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Destabilisation of *Hevea* Latex by Bark Sap: Involvement of High Density Rubber Particles in Latex

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Abstract

Bark sap and latex B-serum of *Hevea brasiliensis* were evaluated for their effectiveness in destabilising suspensions of rubber particles, whole latex and reconstituted latex. The sensitivities of rubber particles from different fractions of centrifuged latex to destabilisation by bark sap were compared. While B-serum was observed to be more potent in inducing flocculation and creaming of a dilute suspension of rubber particles in water, bark sap was far more effective than B-serum in destabilising and coagulating whole latex. A small proportion of the rubber particles in latex was especially sensitive to destabilisation by bark sap. These were the relatively smaller and higher density rubber particles from Zones 2 and 3 of centrifuged latex. It is speculated that destabilised high density rubber particles act as sites of initiation of latex vessel plugs which exert control of latex outflow when the tree is tapped.

Full paper follows

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Bark sap and latex B-serum of Hevea brasiliensis *were evaluated for their effectiveness in destabilising suspensions of rubber particles, whole latex and reconstituted latex. The sensitivities of rubber particles from different fractions of centrifuged latex to destabilisation by bark sap were compared. While B-serum was observed to be more potent in inducing flocculation and creaming of a dilute suspension of rubber particles in water, bark sap was far more effective than B-serum in destabilising and coagulating whole latex. A small proportion of the rubber particles in latex was especially sensitive to rapid destabilisation by bark sap. These were the relatively smaller and higher density rubber particles from* Zones 2 *and* 3 *of centrifuged latex. It is speculated that destabilised high density rubber particles act as sites of initiation of latex vessel plugs which exert control on latex outflow when the tree is tapped.*

When the rubber tree is tapped, latex vessel plugs begin to form almost immediately at the severed ends of latex vessels[1]. Latex exudation ceases from plugged vessels and flow from the tapping cut as a whole slows down and stops eventually. Thus, the destabilisation of latex that initiates plug formation has an important bearing on the flow characteristics of the exuding latex and, ultimately, the yield from the rubber tree.

To a large extent, the stability of rubber particles in latex is dependent upon the presence of net negative charges on the lipo-protein surface of the particles which enable them to mutually repel each other and also to repel the lutoids which are membrane-bound vesicles also present in the latex[3]. While the lutoids are negatively charged on the exterior, the serum contained within them (the B-serum) is strongly cationic due to the presence of proteins of high isoelectric points and divalent inorganic salts such as Mg^{++} and Ca^{++}. In the presence of B-serum, the negative charges on the rubber particle surface are readily annulled by the B-serum cations, thereby negating the stabilising influence the negative charges have on the rubber particles. The destabilising effect of this electrostatic interaction on rubber particles can be shown by their rapid flocculation in a dilute aqueous suspension when B-serum is added[4]. Besides the electrostatic interaction, other complementary mechanisms of rubber particle destabilisation by B-serum proteins and enzymes have also been suggested[3,4], but not as yet amply demonstrated.

As latex exudes from the tree, the fragile lutoid membranes are liable to damage by such factors as osmotic shock[5] and physical shear[6]; this results in the release of B-serum. The latex in the immediate vicinity of the damaged lutoids is consequently destabilised and latex vessel plugging ensues[7].

More recently, bark sap released during tapping of the tree has been examined as another possible factor in the induction of latex

*Some preliminary results from this study were presented at the meeting of the International Rubber Research and Development Board Physiology and Exploitation Group held in Danxian (Hainan), China, December 1986[2]

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destabilisation and latex vessel plugging[2,8–10]. The mechanism involved has not been established but unlike B-serum, bark sap has a net negative charge[11]. Destabilisation of rubber particles is therefore unlikely to arise *via* an electrostatic mechanism.

This paper examines the destabilisation of rubber particle suspensions, whole latex and reconstituted latex by *Hevea* bark sap. The sensitivity to destabilisation of rubber particles derived from the different fractions of centrifuged latex known to be rich in rubber is also examined. The potency of B-serum in these respects is compared with that of bark sap.

MATERIALS AND METHODS

The term 'bark sap' refers to the juices expressed by passing bark shavings (with the outer corky layers removed) between stainless steel pinch rollers *(Figure 1)*. The extract was centrifuged at low speed to obtain a clear supernatant.

All experiments were carried out on clone RRIM 600 tapped on a half-spiral, alternate-day system (½S d/2).

Chilled fresh latex was fractionated by centrifugation at 19 000 r.p.m. (44 000 g max.) at 4°C on a Sorvall RC 2B centrifuge for 1 h. The latex separated into three main fractions: an upper phase of rubber cream *(Zone 1)*, a heavy bottom fraction and a zone of C-serum in between. (The numbering of the centrifugation fractions follows that adopted by Moir[12].) Two minor fractions, *Zones 2* and *3*, were located beneath *Zone 1 (Figure 2)*.

To prepare B-serum, the bottom fraction was removed from the centrifuge tube and washed of traces of C-serum by re-suspending in ten volumes of 0.35 *M* mannitol. The suspension was filtered through muslin and re-centrifuged at 19 000 r.p.m. for 30 min. The supernatant was discarded and the washed bottom fraction was then subjected to alternate freezing and

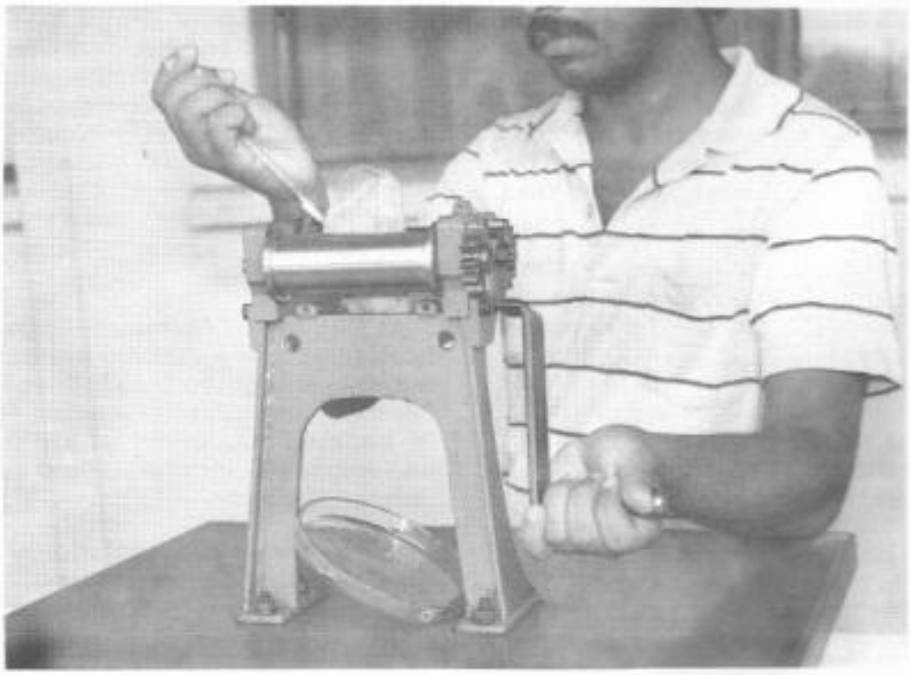

Figure 1. Extraction of bark sap by passing bark shavings (enveloped in muslin cloth) between pinch rollers.

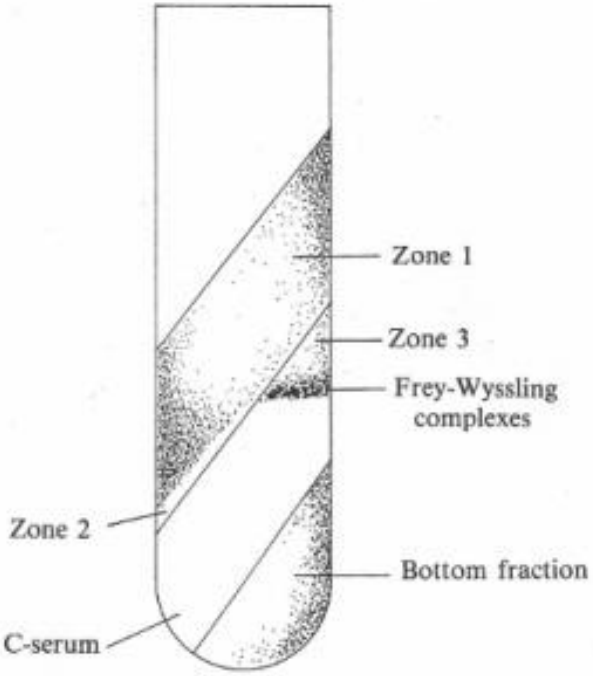

Figure 2. Diagrammatic representation of latex fractionation by high speed centrifugation.

thawing to rupture the lutoids[13]. After re-centrifugation, B-serum was recovered as the clear supernatant.

All percentage values relating to concentrations of rubber particle suspensions, bark sap and B-serum cited in the text refer to their final concentrations in the respective mixtures.

RESULTS AND DISCUSSION

Destabilisation of Dilute Suspensions of Rubber Particles

Rubber particles suspended in water. A dilute suspension of rubber particles in distilled water was prepared from the creamed rubber fraction *(Zone 1)* of centrifuged latex. When B-serum was added to the suspension to give a final concentration of 1% rubber particles and 2%, 4% or 8% B-serum, flocculation of the suspension could be observed within 5 min in all cases. Creaming of destabilised rubber occurred soon thereafter as the flocs rose to the surface of the suspension *(Figure 3)*. In agreement with the findings of Southorn and Edwin[4], therefore, the electrostatic interaction between the positively-charged B-serum and the negatively-charged rubber particle membranes was fast and unequivocal.

When B-serum was replaced by 2% or 4% bark sap in the mixtures, creaming was not observed even after 2 h; only when bark sap concentration in the mixture was increased to 8% and above did this take place *(Figure 3)*. Using the creaming reaction as the criterion, it can be inferred therefore that, volume for volume, B-serum is more powerful than bark sap as a destabilising agent of rubber particles suspended in water.

Rubber particles suspended in C-serum. While the fore-going observations demonstrated the relative strengths of the two latex destabilisers,

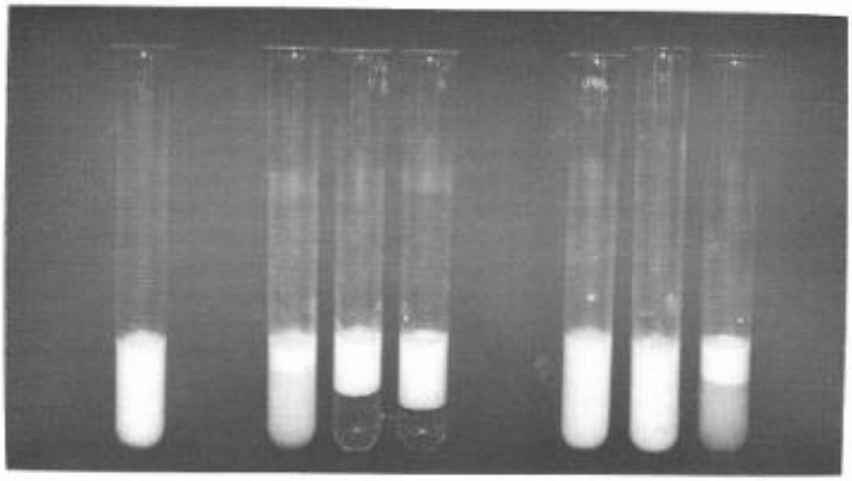

Figure 3. Destabilisation of a 1% rubber particle suspension in water by B-serum and bark sap. (Observation after 35 min.) Left to right: control; + 2%, + 4%, + 8% B-serum; + 2%, + 4%, + 8% bark sap.

B-serum and bark sap, they are arguably unrepresentative of the *in vivo* situation. In whole latex, rubber particles are not suspended in distilled water, but in the C-serum. C-serum is rich in negative ions and so acts as a protective buffer against the cationic B-serum. In whole latex, the release of B-serum through breakage or leakage of lutoids is thought to cause only a localised destabilisation of rubber particles resulting from a temporary high concentration of the serum in the vicinity of damaged lutoids[4]. The proportion of C-serum present in whole latex is relatively large and as such, rapid mass destabilisation or coagulation of the rubber particles would not occur even if B-serum were to be completely released from all the lutoids in a volume of latex[4]. Thus, when a mixture of rubber particles and B-serum was prepared in a medium of C-serum rather than in distilled water, creaming of the rubber particles was markedly inhibited. No creaming was observed after 30 min and only incomplete creaming occurred after 1 h when a higher B-serum concentration of 8% was added *(Figure 4)*.

As the mechanism of rubber particle destabilisation by bark sap is not primarily dependent on ionic charges, there is the likelihood that it would be more effective than B-serum in creaming rubber particles suspended in C-serum. However, experimental results showed otherwise. Whereas 8% B-serum gave rise to incomplete creaming, bark sap under the same conditions failed to invoke creaming altogether *(Figure 4)*.

Although B-serum appeared more powerful than bark sap as a destabiliser of rubber particles in C-serum (just as it was with rubber particles suspended in water), the observations could be misleading. Such assessment of instability had been based specifically on the ability of rubber particles to cream whereas this is not the sole criterion by which latex instability can be assessed. Bark sap reacted with C-serum to give a copious heavy precipitate that could be clearly shown by low-speed centrifugation *(Figure 5)*. This precipitate might encumber the rubber particles in their rising to form a cream. (Though B and C-sera mutually precipitate when mixed[4], the precipitate arising from the amounts of the sera used was very little compared with that from a mixture of bark sap and C-serum.)

Destabilisation of Whole Latex

Further comparison of the latex destabilising effects of bark sap and B-serum involved their

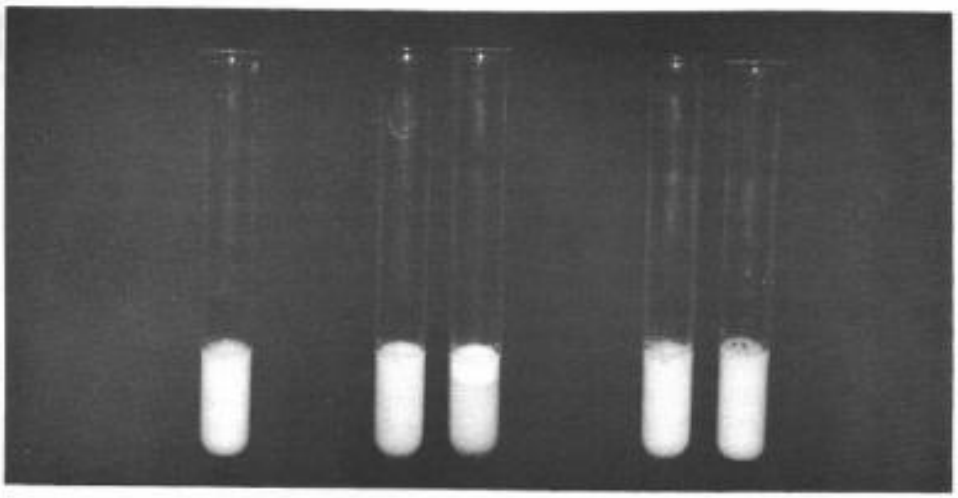

Figure 4. Destabilisation of a 1% rubber particle suspension in C-serum by B-serum and bark sap. (Observation after 1 h.) Left to right: control; + 4%, + 8% B-serum; + 4%, + 8% bark sap.

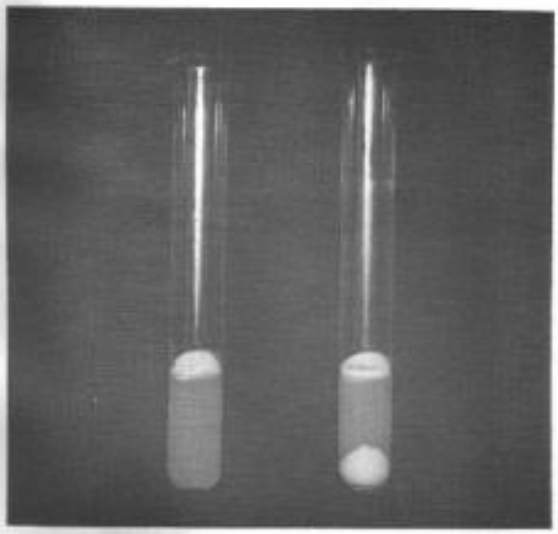

Figure 5. Low-speed centrifugation of a 2% rubber particle suspension in C-serum after addition of 8% B-serum (left) and 8% bark sap (right).

addition to whole latex to obviate the interaction between bark sap and C-serum confounding rubber suspension creaming as a definitive marker of latex instability. Bark sap or B-serum was mixed into whole latex and immediately centrifuged. When up to 8% B-serum was added to whole latex, the patterns obtained after centrifugation were very similar to that of the untreated control latex *(Figure 6)*. This is not surprising since (as observed above) B-serum has only limited ability to destabilise

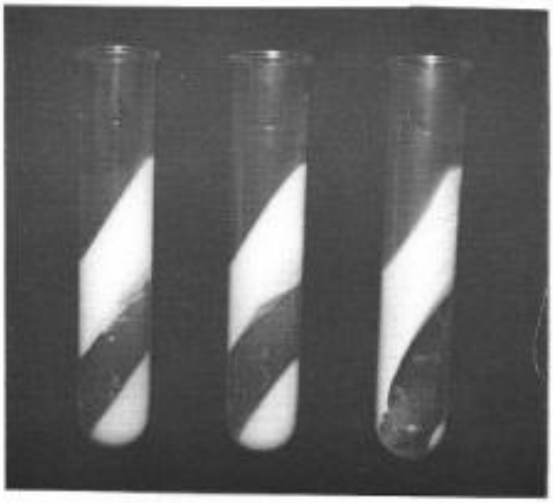

Figure 6. High-speed centrifugation of whole latex following treatment with 8% B-serum and 8% bark sap. Left to right: control; + 8% B-serum; + 8% bark sap.

rubber particles suspended in a medium of C-serum, which is the case with whole latex. Thus, the expected three main centrifugation zones were observed: the upper rubber fraction, the heavy bottom fraction consisting mainly of lutoids, and the serum phase in between. The rubber fraction remained creamy and was not coagulated.

When B-serum was replaced by the same quantities of bark sap added to the latex, there was a reduction in the bottom fraction as bark sap was increased from 2% to 8%. In most instances, the bottom fraction was displaced completely, or almost completely, with the addition of 8% bark sap *(Figure 6)*. The bottom fraction constituents had in fact formed a layer consisting of an agglomeration of lutoids and coagulated rubber. This lay beneath the fraction of uncoagulated rubber cream, the latter decreasing in proportion relative to the coagulated rubber as bark sap concentration was increased. Insofar as *whole latex* stability is concerned, it is clear that bark sap is far more powerful as a destabilising agent than is B-serum, volume for volume.

Although bark sap added to latex reduced the bottom fraction (an observation also made previously[8]), it cannot immediately be assumed that bark sap has acted directly on lutoids to destabilise them. Intact lutoids could have adhered to the lighter destabilised rubber particles and got carried centripetally towards the rubber fraction during centrifugation. The usual method of assessing lutoid damage is to assay for the enzyme acid phosphatase[14] which, in stable latex, is confined almost exclusively to the lutoids. In the presence of bark sap, however, proteins are liable to precipitation and acid phosphatase is not therefore a reliable marker for lutoid damage.

To determine whether the lutoids were damaged by bark sap, 15% sap was added to a mixture of five parts C-serum to one part bottom fraction. Upon centrifugation, the bottom fraction sedimented normally *(Figure 7)*, indicating the absence of gross lutoid damage. In contrast, bottom fraction that had first been subjected to repeated freezing and thawing to rupture the lutoids was not recovered by re-centrifugation and only the lutoid membrane

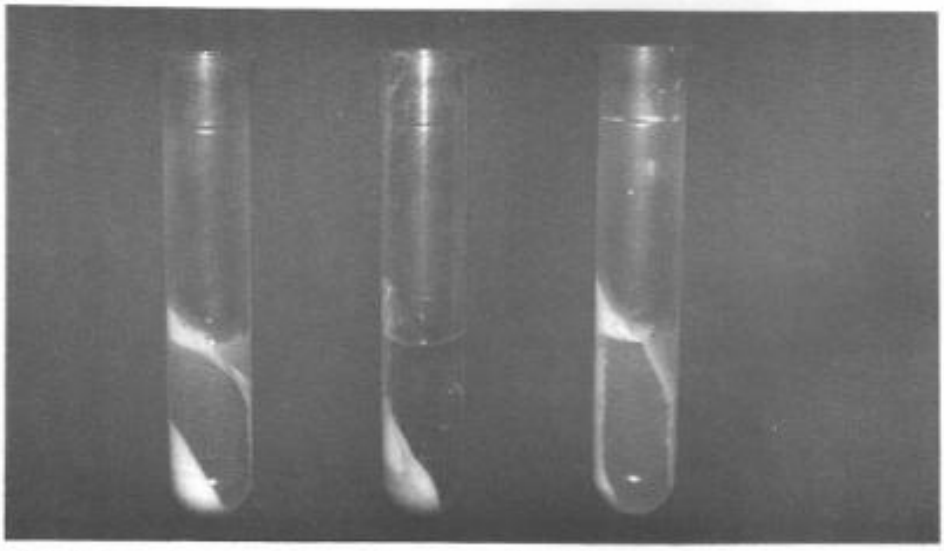

Figure 7. High-speed centrifugation of a suspension of bottom fraction in C-serum. Prior to centrifugation, the bottom fraction had been untreated (control, left), treated with 15% bark sap (centre) or subjected to alternate freezing and thawing (right).

fragments were sedimented *(Figure 7)*. Bark sap did not therefore act directly on lutoids to destabilise them nor, in consequence, to destabilise whole latex.

Destabilisation of Reconstituted Latex

Latex was centrifuged and the rubber cream, C-serum and the entire bottom fraction were carefully removed from several centrifuge tubes taking care not to sample either rubber cream or C-serum close to the interface of the two zones. The latex was re-combined with the rubber cream, C-serum and bottom fraction present in the ratio 15:11:4 to give a re-constituted latex comprising, proportionately, material from the three main centrifugation zones. This was left untreated (control) or had mixed into it 4% or 8% of bark sap. The latices were then centrifuged and compared with centrifuged normal whole latex given the same bark sap treatments. The centrifugation patterns of the normal latices were as expected. The bottom fraction was destabilised and diminished in size where 4% bark sap had been added. The latex treated with 8% bark sap had practically all the bottom fraction displaced *(Figure 8)*.

The centrifugation pattern of the reconstituted latex without bark sap was generally similar to that of the untreated (control) latex, except for the absence or reduction of two minor zones that had not been included during reconstitution of the latex. These were *Zone 3* and the Frey-Wyssling layer. Unlike whole latex, however, treatment of reconstituted latex with either 4% or 8% bark sap gave rise to only small decrements in size of the bottom fraction *(Figure 8)*. The reconstituted latex appeared to be resistant to destabilisation by bark sap compared with whole latex. It might be inferred from this observation, therefore, that certain constituents located either in *Zone 3* or the Frey-Wyssling layer acted as essential co-factors in the destabilisation of whole latex by bark sap. To investigate this proposition, reconstituted latex was prepared as before but this time, treatments were included where material from *Zone 3* or the Frey-Wyssling layer were re-inserted into the latex mixture.

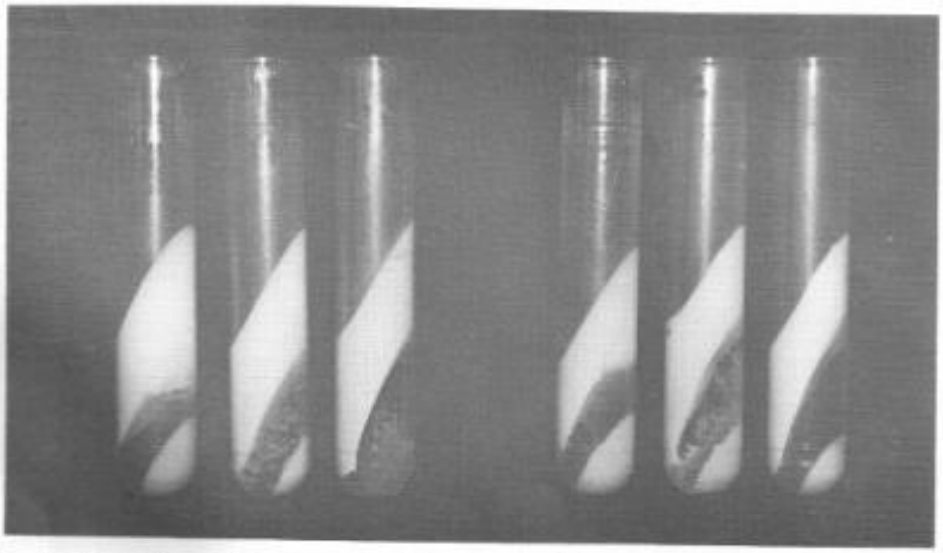

Figure 8. High-speed centrifugation of whole latex and latex reconstituted from Zone 1 rubber particles, C-serum and bottom fraction, following treatment with bark sap. Left to right: whole latex control; + 4% bark sap; + 8% bark sap; reconstituted latex control; + 4% bark sap; + 8% bark sap.

Following high-speed centrifugation, the reconstituted latex with *Zone 3* present showed markedly decreased stability when treated with bark sap. With the addition of 8% bark sap, the bottom fraction was missing, having been completely displaced by the adhesion of its constituent lutoids to destabilised rubber particles *(Figure 9)*.

As noted previously[2], reconstituted latex containing material from the Frey-Wyssling layer (but not from *Zone 3*) was also liable to destabilisation by sap. However, the effect was not as marked. The bottom fraction, while diminished, was not displaced completely and the degree of latex destabilisation was less consistent between repetitions. It seems probable that the latex-destabilising factor was present principally in *Zone 3*, with activity from the thin Frey-Wyssling layer being due to contamination by material from the adjacent *Zone 3*.

Destabilisation of Rubber Particles from *Zones 1, 2* and *3* of Centrifuged Latex

The three uppermost fractions of centrifuged latex — *Zones 1, 2* and *3* — have all been shown by electron microscopy studies to have rubber particles as their main particulate constituents[15]. In elucidating the active co-factor present in *Zone 3* that induced latex destabilisation by bark sap, the rubber particles present in the fraction were hence the obvious object of investigation. These rubber particles are smaller than those from *Zone 1*[15] where most of the rubber particles in fresh latex are sedimented by high-speed centrifugation. The fact that *Zone 3* rubber particles remain in suspension after centrifugation whereas those in *Zone 1* readily cream shows that the former are of a higher density than the latter.

The reactivity of *Zone 3* rubber particles was compared with that of *Zone 1* particles as well as rubber particles from uncentrifuged whole latex. Suspensions of 9% rubber particles from *Zone 1, Zone 3* and whole latex were prepared in C-serum, and B-serum or bark sap was then added to a concentration of 9%. (At this high concentration of rubber particles, creaming did not take place.) Immediately after mixing in a test tube using a vortex mixer, considerable quantities of flocs of destabilised rubber could be seen left on the sides of the test tubes containing bark sap and *Zone 3* rubber

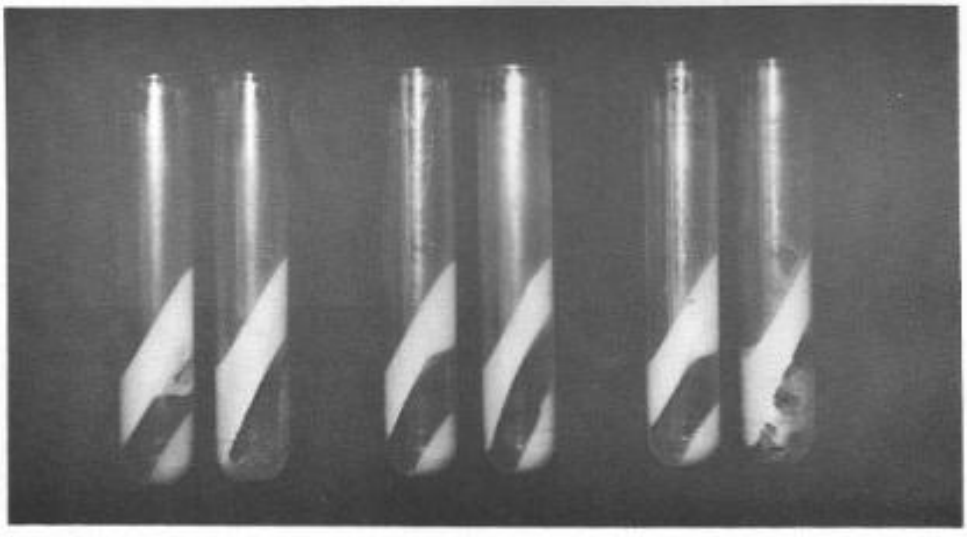

Figure 9. High-speed centrifugation of whole latex and reconstituted latex following treatment with 8% bark sap. Left to right: whole latex untreated; + 8% bark sap; reconstituted latex with rubber particles from Zone 1, untreated; + 8% bark sap; reconstituted latex with rubber particles from Zones 1 and 3, untreated; + 8% bark sap.

particles. Small quantities of flocs could also be seen on the sides of test tubes containing mixtures of bark sap and *Zone 1* rubber particles or particles from whole latex (which were, in any case, mainly *Zone 1* particles). Such flocs were absent or sparse on the sides of test tubes containing the B-serum mixtures *(Figure 10)*. This showed, firstly, that latex destabilisation by bark sap was very fast-acting. The observations also indicated that a suspension of rubber particles in C-serum was much more readily destabilised by bark sap than by B-serum, particularly when the particles were from *Zone 3* of centrifuged latex. When the contents of the test tubes were poured out after 20 min, *Zone 3* rubber particles were seen to have almost completely coagulated whereas the mixtures in the other test tubes flowed out freely *(Figure 11)*.

Having considered the destabilisation of rubber particles from *Zones 1* and *3*, the destabilisation behaviour of *Zone 2* rubber particles by bark sap was next investigated. Rubber particles from *Zone 2* are smaller than those of *Zone 1* but larger than those of *Zone 3*[15]. The particles in *Zones 2* and *3* are thought to be closely similar in that they share an identical staining reaction (differing from that of *Zone 1*), and that with prolonged centrifugation, *Zone 3* decreases in amount while *Zone 2* increases[12]. From its sedimentation behaviour *(Figure 2)*, *Zone 2* particles are intermediate in density with respect to *Zone 1* and *Zone 3* particles, but more resembling the latter.

To compare the reactivity of *Zone 2* rubber particles to bark sap with that of *Zone 1* and *Zone 3* particles, 9% bark sap was added to 9% suspensions of *Zone 1*, *Zone 2* and *Zone 3* rubber particles suspended in C-serum. On pouring out the contents of the test tubes after 20 min, the *Zone 1* rubber particle mixture flowed out freely while the *Zone 3* mixture was coagulated, as was expected. The *Zone 2* mixture was observed to have visibly thickened but not coagulated. In another similar set of test tubes, the contents were poured out only after 50 min. In this instance, *Zone 2* rubber

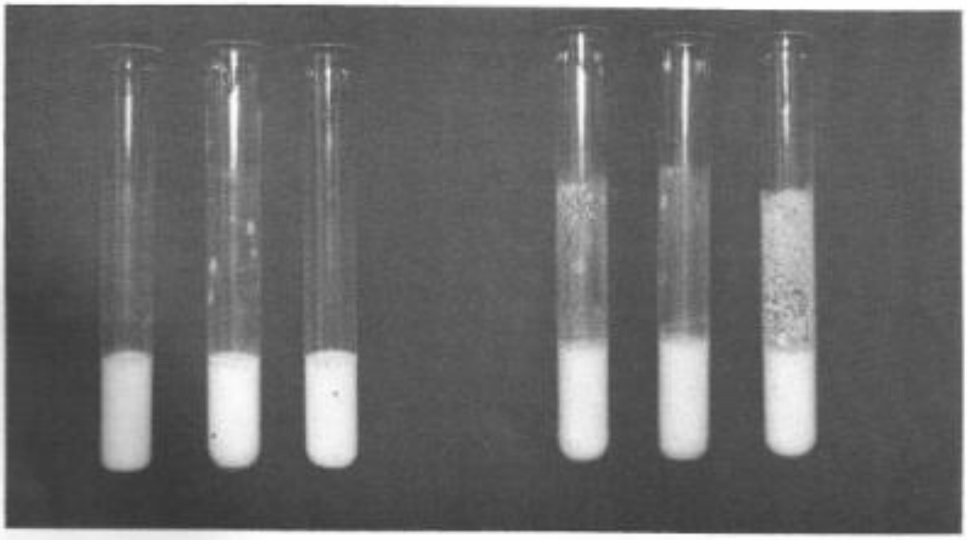

Figure 10. Flocs of destabilised particles on the side of the test tube after treatment of 9% rubber particle suspensions in C-serum with 9% B-serum or bark sap, followed by vortex-mixing. Left to right: rubber particles from whole latex; from Zone 1; from Zone 3 treated with B-serum; rubber particles from whole latex; from Zone 1; from Zone 3 treated with bark sap.

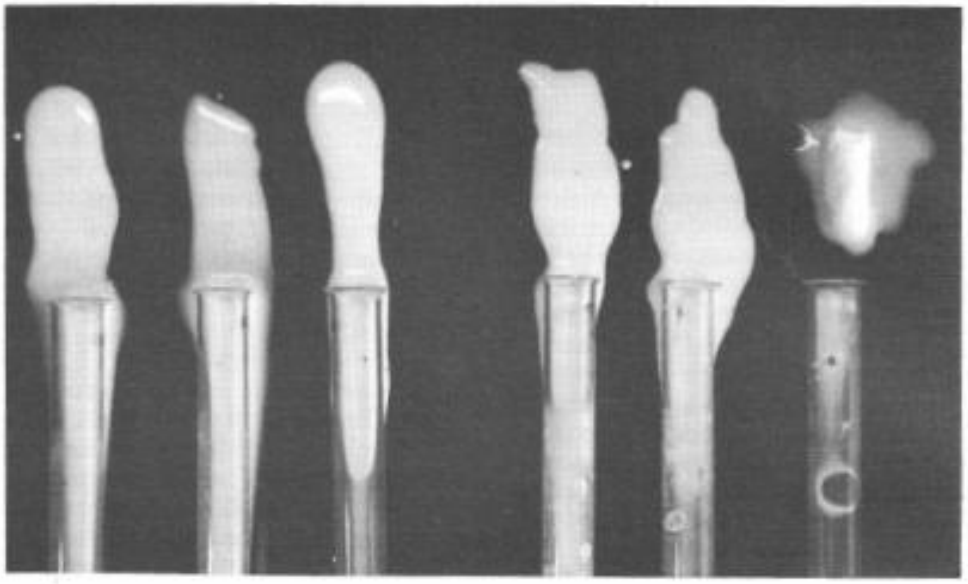

Figure 11. Rubber particle suspensions (9% in C-serum) treated with 9% B-serum or bark sap and poured out of the test tube after 20 min. Left to right: rubber particles from whole latex; from Zone 1; from Zone 3 treated with B-serum; rubber particles from whole latex; from Zone 1; from Zone 3 treated with bark sap.

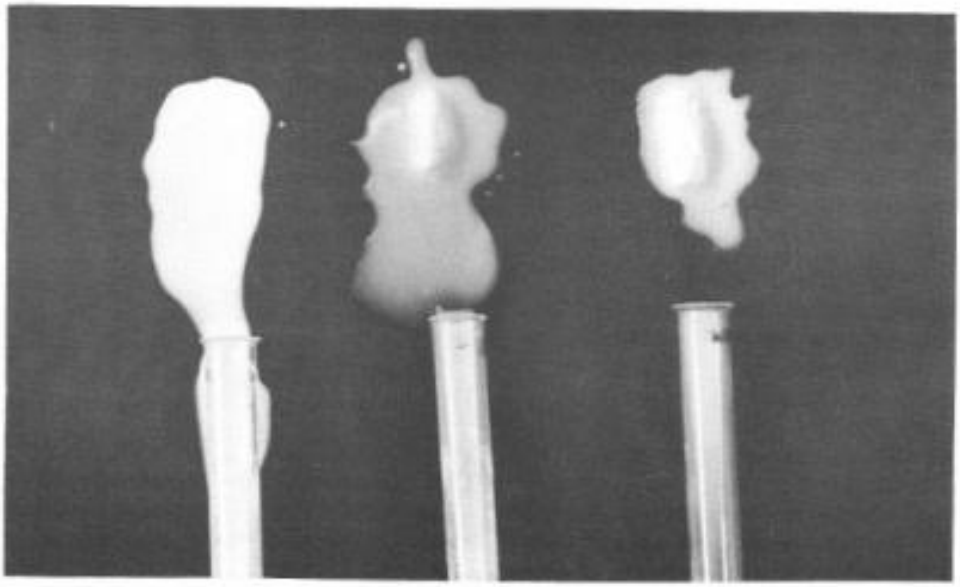

Figure 12. Rubber particle suspensions (9% in C-serum) treated with 9% bark sap and poured out of the test tube after 50 min. Left to right: rubber particles from Zone 1; from Zone 2; from Zone 3 of centrifuged latex.

particles were found to have coagulated although not as extensively as the *Zone 3* particles. *Zone 1* rubber particles were uncoagulated and remained fluid *(Figure 12)*. It would appear, therefore, that just as *Zone 2* rubber particles are intermediate in size and density between *Zone 1* and *Zone 3* rubber particles, their sensitivity to destabilisation by bark sap is also intermediate, but more closely resembling that of *Zone 3* rubber particles.

CONCLUSIONS

Bark sap has been shown to be a powerful destabiliser of latex as distinct from a laboratory preparation of rubber suspension. Whereas B-serum flocculates readily a suspension of rubber in water, its potency in an environment of C-serum — as would be the case in whole latex — is very much inhibited. On the other hand, bark sap destabilises whole latex readily and its activity might therefore be very relevant in terms of plug formation and the regulation of latex flow when the tree is tapped. Indeed, volume for volume, bark sap prepared in the manner that was done for the present series of experiments is far more powerful a destabiliser of latex than is B-serum.

Rubber particles are not all equally reactive towards bark sap. The smaller, higher density particles — those from *Zones 2* and *3* of centrifuged latex — are far more sensitive to destabilisation than the larger, lower density rubber particles from *Zone 1* which constitute the bulk of the rubber particles in whole latex. It might be speculated that destabilised high density rubber particles act as sites of initiation of latex vessel plugs which exert control on latex outflow when the tree is tapped.

The results from recent research pointing to bark sap being an important native latex destabilising agent in *Hevea* depart from the emphasis on lutoids and B-serum in explaining latex vessel plugging. Nevertheless, the postulate that lutoid damage is involved in the initiation of latex vessel plugging is not rejected, but more awareness should be placed on the action of bark sap and high density rubber particles as complementary factors in this respect.

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