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A Diffusion-equilibration Method as a Stage in the Determination of the Deuterium Oxide Content in 1—2 Microlitres of Fluid

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Determination of the deuterium oxide content in fluids is generally performed utilizing the difference in density between pure water and deuterium oxide. Convenient methods have been described which allow an estimation of the deuterium oxide content in very small volumes with sufficient accuracy for most biological purposes. Thus the gradient tube method developed by Linderström-Lang¹ (see also Linderström-Lang, Jacobsen and Johansen²) requires only about 0.5 microlitre for an accurate determination. However, all methods based upon density determinations require that the fluid, the deuterium oxide content of which will be determined, contains only H_2O , HDO and D_2O , respectively. A distillation procedure is thus a necessary step before the actual measurements can be made. If the volume of the fluid to be analyzed is limited to a few microlitres, common distillation procedures become inconvenient.

The present work is a description of a simple method for "purification" of very small samples (1—2 μ l) prior to the density determination in the gradient tube.

Theory. The principle of the method is shown in Fig. 1. Sample 1, a drop of volume V_1 containing an unknown concentration C_1^0 of deuterium oxide, is allowed to attain diffusion-equilibrium in a

small closed space with sample 2, a drop of volume V_2 containing distilled water. (It is assumed, for the sake of simplicity, that the distilled water used contains no deuterium oxide. This assumption introduces a negligible error.) The air space between the drops acts as a semipermeable membrane allowing only volatile substances to pass through. After equilibrium has been attained, any volatile substance has the same activity in both drops. It may be assumed that the activity coefficients of HDO (and D_2O) in water do not significantly differ from the activity coefficients of HDO (and D_2O) in water solutions of the molar and ionic strength present in biological fluids. Therefore

$$C_1^{eq} = C_2^{eq} \quad (1)$$

where the superscripts denote that the concentrations are those at equilibrium.

Furthermore

$$C_1^0 V_1 = (V_1 + V_2) C_2^{eq} \quad (2)$$

and

$$C_2^{eq} = C_1^0 \frac{V_1}{V_1 + V_2} \quad (3)$$

If the only volatile substance present is deuterium oxide, a density determination of sample 2 at equilibrium will give the original concentration of deuterium oxide in sample 1. Equation (2) contains of course the assumption that the amount of deuterium oxide present in the air space

Table 1.

Expt. No.	Original conc. of HDO in sample 1, Mole fraction	Equilibration time, Minutes	Conc. of HDO in sample 1 after equilibration, Mole fraction	Conc. of DHO in sample 1 (calc.), Mole fraction	Recovery %
17.9	0.12298	1 110	0.061494	0.12299	100.0
24.9	0.12298	140	0.061342	0.12268	99.8
25.9	0.12298	160	0.061368	0.12274	99.8
28.9	0.07074	245	0.036004	0.07201	101.8
29.9	0.02337	300	0.011902	0.02380	101.9
2.10	0.02337	125	0.011664	0.02333	99.8
5.10	0.02337	285	0.011802	0.02360	101.0
20.10	0.01774	300	0.008918	0.01784	100.6
21.10	0.01774	180	0.008960	0.01792	101.0
22.10	0.00912	135	0.004520	0.00904	99.1
23.10	0.00912	160	0.004635	0.00927	101.6

is negligible in comparison with that present in V_1 and V_2 .

The basic principle of this method has long been known (*cf.* Linderstrøm-Lang and Holter³ and Conway⁴).

Method. Small glass tubes of the form and dimensions given in Fig. 1 are made from ordinary glass. They are thoroughly cleaned and treated on the inside with Desicote (Beckman) to make the surface non-wettable. After Desicoting they are again rinsed several times with distilled water. The rinsing after the Desicoting procedure is important as it removes traces of water-soluble matter in the Desicote film. The sample to be analyzed is then transferred into the bottom of the tube, preferably by means of a constriction pipette. Sample 2 is then immediately placed in the tube about 1 mm above the bottom drop (see Fig. 1). These operations are conveniently performed using the clamps and stands for pipettes and tubes described by Holter³ for Cartesian diver manometry. Immediately after sample 2 has been delivered, the open end of the tube is dipped into melted paraffin which upon rapid cooling forms an effective seal. The tube is then allowed to stand in room temperature for equilibration. The time necessary for complete equilibration depends of course on the dimensions of the system. The dimensions used in the present investigation are seen in Fig. 1 and complete equilibrium is attained after about 100 minutes.

Results. The results from measurements on different standard solutions containing varying amounts of deuterium oxide is

seen in Table 1. The concentrations are given as mole fractions HDO as if no D_2O molecules are present, an assumption which is nearly true in the dilutions used. The volume of the samples was 1.6 μ l throughout, the same pipette used for both samples. The time for equilibration is also shown, it was always more than 125 minutes. In one instance it was as long as 1 110 minutes without affecting the recovery percentage. The mean recovery percentage from the 11 experiments shown in the table was 100.6 with a range between 99.1 and 101.9.

Discussion. As is seen from Table 1, recovery is obtained with an accuracy sufficient for most biological purposes. It seems therefore that the method described might be useful in those cases where the amount of fluid available for analyses is of the order of a few microlitres. Of course, any volatile substance present in addition to deuterium oxide is liable to affect the density of the recipient drop, and in certain cases this effect might be larger than otherwise tolerated.

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Note (added in proof): After this paper was submitted for publication the author's attention was drawn to a very recent paper by A. Hvidt, G. Johansen, K. Linderstrøm-Lang and F. Vaslow (*Compt. rend. trav. Lab. Carlsberg, Sér. chim.* **29** (1954) 129). The method used by these authors is essentially the same as that presented here, with the exception that it is used for larger volumes ($\sim 15 \mu\text{l}$).

Guaiacylglycerol and its β -Guaiacyl Ether

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According to the views expressed by Erdtman^{1,2} regarding the course of the dehydrogenative dimerization and polymerization of *p*-propenylphenols, guaiacylglycerol- β -aryl ether structures (I), in addition to other structures, can be expected to arise when coniferyl alcohol is subjected to dehydrogenation. The same author discussed the occurrence of such structures in lignin. On the basis of experimental results concerning the behaviour of lignin in sulphonation with sulphite solutions, alkylation with alcoholic hydrochloric acid, and similar reactions, Adler and Lindgren^{3,4} and Adler and Yllner⁵ also suggested the presence of structure I in lignin.

- I. $R_1 = \text{H}$ or the β -C-atom of a propane side-chain; $R_2 = \text{H}$ or a C-atom of a propane side-chain
- II. $R_1 = \text{CH}_3$; $R_2 = R_3 = R_4 = \text{H}$
- III. $R_1 = R_2 = R_3 = R_4 = \text{H}$
- IV. $R_1 = R_2 = R_3 = \text{COCH}_3$; $R_4 = \text{H}$
- V. $R_1 = R_2 = R_3 = \text{H}$; $R_4 = \text{CH}=\text{CH}-\text{CH}_2\text{OH}$

The β -guaiacyl ether of α -(3,4-dimethoxyphenyl)-glycerol (II) was synthesized³ and found to be a promising lignin model. It was sulphonated^{3,4} and alkylated³⁻⁵ in the expected manner, and also yielded formaldehyde on heating with strong sulphuric acid^{3,4,7}. As in the case of α -(3,4-dimethoxyphenyl)-glycerol (VI)^{3,7}, the β -guaiacyl ether II yielded Hibbert's "ethanolysis" products on prolonged heating with ethanolic hydrochloric acid. This reaction cannot be given by other dimeric systems like those present in dehydrodiconiferyl alcohol or in pinoresinol which also are assumed to occur in lignin (*c.f.* Ref.⁸).

Only one phenolic guaiacylglycerol compound, *viz.*, "guaiacylglycerol" itself, *i. e.*, α -(3-methoxy-4-hydroxyphenyl)-glycerol (VII), has been available as yet. It had been obtained⁹ as a syrupy mixture of the two possible pairs of optical antipodes, and yielded two crystalline tetraacetates (VIII), m.p. 84–85° and 113–114°, respectively. A sample of the syrupy product, which contained some water and had been kept in the refrigerator for several months, has now partly crystallized. Inoculation of other samples with the crystalline material induced rapid crystallization. Recrystallization from ethyl acetate, which was saturated with water, yielded needles, m. p. 82–84°. This compound contained one mole of water, which was removed at 60° (0.1 mm Hg). (Found: H_2O 7.71. Calc. for $\text{C}_{10}\text{H}_{14}\text{O}_5 \cdot \text{H}_2\text{O}$: H_2O 7.76.) The anhydrous product was a colourless glass. (Found: C 56.0; H 6.62; OCH_3 14.8. Calc.