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Separation and Estimation of Lindane from a Mixture of Hexachlorocyclohexane Isomers via Formation of an Inclusion Compound with β-Cyclodextrin

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Abstract. The treatment of a mixture of hexachlorocyclohexane isomers containing 16.03% lindane (γ-isomer) with β-cyclodextrin results in an effective separation of lindane as indicated by a 50.4% lindane content in the total included guest. An assembly for a semimicro quantitative extraction for the separation of guest components in the inclusion compounds of cyclodextrins has been designed. The separation by inclusion has been confirmed by the preparation of an inclusion compound of β-cyclodextrin with pure lindane whose guest content has been found to be 10.5% using three independent methods.

Key words: β-Cyclodextrin, lindane, assembly for extraction of guest, spectrophotometry, Schoniger method, GLC.

1. Introduction

Cyclodextrins [1] have been the subject matter of wide ranging studies including crystallographic studies of various cyclodextrins [2], inclusion complexes [3,4] and their role in catalysis as a model for enzyme action [5]. Chromatographic separations of a few inclusion complexes of cyclodextrins have been reported [6]. Their potential in bringing about analytical separations of mixtures of guest molecules has not, however, been fully explored. Though the physiologically active β-cyclodextrin inclusion compound with lindane has been reported in a patent [7], a detailed study of its formation and analysis has not been made. Similarly, though prolific literature exists on isomeric hexachlorocyclohexanes and the insecticidal γ-isomer, the separation and analysis of the latter via inclusion with β-cyclodextrin has not been reported. We report the separation of lindane from its mixture with other isomers, by inclusion in β-cyclodextrin which has been extended to a quantitative analysis of the inclusion compounds of lindane.

2. Experimental

2.1. MATERIALS

β-Cyclodextrin (Aldrich) and lindane were obtained commercially and further purified. Hexane, benzene and dimethyl formamide used were of AR grade. Acetone was purified and dried for use.

Infrared spectra were recorded on a Hilger and Watts, Infrascan, H-900 spectrometer and on a Beckman 4250 instrument. X-ray diffractograms were obtained on a Philips Stabilised D.C. X-ray diffraction generator model No. Type P.W. 1000/30 NRD 1023 and the computer processing was done on a TDC 316(ECI Ltd) System.

Gas liquid chromatographic analysis was done on a CHROM-1 Laboratorni Pristoje N.P. Unit (PRAHA) with FID and BARC unit with ECD.

2.2. PREPARATION OF *o*-TOLUIDINE REAGENT FOR SPECTROPHOTOMETRY

One gramme of freshly distilled *o*-toluidine was dissolved in 115 ml of concentrated hydrochloric acid and diluted up to 1 l with distilled water to give a colourless solution. This was diluted before use.

2.3. PREPARATION OF INCLUSION COMPOUNDS

A saturated solution of the hexachlorocyclohexane isomers in acetone was added to an aqueous solution of 150 mg of β-cyclodextrin at 60° C until a faint precipitate was obtained. The solution was cooled to 15° C and kept for 16 h for complete crystallization of the complex. It was filtered, and washed with 100 ml of dry acetone and ether. The complex was dried at 105° C in an oven to a constant weight. The complex formation was detected by melting point, IR spectra and X-ray diffraction patterns.

2.4. AN ASSEMBLY FOR THE EXTRACTION OF THE GUEST COMPONENT FROM THE β-CYCLODEXTRIN INCLUSION COMPOUND FOR GLC ANALYSIS

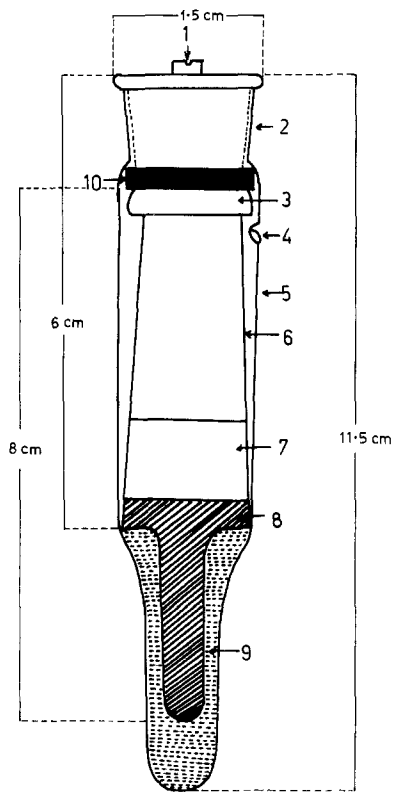

Fig. 1. (1) Inlet for syringe needle; (2) quick fit stopper; (3) cap for inner tube; (4) inlet for chilled bath; (5) outer tube; (6) inner tube; (7) solvent layer; (8) aqueous layer; (9) hot bath; (10) rubber septum.

The inclusion compounds prepared from both pure lindane and the mixture of hexachlorocyclohexanes were then analysed by GLC which needed prior extraction of the guest. This was achieved by means of the extraction assembly shown in Figure 1. The latter consists of a glass tube with a tapered end fitting into another modified centrifuge tube, with a stopper and cap, both with holes and a self-sealing septum of the type used in GLC analysis.

The inlet and outlet are the same. The bath is not of the circulating type but designed for the addition of chilled water to avoid solvent losses. The hot and chilled baths are separated by an air-tight arrangement. The GLC syringe passes through the septum to collect the organic layer. The hot and chilled baths are also used, as shown, to facilitate declathration and extraction.

About 100 mg of the inclusion compound to be extracted was accurately weighed and introduced into the inner tube of the extraction assembly and was then dissolved in the minimum amount of dimethyl formamide, and the tube kept in the outer tube containing the hot bath. To the dissolved complex was then added distilled water, dropwise, a little in excess of that required for the liberation of the host and guest molecules. About 3 ml of AR benzene was introduced into the inner tube and it was stoppered with a cap, above which was placed the rubber septum, and the outer tube was also closed. The chilled water was then introduced through the side hole. The contents were centrifuged at a speed of 3000 rpm with a similar counterbalance. The organic layer was then removed by inserting the needle of the micro syringe through the septum. This procedure was repeated with at least two more benzene extractions, and then collected together.

The GLC analysis was carried out by taking different aliquots of a stock solution containing 1×10^{-9} g lindane per microlitre, in duplicate. Thus, aliquots from one to eight microlitres were injected under the conditions specified below and a calibration plot was obtained by measuring the areas under the curves, which was then used to obtain the lindane content.

Operating conditions for GLC

Column	92 cm × 0.64 cm S.S. column loaded with 3% SE 30
Column temperature	176°C for pure lindane calibration; 144°C for separation of isomers
Injection port temperature	196°C
Carrier gas and its pressure	Nitrogen 0.5–0.6 kg/cm^2
Carrier gas flow rate	40 ml/min
Attenuator	10 mV
Current through cell	1×10^{-9} amp
Detector	Electron capture detector

2.5. ANALYSIS BY SPECTROPHOTOMETRY

Calibrated amounts of pure lindane ranging from 1 to 6 mg were weighed accurately on ashless papers meant for halide estimations. To samples containing more than 3 mg were added 3 mg of β-cyclodextrin as a flux. This was then packed and inserted into the preoxidised, clean platinum loop of the stopper of a Schoniger flask. Fifteen millilitres of the absorption reagent were then added to the flask with about 5 ml distilled water, and oxygen was passed at a rate of 30 ml/min for 40 s and the flask stoppered. The sample was burnt by means of an IR lamp and the time of combustion was noted at zero time. The contents of the flask were transferred

quantitatively to a 25 ml capacity standard flask after exactly 15 min and the optical density readings were taken against the 'cyclodextrin blank' at 20 min at 444 nm which is the absorption maximum for the developed yellow complex. The procedure was repeated with a series of samples so as to get a Beer–Lambert plot on which the complex readings were extrapolated. The analysis was done on a Beckman DU 2 single beam spectrophotometer.

3. Results and Discussion

β-Cyclodextrin decomposes at 297°C while the complex decomposes at 236°C showing a marked depression of 61°C. There are two additional characteristic IR bands at 702 and

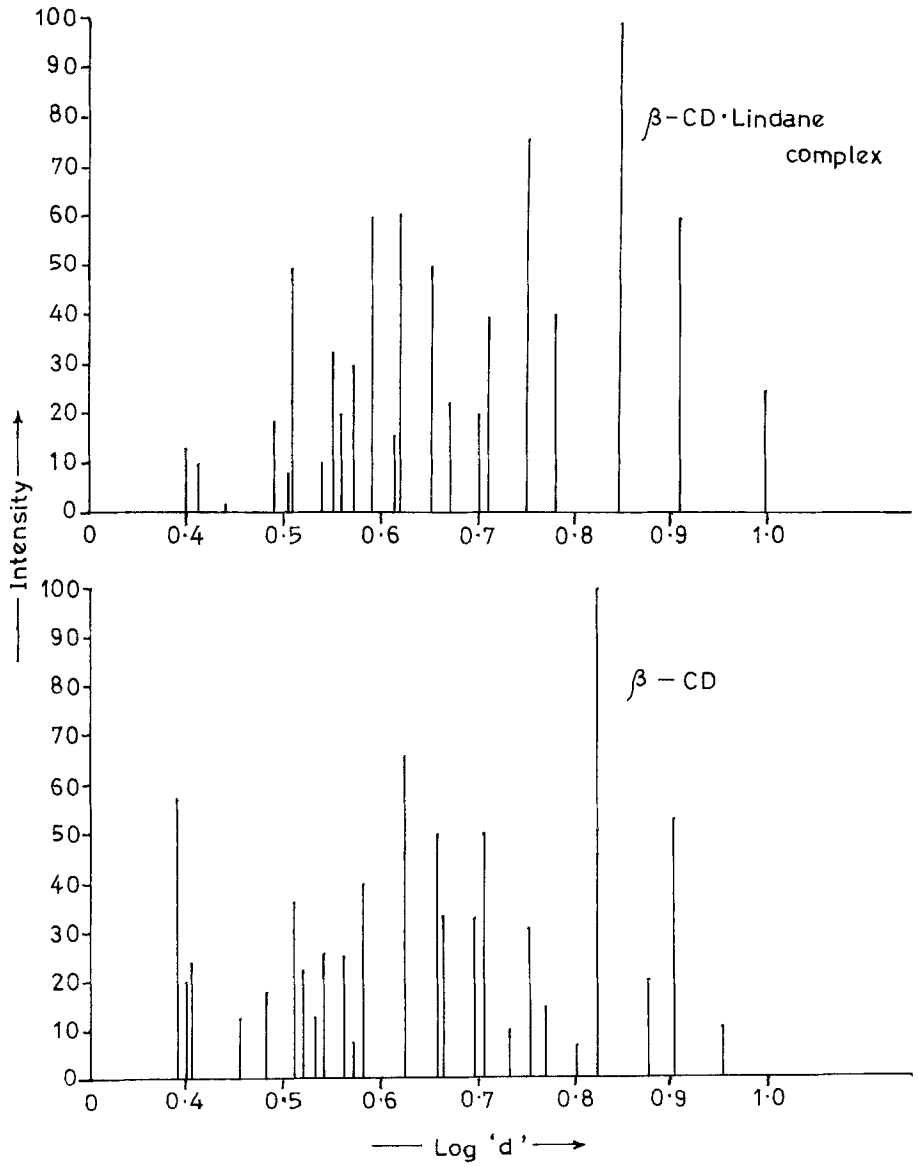

Fig. 2. X-ray powder diffraction pattern (line diagram).

849 cm^{-1} in the spectrum of the complex of lindane with β-CD which are absent in the IR spectrum of β-CD. There is also a positive test for lindane using TLC, indicating the formation of a complex. The X-ray line diagrams of β-cyclodextrin and its inclusion complex with lindane are given in Figure 2. The shifts observed in the latter substantiate the formation of a complex.

The determination of the extent of separation of lindane in the complexes with hexachlorocyclohexane mixtures was the next step. Among the eight well described isomers of hexachlorocyclohexane, the γ-isomer is commercially important due to its insecticidal activity. Separation of this isomer from other isomers is commercially important and is achieved by various techniques like extraction with solvents, percolation etc. Cyclodextrins, due to their spatially restricted cavities, are selective towards the inclusion of one particular conformational isomer. With this perspective in mind, the inclusion compounds of mixtures of isomers of hexachlorocyclohexanes with β-cyclodextrin were prepared, by taking an excess of hexachlorocyclohexanes with β-cyclodextrin in hot water and allowing a complex to form vide experimentally. The determination of the amount of γ-isomer present in the total included hexachlorocyclohexane mixture, was important and interesting. This could be achieved by a separation of the isomers using GLC, which demanded a quantitative separation of the included guest from β-cyclodextrin. In spite of the other methods available for separation, considering the appreciable solubility of cyclodextrins in water and that of lindane in organic phases, a solvent extraction principle was used. Complexes being more soluble in dimethyl formamide are dissolved in it first, and both the host and guest are thrown out of the solution as soon as a little excess of water is added with heating to facilitate declathration. The liberated guest (lindane/hexachlorocyclohexane mixture) can then be extracted into a suitable organic solvent. Considering the milligram quantities of guest involved and the accuracy necessary, and assembly for the quantitative declathration using the centrifugation technique was devised and fabricated (vide experimentally). The efficiency of the extraction was checked by taking known quantities of guest and checking the recovery.

After extraction, suitable aliquots were monitored on the GLC, so as to get on scale, with prior dilutions. The conditions were adjusted so as to get a clear separation of the γ-hexachlorocyclohexane peak from the mixture. The γ-hexachlorocyclohexane peak was identified by the standard addition technique. Thus, a feed mixture containing 16.03% γ-isomer showed 50.4% of the γ-isomer (Table I) in the total included guest showing a clear selectivity towards lindane and suggesting that a clear separation of it from other isomers in a mixture would be possible with a higher percentage of lindane in the feed mixture.

The selective inclusion of lindane rather than the other isomers induced us to determine the lindane content of the complex formed by allowing the β-cyclodextrin in water to mix with pure lindane. The determination of lindane was carried out by spectrophotometry. This entailed separation of cyclodextrin from lindane due to possible interference. This difficulty is overcome by adopting the Schoniger oxygen flask technique by the combustion of

Table I. Lindane content (%) before and after inclusion

Isomer	Feed mixture (%)	Inclusion compound (%)
Lindane	16.03	50.4
Others	83.97	49.6

standards, as well as complexes, followed by a colour development with *o*-toluidine reagent as developed by Scheubeck and Ernst [8]. Based on these, a spectrophotometric method without extraction, using combustion of compounds, both standards and complexes, and absorption of chloride in an absorbing solution containing *o*-toluidine was devised. The complex thus analysed contained 10.4% lindane as obtained by the analysis of ten independent samples (Table II). These results were also confirmed by Schoniger's method for halide determination.

Table II. Analytical data for the inclusion compounds

	Schoniger's determination	Spectro-photometry	GLC ECD
Percent error of method	±0.5%	±2%	±0.5%
Mean lindane (%)	10.4	10.5	10.5
Standard deviation	0.35	0.22	0.15

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