

A Simple Spectrophotometric Method for the Determination of Trace Level of Cadmium in Real, Environmental, Biological, Tobacco, Fertilizer and Soil Samples Using 2', 3, 4', 5, 7 Pentahydroxyflavone

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors have equal contributions in this manuscript. All authors read and approved the final manuscript.

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ABSTRACT

A very simple, ultra-sensitive and highly selective non-extractive spectrophotometric method for the determination of trace amount of cadmium using 2', 3, 4', 5, 7-pentahydroxyflavone (morin) has been developed. Morin reacts in a slightly acidic (0.000001-0.000012M H₂SO₄) in 50% ethanolic solution with cadmium to give a deep greenish- yellow chelate which has an absorption maximum at 422 nm. The reaction is instantaneous and the absorbance remains stable for over 24 h. The average molar absorption co-efficient and Sandell's sensitivity were found to be $4.09 \times 10^6 \text{ L mol}^{-1} \text{ cm}^{-1}$ and 4.0 ng cm^{-2} of cadmium, respectively. Linear calibration graphs were obtained for 0.01 - 25 mg L⁻¹ of cadmium with a correlation co-efficient value 0.9996 for Cd-morin complex. The stoichiometric composition of the chelate is 1:2 (Cd:morin). The detection limit and quantification limit of the reaction system were found $1 \mu\text{g L}^{-1}$ and $10 \mu\text{g L}^{-1}$, respectively. A large excess of over 50 cations, anions and complexing agents (like, chloride, tartrate, azide, citrate, EDTA, SCN⁻ etc.) do not interfere in the determination. The developed method was successfully used in the determination of cadmium in several Standard

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Reference Materials (alloys and steels) as well as in some environmental waters (inland and surface), biological samples (human blood, urine and hair), tobacco, fertilizer, soil samples and complex synthetic mixtures. The results of the proposed method for biological samples were comparable with AAS and were found to be in good agreement. The method has high precision and accuracy ($s = \pm 0.01$ for 0.5 mg L^{-1}).

Keywords: Spectrophotometry; cadmium determination; 2', 3, 4', 5, 7-pentahydroxyflavone; alloy; steel; environmental; biological sample; tobacco; fertilizer; soil sample.

1. INTRODUCTION

Cadmium in trace amounts is important industrially [1], as a toxicant [2] and biological non-essential [3], as an environmental pollutant [4] and as an occupational hazard [5]. The accurate determination of cadmium in agroindustrial, environmental, biological, soil and food samples is of importance because of the toxicity of this heavy metal and related compounds which are extremely toxic and responsible for a number of deaths [6]. Symptoms of cadmium poisoning include instantaneous hypertension, shortening of life-span, kidney damage, bronchitis, retardation of growth, gross abnormalities of the vital organs, alopecia (loss of hair), diabetes, diarrhea, nausea, liver injury, anemia, renal cancer in human and the risk of prostatic cancer [6]. The most serious situation being the disease called Itai-Itai disease which causes gradual weakening of the bone structure, diminution of stature and ultimately the total collapse of the entire skeletal system [7]. Its extreme toxicity towards marine and freshwater organisms is also well known [8]. Cadmium is a potential health hazard due to its presence in urban atmosphere and cigarette smoke [8]. On the other hand, the nutrient role of the metal ion has also recently been recognized and cadmium deficiency in goats caused myasthenia [9]. All these findings cause great concern regarding public health, demanding accurate determination of this metal ion at trace and ultra trace levels.

Many sensitive techniques such as X-ray fluorescence [10], Neutron Activation Analysis (NAA) [11], Graphite furnace atomic absorption spectroscopy (GF-AAS) [12], Inductively coupled plasma-mass spectrometry (ICP-MS) [13], Inductively coupled plasma-optical emission spectroscopy (ICP-OES) [14], High Performance Liquid Chromatography (HPLC), Spectrofluorimetry [15] and Pulse polarography [15a] have been widely applied to the determination of cadmium. These methods are disadvantageous in terms of cost and the instruments used in routine analysis. AAS often lacks of sensitivity, and is affected by the matrix conditions of samples, such as salinity. Catalytic solvent extractive methods are highly sensitive and less expensive, but generally lack simplicity.

Spectrophotometry is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis. 2', 3, 4', 5, 7-pentahydroxyflavone (morin) has been reported as a spectrophotometric reagent for iron(II) [16] and aluminum [17] but has not been used previously for the spectrophotometric determination of cadmium. This paper reports on its use in a very sensitive, highly selective spectrophotometric method for the trace determination of cadmium. The method possesses distinct advantages over existing methods [18-26] with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range, thermal stability, accuracy, precision and ease of operation. The method is based on the reaction of non-absorbent morin in a slightly acidic solution (0.000001 - $0.000012 \text{ M H}_2\text{SO}_4$) with Cd to produce a highly absorbent deep greenish-yellow colored chelate product, followed by a direct measurement of the absorbance in an aqueous

solution. With suitable masking, the reaction can be made to be highly selective and the reagent blank solution does not show any absorbance.

2. EXPERIMENTAL SECTION

2.1 Apparatus

A Shimadzu (Kyoto, Japan) (Model-1800) double-beam UV/VIS spectrophotometer and a Jenway (England, UK) (Model-3010) pH meter with combination of electrodes were used for measurements of the absorbance and pH, respectively. A Thermo (Model-iCE 3000 C093300131v1.30) atomic absorption spectrophotometer equipped with a microcomputer-controlled nitrous oxide-acetylene flame was used to compare of the results. Infrared spectrum was recorded with FTIR Spectrophotometer, Shimadzu (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 cm^{-1} to test the purity of the morin.

2.2 Reagents and Solutions

All chemicals used were of analytical-reagent grade of the highest purity available. Doubly distilled de-ionized water and HPLC-grade absolute ethanol, which is non-absorbent under ultraviolet radiation, were used throughout the experiment. Glass vessels were cleaned by soaking in a acidified solutions of KMnO_4 or $\text{K}_2\text{Cr}_2\text{O}_7$, followed by washing with nitric acid (1+1) and were rinsed several times with high-purity de-ionized water. Stock solutions and environmental water samples (1000 mL each) were kept in polypropylene bottles containing 1 mL of concentrated HNO_3 . More rigorous contamination control was used when the cadmium levels in the specimens were low.

2.2.1 Morin solution, 5.05×10^{-3} M

The reagent solution was prepared by dissolving the requisite amount of morin (BDH Chemicals, Proanalysis grade), in a known volume of distilled ethanol. More dilute solutions of the reagent were prepared as required. The purity of the reagent (morin) was tested by IR spectrum as shown in Fig. 1. Infrared spectrum was recorded in the range 7500-350 cm^{-1} [$\nu_{\text{C}=\text{C}}$ at 1527-1591 cm^{-1} , $\nu_{\text{C}=\text{O}}$ at 1612-1630 cm^{-1} , $\nu_{\text{C}=\text{O}}$ at 1260-1326 cm^{-1}].

2.2.2 Cadmium standard solution (8.89×10^{-3} M)

A 100 mL amount of stock solution (1 mg mL^{-1}) of divalent cadmium was prepared by dissolving 228.2 mg of purified-grade (E Merck, proanalysis grade) hydrated cadmium sulfate $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ (super special grade J. T. Baker) in doubly distilled de-ionized water and aliquots of this solution were standardized by EDTA titration using xylenol orange as an indicator. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with de-ionized water as and when required.

2.2.3 Potassium permanganate solution

A 1% potassium permanganate (Merck) solution was prepared by dissolving in de-ionized water. Aliquots of this solution were standardized with oxalic acid. Sodium azide solution (2.5 % w/v) (Fluka purity > 99%) was also used.

Table 1. Summary of the existing spectrophotometric methods for the determination of cadmium

Reagent	λ_{\max} (nm)	Molar Absorption co-efficient ϵ ($\text{Lmol}^{-1}\text{cm}^{-1}$)	Beer's Law (mgL^{-1})	Detection Limit (ng mL^{-1})	Interference	Remarks	Reference
Arsenazo-1	420	6.2×10^3	0 - 4.0	40	Many	i) pH dependent. ii) Less selective due to much interference. iii) Less sensitive. iv) Solvent extractive method hence lengthy and time consuming.	[18]
2-(5-bromo-2 pyridylazo)-5-(diethylamins) phenol	508	4.5×10^4	0 -5.0	35	Many	i) Less sensitive. ii) Solvent extractive process hence lengthy. iii) Application was not studied. iv) Less selective due to much interference.	[19]
5,7-dibromo-8-hydroxyquinolin-e (DBHQ)	396	5.8×10^3	0.1 -30	50	Cr(III),Cr(VI),Cu(II),Ni(II), V(V),Fe(III),Hg(II), Mo(VI),Se(IV) and Ag Interference at high concentration.	i) Less sensitive. ii) pH dependent. iii) Less selective due to much interference. iv) Limited application.	[20]
Malachite Green	690	1.7×10^4	0 - 20	30	Many	i) pH dependent. ii) Less sensitive. iii) Less selective due to much Interference.	[21]
Cinnamaldehyde-4-hydroxybenzoylhydrazone(CMHBH)	383	5.6×10^4	0.06-0.5	70	Many ions interfere	i) pH dependent. ii) Less selective due to much interference. iii) Solvent extractive method hence lengthy and time consuming.	[22]
Xylenol orange	578	1.6×10^4	0.05-6.0	60	Many	i) pH dependent. ii) Less sensitive. iii) Less selective due to much interference. iv) Application was not studied.	[23]
2',3,4',5,7-pentahydroxyflavone(Morin) (Present method)	422	4.09×10^6	0.01-25.0	1.0	Using suitable masking agents, the reaction can be made highly selective	i) Ultra sensitive. ii) Highly selective. iii) Non-extractive. iv) Aqueous reaction medium. v) Color stable more than 24 h at 25 ± 5 C. vi) Simple and rapid. Carcinogenic solvents have been avoided.	Present method

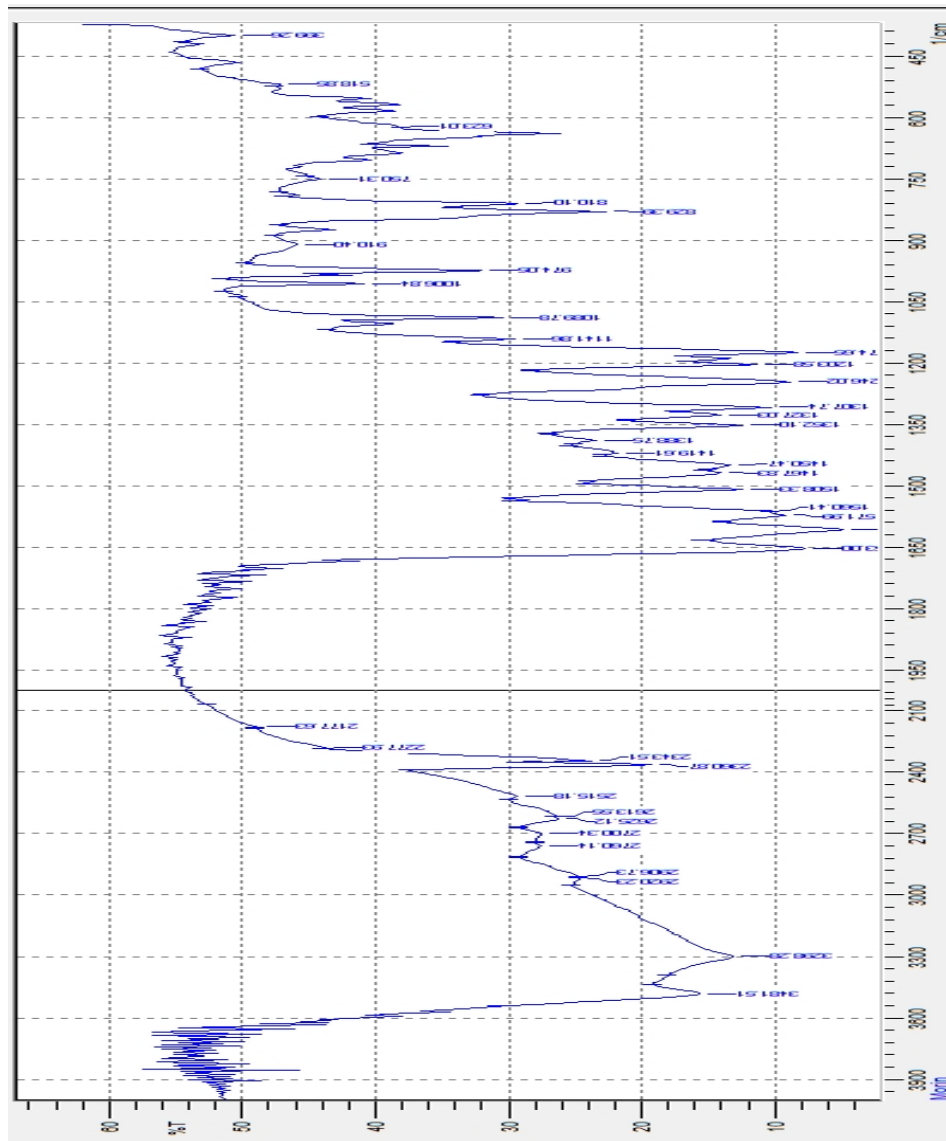


Fig. 1. FTIR spectrum of 2', 3, 4', 5, 7-pentahydroxyflavone (morin)

2.2.4 EDTA solution

A 100 mL stock solution of EDTA (0.01% w/v) was prepared by dissolving 10mg of A.C.S.-grade (≥ 99%) ethylenediaminetetraacetic acid disodium salt dehydrate in (100 mL) deionized water.

2.2.5 Tartrate solution

A 100 mL stock solution of tartrate (0.01 % w/v) was prepared by dissolving 10 mg of A.C.S.-grade (99%) potassium sodium tartrate tetrahydrate in (100 mL) de-ionized water.

2.2.6 Aqueous ammonia solution

A 100 mL solution of an aqueous ammonia solution was prepared by diluting 10 mL concentrated NH_4OH (28-30%, A.C.S.-grade) to 100 mL with de-ionized water. The solution was stored in a polypropylene bottle.

2.2.7 Other Solutions

Solutions of a large number of inorganic ions and complexing agents were prepared from their Anala R grade or equivalent grade water-soluble salts (or the oxides and carbonates in hydrochloric acid); those of niobium, tantalum, titanium, zirconium and hafnium were specially prepared from their corresponding oxides (Specpure, Johnson Matthey) according to the recommended procedures of Mukharjee [27]. In the case of insoluble substances, special dissolution methods were adopted [28].

2.3 Procedure

To 0.1-1.0 mL of a neutral aqueous ($\text{pH} = 6$) solution containing 0.1-250 μg of cadmium in a 10-mL calibrated flask was mixed with a 1:200-1:700 fold molar excess of the morin reagent solution (preferably 1 mL of 5.05×10^{-3} M) followed by the addition of 0.02-0.12 mL (preferably 0.05 mL) of 0.0001 M of sulfuric acid. After 1 min, 5ml of ethanol was added and the mixture was diluted to the mark with de-ionized water. The absorbance was measured at 422 nm against a corresponding reagent blank. The cadmium content in an unknown sample was determined using a concurrently prepared calibration graph.

2.4 Sample Collection and Preservation

Environmental sample: Water and soil samples were collected in polythene bottles from different places of Bangladesh. After collection, HNO_3 (1 mL L^{-1}) was added as preservative.

Blood and Urine: Blood and urine samples were collected in polythene bottles from effected persons of Chittagong Medical College Hospital, Bangladesh. Samples were collected with the consent of patient and also with permission of Doctor by health technician. Immediately after collection they were stored in a salt-ice mixture and latter, at the laboratory, were at 20°C .

Soil: Soil samples were collected from different locations of Bangladesh. Samples were dried in air and homogenized with a mortar.

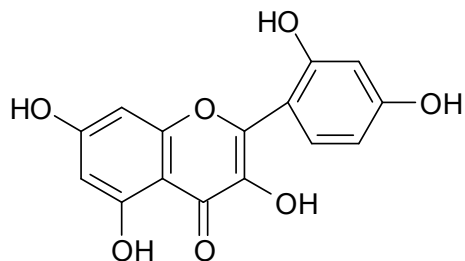
Tobacco and Cigarette: Tobacco and cigarette samples were collected from local market, Chittagong.

3. RESULTS AND DISCUSSION

3.1 Absorption Spectra

The absorption spectra of the Cd-morin system in 0.0001M sulfuric acid medium was recorded using a spectrophotometer. The absorption spectra of the Cd-morin is a symmetric curve with maximum absorbance at 422 nm; an average molar absorption coefficient of $4.09 \times 10^6 \text{ L mol}^{-1}\text{cm}^{-1}$ is shown in Fig. 2. Morin did not show any absorbance. In all

instances, measurements were made at 422 nm against a reagent blank. The reaction mechanism of the present method is as reported earlier [17].



Structure of 2', 3, 4', 5, 7-Pentahydroxyflavone (morin).

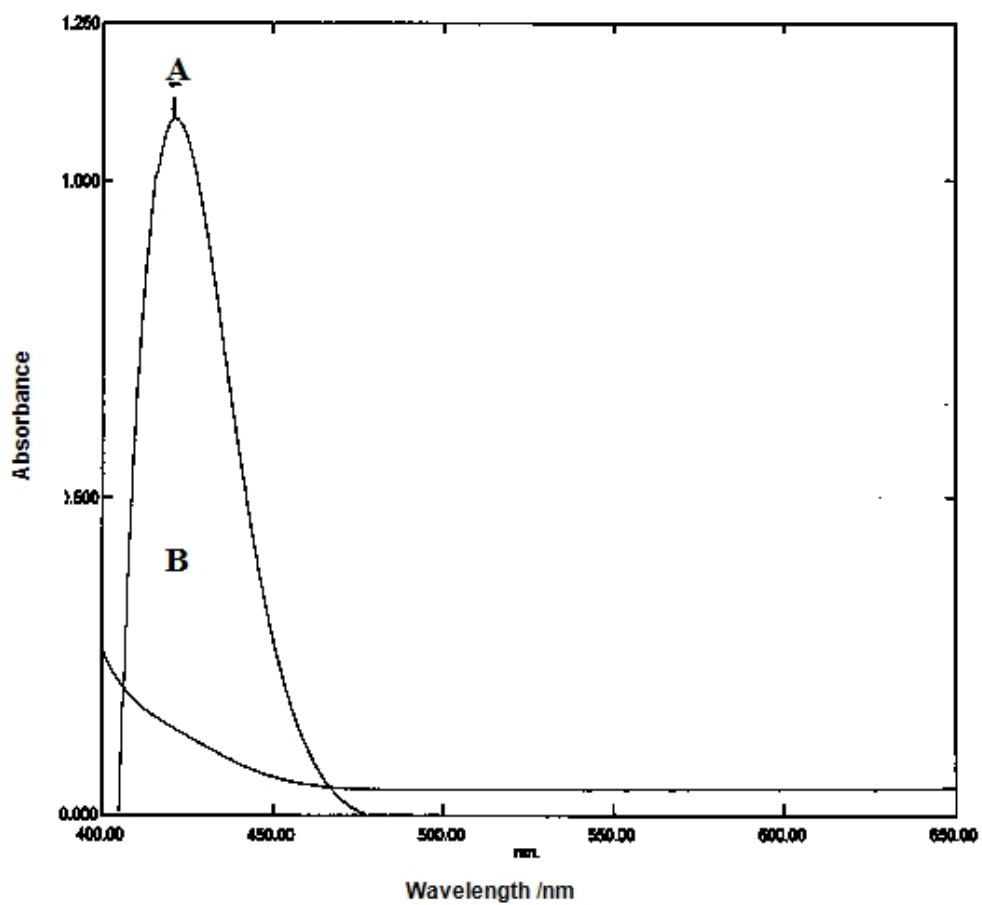


Fig. 2. A and B absorption spectra of Cd-morin system and the reagent blank ($\lambda_{\max}=422$ nm) in aqueous solutions

3.2 Effect of Solvent

Because morin is insoluble in water, an organic solvent was used for the system. Of the various solvents (benzene, chloroform, acetone, carbon tetrachloride, nitrobenzene, isobutyl alcohol, n-butanol, isobutyl methyl ketone, ethanol and 1, 4-dioxane) studied, ethanol was found to be the best solvent for the system. No absorbance was observed in the organic phase with the exception of n-butanol. In 50% (v/v) ethanolic medium, however the maximum absorbance was observed (Fig. 3); hence, a 50% ethanolic solution was used in the determination procedure.

3.3 Effect of Acidity

Of the various acids (nitric, sulfuric, hydrochloric and phosphoric) studied, sulfuric acid was found to be the best acid for the system. The absorbance was at a maximum and constant when the 10 mL of solution (1 mg L^{-1}) contained 0.02-0.12 mL of 0.0001 M sulfuric acid at room temperature ($25 \pm 5^\circ\text{C}$). Outside this range of acidity, the absorbance decreased (Fig. 4). For all subsequent measurements 0.05 mL of 0.0001 M sulfuric acid was added.

3.4 Effect of Temperature

The cadmium-morin system attained maximum and constant absorbance at $(15-40)^\circ\text{C}$ temperature. For all subsequent measurements was done at room temperature ($25 \pm 5^\circ\text{C}$).

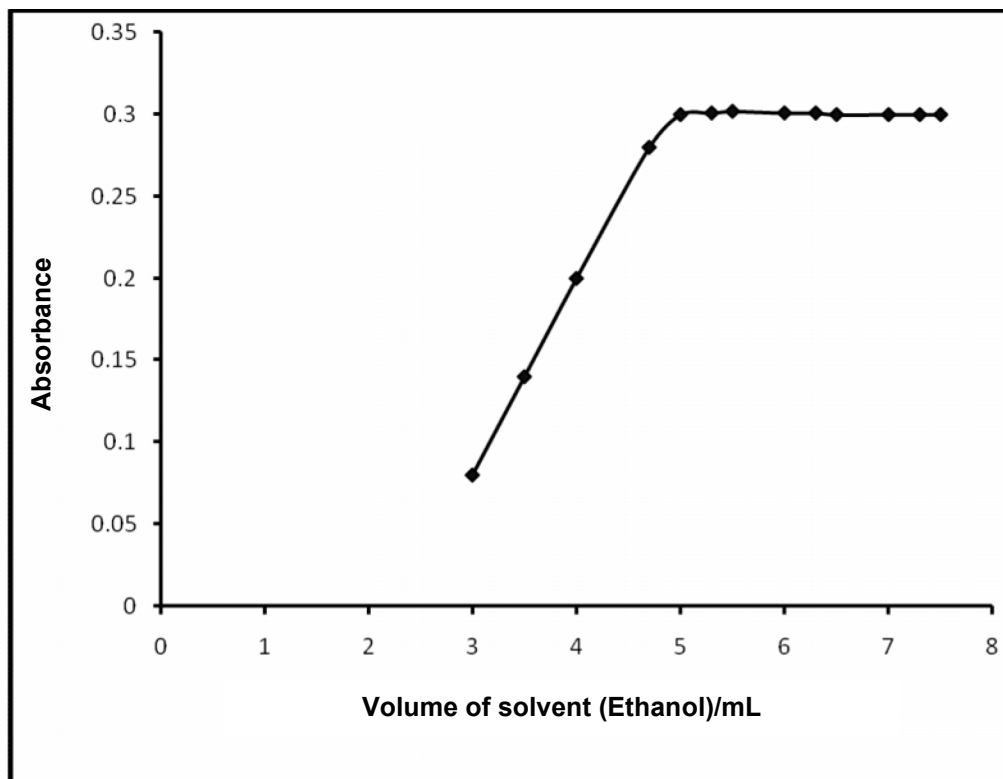


Fig. 3. Effect of solvent on the absorbance of Cd-morin system

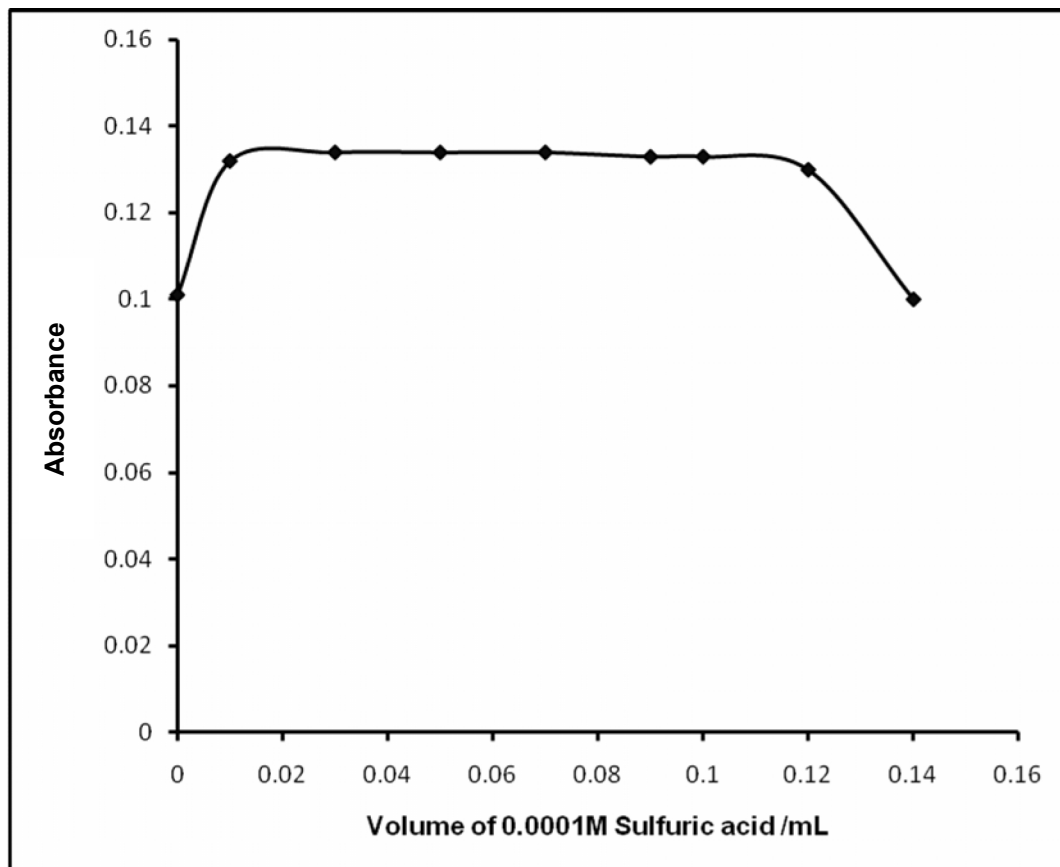


Fig. 4. Effect of acidity on the absorbance of Cd-morin system

3.5 Effect of Time

The reaction is instantaneous. The cadmium-morin system attained maximum and constant absorbance immediately (within 1min) after dilution the solution to the final volume, which then remained strictly unaltered for 24h (Fig. 5).

3.6 Effect of Reagent Concentration

Different molar excesses of morin were added to a fixed metal ion concentration and absorbances were measured according to the standard procedure. It was observed that at 1 mg L^{-1} Cd metal, the reagent molar ratios of 1:200-1:700 produced a constant absorbance of the Cd-chelate (Fig. 6). Greater excesses of reagent were not studied. For all subsequent measurements, 1 mL of $5.05 \times 10^{-3} \text{ M}$ morin reagent was added.

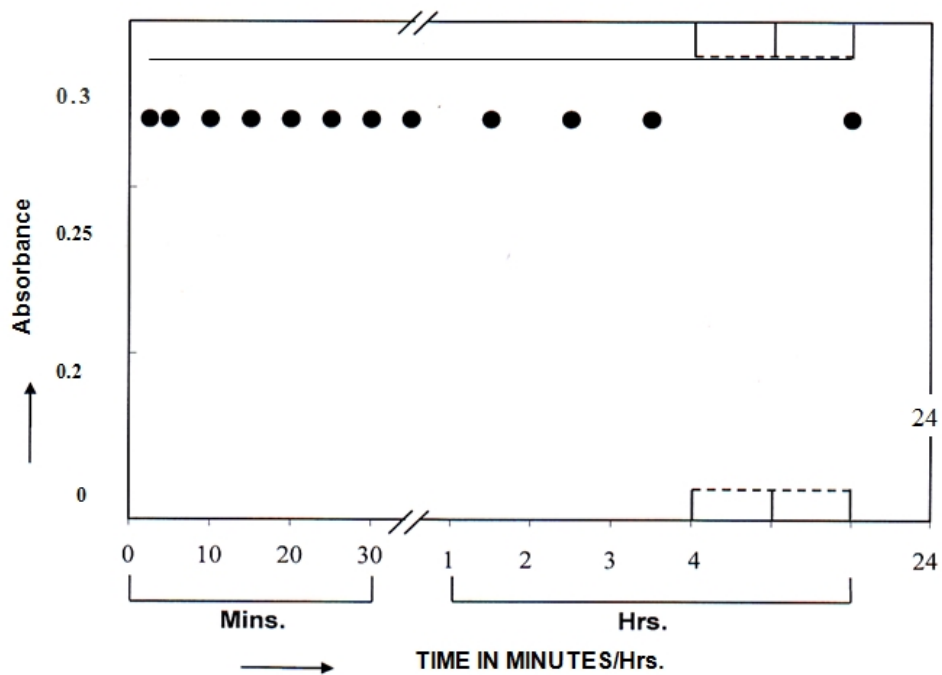


Fig. 5. Effect of time on the absorbance of Cd- morin system

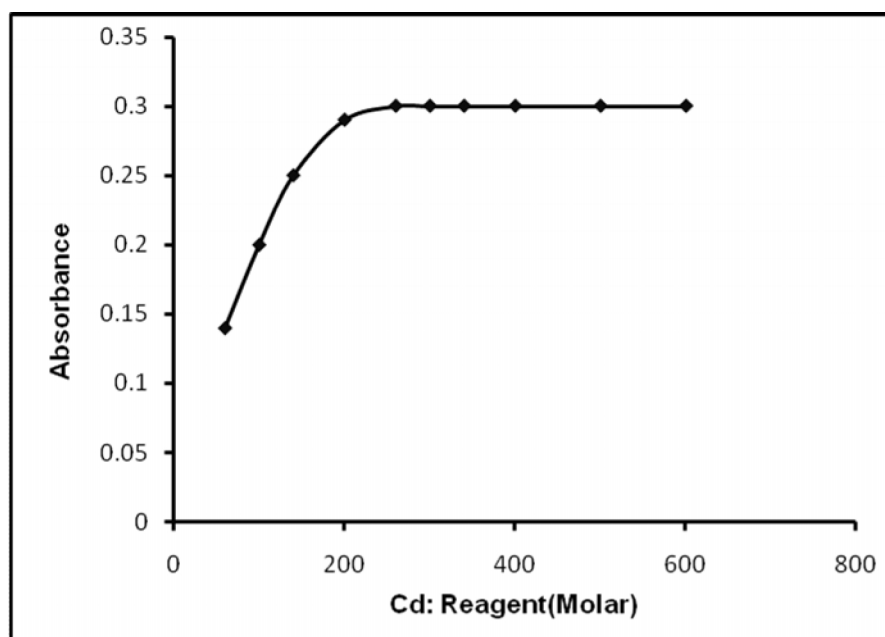


Fig. 6. Effect of reagent [Cd: morin molar concentration ratio] on the absorbance on Cd-morin system

3.7 Calibration Graph (Beer's Law and Sensitivity)

The well-known equation for spectrophotometric analysis in very dilute solutions derived from Beer's law. The effect of metal concentration was studied over 0.01-100 mgL⁻¹ distributed in four different sets (0.01-0.1, 0.1-1, 1-10 and 10-100 mgL⁻¹) for convenience of measurement. The absorbance was linear for 0.01-25 mgL⁻¹ of cadmium at 422 nm representing four graphs (0.01-0.1, 0.1-1, 1-10 and 10-25mgL⁻¹). Of four calibration graphs, the one showing the limit of the linearity range is shown in Fig. 7; the next three were straight-line graphs passing through the origin ($R^2 = 0.9996$). The molar absorption coefficient and Sandell's sensitivity [29] were found to be $4.09 \times 10^6 \text{ L mol}^{-1}\text{cm}^{-1}$ and 4.0 ng cm^{-2} of cadmium, respectively. The selected analytical parameters obtained with the optimization experiments are summarized in Table 2.

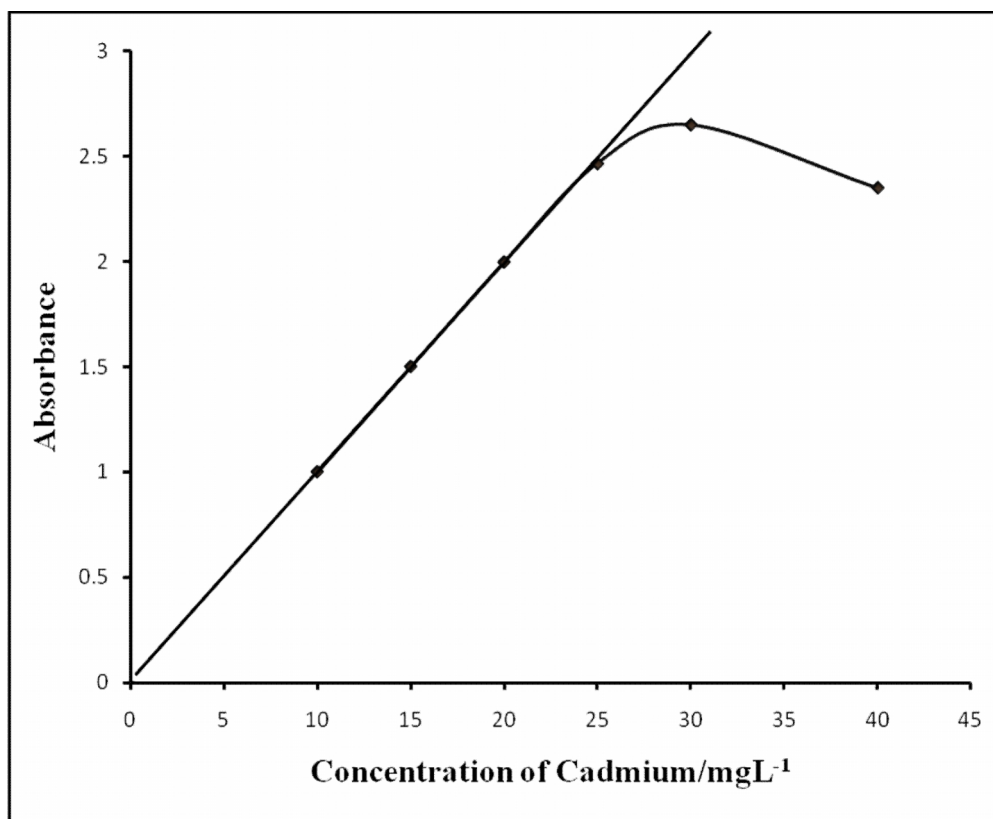


Fig. 7. Calibration graph D: 10 – 25.0 mgL⁻¹ of cadmium

Table 2. Selected analytical parameters obtained with the optimization experiments

Parameters	Studied range	Selected value
Wavelength / λ_{\max} (nm)	200-800	422
Acidity / M H ₂ SO ₄	1×10^{-6} - 1×10^{-5}	0.000001-0.000012 (Preferably 0.000005)
pH	4.50-3.50	4.5-3.80 (Preferably 4)
Solvent / % Ethanol	10-80	50-80 (Preferably 50)
Time / h	0 - 72	1min-24 h (Preferably 2 min)
Temperature / °C	10-70	15-40 (Preferably $25 \pm 5^{\circ}\text{C}$)
Reagent (fold molar excess, M:R)	1:10 - 1:700	1:200 - 1:700 (Preferably 1: 200)
Linear range/mg L ⁻¹	0.001-100	0.01 - 25
Molar absorption coefficient / L mol ⁻¹ cm ⁻¹	5.62×10^6 - 3.16×10^6	4.09×10^6
Sandell's sensitivity/ng cm ⁻²	1-100	4.0
Detection limit / $\mu\text{g L}^{-1}$	0.1-10	1.0
Reproducibility (% RSD)	0 - 10	0 - 3%
Regression Co-efficient (R ²)	0.9992-0.9999	0.9996

3.8 Effect of Foreign Ions

The effect of over 50 ions and complexing agents on the determination of only 1 mgL⁻¹ of cadmium was studied. The criterion for an interference [30] was an absorbance value varying by more than $\pm 5\%$ from the expected value for cadmium alone. The results are summarized in Table 3. As can be seen a large number of ions have no significant effect on the determination of cadmium. The most serious interference were from Al(III) and Fe(II) ions. Interference from these ions are probably due to complex formation with morin.

The greater tolerance limits for these ions can be achieved by using several masking agents. In order to eliminate the interference of Al(III) and Fe(II) ions, EDTA and citric acid can be used as masking agents [31]. A 10-fold excess of Al(III) and Fe(II) ions could be masked with EDTA and citrate. During the interference studies, if a precipitate was formed, it was removed by centrifugation. Al(III) and Fe(II) ions, interfere when present in amounts of excess of cadmium. Interference from these two metal ions have been effectively removed by a short single-step ion-exchange separation process, using an Amberlite XAD-8 resin (100-200 mesh) anion exchanger. The amount mentioned is not the tolerance limit but the actual amounts studied. However, for those ions whose tolerance limit has been studied, Their tolerance ratios are mentioned in Table 3.

Table 3. Table of tolerance limits of foreign ions, *tolerance ratio [species(x)]/Cd(w/w)

Species x	Tolerance ratio x/Cd (w/w)	Species x	Tolerance ratio x/Cd (w/w)
Ammonium (I)	100	Lead (II)	50
Arsenic (III)	25	Magnesium	50
Arsenic (V)	25	Manganese(II)	25
Aluminium	10 ^a	Mercury (II)	50
Azide	100	Molybdenum (VI)	50 ^c
Ascorbic acid	100	Nitrate	100
Beryllium(II)	100	Nickel	20 ^b
Bromide	100	Potassium	100
Bismuth (III)	20 ^a	Phosphate	20
Barium	20 ^a	Selenium (VI)	50
Calcium	20 ^a	Selenium (IV)	50
Chloride	100	Silver	100
Cobalt (II)	25	Sodium	100
Chromium (III)	50	Strontium	100
Chromium (VI)	25	Oxalate	200
Carbonate	100	Tin(II)	50
Cesium	100	Tin(IV)	100
Copper (II)	50	Titanium(IV)	50
Citrate	100	Tellurium (IV)	50
Cyanide	100	Thiocyanate	100
EDTA	100	Thiosulfate	100
Fluoride	100	Tungsten (VI)	50 ^c
Iodide	100	Tartrate	100
Iron (II)	10 ^b	Vanadium (V)	50
Iron (III)	50 ^b	Zinc	50
Lithium	25 ^b		

*Tolerance limit was defined as ratio that causes less than 5 percent interference.

^awith 10 mg L⁻¹ EDTA.

^bwith 10 mg L⁻¹ citric acid.

^cwith 10 mg L⁻¹ tartrate.

3.9 Composition of the Absorbent Complex

Job's method [32] of continuous variation and the molar ratio [33] method were applied to ascertain the stoichiometric composition of the complex under the optimum conditions (Table 1). A Cd-morin (1:2) complex was indicated by Job's method as shown in Fig. 8. Job's method of continuous variation was applied to ascertain the stoichiometric composition of the complex. Experimental data has been presented graphically in Fig. 8 and the stoichiometry was found to be 1:2 (Metal: Ligand).

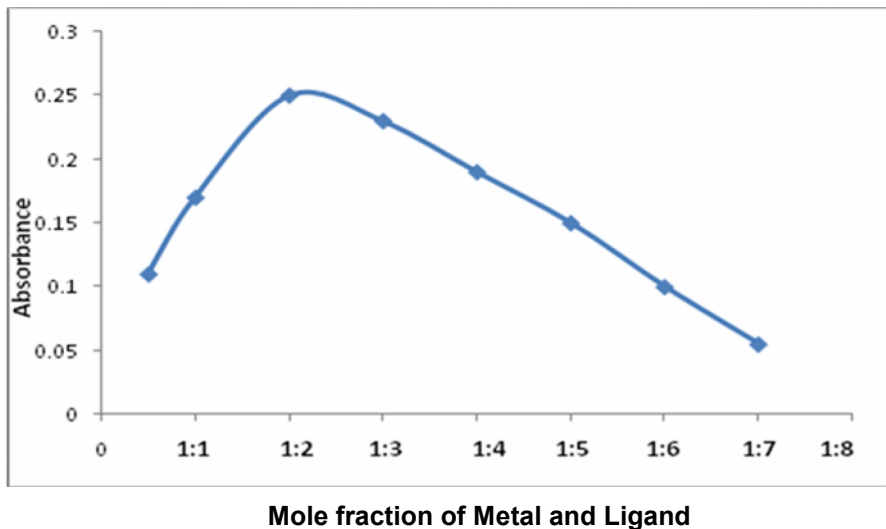


Fig. 8. Job's method for determining the composition of Cd: morin (1:2) complex

3.10 Precision and Accuracy

The precision of the present method was evaluated by determining different concentrations of cadmium (each analyzed at least five times). The relative standard deviation ($n=5$) was 0-3% for 0.1-250 μg of cadmium in 10 mL, indicating that this method is highly precise and reproducible, Table 1. The detection limit ($3s$ of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for cadmium were found to be $1.0 \mu\text{g L}^{-1}$ and 4.0 ng cm^{-2} , respectively. The method was tested by analyzing several synthetic mixtures containing cadmium and diverse ions, Table 3. The method was applied in a number of real samples, the results for total cadmium were in good agreement with certified values Table 4. The reliability of our cadmium-chelate procedure was tested by recovery studies. The average percentage recovery obtained for addition of cadmium spike to some environmental water samples was quantitative, as shown in Table 5. The results of biological sample analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS, Table 6. Hence, the precision and accuracy of the method were found to be excellent.

3.11 Applications

The present method was successfully applied to the determination of cadmium in a series of synthetic mixtures of various compositions (Table 4.) and also in a number of real samples e.g. several Certified Reference Materials (CRM) (Table 5.). The method was also extended to the determination of cadmium in a number of environmental, biological, tobacco, fertilizer and soil samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such samples were analyzed for cadmium content; the recoveries in both the "spiked" (added to the samples before the mineralization or dissolution) and the "unspiked" samples are in good agreement (Table 6.). The results of biological sample analyses by spectrophotometric method were found to be in excellent agreement with those obtained by AAS (Table 7). The results of soil sample, tobacco and fertilizer sample analysis by the spectrophotometric method are shown in Table 8, Table 9. and Table 10, respectively. The precision and accuracy of the method were excellent.

3.11.1 Determination of cadmium in synthetic mixtures

Several synthetic mixtures of varying compositions containing cadmium and diverse ions of known concentrations were determined by the present method using EDTA or tartrate as a masking agent; the results were found to be highly reproducible as shown in Table 4. Accurate recoveries were achieved in all solutions. The method was tested by analyzing several synthetic mixtures containing cadmium and diverse ions. It was found that recoveries are almost 100 % which indicates that the method is highly reproducible.

Table 4. Determination of cadmium in some synthetic mixtures

Sample	Composition of mixtures (mg L ⁻¹)	Cadmium/mg L ⁻¹		
		Added	Found ^a (n=5)	Recovery ± SD ^b (%)
A	Cd	0.50	0.49	98 ± 0.5
		1.00	1.00	100 ± 0.0
B	As in A + Cr ³⁺ (25) + NH ₄ ⁺ (25)+ EDTA(10)	0.50	0.50	100 ± 0.0
		1.00	0.99	98 ± 0.6
C	As in B + Sn ²⁺ (25) + Cu ²⁺ (25)+ Tartrate(10)	0.50	0.51	102 ± 0.7
		1.00	1.02	102 ± 0.8
D	As in C + Hg ²⁺ (25) + K (25)	0.50	0.52	104 ± 1.0
		1.00	1.04	104 ± 1.2
E	As in D +Mg(25) + Zn(25) + Citrate(10)	0.50	0.53	106 ± 1.5
		1.00	1.08	108 ± 1.8

^a Average of five analyses of each sample.

^b The measure of precision is the standard deviation (SD).

3.11.2 Determination of cadmium in alloys and steels (certified reference materials)

A 0.1-g amount of an alloy or steel sample containing 0.26 – 7.0 µg g⁻¹ of cadmium was weighed accurately and placed in a 50 mL Erlenmeyer flask following a method recommended by Parker [34]. To it, 10 mL of 20% sulfuric acid was added while carefully covering with a watch glass until the brisk reaction subsided. The solution was heated and simmered gently after the addition of 10 mL of concentrated HNO₃ until all carbides were decomposed. Then, 2 mL of 1:1 (v/v) H₂SO₄ was added and the solution was carefully evaporated to dense white fumes to drive off the oxides of nitrogen, and then cooled to room temperature (25 ± 5)°C. After suitable dilution with de-ionized water, the contents of the Erlenmeyer flask were warmed so as to dissolve the soluble salts. The solution was then cooled and neutralized with a dilute NH₄OH in the presence of 1-2 ml of 0.01% (w/v) tartrate solution. The resulting solution was filtered, if necessary, through a Whatman No. 40 filter paper into a 25 mL calibrated flask. The residue (silica and tungstenic acid) was washed with a small volume of hot (1 + 99) H₂SO₄, followed by water; the volume was made up to the mark with de-ionized water.

A suitable aliquot (1-2 mL) of the above-mentioned solution was taken into a 10-mL calibrated flask and the cadmium content was determined; as described under Procedure using EDTA or tartrate as masking agent. Based on five replicate analyses, average cadmium concentration determined by our general procedure. The validity of our method was tested by analyzing several certified reference materials (CRMs) of different composition. The results for cadmium found were in good agreement with those of the certified values. The results were highly reproducible. The results are shown in Table 5.

Table 5. Determination of cadmium in certified reference materials

Sample	Certified Reference Materials ^a (Composition $\mu\text{g g}^{-1}$)	Cadmium($\mu\text{g g}^{-1}$)		
		In C.R.M sample	Found (n=5)	RSD ^b
1	GBW01619-Unalloyed steel (Ag = 3.5, As = 17, Bi = 4.2, Ca = 42, Cd = 7.0, Ga = 29, In = 11, Mg = 82, Pb = 12, Sb = 204, Se = 43, Sn = 103, Ti = 8.5, Zn = 24)	7.0	6.97	1.5
2	GBW01620-Unalloyed steel (Ag = 4.6, As = 11, Bi = 0.4, Ca = 21, Cd = 4.6, Ga = 32, Mg = 16, Pb = 4.1, Sb = 95, Se = 16, Sn = 53, Ti = 22, Zn = 32)	4.6	4.55	1.8
3	HZL205- High tensile steel (Cu = 5.50, Si = 0.25, Mn = 0.15, Fe = 0.14, Mg = 0.13, Ti = 0.10, Cd = 0.26, V = 0.076)	0.26	0.27	3.5
4	GBW01622-Unalloyed steel (Ag = 0.3, As = 72, Bi = 0.5, Ca = 22, Cd = 1.9, Ga = 28, In = 0.4, Mg = 53, Pb = 2.2, Sb = 7.4, Se = 43, Sn = 0.10, Ti = 83, Zn = 20)	1.9	2.0	2.5

^aThese CRMs were from Beijing NCS Analytical Instruments CO. Ltd. China.

^bThe measure of precision is the relative standard deviation(RSD).

3.11.3 Determination of cadmium in environmental water samples

Each filtered (with Whatman No. 40) environmental water sample (500 mL) was evaporated nearly to dryness with a mixture of 5-mL concentrated H_2SO_4 and 10 mL of concentrated HNO_3 in a fume cupboard, following a method recommended by Greenberg et al. [35]. and was then cooled to room temperature. The residue was then heated with 10 mL of de-ionized water in order to dissolve the salts. The solution was then cooled and neutralized with dilute NH_4OH solution in presence of 1-2 mL of 0.01% (w/v) EDTA solution. The resulting solution was then filtered and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with de-ionized water.

An aliquot (1-2 mL) of this preconcentrated water sample was pipetted into a 10-mL calibrated flask and the cadmium content was determined as described under the Procedure using EDTA or tartrate as masking agent. The analyses of environmental water samples from various sources of cadmium are shown in Table 6.

Most spectrophotometric methods for determination of cadmium in natural and sea water require preconcentration of cadmium [36]. The concentration of cadmium in natural water and sea water is a few $\mu\text{g L}^{-1}$ in developed countries [37]. The mean concentration of cadmium found in U.S. drinking water is 10-100 $\mu\text{g L}^{-1}$ [38].

Table 6. Determination of cadmium in some environmental water samples

Sample	Cadmium/ $\mu\text{g L}^{-1}$		Recovery \pm s (%)	s_r^b (%)
	Added	Found ^a		
Tap water	0	10.5	± 0.5	0.45
	100	110.0	99.5 ± 0.2	0.33
	500	510.5	100 ± 0.0	0.00
Well water	0	12.0		
	100	112.0	100 ± 0.0	0.00
	500	510.0	99.0 ± 0.5	0.29
River water	Karnaphully (upper)	0	25.5	
		100	125.0	99.8 ± 0.3
		500	525.5	100 ± 0.0
	Karnaphully (lower)	0	28.7	
		100	128.0	99.6 ± 0.6
		500	530.0	100.3 ± 0.5
	Halda (upper)	0	15.8	
		100	115.8	100 ± 0.0
		500	520.0	100.8 ± 0.6
Halda (lower)	0	17.0		
	100	117.0	100 ± 0.0	
	500	520.0	100.6 ± 0.4	
Sea water	Bay of Bengal (upper)	0	5.50	
		100	105.0	99.5 ± 0.8
		500	510.0	100.9 ± 0.7
	Bay of Bengal (lower)	0	6.8	
		100	106.0	99.3 ± 0.6
		500	510.0	100.6 ± 0.5
Drain water	T. S. P. Complex ^c	0	35.5	
		100	135.5	100 ± 0.0
		500	540.0	100.8 ± 0.6
	PHP ^d	0	40.8	
		100	142.0	100.8 ± 0.4
		500	540.8	100 ± 0.0
	BSRM ^e	0	125.0	
		100	225.0	100 ± 0.0
		500	530.0	100.9 ± 0.8
K.P.M water ^f	0	85.0		
	100	186.0	100.5 ± 0.7	
	500	585.0	100 ± 0.0	
Eastern refinery ^g	0	130.5		
	100	230.0	99.8 ± 0.7	
	500	640.0	101.5 ± 0.8	

^aAverage of five replicate determinations of each Sample .^bThe measure precision is the relative standard deviation(s_r).^cT. S. P. complex Ltd., Patenga, Chittagong.^dPHP Steel Mill, Kumira, Chittagong.^eBangladesh Steel Re -rolling Mills Ltd. (BSRM), Baizid Bosthami, Chittagong.^fKarnaphuli Paper Mill, Chandraghona, Chittagong.^gEstern Refinery, North Patenga, Chittagong.

3.11.4 Determination of cadmium in biological samples

Human blood (2-4 mL) or urine (10-20 mL) or hair (2-5 g) was taken into a 100-mL micro-Kjeldahl flask. A glass bead and 10-mL of concentrated nitric acid were added, and the flask was placed on the digester under gentle heating. When the initial brisk reaction was completed, the solution was removed and cooled following a method recommended by Stahr [39]. A 1mL volume of concentrated sulfuric acid was carefully added, followed by the addition of 1mL of 70% perchloric acid; and heating was continued to dense white fumes, while repeating nitric acid addition if necessary. Heating was continued for at least 0.5 hr and then cooling was applied. The content of the flask was filtered and neutralized with dilute NH_4OH solution in presence of 1-2 mL of 0.01% (w/v) tartrate solution. The resultant solution was then filtered and transferred quantitatively into a 10-mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1-2 mL) of the final solution was pipetted out into a 10-mL calibrated flask and the cadmium content was determined as described under procedure using EDTA or tartrate as masking agent. The results of biological analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS. The results are shown in Table 7.

The abnormally high value for the kidney disease patient is probably due to the involvement of high cadmium concentrations with As and Zn. The occurrence of such high cadmium contents is also reported in kidney disease patient from some developed countries [40]. Lowest value of cadmium was found in the blood and urine of smoker which due to low value of cadmium in soil.

Table 7. Determination results of cadmium for human fluids and hair

Serial no.	Sample	Cadmium / $\mu\text{g L}^{-1}$				Sample source*
		AAS (n=5)		Proposed method (n = 5)		
		Found	RSD(%)	Found	RSD(%)	
1	Blood	222.5	1.0	222.5	1.0	Kidney disease patient (Male)
	Urine	59.4	1.5	63.5	1.3	
2	Blood	104.4	1.6	108.5	1.5	Hypertension patient (Female)
	Urine	32.2	1.8	35.5	2.0	
3	Blood	200.5	1.4	205.0	1.2	Oesophagus cancer patient (Female)
	Urine	56.5	1.7	60.3	1.5	
4	Blood	190.5	1.2	192.8	1.4	Liver cirrhosis patient (Male)
	Urine	47.5	1.8	50.5	1.8	
5	Blood	61.3	2.0	64.5	2.2	Diabets patient(Male)
	Urine	16.5	2.5	18.3	2.5	
6	Blood	56.5	2.3	53.5	2.1	Smoker(Male)
	Urine	15.5	2.6	14.8	2.8	
7	Blood	3.71	1.5	3.35	1.8	Normal adult(Male)
	Urine	0.78	2.0	0.71	2.0	
8	Hair**	62.8	1.5	65.5	1.8	Normal human hair (Male)

*Samples were from Chittagong Medical College Hospital.

**Values in ng g^{-1} .

3.11.5 Determination of cadmium in soil samples

An air-dried homogenized soil sample (100 g) was accurately weighed and placed in a 100-mL micro-Kjeldahl flask. The sample was digested in the presence of an oxidizing agent, following a method recommended by Jackson [41]. The content of the flask was filtered through a Whatman No.40 filter paper into a 25-mL calibrated flask and neutralized with dilute NH_4OH solution in presence of 1-2 mL of 0.01% (w/v) EDTA solution. The resulting solution was then filtered and diluted up to mark with de-ionized water.

A suitable aliquot (1-2 mL) of the final solution was pipetted out into a 10-mL calibrated flask and the cadmium content was determined as described under procedure using EDTA or tartrate as masking agent. The cadmium content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results are shown in Table 8. The average value of cadmium in Bangladesh surface soil was found to be 1.95 mg kg^{-1} . The results of the highest value of cadmium found in BSRM (Bangladesh Steel Re-rolling Mills Ltd., Chittagong, Bangladesh) soil because Cd-amalgam was used in Steel Re-rolling Mills to join the iron rod. This film of cadmium was also applied on ferrous metal surfaces to retard corrosion by electroplating technique. The lowest value of cadmium was obtained in the marine soil which was diluted with sea water. The normal value was obtained from agriculture soil which is useful for normal crop cultivation.

3.11.6 Determination of cadmium in tobacco and cigarette

Tobacco or tobacco of cigarette (2 g) was accurately weighed and placed in a 100-mL micro-Kjeldahl flask. The sample was dissolved in 10 mL of concentrated HNO_3 and heating about to dryness. The content of the flask was filtered through a Whatman No. 40 filter paper into a 25-mL calibrated flask and neutralized with dilute NH_4OH solution in presence of 1-2 mL of 0.01% (w/v) EDTA solution. The resulting solution was then filtered and diluted up to the mark with de-ionised water [42].

A suitable aliquot (1-2 mL) of the final solution was pipetted out into a 10-mL calibrated flask and the cadmium content was determined as described under procedure using EDTA or tartrate as masking agent. The cadmium content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results are shown in Table 9. In our analysis the highest cadmium was found in the Gold-Leaf cigarette. A single cigarette typically contains 1-2 mcg of cadmium. When burned, cadmium is present at a level of $1000\text{-}3000 \text{ } \mu\text{g m}^{-3}$ in the smoke. Approximately 40-60 % of the cadmium inhaled from cigarette smoke is able to pass through the lungs and into the body. This means that for each of cigarette smoked, a person can absorb an additional 1-3 mcg of cadmium over what is taken in from other sources in their daily life. Smokers typically have twice as much as cadmium in their bodies as their nonsmoking counterparts. As a result smokers are highly in risk of liver and kidney damage and other cadmium poisoning diseases [43].

Table 8. Determination of cadmium in some surface soil

Serial no.	Cadmium (mg kg ⁻¹) ^a	RSD (%)	Sample Source ^b
S ₁ ^b	0.35 ± 0.1	1.2	Agriculture soil (Chittagong University Campus)
S ₂	0.21 ± 0.1	1.5	Marine soil (Bay of Bengal)
S ₃	1.5 ± 0.5	1.8	Traffic soil (Kadamtali Bus Terminal)
S ₄	1.8 ± 0.8	2.0	Industrial soil (Estern Cables)
S ₅	2.0 ± 0.6	2.2	Industrial soil (T.S.P. Complex, Chittagong)
S ₆	7.2 ± 1.0	2.5	Industrial soil (Bangladesh Steel Re-rolling Mills Ltd., Chittagong, Bangladesh)
S ₇	0.58 ± 0.3	2.1	Road-side soil (Dhaka-Chittagong Highway)

^aAverage of five analyses of each sample^bComposition of the soil samples: C, N, P, K, Na, Ca, Mg, Cu, Mo, Fe, Pb, Cd, V, Zn, Mn, Co, NO₃, SO₄ etc.**Table 9. Determination of cadmium in tobacco and cigarette**

Serial no.	Cadmium (µg g ⁻¹) or ng/Cigarette		Sample/ Brand Name
	Found (n = 5)	RSD(%)	
S ₁	2.95 ± 0.05	2.0	Whole tobacco µg g ⁻¹
S ₂	282 ± 1.2	1.5	Gold-leaf ng/cigarette (Main stream smoke)
S ₃	225 ± 1.5	1.8	Banson ng/cigarette(Main stream smoke)
S ₄	165 ± 1.6	2.5	Navy ng/ cigarette(Main stream smoke)

3.11.7 Determination of cadmium in fertilizer

1.0 g of finely grounded fertilizer sample was accurately weighed and dissolved in 5 mL of concentrated HNO₃ acid in a 100 mL micro-Kjeldahl flask by gently heating following a method recommended by Page et al. [42]. Then it was diluted with de-ionized water. The solution was filtered and neutralized by dilute NH₄OH solution in presence of 1-2 mL of 0.01% (w/v) EDTA solution. The content of the flask was filtered and transferred quantitatively into 25-mL flask. The resulting solution was then filtered and diluted up to the mark with de-ionized water.

A suitable aliquot (1-2 mL) of the final solution was pipette out into a 10-mL calibrated flask and the cadmium content was determined as described under procedure using EDTA or tartrate as masking agent. The cadmium content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results are

shown in Table 10. The highest value of cadmium was found in TSP (Triple super phosphate). From the study it is observed that high cadmium content is found in low quality in TSP fertilizer. For this reason Bangladesh rice has an average of 0.099 mg of cadmium per kg. It is more dangerous for Bangladesh as most of her people eat 400-500 grams of rice each day. Cadmium contamination in Bangladesh rice is massive due to the usage of low quality of imported TSP fertilizers [44].

Table 10. Determination of cadmium in some fertilizer samples

Serial no.	Cadmium / mg kg ⁻¹		Sample
	Found ^a (n = 5)	RSD(%)	
S ₁	2.52 ± 0.5	2.0	T.S.P (Triple super phosphate)
S ₂	1.88 ± 0.3	1.5	M.O.P (Muriate of Potash)

^aAverage of five replicate determinations ±s.

4. CONCLUSIONS

In the present work, a new, simple, sensitive, selective and inexpensive method with the Cd-morin complex was developed successfully for the determination of cadmium in some real, biological, soil, tobacco, fertilizer and environmental samples, for continuous monitoring to establish the trace levels of cadmium in difficult sample matrices. Although many sophisticated techniques such as pulse polarography, HPLC, NAA, AAS, ICP-OES and ICP-MS are available for the determination of cadmium at trace levels in numerous complex materials, factors such as the low cost of the instrument, easy handling, portable, lack of any requirement for consumables and almost no maintenances have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets. The sensitivity in terms of molar absorptivity ($\epsilon = 4.09 \times 10^6 \text{ L mol}^{-1} \text{ cm}^{-1}$) and precision in terms of relative standard deviation of the present method are very reliable for the determination of cadmium in real samples down to ng g^{-1} levels in aqueous medium at room temperature (25 ± 5)°C.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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