

the print, but this does not interfere with detection of spots. For two-dimensional chromatograms, a printing frame is desirable (4). Poor contact gives less well-defined spots and slightly less sensitive detection.

Exposure time is much longer than for photocopy paper and is not very critical,

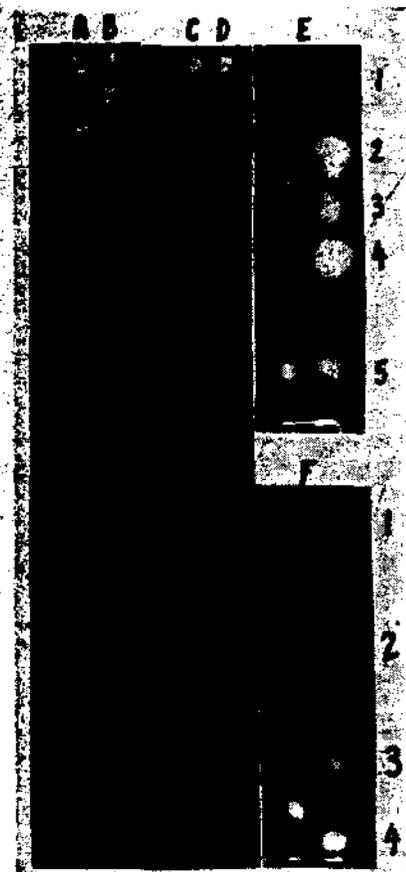


Fig. 1. Blueprints of ultraviolet spots on Whatman No. 4 filter paper. Columns A to D are 2- μ l test spots. Solutions in columns A and C are 0.05M (0.1 μ mole), in columns B and D, 0.005M (0.01 μ mole). A and B blueprinted at 254 m μ , 1 minute, C and D at 360 m μ , 1 minute. Identification: 1, pyrene; 2, phenanthrene; 3 acenaphthene; 4, acenaphthylene; 5, ergosterol; 6, testosterone; 7, estradiol; 8, DDT; 9, DDE; 10, sulphenone; 11, sevin; 12, keithane; 13, mitox; 14, rotenone; 15, 2,4-D; 16, methadone; 17, adenosine; 18, nicotinamide; 19, pyridoxine; 20, ascorbic acid; 21, tryptophan; 22, maleic acid; 23, fumaric acid; 24, potassium ferricyanide; 25, sodium molybdate; 26, copper acetate; 27, ferric chloride. Columns E and F are chromatograms, with β -methoxypropionitrile as the stationary phase and isooctane as the mobile phase, blueprinted at 254 m μ , 3 minutes. The spots on the left are azo dyes used as reference standards. Spots on right are: Chromatogram E (all 0.08 μ mole): 1, fluorene; 2, methyl cholanthrene; 3, 7,8-benzoquinoline; 4, 3,4-benzoquinoline; 5, acenaphthenol. Chromatogram F (all 0.1 μ mole): 1, diazinon; 2, trithion; 3, parathion; 4, guthion.

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cal, but a photographic printing timer is useful. Correct exposure should be determined for the particular lamp, filter paper, and blueprint paper used. With Whatman No. 4 paper, at 254 m μ , a 1-minute exposure gives a very pale blue background, and spots are detected with maximum sensitivity. A 3-minute exposure gives a darker background, against which spots show with maximum contrast and definition but with slightly less sensitivity. Longer exposures simply lower the sensitivity of detection and are useful only if it is desired that weak spots not show on the print. At 360 m μ , exposure time is about half that at 254 m μ . With thick papers a 10-minute exposure may be necessary, unless a solution of 25 percent heavy white mineral oil in isooctane is sprayed or poured on the paper. The increased transparency reduces exposure time by a factor of 2 to 3, and improves the definition of spots. It lowers sensitivity of detection, however, by a factor of 2 to 3, because the intensification ("hyperchromic absorption") of spots on dry filter paper is lost (6).

After exposure, the blueprint paper is washed in cold water under the tap for 5 to 10 seconds. The paper is then pressed flat on a ferrotype plate or other smooth flat surface and allowed to dry for a few minutes. To prevent curling the paper must be removed before it is completely dried and pressed flat between sheets of blotter paper for 10 minutes or more. Starting lines, solvent front lines, and other notations in ink or pencil on the chromatogram are blueprinted like the ultraviolet spots, so that R_f can be measured directly on the print.

If a chromatogram or spot test is to be blueprinted both at 254 and at 360 m μ , the test at 360 m μ must be done first, because the exposure at 254 m μ may cause chemical changes in the absorbing substance that cause the spot to absorb at 360 m μ or even to develop a visible color. Pyridine derivatives, such as nicotinamide, for example, are not detected by blueprinting at 360 m μ , but the decomposition products formed during blueprinting at 254 m μ cause the spot to turn yellow and to absorb strongly at 360 m μ .

Ultraviolet-sensitive papers other than ferric ferricyanide blueprint paper have been tried and found to be less satisfactory. Diazotype papers give dark spots on a white background, but neither the sensitivity nor the contrast is as good as that of blueprint paper. Ferric-silver "Vandyke" papers (such as Dietzgen No. 227) give white spots on a dark brown background but are more expensive and not significantly better than blueprint paper.

By using an intense high-pressure mercury arc and a nickel sulfate-cobalt

sulfate solution in a Lucite cell as a filter, Bernasconi *et al.* (7) obtained a narrow ultraviolet band (300 to 350 m μ) useful for selective photographic detection of compounds with maxima in this region. The filter system, however, would probably transmit too little light to be suitable for the relatively insensitive blueprinting method, although it is satisfactory with silver halide papers.

Sample blueprints of spot test strips and of chromatograms are shown in Fig. 1. If the molecular extinction coefficient of a substance at 254 m μ is of the order of 10,000, a spot of 0.001M solution can be detected. Absorption at 254 m μ has been used for detecting purines and pyrimidines (1) and ketosteroids such as cortisone (3, 8). Figure 1 shows that blueprint paper is also useful for detecting certain sterols, insecticides, acaricides, many aromatic compounds, and some inorganic ions. Absorption at 360 m μ is of more limited use but can be used to detect polynuclear aromatics and other compounds with long sequences of conjugated double bonds, some aromatic nitro compounds, and a few inorganic ions.

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Determination of Deuterium Oxide in Water by Measurement of Freezing Point

A simple, rapid method for determination of deuterium oxide (D₂O) in water has been devised. The most widely used and precise methods involve use of the mass spectrometer and the falling drop. Other less common techniques include measurements of density gradient, phase-contrast refraction, sinking rate of a quartz float, infrared absorption, and thermal conductivity. No reference has been found to a method based on elevation of the freezing point of water.

The freezing point of deuterium oxide

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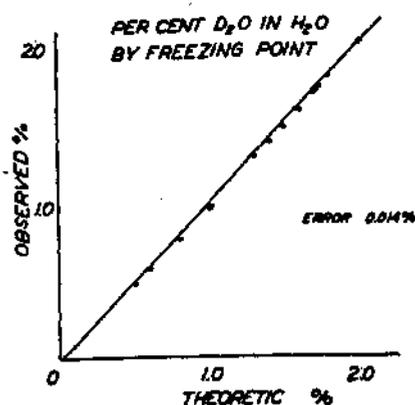


Fig. 1. Observed concentrations, expressed as percentage of D_2O in water, plotted against the theoretic values. The error (± 0.014) is expressed as deviation from the theoretic straight line shown.

Table 1. Evaluation of accuracy of the method.

No. of determinations	Standard error (%) D_2O	D_2O concn. range (%)	Type of measurement
14	± 0.014	0-2	Blind
210	± 0.019	0-5	Repetitive

is $3.802^\circ C$. Hence, a method for measuring freezing points to a thousandth of a degree should permit differentiation of at least 0.01 percent deuterium oxide in water. Such a method is now available in the form of the Fiske osmometer (1). The operation of this instrument is based on the principle that slightly supercooled solutions can be rapidly frozen by agitation and that the temperature of the ice crystals can be measured during the several seconds it remains constant. This temperature is considered to be the freezing point. The apparatus is designed to operate at temperatures below zero, but proper adjustments of the calibration will allow measurements a degree or so above zero. The temperature is measured by means of a thermistor in a bridge circuit. If measurements are confined to a narrow temperature range (a degree or so), potentiometer readings are essentially linear.

When the instrument is used as an osmometer, the thermistor is calibrated by determining the potentiometer readings at which 100 and 500 milliosmolar solutions of sodium chloride freeze. When these are adjusted to provide dial readings of 100 and 500 on the potentiometer, the osmolarity can be read directly.

Since the freezing point of 100 milliosmols of sodium chloride in distilled water is $-0.1858^\circ C$ and that of distilled

water is $0^\circ C$, these liquids were used for the purpose of calibrating the osmometer. One hundred scalar divisions extended over a temperature range of 0° to $0.2044^\circ C$ for 0 to 5 percent solutions of D_2O in distilled water. For assessment of linearity, 0.5, 1, 2, 3, 4, 10, 15, 20, and 25 percent solutions of D_2O in distilled water were studied. Calibration was checked by determining concurrently the 100 milliosmolar solution of sodium chloride and the water with each series of measurements of the freezing points. Thirty separate determinations were made of each dilution.

It was apparent that the precision of the method was limited by the sensitivity of the osmometer, which, incidentally, can be increased. Repeated determinations were reproducible to 0.2 scalar division of the potentiometer. Linearity within the range of 0 to 5 percent D_2O was also within less than 0.2 scalar division. Figure 1 and Table 1 show the sensitivity and accuracy of the method. For a concentration range of 0 to 2 percent D_2O in water, the D_2O was determined to a concentration of 0.05 percent.

The simplicity and reliability of this method ensure its usefulness in the study of many problems involving D_2O in the medical, biologic, physical, and chemical fields, provided that levels of concentration of D_2O are such as to fall within the range of the osmometer. The sensitivity of the method can be increased by more precise determinations of the freezing points (2).

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Notes

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Accumulator Plants and Rock Weathering

Accumulator plants may be defined as those that take up certain specific elements to a much greater degree than most plants. Accumulators that selectively take up elements harmful to agriculture—such as selenium and molybdenum—have been extensively studied, but little has been written about the problems posed by plant accumulators of major constituents of the earth's crust such as silica, aluminum, and iron. Although it is generally recognized that

biochemical factors are important agents in surficial processes, the possibility that accumulator plants may withdraw from the earth geologically significant amounts of various elements has not previously been pointed out so far as I am aware. The amount of silica taken out of the ground by certain plants is sufficiently large to warrant consideration of this process as an important factor in the development of silica-deficient soils such as the laterites. Horsetail or "scouring-rush," some dicotyledons, and many of the monocotyledons such as corn, palm trees, bamboo, reeds, and other grasses take up large amounts of silica (see Table 1).

According to Amos and Dadswell (1), more than 375 species of tropical timber trees must be classed as silicic—that is, they contain more than 0.05 percent SiO_2 (dry weight). Many species of the tropical hardwoods studied by Amos and Dadswell range between 2 and 3 percent SiO_2 (dry weight); some species of bamboo and rush contain 3 times this amount. One order of trees common in Brazil, the Chrysobalanea, especially the genus *Moquilea*, is unusually rich in silica, and the heavy bark of trees of this genus contains slightly more than 50 percent SiO_2 (dry weight) (2).

According to Bear (3), tropical forests produce from 10 to 20 tons dry weight of new growth per acre above ground each year. If we assume that a forest in the tropics containing good silica accumulators averages 3 percent dry weight of silica and produces approximately 13 tons dry weight of new growth per acre per annum, it is readily calculated that such a forest would abstract from the soil about 0.4 ton of silica per acre per year. This is equivalent to 1 ton of silica per acre each $2\frac{1}{2}$ years.

A basaltic lava having a density of about 3 and containing 49 percent SiO_2 would have about 2000 tons of silica per acre foot. It is apparent, then, that a tropical jungle of silica-accumulator vegetation could in 5000 years easily remove silica equivalent to that in 1 acre foot of basalt. The bulk density of the lateritic soils commonly ranges from 30 to 40 percent of the bulk density of the parent basalt. If we assume that 3 to 1 is a fair approximation of the ratio of parent rock to residual lateritic soil, volume for volume the laterite and parent basalt would be nearly equivalent, but the lateritic soil would represent only about one-third the weight of the fresh parent rock. If this lateritic soil contained about 35 weight percent SiO_2 (see Hough and Byers, 4) there would be approximately 475 tons of silica per acre foot residual from the 2000 tons of silica in 1 acre foot of fresh basalt. The hypothetical tropical jungle of silica-ac-