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MULTI-STAGE CHROMATOGRAPHIC PROCEDURE FOR THE ISOLATION OF A SINGLE OLIGOMERIC SPECIES (VINYL CHLORIDE TETRAMER) FROM POLY(VINYL CHLORIDE) RESIN USED FOR FOOD PACKAGING APPLICATIONS

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SUMMARY

A multi-stage chromatographic procedure is described whereby low-molecular-weight material initially obtained by soxhlet diethyl ether extraction from food grade poly(vinyl chloride) (PVC) resin, is fractionated to yield firstly a mixture of vinyl chloride oligomers and then a single species free from other impurities. A number of column chromatographic procedures were evaluated, and a combination of high-performance size exclusion columns using initially tetrahydrofuran solvent followed by toluene solvent were shown to produce the most effective separation. The isolation and purification of a vinyl chloride tetramer was monitored by capillary column gas chromatography with specific chlorine detection (Hall electrolytic conductivity detector) and the identification confirmed by mass spectrometry. Using this newly developed procedure 0.5 mg of vinyl chloride tetramer was isolated from a PVC base resin.

INTRODUCTION

An interest in the low-molecular-weight fraction of poly(vinyl chloride) (PVC) as a potential source for migration from packaging materials into foods, previously led to the isolation of a series of oligomeric species[1,2]. These chlorinated compounds were shown from capillary column gas chromatography–mass spectrometry (GC–MS) data to be two homologous series ranging from trimer to hexamer, each oligomer being represented by species containing a ring system or a double bond and each occurring as a number of structural isomers[2]. Each oligomer was found to be present in the resin at minimum amounts from 1 to 10 mg/kg with both qualitative and quantitative similarities in the pattern of components in a range of commercial food grade resins intended for a variety of food contact applications[2].

For assessment of vinyl chloride oligomers either in terms of designing experiments to monitor their migration or for toxicological evaluation, a precise knowledge of the structure was required. To establish structures unequivocally for such

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novel compounds GC–MS, NMR spectroscopy and microchemical degradative techniques such as ozonolysis (for double bond location) need to be employed, necessitating the separation of single pure individual components in minimum amounts of 0.5 mg.

This requirement led to the development of the multi-chromatographic isolation and clean-up procedure for the purification of a single oligomeric species described in this paper. Of the various oligomers which have been detected, the non-cyclic tetramer was selected for isolation as it appeared to be present in the greatest amount and also exhibited a minimum number of positional isomers.

A number of alternative chromatographic procedures were available for isolation and purification, but of these gas chromatography was discarded because of inherent difficulties of component trapping and because of the risks of thermal decomposition. High-performance liquid chromatography (HPLC) had previously been utilised with some success in conjunction with the Hall electrolytic conductivity detector[3] and in addition high-performance size-exclusion chromatography (HPSEC) was further pursued as both techniques offer the advantages of relatively large sample capacity, simple trapping of separated components and operation at ambient temperature. In this paper the multi-stage chromatographic procedure developed is described and ultimately successfully applied to the isolation of 0.5 mg of non-cyclic vinyl chloride tetramer.

EXPERIMENTAL

Materials

PVC base resin intended for film applications with weight-average molecular weight of 180,000, *K* value of 69 and a viscosity number of 120 (ISO R174) was obtained from Norsk-Hydro (Aycliffe, U.K.).

PL gel HPSEC columns (300 × 7.7 mm) packed with 10 μm poly(styrene–divinylbenzene) of nominal pore size 100 Å were obtained from Polymer Laboratories (Church Stretton, Salop, U.K.). Sephadex LH-60 and SR 100/25 columns were from Pharmacia (Uppsala, Sweden), Biobeads SX-3 (200–400 mesh) from BioRad Labs. (Richmond, U.S.A.) and HPLC columns (Spherisorb S5 ODS and Sperisorb S5W, both 250 × 4.9 mm) were from Hichrom (Reading, U.K.). All solvents were of HPLC grade from Rathburn Chemicals (Walkerburn, U.K.) with the tetrahydrofuran obtained unstabilized, under nitrogen and used within a month of purchase.

Methods

Solvent extraction of base polymer. Base resin (20 g) contained in pre-extracted thimbles was soxhlet extracted for 16 h with re-distilled diethyl ether. After solvent removal on a rotary evaporator and vacuum drying at 40°C the weighed residues were stored at −15°C until required.

Preparative size exclusion chromatography. Sephadex LH-60 columns were prepared and used as previously described[4]. Column lengths were 33 ± 1 cm and two fractions were routinely collected standardised at 0–90 ml and 90–190 ml containing respectively high-molecular-weight and low-molecular-weight material. Total diethyl ether extracts were fractionated in 160–180 mg aliquots dissolved in tetrahydrofuran (THF) (2 ml), the solvent being removed from the collected fractions by evaporation under nitrogen.

Biobeads S-X3 gels were packed by the slurry technique in THF using Pharmacia SR25/45 columns to give a gel bed of 32 ± 2.5 cm. Aliquots (up to 250 mg) of the low-molecular-weight fraction from the Sephadex column in THF (2.0 ml) were subjected to chromatography in the normal manner[4]. From calibration of the column the fraction (53–90 ml) nominally containing material of molecular weight 0–500 was routinely collected for further purification.

Silica gel column chromatography. Purification of the above fractions was carried out using 10 × 1 cm silica gel columns (Merck 7734, 70–230 mesh) packed in hexane. After the sample (up to 80 mg in 2 ml hexane) was loaded onto the column it was washed with hexane (100 ml) and the oligomers eluted with toluene (50 ml). The solvent was removed on a rotary evaporator under vacuum at 60°C prior to further fractionation.

High-performance liquid chromatography. Both HPLC and HPSEC was carried out using a Waters 6000 A pump (Waters Assoc., Milford, MA, U.S.A.) and Rheodyne 7010 injection system (50 μl loop) operating at solvent flows of 1 ml/min. The low-molecular-weight fractions from the preliminary extraction were redissolved in THF (1 ml) and filtered through a 0.45-μm Milipore syringe filter before being evaporated under nitrogen and redissolved in 200 μl fresh THF.

Monitoring of the elution volume of the vinyl chloride tetramer was carried out by collecting 1 ml fractions (Gilson Microcol TDC 80 fraction collector), which were then evaporated under nitrogen and redissolved in THF for GC analysis. Once the approximate elution profile had been established this was further refined by repeating the fractionation but collecting 0.2-ml volumes for GC analysis.

Gas chromatography. Fractions at various stages in the clean-up procedure were analysed by capillary GC using a Carlo Erba 4160 chromatograph. Aliquots (1 μl) in THF solvent were injected using a splitless technique under the following conditions:

Column, 25 m × 0.25 mm fused-silica wall-coated open-tubular column (WCOT) containing 0.12 μm CP SIL 5CB; carrier gas, hydrogen at a flow-rate of 1 ml/min; injector temperature, 200°C; oven temperature, 60°C for 2 min, then programmed at 40°C/min to 130°C at 8°C/min to 300°C followed by 10 min isothermally; detector temperature, 350°C.

Monitoring of fractions was by alternatively a flame ionization detector and/or a Hall electrolytic conductivity detector (Tracor Model 700) operated at a furnace temperature of 760°C, hydrogen make-up gas flow of 60–70 ml/min and isopropanol–water (50:50, v/v) flow-rate of 0.7 ml/min, with a conductivity setting of × 100. The capillary column was interfaced through the side of the GC oven into the furnace reaction tube of the detector. The end of the column projected 15 mm past the hydrogen inlet tube so that the effluent was swept into the detector. Particularly critical was the maintenance of uniform transfer line heating which was achieved by passing the column through a length of copper tubing (75 × 25 mm I.D.) inserted between the detector and the GC exit. The tubing was itself evenly wrapped with heating tape incorporating a thermocouple to monitor the transfer line temperature maintained at 350°C.

Mass spectrometry. GC–MS was carried out using a Carlo Erba 4160 chromatograph directly coupled to a VG 12000 quadrupole mass spectrometer operated under identical chromatographic conditions to those described above. Capillary col-

umns were directly coupled via an all glass system held at 300°C. The mass spectrometer was operated at a source temperature of 200°C with 70eV electron energy and 200 μA current. Spectra were obtained by scanning from m/z 40 to 600 in 1.0 sec. Chemical ionisation spectra were obtained using methane reagent gas.

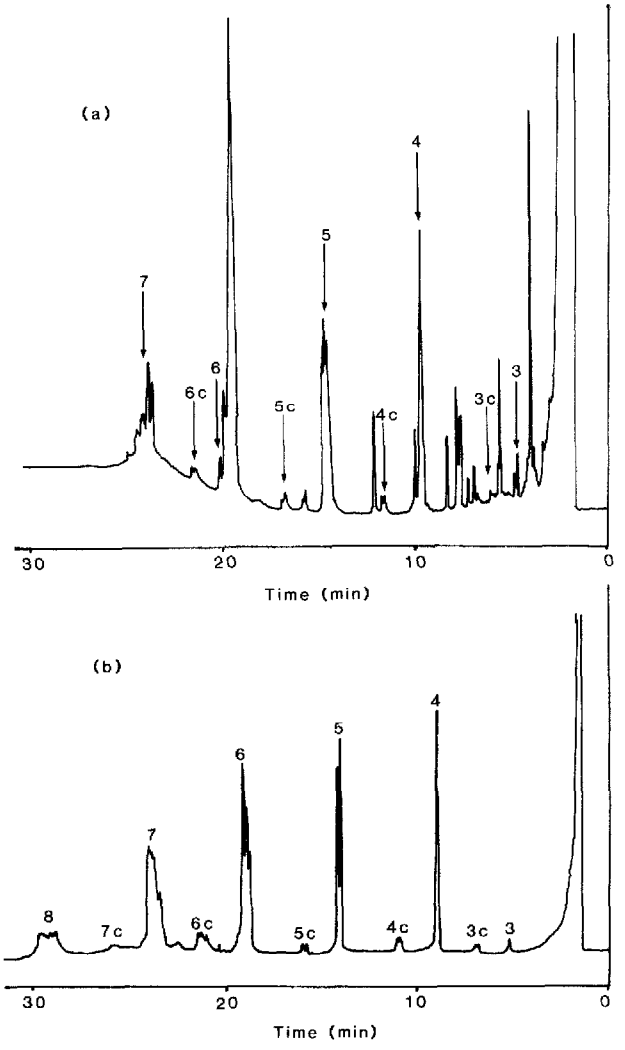

Fig. 1. Gas chromatograms of low-molecular-weight PVC fraction after preliminary clean-up. (a) Flame ionisation detection; (b) chlorine specific Hall electrolytic conductivity detection. Numbering: 3 = VC trimer, 3c = cyclic VC trimer, 4 = tetramer, 4c = cyclic VC tetramer and so forth. Conditions: column, 25 m × 0.25 mm fused-silica (CP SIL 5CB) operated at 1 ml/min hydrogen carrier flow-rate; temperature programme of 60°C for 2 min, 4°C/min to 130°C followed by 8°C/min to 300°C and finally isothermally for 10 min.

RESULTS AND DISCUSSION

The preliminary fractionation procedure consisting of two open-column size exclusion separations followed by clean-up on a silica gel column was essentially unchanged from that previously[3] reported and on average 120 g of the base resin examined yielded 60 mg of low-molecular-weight material (< 500) for further clean-up. Some variations in the amounts collected at this stage were experienced and this was attributed to polymerised THF which could not be evaporatively removed and was difficult to eliminate, despite care in utilising freshly distilled solvent. Similar problems were experienced even when THF was redistilled immediately before use. Fig. 1 illustrates the chromatograms obtained both with flame ionization detection (FID) and chlorine specific detection for this fraction indicating in particular the vinyl chloride tetramer which was selected for further isolation.

Reverse-phase HPLC analysis of the fraction enabled separation of the tetramer from the other oligomers but did not resolve the tetramer from phthalate impurities. Despite modifications in the mobile phase conditions such a resolution could not be achieved. Similar difficulties were encountered with normal phase HPLC although effectively this was only being tested as a chromatographic refinement to the earlier applied silica gel clean-up. With both the HPLC systems examined it was not possible to find a clear chromatographic window for trapping of the oligomer free from other components in the mixture. The time-consuming mode of re-construction

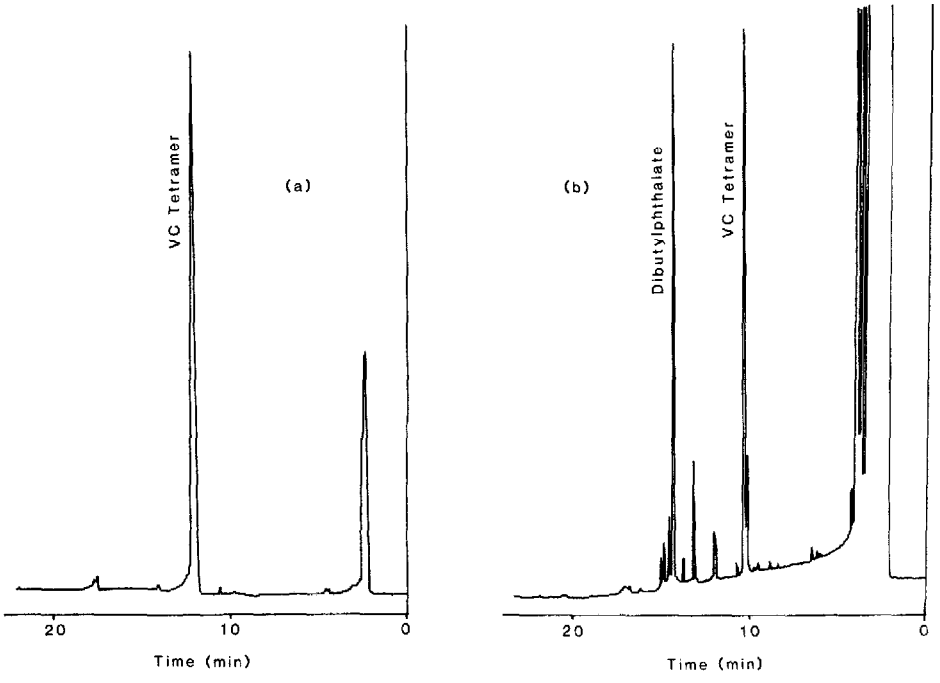

Fig. 2. Gas chromatograms of isolated VC tetramer after HPSEC trapping using THF solvent. (a) Chlorine specific Hall electrolytic conductivity detection; (b) flame ionisation detection. GC conditions identical to Fig. 1.

of the chromatogram by monitoring by GC inevitably restricted the number of LC chromatographic variations that could be examined.

HPSEC gave the best performance in terms both of separating individual oligomers and of separating oligomers from other low-molecular-weight components. With THF as solvent the fraction from 15.4 to 16.0 ml contained non-cyclic VC tetramer which was well resolved from other oligomers. However when examined by GC with FID a second component was evident which was identified by GC–MS as dibutyl phthalate. Typical chromatograms of the vinyl chloride tetramer trapped from HPSEC and analysed by GC with FID and chlorine specific detection are illustrated in Fig. 2.

Separation of tetramer from dibutyl phthalate was achieved by additional HPSEC with toluene as solvent. Both the solvation of solutes and their interaction via π-bonding with the packing material are influenced by solvent choice[5]. In this case the elution volume of the tetramer in toluene was approximately 1.2 ml greater than that in THF. This sugggests that as expected the oligomer was solvated in THF and not in toluene. A similar change in solvation of the phthalate could also be predicted but might have been counteracted in effect by decreased π-bonding of this compound to the gel with toluene as eluent. It was thus possible to achieve a final purification of the tetramer and a chromatogram using FID showing a single species is given in Fig. 3.

The mass spectra for the isolated tetramer are shown in Fig. 4 in both electron impact and chemical ionisation modes both employed for confirmation of identifi-

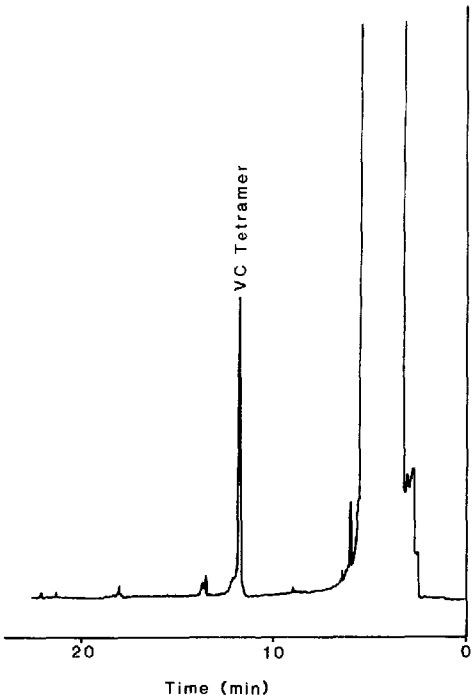

Fig. 3. Gas chromatogram with flame ionisation detection of final purified VC tetramer after trapping from HPSEC column using toluene solvent. GC conditions identical to Fig. 1.

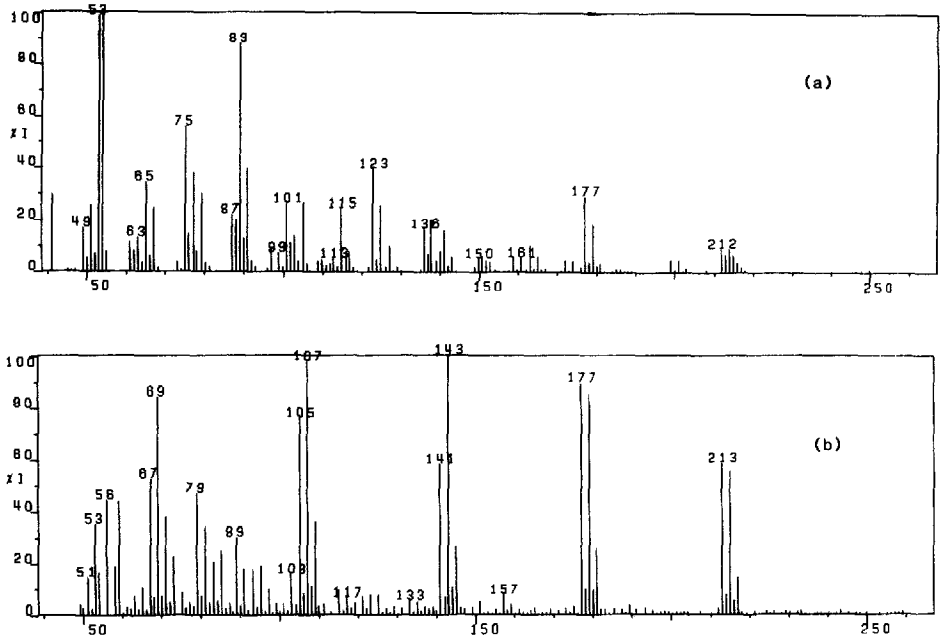

Fig. 4.
Mass spectra of purified non-cyclic VC tetramer obtained by GC–MS. (a) Electron impact mode; (b) chemical ionisation mode using methane reagent gas.

cation. As previously observed[3] the tetramer showed a characteristic pattern of ions due to (M – Cl), (M – HCl) and (M – HCl_2) with a molecular ion present at only low intensity. Chemical ionisation using methane as reagent gas failed to enhance the weak molecular ion and produced intense peaks, for ions appearing to correspond to successive losses of chlorine. This behaviour was typical of chlorinated alkenes and for example an analogous chemical ionisation spectrum was obtained for dichlorobutene.

A schematic illustrating the final overall isolation procedure is shown in Fig. 5 from which acceptable chromatographically pure tetramer could be isolated, and from which other components for further analysis could be collected. The yield of purified material was around 0.1 mg from an initial soxhlet extraction of 120 g of PVC resin, despite earlier indications of amounts of this oligomer occurring at least ten times this level. Not surprisingly the overall recovery of a single component from such a multi-stage chromatographic fractionation is low, not least due to the many evaporative steps involved. Efforts to scale-up the initial extraction were unsuccessful, possibly due to inadequate solvent penetration of a larger bulk of resin, and to achieve the final target of 0.5 mg of purified material multiple batch extractions were carried out combining the isolated material at the end of the procedure. With little modification it is anticipated that the procedure should be adaptable to the isolation of other individual oligomers from the mixture although the chromatographic conditions will need to be established separately.

With the development of the purification procedure reported in this paper the way is now open to the isolation and structural characterisation of a range of indi-

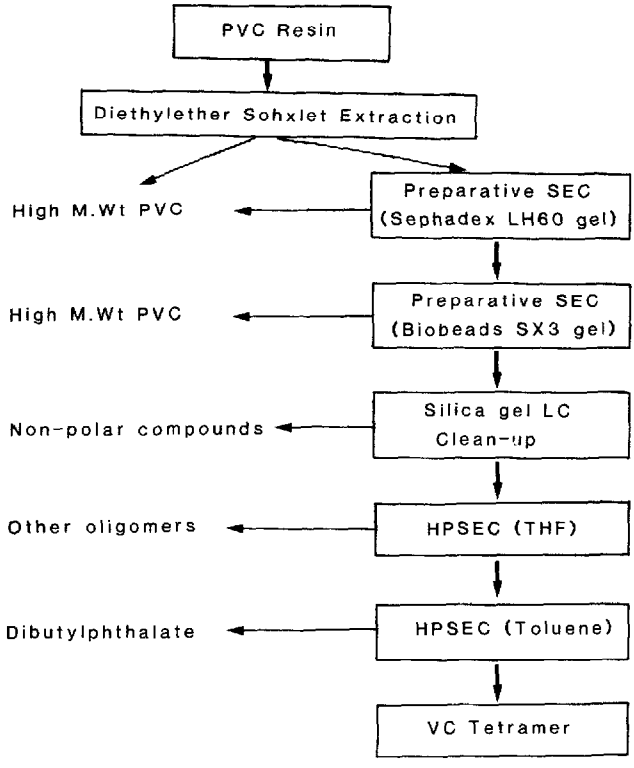

Fig. 5. Schematic illustration of multi-stage chromatographic isolation procedure for VC tetramer.

vidual oligomers. As a first stage vinyl chloride tetramer has been isolated and spectroscopic studies will be reported elsewhere in confirmation of its structure.

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