Determination of molybdenum(VI) by differential pulse polarographic technique using 4-(2-hydroxy phenyl ethaminodiol), benzene-1,3-diol(4-2-HPEDB-1,3,D)

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Abstract A novel analytical reagent 4-(2-hydroxy phenyl ethaminodiol), benzene-1,3-diol(4-2-HPEDB-1,3,D) was synthesized for the determination of molybdenum(VI). The present paper reveals the detailed study of electroanalytical behaviour for [Mo-(4-2-HPEDB-1,3,D)] complex under optimized conditions. The peak obtained for [Mo-(4-2-HPEDB-1,3,D)] prevent the interference of foreign ions which shows the sensitivity of the proposed method. The linearity was maintained at the concentration range of 0.5–200 μg/mL at pH 4.5 with correlation factor 0.9997. The present method was successfully applied for the analysis of molybdenum(VI) in biological fluids and plant material. The results obtained from the proposed method show good agreement with reference method.

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2004; Lis and But, 2000; Taher and Mobarak, 2005; Tunceli and Rehber, 2004; Zaijun et al., 2005; Nukatsuka et al., 1995), Inductively Coupled Plasma Emission Spectrometry (Martynkova et al., 2004; Liang et al., 2004) and Neutron Activation Analysis (Sun et al., 1999). Those above mentioned techniques suffers with some disadvantages such as tedious reaction steps, time taking procedures, high reagent concentration, requires sophisticated laboratory techniques and highly economical. In view of this, author reported a simple, sensitive and novel electroanalytical technique for the determination of molybdenum(VI) in biological fluids and plant material using newly synthesized analytical reagent. Several analytical reagents and techniques have been reported for the determination of molybdenum(VI) in various samples which is shown in (Table 1).

2. Experimental

2.1. Apparatus and reagents

An Elico CL-362 model polarographic system is used for DPP measurements and Elico Li-129 model glass-calomel combined electrode was employed for measuring pH values.

Ag/AgCl (salt KCl) was used as a reference electrode which provide a reversible half reaction with nernstian behaviour be constant over time and easy to assemble and maintain and a platinum wire as an auxiliary electrode which displays negative potential range.

All reagents used were analytical reagent grade. Double distilled water was used throughout the experiment. A stock solution of molybdenum(VI) was prepared by dissolving appropriate amount of ammonium hepta molybdate in double distilled water in volumetric flask. Working standard solution was freshly prepared by diluting the stock solution with double distilled water. Concentration (0.1 M) of chelating reagent was prepared by dissolving 2.84 g of 4-2-HPEDB-1,3,D in 100 mL of methanol.

2.2. Synthesis of 4-2-hydroxy phenyl ethamindiol benzene-1,3-diol(4-2-HPEDB-1,3,D)

Equimolar ratio of 2,4-dihydroxy acetophenone and 2-amino phenol in methanol mixture was refluxed for 3–4 h and the contents were cooled at room temperature, it gives orange-red precipitate. The precipitate was filtered and washed with methanol to pure Schiff base. M.P. – 115/176°C, yield (97%) as shown in Scheme 1.

I.R – (KBr), 1601.8 cm⁻¹ (C=O), 3304.0 cm⁻¹ (N–H), free (OH) 3375.3 cm⁻¹ 1465.9 cm⁻¹ (O–H) and the spectrum was shown in Fig. 1.

2.3. Recommended analytical procedure for the determination of metal ions

An aliquot of working standard solution containing 1–100 µL of metal ion is taken in to 25 mL volumetric flask. To this 5 mL of acetate buffer solution (pH 4.5), 2 mL of reagent solution were added. This mixture was transferred in to polarographic cell and diluted with 9 mL of supporting electrolyte and then deoxygenated with nitrogen gas for ten min. After recording polarogram small increment (0.2 mL) of standard solution is added to the cell treated for 1 min and polarogram
is again recorded under similar conditions. In the same man-
ner, 10 polarograms are recorded for 10 additions. In the pres-
ent study the best precision was obtained at pH 4.5 with a drop
time 2 s, pulse amplitude of 50 mV and an applied potential of
−1044.0 mV The relative standard deviation and correlation
coefficients were found to be 16.65% and 0.9997 for 10
replicates.

2.4. Sample collection

Tirupati is taken as study area in present paper for sample
analysis which is located at south in India and it is a rapidly
developing, upcoming city which is widely known at global
level. The arrival of approximately 50,000–60,000 pilgrims
per day to visit the lord Balaji temple (Tirumala) who are
mainly responsible for the threatening of the natural beauty
of this holy town. Hence, this holy pilgrim centre has been
chosen as the potential collection area for present study. Sev-
eral small and medium scale industries are situated in the
suburban fringes of Tirupati town. The samples were col-
lected from different locations of the study areas of in and
around Tirupati. The necessary and possible precautions were
taken at various stages starting from sample containers, sam-
ple collection and storage, processing and analyzing the
samples.
2.5. Analysis of biological fluids

The human serum or urine samples were centrifuged for 30 min by 1000 g centrifuge. The serum or urine samples were kept at 253 K for storage and the samples were digested with a 5:1 (v/v) mixture of conc. HNO₃ and HClO₄ at temperature of 393 K and then diluted to proper volumes. Suitable volumes of these solutions are taken for the determination of molybdenum(VI) as described in above said procedure and the results were shown in (Table 2).

2.6. Analysis of plant material

Finely chopped fresh potato leaves (5 g each) were placed in a 250 mL beaker, and a solution of concentrated H₂SO₄/HNO₃ 1:1 (v/v) (10 mL) was added. This mixture was heated until the solution is clear. The solution was filtered off and concentrated to 5 mL then cooled and diluted to 50 mL with deionised double distilled water then the general procedure was applied to 1 mL of this solution and results obtained were shown in (Table 3).

3. Results and discussion

3.1. Differential pulse polarographic studies

3.1.1. Effect of pH

The effect of pH on the peak potential $E_p$ and current intensity $i_p$, using differential pulse polarography was examined for [Mo-(4-2-HPEDB-1,3,D)] complex. The pH was varied in the whole pH range 2.5–8.5 for [Mo-(4-2-HPEDB-1,3,D)] complex. It can be observed from Fig. 3, $E_p$ is shifted towards more negative values, indicating proton participation in the reduction process.

3.2. Effect of pulse amplitude and scan rate

The influence of the pulse amplitude was investigated. The results suggested that DPP peak current reached the maximum value which is shown in Fig. 2 when the pulse amplitude was 50 mV. As per the scan rate; the current response with increasing the scan rate of 40 mV s⁻¹ gave the maximum response. Accordingly, the optimum conditions for recording a maximum developed and sharper DPP peak for 0.5 mM [Mo-(4-2-HPEDB-1,3,D)] are scan rate: 40 mV s⁻¹ and pulse amplitude: 50 mV.

### Table 2

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Sample</th>
<th>Source for samples</th>
<th>Molybdenum(VI) content µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DDP method</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Found</td>
</tr>
<tr>
<td>1</td>
<td>Serum-1</td>
<td>Anemia patient (male)</td>
<td>165.1</td>
</tr>
<tr>
<td>2</td>
<td>Urine-1</td>
<td></td>
<td>39.5</td>
</tr>
<tr>
<td>3</td>
<td>Serum-2</td>
<td>Normal adult (male)</td>
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<tr>
<td>4</td>
<td>Urine-2</td>
<td></td>
<td>33.9</td>
</tr>
<tr>
<td>5</td>
<td>Serum-3</td>
<td>Paralysis patient (male)</td>
<td>16.7</td>
</tr>
<tr>
<td>6</td>
<td>Urine-3</td>
<td></td>
<td>5.5</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Added Sample – potato leaves</th>
<th>Proposed method Molybdenum(VI) content (µg/mL)</th>
<th>Found</th>
<th>Mean recovery (%)</th>
<th>Mean recovery (%)</th>
<th>Reference method Molina-Diaz et al. (1996) Molybdenum(VI) content (µg/mL)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>22.25</td>
<td>22.47</td>
<td>99.9</td>
<td>22.55 – – 10 32.59 100.4 – 43.68 105.7 – 30 52.99 101.5</td>
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<td>34.26 104.8</td>
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<td></td>
<td>52.99</td>
<td>101.5</td>
<td>101.5</td>
<td>52.85 101.3</td>
</tr>
</tbody>
</table>

The Standard Reference Material SRM-1573a of tomato leaves were analyzed by the proposed method to determine the accuracy and precision of the present method which is given by National Institute of Standard and Technology. Inter calibration was performed by using tomato leaves – SRM-1573a and it’s certified value is 0.46 ppm. The analytical results obtained from the present method (0.451 ± 0.05) is good agreement with certified value (0.46 ppm).

3.3. Intercomparison of methods

### Table 3

<table>
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<th>Added Sample – potato leaves</th>
<th>Proposed method Molybdenum(VI) content (µg/mL)</th>
<th>Found</th>
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<td>101.5</td>
<td>52.85 101.3</td>
</tr>
</tbody>
</table>

* Samples collected from Acharya N.G. Ranga Agricultural College Tirupati, A.P., India.
3.3. Calibration

The detection limit and the relative standard deviation obtained as 0.29 μg/mL, 16.65 μg/mL, respectively. The linearity is maintained in the concentration range of Mo 0.5–200 μg/mL with correlation coefficient of 0.9997 and calibration curve is prepared according to the general procedure under the optimized conditions as shown in Fig. 4.

The method is validated by analysing the Certified Reference Material of tomato leaves (SRM-1573a) which shows the accuracy, precision and applicability of the present investigation.

Figure 2  Differential pulse polarogram of molybdenum(VI) peak at −1044.0 mV.

Figure 3  Effect of pH on determination of Mo(VI).
3.4. Stoichiometry of the complex

The composition of the complex was found to be $1:2 = \text{Mo}^{6+} : 4\text{-2-HPEDB-1,3,D}$. The stoichiometry of the complex was verified by mole ratio method and their data was shown in Fig. 5.

3.5. Effect of interfering ions

The effect of interfering ions on the determination of molybdenum(VI) in biological fluids and plant material were investigated and the results were shown in (Table 4) which is individually added to the molybdenum(VI) having appropriate concentration and the general procedure was applied. The tolerable limits of various foreign ions are masked using proper masking agents and recovery ranges are shown in (Table 4). The results are almost quantitative in the presence of interfering ions to evaluate the feasibility and sensitivity of the present method.

4. Conclusions

The present investigation for the analysis of molybdenum(VI) by differential pulse polarography provides the simple, sensitive, precise and economically cheap to achieve good enrichment factors. The newly synthesized analytical reagent is very economical and simple at ordinary laboratory conditions. The study of the interfering ions made the method more sensitive and selective and it is successfully applied for the determination of molybdenum(VI) in samples of plant material and

![Figure 4](image1.png) Calibration curve for Mo(VI).

![Figure 5](image2.png) Mole ratio method for the Mo(VI)-4-2-HPEDB-1,3,D.

**Table 4** Effect of interfering ions on the determination of molybdenum(VI).

<table>
<thead>
<tr>
<th>Interfering ions</th>
<th>Tolerance limit (μg/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$^{3+}$, Na$^{2+}$</td>
<td>10,500</td>
<td>98</td>
</tr>
<tr>
<td>Cu$^{2+}$, Co$^{2+}$, Cd$^{2+}$, Ni$^{2+}$, Ca$^{2+}$</td>
<td>4200</td>
<td>98</td>
</tr>
<tr>
<td>Pb$^{2+}$</td>
<td>2500$^a$</td>
<td>97</td>
</tr>
<tr>
<td>Mn$^{2+}$, Zn$^{2+}$, Mg$^{2+}$</td>
<td>1100</td>
<td>99</td>
</tr>
<tr>
<td>K$^+$</td>
<td>800$^b$</td>
<td>98</td>
</tr>
</tbody>
</table>

$^a$ Can be masked up to 2500 μg/mL by the addition of 2 mL of 2% sulfonic acid.

$^b$ Can be masked up to 800 μg/mL by the addition of 2 mL of 5% EDTA.
biological fluids having good accuracy and precision when compared with other technique which is existing in literature and their data is shown in (Table 1). This method is validated using a Certified Reference Material (potato leaves SRM-1573a) which is distributed by National Institute of Standard and Technology shows the accuracy of the proposed method.

References

Zaijun, Li, Yuling, Yang, Jian, Tang, Jiaomai, Pan, 2005. Journal of Food Composition and Analysis 18, 561–569.