CHAPTER 3

3.0 METHODOLOGY

3.1 Introduction.

In this study, there were two main stages involved. The first stage involved the process of creating microencapsulated imipenem in polyethylene glycol materials with a simple technology called freeze drying. Freeze-drying process started by freezing the material. Then when inserting the samples into the freeze drying system, the surrounding pressure was reduced. After that enough heat was added to allow the frozen water in the material to sublime directly from the solid phase to the gas phase.

After that, the second stage involved a thermal analysis studied of the encapsulated product by using differential scanning calorimeter (DSC). Differential Scanning Calorimeter (DSC) is one of the most commonly used techniques in thermal analysis research. It consists a measurement of heat exchanges between the reference and a sample versus temperature during a heating program. Generally the sample and the reference were contained in identical crucibles, which were sealed properly as what the experiment conditions required. Both crucibles were placed in a thermo regulated furnace which imposed the temperature program. The varieties of DSC devices were differed by the way thermal exchanges were measured. In this research we used a Perkin Elmer machine; model DSC 6000 which used a heat flux DSCs principle. In DSC 6000 there was a thermocouple used to measure the temperature difference between the two crucibles at one point of their walls (bottom part).

The information from DSC result had told us about the changes in pure materials and samples heat capacity by tracking the changes in its heat flow. This had allowed us to detect thermal transitions like melts, glass transitions, crystallization, phase changes and curing.

3.2 The production of microencapsulation Imipenem.

3.2.1 Materials.

Imipenem Antibiotics Standard (Catalogue number: 1337809) was purchased from U.S Pharmacopeia. Tienam® was purchased from Merck Sharp & Dohme, USA. Polyethylene glycol (PEG) 2000 (Catalogue number: 84797) was purchased from Sigma Aldrich, Germany and PEG 35000 (Catalogue number: 94646) was purchased from Fulka Sigma Aldrich, Germany. In step by step freezing processed, the refrigerator was from Electrolux, USA, freezer (-20^{0}C) was from Acson, Malaysia

and the ultra low freezer (-20^{0}C) was from Electrolux, USA.The two separate mixtures of Tienam® antibiotic and PEG were freeze dried by using freeze drying system from Labconco Corporation, USA.

3.2.2 Preparation.

The antibiotic powder and PEG were weight and dissolved in ultra pure water separately. After that, both solutions were mixed together. The mixture was stirred for a few minutes. The mixture was transferred into a freeze dryer flask, sealed with a parafilm and store in a refrigerator set at 4^{0}C. The whole process was repeated by using high molecular weight of PEG. The amounts of raw materials used in the above preparation were shown in the below step by step calculations.

(a) We assumed that we were preparing a tablet having a weight of 30 mg each.

(b) Inside the 30 mg of each tablet contained about 64 µg of Tienam® (imipenem: cilastatin, 1:1).

(c) Therefore the calculation would be:-

→ 30 mg = 64 μg

→ 30 mg = 0.064 mg, convert to mg unit on both sides.

→ 30 000mg = 64 mg, pretended to prepared 1000 tablets

→ 30g = 0.064 g

→ 30g = 0.064 g Tienam® + 29.936 g PEG

Finally, another set of preparation was done to create a 1:1 ratio having 50% of Tienam and 50% of both PEGs separately.

3.2.3 The freezing process.

The most important part in this process was to make sure that the sample was really frozen. In order to do that, the cooling and freezing process had to be done step by step. After kept it in the refrigerator at 4^0C for 3 hours, the samples were transferred into a freezer set at -20^0C. The freezing process was continued for about minimum of 24 hours. Than the samples were kept in an ultra low freezer set at -80^0C to further frozen the whole sample outer and inner part of it. Once, finishing the step by step cooling and freezing process, the samples were now ready for the freeze drying process.

3.2.4 The freeze drying process.

To begin with the freeze drying process, the equipment had to be warmed up for about half an hour to make sure the cold trap inside the collecting system reaching temperature -40^0C. After that the frozen sample was plug in onto the machine and the freeze drying process are started. A vacuum pump was turned on to make the whole system in a vacuum condition. At the same time, it was really important to check both setting, which means that the cooling traps and the vacuum pump functions in order to

allow the sublimation to occur. The setting of the vacuum inside the whole system had to be as low as possible.

If it did not subside, there was a slight leak of a vacuum occurred in the system. Therefore, a very little amount of vacuum grease was applied at the cover of the freeze dry system and at the outside of the mouth of the samples container. The freeze drying process was considered completed after the samples had shown very dried and no sign of liquid present. Finally, the dried samples were taken out from the freeze drying container and stored in an amber vials to prevent any degradation by lights. The products were now ready for thermal analysis using Differential Scanning Calorimetry.

3.3 Thermal Analysis by using Differential Scanning Calorimeter.

3.3.1 Materials.

Imipenem standard, Tienam, PEG and the freeze dried products were scanned using DSC-6000 unit from Perkin Elmer, Inc., USA. A 20µl hermetically sealed aluminium pans from Perkin Elmer, Inc., USA were used for the DSC scanning. Indium standard from Perkin Elmer, Norwalk, USA was used to calibrate the DSC unit. The purge gases used were compressed purified nitrogen obtained from Malaysian Oxygen (MOX) Sdn Bhd. All DSC curves were analyzed using Perkin Elmer Pyris software.

3.3.2 The Analysis Process. The various samples scanned using DSC were shown in Table 3.1.

Table 3.1: List of samples scanned using the DSC.

No.	Name of Material	Information of material
1.	Imipenem	Pure Imipenem
2.	Tienam ®	Mixture of Imipenem and Cilastatin; 50% : 50%
3.	PEG 2000	PEG having 2000 molecular weight
4.	PEG 35 000	PEG having 35000 molecular weight
5.	Sample A	Microencapsulated product of Tienam® in PEG 2000
6.	Sample B	Microencapsulated product of Tienam® in PEG 35000
7.	Sample C	Microencapsulated product of Tienam® in PEG 2000 with ratio 1:1(50%:50%)
8.	Sample D	Microencapsulated product of Tienam® in PEG 35000 with ratio 1:1(50%:50%)

About 1-2 milligram of each material was loaded into hermetically sealed aluminium pans. Then, the materials were analysed inside the DSC 6000 instrument at a heating rate of 10.0 $^{\circ}$C/min. The heating process was set from 5°C up to 300°C.