### **CHAPTER 2**

#### 2.0 LITERATURE REVIEW

### 2.6 Thienamycin and Synthesis of Imipenem Antibiotic.

Many of the antibiotics used today for medical, veterinary and agricultural are produced by a group of bacteria called *Streptomyces*. *Streptomycetes* was discovered about 450 million years ago as branched filamentous organisms that could brake-down plant parts and absorb nutrients available inside them. They belong to a class of bacteria of considerable interest to human welfare that were known as *Actinomycetes* (Chater., 2006). *Streptomycetes* covers nearly two-thirds of all naturally occurring antibiotics in clinical use, for examples Thienamycin for bacterial infections. Thienamycin was one of the synthetic antibiotics that had carbapenem nucleus.



Figure 2.1: Molecular Structures of Penicillin G (right) and Thienamycin (left). (Adapted from Hakimelahi *et al.*, 2005).

Carbapenem means that the antibiotics contain 4:5 fused ring lactam of penicillin but the carbon had been replaced by sulphur and this result to a new formation of an unsaturated carbon bond at the five-member ring. This unsaturated formation becomes an enamine system similar to cephalosporin (Jerome *et al.*, 1985).

Thienamycin was the first carbapenemisolated. It was produced by Streptomyces cattleya which also synthesizes N-acetyl thienamycin and cephamycin C and penicillin -N. Besides that, Streptomyces penemifaciens was also known to produce Thienamycin (Nunez et al., 2003). Thienamycin shows its mode of action similar to classic betalactams antibiotics by inhibiting transpeptidases, an important enzyme for peptidoglycan synthesis. Thienamycin is necessary for bacterial cell wall formation and repair. As a result, it causes death to the microorganism because new wall cannot be produced. Compare to other beta-lactams antibiotics, thienamycin reacts with the cell in a different way. As an example, when Escherichia coli were treated with ampicillin, its long filaments were induced followed by the formation of blebs and the cells slowly start to lysis. However, in the case of thienamycin mode of actions, the cells immediately changed to lemon shapes and start to lysis quickly without producing any filaments. This was the main different between thienamycin and other beta-lactams agent in terms of its interaction with penicillin-binding proteins. With this advantage, thienamycin had high an unusually broad spectrum of bacteria including Pseudomonas (Jerome et al., 1985).

In the further development of thienamycin antibiotic, more problems have arisen. Thienamycin is not stable in solid state and in concentrated solutions due to the formation of inactive dimmer. This is caused by the interaction between the amine side chain and the second molecules of beta-lactam .Therefore, when the concentrations of the thienamycin increases, it will become more unstable (Kahan *et al.*, 1979). To overcome this instability profile, rapid chemical derivatizations processed have been done. A lot of amidine derivates had been generated. It ends up with the finding of most suitable one, called crystalline N-formimidoyl thienamycin . As a result, the thienamycin become more and more stable with an adequate solid state. Since there were changes in its chemical properties and structure, the scientists had decided to give

a new name for this antibiotic. This new member in this family was called Imipenem (Jerome *et al.*, 1985).

# 2.2 Tienam® Antibiotic.

Imipenem is one of the Carbapenem antibiotics that have the widest antibacterial spectrum and because of that property there are plenty of products that have been produced. One of the famous products available in the market is called Tienam®.





Figure 2.2: Molecular Structure of Imipenem (top) and Cilastatin (bottom) (Adapted from Myers C.M *et al.*, 1984).

Tienam® is manufactured by Merck and Company Industries. It is an antibiotic supplied as intravenous infusion. There are two major components inside the Tienam, non-antibiotic known as Cilastatin sodium and the antibiotic component which is Imipenem. The Cilastatin sodium is a specific enzyme inhibitor which couple together with Imipenem in order to prevent the antibiotic being metabolised in the kidney.

Imipenem is well known as a bactericidal against a broad spectrum of pathogens, gram positive and gram negative, anaerobic and aerobic. Therefore, it is much easier to look at the bacterial organisms that resistant to Imipenem rather than look at the un-resistant one. Species of bacterial resistant to Imipenem include intracellular organisms such as rickettsiae, mycoplasma, ureaplasma, chlamydiae, *Stenotrophomonas maltophilia*, and *Enteroccus faecium*. Other species include many strains of methicillin-resistant staphylococci and some strains of *Pseudomonas cepacia*, of flavobacteria and of anaerobic corynebacteria known to be resistant to imipenem (Toni., 1999). Although imipenem resistant to the mention species above, it is still having a wide activity against a lot of pathogenic bacteria that cause disease in children and adults.

Studies have shown Imipenem is active against gram-negative respiratory pathogens, such as  $\beta$ -lactamase-negative *Haemophilus influenza* and *Moraxella catarrhalis*, as well as active against *Citrobactor, Proteus, Serratia* and *Pseudomonas*. Furthermore, studies have reported that the Imipenem is active against gram-positive organisms including *Listeria monocytogenes, Enterrococcus faecalis, Streptococcus pyogenes, Staphylococcus aureus* and *Streptococcus pneumoniae* (Toni., 1999).

In recent years, there is a study on the *Acinetobacter sp.* ongoing by the researcher in Malaysia. *Acinetobacter* is one of the genuses in bacteria that present in natural environment and have causes an outbreak of nosocomial infections, causing pneumonia, urinary tract infections, bacteraemia, wound infections and meningitis (Misbah *et al.*, 2004). These bacteria can easily spread by cross-contamination of hospital equipment and hands. It can grow on dry surfaces and all over the hospital environments. At the same time it can become more resistance to many antimicrobial

agents (Misbah *et al.*, 2004). Usually, the antibiotics use for the treatment of *Acinetobacter* infection is Carbapenems.

Nowadays, it is difficult to get sources of Carbarpenems and this has lead to the use of Imipenem on treating bacteraemia and ventilator-associated pneumonia infection which cause by *Acinetobacter*. As a result, Tienam have been used in order to get direct sources of Imipenem antibiotics.

There has been a growing interest in the different way of treating Acinetobacter.

Previous study has indicated (data not shown here) that the best time to treat *Acinetobacter* is when the bacteria start to enter log phase during its grown and incubation on culture and this has lead to more than 2 hours. After 2 hours of incubations, *Acinetobacter* can be easily been treated at a maximum concentration of Tienam. As addition to this, maximum concentration of Tienam® requires to treat *Acinetobacter* is  $64\mu$ g/ml but 2 hours is such a long period. Therefore, this problem has brought up on the need to come out with a new strategy that can help to delay releasing the Tienam® antibiotics from the beginning of the treatment processes. One of the best solutions is to microencapsulate the Tienam® antibiotics within a second material. A quick survey has been done and we have come out with solutions that try to microencapsulate imipenem in polyethylene glycol.

### 2.3 The Science of Microencapsulation.

History has reported that microencapsulation technology begin since 1954 with its first product carbonless copy paper (Wilson *et al.*, 2007). It is a technology that helps to pack very small material and active ingredient of solid, liquid or gas in another material that becomes a shield protection from the surrounding environment. The product of it can be simply call capsules. These capsules have a size range from 5-300 micron in diameter. It has a unique feature where it can have irregular shape containing small particles in a matrix of shell material or the individual's core material being surrounded by a shell material. As a result, the hydrophobic core will be protected by the hydrophilic shell or the hydrophilic material is protected by a hydrophobic shell where the shell can be one or more materials (Wilson *et al.*, 2007). Thus, these capsules will release its inside material at a later time depending on it use and applications.

Furthermore, studies have reported that there are four main processes where the capsules release their inner contents. The processes can be dissolution of the wall, melting of the wall, diffusion through the wall and breaking of the capsule wall. In addition to that sometimes the capsule may go through biodegradation and slow erosion of the wall process in order to release their trapping materials (*Franjione et al., 1995*).

Today, microencapsulation is important in the pharmaceutical industry especially when treatment using slow release drug is required. One of the best examples for this is Aspirin. It has been given to people who suffered fever, arthritis and inflammation. However, this becomes dangerous because direct doses of aspirin can lead to peptic ulcers and bleeding. That is why microencapsulation comes into place. With microencapsulation technology, aspirin have been encapsulated in ethyl cellulose or hydroxypropyl methylcellulose and starch.Furthermore, these microcapsules will be compress and convert it into aspirin tablets. With this microencapsulation and drug formulation, aspirin will be released slowly through the shell rather than being release all at one time (*Franjione et al., 1995*).

The successful of microencapsulation process highly depends on the choice of material being use. Polyethylene glycol (PEG) is one of the regular use shell material in microencapsulation process. PEG is produced in the factory by polymerization of ethylene oxide with either diethylene glycol or mono ethylene glycol, under alkaline catalysis. The production process is stopped by neutralizing the catalyst

with acid after reaching certain molecular weight that required. This is done by monitoring and measuring the viscosity value of the PEG. In addition to the neutralizing process, acetic acid and lactic acid are the two main regularly used acids. As a result, a very simple chemical with a general formula H (OCH<sub>2</sub>CH<sub>2</sub>) <sub>N</sub>OH, have been produced. Where *n* is the average number of repeating an oxyethylene group from 4 to 180(Henning., 1979).

In other words, PEG means a repeating of chemical compound which consist few units of ethylene glycol. Today, commonly available PEG is call purified PEG and it contains a mixture of different oligomer sizes in high or low molecular weight (MW) ranges. As an example, PEG 8000 means a product resulting from the mixture of oligomers having an average MW of 8000 g/mol. Thus, PEG 600 has a low mixture of oligomers having an average MW of 600 g/mol. Studies have shown that PEG has several chemical properties that make it very useful. PEG has very low toxicity, highly soluble in aqueous solutions and extremely low immunogenicity and antigenicity (Samuel Z., 1995). Generally, all PEGs are soluble in water which makes them suitable for use in a lot of applications. Even 50% (w/w) of a PEG 35000 can be dissolved easily in water at room temperature. PEGs are also soluble in many aqueous solutions of various salts as well as soluble in hard water.

Apart from its solubility profile, PEGs can precipitates when react with a chemical such as cresol, salicylic acid, tannin, potassium iodide and phenol. This property will be useful for quantitatively analysis or qualitative detection of polyethylene glycols. Studies have shown polyethylene glycols have an outstanding toxicological safety regarding embryo toxicity, skin compatibility or acute and chronic oral toxicity. Due to these properties, PEGs have been widely used in foodstuffs, pharmaceutical industry and cosmetics. It is also registered in all relevant

pharmacopoeias. In addition to this, PEG has an acceptable daily intake (ADI-value) as maximum 10 mg/kg body weight that have been defined by the World Health Organization (WHO) (Henning., 2002).

In recent years, solid PEGs have been widely use as a suppository base. This is due to a lot of active ingredients that can be dissolved in PEGs and it becomes a good bioavailability product. With the help of PEG, the disintegration of the active ingredient can occur by melting within the body or by dissolving in the body fluids. At the same time by using PEG base, it shows some good stability products, easy to remove from mould and does not need refrigeration during storage. Furthermore, different solidity can be set by choosing the molecular weight and suitable ratio. In this case, 25% PEG 4000 and 75% PEG 6000 will lead to the production of more solids products. Whereas, 25% PEG 1000 and 75% PEG 1500 will produce more soft products. Whereas, in tablet productions, it requires numerous excipients with different functions, a lot of it can be covered by using the PEGs substance. PEGs can be used as carriers, solubilises and it can be used to improve the absorptions of active ingredients. Furthermore, PEGs can be used as binders and lubricants during the tablet productions. With its plasticizing property, PEGs will help on reconstructing the shaping of tablet mass during the compression process, may help on creating a 'cap' for the tablet and will be used in tablet coatings (Henning., 2002).

To date, PEG have a lot of usages where people make it as contact lens fluids, as foam stabilisers, as plasticisers in paint films, as adhesives and as solubilising agents for drugs. In addition, PEG have been used as matrices for fast release dosage forms compared to normal dosage form where it help to increase the dissolution rate of the non-soluble drug after this drug is incorporated within the PEG. Solid PEGs are useful bases for suppository masses. Many types of active ingredients can be mixed into solid PEGs and have then a good bioavailability. The disintegration of the active ingredient occurred not only by melting within the body but also disintegrate in body fluids. During the production process, suppositories made from PEGs show high stability, easy release from mould and no refrigeration is needed during storage. Nowadays, PEG available in the market are in liquid form at room temperature (PEG 200-600), in semisolids (PEG 1500), in semi crystalline solids (PEG 30000-20 000) and in solid form (PEG 30 000 and above) (Craig., 1995).

Sometimes, PEG have been widely used as a type of organic phase change materials (PCMs) which PEG become a thermal energy storage material because it has a better resistance to corrosion, suitable melting point, congruent melting behaviour and most important PEG have relatively high latent heat equal or more than 187 J/g. Moreover, PEG is a type of materials with different molecular weights with different melting temperatures. Therefore, PEG can be used in many applications such as industrial heat utilization, electronic device management and protection as well as in active and passive heating or cooling of building (Wang *et al., 2009*).

# 2.4 Freeze Drying Technology.

Freeze drying has been used in a lot of applications and processes for quite sometime especially in pharmaceutical and food industries. The freeze drying technology has been extended so it can be used even in preservation of whole animal specimens for museum display and restoration of books damaged by water.

Freeze drying or in other word call lyophilisation is a process that removes water from a substance. This dehydration process is performed under vacuum while the substance is in a frozen state. Lyophilisation technology is used to freeze-dry products such as biological, bacterial cultures, analytical chemistry moieties, and therapeutic molecules (antibodies, vaccines, drugs, and heat-sensitive proteins). Lyophilizing such products, particularly liquid formulations, vastly increases their shelf-life and stability (Kamatha., 2006).

Freeze drying technique is often used in pharmaceuticals industries to make a more stable product and prolong storage time especially in protein drugs.

Freeze-dried formulations also have an advantage to provide an easy handling, shipping and storage of a variety of products. Freeze drying could take days or even weeks to finish if the freeze-drying cycle is not properly optimized. We can consider it as a time and energy intensive process. The duration of the cycle is one of the factors needed to be considered for freeze-drying process optimization. A shorter freeze-drying process or cycle has an advantage of producing a much stable product. Therefore, some factories may use fewer small freeze dryers to produce a quality and stable products rather than use one big freeze dryer for a given amount of sample. A non optimum freeze-drying process may compromise drug stability, take longer, and cost more than it is necessary (Tang *et al.*, 2004).

In other words, freeze drying helps to remove a solvent from a frozen material or frozen solution by desorption of the sorbet solvent, under reduced pressure and by sublimation of the solvent. The whole process consists of three main stages: freezing, primary drying, and secondary drying periods. The successful of freeze drying process depend highly on the freezing stage. The material to be freeze dry is cooled down to a temperature below its solidification.

During the freezing stage, it is important to produce a lot of ice crystals formed which later enhance the shape of the pores, the pore size distribution, and the pore connectivity of the porous network of the dried layer formed by the sublimation of the frozen water during the primary drying stage. An excellent freeze dry process can be obtained by a large dendritic ice crystals formed and a homogeneous dispersion due to the high mass transfer rate of the water vapor in the dried layer. The product could then be dried more easily and quickly (Boss *et al.*, 2004).

In the other hand, the solvent is removed by sublimation under vacuum and heat addition during the primary drying stage. Certain amount of sublimation latent heat is also need when the water molecules sublimate and enter to the vapor phase. Therefore, the temperature of the frozen product is reduced.

After that, it is necessary to supply heat to the product, which could be provided by conduction, convection and/or radiation. Followed by the secondary drying stage, the solvent is continually been removed from the chamber. At the same time, a small amount of sorbed water can be removed by desorption. Finally, bound water is removed by heating the product under vacuum (Boss *et al.*, 2004).

In the freeze-dried process there are two main factors which have to be carefully considered. Overall dimensions of the container and good preparation of samples to be freeze-dried are the factors that help on getting the optimum process conditions that need to be set. Other parameters that can be controlled or set in freeze-dried process can be summarized in Table 2.1.

Table 2.1: Parameters that can be controlled in freeze-dried process

Parameters	Descriptions
Container	type (vial, ampoule, syringe), geometry, stoppers
Equipment	freeze-drier model, loading (shelf, trays), probes (position, type)
Process	shelf temperature, chamber pressure, time (cooling, annealing,
	primary/secondary drying cycles)

Adapted from (Franks; 1998).

At the same time, other parameters relating to formulation, processing conditions, cost and other considerations have to be included. As a result, appearance of the final product and its quality are highly depended on the composition, concentration and volume of the material to be dried, several equipment and process parameters as well as the geometry of the containing vessel (Franks., 1998).

### 2.5 Thermal Analysis and Differential Scanning Calorimeter.

Thermal Analysis is a concept used to explain about analytical methods that calculate chemical and physical properties of a sample as a function of time or temperature. Nowadays, thermal analysis has become an important analytical technique for research and development of new pharmaceutical products. It is an addition to the current analytical techniques such as chromatographic and spectroscopy (Roy., 2002).

In other point of view, thermal analysis is used to generate thermodynamic information which is important to have a better understanding on the behaviour of material under reduction or oxidation atmosphere, different heating and cooling rates, or under different gas pressures. Thermal analysis consists of many techniques in which a physical property of a substance is measured to a controlled temperature program. To date, several methods are commonly used such as differential thermal analysis (DTA), dynamic mechanical analysis (DMA), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and dielectric analyse (DEA). Among the above mention thermal analysis techniques, DSC is the first choice because it has wider application as compared to others techniques. Therefore, many researches in material science, pharmacy and food industry use DSC for studying phase transition under different atmospheric influences, heating / cooling rates and temperatures(Klancnik *et al.*, 2010).

Generally, when sample and reference in DSC are exposed to a temperature program, the difference in heat flow between them is measured against temperature and time. Based on this, DSC technique can be used to obtain information about heat of fusion, purity, polymorphism, pseudo-polymorphism, interaction/compatibility, thermal stability, melting point/melting range, crystallization, glass transition and other thermal transition results. All these information are really useful in the development of a stable and effective dosage form as well as can be used in the process of pre-formulation (Roy., 2002).

A lot of information or data can be obtained start from characterization of raw materials up to the interactions between phospholipids and drugs just by doing DSC analysis. At the end of DSC analysis, we can get a lot of information about first order transitions, such as melting and crystallization and the second order transitions, such as the glass transition temperature in polymer samples (Bond et al., 2002). In a typical DSC analysis, there are two pans isolated from the room temperature in a chamber.First pan is an empty sealed aluminium pan called the 'reference' pan are placed in the DSC instrument chamber alongside with a second sealed aluminium pan called the 'sample' pan which contain few milligrams of sample. Underneath them have heaters that will be used to raise or lower the temperature. At the same time, there are sensors under each pan to record the temperature of the pans at any given moment. Each pan is then heated at a pre-determined and constant rate  $(\Delta T / \Delta t)$ , means that the rate of temperature change will be the same for both heaters. Even though both pans heat at same and constant rate, the *heat flow*, which is the rate at thermal energy  $(\Delta Q/\Delta T)$  for both pans, are different. The pan with the sample inside will heat up slower than the empty pan. Therefore, the control computer of the DSC instrument will need to provide higher heat flow to the pan with a sample in order to achieved same rate. Again, the control computer of the DSC instrument will monitor the starting temperature, heating rate and heat flow. As a

result, it records the difference in heat flow between the sample pan and the reference pan which actually the total heat flow for the sample and plots it against temperature as shown in Figure 2.4 (Lukas *et al.*, 2009).



Figure 2.3: Example of DSC profile of Silicon Rubber. Here the endothermic heat flow is measured versus temperature (Adapted from Lukas *et al.*, 2009).

As we know, the heating rate is set and the heat flow is recorded. Thus, it is important for us to calculate the heat capacity,  $C_P$ . Heat capacity is defined as the ratio of a small amount of heat,  $\Delta Q$  added to the sample or material that we test, to the corresponding small increase in its temperature,  $\Delta T$ . If we know the mass (*m*) of the samples or testing materials, we can calculate the specific heat capacity,  $c_p$  as a function of temperature (Lukas *et al.*, 2009).

This explanation can be summarized into simple formula like these:-

Heat flow

Heat capacity = -----

Heating rate

With the previous explanations, we could state that DSC is a tool for measuring the energy applies to a sample and reference as a function of temperature.

In addition to that, the direct measurement signal for any types of DSC is a temperature difference. DSC will calculate the intensity of the exchange of the heat between the sample-reference part and the furnace. The resulting heat flow rate is corresponding to the temperature difference. Nowadays, most of DSC instruments that have been widely use is called heat flux DSC types. There are 3 different types of heat flux DSC which are the disk type measuring system, the turret type measuring system and the cylinder-type measuring system.

In this study, we only use the disk type measuring system. The heat flux within the DSC occurs through heat conduction path with a low thermal resistance from furnace to the samples. In disk type measuring system heat flux DSC, the heat exchange will takes place trough a disk which is solid sample support. Its advantages are high sensitivity

and small sample volume. Actually the disk act as a sample holder and the heat exchange measurement. The main heat flow is supplied from the furnace passes through the disk into the sample with a medium thermal conductivity. This is its main characteristic.



Figure 2.4: Schematic presentation of heat flux DSC with a disk type measuring system (Adapted from Klancnik *et al*; 2010).

In some analysis, the disks are made with combination of more than two materials for example metal such as platinum and the cover made from ceramics. To date, the disk is often called as crucible or pans. Typical crucible materials for the heat flux DSC apparatuses are made of aluminium, stainless steel, graphite, platinum and gold-plated which all use for different types of samples and analysis.

Generally, inside the heat flux DSC there is a connecting metal strip which often used as a sensor to record the temperature difference by measuring a voltage signal.

The corresponding voltage signal is than converted to a heat flow rate (usually in units J  $s^{-1}$ ). Furthermore, heat flux DSC allows only medium heating and cooling rates

because the heat exchange from the furnace to the sample is limited. Commonly use heating or cooling rate is between  $10-20^{\circ}$ C/min. The main advantage of heat flux DSC is that the signal is independent of the thermal properties of the sample (Klancnik *et al.*, 2010).