However, for a fixed set of conditions, the determination of spot size lends itself fairly well for an, at least approximate, quantitative determination of the contents of the spots.

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A thin-layer chromatographic method for distinguishing between natural rubber and synthetic polyisoprene

With the advent of increasing production of synthetic polyisoprene and subsequent use in commercial vulcanizates, there was an obvious need for a simple, reliable method for distinguishing between natural rubber and synthetic polyisoprene, to be used in a programme of research undertaken by the Association.

The present analytical methods, *e.g.*, the Weber test, infra-red spectroscopy etc., will only identify the polymers as *cis*-polyisoprene and will not differentiate between natural rubber and synthetic polyisoprene. Several methods have been published[1,2] which depend on the identification of a minor constituent of natural rubber not found in synthetic polyisoprene. The disadvantages of these methods are that they are time-consuming and present some uncertainty when dealing with rubber of unknown origin.

Natural rubber contains about 1 % w/w of extractable lipid materials[3,4] consisting of phospholipids, sterols, tocopherols, tocotrienols, carotenes, squalene etc., whereas synthetic polyisoprene will only contain ingredients added during manufacture. A new approach to this problem is based on examination of a solvent extract of the rubber by the technique of thin-layer chromatography, in order to characterize and distinguish between the lipid components in natural rubber and the additives in synthetic polyisoprene.

Experimental

Five grams (± 0.01 g) of the rubber sample (raw, gum or vulcanized: thinly sheeted on a mill) were extracted for 12 h with Analar acetone under reflux. After removal of the acetone by distillation the extract was dried at 105° for 10 min, dissolved in Analar carbon tetrachloride and diluted to 5 ml in a graduated flask. Silica gel (50 g) (Kieselgel G nach STAHL; neutral grade) was used as the substrate,

being slurried with distilled water (100 ml) and applied to clean, grease-free plates using a Shandon applicator. The plates were activated by heating in an air-oven at 105° for 30 min, followed by cooling and storing in a desiccator.

Suitable aliquots (5 μl) of the extract were applied to the activated plate using Drummond microcap pipettes and a multiple-spotting template.

A solvent system of 40–60° petroleum ether–ether (50:50, v/v), Analar grades, was used for ascending elution of the plates at a constant temperature of 20 ± 1°. The plates were developed in tanks lined with filter paper and sealed with masking tape, to ensure complete saturation of the tank atmosphere with solvent vapour. The length of the run was 150 ± 5 mm, the time taken for the solvent front to travel this distance being 35 min.

After elution the chromatograms were dried and then sprayed with phosphomolybdic acid solution (10% w/v in methanol). The lipids in natural rubber gave a blue spot on a yellow background. One of these components, β-sitosterol, is only detected after heating at 105° for 10 min and it is detection of this component that forms the basis of the described method.

Results

Raw rubbers. Various grades of natural rubber (RSS1, RSS3, RSS5, pale crepe, SMR 5–50, SMR Heveacrumb 5–50 etc.) and synthetic polyisoprene (Cariflex, Natsyn 200, 400 and 2000) were examined by the described technique. The chromatograms obtained showed that the natural rubber extracts contained a component of R_F value 0.40 ± 0.02 units, only being detected on heating the plate at 105° for 10 min. This component has been identified as being β-sitosterol by several workers[5,6] and confirmed in these laboratories by comparison of the R_F value obtained from an authentic sample of β-sitosterol (recrystallized × 6; m.p. 133°).

No corresponding component was detected in the synthetic polyisoprene samples. However a purple spot was detected ($R_F \approx 0.9$) being due to the presence of a phenolic type of antioxidant added during manufacture. Due to the uncertainty of the antioxidant type used by different manufacturers, no definite R_F values can be quoted to characterize the presence of synthetic polyisoprene.

Gum and vulcanized rubbers. In order to establish whether any interference was observed from extractable compounding ingredients, several samples of gum and vulcanized rubbers containing different ingredient recipes were examined by the described technique, typical examples being:

(1) Polymer	100 parts	(natural rubber or synthetic polyisoprene);
Zinc oxide	5 parts	
Stearic acid	1 part	
Tetramethyl thiuram disulphide	1.2 parts	
Dibenzothiazol-2-yl disulphide	1.1 parts	
Sulphasan R	0.5 parts	
Antioxidant	1.0 parts	

Cured at 140°/40 min.

(2) Polymer	100 parts	(natural rubber or synthetic polyisoprene)
Zinc oxide	5 parts	
Stearic acid	2 parts	
Cyclohexylbenzothiazol-2-yl sulphenamide	0.4 parts	
Sulphur	2.0 parts	
HAF black	50.0 parts	
Antioxidant	2.0 parts	

Cured at 140°/30 min.

In all samples containing natural rubber as the raw polymer, positive identification of β-sitosterol (R_F 0.40 ± 0.02) was possible. No component of corresponding R_F value was detected in the synthetic polyisoprenes, although the phenolic antioxidant could be detected ($R_F \approx 0.9$).

An interesting observation was that the relative concentration of β-sitosterol expressed as the spot area per gram of natural rubber was reasonably constant (3.0 ± 0.5 mg; tracing and weighing), irrespective of the natural rubber grade, compounding ingredients or curing conditions. Even raising the temperature of cure from 140° to 160° for 40 min had little or no effect on the relative concentration of β-sitosterol.

It seemed possible that the technique could be made semi-quantitative by estimating the concentration of β-sitosterol (expressed as spot area) relative to a known weight of natural rubber.

Consequently six blends of natural rubber/synthetic polyisoprene were prepared, the raw polymer composition having the following ratios of natural rubber to synthetic polyisoprene: (1) 100:0 %; (2) 75:25 %; (3) 50:50 %; (4) 25:75 %; (5) 5:95 %; (6) 0:100 %.

Then 100 parts of raw polymer were mixed with 5 parts zinc oxide, 2 parts stearic acid, 2.5 parts sulphur, 0.5 parts cyclohexyl benzothiazol-2-yl sulphenamide, 50.0 parts HAF carbon black, and 2.0 parts antioxidant; and cured at 140°/60 min.

Each sample was examined by the previously described experimental procedure. A typical chromatogram (Fig. 1) shows the presence of the β-sitosterol component.

The thin-layer chromatogram can be evaluated semi-quantitatively by measurement of the spot area of the β-sitosterol component, which is related to the volume of extract applied (and hence weight of natural rubber) by the following expression[7]:

$$\sqrt{A} = m \log W + c$$

where A = spot area; W = weight of extract applied, and m and c are constants for the individual compound.

From the results obtained a linear relationship was found for the square root of the spot area and the $\log_{10}$ of the concentration of natural rubber over the ranges 0–100 % w/w natural rubber, as shown in Fig. 2. The limit of detection is about 5 % natural rubber in a natural rubber/synthetic polyisoprene blend. If the β-sitosterol component cannot be detected and the presence of *cis*-polyisoprene has been shown by other methods, *e.g.*, the Weber test or I.R. spectroscopy, it can be concluded that the natural

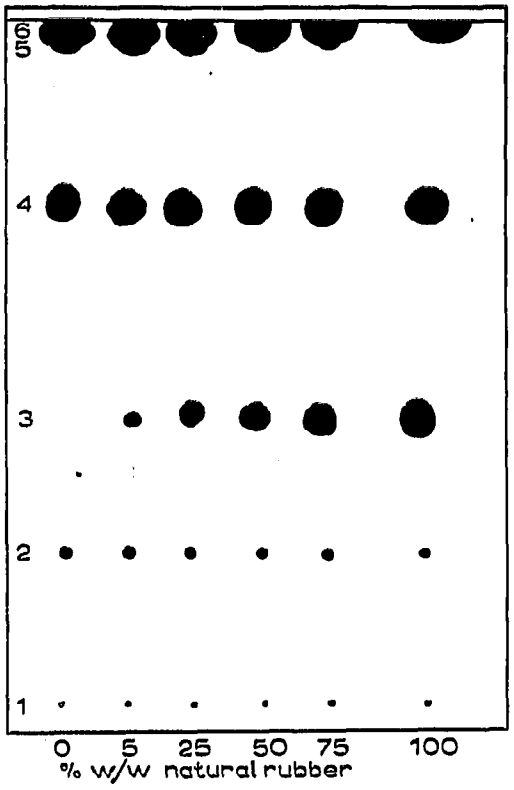

Fig. 1. A chromatogram of solvent extracts of natural rubber/synthetic polyisoprene blends in a vulcanized stock. (1) = Origin; (2) = unknown; (3) = β-sitosterol; (4) = antioxidant of p-phenylenediamine type; (5) = phenolic antioxidant in synthetic polyisoprene; and (6) unresolved components.

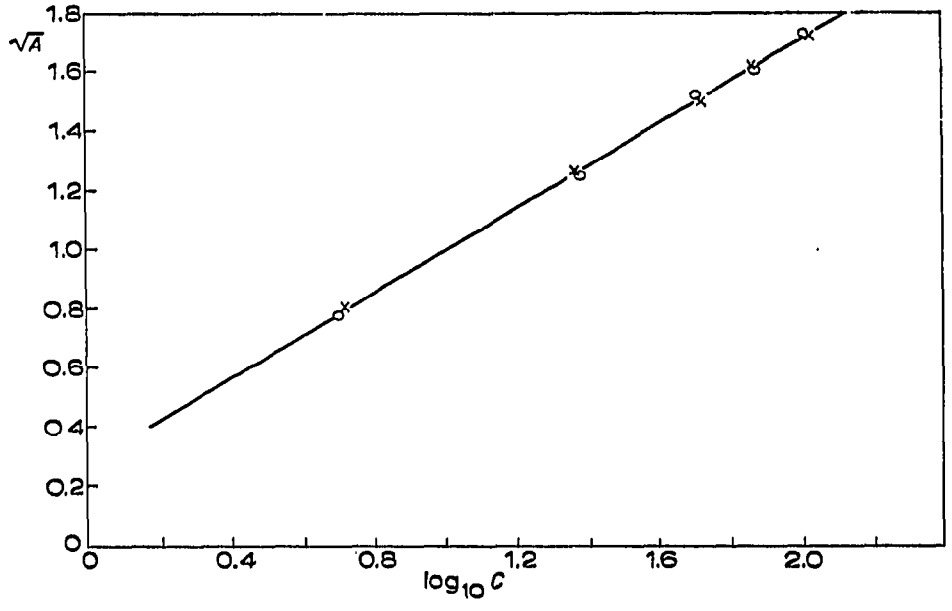

Fig. 2. The relationship between the square root of the spot area (A) of β-sitosterol and the logarithm (base 10) of the concentration (C) expressed as % w/w natural rubber, in a natural rubber/synthetic polyisoprene blend. XO = Duplicate analysis.

rubber content is less than 5 % w/w and that the polymer is substantially synthetic polyisoprene. This will be confirmed by detection of purple spots due to the presence of phenolic antioxidants added to the raw polymer.

Applications

The main applications will be in laboratories dealing with polymer identification and will enable a differentiation of natural rubber (NR)/synthetic polyisoprene to be made. This can be made semi-quantitative if accurate blends of NR/polyisoprene can be prepared.

The technique will also be useful in examination of small rubber samples (50–100 mg), where solvent extraction followed by dissolving of the residue in 1 drop ($\approx$ 0.05 ml) of carbon tetrachloride will enable thin-layer chromatography to be performed on a qualitative basis.

The equipment required is relatively cheap, the technique is simple to perform and the time required for the analysis (excluding the extraction procedure which can be performed overnight) is 40–45 min.

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