Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).
Description

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The invention relates to a method and reagents for the measurement of polyvalent metal ions in aqueous media, in particular, lead ions in physiological fluids.

2. Description of Related Art

[0002] Heavy metals, such as lead, cadmium, mercury, etc., are known to be toxic and to have deleterious effects on humans. Lead and its compounds, which over the centuries have been widely distributed in the environment as a result of their industrial uses, pose a significant health hazard. Lead toxicity is due to its affinity to thiol and cellular phosphate groups of enzymes and proteins. It also inhibits biosynthesis of heme and increases premature red cell destruction. Lead is toxic to the central nervous system, especially in children. It is estimated that over three million preschool children have dangerously elevated lead concentrations. While the largest part of environmental lead results from the combustion of leaded gasoline, the most concentrated source of lead in the environment of children is lead-containing paint. Lead uptake can occur throughout the gastrointestinal tract, lungs, or skin. It accumulates in bones, kidneys, liver and other organs. In addition to exhibiting neurotoxicity, lead has also been classified as a probable carcinogen.

[0003] The determination of lead in whole blood is important for monitoring exposure, particularly in children. In 1991, the Centers for Disease Control and Prevention in the United States lowered the acceptable limit of blood lead concentration from 25 mg/dL to 10 mg/dL and recommended screening for blood lead of all children under the age of six. Thus, the popular indirect method of lead determination by the fluorescence of zinc protoporphyrin became unsuitable due to its inadequate sensitivity. The measurement of lead in whole blood at concentrations below 20 mg/dL poses a challenge due to the complex nature of the matrix. A simple, reliable, and inexpensive method of measuring lead in blood is not available. Atomic absorption spectrophotometry and anodic stripping voltametry are the most commonly used methods. Although they are claimed to be accurate, they are also cumbersome to use, susceptible to contamination, and costly.

[0004] Other methods of lead determination have recently been reported. Use of stable isotope dilution gas chromatography-mass spectrometry (Herold et al., Clin.Chem. 40, 1994) for the determination of lead in urine and whole blood has been described. Spectrophotometric determination of lead in water using a porphyrin system has been reported (Schneider and Horning, Analyst 118 933, 1993). Also, measurement of lead by a lead-selective optode, with subnanomolar detection limit, has been demonstrated (Simon et al., Anal. Chem. 64 1534, 1992). Enzyme-based biodetection systems for lead, which employ δ-aminolevulinic acid dehydrase (Silbergeld, U.S. Patent No. 5,354,652 of October 11, 1994) or isocitrate dehydrogenase (Henkens et al., U.S. Patent No. 5,217,534 of June 8, 1993) have also been proposed.

[0005] WO-A-95 14926 discloses an assay using a first sandwiched chelator linked to a protein immobilized on a solid support. A second sandwiched chelator is linked to an enzyme having a reporter group immobilized thereon on US-A-4 080 171 relates to a system wherein liquid is filtered through a filter paper having an ion exchange functional group and chelating ion binding functional groups.

SUMMARY OF THE INVENTION

[0006] The present invention relates to a method for the determination of polyvalent metal ions using a sandwich aggregation assay. One or more chelating agents capable of forming at least 2:1 or higher ratio stoichiometry complexes with polyvalent metal ions are attached to a suitable carrier such as latex particles through covalent bonds or by non-covalent interactions, such as hydrophobic interactions. Upon complexation with the metal ions, the carriers of the complexing agent aggregate, causing an increase in light absorbance or light scattering which is proportional to the concentration of metal ions in the test sample. The measurement of the light absorbance or light scattering by a suitable instrument, such as a spectrophotometer or nephelometer provides a means for measuring the concentration of metal ions.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0007] The present invention can be used to detect and measure a variety of metal ions including lead, copper, cadmium, cobalt, calcium, chromium, iron, aluminum, manganese, molybdenum, mercury, nickel, uranium, vanadium,
zinc, magnesium, and the like. The sensitivity and selectivity of the system comprising a chelating agent associated with a carrier will depend on the chelating agent used. Furthermore, selectivity can be enhanced by using suitable masks, such as neocuproine to mask copper. The metal ions are most often found in a matrix, such as water, blood, urine, serum, milk and the like. It will also be recognized that anions can also be measured according to the principle of the present invention, for example, sulfide and carbonate, by selecting an appropriate complexing agent.

Chelating agents which form complexes, for example with lead, can be selected from, but are not limited to, a variety of compounds such as mercaptoamides, mercaptoamides, mercaptans, mercaptoamines, mercaptothiones, aminothiones, thiohydroxamic acids, hydroxypyridinethiones, aminothiophenols, aminophenols, and dithiocarbamates.

The chelating agent can be bound or linked to a suitable carrier at the time of formation of the reaction mixture, or it can be in a form capable of becoming linked to the carrier. In the latter instance, for example, an interaction between a ligand and a specific binding partner for the ligand, such as antibody-antigen, hapten-anti-hapten antibodies, or avidin-biotin can be utilized. The binding partner can be attached to the carrier, either by covalent or non-covalent means.

A conjugate which comprises the ligand and the chelating agent which forms at least a 2:1 or higher ratio stoichiometry with the metal ion can be prepared. For all practical purposes the maximum ratio is controlled by the valence of the metal. Thus, most ratios of conjugate to metal ion will vary from 2 to 3:1. Upon contact with the metal ions, the conjugate forms a complex which, in turn, induces aggregation of the carrier due to the ligand-binding partner interaction.

The carrier can be soluble or insoluble in the reaction mixture as long as aggregation of the carrier results in a measurable change in light absorbance or light scattering. The carrier is typically particulate matter, and can be a microparticle such as latex particles made by emulsion or suspension polymerization, fine pigments, particles of oxides, for example, silicon oxide, titanium oxide or zinc oxide, and sulfates such as barium sulfate or calcium sulfate.

The microparticle can also be neutral or synthetic macromolecules, for example, polysaccharides, certain polypeptides or dendrimers. The macromolecule can be linear, globular or crosslinked. The microparticles can have a mean diameter within the range of from about 0.001 µm to about 10 µm, preferably about 0.01 to about 1 µm, and most preferably about 0.1 to about 0.25 µm. The only restriction in the microparticle of choice is that it should not contain ions which will interfere with the specific chelating reaction or specific metal detection.

In a preferred embodiment, a conjugate comprising biotin and thiolactic acid linked together by a polyether linker is contacted with a sample containing lead. A 2:1 conjugate/lead complex is formed. The complex is then contacted with latex particles coated with avidin. Due to high affinity of biotin toward avidin, aggregation occurs, and an increase in absorbance, which is proportional to the concentration of lead and is measured with a suitable analytical device, such as a spectrophotometer.

The following examples set forth various aspects of the subject invention. It will be understood that the formulations and procedures which follow are provided for purposes of illustration only and that other equivalent ingredients, proportions, and procedures can be employed in accordance with the principles of this invention.

**Example 1:** Preparation of reagent 1 for lead determination.

A stock solution (2.0 mL) of 2.5 mg/mL (2-phenyl)ethyl thiolactic acid amide in ethanol was prepared. An aliquot (8 mL) of this solution was added to 10 mL of 0.01 N NaOH and stirred. To this solution was added 0.5 mL of 5% polyvinyltoluene latex and the mixture was stirred for 2 hours to form the reagent 1 composition.

**Example 2:** Determination of lead with reagent 1.

A sample of reagent 1 (0.30 mL) was mixed with 1.7 mL of 0.1 M 2-amino-2-methylpropanol (AMP) buffer (pH 10) containing 0.1 N NaCl, in a 3 mL cuvette. A 10 mL sample of an aqueous solution of lead nitrate (1.0 x 10^{-5} M) was added to obtain 5.0 x 10^{-8} M Pb^{2+} in the cuvette, mixed, and the change in absorbance at 600 nanometers (nm) was recorded after 5 minutes with a Cary 3 spectrophotometer (Varian Analytical Instruments). Other concentrations were obtained by adding 2, 5 and 10 mL of 1.0 x 10^{-4} M lead nitrate to obtain [Pb^{2+}] concentrations of 1.0, 2.5, and 5.0 x 10^{-7}, respectively. Table 1 shows the lead response data obtained from the spectrophotometer:

<table>
<thead>
<tr>
<th>[Pb^{2+}], M</th>
<th>A_{600}(5 \text{ min})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.120</td>
</tr>
<tr>
<td>5.0 x 10^{-8}</td>
<td>0.260</td>
</tr>
</tbody>
</table>
**Example 3:** Preparation of reagent 2 for lead determination.

[0017] A stock solution (2.0 mL) of 2.5 mg/mL (2-phenyl)ethyl amide of N-(2-mercaptopropionyl)glycine in ethanol was prepared. An aliquot (20 mL) of this solution was added to 10 mL of 0.01 N NaOH and stirred. To this solution, 0.5 mL of 5% polyvinyltoluene latex was added and the mixture was stirred for 2 hours to form the reagent 2 composition.

**Example 4:** Table 2 shows lead response data obtained with reagent 2 using the procedure of Example 2.

[0018]

<table>
<thead>
<tr>
<th>[Pb^{2+}], M</th>
<th>A_{600}(5 \text{ min})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.120</td>
</tr>
<tr>
<td>5.0 \times 10^{-8}</td>
<td>0.260</td>
</tr>
<tr>
<td>1.0 \times 10^{-7}</td>
<td>0.313</td>
</tr>
<tr>
<td>2.5 \times 10^{-7}</td>
<td>0.775</td>
</tr>
<tr>
<td>5.0 \times 10^{-7}</td>
<td>0.870</td>
</tr>
</tbody>
</table>

**Example 5:** Lead assay.

[0019] A sample of reagent 2 (0.30 mL) was mixed with 1.3 mL of 0.14 M 2-amino-2-methylpropanol (AMP) buffer (pH 10) containing 0.13 N NaCl, in a 3 mL cuvette. A 0.4 mL sample of an aqueous solution of lead nitrate (0, 10, 15, 20, 30 & 50 mg/dL Pb^{2+}) was added to the buffer in cuvette, mixed, and the absorbance at 600 nm was recorded after 5 minutes with a spectrophotometer. A sample of lead responses is shown in Table 3.

<table>
<thead>
<tr>
<th>[Pb^{2+}], mg/dL</th>
<th>A_{600}(5 \text{ min})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.855</td>
</tr>
<tr>
<td>10.0</td>
<td>0.912</td>
</tr>
<tr>
<td>15.0</td>
<td>0.996</td>
</tr>
<tr>
<td>20.0</td>
<td>1.135</td>
</tr>
<tr>
<td>30.0</td>
<td>1.272</td>
</tr>
<tr>
<td>50.0</td>
<td>1.452</td>
</tr>
</tbody>
</table>

[0020] The lead level in the data shown in Table 3 is comparable to toxic concentrations of lead found in blood in cases of lead poisoning.

**Example 6:** Preparation of reagent 3 for calcium determination.

[0021] A stock solution (2.0 mL) of didecylphosphate in ethanol was prepared. An aliquot (0.10 mL) was added to 10 mL of 0.01 N NaOH and stirred. To this solution was added 0.5 mL of 5% polyvinyltoluene latex and the mixture was stirred for 2 hours to form the reagent 3 composition.
**Example 7:** Determination of calcium with reagent 3.

[0022] A sample of reagent 3 (0.30 mL) was mixed with 1.7 mL of 0.2 N NaOH in a 3 mL cuvette. Samples (5-10 mL) of an aqueous solution of calcium chloride (0.1 M) were added, mixed, and the change in absorbance at 600 nm was recorded with a Cary spectrophotometer after 5 minutes. Responses to different calcium levels are shown in **Table 4**.

**Table 4**

<table>
<thead>
<tr>
<th>[Ca^{2+}], M</th>
<th>(A_{600(5 \text{ min})})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.5 (\times) 10^{-4}</td>
<td>0.120</td>
</tr>
<tr>
<td>3.5 (\times) 10^{-4}</td>
<td>0.220</td>
</tr>
<tr>
<td>5.0 (\times) 10^{-4}</td>
<td>0.660</td>
</tr>
</tbody>
</table>

[0023] In the preparation of chelating agents used in reagents for lead determination, unless specified otherwise, reagent grade reactants and solvents were used as received from chemical suppliers. Dimethylsulfoxide (DMSO) was dried over molecular sieves (4 Å) (Aldrich). 1,17-diamino-3,6,9,12,15-pentaoxa-heptadecane was synthesized according to a published general procedure (Lehn, Tetrahedron 29, 1629, 1979). Melting points were determined on a Thomas-Hoover capillary apparatus. \(^1\)H NMR spectra were recorded with a Varian Gemini 200 Mhz spectrometer and chemical shifts are reported in parts per million (d) downfield from tetramethylsilane.

**Example 8:** Preparation of N-(2'-phenylethyl)-2-mercaptopropanamide

[0024]

![Chemical structure](image)

N-hydroxysuccinimide (0.58 g, 5.0 mmol) and dicyclohexylcarbodiimide (1.03 g, 5.0 mmol) was added to a solution of thiolactic acid (0.53 g, 5.0 mmol) in \(\text{CH}_2\text{Cl}_2\) (8 mL). The mixture was stirred overnight at room temperature and filtered into 2-phenethylamine (0.61 g, 5.0 mmol). The reaction mixture was stirred for 24 hours at room temperature, filtered, and the solvent was removed \(\text{in vacuo}\) from the filtrate. The residue was chromatographed on silica gel with \(\text{CH}_2\text{Cl}_2\) and \(\text{CH}_2\text{Cl}_2\)/MeOH (98/2) as eluent to give 0.68 g (65%) of the N-(2'-phenylethyl)-2-mercaptopropanamide product as a solid with mp 78-79°C. \(^1\)H NMR(\(\text{CDCl}_3\)): \(\delta\) 1.51 (d, 3H), 1.93 (d, 1H), 2.84 (t, 2H), 3.30-3.60 (m, 3H), 6.90 (br s, 1H), 7.15-7.38 (m, 5H).

**Example 9:** Preparation of N-(2-phenylethyl)amide of 2-mercaptopropionylglycine

[0026]

![Chemical structure](image)

N-hydroxysuccinimide (0.58 g, 5.0 mmol) and dicyclohexylcarbodiimide (1.03 g, 5.0 mmol) was added to a solution of 2-mercaptopropionylglycine (0.82 g, 5.0 mmol) in DMSO (16 mL) and the mixture was stirred for 5 hours at room temperature. The mixture was filtered into 2-phenethylamine (0.60 grams, 5.0 mmol) and stirred overnight at room temperature. The solvent was removed \(\text{in vacuo}\) and the residue was column chromatographed on silica gel with \(\text{CHCl}_3\)/MeOH (97/3) as eluent to give 0.55 g (41%) of the product as a solid with mp 116-118.5°C. \(^1\)H NMR (\(\text{CDCl}_3\)): \(\delta\) 1.51 (d, 3H), 2.02 (d, 1H), 2.81 (t, 2H), 3.33-3.60 (m, 3H), 3.86 (d, 2H), 6.29 (br s, 1H), 7.10-7.37 (m, 5H).
**Example 10:** Preparation of biotin-thiolactic acid conjugate.

[0028]

A solution of di-tert-butyl dicarbonate (0.78 g, 3.57 mmol) in ethyl ether (10 mL) was added dropwise, over a 3-hour period, to a solution of 1,17-diamino-3,6,9,12,15-pentaoxaheptadecane (1.00 g, 7.1 mmol) in DMSO (10 mL) at 16-20°C. The reaction mixture was stirred at room temperature for 24 hours. A sufficient amount of water and ethyl ether were added to partition the reaction mixture. The organic layer and aqueous layer were separated. The aqueous layer was extracted first with ethyl ether and then with CH₂Cl₂. The organic layers were combined and dried over K₂CO₃. The solvent was removed *in vacuo* and the residue was column chromatographed on deactivated basic alumina with CHCl₃/MeOH (98/2) to give 0.90 g (33%) of the mono-BOC-protected product NH₂(CH₂CH₂O)₅CH₂CH₂NH-BOC as a pale yellow, viscous liquid. **¹H NMR (CDCl₃ + D₂O):** δ 1.41 (s, 9H), 2.82 (br s, 2H), 3.28 (t, 2H), 3.45-3.67 (m, 22H).

[0030] N-hydroxysuccinimide (0.12 g, 1.04 mmol) and dicyclohexyldiimide (0.21 g, 1.02 mmol) was added to a solution of biotin (0.24 g, 0.98 mmol) in DMSO (2 mL). The mixture was stirred overnight at room temperature and filtered into t-butoxycarbonyl (BOC) protected amine NH₂(CH₂CH₂O)₅CH₂CH₂NH-BOC (0.35 g, 0.96 mmol). The reaction mixture was stirred at room temperature for 20 hours. The solvent was removed *in vacuo* and the residue was column chromatographed on silica gel with CHCl₃/MeOH (9/1) to give 0.60 g (100%) of biotin derivative as a gelatinous solid. **¹H NMR (CDCl₃):** δ 1.43 (s, 9H), 1.57-1.82 (m, 6H), 2.22 (t, 2H), 2.67-2.97 (m, 2H), 3.07-3.68 (m, 25H), 4.26-4.35 (m, 1H), 4.45-4.54 (m, 1H), 5.10 (br s, 1H), 5.38 (br s, 1H), 6.32 (br s, 1H), 6.32 (br s, 1H), 6.70 (br s, 1H).

[0031] The biotin derivative was treated with trifluoroacetic acid (10 mL) and the solution was kept at room temperature for 0.5 hours. The solvent was removed *in vacuo* and the residue was dissolved in dimethyl formamide (2 mL) and made basic with triethylamine. To this solution was added 1 mmol of N-hydroxysuccinimide ester of thiolactic acid. The mixture was stirred overnight at room temperature. The solvent was removed *in vacuo* and the residue was redissolved in CHCl₃ and washed with deoxygenated water to prevent oxidation of sulfhydryl groups. The solvent was removed *in vacuo* and the residue was column chromatographed on silica gel with CHCl₃/MeOH (9/1®8/1) to produce the conjugate,
$^1$H NMR (CDCl$_3$ + D$_2$O): $\delta$ 1.37-1.95 (m, 10H), 2.23 (t, 2H), 2.61-2.97 (m, 3H), 3.16 (q, 1H), 3.37-3.72 (m, 24H), 4.28 (m, 1H), 4.45-4.55 (m, 1H).

Example 11: Preparation of avidin latex particles (60 mg/mg).

[0032] 4.0 ml of 5.0% (w/v) 165 nm core-shell latex particles made of a polyvinyltoluene core and a polyvinylbenzyl chloride shell were prepared in accordance with U.S. patent application Serial No. 08/330,259, filed October 27, 1994 the core-shell latex particles were added to 15.0 ml 0.01 M phosphate buffer pH 9.5 and stirred for 30 minutes. 20 ml 0.01 M phosphate buffer pH 9.5 containing 12.0 mg avidin were then added and stirred an additional 30 minutes. The suspension was incubated at 40°C for 3 hours in an incubator equipped with a mechanical shaker. After 3 hours the suspension was removed from the incubator, 5.0 ml of 2 M ethanolamine added, and the mixture incubated an additional 18 hours at 40°C. Equal volumes of the suspension were added to 2 tubes and centrifuged at 16,000 rpm for one hour at a temperature in the range of 8-15°C in a refrigerated ultracentrifuge. the supernatant was discarded and 10.0 ml solution of 0.9% (v/v) NaCl, 0.02% (w/v) Tween-20 added to the pellet in each tube, sonicated and centrifuged at 16,000 rpm. The resuspension, sonication and centrifugation steps were repeated twice with 10 ml aliquots of NaCl solution. The pellets were then combined and resuspended in 100 ml solution of 0.9% (w/v) NaCl, 0.2% Tween®-20, and 0.5 mM NaOH. This solution was then sonicated to obtain a uniform avidin latex suspension. The avidin particles were stored at 4°C.

Example 12: Determination of lead using biotin thiolactic acid conjugate (reagent 4).

[0033] A stock solution of 5.0 x 10^{-5} M biotin thiolactic acid conjugate was prepared in methanol containing 0.05% v/v tributylphosphine. An aliquot (40 ml) of this solution was added to 2.0 ml of 0.2 N NaCl, 0.05% Triton® X-100 (Rohm and Haas) and stirred to form R1. R2 consisted of a 2 mg/ml solution of 60 mg/mg avidin latex particles (Example 11) in 0.9% (w/v) NaCl, and 0.1% (w/v) Triton X-100.

[0034] The assay of lead was done on an RA-XT analyzer (Bayer Corporation, Tarrytown, New York, USA) at 37°C using zero-order quadratic rate chemistry parameters. RA-XT parameters:

- R1 320 ml
- R2 80 ml
- Sample volume 4 ml
- Filter 600 nm
- Delay time 15 seconds
- R2 delay 1 minute

[0035] Aqueous lead samples were prepared to contain 0.0-0.1 mM lead. The samples were diluted 1:100 on the instrument to yield final concentrations of 0-20 mg/dl.

[0036] The quadratic rate (Q) in (AU/min) reported by the instrument is tabulated in Table 5 and was used to construct the response curve shown in Fig. 1.
As a control experiment, the lead response was also determined in the absence of the biotin conjugate. Example 12 was repeated without addition of biotin thiolactic acid conjugate to show that the agglutination was due to the formation of the 2:1 complex and not to the non-specific agglutination of the avidin latex particles by the divalent cation. The results appear in Table 6.

### Table 5

<table>
<thead>
<tr>
<th>[Pb^{2+}], mg/dl</th>
<th>Q(AU/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.006</td>
</tr>
<tr>
<td>2.0</td>
<td>0.008</td>
</tr>
<tr>
<td>4.0</td>
<td>0.0185</td>
</tr>
<tr>
<td>6.0</td>
<td>0.029</td>
</tr>
<tr>
<td>8.0</td>
<td>0.0436</td>
</tr>
<tr>
<td>10.0</td>
<td>0.0562</td>
</tr>
<tr>
<td>12.0</td>
<td>0.063</td>
</tr>
<tr>
<td>14.0</td>
<td>0.0696</td>
</tr>
<tr>
<td>16.0</td>
<td>0.0756</td>
</tr>
<tr>
<td>20.0</td>
<td>0.0794</td>
</tr>
</tbody>
</table>

### Example 13

Preparation of chelating agent (reagent 5) used in copper determination.

N-hydroxysuccinimide (0.24 g, 2.08 mmol) and dicyclohexylcarbodiimide (0.42 g, 2.04 mmol) was added to a solution of biotin (0.50 g, 2.05 mmol) in DMSO (5.5 mL). The mixture was stirred for 5 hours at room temperature and filtered into N-ethylethylenediamine (0.22 mL, 2.05 mmol). The mixture was stirred for 3 hours at room temperature. The solvent was removed *in vacuo* and the residue was column chromatographed on alumina with CHCl₃ and CHCl₃/MeOH to give 0.25 g (39%) of biotinamine.
1H NMR (CDCl3 + D2O): δ 1.09 (t, 3H), 1.35-1.85 (m, 6H), 2.22 (t, 2H), 2.55-2.97 (m, 5H), 3.05-3.5 (m, 4H), 4.22-4.37 (m, 1H), 4.43-4.57 (m, 1H).

[0039] The biotinamine (143 mg, 0.45 mmol) was dissolved in a mixture of DMSO (1 mL) and acetone (1 mL) at 0-5°C. To this solution was added a mixture of carbon disulfide (27 mL) and acetone (30 mL) and, after stirring for 10 minutes at 0-5°C, a solution of NaOH (19 mg) in water (91 mL) was added and the mixture was stirred at 0-5°C for 20 minutes. Acetone was added to precipitate biotindithiocarbamate, which was separated by filtration and washed with ethyl ether. The product was dried under vacuum and stored under argon. The biotindithiocarbamate can also be used for determination of lead and other heavy metals.

Example 14: Determination of copper using biotin dithiocarbamate conjugate (reagent 5).

[0040] A stock solution of 9.2 x 10⁻⁵ M biotin dithiocarbamate conjugate was prepared in 1.0 x 10⁻⁴ M NaOH. An aliquot of this solution (44 ml) was added to 2 ml of 0.2 N NaCl, 0.05% Tween®-20 (ICN) and stirred (R1). R2 consisted of a 2 mg/ml suspension of 60 mg/mg avidin latex particles (Example 11) in 0.9% (w/v) NaCl, and 0.1% Tween®-20.

[0041] The assay of copper was done on the RA-XT at 37°C using zero-order quadratic rate chemistry parameters.
RA-XT parameters:

[0042]

R1 320 ml
R2 80 ml
Sample volume 4 ml
Filter 600 nm
Delay time 15 seconds
R2 delay 1 minute

[0043] Aqueous samples were prepared to contain 0.0-1.0 x 10^{-4} M copper. The samples were diluted 100 fold on the RA-XT to yield final concentrations of 0.0-6.4 mg/dl copper. The Q rate (AU/min) reported by the instrument is tabulated in Table 7 and was used to plot the response curve shown in Fig. 2.

Table 7

<table>
<thead>
<tr>
<th>[Cu^{2+}], mg/dl</th>
<th>Q(AU/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>-0.0008</td>
</tr>
<tr>
<td>1.27</td>
<td>0.0078</td>
</tr>
<tr>
<td>2.54</td>
<td>0.0186</td>
</tr>
<tr>
<td>3.81</td>
<td>0.0269</td>
</tr>
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<td>5.08</td>
<td>0.0329</td>
</tr>
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<td>6.35</td>
<td>0.0389</td>
</tr>
</tbody>
</table>

Claims

1. A test composition for determining the amount of a polyvalent metal ion in an aqueous medium using a sandwich aggregation assay, consisting essentially of:

   I. a chelating agent capable of forming at least a 2:1 stoichiometric ratio complex of chelating agent to polyvalent metal ion, and
   II. carriers for chelating agent, wherein said chelating agent:

   a) is linked to said carriers, or
   b) is in the form of a conjugate with a ligand, and a specific binding partner for said ligand is linked to said carriers;

   wherein, upon the complexation of the chelating agent with the polyvalent metal ion, the carriers linked to the complexed chelating agents are characterized by the ability to aggregate, thereby causing an increase in light absorbance which is proportional to the concentration of the polyvalent metal ion in the aqueous medium.

2. The test composition of claim 1, wherein the ion is selected from the group consisting of lead, copper, cadmium, cobalt, chromium, iron, aluminum, manganese, molybdenum, mercury, nickel, uranium, vanadium, zinc, magnesium.

3. The test composition of claim 1, wherein the ion is lead.

4. The test composition of any of claims 1 to 3, wherein the carrier is at least one selected from the group consisting of latex particles, fine pigments, oxide particles, and sulfates, preferably wherein the mean particle size of the carriers is between about 0.001 and about 10 μm.

5. The test composition of claim 4, wherein the ligand/specific binding partner pair is selected from the group con-
The test composition of any of claims 1 to 4, wherein the carriers are covalently linked to the chelating agent.

The test composition of any of claims 1 to 6, wherein the chelating agent is selected from the group consisting of mercaptoamides, mercaptothioamides, mercaptans, mercaptoamines, mercaptothiones, aminothiones, thiohydroxamic acids, hydroxypyridinethiones, aminothiophenols, aminophenols, and dithiocarbamates.

A method for determining the amount of an ion in a matrix comprising the steps of (a) contacting the ion with the test composition of any of claims 1 to 7, and (b) measuring the resulting aggregation in the reaction mixture as a function of the amount of the ion in the matrix.

The method of claim 8, wherein the resulting aggregation is measured by measuring the light absorbance or light scattering of the reaction mixture.

Patentansprüche

1. Test-Zusammensetzung zur Bestimmung der Menge eines mehrwertigen Metallions in einem wässrigen Medium unter Anwendung eines Sandwich-Aggregationsassay, bestehend im wesentlichen aus:

I. einem Chelat-bildenden Mittel mit der Fähigkeit zur Bildung eines Komplexes aus dem Chelat-bildenden Mittel und den mehrwertigen Metallionen in einem Stöchiometrie-Verhältnis von mindestens 2:1 und

II. Trägern für das Chelat-bildende Mittel, worin das genannte Chelat-bildende Mittel:

a) an die genannten Träger gebunden ist oder

b) in der Form eines Konjugats mit einem Ligand vorliegt und ein spezifischer Bindungspartner für den genannten Ligand an die genannten Träger gebunden ist,

worin, bei der Komplexierung des Chelat-bildenden Mittels mit dem mehrwertigen Metallion, die an die komplexierten Chelat-bildenden Mittel gebundenen Träger durch die Fähigkeit gekennzeichnet sind, zu aggregieren, um dadurch einen Anstieg der Lichtabsorption zu verursachen, der proportional zur Konzentration des mehrwertigen Metallions im wässrigen Medium ist.

2. Test-Zusammensetzung gemäß Anspruch 1, worin das Ion aus der Gruppe ausgewählt ist, bestehend aus Blei, Kupfer, Cadmium, Kobalt, Chrom, Eisen, Aluminium, Mangan, Molybdän, Quecksilber, Nickel, Uran, Vanadin, Zink und aus Magnesium.

3. Test-Zusammensetzung gemäß Anspruch 1, worin das Ion Blei ist.

4. Test-Zusammensetzung gemäß einem der Ansprüche 1 bis 3, worin der Träger mindestens einer ist, ausgewählt aus der Gruppe, bestehend aus Latexpartikeln, Feinpigmenten, Oxid-Partikeln und aus Sulfaten, worin die mittlere Partikelgröße der Träger vorzugsweise ca. 0.001 bis ca. 10 µm beträgt.

5. Test-Zusammensetzung gemäß Anspruch 4, worin das Paar aus Ligand/spezifischem Bindungspartner aus der Gruppe ausgewählt ist, bestehend aus Biotin/Avidin und Haptenen/anti-Hapten-Antikörpern.

6. Test-Zusammensetzung gemäß einem der Ansprüche 1 bis 4, worin die Träger kovalent an das Chelat-bildende Mittel gebunden sind.


8. Verfahren zur Bestimmung der Menge eines Ions in einer Matrix, umfassend die Stufen (a), wobei man das Ion...
mit der Test-Zusammensetzung gemäß einem der Ansprüche 1 bis 7 in Kontakt bringt, und (b), wobei man die entstandene Aggregation in der Reaktionsmischung als Funktion der Menge des Ions in der Matrix misst.

9. Verfahren gemäß Anspruch 8, wobei die entstandene Aggregation durch Messung der Lichtabsorption oder Lichtstreuung der Reaktionsmischung gemessen wird.

Revendications

1. Composition analytique pour déterminer la quantité d'un ion métallique polyvalent dans un milieu aqueux en utilisant une analyse d'agrégation en sandwich, consistant essentiellement en :

I. un agent chélatant capable de former un complexe, en un rapport stoechiométrique d'au moins 2:1, de l'agent chélatant à un ion métallique polyvalent, et

II. des supports pour l'agent chélatant, dans laquelle ledit agent chélatant :

   a) est lié auxdits supports, ou
   b) est sous forme d'un conjugué avec un ligand, et un partenaire à liaison spécifique pour ledit ligand est lié auxdits supports ;

dans laquelle, par complexation de l'agent chélatant à l'ion métallique polyvalent, les supports liés aux agents chélatants complexés sont caractérisés par l'aptitude à l'agrégation, ce qui provoque une augmentation d'absorbance de la lumière qui est proportionnelle à la concentration d'ion métallique polyvalent dans le milieu aqueux.

2. Composition analytique suivant la revendication 1, dans laquelle l'ion est choisi dans le groupe consistant en les ions plomb, cuivre, cadmium, cobalt, chrome, fer, aluminium, manganèse, molybdène, mercure, nickel, uranium, vanadium, zinc et magnésium.

3. Composition analytique suivant la revendication 1, dans laquelle l'ion est l'ion plomb.

4. Composition analytique suivant l'une quelconque des revendications 1 à 3, dans laquelle le support consiste en au moins un support choisi dans le groupe consistant en des particules de latex, des pigments fins, des particules d'oxydes et des sulfates, le diamètre moyen de particules des supports étant compris de préférence dans l'intervalle d'environ 0,001 à environ 10 µm.

5. Composition analytique suivant la revendication 4, dans laquelle la paire ligand/partenaire à liaison spécifique est choisie dans le groupe consistant en la paire biotine/avidine et des paires haptènes/anticorps antihaptènes.

6. Composition analytique suivant l'une quelconque des revendications 1 à 4, dans laquelle les supports sont liés par covalence à l'agent chélatant.


8. Méthode pour déterminer la quantité d'un ion dans une matrice, comprenant les étapes consistant (a) à mettre en contact l'ion avec la composition analytique suivant l'une quelconque des revendications 1 à 7 et (b) à mesurer l'agrégation résultante dans le mélange réactionnel en fonction de la quantité de l'ion dans la matrice.

9. Méthode suivant la revendication 8, dans laquelle l'agrégation résultante est mesurée en mesurant l'absorbance de la lumière ou la diffusion de la lumière du mélange réactionnel.
Copper response curve:

FIGURE 2
Copper response curve:

FIGURE 2