

Full Length Research Paper

Simultaneous ultraviolet-visible (UV–VIS) spectrophotometric quantitative determination of Pb, Hg, Cd, As and Ni ions in aqueous solutions using cyanidin as a chromogenic reagent

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The use of cyanidin (3, 3¹ 4¹ 5, 7 – pentahydroxyflavylium chloride) extracted from a tropical plant *Hibiscus sabradiffa* L. as a chelating reagent for simultaneous spectrophotometric determinations of ions of Pb, Hg, Cd, As and Ni in mixed aqueous solution is reported. The purified extract of the dried calyces of the Roselle plant was characterized and the results compared with literature values. Complexation of the metals ions with cyanidin and scanning through 200-700 nm in a UV –VIS spectrophotometer gave the wavelength of maximum absorption (λ_{max}) of these metal complexes to be 389.6, 360.0, 357.8, 396.8 and 401.0 nm for Pb(II); As(II); Cd(II); Hg(II) and Ni(II) complexes respectively indicating appreciable bathochromic shifts compared to pure cyanidin absorption at 283.2 nm. The effect of pH on the determinations was studied and a pH value of 5 was found to be the optimal. Calibration curve plots of the complexes showed linearity between concentration of 0.1 to 5.0 ppm. This method offer cheap, simple, rapid, sensitive, and eco-friendly technique for simultaneous determination of trace heavy metals in mixed aqueous solutions and have potentials for environmental and biological samples.

Key words: Cyanidin, ultraviolet (UV) spectroscopy, simultaneous, chromogenic, eco friendly, trace metals.

INTRODUCTION

Determination of trace metals is of interest because while some are essential nutrients some others are toxic. Metals like zinc, manganese, copper, chromium, iron and cobalt are essential trace elements for humans, animals and plants; but become toxic if the homeostatic mechanisms maintaining their physiological limit are disrupted. On the other hand, lead, cadmium, nickel, arsenic and mercury etc are toxic even at low levels. The need to estimate the levels of these metals in materials or samples have increased tremendously after reports on different roles they play in human health and disease. Numerous analytical techniques exist in literature for use in trace heavy metals assay in mixed solutions. Among

the methods, atomic absorption spectrophotometry (AAS) is well recognized because of its attractive features such as sensitivity, reliability, versatility, accuracy and precision (Okoye, 2005; Khamms et al., 2009). However, the use of AAS is limited by the need for high technical skills, huge capital and maintenance cost (Strong and Martin, 1990). Furthermore determinations of metals like arsenic and mercury pose health risk to the analyst using AAS.

Some other methods employed in simultaneous determination of metals include polarography, voltammetry, inductively coupled plasma-mass spectroscopy (ICP–MS), inductively coupled plasma-atomic emission spectroscopy (ICP–AES), liquid chromatography (LC) etc (Rouhollahi et al., 2007). Like AAS, these techniques require very costly equipment and reagents that are not easily available in poor nations of the world coupled with very specialized skills needed.

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Also, most of the reagents are not eco friendly. The use of UV–VIS spectrophotometry in determination of heavy metals in samples is becoming popular in many laboratories because it provides for easy, simple and rapid determination in low to high concentrations at cheap cost (Soomro et al., 2008). Simultaneous determination of metal ions using UV–VIS methods was reported to be difficult without separation due to overlap absorption spectra (Nai-Liang et al., 2005). The problems associated with spectroscopic determination of these trace heavy metals are complicated by the nature of the photochromic or chromogenic ligands which are mainly synthetic and are toxic in the environment. Some of the reported simultaneous determination of metals using UV spectrophotometry were carried out using toxic and expensive reagent (Rouhollahi et al., 2007; Kachbi, 2010; Naguraja et al., 2009). Other works in literature use specific ligands for specific metals at even different pH values for multi-elements determinations.

Cyanidin (3, 3', 4', 5', 7 – pentahydroxyflavylium ion) is found in an intensely coloured flowers. It is a water soluble pigment which has been widely used as a food colourant, titrimetric indicator and in disease treatment (Meiers, 2001; Wang and Jiao, 2000; Francis, 1989; Kong et al., 2003; David, 1998; Odigwe et al., 2003; Aoshima et al., 2007). The potentials of cyanidin as chromogenic ligand for metal complexes have been reported by Ukwueze et al. (2009).

This study reports the simultaneous determination of some trace heavy metals by UV-VIS spectrophotometry using cyaniding as a chelating reagent. We relied on the shifts of maximum absorption wavelength (λ_{\max}) of cyanidin after complexation with the metals. The objective of the work was to develop a rapid, sensitive, specific and simple method for simultaneous determination of metals in environmental and industrial samples.

MATERIALS AND METHODS

Equipment and reagents

Salts of Pb, Cd, Hg, Ni and oxide of Arsenic were purchased from Rie – del – de Haen, Germany in analytical grade. Jenway (6405-model) Spectrophotometer and Jenway (3015-model) pH meter were used for absorbance and pH measurement respectively. The chemicals used to prepare buffer solutions were also of analytical grade and deionized water was used. All glass wares were first washed with detergents and cupiously rinsed with deionized water.

Cyanidin extraction

Cyanidin was extracted from calyces of Roselle plant (*Hibiscus sabdariffa* L.) according to the method of Ukwueze et al. (2009). A 500 g of dry calyces were ground to powder and macerated in 2.5 L methanol: HCl mixture (85:15% v/v) for 72 h and filtered. The filtrate was concentrated to 500 and 100 ml of conc. HCl was added and

content was refluxed for 2 h. The solution was then put in a beaker and cooled in a refrigerator until crystals settled out. The crystals were filtered out under suction and re-crystallized from hot methanol, air dried and weighed.

Preparation of buffer solutions

Buffer solutions of pH 1 to 8 were prepared in accordance with methods described earlier using KCl, HCl, $\text{KHC}_8\text{H}_4\text{O}_4$, KH_2PO_4 in varying concentrations and mixings (Lange, 1973; Meities, 1963).

Determination of λ_{\max} of cyanidin

5% cyanidin solution was prepared by dissolving 5 g of the purified crystals in methanol containing 0.01% conc. HCl and made up to 100 cm^3 in a standard flask. 1 cm^3 of this solution was diluted to 10 cm^3 and its λ_{\max} was determined by scanning from 200-700 nm using Jenway (6405 model) spectrophotometer in a 1 cm^3 cuvette.

Preparation of stock and working solutions of metals

Stock solutions (1000 ppm) of Pb, Cd, Hg, Ni, and As were prepared from lead nitrate, cadmium, mercury and nickel chlorides and arsenic oxide. The solutions were serially diluted to the required working standards. 2.5 cm^3 of each stock solution was diluted in a 250 cm^3 standard flask to give 10 ppm solution.

Determination of wavelength of maximum absorption (λ_{\max}) of metal – cyanidin complexes

5 cm^3 metal solutions (10 ppm) were diluted with 5 cm^3 of the 5% cyanidin solution and the wavelength of absorption was scanned from 200 to 700 nm in a 1 cm^3 cuvette using the spectrophotometer.

Determination of optimum pH (pH_{opt}) of metal-cyanidin complexes

Each of the 8 beakers containing 5 cm^3 of 5% cyanidin in methanol were added 5 cm^3 of standard solutions of one of the studied metals. 50 cm^3 of the solution in each beaker was adjusted to a given pH from 1 to 8 using the buffer solutions prepared. The absorbance of each solution was read at the λ_{\max} of the analyte metal at the adjusted pH.

Simultaneous determination of various metal – cyanidin complexes at pH_{opt}

Working standard solutions from 1.0 - 9.0 ppm of Pb(II); Cd(II); Hg(II), As(III) and Ni(II) solutions were prepared from the 10 ppm solution of each metal by diluting appropriately. These were used to plot a calibration graph for each metal-cyanidin complex by determining the absorbance at λ_{\max} of 5 cm^3 of standard solution diluted by 5 cm^3 of 5% cyanidin solution in a 1 cm^3 cuvette at pH_{opt} determined.

5 cm^3 of 1.0 ppm mixed standard solution was diluted with 5 cm^3 of 5% cyanidin chloride solution adjusted to pH_{opt} and the absorbance read at the λ_{\max} of each metal – cyanidin complex. Similar procedures were repeated for 2-9 ppm mixed metal solutions.

Table 1. R_f values (X 100) of TLC and PC of cyaniding in various solvents.

Variable	TLC			PC		
	Forestal	2MHCI	BAW	Forestal	2MHCI	BAW
Literature values	48	2.5	52	47	2.5	52
Experimental value	45	5	55	49	3	50

Table 2. Spectral results of Cyanidin extract compared with literature values.

Variable	UV region (λ_{max})	VIS region (λ_{max})
Literature values	282.9 nm	530.0 nm
Experimental value	283.2 nm	530.2 nm

Table 3. Effect of metal ions on wavelength of absorption of cyaniding.

Wavelength	Cyanidin (Cy)	Pb(II)-Cy	Hg(II)-Cy	Cd(II)-Cy	As(III)-Cy	Ni(II)-Cy	Pb(II) shift	Hg(II) shift	Cd(II) shift	As(III) shift	Ni(II) shift
UV	283.2	389.6	396.8	357.8	360.0	401	106.4	113.6	74.6	76.8	117.8
VIS	530.2	536.6	538.6	535.6	533.8	555.6	6.4	8.4	5.4	3.6	5.4

RESULTS AND DISCUSSION

Cyanidin extract characterization

The extracted cyanidin was found to conform to the characteristics earlier reported by Ukwueze and others (2009). Table 1 shows the R_f values of thin layer and paper chromatographs (TLC and PC) obtained in various solvents compared to literature values. Both experimental and literature R_f values are very close.

The spectral data obtained from the analysis of the extract in comparison with literature values are shown in Table 2. The R_f values and λ_{max} 's of the extract showed no significant variation from the authentic literature values (Harborne, 1958).

Effects of the metal ions on the absorption wavelength of cyanidin

The result of the investigation of the effects of the metal ions on the λ_{max} of cyanidin is shown in Table 3. From the table, all the metals caused a marked bathochromic shift in cyanidin absorbance wavelength in the UV region. Shifts in VIS region are very small causing an overlap in absorbances when plotted. The simultaneous determination of these metals can be measured with satisfaction in the UV region. The affinity of metal ions for ligands is controlled by size, charge and electronegativity. These metals are highly polarisable, have lower charge density, large ionic size and their d-orbitals are available

for π - bonding and due to these, they form covalent complexes showing absorption in the UV region.

Selection of pH optimum at λ_{max} of metal ions

The effect of pH on the formation of metal-cyaniding complexes was studied at different pH values in methanol and the result shown in Figure 1.

From the graph, Cd – cyanidin complex has pH = 3 as the optimum pH; Pb – cyanidin has pH = 4; Hg – cyanidin has pH = 5; As – cyanidin has pH = 2 and Ni – cyanidin has pH = 6. At these various pH values, these metal-cyaniding complexes showed reproducible results. However, all the metal complexes showed prominent absorbances at pH = 5. As a midway or compromise between selectivity and sensitivity of the developed method, pH value of 5 was chosen as the optimum pH for the simultaneous determination of these metals complexes with cyanidin in aqueous media.

Simultaneous determination of the metal ions

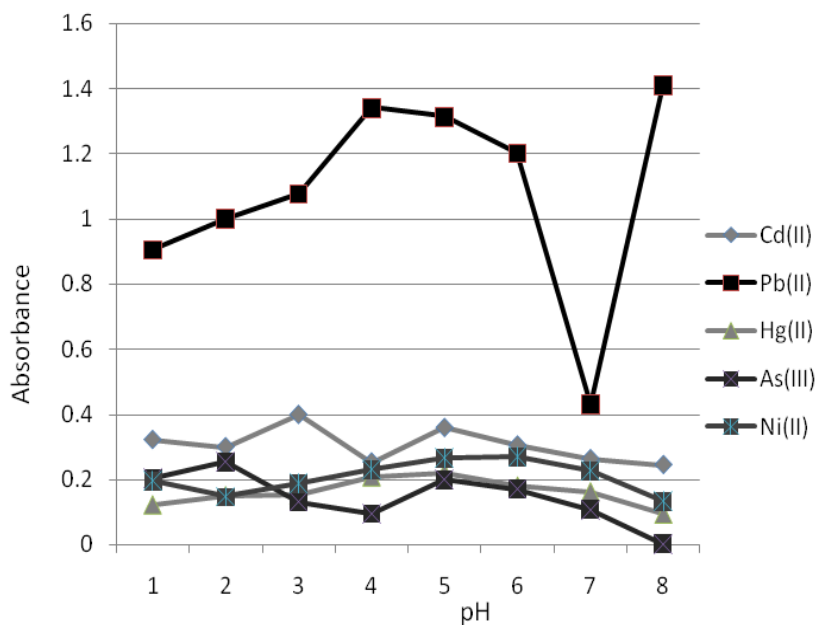
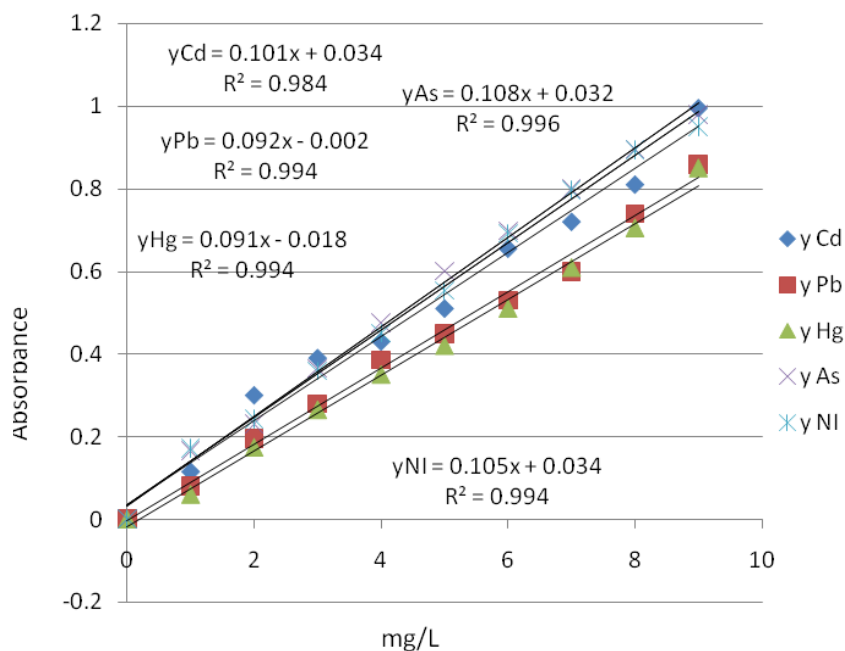
At the optimal pH value, the UV spectra of the mixed metal ions – cyanidin complexes was studied for possible spectral overlaps or interferences. The results are shown in Table 4.

From the table, only Pb(II), Hg(II) and As(III) were absorbed at their original λ_{max} . It was noted that Cd(II) showed no peaks which could be attributed to the closeness of its λ_{max} value to As(III). The absorption

Table 4. λ_{\max} for simultaneous determination of mixed metal – cyanidin complexes.

λ_{\max} (nm)	Pb(II)- Cy	Hg(II)- Cy	Cd(II)- Cy	As(III)- Cy	Ni(II)-Cy
UV	389.6	396.0	—	360.0	496.4
VIS	—	—	—	—	—

Cy = cyaniding.

**Figure 1.** pH variation at λ_{\max} of absorption by metal-cyanidin complexes.**Figure 2.** Mixed graph of Abs vs. Conc. Of the metal complexes.

intensity of As(III) was greatly enhanced in the spectra. Ni(II) absorbed at higher wavelength. Such interferences might result from the formation of mixed oxides or from suppression of ionized gaseous metals pressures which occur in the molecular environment adjacent to the chromophore.

Linear detection range of metal – cyanidin complexes

Absorbance of the five complexes at various concentrations at various λ_{\max} 's and optimum pH (pH = 5) were investigated and the result was shown in Figure 2. The calibration curves obtained showed linearity between concentration ranges of 1.0-10.0 ppm. At this range, Beer-Lambert law was obeyed.

Conclusion

Complexation of cyanidin simultaneously with Pb, Hg, Cd, As and Ni ions markedly altered the wavelength of absorption of the cyanidin and this phenomenon can be utilized in spectrophotometric determination of these metal ions. The complexes were stable and yielded reproducible results at pH = 5. It was found that all the metal ions determined show linearity at concentration range of 1.0 to 10.0 ppm indicating that at these concentrations, UV spectroscopy can conveniently be used for their simultaneous determination.

This offers a simple, rapid, sensitive and cheap method that promotes the spirit of green chemistry in chemical quantitative analysis. It has potentials in evaluation of trace heavy metals in environmental, biological and food samples.

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