Extractive spectrophotometric method for the determination of molybdenum (VI) in environmental samples

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ABSTRACT

An extractive spectrophotometric method has been developed for determination of molybdenum (VI) using newly synthesized reagent, \( \text{N}'(2\text{-hydroxybenzylidene})\text{-}3\text{-}(4\text{-o-tolylpiperazin}-1\text{-yl})\text{propanehydrazide} \) (HTP). Molybdenum (VI) forms a yellow coloured water insoluble complex with HTP reagent in the acidic medium. The formed water insoluble complex is extractable in chloroform solvent and the complex shows maximum absorbance at max 389 nm. Under optimum conditions, Beer’s law is obeyed in the range 1.918-19.18 μg mL\(^{-1}\) of Molybdenum (VI). The molar absorptivity and Sandell’s sensitivity for the complex is found to be 4.903 \( \times \) 10\(^4\) L mol\(^{-1}\) cm\(^{-1}\) and 0.02 μg. cm\(^{-2}\) respectively. The interference effect of various diverse ions has been studied. The complex shows 1:1 [Mo (VI): HTP] stoichiometry with stability constant of 8.29 \( \times \) 10\(^5\). The developed method has been employed for the determination of molybdenum (VI) in synthetic samples and in alloys.

Keywords: Spectrophotometry; molybdenum (VI)-HTP complex and alloy samples.

INTRODUCTION

Molybdenum is an important transition metal in agriculture and metallurgy. Its chemistry is very complex in nature. The compounds containing molybdenum in 0, +2, +3, +5 and +6 oxidation states are known to exist. About 85 percent molybdenum that is being produced is used in manufacture of iron based alloys. Molybdenum is added to the cast iron to increase its strength which is widely used in the manufacture of automobile engines. Because of the properties such as strength, stiffness, electrical and thermal conductivity and corrosion resistance, the alloys of molybdenum find numerous industrial applications. Molybdenum when added in the form of fertilizer, large increase in crop yields, particularly leguminous, have been observed. It is added in trace amounts in vitamin supplements and specialized medicines. Recent studies have shown that molybdenum is an important element in promoting healthy teeth. Molybdenum is being used as catalyst in many petroleum and chemical processes. It is used as activator or promoter of other catalysts.

Prolonged exposure to molybdenum in the environment can cause toxicity to humans, animals and plants (Kononova et al., 2004). Molybdenum (VI) raises the liability of blood pressure and reduces cholesterol transportation. These ions also decrease catalase activity, which causes an increase in uric acid concentrations in organisms, leading to podagra. The presence of molybdenum in water samples at about 5 mg L\(^{-1}\) level inhibits biochemical self-purification processes of water. An even higher content of molybdenum in water prevents the growth of some microorganisms (Tunseli et al., 2004). There are several highly sensitive analytical techniques for determination of molybdenum, such as inductively coupled plasma atomic emission spectrometry (Kalal et al., 2011), inductively coupled plasma mass spectrometry (Reid.H.J et al., 2008), atomic absorption spectrometry (Reddy.G et al., 2011) and neutron activation analysis (Freitas,M.C et al., 2008), these techniques require the use of rather sophisticated and high cost instruments. A literature survey revealed that spectrophotometric methods are most commonly used.

The number of chromogenic reagents available for molybdenum is relatively small. Of these the thiocyanate-tin (II) chloride method is the oldest and most...
widely used. However, even this method suffers from a number of limiting factors (Rao et al., 1986; Sandell.E.B 1959; Marezenko.Z 1976), such as variations of absorbance with thiocyanate, tin (II) chloride and hydrogen ion concentrations, interference from various metal ions and problems of stability and reproducibility of measurements. Among the reagents employed diethiol is popular because of its selectivity, sensitivity. However, the reaction between molybdenum and Diethiol is more rapid at higher temperatures (750 C) and heating is not possible in the presence of tungsten, which reacts with diethiol. Most of the methods are extraction methods (Rodriguez.M.T 1982; Boregowda Keshavan et al., 1986). Only a few methods are reported using benzoyl hydrazone derivatives. (Podcainova et al., 1974); reported spectrophotometric determination of molybdenum(VI) with 2-Hydroxy-1-naphthaldehyde isonicotinoyl hydrazone which suffers from interference of Fe (III). (Sreenivasulu Reddy et al, 1993); determined Mo (VI) with resacophenone-isonicotionolhydrazone spectrometrically; Kaventis in 1986 presented a paper describing the spectrophotometric determination of molybdenum with salicyladehyde isonicotinohydrazone. (Murthy et al 1989); determined molybdenum spectrophotometrically with salicyaldioxime isonicoylhydrazone. Not many P-hydroxy hydrazones have been used for the determination of Mo (IV). Further derivative spectrophotometric methods are very few for the determination of Mo (IV).

In this work the capability of N’-(2-hydroxybenzylidine)-3-(4-o-tolylpiperazin-1-yl) propanehydrazone (HTP) (show in Figure 1) for the extraction and spectrophotometric determination of Molybdenum (VI) is investigated. The influences of analytical parameters including extraction solvent, pH and reagent amounts, etc. on the complex formation were studied. The developed method is simple, selective and free from interference of other ions.

EXPERIMENTAL

APPARATUS

All spectral and absorbance measurements were carried out on a Shimadzu UV-Visible 1601 spectrophotometer (Shimadzu Corporation, Japan) with 1 cm matched quartz cells. The pH of buffer solutions was monitored by using Systronic digital pH meter (India). An electronic micro balance (Sartorius MC 5, Germany) and Afcost electronic balance (Mumbai, India) were used for weighing the solid materials.

Procedure for preparation of N’-(2-hydroxybenzylidene)-3-(4-o-tolylpiperazin-1-yl) propanehydrazone (HTP)

A mixture of Tolylpiperazine (1) (3.52 gms, 0.02 mol), ethyl 3-chloropropanoate (2) (2.72 gms, 0.02mol) and Hydrazene hydrate (3) (1.96gms, 0.04 mol) was taken into ethanol in presence of potassium carbonate for 6 hours at reflux temperature. After completion of the reaction, as monitored by TLC, the reaction mass was cooled to room temperature and filtered. The ethanol layer was distilled off by using vacuum to get 3-(4-O-tolylpiperazin-1-yl) propanehydrazone (4). The crude product of recrystallized from ethanol. A mixture of 4 (2.62, 0.01) and 2-hydroxybenzaldehyde (1.22 gms, 0.01 mol) in Ethanol was refluxed for 4 hrs. After completion of the reaction, as monitored by TLC, the reaction mass was cooled to room temperature and filtered. The ethanol layer was distilled off by using vacuum to get N’-(2-hydroxybenzylidene)-3-(4-o-tolylpiperazin-1-yl) propanehydrazone. The crude product of recrystallized from ethanol. Yield: 2.80 gms, (76.5%). The main intermediate, 3-(4-O-tolylpiperazin-1-yl) propanehydrazone and final product, N’-(2-hydroxybenzylidene)-3-(4-o-tolylpiperazin-1-yl) propanehydrazone are characterized by elemental and spectral analysis. The complete reaction mechanism is depicted in Scheme.1

RESULTS AND DISCUSSIONS

In hydrochloric acid solution, molybdenum, in the hexavalent state, reacts with N’-(2-hydroxybenzylidene)-3-(4-o-tolylpiperazin-1-yl) propanehydrazone (HTP) forming a yellow colored complex which is extractable quantitatively into chloroform. This colour reaction was investigated in detail and developed extractive spectrophotometric method for the determination of
Figure 2: Absorption spectra a) HTP (1x10^-3 M) against chloroform

Scheme.1

Figure 3: Job’s method of continuous variation \([\text{Mo (VI)}] = [\text{HTP}] = 1 \times 10^{-3} \text{ M}\)

Table 1: Effect of solvents on the extraction of Mo (VI)-HTP complex

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoamylalcohol</td>
<td>0.292</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.506</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>0.443</td>
</tr>
<tr>
<td>n-hexanol</td>
<td>0.301</td>
</tr>
<tr>
<td>Xylene</td>
<td>0.281</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>0.331</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>0.224</td>
</tr>
<tr>
<td>Methyliso-butyl ketone</td>
<td>0.157</td>
</tr>
</tbody>
</table>
Figure 4: Molar ratio plot of [Mo (VI)- HTP] complex

Table 2: Effect of HTP concentration on the absorbance

<table>
<thead>
<tr>
<th>M:R</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:3</td>
<td>0.399</td>
</tr>
<tr>
<td>1:5</td>
<td>0.604</td>
</tr>
<tr>
<td>1:10</td>
<td>0.612</td>
</tr>
<tr>
<td>1:15</td>
<td>0.615</td>
</tr>
<tr>
<td>1:20</td>
<td>0.618</td>
</tr>
<tr>
<td>1:25</td>
<td>0.614</td>
</tr>
<tr>
<td>1:30</td>
<td>0.618</td>
</tr>
</tbody>
</table>

Table 3: Effect of metal ions on the determination of 9.594 (µg mL⁻¹) Mo (IV)

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Tolerance limit (µg mL⁻¹)</th>
<th>Metal ion</th>
<th>Tolerance limit (µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodide</td>
<td>953</td>
<td>Mg^{2+}</td>
<td>62</td>
</tr>
<tr>
<td>Tetraborate</td>
<td>869</td>
<td>Sn^{4+}</td>
<td>18</td>
</tr>
<tr>
<td>Citrate</td>
<td>1154</td>
<td>Bi^{3+}</td>
<td>52</td>
</tr>
<tr>
<td>Thiourea</td>
<td>810</td>
<td>Se^{4+}</td>
<td>78</td>
</tr>
<tr>
<td>Tartrate</td>
<td>965</td>
<td>Fe^{2+}</td>
<td>52</td>
</tr>
<tr>
<td>Al (III)</td>
<td>190</td>
<td>Fe^{3+}</td>
<td>31</td>
</tr>
<tr>
<td>Thiosulphate</td>
<td>770</td>
<td>Ni^{2+}</td>
<td>46</td>
</tr>
<tr>
<td>Oxalate</td>
<td>581</td>
<td>Pd^{2+}</td>
<td>36</td>
</tr>
<tr>
<td>Chloride</td>
<td>642</td>
<td>Zn^{2+}</td>
<td>38</td>
</tr>
<tr>
<td>Fluoride</td>
<td>440</td>
<td>Cd^{2+}</td>
<td>19</td>
</tr>
<tr>
<td>Phosphate</td>
<td>920</td>
<td>Cu^{2+}</td>
<td>16</td>
</tr>
<tr>
<td>Ba^{2+}</td>
<td>54</td>
<td>Co^{2+}</td>
<td>41</td>
</tr>
<tr>
<td>Cr(VI)</td>
<td>48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Optical and regression characteristics of the proposed method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ_{max} (nm)</td>
<td>389</td>
</tr>
<tr>
<td>Beers law limit (µg mL⁻¹)</td>
<td>1.9188-19.188</td>
</tr>
<tr>
<td>Molar absorptivity (L/mol cm)</td>
<td>4.903x10⁻³</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg cm⁻²)</td>
<td>0.02</td>
</tr>
<tr>
<td>Regression equation (Y = a + bC) Slope</td>
<td>0.0511</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0033</td>
</tr>
<tr>
<td>Regression coefficient (r²)</td>
<td>0.9994</td>
</tr>
<tr>
<td>Standard deviation of slope (S_b)</td>
<td>0.61x10⁻³</td>
</tr>
<tr>
<td>Standard deviation of intercept (S_a)</td>
<td>0.007</td>
</tr>
<tr>
<td>Detection limit LOD (µg mL⁻¹)</td>
<td>0.46</td>
</tr>
<tr>
<td>Quantification limit LOQ (µg mL⁻¹)</td>
<td>1.40</td>
</tr>
</tbody>
</table>
Molybdenum (VI) in non-aqueous medium.

The Molybdenum (VI) – HTP complex formed in aqueous phase is transferred quickly into chloroform giving a maximum and constant absorbance for the variation of equilibration time from 10 to 150 sec. The absorption spectra shown in Figure 2.

From the Figure 2, it is evident that the colored species shows maximum absorbance at 389 nm. Hence, this wavelength 389 nm is selected for further studies.

In order to optimize the conditions for proposed spectrophotometric methods, the effect of experimental variables was studied by altering each variable in turn while keeping the others constant.

**Effect of Solvents on the Extraction of Mo (VI)-HTP Complex**

Solvents such as isoamyl alcohol, chloroform, dichloromethane, n-hexanol, xylene, carbon tetrachloride, cyclohexane, and methyliso-butylketone through single extraction with equal volumes of solvent. The two phases were allowed to separate and organic phase was collected in 10 ml flask after passing through anhydrous sodium sulphate to remove water droplets. The effect of solvents on the extraction of Mo(VI)-HTP complex are shown in Table 1.

As per the results reported in Table 1, the maximum constant absorbance was observed in chloroform solvent. Hence chloroform is found to be a suitable solvent for the effective extraction of Mo (VI)-HTP complex. Hence, chloroform was chosen for all further studies.

**Effect of reagent concentration on the absorbance of reaction mixture**

The study of the effect of the reagent concentration on the absorbance of the extracted species was carried out in the following procedure by measuring the absorbance of the complex at 389 nm against the similarly prepared reagent blank.

In a 100 ml separate funnel, 2 mL of hydrochloric acid (0.5 M), 1.0 ml of 1x10^-3 M Mo (VI) and different volumes of 1x10^-2 M reagent (HTP) solution were taken. The final volume of aqueous phase was adjusted up to 10 ml with double distilled water. The contents were mixed and equilibrated with 10 ml Chloroform for 60 sec. The two phases were allowed to separate and organic phase was collected in 10 ml flask after passing through anhydrous sodium sulphate to remove water droplets.
through anhydrous sodium sulphate to remove water droplets. The absorbance of extract was measured against at 389 nm against a corresponding reagent blank prepared accordingly. The effect of HTP concentration on the absorbance is shown in Table 2.

From Table 2, it is found that the absorbance reached its maximum when the amount of HTP reagent ratio is 1:10. Also, it does not change with any further increase in the amount of reagent. This clearly indicates that Mo (VI) has been totally formed complex to attain a maximum.

**Time Stability of the Color Reaction**

The absorbance value of the Mo (VI)-HTP complex was measured at different intervals of time at 389 nm against reagent blank which was prepared under identical conditions to ascertain the time stability of the color of the complex. It is found that the color of the complex remained constant for 10 hrs.

**Order of addition of reagents**

The order of addition of constituents (acid, metal and reagent) has no adverse effect on the absorbance.

**Composition and stability constant of complex**

The composition of the complex was determined by Job’s continuous variation method. The stability constant of complex was calculated using the data obtained from Job’s plot.

i. **Job’s continuous variation method**

Equimolar (1 × 10⁻³ M) concentration of molybdenum (VI) and reagent (HTP) were prepared.

Series of the master solutions of molybdenum (VI) with the reagent (HTP) were made up comprising different complementary proportions. 2 ml of hydrochloric acid (0.5 M) added to each flask and the final volume of aqueous phase was adjusted up to 10 ml with double distilled water. The contents were mixed and equilibrated with 10 ml Chloroform for 60 sec. The two phases were allowed to separate and organic phase was collected in 10 ml flask after passing through anhydrous sodium sulphate to remove water droplets. The absorbance of extract was measured against at 389 nm against a corresponding reagent blank prepared similarly. From Figure 4, the molar ratio graph also suggests composition of the complex to be 1:1.

**Tolerance limit of foreign ions**

Various amounts of foreign ions that is generally associated with the Mo (VI) in real samples are added to a fixed amount of Mo (VI) (9.594 µg mL⁻¹) solution and the absorbance is measured at 389 nm under optimal conditions by the developed procedure. The concentration (µg mL⁻¹) at which various ions do not cause an error of more than ± 2% in absorbance is taken as the tolerance limit and the results are presented in Table 3.

The data in Table 3 indicate that many metal ions and anions do not interfere in the determination of Mo (VI).

**Validation**

Under the optimized experimental conditions, a linear relation was obtained between absorbance and concentration of molybdenum (VI) in the proposed method. Regression analysis for the results was carried out using least-square method. Beer’s law plots were linear with good correlation coefficients as shown Table 4. The limits of detection (LOD) and limits of quantification (LOQ) were determined [17] using the formula: LOD or LOQ = kSᵣ/b, where k = 3.3 for LOD and 10 for LOQ, Sᵣ is the standard deviation of the intercept, and b is the slope.

**Precision and accuracy**

The precision and accuracy of the proposed methods were determined at three different concentrations of Mo (IV). At each concentration, six replicate determinations were made. The relative standard deviation as precision and percentage relative error (RE %) as accuracy of the suggested methods was calculated as shown in Table 5.

The results presented in Table 5, reveal that precision and accuracy of the proposed methods are fairly high as indicated by the low values of % RSD and % RE.

**Figure 2:** Absorption spectra a) HTP (1x10⁻³ M) against chloroform

a1) 1x10⁻⁴ M Mo (IV)-HTP complex against reagent blank.

\[ [\text{Mo (IV)}] = 1 \times 10^{-4} \text{ M} \]

Flue dust sample (0.1 g) from copper manufacture, containing no molybdenum, was mixed with the solution of the known molybdenum content (1 mg) and heated to near dryness and nitrate was expelled from the residue using 5 ml of concentrated hydrochloric acid. The residue was dissolved in double-distilled water and made up to 100 ml. This serves as stock solution. The experimental solution was prepared by diluting stock solution with distilled water. A suitable aliquot of the sample solution is taken for analysis. The results are presented in the Table 6 (c).

CONCLUSION
In the present investigation, the authors introduced a new reagent, N’-(2-hydroxybenzylidene)-3-[4-(o-tolyl)piperazin-1-yl] propanehydrazone (HTP) to the field of extractive spectrophotometric determination of molybdenum (VI). The colour reaction between Mo (VI) and HTP is almost instantaneous and the absorbances of complex remain constant for 10 hrs. The order of addition of constituents has no adverse effect. The reagent was found to be selective and sensitive. The selectivity of the reagent was further improved by the use of proper masking agents to suppress the interference of diverse metal ions. The results from the present developed method clearly demonstrate the usefulness of HTP as an extracting agent for the determination of molybdenum (VI) in environmental matrices such as water samples, standard alloy samples and Fuel dust samples.

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