



## CHAPTER II

### THEORITICAL BACKGROUND AND LITERATURE REVIEW

#### 2.1 Air Pollution

Today, air pollution is one of the most important environmental problems, which is made from both natural processes and human activity i.e., industrial activities, power plants, car transport, combustion and gasification process. Air pollution is any chemicals or compounds include:

- Sulfur dioxide ( $\text{SO}_2$ ) is emitted from burning of coal and oil. The primary producers of  $\text{SO}_2$  are power plants and factories.
- Carbon monoxide (CO) is a gas without odor and color but very poisonous, which is produced by incomplete combustion of fuel such as natural gas, coal or wood and vehicular exhaust.
- Carbon dioxide ( $\text{CO}_2$ ) is the primary greenhouse gas released by human activity i.e., burning of fossil fuel.
- Volatile organic compounds (VOCs), such as hydrocarbon fuel vapors and solvents, that are particles of unburned gasoline vapors, paint, glue and other airborne materials.
- Particulate matter (PM) is the general term for a mixture of solid particles and liquid droplets found in the air. PM are usually caused by emissions from cars, trucks, and other vehicles that burn gas.
- Toxic metals, such as lead, cadmium, and copper
- Ammonium ( $\text{NH}_3$ ) emitted from agricultural processes.

Carbon dioxide ( $\text{CO}_2$ ) is one of the major pollutants in the atmosphere, which is toxic to the body and can result in breathing and other health problems (Volzone *et al.*, 2007). The formation of huge volume of  $\text{CO}_2$  which contributes to the creation of the greenhouse effect is presented in the atmosphere like pollutants (Bodzek, 2000). These are creating a gaseous layer that is too dense to allow the heat to escape. Many scientists believe this is causing global warming. Moreover, others

gases contributing to this problem include chlorofluorocarbon (CFC), methane, nitrous oxides, and ozone. To minimize this problem, it is very important to find the way for prevention and controlling air pollution. Design adsorbent materials that would adsorb such gases before being liberated into the environment should be reviewed for reducing air pollution problem.

## 2.2 Adsorption Process and Adsorbent Materials

Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms (the adsorbate). This process is widely used in industrial applications such as activated charcoal, synthetic resins, and water purification e.g. adsorption with charred wood to improve the taste of water and adsorption of gases by a solid charcoal, that was described by C.W. Chele in 1773. Adsorption process may be classified as purification or bulk separation, depending on the concentration in the feed fluid to the component adsorbed. However, based on the nature between the molecule and the surface, adsorption phenomena can be classified as physisorption (characteristic of weak van der Waals forces) and chemisorption (characteristic of covalent bonding). In physical adsorption, the only bonding is by weak van der waals-type forces whereas chemisorption, a chemical bond, involving substantial rearrangement of electron density, is formed between the adsorbate and substrate. The rate of adsorption may be affected by many factors including surface area of the adsorbent, nature of solute (adsorbate) and surface tension fo the solvent. The important classification and characteristics between chemisorption and physisorption is shown in Table 2.1.

Adsorbent materials may be organic compound or inorganic compound, that have some tendency to adsorb fluid medium onto their surface. Adsorbents are used usually in the form of spherical pellets, rods, moldings, or monoliths with hydrodynamic diameters between 0.5 and 10 mm. They must have high abrasion resistance, high thermal stability and small pore diameters, which results in higher exposed surface area and hence high surface capacity for adsorption. The adsorbents must also have a distinct pore structure which enables fast transport of the gaseous

vapors. Most industrial adsorbents fall into one of three classes along with their characteristics, application and disadvantages (see Table 2.2).

**Table 2.1** Typical characteristics of adsorption processes.

<b>Characteristics</b>	<b>Physical Adsorption</b>	<b>Chemical Adsorption</b>
<b>Binding force</b>	Physical force attraction; Van der waal adsorption	Chemical force attraction; Activated Adsorption
<b>Saturation uptake</b>	Multilayer phenomena	Single layer phenomena
<b>Activation Energy</b>	No activation energy involved	May be involved
<b>Temperature range</b>	Adsorption is appreciable at low temperature (below b.p. of adsorbate)	Adsorption can take place even at higher temperature
<b>Nature of sorbate</b>	Amount of adsorbate removed depends more on adsorbate than adsorbent	Depends on both adsorbent and adsorbate
<b>Heat of adsorption</b>	1 kcal/mole	50-100 kcal/mole

Many scientists developed the adsorbent materials for gas adsorption in air treatment process and also gas separations in the chemical industry i.e. by using carbon, zeolites and/or silica as solid adsorbent in gas separation process (Yang *et al.*, 1987; Kapoor *et al.*, 1989; Bae *et al.*, 2004).

The adsorption characteristics on a carbon molecular sieve (CMS) were studied by Bae *et al.*, 2004. They used CMS as an adsorbent in gas separation process in order to produce high purity products. Adsorption equilibrium on carbon molecular sieve was obtained at the wide ranges of temperature and pressure by the volumetric method. The adsorption kinetic characteristics on the CMS were affected by the relative importance of atomic/molecular size, shape, and polar properties. Especially, the interaction properties of adsorbate molecules were proposed as an important factor to estimate the relative adsorption rate.

**Table 2.2** Characteristics of different adsorbents.

Type	Characteristics	Use	Disadvantages
<b>Carbon-based compounds such as activated carbon and graphite</b>	Hydrophobic, favors organics water	Removal of organic pollutants	Difficult to regenerate
<b>Oxygen-containing compounds such as silica gel and zeolites</b>	Hydrophilic, polar, high capacity	Air separation, dehydration	Trace removal not effective
<b>Polymer-based compounds</b>	Polar or non-polar functional groups in a porous polymer matrix.	Air separation	Cannot use at high temperature

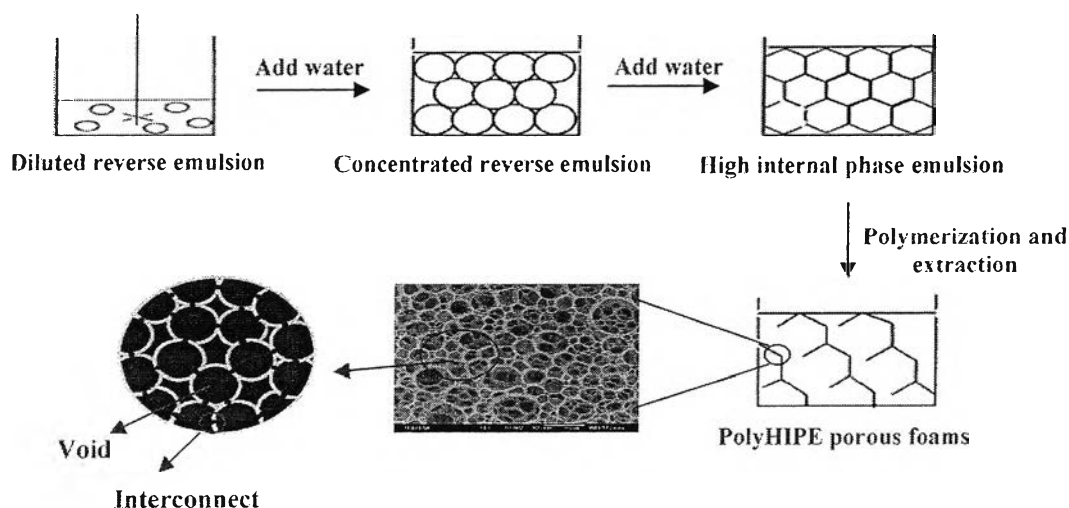
In 2007, Volzone *et al.* reviewed that clay mineral and their modified products i.e., organo-modified clay and acid treated organo-modified clay can be used as solid adsorbents in gas adsorption technology, which help us to remove as much as greenhouse gases emission and pollutants to the atmosphere.

The CO<sub>2</sub> adsorption by X zeolite was investigated by Diaz *et al.* (2008). In this work, they studied the adsorption of CO<sub>2</sub> on alkaline modified X zeolite by using temperature programmed desorption (TPD). Alkaline treatment of X zeolite with using sodium and cesium aqueous solution was prepared and determined

morphological, chemical properties and also adsorption properties. They showed that X-zeolite was suitable material for CO<sub>2</sub> adsorption process at high temperature, which the relative high CO<sub>2</sub> adsorption capacity was observed. This is because of the presence of alkaline species inside of the pore structure of the zeolite. In this work was focused on another materials for using as an adsorbent in gas adsorption for air treatment process. An alternative method for the preparation of highly porous polymer material is polymerization of the continuous phase of a high internal phase emulsion (HIPE), that the obtained materials has an open cellular structure with interconnects pore network.

### 2.3 High Internal Phase Emulsion Polymer (PolyHIPE)

Novel polymeric foams known as polyHIPE (Polymerized High Internal Phase Emulsion) is a new porous polymer produced by polymerization of the continuous phase around the emulsion, leading to highly open porous polymer foam with interconnecting pore networks. The internal phase of the polyHIPE occupies more than 90% of the total volume (Barby *et al.*, 1982; Hainey *et al.*, 1991; Williams *et al.* 1988). Typically, to fabricate polyHIPE porous polymer or polyHIPE foam, the system consists of two phase; the organic phase, generally constitutes less than 26% of the total volume, composes of monomer, suitable surfactant and cross-linking comonomer and the internal phase (aqueous phase  $\geq 90\%$  of total volume), which is usually water, initiator and stabilizer.



**Figure 2.1** Formation of polyHIPE porous polymer foam.

By introducing droplet of aqueous phase in the organic mixture, under mechanical stirring, highly internal phase emulsion is produced. When polymerization of the continuous phase occurred highly porous materials with interconnected and very low density will be formed, ( see Figure 2.1). For polyHIPE porous polymer, styrene (S) with divinylbenzene (DVB) based polyHIPE is the most popular one for produce polyHIPE porous materials. However, many polymer compositions such as methacrylate or acrylic acid are possible such as poly(GMA/EGDMA)polyHIPE and poly(DVB/VBC)polyHIPE (Barbetta *et al.*, 2000; Krajnca *et al.*, 2005). According to their unique properties such as high porosity, high surface area and degree of interconnectivity, polyHIPE porous materials are being considered attractive for many applications such as poly(S/DVB)polyHIPE modified with bioactive molecules are being used as a scaffold for biomedical applications (Akay *et al.*, 2004; Busby *et al.*, 2001; Hayman *et al.*, 2005; Zhang *et al.*, 2005), poly(GMA/EGDMA)polyHIPE are used as a separation media (Krajnca *et al.*, 2005).

In 2004, Akay and co-worker shown that polyHIPE porous materials has been developed as a three-dimensional polymer matrix for in vitro tissue engineering applications. PolyHIPE as porous support has successfully tested the ability to support the growth cells and has good biocompatibility with osteoblasts and solid supports *in vitro* environment. They found that polyHIPE (highly porous) has a potential for used as scaffold for bone tissue engineering application, this is because the osteoblasts cell seeded onto polyHIPE scaffold reveal cellular attachment, proliferation leading to the support of an osteoblastic phenotype.

Additionally, polyHIPE porous materials are used as a catalyst support (Delenze *et al.*, 2002), a filtration media (Bhumgara *et al.*, 1995), and as an ion exchange membrane. In 1998, Wakeman *et al.* was prepared highly porous, low density, open cellular poly(S/DVB)polyHIPE and its surface properties were modified by sulphonation that are being considered as an ion exchange membrane (Wakeman *et al.*, 1998). They found that the effectiveness of polyHIPE porous materials as ion exchange resin was produced with incorporating Na<sup>+</sup> ions onto polyHIPE surface. Moreove, the capacity of polyHIPE increased with the degree of sulphonation.

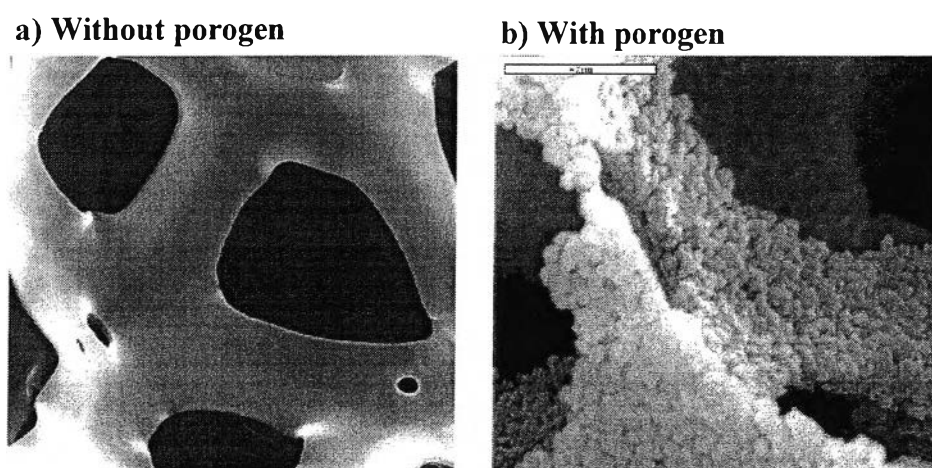
For other applications of polyHIPE porous polymer, it can be used as a superabsorbent materials. Due to the resulting morphology of polyHIPE which exhibit highly permeable structure and small interconnecting pores, it will allow liquid to flow through and rapidly fill in the cavity of materials. The amount solution absorbed by polyHIPE materials is about twice compare with the other commercials product and complete uptake would occur in only a few second (Benson, 2003).

It is possible to optimized and modified the structural and properties of polyHIPE through the regulation of pores sizes, interconnecting holes, surface area and other parameters by controlled the emulsion stability of system. The stability of polyHIPE emulsions is strongly influenced by many factors, a better understanding of these factors will have important impact for selecting type of oils or emulsifiers to obtain suitable system. Until now, there are many researches involve in the ways to increase emulsion stability of polyHIPE foam.

In year 1990, William and co-worker studied the effect of DVB content that influence properties of polyHIPE foam. They found that the number and size of large voids decreased as the level of DVB increased from 0% to 100%. This is because more hydrophobic of DVB would cause the reduction of interfacial tension around emulsion droplet and the enhancement of emulsion stability of the system. The second factor for improving polyHIPE morphology is surfactant concentration. Result from the literature found that slightly higher surfactant levels lead to increase emulsion stability and a small opening in the cell wall, known as interconnected pores, are formed. However, at the surfactant level above 50% of the total organic phase volume, the structure of the resulting materials were not maintained. The polyHIPE foam generated under these conditions would has no walls and the structure of the emulsion would collapse. From this work, they concluded that fine cellular morphology and small interconnected with very smooth surface area were observed at around 20 % surfactant used.

The effect of mixture surfactant on styrene based polyHIPE was also investigated by Cameron *et al.* (2004). Usage of mixture of surfactant lead to reduce interfacial tension of the system and form a more strong interfacial film around each emulsion droplet when compare with the single surfactant system. This caused enhancement in emulsion stability which in turn led to decrease in average void size

and increase the surface properties of the polyHIPE foam. Normally, the surface area of polyHIPE materials is quite low ( 2-30 m<sup>2</sup>/g ). There are methods to increase the surface area of polyHIPE foam by replacing some of monomer with inert porogenic solvent. Porogen is inert porogenic diluents or non-polymerisable solvent such as toluene (T), chlorobenzene (CB), 2-chloroethylbenzene (CEB), and 1-chloro-3-phynyl-propane (CPP). The surface area of the resulting materials might be increased with the reduction of average cell diameters because this solvent was packing with the surfactant at the interface lead to enhance the emulsion stability of polyHIPE system. In addition, porogenic solvent also effect polyHIPE surface properties by generating permanent porosity or secondary pore structure within the cell walls of polyHIPE foam (see Figure 2.2). These situation can be confirmed by TEM micrographs, that presented the direct image of secondary pore structure within the cell walls of polyHIPE foam (Cameron *et al.*, 2000; Barbetta *et al.*, 2004).



**Figure 2.2** Secondary pore structure in polyHIPE foams; a) polyHIPE without porogenic solvent and b) polyHIPE with porogenic solvent.

The nature of the porogen has a strong influence on the surface area, and this is strongly related to the solvent type. Better solvents with good solubility parameter for the growing network give rise to higher surface areas (Cameron *et al.*, 1996). PolyHIPE system with using good porogenic solvent could be produce a large number of small microparticles, which remain discrete until complete conversion



since the residual monomer is low, and results in a material of high surface area. The cell size was reduced as the solvent became a better cosurfactant and this caused the windows connecting adjacent cells to increase. The effect of the different type of porogenic solvent on surface properties of polyHIPE was investigated by Cameron *et al.* (2000). They prepared polyHIPE with using toluene (T), chlorobenzene (CB), 2-chloroethylbenzene (CEB), and 1-chloro-3-phenyl-propane (CPP) as porogenic solvent. The type of porogen added to the continuous phase of HIPE containing divinylbenzene strongly influences the phase morphology and surface area of the resulting polyHIPE foam. The highest surface area was found with using CEB as a solvent due to the the solubility parameter of the solvent approached that of the polymer system, but the structure of porous foam cannot maintain and were very weak mechanically. However, for our study, poly(divinylbenzene; DVB)polyHIPE with various porogenic solvent were prepared and the preliminary result was observed that the suitable solvent for DVB based polyHIPE is toluene, which the highest surface properties was obtained.

On the other hand, this porous materials would exhibit poor mechanical properties, such as brittleness, and low crush strength, therefore their practical use in many industrial applications are being limited. To improve the mechanical performance of polyHIPE foams, addition of filler such as nanosize silica, and carbon black into polymer system were carried out.

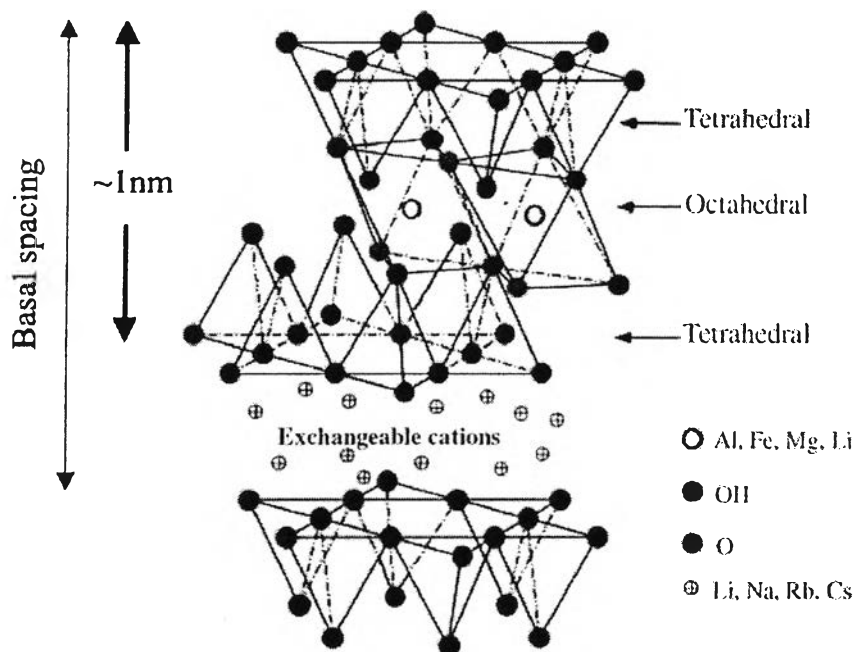
Haibach *et al.* (2006) successfully improved the physical properties of polyHIPE foam using inorganic materials as reinforcement. Highly open porous low-density polymer foam with better mechanical properties was synthesized by using nanosized silica particles as inorganic fillers. Young's modulus of silica reinforced polyHIPE foams was found to increase by 280% and the crush strength by 218% as compared with polyHIPE foam without the reinforcement.

Attempts were also made to increase the density and pore interconnectivity of polyHIPE porous materials by preparing carbon black-filled polyHIPE foam (Menner *et al.*, 2006). The pore interconnectivity was increased by adding *in situ* grafting carbon black into the pore walls of polyHIPE foam; this led to an increase in emulsion stability while its mechanical properties were much improved by increasing the organic phase volume, thus increasing the foam density.

Another inorganic filler which may be suitable for use as a reinforcing phase in polyHIPE foam is clay mineral. Researchers also found that the combination between high surface area and mechanical performance of high internal phase emulsion by using organoclay as inorganic filler would make polymeric materials attractive in many applications such as adsorption technology.

#### **2.4 Clay Mineral and their Modified Products**

Clays are layer silicates (Phyllosilicate) composed of a sheet like structure with a particular stacking sequence which commonly used in polymer nanocomposite. This silicate layered are divided into four main groups, which are illite, smectite, vermiculite and kaolinite. Among these, the one that is found to be useful in the field of polymer composites is a group of expandable clay known as smectite clay. Montmorillonite (MMT) is in the smectite group which is naturally occurring 2:1 phyllosilicate having permanent layer charge because of the isomorphous substitution in either the octahedral sheet or the tetrahedral sheet (see Figure 2.3) and their chemical formula are shown in Table 2.3. The layer thickness is around 1 nm and the lateral dimensions of these layers may vary from 300 Å to several microns or larger depending on the particular clay. The crystal lattice consists of a central octahedral sheet of alumina fused between two external silica tetrahedral sheets and the gap between layers is called interlayer or gallery. The stack of clay layers are held tightly together by weak dipolar and Van der Waals forces. A single layer of aluminum octahedron is sandwiched between two layers of a silicon tetrahedron. Some of the  $\text{Al}^{+3}$  and  $\text{Si}^{+4}$  ions usually are replaced by lower valence ions such as magnesium, iron, manganese and occasionally lithium. This creates a charge imbalance on the surfaces of each layer platelet and the negative charge imbalance is neutralized by adsorption of hydratable cations, most remarkably sodium and calcium between the clay layer platelets. When the hydrated cations are ion-exchanged with organic cations such as more bulky alkylammoniums, it usually results in a larger interlayer spacing.



**Figure 2.3** Structure of Montmorillonite.

**Table 2.3** Chemical formula and characteristic parameter of commonly used 2:1 Phyllosilicates.

2:1 phyllosilicates	Chemical formula	CEC (mequiv/100 g)	Particle length (nm)
Montmorillonite	$M_x(\text{Al}_{4-x}\text{Mg}_x)\text{Si}_8\text{O}_{20}(\text{OH})_4$	110	100–150
Hectorite	$M_x(\text{Mg}_{6-x}\text{Li}_x)\text{Si}_8\text{O}_{20}(\text{OH})_4$	120	200–300
Saponite	$M_x\text{Mg}_6(\text{Si}_{8-x}\text{Al}_x)\text{Si}_8\text{O}_{20}(\text{OH})_4$	86.6	50–60

M: monovalent cation, x: degree of isomorphous substitution (between 0.5 and 1.3).

In the present work, bentonite is a clay mineral used for the preparation of nanocomposites materials. Bentonite is a clay minerals derived from the alteration of volcanic ash, comprising predominantly of montmorillonite. Depending on their sources, bentonite contains a small portion of accessory minerals and metal oxides in addition to montmorillonite. The presence of these compounds can impact the industrial value of bentonite. It has unique water absorbing and swelling

characteristics. These characteristics make bentonite to be a very desirable industrial mineral. By the modification of the clay mineral using an ion exchange reaction with quaternary ammonium cations, the layered silicates in the clay structure would be expanded and the d-spacing of the organo-modified clay increased, which helps in better intercalation and exfoliation (Jin-Ho *et al.*, 1997; Polverejan *et al.*, 2002; Fornes *et al.*, 2004).

Organoclay complexes exhibiting hydrophilic as well as organo-philic natures by using several kinds of quaternary alkylammonium cations, which different molecular structure and functional groups i.e. tearyldimethyl-ammonium chloride (DMDS), methyltriethanoldipalmitylesterammonium methyl-sulfate (DCEM), monostearyltrimethylammonium chloride (ODTM), and methyltriethanolpalmitylesterammonium methylsulfate (DHMC), were prepared by Jin-Ho *et al.* (1997). The intercalation was observed and the basal spacing of organoclay expanded up to 66 Å, indicating the incorporation of large surfactant molecules.

The use of nanometer-sized clay particles as the reinforcement phase with finely dispersed in polymer matrices leading to provide enhancements in stiffness, toughness, tensile strength, thermal stability, gas barrier properties and also adsorption capacity. Polymer nanocomposites with nanoclay as a filler results in a significant improvement in mechanical strength, caused by the nanometric dimensions and high aspect ratio as a report by Jo *et al.* (2007), which studied the effect of nanaclay loading on the foam morphology and mechanical properties of microcellular HDPE-Clay nanocomposite foams. They found that nanoclay can play an important function as inorganic reinforcement leading to improve mechanical properties such as elastic modulus and tensile strength at yield.

Polypropylene/layered silicate nanocomposites were prepared from base polymer (10 MFI) and octadecylamine modified montmorillonite (1.30P nanomer), melt compounded with and without maleic anhydride grafted polypropylene as a compatibilizer (Parija *et al.*, 2004). They studied on physico-mechanical properties of the virgin PP and nanocomposites with different nanomer loadings. Thermal characteristics of polypropylene/ layered silicate nanocomposites and dispersion of nanomer were also carried out. The experimental findings revealed a consistent improvement in the mechanical properties of the polypropylene/ layered silicate

nanocomposite for a relatively lower nanomer loading (3%) due to the reinforcing and toughening effect of nanoparticles. Addition of compatibilizer further enhanced the mechanical properties of nanocomposites because of the compatibilization of the clay and host polymer interface.

In 2005, Ding *et al.* prepared organophilic clay by using cetyl trimethyl ammonium bromide (CTAB) as a surfactant. PP/clay nanocomposites were prepared by using twin screw extruder and the content of Organo- Montmorillonite (OMMT) is relative to 100 phr polymeric matrix. The maximum improvement of the mechanical properties of nanocomposites i.e. tensile strength, flexural strength and impact strength was observed at an Organo- Montmorillonite (OMMT) content of 2 phr. The significantly increased mechanical properties at low OMMT loading may be due to the uniformly dispersed MMT tactoid with intercalated structures. At higher OMMT content, aggregation of the organoclay may take place. Thus, the mechanical properties of the nanocomposite with higher OMMT content were found to decrease.

The Study of porous clay heterostructures (PCHs) and hybrid organic–inorganic PCHs (HPCHs) were also carried out. Porous clay heterostructure (PCH) is a recent class of solid porous materials formed by the intercalation of surfactant within the intragalleries of clays.

In 1995, Galarneau *et al.* discovered a new porous material called a porous clay heterostructure (PCH) which had uniform pore diameters in the supermicropore to small mesopore range (1.5–3.0 nm), with high specific surface areas and high thermal stability. Due to the capability of PCH that form mesoporosity with specific and uniform pore diameter, the adsorption properties of porous clay materials have demonstrated in many researchs (Pires *et al.*, 2004, Pinto *et al.*, 2005).

In year 2004, Pires *et al.* prepared porous clay materials as adsorbent by the gallery templated approach, using a quaternary ammonium cation (CTAB) and natural amine with different chain length. They reported that this porous materials have a possibility to use as adsorbents of volatile organic compound (VOC) because of their textural and hydrophobic characteristic, which the BET surface areas of this materials were in the range 600-700 m<sup>2</sup>g<sup>-1</sup>, was studied by the adsorption of ethane and methyl ethyl ketone and water.

Additionally, in order to improve the chemical properties and hydrophobicity on the porous structure, hybrid organic–inorganic porous clay heterostructures (HPCHs) have been successfully prepared by incorporating organic components via the co-condensation reaction. Organic modification of the mesoporous silica permits precise control over the surface properties and pore size of the mesoporous materials for specific applications (Burkett *et al.*, 1996).

In 2004, Wei *et al.* synthesized a new ordered organic-inorganic hybrid porous clay heterostructure by incorporated phenyl groups into the porous clay heterostructures within the galleries of montmorillonite via co-condensation reaction of tetraethoxysilane and phenylethoxysilane in the presence of surfactant templates (dodecylamine and cetyltrimethylammonium ion) known as “one-pot synthesis”. The surfactant templates were removed from the pores by solvent-extraction. The BET surface area and pore volume of these porous materials are 390-771 m<sup>2</sup>g<sup>-1</sup>, pore volumes of 0.3-0.59 cm<sup>3</sup>g<sup>-1</sup>, respectively and the framework pore sizes in the supermicropore to small mesopore range (1.2-2.6 nm). After modification HPCH exhibit hydrophobic properties that possibly to use this porous materials in catalysis, adsorption and separation chemistry. Since, the high performance of hybrid organic–inorganic porous clay heterostructure, this silicate materials can be applied in to the polymer system as reinforcing phase which they have the ability to impart the combination between tremendous surface area, high adsorption capacity and excellent stress transfer to the reinforcement phase and also improve the compatibility between matrix and reinforcing phase, leading to improvement in the physical properties of the resulting materials.

Moreover, clay mineral would not only provide reinforcement but also adding new properties; i.e. the incorporation of layered silicate in the polymer matrix should lead to improving the surface properties and thermal stability, of the obtained materials (Pakeyangkoon *et al.*, 2009). There is information from many works, which indicated that clay mineral and their modified products i.e. porous clay, organo-modified clay, and acid treated clay are recognized as potential adsorbents. This is because of the ability itself of clay mineral that can exchange interlayer cations for charged organic cations in solution. In addition, they also reported that the N<sub>2</sub> and CO<sub>2</sub> adsorption of organo-montmorillonite increased after treatment with organic

cations. Researchers attempted to increase adsorptive capacity of clay mineral with using acid treatment. Acid treatment of clay minerals (by using mainly HCl or H<sub>2</sub>SO<sub>4</sub> solutions) is chemical treatment of the clays to modify structural, textural and/or acidic properties, which influence the resulting properties of raw material such as acidity, surface area and adsorption capacity as well (Volzone *et al.*, 2000; Venaruzzo *et al.*, 2002; Mills *et al.*, 1950). The octahedral ions are leached out, resulting in improving the surface area and gases adsorption capacity of the clay after acid treatment.

Volzone and co-worker has been prepared acid smectites by using 5.0 N sulphuric acid solution. The structural and chemical composition of smectites after acid treatment were changed. The improvement of the gas adsorption by smectites after acid treatment could be attributed to structural and textural properties. Moreover, the different retentions of gases i.e. O<sub>2</sub>, CH<sub>4</sub>, and CO<sub>2</sub> by acid smectites was also observed. These could be indicated that this materials will be a potential for use as an adsorbent in gas separation process (Volzone *et al.*, 2000).

In 2002, modified bentonite clay mineral was used as an adsorbents of CO, CO<sub>2</sub> and SO<sub>2</sub> gases, which reported by Venaruzzo *et al.* Bentonite clay minerals were modified with 3.0 and 6.0 N HCl solution at boiling temperature during 3 h. The all samples were determined the surface properties, chemical composition and also gas adsorption using BET, XRD, FT-IR and equilibrium adsorption, respectively. After acid treatment, the structural and textural properties of acid-treated bentonite were changed. The increasing of BET surface, micropore volume and also the Si/(Al+Fe+Mg+Ti) ratio of the bentonite clay after acid treatment lead to obtained the high gas retention of the CO<sub>2</sub> and SO<sub>2</sub>. Furthermore, they also found that the retention values of the SO<sub>2</sub> (0.636-1.232 mmole/g) were higher than that CO<sub>2</sub> (0.218-0.516 mmole/g) and CO (0.075-0.119 mmole/g).

Then the first aim of this work was to enhance surface area, mechanical properties and also CO<sub>2</sub> adsorption capacity of polyHIPE foam by using three types of organoclay as inorganic filler for poly(DVB)polyHIPE porous foam, including hybrid organic-inorganic porous clay heterostructure (HPCH) derived from organo-bentonite which prepared through surfactant-directed assembly of tetraethoxysilane (TEOS)/methyltetraethoxysilane (MTS) into galleries of clay mineral, organo-

modified bentonite (MOD) treated with quaternary alkyl ammonium cation by ion exchange reaction, and acid treated organo-modified bentonite (AC-MOD), which treated with acid solution then treated with quaternary alkyl ammonium cation by ion exchange reaction. In order to study the effect of type of organoclay on overall properties of poly(DVB)polyHIPE porous foam.

## **2.5 Scaffold and Tissue Engineering**

### 2.5.1 Tissue Engineering

Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to the development of biological substitutes that restore, maintain, improve or replace biological function. Scientific advances in biomaterials, stem cells, growth and differentiation factors, and biomimetic environments have created unique opportunities to fabricate tissue in the laboratory from combinations of engineered extracellular matrices (scaffold), cells, and biologically active molecules.

### 2.5.2 Scaffold Materials

Scaffold materials play an important role in tissue engineering by serving as matrices for cellular ingrowth, proliferation, and new tissue formation in three-dimensional (3D). Generally, scaffold materials serve at least one of the following purposes: First, it should be allow cell attachment and proliferation. Second, it can deliver and retain cells and biochemical factors. Third, it can enable diffusion of vital cell nutrients and expressed products. Fourth, it preserve apply certain mechanical and biological influence to modified the behaviour of the cell phase. Both natural and synthetic materials have been investigated for tissue engineering application. For natural materials, proteic materials, such as collagen or fibrin, and polysaccharide materials, have all proved suitable in terms of cell compatibility. The commonly used synthetic materials are polylactic acid (PLA), polyglycolic acid (PGA) and polycaprolactone (PCL) and their copolymer. All of them is polyester which degrades within the human body, a naturally occurring chemical which is easily removed from the body. In addition, scaffold materials can



be either permanent and temporary in nature, depending on the application and the function of the neo-tissue. Moreover, an ideal scaffold in order to achieve the goal of tissue reconstruction, it must meet some specific requirements as follow:

1. To facilitate cell attachment, seeding and diffusion throughout the whole structure, this requires that the shape of scaffold should be three-dimensional (3D) with a high porosity with a high surface area to volume ratio and an adequate pore size, which enable growth of cells and tissues into scaffold and permit transport of nutrients and oxygen (Edwards *et al.*, 2004).

2. Biodegradability is often an essential factor since scaffolds should preferably be absorbed by the surrounding tissue without the necessity of a surgical removal.

3. Scaffold materials acquire mechanical properties similar to those of the tissue in which it is implanted so that it can support local stresses until new tissue develops. In addition, the mechanical properties of the scaffold must be sufficient in order to prevent the collapse during the patient's normal activities.

4. The appropriate surface chemistry that can promote attachment, seeding, proliferation and differentiation of cells.

Several processing techniques have been developed over the past two decades to obtain the highly porous suitable scaffold materials for the many different applications of tissue engineering and cell types. The method to fabricate scaffold materials for tissue engineering have been described in literature review as follow:

- Nanofiber self-assembly; Molecular self assembly is one of the few methods to create biomaterials with properties similar in scale and chemistry to that of the natural *in vivo* extracellular matrix (Mikos *et al.*, 1993a).

- Textile technology; non-woven meshes can be prepared by textile technology. Fibrous structure have been found useful to grow different type of cells. However, structure of scaffold from these technique often only have little structural stability and low mechanical properties (Walther *et al.*, 2007).

- Solvent casting and particulate leaching (SCPL); This technique is widely used to fabricate porous scaffold with regular porosity, but with a limited thickness, which involves the use of suitable particulate porogens to form

sponge/foam-like scaffold. The size and the shape of pore structure obtained from these technique depended on type of porogen chosen. With these procedure, organic solvents are used, that may be toxic to the living cell and also the leaching step significantly increases the fabrication time (Mikos *et al.*, 1993b; Mikos *et al.*, 1994).

- Gas foaming; Thses technique use to prepare the porous structure by using gas as a porogen, resulting in a sponge like structure. However, with using this technique, the pores do not form an interconnected structure(Mooney *et al.*, 1996a).

- Emulsification/Freeze-drying; This method is divided into two step. First, formation of an emulsion and then fozen emulsion by immersion into liquid nitrogen. Porous structure obtained from this technique is relatively small and porosity is often irregular (Whang *et al.*, 1995).

- The phase separation technique; This tecnique is based on a thermodynamic demixing of a homogeneous polymer-solvent solution into a polymer-rich phase and a polymer-poor phase. The polymer-poor phase is removd, leaving a highly porous polymer network (Lo *et al.*, 1995; Whang *et al.*, 1995).

- High Internal Phase Emulsion (HIPE technique); by polymerized the continuous phase of a high internal phase emulsion, highly porous materials as called polyHIPE polymer are obtained. PolyHIPE polymers, contain extremely large cavities that are interconnected. Cavities are of micrometer dimensions, rather than angstrom dimensions of conventional polymers. Total pore volume can exceed 90%.

Highly open, porous polymeric foam, called polyHIPE foam, produced from a high internal phase emulsion (HIPE) technique is one of the most widely used synthetic materials in this application (Akay *et al.*, 2004; Hayman *et al.*, 2005). PolyHIPE porous foam has been developed as a three-dimensional polymer matrix for *in vitro* tissue engineering applications.

Akay *et al.* (2004) studied the ability of polyHIPE polymer for using as a scaffold in tissue engineering application. As a porous support, poly(S/DVB)polyHIPE foam has successfully been tested for its ability to support the growth of cells, and it has good biocompatibility with osteoblasts and solid

supports in the *in vitro* environment. PolyHIPE foam has the potential to be used as a scaffold for bone tissue engineering applications because the osteoblast cells seeded onto a polyHIPE scaffold reveal cellular attachment and proliferation, leading to the support of an osteoblastic phenotype.

PolyHIPE porous foam produced from biomaterial has been studied by Busby *et al.* (2001) and Christenson *et al.* (2007). Busby and co-worker prepared highly porous polyHIPE foam containing poly( $\epsilon$ -caprolactam; PCL) by the free radical polymerization of a PCL macro monomer. PolyHIPE porous foam containing the PCL macromonomer can act as a substrate for the growth of human fibroblast. These materials are sufficiently biocompatible to support cell function and growth over a period of 2.5 days.

In 2007, Christenson *et al.* reported that HIPE technique can be used to generate highly porous biodegradable scaffold with interconnected pores for tissue engineering application. Highly porous, fully biodegradable scaffolds were successfully prepared by HIPE technique containing the macromer poly(propylene fumarate) (PPF) and the cross-linker propylene fumarate diacrylate (PFDA). However, polyHIPE foam from HIPE technique usually produces from synthetic materials such as polystyrene, which is hydrophobic in nature and lack of cell recognition signals. This is improper for supporting the cell–scaffold interaction, and poor adhesion between cell and substrate is obtained since an aqueous media has usually been used in cell culturing. Therefore, the investigation of scaffold materials with desirable surface chemistry properties was concerned in order to improve cell adhesion, support cell growth, proliferation and also differentiation. There are several methods have been developed in order to enhance the biological response to tissue fluids, hydrophilic groups and wetting characteristics, including physical, mechanical treatment, conventional coating processes (i.e. spraying or dipping), vacuum deposition technique, wet chemical treatments (i.e. plating, grafting or bonding). However, all of these methods are always involved in many organic reagents lead to reduce biocompatibility between scaffold and living cell. One interest is in plasma surface modification, that is an effective and widely used for modifying the substrate's surface without changing its bulk properties. Through plasma surface modification, biocompatibility as well as biofunctionality can be achieved. Additionally, these

technique also provides device manufactures with a flexible, safe and environmentally friendly process. The principles and utilization of plasma phenomena have been recognized for at least a decade and have been published elsewhere (Fridman *et al.*, 2004).

## 2.6 Plasma Surface Modification

Plasma can be defined as a partially or wholly ionized gas with a roughly equal number of positively and negatively charged particles. Sometime, plasma is often referred to the fourth state of matter, can be divided into three distinct types: First, ablation, or removal of materials from a surface; second, alteration, or chemical modification of a surface by activation or grafting of specific functional groups; and third, accretion, or addition of a new chemical layer (for example, a plasma-deposited film) to a surface. However, some scientists have classified plasma technique into two type including high temperature and low temperature. For high temperature plasma, it can be artificially generated using a high voltage, high temperature arc, which is the basis for the corona discharge process whereas low temperature plasma is ionized gases generated at pressures between 0.1 and 2 torr. In the plasma surface modification process, a glow discharge plasma is created by evacuating a reaction chamber and then refilling it with a low-pressure gas. When the energy is applied to the process gas molecules, the energentic species produced by the inelstic collisions of electrons and gas molecules are a mixture of electrons, free radicals, and highly excited ions consisting of fragmented portions of the parent molecules. Surfaces in contact with the gas plasma are bombarded by these energetic species and their energy is transferred from the plasma to the solid. The energetic states of plasma particles cause them to be highly reactive with the surfaces that they contact to result in a unique type of surface modification that reacts with surfaces without altering the bulk properties of the material (Fridman *et al.*, 2004; Tasanatanachai *et al.*, 2008). For polymeric biomaterial treatment, their energy can crack the organic molecules, consequently creating radicals on the surface. There are many work reported that plasma processes have been developed to obtain a varity of specific surface propertie such as surface cleaning, adhesion promotion, controlling

surface energies, that can be created hydrophobic or hydrophilic surface through interaction with a gas plasma lead to improve biocompatibility for medical application.

In 2005 Ryu *et al.* reported that surface modification of poly(D,L-lactid-co-glycolic acid)—PLGA—with TiO<sub>2</sub>, using the plasma technique has been applied in order to improve substrate/cell interaction. It was found that cell affinity and proliferation increased. This could be due to the improvement of hydrophilicity of the substrate's surface by plasma surface modification (Ryu *et al.*, 2005).

Electrospun polystyrene scaffolds were fabricated and biological studies was reported by Baker *et al.* (2006). In addition, argon plasma treatment was applied in order to improve the wettability of electrospun scaffold. They found that modifying the surface chemistry and bulk architecture resulting in significantly increased wettability and interaction between living cell and substrate. Then the increasing in the cell attachmet was also observed.

## 2.7 MTT assay

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma Aldrich, USA) assay is a quantitative method and standard colorimetric assay (an assay which measures changes in colour) for the measurement of cell viability and growth. The reduction of yellow tetrazolium salt in metabolically active cells to form an insoluble purple formazan crystal product by the dehydrogenase enzymes secreted from the mitochondria of viable cells. This assay can also be used to determine the cytotoxicity of potential medicinal agents and other toxic materials. Firstly, the cell-contained polyHIPE foam was washed two times with PBS to remove any unattached cells, and then a 300 µl MTT stock solution (5 mg/mL in medium without phenol red) was added to each well and incubated at 37°C for 30 min. After incubation of the cells with the MTT solution, a buffer solution containing dimethylsulfoxide (DMSO; 900 µl /well) and glycine buffer (100 µl/well) was placed in each well in order to extract the purple formazan crystal and determine their amount by using a UV visible spectrophotometer at a wavelength of 570 nm.