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THE DETECTION AND ESTIMATION OF PENTACHLOROPHENOL
IN NATURAL LATEX BY THIN-LAYER CHROMATOGRAPHY

J. R. DAVIES AND S. T. THURAISINGHAM

The Natural Rubber Producers' Research Association, 48-56 Tewin Road, Welwyn Garden City, Herts. (Great Britain)

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SUMMARY

A method is described for the detection and estimation of pentachlorophenol (PCP) in natural latex, by separation of PCP from other acetone-soluble substances by thin-layer chromatography on silica gel. The quantitative estimation is based on the measurement of the PCP spot area under specified conditions.

Pentachlorophenol (PCP), a germicidal agent, has been used as a preservative for natural rubber latex. In "low ammonia" latex concentrates the preservative consists of *ca.* 0.2% ammonia with *ca.* 0.2% pentachlorophenol (sodium or ammonium salt) as the secondary bactericide.

Since the introduction of PCP in this application there has been a requirement for an analytical method for the detection and estimation of PCP in natural rubber latex which would be both rapid and applicable to large numbers of samples.

Several methods^{1,2} involving isolation of PCP by either solvent extraction or steam distillation procedures are available but these are rather time consuming when used on a routine basis.

Thin-layer chromatography has been widely applied to the analysis of chlorinated pesticide residues in agricultural products³ because it is generally faster, more sensitive and specific than other methods normally employed. This paper describes the application of thin-layer chromatography to the detection of PCP in natural rubber latex by initially resolving it from the other non-rubber constituents in the latex followed by use of a specific spray reagent to locate the PCP.

EXPERIMENTAL

Natural rubber latex contains a large number of non-rubber components⁴, including squalene, carotene, sterols, esters, fatty acids, tocopherols, phospholipids, a large proportion of which pass into the rubber phase when latex is coagulated and which will be associated with the PCP added to the latex sample.

The basis of the adopted method is that the latex sample is coagulated with 2% acetic acid and a known amount of the coagulated rubber film is extracted with acetone

under reflux for 12 h, followed by examination of the extract by thin-layer chromatography. To obtain the optimum conditions for separation of the PCP from the other acetone soluble materials, preliminary investigations were carried out on silica gel plates using mixtures of solvents of different polarities and a variety of spray reagents. Difficulty was observed in obtaining a specific spray reagent for PCP because the phenolic components in latex, *i.e.* tocopherols/tocotrienols, gave comparable colour reactions to the PCP.

Good separations were obtained using a chloroform-acetic acid (100:1 by vol.) mixture as developing solvent and a spray reagent of copper sulphate-pyridine solution which gives specific and well shaped spots with PCP. A typical chromatogram is shown in Fig. 1.

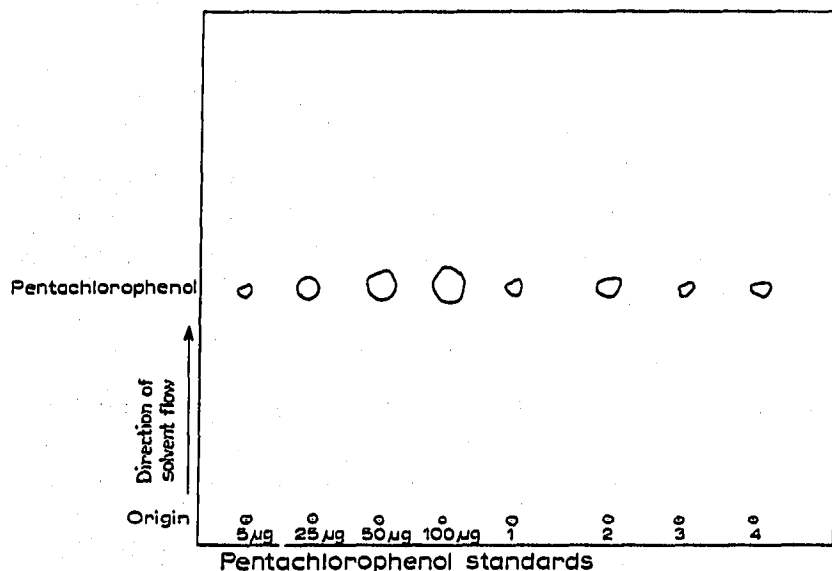


Fig. 1. Typical chromatogram of extracts of natural rubber latex films containing pentachlorophenol. 1, 2, 3, and 4 are extracts of natural latex films.

METHOD

Apparatus

Soxhlet extraction apparatus (as in B.S. 903).

Microcap glass pipettes, Drummond type, 5 μ l capacity.

Thin-layer chromatography spreader, Shandon Scientific Company.

Reagents

All reagents should conform to recognized analytical reagent quality.

Silica gel, Kieselgel G, Merck

Acetone

Chloroform

Acetic acid

Carbon tetrachloride

Copper sulphate-pyridine reagent (freshly prepared): 5 g copper sulphate and 5 g pyridine made up to 100 ml with water-acetone 10:1)

Pentachlorophenol (m.p. 190°)

Standard solution of pentachlorophenol 0.1–2.0% w/v in carbon tetrachloride.

Procedure

The latex concentrate sample (ca. 30 g) is coagulated with 2% acetic acid as described in B.S. 1672⁵ to give a thin uniform film not exceeding 2 mm thick.

Weigh accurately 10 g (± 0.01) of the film and extract with acetone in a vapour jacketed type Soxhlet apparatus for 12–16 h (*i.e.* overnight). After removal of the acetone by distillation the residue is dissolved in carbon tetrachloride and diluted to 2 ml volume in a graduated flask. The silica gel plates are prepared from a slurry of silica gel–water (1:2, w/v) spread onto 20 × 20 cm glass plates using the applicator to give a thickness of 0.25 mm. The plates are then dried for 1 h at 105° and stored in a desiccator prior to use.

Suitable aliquots (normally 5 μ l) of the extract solution are transferred to the silica gel plate, together with the same aliquot of the standard PCP solutions to cover the concentration range 0–100 μ g of PCP. (Ten samples and five standards can be easily accommodated on one 20 × 22 cm plate.) The chromatogram is developed using a solvent system of chloroform–acetic acid (100:1, v/v) in a conventional tank lined with filter paper to ensure saturation of the tank atmosphere. When the solvent front has travelled 15 cm the plate is removed and dried prior to spraying with the copper sulphate–pyridine reagent in a fumehood. PCP gives an intense brown spot on a light blue background showing one component of R_F ca. 0.5. There is little or no interference observed from the non-rubber components normally found in the latex film extract. Comparison of the spot colour and R_F value for the samples and standards will show whether or not PCP is present. The limit of detection is ca. 2 μ g PCP which corresponds to 0.01% w/w PCP in rubber under the conditions previously described.

For the semi-quantitative estimation of the PCP the area of the spot is estimated by tracing onto mm² squared paper and counting the squares under the spot. A cali-

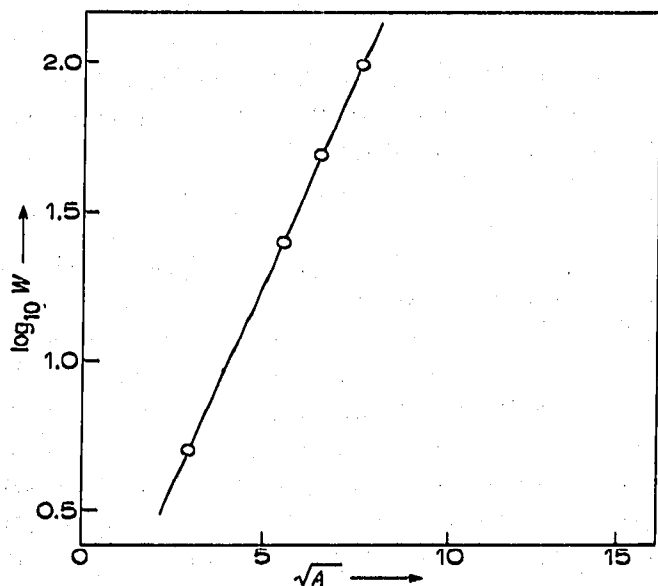


Fig. 2. Calibration graph of pentachlorophenol standards by thin-layer chromatography.

bration graph is prepared from the PCP standards using the relationship⁶:

$$\sqrt{A} = K \log_{10} W,$$

where

A is the spot area (mm^2)

W is the weight of PCP applied (μg)

K is a constant

A plot of \sqrt{A} versus $\log_{10} W$ (Fig. 2) gives a linear relationship over the concentration range 0–100 μg of PCP.

Typical results for PCP in natural rubber latex are given in Table I, the standard deviation being *ca.* $\pm 10\%$ at the 0.2% level of PCP.

TABLE I

PENTACHLOROPHENOL CONTENT IN NATURAL RUBBER LATEX FILMS

Sample	% w/w pentachlorophenol
A ^a	0.04; 0.02; 0.02; 0.02; 0.03
B ^a	0.07; 0.05; 0.05; 0.07; 0.07
C ^b	0.20; 0.17; 0.21; 0.22
D ^c	0.38; 0.39; 0.37; 0.41

^a Commercial samples.

^b Specially prepared containing 0.2% w/w.

^c Specially prepared containing 0.4% w/w.

The results show that PCP can be detected and estimated with reasonable accuracy by this method, giving a considerable reduction in time required for analysis (*ca.* 40 min per sample excluding the extraction procedure). The simplicity and rapidity of the technique will be useful in production control of PCP in natural rubber latex.

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