A Modified Micro-Diffusion Method for the Determination of Ethyl Alcohol in Blood

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A method is described for the micro-determination of ethyl alcohol in blood or urine by means of a new micro-diffusion procedure that gives results in 30 minutes over the range of 80 to 300 μg per 0·1 ml of blood or urine with an accuracy of better than $\pm$3 per cent.

Bahner,[1] working in this laboratory, has devised a convenient micro-diffusion apparatus that permits the separate temperature control of the two chambers. The apparatus, illustrated in Fig. 1, can be applied to many analytical methods in which diffusion at a uniform temperature is slow or incomplete. The lower vessel, which contains the sample for determination, has a flat base with a surface area of 2·5 sq. cm and is attached to the chimney part of the "mushroom" by a B19 standard joint. The "mushroom" is surrounded by a simple form of condenser that allows water at 50° C to circulate over and under it. The flat base of the "mushroom," which has a surface of 10·0 sq. cm, contains the dichromate - sulphuric acid mixture.

The first use made of this apparatus, apart from that by Bahner who designed it for the determination of acetone and "ketone bodies" in blood, was in the determination of ammonia (Scandrett, unpublished). By the boric - hydrochloric acid procedure, full recoveries were regularly obtained in 10 minutes with a solution containing 112 μg of ammonia (NH_3) per 0·5 ml, which demonstrated the efficiency of the micro-diffusion in this apparatus.

Experimental

Preliminary observations indicated that, if the apparatus was to be successfully used for the determination of ethyl alcohol in blood, certain limiting factors would have to be considered and, if necessary, modified to meet the requirements of the unit.

The concentrations of potassium dichromate and sulphuric acid appeared to be critical, especially that of the sulphuric acid, as incomplete absorption and oxidation resulted if the concentration of sulphuric acid fell below 50 per cent.

As the iodine titration depends on the pH of the solution (the optimum value is about pH 1·4), which involves a considerable dilution immediately before titration, and as the capacity of the "mushroom" is limited, only a small quantity, 1·0 ml, of original reagent could be used. This quantity was found just to cover the base of the "mushroom," which from the point of view of absorption is ideal, since the rate of absorption is inversely proportional to the volume (Conway[2]).

Preliminary experiments with 1·0 ml of dichromate - sulphuric acid mixture indicated that the absorption and oxidation is complete in 30 minutes, as against 2 hours by the Widmark method.[3]

A 0·005 *N* solution of sodium thiosulphate is used for alcohol concentrations up to 200 mg per 100 ml; for higher concentrations, *i.e.*, between 200 and 500 mg per 100 ml, 0·01 *N* sodium thiosulphate is used.

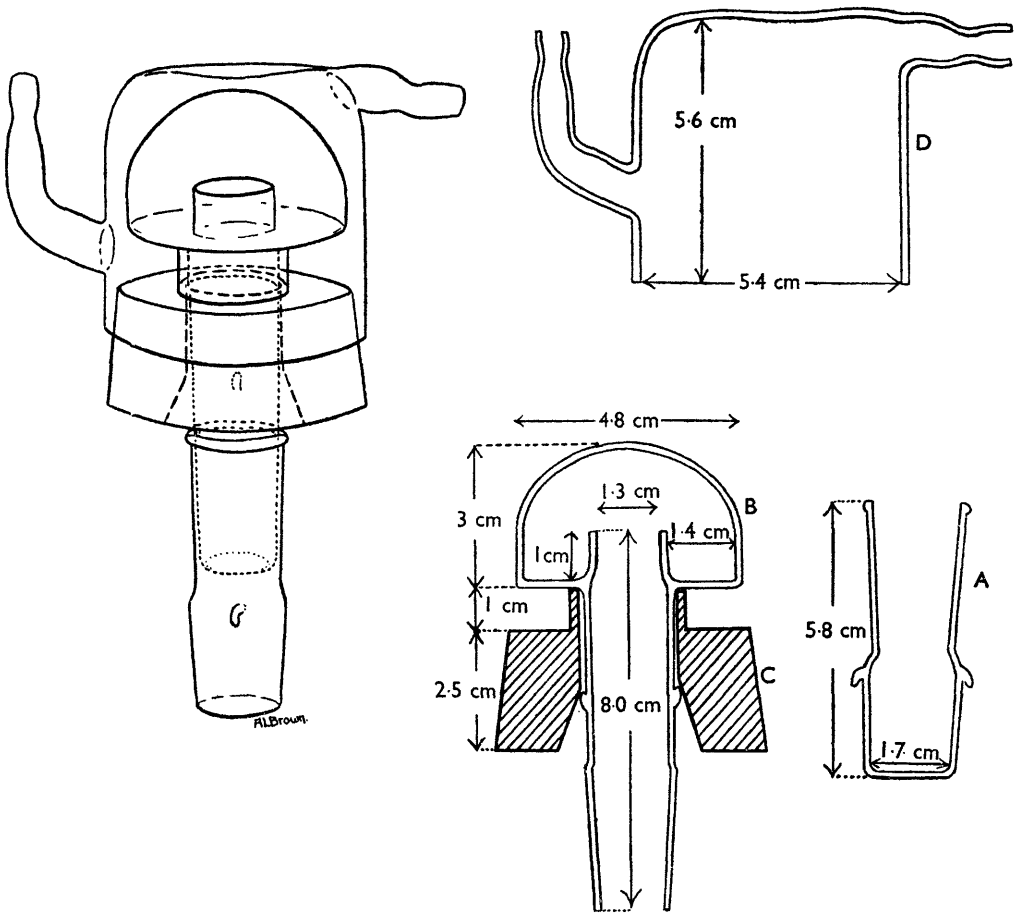

Fig. 1. View and sections of apparatus

A, lower vessel; B, "mushroom" receiver; C, rubber bung; D, condenser jacket

From a consideration of the above conditions and a large number of trials it was evident that the quantities and concentrations of reagents used by Widmark[3] were the most suitable, and this was proved by subsequent experiments.

Method

Reagents—

Potassium dichromate, 0·02 N, in concentrated sulphuric acid—This concentration is used for blood containing less than 200 mg of ethyl alcohol per 100 ml.

Potassium dichromate, 0·05 N, in concentrated sulphuric acid—This concentration is used for blood containing between 200 and 500 mg of ethyl alcohol per 100 ml.

Potassium iodide—A 5 per cent. solution.

Sodium thiosulphate—0·01 *N* and 0·005 *N* solutions.

Starch—A 1 per cent. solution.

All reagents should be of recognised analytical quality. With a Bang burette, which is available in most analytical laboratories, the strengths of the sodium thiosulphate solutions can be reduced. This was not done in the investigation described here.

PROCEDURE—

Transfer 1·0 ml of the dichromate - sulphuric acid mixture to the inverted "mushroom" by means of a 1·0-ml slow-delivery bulb pipette. It is advisable to blow the liquid out and to touch the surface of the dichromate - sulphuric acid mixture with the tip six times, to hold the "mushroom" against a white background and to watch the introduction of the liquid and the withdrawal of the pipette by looking down the chimney. Preliminary experiments with this procedure for delivery of 1·0 ml of dichromate - sulphuric acid mixture showed that the average error of ten such measurements is less than 1 per cent.

Using pipettes, place in the bottom vessel 0·2 ml of water and 0·1 ml of blood, the 0·1-ml blood pipette being rinsed out twice and the water and blood thoroughly mixed. Invert the "mushroom" and, with a cork-screw motion, attach the bottom vessel tightly to the chimney,

TABLE I

RECOVERY OF ETHYL ALCOHOL ADDED TO FRESHLY WITHDRAWN CITRATED BLOOD

Additions made as aldehyde-free ethyl alcohol in distilled water

Alcohol per 0·1 ml of blood			
Added, μg	Found, μg	Mean, μg	Standard deviation of mean
80·0	78·0, 83·0, 80·8, 82·0, 82·0, 84·2, 80·8, 76·8, 77·5, 76·3, 77·5, 84·6	80·3	±2·97
160·0	161·5, 158·0, 161·5, 160·4, 159·2, 162·0	160·4	±1·56
300·0	300·0, 305·0, 300·0, 300·0, 305·0, 305·0	302·5	±2·70

Blanks at each level were: 0 μg added, 0 μg found.

TABLE II

RECOVERY OF ETHYL ALCOHOL ADDED TO FRESHLY VOIDED URINE MADE ALKALINE WITH SODIUM HYDROXIDE

Alcohol per 0·1 ml of urine			
Added, μg	Found, μg	Mean, μg	Standard deviation of mean
300·0	301·7, 299·5, 296·0, 301·7, 304·0, 298·0, 294·0, 296·0, 296·0, 298·4	298·5	±2·80

Blank: 0 μg added, 0 μg found.

taking care to see that the joints are perfectly dry. Suspend the bottom vessel, held in position in this way, in a bath of boiling water almost up to the joint, while the "mushroom," enclosed by the condenser, has water at 50° C flowing over and under it. The rate of flow through the condenser may conveniently be 1 litre every 10 minutes, but this is not critical. After 30 minutes disconnect the unit, drain the condenser of water and take it off. Disconnect the bottom vessel, invert the "mushroom" and cool it under the tap. Add 25·0 ml of water to the "mushroom," again cool (preferably in ice) and add 0·5 ml of 5 per cent. potassium iodide before titrating with sodium thiosulphate in a 5·0-ml micro-burette.

Experiments were also made to determine the effect of added water on the rate of absorption and oxidation by the dichromate - sulphuric acid mixture. The following figures show that additions of water did not affect the acid mixture, as full recoveries in each experiment were obtained at the end of 30 minutes. To 1·0-ml portions of dichromate - sulphuric acid mixture were added 0·1, 0·2 or 0·3 ml of water. The amounts of alcohol found in 0·1-ml portions of blood containing 80·0 μg of ethyl alcohol per 0·1 ml, when acted upon by these solutions, were 78·8, 79·3 and 80·0 μg, respectively. It was not possible to use 0·2 ml of blood, as recoveries were always poor, probably because the heat-coagulated protein formed too great a barrier for the free diffusion of the alcohol vapour through the mixture.

The "mushroom" easily holds 25·0 ml of water and the constricted opening and the chimney are advantageous in iodimetric titrations.

Results and discussion

Accurate results were obtained throughout the range of 80 to 300 μg of alcohol per 0·1 ml of blood and urine, as shown in Tables I and II, the standard deviation being less than $\pm 3{\cdot}0$ μg per 0·1 ml of blood.

The method reduces the time for a single diffusion to 30 minutes, as against 2 hours with the Widmark[3] and Winnick[4,5,6] methods. A large number of determinations can be carried out simultaneously.

Further advantages are that, as a result of the large excess of water in the "mushroom" receiver, there is no possibility of losing the liberated iodine, the end-point is not so abrupt and the subsequent titration and shaking are, therefore, easy to control and manipulate.

By increasing the temperature gradient over which absorption takes place, it is possible to increase the range over which diffusion techniques can be used, so that the versatility of the apparatus is increased.

Experiments made with blood containing 300 mg of alcohol per 100 ml, stored at room temperature for one week, did not show any diminution of alcohol concentration. Normal blood used as a control and kept under the same conditions did not give a "blank."

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References

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