

Determination of Cobalt in Beer

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COBALT IS PRESENT in trace amounts in virtually all living tissues. The raw materials used for brewing—barley, corn, rice, and hops—are no exception. As a consequence, commercial beer normally contains a minute concentration of cobaltous ion, usually a few hundredths of a part per million. Such trace amounts of cobalt are of no consequence to the properties of beer, and brewers have, until recently, had no particular reason, other than scientific curiosity, for analyzing beer for its cobalt content. Although a number of assay procedures have been available for the quantitative estimation of low concentrations of cobalt in various materials, these had not generally been applied to beer.

This situation was changed by Thorne and Helm's discovery (5) that the addition of cobaltous salts to beer to give about 1 ppm. cobalt is beneficial, improving foam properties and inhibiting gushing.

Since that discovery, millions of barrels of beer have been treated with cobalt salts by brewers outside the U. S., and, as an obvious consequence, the brewing chemist abroad required an analytical method for estimation of the cobalt content in beer.

Harold and Szobolotzky (2) of Carlton and United Breweries in Melbourne, Australia, recently published a method for the determination of cobalt concentrations in beer ranging from 0 to 2 ppm., with a claimed accuracy of ± 0.02 ppm.

Until recently, because of the stringent requirements of the 1958 Amendment to the U. S. Food and Drug Act, it was not permissible to add cobalt to beer sold in the United States. This restriction was not a result of any known harmful effect of cobalt salts. Under the

law, nothing new can be added to foods until data are presented to the Food and Drug Administration proving that the addition is both useful and safe. This evidence was submitted to F.D.A., and approval for the addition of up to 1.2 ppm. cobalt (calculated as the element) to beer has now been granted by the U. S. government (6).

A substantial quantity of beer brewed in the United States is now being treated with cobalt, under Thorne's patent (5). Accordingly, the interest in analytical methods for cobalt in beer has spread from foreign to domestic brewers.

The brewer has two needs as to cobalt methods: 1) a qualitative method to check whether a beer sample does or does not contain added cobalt, and

2) a quantitative method to determine how much cobalt is present. The quantitative method is of special importance, in that too much cobalt (over 1.2 ppm.) is not allowed, and too little is relatively ineffective.

In this paper we present several qualitative and quantitative procedures, together with their advantages and shortcomings, to help the brewing chemist select a method suitable for his needs.

Qualitative Methods

It is sometimes useful for the brewer to have a simple procedure whereby he can determine quickly whether or not a given sample of beer contains added cobalt. Since beers to which cobalt has not been introduced contain only a few hundredths of a ppm. of cobalt, in sharp contrast to the 1 ppm. when cobalt is added, it is feasible to find reagents which give a color in the latter case and not in the former. Doubtlessly,

many suitable procedures could be devised, since the literature contains a variety of colorimetric reagents for cobalt (2). We present two methods that we have found useful.

The first such method was devised by Thorne and Helm of the Alfred Jorgensen Laboratory for Fermentation'. This

method depends on the pink color of 'a trivalent cobalt complex, 2-nitroso-1-naphthol (7), and extraction of the colored complex from beer (which masks its color) with toluene. Method

Reagents: (1) 2-Nitroso-1-naphthol freshly recrystallized from water; 0.2 gm. dissolved in 20 ml, glacial acetic acid.

(2) Sodium hydroxide, 2N.

(3) Toluene. Technical grade is satisfactory.

(4) Teepol. Secondary sodium alkyl sulfate, available from Shell Chemical Corporation.

Procedure: Place 5 ml. of decarbonated beer in a 50-ml. glass-stoppered separatory funnel and mix thoroughly with 1 ml. 2-nitroso-1-naphthol solution. After a waiting period of at least 3 min. to allow for oxidation, add 4 ml. toluene and 1 drop Teepol and shake the mixture vigorously for 1 min. After the phases have separated, discard the lower aqueous phase. Then shake the toluene layer with 15 ml. 2N NaOH solution. (The phases separate quickly.)

If the beer sample contains added cobalt, the upper (toluene) layer will be colored pink. Since the pink color is not intense, it is desirable to run a beer known not to contain cobalt as a blank, side by side with the sample, for comparison purposes.

The second method is a modification of the quantitative procedure of Harold and Szobolotzky (2). This

method is faster than Thorne and

Helm's, and because of the relatively intense color of the complex formed by the interaction of cobalt and 3-meth

oxy-2-nitrosophenol, it can be used without a blank.

By using either of these methods, the laboratory can answer the question of whether a beer does or does not contain added cobalt, in about 20 or 25 min.

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Method

Reagents: (1) 2-Methoxy-2-nitrosophenol (available from K & K Laboratories, Plainview, New York). The dry solid should be purified by ether extraction before use. Use a saturated aqueous solution.

(2) NH₄OH: concentrated.

(3) CCl₄. Technical grade is satisfactory.

Procedure: Place 25 ml. of decarbonated beer in a glass-stoppered graduated cylinder. Add 5 ml. of saturated aqueous 3-methoxy-2-nitrosophenol and 8 drops of NH₄OH. Shake the mixture vigorously for 1 min. After a 10-min. wait, add 4 ml. CCl₄, and shake the mixture again. (The phases separate quickly.) An orange-red

color appears in the

CCI₄ (bottom) phase, if added cobalt

is present in the beer sample. The color is sufficiently intense that a blank for visual comparison is not essential.

Both of the qualitative methods described above are dependent upon the presence of free cobaltous ion. If the beer contains an added chelating agent which binds cobaltous ion more tightly than the reagent used to develop color, a negative test will result. It should be noted that, from a practical point of view, addition of both cobalt and chelating agent to the same beer is pointless, as the two additives will more or less cancel each other out. In other words, chelating agents will negate the beneficial effects of cobalt on foam and gushing. In effect, then, the qualitative test will determine whether "effective cobalt" is present.

Iron in the amounts normally present in beer does not interfere with either of the above tests.

Quantitative Methods

For control purposes, the brewer desires a simple, accurate, and reliable assay method.

We shall present here two such methods. One of these, the thiocyanate method, was submitted to, and was accepted by, the U. S. Food and Drug Administration in our petition to that agency for approval of the use of cobalt salts in beer. In our opinion a method utilizing cyclohexanetrione trioxime, developed subsequently, offers definite advantages for routine work over other available methods.

Potassium Thiocyanate Procedures

The formation of a blue complex by the reaction of cobalt ion with thiocyanate ion is well known, and has formed the basis for numerous quantitative analytical procedures. The intensity of color of this complex increases very rapidly with increased concentration of water-miscible organic solvent; we have adopted and modified the procedure of Kitson (3), in which color absorbency is measured in 50% acetone.

The major disadvantage of the thiocyanate method is that ions normally present in beer, other than cobalt, interfere with the determination, so the method is unsatisfactory unless these interfering ions are removed.

We eliminated such interference by selectively absorbing and then eluting cobaltous ion, using a Dowex 2 (Cl) anion-exchange resin. While this method is relatively lengthy because of the ion-exchange step, it has the advantage of complete specificity. For this reason it was the method chosen by us for submission to the Food and Drug Administration.

Experimental

Reagents: (1) Standard cobalt solution. Cobalt chloride hexahydrate. Convert Baker Analytical Reagent Grade of known assay to the anhydrous form by heating to 140°C. for 4 hr. Dissolve the anhydrous salt (0.554 gm. for 99.341 CoCl₂) in water and make up to 1 liter. One ml. of this solution contains 0.250 mg. Co. Dilution of 10 ml. of this solution to 100 ml. gives a working solution containing 0.025 mg. per ml. Use aliquots of the working solution in preparing the standard calibration curve. (2) Potassium thiocyanate, A.R. Use a 60% aqueous solution of the dried salt. (3) Dowex 2

(Cl) . Prepare the exchange column by packing a 10-mm. i.d. glass tube with Dowex 2 (Cl) to a height of 150 mm. and passing 30 ml. 0.01N HCl followed by 5 ml. 9N HCl through the column. (4) HCl: conc., 9N, 4N, and 0.01N. (5) Ammonium acetate, A.R., 50% aqueous solution. (6) Acetone. A.R.

Preparation of Standard Curve: Place aliquots of the working solution, chosen to contain amounts of cobalt giving concentrations up to about 2 ppm. cobalt, if present in 50-ml. portions of beer, in 50-ml. beakers and evaporate to dryness over a steam bath. To each beaker add 5.0 ml. water, 2.5 ml. 50% ammonium acetate, and 2.5 ml. 60% potassium thiocyanate. After mixing well, add 10.0 ml. acetone and again mix the solution. Read the optical density of each mixture at 325 mμ in a Beckman Model 1)U spectrophotometer, using a blue phototube. Use a similar mixture containing all reagents except the cobalt chloride solution to zero the apparatus. Results obtained are shown graphically in Fig. 1.

Procedure: Degas the beer to be analyzed and add (1.1 ml. conc. HCl). Evaporate the acidified beer to dryness in a Vycor evaporating dish over a steam bath, or under an infrared heat lamp, or over a ring gas burner under an aluminum reflector.

Ignite the residue at low heat (650°C.) in a muffle furnace until free of carbon. usually in about 1/2 hr., and allow to

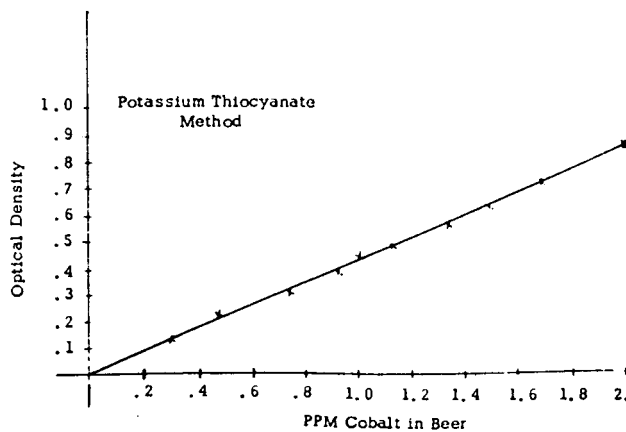


Fig. 1. Potassium thiocyanate method.

cool. If difficulty is encountered removing the last traces of carbon, allow the dish to cool, moisten the ash with 4N HCl, dry the mixture over a small flame, and return the dish to the muffle for about 15 min. This treatment should yield a carbon-free ash.

Take up the cool ash with 4 ml. of 9N HCl, and pass this solution through a prepared exchange column of Dowex 2 (Cl). Use four additional 5-ml. portions of 9N HCl to rinse the evaporating dish, and pass the washings also through the column. To ensure complete absorption of cobalt, the flow rate through the column should not exceed 2 drops per sec.

At this same flow rate, elute cobalt from the column by passing through first 30 ml. 4N HCl and then 10-15 ml. of 0.01N HCl. Collect the eluate in a 100-ml. beaker and evaporate just to dryness over a small flame.

To the residue add 5.0 ml. water, 2.5 ml. 50% ammonium acetate solution, and 2.5 ml. 60% potassium thiocyanate solution. After mixing well, add 10.0 ml. acetone and mix the solution.

Determine optical density of the resultant blue solution, just as with the standard cobalt solutions. To zero the spectrometer, use as a blank a mixture of 5.0 ml. water, 2.5 ml. 50% ammonium acetate solution, 2.5 ml. 60% potassium thiocyanate solution, and 10.0 ml. acetone.

Validation Data. Analysis of Beer Containing Known Amounts of Added Cobalt, Aliquots of the "working solution containing a known concentration of cobalt chloride were placed in 50-ml samples of beer containing no previous

ly added cobalt. Cobalt concentration were determined by the KCNS method described above. Results are shown in Table I.

1,2,3-Cyclohexanetrione trioxime (CHTT) has recently been used as a

reagent to determine cobalt colorimetrically (1). In the presence of air, which acts as an oxidizing agent, cobalt forms a stable yellow trivalent complex with CHTT. This complex is suitable for precise analytical measurement of cobalt concentrations, by determination of light absorbance of the complex at pH 3 to 4 at 400 m μ .

This method has been found to be readily adaptable to the relevant range of cobalt concentrations in beer. There is no significant interference from other metal ions at concentrations normally present in beer. Iron at concentrations of about 0.4 ppm. and above will give slightly high results. In the rare instance of a beer containing above 0.4 ppm. iron, the accuracy of the cobalt

ISO determination will be improved by isolation of cobalt by the ion-exchange technique described for the KCNS method.

Use of CHTT, then, has a marked advantage over the KCNS method in that the ion-exchange step can be eliminated.

Experimental

Reagents: (1) Standard cobalt solution. Cobalt chloride hexahydrate. Convert Baker Analytical Reagent Grade of known assay to the anhydrous form by heating to 140°C. for 4 hr. Dissolve the anhydrous salt (55.4 mg. for 99.3% CoCl₂) in water and make up to 250 ml., giving a concentration of 0.004 mg. Co/ml. Dilute 10 ml. of this solution to 100 ml., giving a working solution containing 0.010 mg. Co per ml. (2) 1,2,3-Cyclohexanetrione trioxime. Eastman Kodak No. 7660, white label. Dissolve 147.3 mg. in 100 ml. 95% ethanol to give a colorless 8.6 X 10⁻³ M solution. (3) HCl, 6N, 4N, and 0.1N. (4) H₂SO₄, 18N. (5) NaOH, 0.1N.

Preparation of the Standard Curve:

Choose aliquots of the working solution to contain amounts of cobalt giving concentrations up to about 2 ppm. cobalt, if present in 50-ml. portions of beer. Add each aliquot to 1.6 ml. CHTT solution in a 25-ml. graduated cylinder. adjust the pH to 3.5, using 0.1N HCl, and then bring up the total volume to 20.0 ml. with distilled water. Since air oxidation of cobalt is necessary for color development, optical density measurements should not be made less than 0.5 hr. from the time the reagents are mixed. Determine absorbencies with a Beckman Model DU spec-

TABLE I
Potassium Thiocyanate Recovery Data

COBALT		
Added to Beer	Found by Analysis	RECOVERY
ppm.	ppm.	%
0.750	0.750	100
0.875	0.860	98.3
1.00	1.02	102
1.125	1.12	99.6
1.250	1.25	100
Av. % recovery = 100%		

1,2,3-Cyclohexanetrione Trioxime Procedures

trophotometer at 400 mmu, using a blue phototube. Use a similar mixture containing no cobalt chloride to zero the apparatus.

ceding method.

Results of these determinations are

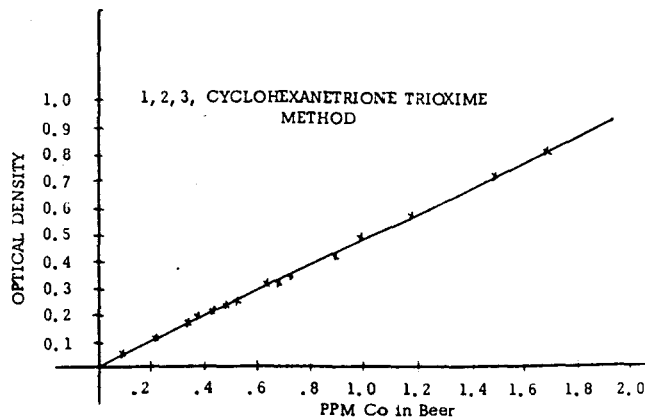


Fig. 2. 1,2,3-Cyclohexanetrione trioxime method.

Results of the calibration are presented graphically in Fig. 2.

Procedure: Add 1 ml. of 18N H₂SO₄ to 50 ml. degassed beer in a 100-ml. Vycor evaporating dish. Evaporate the acidified beer to dryness over a steam bath, or under an infrared heat lamp, or over a ring gas burner under an aluminum reflector. Convert the residue to a white ash, free of carbon, in a muffle furnace at 650°C. If necessary, complete carbon removal by moistening the cooled ash with 4N HCl and reigniting.

Moisten the carbon-free ash with 1 ml. of 6N HCl and dry under a heat lamp or over a very low flame. Add 10 ml. distilled water, cover the dish with a watch glass, warm below the boiling point for 5 min., and allow to cool. Add CHTT solution (1.6 ml.) and adjust the pH to 3.5, using either 0.1N HCl or 0.1N NaOH. Filter this solution through a small retentive paper (9 mm. S & S No. 589, Blue Ribbon) into a 25-ml. graduated cylinder. Rinse the dish and filter with distilled water, and combine the washings with the filtrate. Bring total volume to 20.0 ml. Determine optical density at 400 mmu on a Beckman Model DU spec tophtome ter, using a blue phototube. Convert optical density to cobalt concentration from Fig. 2.

Validation Data. Analysis of Beers Containing Known Amounts of Cobalt. A beer known not to contain added cobalt was chosen. Aliquots of the working solution were added to 50-ml. samples of this beer, to give known concentrations of added cobalt ranging from about 0.5 to 1.2 ppm. The con

centration of added cobalt was then determined for each sample by the pre-

presented in Table II.

Summary

Cobaltous salts are being added to beer

TABLE II
1,2,3-Cyclohexanetrione Trioxime
Recovery Data

COBALT		
Added to Beer	Found by Analysis	RECOVERY
ppm.	ppm.	%
0.51	0.51	101
0.65	0.66	101
0.90	0.90	100
1.00	1.00	100
1.10	1.09	99
1.20	1.20	100

to improve foam and to prevent gushing. Methods of detecting such addition and of quantitatively determining the amount added are therefore of interest to brewers.

Two qualitative procedures are presented by which to check quickly whether beer does or does not contain added cobalt salts in the range added by brewers, namely, about 1 ppm. cobalt calculated as the element.

Two procedures for quantitative determination of cobalt in beer are described. One procedure requires isolation of cobalt from other cations by ion exchange before colorimetric determination. The other procedure does

not require such isolation except in those rare instances where the iron content of the beer is over 0.4 ppm.

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