CHAPTER II

LITERATURE REVIEWS

Characteristics of natural rubber latex

The fresh latex is tapped from the para rubber tree which is known by scientific name as *Hevea brasiliensis*. It is a milky white or slightly yellowish opaque liquid and contains 25 to 50 % dry rubber content. The factors that affect characteristics of natural rubber latex are rubber clones, tapping system, age of rubber tree, climate, soils and many other conditions. The composition of fresh latex is shown in Table 2.1.

Table 2.1 The composition of fresh latex (Fong, 1992)

Composition	per cent
Rubber Hydrocarbon	30-45
Proteinous substance	2.0-2.5
Water	58.5
Neutral lipids	1.4
Phospholipids	0.6
Ash	0.5
Inositols and carbohydrates	1.6
Other nitrogenous compounds	0.3

The latex contains rubber particles of different sizes having diameters in the range of about 0.02 μm to 3 μm. The rubber particles are mostly spherical but the small amounts of particles may be pear-shaped. The rubber particles in fresh latex are protected by a complex film containing proteins and lipids. (Fong, 1992)

The latex consists of three main constituents, the rubber hydrocarbon (30-45 %), non-rubber constituents (6.4 - 6.9%) and water (58.5 %). Apart from proteins which play an important role, other non-rubber components in natural rubber latex have been presumed to affect the properties of solid rubber prepared from latex, and compound rubber before and after vulcanization.

The fractionation of Hevea latex

Fresh latex can be separated into four major fractions by high speed centrifugation (e.g. 59,000 × g for 1 h). There are three particulate fractions and serum phase which are listed below and shown in Figure 2.1 (Moir 1959) :

1. The uppermost layer consists of rubber particles which comprises 25% - 45 % of total volume of fresh latex. The rubber fraction contains rubber hydrocarbon and small amounts of other non-rubber substances , mainly proteins and phospholipids forming surface around the rubber particles.

2. The next layer are Frey Wyssling particles. These particles are mostly lipids which are spherical and have bright yellow color. They are large sizes and have high density which are often in form of the clusters. The color of these Frey-Wyssling particles is due to the presence of carotenoid pigments which is the cause of the yellow color of some latics.

3. The middle layer is a serum phase which contains mostly water. In addition to water, the serum phase contains small amounts of soluble compounds including inositols, carbohydrates , amino acids, proteins, inorganic anions and metal ions.

4. The bottom fraction contains mainly of the lutoids which comprises 10%-20% volume of the latex. Lutoids are spherical membrane-bounded bodies typically 2-5 μm in diameter. These lutoids are heavier than rubber particles and form the bulk of the bottom fraction.

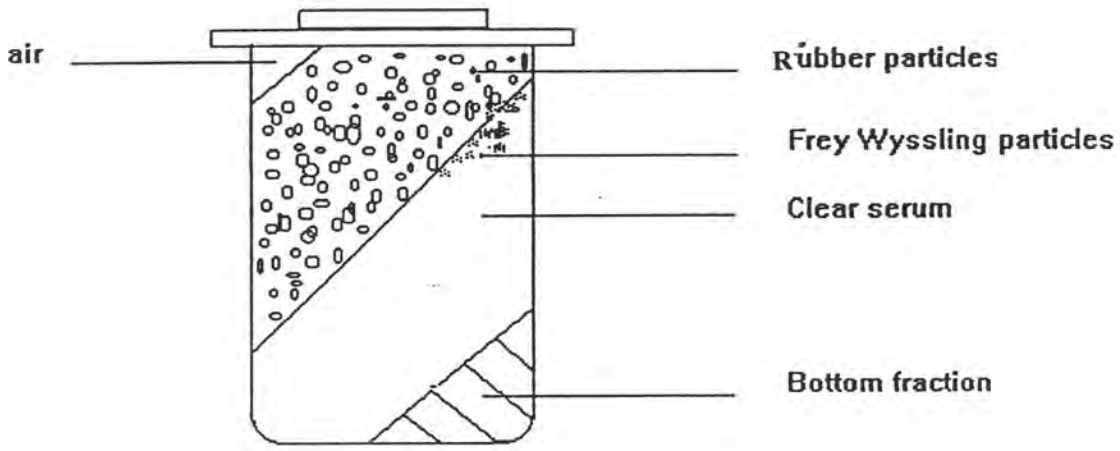

Figure 2.1 Fractionation of Hevea latex after ultracentrifugation

Classification of clones

According to the RRIM Recommendations for conventional planting, rubber clones are classified based on the growth and yield characteristics of the rubber tree which was listed below: (Yip, 1990)

Class I clones: High performance materials recommended for large scale plantings: GT 1, RRIM 600, RRIM 712, PR 255, PR 261and PB 217.

Class II clones : Promising materials, suitable for moderate-scale planting such as RRIM 623, RRIM 628, RRIM 701, RRIM 703, RRIM 729, RRIC 100 and PB 28/59.

Class IIIA clones: Experimental materials planted up to 10 ha per clone such RRIM 803, RRIM 804, RRIM 806, RRIC 100 and PB 312.

Class IIIB clones : New materials planted in one-task-size blocks only selections of which have only been tested in small-scale trials.

Structure and molecular weight of natural rubber

Chemical structure of natural rubber

The structural formula of natural rubber molecules are $(C_5H_8)_n$, where the isoprene monomer is represented by formula, C_5H_8 and n is about 20,000. About 90% of the monomers are cis-1,4- polyisoprene with only 2-3 trans-form isoprene as shown in Figure 2.2.

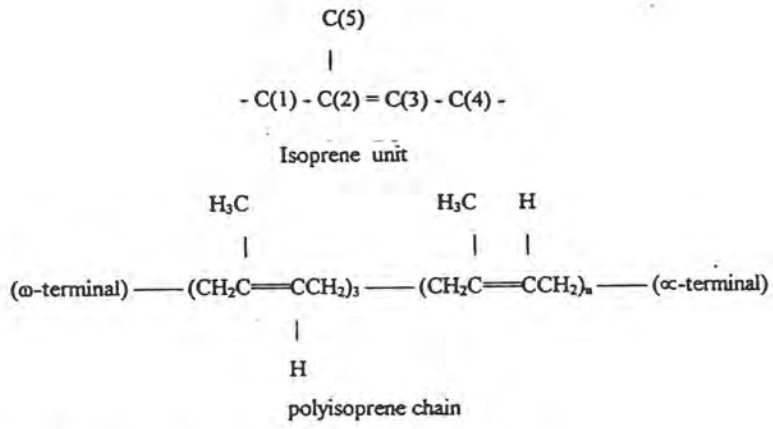

Figure 2.2 The chemical structure of natural rubber.

Gel phase

NR consists of 5-50% of gel phase which can be classified in two types: macrogel and microgel. Macrogel is insoluble in solvents but microgel can be soluble in solvents. When NR is dissolved in a solvent, macrogel is in the form of swollen rubber floated in the solution. Microgel is not visible to the naked eyes but the diameters of 50-200 nm can be estimated by electron microscopy.

Protein and its effect

The total protein content of fresh latex is about 1.0%-1.5%. About 20% of total protein is adsorbed on the rubber particles and the other 20% is associated with the bottom fraction. The rest are soluble proteins in aqueous serum phase. The proteins adsorbed on the rubber particles have not been studied in much details due to the difficulties in removing them from the rubber particles. Proteins do not confer any significant advantages. The presence of proteins in the natural rubber has been reported to have resulted in some undesirable properties such as poor creep and stress-relaxation, reduced modulus and increasing sensitivity to water and moisture.

Molecular weight and molecular weight distribution

In 1971 gel permeation chromatrography (GPC) was used to determine the molecular weight and molecular weight distribution (MWD)of rubbers. The MWD of rubber clones were either bimodal or unimodal. The bimodal distribution may be of two types: one where the lower and higher molecular weight peaks are of nearly the same height, and the other where the higher molecular weight peak height is larger than of the lower molecular weight peak. Figure 2.2 shows examples of the MWD of rubber in three different types.

Different clones of rubber show variation in the average molecular weight and patterns of MWD. The higher viscosity rubber clones such as PB 5/51, PB 28/59 usually have unimodal type of distribution and the lower viscosity rubber clones such as RRIM 600, GT 1 mostly have a bimodal type. The polydispersity expressed by

Mw/Mn show a wide distribution of rubber clones in range from about 4 to 10. (Subramaniam, 1993)

Comparison among different clones, show great variations in the average molecular weight. The weight average molecular weight of rubber is in the range of 3 $\times 10^4$ and 10^7, the peak of high molecular weight fraction is about 1-1.25 $\times 10^6$ and the low molecular weight peak at 1-1.25 $\times 10^5$. (Table 2.2)

Table 2.2 Weight average molecular weight (Mw), number average molecular weight (Mn) and type of molecular weight distribution of some clonal rubber. (Subramaniam, 1975)

Clones	$Mw \times 10^{-6}$ from GPC	$Mn \times 10^{-5}$ from GPC	Type of MWD curve
RRIM 600	1.93	2.58	2
PB 28/59	2.15	5.20	3
PB 5/51	2.18	5.21	3
GT 1	1.85	2.65	2

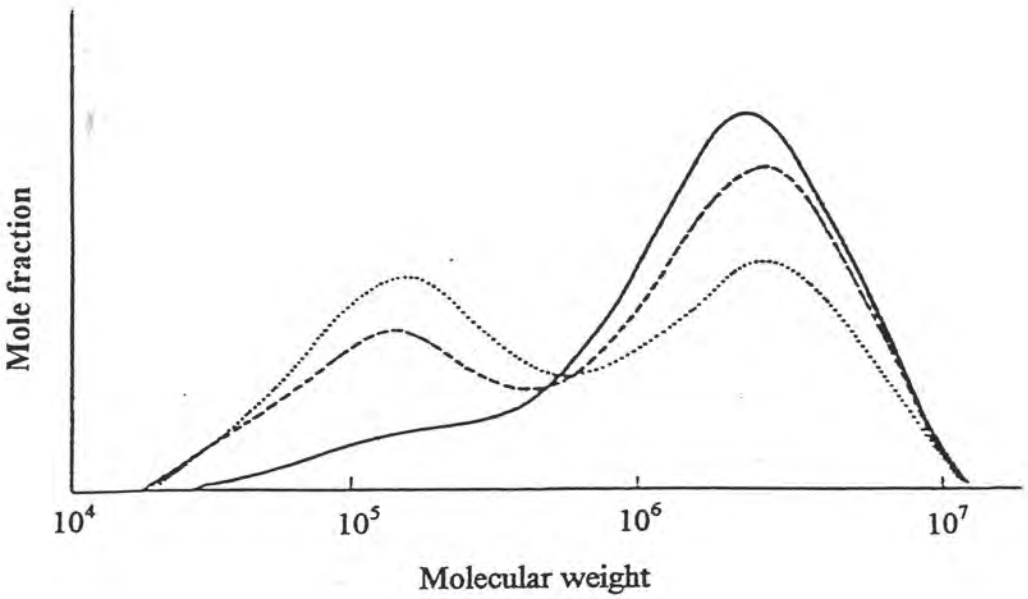

Figure 2.3 Types of molecular weight distribution curves of natural rubber. (Subramaniam, 1980)

Type 1(.....): Distinctly bimodal distribution where the peaks are of nearly the same height : RRIM 501, RRIM 605, RRIM 703

Type 2(----): Distinctly bimodal distribution where the peak in the low molecular weight region is smaller : RRIM 600, GT 1.

Type 3(___): Skewed unimodal distribution with a "shoulder" of a "plateau"in the low molecular weight region : PB 28/59, PB 5/51.

Storage-hardening phenomenon

The increase in viscosity of natural rubber upon storage after preparation in solid rubber form commonly known as storage-hardening is an important physico-chemical change. Storage-hardening phenomenon is brought about by a slow crosslinking process while natural rubber was kept over a period of several years (Bristow, 1974). This increase can be accelerated by conditions of high temperature and low humidity. Storage-hardening leads to variability in viscosity and processing behaviour.

Assumptions of storage-hardening is the result of condensations between aldehyde groups on the polyisoprene chains and some aldehyde condensing groups on the other polyisoprene chains or on proteins contained in the latex.

Defect of natural rubber is that it results in an increase in viscosities of rubber and needs pre-mastication which demand longer time and higher energy consumption. These problems can be solved by adding inhibitor such as hydroxylamine hydrochloride, hydroxylamine sulphate and semicarbazide hydrochloride to prevent hardening.

Constant-viscosity natural rubbers or CV-NR are produced by adding hydroxylamine hydrochloride in latex before acid coagulation to react with the aldehyde groups. The storage-hardening of natural rubber was suppressed by the most efficient inhibitor such as hydroxylamine hydrochloride before coagulation. (John, 1970).

Coagulation of latex

The dynamic properties of rubber depend on how it coagulate. Some methods of rubber coagulation are listed below ;

1. Acid coagulation ; by adding 2% v/v formic acid at pH 5.0.
2. Auto-coagulation ; by storing in open container for 24 hours.
3. Biological coagulation ; by adding anionic surfactant (Aerosol GPG) and molasses.

4. Instant coagulation ; by adding Aerosol GPG and calcium chloride within 5 minutes.

5. Heat gelation ; by heating in oil bath at 110°C.

6. Steam coagulation ; by heating with steam (0.1 $MN.m^{-2}$) for 15 minutes in the autoclave.

The auto-coagulation is usually complete in about 48 hours. The disadvantages of natural coagulation is time-consuming, often incomplete coagulation, offensive odour in the finished dry rubber and unsatisfactory properties. Biological coagulation reduces coagulation time from 48 to 16 hours in comparison with auto-coagulation. Biological coagulation process is attractive since the speed of coagulation, low price and local availability of carbohydrate additives (John, 1966).

The use of acid coagulation is the most popular because it is well-known and convenient. Dry rubber is prepared from Hevea latex by coagulation with formic or other acids to reduce its pH. The advantage of using steam coagulation instead of acid coagulation is time-saving and pollution-free. Apart from those, the solid rubber yield from steam coagulation is higher than that from acid coagulation.

The removal of proteins from natural rubber

The removal of proteins from natural rubber has been studied to improve technological and dynamic properties. There are three methods in separation of proteins from natural rubber which can be summarized as below:

1) Treatment by using surfactant or detergent by which protein was eluted out from rubber surface.

2) Chemical or alkaline treatment by immersing in sodium hydroxide solution for 24 hours by which protein was hydrolysed by chemical reaction.

3) Biochemical or enzymatic treatment by using proteolytic enzymes such as Papain, Alcalase, Trypsin, Superase etc. by which protein was hydrolysed into small peptides and amino acids. These can be leached out easily. This method is the most suitable for removal of proteins due to its mild operating condition.

Deproteinized natural rubber

Deproteinized natural rubber was introduced in 1974 and developed form of natural rubber. DPNR is very closely similar in physical and chemical characteristics to synthetic cis-1,4-polyisoprene. DPNR has better green strength and building tack than its synthetic quality. DPNR shows excellent dynamic properties and improved stress relaxation behaviour when compounded with rubber-soluble vulcanizing ingredient, particularly suitable for use in engineering applications.

Properties of DPNR

The properties of DPNR produced at the RRIM Experiment Station.

Table 2.3 Specifications of DPNR

Properties	DPNR from latex concentrate	DPNR from clarified field latex	Proposed specification
Dirt (% wt)	0.006	0.005	No value >0.015
Nitrogen (% wt)	0.066	0.120	No sample >0.15
Ash (% wt)	0.052	0.130	No sample >0.15
Volatile matter (%wt)	0.280	51	No value > 0.5
Mooney viscosity,	-	32	45-55
ML 1-4 (100°C)			55-65
P_0	39	85	-
PRI	66	70	60 (min)

Development of DPNR manufacture

There are several methods used for removing protein from latex in different types of latex which can be summarized in Table 2.4.

Table 2.4 Developments in DPNR manufacture.

Raw material	Removing agent	Reference
Skim latex	Sodium hydroxide	Firestone Tyrerubber Co, 1955
Field latex	Dioctyl-octyl sodium sulfursuccinate	John, 1971
Skim latex	Sodium sulfosuccinate and calcium chloride	John and Sin, 1973
Field latex	Papain	Nadarajah et al., 1973
Latex concentrate	Superase	Chin & Smith, 1974
Skim latex	Trypsin	Ong, 1974
Skim crumbs	Sodium hydroxide and oxalic acid	Ong, 1974
Field latex	Papain/$NH_2OH.HCl$	Yapa, 1975
Clarified latex	Superase or Alcalase	Chang et.al., 1977
Clarified latex	Superase/$NH_2OH.HCl$	Chang et al., 1977
Field latex	Papain/Alkaline treatment	Yapa, 1977
Field latex	Papain/Alkaline treatment/ $NH_2OH.HCl$	Yapa, 1977
Field latex	BPN/Alkaline treatment/ $NH_2OH.HCl$	Yapa, 1977
Field latex	Superase/Alkaline treatment/$NH_2OH.HCl$	Yapa, 1977
Skim latex	Papain	Yapa et al., 1978
Field latex	Pineapple juice (bromelain)	Yapa et al., 1980
Field latex	Papain	Yapa, 1984
Field latex	Pineapple juice (bromelain)	Yapa, 1984
Field latex/ Latex concentrate	Papain or Alcalase	Visessanguan, 1992

Use of Papain in DPNR production

Proteolytic enzymes was obtained from both vegetable (e.g. Papain,ficin and bromelain) and animal sources (e.g. pepsin, trypsin). Trypsin is the most effective of the three enzymes; trypsin, pepsin and Papain but Papain is of most interest because Papain is a locally available raw material (Yapa and Balasingham, 1974).

Papain is oven-dried latex obtained from the skins of the immature fruit of papaya, Carica papaya. Papain is a cysteine proteinase which is used for the solubilitization of membrane proteins. The two grades of Papain (white and brown) are available commercially. The isoelectric point of Papain is at pH 8.75 and has limited solubility above pH 7 while its optimum pH of activity is between 6-8. For the optimum pH of commercial grade depends on the substrate and for serum globulin which is the main protein of field latex.

The coagulants commonly used for natural rubber latex are acids. In which acids normally was used as coagulants are formic, acetic acid and sulfuric acid. Organic acids agglomerate the rubber particle by bringing down the pH of latex to near that of the isoelectric point of the proteins which protects around the rubber particles. The pH of acid coagulation is approximately 4.6. Treatment with proteolytic enzymes like Papain have been introduced because it can destabilize latex resulting in coagulation. For several years, enzyme deproteinization of field latex was used to produce deproteinized rubber which gives a low protein with low water absorption for specialized use in industry (Nadarajah and Yapa, 1973).

Two purposes of Papain treatment are that it acts as a coagulant and reduces the nitrogen content of resulting rubber. Papain break down Hevea proteins to the several amino acids namely glutamic acid, serine, alanine, tryptophan, phenylalanine, valine and leucines present in the serum after enzyme treatment. The only disadvantage of enzyme treatment is an increase in the ash content retained in the deproteinized rubber .

DPNR production

Enzymatic deproteinization is the incubation of latex with enzyme resulting in protein reduction. Proteins in natural rubber have the effect on physical and mechanical properties of natural rubber. During the processing of DPNR, other non-rubber constituents could be washed away together with the nitrogenous substances and the removal of these constituents could affect the rubber properties. The removal of protein which is the non-rubber constitutes or contaminants reduction, therefore properties of DPNR become similar to properties of synthetic cis-1,4-polyisoprene. DPNR has been found to have significantly improved rubber properties.

Defection of manufacture of NR by using centrifuged latex is that the DPNR obtained has low PRI since the leaching by ammonia of alkali soluble antioxidants present in the rubber particles. This defect does not occur with enzyme deproteinization. The method of enzyme deproteinization is therefore the most suitable.

Deproteinized rubber has low level of nitrogen contents (0.06%) which reduces ability to absorb moisture and improve characteristics (Bernard, 1973).

Low nitrogen CV rubber or CV-DPNR was prepared by a combination of two processes;viscosity stabilization and deproteinization. Hydroxylamine hydrochloride prevents storage hardening while Papain reduces the nitrogen content (Yapa, 1975). The advantage of CV-DPNR is that it requires very little or no mastication prior to mixing.

Main advantages of low protein rubber (Bernard, 1973)

1. improved heat-build up.

2. low affinity for water due to removal of naturally occurring hydrophilic substances.

3. enhanced resilience.

4. superior fatigue life.

5. uniformity in cure behavior.

Theoretical considerations

Enzyme-catalyzed reaction

Enzymes, like all proteins, are composed of α-amino acid residues joined by peptide bonds in a specific sequence. The primary structure is referred to linear sequence of residues in the peptide chain. The coiling or folding of the peptide chain is referred to as the secondary structure (Harper & Row, 1968). Enzymes enhance reaction rates by lowering the activation energy required. The function of the enzyme is to catalyze a reaction. The important factor affecting the rates of enzyme-catalyzed reactions are : enzyme concentration, ligand concentrations (substrates, products, inhibitors, and activators), pH, ionic strength, and temperature. The reaction by enzyme occurs in the area of contact which is called the active site. The active site is a small portion of the protein surface. A small amounts of chemical agents or co-factor was used to start the catalyzed reaction and remain attached to the enzyme throughout its catalytic cycle. Co-foctors may be metal ions or organic residues which their function is to provide a specific chemical property not easily attained with the amino acid residues alone. Enzyme or catalyst is unchanged chemically after the reaction but may be changed physically so that it no longer behaves as a catalyst.

The enzyme-catalyzed reaction involves two steps;

(1) the reversible, rapid combination of enzyme (E) and reactant or substrate (S) to form a complex (ES).

(2) the breakdown of ES to give product (P) and regenerate free enzyme. The kinetic model is given as

The simplest enzyme catalyzed reaction, represented by

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Effect on the rate of enzyme-catalyzed reaction

pH

The pH affects the stability of an enzyme and influences the velocity of an enzyme-catalyzed reaction. The effect of pH on the rate of enzyme catalyzed reactions is due to the changes it causes in the ratio of the hydrogen ion-free, and hydrogen ion-bound, forms of the enzyme and its substrates. The active sites on enzymes are composed of ionizable groups which must be in the proper ionic form to maintain the conformation of the active site, bind the substrates, or catalyze the reaction. The one or more of the substrates themselves may contain ionizable groups. The pH stability of an enzyme depends on many factors including temperature, ionic strength, chemical nature of the buffer, concentration of various preservatives, concentration of substrates or cofactors of the enzyme and enzyme concentration. At low enzyme concentrations, the enzyme may dissociate into smaller oligomers or monomers.

Temperature

The variation of the rate constant k with the absolute temperature could be described by Arrhenius equation. The constant A is called the frequency factor and the term $e^{-Ea/RT}$ Is the Boltzmann expression for the fraction of molecules having an energy in excess of the value E_a. The energy E_a is the minimum energy that must be acquired by the reactant molecules before a reaction can take place. At given temperature, the enhancement of the rate of a reaction can be explained by an increase in the term $e^{-Ea/RT}$ or by a reduction in E_a by an increase in the value of A or by a combination of the two effects. An increase in temperature will affect this reaction by changing the rate constants for each steps. In practical terms, an enzyme molecule is a very delicate and fragile structure. The tertiary structure of an enzyme is maintained primarily by a large number of weak noncovalent bond. If the molecule absorbs too much energy, the tertiary structure will disrupt and the enzyme will be denatured resulting in lose catalytic activity. The temperature of an enzyme depends on a number of factors including the pH and ionic strength the medium and the presence or absence of ligands.

Collision of reactants

The velocity of any homogeneous chemical reaction depends on the frequency of collisions between reactant molecules. The collision frequency is influenced by the concentrations of reactant molecules and does not equal the reaction velocity because only a small proportion of the collisions occur with sufficient energy to promote the reaction. This minimum energy required for a fruitful reaction is called the energy of activation, E*a*. Arrhenius (1889) proposed that molecules could be divided into two categories; normal molecules which do not take part in reactions and molecules which required a level of energy and have become activated. An increase in molecular motion and hence to an increase in the number of collisions that produce activated molecules. An increase in temperature will lead to an increase in reaction rate. The collision theory proposed that the reaction rate is dependent on the number of collisions giving rise to activated molecules.

Mixing

Mixing action is not only promoted to produce more uniform mixtures of components. An important part of the mixing operation is movement or treatment or transfer of materials to or from surfaces of particles or phases. Examples of such operations are dissolution, leaching, gas absorption, crystallization, and liquid-liquid extraction. The fluid motion reduces the thickness of the resisting "film", it effectively increases the concentration gradient immediately adjacent to the particle or phase surfaces of the transferring components in the fluid. A very common and important mixing operation is bringing different molecular species together to obtain a chemical reaction. The components may be (a) miscible liquids, (b) immiscible liquids, (c) solid particles and a liquid, (d) a gas and a liquid, (e) a gas and solid particles, or (f) two gases.

In vessel-type equipment, there is a circulation or back-flow that moves fluid into all parts of the vessel or chamber. Examples of the vessels are cylindrical tanks stirred by rotating turbines or propellers, by jets of liquid or by gas bubbles. Impellers

produce circulation flows in a cylindrical flows in a cylindrical mixing vessel and a similar combined vortex was occurred. The movements of fluids or particles which are required to carry out the types of mixing operations require that external forces be imposed to overcome resisting forces in the fluid. For viscous materials like polymers and polymer solutions, shear forces are not only the major resistance to moving a stirrer, but also provide the mechanism for moving the fluid in a desired flow pattern. Some high molecular weight polymers have an elastic as well as viscous resistance to motion.

In high viscosity liquids, mixing is carried out in a laminar state and the molecular diffusion is very small. Mixing in the laminar flow regime should be avoided since the relatively poor mixing. For liquids, it required more time because of the low rate of molecular diffusion. In laminar flow, the state of mixing proceeds as follows; (1) mixing by convection flow, (2) mixing by turbulent diffusion, (3) mixing by a local convection flow which causes the disintegration of turbulent eddies, (4) mixing by molecular diffusion. For turbulent mixing, the nature of the turbulent mixing problem, little is known about the actual mechanism. But the mechanism of turbulent mixing is not essentially different from that of laminar mixing. The turbulent diffusion is assumed for the mixing phenomena in a large space compared with the average size of turbulent eddies. The turbulence in mixing vessels is composed of an average convection flow with a turbulent fluctuation. For mixing in a large space volume is accomplished by (1) convection due to a mean flow and (2) turbulent diffusion. Mixing by the disintegration of turbulent eddies and by the molecular diffusion is important for micro mixing, but is negligible for macro mixing in a large space (Nagata, 1975). There are two important aspects of the problem of turbulent mixing. First, experimental information interpreted in terms of the theory may provide some insight into the actual mechanistic contribution of turbulence and of molecular diffusion to mixing, and second, with reasonable approximations for the mixing spectrum and boundary conditions (Vincent and Gray, 1966).