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Effect of Hydroxylamine Hydrochloride on Micro-Organisms in Hevea Latex

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Viscosity-stabilised (CV) rubber can, with some advantages, be produced by the assisted biological coagulation (ABC) process, though the coagulation time is thereby prolonged and also the coagulum becomes hard and non-porous. The inhibitory effect of hydroxylamine hydrochloride on Hevea *latex bacteria, resulting in less acid formation has been found to prolong the coagulation time while the non-porosity of the coagulum due to interference with the metabolic activities of gas-producing bacteria caused by the chemical. A porous CV rubber coagulum can, however, be obtained through seeding latex serum.*

Latex is coagulated by its indigenous bacteria and yeasts which produce acids by metabolising the non-rubber substances in it (JOHN, 1966 a and b). This process normally takes about 48 hours to complete, but can be reduced to about 16 hours by assisted biological coagulation (ABC process) mediated through the addition of a carbohydrate source such as molasses (JOHN, 1966c). The resulting coagulum is porous enough to increase by about 50% the throughput in granulation and pelletisation (SMITH, 1969). The dry rubber is lighter in colour and has better dynamic properties as compared with acid-coagulated rubber.

Viscosity-stabilised (CV) rubber, produced by treating latex with 0.15% hydroxylamine hydrochloride (HH) and coagulating it with formic acid at pH 5.2 (SEKHAR, 1960), has many technological advantages over conventional rubber. But, it is slow-curing and darker in colour (CHIN, 1969), and the coagulum is non-porous and hard. To overcome these disadvantages, attempts were made to make CV rubber by ABC process, but the time of coagulation was found to be inconveniently long (RUBBER RESEARCH INSTITUTE OF MALAYA, 1968) suggesting that HH has some inhibitory effect on the activities of micro-organisms in latex.

This paper discusses the results of studies on the influence of HH on the metabolic activities of bacterial and yeast populations of *Hevea* latex and its effect on coagulation.

MATERIALS AND METHODS

Media

The culture media used to enumerate bacteria were: (1) molasses/yeast extract agar consisting of 0.5% molasses, 0.5% yeast extract, 0.005% bromocresol purple and 1.25% Oxoid agar No. 3 (JOHN, 1968); (2) molasses/yeast extract broth, which is similar but without any agar and (3) Oxoid nutrient broth No. 2 consisting of 1% beef extract, 1% peptone and 0.5% sodium chloride. For the enumeration of yeasts, a modified Martin's medium consisting of 4.0% Oxoid malt extract agar, 75 mg/ml of dihydrostreptomycin sulphate and 0.0033% of rose bengal was used (JOHN AND TAYSUM, 1963).

Enumeration of Bacteria and Yeasts

Bacteria and yeasts were enumerated in solid medium by pour-plating or surface-plating tenfold serial dilutions of the test material, incubating at 30°C for three to four days and counting the colonies by the method of JOHN AND TAYSUM (1963); in liquid medium, they were estimated by light scattered in a nephelometer after a period of incubation at 30°C.

Organisms

The effect of hydroxylamine hydrochloride on five of the bacteria and three of the yeasts

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TABLE 1. EFFECT OF HH ON TIME OF COAGULATION OF LATEX AND POROSITY OF RESULTING COAGULUM

Coagulation system	Additive	Initial pH	Time of coagulation (h)	Porosity* of coagulum
Auto	—	6.2	48	+++
Auto	HH	6.0	96	Nil
ABC	Molasses	6.2	16	+++++
ABC	Molasses + HH	6.0	48	Nil
Acid	Formic acid	5.2	8	+
Acid	Formic acid + HH	5.2	8	Nil

*Porosity was assessed on a scale of O-5 units visually, based on estimation of the amount of gas entrapped in the coagulum.

present in *Hevea* latex in large numbers were studied, since they are widely regarded as being fairly representative of its microflora. The bacteria were: *Aerobacter* sp, *Listeria* sp, *Chromobacterium* sp and two strains of *Corynebacterium*. They were maintained on molasses/yeast extract agar or on modified Martin's medium. Investigations on the identity of the yeasts reacted with HH are in progress.

Gas Formation by Bacteria

The gas-producing ability of a pure culture of bacteria was tested by inoculating a standard test tube containing 10 ml of melted medium, shaking gently until the medium solidified and incubating at 30°C for two days; gas production was indicated by the splitting of the solid medium in the tube.

Coagulation

Latex was coagulated in three ways: (a) with formic acid at pH 5.2; (b) by assisted biological coagulation with 0.4% molasses added on the basis of d.r.c.; and (c) by auto-coagulation.

Latex Serum Seeding

Serum extracted from one-day-old coagulum and filtered to remove any crumbs was added to fresh latex at 15% w/w.

RESULTS

Effect of HH on Coagulation

Factory bulk latex was distributed into six coagulating tanks and the latex in three was treated with 0.15% HH (see *Table 1*). One each of these tanks was coagulated in the presence of either acid or molasses or with no additives. The time to obtain a firm coagulum and its porosity were noted. Despite its ability to slightly lower the pH of latex, HH considerably prolonged the time to complete coagulation by the ABC and autocoagulation. It also completely prevented the formation of gas bubbles responsible for producing a porous coagulum.

Inhibition of Bacteria and Yeasts in Latex

Factory bulk latex was separately treated with various levels of HH and viable bacterial and yeast populations enumerated at various intervals (0, 4, 8 and 12 hours). *Figure 1* shows that 0.3% HH markedly inhibited the bacterial population, while 0.15% did not allow any further proliferation during the first 12 hours. The inhibitory effects of 0.1 and 0.05%, however, were less marked. By the twelfth hour, the bacterial population in the latex treated with 0.15% HH (concentration normally used

in commercial production of CV rubber) was reduced from 10^9 to 10^7 per ml.

Figure 2 shows that the yeast population of latex with HH was only marginally different from that of the control for the first eight hours; thereafter, if anything, HH sustained the growth rather than inhibiting it.

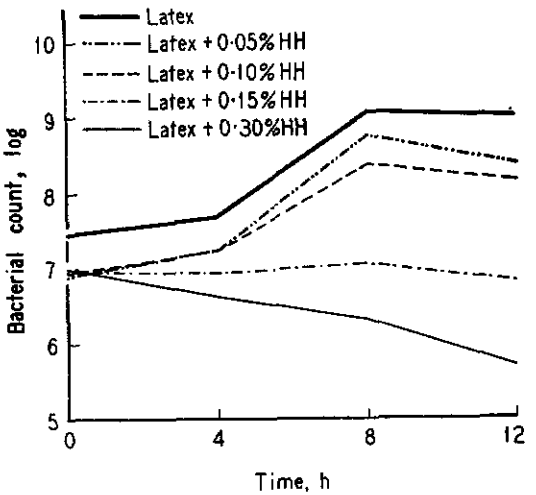

Figure 1. Inhibitory effect of HH on latex bacteria.

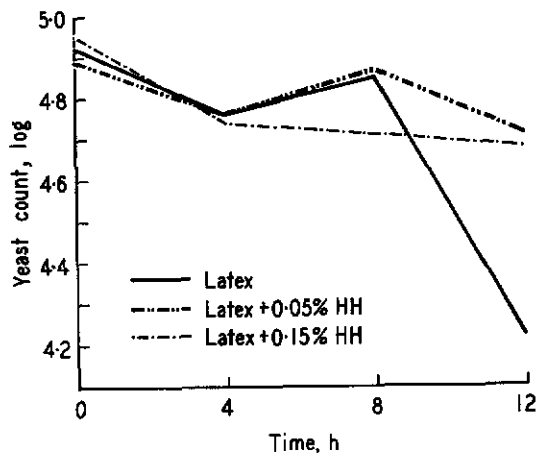

Figure 2. Effect of HH on yeasts.

Effect of HH on Pure Cultures of Bacteria and Yeasts

Bacterial growth. The five bacterial isolates from fresh field latex were separately inoculated into Oxoid nutrient broth, with and without HH. The cultures were incubated at 30°C and bacterial growth estimated at various intervals using a nephelometer. The growth pattern of these organisms is shown in *Figure 3.* In the absence of HH, a typical growth curve is obtained in all five organisms. In the presence of 0.015% HH, a highly protracted lag phase is observed in all with a rise in population to varying degree after 25–30 hours. With 0.035%, however, hardly any growth was observed even in 48 hours. Only one of the *Corynebacterium* sp behaved somewhat differently, showing a rise after 10 hours with 0.015% and some weak growth after 30 hours with 0.035%.

Yeast growth. Three yeast cultures originally isolated from latex were separately inoculated into broth containing three levels (0.015, 0.035 and 0.05%) of HH. The growth estimated at various intervals after being incubated at 30°C showed that HH had no effect on their growth at any of the concentrations used.

Porosity of Coagulum

Factory bulk latex was separately treated with four levels (0.05, 0.1, 0.15 and 0.3%) of HH followed by coagulation with acid, molasses or with no additives. The porosity of coagula was assessed visually on a scoring scale ranging from 0–5 units. The experiment was repeated six times using latices from various sources; the results obtained are given in *Figure 4.*

Reduction in porosity was negligible or none at 0.05% level; as the concentration increased, however, it became marked, irrespective of the coagulation method used.

Interference of HH in Metabolic Activity

Fifty colonies of bacteria growing in a surface plate derived from a ten-fold serial dilution of a fresh field latex sample were purified. The cultures were inoculated into deep tubes and their ability to produce gas was examined. Only seven cultures which produced gas were then inoculated into two sets of molasses/yeast extract agar deep tubes, one set containing

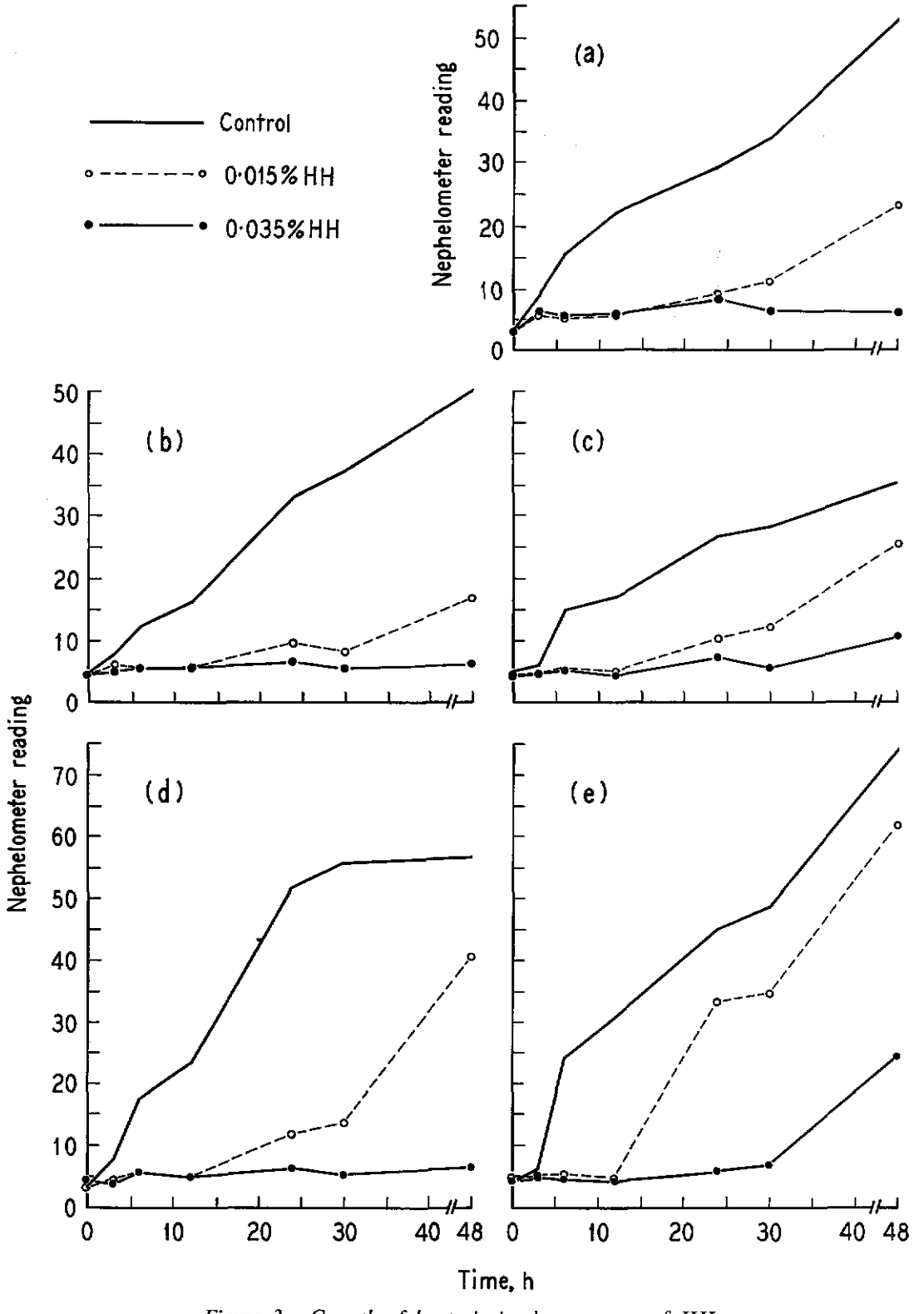

Figure 3. Growth of bacteria in the presence of HH.
(a) Acrobacter *sp; (b)* Listeria *sp; (c)* Corynebacterium *sp; (d)* Chromobacterium *sp; (e)* Corynebacterium *sp.*

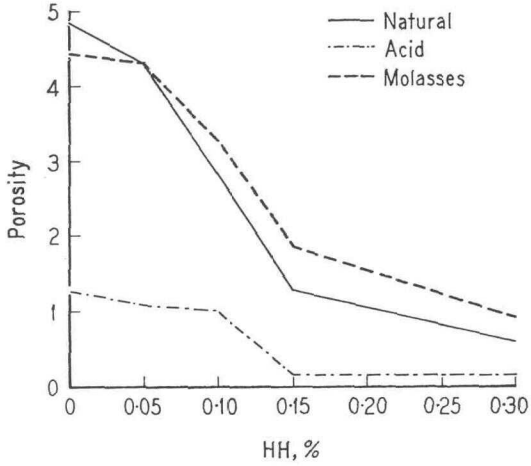

Figure 4. Effect of HH on the porosity of coagulum (mean of seven experiments).

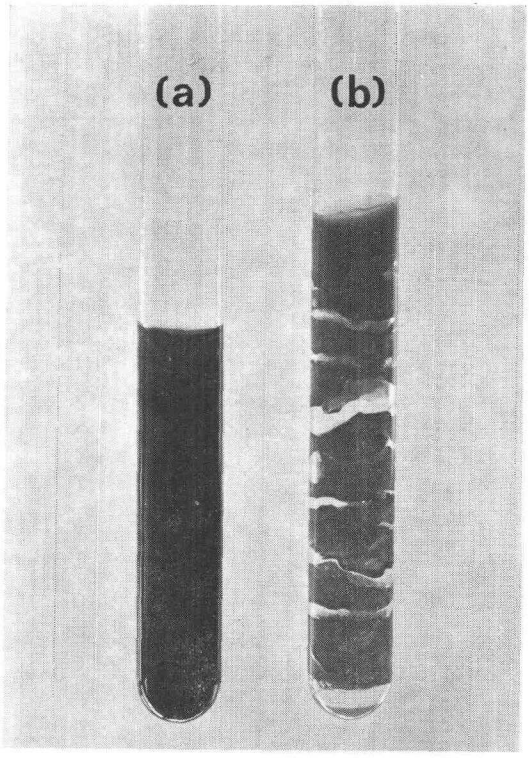

Figure 5. Effect of HH on gas production of bacteria. (a) Treated with HH; (b) Without HH.

0.05% HH and the other without HH. Both sets were incubated at 30°C. All seven organisms grown in the absence of HH produced copious amounts of gas, while no gas was produced in its presence *(Figure 5)*.

The seven deep tube cultures grown in the presence of HH were transferred to fresh deep tubes without HH and incubated at 30°C to find out whether the absence of gas was due to destruction of these organisms or to inhibition of their metabolic activity by HH. In 24 hours all the cultures had grown profusely and produced gas, proving that HH merely interfered with the metabolic activity of these organisms but did not destroy them.

Effect of Latex Serum Seeding on HH Resistance

With a view to producing a porous CV rubber coagulum, the following experiment was carried out. Fresh field latex treated with 0.4% molasses and 0.05% HH was left to coagulate. The serum was extracted after 48 hours and used to seed a fresh sample of latex treated with 0.4% molasses and 0.1% HH. A semi-porous coagulum which resulted after 48 hours was pressed and the serum was used to seed another fresh latex sample containing 0.4% molasses and 0.15% HH. The resulting coagulum was found to be more porous having more air bubbles, whereas the coagulum obtained from the same bulk of latex without the latex serum seeding was devoid of air bubbles. Daily seeding passages were carried out using latex serum expressed from the previous batch. The resulting coagula were porous, harbouring numerous air bubbles.

DISCUSSION

The investigations have shown that latex bacteria are inhibited by hydroxylamine hydrochloride, the extent of inhibition, however, depending upon its concentration. This occurs with bacteria indigenously present in latex as well as pure cultures, isolated from it and grown in synthetic media. A higher rate of inhibition appears to occur in cultures grown in synthetic media as compared to bacteria in latex. This is appreciated as latex is a complex biological system consisting of resins, sugars, proteins, lipids, mineral salts, carotenoids etc

(ARCHER, BARNARD, COCKBAIN, DICKENSON AND MCMULLEN, 1963; and DUNPHY, WHITTLE, PENNOCK AND MORTON, 1965), also capable of enhancing the growth of bacteria (JOHN, 1966a and b). Another possibility is that monocultures may be more sensitive to hydroxylamine hydrochloride. GILLISSEN AND HEUSEL (1961), who screened a number of organisms for the minimum bacteriostatic concentration of hydroxylamine hydrochloride, found that there is a remarkable variation in the sensitivity of the organisms, with *Bacillus subtilis* needing the minimum and *Escherichia coli* needing the maximum concentration among the organisms tested. ALLAN GRAY AND LAMBERT (1948), while testing the ability of oximes and their derivatives, likewise found HH inhibiting the growth of *Staphylococcus aureus*, *Streptococcus*, *Salmonella typhi*, *Bacterium coli*, *Proteus vulgaris* and *Bacillus subtilis*.

The inhibition of bacteria, though relatively less in latex than in pure cultures, is still sufficient to result in less acid production within a reasonable time. Consequently latex containing HH takes about 96 hours to coagulate completely, instead of the normal 48 hours. Similar reaction is also observed when latex is coagulated with molasses, the time of coagulation being prolonged from about 16 to 48 hours. HH, however, has little effect on the time of coagulation with acid; if anything, it enhances the coagulation slightly, presumably due to its acidic nature. The porosity of coagulum is extremely important as it can dictate the throughput of granulation and pelletisation; a porous coagulum harbouring gas bubbles gives about 50% greater throughput and dries faster compared to a non-porous hard coagulum (SMITH, 1969).

It is evident that HH interferes with the metabolic activities of latex bacteria preventing the formation of gas. This would adequately explain why a non-porous coagulum was obtained when latex containing HH was coagulated spontaneously or in the presence of molasses.

Although the presence of HH leads to a non-porous coagulum, it is possible to obtain a porous coagulum through a process of seeding latex with a serum extracted from the coagulum. The seeding involves treatment of latex with a small quantity of HH initially and then gradually increasing the level to that required in the preparation of CV rubbers.

Investigations on the possible inhibitory effect of HH on latex yeasts have shown that HH, at the concentration used for CV rubber production and at higher levels tested, does not inhibit the yeast population; if anything, it sustains their growth *(Figure 2)*. This is in agreement with the findings of GILLISSEN AND HEUSEL (1961) who reported that the minimum bacteriostatic concentration of HH for baker's yeast is about hundred times more than that required for bacterial cultures.

In conclusion, HH affects the growth of latex bacteria causing a delay in the time required for spontaneous coagulation. The resulting coagulum is non-porous as HH interfers with the metabolic activities of gas-producing bacteria and prevents formation of gas bubbles. However, a porous coagulum enhancing the throughput of granulation and pelletisation can be obtained from latex treated with HH by gradually building up a bacterial population resistant to it.

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REFERENCES

ALLAN GRAY, J.D. AND LAMBERT, R.A. (1948) Bacteriostatic action of oximes. *Nature, Lond.*, **162(4123)**, 733.

ARCHER, B.L., BARNARD, D., COCKBAIN, E.G., DICKENSON, P.B. AND MCMULLEN, A.I. (1963) Structure, composition and biochemistry of *Hevea* latex. *The Chemistry and Physics and Rubber-like Substances (Bateman, L., ed.)*, 41. London: Maclaren and Sons Ltd.

CHIN, P.S. (1969) Viscosity-stabilised Heveacrumb. *J. Rubb. Res. Inst. Malaya*, **22(1)**, 56.

DUNPHY, P.J., WHITTLE, K.J., PENNOCK, J.F., AND MORTON, R.A. (1965) Identification and estimation of tocotrienols in *Hevea* latex. *Nature, Lond.*, **207(4996)**, 521.

GILLISSEN, G. AND HEUSEL, E. (1961) A criticism of the sterility test of penicillin-containing preparations. *Arch. Hyg. Bakt.*, **145**, 32.

JOHN, C.K. (1966a) Breakdown of amino by *Hevea* latex bacteria. *J. Rubb. Res. Inst. Malaya*, **19(4)**, 214.

JOHN, C.K. (1966b) Metabolism of quebrachitol and other carbohydrates by *Hevea* latex bacteria. *J. Rubb. Res. Inst. Malaya*, **19(4)**, 219.

JOHN, C.K. (1966c) Biological coagulation of *Hevea* latex using waste carbohydrate substrates. *J. Rubb. Res. Inst. Malaya*, **19(5)**, 286.

JOHN, C.K. (1968) A medium for isolation and cultivation of *Hevea* latex bacteria. *J. Rubb. Res. Inst. Malaya*, **20(5)**, 285.

JOHN, C.K. AND TAYSUM, D.H. (1963) The enumeration of yeasts in *Hevea* latices. *J. Rubb. Res. Inst. Malaya*, **18(1)**, 1.

RUBBER RESEARCH INSTITUTE OF MALAYA (1968) *Rep. Rubb. Res. Inst. Malaya 1967*, 64.

SEKHAR, B.C. (1960) Degradation and cross-linking of polyisoprene in *Hevea brasiliensis* latex during processing and storage. *J. Polym. Sci.*, **48**, 133.

SMITH, M.G. (1969) Recent aspects of block natural rubber production by mechanical methods. *J. Rubb. Res. Inst. Malaya*, **22(1)**, 78.