CHAPTER 5

5.0 DISCUSSION

5.1 Preparation of Microencapsulation Imipenem.

5.1.1 The freeze drying process.

In our study we have chosen manifold method as a method of freeze drying. Manifold methods mean it uses a turret with multiple ports (a manifold) where vials and flasks are individually attached via suitable valves. The product inside the vial or flask is previously frozen inside a freezer. Then, the pre-frozen product must be quickly attached to the drying chamber. The product container has to be rapidly evacuated and at this point, we can only rely on the evaporative cooling to maintain the product temperature. (Luque de Castro, M. D., *et al., 2002).* This method is chosen because small amount of samples can be used to start the freeze-dried process.

The manifold method has an advantage as compare to batch method where each flask has a direct path to the collector. This will minimize the competition for molecular space created in a batch system. (Luque de Castro, M. D., *et al., 2002).* Therefore, we can maximize drying efficiency. At the same time we can remove each flask from the drying chamber separately when its drying process is completed.

Basically, microencapsulation is a technology of packaging solids, liquids, or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions. The packaged materials can be pure materials or a mixture, which are also called coated material, core material, actives ingredient, fill ingredient, internal phase or payload. Whereas, the packaging materials are called coating material, wall material, capsule, membrane, carrier or shell, which can be made of sugars, proteins, natural and modified polysaccharides, lipids and polymers. (Fang *et al., 2010).*

Our study has clearly showed we manage to create a microencapsulated product via freeze-dried manifold method.

The microencapsulated product that we obtain is in solid powder form. Tienam ® antibiotic has colourless and when reconstituted in water it has turn into yellowish colour (Dohme. *2010)*. Whereas, PEG 2000 has a very light whitish in colour and PEG 35000 has white colour. The microencapsulated product has shown very light yellow colour which carry the Tienam ® antibiotic (packaged material) properties and confirm that the Tienam ® have been successfully encapsulated inside the PEGs (packaging material).

There are so many technique used for encapsulation. In general, three steps are involved in the encapsulation of bioactive agents: (i) the formation of the wall around the material to be encapsulated; (ii) ensuring that undesired leakage does not occur; (iii) ensuring that undesired materials are kept out. Some of the current encapsulation techniques include spray drying, , extrusion, fluidized bed coating, coacervation, spray cooling/chilling liposome entrapment, inclusion complexation, cocrystallization centrifugal suspension separation, emulsion , and freeze-drying (Fang *et al., 2010).*

5.2 DSC Analysis.

5.2.1 Sample preparations and operating conditions.

As mention by Coleman *et al., 1996, DSC use to detect t*hermal events such as endothermic (e.g. melting, dehydration) and exothermic (e.g. crystallisation) or changes in the heat capacity of a sample (e.g. glass transition phenomena). In this study, we have used a heat-flux DSC since it is the most common form of the technique and consists of a sample and reference positioned symmetrically in a furnace. The sample and reference pans are heated from the same source and the

differential temperature is measured. The resultant voltage signal is converted to heat flow rate usually in unit Js^{-1}.

Previous studies (e.g Coleman *et al., 1996) coded that*, nature of sample and the instrumental condition such as scan rate, atmosphere, pan configuration and reference system are the main factors that we have to take into account in order to generate good results from a DSC scanned that we performed. Heat flow is accurately measured by using small quantities of sample because it minimised thermal gradients within the samples. Small amount of samples have gave better resolution and better heat transfer within sample but it create a low detection limit which produce small peak. Whereas, large sample size can give better detection limit which is represented by larger peak but it can reduce resolution and reduce heat transfer within sample. *Therefore*, in our study, we try to use small amount of materials and samples loaded into the pans. We carefully weighing out the sample and loaded into the pan and make sure each of the samples sit at the bottom of the pan. Than we make sure that the pan is closed and sealed properly by the crimper. In addition to this, a small and shallow pan was recommended as a sample vessel and we have to make sure that the samples were fully covered at the bottom part of the pan in order to obtain a maximum thermal contact during scanning.

In our study, the reference system we use is an empty pan of the same type as the sample pan. We used hermetically sealed aluminium pan because aluminium material could stand heat up to 600^0C. We also chose it because it has less reaction with the sample. Aluminium only reacts with certain materials which are not used by us in this study(Coleman *et al., 1996)*. Typically, the heating rate use in normal DSC analysis is between 10^0C to 20^0C per minute. The heating rate can highly influence the temperature distribution inside the sample. It can influence the phase transition of polymers where usually sometimes we need to set at high heating rate in order to speed up the reaction especially in the higher temperature region. However, in this experiment

we have chosen 10^0C/min as a minimum value of heating rate in order to perform the experiment at a condition closer to equilibrium conditions.

Finally, we have chosen nitrogen as the choice of atmosphere in this experiment because we tried to minimize oxidation or burning of the samples and materials that we tested. At the same time we wanted to keep the furnace dry without any moister.

5.2.2 The DSC plots (thermogram) analysis.

In our study, all the tested material does not show a glass transition profile. For example, PEGs exhibit a glass transition temperature which depends on the molecular weight of the sample. Previous studies have indicated that, PEGs who have a molecular weight range from 10^2 to 10^7 will have the glass transition temperature rises from -98 °C to a maximum of - 17 °C. (Craig., 1995). Therefore we assume that all the tested materials might have the glass transition profile at very low temperature region.

Apart from that, there are two main thermal transition profiles that can be seen clearly from DSC plot that we obtain. They are called crystallization and melting. When the individual materials and samples are heated, its molecules started to move. After certain period of time they gain a lot of mobility which causes them to wiggle and squirm. At the same time, they cannot stay at one place for very long time because they cannot move anywhere. When they reach right temperature, they start to arrange themselves into a better order. At the same time, they have gain a lot of energy. This phenomenon is called crystals. When the samples are in crystalline arrangements, they are releasing their energy. This event is shown by the depression in the graph. The crystallization temperature (T_C) represent by the peak of the depression in the DSC plot. Furthermore, the area of the depression could be calculated. By using this value, mass of sample and heating rate, the latent heat of crystallization can be calculated. Since the crystallization process released out energy, we could call it as an *exothermic* transition.

In our experiment, only Imipenem and Tienam® have shown crystallization properties. From the Table 4.1, we could see the T_C for Imipenem in its pure form is at 171.77 ± 1.14^{0}C, but the T_C of Imipenem in Tienam® is at 157.36 ± 5.64^{0}C. The T_C of Imipenem has decreased about 14.41^{0}C. When heating process is continued and as the material past its T_C, its molecules start to vibrate. Further increment of the temperature will create more vibrations on the molecules and atoms. This will causes the crystals ordered started to fall apart and at this point we can say the molecules start to melt. The molecules are come out from the previous ordered arrangement and begin to move freely. From Table 4.2, we can see that the melting temperature, T_m for Imipenem is 195.90 ± 6.03 in its pure form but the T_m has decrease about 5.44 ^{0}C to 190.46 ± 3.99 ^{0}C went Imipenem is inside Tienam®. Tienam® consists of Imipenem and Cilastatin sodium, in one to one ratio (1:1). Therefore, we think the present of Cilastatin material that have been coupled together with the Imipenem in Tienam® does causes some minor change on the T_C and T_m of Imipenem. Later, as we supply more heat to Imipenem, there are something happened and we can see it clearly as the temperature passes 200^{0}C. We can see the generating of many small peaks and this show to us that the Imipenem have started to decompose as it passes above 200^{0}C.

In Tienam®, we can say that the melting profile appear in the thermogram is belong to the Imipenem melting profile because it appear within temperature range of 150-200^{0}C very similar to the melting profile of pure Imipenem. As we continued to heat up the Tienam®, we could see again many small peaks start to appear at a temperature above 250^{0}C and this shows to us the starting process of decomposition of Imipenem inside the Tienam®.

During our DSC scan of PEGs material, we can see PEG 2000 have T_m of 52.77 ± 1.26 ^{0}C with a heat of fusion (ΔH) of 212.77 J g^{-1}. PEG 35000 have T_m of 66.92 ±

1.01 ^{0}C with a ΔH of 228.05 J g^{-1}. These have shown to us that different molecular weights of PEGs will have different melting temperatures and higher ΔH which above 200 J g^{-1}. Previous study has mentioned that Polyethylene glycol can be used as a thermal energy storage material due to its relatively high latent heat of 187 Jg^{-1}, suitable melting point, and congruent melting behaviour. Moreover, because PEG was a type of materials with different molecular weights with different melting temperatures; they can be used in many applications (Wang *et al., 2009*).There is no sign of decomposition for both PEGs even though we continued to heat it at temperature up to 300^{0}C. As we mention previously, Sample A is a result from microencapsulation of Tienam® antibiotic and the PEG 2000. From the DSC plots of Sample A, we can say that the melting profile appears definitely belong to PEG 2000 since its similar to pure PEG 2000 melting profile. Same thing happen in Sample B where the melting profile appear definitely belong to the melting profile of PEG 35000 because of the similarity of the thermogram generate at that temperature. Sample A and Sample B do not show additional melting profile that similar to Tienam® melting profile. From Table 4.2, we can see melting profile of PEG 2000 contain in Sample A have shifted ±8.22 ^{0}C higher than the melting profile of pure PEG 2000. Whereas, in Sample B, the melting profile for PEG 35000 have shifted ±1.92 ^{0}C lower as compare to melting profile of pure PEG35000. Both events are causes by the introduction of Tienam® inside the PEGs which have made changes in the arrangement of the molecules and its structure that lead to the shift of its melting profile. This appearance also indicates to us the formation of solid solution between the antibiotics (Tienam®) and the carrier (PEGs).

In our study, we can see the appearances of an exothermic reaction occur at a temperature above 120 ^{0}C especially in PEG 2000, Sample A, PEG 35000 and Sample B. These reactions happen due to the use of hermetically sealed pans in the experiment. When the material and samples are sealed in air, some air is trapped and cannot be

removed by the purging gas. The presence of air has caused oxidation of PEG at that particular temperature which produces by the dip that leads to the exothermic reaction to happen (Anguiano-Igea *et al., 1995*).. These situations are confirmed by Figure 4.7 & Figure 4.8 where the exothermic reaction suddenly disappeared when we load both PEG samples in non-hermetically sealed pans. However, we cannot use non-hermetically sealed pans because the PEG material shown a little bit of volatile characteristic and it tended to come out from the pan at high temperature which caused the heating chamber to become dirty and need to be cleaned each time we scan the sample.

Sample C and Sample D have been prepared with a 1:1 ratio where the product have 50% of Tienam® antibiotic encapsulated in 50% of PEG. During the analysis of Sample C as shown in Table 4.3, we can see that the first melting profile belong to the PEG 2000 since it similar to PEG 2000. Whereas in Sample D the melting profile belong to the PEG 35000 since it similar to PEG 35000. As the temperature increase above 200^0C, Sample C and Sample D have shown another melting profile which belong to the Imipenem since it similar to the Imipenem melting profile. This have prove to us that the Imipenem is actually present in both Sample A and Sample B but we cannot see clearly its thermal transition profile because the amount of Imipenem in both samples are very little as compared to the amount of PEGs. The Imipenem melting have been hidden by the PEGs melting profile. In addition to this, the results have given us proved of the successfulness of our microencapsulated of Tienam® into PEGs by using freeze drying method.