Analytical Application of the Pigment Isolated from Iraqi Red Radish Peels (Raphanus Sativus L)

N. J. Al-Salihi, I. A.A. Al-Hassan and F. S. Sebah
Department of Chemistry, College of Science, University of Basrah

Abstract

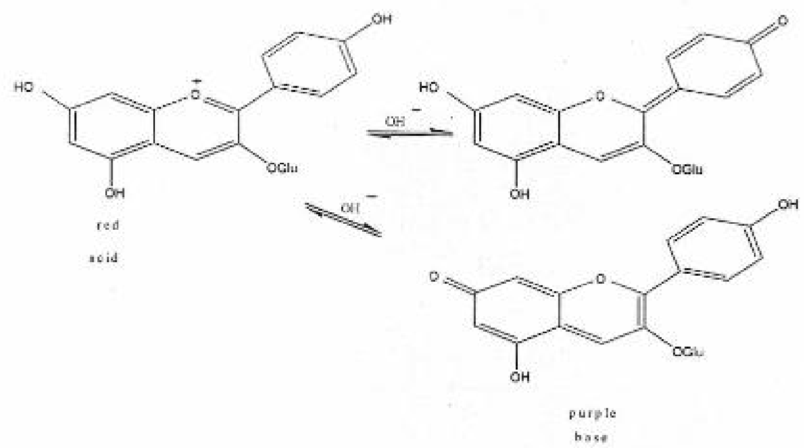
The anthocyanin flavonoid pigment isolated from Iraqi red radish peels (Raphanus Sativus L) was used as an acid – base indicator with a pk equal 7.0 and pH equal (6-8). The acidic water extract was successfully used instead of conventional litmus papers.

Introduction

Color is an important attribute of most fruits and vegetables , the identification of compounds responsible for color is of interest .The red and blue colors of plants are due to the presence of anthocyanin pigments (1) .Anthocyanin are major natural phenol compounds which belong to a large and widespread group of plant constituents collectively known as flavonoids . The major importance comes from their potential application as natural colorants to replace synthetic dyes foods. Natural anthocyanins are prescribed as medicine in many countries.Anthocyanins are also potent antioxidants in vitro against various reactive oxygen species (2), (3).

Other important aspects of anthocyanins are their analytical applications. A visual acid – base indicator is just a weak acid with differently colored acid and conjugate base forms. Flower of leaf pigments often fit this description. Sense (4) use the rose petals and the red cabbage as acid-base indicator solution .In the teaching of the principles of acid and bases, experiments on pH color-indicator have been carried out by Senese(5) using fruit and vegetable juice as the indicators. The mechanism of pH –indicator and buffer action of juices as pH –indicators were discussed in detail by Kogenlnik and Joachim(6).

Willastter attributed the variety of flower colors to different pH values in solution(7). Indeed, anthocyanin changes its color with pH. Interest in anthocyanin has increases, since it is a safe indicator in volumetric analysis like litmus, anthocyanin changes its color to a strongly acidic medium which appears orange to red (flavylium ion), whereas in weakly acidic to neutral solution it appears purple to violet. The blue color can be produced only in alkaline solution(7) .



Our objective was concentrated on the application of the isolated pigment from the peels of red radish (Raphanus Sativus L.) as acid–base indicator in neutralization reaction. The procedures of extraction and purification have been explained in detail by Al-Derawi(8) .

Materials and Methods

Plant materials: Red radish (Raphanus Sativus L.) were obtained from Basrah market. They were cleand and the peels were collected carefully and allowed to dry at room temperature. The dried peels were blended by using (Electrical mill blender), the powder of the peels were kept until required(8) .

Chemicals and materials: All chemicals were of purely analytical grades. Methyl alcohol ,sodium hydroxide were obtained from Fluka; potassium dihydrogen phosphate , disodium hydrogen phosphate and phenolphthalein indicator from Merck; Methyl orange indicator and sodium carbonate from RDH; Hydrochloric acid (analar) acetic acid from BDH; 95% ethanol from Baghdad factory for drugs and cosmetics.

Instruments:

- JASCO uv – visible spectrophotometer.
- pH-meter HI 8417 HANNA instruments

Extraction method: Extraction and isolation of pigment was performed according to the method explained by AL-Derawi (8).

Analytical application: To study the possibility of using the pigment as an acid – base indicator in volumetric analysis, many experiments has been done to determine the best solvent used, the effect of the temperature and the pH as well.

Choosing the solvent: (0.020gm) of the pigment were dissolved in each 3 ml of distilled water, absolute ethanol, 50% ethanol, methanol and dimethyl sulfoxide. The visible spectrum of each solution was recorded from 450 – 620 nm wave length directly and after 24 and 72 hrs. the spectra were shown in fig. (1, 2, 3, 4, 5).

Temperature Effect: The solution of (0.020 gm) of the pigment in 3 ml distilled water was prepared and the effect of the temperature was studied from 20 –70 °C by increasing the temperature 10 degrees in each time for 10 min. The absorbance values were measured at maximum wave length 550 nm.

Spectrophotometric determination of the stability constant:

pK values were determined according to the method explained byVogel(9). Precision spectrophotometer JASCO uv – visible spectrophotometer was used to determine the wave length at which the acidic and the basic forms of the pigment (indicator) exhibit absorption maxima. The absorbances were measured from 200 -600 nm wave length and the isosbestic point was recorded as shown in fig. (6). Eight solutions of different pH (5 – 8.2) were prepared , figs.(7, 8), and the absorbances were measured at two maximum wave length 440 nm and 520 nm(10).

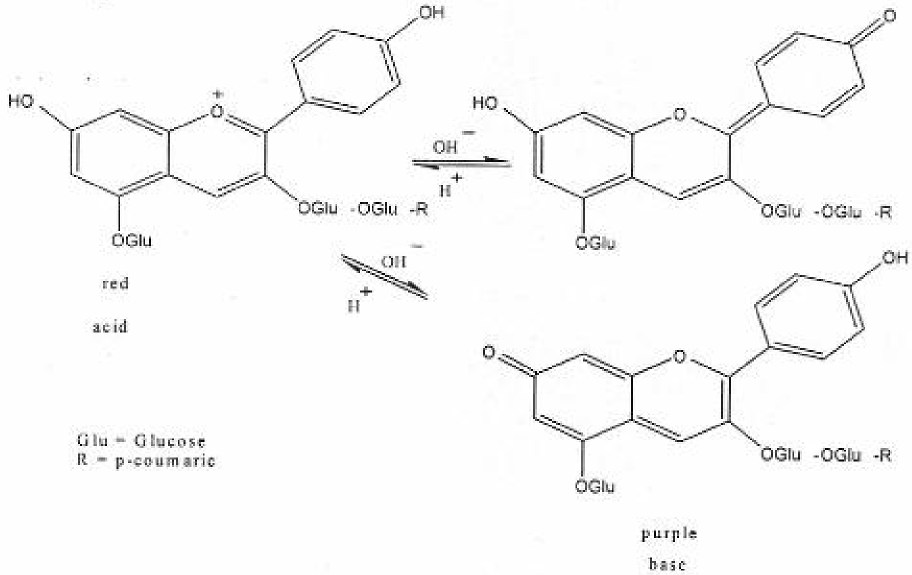
Results and Discussion

Analytical application

Figs. (1, 2, 3, 4, 5) show the effect of different solvents on the stability of pigment color with time, from the figuers it appears that the water was the best solvent which was the pigment remain stable after 72 hrs. The effect of the temperature was shown that the pigment was stable with increasing the temperature up to 60 °C.

The pK value were determined from the absorbance versus pH at λ_{max} = 440 nm and 520 nm and from the relation between log $[In^-]$ / [HIn] versus pH and from the calculated value figs. (7, 8, 9), the value of pK found to be = 7.0 and $\Delta pH = pK \pm 1 = 7.0 \pm 1 = 6.0 - 8.0$, the behavior of pigment as an acid-base indicator found to be similar to the behavior of the anthocyanin pigments found in the literature(11).

The proposed mechanism of the color change of the pigment was explained as follows:



which is the same for that found by Goto and Kondo(11).

To improve the behaviour of the pigment as indicator, two acid-base indicators have been chosen for comparison, phenolphthation and methyl orange. 10 successive titration experemints have been done using 0.1 N HCl against primary standard solution of 0.1N Na_2CO_3 using methyl orange indicator, the results show 3.4 % percent error and 10 successive titration experemints in the reaction of 0.1N HCl against 0.1 N NaOH gave 2.4% percent error.

The results show good agreement between pigment as indicator and the popular indicators used in volumetric analysis (neutralization reaction).

Preparation of indicator paper

Filter papers (Whatman No. 1) were prepared (1 x 6 cm) and immerse in the water extract of the red radish peels. The paper was used as Litmus paper to indicate the acidity of the solution. The color of the paper changed from red in acid medium to violet color in alkaline. The method found to be economic in preparing such papers instead of litmus paper. The same idea was used by others by preparing papers similar to litmus paper from the aqueous extract of red rose, cherry and red cabbage(5)(6)(11).

Refrences

1. Fuleki,T.(1969) , The anthocynine of strawberry Rhubarb, Radish and Onion. J. of Food Science, 34:365-369 .
2. Hammouri, M.;Al-Samdi, M; Bataineh, M. and Ou, B. (2001). Separation and characterization of an anthocyanin, cyaniding-3-O-arabinosyl gluoside from petals of flowers of pheasant's eye (Adonis aestivalist L.) International J. of Biochromatography, 6:173-183.
3.Giuisti ,M.and Wrolstad, R.(2001). Characterization and measurement of anthocyanins by uv-visible spectroscopy, current protocols in food analytical chemistry, p. 1-12.
4. Senese, F.(2001b). "What are some natural acid /base indicators?", General chemistryonline,FAQ. (Internet) http://antonine.fsu.umd.edu/chem/senese/101/acid-base/faq/household-indicators.shtm/)
5. Senese, F. (2001a). "What hous hold substances can be used as acid / base indicators ?", General chemistry on line, FAQ, p. 1-3 (Internet) http //,antoine. fus. umd. edu/chem./senese/101/ acid – base/ faq/house hold – indicators .Shtm/).
6. Kogelnik and Joachim, H. (1982). "Fruit and vegetable juices as indicator in chemistry instruction", prox nature wiss, Chem., 31(4): 123-125 (chem.. Abs.) 96 : 216800t.
7. R. Willstter, Justus Liebijs Ann, Chem., 401 (1913), 189, In "perilia", (Yu, H.; Kosuan, K. and Haga, M.), Harwood academic

publishers, The Netherland, p.149.

8. Al-Derawi, F. S.(1996) Isolation and identification of some pigments from red peels of Iraqi red radish (Raphanus Sativus L.) and study some of its analytical and biological applications.M.SC.Thesis , College of Science , University of Basrah.
9. Vogel,I.(1961). A Text-Book of quantitive inorganic analysis including elementary instrumental analysis3rd edition,Lowe@ Bry done ,LTD,London.
10. Donald,T. S.; William, R.h.and Janice,M.Beebe (1989) Chemistry experiments for instrumental method ,John Wiley and Sons, New York.
11. Goto, T. and Kondo, T. (1991) "Structure and molecular stocking of anthocyanins flower color variation", A ngew, Chem. Int., Engl., 30 :17-33.

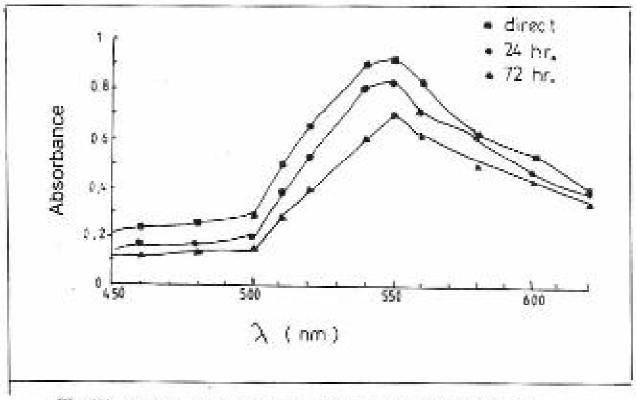


Fig.(1): Visible spectra of pigment using water as solvent

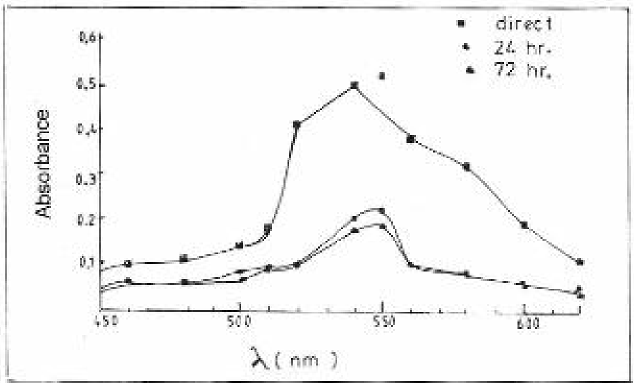


Fig.(2): Visible spectra of pigment using absolute ethanol as solvent

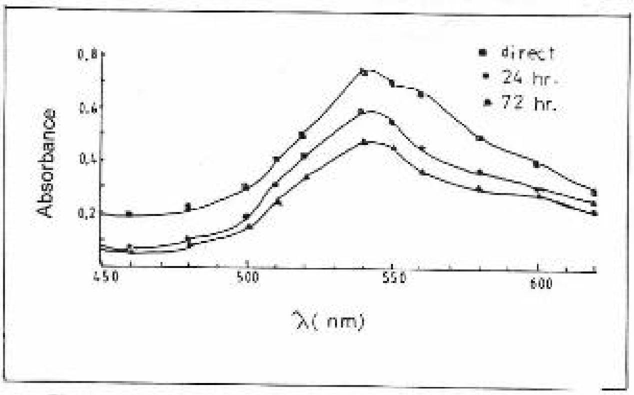


Fig.(3): Visible spectra of pigment using 50% ethanol as solvent

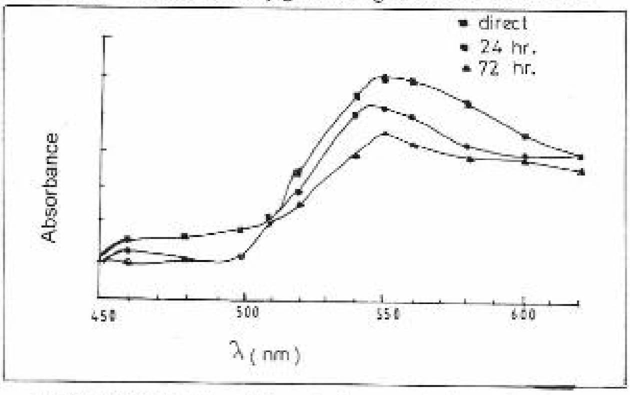


Fig.(4): Visible spectra of pigment using methanol as solvent

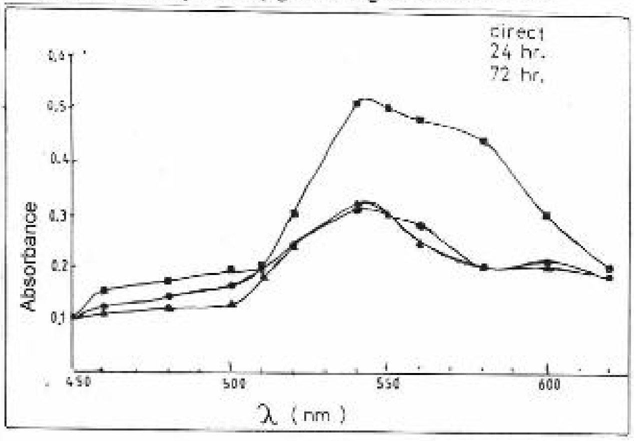


Fig.(5): Visible spectra of pigment using DMSO as solvent

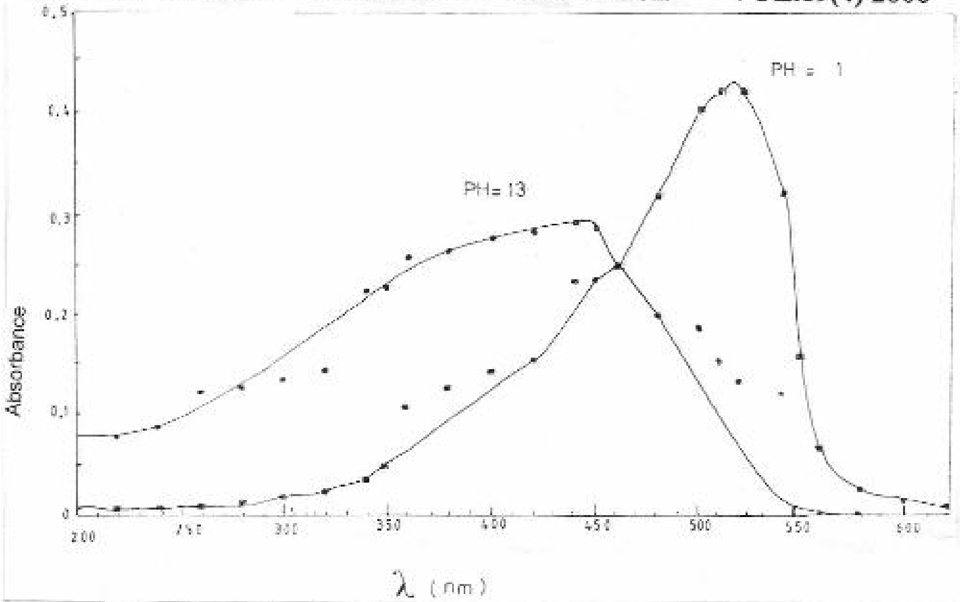


Fig.(6): Isobestic point for pigment

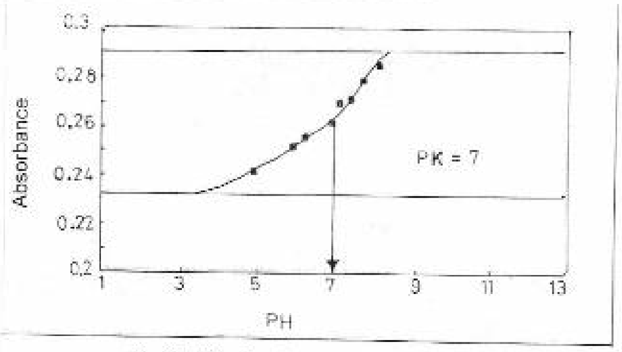


Fig.(7): PK value for pigment at 440nm

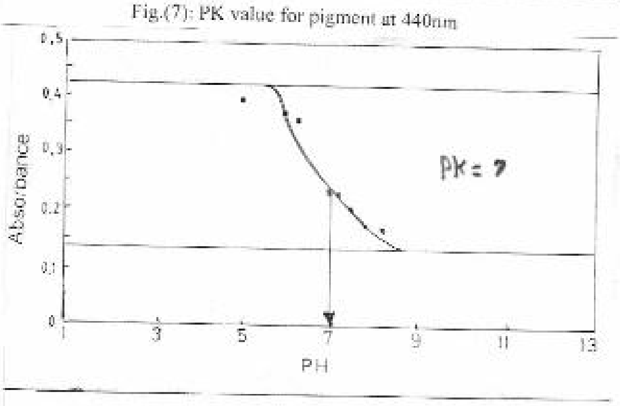


Fig.(8): PK value for pigment at 520nm

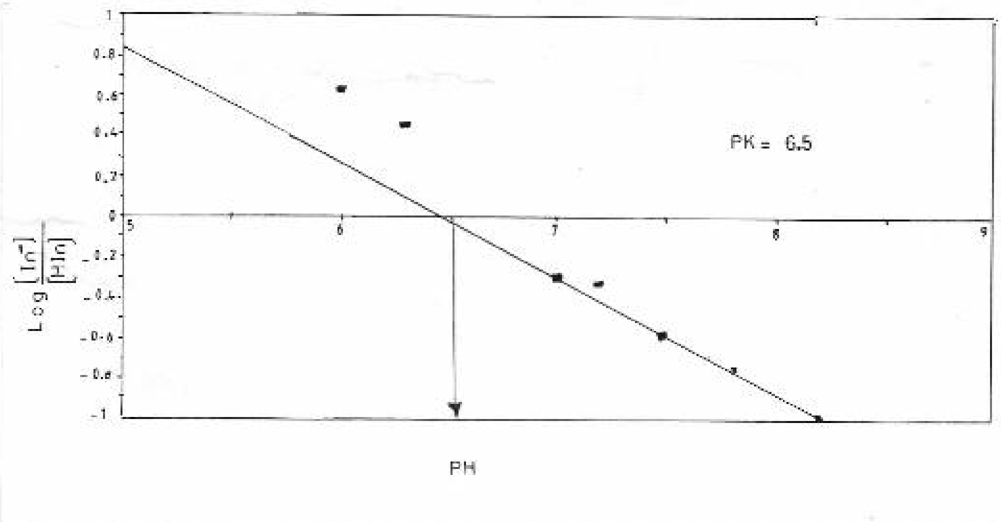


Fig.(9) PK value from log $\frac{[In^-]}{[HIn]}$ and PH value for pigment

مجلة ابن الهيثم للعلوم الصرفة والتطبيقية المجلد 19 (4) 2006

تطبيقات تحليلية للصبغة المستخلصة من قشور نبات الفجل الأحمر العراقي

نيران جاسم الصالحي ، عيسى عبد عبدالحسن، فاطمة صيوان صباح
قسم الكيمياء ، كلية التربية- ابن الهيثم،جامعة بغداد

الخلاصة

تم دراسة التطبيقات التحليلية لصبغة المعزولة من قشور درنات الفجل الأحمر العراقي red radish peels(Raphanus Sativus L) واقتراحها كدليل حامض- قاعدة وحساب قيمة الثابت Pk لها ومدى تغيير الدالة الحامضية لاثبات امكانية استخدامها كدليل في تفاعلات التعادل كما استخدم المستخلص المائي المحمض لقشرة درنات الفجل الأحمر لعمل أوراق PC لمعرفة حامضية و قاعدية المحاليل كبديل عن أوراق زهرة الشمس Litmus papers.