

## A Simple Pre-concentration Method for the Determination of Nickel(II) in Urine Samples Using UV-Vis Spectrophotometry and Flame Atomic Absorption Spectrometry Techniques

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**Abstract:** The cloud point technique was effectively utilized for extraction and pre-concentration of nickel(II) in urine samples before measurement by UV-Vis spectrophotometer and AAS techniques. The metal response to a para-aminophenol (PAP) reagent in a non-ionic surfactant Triton X-114 medium was to form the Ni-PAP complex. The adopted concentration for PAP, concentration of Triton X-114, pH effect and water bath temperature, incubation time, salt effect, and interference effects were all optimized. The calibration curve was linear over the range of (0.0625–1.25) mg.L<sup>-1</sup> with a correlation coefficient  $r^2$  of 0.9682 for the UV-Vis spectrophotometer at a  $\lambda_{max}$  of 629 nm. The limit of detection was 0.005 mg/L. The relative standard deviation for six replicates was 1.07%. This method was applied successfully to determine copper (II) concentrations in 44 urine samples of occupational worker samples as determined by UV-Vis spectrophotometry and FAAS techniques.

**Keywords:** Ni(II); cloud point extraction; non-ionic surfactant; urine sample, UV-Vis spectrophotometer; FAAS

### ■ INTRODUCTION

Estimating trace elements in biological samples is an important area of study to evaluate occupational and environmental exposure [1]. Nickel is considered to be moderately toxic compared to other elements. However, the inhalation of fumes containing nickel may lead to serious health problems [2]. Nickel has several industrial uses, such as its use in electroplating, ceramics, magnetic tapes, computer components as well as in the manufacture of arc-welding rods. Nickel is present in water, soil and the atmosphere as a result of industrial processes and improper disposal of industrial waste [3]. Nickel plays a role in the formation of certain enzymes such as the urease enzyme which is necessary for animals and plants. Considerable attention has been paid to the toxicity of nickel in low concentrations because nickel can cause an allergic reaction and certain nickel compounds may be carcinogenic [4]. Moreover, nickel can cause a disorder

known as nickel-eczema [2]. The concentration range of nickel in urine is 0.27–3.68 ng/mL with a median value of 1.7 ng/mL [5]. There are commonly used analytical methods in which nickel concentrations have been determined, such as FAAS [6-10]. Inductively coupled plasma mass spectrometry (ICP-MS) [11-13], graphite furnace-atomic absorption spectrometry (GFAAS) [14], UV-VIS spectrometry [15-16], inductively coupled plasma optical emission spectrometry [17-18]. The cloud point extraction method has become an important method that is commonly used for pre-concentration and element estimation [19]. Cloud point extraction processes occur depending on the use the phase behavior of non-ionic surfactants in aqueous solutions, where micelles are formed, and the solution becomes cloudy when heating to a certain temperature. At higher temperatures, phase separation will occur [20]. The typical surfactants used in the CPE processes mostly

include (1) anionic surfactants, such as sodium dodecyl sulphate (SDS). (2) Non-ionic surfactants, such as Triton X-100, Triton X-114. (3) Cationic surfactants, for example cetyl trimethyl ammonium bromide (CTAB). (4) Zwitterionic surfactants have one cationic center and one anionic part [21]. CPE is applied to determine trace element concentrations such as Fe(III) [22] and Se(II) [23].

The aim of this study was the development of a sensitive, selective and environmentally friendly spectrometric method of determination of Ni(II) concentration using para-aminophenol (PAP) as a complexing agent and cloud point extraction. A UV-Vis spectrophotometer was used as detection technique and the proposed method was applied to a number of samples. In the present work, the CPE method has been used for separation and pre-concentration of nickel(II) after the formation of a complex with PAP, using Triton X-114 as the surfactant and then measurement by using a UV-VIS spectrophotometer at 629 nm and FAAS techniques. The analytical conditions for the pre-concentration of nickel were investigated.

## ■ EXPERIMENTAL SECTION

### Apparatus

The pH of the solution was adjusted by using a WTW (model 720) pH meter with a combined glass electrode. A Hettich centrifuge (model EBA - 20, Germany) with 10 mL calibrated centrifuge tubes was used for phase separation at 3600 rpm for 5 min. A water bath (Lab companion BS-11 Shaking Water bath) with an excellent temperature control was used to accelerate CPE experiments. Micropipettes ((10–100)  $\mu$ L and (100–1000)  $\mu$ L) were used to deliver accurate volumes. A Lab Line Super Mixer (model 129) Hitech Trader, U.S.A. was used to mix the solution. All absorption spectra were recorded on a Shimadzu double beam UV-VIS. spectrophotometer (UV-1800, Japan) equipped with deuterium and tungsten lamps and using 1 cm quartz cells (0.5 mL) for all measurements. The trace nickel was measured by using atomic absorption techniques (Atomic Absorption -AA-7000- Shimadzu-Japan).

### Reagent and Solutions

Solutions were prepared using analytical grade chemicals and deionized water. 1% (w/v) of para-aminophenol (PAP) was prepared by dissolving 1 g PAP in (1–2) mL dimethylsulphoxide (DMSO) and then adding absolute ethanol to a 100 mL volumetric flask. Solutions of non-ionic surfactant Triton X-114 were prepared at a 20% (v/v) concentration by diluting 20 mL of Triton X-114 to 100 mL (hot deionized water) in a volumetric flask. A stock solution of 1000 mg/L of Ni(II) was prepared by dissolving 0.3 g Ni(NO<sub>3</sub>)<sub>2</sub> in 100 mL deionized water.

### Sample Collection

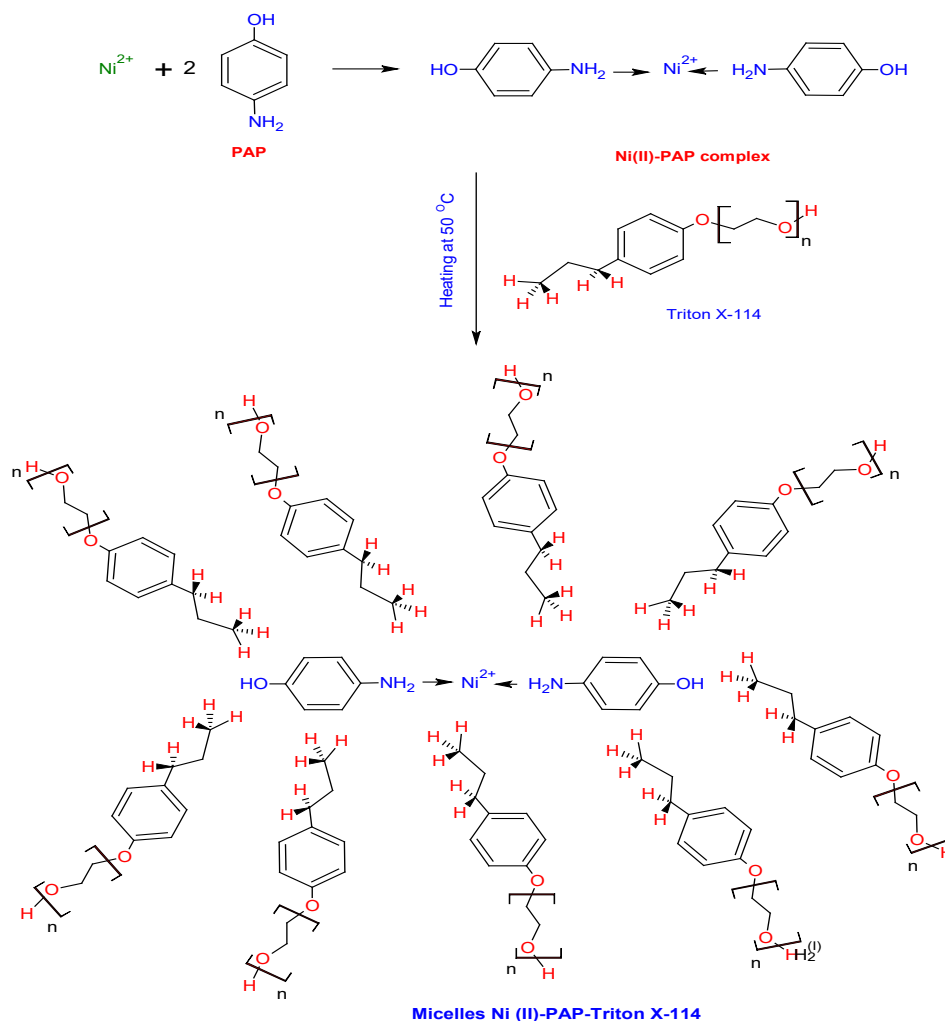
Urine samples were collected from people working in different professions such as dyeing, welding, casting alloys, and oil refineries, and then terminals to provide fuel and quality control within the oil sector. Urine samples were collected in a fresh polyethylene bottles (120 mL) and stored in a fridge at (0–5) °C to keep the models from damage after that are initialized urine samples for subsequent operations.

### Urine Sample Digestion

Digestion of urine samples was carried out via the following steps [24-25]. In a 100 mL beaker, 25 mL of urine sample was treated with a mixture of 5 mL H<sub>2</sub>O<sub>2</sub> and 2.5 mL of conc. HNO<sub>3</sub>, and placed on a hot plate. The samples were then heated until the dark color turned light. Then, the samples were heated until completely dry. 2.5 mL of conc. HNO<sub>3</sub> was added to the resultant dark residue and reheated until dry; this step was repeated several times until white ashes were produced. These were dissolved in the final step using 2.5 mL of 3 M HCl.

### Cloud Point Extraction Procedure

CPE has been carried out according to the following aliquots of 10 mL of the working solution containing 0.5 ppm of Ni(II), 0.25 mL of 1% (w/v) PAP reagent and then left for 30 min to complete the reaction with 0.5 mL of 20% (v/v) triton X-114. The mixture was



**Fig 1.** Schematic diagram for proposed mechanism of Ni(II)-PAP complex separation via the CPE method [22]

diluted to 10 mL using deionized water then placed in the super mixer to mix the solution. After this, the mixture was held in a water bath at 50 °C for 10 min. The separation into two phases was achieved by centrifugation for 5 min. at 3500 rpm. The solution was then cooled in an ice bath at (0–5) °C for 5 min to increase the viscosity so that the surfactant-rich phase separated easily by converting tube. The surfactant-rich phase of this procedure was dissolved and diluted to 0.75 mL with absolute ethanol and transferred into 1.0 cm quartz cell (1 mL). The absorbance of the solution was measured at  $\lambda_{\text{max}} = 629 \text{ nm}$  against a blank solution prepared in the same manner. Fig. 1 shows the CPE scheme and the proposed reaction mechanism for the nickel(II)-PAP complex.

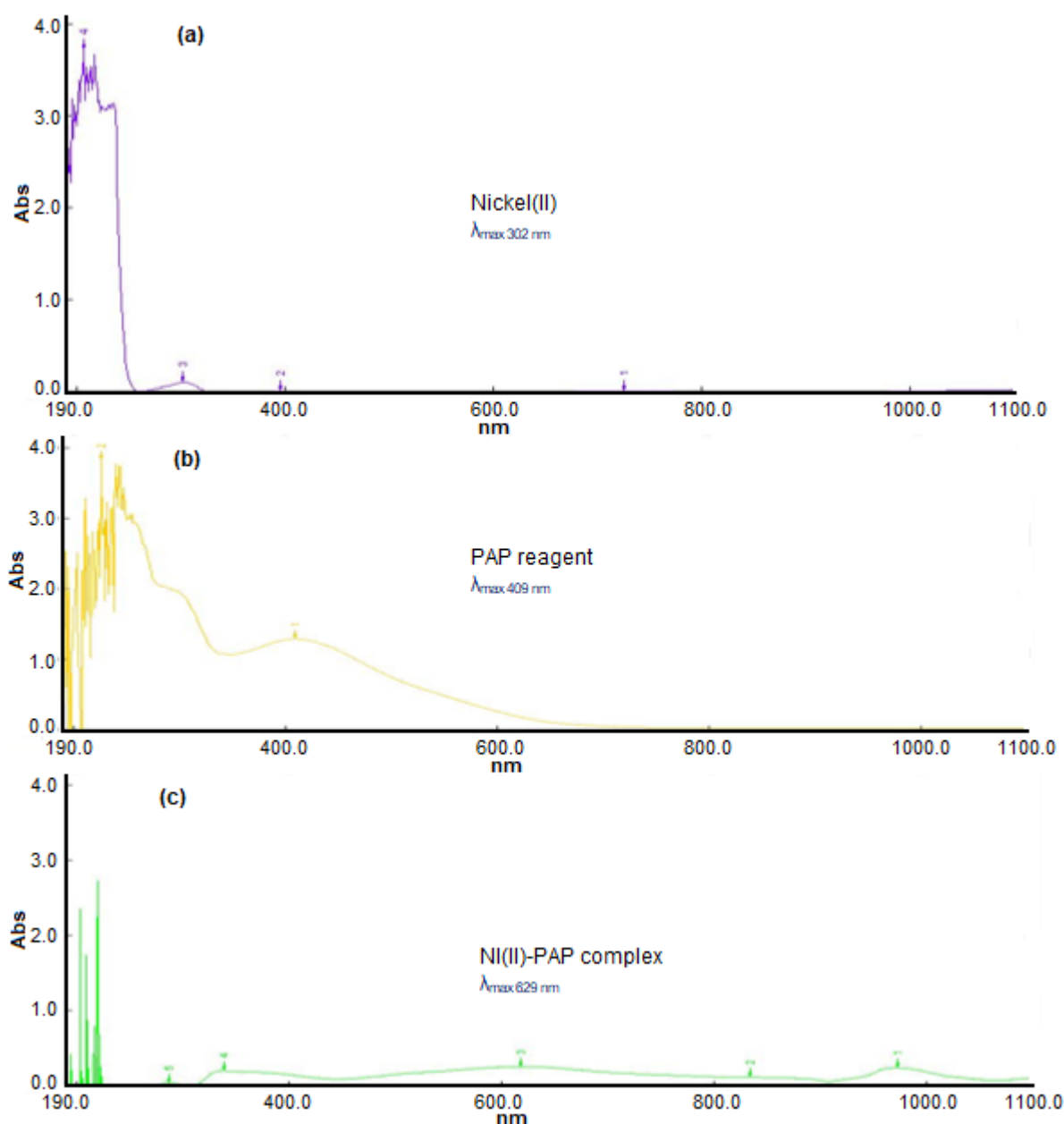
### Absorption Spectra

Fig. 2 shows the absorbance spectrum for the PAP reagent, standard solution of nickel(II) and the nickel(II)-PAP complex with a surfactant-rich phase against a reagent blank prepared under similar conditions. The absorbance spectrum of PAP were at 409 nm, the absorbance spectra of standard solution of nickel(II) were at 302 nm, while the absorbance spectra of the Ni(II)-PAP complex was at 629 nm.

## RESULTS AND DISCUSSION

### Optimization of the Experimental Conditions

To achieve the best performance for the cloud point extraction procedure, effects including order of



**Fig 2.** Absorption spectra for (a) Ni(II) metal solution, (b) Para-aminophenol (PAP) reagent, and (c) Ni(II)-PAP complex

addition, pH, concentration of reagent, types of surfactant, concentration of surfactant, equilibrium temperature, time of incubation, salt out and interfering ions were evaluated and optimized.

#### **Order of addition effect**

The most important factors that have been studied are the order of additions where it was change the order of

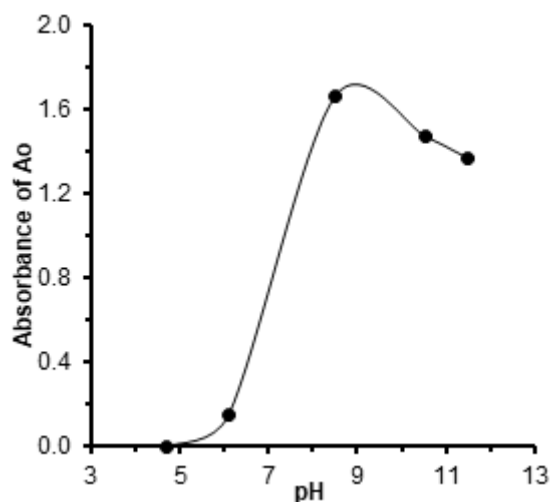
**Table 1.** Effect of order addition on the absorbance of the result

Order addition	Ao	Aaq	As
M + R + T	0.000	0.000	1.215
R + M + T	0.000	0.000	1.158

Ao : Absorbance of an original solution

As: Absorbance of a rich phase surfactant

Aaq: Absorbance of an aqueous



**Fig 3.** The effects of pH on the absorbance of Ni(II)-PAP complex

**Table 2.** Effect of concentration of PAP on the signal output

Volume added (mL)	PAP concentration mmol.L <sup>-1</sup>	Ao	Aaq	As
1.00	0.00796	0.394	0.136	1.579
0.50	0.00416	0.286	0.042	1.560
0.25	0.00254	0.244	0.034	1.329
0.10	0.00086	0.111	0.000	0.986
0.05	0.00043	0.000	0.000	0.355
0.03	0.00026	0.000	0.000	0.192

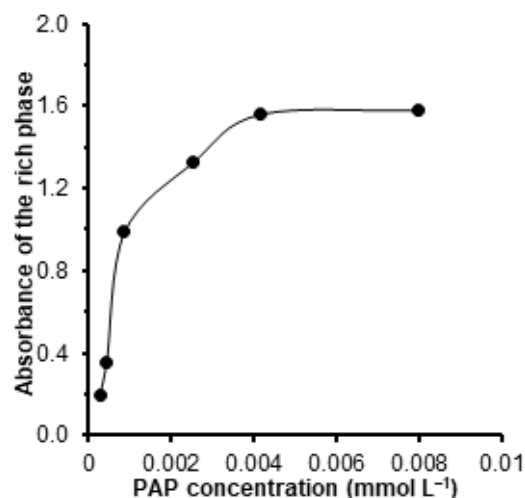
**Table 3.** Type of surfactant effect on the absorbance of Ni(II)-PAP

Type of surfactant	Ao	Aaq	As
SDS	0.377	0.476	0.475
Triton-X100	0.377	0.286	1.011
Triton-X114	0.377	0.115	1.751

(0.5 ppm/10 mL) of the Ni(II) and (0.0916 M/0.25 mL) of the reagent (PAP) as shown through the results in Table 1 that produced the greatest absorbance value from the first test.

#### Effect of pH

The formation of metal complexes and their chemical stabilities are two important influencing factors in cloud point extraction. The pH plays a critical role on metallic complex formation and subsequent extraction and has found to be a significant parameter for



**Fig 4.** Effect of amount of PAP on the absorbance of the produced complex

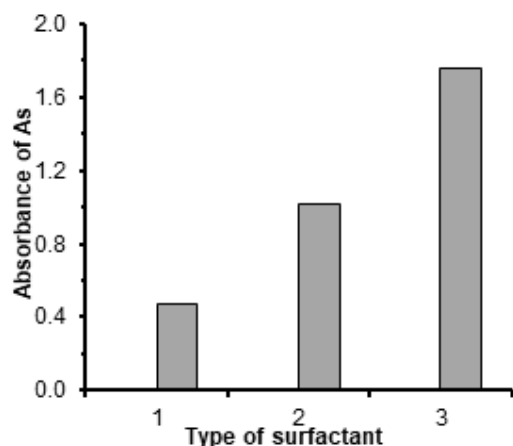
CPE. The pH of the sample solution was adjusted to lie in the range (4.7–11.5) using 0.1 M NaOH and 0.1 M HCl solution using a pH meter. The result in Fig. 3 shows an increase of absorbance when the pH was equal to 8.5, the complexation reaction at pH values lower than 8.5 is incomplete, probably due to the protonation of PAP; on the other hand, at pH values greater than 8.5 hydrolysis of Ni(II) occurs.

#### Reagent concentration effect

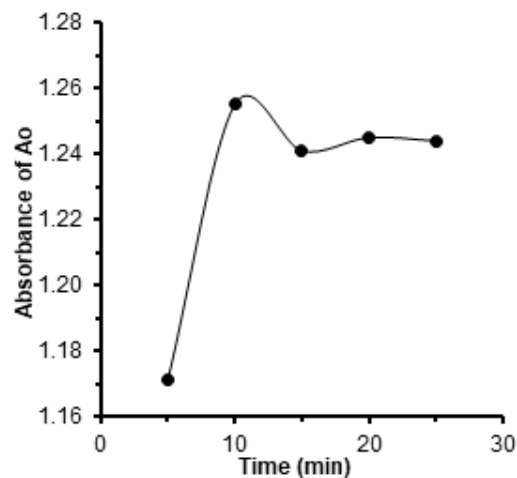
The concentration effect of chelating agent para-amino phenol (PAP) on absorbance value was studied by using various volumes from (0.01–1.00) mL of 1% w/v PAP, whilst keeping other factors constant. The results in Table 2 and Fig. 4 show that the absorbance value increased with increasing amounts of reagent (PAP). The best results relied upon when the amount of reagent is 0.25 mL, while other results and that's were neglected for many reasons, including Precipitates be when added large quantities of reagent PAP.

#### Influence of type of surfactant

The efficiency of the cloud point extraction is dependent on the type of surfactant used. In this study, this was examined using certain concentrations of various types of surfactant such as Triton X-100, Triton X-114, or sodium dodecyl sulphate (SDS). The results in Table 3 and Fig. 5 show that the absorbance value increased with Triton X-114.



**Fig 5.** Effects of types of surfactant in the rich phase formation: (1) SDS, (2) Triton X-100 and (3) Triton X-114



**Fig 6.** Effect of incubation time on the rich phase formation

**Table 4.** Amount of Triton X-114 effect on the rich phase formation

Volume added of Triton X-114 mL	Ao	Aaq	As
1.00	0.130	0.000	1.118
0.75	0.145	0.000	1.190
0.50	0.122	0.000	1.716
0.25	0.131	0.000	1.689
0.10	0.112	0.000	1.680
0.05	0.122	0.000	1.614
0.02	0.134	0.000	1.509

#### Amount of Triton X-114 effect

The Triton X-114 is one of the non-ionic surfactants extensively used in CPE. This is due to its advantages such as commercial availability in high purity, relatively low cloud point temperature, low toxicity, low cost and high density of the surfactant-rich phase which facilitates phase separation by centrifugation. Table 4 tabulated the results of the effect of amount of non-ionic surfactant, the volume range of 20% (v/v) of Triton X-114 from 0.02–1.00 mL. The absorbance of the complex increased by increasing the volume of Triton X-114 added.

#### Effect of equilibrium temperature

The cloud point extraction efficiency is strongly affected by temperature. The effect of the equilibrium temperature was examined due to its importance in driving the reaction to completion and the efficient

**Table 5.** Effect of equilibrium temperature on the absorbance of the Ni(II)-PAP complex

Temperature °C	Ao	Aaq	As
50	0.123	0.000	1.477
55	0.119	0.000	1.390
60	0.122	0.000	1.226
65	0.120	0.000	1.300
70	0.132	0.000	1.079
75	0.139	0.000	1.271
80	0.125	0.000	1.252
85	0.119	0.000	1.174

separation of phases. The temperature of the water bath was varied from (50–85) °C. The results were listed in Table 5 that shows the highest absorbance signals when the temperature at 50 °C.

#### Incubation time effect

In order to achieve easy phase separation and efficient pre-concentration in cloud point extraction processes, it was desirable to employ the shortest incubation time. The effect of incubation time was investigated over times ranging from (1–25) min. The results demonstrate that an incubation time of 10 min was optimal as shown as in Fig. 6, and was thus chosen for further experiments.

#### Salt out effect

In the cloud point extraction, the addition of salt to the sample solution helps to achieve phase separation

and increased mass transfer of the analyte from the aqueous phase to the surfactant-rich phase, the results listed in Table 6. The effects of salts on CPE were studied by added 0.5 mL of 15% w/v of NaCl, KCl, Na<sub>2</sub>CO<sub>3</sub> and Na<sub>2</sub>SO<sub>4</sub> to the solution of Ni(II) and were subjected to the complete procedure. The results demonstrate that the presence some salts led to increasing the absorbance due to increasing CPE efficiency because of these salts water molecules will pull and destroy the hydration shell of nickel ion which leads to increase the bonding between the reagent (PAP) and nickel, where the Na<sub>2</sub>CO<sub>3</sub> salt is the best to increase the sensitivity of the method.

### Effect of interferences

The effects of foreign ions on the extraction of 2 mg.L<sup>-1</sup> of Ni(II) were studied, including cations that may react with PAP or species that may react with analyte and decrease the extraction efficiency to perform this

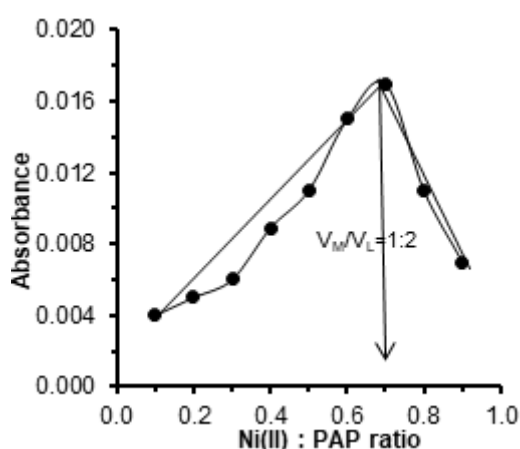
study. To this end, 100 mg.L<sup>-1</sup> of 'interfering' ions was added to a solution of 2 ppm Ni(II) and was subjected to an identical procedure. The results demonstrate that the presence of large amounts of the species commonly present in water samples have a significant effect on the cloud point extraction efficiency. Table 7 tabulated the results of interferences effect, from that can be seen some ions interfering with nickel ion like Cr(III) and Al(III)

**Table 6.** Effect of salts out on the absorbance of Ni(II)-PAP

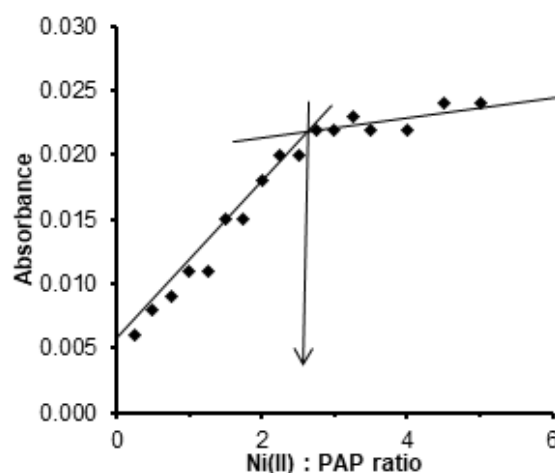
Type of salts	As
NaCl	1.025
KCl	1.122
Na <sub>2</sub> CO <sub>3</sub>	2.575
Na <sub>2</sub> SO <sub>4</sub>	1.296
without salt	0.932

**Table 7.** Effect of interference on the absorbance of Ni(II)-PAP

Interfering ion	Added as	Conc. of Interfering ion ppm/Conc. of Cu(II) mg.L <sup>-1</sup> ratio	Abs.
Co(II)	Co(NO <sub>3</sub> ) <sub>2</sub>	50	1.762
Fe(III)	FeCl <sub>3</sub>	50	0.632
Cu(II)	Cu (NO <sub>3</sub> ) <sub>2</sub> . 3H <sub>2</sub> O	50	1.381
Cr(III)	Cr(NO <sub>3</sub> ) <sub>3</sub>	50	0.712
Al(III)	Al (NO <sub>3</sub> ) <sub>3</sub> . 9H <sub>2</sub> O	50	0.494
Pb(II)	Pb(NO <sub>3</sub> ) <sub>2</sub>	50	1.068
Zn(II)	ZnCl <sub>2</sub>	50	1.244
without	---	---	1.477



**Fig 7.** Job's plot of absorbance at 629 nm versus mole fraction of Ni(II) ion



**Fig 8.** Mole ratio plots of the PAP-Ni(II) complex

**Table 8.** Summary of results for calibration graph for Ni (II) by using UV-Vis - CPE

Conc. of Ni(II) $\mu\text{g.mL}^{-1}$	Mean $\bar{y}$	SD $\sigma_{n-1}$	RSD%	C.I $\bar{y} \pm t_{0.05/2} \frac{\sigma_{n-1}}{\sqrt{n}}$
10	2.310	0.0070	0.003030	$2.310 \pm 0.017$
8	2.300	0.0050	0.002187	$2.300 \pm 0.013$
6	2.156	0.1360	0.063106	$2.156 \pm 0.338$
4	1.598	0.0080	0.005056	$1.598 \pm 0.020$
2	1.307	0.0210	0.016174	$1.307 \pm 0.052$
1	0.605	0.1209	0.179942	$0.605 \pm 0.300$
0.5	0.517	0.1082	0.209389	$0.517 \pm 0.269$
0.25	0.497	0.0040	0.007762	$0.497 \pm 0.010$
0.1	0.387	0.0310	0.080103	$0.387 \pm 0.077$
0.08	0.326	0.0470	0.144051	$0.326 \pm 0.117$
0.05	0.264	0.0262	0.099355	$0.264 \pm 0.065$
0.01	0.146	0.0255	0.174271	$0.146 \pm 0.063$
0.005	0.076	0.0236	0.308205	$0.076 \pm 0.059$

**Table 9.** Results for linear regression analysis of nickel(II) using CPE

Range of conc. $\mu\text{g.mL}^{-1}$	Linear regression equation	R $r^2$ $r^2\%$	$t_{\text{critical}}$ at 95% confidence level, n- 2	Calculated t-value $t_{\text{cal}} = ( r \sqrt{n} - 2) / \sqrt{(1-r^2)}$
Ni(II) n = 12	0.005 – 10.000 $Y = 0.3001 X + 0.0927$	0.9839 0.9682 96.82		2.228 <<< 7.8977

ion but the unnecessary effect because the concentration of these ions is very low in urine samples. In comparison with the concentration of Ni(II) ion.

### Stoichiometry of the Complex

The stoichiometry of a complex can be determined by using either of two methods. In Job's method (Fig. 7), a solution of reagent (PAP) and nickel(II) ion ( $1 \times 10^{-3}$  M) were prepared and mixed in continuous variation then diluted to 10 mL of DDW after this measurement the solution by UV-Vis Spectrophotometer at 629 nm. The stoichiometry of the complex determined by Job's method was found to be 1:2 ratio of M:L.

In the mole ratio method (Fig. 8), a solution of reagent (PAP) and nickel(II) ion ( $1 \times 10^{-3}$  M) were prepared. There are two ways that can be followed in order to determine the mole ratio, namely when the concentration of nickel(II) ion is fixed and the volume of reagent (PAP) is changed, or vice versa, then diluted to 10 mL of deionized water after this measurement the

solution by UV-Vis Spectrophotometer at 629 nm. The stoichiometry of the complex, as determined by the mole ratio method, was found to be 1: 2 as M:L ratio.

### Calibration Curve of Complexes by Coupling the UV-Vis Method with CPE

A calibration curve (Fig. 9) of absorbance versus concentration was constructed by collecting the analytical signals of different concentrations of Ni(II) standard solutions. Under the optimum experimental conditions, the calibration curve for Ni(II) ion is linear from (0.005–0.1)  $\text{mg.mL}^{-1}$  with a correlation coefficient (R) of 0.9682. Table 8 shows the summary of results for calibration graph while Table 9 listed the results for linear regression analysis of nickel(II) using CPE.

### Precision and Accuracy

#### Repeatability

The test of the precision of the UV-Vis-CPE method for nickel(II) ion determination can be shown by



calculating the relative standard deviation (RSD%) values for the resulting repeatability test. Concentrations of (0.25 and 2.0)  $\mu\text{g.mL}^{-1}$  of Ni(II) were used. The relative standard deviations (RSD%) determined for the Ni(II) ion are tabulated in Table 10. A percentage relative standard deviation of less than 5% can be achieved using this method.

#### Calibration curve of Ni(II) by FAAS

A series of standard nickel(II) solutions ranging from 0 to 4  $\mu\text{g.mL}^{-1}$  were recorded using  $\lambda_{\text{max}}$  232.0 nm to determine an appropriate calibration curve for Ni(II) using the FAAS technique.

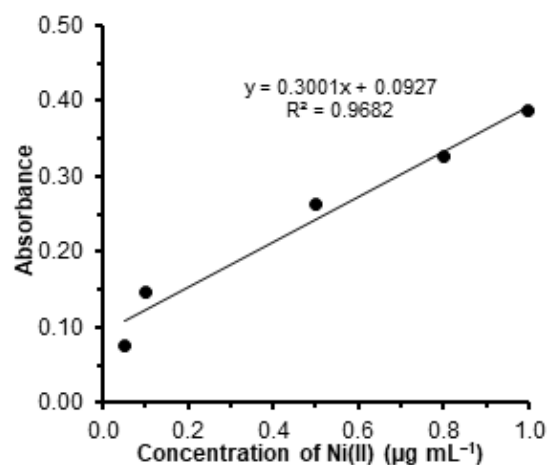


Fig 9. Calibration curve for Ni(II)-PAP complex

Table 10. Repeatability of Ni(II) complex at optimum parameters

No. of repeating	Conc. of Ni(II) ( $\mu\text{g.mL}^{-1}$ )	Abs.	Mean $\pm$ SD	RSD%
6	0.25	0.525, 0.520,	0.5095 $\pm$ 0.01870	3.67
		0.520, 0.517,		
		0.499, 0.476		
6	2	1.322, 1.299,	1.2983 $\pm$ 0.01392	1.07
		1.280, 1.290,		
		1.300, 1.299		

Table 11. Results of cloud point determination of nickel (II) concentrations in urine samples by coupling with the UV-Vis spectrophotometry and AAS techniques

Occupation	Method	No. of sample	Mean $\pm$ SD	C.I $t_{0.05/2} \frac{\sigma_{n-1}}{\sqrt{n}}$
Oil refineries	UV-Vis-CPE	14	0.01689 $\pm$ 0.00637	0.003670
	AAS-CPE	16	0.02327 $\pm$ 0.01679	0.008946
Welding	UV-Vis -CPE	10	0.01795 $\pm$ 0.00947	0.006770
	AAS-CPE	11	0.01491 $\pm$ 0.00836	0.005622
Casting alloys	UV-Vis-CPE	5	0.01393 $\pm$ 0.00156	0.001930
	AAS-CPE	5	0.00742 $\pm$ 0.00452	0.005623
Terminals to provide fuel	UV-Vis-CPE	3	0.02243 $\pm$ 0.00325	0.008000
	AAS-CPE	5	0.044710 $\pm$ 0.03100	0.049600
Dyeing	UV-Vis-CPE	5	0.01856 $\pm$ 0.01240	0.015400
	AAS-CPE	5	0.01217 $\pm$ 0.0045	0.005638
Total	UV-Vis-CPE	37	0.01745 $\pm$ 0.00770	0.002100
	Direct AAS	42	0.020425 $\pm$ 0.01981	0.006170

Table 12. Results of Grubbs-test applied on the Ni(II) ions concentration results

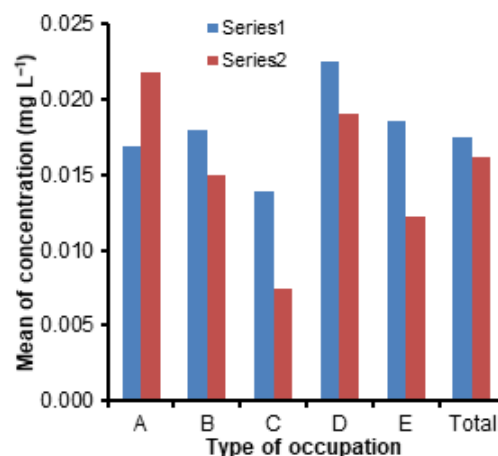
No. of sample	Readings excluded		C.F	Range of concentration $\mu\text{g.mL}^{-1}$	
	UV-Vis-CPE	FAAS-CPE		UV-Vis-CPE	FAAS-CPE
Ni(II) 44	1	1	22.5	0.0026-0.0444	0.0020-0.1097

### Application of method UV-Vis and AAS

These methods were applied successfully to determine the Ni(II) ion in urine samples after pre-concentration by cloud point extraction with different concentration are tabulated in Table 11; also, from the results presented in Fig. 10, the concentration of Ni(II) can be seen.

### Statistical treatment of data results

The results of the application of the Grubbs test to the outliers of the 44 sample concentrations after estimation by UV-Vis spectrophotometry and FAAS techniques, gave the excluded values shown in Table 12. When excluding such outliers, the significance tests described so far are used for comparing means and hence for detecting systematic errors. The F-test was used to compare the variances between the concentrations of copper



**Fig 10.** The nickel(II) concentration in urine samples by occupation as determined via UV-Vis-CPE (series 1) and FAAS-CPE (series 2). A: Oil refineries, B: Welding, C: Casting alloys, D: Terminals providing fuels, E: Dyeing

**Table 13.** Application F-test on the Ni(II) concentration in samples to compare between the results given by the two methods (UV-Vis-CPE and AAS-CPE)

Occupation	Type of measurement	d.f = n-1	SD n-1	Variance $\sigma^2$	F-calculate; $F = \sigma_2^2 / \sigma_1^2; \sigma_2^2 > \sigma_1^2$	F-critical value	P-value
Oil refineries	UV-Vis-CPE	13	0.0063	0.07937	4.5292	2.4481	0.0033
	AAS-CPE	15	0.0167	0.35948			
Welding	UV-Vis-CPE	9	0.0094	0.09695	3.1149	3.0203	0.0456
	AAS-CPE	10	0.0083	0.30183			
Casting alloys	UV-Vis-CPE	4	0.0015	0.03872	1.7324	6.3882	0.3037
	AAS-CPE	4	0.0045	0.06708			
Terminals to provide fuel	UV-Vis-CPE	2	0.0032	0.05656	3.5316	6.9442	0.1307
	AAS-CPE	4	0.0399	0.19974			
Dyeing	UV-Vis-CPE	4	0.0124	0.11135	1.6599	6.3882	0.3177
	AAS-CPE	4	0.0045	0.06708			
Total	UV-Vis-CPE	36	0.0077	0.08774	1.6037	1.7032	0.0720
	AAS-CPE	41	0.0198	0.14071			

P-value < 0.05 sig. difference, P-value > 0.05 non-significant difference

**Table 14.** The results of t-test (one tail) to compare with the concentration of Ni(II) in urine samples with the literature values

Occupation	Method	d.f; n-1	t-calculated; $t = \bar{y} - \mu \frac{\sqrt{n}}{\sigma_{n-1}}$	P-Value
Oil refineries	UV-Vis-CPE	13	8.9680	0.00001
	FAAS-CPE	15	5.1220	0.00006
Welding	UV-Vis-CPE	9	5.4095	0.00021
	FAAS-CPE	10	5.2367	0.00019
Casting alloys	UV-Vis-CPE	4	17.5300	0.00003
	FAAS-CPE	4	2.8323	0.02361
Terminals to provide fuel	UV-Vis-CPE	2	11.2040	0.00393
	FAAS-CPE	4	2.4090	0.03681
Dyeing	UV-Vis-CPE	4	3.0403	0.01919
	FAAS-CPE	4	5.1678	0.00333
Total	UV-Vis-CPE	36	12.4420	0.00001
	FAAS-CPE	41	6.1206	0.00001

using the UV-Vis-CPE and FAAS-CPE methods to determine any significance or otherwise between them. Table 13 shows the results of the F-test. A comparison of the sample mean with the true mean must be based on Student's t-test to allow comparison of the mean nickel concentrations found by UV-Vis-CPE and FAAS-CPE. Table 14 shows the results of the t-test.

## ■ CONCLUSION

Her, a simple, a successful and inexpensive pre-concentration method to determine Ni(II) ion in urine samples that depended on the cloud point extraction method which is deals with very small quantity of urine sample, while the concentration factor (C.F) is more than 50, so that increase of the sensitivity of measurement by UV-Vis spectrophotometry and Flame Atomic Absorption Spectrometry Technique.

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