

PARTIAL SUBTRACTION CHROMATOGRAPHY

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The qualitative and quantitative analysis of certain mixtures can be carried out advantageously by combining gas chromatography with chemical treatment. One of the variants of this mode of operation is subtraction chromatography, in which one or more components are eliminated selectively, resulting in the disappearance of the corresponding peaks. By comparison with a conventional chromatogram, identification and quantitative analysis of the reacting compounds can be carried out readily. The reagents** employed in this method act rapidly; they are coated on the solid support and filled into a short piece of tubing attached to the main partition column. The emergence time of the non-reacting components is practically unaffected under these circumstances. Examples of suitable reagents are concentrated sulphuric acid, which is useful for the subtraction of olefins in mixtures with other hydrocarbons, or silver nitrate, which permits the analysis of secondary and tertiary alkyl bromides in the presence of primary isomers¹.

The procedure is of particular value when separation cannot be achieved by the usual gas chromatographic techniques. The analysis of argon and oxygen, for instance, has been carried out successfully only when the oxygen was burned in a precolumn loaded with palladium catalyst, using hydrogen as the carrier².

On the other hand, JANÁK AND NOVÁK⁴, in developing an accurate procedure for the analysis of impurities accompanying butadiene, used the relatively slow acting maleic anhydride for subtracting the diene. A column containing about 10 g of the dienophile on 22 g of support was necessary to complete the reaction in 23 sec at 100°. In such a case the retention volume of the non-reacting components is considerably changed by the precolumn.

In the present paper we wish to discuss a procedure involving only partial subtraction of the reacting components. In this method chromatographic peaks are identified by observing either the occurrence or the rate of reaction with the stationary phase. The rate is determined from the peak areas corresponding to the residual amounts of components emerging from the column at different flows of the carrier gas.

The method is illustrated by experiments with conjugated dienes as the subtracted components, and the dienophilic chloromaleic anhydride (CIMA) as the stationary phase. The resulting adducts are relatively non-volatile and remain on the column.

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** It should be noted that molecular sieves³ can be used effectively for the selective and irreversible absorption of *n*-aliphatic compounds.

The commercial CIMA was distilled before use, but, in general, not further purified by crystallization, since a small amount of residual impurities conveniently depresses the melting point below room temperature. When relative values only are required, the effect of the impurities on the rate of reaction can be disregarded, as long as care is taken to work with the same batch of reagent.

It has been shown^{5,6} that for a pseudo-first-order reaction the rate of disappearance of the reacting component is given by $dx/dt = -kxH$, where x is the total amount of the reacting component at time t in the plug passing through the column, and H is a factor by which x has to be multiplied in order to give the amount of component dissolved in the stationary phase.

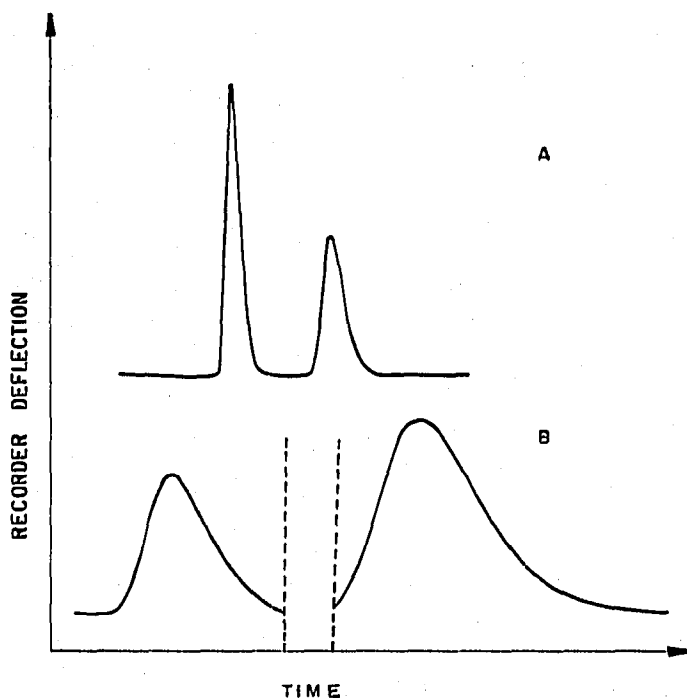


Fig. 1. Chromatography of commercial 1,3-pentadiene on a dual column containing silver nitrate and chloromaleic anhydride, respectively, as the stationary phases (see Table I). Temperature 40°. Curve A—flow rate of helium 77 ml/min, area of 1st peak (*trans* isomer) 60%; curve B—flow rate of helium 11.4 ml/min, area of 1st peak (*trans* isomer) 31%.

The conditions necessary for subtracting can be calculated by combining the equation $\log x^0/x = 0.4343 k t H$ with the expression for the contact time $t = V_R/F$ where V_R is the uncorrected retention volume of the solute on the subtracting column, and F is the rate of flow of the mobile phase; hence

$$k = 2.3 \log x^0/x F/V_R H \quad (1)$$

For complete subtraction $\log x^0/x$ can be taken as equal to 5, whereas for partial subtraction $\log x^0/x$ should be about 0.04 to 1.0. The rates of the reactions, which can be conveniently used in partial subtraction chromatography, cover a wide range under the chromatographic conditions employed (see Tables I and II for flow rates and description of columns, Fig. 2 for retention volumes, and EXPERIMENTAL for H

TABLE I
PARTIAL SUBTRACTION CHROMATOGRAPHY OF CONJUGATED DIENES^a

Column	1st Peak (% of total area)	2nd Peak (% of total area)	3rd Peak (% of total area)	Helium flow rate (ml/min)
<i>1,3-Pentadiene</i>				
AgNO ₃ -glycol ^b	65	35		
AgNO ₃ -glycol ^b and CIMA ^c	60 31	40 69		77 11.4
<i>Crude 1,3-hexadiene-5-yne</i>				
Tri- <i>o</i> -tolyl phosphate ^d	55	45	0	
CIMA ^f	60	40	0	69
"	65	35	0	31.5
"	83	27	0	13
<i>Crude 2,4-hexadiene</i>				
AgNO ₃ -glycol ^g	40	18	42	
<i>(area/area n-hexane^h)</i>				
CIMA ^e	0.23	0.32	1.10	116
"	0.09	0.26	1.13	58
"	0.04	0.18	1.11	31

^a Temperature 40°.

^b Saturated silver nitrate-glycol solution; column length 1 m; weight ratio solid support to stationary phase 2:1.

^c Non-crystalline chloromaleic anhydride; column length 1 m; i.d. 4 mm; weight ratio solid support to liquid phase 2:1.

^d Column length 2 m; weight ratio solid support to liquid phase 2:1.

^e The third peak, corresponding to the starting material, 1,5-hexadiyne (about 10%) was not taken into account in the above area calculations.

^f As c, but column length 2 m.

^g As b, but column length 2 m.

^h Added inert reference substance.

TABLE II
SUBTRACTION CHROMATOGRAPHY^a OF A MIXTURE OF ISOPRENE (I), *trans* (II)- AND
cis (III)-1,3-PENTADIENE AND CYCLOPENTADIENE (IV)

Compound	1st Peak	2nd Peak	3rd Peak	4th Peak	5th Peak	Helium flow rate (ml/min)
	<i>n</i> -hexane	I	II	III	IV	
<i>Stationary phase—β,β'-oxydipropionitrile^b</i>						
% of total area	31.2	30	14	7.6	16.8	
<i>Stationary phase—chloromaleic anhydride^c</i>						
% of total area	48	32.2	8.8	11	nil	42.2
Area/area <i>n</i> -hexane		0.67	0.18	0.23		
% of total area	53.5	30.7	3.1	12.7	nil	14.8
Area/area <i>n</i> -hexane		0.58	0.058	0.24		

^a Temperature 40°.

^b Column length 1 m; weight ratio solid support to liquid phase 3:1.

^c Crystalline chloromaleic anhydride; column length 1 m; weight ratio solid support to liquid phase 2:1.

values). In the present examples, for instance, the kinetic constants varied from $2.0 \cdot 10^{-2}$ to $1.4 \cdot 10^{-4} \text{ sec}^{-1}$ (half life time 30 sec to 100 min respectively)⁶.

Many reagents should be suitable for use in subtraction chromatography, provided conditions in the subtracting column are adjusted in accordance with the requirements of eqn. (1). H can be taken as approximately equal to unity, except where the retention volume is relatively small (see EXPERIMENTAL). V_R can be varied

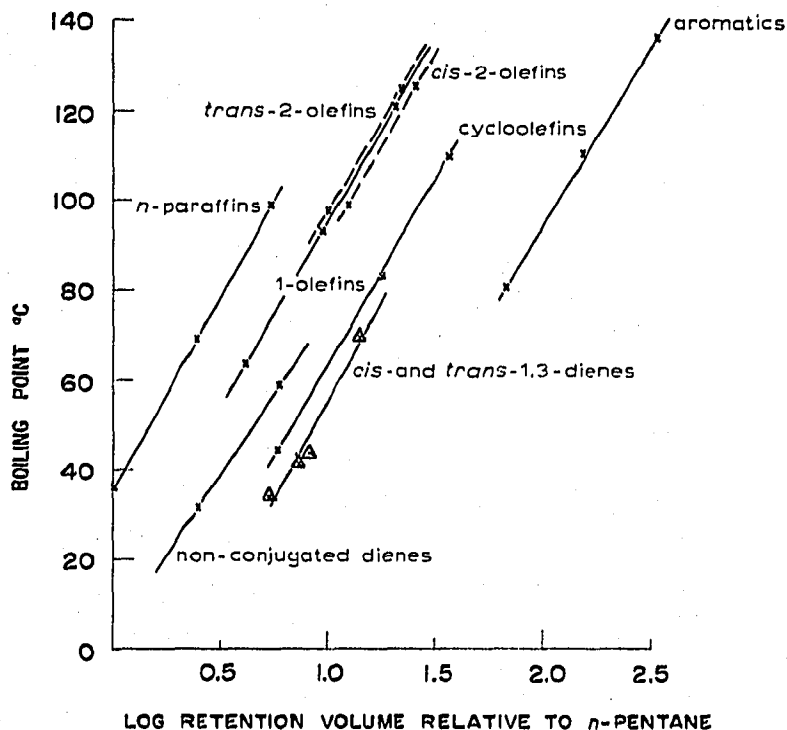


Fig. 2. Plot of log retention volume (relative to *n*-pentane) versus boiling point for different classes of hydrocarbons on non-crystalline chloromaleic anhydride at 40°. Net retention volume of *n*-pentane 11.3 ml, on a 1 m × 4 mm column containing 7.4 g of a mixture of solid support and CIMA in the ratio 2:1.

by the amount of stationary phase used, and the effective value of the kinetic constant can be modified by choice of temperature, by the addition of catalysts or by dilution. For example, when experimenting with mercury acetate as a reagent for the partial subtraction of mono-olefins⁷, it was found necessary to dilute the acetate considerably with ethylene glycol in order to bring the half life time into a proper range (25 sec and 100 sec for 1-hexene and cyclohexene, respectively).

DISCUSSION OF RESULTS

Differentiation between cis- and trans-1,3-dienes

Chromatographic peaks due to *cis*- and *trans*-1,3-dienes can be distinguished readily from each other by partial subtraction with dienophiles. The *trans*-isomers, indeed, easily undergo Diels-Alder addition, whereas the *cis*-isomers^{8,9} react very much more slowly or not at all.

Piperylene is the simplest example of a 1,3-diene showing geometric isomerism.

The commercial product gives two peaks on a column with silver nitrate-glycol as the stationary phase. When the mixture was run on a dual column containing silver nitrate-glycol and CIMA, respectively, the proportion of the first peak decreased relatively to the other, and with a higher contact time (helium flow rate, 11.4 ml/min) dropped below that of the second peak (see Table I and Fig. 1). It is easily seen from these data that the pentadienes emerge in the same order on both chromatograms, and that the first peak corresponds to the *trans*-isomer and the second peak to the *cis*-compound. In the same way it was possible to distinguish between the peaks due to *cis*- and *trans*-1,3-hexadiene. In both cases, as the flow rate altered, areas corresponding to the *cis*-isomer did not change with respect to an added inert substance.

The method has been used to prove the formation of *trans*-1,3-pentadiene in the elimination reaction of the tosylate of *trans*-2-methylcyclobutanol¹⁰. The nature of the by-products, accompanying the expected 3-methylcyclobutene, could thus be ascertained unambiguously.

Another interesting application is the identification of the geometric isomers of 1,3-hexadien-5-yne, prepared from 1,5-hexadiyne by action of potassium *tert*-butoxide in *tert*-butanol¹¹. As shown by chromatography with a stationary phase such as tri-*o*-tolyl phosphate or Octoil, the product was found to consist of a small amount of unchanged starting material and two major components with peak areas in the ratio 5.5:4.5. On a CIMA column the peaks emerged in the same order, as could be seen by their respective areas. When the flow rate of the mobile phase was reduced (see Table I), the second of the large peaks gradually decreased relative to the other and, thus, was ascribed to *trans*-1,3-hexadien-5-yne*. The assignment was confirmed by preparative G.L.P.C. and infrared spectroscopy of the isolated fractions¹¹. It is to be pointed out that the *trans*-form of the 1,3-hexadien-5-yne has the larger retention volume, contrary to the behaviour of the 1,3-penta- and hexadienes, as well as of some monoolefins on polar stationary phases¹².

Differentiation of structural types of conjugated dienes

Structural factors may markedly influence the rate of reaction of conjugated dienes with dienophilic reagents. In particular, alkyl substitution and inclusion of the diene system into a five-membered ring increase reactivity considerably. Available information in the literature^{6,9,13} on the direction and magnitude of such effects may be used with advantage for the identification of chromatographic peaks, as shown below.

(a) Crude 2,4-hexadiene, prepared from crotonaldehyde and ethyl magnesium bromide¹⁴, gave on a silver nitrate-glycol column a chromatogram consisting of three peaks. According to ALDER AND VOGT¹⁴ there is formed, in fact, in addition to the expected *trans*, *trans*- and *trans*, *cis*-2,4-hexadienes, also *trans*-1,3-hexadiene. When the product was chromatographed at different flow rates on a column containing CIMA, it was found that the first peak decreased rapidly, the second peak more slowly, while the third peak remained unchanged (Table I). The last peak clearly corresponded to *cis*, *trans*-2,4-hexadiene, which does not react under mild conditions. The other peaks were due to *trans*, *trans*-2,4-hexadiene and *trans*-1,3-hexadiene; their assignment could be made on the ground that substitution at both ends of a diene

* It has been reported¹¹ that with maleic anhydride the dien-yne forms 1,2,3,6-tetrahydro-3-ethynyl phthalic anhydride.

system (*trans, trans*-2,4-hexadiene, first peak) more effectively promotes Diels-Alder addition than a similar substitution at one end only.

(b) To illustrate the distinctive behaviour of cyclopentadiene, a mixture consisting of isoprene, *cis*- and *trans*-1,3-pentadiene, cyclopentadiene, and *n*-hexane (reference substance) was passed over a CIMA column at 40° (Table II). Only four peaks were found on the chromatogram even at the higher rate of flow of the carrier gas, as the cyclopentadiene had reacted completely on passage through the column. The non-appearance of a fifth peak was not due to low efficiency of CIMA as a stationary phase, since, as shown on Figs. 2 and 3, the polar CIMA has about the same

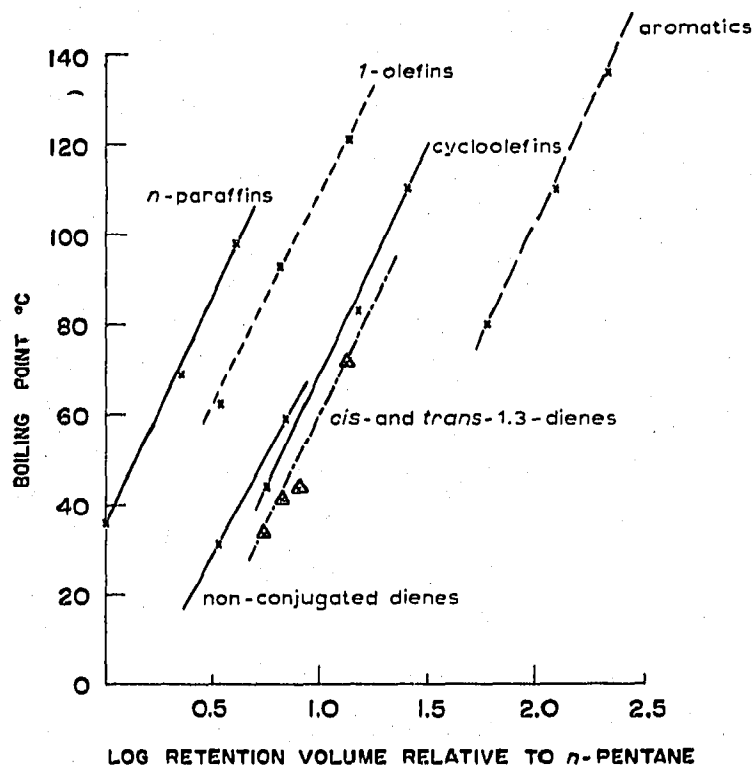


Fig. 3. Plot of log retention volume (relative to *n*-pentane) versus boiling point for different classes of hydrocarbons on β, β' -oxydipropionitrile at 40°.

selectivity towards various groups of hydrocarbons as β, β' -oxydipropionitrile, and all five compounds present in the sample were readily separated on the latter phase.

Peak identification by accurate kinetic data

Accurate measurement of the rate of subtraction of a peak, and comparison with that of the component assumed to be present, can be used as a method of identification. Such a procedure is of interest in cases in which preliminary peak assignment has been made, *e.g.* on the basis of the retention volume, but further confirmatory evidence is required. The reproducibility and accuracy of the method is seen in Fig. 4, in which comparison is made of the isoprene and *trans*-1,3-pentadiene peaks appearing in the chromatogram of the mixture described in Table II. The values of $\log x^\circ/x$ for the second and third peak of the sample closely fit the curves of the respective pure components. Special care must of course be taken to operate under the same con-

ditions; thus in all cases CIMA purified by crystallization was used, and the temperature was kept at 40°.

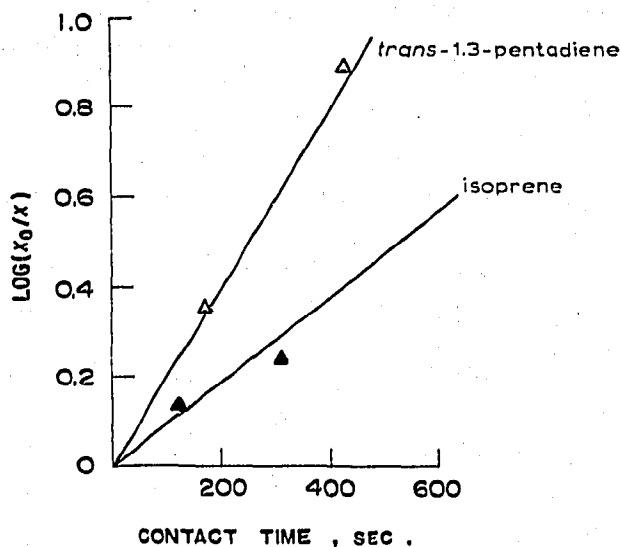


Fig. 4. Plot of $\log x^0/x$ versus contact time for isoprene and *trans*-1,3-pentadiene on a chloromaleic anhydride column. (—) Pure isoprene and pure *trans*-1,3-pentadiene; points correspond to 2nd peak (▲) and 3rd peak (Δ) of the mixture described in Table II. Stationary phase crystalline chloromaleic anhydride; column length 1 m; weight ratio of solid support to liquid phase 2:1; temperature 40°.

Quantitative analysis

For quantitative analysis a parallel run has to be made on a non-reacting stationary phase, and the order of emergence of the peaks, as compared with that on the subtracting column, has to be checked. Such a comparison of peaks corresponding to non-reacting substances can be made on the basis of their respective areas. As to reacting components, no difficulty arises if only one of the components is partially subtracted. If, however, more than one peak varies, assignment can be made after estimating, to a first approximation, the initial amounts of subtracted components by extrapolating to zero time the corresponding values of $\log x$ (with respect to an inert reference substance). An example of such a case is given in the experimental section.

EXPERIMENTAL

Materials

1,3- and 2,4-Hexadiene were prepared according to ALDER AND VOGT¹⁴. Samples of 1,3-hexadien-5-yne were kindly supplied by Dr. F. SONDEIMER and Dr. D. BENEFRAIM. All other dienes used were commercial products; cyclopentadiene was obtained from dicyclopentadiene by distillation.

Chloromaleic anhydride was distilled at 96°/25 mm, and, where necessary, purified by crystallization from ether¹⁵ at -20°, m.p. 31-32°.

Apparatus and procedure

A Perkin-Elmer Model 154 A Fractometer was used in the experiments. The solid support was powdered Johns-Manville C-22 firebrick of 50-80 mesh, prepared as described previously¹⁶. The firebrick powder was coated with CIMA, using a small

amount of ether as solvent, and immediately poured into the chromatographic column. The ether was evaporated by passing carrier gas through the column after installation in the apparatus, care being taken to avoid moisture.

The quantities of material injected into the column were adjusted so as to obtain symmetrical peaks, and the areas were calculated by multiplying heights by peak width at half height.

In experiments in which only a CIMA column was employed, the contact time (t) was obtained directly from the chromatogram by dividing the distance from the injection point to the peak maximum by the speed of the recorder paper. For dual columns t was calculated from the equation $V^{\circ}_R = j Ft$. V°_R , the corrected retention volume was determined by a separate measurement using the CIMA column only; j , the pressure gradient factor, was obtained by estimating the pressure (p_t) at the head of the CIMA section of the dual column on the assumption that resistance to flow was proportional to length¹⁷; F , the flow of carrier gas at the exit from the CIMA column at atmospheric pressure (p_0), was measured with a soap bubble meter (the CIMA was always placed at the exit side of the dual column system).

Determination of the peak order of the components of crude 2,4-hexadiene

This was carried out on a 2 m column with a saturated silver nitrate-glycol solution as the stationary phase (chromatogram A) and on a 1 m CIMA column (chromatogram B). The ratio of solid support to liquid phase was 2:1 on both columns. The CIMA was not purified by crystallization and was liquid at room temperature. On A the areas of the peaks with respect to *n*-hexane were in their order of emergence, (1) 0.95, (2) 0.42, (3) 1.02. The first peak on B had the same relative area with respect to the inert reference substance as the third peak on A, and was therefore ascribed to the non-reacting *cis, trans*-2,4-hexadiene. As to the first and second peaks on B, extrapolation of the values of log area x /area *n*-hexane (Table I) showed that at zero time the first component had a larger area than the second component (log relative area ~ 0 and -0.4 , respectively). Hence it followed that on both chromatograms the order of emergence of the peaks was the same.

TABLE III

H VALUES OF DIENES ON A CHLOROMALEIC ANHYDRIDE COLUMN AT 40°*

Compound	<i>H</i>
Butadiene	0.68
Isoprene	0.84
<i>trans</i> -1,3-Pentadiene	0.89
<i>trans</i> -1,3-Hexadiene	0.89
<i>trans,trans</i> -2,4-Hexadiene	0.94
<i>trans</i> -1,3-Hexadien-5-yne	0.98

* For details on column see Table I, footnote f.

H values of dienes on a chloromaleic anhydride column at 40°

H is calculated from the retention volume according to the equation:

$$H = \frac{V'_R}{V_R} \quad (2)$$

where V'_R = adjusted retention volume and V_R = uncorrected retention volume. The values are given in Table III.

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SUMMARY

Partial subtraction chromatography is defined as a procedure in which stationary phases are employed capable, under the chromatographic conditions, of reacting at a measurable rate with certain classes of compounds. The reaction occurring can be followed as a function of time by determining the areas of the corresponding peaks emerging from the column at different flow rates of carrier gas.

Partial subtraction is proposed as a method of peak identification. Its application is illustrated by a series of examples in which conjugated dienes are the reacting components and chloromaleic anhydride is the stationary phase.

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