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SEPARATION OF POLYCHLORINATED BIPHENYLS FROM DDT AND ITS ANALOGUES ON A MINIATURE SILICA GEL COLUMN

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SUMMARY

A simple method for the separation of polychlorinated biphenyls from DDT (and its analogues DDE and TDE), dieldrin and BHC on a miniature silica gel column is described. Results of separations on different activated and deactivated silica gels are presented. The best results were obtained when silica gel (activated by heating at 200°) was used with *n*-pentane and benzene as eluents. The routine use of the method and necessary precautions are discussed.

INTRODUCTION

The presence of polychlorinated biphenyls (PCBs) in numerous environmental samples (notably fish and fish-eating birds) has been reported by workers studying residues of chlorinated pesticides[1–3]. PCBs in a sample being examined for pesticide residues can interfere in or even make impossible the identification and measurements of some chlorinated pesticides owing to the similarity of their gas–liquid chromatographic (GLC) retention times. Hence a separation prior to GLC analysis is necessary, and there are already several published procedures for the separation of PCBs from DDT and other chlorinated pesticides by means of thin-layer chromatography[4,5] and liquid chromatography. In liquid chromatographic separations the column packings used are Fluorisil[6] and activated carbon[7], but the most popular column adsorbent for the separation of PCBs and chlorinated pesticides is silica gel. There are several procedures based on different activations and/or deactivations of this adsorbent[8–13].

The aim of this work was to investigate the use of a simple miniature silica gel column for the efficient separation of PCBs from DDT and its analogues by means of gravity elution with simple solvents and without the use of complicated pressure systems and/or solvent systems.

EXPERIMENTAL

Materials and reagents

Silica gel, porosity 40 Å, 70–230 mesh (ASTM), was obtained from Merck

(Darmstadt, G.R.F., Cat. No. 10180, analytical-reagent grade). For simplicity this silica gel is designated here as SG 40. Silica gel, porosity 60 Å, 70–230 mesh (ASTM), was also obtained from Merck (Cat. No. 7734, analytical-reagent grade). This silica gel is designated here as SG 60. Activation and deactivation of the silica gel were performed as specified by Armour and Burke[8], Snyder and Reinert[10] and McClure[11].

Alumina 90 active, neutral for column chromatography (Merck, Cat. No. 1077) (Brockman activity I) was heated at 500° for 12 h and partially deactivated by addition of 5% (w/w) of distilled water.

All solvents were of analytical-reagent grade (mostly from Merck) and were freshly distilled in glass prior to use. A commercial PCB compound containing 54% of chlorine (Aroclor 1254) was obtained from the Monsanto Organic Chemicals Division of the Monsanto Co. (St. Louis, Mo., U.S.A.). Standards from chlorinated insecticides were obtained from PolyScience Corp. (Evanston, Ill., U.S.A.).

Apparatus

Chromatographic columns (200 × 6 mm I.D. and 200 × 10 mm I.D.) with coarse fritted discs and glass stopcocks at the bottom were used for separations on silica gel. A glass column (100 × 6 mm I.D.) with a coarse fritted disc at the bottom was used for cleaning biota extracts.

A Hewlett-Packard Series 7620 gas chromatograph equipped with a nickel-63 electron-capture detector and a 1.5 m × 4 mm I.D. column containing 4% SE-30 + 6% OV-210 on 100–200-mesh Gas-Chrom Q was used. The flow-rate of the carrier gas (5% methane in argon) was 30 ml/min, the injector and detector temperatures were 250° and the column temperature was 210°.

Procedure

The columns were filled with either dry silica gel or a slurry prepared with a solvent. The silica gel was carefully packed into the column until it reached the 100 ± 2 mm or 200 ± 2 mm level. Gentle tapping of the column facilitated packing. It is good practice to keep a sufficient amount of solvent in the column to ensure that the added silica gel will filter through the solvent, eliminating air bubbles. The column must be free of air bubbles and breaks. In practice, it is difficult to obtain a well conditioned column in a hot room when the silica gel slurry is prepared with *n*-pentane.

A 1.0-ml aliquot of a concentrated sample extract was pipetted into the column. When the last of the sample has entered into the column of silica gel, the internal wall of the column was rinsed with 0.25 ml of *n*-hexane. In order to elute all chlorinated hydrocarbons present in a sample, a sufficient amount of solvent or a solvent mixture (pre-determined volume) was added to the column. Chlorinated hydrocarbons were collected in an accurately calibrated vessel and each eluate was concentrated to the volume needed for GC analysis. It is well known that if large amounts of chlorinated hydrocarbons are placed on a silica gel column poor separation results are obtained. Therefore, in all separations of spiked sample extracts or known organochlorine mixtures in *n*-hexane, the amounts of PCBs were kept at 0.2 μg and chlorinated insecticides at 0.1 μg.

Clean-up of the samples was carried out according to Holden and Marsden[14,15]. Mirex was added as an internal standard prior to concentration of the sample extract with 50–100 mg of lipid residue. The sample extract was concentrated to 1 ml under

vacuum by means of a rotatory evaporator and applied to a column of alumina (I.D. 6 mm, 2 g of alumina). Elution was performed with 15 ml of *n*-hexane and the eluates were concentrated to 1 ml and applied to a column of silica gel or, if necessary, diluted to the concentration applicable to the separation column.

For the determination of chlorinated hydrocarbons in the eluates, all eluates were concentrated to 1 ml under vacuum by means of a rotatory evaporator and a 3-μl aliquot was injected into the gas chromatograph. The organochlorine compounds were quantified by comparison of the peak areas of the sample with standard chromatograms.

RESULTS AND DISCUSSION

The results obtained when silica gel was prepared as described by McClure[11] are presented in Fig. 1. The column (6 mm I.D.) was packed with silica gel to a height of 100 mm and the flow-rate of the eluent was 1 ml/min. It can be seen that the separation of Aroclor 1254 from the chlorinated insecticides investigated was not successful on a column of silica gel of porosity either 60 Å or 40 Å.

Silica gel prepared as described by Armour and Burke[8] was also examined for the separation of Aroclor 1254 from some chlorinated insecticides. The columns were packed with silica gel without the addition of Celite. The height of the silica gel was 200 mm and elutions were performed at the rate of 0.6 ml/min. The results are presented in Fig. 2. No successful separation of PCBs from DDE was obtained although three different water-deactivated silica gels were used (containing 2.0, 2.5 and 3.0% of water).

Results for the separation of PCBs from insecticides on a column packed with

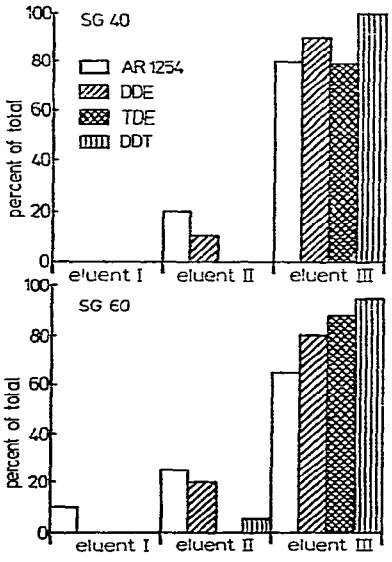

Fig. 1. Elution of Aroclor 1254, DDT, DDE and TDE from silica gel column. Eluent I, 5 ml of *n*-hexane; eluent II, 10 ml of *n*-hexane; eluent III, 20 ml of 0.5% diethyl ether in benzene.

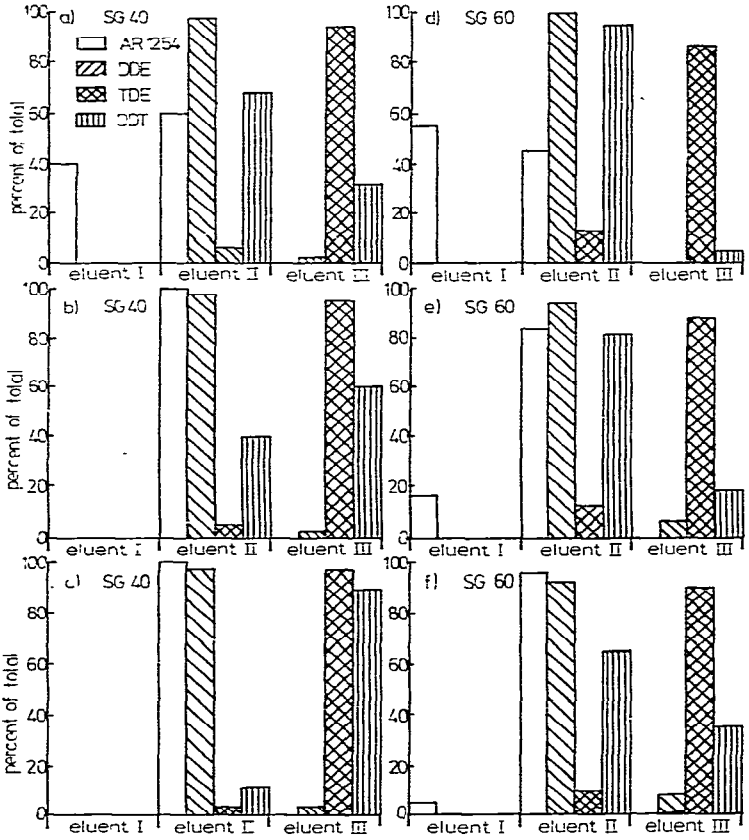

Fig. 2. Elution of Aroclor 1254, DDT, DDE and TDE from silica gel column. Eluent I, 10 ml of *n*-hexane; eluent II, 10 ml of *n*-hexane; eluent III, 10 ml of 10% diethyl ether in *n*-hexane. Silica gel deactivated with: (a) and (d) 2.0% of water; (b) and (e) 2.5% of water; (c) and (f) 3.0% of water.

3% water-deactivated silica gel are shown in Fig. 3, both a dry-packed column (Figs. 3a and 3b) and a wet-packed column (Figs. 3c and 3d) being used. The column (6 mm I.D.) was packed with silica gel to a height of 200 mm and the elution rate was 0.7 ml/min. Results of the elution with a column (6 mm I.D.) containing a 100-mm bed of wet-packed silica gel are presented in Figs. 3e and 3f. The elution rate was 1 ml/min. These last results show the complete separation of Aroclor 1254 from DDT and TDE but a poor separation from DDE on SG 60, while on SG 40 the separation of Aroclor 1254 from DDE was also good.

In Fig. 4 are presented the results of the separation of Aroclor 1254 from DDT and its analogues on a silica gel column when the silica gel was prepared as described by Snyder and Reinert[10]. The columns (6 and 10 mm I.D.) were packed with silica gel to a height of 100 mm, the elution rate being 1 ml/min for the former column and 3 ml/min for the latter. A reasonably good separation of DDE from Aroclor 1254 on silica gel with a porosity of 60 Å in the 10-mm I.D. column was obtained.

The above separations of PCBs from chlorinated insecticides were tested as separate mixtures of insecticides and Aroclor 1254 on two parallel columns. Fig. 5

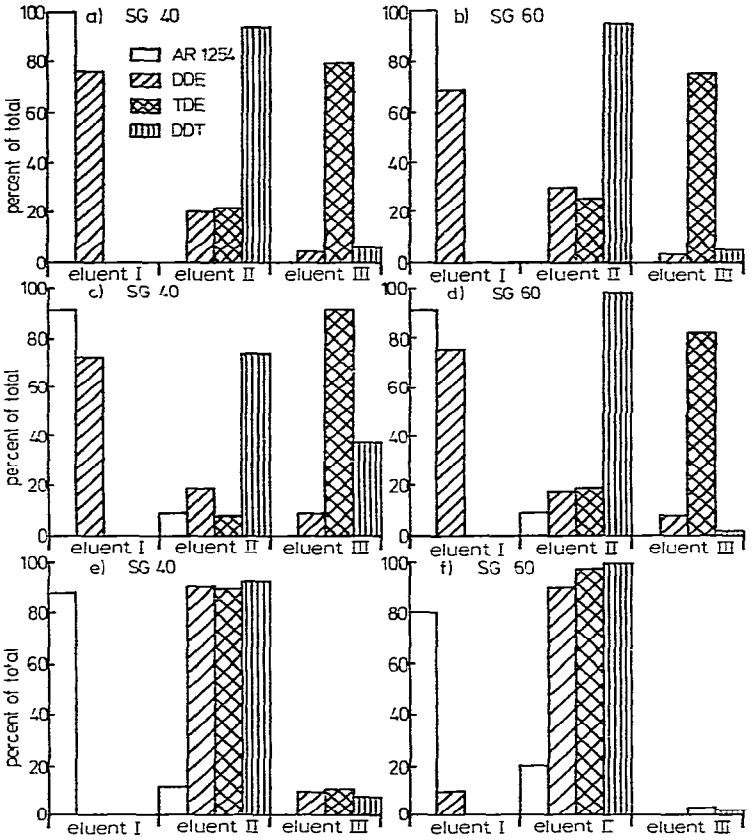

Fig. 3. Elution of Aroclor 1254, DDT, DDE and TDE from silica gel column. Eluent I, 10 ml of *n*-hexane; eluent II, 10 ml of *n*-hexane; eluent III, 10 ml of 10% diethyl ether in *n*-hexane. Dry silica gel in (a) and (b); wet silica gel in (c), (d), (e) and (f). Column height, 200 mm in (a), (b), (c) and (d), 100 mm in (e) and (f).

gives the results of a separation in which mixtures of Aroclor 1254 and insecticides were applied on the same column, Figs. 5a and 5c with silica gel prepared according to the Armour and Burke procedure[8] with 3% of water and Figs. 5c and 5d with silica gel prepared according to Snyder and Reinert[10]. It can be concluded that the Snyder and Reinert procedure for the preparation of silica gel and elution of chlorinated hydrocarbons for the miniature column gives the best separation of PCBs from DDE and the other chlorinated insecticides investigated.

The dynamics of the elution for several pesticides and Aroclor 1254 on the SG 60 column are presented in Fig. 6. The results indicate that, in addition to DDT and its analogues, on this column BHC and dieldrin can also be separated from Aroclor 1254.

The influence of the elution rate on the separation of insecticides from PCBs on SG 60 columns (silica gel height 100 mm; I.D. 6 and 10 mm) are presented in Fig. 7. Aroclor 1254 is eluted mainly in the *n*-pentane fraction (eluent I) and insecticides in the benzene fraction (eluent II). At low elution rates some of the insecticides are eluted

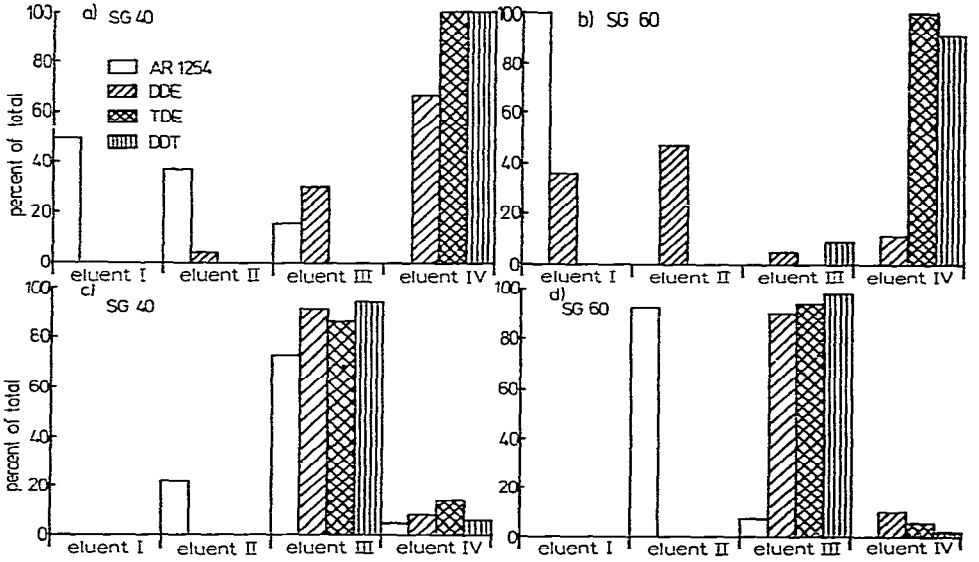

Fig. 4. Elution of Aroclor 1254, DDT, DDE and TDE from silica gel column. Eluent I, 16 ml of *n*-pentane; eluent II, 16 ml of *n*-pentane; eluent III, 20 ml of benzene; eluent IV, 20 ml of benzene. (a) and (b), column I.D. 6 mm; (c) and (d), column I.D. 10 mm.

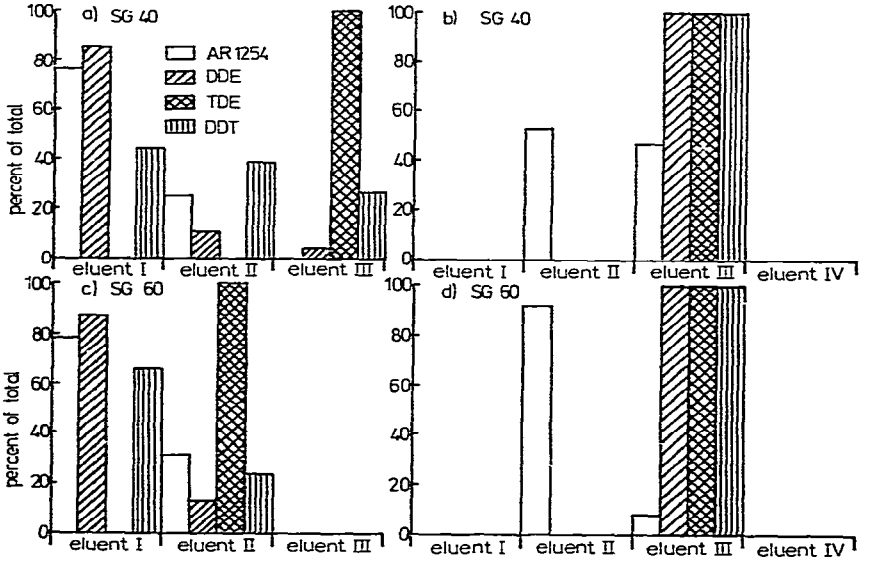

Fig. 5. Elution of Aroclor 1254, DDT, DDE and TDE from silica gel column. (a) and (c): eluent I, 10 ml of *n*-hexane; eluent II, 10 ml of *n*-hexane; eluent III, 10 ml of 10% diethyl ether in *n*-hexane. (b) and (d): eluent I, 16 ml of *n*-pentane; eluent II, 16 ml of *n*-pentane; eluent III, 20 ml of benzene; eluent IV, 20 ml of benzene.

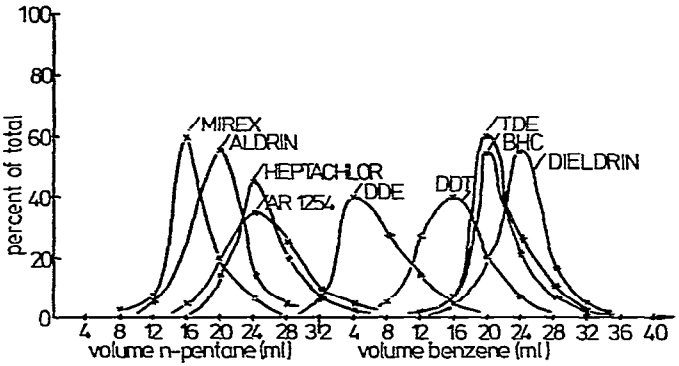

Fig. 6. Dynamics of Aroclor and pesticide separation on silica gel column.

in the *n*-pentane fraction together with PCBs. Differences in the slope of the curve for columns with different inner diameters are also significant.

For routine analysis, it is important to be able to use a column after the silica gel has been regenerated. Regeneration of the silica gel in the column was performed with 16 ml of *n*-pentane and results for the separation of mixtures of insecticides and Aroclor 1254 in *n*-hexane on the regenerated silica gel column are presented in Fig. 8a. After a second regeneration of the column, the separation of PCBs and DDE was poor; DDT was not completely eluted in the benzene fraction (eluate II) but appeared partially in the *n*-pentane fraction together with PCBs. Investigation of the column regeneration possibilities for the separation of PCBs from insecticides in extracts obtained from fish and mussel samples showed a different picture of the separation. The results in Fig. 8b indicate that even after four regenerations a column retained good characteristics, separating Aroclor well from DDE and other pesticides. The practice in our laboratory was to make three regenerations, i.e. four separations were performed on the same column. Naturally, if air bubbles or breaks appear in the column during the separation or the regeneration process, the column is discarded.

Separations of PCBs from pesticides have been performed with numerous fish samples (14 species of fish from the Adriatic sea) and with numerous samples of mus-

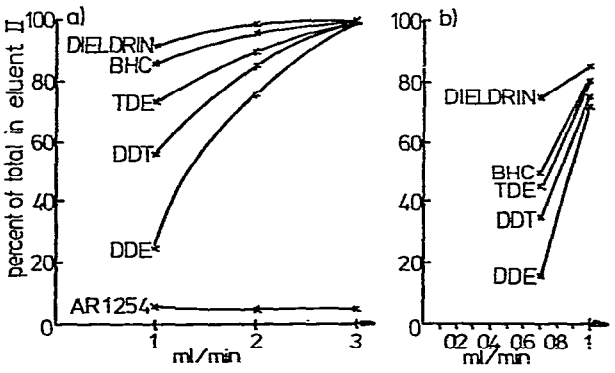

Fig. 7. Influence of flow-rate on separation efficiencies: (a) 10 mm I.D. column; (b) 6 mm I.D. column.

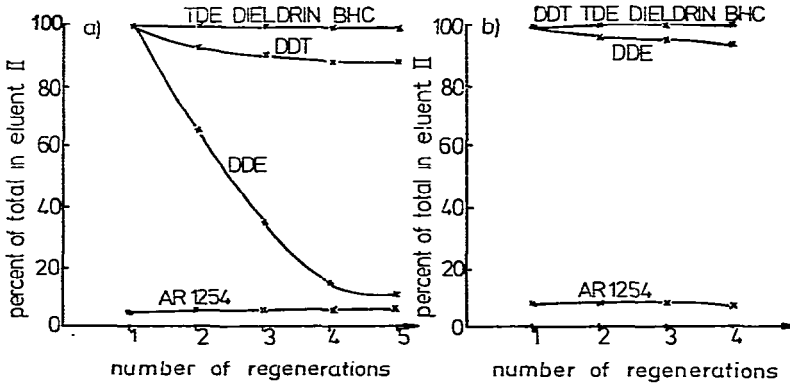

Fig. 8. Influence of number of regenerations on separation efficiency. (a) Chlorinated hydrocarbons added in *n*-hexane on column; (b) chlorinated hydrocarbons added in purified mussel extract on column.

sels and oysters. In most of the samples no serious problem was encountered during the separation processes. Separation problems appeared only in samples that were badly contaminated with chlorinated hydrocarbons (concentrations up to several hundred parts per billion on wet-weight basis). In such cases the extract was diluted with *n*-hexane to the required concentration of pollutant and the separation process then proceeded smoothly.

CONCLUSION

The method described for the separation of PCBs from DDT and its analogues has several advantages compared with other published methods. Significantly smaller amounts of silica gel for column preparation, small amounts of solvent for elution and the simple column are advantages in comparison with the widely used Armour and Burke method[8]. In contrast to another miniature silica gel column separation described by Erney[13], our method makes possible the separation of DDE from PCBs.

Compared with the method of Snyder and Reinert[10], our method is more rapid (about two-fold) and introduces the possibility of running several samples consecutively through the same column. When numerous samples have to be run simultaneously, the possibility of using the same column for four separations after simple and rapid regeneration of the silica gel in the column appears to be the most important advantage of our procedure.

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