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(21) International Application Number: PCT/US95/06017 (22) International Filing Date: 15 May 1995 (15.05.95) (71) Applicant: AQUARIUM PHARMACEUTICALS, INC. [US/US]; 50 E. Hamilton Street, Chalfont, PA 18914 (US). (72) Inventors: ELSON, Jesse; P.O. Box 484, Doylestown, PA 18901 (US). YOSHPA, Michael; 4206 Grey Friars Terrace, Chalfont, PA 18914 (US). (74) Agents: NADEL, Alan, S. et al.; Panitch Schwarze Jacobs & Nadel, P.C., 36th floor, 1601 Market Street, Philadelphia, PA 19103 (US).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UZ, VN, ARIPO patent (KE, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: METHOD FOR DETERMINING DISSOLVED OXYGEN IN WATER (57) Abstract <p>The invention relates to a method for determining the concentration of dissolved oxygen in a liquid water sample comprising the steps: (a) providing a liquid water sample of predetermined volume, the oxygen content of which is to be determined; (b) mixing additives with the liquid water sample to form a mixture, the additives comprising an oxidizable metal salt comprising a metal cation and an anion selected from an inorganic anion or an organic anion which, with the cation, results in a water soluble oxidizable metal salt, at least one water soluble alkali metal compound selected from an alkali metal oxide, or an alkali metal hydroxide or a salt of an alkali metal and a weak acid; and at least one oxidation-reduction indicator capable of a color change upon oxidation of the oxidizable metal salt during contact with dissolved oxygen in the liquid sample to a color indicative of the concentration of dissolved oxygen in the liquid sample; (c) preventing any substantial contact of the mixture with atmospheric oxygen; and (d) determining the concentration of dissolved oxygen in the liquid sample by comparing the color of the mixture to a predetermined color indicative of the concentration of dissolved oxygen in the water sample.</p>		

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METHOD FOR DETERMINING DISSOLVED OXYGEN IN WATER**Field of the Invention**

The invention relates to a method for determining the concentration of dissolved oxygen in water and, more particularly, a method including the use of additives to determine the amount of dissolved oxygen in a liquid water sample wherein the additives comprise an oxidizable metal salt, at least one alkali metal compound, and at least one oxidation-reduction indicator capable of a color change upon oxidation of the metal salt.

Background of the Invention

Water quality management is of particular concern in the cultivation of aquatic organisms for such purposes as food, recreation, education, research and hobbies. Fish and other aquatic animals require an adequate supply of oxygen in order to ensure survival. In addition, aerobic bacterial processes require an adequate supply of oxygen to convert toxic waste into nontoxic by-products. It is crucial to maintain a minimum level of dissolved oxygen in water in order to maintain this aquatic life.

When water is free of all aquatic life, at a water temperature of approximately 70°F (21.1°C), the dissolved oxygen level in water is approximately 9 parts per million (ppm). However, in the presence of aquatic animals and accompanying aerobic bacterial processes, the oxygen level may become insufficient to support such life. Therefore, it is desirable for aquaculturists to have a simple, reliable method for testing the concentration of dissolved oxygen in a water sample from a water source such as an aquarium or a fish pond, in order to ensure an adequate concentration of dissolved oxygen for maintaining such aquatic life.

The prior art discloses various methods for determining the concentration of dissolved oxygen in

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liquids. However, these methods generally are inconvenient, inaccurate, or prohibitively expensive, both in terms of time and money to the home aquaculturist, as they require specialized titration equipment and solutions or electronic equipment and complicated procedures for use, or these methods may be adversely influenced by various factors associated with the water sample such as depth and turbulence of the water in the area from which the sample is taken, as well as temperature, light, sludge deposits or other components of the sample, microbial action, travel time and mixing. Moreover, techniques involving detecting oxygen in gaseous mixtures are not applicable to determining the concentration of oxygen in water.

Compared to the prior art methods for determining the dissolved oxygen concentration in liquids or detecting the presence of oxygen in gases, the present invention is a simple, reliable method for accurately determining the concentration of dissolved oxygen in a water sample, such as an aquarium or fish pond, for example, capable of use by the home aquaculture enthusiast.

Definitions

As used herein, an "alkali metal compound" is defined to mean any compound comprising a metal of Group IA of the Periodic Table.

As used herein, an "oxidizable metal salt" is defined to mean a salt of any metal except alkali metals (Group IA of the Periodic Table) and alkaline earth metals (Group IIA of the Periodic Table).

As used herein, "percent" or "%" is defined to mean percent by weight of the total mixture of ingredients in a mixture, unless otherwise indicated.

The term "redox", as used herein, is defined to mean oxidation-reduction.

The term "substantial absence of atmospheric oxygen", as used herein, is defined to mean that the

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additives and water sample are mixed under atmospheric conditions in which the level of atmospheric oxygen does not significantly influence the color change of the oxidation-reduction indicator upon oxidation of the metal salt.

5 The term "substantially colorless", as used herein, is defined to mean that the oxidation-reduction indicator is without color or has a color which does not affect the color change for determining the concentration of oxygen in the sample upon oxidation of the metal salt.

10 Summary of the Invention

The present invention relates to a method for determining the concentration of dissolved oxygen in a liquid water sample comprising the steps: (a) providing a liquid water sample of predetermined volume, the oxygen
15 content of which is to be determined; (b) mixing additives with the liquid water sample to form a mixture, the additives comprising an oxidizable metal salt comprising a metal cation and an anion selected from an inorganic anion or an organic anion which, with the cation, results in a
20 water soluble oxidizable metal salt; at least one water soluble alkali metal compound selected from an alkali metal oxide, or an alkali metal hydroxide, or a salt of an alkali metal and a weak acid; and at least one oxidation-reduction indicator capable of a color change upon oxidation of the
25 oxidizable metal salt during contact with dissolved oxygen in the liquid sample to a color indicative of the concentration of dissolved oxygen in the liquid sample; (c) preventing any substantial contact of the mixture with atmospheric oxygen; and (d) determining the concentration of
30 dissolved oxygen in the liquid sample by comparing the color of the mixture to a predetermined color indicative of the concentration of dissolved oxygen in the water sample.

Detailed Description of the Preferred Embodiment

Generally, the present invention involves the use of additives which, when added to a water sample, produces an electrochemical potential. The magnitude of the electrochemical potential is indicated by a color change of an oxidation-reduction indicator component of the additives. The color change is directly proportional to the concentration of dissolved oxygen in the water sample.

The additives of the present invention are superior to those of the prior art in that the individual components of the present additives need not be prepared or maintained under oxygen-free conditions prior to combination. In fact, two of the components may be combined prior to use. Even when the method is performed on a water sample, absolute absence of atmospheric oxygen is not required, as explained below.

One component of the present additives comprises an oxidizable metal salt comprising a suitable cation and an anion which may be an inorganic anion or an organic anion which, with the cation, results in a water soluble oxidizable metal salt.

The oxidizable metal salt is preferably a salt having a cation from any metal except alkali metals (Group IA of the Periodic Table) and alkaline earth metals (Group IIA of the Periodic Table). Transition metal cations are preferred. The transition metal of the transition metal salt is preferably selected from scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel or copper. The metal is preferably in its lowest oxidation state, although one of ordinary skill in the art in view of this disclosure would understand that the metal may be in any oxidation state as long as it is capable of being oxidized by oxygen to a higher oxidation state. The metal salt is also preferably colorless in the lower oxidation state so as not to interfere with any color change of the indicator when the additives are added to the water sample.

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Preferably the metal salt is in solid form, although one or ordinary skill in the art would understand that the metal salt may be in liquid form, such as an aqueous solution, as long as the effect of any dissolved oxygen in the water component of the aqueous solution is accounted for in the color change of the indicator when the additives are combined with the water sample.

The salt anion may be any suitable anion that results in a water soluble oxidizable salt when combined with the cation-containing component. Suitable anions include, for example and without limitation, inorganic anions like halides (such as chloride) or sulfates, and organic anions like citrates and acetates (such as ethylenediaminetetraacetate). Other suitable anions would be well known to those skilled in the art in view of this disclosure.

Preferably, the oxidizable metal salt is a salt of a transition metal. In an alternative preferred embodiment, the transition metal salt is preferably a soluble ferrous salt. The soluble ferrous salt may be organic or inorganic. Examples of suitable oxidizable transition metal salts include ferrous sulfate, ferrous chloride, ferrous ammonium sulfate, ferrous citrate, or ferrous ethylenediaminetetraacetate. As presently preferred, the transition metal salt is ferrous ammonium sulfate, otherwise known as Mohr's salt.

Another component of the additives used in the present invention comprises at least one water soluble alkali metal compound. The alkali metal compound may be selected from an alkali hydroxide, or an alkali metal oxide, or a salt of an alkali metal and a weak acid. Preferably, the alkali metal compound is selected from lithium hydroxide, sodium hydroxide, potassium hydroxide, rubidium hydroxide or cesium hydroxide. As presently preferred, the alkali metal compound is sodium hydroxide. Hydroxide forms of the alkali metal compound facilitate the oxidation

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reaction by raising the pH of the mixture of the additives and sample so that the mixture is basic.

The salt of an alkali metal and a weak acid may be any number of salts that, upon hydrolysis, will give an alkaline or basic reaction. A typical salt would be one such as an alkali metal carbonate, an alkali metal bicarbonate, an alkali metal acetate, an alkali metal citrate, an alkali metal lactate or an alkali metal succinate. These are water soluble salts resulting from the reaction of the alkali metal and the corresponding weak acid.

The alkali metal compound is preferably used in solid form, however, a liquid form, such as an aqueous solution, may be used as long as the effect of any dissolved oxygen in the water component of the aqueous solution is accounted for in the color change of the indicator when the additives are combined with the water sample.

Another component of the additives comprises at least one oxidation-reduction indicator capable of a color change upon oxidation of the transition metal salt. Preferably, the oxidation-reduction indicator is substantially colorless when the metal salt is in a lower oxidation state and is colored when the metal salt is in a higher oxidation state, although one skilled in the art would understand that the oxidation-reduction indicator need not be substantially colorless when the metal salt is in a lower oxidation state, as long as the oxidation-reduction indicator undergoes a color change when the metal salt is in a higher oxidation state. The color of the oxidation-reduction indicator changes at a specific electrochemical potential value which is characteristic for each oxidation-reduction indicator.

While the inventors do not wish to be bound by any specific theory, it is believed that the oxidation-reduction indicator is not directly involved in the oxidation reaction by which the oxidizable metal salt is elevated to a higher

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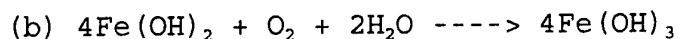
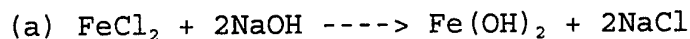
oxidation state. The action of the indicator is believed not to depend on the specific nature of the oxidant or reductant involved in the oxidation-reduction reaction, but upon the oxidation-reduction reaction achieving a reduction potential numerically equivalent to that at which the indicator undergoes a color change. The oxidized form of the indicator is preferably a different color than the reduced form or it may be colored in one form and colorless in another.

Preferably, the oxidation-reduction indicator is selected from the group consisting of indigo monosulfonate, phenosafranine, indigo tetrasulfonate, methylene blue, safranine O and mixtures thereof. In order to cover a broader range of possible values for reduction potential (i.e., dissolved oxygen concentration), a plurality of oxidation-reduction indicators may be combined for use in the present invention. Each indicator would be capable of changing color at a different reduction potential to thereby cover a broader range of dissolved oxygen concentrations than could be determined by use of a single indicator.

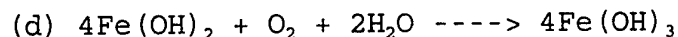
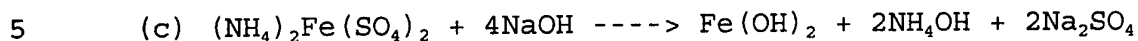
The reaction of the components in producing the color change is illustrated by the following exemplary consecutive reactions. One skilled in the art would understand in view of the present disclosure that different oxidizable metal salts, alkali metal compounds, and oxidation-reduction indicators may be used in accordance with the present invention other than those specifically set forth herein. In the first exemplary consecutive reactions, the oxidizable metal salt is ferrous chloride and in the second exemplary consecutive reactions, the oxidizable metal salt is ammonium ferrous sulfate. In each exemplary reaction, the alkali metal compound is sodium hydroxide and the oxidation-reduction indicator is preferably a combination of 80 weight percent methylene blue and 20 weight percent safranine O.

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First example of consecutive reactions:



Second example of consecutive reactions:



Methylene blue and safranine O are preferred because the reduction potentials at which these indicators change color corresponds to the reduction potential developed by the oxidation-reduction reaction of the preferred transition metal salt, for example, the oxidation by oxygen of a ferrous hydroxide to ferric hydroxide. For reactions such as those set forth above, the reduction potential may be calculated by the ratio of the concentration of Fe(OH)_3 to the concentration of Fe(OH)_2 . The value of the reduction potential developed by the reaction depends upon the degree to which the oxygen has oxidized the ferrous form to the ferric form. Thus, the color change of the oxidation-reduction indicator depends upon the dissolved oxygen content of the water. For example, in their reduced form, both methylene blue and safranine O are colorless. However, at a reduction potential of 0.36 volts, methylene blue indicator exhibits a green-blue color. At 0.28 volts, safranine O exhibits a red color.

Table 1 provides other examples of different formulations of the additives which may be used in accordance with the present invention.

TABLE 1

	<u>Oxidizable Metal Salt</u>	<u>Alkali Metal Compound</u>	<u>Oxidation-Reduction Indicator(s)</u>
5	FeSO ₄	NaOH	Methylene Blue and Safranin O
	FeCl ₂	NaOH	Methylene Blue and Safranin O
	(NH ₄) ₂ Fe(SO ₄) ₂	NaOH	Methylene Blue and Safranin O
10	FeEDTA	NaOH	Methylene Blue and Safranin O
	Fe Citrate	NaOH	Methylene Blue and Safranin O

15 The method according to the present invention for
determining the concentration of dissolved oxygen in a water
sample using the present composition will now be described
generally. The method generally comprises a first step of
obtaining a water sample of predetermined volume, for
example, 10 milliliters, in a convenient vessel, such as a
20 test tube capable of being covered, and of such volume that
there is not a considerable air space when all ingredients
have been added. Thus, the test tube or other vessel should
be almost completely full, so that when it is capped, the
contents of the tube are in the substantial absence of
25 atmospheric oxygen. One skilled in the art would understand
that the water sample may be of any predetermined volume so
long as a proportionate amount of the additives and water
are used for testing.

30 The method further comprises a second step of
mixing the additives with the liquid water sample in the
substantial absence of atmospheric oxygen to form a mixture.
The additives comprise, as discussed above, an oxidizable
metal salt preferably comprising a transition metal cation
and an anion that is an inorganic anion or an organic anion,
35 which, with the cation, results in a water soluble
oxidizable metal salt; at least one alkali metal compound as

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described above; and at least one oxidation-reduction indicator capable of undergoing a color change upon oxidation of the oxidizable metal salt. Any substantial contact of the mixture of the sample and additives with atmospheric oxygen should be prevented.

The components of the additive used in the present invention may be in a solid or liquid state, for example in the form of powder, tablets or prepared solutions.

Generally, in solid form, the oxidizable metal salt comprises about 65 to about 75, and preferably about 70 to about 73, weight percent of the additive mixture (not including water). The alkali metal compound comprises about 20 to about 25, and preferably about 22 to about 24, weight percent of the additive mixture. The oxidation-reduction indicator generally comprises about 1 to about 8, and preferably about 4 to about 6, weight percent of the additive mixture.

More preferably, in solid form, the additives comprise about 1.5 milligrams of the oxidizable metal salt per milliliter of the water sample, about 0.5 milligrams of the alkali metal compound per milliliter of the water sample and about 0.1 milligrams of the oxidation-reduction indicator per milliliter of the water sample.

In liquid form, the additives preferably comprise about 0.075 milliliters of a first aqueous solution comprising about 20 percent by weight of the oxidizable metal salt, about 0.10 milliliters of a second aqueous solution comprising about 5 percent by weight of the alkali metal compound and about 0.20 milliliters of a third aqueous solution comprising about 0.5 percent by weight of the oxidation-reduction indicator per 10 milliliters of water sample. In view of the present disclosure, one skilled in the art would understand that these preferred amounts are only approximations and that these amounts may vary.

Preferably, the additives are used in the form of dry tablets. The oxidizable metal salt and oxidation-

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reduction indicator may be combined in a single tablet. A second tablet may contain the alkali metal compound, which, due to its reactivity, generally must be kept separate from the other components of the additive mixture until added to the water sample. Various methods of forming the tablets using conventional equipment and techniques is well within the knowledge of one skilled in the art and further discussion is therefore not believed to be necessary. The tablet form is particularly advantageous for use by the home aquaculturist because of its simplicity and ease of handling.

The additives and sample may be mixed in a variety of ways. Preferably, a container or test tube of predetermined volume sufficient to accommodate the water sample and additives is substantially filled with a water sample. The oxidizable metal salt and indicator (in tablet form or otherwise) is added to the sample in the container and then the alkali metal compound is added. The container containing the sample and all additive components of the present invention is covered and the mixture may be agitated in the substantial absence of oxygen. The next step of the method may then be carried out.

The next method step is determining the concentration of dissolved oxygen in the liquid sample, rather than merely detecting an undetermined concentration of oxygen in the liquid sample. The oxygen concentration is determined by comparing the color of the mixture to a predetermined color indicative of the concentration of dissolved oxygen in the water sample. For example, the color of the mixture may be compared to a standard reference color chart, the colors of which have been correlated to the concentration of oxygen in parts per million with respect to each indicator or combination of indicators, thus creating a standard color comparison chart.

The use of a simple, standard, color comparison chart eliminates the need for sophisticated and costly

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testing equipment which is not easily adaptable to home use. Use of the present invention is not limited to the home aquaculturist for aquariums and fish ponds, but it may also be used in industrial and commercial applications, to name a few.

5 Table 2 shows the color change exhibited by the indicators methylene blue, safranine O and the preferred combination of 80 weight percent methylene blue and 20 weight percent safranine O based upon different
10 concentrations of oxygen in test samples. The additives include the indicator, ammonium ferrous sulfate and sodium hydroxide in the following proportions. Approximately 15.0 milligrams of ammonium ferrous sulfate, 5.0 milligrams of sodium hydroxide and 1.0 milligram of indicator, each in
15 solid form were added to each 10 ml water sample to form a mixture. The indicator and ammonium ferrous sulfate may be combined prior to addition to the test sample.

Each mixture was shaken and the resulting color exhibited was compared to a standard color chart, namely,
20 Pantone® Color Formula Guide 747XR, 2nd Edition (1990-91), which is commercially available from Pantone, Inc. of Moonachie, New Jersey, U.S.A. However, one skilled in the art would understand that any standard color chart may be used to determine the quantity of oxygen in parts per
25 million based upon the chosen indicator, as long as it is used consistently so that comparison standards can be established.

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TABLE 2

	<u>Concentration of Oxygen (ppm)</u>	<u>Methylene Blue</u>	<u>Safranin O</u>	<u>Methylene Blue and Safranin O</u>
	0	317-C*	317-C*	317-C*
5	2	317-C*	176-c (pinkish)	176-C (pinkish)
	4	317-C*	177-C (pinkish)	177-C (pinkish)
	6	284-C (blue)	178-C (dark pink)	2583-C (red)
	8	286-C (dark blue)	179-C (dark red)	2603-C (purplish)
	10	288-C (dark blue)	180-C (dark red)	2623-C (dark Purple)
10	12	484-C**	180-C (dark red)	175-C (dark brown)

* Indicators (methylene blue & safranin O) are colorless, but the test sample had color 317-C (light blue) corresponding to the iron salt in solution.

15 ** Color corresponds to that of precipitate of ferric hydroxide (brown).

Referring to Table 2, at a dissolved oxygen concentration of 0 ppm, the combined indicator of methylene blue and safranin O exhibited a light blue color corresponding to color 317-C of the Pantone® chart. The color of the test sample may be attributed to the iron salt in solution and does not adversely affect the test results, which are based upon changes in color. At a dissolved oxygen content of 6 ppm, the sample exhibited a red color corresponding to color 2583-C of the Pantone® chart.

25 The color comparison is generally not carried out until the color of the solution remains virtually unchanged. This steady state color may be achieved in as short a period of time as one minute, although one skilled in the art would understand in view of this disclosure that the time for the color change to reach steady state may vary depending on such factors as the concentration of the dissolved oxygen in the test sample and the choice of components in the additive mixture.

35 The present invention is simple enough for the home aquaculturist to employ yet meets the rigorous accuracy and precision requirements necessary to determine low concentrations of dissolved oxygen in water. The additives may be formed in a convenient solid form, such as tablets. In addition, the present method eliminates the need for

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expensive analysis equipment such as spectrophotometers and titrators. The present method reduces the possibility of injury to the tester since it is not necessary to the method to use an ampul (typically glass) having a fracturable tip. Therefore, the present invention fulfills a long-felt need in the art for a simple, reliable and accurate test to determine the concentration of dissolved oxygen in a water sample.

It will be appreciated by those skilled in the art in view of this disclosure that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but is intended to cover modifications which are within the spirit and scope of the present invention as described by the appended claims.

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CLAIMS

1. A method for determining the concentration of dissolved oxygen in a liquid water sample comprising the steps:

(a) providing a liquid water sample of predetermined volume, the oxygen content of which is to be determined;

(b) mixing additives with the liquid water sample to form a mixture, the additives comprising an oxidizable metal salt comprising a metal cation and an anion selected from an inorganic anion or an organic anion which, with the cation, results in a water soluble oxidizable metal salt, at least one water soluble alkali metal compound selected from an alkali metal oxide, or an alkali metal hydroxide or a salt of an alkali metal and weak acid; and at least one oxidation-reduction indicator capable of a color change upon oxidation of the oxidizable metal salt during contact with dissolved oxygen in the liquid sample to a color indicative of the concentration of dissolved oxygen in the liquid sample;

(c) preventing any substantial contact of the mixture with atmospheric oxygen; and

(d) determining the concentration of dissolved oxygen in the liquid sample by comparing the color of the mixture to a predetermined color indicative of the concentration of dissolved oxygen in the water sample.

2. A method according to claim 15 wherein the additives comprise an additive mixture of solids comprising about 65 to about 75 weight percent of the oxidizable metal salt, about 20 to about 25 weight percent of the alkali metal compound and about 1 to about 8 weight percent of the oxidation-reduction indicator, the weight percents being weight percents of the additive mixture.

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3. A method according to claim 2 wherein the additives comprise about 70 to about 73 weight percent of the oxidizable metal salt, about 22 to about 24 weight percent of the alkali metal compound and about 4 to about 6 weight percent of the oxidation-reduction indicator, the weight percents being weight percents of the additive mixture.

4. A method according to claim 1 wherein the mixture is formed by mixing with the liquid water sample about 1.5 milligrams of the oxidizable metal salt in solid form per milliliter of the liquid water sample, about 0.5 milligrams of the alkali metal compound in solid form per milliliter of the liquid water sample, and about 0.1 milligrams of the oxidation-reduction indicator per milliliter of liquid the water sample.

5. A method according to claim 1 wherein the mixture is formed by mixing, at a concentration per about 10 milliliters of the liquid water sample, about 0.075 milliliters of a first aqueous solution comprising about 20 percent by weight oxidizable metal salt, about 0.10 milliliters of a second aqueous solution comprising about 5 percent by weight alkali metal compound and about 0.20 milliliters of a third aqueous solution comprising about 0.5 percent by weight oxidation-reduction indicator per 10 milliliters of the liquid water sample.

6. A method according to claim 1 wherein the metal salt is a soluble ferrous salt.

7. A method according to claim 6 wherein the metal salt is ammonium ferrous sulfate.

8. A method according to claim 1 wherein the alkali metal compound is selected from lithium hydroxide,

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sodium hydroxide, potassium hydroxide, rubidium hydroxide or cesium hydroxide.

9. A method according to claim 1 wherein the oxidation-reduction indicator is selected from the group consisting of indigo monosulfonate, phenosafranine, indigo tetrasulfonate, methylene blue, safranine O and mixtures thereof.

10. A method according to claim 1 wherein the oxidation-reduction indicator is substantially colorless when the metal salt is in a lower oxidation state and is colored when the metal salt is in a higher oxidation state.

11. A method according to claim 1 wherein a plurality of oxidation-reduction indicators are present, each indicator having a different color upon oxidation of the metal salt, wherein different colors indicate different concentrations of dissolved oxygen in the liquid water sample.

12. A method according to claim 1 wherein the metal cation is a transition metal cation selected from scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel or copper.

13. A method according to claim 1 wherein the anion is selected from chloride, sulfate, ammonium sulfate, citrate or ethylenediaminetetraacetate.

14. A method according to claim 1 wherein the oxidizable metal salt is in tablet form.

15. A method according to claim 1 wherein the alkali metal compound is in the form of a tablet.

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16. A method according to claim 1 wherein first and second components are mixed with the liquid water sample, the first component comprising the oxidizable metal salt and the indicator, and the second component comprising the alkali metal compound.

17. A method according to claim 16 wherein each of the first and second components is in tablet form.

18. The method of claim 1 wherein the liquid water sample is representative of a body of water for which the concentration of dissolved oxygen in the body of water is to be determined.

19. A method according to claim 18 wherein the body of water is an aquarium or a fish pond.

INTERNATIONAL SEARCH REPORT

 International application No.
 PCT/US95/06017
A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G01N 21/00, 21/75, 33/00, 33/18

US CL : 436/62, 127, 136, 138, 164, 166

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/62, 127, 136, 138, 164, 166

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,169,811 (YOSHIKAWA ET AL.) 02 October 1979, column 1, lines 5-14, 40-41; column 2, lines 11-20; column 4, lines 63-68; column 5, lines 19-20.	1, 6-9, 12
Y	JP, A, 54-46186 (TOPPAN PRINTING KK) 11 April 1979, see Abstract.	1, 12
X	US, A, 4,349,509 (YOSHIKAWA ET AL.) 14 September 1982, column 1, lines 65-68; column 2, lines 12-19, 26-32; column 3, lines 2-10, 25-29.	1, 6-9, 12, 14-17
Y	"WATER ANALYSIS HANDBOOK" published 1989 by Hach Company (Loveland, Colorado), pages 442-452.	1



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

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Date of mailing of the international search report

21 AUG 1995

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/06017

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, -3,626,742 (HOGAN ET AL.) 14 December 1971, column 1, lines 41-51; column 2, lines 8-10.	1, 12
Y	US, A, 3,663,176 (CAGLE ET AL.) 16 May 1972, column 1, lines 34-40; column 2, lines 17-22.	1, 12, 13
Y	US, A, 2,823,985 (STRANGE) 18 February 1958, column 1, lines 63-72; column 2, lines 1-8.	1, 10-11, 13
Y	US, A, 2,967,092 (BUCHOFF ET AL.) 03 January 1961, column 2, lines 64-71.	1, 8, 11
Y	US, A, 3,119,670 (MITCHELL ET AL.) 28 January 1964, column 1, lines 63-69.	1
Y	US, A, 5,358,876 (INOUE ET AL.) 25 October 1994, column 2, lines 61-68; column 3, lines 23-34.	9
Y	US, A, 4,023,934 (SPINNER ET AL.) 17 May 1977, column 3, lines 32-33.	9
Y	US, A, 2,387,244 (COMPTON ET AL.) 23 October 1945, column 1, lines 4-7, 40-43.	15
Y	US, A, 3,375,078 (DENDY) 26 March 1968, column 1, lines 18-27.	18-19
X,E	US, A, 5,415,809 (ELSON ET AL.) 16 May 1995, see entire document.	1-19

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/06017

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE (elson, yoshpa, oxygen, metal salt, oxidation, ferrous, ammonium ferrous sulfate, indicator, dye, tracer, tag, indigo monosulfonate, phenosafranine, indigo tetrasulfonate, methylene blue, safranine O)