UNIVERSITY OF NIŠ
The scientific journal **FACTA UNIVERSITATIS**
Series: **Physics, Chemistry and Technology** Vol. 1, N° 5, 1998 pp. 129 - 134
Editor of series: Momčilo Pejović, e-mail: pejovic@elfak.ni.ac.yu
Address: Univerzitetski trg 2, 18000 Niš, YU
Tel: +381 18 547-095, Fax: +381 18 547-950

A METHOD FOR SPECTROPHOTOMETRIC DETERMINATION OF ACETALDEHYDE WITH THIOSEMICARBAZIDE IN BLOOD

UDC: 535.243+661.727.2+631.867

Vidosava Djurdjić, Srdjan Stojanović

Faculty of Chemistry, University of Belgrade
Studentski trg 16, 11000 Belgrade, Yugoslavia

Abstract. *The thiosemicarbazide method for spectrophotometric determination of acetaldehyde (AcH) in whole blood is presented. The concentration of thiosemicarbazone formed in slightly acidic medium was determined at 260 nm. The influence of non-specific chromogens present in blood was eliminated by simultaneous AcH distillation and thiosemicarbazone formation. The oxidation of AcH in blood samples was prevented by chloral hydrate addition. The presented method was found two folds more sensitive than a spectrophotometric determination of AcH by semicarbazide. Due to its simplicity and sensitivity as well as to the use of low cost equipment, this method should be a valuable complement to expensive GLC or HPLC determinations of AcH.*

Keywords: *acetaldehyde (AcH), thiosemicarbazide, spectrophotometric determination, thiosemicarbazone*

1. INTRODUCTION

Acetaldehyde (AcH) is the principal metabolite of ethanol metabolism. It is produced by enzymatic oxidation with alcohol dehydrogenase, as well as, by non-alcoholic dehydrogenase systems [1].

Because of its chemical reactivity AcH is likely to contribute to the manifestations of acute and chronic alcohol intoxication [2]. It has also been suggested that AcH concentration may be the discriminatory factor of alcoholism and that an inherited abnormal metabolic response in some individuals may have ethiological importance [3].

The studies of metabolism and the effects of AcH have been hampered by analytical difficulties related to its assays. Various difficulties are inherent in the attempt to determine blood AcH levels. Some of AcH can bind to erythrocytes and be oxidized by

Received Avgust 11, 1997; in revised form February 10, 1998; accepted April 2, 1998

erythrocyte aldehyde dehydrogenase [4] leading to AcH disappearance in treating blood samples. After taking ethanol, the level of AcH is extremely low in healthy individuals and the presence of many compounds may influence the determination, rendering it nonspecific.

Numerous methods were proposed for AcH determination. Older, enzymatic [5] and spectrophotometric methods [6] are lacking specificity, sensitivity and reliability. The most commonly used spectrophotometric method based on the reaction with semicarbazide, was found insufficiently sensitive. Specific gas-chromatographic (GLC) and high-pressure liquid chromatographic (HPLC) determinations are time consuming, needing sample pretreatment and expensive equipment [7]. Thus, there has, so far, no reliable method for AcH determination [8].

It is therefore necessary to develop a simple, inexpensive, but sensitive method to quantitate the blood AcH level. The simultaneous distillation and spectrophotometric determination of blood AcH levels based on reaction of AcH with thiosemicarbazide was proposed.

All relevant factors that may influence the spectrophotometric analysis were investigated. The conditions for elimination of nonspecific influences and the stability of blood AcH level were determined too.

2. EXPERIMENTAL

All reagents used were of analytical grade purchased from Merck (Darmsradt) or Sigma Chemical Co.

Blood specimens were prepared by adding 4.00 mL of fresh blood to 1.00 mL of solution containing citric acid (4.7 g/L), sodium citrate (16 g/L), glucose (25 g/L) and chloral hydrate (0.25 mol/L).

Thiosemicarbazide reagent (4 μmol/L) was prepared in sodium phosphate buffer pH = 6 (0.2 mol/L). The thiosemicarbazide reagent was found stable for 30 days at +4 °C. AcH stock standard solutions (1 g/L) were prepared by freshly distilled AcH and stored at –23 °C. Shortly before use, stock solutions were diluted with cold water (10 °C).

Calibration graph was constructed by mixing 0.5 mL of AcH solutions (0.5-7.0 mg/L) and 3.0 ml of thiosemicarbazide reagent. After incubation for 30 minute at 25 °C the absorbance at 260 nm was measured against a reagent blank.

Concentration of AcH in blood was determined by AcH distillation from 1.00 mL of blood samples, heated at 45 °C in vacuum, into 5.00 mL of buffered thiosemicarbazide solution. Distillation was performed within 45 minute and the concentration of the formed thiosemicarbazone was determined, after dilution to 10.00 mL with sodium phosphate buffer at 260 nm against the reagent blank.

The semicarbazide spectrophotometric determination of AcH was performed by the use of semicarbazide solution (6.7 μmol/L) in sodium phosphate buffer pH = 7 (0.2 mol/L). The concentration of semicarbazone was determined at 226 nm [6].

The spectrophotometric measurements were performed by the use of Beckman DU-50 spectrophotometer.

3. RESULTS AND DISCUSSION

By distillation of blood AcH into thiosemicarbazide solution, thiosemicarbazones of AcH were formed, exhibiting absorption maximum at 260 nm (Fig. l).

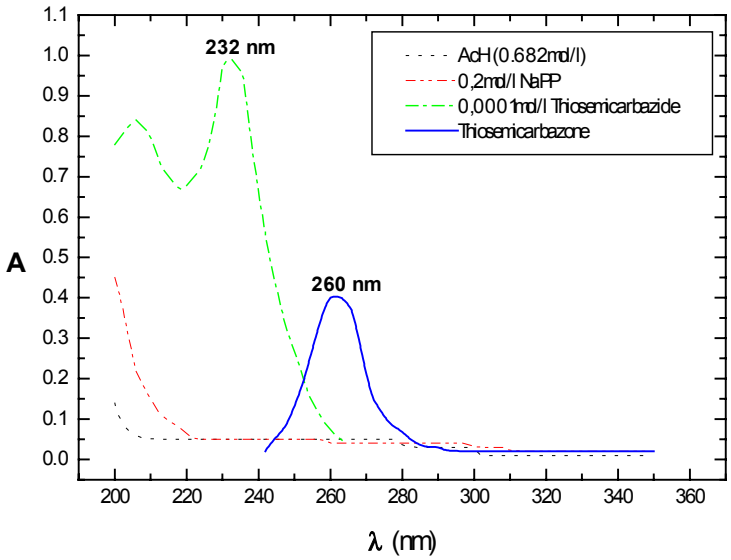

Fig.1. Absorption spectra of AcH (_._._.-), sodium phosphate buffer (.....), thisemicarbazide (_____) and thiosemicarbazone (____).

Many parameters that may cause some influences upon the spectrophotometric determination of AcH were investigated, such as the pH-optimum , thiosemicarbazide concentration, and rate of thiosemicarbazone formation, elimination of nonspecific effects of other blood constituents, as well as the stability of blood AcH. The correlation with semicarbazide AcH spectrophotometric determination [6] was made, too. All experiments were performed by the use of standard AcH solution as well as with as with blood samples with AcH addition.

3.1. The optimum pH-region, thiosemicarbazide concentration and the rate of AcH-thiosemicarbazones formation

As the formation of thiosemicarbazones of AcH is pH dependent the optimum pH-region for AcH-thiosemicarbazone formation was determined. The experiments were performed in a pH-region between 2-7 by the use of AcH solutions (0.5-7.0 mg/L) and buffered thiosemicarbazide solutions. Simultaneous optimum thiosemicarbazide concentration was determined by the use of thiosemicarbazide solutions of various concentrations (0.5-5.0 μmol/L).

As can be seen from results presented in figs 2 and 3 the maximum AcH-thiosemicarbazone formation was obtained by the use 4 mmol/L of buffered thiosemicarbazide solution pH = 6.

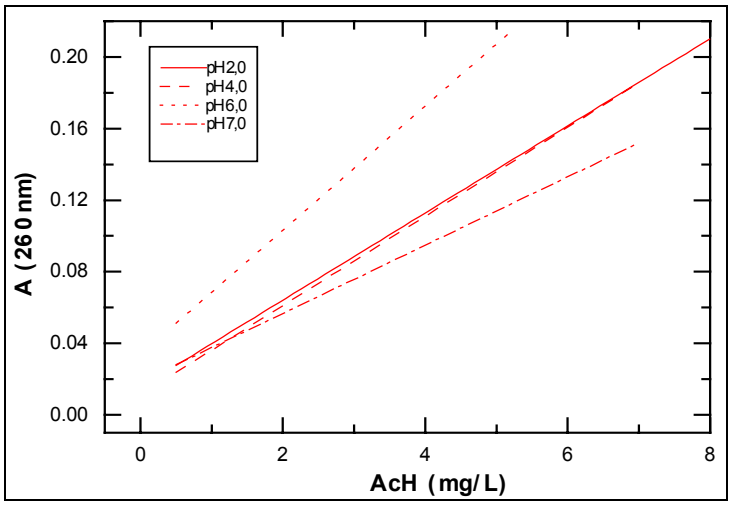

Fig. 2. Effects of pH on AcH-thiosemicarbazones formation by the use thiosemiocarbazide (4 mmol/L): pH=2 (____), pH=4 (_ _ _), pH=6 (.....) and pH=7 (-.-.-.)

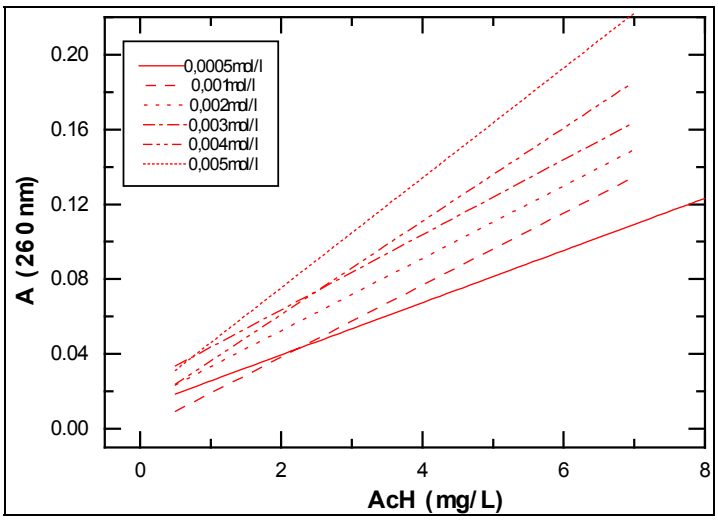

Fig. 3. Effects of thiosemicarbazide concentration on AcH thiosemicarbazones formation: 0.5 μmol/L (-----), 1.0 μmol/L (- - -), 2.0 μmol/L (.....), 3.0 μmol/L (-.-.-), 4.0 μmol/L (-..-.) and 5.0 μmol/L(.....)

The rate of AcH-thiosemicarbazone formation was analyzed by the use of AcH solution (30 mg/L) and buffered thiosemicarbazide solution (pH 6 and 7, concentration 4 mmol/L). The intensity of absorbance was determined in 30 s intervals during 100 minutes. On the basis of experimental data AcH-thiosemicarbazone formation was complete within 30 minutes. The absorption must be presently determined.

3.2. Determination of AcH in blood samples

Since numerous blood constituents form thiosemicarbazones, the effect of all these compounds upon the spectrophotometric determination of AcH was investigated. The most common interference of volatile blood compounds is the presence of increased concentration of acetone.

In experiments dealing with the addition of various amounts of acetone to blood samples containing AcH (0.5-7.0 mg/L) the optimum separation of AcH was obtained by fractional distillation during 45 minutes at 30 °C with air flow of 2.0 ± 0.2 m/s, with recoveries of $98.7 \pm 2.7\%$.

A quickly decrease of AcH concentrations in blood specimens as a consequence of AcH oxidation or/and interaction with various biomolecules [9]. This decrease is especially evident in the presence of erythrocytes, namely erythrocyte's aldehyde dehydrogenase (ALDH). In order to prevent AcH decrease protein precipitation, cyanamid or calcium carbide addition [10,11] and ALDH inhibition with chloral hydrate [12] were proposed.

By analyzing AcH in blood samples, with and without chloral hydrate addition, it was experimentally confirmed that chloral hydrate addition ensures the AcH blood level stability. By analyzing blood specimens containing AcH between 5-30 mg/L in a described manner, the level of AcH was found stable within 24 hours and the recoveries of 99.7% were obtained.

3.3. Comparison of thiosemicarbazide and semicarbazide spectrophotometric methods for AcH determination

The proposed thiosemicarbazide method was correlated with the most commonly used semicarbazide spectrophotometric determination [6] of AcH in blood. AcH solutions in concentration ranges of 1.0-7.0 and 0.1-1.0 mg/L were analyzed by both methods. The results obtained are presented in fig. 4.

On the basis of results presented it can be concluded that the described thiosemicarbazide method was found two folds more sensitive as compared to previously reported semicarbazide method for AcH determinations. At the same time thio-

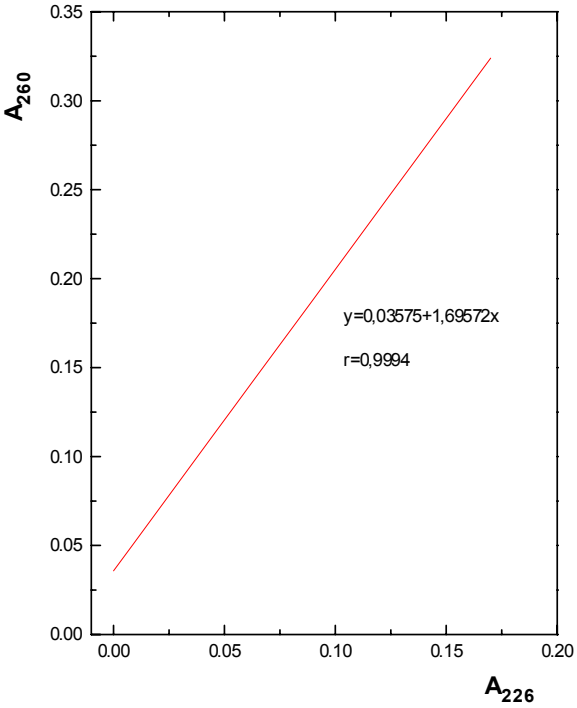

Fig. 4. Correlations of semicarbazide (x) and thiosemicarbazide (y) methods.

semicarbazide method was found more reproductive (r = 0.999) as compared to r = 0.951 for semicarbazide method. The calibration graph was found to be linear between 0.1-200 mg/L of AcH, with a linear regression equation being y = 0.0112 x + 0.00033.

4. CONCLUSION

On the basis of all the results, the proposed thiosemicarbazide spectrophotometric method for blood AcH determination was found simple, reproductive, sensitive and specific, being superior to semicarbazide or other spectrophotometric methods previously reported. Also, it can be realized with inexpensive instrumentation, thus being a valuable complement to expensive GLC or HPLC determinations of AcH.

REFERENCES

1. C. S. Lieber, *Medical disorder of alcoholism*, W.B. Saunders, Philadelphia, 1987.
2. C. S. Lieber, *Semin. Hematol*., **17,** 85-99 (1980).
3. M. A. Korsten, S. Matsusuki, L. Feinman and C. S. Lieber, *N. Engl. J. Med*. **292**, 386-389 (1975).
4. K. Inoue, Y. Ohbora, M. Fukunaya and K. Yamasawa, *Alcohol: Clin. Exp*. *Res*. **6,** 433-438 (1982).
5. H. Theorell, A. Nygaard and R. Bonnichsen, *Acta Chem. Scand*. **9,** 1148-1151 (1955).
6. T. N. Burbridge, C. H. Hine and A. F. Schichk, *J. Lab. Clin. Med*. **35,** 983-987 (1950).
7. M. Tomita, I. Ijiri and K. Shimosato, *J. Chromatog.* **414**, 454- 459 (1987).
8. C. J. P. Eriksson, *Pharmacol. Biochem. Behav***. 18** (Suppl. l), 141-150 (1983).
9. C. J. P. Eriksson, *Alcohol: Clin. Exp. Res***. 4**, 22-29 (1980).
10. H. Marchner and O. Tottmar, *Acta Pharmacol. Toxicol.* **43,** 219-232 (1978).
11. J. F. Brien, J. E. Peachey, C.W. Loomis and B. J. Rogers, *Clin. Pharmacol. Therap*. **5**, 454-463 (1978).
12. J. P. Von Wartburg and M. M. Ris, *Experientia* **35**, 1682-1683 (1979).

SPEKTROFOTOMETRIJSKO ODREĐIVANJE ACETALDEHIDA U KRVI SA TIOSEMIKARBAZIDOM

V. Djurdjić, S. Stojanović

U radu je opisana metoda za spektrofotometrijsko određivanje acetaldehida (AcH) u krvi. Kao reagens upotrebljen je tiosemikarbazid a apsorpcija nastalih u slabo kiseloj sredini tiosemikarbazona određuje se na 260 nm. Uticaj nespecifičnih hromogena prisutnih u krvi eliminiše se destilacijom AcH sa istovremenim građenjem tiosemikarbazona. Oksidacija AcH u uzorcima krvi sprečena je dodatkom hloral hidrata. Opisana metoda se pokazala osetljivom u odnosu na spektrofotometrijsko određivanje AcH sa semikarbazidom. Zbog svoje jednostavnosti, osetljivosti i ekonomičnosti ova metoda može predstavljati korisnu zamenu za GLC i HPLC određivanja AcH.

Ključne reči: *acetildehid (AcH), tiosemikarbazid, spektrofotometrijsko određivanje, tiosemikarbazon*