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ON THE PROTEIN CONTENT OF CONCENTRATED LATEX

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INTRODUCTION

Natural rubber latex is a complex colloidal dispersion of rubber particles in an aqueous medium, the colloidal system being stabilised by some non rubber materials. The major non rubber constituents are proteins, lipids, phospholipids, carbohydrates, amino acids and inorganic ions. The total non rubber materials in Hevea Latex is about 3-5% by weight, of which about 1% is proteins(1,2). About half of the proteins is dissolved in the aqueous phase, one quarter is adsorbed on the surface of the rubber particles and the remaining associated with larger particle bodies, such as Lutoids(1,3).

The importance of proteins in determining the colloidal stability of natural rubber field latex was understood much early. Kemp and Teoiss making use of the assumption that the resultant surface charge density of a composite colloidal particle depends on the surface charge densities of the surface substances adsorbed at the particle demonstrated that above a total solids content of 12%, the rubber particles are completely covered with a proteins(4). Proteins in latex behave as amphoteric polyelectrolytis. The behaviour of any given protein is determined not so much by any free carboxyl and amine groups which may be situated at the ends of the molecules, but by the presence of free acidic and basic functional groups in the main chain substituents(5). The zwitter-ion effect indeed by the presence of free amino and carboxyl groups in the latex proteins is of fundamental importance in determining colloidal the characteristics of latex(6).

Proteins in Hevea Latex

Attempts to characterise the latex proteins were reported from 1930s onwards. Bondy and Freundlich(7) have separated two proteins from the centrifugally separated serum of ammonia preserved NR latex, which they called protein A and B. The proteins were distinguished by their electrokinetic behaviour and their solubilities in aqueous solutions and in alcohol. Proteins A and B had isoelectric points of 4.55 and 3.9 respectively. The former was insoluble in water and alcohol, while the latter was soluble in water and 70% alcohol.

Kemp and Straitiff(6) isolated 3 proteins from ammoniated latex serum by ammonium sulphate precipitation method - Protein A, Protein B and Protein C. However Protein C was found to be a heat-denatured product of protein B.

Archer and Sekhar(3) have studied the aqueous serum of unpreserved NR latex, obtained by high speed centrifuging and by freezing at -25°C. In both the types of serum, electrophoritically distinct protein components have been detected. However in the serum of ammonia preserved field latex, only two of the above seven proteins were readily resolvable electrophoritically. From the freeze dried serum solids, Archer and Cockbain(8) separated & -globulin by an isoelectric ammonium sulphate precipitation method. It has an isoelectric point of 4.8 and molecular weight of the order of 2×10^5 . The similarity in the electro phoritic and colloidal behaviour between dissolved ≪-globulin and the particles of Hevea Latex suggest that this protein is an component of the protein layer which is adsorbed on the rubber particles. The nature of ∝-globulin corresponds to 'Protein A' reported earlier(6,7).

Hevein, the second principal protein, which is dissolved in latex serum was isolated by ammonium sulphate fractionation of cold aqueous extract of the freeze-dried solids derived from the so called 'bottom fraction'(9). It was a low molecular weight of about 10000 and isoelectric point of 4.5. Hevein is water soluble at all pHs and does not contribute much to the colloidal behaviour of NR latex.

Paper electrophoritic studies(10) ion exchange chromatographic and starch gel electrophoric studies(11) of 'Bottom Fraction', Starch Gel Electrophoric studies of C serum proteins(12) have detected several protein components. Recently it is shown that proteolipids are associated with the rubber particles in latex(13).

Even though a lot of studies have been carried out on proteins in natural rubber field latex, the volume of work on proteins in concentrated latex is much less despite the fact that concentrated latex is the main raw material for almost all latex products. Recently some work has been done on the proteins in high ammonia (HA) preserved NR latex concentrate(14). Such studies are very relevant in the present circumstances because some proteins in NR latex gloves have been identified as source of some allergic problems in sensitive human beings.

Proteins of HA latex concentrate are distributed between two main fractions - the serum fraction and the rubber fraction.

The serum contains mainly six proteins and probably these are the ones that can be leached out of latex products. The rubber particles are associated with one main protein of molecular weight 14000, along with small quantities of another one of molecular weight 24000. A small fraction of the rubber

particle proteins can be extracted with ammonia solution. The rest is tightly bound to rubber particles and are retained even after leaching (14).

Several attempts have been made to control the level of extractable protein(15) like wet gel leaching, dry film washing, steam treatment, chlorination etc. Recently some reports have come on the production and technological evaluation of a low protein latex(16).

Protein Removal during Latex Concentration

As reported elsewhere, the non rubber content of field latex is about 3-5%, whereas that of concentrated latex and skim latex are about 1.2-1.6% and 5-10% respectively. The particle size distribution in NR latex has a profound influence on the above non rubber percentages.

The particle size distribution of NR latex covers a wide range - a typical order being 0.2 to 20 microns(17). About 4% of particles have diameters above 4 microns. However this small fraction accounts for some 85% by mass of the total dispersed rubber.

When field latex is centrifugally concentrated, the association of high levels of non-rubber materials in skim latex can be ascribed to

- (a) high serum to rubber ratio means that a large proportion of soluble proteins and other non rubber materials are carried into skim.
- (b) skim latex consists of particles of very small diameters, and high specific surface area, so that the quantity of adsorbed non rubber materals per unit mass of rubber is higher.

During centrifuging if a latex particle is initially at distance of Ro from the axis of rotation, and if R is the limiting position, such that the particle be carried to the cream after centrifuging for a time t, then the limiting radius of the largest skim latex particle is given by

$$x_{L} = \left[\frac{9 \, \text{m}}{2 \, \text{w}^{2} (p'-p) t} \quad \ln \left(\frac{\text{Ro}}{R} \right) \right]^{\frac{1}{2}} \tag{1}$$

where of is the viscosity the aqueous medium w the argular velocity of the particle, p' and p are the densities of serum and rubber partice respectively.

The above equation can be rearranged as

$$x_L^2 t = \frac{9\sigma}{2w^2(p'-p)} \ln \left(\frac{Ro}{R}\right)$$
 (2)

Under a given set of operating conditions the right hand side of equation (2) is a constant, so that x_L^2t = a constant.

or x_L
$$\propto \sqrt{\frac{1}{t}}$$

ie. as the time t a particle is subjected to centrifugal force, is increased, the limiting radius \mathbf{x}_{L} decreases. During centrifugal concentration of latex, t can be varied by changing the skim screw - the longer the screw, the higher will be the t value.

It seems that the protein distribution in latex as soluble protein, interfacial protein, and those associated with bloid particles is at an equilibrium. If the field latex is

diluted, then it is likely that this equilibrium is disturbed and the soluble protein content increases so that protein removal in skim latex should be higher. Further ammonia solution can extract some rubber particle protein.

Hence it was decided to study

- (1) effect of skim screw length on the non-rubber content, and thereby the protein content of cenex.
- (2) the effect of dilution of field latex on protein removal

EXPERIMENTAL

Effect of Skim Screw Length

Preserved field latex was centrifuged in an Alpha Laval LRB 510 model latex separator using 10.5 mm feed tube 6 inch float valve level and skim screws of different lengths. The trial was conducted using HA and LATZ preserved field latex. The field latex used and cenex obtained in each case was subjected to the following tests:

- 1. Total Solids
- 2. Dry rubber content
- 3. N Content in TS
- 4. N content in dried coagulum.

The above estimations were made by following the relevant Indian Standards. Non rubber content was estimated as a difference between TS and DRC.

The properties of the field latices used are given in Table 1. The characteristics of the cenex obtained are given in Tables 2 and 3.

Effect of Dilution

Field latex was diluted with water to different extents and diluted latex allowed to equilibrate for 24 hrs. In the case of HA latex, dilution was done by 1% ammonia water. For LA latex 0.3% ammonia water containing 0.025% ZnO and TMTD was used. The cenex obtained in each case was tested and the results are given in Tables 4 and 5.

Results and Discussions

The variations of NRS given in Tables 2 and 3 show almost a linear relation with the skim screw length both in the case of LA and HA latex. Similarly the approximate protein content in the dry cenex coagulum varies linearly with the skim screw length. The increase in NRS and protein content is due to the inclusion of more of smaller rubber particles into cenex, rather than their going in skim. Also some more water soluble components are introduced into the cenex. (Figs. 1, 2)

The results obtained on diluting the latex prior to centrifuging are given in Tables 4 and 5 and in figures 3 and 4. Effect of dilution is more marked in the range 0-20% in reducing the NRS and protein content in cenex. This is due to the preferential loss of the water soluble fractions into skim, which may reside with cenex in a normal centrifuging. Some contribution is made by the ammonical medium in solubilising some more of adsorbed proteins. As dilution increases beyond

20% the contribution from soluble materials decreases. It is likely that protein solubilisation may increase, but its rate decreases, as it becomes more and more difficult to remove the very strongly adsorbed proteins.

Conclusions

- The increase in skim screw length, with a view to enhance the processing efficiency, results in a simultaneous increase in non rubber solids and protein content in cenex.
- Diluting the field latex reduces the NRS and protein content in cenex. The rate of reduction decreases as the dilution increases.
- 3. Dilution by 10-20% is found to be the optimum.

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REFERENCES

- Altman RFA. Organic Analysis of Hevea Latex.IX. Some Details of the Main Fractions. Recueil des Travaux Chimiques Pays - Bas. 65(12), 919 (1946).
- Tata SJ. Distribution of Proteins Between the Fractions of Hevea Latex Separated.by Ultracentrifugation. J. Rubber Res. Inst. Malaysia 28(2) 77 (1980)
- 3. Archer BL and Sekhar BC. The Proteins of Hevea Brasiliensis Latex.I. Protien Components of Fresh Latex Serum. Biochemical J. 61(3), 503 (1955).
- Kemp.I and Twiss DF. The Surface Composition of the Rubber Globules in Hevea Latex. Trans. Faraday Soc. 23(6) 890 (1936).
- Blackley DC. High Polymer Lattices. Vol.I p 118.
 Maclaren and Sons Ltd., London. 1965.
- 6. Kemp AR and Straitiff WG. Hevea Latex: Effect of Proteins and Electrolytes on Colloidal Behaviour. J. Phy. Chem. 44(6) 788 (1940)

- 7. Bondy C and Freundlich H. The Proteins in Preserved Hevea Latex. India Rubber J. 95 (17), 513 (1938)
- 8. Archer BL and Cockbain EG. The Proteins of Hevea
 Brasiliensis Latex II. Isolation of

 ✓ Globulin of
 Fresh Latex Serum. Biochemical J.61 (3) 508 (1955).
- 9. Archer BL. The Proteins of Hevea Brasiliensis
 Latex.4. Isolation and Characterization of
 Crystalline Hevein. Biochemical J. 75, 236 (1960).
 - 10. Moir GFJ and Tata SJ. The Proteins of Hevea brasiliensis Latex. Part 3. The soluble Proteins of 'Bottom Fraction'. J.Rubber Res. Inst. Malaysia 16(4), 155 (1960).
 - 11. Karunakaran A, Moir GFA and Tata SJ. The Proteins of Hevea Latex: Ion Exchange Chromatography and Starch Gel Electrophoresis. Proc. Natural Rubber Res. Conf. Kuala Lumpur, 1960. p. 798.
 - 12. Tata SJ and Moir GFJ. The protiens of Hevea brasiliensis latex. Part 5. Starch Gel Electrophoresis of C Serum Proteins. J. Rubber Res. Inst. of Malayisa 18(3), 97 (1964).
 - 13. Hasma H. Proteolipids of Natural Rubber Particles.

- J. nat. Rubber Res. 2(2), 129 (1987)
- 14. Hassma H. Proteins of Natural Rubber latex

 Concentrate.J. nat. Rubber Res. 7(2), 102 (1992)
- 15. Dalrymple SJ and Andley BG. Allergenic Proteins in Dipped Products: Factors Influencing Extractable Protein Levels. Rubber Developments 45, 51 (1992)
- 16. Ghazaly, Hafsah bte Mohd. Properties of Natural Rubber Low Protein latex. International Rubber Technology Conference. June 1993. Kuala Lumpur Latex Protein Workshop. Paper No.7.
- 17. M.Van den Temple. Electron Microscopy of Rubber Globules in Hevea latex. Trans. Inst. Rubber Ind. 28, 303 (1952)

Table 1
Properties of Field Latex

S1.No. characteristics		Preservation System		
٠		LATZ	НА	
1	Total Solids %	35.4863	30.9436	
2	Dry Rubber Content %	32.7146	28.1451	
3	Non rubber solids %	2.7717	2.7985	
4	N on TS %	0.649	0.694	
5	N on dry coagulum %	0.413	0.424	
6	Approximate Protein			
	content in dry coagu-			
	lam %	2.58	2.65	

Table 2

Effect of skim Screw Length on NRS and Protein Content of

LA Latex Concentrate

S1.No. Characteristics		Skim Screw length (mm)					
	of Cenex	10.5	11.0	11.5	12.0		
1.	Total Solids %	63.7243	62.9573	63.2880	63.1142		
2.	Dry Rubber						
	Condent %	62.4368	61.5689	61.9202	61.5354		
3.	Non rubber solids	% 1.2875	1.3884	1.4678	1.6788		
4.	N on TS %	0.2593	0.2933	0.3021	0.3271		
5.	N on dry coagulum	% 0.2172	0.2453	0.2534	0.2754		
6.	Approximate Protein						
	Content in dry						
	coagulum %	1.36	1.53	1.58	1.72		

Table 3

Effect of Skim Screw Length on NRS and Protein content

of HA Latex Concentrate

Sl.No. Characteristics of		Skim Screw Length (mm)				
	Cenex	9.5	10.5	11.0	12.0	
1	Total Solids %	64.1730	62.5728	62.4586	61.8266	
2	Dry Rubber Condent %	63.2077	61.2488	61.0577	60.1051	
3	Non rubber solids %	0.9653	1.3240	1.4009	1.7215	
4	N on TS %	0.3338	0.4004	0.4086	0.3756	
5	N on dry Coagulum %	0.2687	0.2784	0.2911	0.3366	
6	Approximate Protein		*			
	Content in dry					
	coagulum %	1.68	1.74	1.82	2.10	

Table 4

Effect of Dilution of LA field latex on

S1	Characteristics	Dilution as % of field latex					
No	of Cenex	0	10	20	30	40	
	Total Solids %	45 5512	64.2618	45 D2125	64.2469	61. 2715	
	Dry Rubber	63.3313	04.2016	03.02133	04.2409	04.3/13	
	Content %	64.0183	62.9913	64.0630	63.3325	63.5302	
3.	Non rubber	4					
	solids %	1.5332	1.2705	0.9584	0.9144	0.8413	
4.	N on TS %	0.2837	0.2549	0.2273	0.2226	0.2178	
5.	N on dry coag-						
	ulam %	0.2627	0.2445	0.2034	0.1821	0.1767	
6.	Approximate						
	Protein content	1					
	in dry			1			
	coagulum %	1.64	1.53	1.27	1.14	1.10	

NRS and Protein content in Cenex

Table 5

Effect of Dilution of HA field latex
on NRS and Protein Content in Cenex

S1 No	Characteristics	Dilut	Dilution as % of field latex				
	of Cenex	0	10	20.	. 40		
1	Total Solids %	62.4553	65.1730	63.8258	64.4504		
2	Dry Rubber						
	content %	61.1851	64.2077	62.9962	63.6880		
3	Non rubber						
	solids %	1.2702	0.9653	0.8296	0.7.624		
4	N on TS%	0.3459	0.3338	0.3131	0.3012		
5	N on dry						
	coagulum%	0.3267	0.2898	0.2898	0.2606		
6	Approximate Protein						
	Content in dry						
	coagulum %	2.04	1.81	1.81	1.63		

Fig.1 Effect of Variation of Skim Screw length on NRS in Cenex

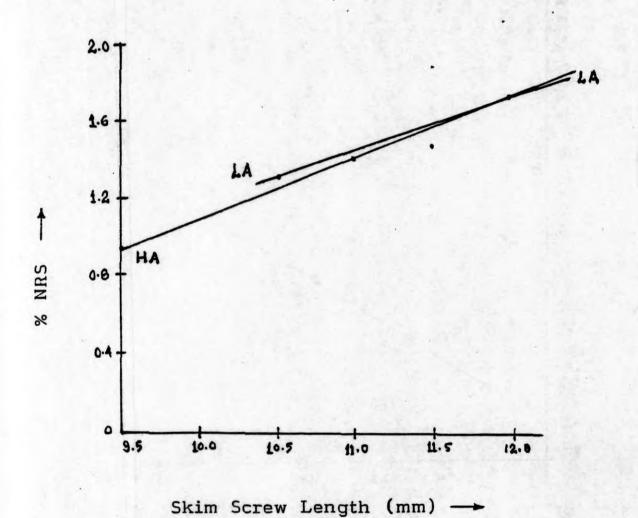


Fig. 2 - Effect of variation of skim screw length on Protein Content in Cenex

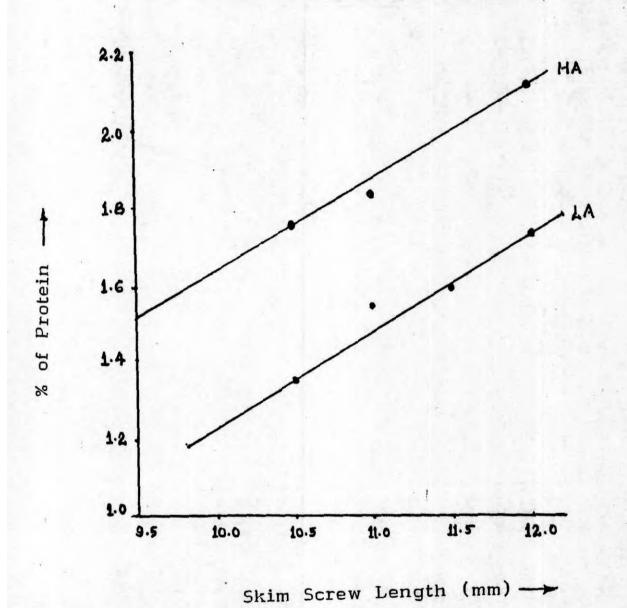


Fig.3 - Effect of Dilution on the NRS in Cenex

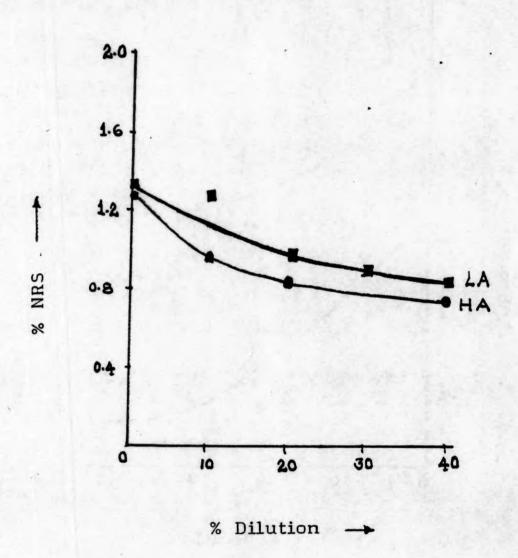


Fig.4 - Effect of Dilution on the Protein Content
in Cenex

