

Research Article

Reversed-Phase Liquid Chromatographic Separation and Determination of Ni(II), Cu(II), Pd(II), and Ag(I) Using 2-Pyrrolecarboxaldehyde-4-phenylsemicarbazone as a Complexing Reagent

Arfana Mallah,¹ Amber R. Solangi,² Najma Memon,²
Rabia A. Memon,³ and Muhammad Y. Khuhawar¹

¹ M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro 76080, Pakistan

² National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080, Pakistan

³ Institute of Plant Sciences, University of Sindh, Jamshoro 76080, Pakistan

Correspondence should be addressed to Arfana Mallah; arfana30@gmail.com

Received 12 June 2012; Accepted 3 October 2012

Academic Editor: Aleš Imramovsky

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This paper reports the utilization of 2-pyrrolecarboxaldehyde-4-phenylsemicarbazone (PPS) as a complexing reagent for the simultaneous determination and separation of Ni(II), Cu(II), Pd(II), and Ag(I) by reversed-phase high-performance liquid chromatography with UV detector. A good separation was achieved using Microsorb C18 column (150 × 4.6 mm i.d.) with a mobile phase consisted of methanol : acetonitrile : water : sodium acetate (1 mM) (68 : 6.5 : 25 : 0.5 v/v/v/v) at a flow rate of 1 mL/min. The detection was performed at 280 nm. The linear calibration range was 2–10 µg/mL for all metal ions. The detection limits (S/N = 3) were 80 pg/mL for Ni(II), 0.8 ng/mL for Cu(II), 0.16 ng/mL for Pd(II), and 0.8 ng/mL for Ag(I). The applicability and the accuracy of the developed method were estimated by the analysis of Ni(II) in hydrogenated oil (ghee) samples and Pd(II) in palladium charcoal.

1. Introduction

Thiosemicarbazones bonded through the sulphur and hydrazine nitrogen atom generally behave as a chelating ligand with transition metal ions. Free thiosemicarbazones and semicarbazones are less active in comparison to metal complexes. Thiosemicarbazones and their complexes have been considered extensively because of their pharmacological activities like anticancer, antibacterial, antiviral, antifungal, anti-HIV, antitumour, and many others [1, 2].

The important part of analytical chemistry is the continuous monitoring of the level of heavy metal ions in the environmental samples because of their positive and/or negative effects on the human body [3]. Few metal ions are toxic and carcinogenic, and they assert dangerous effects. These metal ions can easily be accumulated in the tissues of organisms and cause serious physiological disorders [4]. They can affect the respiratory system and lungs, causing asthma,

pneumonia, wheezing, and also nasal and throat cancers due to acute toxicity [5, 6]. Due to their hazardous effects, the researchers have increasing interest in the determination of these metal ions in biological and environmental samples. A number of methods have been reported employing various separations and detection techniques including atomic absorption spectrometry [7], ion chromatography [8], HPLC with solid-phase microextraction [9], inductively coupled plasma-atomic emission spectrometry [10], inductively coupled plasma-mass spectrometry (ICP-MS) [11], and capillary electrophoresis [12]. Although the most sensitive and reliable techniques reported so far are the ICP-MS and AAS, these are very costly. However, HPLC is the most commonly used technique, which fulfills all the requirements for analytical determination/separation of metals at very small scale. The purpose of this work is not only to synthesize and characterize the transition metal complexes with some ligands but also to develop a simple method to determine hazardous as well as

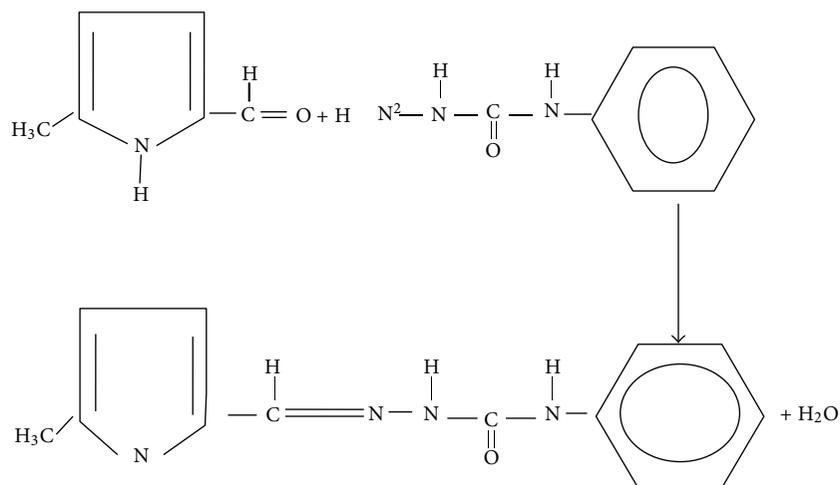


FIGURE 1: 2-Pyrrolicarboxaldehyde-4-phenylsemicarbazone.

essential metal ions at a very low concentration. The current study highlights a new loom for separation and simultaneous determination of Ni(II), Cu(II), Pd(II), and Ag(I) using 2-pyrrolicarboxaldehyde-4-phenylsemicarbazone (PPS) as a complexing reagent.

2. Experimental

2.1. Chemicals. Nitrate/sulfate or chloride salts of Co(II), Cu(II), Ni(II), Fe(II), Pd(II), Hg(II), Pb(II), Zn(II), and Ag(I) containing 1 mg/mL of a corresponding metal ion were prepared in deionized water and were used as stock solutions. Palladium(II) chloride was dissolved by adding warm hydrochloric acid solution, and volume was made up to mark with deionized water. Chloroform methanol, acetonitrile, tetrabutylammonium bromide (Merck), hydrochloric acid (Fluka), nitric acid (Fluka), 6-methyl-2-pyridinecarboxaldehyde (Aldrich), and 4-phenyl-3-thiosemicarbazide (Aldrich) were used as received without further purification. The following range of buffer solutions was used to adjust the pHs of the solutions: hydrochloric acid (0.1 M) and potassium chloride (1 M) for pH 1-2; acetic acid (1 M) and sodium acetate (1M) for pH 4-7; bicarbonate (1 M) and sodium carbonate (saturated) for pH 8-10.

2.2. Instrumentation. Hitachi 655A liquid chromatograph connected with a variable wavelength UV detector was used for the analysis of metal ions. Samples (20 μ L) were injected by rheodyne sampling valve and chromatointegrator D-2500, using Microsorb C18, (150 \times 4.6 mm i.d., 5 μ m) (Ranin Instruments, Woburn, MA, USA) column. Orion 420 A pH meter connected with combined glass electrode was used to measure the pH. A double beam spectrophotometer Hitachi 220 (Hitachi (Pvt.), Tokyo, Japan) with dual 1 cm cuvette was used to study absorption spectra for metal complexes.

2.3. Synthesis of the Reagent. 1.0 g of 2-pyrrolicarboxaldehyde was dissolved in methanol (10 mL), and 1.4 g of

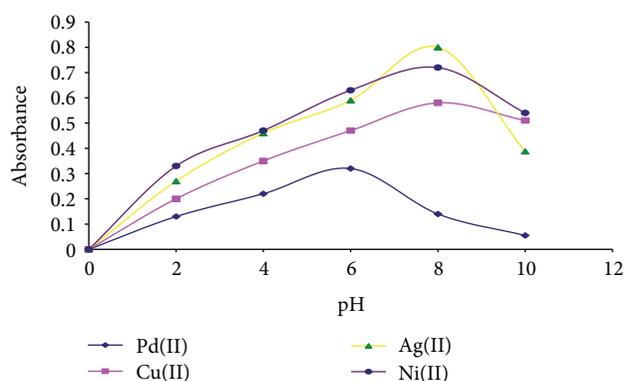


FIGURE 2: The effect of variation of pH on the formation of Pd(II), Ag(I), Ni(II), and Cu(II) as complexes of PPS against reagent blank. The final concentrations were 2, 1, 1, and 1 μ g/mL, respectively.

4-phenylsemicarbazone was dissolved in methanol:water (1:1) (20 mL). Both solutions were mixed well by adding glacial acetic acid (1 mL). The mixture was refluxed for 30 minutes, concentrated to 10 mL, and cooled at -5°C overnight. Slightly yellow-colored crystals of PPS were obtained, and product was recrystallized twice from methanol. The PPS melted at 194°C (Figure 1).

2.4. Spectrophotometric Procedure. The appropriate volume of Ni(II), Cu(II), Pd(II), and Ag(I) solutions containing 1.0–70 μ g of metal ion was transferred to a volumetric flask (10 mL), and reagent PPS solution (1 mL, 0.2% w/v in methanol) was added. 2 mL of buffer solution (pH 6-7) was added and made up to mark with methanol. The solutions were scanned in the wavelength range of 700–300 nm against reagent blank in the same solvent. The same procedure was adopted to study the effect of pH (1–10) on absorption spectra of metal PPS complexes. The variation in absorbance with pH was investigated (Figure 2). Similarly, the stability of metal PPS complexes was investigated by recording the

TABLE 1: Quantitative spectrophotometric data of color reaction of PPS with metal ions.

S. no.	Metal ions	Solvent	pH	λ_{\max} (nm)	$\epsilon = 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$	Solution stability
1	Ni(II)	Methanol : Water	8.0	380	45.0	>24 hr
2	Cu(II)	Methanol : Water	8.0	370	30.0	>24 hr
3	Pd(II)	Methanol : Water	5.0	370	20.0	>24 hr
4	Ag(I)	Methanol : Water	6.0	385	8.5	>6 hr

absorption spectra immediately after preparation and after lapse of different intervals. The variations in absorption spectra at different time intervals and the effects of time on the characteristic of absorption spectra were evaluated and shown in Table 1.

2.5. HPLC Procedure. A solution (1–5 mL) containing Ni(II), Cu(II), Pd(II), and Ag(I) (1–100 μg) was transferred to a 10 mL volumetric flask and was added reagent PPS solution (0.5 mL, 0.2% w/v in methanol) and 1 mL of bicarbonate buffer solution of pH 8.0. The contents were mixed well, and the volume was adjusted to the mark with methanol. The solution (2 μL) was injected on column Microsorb C18, 5 μm (150 \times 4.6 mm i.d.), and complexes were eluted with a mixture of methanol : water : acetonitrile : sodium acetate (68 : 25 : 6.5 : 0.5 v/v/v/v) using a flow rate of 1 mL/min. Detection was performed at 280 nm. The analytical column was re-equilibrated for 5 min after each run, and the chromatographic peak height was used for quantification purpose.

2.6. Determination of Pd(II) in Palladium Charcoal. 5 mL of hydrochloric acid (37%) was added to 0.3 g of palladium charcoal with Pd 10% (E. Merck) and refluxed for half an hour, and then 5 mL of water was added to the solution, cooled, and filtered. The final solution was adjusted to 25 mL. The solution (0.5 mL) was transferred to 10 mL of volumetric flask, and the above analytical procedure was followed. The amount of Pd(II) in palladium charcoal was calculated from the calibration curve prepared from the standard Pd solution.

2.7. Analysis of Ni(II) in a Hydrogenated Vegetable Oil (Ghee) Sample. 20 g of a hydrogenated vegetable oil sample (Pakistan Oil Mills, Pvt. Ltd., Karachi and Tullow Oil Mills, Hyderabad, Pakistan) was transferred to conical flask, and 30 mL of HNO_3 (1 M) was added. After shaking on mechanical shaker for one hour, the layers were allowed to separate, and aqueous layer was collected and concentrated to nearly 5–7 mL, and the final volume was adjusted to 10 mL with water. In a 10 mL flask, 0.5 mL of solution was transferred, pH was adjusted to 6.0, and analytical procedure was followed as mentioned in a previous section. External calibration curve was used for the quantification of Ni. To compare the results obtained with the proposed method, content of Ni in aqueous extract was also determined by using Varian AA-20 flame atomic absorption spectrophotometer. Samples were run in triplicate with delay and integration time of 3 seconds.

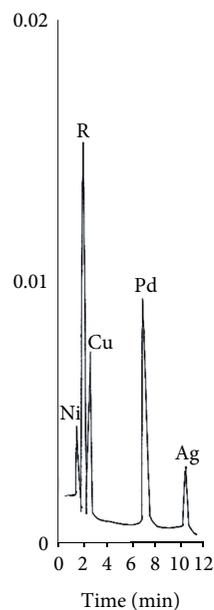


FIGURE 3: HPLC separation of Ni, Cu, Pd, and Ag complexes on Microsorb C18, 5 μm (150 \times 4.6 mm i.d.), with a mixture of methanol : water : acetonitrile : sodium acetate (1 mM) (68 : 25 : 6.5 : 0.5 v/v/v/v) with a flow rate of 1 mL/min and at a wavelength of 280 nm.

3. Results and Discussion

The reagent PPS was prepared as reported and reacted with Cu(II), Ni(II), Co(II), Fe(II), Hg(II), Pd(II), and Ag(I) to form colored complexes. The maximum color reaction was observed within pH 5–8 and was stable for 6 to 24 hrs. Ag(I) complex developed turbidity and required solvent extraction procedure in chloroform. All complexes were absorbed maximally within 370–380 nm with molar absorptivity in the range of 8.5 to 45 $\times 10^3 \text{ L mole}^{-1} \text{ cm}^{-1}$. The Ag(I) indicated the minimum and Ni(II) the maximum value of molar absorptivity. The Beer's law was obeyed with 0.1–7.0 $\mu\text{g/mL}$ of the metal ions (Table 1). The reagent PPS has high a spectrophotometric permittivity, but the complexes were absorbed within the narrow range of 377–390 nm in visible region; therefore, HPLC was examined for their simultaneous determinations. The complexes were injected on Microsorb C18 column and eluted isocratically with a mixture of methanol water and acetonitrile. However, a separation between Ni(II), PPS, Cu(II), Pd(II), and Ag(I) was obtained when eluted with a mixture of methanol : water : acetonitrile : sodium acetate (1 mM) (68 : 25 : 6.5 : 0.5 v/v/v/v) with a flow rate of 1 mL/min at 280 nm wavelength (Figure 3). The retention times for

TABLE 2: Quantitative HPLC data for metal ions Cu(II), Ni(II), Pd(II), and Ag(I) using PPS complexing reagent.

S. no.	Metal ion	Eluent	pH	Calibration range $\mu\text{g/mL}$	Detection limits
1	Ni(II)	M : W : A : Sod.Acet.*	8.0	2–10 $\mu\text{g/mL}$	80 pg/mL
2	Cu(II)	M : W : A : Sod.Acet.*	8.0	2–10 $\mu\text{g/mL}$	0.8 ng/mL
3	Pd(II)	M : W : A : Sod.Acet.*	5.0	2–10 $\mu\text{g/mL}$	0.16 ng/mL
4	Ag(I)	M : W : A : Sod.Acet.*	6.0	2–10 $\mu\text{g/mL}$	0.8 ng/mL

*M: methanol, W: water, A: acetonitrile, Sod.Acet: sodium acetate.

TABLE 3: Analysis of Ni(II), in hydrogenated oil (ghee), and Pd(II), in palladium charcoal.

S. no.	Sample	Metal ion	Amount found with HPLC	Amount found with AAS	Std. Error (%)
1	Pak. ghee oil mills	Ni(II)	2.40 $\mu\text{g}/20\text{ g}$	2.31 $\mu\text{g}/20\text{ g}$	3.8
2	Tullow oil mills	Ni(II)	3.04 $\mu\text{g}/20\text{ g}$	2.96 $\mu\text{g}/20\text{ g}$	2.7
3	Palladium charcoal	Pd(II)	9.80%	*10%	2.0

*Palladium was not analyzed by AAS; The result was compared with the standard value provided by E. Merck.

Ni(II), Cu(II), Pd(II), and Ag(I) were observed as 2.0, 2.9, 3.9, and 12 min, respectively. Linear calibration curves were plotted by recording the average peak height ($n = 3$) versus concentration and were obtained with 2.0–10 $\mu\text{g/mL}$ of each metal ion, corresponding to 5–50 ng/injection. The detection limits remained as three times the signal-to-noise ratio (3 : 1) and were obtained as 0.8 ng/mL for Ag(I), 0.16 ng/mL for Pd, 0.8 ng/mL for Cu, and 80 pg/mL for Ni. The coefficient of determination (R^2) for Ag, Ni, Cu, and Pd with six-point calibration was observed as 0.995, 0.994, 0.998, and 0.996, respectively (Table 2).

Nickel(II) eluted before the complexing reagent PPS with retention time of 2.0 min and palladium after the reagent show very good separation and indicated high permittivity. Therefore, the determination of Ni(II) and palladium from real samples was considered. The method was used for the determination of palladium in palladium charcoal and nickel in hydrogenated oil (ghee) samples. The results of Ni are also compared with AAS method and summarized in Table 3. The effect of different ions at least twice the concentration of Ni(II) and Pd(II) was also examined. Cu(II), Fe(II), Cd(II), Zn(II), Pb(II), Mn(II), Cr(III), Ca(II), Mg(II), Bi(III), Cl^- , Br^- , I^- , SO_4^{2-} , citrate, and tartrate did not interfere.

4. Conclusion

A rapid, precise, reproducible, and sensitive method for simultaneous determination and separation of metal complexes using RP-LC was developed. Enhanced detection sensitivity (pg to ng levels) with quantitative data was successfully demonstrated. PPS was found to be a very efficient reagent for the determination of these metal ions after complex formation. PPS reacts with the number of metal ions but is more selective and sensitive for Ni(II), Cu(II), Pd(II), and Ag(I). The applicability of method to a variety of matrices reveals the ruggedness of the proposed method.

Conflict of Interests

All the authors of the paper declare that they do not have a direct financial relation with any commercial identity

mentioned in the paper that might lead to a conflict of interests for any of the authors.

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