Chapter 5

Summary and conclusion

Once a new drug is developed, little can be said about the thermodynamic properties of the drug including the solubility, thermodynamic behaviour and stability (Marini et al., 2003). In the solid state an API may exist in different physical forms; each with its own physico-chemical characteristics that may influence drug performance. The aim of the screening of the solid form is to identify and characterise all of the physical forms in which a given drug may present itself. The challenge brought to the pharmaceutical scientist is to identify the ultimate drug form for further developmental purposes. In most instances the stable form of the drug is the obvious choice; having a lesser chance of solid-state transformations in the later stages of drug development (Saunders & Gabbot, 2011; Lu & Rohani, 2009; Yu et al., 2003). In some instances, though, the unfavourable characteristics including the poor solubility and/bioavailability of the stable form may necessitate the incorporation of a less stable drug form (e.g. metastable or amorphous forms). In such cases the thermodynamic and stability properties of the drug will form the basis of the selection process and will determine the framework critical for operation during processing and storage (Bernstein, 2002).

The aim of this study was to prepare different solid-state forms (polymorphic or amorphous) of macrolide antibiotics from organic solvents commonly used in manufacturing processes. The next step was to characterise the prepared forms using a range of thermal, spectroscopic and crystallographic techniques as required by regulatory authorities. Additionally stability and solubility studies were performed in the hope of finding a solid form with improved stability and solubility characteristics.

**Spiramycin:**

Spiramycin is a poorly water-soluble 14-membered ring compound of the macrolide family. The recrystallisation of spiramycin by slow evaporation did not yield any crystalline products. The aim of generating new polymorphic forms of spiramycin was therefore discarded. In each case an amorphous, resin-like substance was formed. In the course of time, as more and more of the solvent started to evaporate, a morphological change to a solid amber-like material was observed. Finally once all of the solvent had evaporated, these materials tended to revert back to the powdered form. The SEM photomicrographs and X-ray results
later confirmed the amorphous character of the raw material and the recrystallisation products.

The DSC proved to be of little value in the characterisation of spiramycin since wide intersample variations were mostly obtained. All of the tested samples were binary mixtures comprising of the raw material and the liquid solvent trapped inside the amorphous product. The entrapped solvent hampered the determination of the glass transition temperature ($T_g$) since numerous endothermic events associated with gradual evaporation of the solvent made it impossible to differentiate between these events on the DSC. Thermal events associated with the physical aging of the substance could also have played a role. TGA results showed higher solvent uptake than expected. This was ascribed to the amorphous, sponge-like character of this drug.

For the sake of reproducibility and quality of the results, characterisation of spiramycin was more reliant on the spectroscopic and crystallographic methods. Samples generated from 2-butanol, chloroform, ethyl acetate, 1.4-dioxane, methanol, n-propanol, iso-propanol and tetrahydrofuran showed characteristic peaks in the range of 2000-2400 cm$^{-1}$ that were not present in the IR spectrum of the raw material. Conversely, the XRPD patterns were all identical, exhibiting a characteristic “halo” pattern with no detectable Bragg diffraction peaks.

A solubility assessment of selected amorphous samples revealed less favourable water solubility characteristics in comparison with the raw material. Since all the attained forms seemed to be similar to the raw material, this was ascribed to poor wettability of the samples or particle-particle interactions. In addition structural relaxation associated with sample annealing could have played a role, since the lowering of the overall potential-energy levels associated with this phenomenon generally leads to decreased solubilities. Also the recrystallisation products could have been more crystalline than the amorphous raw material. The XRPD diffractograms show that all the recrystallisation products were amorphous, but the degree of amorphism was not quantified. Amorphous materials tend to be more soluble than their crystalline counterparts.

The characterisation study conducted on spiramycin highlighted the need for effective, reproducible characterisation methods to assess amorphous substances. The lack of understanding of this form has greatly limited its production by pharmaceutical companies, even though it offers a potential means of improving the bioavailability of drug products (Craig et al., 1999). Also strategies for stabilising the amorphous forms are needed, if they are to be approved by regulatory authorities.
**Clarithromycin:**

According to the literature, clarithromycin exists in five forms. Form 0 exists as a solvate, form I is a metastable form, form II is the stable form, form III is a solvate of acetonitrile and form IV is a hydrate. The stable form, form II, is used in formulations currently on the market. A follow-up investigation was done relating to a study conducted by De Jager (2005).

Recrystallisation of the raw material (clarithromycin form II) from acetonitrile delivered single crystals with a prismatic-like crystal habit. In contrast to what was reported in literature, thermal analysis showed no evidence of solvate formation. Thermal analysis indicated an exothermic crystallisation event at 133°C, not reported in any of the previous characterisation studies. The XRPD pattern, although having similarities to form III, presented with unique peaks at 4.4, 6.4, 10.1 and 10.2°2θ. Evidence from the IR suggests that this form may convert to the thermodynamically stable form when the necessary driving force for polymorphic transition is supplied.

Recrystallisations from chloroform produced a solvate with an equant-like crystal habit. XRPD did not match any of the solvates (form 0 and form III) that were previously reported. DSC thermogram showed desolvation of chloroform at 118°C also seen on the TM in the form of gas evolution in the silicone oil and subsequent loss of transparency of the crystals. The TGA weight loss suggested a 2:1 chloroform:clarithromycin solvate. A twelve-week stability study (40 °C and 75% RH) was performed to determine the stability of the chloroform solvate. After the first weekly analysis, the results from thermal analysis showed that the crystals had desolvated completely. XRPD patterns indicated a conversion to form II. XRPD performed on subsequent weeks showed the crystal persisting in the stable form for the remainder of the 12 weeks.

Crystals of form II were directly recrystallised from ethyl acetate, as reported in the literature. This proves that the stable form can be directly isolated from this solvent without requiring the additional conversion step as was the case in the study conducted by De Jager (2005). Clarithromycin is poorly soluble in water. A solubility study was performed to determine the relative solubility of the prepared crystal forms. Solvated crystals from chloroform had the most favourable water-solubility characteristics, showing a 4-fold increase in solubility followed by the corresponding desolvate with a 1.5-fold increase in solubility when compared to the raw material. The fact that the desolvated crystals resembled the stable form explains the reduced solubility when compared to the solvated form.
The differences in physical properties including solubility, stability, melting point etc, of the different crystal forms of clarithromycin accentuated the importance of identifying and characterising all possible forms of the API. The success or failure of the pharmaceutical product will ultimately depend on the choice of crystal form. Since all the prepared forms in this study and in previous studies seem to convert eventually to the stable form II, it is clear why this form is preferred by pharmaceutical companies.

To conclude, the physico-chemical characterisation of a substance forms an integral part of any drug development process. Characterisation studies provide a means for better design of dosage forms and improved control over drug performance (Byrn et al., 1994). The selected form should not only adhere to stability and solubility standards, but should also be a form that can be readily reproduced. Failure to identify irregularities during the research and developmental stages could prove costly for a pharmaceutical company, as was seen in the case of ritonavir.
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“Trust in the Lord with all your heart and lean not on your own understanding; in all your ways submit to him, and he will make your paths straight.” Proverbs 3: 5-6.