The Semimicro Chromatographic Determination of Gamma Hexachlorocyclohexane

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Numerous methods have been proposed for the determination of the gamma isomer of the hexachlorocyclohexane, some of them applying spectroscopic, isotopic, polarographic and X-ray techniques respectively. Others depend upon gravimetric[1]), hydrolytic[2]) or cryoscopic[3]) operations. All these methods suffer from various disadvantages, such as low accuracy, expensive apparatus or they are time-consuming. Alternative methods,

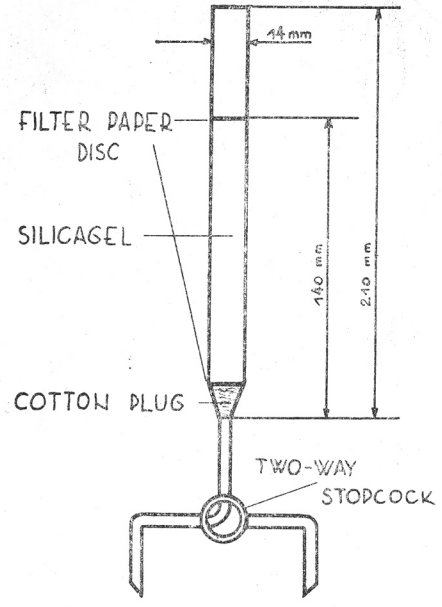

Fig. 1. Semimicro chromatographic column.

e. g. the microscopic[4]) one, can be used only with a rather pure gamma isomer (Lindane). The most promising method is the chromatographic method. This method was modified recently by Harris[5]) and Fontana[6]) who use some dyes for the marking of the position of the gamma isomer band in the chromatographic column. Both of them prepared their columns with nitromethane. Granger and Zwilling[7]) modified with water the adsorption properties of the silicagel. This method is still time- and reagentconsuming, besides of being unsufficiently accurate. Because a faster and simpler method is desirable, a chromatographic method suggested by one of us[8]) was modified to meet those requirements. The method described here depends upon the determination of the labile

chlorine in the gamma isomer, previously separated in the semimicro column.

Preliminary experiments in which nitromethane was used on a semimicro scale were unsuccesful. When the adsorptive properties of the silicagel were modified with water very satisfactory results were obtained. A commercial grade of silicagel was employed. The particular advantage in using this substance lies in the fact that it is available in standardised quality. The silicagel prepared according to Gordon batchwise on a laboratory scale shows a high but inequal adsorptive power[9] [10]. To study the optimal conditions of the fractionation, artificial mixtures of pure alpha and gamma isomers were employed. The best results in separating the alpha and gamma isomers were achieved with a silicagel contaning 10% of water. Under the conditions described the water content has to be reduced to 6,5% when analysing crude products. By applying such a silicagel one may separate the alpha and the gamma isomer and also the gamma isomer and the heptachlorocyclohexane (m. p. 157°C). The substance mentioned was observed to follow closely the gamma isomer. This rendered the separation more difficult.

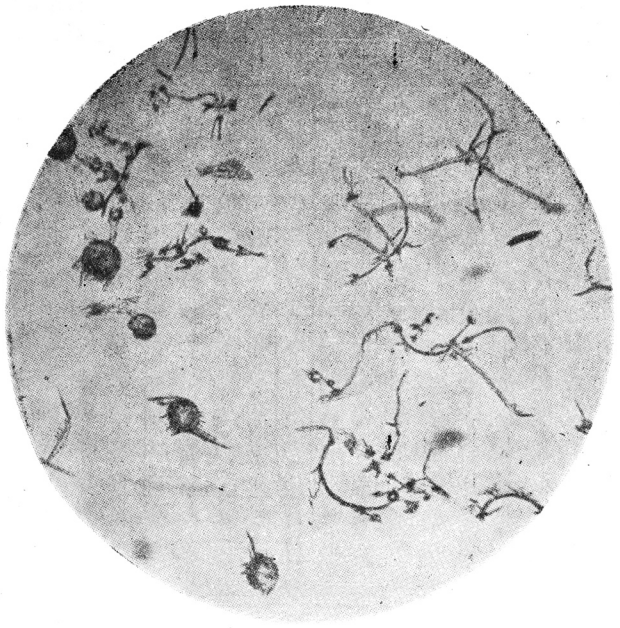

Fig. 2. Isomer alpha, 60 × magn.

The development was carried out with petroleum ether. To prepare the working developer we carried out the fractional distillation of a commercial grade aviation gasoline (octane number 100) and of a commercial grade ordinary gasoline. The boiling range and the percentage of olefines in the gasoline influenced the separation. The rate the single fractions are mowing down the column is growing proportionally with the percentage of the unsaturated compounds contained in the petroleum ether.

The fraction of the ordinary gasoline which distilled beween 30 and 70°C containing 11% olefines was found most suitable. The optimal rate of flow which still permits the identification of single fractions is 4 ml per minute. The identification was carried out by evaporating a few drops of the solution and examinig the residue under the microscope.

When folowing the suggestions made above no previous extraction of the gamma isomer from the sample was necessary, because our column showed a separation capacity which was great enough to carry out the separation satisfactorily.

For the determination of the labile chlorine the potentiometric method was employed. We modified the differential titration method[11]) to make it more suitable for our purposes.

procedure repeated. The silicagel is filtered, dryed at 125°C to constant weight and kept in a dessicator.

18 g. are weighed in a narrow-necked flask and 1,2 ml. of distilled water added. The flask is then stoppered and shaken till the silicagel has cooled down to room temperature. The bottom of the column is ploughed with cotton wool and covered with a round piece of filter paper. Petroleum ether is then poured onto the column just to cover the filter paper (the stopcock must be shut). The silicagel is mixed with 30 ml. of petrolem ether and poured into the column through a glass funnel. After that the stopcock is opened and the silicagel allowed to settle to form a homogenous layer. Then nitrogen is admitted to pack the bed to a height of 14 cm. Care must be taken that the top of the column is co-

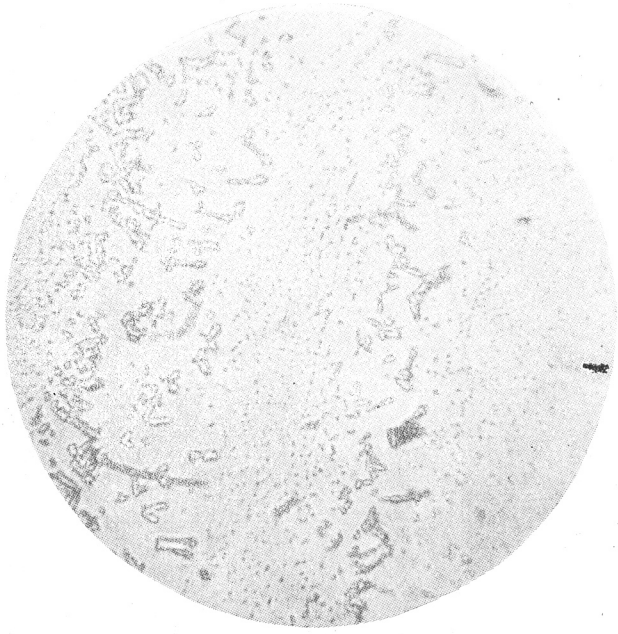

Fig.4. Fraction coming down next after the gamma isomer, 60 × magn.

vered with the solvent. When the packing desired is obtained the solvent is allowed to drain and the silicagel is covered with a tightly fitting piece of filter paper.

50 mg. of the pulverized crude sample are weighed in a 10 ml. test tube, then 1,5 ml. of petroleum ether is added and slowly heated. The sample should be heated until the bulk is dissolved. The test tube is then placed in a vertical position on the column and the bottom of the tube broken with a glass rod to bring the solution into the column. The rod and the test tube are washed with 2 ml. of the solvent, which is left to penetrate the column. The sides of the column are washed down with a small amount of solvent. The column is then filled with the solvent leaving the upper 2 cm. empty. The solvent reservoir is connected with

the column and the rate of flow adjusted to about 4 ml. per minute by varrying the pressure of nitrogen.

Applying the conditions mentioned above and using petroleum ether with a boiling range 30—70° and containing 11% of olefines, we get the following fractions.

I. 110—120 ml.: octachlorocyclohexane + heptachlorocyclohexane
II. 160—250 ml.: alpha hexachlorocyclohexane
III. 300—425 ml.: gamma hexachlorocyclohexane
IV. 460 ml. and further: heptachlorocyclohexane + beta hexachlorocyclohexane.

The identification of the fractions is carried out by catching a drop or two on a watch glass previously heated at about 70°C. The residue is then examined under a microscope using a 50 fold magnification. At

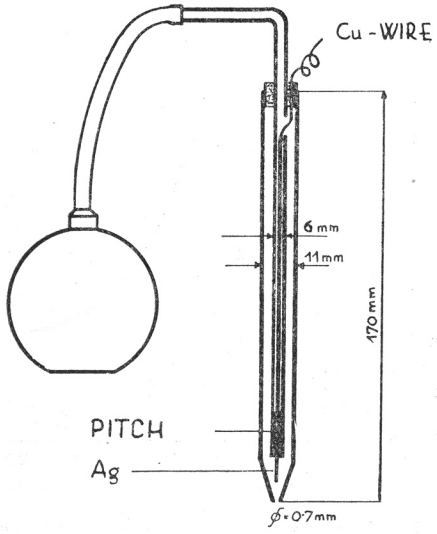

Fig. 5. Silver electrode for differential titration

a higher concentration of the component the crystals appear as soon as the solvent has evaporated. Otherwise the crystalisation may be induced by sctatching with a thin glas rod or by seeding with a small crystal of the product. Figures 2, 3 and 4 illustrate the characteristic differences between the various fractions. When the alpha isomer has passed for about 20 ml., the stopcock is turned to collect the gamma isomer in a graduated cylinder. After the gamma isomer leaves the column, wait for about 5—10 ml and turn the stopcock again. The outlet of the column is then washed down with petroleum ether and so are the watch glasses. The solvent is evaporated in a vacuum to the volume of 2—3 ml. at a bath temperature not above 30°C. Then 10 ml 0,5 N alcoholic KOH are added. The solution is refluxed for 10 minutes, then neutralized with 0,5 N HNO_3 until neutral to bromothymolblue (pH value about 7). The solution is evaporated to dryness and the residue dissolved in 80 ml. of water.

Determination of the chlorine ion. The apparatus for the differential titration consists of a 150 ml. beaker, two silver electrodes, a glass stirrer and a 10 ml. buret, graduated in 0,02 ml. divisions. A Phillips pH meter GM 4494 was used for all measurements of the electrode potentials. The electrode is made of Ag foil welded to a Cu wire. The details are shown in figure 5.

The electrodes were chlorinated before use to attain constant potentials. The surface of the electrodes is cautiously cleaned with emery cloth, then both electrodes are dipped simultaneously in a 5% NaCl solution. As a source of the electric current a lead storage battery was used. The voltage was raised un till small H_2 bubbles appeared on the

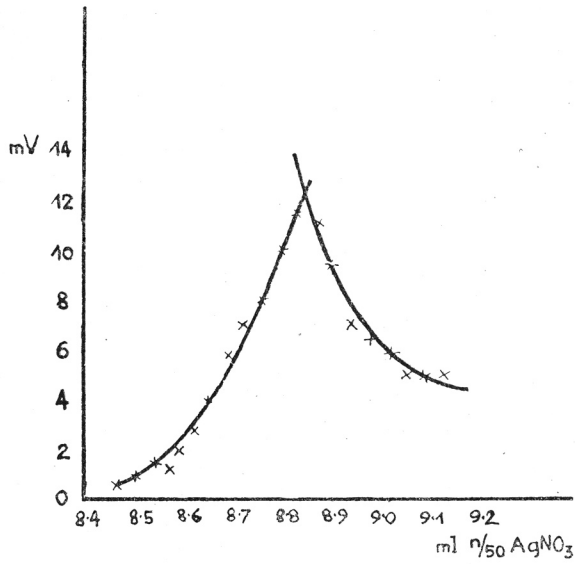

Fig. 6. Titration curve (Trial No. 48 — gamma)

Pt electrode. This occured at 1,8 V and a current intensity of 0,3 mA. During the chlorination the Ag electrodes get chocolate brown and the he current decreases as far as 0,02 mA at a constant voltage. This was attained in 20 do 30 minutes. The chlorination is then discontinued, the two electrodes are placed in the titration apparatus and short-connected for a while. The electrodes in this manner prepared remained unchanged for some months, if kept in a dark glass vessel filled with distilled water. For titration 0,05 N $AgNO_3$ is used and the solution added in 0,1 ml. portions. The cathode is a platinum wire. The figure 6 is a typical example of obtained titration curves.

DISCUSSION

The precision of the experimental procedure described was checked by the data obtained for pure gamma and alpha isomers. Table I presents the data obtained from the determination of the labile chlorine

content of the gamma isomer according to the procedure described above. All these determinations were carried out with 0,05 N $AgNO_3$ with a factor 1,0034.

Table I

Differential titrations of the labile chlorine content of the gamma isomer

Weight of the gamma isomer in mg.	Used ml. of 0.05N $AgNO_3$	Required gamma isomer in mg.	Determined gamma isomer in mg.	Deviation in mg.	Deviation in %
13,780 (diluted to 100 ml. and 25 ml. of the dilution titrated)	1,815	3,445	3,525	+0,080	+2,27
18,250 (diluted to 100 ml. and 25 ml. of the dilution titrated)	2,350	4,562	4,572	+0,010	+0,22
	2,318	4,562	4,510	0,052	—1,14
	2,312	4,562	4,498	—0,064	—1,40
14,090 (diluted to 100 ml. and 25 ml. of the dilution titrated)	1,796	3,522	3,494	—0,028	—0,79
	1,832	3,522	3,506	—0,016	—0,45
	1,802	3,522	3,506	—0,016	—0,45

The results of the tests with the mixtures of pure gamma and alpha isomers, which were separated in the chromatographic column and the chlorine ion estimated, are shown in Table II.

Table II

Chromatographic fractionation and the differential titration of the mixtures of the pure gama and alpha isomers

Trial No.	Weight of the isomer in mg		Used ml. of 0,05 N $AgNO_3$	Found in mg.	Deviation in mg.	Deviation in %
38	gamma:	8,750	1,130	8,790	+0,040	+0,46
	alpha:	3,6	—	—	—	—
46	gamma:	19,680	10,083	19,620	—0,060	—0,31
	alpha:	5,710	2,950	5,739	+0,029	+0,51
47	gamma:	29,7	—	—	—	—
	alpha:	3,890	1,985	3,862	—0,028	—0,73
48	gamma:	17,200	8,845	17,208	+0,008	%0,05
	alpha:	5,660	2,909	5,659	—0,001	—0,02

Results of tests with the crude hexachlorocyclohexane are shown in Table III.

Table III

Determination of gamma isomer in crude hexachlorocyclohexane

Trial No.	Weight of sample in mg.	Used ml. of 0,05 N $AgNO_3$	Gamma isomer found in mg.	Gamma isomer in %	Deviation from mean
1	50,0	3.950	7,685	15,36	+0,06
2	50,0	3.942	7,673	15,34	+0,04
3	50,0	3.902	7,559	15,20	—0,10
4	50,0	3.938	7,658	15,32	+0,02

mean, 15,30% of gamma isomer

Comparison of our results with the results obtained by other authors reveal that the method presented is of a greater precision allthough the amount of the sample is reduced 20 — 30 times. The procedure requires 90 minutes for the chromatography, the procedure described by Granger requires 80 min. and the method of Harris 45 min. But we have succesfully eliminated the operation of extracting 3 times the sample. The time required for this operation is 90 min. plus the time necessary for the drying of the collected gamma isomer to cons'ant weight. No time requirements are given for this operation. Accordingly to our observations, this operation must be very lenghty, if any loss of the gamma isomer should be avoided.

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IZVOD

Semimikro kromatografsko določevanje gama heksaklorcikloheksana

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Doslej opisane metode za določevanje gama izomere heksaklorcikloheksana imajo razne pomanjkljivosti kot n. pr. komplicirana aparatura, nezadovoljiva natančnost ali, pa prevelika poraba časa. Večina tega je pri opisani metodi odpravljena. Uporabljena je bila komercijalna vrsta silikagela in tako dosežena enakost adsorbtivnih lastnosti pri vseh šaržah Namesto nitrometana bila je za prepariranje silikagela uporabljena voda, ki omogoča boljšo ločbo posameznih izomer v semimikro merilu. Optimalni odstotek vode v silikagelu je za umetne zmesi alfa in gama izomere 10%, pri surovih produktih pa 6,25%. Na potek ločbe vpliva razmerje večjih in manjših delcev silikagela. Kot razvijalno topilo se je najbolje obnesel navadni avtomobilni bencin z vreliščem od 30—70°C in 11% olefinov. Optimalna pretočna hitrost je 4 ml na minuto. Za titracijo labilnega klora je bila uporabljena potenciometrična metoda v izvedbi diferenčne titracije, ki je bila prikrojena danim zahtevam. Pri zahtevi 50 mg surovega heksaklorcikloheksana petroletru z 11% olefinov ter vreliščem 30—70°C se nahaja gama izomera v tekočem kromatogramu od 300—425 ml. Izkazalo se je, da ima opisana kromatografska kolona večjo razdelilno sposobnost od doslej opisanih in je zato lahko odpadla predhodna ekstrakcija gama-izomere iz vzorca, kar zelo skrajša čas analize. Prav tako odpade po tej metodi sušenje kromatografirane gama-izomere do konstantne teže.

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