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AN ADAPTATION OF THE BECKMAN "B" SPECTROPHOTOMETER TO THE SHUPE AND DUBOWSKI METHOD OF ALCOHOL DETERMINATION IN BODY FLUIDS

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Described in this paper is an adaptation of the Beckman model "B" spectrophotometer to the alcohol determination method of Shupe and Dubowski. This method, as herein modified, is in routine use in the Indiana State Police Laboratory and has proved to be adequately rapid and reliable.

The idea of colorimetric determination of blood alcohol concentration is not new. The technique of Strassman (1) (1891) of preparing a set of color standards using known amounts of alcohol in dichromate-sulfuric acid solution, and comparing unknowns to these standards was also used by Heise (2) (1934), Abels (3) (1936), and Jetter (4) (1936). In 1937 A. G. Sheftel (5) outlined a method for alcohol determination using a photoelectric colorimeter. Gibson and Blotner (6) followed with their method in 1939. Anderson (7) (1942) and Shapiro (8) (1942) also used a spectrophotometer in blood alcohol determination.

After a survey of the literature was made, the method of Shupe and Dubowski (10) (1952) was selected for experimentation. These experiments were conducted using a Beckman model "DU" and Beckman model "B" spectrophotometers.

This experimentation indicated that while the method of Shupe and Dubowski as reported was quite satisfactory with null-type instruments with a long scale, it did not adapt satisfactorily to small instruments such as the Beckman "B". In a personal communication with Dr. Dubowski, he stated that his original work had been done on the Beckman model "DU", and that he had not fully considered the problems encountered when using a spectrophotometer such as the Beckman "B" or Coleman Jr. where the advantages of the "null" arrangement for balancing the instrument and the long scale cannot be employed. These problems have been satisfactorily solved by the modified method herein described.

THE METHOD

Reagents

1. 10% Sodium Tungstate Solution (100 gms. diluted with distilled water to make one liter).
2. 2/3 N Sulfuric acid (20 ml. concentrated sulfuric acid dissolved in 980 ml. distilled water).

3. Stock potassium dichromate solution .0434 Normal (2.129 gm. potassium dichromate dissolved in distilled water to make one liter).
   a. Oxidizing reagent #1 (200 ml. stock dichromate, 300 ml. distilled water; add sufficient concentrated sulfuric acid to make one liter when cool).
   b. Oxidizing reagent #2 (400 ml. stock dichromate, 100 ml. distilled water; add sufficient concentrated sulfuric acid to make one liter when cool).
4. Alcohol Standard .40 mg./ml. (To 50 ml. of distilled water add 4 ml. of 10% ethyl alcohol by weight in distilled water and make up to 100 ml. with distilled water.) This can be made up in 5 ml. ampoules for convenience.

Apparatus

1. Shupe and Dubowski distilling apparatus.[1]
2. One, two, five, and ten ml. Ostwald-Folin pipettes.
3. Hot water bath.
4. Beckman model "B" Spectrophotometer.

Procedure

1. Place 10 ml. of distilled water in the distilling flask. Pipette exactly 2.00 ml. of the blood sample to be analyzed into the flask. Wash down the pipette with two portions of distilled water. Add 5 ml. of 2/3 N Sulfuric acid and 5 ml. 10% sodium tungstate solution. Mix. This will cause the proteins in the blood to precipitate.
2. Attach the flask to the distilling apparatus. A silicone lubricant should be used on the glass ball joint to insure a tight seal. Be sure the clamp is screwed tight. Heat the solution slowly at first. When the precipitate floats to the top, more heat may be applied. This prevents frothing and bumping, and no further precautions are necessary. Collect 10 ml. of the distillate in a 10 ml. volumetric flask, and shake well. The total distilling time should be about 10 minutes.
3. To one test tube add 1 ml. distilled water (this will be the "water blank"), to another, 1 ml. of the alcohol standard (this will be the .20% alcohol standard solution), and to another 1 ml. of the distillate being analyzed (this will be the unknown solution). Add 5 ml. of the Oxidizing Reagent #1 (.0%–.20%) to each of the test tubes. If, after 2 or 3 minutes, the yellow color remaining in the unknown is less than in the alcohol standard, prepare a new unknown test tube using 5 ml. of Oxidizing Reagent #2 (.20%–.45%). Stopper with metal foil cover to prevent contamination. Heat in a boiling water bath for ten minutes. (To save time, unknown test tubes may be made up in duplicate, one using Oxidizing Reagent #1 and the other Oxidizing Reagent #2. Use the tube that applies, and discard the other.)
4. Cool in an ice bath, and transfer to 10 mm. Pyrex glass cuvettes.
5. With a Beckman "B" Spectrophotometer, using the slit control to set the low end and the dark current control to set the high end of the scale, balance so that the alcohol standard solution absorbs O and the distilled water solution ("water blank") absorbs .35 at 450 millimicrons.

[1] This may be obtained from the Scientific Glass Apparatus Company, Bloomfield, New Jersey, at the approximate cost of $15.

TABLE OF ABSORBENCE—% BLOOD ALCOHOL

Range I		Range II	
Absorbence	% Blood Alcohol	Absorbence	% Blood Alcohol
.0000	.20%	.131	.40%
.0175	.19%	.149	.39%
.0350	.18%	.166	.38%
.0525	.17%	.184	.37%
.0700	.16%	.201	.36%
.0875	.15%	.219	.35%
.1050	.14%	.236	.34%
.1225	.13%	.254	.33%
.140	.12%	.271	.32%
.157	.11%	.289	.31%
.175	.10%	.306	.30%
.192	.09%	.324	.29%
.210	.08%	.341	.28%
.227	.07%	.359	.27%
.245	.06%	.376	.26%
.262	.05%	.394	.25%
280	.04%	.411	.24%
.297	.03%	.429	.23%
.315	.02%	.446	.22%
.332	.01%	.464	.21%
.350	.00%	.481	.20%

6. Determine the absorbency of the unknown and read the percent blood alcohol from the table which applies.

Notations About the Method

1. The wavelength setting of 450 Mμ was selected because at this wavelength dichromate absorbs strongly, and there is no interference from the green chromic color which is formed.
2. At the wavelength, concentration, and pH selected for this method, Beer's law applies. Thus, because both ends of the curve are set against dichromate, the intervals are constant; concentration-absorbency points on the curve are fixed and predetermined.
3. Since two concentration ranges are used, an adequate split is obtained so that the meter readings can be accurately determined even on a short scale. Fewer interpolations of meter readings are necessary.
4. Since the concentration of the dichromate solution No. 1 is the same as that used in Dr. R. N. Harger's (9) method, independent answers may be obtained by titrating with the red reducing fluid described in his work without additional preparation.
5. The strength of the alcohol standard solution (.40 mg./ml.) should always be determined by direct titration when it is prepared. Add 4 ml. of distilled water, 1 ml. of alcohol standard solution (.40 mg./ml.), 1 ml. of the stock potassium dichromate solution, and 5 ml. of concentrated sulfuric acid. Titrate with the Harger red re-

RECOVERY OF ADDED ETHANOL FROM WATER SOLUTIONS

Test No.	Per cent Ethanol Added	Per cent Ethanol Found	Per cent Recovery
1	.050	.050	100. %
2	.100	.103	103.0
3	.150	.148	98.6
4	.200	.196	98.0
5	.250	.243	97.2
6	.300	.296	98.6
Mean			98.9 %

ducing fluid. Make up a similar tube substituting 1 ml. of distilled water for the alcohol standard solution. Titrate the "water blank". Titrate an extra ml. of the dichromate standard solution, and solve the Harger blood-alcohol formula. The alcohol standard solution must check at exactly .20%.

Of 50 tests on blood and urine using both the Harger titration method and the Spectrophotometric method, the results in 45 showed perfect agreement. Five deviated by ±.01%. Blood alcohol is reported as percent with the third significant figure dropped in rounding off.

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