

A Polyethylene-Alumina Column for Purification of Tissue Extracts before Analysis

W. M. HOSKINS, W. R. ERWIN,
and RAYMOND MISKUS

Department of Entomology and
Parasitology, University of Cali-
fornia, Berkeley, Calif.

W. W. THORNBURG and L. N.
WERUM

California Packing Corp.,
Emeryville, Calif.

Alumina granules coated with polyethylene, when used in a column with acetonitrile-water mixtures as eluents, hold back interfering extractives from samples and permit residual insecticides to pass. Performance with a number of plant products and insecticides is shown. The behavior with dyes indicates a partition effect in addition to effective filtration.

ANALYSIS FOR A RESIDUAL ORGANIC PESTICIDE in plant or animal tissues is possible only after an extract obtained with an organic solvent is partially purified by removal of the interfering extractives. Successive shaking of the extract with acetonitrile transfers the pesticide to the acetonitrile while leaving much of the extractives behind (2). For purification Erwin *et al.* (1) passed the acetonitrile solution diluted from 40 to 75% by volume with water through a column containing chromatographic alumina coated with 15% by weight of a 2 to 1 petrolatum-paraffin wax mixture. The wax column was devised on the theory that fats, oils, and waxes from the sample would be retained by a stationary phase of a similar nonpolar nature, whereas the more polar pesticides would be carried through by the acetonitrile-water moving phase. It has been used extensively for purification of extracts from many kinds of plant and animal tissues prior to determination of the residual pesticides by chemical tests and (or) bioassay.

An objection to the wax column is that the petrolatum-paraffin wax coating is slightly soluble in the aqueous acetonitrile eluent, especially in the acetonitrile-rich solution needed to carry through aldrin and some other pesticides. Inclusion of even very small amounts of the typical organic solvents such as may be needed for some separations removed much more of the coating. This behavior lessened the efficiency of the column, because the extract became slightly contaminated with petrolatum and paraffin wax, which may interfere with subsequent analyses in the same manner as the original extractives. The column is usually so altered by the single passage of eluent that it cannot be used again. With a nonpolar coating insoluble in acetonitrile and in other solvents, the column would remove these objections and be usable with a wide range of solvents as the moving phase.

Column Materials and Eluents

The hydrocarbon polymers have the desired properties. Extensive trials were

made with polystyrene and several grades of polyethylene coated by the use of suitable solvents upon silica, calcium carbonate, powdered cellulose, chromatographic alumina, and ignited alumina. Performance was judged by the removal of extractives from the extracts of various plant products and by the efficient passage of the contaminating insecticide. Best results were obtained with polyethylene, grade DYNH, on either chromatographic alumina or on the purified, ignited grade. The latter is now used in routine work, as it is cheaper, but could not be used in the wax column (1), as the components separated.

Using spinach as a typical plant product and DDT, methoxychlor, lindane, and parathion as insecticides, numerous eluent systems were tested including methanol, methylal, acetone, and ethylene chlorohydrin at various concentrations in water. None proved as satisfactory, however, as the acetonitrile-water eluents used previously with the wax column.

Special Materials and Reagents

Polyethylene, Bakelite DYNH, granular. This material contains plasticizer and perhaps lower polymers which must be removed as described later.

Polyethylene powder, no plasticizer (Heisler Corp., Wilmington, Del.).

Polyethylene, reground (Automatic Plastics Molding Co., Berkeley, Calif.).

Alumina, chromatographic, 80 to 200 mesh (Fisher Scientific Co.).

Alumina, purified ignited. The material used was 100-mesh and less (Baker & Adamson).

Acetonitrile (Union Carbide Chemicals Co.).

Toluene, reagent grade.

Preparation of Column

By empirical trials the following procedure has been found to give coated particles of uniform appearance and properties. Two hundred grams of granular polyethylene are dissolved in about 2 liters of boiling toluene with continued heating and stirring. One

thousand grams of either grade of alumina are added and the mixture is allowed to cool with continuous stirring. A stream of warm air directed across the surface hastens removal of the toluene and a Kitchen-Aid mixer or other similar device is convenient, but not necessary. When the mixture has become a fine, white powder, dry to the touch, it is spread in a thin layer on a tray and allowed to stand in a ventilated hood for several hours to remove the last traces of toluene. After screening through cheesecloth to remove any large chunks, the powder is ready and may be kept in a closed container indefinitely.

The glass tube used to hold the polyethylene-alumina granules was 20 mm. in outside diameter and 26 cm. long. It has a coarse, sintered glass filter and a drip tube at the bottom and a 150-ml. reservoir at the top. The usual weight of column material was 35 grams. A small cotton pad is placed on top of the granules. If the DYNH polyethylene has been used, it is necessary to flush out plasticizer and other low molecular weight materials with 150 to 200 ml. of petroleum ether, followed by 200 ml. of the acetonitrile-water eluent to be used with the extract. It is, of course, possible to wash the granules at the time of preparation. Flushing with petroleum ether is not necessary with either the reground polyethylene or the Heisler product, but passage of a volume of the appropriate acetonitrile solution increases uniformity of packing and removes a small amount of colored matter which tends to interfere with the Schechter-Haller method for the estimation of the DDT group.

Treatment of Extracts. Extracts corresponding to 50-gram samples in whatever solvent is best suited to the contaminating insecticide and the product concerned are prepared and transferred to acetonitrile, by evaporation to 1 to 2 ml. at room temperature, adding 0.5 gram of Celite, and completing the evaporation under a gentle air stream. The residue is then redissolved in warm acetonitrile. Evaporation of the original extract upon Celite is an improvement on the use of fine sand as described

by Erwin *et al.* (7). In this earlier article emphasis was laid on the necessity for adding the extract to the column in a small volume—e.g., not over 10 ml.—when residual insecticides were being separated or partly identified by the portion of the eluate in which they appear. If this is not necessary, it has been found convenient to add the extract to the column in a mixture of 24 ml. of acetonitrile and 36 ml. of water for the 40% eluent or with correspondingly less water for the 60 or other % eluent. Residual insecticides are recovered at reasonably high yields in 50 to 100 ml. of eluate, but for routine work 200 ml. of eluate is collected and data given refer to this volume.

Purification of Extracts and Recovery of Added Insecticides

Table I contains data on the weights of extractives obtained during treatment of 50-gram samples of a number of plant products and the percentage removed—i.e., the degree of purification, by a single passage through the column of the eluent best suited for the insecticide. Data are also included on the percentage of various insecticides recovered after addition to the plant products, before extraction or to the extract before passage. Schechter-Haller analysis was used for DDT and analogs, and Averell-Norris analysis for parathion. All other compounds were analyzed by bioassay with houseflies (7). No further purification was necessary in any case. The amounts added were arbitrary, but of the magnitude often encountered in residue analysis. In general, the three types of columns gave comparable purifications, sufficient in all cases for quantitative determination of the insecticide by bioassay and in most cases for chemical analysis. The various extracts are purified to widely different extents—e.g., the heavily contaminated alfalfa extracts containing much inert material came out almost entirely free of extractives, whereas about two thirds of the tomato extractives, mostly the pigment lycopene, came through, but did not interfere with subsequent analysis.

The combinations of product and insecticide shown in Table I are only those for which a complete study has been made. The column is used whenever preliminary tests show a satisfactory recovery of added insecticide. If excessive amounts of fatty materials must be taken as a sample—e.g., butter, body fat, or olive oil containing only minute amounts of residual insecticides—a preliminary partition between petroleum ether and acetonitrile may be needed (2) to reduce the extractives to an amount which the column can handle. This amount has not been determined quantitatively and it doubtless varies with the product and the type of column used,

Table I. Extractives Removed and Insecticides Passed by Polyethylene Columns

Product, Fresh Unless Noted	Weight of Extractives, Mg.	% Purification	% CH ₃ CN ^a in Eluate	Insecticide, P.P.M. Added, % Recovered
A. Reground Polyethylene on Ignited Alumina				
Beans, dry kidney ^b	30	80	40 ^a 60 ^a	Lindane (0.3, 88), parathion (0.05, 96), dieldrin (0.2, 83)
Grapes ^b	28	78	40 ^a 60 ^a	Lindane (0.3, 107), parathion (0.05, 110), dieldrin (0.2, 81)
Prunes ^b	59	91	40 ^a 60 ^a	Lindane (0.3, 98), parathion (0.05, 92), dieldrin (0.2, 81)
B. DYNH Polyethylene ^d on Ignited Alumina				
Alfalfa	700	99	40 60	Parathion (0.05, 88), DDT (1.0, 90), dieldrin (0.14, 90)
Carrots ^c	34	94	40 60	Parathion (0.05, 91), DDT (1.0, 83), dieldrin (0.14, 90)
Pears ^c	15	69	40 60	Parathion (0.05, 84), DDT (1.0, 79), dieldrin (0.14, 78)
Spinach ^c	163	95	40 60	Parathion (0.05, 84), DDT (1.0, 81), dieldrin (0.14, 86)
C. Reground Polyethylene on Chromatographic Alumina				
Alfalfa	700	95 93	40 60	Parathion (8.9, 93), DDT (7.6, 87.5), DDD (8.0, 88), methoxychlor (4.9, 93)
Beans, Blue Lake ^c	10	87	40 65	Perthane (6.7, 109), Diazinon (0.42, 87), Chlorthion (1.7, 89), Dow ET-14 (0.64, 75), Ethion (8.0, 69), Guthion (3.2, 82), Trithion (4.9, 110)
Beans, pole ^c	28	93	65	Toxaphene (7.7, 88)
Cabbage ^c	54	94	65	Endrin (0.3, 107)
Carrots ^c	34	70 64	40 60	Parathion (4.4, 95), DDT (3.9, 84), DDD (4.0, 84), methoxychlor (4.1, 99)
Corn, cream ^c	102	65	40 65	Perthane (6.7, 87), Diazinon (0.42, 89), Chlorthion (1.7, 87), Dow ET-14 (0.69, 81), Ethion (8.0, 88), Guthion (3.2, 82), Trithion (4.9, 102)
Pears ^c	14	59 45	40 60	Parathion (4.4, 96), DDT (3.9, 84), DDD (4.0, 80), methoxychlor (4.1, 95)
Peas ^c	131	90	45	Chlorthion (2.7, 100)
Raisins	58	96	65	Chlordan (0.73, 89)
Spinach ^c	163	84 76	40 60	Parathion (4.4, 94), DDT (3.8, 83), DDD (4.0, 79), methoxychlor (4.1, 98), dieldrin (4.0, 85)
Tomato puree ^c	80	30	40 65	Perthane (6.7, 99), Diazinon (0.42, 84), Chlorthion (1.7, 92), Dow ET-14 (0.64, 84), Ethion (8.0, 70), Guthion (3.2, 79), Trithion (4.9, 95)

^a Indicates 125 ml. eluate; all others 200 ml. ^b Insecticides added to extract before passing through column. ^c Sample canned. ^d Columns washed with 100 ml. petroleum ether and 200 ml. 40 or 60% acetonitrile before use. Samples weighed 50 grams, except as noted all insecticides were added to samples before extraction.

but from 2 grams of olive oil put through a 20-gram column of reground polyethylene on ignited alumina only 57 mg. were eluted in 100 ml. of 40% acetonitrile, and further passage of 100 ml. of 50% and 100 ml. of 60% acetonitrile still left 93% of the oil on the column. Similar results were obtained with dogfish liver oil. With the polyethylene column, as with the wax column, higher concentrations of acetonitrile in the eluent give lower purification (Table I, C), but even the 75% concentration regularly used with aldrin does not

result in unmanageable amounts of extractives in the eluates from the usual plant samples. A very useful property is that the extractives from a sample held by the column may be removed by flushing with petroleum ether. After the column has been equilibrated once more with the appropriate acetonitrile-water mixture, it is ready for use with another sample. This process may be repeated several times.

The rate of flow may be expected to have an effect upon the cleanup of extracts. Because the ignited alumina

is considerably more finely subdivided than the chromatographic grade, columns containing polyethylene upon the former operate comparatively slowly—e.g., up to 1.5 hours is required, unless pressure is applied on the eluting liquid. For routine use about 1-pound air pressure is applied. In a quantitative test, five extracts from 50-gram samples of canned corn were allowed to run through a regular column of re-ground polyethylene on chromatographic alumina with 200 ml. of 40% acetonitrile as the eluting agent. The periods required varied from 50 to 91 minutes, the purification ranged from 93 to 97%, and the recovery of parathion added at 7 p.p.m. was from 86 to 91%. A similar set of five extracts put through under 5 pounds of air pressure required 1.5 to 4.9 minutes, giving a purification of 76 to 86% and a recovery of parathion of 86 to 91%.

Successful use of the Heisler polyethylene powder directly as a column material—i.e., without precipitating upon alumina granules—has been reported from the research laboratories of the National Canners Association (3). This result has been confirmed in the case of DDT and methoxychlor added at the rate of 1.9 p.p.m. to green beans, carrots, and grapes. With 60% acetonitrile as eluent the purifications with the same order of products were 87, 94, and 78% and the recoveries (DDT, methoxychlor) were 106, 105; 87, 100; 69, 101. The sample of powdered polyethylene which was supplied for the present work varied widely in size and shape of the particles and tended to pack in the column so tightly, that excessively long periods were required to force the eluent through. With heavily contaminated samples there was a tendency to plug completely. Because the coated granules are free from this objection they are preferred, but it may be possible to secure pure polyethylene powder which would perform satisfactorily.

Mode of Operation of Column. At least two processes are concerned in the action of the polyethylene column as

it has been used for cleanup of extracts. It is a filter with very fine passages and hence it retains the part of the extractives which is thrown out of solution during the transfer into 40 or 60% acetonitrile. It also is an effective partition column. When small amounts of the three dyes Oil Blue, Oil Orange, and Sudan Yellow, which are somewhat soluble in strong acetonitrile solutions, are put on the column they move in the following decreasing order: Sudan Yellow, Oil Orange, Oil Blue. This is the same order as on a reverse phase paper strip. Thus the more polar the dye the faster it passes through the column, which is in agreement with the interpretation that partition occurs between the highly nonpolar stationary polyethylene phase and the somewhat polar moving acetonitrile phase.

It is unlikely that the alumina plays any part in the action of the column when aqueous acetonitrile solutions are used as eluents, but it might be of importance with nonaqueous systems. Hence a few simple tests were made to detect noncoated alumina granules. In the first place, the untreated alumina powder sinks almost instantly when agitated in water, but the polyethylene-alumina granules are not wetted, since the finer ones persist in floating upon the surface and only the larger ones sink under the influence of gravity. Thus the granules appear to have a polyethylene surface.

Under the microscope the particles may be seen to vary in size and shape in the same manner as the original alumina used. They are of uniform appearance, except a few rather coarse, translucent chunks which probably are pure polyethylene. A typical batch made to contain approximately 20% polyethylene by weight was separated by screening into the following fractions: held on 35 mesh, 10% by weight; held on 60 mesh, 10%; held on 100 mesh, 22%; held on 150 mesh, 33%; fines—i.e., through 150 mesh, 25%. The polyethylene content of each fraction was determined by dissolving in

boiling toluene with the following results in the same order: 31, 33, 20, 20, 19.5% by weight. The chunks of polyethylene in the coarse fractions would account for the excess found in them. The very close agreement with the theoretical content in the fine fractions indicates that polyethylene was present as a coating on the alumina particles as their appearance also indicated.

If substantially all the alumina granules are covered by a layer of polyethylene, the addition of a relatively small amount of untreated alumina should change the behavior of the column with nonaqueous eluents, but if noncoated alumina is already present, an extra amount should have relatively little effect. The experiment was made of adding the dye Sudan Yellow and eluting with iso-octane. The dye came through a regular column in the 55- to 95-ml. cut. But when 20% free alumina was mixed in the column, the dye moved very slowly and did not even start to emerge when over 200 ml. of eluent had passed. Thus by these four rather different criteria the column acts as if it consists of granules presenting only a polyethylene surface to the eluting liquids.

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RUMINANT NUTRITION

Ammonia Formation in the Bovine Rumen with Various Diets

DURING the past decade research has been conducted on the fate of protein and other nitrogenous materials consumed by ruminants. The nitrogen nutrition of the animal is largely controlled by the balance of three processes: the net conversion of dietary protein to

microbial protein; breakdown of ammonia from dietary protein and non-protein nitrogen sources to ammonia and the extent to which the ammonia is absorbed through the rumen wall; and the synthesis of microbial protein from the nonprotein nitrogen of the

rumen (7). One of the important items in ruminant nutrition is to determine, with certain protein sources and practical diets, which of these processes are dominant (79). The interaction of these opposing tendencies is probably a major factor leading to the relative

O. T. STALLCUP and C. G. LOOPER
 Department of Animal Industry and
 Veterinary Science, Arkansas Agri-
 cultural Experiment Station,
 Fayetteville, Ark.