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Note

The determination of ^2H_2O in water and biological fluids by gas chromatography

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The determination of ^2H_2O in water or in biological fluids is interesting both from the viewpoint of analytical methods and for the investigation of some physiological processes in living organisms. The methods for the determination of ^2H_2O can essentially be divided into direct methods, where ^2H_2O itself is determined, and indirect methods, where the sample containing ^2H_2O is decomposed into a gaseous mixture of hydrogen isotopes which is then analyzed for deuterium content. If the mixture obtained contains isotopes of hydrogen and deuterium only, the determination of deuterium content can be carried out by measuring the heat conductivity of this mixture[1]. However, if the sample also contains other compounds, *e.g.* oxygen and nitrogen from air, a separation method must be employed for the analysis. Gas chromatography (GC) is advantageous for this purpose. Publications dealing with GC separation and determination of individual isotopes of hydrogen can be divided into two groups. The determinations based on separation of individual isotopes[2–9], followed by a non-selective detection, are included in the first group; the second group includes methods where a mixture consisting of hydrogen isotopes only is first separated from other gases, and then deuterium is detected selectively in the isotopic mixture[1,10,11].

It follows from the above that indirect methods based on the generation of a H_2-H^2H isotopic mixture from a sample are more convenient for routine determinations of ^2H_2O in liquid samples, as they eliminate the interference of inorganic and organic admixtures. For the routine determination of deuterium in the isotopic mixture, the use of selective detection affords a considerable advantage as it does not require the constant low temperature which is necessary for the separation of H_2 and H^2H. Gaseous isotopic H_2–H^2H mixtures can be generated from a liquid sample containing ^2H_2O by electrolysis or by reaction with substances that decompose water to generate hydrogen. The use of electrolysis for the sample decomposition is only apparently advantageous. Uneven rates of decomposition of H_2O and ^2H_2O, the ratio of which is between 3 and 30, according to the concentration of ^2H_2O in the liquid sample, make this procedure complicated.

During electrolysis, deuterium is concentrated in the liquid residue and the gaseous mixture produced has a variable composition. This difficulty can be eliminated by total decomposition of the sample, which can, however, be carried out only in

an electrolyzer with non-separated electrodes. The mixture produced in such a way contains, however, an equivalent amount of oxygen and thus makes heavy demands on the separation system. The second procedure for the evolution of both the isotopes from the sample is the reaction with some substances that decompose water and release hydrogen, *e.g.* the reaction with CaH_2 or $LiAlH_4$. These substances react with a mixture of H_2O and ^2H_2O practically instantaneously and non-selectively, generating a gaseous mixture of H_2 and H^2H, in which the isotopic ratio is the same as that in the original liquid sample (*cf.* refs. 10–12). The decomposition of the sample can be carried out either by direct injection into a pre-column, filled with the respective hydride, in a stream of carrier gas, or, in a separate independent vessel serving simultaneously as a container of the gaseous sample from which the sample is taken and injected by means of a syringe. The choice of the column is governed by the method of preparation of the isotopic mixture and by the type of the detector used. The separation of the isotopic mixture from other components present in the sample, especially from oxygen and hydrogen, is the main problem with the indirect method. As relatively low concentrations should be determined in most instances, it is necessary to inject volumes as large as possible, *i.e.* approx. 5 ml. Hence, a large column capacity is required both to damp sufficiently pressure pulses caused by the injection of the sample and to separate the hydrogen peak from the peaks of other components.

EXPERIMENTAL

Standard deuterium samples were prepared from 91 wt.% ^2H_2O which was kindly provided by the Research Institute of Macromolecular Chemistry, Brno, Czechoslovakia; $LiAlH_4$ and CaH_2 were obtained from Lachema, N.E., Brno, Czechoslovakia. Hydrogen used as the carrier gas was of standard electrolytic grade, obtained in pressure cylinders from Technoplyn, N.E., Prague, Czechoslovakia. The column was packed with Supersorbon activated charcoal, a product of Moravian Chemical Works, N.E., Ostrava-Hrušov, Czechoslovakia. The charcoal was of grain size 0.2–0.25 mm and activated at 300° under a nitrogen atmosphere. The chromatograph was an in-house instrument and its injection port was modified to permit the insertion of 3–4 g of $LiAlH_4$ when employing the pre-column decomposition technique. The injection port was provided with an electric heating wire capable of generating temperatures of 130–150°. The column was made of a thick-walled silicone rubber tube, 1.2 m long, 6 mm I.D. Fractions leaving the column were detected by a katharometer of standard type from Gow-Mac (Madison, N.J., U.S.A.) equipped with a double filament of gold-plated tungsten. The detector signal was recorded by an EZ 11 line recorder from Laboratory Instruments, N.E., Prague, Czechoslovakia. The following working procedures were tested.

Decomposition of the sample by electrolysis

Electrolytic decomposition of the sample was carried out in a microelectrolyzer with a volume of approx. 0.15 ml. A 10-μl amount of sample, acidified with a small amount of concentrated sulphuric acid, was placed into the electrolyzer. Electrolysis was carried out at 8 V d.c. and the gas generated was led by means of a capillary coupling into an eudiometer with a volume of 10 ml. The eudiometer was closed with a rubber septum to enable sampling by means of an injection syringe.

Decomposition by means of a pre-column filled with $LiAlH_4$

In order to test the function of the pre-column decomposition technique (the "on-line" system), the injection port was filled with 3 ml of a powdery mixture consisting of one part of Chromosorb P and two parts of $LiAlH_4$. Chromosorb was added in order to decrease pneumatic resistance of the packing and to suppress its sintering. The injection port was heated to a temperature of 130°. Defined sample amounts of 1–2 μl were injected directly into the pre-column.

Decomposition of the sample by means of $LiAlH_4$ in a generating tube

The device used for the decomposition of an isotopic water sample is shown in Fig. 1. It is a glass tube, with a diameter of 12 mm and total length of 40 cm, which is bent into an acute angle in such a way that one arm is about 12 cm long. Another glass tube with a larger diameter serves as a reservoir and is sealed to this arm. Both ends of the tube are made in such a way that they can be closed by rubber stoppers. The tube was filled in the horizontal position with the sample under analysis so that the longer arm was filled entirely with the liquid and its level reached the opening of the reservoir. On turning the tube quickly into a vertical position, the sample remained in the longer arm as the liquid was prevented from coming out by air pressure. About 10 mg of $LiAlH_4$, closed in a cellophane capsule, were inserted through the lower opening into the bent tube and the opening was then closed by a rubber stopper. After several seconds, the liquid sample permeated the cellophane and reacted with the $LiAlH_4$. The gaseous H_2–H^2H mixture formed by the reaction is accumulated under the upper rubber stopper and thus displaces the liquid into the reservoir. When the reaction is complete the H_2–H^2H sample mixture is stored in the tube and the rest of the liquid sample serves as a liquid seal. Through the upper rubber septum, a gaseous H_2–H^2H sample can be taken for an analysis as required.

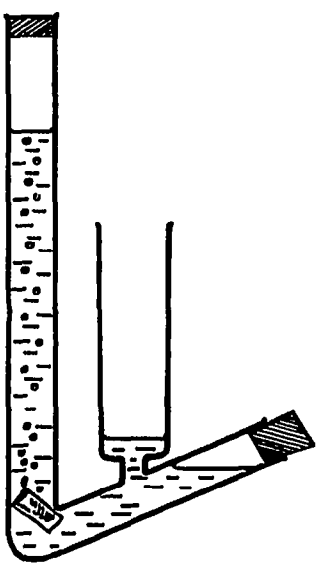

Fig. 1. Tube for generating a H_2-H^2H mixture by the decomposition of liquid samples by $LiAlH_4$.

RESULTS AND DISCUSSION

The generation of a gaseous H_2–H^2H mixture by electrolysis is reliable, but the time required for the quantitative decomposition of a liquid sample is relatively long. About 20 min are necessary for decomposing 10 μl. An incomplete electrolysis, on the other hand, diminishes the amount of H^2H in the gaseous sample, thus decreasing the sensitivity of the method.

The results obtained by the direct injection of the liquid sample into the precolumn containing $LiAlH_4$ showed that the evolution of H^2H proceeds slowly and, moreover, the decomposition time of the liquid sample differed considerably for injections repeated several times. All these effects resulted in unreproducibility of the analysis.

The decomposition of the sample in a separate generating tube proved to be an optimal and entirely reliable procedure. The reproducibility and sensitivity of this procedure were proved for a series of model mixtures containing 0.05–0.25 wt.% of ^2H_2O in water or in urine. Always 5-ml volumes of the gaseous mixture in question were analyzed. Corresponding peak heights in the chromatograms obtained varied in a range of approx. 10 to 50 mm. The dependence of the peak height on the concentration of ^2H_2O in the liquid sample was linear and the reproducilibity of one determination, expressed by the relative standard deviation, was 5.2%. The sensitivity of the method, expressed as the minimum detectable concentration of ^2H_2O in a liquid sample, was 0.03 wt.% of ^2H_2O. The advantage of the procedure is the fact that the liquid sample is always in large excess and that the reaction thus proceeds reliably. The gaseous sample, accumulated in the generation tube, can be stored in this tube for an unlimited period and its volume is sufficient for several duplicated analyses. Moreover, the decomposition can be repeated with the same liquid sample by merely sampling another hydride capsule. The simplicity of the whole procedure enables serial analyses of liquid samples and calibration mixtures to be carried out quickly.

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