

## Note

### **Selected-ion monitoring of 4-vinyl-1-cyclohexene in acrylonitrile-butadiene-styrene polymer products and food simulants**

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In recent years, there has been increasing concern with the migration of substances from packaging materials into foods, particularly that of monomers from plastics. Because the extent of monomer migration is related to its concentration in a polymer, it is important to determine residual levels. As evidence has been presented linking the inhalation of vinyl chloride with the occurrence of angiosarcoma<sup>1,2</sup> of the liver, attention has been directed to the possible presence of other monomer residues in food packaging materials. Residual data have been published for styrene<sup>3,4</sup>, vinylidene chloride<sup>5–8</sup>, acrylonitrile<sup>9,10</sup> and butadiene<sup>11,12</sup>.

1,3-Butadiene (BD) is widely used industrially, especially as a polymer component in the manufacture of synthetic rubber and copolymers such as acrylonitrile-butadiene-styrene (ABS) resins, which are regularly used for packaging foodstuffs.

BD is a vinyl-substituted ethylene and, like vinyl chloride, it is believed to be metabolized in humans via an epoxide intermediate<sup>13</sup>. BD monoxide has been found to have mutagenic activity in bacteria<sup>13</sup>. Moreover, in an inhalation test<sup>14</sup> with BD and 4-vinyl-1-cyclohexene (VCh), a BD dimer, the ciliated epithelium of rat trachea was considerably stripped and the cilia themselves were greatly stunted.

Although the determination of BD in plastics by gas chromatography with flame ionization detection (GC-FID) has been described previously<sup>11,12</sup>, there are no reports of the chromatographic determination of VCh in plastics. When we previously analysed BD-based polymers for VCh<sup>15</sup>, none could be detected in polybutadiene household wrapping films, but ABS products were found to contain VCh. Retail ABS polymer products can be analysed for VCh by selected-ion monitoring (SIM), and this technique is now routinely used in many laboratories as a highly specific chromatographic detector for quantitative measurements in the selected-ion mode. It offers the additional advantage of much greater sensitivity than FID.

Because of its possible toxicity, we were interested in measuring residual VCh in ABS polymer products and establishing its potential for migration into foods using two lots of food-grade ABS sheets. In testing extractables from food packaging materials, it is customary to use food-simulating solvents rather than foods themselves. This practice was followed in the procedures described here, using water, 4% acetic acid, 20% ethanol and *n*-heptane as food simulants.

## EXPERIMENTAL

*Materials*

Retail ABS products were purchased from supermarkets and food-grade ABS sheets were prepared and their surface areas were simply measured for migration experiments; the sheet thickness was 1.05 mm.

4-Vinyl-1-cyclohexene was obtained from Tokyo Chemical Industries (Tokyo, Japan), UV-grade N,N-dimethylformamide (DMF) from Wako (Osaka, Japan) and organic solvents were of analytical-reagent grade.

*Determination of VCh in ABS products*

*Sample preparation.* ABS products were cut into narrow strips (ca. 1 cm × 0.2 cm). Samples of about 1.0 g were weighed accurately into volumetric flasks and the volume was adjusted to 20 ml with DMF. The volumetric flasks were then capped and left overnight to dissolve the plastic.

*Selected-ion monitoring.* A Shimadzu GC-9A gas chromatograph was coupled through an all-glass jet separator to a Shimadzu QP-1000 quadrupole mass spectrometer. Chromatography was carried out on a 0.5 m × 3 mm I.D. glass column packed with 15% DC-550 silicone on 60–80-mesh Gasport A (Gasukuro Kogyo, Tokyo, Japan) held at 60°C for 3.5 min and then programmed to 120°C at 20°C/min to remove DMF, using helium as the carrier gas at a flow-rate of 40 ml/min. The injection temperature was 200°C, the analysis time 7.5 min and the stabilization time about 10 min.

The mass spectrometer was operated in the electron-impact mode (electron energy 70 eV, trap current 60  $\mu$ A) and maintained at 250°C. Multiple ion monitoring of the ions at  $m/z$  54 (base peak of VCh) and 79 was done under computer control.

*ABS products analysis.* Volumes of 5  $\mu$ l of polymer solutions in DMF were injected into the gas chromatograph–mass spectrometer under the conditions described above. Determination of VCh in the ABS products was based on peak-area measurement and from a calibration graph for a VCh in DMF standard analysed immediately prior to the sample.

*Determination of VCh in food simulants*

*Sample preparation.* An extractability test was adapted from the food sanitation law of Japan<sup>16</sup> for use in this study. The four food simulants used were as follows: water to simulate non-acid aqueous food products, 4% acetic acid as the solvent for acidic food products, 20% ethanol to simulate alcoholic food products and *n*-heptane to simulate foods containing free oil or fat.

ABS sheets of area 100 cm<sup>2</sup> were placed with 200 ml of food simulants (a rate of 2 ml per cm<sup>2</sup>) in a closed container. When water, 4% acetic acid and 20% ethanol were used, each container was maintained for 30 min in a water-bath at 60°C, and when *n*-heptane was used, for 1 h in a forced-air oven at 25°C. After being in contact with each other, the ABS sheets in the container were removed and each eluate was allowed to cool to room temperature, and then treated as follows.

*Water and 4% acetic acid.* A 50-ml aliquot of the eluate was extracted with 20 ml of *n*-hexane for 10 min in a separating funnel, the aqueous layer was shaken with 20 ml of *n*-hexane and the combined organic layers were dried over 2 g of anhydrous

sodium sulphate. The organic layer was transferred into volumetric flasks and the volume was adjusted to 50 ml with *n*-hexane.

*20% Ethanol.* To avoid emulsion formation, 2.5 g of sodium chloride were added to 50 ml of eluate and the procedure as described above was followed for the water and 4% acetic acid sample preparation.

*n-Heptane.* The eluates were used directly as sample solutions.

*Selected-ion monitoring.* Identical apparatus and conditions to those already described were used.

*Food simulant analysis.* Volumes of 10  $\mu$ l of *n*-hexane extract solutions in volumetric flasks and *n*-heptane eluates were injected into the gas chromatograph-mass spectrometer system under the conditions described above. Determination of VCh in the food simulants that had migrated from ABS sheets was based on peak-area measurement and from the calibration graph for the VCh in *n*-hexane or *n*-heptane standard analysed immediately prior to the sample.

## RESULTS AND DISCUSSION

VCh (b.p. 126–127°C) is well suited for liquid sampling. As a liquid at ambient temperature, it is readily handled with volumetric glassware. It is readily soluble in all the organic solvents used in this study. VCh elutes well ahead of DMF on a DC-550 silicone GC column and is well separated from any interferences present.

Gas chromatographic-mass spectrometric confirmation of VCh requires a high level of specificity at low sample concentrations to be maintained. The full mass scan of VCh revealed that the two ions at  $m/z$  54 and 79 constituted the base peak and *ca.* 60% of the relative intensity. Multiple ion monitoring, *i.e.*, monitoring of peaks from a limited number of ions, of these two ions was carried out to enhance the sensitivity beyond that obtainable by full mass scans. The specificity for VCh was maintained in the multiple ion monitoring mode by careful attention to the relative areas of the monitored ions.

### *Determination of VCh levels in polymers*

VCh levels in ABS products purchased from retail outlets were determined by injecting solutions of the polymer. The retention time for VCh was 2.9 min. The VCh levels found in ABS products in this study varied with each sample (Table I), ranging from 29 to about 200 mg/kg. VCh in ABS sheet was confirmed by correspondence of

TABLE I  
OBSERVED LEVELS OF 4-VINYL-1-CYCLOHEXENE IN RETAIL ABS PRODUCTS

<i>Product</i>		<i>4 Vinyl-1-cyclohexene</i> (mg/kg)
Food-grade sheet:	1	104
	2	205
Ladle		132
Grater		88
Lunch tray:	1	110
	2	29

TABLE II  
MIGRATION OF 4-VINYL-1-CYCLOHEXENE (VCh) FROM TWO LOTS OF FOOD-GRADE ABS SHEETS TO *n*-HEPTANE FROM 1 TO 10 DAYS AT 25°C

Lot No.	Surface area (cm <sup>2</sup> )	Weight (g)	Volume of <i>n</i> -heptane (ml)	Level of VCh (mg/kg)	Total VCh found (mg/kg)	Day	Concentration of VCh found in solvent (mg/kg)	Calculated total VCh extracted (μg)	Total VCh extracted (%)
1	100	5.7	200	104	593	1	0.30	60	10
						5	0.62	124	21
						8	0.69	138	23
						10	0.72	144	24
2	100	5.7	200	205	1169	1	0.46	92	8
						5	0.88	176	15
						8	0.96	192	16
						10	1.02	204	17

the retention times, ion profiles and relative areas of the monitored ions with those of VCh standards.

A 5% solution of polymer in DMF was used because higher concentrations became cloudy when cooled to room temperature. The limit of quantification on direct injection of about 5  $\mu$ l of these 5% solutions was about 0.5 mg/kg for VCh, equivalent to about 10 mg/kg of VCh in the polymer. The recovery of VCh added to 5% ABS polymer solution was 89–103% in the range of 40–80  $\mu$ g per sample and the coefficient of variation was 5.4%.

#### *Determination of VCh in food simulants*

This SIM procedure is very useful in migration studies with food simulants where the concentrations of VCh may be extremely low. Such studies often require higher analytical sensitivity and much lower noise than can be achieved by GC-FID. Food simulants spiked with VCh at concentrations of 2.5 and 5.0  $\mu$ g were prepared. The recoveries of VCh were found to be 98–100, 102–97 and 94–91% from water, 4% acetic acid and 20% ethanol, respectively. Blank food simulants should be tested to ensure the absence of extraneous chromatographable compounds eluting at the retention time of VCh.

#### *Migration experiments*

To illustrate the applicability of the method, experiments were performed to determine the extent of migration of VCh into water, 4% acetic acid, 20% ethanol and *n*-heptane and the approximate rates. VCh levels were determined in two lots of food-grade ABS sheets. ABS lots 1 and 2 contained 104 and 205 mg/kg of residual VCh, respectively. Duplicate sheets of each lot, measuring approximately 100 cm<sup>2</sup>, weighing 5.7 g and 1.05 mm in thickness, were then immersed in water, 4% acetic acid or 20% ethanol for 30 min at 60°C and in *n*-heptane from 2 h up to 10 days at 25°C.

No migration of VCh from the two lots of food-grade ABS sheets into the four food simulants was found, with a detection limit of 0.05 mg/kg, under the effluent conditions of the sanitation law of Japan for total migration from various polymers. Migration levels into *n*-heptane were monitored periodically for up to 10 days because VCh itself shows some tendency to migrate into oily foodstuffs. These analyses, given in Table II, gave concentrations of 0.72 and 1.02 mg/kg of VCh in *n*-heptane for lots 1 and 2, respectively. These concentrations represent total migrations of approximately 24 and 17%, respectively, of the residual VCh from ABS sheets into *n*-heptane.

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