NOTE ON THE PRESERVATION OF LATEX BY STERILISATION IN THE PRESENCE OF A BUFFER SOLUTION

BY

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Summary

A method of preserving latex has recently been suggested by Spence which consists of the sterilisation of latex by autoclaving in the presence of a buffer reagent. In view of the improved properties claimed for the material, together with the lower cost of production, both of which are of interest to producers of latex, the method has been investigated. It is concluded that the process is not of value as a practical method for the commercial preservation of fresh Hevea latex.

It is well known that commercial latex is preserved with either a fixed alkali such as caustic potash or a volatile alkali such as ammonia although various objections may be cited against each of these types of material. The fixed alkali remains in the rubber if the latex is dried by evaporation and may affect materials with which it comes into contact, or it may introduce other features such as an increase in the water-absorption capacity of the rubber. On the other hand the volatility of ammonia is objectionable to operatives on account of its odour; it has to be removed by air-blowing before the latex can be utilised in certain processes, otherwise inferior products are obtained; it is also relatively expensive to the producer.

For these reasons the problem of finding alternative materials as preservatives of latex has occupied the attention of the producers' research organisations for a considerable time, and the properties of a number of bactericides have been investigated in the laboratories of the Rubber Research Institute of Malaya. The results of these investigations have been published periodically in this *Journal*.

It was found by Rhodes that materials such as the alkyl salts of mercury (1935,1), compounds of arsenious oxide (1935,2) and the sodium salts of chlorinated phenols (1938) are efficient preservatives of Hevea latex, but in all cases only if a small amount of ammonia is present which raises the pH of the latex to the

Fig. 1.

FLUCTUATION IN BACTERIAL POPULATION OF FRESHLY AMMONIATED FIELD LATEX.

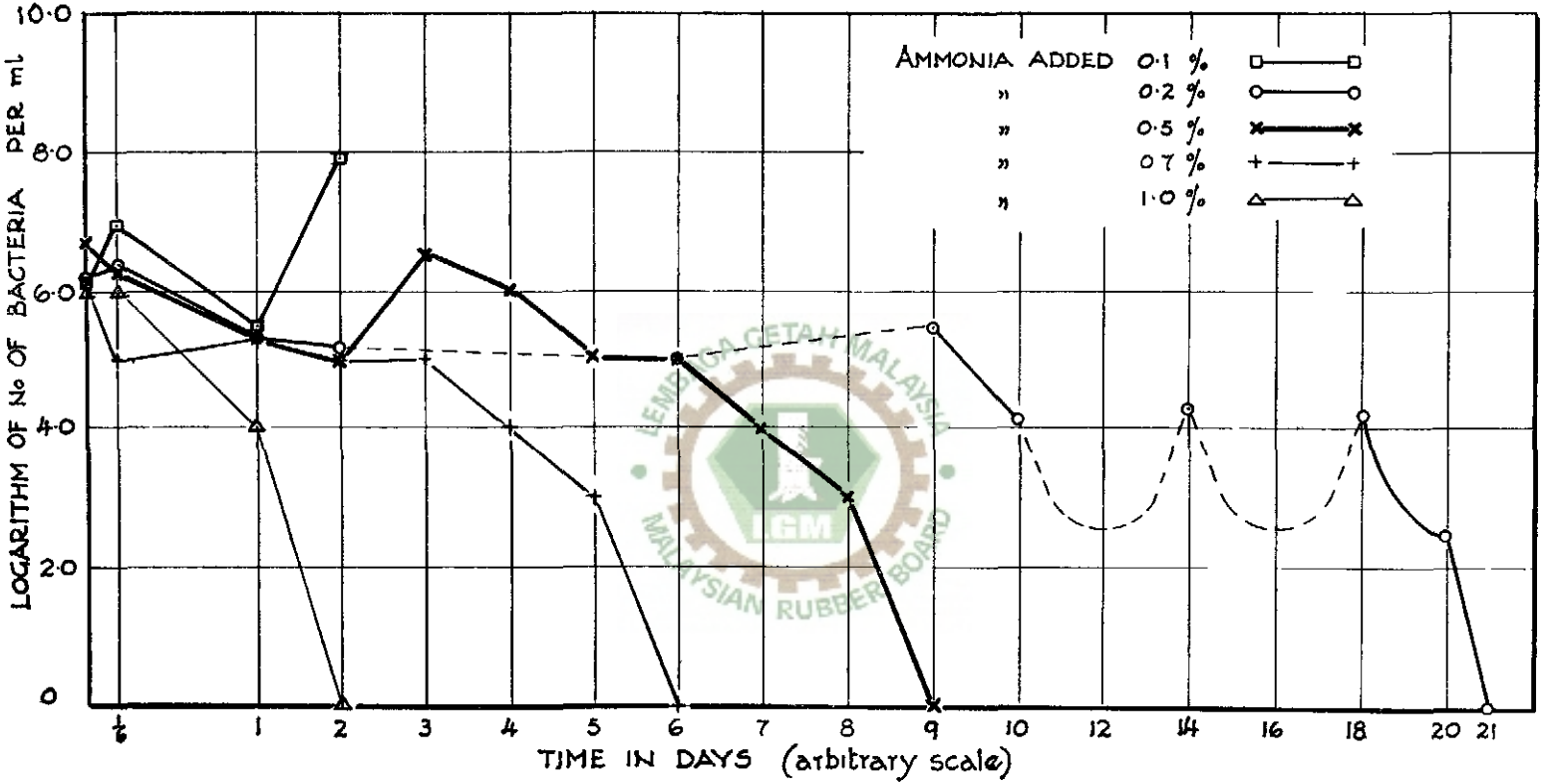

TABLE I

Bacterial Population (in thousands) per ml. of Latex preserved with various quantities of Ammonia

Days after Preservation		Latex A		Latex B		Latex A
		Ammonia (per cent)				
		0.1	0.2	0.5	0.7	1.0
0	11 a.m.	1,300	1,500	5,000	1,300	1,000
	3 p.m.	9,000	2,500	2,000	100	1,000
1		300	200	200	200	< 10
2		83,600	150	100	100	0
3		Coagulated	< 100	3,300	100	Sterile
4			-do-	1,000	10	
5			-do-	100	1	
6			-do-	100	0	
7			-do-	10	Sterile	
8			30	1		
9			13	0		
10			< 1	Sterile		
11			-do-			
12			-do-			
13			20			
14			< 1			
15			-do-			
16			-do-			
17			16			
18			< 1			
19			0.3			
20			0			
21			Sterile			

neighbourhood of 8.0, or alternatively if a sufficient quantity of the preservative is used, as in the case of sodium pentachlorphenate, to attain approximately the same pH value. Owing to their

poisonous nature the use of some of the materials mentioned above is impracticable, but sodium pentachlorphenate is known to have commercial possibilities.

Spence (1938) has criticised the use of alkalies such as ammonia on the grounds that "they merely delayed or lessened the natural tendency of fresh latex to decompose and coagulate without eliminating the cause for those chemical and physical changes which find their expression in the constantly changing properties of the final coagulum" adding that "latex is still far from being a stable article of commerce and there are many practical difficulties in connection with its use." Presumably in support of the above, Spence uses the following extract from an address by Rhodes before the Rubber Section of the American Chemical Society (1935,3)—"When latex is won from a tree under practical conditions, it is contaminated by bacteria. This contamination cannot be prevented in practice . . . The acidity which the bacteria produce causes premature coagulation in the field which can be prevented by the addition of small quantities of either sodium sulphite or ammonia. But the amounts in which they are used are not sufficient to kill the bacteria. *They do not sterilize the latex, and although the latex may appear to be satisfactory, the bacteria are still at work, producing decomposition in the latex constituents.* Varying degrees of bacterial contamination and activity from day to day can, therefore, make only for a further adventitious variability in latex composition."

The italics in the above quotation are Spence's and the inference which one might draw is that active bacteria are present in all ammonia-preserved latex, whereas the above extract refers to latex treated in the field with small quantities of ammonia which are insufficient to sterilize the latex. The existence of bacteria in latex depends upon the extent of ammoniation and although periodical infection may occur through contact with the atmosphere, if it is adequately preserved bacteria cannot survive and for practical purposes sterility is maintained.

Data taken from our records showing the fluctuations in the bacterial populations of 1 ml. of latex in the period after collection and preservation with different amounts of ammonia are shown in Table I and also in Figure I in which the logarithms of the bacterial populations are plotted. The figures in the columns of the Table under 0.1, 0.2 and 1.0 per cent ammonia were derived from identical latex (A) taken from the same task at the same time. This was a latex having a low initial bacterial population. On the other hand, the figures in the columns under the headings 0.5 and 0.7 per cent have been obtained from an average type of field latex (B) with an infection higher than A at the time of ammoniation.

In the case of latex A, 0.1 per cent ammonia was insufficient to prevent a large outburst of bacterial activity on the third day, resulting in coagulation. With 0.2 per cent ammonia a small increase in bacterial content occurred on the afternoon of the day of collection and it will be observed that renewed activity occurred on the fourteenth and eighteenth days before sterility was attained after 21 days. If this latex had had a higher initial infection, produced, for example, by delaying ammoniation, the initial outburst would have been greater and probably would have resulted in early coagulation. This is illustrated in the case of latex B preserved with 0.5 per cent ammonia. Here the initial infection was higher and there was a relatively larger outburst of activity on the fourth day before sterility was attained on the tenth day. Larger amounts of ammonia prevent this activity as shown in the examples of the latex preserved with 0.7 and 1.0 per cent, where apart from a relatively small increase in the case of the former on the second day, a gradual decrease in bacterial numbers occurred and sterility was attained on the seventh and third days respectively. Changes were observed by Beeley (1934) in the bacterial population of a bulked estate latex which was not very clean and in which further contamination, possibly from the collecting buckets and other utensils, caused an even greater increase in bacteria numbers attended by a greater accentuation of the bursts in bacteria development. In such cases the risk of coagulation after ammoniation is correspondingly greater, particularly if only a small amount of ammonia is added. Hence the recommendations to producers to use not less than 0.70 per cent ammonia for the preservation of estate latex.

The sterility of efficiently preserved latex is confirmed also by the small quantitative changes which take place in its constituents over a period of storage. It has been ascertained that the changes in rubber and ammonia contents over a period of storage are very small (unpublished London Advisory Committee Report). Davey & Coker (1938) have shown that a latex having an original ammonia-content of 0.77 per cent and a dry-rubber-content of 36.2 per cent had after keeping for one year an ammonia-content of 0.79 per cent and a dry-rubber-content of 35.8 per cent. The change in the dry-rubber-content is negligible compared with that which takes place when rubber is broken down into " water-soluble products " by mould. Bishop and Fullerton (1932) however observed an appreciable change in ammonia-content during a similar period when the latex was not sterile.

It is realised that considerable changes in the stability of latex take place during storage, justifying to some extent the description that it is not a " stable article of commerce." The stability of latex increases during a period of several months after preparation

and then gradually decreases. These changes are not a result of the presence of active bacteria, but are caused by the presence of either ammonia, or naturally occurring enzymes, or enzymes formed *in situ* from bacteria before the latter are destroyed, each of which may react with the non-rubber substances, gradually altering the nature of the protective coating on the rubber particles. Moreover evidence has been accumulated by Roberts (1938) showing that the changes which occur in latex are very marked in the period subsequent to ammoniation and further that it is the natural variation inherent in a plant product such as latex which causes the chief difficulties to the manufacturer during processing.

Spence sought to eliminate " the objectionable changes known to develop in ammonia-preserved latex and to produce a stable product, free from the practical difficulties resulting from the presence of alkali in latex " by investigating the factors which affect the stability of Hevea latex de-ammoniated by dialysis, and by making studies with latex from the Guayule plant. The method adopted was to buffer the latex with a solution of M/20 sodium dihydrogen phosphate containing the requisite amount of M/20 caustic soda to produce a mixture having a pH of approximately 7.0. It was shown that " the stability of the latex was largely dependent on its reserve buffer strength and that by increasing this strength by the use of an artificial reserve of buffer solution having the same pH as that of the latex itself, the stability of the latex could be maintained over a prolonged period of time without coagulation taking place; that fresh latex adequately buffered in this way could be heated to temperatures well above those employed in modern bacteriological practice without coagulation ensuing. These observations paved the way for a permanently stable and neutral sterile latex."

A method claiming such results must command the attention of those interested in the preservation of latex. The method claims to eliminate the use of alkalies and bactericides, either or both of which have hitherto been and, in view of the experiments described below, still are considered indispensable for the satisfactory preservation of latex. It is uncertain as to how far experiments were made with fresh latex but in view of the statements that " sterile latex prepared in the East remained uncoagulated as far as it is possible to determine " and later that " at long last fresh Hevea latex can be shipped to any part of the world " the authors felt justified in assuming that the method was suitable for the preservation of fresh latex and have endeavoured to ascertain if this process had any commercial possibilities.

Experimental

The buffer solution was prepared by adjusting the proportions of M/20 solutions of caustic soda and sodium dihydrogen phosphate to obtain a solution of pH 7.0. The proportions required were found to be 500 ml. of M/20 NaH_2PO_4,H_2O and 230 ml. of M/20 NaOH, and therefore 1 ml. of buffer solution contained 0.00473 *gm. of* NaH_2PO_4,H_2O *and* 000630 *gm.* NaOH.

Varying proportions of the buffer solution were added to 100 ml. quantities of fresh latex (d.r.c. 38 per cent) from the Rubber Research Institute Experiment Station, and the mixtures were (a) allowed to stand without having been heated and (b) heated in an air oven for 2 hours at 100°C. In every case after standing for a period of 24 hours the mixtures showed a tendency to coagulate or curdle and were very unstable.

The particulars of the mixtures used are shown in Table II.

TABLE II

Date	Sample No.	Volume Latex (ml)	Volume Buffer (ml)	NaH_2PO_4,H_2O in Mix (per cent)	NaOH in Mix (per cent)
2.3.39	1	100	10	0.0430	0.0057
„	2	100	20	0.0788	0.0105
„	3	100	30	0.1014	0.0145
„	4	100	40	0.1351	0.0180
„	5	100	50	0.1576	0.0210
„	6	100	60	0.1773	0.0236
„	7	100	70	0.1948	0.0260
„	8	100	80	0.2102	0.0280
„	9	100	90	0.2240	0.0298
„	10	100	100	0.2365	0.0315

A second series of samples were prepared, one half of which was stored at room temperatures and the other half heated in an autoclave at 15 lb. pressure for 20 minutes. Again the resultant mixtures were unstable and either clotted or coagulated within a short time. It appears that the method is unsatisfactory for the preservation of fresh latex with the quantities of buffer solution used. With the larger amounts of buffer solution it was observed

that coagulation was not so marked, probably due to the dilution effect. The addition of still larger amounts would necessitate further dilution and would therefore be outside the bounds of practical considerations.

The data relating to the second series of experiments are shown in Table III.

TABLE III

Sample No.	Volume Latex (ml)	Volume Buffer (ml)	NaH_2PO_4, H_2O in mix (per cent)	NaOH in mix (per cent)	pH, mix	Condition on Storage	
						Not sterilised	Sterilised
1	100	25	0.0946	0.0126	6.5	Coagulated next day	Clotted on autoclaving
2	100	50	0.1576	0.0210	6.6	- do. -	- do. -
3	100	75	0.2028	0.0270	6.6	- do. -	- do. -
4	100	100	0.2365	0.0315	6.6	- do. -	- do. -

Spence has stated that the variation which occurs in the pH of latex of the same species is slight, and in the case of Hevea does not extend beyond the range of substantial neutrality. There is by no means unanimity as to the actual pH of fresh latex, values ranging from 5.8 to 7.2 having been indicated by different workers e.g. Belgrave (1923), Bobilioff (1924), Hauser and Scholz (1927) and Van Harpen (1930). This variation is probably due to the use of different methods of determination rather than to inherent differences in Hevea latex from different sources. The pH of the latex used in the above experiments (Table III) was 6.5 (as determined by the glass electrode) and in order to simulate Spence's conditions it was brought nearer to neutrality by the addition of a small amount of ammonia.

Three samples of latex thus treated were buffered and sterilised in a manner similar to that of the previous series. The results are shown in Table IV.

Sample No. 3, autoclaved, was still fluid after seven days, and on opening the container, it was observed that the latex was also sweet. After a further five days, however, the latex clotted, probably owing to its being non-resistant to bacteria which were admitted when the container was opened. The results of this series were therefore slightly less unsatisfactory than those of the unammoniated samples, but could not be considered of practical value.

TABLE IV

Sample No.	Volume Latex (ml)	Volume Buffer (ml)	NaH_2PO_4, H_2O in mix (per cent)	NaOH in mix (per cent)	pH. mix	Condition on Storage	
						Not sterilised	Sterilised
1	100	25	0.0946	0 0126	6.9	Coagulated 1 day	Coagulated on auto-claving
2	100	50	0.1573	0.0210	6.9	- do. -	- do. -
3	100	100	0.2365	0.0315	6 9	- do. -	Curdy after 12 days

It is known that ammoniated latex, even after de-ammoniation, behaves in many ways differently from fresh latex. The complete de-ammoniation of latex is difficult but it was considered advisable to ascertain the effect of the sterilisation-buffer process on this type of latex as used by Spence. Latex which had been stored for two months was de-ammoniated to a methyl red titre of 0.03 per cent and subsequently sterilised (autoclaved) after buffering with various amounts of buffer solution. The results are shown in Table V.

TABLE V

Sample No.	Volume Latex (ml)	Volume Buffer (ml)	NaH_2PO_4, H_2O in mix (per cent)	NaOH in mix (per cent)	pH. mix	Condition on Storage	
						Not sterilised	Sterilised
Control							
1	100	Nil	Nil	Nil	7.3	Coagulated 2 days	Coagulated 4 weeks
2	100	25	0.0946	0.0126	7.8	- do. -	Clot present in 4 weeks
3	100	50	0.1576	0.0210	8.0	- do. -	Big clot in 4 weeks
4	100	75	0.2028	0.0270	7.7	- do. -	Very big clot in 4 weeks
5	100	100	0.2365	0.0315	7.5	- do. -	Big clot. Bad smell in 4 weeks

The results shown in this series are not so unsatisfactory as those of the previous series. All the latices were in a fairly good condition after storage for periods up to 3 weeks, but after this they showed signs of deterioration and after 4 weeks all the samples had coagulated. It was observed also that the buffered de-ammoniated latices were not superior to the unbuffered de-ammoniated latices, indicating that the buffering was not of material value.

As a result of these experiments the following general conclusions are drawn.

(1) That diluted fresh latex, buffered or unbuffered, is more resistant than concentrated latex to clotting and coagulation on sterilisation by autoclaving.

(2) That de-ammoniated latex is more resistant than fresh latex to clotting or coagulation on sterilisation by autoclaving.

(3) That buffered latex sterilised by autoclaving is non-resistant to bacteria and is therefore unstable and coagulates when exposed to infection. All the samples described above would be contaminated when the containers were opened and the contents examined periodically.

Spence (1939), in a notable contribution to the subject of the preparation from plantation rubber of a modified rubber with improved oil-resistant properties, mentions the determination of the amounts of the sol and gel fractions of latex buffered and sterilised in sealed containers immediately after collection in the Far East. Some of this latex was stated to be three years old and it is presumed that it had been kept in the sealed containers during this period in order to retain its fluidity. Such conditions would be difficult to attain in commercial practice and in view of the observations recorded above it is concluded that the method is of academic interest only and that it would have no value as a practical method for the large-scale preparation and shipment of latex from the producing countries.

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