DETERMINATION OF CASTOR OIL IN HEVEACRUMB RUBBER BY THIN-LAYER CHROMATOGRAPHY

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INTRODUCTION

Heveacrumb rubber is a natural rubber prepared by a process in which coagulated latex is crumbled by the addition of 0.7 % (based on the weight of rubber) of castor oil prior to milling. The rubber is then washed with water to remove the excess castor oil, dried and compressed into bales. A precise method was required for process control of the residual level of castor oil both at the plantations and in the customers' laboratories.

The main components in castor oil are the unsaturated hydroxy fatty acid glycerides[1]

triricinolein 75 % w/w
diricinolein 25 % w/w

as well as traces of free acids (C_{16}, C_{18} saturated, oleic, linoleic, ricinoleic, dihydroxystearic). Natural rubber also contains about 1 % of lipid materials[2] such as phospholipids, sterols, tocopherols, glycerides, carotenes, squalene etc., and it is obvious that any analytical procedure must be separative, in order to distinguish between the naturally occurring glycerides in rubber and those derived from added castor oil. The technique of thin-layer chromatography seemed ideally suited to this problem in terms of simplicity, time required and subsequent use on a routine basis.

EXPERIMENTAL

To establish the optimum conditions for the separation of castor oil from the other acetone-soluble materials in natural rubber, preliminary investigations were carried out on silica gel using mixtures of solvents of different polarity. Good separations were obtained using as developing solvent a light petroleum ether–ether–acetic acid mixture (62:37:1, v/v) and satisfactory spots were obtained with a phosphomolybdic acid spray, followed by heating to 105°. Methanolic iodine solution or a 50 % (v/v) sulphuric acid were also suitable spray reagents. A typical chromatogram is shown in Fig. 1.

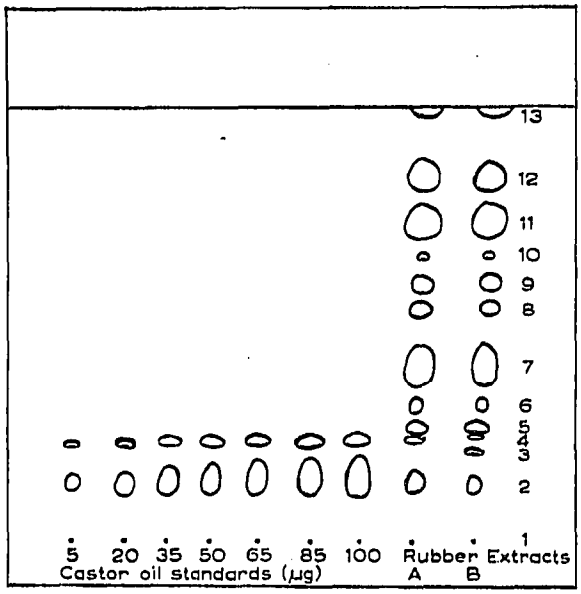

Fig. 1. Typical chromatogram of standard castor oil solutions (5–100 μg) and Heveacrumb rubber extracts (A and B) on silica gel plates. Solvent: petroleum ether (40–60°)–ether–acetic acid 62:37:1. Spray: 10% methanolic phosphomolybdic acid. 1 = Origin, 2 = castor oil (triglyceride); 3 = unknown; 4 = castor oil (diglyceride); 5 = β-sitosterol; 6–13 = lipid components in natural rubber.

METHOD

Apparatus

Soxhlet extraction apparatus
Hypodermic syringe—glass, Hamilton type, 10 μl capacity
Microcap pipettes—glass, Drummond type, 2 or 5 μl
TLC spreader

Reagents

Silica gel—Kieselgel G, Merck
Acetone, Analar
Petroleum ether (40–60°), Analar
Diethyl ether, Analar
Acetic acid, glacial, Analar
Carbon tetrachloride, Analar
Phosphomolybdic acid, reagent grade—10% methanolic solution
Castor oil, B.P.
Castor oil reference solutions in carbon tetrachloride, 0.1–1.0% w/v
Alumina, chromatography grade
Petroleum ether (40–60°)–diethyl ether–acetic acid, glacial (62:37:1, v/v)

PROCEDURE

A representative sample of the Heveacrumb rubber is lightly milled and cut into small pieces and 10.00 ± 0.01 g are extracted in the Soxhlet apparatus with

100 ml of acetone for 12–16 h (*i.e.* overnight). After removal of the acetone by distillation the residue is dried for 20 min at 105° and then dissolved in 1–2 ml of carbon tetrachloride. The solution is then applied to a short alumina column (12–15 cm by 6 mm diameter) and the first fraction eluted with 20 ml of carbon tetrachloride. The second fraction is eluted with 30 ml of ether and the elution is then continued with 20 ml of acetone. The ether fraction is taken to dryness and dissolved in a known volume of carbon tetrachloride (normally 10 ml). As a check on the separation of the castor oil and to standardise on procedure, the other two fractions may be treated similarly and chromatographed concurrently with the ether-eluted fraction.

The silica gel plates are prepared from a slurry of silica gel–water (1:2, w/v), spread onto 20 × 20 cm or 8 × 20 cm glass plates with a Shandon applicator or other levelling device, to give a thickness of 0.25 mm, followed by drying in an oven at 105° for one hour and storing in a desiccator prior to use.

The solvent mixture is contained in a conventional developing tank lined with filter paper soaked separately in the solvent mixture to ensure saturation of the tank atmosphere and sealed securely to minimise loss by evaporation.

Suitable aliquots (2–5 μl) of the extract solutions are transferred to the silica plates, ensuring that the size of the spot is as constant as possible. Aliquots (2–5 μl) of standard castor oil solutions in carbon tetrachloride containing 5–50 μg of castor oil are transferred simultaneously onto the plate (normally ten samples and five standards can be accommodated on one 20 × 20 cm plate). The plate is then placed in the solvent tank until the front has travelled 15 cm and is then removed and dried. The plate is then sprayed with 10 % methanolic phosphomolybdic acid and heated for ten minutes at 105°, revealing intense blue spots on a yellow background. Castor oil shows two main components which correspond to the triglyceride (R_F 0.19) and the diglyceride (R_F 0.28). Comparison of the R_F values for the standards and rubber extracts will show whether or not castor oil is present.

For the quantitative determination of castor oil the phosphomolybdic acid spray should be applied thoroughly and evenly and the heating at 105° continued to the point of optimum differentiation so that intense blue spots have well-defined edges against the yellow background. The area of the glyceride spot (R_F 0.19) is estimated by illuminating the plate from below and drawing over a second glass plate onto tracing paper marked in mm squares. The area is obtained by either counting the squares or weighing the cut-out areas on a micro balance. The castor oil content of the rubber is calculated from the relationship[3]

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

which was found to be valid over the range of 1–100 μg of castor oil, where

A = area of the triglyceride spot,
W = weight of castor oil applied,
K = a constant determined from the castor oil standards.

RESULTS

Results obtained using standard solutions of castor oil are given in Table I, showing that there is a direct relationship between the logarithm of the sample weight and the square root of the spot area.

Typical results obtained for Heveacrumb rubbers are given in Table II.
The precision of the method expressed as twice the standard deviation is better than ± 5 %.

TABLE I

RELATIONSHIP BETWEEN CASTOR OIL SAMPLE SIZE AND SPOT AREA

Plate No.	*Weight of castor oil (μg)*	*Log_{10} weight*	*Area from TLC (mm^2)*	*$\sqrt{Area}$*	*$K = \frac{\sqrt{Area}}{Log_{10}\ weight}$*
1	10	1.00	20	4.47	4.47
	30	1.47_7	44	6.62	4.49
	50	1.69_8	58	7.60	4.49
2	10	1.00	17.5	4.13	4.13
	30	1.47_7	38	6.16	4.18
	50	1.69_8	50	7.07	4.16
	75	1.87_5	60	7.75	4.14
	100	2.00	68	8.25	4.12

TABLE II

CASTOR OIL CONTENT OF HEVEACRUMB RUBBERS

Sample	*Castor oil (% w/w)*
SMR 5L	0.52; 0.50; 0.53; 0.50
SMR 5L	0.34; 0.34
SMR 5	0.24; 0.23
SMR 5	0.22; 0.22; 0.21; 0.23
SMR 20	<0.05

DISCUSSION

The results show that the residual castor oil in Heveacrumb rubbers can be determined with a level of accuracy (± 5 % at the 0.2–0.5 % level) which is adequate for control purposes. The critical factors in maintaining the accuracy are the construction of even layered plates, the accurate dispensing of the sample aliquot to the plate and the estimation of the spot areas.

The preliminary column separation was introduced because it was found when working with rubber extracts that a considerable amount of polar material had a lower R_F value than the castor oil components, the chromatograms showing material at the origin and a fairly wide streak enveloping the castor oil spot. This material affects the spot area since radial chromatography in the original spot will place the castor oil in a ring round the material of lower R_F value. Removal of this material using column chromatography showed the castor oil standard and the rubber extract fraction to be very similar in the lower R_F region.

Occasionally a rubber extract shows a spot of similar R_F value to the main castor oil spot and barely separated from it. This spot appears to be associated with

the lipid components in natural rubber, as on coincidental spotting of the sample extract and castor oil two distinct spots are detected on development. Castor oil can be differentiated from this component at the 0.1 % level of castor oil.

If interference is observed, the thin-layer chromatography may be more usefully performed on silica gel impregnated with boric acid. The R_F values of the castor oil components are then altered due to complexing of the hydroxyl groups, an effect observed by MORRIS[4]. Thus a separation from an interfering spot can be achieved, enabling the quantitative measurement to be made as in the normal procedure.

This work forms part of a joint investigation by the Natural Rubber Producers' Research Association and the Dunlop Company.

SUMMARY

A method is described for determining the residual castor oil in Heveacrumb rubber, by the separation of castor oil from the other acetone-soluble substances by thin-layer chromatography on silica gel. The quantitative estimation is based on measurement of the area of the triglyceride component of castor oil under specified conditions. Typical results are given for Heveacrumb rubbers showing good reproducibility.

REFERENCES

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