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Synergism Between B-serum and Bark Sap in the Destabilisation of High Density Rubber Particles in Hevea *Latex*

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Hevea *B-serum and bark sap acted synergistically to destabilise the high-density* (Zone 2) *rubber particles of latex. When bark sap was supplemented to B-serum and* vice versa, *flocculation and coagulation of the rubber particles were greatly activated. Whereas coagulation by bark sap occurred only in the substantial presence of C-serum and that by B-serum required its absence, the coagulation induced by various combinations of the two destabilisers had intermediate C-serum requirements. Hence, given the appropriate combination of B-serum and bark sap, coagulation could be evoked in the presence of practically any concentration of C-serum. The synergistic interaction of B-serum with bark sap serves as a mechanism by which B-serum can contribute actively in the destabilisation of rubber particles even in the presence of considerable amounts of C-serum, as is the situation in latex.*

Destabilisation of *Hevea brasiliensis* latex as it exudes from the tree is closely involved in the formation of latex vessel plugs which stop outflow from the plugged vessels. Electron microscopic studies have revealed that latex vessel plugs comprise principally of agglomerations of destabilised rubber particles and lutoids[1]. From other studies[2], destabilised rubber particles have also been found within latex vessels of partially yielding or non-yielding (dry) trees. As in the case of latex vessel plugs, these flocculated or coagulated rubber particles were frequently seen adhering to lutoids. The close association of lutoids with latex destabilisation stems from the release of B-serum from damaged lutoids. B-serum has a strong flocculating effect on rubber particles[3,4] and can induce their coagulation under certain circumstances[5,6].

While B-serum is an effective and fast-acting destabiliser of rubber particles, it has one major inherent limitation: its activity is strongly inhibited by C-serum[3]. This is an important factor when considering latex destabilisation *in vivo* since the rubber particles in latex are suspended in C-serum.

Besides B-serum, *Hevea* bark sap is another native destabiliser of rubber particles[6–9]. Bark sap activity is largely uninhibited by C-serum, and in fact, its presence is required for the coagulation of high-density rubber particles[6].

In preceding reports[6,9], comparisons have been made between the potencies of B-serum and bark sap acting individually as latex or rubber particle destabilisers. The present paper describes an interaction between B-serum and bark sap that generates a powerful destabilising effect surpassing the effects of either destabiliser acting alone.

MATERIALS AND METHODS

Latex and bark shavings from the tapping cut were collected from RRIM 600 trees tapped ½S d/2 on *Panel BO-2*. High-density *(Zone 2)* rubber particles[10], B-serum and C-serum were prepared from centrifuged latex and bark sap from bark shavings as described previously[6]. *Zone 2* rubber cream was dispersed in various dilutions of C-serum (3% - 90%) to give 6% rubber particle suspensions. The rubber particle destabilisation reactions were initiated by

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adding B-serum or bark sap to the suspension and mixing immediately with a vortex mixer for 2 – 3 s. In treatments where both B-serum and bark sap were added, the two destabilisers were either pre-mixed or were added one after the other as indicated in the text, with vortex mixing after each addition. All reaction mixtures were unbuffered.

Destabilisation of the suspensions was quantified by the extent of flocculation and coagulation of the rubber particles that took place. The intensity of flocculation was determined from the amount of rubber floccules (scored on a scale of 0 to 5) left on the side of the test tube after vortex mixing[6]. To determine the extent of coagulation, the lump of rubber which coagulated 20 min after adding the destabiliser was picked up using a short length of wire mounted on a glass rod. The rubber coagulum and the uncoagulated portion of the suspension were dried and weighed separately. The coagulum dry weight was then expressed as a percentage of the total dry weight of the suspension.

The concentration of C-serum in the rubber particle suspensions refers to the proportion of C-serum in the suspending medium after addition of the destabiliser. Concentrations of rubber particles, B-serum and bark sap refer to the final concentrations in 2 ml reaction mixtures.

RESULTS

Separate and Combined Destabilising Effects of B-serum and Bark Sap

The destabilising effects of B-serum and bark sap were tested on 6% suspensions of high-density *(Zone 2)* rubber particles which have been shown[6,9] to be sensitive to destabilisation. The individual effects of B-serum and bark sap on the suspensions were tested by the addition of the undiluted destabilisers to attain a final concentration of 8%. To assess their combined effects (*i.e.* the interaction between B-serum and bark sap), half the amount (*i.e.* 4%) of each destabiliser was added.

The results showed that the flocculation of rubber particles by 8% B-serum was most active when C-serum was substantially absent. When the C-serum concentration in the suspending medium exceeded about 50%, flocculation was almost completely suppressed. On the other hand, flocculation induced by 8% bark sap was not as much affected by the presence of C-serum *(Figure 1)*.

When 4% each of B-serum and bark sap were added one after the other to the rubber suspension, the flocculation that ensued was generally comparable to that induced by 8% bark sap. However, flocculation was less intense at the higher C-serum concentrations, especially where the addition of bark sap had preceded B-serum *(Figure 1)*. When the two destabilisers were pre-mixed prior to addition to the suspension, there was a very marked reduction in flocculation *(Figure 2)*.

The test suspensions were checked for coagulation 20 min after addition of the destabilisers. Coagulation was induced by 8% B-serum only when the C-serum content was very low (3%) and was completely inhibited at all the higher C-serum dilutions attempted *(Figure 3)*. Unlike B-serum, bark sap-induced coagulation was not inhibited by C-serum; indeed, the presence of C-serum was obligatory. Coagulation was strongest when C-serum concentration in the suspending medium was 70%. Bark sap did not induce coagulation at C-serum concentrations below 50% *(Figure 3)*.

Because B-serum and bark sap had opposite C-serum requirements for coagulation, there was a middle band of C-serum concentrations (generally from 20% to 50%, but the exact range varying slightly between experiments) where neither destabiliser acting on its own could induce coagulation. When 4% each of B-serum and bark sap were added to the rubber particle suspension, however, coagulation was observed over the entire range of C-serum dilutions. The reaction was especially strong in the middle range of 20% to 70% C-serum, being weaker and less consistent where C-serum levels were very high (90%) or very low (3%) *(Figure 3)*. Thus, the combined effect of B-serum and bark sap effectively induced coagulation even where the concentration of C-serum precluded coagulation by either

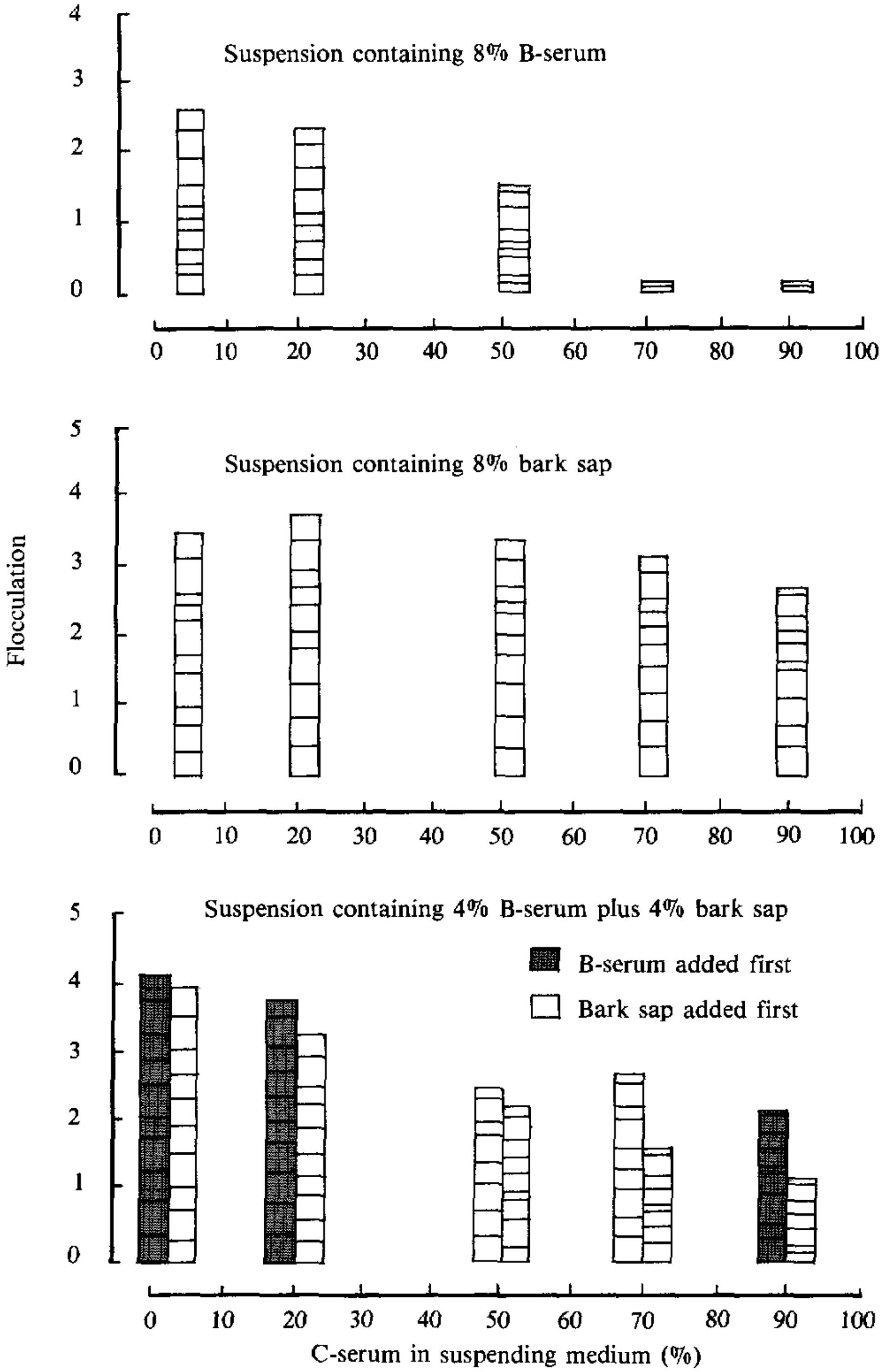

Figure 1. Induction of flocculation in Zone 2 rubber particles (6%) suspended in various concentrations of C-serum by the separate and combined effects of B-serum and bark sap. Column heights represent the means of ten experiments. Each segment in a column represents the proportional contribution of one experiment.

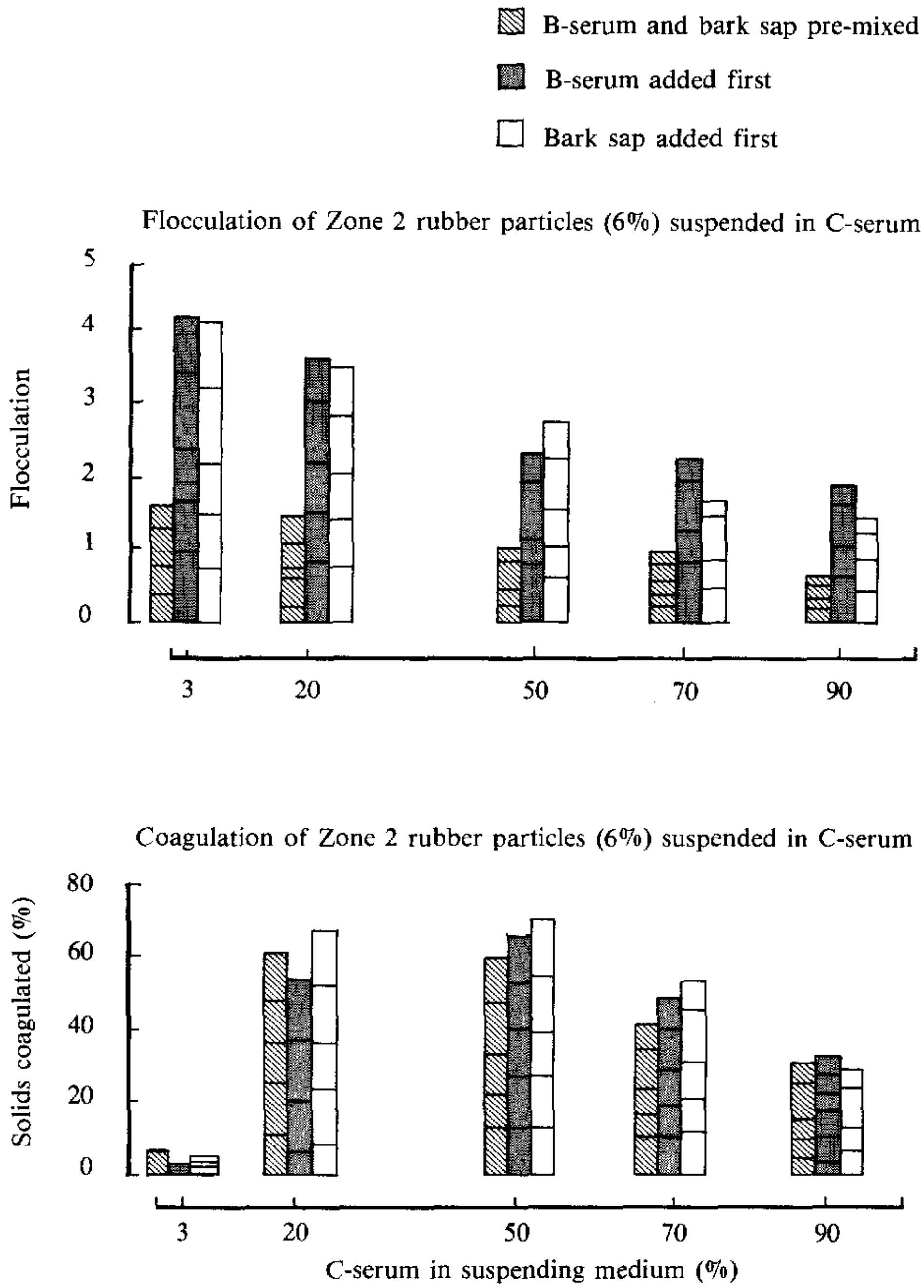

Figure 2. Effect of pre-mixing B-serum and bark sap on the flocculation and coagulation of Zone 2 *rubber particles (6%) suspended in various concentrations of C-serum. The suspensions contained 4% B-serum plus 4% bark sap, with the B-serum and bark sap pre-mixed or with B-serum added first or with bark sap added first. Column heights represent the means of five experiments. Each segment in a column represents the proportional contribution of one experiment.*

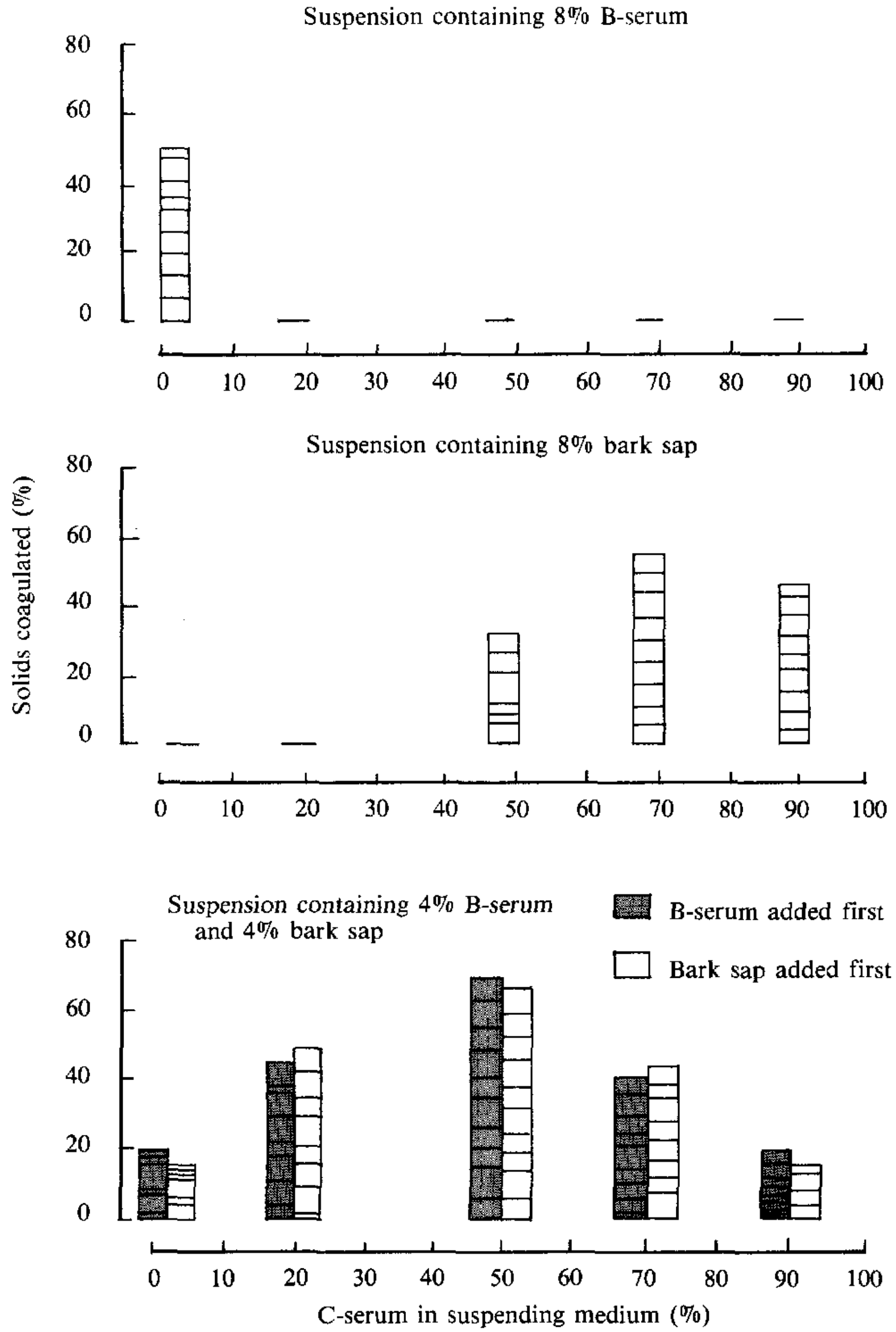

Figure 3. Induction of coagulation in Zone 2 rubber particles (6%) suspended in various concentrations of C-serum by the separate and combined effects of B-serum and bark sap. Column heights represent the means of ten experiments. Each segment in a column represents the proportional contribution of one experiment.

destabiliser acting separately. For example, neither 8% B-serum nor 8% bark sap evoked the coagulation of the rubber particles suspended in 20% C-serum, but a combination of 4% each of B-serum and bark sap did so effectively (*Figures 3* and *4*). Unlike the flocculation reaction, there was no difference whichever destabiliser was added to the suspension first or if the destabilisers had been pre-mixed (*Figure 2*).

The above trends in flocculation and coagulation were consistently repeatable although the absolute C-serum concentrations for maximal and minimal activities shifted slightly between experiments. Similar trends were also observed with rubber particle suspensions prepared from latices of clones GT 1 and PR 261 (results not presented).

As the destabilisation of latex or rubber particle suspensions by B-serum[3], bark sap[8] or cations[5] is enhanced under acidic conditions, a check was made to determine if the destabilisation induced by B-serum and/or bark sap arose primarily from a depression in pH. Accordingly, pH was determined in suspensions containing 3%, 70% and 50% C-serum (being optimal for destabilisation by B-serum, bark sap and the two destabilisers combined) after the respective destabilisers were added. The pH readings (*Table 1*) showed that C-serum, which had a pH close to neutral, had a high buffering capacity. Although B-serum and bark sap were both acidic, rubber particle suspensions containing 50% or 70% C-serum had pH only slightly below neutral when the destabilisers were added. It was only when C-serum was reduced to 3% in the suspending medium that

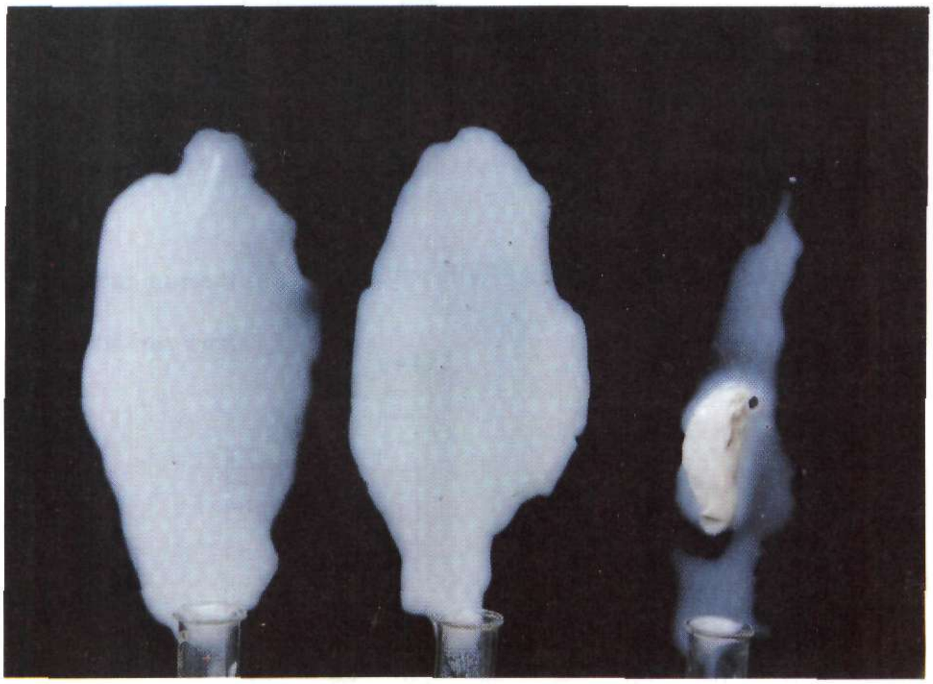

Figure 4. Separate and combined effects of B-serum and bark sap on the coagulation of Zone 2 *rubber particles (6%) suspended in 20% C-serum. The suspensions contained (left) 8% B-serum, (centre) 8% bark sap and (right) a mixture of 4% B-serum plus 4% bark sap. The test tube contents were poured out 20 min after addition of the destabilisers to show coagulation induced by the interaction between B-serum and bark sap.*

TABLE 1. pH OF DESTABILISERS AND REACTION MIXTURES

Destabiliser/Reaction mixture	pH
Bark sap	5.5
B-serum	5.4
C-serum	6.9
6% Zone 2 rubber particle suspensions containing:	
8% B-serum, 3% C-serum	5.8
8% bark sap, 70% C-serum	6.7
4% B-serum, 4% bark sap, 50% C-serum	6.6

the pH decreased to almost that of the B-serum that was added. Thus, while decreased pH might possibly have contributed to the destabilisation effected by B-serum, that by bark sap or the mixture of bark sap and B-serum was not primarily mediated through a decrease in pH.

Destabilising Effects of B-serum and Bark Sap Combined in Varying Proportions

In the foregoing experiments, the interaction between B-serum and bark sap had been investigated by adding equal proportions (4%) of B-serum and bark sap to the rubber particle suspensions. In subsequent experiments, the combined effects of the two destabilisers present in varying proportions were investigated by holding the concentration of one destabiliser constant at 4% while the concentration of the other was varied between 0% and 6%. In these experiments, bark sap was always added before B-serum to the rubber particle suspensions.

The results presented in *Figure 5* show that 4% B-serum or 4% bark sap, each acting separately, was not sufficient to induce rubber particle flocculation. However, when 2% bark sap was supplemented to the B-serum or, *vice versa,* when 2% B-serum was supplemented to bark sap, active flocculation was observed. It was apparent that irrespective of which destabiliser was held constant and which was varied in concentration, the trend was similar. Rubber particle flocculation was progressively enhanced by the increase in the *total* destabiliser content in the suspension, irrespective of the ratio of each destabiliser present. For any given mixture of the two destabilisers, flocculation was always strongest at the lowest C-serum concentration (3%), decreasing progressively as C-serum concentration increased to 90% *(Figure 5).*

Whereas rubber particle flocculation was not influenced by the ratio of B-serum and bark sap added, coagulation of the rubber particles was very much so. No coagulation was induced by 4% B-serum alone but coagulation activity was evident when 2% bark sap was supplemented *(Figure 6).* As more bark sap was added, coagulation activity increased, especially at the higher C-serum concentrations. When finally 6% bark sap was supplemented to the 4% B-serum, coagulation was observed to be strongest at 70% C-serum. Thus, when there was a predominance of bark sap over B-serum, the combined effect on coagulation resembled that of 8% bark sap added alone *(cf. Figures 3* and *6).*

A corresponding relationship was observed when B-serum was added in excess of bark sap in that coagulation behaviour tended towards that of B-serum acting alone. Thus, when 6% B-serum was supplemented to 4% bark sap, the strongest coagulation activity was observed when the amount of C-serum present was lowest *(cf. Figures 3* and *6).*

Rate of Destabilisation by a Mixture of B-serum and Bark Sap

Flocculation of high-density rubber particles induced by B-serum, bark sap or their mixtures was instantaneous upon addition of the destabilisers. Coagulation required a lapse of time, but under optimal conditions, the period required would be far shorter than the 20 min that had been adopted in the standardised assays in the present study. In the experiments described, the concentrations of the destabilisers and the rubber particles in the suspensions had been deliberately kept low so that a gradation of the destabilisation responses could be discerned between treatments.

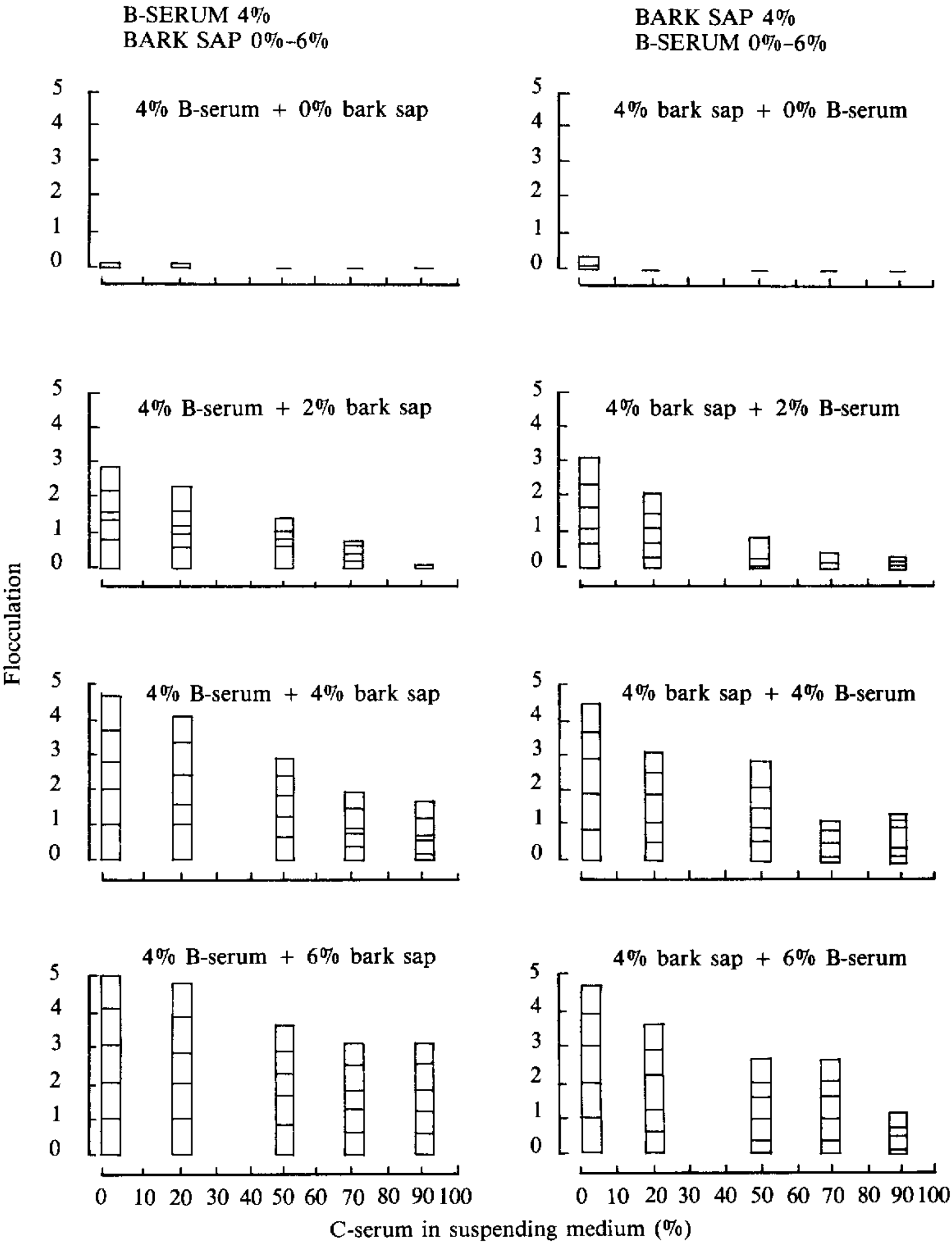

Figure 5. Effect of varying proportions of B-serum and bark sap on flocculation of Zone 2 *rubber particles (6%) suspended in various concentrations of C-serum. Column heights represent the means of five experiments. Each segment in a column represents the proportional contribution of one experiment.*

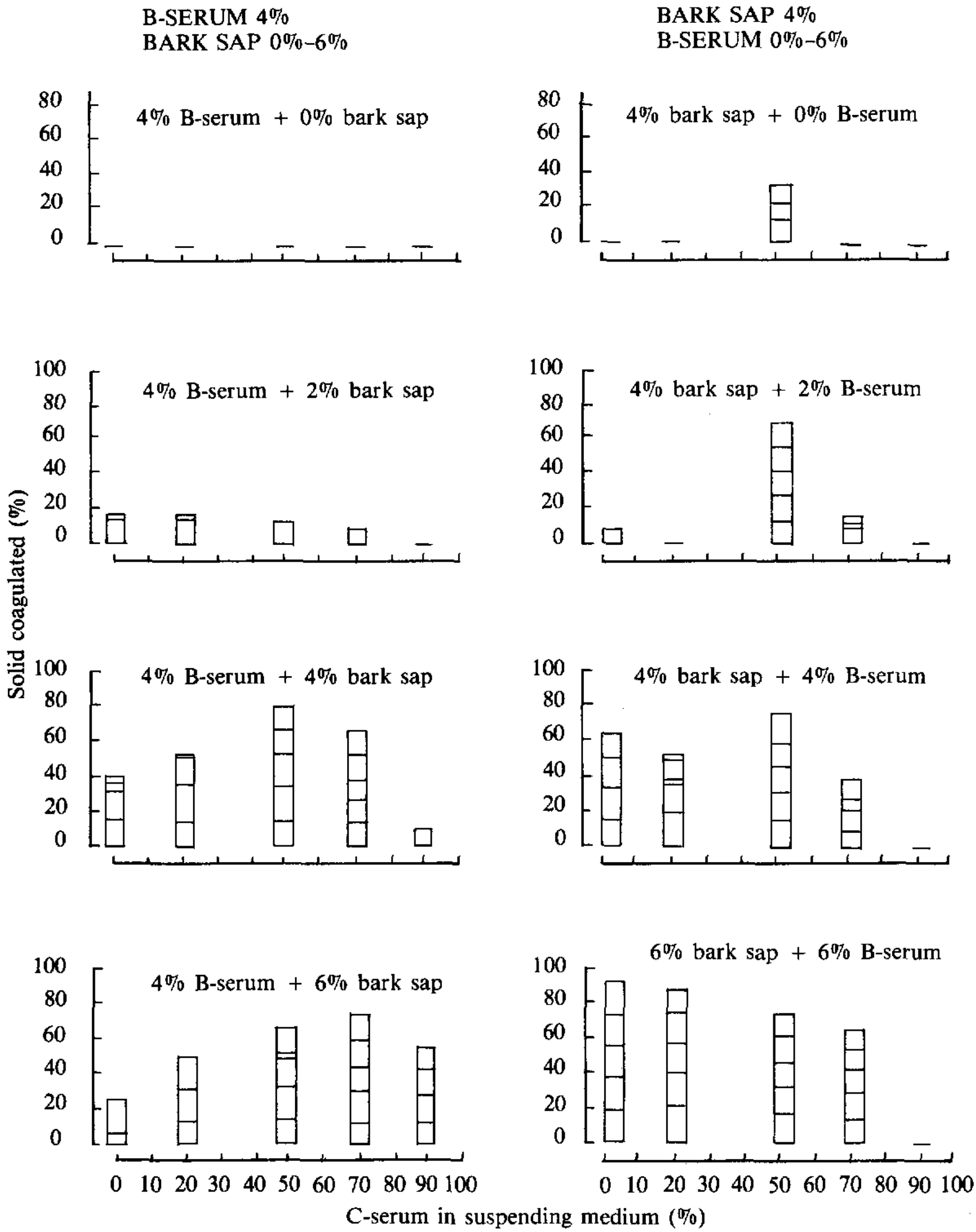

Figure 6. Effect of varying proportions of B-serum and bark sap on coagulations of Zone 2 *rubber particles (6%) suspended in various concentration of C-serum. Column heights represent the means of five experiments. Each segment in a column represents the proportional contribution of one experiment.*

To determine how fast coagulation could be effected when the concentrations of the destabilisers and rubber particles were increased, B-serum and bark sap where pre-mixed in equal proportions and added to *Zone 2* rubber particles suspended in 50% C-serum. The rubber particle suspension was checked for the onset of coagulation every 15 s. When the rubber content of *Zone 2* was increased from 6% to 20%, mass coagulation (gellation) of the entire suspension was induced by a mixture of 4% B-serum and 4% bark sap after an average of only 1.7 min in five replicate observations. When the concentrations of the destabilisers were increased to 10% B-serum plus 10% bark sap, coagulation in the 20% rubber suspension took an average of 48 s.

DISCUSSION

When lutoids in latex flowing out of the tree are damaged, the compartmentalised B-serum is released into the ambient C-serum which, theoretically, ought to strongly inhibit its activity. To explain B-serum activity *in vivo,* it is commonly held that when lutoids burst, the sudden release of B-serum results in its temporary high concentration in the immediate vicinity of the ruptured lutoids. Rubber destabilisation can then take place before much mixing can occur between the B-serum and the ambient C-serum[3]. While this explanation is reasonable, observations on lutoid behaviour under the microscope have suggested that the deterioration of lutoid membranes is often gradual. The lutoid membranes become progressively more permeable, leading to a slow leakage of B-serum into the exterior[11]. Yet if B-serum were to diffuse *gradually* out of membrane-damaged but unruptured lutoids rather than be released *suddenly* by the lutoids bursting, it would be difficult to avoid extensive mixing with the preponderance of ambient C-serum that would inactivate it. In this situation, it might be expected that the potency of B-serum as a destabiliser of rubber particles would be severely curtailed. The synergism between B-serum and bark sap demonstrated in the present study provides a mechanism by which B-serum can contribute actively to the destabilisation of rubber particles even in the presence of considerable amounts of C-serum.

The behaviour of B-serum and bark sap in inducing coagulation gave rise to the situation where induction of coagulation by either destabiliser separately involved two extremes in C-serum requirement. As previously noted[6], its substantial absence was essential in coagulation by B-serum while its substantial presence was required in coagulation by bark sap. In between these two extremes (around 20% - 50% C-serum), neither destabiliser could actively induce coagulation. This was however readily achieved by the combined effect of the two destabilisers. Coagulation so induced was optimal at varying concentrations of C-serum, depending on the relative proportions of each destabiliser present. Thus, given the appropriate combination of B-serum and bark sap (ranging from a mixture high in B-serum content to one high in bark sap), coagulation could be strongly induced in the presence of practically *any* concentration of C-serum. The dependence of each individual destabiliser on the presence or absence of C-serum and the limitations imposed thereby are hence largely overcome by their synergistic interaction. Moreover, the flocculation and coagulation that ensued required less amounts of each destabiliser compared with the destabilisers acting separately.

The mechanism of the interaction between B-serum and bark sap has yet to be elicited. It appears to be more complex than the simple additive effects of the two destabilisers since the interaction could induce destabilisation under conditions where neither destabiliser was effective. The pH measurements of destabilisers and test mixtures indicated that the mechanism was not principally related to changes in pH.

To what extent are the rubber particle destabilisation reactions described relevant to the *in situ* latex coagulation in dry trees and to the formation of latex vessel plugs? It is possible that, through a physiological disorder, bark sap infuses into latex vessels to destabilise rubber particles jointly with B-serum from lutoids in the latex. Tree dryness may subsequently result. In the case of latex vessel plugging, two considerations need to be examined:

- Whether both B-serum and bark sap are likely to be released at or near the surface of the tapping cut where plug formation takes place
- Whether the destabilising effects of the interaction between B-serum and bark sap are sufficiently rapid.

Increased plugging was observed when the release of bark sap at the tapping cut was putatively increased[12]. It might be expected that ample opportunity exists for damaged and unstable lutoids in the exuding latex to come into contact with bark sap released at the tapping cut. It is still unresolved, however, whether any bark sap that is released *in vivo* is similar in nature and composition to that prepared in the laboratory.

The formation of latex plugs has to be very rapid as otherwise the incipient plugs would be washed out with the out-flowing latex[3]. In this connection, latex flocculation, which is practically instantaneous, meets this requirement readily. It is shown in the present study that coagulation (mass gellation) induced by a combination of B-serum and bark sap, although not instantaneous, can occur in less than 1 min. The coagulation reaction might therefore serve to consolidate the incipient plugs initially laid down by the flocculation mechanism.

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