# THE CONSTITUENTS OF HEVEA LATEX

## PART I

## THE ISOLATION AND QUANTITATIVE DETERMINATION OF THE CONSTITUENTS

BY

K. C. ROBERTS

---

### Contents



### Introduction

It is noteworthy that the very considerable body of published work on the chemistry of Hevea latex contains no record of attempts at a definitive analysis of this complex mixture of substances. This is the more remarkable since it is well known that certain non-caoutchouc constituents, which have been repeatedly shown to occur in latex, exert a profound influence on the properties of plantation rubber.

A review of the literature shows that evidence has been adduced for the presence in latex of no fewer than sixty different substances, but of these, very few have actually been isolated from latex and submitted to a severely critical examination. In the discussion even of the better-known non-caoutchouc constituents, such as the sugars, the proteins, the inorganic salts, and above all, the so-called "resins", there is always a certain amount of assumption and inference; and although more or less reliable determinations of the amounts of these constituents have been made, little is known of their mode of occurrence, or in most cases of their chemical composition. Moreover, an important group of ten to twelve substances, including the fatty acids and the natural anti-oxidants of rubber, isolable from the acetone extract of crepe rubber, have not up to the present been isolated from latex itself, although their presence therein must clearly be inferred.

The present work describes a method for the isolation and quantitative determination of the constituents of Hevea latex (including the caoutchouc hydrocarbon) in the form in which they occur naturally. Such an analysis is necessarily a long and tedious process, involving as it does the isolation in a pure state of a considerable number of highly complex substances, but already the broad outlines of the constitution of this latex have been determined, and the existence of three hitherto unknown constituents has been established.

It is hoped in the near future to report the results of more detailed chemical investigations of the various constituents. Further it may be mentioned that applications of the method which are now in hand promise to throw light on a number of vexed questions. These include not only matters of immediate interest to the producer and the consumer of plantation rubber, such as the causes of variability, the constitution of clonal latices, and the effect of ammoniation, but also a group of fundamental problems including the structure of the caoutchouc hydrocarbon and the place of latex in the metabolism of the Hevea tree.

## Experimental

### General

Two fundamental requirements in undertaking this analysis are, first, that the raw material should be exposed to the least possible risk of undergoing fermentative changes, and second, that throughout the process any treatment, chemical or physical, which might be suspected of effecting chemical change in the latex or in its constituents should be avoided. It is felt that the procedure now to be described meets these requirements with a considerably greater degree of success than has hitherto been attained in the handling of the "total solids" of latex.

### Drying of Latex

By arrangement with a nearby estate, the latex from a particular "task" is being submitted regularly in an untreated condition directly from the tapper to the Institute. On days when an analysis is commenced, this latex, immediately on arrival, is passed through a Monel metal sieve, and 1000 grams, in 100-gram portions, are immediately transferred through a burette to ten sheets of plate glass, each 54 cms. square. Each sheet is mounted in a substantial wooden frame to facilitate handling and levelling operations. The latex is then carefully distributed so as to cover almost the entire surface of the glass in a more or less uniform film which thus averages 0.34 mm. in thickness. The frames are then exposed in the shade to a brisk current of air from a portable fan until the contents have reached a uniform translucence. As each frame attains this state, it is placed in direct sunlight for half-an-hour to complete removal of water and give a tough transparent film. By this procedure the entire operation of drying one litre of fresh field latex at atmospheric temperature is usually complete within three and one-half hours of receipt of the latex.

### Dispersion of the dried Film

The dried films are removed from the frames in narrow strips, care being taken to avoid massing. As each strip is removed, it is placed in an aluminium or a glass vessel containing a mixture of carbon tetrachloride (7000 ccs.) and acetone (4500ccs.) which is kept well stirred during the operation and at intervals for some time afterwards. In this way a uniform dispersion of the total solids from the original litre of latex is obtained in a remarkably short time, the process being virtually complete in 1--2 hours. At this stage the dispersion is conveniently allowed to stand overnight in the closed vessel.

The use of the above mixed solvent has the additional advantage that the resulting dispersion is markedly less viscous than those obtained with the usual rubber solvents.

The dispersions thus obtained normally vary in concentration between the limits of 2.6 per cent (latex total solids 30 per cent) and 4 per cent (latex total solids 46 per cent), but it was judged advisable to use the above standardised procedure rather than to prepare day by day solutions of a fixed concentration and varying volume. The justification for this procedure will appear in the following.

### Removal of Rubber

Acetone (4500 ccs.) is now added slowly in a uniform stream to the homogeneous dispersion, which is kept vigorously stirred. The caoutchouc is thus quantitatively precipitated in a fine flocculent form, carrying with it all the proteins, sugar, quebrachitol and inorganic constituents of the latex. The precipitate is allowed to agglutinate during 24 hours, at the end of which time it is readily separated as an opaque white lump from the supernatant clear yellow liquid. After being allowed to drain, it is carefully washed twice with a total of 500 ccs. of acetone, and the drainings and washings are added to the carbon tetrachloride-acetone solution. The further treatment of this precipitated rubber is described later.

### Isolation of the dissolved Matter

The carbon tetrachloride-acetone solution is now concentrated to small bulk by distillation from a steam-bath, and the residue is transferred quantitatively to a porcelain basin from which the remaining solvent is allowed to evaporate. The residue, weighing 10—20 grams, is a tacky, semi-fluid mass which varies in colour from yellow to deep red in different specimens. It will be referred to for brevity as "the organic-soluble constituents".

The mixed solvent readily lends itself to the recovery of carbon tetrachloride.

### Isolation of the individual Organic-soluble Constituents

It has been found that this complex mixture of substances not previously isolated from latex is most readily separated into its primary components by selected solvent action. In outline, the method adopted involves extraction of the material first with water and then with rectified spirits. In this way are obtained (a) the water extract, (b) the alcohol extract, and (c) the residue, now to be described. As the detailed chemical examination of the resulting components is not yet complete, only a brief indication of their general nature is now given.

(a) *The Water Extract.* The organic-soluble constituents from one litre of latex are thoroughly extracted three times at room temperature by vigorous stirring with distilled water totalling 100 ccs. On drying the extract to constant weight on the water-bath a colourless crystalline residue is obtained which represents approximately 0.02 per cent by weight of the latex. This sometimes contains a small amount of quebrachitol, but in the main consists of the ammonium salt of a water-soluble aldehydic or ketonic acid. Pending its more complete description it will be referred to as the "ammonium salt".

(b) *The Alcohol Extract.* The material remaining after the water extraction is carefully kneaded five times at room temperature with a total volume of 210 ccs. of rectified spirits ($3 \times 50$ and $2 \times 30$ ccs.). Evaporation of the combined extracts on the water-bath leaves a deep red oil containing more or less crystalline matter and constituting on an average 0.5 per cent by weight of the latex.

This material is now divided into components by treating it three times with petroleum ether (total volume 100 ccs.).

(i) *The petroleum ether-insoluble portion or "Ester."* This substance is relatively small in amount (approximately 0.05 per cent by weight of the latex) and when first isolated is a crystalline solid white or pale brown in colour. Its components are (a) a water-soluble aldehydic or ketonic acid and (b) a higher aliphatic alcohol. It is thought that the acid may prove to be the same as that occurring in the "ammonium salt" (above), while the alcohol gives evidence of being identical with that isolated from crepe rubber by Bruson, Sebrell and Vogt (1) and diagnosed by them as octadecyl alcohol. A small amount of quebrachitol is at times associated with this ester, and may be removed in virtue of its insolubility in absolute alcohol.

(ii) *The petroleum ether-soluble portion or "Fatty Acid Complex."* This comprises on an average 0.45 per cent by weight of the latex, that is 90 per cent of the alcohol-extractable material. As first obtained it is a deep red oil and is weighed in this form, but an alcohol solution of the material deposits almost quantitatively a homogeneous crystalline mass. Preliminary work indicates that the eight to ten component substances are isolable from the mixture only after hydrolysis. These facts, together with the mode of isolation of the material, provide the justification for classing it as a single latex constituent of the type of a "chemical complex" of considerable stability. The preliminary work further shows that the complex consists in the main of fatty acids, saturated and unsaturated, which are presumably identical with those isolated from the acetone extract of crepe rubber by Sebrell *loc. cit.* and by Whitby, Dolid and Yorston (2), and identified by them as oleic, linoleic and stearic acids. The remaining material is terpenic in character, and it is anticipated that further work will show it to consist of the substances diagnosed, in part by Sebrell and in part by Whitby (*loc. cit.*), as phytosterol, phytosterol glucoside, a hydrocarbon $C_{15}H_{24}$, a ketone $C_{15}H_{24}O$, and sterols $C_{27}H_{42}O_3$ and $C_{20}H_{30}O$.

(c) *The Residue.* This extremely interesting material, the existence of which either in latex or in rubber has hitherto been unsuspected, is one of the major non-caoutchouc constituents. It is widely variable from specimen to specimen in amount, in colour and in texture, but is in general a cream-coloured or brown plastic material constituting approximately 1 per cent by weight of the latex. It is weighed after the adhering alcohol has been allowed to evaporate at room temperature. Since its composition and characteristics are still being studied, mention will be made in this preliminary paper only of three observations: first, that the material is freely soluble in ether to a mobile solution; second, that it melts to a viscous liquid below 100°C; and third, that it holds in combination a sulphur-containing acid. For convenience it will be called the " sulphur complex ". It is interesting that a somewhat similar substance results when crepe rubber is allowed to react with thioglycollic acid at room temperature (3).

*Note.* In preliminary work, the water extraction (above) was followed by an extraction of the residue with dilute aqueous sodium carbonate. As this operation on no occasion gave evidence of removing acidic substances, it was discontinued.

### Drying of the Precipitated Rubber

The acetone-washed mass of precipitated rubber (above), after being allowed to drain thoroughly, is repeatedly passed between cold rolls, the nip being gradually reduced until a thin pale-yellow sheet results. This is allowed to dry in air to constant weight. This product has the interesting property of dissolving almost without swelling in the usual rubber solvents to form opalescent, relatively mobile solutions. An investigation of its physical and mechanical properties is being made.

### Removal of Non-Caoutchouc Substances from the Precipitated Rubber

The dried sheet of precipitated rubber contains the whole of the sugar, quebrachitol*, inorganic matter and protein of the original latex. The following method has been developed for the almost complete removal of these substances in the form of a single water-soluble complex, to be known as the "protein complex".

Ten grams of the precipitated rubber are dissolved in carbon tetrachloride (300 ccs.) in a two-litre round-bottomed flask, distilled water (1000 ccs.) is added, and the whole is vigorously shaken for some time. The flask is then placed in a water-bath until the carbon tetrachloride begins to boil, at which stage a current of steam is passed through the mixture until all the carbon tetrachloride is removed. The resulting aqueous solution and the rubber are now separated as completely as possible (the voluminous rubber phase contains much solution which can be removed by pressure), and the water is removed from the solution until the residue is of constant weight.

### The Protein Complex

The above residue is a pale brown, somewhat hygroscopic syrup with a biscuit-like odour, which slowly crystallises in air in well-formed elongated plates of considerable size. It is almost

*With the exception of the very small amounts sometimes found with the organic-soluble constituents.

completely soluble in a small amount of cold water, and the solution gives the ninhydrin test for protein. Fehling's solution is however unaffected owing to the absence of free reducing sugars, while the reactions with ammoniacal silver nitrate and ammonium molybdate are masked by the fact that quebrachitol and the metallic phosphates are also not present as such, but as components of a chemical complex.

All four reagents however give normal positive tests if the aqueous solution of the complex is first hydrolysed by boiling it for half-an-hour with its own volume of 5N sulphuric acid. Phenylglucosazone (m.p. 203°) has been obtained by suitable treatment of the hydrolysate.

It may therefore be stated that the components of the complex include protein, phosphate, glucose or fructose (or polysaccharides from which they are derivable), and quebrachitol. The observations noted in the ensuing section indicate that this complex also comprises the remaining inorganic constituents which have been recorded by previous workers. It is anticipated that the material will readily lend itself to the determination of its major constituents.

THE PURE RUBBER HYDROCARBON

The rubber remaining after the above water extraction is milled to the thinnest possible sheet in a stream of water, surface water is removed, and the product is then dried to constant weight *in vacuo* over sulphuric acid. This operation is usually complete in 48—72 hours, but on occasion the rubber at this stage is too tacky to be handled quantitatively.

This product consists of the rubber hydrocarbon in a highly purified state. It retains a relatively small amount of protein and when burnt yields only an imponderable residue which is phosphate-free. Other features of the substance including the above-mentioned variability will be discussed in a subsequent paper.

## Typical Results

It will be noticed that the total amount of non-caoutchouc constituents (excluding water) is about 4 per cent of the latex and 10—15 per cent of the latex total solids. These figures are appreciably higher than have been observed hitherto, and are mainly attributable to the inclusion for the first time in a table of this kind of the "sulphur complex", which is seen to be a major non-caoutchouc constituent.

The percentage of water is obtained from the "Total Solids" determination, which is a routine part of each analysis.

TABLE I

| Latex Constituent | Latex A per cent | Latex B per cent | Latex C per cent |
|---|---|---|---|
| Ammonium Salt ... | 0.02 | 0.03 | 0.02 |
| Ester ... ... | 0.06 | 0.06 | 0.02 |
| Fatty Acid Complex ... | 0.41 | 0.33 | 0.47 |
| Sulphur Complex ... | 0.92 | 0.94 | 1.16 |
| Protein Complex ... | 2.56 | 1.45 | 2.05 |
| Caoutchouc ... ... | 32.92 | 27.17 | 32.98 |
| Water ... ... | 62.75 | 69.78 | 63.68 |
| Total ... ... | 99.64 | 99.76 | 100.38 |

## Additional Considerations

(i) In most cases the amount of a given constituent isolated by the above procedure, though definite, is admittedly small. For the more detailed investigations which are in progress, these small amounts are allowed to accumulate over a period, and are stored in solution in well-stoppered bottles which are kept in the dark.

(ii) It will be appreciated that, in dealing with a mixture of this degree of complexity which shows wide variations in composition from day to day, technical difficulties are encountered from time to time in applying the above method of analysis. All such difficulties to date have been readily dealt with by using ordinary measures, and particulars are therefore omitted from the above account.

(iii) Objection may be raised that the above method does not ensure that latex constituents of high volatility would be observed. This point has been met by subjecting the fresh latex from time to time to distillation with steam. On no occasion has any evidence thus been obtained of the existence in latex of detectable amounts of volatile acids, bases, or aldehydes such as hydrocyanic, hydrosulphuric, formic and butyric acids, ammonia, methylamine, and acetaldehyde, claims for all of which have been put forward in the earlier literature.

## Summary

(i) A method is described for the quantitative isolation of the chief constituents of Hevea latex *in the form in which they occur in nature*.

(ii) Several hitherto unknown constituents have been observed and receive a preliminary description. They include a sulphur-containing plastic material which is of major importance.

(iii) The known major constituents—sugar, quebrachitol, phosphates, protein, fatty acids—are shown to exist in fresh latex in the main as components of stable chemical complexes.

(iv) It is hoped in the near future to report the results (a) of more detailed investigations, and (b) of applications of the method in the study of some fundamental problems.

**Acknowledgement**

Grateful acknowledgement is made to Dr. E. Rhodes, Senior Officer of the Chemical Division for his active interest and encouragement throughout the course of the work.

**Literature Cited**

(1) Bruson, Sebrell and Vogt, Ind. Eng. Chem., **19** (1927), 1187

(2) Whitby, Dolid and Yorston, J.C.S., **128** (1926), 1448

(3) Holmberg, Ber., **65** (1932), 1349.

Kuala Lumpur,

20th May, 1936.