

CHAPTER II

THEORY

2.1 Poly(β -hydroxybutyrate)

Poly(β -hydroxybutyrate) or PHB is a member of polyhydroxyalkanoates or (PHAs) which are biodegradable polyesters [Doi, 1990]. Figure 2.1 illustrates chemical structure of PHAs and PHB.



n = 1	R = hydrogen	Poly(β -hydroxypropionate)
	R = methyl	Poly(β -hydroxybutyrate)
	R = ethyl	Poly(β -hydroxyvalerate)
	R = propyl	Poly(β -hydroxyhexanoate)
	R = pentyl	Poly(β -hydroxyoctanoate)
	R = nonyl	Poly(β -hydroxydodecanoate)
n = 2	R = hydrogen	Poly(4-hydroxybutyrate)
n = 3	R = hydrogen	Poly(5-hydroxyvalerate)

Figure 2.1 Typical chemical structure of PHAs and PHB [Lee, 1996]

Nomenclature of various types of PHAs is also shown in Figure 2.1. PHB is one of the members of PHAs of which R position is substituted by methyl group and n is equal to 1. PHB (and PHAs) can be synthesized and accumulated by various types of microorganisms as carbon and energy sources during the starvation [Lee, 1996]. Types of microorganisms that accumulate PHB are shown in Table 2.1.

Table 2.1 PHB accumulating microorganisms [Doi, 1990]

Acinetobacter	Gamphosphaeria	Photobacterium
Actinomycetes	Haemophilus	Pseudomonas
Alcaligenes	Halobacterium	Rhizobium
Aphanotece	Hyphomicrobium	Rhodobacter
Aquaspirillum	Lamprocystis	Rhodospirillum
Azospirillum	Lampropedia	Sphaerotilus
Azotobacter	Leptothrix	Spirillum
Bacillus	Methylobacterium	Spirulina
Beggiatoa	Methylocystis	Streptomyces
Beijerinckia	Methylosinus	Syntrophomonas
Caulobacter	Micrococcus	Thiobacillus
Chlorofrexeus	Microcoleus	Thiocapsa
Chlorogloea	Microcystis	Thiocystis
Chromatium	Moraxella	Thiodictyon
Chromobacterium	Mycoplana	Thiopedia
Clostridium	Nitrobacter	Thiosphaera
Derxia	Nitrococcus	Vibrio
Ectothiorhodospira	Nocardia	Xanthobacter
Escherichia	Oceanospirillum	Zoogloea
Ferrobacillus	Paracoccus	

For normal growth, microorganisms need a carbon source, an energy source, trace elements, oxygen and nutrients such as nitrogen, sulfur and phosphorus. If there is a limitation of nutrients that are necessary for growth, microorganisms will synthesize PHB (or PHAs) by converting excessive carbon source to PHB and use PHB as energy and carbon source during the starvation [Luzier, 1992].

PHB was first mentioned in literature as early as 1901 [Griffin, 1994]. Detailed studies were reported by Maurice Lemoigne, a microbiologist of Pasteur Institute, Paris [Doi, 1990]. He observed granule-like inclusions in cytoplasm of bacteria. By chloroform extraction, granule-like inclusions were isolated and he had concluded that it was polyester polymer having the empirical formula of $C_4H_6O_2$. Many reports from various groups of researcher were made in the next thirty years. In the late 1950s and early 1960s, PHB was produced commercially and manufactured as sutures and prosthetic devices [Griffin, 1994]. However, PHB fermentation yields were relatively low and the PHB purification process was very expensive. Particularly, the polymer produced was heavily contaminated with bacteria residue. As the result, the commercial PHB producing project was abandoned for over a decade [Griffin, 1994].

ICI was the first company to solve these problems. By combining the specialities in large scale fermentation from agricultural division with skills in polymer processing from the plastics division, ICI found that PHB can be produced up to 70% of dry mass of bacterium *Alcaligenes eutrophus* which was used in fermentation process. ICI had made an advance in PHB production by patenting a procedure to produce the copolymer of β -hydroxybutyrate and β -hydroxyvalerate (PHBV) which is known in the tradename of "Biopol" [Griffin, 1994]. The chemical structure of PHBV is shown in Figure 2.2.

Fundamental characteristics and properties of PHB are summarized in Table 2.2. Properties of PHB are often compared to those of polypropylene (PP) because both of them

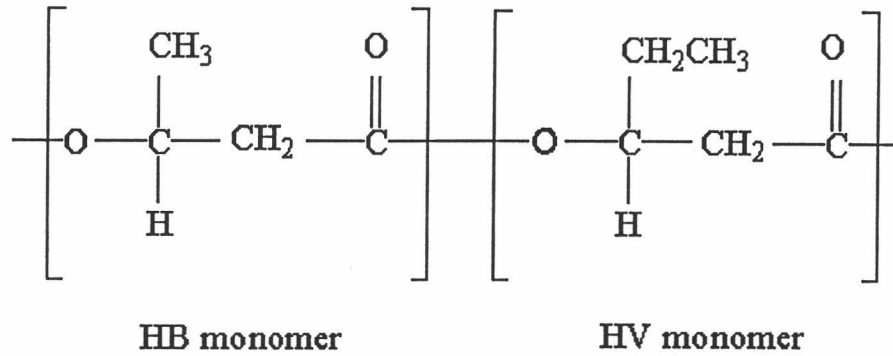


Figure 2.2 Chemical structure of Poly(β -hydroxybutyrate-co-hydroxyvalerate) or PHBV
[Griffin, 1994]

have similar melting temperatures (T_m), degrees of crystallinity and glass transition temperatures (T_g). However, PHB is stiffer and more brittle than PP [Jogdand, 1999]. There are a lot of researches studying the brittle nature of PHB and trying to improve the properties of PHB. Each group of researchers has their own conclusion about the brittleness mechanism of PHB. The results of the researches are summarized in chapter III.

Table 2.2 Fundamental characteristics and properties of PHB and PP [Jogdand, 1999]

Characteristics and properties	PP	PHB
Melting temperature ($^{\circ}\text{C}$)	171-186	171-182
Glass transition temperature ($^{\circ}\text{C}$)	-15	5-10
Degree of crystallinity (%)	65-70	65-80
Flexural Modulus (GPa)	1.7	3.5-4
Tensile Strength (MPa)	39	40
Extension at break (%)	400	6-8
UV resistance	Poor	Good
Solvent resistance	Good	Poor
Biodegradability	-	Good

One of the most attractive properties of PHB is its biodegradability. PHB can be biodegraded in soil, sludge, lake water and seawater [Sudesh *et al.*, 2000]. PHB and PHAs can be completely biodegraded to carbon dioxide and water [Eggink *et al.*, 1994]. When PHB was discarded to the environment, biodegradation was proceeded by microorganisms such as bacteria, fungi and algae at the surface of the polymer [Griffin, 1994]. The microorganisms excrete extracellular PHB depolymerase to hydrolyze the PHB surface into the dimers and/or monomers. The resulting products are absorbed and metabolized by microorganisms as nutrient. The extracellular PHB depolymerase can hydrolyze PHB which has a high degree of crystallinity. This mechanism is completely different to that of intracellular PHB depolymerase which can hydrolyze only PHB granules on an amorphous elastomer in the cytoplasm of microorganisms [Griffin, 1994]. Some microorganisms such as fungi which are incapable of accumulating PHB also excrete enzyme to biodegrade PHB [Doi, 1990]. There are many factors affecting the biodegradation rate [Jogdand, 1999] including:

1. Conditions of environment: temperature, moisture level, pH level and nutrient supply.
2. Properties and characteristics of PHB: composition, crystallinity, additives, surface area, surface texture and dimension of specimen.

2.2 Characterization of molecular weight and molecular weight distribution

Two of the most important characteristics of polymer are molecular weight and molecular weight distribution. These characteristics affect the processing and the strength of polymer. Molecular weight of polymer differs from that of the low molecular weight compound. The molecular weight of the latter is identical in every molecule but that of polymer are polydispersed or heterogeneous. The molecular weight of polymer is expressed in average value. The average molecular weight of polymer can be defined in many ways. Two of the most conventional used averages are the number average molecular weight, M_n (based on the number of molecules presented) and the weight average molecular weight, M_w (based on the total weight of the molecules of each size). The formulas of these averages are defined in equation 2.1 and 2.2, respectively.

Molecular weight distribution (MWD) is defined as the ratio of the weight average molecular weight to the number average molecular weight.

$$M_n = \frac{\text{total weight of all molecules}}{\text{total number of molecules}} \quad (2.1)$$

$$M_w = \frac{\sum(\text{weight of all molecules of each size} \times \text{its molecular weight})}{\text{total weight of all molecules}} \quad (2.2)$$

Gel permeation chromatography (GPC) or size exclusion chromatography (SEC) is the principle method in determining the molecular weight and molecular weight distribution of the polymers. The separation of polymer molecules by GPC method is based on the difference in their molecular size. A schematic diagram of a typical GPC is shown in Figure 2.3. SEC column is filled with beads, which have pores with various size and distribution, commonly made from polystyrene cross-linked with divinyl benzene (or many types of glass in the case of SEC) [Cheremisinoff, 1990].

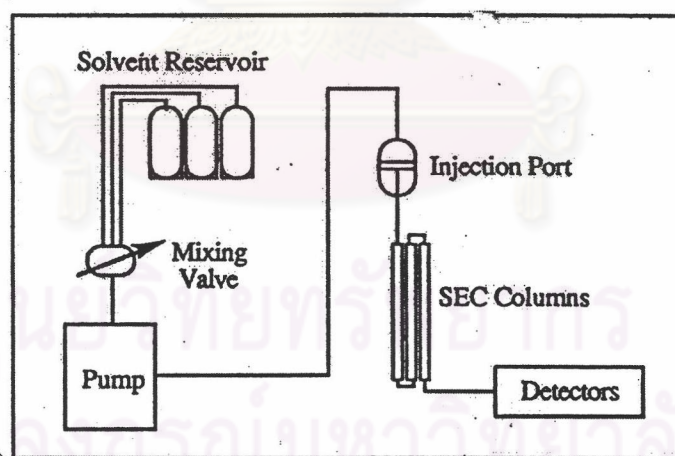


Figure 2.3 Schematic diagram of typical GPC [Painter and Coleman, 1994]

From the schematic diagram shown in Figure 2.3, a polymer solution is pumped through the set of columns. Molecules of different size are eluted from the column and the detectors measure the amount of the different species as they pass by. While the

small molecules diffuse into the pores, large molecules may be excluded from the pores and will be swept through the column first, followed by others of decreasing size. Figure 2.4 illustrates the schematic diagram of size separation in GPC technique.



Figure 2.4 Schematic diagram of polymer size separation in GPC [Hadad *et al.*, 1988]

2.3 Tensile properties

Tensile or stress-strain testing is one of the most widely used methods for testing mechanical properties of polymers. The test is usually done by measuring the force that generates as the sample is elongated at a constant rate of extension. The tensile force is recorded as a function of elongation. The standard tensile test for plastics is outlined in ASTM D638 and ASTM D882. The tensile test according to ASTM D638 is performed on the dumb-bell shaped specimens. ASTM D882 is used as the standard procedure for tensile testing of materials that are in the form of film or thin sheet.

Test specimens can be prepared by many ways such as injection moulding, compression moulding and solution casting. The specimens which prepared by injection moulding method may exhibit various tensile properties because of polymer orientation.

Figure 2.5 shows the translation of stress-strain curves when the temperature decreases or strain rate increases [Gruenwald, 1993]. The graphs vary from rubbery behavior to glassy behavior. The change in tensile behavior when the strain rate is increased is also similar to the tensile behavior of a polymer with decreasing temperature.

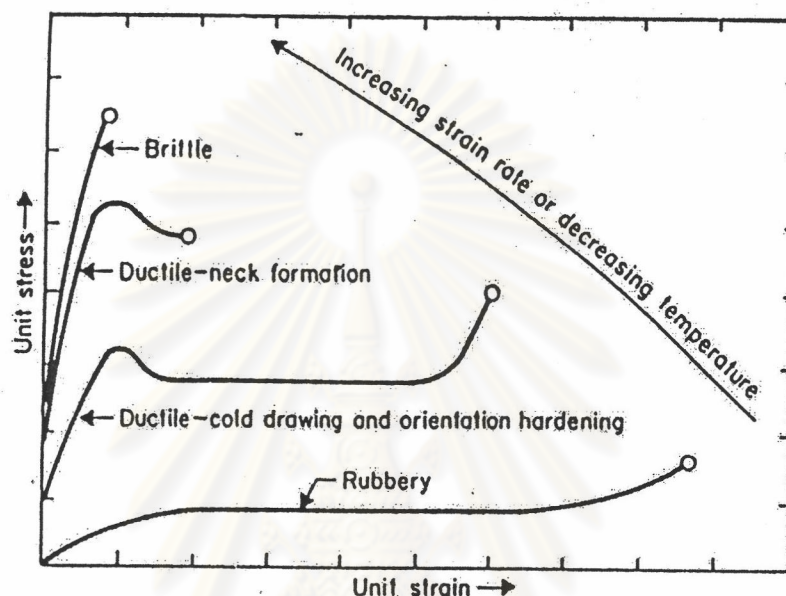


Figure 2.5 The effects of temperature and strain rate on stress-strain curves [Gruenwald, 1993]

A typical stress-strain curve for plastics is shown in Figure 2.6. For a better understanding of this curve, explanation of terminology used in tensile testing is as follows:

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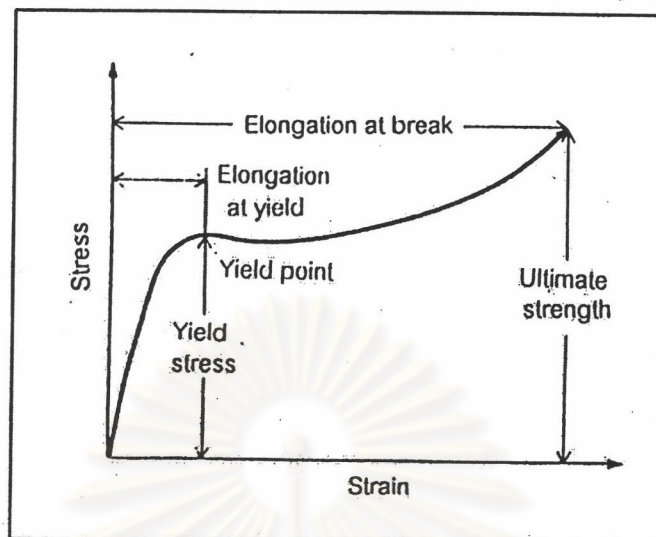


Figure 2.6 A typical stress-strain curve for plastics [Haung, 1988]

2.3.1 Stress

Stress is the tensile load applied to produce deformation per unit of cross-sectional area of testing specimen at a given moment. The standard unit measuring in Pascal (Pa) or pound per square inch (psi). “Ultimate tensile strength” or “ultimate tensile strength at break” is calculated by dividing the load at break by cross-sectional area of the specimen.

2.3.2 Strain

Strain is the ration of elongation or deformation to the gauge length of the test specimen; that is, the change in length per unit of original length. This term is expressed as a dimensionless ratio (mm/mm or in/in).

The specimen shows elastic behavior when it is elongated below the elastic limit, that is, the deformation is completely and instantaneous recovered when the specimen

is unloaded. Stressing the specimen beyond its elastic limit results in a degree of permanent set. This unrecoverable stress is called plastic strain. This strain is usually associated with plastics, but it is also seen in other materials.

2.3.3 Young's modulus or modulus of elasticity

Modulus is defined as stress divided by the strain. This value is slope of stress-strain curve. Modulus values for plastics are determined at very low extension which stress-strain curve is straight line. This constant modulus is called modulus of elasticity or young's modulus. Young's modulus is normally expressed in MPa or GPa. Young's modulus is applied to describe the stiffness or rigidity of plastics. Figure 2.7 shows an example of the calculation of young's modulus on the initial straight portion of the straight strain curve.

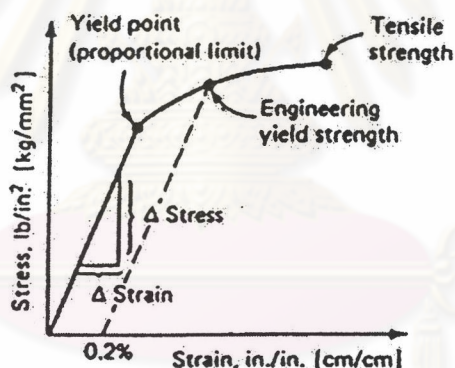


Figure 2.7 Example of the young's modulus determined on the initial portion of the stress strain curve [Rosato, 1991]

2.3.4 Yield point

Yield point is the first point of the stress-strain curve that the strain is increased without any increase in stress. After the yield point the specimen exhibits unrecoverable behavior. Normally this point represents the limit of elasticity of polymer. The stress at the yield point is defined as tensile strength at yield or yield stress.

2.3.5 Elongation

Elongation is expressed as a percentage of length increase of the test specimen, normally called the percentage of elongation.

2.3.6 Area under the stress strain curve

Generally, the area under the stress-strain curve from the origin to breaking point is proportional to energy required to break the plastics and is sometimes referred to the toughness of plastics. Figure 2.8 illustrates typical stress-strain curve of several types of polymer [Haug, 1988]. Plastics can be classified according to the stress-strain behavior.

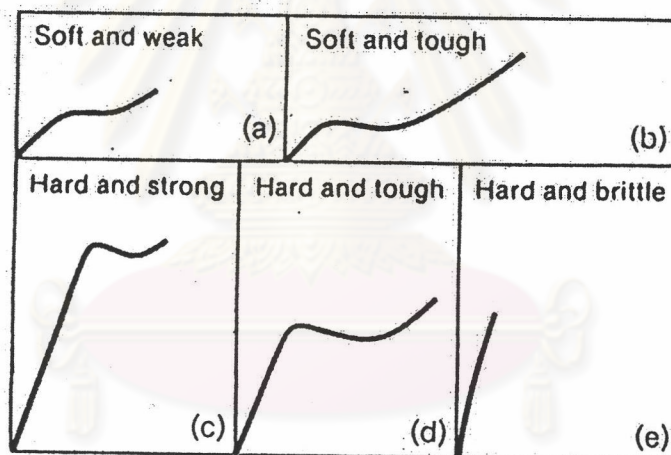


Figure 2.8 Typical stress-strain curves for several types of polymers [Haug, 1988].

In Figure 2.8, a soft and weak is characterized by low modulus, low yield strength and moderate elongation at break (a). Soft and tough materials as polyethylene show low modulus, low yield stress, but very high elongation at break (b). Hard and strong materials as polyacetal has high modulus, high yield stress, usually high ultimate strength and low elongation (c). Hard and tough materials such as polycarbonate is characterized by high modulus, high yield stress, high elongation at break and high

ultimate strength (d). Hard and brittle material such as general purpose phenolic is characterized by high modulus and low elongation (e).

2.4 Amorphous and crystalline polymer [Sperling, 2001; Billmeyer, 1984; and Carraher 1996]

Amorphous polymer contains chains that are arranged in a disorder manner. Amorphous polymer exhibits a glass transition temperature and does not give a clear X-ray diffraction pattern. Conformation of amorphous polymer can be described by freely jointed chain model and random coil model. Freely jointed chain model is the simplest model of a polymer chain in space. This model describes a random flight of polymer chain in three dimension. Figure 2.9 presents chain of amorphous polymer which described by freely jointed chain model.

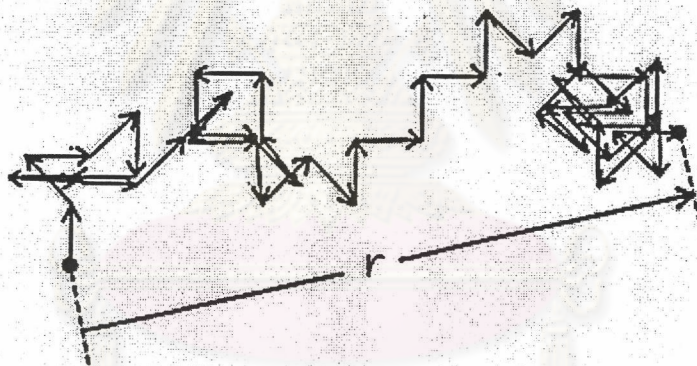


Figure 2.9 A chain of amorphous polymer which described by freely joint chain model [Sperling, 2001]

Random coil model is used to describe the unperturbed shape of the polymer chain in both dilute solutions and in the bulk amorphous state. In dilute solution the polymer-solvent interactions and the excluded volume term cancel each other. In the bulk amorphous polymer the sum of interaction between mer and mer is zero. In this model the polymer chains are permitted to wander about in a space-filling way as long

as they do not pass through themselves or another chain. Random coil model of bulk amorphous polymer is showed in Figure 2.10



Figure 2.10 Random coil model of bulk amorphous polymer [Grassino, 2000]

Glass transition is a transition which happens to amorphous polymer. Glass transition temperature (T_g) is the temperature at which a polymer changes from hard and brittle to soft and pliable. Hard plastics like polystyrene and poly(methyl methacrylate) are used below their T_g which they are glassy. Rubber elastomer like polyisoprene and polyisobutylene are used above their T_g which they are soft and flexible.

A crystalline polymer is a polymer that is arranged in a neat orderly structure. The crystalline polymer diffracts X rays and exhibits the first-order transition known as melting. The first model that describes the structure of partially crystalline polymer was fringed micelle model. From this model, the polymer chain was believed that they passed in and out of many crystallites. Figure 2.11 shows the schematic diagram of the fringed micelle model.

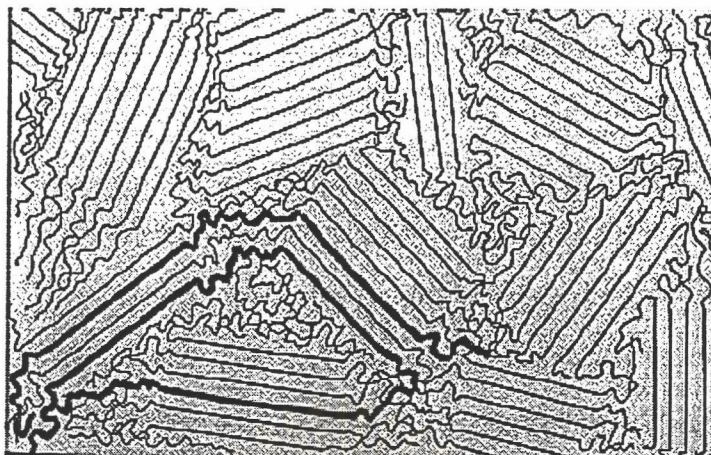


Figure 2.11 the schematic diagram of the fringed micelle model [Koch, 2000]

According to the fringed micelle model, the disorder regions separating the crystallites are amorphous. The chains wander from the amorphous region through a crystallite, and back out into the amorphous region. The chains are long enough to pass through several crystallites, binding them together. The idea about polymer crystallinity was changed when the single crystal was prepared by Keller. He concluded that the polymer chains in the crystals had to be folded upon themselves. This led to the folded-chain model which showed that the polymer molecules folded back and forth with hairpin turns and exhibited adjacent reentry. The schematic diagram of the folded-chain model is presented in Figure 2.12.

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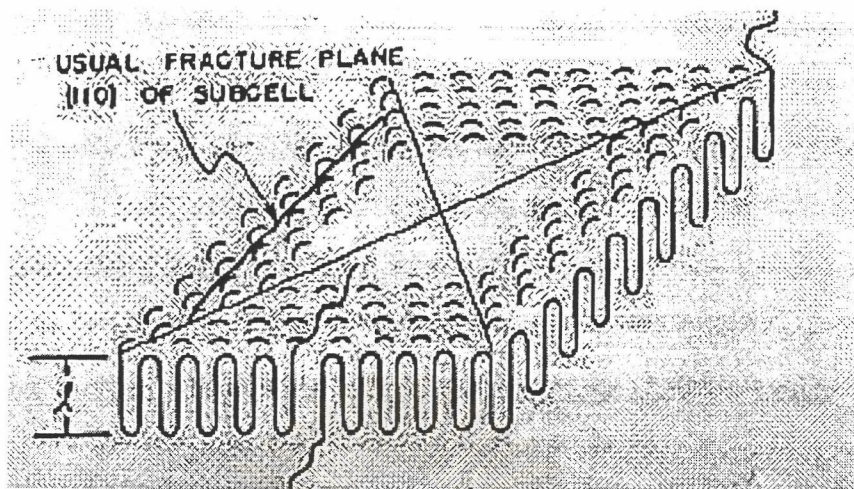


Figure 2.12 schematic diagram of the folded-chain model [Marand, 2001]

Another model which used to describe the structure of crystallites is the switchboard model. In the switchboard model the polymer chains do not have a reentry into the lamellae by regular folding; they rather reenter more or less randomly. The model more or less resembles an old-time telephone switchboard. The schematic diagram of the switchboard model is showed in Figure 2.13.

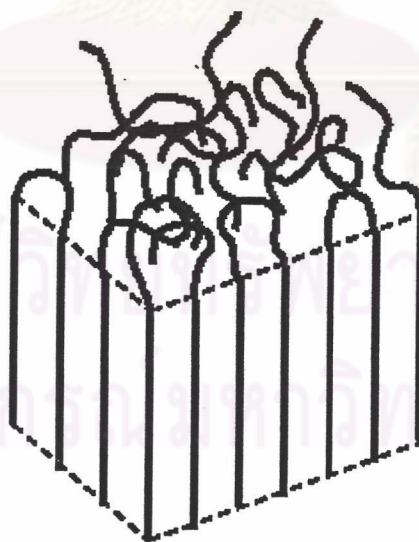


Figure 2.13 The schematic diagram of the switchboard model

[Beaucage, 1998]

Folded-chain and switchboard model are used to describe the structure of a single crystal which crystallized from dilute solutions. From somewhat more concentrated solutions, various multilayered dendritic structures are observed. When polymer samples are crystallized from the melt, the most obvious if observed structures are the spherulites. The spherulites are sphere shaped crystalline structures that form in the bulk. A model of spherulite structure is illustrates in Figure 2.14. The chain direction in the bulk crystallized lamellae is perpendicular to the board plane of the structure, just like the dilute solution crystallized material. The spherulite lamellae also contain low-angle branch points where new lamellar structures are initiated. The new lamellae tend to keep the spacing between crystallites constant.

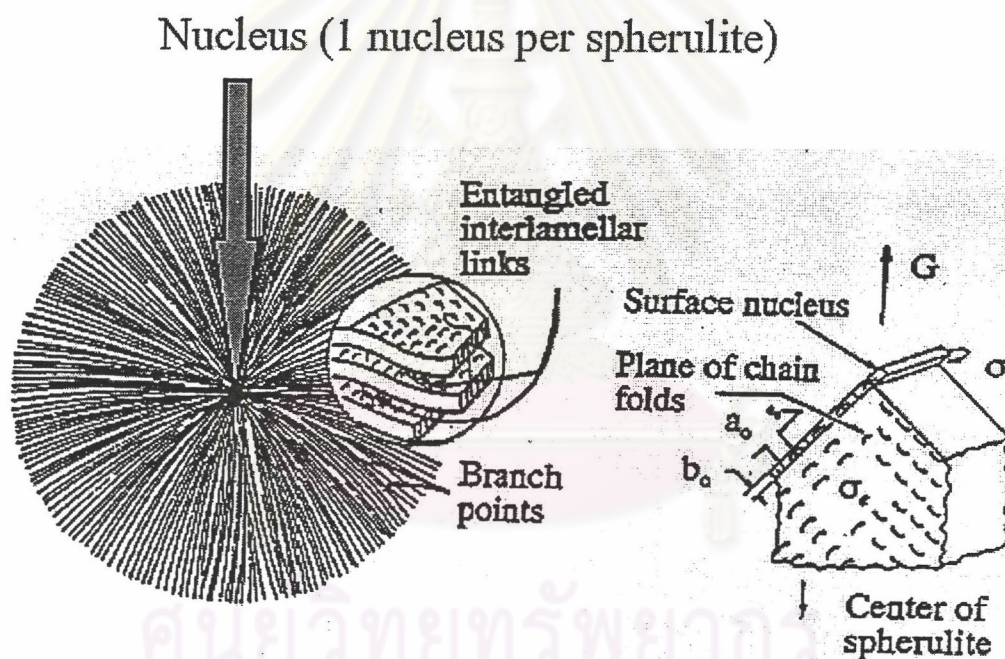


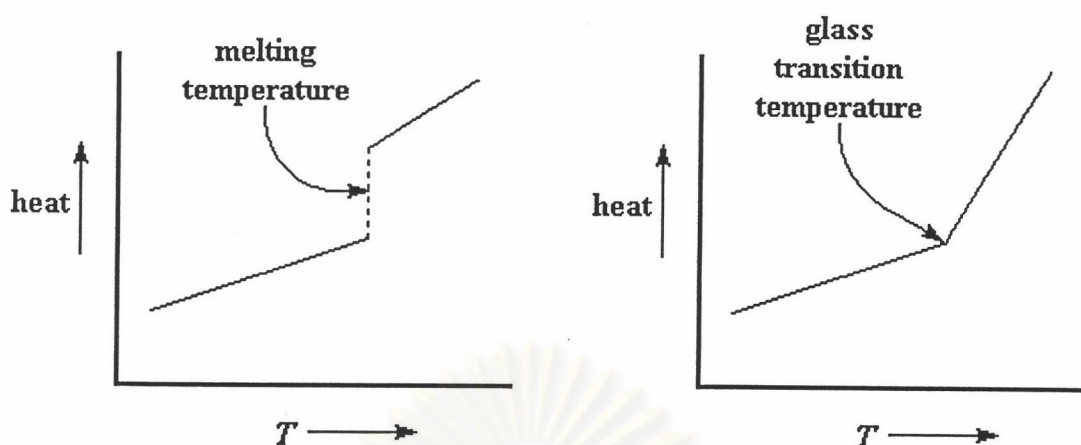
Figure 2.14 Model of spherulite structure [Koch, 2000]

Melting is a transition which occurs in crystalline polymer. Melting temperature is a temperature the polymer falls out of their crystal structures and become a disorder liquid. Most polymers consist of a combination of crystalline and amorphous regions so that they have both glass transition temperature and melting temperature.

There are a lot of different between glass transition and melting. When the 100% crystalline polymer is heated at a constant rate, the temperature will increase at a constant rate. The amount of heat required to raise the temperature of one gram of the polymer one degree Celsius is called the *heat capacity*. When the temperature reaches the polymer melting temperature, the temperature will hold steady until the polymer is completely melt, even though the heat is continue added. The heat that added to melt the polymer, not to increase the temperature at the melting point, is called *the latent heat of melting*. When the 100% amorphous polymer is heated at a constant rate, the temperature increases at a rate determined by the heat capacity of the polymer. When the temperature reaches the T_g of the polymer, it does not increase at the same rate as that of below T_g . The polymer undergoes an increase in its heat capacity when the temperature is beyond its T_g . The plot between heat that is added and the temperature of glass transition and melting process is presented in Figure 2.15.

The left plot demonstrates the heating process of 100% crystalline polymer. The brake in the graph is occurred at the melting temperature. The slope of the graph is the heat capacity. The change in the slope of the graph is attributed to the change in the heat capacity. At the brake, a lot of heat added without any increasing of temperature is called *the latent heat of melting* or the enthalpy of fusion, ΔH_f . This plot is also called a first-order transition.

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A heat vs. temperature plot for a crystalline polymer, on the left; and a amorphous polymer on the right.

Figure 2.15 The plot between heat added and temperature of glass transition and melting process [University of Southern Mississippi, 2000]

The right plot demonstrates the heating process of 100% amorphous polymer. The change in slope which means the increase in heat capacity is observed only. There is no latent heat involved with the glass transition. This plot is also called the second-order transition.

2.5 Differential scanning calorimetry

Differential scanning calorimetry (DSC) is generally used for measuring the melting temperature, glass transition temperature, crystallization temperature, oxidation temperature of polymers. This test measures the difference of the thermal energy absorbed (endothermic) or given off (exothermic) by the sample and the reference substance at a constant rate of heating or cooling in an identical (normally nitrogen) environment. Figure 2.16 illustrates the operating principle of DSC. The sample and the reference substance are placed in the thin aluminium pan, with the thermocouple sensor below the pans. DSC measurement can be made in two ways: by measuring the electrical energy provided from heaters below the pans necessary to maintain two pans

at the same temperature or by measuring the heat flow as a function of sample temperature.

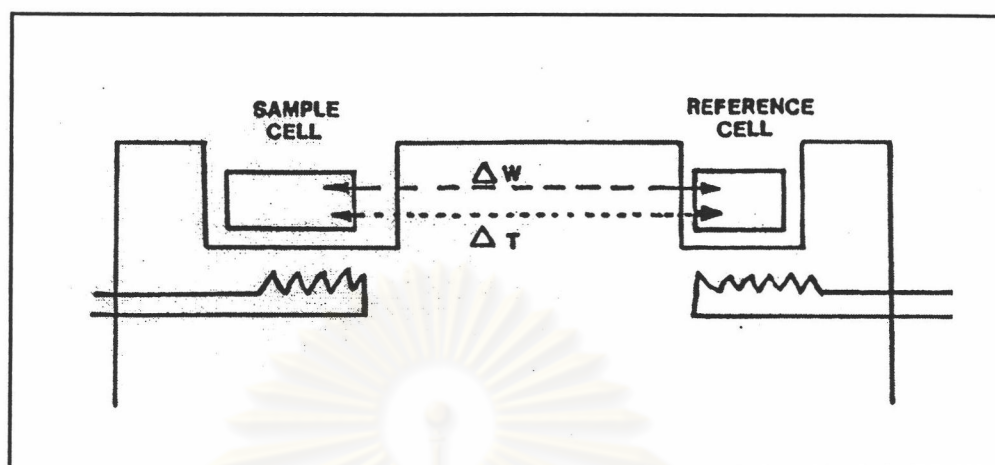


Figure 2.16 Operation principle of DSC; differential energy (dashed line); differential temperature (dotted line) [Cheremisinoff, 1990]

Although DSC is always used in polymer characterization studies, care must be taken to be aware of variable factor that can distort results such as conditions of polymer processing. It is well known that the first DSC heating curve is significantly influenced by the thermal history of the sample that is subjected to the processing. Therefore, The second heating scan must be performed to introduce constant thermal history to the samples.

2.6 Scanning Electron Microscopy (SEM)

Scanning electron microscopy is the method for observing the sample surface by focusing the electron beam to the surface. Non-conductive materials such as polymers have to be coated by thin layer of gold to provide the conductive layer [Woodward, 1995]. Principle of SEM is different from that of light microscopy. In light microscopy, a specimen is viewed through a series of lens that magnify the visible-light image. SEM does not actually view a true image of specimen, but rather produces an electron map of the sample surface that was displayed by cathode ray tube (CRT) [George Mason University, 1998]. Figure 2.17 illustrates the principle of SEM. SEM is

becoming the most popular method of observation of polymer blends. SEM provides more detailed information on the morphology; domain down to a size of 10 nm can be resolved. The great advantages of this technique are: rapidity, great depth of focus, relatively simple image interpretation, and easy sample preparation. But there are limitations which are caused by specimen charging that occurs in the SEM when the subject is non conductive, and by structural damage caused by the high energy electron beam when it impinges on the specimen.

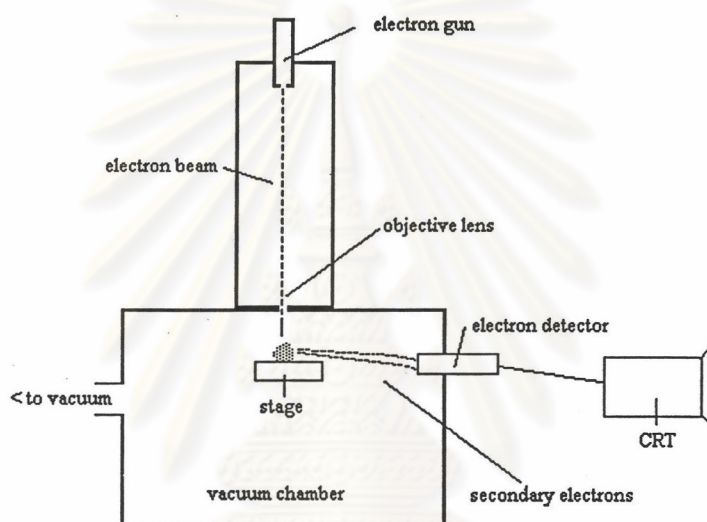


Figure 2.17 Schematic diagram of typical SEM [George Mason University, 1998]