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# A Simple Spectrophotometric Determination of Trace Level Mercury Using 1,5-Diphenylthiocarbazone Solubilized in Micelle

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A very simple, ultra-sensitive and fairly selective non-extractive spectrophotmetric method is presented for the rapid determination of mercury(II) at ultra-trace level using 1,5-diphenylthiocarbazone (dithizone) as a new micellar spectrophotometric reagent ($\lambda_{max}$ = 490 nm) in a slightly acidic (0.07 – 0.17 M $H_2SO_4$) aqueous solution. The presence of a micellar system avoids the previous steps of solvent extraction and reduces the cost, toxicity while enhancing the sensitivity, selectivity and the molar absorptivity. The reaction is instantaneous and the absorbance remains stable for over 24 h. The average molar absorption coefficient and Sandell's sensitivity were found to be $5.02 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ and 10 ng cm$^{-2}$ of Hg, respectively. Linear calibration graphs were obtained for 0.05 – 10 mg L$^{-1}$ of Hg; the stoichiometric composition of the chelate is 1:2 (Hg:dithizone). The method is characterized by a detection limit of 1 μg L$^{-1}$ of Hg. Large excesses of over 60 cations, anions and complexing agents (*e.g.* EDTA, tartrate, oxalate, citrate, phosphate, thiourea, azide, SCN$^-$) do not interfere in the determination. The method was successfully applied to a number of environmental water samples (potable and polluted), biological samples (human blood and urine; milk and fish) and soils; solutions contained both mercury(I) and mercury(II) as well as complex synthetic mixtures. The method has high precision and accuracy ($s$ = ±0.01 for 0.1 mg L$^{-1}$).

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## Introduction

The analysis and monitoring of mercury in environmental, biological, industrial and food samples is extremely important because of the high toxicity of this metal both in its inorganic and organic compounds.[1] The symptoms of mercury (*e.g.* methyl mercury) poisoning include instantaneous neurological damage, particularly irritability, paralysis, insanity or blindness; chromosome breakage and birth defects; liver and brain damage.[2] One example of acute mercury poisoning is "Minamata disease" which causes mental disturbance; a loss of balance, speech, sight and hearing difficulty; in swallowing; and finally coma and death.[2] The toxicity of mercury depends on its chemical state.[2] Inorganic mercury has a very high affinity for protein sulfhydryl groups, which is hence accumulated in the kidneys, whereas organic mercury has a greater affinity for the brain.[2] The ability of living organisms to convert inorganic mercury to organic mercury compounds, which are more toxic and accumulate to a greater extent in living organisms, additionally increases the danger of mercury exposure, even at trace levels.[3] However, people who eat a lot of fish may consume much more; for instance, a level of 0.6 mg Hg kg$^{-1}$ fish could provide 0.15 mg of methyl mercury in one meal.[3] All these findings cause great concern regarding public health, demanding an accurate determination of this metal ion at trace and ultra-trace levels.

1,5-Diphenylthiocarbazone (dithizone) is one of the most widely used photometric reagents and forms colored water-insoluble complexes with a large number of metal ions.[4] Metal-dithizone complexes are water insoluble, and thus their determination requires a prior solvent extraction step into chloroform or carbon tetrachloride,[4,5] followed by spectrophotometric measurements. Since these methods involve solvent extraction, are lengthy and time-consuming and lack selectivity due to much interference.[6] Carbon tetrachloride and chloroform had been used as solvents for these extractions, which can be classified as toxic and as environmental pollutants.[2] They have been listed as carcinogens by the ATSDR[7] and EPA.[8] This problem has been overcome in recent years by introducing a hydrophobic micellar system generated by a surfactant similar to that employed in phase-transfer reactions.[9,10] Micellar systems are convenient to use because they are optically transparent, readily available, relatively non-toxic and stable.[11] Nevertheless, the addition of surfactants at concentrations above the CMC to an aqueous medium to form a miceller solution is the most commonly preferred procedure today. A non-ionic surfactant, like Triton X-100[12,19] and Tween-80,[13] have been used for the spectrophotometric determination of several metal ions. Similarly, a few anionic surfactants have been used.[14]

The aim of the present study is to develop a simpler direct spectrophotometric method for the trace determination of mercury with dithizone in the presence of inexpensive anionic micelles, such as sodium dodecyl sulfate, in aqueous solutions. This method does not require a solvent-extraction step; hence, the use of carcinogenic carbon tetrachloride or chloroform is avoided. The method described here has recorded for the first time the non-extractive direct spectrophotometric determination of mercury(II) in aqueous media without the recourse of any "clean-up" step. This method is far more selective, sensitive,

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non-extractive, simple and rapid than all of the existing spectrophotometric methods.[15-19] The method is very reliable, and a concentration in the ng $g^{-1}$ range in an aqueous medium at room temperature (25 ± 5°C) can be measured in a very simple and rapid way.

## Experimental

*Apparatus*

A Perkin Elmer (Germany) (Model: Lambda-2) double-beam UV/VIS spectrophotometer and a WTW Inolab (Germany) (Model: Level-1) pH-meter with a combination of electrodes were used for measurements of the absorbance and pH, respectively. A polarized Zeeman (Model-Z 5000) atomic-absorption spectrometer equipped with a mercury hollow-cathode lamp and mercury analyzer accessory (hydride vapor generator) was used for comparing the results. The experimental conditions were: slit width, 1.3 mm; lamp current, 6 mA; wavelength, 253.7 nm; time constant, 1 s; PMT voltage, 625 V.

A EG&G Princeton Applied Research (USA) (Model-174A) polarographic analyzer equipped with the differential pulse mode was also used for comparing the results. The experimental conditions were: sensitivity, 1 μA; amplitude mode, 5 mV; chart speed, 10 mV $s^{-1}$; initial potential, 1.0 V; potential charge, 3.0 V; low pass fitted, 1; cell assembly at, HDME; electrode (working), glassy carbon; purge time ($N_2$), 4 min; quiescent time, 30 s.

*Reagents and solutions*

All chemicals used were of analytical reagent grade or the highest purity available. Doubly distilled deionized water, which is non-absorbent under ultraviolet radiation, was used throughout. Glass vassals were cleaned by soaking in acidified solutions of $KMnO_4$ or $K_2Cr_2O_7$, followed by washing with concentrated $HNO_3$ and rinsed several times with deionized water. Stock solutions and environmental water samples (1000 mL each) were kept in polypropylene bottles containing 1 mL of concentrated nitric acid. Human fluids were collected in polyethane bottles from affected persons. Immediately after collection, they were stored in a salt-ice mixture and later, at the laboratory, were kept at –20°C.[20] More rigorous contamination control was used when one mercury levels in the specimens were low.

*Sodium dodecyl sulfate (SDS) solution 0.6 M*. A 500 mL of SDS solution was prepared by dissolving 86.4 g of pure sodium dodecyl sulfate (Merck Darmstadt, Germany) in 250 – 300 mL if doubly distilled deionized water, sonicated for 15 min and diluted with deionized water when it became transparent.

*1,5-Diphenylthiocarbazone (dithizone) 1.95 × $10^{-4}$ M*. Prepared by dissolving the requisite amount (0.005%) of diphenylthiocarbazone (Merck, Darmstadt) in a known volume of isoamylalcohol (Merck-Schuchardt). More dilute solutions of the reagent were prepared as required.

*Mercury(II) standard solutions (4.99 × $10^{-3}$ M)*. A 100 ml stock solution (1 mg $mL^{-1}$) of divalent mercury was prepared by dissolving 135 mg of mercuric chloride (Merck, Darmstadt) in deionized water containing 1 – 2 mL of nitric acid (1+1). Aliquots of this solution were standardized with EDTA using Xylenol Orange as an indicator. More dilute standard solutions were prepared from this stock solution, as and when required.

*Mercury(I) stock solutions (4.23 × $10^{-3}$ M)*. A 100 ml of mercury(I) stock solution (1 mg $mL^{-1}$) was prepared by dissolving 117.68 mg of purified-grade mercury(I) chloride (Merck, Darmstadt) in deionized water. The working standard of mercury(I) was prepared by appropriate dilution of this solution

*Potassium permanganate solution*. A 1% potassium permanganate solution (Merck) was prepared by dissolving the requisite amount in deionized water. A sodium azide solution (2.5% w/v) (Merck) was also used.

*Tartrate solution*. A 100 ml stock solution of tartrate (0.1% w/v) was prepared by dissolving 190.6 mg of potassium sodium tartrate tetrahydrate (Merck, Darmstadt) in (100 mL) deionized water.

*Aqueous ammonia solution*. A 100 mL solution of aqueous ammonia was prepared by diluting 10 mL of concentrated $NH_3$ (28 – 30%) ACS grade to 100 mL with deionized water. The solution was stored in a polypropylene bottle.

*EDTA solution*. A 100 mL stock solution of EDTA (0.1% w/v) was prepared by dissolving 128 mg of ethylenediaminetetraacetic acid, disodium salt dehydrate (Merck, Darmstadt) in (100 mL) deionized water.

*Other solutions*. Solutions of a large number of inorganic ions and complexing agents were prepared from their AnalaR grade, or equivalent grade, water-soluble salts. In the case of insoluble substances, a special dissolution method was adopted.[21]

*Procedure*

To 0.1 – 1 mL of a slightly acidic solution containing 0.5 – 100 μg of mercury(II) in a 10 mL calibrated flask was mixed 5 – 8 mL (preferably 5 mL) of 0.6 M SDS and 0.7 – 1.7 (preferably 1 mL) of 1 M $H_2SO_4$, followed by the addition of a 20 – 100 fold molar excess of a dithizone solution (preferably 1 mL of 1.95 × $10^{-4}$ M). The mixture was diluted to the mark with deionized water. The absorbance was measured at 490 nm against a corresponding reagent blank. The mercury content in an unknown sample was determined using a concurrently prepared calibration graph.

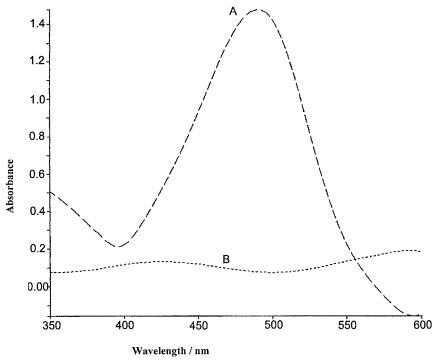


Fig. 1 A and B absorption spectra of Hg(II)-dithizone system and reagent blank ($\lambda_{max}$ = 490 nm) in anionic micellar media of sodium dodecyl sulfate.

## Results and Discussion

*Factors affecting the absorbance*

*Absorption spectra*. The absorption spectra of the mercury(II)-dithizone system in a 1 M sulfuric acid medium were recorded using a spectrophotometer. The absorption spectra of the mercury(II)-dithizone is a symmetric curve with the maximum absorbance at 490 nm and an average molar absorption coefficient of 5.02 × $10^4$ L $mol^{-1}$ $cm^{-1}$ (Fig. 1). The reagent

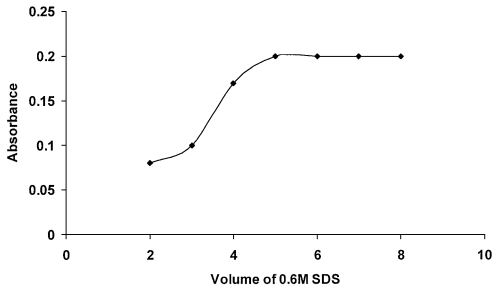


Fig. 2 Effect of a surfactant on the aborbance of the Hg(II)-dithizone system.

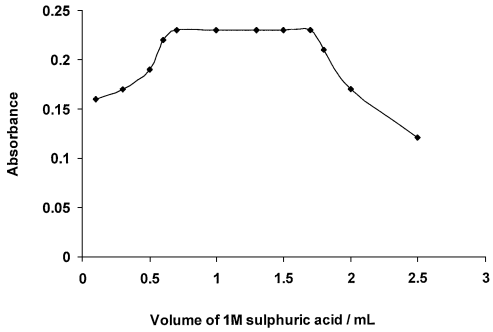


Fig. 3 Effect of the acidity on the absorbance of the Hg(II)-dithizone system.

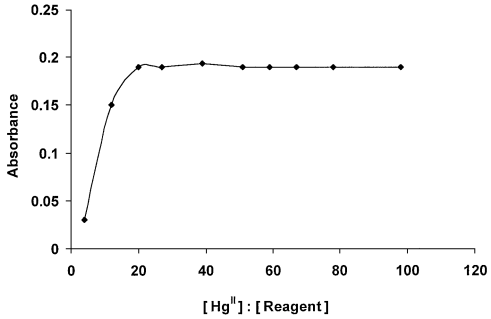


Fig. 4 Effect of a reagent [dithizone:Hg$^{II}$ molar concentration ratio] on the absorbance of the Hg(II)-dithizone system.

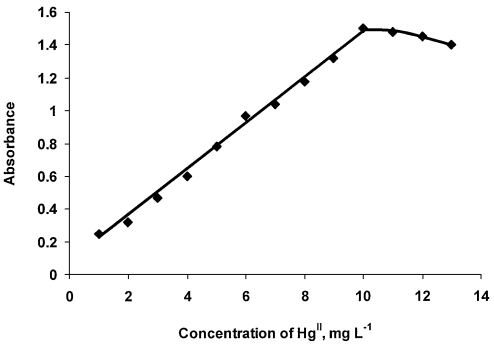


Fig. 5 Calibration graph: *C*, 1 – 10 mg L$^{-1}$ of Hg$^{II}$.

blank exhibited negligible absorbance, despite having a wavelength in the same region. In all instances, measurements were made at 490 nm against a reagent blank. The reaction mechanism of the present method is as reported earlier.[22]

*Effect of surfactant*. Of the various surfactants [nonionic {polyoxyethylenedodecylether (Brij-35), polyoxyethylene sorbitan monopalmitate (Tween-40), polyoxyethylene sorbitan mono-oleate (Tween-80), Triton X-100}; cationic {cetyltrimethylammonium bromide (CTAB)}; and anionic {cetylpyridinum chloride (CPC), sodium dodecyl sulfate (SDS)}] studied, SDS was found to be the best surfactant for the system. In a 0.6 M SDS medium, however, the maximum absorbance was observed; hence, a 0.6 M SDS solution was used in the determination procedure.

Different volumes of 0.6 M SDS were added to a fixed metal ion concentration, and the absorbance was measured according to the standard procedure. It was observed that at 1 mg L$^{-1}$ Hg-chelate metal, 5 – 8 mL of 0.6 M SDS produced a constant absorbance of the Hg-chelate (Fig. 2). A greater excess of SDS were not studied. For all subsequent measurements, 5 mL of 0.6 M SDS was added.

*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 a 10 mL of solution (1 mg L$^{-1}$; path length, 1) contained 0.7 – 1.7 mL of 1 M sulfuric acid (or pH 0.8 – 1.2) at room temperature (25 ± 5°C). Outside this range of acidity, the absorbance decreased (Fig. 3). For all subsequent measurements, 1 mL of 1 M sulfuric acid (or pH 1) was added.

*Effect of time*. The reaction is very fast. Constant maximum absorbance was obtained just after dilution to volume, and remained strictly unaltered for 24 h.

*Effect of temperature*. The absorbance at different temperatures, 0 – 70°C, of a 10 mL solution (1 mg L$^{-1}$) was measured according to the standard procedure. The absorbance was found to be strictly unaltered throughout the temperature range of 5 – 60°C. Therefore, all measurements were performed at room temperature (25 ± 5°C).

*Effect of the reagent concentration*. Different molar excesses of dithizone were added to a fixed metal-ion concentration, and the absorbances were measured according to the standard procedure. It was observed that at 1 mg mL$^{-1}$ Hg metal (optical path length, 1 cm), reagent molar ratios 1:20 and 1:100 produced a constant absorbance of the Hg-chelate (Fig. 4). A greater excess of the reagent was not studied. For all subsequent measurements, 1 ml of $1.95 \times 10^{-4}$ M dithizone reagent was added.

*Calibration graph (Beer's law and sensitivity)*. The effect of metal concentration was studied over 0.01 – 10 mg L$^{-1}$, distributed in three different sets (0.01 – 0.1, 0.1 – 1, 1 – 10 mg L$^{-1}$) for convenience of the measurement. The absorbance was linear for 0.05 – 10 mg L$^{-1}$ of mercury at 490 nm. From the slope of the calibration graph, the average molar absorption coefficient was found to be $5.02 \times 10^4$ L mol$^{-1}$ cm$^{-1}$. The Sandell's sensitivity[23] (concentration for 0.001 absorbance unit) was found to be 10 ng cm$^{-2}$. Of the three calibration graphs, the one showing the limit of the linearity range is given in Fig. 5; the next two were straight-line graphs passing through the origin ($R^2 = 0.99$). The selected analytical parameters obtained with the optimization experiments are summarized in Table 1.

*Effect of foreign ions*. The effect of over 60 cations, anions and complexing agents on the determination of only 1 mg L$^{-1}$ of Hg$^{II}$ was studied. The criterion for interference[24] was an absorbance value varying by more than 5% from the expected value for Hg$^{II}$ alone. There was no interference from the following 1000 fold amount of EDTA or tartrate; a 500-fold

Table 1 Selected analytical parameters obtained by optimization experiments

| Parameter | Selected value |
|---|---|
| Wavelength, $\lambda$/nm | 490 |
| Acidity/M $H_2SO_4$ | 0.07 – 0.17 (preferably 0.1) |
| pH | 0.8 – 1.2 (preferably 1) |
| Surfactant/M sodium dodecyl sulfate (SDS) | 0.3 |
| Time/h | 24 |
| Temperature/°C | 5 – 60 (preferably 25 ± 5) |
| Reagent (fold molar excess, M:R) | 1:20 – 1:100 (preferably 1:50) |
| Linear range/mg $L^{-1}$ | 0.05 – 10 |
| Molar extinction coefficient/L $mol^{-1}$ $cm^{-1}$ | $5.02 \times 10^4$ |
| Detection limit/μg $L^{-1}$ | 1 |
| Reproducibility, %RSD | 0 – 2 |

amount of acetate, chloride, oxalate or ammonium(I). EDTA prevented the interference of 50-fold amounts of cerium(III and IV) or chromium(VI). During interference studies, if a precipitate was formed, it was removed by centrifugation. The amount mentioned is not the tolerance limit, but the actual amount studied. However, for those ions whose tolerance limit has been studied, their tolerance ratios are mentioned in Table 2.

*Composition of the absorbance*

Job's method[25] of continuous variation and the molar-ratio[26] method were applied to ascertain the stoichiometric composition of the complex. A Hg-dithizone (1:2) complex was indicated by both methods.

*Precision and accuracy*

The precision of the present method was evaluated by determining different concentrations of mercury (each analyzed at least five times). The relative standard deviation ($n$ = 5) was 2 – 0% for 0.5 – 100 μg of $Hg^{II}$ in 10.0 mL, indicating that this method is highly precise and reproducible. The detection limit[27] (3 s of the blank) and Sandell's sensitivity[23] (concentration for 0.001 absorbance unit) for mercury(II) were found to be 1 μg $L^{-1}$ and 10.0 ng $cm^{-2}$, respectively. The results of the total mercury in a number of real samples were in good agreement with the expected values. The reliability of our Hg-chelate procedure was tested by recovery studies. The average percentage recovery obtained for the addition of a mercury(II) spike to some environmental water samples was quantitative, as shown in Table 3. The method was also tested by analyzing several synthetic mixtures containing mercury(II) and diverse ions. The results of biological (human fluid) analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS (Table 4). The results of milk analyses by the spectrophotometric method were also in excellent agreement with those obtained by DPASV (differential pulse anodic stripping voltammetry), which confirmed the validity of the micellar spectrophotometric method. The results for the speciation of mercury(I) and mercury(II) in mixtures were highly reproducible. Hence, the precision and accuracy of the method were excellent.

*Applications*

The present method was successfully applied to the determination of mercury in a series of synthetic mixtures of various compositions, and also in a number of real samples. The results for the speciation of mercury(I) and mercury(II) were highly reproducible. The method was also extended to the determination of mercury in a number of environmental, biological and soil samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each sample were analyzed for mercury 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 3). The results of biological analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS (Table 4). The results of soil-samples analyses by the spectrophotometric method were found to be highly reproducible.

*Determination of mercury in synthetic mixtures.* Several synthetic mixtures of varying compositions containing mercury(II) and diverse ions of known concentrations were determined by the present method using EDTA or tartrate as a masking agent; and the results were found to be highly reproducible. Accurate recoveries were achieved in all solutions.

*Determination of mercury(I) and mercury(II) speciation in mixtures.* Suitable aliquots (1 – 2 mL) of mercury(I+II) mixtures (preferably 1:1, 1:5, 1:10) were taken in a 25-mL conical flask. A few drops of 1 M sulfuric acid and 1 – 2 mL of 1% (w/v) potassium permanganate solution were added to oxidize the mono-valent mercury. Then, a 5-mL volume of

Table 2 Tolerance limits of foreign ions[a], tolerance ratio, [species (x)]/$Hg^{II}$ (w/w)

| Species x | Tolerance ratio x/$Hg^{II}$ | Species x | Tolerance ratio x/$Hg^{II}$ |
|---|---|---|---|
| Ascorbic acid | 200 | Cobalt(II & III) | 100 |
| Azide | 200 | Calcium | 100 |
| Acetate | 500 | Cerium(III & IV) | 50[a] |
| Bromide | 200 | Copper(II) | 100 |
| Citrate | 200 | Cesium | 100 |
| Chloride | 500 | Gallium | 100 |
| Carbonate | 200 | Gold | 50 |
| EDTA | 1000 | Indium(III) | 100 |
| Iodide | 200 | Iron(II) | 50 |
| Nitrate | 200 | Iron(III) | 100 |
| Oxalate | 500 | Lead(II) | 50 |
| Phosphate | 200 | Manganese(II) | 100 |
| Persulfate | 200 | Manganese(VII) | 50 |
| Sulfite | 200 | Mercury(I) | 50 |
| Sulfate | 200 | Molybdenum(VI) | 100 |
| Tartrate | 1000 | Magnesium | 100 |
| Thiocyanide | 200 | Nickel(II) | 100 |
| Ammonium(I) | 500 | Potassium | 100 |
| Antimony(III) | 100 | Palladium(II) | 75 |
| Aluminum | 100 | Selenium(IV) | 100 |
| Arsenic(III) | 100 | Silver(I) | 50 |
| Arsenic(V) | 100 | Sodium | 200 |
| Beryllium(II) | 100 | Strontium | 100 |
| Barium | 100 | Thallium(I) | 100 |
| Bismuth(III) | 100 | Thorium | 100 |
| Cadmium | 100 | Tungsten(VI) | 100 |
| Chromium(III) | 100 | Vanadium(V) | 100 |
| Chromium(VI) | 50[b] | Zinc | 100 |

a. Tolerance limit defined as ratio that causes less than 5% interference.
b. With 10 μg $mL^{-1}$ EDTA.

Table 3 Determination of mercury in some environmental water samples

| Sample | Mercury/μg $L^{-1}$ | | Recovery ± $s$,[a] % | $s_r$, %[b] |
|---|---|---|---|---|
| | Added | Found | | |
| Tap water | 0 | 1.6 | | |
| | 100 | 101.5 | 99.9 ± 0.2 | 0.21 |
| | 500 | 502.0 | 100 ± 0.1 | 0.25 |
| Well water | 0 | 2.5 | | |
| | 100 | 102.0 | 99.5 ± 0.4 | 0.29 |
| | 500 | 504.0 | 100.3 ± 0.2 | 0.15 |
| Lake water[c] | 0 | 131.0 | | |
| | 100 | 228.0 | 98.7 ± 0.3 | 0.24 |
| | 500 | 635.0 | 100.6 ± 0.5 | 0.27 |
| River water | | | | |
| Indus (upper stream) | 0 | 4.0 | | |
| | 100 | 103.0 | 99 ± 0.2 | 0.13 |
| | 500 | 504.0 | 100 ± 0.0 | 0.00 |
| Indus (lower stream) | 0 | 5.6 | | |
| | 100 | 106.0 | 100.4 ± 0.3 | 0.16 |
| | 500 | 504.0 | 99.6 ± 0.1 | 0.10 |
| Sea water | | | | |
| Arabian sea (upper) | 0 | 3.4 | | |
| | 100 | 104.0 | 100.5 ± 0.6 | 0.08 |
| | 500 | 505.0 | 100.3 ± 0.4 | 0.10 |
| Arabian sea (lower) | 0 | 4.5 | | |
| | 100 | 104.0 | 99.5 ± 0.5 | 0.04 |
| | 500 | 504.5 | 100 ± 0.0 | 0.00 |
| Drain water | | | | |
| MNV drain[d] | 0 | 77.0 | | |
| | 100 | 175.0 | 99 ± 0.5 | 0.16 |
| | 500 | 580.0 | 100.5 ± 0.6 | 0.23 |
| Pulp industry[e] | 0 | 193.0 | | |
| | 100 | 295.0 | 100.7 ± 0.5 | 0.29 |
| | 500 | 686.0 | 99 ± 0.8 | 0.48 |

a. Average of five replicate determinations.
b. The measure precision is the relative standard derivation ($s_r$).
c. The Manchar Lake, Hyderabad, Sindh.
d. MNV drain, Dadu District, Sindh.
e. Oriented Pulp Industry, Karachi.

Table 4 Determination results for human fluids

| | Sample | Mercury/μg $L^{-1}$ | | | | Sample source[a] |
|---|---|---|---|---|---|---|
| | | Proposed method ($n$ = 5) | | AAS ($n$ = 5) | | |
| | | Found | RSD, % | Found | RSD, % | |
| 1 | Blood | 94.85 | 1.5 | 91.75 | 2.0 | Kidney damage patient (M) |
| 2 | Blood | 232.14 | 1.2 | 234.37 | 1.8 | Paralysis patient (M) |
| 3 | Urine | 54.33 | 1.8 | 52.65 | 2.3 | Brain damage patient (M) |
| 4 | Blood | 9.70 | 1.3 | 7.80 | 1.5 | Normal adult |
| | Urine | 3.33 | 1.2 | ND[b] | — | (M) |

a. Samples were from LUMHS Hospital, Hyderabad.
b. Not detectable.

water was added to the mixture, which was then heated on a steam bath for 10 – 15 min, with occasional gentle shaking, and then cooled to room temperature. Then, 3 – 4 drops of a freshly prepared sodium azide solution (2.5% w/v) were added and heated gently with the further addition of 2 – 3 mL of water, if necessary, for 5 min to drive off the azide cooled to room temperature. The reaction mixtures were transferred quantitatively into a 10 mL volumetric flask, 5 mL of 0.6 M SDS was added, followed by the addition 1 mL of 1 M $H_2SO_4$ and 1 mL of $1.95 \times 10^{-4}$ M dithizone reagent solution. It was made up to the mark with deionized water. The absorbance was measured at 490 nm against a reagent blank. The total mercury content was calculated with the help of a calibration graph.

An equal aliquot of the above mercury(I+II) mixture was taken into a 10-mL volumetric flask; then, 5 mL of 0.6 M SDS was added, followed by the addition of 1 mL of 1 M $H_2SO_4$ and 1 mL of $1.95 \times 10^{-4}$ M reagent, and made up to the volume with deionized water. The absorbance was measured against a reagent blank, as before. The mercury concentration was calculated in mg $L^{-1}$ or μg $L^{-1}$ with the aid of a calibration graph. This gave a measure of the mercury(II) originally present in the mixture. The value was subtracted from that of the total mercury to determine the mercury(I) present in the mixture. The results were found to be highly reproducible. The mean errors for Hg(II) and Hg(I) were found to be ±0.01 and ±0.016, respectively, and corresponding standard deviations for Hg(II) and Hg(I) were found ±0.004 and ±0.007, respectively. The occurrence of such reproducible results is also reported for different oxidation states of mercury.[16]

*Determination of mercury in environmental waters*. Each filtered (with Whatman No. 40) environmental water sample (250 ml) was mixed with 10 ml of concentrated nitric acid in a 500-ml distillation flask. The sample was digested in the presence of an excess potassium permanganate solution according to the method recommended by Fifield *et al*.[28] The solution was cooled and neutralized with a dilute $NH_4OH$ solution. The digest was transferred into a 25-ml calibrated flask and diluted up to the mark with deionized water.

An aliquot (1 – 2 mL) of this solution was pipetted into a 10-mL calibrated flask, and the mercury content was determined as described under a procedure using EDTA as a masking agent. The analysis of environmental water samples from various sources for mercury and the results are given in Table 3.

Most spectrophotometric methods for the determination of mercury in natural water and seawater require the preconcentration of mercury.[17] The concentration of mercury in natural water and seawater is a few μg $L^{-1}$ in Australia.[18] The mean concentration of mercury found in US drinking water is less than 2 μg $L^{-1}$.[28]

*Determination of mercury in biological samples*. Human blood (5 – 10 mL), urine (10 – 20 mL), milk (10 – 20 mL) or 10 – 20 g of fish sample was taken in a 100 mL micro-Kjeldahl flask with a B24 socket attached to a standard double surface reflux condenser. The sample was digested in the presence of an excess potassium permanganate solution according to a method recommended by the Analytical Methods Committee.[29] The digest was filtered (if necessary) and neutralized with dilute ammonia in the presence of a 1 – 2 ml 0.01% (w/v) EDTA solution. The solution was transferred quantitatively into a 25-mL calibrated flask and made up to the mark with deionized water.

A suitable aliquot (1 – 2 mL) of the final solution was pipetted out into a 10-mL calibrated flask, and the mercury content was determined as described under *Procedure* using tartrate as a masking agent. The results of biological (human fluids) analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS (cold vapor technique). The results are given in Table 4. The results of a milk analysis by the spectrophotometric method were also

found to be excellent agreement with those obtained by DPASV.

The abnormally high values for paralysis and kidney damage patients are probably due to the involvement of high mercury concentrations in fish. The occurrence of such high mercury contents are also reported concerning paralysis and kidney-damage patients from some developed countries.[2]

*Determination of mercury in soil samples*. The method was applied to the determination of micro-quantities of mercury in various types of soils. An air-dried homogenized soil sample (10 – 20 g) was accurately weighed and placed in a 100 mL micro-Kjeldahl flask equipped with a reflux condenser. The sample was digested following a method recommended by Kumburova.[30] The content of the flask was filtered and neutralized with dilute $NH_4OH$ in the presence of 1 – 2 mL of a 0.01% (w/v) EDTA solution, transferred quantitatively into a 25-mL calibrated flask and made up to the mark with deionized water. Suitable aliquots (1 – 2 mL) were transferred into a 10-mL calibrated flask and the mercury content was determined, as described under *Procedure*, using tartrate as a masking agent. The average value of the total mercury in five different surface soil samples was found to be 0.29 mg $kg^{-1}$.

## Conclusions

In the present work, a simple, sensitive, selective and inexpensive micellar method with the Hg(II)-dithizone complex was develop for the determination of mercury in industrial, environmental, biological, pharmaceutical, food and soil samples, for continuous monitoring to establish trace levels of mercury in difficult sample matrices. The presence of a micellar system (altered environment) avoids the previous steps of solvent extraction, and reduces the cost and toxicity while enhancing the sensitivity, selectivity and molar absorptivity. It also offers a very efficient procedure for speciation analysis. Although many sophisticated techniques, such as pulse polarography, HPLC, NAA, AAS and ICP-MS, are available for the determination of mercury 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 maintenance, have caused spectrophotometry to remain a popular technique, particularly in the laboratories of developing countries with limited budgets. The sensitivity in terms of molar the absorptivity ($\varepsilon = 5.02 \times 10^4$ L $mol^{-1}$ $cm^{-1}$) and precision in terms of the relative standard deviation of the present method are very reliable for the determination mercury in real samples down to (g $kg^{-1}$ levels in an aqueous medium at room temperature (25 ± 5°C).

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### References

1. ATSDR (Agency for Toxic Substance and Disease Registry), "*Toxicology Profile for Mercury*", **1999**, Public Health Service, Atlanta, GA.
2. "*Casarette and Doull's Toxicology*", ed. C. D. Klaassen, **2001**, McGraw-Hill, New York, 834.
3. National Health and Medical Research Council, Australian Drinking Water Guidelines, **1996**, http://www.health.Gov.au/nhmrc/publications/pdf/eh19.pdh.
4. J. Fries and H. Getrost, "*Organic Reagents for Trace Analysis*", **1977**, Merck Darmstadt, 243.
5. L. Hageman, L. Torma, and B. E. Ginther, *J. Assoc. Off. Anal. Chem*., **1975**, *58*, 990.
6. B. Kumar, H. B. Singh, M. Katyal, and R. L. Sharma, *Mikrochim. Acta* [Wien], **1991**, *111*, 79.
7. ATSDR (Agency for Toxic Substances and Disease Registry), "*Toxicology Profile for Carbon Tetrachloride*", **1994**, Public Health Service, Atlanta, GA.
8. EPA (U.S. Environmental Protection Agency), "*Proposed Guidelines for Carcinogen Risk Assessment*", **1999**, Office of Research and Development, NCEA-F-0644.
9. X. Jin, M. Zhu, and E. D. Conte, *Anal. Chem*., **1999**, *71*, 514.
10. H. Tani, T. Kamidate, and H. Watanabe, *Anal. Sci*., **1998**, *14*, 875.
11. M. E. D. Garcia and A. S. Medel, *Talanta*, **1986**, *33*, 255.
12. M. C. G. Alvarez-Coque, R. M. V. Camanas, M. C. M. Vaya, G. Ramos, and C. M. Fernandez, *Talanta*, **1986**, *33(8)*, 697.
13. J. Yun and H. Choi, *Talanta*, **2000**, *52*, 893.
14. G. A. Shar and M. I. Bhanger, *J. Chem. Soc. Pak*., **2001**, *23(2)*, 74.
15. P. Padmaja, N. Balasurbramanian, and T. V. Ramakrishna, *Talanta*, **1994**, *41(2)*, 255.
16. M. J. Ahmed and M. S. Alam, *Spectroscopy*, **2003**, *17*, 45.
17. V. P. Dedkova, O. P Shoeva, and S. B. Savvin, *J. Anal. Chem*., **2004**, *59(4)*, 381.
18. N. Amini, I. J. Cardwell, R. W. Cattrall, R. J. S. Morrison, and S. D. Kolev, *Talanta*, **2004**, *63*, 1069.
19. K. Ueno, K. Shiraishi, T. Togo, T. Yano, I. Yoshida, and H. Kobayashi, *Anal. Chim. Acta*, **1979**, *105*, 289.
20. M. J. Ahmed and M.-A. Momun, *Talanta*, **2001**, *55*, 43.
21. B. K. Pal and B. Chowdhury, *Mikrochim. Acta*, **1984**, *11*, 121.
22. N. S. Murcia, E. G. Lundquist, R. O. Russo, and D. G. Peters, *J. Chem. Ed*., **1990**, *67*, 7.
23. E. B. Sandell, "*Colorimetric Determination of Traces of Metals*", **1965**, Interscience, New York, 269.
24. C. B. Ojeda, A. G. de Torres, F. S. Rojas, and J. M. C. Pavon, *Analyst*, **1987**, *112*, 1499.
25. P. Job, *Ann. Chim*. [Paris], **1928**, *9*, 113.
26. J. A. Yoe and A. L. Jones, *Ind. Eng. Chem. Anal. Ed*., **1944**, *16*, 11.
27. S. Mitra (ed.), "*Sample Preparation Techniques in Analytical Chemistry*", **2003**, Wiley-Interscience, New Jersey, 14.
28. F. W. Fifield and P. J. Haines (ed.), "*Environmental Analytical Chemistry*", **2000**, Blackwell Science, 378.
29. Analytical Method Committee, *Analyst*, **1977**, *102*, 769.
30. M. Kamburova, *Talanta*, **1993**, *40(5)*, 719.