CHAPTER II

THEORITICAL CONSIDERATION AND LITERATURE REVIEWS

2.1 Constitution of Fresh and Ammonia-preserve Natural Rubber [3]

Freshly tapped natural rubber latex is a whitish fluid having density in range 0.975-0.980 Mg m^{-3}, pH 6.0-7.0, and surface free energy 40-45 mJ m^{-2}. Its viscosity is variable. Being a natural product, the composition of fresh natural rubber latex varies between wide limits. The composition given in Table 2.1 is typical.

The substances present in fresh natural rubber latex are distributed between the following three principle phases:

1. the rubber particles, which account for *ca.* 35% w/w of the latex;
2. the aqueous phase, which accounts for *ca.* 55% w/w of the latex; and
3. the lutoid phase; which accounts for most of the remaining *ca.* 10% w/w of the latex.

There are also several minor phases present in the latex such as Frey-Wyssling particles.

The rubber content of fresh natural rubber latex varies over the range 25-35% w/w. The view of 33% of rubber content of fresh natural rubber latex in Table 2.1 is an average. The difference between the total solid content and the dry rubber content of fresh latex is *ca.* 3% w/w; this compares with *ca.*1.5% w/w for the concentrate obtained by centrifugation once. The aggregate amount of non-aqueous, non-rubber constituents is *ca.* 5% w/w of the whole latex. Of the content of proteinic substances, about half is dissolved in the aqueous phase, quarter is adsorbed on the surface of the rubber particles, and the remaining quarter is associated with the larger particulate bodies such as the lutoids.

Table 2.1 Typical composition of fresh natural rubber latex [3].

Constituent	Proportion / % w/w on the whole latex
Total solids	36
Dry rubber	33
Proteinic substances	1-1.5
Resinous substances	1-2.5
Ash	Up to 1
Sugar	1
Water	*ad.* 100

2.1.1 The Rubber Phase

2.1.1.1 Shape, Size, Size distribution and Physical Structure of the Rubber Particles in Natural Rubber Latex [3].

The first important microscopic study of natural rubber latex is by Hauser. He came to the conclusion that the rubber particles are predominantly pear-shaped rather than spherical, and that they consist of a tough, hard elastic shell which encloses a viscous liquid. He reached this later conclusion from a series of elegant studies using a micro - manipulator, by means of which the particles were pierced with a fine needle. The Hauser's two-phase model for the particles in natural rubber latex accorded well with the observation. It is a widely accepted view that solid natural rubber comprises two separate fractions, a sol fraction and a gel fraction. While the existence of sol and gel fractions in solid natural rubber is still recognized (see Section 2.2.1.2), these fractions are now regarded as being mixed on the molecular scale, rather than separated. Most of other observers have found the rubber particles in natural rubber latex to be predominantly spherical in shape, especially in the latex from young trees. There is a general agreement that the shape varies to some extent with the age and type of tree from which the latex is obtained.

The size of the particles in fresh natural rubber latex varies over wide range from 20 to 5000 nm. Results have been reported by van den Tempel for the distribution of particle sizes in unconcentrated natural rubber latex. In this measurement he observed by electron microscopy for rubber particles hardened by bromination,. The cumulative particle-size distributions shown in Figure 2.1 (a) have been derived from these results making various assumptions. These distributions are respect to particle diameter for (I) aggregate particle number, (ii) aggregate particle surface area, and (iii) aggregate particle volume. The corresponding differential distributions have also been derived, as shown in Figure 2.1(b) (i), (ii) and (iii), respectively. Although these distributions undoubtedly lack accuracy, they probably give correct impressions. The distribution of particle sizes in fresh natural rubber latex is so wide that most of the volume of the dispersed rubber in the latex is present in form of a small minority of large particles. Thus, less than 4% of the particles have diameters larger than 400 nm; this is roughly the fraction, which is visible through an optical microscope. This minority of particle accounts for *ca.* 85% v/v of the total dispersed rubber, It is possible that this minority of very large particles is formed by the agglomeration and coalescence of smaller particles.

Schoon and van der Bie and Phoa using electron microscope and technique of phase-contrast microscopy observed a highly asymmetric distribution of particle sizes, which they interpreted to be arisen from the superimposition of several relatively narrow Gaussian distributions. Spherical, oval and pear-shaped particles were reported, and their appearance was such as to lead them to conclude that they were formed from much smaller primary particles. The addition of dilute strong alkali to the latex was found to enhance the clustered appearance.

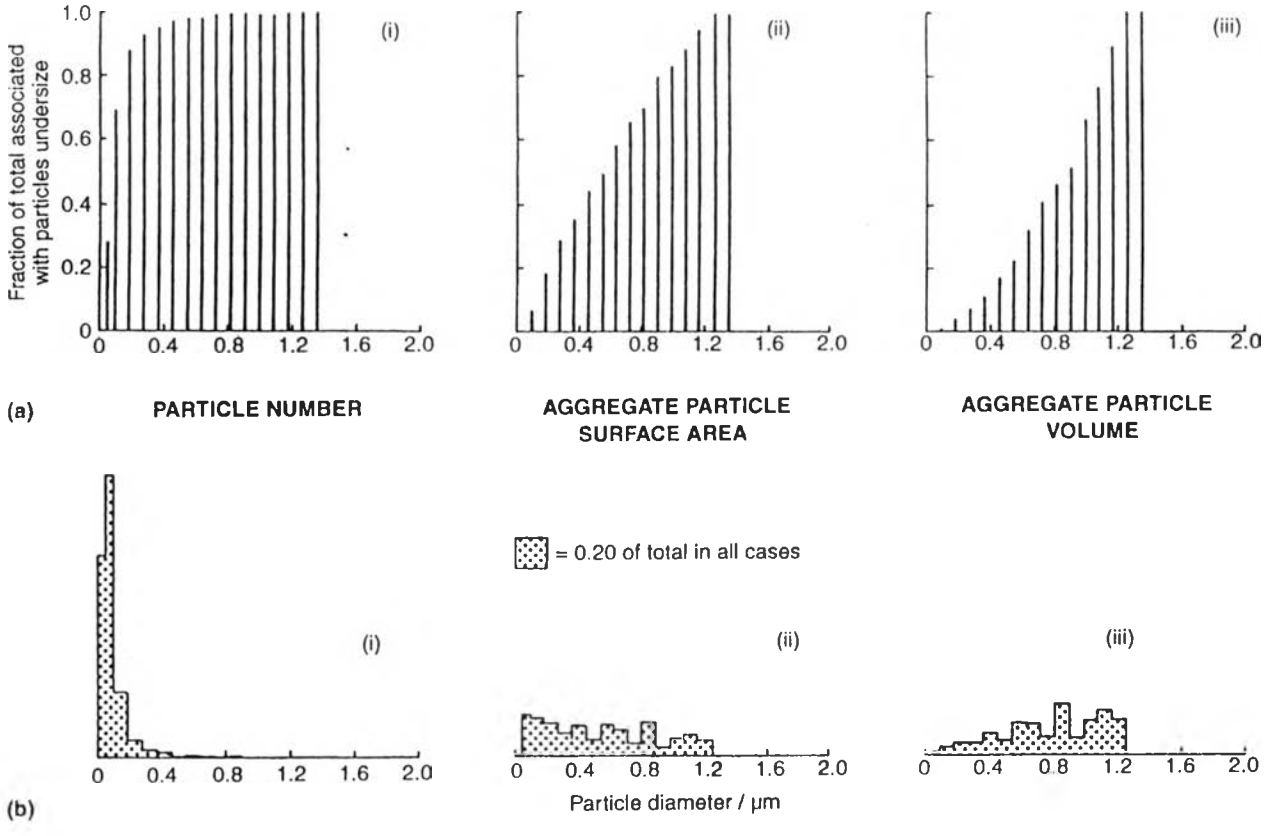

Figure 2.1 Particle-size distributions for unconcentrated natural rubber latex (a) cumulative distribution for (i) particle number, (ii) aggregate particle surface area, and (iii) aggregate particle volume with respect to particle diameter; (b) corresponding differential distributions [5].

Pendle and Swinyard recently reported the distribution of particle sizes in ammonia-preserved natural rubber latex concentrates. The principal technique used was photon correlation spectroscopy. Average particle diameters calculated from the results obtained by this technique are said to be 'hydrodynamic' average particle diameters. These are the diameter of the particle which, if it replaced all the particles in the actual sample, would give hydrodynamic behavior equivalent to that of the actual sample. The average particle diameter calculated in this way is said to approximate to the volume-average particle diameter. Samples taken from some 40 shipments of centrifuged concentrates were investigated using this technique. The average particle diameters obtained from the high-ammonia concentrates were in the range 510-600 nm, whereas those for the low–ammonia concentrates (LATZ type) tended to be somewhat lower, being in the range 480-550 nm.

They have also presented information concerning the distribution of particle sizes in the two types of ammonia-preserved natural rubber latex concentrate. Typical distributions are shown in Figures 2.2 (a) and (b) for high-ammonia and LATZ low-ammonia latices, respectively. An interesting feature of the distributions obtained by them is that most of the distributions are clearly bimodal. The peak diameters vary somewhat, the ranges being 200-300 nm for the lower peak, and 700-1500 nm for the higher peak. The bimodality is also reported for a sample of field natural rubber latex, although, as expected, the peaks are observed at lower particle diameters. Pendle and Swinyard stated that the bimodality in natural rubber latices has been confirmed by measurements made by the Du Pong Company using the technique of sedimentation field flow fractionation.

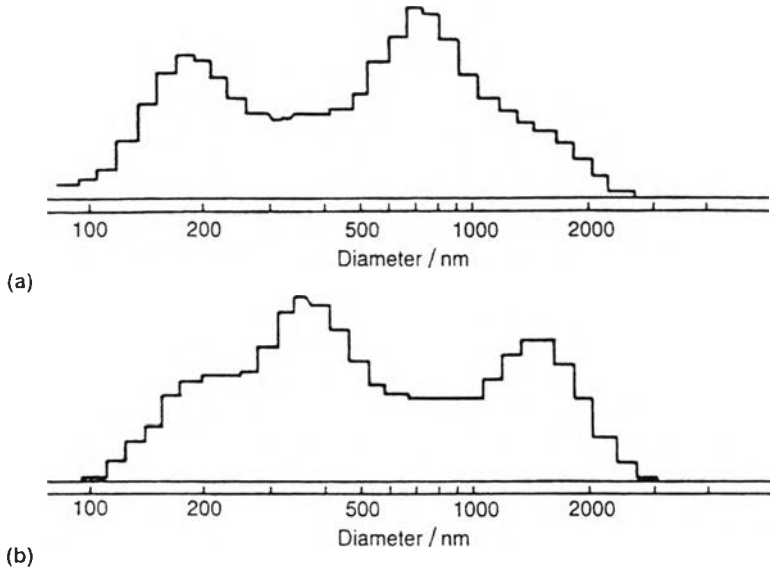

Figure 2.2 Typical particle size distributions for (a) high-ammonia and (b) LATZ low-ammonia latices.

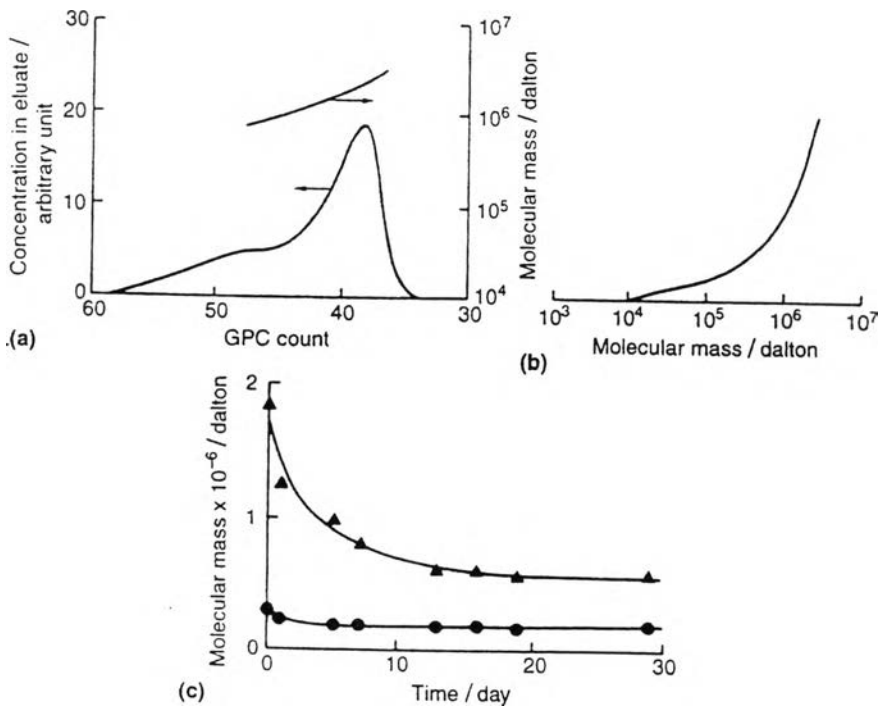

Figure 2.3 Molecular weight-size distribution [4].

Table 2.2 Typical composition of rubber particles in fresh natural rubber latex [3].

Constituent	Proportion / % w/w on whole latex
Rubber hydrocarbon	86
Water (possibly dispersed in the rubber hydrocarbon)	10
Proteinic substances	1
Lipid substances	3

Table 2.3 Apparent gel contents of natural rubber (pale crepe grade) as determined using various solvents.

Solvent	Experimentally determined gel content ,%w/w	Rubber-solvent interaction parameter, χ_1	Diffusion coefficient for diffusion of solvent through rubber at 25^{0}C, $cm^2\ s^{-1}$
Carbon tetrachloride	29	0.334	2.16×10^{-6}
Chloroform	22	0.383	3.90×10^{-6}
Toluene	18	0.391	3.28×10^{-6}
Cyclohexane	46	0.399	2.05×10^{-6}
Tetrahydrofuran	16	0.452	3.85×10^{-6}
2,2,4-trimethylpentane	40	0.513	2.34×10^{-6}
n-butyl acetate	30	0.561	2.50×10^{-6}
n-propyl acetate	68	0.649	2.05×10^{-6}

2.1.1.2 Chemical Constitution and Structure of the Particles in Natural Rubber Latex

A typical composition for the rubber phase in fresh natural rubber latex is shown in Table 2.2. Trace metals, notably magnesium, potassium and copper, are also associated with the rubber particles to an aggregate extent of *ca*. 0.05% w/w. The density of the rubber particles is *ca* 0.920 Mg m^{-3}, this being determined principally by the density of the rubber hydrocarbon.

The rubber hydrocarbon in natural rubber latex is predominantly linear *cis*-1, 4-polyisoprene. Chemical analysis shows the overall composition of natural rubber to correspond to C_5H_8 unit. Bromination and other tests revealed that the olefin unsaturation is present one carbon-carbon double bond for each C_5H_8 unit. Ozonolysis of natural rubber formed levulinic aldehyde and acid. This reveals that the molecule of natural rubber is based upon the chemical unit 2-methyl-2,3-butene. Physical properties indicate that natural rubber is a polymer of high molecular weight. The average molecular mass of the soluble fraction can be determined by techniques such as osmometry. Result of X-ray diffraction studies of crystallized natural rubber is consistent with the distance of isoprene unit corresponding to the *cis* configuration, rather than with the *trans* configuration.

It is well known that the rubber hydrocarbon in dry solid natural comprises two fractions; **sol fraction** and **gel fraction**, showing differences in behavior towards good solvents. The former is soluble in such solvents and the latter is insoluble in, but highly swollen in good solvents. It is well known that the experimentally determined proportion of the gel fraction in solid natural rubber hydrocarbon depends upon the solvent, which was used to extract the sol. The results reported by Allen and Bristow demonstrate this phenomenon for a particular grade of natural rubber, e.g., pale crepe. From these results, shown in Table 2.3, it is evident that the gel content can vary over a wide range, depending upon the solvent. The gel content does, however, correlate with diffusion coefficient of the liquid through rubber: the gel content decreases as the diffusion coefficient increases. Gel content has associated with

proportionately more of the nitrogenous substances from the latex than does the sol material. The addition of small amounts of polar liquids such as aliphatic alcohol to the extraction liquid can greatly reduce the apparent gel content, and the apparent gel content is also greatly reduced when the latex is enzymatically deproteinized. Thus, concerning the latter phenomenon, Shiibashi has reported that the apparent gel content of natural rubber could be reduced from *ca.* 40% w/w to almost zero when treating the latex with a deproteinizing enzyme for *ca.* 3 weeks.

Gel content of the rubber hydrocarbon in very fresh latex is small but variable. For very fresh latex from trees in regular tapping, it may be effectively zero. It also seems to be generally agreed that the gel content of the rubber particles increases as the latex ages, whether the aging takes place in the tree or outside of it. It appears that a crosslinking reaction commences immediately after tapping. Allen and Bristow postulated that the rate of crosslinking is dependent upon the entry of some chemical species into the latex particles from the aqueous phase, so that the level of crosslinking is greatest in the smallest particles. There is certainly much experimental evidence that the gel in natural rubber hydrocarbon is predominantly present in very small particles. Thus, Freeman has reported that the size of the gel particles in the rubber from fresh latex is *ca.* 100 nm, whereas the average size of the particles is *ca.* 1000 nm.

It remains to consider the nature of the crosslinks, which cause a variable proportion of the rubber hydrocarbon to be insoluble. These crosslinks clearly differ in chemical nature from those, which are normally present in crosslinked synthetic polymers. In this case, it typified by the carbon-carbon crosslinks, which form when a diene rubber is crosslinked by heating with a peroxide. It has long been recognized that the natural rubber molecule does not consist exclusively of carbon and hydrogen atoms, but also contains minor amounts of oxygen, probably in the from of carbonyl and ester groups. The present view is that a substantial proportion of the microgel in natural rubber forms by polar interaction between these groups attached to the rubber hydrocarbon macromolecules and the proteinic molecules. Allen and Bristow have suggested that the microgel particles in solid dry natural rubber are themselves bound

together in a loose network by association with proteinic substance. This view of the nature of the crosslinks in the gel phase of natural rubber appears to be consistent with the observations. The disproportionate presence of nitrogenous substances in the gel is then readily explained. The dependence of the apparent gel content upon the nature of extracting solvent is explicable as a consequence of differing abilities to disrupt the polar associations between the rubber and proteinic macromolecules. Enzymatic deproteinization reduces the apparent gel content to very low levels because the proteinic crosslinks between the rubber macromolecules are destroyed.

Figure 2.3 (a) shows a molecular-size distribution for the sol fraction of natural rubber hydrocarbon reported by Shiibashi. The distribution is unimodal, having a peak at a molecular mass of *ca.* 10^6 daltons. Shiibashi's results are consistent with the generally accepted view that the number-average molecular mass for the sol fraction of natural rubber hydrocarbon is *ca.* 3×10^5 daltons. Thus the ratio $\bar{M}_w/\bar{M}_n$ is *ca.* 6. This is high, and it indicates that the hydrocarbon macromolecules in natural rubber have high polydispersity. The cumulative molecular-size distribution shown in Figure 2.3 (b) for the rubber hydrocarbon in natural rubber latex concentrate has been published by Gazeley and Mente [4]. It shows the cumulative mass fraction of macromolecules as a fraction of molecular mass. The curve representing this distribution terminates at a mass fraction, which is far short of unity, indicating that the rubber contained gel. Shiibishi, Gazeley and Mente show that the molecular-size distribution for the sol fraction of the rubber hydrocarbon in natural rubber latex effectively terminates when molecular mass exceeds *ca.* 5×10^6 daltons, corresponding to a degree of polymerization of *ca.*10^5 and that most macromolecules of higher molecular mass have become incorporated in the gel fraction of the polymer.

Shiibashi has also reported the interesting observation that the weight-average molecular mass for the sol fraction of natural rubber hydrocarbon (but not the number-average molecular mass) is greatly reduced when natural rubber latex is subjected to enzymatic deproteinization, as well as the gel content of the rubber. The results are reproduced here as Figure 2.3 (c). Over a period of *ca.* 3 weeks treatment,

$\bar{M}_w$ was reduced to a value of *ca.* 6×10^5 daltons, which remained approximately constant thereafter. The polydispersity, as quantified by the ratio $\bar{M}_w/\bar{M}_n$, was reduced to *ca.* 3. The implication of these observations is that the hydrocarbon macromolecules in natural rubber of apparently very high molecular mass are formed by interaction between smaller hydrocarbon macromolecules and certain proteinic substances in the latex.

Another consequence of the presence of minority of polar groups attached to the natural rubber hydrocarbon is that further crosslinking of the rubber hydrocarbon gradually occurs in the latex and dry-rubber states, possibly by interaction between the polar group and active methylene groups on adjacent polymer chains. This crosslinking contributes significantly to the irreversible hardening, which occurs in natural rubber during prolonged storage.

2.2.2 The Aqueous Phase

2.2.2.1 Carbohydrates

The aqueous phase of fresh natural rubber latex is a dilute aqueous solution of density *ca.*1.020 Mg m^{-3}which contains many different chemical species, of which the principal types are carbohydrates, electrolyte proteins and amino acids.

The principal carbohydrate present in the aqueous phase of fresh natural rubber latex is a substance known as l-methyl inositol or quebranchitol. It occurs to the extent of *ca.* 1% w/w on the whole latex. It is noteworthy that, so far, no use has been found for it. Large amounts of this substance are thrown away annually. Other carbohydrates, which are present in small amounts, include galactose, sucrose, glucose, fructose and various other inositols. The biological significance of these carbohydrates (and of quebrachitol in particular) is obscure. They have little influence upon the properties of the latex or of the rubber with it contains. In the absence of adequate preservation, the carbohydrates become microbiologically oxidized to so-called volatile fatty acids, which comprise mainly formic, acetic and propionic acids.

2.2.2.2 Protein and Amino Acids

The aqueous phase of fresh natural rubber latex contains several proteins of differing isoelectric points. The principle proteins are known as α-globulin and hevein. α-globulin can be obtained by precipitation from a dialysed solution of freeze-dried aqueous phase in sodium citrate buffer solution of pH 4.5 and ionic strength 4×10^{-2} moldm^{-3}. To prevent denaturation of the protein, it is necessary to work at 0°C and to avoid keeping the protein at low pH. α-globulin is a surface-active protein of molecular mass *ca.* 2×10^{5} daltons. It is readily adsorbed at air-aqueous phase and oil-aqueous phase interfaces, with concomitant lowering of the respective interfacial free energies. It is insoluble in distilled water, but is soluble in neutral salt solution, acid solutions and alkaline solutions. Its isoelectric point is at pH 4.8, which is close to that for the particles in fresh natural rubber latex. Ammoniation increases the electrophoretic mobility of both α-globulin and latex particles. Fresh natural rubber latex undergoes gross colloidal destabilization under conditions of pH at which α-globulin is least soluble in aqueous media. The similarities between the electrophoretic behavior of dissolved α-globulin and that of the particles in fresh natural rubber latex provide the principal evidence for believing that this protein is an important component of the protein layer which is bound to surface of the rubber particles. α-globulin is denatured by heating and by storage in the dry state.

Hevein has been isolated by ammonium sulfate fractionation of the freeze-dried solids, which are derived from the so-called bottom fraction of natural rubber latex. After reprecipitation and the removal of salt by electrodialysis, the hevein can be freeze-dried and crystallized from cold water. It is a crystallized protein of abnormally low molecular mass (*ca.* 1×10^{4} daltons). Its isoelectric point is at pH 4.5. It contains *ca.* 5% of sulfur as cystine-type linkages. Hevein displays little surface-activity, is soluble in water at all pH values, and is not precipitated from water by boiling. It is likely to affect the colloidal properties of natural rubber latex significantly.

Several other proteins are present in the aqueous phase of fresh natural rubber latex besides α-globulin and hevein. Thus the presence of three protein fractions

was demonstrated by Bishop and confirmed by Kemp and Straitiff. Roe and Ewart have reported an electrophoretic analysis of the aqueous phase of fresh natural rubber latex. They showed the presence of at least seven district protein components having various isoelectric points. The finding has been confirmed by Moir and Tata, who used the technique of paper electrophoresis. These workers have also investigated the proteins, which are contained in the bottom fraction of fresh natural rubber latex. Especial interest attaches to the proteins of high isoelectric point, because these may be cationic under the conditions of pH, which reveal in aqueous phase of fresh natural rubber latex. They will therefore tend to reduce the colloid stability of the latex.

Archer and McMullen have shown that at least one nucleoprotein, known as *hevea* ribonucleoprotein, present in aqueous phase of fresh natural rubber latex. It can be extracted by neutral salt solution from fresh latex serum solids after solids after acid-soluble nucleotides. Other substances of low molecular mass have been removed. It can be purified by repeated precipitation in 66% (v/v) ethanol and also by treatment with acid at pH 3.5-4.0. The extracts contaminating proteins can be removed by precipitation with cetyltrimethylammonium bromide. The final product is homogeneous in the ultracentifuge, comprising of *ca.* 70% w/w of a polynucleotide of molecular mass *ca.* 2.8×10^4 daltons and *ca.* 30% w/w of a protein of molecular mass *ca.* 11.4×10^3 daltons. The base composition is an unusual combination of adenide, uracil, cytosine and guanine, with no fifth component.

A multiplicity of polypeptides and simple amino acids has been observed in the aqueous phase of fresh natural rubber latex, but whether these are the precursors or the degradation product of the latex proteins (or possibly both) is not clear. A useful review of this subject, with some extension, was provided by Ng in 1960, from which Tables 2.4 and 2.5 have been reproduced in modified from Table 2.4 the summarizes the amino acids which had been identified in the aqueous phase of natural rubber latex up to that time. Table 2.5 shows the amino acids that had been identified in the hydrolysis products of latex proteins. The free amino acids comprise together *ca.* 0.1% w/w of the mass of the whole latex.

There has been increasing concern in recent years regarding human allergenic reaction to certain proteinic substances, which are present in films, obtained from natural rubber latex. The most serious reaction attributed to contact with latex proteins is that know as **anaphylactic shock**. It is characterized by a severe reduction in blood pressure, breathing difficulties, increased heartbeat rate and unconsciousness. It can result in death. Other reactions include **contact urticaria**, which is contemporary eczematous reaction in which weal or flares at the contact site, with itching or stinging. These reactions are quite distinct from those attributable to rubber compounding ingredients, which may have been incorporated during the manufacture of the product. They are, as it were, inherent to natural rubber latex itself, being attributed to the presence of water-soluble proteinic substances in the final product. Thus, extensive leaching of vulcanized latex films considerably alleviates the problem. Natural rubber articles manufactured from dry rubber do not appear to give rise to these problems.

Several possible reasons are recognized for the recent increase in the number of patients from natural rubber in latex form. Greater use of films from natural rubber latex in the form of gloves and condoms for protection of humans against life-threatening microorganisms has increased exposure to allergenic substances in the films. Greater demand for protective products may have led to more rapid manufacture, with less-thorough leaching. Increasingly, such products are being manufactured in countries where natural rubber latex is produced. Exposure of proteinic substances to the ammonia preservative is therefore reduced. It is possible that prolonged exposure to ammonia may render such substances less allergenic. Variations in latex composition caused by clonal, seasonal and environmental changes may also be a factor, as may improve skill on diagnosis of allergenic reactions. There is now a large number of literatures relating to this matter. One of the most importance will be a review by Levy et al. [19] for the information concerning medical aspects, and a paper by Dalrymple and Audley for information concerning the factors which influence the level of extractable proteinic substances present in products manufactured from natural rubber latex.

Table 2.4 Free amino acids, which have been identified in the aqueous phase of fresh natural rubber latex.

Amino acid	Mc Gavack and Rumbold	Altman	Whitby and Greenberg	Drake	Ng
Glycine	No	Yes	No	Yes	Yes
Alanine	Yes	Yes	No	Yes	Yes
Valine	No	Yes	Yes	No	Trace
Leucine	No	Yes	Yes	Yes	Yes
Isoleucine	No	Yes	Yes	Yes	Yes
Phenylalanine	Yes	Yes	Yes	No	Yes
Tyrosine	No	Yes	Yes	No	Yes
Aspatic acid	No	No	Yes	Yes	Yes
Glutamic acid	No	Yes	No	Yes	Yes
Arginine	No	No	Yes	No	Trace
Proline	No	Yes	Yes	No	Trace
Lysine	No	No	No	No	Yes
Crystine	No	Yes	No	No	Yes
Serine	No	No	No	Yes	Trace
Tryptophan	No	No	No	No	Yes
Threonine	No	No	No	No	Trace

Table 2.5 Amino acids, which have been identified in the hydrolysis products of natural rubber latex proteins.

Amino acid	Belgrave	Midgely, Henne and Recoll	Altman	Tristram	Whitby And Greenberg	Drake
Glycine	Yes	No	No	Yes	Yes	Yes
Alanine	No	No	Yes	No	No	Yes
Valine	No	No	Yes	No	No	Yes
Leucine	No	Yes	Yes	Yes	No	Yes
Isoleucine	No	No	No	No	No	Yes
Phenylalanine	No	No	No	No	No	Yes
Dihydroxyphenylalanine	No	No	No	No	No	No
Tyrosine	No	Yes	Yes	No	Yes	Yes
Aspartic acid	No	Yes	Yes	Yes	Yes	Yes
Glutamic acid	No	Yes	Yes	No	Yes	Yes
Arginine	Yes	Yes	Yes	Yes	Yes	Yes
Histidine	Yes	Yes	Yes	Yes	Yes	Yes
Lysine	Yes	No	No	Yes	Yes	Yes
Proline	Yes	No	No	Yes	Yes	Yes
Hydroxyproline	No	No	No	No	No	No
Tryptophan	No	Yes	Yes	No	No	No
Cystine	Yes	Yes	Yes	No	No	No
Ornithine	No	No	No	No	No	Yes

2.3 Biosynthesis of Natural Polyisoprene

2.3.1 Occurrence of Natural Cis- and Trans-Polyisoprenes

Naturally produced polyisoprene occurs often, either in a network of interconnected cells called latex cells in the form of latex, or in single thin-walled parenchyma cells. In general, polyisoprene consisting of isoprene units in the *cis* configuration is referred to as rubber and that consisting of *trans*-isoprene units as gutta. Over 2000 species of higher plants (trees, shubs and herbs) have been shown to contain polyisoprene. The location of polyisoprene in plant tissues has been shown for twenty typical species bearing rubber or gutta. Rubber content has been analyzed for a number of South Africa plants, northern temperate zone plants and arid land plants.

Hevea brasiliensis is established as practically the only important natural source of rubber due to its high productivity of rubber having excellent physical properties. Guayule (*Parthenium argentatum*) and Russian dandelion (*Taraxacum kok-saghyz)* are typical rubber-bearing plants in the temperate zones. Guayule is expected to be an economical future source of natural rubber in semi-arid regions of Mexico and the United States. In addition to polyisoprene, guayule rubber contains resin, rubber soluble triglycerides and higher terpenes in amount as high as 20-25%. Jelutong (*Dyera costulata*), sorva (Coma macrocarpa) and sorvinha (Coma utilis) produce rubber containing a large amount of resins. Rubber from 33 northern temperate zone plants have lower weight –average molecular weight values than *Hevea* or guayule rubbers.

Gutta percha (*Plaguium gutta*) and balata (*Mimusops balata*) are sources of high molecular weight *trans*-polyisoprene. Recently, various species of grasses growing in the temperate zone were found to bear *trans*-polyisoprene. Chicle (*Achras sapota*) is an exceptional tree which produces a mixture of *cis*-polyisoprene and low molecular weight *trans*-polyisoprene together with resins. Some species of fungal genera are also known to produce low molecular weight *cis*-polyisoprene.

2.3.2 Biosynthesis Process

The individual steps in the biosynthesis of natural polyisoprene have been of considerable theoretical interest. The elucidation of the biosynthesis process is also of great practical importance because it may be enable the molecular weight of natural rubber to be controlled and the productivity of rubber-bearing plants to be increased. It is commonly considered that fundamentally the biosynthesis of rubber is quite well understood. As a matter of fact little is known about the mechanism of polymerization from the viewpoint of polymer chemistry. For example, the process of formation of new rubber molecules and the mechanism of controlling the geometric isomerism and molecular weight have not been elucidated. These are the fundamental steps corresponding to the initiation, propagation and termination reactions in polymer synthesis. The biosynthesis of natural rubber has been reviewed from the viewpoint of biosynthetic pathways from sucrose to the polyisoprene molecule. On the other hand, the formation of *trans*-polyisoprene has not been covered in these reviews.

2.3.2.1 Formation of Isopentenyl Pyrophosphate

Isopentenyl pyrophosphate, which polymerized to give polyisoprene, is synthesized from carbohydrates. A presumed pathway from carbohydrates, acetyl-CoA, mevalonate and isopentenyl pyrophosphate is shown in Figure 2.4. Radioactive tracer studies have been used to prove the incorporation of individual precursors. It is generally assumed that sugars are the main source of carbon for rubber formation. Although it has been elucidated that *Hevea* latex can convert sugars to pyruvate [30,31], there is little direct proof of incorporation of ^{14}C into rubber molecules when *Hevea* latex is incubated with ^{14}C-labeled sucrose, glucose or fructose. In the case of tissue culture of guayule, however, rubber and resin contents in callus cultures showed an increase with increasing levels of sucrose in the culture medium [5]

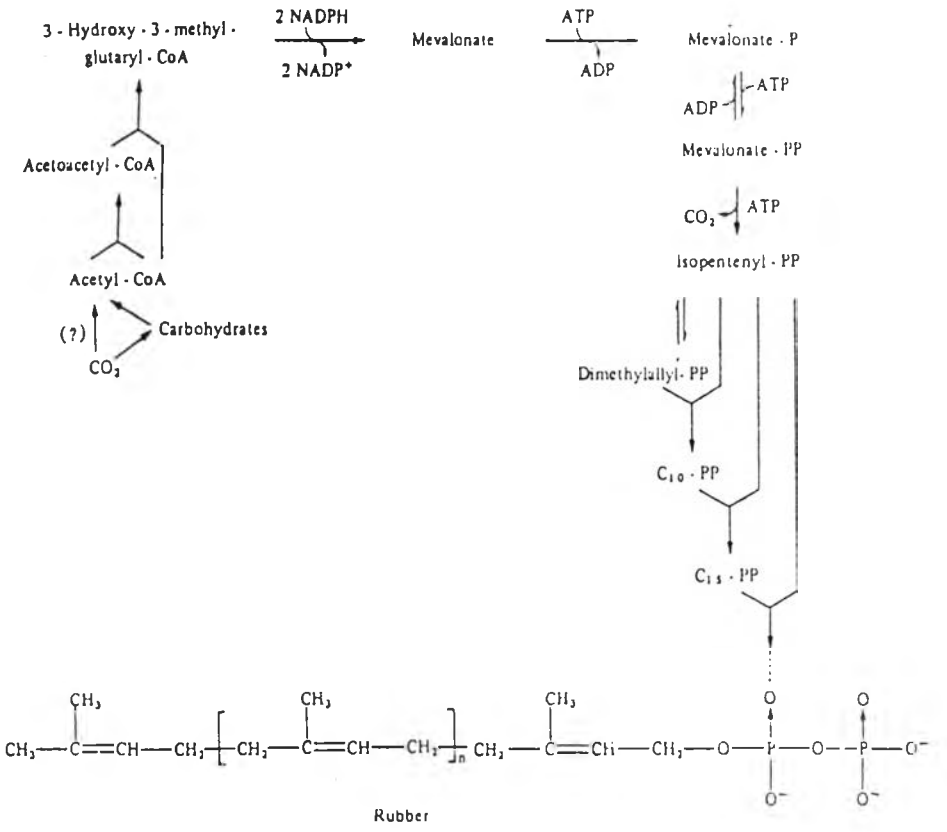

Figure 2.4 Presumed pathway for biosynthesis of rubber.

The conversion of pyruvate or acetate to rubber was demonstrated by incubation of the ^{14}C-labeled compounds with *Hevea* latex, although the conversion efficiency was very low. Similarly, ^{14}C-labeled acetate was incorporated into guayule rubber. However, the conversion of acetate to rubber is estimated to be as low as 1%. This poor conversion of pyruvate and acetate is thought to be partly due to low activity of enzymes necessary for the consumption of these compounds and also to the rapid conversion of pyruvate to ethanol in *Hevea* latex. The incorporation of acetyl-CoA and acetoacetyl-CoA into rubber was estimated in a similar way, although there is no direct evidence to prove the conversion of these materials to polyisoprene.

On the other hand, 3-hydroxyl-3-methylglutary-CoA (HMG-CoA) was found to be incorporated directly into rubber in proportion as high as 50% when incubated with *Hevea* latex. This finding was confirmed by ozonolysis of rubber from ^{14}C labeled HMG-CoA. Similarly, it was demonstrated that mevalonate is converted to high molecular

weight rubber in *Hevea* latex. The formation of mavalonate from HMG-CoA was estimated to be an important rate-controlling step in rubber formation. The degree of incorporation of ^{14}C-isopentenyl pyrophosphate (IPP) into the rubber skeleton was found to be as high as 97% and the incorporation was faster than that of mevalonate. The conversion of mevalonate to IPP was confirmed to proceed via 5-phosphomevalonate and 5-pyrophosphomevalonate in *Hevea* latex. These findings demonstrate that IPP is the direct biological precursor of the *cis*-polyisoprene molecule.

2.3.2.2 Initiation of Polymerization

The initiation step, which forms a new rubber molecule, has been assumed to start from dimethylallyl pyrophosphate (DMAPP). This is analogous with the known biosynthesis mechanism of acyclicterpenes such as geraniol (*trans*-C_{10}), farnesol (*trans. trans*-C_{15}) and geranylgeranoil (*trans, trans, trans*-C_{20})

One of the major problems of the proposed initiation step is the incorporation of the dimethylallyl skeleton molecule. In early works, it was found that the addition of DMAPP to *Hevea* latex forms farnesyl pyrophosphate, which inhibits rubber formation. However, DMAPP is incorporated into rubber when incubated with a suspension of rubber particles washed with detergent. On the other hand, neryl pyrophosphate (*cis*-C_{10}) was found to stimulate rubber formation in washed latex, as would be expected from the similarity of its geometric isomerism to that of rubber [6].

The initiation step must be preceded by the isomerization of IPP to DMAPP, which is well established in the case of biosynthesis of acyclic terpenes. The existence of an enzyme, isopentenyldiphosphate isomerase, in *Hevea* latex was suggested by several experiments. Ozonolysis of rubber obtained by incubation of *Hevea* latex with 4-^{14}C-IPP yielded radioactive levulinic acid while it gave no ^{14}C-acetone, which would be derived from the dimethylallyl group. This was attributed to extremely small mass fraction of the terminal group in comparison with the internal isoprene units.

Recently, various types of allylic pyrophosphate have been examined as initiators of the incorporation of ^{14}C-IPP into washed rubber particles on incubation. This activity is independent on the concentration of the initiator and also on the chain length. It is remarkable that the activity increased as the chain length of the initiator was increased from C_5 to C_{20}, practically independently of the configuration of the oligoisoprene unit combined with the pyrophosphate moiety. These findings suggest that the substrate specificity for the addition of IPP is not high in *Hevea* latex. The enzymatic incorporation of ^{14}C-IPP was also demonstrated for the washed rubber particles from the latex of *Ficus elastica*, on incubation with the enzyme in extracts of grayule leaves. In this experiment, the addition of DMAPP resulted in a two-fold increase incorporation of IPP into rubber.

2.3.2.3 Chain Elongation Step

The conversion of IPP to rubber was observed. This indicates that the enzyme so-called rubber transferase is adsorbed onto rubber particles and is still present on them after the particles are washed. Rubber transferase is also obtained from the leaves and stems of guayule, a non-laticiferous plant. It is noteworthy that the activity of the enzyme increase with an increment in the concentration of washed rubber particles. This finding suggests that the amount of 'living' polyisoprenyl pyrophosphate is proportional to the concentration of washed rubber particles. The rubber transferase from *Hevea* latex was partially purified and was estimated to have a molecular weight of 6×10^4. Recently, purification and systematic examination have been performed on the rubber transferase from guayule and from *Hevea* latex, but the structure has yet to be investigated.

The stereochemistry of rubber formation has been studied by using doubly labeled (2-^{14}C-(4R)-4-$^{3}H_1$) and (2-^{14}C-(4S)-4-$^{3}H_1$) mevalonates as in the case of acyclic isoprenoid compounds. The geometric isomerism of the double bonds can be determined from the $^{3}H/^{14}C$ ratio in the product. The ratio in the rubber was precisely the opposite of that in the *trans,trans*-farnesyl pyrophosphate, showing that the isoprene

units are formed by elimination of 4-^{3}H in the R configuration in rubber synthesis as shown in Figure 2.5. The result indicates that the *cis* configuration is biogenetically determined with respect to proton elimination from C-2 of IPP, without the intermediate formation of the *trans* configuration.

The mechanism of controlling the geometric isomerism is not yet known. It has been assumed that IPP attached to the active site of rubber transferase may interact with the living polyisoprenyl pyrophosphate terminal group of rubber molecule on the surface of a rubber particle, as illustrated in Figure 2.6.

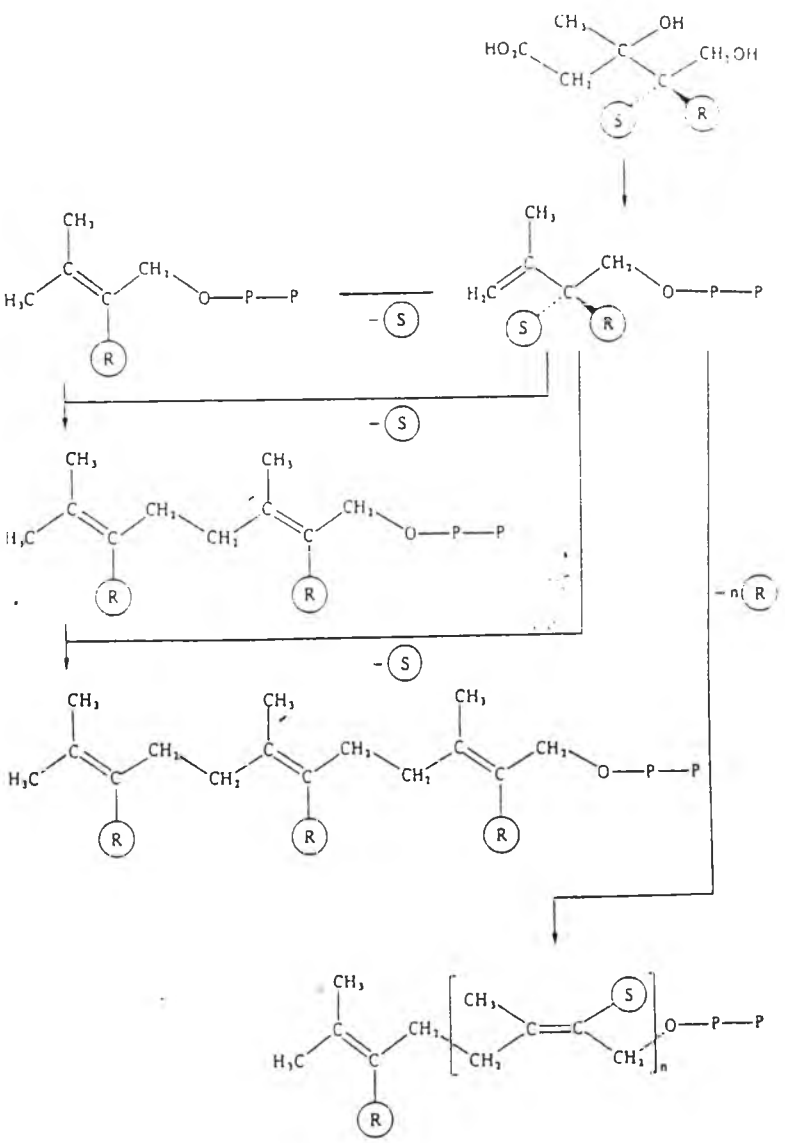

Figure 2.5 Stereochemistry of rubber synthesis from mevalonic acid.

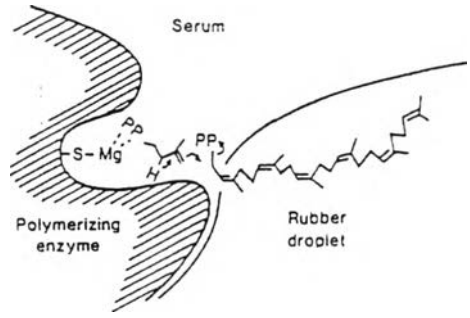

Figure 2.6 Hypothetical scheme of the chain extension mechanism on the surface of a rubber particle.

2.3.2.4 Termination of Polymerization

The molecular weight of natural polyisoprenes may be determined by the ratio of IPP units to initiating molecules. This is because the proposed chain extension process has a character of a living polymerization, although it proceeds by a condensation mechanism. However, natural polyisoprenes always exhibit a broad molecular weight distribution far from monodispersed distribuition.

In the case of grayule rubber, the molecular weight distribution can be adequately approximated by the relation $\overline{M}_z:\overline{M}_w:\overline{M}_n$ = 3:2:1. On the basis of this most probable molecular weight distribution, one of the possible mechanisms of molecular weight control was proposed as follows. The size of the rubber droplet reaches a point where it dose not allow further polymerization, and the repeated polymerizations up to that molecular weight tend to yield a most probable molecular weight distribution. On the other hand, the molecular weight of *Hevea* rubber has been shown to be not directly related to the size of the rubber particles in the latex, which can vary from 0.02 to 3 μm.

The occurrence of some special chain termination reactions is expected because of the low stability of the terminal polymer-pyrophosphate linkage. It can be hydrolyzed, esterified or cyclized under appropriate conditions. However, no biochemical exploration of the termination mechanism has been carried out.

2.4 Molecular Weight of Natural Rubbers [5]

2.4.1 Molecular Weight Distribution in Freshly Tapped Latex

Bristow [7], Westall [8] and Nair [9] first suggested that natural rubber might have a biomodal distribution. Exhaustive studies of the MWD of natural rubber in freshly tapped latex by Subramaniam [5] using GPC have confirmed that the distribution is, in general, bimodal. The distributions of all clonal rubbers can be classified into one of three types as shown in Figure 2.7 (these are actual tracings from the GPC):

- Type 1. Distinctly bimodal distribution where the peak height in the low molecular weight region is nearly equal or slightly less than the peak height at high molecular weight region.
- Type 2. Distinctly bimodal distribution where the height of the low molecular weight peak is only half or less than the height of the high molecular weight peak.
- Type 3. Skewed unimodal distribution with a 'shoulder' or a 'plateau' in the low molecular weight region.

As it is to be expected, slight differences in the shapes of the distributions between different samples from the same clone are observed. The high average molecular weight is of Type1.

The MWD is extremely wide: the $\bar{M}_w/\bar{M}_n$ ratio, where $\bar{M}_w$ and $\bar{M}_n$ are the weight and number average molecular weights, respectively, ranges from 2.5 to 10. Although the shapes of the distribution curves are different, the range of molecular weight is approximate the same in rubber from all clones, usually from about 3×10^4 to about 10^7:in some cases even wider, viz., from 10^4 to 1.2×10^7. (The values quoted for the upper limit of the molecular weight range are only good estimates). The high molecular weight peak appears between 1×10^6 and 2×10^6 for different rubbers. The position of the low molecular weight peak or 'shoulder' is less variable and appears between 1×10^5 and 2×10^5 for all the clonal rubbers studied.

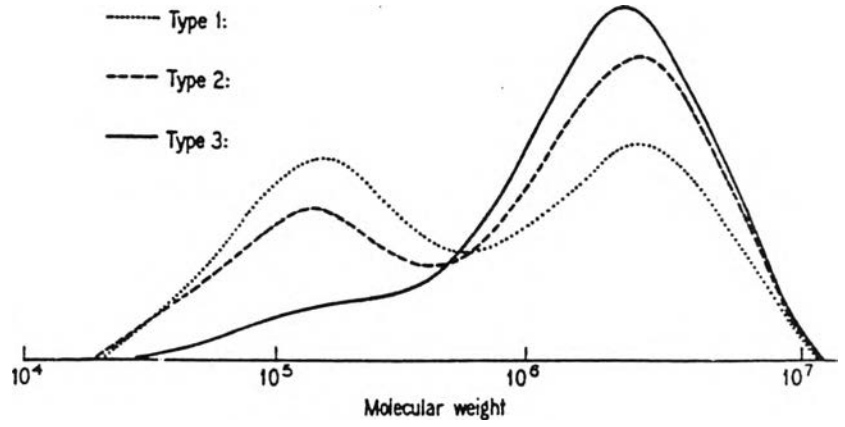

Figure 2.7 Types of molecular weight distribution curves of natural rubber [10]

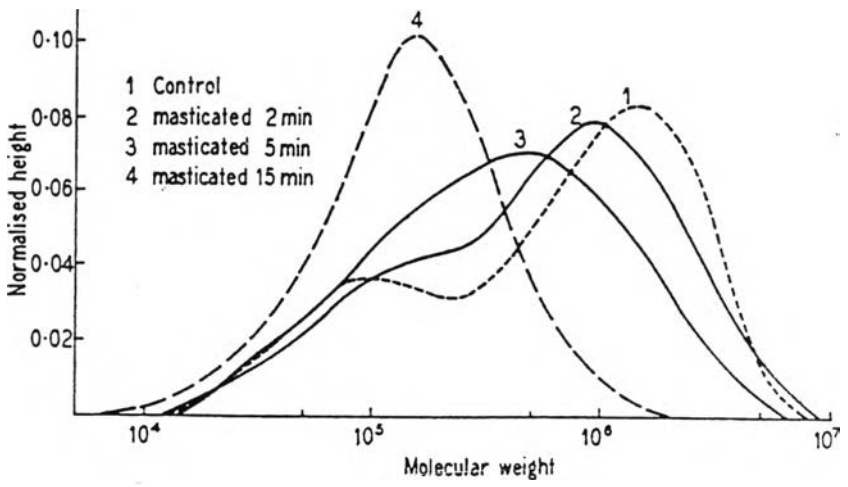

Figure 2.8 Molecular weight distribution of rubber masticated at 30°C [11].

2.4.2 Effect of Mastication, Sunlight and Heat on Molecular Weight

2.4.2.1 Mastication

The mechanical breakdown of rubber during premastication and while compounding is a non-random process, affecting the higher molecular weight species preferentially. The bimodal distribution changes to a unimodal distribution becomes narrower and the average molecular weight is reduced (Figure 2.8 and Table 2.8)

Table 2.8 Molecular weight distribution of rubber masticated for different periods [11].

Sample		$M_w \times 10^{-5}$ from GPC	$M_n \times 10^{-5}$ from GPC
Control		11.9	3.10
Masticated	(2 min)	10.9	2.46
at 30 °C	(5 min)	6.98	1.85
	(15 min)	2.60	1.19
Masticated	(2 min)	6.37	2.07
at 30 °C	(5 min)	4.88	1.71
Masticated	(2 min)	8.15	2.46
at 30 °C	(5 min)	6.92	2.24
	(30 min)	4.99	2.13

Below about 100 °C where oxidative degradation is not severe, mastication at a lower temperature causes a faster breakdown of the rubber. Harmon and Jacobs [11] have reported the appearance of a hump at a very high molecular weight during the initial stages of mastication. This observation has not been confirmed, but as shown in Figure 2.8 some very high molecular weight components are present in the rubber masticated for two minutes.

SMR CV has lower Mooney viscosity than the non-stabilized SMR WF or SMR 5 grades prepared from the same bulk of latex. Figure 2.8 shows the MWD of SMR 5 (Initial Mooney Viscosity V_R = 73) after it has been masticated to approximately the same V_R as SMR CV (VR of masticated SMR 5 =61, VR of masticated SMR 5 =62). The main differences between masticated SMR 5 and unmasticated SMR CV are:

(i) The masticated rubber has a lower weight average molecular weight $\bar{M}_w = 7.3\times10^5$; $\bar{M}_w$ of SMR CV = 1.2×10^6

(ii) The masticated rubber has a narrower MWD $\bar{M}_w/\bar{M}_n = 3.1$; $\bar{M}_w/\bar{M}_n$ of SMR CV = 6.2

(iii) The lower molecular weight peak in the bimodal distribution of the CV rubber only appears as a 'shoulder' in the masticated rubber.

Prolong mastication of rubber in a 12 in. laboratory two-roll mill with tight nip setting showed that the limiting $\bar{M}_n$ reached was about 10^5 .The limiting $\bar{M}_w/\bar{M}_n$ value was about 2.

2.4.2.2 Sunlight and Heat

When rubber is exposed to sunlight, The surfaces become tacky initial due to oxidation. On long exposure, the molecular weight decrease gradually. The bimodal distribution changes to a distribution with a broad plateau as shown in Figure 2.9. The bulk rubber away from the surface is usually unaffected. Heat aging causes a similar change. The MWD becomes a broad plateau though the bimodal distribution is still detectable after 1 h at 140 °C.

Sunlight degradation in solution is very rapid. The molecular weight may decrease by a factor of 10 in a matter of hours depending on the concentration of the solution and the intensity of sunlight. The MWD becomes unimodal and narrower as shown in Figure 2.10.

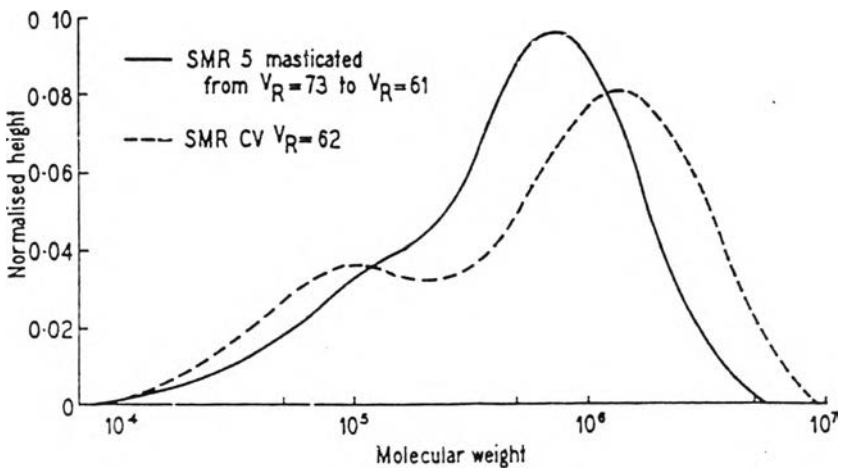

Figure 2.9 Molecular weight distribution of SMR 5 masticated to the same Mooney viscosity as SMR CV [11].

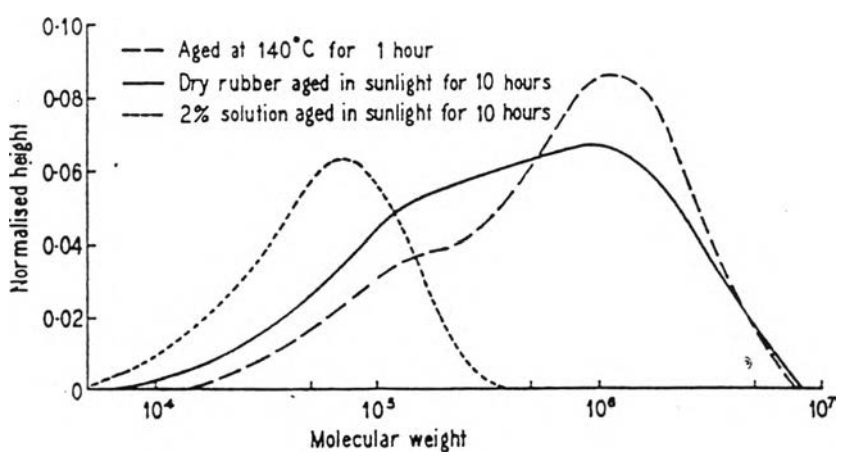

Figure 2.10 Molecular weight distribution curves for heat and sunlight degraded rubber [11].

2.5 Literature Review

Tanaka et al.[12] studied the synthesis of graft copolymers from highly deproteinized natural rubber (HDPNR) which was prepared by incubation of high ammonia (HA) latex with a proteolytic enzyme in the presence of surfactant followed by double centrifugation. Grafting of styrene onto untreated HA-latex and HDPNR latex was made, ter-butyl hydroperoxide/tetraethylenepentamide was used as a redox initiator. The grafting efficiency and average number of grafted sites were obtained by extraction and ozonolysis. The number of grafting sites for graft copolymer from HDPNR latex was about three times larger than that determined for graft copolymer from untreated natural rubber latex.

Sakaki et al. [13] studied the preparation of latex stabilized with anionic surfactants and their physical properties. Highly deproteinized natural rubber (HDPNR) latex was prepared by incubation of natural rubber latex with a proteolytic enzyme and anionic surfactants. Green strength and processability for a coagulant dipping process of HDPNR were investigated. Tensile strength, modulus, permanent set, and water absorption of cured film from HDPNR were also studied.

Tanaka et al. [14] studied the effect of small rubber particles from highly purified natural rubber on the latex stability. HDPNR latex was obtained by incubation with a proteolytic enzyme in the presence of surfactants followed by washing, using a rotary disk membrane module. The particle-size distribution and mechanical stability were investigated.

Hayashi et al. [15] studied the biological properties of highly purified natural rubber (HDPNR). HDPNR latex was prepared by incubation of HA latex with a proteolytic enzyme in the presence of surfactants followed by concentration with centrifuge. The nitrogen content of HDPNR was less than 0.02%. The potential of Type I allergy was evaluated by passive cutaneous anaphylaxis reaction test (PCA tests) and Enzyme-linked immunosorbent assay (ELISA). Antibody was not detected in the serum of animals sensitized with HDPNR latex by these two tests.

Tanaka [16] studied deproteinization of latex by saponification. Properties of DPNR prepared by saponification was analyzed for nitrogen content, fatty acid ester content, and gel content in toluene.

Hermann Miedel et al. [17] reported the process for producing rubber poor in albumin. Natural rubber latex was treat with dilute alkali solution to remove albumin from rubber lattices and to obtain therefore pure rubber hydrocarbon. Treated latex was allowed to cream and remove the alkali from sample by dialysis. Finally $ZnCl_2$ was added to yield finely flocculent coagulum to facilitate the washing out of the rubber coagulant. The finishing product has the nitrogen content of less than 0.05%. The rubber obtained in accordance with this invention can be employed in its vulcanize, or unvulcanized state as an electrical insulating material, for example, for submarine cables.

Melvin John et al. [18] studied the latex coagulation process using lignin compound. Synthetic styrene-conjugated diolefin polymer, e.g., SBR rubber, is recovered from their aqueous polymerization emulsion by coagulation with acid and a lignin compound. The conventional use of sodium chloride in as a coagulant is avoided so as to overcome the problem of sodium chloride in the effluent. By this process of the invention, a rubber crumb of increased porosity was obtained, which is easier to dry. They also reported that lignate coagulated rubber vulcanized showed slight advantages over the control (brine/acid coagulation process). It showed that the presence of the lignin in the rubber produced according to the invention has no deterious effect on the vulcanizate properties.

Gan Seng-Neon [19] reported FTIR studied on amino groups in purified *Hevea* rubber. Purification of fresh field latex was carried out by treated with enzyme followed by washing in the presence of surfactant. FTIR spectrum of natural rubber shows characteristic bands of attached nitrogenic compounds at 3280 cm^{-1} and 1540 cm^{-1}. These bands diminish if the fresh field latex is treated; a band at 3316 cm^{-1} is noticeable after the treatments suggests the presence of residual amino acids bonded to the rubber molecule.

Kawahara Seiichi et al. [20] studied in thermal properties and crystallization behavior of highly deproteinized natural rubber. Natural rubber was deproteinized with a proteolytic enzyme and surfactant to reduce nitrogen content to less than 0.02%. The deproteinized rubber was further purified by acetone-extraction followed by tranesterification with sodium methoxide. The degradation of rubber decreased and the thermal expansion coefficient increase by deproteinization and tranesterification. The glass transition temperature, T_g, was slightly reduced after deproteinization and tranesterification. These were explained in relation to the decrease in gel content of the rubber.

Bittencourt F. et al. [21] studied in the aggregation stability of natural rubber latex with low dry rubber content in acidic medium. Natural rubber latex was diluted from it original, 61% DRC, to 10% and the resulting material was studied with respect to its aggregation stability. Two nonionic surfactant with district HLB (hydrophilic-lyophilic balance) and cloud point values were used to stabilize the diluted latex. The stability of the obtained materials was evaluated through the addition of acetic acid solution of different concentration, and measure of the pH and coagulation times of the resulting mixture. Independent of the nature and concentration of surfactants, the sane behavior was observed for all the system. All the latex solution was stable after the addition of the acetic acid solution for at least 30 days.