AIDS FOR THE ANALYST . . . . .

Apparatus for Liquid-Liquid Extraction without Formation of Emulsions. Frederic E. Holmes, Clinical Laboratory, Christ Hospital, Cincinnati, Ohio.

THE apparatus shown in Figure 1 prevents formation of emulsions in liquid-liquid extractions, maintains an adequate area of interface between the two solvents, and is simple in construction and operation. It has been used for extraction by ethyl ether of fats and fatty acids, of hippuric acid and barbiturates from urine and other body fluids and from feces and ground tissues suspended in an aqueous medium, and for extraction by benzene of machine oil held in boiler feed water by emulsifying agents. The process is similar to that in the conventional extractor, except that the refluxed solvent, instead of passing through the aqueous layer, flows across its surface.

Some specimens form relatively stable emulsions with the solvents. Even large drops of solvent passing through an interface, or slight turbulence where aqueous and nonaqueous layers flow around the shaft of a stirrer, may produce stubbornly persistent coarse emulsions, or suspensions of finely divided solids, which are carried over into the final extract. Washing or settling chambers have been used to promote separation of such entrained matter (*1*, *4*, *6*, *8*). However, use of the horizontal interface alone, and of the completely submerged, magnetically operated stirrer, prevents formation of emulsions.

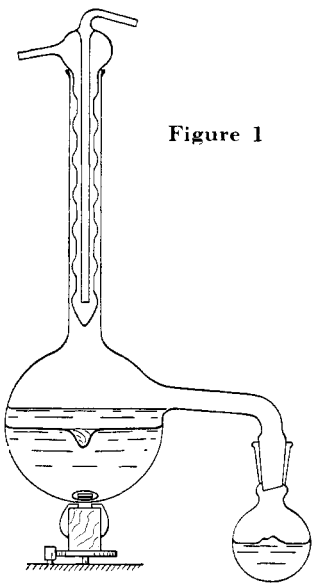

Figure 1

Several devices provide more area of contact than the horizontal interface, but may be otherwise unsatisfactory. The vertically disposed interface formed by a heavier immiscible solvent flowing down the walls of the extraction chamber (*7*) is inherently less stable than the horizontal interface. Dispersal of solvent in minute droplets by passage through many fine orifices, or by vigorous stirring or shaking, makes possible practically unlimited increase of interfacial area, but aggravates the difficulty of separating the extract. On the other hand, the area of interface between the solvent and the sample provided in the middle of the spherical extraction chamber of the present apparatus is considerably greater than the area of the corresponding plane of contact plus that of the surface of drops of solvent rising through the sample in a conventional extractor of the same capacity, even when drops are forced to follow a spiral (*5*) or devious (*3*) path.

In an alternative form the round extraction chamber consists of a standard short-necked round-bottomed flask with standard-taper joint for attachment of a standard condenser. An extractor with capacity for 500 ml. of sample requires a 1-liter flask. In both forms, on extractors of 500-ml. capacity or less, the side tube connecting with the boiler receiver is fused to the chamber at a point such that solvent will overflow into it when the level reaches 15 to 20 mm. above the mid-line. For extractors of 3-liter capacity, the distance above the mid-line is doubled.

Depth of the supernatant solvent is initially controlled by addition of water to the chamber. It may be adjusted by tipping the extractor to raise or lower the side tube. When the aqueous phase increases in volume through absorption of solvent, or wave-like surges occur due to asymmetry in the stirring motion, the side tube may be raised slightly. When circulation is smooth, the side tube may be lowered to decrease the depth and volume of the solvent pool and effect a more rapid turnover (*2*).

The apparatus may be tipped to decant off the last portion of extract into the boiler receiver. Extracts too large to be handled in a small evaporating dish may be concentrated without exposure to oxidation, danger of fire, or risk of spattering, by raising the side tube and distilling the solvent back into the chamber.

Batch extraction may be preferred when continuous extraction results in accumulation in the extract of unwanted substances which are slightly soluble in the solvent; substances such as hippuric acid may interfere in the gravimetric determination of fats. During prolonged batch extractions, the condenser prevents loss of solvent. At the end of the extraction, the extract is decanted into the boiler receiver. Solvent may be distilled back, decanted over to wash the last traces of extract into that previously collected, and then concentrated in the apparatus without danger of oxidation, fire, or loss.

The stirrers are made by sealing short pieces of soft iron rod into glass tubes which are tapered to form stubby or slender spindles 15 to 35 mm. long for use with small or large volumes of aqueous sample, different viscosities of solutions, and different curvatures of the round extraction chambers. The magnet for actuating the stirrers is mounted in a wooden block on the idling wheel of a phonograph turntable. The speed is controlled by the wall thickness of the rubber tubing placed over the shaft of the motor to drive the idling wheel.

The performance of the extractor was compared with that of a common type of laboratory extractor (Table I).

The conventional extractor consists of a cylindrical body with a side tube connecting with the boiler receiver, the space below the side tube serving as the extraction chamber. A condenser of the type shown in Figure 1 hangs in the part above the side tube, and a 7-mm. glass tube with funnel top conducts the solvent to the bottom of the chamber, where it escapes through notches in the flared end. The method used to measure rate of extraction was suggested by that of Bewick, Currah, and Beamish (*1*). Time of extraction of approximately 40% of the substance sought at equal rates of reflux was taken as a measure of rate of extraction, and was controlled by identical conditions of heating. Turnover of ether was estimated by counting drops. Hippuric acid was introduced in a solution of known concentration. The fatty specimen was prepared by grinding together skim milk, casamino acid (Difco), acacia, starch, soluble starch, olive oil fatty acids, olive oil neutral fat, Turgatol 7, Permutit, and finally gradually increasing amounts of water until a smooth paste and then a uniform thick emulsion suspension were obtained. On direct titration, 10 ml.

Table I. Performances of Extractors

Substance extracted	Hippuric Acid		Artificial Fatty Specimen	
Apparatus	Conventional	Present	Conventional	Present
Solute or sample, g.	0.087	0.087	10 ml.	10 ml.
NaOH in receiver, ml.	2 (0.1 *N*)	2 (0.1 *N*)	1 (1 *N*)	1 (1 *N*)
$CaCl_2$ in receiver, ml.	...	...	5 (10%)	5 (10%)
Aqueous layer, ml.	600	650	625	675
Ether layer, ml.	100	100	75	75
Estimated turnover, of ether, ml./hour	100–150	100–150	250–350	250–350
Aqueous layer, depth, mm.	205	60	215	63
Interfacial areas, sq. cm.				
Sum of drops	8	None	Estd. 20	None
Horizontal plane	28	120	28	120
Total	36	120	Estd. 48	120
Extraction of "40%," min.	378	347	992	497

were equivalent to 2.5 ml. of 1 *N* sodium hydroxide. Extraction was continued until the phenolphthalein in the standard aqueous alkaline solution in the boiler receiver became decolorized. (Calcium chloride was used with the sodium hydroxide to form the calcium soaps, which are not alkaline to phenolphthalein in aqueous solution.)

LITERATURE CITED

(1) Bewick, H. A., Currah, J. E., and Beamish, F. E., ANAL. CHEM., 20, 740 (1948).
(2) Dubbs, C. A., private communication.
(3) Francis, A. W., IND. ENG. CHEM., ANAL. ED., 1, 15 (1929).
(4) Knox, W. T., Weeks, R. L., Hibshman, H. J., and McAtear, J. H., *Ind. Eng. Chem.*, 39, 1579 (1947).
(5) Mathews, A. P., "Physiological Chemistry," 4th ed., p. 1006, New York, Wm. Wood and Co., 1925.
(6) Pearl, I. A., IND. ENG. CHEM., ANAL. ED., 16, 62 (1944).
(7) Stracy, L. C., Hunter, T. G., and Nash, A. W., *Ind. Eng. Chem.*, 29, 278 (1937).
(8) Wylie, A. B., and Schmulovitz, M. J., *Science*, 83, 398 (1936).

Use of pH Meters in Conjunction with Chromatographic Columns. R. N. Jeffrey, Division of Tobacco, Medicinal, and Special Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.

ONE of the drawbacks to the use of various chromatographic procedures in the isolation of colorless substances, whether the procedures are based on adsorption, partition, or ion exchange, has been the difficulty of determining which portion or portions of the separated material contain the interesting compounds. This is particularly true when a flowing column is used, from which the solution is collected continuously. However, this type of procedure has certain advantages in analytical methods for the determination of compounds which can be measured readily when relatively pure, but cannot be determined accurately in the presence of some of the other constituents of the original mixture. Quantities of material can be used which permit the accurate determination of the compounds separated, and this is not always true when a single sheet of paper is used instead of a column. The flowing column also has advantages from a manipulative standpoint over the older procedure of extrusion and separation of various portions of the solid material in the column.

The best method of selecting a fraction from the flowing column which contains all of the desired compound without contamination with interfering substances naturally depends on the compounds being studied. When weak acids or bases are determined, the use of a pH meter with electrodes in the effluent from the column assists in this selection.

In this laboratory the composition of cured tobacco leaves grown using different cultural practices is being studied. An effort is being made to determine separately each of the alkaloids and the organic acids present in these samples. The quantities of various other substances in these complicated mixtures affect the volume in which a given substance is eluted. Some evidence has been obtained that a continuous indication of the pH of the effluent assists materially in selecting a fraction that will contain the maximum amount of the ingredient for which the particular analysis is being conducted, with the minimum amount of interfering substances. The use of pH values, as such, applies only to water solutions, such as compose the effluents from ion exchange columns and partition columns in which water is the solvent for the moving phase. In the more common type of partition column, in which the solvent of the moving phase is an organic liquid, variations sometimes occur in the potential read on the pH meter as the effluent moves through the cell, but these should not be referred to the pH scale.

The cell in use in this laboratory was made from a 50-ml. distilling flask. The neck was cut off 2 cm. below and 1 cm. above the side arm and the side arm was cut to a length of 5 cm. The bottom of this piece was flared slightly and the top made oval in cross section, to allow the two standard 2.5-inch Beckman electrodes to enter it while they are mounted in the usual way on the door of the Model G meter. A small-diameter glass tube was bent into U-shape, connected to the bottom of the ion exchange column, and passed up through the hole in the bottom of the Bakelite beaker holder, where it was inserted into a short rubber stopper which fits the bottom end of the cell. The side arm was bent downward about 2 cm. from its end, which allows a 100-ml. graduate to be placed under it, so that records of the volume of effluent can be kept along with records of pH value, and the graduate can be replaced when desired. The cell thus formed has a net volume of 1.3 ml. when the electrodes are in place.

One can also obtain a commercial flow-type electrode assembly or can make a flowing microcell modified from the one described by Dietz [*Science*, **108**, 338–9 (1948)].

Micro Still Pot Suitable for Column Calibration. T. J. Walsh and E. H. Phelps, Case Institute of Technology, Cleveland, Ohio.

THE most satisfactory method of measuring the efficiency of a distillation column is to take spot samples of the column overhead and pot liquids while the column is operating at equilibrium conditions on a test mixture of known properties. Securing a pot sample from a microstill without disturbing the thermal equilibrium of the still is possible, using a hypodermic syringe with a 6-inch needle attached permanently to the still pot.

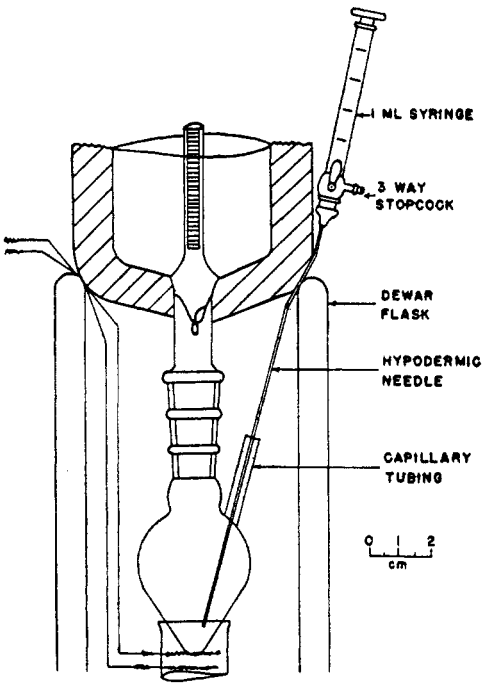

Figure 1. Syringe Attachment for Obtaining Still Pot Samples

The hypodermic needle is inserted through a piece of capillary borosilicate glass tubing before the tubing is sealed to the shoulder of the still pot. In sealing, the glass flows around the needle forming a tight seal. The seal may be ensured by flowing de Khotinsky cement into the capillary above the glass constriction.

Outside the still pot, the needle should be long enough to extend beyond the insulating flask. The outer end is connected to a 1-ml. hypodermic syringe through a capillary three-way stopcock. Samples as small as 2 drops may be taken by drawing a few tenths of a milliliter of liquid into the syringe and discharging through the third port of the stopcock. Excess material may be returned to the still pot.

This arrangement also permits changing the still pot composition without dismantling the still.