Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Separation of Biological Solutes by Liquid Thermal Diffusion

Joseph C. Touchstone[a]; Murrell F. Dobbins[a]
[a] DEPARTMENT OF OBSTETRICS AND GYNECOLOGY AND HARRISON DEPARTMENT OF SURGICAL RESEARCH SCHOOL OF MEDICINE, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA, PENNSYLVANIA

To cite this Article Touchstone, Joseph C. and Dobbins, Murrell F.(1975) 'Separation of Biological Solutes by Liquid Thermal Diffusion', Separation Science and Technology, 10: 5, 617 — 621
To link to this Article: DOI: 10.1080/00372367508058043
URL: http://dx.doi.org/10.1080/00372367508058043

SEPARATION SCIENCE, 10(5), pp. 617–621, 1975

NOTE

Separation of Biological Solutes by Liquid Thermal Diffusion

JOSEPH C. TOUCHSTONE and MURRELL F. DOBBINS

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
AND
HARRISON DEPARTMENT OF SURGICAL RESEARCH
SCHOOL OF MEDICINE
UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA, PENNSYLVANIA 19174

Abstract

A number of phenolic components of urine reactive to phosphomolybic acid were separated by the use of liquid thermal diffusion.

The chromatographic data reported here provide evidence that thermal diffusion (Soret effect) may be successfully used to separate solutes from biological media. Six different phenolic substances were separated and concentrated directly in the urine as a result of thermal diffusion in a 48-hr experiment without any treatment or supervision during the operation.

Although molecular movement due to thermal gradient has been known since 1856 (*1*), it has not been used extensively except in the petrochemical field. Soret (*2*) in 1879 described thermal diffusion in the total absence of convection. Kramers and Broeder (*3*) described analytical separation of hydrocarbon oils using thermal diffusion. Jones (*4*) showed separation of *o*- and *m*-xylene by thermal diffusion. He also showed the separation of *cis*- and *trans*-1,2-dimethylcyclohexane. More recently Grodzka and Bannister (*5*) reviewed the principles of thermal diffusion and its use in low *g* environments.

Most data available on the Soret effect are for aqueous solutions of inorganics, and few experiments have involved solutions of organic compounds. The present work was confined to a study of the Soret effect on media from biological sources. The example given here describes the results obtained on separation of phenolic components of urine from pregnant women.

The thermal diffusion cell used in this work consists of three concentric tubes of 30 cm length. The annular space containing the sample was 0.3 mm. The inner tube consisted of 316 stainless steel. The two outer tubes were of precision bore glass. The tubes were provided with ports for entrance and exit of the cooling and heating liquids. The capacity of the annular space was 5.2 ml.

The schematic for the apparatus is pictured in Fig. 1. Exit ports fitted with rubber septa (as used in gas chromatography) are positioned at the top, bottom, and middle of the tube for withdrawal of samples at comple-

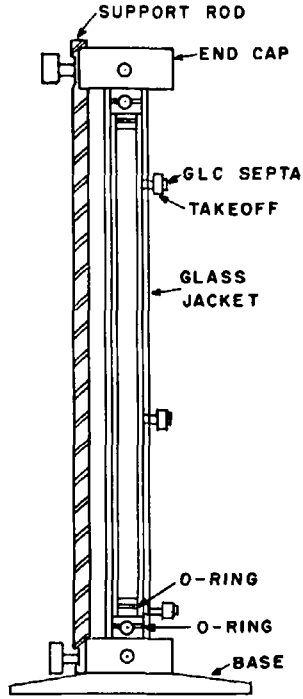

FIG. 1. Assembly of the thermal diffusion cell.

tion of the experiment. The samples were withdrawn by use of hypodermic needles. In the present work, water cooled to 0 C was circulated through the outer tube (glass tubing). Water of 37 C was circulated through the inner tube (steel tubing).

Untreated urine from a pregnant woman was injected by hypodermic needle through the septum in the bottom port. Thermal diffusion was carried out for 48 hr. At the end of this period, 0.25 ml samples were drawn from the top, middle, and bottom ports in that order.

To each sample was added 4.75 ml of water and 0.75 ml of concentrated hydrochloric acid. These were refluxed for 15 min. Each was extracted twice with 10 ml of ether. The ether was combined and washed two times with 2 ml of 8% sodium bicarbonate solution. Removal of phenols was achieved by extraction twice with *N* sodium hydroxide solution, 5 ml each time. After acidification the phenols were removed by extraction with ether. The extracts so obtained were spotted on a silica gel G thin-layer plate and developed with a solvent of 15% ethanol in benzene until the front was 1 cm from the top of the 20 × 20 cm plate. After drying, the plate was sprayed with 5% phosphomolybdic acid in ethanol and heated in an oven at 120 C for 15 min.

Figure 2 shows the result of the separations obtained by thermal diffusion. The sample removed from the bottom portion of the tube shows the concentration (separation) of five different components by the procedure. In the case of the next to lowest component, the concentration of the sample in the bottom of the annular space was incomplete since the component was present in the fractions from the middle and bottom ports. The four components in the reference lane on the chromatogram show that the materials may be different from the known identity of the reference estrone, estradiol-17β, epiestriol, and estriol (positioned from top to bottom). The identity of the separated phenols is not yet known. Since acid hydrolysis was necessary to release the phenols separated, it appears that the thermal separation took place with the glucuronides. It is generally known that this is the major form of urinary metabolites. The present report is presented to show that separations do occur under the conditions described. There was also a noticeable concentration of the more polar substance which did not move from the starting line of the chromatogram. These separations seem to be sharp, considering only 0.25 ml was removed from each area of the annular space.

Since separations of organic materials in thermal diffusion have been based on molecular configurations, it appears that this technique may be useful for separations and concentrations of biological solutes that previously were considered impossible.

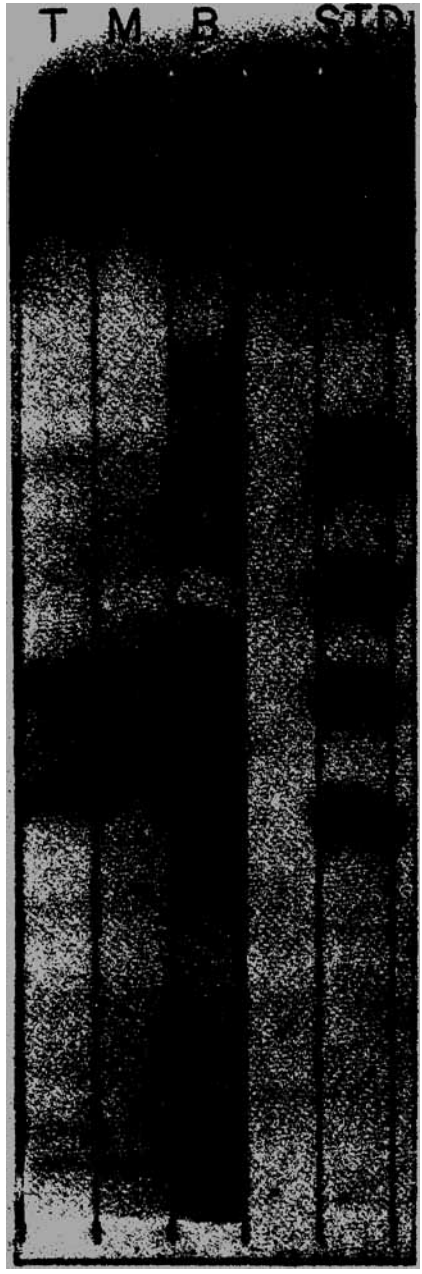

Fig. 2. Thin-layer chromatogram showing separation of components of pregnancy urine in the bottom layer of the thermal diffusion cell. T, top; M, middle; B, bottom; and STD, reference compounds (estrone, estradiol, 16-epiestriol, and estriol; positioned from top to bottom on the chromatogram).

REFERENCES

1. C. Ludwig, *Wien Akad. Ber.*, *20*, 39 (1856).
2. C. Soret, *Arch. Sci. Phys. Nat.*, *2*, 48 (1879).
3. H. Kramers and J. J. Broeder, *Anal. Chim. Acta*, *2*, 687 (1948).
4. A. L. Jones, *Technique of Organic Chemistry, Part 1. Separation and Purification*, Vol. 3 (A. Weissburger, ed.), Wiley-Interscience, New York, 1956, p. 1.
5. P. G. Grodzka and T. C. Bannister, Paper presented at 12th AIAA Aerospace Sciences Meeting, Washington, D.C., January 30, 1974.

Received by editor November 25, 1974