



Synthesis and solvatochromic studies of 2- amino-3-phenolazo1-(4-sulfophenyl)-3-methyl-5-pyrazolone and use it for the determination of trace amount of Nickel (II) in blood samples

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Abstract: A new, simple, sensitive and rapid spectrophotometric method is proposed for the determination of trace amount of Nickel (II). The method is based on the formation of a 1:2 complex with 4-(4-((2-hydroxy-6-nitrophenyl) diazenyl) -3-methyl-5-oxo-2, 5-dihydro-1H-pyrazol-1-yl) benzenesulfonic acid (2-ANASP) as a new reagent is developed. The complex has a maximum absorption at 516 nm and ϵ_{\max} of $1.84 \times 10^5 \text{ L. mol}^{-1} \text{ cm}^{-1}$. A linear correlation (0.25 – 4.0 $\mu\text{g. ml}^{-1}$) was found between absorbance at λ_{\max} and concentration. The accuracy and reproducibility of the determination method for various known amounts of Nickel (II) were tested. The results obtained are both precise (RSD was 1.2 %) and accurate (relative error was 0.787 %). The effect of diverse ions on the determination of Nickel (II) to investigate the selectivity of the method were also studied. The stability constant of the product was $0.399 \times 10^6 \text{ L. mol}^{-1}$. The proposed method was successfully applied to the analysis of diabetes blood and normal human blood.

Key word: Nickel (II) determination, Solvatochromic; 4-(4-((2-hydroxy-6-nitrophenyl) diazenyl)-3-methyl-5-oxo-2; 5-dihydro-1H-pyrazol-1-yl) benzenesulfonic acid

Introduction

The determination of metal ion at trace amount have been shown significant important in the fields of environment and biological studies. Literature date show that pyrazolone derivative have been widely used in the determination metal ions due to sensitive colour, stability and very good chelatogenic characteristics [1-5].

Nickel play great role in the biological systems and used in many industries. Nickel forms many complexes the biological systems, ingredient of some bioactive molecules of enzymes and also in the storage and transport of active substances [6-8]. The biological complexes of nickel are important bioenzymes and have role in the biological systems. Important coenzymes studied are: methyl coenzyme, urease and acetyl coenzyme [9]. Several instruments method are present in the literature used to determine ion, and these method inductively coupled plasma atomic emissions spectrometry [10, 11], flame and electro thermal absorption spectrometry [12-17], electrochemical methods [18, 19] and spectrophotometric techniques [20, 21] show good sensitivity but is limited because of expensive instrumentation and high cost for routine analysis.

According to the best of our knowledge, this reagent has not been reported in the literature as being used for any cation determination. In this

study, we wish to report the 4-(4-((2-hydroxy-6-nitrophenyl) diazenyl)-3-methyl-5-oxo-2, 5-dihydro-1H-pyrazol-1-yl) benzenesulfonic acid (2-ANASP) reagent as a selective reagent in spectrophotometric determination of micro amounts of Nickel (II).

Materials and Methods

I/ Preparation of the reagent ((2-ANASP))

The reagent was prepared by coupling 1-(4-sulfophenyl)-3-methyl-5-pyrazolone with diazotate 2-amino-3-nitrophenol alcoholic solution. A diazonium solution was prepared by taking 1 g 2-amino-3-nitrophenol in 15mL of ethanol and concentrated hydrochloric acid with 5 mL of distilled water and adding sodium nitrite solution drop wise at 0-5°C. 1-(4-sulfophenyl)-3-methyl-5-pyrazolone 1.6510 g was dissolved in 50 mL of ethanol and 30 mL of 0.1 M were added at (-5°C). The mixture was left to stand overnight. The precipitate was filtered off and recrystallized from ethanol [22] Scheme 1.

Preparation of Nickel(II)complex

The complex was prepared by stoichiometric amount from ligand in 50 mL of ethanol then added drop wise with stirring to a stoichiometric amount 1:2 for Nicke salt in 25 mL hot distilled water. The solid greenish black product thus formed off, washed with ethanol and dried.

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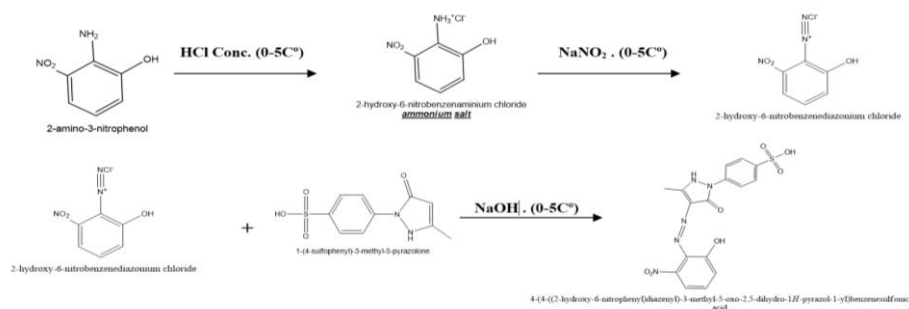
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Scheme 1: preparation of reagent (2-ANASP)

Apparatus

Spectrophotometric measurement were made with Shimadzu UV – visible – 1700 double beam spectrophotometer using 1.00 cm glass cells. Vibrational spectra were recorded on Test scan Shimadzu FT. IR 8000 series. Measurements of pH were made using an Hanna, HI9811-5 pH – meter equipped with a glass – saturated calomel combined electrode. Melting points of both ligand and complex were obtained with an electrothermal melting point apparatus. Conductivity was measured in DMSO (10-3) solution with an Alpha digital conductivity model -800.

Reagents

All chemicals used were of analytical grades
Nickel (II) stock solution (100 µg. ml⁻¹)

prepared by dissolving 0.05 g of Nickel chloride in 500 ml of distilled water, working standard of Ni (II) solutions were prepared by simple dilution of the appropriate volume of the standard Ni (II) solution (100 µg. ml⁻¹) with distilled water. 4-(4-((2-hydroxy-6-nitrophenyl) diazenyl)-3-methyl-5-oxo-2,5-dihydro-1H-pyrazol-1-yl) benzenesulfonic acid (2-ANASP) (1 mM) 0.1048 g of reagent was dissolved in 250 ml of ethanol.

Foreign ion solutions (10 µg. ml⁻¹)

These solutions were prepared by dissolved an amount of the compound in distilled water completing the volume in a volumetric flask.

General Procedure

In to a series of 10 ml calibrated flask, transfer increasing volumes of Ni(II) working solution 10 ppm to cover the range of calibration curve, add 2.0 ml of 1Mm of (2-ANASP) solution and pH was adjusted to 10. The complexes formed were solubilized in water and diluted up to 10 ml in a standard flask. The absorbance of the resulting solution was measured at the respective absorption maxima against a reagent blank prepared under similar condition.

Results and Discussion

Spectra

The result of this work indicated that the reaction of Ni (II) with (2-ANASP) at pH 6 yield highly soluble product which can be utilized as a suitable assay procedure for Ni (II). This product has a maximum absorption at 516 nm at which the blank at this wavelength shows zero absorbance. The spectrum of (2-ANASP) solution gives strong absorption band of ligand –to ligand charge transfer LLCT due to $\pi \rightarrow \pi^*$ of nitrogen atom of azo group at 466.0 nm and with shoulder band due to transition $\pi \rightarrow \pi^*$ of C=N group Fig. 1.

The bands of N=N and C=N were shifted to broad peak at high wavelength with high intensity at 516.0 nm upon complexation due to transition of $\pi \rightarrow \pi^*$ charge transfer. Fig. 2 [23]

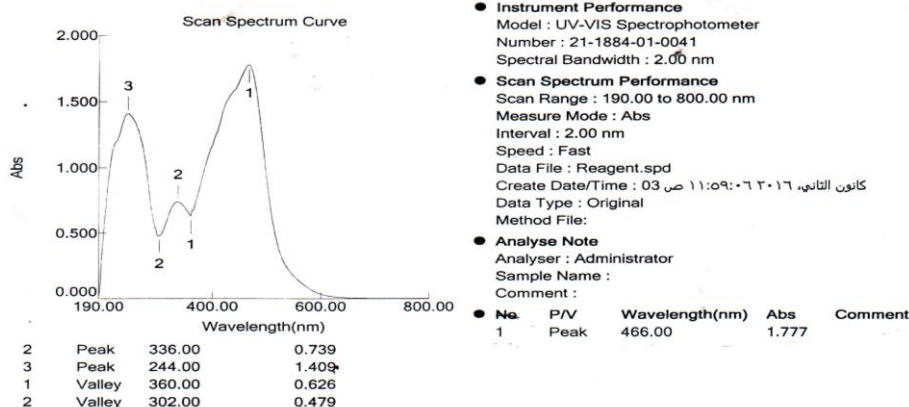


Figure 1: Absorption spectrum of the reagent (2-ANASP) against ethanol as blank

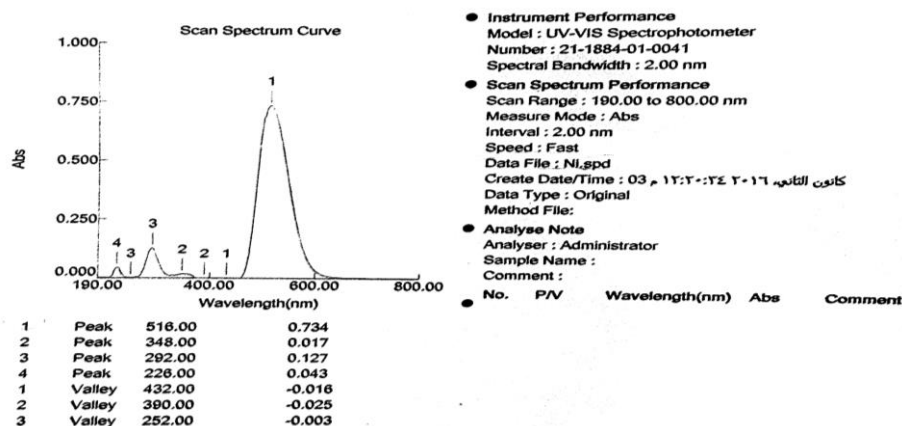


Figure 2: Absorption spectrum of [Ni (II) + (2-ANASP)] treated as described under procedure and against a reagent blank.

Solvatochromism study of 2 - amino - 3 - phenolazo1 - (4-sulfophenyl) - 3 - methyi - 5 - pyrozone Compound:

To determination of solvate chromic range of azo dyes according to sensitivity to solvent change by using UV-Visible, more than (18) empirical scales of solvent polarity has been used, i. e Et (30) scale has more comprehensive applications for characterization of the chromatographic materials and polarity of polymers in addition of measurements of the polarity of all type of solvents, electrolyte solution and binary solvent mixture [24-27]. The popular comparison methods of solvatochromic, demonstrated by Kamlet and Taft and improved by Abraham, Carr and Abboud has been to explain the solvent effect. A solvatochromic equation with three parameters Λ^* , β and, α can be used to determine two type hydrogen bonding [28]. The absorption spectra of dye carried out over the range between 190-800 nm in various selected solvent and the results are summarized in Table 1.

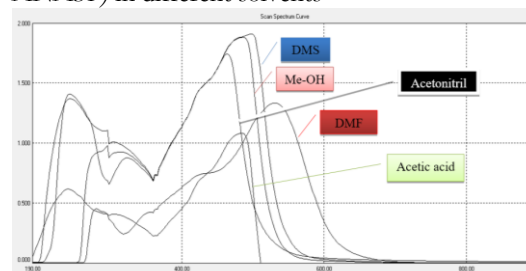
Dye of 4-(4-((2-hydroxy-6-nitrophenyl) diazenyl)-3-methyl-5-oxo-2, 5-dihydro-1H-pyrazol-1-yl) benzenesulfonic acid showed a weak peak at region 190-300 nm assigned to hydrazone tautomeric form at 370-600 nm [29]. Dye showed the positive solvatochromism due to the absorbance of the first band was decreased via increasing solvent polarity. This mean that the excitation state more stable and have polarity different form ground state. It was also observed that the negative solvatochromism exhibit by the second band absorbance it was increase via increasing solvent polarity and it shifted to lower wavelength [30] Fig. 3.

Table 1: Influence of solvent on maximum wavelength of (2-ANASP).

Solvent	Absorption maximum band nm	
	Peak 1	Peak 2
Methanol	242.00	486.00
Acetic acid	240.00	484.00
DMF	280.00	530.00
DMSO	296.00	498.00
Acetonitrile	242.00	464.00

DMF = (N, N dimethyl formamide), DMSO = dimethyl sulfoxide

Figure 3: Absorption spectra of the reagent (2-ANASP) in different solvents



The effect of various parameters on the absorbance intensity of the formed products were studied and the reaction conditions were optimized.

Effect of pH

The pH of metal complex solutions was adjusted using dilute solutions (0.05M) NaOH and (0.05 M) HCl, and the effect on absorbance was studied Fig. 4. The absorbance of the complex was maximum and constant in the pH range given in Table 2.

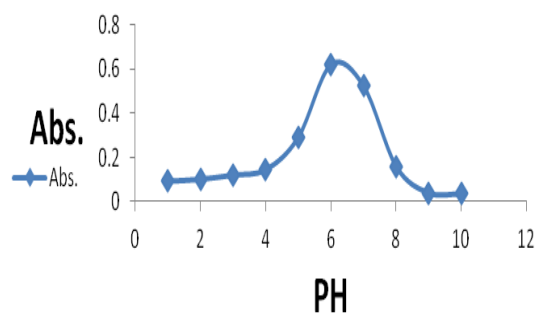


Figure 4: Effect of pH

Table 2: Analytical characteristics of Ni[(2-ANASP)₂] complex.

Characteristic	Ni (II) – complex
Absorption maximum (nm)	516
Beer's law range (ppm)	(0. 25– 4)
pH range	(5. 5-7. 0)
Sandell's sensitivity $\mu\text{g. cm}^{-2}$	$2. 59 \times 10^{-3}$
Molar absorptivity ($\text{L. mol}^{-1} \cdot \text{cm}^{-1}$)	$1. 84 \times 10^5$
Stability constant (L. mol^{-1})	$0. 399 \times 10^6$
Melting point for reagent	270 C^0
Melting point for Ni (II) – complex	290 C^0

Effect of (2-ANASP) concentration

Various concentration of (2-ANASP) was added to fixed concentration of Ni (II). 2. 0 ml of 1. 0 mM (2-ANASP) solution was sufficient and gave minimum blank value was increased causing a decrease in the absorbance of the sample. Therefore, 2ml of 1 mM of (2-ANASP) was used in all subsequent experiment Fig. 5.

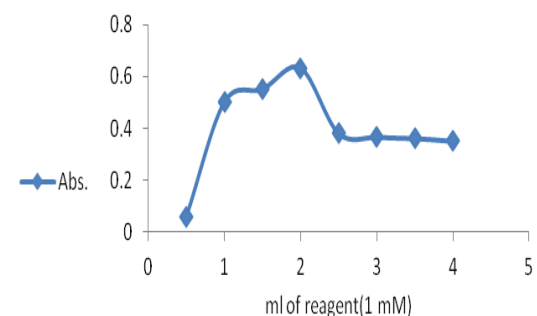


Figure 5: Effect of (2-ANASP) concentration

Effect of reaction time

The colour intensity reached a maximum after the Ni (II) has been reacted immediately with (2-ANASP) and became stable after one minute, therefore one-minute development time was selected as optimum in the general procedure. The colour obtained was stable for a least 24 hours.

Effect of temperature

The effect of temperature on the colour intensity of the product was studied. In practice, the same absorbance was obtained when the colour was developed at room temperature (25 – 30°C), but when the volumetric flask were placed in a water – bath at (40-60°C) a loss in colour intensity and stability were observed, therefore it is

recommended that the colour reaction should be carried out at room temperature form 25-35 C° for complex Figure 6.

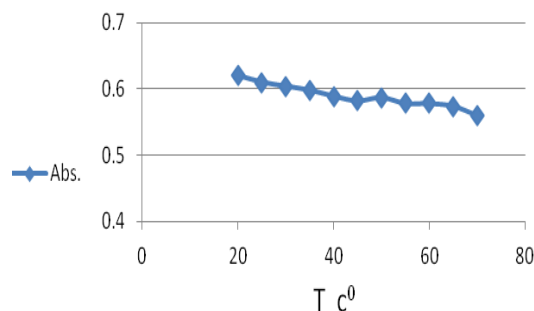


Figure 6: Effect of temperatures on complex Ni [(2-ANASP)₂] complex

Calibration graph

The calibration equation for (0. 25– 4 μg per ml) Ni (II) is $Y = 0. 385x + 0. 099$ ($R^2 = 0. 982$). Since the coloured complex is stable for 24 hrs, the method can be applied to large series of samples. The molar absorptivity and sandell' sensitivity are given in Table 2.

Composition of the complex

The composition of complex was studied in the excess of reagent solution by the mole-ratio and Job s methods Fig. 7, 8. A break at a 1:2 (M:L) mole ratio suggested the formation of complex where M= Ni(II) and L= (2-ANASP) under the given condition.

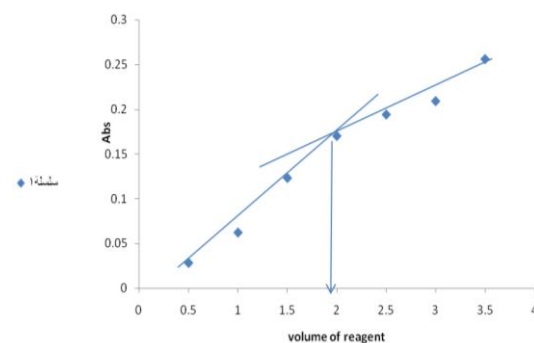


Figure 7: mole-ratio method for Ni [(2-ANASP)₂] complex

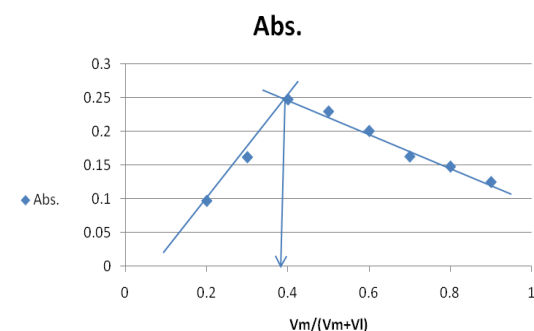


Figure 8: Job s method for Ni [(2-ANASP)₂] complex

Conductivity measurements

The solubility of the complexes in dimethyl sulfoxide and methanol permitted of the molar conductivity of 10^{-3} M solution at 25°C and by

comparison, the electrolytic nature for complexes. The low values of the molar conductance data listed in Table. 3 indicate that the complexes are non-electrolytes.

Table 3: Conductivity values of $\text{Ni}[(2\text{-ANASP})_3]$ complex

Molar conductivity, $\mu\text{S mole}^{-1} \text{cm}^2$ Methanol	Molar conductivity, $\mu\text{S mole}^{-1} \text{cm}^2$ Ethanol	Molar conductivity, $\mu\text{S mole}^{-1} \text{cm}^2$ DMSO	Complex
0. 397	0. 091	0. 235	Ni [(2-ANASP)]

Table 4: Effect of foreign ions

Seq	foreign ions Conc. $50\mu\text{g. mL}^{-1}$	Best volume of masking reagent Oxalic acid (0. 1M), Urea (0. 1M) and Thiourea (0. 1M)	E% without masking agent	E% with masking agent
1-	Hg ⁺⁺	0. 5 ml	- 44. 60%	4. 76%
2-	Pb ⁺⁺	0. 5 ml	-73. 65%	4. 92%
3-	Cs ⁺	=	-40. 79%	0. 0%
4-	Cr ³⁺	=	32. 69%	-3. 17%

Interferences

The effect of diverse ions in the determination metal ion was studied. To test of diverse ions were determined by the general procedure, in the presence of their respective foreign ions. The metal ion can be determined in the presence of a 5 or more-fold excess of cation and anion Table 4.

In the experiment, a certain amount of standard Ni (II) solution, coexisting ion solution and masking agent (or absence of masking agent) were added. It is found that all the studied ions interfere seriously. However, their interferences are masked efficiently by addition 0. 5 ml of 0. 1 M of (NaNO_2 , thiourea and citric acid).

FT. IR of reagent and its complex

The FT. IR of the free ligand and it's metal chelate were carried out in the (400-4000) cm^{-1} Range. The IR bands of the (2-ANASP) and its Ni (II)

complex with their probable assignment are given in Table. 5. The IR spectrum of ligand shows a broad band at $3380. 27 \text{ cm}^{-1}$ corresponding to $\nu\text{C-O}$ and $\delta\text{O-H}$. This band drastic disturbance in all the frequencies of the hydroxyl group refers to coordination of the deprotonated phenolic group of the (2-ANASP) with the Ni (II) [31] Fig. 9, 10. However, the $\nu(\text{N}=\text{N})$ stretching band in the free ligand is observed at $1499. 04 \text{ cm}^{-1}$. This band is shifted to lower with low intensity at $1440. 21 \text{ cm}^{-1}$ frequency value upon complexation suggesting chelation via the (M-N) [32]. The IR spectrum of the ligand revealed a sharp band at $1634. 69 \text{ cm}^{-1}$ due to $\nu(\text{C}=\text{O})$ of pyrazole azo. This band is shifted to lower with low intensity at $1595. 66 \text{ cm}^{-1}$ frequency value upon complexation [33] Fig. 10. The bonding of oxygen to the metal ion is provided by the occurrence of band at $533. 29 \text{ cm}^{-1}$ as the result of $\nu(\text{M-O})$ [34, 35].

Table 5: Selected FT. IR data of (2-ANASP) and it's complex with Ni (II)

Compound	$\nu(\text{OH})$	$\nu(\text{C}=\text{N})$	$\nu(\text{N}=\text{N})$	$\nu(\text{C}=\text{O})$	$\nu(\text{M-O})$	$\nu(\text{M-N})$
(2-ANASP)	3380. 27	1616	1499.04	1634. 69	-	-
[Ni (L) ₃]	-	1595. 51	1440.21	1595. 66	533. 29	-

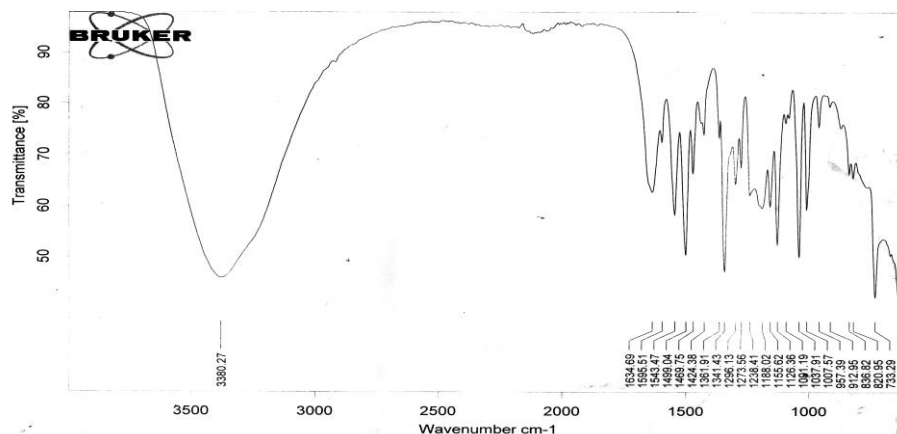


Figure 9: FTIR of the reagent (2-ANASP)

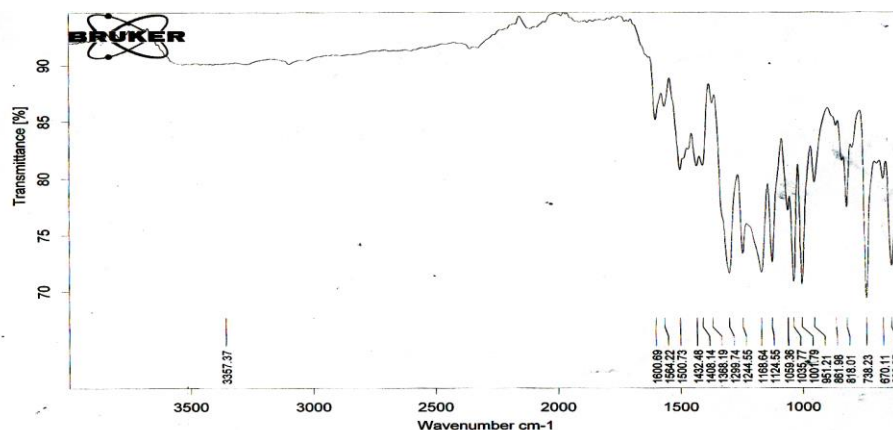
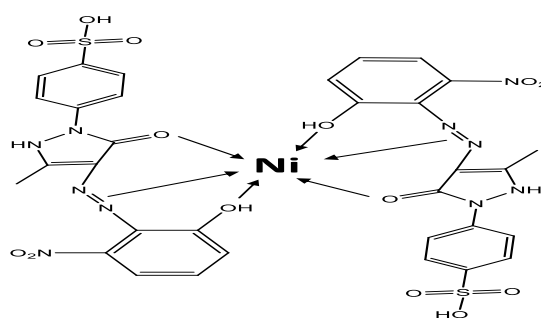


Figure 10: FTIR of the complex of Ni[(2-ANASP)₃] complex

On the basis of the FT.IR, stoichiometric, and molar conductivity data the structure of complex can be suggested as the following:



Structure of [Ni(2-ANASP)₃] complex

Table (6): Ni(II) levels in bloods of human

Blood Samples	A mount found by our spectrophotometric method ($\mu\text{g. ml}^{-1}$)	A mount found by Atomic Absorption ($\mu\text{g. ml}^{-1}$)
Normal adult (male)	0. 959	0. 664
Diabetes patient	0. 774	0. 615

Applications

Estimation of Ni(II) in the blood samples taken from patients with diabetes and adults:

After collecting blood samples in tubes made of polyethylene saves at (-20 °C) temperature. First transferred to baker, a concentrated Nitric acid (10 ml) was added and heating quietly, when the reaction starts to be activity, sample solution was removed and left to cool well. Then, (1ml) of H₂SO₄ concentrated was added. Next, (1. 5ml) of 1% KMnO₄ was added also and mixed well. After

this (1ml) of Sodium azid (NaN₃) (2. 5%) and (0. 5 ml) of 70%HClO₄ were added with heating under 70 °C for 30 min to remove any excess of NaN₃. Finally, the solution was left to cool then neutralization with dilute Ammonium hydroxide (NH₄OH), next the solution transferred to volumetric flask (200 ml) and diluted to the mark with deionized water. All samples were ready for UV-Visible and atomic absorption spectrometric analysis [36]. The results are shown in Table 6, indicate that satisfactory precision and accuracy could be attained with proposed method.

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