A CALORIMETRY STUDY OF DRUG/POLYMER INTERACTION

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Abstract

Many drugs are poorly water soluble and their dissolution rate can limit bioavailability. Preparing drugs in dispersed systems can enable them to be readily absorbed when they enter the Gastrointestinal Tract. However, the stability of such systems is not always desirable. The aims of this work were to investigate the use of calorimetry as a method for investigating the stability of dispersed systems in a thermodynamic perspective. This work was also aimed at investigating the possibility of predicting the stability of solid dispersions by studying the interactions between drug and components in their liquid state.

Isothermal Titration Calorimetry (ITC) was employed to study the interactions between poloxamer and ibuprofen/ketoprofen suspensions. The significant variation shown from the interactions led to the study on the equilibration of poloxamer solutions and their association behaviour in water. It was found that poloxamer solutions were concentration dependent and poloxamer formed aggregates following the open association model. The investigation suggested that a large time scale was required for poloxamer solution to reach equilibrium which was essential in ensuring consistent performance and solutions were provided as to how to efficiently achieve equilibrium state of poloxamer solutions at different concentrations. All these findings significantly improved the poor reproducibility, enabled better application of poloxamer solutions and better understanding of how to handle poloxamer solutions.

A calorimetric study was carried out to investigate the effect of a second excipient Poly(2-hydroxypropylmethacrylate) (PHPMA) on the stability of Griseofulvin / Indomethacin – Polyvinylpyrrolidone (PVP) solid dispersions. Studies were carried out in liquid state. It was found that the addition of PHPMA significantly increased the stability of solid dispersions in both binary and ternary systems. Systems in which PHPMA was added last showed the greatest stability indicating its order of addition was essential to the stability of the system. Correlation with previous studies suggested that calorimetry can be used to predict the stability of solid dispersions in their solid states by studying their properties in their liquid states and hence avoid of many uncertainties introduced during the manufacture processes.
Acknowledgements

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I would like to dedicate this thesis to my mum and my late grandmother for their unconditional love!
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<tr>
<td>$\varepsilon$</td>
<td>proportionality constant</td>
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<tr>
<td>$\eta$</td>
<td>viscosity of fluids</td>
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<tr>
<td>$\theta$</td>
<td>Contact Angle</td>
</tr>
<tr>
<td>$\sigma, \gamma, \text{or } \Gamma$</td>
<td>Surface Tension</td>
</tr>
<tr>
<td>$\rho_f$</td>
<td>Density of the fluid</td>
</tr>
<tr>
<td>$\rho_p$</td>
<td>Density of the drug particles</td>
</tr>
<tr>
<td>$\tau_2$</td>
<td>Slow Relaxation Time</td>
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<tr>
<td>% w/v</td>
<td>Percentage Weight in Volume</td>
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<tr>
<td>CAC</td>
<td>Critical Aggregation Concentration</td>
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<tr>
<td>CMC</td>
<td>Critical Micelle Concentration</td>
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<td>CMT</td>
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<td>$C_p$</td>
<td>Heat Capacity</td>
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<td>DCA</td>
<td>Dynamic Contact Angle</td>
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<td>Differential Thermal Analysis</td>
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<td>Ed.</td>
<td>Editors</td>
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<td>FTIR</td>
<td>Fourier Transform Infrared</td>
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<td>G</td>
<td>Gibbs Energy</td>
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<td>GIT</td>
<td>Gastrointestinal Tract</td>
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<td>GPC</td>
<td>Gel Permeation Chromatography</td>
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<tr>
<td>$h$</td>
<td>Hours</td>
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<td>H</td>
<td>Enthalpy</td>
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<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<td>ITC</td>
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<td>$k$</td>
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<td>K</td>
<td>Kelvin</td>
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<td>$K_b$</td>
<td>Binding Constant</td>
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<td>min</td>
<td>Minutes</td>
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<td>Molecular Weight</td>
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<td>$p$</td>
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P  Pressure
PHPMA  Poly(2-hydroxypropylmethacrylate)
PR  Raw Power Signal
Ps  Saturation Pressure of the Absorbate
PVP  Poly(Vinylpyrrolidone)
q  Heat
r  Radius of Drug Particles
S  Entropy
SDS  Sodium Dodecyl Sulphate
TAM  Thermal Activity Monitor
TGA  Thermal Gravimetric Analysis
U  Internal Energy
Ue  Electrical Potential
V_{ads}  Volume of Gas Absorbed
V_m  Volume Absorbed by a Monolayer of Gas Molecules
Vs  Particle Settling Velocity
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Chapter One

Introduction
1.1 Dispersed Systems

The liquid system has proved to be the most difficult system to study when compared to gas and solid systems. In the gas state, molecules move in a random and translational way and the interactions between gaseous molecules are very limited. In the solid state, the interaction sites are confined within vibrational range due to the immobility of the atoms and molecules if any interaction occurs and this enables the predictability. A lot of effort has been put into the study of the gaseous and solid states and a number of problems have been solved with great satisfaction, a lot more was understood about these two systems (Florence and Attwood, 1981a). Yet, more attention needs to be paid to the liquid system since the understanding is still very limited and the system is more complicated as some of the issues encountered in the other two systems can occur in this system too, moreover, there are still many problems unsolved.

A drug has to be in solution in order to cross the Gastrointestinal (GI) membranes and reach the general circulation for absorption. The dissolution rate of the drug is among the many physicochemical properties of the drug that will influence its passage into the solution and be transferred across the membranes, other factors include the $pK_a$, the lipid solubility, chemical stability and complexation potential of the drugs. Since many drugs are hydrophobic, how to improve the solubility has great significance on the absorption profiles of these drugs. Although higher dissolution rate does not necessarily indicate better bioavailability, dissolution is usually regarded as the rate-limiting step for most of the drugs. Scientists have been trying all sorts of methods to prepare drugs in solution forms which are thermodynamically stable and can be readily absorbed. Such methods include transforming the drugs to salts, adding excipients or complexation to aid the dissolution process of the drugs and subsequently the absorption process to make the formulations more desirable. However, not all the drugs can be formulated in solutions, alternatively, they are formulated in dispersions. After appropriate modification, dispersions can also show desirable performance (Jackson, et al., 2000; Ashford, 2002a and b).
Dispersion is defined as a heterogeneous system in which one phase is dispersed (with certain degree of uniformity) in a second phase. Based on the dispersed phase and the dispersion medium, dispersions can be classified as listed in Table 1.1 (Nash, 1996).

<table>
<thead>
<tr>
<th>Dispersed Phase</th>
<th>Dispersion medium</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas</td>
<td>Liquid</td>
<td>Foam</td>
</tr>
<tr>
<td>Liquid</td>
<td>Gas</td>
<td>Aerosol</td>
</tr>
<tr>
<td>Liquid</td>
<td>Liquid</td>
<td>Emulsion</td>
</tr>
<tr>
<td>Solid</td>
<td>Gas</td>
<td>Aerosol</td>
</tr>
<tr>
<td>Solid</td>
<td>Liquid</td>
<td>Suspension</td>
</tr>
<tr>
<td>Solid</td>
<td>Solid</td>
<td>Solid Dispersion</td>
</tr>
</tbody>
</table>

Table 1.1 Classification of dispersions based on the dispersed and continuous phases

1.1.1 Suspension

Suspension is dispersion with solid particles within a specific range of sizes uniformly distributed in the suspending vehicle with the aid of suspending agent(s) through agitation. The system is called colloidal suspension if the average suspended particle size is smaller than 1 μm, and is called coarse suspension otherwise. Suspensions can be administered orally, topically and parenterally, the wide application has endowed suspension with promising potential in the pharmaceutical industry.

When a drug is administered orally as in a solid dosage form, its absorption largely relies on the dissolution of the drug from the capsule or tablet. If the drug is insoluble or poorly soluble, the absorption is hindered by this process. Compared to those solid dosage forms, suspension shows great advantages because the surface area of the drug is profoundly increased when dispersed in the solvent and hence a higher degree of absorption and bioavailability is ensured (Nash, 1996).
However, the stability is a big issue for suspensions. Problems such as sedimentation, caking and flocculation arise due to the nature of suspensions. By minimising the particle size and/or by increasing the viscosity can help improve the stability. However, there is always a limit and it is necessary to resort to other means to improve the stability situation. A lot of components have been added in the formulation to improve the stability of the suspension and polymer is one of the most commonly employed excipient as they can enhance the dissolution of the suspending particles, improve the flocculation and hence the caking and to increase the viscosity and therefore decrease the sedimentation rate. It is suggested that an ideal suspending agent should have the following characteristics (Farley and Lund, 1976):

(a) can be readily and uniformly incorporated in the system  
(b) be readily dissolved or dispersed in solvent without resorting to special techniques  
(c) ensure the suspension of a loosely flocculated system which can be easily re-dispersed and does not cake  
(d) does not negatively influence the dissolution and absorption rate of the drug  
(e) non-toxic  
(f) inert  
(g) compatible with drug and excipients

1.1.2 Solid Dispersion

The bioavailability of many pharmacologically active drugs with poor water solubility is very often limited by their dissolution rate. It was found that the dissolution rate of the particles could be increased by reducing the size of the particles, which can be achieved by conventional trituration and grinding; milling; micronisation and spray drying upon controlled precipitation by change of solvents and temperature. Although size reduction can be easily achieved by the techniques stated above, it is not necessary that the bioavailability of the drugs will be improved. This could be resulted from two possibilities (1) the precipitation of the fine particles when introduced to the GI tract; or (2) the agglomeration and aggregation of the particles due to the increased surface
energy. Also, the poor wettability in water may not have been improved despite the reduction in particle size and hence the dissolution in water was not improved.

A novel technique is needed to further improve the dissolution rate and the bioavailability of the water insoluble compounds. The idea of solid dispersion, which was intended to reduce the particle size and to increase the rate of dissolution and absorption, was first proposed by Sekiguchi and Obi in 1961.

Solid dispersion is defined as a drug dispersed in a biologically inert matrix or carrier at solid state. Solid dispersion provides a new technique in the pharmaceutical industry to efficiently increase the dissolution, absorption and the therapeutic effects.

Solid dispersion was prepared by dispersing one or more active ingredients, usually with poor solubility, in an inert matrix in order to achieve increased dissolution rate, sustained release of drugs, desirable solid-state properties, enhanced release of drugs and improved solubility and stability. Solid dispersion technique was not limited to increasing the dissolution and absorption rate of water-insoluble drugs, it can also be utilised to obtain a homogeneous distribution of a small amount of drugs at solid state, to stabilise unstable drugs and to formulate controlled-release dosage forms by selecting carriers with different solubility (Habib, 2001). It is reported that the bioavailability of many drugs with desirable therapeutic effects but poor water solubility has been significantly improved by applying the solid dispersion technique. When the drug is in contact with the aqueous solvent, because the drug is intimately surrounded by the water soluble compounds, the carrier can be readily dissolved and leads the drug to be wetted by the solvent and hence dissolve more readily. As a consequence, a homogeneous suspension is obtained with reduced drug particle size and this in turn benefits the dissolution.
1.2 Biopharmaceutics Classification System (BCS)

The biopharmaceutics Classification System (BCS) provides recommended methods for classification according to dosage form dissolution and characteristics of the drug substances, and a class of immediate-release solid oral dosage forms for which the clinical bioequivalence test, which could also be identified by the BCS, can be assessed based on in vitro dissolution tests. The BCS was introduced by the U.S. Food and Drug Administration (FDA) in order to improve the drug development and the review process.

The BCS considers three main factors that contribute the drug adsorption: dissolution, solubility and intestinal permeability and it classify the drug substances based on their aqueous solubility and intestinal permeability. According to BCS, drug substances are classified as follows (Food and Drug Administration, http://www.fda.gov/cder/OPS/BCS_guidance.htm):

- Class I - High Permeability, High Solubility
- Class II - High Permeability, Low Solubility
- Class III - Low Permeability, High Solubility
- Class IV - Low Permeability, Low Solubility

The drugs covered in this study included ibuprofen, ketoprofen, griseofulvin and indomethacin. They all belong to Class II, which has high permeability and low solubility, indicating the dissolution of these drug substances in aqueous solvent is the rate limiting step in adsorption. These four drugs are to be dispersed in dispersions and surfactants are added to enhance their solubility and stability in the dispersions.

1.3 Surfactants

Surfactants are characterized by containing two opposing parts, lyophilic, which is affinitive to solvent; and lyophobic, which is antipathic to solvent. While lyophilic parts enhance the solubility in the solvent, the lyophobic parts improve the immiscibility. If the solvent is water, the two parts can be rephrased as hydrophilic and hydrophobic.
Due to the unique nature of their chemical structures, surfactants tend to concentrate at the surface of a solution or interfaces of two immiscible phases and as a result, altering the free energy of the surfaces or interfaces to a significant degree even when presented at a low concentration in the system. Therefore, they are often referred to as surface active agents and that is the origin of the name surfactant (Pickett, 1977). The term interface denotes the boundary between any two immiscible phases, it could be two immiscible liquids or between solid and liquid. Whereas the term surface often refers to an interface where one of the phases is gas, usually air. The driving force for surfactants to adsorb to the surfaces or interfaces is the lowering of the free energy of that phase boundary. When the boundary is covered with surfactant molecules, the surface tension is reduced. The more surfactant molecules packed at the surface or interface, the more reduction in surface tension. However, surfactants can not infinitely reduce the surface or interface tension. The limit is reached when micelles start to form, i.e., at the critical micelle concentration, for conventional surfactants.

Surfactants have also been used to facilitate the interfacial tension reduction between the oil and water phase in emulsions. In some cases, the interfacial energy is reduced to such a low level that two formerly immiscible phases spontaneously emulsified and form very stable emulsions. Such effect on the interfacial tension is also applied in some transdermal formulations to enhance their membrane absorption, by lowering the surface tension of the formulation closer to that of human skin, which is at approximately 22-30 mN·m⁻¹ (Florence and Attwood, 1981c).

The hydrophobic moieties of a surfactant are often, but not always, long linear hydrocarbon chains. The length of the chain is usually within the range of 8-20 carbon atoms (Holmberg et al., 2003). The degree of the chain branching, the position of the hydrophilic moiety and the length of the chain are all important to the physico-chemical properties of the surfactants.
1.3.1 Classification of Surfactants

Generally, hydrophobic moieties of surfactants are long hydrocarbon chains, saturated or unsaturated; in some rare cases, they can also be heterocyclic or aromatic ring systems; therefore, differences in the nature of hydrophilic moiety are more pronounced than in the nature of hydrophobic moiety. Hence, the primary classification of surfactants is based on the charge of the hydrophilic head group. Basically, the classification is divided into ionised and non-ionised. Based on the charge carried, the ionised group is sub-divided into anionic, cationic and amphoteric (Pickett, 1977).

1.3.1.1 Anionic

The surfactant molecules in this group bear a negative charge. They are by far the largest surfactant class and are used in greater volume than any other three classes. Although there are important exceptions, they are generally not compatible with cationics. Sodium dodecyl sulphate (SDS) is by far the most important surfactant within this category and has been studied at length. This surfactant has been utilised in part of this research.

\[ \text{CH}_3(\text{CH}_2)_{11}\cdot\text{SO}_3\text{Na}^+ \]

1.3.1.2 Cationic

The surfactant molecules in this group bear a positive charge. The majority of cationic surfactants are based on the nitrogen atom carrying the positive charge. Surfactants within this category are mostly either amine or quaternary based. A well-known example is hexadecyl trimethyl ammonium bromide:

\[ \text{CH}_3(\text{CH}_2)_{15}^+\text{N}^+(\text{CH}_3)_3\text{Br}^- \]

Another well known cationic surfactant is benzalkonium chloride being used as a preservative in multi-dose injections and ophthalmic formulations.

1.3.1.3 Amphoteric

Amphoteric surfactants contain two differently charged groups. They are often referred to as zwitterionic surfactants. Depending on pH, amphoteric surfactants can be anionic,
cationic or zwitterionic. The positive charge is almost invariably ammonium and carboxylate is by far the most common source of negative charge. One typical example is N-dodecyl-N,N-dimethylbetaine:

\[ \text{C}_{12}\text{H}_{25}\text{N}^+(\text{CH}_3)_2\text{CH}_2\text{COO}^- \]

1.3.1.4 Non-ionic

Surfactants within this category hold no apparent charge. They are the second largest surfactant class. One great advantage of surfactants in this class is that they are not affected by the pH of the environment and they do not disturb the electric balance of the system. Another advantage is that desirable physico-chemical properties, such as hydrophobicity, can be obtained by altering the hydrophobic and the hydrophilic chains of the surfactants. Therefore, in an ideal way, an optimum surfactant can be selected according to the desired result to produce a marked effect in different applications. More and more non-ionic surfactants have been introduced and widely used on a large scale.

Examples:

\[ \text{CH}_3(\text{CH}_2)_{15}(\text{OCH}_2\text{CH}_2)_7\text{OH} \]

Heptaoxyethylene monohexadecyl ether

\[ \text{C}_{16}\text{H}_{33}(\text{OCH}_2\text{CH}_2)_{21}\text{OH} \]

Cetomacrogol 1000

1.3.2 Micelle

As mentioned at the beginning of this section that surfactants tend to adsorb at the surface and inter-surface and the driving force for this phenomenon is the reduction in free energy.

Take a simple surfactant as an example, when it is scarcely present in water, the surfactant molecules adsorb to the surface of water driven by the Gibbs free energy with the hydrophilic group facing the bulk of the solution while the hydrophobic group
extending to the vapour of the solution. This is in favour of the hydrophobic moiety because they are distanced from the ‘hostile’ aqueous phase. The hydrophobic groups will not enter the bulk of the solution as readily as they enter the surface of the solution. The re-entering to the bulk would not take place until the molecules are provided dynamic energy with suitable strength and direction and this depends on the structure of the molecule and the condition of the solution at that particular moment (Florence and Attwood, 1981c).

When the amount of surfactant molecules at the surface reaches a certain degree, more energy is required to push the surfactant molecules into the surface than the lowering of the free energy from the surfactant adsorption and the adsorption to the surface is no longer in favour. On the other hand, the aggregation of the surfactant molecules in the bulk can reduce the free energy profoundly. Consequently, when more and more surfactant is introduced to the system, instead of adsorbing to the surface, surfactant molecules start to aggregate with the hydrophobic group in the centre surrounded by the hydrophilic groups orienting outwards in the bulk. These aggregates are known as micelles. There are different interpretations found in the literature, but it is generally agreed that the lowest concentration of micelle forming is called Critical Micelle Concentration (CMC). Micelles are generally formed within a narrow size distribution and the average number of surfactant molecules forming one micelle is called the aggregation number. There is a dynamic balance between the molecules at the surface, in the micelle and in the bulk. The micelles are simultaneously and continuously breaking down and reforming. The molecules are constantly inter-changing with one another but the equilibrium between the three phases is achieved at all times.

Micellisation is one of the most important properties of surfactants because it can be best used for dissolving substances which are insoluble or sparingly soluble in the solvent. The insoluble substances are incorporated in the hydrophobic core of the micelles and hence improve the solubility and stability. Solubilisation only occurs when micelles are formed, i.e. above CMC. The way the substances are incorporated in the
micelles is dependent on the chemical structure of the substances. It is generally believed that the aggregation number of the micelles is constant and the size of micelles remains the same. However, micelles might change in order to form a bigger and more stable structure to better accommodate the foreign substance. More surfactant molecules would join to form one micelle and hence the size of the micelle tends to grow bigger and fewer micelles are formed. The size of the micelle increases when substance is incorporated into the micelle resulting from the core being enlarged by the solubilisation and also from the increase of the aggregation number. The size of the micelle changes solely when incorporation occurs (Florence and Attwood, 1981b).

As a consequence, surfactants have been greatly employed in various formulations to overcome the restrictions of drugs, the limited solubility, for both internal and external use. Due to the toxicity consideration, non-ionic surfactants have out-performed surfactants in other categories and have been used most widely.

A few polymers, which belong to the non-ionic surfactants, are employed in this study and the introduction is given below.

1.4 Poloxamers
Copolymers, in contrast to polymers which are composed of the same type of monomers, are synthesized by simultaneous or consecutive polymerization of different types of monomers. The product of such a synthesis is called a block copolymer when the individual monomers of various lengths are in linear and/or radial arrangements in the copolymer molecule.

Poloxamers, also known as the Pluronics ®(BASF) or Synperonics (ICI), are just one example of triblock copolymer surfactants. Water soluble triblock copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), often denoted as PEO-PPO-PEO, are commercially available non-ionic surfactants. Products of various molecular weights and hydrophobicities are accessible by simply altering the chain
length or the PPO/PEO ratio. As a result, PEO-PPO-PEO block copolymers are an important class of surfactants and find widespread applications in industry and in pharmaceutical sciences. General structure of a PEO-PPO-PEO triblock copolymer is as follows:

\[ \text{HO}(\text{CH}_2\text{-CH}_2\text{-O})_a (\text{CH}_2\text{-CH}_2(\text{CH}_3)\text{O})_b(\text{CH}_2\text{-CH}_2\text{-O})_a \text{H} \]

Such poloxamers are also termed ABA block copolymers. They are used in a wide range of industrial applications as anti-foamers, emulsifiers, wetting agents, solubilising agents and numerous other applications (Hellsten, 1987; Alexandridis and Hatton, 1995; Edens, 1996; Ali, 2000).

1.4.1 Synthesis of Poloxamers

The PEO-PPO-PEO triblock copolymers are synthesized by the sequential addition of propylene oxide (PO) to a low molecular weight water-soluble propylene glycol initiator followed by the addition of ethylene oxide (EO) to both ends of the poly (propylene oxide) oligomer. The latter oxilation steps are carried out in the presence of an alkaline catalyst, usually sodium or potassium hydroxide, which is then neutralized and removed from the final product (Schmolka, 1994; Alexandridis and Hatton, 1995; Ali, 2000).

The synthesis processes are represented by the equations below:

*Addition of PO to form the PPO block:*

\[ \text{HOCH(CH}_3\text{)CH}_2\text{OH} + (m-1)\text{CH}_3\text{CH(O)CH}_2 \rightarrow \text{HO(CH(CH}_3\text{)CH}_2\text{O)}_m \text{H} \]

*Addition of EO to form the PEO end blocks:*

\[ \text{HO(CH(CH}_3\text{)CH}_2\text{O)}_m \text{H} + (2n)\text{CH}_2\text{(O)CH}_2 \rightarrow \text{HO(CH}_2\text{CH}_2\text{O)}_n (\text{CH(CH}_3\text{)CH}_2\text{O)}_m (\text{CH}_2\text{CH}_2\text{O)}_n \text{H} \]

**Figure 1.1 Synthesis of the PEO-PPO-PEO copolymers**
1.4.2 Nomenclature

There are two systems for naming the copolymers. The first system for naming the Pluronic triblock copolymers starts with the letters L, P or F, which stands for liquid, paste or flakes respectively. Comparatively, the second system is more direct and informative. It always starts with P, which stands for Polymer. The first one or two numbers when multiplied by 100 are indicative of the approximate molecular weight of the hydrophobic block and the last digit indicates the weight fraction of the hydrophilic block. For example, the molecular weight of the PPO block of poloxamer P188 is about 1800 g mol\(^{-1}\), and the molecular weight of the two PEO blocks comprises 80 percent of the whole molecule.

With their unique chemical structure, which can be controlled by the synthesis procedure, block copolymers are found to have a wide range of useful properties. These properties are utilised by industries since the early 1950's. Application areas for block copolymers range from coal and petroleum deemulsifier, to plastics compatibilisers, agricultural chemicals, detergents and medical, biomedical and pharmaceuticals (Edens, 1996; Kabanov et al., 2002). Names in both nomenclature systems and the structural information for some of the most often used block copolymers are listed in Table 1.2 (Alexandridis and Hatton, 1995; Alexandridis et al., 1994).
<table>
<thead>
<tr>
<th>Pluronic</th>
<th>Poloxamer</th>
<th>MW</th>
<th>PPO wt</th>
<th>PO units</th>
<th>EO units</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-68</td>
<td>188</td>
<td>8350</td>
<td>1750</td>
<td>30</td>
<td>2×75</td>
</tr>
<tr>
<td>F-87</td>
<td>237</td>
<td>7700</td>
<td>2250</td>
<td>39</td>
<td>2×62</td>
</tr>
<tr>
<td>F-88</td>
<td>238</td>
<td>11800</td>
<td>2250</td>
<td>39</td>
<td>2×97</td>
</tr>
<tr>
<td>F-108</td>
<td>338</td>
<td>14000</td>
<td>3250</td>
<td>54</td>
<td>2×128</td>
</tr>
<tr>
<td>F-127</td>
<td>407</td>
<td>12000</td>
<td>4000</td>
<td>67</td>
<td>2×98</td>
</tr>
<tr>
<td>L-64</td>
<td>184</td>
<td>2900</td>
<td>1750</td>
<td>30</td>
<td>2×13</td>
</tr>
<tr>
<td>P-85</td>
<td>235</td>
<td>4650</td>
<td>2250</td>
<td>39</td>
<td>2×27</td>
</tr>
<tr>
<td>P-103</td>
<td>333</td>
<td>4950</td>
<td>3250</td>
<td>54</td>
<td>2×20</td>
</tr>
<tr>
<td>P-105</td>
<td>335</td>
<td>6500</td>
<td>3250</td>
<td>54</td>
<td>2×38</td>
</tr>
</tbody>
</table>

Table 1.2 Nomenclature and Properties of some Poloxamers

1.4.3 Micellisation of Poloxamers

The ever increasing interest in block copolymers stems mainly from their unique associative properties in solution as a consequence of their molecular structure. Particularly, their surface active and self-assembly characteristics, which lead to micellisation in aqueous solutions, are directly related to their segmental incompatibility.

The formation of micelles occurs when a block copolymer is dissolved in a liquid which is a good solvent for one block and a precipitant for the other in terms of thermodynamics. Micelles are formed by association of molecules in a selective solvent above a specific concentration well known as Critical Micelle Concentration (CMC), which is usually determined from the sharp decrease in the surface tension as a function of concentration. Micelles are a stable system. When micelles are formed, thermodynamically equilibrium is reached. A typical structure of a micelle is illustrated in Figure 1.2 (Hamley, 1998). The micellisation is one of the most important and useful properties of block copolymers. The micellisation of block copolymers resembles but is inherently more complicated than that of the conventional surfactants of low molecular weight (Nagaranjan, 1996).
Figure 1.2 Schematic of a block copolymer micelle

It is generally believed that at very dilute concentration, the poloxamer molecules exist as unimers that are unassociated with each other. As the concentration increases, the surfactants change to a self-assembled or self-associated state with a swollen core of the insoluble blocks surrounded by the hydrophilic chains which form the corona. As the concentration increases even further, micelles themselves will associate with each other and form gels. However, the gel formation and its properties are beyond discussion in this study.

One of the most useful properties of the micellar associates is their ability to enhance substantially the aqueous solubility of hydrophobic substances, which are otherwise hardly or sparingly soluble in water. The enhancement of the aqueous solubility is attributed to the micellar cores which contain the hydrophobic blocks. The hydrophobic blocks which are compatible for the water-insoluble solute can non-covalently bound to hydrophobic drugs and serve as a micro environment and hence increase the solubility (Yokoyama, 1992; Alexandridis and Hatton, 1995; Alexandridis and Lindman, 2002; Torchilin, 2001; Barreiro-Iglesias et al., 2003, 2004; Oh et al., 2004). The presence of the block copolymers shielded the hydrophobic drugs from the ‘hostile’ aqueous solvent and avoided the direct contact of the two phases, making the system thermodynamically more stable.
Aggregates of block copolymers can form in two ways with minor differences in how
the solute is contained inside them. In type (a), the core of the aggregate is formed by
the solvent incompatible solute and hydrophobic block of the copolymer. The core is
covered by the corona formed by the solvent compatible hydrophilic block of the
copolymer and the solvent. In type (b), the core of the aggregate is separated into two
parts with the inner core of the aggregate formed by the pure solute and immediately
surrounded by the outer core region comprised of the hydrophobic block and the solute.
The corona of the aggregate is the same as that in type (a). However, type (a) structure
is thermodynamically more favourable due to the zero size for the solute pool
(Nagarajan, 1999).

Figure 1.3 Schematics of spherical micelles containing hydrophobic substance
The dark lines denote the hydrophobic block while
the light lines denote the hydrophilic block
(a) all of the hydrophobic substances retain in the core of the micelle
(b) the hydrophobic substances interact both with the core and the corona block
(adapted from Nagarajan, 1996)
Poloxamers are versatile pharmaceutical excipients capable of increasing aqueous solubility and stability of drugs (Kabanov et al., 2002). The versatility of poloxamers, resulting from the wide range of molecular weights and PPO/PEO ratio which determines its hydrophilicity, makes it an ideal excipient in a drug formulation. Some characteristics of the formulation such as the blood circulation time, drug release profile and targeting capability can be achieved by choosing the right poloxamer,

1.5 PVP

1.5.1 Applications of PVP
Polyvinylpyrrolidone (PVP) finds a large number of uses in various industries, such as the food industry, cosmetic industry and pharmaceutical industry. Its wide applications in pharmaceutical industry derive from its biological compatibility, low toxicity, stability over a wide pH range and resistance to thermal degradation. Due to its adhesive characteristics, PVP has been extensively used as tablet binder. Its inclusion in tablet formulations exhibit reliable dissolution rates. PVP was also employed to achieve sustained-release profiles of drugs. PVP has also been used in various other formulations to enhance the solubility and hence the bioavailability of the drugs, it is also used in aqueous suspensions, emulsions and dispersions as suspending agent and viscosity enhancer to improve the stability of these formulations. The wide solubility of PVP in organic solvents also makes it popular in the pharmaceutical industry (Robinson et al., 1990; Leuner and Dressman, 2000).

1.5.2 Synthesis of PVP
PVP is synthesised through the polymerisation of N-vinyl-2-pyrrolidone (NVP). The coupling of the unsaturated –CH=CH₂ groups forms the chain of carbon atoms, to which the pyrrolidone rings are attached through the nitrogen atoms. The yielded PVP is in solution form, the solution is then dried to produce PVP in its powder form (Robinson et al., 1990).
1.5.3 Classification of PVP

PVP comes in a wide range, they are commonly distinguished by a K-number which is an indication of the molecular weight. More frequently used ones range from K15 to K90. The approximate molecular weight is given in Table 1.3 as a function of the K values (Leung and Dressman, 2000).

<table>
<thead>
<tr>
<th>K Values</th>
<th>Approximate Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>2500</td>
</tr>
<tr>
<td>15</td>
<td>8000</td>
</tr>
<tr>
<td>25</td>
<td>30000</td>
</tr>
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<td>60</td>
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<tr>
<td>90</td>
<td>1000000</td>
</tr>
<tr>
<td>120</td>
<td>3000000</td>
</tr>
</tbody>
</table>

Table 1.3 Various PVP products and their corresponding molecular weight

1.5.4 Physical Properties of PVP

PVP is soluble in a variety of organic solvents including ethanol, methanol and chloroform. PVP is also soluble in water, but its solubility in water is restricted by the viscosity of the resulting solutions. PVP is stable in either solid or solution form. It is resistant to heat and strong acid. The glass transition temperature of PVP is dependent on the MW, its T_g increases as the MW increases (Sanner, et al., 1983).

1.6 Calorimetry

When a reaction takes place, either chemical or physical, it is almost inevitable that a change in heat content or enthalpy would occur. Calorimetry, which measures such heat change, has found increasing applications in pharmaceutical industry in areas such as discovery, development and characterisation. One of the greatest advantages of calorimetry is that its quantification of the heat change is not restricted to that resulted
from chemical reactions, and hence it is capable of detecting traces of heat produced from physical reactions such as crystallisation and micellisation. Calorimetry measurements allow both thermodynamic (heat output $q$ from a reaction) and thermo kinetic (heat output as a function of time $dq/dt$) analysis (Gaisford and Buckton, 2001; Gaisford and O’Neil, 2006).

1.6.1 Thermodynamics

Work, energy and heat are basic concepts of thermodynamics. The object being studied is referred to as the system, around which are surroundings where measurements are carried out. If a system loses heat, the system is said to have undergone exothermic process; on the other hand, if a system gains heat, the system is said to have undergone an endothermic process. The total energy content of a system is known as the internal energy ($U$). Thermodynamics could not determine the internal energy of a system but can only measures the changes of internal energy of such system.

1.6.1.1 First Law of Thermodynamics

The internal energy of a system can be changed through heat or work. If a system is isolated, neither matter nor energy could be transferred between the surroundings and the system, the internal energy of such system is constant. In other words, unless work has been done or heat has been transferred, the internal energy of a system is constant, it is expressed as:

$$\Delta U = U_f - U_i = q + w$$

where $\Delta U$ is the change in internal energy, $U_i$ and $U_f$ are the internal energies of a system at its initial and final states respectively, $q$ is the heat being transferred to and from the surroundings, $w$ is the work being done by the system.

1.6.1.2 Second Law of Thermodynamics

Entropy ($S$) indicates the degree of disorder of a system. The more disordered a system is, the higher the entropy. The entropy of an isolated system must increase mathematically during a spontaneous process.
\[ \Delta S_{\text{tot}} > 0, \]

where \( \Delta S_{\text{tot}} \) is the total entropy of a system.

### 1.6.1.3 Third Law of Thermodynamics

The third law of thermodynamics define the conditions which must be met for a process to take place spontaneously and it is expressed as:

\[ \Delta G = \Delta H - T \Delta S \]

where \( G \) is the Gibbs energy, \( H \) is the enthalpy, \( S \) is the entropy, \( T \) is the temperature and \( \Delta \) indicates the measurable changes.

\( \Delta G \) must be negative if a spontaneous process is to occur. As \( \Delta S \) must be positive, a spontaneous process can easily take place if \( \Delta H \) is negative, in this case, such process is enthalpically driven; however, even when \( \Delta H \) is positive, if the contribution from \( \Delta S \) can overcome the increase in \( \Delta H \), the process could still take place spontaneously, such process is called entropically driven (Gaisford and O’Neill, 2006).

### 1.6.2 Classification of Calorimetry

Calorimetry can be classified into three types, depending on their measuring principles (Wadsô and Goldberg, 2001; Clas et al., 2002; Gaisford and O’Neill, 2006).

#### 1.6.2.1 Power Compensation Calorimetry

As the reaction takes place in the sample, heat is given out or absorbed. In order to maintain the samples at a constant temperature, heat is added to or removed from the sample through electrical power. The heat output from the sample is equivalent to the electrical power supplied. A typical power compensation calorimetry is the power compensation DSC.

#### 1.6.2.2 Adiabatic Calorimetry

In adiabatic calorimetry the system is isolated, no heat exchanges between the vessels and its surroundings. Therefore, the heat content of the sample changes when a reaction
takes place, causing an increase or decrease in temperature in the vessel. The change in heat is thus equal to the change in the sample heat content throughout the process and an experimentally determined calibration constant, which is pre-determined by electrical calibration.

1.6.2.3 Heat Conduction Calorimetry
In heat conduction calorimetry, the sample is surrounded with heat-sinks, which are designed to help maintain the system at a constant temperature. Thermopile walls are placed between the sample vessels and the heat-sinks. The thermopiles, through which the heat flows to / from the heat sinks, generate a voltage signal proportional to the heat flow. The voltage signal is then amplified, multiplied by the cell constant, which is determined by the electrical calibration. The power signal was then recorded as a function of time.

1.6.3 Instrumentation
There are many types of calorimetry, each of which has its own features designed to suit different needs. They include: a) Ampoule Calorimetry; b) Batch Calorimetry; c) Flow Calorimetry; d) Titration Calorimetry; e) Gas Perfusion Calorimetry; f) Solution Calorimetry; g) Differential Scanning Calorimetry (Gaisford and O'Neill, 2006).

Some of the instruments listed above have been employed in this study. The operation principle of the calorimetry which have been employed in this study is discussed in detail in Chapter 2, and the differences between each type of calorimetry are not illustrated here.

1.6.4 Applications of Calorimetry in Pharmaceutical Industry
Calorimetry found increasing application in Pharmaceutical systems because usually only very small amount of sample was required for the study and the high sensitivity of calorimetry enabled the detection of small traces of changes such as the crystallisation and micellisation of surfactants.
1.6.4.1 Compatibility
The compatibility between drug and excipients can have significant influence on the stability and bioavailability of drugs and hence their safety and efficacy. Screening of suitable excipient at an early stage increases the probability of developing a stable dosage form. By subjecting the formulation to thermal analysis, information such as the $T_g$ and crystallisation can be achieved and hence the physico-chemical interaction between the two components can be evaluated (Schimitt et al., 2001; Verma and Garg, 2005).

1.6.4.2 Measurement of Binding Enthalpy/Stoichiometry/Affinity
Calorimetry has also been used to measure the enthalpy associated with an interaction and hence to provide rich thermodynamic information. It can be used to determine the binding constant, $K_b$ and the enthalpy of an interaction. Such parameters can then be used to evaluate the thermokinetic model the interaction follows, to calculate the $\Delta G$ and $\Delta S$ (Wesemeyer et al., 1993; Ladbury and Chowdhry, 1996; Doyle, 1997; Leavitt and Freire, 2001; Gaisford and Buckton, 2001).

1.6.4.3 Stability Assessment
The stability of a formulation is essential as it ensures the safety and efficacy of the drug. The stabilities of the components in a formulation individually and in combination are of equal importance. Calorimetry enables the prediction of the long-term stability of a formulation by short-term experiment, avoiding of the time and labour consuming trials which are also expensive (Oliyai and Lindenbaum, 1991; Koenigbauer et al., 1992; Zaman et al., 2001).

Calorimetry could also be employed in other areas such as quantification, characterisation and rational drug design (Gaisford and Buckton, 2001; Gaisford and O’Neill, 2006). With the continuous improvement in the instrument and the technique, calorimetry will undoubtedly find even greater application in pharmaceutical industry.
1.7 Aims

Most of the drugs that have proven to have desirable therapeutic effects are water insoluble or practically insoluble in water. How to improve the solubility of these compounds is of fundamental importance. Upon achieving the solubility, many other essential aspects can also be improved or attained, such as the bioavailability and stability. The interactions between the drug and the excipient could have marked impact on the solubility and stability of the drug. It was agreed that by introducing surfactants, the solubility of the drug can be pronouncedly improved and as a result, optimising the whole formulation. However, this can only be achieved when the right surfactant is chosen and is added at the right amount. In this study, polymer is chosen as the excipient.

Traditionally, researchers have been following the tedious yet inefficient way to optimise formulations by first preparing numerous formulations with different combinations of excipients and at various concentrations, stability tests are then carried out. In this way, a lot of time and labour is consumed and a large amount of substance is required. This is extremely inappropriate if some expensive material in included, yet it is also possible that the optimum combination could be missed out. The many considerations, such as the method of manufacture, selection of component material, and the effect of environmental factors, such as temperature and pH has made this process even more difficult. In addition, the very few successful examples of novel formulations and the substantial time and money that have been invested in have made it daunting for scientists in these areas (Lieberman et al., 2006). However, a reliable and efficient technique is strongly required to replace the traditional trial and fail method to avoid of the wastage of time, labour and expenses in order to carry out the formulation development process in a more reasonable and systematic manner.

Calorimetry is chosen as the main technique to study the interactions between drug and excipients in a thermodynamic way in order to investigate how to best exploit the
excipients(s) to maximise the stability of a drug in a formulation by carrying out studies of the interactions between the drugs and excipients.

Liquid systems are chosen for this study. As mentioned at the beginning of this chapter, they are more challenging due to their complicated nature, inadequate techniques and also the lack of understanding when compared to the gaseous and solid systems. A lot of effort needs to be put in to understand the liquid system as it would contribute significantly to the pharmaceutical science. Two liquid systems are chosen for the study. Suspension is chosen as the first model for its broad application and water is chosen as the medium as it is the most commonly used solvent in pharmaceutical and biological systems and also because of its simple nature. The second model is a solid dispersion system. This study is to be carried in the liquid state before the drug-excipient mixture subjects to the solvent removal process which turns the mixture into solids. This system comprised of organic solvent and is a three component system. This is a much more complicated system compared to the suspension system in the first part of this study, in which a two component system is dispersed in water.

The overall aims of the thesis were:

- To enhance the understanding of the association behaviour of poloxamer in water and how it may be used to improve the stability of suspensions containing hydrophobic drugs by decreasing their sedimentation rate via adsorption
- To enhance the understanding of the interaction mechanisms between hydrophobic drugs and polymer(s) in solid dispersions and how polymer(s) maybe be used to improve the physical stability of poorly water soluble drugs
- To investigate the possibility of predicting the properties of solid dispersions by studying the system in solution during manufacture in order to build up a connection between the solid and liquid properties of a formulation
Chapter Two

Methods and Materials
2.1 METHODS

2.1.1 Calorimetry

The word calorimetry is composed of two parts, the former half of the word, calor, which derived from Latin, meaning heat; and the second half of the word, metry, which derived from Greek, meaning to measure. Calorimetry is the measurement of the amount of heat involved in a process. Heat generation (exothermic processes) or consumption (endothermic processes) exists in chemical and/or physical reactions. As a result, calorimetry has found wide application in various fields including microbiology, biochemistry, chemistry and the pharmaceutical industry (Wadsö, 1997a and b; Gaisford and Buckton, 2001; Clas et al., 2002; Gaisford and O’Neill, 2006). Since the advent of calorimetry in the mid 18th century, a number of techniques have been developed to serve different needs under different conditions and have aroused a lot of interests in calorimetric studies. Below are introductions to the different types of calorimetry used throughout this study.

2.1.1.1 Isothermal Titration Calorimetry (ITC)

2.1.1.1.1 Introduction

Isothermal Titration Calorimetry (ITC) is a technique that can directly monitor reactions in real time and at constant temperature. ITC measures the differential enthalpy changes when either a physical or a chemical reaction occurs. ITC is rich in thermodynamic information and has the advantage of general applicability. It is anticipated that ITC will play an expanding role in understanding of chemical reactions and the properties of chemical substances (Gaisford and Buckton, 2001; Gaisford and O’Neill, 2006). The titration unit is within the Thermometric 2250 range of Micro Reaction Systems. The Thermometric 2250 range can be used for applications such as titration, continuous perfusion and controlled relative humidity (RH) perfusion experiments (Thermometric AB, 1993). Throughout this work, only the titration application is discussed.

2.1.1.1.2 Principle

The titration unit is designed specifically for use in a standard calorimetric unit
operating in the Thermometric 2277 Thermal Activity Monitor (TAM, Thermometric AB, Järfälla, Sweden). The TAM has four sets of twin channels and therefore is capable of running four experiments simultaneously. Each set of channels includes one reference ampoule and one sample ampoule. In this work, two stainless steel ampoules of 4mL capacity were used. Both the reference and the sample ampoules are loaded with the same solution of the same amount and fitted into the TAM. The main tube which houses the titration shaft is the back bone of the system. It is a straight hollow tube with a smooth inner surface while the outer surface has alternating thin and thick wall sections. A cylindrical heat sink is attached to each of the thick wall sections. After the titration unit is installed in the TAM, which operates under isothermal and isobaric conditions, the upper heat sinks are closely thermally connected through the stainless steel neck tubes of the calorimetric measuring unit with the water in the precision thermostat and the lower heat sink is in thermal contact with the main heat sink. A liquid titrant is then added to the reaction ampoule through a fine bore cannula housed by the shaft using a motor driven syringe pump under computer control following a pre-programmed schedule. A stirrer, fitted to the lower end of the shaft is rotated at the speed of 30 rpm during the titration experiments. The experiment is set up using interactive computer software Digitam®, which controls the injection schedule (number of injections, volume of each injection and time intervals between injections), titration data collection and data analysis. The heat conduction from the vessel to the heat sink requires quite a large time constant and this can be a big disadvantage. However, with the use of a “dynamic correction”, this shortage can be overcome. With this solution, it is possible to shorten the duration of the experiment dramatically without any loss of accuracy, especially for a series of titration experiments. Dynamically corrected data were chosen for analysis throughout this study. Dynamically corrected data will be discussed in greater detail in the following section.

The Isothermal Titration Calorimeter operates at 25.00±0.01°C. When any thermal energy changes occur in the sample, a small temperature difference is created relative to the heat sink surrounding the ampoule. As a result, the heat flows either to or from the
heat sink which is maintained at a constant temperature. Very sensitive thermopiles are
placed around the sample ampoule to measure and quantify the heat flow which is
directly proportional to the temperature difference created. After each injection, the
energy required to offset the temperature differences induced by the injection is
measured. The potential generated in the thermopiles is then amplified and recorded as a
signal, which is integrated by the computer-based control unit to yield the heat \( q \)
associated with each injection and is usually recorded as a function of time
(Thermometric AB, 1993; Wadsö and Goldberg, 2001; Gaisford and O’Neill, 2006). If
the reactions reach equilibrium, enthalpy and the rate constant of the interaction can be
obtained from the calorimetry data and the \( \Delta S \) and \( \Delta G \) can be easily determined using
the third law of thermodynamics.

### 2.1.1.3 Dynamically Corrected Data

When any interaction takes place, the heat released (or absorbed) in the reaction sample
is quantitatively exchanged with the surrounding heat sink through the thermopiles. The
thermopiles then generate an electrical signal which is proportional to the heat flow
running through the thermopiles. The electrical potential \( U_e \) is then multiplied by a
proportionality constant \( (\varepsilon) \), which is determined by an electrical calibration, to give the
raw power signal \( (P_R) \)

\[
P_R = \frac{dq}{dt} = \varepsilon U_e
\]

and

\[
U_e = g \Delta T = g (T-T_0)
\]

where \( g \) is the Seebeck coefficient \((V K^{-1})\), which stems from the thermopiles from
which the electrical potential is generated; \( T_0 \) is the equilibrium temperature of the
vessel before the reaction takes place and \( T \) is the temperature of the vessel after the
reaction takes place. From this:

\[
T = \frac{U_e}{g} + T_0
\]

And the time derivative of \( dT \) is given by \( dT/dt = (dU_e/dt)/g \)

However, there is always a time delay when the measurement of the heat released or
adsorbed from the interaction was made. For example, when an exothermic reaction
takes place in the ampoule, heat is released and causes the temperature in the ampoule
to rise, which is dependent on the capacity of the ampoule C. As the temperature of the sample ampoule rises, a temperature gradient is created between the ampoule and the heat-sink and heat flow between the two was generated in order to restore the thermal equilibrium. The rate of the heat transfer between the two is dependent on the heat transfer coefficient $k$ (W K$^{-1}$). Therefore, the total heat generated from the reactions equals to the heat accumulated in the ampoule and the heat transferred to the heat sink which gives the raw power signal. Hence:

$$\frac{dq}{dt} = k(T-T_0) + C \frac{dT}{dt}$$

From the discussion above, the equation above can be constructed:

$$\frac{dq}{dt} = U_e k / g + (dU_e / dt) \times (C / k)$$

By defining $\varepsilon = k / g$ and $\tau = C / k$, where $\tau$ is the time constant of the instrument, the equation above can be reconstructed as:

$$\frac{dq}{dt} = \varepsilon (U_e + \tau dU_e / dt)$$

As mentioned earlier in this section, $P_t = \varepsilon U_e$, dynamically corrected power signal $P_c$ is obtained by:

$$P_c = P_t + \tau dP_t / dt$$

(Wadsö, 1997a; Gaisford and O’Neill, 2006)

2.1.1.4 Method

Samples were equilibrated in a stainless steel ampoule of the isothermal calorimeter (Thermal Activity Monitor, TAM, Thermometric AB, Järfälla, Sweden) at 298.15K. After loading the titration unit down the channel, the ampoule sat in the measuring site of the calorimeter until a stable baseline was obtained. When the signal difference over 8 hours was not more than \(\pm 1.0\mu W\) and the baseline noise was not more than \(\pm 0.5\mu W\), the baseline was considered to be stabilized. Titrant was then injected from a 500 \(\mu L\) gas-tight motor driven syringe. Blank experiments were undertaken to allow for the correction of the heat produced from the physical reaction when droplets were injected into the ampoule. The heat change for each injection was recorded and measured as power as a function of time. The dynamically corrected results for the enthalpy change were then analysed using the software Origin® (Patel, 2000; Gaisford, 1997). The
external appearance of the TAM, the schematic diagrams of the titration unit, the diagram of the TAM, the close-up view of the thermopile arrangements and the schematic view of the calorimetric chamber are shown in Figure 2.1-2.4 respectively.

Figure 2.1 The External Appearance of TAM (above; taken from Thermometric AB, 1993)

Figure 2.2 Schematic diagram of the titration unit (on the right; taken from Thermometric AB, 1993)
Figure 2.3 A diagram of the TAM, and a close-up view of the thermopile arrangements.

Figure 2.4 A diagram of the TAM, a schematic view of the calorimetric chamber.
2.1.1.1.5 Pump and Syringe Testing

As introduced in section 2.1.1.1.4, the ITC experiments were carried out by injecting the titrant from the gas-tight 500μl syringe to the ampoule which contained the titrand. To avoid any unnecessary artefacts brought to the data analysis, a pump test was carried out to see if the amount of each injection was constant. Distilled deionised water was injected through the same syringe driven by a pulse free motor (Lund pump, Thermometric AB). An injection of 15μl of water was delivered to a beaker covered by parafilm to prevent evaporation during the test. The weight of the beaker was recorded after each injection. Before the experiment started, the beaker was filled with a small quantity of water, the balance where the beaker sat on was then tared. Hence, the reading weight reflected the water added to the beaker only. The cannula which attached to the tip of the syringe was inserted beneath the water surface. All experiments were carried out in an air-conditioned room at a temperature of 20°C. This experiment comprised 30 injections. The weight of water after each injection is shown in Figure 2.5 for inspection.

![Pump and Syringe Test](image)

**Figure 2.5 Pump and syringe test**

\[ y = 0.0151x + 0.0005 \]

\[ R^2 = 1 \]
Figure 2.5 showed a well-fitted linear trendline going through all data points, indicating each injection has equal weight with little error. As the equation implied, the average weight of 15μl water in one injection was 0.01515g, therefore, the experimental density of water was 1.01g·mL⁻¹, which correlated well with the literature value 0.998207g·mL⁻¹ at 20°C (Harris, 1995). It was concluded that the amount of water delivered in each injection was accurate and both the pump and the syringe were suitable to use in the experiments thereafter.

2.1.1.1.6 Control Experiment

Control experiments were carried out when distilled deionised water was titrated into the ampoule containing 2mL of the same water in aliquots of 15μl in order to determine the physical reaction enthalpy from titration. Each experiment comprised 30 injections and each experiment was carried out three times. The results were analysed using software Origin® (MicroCal Inc., USA) and the mean enthalpy involved in each injection was then determined. The power-time data of the control experiment is shown in Figure 2.6, a few injections were chosen for closer examination and are shown in Figure 2.7.

![Figure 2.6 A typical Power-time data set for dilution of water into water using Isothermal Titration Microcalorimetry at 25°C](image-url)
The purpose of carrying out this experiment, as mentioned above, was to determine the enthalpy involved in each injection resulting from the physical interaction when the titrant in the syringe was titrated to the same solution. As can be seen from Figure 2.7, the noise in the baseline interfered with the data analysis, which made the integration of the area under the peak difficult, and error was hence introduced to the data analysis. Since what was measured was the result of the physical interaction when a drop of liquid was titrated into the same solvent, the result of this study should be taken into account in all the following studies carried out in ITC. The average enthalpy from all 30 injections was 73.3 ± 8.7\(\mu\)J. Also, judging by Figure 2.7, the heat signal produced from each injection seemed to be even despite the noise present and its capability of detecting such small heat signal demonstrated the strength of ITC for this study.

Figure 2.7 Enlarged image of the power-time data presented in Figure 2.6 for dilution of water into water
2.1.1.2 Micro Solution Ampoule

2.1.1.2.1 Introduction

The Micro Solution Ampoule is used to measure the heat and kinetics of dissolution when a solid compound is dissolved in a liquid.

The Micro Solution Ampoule is utilised with a 20 mL Twin Ampoule Calorimetric Unit (2265) operating in the Thermometric 2277 Thermal Activity Monitor (TAM, Thermometric AB, Järfälla, Sweden). The main tube, similar to that of ITC, also has three cylindrical heat sinks attached at equal intervals. During the equilibrium state and experiment, these heat sinks are directly in contact with the stainless steel neck tubes of the calorimetric unit, which are also in direct contact with the water in the TAM thermostat. The stirrer, differs from that of the ITC in that it is a combination of turbine and propeller. The stirrer is attached to the lower end of the stirrer shaft housed in the hollow main tube. The distance between the turbine and the propeller can be adjusted by a screw thread to obtain optimum distance and therefore for better stirring effect. The whole stirrer, including the turbine and the propeller, is immersed in the solution in all experiments. A 2277-401 Stirrer Power Supply motor, located into the top of the flow divider which fits inside the top of the main tube, is used to drive the stirrer shaft. The Micro Solution Ampoule is illustrated in Figure 2.8.

The 20 mL ampoule comes in stainless steel and glass. The glass ampoule in some cases are more advantageous since some of the chemicals might be corrosive to the stainless steel ampoule. The disadvantage is that the glass ampoules are also more fragile and easy to break. In this study, stainless steel ampoules are chosen as the materials used in this study are not corrosive.
Figure 2.8 Schematic Diagram of the Micro Solution Ampoule
(Adapted from Thermometric AB, 1996)
Solid samples are loaded in the cartridges which are hidden into the underside of the ampoule lid before the experiments are initiated. Up to three solid samples can be loaded in the three individual cartridges in each experiment, as shown in Figure 2.9. Each cartridge holds solid sample for up to 40μL. After the instrument has reached equilibrium, the experiments are initiated by injecting the cartridges into the constantly stirring solvent. The sample cartridge, as shown in Figure 2.10, consist of one base cap and two cartridge sides, all of which form a tube for holding the samples. The base cap is sealed with an O-ring to keep the samples from exposing to the solvent. As seen from the illustrations, liquid would not be suitable as solute for this technique. The base caps and the cartridges sides are all marked with either I, II or III, and are designed for the corresponding cartridges. The same cartridge is injected from the same position each time, therefore, the friction so produced is the same and can be offset from each experiment; as a result, the systematic error is minimised. The samples can be released by pushing the cartridge plungers which are housed in the ampoule lid and controlled by the injector (Thermometric AB, 1996).

2.1.1.2 Principle and Method

As both the Micro Solution Ampoule and the ITC operates on the same heat conduction principle, the operation of the two techniques is very similar to each other; therefore, the principle and method of Micro Solution Ampoule are not discussed here. Note that the volume of sample used in this study was 15ml as recommended by the manufacturer and the stirrer was operated at 60rpm.
2.1.1.3 Differential Scanning Calorimetry (DSC)

2.1.1.3.1 Introduction

Differential Scanning Calorimetry (DSC) is one of the most widely used thermal analytical techniques. DSC is often employed to measure the reaction enthalpy or to characterise the transition temperature of a reaction or a transition process. DSC has found application in a number of fields including the characterisation of materials such as polymers and proteins, quality control, the identification of substances on their own and in a mixture, stability investigations, phase transition investigations and purity determinations. (Clas et al., 2002; Höhne et al., 2003; Gaisford and O'Neill, 2006). In this work, a DSC for fluids was employed to better suit the purpose of the work

2.1.1.3.2 Principle

The reference and sample ampoules are maintained at nearly the same temperature throughout the experiment. Heat is supplied to the sample cell and the reference cell linearly as a function of time when they are not at equilibrium. The difference is measured between the heat required to raise the temperature of the reference and the sample ampoules as a function of temperature. When a sample is undergoing an endothermic or exothermic reaction, more or less heat is required to raise the
temperature of the sample relative to that of the reference. By measuring the difference between the two, the energy generated or consumed during this reaction and/or the characterisation temperature of a process is known (Höhne et al., 2003; Gaisford and O’Neill, 2006).

The fluid DSC MC-2 employed in this study is a product of MicroCal, Inc. Two 1.2 ml coin shape cells are used as reference and sample cells instead of pans which are normally used for solid samples. A resistant cascade is employed to control the scanning rate. The cells are surrounded by an aluminium jacket seated in an adiabatic chamber in order to obtain high sensitivity. The jacket is powered by the main heater and the feedback heater which is driven by the thermopile sitting between the cells and the jacket to ensure that the jacket temperature is always the same as that of the cells. The signal (0-100 microvolt) between the cells and the jacket is amplified and then fed back into the feedback heater. If heat is required to supply to the cells, the temperature of the jacket is increased accordingly to keep up with that of the cells. Each cell is surrounded by its own set of heaters, the main and the cell feedback (CFB) heater. Another thermopile is placed between the two cells in order to record the off balance ΔT between the two and the voltage is produced based on the ΔT recorded. The voltage (0-5 microvolt) is then amplified and fed back to the cell feedback heaters (CFB) on the sample side, which drives the off-balance ΔT to zero. The auxiliary heater on the reference side, the corresponding CFB of the sample cell, gives the corresponding signal proportional to the differential power (dCp) when supplied with a voltage signal (MicroCal, Inc., 1993). Therefore, a typical display of a DSC scanning would be a record of the differential power as a function of the temperature of the reference cell and the sample cell. The coolant shown in the graph is for down scanning purpose, however, as down scanning was not included in this study its principle is not described here. A simplified schematic diagram of the fluid DSC chamber is shown in Figure 2.11 (MicroCal Inc., 1993).
2.1.1.3.3 Calibration

Before any experiments are carried out, calibrations are required to ensure the accuracy of the results. Two sets of calibrations were carried out, the electronic calibration and the chemical calibration.

**Electrical Calibration:**

The electrical calibration was carried out by supplying a precisely known current, \( I \), through a wire of precisely known resistance, \( R \), located very close to the calorimetric channel, for a given period of time. The total heat generated can be calculated by:

\[
Q = IRt
\]

The heat generated hence initiated the generation of an electrical signal \( (U_e) \) which is proportional to the heat flow and a raw calorimetric signal \( P_r \) is produced by multiplying \( U_e \) with a proportionality constant \( (\epsilon) \), which is experimentally determined:

\[
P_r = dq/dt = \epsilon U_e
\]

And the characteristic form of calorimetric data \( dq/dt \) is obtained.
Chemical Calibration:
The chemical calibration was carried out by subjecting a 0.1% (w/v) 4-nitrotoluene solution (dissolved in acetonitrile) to DSC examination and its transition temperature was recorded as 53.7 ± 0.1 °C (n = 5). The transition temperature was considered to have correlated well with the literature value 51.61°C (Price, 1995). The difference from the literature value was considered to have resulted from the systematic error.

2.1.3.4 Method
Since only fluid samples are used in this DSC, special precautions should be taken when compared to the conventional DSC which handles solid samples. When handling aqueous samples, it is almost inevitable that air bubbles are produced when extracting or releasing the samples through the syringe. If air bubbles exist in the samples, they would certainly bring unpredictable interference to the results when undergoing a heating up process. To prevent this, a degassing system is introduced. The degassing system is used to obtain optimum sample preparation.

A degassing system consists mainly of a vacuum pump and a magnetic stirrer. Before introduced to the cells, samples are placed in the vacuum jar and with a magnetic stirrer inside the sample container. Samples are stirred for 5 minutes with the vacuum pump on. Being agitated, the air bubbles are forced to come out of the solution and are removed by vacuum. The degassed sample is then loaded slowly through a syringe to the sample cell to avoid the formation of any air bubbles. When approximately 10μl of sample was remained in the syringe, a thrust is applied to the plunger to load the remaining sample to the cell over a very short period of time to force the bubbles formed during the sample loading out from the cell.

After the samples are properly degassed and loaded, the initial temperature, the final temperature, and the scan rate (°C/hr) are chosen. The samples go through a pre-start and then a post-start section before actually undergoing the scanning process. When the pre-start section starts, the solenoid valves of the cells situated inside the cell chamber
will open and the coolant from the external water bath will run through the jacket for the assigned time. The external water bath is set at a lower temperature to allow the scan starting from a more appropriate temperature and it enables the samples to cool faster when the scan finishes. This allows the experiments to run more efficiently. The post-start section starts after the pre-start section elapses, and the solenoids of the cells close and the heaters start supplying heat to the cells. The temperature during this section would approach the initial temperature but will get no higher than that. The purpose of the post-start section is for the cells and the jacket to reach equilibrium after the acute disruption caused by the coolant and the following heat supply before they undergo scanning.

During this study, the samples were degassed before being loaded into the cells. The scans started at 15°C and ended at 80°C at a scanning rate of 60°C/hr. The results are analysed using Origin® software (MicroCal, Inc., USA).

2.1.2 Surface Tension Measurement

2.1.2.1 Introduction

The CAHN Dynamic Contact Angle Analyzer (DCA-312) was employed for the surface tension measurement throughout this study. The principle behind this technique is the Wilhelmy plate method. This method has the advantage of measuring the surface tension over a long period of time when compared to Nouy Ring (Adamson, 1990; Tsujii, 1998).

2.1.2.2 Principle

In the bulk of the liquid, each molecule is under the force from the neighbouring molecules and the forces are equalised in all directions. As a result, the net intermolecular force is zero. Whereas the molecules at the surface are subject to very little attraction from the vapour but at the same time are pulled inwards by the molecules from the bulk, therefore, excess energy is induced and surface tension is formed. Figure 2.12 illustrates the molecules at the surface and in the bulk of the
solution. The molecules in the upper layer represent the molecules at the liquid-air interface while the molecules in the lower layer represent molecules in the bulk. However, it should be noted that the molecules very near the surface also experience imbalance of the forces from the vapour and the molecules from the bulk, and consequently the surface of the solution should be considered as a layer of two or three molecules thick rather than a monomolecular layer. This layer, namely, the surface of the solution tends to contract simultaneously and form a spherical shape under the drawing force from the bulk (Pickett, 1977). Surface tension, measured in Newton per meter (N·m⁻¹), is represented by the symbols σ, γ or Γ (Tsujii, 1998).

The measurement is carried out using Wilhelmy plate method, which most often is a platinum plate, a microscope cover slide or a thin wire. Microscope cover slides were employed throughout this study. The principle of the surface tension measurement using Wilhelmy plate is illustrated below in Figure 2.13. A probe is suspended from a balance and is kept immobilised before an experiment starts. The weight of the probe $W_{\text{plate}}$ is then determined. When the probe is in contact with the solution, a meniscus is formed and there forms the force $F_\gamma$ from the meniscus pulling the probe into the liquid. The weight of liquid directly under the probe is omitted. The force the balance detected is expressed as:

$$W_{\text{tot}} = W_{\text{plate}} + F_\gamma \cos \theta \cdot p$$

Where $\theta$ being the contact angle between plate, liquid and air; $p$ being the perimeter of
the plane of the cover glass being in contact with the liquid. Provided the probe is
cleaned to the maximum extent and since nearly all the liquid has a lower surface
tension than water, contact angle $\theta$ may be considered as zero for the convenience of
surface tension determination (Bikerman, 1958; Adamson, 1990; CAHN Instrument,

![Figure 2.13 Illustration of the Wilhelmy plate method principle](taken from CAHN Instrument Inc., 1990)

**2.1.2.3 Method**

The solution for study was poured into a clean beaker seated in the water bath in the
inner chamber right beneath the sample stirrup. Measurement should not start until after
the sample solution has reached the desired temperature which is pre-set as the water
bath temperature. The length and the width of the side of the cover glassing going to be
in contact with the solution are precisely measured. The values obtained are then used to
calculate the perimeter of the cover glass. The cover glass is then flamed carefully using
the oxidizing blue flame before measurement to acquire perfect cleanliness. Cover glass
is then allowed to cool down on the stirrup before the experiments start. The solution
position is adjusted so that the surface of the solution sample is reasonably close
(approx. 4-5 mm) to the lower level of the cover glass. A minimum distance of 4 mm is
required to ensure a clean break of the meniscus upon the contact of the liquid and the
cover glass. The sample perimeters (mm), calibration weight value (mg) and the wetting
medium surface tension (N·m⁻¹) are then input in the software for the calculation of contact angle or surface tension. The platform where the solution sample sits on is then moved upwards through a designated distance (10mm) to be in contact with the solution at the speed of 151.7 μm·sec⁻¹. The designated distance indicates the absolute distance relative to the starting point of the platform at the beginning of the run. The platform moves down to its initial position after it reached its designated distance. The weight changes of the cover glass are recorded during the run, the data points are collected every second. The advancing and receding surface tension are measured when the cover glass enters and leaves the solution respectively.

**Calibration**

Calibration with water is carried out to ensure the accuracy of the measurements. HPLC grade water was used for calibration. The surface tension of water at 20.0°C in literature is listed in Table 2.1, and the experimental results at the same temperature carried out in this study are listed in Table 2.2. Since the results obtained correlated with the literature values, all the following measurements are carried out with samples prepared in HPLC grade water. Default calibrations were carried out on a daily basis before measurements.

<table>
<thead>
<tr>
<th>Reference Source</th>
<th>Surface Tension (N·m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lide (2005)</td>
<td>72.75</td>
</tr>
<tr>
<td>Bikerman (1958)</td>
<td>72.58-72.91</td>
</tr>
<tr>
<td>Davies and Rideal (1963)</td>
<td>72.8</td>
</tr>
<tr>
<td>Gaonkar and Neuman (1984)</td>
<td>72.94</td>
</tr>
</tbody>
</table>

**Table 2.1 Literature values of water surface tension at 20.0°C**

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Mean ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface tension (N·m⁻¹)</td>
<td>72.5</td>
<td>72.8</td>
<td>72.8</td>
<td>72.7 ± 0.17</td>
</tr>
</tbody>
</table>

**Table 2.2 Surface tension values of HPLC Grade water at 20.0°C**
As soon as the cover glass is in contact with the solvent, a huge rise appears in the force detected by the balance. As the cover glass moves further down, a slight decrease in the force is detected because of the increasing buoyancy. An advancing and a receding value are obtained when the experiment finishes. Often the liquid does not spread smoothly onto the cover glass as it advances to the solution, an uneven meniscus is formed and the advancing value is invalid. Hence, the receding values are used for calculation because the values are determined under the circumstances when the contact angle is closest to 0 and they show better reproducibility and are more reliable. Data are considered valid only when the advancing and the receding curves retrace each other.

Results are obtained through the software based on:

\[ F = \gamma \times P \times \cos \theta / 0.981 \]

Where F (N) is the force detected by the balance, 0.981 is a constant of conversion factor between weight and force and \( \gamma \) as the Surface Tension (N·m\(^{-1}\)).

2.1.3 Gel Permeation Chromatography (GPC) / Size Exclusion Chromatography (SEC)

2.1.3.1 Introduction

Gel Permeation Chromatography (GPC) is used for the separation of large molecules like proteins, polymers and poly-saccharides based on their molecular size. Polydispersity in these molecules can affect their performances and the information on the size distribution can be essential. GPC is widely used because this technique does not change the nature of the substances during analysis, it evaluates the properties of the samples as they exist in the solution when subject to analysis. This merit of the techniques is extremely important when analysing proteins.

The polydisperse phase detected by the technique is described by three parameters, which are (i) the number average, \( M_n \); (ii) the weight average, \( M_w \) and (iii) z-average, \( M_z \). When \( M_n = M_w \), the substance is monodisperse. In other words, the molecules in the sample have the same molar mass. \( M_w / M_z \) is used to describe the polydispersity of
the molecules existing in the sample.

\[
M_n = \frac{\sum N_i M_i}{\sum W_i}
\]

\[
M_w = \frac{\sum M_i W_i}{\sum W_i}
\]

\[
M_z = \frac{\sum W_i M_i^2}{\sum W_i M_i}
\]

Where \(N_i\) is the number of molecules of molecular weight \(M_i\) and \(W_i\) is the weight or concentration of \(M_i\). The ratio of \(M_w/M_n\) or \(M_z/M_w\) indicates the distribution range (Raghavan and Joseph, 2002). GPC is mainly used to determine the polydispersity of polymers soluble in organic solvents. The range of the sizes and the efficiency of separation are determined by the types of gels, the method of packing, the number of columns containing gels, the mobile phase and the operation conditions of the instrument (Billingham, 1977b; Holthuis and Driebergen, 1995; Raghavan and Joseph, 2002).

2.1.3.2 Principle

The sample is dissolved at a low concentration ranging from 0.1 to 1.0% and is then injected into a stream of pure solvent which runs through a set of columns containing porous gels with a range of different known porous sizes. At the beginning, the molecules with a smaller size are stuck in the smaller pores and are held back by the penetration in the pores; whilst the bigger molecules are too big to enter the smaller pores and are pushed forward by the mobile phase solvent. The remaining molecules go through the same process and are subdivided into different categories according to their sizes and shapes. The bigger molecules not trapped in the gel pores come out of the column first followed by smaller molecules. A detector is placed at the end of the column set to detect the size and the concentration of the polymer molecules (Billingham, 1977a; Holthuis and Driebergen, 1995).

2.1.3.3 Method

In this study the poloxamer characterisation was performed by the Gel Permeation Chromatography (Viscotek, USA). 0.2mg of each poloxamer was dissolved in 1ml of DMF (Dimethyl-formamide). The poloxamer solutions were filtered through a 0.22μl
filter and 0.3ml of the filtrate was then loaded into the GPC column for measurement.

2.1.4 Surface Area Analysis

The determination of the surface area of powders has been essential in research and in industry. The gas adsorption method has been widely used because of its wide applicability and high sensitivity. BET theory, which is named after Brunauer, Emmett and Teller in 1938, is the most widely used model because of its general applicability and high reproducibility (Rose, 1953; Orr and Dallavalle, 1959; Lowell, 1984; Kaye, 1999; Beckman Coulter Particle Characterisation Group, 2006). The instrument employed in this study for surface area determination is based on this theory.

2.1.4.1 Introduction

The molecules at the surface of a solid are attracted to the molecules inside the solid and therefore are not balanced under the forces from molecules around. In order to stabilise this imbalance situation, the molecules tend to attract the molecules available like gas, vapour or liquid molecules. When gas adsorption happens, that is when the molecules at the surface attract the surrounding gas molecules to balance the force they are borne. Therefore, with known size of the gas molecules, the surface area can be easily calculated by determining the quantity of gas adsorbed. The interaction between the solid and the gas comes in two forms, physical and chemical interaction. The physical interactions between gas and solid are dominated by van der Waal’s forces and are relatively weak. The interaction between the two, i.e., the van der Waal’s forces, can be easily removed at the same temperature when it happens. At a given pressure, the adsorption of gas onto the solid increases as the temperature decreases. Chemical interactions on the other hand are stronger and can not be removed unless the temperature is increased. Provided the pressure is the same, the strength of an interaction decreases as the temperature increases. As a consequence, measurements are made at low temperatures and liquid nitrogen or liquid oxygen is usually employed to allow gas adsorption to take place at low temperature (Orr and Dallavalle, 1959).
2.1.4.2 Principle
Two most widely used theories have been developed based on the principle described above, one of them is the Langmuir theory. The Langmuir theory is developed based on the assumption that the gas adsorbs onto the surface and forms a monolayer only. This theory is the cornerstone for many of the later theories. However, only certain isotherm models conform to the Langmuir theory due to the assumption the theory is based on, which also limited the applicability of this theory, and the BET theory is developed. It is believed that adsorbed molecules can hold more than one molecule and as a consequence, multi-molecular layer of gas molecules is formed. BET theory has found wider application. However, it is important to bear in mind that BET theory is confined to certain models but not all (Rose, 1953; Orr, 1959; Lowell, 1984; Kaye, 1999; Beckman Coulter Particle Characterisation Group, 2006)

The BET theory is formulated on the assumption that i) the energy for a gas molecule to adsorb onto the solid molecule surface is different from that of a gas molecule adsorbing onto another gas molecule, which happens when the solid molecule surface has been pre-occupied by another gas molecule; ii) the attractions between the solid molecule and the gas molecules in different layers are the same iii) the attractions between the solid molecule and the gas molecules are vertical. The BET theory is explained as:

$$\frac{P}{V_{ads}(P_0 - P)} = \frac{C - 1}{V_m C} \times \frac{P}{P_0} + \frac{1}{V_m C}$$

Where $V_{ads}$ is the volume of gas adsorbed at pressure $P$, the sample pressure, $V_m$ is the volume adsorbed when the entire solid molecule is covered by a mono-molecular layer of gas molecules, $P_s$ the saturation vapour pressure of the adsorbate, which is gas in this case, $C$ is the adsorption energy constant.

Results are recorded as the quantity of gas adsorption against the pressure at a given temperature, such plots are termed adsorption isotherms. The plots are mainly grouped into five categories, as shown in Figure 2.14. The Langmuir theory can only fit into Type I, while BET theory has been found to be most applicable to Type II and Type IV.
Figure 2.14 Five types of the typical isotherms
(Adapted from Beckman Coulter Particle Characterisation Group, 2006)
Figure 2.15 Illustration of a simplified instrumentation for investigating solid particle surface area

A, B, C, D, E are mercury reservoirs. V₁, V₂, and V₃ are valves to adjust the inflow or outflow of the air. By adjusting the values, the mercury is raised to the five different levels to change the volume and the pressure inside the sample tube.

(Adapted from Kaye, 1999)

An illustration of simplified instrumentation is shown in Figure 2.15. The apparatus is mainly composed of a flat-bottom tube which holds the sample (a flat-bottom tube is used in this study to hasten the equilibrium process and increase the contact area with cooling medium), a Dewar flask for liquid nitrogen as a bath providing low temperature, a manometer for determining the pressure, a mercury reservoir connected with a vacuum pump to attain high-vacuum condition, burettes for gas volume adjustment and temperature measurement devices.
2.1.4.3 Method

Liquid nitrogen is used because it is available in high purity at reasonable cost. Before the experimental investigation, the powder under study went through an out-gassing process to ensure that the powder has been cleared of all previously adsorbed moisture and gases.

Samples are immersed in the Dewar flask filled with liquid nitrogen at -195 °C after out-gassing. Nitrogen is emitted in doses of known volume to the sample tube when the sample is ready to adsorb the gas molecules. The amount of gas adsorbed is recorded as a function of pressure. The operation, data acquisition and data analysis is achieved by the software provided from the manufacturer. The results are analysed based on the 2nd and 4th isotherm. As shown in Figure 2.14, the linear section of the isotherm was considered to be the monolayer adsorption of gas molecules onto the solid molecule surface, the determination of the slope and the intercept of this part enables the calculation of $V_m$, which is the volume adsorbed when the entire solid molecule is covered by a mono-molecular layer of gas molecules:

$$V_m = \frac{1}{\text{slope} + \text{intercept}}$$

Assuming that the nitrogen molecules pack closely and regularly on the solid molecules surface, the total area hence equals to the number of molecules in the monolayer multiplied by the area that each molecule takes up. Knowing that a mole of gas at saturation pressure occupies 22414ml, 1 mole of gas has $6 \times 10^{23}$ molecules and the nitrogen molecule has an area of 16.2Å², the specific surface area (SA) given in m²·g⁻¹ can be obtained by:

$$SA = \frac{(V_m/22414) \times (6 \times 10^{23}) \times 16.2 \times 10^{-10}}{(\text{sample mass})}$$

2.1.5 Analysis of Size Using MasterSizer

The mean size and size distributions of particles were analysed via laser diffraction on a Mastersizer (Malvern Instruments, UK) equipped with a 5mW Helium-Neon laser (632.8 nm). The machine measures particles with a diameter range of between 0.02 μm to 2000 μm and uses Mie theory to calculate the diameter. Software Mastersizer-S v2.18,
was used to collect and analyse the data.

The samples were analysed at an obscuration of 10-20% using the following parameters: 300RF lens – 0.5-90μm; beam width 14.3nm; Magnetic Cell Stirrer MS7 and with the stirrer rate set at the 3\textsuperscript{rd} or 4\textsuperscript{th} demarcation.

2.1.6 Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopy was used to analyse the size and morphology of the particles produced. Dried particles were mounted onto an SEM stub with double-sided carbon impregnated discs using a fine hair paintbrush. Samples were then sputter-coated with gold using an Emitech K550 Sputter Coater for 2 minutes at 30 mA. The surface morphology of the samples is studied by SEM (Philips / FEI XL30, Philips, The Netherland).
### 2.2 Materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
<th>Address of Supplier</th>
<th>Batch</th>
<th>Grade</th>
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<tr>
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<td>Uniqema</td>
<td>Uniqema Everslaan 45</td>
<td>1108BS0686</td>
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<td>201, rue Carnot-F- 94126</td>
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<td></td>
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<tr>
<td>4-nitrotoluene</td>
<td>SUPELCO</td>
<td>SUPELCO 595 North Harrison Road, Bellefonte, PA 16823-0048, USA</td>
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<td>(1000μm/ml in acetonitrile)</td>
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Chapter Three

Study on Poloxamer Suspensions of Ibuprofen / Ketoprofen
3.1 Model drugs
Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and ketoprofen, have been widely used for the treatment of headaches, muscle aches, arthritis and soft tissue inflammation (Patel and Leswell, 1996). Recent research suggests that NSAIDs have preventative effects against colon, breast, prostate and skin cancers (Godwin et al., 2006). They are normally administered orally or topically. However, like many other drugs with therapeutic effects, they are both water insoluble and their applications are restricted in pharmaceutical products.

A certain degree of aqueous solubility is required to obtain therapeutic effects. Poor solubility results in slow dissolution rate, which is the limiting step in the absorption process and hence can affect bioavailability. Poor solubility also limits the dosage forms that can be used. It has been known that nearly half of all pharmacologically active substances have been identified as either water insoluble or poorly water-soluble. Therefore, optimising the drug solubility and hence the stability of formulations have always remained as a great challenge and one of the biggest issues in drug development. In this study, ibuprofen and ketoprofen were chosen as model drugs.

![Figure 3.1 Chemical Structure of Ibuprofen](image1.png)

![Figure 3.2 Chemical Structure of Ketoprofen](image2.png)
3.2 Solvent system

In this study, suspension was chosen to be the model dosage form because they have been widely used as one of the most popular dispersed systems. They are easy to prepare and also show better therapeutic effects when compared to other dosage forms. Ibuprofen suspension has been administered orally as an antipyretic and analgesic agent in paediatric treatment. In the pharmacokinetic study of ibuprofen in three different dosage forms, ibuprofen suspension was recommended over chewable tablets and tablets for children with cystic fibrosis (Scott et al., 1999). In a bioavailability study, the oral suspension of ibuprofen also showed greater release and absorption speed over effervescent granules (Portoles et al., 2001). Ibuprofen suspension has also been used in various other treatments due to the simplicity of the manufacturing procedure. All these examples show the great potential of suspensions as a dosage form. It was the aim of this study to further improve the physical stability, which has been an obstinate problem for almost all suspensions, in order to enhance the performance of NSAIDs.

Aqueous solvents are the most commonly used in pharmaceutical and biological systems (Florence and Attwood, 1981a) and water was employed as the suspension solvent in this study. Normally, suspending agents and other excipients would be employed in the suspension to improve the stability of the system. However, in this study, the system was confined to three components only in order to study the system when it was most simple. Poloxamers were chosen as the suspending agent as it was capable of increasing the stability of the suspension.

3.3 Poloxamer

As introduced in Chapter 1, poloxamers are also known as Pluronics®. They are a family of amphiphilic nonionic block copolymers, consisting of hydrophobic blocks of poly(propylene oxide) (PPO) and hydrophilic blocks of poly(ethylene oxide) (PEO), with the PPO block in the centre and connecting PEO blocks on either side.

Poloxamers have attracted tremendous attention in the past two decades and showed
much promise. Poloxamers have been widely used in formulations, particularly in gels
(Paavola et al., 1998; Veyries et al., 1999), liposome stabilisation (Bergstrand and
Katarina, 2004) and solid dispersions (Vippagunta, et al., 2002). Poloxamers have been
employed in various drug delivery and gene delivery systems as they were found to be
effective in achieving some desirable properties, such as increased solubility, stability
and permeability (Kabanov et al., 2002).

Poloxamers can be designed intrinsically to meet the requirement of a formulation by
varying the length of the blocks and the ratio of the hydrophobic and hydrophilic
moieties (Alexandridis et al., 1994). One of the main applications of poloxamers is their
strong adsorbance to hydrophobic particles via their hydrophobic blocks, leaving the
hydrophilic blocks stretching outwards from the particle surface in a mobile state. This
significantly decreases the interfacial energy between the hydrophobic substances and
the solvent and at the same time improves the stability of the particle suspensions by
introducing a steric mechanism provided by the hydrophilic blocks (Moghimi and
Hunter, 2000).

The aim of this study was to improve the solubility of the two model drug, ibuprofen
and ketoprofen and the physical stability of the suspensions which they were dispersed
in. It was hoped that the methodology and the technique could be further applied to
other poorly water-soluble drugs.

3.4 Study of the micellisation of Sodium Dodecyl Sulphate (SDS)
SDS, a conventional surfactant, has been well studied. It is generally accepted that SDS
follows the close association model to form micelles. Its Critical Micelle Concentration
(CMC) has been widely reported (Table 3.1). This study was to investigate the
micellisation process of SDS, to observe a close association model, and to test the
reliability of the ITC.
In order to investigate the whole micellisation process, including the pre- and post-micellisation behaviour of SDS when diluted into water, the concentration of the SDS solution in the syringe was chosen to be above the CMC so that the concentration in the ampoule would go past the CMC during the experiment. Using the CMC values of SDS reported in the literature (Table 3.1), the concentration of SDS for this experiment was selected to be 5% w/v, i.e., 0.17Mol·L⁻¹. The SDS solution was prepared by dissolving the known amount of SDS in distilled deionised water. The solution was then stored at 25°C overnight before use.

### Table 3.1 Literature values for the CMC of SDS at 25°C

<table>
<thead>
<tr>
<th>Authors</th>
<th>CMC (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paredes et al., (1976)</td>
<td>8.1</td>
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<td>Sharma et al., (1987)</td>
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<tr>
<td>Wang and Olofsson (1995)</td>
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<td>Paula et al., (1995)</td>
<td>8.0</td>
</tr>
<tr>
<td>Holmberg (2003)</td>
<td>8.2</td>
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In order to investigate the whole micellisation process, including the pre- and post-micellisation behaviour of SDS when diluted into water, the concentration of the SDS solution in the syringe was chosen to be above the CMC so that the concentration in the ampoule would go past the CMC during the experiment. Using the CMC values of SDS reported in the literature (Table 3.1), the concentration of SDS for this experiment was selected to be 5% w/v, i.e., 0.17Mol·L⁻¹. The SDS solution was prepared by dissolving the known amount of SDS in distilled deionised water. The solution was then stored at 25°C overnight before use.

### 3.4.1 Dilution of SDS into water

30 injections in aliquots of 15μL of 0.17M SDS were made into an ampoule containing 2mL of distilled deionised water at 15min intervals. A mean value was obtained from data of triplicate experiments.

The peak area was integrated by software Origin® (MicroCal software, Inc., USA). Both the starting point and the finishing point of the peak were set on the baseline, the starting point was defined as the point at which the power signal was about to rise and the finishing point was when the power signal immediately came back to baseline. The

- 79 -
enthalpy of each injection was integrated from the dynamically corrected data initiated from the starting to the finishing point. The enthalpy of each injection as a function of the concentration of SDS in the ampoule is shown in Figure 3.3.

![Micellisation of SDS in water](image)

**Figure 3.3** Reaction enthalpy for the dilution of 0.17M SDS into water as a function of SDS concentration in the ampoule (mean ± Standard Deviation)

The CMC was determined by identifying the summit of the enthalpy peak and its corresponding concentration. The mean CMC value for SDS obtained by ITC was 8.2mM and was in accordance with those in the literature (Table 3.1). Although the CMC correlated well with the literature value, the micellisation curve was not ideal as there were no plateaux shown before and after the peak. This was contradictory to what was predicted:

As the concentration of SDS solution in the syringe was higher than the CMC, the SDS in the titrant existed as micelles. When they were titrated into the ampoule containing water, the micelles underwent dilution and demicellisation process and formed
monomers in the ampoule, provided that the concentration of SDS in the ampoule is below CMC. Large amount of heat, largely contributed from the enthalpy of demicellisation ($\Delta_{\text{demic}}H$) was given out. As the number of micelles in each injection was the same, the enthalpy from each injection was the same, and a pre-micellisation plateau was formed. After the CMC was reached in the ampoule, both the SDS solutions in the syringe and ampoule were composed of SDS micelles. When the titration continued to take place, the SDS in the titrant underwent dilution, which is the main reaction taken place and the heat given out is small. Since the enthalpy of each injection reflected $\Delta_{\text{dil}}H$ only and the power change per injection was constant, thus forming a second plateau. Due to the significant difference between $\Delta_{\text{demic}}H$ and $\Delta_{\text{dil}}H$, a large drop in the enthalpy was observed and consequently, the plot of enthalpy per injection versus concentration of SDS in the ampoule was expected to be S-shaped.

As what was observed did not agree with what was predicted, a further study was carried out.

3.4.2 Dilution of SDS into buffer

Since SDS is an anionic surfactant, it is speculated that the pre-and post-micellisation behaviour of SDS could be improved in buffer. 0.17M SDS solutions were injected in aliquots of 15µL into the ampoule containing 2mL of 0.01M pH7.0 Phosphate buffer (Na+ salt). The injections were carried out at a 15 minute time interval. The enthalpy was shown as a function of SDS concentration in the ampoule. The micellisation process is shown in Figure 3.4.
Figure 3.4 Reaction enthalpy for the dilution of 0.17M SDS into 0.01M phosphate buffer (pH 7.0) as a function of SDS concentration in the ampoule (mean ± Standard Deviation)

It was observed in Figure 3.4 that the reaction enthalpy for each injection in the first half of the experiment remained constant and the enthalpy significantly decreased as the concentration of SDS in the ampoule was approaching 5mM, which was the CMC of SDS in buffer (Patel et al., In 2007). The reaction enthalpy remained constant afterwards. The micellisation of SDS in buffer agreed with what was predicted earlier in 3.4.1.

As SDS is an anionic surfactant, the counterion balance in the ampoule was disrupted each time after the SDS solution was titrated into water and the solution state was changing constantly. The disruption of the counterion balance interfered with the de-micellisation and micellisation processes and excess energy was required to keep the system at equilibrium, therefore, the pre- and post- micellisation processes observed in the previous study deviated from what was expected. However, when SDS solution was
titrated into a buffer solution here, the large amount of counterion (Na\(^+\)) present in the electrolyte solution provided excess counterion and it enabled the system to be at a constant balance throughout the whole titration process and distinct micellisation behaviour was observed (Patel, 1999).

As the measured CMC value correlated very well with that reported in the literature (Patel et al., 2007), together with the typical micellisation behaviour observed when SDS was titrated into buffer, it was reasonable to conclude that ITC was sensitive and reliable.

### 3.5 Study of interactions between Drugs and Poloxamers

After the study of SDS micellisation behaviour, ITC was proven to be sensitive and reliable, thus it was chosen to carry out studies on how to improve the stability of ibuprofen and ketoprofen suspensions by use of poloxamers.

Poloxamers PE/F 68, PE/F 87, PE/F 108 and PE/F 127 were chosen for this study as they all have a high percentage of PEO, which is very beneficial in enhancing the solubility of the hydrophobic drugs, as the hydrophilic blocks enable the hydrophobic drug particles to be wetted more readily and hence enhance their solubility. These four poloxamers were chosen also because they cover four different molecular weights as poloxamers with different molecular weights and PEO% present different properties, such as the Hydrophobic Hydrophilic Balance (HLB) (Bahadur and Pandya, 1992; Brown et al., 1992; Alexandridis et al., 1994; Kabanov et al., 1995; Waton et al., 1999). By comparing their interactions with the same drug, it was hoped that a better understanding of the effect of the MW and PEO% of the poloxamers on their behaviour could be achieved. Poloxamer solutions of high concentration (15% w/v) were chosen to cover a large range of concentration during titration.

### 3.5.1 Method

Poloxamer solutions of PE/F 68, PE/F 87, PE/F 108 and PE/F 127 were prepared by
dispensing the known amount of poloxamers in fresh distilled deionised water. All the poloxamer solutions were made at 15% w/v and were left in the fridge for 24 hours before use. Drug suspensions were made in the ITC ampoule by dispensing 0.02g of ibuprofen and ketoprofen in 2mL fresh distilled deionised water. ITC was then loaded in the TAM with the motor driven stirrer at 30rpm. After the ITC reached equilibrium, the poloxamer solutions, which were loaded in the syringe, were titrated into the drug suspension in aliquots of 15μL with a time interval of 15min at the rate of 1μL·s⁻¹. 30 injections were made in each experiment. Each combination was repeated three times.

3.5.2 Results and Discussion

The power-time data of each combination between one poloxamer and one drug are shown below with A indicating the power-time data of the interaction and B the integrated enthalpy as a function of poloxamer concentration in the suspension. a, b and c in the legends indicated the triplicates. The first peak appeared in all the power-time data was the result of the dynamic correction signal produced by the Digitam® software for calibration. The power-time data of the three interactions were fitted in one graph for the convenience of comparison and the y-axis of the following power-time figures (Figure 3.5A, Figure 3.7A to Figure 3.13A) were offset for clarity.
Figure 3.5A Power-time data of interactions of PE/F 68 and Ibuprofen

Figure 3.5B Enthalpy per injection versus Concentration from Titration of PE/F 68 Solutions to Ibuprofen Suspensions

The power-time data of interactions between PE/F 68 and ibuprofen are shown in
Figure 3.5A and the enthalpy of each injection as a function of total poloxamer concentration in the suspension is shown in Figure 3.5B. As can be seen from both figures, the interactions were all exothermic and the enthalpy was generally following a decreasing trend as more and more poloxamer solution was titrated into the ibuprofen suspension followed by a plateau. What was observed was speculated to be the result of the adsorption of poloxamer molecules onto ibuprofen drug particle surface, which took place via the hydrophobic block while the hydrophilic blocks were left protruding into the solvent phase. As a result, the surface energy between the drug and the solvent was reduced. At low concentrations, it was speculated that the poloxamers adsorbed on the drug particle surface in a flat configuration for greater coverage of the surface. As more and more poloxamer molecules were adsorbed, the adsorption between the increasingly hydrophilic drug particle and the PPO blocks of poloxamer showed less affinity and a decrease in enthalpy was seen, which constitute the first part of the curve. When the concentration of poloxamer was increased further in the suspension, monolayer coverage of the drug particles was achieved and the enthalpy became relatively constant and a plateau was formed, which was seen in all three triplicates starting at concentration of approximately 0.005g/mL. However, in order to accommodate more poloxamer molecules when the poloxamer concentration was increased further and to minimise the area the hydrophobic blocks exposed to the solvent, poloxamer molecule changed from the flat into the loop and tail configuration thanks to their flexible structure (Adamson and Gast, 1997; Göppert and Müller, 2005). Their conformational change is shown in Figure 3.6. The variation in drug particle size could also affect the conformational change, as the configuration of poloxamer depends on the degree of the particle curvature. Hence, the slight increase in enthalpy was speculated to be the result of the conformational change of poloxamer molecules and the fluctuation was the different degree of conformation change due to the drug particle size variation. This explained the observation in the first half of the plateau in (a) and (b). After the configuration change was complete and the drug particles were all covered by poloxamer molecules and the poloxamer molecules were at their most stable and compact state, no more changes took place and the enthalpy became constant, which
was reflected in the second half of (a) and (b). Similarity was shown between (a) and (b) and (c) was deemed to be the odd one as significant fluctuation was shown in the second half of the plateau and the interaction enthalpy was also different from the other two. Considering that all the experiments were carried out in the same way, question was raised concerning the source of the differences observed.

Figure 3.6 Configuration of poloxamer molecule when adsorbed onto hydrophobic particles in dependence on the poloxamer concentration (Adapted from Göppert and Müller, 2005)
Figure 3.7A Power-time data of interactions of PE/F 87 and Ibuprofen

Figure 3.7B Enthalpy per injection versus Concentration from Titration of PE/F 87 Solutions to Ibuprofen Suspensions
The interaction between PE/F 87 and ibuprofen are shown in Figure 3.7A and 3.7B. From Figure 3.7A, where the power-time data was shown, both endothermic and exothermic processes were seen in each data set but to different extents. The endothermic reactions were speculated to be the poloxamer aggregation, as the aggregation process was suggested to be the entropy driven (Alexandridis et al., 1994; Paterson et al., 1997; Gaisford et al., 1998). A trend of enthalpy reduction was shown at the beginning of all triplicates, which was suggested to be the result of the accumulating adsorption of poloxamer molecules onto drug particles. The enthalpy-concentration curve of (c) was similar to what was observed from interaction PE/F 68 and ibuprofen, as the reduction in enthalpy, the fluctuation from the conformation change followed by the plateau were all shown. However, no plateau was shown in (a), and instead the enthalpy was seen to decrease gradually and a steady plateau was seen in (b). The gradual increase in enthalpy in (a) was considered to be the joint result of the continuous configuration change of poloxamer molecules and adsorption of poloxamer onto drug particles. As discussed before, the conformation change of poloxamer depends on the degree of curvature (Göppert and Müller, 2005), the less variation in drug particles, the less variation in the configuration of the poloxamer molecules. The enthalpy of the three experiments started at the same level; however, significant differences started to show between the three towards the end of the interaction. The large variation in interaction enthalpy and the dissimilar adsorption behaviour shown indicated that significant difference was present between the three experiments.
Figure 3.8A Power-time data of interactions between PE/F 108 with Ibuprofen

Figure 3.8B Enthalpy per injection versus Concentration from Titration of PE/F 108 Solutions to Ibuprofen Suspensions
Power-time data of interactions between PE/F 108 and ibuprofen are shown in Figure 3.8A. Endothermic reactions, which were speculated to be the aggregation of poloxamers, were seen in the first half of experiment (b) and throughout experiment (a), but were not seen in (c). The integrated enthalpy as a function of poloxamer concentration in the suspension is shown in Figure 3.8B. Experiment (a) was observed to be significantly different from the other two in terms of the intensity of the interaction and its adsorption behaviour. The enthalpy of each injection in experiment (b) was seen to be constant throughout the whole process, and this was suggested to be the result of the monolayer coverage of poloxamer on ibuprofen drug particles. The adsorption of poloxamer molecules was achieved through the PPO block, therefore, the larger the PPO block, the fewer molecules were required for the complete coverage of the drug particles, and the plateau would reach at a lower poloxamer concentration. Although the reduction in enthalpy was not observed at the beginning of the curve, the enthalpy-concentration curve of experiment B was considered to be reasonable. An endothermic transition was seen at the beginning of experiment C; however, such observation was considered to be rather the result of artefacts. During the first few injections, the baseline did not come back to zero before the next injection started and artefacts were introduced into the enthalpy integration and they were hence significant. Contrary to what was observed in the previous two experiments, two plateaux were formed in (a). An increase in exothermic enthalpy was seen from the first to the second plateau, indicating that the system was further stabilised. The first plateau is suggested to be the adsorption of poloxamer molecules in their flat conformation at low concentrations and the second plateau was the adsorption of poloxamers in their loop and tail configuration. As more and more poloxamer molecules adsorbed onto the drug particles in loop and tail form, the hydrophobic parts, the PPO blocks from the poloxamers and the drug particles, were shielded from the aqueous solvent and the whole system was better stabilised. As more adsorption sites were exposed and adsorption continued to take place due to the conformation change, more heat was produced from the system and the system was driven to a more stable state. The enthalpy change between the two plateaux was more significant compared to what was
observed in the previous interactions between ibuprofen and PE/F 68 and 87, such observation was suggested to be the result of the larger poloxamer molecule. As PE/F 108 is composed of a larger PPO and PEO block compared to PE/F 68 and 87 (see Chapter One), the conformation change was more beneficial to the stability of the system as it reduced the contact of the PPO block with the aqueous solvent and the steric hindrance introduced by the PEO blocks. The two plateaux regions in (a) as opposed to one in (b) and (c) possibly resulted from the variation in drug particle size and homogeneity of the poloxamers. The significant differences in the adsorption pattern and interaction enthalpy once again demonstrated that different reactions were involved during the interaction of Ibuprofen and PE/F 108.

Figure 3.9A Power-time data of interactions of PE/F 127 with Ibuprofen
Power-time data of interactions between PE/F 127 and ibuprofen are shown in Figure 3.9A. Again, the power-time data indicated that different processes were involved in the interactions. Under close examination, both endothermic and exothermic reactions were involved in all three of the experiments, but as seen in Figure 3.9A, the intensity of the endothermic reactions in experiment B was much greater than the other two. Due to the limited time between each injection, the baseline did not come back to zero before the next titration and this introduced artefacts into some of the enthalpy integration. This was also taken into account when analysing the enthalpy-concentration curve. In (a), a plateau was formed (the 2nd and 3rd data point was ignored due to the integration problem stated above) followed by a second plateau at a lower energy level. The first plateau was caused by the monolayer adsorption of poloxamer and the following increase in enthalpy was the joint result of the continuous adsorption and conformation change, which was discussed in detail in the interaction between Ibuprofen and PE/F 108 (a) and was not repeated here. However, the second plateau was formed at a lower energy state, which was contrary to what was observed earlier. This was speculated to
be the result of a multi-layer poloxamer adsorption (Santander-Ortega et al., 2006). This was not seen in the previous interactions between ibuprofen and the other three poloxamers. As PE/F 127 has the largest PPO block, the multi-layer adsorption took place at a lower poloxamer concentration, and was speculated to be too low to be covered in the concentration range studied.

After the monolayer poloxamer adsorption was complete, the extra poloxamer molecules which were not adsorbed onto the drug particles could form aggregates in the suspension and also adsorbed onto poloxamer molecules which were already on the drug particle surface. The endothermic peaks shown in Figure 3.9B were possibly a result from the former process. As the adsorption between poloxamer and poloxamer was not as strong as that between drug and poloxamer, less heat was produced and the enthalpy was reduced. The enthalpy from the adsorption of poloxamer molecules onto poloxamer molecules was constant and a second plateau was formed at a higher energy state. (b) showed a similar adsorption behaviour without the first plateau being seen. The first plateau and the increase in enthalpy were shown in (c) whilst the second plateau was not seen. Such observation could be the result of the polydispersity of the poloxamers.
Figure 3.10A Power-time data of interactions of PE/F 68 with Ketoprofen

Figure 3.10B Enthalpy per injection versus Concentration from Titration of PE/F 68 Solutions to Ketoprofen Suspensions
The Power-time data of interactions between PE/F 68 and ketoprofen is shown in Figure 3.10A, showing that the interactions between PE/F 68 and ketoprofen were mainly exothermic, although some small endothermic peaks were present in (a). As can be seen from Figure 3.10B, a significant decrease in the enthalpy was observed within the first few injections followed by a gradual decrease. It was suggested that two plateaux were formed in (a) at concentration of about 0.012 and 0.02g/mL. It was speculated that the decrease in enthalpy was the continuous adsorption of poloxamer onto ketoprofen particles; the first plateau was the reflection of the monolayer poloxamer adsorption and the second plateau was the multi-layer poloxamer adsorption. Such observation was very close to what was expected. The mechanism was discussed in interaction of Ibuprofen and PE/F 127 and was not repeated here. Compared to the interaction between the same poloxamer and ibuprofen, the formation of the first plateau was formed at a higher concentration, indicating that more poloxamers were required to cover the same amount of drug particles and ketoprofen particles had larger surface area. However, (b) was different from (a), as only one plateau was seen in the enthalpy-concentration curve as opposed to two and the plateau started at a lower concentration. What was observed in (c) was similar to (a), where two plateaux were formed, the difference being that the concentrations at which the plateaux were formed were both lower than those in (a). The difference of the triplicates was speculated to be the result of the variation in surface area available for adsorption. The interaction enthalpy for the three experiments all started at more or less the same level; however, the enthalpy at the final stage was very different. Therefore, the total interaction enthalpy was different and the interaction mechanism was different, indicating different processes were involved in the interaction between PE/F68 and ketoprofen.
Figure 3.11A Power-time data of interactions of PE/F 87 with ketoprofen

Figure 3.11B Enthalpy per injection versus Concentration from Titration of PE/F 87 Solutions to Ketoprofen Suspensions
The interactions between PE/F 87 and ketoprofen are shown in Figure 3.11A. Only exothermic reactions were shown in (a) while both exothermic and endothermic reactions were shown in the other two. However, the occurrence of the endothermic reactions was also different between the two. Endothermic reactions were seen throughout almost the whole process in (b), while they were only seen in the latter part of (c). The interaction enthalpy (Figure 3.11B) was also very different between the three experiments. One plateau was clearly formed in (a), which was suggested to be the monolayer adsorption of poloxamer molecules onto ketoprofen particles. This was also seen in (b), but the interaction enthalpy was smaller compared to (a). It was speculated that a second plateau was formed at the end of the interaction, which was the multilayer adsorption of poloxamer. Two plateaux were seen in (c), the concentration at which the first plateau was formed was close to that observed in the interaction between PE/F 68 and ketoprofen. This was considered to be reasonable, as the size of the PPO blocks in PE/F 68 and PE/F 87 was similar (30 unit in PE/F 68, 35 units in PE/F 87); the second plateau was reached towards the end of the interaction. The appearance of a second plateau was contrary to what was expected. The discussion on interactions of PE/F 68 with the two drugs suggested that ketoprofen had a greater surface area for adsorption and the second plateau was expected to be reached at a higher poloxamer concentration. However, no second plateau was formed from interaction of PE/F 87 and ibuprofen but was seen in two of the triplicates in interaction of PE/F 87 and ketoprofen. Such observation was also seen from interactions of PE/F 68. Therefore, it was speculated that the curvature of the drug particles affected the poloxamer adsorption. Ketoprofen has a smaller particle size when compared to Ibuprofen and hence a greater curvature. As a result, the poloxamer molecules already adsorbed could hinder the following adsorption dramatically as the steric hindrance it produced. At certain concentration when the adsorption became too difficult and the adsorption of poloxamer molecules onto poloxamer molecules was favoured over the adsorption onto drug particles. The interaction enthalpy between the three started the same, but significant difference was shown between the three at the end of the interaction. It was believed that significant difference exist between the triplicates.
Figure 3.12A Power-time data of interactions of PE/F 108 with ketoprofen

Figure 3.12B Enthalpy per injection versus Concentration from Titration of PE/F 108 Solutions to Ketoprofen Suspensions
The triplicate power-time data of the interactions between PE/F 108 and ketoprofen are shown in Figure 3.12A. Only exothermic reactions were observed in experiment (b), while both exothermic and endothermic reactions were seen in both (a) and (c), however, the strength of the endothermic reactions in (c) was seen to be greater than that in (b). PE/F 108 consist of 54 units of PPO, nearly twice that of the PE/F 68 and 87. As the adsorption took place via the PPO block, the larger the PPO block, the fewer molecules were required to cover the drug particles; hence, the first stage of the adsorption, the significant drop in enthalpy, was not shown in Figure 3.12B. Two plateaux were seen in (b) with the second at a higher energy level, which was close to what was expected. Two plateaux were seen at the start and at the end in both (b) and (c) with the second plateaux in a lower energy state. The increase in enthalpy between the two plateaux was attributed to the configuration change of poloxamer molecules while the first and second plateau reflected the monolayer adsorption of poloxamer in flat and loop-and-tail configuration respectively. The configuration change was necessary as it shielded the large hydrophobic block from the aqueous solvent and reduced the steric hindrance resulting from the large molecule size. This was not shown in PE/F 68 and 87 interactions because PE/F 108 is a much larger molecule, the MW of PE/F 108, 68 and 87 is 14 000, 8350 and 7700 respectively. However, it is worth noting that the possibility of configuration change in interactions of PE/F 68 and 87 was not excluded. It was speculated that the change in enthalpy resulting from the change of poloxamer conformation was more prominent for PE/F 108 than for PE/F 68 and 87 due to its larger structure. The different adsorption behaviour between (b) and the other two was speculated to be the result of poloxamer polydispersity. The significant difference in the adsorption behaviour and interaction enthalpy suggested that different reactions were taking place in the triplicates.
Figure 3.13A Power-time data of interactions of PE/F 127 with ketoprofen

Figure 3.13B Enthalpy per injection versus Concentration from Titration of PE/F 127 Solutions to Ketoprofen Suspensions
The power-time data of PE/F 127 interacting with ketoprofen are shown in Figure 3.13A. Great similarity was shown in this interaction as only exothermic reactions were shown in all three experiments. The total interaction enthalpy was also shown to be similar as the interaction started at the same level and ended at a similar level. One plateau was formed in (a) while two plateaux were formed in both (b) and (c), albeit, at different concentrations. The first plateau of (b) and (c) was formed at concentration of about 0.005g/mL, which was contrary to what was expected. The larger the PPG blocks of the poloxamers, the fewer molecules were required to cover the drug particles, and the plateau at which the monolayer coverage was achieved should appear at a lower concentration. PE/F 127 consist of the largest PPG block among the four poloxamers studied and it was speculated that the concentration at which the plateau formed should be the lowest. In this case, the plateau was formed at a similar concentration to that of PE/F 68 and 87. However, applying by the same theory stated earlier in the interaction of PE/F 108 and ketoprofen, the observation of the first plateau was considered to be reasonable when compared to the results of interaction of ibuprofen with the same poloxamer. After reaching the first plateau, the enthalpy change was different among the three, the interaction enthalpy was observed to be constant for (a), an increase followed by a formation of a second plateau was seen in (b) and a gradual decrease and finally reaching a second plateau was seen in (c). (a) reflected the continuous poloxamer adsorption at a monolayer level, while (b) and (c) both showing the adsorption but changing from a monolayer to a multi-layer level. Significant configuration change was also shown in (b).

3.5.3 Conclusion

All the interactions between four poloxamers and ibuprofen and ketoprofen are shown above from Figure 3.5, 3.7 to 3.13. Different degree of irreproducibility was shown in the interactions between different combinations. The irreproducibility was embodied in (i) the difference in the total enthalpy output among the three repeats; (ii) the different patterns shown by the power-time data indicating different reactions (exothermic/endothermic) were taken part (iii) the different adsorption behaviour of
poloxamer.

The adsorption behaviour was dependent on the concentration of the poloxamer, curvature of the particle surface and the variation of the particle size. At low poloxamer concentrations, poloxamer molecules adsorbed in a flat conformation; as the number of poloxamer molecules increased in the suspension, the poloxamers changed from the flat to the loop and tail configuration, when the concentration of poloxamer increased even further, multi-layer adsorption took place. The curvature of the particle surface was decided by the size of the particles, as it determined the conformation and the amount of poloxamer molecules adsorbed on the drug particle surface. The size of the particles which the poloxamer adsorbed onto also had influence on the adsorption behaviour. If the particle size was small, the curvature of the particle surface was great, and it might introduce greater steric hindrance to the poloxamer adsorption, which explained the different adsorption behaviour observed between the two drugs. The variation in drug particle size could cause poloxamer molecules to adsorb onto the drug particle surface in different conformation and hence exhibiting different adsorption behaviour.

Since the materials used were from the same batch and the experiments were conducted in the same way, the significant difference from the observations above sufficiently demonstrated the poor irreproducibility of the interactions between poloxamers and drugs. The observation raised the question: why would this happen and how to solve it?

3.6 Bench study of the interactions between Poloxamers and ibuprofen/ketoprofen
The ITC study on the micellisation behaviour of SDS demonstrated the accuracy of the technique. Therefore, it was reasonable to believe that the irreproducibility shown in the previous section where poloxamer solutions were titrated into ibuprofen/ketoprofen suspensions was not brought in by the technique used, rather, it was highly possible that the problem arose from the system itself. However, as the experiments were carried out in the TAM, no visual observation can be made during the process. A further study was required to understand the interactions occurring in the ampoules and more experiments
were carried out on a larger scale to observe the interaction during the whole process.

3.6.1 Method
The experiments were carried out in 28ml glass vials (height 7.2cm, diameter 2.5cm) for the convenience of observation and measurement. These glass vials were rinsed with distilled deionised water and then with the poloxamer solution that was intended for the experiment. The vials were then dried and 0.01g +/- 1% of ketoprofen or ibuprofen was placed in the vial and 10ml of distilled deionised water was added. The suspension was stirred for 15 minutes and then titrated five times with 0.45ml of the poloxamer aqueous solution every 15 minutes; hence the total volume of the poloxamer aqueous solution added was 2.25ml (in 10ml drug suspension), which was proportional to the original system where a total of 0.45ml of poloxamer was titrated into 2ml of drug suspension. The experiments were carried out by titrating the poloxamer suspensions 5 times instead of the original 30 times due to the large time scale it required. The suspensions were placed in a water bath at 25°C, the same temperature at which the ITC study was carried out. Observation was made to see if the discrepancies between the three suspensions were also present and to quantify the differences. The sediment height was measured at time intervals of 0.15, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hours starting from the first titration. Observations were made during the interaction process in order to correlation with those from the ITC study; therefore, no stirring could be applied during and after the titration of poloxamer solutions. Poloxamer solutions were also prepared at various concentrations to see if the reproducibility could be improved by varying the poloxamer concentration.

3.6.2 Results and Discussion
3.6.2.1 Sedimentation behaviour of ketoprofen suspensions in the presence of poloxamers at various concentrations
The sediment of the ketoprofen suspension without any poloxamer present was measured and used as a control. Distilled deionised water was titrated instead of poloxamer solutions to the ketoprofen suspension. Three suspensions were prepared at
the same time. The height of the sediment was measured showing the sedimentation process as a function of time. The results are shown below in Figure 3.14. The results of ketoprofen suspension titrated with poloxamer solutions at concentrations of 1, 2.5, 5, 10, 15% (w/v) were shown from Figures 3.15-3.19.

Figure 3.14 Sedimentation behaviour of the ketoprofen control suspensions
Figure 3.15 Sedimentation behaviour of ketoprofen suspensions titrated with various Poloxamer solutions at 1% (w/v)

Figure 3.16 Sedimentation behaviour of ketoprofen suspensions titrated with various Poloxamer solutions at 2.5% (w/v)
Figure 3.17 Sedimentation behaviour of ketoprofen suspensions titrated with various Poloxamer solutions at 5% (w/v)

Figure 3.18 Sedimentation behaviour of ketoprofen suspension titrated with various Poloxamer solution at 10% (w/v)
The sedimentation rates of the suspensions were calculated based on their sedimentation behaviour in the first 15 minutes when they followed the zero order reaction, the rate of which was considered to be the corresponding sedimentation rate. Compared to the control experiment, the sedimentation rate for suspensions containing poloxamer had all been greatly reduced from 6.7 mm·h^{-1} to 0.6, 0.8, 1.0 and 1.3 mm·h^{-1} for PE/F 68, 87, 108 and 127 respectively. In other words, PE/F 68 is the most efficient poloxamer in reducing the sedimentation rate of ketoprofen suspensions. A decrease in sedimentation rate was observed as the concentration of poloxamer was increased for all poloxamers. However, greater reduction in sedimentation rate was seen from PE/F 108 and 127, the sedimentation rates of which dropped from 1.0 and 1.3 to 0.6 to 0.8 mm·h^{-1} respectively when the concentration of poloxamer solutions was increased to 15%, whilst the sedimentation rate for PE/F 68 and 87 dropped from 0.6 and 0.8 to 0.5 and 0.7 mm·h^{-1} respectively. It was speculated that the restrained sedimentation was a result of the increase in suspension viscosity. This showed that poloxamers, regardless of the concentration they were at, were an efficient ingredient to slow down the sedimentation.
rate and therefore, to increase the stability of the suspension.

The average sediment height was measured at the end of the study, which was 8 hours after the sedimentation started. However, it is worth noting that the sediment height was measured before the sedimentation was complete (such experiment design would be discussed later in this section); as a result, the discussion on sediment height was based on comparison of the measurements at 8 hours. The average sediment height was shown to have reduced as the concentration of poloxamer solution increased for all four poloxamers. PE/F 68 proved to be the most efficient among the four poloxamers on reducing the sedimentation rate (see Figures 3.15-3.19), the height of sediment in suspensions containing PE/F 68 was the least at all concentrations. The general order of sedimentation height at the end of the test, from the least to the most, was PE/F 68, PE/F 87, PE/F 108 and finally PE/F 127 at all concentrations. The sedimentation rate, from the slowest to the fastest, was also following the same order, and the poloxamer efficiency of increasing the suspension stability was suggested to follow the same order. For PE/F 68, the height of the sediment significantly decreased from 2.5 to 0.6mm once poloxamer solution was introduced to the system. When the concentration of poloxamer was increased further, no dramatic change in sediment height was observed until the concentration reached 15%, at which the final sediment height was 0.4mm. However, the increase in the concentration had greater effect on the sedimentation behaviour for other poloxamers. A gradual decrease in sediment height was seen in both PE/F 127 and PE/F 108, but remained constant when the concentration was increased to 5% and beyond. The sediment height in suspensions containing PE/F 68 after 8 hours of observation was the least at each poloxamer concentration tested in this study. Even at concentration as low as 1%, the effect of PE/F 68 on sedimentation was equivalent or even better than those achieved by other poloxamers at high concentrations. Therefore, it was reasonable to conclude that PE/F 68 was the best stabiliser of the four poloxamers in the case of ketoprofen suspensions.

However, it is very important to point out that the aim of this study was to observe the
interaction process between the drug and the poloxamers in order to solve the problem discovered in the calorimetry study, in which no visualisation could be made during the interaction process, and the study of suspension sedimentation behaviour was chosen as a means to provide quantitative data for the observation. Hence, the study was focused on the duration of interaction and the study was ended after 8 hours of observation. As can be seen from Figures 3.15-19, the sedimentation did not complete within the observation time as an increasing trend in the sediment height was shown at the end of the curves. As a result, the sediment height was much smaller when compared to the control experiments at the same time interval. It is worth noting that the addition of poloxamer did not reduce the total sediment volume and the reduction in the sediment height was rather a result of the decrease in sedimentation rate rather than the decrease in sediment volume. Because the observation was made up to 8 hours, the sedimentation was not complete at the time and sediment height may continue to increase after 8 hours, but slowly.

3.6.2.2 Sedimentation behaviour of ibuprofen suspensions in the presence of Poloxamers at various concentrations

The sedimentation study of ibuprofen suspensions were carried out as described in 3.6.1 and 3.6.2.1. The control experiment, which studied the sedimentation behaviour of ibuprofen suspension without poloxamers, is shown below in Figure 3.20. The sedimentation behaviour study of ibuprofen suspensions titrated with four poloxamer solutions at 1, 2.5, 5, 10 and 15% are shown in Figures 3.21-3.25.
Sedimentation Study of Ibuprofen Suspensions without Poloxamers

Figure 3.20 Sedimentation behaviour of the ibuprofen control suspensions

Sedimentation Study of Ibuprofen Suspensions Titrated with 1% (w/v) Poloxamer Solutions

Figure 3.21 Sedimentation behaviour of ibuprofen suspensions titrated with various Poloxamer solutions at 1% (w/v)
Sedimentation Study on Ibuprofen Suspensions Titrated with 2.5% (w/v) Poloxamer Solutions

![Graph showing sedimentation behaviour of ibuprofen suspensions titrated with various Poloxamer solutions at 2.5% (w/v).](image)

**Figure 3.22** Sedimentation behaviour of ibuprofen suspension titrated with various Poloxamer solutions at 2.5% (w/v)

Sedimentation Study on Ibuprofen Suspensions Titrated with 5% (w/v) Poloxamer Solutions

![Graph showing sedimentation behaviour of ibuprofen suspensions titrated with various Poloxamer solutions at 5% (w/v).](image)

**Figure 3.23** Sedimentation behaviour of ibuprofen suspensions titrated with various Poloxamer solutions at 5% (w/v)
Figure 3.24 Sedimentation behaviour of ibuprofen suspensions titrated with various Poloxamer solutions at 10% (w/v)

Figure 3.25 Sedimentation behaviour of ibuprofen suspensions titrated with Poloxamer solutions at 15% (w/v)
Similar sedimentation behaviour was observed in the ibuprofen suspensions, where the effect of poloxamer on reducing the sedimentation rate was generally following the same order, from the most effective to the least effective: PE/F 68, PE/F 87, PE/F 108 and PE/F 127. The sedimentation rate, which was determined as described in 3.6.2.1, dropped from 4.3mm·h\(^{-1}\) to 0.4, 0.6, 0.7 and 0.8mm·h\(^{-1}\) for PE/F 68, 87, 108 and 127 respectively. A gradual decrease in sedimentation rate was observed upon the increase in poloxamer concentration (Figure 3.21-3.25). The reason for not carrying out the study until the sedimentation was complete was discussed in 3.6.2.1. Upon the addition of poloxamer, the sediment height dropped from 2.3mm to 1.1.1mm at the end of the study (Figure 3.20, 3.21). As the concentration of poloxamer solution increased, the sediment height at the end of the study was seen constant until the concentration reached 15% for PE/F 108 and PE/F 127 and the sediment height was dropped from 1.06 to 0.93 and 0.88mm respectively. A very slight decrease in sediment height was observed in PE/F 87 as the concentration of poloxamer increased and finally reached 0.77mm at the concentration of 15%. When the concentration of poloxamer PE/F 68 reached 10% and further, no change in sediment height was seen, and the sediment height remained at 0.66mm.

### 3.6.3 Conclusion

One of the purposes of this study was to see if the irreproducible results shown in the ITC can be reflected on a larger scale in order to better understand the ITC data. Due to the intrinsic differences between the two studies, as ITC monitored the whole process by measuring the heat involved in the interaction whilst the observation of the sedimentation behaviour study was focused on the effect of the interaction, comparisons were made based on the variation of the sediment behaviour between the triplicates to see if the sedimentation study was reflecting what was observed from the ITC study.

At the concentration of 15%, the same concentration employed in the calorimetric study, an error range of approximately 0.2mm sediment height was observed for ketoprofen suspensions. Variations were also observed for ibuprofen suspensions. An error range of
approximately 0.1mm was observed for all poloxamers. The sedimentation rate was significantly reduced as soon as poloxamer solutions were introduced to both suspensions.

As mentioned above, the correlation of data from ITC and sedimentation study can only be made on the basis of the standard deviation comparison. In general, greater variation was shown in ketoprofen suspensions than in ibuprofen suspensions, which coincided with the observation from the calorimetric study. Greater variation was also shown in suspensions containing PE/F 108 and PE/F 127 in this sedimentation study whilst greater variation was seen from PE/F 87 and 108 from the ITC data.

The different patterns shown in the ITC power-time data indicated that different reactions were taking place. It was speculated that the exothermic reactions were the results of the adsorption of poloxamers on drug particles, which was the dominating reactions taken place when poloxamers were introduced to the suspension system as it reduced the interfacial free energy between the drug and the medium. The nature of the endothermic reactions, however, was not clear at this stage. The differences shown in the power-time data reflected that different reactions were taking place and the intensity of the reactions was also different. The sedimentation behaviour of the two suspensions also showed significant variation, which reflected the irreproducibility in a different perspective. As mentioned before, when the poloxamers were introduced to the system, they first adsorbed onto the drug particles, some other poloxamers may form aggregates in the bulk, aggregation might continue to take place and led to multi-layer adsorption. Relaxation and orientation of poloxamer molecules could have been involved in the interaction as well but were not reflected in the sedimentation study, as the sedimentation was rather a study on the effects of poloxamers on the drug particles. The aggregation of poloxamer molecules in the bulk increased the viscosity of the suspension and hence withhold the sedimentation. The viscosity of the suspension, dependent on the poloxamer aggregates in the bulk, was determined by the amount of poloxamer adsorbed on drug particles. Therefore, as the total amount of drug was
known, information on the interactions between the drug and the poloxamer could be obtained by studying the sedimentation behaviour of the suspension. In other words, the sedimentation study also reflected what was happening in the ampoule but from a different aspect. It was considered that the deviation observed in sediment behaviour agreed with what was observed from ITC, indicating that irreproducibility was possibly originated from the system.

3.7 Surface Tension Measurement of Ketoprofen and Ibuprofen Suspensions

When conventional surfactants are introduced to water, surfactant molecules are distributed at the surface with the hydrophobic part facing outwards and the hydrophilic part facing inwards at low concentrations. The surface tension is reduced due to the presence of surfactant molecules and the reduction in surface tension is determined by the number of surfactant molecules at the surface. As the number of surfactant molecules at the surface increases with concentration, the surface becomes saturated with surfactant molecules. Once the concentration reaches the Critical Micelle Concentration (CMC) micelles start to form in the bulk, as the Gibbs free energy favours the formation of micelles over the competition between surfactant molecules for the adsorption at the surface. Surface tension remains constant once micelles are formed, as the surface is occupied by the same amount of surfactants (Tsujii, 1998). Therefore, the study of surface tension may provide information on the surfactant behaviour in the bulk.

However, the poloxamer behaviour in drug suspensions would be affected by the presence of the hydrophobic drug particles. Poloxamer would be driven to adsorb at the air-water interface and the drug surface to lower the Gibbs free energy. Consequently, the behaviour of poloxamer in the system was therefore more complicated to predict. Surface tension measurement was chosen as it may provide information on the poloxamer behaviour on the suspension surface and therefore gives insights on the interactions between the poloxamers and drugs in the bulk.
Surface tension measurements were carried out for ketoprofen and ibuprofen suspensions titrated with four different poloxamer solutions and at different concentrations following the same procedure carried out as described in 3.6.1. The suspensions were stirred during the whole titration process and surface tension measurements were carried out 15 min after the last titration, stirring was not applied during this 15 minutes. The results are shown below with error within 0.5 mN·m⁻¹.

Surface tension of ketoprofen and ibuprofen suspensions titrated with poloxamer solutions at concentrations of 1, 2.5, 5, 10 and 15% are shown in Table 3.2 and 3.3 respectively.

<table>
<thead>
<tr>
<th>Concentration (w/v)</th>
<th>PE/F 68</th>
<th>PE/F 87</th>
<th>PE/F 108</th>
<th>PE/F 127</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>43.4 ± 0.6</td>
<td>40.5 ± 0.2</td>
<td>40.6 ± 2.0</td>
<td>41.2 ± 1.2</td>
</tr>
<tr>
<td>2.5%</td>
<td>41.1 ± 0.3</td>
<td>38.8 ± 0.3</td>
<td>39.8 ± 1.5</td>
<td>41.0 ± 1.3</td>
</tr>
<tr>
<td>5%</td>
<td>39.8 ± 0.6</td>
<td>37.7 ± 0.2</td>
<td>37.2 ± 1.5</td>
<td>37.8 ± 1.0</td>
</tr>
<tr>
<td>10%</td>
<td>36.4 ± 0.6</td>
<td>36.8 ± 0.1</td>
<td>35.0 ± 0.3</td>
<td>36.5 ± 0.6</td>
</tr>
<tr>
<td>15%</td>
<td>36.0 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>36.0 ± 1.8</td>
<td>36.4 ± 0.9</td>
</tr>
</tbody>
</table>

Table 3.2 Surface Tension Measurement of Ketoprofen suspensions containing Poloxamers PE/F 68, PE/F87, PE/F108 and PE/F127 at concentrations of 1, 2.5, 5, 10 and 15% (w/v)
Table 3.3 Surface Tension Measurement of Ibuprofen suspensions containing Poloxamers PE/F 68, PE/F87, PE/F108 and PE/F127 at concentrations of 1, 2.5, 5, 10 and 15% (w/v)

<table>
<thead>
<tr>
<th>Concentration (w/v)</th>
<th>Surface Tension (mN·m⁻¹) of different ibuprofen suspensions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE/F 68</td>
</tr>
<tr>
<td>1%</td>
<td>43.1 ± 0.6</td>
</tr>
<tr>
<td>2.5%</td>
<td>41.1 ± 0.3</td>
</tr>
<tr>
<td>5%</td>
<td>40.0 ± 0.2</td>
</tr>
<tr>
<td>10%</td>
<td>37.1 ± 0.3</td>
</tr>
<tr>
<td>15%</td>
<td>36.7 ± 0.1</td>
</tr>
</tbody>
</table>

The interactions between poloxamers and drugs were very complicated. When introduced to the suspension, the poloxamer molecules could behave in the following ways: a) adsorption at the surface of the suspension, b) adsorption at the surface of the drug molecules and c) aggregation in the bulk of the suspension. Each aggregation process has influence on the other two processes. However, it was speculated that at low concentrations, the two adsorption processes were more favoured as it reduced the interfacial energy to a greater extent and hence increased the stability of the system. Although a comprehensive understanding of the interaction mechanism between the drug and the poloxamer was considered to be difficult by use of surface tension measurement alone, it was hoped that by investigating the water-air interfacial properties of the suspensions, which reflected the adsorption of poloxamer at the surface, a better understanding of the interactions in the bulk could be achieved especially at lower concentrations when adsorption was the dominating process. As mentioned in the previous section, due to the fundamental differences between the ITC and the sedimentation study, the evidence provided in the sedimentation study was not convincing enough to prove the irreproducibility shown in the ITC. Therefore, it was also the aim of this study to study the suspension from another angle and to provide more evidence to prove that the irreproducibility originates from the system itself.
The surface tension of the suspensions was observed to decrease when the concentration of poloxamer increased as seen from Table 3.2 and 3.3. Due to the increasing number of poloxamer molecules accumulating in the suspension, the conformation of the poloxamer changed to accommodate more molecules at the surface (Göppert and Müller, 2005) and hence decrease in surface tension was observed even when the concentration reached 15% which was considered to be a very high concentration. The two long PEO tails endow the molecule with high flexibility and the change in conformation was highly attainable. However, saturation at the air-water interface could be reached after the concentration of poloxamer reached certain level when gel is formed.

Greater variation was shown in ketoprofen suspensions and suspensions containing PE/F 108 and 127 followed by PE/F 87 and 68. The greater variation in ketoprofen suspensions than in ibuprofen suspensions agreed with what was observed from the calorimetric and sedimentation study. Observation of significant discrepancies from this surface tension study correlated very well with both the calorimetric and sedimentation study. The calorimetry, sedimentation and surface tension study were carried out to study different aspects of the same system and direct comparison between the three was difficult. However, significant discrepancies were shown in all three studies and it once again proved that the incoherent results from the ITC originated from the system itself and this remained a problem to be solved.

3.8 Polydispersity of Poloxamers

Polydispersity, which is the distribution of the molecular weight in a given polymer sample, arises due to the variation in the degree of polymerisation. It is almost inevitable and yet should be controlled within a reasonable range to ensure the desirable properties of the polymers. Gel Permeation Chromatography (GPC) has been frequently employed to characterise the physical properties of polymers and it was used in this study to determine the polydispersity of the four polymers. Results of PE/F 127, 108, 87 and 68 are shown below in Figure 3.26-29 respectively.
Figure 3.26 Polydispersity analysis of PEIF 118 by GPC

Figure 3.27 Polydispersity analysis of PEIF 17 by GPC
Figure 3.28 Polydispersity analysis of PE/F 87 by GPC

Figure 3.29 Polydispersity analysis of PE/F 68 by GPC
PE/F 127 (Figure 3.26) was considered to be the most heterogeneous among the four poloxamers. Two large peaks and one small peak were observed, giving a total of 3 peaks. The two large peaks were polymers with MW of 21051 and 7357 with polydispersity of 1.069 and 1.158 respectively. The MW of the two large peaks did not correlate with the theoretical MW 12,000.

PE/F 108 was the second most heterogeneous among the four poloxamers as seen from Figure 3.27. Two large peaks were detected, indicating the existence of two substances, the molecular weights (MW) of which were 30997 and 11674 and their polydispersities were 1.04 and 1.116 respectively. The MW of the two detected polymers deviated from the theoretical MW 14,000. Another two small peaks appeared at 14.30 and 15.30mL were also observed.

The two polymers detected in PE/F 127 and 108, the molecular weight of which were smaller than their theoretical molecular weight, were considered to be the degraded products; whilst the other two, which were greater than the theoretical molecular weight, were considered to be contaminants. It was speculated that the contaminants were products of polymerisation of degraded products. Such speculation requires further studies, however, as it was not the aim of this study and they were not carried out.

It was suggested that PE/F 87 and 68 were less heterogeneous when compared to the other two poloxamers, as only one large peak was observed in each study (Figure 3.28 and 3.29). Polymers with MW of 9789 and 10499 were measured for PE/F 87 and 68 with polydispersity of 1.080 and 1.034 while their theoretical MWs were 7700 and 8350 respectively. Small peaks were also observed for these poloxamers, their retention volume was very similar to those which appeared in PE/F 108 and 127 and they were considered as impurities. The size of these two peaks was much smaller than the dominating peak, indicating that the impurities composed only a very small fraction in the whole sample. In addition to the small MW distribution of the main peak, these two poloxamers were considered to be essentially mono-dispersed despite of the presence of
impurities. The homogeneities of PE/F 108 and 127 were poor as more than one large peak was observed, suggesting that the polymer samples consisted of more than one material the properties of which were unknown. It was suggested that the heterogeneity of such poloxamers contributed to the poor reproducibility evidenced in the calorimetric study.

The experimental MW did not correlate with the theoretical MW, 9789 (experimental) as opposed to 7700 (theoretical) for PE/F 87 and 10499 (experimental) as opposed to 8350 (theoretical) for PE/F 68. However, it was speculated that such difference between the two values was mainly introduced by the technique itself. The investigation of the poloxamer polydispersity was carried out by dissolving poloxamer in DMF and then subject to the GPC examination. Solvent molecules were attracted to the surface of the poloxamer molecules when they underwent the detector. As a result, the measured molecules were rather poloxamer molecules surrounded with solvent molecules and the apparent size of the molecules was increased (Meyer, 1993). As the determination of MW in GPC was carried out based on the size of the molecules, the MW of poloxamers so obtained was hence bigger than the theoretical MW. The experimental MW was 27.1% and 25.7% larger than the theoretical MW for PE/F 87 and 68 respectively. Therefore, even though the measured MW did not correlate with the theoretical MW, it was considered that PE/F 68 and PE/F 87 were both mono-dispersed.

The sedimentation study and the surface tension study of the suspensions showed that the reproducibility was poorer for suspensions containing PE/F 108 and 127 than for PE/F 68 and 87. Such observation was explained by the GPC results as both PE/F 108 and 127 were shown to be heterogeneous whilst the other two were considered to be mono-dispersed. However, it was considered to be unlikely that the polydispersity of the poloxamers was the sole reason, as the irreproducibility of the ITC data was shown in all interactions including those containing mono-dispersed poloxamers. Therefore, it was concluded that the heterogeneity of the samples could have introduced variation when interacting with drugs, but could not have been the only and/or the main reason
for the poor reproducibility.

One might argue that the impurities shown at elution time of approximately 14.5 and 15.5mL from Figures 3.26-3.29 could have led to the irreproducibility. More investigations were also carried out to see if the impurities were evenly distributed in the poloxamer samples. Investigations were carried out by taking PE/F 68 and 87 samples from different parts in the poloxamer containers and subjecting the samples to GPC examination using the same methodology. The results showed that the occurrence of the impurities and the size of the peaks were the same as those shown in the Figures 3.28 and 3.29, indicating the impurities were evenly distributed. In addition, the size of the peak suggested that the impurities comprised only a small fraction of the sample. Given that the impurities were evenly distributed in the poloxamer sample and that they presented in a very small quantity, it is unlikely that the impurities caused the variation in the previous studies.

Since the reason for the irreproducibility did not seem to lie in the polydispersity of the polymers alone, further investigations were carried out.

3.9 SEM Analysis of Drug Particles and Surface Area measurement by BET

More investigations were carried out on drug particles, including the screening of the drug particles by SEM and the surface area measurement of the drug particles by BET.

SEM does not only have the advantage of measuring the size but also enables the visualisation of the drug particles, and therefore, provides information on the size, morphology and the diversity of the drug particles. Ketoprofen and ibuprofen particles were examined under SEM. The images of ketoprofen and ibuprofen are shown in Figures 3.30 and 3.31 respectively.
Figure 3.30 SEM Images of Ketoprofen

(a) Low magnification image of Ketoprofen;
(b) High magnification image of Ketoprofen
Figure 3.31 SEM Images of Ibuprofen

(a) Low magnification image of Ibuprofen

(b) High Magnification image of Ibuprofen
Judging by the SEM images of ketoprofen and ibuprofen, which are shown in Figure 3.30 and 3.31 respectively, the size of the particles was shown to be diverse for both drugs; the size of the ketoprofen particles ranged from 2 to 10μm whilst ibuprofen particles ranged from 50 to 250μm. However, as the images only represented a small fraction of samples, the conclusion on the average and the range of the particle size could be arbitrary and inaccurate. The SEM results suggested that the variation shown in the sedimentation study could be due to the different drug particle size according to Stokes’ Law.

\[ V_s = \frac{2 r^2 g (\rho_p - \rho_f)}{9 \eta} \]

Where \( V_s \) is the particle settling velocity, \( r \) is the radius of drug particles, \( g \) is the gravity, \( \rho_p \) is the density of the drug particles, \( \rho_f \) is the density of the fluid and \( \eta \) is the viscosity of fluids. As all the formulations comprised of the same amount of drug and the same amount of poloxamer solution at the same concentration, the variation in sedimentation velocity came from \( r \), the drug particle size and \( \rho_p \), the density of the drug particles, which reflected the difference in drug particle size. Such variation in drug particle size was evidenced by SEM images from Figures 3.30 and 3.31. The SEM images suggested that the observation of the varied sedimentation behaviour could be (partially) resulted from the drug particle size variation, which explained the variation in interaction enthalpy; however, the observation from the ITC where different reactions were taken place still could not be explained.

Another advantage of SEM images is that they provide information on the morphology of the drug particles. A smooth surface was shown in ketoprofen images while pleats were seen on the surface of ibuprofen particles (Figure 3.30-31). The presence of the pleats would significantly increase the surface area of ibuprofen and the surface area of them could not be simply based on the size of the drug particles. Studies were carried out to measure the surface area of the drug particles.

The surface area of drugs is an essential factor in their interaction with poloxamers, as
mentioned previously that the adsorption of poloxamer molecules onto drug particles was the dominating reaction taken place affecting the aggregation behaviour of poloxamers in the bulk and at the surface of the suspensions. The larger the surface area, the more poloxamer molecules can adsorb onto the drug particle surface. If large variations in surface area were shown between the different samples, the interaction enthalpy from the interactions between the drug and the poloxamer would be expected to vary as well.

Drugs were used as received without further sieving. Samples of the drugs were randomly chosen by sampling from different parts of the container and subject to BET surface area measurement. Three samples were chosen for each drug and the same sample was measured five times to obtain a mean value. The results are shown in Table 3.4. The drug samples used throughout the study were from the same batch; therefore, it was believed that the size and the surface area results of the drug particles were consistent.

<table>
<thead>
<tr>
<th>Surface Area of Drug Particles (m²·g⁻¹)</th>
<th>Ketoprofen</th>
<th>Ibuprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples (n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ketoprofen</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>Sample 1</td>
<td>1.40 ± 0.08</td>
<td>5.7%</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1.37 ± 0.06</td>
<td>4.4%</td>
</tr>
<tr>
<td>Sample 3</td>
<td>1.33 ± 0.04</td>
<td>3.0%</td>
</tr>
</tbody>
</table>

Table 3.4 Summary of the surface area measurements of ketoprofen and ibuprofen

The BET measurement provided information on the surface area of the drug particles. The results were analysed for significance using One-way Anova followed by post-hoc Tukey's Test. Significance was stated where p<0.05. SPSS Version 15.0 (SPSS Inc.) software was used for the analysis. The results suggested the surface area varied from
sample to sample in the ibuprofen group but not in the ketoprofen group. It is possible that the variation in surface area led to different extents of adsorption of poloxamer onto drug particles and hence variation was shown in interaction enthalpy. It was reasonable to conclude that the irreproducibility from the interactions of drug and poloxamer can be improved by limiting the variation of the drug particle size in order to have surface area of smaller distribution.

But again, the variation in particle size could not be the only or main reason for the irreproducibility from ITC as significant variation was not shown from ketoprofen particles. Also, the different patterns shown in ITC indicated that the adsorption behaviour of poloxamers was different and the reactions involved were also different between the triplicates from the same combinations. The diversity of the drug particle size and surface area could only influence the intensity of the interactions but not the type of the reactions (exothermic / endothermic) involved.

### 3.10 Measurement of Drug Particle Size in Suspensions by MasterSizer

The previous measurements of the drug particle size were made on the drug alone without the presence of poloxamers, more studies were carried out to measure the particle size in the suspensions by using a Malvern MasterSizer (Malvern Instruments, UK).

The original suspension with the drug concentration at 0.01\( \text{g-mL}^{-1} \) was titrated with 15\% (w/v) poloxamer solution. The poloxamer reached approximately 2\% (w/v) in the end. However, the concentration of the suspension was too high for the MasterSizer analysis and a poloxamer solution of a much lower concentration was chosen instead to prepare the suspension. The drug concentration was kept the same in order to maintain the particle population to be detected. PE/F 68 was chosen for this study as it was the least polydispersed among the four poloxamers. Therefore, the drug suspensions (0.01\( \text{g-mL}^{-1} \)) were prepared with 0.01\% (w/v) poloxamer solutions. Three samples were prepared for each drug suspension and were then loaded to the sample chamber for
measurement when stirred. The results of ketoprofen and ibuprofen are shown in Table 3.5 and 3.6 respectively.

<table>
<thead>
<tr>
<th></th>
<th>$D_{v0.1}$</th>
<th>$D_{v0.5}$</th>
<th>$D_{v0.9}$</th>
<th>Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.67</td>
<td>43.54</td>
<td>201.89</td>
<td>4.323</td>
</tr>
<tr>
<td>2</td>
<td>12.00</td>
<td>39.63</td>
<td>250.02</td>
<td>6.006</td>
</tr>
<tr>
<td>3</td>
<td>22.53</td>
<td>37.24</td>
<td>241.39</td>
<td>5.877</td>
</tr>
</tbody>
</table>

Table 3.5 Particle size measurements of Ketoprofen Suspensions (µm)

<table>
<thead>
<tr>
<th></th>
<th>$D_{v0.1}$</th>
<th>$D_{v0.5}$</th>
<th>$D_{v0.9}$</th>
<th>Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29.09</td>
<td>86.38</td>
<td>194.51</td>
<td>1.915</td>
</tr>
<tr>
<td>2</td>
<td>33.87</td>
<td>89.74</td>
<td>201.32</td>
<td>1.886</td>
</tr>
<tr>
<td>3</td>
<td>27.29</td>
<td>75.46</td>
<td>164.17</td>
<td>1.814</td>
</tr>
</tbody>
</table>

Table 3.6 Particle size measurements of Ibuprofen Suspensions (µm)

$D_{v0.1}$, $D_{v0.5}$ and $D_{v0.9}$ are characteristic values for which, respectively, 10%, 50% and 90% (v/v) of the particles having the size lower than the measured diameter. $D_{v0.5}$ was considered as the mean value. Distribution of the particle size (SPAN) was determined by the following:

$$\text{SPAN} = \frac{(D_{v0.9} - D_{v0.1})}{D_{v0.5}}$$

The SPAN values shown in Table 3.5 and 3.6 provided information on the distribution of the drug particle size in suspensions. Variations were shown in both suspensions, a mean SPAN value of 1.872 and 5.402 was shown for ibuprofen and ketoprofen suspensions respectively, suggesting greater particle size distribution in ketoprofen suspensions.

It is worth noting that the measurements carried out by MasterSizer were made on the assumption that the particles were spherical, which was opposed to what was seen from the SEM images. Therefore, the size of poloxamer molecules adsorbed around drug
particles by simply comparing the particle size obtained from MasterSizer and SEM was not accurate. The particles size of ibuprofen and ketoprofen were also measured by Light Scattering techniques. $D_{V0.1}$, $D_{V0.5}$, $D_{V0.9}$ and SPAN values for ibuprofen particles in water measured by MasterSizer (Malvern Instruments Ltd, Malvern, UK) were 24.21, 43.43, 74.12 μm and 1.149 respectively. However, the particle size of ketoprofen was too small for MasterSizer measurement and its measurement in ZetaMaster (Malvern Instruments Ltd, Malvern, UK), which was designed for particles with smaller size, was very difficult without the aid of stirring as the drug particles precipitated before measurement could be made. As a result, no particle size comparison was made based on the results from Light Scattering techniques between ketoprofen in water and in suspension. It is also worth noting that the particle size measured could be a joint result of the drug particles with poloxamer molecules adsorbed on the surface and the aggregates of poloxamer molecules in the bulk.

In the case of ibuprofen, drug particle size in water was shown to be comparatively uniform with SPAN of 1.149, the size distribution of particles in poloxamer suspensions increased slightly to 1.872, surface area of drug particles was $0.094 \pm 0.011 \text{ m}^2\text{g}^{-1}$. For ketoprofen, due to its fast precipitation rate in water, no drug particle size was measured by use of Light Scattering techniques. As a result, SEM measurement results were employed instead for the comparison. SEM images indicated that the particle size ranged from 2 to 10 μm and was reasonably distributed within a narrow range; SPAN of ketoprofen in poloxamer suspensions was 5.402, surface area of ketoprofen was $1.34 \pm 0.12 \text{ m}^2\text{g}^{-1}$. By comparing the two suspensions, it was shown that the drug particle size and drug surface area of ibuprofen was greater distributed than ketoprofen. However, when it was dispersed in poloxamer suspensions, the particle size distribution was significantly reduced. Therefore, it was speculated that the slight differences between the two drugs could lead to the observed significant differences in their behaviour in poloxamer suspensions.

Although both of them are hydrophobic, ketoprofen showed higher hydrophobicity due
to the presence of an extra benzene ring. As the poloxamer molecules adsorb to the drug through the hydrophobic PPO blocks while the hydrophilic PEO blocks extending in the bulk of the hydrophilic solvents, higher drug hydrophobicity favours the poloxamer adsorption. The surface area of ketoprofen was also shown to be more than ten times that of ibuprofen. These two factors together made it more advantageous for poloxamers to adsorb onto ketoprofen particles. In other words, the adsorption of poloxamer onto ketoprofen particles was favoured due to the higher hydrophobicity and larger surface area; therefore the interaction would be more intense as poloxamer molecules competing for the absorption sites. However, the steric effects also played an important role in this interaction. The two benzene rings in ketoprofen being so close to each other create an environment that disfavoured the adsorption of large molecules like poloxamer, each of which comprised of 30 PO units and 150 EO units. Therefore, the poloxamer molecules in the suspension were in a contradictory situation, where the spontaneous adsorption of poloxamer onto drug surface was hindered by the steric effects created by the chemical structure of the drug, extra energy was required for poloxamer molecules to overcome the hindrance and to stabilise the system. The speculation explained the ITC results, with the positive peaks indicating the exothermic process of poloxamer molecules adsorbing onto the drug surface while the negative peaks indicating the endothermic process when extra energy was required to overcome the steric hindrance. The two events were shown separately in the ITC was suggested to be the difference in their time scale. It was speculated that the adsorption process was an instant interaction while the stabilising process (the endothermic process) was a comparatively long-term process as more time was required for the poloxamer molecules to adjust themselves (such as molecule relaxation and conformation change) in order to reach a stabilised state. The enthalpy from the beginning of the endothermic reaction was offset by the exothermic reaction.

The variation in the ITC results was possibly a reflection of the different degree of steric hindrance which was mainly induced by the variation in the surface area. As the variation shown in the ITC results was much greater than the variation shown in the
surface area of the drug particles, it was believed that by restricting the surface area
distribution would contribute to the variation improvement but it could not be the
ultimate reason for the significant deviation shown in calorimetric study.

Since the concentration of poloxamer in the suspension, which subject to MasterSizer
measurement (0.01% w/v), was much lower than the one employed in the ITC study
(2% w/v), the results above can only be an indication. It was speculated that due to the
increase in poloxamer molecules and even more limited space, the competition between
poloxamer for adsorption sites would be even fiercer and the steric hindrance would be
even greater and hence greater variation would show as the concentration of poloxamer
solution increased.

3.11 Conclusion
Poloxamers PE/F68, 87, 108 and 127 were chosen to interact with NSAID drugs
ibuprofen and ketoprofen in the hope of improving their suspension stability.

Isothermal Titration Calorimetry (ITC) was chosen to study the interaction enthalpy
between poloxamers and drugs in suspensions. However, poor reproducibility was
observed from the triplicates for all interactions, significant variation was shown to
different extents in different combinations. Therefore, investigations were carried out to
solve the irreproducibility problem.

Studies were carried out to observe the sedimentation behaviour of the suspensions as a
function of time but on a larger scale. Surface tension measurement was also employed
to study the surface properties of the suspensions. Poloxamer solutions at 5 different
concentrations were used as against one used in the ITC study. Variations were shown
from both of the studies, suggesting the poor reproducibility originated from the
suspension systems.

Gel Permeation Chromatography (GPC) was chosen to test the heterogeneity of all four
poloxamers. It was shown that PE/F 127 and 108 were heterogeneous and PE/F 87 and 68 were mono-dispersed. As not all the poloxamers were poly-dispersed, the polydispersity of the poloxamers did not explain the discrepancies shown in all combinations.

Scanning Electron Microscopy (SEM) was employed to examine the size and morphology of the drug particles. Surface Area Analysis was used to measure the surface area of the drugs on its own. Variation was shown in both drugs. MasterSizer was employed to measure the particle size of ibuprofen and ketoprofen in poloxamer suspensions. Greater variation was shown from ketoprofen suspensions although better uniformity was observed in the drug particle size and drug surface area. Hence, speculation was raised that the variation was introduced upon the adsorption of poloxamer on drug particles and it was mainly a result of the limited surface area for adsorption induced by the steric effect of the large poloxamer molecules. The surface area measurement by BET suggested that the variation in the surface area contributed to the irreproducibility results from the calorimetric study but could not be the only and/or main reason.

It was concluded that the variation shown in the ITC could be the results of the following reasons: (i) the heterogeneity of the poloxamers, as some of the poloxamers were heterogeneous; (ii) the variation of surface area in each sampling. Improvements could be made by using mono-dispersed poloxamer and by restricting the size of the drug particles to a much narrower range in order to have less variation in the surface area. Further more, better understanding of the interaction mechanism between the drug and poloxamer was achieved. However, it was believed that the two reasons above could not sufficiently account for the significant variation observed from ITC and further investigation was required.
Chapter Four

Study on Association Behaviour of Poloxamer PE/F 68 and Formulation Optimisation of Ketoprofen and Ibuprofen
4.1 Introduction

Although the original suspension that contained the drug with a known mass and poloxamer solution at concentration of 15% (w/v) seemed very simple, the whole interaction process between the two was actually very complex. This was evidenced by the variability between the triplicates when such systems were undergone ITC investigation.

The poloxamer solution used to interact with the drug suspension was at 15% (w/v). The concentration was so high it was assumed that aggregates were formed. Upon the titration of poloxamer into the drug suspension, the poloxamer in the titrant went through processes involving dilution, deassociation, adsorption and relaxation. Adsorption can take place at the surface of the suspension and also at the surface of the drug particles. Therefore, the heat exchange measured was the sum of the interactions between the drug and the poloxamer, the dilution, deassociation, adsorption and relaxation of poloxamers in the suspension. As a result, it was difficult to predict exactly how poloxamer molecules interacted with drugs in the suspensions.

The results of poor reproducibility from the interactions between poloxamers and model drugs ibuprofen and ketoprofen obtained from ITC were validated by the sedimentation and surface tension study on a larger scale. The GPC test on poloxamers excluded the possibility that the irreproducibility was induced solely from the polydispersity of poloxamers. The particle size and surface area measurement of the drug particles together with the particle size measurement in suspension suggested that the performance consistency could be improved by limiting the distribution of the drug surface area. It was suggested that by narrowing the drug particle size and particle surface area and also by use of poloxamers with mono-dispersion, variation in interactions between drugs and poloxamers would be improved. However, the observation from ITC that different reactions were taking place in the interactions between the same two species while experiments were carried out in exactly the same way still remained a problem unsolved and more detailed study was required.
As the total number of poloxamer molecules in the system remained constant after the completion of the titration, the three processes taking place in the system, there being the adsorption of poloxamer molecules onto the drug particle surface, in the bulk and at the surface of the system, had influence on each other to certain extents. Therefore, by studying the adsorption of poloxamer in the solution may elucidate their adsorption onto drug particle surface. More investigations were carried out in order to understand better the poloxamer behaviour in solution in a simpler system before understanding can be achieved on the complex interactions between poloxamer and drugs.

4.2 Dilution of Poloxamer solution in water using ITC

4.2.1 Dilution of Poloxamer PE/F 68 in water from various concentrations

Poloxamers were prepared at concentrations of 1%, 2.5%, 5%, 10% and 15% (w/v). The poloxamer solutions were prepared in the same way as in the previous study on drug-poloxamer interactions. The solutions were prepared by dissolving a certain amount of poloxamer in distilled deionised water and then storing in the fridge for 24 hours. The solution was then rotated until it visually resembled a homogenised solution. For titration calorimetry, the poloxamer solutions were used to titrate into the ampoule containing distilled deionised water when thermal equilibrium was obtained. PE/F 68 was chosen as the model poloxamer for the study as it was the least poly-dispersed among the four poloxamers suggested by GPC results shown in Chapter 3.

30 injections in aliquots of 15μL of PE/F 68 solutions were injected into an ampoule containing 2mL distilled deionised water at time interval of 15 minutes between each injection. Signals were recorded as a function of time. The enthalpy of each injection was plotted as a function of the final concentration in the ampoule. g·mL⁻¹ was chosen as the unit of concentration for better accuracy since the molar mass of polymers covered a wide range due to polydispersity.

Dilutions of poloxamer PE/F 68 solutions from concentrations of 1, 2.5, 5, 10, 15% (w/v) into water are shown in Figure 4.1. Dilutions of poloxamer solutions from
different concentrations are also shown in Figure 4.2-4.6 for close examinations.

**Figure 4.1 Dilution Enthalpy of PE/F 68 solutions into water from concentrations of 1%, 2.5%, 5%, 10% and 15% (w/v)**

**Figure 4.2 Dilution Enthalpy of 1% (w/v) PE/F 68 into water as a function of concentration in the ampoule**
Figure 4.3 Dilution Enthalpy of 2.5% (w/v) PE/F 68 solution into water as a function of concentration in the ampoule

Figure 4.4 Dilution Enthalpy of 5% (w/v) PE/F 68 solution into water as a function of concentration in the ampoule
Figure 4.5 Dilution Enthalpy of 10% (w/v) PE/F 68 solution into water as a function of concentration in the ampoule.

Figure 4.6 Dilution Enthalpy of 15% (w/v) PE/F 68 solution into water as a function of concentration in the ampoule.
Figure 4.1 shown above was different from those of some conventional surfactants such as sodium dodecyl sulphate (SDS). As discussed before for some surfactants, like SDS, the molecules in the solution behave differently before and after Critical Micelle Concentration (CMC). Before the concentration in the ampoule reaches CMC, molecules exist as monomers while SDS in the titrant exist as micellar molecules. When micellar SDS solution is titrated into dilute monomer SDS solutions, deaggregation takes place and a lot of heat is given out. The enthalpy of deaggregation is proportional to the number of micelles in the titrant and the curve at this stage is linear. When more molecules are added to the solution, no more micellisation occurs as micelles exist in both the titrant and the solution in the ampoule and dilution becomes the main reaction taken place, the enthalpy change remains the same and the curve becomes linear again. Due to the difference in the reaction enthalpy from deaggregation (large heat signal) to dilution enthalpy (small heat signal), the whole dilution curve of enthalpy as a function of concentration would be S-shaped, as shown in chapter 3. The dilution curves shown above are not similar to that of SDS. PE/F 68 solutions, when diluted with water, showed no abrupt enthalpy change. Therefore, it was reasonable to deduce that PE/F 68 followed a different association model from that of SDS.

There are two possible association models according to Elias (1972, 1973) and Tuzar et al., (1976). One is called open association. In this model, coalescence of molecules into a micelle continuously occurs, in other words, the number of monomers to form a micelle is not restricted to a certain number. Hence, a series of continuous equilibrium states exist and the CMC is not observable. Since CMCs of many surfactants are detected, this model is not supported by most of the researchers working in this field. However, according to Elias (1973), the concept of CMC can be defined as a phenomenological parameter which denotes the concentration at which the number of micelles is large enough to be detected by a given technique. It is therefore implied that even though ‘CMC’ of a certain surfactant could not be detected by certain technique(s), it does not exclude the possibility of its existence. The second model is termed close association where number of monomers to form a micelle is fixed and the CMC is
detectable. In this model, micelles and monomers are considered to be distinct phases.

Studies showed that the number of monomers to form one micelle following the close association model was usually between 20-80, while the number of monomers forming one aggregate in the open association model was normally between 2 and 10. It was indicated that if the number of monomers to form aggregates in the open association model was large enough to be close to the number of monomers forming micelles in the close association model, the two models approximated. The aggregation number was determined by the size of the molecule and the chemical structure of the molecules (Hvidt et al, 2002).

The micellisation of SDS has been well studied and it has been widely accepted that SDS forms micelles following the close association model. SDS is a small molecule with molecular weight at 288.38. For SDS, the number of monomers to form a micelle was fixed and it was agreed that 64 monomers form an SDS micelle (Bales et al., 1998). However, it was a different matter for PE/F 68. Judging by the dilution process of PE/F 68 into water shown in Figure 4.1, no S-shaped dilution curve was observed and it was hence reasonable to believe that PE/F 68 followed a different association model from that of the SDS. The molecular weight for PE/F 68 is 8350, which is nearly 30 times that of SDS. The enormous structure of the poloxamer molecule, a hydrophobic PPO block in the middle connected with two hydrophilic PEO blocks on both ends, played an important role. The collective effects made it possible for even one single molecule to form an aggregate by itself, with the hydrophobic block in the centre surrounded by the two hydrophilic groups. As a result, the number of monomers forming a micelle was not fixed, the number of aggregates was not dependent on the number of monomers and aggregation could continue until the concentration was too high that gel was formed. A lot of complexities were introduced to the system because of the uncertainties about the number of molecules forming one aggregate and the mechanism how molecules formed aggregates. The word aggregate was employed instead of micelle for a more accurate description because of the differences in their properties.
Figure 4.1 showed dilution enthalpy of PE/F 68 solution starting from different concentrations. Titrant of 1% (w/v) was employed to start with, the concentration in the ampoule after the first injection reached as low as 0.005% (w/v) and reached 0.13% (w/v) after 30 injections. As no conventional S-shaped curve was observed from Figure 4.2, a titrant of higher concentration was used. The concentration of the titrant reached as high as 15% (w/v) and the concentration in the ampoule was nearly 2% (w/v) after 30 injections. However, no S-shaped curve was shown, as can be seen from Figures 4.3 to 4.6, which showed dilution of poloxamer PE/F 68 solutions from concentrations of 2.5, 5, 10, 15% (w/v) respectively, and in Figure 4.1 in which all five dilution processes were shown. As can be seen in Figure 4.2, the standard error was comparatively large. This was a result from the noise of the baseline, which introduced artefacts when integration of the peak area was made; rendering it meaningless to dilute a poloxamer solution of concentration lower than 1% into water, as it was highly possible that the result could be misinterpreted. Although the concentration in the ampoule has reached a much higher level compared to the CMCs of other conventional surfactants, one might argue that the concentration range was incomprehensive to cover that of poloxamers if it exists. In order to cover a wider range, a higher concentration of titrant was required. However, too viscous a titrant in the syringe might damage the cannula and the design of the experiment needed modification.

Another conclusion drawn was that the state of aggregates in solution was concentration dependent. The solution in the syringe before titration was the initial state and the solution in the ampoule after titration was the final state. The same concentration can be reached in the ampoule from titrants at different concentrations. The final state should be the same if the final concentration was the same regardless of the concentration at which the dilution started from. If the aggregation follows the close association model and the poloxamer molecules only exit in two states, micelles or monomers, the enthalpy involved in one titration, including dilution, disassociation, aggregation and relaxation, should remain constant for dilution from micelles to monomers because micelles were all formed by a fixed number of monomers. Therefore, enthalpy of
dilution from a higher concentration (micellised state) to a lower concentration (monomer state) should be proportional to the number of micelles and also the concentration of the solution, as the number of micelles was dependent on the concentration of the solution. If this assumption was true, the dilution enthalpy should be proportional to the concentration of the titrant and the dilution curve should be parallel. Linear fit trendlines were added to the five dilution curves for the convenience of comparison and their equations are listed in Table 4.1.

<table>
<thead>
<tr>
<th>Concentration of poloxamer solutions (w/v)</th>
<th>Equation of the trendline</th>
<th>R² Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%</td>
<td>Y = 450386X - 18773</td>
<td>R² = 0.9762</td>
</tr>
<tr>
<td>10%</td>
<td>Y = 236103X - 6707</td>
<td>R² = 0.99</td>
</tr>
<tr>
<td>5%</td>
<td>Y = 95310 X - 1536</td>
<td>R² = 0.7178</td>
</tr>
<tr>
<td>2.5%</td>
<td>Y = 52742 X - 553</td>
<td>R² = 0.2618</td>
</tr>
<tr>
<td>1%</td>
<td>Y = 14047 X - 54</td>
<td>R² = 0.1197</td>
</tr>
</tbody>
</table>

Table 4.1 Trendlines for the dilutions of different poloxamer solutions at different concentrations into water

Specify Cᵢ as the initial state from which the titration started, Cᵣ as the final state and H_dil as the dilution enthalpy. If the concentration at Cᵣ was the same, the final state in the ampoule was the same. The dilution enthalpy from Cᵢ to concentration Cᵣ can be easily obtained by displacing (Cᵢ-Cᵣ) with X in the corresponding equation listed in Table 4.1. The resulting Δ_dilH, however, was not proportional to the concentration of the poloxamer solutions. Because the final concentration was the same, the final state was also the same but the dilution enthalpy was different, leading to the conclusion that the initial states were different. Therefore, the dilution of poloxamer solutions was dependent on the initial states, which were the concentration of the titrant and the aggregation of poloxamer in aqueous solutions was concentration dependent.
As can be seen from Figures 4.2 to 4.6, the dilutions from 1 and 2.5% solutions showed poor consistency between the triplicate, minor standard error was shown for 5% solution while small deviation was shown in dilutions from 10 and 15% solutions with square of regression coefficient of 0.99 and 0.9762 respectively. The considerable error of the dilution from 1% and 2.5% was due to the signals produced being not sufficiently large enough when compared to the baseline noise which might have contributed significantly to the integration of the peak areas. The dilution from 15% has a larger error range when compared to that from the 10% solution which could be due to the limited time for the aggregation and relaxation. This will be further discussed later in this chapter.

4.2.2 Serial Dilution of PE/F68 in water from 15% (w/v)
As discussed above, the design of the experiment required modification in order to study the dilution process of PE/F 68 into water covering a comprehensive concentration range. A 15% PE/F 68 solution was titrated into water continuously until a high concentration of poloxamer solution was obtained.

PE/F 68 was prepared at the concentration of 15% (w/v) and was titrated in aliquots of 15μL into 2mL of distilled deionised water 24 hours after the poloxamer solution was made. After every 30 injections, the ampoule was taken out and refilled with PE/F 68 solution at appropriate concentration and the syringe was refilled with the same PE/F 68 solution to continue the titration. The enthalpy was recorded as a function of poloxamer concentration in the ampoule and is shown in Figure 4.7. Some small discrepancies presented in the curve were a result of the replacement of the syringe and ampoule.
Figure 4.7 Dilution enthalpy of continuous titration of 15% (w/v) PE/F 68 solution into distilled and deionised water

Compared to the dilution of poloxamer solutions from different concentrations prior to this section, this dilution process covered a much wider concentration range, from as low as 0.07% (w/v) to 9.4% (w/v). Based on the discussion on the two association models earlier, it seemed unlikely that the poloxamer formed aggregates following the close association model as no S-shaped curve was observed. On the other hand, if poloxamer form aggregates following the open association model, aggregates can form by as few as one molecule, critical aggregation concentration (CAC) could occur at very low concentration and be covered by the baseline noise and not be detected, as suggested by the dilution of 1% PE/F 68 into water. Although the CAC was not detected from this study, it was reasonable to believe that the concentration range of the dilution would be sufficient to cover the CAC of PE/F 68 if it exited.

In the close association model, molecules exit as either monomers or micelles, the CMC between the two phases is apparent. While in the open association model, aggregates start to form at very low concentration and aggregates are continuously formed as more
and more surfactant molecules are introduced to the system. A number of continuous equilibria were created. Each equilibrium state was discrete but the difference was not as distinct as monomers and micelles and hence the changes were seen to be continuous. Based on the smooth curve observed from Figure 4.7, it was speculated that PE/F 68 follows the open association model and that the equilibrium state of the poloxamer solution was dependent on the state of the molecules in the bulk at the specific time, temperature and concentration.

Since no CMC could be detected during the dilution even though a wide range of concentrations were covered, and the observed dilution curve was significantly different from that of the conventional association model in that the physico-chemical properties change rapidly but continuously, it was therefore reasonable to conclude that PE/F 68 formed aggregates following the open association model. Such conclusion was also supported by Ruckenstein and Nagarajan (1975).

4.2.3 Dilution of PE/F 68 from 15% to 5% (w/v)

Further investigations were carried out to study the dilution process when the poloxamer solution was titrated to the same solution but at a lower concentration instead of water. Solution of PE/F 68 at 15% was titrated into the ampoule containing 2mL of PE/F 68 solution at 5% (w/v) in aliquots of 15μl with the presence of stirrer at 30rpm. 30 injections were carried out. The enthalpy for each injection was plotted against the concentration of poloxamer in the ampoule. The enthalpy resulting from the physical reaction, solvent into solvent, was also taken into account. The experiment was carried out in triplicate and is shown in Figure 4.8. The results were also used to compare with the study above, in which 15% PE/F 68 solution was titrated into water, both of the dilution processes are shown in Figure 4.9. Trendlines were added to both dilution curves covering 30 injections commencing from 5%, their equations and the regression coefficient are listed in Table 4.2.
Figure 4.8 Dilution of PE/F 68 from 15% to 5% (w/v) solution

Figure 4.9 Dilution of PE/F 68 solution from 15% to 5% (w/v) and to water
Table 4.2 Equations and regression coefficients of trendlines fitted to the dilution curve from 15% PE/F 68 solution to 5% solution and to water

As shown in Figure 4.8, the dilution of 15% poloxamer solution to 5% solution was similar to the dilution processes of poloxamer solution into water observed previously, all appearing to be linear or very close to linear. By applying the two equations listed in Table 4.2, no significant difference was shown in reaction enthalpy between the two dilution processes when taken into account their error range. Therefore, it was suggested that the two dilution processes can be regarded as the same. The result correlated very well with the conclusion drawn above that the states of the poloxamer solution were concentration dependent. When the initial and the final states of the solutions were the same, the $\Delta_{\text{dis}}H$ was the same.

4.3 Dissolution of PE/F 68 into water using Micro Solution Ampoule

As mentioned at the beginning of this chapter, the dilution of poloxamers was actually a complex process. In order to understand it better, the whole process needed to be examined closely starting from the dissolution of the material. Experiments were carried out to investigate the time needed to dissolve PE/F 68 and the time needed for the solution to reach equilibrium.

Micro Solution Ampoule was chosen for this study. The principle of Micro Solution Ampoule is similar to that of ITC (see Chapter 2), except that cartridges were used instead of syringe to load the titrant, as a result, the titrant was restricted to solid state. All experiments carried out in the Micro Solution Ampoule were carried out at 25°C. 15mL of distilled deionised water was loaded in the ampoule and the whole Micro Solution Ampoule unit was loaded in TAM with the stirrer present at a speed of 30rpm. Once the equilibrium of the Micro Solution Ampoule unit was reached, that is when no
heat was transferred between the system and its surroundings, i.e. no heat signal was shown, cartridges loaded with PE/F 68 of known amount mass before the experiment started were injected into the ampoule containing water. The enthalpy changes since the exposure of poloxamer to water were recorded as a function of time. The dissolution curve of 0.045g of PE/F 68 in 15mL of distilled deionised water is shown in Figure 4.10. All experiments were repeated three times to give mean and standard deviation values.

Figure 4.10 Close up Power-Time dissolution curve of PE/F 68 into water using Micro Solution Ampoule. Inset graph shows the same dissolution process over a long period of time

The mean dissolution enthalpy was 35.8 ± 0.112J·g⁻¹. The results showed good consistency and proved to be reproducible. As can be seen from Figure 4.10, the dissolution completed within an hour since the poloxamer was exposed to water when the solution was stirred at the speed of 30 rpm during the whole process. It was assumed that equilibrium was achieved within one hour as no heat exchange was detected after
that and the baseline remained constant.

The study above shed light on how to efficiently prepare poloxamer solutions at low concentrations. It was shown in Figure 4.10 that the equilibrium of 0.3% (w/v) PE/F 68 solution can be attained within an hour when stirred. The result gave an indication as to the time required to prepare equilibrated poloxamer solutions at 25°C at a comparatively low concentration under stirring. However, the preparation method for the poloxamer solutions used in the previous dilution studies was very different. Those poloxamer solutions were prepared at a much higher concentration and they were prepared by storing in the fridge to hasten the dissolution process, no stirring or agitation was applied during this time. Initially, the upper part of the solution was clear while flakes of poloxamers settled at the bottom. Clear solutions could be obtained after some agitation by reversing the flasks but no equilibrium was ensured. Therefore, further investigation was required as to how to attain equilibrium for solutions at higher concentrations in large volume. Due to the limited capacity of Micro Solution Ampoule, another technique was required to carry out this investigation.

The dissolution curve observed in this study turned out to be very different from that reported in the literature (Irwin et al., 1993). It would be interesting to investigate the reason for the dramatic differences between the two dissolution curves. The dissolution curve produced by Irwin is shown in Figure 4.11.
In Irwin's system, the study was carried out by recording the heat signal produced from the 0.5% PE/F87 solution already made and the first 30 minutes worth of data preceding the measurement was missing due to the equilibration time required by the instrument. The study was carried out in Microcalorimetry. In order to imitate the conditions under which the experiment was carried out by Irwin, poloxamer PE/F87 at the same concentration was used. Micro Solution Ampoule was chosen over Microcalorimetry to overcome the disadvantages of the data being lost during the first few hours. Due to the obscure descriptions of how the experiment was carried out in Irwin's paper, for example, how the solution was prepared and how long the solution had been prepared before subjecting to the measurement were not stated in the paper, it was impossible to repeat the experiment in exactly the same way since the initial state was not well defined. Micro Solution Ampoule enabled the detection of the enthalpy change during the whole process starting from the dissolution of poloxamer in water, making the study more comprehensive compared to that of Irwin's system, in which only the process after the solution was made was studied. Since stirring was not
mentioned in Irwin’s paper and based on the observation in the previous section that equilibrium was achieved in about one hour when stirred compared to the 25 hours in Irwin’s paper, it was assumed that no stirring was applied and the study below was carried out without stirring. In summary, Micro Solution Ampoule was chosen to repeat the experiment carried out by Irwin. Due to the restrictions of the cartridge capacity, a total of 0.05g of PE/F 87 was loaded in three cartridges, they were injected into the ampoule containing 10mL of distilled deionised water after equilibration was achieved. No stirring was applied during the whole process. Enthalpy change was recorded as a function of time since the exposure of poloxamer to water and the power-time data is shown in Figure 4.12.

It was suggested by Irwin that the enthalpy change remained relatively constant after approximately 25 hours since the system was introduced to the Microcalorimeter. As stated in the paper, the complicated relaxation process involved an endothermic process at the beginning followed by two distinct endothermic processes. It was speculated that the raw data were processed by multiplying the power signals by -1 to depict what was happening in the ampoule rather than what was recorded in the instrument. For example, a positive signal indicated exothermic reaction, while in Irwin’s paper a positive signal at the beginning of the reaction was identified as an endothermic reaction. This explained the different observations, the positive and negative peaks, between Irwin’s study and the investigation carried out in this study.

It was concluded in Irwin’s paper that the latter half of the thermal processes were associated with the slow hydration of the hydrophobic moiety and followed by orientation. It was also concluded that the equilibration was reached after 25 hours (Figure 4.11). However, as the baseline after 25 hours continued to show fluctuation, which implied that reactions were still occurring, it was inappropriate to conclude that equilibrium was reached after 25 hours, especially when considering that the time before the study was not taken into account either. The results of the experiments to imitate Irwin’s system are shown below in Figure 4.12.
Figure 4.12 Power-time dilution curve of PE/F 87 into water by Micro Solution Ampoule

The dissolution curve shown in Figures 4.12 was not similar to what Irwin observed. It was speculated that the large peak shown in the power-time data was a result of the dissolution process because of the large signal produced within such a short time scale.

The enthalpy measured in this study did not correlate with that reported by Irwin. The baseline after the dissolution peak fluctuated within a comparatively narrow range yet showed greater signal than the noise specification of the instrument, it was hence speculated that the solution has not reached equilibrium even after 80 hours. The fluctuation in the baseline was possibly caused by a combination of different reactions. Since no stirrer was present, diffusion and aggregation might dominate at the first stage along with relaxation and it is suggested that orientation was taking part at the latter stage.

Based on the observation from the previous section that equilibrium was achieved within one hour after the exposure of PE/F 68 to water when stirred, it was speculated
that the equilibrium of the PE/F 87 solution in this study could also be achieved within one hour by applying a stirrer at 30rpm.

All in all, the study carried out above covered a much larger time scale than that in Irwin's study (Figure 4.12), the data was collected since the solid poloxamer was exposed to the solvent no data were lost during the whole process. Therefore, with the optimised methodology, the conclusion made here was more accurate. The equilibration time of poloxamer solutions, even at low concentrations, would require hours to complete if not stirred. However, with the presence of a stirrer at a moderate speed, such as 30rpm, the equilibration time can be significantly reduced to less than 1 hour at the concentration of 0.3% (w/v). Nevertheless, it is worth noting that such conclusion was drawn based on the studies carried out on poloxamer solutions at low concentrations. Due to the limitation of the technique, it still remain unknown how to efficiently achieve equilibrium for poloxamer solutions at higher concentrations.

**4.4 Dissolution of PE/F 68 into water**

PE/F 68 of known mass was loaded in three cartridges before the experiment started. The Micro Solution Ampoule, containing 15mL distilled deionised water, was then loaded in the TAM operating at 25°C. After the equilibration of the instrument was achieved, the poloxamer in the cartridges was injected into the water with the presence of a stirrer at 30rpm. The experiment was ended only after the power signal came back to baseline. The enthalpy resulted from the injection of cartridges into water was also taken into account. The study was carried out by dissolving PE/F 68 of different masses into the same amount of water. The dissolution enthalpy was then recorded and is shown in Table 4.3. The dissolution enthalpy against the mass of poloxamer is shown in Figure 4.13. Measurements were carried out in triplicate.
Concentration of final PE/F 68 solutions (w/v) & 0.3% & 0.2% & 0.1% \\
Dissolution Enthalpy/Mass (J/g) & 35.8 ± 0.112 & 41.8 ± 1.70 & 48.3 ± 2.02 \\

<table>
<thead>
<tr>
<th>Table 4.3 Dissolution Enthalpy of PE/F 68 in different masses</th>
</tr>
</thead>
</table>

Assuming that poloxamers formed aggregates in the closed association model and the states of the solutions at different concentrations were the same, the dissolution enthalpy should be proportional to the mass of the solute. However, as seen from Table 4.3, the dissolution enthalpy measured from solutions at various concentrations was not proportional to their mass (as seen from Table 4.3). Therefore, the assumption made above was not true and it was reasonable to conclude that the behaviour and performance of the poloxamer solutions were dependent on their concentrations and poloxamer formed aggregates in the open association model.

The results from the three solutions fitted very well to the trendline added in Figure 4.13.
It was suggested that a linear relationship might exist between the dissolution enthalpy and the concentration of the solution. However, further investigation was required to provide a greater range of data to determine if the relationship was linear.

4.5 Study on the phase transition temperature using Solution DSC
The conclusion drawn above that the state of poloxamers in the solution was concentration dependent was inconclusive and it raised the question as to the exact meaning of the word 'state', if it referred to the fundamental properties or the orientation state of the poloxamers in the solution?

It has been widely accepted that the phase transitions of poloxamers were temperature dependent and DSC has proved to be an efficient technique to detect minor differences (Beezer et al., 1991; Alexandridis and Hatton, 1995; Gaisford et al., 1998), therefore DSC was chosen for this study in order to detect the differences of solutions at different concentrations.

4.5.1 Phase Transition Temperature of Poloxamer solutions at different concentrations
The temperature coverage of DSC was confined by the solvent, which was water in this case. For the sake of instrument maintenance, the scanning temperature was set from 15 to 80°C. PE/F 87 was chosen instead of PE/F 68 because the phase transition of PE/F 87 occurs at a lower temperature (10°C lower at 10% Dwyer et al., 2004). When the temperature of the poloxamer solution was raised from room temperature to the phase transition temperature, the heat applied to the solution might hasten the equilibration process and lead to misinterpretation of the results. Therefore, the shorter time it took for the solution to reach the Critical Micellisation Temperature (CMT), the less effect heat had on the equilibration of the solution and more accurate the results were. This would be essential when carrying out the time dependence study later on in this section, as it was expected that only slight differences would show between samples prepared at different times. In order to maximise the possibility of seeing the differences, a
poloxamer of lower CMT would be preferred. Moreover, due to the limited DSC studies carried out on PE/F 68, correlation with published data was difficult. Consequently, PE/F 87 was chosen for the DSC study instead of PE/F 68.

The published CMT for PE/F 87 at concentrations employed in this study are listed in Table 4.4.

<table>
<thead>
<tr>
<th>Concentration (% w/v)</th>
<th>Paterson, et al., 1997</th>
<th>Gaisford et al., 1998</th>
<th>Dewyer et al., 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>308.1 K</td>
<td>311.6 K</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>307.1 K</td>
<td>309.9 K</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>307.5 K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>306.3 K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>305.3 K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>303.8 K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td>300.2 K</td>
</tr>
</tbody>
</table>

Table 4.4 Literature values of PE/F 87 Phase Transition Temperatures at various concentrations

4.5.1.1 Methods
The PE/F 87 solutions employed for this study were prepared by the commonly used cold method. Known amount of PE/F 87 was dissolved in distilled deionised water in volumetric flasks and were then stored in the fridge. The volumetric flasks were rotated end over head from time to time to hasten the dissolution and homogenisation. The rotation was carried out gently in order not to produce foams. The PE/F 87 solutions were subject to the fluid DSC (MicroCal, Inc., USA) examination 3 days after they were prepared. All the solutions were ensured that they visually resembled homogenised solutions before examination. The poloxamers were loaded in the sample cell while distilled deionised water was loaded in the reference cell. The samples were heated from
15°C to 80°C at the rate of 60°C/h. The heat capacity $C_p$ was recorded as a function of temperature. The DSC traces were then analysed using Origin® software (MicroCal software, Inc., USA). The CMT was identified at which a tangent drawn from the ascending linear portion of the plot intersects the extrapolated baseline before an increase in the $C_p$ was seen. All the experiments were run three times by using different samples.

4.5.1.2 Results and Discussion

The DSC traces of PE/F 87 at various concentration obtained from this study are shown in Figure 4.14 and their mean values and standard errors are given in Table 4.5 below.

![DSC curves for Poloxamer PE/F 87 aqueous solutions at various concentrations](image)

**Figure 4.14** Differential Scanning Calorimetry trances for Poloxamer PE/F 87 aqueous solutions at various concentrations
Concentration (w/v) | 1% | 2% | 3% | 4% | 5% | 10%
---|---|---|---|---|---|---
CMT (K) | 309.7 | 309.0 | 308.3 | 307.3 | 306.7 | 302.3
Mean ± S.D. | ±0.2 | ±0.1 | ±0.0 | ±0.1 | ±0.0 | ±0.1

**Table 4.5 Phase transition temperatures for PE/F 87 at different concentrations**

As can be seen from Table 4.4 and 4.5, small discrepancies were shown between the published CMT data and those obtained from this study. However, due to the lack of details such as how the poloxamer solutions were prepared and the polydispersity of the samples, it was difficult to correlate the results obtained from this study with those published data. Therefore, the literature value was rather served as a guide to see if the concentration dependence behaviour could be observed, if the difference in CMT between different concentrations would be reasonable compared to the literature values and if the data obtained were reliable.

It was suggested that the phase transition was the reflection of the intra-molecular conformational change, which was caused by the dehydration of the hydrophobic moiety (Attwood *et al.*, 1985; Beezer *et al.*, 1991; Paterson *et al.*, 1997; Gaisford *et al.*, 1998; Su *et al.*, 2003). The results showed that the phase transition temperatures for PE/F 87 at different concentrations were significantly different, which characterised the poloxamer solution at a specific concentration. The results above provided more evidence that the poloxamer solutions were concentration dependent.

**4.5.2 Time dependence study of PE/F 87 solution at 5% (w/v)**

Further investigations were carried out to see if the performance of poloxamer solutions was time dependent. In the previous study, samples of PE/F 87 solutions at 5% (w/v) were examined 3 days after preparation. However, it still remained uncertain if the samples had reached equilibrium at that time and more importantly, how much time was required for poloxamer solutions to reach equilibrium. Poloxamer solutions were subject to DSC at different times after the samples were made to see if any aging
processes could be detected.

4.5.2.1 Methods

5% (w/v) solutions were chosen for this study and they were subject to fluid DSC (MicroCal, Inc., USA) examination at different times after they were prepared. The time intervals were chosen to be 15, 18, 21, 24, 27, 40 and 43 hours after the solutions were made. All the solutions were ensured they visually resembled homogenised solutions before examination. The poloxamers were loaded in the sample cell while distilled deionised water was loaded in the reference cell. The samples were heated from 15°C to 80°C at the rate of 60°C/h. The heat capacity $C_p$ was recorded as a function of temperature. The DSC traces were then analysed using Origin® software (MicroCal software, Inc., USA). The CMT was identified as described in section 4.5.1.1.

4.5.2.2 Results and Discussions

The mean and the standard deviation of the triplicate is listed below in Table 4.6.

<table>
<thead>
<tr>
<th>Time after sample preparation (hr)</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>24</th>
<th>27</th>
<th>40</th>
<th>43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Temperature (K)</td>
<td>306.5</td>
<td>306.5</td>
<td>306.4</td>
<td>306.5</td>
<td>306.5</td>
<td>306.6</td>
<td>306.6</td>
</tr>
<tr>
<td>±0.0</td>
<td>±0.1</td>
<td>±0.2</td>
<td>±0.1</td>
<td>±0.0</td>
<td>±0.1</td>
<td>±0.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.6 Phase Transition Temperature of PE/F 87 5% (w/v) at different times

The results of the phase transition temperatures were analysed for significance using Kruskall-Wallis followed by post-hoc Nemenyi’s Test. Significance was stated where $p<0.05$. SPSS Version 15.0 (SPSS Inc.) software was used for the analysis. After the statistic analysis, no significant difference was shown between all samples.

4.5.3 Dilution from 10% to 5%

4.5.3.1 Methods

In order to further prove that the properties of the poloxamer solutions were
concentration dependent, 5% solution diluted from 10% solution was subject to phase transition temperature measurement using fluid DSC (MicroCal, Inc., USA). Samples were obtained by taking the 10% solution which was prepared for 3 days, mixed with the same amount of fresh distilled deionised water. The diluted sample was subject to DSC measurement immediately after vigorous shaking. The sample was ensured they visually resembled homogenised solutions before examination. The samples were heated from 15°C to 80°C at the rate of 60°C/h. The heat capacity $C_p$ was recorded as a function of temperature. The DSC traces were then analysed using Origin® software (MicroCal software, Inc., USA). The CMT was identified as described in section 4.5.1.1.

4.5.3.2 Results and Discussions

The phase transition temperatures of the 5% and 10% solutions obtained from the previous study were reproduced here for convenience. These solutions were subject to DSC measurements 3 days after they were prepared and they were referred to as readily made solutions in this section. The mean and standard errors of phase transition temperatures for the 10% and 5% readily made solutions and 5% solution obtained from dilution are listed below in Table 4.7. The DSC traces of 5% readily made solution and the 5% solution made from dilution are shown in Figure 4.15. In order to see the minor difference between the two 5% PE/F 87 solutions better, the DSC trace of 10% readily made solution was not included in Figure 4.15.

<table>
<thead>
<tr>
<th>Samples</th>
<th>10%</th>
<th>5% readily made solution</th>
<th>5% from dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMT (K)</td>
<td>302.3 ± 0.1</td>
<td>306.7 ± 0.0</td>
<td>305.8 ± 0.1</td>
</tr>
</tbody>
</table>

Table 4.7 Phase Transition Temperature of PE/F 87 solutions at 10%, 5%, and 5% diluted from 10% (w/v)
Significant difference was shown among the three samples. The difference between the 5% readily made solution and the solution obtained from dilution suggested a transition process between the two 5% solutions. As the CMT was concentration dependent, it was speculated that given enough time, the 5% solution from dilution would finally reach the same state as the 5% readily made solution was in and give the same CMT. It was also speculated that the aggregates in the 10% solution were organised distinctly different from those in 5% solution, after the aggregates were introduced to the new environment, the aggregates disassociate themselves and re-associate again in the way aggregates were formed in a 5% solution. The difference in CMT shown in Table 4.7 suggested the aggregates in the 5% solution obtained from dilution were approaching the equilibrium state, which was the state the 5% readily made solution was in.

Further studies were carried out to investigate how to hasten the equilibration process and the time required for a freshly prepared poloxamer solution to reach equilibrium,
which might explain the irreproducible interactions between the drug and the poloxamers presented in Chapter 3.

4.5.4 Heating effect on the equilibration of solutions

As it was widely accepted that the micellisation process could be induced upon increasing the temperature (Hecht and Hoffman, 1994), it was hence speculated that by subjecting the solution to heat could increase the micellisation and relaxation rate of the molecules in solution and therefore hasten the equilibration process. The aim of this study was to prove the speculation made above, to investigate the effect of heat on the equilibration state of poloxamer solution.

4.5.4.1 Methods

A 5% (w/v) solution was freshly prepared in the cold method by dissolving the required amount of poloxamer PE/F 87 in distilled deionised water and the solution was then stored in the fridge. After complete dissolution was obtained, the poloxamer solution was heated up in a boiling water bath until the sample has reached 80°C. The sample was then cooled down to room temperature and subject to fluid DSC (MicroCal, Inc., USA) examination. During the DSC examination, the samples were heated from 15°C to 80°C at the rate of 60°C/h. The heat capacity $C_p$ was recorded as a function of temperature. The experiments were carried out three times by using three different samples. The DSC traces were then analysed using Origin® software (MicroCal software, Inc., USA). The CMT was identified as described in 4.5.1.1.

4.5.4.2 Results and Discussions

The mean CMT measured for 5% PE/F 87 solutions which were heated up in a water bath after they were freshly prepared in the conventional cold method was 306.7 ± 0.0K. The results of the phase transition temperatures were analysed for significance using t-Test. Significance was stated where $p<0.05$. SPSS Version 15.0 (SPSS Inc.) software was used for the analysis. After the statistic analysis, no significant difference was shown between the heated up samples and samples prepared for 3 days. The
comparisons made among the 3 kinds of samples, including freshly prepared samples, samples prepared for 3 days and samples prepared by heating up, suggested that heating up samples could hasten the equilibration process of the poloxamer solutions.

It has been widely reported that the poloxamer aggregation is thermally reversible (Alexandridis et al., 1994; Gaisford et al., 1998; Dwyer et al., 2005). Therefore, one might argue that when the heated solution was cooled down it would turn back to its original state and hence the heating effect was not permanent. Consequently, it is worthwhile to point out that the original states of the two processes were different. By subjecting the solution to heat, water became an even poorer solvent for the hydrophobic part of the poloxamer, hence the hydrophobic chain PPO coiled up and formed the core of the aggregates and shielded by the hydrophilic PEO chains which formed the corona of the aggregates; at the same time the solvent was excluded from the PPO blocks (Alexandridis et al., 1994; Gaisford et al., 1998). For the convenience of discussion, such state is referred to as the dehydrated state. Such observation was the base of the speculation that subjecting the poloxamer solution to heat could hasten its equilibration process. If a solution had reached equilibrium before subjecting to heat, upon the heating and cooling, the solution would reach the dehydrated state and reverse to its original state which was the equilibrated state. However, if the solution had not yet reached its equilibrium state, equilibrium of the solution was achieved during the heating process and finally reaching the dehydrated state; upon cooling, the solution would turn back to the equilibrium state rather than its original state which was unequilibrated, as the former was more stable.

4.6 Surface Tension measurements of PE/F 68 solutions at different concentrations
Equilibrium surface tension measurement was employed as an alternative method to provide more information on the polymer properties. Calibration of the instrument was carried out on a daily basis and the calibration of water was carried out to ensure the accuracy of the instrument. All the calibration procedures and the results of water calibration were mentioned in Chapter 2.
In order to maximise the accuracy of the measurements, glassware such as volumetric flasks and beakers, which were used for preparing the solutions and the measurements, underwent strict cleaning processes. All the glassware was rinsed with distilled and deionised water until no foams were produced after vigorous shaking, indicating that no surfactants were present. The glassware was then rinsed with hydrochloric acid and again rinsed with distilled and deionised water. Finally, glassware was rinsed with HPLC grade water. Rinsed water was then subjected to surface tension measurement to ensure it correlated well with the literature value of water before the glassware was used.

Solutions of PE/F 68 were prepared at different concentrations, ranging from 0.001% to 15% (w/v), with HPLC grade water. The required amount of PE/F 68 was dissolved in HPLC grade water in volumetric flasks which underwent the strict cleaning process mentioned earlier. The solutions were then stored in the fridge. Such preparation method was called cold method. Surface tension of the solutions was measured 15 hours after they were prepared using CAHN Dynamic Contact Angle analyser. The operation procedure was described in detail in Chapter 2. The poloxamer solutions were taken out of the fridge 14.5 hours after they were made. The 0.5 hour was set aside for measurement preparation. After the samples were taken out from the fridge, gentle agitation was applied to the solutions by rotating the volumetric flasks end over head several times until they visually resembled homogenised solutions; at the mean time, special care was taken to ensure that no foams were produced during the rotation. The solutions were then put in a beaker which has gone through the strict cleaning processes mentioned above. The samples were placed in the water bath in the instrument. The temperature of the water bath was ensured to reach 25°C before the measurement preparation. After ensuring the sample has reached the desired temperature, the samples underwent surface tension measurement as described in Chapter 2. All samples were measured three times. The mean value of the surface tension as a function of concentration is plotted in Figure 4.16 below. The error, which was within 0.5 mN·m⁻¹, was also shown in the graph.
In order to focus more on the surface tension at lower concentrations, graphs were drawn to cover a specific range of concentrations and they are shown in Figure 4.17 and 4.18 respectively.

Figure 4.16 Surface Tension of PE/F 68 at various concentrations

Figure 4.17 Surface Tension of PE/F 68 solutions from 0.001% to 0.05% (w/v)
It was shown in Figure 4.16 that the surface tension decreased as the concentration increased. This was different from that observed from conventional surfactants (Mysels, 1986). For conventional surfactants, after the surface has been fully occupied, surfactant molecules start to enter the bulk of the solution and form micelles and the CMC is reached. Surface tension remains constant after CMC is reached because the surface is occupied by the same amount of surfactant molecules. As the results implied, the surface tension continued to decrease after 15% (w/v), which was considered to be too high a concentration to be the CMC. As the observed association behaviour was dramatically different from that of conventional surfactants, it was reasonable to conclude that the PE/F 68 followed the open association model, which is also supported by other researchers (Chu, 1995; Cho, et al., 1997; Gaisford et al., 1998).

The constant decrease in surface tension suggested that the accumulation of the poloxamer molecules continued even when the concentration of the solution reached as high as 15% (w/v). Therefore, it was speculated that it was more advantageous for the poloxamer molecules to form aggregates in the bulk than accumulating at the surface, in
other words, forming aggregates in the bulk can reduce the system Gibbs free energy more compared to accumulating at the surface. This could be the joint result of the enormous size of the molecules and the size imbalance between the PPO and the PEO blocks. PPO comprises only 20% of the whole molecule weight. 75 PEO units are on either side of the hydrophobic block which consist of 30 PPO units. As a result, aggregate formation was preferable to the system stability. The triblock structure also made the poloxamer molecules very flexible. It was speculated that at low concentration, the poloxamer molecules aggregated at the surface by spreading out the PPO blocks. The area of which the PPO exposed to air was maximised and the interfacial tension was minimised under the circumstances when limited surfactants were present. When the concentration in the solution increased, more and more molecules aggregated at the surface, in order to accommodate more molecules, the structure of the molecules changed. The PPO blocks might coil themselves up in order to reduce the space one molecule took up (Göppert and Müller, 2005). As a result, more molecules could aggregate at the surface and the surface tension decreased correspondingly. It was speculated that the surface and the bulk could become so fully packed as aggregation continued to take place that a gel was formed.

Two inflections at the concentrations of approximately 0.006% and 0.07% (w/v) can be seen from Figure 4.17 and 4.18 respectively. The observation of the two inflections in surface tension-concentration curve was reported in the literature. However, due to the significant variation in CMC/CAC vaules reported in the literature (1.5×10⁻³ %, 20°C, Prasad, et al., 1979; 5.5×10⁻³ %, 25°C, Schmolka and Raymond, 1965; 0.1 %, 25°C (with first inflection at 0.05%), Sasaki and Shah, 1965; no observation under 40°C, Alexandridis, et al., 1994), also the lack of details on how the poloxamer solutions were prepared before subject to surface tension measurement, direct correlation between this study and the literature results was very difficult.

Different opinions were held as to the sighting of the inflection and what it signified. The literature debate was illustrated as follows:
It was suggested that only monomers existed in PE/F 68 solutions under 40°C as evidenced by the light scattering and fluorescent measurements (McDonald and Wong, 1977; Bhadur and Pandya, 1992; Kabanov et al., 1995). No CMC was reported for PE/F 68 solutions under 40°C and the CMC value for PE/F 68 was measured to be 7% (w/v) at 40°C (Alexandridis, et al., 1994), which was considered to be doubtful as it was much higher when compared with CMC values of other poloxamers of the same PEO ratio or of similar molecular weight. To conclude, these studies suggested that no aggregation occurred under 40°C and hence no CMC/CAC could be detected under this temperature.

On the other hand, the two inflections in the surface tension-concentration curve were observed by many colleagues. In Prasad's study in 1979, the first inflection was obtained at 1.5×10⁻³ % (w/v), while the second inflection was ignored. Judging by the figure presented in the article, the second inflection was at approximately 0.3% (w/v). As no multi-molecular micelles were observed at low concentration by light scattering and ultracentrifugation study, the first inflection was suggested to be a result of the unimolecular aggregation. The unimolecular aggregate hypothesis was supported by a few scientists (Dobry and Boyer-Kawenski, 1947; Tuzar and Kratochvil, 1976; Chu, 1995). Such conclusion that aggregation occurred at 20°C contradicted to the previous conclusion.

As mentioned above, the first inflection was believed by some workers to be the CMC and it was also suggested to be caused by the formation of 'unimolecular' micelles; the following decrease in surface tension and the second inflection were often ignored (Prasad et al., 1979). It was pointed out by Alexandridis (1995) that it was the second inflection that signified the CMC instead of the first one and the first inflection was possibly caused by impurities. The reasons for suggesting the second inflection being the CMC were (i) the constant surface tension observed after the second break, and (ii) the effect of temperature on the copolymer concentration at which the second break occurs.
In the first interpretation, the first inflection was regarded as the CMC whereas the second inflection was ignored. Since the second inflection was prominent and was also sighted by other scientist, choosing to ignore such an observation was considered to be inappropriate. In the second interpretation, the first inflection was suggested to be caused by impurities while the second inflection was considered to be the real CMC. As the constant surface tension after the second break, which was one of the reasons for the second inflection being the CMC, was not shown in this and some other studies (Saski and Shah, 1965; McDonald and Wong, 1977; Prasad et al., 1979), the conclusion that the second break being the real CMC was arguable. What’s more, CMC is a parameter determined by the change in surface tension as a function of concentration rather than the occurrence of the constant surface tension. Although the constant surface tension was observed for most conventional surfactants and hence became a convenient and effective method in terms of CMC determination, it should be emphasised here that a constant surface tension is not the criteria for CMC determination. To summarise, the second interpretation that the second inflection in the surface tension-concentration curve being the CMC was untenable.

As discussed before, one poloxamer molecule would be able to form a micelle itself due to its enormous size and its unique chemical structure. As more and more poloxamers were present in the solution, the “unimolecular” micelles disassociated themselves and form aggregates at the inter-molecular level. Based on such speculation, it is suggested that the first inflection in the surface tension-concentration curve signifies the formation of ‘unimolecular’ micelles and the second inflection signifies the formation of multi-molecular aggregates. Since the structure of the “unimolecular” micelle was significantly different from that of the multi-molecular aggregates, such structural change caused the formation of the second inflection. The slow changes before and after the two inflections also appeared to be different from the dramatic changes observed from conventional surfactants. Such observation was better explained by the step-wise association model, i.e., open association model. In the region close to the concentration at which aggregates start to form, the physico-chemical properties change rapidly but
continuously (Ruckenstein and Nagarajan, 1975).

It was observed that the concentration at which the second inflection occurred decreased significantly when the temperature increased whereas the first break remained roughly the same at those two temperatures (25°C and 35°C) studied. Such observation was also seen for a number of copolymers (Alexandridis and Hatton, 1995). The discussion above was also able to explain the variation in the inflection shifting upon the temperature increase. When the solution temperature was increased, it hastened the PEO chain entanglement and the PPO dehydration (Alexandridis et al., 1994; Gaisford et al., 1998), in other words, the micellisation. Fewer monomers were required to form one aggregate and hence the concentration at which the aggregates formed was shifted to a lower concentration. On the other hand, the first inflection was induced by the formation of unimolecular micelles, the increase in temperature could only reduce the time for the unimolecular formation but could not influence the number of molecules to form the micelle. As a result, the first inflection remained the same when the temperature increased.

It was suggested by Alexandridis and Hatton (1995) that the first inflection observed was caused by impurities and their occurrence were very reproducible. However, the speculation and the observation were found to be conflicting. Since the nature of the impurities were not mentioned in the article, it was assumed that the impurities were also surfactants and most possibly by-products from the synthesis such as di-block copolymers; otherwise, no micelles can be formed and no inflection could be observed in the surface tension curve. Assuming that the first inflection was induced by micelles formed by multi-molecules, which was the opinion held by the authors, the inflection would shift as temperature increased as discussed above and it would not be reproducible since impurities only present in a small amount and the impurities would differ from batch to batch and high reproducibility was difficult to ensure. Therefore, such speculation that the first inflection in surface tension-concentration curve was formed by impurities was arguable.
There are also reports suggesting that no aggregates existed at lower concentrations at lower temperatures (<25°C) (McDonald and Wong, 1977; Bhadur and Pandya, 1992; Brown et al., 1992) and the concentration at which the existence of aggregates was detected was considered to be the CMC. However, the conclusion drawn from those reports were considered to be rather a misinterpretation. No detection of aggregates at low concentration at lower temperature was rather a failure to detect the aggregates than the non-existence of the aggregates. At very low concentration, such as 0.006% (w/v), poloxamer existed as “unimolecular” aggregates; at a slightly higher concentration, poloxamer existed as aggregates formed by a few molecules. Hence it was highly possible that the size of these aggregates or the population of such aggregates were too small to be detected. As suggested by Elias (1973), the CMC can be defined as a phenomenological parameter which indicates the concentration at which the number of micelles is large enough to be detected by a given technique. Therefore, failing to detect the existence of aggregates should not lead to the conclusion that no aggregates exist. As the concentration increased, it was speculated that the aggregation number increased as well and the size of the aggregates grew accordingly. Such phenomenon was evidenced by many researchers using light scattering and fluorescent quenching techniques (Al-Saden et al., 1982; Zhou and Chu, 1988; Mortensen and Pedersen, 1993; Molpeceres et al., 1996).

All in all, the inflection at the lower concentration shown in the surface tension-concentration curve was speculated to signify the formation of ‘unimolecular’ micelle while the second inflection which was at a higher concentration was suggested to signify the formation of multi-molecular aggregates.

4.7 Aging study of PE/F 68 by Surface Tension Measurement

As soon as a surface is created, the equilibrium surface shows time dependence and this phenomenon is called surface aging. It is suggested that there are two distinct models during the surface aging process, the rapid and the slow aging. The former mode of surface aging is controlled by diffusion and the latter one is controlled by steric factors.
The surface aging could be dominated by one of the two models or both of them and it is dependent on the electrolyte, the surfactant concentration in the solution and also the temperature (Rawlins, 1977). A generalised illustration of the aging process is shown below in Figure 4.19.

![Figure 4.19 Change of interfacial tension with age](taken from Rawlins, 1977)

The rapid aging process is controlled by the diffusion of the surfactant molecules towards the surface. The diffusion rate dramatically slows down when the surface of the solution becomes too crowded; after aggregates are formed, the size of the aggregates are profoundly larger than a single molecule and this also hindered the molecules from moving. The surface aging then turns to the slow aging process which resulted from the reorientation and penetration of the molecules onto the surface and this is greatly influenced by the steric effects of the molecules (Rawlins, 1977). The bigger the molecules and the aggregates, the more time it takes for the surface to reach equilibrium.
4.7.1 Surface tension measurement of PE/F 68 as a function of time

5% (w/v) PE/F 68 solution was chosen for the study. Solutions were freshly prepared by dissolving the proper amount of poloxamer in water (HPLC grade). The solution was then rotated several times to prevent the solid poloxamer from precipitating at the bottom of the volumetric flasks before it was stored in the fridge. In order to start the measurement 15 hours after the poloxamer solution was prepared (because complete dissolution of poloxamer could be ensured at such time interval and comparison could be made between the time study carried out by DSC and surface tension), 0.5 hour was set aside in order to allow time for the solutions to be ready for measurement. Hence, solution was then taken out from the fridge 14.5 hours after preparation and was then rotated until it visually resembled a homogenised solution. The clear solution was transferred from the volumetric flask to the beaker, which underwent strict cleaning processes as mentioned in 4.6, and was then stored in the water bath in the CAHN Dynamic Contact Angle analyser (DCA-312) for the solution to reach the required temperature (25°C) at which measurements were carried out. The measurements were made after the surface of the solution became visually motionless. The surface tension measurement of the poloxamer solution was made every one hour and measurement was repeated three times to give mean values. Measurements were made for three solutions and every solution was prepared in exactly the same way as described above. Results are shown in Figure 4.20 with errors within 0.5 mN·m⁻¹ and the actual concentration of the solutions was also taken into account.
The first measurements were made shortly after the solutions were transferred to the measuring beakers; consequently, the poloxamer molecules were still migrating and were not equilibrated at the time being. This accounted for the large discrepancies seen between the first measurements (Figure 4.20). The great variation appeared from the first measurements was hence considered to be the result of the agitation. Although the agitation was gentle, the force applied was large enough to cause dramatic movement of molecules and hence influence the surface tension measurement.

A trendline was fitted to the surface tension-time curve in order to predict the time required for the system to reach equilibrium. It was assumed that the reactions between the two states, the initial state A, i.e., the un-equilibrated state, and the final state B, i.e., the equilibrated state, were both first order and reversible; therefore, the scheme is shown as below (Atkins, 1994):
A → B \quad v = k[A]
\quad B → A \quad v = k'[B]

The net rate of change was therefore
\[
d[A]/dt = -k[A] + k'[B]
\]
Suppose the initial concentration of A is [A]₀, then at all times, [A] + [B] = [A]₀, therefore,
\[
\]
\[
[A] = (k'+k'\text{e}^{(k+k')}[A]₀)/{(k+k')}
\]

Such a reaction model was fitted to the surface tension measurement obtained above, the relation of the surface tension as a function of time is expressed in Equation 4.1 and the fitted trendline is shown in Figure 4.21.

![Surface Tension measurement as a function of time](image)

**Figure 4.21** Surface Tension measurement of the solutions and the predicted time of equilibration
\[ Y = C_0 \cdot \exp(-C_1) \cdot X + C_2 \]  
\textbf{Eqn.4.1}

Where \( C_0 \) is the difference in surface tension between the starting state and the equilibrium state, \( C_1 \) is the total rate constant, \( C_2 \) is the displacement on the y-axis.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Value</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_0 )</td>
<td>2.90</td>
<td>0.14</td>
</tr>
<tr>
<td>( C_1 )</td>
<td>0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>( C_2 )</td>
<td>42.47</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\textbf{Table 4.8 Values and Standard errors of coefficients in the first order kinetic reaction approaching equilibrium}

The residual sum of squares and the correlation coefficient of the fitted trendline were 0.36 and 0.99 respectively. Hence, the reaction model was considered to fit very well to the experimental data. According to the fitted model, approximately 45 hours was required for the solution to reach equilibrium.

As suggested by the results from section 4.5.2, no time dependence of poloxamer solution was detected in the DSC study. It is worth noting that such an observation did not contradict with the observation from the surface tension study and it was not resulting from the sensitivity of DSC. The time dependence of poloxamer solution could not be detected was simply because the DSC was not suitable for such study. The surface tension study measured the surface properties of the poloxamer solutions as they were without applying extra forces; on the other hand, when the poloxamer solutions were subject to DSC measurements, heat was supplied to the samples and hence influenced their equilibrium states. Heat, as suggested by the previous studies, had positive effect on the equilibrium state of the solution as it hastened the equilibration process. Therefore, the trace difference between the states the solution were in at different times was further minimised due to the heat the solution was subjected to in the DSC study and the time dependence of poloxamer solution was hence not detected.
4.7.2 Surface tension measurement of PE/F 68 after agitation

The total time measured, 15 hours of preparation time and 45 hours of equilibration time since measurement, involved the dissolution of poloxamers, aggregation, relaxation and orientation. However, the transfer of the solution and the agitation done to the solution before the measurement started created a new surface and affected the equilibrium state of the solution due to the dynamic movements of the molecules, leaving and entering from both the bulk and the vapour. Therefore, the result that the solution did not reach equilibrium until after approximately 45 hours could have been elongated. Consequently, the following study was to investigate the time required for a surface to reach a definable position after agitation. The solutions employed in the previous study were left untouched over 45 hours after they were first subject to measurements in order to achieve the equilibrium state. The solutions were then agitated and transferred to another beaker and the surface tension was measured again as a function of time. The results are shown in Figure 4.22. Hence, the time for a solution to reach equilibrium could be easily obtained by subtracting the result from this study from that obtained from the previous study.

![Figure 4.22 Surface Tension measurement of solution PE/F 68 5% (w/v) after agitation](image-url)
The surface tension of the three solutions was different at the beginning. Such observation was similar to what was observed in the previous study and was suggested to result from the same reason, the transfer and agitation of the solution. The surface tension of all three samples remained constant after approximately 4 hours. No rapid aging was shown at the start and this was because the diffusion happens so fast that no surface tension measurements could be made with errors within acceptable range.

Therefore, it was concluded that 4 hours was required for an equilibrated poloxamer solution to re-reach equilibrium after the equilibration state was disrupted As suggested by Hecht and Hoffmann (1995), a slow relaxation time of 4 hours was considered to be reasonable for long non-ionic surfactants with long hydrophobic chains, as the slow relaxation time might take several hundred minutes.

There were many literature reports about the slow relaxation time \( \tau_2 \) of many non-ionic surfactant solutions, many of them were on the order of milliseconds. The slow relaxation process with relaxation time \( \tau_2 \) is attributed to the micelle formation and dissolution. The slow relaxation time \( \tau_2 \) reflect the stability of the micelles, it is the reflection of the formation or disintegration kinetics of micelles under equilibrium conditions. On the other hand, the study above was the measurement of time required for freshly prepared poloxamer solutions to reach equilibrium. For many years, no correlation was found between the relaxation time \( \tau_2 \) with the equilibration time (Patist \textit{et al.}, 2002). It is worth noting that there are fundamental differences between the two parameters, and it is important to look at them with prudence. Consequently, no comparisons can be made between them.

Riess (2003) suggested that the equilibration of block copolymer micellar systems was not necessarily reached. However, it was assumed that such conclusion was made resulting from the failure to detect the equilibrium of such systems and the large time scale could be one of the reasons. Taking into account the large molecular weight, the size of molecules and the concentration of the solution, the time measured for 5% (w/v)
PE/F 68 to reach equilibrium was deemed to be reasonable.

In conclusion, approximately 45 hours was required for a 5% (w/v) PE/F 68 solution to reach equilibrium, in which the 15 hour preparation time and the time contributed from agitation were not taken into account. Approximately 4 hours was required for an equilibrated 5% (w/v) PE/F 68 solution to re-reach equilibrium once the equilibrium was disrupted.

Such findings could explain the irreproducible results between poloxamers and drugs from ITC shown in Chapter 3. In the calorimetric study, the solutions were subject to interact with drug ibuprofen and ketoprofen 24 hours after they were prepared and were added to the drug suspensions at a time interval of 15 minutes. In other words, the poloxamer solutions did not reach equilibrium before they were introduced to interact with the drugs and not enough time was allowed for the interaction between ibuprofen / ketoprofen and poloxamer to complete. Poloxamer molecules did not reach their most stable state throughout the whole interaction process and the actual state they were in were not well defined, a lot of uncertainties were hence involved and led to significant variation.

4.8 Optimum formulation study of PE/F 68 and Ibuprofen / Ketoprofen

It was speculated that the poloxamer solution had not yet reached equilibrium before interacting with the drug solution and this could be one of the reasons for the irreproducibility discovered at the beginning of Chapter 3. However, as also suggested in Chapter 3, better consistency in performance could be achieved by restricting the drug particle size distribution in order to limit variation in drug particle size and drug surface area. In addition, the concentration dependence of the poloxamer solution and that the poloxamer formed aggregates in open association model discovered in this chapter could also be employed to improve the irreproducibility. Aggregates were formed in different manners at different concentrations, the mean size and size distributions of aggregates were also dependent on the concentration of the solution.
Choosing the right concentration of poloxamer solution could aid the irreproducibility improvement. In this study, it was aimed to optimise all the factors in the formulation to improve the reproducibility.

A range of experiments were designed by varying the drug particle size, drug concentration and the poloxamer concentration. More importantly, the equilibrium of the poloxamer solutions used was ensured. The design of the experiments was listed in Table 4.9 and 4.10. Letters and numbers were given to the ibuprofen and ketoprofen suspensions respectively in order to correlate with the results shown later. Each experiment was performed in triplicate.

PE/F 68 solutions were prepared at concentrations of 5, 10 and 15% (w/v) by dissolving the right amount of poloxamer in distilled deionised water, the solutions were then stored in the fridge. The solutions were used 3 days after they were prepared. During this time, the volumetric flasks in which the solutions were prepared were rotated from time to time in order to improve the homogeneity and hasten the equilibration process. Suspensions were then prepared by adding the proper amount of ibuprofen / ketoprofen to 10mL poloxamer solutions in 28ml glass vials (height 7.2cm, diameter 2.5cm) for the convenience of observation and measurement. The amount of drug and the concentration of the poloxamer solution were added according to Table 4.9 and Table 4.10. The suspensions were stirred for 1 hour at the speed of 60 rpm and were then left on the bench untouched for two hours before observations were made. Conclusions were drawn based on the sedimentation rate and uniformity. The aim of this study was not to investigate the optimum combination, rather, it was to study the influence of different factors on the stability and the reproducibility of the formulations.

The size effect of ketoprofen particles were not taken into account in this study because its particle size was shown to be very small and within a comparatively narrow range as suggested by SEM shown in Chapter 3. Therefore, only the drug concentration and the poloxamer solution concentration were employed as variables for ketoprofen
suspensions.

<table>
<thead>
<tr>
<th>Drug Particle Size</th>
<th>Drug Concentration (g·mL⁻¹)</th>
<th>Poloxamer Solution Concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td>75-90 µm</td>
<td>0.01</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>P</td>
</tr>
<tr>
<td>125-150 µm</td>
<td>0.01</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>V</td>
</tr>
</tbody>
</table>

Table 4.9 Formulation design for suspensions containing Ibuprofen and Poloxamer

<table>
<thead>
<tr>
<th>Drug Concentration (g·mL⁻¹)</th>
<th>Poloxamer Solution Concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>5%</td>
</tr>
<tr>
<td>0.005</td>
<td>10%</td>
</tr>
</tbody>
</table>

Table 4.10 Formulation design for suspensions containing Ketoprofen and Poloxamer

4.8.1 Ibuprofen Suspensions

Observations were made two hours after the suspensions have been stirred for one hour and are shown in Figure 4.23 below. The numbers and letters on the vials shown in the photos correlate with those in Table 4.9 and Table 4.10 whilst the subscripts indicated the triplicate sample. Additional information was added for reading convenience. The variables of the formulations were provided in the caption in the order of type of drug, drug concentration, drug particle size and poloxamer solution concentration.
Formulation M (ibuprofen 0.01 g·mL\(^{-1}\), 75-90\(\mu\)m, 5%)

Formulation N (ibuprofen 0.01 g·mL\(^{-1}\), 75-90 \(\mu\)m, 10%)
Formulation O (ibuprofen, 0.01 g·mL⁻¹, 75-90 μm, 15%)

Formulation P (ibuprofen 0.005 g·mL⁻¹, 75-90 μm, 5%)
Formulation Q (ibuprofen, 0.005 g·mL⁻¹, 75-90 μm, 10%)

Formulation R (ibuprofen, 0.005 g·mL⁻¹, 75-90 μm, 15%)
Formulation S (ibuprofen, 0.01 g·mL⁻¹, 125-150 μm, 5%)
Formulation U (ibuprofen, 0.01 g·mL⁻¹, 125-150 µm, 15%)

Formulation V (ibuprofen, 0.005 g·mL⁻¹, 125-150 µm, 5%)
Formulation W (ibuprofen, 0.005 g·mL$^{-1}$, 125-150 μm, 10%)

Formulation X (ibuprofen, 0.005 g·mL$^{-1}$, 125-150 μm, 15%)

Figure 4.23 Observations of Ibuprofen Suspension in Optimisation Study
The formulations were ranked based on the performance consistency between the three samples and also their precipitation behaviour. However, due to the size of the samples, accurate measurement of sedimentation rate was difficult, instead, discussions were to focus on the comparisons of sedimentation behaviour between samples. Comparisons of sedimentation behaviour were made based on the visual assessment of clarity of the supernatant. The clearer the supernatants were, the greater drug particles sedimentation. Based on these two criteria, the best formulation among these was formulation T, which was comprised of ibuprofen particles ranging from 125-150μm at the concentration of 0.01 g·mL⁻¹ in 10% (w/v) poloxamer solution, followed by formulations composed of 15% poloxamer solutions. It was not surprising to see that the more desirable suspensions were mainly composed of poloxamer solutions at a higher concentration.

The factors influencing the precipitation behaviour was shown in the Stokes’ Law:

\[ V_s = \frac{2r^2g(p_p - p_f)}{9\eta} \]

Where \( V_s \) is the particle settling velocity, \( r \) is the radius of drug particles, \( g \) is the gravity, \( p_p \) is the density of the drug particles, \( p_f \) is the density of the fluid, \( \eta \) being the viscosity of fluids.

As implied by the Stokes’ Law, by minimising the radius of drug particles, the density difference between the drug particles and the fluid and increasing the viscosity of fluids can minimise the precipitation to the greatest extent. The purpose of this study was to investigate how much these three factors contributed to the stability of the system and if they could improve the performance consistency of the formulations.

For suspensions O and R, the poloxamer solutions were at the same concentration, 15% (w/v), and the drug particle size was between 75-90μm, the only difference was the drug concentration in the suspension, with O at 0.01 g·mL⁻¹ and R at 0.005 g·mL⁻¹. As observed from the results shown in Figure 4.23, Formulation R was observed to be slightly clearer than Formulation O, indicating that the precipitation of formulations R was slightly greater than that of formulation O. However, better consistency was shown
in R. Since precipitation was inevitable for suspensions, it was considered that consistent performance would be more desirable and formulation R was considered to be a more desirable formulation than O. Similar precipitation behaviour was observed from formulation U and X. Both of Formulations U and X were composed of ibuprofen particles ranging from 125-150μm in size and poloxamer solution at concentration of 15% (w/v). The drug concentration for Formulation U was at 0.01g·mL⁻¹ while the concentration was at 0.005g·mL⁻¹ for Formulation X. Judging by the same criteria as described above, the precipitation of Formulation X was observed to be greater than Formulation U. Significant variations were shown in both formulations. In Formulation U, one triplicate was seen to be significantly different from the other two while slight difference was seen among all three in Formulation X. Therefore, it was difficult to draw a conclusion as to which formulation was better. Based on the comparisons made among the four formulations, which comprised of the same poloxamer solution and drug particles of the same size, formulations of lower drug concentration showed greater precipitation but better consistency. Such observation was considered to be reasonable as the presence of other drug particles restrained their movement in the suspension and the sedimentation was hence restrained. The sedimentation is slower when the drug concentration is higher.

Comparisons were also made between formulations comprised of the same drug concentration and poloxamer concentration but of different drug particle size. R, comprised of smaller drug particles, showed more desirable precipitation behaviour when compared to X which comprised of larger drug particles, as better consistency and less precipitation was observed. Such observation could be easily explained by Stokes’ Law. In these two formulations, the viscosity of the fluid, the density of the fluid and the drug particles can be deemed as constant, therefore, the larger the drug particle size, the greater the sedimentation. It was expected that Formulation U and O would show similar precipitation behaviour, however, as significant differences were shown in both formulations, comparison between the two was difficult.
Formulation T, comprised of drug particles ranging from 125-150μm in size at the concentration of 0.01g·mL⁻¹ and poloxamer solution concentration at 10% (w/v) showed moderate precipitation and the best consistency among the 12 suspensions and was therefore considered to be the most desirable formulation. If the poloxamer concentration was too low, the adsorption of poloxamer molecules on the drug particle surface was hindered by the number of poloxamer molecules available, the interfacial energy between the two phases, the drug particles and the poloxamer solutions, remained high and the stability of such suspension was poor. However, if poloxamer concentration was too high, the aggregation took place continuously, as described above, it could increase the precipitation which was also not desirable. Therefore, it was important to reach a balance when sufficient poloxamer molecules could aggregate on the surface of the drug particles and the balance was determined by the three elements, the drug concentration, the drug particle size and the concentration of the poloxamer solution.

For the rest of the formulations which were not included in the discussions above, the great precipitation or the significant difference between the triplicate samples made the comparisons between these formulations very difficult. It was also assumed that these formulations would show similar behaviour to those covered in the discussion above and the discussions on the ibuprofen suspensions were only focused on a few formulations.

To summarise, Formulation T was considered to be the most desirable formulation out of the 12. It was difficult to conclude which factor contributed more to the stability of the formulations because they were also under the influence of the other two factors. It was speculated that poloxamer acted as a viscosity enhancer and wetting agent in these suspensions. Its role as wetting agent and/or viscosity enhancer depended on the amount of drug and poloxamer in the suspension and the available drug surface area. Discussions were made based on the formulations comprised of poloxamer solutions at a higher concentration as the precipitation of these suspensions was not as great when compared to those comprised of lower poloxamer concentrations and the comparison.
between these formulations would be easier and more accurate.

4.8.2 Ketoprofen Suspensions

The ketoprofen suspensions were prepared in the same way as described in 4.8.1. The suspensions were prepared according to Table 4.10. However, very little precipitation took place in ketoprofen suspensions within 2 hours. In order to see greater differences between the ketoprofen suspensions of various combinations for the convenience of observation and comparison, the observation time was extended to 24 hours after the stirring. The observations are shown in Figure 4.24.

Formulation 1 (ketoprofen, 0.01g·mL⁻¹, 5%)
Formulation 2 (ketoprofen, 0.01 g·mL$^{-1}$, 10%)

Formulation 3 (ketoprofen, 0.01 g·mL$^{-1}$, 15%)
Formulation 4 (ketoprofen, 0.005 g·mL⁻¹, 5%)

Formulation 5 (ketoprofen, 0.005 g·mL⁻¹, 10%)
Judging by the conformity among the samples and the general clarity of the suspensions, which reflected the sedimentation behaviour of the suspensions, comparisons were made between the different combinations in order to investigate the effects of poloxamer concentration and drug concentration on the stability of the formulations. The size of the drug particles was not taken into account as the size was very small and comparatively evenly distributed (2-10μm), as shown from SEM images, further classification of the drug particle size was not necessary.

The consistency for Formulation (1) was not good, as greater precipitation was observed in one of the samples than the other two. The sedimentation behaviour and consistency of Formulation (2) seemed to be more desirable compared to Formulation (1) as the supernatant was not as transparent indicating that less sedimentation took place; also, no significant differences were seen among the samples and better consistency was suggested. The sedimentation for Formulation (3) was greater than (2) but better
consistency was observed with only small differences shown among the samples. Greater precipitation took place in Formulation (4) than in Formulation (3) and was shown to be less consistent as far less precipitation was observed in one sample. Formulation (5) showed much greater sedimentation when compared to Formulation (2), which comprised of poloxamer solution at the same concentration. One of the samples in Formulation (5) showed much less sedimentation resulted in poor consistency. Formulation (6) was considered to be the second best formulation among all 6 formulations as the consistency in performance was slightly inferior to that of Formulation (3) although less sedimentation was observed.

The performance of the 6 formulations were evaluated and put in an order from the best to the worst. The judgement was made based on the sedimentation behaviour and the consistency of their performances. The conformity of the performance was considered to be of higher priority as sedimentation could be easily solved by simply redisperse the suspension, especially in the case of ketoprofen when a much large time scale was required for the sedimentation to complete compared to ibuprofen suspensions. Hence, the performance of the 6 formulations follows the order, from the best to the worst, of: 3, 6, 2, 4, 5, 1.

It was suggested that there were two main processes taking place in the suspensions, the adsorption of poloxamer molecules at the drug particle surface and the aggregation of poloxamers in the bulk. The adsorption reduced the interfacial tension between the drug particles and the medium and was a spontaneous process while the aggregation in the bulk restrained the movement of the drug particles in the suspension and hence withheld the sedimentation.

Formulation (6) and (3) were considered to be the best two formulations out of 6 as better consistency and less sedimentation were shown; the abundant poloxamer present in the suspension reduced the interfacial tension and the sedimentation. However, Formulation (3) was considered to have outperformed Formulation (6) as better
consistency was observed. Formulation (2) and (3) had the same drug concentration, but (3) had a higher poloxamer concentration. (3) was considered to have outperformed (2) because better consistency was shown, although the sedimentation was greater. It was speculated that the greater sedimentation in Formulation (3) was a result of more aggregation taking place on the drug particle surface which was due to the continuous poloxamer adsorption. Inconsistency was shown in both Formulation (2) and (4), sedimentation was shown to be greater in Formulation (4) which was a result of a lower viscosity in the suspension as the poloxamer solution was at a lower concentration. Both Formulation (1) and (5) showed poor consistency and great sedimentation and no significant difference was seen between these two formulations. By comparing formulations, it was suggested that by increasing the poloxamer solution or decreasing the drug concentration, more poloxamer molecules were made available for the adsorption on drug particle surface to reduce the interfacial tension, better stability was hence achieved. Poloxamer molecules can also aggregate in the bulk to increase the viscosity of the system, enhancing the system sedimentation behaviour. However, if poloxamer molecules outbalance the drug particles, too much aggregation could also lead to greater sedimentation which is not desirable.

4.8.3 Summary on Ibuprofen and Ketoprofen Suspensions

Despite of the comprehensive study on the association behaviour of poloxamer in solution, the irreproducibility still persisted.

It was speculated that the irreproducibility was a result of the various aggregate sizes, however, the diversity in size was not caused by impurities. It was suggested by Ruckenstein and Nagarajan (1975) that the addition of poloxamer above the CAC not only led to an increase in the aggregate number but also gave rise to aggregates of larger average sizes. Aggregates of various sizes existed at all concentrations. At low concentration, the number and size of aggregates were small; they increased as the concentration increased. At low concentration, the aggregate size was monodispersed, the distribution of the aggregate size increased as a function of concentration.
It was also speculated that the compatibility between the drug particle size and poloxamer aggregate size could also improve the irreproducibility. Based on the observation from the two suspensions, suspensions comprised of poloxamers at a lower concentration (e.g. 5%) did not show better consistency than those comprised of poloxamers at higher concentrations (e.g. 15%) despite that the size distribution was expected to be smaller at lower concentrations. As discussed in Chapter 3, the steric hindrance also played an important role when the poloxamer adsorbed onto the drug surface. By choosing compatible drug particle size and poloxamer aggregate size, which was concentration dependent, the steric hindrance could be reduced and hence the stability of the suspension could be increased.

It was also important to control the amount of poloxamer to be sufficient but not excess for the adsorption onto drug particles. If excess poloxamer molecules were present in the suspensions, continuous aggregation might lead to greater sedimentation, which was not desirable.

To summarise, it was essential to reach a balance between the drug and the poloxamers in size and in amount. It would be beneficial to choose poloxamer solutions at a lower concentration, as the variation of aggregate size was smaller, better consistency was expected. However, as poloxamer solutions are concentration dependent, indicating that the average aggregate size and association behaviour were concentration dependent, hence it was also important to choose the right concentration of poloxamer solution in order to have compatible size and amount of poloxamer aggregates with those of the drug particles.

4.9 ITC studies of the original system

After the comprehensive study of the poloxamer association behaviour in water and also the optimum formulation study, it is suggested that the result of poor reproducibility shown in Chapter 3 could be improved by amending the original design of the experiments carried out in ITC.
As shown in Figure 4.1, when poloxamer solutions were diluted with water, the error was larger for dilution starting from lower concentrations such as 1% and 2.5% (w/v) and the error range was considered to be acceptable for dilution starting from 5%, 10% and 15% (w/v). The error range for dilution starting at lower concentrations was large because the signal was so small that the noise of the baseline was interfering with the data analysis. For dilution starting from high concentration, such as 15% (w/v), the aggregation was far more complicated and the solution, as suggested before, was composed of more poloxamer aggregates the size of which were more diverse, more time was required for relaxation. As a result, the error was large. Therefore, solutions at such concentrations were not suitable for this study and 5% (w/v) was chosen in the following study considering the accuracy of measurements and the time taken for the study.

Based on the results from previous study, the poloxamer solutions were prepared in a different way. The known amount of poloxamer was weighed in a volumetric flask and was filled with distilled deionised water to nominal volume. The solution was left sitting on the bench for 2 days after the poloxamer was all dissolved, agitation was applied to the solution occasionally in order to hasten the equilibration process. The solution was then heated in a boiling water bath to 80°C and was cooled down to room temperature. At the same time, the drug suspensions were prepared by simply dispensing the proper amount of drug particles in distilled deionised water and the ITC was set up ready for the study. According to the time dependence study by surface tension measurement, a minimum of 4 hours was needed for the solution to re-reach equilibrium once the equilibrium state was disrupted. Therefore, the poloxamer solution was then placed in the syringe for at least 4 hours before any titration was carried out and the time interval between each injection was set to 4 hours. As mentioned above, due to the time limit and the concentration consideration, the following study was not the optimum but an improved design with compromise.

Based on the optimum formulation selection results, ketoprofen was used as received
because the size of the particles was fine enough while 75-90 μm was chosen for ibuprofen. 0.01 g of ketoprofen and 0.02 g of ibuprofen was suspended in 2 mL of distilled deionised water in the ampoule and was titrated with PE/F68 5% (w/v) solution in aliquots of 15 μl at the time interval of 4 hours with the presence of stirrer at 30 rpm. Injections were made 10 times instead of 30 due to the time consideration. Power-time data for interactions between PE/F 68 and ibuprofen and ketoprofen are shown in Figures 4.25 and 4.26 respectively, with a, b and c indicating the three samples. Dilution enthalpy was plotted as a function of the poloxamer concentration in the ampoule. The results of ibuprofen and ketoprofen are shown in Figures 4.27 and 4.28 respectively.

Figure 4.25 Power-time data for interactions between ibuprofen and PE/F 68
Power-time data of interactions between Ketoprofen and PE/F 68 5% (w/v)

Figure 4.26 Power-time data of interactions between Ketoprofen and PE/F 68 5%

Ibuprofen with PE/F 68 5% (w/v)

Figure 4.27 PE/F 68 5% (w/v) solution interact with Ibuprofen suspension with improved parameters using ITC
Figure 4.28 PE/F 68.5% (w/v) solution interact with ketoprofen suspension with improved parameters using ITC

4.10 Conclusion

1. The initial irreproducibility problem has been greatly improved. Because of the large time period between each titration, only 10 injections were carried out. Due to the limited data, it was difficult to predict the trend of the interactions between the drug and poloxamer by the observation from Figure 4.27 and 4.28 alone and speculations were made based on the investigation findings from Chapter 3 and 4. It was speculated that the addition of poloxamer improved the stability of the ibuprofen / ketoprofen suspensions as exothermic reactions were observed, which drove the system to a lower energy state. Such observation was expected as poloxamer can reduce the interfacial energy between the drug particles and the solvent. It is suggested that when poloxamer was added to the suspensions, adsorption on the drug particles took place. When the surface of the drug particles were fully covered by poloxamer molecules, the extra poloxamer molecules added would continue to aggregate onto those poloxamers adsorbed onto drug particles. As poloxamer follows the open association model,
aggregation would continue to take place until the concentration of poloxamer was too high that gel was formed. As a result, no plateau was expected from the enthalpy-concentration curve indicating the balance was reached between the drug and the poloxamers. However, a minor change in the curve might be detected between the adsorption of poloxamer on drug particles and poloxamer aggregates. Poloxamer aggregates are formed with the PPO hydrophobic blocks in the core surrounded by the PEO hydrophilic blocks and hence exhibited hydrophilicity when compared to the drug. Hence, it was speculated that more heat was given out upon the adsorption of poloxamer molecules onto drug particles as the interfacial energy between the drug particles and the medium was much higher compared to that between the poloxamer aggregates and the solvent and the stability of the whole system can be significantly improved upon the adsorption of poloxamer molecules onto drug particles. Therefore, a decrease in enthalpy was expected when the adsorption change from the drug particles to poloxamer aggregates. As the concentration of poloxamer increased in the suspension, the enthalpy would continue to decrease as the adsorption of poloxamer onto poloxamer aggregates became the dominant process. As can be seen from Figure 4.28, a plateau was seen in the first half of the curve followed by a decrease in enthalpy. It was speculated that such observation reflected the adsorption of poloxamer onto ketoprofen particles followed by the adsorption of poloxamer onto poloxamer aggregates. On the contrary, the ibuprofen suspension was showing a different process. No plateau was seen at the beginning of the curve (Figure 4.27), the enthalpy continuously decreased as the concentration of poloxamer increased. It was speculated that what was shown in the figure was the adsorption of poloxamer onto poloxamer aggregates, indicating that the surface area of ibuprofen particles was covered by the poloxamer molecules after the first injection. To summarise, ITC was capable of detecting the monolayer coverage of poloxamers on drug particles, but it could not determine the balance between the drug and the poloxamers when the suspension was the most stable. However, such a drawback was a result from the
poloxamers following the open association model to form aggregates.

2. The dilution study of poloxamer PE/F 68 solutions from different concentrations to distilled deionised water carried out in ITC showed that poloxamer PE/F 68 followed the open association model as no phase transition was observed. In other words, the aggregation was a step-wise process and the aggregation number was not fixed. It was hypothesised that aggregates can be formed even at a very low concentration due to its size and its chemical structure which consists of both hydrophobic and hydrophilic moieties. The continuous decrease in surface tension also suggested that aggregation continued to take place even after concentration reached as high as 15% (w/v). Although only PE/F 68 was chosen as the main model for the study, it was reasonable to believe that poloxamers followed the open association model. This provided useful information as to how to handle and better use this material when applied in a formulation.

3. The dilution of PE/F 68 from various concentrations also proved that the properties of poloxamer solutions were concentration dependent. The result implied that it was essential to choose appropriate concentration of poloxamer solution when it is included in a pharmaceutical formulation. This conclusion was also supported by the DSC study and the surface tension measurement study of poloxamer solutions at different concentrations.

4. The time dependence study carried out by surface tension measurement indicated that 5% (w/v) PE/F 68 solution reached equilibrium approximately 60 hours after preparation. The equilibrium was reached 45 hours after the measurement, which was made 15 hours after the solution was prepared. Minimum 4 hours was required for equilibrated solution to re-reach equilibrium after its equilibrium state was disrupted. It was suggested by the results from Micro Solution Ampoule that by subjecting the solution at a lower concentration to constant stirring can be effective in hastening its equilibration. It was also
proved by DSC and surface tension measurement that by heating up solutions at higher concentration can also be an effective method to hasten the equilibration. It was suggested in this study that it was essential to make sure the polymer solution has reached equilibrium before use to ensure consistent performance.

5. Based on the findings from Chapter 3 and 4, the study of ibuprofen and ketoprofen interacting with 5% (w/v) PE/F68 solution at the end of this chapter was based on the original study shown at the beginning of Chapter 3 with amendment. The reproducibility, as seen at the end of this chapter, has been greatly improved. However, as the design of the study was made with compromise due to the time and technique consideration, the error range was still not as ideal.

6. What was discovered here required the attention from all scientists who deal with polymers. When dealing with polymer solutions, the equilibration state of the polymer solutions has always been ignored before the solution was used. It seldom occurs to researchers the importance of the way the polymer solutions were prepared and the importance to ensure the equilibrium of the solution before they were used. Performance and behaviour of a solution before and after it reaches equilibrium could be different, as evidenced in this study; more importantly, the performance of the solution could be inconsistent. Throughout the literature, not many publications illustrated the way the solution was prepared and no equilibrium of the polymer solutions was ensured; as a result no experiments can be repeated in exactly the same way.

7. This study also suggested that calorimetry as a sensitive and reliable technique should be widely applied to study materials from a brand new angle. The poloxamer solutions only act as a model in this study, the study on the poloxamer solution demonstrated the high sensitivity of ITC, Micro Solution Ampoule and DSC and that they are capable of detecting traces of differences.
Calorimetry can be applied whenever heat is involved. Their sensitivity, reliability and the wide applicability enabled calorimetry to be employed in many other fields, such as characterisation, stability study, physical and chemical interactions study.
Chapter Five

The Use of PHPMA to Improve the Stability of Griseofulvin/Indomethacin-PVP Solid Dispersions
5.1 Solid Dispersion

As stated in chapter one, many drugs on the market are water insoluble. Their bioavailability is largely limited by their dissolution rate. A lot of efforts are put in in order to improve their dissolution rate and hence their bioavailability. The absorption rate, which characterises the transport of drug molecules across the Gastrointestinal (GI) membrane, can be significantly increased upon the increase in the drug dissolution rate which can be achieved by decreasing the particle size of the drugs. Reduction in particle size can be achieved by (1) trituration and grinding; (2) milling; (3) micronisation; (4) spray drying; (5) ultrasonic waves; (6) precipitation by change of solvent or temperature and so on. However, due to the increased surface energy and van der waal’s attraction between non polar molecules, the fine particles achieved by using the techniques above tend to aggregate or agglomerate; also, the wettability of these particles remains unimproved and hence, their dissolution can be restrained (Florence and Attwood, 1981b; Habib, 2002).

Sekiguchi and Obi (1961) proposed the formation of a eutectic mixture of a poorly water soluble drug with a physiologically inert water soluble carrier. The eutectic mixture was prepared by melting the physical mixture of the drug and the carrier followed by a rapid solidification process. After the formulation is complete, the solid mixture of the drug and the carrier(s) can be milled or ground to produce very small size particles and the surface area of the compound is hence increased. Under certain circumstances, the size of the drugs can be reduced to a molecular level. The wettability of the drug is improved by encircling the drug with water soluble carriers, which makes it easy when the drug is in contact with the aqueous solvent. As a result, the dissolution rate is optimised and also apparent saturation solubility is increased (Leuner and Dressman, 2000; Weuts et al., 2005; Urbanetz and Lippold, 2005).

The enhanced dissolution rates from solid dispersions is a result of: a) the metastable form of the drug that can be stabilised by interacting with the polymer, such as through hydrogen bonding; b) the reduction in particle size of particle aggregation or
agglomeration due to the presence of the polymer; c) the increase in the wettability of the drug and d) the water soluble complexes formed by the drug and polymer.

The amorphous form is adopted to increase the apparent solubility of a drug because it does not require the energy to break up the crystalline lattice. However, the amorphous form is not stable and it tends to crystallise especially stored at the glass transition temperature ($T_g$). By preparing the drug in a solid dispersion with polymer, the $T_g$ of which is a lot higher than that of the drug, the $T_g$ of the mixture is hence increased compared to that of the drug alone; the drug can also be transformed from crystalline to amorphous form. As a result, the stability and the apparent solubility of the drug is increased (Weuts et al., 2005).

The physical form of the drug and the dispersion depends on a) the preparation method; b) the solvent used; c) the properties of the polymer; d) the drug loading and e) the physico-chemical properties of the drug.

Solid dispersion refers to the dispersion of one or more active ingredients in an inert carrier or matrix in the solid state, prepared by melting (fusion), solvent or the melting-solvent method. By choosing the appropriate carrier or combination of carriers, the release rate can also be slowed down and fit into the desired release pattern and so to achieve sustained-release profiles of drugs. Solid dispersion can also be used to achieve altered solid-state properties, enhanced release of drugs and improved solubility and stability.

Easy to manufacture, low expense and low toxicity, all these advantages have favoured solid dispersion; coupled with the large number of polymer available as carriers and the increasing number of water-insoluble drugs with desirable therapeutic effects the potential of solid dispersion is to be recognised in the pharmaceutical sciences.
5.1.1 Classification of solid dispersions

Solid dispersions are mainly classified into five categories as suggested by Chiou and Riegelmen (1971), (1) simple eutectic mixtures; (2) solid solutions; (3) glass solutions of suspensions; (4) compound or complex formations between the drug and the carriers; and (5) any combinations of above.

5.1.1.1 Simple Eutectic Mixtures

The two crystalline components, a sparingly water soluble drug and a highly water soluble carrier, are physically blended together and the two components crystallise simultaneously in very small particle sizes. The increased dissolution rate was mainly a result of the increase in specific surface area (Habib et al., 2002).

5.1.1.2 Solid Solutions

Solid solutions are composed of a solid solute (water insoluble drug) dissolved in a solid solvent. A mixed crystal is formed if the carrier is crystalline because the two components crystallise in a one-phase system. The increased dissolution rate is achieved by the formation of colloidal or molecular dispersion of the drug in the carrier (Chiou and Riegelmen, 1971).

5.1.1.3 Glass Solution of Suspension

A glass solution is a homogeneous system where a glassy form of a carrier solubilises drug particles in its matrix in the molecular level. For example, PVP demonstrates glassy properties when the organic solvent it is dissolved in undergoes evaporation (Chiou and Riegelmen, 1969) and thus the solid dispersion formed constitutes the glass solution suspension.

5.1.1.4 Compound and Complex formation

This solid dispersion system is formed by the complexation between the two components in a binary system during the preparation. The bioavailability of the drug is dependent on the solubility, dissociation rate and the intrinsic absorption rate of the
5.1.2 Methods of preparing solid dispersions

5.1.2.1 Melting method

The melting method has been exclusively used when the notion of solid dispersion came to light. The melting method is also known as the fusion method. The carrier is heated to a temperature just above its melting point and the drug is incorporated into the matrix. The melted mixture is cooled under constant stirring to homogeneously disperse the drug throughout the matrix. Alternatively, the mixture can be placed in an ice bath after ensuring the uniformity of the mixture. The solidified end product is then milled to desirable small sizes. The cooling leads to supersaturation and the solidification results in the drug being trapped within the carrier matrix. The preparation of the dispersion depends on the degree of the supersaturation and also the rate of cooling during the process (Leuner and Dressman, 2000; Habib et al., 2002).

Several mechanisms may have been involved during the preparation process. If the solubility of the drug is high in the carrier, the drug is presumably dispersed in the matrix at a molecular level and would remain ‘dissolved’ in the solid state, a solid solution is hence formed. If the solubility of the drug in the carrier is not so high, the drug is dispersed in the matrix in the crystalline form. A moderate increase in dissolution rate is expected. A third possibility is the conversion of the drug to an amorphous form. Due to the higher energy state it is in, higher dissolution rate is expected. During the preparation process, increased wettability (or decreased hydrophobicity) may also be achieved endowed by the carrier (Habib et al., 2002).

However, there are a few limitations for this method. Firstly, since the mixture of the drug and the carrier has to be melted at a high temperature, it is essential that the drug is thermally stable. Potential problems include thermal degradation, sublimation and polymorphic transformation. Although such problems could be avoided by preparing under vacuum or in a closed or inert system, it increases the difficulty and it denies the
accessibility of this method. Secondly, the molten mixture needs to be miscible; otherwise, no molecular dispersion can be formed. Thirdly, decomposition may take place during fusion. In order to avoid decomposition or control decomposition to an acceptable level, the fusion temperature should be limited to that which is just capable of melting both the drug and the carrier. Fourthly, crystallites may form during quench cooling. (Leuner and Dressman, 2000; Habib et al., 2002)

This method has been optimised in recent years by using hot melt extrusion and hot-spin-melting. The advantage of these two methods is that the drug/carrier mix is only subjected to elevated temperature over a very short time and it minimises the problem for thermolabile substances (Leuner and Dressman, 2000).

Spray congealing from a modified spray drier onto cold metal surfaces is also suggested. This method has the advantage of producing pellets of the dispersion without grinding and altering the crystalline structure of the drug. (Froemming et al., 1978)

5.1.2.2 Solvent method
The solvent method was first introduced by Tachibani and Nakumara in 1965. This method was also termed coprecipitation and coevaporation. The process is to dissolve both the drug and the carrier in a common organic solvent and then evaporate the solvent at an elevated temperature or under vacuum to produce a solid dispersion. The operating temperature for the solvent evaporation is usually at a lower temperature than that the samples subject to using the melting method. Upon the solvent evaporation, supersaturation occurs followed by simultaneous precipitation. The prerequisite of this method is that both the drug and the carrier have to be readily dissolved in the solvent and the solvent can be easily removed. Freeze drying and spray drying are the two most frequently used methods for solvent evaporation.

In order to employ this method, a solvent that both the drug and the carrier can be sufficiently dissolved in is a must. Whether or not the organic solvent can be completely
removed is also another one important consideration, as most of the organic solvents used are toxic to certain extents. Removal of even trace amount of solvent is essential. However, it is possible that some solvent is included within the crystal lattice, which makes the solvent removal even more difficult (Leuner and Dressman. 2000; Habib et al., 2002). Highly sensitive techniques such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and differential thermal analysis (DTA) are often employed to detect the solvent removal efficiency. More rapid solvent removal may be achieved by freeze drying or spray drying (Habib et al., 2002). Since the increase in dissolution rate is accomplished by dispersing drug at the molecular level, the drug-to-carrier ratio is particularly important (Sekikawa et al., 1978). Thanks to the solvent method, a lot of materials that could not be utilised in a solid dispersion by the melting method due to the problems associated such as the high melting point or the thermo instability can be reconsidered again (Leuner and Dressman, 2000). There are also reports in the literature that dissolution rate is faster for dispersions prepared using the solvent method compared to the melting method (Arias et al., 1995).

5.1.2.3 Melting-Solvent method
First dissolve a drug in a suitable liquid solvent and then incorporate the solution into the melt of the carrier. The melting-solvent method is a combination of the previous two methods. It is expected to possess the advantages of both methods but it is also restricted by their drawbacks such as the small amount of drug that can be dissolved in the solvent and the miscibility of the drug solvent with the molten carrier. However, if the carrier is capable of holding a certain proportion of liquid yet maintaining its solid properties and if the liquid the drug was dissolved in is innocuous, the removal of the solvent is unnecessary.

5.2 Aim
A typical solid dispersion is prepared by dispersing the drug in one polymer. A hydrophobic drug dispersed in one carrier, such a system is called a binary system. A binary system is better known and more widely used. However, a ternary system can
also be formulated, in which a secondary excipient is added to further improve the solubility and stability. The secondary excipient is usually a surfactant. It was hypothesised that the stability of a solid dispersion may be improved by adding a second excipient (Serajuddin et al., 1990). Fletcher (2006) prepared solid dispersions of Griseofulvin and PVP with a variety of second excipients including poly(acrylic acid) (PAA), sucrose and poly(2-hydroxypropylmethacrylate) (PHPMA) by spray drying. The stability study showed that PHPMA has the most profound and consistent effect among the three excipients on improving the amorphous stability of the drug. It was found that the order in which the components were dissolved in the feed solution prior to spray drying also had an effect on the amorphous stability of the drug. It was speculated that the increase in stability was achieved by hydrogen bonding.

However, the mechanism of how the second excipient contributed to the stability of the drug was not clear. Many researchers working on solid dispersions very often adopted Scanning Electron Microscopy (SEM), X-ray powder diffraction (XPRD), and Differential Scanning Calorimetry (DSC) to study the difference between different preparation methods or the solid dispersion prepared with different carriers. However, such methods can only provide very limited information on the solid dispersion formation mechanism as the information was obtained after the formulation was formed but not during the preparation process. Sometimes, the influence of the second excipient on the interactions between the drug and the carrier was not detectable from DSC (Veiga et al., 1993; Arias et al., 1995); and a long time, days up to months, was often required for the stability study.

In this study, solid dispersions were prepared by using Griseofulvin (Gris) and PVP, and PHPMA was chosen as the second excipient. Another model drug Indomethacin (Indo) was also chosen for this study. ITC was chosen to study the stability of the solid dispersions in liquid state. By correlating the results obtained from this study with those from Fletcher's work (2006), it was aimed to enhance the understanding of the mechanism on how the second excipient influenced the stability of the solid dispersion;
to investigate the feasibility of employing ITC as a new technique to study the stability of solid dispersions; more importantly; to predict the component compatibility, the stability of the solid dispersions in solid state by studying systems in their liquid state. The prospect of the last objective is promising, as it could benefit the research efficiently and economically.

5.3 Model Drugs

5.3.1 Griseofulvin

![Chemical Structure of Griseofulvin](image)

Griseofulvin, 7-chloro-2',4,6'-trimethoxy-4'-methylspiro[benzofuran-2(3H), 1-(2)cyclohexene]-3,4'-dione, was first isolated from the fungus *Penicillium griseofulvum*. Its chemical structure is shown in Figure 5.1. However, its therapeutic effect against fungus was not noticed until 10 years after it was first discovered in 1939. Although it has been widely used in treatments of dermatological diseases, it was usually administered orally (Finkelstein *et al.*, 1996).

However, the use of griseofulvin in treatment of antifungus was limited due to its physicochemical properties as a hydrophobic drug. Griseofulvin is practically insoluble in water, which largely increased the difficulty of its formulation and delivery. However, attempts have been made to improve its bioavailability by various techniques such as micronisation (Wong *et al.*, 2006), complexation of griseofulvin with cyclodextrins (Wulff and Alden, 1999) and preparation of griseofulvin in nanoparticles (Zili *et al.*, 2005).
5.3.2 Indomethacin

![Chemical Structure of Indomethacin](image)

Figure 5.2 Chemical Structure of Indomethacin

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that exhibits antipyretic and analgesic effects. As an anti-inflammatory agent, indomethacin was also proved to be effective for long-term use in rheumatoid arthritis, ankylosing spondylitis and osteoarthritis. It was found to be effective in relief of pain, reduction of fever, swelling and tenderness, along with other symptoms of rheumatoid arthritis and gouty arthritis. About 99% of indomethacin is bound to protein in plasma over the expected range of therapeutic plasma concentrations. Indomethacin has also been found to cross the blood-brain barrier and the placenta.

The stabilising effect of indomethacin / PVP solid dispersion was achieved through the specific interaction between the hydroxyl section of the carboxylic acid group of indomethacin, as shown in Figure 5.2, and the carbonyl group of PVP. Such interaction was believed to prevent indomethacin from recrystallisation. Dimers, formed between indomethacin molecules, were the base unit of the crystals (Taylor and Zografi, 1997; Matsumoto and Zografi, 1999)

5.4 Polyvinylpyrrolidone (PVP)

In the solid dispersion technology, an ideal carrier should be a) freely water soluble with intrinsic rapid dissolution properties; b) non-toxic; c) compatible with the drug; d) pharmacologically inert and 5) should not form complexes with slow disassociation rate constant that decreases the dissolution rate (Akin, et al., 1998).
Ever since solid dispersions came to light, their use has been studied extensively, including the method of preparation, the mechanisms to improve the dissolution rates or solubility and ultimately bioavailability of the drugs, and also the exploration of carrier candidates. Carriers of different water solubility can be chosen to achieve different drug release profiles. PVP is one of the most used water-soluble polymers.

![Chemical Structure of Polyvinylpyrrolidone (PVP)](image)

**Figure 5.3 Chemical Structure of Polyvinylpyrrolidone (PVP)**

PVP has been one of the most widely used carriers in solid dispersions. PVP is chosen in this study as the carrier as it is a water-soluble tertiary amide and a strong Lewis base. PVP is a good carrier for solid dispersion because it undergoes rapid dissolution, is non-toxic and pharmacologically inert and it is compatible with many drugs.

The average molecular weight of PVP ranges from 10,000 to 700,000. It is soluble in various solvents, including water, ethanol and chloroform. The high $T_g$ of PVP made it unsuitable for preparing solid dispersions by the fusion method but only the solvent method (Leuner and Dressman, 2000).

The glass transition temperature of a given PVP is dependent not only on its MW but also on its moisture content. In general, the glass transition temperature for PVPs is quite high. Such property has limited the usage of PVP in solid dispersions prepared by the melting method but it also helps increase the stability of the solid dispersion. When compared to many other carriers, PVP is reported to improve the release rate of the drugs more efficiently in many studies. Its solubility in a wide range of organic solvents, its good water solubility and good wettability of the dispersed compounds, combined with its low toxicity when administered orally, PVP has demonstrated its great potential
as the carrier in solid dispersions (Leuner and Dressman, 2000).

As the molecular weight of PVP increases, the dissolution rate of most of the drugs decreases which is a result from the increase in the viscosity and swelling of the PVP. As the thickness of the diffusion boundary increases, the diffusion rate of drug molecules from matrix to the dissolution medium decreases. However, PVP of high molecular weight can slow down the crystallisation (Owusu-Ababio, 2002). A study on the dissolution behaviour of probucol from solid dispersion systems consisting of various PVPs as carriers show the order of their release rate was PVP K30>K25>K90 (Yagi et al., 1996). As the result implies, the dissolution rate of the drug from the solid dispersion relies on the PVP employed in the formulation. A PVP K30, considered to have a moderate MW, was chosen for this study.

5.5 Poly(2-hydroxypropylmethacrylate) (PHPMA)

Very few reports have been found about the use of PHPMA as it was only employed as a carrier for solid dispersions in recent years. PHPMA has a special chemical structure that includes both the hydrogen donor group, hydroxyl group, and the hydrogen acceptor group, the carbonyl group. It was reported that PHPMA / PVP were totally miscible at various composition range (Kuo et al., 2004). PHPMA, MW 20 000, was chosen for this study.

5.6 Experimental Description

PHPMA was found to be very poorly soluble in acetone and PVP was relatively
insoluble in acetone, which can dissolve Griseofulvin. Also, in order to correlate the findings from Fletcher (2006), a combination of water and acetone was chosen as the solvent system. Increasing the amount of acetone in the solvent combination tends to precipitate some of the components while increasing the amount of water tends to minimise the precipitation. Hence, a mixture of acetone and distilled deionised water (19:9) was chosen as the solvent. The three components, the model drug, PVP and PHPMA were used as received. The three components were dissolved in the solvent mixture at concentrations of 0.2\%, 0.1\% 0.1\% (w/v) respectively. 0.5ml of the titrant loaded in the syringe was titrated to the sample ampoule containing 0.5ml of reactant in ITC. The titrant was dispensed at the rate of 1\mu\text{l}\text{s}^{-1}. The reactant, 0.5mL, in the ampoule was stirred at 30rpm during the whole process. The experiments were carried out in the Thermometric 2277 Thermal Activity Monitor (TAM, Thermometric AB, Järfälla, Sweden) which was operated at 25.00±0.01°C. The experiment was controlled and monitored by the Digitam® software, the results were then analysed by the software Origin® (MicroCal Software, Inc., USA). All experiments were repeated three times and the mean and standard deviation were obtained.

However, contrary to the study of poloxamer in Chapter 4, which required 4 hours for poloxamer solution to reach equilibrium once its equilibrium state was disrupted. The experiment was carried out in a way to minimise the time duration to prevent the solvent evaporation. Therefore, the titrant was loaded to the syringe only after the instrument had reached equilibrium and the experiment was started once the baseline had re-reached equilibrium. The disruption of the baseline was caused by the insertion of the syringe cannula.

5.7 Griseofulvin/PVP/PHPMA

5.7.1 Griseofulvin Binary system

Although the effect of a second excipient on solid dispersion was reported to be positive (Serajuddin et al., 1990; Fletcher, 2006), the study was purposefully limited to a binary system initially in order to achieve better understanding of interactions between the
drug, the carrier and the second excipient and so to provide the base for discussion of the ternary system.

Therefore, the studies were carried out to measure the reaction enthalpy between any two components, Griseofulvin and PVP, Griseofulvin and PHPMA, PVP and PHPMA. Such results and the corresponding blank experiment results are shown in Table 5.1. Positive results indicated endothermic processes whilst negative results indicated exothermic processes.

<table>
<thead>
<tr>
<th>Titrant</th>
<th>Reactant</th>
<th>Enthalpy (μJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP</td>
<td>Griseofulvin</td>
<td>-44100 ± 2200</td>
</tr>
<tr>
<td>PVP</td>
<td>Solvent</td>
<td>275 ± 20</td>
</tr>
<tr>
<td>Solvent</td>
<td>Griseofulvin</td>
<td>35100 ± 1500</td>
</tr>
<tr>
<td>PHPMA</td>
<td>Griseofulvin</td>
<td>-22700 ± 1800</td>
</tr>
<tr>
<td>PHPMA</td>
<td>Solvent</td>
<td>353000 ± 36000</td>
</tr>
<tr>
<td>PHPMA</td>
<td>PVP</td>
<td>129000 ± 1600</td>
</tr>
<tr>
<td>Solvent</td>
<td>PVP</td>
<td>488 ± 35</td>
</tr>
<tr>
<td>PVP</td>
<td>PHPMA</td>
<td>177000 ± 16000</td>
</tr>
<tr>
<td>Solvent</td>
<td>PHPMA</td>
<td>496 ± 22</td>
</tr>
</tbody>
</table>

Table 5.1 Reaction enthalpy of Griseofulvin Binary System

The reaction enthalpy between any two main components of the Griseofulvin system can be obtained by subtracting the control experiments, the dilution of the titrant and the dilution of the reactant, from the total reaction enthalpy. For example, the interaction enthalpy between Griseofulvin and PVP was obtained by subtracting the enthalpy from the titration of PVP into Solvent (275 ± 20) and Solvent into Griseofulvin (35100 ± 1500) from that of PVP into Griseofulvin (-44100 ± 2200). The standard error was obtained by adding up the standard deviations of all interactions involved.
The results were shown as the enthalpy involved in each interaction (J) rather than in the common practice as J·g⁻¹ or J·mol⁻¹ to avoid misinterpretation and introducing complexities to the discussion, which stems from the choice of the control material. For example, when PVP was titrated to Griseofulvin solution, the enthalpy involved in this process was expected to be different from that of the titration of Griseofulvin into PVP solution. However, when taken into account the blank experiments, the dilution of the titrant and the titrand, the interaction between Griseofulvin and PVP was expected to be the same, regardless of which was titrated into which. However, if the titrand was chosen as the control, the resulted enthalpy/weight (mole) would be very different as the amount of PVP and Griseofulvin used was different, and the MW was also different. As the amount of the three components used was consistent in all dispersions studied, the correction for the amount of components on the weight or mole basis was not necessary, and such way of expression was used throughout this chapter for the convenience of discussion.

The net interaction enthalpy of the Griseofulvin binary system is listed in Table 5.2.

<table>
<thead>
<tr>
<th>Dispersion number</th>
<th>Reactions</th>
<th>Interaction Enthalpy (mJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1.1</td>
<td>Griseofulvin / PVP</td>
<td>-79.5 ± 3.7</td>
</tr>
<tr>
<td>5.1.2</td>
<td>Griseofulvin / PHPMA</td>
<td>-411 ± 39</td>
</tr>
<tr>
<td>5.1.3</td>
<td>PVP / PHPMA</td>
<td>176 ± 20</td>
</tr>
</tbody>
</table>

Table 5.2 Interaction enthalpy between Griseofulvin / PVP, Griseofulvin / PHPMA and PHPMA / PVP

As shown from Table 5.2, the interactions from dispersion 5.1.1 (Griseofulvin and PVP) and 5.1.2 (Griseofulvin and PHPMA) were both exothermic, and the interaction from 5.1.3 (PVP and PHPMA) was endothermic. The enthalpy of 5.1.2 was approximately 5 times that of 5.1.1, indicating the interaction between Griseofulvin and PHPMA was much more intense than that between Griseofulvin and PVP. It was speculated that hydrogen bonding was formed between Griseofulvin and PHPMA, which stabilised the
system, as the exothermic reaction drove the system to a lower energy state.

On the other hand, the interaction between Griseofulvin and PVP was not as strong, as both of them are proton acceptors, no hydrogen bonding was formed. However, the exothermic interaction between the two indicated that the presence of PVP contributed to the increase in Griseofulvin stability. As no hydrogen bonding could be formed between PVP and Griseofulvin due to the lack of proton donors, it was speculated that the exothermic reaction was a result of the physical bonding of PVP with Griseofulvin. Although Griseofulvin was dissolved in the solvent mixture, due to the presence of water in the solvent, Griseofulvin was not at its most stable state; PVP molecules, containing both the hydrophobic and hydrophilic parts, were thus driven to migrate and bond with Griseofulvin at a molecular level. As a result, the interfacial energy between the Griseofulvin and the solvent was further lowered. As the bonding took place at the physical level, the reaction enthalpy involved was comparatively small.

The interaction between PVP and PHPMA was endothermic, indicated that extra energy was required to stabilise the system and the interaction was likely to be entropy driven. It was suggested that hydrogen bonding was formed between PHPMA molecules before the interaction with PVP as PHPMA has a unique chemical structure which includes both the hydrogen donors and acceptors (Kuo et al., 2004). Upon the introduction of PHPMA to PVP solutions, the hydrogen bonding formed within PHPMA molecules were broken down and was re-formed between PHPMA and PVP molecules. Infrared spectra, FTIR and DSC provided evidence that the inter-association between PHPMA and PVP through hydrogen bonding dominates the intra-hydrogen bonding of the pure PHPMA in the mixture (Kuo et al., 2004). However, the hydrogen bonding between the two was hindered due to the steric effects resulted from the large alkyl groups, therefore, the bonding between PHPMA and PVP was not favoured. As a result, extra energy was required to complete the hydrogen bonding between the two. Therefore, it was concluded that the interaction between PHPMA and PVP was not enthalpy favoured, rather, it was an entropically driven process. It is also worth noting that the hydrogen
bonding could also retain within some PHPMA molecules. As PHPMA and PVP was at a 1:1 (w/w) ratio, the MW of PHPMA was 20 000 whilst the MW of PVP K30 was 50 000.

5.7.2 The effect of order of addition in Griseofulvin Ternary Systems

After the study on Griseofulvin binary system, further studies were carried out on ternary system in order to investigate the effect of a secondary excipient and also the effect of its order of addition on the stability of the system. PHPMA was chosen as the secondary excipient to form the ternary system. In order to study the effect of the secondary excipient and its order of addition, the study was carried out by preparing different solutions containing Griseofulvin and PHPMA, Griseofulvin and PVP and PHPMA and PVP at the original ratio which were then subject to interaction with the third component. Such experiments were aimed to study the effect of the addition order of secondary excipient/carrier. The titrant and the reactant were both 0.5mL, the weight ratio of three components was maintained at the original ratio (Drug: Carrier: Second Excipient = 2:1:1). The measured enthalpy of the reactions is listed in Table 5.3.

<table>
<thead>
<tr>
<th>Titrant</th>
<th>Reactant</th>
<th>Enthalpy (μJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHPMA + PVP</td>
<td>Griseofulvin</td>
<td>-28300 ± 2500</td>
</tr>
<tr>
<td>PHPMA + PVP</td>
<td>Solvent</td>
<td>-15000 ± 1600</td>
</tr>
<tr>
<td>Solvent</td>
<td>Griseofulvin</td>
<td>35100 ± 1500</td>
</tr>
<tr>
<td>PHPMA</td>
<td>Griseofulvin + PVP</td>
<td>-17200 ± 390</td>
</tr>
<tr>
<td>PHPMA</td>
<td>Solvent</td>
<td>353000 ± 36000</td>
</tr>
<tr>
<td>Solvent</td>
<td>Griseofulvin + PVP</td>
<td>-14000 ± 1400</td>
</tr>
<tr>
<td>PVP</td>
<td>PHPMA + Griseofulvin</td>
<td>109000 ± 12000</td>
</tr>
<tr>
<td>PVP</td>
<td>Solvent</td>
<td>275 ± 20</td>
</tr>
<tr>
<td>Solvent</td>
<td>PHPMA + Griseofulvin</td>
<td>-14800 ± 1100</td>
</tr>
</tbody>
</table>

Table 5.3 Reaction enthalpy of Griseofulvin Ternary System
The analysis was processed in the same way as described in section 5.7.1. The enthalpy of ternary interactions is shown in Table 5.4.

<table>
<thead>
<tr>
<th>Dispersion Number</th>
<th>Reactions</th>
<th>Interaction Enthalpy (mJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1.4</td>
<td>PVP / (Griseofulvin + PHPMA)</td>
<td>124 ± 13</td>
</tr>
<tr>
<td>5.1.5</td>
<td>PHPMA / (Griseofulvin + PVP)</td>
<td>-356 ± 38</td>
</tr>
<tr>
<td>5.1.6</td>
<td>(PHPMA + PVP) / Griseofulvin</td>
<td>-48.4 ± 5.6</td>
</tr>
</tbody>
</table>

Table 5.4 Interaction enthalpy between PVP / (Griseofulvin + PHPMA), PHPMA / (Griseofulvin + PVP) and Griseofulvin / (PHPMA + PVP)

The reaction enthalpy shown in Table 5.4 was the interaction enthalpy involved between the titrant and the reactant(s). As seen from Table 5.4, when PVP interacted with the Griseofulvin and PHPMA mixture, the interaction was endothermic with interaction enthalpy of 124 ± 13mJ, indicating that the interaction between PVP and the Griseofulvin / PHPMA mixture was not enthalpy favoured. It was hypothesised that hydrogen bonding was formed between Griseofulvin and PHPMA; when PVP was added to the mixture, the bonding of PVP with the drug was not favoured as the bonding site has been occupied by PHPMA and also the steric hindrance introduced by PHPMA. It was also hypothesised that the introduction of PVP forced the hydrogen bond breakage between Griseofulvin and PHPMA, as the proton acceptors outnumbered the proton donors because both Griseofulvin and PVP are proton acceptors and only PHPMA is a proton donor. The deassociated PVP in the solution forced some of the PHPMA molecules to free themselves from Griseofulvin and bonded with PVP. Hence, the process was entropy driven.

The addition of PHPMA to the Griseofulvin and PVP mixture was shown to be favourable to the stability of the system as a lot of heat was given out (-356 ± 38mJ). Such observation was supported by the findings of Fletcher (2006). The bonding of PVP with Griseofulvin took place as the two were mixed together. As discussed before, the introduction of PVP could increase the stability and dissolution rate of Griseofulvin by...
reducing the interfacial energy and increasing the wettability. When PHPMA was introduced to the system, hydrogen bonding was formed with both PVP and Griseofulvin. This further stabilised the system as PHPMA was able to interact with both Griseofulvin and PVP equally and it acted as a bridge to strengthen the bonding between Griseofulvin and PVP.

The interaction enthalpy between Griseofulvin and the PHPMA and PVP mixture was $-48.4 \pm 5.6 \text{mJ}$. It was speculated that hydrogen bonds was formed between PHPMA and PVP before their introduction to the Griseofulvin solution, however, as there were more PHPMA molecules than PVP molecules in the solution, extra PHPMA molecules which were not bonded were present in the solution. When the mixture was added to the Griseofulvin solution, hydrogen bonds were formed between Griseofulvin and the extra PHPMA molecules and the measured enthalpy was negative, indicating that the bonding was beneficial to the stability of the system. As the bonding was limited due to the limited available proton donors, the total net heat output was small.

5.7.3 Stability of the Griseofulvin Systems

The discussion above was rather aimed to elucidate the mechanism of how the carrier (PVP) and secondary excipient (PHPMA) improved the dissolution and increased the physical stability of the water-insoluble drug Griseofulvin. The discussion was confined to the interaction between a mixture of two components with a third component. The following section was to investigate the stability of Griseofulvin system as a whole by taking into account the enthalpy involved when preparing the mixture. The data presented earlier are reproduced here for convenience.
Table 5.5 Total enthalpy of Griseofulvin Ternary systems

<table>
<thead>
<tr>
<th>Dispersion Number</th>
<th>5.1.4</th>
<th>5.1.5</th>
<th>5.1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture</td>
<td>Gris + PHPMA</td>
<td>Gris + PVP</td>
<td>PHPMA + PVP</td>
</tr>
<tr>
<td>Interaction Enthalpy of the mixture (mJ)</td>
<td>-411 ± 39</td>
<td>-79.5 ± 3.7</td>
<td>176 ± 20</td>
</tr>
<tr>
<td>Third Component</td>
<td>PVP</td>
<td>PHPMA</td>
<td>Griseofulvin</td>
</tr>
<tr>
<td>Enthalpy upon addition of 3rd component</td>
<td>124 ± 13</td>
<td>-356 ± 38</td>
<td>-48.4 ± 5.6</td>
</tr>
<tr>
<td>Total system enthalpy (mJ)</td>
<td>-287 ± 52</td>
<td>-436 ± 42</td>
<td>128 ± 26</td>
</tr>
</tbody>
</table>

When the system gave out heat, it was driven to a lower energy state and such system tends to be more stable. Therefore, the more heat was given out, the more stable the system was. As indicated by Table 5.5, both systems 5.1.4 and 5.1.5 were exothermic, and system 5.1.6 was endothermic. Therefore, the system in which the secondary excipient and carrier mixture was added to the drug solution was the least stable of all three systems and the system when Griseofulvin was first interacted with carrier PVP followed by addition of PHPMA was the most stable. This finding agreed very well with previous work (Fletcher, 2006). Discussions of mechanisms were included in the previous sections 5.7.1 and 5.7.2.

It is also worth noting that the enthalpy involved when PHPMA and Griseofulvin are mixed together was -411 ± 39mJ, whilst the total heat output for system 5.1.5 was -436 ± 42mJ, no significant difference was shown between the two systems, partly resulted from the large error range. It was suggested that the stability of the Griseofulvin was
mainly achieved by the addition of PHPMA. Due to the large error range, it was
difficult to conclude which system was more stabilised.

Judging by the results presented in Table 5.5, it was reasonable to conclude that the
order of addition of the components could significantly influence the stability of the
systems as different mechanisms were involved. In the case of Griseofulvin, adding
secondary excipient PHPMA after Griseofulvin and PVP were mixed was proved to be
the most stable. Such findings correlated very well with Fletcher’s work (2006).
Therefore, it was indicated that the properties of the solid dispersions at their liquid state
related to those at their solid state and the stability study of the solid dispersions by ITC
at solution state could predict accurately the stability of the systems at solid state.

5.8 Indomethacin/PVP/PHPMA
5.8.1 Indomethacin Binary System
Indomethacin was chosen as a model drug as it has been well studied in solid
dispersions. Indomethacin differs from Griseofulvin as it has the hydroxyl group which
makes hydrogen bonding between indomethacin and PVP possible. Therefore, studies
were carried out to investigate if hydrogen bonds were formed between indomethacin
and PVP and if they could be detected by calorimetry. Studies were also carried out to
investigate whether indomethacin could be better stabilised by PHPMA which also have
both carbonyl and hydroxyl groups.

The studies were carried out as described in section 5.6. Indomethacin, PVP and
PHPMA were prepared at the same concentration and the same ratio as the Griseofulvin
systems, Indomethacin: PVP: PHPMA = 2:1:1 (w/w/w). The solvent system was also
the same acetone and water mixture (19:9). Hence, the results of some blank
experiments were reproduced in this section for convenience. Interaction enthalpy
results of the indomethacin binary system are shown in Table 5.6.
Table 5.6 Reaction enthalpy of Indomethacin Binary System

<table>
<thead>
<tr>
<th>Titrant</th>
<th>Reactant</th>
<th>Enthalpy (µJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP</td>
<td>Indomethacin</td>
<td>-70700 ± 8300</td>
</tr>
<tr>
<td>PVP</td>
<td>Solvent</td>
<td>275 ± 20</td>
</tr>
<tr>
<td>Solvent</td>
<td>Indomethacin</td>
<td>-8810 ± 1100</td>
</tr>
<tr>
<td>PHPMA</td>
<td>Indomethacin</td>
<td>-13800 ± 700</td>
</tr>
<tr>
<td>PHPMA</td>
<td>Solvent</td>
<td>353000 ± 36000</td>
</tr>
<tr>
<td>PHPMA</td>
<td>PVP</td>
<td>129000 ± 12000</td>
</tr>
<tr>
<td>Solvent</td>
<td>PVP</td>
<td>488 ± 35</td>
</tr>
<tr>
<td>Solvent</td>
<td>PHPMA</td>
<td>496 ± 22</td>
</tr>
</tbody>
</table>

Table 5.7 Interaction Enthalpy between Indomethacin /PHPMA and Indomethacin / PVP

Hydrogen bonds were formed through the hydroxyl section of the indomethacin carboxylic acid group and the carbonyl group from PVP. Such hydrogen bonding was suggested to prevent the indomethacin from recrystallisation as it stopped the formation of the dimer which is the base of the indomethacin crystals (Taylor and Zografi, 1997; Matsumoto and Zografi, 1999). The enthalpy from systems 5.2.1 and 5.2.2 are both exothermic, indicating that the addition of either PHPMA or PVP can increase the stability of indomethacin and such effect was suggested to have achieved by the hydrogen bonding. The enthalpy involved from system 5.2.1 (indomethacin with
PHPMA) was nearly six times that of system 5.2.2 (indomethacin with PVP), which was suggested to be the result of the extra hydroxyl groups introduced upon the addition of PHPMA. The MW of PVP K30 was 50 000 whilst the MW of PHPMA was 20 000, consequently, more hydrogen bonding sites were present when PHPMA was introduced to the system when the amount of PHPMA and PVP added to the indomethacin solution was the same. What’s more, the large alkyl group from PVP introduced steric hindrance which did not favour the hydrogen bonding. These resulted in PHPMA being more efficient at increasing the indomethacin stability when the same amount of PHPMA and PVP was added to the indomethacin solution.

Despite the evidence provided by many researchers that PVP was very effective at increasing the stability of indomethacin (Imaizumi et al., 1983; Lu and Zografi, 1998; Crowley and Zografi, 2003; Watanabe et al., 2003), the reaction enthalpy between indomethacin and PVP was surprisingly small. The small interaction enthalpy between Indomethacin and PVP was speculated to be the result of the breakage of hydrogen bonds from Indomethacin dimers. Upon the addition, the hydrogen bonds formed between the dimers were broken in order to bond with PVP at the intra-molecular level. As a consequence, the endothermic enthalpy resulted from the hydrogen bond breakage also contributed to the whole interaction enthalpy and the measured net enthalpy was comparatively small. It is worth noting that despite of the increased number of proton donors in the system, the enthalpy between PHPMA and indomethacin (-358 ± 38 mJ) was still smaller than that between Griseofulvin (-411 ± 39 mJ), which was also suggested to be the result of the hydrogen breakage from the indomethacin and PHPMA dimers.

As indicated from the results of the indomethacin binary systems, both PVP and PHPMA have positive effects on increasing the stability of indomethacin in the solvent and PHPMA was shown to have more intense bonding with indomethacin. The following discussion was aimed to investigate if a tertiary component could further increase the stability of the system and if the order of addition of excipients would play
5.8.2 The effect of order of addition in Indomethacin Ternary Systems

The investigation was carried out in the same fashion as described in section 5.7.2. The titrants and reactants were prepared in accordance to Table 5.8. Indomethacin, PVP and PHPMA were prepared at concentrations of 0.2%, 0.1% and 0.1% (w/v) respectively. Triplicate measurements were carried out. The mean enthalpy and the standard error of these interactions are shown in Table 5.8.

<table>
<thead>
<tr>
<th>Titrant</th>
<th>Reactant</th>
<th>Enthalpy (μJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHPMA + PVP</td>
<td>Indomethacin</td>
<td>-3060 ± 140</td>
</tr>
<tr>
<td>PHPMA + PVP</td>
<td>Solvent</td>
<td>-15000 ± 1600</td>
</tr>
<tr>
<td>Solvent</td>
<td>Indomethacin</td>
<td>-8810 ± 1100</td>
</tr>
<tr>
<td>PHPMA</td>
<td>Indomethacin + PVP</td>
<td>-18500 ± 1600</td>
</tr>
<tr>
<td>PHPMA</td>
<td>Solvent</td>
<td>353000 ± 36000</td>
</tr>
<tr>
<td>Solvent</td>
<td>Indomethacin + PVP</td>
<td>-10400 ± 600</td>
</tr>
<tr>
<td>PVP</td>
<td>PHPMA + Indomethacin</td>
<td>-73400 ± 8600</td>
</tr>
<tr>
<td>PVP</td>
<td>Solvent</td>
<td>275 ± 20</td>
</tr>
<tr>
<td>Solvent</td>
<td>PHPMA + Indomethacin</td>
<td>-101000 ± 6600</td>
</tr>
</tbody>
</table>

Table 5.8 Reaction Enthalpy of Indomethacin Ternary System

The data analysis was carried out as described in section 5.7.1. The calculated interaction enthalpy between the titrant and the reactant is shown in Table 5.9.

<table>
<thead>
<tr>
<th>Dispersion Number</th>
<th>Reactions</th>
<th>Interaction Enthalpy (mJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2.3</td>
<td>PVP / (Indomethacin + PHPMA)</td>
<td>27.3 ± 15</td>
</tr>
<tr>
<td>5.2.4</td>
<td>PHPMA / (Indomethacin + PVP)</td>
<td>-361 ± 38</td>
</tr>
<tr>
<td>5.2.5</td>
<td>(PHPMA + PVP) / Indomethacin</td>
<td>20.8 ± 2.8</td>
</tr>
</tbody>
</table>

Table 5.9 Reaction Enthalpy of Indomethacin Ternary Systems
As seen from Table 5.9, only one system out of three showed an exothermic reaction. The interaction between PHPMA and the mixture of Indomethacin and PVP produced a lot of heat upon mixing, which favoured the stabilisation of the system. However, when the mixture of PHPMA and PVP was introduced to the indomethacin solution or when the PVP solution was introduced to the Indomethacin and PHPMA mixture, both interactions were endothermic.

It was suggested that hydrogen bonding was formed between indomethacin and PHPMA and indomethacin dimers in dispersion 5.2.3, (as the number of indomethacin molecules outnumbered the PHPMA molecules). When PVP was introduced to the mixture, it was hypothesised that the hydrogen bonds between indomethacin dimers were broken in order to bond with PVP. A lot of energy was required to break the hydrogen bonds between indomethacin dimers, however, the hydrogen bonds formed with PVP was hindered due to the steric hindrance introduced by the repeated units of PVP. The total interaction enthalpy was therefore endothermic and the interaction was entropy driven.

The same applied to system 5.2.5, in the PHPMA and PVP mixture, hydrogen bonds were formed between the two and PHPMA dimers. When the mixture was introduced to the indomethacin solution, it was speculated the entropy of the system drove the hydrogen bonds between the PHPMA dimers and some of the Indomethacin dimers to break and re-form between PHPMA and Indomethacin. As PHPMA was preoccupied by PVP molecules, only limited hydrogen bonds could form between PHPMA and indomethacin. Thus, the interaction enthalpy between the PHPMA and PVP mixture and indomethacin was small and the interaction was entropy driven.

In dispersion 5.2.4, Indomethacin and PVP was bonded through hydrogen bonds before they were mixed with PHPMA. When PHPMA was introduced to the system, hydrogen bonds were formed between PHPMA and indomethacin. The large amount of heat produced from such bonding indicated that the interaction between indomethacin and
PHPMA was very beneficial to the stability of the system.

### 5.8.3 Stability of Indomethacin Ternary Systems

The stability of the Indomethacin Ternary Systems was assessed by taking into account the interaction enthalpy from preparing the mixture carried out in section 5.8.1. The analysis was carried out in the same manner as described in section 5.7.3. The results are shown in Table 5.10.

<table>
<thead>
<tr>
<th>Dispersion Number</th>
<th>5.2.3</th>
<th>5.2.4</th>
<th>5.2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture</td>
<td>Indo + PHPMA</td>
<td>Indo + PVP</td>
<td>PHPMA + PVP</td>
</tr>
<tr>
<td>Interaction Enthalpy of the mixture (mJ)</td>
<td>-358± 38</td>
<td>-62.2 ± 9.4</td>
<td>176 ± 20</td>
</tr>
<tr>
<td>Third Component</td>
<td>PVP</td>
<td>PHPMA</td>
<td>Indo</td>
</tr>
<tr>
<td>Enthalpy upon addition of 3rd component</td>
<td>27.3 ± 15</td>
<td>-361 ± 38</td>
<td>20.8 ± 2.8</td>
</tr>
<tr>
<td>Total system enthalpy (mJ)</td>
<td>-331 ± 53</td>
<td>-423 ± 47</td>
<td>197 ± 23</td>
</tr>
</tbody>
</table>

**Table 5.10 Total enthalpy of Indomethacin Ternary Systems**

Despite of the endothermic reaction between PHPMA and the Indomethacin and PVP mixture, the total enthalpy output for system 5.2.3 was actually exothermic, a lot of heat was produced from this system. The result suggested that adding the secondary excipient to the drug before the carrier could also stabilise the system. However, when PHPMA and PVP were mixed before interacting with Indomethacin, a lot of energy was required to stabilise the system and such a system was the least stable among the three. The addition of PHPMA after the matrix of Indomethacin and PVP was formed was the most stable system as the most heat was produced.

In dispersion 5.2.5, as both PHPMA and PVP were preoccupied with each other via hydrogen bonding, extra energy was required to break up the bonding in order to
associate indomethacin with both of the polymers, therefore, dispersion 5.2.5 was the least stable system among the three. In dispersion 5.2.3, more hydrogen bondings were formed between indomethacin and the polymers when compared to dispersion 5.2.5, and they drove the system to a more stable state. In dispersion 5.2.4, hydrogen bonding was formed with the hydroxyl group in indomethacin and the carbonyl group in PVP while the carbonyl group in indomethacin was unassociated. It was speculated that when PHPMA was introduced to the system, hydrogen bonding could also be formed between the unassociated carbonyl group in indomethacin and the hydroxyl group in PHPMA, as a result, the three components were bound together through hydrogen bonding and hence, such system was the most stable among the three. To summarise, in addition to maximising the hydrogen bondings between indomethacin and both PHPMA and PVP, a bridge was built between the three components, rendering dispersion 5.2.4 to be the most stable system among the three. On the other hand, as the hydrogen bonding between indomethacin and PHPMA was stronger than that between indomethacin and PVP, as seen from Table 5.7, the bonding of PVP with indomethacin was not favoured, and dispersion 5.2.3 was less stable compared to dispersion 5.2.4.

The interaction enthalpy of indomethacin and PHPMA was \(-358 \pm 38\text{mJ}\), the total system enthalpy of dispersion 5.2.3 and 5.2.4, the ternary systems in which PVP was also included, was \(-331 \pm 53\) and \(-423 \pm 47\text{mJ}\) respectively. Therefore, it was reasonable to conclude that the stability of the indomethacin system was achieved mainly through the addition of PHPMA. Due to the large error range, no significant difference was shown between the three systems. However, it was suggested that the system was the most stable when PHPMA was last added to the indomethacin ternary system. As no significant variation was shown between the indomethacin/PHPMA binary system and the indomethacin ternary system 5.2.3, it was difficult to conclude which system had greater stability and this required further study.

5.9 Conclusion
PVP and PHPMA were chosen as carrier and secondary excipient to stabilise the
Griseofulvin and Indomethacin systems. Binary and Ternary systems were prepared in order to understand the mechanism of how these two components increase the stability of the systems. Studies were also carried out to investigate the effects of the addition order of the components.

It was reasonable to conclude that PHPMA significantly increased the stability of both drugs, in either binary or ternary systems. The stability of the systems was mainly achieved through hydrogen bonding. The order of addition also played a very important role. In both ternary systems, adding PHPMA after the matrix of the drug and PVP was formed was the most stabilised system, which correlated very well with previous work (Fletcher, 2006).

In the Griseofulvin system, the binary system with PHPMA was the second stabilised system, followed by the ternary system in which PVP was added the last. The same held true for the indomethacin systems.

However, it is worth noting that the conclusion was based on the systems when the drug, carrier and secondary excipient was prepared at the ratio of 2:1:1 (w/w/w). The result might change dramatically if the ratio was changed, as the hydrogen bonding through which the system was stabilised, was based on the number of acceptors and donors available.
Chapter Six

Conclusions and Future Work
A drug has to be in solution in order to cross the Gastrointestinal (GI) membranes and reach the general circulation for absorption. However, many drugs that have therapeutic effects are water-insoluble and their dissolution rate very often becomes the rate-limiting step. Many methods were proposed to increase the dissolution rate of these drugs, such as formulating the hydrophobic drugs in dispersed systems which showed desirable dissolution rate after appropriate modification. However, these systems are generally associated with one problem - the stability, which stems from the hydrophobic nature of the drug. It was suggested that by introducing polymer, the dissolution profile and stability of the drug can both be increased. Therefore, two dispersed systems were chosen for this study, which were suspension and solid dispersion.

In this study, calorimetry as a sensitive, reliable and efficient technique which provides rich thermodynamic information was chosen to study the interaction between hydrophobic drug and the polymer(s) during the interaction process. The overall aims of this thesis were:

- To enhance the understanding of the association behaviour of poloxamer in water and how it may be used to improve the stability of suspensions containing hydrophobic drugs by decreasing their sedimentation rate via adsorption
- To enhance the understanding of the interaction mechanisms between hydrophobic drugs and polymer(s) in solid dispersions and how polymer(s) maybe be used to improve the physical stability of poorly water soluble drugs
- To investigate the possibility of predicting the properties of solid dispersions by studying the system in solution during manufacture in order to build up a connection between the solid and liquid properties of a formulation

Investigations were carried out on the association behaviour of the poloxamers and PE/F 68 was chosen for the study. The association behaviour of PE/F 68 was first studied by diluting different concentrations of poloxamer solutions into water using Isothermal Titration Calorimetry (ITC). No phase transition was observed during the
dilution process and the dilution enthalpy from different concentrations to the same concentration was not proportional to the concentration the dilution started from. Conclusions based on such observation were drawn: a) poloxamer solutions were concentration dependent and b) poloxamer formed aggregates following the open association model which was contrary to the opinion held by most of the researchers in this field.

The concentration dependence of poloxamer solutions was also supported by the Micro Solution Ampoule study and the Differential Scanning Calorimetry (DSC) study. In the Micro Solution Ampoule study, different masses of poloxamer (solid) was dissolved in water and the dissolution enthalpy measured was not proportional to the mass. A poloxamer solution of higher concentration was subject to DSC measurement immediately after it was diluted, the Critical Micellisation Temperature (CMT) obtained was different from that of the poloxamer solution at the concentration after dilution; indicating the two solutions were at two different states even though their concentrations were the same. Also, the different CMT obtained from poloxamer solutions at different concentrations suggested the same.

The open association behaviour of poloxamer was evidenced by the continuous decrease in surface tension as opposed to the constant surface tension after Critical Micelle Concentration (CMC) seen from many conventional surfactants. Inflections at 0.006% and 0.07% (w/v) were seen from the surface tension-concentration curve. Such observation was also different from that of conventional surfactants and it was suggested that the first inflection indicated the formation of ‘unimolecular’ micelles while the second inflection reflected the formation of multi-molecular aggregates.

A study on the time required for a freshly prepared poloxamer solution to reach equilibrium was carried out by measuring the surface tension of 5% poloxamer solution as a function of time. Measurement was carried out 15 hours after the solution was made. Results suggested that 45 hours was required for the poloxamer solution to reach
equilibrium as the surface tension gradually decreased and finally became constant; approximately 4 hours was required for an equilibrated solution to re-reach equilibrium once its equilibrium state was disrupted.

Studies were also carried out on how to efficiently achieve the equilibrium state of poloxamer solutions. Experiments carried out in Micro Solution Ampoule suggested that the equilibrium of the solution was achieved within 1 hour under constant stirring as no heat signal was seen after that. Due to the limitation of the technique, the study on solution of higher concentration was carried out by DSC. Heating up freshly prepared poloxamer solution proved to be an efficient way of hastening the equilibration process for poloxamer solutions at higher concentrations as the CMT of such solutions coincided with that of the equilibrated solution at the same concentration.

Such findings are essential as they enable better application of poloxamers. As poloxamers are concentration dependent and they follow open association models to form aggregates, desired poloxamer properties could be obtained by choosing the right concentration of poloxamer solutions and preparing formulations in a proper way. The findings also provided guidance as to how to achieve the equilibration state for poloxamer solutions at different concentrations and the time required for a poloxamer solution to reach equilibrium. This is also very important when handling poloxamer solutions. The equilibrium state of poloxamer solution is very often neglected by many researchers and so as other polymers and large molecules. However, the results indicated that a long time may be required for poloxamer solutions to reach equilibrium and special care should be taken in order to achieve consistent performance and desirable properties of poloxamers. Hence, the results from this study provided alternative methods to efficiently hasten the equilibration process and the time required for poloxamer solutions to reach equilibrium was significantly reduced and the equilibrium state of the solution was also ensured.

Calorimetric studies were carried out in ITC to investigate how a second excipient
Poly(2-hydroxypropylmethacrylate) (PHPMA) could increase the stability of Griseofulvin / Indomethacin – Polyvinylpyrrolidone (PVP) solid dispersions by studying the interactions between these components in a solvent of acetone and water mixture (19:9). The solid dispersions were carried out at a Drug: PVP: PHPMA = 2:1:1 ratio. Studies were carried out on binary and ternary systems and also the effect of the order of addition of the secondary excipient. Results suggested that the addition of PHPMA can significantly increase the stability of both Griseofulvin and Indomethacin solid dispersions in either binary or ternary systems. The increase in stability was ascribed to the hydrogen bonding. The most stable solid dispersions were achieved by adding PHPMA to the mixture of PVP and drug, suggesting the order of addition of the secondary excipient was essential in achieving solid dispersions of desirable stability. The results of the Griseofulvin system were supported by Fletcher (2006). In his study, the stability test was carried out on Griseofulvin solid dispersions (containing PVP and PHPMA) which were prepared by spray drying.

The calorimetric results from above brought insights into the mechanism of interactions between drug and carrier(s), the stabilising effect of the second excipient and its order of addition. Such studies were carried out in liquid state before the manufacture process was carried out, which turns the dispersion into solid state such as by spray drying. This is different from common practice where a lot of uncertainties were involved by the manufacture process. The correlation between the two studies suggested a connection of the properties of solid dispersions between its solid and liquid state and hence a lot of time, labour and expense could be saved as it could avoid of some unnecessary manufacture processes which are carried out on a larger scale.

The use of calorimetry in both these studies strongly demonstrated its strength in studying polymer properties, interactions between drug and polymers and stability of dispersed systems by providing rich thermodynamic information. The small amount of sample required for carrying out calorimetric studies, the efficiency in data acquisition, the general applicability together with the rich thermodynamic information it provides
lead to the conclusion that the merit of calorimetry is yet to be recognised and calorimetry, as a sensitive and reliable technique, should be widely employed in many other areas.

The work has identified a number of directions further work could follow.

Due to the complexity of the suspensions system, the interaction mechanism of drug and poloxamer was not clearly understood. Therefore, Photon Correlation Spectroscopy (PCS) is required to investigate the adsorption behaviour of poloxamer onto drug particles. Also by rationalising the ratio between the drug and the poloxamers, more thermodynamic information could be obtained, such as the interaction rate constant, $\Delta H$, $\Delta G$, and $\Delta S$. The interaction between drug and four different poloxamers suggested that different mechanisms may have been involved due to their different MW and PPO/PEO ratio. Further studies can be carried out to investigate if the interaction mechanism was dependent on those two parameters.

Further studies are required to test the stability of Indomethacin solid dispersions containing PVP and PHPMA prepared in acetone and water mixture to provide more evidence for the connection of the solid dispersion properties between its solid state and its liquid state. Further studies may also be carried out by varying the ratio between the three components, the ratio between acetone and water in order to optimise the system to achieve the greatest stability. Different polymorphic forms of the same drug may be obtained if different solvents are used. Therefore, different solvents in which the solid dispersion is prepared can be studied in the future and comparisons can be made between them. Also, more studies can be carried out to explore more combinations of polymers.
References


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