SHORT COMMUNICATIONS

The use of iodine for the detection of lipids

The detection of complex lipids and terpenoids after electrophoretic or chromatographic separation on filter paper often presents some difficulty due to absence of markedly reactive groups in these compounds. We have found that treatment of the developed papers with iodine often affords a satisfactory means for locating many lipids including steroids, unsaturated fatty acids and terpenoid hydrocarbons.

BRANTE[1] introduced the use of iodine vapor for the detection of nitrogenous compounds and it has been subsequently adopted for the observation of a number of relatively unreactive organic compounds (non-reducing carbohydrates, etc.) on paper[2,3].

The paper chromatogram or electrophoretic strip is thoroughly dried and exposed overnight

TABLE I

SENSITIVITY OF LIPIDS TO IODINE VAPOR (24 HOUR EXPOSURE) ON WHATMAN NO. 1 PAPER

Compound	*Quantity in μg*		
	50	*100*	*200*
Triolein	++	+++	+++
Tristearin	±	±	+
Monomyristin	—	±	±
Phrenosine	+	+	++
Sphingomyelin	++	+++	+++
Phosphatidyl choline	++	+++	+++
Phosphatidyl ethanolamine	+	+++	+++
Lipositol	+	+	++
Cholid acid	+	+	+
Desoxycholic acid*	+	+	+
Lithocholic acid	+	+	+
Trihydroxycoprostane	+	+	+
Cholesterol	++	++	++
Lanosterol	+	+	+
Ergosterol	+++	+++	+++
β-Sitosterol	+	++	++
Progesterone	+++	+++	+++
Testosterone	+++	+++	+++
Estradiol	+	+	+
Androstenedione	+	+	+
Cortisone	+	+	+
Hydrocortisone	++	++	++
Corticosterone	+	++	++
Desoxycorticosterone	++	++	++
Kryptogenin	++	++	++
Diosgenin	++	++	++
Pimaric acid	+	++	++
Abietic acid	++	++	++
β-Carotene	+	++	++
Lycopene	±	+	+
Squalene	+	+	+
Vitamin A	+++	+++	+++

* Desoxycholic acid required a long period of time (48 hours) in the iodine before reaction was noticeable.

in a closed chamber containing a few crystals of iodine. The presence of the lipids (Table I) is usually revealed within a few minutes as characteristically colored zones (brown or yellow). The precise coloration appears to vary with the acidity or alkalinity of the dry paper. Inspection under ultra-violet light facilitates the detection of feebly staining compounds.

Alternatively, the dried papers may be sprayed or dipped in a 0.2% solution of iodine in petroleum ether or diethyl ether and the excess iodine allowed to volatilize in a gentle draught of air.

The use of iodine has been previously reported for the detection of certain steroids[4,5].

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Department of Biochemistry, The School of Medicine, University of Pennsylvania, Philadelphia, Pa. (U.S.A.)

MICHAEL W. WHITEHOUSE
ANN E. BRESLER
EZRA STAPLE

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Correlation of ion exchange column and equilibration experiments under non-ideal conditions

In devising separative procedures involving ion exchange chromatography, an investigator is ordinarily faced with the problem of monitoring effluent from the column. Since many experiments may be necessary to delimit conditions and to check on completeness of recovery, apparatus for automatic monitoring of column effluent is necessary for efficient operation. This represents a severe disadvantage, particularly for the investigator who desires to consider only a single separation in the course of other work and who may not have the necessary monitoring equipment readily available. The problem would be somewhat simplified if the initial experiments could be performed using a batchwise equilibration technique with these results applied to design of a column separation.

Procedures for correlating column and equilibration experiments have been presented; however, they refer to exchange reactions involving only monovalent ions in which the influent ion is present only in trace concentrations, allowing the approximation of ideal behavior both in solution and resin phases to be made. For practical purposes it is often necessary to perform separations of polyvalent ions in concentrations too high to be considered ideal. This paper reports the application of data obtained in shaking experiments to column experiments involving divalent ions in concentrations up to 0.5 *M* without any attempt to calculate activity coefficients, to determine to what extent failure of ideal behaviour would distort the results. The exchange of lead(II) nitrate in 2 *N* nitric acid with a cation resin in the hydrogen from has suitable elution characteristics.

Apparatus and reagents

Ion exchange resin. Dowex 50-X12, 100–200 mesh, analytical grade cation resin was used. This resin was obtained from Bio-Rad Laboratories, Berkeley, Calif., and had been washed repeatedly with acid and alkali and finally converted to the hydrogen form. It contained approximately 45% water and had a capacity of 5.01 mequiv. per oven-dried gram. The void space in water averaged 48%, the density 0.46 g/ml.

Standard lead solution. Lead solutions were prepared from analytical reagent grade $Pb(NO_3)_2$ and were standardized by precipitation of $PbSO_4$ or by amperometric titration with $K_2Cr_2O_7$ in acetic acid–sodium acetate buffer, using a D. M. E. at an applied voltage of zero *vs.* S. C. E.

Other reagents. Other reagents, with one exception, were analytical grade and were used without further purification. Gelatin, for maximum suppression in polarographic determination of lead, showed no blank.

Polarograph. A Sargent Model XXI, pen-recording polarograph and a Sargent "Ampot" amperometric titrimeter were used for lead determinations. Polarographic determinations were carried out using a controlled head of mercury above the D. M. E. and controlled temperature to allow use of a calibration curve.