ADSORPTIVE PROPERTIES OF COLUMN TUBING AND SOLID SUPPORT IN THE GAS LIQUID CHROMATOGRAPHIC METHOD OF CHLORINATED PESTICIDE ANALYSES

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ABSTRACT

ADSORPTIVE PROPERTIES OF COLUMN TUBING AND SOLID SUPPORT IN THE GAS LIQUID CHROMATOGRAPHIC METHOD OF CHLORINATED PESTICIDE ANALYSES

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This study involved consideration of column technology for use in the gas liquid chromatographic method of analysis of chlorinated pesticides, especially as it pertained to the adsorptive properties of column tubing and solid support material used in column manufacture. The preliminary work was devoted to the choice of a liquid phase, either 1.95 % w/w QF-1 + 1.5 % w/w OV-17 or 3.5 % w/w OV-17, based on the adequacy of separation of methoxychlor using Chromosorb W HP, 80-100 mesh, as the solid support. Thermal Conductivity Detection (TCD) and Flame Ionization Detection (FID) methods were both used for this purpose, the latter eventually being chosen for the bulk of the subsequent analyses carried out.

The preferred liquid phase, 3.5 % w/w OV-17 was then used in the manufacture of all columns prepared to study the adsorptive properties of column tubing materials,

including pyrex glass, nickel, stainless steel, copper and aluminum, and of twelve solid supports, including Chromosorb 750, and treated and untreated Chromosorbs W, P and G, all 80-100 mesh in particle size. Tests were carried out using a mixture of lindane and aldrin as the test sample, at different column temperatures (185 to 252 ^{o}C), and for different sample sizes (15 to 50 µg). The relationship between column efficiency and degree of adsorption of the columns studied was evaluated from the experimental results. Brief consideration only was given to the effect of column temperature on peak area responses of lindane and aldrin, primarily as measured with the FID.

ACKNOWLEDGEMENTS

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1. Introduction

1.1 General

Chlorinated pesticide analysis is one of many pesticide residue analyses commonly performed. The importance of such analyses has been recognized by agencies responsible for controlling the use of pesticides. Pesticides are found in food, dairy and meat products. Effects of their widespread occurrence on natural resources in the environment, such as water, fish and wildlife, have become a subject of great concern. Analytical techniques that are useful for determination of trace amounts of pesticides are a great aid in present attempts to control the pesticides in the environment and in foods. Among chlorinated pesticides are lindane, aldrin, dieldrin, endrin, DDT, methoxychlor and many others.

The following methods are widely used in chlorinated pesticide analyses.

(i) Thin Layer Chromatography (TLC)

TLC is a relatively simple but versatile method. It is used for separation, identification, clean up and semi-quantitative determinations. TLC is so versatile that it is applicable to most analytical problems where chromatographic methods are involved.

(ii) Liquid Chromatography (LC)

In the classical method, LC is better known as column chromatography. In the modern method, it is referred to as High Pressure Liquid Chromatography or High Performance Liquid Chromotography (HPLC). This method is used for clean up, separation, identification and quantitative determination of chlorinated pesticides.

(iii) Infrared Spectrophotometry

It is primarily useful for identification purposes. In some cases, it is also used for quantitative analyses of chlorinated pesticides.

(iv) Chemical Methods

A chemical method involves altering the original compound to another form of compound and subsequent measurement of this product. These methods include colorimetric analysis and chlorine determinations.

(v) Mass Spectrometry (MS)

MS is a very useful method for structure identification. Combined with Gas Chromatography (GC-MS) it is likely the most useful method in chlorinated pesticide analysis.

(vi) Gas Chromatography (GC)

It is generally referred to as Gas Liquid Chromatography (GLC). It is by far the most widely used method for separation, identification and quantitation. Because of

the existing column technology and the Electron Capture Detector (ECD) which is very sensitive towards chlorine, GLC provides both very unique separation powers and the capability of detecting trace amount of chlorinated pesticides (i.e. nanograms to picograms range).

Column technology for use in the gas liquid chromatographic method of chlorinated pesticides was the subject of this thesis, especially as it pertains to the adsorptive properties of column tubing and solid support material used in column manufacture. The preliminary work was devoted to the choice of liquid phase most suitable for chlorinated pesticide analysis. This involved consideration of two liquid phase systems only that were known from literature (7,8) to be useful for this type of anlysis. Upon completion of this work, one of the two liquid phases was used to study the adsorptive properties of different column tubing materials and different types of solid supports. Thermal Conductivity Detection (TCD) and Flame Ionization Detection (FID) methods were both used for this purpose, the latter eventually being chosen for the bulk of the anlysis carried out. Finally, the effect of column temperature on the response of chlorinated pesticides using both FID and TCD was studied.

It is hoped that the results presented in this thesis will be useful in helping to achieve a more complete understanding of column behavior in gas liquid chromatographic analyses of chlorinated pesticides, and in illustrating the pitfalls and sources of error that such analyses entail.

1.2 Gas Liquid Chromatography (GLC)

The gas liquid chromatographic method of analysis originated from the early work of James and Martin (1,2). The technique is also known as Vapour Phase Chromatography, Gas Liquid Partition Chromatography, and Gas Partition Chromatography. Because of its versatility, GLC has become a widely accepted method and is used in many applications.

In GLC separation is achieved through partitioning of the sample into two phases, an inert gas (the carrier gas), and stationary (liquid) phase. The stationary phase is coated on the inert surface of a solid support. Because of the selectivity of a liquid phase to retard sample components according to their partition coefficients, each component of a sample elutes from a column as an individual band separated from bands of other components by zones of carrier gas. This process is called elution chromatography. The eluted bands are

detected by a suitable detection system, and after amplication, are recorded as a series of peaks on a strip chart recorder. The retention time of each peak gives qualitative information about the sample components.

The basic components of a gas chromatograph are : (i) carrier gas source equipped with pressure and flow controls; (ii) heated sample injection port; (iii) thermostated column oven where the analytical column(s) is installed; (iv) the detector; (v) an electrometer to provide amplification of the input and output signals; and (vi) the recorder. Figure 1 schematically illustrates a gas chromatographic system.

GLC has two main functions (i) separation of samples into individual components; and (ii) their detection.

(i) Separation

The separating power of the column depends on a number of factors including :

(a) the nature and amount of the liquid phase
(b) the nature and particle size of the solid support
(c) degree of uniformity of the packing in the column
(d) the length and diameter of the column

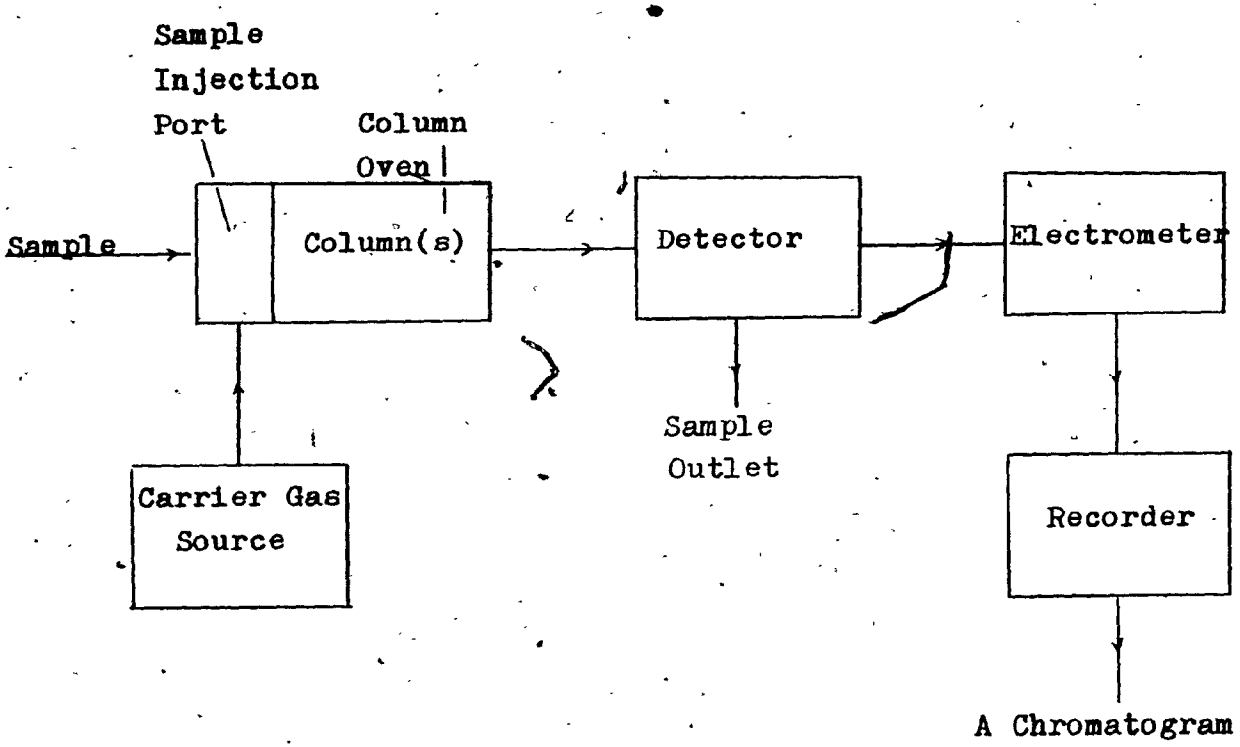


Figure 1 A Block Diagram of a Gas Chromotographic System

(e) the temperature (isothermal or temperature programming) of analysis

(f) the flow rate of the carrier gas

(g) the type of sample mixture and the size of sample to be separated.

These factors are interrelated, and are commonly referred to as the operating conditions for any given analysis.

(ii) Detection

The function of the detector is to sense the elution of the separated sample component from the column, measure its amount, and send a signal to an electrometer. The desirable characteristics of a detector are sensitivity, low noise level, a large linear range of response, and insensitivity to flow and temperature changes. A large number of detectors are currently in use differring in sensitivity and specificity. The operating principle of each kind of detector is generally unique. In practice, there is no ideal detector that can respond to all type of compounds, and at very low concentrations (i.e picograms level). The most commonly used detectors are the following :

(a) Thermal Conductivity Detector (TCD)

Because of its simplicity and versatility, TCD is considered to be a universal detector. It responds to all organic compounds to extents that depend on how much their

thermal conductivities differ from that of the carrier gas which has a comparatively high thermal conductivity. It is a nonspecific detector. The detection limit is about 10 ng of sample weight and linear range is about 10^5 (3).

(b) Flame Ionization Detector (FID)

The FID has been described as being the carbon atom counting detector. Its response is proportional to the effective number of carbon atoms in the sample. This detector is insensitive to carbon dioxide and water vapour. It responds only to oxidizable carbon atoms. There is no response from fully oxidized carbon groups such as carbonyl or carboxyl groups, and its response diminishes with increasing substitution of halogens, amino and hydroxyl groups. It is therefore a specific detector for hydrocarbons. Its detection limit is about 20 pg of sample weight, and its linear range of response is about 10^7 (3).

(c) Electron Capture Detector (ECD)

The ECD is also known as the electron affinity, or electron adsorption detector. ECD responds to electron capturing groups such as : -CO·CO-, -CO·CH:CH·CO-, quinone, $-NO_2$, and halogens. The affinity of the halogens are in the order of I > Br > Cl > F. The ECD is generally used in GLC analysis of traces of chlorinated and organometallic compounds. It is virtually

insensitive to hydrocarbons and amines. Its detection limit is about 0.1 pg of sample weight. The linear range of detection is dependent on the type and design of the ionization radiation source of electrons within the detector. As an example, for the tritium (H^3) source, it is only about 500 (4).

1.3 Column Technology

1.3.1 Liquid Phase

In the gas chromatographic method of analysis of chlorinated pesticides, the choice of the stationary (liquid) phase plays an essential role in determining the degree of separation achievable for any given mixture of chlorinated pesticides. There are several requirements necessary for a stationary phase to yield a good separation.

(i) It should be chemically inert towards the sample.

(ii) It should act as a good solvent for sample components.

(iii) It should show differential solubility for the sample components.

(iv) Its vapour pressure should be less than 0.1 mm Hg (nonvolatile) at the given operating temperature.

(v) It should be thermally stable.

The choice of the right liquid phase for a given separation is an art as much as a science. There are a

large number of liquid phases, and mixed liquid phases, having different polarities, that have been found to be suitable for chlorinated pesticide analysis depending on the problem under investigation. There is no universal liquid phase for pesticide analysis.

Stanley (5) employed 5 % DC-2000 oil, and 5 % QF-1 coatings, respectively, on Chromosorb G, 70-80 mesh. Mixtures of these liquid phases in different proportions have been tested as well. A mixture of 2.5 % of each liquid phase was concluded to be the optimum for the separation of a series of chlorinated pesticides, i.e lindane, heptachlor, aldrin, dehydrochlorinated DDD, heptachlorepoxide, DDE, dieldrin; o,p'-DDT; p,p'-DDD; p,p'-DDT and methoxychlor. Liquid phase loading is given in percent by weight unless otherwise specified. Other stationary phases that have been used for chlorinated pesticide analysis as reported in the review by Sherma (6) are

(i) 4 % SE-30 + 6 % OV-210 (or QF-1) coated on Chromosorb W HP, 80-100 mesh.

(ii) 5 % OV-210 (or QF-1) on Chromosorb W HP, 100-120 mesh.

(iii) 3 % DEGS on Gas Chrom ® P, 80-100 mesh.

Prior to being used these columns were subjected to conditioning by passing nitrogen carrier gas through at a

flow rate of 60 ml/min and the following conditions, 245°C for 72 hours; 275°C, 20 hours; 237°C, 20 hours, followed by on column silylation by injecting 25 ul of Silyl-8 at 30 minutes intervals. For special purposes mixtures of three stationary phases have been used. For example:

(i) 10% DC-200 / 7.5% QF-1 / 3% XE-60 (a mixture of 1:1:1, previously coated on individual supports).

(ii) 3% OV-61 / 7.5% QF-1 / 3% XE-60 (1:1:0.5) for separation of hexachlorobenzene and hexachlorocyclohexane isomers.

A mixed phase of 1.95 % QF-1 and 1.5 % OV-17 (7) was succesfully used to separate all the usual chlorinated pesticides found in tissue samples, but there was no methoxychlor among the mixtures. The column was conditioned with the procedure as described for liquid phases (i) to (iii), but for only 20 hours at 245 °C.

A single phase of 5 % OV-17 was recommended by the Federal Water Pollution Control Administration (FWPCA) (8). Methoxychlor was effectively separated from the other chlorinated pesticides. The column was subjected to conditioning prior to its use.

1.3.2 Column Tubing

In the gas chromatographic method of pesticides

analysis, the most commonly used materials for column tubing are glass (pyrex), stainless steel, copper and aluminum. The superiority of one material over the other has been the subject of a controversy that has not been fully resolved (9). The FWPCA (8) method recommends that columns be made from either aluminum or borosilicate glass. For heat sensitive pesticides, a glass column is considered more reliable.

Glass columns have replaced metal columns to a large extent in recent applications. The obvious advantage of a glass column is that it is possible to see the packing inside the column and thereby ensure that it is well packed. In addition glass columns are considered to be the most inert for many applications (10). Its greatest disadvantage is that glass is very fragile and requires careful handling when it is mounted in or removed from the column oven. Prior to being used a glass column is chemically treated to reduce its surface activity due to the presence of silanol groups. The common method of treatment is to fill the glass tubing with a solution of 5 % dimethyldichlorosilane in toluene and allow it to stand for a few minutes. The solution is then flushed out with toluene and finally with methanol.

In the case of stainless steel, the major advantage is that it is easy to handle, and is as inert as

glass tubing for many applications. Copper and aluminum have disadvantages in that they form oxides which can act as adsorbents (10). In a recent publication, Zlatkis et al (11) reported the use of nickel tubing as an alternative to glass tubing. The advantages of using nickel are that it is durable, economical relative to glass, has the inertness of glass in many applications, and is not subject to breakage.

In packed column gas chromatography, tubing dimensions are either 1/8, 3/16 or 1/4" outside diameter (o.d). The FWPCA (8), reported that the most useful length of column is about 6 feet with a diameter of 1/4" o.d to 1/8" o.d depending on the detector and sample employed. Bevenue (9) classified tubing sizes in two general catagories, 6 feet long by 4.5 mm i.d, or 4 to 5 feet long by 1/8" o.d. Supina (10) has suggested the use of 1/8" o.d column when sensitive detectors are used.

1.3.3 Solid Support

The primary function of the solid support is to provide a surface to hold the stationary (liquid) phase in the column. Ideally, it should be an inert material but this is frequently not the case. The structure of the solid support onto which the liquid phase is coated is a very important factor in determining the efficiency of the column.

An asymmetric or tailing peak is caused by an interaction between the sample and the solid support. The degree of tailing increases with the degree of hydrogen bonding in the order :

Hydrocarbon < ethers < esters < amines < alcohols < acids < glycols < hydrazines < water < amonia (12).

Generally, for homologeous series of compounds, the lowest member tails the most severely (e.g methanol, amongst alcohols). This tailing will be increased as the loading of the liquid phase is lowered or as the sample size is reduced.

Diatomite, which is also called diatomaceous earth, is the most widely used solid support in gas chromatographic work. A detailed description of diatomite supports has been given by Ottenstein in different publications (13, 14, 15). Diatomite consists of skeletons of diatoms, single cell algae. The surface of the diatom is composed of many pores approximately one micron in diameter. This pore structure results in a surface area of approximately 20 m^2/gm. Most diatomite supports are derived from the production of filter aids or fire brick. Johns-Manville diatomaceous earth products, Chromosorbs, W, P and G are briefly described in Table 1.

Table 1 Physical Properties of Chromosorbs, W, P and G (60 - 80 mesh)*

Properties	Chromosorb W	Chromosorb P	Chromosorb G
Color	White	Pink	Oyster White
Type	Flux - Calcined	Calcined	Flux - Calcined
Density, gm/cc :			
(i) Loose Weight	0.18	0.38	0.47
(ii) Packed	0.24	0.47	0.58
Surface Area, m^2/gm	1.0	4.0	0.50
Surface Area, m^2/cc	0.29	1.88	0.29
Maximum Liquid Phase Loading	15%	30%	5%
Handling Characteristic	Slightly Friable	Good	Good

* Johns - Manville, Bulletin, Chromosorb Diatomite Supports for Gas Liquid Chromatography, 22E. 40 ST; N.Y 10016 U.S.A

Chromosorb W is a flux-calcined diatomite support specially processed from the production of Johns-Manville's celite diatomaceous silica. It is used for the separation of polar compounds and its surface is relatively nonadsorptive.

Chromosorb P is prepared from Johns-Manville's SIL-O-CEL C-22 insulating brick. It is a calcined diatomite and used primarily for hydrocarbons and moderately polar compounds. It has been found to be capable of high column efficiency, but its surface is more adsorptive than that of other types of Chromosorbs.

Chromosorb G is a flux-calcined diatomite, used primarily for the separation of polar compounds. It has the characteristics of the high column efficiency of Chromosorb P and the comparatively nonadsorptive surface of Chromosorb W. Its capacity, however, is relatively low.

Untreated Chromosorb is referred to as Chromosorb non-acid washed (NAW). The differences in the adsorptive natures of Chromosorbs W, P and G are due to differences in surface area. The surface area is generally reported in m^2/gm, but in a study of solid supports using a standard size column the unit m^2/cc is more relevant. A comparison is normally made on the basis of equal volumes of solid support, not equal weights of solid support. It is

difficult to determine whether the flux calcination of Chromosorbs W and G have really changed the nature of the surface, or merely altered the size of surface area. Generally, the greater the surface area available in the column, the greater will be the adsorption.

In addition to this, the active sites on the solid support surface also play an important role in causing adsorption or tailing effects. Three types of active sites on the solid support surface are identified as :

(i) Basic sites

(ii) Acidic sites

(iii) Hydrogen bonding sites

Both basic and acidic sites are due to mineral impurities in diatomite supports. Hydrogen bonding sites are due to the presence of silanol groups. To deactive the sites, a number of deactivation methods are known.

(i) Removal of mineral impurities by acid or base washing of the solid support.

(ii) Removal of surface silanol groups by reaction with a silanizing agent.

(iii) Saturation of active sites with an active agent.

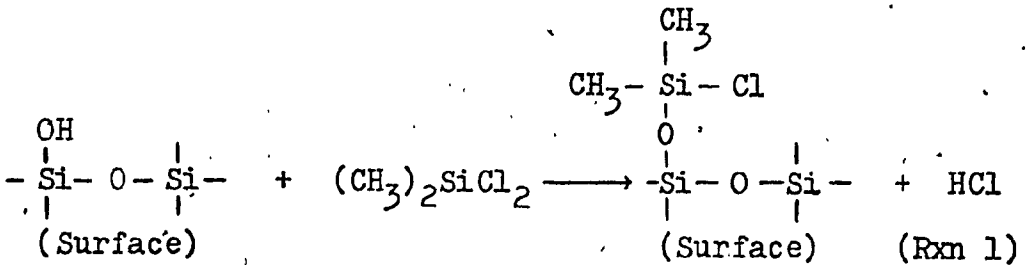
Acid and Base Washing

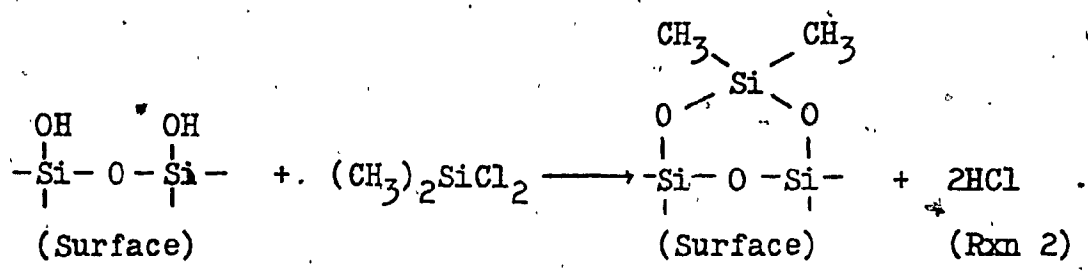
Both are very effective in removing the mineral impurities from solid support surfaces. However,

comparing acid and base washing (15), in most cases, acid washing (AW) is more effective than base washing (BW). In Johns-Manville's AW treated Chromosorb, hydrochloric acid is used to wash the support. The support is then washed to nearly neutral with deionized water.

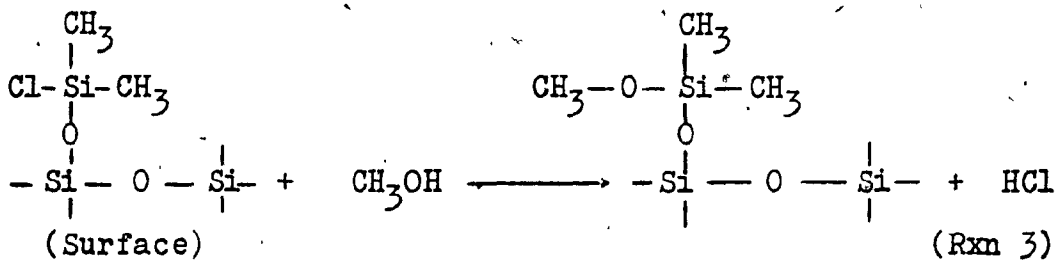
Silanization

Two types of silanizing agents, hexamethyldisilazane (HMDS) and dimethyldichlorosilane (DMCS), are commonly used to remove the active sites. The reactions of both DMCS and HMDS with the solid support surface silanol group have been described by Bohemen et al (16). DMCS reacts with surface hydroxyl groups according to reactions, Rxn 1 and Rxn 2.



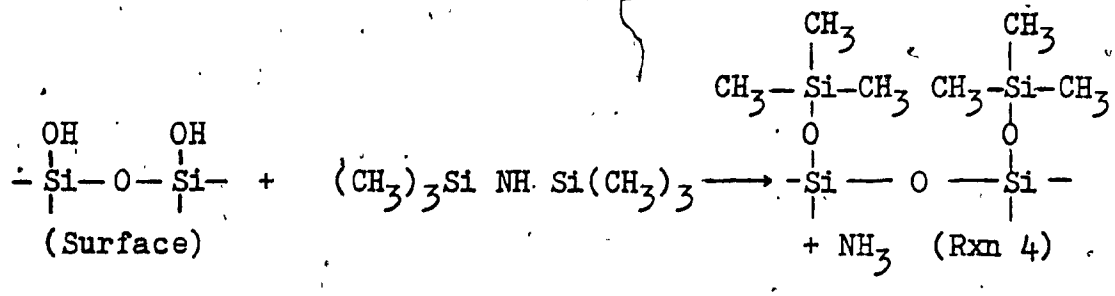


In most cases, when DMCS treatment is carried out it has to be followed by methanol washing to remove the HCl. The Si-Cl bond produced in reaction, Rxn 1, is not desirable because it can hydrolyze to give a Si-OH surface hydroxyl group. Washing with methanol gives :



(Surface) (Rxn 3)

The reaction of HMDS with surface silanol groups proceeds as follows :



(Surface) (Rxn 4)

It has been reported that a comparison between DMCS and HMDS treatment indicated DMCS was more effective than HMDS in silanization (15). In practice, it is most effective to combine acid washing and DMCS treatment (AW DMCS).

Saturation of the Sites with an Active Agent

The active agent can be a stationary phase, an additive to the stationary phase, an agent introduced into the column with the carrier gas (i.e a tail reducer) or the sample itself (column priming). An excellent discussion of this topic is given by Ottenstein (13, 15).

(i) Stationary Phase

On a selective basis, a stationary phase having certain functional groups can be used effectively to deactivate the active sites on a solid support surface. These functional groups are the hydroxyl, carbonyl, carboxyl, ether, primary and secondary amine, and amide groups. Stationary phases having such functional groups are frequently referred to as being polar stationary phases. Nonpolar phases (i.e squalane) give little deactivation of the solid support, but deactivation increases with increasing concentration of liquid phase. Hence, in addition to giving the desired separation, a stationary phase acts also to deactivate the active sites of the solid support. The silicone type of stationary phases are known to exhibit tailing when used with polar compounds. For these silicones, the trifluropropyl silicone (i.e QF-1) shows more tailing than methyl phenyl silicone (i.e OV-17).

(ii) Tail Reducers

In situations where tailing is severe and silanization of the solid support is not adequate to eliminate tailing, an active agent is added to the stationary phase to obtain deactivation. The active agent is referred to as a tail reducer. It can be a polar stationary phase, an inorganic material, or a material introduced into the column with the carrier gas. In general, when a tail reducer is introduced, its contribution towards selectivity of the column is considered very minimal, and the selectivity is that of the nonpolar stationary phase. Examples of commonly used tail reducers are Alkaterge T, and polar stationary phases(i.e carbowax 20M),which are introduced in a relatively smaller amount than the actual liquid phase used for the separations, and Rejuv-8 and Silyl-8, both silanating active agents. The latter tail reducers are introduced into the column with the carrier gas and their use is commonly known as on-column silylation.

(iii) Column Priming

Column priming is a method of reducing severe tailing by repeatedly injecting the sample to be analyzed into the column until the response is reproducible. The main problem with this method is that it is only a temporary basis of deactivation. When the

priming effect deteriorates the tailing will appear again. Therefore, constant checking of the column with a standard prior to analysis is required. This method has been used for steroids and pesticides analyses for the purpose of making up for the inadequacies of the inert solid supports. In the early methods of pesticides analysis, most procedures required that the column be conditioned for several weeks and primed by injections of milligram quantities of pesticide samples because of the poorer quality of solid supports available at that period in time. This procedure is no longer used as extensively as it was before with the better quality of solid supports currently available (17).

Preconditioning of Columns

White, in private communication to Bevenue (18), stated that it is not necessary to precondition columns for long periods of time, nor is it necessary to introduce pesticide samples into columns for conditioning. However, these results were only partially confirmed by Bevenue, with columns prepared under similar conditions. The FWPCA (8) procedure emphasizes the importance of conditioning columns to yield good quality chromatograms. To condition a column, it is heated to 270° C without gas flowing through for two hours; then cooled to 225° C and kept at this temperature for a minimum of 30 minutes. At this point.

carrier gas is introduced, 50 ml/min of carrier gas for a 1/4" column, or 25 ml/min for a 1/8" column. After one hour the column temperature is increased to 240 °C and maintained for 24 to 48 hours. Johns-Manville's bulletin (19) on high performance (HP) Chromosorbs W and G for analyses of steroids reported no requirement for a long period of column conditioning.

Particle Size of the Solid Support

The particle size of the solid support is expressed in terms of mesh (Table 2). The efficiency of a column is affected by the particle size of the solid support used. As the particle size is reduced, the column efficiency is increased. In gas chromatography the commonly used support particle sizes are 60-80, 80-100 and 100-120 mesh. The latter provides the highest column efficiency in term of particle size (Van Deemter equation).

Coating of the Solid Support

Column efficiency is dependent on how the solid support is coated with the liquid phase to be used for separation and how well the coated support is packed in the tubing used. The commonly used method for coating solid supports are by the solution-evaporation (slurry) technique and by the solution-coating (filtration) technique. The advantages and disadvantages of these two methods have been described by Supina (20).

Table 2. Relationship of Particle Size to Mesh (26)

Mesh Range	Top Screen Opening (µm)	Bottom Screen Opening (µm)	Spread (µm)	Range Ratio
60 - 80	250	177	73	1.41
80 - 100	177	149	28	1.19
100 - 120	149	125	24	1.19

In the solution evaporation method, the required amount of liquid phase is dissolved in a volatile solvent, and a known weight of solid support is slowly poured into the solution. To ensure mixing, the container is swirled constantly, or the slurry is stirred if the solid support is not friable. Solvent is then slowly evaporated off by using either a rotary evaporator, or a heated oil bath or hot plate. In the solution coating method, a known weight of solid support is soaked in a known concentration of liquid phase solution. The excess of the solution is filtered off with a Buchner funnel, followed by drying of the wet coated supports.

Deactivation Studies of Solid Supports

Deactivation studies are normally carried out using nonpolar liquid phases which themselves have very little deactivating effect on the solid support, and using oxygenated compounds as test samples. These chosen conditions are used to emphasize the adsorptive properties of the solid support rather than to minimize them. By employing this method, differences in adsorptivity are readily seen. Johns-Manville (21) has published a brochure describing the results of such studies with Chromosorbs W, P and G. In the following description of solid supports, the word Chromosorb has been omitted in some cases when referring to a certain type of Chromosorb.

As a typical example, "Chromosorb W NAW" is referred to as "W NAW".

In a deactivation study of the solid support surface of Chromosorbs W NAW, W AW and W AW DMCS, the adsorptive character of these Chromosorbs followed the order :

W NAW > W AW > W AW DMCS

The least adsorptive support was W AW DMCS. A nonpolar 5 % squalane liquid phase, and a mixture of a 1:1 ratio of methanol to toluene were used in obtaining the above results. Adsorptivity was evaluated in terms of the methanol-toluene peak height ratio. The higher the ratio, the less adsorptive was the solid support. When a polar mixture (22) consisting of

ethanol	40 parts by volume
methyl ethyl ketone (MEK)	20 parts by volume
benzene	10 parts by volume
cyclohexane	5 parts by volume

was used, it was found that the performances of W AW and W NAW were very similar. However, considerably less adsorptivity was indicated by W AW DMCS (23).

For Chromosorb P, the adsorptivities of P NAW, P AW and P AW DMCS followed the order

P NAW ≈ P AW > P AW DMCS

A comparison of deactivation of Chromosorb P and Chromosorb W with HMDS treatment (24) showed that the treated W was more inert than the treated P. It was also found that for Chromosorb P, the combination of HMDS as a silanating agent and 0.2 % Alkaterge T gave greater deactivation than either HMDS or 0.2 % Alkaterge T individually. On the other hand, for Chromosorb W, the combination of HMDS and 0.2 % Alkaterge T showed no significant improvement over the HMDS or 0.2 % Alkaterge T used alone. It was clearly implied that the deactivation effect of HMDS treatment was more effective on treated Chromosorb P than on treated Chromosorb W. In the above results, a 20 % squalane liquid phase, and a sample consisting of methanol (1 part), acetone (1 part), ethylacetate (2 parts) and n-hexane (2 parts) were used.

Deactivation studies of Chromosorbs G NAW and AW DMCS (25), showed that G AW DMCS was less adsorptive than G NAW based on the sharper ethanol and methyl ethyl ketone peaks obtained (peak area was not mentioned) when the liquid phase was 2.5 % squalane and the sample was a polar mixture as described in reference (22). A comparison of untreated Chromosorbs W and G coated with liquid phase loadings of 6 % and 2.5 % squalane, respectively, indicated that Chromosorb G was less adsorptive than W with respect to methanol as the sample.

1.4 Column Performance (29)

Column Efficiency

Column efficiency is generally expressed in terms of theoretical plates (n) calculated for a specific peak (Figure 2).

$$n \cong 8 \ln 2 \left(\frac{t_R}{W_h}\right)^2 \qquad \text{(Eq. 1)}$$

or

$$n = 16 \left(\frac{t_R}{W_b}\right)^2 \qquad \text{(Eq. 2)}$$

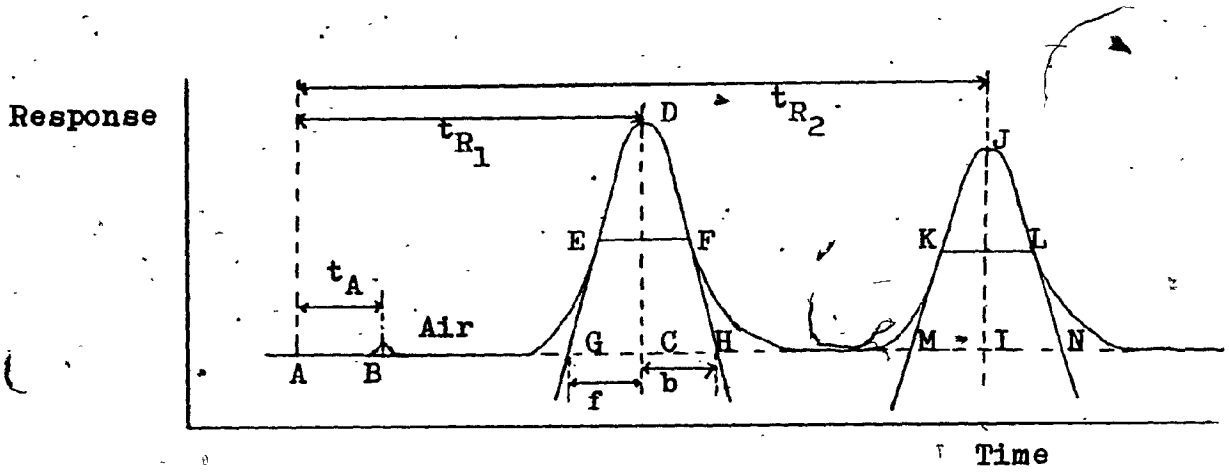
where

$$W_b = 1.699\ W_h \qquad \text{(Eq. 3)}$$

Another means of expressing column efficiency is to use the Height Equivalent to one Theoretical Plate (HETP) concept, given by

$$HETP = \frac{L}{n} \qquad \text{(Eq. 4)}$$

where L is the length of the column in centimeters or millimeters. HETP is related to Van Deemter equation by



A = Injection point

t_A = Retention time of air

$t_{R_{(i)}}$ = Retention time of peak (i), i = 1, 2

$t'_{R_{(i)}}$ = Corrected retention time, $t_{R_{(i)}} - t_A$

CD = Peak height (h)

EF = Peak width at half peak height (W_h)

GH = Peak width at the base (W_b)

Figure 2 A Typical Chromatogram Illustrating the Separation of Two Compounds

$$HETP = A + \frac{B}{\bar{\mu}} + C\bar{\mu} \qquad \text{(Eq. 5)}$$

where A, B and C are constants associated with eddy diffusion, molecular diffusion and resistance to mass transfer effects, respectively. Equation, Eq. 5, can also be written in more detailed form.

$$HETP = 2\lambda d_p + \frac{2\gamma D_{gas}}{\bar{\mu}} + \frac{8k}{\pi^2 (k+1)^2} \cdot \frac{d_f^2 \bar{\mu}}{D_{liq}} \qquad \text{(Eq. 6)}$$

Where :

λ = a constant due to packing irregularity

d_p = average particle diameter of the solid support

γ = a correction for tortuosity factor

D_{gas} = diffusivity of the solute in the gas phase

$\bar{\mu}$ = average linear carrier gas velocity, cm/sec

k = capacity (partition) ratio = the ratio of the amount of the solute in the liquid phase to that in carrier gas.

d_f = effective film thickness of liquid phase coated on the solid support

D_{liq} = diffusivity of the sample in the liquid phase

The above equation shows the importance of particle size of the solid support and of film thickness of liquid

phase in affecting the efficiency of the column.

Separation and Resolution

Separation refers to the distance between two peak maxima, regardless of the shape of the peaks, and measures the column's selectivity. On the other hand, resolution describes the separation of two peaks taking into account the peak widths. The simplest expression for separation is Relative Retention (r) (also called Separation Factor), defined as the ratio of the two adjusted retention times, or, the ratio of the two capacity ratios

$$r = \frac{t'_{R_2}}{t'_{R_1}} = \frac{k_2}{k_1} \qquad \text{(Eq. 7)}$$

Capacity ratio (k) is defined as the ratio of the amount of sample in the liquid phase to that in the gas phase and is given by

$$k = \frac{t_R - t_A}{t_A} \qquad \text{(Eq. 8)}$$

Resolution (R) is expressed by the ratio of the difference in the retention times and the average peak widths at the base :

$$R = \frac{t_{R_2} - t_{R_1}}{1/2\ (W_{b_1} + W_{b_2})} \qquad \text{(Eq. 9)}$$

In terms of peak widths at half height, resolution is given by

$$R_h = \frac{t_{R_2} - t_{R_1}}{1/2\ (W_{h_1} + W_{h_2})} \qquad \text{(Eq. 10)}$$

where the sub-indices 1 and 2 refer to the two peaks in the order of their elution. R_h is related to R by

$$R_h = 1.699\ R \qquad \text{(Eq. 11)}$$

Peak Symmetry

Peak symmetry measures the degree to which the system used limits the realization of good column performance. By defination, peak asymmetry (AS) is given by

$$AS = \frac{b - f}{b + f} \qquad \text{(Eq. 12)}$$

where a negative value indicates leading and a positive value tailing.

1.5 Peak Height and Peak Area Measurements

In gas chromatographic practice, it is very common

to use peak height measurement for well resolved and sharp peaks, for quantitative analysis because of simplicity and accuracy. Peak height is expressed in units of millimeters (mm). Peak area measurement is normally used when the peak is either not well resolved, or too broad, or where the detector response is a simple function of a stoichiometric property of the compounds (30). Various methods of measuring the peak area have been used. These include triangulation, planimetry, cutting and weighing the chart paper, peak height multiplied by peak width at half height, Disc Integration, and Electronic Digital Integration. The advantages and precisions of these methods have been described in numerous references (30a, 30b).

Scott and Grant (31) reported the results of a study on the precision of three of the commonly used methods for the measurement of peak area; peak height multiplied by peak width at half height (referred to as the general method), triangulation, and planimetry. Replication tests proved conclusively that the general method was the most precise. The general method is strictly valid for Gaussian peaks, and hence the precision of its application depends on how nearly this condition is realized in practice. The accuracy is also dependent on the stability of the base line.

2. Experimental

2.1 Instrumentation

A Microtek GC 2000-R gas chromatograph having dual columns with a Thermal Conductivity Detector (TCD) and a Flame Ionization Detector (FID) was used. The principles of TCD and FID operations are given in Appendix A. In most cases, the analytical (sample) column and reference column were different. Since analyses were carried out under isothermal conditions, the use of a different column as a reference column was justified. Sample injections were performed by using a 10 µl Hamilton syringe. All gases were passed through a gas-dry filter trap, followed by a hydrocarbon trap, before entering the chromatographic system. The flow rates of carrier gas were measured at room temperature with a soap bubble flowmeter. The flow rates of the hydrogen gas and air were also measured at room temperature, but using a piston type flowmeter.

2.2 Materials

All materials for the columns, and the standard chlorinated pesticide sample were bought from a commercial source. The standard chlorinated pesticides used were lindane (100 %), aldrin (99 %), dieldrin (100 %) and methoxychlor (99 %) where the percentage value in

brackets indicates the purity of the pesticide (Table 2a). The solvent was pesticide grade hexane. Liquid phases were QF-1 and OV-17 (Table 2b).

2.3 Sample and Column Preparation

Sample Preparation

The following sample concentrations in hexane were prepared, unless otherwise specified.

(i) Individual pesticide solutions : lindane, aldrin, dieldrin and methoxychlor, 5.00 mg/ml, and 10.00 mg/ml.

(ii) A mixture of dieldrin and methoxychlor 5.00 mg/ml of each.

(iii) A mixture of lindane and aldrin 5.00 mg/ml of each.

All samples prepared were stored in 6 ml Hypo-Vials sealed with Teflon® discs.

Column Preparation

Solid supports were coated with the liquid phase using the solution evaporation method. All 4 feet long columns, with the exception of the glass column, were packed using the funnel packing method, and the rest of the columns were packed by the Column-Pak packing method. A more detailed procedure for sample and column preparation is described in Appendix B. Prior to testing, the columns were subjected to conditioning at 220 ^{o}C by

passing nitrogen gas through at the rate of about 10 ml/min for 22 hours.

2.4 Experimental Procedure

2.4.1 Establishing the Sensitivity of the FID

The sensitivity of the FID was determined at different hydrogen flow rates keeping the air and carrier gas flow rates constant. Figures 3 and 4 illustrate the response profile of lindane and aldrin in terms of peak height and peak area at the operating conditions given in Table 3.

2.4.2 Liquid Phases for the Separation of Methoxychlor

A selective study of liquid phases was carried out by concentrating on the two liquid phases described in references (7) and (8). The feasibility of the mixed phase of 1.95 % w/w QF-1 and 1.5 % w/w OV-17 to separate methoxychlor was tested. Tests with the liquid phase OV-17 were made using a 3.5 % w/w loading. The use of a 3.5 % loading, rather than a 5 % loading, was to improve column efficiency as predicted by the Van Deemter equation (Eq. 6), and also to reduce analysis time.

Four common chlorinated pesticides, lindane, aldrin, dieldrin and methoxychlor, were used for the analyses with the following columns.

Column A : Stainless steel column (6' x 1/8" o.d, 0.020" wall thickness), 1.95 % w/w QF-1 + 1.5 % w/w OV-17 on Chromosorb W HP, 80-100 mesh.

Column B : Nickel column (6' x 1/8" o.d, 0.020" wall thickness), 1.95 % w/w QF-1 + 1.5 % w/w OV-17 on Chromosorb W HP, 80-100 mesh.

Column C : Glass column (4' x 4 mm i.d), 3.5 % w/w OV-17 on Chromosorb W HP, 80-100 mesh.

Operating conditions for the separation of methoxychlor with column A and B are given in Table 4. Table 5 lists the operating conditions used for the separation of methoxychlor with column C.

2.4.3 Adsorptive Porperties of Column Tubings

In this study, five types of column tubing materials varying in length and diameter were tested to determine their adsorptive properties. The column tubing materials tested were glass (pyrex), nickel, stainless steel, copper and aluminum. The adsorptive properties of these column tubings were studied, using lindane and aldrin as the test samples, at different column temperatures, and for different sample sizes. A 3.5 % w/w OV-17 on Chromosorb W HP, 80-100 mesh column packing was used in each case. Columns packed in the tubing specified as follows were studied.

Column C : Glass column (4' x 4 mm i.d), packing density (ρ_p) 0.2044 gm/cm^3

Column D : Nickel column (4' x 1/8" o.d, 0.020" wall thickness), ρ_p 0.2724 gm/cm^3

Column E : Stainless steel column (4' x 1/8" o.d, 0.020" wall thickness), ρ_p 0.2871 gm/cm^3

Column F : Copper column (5' x 3/16" o.d, 0.030" wall thickness), ρ_p 0.2003 gm/cm^3

Column G : Aluminum column (5' x 3/16" o.d, 0.028" wall thickness), ρ_p 0.1953 gm/cm^3

Operating conditions used are given in Table 6, where the flow rate of the carrier gas was measured only at the beginning of each run. Table 7 lists the volumes and concentrations of pesticide samples introduced for various columns, along with column temperatures used to determine responses for different sample sizes.

2.4.4 Adsorptive Properties of Solid Supports

In this study, twelve kinds of Chromosorbs (Table 8) were tested for adsorptive properties at different column temperatures and sample sizes using a mixture of lindane and aldrin as the test sample. The physical properties of these Chromosorbs W, P and G before treatment, are presented in Table 1. It was assumed that treated Chromosorbs would have the same physical properties as the

untreated Chromosorbs, but would differ in surface activity. Chromosorb 750 has a loose weight density between 0.32 and 0.35 gm/cc and a surface area between 0.5 and 1.0 m^2/gm (28). The surface area in terms of m^2/cc is approximated to be between 0.16 to 0.35 m^2/cc. For all the columns, the tubing material was nickel 4' x 1/8" o.d (0.020" wall thickness) and the liquid phase was 3.5 % w/w OV-17. The following columns, referring to various solid supports (80-100 mesh) were used.

Column H : Chromosorb 750

Column D : Chromosorb W HP

Column I : Chromosorb W AW DMCS

Column J : Chromosorb W AW

Column K : Chromosorb W NAW

Column L : Chromosorb P AW DMCS

Column M : Chromosorb P AW

Column N : Chromosorb P NAW

Column O : Chromosorb G HP

Column P : Chromosorb G AW DMCS

Column Q : Chromosorb G AW

Column R : Chromosorb G NAW

Operating conditions are given in Table 9. The flow rate of the carrier gas was measured only at the beginning of each run. Table 10 lists the volumes and concentrations

of pesticide samples introduced for various columns, along with the column temperatures, and output attenuations used for measurement of responses for different sample sizes.

2.4.5 Effect of Column Temperature on Responses of Lindane and Aldrin with FID and TCD

This study involved the interpretation of the responses obtained in 2.4.3 and 2.4.4 in terms of peak area for the FID. With the TCD, the effect of column temperature on responses on lindane and aldrin was studied only with column D in terms of peak area. Operating conditions are given in Table 11 where the flow rate of the carrier gas was measured only at the beginning of the run.

Table 2a Structure of Lindane, Aldrin, Dieldrin and Methoxychlor

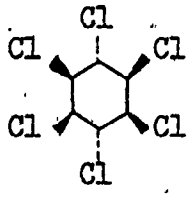
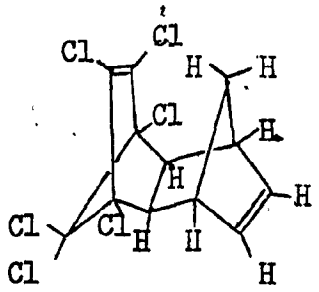
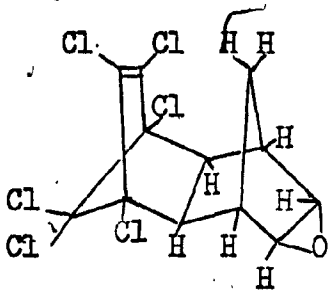
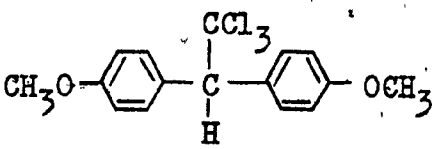
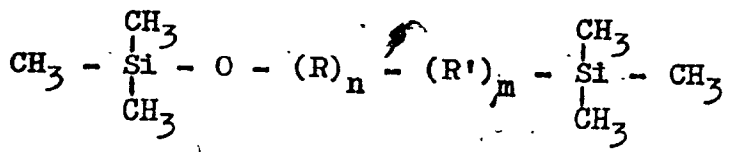
Pesticide	Synonym	Structure
Lindane	Gamma - 1,2,3,4,5,6 - hexachlorocyclohexane	
Aldrin	1,2,3,4,10,10, - Hexa - chloro - 1, 4 ,5,8,8 - hexahydro - 1,4 endo - exo - 5,8 - dimethano - naphthalene (HHDN)	
Dieldrin	1,2,3,4,10,10 - Hexa - chloro - 6,7 - epoxy - 1,4,4 ,5,6,7,8,8 - octahydro - endo,exo - 1, 4:5,8 - dimethano - naphthalene (HEOD)	
Methoxy-chlor	1,1,1 - Trichloro - 2,2 - (p-methoxyphenyl) ethane	

Table 2b Structure of QF-1 and OV-17 Liquid Phases (52)



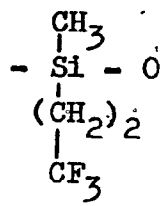
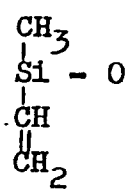
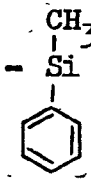
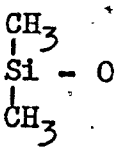
Liquid Phase	Type	R	R'	n/m	Remarks
QF-1 (max temp 250 °C)	Fluoro Alkyl			∞	50% Trifluoro-propyl
OV-17 (max temp 350 °C)	Methyl Phenyl Dimethyl			∞	50% Phenyl

Figure 3 Effect of Hydrogen Flow Rate on Peak Height Response of Lindane and Aldrin

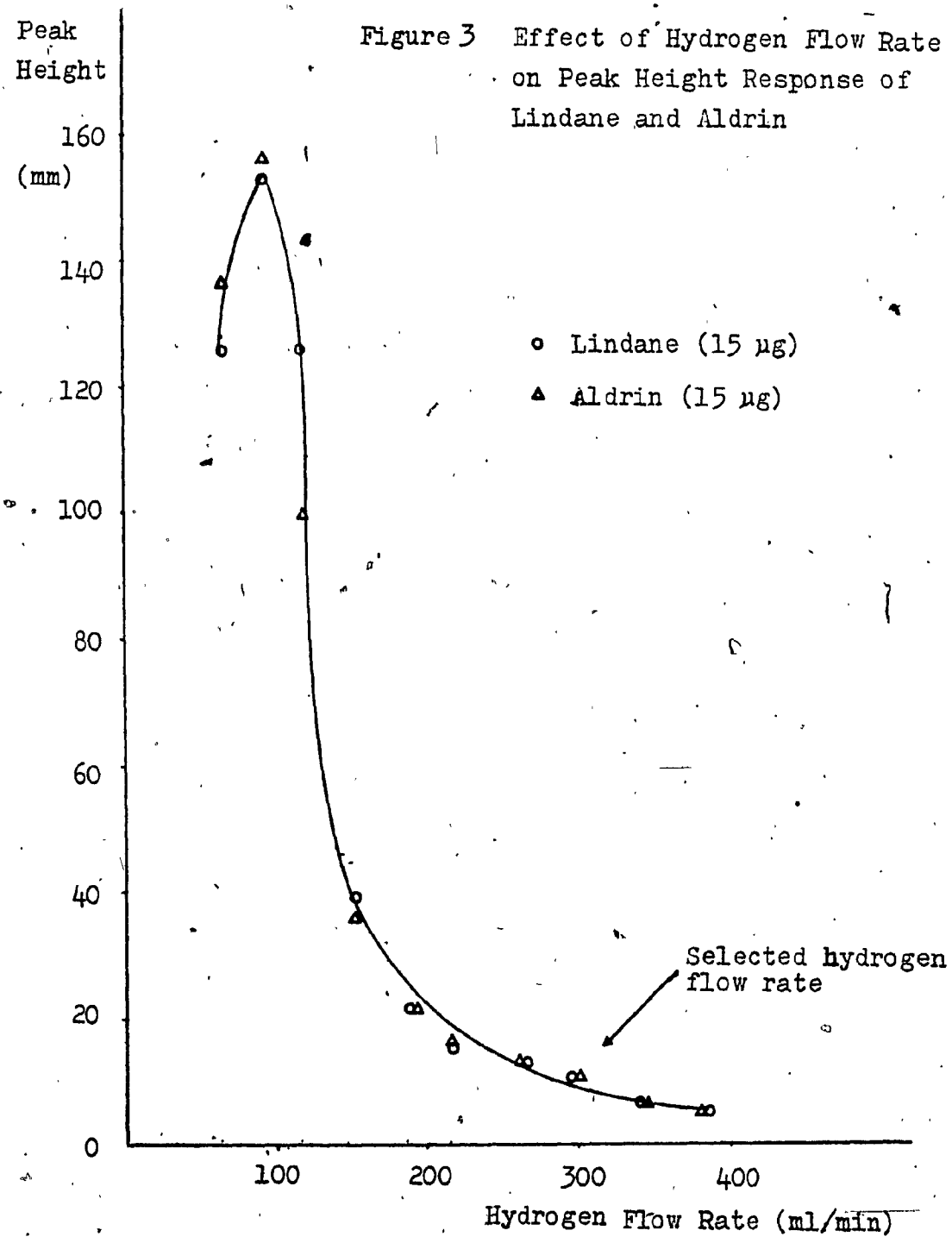


Figure 4 Effect of Hydrogen Flow Rate on Peak Area Response of Lindane and Aldrin

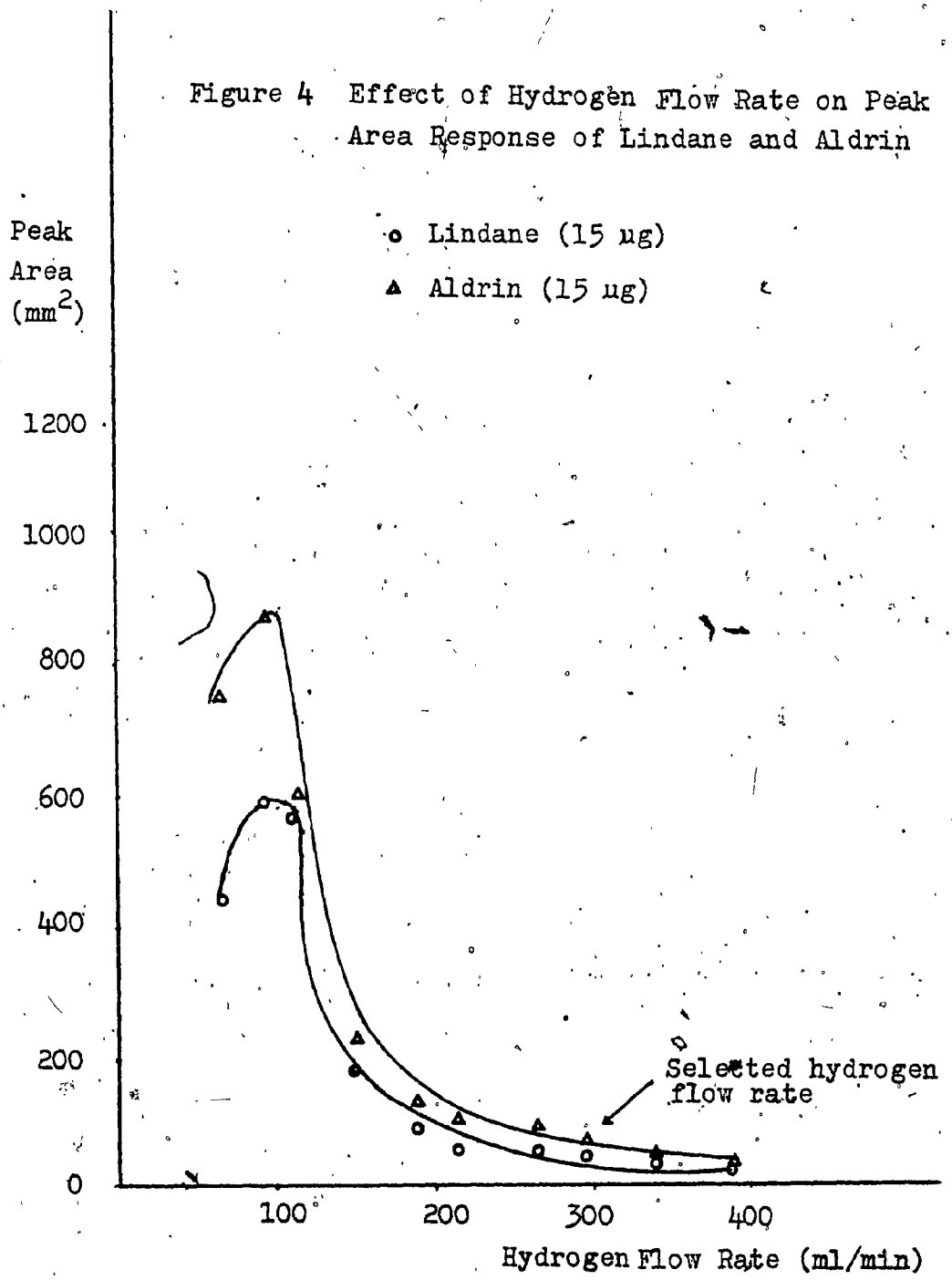


Table 3 Operating Conditions for Establishing the Sensitivity of the FID detector at Different Hydrogen Flow Rates

Analytical column	: Nickel column 4' x 1/8" o.d, 0.020" wall thickness, 3.5 % OV-17 on Chromosorb W HP, 80-100 mesh
Reference column	: Nickel column 4' x 1/8" o.d, 0.020" wall thickness, 3.5 % OV-17 on Chromosorb W HP, 80-100 mesh
Detector	: FID
Attenuation	
Output	: 4
Input	: 10^2
Temperature (^oC) :	
Detector	: 245
Injection port	: 240
Column	: 205
Flow rate :	
Carrier gas	: Helium, 50 ml/min
Flame	: Air, 330 ml/min
Samples	: Each injection consisted of a 3 µl of a mixture containing 5.00 mg/ml Lindane + 5.00 mg/ml Aldrin

Table 4 Operating Conditions for the Separation of Methoxychlor with Columns A and B

Analytical column	: Columns A and B
Reference column	: Stainless steel column 4'x 1/4" o.d , 20 % SE-30 on Chromosorb W HP, 80-100 mesh
Detector	: TCD
DC filament current	: 235 mA
Attenuation	: 4
Temperature (^{o}C) :	
Detector block	: 245
Injection port	: 242
Column	: 195
Flow rate, carrier gas	: Helium, 100 ml/min.
Samples	: 5 µl each of 10.00 mg/ml Lindane, 10.00 mg/ml Aldrin and 10.00 mg/ml Dieldrin. 10 µl of 10.00 mg/ml Methoxychlor

Table 5. Operating Conditions for the Separation of Methoxychlor with Column C

Analytical column		: Column C
Reference column		: Column A
Attenuation :		
	Output	: 4
	Input	: 10^2
Temperature (^{o}C) :		
	Detector	: 245
	Injection port	: 240
	Column	: 215
Flow rate :		
	Carrier gas	: Helium, 93 ml/min
	Flame	: Hydrogen, 295 ml/min
		Air, 330 ml/min

Samples :

A = 5.00 mg/ml Lindane + 5.00 mg/ml Aldrin

B = 5.00 mg/ml Dieldrin + 5.00 mg/ml Methoxychlor

5 µl (2 µl A + 3 µl B) were introduced.

Table 6. Operating Conditions for the Study of Adsorptive Properties of Column Tubings Using Lindane and Aldrin as the Test Samples

Analytical column		: Columns C to G
Reference column		: Column A
Detector		: FID
Attenuation	:	
	Output	: 4
	Input	: 10^2
Temperature (^{o}C)		
	Detector	: 245
	Injection port	: 240
	Column	: (i) Response at different column temperatures: 185,195,205,215,225, 235,242 and 252.
		(ii) Response for different sample sizes : 215, unless otherwise specified (see Table 7)
Flow rate	:	
	Carrier gas	: Helium, 50 ml/min
	Flame	: Hydrogen, 295 ml/min
		Air, 330 ml/min.

Table 7 Volume of Pesticide Sample Introduced for Column C to Column G

Column	Tubing Material	Sample A Mixture Containing	Volume* (µl)	Volume** (µl)	Column Temp** (°C)
C	Glass	5.00 mg/ml Lindane and 5.00 mg/ml Aldrin	3	3 - 9	215
D	Nickel	5.04 mg/ml Lindane and 5.00 mg/ml Aldrin	3	2 - 9	215
E	Stainless Steel	5.04 mg/ml Lindane and 5.05 mg/ml Aldrin	3	2 - 9	215
F	Copper	5.04 mg/ml Lindane and 5.05 mg/ml Aldrin	3	2 - 9	225
G	Aluminum	5.04 mg/ml Lindane and 5.05 mg/ml Aldrin	3	2 - 9	225

* To measure response at different column temperatures

** To measure response for different sample sizes

Table 8 Type of Chromosorbs Studied (80 - 100 mesh)

Solid Support	Treatment				
	HP**	AW DMCS	AW	NAW	Other
Chromosorb^{R} 750*					x
Chromosorb W	x	x	x	x	
Chromosorb P		x	x	x	
Chromosorb G	x	x	x	x	

* Chromosorb^{R} 750 is highly efficient solid support designed specifically for biomedical and pesticides analyses (28).

** Chromosorb HP is extra treated regular grade Chromosorb AW DMCS (27).

Table 9 Operating Conditions Used in the Study of Adsorptive Properties of Various Solid Supports Using Lindane and Aldrin as the Test Sample

Analytical column	: Column D, and columns H to R
Reference column	: Nickel column 4' x 1/8" o.d, 0.020" wall thickness, 3.5 % w/w OV-17 on Chromosorb W HP, 80-100 mesh
Detector	: FID
Attenuation	:
Output	: (i) Response at different column temperatures : 4
	(ii) Response for different sample sizes : 4, unless otherwise specified (see Table 10)
Input	: 10^2
Temperature (^oC)	:
Detector	: 245
Injection port	: 240
Column	: (i) Response at different column temperatures 185, 195, 205, 215, 225, 235, 242 and 252
	(ii) Response for different sample sizes : 215, unless otherwise specified (see Table 10)

Continued

Continued

Flow rate :

Carrier gas : Helium, 40 ml/min

Flame : Hydrogen, 295 ml/min

Air, 330 ml/min

Table 10 Volume of Pesticide Sample Used for Column D, and Column H to Column R, Along with Column Temperatures and Output Attenuation Settings Used

Column	Chromosorb	Sample A Mixture Containing	Volume* (µl)	Column Temp** (°C)	Output Att**
H	750	5.00 mg/ml Lindane and 5.00 mg/ml Aldrin	5	215	4
D	W HP	5.00 mg/ml Lindane and 5.00 mg/ml Aldrin	3	205	4
I	W AW DMCS	5.12 mg/ml Lindane and 5.18 mg/ml Aldrin	5	215	2
J	W AW	5.12 mg/ml Lindane and 5.18 mg/ml Aldrin	3	205	4
K	W NAW	5.00 mg/ml Lindane and 5.17 mg/ml Aldrin	3	205	4
L	P AW DMCS	5.00 mg/ml Lindane and 5.08 mg/ml Aldrin	5	215	2

Continued

Continued

M	P AW	5.00 mg/ml Lindane and 5.08 mg/ml Aldrin	5	215	2
N	P NAW	5.08 mg/ml Lindane and 5.00 mg/ml Aldrin	5	215	2
O	G HP	5.00 mg/ml Lindane and 5.08 mg/ml Aldrin	5	235	2
P	G AW DMCS	5.00 mg/ml Lindane and 5.00 mg/ml Aldrin	5	235	2
Q	G AW	5.00 mg/ml Lindane and 5.00 mg/ml Aldrin	5	235	2
R	G NAW	5.00 mg/ml Lindane and 5.00 mg/ml Aldrin	5	235	2

* To measure response at different column temperatures

** To measure response for different sample sizes (sample volumes of 3 to 10 µl)

Table 11 Operating Conditions for the Effect of Column Temperature on TCD Responses of Lindane and Aldrin

Analytical column	: Column D
Reference column	: Nickel column 4' x 1/8" o.d, 0.020" wall thickness, 3.5 % w/w OV-17 on Chromosorb W HP, 80-100 mesh
Temperature (^{o}C)	:
Detector	: 240
Injection port	: 230
Column	: 185, 195, 205, 215, 225, 235, 242 and 252
DC filament current	: 210 mA
Attenuation	: 8
Flow rate, carrier gas	: Helium, 65 ml/min
Samples	: Each injection 5 µl of a mixture containing 5.00 mg/ml Lindane + 5.46 mg/ml Aldrin

3. Results and Discussion

3.1 Liquid Phase for the Separation of Methoxychlor

The test for the mixed phase, 1.95 % w/w QF-1 + 1.5 % w/w OV-17 on Chromosorb W HP, 80-100 mesh, to separate methoxychlor was carried out by employing TCD. Bevenue (32) has stated the importance of TCD for pesticide analysis in the parts-per-million range. A chromatogram of a separation of 50 µg lindane, and 100 µg each of aldrin, dieldrin and endrin was included in his paper.

Methoxychlor was used as the test pesticide in choosing a liquid phase because it belongs to a class of more strongly retained chlorinated pesticides. Its retention time is longer than either that of lindane or aldrin. Therefore, methoxychlor will be significantly affected by the adsorptive properties of the column tubing and solid support used for the analysis, which is the subject of the following studies.

Using columns A and B, individual pesticides, 50 µg each of lindane, aldrin and dieldrin, were eluted from the columns using operating conditions given in Table 4. When 10 µl of 10.00 mg/ml methoxychlor in hexane was introduced into column A, no peaks were observed for 50 minutes after the injection point (duplicate determinations). The

procedure was repeated with column B, and the same result was obtained. Retention of methoxychlor by the columns could have been caused by either (i) the columns were too long, or (ii) the liquid phase used strongly retained methoxychlor. Factors (i) and (ii) were considered separately. To eliminate the possibility of factor (i), column B was reduced from 6 feet to 5 feet. After conditioning for about two hours at the same experimental conditions, the same amount of methoxychlor was injected, and no peaks were observed for 60 minutes, even though injections were repeated four times at 15 minutes intervals. Reduction of the column length to 4 feet did not improve the results. Again, no peaks were observed, triplicate determinations being recorded for 30 minutes. At this stage, it was considered that methoxychlor might be undergoing complete adsorption on active sites on the support. To eliminate this possibility, triplicate 10 µl portions of Silyl-8, a tail reducing reagent, were introduced into the column at 10 minute-intervals to obtain some deactivation of the coated support. However, subsequent injection of 10 µl of 10.00 mg/ml methoxychlor gave no observable peaks for 30 minutes. Hence, the possibility of surface activity of the coated support being responsible for non-elution of methoxychlor was not confirmed. The column length was further reduced to 2 feet. When the test was carried out with the same amount

of sample at column temperature 220 °C, a tailing methoxychlor peak was observed with retention time 3.35 minutes (Figure 5). However, from a practical point of view this was not a satisfactory result. This 2 foot column can not be relied upon too much since most analyses involve separations of mixtures consisting of a large number of chlorinated pesticides with various retention times, and not only methoxychlor.

To consider factor (ii), it was necessary to change the mixed phase to a liquid phase that was more suitable for separation of methoxychlor. In this case, column C was used together with the FID detector. A satisfactory result was obtained. Figure 6 illustrates the chromatogram of the separated four pesticides at a column temperature of 215 °C with operating conditions as given in Table 5. Studying Figure 6 carefully, a small hump was found at retention time 25.4 minutes. The chromatogram that was published by FWPCA (8) also indicated a small hump present at the same region of retention time. It was due to impurities from methoxychlor. These results clearly showed the effectiveness of 3.5 % w/w OV-17 on Chromosorb W HP, 80-100 mesh, in separating the four pesticides, particularly methoxychlor. The glass tubing of column C might also be contributing to the effectiveness of this

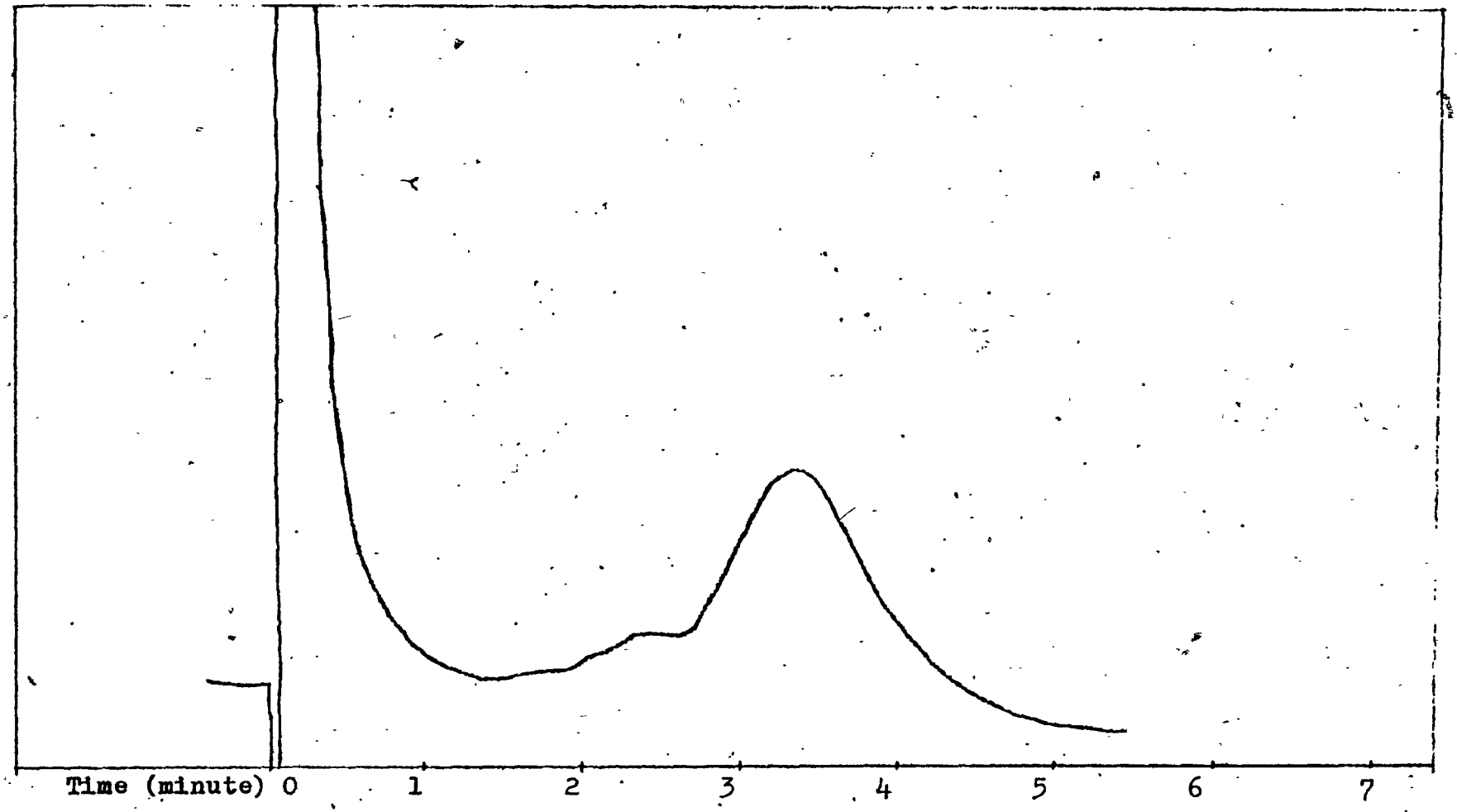


Figure 5 Chromatogram of 100 µg. Methoxychlor Obtained from Nickel Column (2' x 1/8" o.d), 1.95% w/w QF-1 + 1.5 % w/w OV-17 on Chromosorb W HP, 80-100, at 215 °C. The Operating Conditions Are Given in Table 4. Chart Speed : 1 inch/minute.

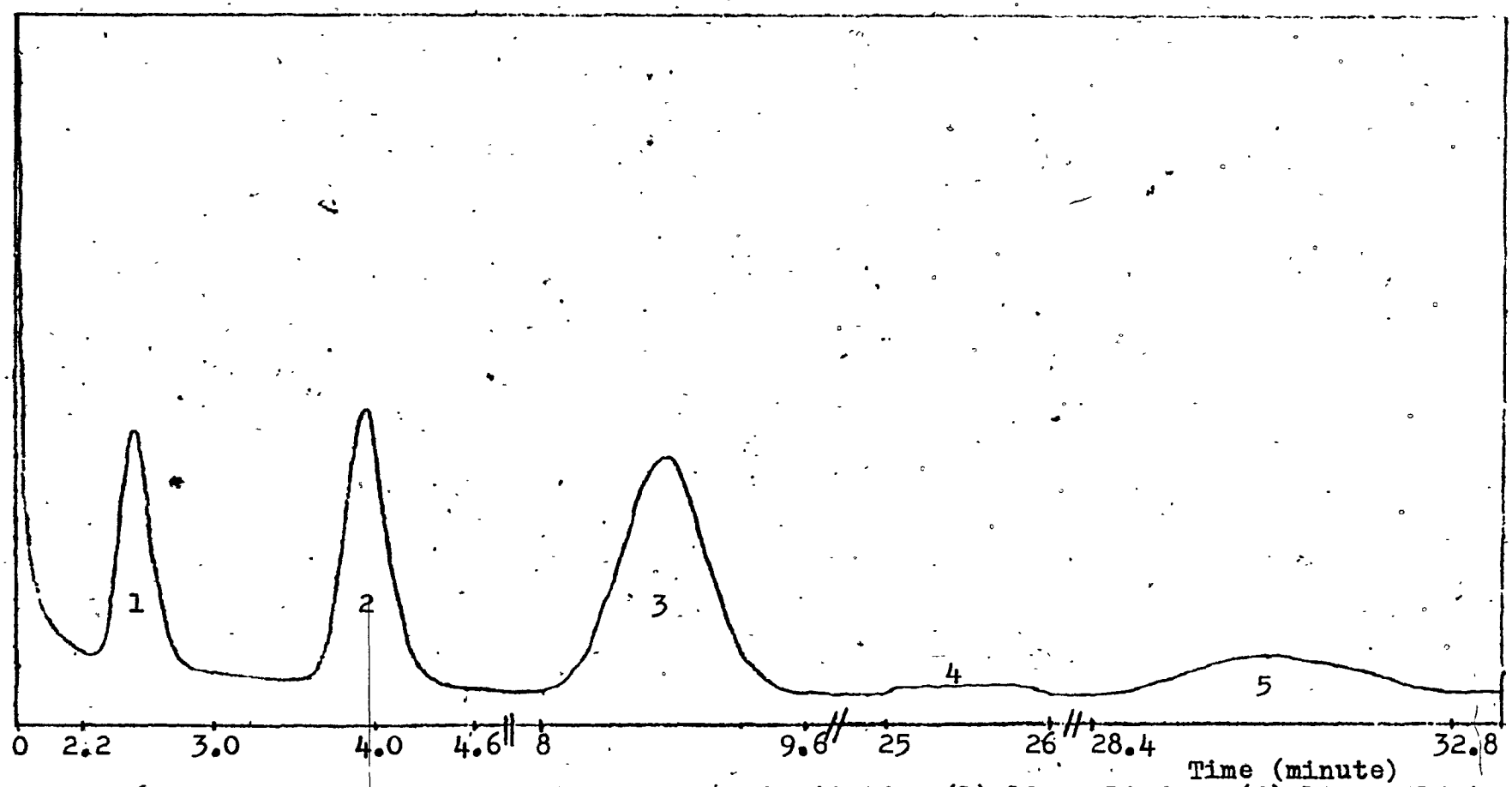


Figure 6 Chromatogram of Separation of Four Pesticides,(1) 10 µg Lindane,(2) 10 µg Aldrin, (3) 15 µg Dieldrin,(4) Impurities from Methoxychlor and (5) 15 µg Methoxychlor Column : Column C. Operating Conditions Are Given in Table 5. Chart Speed : 1 inch/minute.

separation since the previous tests with columns A and B used stainless steel and nickel, respectively, as column tubing materials.

3.2 Adsorptive Properties of Column Tubings

A comparison of adsorptive properties of different column tubing materials was carried out using 3.5 % w/w OV-17 liquid phase on Chromosorb W HP, 80-100 mesh, as the column packing. Even though the choice of the liquid phase was targeted to separations of more strongly retained chlorinated pesticides, the use of methoxychlor as the test sample was not practical because of its relatively long retention time. Therefore, lindane and aldrin were chosen as the test samples. Lindane was convenient to work with and aldrin is commonly used as an internal standard compound in gas chromatographic analyses of chlorinated pesticides. Aldrin, if it is used as an internal standard, should give a predictable and constant response in terms of peak area for any given set of experimental conditions on any given column. By knowing the adsorptive character of the column for aldrin, the actual effects on chlorinated pesticides in general can be deduced as a rough estimation. The sample sizes were in the range of 10 to 50 micrograms which were relatively large in comparison to common chlorinated pesticides residue analyses carried out using the electron

capture detector (ECD). Any undesirable effect will be magnified when a smaller size of sample is analyzed. Since the nature of this study was not to find out the lowest detection limit obtainable with each column and detector, the use of a flame ionization detector (FID) not at optimum conditions was justified (Figures 3 and 4). An electron capture detector was not available when this project was undertaken. At the selected hydrogen gas flow rate, it was possible to study the response pattern over the column temperature range of 185 to 252 ^{o}C without necessarily changing the sensitivity of detection through changing output or input attenuations. It should also be noticed that the same hydrogen flow rate was used previously in the separation of four pesticides, lindane, aldrin, dieldrin and methoxychlor (see Table 5).

The comparison of adsorptive properties of different column tubings was based on :

(i) Peak height and peak area responses at different column temperatures

(ii) Peak height and peak area responses for different sample sizes.

It was expected that the largest responses, peak height or peak area, would be obtained using the least adsorptive column, and the smallest responses using the most adsorptive column.

All results presented for discussion were results obtained after column priming with the sample itself. The degree of column priming was the same from one column to another, with the exception of column D (nickel) which was primed to a greater extent, having been used to establish the sensitivity of FID detection at various hydrogen flow rates. Priming was accomplished by carrying out a typical run i.e., injecting the sample into the columns at various column temperatures (185-252° C). The purpose of column priming was to provide a better consistency of the responses. Hence, a more satisfactory evaluation of the adsorptive properties of each column studied could be obtained. Every result reported was the average response from three determinations. Using the Q test (33), as a criterion for rejection of data, for three sample determinations, all results were acceptable.

Parameters for column efficiency were calculated to evaluate the column performance of columns studies.

3.2.1 Response at Different Column Temperatures

A comparison was made in the column temperature range 185 to 252 C. The results of responses for the second runs in terms of both peak height and peak area of lindane and aldrin were plotted against column temperature in Figures 7 to 10 (Appendix C) for various columns where

the amount of sample is indicated in the bracket. Table 12 lists responses obtained on different columns at one selected column temperature, 215°C. The chromatograms are illustrated in Figures 11 and 12. The response at 215 °C was chosen because it lay approximately in the middle range of column temperatures under consideration. A precision of 5 percent or better was obtained for both peak height and peak area measurements.

As is indicated from Table 12, the order of the responses in terms of peak height and peak area is different to some extent. Therefore, a separate evaluation is necessary. The following orders of adsorptivity are obtained, based on Figures 7 to 10.

(i) Based on Peak Height

(a) Adsorptivity Towards Lindane

Column F (copper) ≈ column G (aluminum) < column D (nickel) < column E (stainless steel) < column C (glass)

(b) Adsorptivity Towards Aldrin

Column F (copper) ≈ column G (aluminum) < column D (nickel) < column E (stainless steel) < column C (glass)

(ii) Based on Peak Area

Adsorptivity Towards Lindane and Aldrin

Table 12 Peak Height and Peak Area of Lindane and Aldrin for Columns C to G at Column Temperature 215 °C/ Taken from the Results of Response at Different Temperatures (Second Run) (Figures 7 to 10)

Column	Tubing Material	Lindane		Aldrin	
		Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
C	Glass	11.5 ± 0.0	86 ± 0	12.0 ± 0.0	126 ± 2
D	Nickel	13.0 ± 0.6	52 ± 3	13.7 ± 0.3	75 ± 2
E	Stainless Steel	12.5 ± 0.5	55 ± 1	14.0 ± 0.3	77 ± 2
F	Copper	13.0 ± 0.0	110 ± 4	13.8 ± 0.2	159 ± 3
G	Aluminum	13.2 ± 0.2	106 ± 2	14.2 ± 0.4	159 ± 6

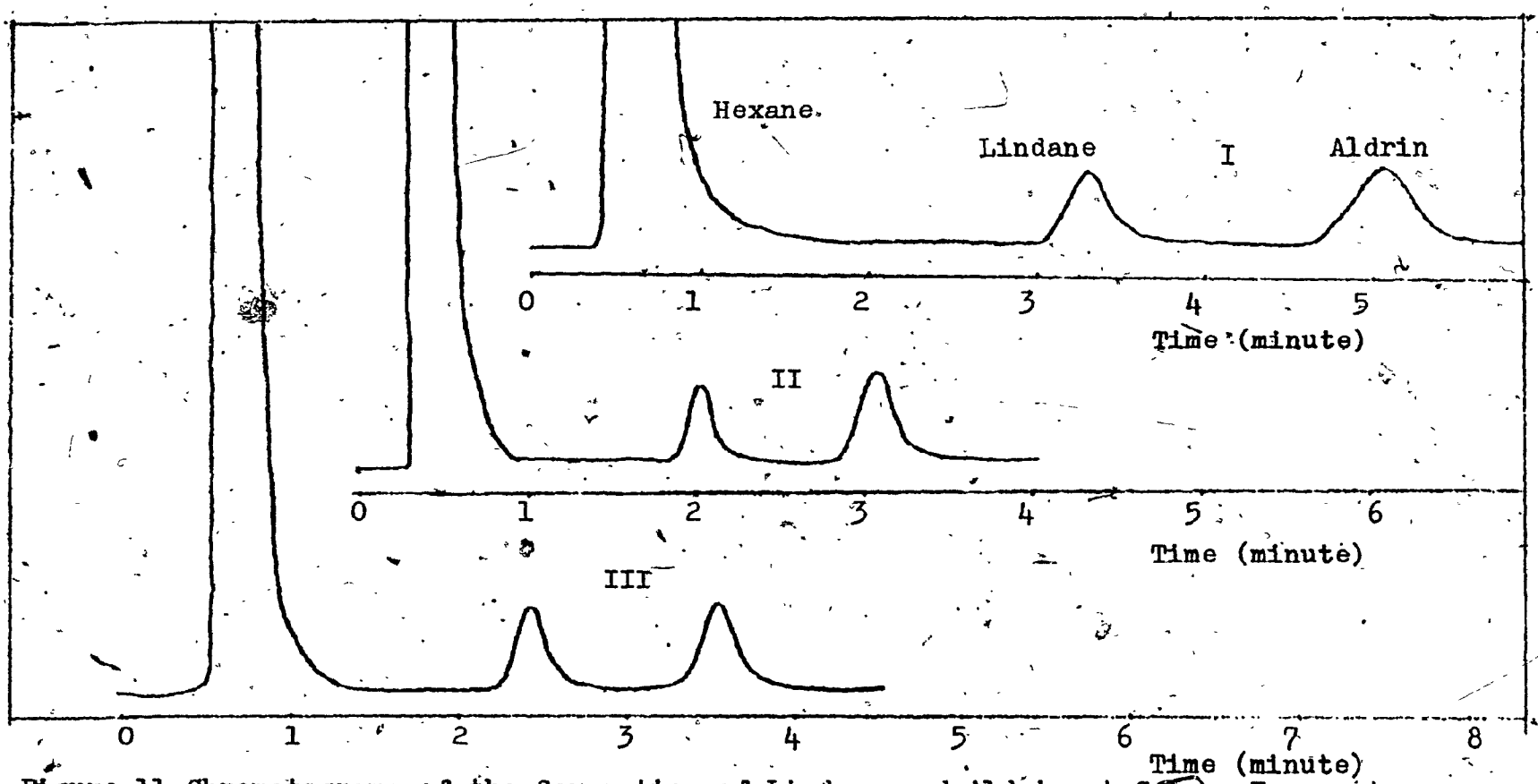


Figure 11 Chromatograms of the Separation of Lindane and Aldrin at Column Temperature 215 °C .(I)Column C :15 µg Lindane and 15 µg Aldrin,(II) Column D : 15.12 µg Lindane and 15 µg Aldrin, (III) Column E : 15.12 µg Lindane and 15.15 µg Aldrin. Operating Conditions Are Given in Table 6. Chart Speed : 1 inch/min.

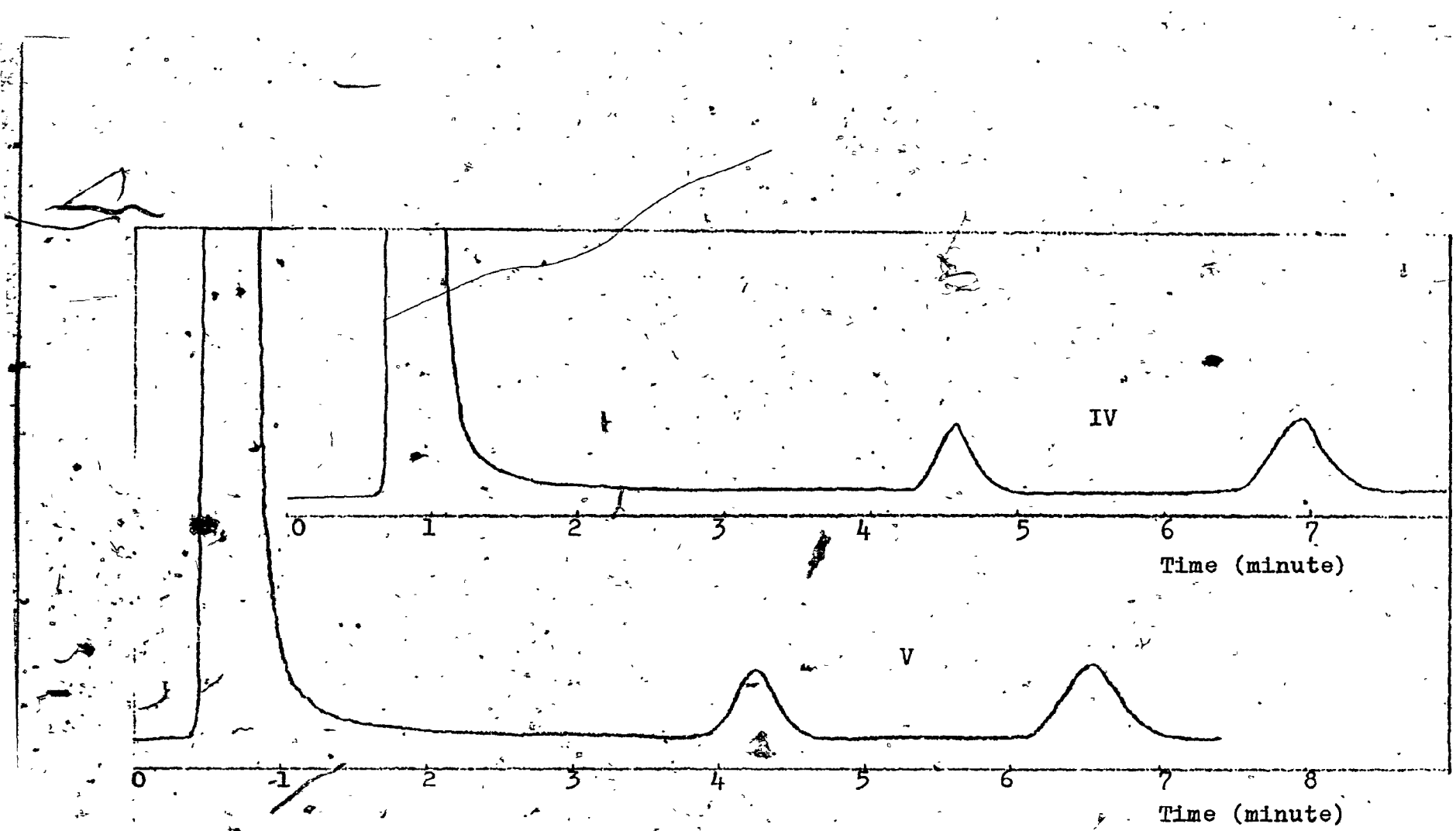


Figure 12 Chromatograms of the Separation of Lindane and Aldrin at Column Temperature 215 °C . (IV) Column F : 15.12 µg Lindane and 15.15 µg Aldrin, (V) Column G : 15.12 µg Lindane and 15.12 µg Aldrin. Operating Conditions Are Given in Table 6. Chart Speed 1 inch/min.

Column F (copper) < column G (aluminum) < column C (glass) < column E (stainless steel) < column D (nickel)

The degree of difference in the orders given above, may or may not be so significant, but it is always significant for the extreme ends in the order.

Column F (copper) and column G (aluminum) were equal, being least adsorptive, in terms of both peak height and peak area. The adsorptive property of column D (nickel) was comparable to those of columns F and G in terms of peak height, but it was the most adsorptive column in terms of peak area. In terms of peak height, the adsorptive property of column E (stainless steel) was comparable to those of column D. The most adsorptive column was column C (glass). The high adsorptivity of column C could be due to only partial removal of active sites on the glass tubing by washing with DMCS. More about this will be given in the discussion of results of adsorptive properties of various solid supports.

Adsorption in a column is contributed by two sources, surface of column tubing and coated supports inside the column, as described by the following equation.

$$A = A_w S + A_s \rho_p V$$

where A = total adsorption

A_w = adsorption due to surface of column tubing

S = surface area of column tubing

A_s = adsorption due to coated supports

ρ_p = packing density

V = volume of column tubing

By defining a_o and a as response with no adsorption and response after adsorption, respectively, the equation can be written as

$$a_o - a = A_w S + A_s \rho_p V$$

or $$(a_o - a)/V = A_w/L + A_s \rho_p$$

where L is the length of the column.

Using this equation, the peak area response on any given column can be interpreted in terms of a given reference column, for which response is assumed to be free of adsorption effects, i.e a_o. Peak area data in Table 12 were treated in this fashion with the response of column F (copper) taken as a_o (Table 12a). Peak area adsorbed per unit volume, $(a_o - a)/V$, was obtained by dividing peak area lost by adsorption, $(a_o - a)$, by the volume of the column tubing. (i) 15.32 cm^3, column C (glass)

(ii) 4.46 cm^3, column D (nickel)

(iii) 4.46 cm^3, column E (stainless steel)

(iv) 12.55 cm^3, column F (copper)

(v) 13.55 cm^3, column G (aluminum)

Table 12a Peak Area Lost by Adsorption ($a_o - a$) and Peak Area Adsorbed per Unit Volume, $(a_o - a)/V$, of Lindane and Aldrin for Columns C to G at Column Temperature 215 °C Taken from Results of Response at Different Column Temperatures (Second Run)

	Lindane			Aldrin		
Column	Peak Area (mm^2)	$(a_o - a)$ (mm^2)	$(a_o - a)/V$ (mm^2/cm^3)	Peak Area (mm^2)	$(a_o - a)$ (mm^2)	$(a_o - a)/V$ (mm^2/cm^3)
C	86 ± 0	24	1.6	126 ± 2	33	2.15
D	52 ± 3	58	13	75 ± 2	84	18.8
E	55 ± 1	55	12	77 ± 2	82	18.4
F	110 ± 4	0	0	159 ± 3	0	0
G	106 ± 2	4	0.30	159 ± 6	0	0

The adsorptive order of the columns deduced from this interpretation was the same as that obtained by directly comparing peak area responses. It does not matter which column's response is taken as a_o, the adsorptive order will be the same. The major drawback of this treatment is that the column assigned the value a_o may itself be subject to a certain degree of adsorption.

Table 13 lists responses at column temperature 215 °C on different columns, obtained during the third run to determine any priming effect of the second run. Comparing Tables 12 and 13, it is interesting to note that columns D and E showed improvement of responses, while columns F and G, with the exception of the lindane response on column F, showed no improvement over the previous responses. When the overall responses of the third run results were compared, no changes in the order of response, or of adsorptivity, were observed.

The responses of lindane and aldrin in terms of peak area decreased with increasing column temperature. A more detailed discussion will be given later on the effect of column temperature on responses. This decreasing pattern of responses was evaluated by applying linear regression analysis (Table 14). From Table 14, the orders of increasing negative slope were obtained based

Table 13 Peak Height and Peak Area of Lindane and Aldrin for Columns C to G at Column Temperature 215 °C Taken from the Results of Response at Different Temperatures (Third Run)

Column	Tubing Material	Lindane		Aldrin	
		Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
C	Glass	*			
D	Nickel	14.3 ± 0.4	54 ± 2	15.8 ± 0.2	87 ± 2
E	Stainless Steel	14.3 ± 0.2	64 ± 1	16.0 ± 0.4	101 ± 6
F	Copper	15.5 ± 0.0	121 ± 4	13.8 ± 0.2	154 ± 7
G	Aluminum	12.5 ± 0.0	100 ± 0	14.0 ± 0.0	147 ± 5

* The third run was not carried out.

Table 14 Slopes and Regression Coefficients(r) of Response at Different Column Temperatures for Columns C to G Listed in the Order of Second and Third Runs

Column	Tubing Material	Lindane		Aldrin	
		Slope	r	Slope	r
C	Glass	- 0.7372	- 0.9820	- 0.9113	- 0.9767
		*			
D	Nickel	- 0.0793	- 0.4856	0.0295	0.1440
		- 0.2296	- 0.8875	- 0.2247	- 0.7750
E	Stainless Steel	- 0.4247	- 0.9707	- 0.3312	- 0.8183
		- 0.3932	- 0.9732	- 0.3019	- 0.9167
F	Copper	- 0.6238	- 0.9814	- 0.6477	- 0.9959
		- 0.7090	- 0.9348	- 0.4598	- 0.7645
G	Aluminum	- 0.8824	- 0.9939	- 0.7466	- 0.9634
		- 0.6245	- 0.9687	- 0.6827	- 0.9517

* The third run was not carried out

only on the second run results.

(i) Slope of Lindane

Column D (nickel) < column E (stainless steel) < column F (copper) < column C (glass) < column G (aluminum)

(ii) Slope of Aldrin

Column D (nickel) < column E (stainless steel) < column F (copper) < column G (aluminum) < column C (glass)

Based on the third run results, with the exception of reversed order between columns F and G for lindane, the orders of slopes were the same as before.

In some cases (four out of eighteen), regression coefficients were so far from $\pm$ 1 that there was no linear correlation between peak area response and column temperature.

3.2.2 Response for Different Sample Sizes

For columns F and G, the experiments were carried out at column temperature 225 °C (Table 7), rather than 215 °C, and therefore it was necessary to correct responses at 225 °C to what they would have been at 215 °C, before comparing responses of various columns. This was

carried out by correlating the responses at column temperature 225 °C to responses at column temperature 215 °C, using calibration factors obtained from response at different column temperature curves (Figures 7 to 10). For example, for column G (aluminum), the peak area response for 15 µg lindane was 92 mm^2 at 225 °C. From Figure 9, the response at 215 °C was found to be 15.2 % greater than that at 225 °C. Accordingly, the following correlation equation was used to calculate responses at 215 °C from those measured at 225 °C

$$R_{215} = R_{225} + (15.2/100 \times R_{225})$$

eg. $R_{215} = 92\ mm^2 + (15.2/100 \times 92\ mm^2) = 106\ mm^2$.

The notation * is used to indicate that this correlation procedure was used.

The plots of peak height and peak area of lindane and aldrin against sample size for the second run results on various columns are presented in Figures 13 to 16, with the regression coefficients (r) given in brackets (Appendix C). When drawing the graphs, linear regression analyses were applied. Selected responses at column temperature 215 °C are listed in Table 15. A comparison of Table 15 and Table 12, indicated the same patterns of response.

Table 15 Peak height and Peak Area of Lindane and Aldrin for Columns C to G at Column Temperature 215 °C Taken from the Results of Response for Different Sample Sizes (Second Run) (Figures 13 to 16)

Column	Tubing Material	Lindane		Aldrin	
		Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
C	Glass	11.3 ± 0.2	89 ± 2	12.2 ± 0.2	128 ± 2
D	Nickel	12.8 ± 0.6	53 ± 2	13.5 ± 0.2	75 ± 1
E	Stainless Steel	12.7 ± 0.4	57 ± 2	14.0 ± 0.4	84 ± 2
F	Copper	14.3 ± 0.2	120 ± 2	15.5 ± 0.3	174 ± 3
G	Aluminum	13.0 ± 0.3	106 ± 4	14.1 ± 0.4	159 ± 6

From Figures 13 to 16, the following orders were obtained describing the adsorptive properties of various columns.

(i) Based on Peak Height

Adsorptivity Towards Lindane and Aldrin

Column F (copper) < column D (nickel) < column G (aluminum) < column E (stainless steel) < column C (glass)

(ii) Based on Peak Area

Adsorptivity Towards Lindane and Aldrin

Column F (copper) < column G (aluminum) < column C (glass) < column E (stainless steel) ≈ column D (nickel)

The above orders of adsorptivity agreed with those found previously using data from response at different column temperatures, except for a small difference for the nickel column. From these results, only column D (nickel) changed in the order while the rest of the columns maintained the same response patterns over sample sizes 15 to 45 micrograms.

3.2.3 Column Performance

Selected calculations of parameters for column efficiency were made at column temperatures 185, 215 and 252 °C on responses of various columns (second run) to evaluate column performances. The parameters are

n = number of theoretical plates

r = relative retention

R = resolution

and equations, Eq.1; Eq.7 ; Eq.8 ; Eq.10 and Eq.11 were used in calculating these parameters. Table 16 lists these parameters for various columns. From Table 16 and results of response at different column temperatures and response for different sample sizes, the following information could be obtained.

(i) There was no clear pattern for the relationship between the order of adsorptivity and n (i.e the least adsorptive column did not give the largest n value).

(ii) Relative retention for every column can be considered to be practically the same.

(iii) The higher the n value of both lindane and aldrin, the higher was the resolution value. In the case of overlapping between n of lindane and aldrin, the pattern was less clear.

Table 16 Parameters for Column Efficiencies with Lindane and Aldrin Used as the Test Samples for Columns C to G (Second Run)

Column	Pesticide	n at Column Temperature (°C)			r at Column Temperature (°C)			R at Column Temperature (°C)		
		185	215	252	185	215	252	185	215	252
C	Lindane	824	724	735	1.65	1.62	1.60	3.50	2.83	1.92
	Aldrin	930	837	554						
D	Lindane	1343	894	482	1.66	1.63	1.58	4.62	3.33	1.73
	Aldrin	1764	1107	544						
E	Lindane	1027	964	614	1.66	1.64	1.65	4.23	3.48	2.14
	Aldrin	1540	1346	812						
F	Lindane	1272	1003	798	1.69	1.63	1.60	4.44	3.53	2.39
	Aldrin	1548	1274	1012						
G	Lindane	1283	1081	948	1.66	1.63	1.62	4.37	3.59	2.53
	Aldrin	1456	1282	969						

Calculation of the asymmetric factor to describe the tailing or fronting of peaks, with equation Eq.12, failed to really account for these effects. As a typical example, a value of peak asymmetry equal to zero, which describes a perfectly Gaussian peak with no tailing or fronting, was obtained for peaks where tailing was evident to the eye. This matter will be described briefly later in conjunction with the results and discussion of adsorptive properties of various solid supports.

3.3 Adsorptive Properties of Solid Supports

3.3.1 Chromatographic Criteria

A comparison of adsorptive properties of solid supports studied was carried out using 3.5 % w/w OV-17 as liquid phase, and test samples, lindane and aldrin. The significant effect of 3.5 % w/w OV-17 loading has to be considered appropriately. Liquid phase OV-17 does cause deactivation of solid supports to some degree, like all of the liquid phases normally used for coating the solid supports in gas chromatographic work. But it is not equally effective for all Chromosorbs. In fact, with 3.5 % w/w OV-17 on each support, Chromosorbs W, P and G, neither the film thickness nor the total weight of liquid phase per column were the same because of the differences in the properties of the Chromosorbs themselves; i.e density and surface area (see Table 1). Therefore,

several points can be made as follows.

(i) Due to differences in density of the solid supports, the total loading of liquid phase on a particular solid support, on an equal column volume basis, will vary from one type of solid support to another.

(ii) The differences in surface area of the solid supports cause the thickness of liquid phase layer on a given solid support, on an equal column volume basis, to vary from one type of solid support to another.

(iii) As the consequence of (ii), the degree of deactivation of the solid support to be expected from the liquid phase will vary from one type of solid support to another.

From the above statements and Table 1, on an equal column volume basis, the following situations are expected to exist :

(i) Chromosorb P received the least deactivation effect from the liquid phase due to its largest surface area (m^2/cc) compared to either Chromosorb W or G. Therefore, tailing should be the greatest on Chromosorb P per unit of surface area.

(ii) The total amount of liquid phase is in proportion to density of the Chromosorbs; i.e

$$G > P > W$$

being the smallest amount on Chromosorb W. As a result, according to the Van Deemter equation (Eq.6), Chromosorb W should give the highest column efficiency when compared with Chromosorbs P or G, and resolution on Chromosorbs P and G should be better than on Chromosorb W. By reference to the physical properties of Chromosorb 750, it is seen that its density in gm/cc is close to the density of Chromosorb P. Therefore the column efficiency of Chromosorb 750 is expected to be approximately equal to that of Chromosorb P.

(iii) The overall deactivation of the solid support contributed from the liquid phase is expected to follow the order

$$P < \text{Chro } 750 < G < W$$

The surface area (m^2/cc) of Chromosorb 750 is close to those of Chromosorbs G and W.

Concerning the presentation of results, the first run tests were used for the purpose of column priming, and discussion of adsorptive properties of solid supports was based on the second run results. However, selected responses of the first run tests are also presented to show the effects of column priming on responses. With the exception of column D (W HP) which received extra treatment, the degree of column priming of each column

was the same.

Due to differences in the degree of deactivation by liquid phase of Chromosorbs W, P and G under conditions used, the comparison of adsorptive properties of solid supports was confined to Chromosorbs belonging to the same group, with the exception of Chromosorb 750. It was included in the Chromosorb W group for practical purposes. The comparisons served to test HP (not available on Chromosorb P), AW DMCS and AW treated Chromosorbs with respect to NAW or untreated Chromosorbs.

The comparison of adsorptive properties of differently treated solid supports within the same group was based on :

(i) Peak height and peak area responses at different column temperatures.

(ii) Peak height and peak area responses for different sample sizes.

It was expected that the largest responses, peak height or peak area, would be obtained using the least adsorptive column, and the smallest responses using the most adsorptive column.

Column efficiency parameters of all columns studied were calculated to evaluate column performances.

3.3.2 Response at Different Column Temperatures

A comparison was made in the column temperature range 185 to 252 °C. Since there was a variation in sample size used, all responses were converted to the equivalent response for 25 µg each of lindane and aldrin, to permit simple direct comparisons. The following notation is used to identify the correlation procedure.

* A linear calibration

It was applied only for small differences in sample size. i.e for Chromosorb W AW DMCS, the peak area response for 25.9 µg lindane at 215 °C was 51 mm^2. Therefore, for 25 µg aldrin, the peak area response was calculated to be 25/25.9 x 51 mm^2 = 49 mm^2.

** A calibration which took into account possible adsorption effects as determined from response for different sample size curves (Figures 29 to 40). i.e for Chromosorb W HP, the peak area response for 15 µg aldrin at 215 °C was 52 mm^2. Therefore, given a linear relationship between response and sample size, the response for 25 µg aldrin would be (25/15 x 52) mm^2. However, Figure 38 showed that the response for 25 µg lindane was in fact 11.2 % less than predicted by the above calculation. Therefore the following equation was used to convert

responses for 15 µg lindane to equivalent responses for 25 µg lindane.

$(R_{15\ \mu g} \times 25/15) - (11.2/100 \times R_{15\ \mu g} \times 25/15) = R_{25\ \mu g}$

eg $(52\ mm^2 \times 25/15) - (11.2/100 \times 52\ mm^2 \times 25/15) = 77\ mm^2$.

*** A calibration which incorporated both calibration procedures ** and *.

Reference should be made to Table 10 in the experimental section for the actual sample concentration and sample size introduced for each column.

The plots of peak height and peak area of lindane and aldrin against column temperature (second run results) are presented in Figures 17 to 28 (see Appendix D-1) for various columns differing in solid support only. A selected presentation of responses in terms of peak height and peak area at column temperature 215 °C for Chromosorbs W, P and G are given in Tables 17, 18 and 19 respectively. A precision of 5 percent or better was obtained for measurements of peak height and peak area. The adsorptive properties of the solid supports were considered separately in terms of peak height and peak area. From Figures 17 to 28, the following orders of adsorptivity were obtained for solid supports within groups (or belonging to the same type), based on the overall responses.

Table 17 Peak Height and Peak Area of Lindane and Aldrin for Various Chromosorbs W at Column Temperature 215 °C and Sample Size 25 µg Taken from the Results of Response at Different Column Temperatures (Second Run). (Figures 17, 20, 23 and 26)

		Lindane		Aldrin	
Column	Solid Support	Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
H	Chro 750	13.8 ± 0.2	90 ± 1	13.0 ± 0.3	123 ± 0
D	W HP	11.6 ± 0.3	53 ± 1	12.8 ± 0.3	77 ± 3
I	W AW DMCS	5.8 ± 0.0	29 ± 0	7.0 ± 0.2	49 ± 2
J	W AW	17.4 ± 0.0	91 ± 1	20.6 ± 0.2	133 ± 6
K	W NAW	10.6 ± 0.4	58 ± 2	14.0 ± 0.4	94 ± 3

Table 18 Peak Height and Peak Area of Lindane and Aldrin for Various Chromosorbs P at Column Temperature 215 °C and Sample Size 25 µg Taken from the Results of Response at Different Column Temperatures (Second Run) (Figures 18, 21, 24 and 27)

Column	Solid Support	Lindane		Aldrin	
		Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
L	P AW DMCS	10.0 ± 0.0	57 ± 2	9.6 ± 0.2	74 ± 3
M	P AW	10.0 ± 0.0	48 ± 2	10.3 ± 0.3	71 ± 1
N	P NAW	5.1 ± 0.2	28 ± 1	5.5 ± 0.2	48 ± 2

Table 19 Peak Height and Peak Area of Lindane and Aldrin for Various Chromosorbs G at Column Temperature 215 °C and Sample Size 25 µg Taken from the Results of Response at Different Column Temperatures (Second Run) (Figures 19, 22, 25 and 28)

Column	Solid Support	Lindane		Aldrin	
		Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
O	G HP	6.8 ± 0.2	56 ± 0	6.0 ± 0.0	70 ± 3
P	G AW DMCS	5.7 ± 0.2	57 ± 2	5.3 ± 0.2	80 ± 3
Q	G AW	8.5 ± 0.0	76 ± 0	7.5 ± 0.0	100 ± 3
R	G NAW	11.3 ± 0.2	79 ± 1	10.0 ± 0.0	100 ± 0

1. Chromosorbs W

(i) Based on Peak Height

(a) Adsorptivity Towards Lindane

W AW < Chro 750 < W HP < W NAW < W AW DMCS

(b) Adsorptivity Towards Aldrin

W AW < W NAW < Chro 750 ≈ W HP < W AW DMCS

(ii) Based on Peak Area

(a) Adsorptivity Towards Lindane

Chro 750 ≈ W AW < W NAW < W HP < W AW DMCS

(b) Adsorptivity Towards Aldrin

W AW < Chro 750 < W NAW < W HP < W AW DMCS

2. Chromosorbs P

(i) Based on Peak Height

Adsorptivity Towards Lindane and Aldrin

P AW < P AW DMCS < P NAW

(ii) Based on Peak Area

Adsorptivity Towards Lindane and Aldrin

P AW DMCS < P AW < P NAW

3. Chromosorbs G

(i) Based on Peak Height

Adsorptivity Towards Lindane and Aldrin

G NAW < G AW < G HP < G AW DMCS

(ii) Based on Peak Area

Adsorptivity Towards Lindane and Aldrin

G NAW ≈ G AW < G AW DMCS < G HP

For Chromosorbs W, W AW showed consistently the least adsorption for both lindane and aldrin in terms of peak

height and peak area. Chromosorb W AW DMCS was more adsorptive than the other Chromosorbs within the group. It is interesting to note that W NAW, untreated Chromosorb W, which is known to be the most adsorptive towards oxygenated compounds, showed less adsorption than supposedly more inert W AW DMCS. For this reason, deactivation studies on Chromosorb W NAW, W AW and W AW DMCS, described previously (21, 23), indicating AW DMCS treatment as being most effective in reducing surface activity of Chromosorb W, were not substantiated for the chlorinated pesticides lindane and aldrin.

Supina (27) has explained that failure of a silane treated solid support may be caused by one or more of the following factors.

(i) Partial deactivation

In this case, not all the silanol groups are converted to silyl ethers (see Rxn. 1, 2 and 3).

(ii) Negative deactivation

Here, some of the residual chlorosilane groups, introduced during the silanization steps, are not converted to methyl ethers by washing throughly with methanol (see Rxn. 1, 2 and 3). The result is a solid support more active than the original.

Similarly, in the context of surface activity, the above statements are applicable in general to the case of the

glass column described briefly in the study of column tubing materials, because both the surfaces of glass tubing and diatomite supports are covered with active silanol (Si-OH) groups which contribute most to the observed adsorption.

AW treatment was apparently the most effective. Acid washing succeeded in removing some of the active sites on Chromosorb W without introducing any new sites of activity.

In terms of peak height and peak area, W HP was less adsorptive than W AW DMCS. It was variable if compared to W NAW in terms of peak height, being less adsorptive towards lindane but more adsorptive towards aldrin. In terms of peak area, it was more adsorptive than W NAW. The results clearly suggested AW DMCS treated Chromosorb W was no better towards lindane and aldrin than untreated W because Chromosorb W HP is prepared by further processing of Chromosorb W AW DMCS (27). The failure of silane treatment on W AW DMCS would be carried over to some extent to W HP if the same batch of W AW DMCS was used to prepare Chromosorb W HP.

The discrepancies between these results and those reported in references (21, 23) may be accounted for on

the following basis.

(i) Failure of DMCS treatment used in the preparation of W AW DMCS and W HP supports.

(ii) Differences in polarity of the liquid phase used in this study (OV-17) and that cited in the references (squalane).

(iii) Differences in types of samples tested, i.e chlorinated pesticides versus simple oxygenated compounds.

(iv) Differences in the mesh of Chromosorb supports used, 80-100 mesh compared to 60-80 mesh in the references.

(v) It is also necessary to note that the deactivation studies as reported by Johns-Manville (21, 23, 24 and 25) were carried out at one particular column temperature and not over a 185 to 252 °C temperature range as in this study.

Chromosorb 750 was more adsorptive than W AW, based on peak height, but it was variable on the basis of peak area. If it was compared to W NAW, it was variable, based on peak height but less adsorptive, based on peak area. Chromosorb 750 is also an acid washed and silane treated Chromosorb, but it is not known whether the treatment is the same as those for Chromosorbs W AW DMCS and W HP. Either the treatment for Chromosorb 750 was not the same

or else it was more effectively carried out, based on the results in this study.

The adsorptive character of Chromosorbs P showed a slightly different pattern compared to those found in deactivation studies cited in reference (23). In terms of peak height and peak area, P NAW was the most adsorptive. While P AW was less adsorptive than P AW DMCS, based on peak height, it was more adsorptive than P AW DMCS, based on peak area. The question arose whether AW DMCS treatment of Chromosorb P was the same as of Chromosorb W since the outcome of the results was completely different. The obvious difference lies in the surface area of the Chromosorbs. Since the surface area of the Chromosorb P NAW is larger than those of W NAW, therefore, P NAW is more reactive than W NAW. As the result, P NAW should benefit more from deactivation treatments.

The order of adsorptive properties of Chromosorbs G was reversed completely from what was expected from various treatments to reduce the surface activity. In terms of peak height and peak area, G NAW was the least adsorptive, and G AW was the second least adsorptive. Chromosorb G HP was less adsorptive than G AW DMCS, based on peak height, but it was more adsorptive, based on peak area. The more adsorptive properties of both G AW DMCS

and G HP could be attributed, as before, to the failure of the DMCS (silane) treatment. In this case acid washing was not as effective as anticipated, but it was better than the combination of both acid washing and DMCS treatments. The failure of both these treatments made G NAW predominantly least adsorptive.

To illustrate the extent of possible priming effect of the first run on the responses of the second run, and therefore the adsorptive orders described above, Tables 20, 21 and 22 were prepared, listing selected first run results, at 215 ^{o}C, for Chromosorbs W, P and G, respectively. Comparison of Tables 17 and 20, Tables 18 and 21, and Tables 19 and 22 indicate that

(i) Second run responses for Chromosorbs W AW DMCS, W AW and W NAW were lower than first run responses, but adsorptive order was maintained.

(ii) Second run responses for Chromosorbs P AW DMCS, P AW and P NAW were lower than first run responses, and the adsorptive order was slightly altered; i.e. Chromosorb P AW DMCS now appeared to be more adsorptive than P AW based on peak height.

(iii) Second run responses for Chromosorbs G HP, G AW DMCS and G AW were slightly lower than first run responses, but the adsorptive order for these Chromosorbs was approximately the same. The second

Table 20 Peak Height and Peak Area of Lindane and Aldrin for Various Chromosorbs W at Column Temperature 215 °C and Sample Size 25 µg Taken from the Results of Response at Different Column Temperatures (First Run)

Column	Solid Support	Lindane		Aldrin	
		Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
H	Chro 750	*			
D	W HP	*			
I	W AW DMCS	7.3 ± 0.3	41 ± 2	7.7 ± 0.2	54 ± 1
J	W AW	17.2 ± 0.3	93 ± 2	19.3 ± 0.3	148 ± 3
K	W NAW	11.3 ± 0.5	57 ± 2	13.6 ± 0.3	101 ± 3

* The first run was carried out on a different recorder.

Table 21 Peak Height and Peak Area of Lindane and Aldrin for Various Chromosorbs P at Column Temperature 215 °C and Sample Size 25 µg Taken from the Results of Response at Different Column Temperatures (First Run)

Column	Solid Support	Lindane		Aldrin	
		Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
L	P AW DMCS	12.3 ± 0.4	70 ± 3	12.1 ± 0.4	95 ± 4
M	P AW	10.5 ± 0.3	58 ± 2	10.3 ± 0.3	73 ± 1
N	P NAW	6.1 ± 0.2	38 ± 0	6.5 ± 0.0	55 ± 0

Table 22 Peak Height and Peak Area of Lindane and Aldrin for Various Chromosorbs G at Column Temperature 215 °C and Sample Size 25 µg Taken from the Results of Response at Different Column Temperatures (First Run)

Column	Solid Support	Lindane		Aldrin	
		Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
O	G HP	7.7 ± 0.2	61 ± 2	6.6 ± 0.3	75 ± 3
P	G AW DMCS	6.0 ± 0.0	60 ± 0	5.5 ± 0.0	82 ± 0
Q	G AW	9.0 ± 0.0	79 ± 2	7.5 ± 0.3	98 ± 4
R	G NAW	7.0 ± 0.0	51 ± 2	6.7 ± 0.2	74 ± 2

run response of G NAW was much greater and consequently in the adsorptive order for Chromosorbs G, G NAW, instead of being comparable to G HP, was now the least adsorptive.

From the above results, it is clear that a significant effect of column priming is evident only for Chromosorb G NAW.

The decreasing pattern of peak area response with increasing column temperature was evaluated by applying linear regression analysis: (Tables 23, 24 and 25). From these Tables, the following orders of increasing negative slope were obtained, based only on the second run results.

(i) Chromosorbs W

Slopes of Lindane and Aldrin

W AW DMCS < W HP < W AW < W NAW < Chro 750

(ii) Chromosorbs P

Slopes of Lindane and Aldrin

P NAW < P AW < P AW DMCS

(iii) Chromosorbs G

(a) Slope of Lindane

G AW DMCS < G AW < G HP < G NAW

(b) Slope of Aldrin

G HP < G AW < G NAW < G AW DMCS

In some cases, no linear correlation between peak area response and column temperature was indicated by the

Table 23 Slopes and Regression Coefficients(r) of Response at Different Column Temperatures for Various Chromosorbs W Listed in the Order of Second and First Runs

Column	Solid Support	Lindane		Aldrin	
		Slope	r	Slope	r
H	Chro 750	- 0.7341 *	- 0.9954	- 0.7754	- 0.9834
D	W HP	- 0.3745 *	- 0.9443	- 0.3381	- 0.9015
I	W AW DMCS	- 0.1550	- 0.9371	- 0.1547	- 0.8838
		- 0.2478	- 0.9345	- 0.0790	- 0.4153
J	W AW	- 0.5134	- 0.8708	- 0.4585	- 0.9001
		- 0.7642	- 0.9458	- 0.5628	- 0.9082
K	W NAW	- 0.6087	- 0.9702	- 0.4897	- 0.9586
		- 0.5622	- 0.9674	- 0.4384	- 0.9461

* The first run was carried out on a different recorder.*

Table 24 Slopes and Regression Coefficients (r) of Response at Different Column Temperatures for Various Chromosorbs P Listed in the Order of Second and First Runs

Column	Solid Support	Lindane		Aldrin	
		Slope	r	Slope	r
L	P AW DMCS	- 0.3175	- 0.9532	- 0.2582	- 0.9511
		- 0.3309	- 0.8451	- 0.3014	- 0.8905
M	P AW	- 0.2782	- 0.9080	- 0.2134	- 0.7904
		- 0.3496	- 0.9516	- 0.2164	- 0.7630
N	P NAW	0.0129	0.1896	0.0862	0.6496
		- 0.1084	- 0.7653	- 0.1482	- 0.7457

Table 25 Slopes and Regression Coefficients(r) of Response at Different Column Temperatures for Various Chromosorbs G Listed in the Order of Second and First Runs

Column	Solid Support	Lindane		Aldrin	
		Slope	r	Slope	r
O	G HP	- 0.3747	- 0.9812	- 0.2289	- 0.8850
		- 0.5091	- 0.9556	- 0.5163	- 0.9234
P	G AW DMCS	- 0.2616	- 0.9810	- 0.3688	- 0.9294
		- 0.3610	- 0.9877	- 0.2926	- 0.8209
Q	G AW	- 0.3374	- 0.9237	- 0.2884	- 0.8541
		- 0.3144	- 0.9144	- 0.1418	- 0.5288
R	G NAW	- 0.5309	- 0.9619	- 0.3466	- 0.9653
		- 0.3002	- 0.9474	- 0.1876	- 0.8037

regression coefficient values.

3.3.3 Response for Different Sample Sizes

For Chromosorbs W, the determinations of response for different sample sizes were carried out under two different column temperatures and output attenuation settings, and therefore it was necessary to correlate these conditions, to compare responses of different Chromosorbs W. The experimental conditions for Chromosorbs G were also standardized, to be comparable with Chromosorbs W, even though they were the same within the group. The standardizations, which were applied to Chromosorbs W and G, were done by correlating all responses to column temperature 215 °C and output attenuation 2. The responses for Chromosorbs P were obtained experimentally under these conditions. The following notation is used to identify the calibration procedure.

* A conversion of responses at attenuation 4 to responses at output attenuation 2 by multiplying the responses by a factor of 2. It involved a linear correlation.

** A correlation of responses at column temperature 235 °C to responses at column temperature 215 °C using conversion factors obtained from the actual

experimental results of response at different column temperature tests.

i.e for Chromosorb G NAW :

Peak area response of 25 µg aldrin at 235 °C was 181 mm^2. Peak area data from response at different column temperature curves (Figure 28) indicate that response at 215 °C was 7.5 % higher than the response at 235 °C. Using this factor, the peak area response at 215 °C was calculated to be 195 mm^2 (7.5/100 x 181 + 181).

*** A correlation of responses at column temperature 205 °C to responses at column temperature 215 °C using calibration factors as obtained from the actual experimental results of response at different column temperatures, and followed by calibration procedure *.

For more detailed information about column temperature and output attenuation settings used to measure responses for different sample sizes for various columns, reference should be made to Table 10 in the experimental section.

The plots of peak height and peak area of lindane and aldrin versus sample size (second run results) for various solid supports studied are given in Figures 29 to 40 (see Appendix D-2). Regression analyses were applied to the straight lines drawn and the values of regression coefficients are included in the graphs for

each solid support. The responses of peak height and peak area of lindane and aldrin at column temperature 215 °C and sample size 25 µg on various Chromosorbs W, P and G are presented in Tables 26, 27 and 28 respectively. A precision of 5 percent or better was indicated for each peak height and peak area measurement. A comparison of Tables 26 and 17, Tables 27 and 18 and Tables 28 and 19, taking into account the difference in output attenuation (2 versus 4, respectively) indicated that generally similar response results were obtained in these two sets of experiments. From Figures 29 to 40, the order of adsorptive character of various Chromosorbs within groups is as follows.

1. Chromosorbs W

   (i) Based on Peak Height

      (a) Adsorptivity Towards Lindane

         W AW < Chro 750 < W HP < W NAW < W AW DMCS

      (b) Adsorptivity Towards Aldrin

         W AW < Chro 750 ≈ W HP ≈ W NAW < W AW DMCS

   (ii) Based on Peak Area

      (a) Adsorptivity Towards Lindane

         Chro 750 ≈ W AW < W NAW < W HP < W AW DMCS

      (b) Adsorptivity Towards Aldrin

         W AW < Chro 750 < W NAW < W HP < W AW DMCS

2. Chromosorbs P

Table 26 Peak Height and Peak Area of Lindane and Aldrin for Various Chromosorbs W at Column Temperature 215 °C and Sample Size 25 ug Taken from the Results of Response for Different Sample Sizes (Second Run) (Figures 29, 32, 35 and 38)

Column	Solid Support	Lindane		Aldrin	
		Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
H	Chro 750	28.0 ± 0.7	182 ± 4	26.4 ± 0.5	250 ± 3
D	W HP	23.5 ± 1.0	108 ± 5	26.6 ± 0.0	151 ± 0
I	W AW DMCS	11.7 ± 0.2	58 ± 0	14.0 ± 0.3	85 ± 3
J	W AW	34.8 ± 0.2	181 ± 1	40.9 ± 0.2	264 ± 5
K	W NAW	21.3 ± 0.3	116 ± 2	27.8 ± 0.3	187 ± 2

Table 27 Peak Height and Peak Area of Lindane and Aldrin for Various Chromosorbs P at Column Temperature 215 °C and Sample Size 25 µg Taken from the Results of Response for Different Sample Sizes (Second Run) (Figures 30, 33, 36 and 39)

		Lindane		Aldrin	
Column	Solid Support	Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
L	P AW DMCS	20.5 ± 0.3	113 ± 2	19.9 ± 0.2	149 ± 2
M	P AW	21.7 ± 0.4	119 ± 2	21.8 ± 0.6	163 ± 4
N	P NAW	13.6 ± 0.6	90 ± 3	11.3 ± 0.4	107 ± 4

Table 28 Peak Height and Peak Area of Lindane and Aldrin for Various Chromosorbs G at Column Temperature 215 °C and Sample Size 25 ug Taken from the Results of Response for Different Sample Sizes (Second Run). (Figures 31, 34, 37 and 40).

Column	Solid Support	Lindane		Aldrin	
		Peak Height (mm)	Peak Area (mm^2)	Peak Height (mm)	Peak Area (mm^2)
O	G HP	13.8 ± 0.3	106 ± 1	12.0 ± 0.4	144 ± 4
P	G AW DMCS	10.8 ± 0.2	108 ± 1	10.3 ± 0.0	157 ± 0
Q	G AW	17.4 ± 0.0	143 ± 6	15.7 ± 0.3	210 ± 3
R	G NAW	22.8 ± 0.2	158 ± 5	19.8 ± 0.2	195 ± 5

(i) Based on Peak Height

Adsorptivity Towards Lindane and Aldrin

P AW < P AW DMCS < P NAW

(ii) Based on Peak Area

(a) Adsorptivity Towards Lindane

P AW ≈ P AW DMCS < P NAW

(b) Adsorptivity Towards Aldrin

P AW < P AW DMCS < P NAW

3. Chromosorbs G

(i) Based on Peak Height

Adsorptivity Towards Lindane and Aldrin

G NAW < G AW < G HP < G AW DMCS

(ii) Based on Peak Area

(a) Adsorptivity Towards Lindane

G NAW ≈ G AW < G HP < G AW DMCS

(b) Adsorptivity Towards Aldrin

G AW < G NAW < G AW DMCS < G HP

A comparison of the orders given above based on peak height with the orders obtained previously from the results of response at different column temperatures, showed no significant differences, except that the response of aldrin on W NAW was now rather comparable to the responses given by either W HP or Chromosorb 750. Hence the response patterns were approximately maintained over the sample range 15 to 50 ug. Based on peak area,

there was a difference, but not a significant one, in the adsorptive properties of Chromosorbs P and G within the respective groups. Chromatograms showing separation of lindane and aldrin on columns of different Chromosorbs P are illustrated in Figure 41 at column temperature 215 °C.

3.3.4 Column Performance

To measure the performance of various columns prepared with different solid supports, selected parameters for column efficiency were calculated as before at column temperatures 185, 215 and 252 °C for the second run results. The parameters were, number of theoretical plates (n), relative retention (r) and resolution (R). The calculated values of these parameters are presented in Tables 29, 30 and 31 for various Chromosorbs W, P and G, respectively. From the Tables, the following information was obtained..

(i) The least adsorptive Chromosorb did not give the highest number of theoretical plates. There was no clear relationship between adsorptive character of the Chromosorbs and n.

(ii) As in the case of column tubing, relative retention was relatively the same from one solid support to another.

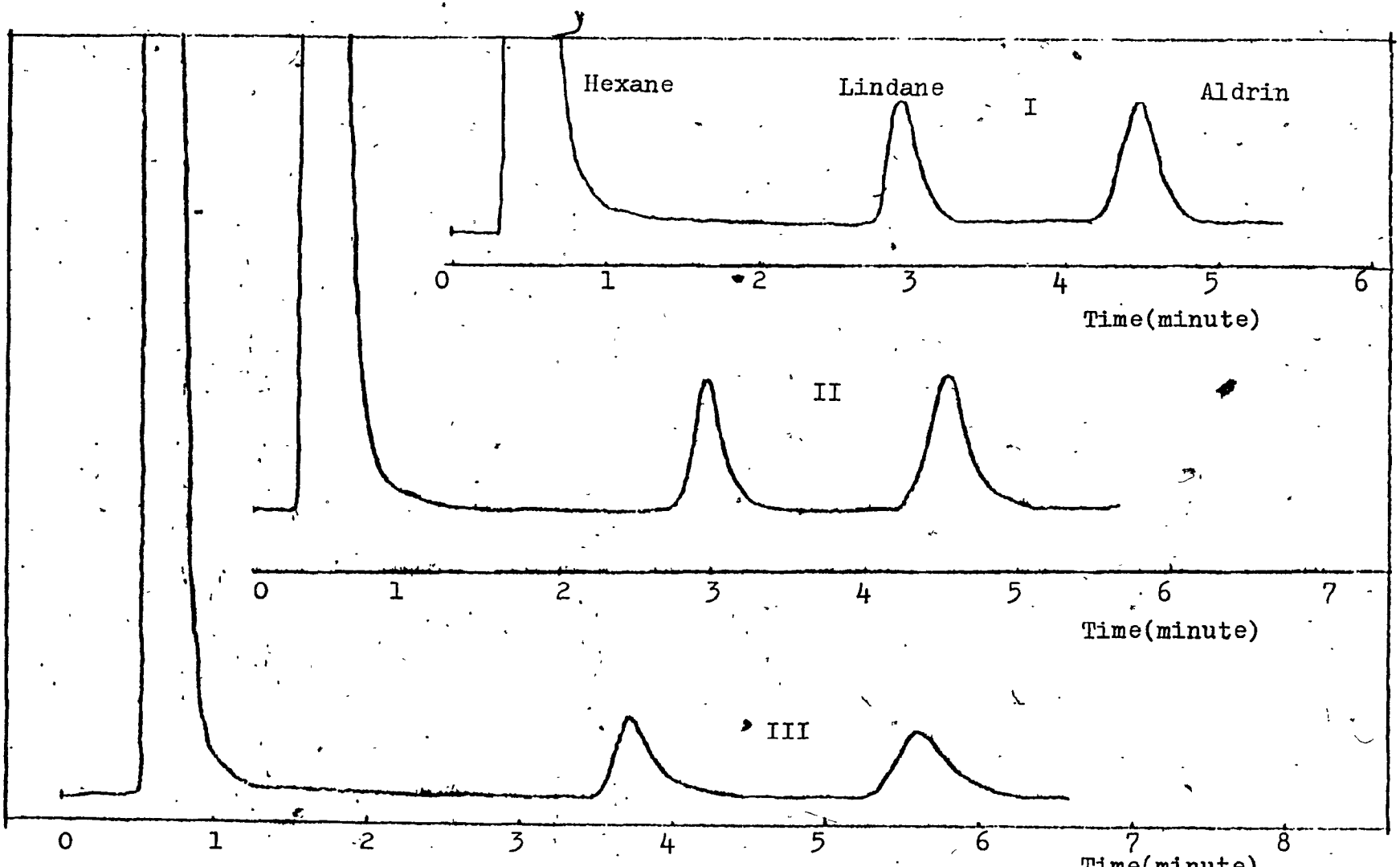


Figure 41 Chromatograms of the Separation of Lindane and Aldrin, (I) Column L : 25 µg Lindane and 25.40 µg Aldrin, (II) Column M : 25 µg Lindane and 25.40 µg Aldrin, (III) Column N : 25.40 µg Lindane and 25 µg Aldrin. Operating Conditions Are Given in Tables 9 and 10. Chart Speed : 1 inch/min.

Table 29 Parameters for Column Efficiencies with Lindane and Aldrin Used as the Test Samples for Various Columns Referring to Different Chromosorbs W (Second Run)

Column	Pesticide	n at Column Temperature (°C)			r at Column Temperature (°C)			R at Column Temperature (°C)		
		185	215	252	185	215	252	185	215	252
H	Lindane	738	538	373	1.65	1.65	1.68	4.92	2.65	1.85
	Aldrin	841	614	467						
D	Lindane	1144	899	483	2.81	1.62	1.63	4.35	3.3	1.81
	Aldrin	1607	1158	654						
I	Lindane	1374	928	381	1.68	1.66	1.60	4.45	3.3	1.77
	Aldrin	1491	1094	614						
J	Lindane	922	636	394	1.65	1.63	1.60	3.64	2.65	1.58
	Aldrin	1073	820	523						
K	Lindane	933	675	529	1.72	1.63	1.62	3.53	2.85	1.73
	Aldrin	1157	937	593						

Table 30 Parameters for Column Efficiencies with Lindane and Aldrin Used as the Test Samples for Various Columns Referring to Different Chromosorbs P (Second Run)

Column	Pesticide	n at Column Temperature (°C)			r at Column Temperature (°C)			R at Column Temperature (°C)		
		185	215	252	185	215	252	185	215	252
L	Lindane	1105	994	589	1.65	1.63	1.57	4.17	3.58	2.10
	Aldrin	1420	1280	766						
M	Lindane	1475	1213	643	1.67	2.34	1.59	4.76	3.93	2.17
	Aldrin	1801	1443	888						
N	Lindane	1606	1272	643	1.58	1.63	1.62	4.72	3.76	2.35
	Aldrin	1681	1213	790						

Table 31 Parameters for Column Efficiencies with Lindane and Aldrin Used as the Test Samples for Various Columns Referring to Different Chromosorbs G (Second Run)

Column	Pesticide	n at Column Temperature (°C)			r at Column Temperature (°C)			R at Column Temperature (°C)		
		185	215	252	185	215	252	185	215	252
O	Lindane	1420	1317	891	1.65	1.63	1.59	5.02	4.24	2.85
	Aldrin	2054	1529	996						
P	Lindane	1272	1051	787	1.65	1.51	1.59	4.36	3.75	2.86
	Aldrin	1327	1164	956						
Q	Lindane	1389	1116	712	1.66	1.63	1.59	4.84	3.91	2.82
	Aldrin	1782	1275	1031						
R	Lindane	1502	1320	846	1.69	1.63	1.60	4.88	4.27	2.79
	Aldrin	1791	1597	975						

(iii) The higher the n value of a column the better was the resolution. In the case of overlapping between n values of lindane and aldrin, the pattern was less clear reflecting the complex relationship between R and n.

(iv) Within each group of Chromosorbs, the Chromosorbs could not be rated according to parameters for column efficiency which could be associated to the nature of the Chromosorbs after various treatments to reduce surface activities.

(v) Among the different types of Chromosorbs, the number of theoretical plates and resolution for Chromosorbs P and G were comparable. For Chromosorbs W, n and R values were generally lower (with the exception of Chromosorbs W AW DMCS, W HP and Chromosorb 750 at column temperature 185°C) as is shown in Tables 29 to 31.

Statements (i) to (iii) were previously found to be true in the study of adsorptive properties of column tubings. Statement (v) could be attributed to the higher density and consequently larger amount of liquid phase on Chromosorbs P and G. The prediction that Chromosorb W would show the best efficiency, according to the Van Deemter equation (Eq. 6), failed. The prediction that resolution on Chromosorbs P and G was better than that on Chromosorbs W, was fulfilled, as

indicated by their higher values of R. The prediction for Chromosorb 750 also failed. It did not show column efficiency comparable to that of Chromosorb P even though its density was close to that of Chromosorb P.

Tailing

As in the study of adsorptive properties of column tubings, the tailing effect produced by each Chromosorb was calculated from the peak asymmetry for lindane and aldrin peaks. Calculations failed to show any clear pattern or relationship between asymmetry and type of Chromosorb treatment. One of the reasons for this has been described previously; i.e values of peak asymmetry equal to zero, suggesting truly Gaussian peaks with no tailing, were obtained for tailing peaks. Another was that chromatograms showed different responses (adsorptivities) without a marked alteration in peak symmetry. The prediction that Chromosorbs P would show the greatest tailing because of their large surface area per unit volume could not be confirmed.

To improve the meaningfulness of calculations of asymmetric factors, a smaller sample size was analyzed, with more sensitive detection conditions using a lower hydrogen flow rate. The tailing effect should be greater, the smaller the sample size. Figures 42 and 43 illustrate the chromatograms of various Chromosorb G

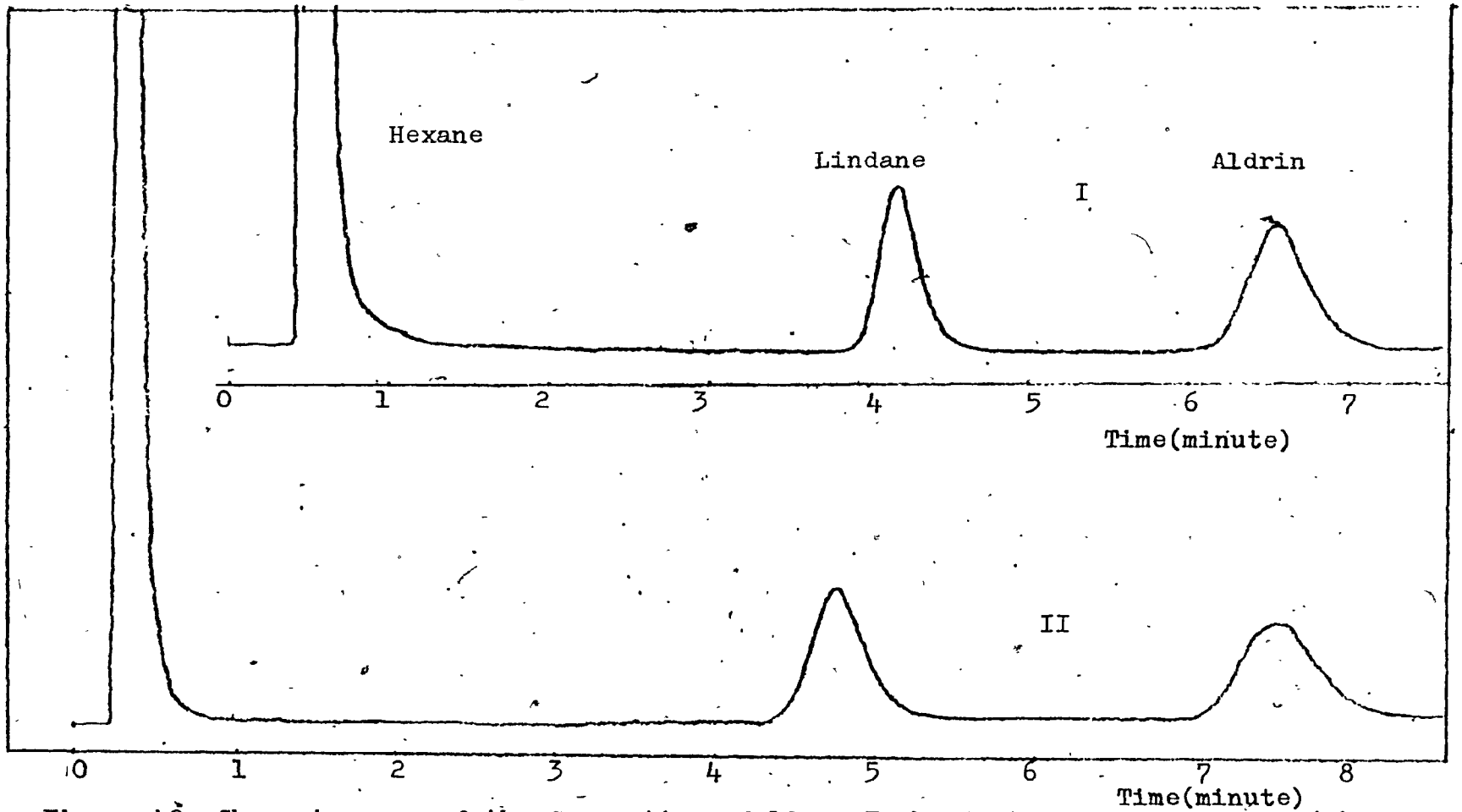


Figure 42 Chromatograms of the Separation of 10 µg Each of Lindane and Aldrin. (I) Column O , (II) Column P . Operating Conditions : Detector 235 °C , Injection Port 235 °C, Column 215 °C ; Attenuation : 2 x 10^2 ,Flow Rate : He, 40 ml/min; H_2 , 120 ml/min and Air, 330 ml/min. Chart Speed : 1 inch/min.

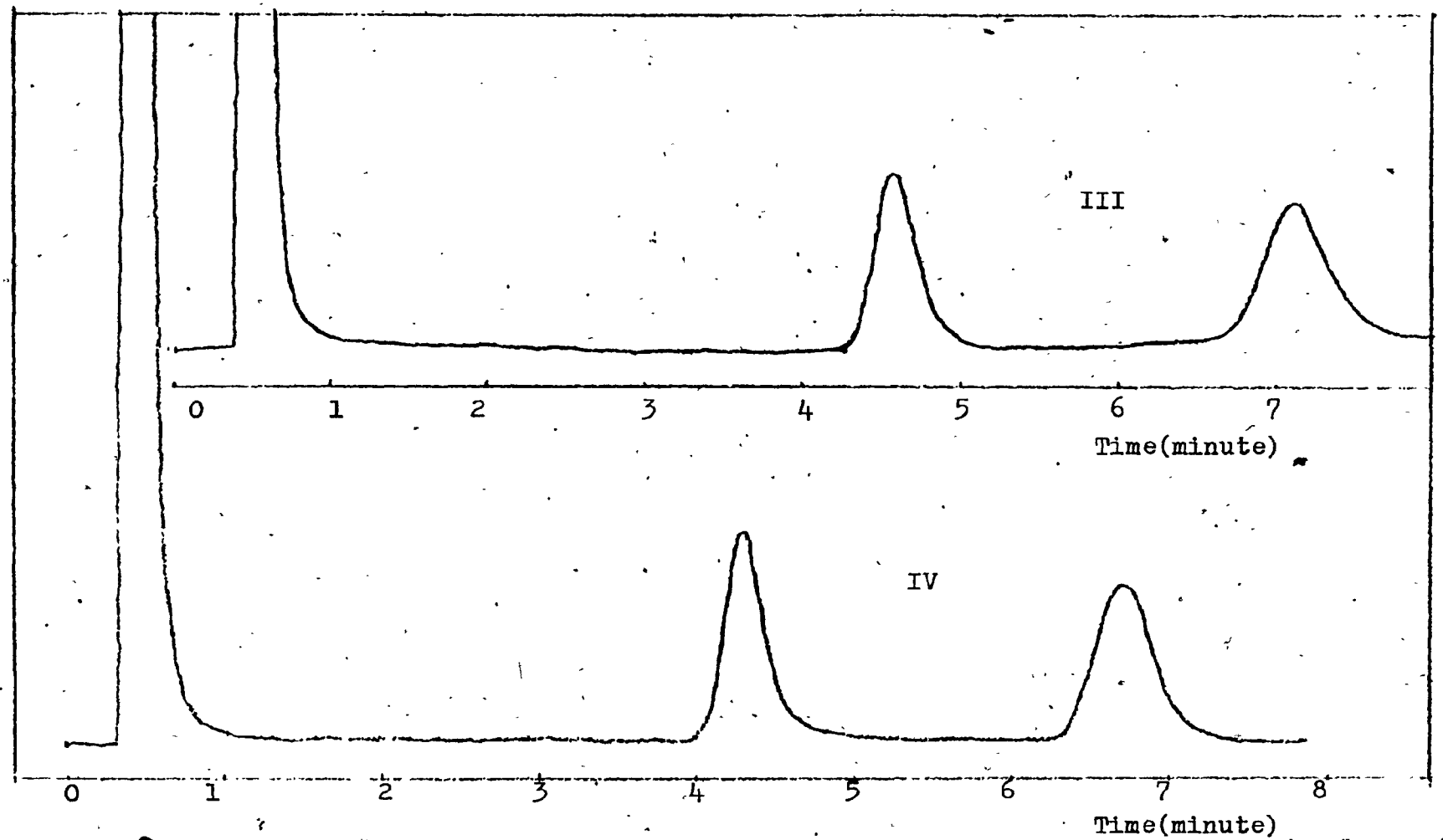


Figure 43 Chromatograms of the Separation of 10 µg Each of Lindane and Aldrin. (III) Column Q , (IV) Column R . Operating Conditions Are the Same As Given in Figure 42 .

columns at a temperature 215 °C, and using a sample size of 10 µg each of lindane and aldrin. Recalculation of the asymmetric factor using equation 12, gave practically the same result as before, with no clear relationship between peak asymmetry and various treatments used to reduce the surface activity of Chromosorbs G. From the above results, one question that can be raised is to what extent does a relationship between adsorption and tailing effects really exist. The solution to this problem may be to carry out the analyses of chlorinated pesticides having very close retention times. In this case, if there is severe tailing, then peaks will be unresolved.

3.4 Effect of Column Temperature on Responses of Lindane and Aldrin with FID and TCD

In this study using an FID, as the column temperature was increased over the range 185 to 252 °C the peak height also increased up to a point, but the peak area decreased (see Figures 9 and 10, and 23 to 28; Appendices C and D-1). The increase in peak height with increasing column temperature was anticipated due to the reduction in the retention time. Peak width and thus peak height was affected. On the other hand, the decrease in peak area with increasing column temperature is considered to be a very unusual result. If the flame ionization detector

response is a simple function of the mass of chlorinated pesticide samples injected it should give the same peak area, either at column temperature 185 or 252 °C, as long as nothing happens to the sample in the column or detector.

McWilliam and Dewar (35) reported on the characteristics of the FID. It was described as being insensitive to flow and ambient temperature variations. A study on characteristic of the FID by Gill and Hartmann (36) showed response to be independent of detector temperature. In their study, the detector was operated at three elevated temperatures : 200, 300 and 390 °C, using propane as the sample.

It was noted during the work with column tubings and solid supports that there was a maximum increase of 17 °C in detector temperature, from 245 to 262 °C, and 5 °C in injection port temperature from 240 to 245 °C (as indicated by the pyrometer) when a seven step increase in column temperature was made from 185 to 252 °C. To ensure that the detector temperature did not affect the peak area response, experiments at a constant column temperature were carried out with columns H and P. The variation of detector and injection port temperatures over a 17 °C temperature range had no effect on peak area or

peak height responses. It is definite, based on the studies made on characteristics of the FID by McWilliam and Dewar, and Gill and Hartmann (35, 36), and on the experimental results, that response is really independent of detector temperature.

The possibility that changes in carrier gas flow rate as column temperatures were increased occured and affected the FID were considered next. However, Novák (37) described that with mass-sensitive destructive detectors, such as the FID, the peak area response is theoretically independent of the rate of introduction of the sample chromatographed into the sensing element.

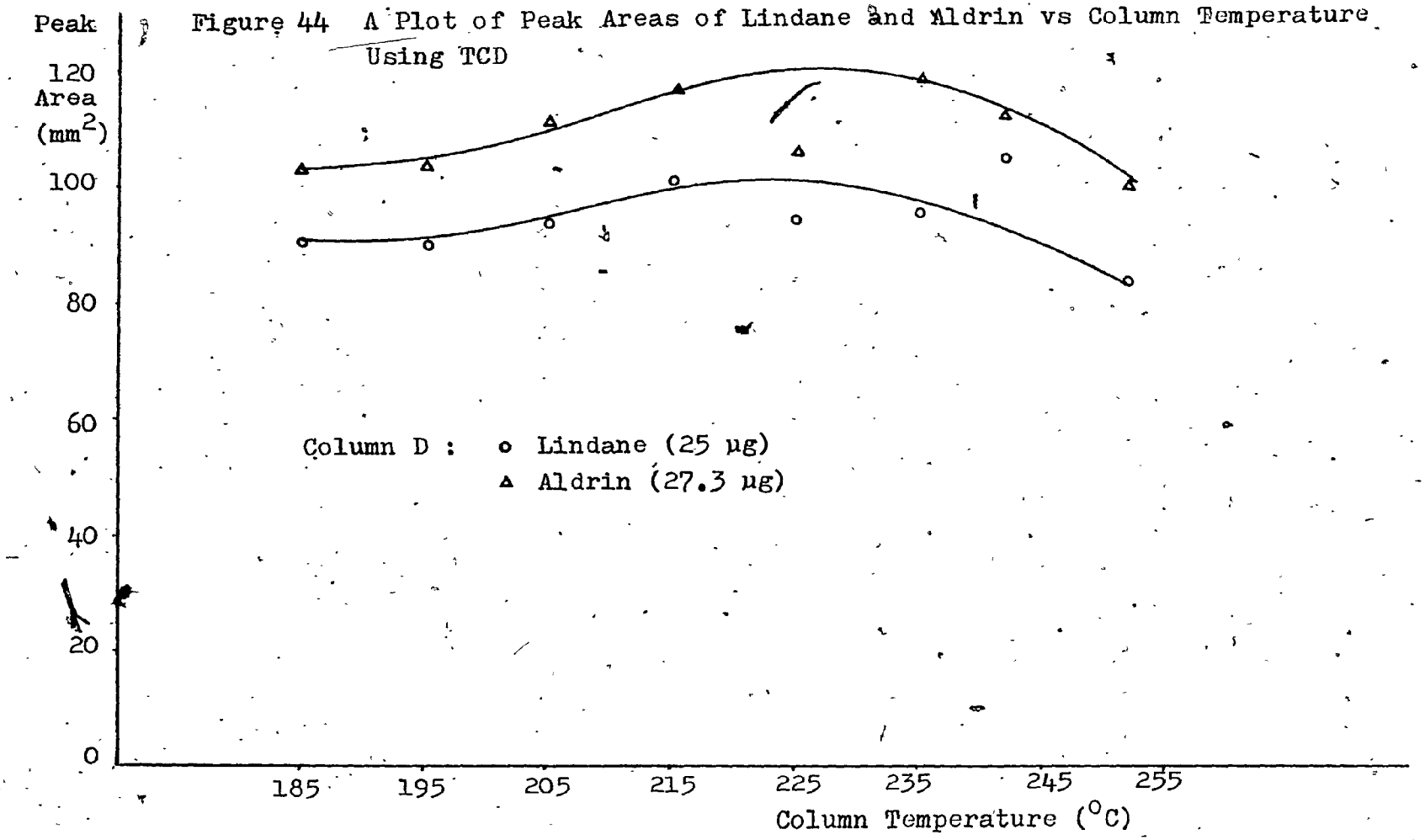
A literature survey on FID indicated that most studies to determine relative molar responses and effective carbon atoms were carried out with hydrocarbons, oxygenated compounds, and amines (38-43). Only a few papers described the study of some chlorinated compounds (42, 43). Hainová et al (42) reported that the relative molar response (RMR) of tetrachloromethane was not significantly influenced by the flow rate variations of carrier gas (N_2) and hydrogen which were independently varied in the range from 0.5 to 2.0 ml/sec, at a constant air flow rate of 800 ml/min. This RMR value was calculated using nonane as a reference substance. Ettre (43), in a short communication with data taken from different sources,

described relative molar responses of chlorinated compounds containing one or two carbon atoms relative to n-heptane. The RMR decreased with increasing substitution of chlorine atoms on methane, but it increases if the hydrocarbon is ethylene. The RMR of substituted (on carbon atom no 1) n-paraffins C_3 to C_7 increases with the number of carbon atoms in the n-paraffins, and is always smaller than that of the corresponding n-paraffins. The RMR values were reported relative to propane.

Based on the RMR values for chlorinated compounds described above, the FID response decreases as the ratio of CH/Cl in a compound decreases. It could be possible that the observed decreasing patterns of peak area with increasing column temperature were due to some kind of rearrangement or reaction of lindane, and aldrin on the column to give compounds with lower CH/Cl ratio and consequently lower FID responses. To confirm this idea, it would be necessary to isolate and identify the samples as they elute from the column.

With the TCD, the response patterns of peak area with increasing column temperature are slightly different than were obtained with the FID (Figure 44). The response patterns approximately increased up to column temperature 215 °C and then slowly decreased to a response lower than

Figure 44 A Plot of Peak Areas of Lindane and Aldrin vs Column Temperature Using TCD



that at column temperature 185°C.

The difference in response patterns of peak area with increasing column temperatures using FID and TCD may be attributed to the different principles of operation of the two detection systems. FID is a mass-sensitive destructive detector while TCD is a concentration sensitive detector. A mass sensitive detector responds to the total amount of the material detected in the sensing element. On the other hand, the concentration sensitive detector responds to the concentration of a compound chromatographed in the column effluent rather than to the total amount of that compound that reaches the sensing element (37). In fact, with TCD the peak area is proportional to the sample concentration in the column effluent, and inversely proportional to the flow rate. From this fact, there is an indication that stability of the flow rate of the carrier gas was not achieved during the experiment. The flow rate which was set at the beginning of the test might have undergone some changes when column temperature was increased.

Novák et al (37a) have studied the effect of column temperature on the sensitivity of Katharometer (TCD) response. They reported that for a given sample size, under constant experimental conditions, the peak

area will be proportional to the column temperature. This relationship is described by the equation

$$\frac{A_1}{A_2} = \frac{n_1}{n_2} \times \frac{T_1}{T_2}$$

where A = peak area, subscripts 1 and 2 refer to column temperatures 1 and 2 respectively

n = sample size in moles or in terms of weight

T = column temperature in degree Kelvin.

To examine how closely the experimental results obtained obey the above equation, Table 32 was prepared. It can be seen that ratios of peak areas at different column temperatures, $A(T^{o})/A(458)$ are not in agreement with the corresponding ratios of column temperatures $(T^{o}/458)$. This discrepancy may arise from possible instability of the carrier gas flow rate, which was measured only at the beginning of each run (at room temperature).

Table 32 Ratio of Peak Areas of Lindane and Aldrin, Relative to the Corresponding Ratios of Column Temperatures

Column Temperature (°C)	Column Temperature T(°K)	$\frac{T}{458}$	Lindane $\frac{A(T)}{A(458)}$	Aldrin $\frac{A(T)}{A(458)}$
185	458	1.00	1.00	1.00
195	468	1.02	1.01	1.01
205	478	1.04	1.06	1.08
215	488	1.06	1.15	1.14
225	498	1.09	1.06	1.04
235	508	1.11	1.07	1.16
242	515	1.12	1.18	1.10
252	525	1.15	0.94	0.98

4. Conclusions

The choice of the liquid phase OV-17 for the study of adsorptive properties of column tubings and solid supports was based on the separation of methoxychlor. It has been shown that a 3.5 % w/w loading was satisfactory for this purpose.

The importance of conditioning the column should be emphasized, to avoid any erratic results caused by bleeding of liquid phase. Since column priming, with one exception, did not significantly affect the subsequent responses observed, evidently column priming was not generally required. A significant improvement in response was found only with Chromosorb G NAW. The method of comparison used absolute response values whose accuracy was dependent upon the operating conditions. Therefore, a particularly careful control of these conditions was necessary.

Adsorptive Properties of Column Tubings

Copper and aluminum tubings (columns F and G) are less adsorptive than column tubings made from glass, stainless steel or nickel (columns C, E and D). Based on peak area measurements, glass tubing is less adsorptive than stainless steel or nickel tubings. The adsorptive properties of the glass tubing tested may be

due to failure of the DMCS treatment to adequately eliminate silanol active sites.

Adsorptive Properties of Solid Supports

The comparison of adsorptive properties of solid supports belonging to the same group, Chromosorbs W, P or G, based on peak area or peak height responses, suggested that AW DMCS and also HP treated Chromosorbs W and G were more adsorptive than corresponding untreated Chromosorbs. The reason for the more adsorptive properties of these Chromosorbs was likely due to failure of the DMCS treatment that was used. These results were considered to be unusual. However, the poor performance of a number of commercially available, acid washed, silane-treated solid supports was previously reported by Roman et al (37b). The majority of the solid supports tested by them were reported as being unsatisfactory for gas chromatographic analysis of esterified estrogens. Roman et al further observed variations in performance for different batches of acid washed, silane-treated solid supports.

Chromosorb 750, which is also an acid washed and silane-treated solid support, was found to be less adsorptive than DMCS treated Chromosorbs W, P and G. Its acid washing and silane treatment must have been carried

out either differently or more effectively.

The results on Chromosorbs P indicated that adsorption was decreased by acid washing and further decreased by silanization.

The results with Chromosorbs W, P and G indicated that acid washed Chromosorbs were consistently better (less adsorptive) than the respective untreated Chromosorbs. An exception was Chromosorb G NAW which became less adsorptive than Chromosorb G AW as a result of column priming.

Column Performance

Under conditions used, the number of theoretical plates (n) could not be used as a criterion to evaluate orders of adsorption for columns prepared with various column tubings and Chromosorbs belonging to the same group. There was no clear relationship between n and the adsorptive order of the columns. The number of theoretical plates and resolution (R) of Chromosorbs G were comparable to those of Chromosorbs P, and they were higher than those of Chromosorbs W. The relative retention (r) of lindane to aldrin was practically the same for all columns tested. The tailing effect calculated from the peak asymmetry (Eq. 12) could not be used to classify the adsorptive properties of the columns

tested. There was no clear relationship between adsorptivity and tailing effects.

Effect of Column Temperature on Responses of Lindane and Aldrin with FID and TCD

The observed patterns of decreasing peak area response with increasing column temperature using FID could be attributed to the test samples, lindane and aldrin, undergoing some kind of rearrangement or reaction on the column to give compounds of lower CH/Cl ratio.

The different patterns of peak area response versus column temperature that were obtained using FID and TCD were due to the different operating principles of FID and TCD. The observed patterns of peak area response using TCD may be attributable to instability of carrier gas flow rate as column temperatures were increased. The flow rates were uncorrected when column temperatures were changed.

5. Suggestions for Further Study

(i) To study the relationship between adsorptivity of a column and its tailing effects by using a smaller sample size (i.e nano to picograms range), and using a more sensitive electron capture detector (ECD), or by employing two chlorinated pesticides having very close retention times. Kelthane seems to be a good test sample since it also contains the hydroxyl group.

(ii) To test the observed decreasing pattern of peak area response with increasing column temperature using FID by employing an electronic integrator to measure the peak area.

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Appendix A

The Principles of TCD and FID Operations

Thermal Conductivity Detector (TCD)

The thermal conductivity detector is based on the principle that a hot body will lose heat at a rate dependent upon the thermal conductivity of the surrounding gas mixture. Thermal conductivity is a process that involves a transfer of kinetic energy caused by a temperature gradient, and is interrelated to transport phenomena such as diffusion and viscosity. Hence the rate of heat loss can be used to measure the amount of a given gas.

A TCD consists of filaments situated inside the cavities of a metal block. Each filament acts as a hot body which is heated by passage of a constant current. Under an equilibrium condition, the heat loss by the heated filament with respect to the heated metal block is constant. When the sample effluent emerges from the column and enters the cavity the gas composition changes in thermal conductivity. This changes the temperature of the filament which in turn changes its resistance causing an imbalance in the bridge circuit. The degree of imbalance is proportional to sample effluent concentration. An analog signal corresponding to this imbalance is then sent to the recorder. Since the absolute measurement of thermal conductivity is difficult, a differential

measurement is used in which the carrier gas passes through one cavity and the sample effluent passes through the other. In most TCD four filaments are used. A Wheatstone bridge circuit and the wiring of the four filaments are shown in Figure 45. Figure 46 illustrates the filaments inside the cavities of a cylindrical block (45).

Littlewood (46) has described the heat loss as being caused by the following processes in approximate order of magnitude.

(i) Mass transfer

(ii) Thermal conductivity

(iii) Convection

(iv) Radiation

The sensitivity of a TCD (47) can be calculated as follows :

$$S = \frac{A \times C_1 \times C_2 \times C_3}{W}$$

where S = sensitivity ($mv \times ml \times mg^{-1}$)

A = peak area in cm^2

C_1 = recorder sensitivity in mv/cm of chart

C_2 = reciprocal of chart speed in minute per cm

C_3 = flow rate of the carrier gas, ml/min

W = weight of the sample introduced, mg.

Flame Ionization Detector (FID)

When a hydrogen flame is burning in an excess of air, only a few ions are formed. However, if an organic compound is introduced into the flame, the number of ions formed is increased greatly. This is largely due to the fact that the actual degree of ionization of an organic material in a hydrogen flame is much greater than would be expected from its normal ionization potentials. That is to say, its effective ionization potential value is lower in the flame. Stern (48) has given a theoretical account of this phenomenon in more detail.

FID consists of a hydrogen flame surrounded by an electrical field, and the ion collector electrode. When column effluent enters the flame, the sample is burned producing ionic fragments and free electrons. The presence of the electrical field is to provide the polarization for charge separation. The charged fragments are then collected to give the ion current which is proportional to the rate of sample entry into the flame. The electrical signal is registered on a strip chart recorder after amplification by an electrometer.

When only carrier gas is flowing, a constant current is produced, referred to as background current. This background current can be compensated for through

the bucking control provided in most gas chromatographs equipped with FID. Hence, under no signal conditions, no electrical current flows and a straight baseline is traced by the recorder. The electrical field is normally supplied by a D.C. voltage. Under applied voltages below 150 V, the ion current obeys Ohm's law. It is saturated between 150 V and 200 V, and remains constant for voltages up to 500 V. The geometry of the ion collector electrode is also important in determining the response. Gill and Hartmann (36) have suggested the use of a cylindrical collector which leads to more sensitivity than the simple loop collector.

Most commercial gas chromatographs, are equipped with dual flames. Figure 47 illustrates the flame compartment of the Microtek GC-2000 R (45). The advantages of dual flames are (i) higher sensitivity and (ii) allowing the use of a differential column system. Figure 48 illustrates a circuit diagram of an actual FID system (49). The ion currents produced in the two flames, oppose each other. As a result, the voltage produced is proportional to the difference of the ion currents in the two flames. The Microtek GC-2000 R gas chromatograph with dual flames employs ± a 300 V D.C. applied voltage, and has cylindrical collector electrodes.

The sensitivity of a FID is given by the equation

$$S = \frac{C_1 \times C_2 \times A}{W}$$

where S = detector sensitivity ($mv.sec.mg^{-1}$)

C_1 = recorder sensitivity in mv/cm of chart

C_2 = reciprocal of chart speed, sec/cm

A = peak area in cm^2

W = weight of sample in mg.

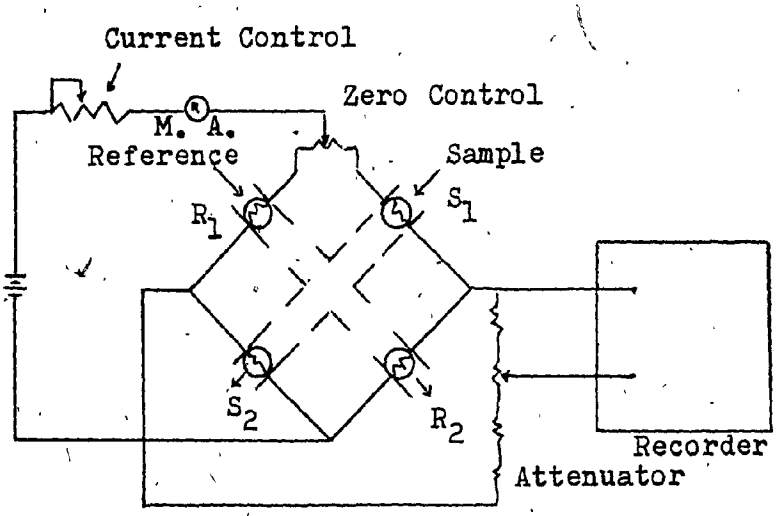


Figure 45 Wheatstone Bridge Circuit for T.C. Cells (Reference 44)

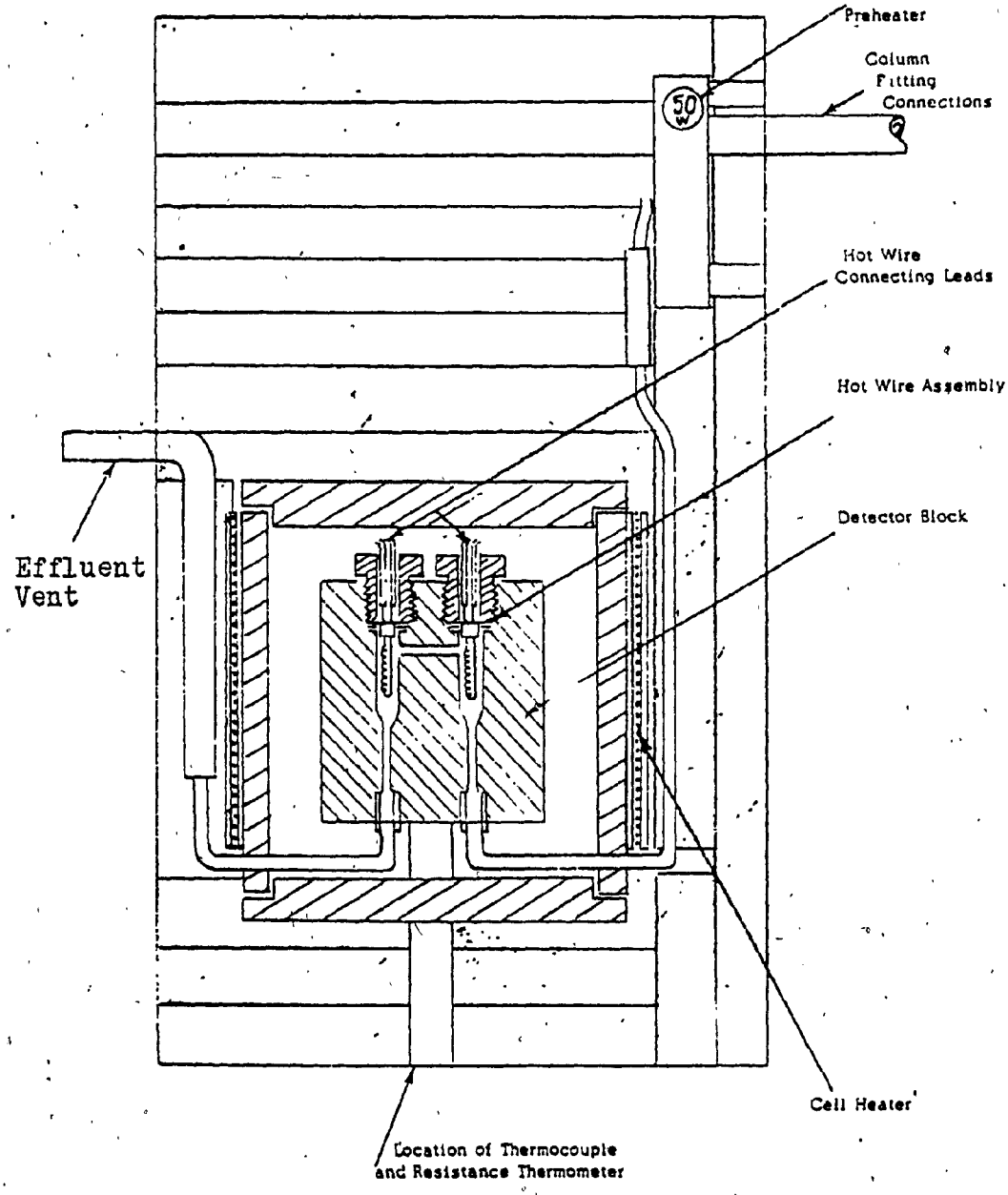


Figure 46 A Typical Thermal Conductivity Detector Assembly (Reference 45)

IDENTIFICATION KEY FOR FIGURE

1 Removable Stack Top
2 Removable Stack
3 Target
4 Radiator Fins
5 Insulator Stuffing
6 Stuffing Compressor Disc
7 Insulating Washer
8 Polarizing Voltage Connector
9 Polarizing Voltage Spring
10 Glass Sleeve with Metallized Band
11 Cone Air Retainer
12 Burner Tip
13 Air Disperser
14 Burner Air
15 Column Effluent
16 Hydrogen
17 Scavenger Air
18 Thermocouple Hole
19 Cartridge Heater Hole
20 Target Hole Screw
21 Coax Connector Fitting
22 Removable Insert
23 Removable Flame Back

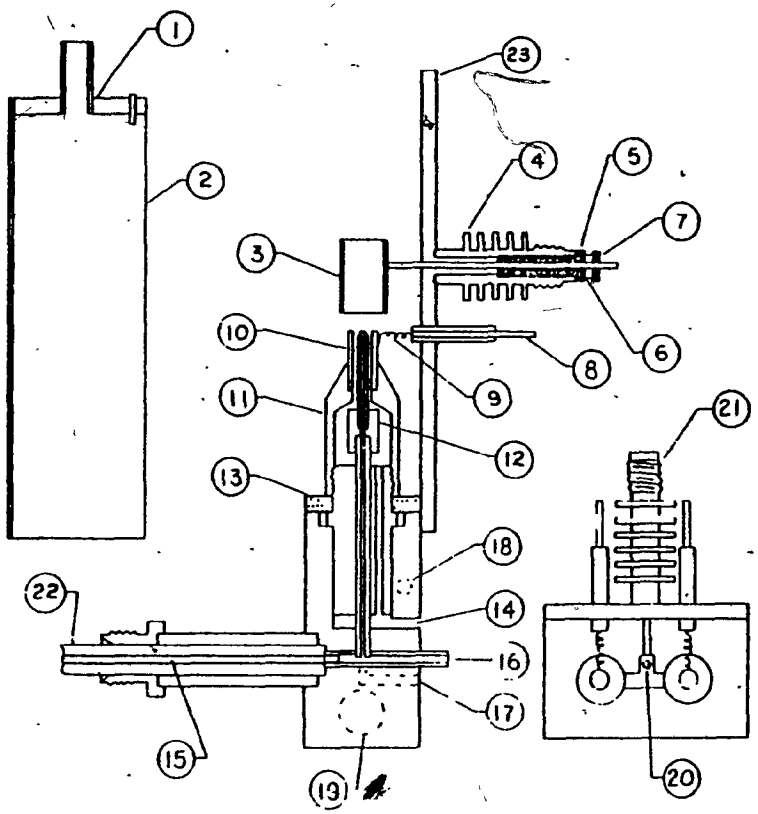


Figure 47 Duo Cone Flame Ionization Detector (Reference 45)

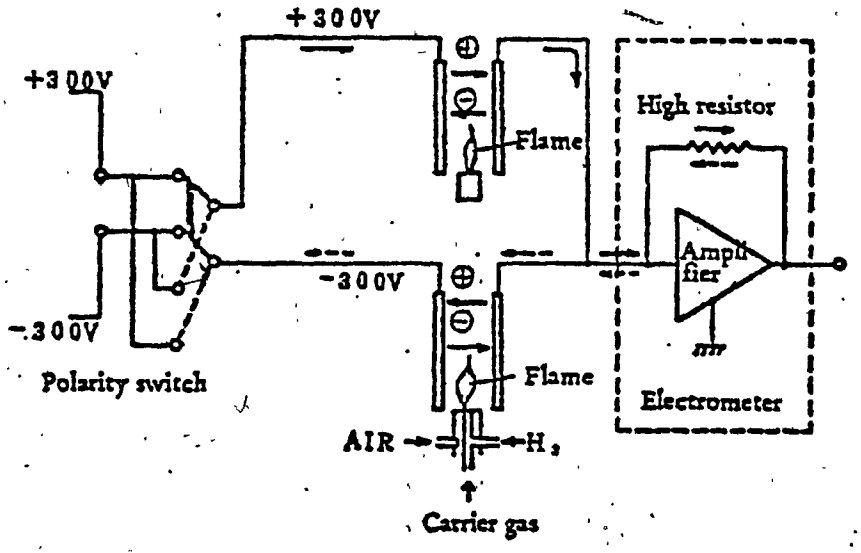


Figure 48 Circuit Diagram of a Dual Flame System (Reference 49)

Appendix B

Sample and Column Preparation

Sample Preparation

The following solutions were prepared.

(i) Pesticide solutions of concentrations 5.00 mg/ml and 10.00 mg/ml were prepared by dissolving 50.0 mg and 100.0 mg of individual pesticides, respectively, in hexane, in 10 ml volumetric flasks.

(ii) A mixture of dieldrin and methoxychlor containing 5.00 mg/ml of each was prepared by weighing 50.0 mg of the individual pesticides into a 30 ml beaker, dissolving in hexane, and making up to 10 ml volume using a 10 ml volumetric flask. To ensure the complete transfer of the pesticide sample from the beaker, the 30 ml beaker was rinsed and the rinsings were used to make up the required 10 ml volume.

(iii) A mixture of lindane and aldrin containing 5.00 mg/ml of each was prepared by using the same procedure as outlined in (ii).

All samples prepared were stored in 6 ml Hypo vials sealed with Teflon ® discs.

Note : All weighings were carried out with a precision of $\pm$ 0.2 mg. The precision of the measured volumes with 10 ml volumetric flasks was assumed to be $\pm$ 0.01 ml.

Column Preparations

The solution evaporation method was used to coat the liquid phase on the solid supports. The organic solvents for the liquid phases used were as recommended by Chromatographic Specialties (50), and the amount of organic solvents used was adopted according to the method suggested by McNair and Bonelli (51).

Preparation of Coated Supports

The coated solid supports were prepared according to the following procedures.

(i) Preparation of mixed phases, 1.95 % w/w QF-1 + 1.5 % w/w OV-17 on Chromosorb W HP, 80-100 mesh, for columns A and B.

A 0.3900 gram portion of liquid phase QF-1 was dissolved in a 150 ml beaker in 40 ml of ethyl-acetate. A 20.0000 gram portion of Chromosorb W HP, 80-100 mesh, was then poured into the solution. The beaker containing the support was heated slowly on a hot plate with periodic swirling. After the solvent was evaporated, the coated support was poured into another beaker containing 0.3000 grams of liquid phase OV-17 in 40 ml of toluene. The evaporation process was repeated as before.

Note : The FDA method for preparing of mixed phases involves coating the liquid phases on the

solid support individually, and then mixing the two coated supports in a 1:1 ratio by weight (6).

(ii) Preparation of 3.5 % w/w OV-17 on Chromosorb W HP, 80-100 mesh for column C, and column E to column G.

20.0000 grams of Chromosorb W HP, 80-100 mesh was poured into a 150 ml beaker containing 0.7000 grams of liquid phase OV-17 in 40 ml of toluene. The beaker was then heated slowly on a hot plate with periodic swirling to ensure a uniform coating of the solid support until the solvent was completely removed by evaporation.

(iii) Preparation of 3.5 % w/w OV-17 on various Chromosorbs untreated and treated in different ways for column D, and column H to column R.

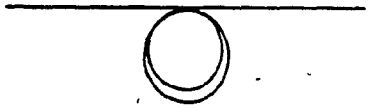
Each packing was prepared by pouring 5.0000 grams of solid support into a 100 ml beaker containing 0.1750 grams of liquid phase OV-17 in toluene. The amount of toluene used was varied depending on the solid support as follows

Solid Support	Volume of Toluene(ml)
Chromosorb W	10
Chromosorb P	7.5
Chromosorb G	2.5

From this point onwards, the procedure used was as is described in (ii).

Packing of Coated Support into Column Tubing

All 4 ft columns, with the exception of the glass column, were packed by using the funnel packing method. The rest of the columns were packed using the Column-Pak packing method. The shape of the column as it was fitted into the column oven of the gas chromatograph but positioned vertically, is illustrated below.



With the funnel packing method, the column tubings were packed straight and after packing they were shaped. For the Column-Pak packing method, the column tubings were shaped prior to packing with the coated supports. All column tubings, with the exception of the glass column, were used directly without prior washing. The glass tubing was washed with a solution of 5 % DMCS in toluene. The tubing was filled with the solution, and allowed to stand for a few minutes. The solution was then flushed out with toluene and finally with methanol. This method of washing glass tubing to reduce its surface activity due to silanol groups was suggested by Supina (10).

Funnel Packing Method

A funnel was attached onto one end of the column tubing and the other end was plugged with silanized glass wool. The supports were then introduced into the column tubing through the funnel with continuous tapping of the tubing to ensure a tight packing. After packing, a silanized glass wool plug was inserted in the top end of the column.

Column-Pak Packing Method

The Column-Pak is designed to fill precoiled columns. The support is held in a cylindrical tube, one end of which is attached to a pressure source and the other onto one end of the column tubing. Pressure from a nitrogen gas cylinder was applied forcing the support into the column tubing, whose other end was plugged with a small amount of silanized glass wool. When the packing was completed, the front end of the column tubing was disconnected from the Column-Pak and a plug of silanized glass wool was inserted.

Note : With the exception of the glass column, all columns prepared had empty spaces of one to two inches on both ends of the column tubing after insertion of silanized glass wool plugs. The glass column had empty spaces of three to four inches.

Appendix C

Figure 7 A Plot of Peak Height of Lindane vs Column Temperature (Second Run)

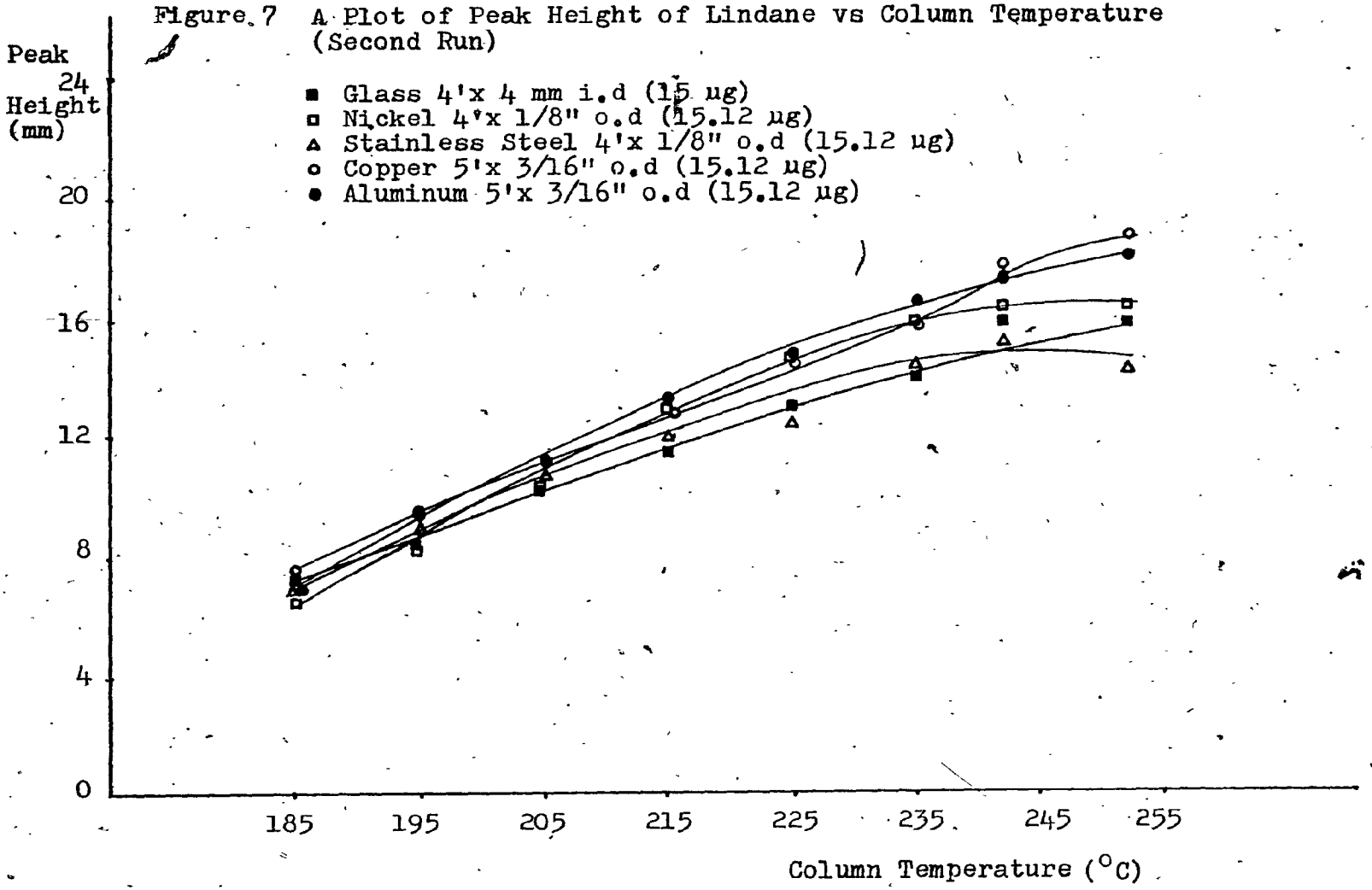


Figure 8 A Plot of Peak Height of Aldrin vs Column Temperature (Second Run)

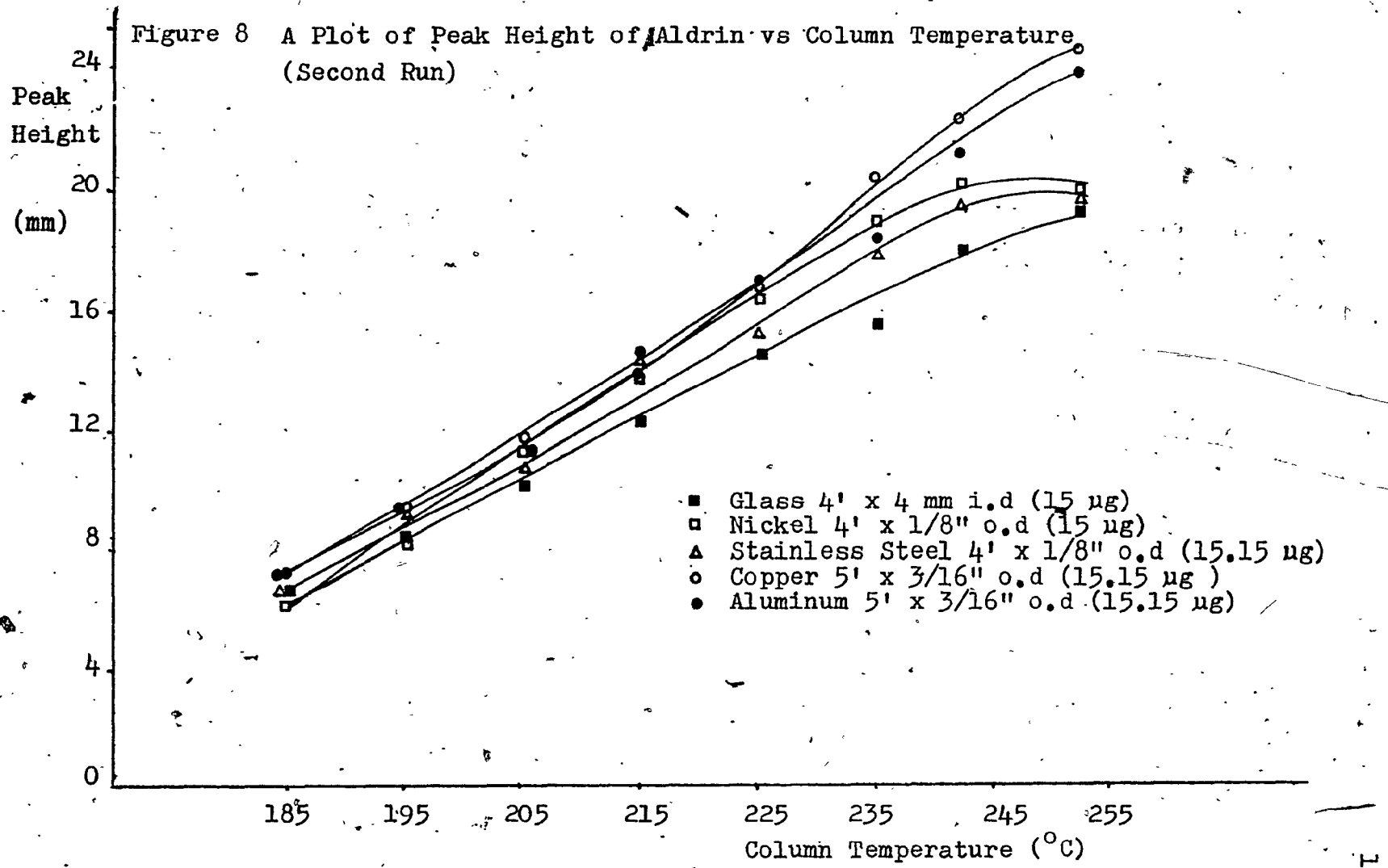


Figure 9 A Plot of Peak Area of Lindane vs Column Temperature (Second Run)

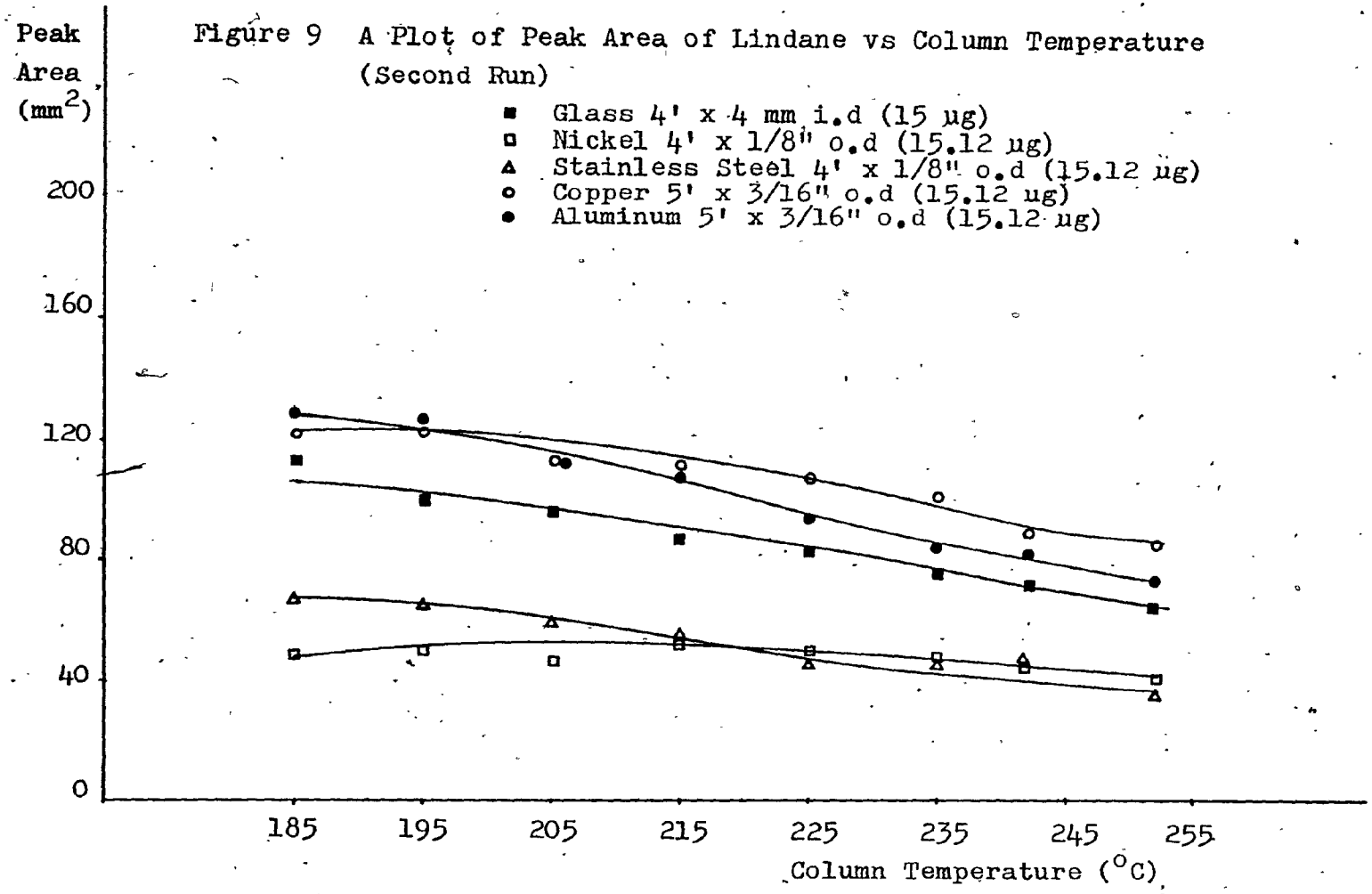


Figure 10 A Plot of Peak Area of Aldrin vs Column Temperature (Second Run)

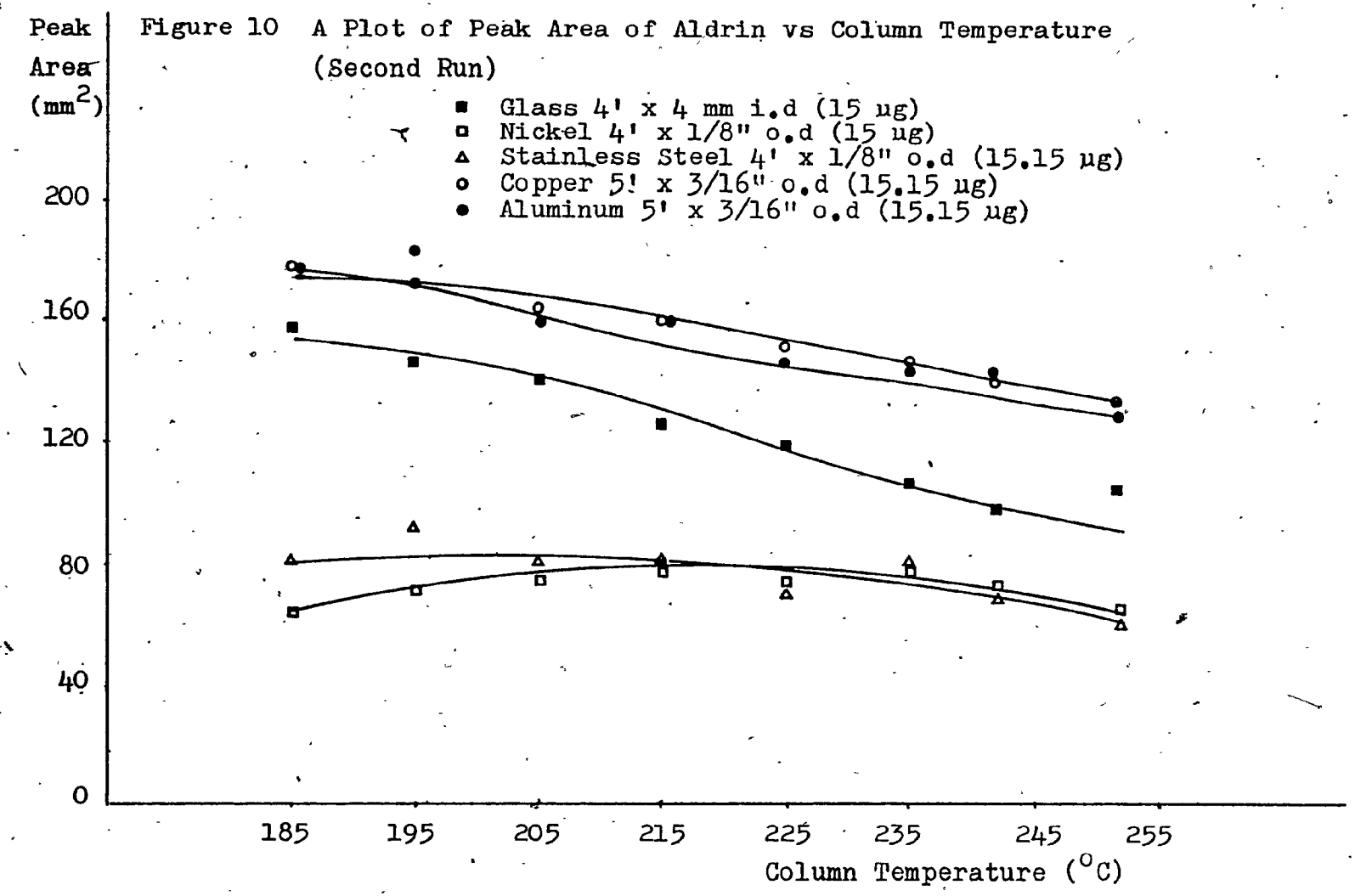


Figure 13 A Plot of Peak Height of Lindane vs Sample Size (Second Run)

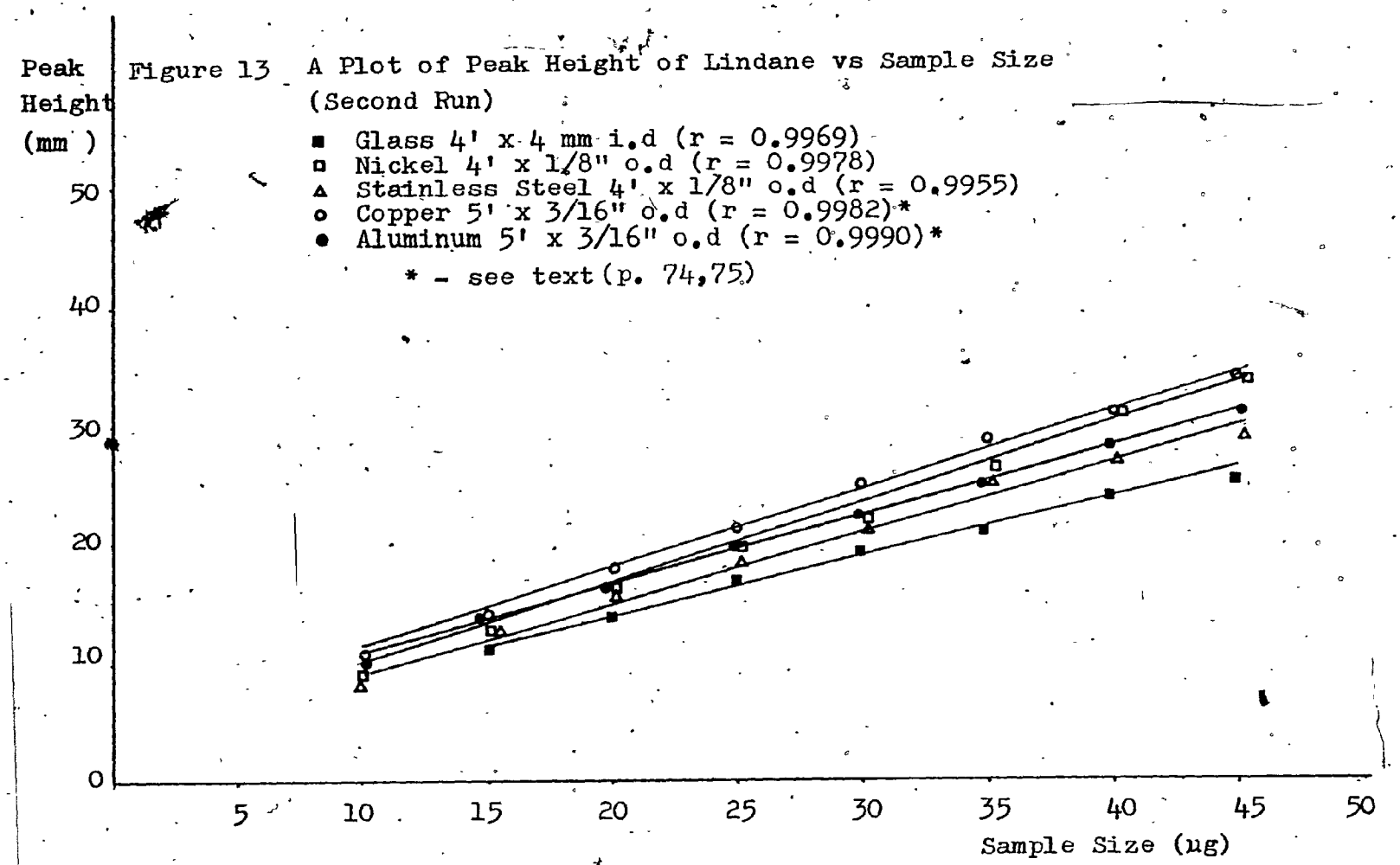


Figure 14 A Plot of Peak Height of Aldrin vs Sample Size (Second Run)

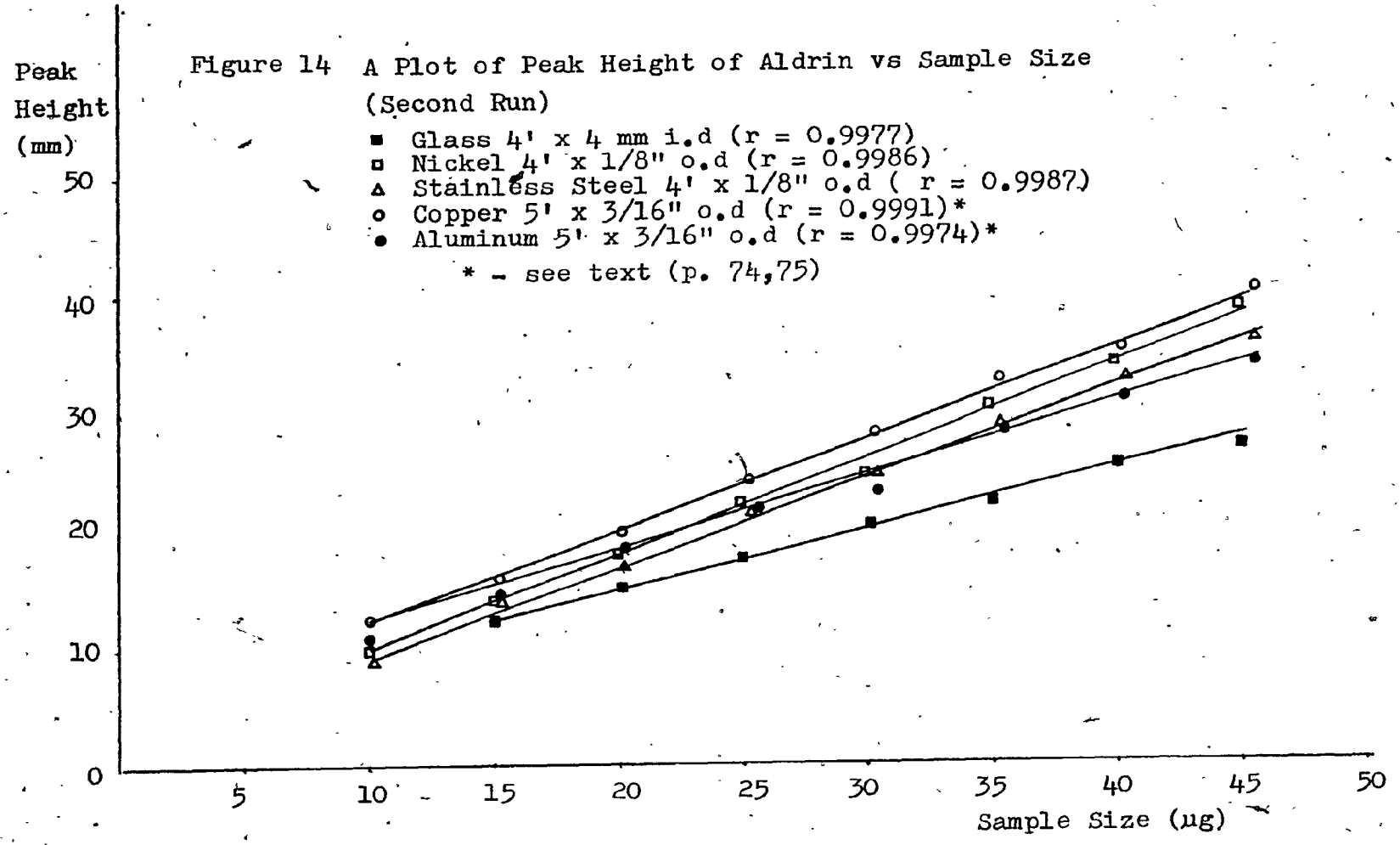


Figure 15 A Plot of Peak Area of Lindane vs. Sample Size (Second Run)

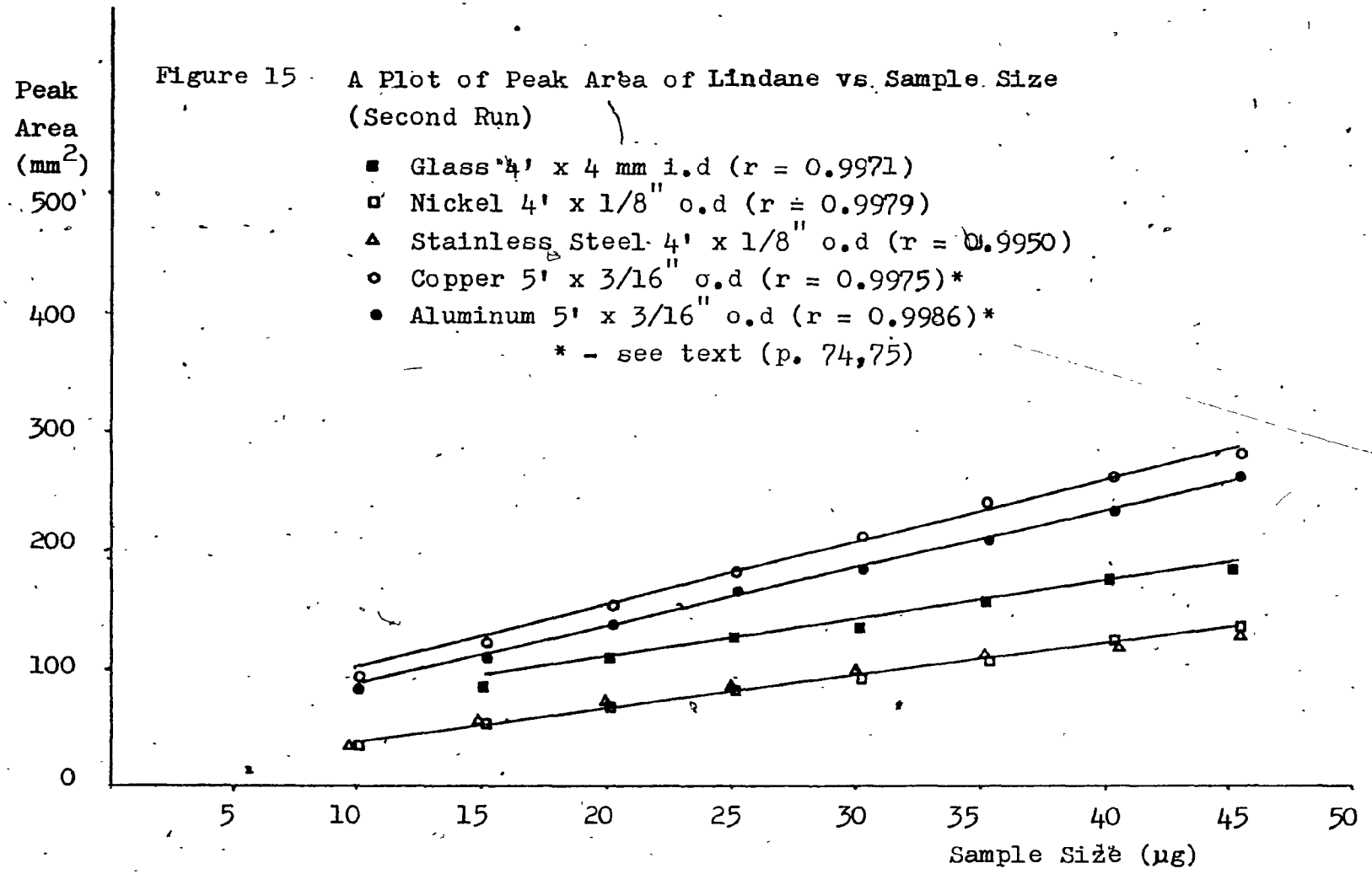
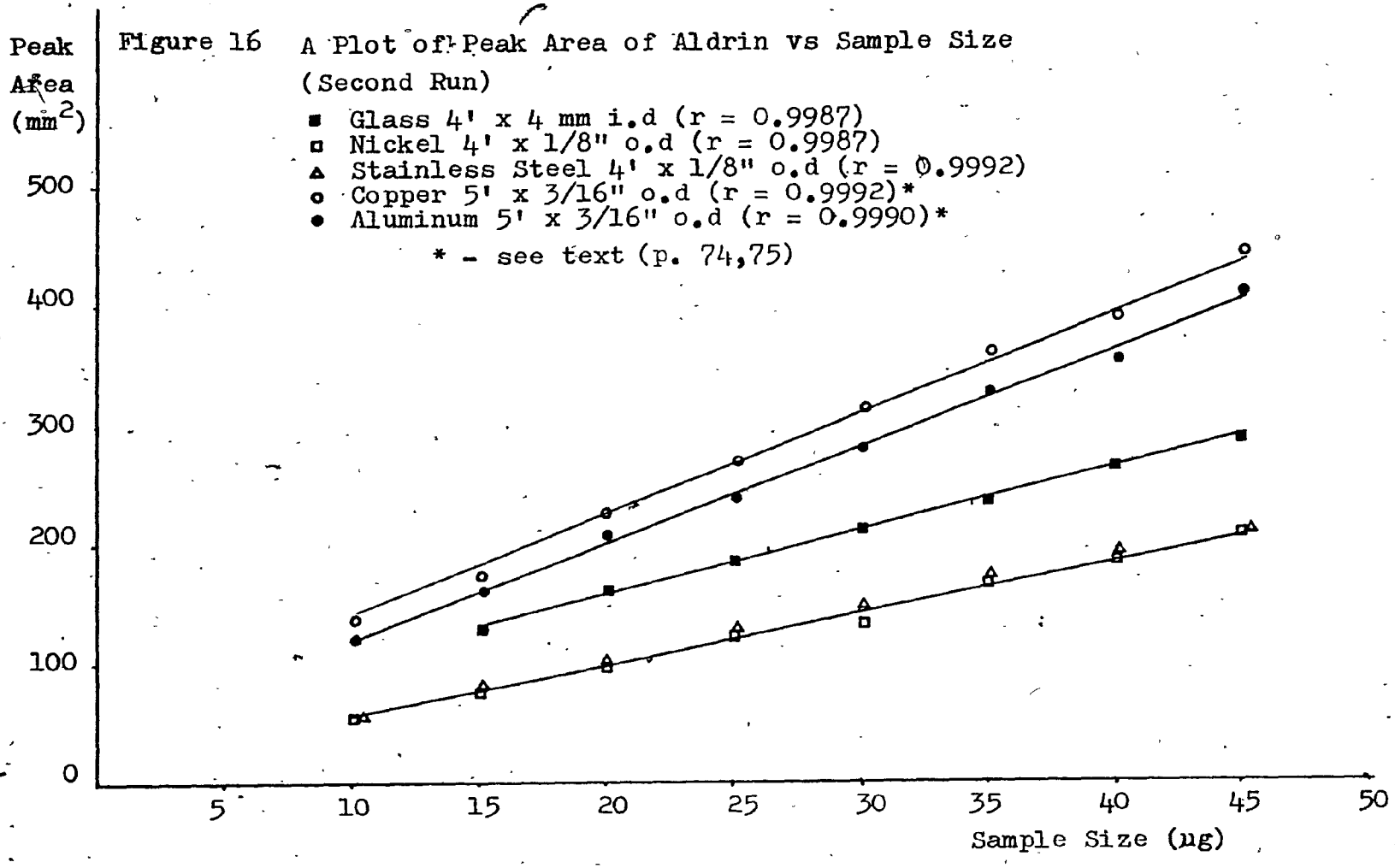


Figure 16 A Plot of Peak Area of Aldrin vs Sample Size (Second Run)



Appendix D-1

Figure 17 A Plot of Peak Height of Lindane vs Column Temperature (Second Run)

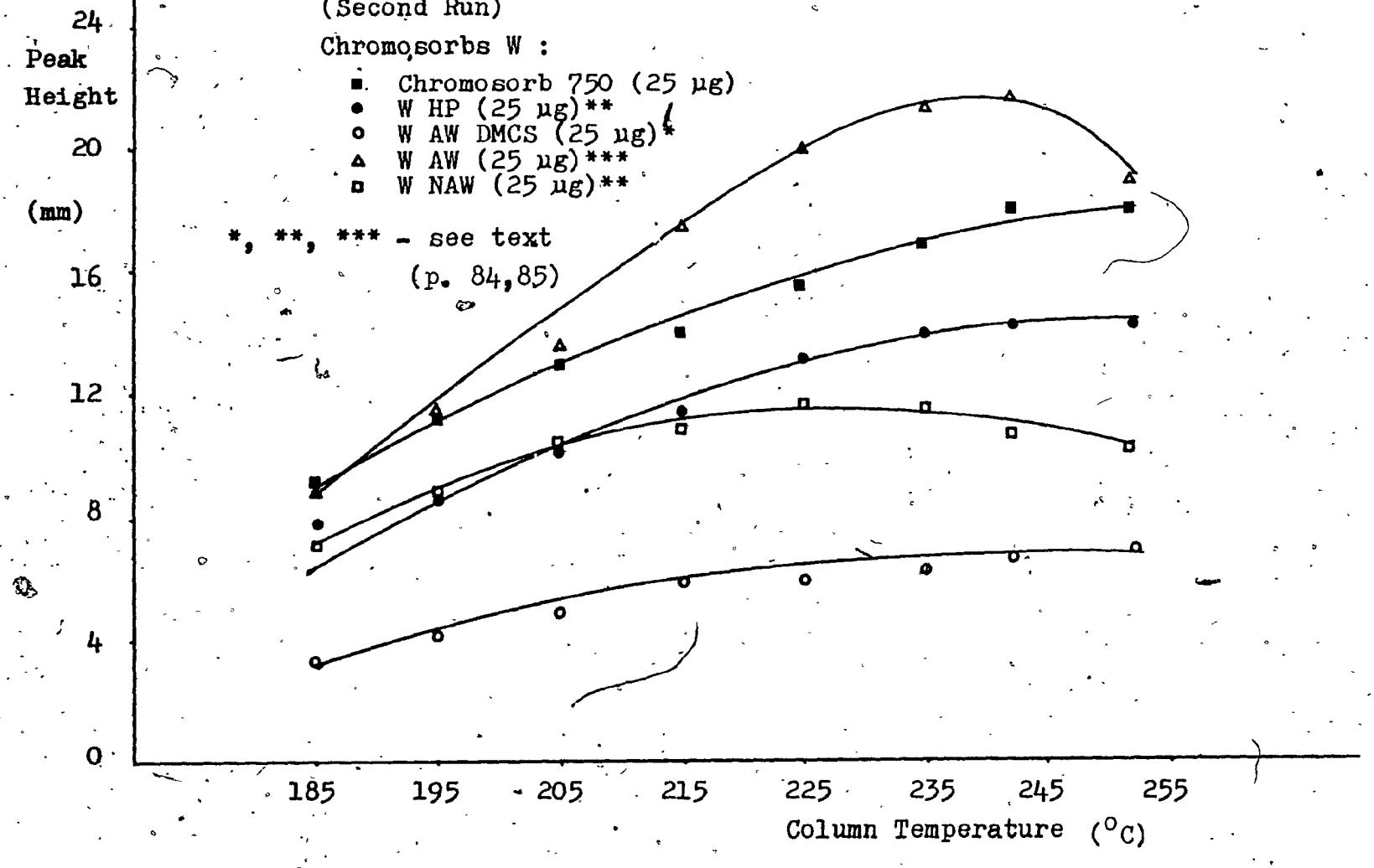


Figure 18 A Plot of Peak Height of Lindane vs Column Temperature (Second Run)

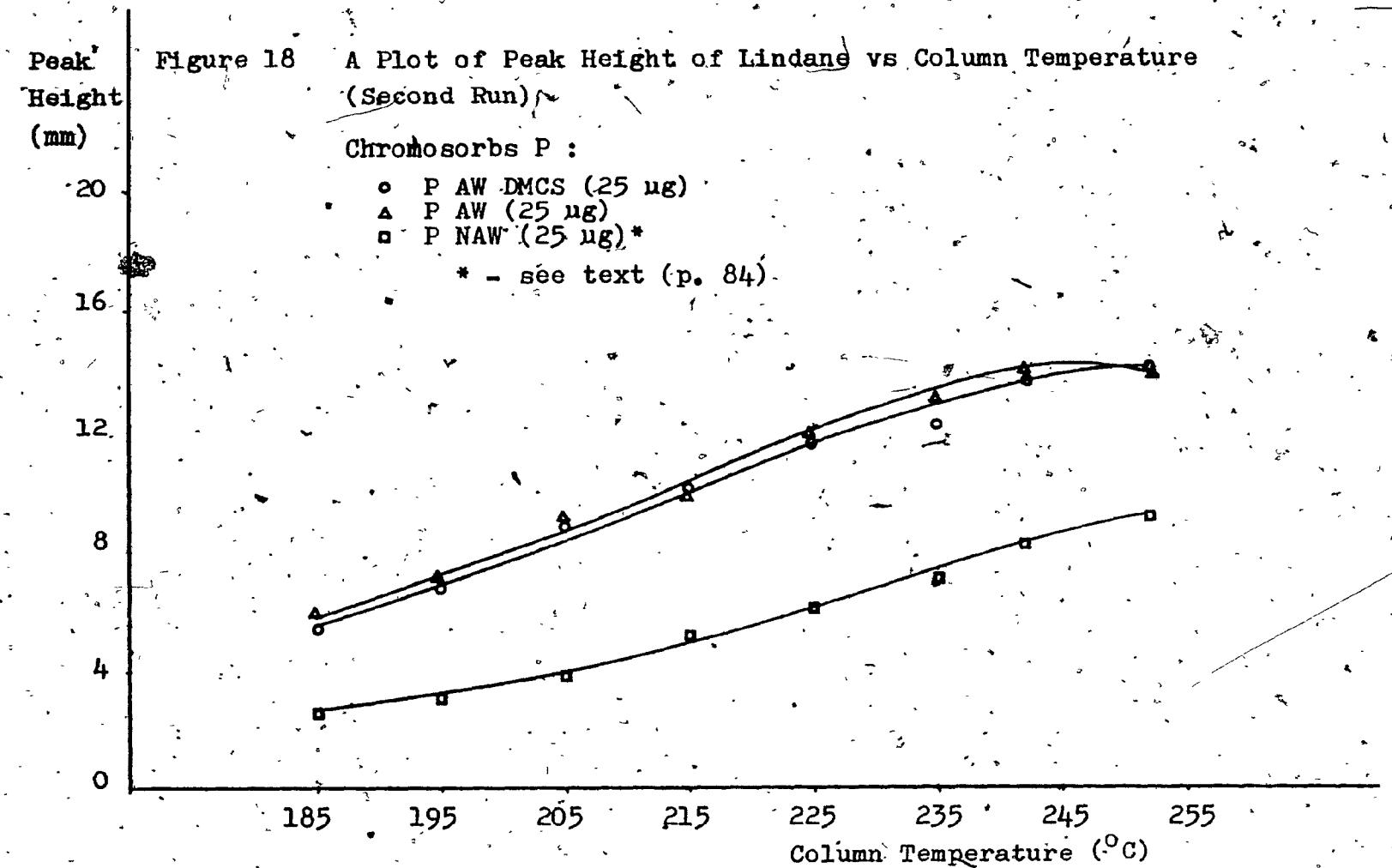


Figure 19 A Plot of Peak Height of Lindane vs Column Temperature (Second Run)

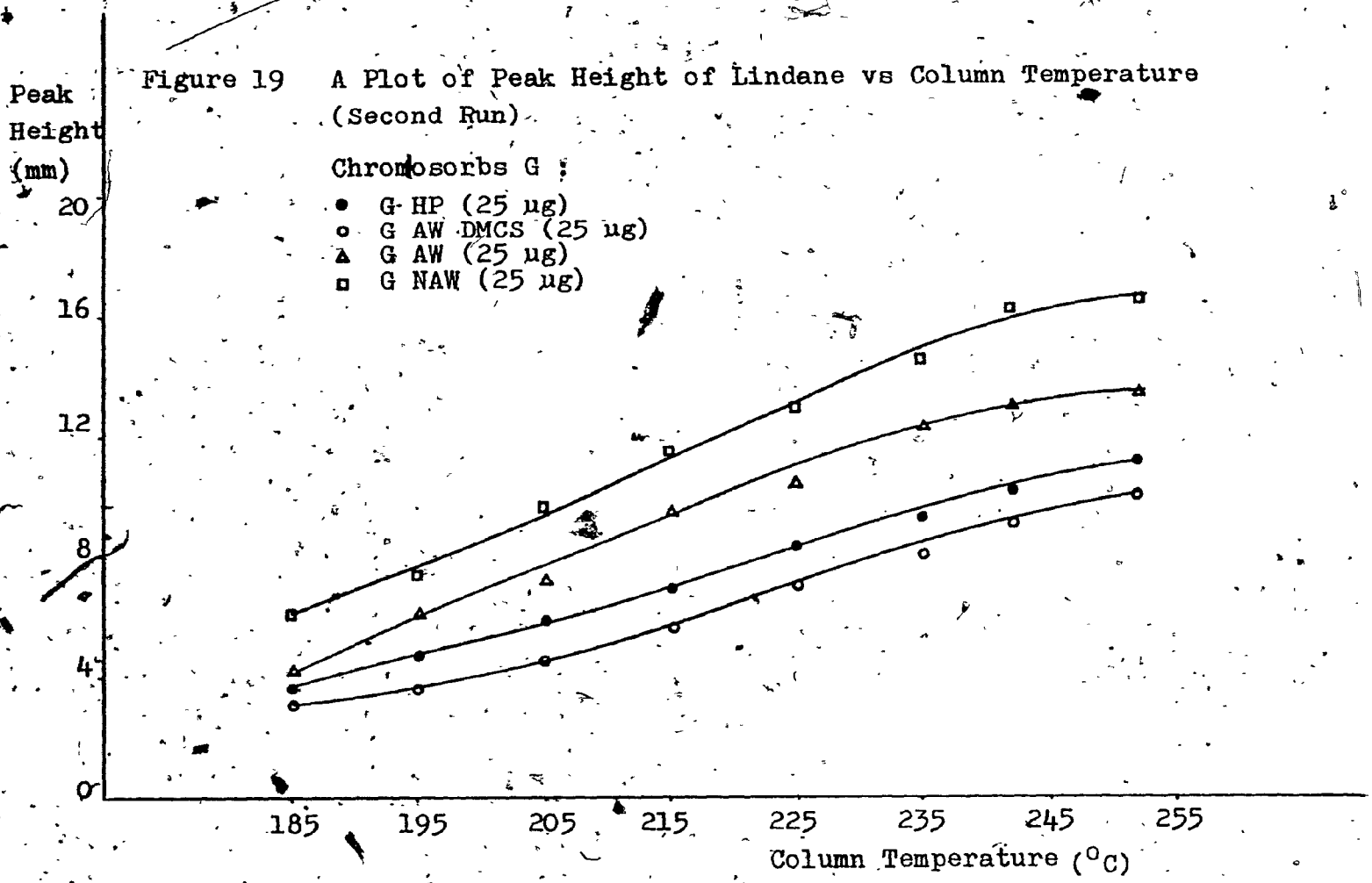


Figure 20 A Plot of Peak Height of Aldrin vs Column Temperature (Second Run)

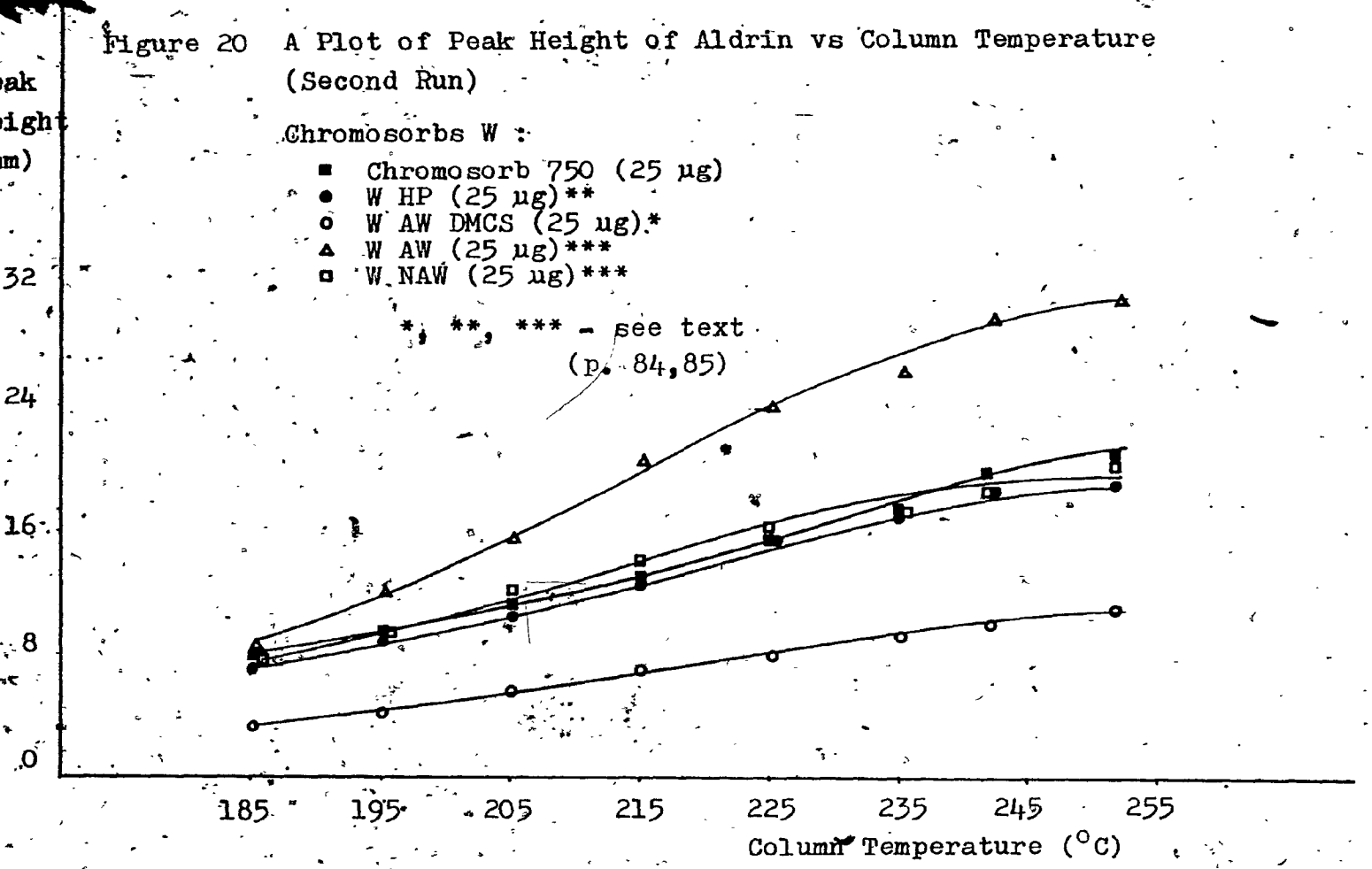


Figure 21 A Plot of Peak Height of Aldrin vs Column Temperature (Second Run)

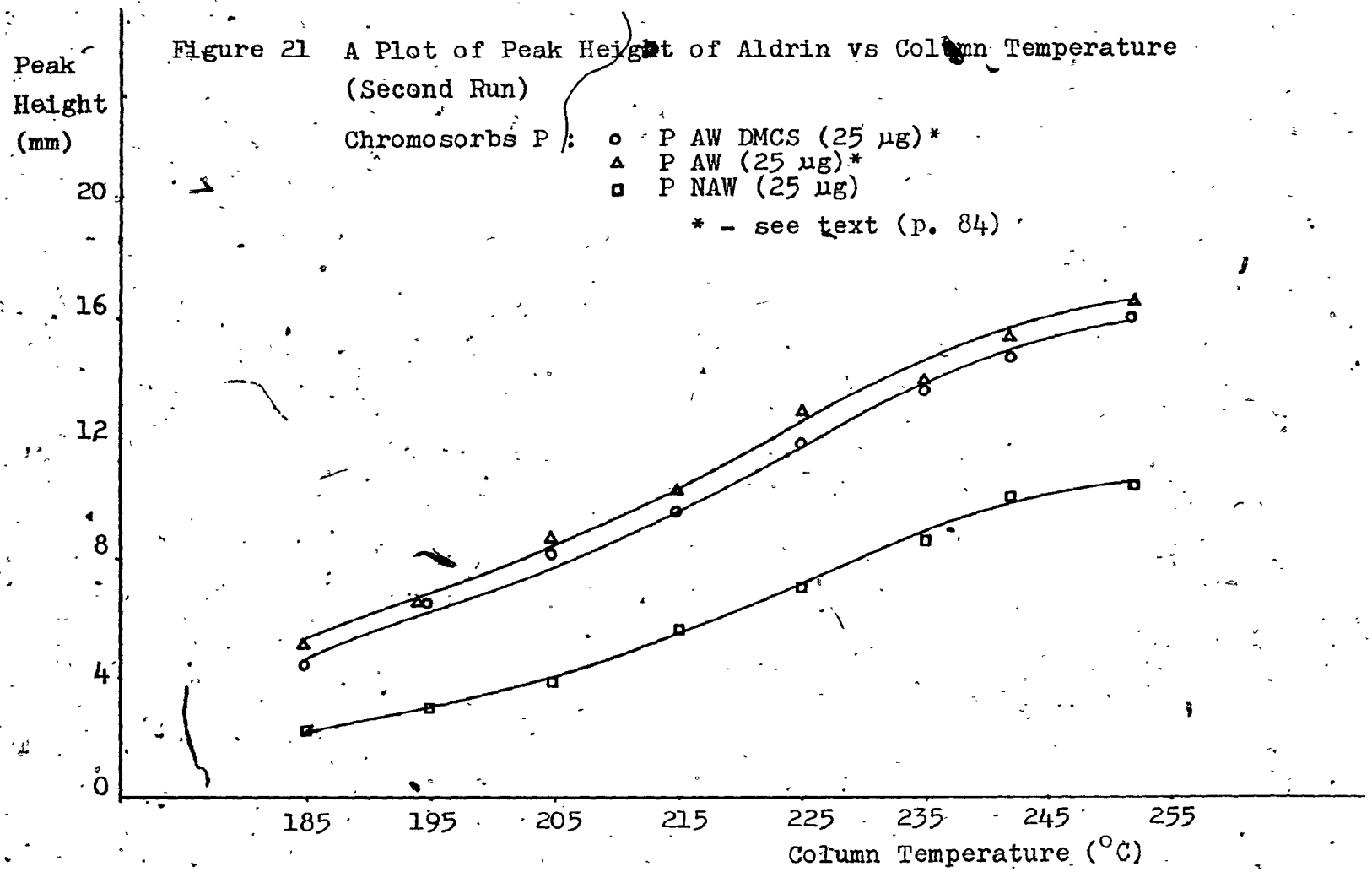


Figure 22 A Plot of Peak Height of Aldrin vs Column Temperature (Second Run)

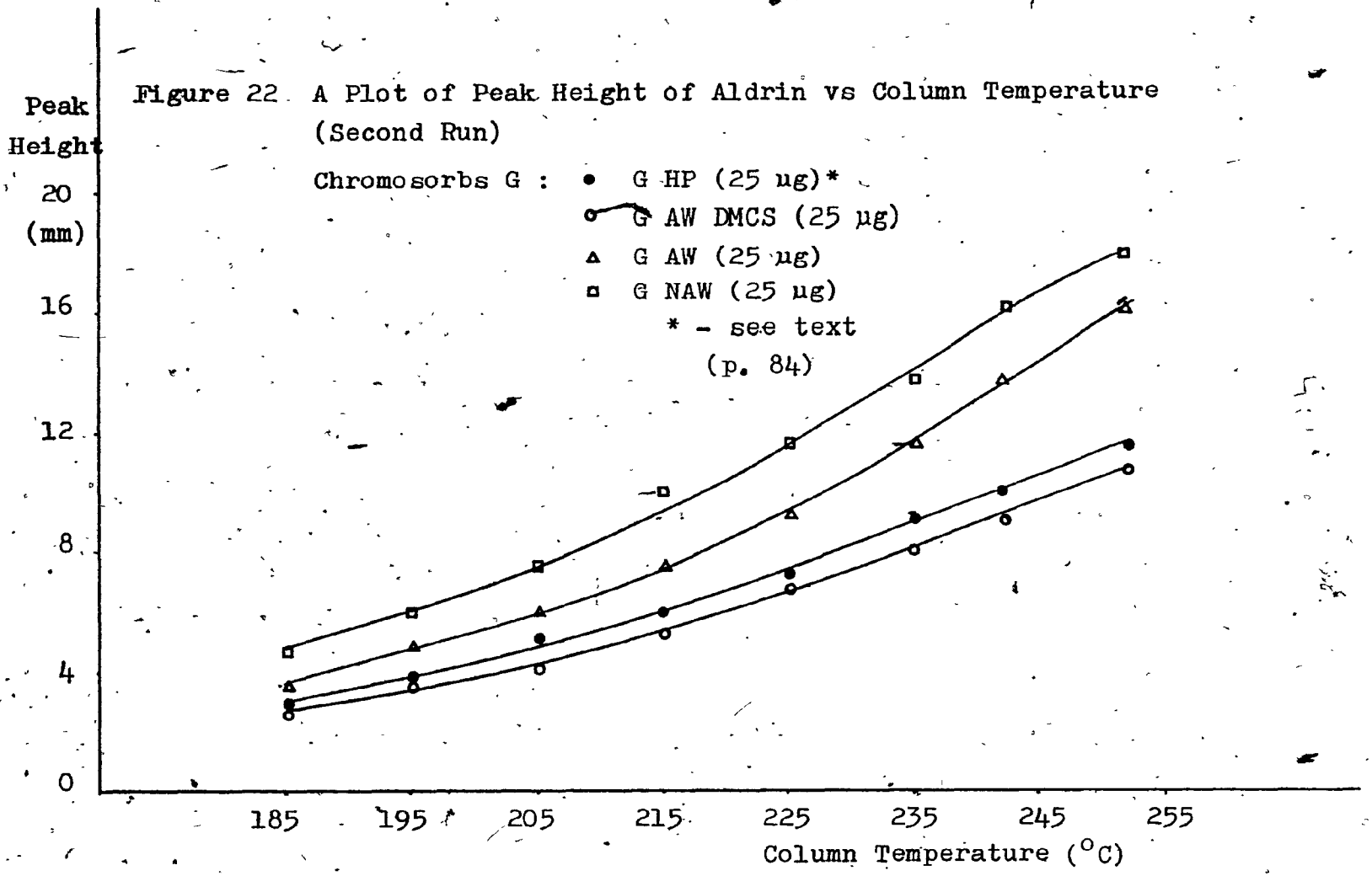


Figure 23 A Plot of Peak Area of Lindane vs Column Temperature (Second Run)

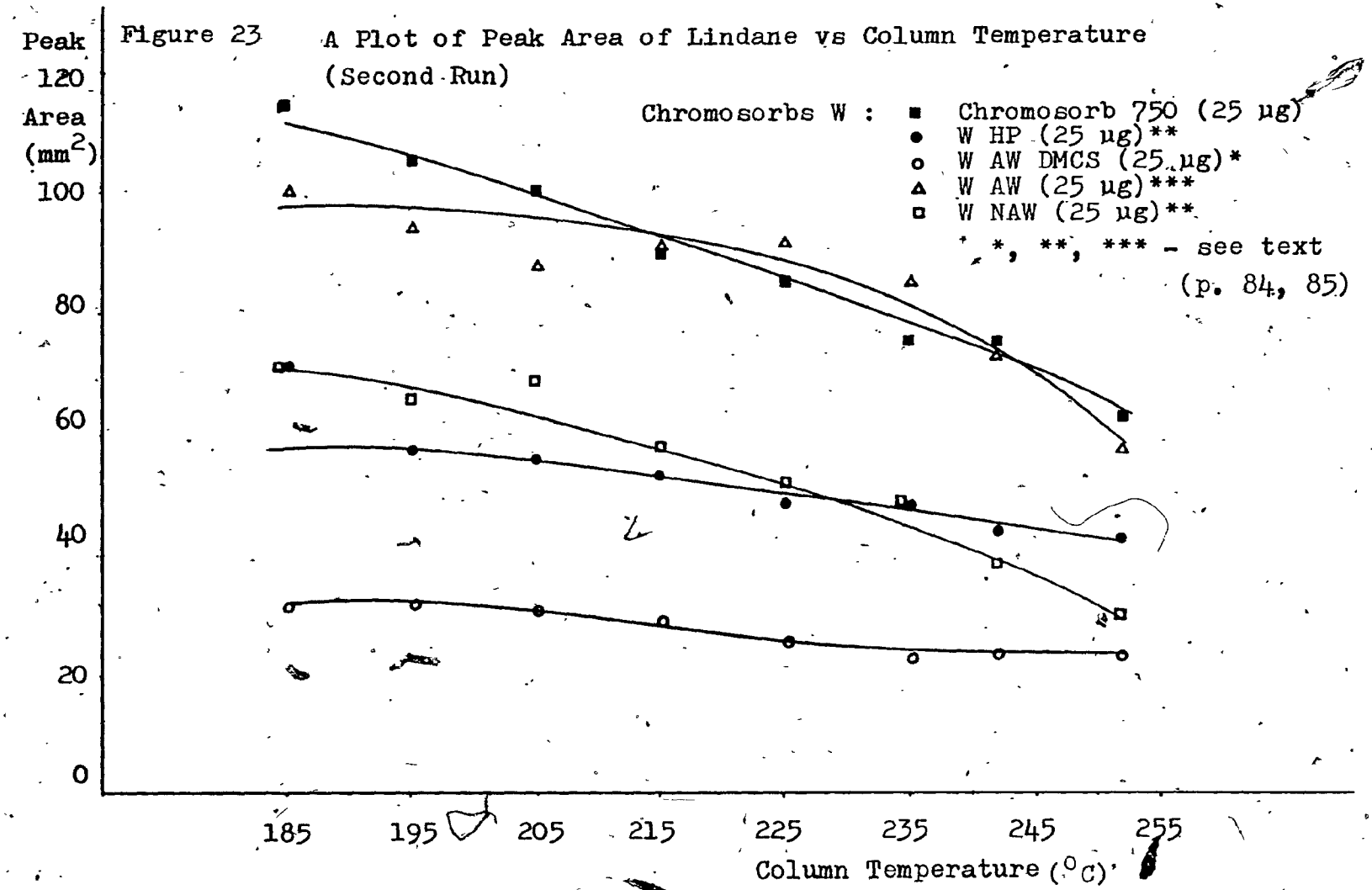


Figure 24 A Plot of Peak Area of Lindane vs Column Temperature (Second Run)

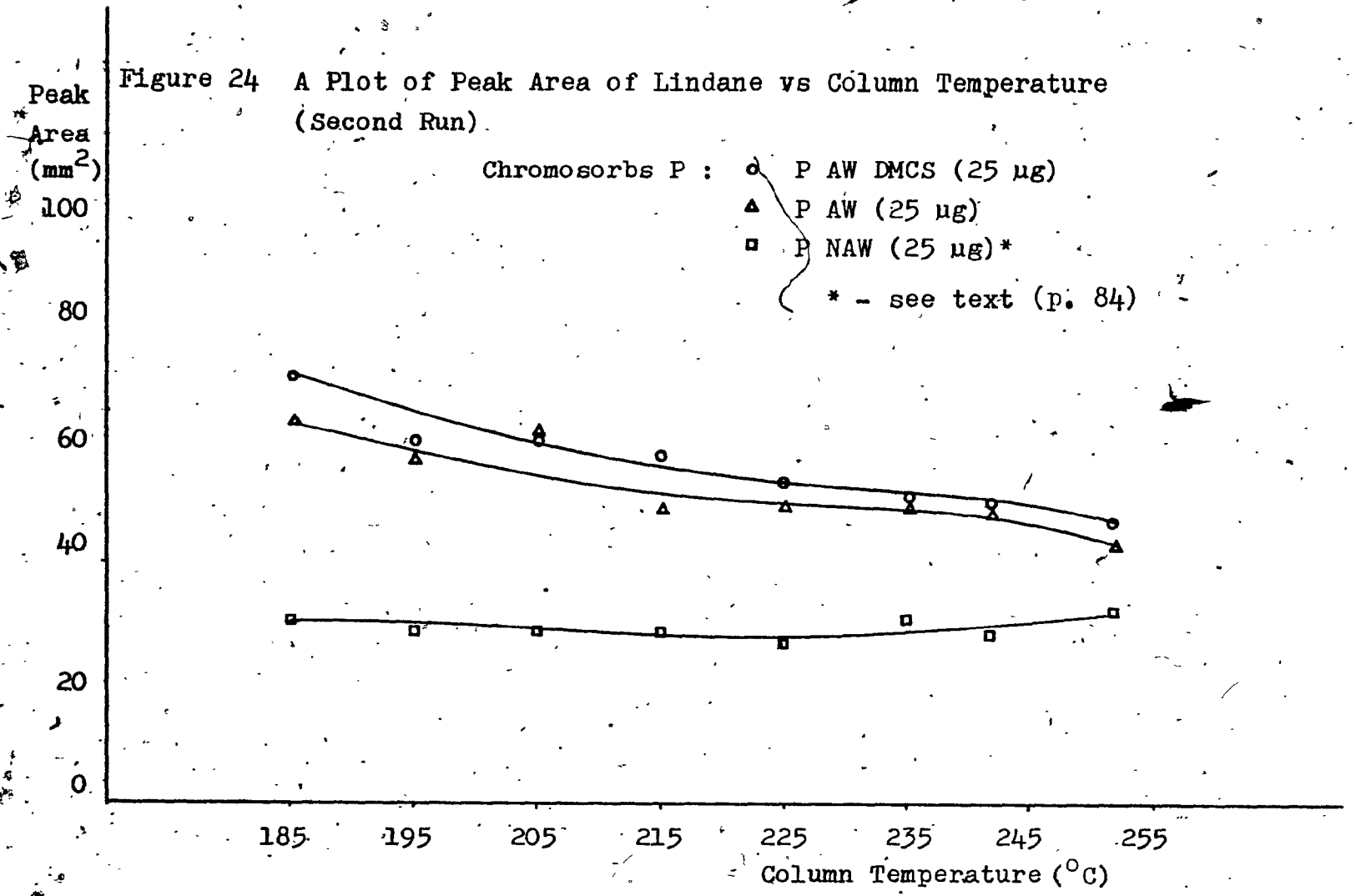


Figure 25 A Plot of Peak Area of Lindane vs Column Temperature (Second Run)

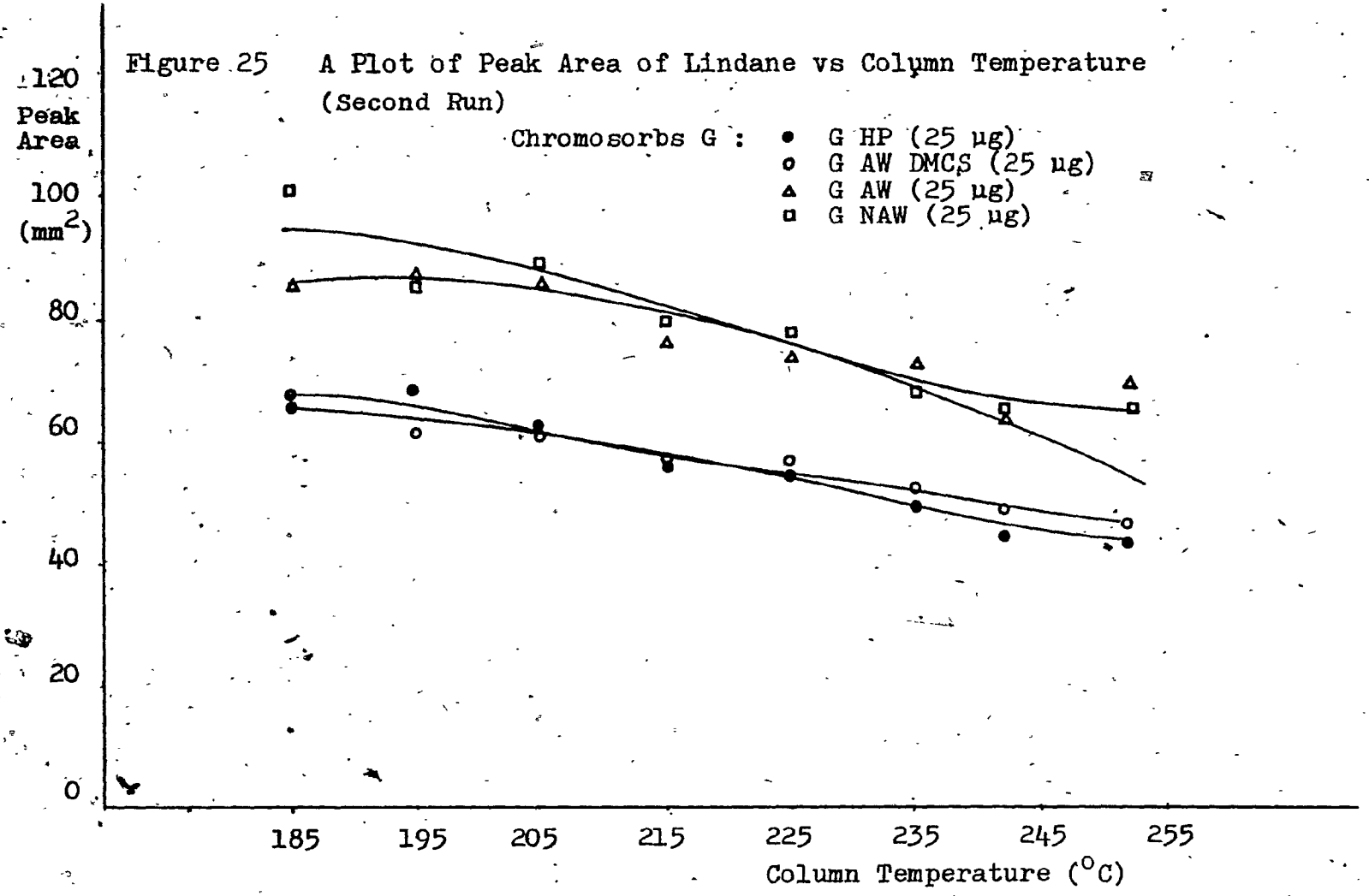


Figure 26 A Plot of Peak Area of Aldrin vs Column Temperature (Second Run)

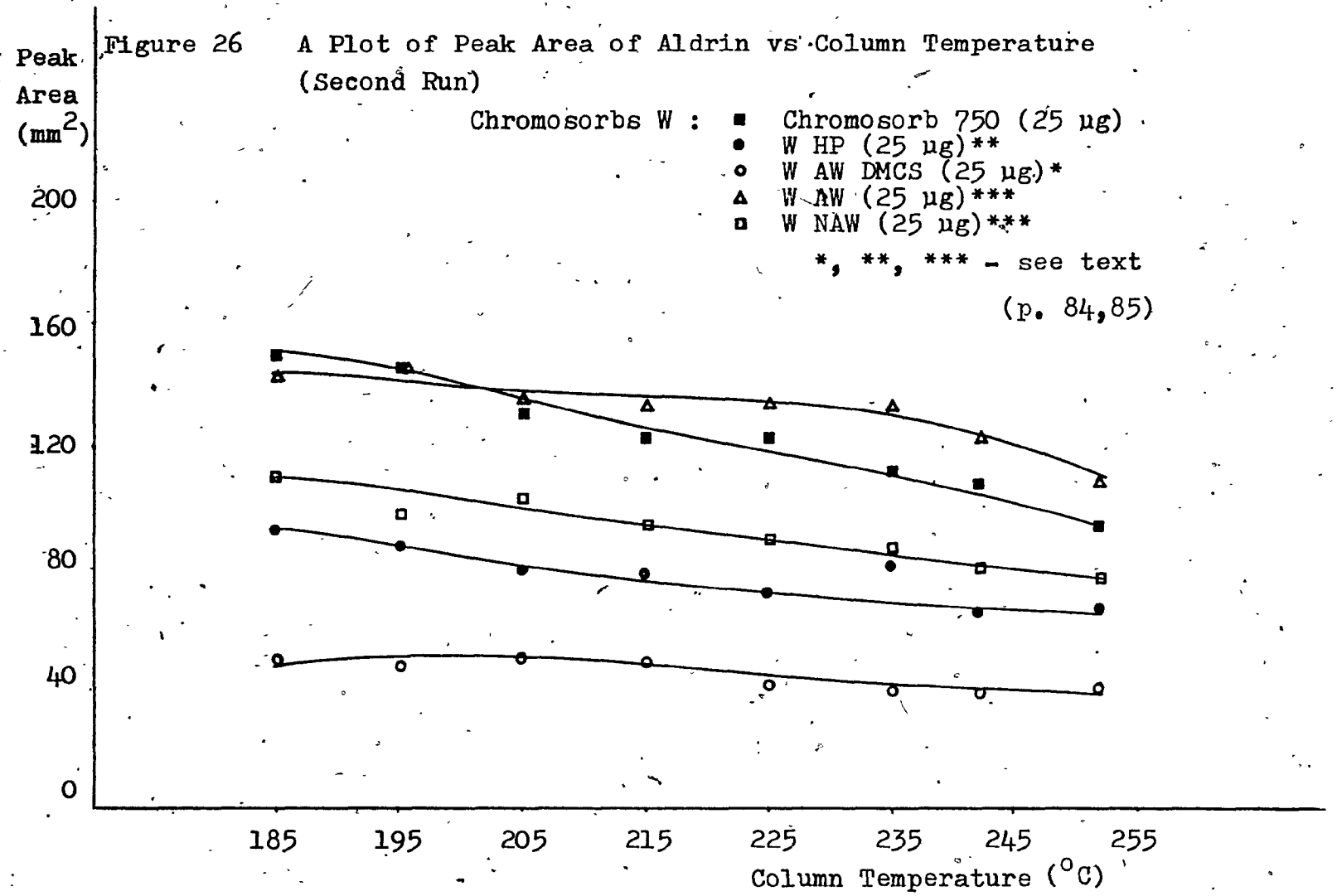


Figure 27 A Plot of Peak Area of Aldrin vs Column Temperature (Second Run)

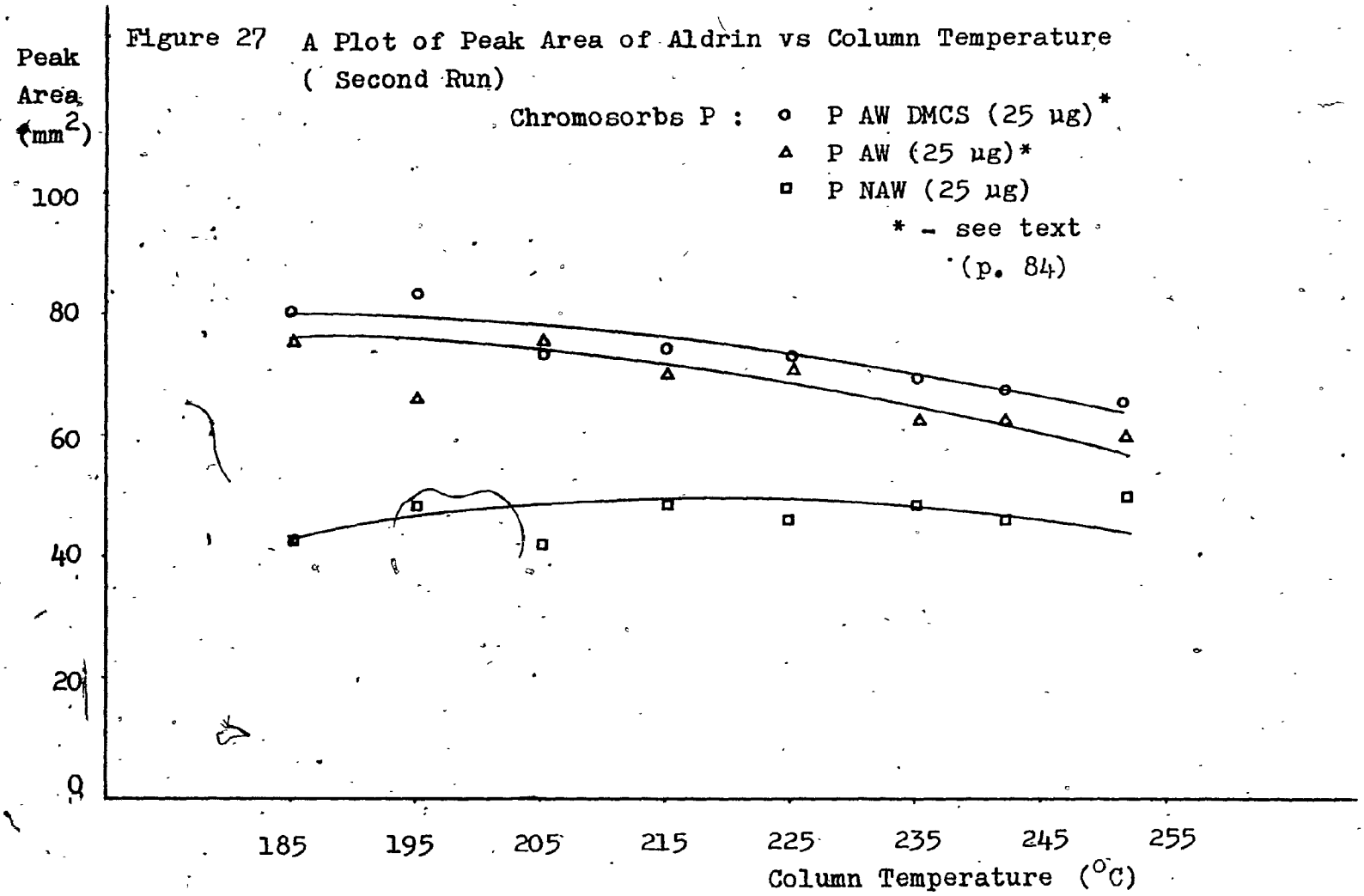
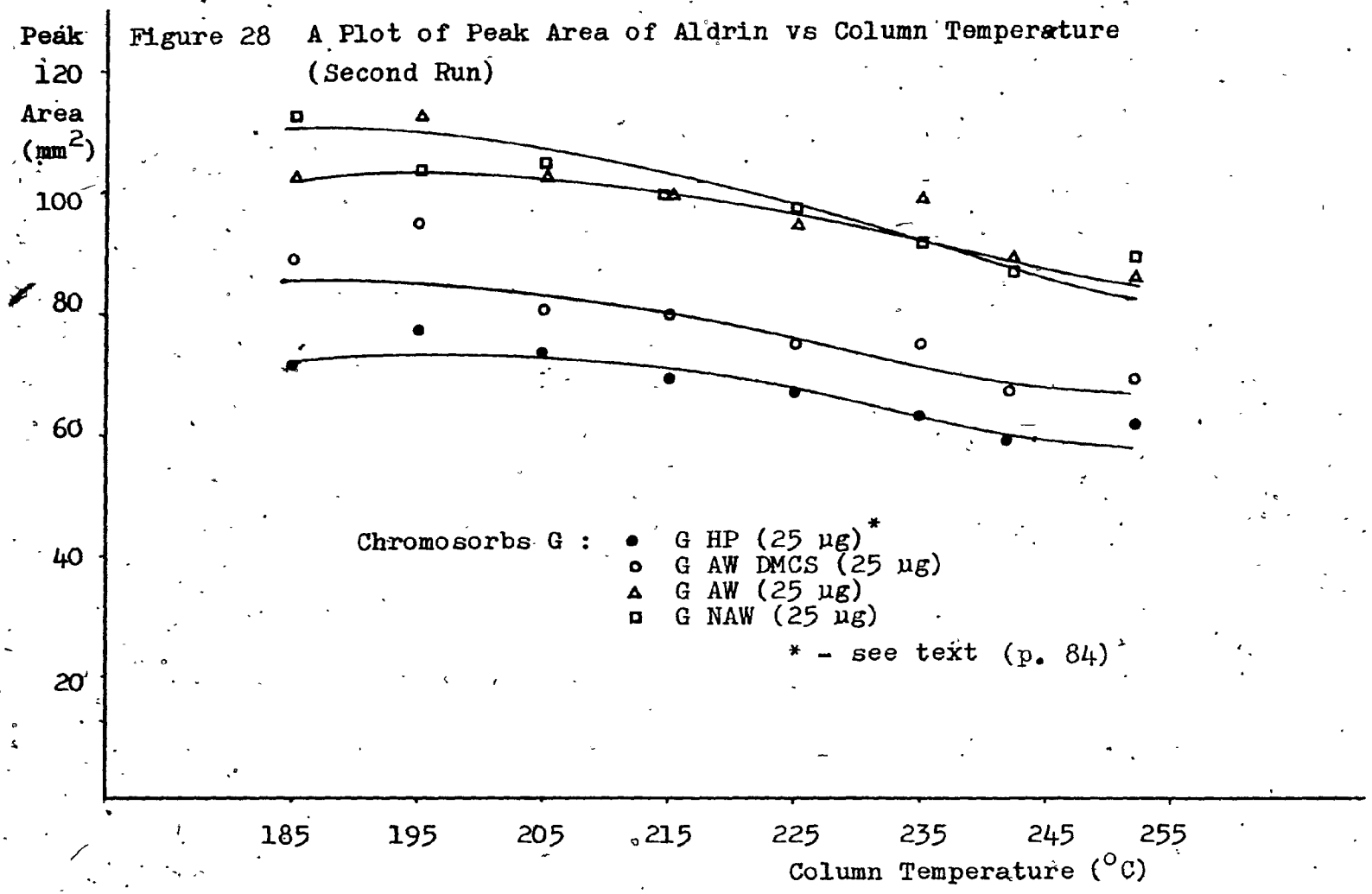


Figure 28 A Plot of Peak Area of Aldrin vs Column Temperature (Second Run)



Appendix D-2

Figure 29 A Plot of Peak Height of Lindane vs Sample Size (Second Run)

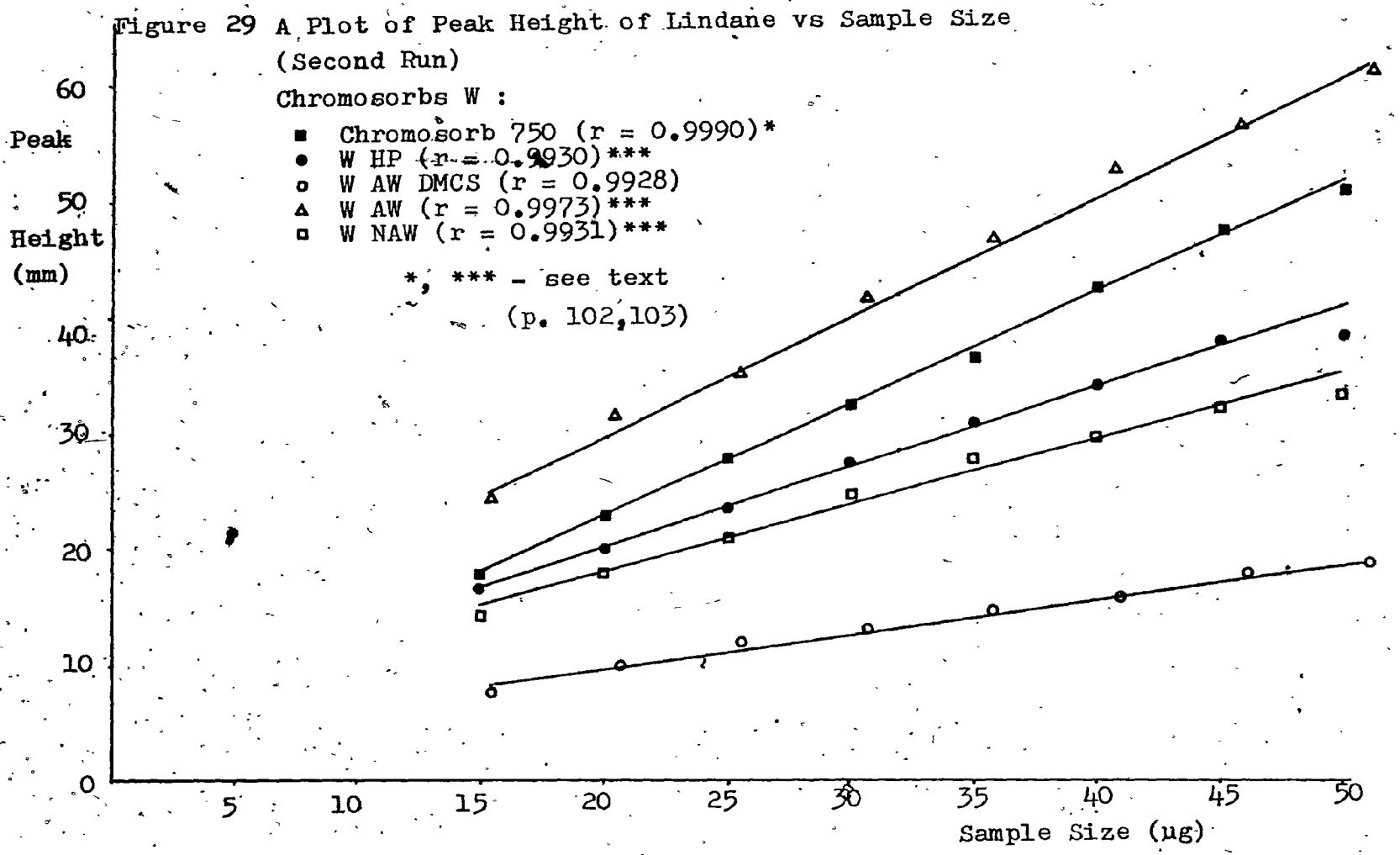


Figure 30 A Plot of Peak Height of Lindane vs Sample Size (Second Run)

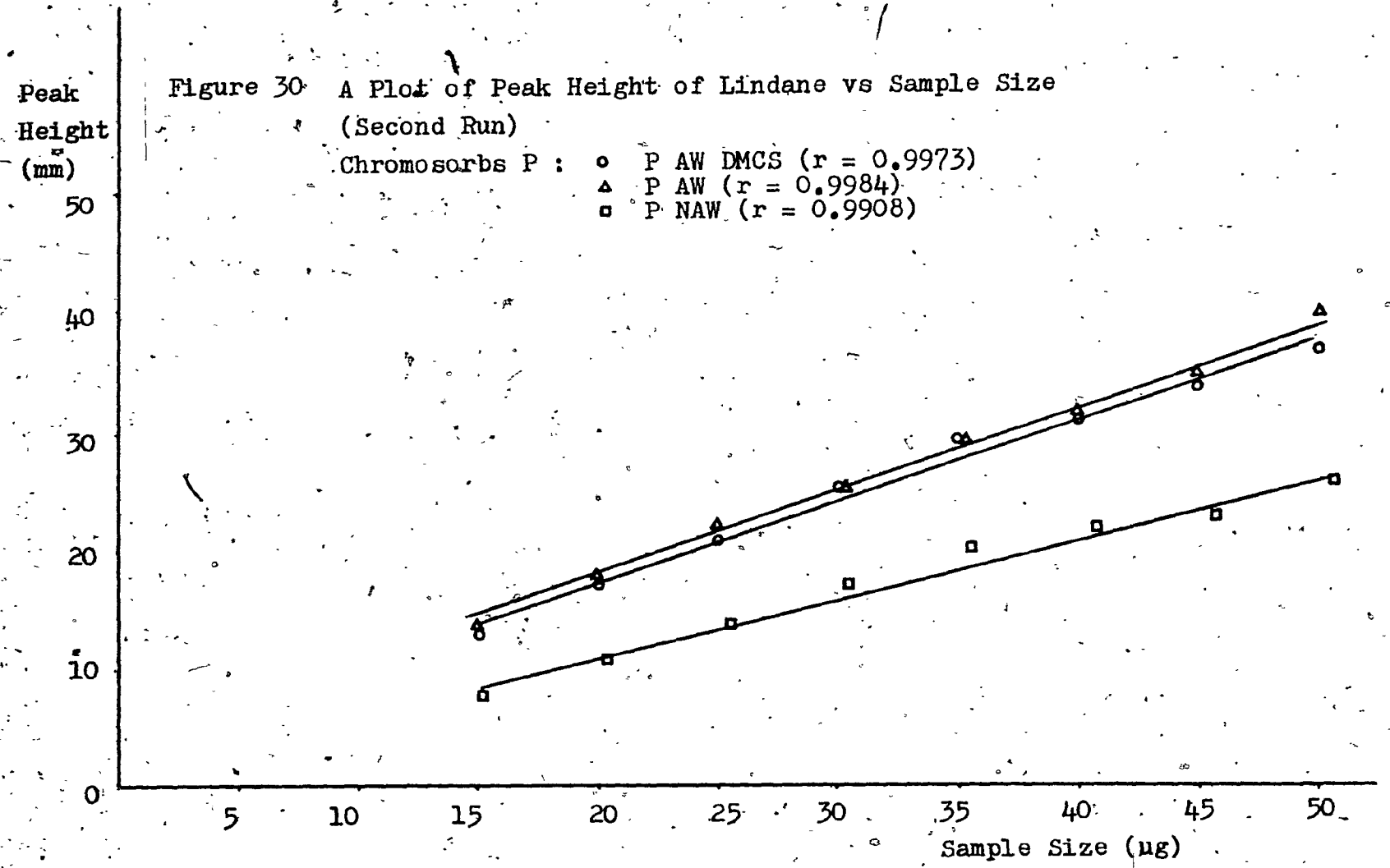


Figure 31 A Plot of Peak Height of Lindane vs Sample Size (Second Run)

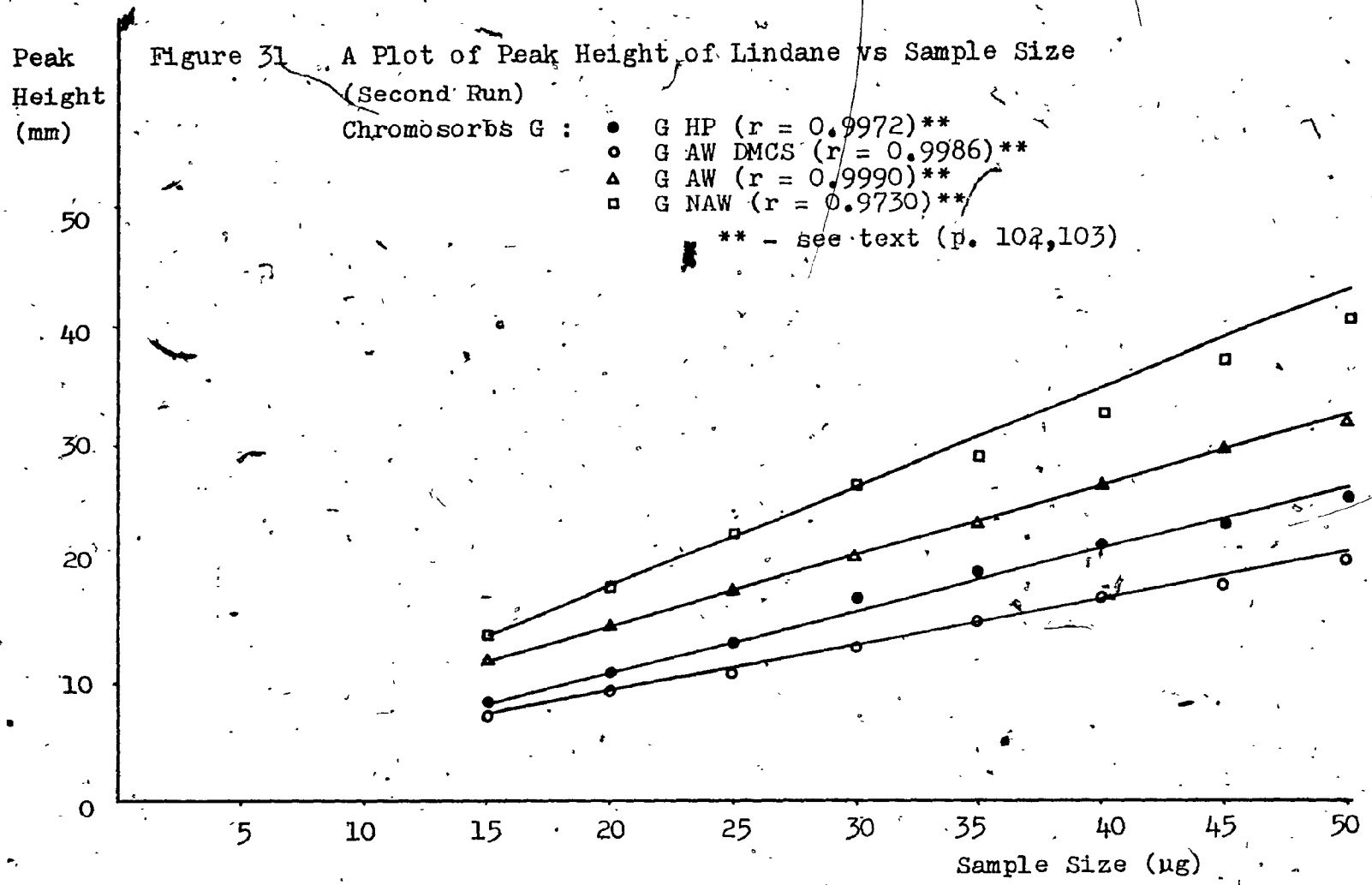


Figure 32 A Plot of Peak Height of Aldrin vs Sample Size (Second Run)

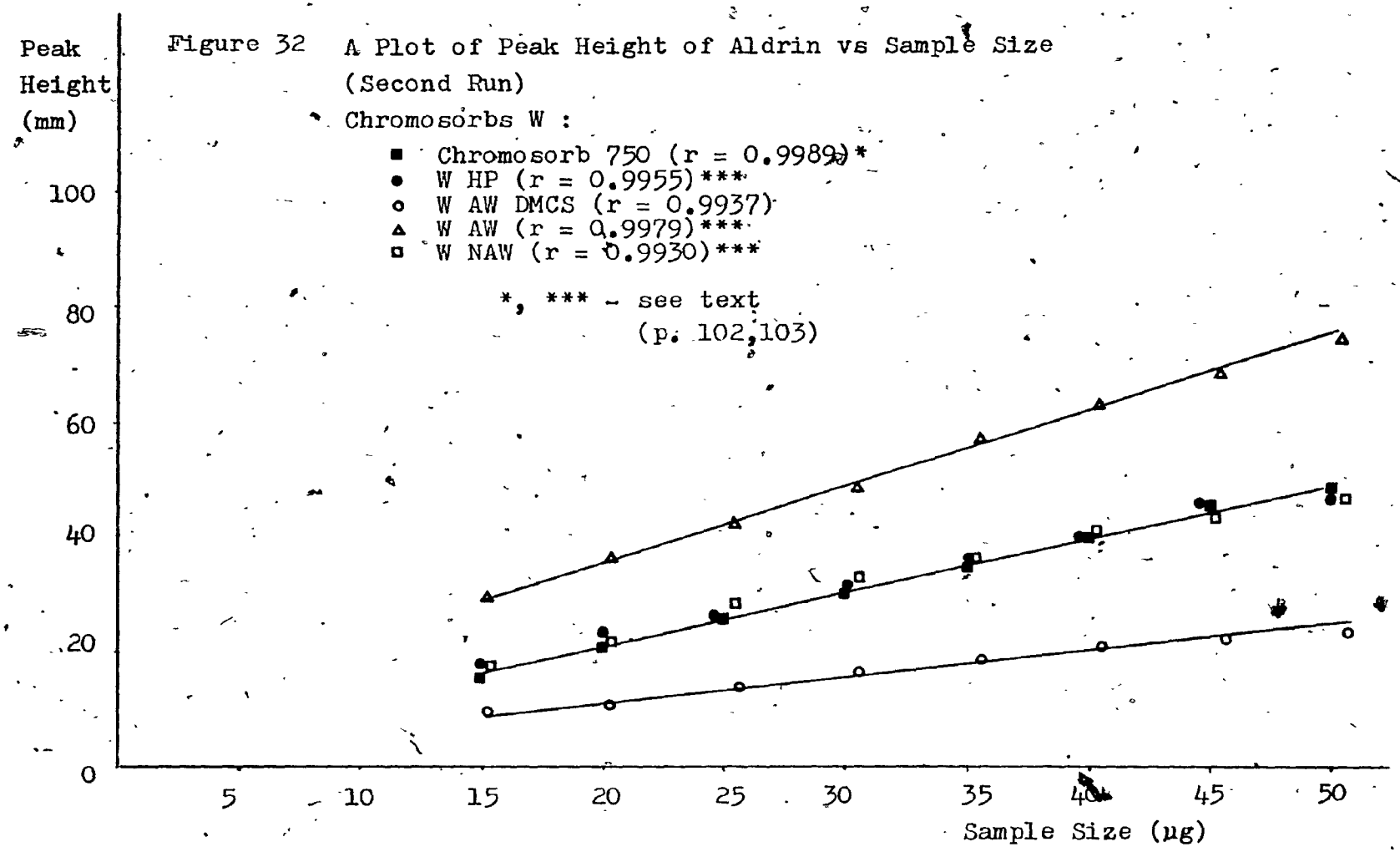


Figure 33 A Plot of Peak Height of Aldrin vs Sample Size (Second Run)

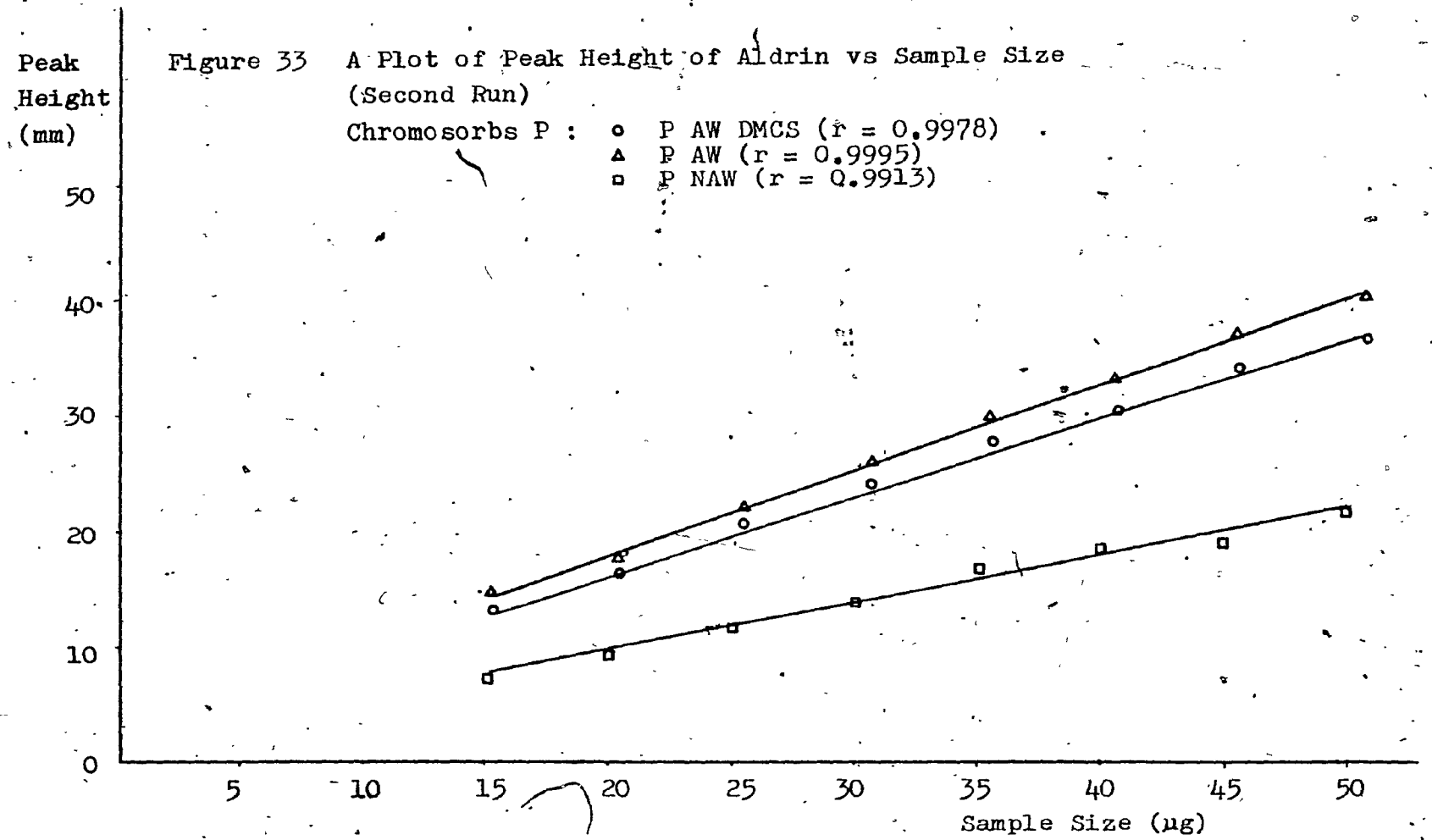


Figure 34 A Plot of Peak Height of Aldrin vs Sample Size (Second Run)

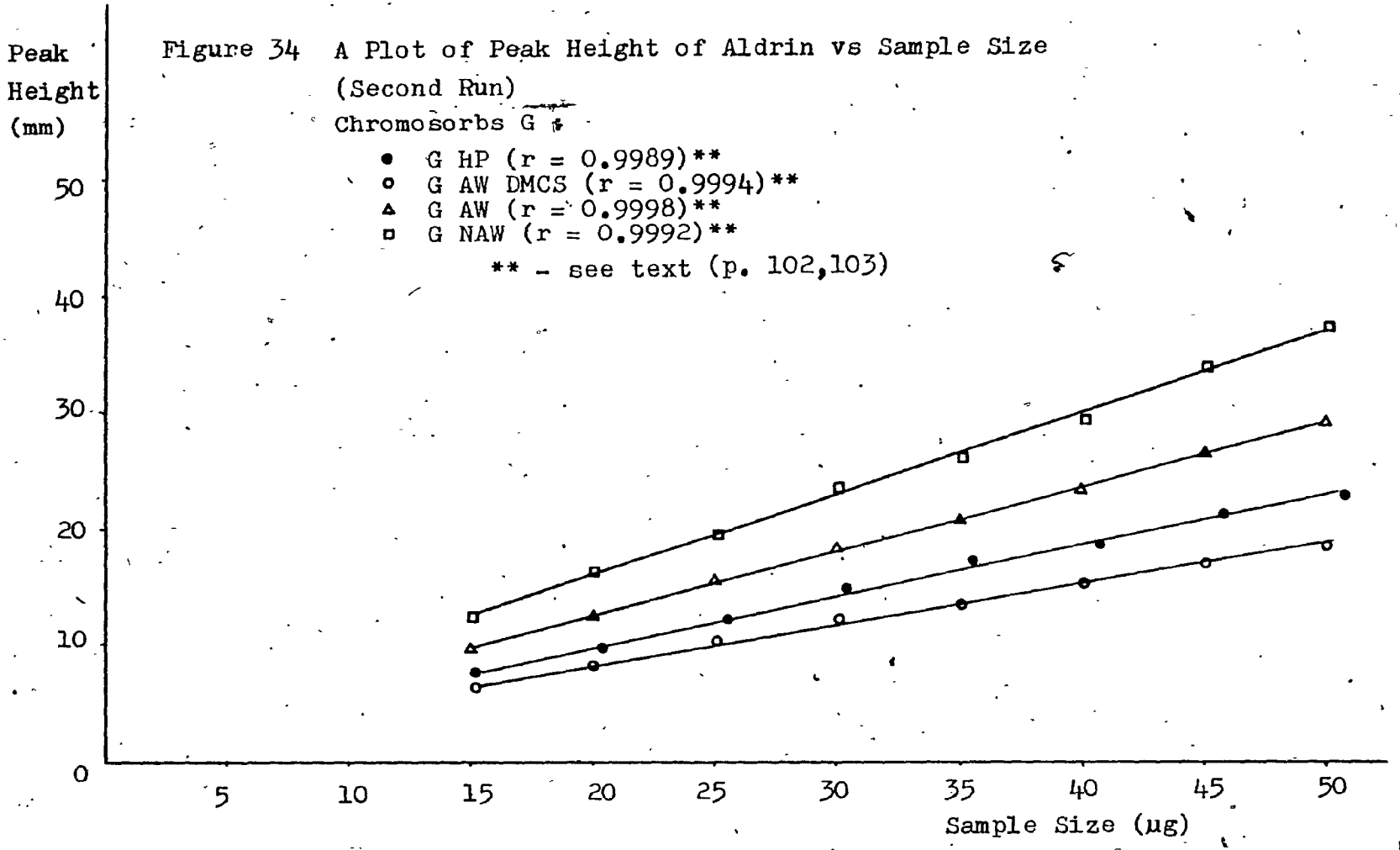


Figure 35 A Plot of Peak Area of Lindane vs Sample Size (Second Run)

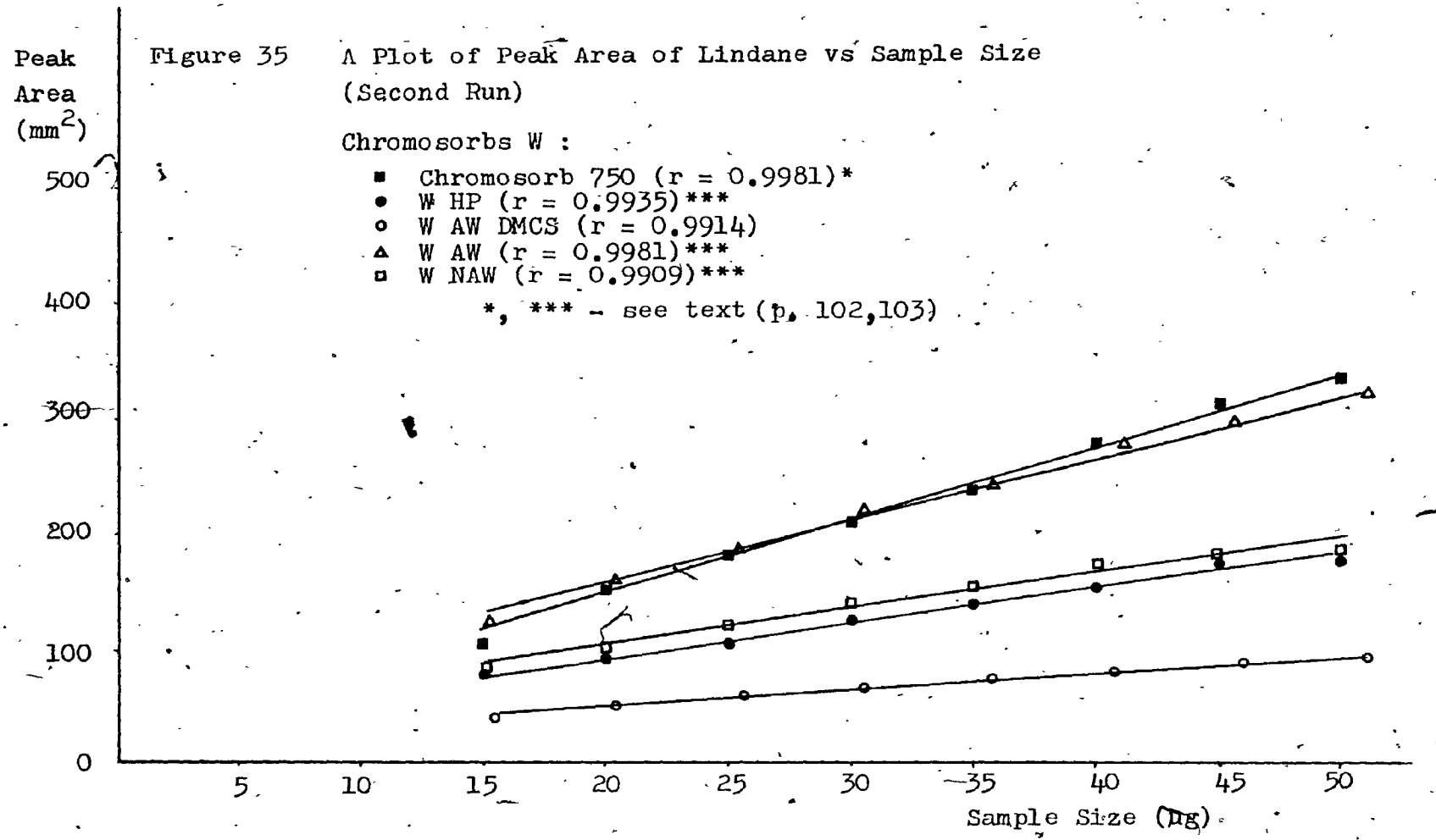


Figure 36 A Plot of Peak Area of Lindane vs Sample Size (Second Run)

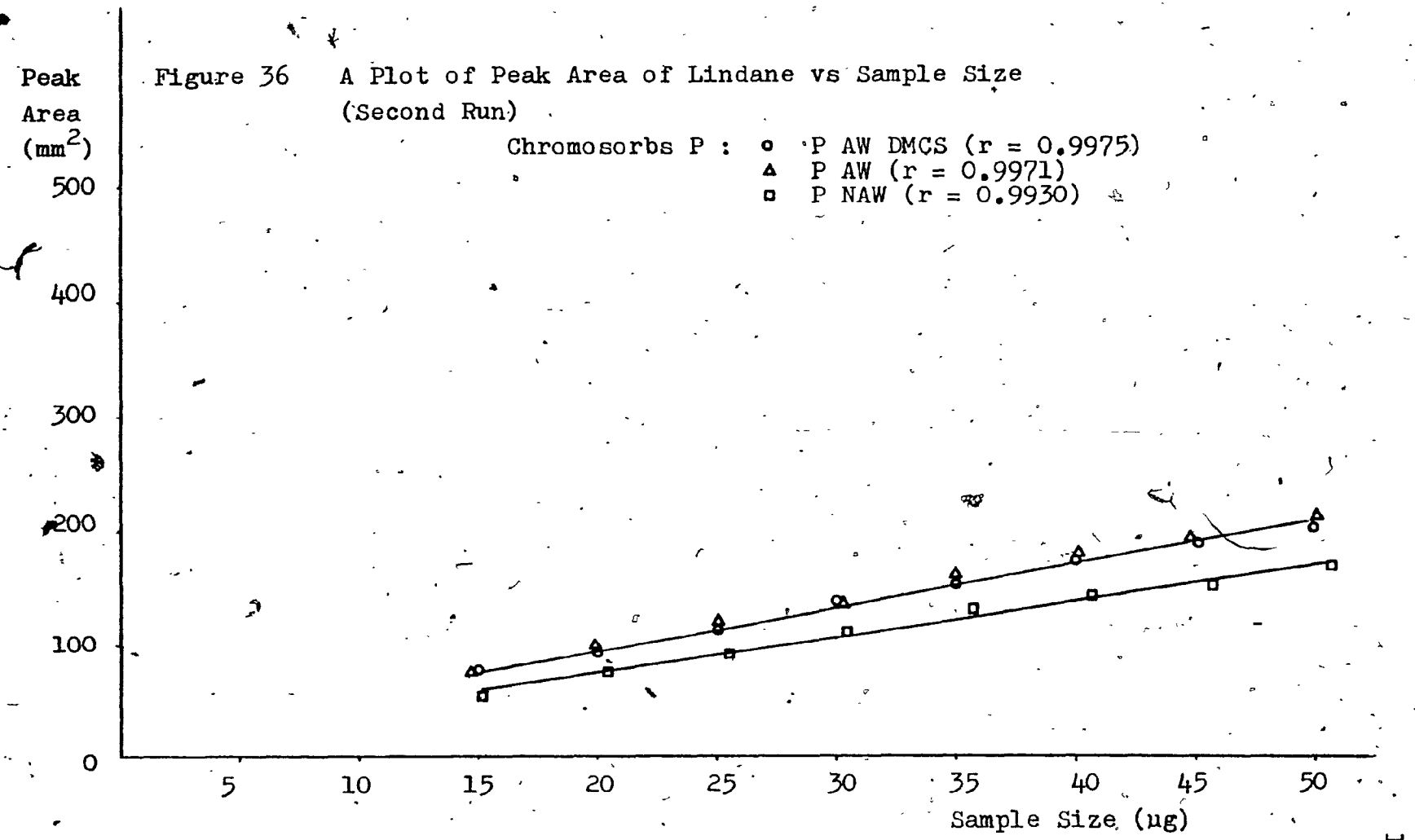


Figure 37 A Plot of Peak Area of Lindane vs Sample Size (Second Run)

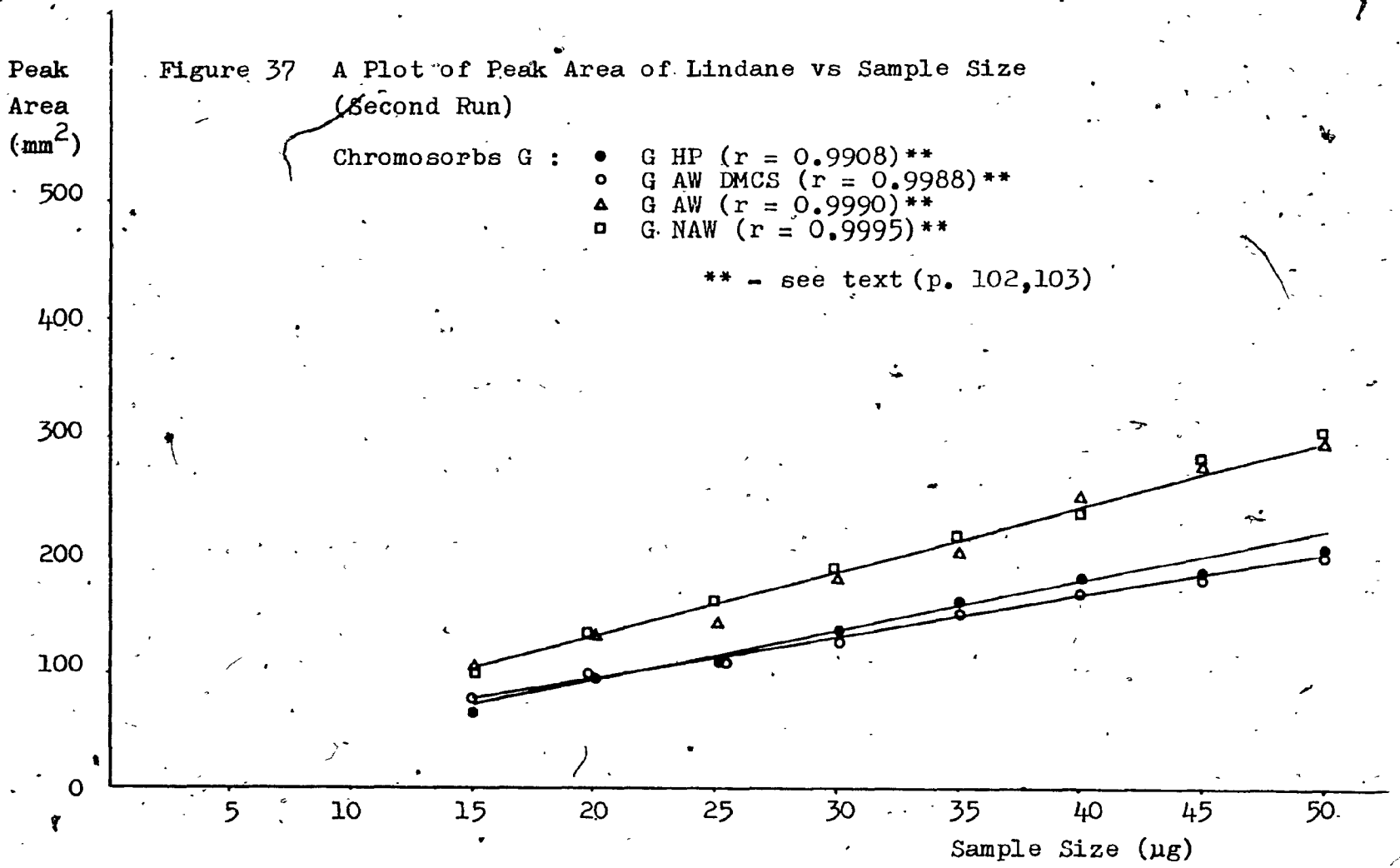


Figure 38 A Plot of Peak Area of Aldrin vs Sample Size (Second Run)

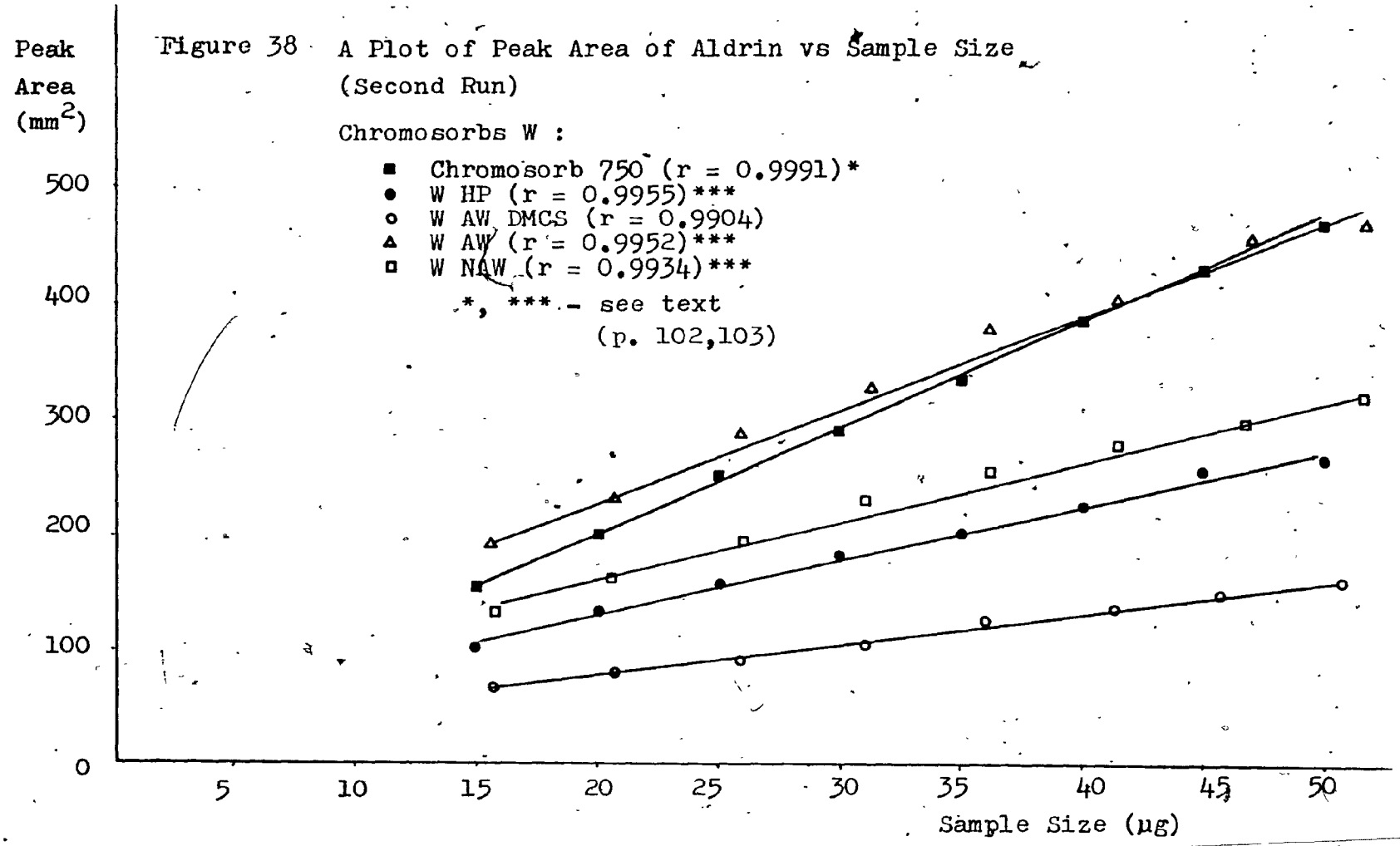


Figure 39 A Plot of Peak Area of Aldrin vs Sample Size (Second Run)

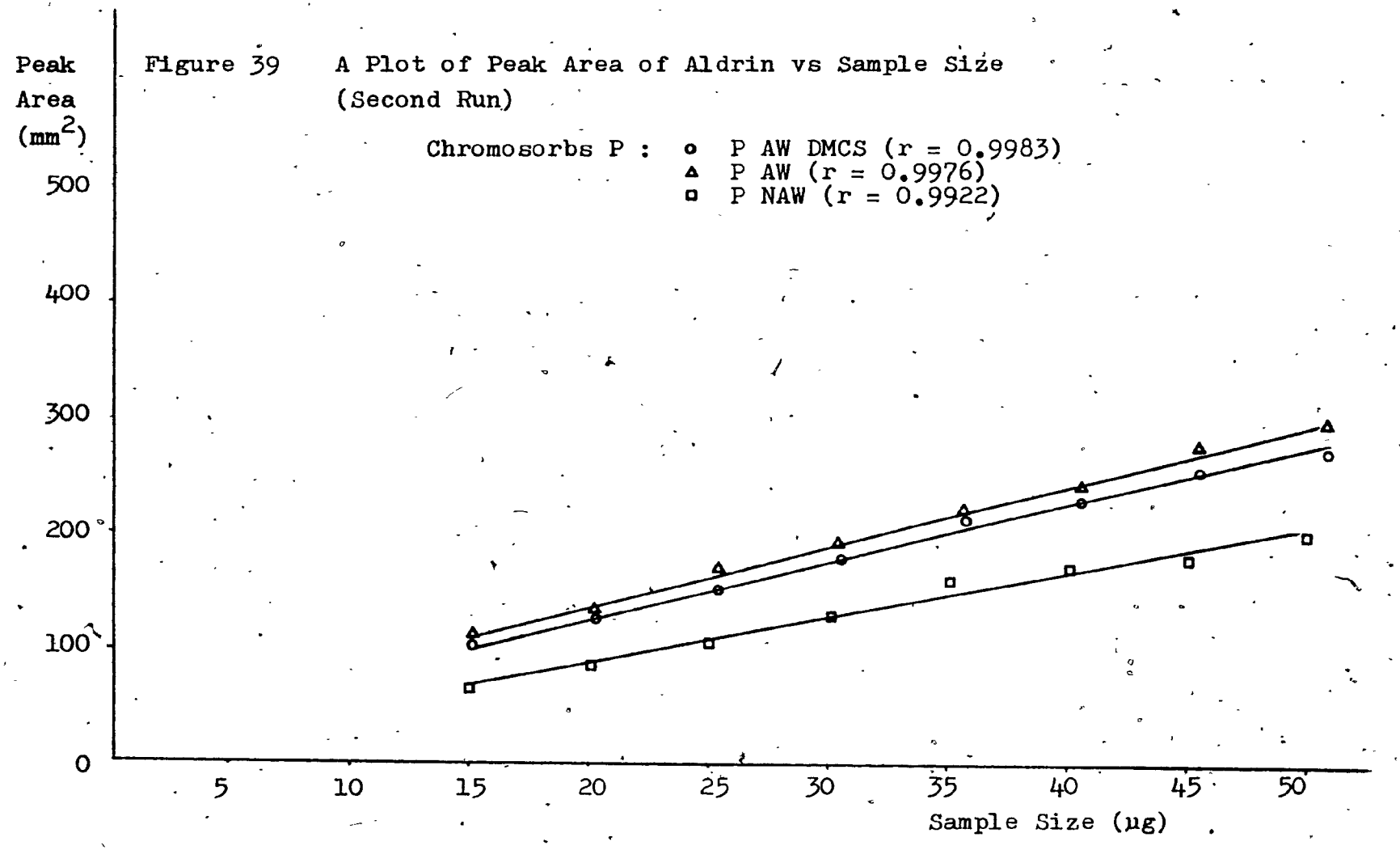


Figure 40 A Plot of Peak Area of Aldrin vs Sample Size (Second Run)