na_2HPO_4 solution

1.419 g of di-sodium hydrogen orthophosphate (mol wt. 141.96 g/mol) was weighed and transferred into 100 mL volumetric flask and distilled water was added up to the mark.

e) 100 mL 0.1 M KH_2PO_4 solution

1.361 g of potassium dihydrogen orthophosphate (mol wt. 136.09 g/mol) was weighed and transferred into 100 mL volumetric flask and distilled water was added up to the mark.

f) 100 mL 0.1 M NaOH solution

0.4 g of NaOH (mol wt. 40 g/mol) was weighed and transferred into 100 mL volumetric flask and distilled water was added up to the mark.

g) 100 mL 0.2 M KCl solution

1.491 g of KCl (mol wt. 40 g/mol) was weighed and transferred into 100 mL volumetric flask and the solution was made by dissolving in the distilled water added up to the mark.

h) 100 mL 0.2 M CH_3COONa solution

0.641 g of NaOH (mol wt. 82.03 g/mol) was weighed and transferred into 100 mL volumetric flask and solution was made by dissolving in the distilled water added up to the mark.

4.6 Determination of concentration of anthocyanin in dye extracts

The concentration of anthocyanins in the dye-extract was determined by pH differential method. Two separate dye solutions of pH 1 and 4.5 were prepared using buffer reagents. Buffered dye solutions are prepared by mixing different buffer reagents and dye extract as shown in the table 1. Each solution was prepared by mixing 5 mL of the original dye-extract with a total of 15 mL of different buffer reagents to obtain desired pH value. The final buffered dye solution thus has the dilution factor of four.

Table 1: Preparation of study sample for the determination of concentration of anthocyanins

pH	Volume of buffer reagents required (Added to 5 mL dye-extract solution)
1	a) 12.3 mL of 0.2M HCl b) 2.7 mL of 0.2M KCl
4.5	a) 11.9 mL of 0.2M CH_3COONa b) 1.8 mL of 0.2M HCl c) 1.3 mL of distilled water

Once the study sample of pH 1 and 4.5 were prepared, the absorbance value of each sample was recorded at 530 nm and 700 nm. All the absorbance values here, as well as all the UV-VIS data, wherever mentioned hereafter too, were measured by double beam spectrophotometer (LABTRONICS LT-2802). The cuvettes used for the measurements were Glass Cuvette (MCG4/104 - Type 1).

Five absorbance values were taken in each case and the average was determined. The concentration of anthocyanins was determined by necessary calculations using the equation devised by Fuleki & Francis (1968).

The equation used was:

$$\text{Anthocyanin pigment concentration (mg/mL)} = \frac{\mathrm{A} \times \mathrm{Mw} \times \mathrm{DF}}{\varepsilon} \times \mathrm{L}$$

Where, $A = (A_{530} - A_{700})_{pH\ 1.0} - (A_{530} - A_{700})_{pH\ 4.5}$, Mw is the molecular weight of anthocyanin (449.2 g mol^{-1}), DF is the dilution factor, ε is the extinction coefficient (26,900 L mol^{-1} cm^{-1}) and L is the path length (1 cm).

4.7 Preparation of buffered dye solutions for absorption and degradation kinetics analysis

The absorption spectra and kinetic degradation study of the dye at pH 2, 4, 6, 8 and 10 were done. The buffered dye solution of each pH was prepared by taking 10 mL of dye-extract solution and mixing it with a total of 10 mL of buffer reagents as listed in left column of the table 2. For spectral and kinetic analysis, buffered aqueous solution of each pH was also prepared to dilute the buffered dye solution as listed in the right column of the table. The use of distilled water to dilute the buffered dye was avoided to discard the change in pH after dilution.

Table 2: Preparation of buffered dye solution for absorption and kinetic study

pH	Buffer reagent for **buffered dye solution** (Added to 10 mL dye extract)	Buffer reagent for **buffered aqueous solutions**
2	a) 6.2 mL of 0.1M pot. hydrogen phthalate b) 3.8 mL of 0.2M HCl	a) 15 mL of 0.1M pot. hydrogen phthalate b) 12.3 mL of 0.2M HCl
4	a) 9.7 mL of 0.1M pot. hydrogen phthalate b) 0.3 mL of 0.1M HCl	a) 20 mL of 0.1M pot. hydrogen phthalate
6	a) 4.3 mL of 0.1M Na_2HPO_4 b) 5.7 mL of 0.1M KH_2PO_4	a) 10 mL of 0.1M Na_2HPO_4 b) 10.4 mL of 0.1M KH_2PO_4
8	a) 6.9 mL of 0.1M Na_2HPO_4 b) 2.1 mL of 0.1M KH_2PO_4 c) 1.0 mL of distilled water	a) 15 mL of 0.1M Na_2HPO_4 b) 6.6 mL of 0.1M KH_2PO_4
10	a) 6.6 mL of 0.1M Na_2HPO_4 b) 2.6 mL of 0.1M NaOH c) 0.8 mL of distilled water	a) 20 mL of 0.1M Na_2HPO_4 b) 0.5 mL of 0.1M NaOH

4.8 Absorption spectra of the dye at different pH

The absorbance spectra of the extracted dye were determined at pH 2, 4, 6, 8 and 10 by using UV-VIS spectrophotometry. The sample solution for each pH case was prepared by taking a fixed volume of dye solution and mixed with buffered aqueous solution of same pH to stabilize pH and to have proper dilution to obtain clear peak in the spectra. For each pH case, 1 mL of the buffered extract solution was taken and mixed with 2 mL buffered aqueous solution of the same pH. Hence it was ensured that each sample solution has the same concentration of dye.

The sample solution was then transferred to cuvette and the cuvette was mounted into the spectrophotometer with a proper blank solution. The absorption spectra at each pH case were obtained by choosing the wavelength window of 400-700 nm setting the wavelength difference of 1 nm.

4.9 Kinetics degradation study of the dye

The kinetics of degradation of dye in treatment with sodium hypochlorite was studied by spectrophotometric method. The study was done at different pH and in 1 M HCl medium. It was done by recording the decay in absorbance of the dye sample over time in treatment with sodium hypochlorite. The concentration of the sodium hypochlorite solution taken in each case was 10% (v/v) of the original solution (available chlorine = 1.12 g).

The decay in absorbance was recorded at a specific chosen wavelength for each pH case. But it was found that the pH of the dye sample changed drastically when reacted with the sodium hypochlorite solution. Consequently, the wavelength of maximum absorbance (λ_{max}) also changed. The comparison of the change in pH of the buffered and non-buffered dye solution in treatment with sodium hypochlorite is shown in annex 1. It shows that, the change in pH in treatment with hypochlorite was greatly minimized by employing the buffered system. It also summarizes resistance offered by the buffered solution to change in pH due to addition of hypochlorite in comparison to addition of distilled water. Annex 2 shows the change in λ_{max} of the buffered dye before and after the treatment with sodium hypochlorite for kinetic study for each pH case. This change could be minimized using buffered solution.

With the help of these studies, the specific wavelength at which absorption decay is recorded for each pH case is selected. The specific wavelength for a particular pH was chosen so that it lies midway between the λ_{max} obtained for buffered dye solution before and after the treatment with hypochlorite at that pH. The wavelength selected for kinetic degradation study are summarized in table no. 3.

Table 3: Selection of wavelength for kinetic degradation study at different pH

pH	Wavelength selected
2	535 nm
4	545 nm
6	550 nm
8	575 nm
10	605 nm
1 M HCl medium	530 nm

4.9.1 Study at different buffered pH medium

The comparative study of kinetic degradation of dye at pH 2, 4, 6, 8 and 10 was done. Each study sample of a particular pH was prepared by mixing 1 mL buffered dye solution with 1 mL buffered aqueous solution of the same pH prepared according to table 2. To this sample 1 mL 10% v/v original hypochlorite solution was added. Hence, the final volume of the study sample prepared in each case was three times the original volume of the buffered dye taken including 1 mL of hypochlorite solution in each case.

When hypochlorite solution was added, the timer was started in a stopwatch alongside to mark the time of mixing. As soon as these solutions are mixed, it is transferred to cuvette by rinsing it once. Then the cuvette was mounted onto the spectrophotometer which is already set with the desired kinetic study mode.

The kinetic study was carried out by setting up all the essentials in spectrophotometer beforehand mixing of the reaction mixture and introduction of the study sample into the spectrophotometer. The setup chosen recorded the absorbance decay at 30 sec intervals at chosen wavelength for different pH case as per table 3. After mounting the study sample cuvette into the spectrophotometer with all the necessary adjustments, degradation in absorbance was initiated to record exactly when the stopwatch marked 15 seconds in each case. The data thus obtained was stored, formatted, and made ready for the analysis.

4.7.2 Study in HCl medium

The direct effect of acid concentration on kinetic degradation of the dye was studied. It was carried out in 1M HCl medium without the use of buffer solution. The buffering is avoided in this study because the pH and λ_{max} of the dye in 1 M HCl medium didn't change significantly in treatment with sodium hypochlorite. Two test samples, one with a high concentration of acid (S2) and the other with the low concentration of acid (S1) were prepared. The difference in volume of 1 M HCl was 2 mL in them. But, volume of 10 % (v/v) hypochlorite solution and original dye-extract solution taken in both samples were equal. The total volume of both samples was made 7.5 mL with distilled water as shown in table no. 4.

Table 4: Preparation of test sample solution in 1M HCl medium

Test solutions	Vol^{m}. of extract taken	Vol^{m}. of 1M HCl taken	Vol^{m}. of hypochlorite solution taken	Vol^{m}. of distilled water
S1	1.5 mL	1.5 mL	2.5 mL	2.0 mL
S2	1.5 mL	3.5 mL	2.5 mL	0.0 mL

The kinetic decay in the absorbance was studied at the wavelength of 530 nm which was selected in the same way as explained above as per annex 2. The experimental setup and the procedure followed was fundamentally same as before.

CHAPTER V

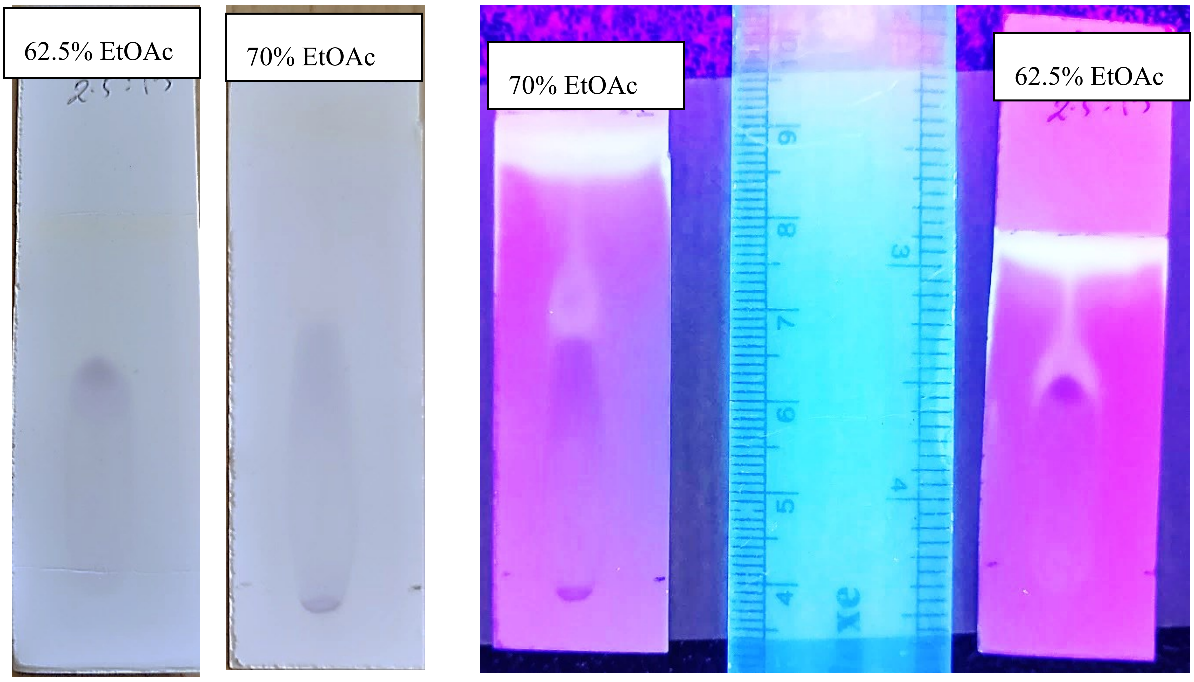
RESULTS AND DISCUSSION

5.1 Extraction of dye from red cabbage

The dye extract was obtained by the extraction method explained in experimental section. The extracted dye was pink in colour with pH 6.06. The dye extract was analysed by TLC and the concentration of anthocyanins in the extracted dye solution was determined.

5.1.1 TLC analysis of the dye extract

Thin layer chromatography was performed to confirm the presence of multicomponent of anthocyanins in the methanol-extracted sample. The best separation was obtained on the solvent system obtained by combination of ethyl acetate (EtOAc) and methanol (MeOH). TLC was run on two solvent system prepared by mixing EtOAc and MeOH at the ratio of 5:3 (62.5% EtOAc) and 7:3 (70% EtOAc). The result obtained is shown in the photos in figure no. 4.



a. Visualized under normal light

b. Visualized under the UV light

Figure 4: TLC of the dye obtained in methanol extract

The retention factor (R_f) was calculated based on the farthest distance the dye was carried up by the solvent using the formula:

$$R_f = \frac{\text{Distance travelled by the dye}}{\text{Distance travelled by the solvent front}}$$

Following are the calculated R_f values for two solvent systems.

62.5% EtOAc = 0.63

70% EtOAc = 0.50

The solvent system with 62.5% EtOAc is more polar because it has greater percentage of methanol which is more polar than the other component ethyl acetate. Higher R_f value for more polar solvent suggests that the extracted dye has greater affinity towards the polar solvent. This is because the dye is composed of anthocyanins which has poly hydroxy groups making it fairly polar.

Due to the interference of water (highly polar) appearing in the dye extract from fresh leaves, the clear separation of spots couldn't be observed. But still from the observation of the graded spread of colour, several anthocyanin compounds can be suspected in the dye extract.

5.1.2 Concentration of anthocyanins in the extracted dye solution

The concentration of anthocyanins was determined by pH differential method. Absorbance values of the dye at pH 1 and 4.5 were determined at the wavelength of 530 and 700 nm. The averaged mean of the five absorbance values recorded for each pH case are tabulated in table no. 5.

Table 5: Average absorbance values for the determination of anthocyanins concentration

pH of the buffered dye extract	Absorbance at 530 nm	Absorbance at 700 nm
pH 1	2.025	0.008
pH 4.5	0.869	0.021

The mathematical equation (Chandrasekhar et al., 2012) to determine the anthocyanins concentration is:

$$\text{Anthocyanin pigment concentration (mg mL}^{-1}) = \frac{\text{A} \times \text{MW} \times \text{DF}}{\varepsilon} \times \text{L}$$

Where, $A = (A_{530} - A_{700})_{\text{pH } 1.0} - (A_{530} - A_{700})_{\text{pH } 4.5}$

MW = Molecular weight of anthocyanins = 449.2 g mol^{-1}

DF = Dilution factor

ε = Molar extinction coefficient = 26,900 L mol cm^{-1}

L = Path length

In our case, A = (2.025 - 0.008) - (0.869 - 0.021) = 1.169, DF = 4 (Since, 5 mL of original dye solution was taken and made 20 mL adding buffer to maintain pH), L = 1cm.

By putting all the values in the formula, we get,

$$\text{Concentration of anthocyanins} = \frac{1.169 \times 449.2 \times 4}{26{,}900} = 0.07808 \text{ mg mL}^{-1}$$

Hence, the concentration of anthocyanins in the original dye extract solution is 0.07808 mg/mL. Since, 100 grams of the fresh leaves of red cabbage was soaked in 100 mL of distilled water for extraction, it showed that 7.808 mg of anthocyanins is extracted from 100 grams fresh leaves by the extraction method employed. The amount of the anthocyanins extracted is not very high as the extraction procedure was simple percolation method rather than any sophisticated methods.

5.2 Concentration of available chlorine in the NaOCl solution

The available chlorine in sodium hypochlorite solution was found to be 1.12 g (% w/v) as determined by iodometric method.

5.3 Colour of extracted dye at different pH

The colour of the extracted dye was observed by adjusting pH from 2 to 12. The photograph in the following figure no. 5 shows the colour of the dye at different pH. The colour of the dye extract at different pH match with the colour reported in the literature.

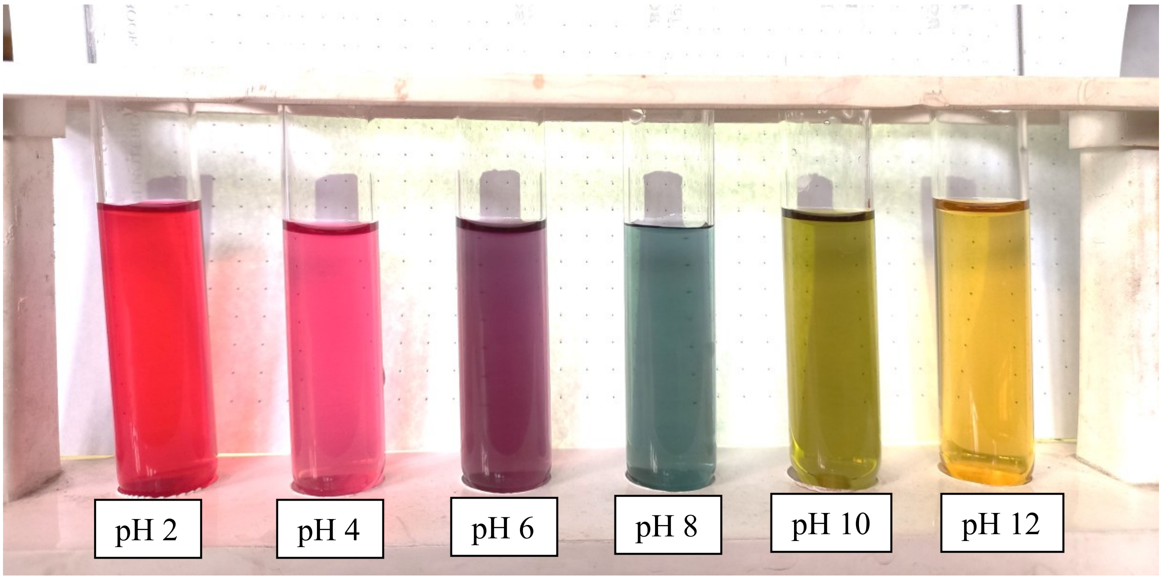


Figure 5: Colour of the dye at different pH

5.4 Absorption spectra of the buffered dye at different pH

The absorption spectra of the dye at pH 2, 4, 6, 8 and 10 were measured with proper dilution. The absorption spectra obtained are shown in figure no. 6 below.

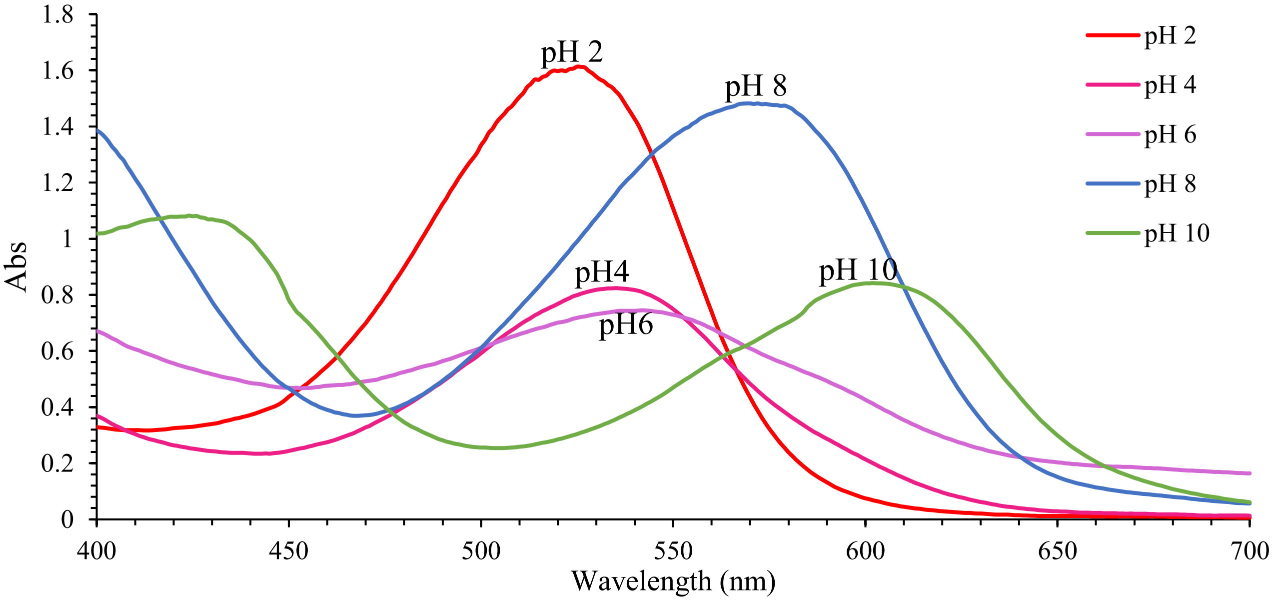


Figure 6: Absorption spectra of the dye solution buffered at different pH

The λ_{max} of the dye solution were found to be 525, 535, 542, 573 and 602 nm at pH 2, 4, 6, 8 and 10 respectively. It showed that the λ_{max} shift to the higher wavelength with the increase in pH, i.e., the bathochromic shift occurs as seen in figure no. 7.

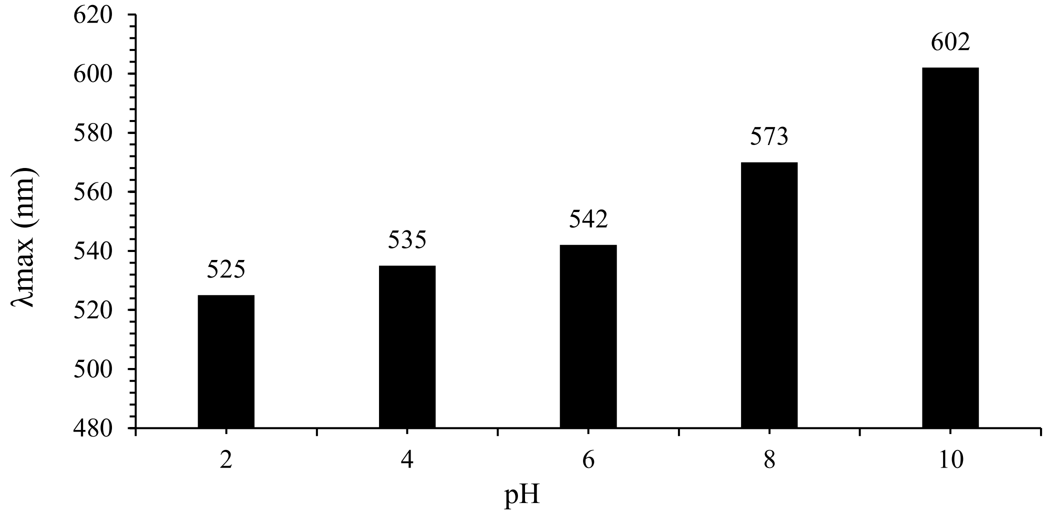


Figure 7: Wavelength of maximum absorption of the dye at different pH

The following figure no. 8 shows the absorption value at λ_{max} of the dye at different pH.

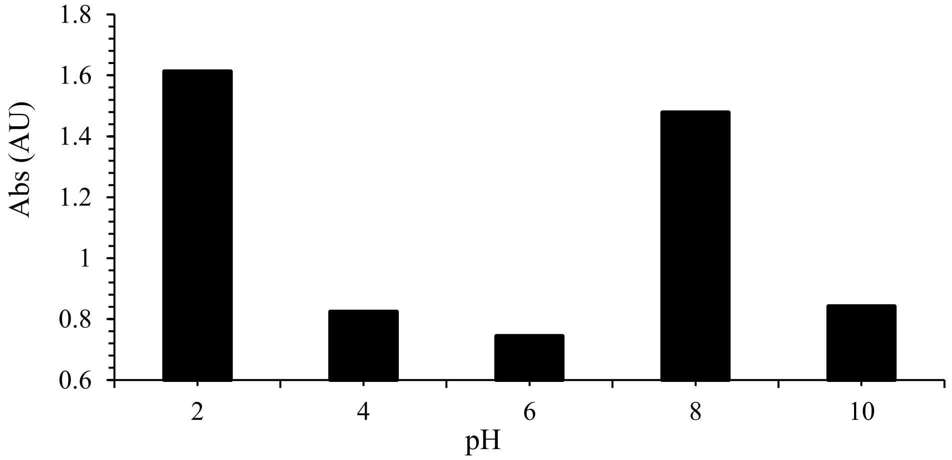


Figure 8: Maximum absorptivity of the dye at different pH

The specific absorptivity is found to be maximum in acidic solution at around pH 2 and drops to the minimum in the pH range of 4 - 6. The minimum absorption in the pH range of 4-6 is due to the predominance of non-absorbing hemiketal form in this pH range as reported in the literature (Ahmadiani et al., 2016; Santos-Buelga & González-Paramás, 2019).

The wide range of colour and spectra of the dye clearly showed that the dye extracted from red cabbage has distinct colors and absorbs at different wavelength at different pH. Hence, the aqueous extract of the dye can be used to monitor pH.

5.5 Kinetic study of degradation of dye

5.6.1 Degradation of dye in different pH buffered medium

The degradation studies of the dye at five different pH were investigated spectrophotometrically. The kinetic studies were done by taking the same amount of dye and a fixed concentration of sodium hypochlorite in all pH cases. The decay in absorbance of the dye with time in each pH case is shown in the figure no. 9.

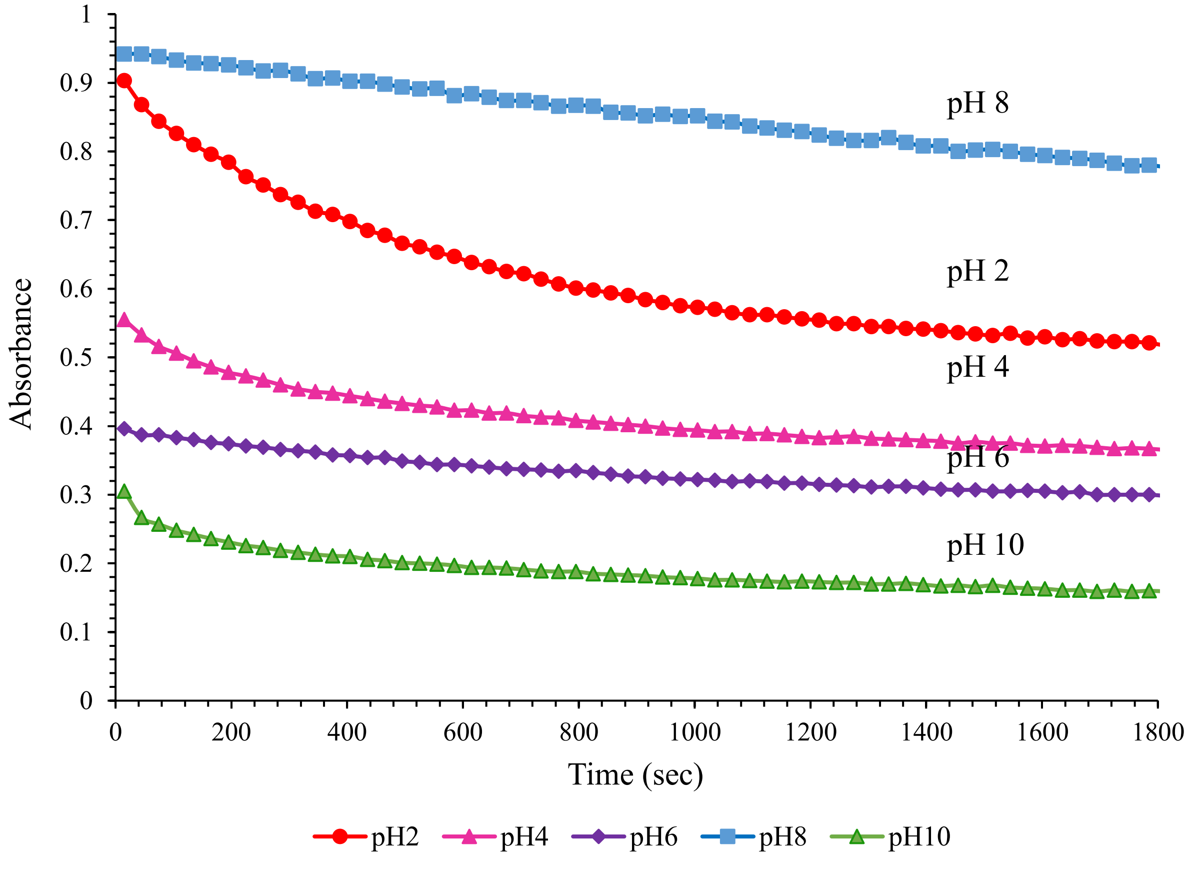


Figure 9: Decay in absorbance over time at different pH condition

Three kinds of degradation pattern can be vividly observed in above curves. Firstly, at pH 2, the degradation curve is somewhat of exponential nature with continuous degradation over the duration of study. Secondly, at pH 4 and 10, the degradation curves seem to be exponential but most of the degradation has occurred within a few seconds. Third kind is the degradation at pH 6 and 8 which seems to be linear. Roughly it looks like the rate of degradation goes on decreasing with the increase in pH.

The first absorbance was recorded exactly after 15 seconds of mixing of dye with hypochlorite and studied for next 30 minutes. Following table summarizes the percentage decay in absorbance value obtained at 15 seconds of the initiation of reaction within 30 minutes.

Table 6: Percentage decay in absorbance within 30 minutes at different pH

pH	**Abs at 15 s**	**Abs at 1815 s**	**% decay in abs**
2	0.903	0.517	42.74 %
4	0.555	0.365	34.23 %
6	0.396	0.298	24.74 %
8	0.942	0.777	17.51 %
10	0.305	0.159	47.86 %

Table no. 6 shows that the degradation of dye at pH 6 and 8 is very minimal. Though degradation at pH 4 and 10 is quite high, further kinetic study cannot be reliable for pH 10 as the degradation has occurred withing first few seconds but the data for first 15 seconds is not available for the study.

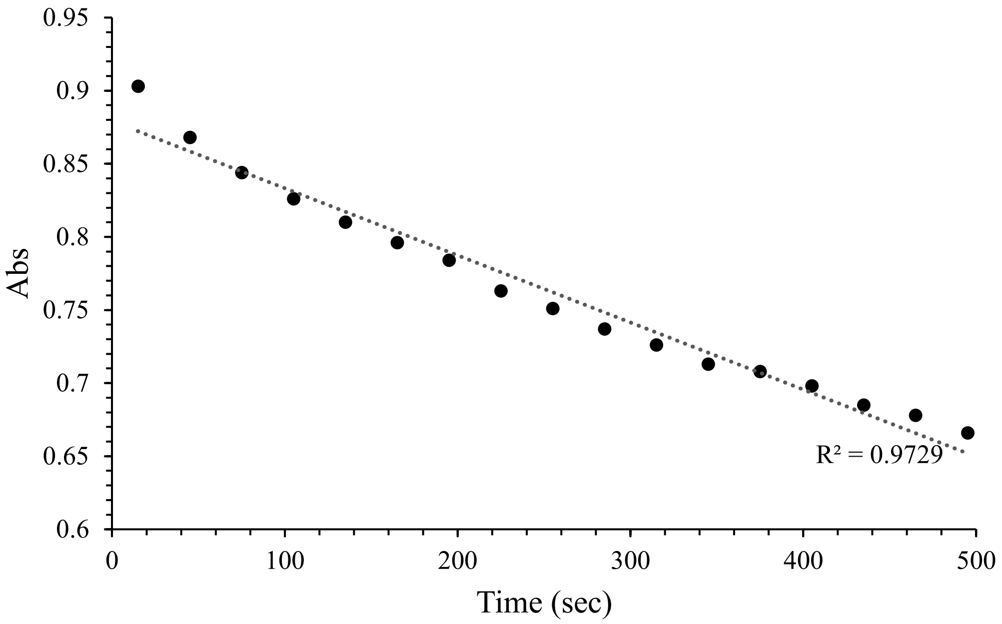
The degradation at pH 2 is significant in comparison to other pH cases and the continuous degradation occurred for a longer period in this case. The degradation at pH 2 condition was chosen for further investigation of reaction kinetics.

5.6.2 Kinetic analysis of degradation of dye at pH 2

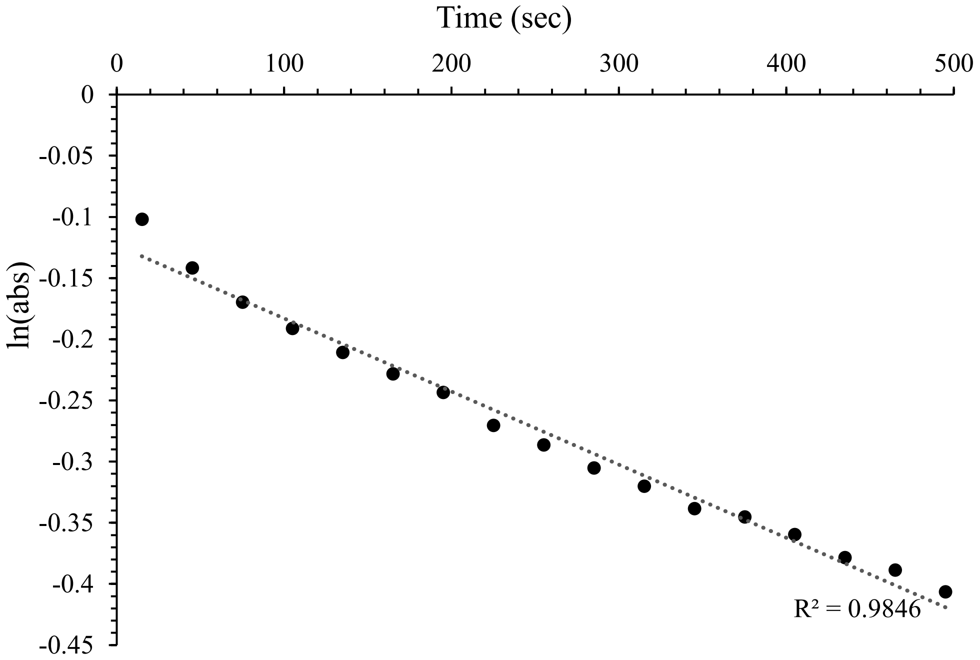
The degradation curve obtained at pH 2 in figure 9 was further analyzed to determine order of the reaction with respect to dye. Graphical method was employed to find whether the reaction followed the zeroth, first and second order kinetics. The zeroth, first and second order can be verified if a straight line is obtained for 'concentration vs time', 'natural log of concentration vs time' and 'inverse of concentration vs time' plot respectively.

In this case absolute concentration of the dye at different time was not available. But, as per Beer-lambert's law, concentration is proportional to absorbance and further, a linear relationship exists between concentration and absorbance in the absorbance range of 0.2-1.4 AU (Lee et al., 2005) and also in the anthocyanin concentration range of 100-500 µg mL^{-1} (Şakar et al., 2008). Hence, it is assumed that the concentration of the dye is proportional to the absorbance in the range of absorbance obtained throughout the kinetic study. Based on this, absorbance has been used as concentration value to develop the plot to investigate the order of reaction.

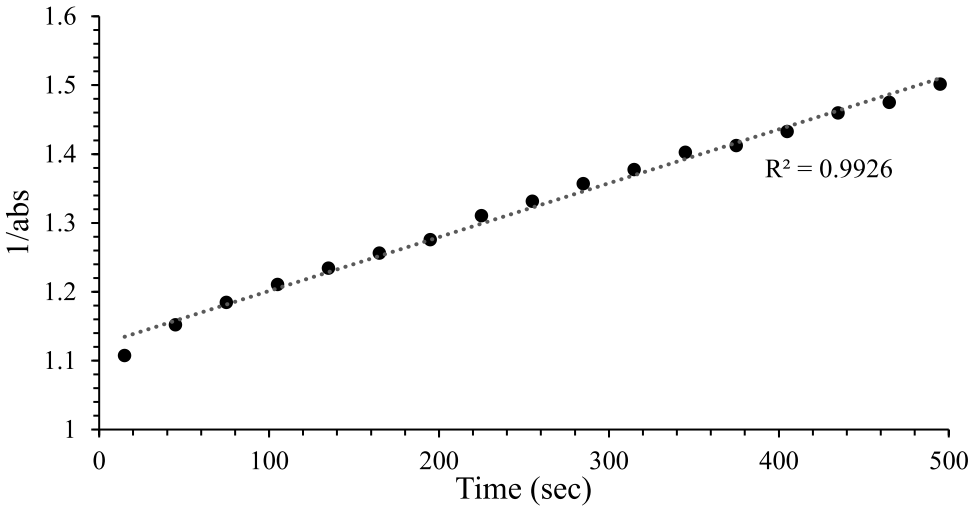
It was found that the best fitting was achieved with the degradation data for first 500 seconds. So, only the data obtained within the first 500 seconds have been used. Three plots, namely, absorbance vs time, natural log of absorbance vs time and inverse of absorbance vs time plots have been developed as shown in the following figure. Linear fitting of these curves is analyzed by using the R-squared value obtained from Microsoft excel 2019 software.



a) Absorbance vs time plot



b) Natural log of absorbance vs time plot



c) Inverse of absorbance vs time plot

Figure 10: Plots for the investigation of order at pH 2

The comparison of above three plots revealed that the inverse of absorbance vs time plot produced the regression line with R-squared value of 0.9926 which is the best fit for the linearity among three cases. It means that, at pH 2, the order of the degradation reaction with respect to dye concentration is close to 2. But the regression line produced in other two cases also have high R-squared value which means the possibilities of the reaction to have followed zeroth or first order kinetics cannot be discarded completely.

It is evident from the data in table 6, the absorbance in decay is not 50 % in any of the pH cases after 15 seconds. The reaction was extremely fast, so half-life method could not be employed to determine the order of the reaction since more than half of the dye is degraded in presence of hypochlorite within a few seconds.

Both the degradation curve and the data obtained at acidic condition (i.e., pH 2) were worth studying further kinetics. Hence, it is desirable to study the kinetics degradation of dye at very acidic condition. So further investigation was done in 1M HCl condition as explained in the experimental section.

5.6.3 Effect of acid concentration on degradation of the dye by hypochlorite

The effect of acid concentration in degradation kinetics was studied in 1M HCl medium. Two studies were done by varying the concentration of acid while keeping the concentration of dye and sodium hypochlorite fixed. The result is shown in the figure below.

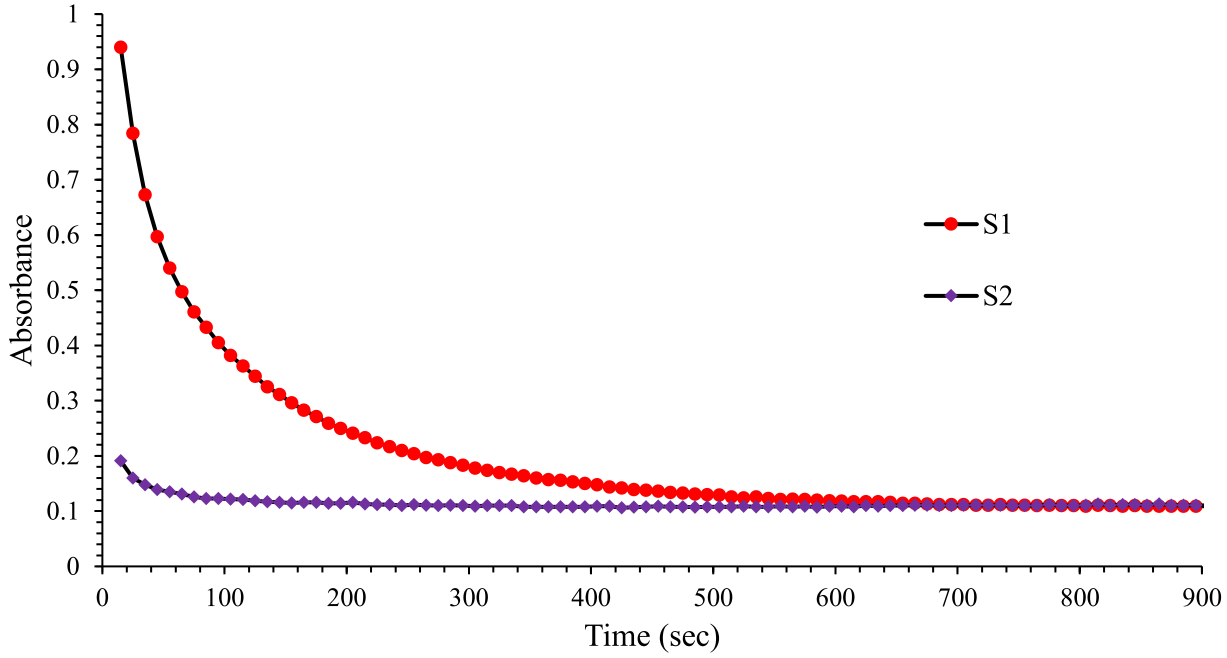


Figure 11: Effect of acid concentration on decay of absorbance over time

The initial concentration of HCl taken for these two study samples are:

S1 = 0.2 M

S2 = 0.47 M

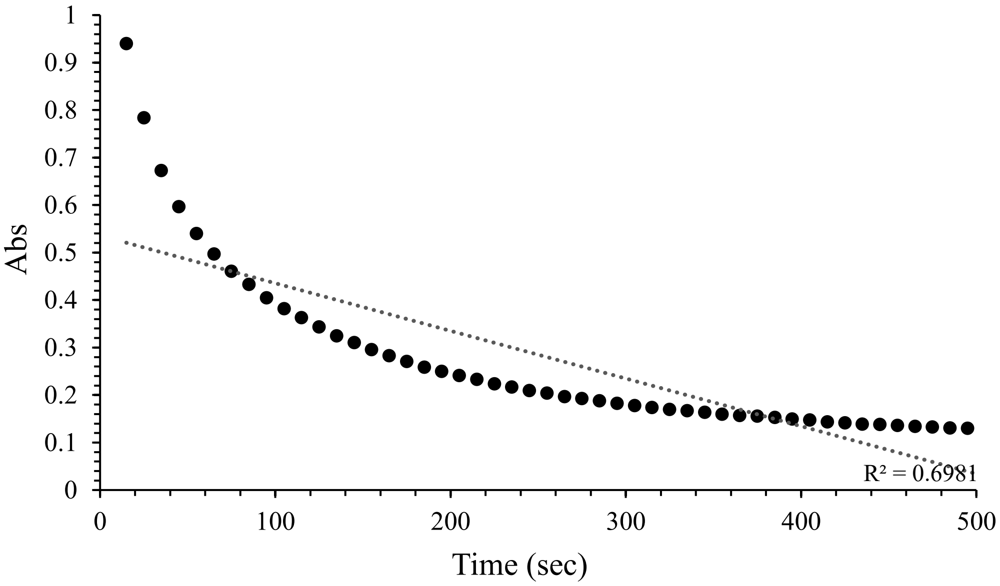
It clearly showed that when the concentration of acid is varied without changing the concentration of hypochlorite and dye, the rate of degradation is greatly enhanced with the increase in acid concentration. Since, the extent of degradation is eventually same in both cases, the amount of hypochlorite controls the extent of degradation but rate of degradation is greatly enhanced by the increase in concentration of acid.

The reason for that can be explained from the pH dependent equilibria existing among hypochlorite, hypochlorous acid and elemental chlorine in sodium hypochlorite solution. In the equilibria, free elemental chlorine gas is dominant in acidic side. Also, chlorine gas is a stronger oxidant in comparison to other species. The faster degradation of dye by sodium hypochlorite with the increase in acid concentration may be due to the presence of stronger oxidizing agent, i.e., chlorine gas.

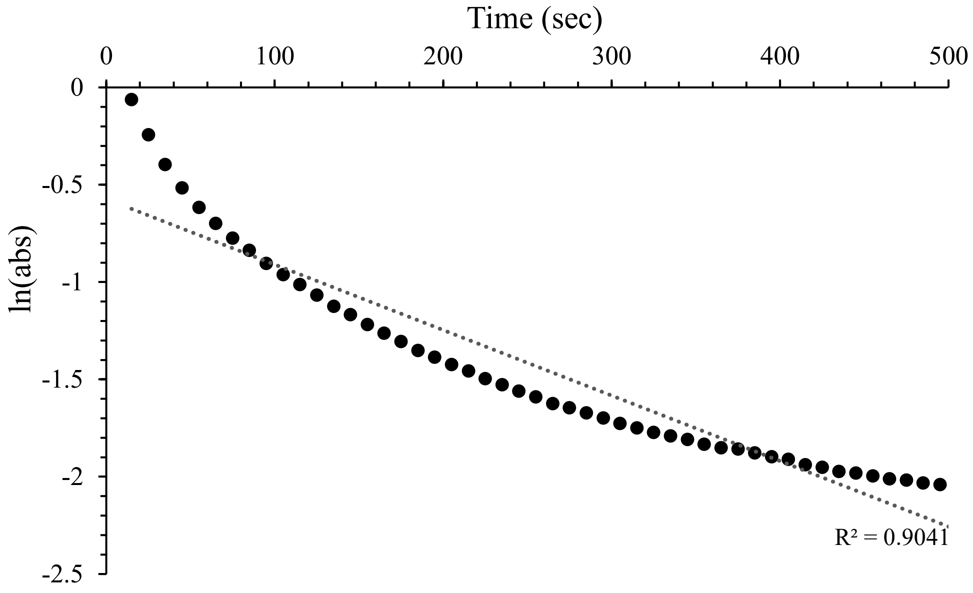
5.6.4 Analysis of order of reaction in acidic medium

When concentration of acid is higher (S2), the dye has degraded almost completely within a few seconds. Hence, further kinetic analysis could not be done in this case. But, the order of the reaction with respect to dye concentration can be studied here as well in case of the lower concentration of acid (S1). Here, order has been studied by graphical method and half-life method.

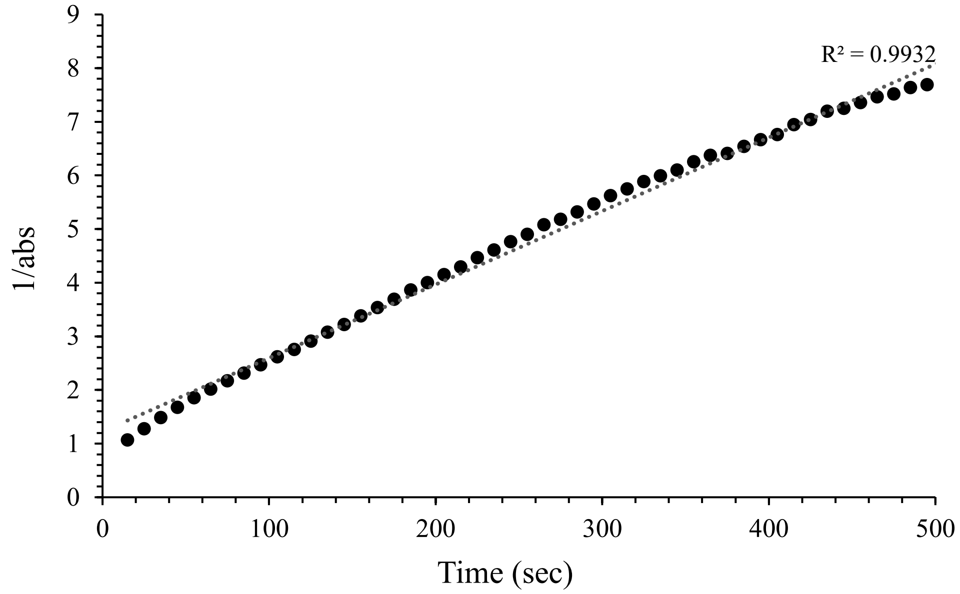
The data obtained within first 500 seconds have been used here as well to study the order of the reaction graphically. Following are the absorbance vs time, natural log of absorbance vs time and inverse of absorbance vs time plots with their respective R-squared value.



a) Absorbance vs time plot



b) Natural log of absorbance vs time plot



c) Inverse of absorbance vs time plot

Figure 12: Analysis of order for the reaction in acidic medium

Figure no. 12 shows that, among three plots, inverse of absorbance vs time plot produced a straight line with R-squared value 0.9932. Hence, the order of the reaction with respect to dye concentration is 2 when dye is bleached by sodium hypochlorite in acidic medium. The order of the reaction can be further confirmed by half-life method in this case. For this, let's tabulate the absorbance value of the dye at successive half-lives for the above cure as below.

Table 7: Investigation of order in acidic medium by half-life method

S.N.	Approximate absorbance at successive half-life [A]	Time taken to reach the absorption value (sec)	Half-life (sec) (t)
1.	0.94	15	60
2.	0.461	75	140
3.	0.233	215	410
4.	0.117	625	-

We can calculate the order of the reaction (n) by a set of two absorption value [A] and their respective half-life (t). We have the relation as:

$$n = 1 + \frac{\log \frac{t_1}{t_2}}{\log \frac{[A]_2}{[A]_1}}$$

For the set of first two, $[A]_1 = 0.94$, $t_1 = 60$ s and $[A]_2 = 0.461$, $t_2 = 140$ s. Putting these values in the relation above, we get,

$$n = 1 + \frac{\log \frac{60}{140}}{\log \frac{0.461}{0.94}}$$

Or, $n = 2.189$

Similarly, for the set of second and third, $n = 2.377$.

Here as well, the order of the reaction is determined to be close to 2 which is in good agreement with the result obtained from graphical method. Hence, this study concludes that oxidative reaction of the dye by sodium hypochlorite either follows second order kinetics or some complex order close to 2.

The oxidative degradation of dye by sodium hypochlorite is very fast and most of the degradation completes within few seconds. As more than half of the dye degrades before the reaction mixture in the cuvette could be mounted to spectrophotometer and reading could be taken, the kinetics cannot be studied by conventional spectrophotometric method. An advanced techniques to study fast reaction is required to study such reaction which at present is not possible in the facilities available in our context. But however, it can be concluded that the oxidation of the dye by hypochlorite is very fast and follows a complex kinetics.

Moreover, the dye extracted is not a single component and in presence of multiple species it is not worthwhile to investigate the detail kinetic analysis of oxidation of the vegetable dye by hypochlorite ion. The TLC revealed that the dye extracted contains multiple components of almost same R_f value and it is not easy to isolate a particular component of the dye. Despite many investigations on the dye from red cabbage, the detailed chemistry of the anthocyanins has been reported and the application of dyes in food coloring and other application is abundantly available, the detailed study of stability of dyes against oxidizing agents like hypochlorite has not been reported (Ramos et al., 2005; Santos-Buelga & González-Paramás, 2019). This study concludes that a further study on kinetics of the degradation of the vegetable dyes by hypochlorite is needed with advanced technique.

CHAPTER VI

CONCLUSION

The anthocyanin-rich dye solution can be extracted from fresh red cabbage leaves in aqueous medium. A promising anthocyanins concentration of 0.07808 mg mL^{-1} can be easily achieved by percolation method at a slightly elevated temperature.

The clear absorption spectra of the buffered dye solution at different pH can be developed in the visible range. The spectra revealed that bathochromic shift occurs if pH is increased as the wavelength of maximum absorbance (λ_{max}) was found to be 525, 535, 542, 573 and 602 nm at pH 2, 4, 6, 8 and 10 is respectively. The minimum specific absorptivity in the pH range of 4-6 can be attributed to predominant non-absorbing hemiketal group formation in the pH range of 4-6 as reported in the literature. Owing to the distinct colour of the dye and radical shifting of the λ_{max} at different pH, it can be concluded that the simple aqueous extract of the dye from red cabbage can be used as an excellent pH indicator.

The degradation of natural dye extracted from red cabbage upon treatment with sodium hypochlorite solution over time can be studied by spectrophotometric method. The kinetic degradation curves could also be obtained at different pH. Form the kinetic investigation, it could be concluded with certainty that the increase in concentration of acid significantly increases the degradation of dye by hypochlorite. The order of the reaction with respect to dye concentration was determined by graphical and half-life method for acidic condition. The order was concluded to be either 2 or it follows some complex order close to 2.

The extraction method employed was found to extract multiple components of anthocyanins as dye which was revealed by TLC analysis. It could be concluded that the oxidation of dye extract by hypochlorite was complex reaction due to multiple reasons like sufficiently fast reaction, multiple components in the reaction matrix and pH dependent equilibria existing parallelly in anthocyanins and hypochlorite. As the degradation reaction of the dye by hypochlorite is extremely fast and involves multiple components in the aqueous extract, a detailed kinetic study using advanced technique is needed in future to understand the mode of the reaction.

Suggestion for the further work

This research concludes that the kinetic degradation of the anthocyanins-based natural dyes by sodium hypochlorite is greatly affected by the pH of the reaction medium. It is hard to assess the action of which species from hypochlorite among elemental chlorine gas, hypochlorous acid or hypochlorite ion oxidizes which of the dye compound in the reaction matrix. The detailed kinetic study is desirable to establish the mechanism of the degradation of natural dyes by oxidizing agents like hypochlorite. It is advised that a single component of anthocyanins be separated from the dye extract, and the kinetics of degradation be studied using advanced technique capable of monitoring fast reactions aided by the compound characterization techniques.

ANNEXES

Annex-1

(Study of change in pH of the extracted dye solution in treatment with sodium hypochlorite)

A. Comparative study of change in pH of original dye-extract vs buffered extract in treatment with sodium hypochlorite

i. pH of ‘5 mL extract + 10 mL distilled water initially = 6.18

ii. pH of ‘5 mL extract + 10 mL 9.2 pH buffer initially = 8.57

To each of these solutions 10% v/v NaOCl solution was added mL wise separately and change in pH was noted.

Volume of NaOCl added	pH change of non-buffered solution (i)	pH change of buffered solution (ii)
1.0 mL	6.63	8.58
2.0 mL	7.00	8.61
3.0 mL	7.39	8.62
4.0 mL	7.75	8.64
5.0 mL	8.25	8.65
6.0 mL	8.78	8.68
7.0 mL	9.18	8.70
8.0 mL	9.49	8.73
9.0 mL	9.86	8.74
10.0 mL	10.23	8.77

B. Comparative study of resistance offered by buffered dye solution in pH change on treatment with hypochlorite vs distilled water in acid, basic and neutral condition:

pH of 10 % v/v NaOCl solution = 11.03

pH of distilled water produced in the lab = 6.46

(10 ml buffered extract solution was taken and to it NaOCl solution and distilled water was added mL wise separately by noting down the change in pH)

A. Acidic condition

Initial pH of 10 mL buffered extract (1:1 extract and buffer reagent) = 1.94

Effect of addition of water		Effect of addition of water NaOCl	
Volume of water added	pH	Volume of NaOCl added	pH
1.0 mL	1.94	1.0 mL	1.98
2.0 mL	1.95	2.0 mL	2.04
3.0 mL	1.97	3.0 mL	2.10
4.0 mL	1.98	4.0 mL	2.17
5.0 mL	1.99	5.0 mL	2.24
6.0 mL	2.01	6.0 mL	2.30
7.0 mL	2.02	7.0 mL	2.37
8.0 mL	2.04	8.0 mL	2.44
9.0 mL	2.04	9.0 mL	2.51
10.0 mL	2.05	10.0 mL	2.58

B. Basic condition

Initial pH of 10 mL buffered extract (1:1 extract and buffer reagent) = 11.42

Effect of addition of water		Effect of addition of water NaOCl	
Volume of water added	pH	Volume of NaOCl added	pH
1.0 mL	11.40	1.0 mL	11.40
2.0 mL	11.40	2.0 mL	11.39
3.0 mL	11.39	3.0 mL	11.37
4.0 mL	11.37	4.0 mL	11.35
5.0 mL	11.37	5.0 mL	11.33
6.0 mL	11.37	6.0 mL	11.32
7.0 mL	11.36	7.0 mL	11.30
8.0 mL	11.36	8.0 mL	11.28
9.0 mL	11.35	9.0 mL	11.27
10.0 mL	11.35	10.0 mL	11.25

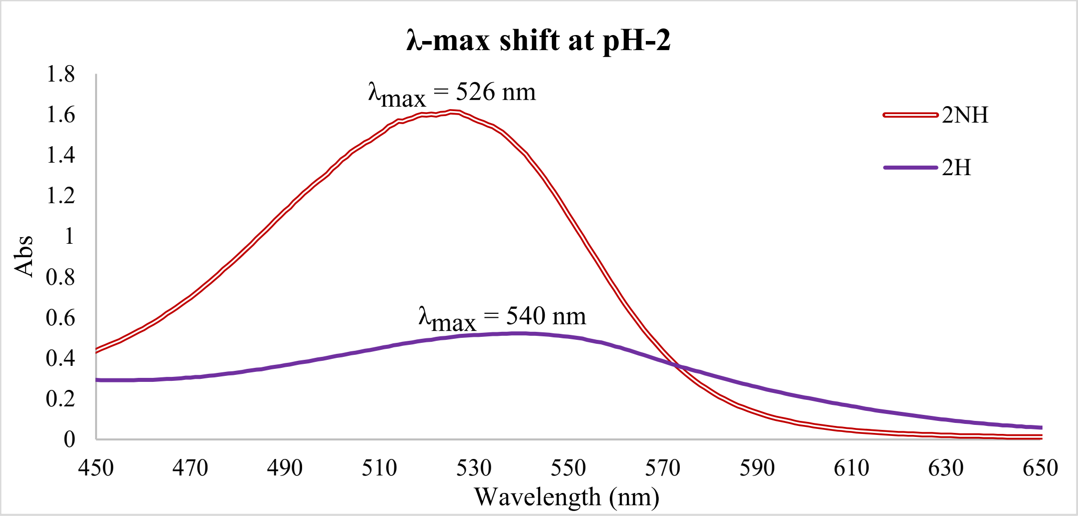
C. Neutral condition

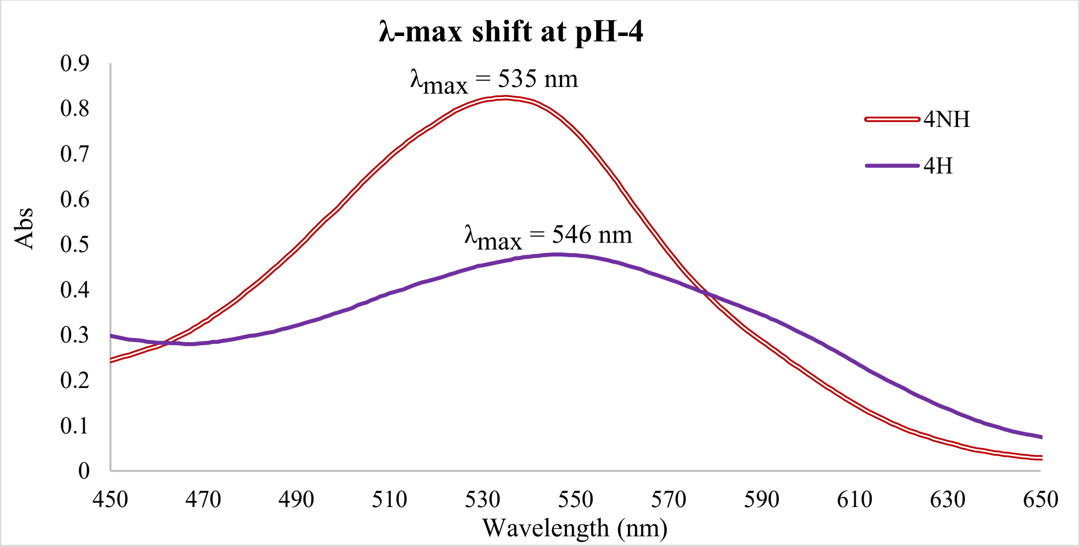
Initial pH of 10 mL buffered dye solution (1:1 dye-extract and buffer reagent) = 7.12

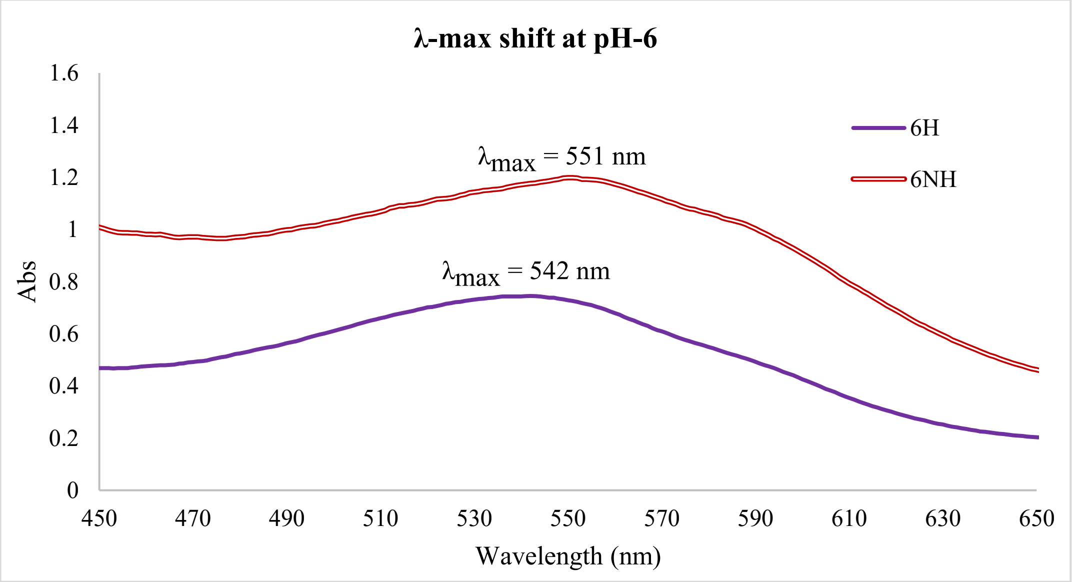
Effect of addition of water		Effect of addition of water NaOCl	
Volume of water added	pH	Volume of NaOCl added	pH
1.0 mL	7.12	1.0 mL	7.16
2.0 mL	7.13	2.0 mL	7.19
3.0 mL	7.15	3.0 mL	7.22
4.0 mL	7.16	4.0 mL	7.25
5.0 mL	7.16	5.0 mL	7.28
6.0 mL	7.17	6.0 mL	7.31
7.0 mL	7.17	7.0 mL	7.34
8.0 mL	7.17	8.0 mL	7.36
9.0 mL	7.18	9.0 mL	7.39
10.0 mL	7.18	10.0 mL	7.41

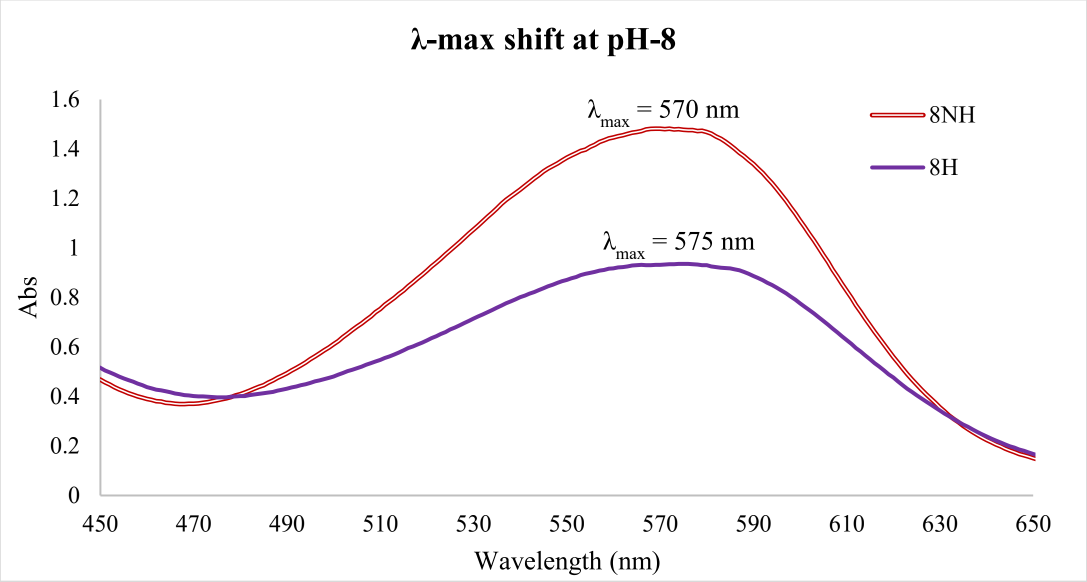
Annex-2

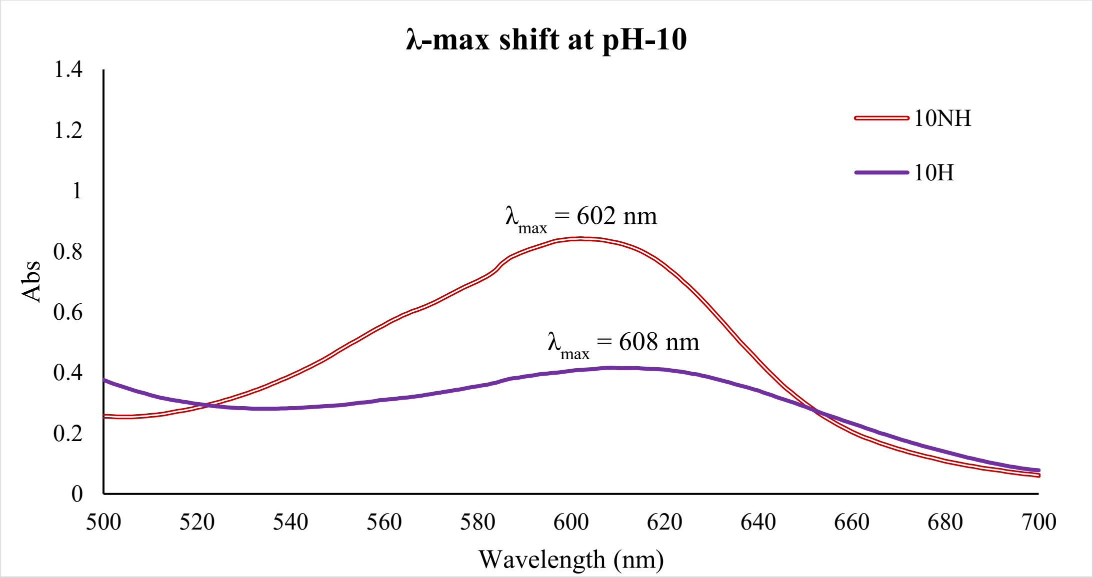
(Study of shift in λ_{max} of the buffered dye in treatment with sodium hypochlorite solution)

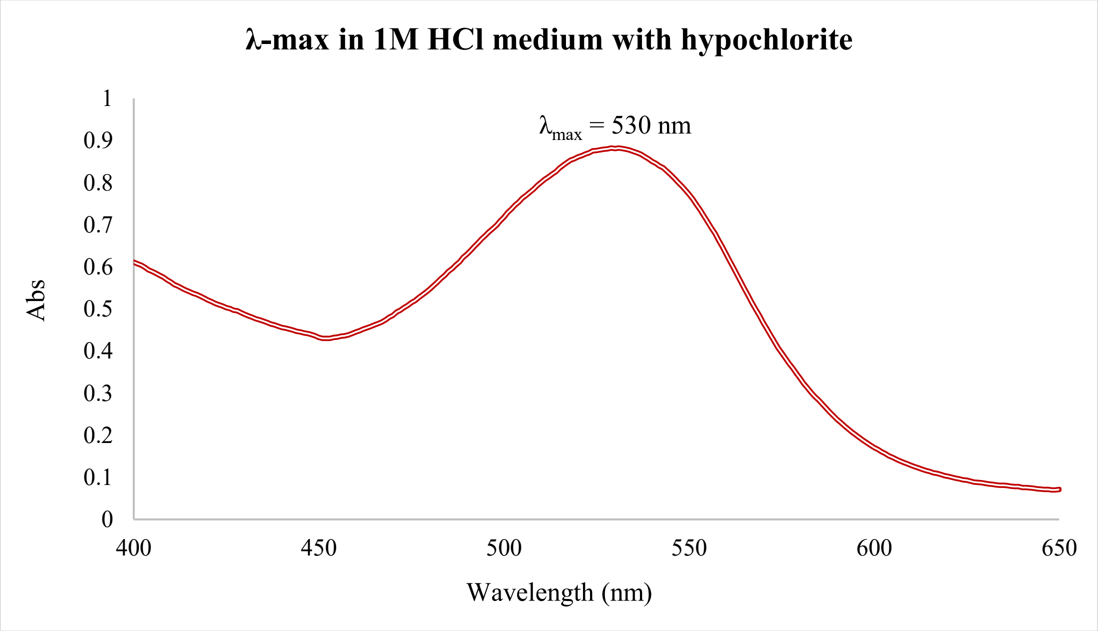







λ-max shift at pH-8
Abs
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
450
470
490
510
530
550
570
590
610
630
650
Wavelength (nm)
λmax = 570 nm
λmax = 575 nm
8NH
8H


λ-max shift at pH-10
Abs
1.4
1.2
1
0.8
0.6
0.4
0.2
0
500
520
540
560
580
600
620
640
660
680
700
Wavelength (nm)
λmax = 602 nm
λmax = 608 nm
10NH
10H


λ-max in 1M HCl medium with hypochlorite
Abs
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
Wavelength (nm)
λmax = 530 nm

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KINETICS OF DEGRADATION OF DYE EXTRACTED FROM RED CABBAGE A DISSERTATION SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY Submitted by: NIRMAL PHUYAL Symbol no: CHEM 1201/074 T.U. Registration No: 5-2-242-17-2012 Submitted to: DEPARTMENT OF CHEMISTRY AMRIT CAMPUS INSTITUTE OF SCIENCE AND TECHNOLOGY TRIBHUVAN UNIVERSITY, KATHMANDU, NEPAL August, 2023 CHAPTER I INTRODUCTION 1.1 Background Red cabbage is used as a leafy vegetable and found in almost every region of the world. It is scientifically known as Brassica oleracea var. capitata f. rubra, that belongs to the Brassicaceae family. It is closely related to other cabbage varieties, such as green cabbage, broccoli, and Brussels sprouts. It is recognized for its striking purplish-red color. The colour of this plant gives a noticeable contrast to it as well as it adds value of immense importance to this plant. The colour of this plant is due to the presence of a group of pigments called anthocyanins (Haddar et al., 2018). Figure 1: Red cabbage and its sliced view Red cabbage is