DETERMINATION OF COPPER, NICKEL AND COBALT IN WATER AND FOOD SAMPLES BY FAAS AFTER SEPARATION AND PRECONCENTRATION USING MULTIWALLED CARBON NANOTUBES MODIFIED BY METHYL-(2-PYRIDYL) KETONE OXIME

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ABSTRACT

A solid phase extraction (SPE) procedure was presented for the preconcentration of copper, nickel and cobalt using multiwalled carbon nanotubes (MWCNTs) modified by methyl-(2-pyridyl) ketone oxime (MPKO, as a new complexing agent) as a solid sorbent prior to their determination by flame atomic absorption spectroscopy (FAAS). Some analytical parameters including pH, sample volume, concentration of MPKO and adsorbent, elution conditions such as volume and concentration of eluent, flow rate of solution and matrix ions, were optimized. At optimum conditions, the detection limits (3 SD/n) of 0.18, 0.28, and 0.47 µg L\textsuperscript{-1} for Cu(II), Ni(II), and Co(II) along with preconcentration factors of 90 respectively, were obtained. The method has been successfully applied for the determination of the aforementioned ions in food and water samples.

KEYWORDS: Flame Atomic Absorption Spectrometry; Solid Phase Extraction; Methyl-(2-Pyridyl) Ketone Oxime (MPKO); Multiwalled Carbon Nanotubes; Food and Water Samples

Heavy metal stress is one of the numerous environmental perturbations with two opposite facets: excess (e.g., heavy metal pollution) or depletion/deprivation (case when the essential elements are not present in the environment in sufficient amounts to support normal growth and metabolism) (Oprea et al., 2014). So, monitoring amounts of heavy metals in food, water and environmental is essential. Copper is one of the most widespread heavy metal contaminants in environment. It is an important element for most life forms as a micronutrient, but is also toxic at high concentrations (Gouda and Amin; 2014). Copper is suspected to cause infant liver damages. Drinking water can be a potential source of copper intake. Therefore, determination of trace amounts of copper in water samples is an important analytical task (Khani et al., 2011). Copper at nearly 40 µg L\textsuperscript{-1} is required for normal metabolism of many living organisms (Durukan et al., 2011). Ingesting high levels of copper can cause vomiting, nausea, diarrhea, liver or kidney damage or even death (Durukan et al., 2011). Nickel has been the subject of many investigations due to it is essential for living organism but toxic when it is excessive (Zeng et al., 2012). General population is exposed to nickel from various sources. Significant sources of nickel exposure for occupationally unexposed population are foods and the inhalation of tobacco smoke (Talio et al., 2011). Totally, nickel is a moderately toxic element and it is known that inhalation of this metal and its compounds can lead to serious problems, including respiratory system cancer (Luiz Silva et al., 2009).

The toxicity of cobalt is low and it is considered as an essential element, which is required in the normal human diet in the form of vitamin B12 (cyanocobalamin). For this reason, Co has been used in the treatment of anemia (Gil et al., 2008). However, the ingestion or inhalation of large doses of this analyte may lead toxic effects (Gil et al., 2008). The recommended dietary allowance (RDA) for vitamin B 12 for adults is 2.4 µg day\textsuperscript{-1}, which contains 0.1 µg of cobalt, and its deficiency can lead to pernicious anemia (Donati et al., 2006). The levels of heavy metals circulating in the environment have seriously increased during the last few decades due to human activity. So, more attention has been focused on environmental and human safety, especially on the pollutions of heavy metals by researchers around the world. Thus, researchers have tried to apply different methods and instruments for preconcentration and determination of heavy metals (Bartos et al., 2014; Wen et al., 2013; Zhao et al., 2012; Yang et al., 2014; Shokoufi et al., 2007).

Recently, Carbon nanotubes as an adsorbent for the preconcentration of traces heavy metals have become very popular (El-Sheikh et al., 2007; Tuzen et al., 2008a; Tuzen et al., 2008b; Liang et al., 2004; Duran et al., 2009). MWCNTs modified with some organic compounds are expected to be more selective than untreated and
oxidized-MWCNTs for the solid phase extraction of metal ions.

This paper reports a solid phase extraction (SPE) method for simultaneous preconcentration and determination of some heavy metals including Cu(II), Ni(II), and Co(II) on multiwalled carbon nanotubes using methyl(2-pyridyl) ketone oxime (MPKO) coupled with flame atomic absorption spectroscopy (FAAS), was developed. Analytical parameters such as pH, amounts of MPKO and adsorbent, sample volume, elution conditions such as volume and concentration of eluent, flow rates of solution and matrix ions, were investigated.

**EXPERIMENTAL**

**Instrumentation**

A Metrohm pH-meter (model 691, Switzerland) was used in order to adjust the pH at desirable values. A Chemtech Analytical Instrument model CTA-3000 atomic absorption spectrometer (Bedford, England) equipped with a flame burner was used for analysis of the understudy metals, including lamp currents and wavelength were those recommended by the manufacturer. All metals were measured under optimized operating conditions by FAAS with an air–acetylene flame.

**Standard solutions and reagents**

All chemicals used were of analytical reagent grade and ultra pure water was used through the work. In order to clean all plastic and glassware, they were soaked in 10% HNO₃ and rinsed by deionized water. Standard solution (1000 mg L⁻¹) of Cu(II), Ni(II), and Co(II) ions were prepared by dissolving appropriate amounts of them (Merck, Darmstadt, Germany) in deionized water and diluting to 100 mL in volumetric flasks. The working standard solutions were prepared by diluting stock standard solution. The ligand MPKO was synthesized according to literature (Furniss et al., 1987). Britton-Robinson buffer solution in the pH range of 3-12 was used to adjust pH values and made by mixing phosphoric acid 0.04 mol L⁻¹, Acetic acid 0.04 mol L⁻¹ and Boric acid 0.04 mol L⁻¹ with appropriate volumes of 0.2 mol L⁻¹ solution of sodium hydroxide (Britton and Robinson; 1931). Multiwalled carbon nanotube was purchased from Aldrich, (Milwaukee, WI, USA). The BET (Brunauer–Emmett-Teller) surface area and density of nanotubes were 300 m² g⁻¹ and 2.1 g mL⁻¹, respectively.

**Preparation of the column**

200 mg of Multiwalled nanotube was packed into a glass column (10 mm x 150 mm) with a glass frit resin support after washing with 1.0 M HNO₃ and water successively. The thickness of resin bed was approximately 1.5 cm. In order to clean the column, before each use, 1.0 mol L⁻¹ HNO₃ solution and water were passed through the column. Then, the column was conditioned to the desired pH values with buffer solutions. The column was conditioned with distilled water during the passing time for the next experiment.

**Recommended procedure**

After preparing the column and preconditioned as described in Section 2.3, an aliquot of the solution containing 0.5-90 μg L⁻¹ of Cu(II), 0.8-280 μg L⁻¹ of Ni(II), and 1.2-170 μg L⁻¹ of Co(II) was prepared, and then MPKO was added to form the metal MPKO chelates. After adjusting to the desired pH values, the solutions were passed through the column gravitationally. After that, the retained analytes on the MWNTs, were eluted with the desired volume and concentration of nitric acid solution and determined by FAAS.

**Samples and sample pretreatment**

The proposed method was applied to different food and environmental water samples. Different environmental water samples (well, river, tap and spring) were taken from around Gachsaran City. The water samples were filtered through a cellulose membrane filter (Millipore) of 0.45 μm pore size and acidified to 1.0 % with nitric acid and were subsequently stored at 4.0 °C in a refrigerator. The organic content of the water samples were oxidized in the presence of 1.0% H₂O₂ and by addition of concentrated nitric acid. The pH of the samples was adjusted to 9.5 with buffer solution. After preparing the water samples, the proposed method was applied to the understudy water samples. Finally, the concentrations of analytes in the water samples were determined by flame atomic absorption spectrometry.

All vegetables (Cabbage, Spinach, Tomato, lettuce and carrot) were purchased from a supermarket in Gachsaran, Iran. Then, they were taken in a small mesh and dried in an oven at 90 °C to constant weight. In order to digest the samples, 1.0 g of sample was digested with 10 mL concentrated HNO₃ and 2.0 mL 30% H₂O₂ in microwave system, then again evaporated to near dryness. After evaporation, 10 mL of deionized water was added.
and the sample was mixed. The resulting mixture was filtered through a blue band filter paper. The filtrate was diluted to 50 mL with deionized water. All the samples were stored in polyethylene bottles. The blanks were prepared in the same way as the sample, but omitting the sample. The preconcentration procedure given above was applied to the samples. The analytes in final solution were determined by FAAS. Also, a 50 g cow liver were purchased and digested according to literature (Ghaedi et al., 2008).

RESULTS and DISCUSSION

In order to study some heavy metals, in the presented work, a solid phase extraction procedure is adopted which Cu(II), Ni(II), and Co(II) ions are used in the presence of MPKO as a chelating agent. The interaction between Cu(II), Ni(II), and Co(II) ions and MPKO led to the formation of metal MPKO chelates. Then, metal ions retained on the MWNT, were eluted with eluent. Finally, the eluent analyzed for the determination of metal concentrations by flame atomic absorption spectrometry.

Effect of pH on sorption

During the solid phase extraction method, the pH value has a significant role for the quantitative recoveries of metal ions (Tuzen et al., 2008a; Tuzen et al., 2008b; Liang et al., 2004). The effect of pH values on the recoveries of Cu(II), Ni(II), and Co(II) as MPKO chelates adsorb on multiwalled carbon nanotubes, from sample solutions, were investigated in the range of 3.0–12. It is observable in Fig. 1, quantitative recoveries (>90%) were obtained for all studied ions at the pH range of 9.0–10. So, the pH 9.5 was selected for all subsequent studies.

Figure 1: The open structure of MPKO

Effect of eluent type

The effects of various eluents (nitric acid, hydrochloric acid and acetic acid) on the recoveries of Cu(II), Ni(II), and Co(II) ions from multiwalled carbon nanotubes modified with MPKO were also investigated. For this aim, three type of eluents including HNO₃, HCl and CH₃COOH (0.5, 1.0 and 1.5 M) were used. The results indicated that 1.0 mol L⁻¹ HNO₃ was sufficient for quantitative elution (>95%). Furthermore, the influence of eluent volume (2.0–10 mL) on the recoveries was studied by using 1.0 mol L⁻¹ HNO₃. By adding 5.0 mL of 1.0 mol L⁻¹ HNO₃ quantitative recoveries were obtained for all analyte ions. The recoveries were not quantitative for the other eluents. Therefore, 5.0 mL of 1.0 mol L⁻¹ HNO₃ was used as eluent for further work.

Effect of amount of chelating agent

One of the most important factors on the quantitative recoveries of analysis in solid phase extraction techniques is the amounts of the ligand (Tuzen et al., 2008a; Duran et al., 2009). The effect of amounts of MPKO on the quantitative recoveries of the analyte ions was examined by varying the amounts of MPKO solution, ranging from 0–7 mmol L⁻¹ (mM). As presented in Fig. 2, the recoveries of analyte ions increased with increasing amounts of MPKO added and reached a constant value over 90% with at least 4.0 mM. The recovery values of analytes were quantitative at the amounts of ligand range of 5.0–7.0 mM. Hence, 5.0 mM of ligand was selected for subsequent studies.

Figure 2: The effect of pH on the recoveries of the metal ions (N= 3.0.)

Effect of amounts of adsorbent (MWNTs)

In order to obtain the optimum adsorbent quantity, different amounts of multiwalled carbon nanotubes on the adsorption of analytes were investigated. The recovery values for analyte ions were found quantitative in the resin amounts of 150-300 mg. In this method, 200 mg of adsorbent was selected as the
optimum. So, in the following studies, the glass column was packed with 200 mg of MWNTs.

**Effect of the sample volume**

In order to obtain high preconcentration factor and investigate the possibility of enrichment of trace quantities of understudy analytes, the effect of sample volume on the recovery of metal ions was also studied by passing 100–600 mL of sample solutions. As presented in Fig. 3, the recoveries for analytes were not quantitative at the higher volumes than 450 mL. The preconcentration factor is calculated by the ratio of the highest sample volume for each analyte (450 mL) and the lowest final eluent volume (5.0 mL). In this study, a preconcentration factor of 90.0 was achieved.

**Flow rates of sample and eluent solutions**

The effect of the flow rates of sample and eluent solutions were investigated under the optimum conditions (pH and eluent type). The effect of the flow rate of the sample and eluent solutions on the recoveries of Cu(II), Ni(II), and Co(II) and on modified MWNTs was examined in the range of 1.0–7.0 mL min$^{-1}$. It was found when the flow rates of the sample and eluent solutions were at the range of 1.0-6.0 mL min$^{-1}$, the recoveries of analytes were quantitative. At higher flow rates, there was a decrease in the recovery in that there is not sufficient contact time between MWNTs and sample solution. Therefore, a flow rate of six milliliters per minute was chosen as the optimum flow rates of the sample and eluent solutions.

**Tolerance studies**

The effect of foreign ions on the recoveries of the analyte ions on multiwalled carbon nanotubes was also investigated. The results are presented in Table 1. The tolerance limit is defined as the ion concentration causing a relative error smaller than ±5% related to the preconcentration and determination of analytes. It is observable in Table 1 that the most common coexisting ions do not interfere seriously with the determination suggesting the highly selectivity of the proposed method.

**Table 1: Effect of interfering ions on the recoveries of the analytes (N= 3)**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration (mg L$^{-1}$)</th>
<th>Cu$^{2+}$ Recovery (%)</th>
<th>Ni$^{2+}$</th>
<th>Co$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$, Cl$^-$, Li$^+$</td>
<td>20000</td>
<td>98±2</td>
<td>96±3</td>
<td>95±2</td>
</tr>
<tr>
<td>I$^-$, K$^+$</td>
<td>17000</td>
<td>96±2</td>
<td>97±1</td>
<td>98±2</td>
</tr>
<tr>
<td>Mg$^{2+}$, Ca$^{2+}$</td>
<td>15000</td>
<td>98±1</td>
<td>98±1</td>
<td>98±2</td>
</tr>
<tr>
<td>Ag$^{+}$, Br$^-$, C$_2$O$_4$$^{2-}$</td>
<td>10000</td>
<td>95±3</td>
<td>96±2</td>
<td>98±1</td>
</tr>
<tr>
<td>CO$_3$$^{2-}$, F$^-$</td>
<td>7000</td>
<td>95±2</td>
<td>98±2</td>
<td>97±1</td>
</tr>
<tr>
<td>SO$_4$$^{2-}$,NO$_3^-$, PO$_4$$^{3-}$</td>
<td>5000</td>
<td>96±1</td>
<td>96±2</td>
<td>96±2</td>
</tr>
<tr>
<td>HCO$_3$$^{−}$, CH$_3$COO$^-$</td>
<td>3000</td>
<td>97±1</td>
<td>98±3</td>
<td>99±1</td>
</tr>
<tr>
<td>Al$^{3+}$, Hg$^{2+}$</td>
<td>100</td>
<td>95±3</td>
<td>96±3</td>
<td>95±3</td>
</tr>
<tr>
<td>V$^{3+}$, Mn$^{2+}$, Sn$^{4+}$</td>
<td>70</td>
<td>98±2</td>
<td>98±1</td>
<td>99±1</td>
</tr>
<tr>
<td>Cr$^{3+}$, Cd$^{2+}$, Pb$^{2+}$</td>
<td>40</td>
<td>98±1</td>
<td>99±2</td>
<td>94±2</td>
</tr>
</tbody>
</table>

*Mean ± standard deviations.

**Adsorption capacity**

For the evaluation of the maximum adsorption capacity of MWNTs, batch method was used under optimum conditions. A 50 mL of aqueous solution containing 1.0 mg of analyte ions at pH 9.5 was added to 0.1 g MWNTs after addition of optimum volume of MPKO. After shaking for 2.0 hr, the mixture was filtered.

Ten milliliters of the supernatant solution was diluted to 100 mL and the analytes were determined by flame atomic absorption spectrometry. This procedure was repeated for each analyte ions separately. The capacity of modified MWNTs for Cu(II), Ni(II), and Co(II) was found to be 31.5, 27.7, and 16.6 mg g$^{-1}$, respectively.
The amounts of sorbed mentioned metal ions were calculated as follows:

$$q_e = (C_0 - C_t) \times \frac{V}{m}$$  \hspace{1cm} (1)

where $q_e$ is the amount of metal ions sorbed onto MWCNTs-MPKO at equilibrium (mg g$^{-1}$); $C_0$ is the initial concentration (mg L$^{-1}$); $C_t$ is the concentration after a certain period of time (mg L$^{-1}$); $V$ is the initial solution volume (L); and $m$ is the MWCNTs-MPKO mass (g). The stability of multiwalled carbon nanotubes was excellent. On storing for six mounts its properties and sorption capacity do not change significantly. The adsorption of metal-MPKO chelates is related with the physical interaction of the surface of the nanotubes and the metal-MPKO chelates.

**Characteristics of the method**

Under the specified experimental conditions, the characteristics data for the performance of the preconcentration method were examined. Linear calibration graphs were obtained in the range of 0.5-90 µg L$^{-1}$ for Cu(II), 1.1-280 µg L$^{-1}$ for Ni(II), and 1.5-170 µg L$^{-1}$ for Co(II) in initial solution by applying the optimum conditions. The equations of lines are $A = 1.22 \times 10^2 C + 0.031$, $A = 2.34 \times 10^3 C + 0.061$, and $A = 4.44 \times 10^3 C + 0.011$ for Cu(II), Ni(II), and Co(II) respectively, where $A$ is the absorbance and $C$ is the concentration of metal ions in µg L$^{-1}$. The regression coefficients for the lines are 0.9996, 0.9996, and 0.9997 for Cu(II), Ni(II), and Co(II) respectively. The detection limits, defined as the concentration equivalent to three times the standard deviation ($N=10$) of the reagent blank solutions of the presented preconcentration study for Cu, Ni, and Co were found as: 0.18, 0.28, and 0.47 µg L$^{-1}$, respectively. The relative standard deviations (R.S.D) for ten replicate measurements of 30 ng ml$^{-1}$ of Cu(II), 150 ng ml$^{-1}$ of Ni(II), and 80 ng ml$^{-1}$ of Co(II) were 1.76, 3.85, and 3.45%, respectively. The preconcentration factor for the proposed method was 90.

**ANALYTICAL APPLICATIONS**

In order to evaluate the validity and accuracy of the presented method, the proposed method was applied to the determination of Cu(II), Ni(II), and Co(II) in different environmental water samples including: well water, river water, tap and spring samples. Various amounts of analytes were also spiked to these water samples. The results are presented in Table 2. A good agreement was obtained between the added and measured analyte amounts. Also, the proposed method has been successfully applied to the determination of Cu(II), Ni(II), and Co(II) in food samples. The results are given in Table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added (µg L$^{-1}$)</th>
<th>Found a (µg L$^{-1}$)</th>
<th>R.S.D (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cu$^{2+}$</td>
<td>Ni$^{2+}$</td>
<td>Co$^{2+}$</td>
<td>Cu$^{2+}$</td>
</tr>
<tr>
<td>Tap</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.4</td>
</tr>
<tr>
<td>Water</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>7.25</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>12.4</td>
</tr>
<tr>
<td>Well</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.2</td>
</tr>
<tr>
<td>Water</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>30.9</td>
</tr>
<tr>
<td>River</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.8</td>
</tr>
<tr>
<td>Water b</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>9.50</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>15.2</td>
</tr>
<tr>
<td>Spring c</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40.5</td>
</tr>
<tr>
<td>Water</td>
<td>50</td>
<td>5</td>
<td>5</td>
<td>93.23</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>10</td>
<td>138.0</td>
</tr>
</tbody>
</table>

a ± t is at 95% confidence (n = 5)
b A river in a non-industrial city, Gachsaran
c taken from a spring in Yasuj.
Table 3. The levels of analytes in vegetables after application of proposed method (sample quantity (g): 0.25, N=6)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cu$^{2+}$ (µg g$^{-1}$)</th>
<th>Ni$^{2+}$ (µg g$^{-1}$)</th>
<th>Co$^{2+}$ (µg g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>7.45±0.5</td>
<td>3.0±0.2</td>
<td>5.69±0.4</td>
</tr>
<tr>
<td>Cabbage</td>
<td>4.10±0.15</td>
<td>1.4±0.1</td>
<td>0.85±0.1</td>
</tr>
<tr>
<td>Spinach</td>
<td>72.04±1.0</td>
<td>81.50±1.0</td>
<td>50.12±1.0</td>
</tr>
<tr>
<td>Lettuce</td>
<td>3.66±0.15</td>
<td>15.1±0.1</td>
<td>8.36±0.15</td>
</tr>
<tr>
<td>Carrot</td>
<td>6.66±0.4</td>
<td>3.94±0.2</td>
<td>1.1±0.15</td>
</tr>
<tr>
<td>Cow liver</td>
<td>5.96±0.3</td>
<td>1.85±0.1</td>
<td>1.44±0.2</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation.

CONCLUSION

In this work, a solid phase extraction procedure for the separation and preconcentration of Cu(II), Ni(II), and Co(II) by using multiwalled carbon nanotubes (MWCNTs) as a solid sorbent and MPKO methyl-(2-pyridyl) ketone oxime as a chelating agent prior to their atomic absorption spectrometric determinations with approximate quantitative recoveries. The limit of detection and preconcentration factor of the presented method seems to be satisfactory in contrast to some recent studies using multiwalled carbon nanotubes (Soylak et al., 2012; Ozcan et al., 2010; Wang et al., 2011; Zang et al., 2009; Cui et al., 2012; Mohammadi et al., 2010).

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