

Synthesis of meso-tetra-(3,5-dibromo-4-hydroxyphenyl)-porphyrin and its application to second-derivative spectrophotometric determination of lead in clinical samples

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A new very sensitive and selective chromogenic reagent, meso-tetra-(3,5-dibromo-4-hydroxyphenyl)porphyrin [T(DBHP)P], was synthesized and studied for the determination of trace lead in detail. In 0.10 mol l⁻¹ NaOH medium, lead reacts with T(DBHP)P to form a 1:2 yellow complex, which gives a maximum absorption at 479 nm; 0–0.48 µg ml⁻¹ Pb(II) obeyed Beer's law. The molar absorptivity of the complex and Sandell's sensitivity are 2.5 × 10⁵ l mol⁻¹ cm⁻¹ and 0.000812 µg cm⁻², respectively. Second-derivative spectrophotometry is better than conventional spectrophotometry in sensitivity and selectivity, and its limit of quantification, limit of detection and relative standard deviation are 0.70 ng ml⁻¹, 0.21 ng ml⁻¹ and 1.0%, respectively. Ca (3250-fold), Mg (2000-fold), Sr (1000-fold), Ba (750-fold), Al (1000-fold), Bi (500-fold), Fe (2000-fold), Co (750-fold), Ni (1000-fold), Cu (750-fold), Zn (1250-fold), Cd (2500-fold) and Ag (550-fold) do not interfere with the determination of lead. The chromogenic system is remarkably superior to other reagents, especially porphyrin compounds. The influence caused by oxygen in air or in solution can be easily eliminated by adding Na₂SO₃. The reaction is very stable, the stability constant of the complex being 1.2 × 10⁴⁵. The chromogenic reaction is completed within 1 min at room temperature when 8-hydroxyquinoline is used as catalyst. The proposed method has been applied to the direct determination of trace lead in clinical samples. The accuracy and precision are both very satisfactory.

Lead and its compounds are hazardous. Lead poisoning is cumulative and its toxic effects are many and severe.¹ Because of environmental pollution (especially in developing countries), clinical lead analysis is becoming increasingly important. Analysis of clinical samples must be reliable and as simple and quick as possible. Flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS) and flow injection (FI)^{2–4} are used for the analysis of lead. However, FAAS is reliable down to about 0.2 mg l⁻¹ and so is not always suitable for the analysis of lead in clinical samples. ETAAS and FI are generally used to determine lead at low concentrations, but they are complex to use; moreover, some type of preconcentration or extraction step must be used to

meet the requirement of accuracy and precision. Spectrophotometric methods are versatile and economical. A large number of spectrophotometric methods for the analysis of lead are available as shown in Table 1. Dithiazone,⁵ diethyldithiocarbamate,^{6,7} arsenazo III,⁸ 4-(2-pyridylazo)-resorcinol (PAR),⁹ arsenazo-TB,⁷ malachite green,¹⁰ 1-(2-thiazolylazo)-2-naphthol,¹¹ pyridine-2-acetaldehyde salicyloylhydrozone,¹² butylrhodamine-B,¹³ monothioethenyltrifluoroacetone,¹⁴ diphenylcarbazone,¹⁵ xylenol orange¹⁶ and 2-(2-thiazolylazo)-*p*-cresol¹⁷ are not suitable for the trace analysis of lead in clinical samples due to their low sensitivity. Dibromo-*p*-methylsulfonazo¹⁸ is sensitive, but Ca(II) and Mg(II), which exist in clinical samples in reasonable amounts, interfere with the determination of lead

Table 1 Review of the spectrophotometric methods for the determination of lead(II)

Reagent	Phase	λ_{max} /nm	Remarks	Reference
Dithiazone	CCl ₄	520	Light sensitive, Hg, Cd, Zn, Cu and Fe interfere	5
Diethyldithiocarbamate	CCl ₄	435	Light sensitive	7
Arsenazo III	aqueous pH 2.5–4.5	660	Light sensitive Fe, Al, Cu, Th, Ti and U interfere	8
4-(2-Pyridylazo)-resorcinol	Aqueous	520	Ag, Cd, Co, Cu, Ni and Zn seriously interfere	9
Arsenazo-TB	Aqueous	620	Light sensitive, Th and Ti seriously interfere	7
Malachite green	Toluene	642	Ca, Hg, Sb, Bi, Co, Sn and Ti interfere	10
1-(2-Thiazolylazo)-2-naphthol	Aqueous	575	Light sensitive, Mn, Fe, Co, Zn and EDTA interfere	11
Pyridine-2-acetaldehyde salicyloylhydrozone	CHCl ₃	380	Salting out agent used	12
Butylrhodamine-B	C ₆ H ₆	570	Hg, Bi, Ti and Ag interfere	13
Monothioethenyltrifluoroacetone	C ₆ H ₆	384	Equilibration time 30 min, Ni, Ca, Co, Cu, Zn and Pd interfere	14
Diphenylcarbazone	Aqueous pH 9.0–10.4	525		15
2-(2-Thiazolylazo)- <i>p</i> -cresol	Aqueous, pH 9.0–10.0	650	Light sensitive, Cu, Ni, Co, Zn, Fe and Cd interfere	17
Xylenol orange	pH 4.5–5.5	580	Light sensitive, Al, Bi, Hg, Ni, RE and Zn interfere	16
Dibromo- <i>p</i> -methylsulfonazo	H ₃ PO ₄	642	Potassium iodide–methylisobutylketone extraction, Ca, Ba, Sr and RE seriously interfere	18

EDTA, ethylenediaminetetraacetic acid; RE, rare earth.

and require prior separation with potassium iodide–methylisobutylketone extraction.

Porphyrin compounds^{19–25} have attracted much attention as analytical reagents owing to their very high sensitivity. However, certain problems seriously affect their application, such as water solubility, stability, selectivity (especially transition metal ions interfere with the determination of lead) and time of reaction. In order to continue the search for new sensitive and selective chromogenic reagents and to study the influence of substituent groups on the analytical characteristics of the reagents, we have attempted to improve the water solubility of porphyrin compounds by introducing an OH into the 4-position of the phenyl of the porphyrin ring, and the selectivity of the chromogenic reagent by introducing two assisting functional groups, Br, into the 3- and 5-positions of the phenyl of the porphyrin ring. The new compound, meso-tetra-(3,5-dibromo-4-hydroxyphenyl)porphyrin [T(DBHP)P] (Fig. 1) was successfully synthesized in our laboratory. The results confirm that the design is successful. In NaOH medium, lead reacts with the reagent to form a stable 1 : 2 yellow complex with an absorption maximum at 479 nm. Its molar absorptivity is $2.5 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$. Beer's law is obeyed over 0–0.48 $\mu\text{g ml}^{-1}$ lead. The influence caused by oxygen in air or in solution is completely eliminated by adding Na_2SO_3 . In the presence of 8-hydroxyquinoline, the chromogenic reaction is greatly accelerated and completed within 1 min. Second-derivative spectrophotometry is better than conventional spectrophotometry in sensitivity and selectivity; its limit of quantification, the limit of detection and relative standard deviation were 0.70 ng ml^{-1} , 0.21 ng ml^{-1} and 1.0%, respectively. All foreign ions studied in reasonable amounts do not interfere with the determination of trace lead. The selectivity is superior to that obtained previously,^{5–18} especially for other porphyrin compounds (see Table 2). The proposed method has been applied to the direct determination of lead in clinical samples with good precision and accuracy. The method is one of the most sensitive

and efficient techniques for the determination of trace lead at the present time.

Experimental

Apparatus

The absorption spectra and second-derivative spectra were recorded and measured on a Beckman DU-7HS (Beckman Instruments, CA, USA) spectrophotometer with a 3 cm cell.

Reagents

Unless otherwise stated, all reagents used were of analytical grade and all solutions were prepared with distilled water.

Standard lead solution. A stock solution of lead(II) was prepared by dissolving 2.0 g of lead nitrate in 250 ml of distilled water containing 0.5 ml of concentrated nitric acid. The solution was standardized by complexometry using ethylenediamine-tetraacetic acid (EDTA), and a working solution of 10 $\mu\text{g Pb(II) ml}^{-1}$ was prepared by suitable dilutions.

T(DBHP)P solution. 0.02% T(DBHP)P solution was prepared by dissolving 0.05 g of T(DBHP)P in 250 ml of *N,N*-dimethylformamide (DMF).

8-hydroxyquinoline (HQ). 2% HQ solution was prepared by dissolving 10 g of HQ in 500 ml of 95% alcohol.

Triton X-100 solution. 2% (w/v) aqueous solution.

General procedure

A solution containing no more than 4 μg of lead(II) was transferred into a 10 ml calibrated flask; 0.5 ml of 2 mol l^{-1} NaOH, 1.0 ml of 2% HQ solution, 0.50 ml of 2% Na_2SO_3 and 0.5 ml of 0.02% T(DBHP)P were added successively; after standing for 1 min, 0.5 ml of 2% Triton X-100 solution was added and diluted to the mark with water and mixed well. The absorbance at 479 nm or the second-derivative value (peak-to-trough value between 479 and 491 nm) against the reagent blank in a 3 cm cell was recorded.

Synthesis of T(DBHP)P

3,5-Dibromo-4-hydroxyphenylmethanal. Bromine (26 g) was dissolved in 26 ml of acetic acid; it was then added to 10 g

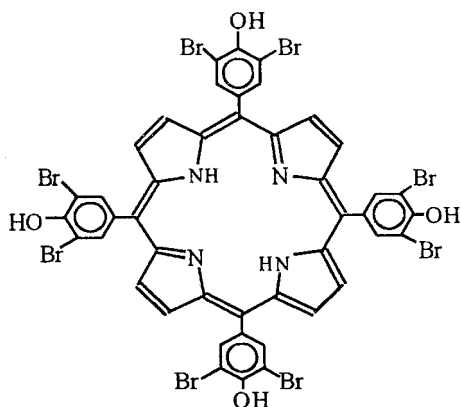


Fig. 1 The structure of T(DBHP)P.

Table 2 Porphyrin compound selectivity comparison

Reagent	Ions tolerated amounts/ μg							Remark	Reference
	Cr(III)	Fe(III)	Co(II)	Ni(II)	Cu(II)	Zn(II)	Cd(II)		
T(4-MOP)P	<15	2	<2	<8.5	<10	<3	<5		25
T(4SP)P	<5	10	<5	<5	<5	<5	<5	Add KCN	20
T(3-Mpy)P	8	5	10	10	12	20	12		21
T(4-TMAP)P	<5	<5	<5	<5	<5	<5	<5	Add KCN	22
T(4HP)P	<1	<1	10	10	5	<5	10		23
T(3-M-4HP)P	30	8	90	35	30	20	50		24
T(DBHP)P	4000	4000	1500	2000	1500	2500	5000	Add HQ	Present method

HQ, 8-hydroxyquinoline; T(4-MOP)P, meso-tetra-(4-methoxyphenyl)porphyrin; T(4SP)P, meso-tetra-(4-sulfonphenyl)porphyrin; T(3-Mpy)P, meso-tetra-(3-methylpyridyl)porphyrin; T(4-TMAP)P, meso-tetra-(4-trimethylaminophenyl)porphyrin; T(4HP)P, meso-tetra-(4-hydroxyphenyl)porphyrin; T(3-M-4HP)P, meso-tetra-(3-methyl-4-hydroxyphenyl)porphyrin; T(DBHP)P, meso-tetra-(3,5-dibromo-4-hydroxyphenyl)porphyrin.

of 4-hydroxyphenylmethanal (The Third Chemical Reagents Factory of Shanghai, China), stirred well and cooled with ice-water; it was left to stand for 10 min; after the bromination was completed, 60 ml of water was added to crystallize; the precipitate was washed with water and then filtered; crude 3,5-dibromo-4-hydroxyphenylmethanal was obtained. The crude 3,5-dibromo-4-hydroxyphenylmethanal (5 g) was dissolved in 50 ml of 95% alcohol, heated on a boiling water bath until crystals formed, left to stand for 30 min and filtered; the pure 3,5-dibromo-4-hydroxyphenylmethanal was obtained with a yield of 90%. Its structure was verified by IR and ^1H NMR spectrometry and the elemental analysis confirmed the purity (calculated: C, 30.03%; H, 1.44%; N, 57.09%).

Meso-tetra-(3,5-dibromo-4-hydroxyphenyl)porphyrin (T(DBHP)P). 3,5-Dibromo-4-hydroxyphenylmethanal (5.6 g), 150 ml of propionic acid and some fluorite (zeolite) were added to a three-necked flask. To this solution was added dropwise a solution of 1.75 ml of pyrrole which had recently been distilled. Reflux was performed for 30 min at 165 °C on an oil-bath. Reflux was continued for another 30 min, and the product was allowed to stand overnight. After filtering and washing with alcohol and ethyl ether separately, the crude product was obtained. The crude product was washed by alcohol, ethyl ether and acetone separately and dried at 100 °C. Pure T(DBHP)P was obtained with a yield of 30%. Elemental analysis confirmed the purity (calculated: C 44.32%; H, 1.67%; N, 4.22%; Br, 48.73%).

Results and discussion

Physical and chemical properties of T(DBHP)P

T(DBHP)P is a blue-violet solid with a melting point of 320 °C. It is easily soluble in basic aqueous solution and in organic solvents such as DMF and formic acid. The absorption spectra of T(DBHP)P at different pH values are shown in Fig. 2. The dissociation constants were evaluated by a spectrophotometric method.²⁶ The values of $\text{p}K_{\text{a}1}$, $\text{p}K_{\text{a}2}$, $\text{p}K_{\text{a}3}$ and $\text{p}K_{\text{a}4}$ were found to be 0.81, 1.21, 8.75 and 9.01, respectively. The equilibrium of the dissociation in water is shown in Fig. 3.

Chromogenic reaction of T(DBHP)P with metal ions

T(DBHP)P was found to react mainly with zinc and lead. Zinc reacts with the reagent to form a 1 : 1 complex with a maximum absorption peak at 447 nm in pH 10.2 borax buffer solution; its molar absorptivity is $2.6 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$. The highly sensitive reaction with lead is examined below.

Absorption spectra and second-derivative spectra

The absorption spectra and second-derivative spectra are shown in Fig. 4. In NaOH medium, the maximum absorption of T(DBHP)P is 445 nm, whereas the Pb-T(DBHP)P complex shows an absorption peak at 479 nm; the wavelength difference is 35 nm.

Effect of acidity

The influence of the pH on the absorbance of the Pb(II)-T(DBHP)P complex was studied. At $\text{pH} > 9$, the complex absorbance remained almost constant and at a maximum. We controlled the final pH by adding 0.5 ml of 2 mol l^{-1} NaOH to the 10 ml calibrated flask in order to keep the colour reaction reproducible, constant and sensitive.

Effect of amount of T(DBHP)P

Under the conditions employed, the volume of 0.04% T(DBHP)P solution required to obtain a maximum and constant absorbance for $4 \mu\text{g}$ of lead was 0.2–1.0 ml. Therefore, 0.5 ml of T(DBHP)P solution was introduced.

Surfactants and organic solvent

The effects of various surfactants and organic solvents on the Pb-T(DBHP)P chromogenic system were studied. It was found that the absorbance of Pb-T(DBHP)P increased remarkably in the presence of non-ionic surfactants. The order of the increase was Triton X-100 > polyethyleneglycol > octylphenyl ether. Cationic surfactants such as cetyltrimethylammonium bromide and cetylpyridinium chloride were added; the absorbance of the Pb-T(DBHP)P complex obviously diminished.

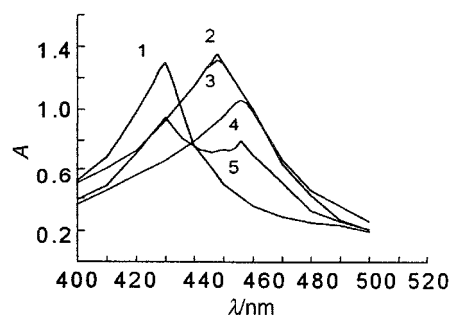


Fig. 2 The absorption spectra of T(DBHP)P at different pH values: 1, 2 mol l^{-1} HCl; 2, HCl + KCl (pH 1); 3, 0.08 mol l^{-1} NaOH; 4, 1.0 mol l^{-1} NaOH; 5, 0.5 mol l^{-1} HCl. Triton X-100, 0.2%; T(DBHP)P, $1.2 \times 10^{-5} \text{ mol l}^{-1}$; 1 cm cell; against water.

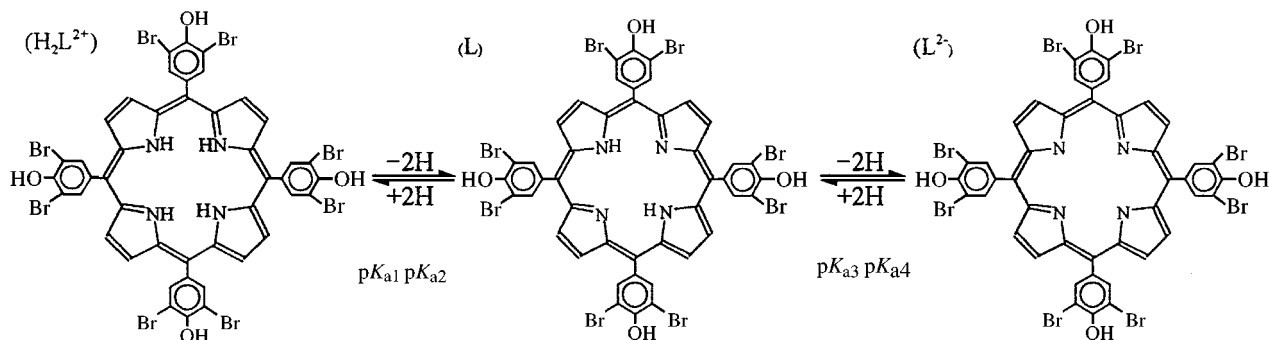


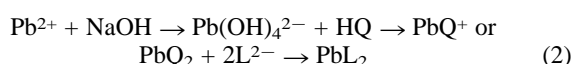
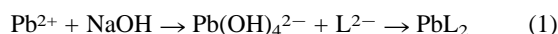
Fig. 3 The equilibrium of the dissociation of T(DBHP)P in water.

The addition of appropriate amounts of various organic solvents did not affect the absorbance of the Pb–T(DBHP)P complex. Hence, Triton X-100 is the best sensitizing agent and 0.1–1.0 ml of 2% Triton X-100 solution gives a constant and maximum absorbance. Therefore, 0.5 ml 2% Triton X-100 was adopted.

Selection of catalyst

As the chromogenic reaction of lead with T(DBHP)P is not complete at room temperature, we studied the effect of alcohol, *o*-phenanthroline, hydroxylamine sulfate, ascorbic acid and HQ on the colour reaction rate. The results show that HQ has obvious catalytic activity; the chromogenic reaction with lead is complete within 1 min at room temperature. Moreover, HQ is also a good masking agent, and improves the reaction system's selectivity.

In 10 ml of solution, the optimum volume of 2% HQ was 0.3–2.0 ml. Thus, an addition of 1.0 ml of 2% HQ was recommended. The chromogenic reaction of lead with T(DBHP)P was proposed as follows:



In NaOH medium, lead forms $\text{Pb}(\text{OH})_4^{2-}$ (maximum peak at 217 nm). As both $\text{Pb}(\text{OH})_4^{2-}$ and L^{2-} [eqn. (1)] have a negative charge, the static repellent effect causes them to remain apart so that the chromogenic reaction does not take place readily at room temperature. Only on heating have $\text{Pb}(\text{OH})_4^{2-}$ and L^{2-} got enough energy to collide to yield the colour reaction. In the presence of HQ, as $\text{Pb}(\text{OH})_4^{2-}$ gives PbQ^+ , which has a positive charge, or neutral PbQ_2 [eqn. (2)] (absorption peak at 434 nm), static attraction causes L^{2-} and PbQ^+ or PbQ_2 to approach each other, thus facilitating the chromogenic reaction at room temperature.

Conditions for measurement of the second-derivative value

The effects of the scan speed on the measurement of the second-derivative value (the vertical distance from a peak to a trough or from the baseline to a trough of the spectrum) were examined. Because the results showed that the scan speed did not affect the value, the highest scan speed of 1200 nm min^{−1} was chosen. It was also found that an increase in $\Delta\lambda$ corresponds to an increase in sensitivity. However, there was no significant difference in selectivity amongst the various $\Delta\lambda$ values (4, 8 and 16 nm), and therefore a value of 16 nm was selected in all subsequent work.

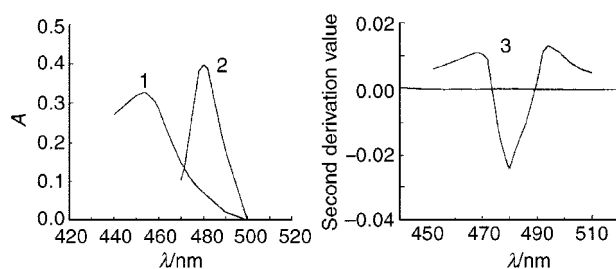


Fig. 4 Absorption spectra: 1, T(DBHP)P against water; 2 and 3, Pb–T(DBHP)P against the reagent blank. Pb, 0.133 μg ml^{−1}; T(DBHP)P, 1.2 × 10^{−5} mol l^{−1}; scan speed, 1200 nm min^{−1}; $\Delta\lambda$ = 16 nm.

Stability of the system

Oxygen in air or in the solution can affect the absorbance of the reagent and the complex. We have found that this can easily be eliminated by adding 0.5 ml of 2% Na₂SO₃ solution. The absorbance of the Pb–T(DBHP)P complex then remains stable for at least 100 h at room temperature (0–40 °C).

Calibration graph

According to the properties of the Pb(II)–T(DBHP)P complex, Beer's law was obeyed for 0–4 μg of lead in 10 ml of solution; the molar absorptivity of the complex is 2.5 × 10⁵ l mol^{−1} cm^{−1}. The calibration graph prepared by plotting the second-derivative value *versus* lead concentration was linear over the range of 0–4 μg of lead in 10 ml of solution (see Fig. 5) when either the peak-to-trough values or the baseline-to-trough values were plotted. The former plot gives a higher sensitivity and is thus recommended. The equation of the line obtained by a least-squares treatment is:

$$C = 33.5 D - 0.009 \quad (3)$$

for the peak-to-trough measurements, where *C* is the number of micrograms of lead in 10 ml of solution and *D* is the second-derivative peak-to-trough value. The limit of detection *C_L*, as defined by IUPAC,²⁷ and the limit of quantification *C_Q*^{28,29} are reported in Table 3. Obviously, the derivative method is more sensitive than conventional spectrophotometry. Ten replicate analyses of a test solution containing 2.0 μg of lead using the general procedure gave a mean of 2.01 μg of lead with a relative standard deviation of 1.0%.

Effect of interfering ions

Under the optimum conditions, the effects of various foreign ions on the determination of 2 μg Pb(II) were examined separately, with a relative error of less than ±5%. The tolerance

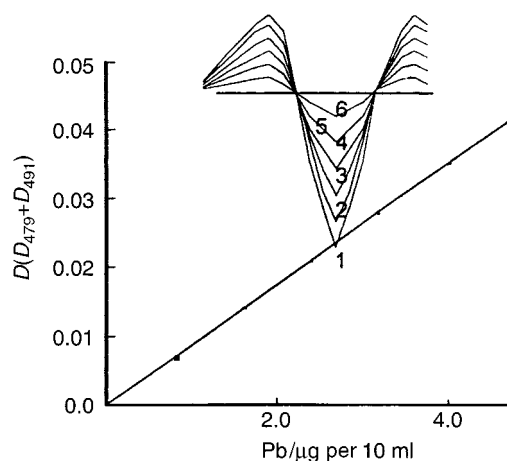


Fig. 5 Calibration graph. Pb/μg in 10 ml of solution: 1, 4.8; 2, 4.0; 3, 3.2; 4, 2.4; 5, 1.6; 6, 0.8. Scan speed, 1200 nm min^{−1}; $\Delta\lambda$, 16 nm; other conditions as in Fig. 4.

Table 3 A comparison of sensitivity

Method	<i>C_L</i> (<i>K</i> ^a = 3)/ ng ml ^{−1}	<i>C_Q</i> (<i>K</i> = 10)/ ng ml ^{−1}
Conventional spectrophotometry	1.6	5.2
Second-derivative spectrophotometry	0.21	0.70

K is a numerical factor chosen in accordance with the confidence level desired.

Table 4 Effect of foreign ions

Foreign ions	Second-derivative spectrophotometry/ μg	Conventional spectrophotometry/ μg
Na(I)	20000	20000
Mg(II)	4000	4000
K(I)	50000	50000
Ca(II)	6500	1500
Sr(II)	2000	900
Ba(II)	1500	1000
Al(III)	2000	2000
Sb(III)	2000	2000
Bi(III)	1000	500
Sn(IV)	1000	850
Ti(IV)	550	100
V(V)	400	200
Cr(III)	4000	2000
Mn(II)	150	100
Fe(III)	4000	1500
Co(II)	1500	1400
Ni(II)	2000	1000
Cu(II)	1500	1000
Zn(II)	2500	2000
Cd(II)	5000	1000
Ag(I)	1100	500
Hg(II)	750	250
Cl ⁻	40000	40000
I ⁻	20000	10000
CO ₃ ²⁻	50000	50000
P ₂ O ₇ ²⁻	20000	20000

limits for various foreign ions are listed in Table 4. All the foreign ions studied can be tolerated in considerable amounts. Second-derivative spectrophotometry shows better selectivity than conventional spectrophotometry. Thus, second-derivative spectrophotometry is more suitable for the direct determination of lead in clinical samples.

Composition of the complex

The composition and apparent stability constant were evaluated by the continuous variation and molar ratio methods. Both showed a molar ratio of lead(II) to T(DBHP)P of 1 : 2, in NaOH medium. The major species of the reagent (L²⁻) reacts with lead(II) to form the complex, which probably has a semimetallic porphyrin structure.³⁰ The average stability constant of the complex is 1.2×10^{45} at 25 °C.

Application

The proposed method was applied to the determination of Pb(II) in clinical samples. Prior to the determination, the samples were pretreated in the following way. Clinical samples were weighed (blood samples, 1 g; hair samples, 0.5 g; urine samples, 5.0 g), dried and carbonized in an electric furnace; this was followed by ashing in a muffle furnace at 550–600 °C and dissolving the ash in 3 ml of 0.1 mol l⁻¹ HNO₃. This solution was transferred into a 10 ml calibrated flask; 0.5 ml 2 mol l⁻¹ NaOH, 1.0 ml of 2% HQ solution, 0.50 ml of 2% Na₂SO₃ and 0.5 ml of 0.02% T(DBHP)P were added successively; after standing for 5 min, 0.5 ml of 2% Triton X-100 solution was added and diluted to the mark with water and mixed well. The second-derivative value between 479 and 491 nm was recorded against the reagent blank in a 3 cm cell. The results are in good agreement with those

Table 5 Determination of Pb(II) in clinical samples

Samples ^a	Found by the proposed method ^b / $\mu\text{g g}$	Found by ETAAS with prior separation by KI-MIBK ^b / $\mu\text{g g}$
Serum	0.16 \pm 0.002	0.15 \pm 0.004
Blood plasma	0.21 \pm 0.003	0.22 \pm 0.005
Urine	0.11 \pm 0.001	0.11 \pm 0.007
Hair	0.45 \pm 0.005	0.44 \pm 0.01

^a The samples, which were taken from persons who were seriously suffering from lead poisoning, were provided by Wuxi Second People's

Hospital. ^b Results expressed as: $\bar{x} \pm \frac{st}{\sqrt{n}}$ where \bar{x} is the mean of n observations of x , s is the standard deviation, t is a distribution value chosen for the desired confidence level.

found for lead by ETAAS with a prior separation by potassium iodide–methylisobutyl-ketone (KI-MIBK) (see Table 5).

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