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3,689,223

DETECTION METHOD

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7 Claims

ABSTRACT OF THE DISCLOSURE

A method for indicating the presence of non-volatile compounds comprising reacting a solution containing non-volatile nucleophiles and reducing agents, and a detecting solution comprising hexamminecobalt (III) perchlorate and a fluid selected from the group consisting of a borate buffer and dimethylsulfoxide, producing ammonia as the indicator for the presence of the non-volatile compounds.

The invention described herein may be manufactured, used, and licensed by or for the Government for governmental purposes without the payment to us of any royalty thereon.

This invention is directed to detect relatively non-volatile substances by means of a volatile compound.

It is the further object of this invention to detect various compounds with hexamminecobalt (III) perchlorate, thus liberating ammonia which is readily detectable.

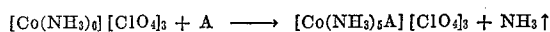
The method of this invention is directed to the qualitative testing of relatively non-volatile compounds which liberate a volatile component, ammonia, after its interaction with the hexamminecobalt (III) perchlorate.

One of the advantages of our detecting system is the use of simple equipment for indicating the presence of relatively non-volatile nucleophiles and reducing agents; for example, arylamines, hydroxyaromatics, aliphatic amines, amino acids, heterocyclics, organophosphorus compounds, enzymes or haloarylaliphatics. The detecting solution can be carried in two pre-measured separate glass vial containers. The analysis is achieved by adding the suspected sample to one of the containers then adding the contents of the second container forming the final testing fluid and then observing the color change in the pH paper. This method can be performed by individuals with no laboratory training in detecting such compounds as 1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane.

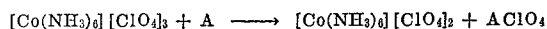
Mestres et al., Bull. Environ. Contam. Toxicol., 1, 245 (1966) determined the residues of insecticide 1,1,1 trichloro-2,2-bis(p-chlorophenyl)ethane by electron capture or microcoulometric GLC (Gas Liquid Chromatography) which requires specialized training and complicated procedures.

The compounds detected in accordance with this invention are operative in either the substitution or redox scheme below:

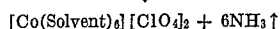
Substitution



Redox



↓
Solvent



A is detected compound.

The results of our investigation seem to indicate that displacement reactions involving the cobalt complex occur more readily in dimethyl sulfoxide, and the redox

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reactions occur more readily in the aqueous buffer. It also appears that the easily oxidizable amines give negative results in the dimethyl sulfoxide.

In a container, for example, 4 dram vial, at ambient temperatures, a solution comprising 1–10 mg. relatively non-volatile nucleophiles and reducing agents is contacted with 0.8–1.2 ml. of a borate buffer pH 9.5 and 0.8–1.2 ml. of aqueous 0.02 M hexamminecobalt (III) perchlorate. A strip of indicating paper (Hydrion paper) in a 1–10 pH range previously moistened with distilled water is suspended about 0.8–1.2 cm. over the solution and then capping the container, thus sealing the container and its contents. The paper changes color toward a more alkaline pH in a time frame up to 30 minutes thus indicating the liberation of ammonia. The pH paper does not have to be moistened with water, however, there is increased sensitivity in the ammonia detection when the paper contains water prior to its positioning over the sample containing the nucleophiles and reducing agents. We consider a negative test for the presence of nucleophiles and reducing agents when no detectable ammonia can be observed within a 30 minute time frame after sealing the container and its contents.

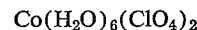
Various detecting solutions may be prepared as follows:

(a) 1 ml. of aqueous borate buffer, pH 9.55 and 1 ml. 0.02 M of aqueous hexamminecobalt (III) perchlorate; (b) 1 ml. of aqueous borate buffer, pH 9.55, and 1 ml. 0.02 M hexamminecobalt (III) perchlorate in dimethylsulfoxide; and (c) 1 ml. 0.02 M hexamminecobalt (III) in dimethylsulfoxide and 1 ml. of dimethylsulfoxide. The final concentration of the hexamminecobalt (III) perchlorate in the detecting solution is 0.008 M to 0.012 M for the liberation of ammonia.

An aqueous stock solution comprises 0.1 M boric acid and 0.1 M KCl made to volume of 1.0 liter. An aqueous solution comprises 0.1 M NaOH. The pH 9.55 borate buffer is prepared by mixing 50 ml. of said stock solution with 36.0 ml. of said aqueous 0.1 M NaOH. Other borate buffers at pH 10.00 and 10.55 were utilized in our procedure but were found to be undesirable in view of the fact that high blanks resulted.

Dimethylsulfoxide, organic solvent, was found to increase the stability of the aqueous metal complex without any detection of ammonia for at least eight days at room temperature. Other solvents such as acetonitrile and methanol were not suitable in our process since they did not sufficiently dissolve the metal complex and, in addition, N,N-dimethylformamide was not satisfactory since it interfered with the test for ammonia. The stability of the aqueous metal complex can be increased to thirteen days with aqueous 0.02 M hexamminecobalt (III) in combination with dimethylsulfoxide in a ratio of 3:1 respectively and stability to seventeen days with the 1:1 ratio of the said aqueous hexamminecobalt (III) with the dimethylsulfoxide.

The Bejerrum, J. et al. general method described in Inorg. Syn. II, 216 (1946) for the preparation of hexamminecobalt (III) salt was utilized to prepare the corresponding perchlorate. A mixture composed of 150 ml. aqueous solution comprising 23.5 g. (0.2 mole) of NH_4ClO_4 was heated, then adding a 25 ml. aqueous solution containing 36.6 g. (0.1 mole) of



followed with the addition of 1.0 g. of activated charcoal, adding 45 ml. of concentrated ammonium hydroxide, forming some precipitate which was not separated, and thus slowly adding with stirring 20 ml. of 30 percent H_2O_2 (0.2 mole) with subsequent heating of about 5.0 minutes following by standing for about 1 hour forming a precipitate containing the desired product. The latter

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precipitate was separated and washed with cold water (5° C.). The said latter precipitate was dissolved by heating in about 1500 ml. aqueous solution at pH 3-5 containing sufficient HClO₄ to produce said pH and the charcoal was then filtered off from the hot solution. To the latter solution about 100 ml. of concentrated HClO₄ was added thus precipitating the desired product. The entire solution was cooled to about 5° C., the product separated and then washed with cold water, ethanol and dried. The product was recrystallized from water.

Analysis.—Calc'd for Co(NH₃)₆(ClO₄)₃ (percent): N, 18.3; Cl, 23.2; Co, 12.8. Found (percent): N, 18.8; Cl, 23.1; Co, 12.5.

Compounds detected with the detecting solution comprising aqueous borate buffer at pH 9.55 and aqueous hexamminecobalt (III) perchlorate in a time range of (a) 15-30 minutes are m-aminobenzoic acid, 2-amino-4-nitrophenol, 2-amino-5-nitropyridine, aniline, N,N-diethyl-2-mercaptoethylamine, 1,6-hexanediamine, phenol, 3-quinuclidinol, starch, N,N,N',N'-tetramethyl-p-phenylenediamine, uric acid and O,O-diethyl-p-nitrophenyl thiophosphate (Parathion) (b) less than 15 minutes are $\pm$ aminocaproic acid, 4-amino-2,6-dibromophenol, 5-amino-1,3-dimethylbenzene (3,5-xylidine), m-aminophenol, p-aminophenol, 2-aminoresorcinol, p-(1-ethylpropyl) aniline, 2-methoxyaniline (O-anisidine), 2,6-dichloroindophenol sodium salt, N,N-diethyl-p-phenylenediamine, N, N-dimethyl-p-phenylenediamine, hydroquinone, pancreatin, peptone, p-phenylenediamine, sodium sulfite, trypsin, L-tyrosine, S-(1,2-dicarbethoxyethyl)-O,O-dimethyldithiophosphate (Malathion) 25% ethyl par-nitrophenylthionobenzene phosphonate (EPN 300), 1,1,1-trichloro-2, 2-bis(p-chlorophenyl)ethane (DDT).

We found that some compounds not detectable in the detecting solution containing the aqueous hexamminecobalt (III) perchlorate and aqueous borate buffer were detected when substituting the dimethylsulfoxide for the aqueous borate buffer in the detecting solution. The detected compounds are p-aminoacetanilide^b, 4'-aminoacetophenone^a, 3'-aminoacetophenone^a, 2-aminobiphenyl(O-phenylaniline)^b, m-bromoaniline^b, didodecylamine^a, ethyl p-aminobenzoate^b. The superscripts *a* and *b* indicate the detection time of less than 15 minutes and 15 to 30 minutes respectively.

Some compounds are detected when either the aqueous borate buffer or dimethylsulfoxide is used with the aqueous hexamminecobalt (III) perchlorate in the detecting solution. The compounds are 5-amino-1,3-dimethylbenzene (3,5-xylidine)^{x,z}, p-aminophenol^{x,y}, p-phenylenediamine^{x,y} or sodium sulfite^{x,y}. The superscripts *x* indicate a detection time of less than 15 minutes with the borate buffer, *y* a detection time of less than 15 minutes with dimethylsulfoxide, and *z* indicates a detection time of 15-30 minutes with dimethylsulfoxide.

A detecting solution comprising aqueous hexamminecobalt (III) perchlorate with either borate buffer or dimethylsulfoxide can be utilized to detect 5-amino-1,3-dimethylbenzene (3,5-xylidine), p-aminophenol, p-phenylenediamine and sodium sulfite.

The qualitative test of this invention can be employed to differentiate between compounds, that is, some compounds detectable with the utilization of borate buffer while not detectable when dimethylsulfoxide is employed in place of the borate buffer with the aqueous hexamminecobalt (III) perchlorate. The compounds detectable in less than 15 minutes of time are $\pm$ α -aminocaproic acid, 4-amino-2,6-dibromophenol, m-aminophenol, 2-aminoresorcinol, p-(1-ethylpropyl) aniline, 2-methoxyaniline, N,N-diethyl-p-phenylenediamine, N,N-diethyl-p-phenylenediamine, pancreatin, peptone, trypsin or L-tyrosine. The compounds detectable in less than 30 minutes are m-aminobenzoic acid, 2-amino-4-nitrophenol, aniline, phenol, starch, N,N,N',N'-tetramethyl-p-phenylenediamine or uric acid.

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Table 1, below, illustrates the effect of water, based on volume, in the final testing fluid utilizing the detecting solution comprising hexamminecobalt (III) perchlorate in dimethylsulfoxide for the liberation of ammonia with various compounds.

Compound tested	Water added		
	0%	25%	50%
10 p-Aminoacetanilidine.....	2	N	N
3-aminoacetophenone.....	1	1	N
4-aminoacetophenone.....	1	1	N
2-aminobiphenyl.....	2	2	N
5-amino-1,3-dimethylbenzene(3,5-xylidine).....	2	2	N
p-Aminophenol.....	1	1	1
m-Bromoaniline.....	2	N	N
Di-n-dodecylamine.....	1	N	N
15 p-Phenylenediamine.....	1	1	1
Potassium iodide.....	1	2	N
Sodium sulfite.....	1	1	1

NOTE.—N refers to no ammonia detection in 30 min.; 2 means ammonia was detected in 15-30 min.; 1 indicates ammonia was detected in less than 15 min.

Example 1

(a) In a container, an unknown sample containing 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane is contacted with a detecting solution comprising 1 ml. of borate buffer (pH 9.55) and 1 ml. of 0.02 M hexamminecobalt (III) perchlorate forming a final solution. A strip of water moistened pH paper is suspended about 1 cm. over the fluid and then capping the container. The paper changes color to a more alkaline pH within less than 15 minutes indicating at least 15% of ammonia liberated which indicates the presence of the insecticide 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane.

(b) The procedure in (a) supra, was repeated with the exception of substituting the insecticides O,O-diethyl-p-nitrophenylthiophosphate or O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate for the 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane giving rise to the presence of the insecticides.

Example 2

(a) In a container, an unknown sample containing L-tyrosine is contacted with a detecting solution comprising 1 ml. of borate buffer (pH 9.55) and 1 ml. of 0.02 M hexamminecobalt (III) perchlorate forming a final fluid. A strip of water moistened pH paper is suspended about 0.8 cm. over the fluid and then sealing the container. The paper changes color to a more alkaline pH indicating the liberation of ammonia in less than 15 minutes after sealing the container.

(b) The procedure in (a), supra, was repeated with the exception of substituting 1 ml. dimethylsulfoxide for the borate buffer (pH 9.55) in the detecting solution. There was no color change in the pH paper in a 30 minute period after sealing the container, thus indicating no liberation of ammonia.

(c) The procedures in (a) and (b), supra, were followed with the exception of uric acid, phenol, M-aminophenol or peptone for the L-tyrosine giving rise to positive results with the utilization of the borate buffer under (a) and negative results with the employment of the dimethyl sulfoxide under (b).

Example 3

(a) In a container, an unknown sample containing p-aminoacetanilide is contacted with a detecting solution comprising 1 ml. of borate buffer (pH 9.55) and 1 ml. of 0.02 M hexamminecobalt (III) perchlorate forming a final fluid. A strip of water moistened pH paper is suspended about 1.2 cm. over the fluid and then sealing the container. The paper did not change color to a more alkaline pH indicating no liberation of ammonia in less than 30 minutes thus a negative test for p-aminoacetanilide.

(b) The procedure in (a), supra, was repeated with the exception of substituting 1 ml. dimethylsulfoxide for the borate buffer in the detecting solution giving a posi-

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tive test, that is, changing the moistened paper to a more alkaline pH in less than 30 minutes.

(c) The procedures in (a) and (b), supra, were followed with the exception of 4'-aminoacetophenone or m-bromoaniline for the p-aminoacetanilide giving rise to negative results with the employment of the borate buffer under (a) and positive results with the utilization of dimethylsulfoxide under (b).

Example 4

(a) A container containing an unknown sample comprising 5-amino-1,3-dimethylbenzene is contacted with a detecting solution comprising 1.0 ml. of borate buffer and 1.0 ml. of 0.02 M aqueous hexamminecobalt (III) perchlorate forming a final fluid. A strip of moistened pH paper is suspended about 2.0 cm. above the fluid and then capping the container. The paper changes color to a more alkaline pH in less than 15 minutes.

(b) The procedure in (a), supra, was followed with the exception of substituting 1.0 ml. of dimethylsulfoxide for the borate buffer in the detecting solution giving rise to a color change in the paper to a more alkaline pH in 15 to 30 minutes.

Example 5

(a) A container containing an unknown sample comprising potassium iodide is contacted with a detecting solution comprising 1 ml. dimethylsulfoxide and 1 ml. 0.02 M hexamminecobalt (III) perchlorate in dimethylsulfoxide forming a final fluid. A strip of pH paper previously moistened with water is suspended about 2.0 cm. above the final solution and then sealing the container and its contents. The paper changed to a more alkaline pH in less than 15 minutes.

(b) The procedure in (a), supra, was followed with the exception of a detecting solution comprising 0.75 ml. dimethylsulfoxide, 0.25 ml. of water, and 1 ml. of 0.02 M hexamminecobalt (III) perchlorate in dimethylsulfoxide. The paper changed to a more alkaline pH between 15-30 minutes.

(c) The procedure in (a), supra, was followed with the exception of a detecting solution comprising 0.50 ml. dimethylsulfoxide, 0.50 ml. water, and 1 ml. of 0.02 M hexamminecobalt (III) perchlorate in dimethylsulfoxide. The paper did not change to a more alkaline pH in 30 minutes.

We claim:

1. In a method for indicating the presence of non-volatile compounds selected from the group consisting of

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arylamines, hydroxyaromatics, aliphatic amines, amino acids, heterocyclics, haloaryl aliphatics, organophosphorus and enzymes, the steps comprising reacting the non-volatile compound with a detecting solution containing hexamminecobalt perchlorate and a fluid selected from the group consisting of borate buffer and dimethylsulfoxide, said reaction step producing ammonia, said ammonia contacting an indicator paper and changing color of the paper to indicate an increased pH value.

2. The method according to claim 1, wherein the organophosphorus compounds are O,O-diethyl-p-nitrophenyl thiophosphate, S(1,2-dicarbethoxyethyl)-O,O-dimethyldithiophosphate or ethyl-p-nitro-phenylthionobenzene phosphonate and the haloarylaliphatics of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane.

3. The method according to claim 1, wherein the hydroxyaromatics are 2-amino-4-nitrophenol, phenol, 4-amino-2,6-dibromophenol, aminophenol, 2-amino-resorcinol, 2,6-dichloroindophenol sodium salt, hydroquinone, or quinuclidinol.

4. The method according to claim 1, wherein the aliphatic amines are 2-mercaptoethylamine, 1,6-hexanediamine, or didodecylamine.

5. The method according to claim 1, wherein the arylamines are N,N,N',N'-tetramethyl-p-phenylenediamine, dimethylbenzene, N,N-diethyl-p-phenylenediamine, N,N-dimethyl-p-phenylenediamine, p-phenylenediamine, aniline, 2-methoxyaniline, 2-aminobiphenyl m-bromoaniline, 5-amino-1, 3-dimethylbenzene, p-aminoacetanilide or aminoacetophenone.

6. The method according to claim 1, wherein the amino acids are aminobenzoic acid or tyrosine.

7. The methods according to claim 1, wherein the heterocyclics are 2-amino-5-nitropyridine or uric acid.

References Cited

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