Roxithromycin

A solubility and stability study

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Table of Contents

Table of contents i
Voorwoord viii
List of tables ix
List of figures xi
Aims and objectives xvii
Abstract xviii
Uittreksel xx

Chapter 1: Polymorphism and amorphism

1.1 Introduction 1
1.2 Amorphous solids 2
1.2.1 The importance of amorphous forms in the pharmaceutical industry 2
1.2.2 Preparation of amorphous solids 5
1.2.3 Physical and chemical properties of amorphous solids 7
1.2.4 Solubility of amorphous forms 9
1.2.5 Stability of amorphous forms 10
1.2.6 Influence of water content on the glass transition temperature 13
1.3 Polymorphism 13
1.3.1 Polymorphic forms 13
1.3.2 Solvates 15
1.3.3 Hydrates 17
1.4 Conclusion 18
References 19

Chapter 2: Roxithromycin

2.1 Introduction 22

2.2 Physico-chemical information 23

2.3 Activity 24

2.3.1 *In vitro* activity 25

2.3.2 Partial *in vitro* activity 25

2.3.3 Resistance 26

2.4 Pharmacodynamics 26

2.5 Indications 27

2.6 Dosage 28

2.7 Contraindications 28

2.8 Brand names 29

2.9 Solubility 29

2.10 Stability 30

2.11 Conclusion 30

References 31

Chapter 3: Characterisation methods

3.1 Introduction 35

3.2 Glass preparation method 35

3.3 Glass powder preparation method 36

3.4 Recrystallisation method 36

3.5 Thermal analysis 37

3.5.1 Differential scanning calorimetry (DSC) 37

3.5.2 Thermogravimetric analysis (TGA) 38

3.6 Thermal microscopy (TM) 39
3.7 X-ray powder diffraction (XRPD) 40
3.8 Infrared spectroscopy (IR) 41
3.9 Karl Fischer titration (KF) 42
3.10 Solubility studies 42
3.10.1 Solubility study A 42
3.10.2 Solubility study B 43
3.11 Powder dissolution 44
3.12 Stability studies 45
3.12.1 Stability study at 40°C / 45% RH 45
3.12.2 Stability study at 25°C / 75 % RH and 30°C / 75 % RH 46
3.12.3 Stability study of desolvated chloroform and ethyl acetate forms at 40°C / 75% RH 46
3.13 The effect of moisture on amorphous roxithromycin 46
3.14 Conclusion 47
References 48

Chapter 4: Characterisation of polymorphic and amorphous forms of roxithromycin

4.1 Introduction 49
4.2 Characterisation of roxithromycin raw material 50
4.2.1 Differential scanning calorimetry (DSC) 50
4.2.2 Thermogravimetric analysis (TGA) 51
4.2.3 Thermal microscopy (TM) 51
4.2.4 X-ray powder diffraction (XRPD) 52

4.2.5 Infrared spectroscopy 54
4.3 Roxithromycin glass 54
4.3.1 Differential scanning calorimetry (DSC) 54
4.3.2 Thermogravimetric analysis (TGA) 55
4.3.3 Thermal microscopy (TM) 56
4.3.4 X-ray powder diffraction (XRPD) 57
4.3.5 Infrared analysis (IR) 57
4.4 Ground roxithromycin – glass powder 58
4.4.1 Differential scanning calorimetry (DSC) 58
4.4.2 Thermogravimetric analysis (TGA) 60
4.4.3 X-ray powder diffraction (XRPD) 60
4.4.4 Infrared spectroscopy (IR) 61
4.5 Roxithromycin chloroform solvate 62
4.5.1 Differential scanning calorimetry (DSC) 62
4.5.2 Thermogravimetric analysis (TGA) 62
4.5.3 Thermal microscopy (TM) 63
4.5.4 X-ray powder diffraction (XRPD) 64
4.5.5 Infrared spectroscopy 66
4.6 Roxithromycin chloroform desolvate 66
4.6.1 Differential scanning calorimetry (DSC) 66
4.6.2 Thermogravimetric analysis (TGA) 67
4.6.3 X-ray powder diffraction (XRPD) 68
4.6.4 Infrared spectroscopy (IR) 69
4.7 Roxithromycin ethyl acetate solvate 69
4.7.1 Differential scanning calorimetry (DSC) 70

4.7.2 Thermogravimetric analysis (TGA) 70
4.7.3 Thermal microscopy (TM) 71
4.7.4 X-ray powder diffraction (XRPD) 72
4.7.5 Infrared spectroscopy (IR) 74
4.8 Roxithromycin ethyl acetate desolvate 74
4.8.1 Differential scanning calorimetry (DSC) 75
4.8.2 Thermogravimetric analysis (TGA) 75
4.8.3 X-ray powder diffraction (XRPD) 76
4.8.4 Infrared spectroscopy (IR) 78
4.9 Conclusion 80
References 82

Chapter 5: Stability studies of roxithromycin amorphous forms

5.1 Introduction 83
5.2 Stability study at 40°C/ 75% RH 83
5.2.1 Thermal analysis (DSC and TGA) 84
5.2.2 Karl Fischer analyses (KF) 89
5.2.3 Infrared analyses (IR) 90
5.2.4 X-ray powder diffraction (XRPD) 92
5.2.5 Discussion 96
5.3 Stability studies at 25°C / 75% RH and 30°C / 75% RH 96
5.3.1 Thermal analysis (DSC and TGA) 97
5.3.2 Infrared spectroscopy (IR) 99
5.3.3 X-ray powder diffraction (XRPD) 99
5.3.4 Conclusion 100

5.4. Stability studies of the chloroform- and ethyl acetate desolvates at 40°C / 75% RH 100
5.4.1 Roxithromycin chloroform desolvate stability study 101
5.4.1.1 Thermal analysis (DSC and TGA) 101
5.4.1.2 Infrared analyses (IR) 102
5.4.1.3 X-ray powder diffraction (XRPD) 102
5.4.2 Roxithromycin ethyl acetate desolvate stability study 103
5.4.2.1 Thermal analysis (DSC and TGA) 103
5.4.2.2 Infrared analyses (IR) 104
5.4.2.3 X-ray powder diffraction (XRPD) 105
5.5 The effect of moisture on amorphous roxithromycin 106
5.5 Conclusion 107
References 108

Chapter 6: Solubility study of roxithromycin amorphous forms

6.1 Introduction 109
6.2 Solubility study A 109
6.2.1 Roxithromycin monohydrate 112
6.2.2 Glass 113
6.2.3 Glass powder 113
6.2.4 Chloroform desolvate 116
6.3 Solubility study B 118
6.3.1 Solubility results 118
6.3.2 Infrared analysis (IR) of dissolved roxithromycin glass 122
6.4 Powder dissolution 125

6.5 Conclusion 126

References 127

Chapter 7: Conclusion 128

Annexure 131

Concept publication 134

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List of Tables

Chapter 2: Roxithromycin

Table 2.1: Physico-chemical properties of roxithromycin 23

Table 2.2: Indications of roxithromycin 27

Chapter 3: Characterisation methods

Table 3.1: Experimental setup and conditions for DSC analysis 38

Table 3.2: Experimental setup and conditions for TGA analysis 39

Table 3.3: Experimental setup and conditions for XRPD analysis (Bruker D8 apparatus) 40

Table 3.4: Experimental setup and conditions for XRPD analysis (PANalytical X'Pert PRO) 41

Table 3.5: Extraction times 44

Table 3.6: Dissolution parameters 45

Table 3.7: Amount of water added to each roxithromycin glass sample 47

Chapter 4: Characterisation of polymorphic and amorphous forms of roxithromycin

Table 4.1: TM micrographs of roxithromycin monohydrate 52

Table 4.2: XRPD data for roxithromycin monohydrate 53

Table 4.3: TM micrographs of roxithromycin glass 56

Table 4.4: TM micrographs of roxithromycin chloroform solvate 64

Table 4.5: XRPD data for roxithromycin chloroform solvate 65

Table 4.6: TM micrographs of roxithromycin ethyl acetate solvate 72

Table 4.7: XRPD data for roxithromycin ethyl acetate solvate 73

Table 4.8: XRPD data for roxithromycin ethyl acetate desolvate 78

Chapter 5: Stability studies of roxithromycin amorphous forms

Table 5.1: TGA results of roxithromycin monohydrate, roxithromycin glass and glass powder 88

Table 5.2: KF results of roxithromycin monohydrate, glass and glass powder 89

Table 5.3: TGA results of stability studies performed at 25°C, 30°C and 40°C and at 75% RH 98

Table 5.4: TGA results of roxithromycin chloroform desolvate 102

Table 5.5: TGA results of roxithromycin ethyl acetate desolvate 104

Chapter 6: Solubility study of roxithromycin monohydrate and amorphous forms

Table 6.1: TGA results before and after the solubility study of the different roxithromycin forms 118

Table 6.2: Solubility study results of roxithromycin monohydrate in water 119

Table 6.3: Solubility study results of roxithromycin glass in water 120

List of Figures

Chapter 1: Polymorphism and amorphism

Figure 1.1: Molecular packing differences between a crystalline state (A) and an amorphous (glassy) state (B) 3

Figure 1.2: XRPD patterns of roxithromycin crystalline form (red) and roxithromycin amorphous form (blue) 3

Chapter 4: Characterisation of polymorphic and amorphous forms of roxithromycin

Figure 4.1: DSC thermogram of roxithromycin monohydrate 50

Figure 4.2: TGA thermogram of roxithromycin monohydrate 51

Figure 4.3: X-ray powder diffractogram of roxithromycin monohydrate 53

Figure 4.4: IR spectrum of roxithromycin monohydrate 54

Figure 4.5: DSC thermogram of roxithromycin glass, prepared through quench cooling from a melt 55

Figure 4.6: TGA thermogram of roxithromycin glass, prepared through quench cooling from a melt 55

Figure 4.7: X-ray powder diffractogram of roxithromycin glass 57

Figure 4.8: DSC thermogram of roxithromycin glass powder 58

Figure 4.9: IR spectra of a) roxithromycin raw material and b) roxithromycin glass 59

Figure 4.10: TGA thermogram of roxithromycin glass powder 60

Figure 4.11: X-ray powder diffractogram of roxithromycin glass powder 61

Figure 4.12: IR spectrum of roxithromycin glass powder 61

Figure 4.13: DSC thermogram of the roxithromycin chloroform solvate 62

Figure 4.14: TGA thermogram of roxithromycin chloroform solvate 63

Figure 4.15: X-ray powder diffractogram of the roxithromycin chloroform solvate 65

Figure 4.16: IR spectrum of roxithromycin chloroform solvate 66

Figure 4.17: Overlay of DSC thermograms of the roxithromycin chloroform solvate (blue) and desolvate (red) 67

Figure 4.18: TGA thermogram of roxithromycin chloroform desolvate 68

Figure 4.19: X-ray powder diffractogram of roxithromycin chloroform desolvate 68

Figure 4.20: IR spectrum of amorphous roxithromycin chloroform desolvate 69

Figure 4.21: DSC thermogram of roxithromycin ethyl acetate solvate 70

Figure 4.22: TGA thermogram for roxithromycin ethyl acetate solvate 71

Figure 4.23: X-ray powder diffractogram of roxithromycin ethyl acetate solvate 73

Figure 4.24: IR spectrum of roxithromycin ethyl acetate solvate 74

Figure 4.25: Overlay of DSC thermograms of the roxithromycin ethyl acetate solvate (blue) and roxithromycin desolvate (red) 75

Figure 4.26: TGA thermogram of roxithromycin ethyl acetate desolvate 76

Figure 4.27: X-ray powder diffractogram of roxithromycin ethyl acetate desolvate 77

Figure 4.28: Overlay of X-ray powder diffractograms of roxithromycin ethyl acetate solvate and desolvate 77

Figure 4.29: IR spectrum of roxithromycin ethyl acetate desolvate 79

Figure 4.30: Overlay of the IR spectra of roxithromycin ethyl acetate solvate and desolvate 79

Figure 4.31: Overlay of IR spectra of the roxithromycin monohydrate, glass, glass powder, chloroform desolvate and ethyl acetate desolvate 80

Chapter 5: Stability studies of roxithromycin amorphous forms

Figure 5.1: DSC thermograms of roxithromycin monohydrate 85

Figure 5.2: DSC thermograms of roxithromycin glass 86

Figure 5.3: DSC thermograms of roxithromycin glass powder 87

Figure 5.4: DSC thermogram of roxithromycin glass powder at week 5 87

Figure 5.5: TGA % weight loss of roxithromycin monohydrate, roxithromycin glass and glass powder, over a period of 4 – 5 weeks 88

Figure 5.6: KF results of roxithromycin monohydrate, roxithromycin glass and glass powder over a period of 4 weeks 89

Figure 5.7: IR spectra of roxithromycin monohydrate 90

Figure 5.8: IR spectra of roxithromycin glass 91

Figure 5.9a: IR spectra of roxithromycin glass powder over a period of 4 weeks 91

Figure 5.9b: IR spectra (3472 cm^{-1}) of roxithromycin glass powder over a period of 4 weeks (enlarged) 92

Figure 5.10: Overlay of the X-ray powder diffractograms of roxithromycin monohydrate over a period of 4 weeks 92

Figure 5.11: X-ray powder diffractograms (staggered) of roxithromycin monohydrate over a period of 4 weeks 93

Figure 5.12: Overlay of the X-ray powder diffractograms of roxithromycin glass over a period of 4 weeks 94

Figure 5.13: X-ray powder diffractograms (staggered) of roxithromycin glass over a period of 4 weeks 94

Figure 5.14: Overlay of the X-ray powder diffractograms of roxithromycin glass powder over a period of 5 weeks 95

Figure 5.15: X-ray powder diffractograms (staggered) of roxithromycin glass powder over a period of 5 weeks.....95

Figure 5.16: Overlay of the DSC thermograms of roxithromycin glass powder at initial and at week 4 (25°C and 30°C, at 75% RH).....97

Figure 5.17: TGA % weight loss of roxithromycin glass powder at 25°C, 30°C and 40°C and at 75% RH 98

Figure 5.18: IR spectra of roxithromycin glass powder at week 4 at 25°C and 30°C and at 75% RH 99

Figure 5.19: Overlay of the X-ray powder diffractograms of the roxithromycin glass powder at week 4 at 25°C (black) and 30°C (blue) at 75% RH, compared with the initial diffractogram (red).....100

Figure 5.20: An overlay of the DSC thermograms of the roxithromycin chloroform desolvate 101

Figure 5.21: IR spectra of roxithromycin chloroform desolvate over a period of 4 weeks 102

Figure 5.22: XRPD overlay of roxithromycin chloroform desolvate at initial (red) and at week 4 (blue) 103

Figure 5.23: An overlay of the DSC thermograms of roxithromycin ethyl acetate desolvate over a period of 4 weeks.....104

Figure 5.24: An overlay of the IR spectra of the roxithromycin ethyl acetate desolvate over a period of 4 weeks 105

Figure 5.25: Overlay of the X-ray powder diffractograms of roxithromycin ethyl acetate desolvate at initial (red) and at week 4 (blue) 105

Figure 5.26: Overlay of the DSC thermograms of roxithromycin glass exposed to different weight fractions of water.....106

Chapter 6: Solubility study of roxithromycin amorphous forms

Figure 6.1: Comparison of the solubility profiles of roxithromycin monohydrate with the amorphous roxithromycin forms (glass, glass powder and chloroform desolvate) 110

Figure 6.2: SEM images of (a) roxithromycin monohydrate, (b) roxithromycin chloroform desolvate and (c) roxithromycin glass 111

Figure 6.3: Overlay of the DSC thermograms of the roxithromycin monohydrate before and after the solubility test 112

Figure 6.4: TGA thermogram of roxithromycin monohydrate after the solubility test 113

Figure 6.5: Overlay of the DSC traces of the roxithromycin glass before and after the solubility test 114

Figure 6.6: TGA thermogram of the roxithromycin glass after the solubility test 115

Figure 6.7: Overlay of the DSC thermograms of the roxithromycin glass powder before and after the solubility test 115

Figure 6.8: TGA thermogram of roxithromycin glass powder after the solubility test 116

Figure 6.9: Overlay of the DSC traces of the amorphous roxithromycin chloroform desolvate after the solubility test 117

Figure 6.10: TGA thermogram of the amorphous roxithromycin chloroform desolvate after the solubility test 117

Figure 6.11: Solubility study results of roxithromycin monohydrate and roxithromycin glass in water 121

Figure 6.12: Overlay of the IR spectra of undissolved roxithromycin glass at 55 minutes (green), 105 minutes (black) and 180 minutes (red) 122

Figure 6.13: Overlay of the enlarged IR spectra of roxithromycin glass over the 4100 – 3100 cm^{-1} range, illustrating the differences in the

spectra at 55 minutes (green), 105 minutes (black) and 180 minutes (red) 123

Figure 6.14: X-ray powder diffractogram of the roxithromycin glass after 55 minutes of the solubility study in water 123

Figure 6.15: XRPD trace of roxithromycin glass after 180 minutes of the solubility study in water 124

Figure 6.16: Powder dissolution results of the roxithromycin monohydrate and the roxithromycin glass powder 125

Aims and Objectives

Polymorphism and amorphism are well known phrases among pharmaceutical scientists and are important in any pre-formulation or formulation study. Solid-state properties of active pharmaceutical ingredients (APIs) is one of the most important and often most neglected fields of a pre-formulation study. Different polymorphic forms of an API display different physico-chemical properties, whilst amorphous forms also differ significantly from their crystalline counterparts. It is imperative for dosage form design that the physico-chemical properties of an active pharmaceutical ingredient (API) are known to the pharmaceutical scientist.

Several studies over the past decade have reported on polymorphism, solvatism and amorphism of roxithromycin. Current literature also reports significant solubility differences between the roxithromycin crystalline and amorphous forms. However, the available stability results on roxithromycin are still limited.

Furthermore, although several studies have been conducted on the polymorphic forms for roxithromycin, none emphasised, nor focused on the amorphous forms of roxithromycin.

For this study, the aim was therefore to prepare an amorphous form of roxithromycin, which would be stable under normal storage conditions and which would demonstrate enhanced solubility and dissolution properties.

The objectives of this study were to:

- Prepare different amorphous forms of roxithromycin;
- To characterise the different forms prepared;
- To evaluate the amorphous forms with regards to stability and solubility; and
- To determine which, if any, amorphous form would be suitable for use in a pharmaceutical dosage form.

Abstract

Roxithromycin
A solubility and stability study

Roxithromycin is a semi-synthetic, macrolide antibiotic, derived from erythromycin A. It acts as a bacteriostatic drug at low concentrations and a bactericidal drug at high concentrations. It binds to the 50S subunit of the 70S ribosome, which causes the reversible inhibition of RNA-dependent bacterial protein synthesis.

It is well known that active pharmaceutical ingredients (APIs) may exist in numerous solid states. Differences in the solid state significantly influence the physical and chemical properties of an API. The *in vivo* performance of a dosage form will also be influenced by the solid state properties of a given pharmaceutical active. The amorphous characteristics of APIs have a significant impact on their performance and thus offer the potential for exciting new pharmaceuticals. Whilst amorphous forms of poorly soluble APIs are more soluble than their crystalline counterparts, they tend to be physically unstable, which makes their formulation into solid dosage forms quite challenging.

Roxithromycin has only 50% oral bioavailability due to its poor aqueous solubility and for this reason, its potential for optimal therapeutic effect are limited. Poor solubility is thus an important obstacle in formulation development.

During this study, amorphous forms of roxithromycin were prepared *via* quench cooling, and desolvation of chloroform- and ethyl acetate solvates. These amorphous forms were characterised by means of several techniques, whilst their solubilities and stabilities were also investigated.

The outcomes of the solubility studies illustrated the complexity of this API and its amorphous forms with regards to their interactions with water. Solubility

studies confirmed the superior solubility of the roxithromycin glass (prepared through quench cooling) and amorphous forms (desolvation of solvates) over the roxithromycin monohydrate in water. The solubility in water improved in the order of roxithromycin monohydrate < roxithromycin glass < roxithromycin glass powder < amorphous chloroform desolvate.

The roxithromycin monohydrate, as well as the amorphous forms of roxithromycin demonstrated stability over a one-month period of exposure 40°C and relative humidity (RH) of 75%. The roxithromycin glass powder tended to revert to the more stable crystalline monohydrate after week 3 of stability testing. The roxithromycin glass at lower temperatures of 25°C and 30°C (both at 75% RH) tended to transform into the more crystalline form at week 4 of the study. These transformations were, however, not as significant as during the 40°C / 75% RH study. The conclusion could therefore be made that this transformation into the crystalline form was more temperature – than moisture dependant. At a higher temperature (at identical humidity conditions), the transformation into the crystalline form was much faster.

Stability studies on the two roxithromycin desolvates were also performed in order to determine whether these amorphous forms, would differ, with regards to their stability, from the glass prepared through heating and cooling. It was determined that the desolvates were more stable than the roxithromycin glass.

Keywords: Roxithromycin, amorphous, glassy, stability, elevated temperatures, relative humidity, solubility.

Uittreksel

Roksitromisien
‘n Oplosbaarheid - en Stabiliteitstudie

Roksitromisien is ‘n semi-sintetiese, makroliede antibiotikum, wat vanaf eritromisien A gederivatiseer word. Hierdie geneesmiddel tree as ‘n bakteriostatiese geneesmiddel by lae konsentrasies op, en as ‘n bakterisidiese geneesmiddel by hoë konsentrasies. Roksitromisien bind aan die 50S subeenheid van die 70S ribosoom, wat dan tot ‘n omkeerbare inhibering van RNA-afhanklike bakterisidiese proteïensintese lei.

Dit is alombekend dat aktiewe farmaseutiese bestanddele (geneesmiddels) in verskeie soliede vorme kan voorkom. Die fisies-chemiese eienskappe van ‘n geneesmiddel word aansienlik deur verskille in die vastetoestand beïnvloed. Die *in vivo* werking van ‘n doseervorm word voorts ook deur die vastetoestand eienskappe van die geneesmiddel affekteer. Die amorfe karakter van geneesmiddels het ‘n groot impak op hul werking en bied dit die geleentheid vir opwindende nuwe geneesmiddels. Alhoewel amorfe vorme die wateroplosbaarheid van swak oplosbare geneesmiddels kan verbeter, is hulle tot fisiese onstabiliteit geneig en kan formulering van hierdie vorme in soliede doseervorme ‘n groot uitdaging wees.

Die biobeskikbaarheid van roksitromisien is slegs 50%, as gevolg van die swak wateroplosbaarheid van hierdie geneesmiddel. Hierdie eienskap beperk die moontlikheid van optimale geneesmiddelaflewering en gevolglike optimale terapeutiese effek. Swak oplosbaarheid is dus ‘n belangrike struikelblok tydens suksesvolle doseervormontwikkeling.

Tydens hierdie studie is amorfe vorme van roksitromisien deur die proses van vinnige afkoeling na smelting berei, sowel as deur desolvering van chloroform- en etielasetaatsolvate. Hierdie amorfe vorme is met behulp van verskeie

tegnieke gekarakteriseer, terwyl hulle oplosbaarhede en stabiliteite ook ondersoek is.

Die uitkomstes van die oplosbaarheidstudies het die kompleksiteit van beide roksitromisien en sy amorfe vorme in terme van hul interaksies met water beklemtoon. Hierdie studies het ook die beter oplosbaarheid van die glasagtige roksitromisien (berei deur vinnige afkoeling na smelting), sowel as van die amorfe vorm teenoor díe van die monohidraat (grondstof) bevestig. Wateroplosbaarheid het in die volgorde roksitromisien monohidraat < glasagtige roksitromisien < verpoeierde glasagtige roksitromisien < amorfe chloroform desolvaat, verbeter.

Tydens ‘n maandlange stabiliteitstudie by bergingskondisies van 40°C en 75% relatiewe humiditeit (RH), het beide die roksitromisien grondstof, sowel as al die bereide amorfe vorme van roksitromisien stabiliteit getoon. Die glasagtige roksitromisienpoeier was geneig om na die meer stabiele kristallyne vorm (monohidraat) van roksitromisien, na ‘n tydperk van 3 weke by storing teen 40°C / 75% RH, te verander. Glasagtige roksitromisien het tydens bergingskondisies by 25°C en 30°C (beide by 75% RH) eers na die vierde week van die studie na die meer stabiele monohidraat van roksitromisien begin rekristalliseer. Om hierdie rede kon die afleiding dus gemaak word dat die verandering na die kristallyne vorm van roksitromisien eerder temperatuur – as vogafhanklik was. By ‘n hoër temperatuur (by identiese humiditeitskondisies) het die verandering na die kristallyne vorm vinniger plaasgevind.

‘n Stabiliteitstudie van die twee gedesolveerde amorfe vorme van roksitromisien is ook uitgevoer, ten einde die stabiliteit van hierdie amorfe vorme (berei vanuit organiese oplosmiddels) met díe van glasagtige roksitromisien (berei deur vinnige afkoeling na smelting) te vergelyk. Dit het voorgekom asof hierdie twee vorme meer stabiel as die glasagtige vorm van roksitromisien was.

Sleutelwoorde: Roksitromisien, amorfe, glasagtig, stabiliteit, hoër temperature, relatiewe humiditeit, oplosbaarheid.

Chapter 1

Polymorphism and amorphism

1.1 Introduction

It is well known that active pharmaceutical ingredients (APIs) may exist in numerous solid states. Differences in the solid state (molecular arrangement) significantly influence the physical and chemical properties of an API. The *in vivo* performance of a dosage form will also be influenced by the solid state properties of a given pharmaceutical active (Han & Suryanarayanan, 1999). The manufacture of an API(s) in a solid form that would exhibit optimal performance of the compound is considered the greatest challenge within the pharmaceutical industry.

A number of authors over the past decades have formulated definitions for polymorphism, but the definition by Brittain and Grant (1999) is probably the best suited for this study, i.e. 'Polymorphism is the ability of a pure substance to exist as two or more crystalline phases, being the result of different molecular conformation. The differences between such two substances can result in different physical properties, which include molecular packing, and thermo dynamic -, spectroscopic - and mechanical properties' (Brittain & Grant, 1999).

Alternatively, polyamorphism is the ability of an API to exist in several different amorphous forms (Hung & Nhan, 2010). An amorphous solid has no long range order of molecular packing, and is not regarded as a crystalline solid (Yu, 2001).

The different solid state forms of drugs, i.e. polymorphs, solvates and hydrates are of great interest to pharmaceutical scientists, and lately much attention has been given to amorphous forms. The identification of different solid state forms is crucial to the pharmaceutical industry, since it is imperative to anticipate

changes in the physical state of an API during storage and handling (Shah *et al.,* 2006).

The amorphous characteristics of APIs largely impact on their performances and thus offer the opportunity for exciting new pharmaceuticals (Hancock *et al.,* 2002).

The following paragraphs will focus on amorphous forms and their crystalline counterparts. Emphasis will be placed on the importance of solid states within the pharmaceutical industry.

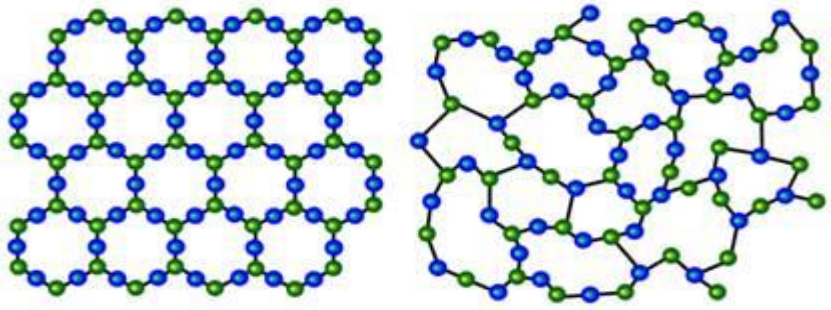
1.2 Amorphous solids

1.2.1 The importance of amorphous forms in the pharmaceutical industry

Many pharmaceutical solids can exist in an amorphous form, which, because of its distinctive properties, is sometimes regarded as a polymorph. However, unlike polymorphs, amorphous forms are not crystalline. Amorphous solids consist of disordered arrangements of molecules and therefore posses no crystalline lattice, nor unit cell and therefore have zero crystallinity, as illustrated in figure 1.1 (Brittain & Grant, 1999).

Both amorphous and crystalline solids have short-range molecular order, but unlike a crystalline solid, an amorphous solid has no long range order of molecular packing. The arrangement of molecules is random and has no structured molecular conformation.

Amorphous solids may also be called disordered systems, due to the randomness in the molecular packing (Shah *et al.,* 2006). Amorphous solids can hence be considered as solidified liquids, caused by the removal of thermal energy or a solvent, without subsequent crystallisation (Hancock, 2002).



(A) Crystalline state **(B) Amorphous state (glass)**

Figure 1.1: Molecular packing differences between a crystalline state (A) and an amorphous (glassy) state (B) (Anon 1, 2010).

No three-dimensional long range order exist between the molecules. An amorphous solid is unable to diffract X-rays like crystalline solids (figure 1.2). X-ray powder diffractometry (XRPD), show broad, diffuse haloes, for amorphous solids, instead of well defined peaks, as shown by crystalline solids (Yu, 2001; Hilden & Morris, 2004).

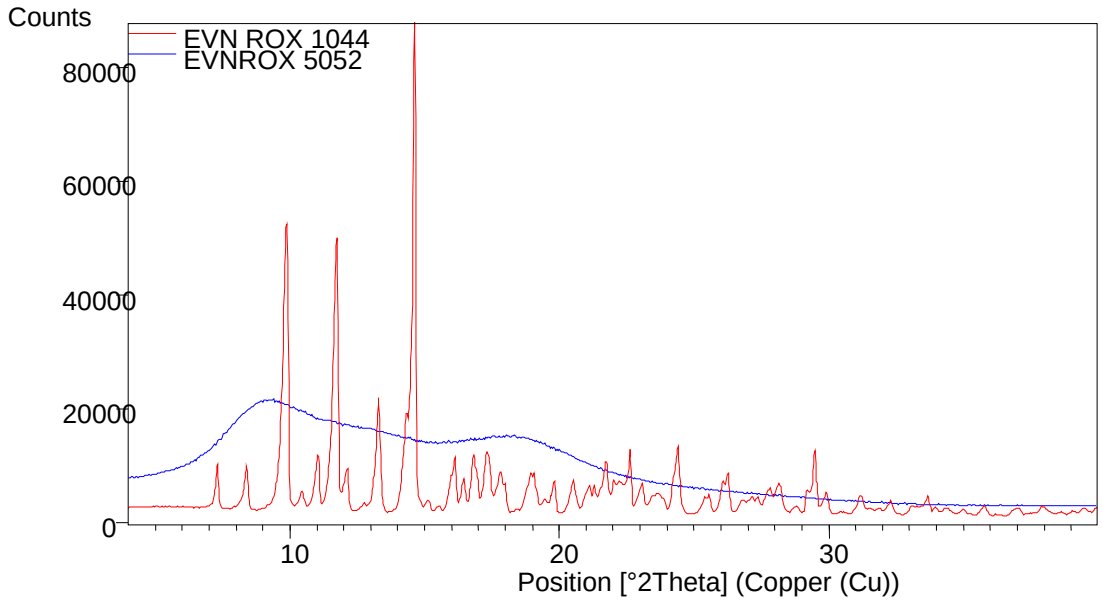


Figure 1.2: XRPD patterns of roxithromycin crystalline form (red) and roxithromycin amorphous form (blue).

For decades, the use of crystalline pharmaceutical materials has been encouraged for obtaining optimal chemical and physical stability. This has resulted in limited knowledge about the structure and molecular behaviour of amorphous APIs (Hancock, 2002). Lately, though, amorphous solids and the application of these forms have attracted the interest of many pharmaceutical scientists. The preparation of amorphous solids is now seen as an innovative approach to improve the performance of APIs.

Dosage form design is mainly influenced by the manufacturability, stability and bioavailability of APIs. The majority of APIs are in the crystalline form (Hancock & Zografi, 1997). However, in the past decade there has been a significant increase in the number of poorly soluble pharmaceutical actives, resulting in a heightened interest in methods to increase their water solubility. Amorphous drugs are known to be more soluble and therefore exhibit higher bioavailability (Bahl & Bogner, 2006).

The importance of amorphous forms in the pharmaceutical industry hence stems from their significant impact on the performance of pharmaceutical dosage forms, focusing scientists' attention increasingly on the differences in their physical and chemical characteristics. One such study was the preparation of a glass of an anti-inflammatory agent through fast cooling of the molten material (Yoshioka *et al.,* 1994).

According to Yu (2001), the term polyamorphism is further used to describe the different annealing times, or preparation routes of amorphous forms. The ability of APIs to exist in different physical forms enhances the opportunity for scientists to use the most advantageous form in formulating pharmaceutical dosage forms. Particle size distribution and solubility vary between different pharmaceutical forms and could proof useful in formulations (Hancock & Parks, 2000).

The advantage of amorphous forms is that they tend to be more soluble with higher dissolution rates, due to their disordered structures making them more energetic than crystalline forms (Bernstein, 2002).

A disadvantage of amorphous forms, is their tendency to be thermodynamically unstable. It therefore is important to ascertain the stability of an amorphous form, by determining its melting point, glass transition point, heat of fusion and crystallinity. The determination of the solubility of amorphous forms is difficult, because amorphous forms tend to revert to the more stable crystalline form when exposed to solvents (Hancock & Parks, 1999). The challenges associated with inclusion of amorphous APIs in pharmaceutical dosage forms have led to renewed interest in preparing, characterising and stabilising amorphous forms (Bernstein, 2002).

1.2.2 Preparation of amorphous solids

Due to thermodynamic factors, the preparation of amorphous solids could be a challenging task. During the preparation of glasses either good glass formers or poor glass formers can be identified. Good glass formers comprise a more spontaneous process, whilst the process for poor glass formers is more complex. The ability for a glass to form thermodynamically originates from a crystalline state that isn't necessarily more stable than the amorphous state, but rather because the molecules are poorly packed, with long distances between them (Yu, 2001). The preparation of amorphous forms theoretically involves three steps, namely (i) energisation of the material, followed by (ii) de-energisation of the material and lastly the (iii) kinetic trapping of the amorphous form (Shah *et al.,* 2006). Several methods can be applied in the preparation of amorphous solids, as described in the following paragraphs.

a) Dehydration / desolvation

Desolvation of a crystalline can be achieved by heating the substance at a certain temperature to release all liquid, causing it in some instances to convert from a crystalline form into an amorphous form. Studies have indicated that dehydration can be one possible route to the formation of amorphous solids, whilst the drying process can also reduce the physico-chemical stability through the loss of crystallinity (Yu, 2001).

b) Freeze drying

To enhance product characteristics, a drug can be produced in an amorphous form by processes, such as freeze - or spray drying, which could lead to enhanced dissolution rates. Freeze drying is the dehydration of a substance through crystallisation of ice, whereafter the water of the substance is sublimated and dried at low temperatures, leading to an open, porous structure. Whilst the crystallisation rate of amorphous forms usually is higher *above* Tg (the transition point where a liquid or rubbery state changes into a brittle state), freeze dried systems are less likely to collapse if stored *under* Tg. It is important to be aware that freeze dried systems are heat sensitive. Since a drug may be partially amorphous at room - or body temperature, an amorphous form can be prepared accidentally by compression, drying, or milling (Craig *et al.,* 1999).

c) Quench cooling from a melt (solidification of melt)

Most glassy systems are prepared through rapid cooling from the melt. The cooling rate is too fast for the crystallisation process to occur and no discontinuity in enthalpy is seen on cooling the material below melting temperature, therefore forming a super cooled liquid (Craig *et al.,* 1999*).* Quench cooling, however, is not always necessary, since an amorphous solid could be produced through slow cooling of the melt to room temperature as well.

d) Particle size reduction

This process mainly includes grinding and milling. These methods can be applied during the different stages of product development and manufacturing. Factors, such as the type of milling apparatus, milling duration and intensity, temperature and the addition of excipients could influence the final amorphous product (Petit & Coquerel, 2006). Care should be taken during this process though, because although grinding and milling can remove all traces of crystallinity, the possibility may exist that the material achieves a microcrystalline state. Subsequently, the solid will contain very small crystals. It is hence crucial to determine the occurrence of glass transition (Yu, 2001).

e) Precipitation from solution

Precipitation of acids and bases, or the removal of solvent, is considered a standard recrystallisation process in solid formation, showing notable low crystallinity (Petit & Coquerel, 2006).

1.2.3 Physical and chemical properties of amorphous solids

The physico-chemical properties of an API have a direct influence on the bioavailability of pharmaceutical systems. As has become evident, amorphous solids have useful properties that make them favourable for use in pharmaceutical preparations. As was discussed above, amorphous solids are more soluble and have higher dissolution rates than crystalline materials, and hence show better bioavailability. The main disadvantage of amorphous forms, however, is that they usually are less stable than their crystal counterparts (Yu, 2001).

As described in paragraph 1.2.1, no long range, three-dimensional, molecular order exists within the amorphous state, and hence no long range, orientational symmetry, as in crystals. Due to this characteristic, the structural mobility and changes thereof are imperative. According to Yu (2001), the physical characterisation of amorphous solids can be derived from different types of

information. Amorphous solids may possess short range orders, residual crystallinity, polymorphic states and regions of different densities. In terms of thermodynamics, amorphous solids possess higher energy, entropy and importantly, more free energy than their crystalline counterparts. Changes in amorphous solids can occur due to crystallisation or structural relaxation, owing to the instability of some amorphous solids. If a more stable crystalline state of a drug exists, the amorphous form can crystallise when sufficient molecular mobility exists.

The ability of an amorphous solid to absorb water (hygroscopicity) could be considered a major disadvantage. Absorbed moisture can change the physico-chemical properties of a pharmaceutical system, which could change its thermodynamic and dynamic properties. This may lead to a decrease in the glass transition temperature and an increase in molecular mobility, causing the physico-chemical stability to decrease (Newman & Byrn, 2003). Absorbed water can further affect the crystallisation of an amorphous solid, due to the fact that the plasticising effect of water enhances structural mobility. Moisture sorption analysis can be performed in the early stages of pharmaceutical development in order to determine the hygroscopicity of such material. The level of hygroscopicity of a solid can be determined by observing the rate of water uptake and changes in relative humidity (RH).

If an amorphous solid exhibits a glass transition, it can be defined as a glass. Glass transition is a phase where the solid amorphous phase undergoes a change in its thermodynamic properties, which includes a change in temperature (Hilden & Morris, 2004).

The glass transition temperature of an amorphous form determines its chemical -, physical - and viscoelastic properties. This is of special importance where these forms are stored or dried at temperatures above the glass transition temperature, as these temperatures could influence the stability of amorphous forms (Hancock & Zografi, 1994).

1.2.4 Solubility of amorphous forms

Understandably, poor solubility of certain APIs may cause problems with their bioavailability. Usually, solubility enhancers are used to improve the solubility and/or dissolution of poorly soluble APIs. Amorphous solids can be good alternatives, as they contain higher free energy, resulting in a higher solubility, thus faster dissolution rate. Thus, if taken orally, drug bioavailability can be drastically improved, when taking into consideration the limiting step for absorption, namely the solubility or dissolution rate in the gastro-intestinal tract (Bhugra & Pikal, 2008; Gao, 2008).

The prediction of the solubility of an amorphous form is a difficult process, as the amorphous state is a non-equilibrium state that tends to convert spontaneously into the more stable crystalline state. This complicates thermodynamic analysis and solubility measurements, because of the conversion to the crystalline state as a function of time. To determine the solubility of amorphous solids, solvents, such as ethanol, methanol and iso-propanol on glass, that don't change significantly with time, are used. Such measurements have shown a direct correlation between the solubility and the free energy differences of the amorphous and crystalline phases (Bhugra & Pikal, 2008).

The following equations can be used for the prediction of solubility of an amorphous material:

$$\Delta G^{a,c}(T) = -RT\ln\left[\frac{c^{a}_{s}(T)}{c^{c}_{s}(T)}\right]$$

and

$$\Delta G^{a,c}(T) = \Delta H^{a,c}(T) - T\Delta S^{a,c}(T)$$

Where:

R = gas constant,

T = temperature,

c^a_s = solubility of the amorphous state,

c^c_s = solubility of the crystalline state, and

$\Delta G^{a,c}(T)$, $\Delta H^{a,c}(T)$, $\Delta S^{a,c}(T)$ = free energy difference, enthalpy and entropy differences between the amorphous and crystalline phases, respectively.

Although an increased solubility of the amorphous phase can be illustrated, the exact ratio is difficult to determine, because of the spontaneous conversion into the crystalline state. The above estimation does not include the effect of water sorption of the amorphous material during solubility measurement (Bhugra & Pikal, 2008).

1.2.5 Stability of amorphous forms

According to Bhugra and Pikal (2008), whilst amorphous solids can improve the solubility of poorly soluble pharmaceuticals, it can impact negatively on the physical stability of oral dosage forms. It is thus important to observe the factors that affect the crystallisation from the amorphous state. Important factors that should be taken into consideration in determining the use of an amorphous state in solid oral dosage forms include:

- Thermodynamic factors: Amorphous forms are usually thermodynamically unstable in comparison with the crystalline state.
- Molecular mobility: A higher molecular mobility could lead to higher chemical degradation rates and spontaneous recrystallisation. Chemical degradation follows the Arrhenius temperature dependence. Accelerated temperature stability testing is used to predict stability.
- Nucleation: If the nucleation barrier is crossed, the amorphous system is physically unstable, because it is ready to revert to the more stable crystalline form, causing crystalline growth (Bhugra & Pikal, 2008).

Since amorphous forms are thermodynamically unstable and tend to revert to the crystalline form when stored, it is important to characterise the glass transition of an amorphous form, in order to enhance the predictability of the stability of the pharmaceutical system (Craig *et al.,* 1999).

Thermodynamic instability and higher molecular mobility increase the instability of amorphous solids and thus increase chemical degradation rates and recrystallisation. Therefore, in order to develop a successful formulation using amorphous drugs, it is important to stabilise the amorphous drug below its glass transition temperature (Tg) (Bhugra & Pikal, 2008).

As expected, different preparation methods would thus have different effects on the physical stability of amorphous forms and are important in stabilising them. Amorphous forms of trehalose were formed by freeze drying, spray drying, dehydration and quenching from a melt. Whilst the different methods showed no impact on the glass transition temperature and fragility of the glass, the enthalpy relaxation, crystallisation and water sorption were altered. For experiments above the glass transition temperature, freeze dried and spray dried trehalose were similar, whereas the trehalose, quenched from the melt, was the most resistant to recrystallisation, whilst the amorphous form through dehydration was sure to lead to crystallisation (Bhugra & Pikal, 2008).

As observed above, the behaviour of an amorphous system would differ below and above the glass transition temperature (Tg). The crystallisation rate is higher above Tg, whilst through freeze drying, the drug is less vulnerable to physical collapse if stored below Tg (Craig *et al.,* 1999*).*

Crystallisation of the amorphous state is more dependent on the onset time of crystallisation than on crystal growth. The temperature dependence is a complicated process, because when the nucleation barrier is crossed, the amorphous system is very unstable, since the system wants to be more stable and is it in the process of transforming to the more stable crystalline state. The temperature has a significant impact on crystallisation, because if the temperature is favourable during storage, growth of the crystals would proceed.

Nucleation, crystal growth, temperature history and the current temperature all impact on stability and thus define the physical stability of amorphous pharmaceuticals (Bhugra & Pikal, 2008).

To formulate a stable glass, it is important that the glass transition temperature is low, that it contains a high viscosity (>10^{14}) at a temperature that is thermodynamically stable and that has the smallest crystal lattice energy. Furthermore, it could be observed that a lack in long range order of molecules can be as a result of different melting points. It is therefore important that all these factors are considered in formulating an amorphous compound with a good physical stability (Bhugra & Pikal, 2008). Temperatures below the glass transition temperature would lead to lower molecular mobility and thus to a more stable substance. For an amorphous form to be stable there must be a high correlation between the glass transition and the storage temperatures.

According to Bhugra and Pikal (2008), when molecules are in a glassy state, there are a few factors that impact on crystallisation, such as:

- Long range diffusion: This factor has an impact on structural relaxation, a decrease in volume, entropy and enthalpy and through this slows mobility and stabilisation.
- Molecular mobility: This factor may lead to nucleation, which may cause an unstable amorphous form, because the unstable form would tend to convert to the more stable crystallisation form.
- Distribution and relaxation times: The distribution and relaxation times of a substrate is characterised by a higher mobility, which also leads to nucleation of the glassy form.

All these factors impact on the stability of amorphous substances and contribute largely to obtaining an overall, physically stable system below glass transition temperature (Bhugra & Pikal, 2008).

1.2.6 The influence of water content on the glass transition temperature

The glass transition temperature of an amorphous solid has a large impact on its chemical -, physical - and viscoelastic properties. When a compound contains water as an additive, it usually acts as a plasticiser, meaning that it lowers the glass transition temperature of the solid. Contrary, if an additive increases the glass transition temperature, it is known as an anti-plasticiser. The importance thereof is that many amorphous pharmaceutical solids spontaneously absorb water, which would impact on the glass transition temperature, causing changes in the chemical -, physical - and viscoelastic properties. The effect of water on the physico-chemical properties of amorphous solids can thus be derived from three important parameters:

- Glass transition temperature of a dry solid;
- Density of an amorphous solid; and
- The ability of an amorphous solid to take up water (Hancock & Zografi, 1994).

1.3 Polymorphism

1.3.1 Polymorphic forms

The relevance of polymorphism to the pharmaceutical industry is mainly to determine which form of a given API is the most stable for a particular pharmaceutical dosage form. Different crystalline polymorphs and solvates have different crystal packing, molecular conformation and lattice energy. These result in differences in the physico-chemical properties of a drug, for instance density, hardness, tabletability, melting point, heat of fusion, solubility and dissolution rates. The differences in physical properties affect the preparation of drugs in dosage forms. Because of all these differences, it is important to understand the solid state properties of polymorphic forms, as well as understand polymorphism, in order to gain control over the crystallisation process. This knowledge helps enable the researcher to obtain a specific stable polymorphic form, suitable for a specific dosage form.

Polymorphism can be classified into two different categories, because of their differences in thermodynamic properties. *Enantiotropes* can reversibly transit between different polymorphs at a certain transition temperature below the melting point, while this is not possible for *monotropes.* In order to determine the nature of enantiotropes and monotropes, four very important factors are defined next (Vippagunta *et al.,* 2001):

- Heat of fusion rule: The rule states that if the polymorph with the higher melting point has the lower heat of fusion, the two polymorphs are enantiotropes and if not, they are monotropes. Heat of fusion is a thermodynamic parameter that largely influences the forming ability and stability of the glass, because it affects the nucleation rate and leads to a higher enthalpy or entropy of fusion (greater thermodynamic driving force). When the heat of fusion is high, crystallisation is faster (Lohani & Grant, 2006).
- Heat of transition rule: This rule states that if an endothermic phase change takes place at a specific temperature and the transition point lies beneath that temperature, the two polymorphs are enantiotropes. Contrary, if an exothermic phase change takes place and no transition point can be observed below that temperature, the polymorphs are monotropes (Bernstein, 2002).
- Infrared rule: The rule states that the hydrogen bonded polymorphic form with a higher frequency in the bond stretching modes, may be assumed to have larger entropy. Of importance is that the successful application of the infrared rule requires detailed information of the nature of the hydrogen bonds in the solid state. More studies on these correlations are needed when utilising this rule (Bernstein, 2002).
- Density rule: According to the density rule, the more thermodynamically stable polymorph is more chemically stable than the metastable polymorph. This could be attributed to the higher crystal packing density of the thermodynamically stable polymorph. The rule applies generally to ordered

molecular solids that are dominated by van der Waals interactions (Bernstein, 2002).

Different polymorphs can also be characterised by the structural differences in their crystal lattices, which can be done through two mechanisms. The first mechanism is *packing polymorphism,* where one observes how molecules are packed in different, three-dimensional structures, because of their different intermolecular actions. The second mechanism is *conformational polymorphism*, where molecules are arranged differently and from where it can be packed in different crystal forms (Vippagunta *et al.*, 2001).

1.3.2 Solvates

It is important to note that crystalline solids exist in different forms and the three types of crystalline solids comprise polymorphs, solvates and hydrates (Vippagunta *et al.,* 2001).

Solvates are characterised as crystalline structures that contain solvent molecules, which cause significant differences in the physico-chemical properties of a drug. Solvates and crystalline solids have different molecular conformation and packing, hence different physical properties, such as melting points, dissolution rates, solubility, thermodynamic and kinetic properties. Solubility differences can impact on the absorption of these compounds (Vippagunta *et al*., 2001).

Desolvated solvates are unsolvated compounds. These solvates have no distinctive crystalline form and therefore the molecules are not structured like their crystalline counterparts (Vippagunta *et al.,* 2001).

There are different ways for a solvent to interact with a crystalline solid, i.e.:

- The solvent molecules can form weak interactions, namely van der Waals, dipole-dipole, or hydrogen bonding;
- The physical entrapment of the solvent in the growing crystal; and

- The adsorbing of solvent in a disordered manner in different regions of the crystal (Brittain & Grant, 1999).

The different crystal faces of a substance have different affinities, therefore the amount of solvent or water absorbed in crystalline materials depends on their morphology and also on many other parameters. The solvent can also be physically entrapped in a crystal, called liquid inclusion. Large amounts of solvent may be adsorbed on the surface of the crystal, which can cause problems with grinding and granulation of these solvates. In an amorphous state the molecular entities are packed more closely, resulting in stronger intermolecular interactions and providing no space for solvent intake. Compounds can crystallise and bind with the solvent to form a solvate, in which case the solvent is a part of the crystalline structure (Guillory, 1999).

Solvates can be divided into two categories, namely stoichiometric and non-stoichiometric solvates (Brittain, 1999). Stoichiometric solvates are known as molecular compounds and the solvent is a part of the crystalline structure. The desolvation of a stoichiometric solvate usually results in a different crystalline structure, or leads to a disordered or amorphous state (Brittain, 1999). Non-stoichiometric solvates are inclusion compounds and the solvent is usually captured in channels of the crystalline structure. These solvates have large, awkward, crystal shapes, which cannot pack close together (Brittain & Grant, 1999).

The ability of a solvate to form or to desolvate has a significant impact on the phase stability of these structures, because it is different for every form. The stability of solvates also depends on the temperatures and the partial pressure of the solvent. The formation of solvates is best when the crystallisation takes place at lower temperatures. The partial pressure of solvates also becomes practically zero for solvates with organic solvents, but not for hydrates, because of the atmospheric moisture. Stability depends on many factors, including the size of the crystals, crystal defects, dynamics of the atmosphere and the desolvation mechanism. To characterise and determine the stability of solvates, thermogravimetric analysis, like differential scanning calorimetry

(DSC) can be used, because it analyses the thermal stability at dry atmospheric conditions, whilst it can be done at elevated humidity. The stability range of solvated and unsolvated forms can be determined through solubility studies at different temperatures. This is also used to determine the transition temperature between the different phases of the solvate (Brittain & Grant, 1999).

1.3.3 Hydrates

When a crystalline structure or compound is combined with water or a water element, this solvent is called a *hydrate*. It is easy for most pharmaceutical substances to form crystalline hydrates, since water is a small molecule that is capable of forming hydrogen bonds in multiple directions. This small molecule can also easily fill voids within the molecular packing of a solid. Water can therefore combine drug molecules in structured crystalline forms. It is important to note that the activity of water in a medium is the only reason why a hydrate structure will form. A monohydrate is a compound that contains one water molecule, whereas a dihydrate contains two water molecules (Vippagunta *et al.,* 2001). According to Vippagunta *et al.* (2001), hydrates are characterised in three different categories:

- Isolated site hydrates: Water molecules in this compound are isolated from others by combining with drug molecules, e.g. cephradine dihydrate;
- Channel hydrates: Water molecules lie next to others and form channels through the crystal, e.g. ampicillin trihydrate; and
- Ion-associated hydrates: Metal ions are combined with water, e.g. calteridol calcium.

Phase changes can occur, for example when a hydrated compound converts into an amorphous phase, because of dehydration. This can cause a poorly soluble drug to convert into a compound that is much more soluble, whilst impacting negatively on the stability. Humidity, temperature and pressure can cause such phase changes (Vippagunta *et al.,* 2001).

1.4 Conclusion

An understanding of the solid state properties of active pharmaceutical ingredients is an important step in the development process of any given pharmaceutical dosage form. The physico-chemical properties of different polymorphic forms, amorphous forms, solvates and hydrates differ significantly and these differences may directly impact on the stability and solubility of a drug. Over the past decade, significant progress has been made to understand the physico-chemical properties of amorphous solids.

Amorphous solids are of great interest to scientists and their better solubility properties make them very promising solid state forms for future usage in pharmaceutical dosage forms.

In order to understand the different physico-chemical properties of an amorphous material, intensive studies, including the screening for different forms, stability studies and solubility studies are necessary to bring about a solid dosage form that will be safe, stable and effective to the end user.

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Chapter 2

Roxithromycin

2.1 Introduction

Roxithromycin is an ether oxime derivative of erythromycin and is classified as a macrolide antibiotic (Chan & Luft, 1986). More accurately, roxithromycin is characterised, together with several other antimicrobials, as erythromycin-like, macrolide, antimicrobial agents (Barry *et al.,* 1988). Macrolide antibiotics are primarily bacteriostatic and bind to the 50S subunit of the ribosome to inhibit bacterial protein synthesis (Miroshnyk *et al.,* 2008). Furthermore, macrolides are clinically used against susceptible organisms, which infect the skin, soft tissues, and respiratory -, genital - and gastrointestinal tracts (Kirst & Sides, 1989). These antibiotics are used extensively, because they are well tolerated and safe. Macrolides are the antimicrobial agents of choice for respiratory infections, because of their anti-inflammatory effects (Ferrara *et al.,* 2005).

Macrolides are active against aerobic and anaerobic, gram-positive cocci, with the exclusion of most eterococci and many *Staphylococcus aureus* strains, including methicillin-resistant strains, *Mycoplasma pneumonia, Chlamydia trachomatis, Chlamydophila pneumonia, Legionella* species, *Cornyebacterium diphtheria, Campylobacter* species, *Treponema pallidum, Propionibacterium acnes* and *Borrelia burgdorferi* (Bryskier, 1998).

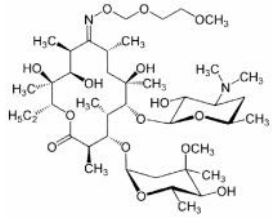
Roxithromycin is a semi-synthetic, macrolide antibiotic, derived from erythromycin A. This derivative of erythromycin A was synthetically prepared in order to improve its pharmacokinetic properties, as well as to inhibit its relatively easy degradation properties. The synthetic preparation steps of roxithromycin include the modification of the ketone at C-9. The resulting oxime derivative was first synthesised at Roussel-Uclaf. The oxime derivative, named roxithromycin, is superior to erythromycin A, since this derivatisation is less

prone to intramolecular cyclisation (Kirst & Sides, 1989). Roxithromycin exhibits multi-functionality, due to the fact that it acts as a bacteriostatic drug at low concentrations and as a bactericidal drug at high concentrations. It binds to the 50S subunit of the 70S ribosome, which leads to the reversible inhibition of RNA-dependent, bacterial protein synthesis (Medsafe, 2010). Because of roxithromycin's lower cytochrome P450 activity, it has fewer undesired interaction than erythromycin (Ostrowski *et al.,* 2009).

2.2 Physico-chemical information

The physico-chemical information of roxithromycin is summarised in table 2.1.

Table 2.1: Physico-chemical properties of roxithromycin

Structure (Wikimedia, 2011)	
Chemical / Empirical formulation (Merck, 2006)	Monohydrate: $C_{41}H_{76}N_2O_{15}$ Anhydrate: $C_{41}H_{74}N_2O_{14}$ Dihydrate: $C_{41}H_{78}N_2O_{16}$
Contents (BP, 2010)	96.0% - 102.0% (anhydrous substance)
Appearance	White or almost white, crystalline powder
Chemical name (Merck, 2006)	Erythromycin 9-[O-[(2-methoxyethoxy)methyl]oxime]
Relative molecular mass (Merck, 2006)	Monohydrate: 837.05 g/mol Anhydrate: 819.3 g/mol Dihydrate: 854.06 g/mol

Description (Merck, 2006)	Roxithromycin is a semi-synthetic, erythromycin derivate, with an *in vitro* antibacterial spectrum.
Solubility (BP, 2010)	Very slightly soluble in water, freely soluble in acetone, in alcohol and in methylene chloride. It is slightly soluble in dilute hydrochloric acid.
Category (Merck, 2006; Chemicalland21, 2011)	Antibacterial, macrolide, anti-infective
Melting point (Chemicalland21, 2011)	Monohydrate: 115°C - 120°C
Moisture content (Chemicalland21, 2011)	Monohydrate: Maximum 3.0%
Storage (BP, 2010)	Preserve in well-closed, light-resistant and tight containers. Store in cool and dry place.

2.3 Activity

Macrolides, including roxithromycin, are relatively safe antibiotics, having a broad spectrum of activity. Their effect on the immune system is important in patients with impaired immune system, like AIDS patients. Since roxithromycin is a derivative of erythromycin, it exhibits antimicrobial activity, similar to that of erythromycin (Chan & Luft, 1986). Roxithromycin is active against infections caused by *Pneumocystis carinii, Toxoplasma gondii,* and *Mycobacterium avium* complex (MAC), all of which are associated with immune compromised (AIDS) patients (Rastogi *et al.,* 1995).

The *in vitro* activities of APIs are mainly determined by using minimal inhibitory and bactericidal concentrations (MIC and MBC) (Danias *et al.,* 1998). The *in vitro* and partial *in vitro* activities of roxithromycin are discussed next.

2.3.1 *In vitro* activity

Roxithromycin is stated to possess significant *in vitro* activity against a wide range of Mycobacteria species, including *Mycobacterium avium* complex (MAC). Its activity can be enhanced by using two to three drug combinations of ethambutol, rifampin, amikacin, ofloxacin and clofazimine (Rastogi *et al.,* 1995).

Human immunodeficiency virus (HIV) patients are prone to develop tuberculosis, a serious problem in the early stages of AIDS. MAC starts in the later stages of the disease, when low CD4 levels are reached. Roxithromycin is used in the chemoprophylaxis of MAC infections in HIV patients and therefore is it necessary to compare its *in vitro* activity. Although results have shown that roxithromycin is not the drug of choice for MAC, it achieves high intra - and extracellular concentrations within lysosomes. Consequently, roxithromycin can be used in chemoprophylactic regimes for the prevention of opportunistic infections in patients with AIDS, but should be carefully monitored, whilst infections, caused by underlying M. *tuberculosis*, should be excluded (Rastogi *et al*., 1995). During *in vitro* studies conducted by Barry *et al.* (1988), it was confirmed that roxithromycin also exhibits activity against *Streptococcus agalactiae, S. pyogenes, S. pneumonia, Enterococcus* spp., *Lysteria monocytogenes, Neisseria meningitides, N. gonorrhoeae and Haemophillus influenza.*

2.3.2 Partial *in vitro* activity

Macrolide antibiotics, including roxithromycin, are weak anti-staphylococcal agents and are not frequently used for therapy. With standard minimal inhibitory concentrations (MIC), minimal bactericidal concentrations (MBC) and time-kill kinetic tests, roxithromycin has shown *in vitro* bacteriostatic activity. Roxithromycin shows poor efficiency against methicillin-susceptible *Staphylococcus epidermidis*, inferior to that of erythromycin (Danias *et al.,* 1998).

Roxithromycin is a promising macrolide antibiotic and its usefulness against *Haeomophilis influenza* relies heavily upon its improved pharmacokinetics (predictable absorption and prolonged serum and tissue levels), since less inhibitory activity takes place than with erythromycin (Jorgensen *et al.,* 1986).

2.3.3 Resistance

Roxithromycin shows resistance against multi-resistant *Styphylococcus aureus, Enterobacteriaceae, Pseudomonas* species and *Acinetobacter* species (Medsafe, 2010).

2.4 Pharmacodynamics

After oral administration of roxithromycin, its bioavailability is approximately 50% and its peak plasma level is reached within 1-2 hours after dosage. Food intake delays absorption and should it therefore be taken 15 minutes before food intake, or on an empty stomach (Medsafe, 2010).

At a concentration of 4.2 mg/L, approximately 92 - 96% of roxithromycin is bound to plasma protein. At a higher concentration of 8.4 mg/L, approximately 87% of roxithromycin is bound to plasma protein (Medsafe, 2010).

With a single dose of 150 mg, efficient concentrations of roxithromycin is found in the respiratory tract and secretions, in the male and female genital tracts, tonsils, paranasal sinuses, synovial fluid and the skin, but not in saliva (Medsafe, 2010). Efficient concentrations are as follows:

- Half-life ($T^1/_2$) is 12 hours for young adults, 20 hours for children and 27 hours for elderly patients.
- For impaired hepatic function the half-life is 25 hours.
- Renal insufficiency is 18 hours (Medsafe, 2010).

7% of roxithromycin is excreted in the urine, 13% through the lungs, 53% in faeces and the remainder is unknown (Medsafe, 2010).

2.5 Indications

Roxithromycin is indicated for respiratory and genitourinary tract infections, skin, soft tissue and orodental infections. Roxithromycin is effective and safe and therefore it is frequently used for pediatric infections (Ostrowski *et al*., 2009). Some of the indications of roxithromycin are listed in table 2.2.

Table 2.2: Indications of roxithromycin

Main indication	**Specific indication / Targeted bacteria**	**Mechanism**
Upper respiratory tract infections (Barreto, 1996)	Acute pharyngitis, tonsillitis and sinusitis	Not described
Lower respiratory tract infections (Ogrendik, 2009)	Acute bronchitis, acute exacerbation of chronic bronchitis and community acquired infections	Not described
Tuberculosis (Rastogi *et al.,*1995)	*Mycobacterium avium* complex	Enhanced by two - or three-drug combinations with ethambutol, rifampicin, amikacin, ofloxacin and clofazimine
Infectious diseases, *Mycoplasma hominis* (Bryskier, 1998)	Urogenital tract infections – pyelonephritis, pelvic inflammatory disease and bacterial vaginosis	*In vitro* activity tested, but generally resistant to macrolides
Anti-androgenic activity (Inui *et al.,* 2001)	Acne	Not described
Atopic dermatitis (Adachi, 2002)	*Staphylococcus aureus*	Replication of DNA coding of super-antigen produced by *S. aureus* suppressed by roxithromycin

Asthma and cystic fibrosis (Ferrara *et al.,* 2005)	Anti-inflammatory effects	Increasing evidence of macrolide anti-inflammatory effects are being found
Malaria (Min *et al.,* 2007)	Activity against *Plasmodium falciparum*	P. *falciparum* is resistant to mefloquine and chloroquine. Roxithromycin contains intrinsic anti-malarial activity and therefore enhances potency of mefloquine and chloroquine and resistance is minimised.
Diarrhoea in AIDS patients (Uip *et al.,* 1998)	*Cryptosporidium species*	Not described

2.6 Dosage

The daily dosage for adults is one dosage of 300 mg/day, or a split dosage of 150 mg twice daily. Patients with impaired renal function use the same dosage, whereas patients with impaired hepatic function may only administer one 150 mg tablet per day. Patients with atypical pneumonia also administer 150 mg tablets twice daily. The daily dosage for children is 5-8 mg/kg/day in two split dosages (Medsafe, 2010).

Roxithromycin therapy usually lasts for 5 - 10 days, depending on response. Streptococcal throat infections require at least 10 days of therapy, whilst non-gonococcal genital infections require 20 days of therapy for complete cure (Medsafe, 2010).

2.7 Contra-indications

The contra-indications for roxithromycin include severely impaired hepatic function, known hypersensitivity to macrolides, including erythromycin, and concomitant therapy with vasoconstrictive ergot alkaloids (Medsafe, 2010).

2.8 Brand names

- Rulide® (Gibbon, 2003);
- Roxithromycin-Hexal® (Gibbon, 2003);
- Throsyn® (Gibbon, 2003);
- ROX® (Drugbank, 2010); and
- Surlid® (Drugbank, 2010).

2.9 Solubility

Roxithromycin only has a 50% oral bioavailability, due to its poor aqueous solubility, which limits its potential for optimal drug delivery and therapeutic effect. Its poor solubility is thus an obstacle in formulation development. As it is more cost effective to chemically re-design a molecule than to move through the whole development process, it is crucial to develop a formulation that overcomes problems of insolubility (Biradar *et al.*, 2006).

Experimental water solubility of roxithromycin is 0.0189 mg/L at 25°C (Drugbank, 2010).

Predicted water solubility is 1.87e-01 mg/ml (Drugbank, 2010).

According to Aucamp (2009), the solubility profile of roxithromycin monohydrate in distilled water was 1.7 ±0.6 µg/ml, whereas the solubility of roxithromycin glassy form indicated significant improvement by exhibiting a solubility value of 32.9 ±3.5 µg/ml in water. The solubility of the chloroform desolvate was 8.6 ±1.8 µg/ml in water.

Furthermore, the more amorphous the form was, the better the solubility profile became. It was also concluded that the crystalline structure had a negative effect on the solubility of roxithromycin (Aucamp, 2009). It thus appears that the most important physico-chemical properties that influence solubility, stability and absorption include particle size and crystallinity.

2.10 Stability

Literature states that roxithromycin is more stable under acidic conditions. The reason for this being that roxithromycin contains an erythronolide lactone ring, which has been modified to differ from erythromycin, through the inclusion of an oxime group on either side of the chain at position 9. This prevents enolic ether formation and prevents inactivation by gastric acid (Ostrowski *et al.,* 2009; Bryskier, 1998). According to Zhang *et al.* (2004), roxithromycin transforms into its Z-isomer in simulated gastric fluid (37°C), resulting in the degradation of roxithromycin. According to first-order kinetics, it was found that roxithromycin consists of a half-life (T½) of 6.5 minutes at pH 1.0; 17 minutes at pH 1.3; 51 minutes at pH 1.8; and 315 minutes at pH 3.0. Therefore, it was concluded that roxithromycin would degrade at a pH of 1.2, the pH of the stomach, and that neither NaCl, nor pepsin would alter the degradation process (Zhang *et al.,* 2004).

2.11 Conclusion

Roxithromycin is a semi-synthetic, macrolide antibiotic, which shows a broad spectrum of activity against Mycobacteria species.

The physico-chemical properties of roxithromycin cause it to be poorly soluble in water, and thus to exhibit poor bioavailability. Any roxithromycin polymorphic form, or amorphous form with better water solubility would thus lead to an improvement in the bioavailability of this active ingredient.

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Chapter 3

Characterisation methods

3.1 Introduction

In this chapter, the different apparatus and methods used to characterise the physico-chemical properties of roxithromycin raw material, as well as the different polymorphic and amorphous forms being prepared during this study, are described. The preparation methods employed to obtain the different polymorphic and amorphous forms are also discussed. The techniques used to characterise the roxithromycin forms can be divided into three sections, namely thermal analysis, X-ray crystallography and sample analysis. The thermal analysis section includes descriptions of differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA) and thermal microscopy (TM) procedures. X-ray powder diffraction (XRPD) is described under X-ray crystallography, whilst sample analyses comprise of infrared (IR) spectroscopy, Karl Fischer (KF) titration, high performance liquid chromatography (HPLC), solubility, dissolution and stability studies.

3.2 Glass preparation method

By using the quench cooling method, a glassy form of roxithromycin was prepared during this study. The raw material was placed in a Binder® E28 (GmBH - Germany) oven at 125°C to form a melt. The melt was removed from the oven and placed on a cool surface and allowed to cool down. This prepared glass was used in all subsequent analyses being performed.

3.3 Glass powder preparation method

The same method as described in paragraph 3.2 was followed to prepare another roxithromycin glass sample. After the melt had reached room temperature, it was transferred into a mortar and pestle and ground into a homogenous powder.

3.4 Recrystallisation method

Slow evaporation of saturated solutions containing roxithromycin was used to prepare various crystals. Saturated solutions were prepared by adding a sufficient amount of roxithromycin raw material into a suitable sized glass beaker or polytop and by adding different organic solvents to each. Each solution was stirred by using a heating magnetic stirrer (Velp® Scientifica – Italy) that was warmed up to just below the boiling point of the relevant solvent. Additional solvent was added as necessary with a pasteur pipette. Once all the roxithromycin raw material had dissolved, the sample holder was removed from the stirrer, covered with Parafilm® (Pechiney Plastic Packing, USA) or a polytop cap and stored in a dark, closed cupboard. These saturated solutions were left to recrystallise, after which the crystals were analysed and characterised.

The following organic solvents were used for the recrystallisations:

- Chloroform;
- Ethyl acetate; and
- 100% ethanol.

During this study, recrystallisations from ethanol and water mixtures were also performed in order to investigate the influence of this binary system on the crystal forms (Annexure A):

- 90% v/v ethanol:water;
- 80% v/v ethanol:water;
- 70% v/v ethanol:water;

- 60% v/v ethanol:water;
- 50% v/v ethanol:water; and
- 40% v/v ethanol:water.

3.5 Thermal analysis

3.5.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry is a method of thermal analysis that is widely accepted in the pharmaceutical industry and which is regularly used on a quantitative basis. The temperature of the sample and reference material is controlled, while the heat flow is measured and plotted as the differential rate of heating (W/s, cal/s or J/s) against temperature (°C). The area under the peak of a DSC thermogram is directly equivalent to the absorbed heat or thermal event and by integrating these peak areas, it yields the heat of the reaction (Wendlandt, 1986). DSC analyses are mainly used to determine the melting point and to illustrate any thermal events. When heat is absorbed through solvent loss, phase transition, or melting, it is defined as an endothermic process, whereas crystallisation and chemical reactions are defined as exothermic processes, where heat is evolved (Byrn *et al.,* 1999).

During this study, data was collected using a Shimadzu DSC-60 (Shimadzu, Japan) system. Sample preparation started by weighing approximately 6-9 mg of each sample into aluminium crimp cells, each covered with an aluminium lid and sealed with a crimping tool. For the purpose of this study the cell lids were not pierced. The samples were placed onto the auto sampler. This process was repeated for the preparation of an empty reference cell that was manually placed on the left hand side of the DSC furnace. The DSC system parameters were set as shown in table 3.1 and the thermal process was started. A DSC trace was produced and analysed, using the Shimadzu software (Version 2.1.1.0).

Table 3.1: Experimental setup and conditions for DSC analysis

Starting temperature	25°C
Maximum temperature for roxithromycin	200°C
Heating rate	10°C/minute
Nitrogen flow rate	35 ml/minute

In some instances a lower heating rate (5°C) was used to investigate thermal events, other than melting.

3.5.2 Thermogravimetric analysis (TGA)

TGA is a technique used to measure the change in weight, mostly due to the loss of moisture, of a substance, as a function of temperature, subjected to a controlled temperature program (Haines, 2002). A sample is prepared and placed on a thermobalance in combination with a microbalance with a furnace, temperature program and a computer control system (Haines, 2002).

The TGA system simultaneously weighs and heats or cools a sample at an ambient temperature in an inert atmosphere (Brown, 2001), in order to plot a thermogram of weight against temperature over a specific time period (Haines, 2002).

A Shimadzu DTG-60 (Shimadzu, Japan) system was used during this study. Firstly, two empty aluminium cells were placed on the TGA furnace and their weights manually zeroed on the microbalance. Data was collected from an amount of sample that was transferred into the one aluminium cell and placed on the right hand side of the furnace, with the empty reference cell on the left, after which the system was closed. The system parameters were set as per table 3.2 and each sample was weighed automatically before starting the thermal process.

Table 3.2: Experimental setup and conditions for TGA analysis

Starting temperature	25°C
Maximum temperature for roxithromycin	200°C
Heating rate	3, 5, 7 and 10°C/minute
Nitrogen flow rate	35 ml/minute

A TGA trace was produced and analysed. Equation 3.1 was used to determine the theoretical weight loss and the stoichiometry of the solvates / hydrates obtained in this study.

$$\% \text{ Weight loss} = \frac{\text{Molecular weight}_{solvent}}{\text{Molecular weight}_{solvent} + \text{Molecular weight}_{roxithromycin}} \times 100 \qquad (3.1)$$

3.6 Thermal microscopy (TM)

For the investigation of polymorphism, a polarising microscope, fitted with a hot stage, has proven very useful. This allows the researcher to document thermal events, like dehydration, desolvation, the melting point and transition temperatures, as well as to determine the degree of stability of forms. TM is a useful technique to use in conjunction with DSC and TGA.

A small amount of sample was placed on a microscope slide and covered with silicon oil (from Fluka Chemika, Switzerland). A cover slide was then placed over this sample.

The thermo microscope being used was a Nikon Eclipse E400 (Nikon, Japan), equipped with a Leitz 350 (Leitz, Germany) heating unit and a Metratherm 1200d thermostat (Metratherm, Germany). Micrographs were taken with a Nikon DS-Fi1 digital camera, fixed to the microscope.

3.7 X-ray powder diffraction (XRPD)

XRPD has the ability to qualitatively determine the crystallinity of a substance, with each substance or polymorph having a unique diffraction pattern. This experimental method usually produces unmistakable results (Brittain & Grant, 1999). Specific fingerprints of microcrystalline powders make X-ray crystallography the preferred method for identifying polymorphic phases (Hilfiker, 2006).

Sample readings were prepared on two instruments, i.e. a Philips XPert-Pro (Netherlands) and a Bruker D8 Advanced (Bruker, Germany). The polymorphic forms of the APIs being prepared during this study were classified according to the measured XRPD traces.

The X-ray powder diffraction profiles were obtained at room temperature. The measurement conditions for the Bruker D8 XRPD apparatus are illustrated in table 3.3 and for the Philips XPert-Pro XRPD apparatus in table 3.4.

Approximately 200 mg of sample was transferred into an aluminium sample holder, taking care not to introduce a preferential orientation of the crystals.

Table 3.3: Experimental setup and conditions for XRPD analysis (Bruker D8)

Target	Cu
Voltage	40 kV
Current	30 mA
Divergence slit	2 mm
Anti scatter slit	0.6 mm
Detector slit	0.2 mm
Monochromator scanning speed	2°/min (step size 0.025°; step time 1.0 sec)

Table 3.4: Experimental setup and conditions for XRPD (PANalytical X'Pert-PRO)

Target	Cu
Voltage	40 kV
Current	45 mA
Divergence slit	0.9570 mm
Anti scatter slit	Fixed
Detector slit	0.2 mm
Monochromator scanning speed	2°/min (step size 0.0170°; step time 5.8142 sec)

The XRPD pattern displays a series of peaks, detected at characteristic scattering angles. These angles and their intensities provide a full crystallographic characterisation of the powdered sample. The higher the intensity counts of the peak, the more crystalline the structure. When comparing the generated XRPD pattern with that of a reference, a new polymorphic identity could be established (Brittain & Grant, 1999).

3.8 Infrared spectroscopy (IR)

IR spectroscopy is a well established, vibrational, spectroscopic method, used as a molecular structure fingerprint. It plays an important role in the solid state characterisation of active pharmaceutical ingredients (APIs). The IR spectrum is conclusive evidence for substance identification, because by comparing an IR absorption spectrum of a substance to a correlating reference standard, the identity of a substance can be obtained with a single test (Hilfiker, 2006).

Potassium bromide (KBr) was used as a background, by dispersing the sample in a matrix of powdered KBr. The IR-spectra were measured in a reflectance cell, using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS).

The IR sample holder was filled with the ground specimen and measurements done using a Shimadzu IRPrestige-21 Fourier Transform Infrared Spectrophotometer (Shimadzu, Japan). The readings were processed by the onboard computer system.

3.9 Karl Fischer titration (KF)

Karl Fischer titration is a method used for quantifying the water content of samples and it is based on the Bunsen reaction between iodine and sulphur dioxide in a water medium (Bruttel & Schlink, 2006).

$ROH + SO_2 + R'N \longrightarrow [R'NH]SO_3R + H_2O + I_2 + 2R'N \longrightarrow 2[R'NH]I + [R'NH]I + [R'NH]SO_4R$

[Alcohol] [base] [alkylsulfite salt] [water] [iodine] [hydroiodic acid salt] [alkylsulphate salt]

The reactive alcohol (ROH) is usually methanol or 2-(2-ethoxyethoxy)ethanol, whilst the Karl Fischer reagent (base) is either pyridine, imidazole, or a primary amine. Water and iodine are consumed in a 1:1 ratio in the Bunsen reaction and once all the water is consumed, the excess iodine is detected by an indicator electrode. The amount of water present in the sample is calculated, based on the concentration of the excess iodine and the amount of Karl Fischer reagent used in the titration. A Metrohm 870 KF Titrino Plus (Metrohm, Switzerland) with Metrohm 803 Ti Stand (Metrohm, Switzerland), calibrated with purified water and sodium tartrate dihydrate were used in the water determinations during this study.

3.10 Solubility studies

3.10.1 Solubility study A

Approximately 500 mg of each of the different roxithromycin preparations was weighed into five 20 ml amber test tubes with screw caps. 10 ml of distilled water was pipetted into each test tube. The test tubes were fixed to a rotating

axis (54 rpm) and submerged in a water bath, set at 37°C ±2°C. Since it is suggested that after 24 hours equilibrium of a solution should be reached, the solutions were then removed and filtered through 0.45 µm (Millipore®) filters to exclude all remaining solids. High performance liquid chromatography (HPLC) analysis was used to determine the concentration of the filtrates. A mobile phase of 30 g/L ammonium dihydrogen phosphate buffer was used and the pH adjusted to 5.3 with sodium hydroxide and acetonitrile. The ratio of buffer to acetonitrile for the mobile phase was 6:4. A Luna C_{18}, 150 mm x 4.6 mm column was used. The flow rate was 1.0 ml/min and the wavelength 205 nm. Validation of this method generated a linear regression of $R^2 = 0.9984$.

DSC, TGA, XRPD and IR analyses were done on the remaining sample powder in the test tubes, to determine if the samples had undergone transformation into the monohydrated form of roxithromycin after the 24 hours experimental run time.

3.10.2 Solubility study B

For this solubility study, 900 ml of distilled water was added to a 1,000 ml beaker and placed in a water bath, set at a temperature of 37°C ±2°C. An overhead stirrer was placed in the beaker and stirred at 200 rpm. Approximately 500 mg of roxithromycin glass was added to the beaker. 1 ml of the solution was extracted every 5 minutes for up to 105 minutes and filtered through 0.45 µm (Millipore®) filters to exclude all remaining solids. The concentrations of the filtrates were determined by HPLC analysis.

The study was repeated over a longer period of 435 minutes in order to investigate whether any monohydrate had formed over this extended period. Samples were extracted at given times as per table 3.5. With each extraction, IR analyses were done to determine if crystal form transformations had occurred.

The percentage of dissolved active was calculated by taking into account the concentration of the standard solution, as well as all the relevant dilution factors.

Table 3.5: Extraction times

Extraction no	Extraction times (min)
1	5
2	55
3	105
4	135
5	165
6	195
7	225
8	255
9	285
10	315
11	345
12	375
13	375
14	405
15	435

3.11 Powder dissolution

A powder dissolution study was performed on roxithromycin raw material (62206005RM) and roxithromycin glass powder (62206005GP), according to the method described by Lötter *et al.* (1983). 200 mg of each sample was separately added into 10 ml test tubes, to which 100 mg of accurately weighed glass beads was added. 3 ml of dissolution medium (distilled water) was withdrawn from each corresponding vessel and added to each test tube. The mixtures were agitated for 120 seconds, using a vortex mixer. The contents of the test tubes were then each transferred into the 500 ml dissolution medium of the corresponding vessels and the dissolution rates measured. This study was performed in triplicate.

Table 3.6: Dissolution parameters

Temperature	37°C
Volume	500 ml
Agitation	Paddles
Paddle speed	75 rates per minute (rpm)
Medium	Distilled water
Withdrawal times	7.5, 15, 30, 60 minutes, 4 hours, 7 hours, 24 hours

After each withdrawal, samples were filtered directly into accurately labelled HPLC vials through 0.45 µm (Millipore®) filters, to exclude remaining solids.

3.12 Stability studies

A Binder KBF 115 (GmBH, Germany) climate control chamber was used for the temperature controlled stability studies. Three stability studies were performed during this study, as discussed next.

3.12.1 Stability study at 40°C / 75% RH

Three forms of roxithromycin were exposed to 40°C and a relative humidity (RH) of 75%. The three forms were:

- Roxithromycin raw material (monohydrate);
- Amorphous (glassy) roxithromycin form; and
- Ground amorphous roxithromycin form (powder).

Samples were taken at onset (initial) and weekly thereafter over a period of one month (four weeks) and were analysed by DSC, TGA, IR, XRPD and Karl Fischer, in order to determine any possible changes caused by exposure to elevated temperature and humidity.

3.12.2 Stability study at 25°C / 75% RH and 30°C / 75% RH

Roxithromycin glass powder was further exposed to two more temperatures, i.e. 25°C and 30°C, both at 75% RH, in order to determine whether the form had remained stable over the period of four weeks at these lower temperatures.

Samples were also taken at onset (initial) and weekly thereafter over a period of one month, and were analysed only by DSC, TGA, IR and XRPD, to determine whether the forms had converted into the monohydrate (recrystallised) with exposure to elevated temperatures and relative humidity.

3.12.3 Stability study of desolvated chloroform and ethyl acetate forms at 40°C / 75% RH

Two desolvated forms of roxithromycin were exposed to 25°C / 75% RH. The two forms were:

- Roxithromycin chloroform desolvate (an amorphous form); and
- Roxithromycin ethyl acetate desolvate.

Samples were taken at onset (initial) and weekly thereafter over a period of one month, and were analysed by DSC, TGA, IR and XRPD, to determine any possible changes caused by the exposure to elevated temperature and humidity.

3.13 The effect of moisture on amorphous roxithromycin

According to Craig *et al. (*1999), if the concentration of water in a solid increases, the glass transition temperature (Tg) decreases and therefore, at any given temperature, the system may change from the glassy to the rubbery state, if water uptake takes place. In this study, different concentrations of water were added to the roxithromycin amorphous form to determine if moisture would have an effect on the glass transition temperature. The amounts of water added to

each roxithromycin glass sample are listed in table 3.7. After the addition of water, DSC and IR analyses were performed.

Table 3.7: Amount of water added to each roxithromycin glass sample

Sample no	Roxithromycin weight (g)	Water weight (g)
1	0.25	0.0025
2	0.25	0.0050
3	0.25	0.0075
4	0.25	0.0010
5	0.25	0.0125
10	0.25	0.025
20	0.25	0.050
30	0.25	0.075
40	0.25	0.100
50	0.25	0.125
60	0.25	0.150
70	0.25	0.175
80	0.25	0.200
90	0.25	0.225
100	0.25	0.250

3.14 Conclusion

The diversity of the analytical methods used in any solid state study is a crucial factor in obtaining useful and accurate test results. In this chapter, the various analytical methods and techniques that were employed during this polymorphic study were described.

In the next chapters, the test results are discussed, from which valuable conclusions regarding the amorphous forms of roxithromycin will be drawn.

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Chapter 4

Characterisation of polymorphic and amorphous forms of roxithromycin

4.1 Introduction

While many drugs exist in their crystalline state, the development of amorphous pharmaceutical solids has lately received much interest. Unlike crystalline solids, amorphous solids have no long range, molecular order, nor a well defined molecular conformation or structure. As expected, the properties of these amorphous solids, such as a higher solubility, enhanced chemical properties and water absorption, would differ from those of their crystalline counterparts (Byrn *et al.,* 1999).

As discussed in chapter 1, the preparation of amorphous solids is of high importance to the pharmaceutical industry, since they contain high amounts of free energy, which may enhance the solubility and dissolution of their otherwise poorly soluble, crystalline counterparts. Consequently, the absorption of these drugs from the gastrointestinal (GI) tract would be higher and therefore may lead to the enhanced bioavailability of an API. The utilisation of APIs in their amorphous states in solid oral dosage forms, have unfortunately been limited, as the amorphous form is thermodynamically unstable at elevated temperatures and tends to recrystallise spontaneously (Bhugra *et al.,* 2008).

For this study, therefore, the aim was to prepare an amorphous form of roxithromycin, which would be stable at elevated temperatures and which would demonstrate enhanced solubility and dissolution properties.

Functional terms used in this chapter:

1. Roxithromycin raw material is referred to as *monohydrate*;
2. Amorphous (glassy) roxithromycin is referred to as *glass*; and
3. Ground amorphous (glassy) roxithromycin is referred to as *glass powder*.

4.2 Characterisation of roxithromycin monohydrate

The standard analytical techniques, as discussed in chapter 3, were employed to study and characterise the solid state forms of roxithromycin. X-ray powder diffraction (XRPD) was especially useful in the characterisation of these crystalline and amorphous forms.

The roxithromycin monohydrate being used in this study was obtained from DB Fine Chemicals (South Africa), with batch number 62206005 and manufactured by Alembic Limited (API division).

4.2.1 Differential scanning calorimetry (DSC)

Figure 4.1 illustrates the DSC thermogram obtained for the roxithromycin monohydrate. A single melting endotherm was observed at 123.9°C. This data correlated well with studies previously conducted by Du Plessis (2004), Bawa (2007) and Aucamp (2009).

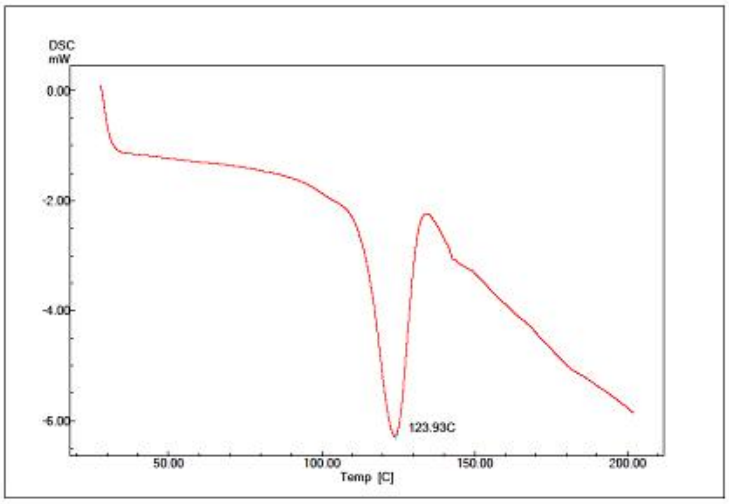


Figure 4.1: DSC thermogram of roxithromycin monohydrate.

4.2.2 Thermogravimetric analysis (TGA)

A theoretical weight loss of 2.1% is indicative of a monohydrated form, i.e. one molecule of water for every roxithromycin molecule. TG measurement indicated a weight loss of approximately 2.0%, which confirmed that the raw material used was a monohydrate (figure 4.2).

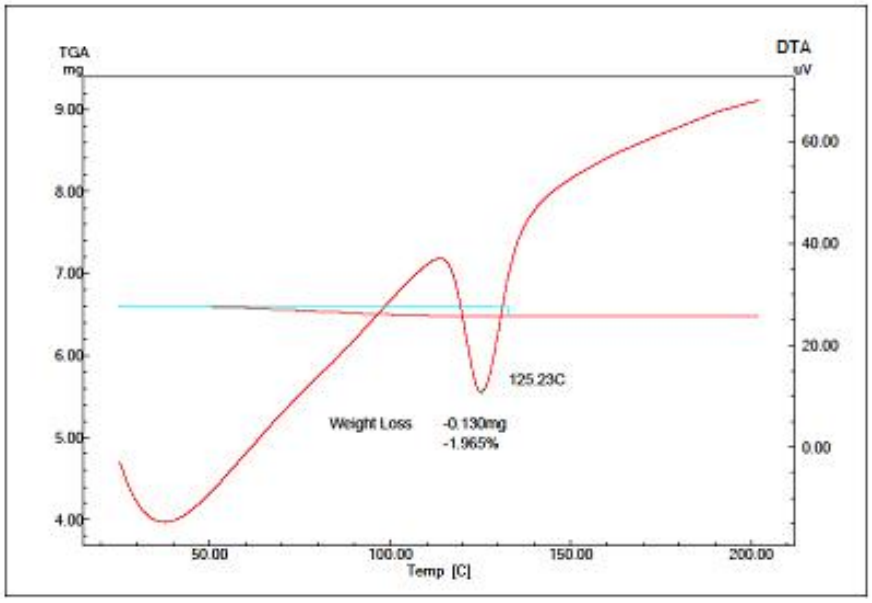
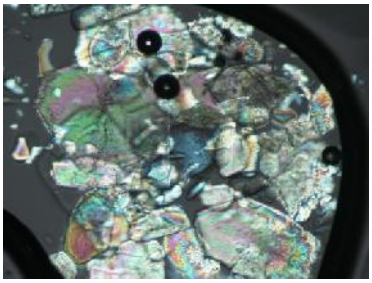
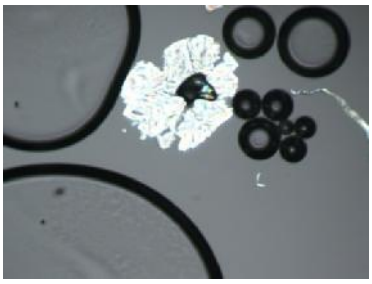
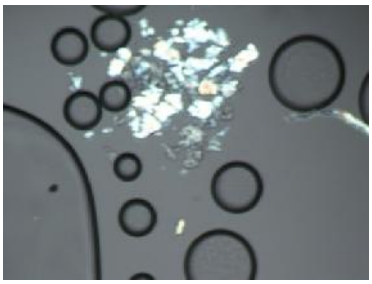


Figure 4.2: TGA thermogram of roxithromycin raw material (monohydrate).

4.2.3 Thermal microscopy (TM)

Thermal microscopy was used as a complementary technique to the other thermal analysis techniques. The micrographs depicted in table 4.1 confirmed the thermal results obtained with DSC and TG analyses. Table 4.1 illustrates the TM micrographs obtained for roxithromycin monohydrate.

Table 4.1: TM micrographs of roxithromycin monohydrate

	Dehydration starts at 121.0°C
	Dehydration and melting at 126.0°C
	End of dehydration and melting at 126.2°C

4.2.4 X-ray powder diffraction (XRPD)

The roxithromycin monohydrate exhibited a crystalline habit, which could be observed from the high intensity counts (715 000) of the X-ray powder diffractogram (figure 4.3). The peak positions and intensities are listed in table 4.2.

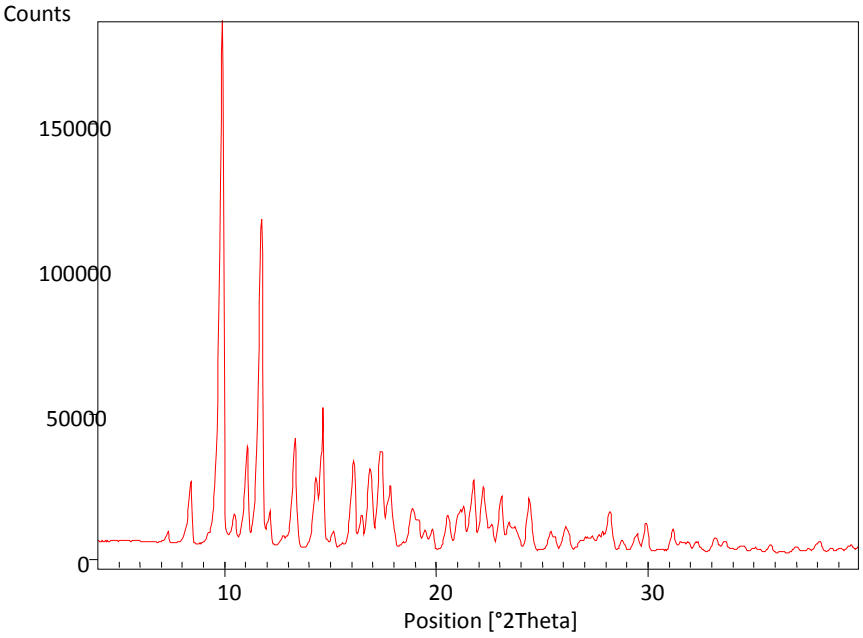


Figure 4.3: X-ray powder diffractogram of roxithromycin monohydrate.

Table 4.2: XRPD data for roxithromycin monohydrate

Position [°2θ]	Intensity [%]
8.4	12.5
9.9	100.0
11.1	18.0
11.7	62.6
13.3	21.2
14.3	13.1
14.6	27.4
16.1	15.7
16.8	13.9
17.4	17.6
17.8	11.3
21.7	11.3

4.2.5 Infrared spectroscopy (IR)

Infrared analysis of the roxithromycin monohydrate was performed, as described in paragraph 3.8. According to Gao *et al.* (2006), roxithromycin illustrates characteristic IR absorption peaks at 3472, 1735 and 1687 cm^{-1}. The IR spectrum (figure 4.4) obtained for the roxithromycin monohydrate correlated well with that stated in the literature (Gao *et al.*, 2006).

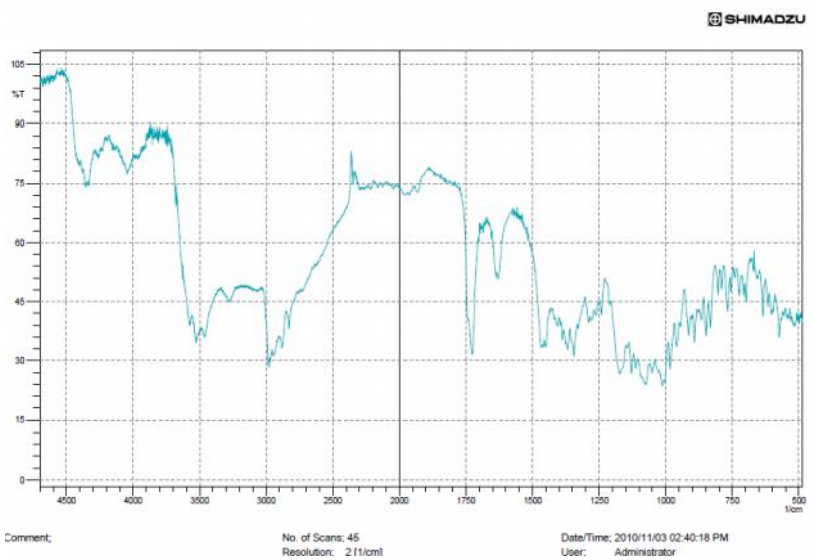


Figure 4.4: IR spectrum of roxithromycin monohydrate.

4.3 Roxithromycin glass

The glass form of roxithromycin was prepared during this study, as described in paragraph 3.2, through quench cooling from a melt.

4.3.1 Differential scanning calorimetry (DSC)

Figure 4.5 illustrates the DSC thermogram of the roxithromycin glass. The glass transition temperature (T_g) can be observed at approximately 89.9°C, which correlated well with the study by Aucamp (2009). This glass could be considered as a super cooled liquid above T_g and a glass below T_g. The reason being that no sign of a melting, nor a recrystallisation event was observed up to 200.0°C.

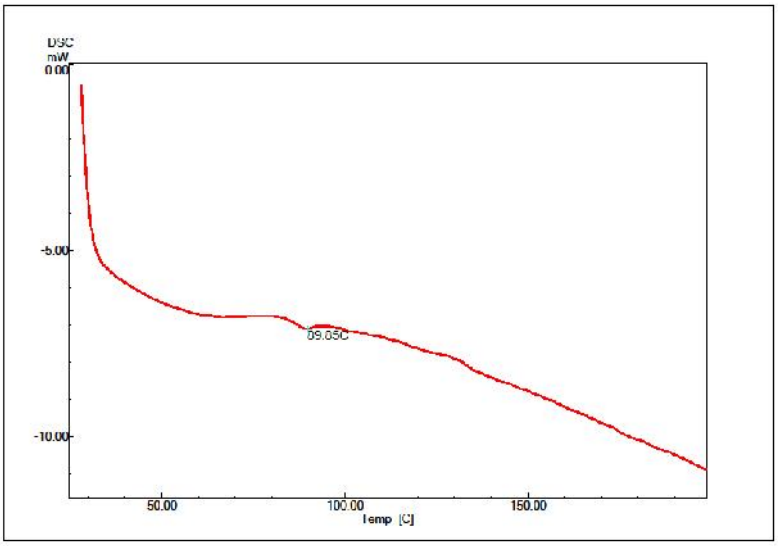


Figure 4.5: DSC thermogram of roxithromycin glass, prepared through quench cooling from a melt.

4.3.2 Thermogravimetric analysis (TGA)

The TGA trace of the glass showed a minimal weight loss of approximately 0.6% (figure 4.6), compared to the roxithromycin monohydrate having a weight loss of 2.0% (figure 4.2). A weight loss of only 0.6% indicated that the amorphous form being prepared was not a monohydrate.

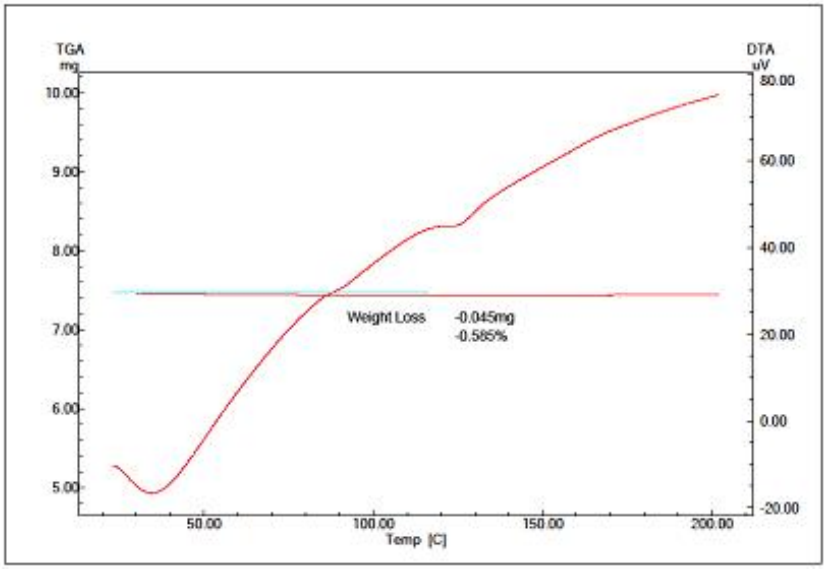
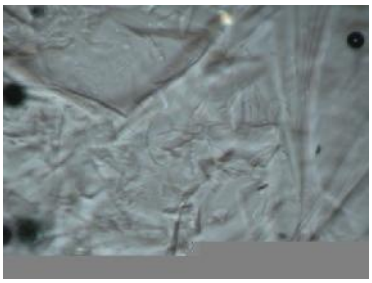
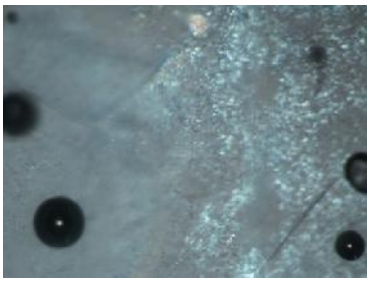


Figure 4.6: TGA thermogram of roxithromycin glass, prepared through quench cooling from a melt.

4.3.3 Thermal microscopy (TM)

In table 4.3, the TM micrographs of the roxithromycin glass, prepared through quench cooling from a melt, are illustrated.

Table 4.3: TM micrographs of roxithromycin glass

	Amorphous form at 25°C
	Glass transition at 89°C
	Melting / Degradation at 118°C

4.3.4 X-ray powder diffraction (XRPD)

With reference to the XRPD pattern of the roxithromycin monohydrate (figure 4.3), a clear difference in crystallinity was observed. Figure 4.7 illustrates the characteristic amorphous halo being obtained with the roxithromycin glass.

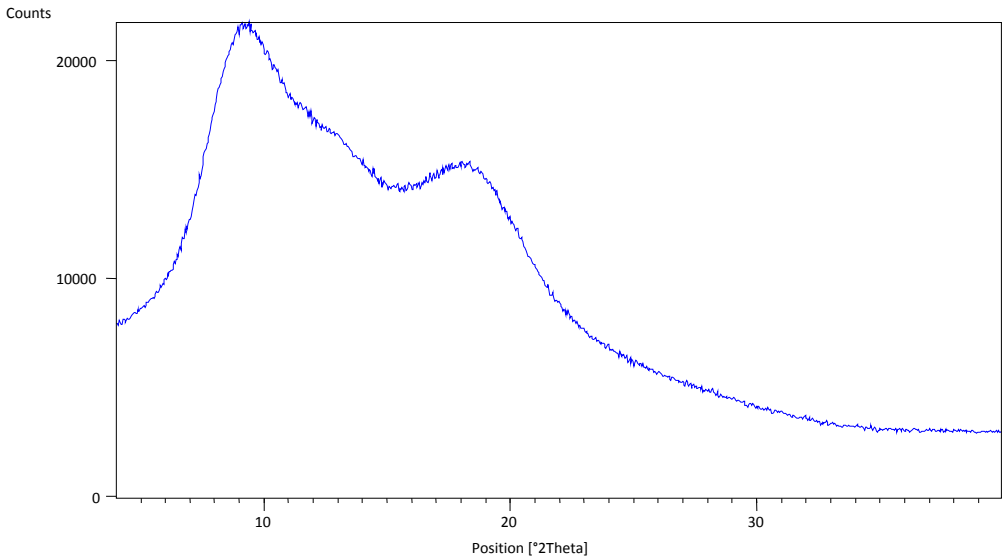


Figure 4.7: X-ray powder diffractogram of roxithromycin glass.

4.3.5 Infrared analyses (IR)

Infrared analysis of the roxithromycin glass was performed, as described in paragraph 3.8. As mentioned in paragraph 4.2.5, roxithromycin monohydrate illustrates a characteristic IR absorption peak at approximately 3472 cm^{-1}. Furthermore, literature states that the –OH stretching mode for free water in the gaseous phase has a characteristic peak at 3655 cm^{-1}. The frequency of this peak is, however, lowered when the water is condensed and/or bound (Hollenbeck, 2007). IR analysis, therefore, confirmed the characteristic –OH stretch in the area of 3472 – 3465 cm^{-1} for the roxithromycin monohydrate. Furthermore, if water is present in several states, it could be identified through the presence of multiple bands on the IR spectrum (Hollenbeck, 2007). From figure 4.9 it was evident that the prepared roxithromycin glass did not consist of any –OH stretch absorption peaks. Only one

characteristic peak at 3490 cm^{-1} was present, while the monohydrate raw material of roxithromycin presented –OH stretching bands at 3577, 3526 and 3465 cm^{-1}.

4.4 Ground roxithromycin glass powder

As described in paragraph 3.3, amorphous roxithromycin powder was prepared by grinding with a mortar and pestle to form a homogenous powder.

4.4.1 Differential scanning calorimetry (DSC)

Figure 4.8 illustrates the DSC thermogram of roxithromycin glass powder. When compared with the DSC trace (figure 4.5) of roxithromycin glass, no changes were observed.

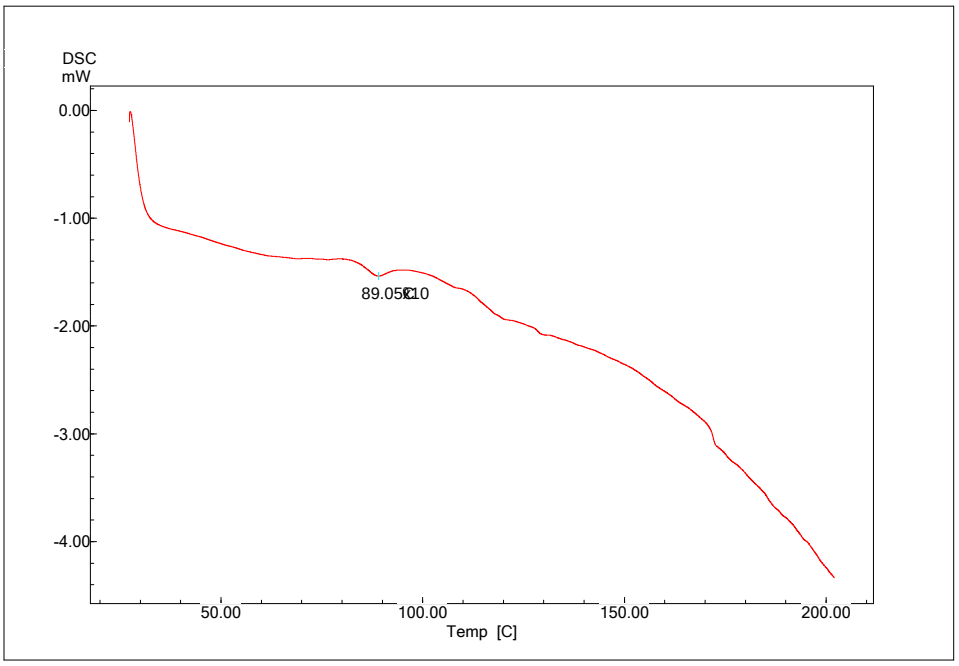
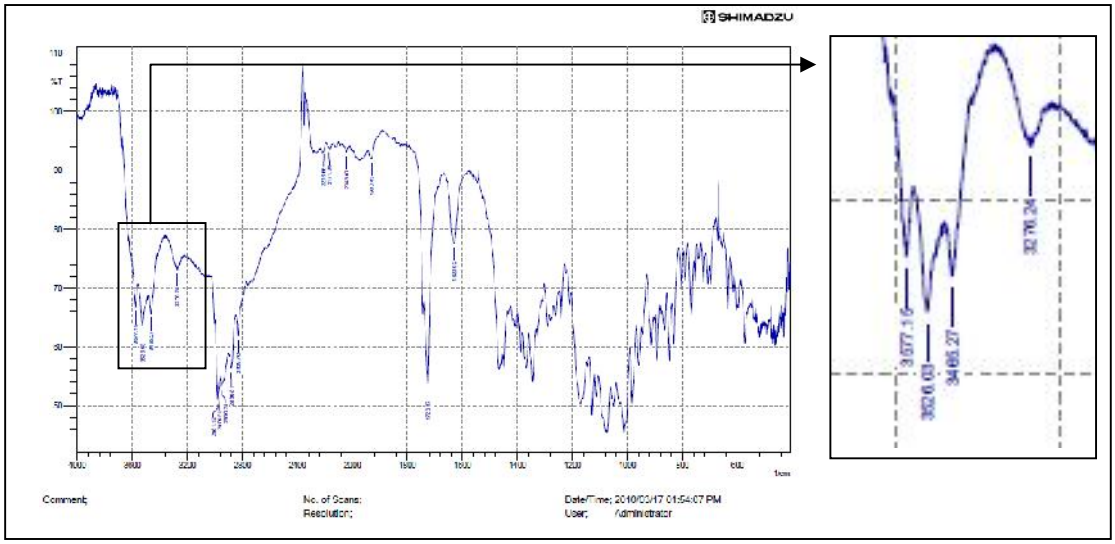


Figure 4.8: DSC thermogram of roxithromycin glass powder.

a)



b)

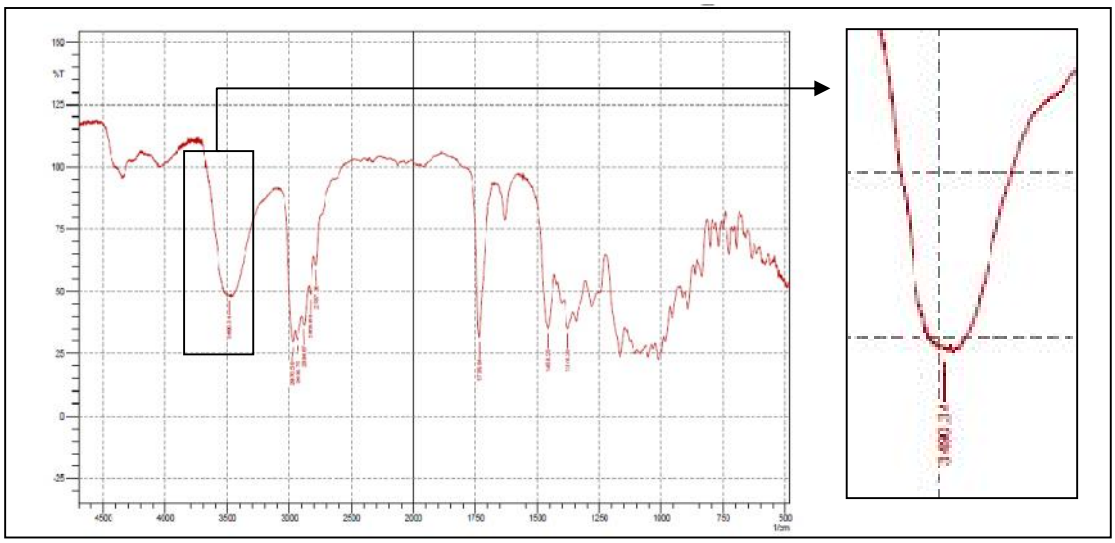


Figure 4.9: IR spectra of a) roxithromycin monohydrate and b) roxithromycin glass.

It therefore could be concluded that grinding of roxithromycin glass had no effect on the stability of the original glass form. The glass transition of the two forms was equal at ≈89°C.

4.4.2 Thermogravimetric analyses (TGA)

Figure 4.10 shows the TG trace of roxithromycin glass powder. In comparison with the roxithromycin monohydrate with a weight loss of 2.0%, the weight loss of 0.325% indicated that the roxithromycin glass powder was not a monohydrate. This low percentage weight loss probably represented adsorbed moisture from the atmosphere.

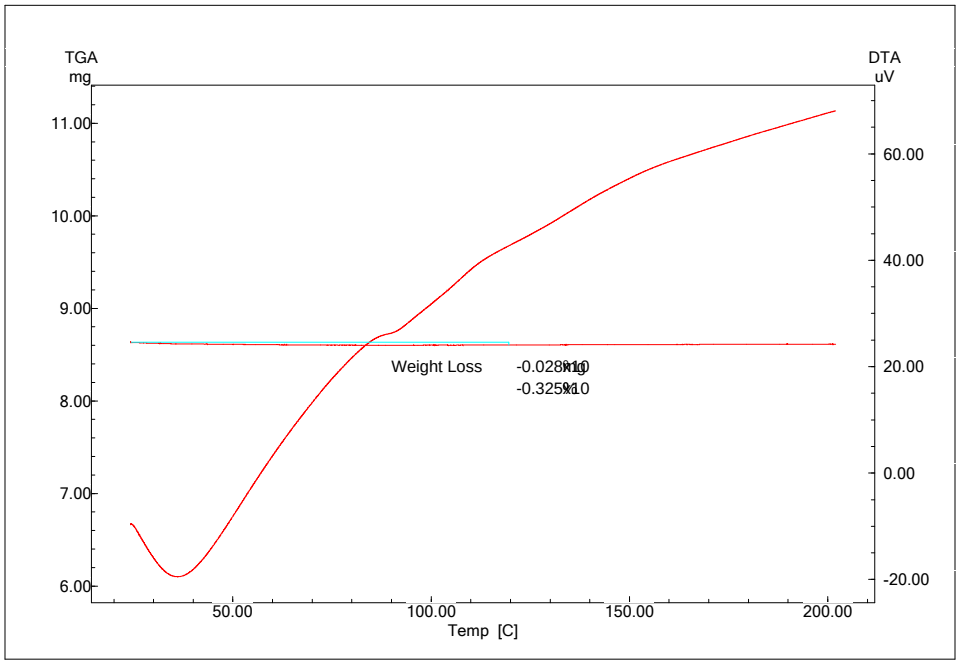


Figure 4.10: TGA thermogram of roxithromycin glass powder.

4.4.3 X-ray powder diffraction (XRPD)

A comparison between the roxithromycin monohydrate XRPD pattern (figure 4.3) and the amorphous halo of the roxithromycin glass powder (figure 4.11) showed differences in crystallinity. When compared with the roxithromycin glass form (figure 4.7), however, the halo shape of the powdered glass showed no change, and could it therefore be concluded that grinding of the glass had no effect on the stability of this amorphous form.

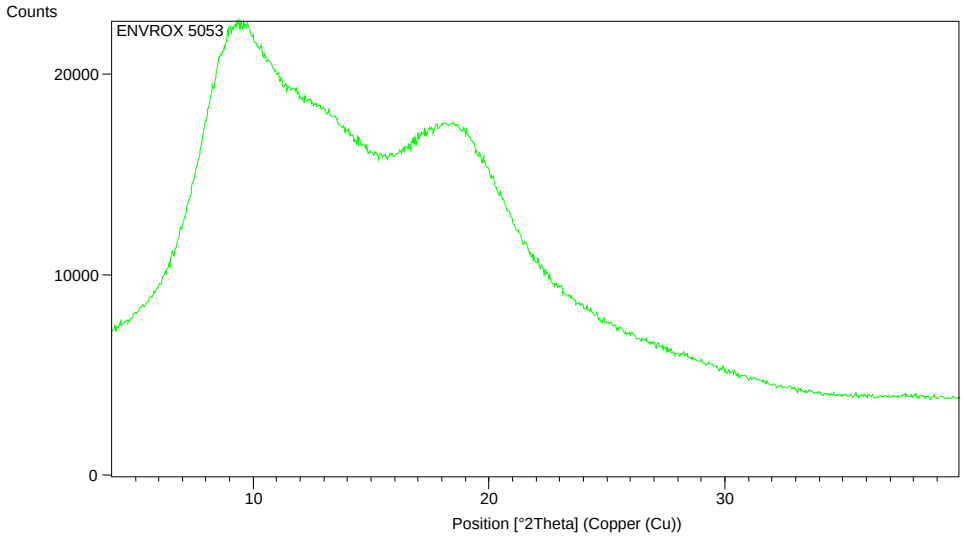


Figure 4.11: X-ray powder diffractogram of roxithromycin glass powder.

4.4.4 Infrared spectroscopy (IR)

IR analysis of roxithromycin glass powder was performed, as described in paragraph 3.8. As mentioned in paragraph 4.3.5, roxithromycin monohydrate illustrates a characteristic IR absorption peak at approximately 3472 cm^{-1}. Figure 4.12 clearly shows that this peak was absent in the roxithromycin glass powder and therefore it could be concluded that it was not a monohydrate.

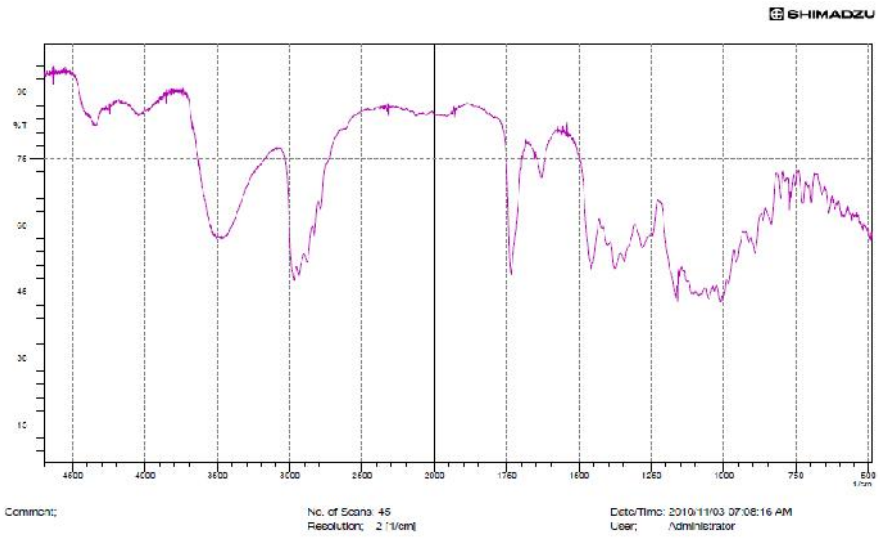


Figure 4.12: IR spectrum of roxithromycin glass powder.

4.5 Roxithromycin chloroform solvate

As described in paragraph 3.4, a roxithromycin solvate was formed through chloroform recrystallisation.

4.5.1 Differential scanning calorimetry (DSC)

Figure 4.13 illustrates the DSC thermogram of the roxithromycin chloroform solvate. Two endothermic peaks can be observed at approximately 58.0°C and 86.7°C. The chloroform solvate thus exhibited a much lower melting point (86.7°C) than the roxithromycin monohydrate (123.9°C).

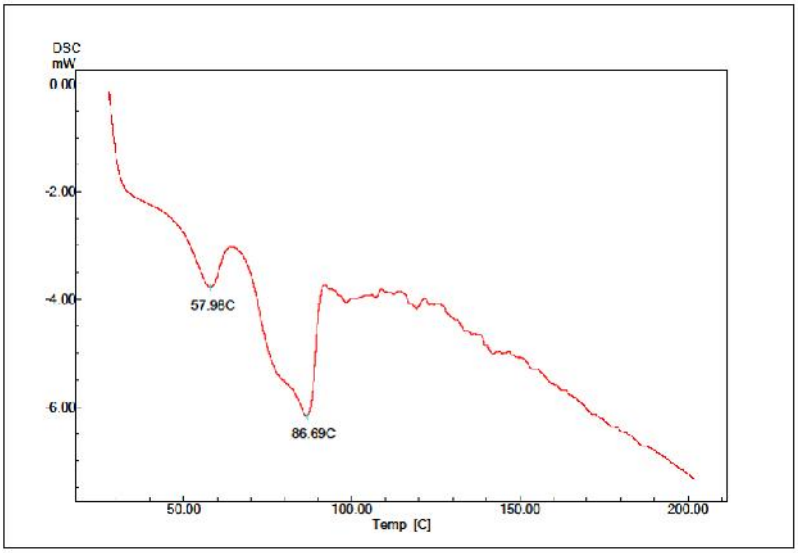


Figure 4.13: DSC thermogram of the roxithromycin chloroform solvate.

4.5.2 Thermogravimetric analysis (TGA)

A weight loss of approximately 10.8% was obtained during TG analysis (figure 4.14). For a 1:1 chloroform solvate, a theoretical weight loss of 12.5% was calculated. The lower value obtained could possibly be attributed to partial desolvation and overlapping of the desolvation and melting endothermic events, which made it difficult to accurately determine the weight loss percentage.

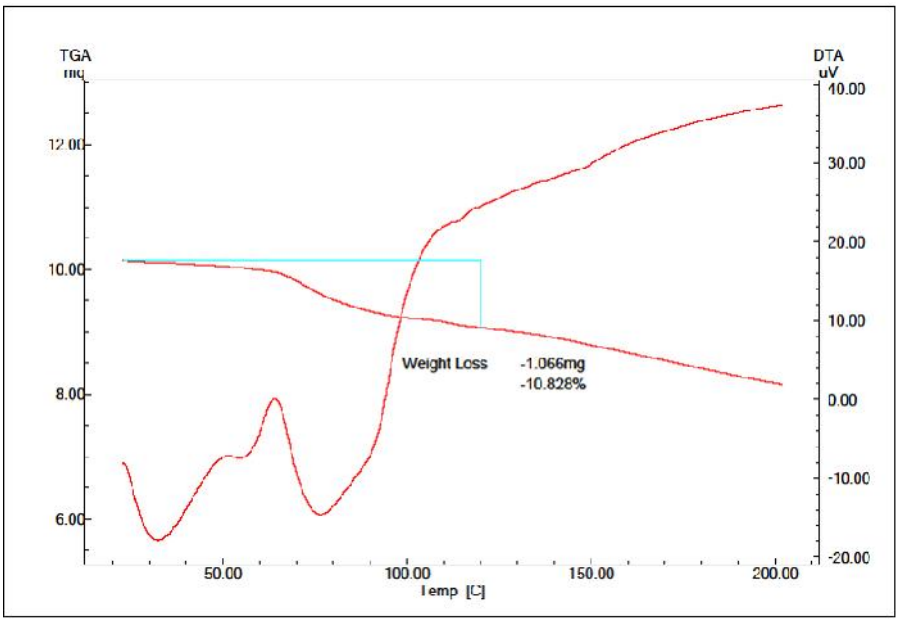
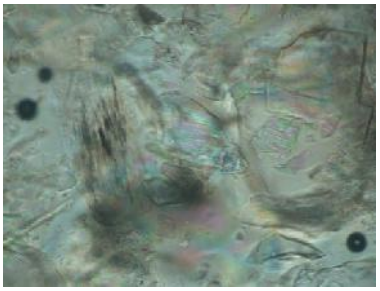
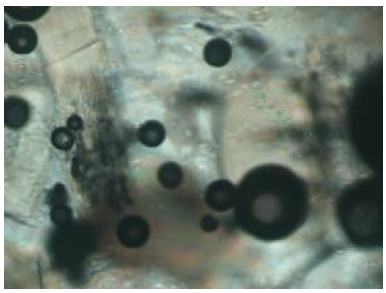


Figure 4.14: TGA thermogram of roxithromycin chloroform solvate.

4.5.3 Thermal microscopy (TM)

Table 4.4 illustrates the images obtained during TM analysis of the chloroform solvate.

Table 4.4: TM micrographs of roxithromycin chloroform solvate

	Amorphous chloroform solvate at 25.0°C
	Starting of desolvation at 56.0°C
	End of desolvation and melting at 82.0°C

4.5.4 X-ray powder diffraction (XRPD)

XRPD traces were obtained, as described in paragraph 3.7. The roxithromycin chloroform solvate had a distinct pattern, but was not as crystalline as the monohydrate. Figure 4.15 represents the diffractogram, whereas the peak angles are listed in table 4.5.

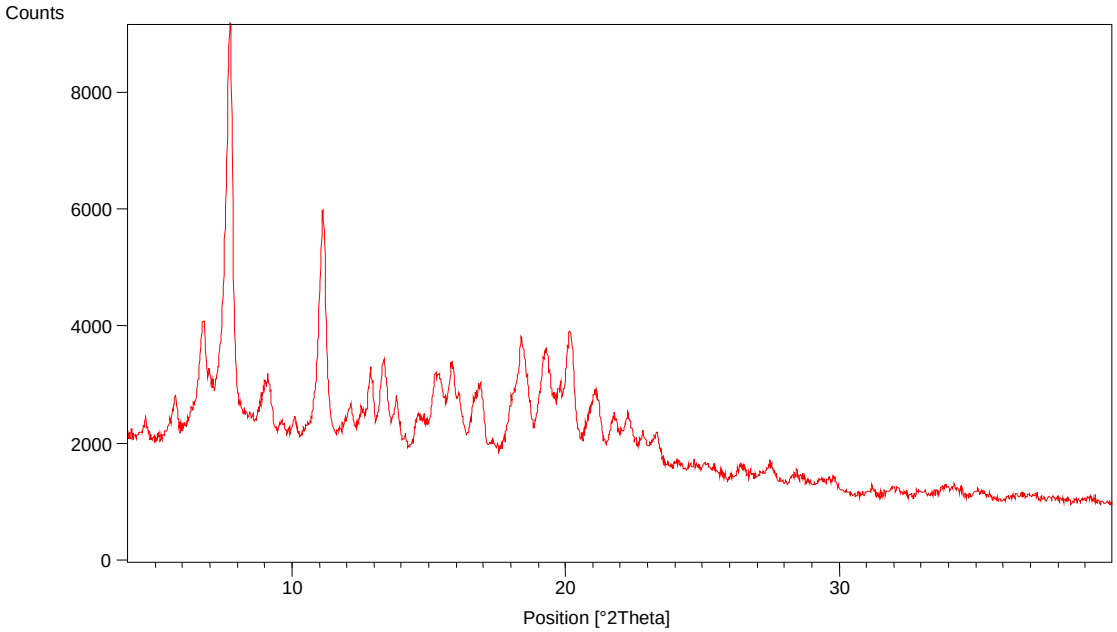


Figure 4.15: X-ray powder diffractogram of the roxithromycin chloroform solvate.

Table 4.5: XRPD data for roxithromycin chloroform solvate

Position [°Th]	Intensity [%]
6.8	24.1
7.7	100.0
9.1	11.2
12.8	16.0
13.3	19.3
13.8	11.4
15.2	15.6
15.8	19.1
16.9	25.3
18.3	13.1
19.2	20.8
20.1	25.5
35.1	56.0

4.5.5 Infrared spectroscopy (IR)

The IR spectrum of roxithromycin chloroform solvate is illustrated in figure 4.16. It shows no absorption peaks in the 3472 cm^{-1} region. It could therefore be concluded that this form was not a monohydrate.

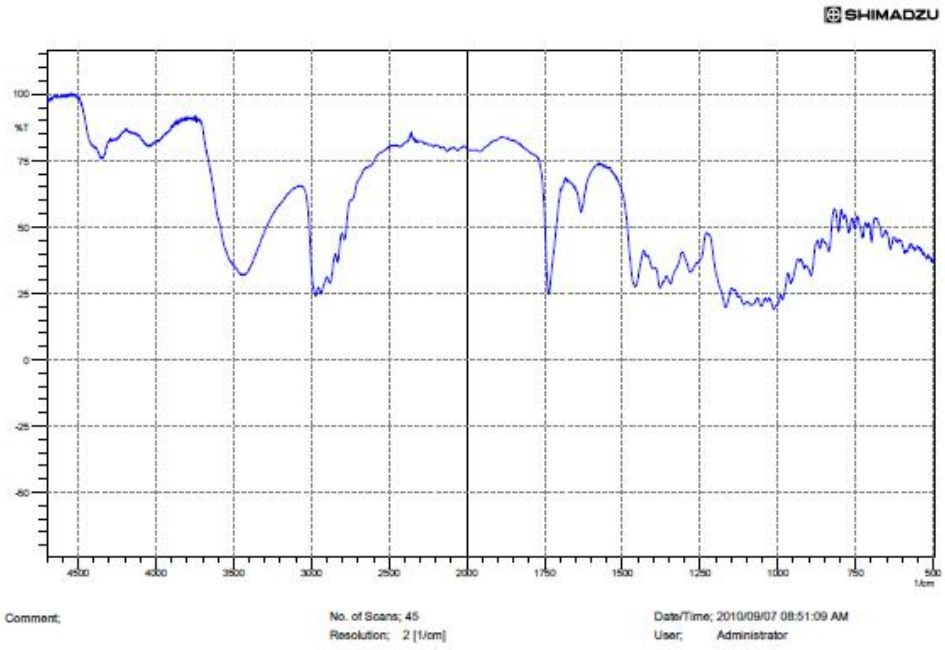


Figure 4.16: IR spectrum of roxithromycin chloroform solvate.

4.6 Roxithromycin chloroform desolvate

By placing the roxithromycin chloroform solvate in a Binder® (GmBH, Germany) oven at 50.0°C for five days, desolvation of the chloroform solvate took place and subsequently an amorphous form was formed.

4.6.1 Differential scanning calorimetry (DSC)

The DSC trace of the roxithromycin desolvated form differed from that of the roxithromycin chloroform solvate. The desolvation endotherm at 58.0°C was absent, whilst a shift in the melting point (79.3°C) (86.7°C for roxithromycin chloroform solvate) was also observed. Figure 4.17 shows an overlay of the roxithromycin chloroform solvate and the roxithromycin chloroform desolvate DSC thermograms.

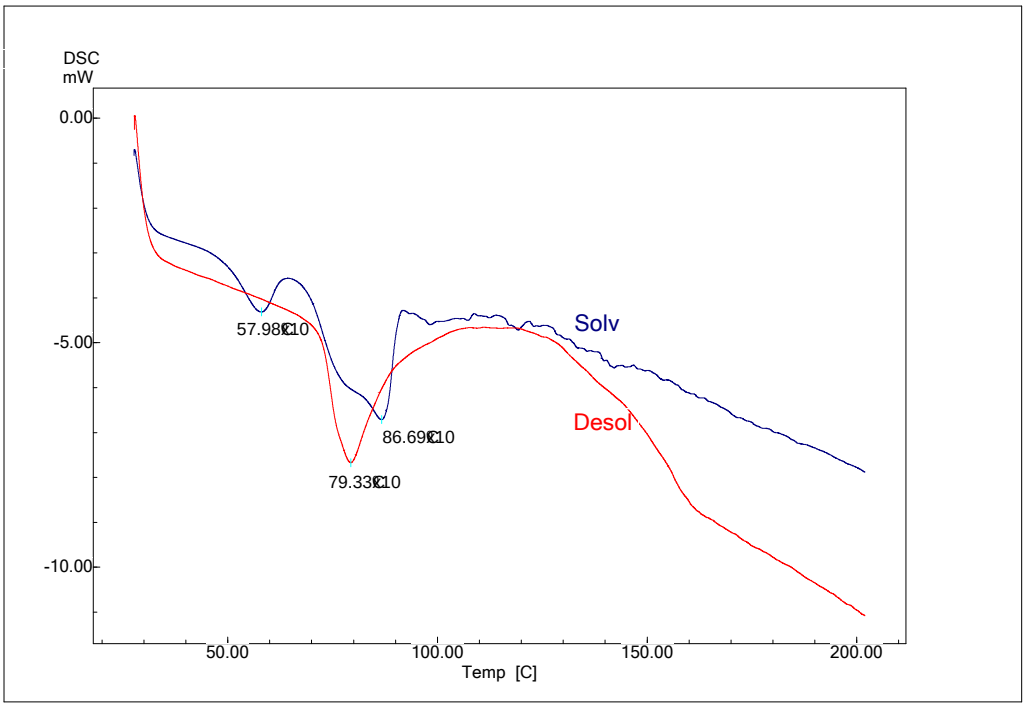


Figure 4.17: Overlay of DSC thermograms of the roxithromycin chloroform solvate (blue) and desolvate (red).

4.6.2 Thermogravimetric analysis (TGA)

The TGA trace showed that complete desolvation was achieved. The weight loss observed was ±2.0%, which showed that the desolvate had transformed into a monohydrate after the desolvation process.

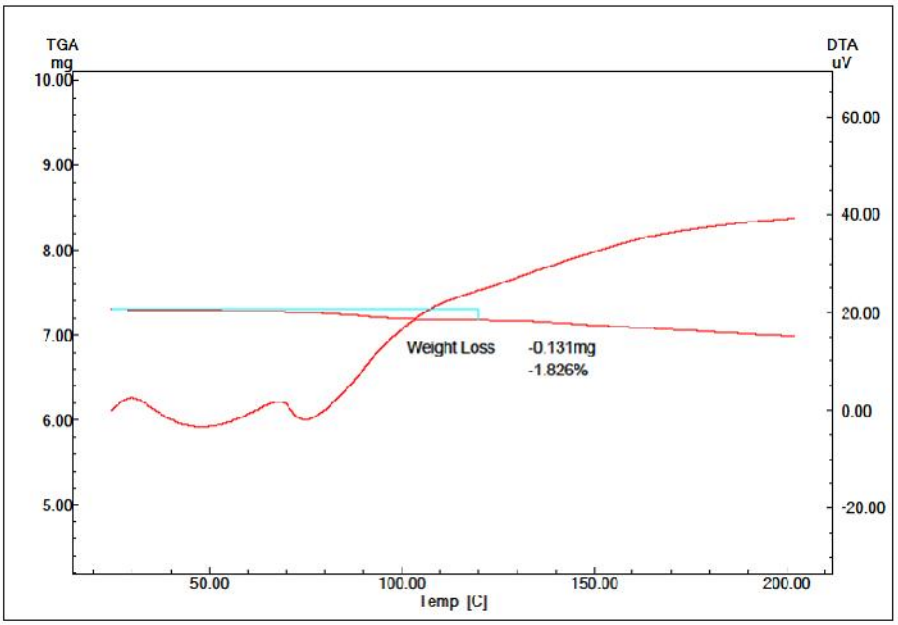


Figure 4.18: TGA thermogram of roxithromycin chloroform desolvate.

4.6.3 X-ray powder diffraction (XRPD)

The X-ray powder diffractogram displayed a halo shape, typical of an amorphous material (figure 4.19).

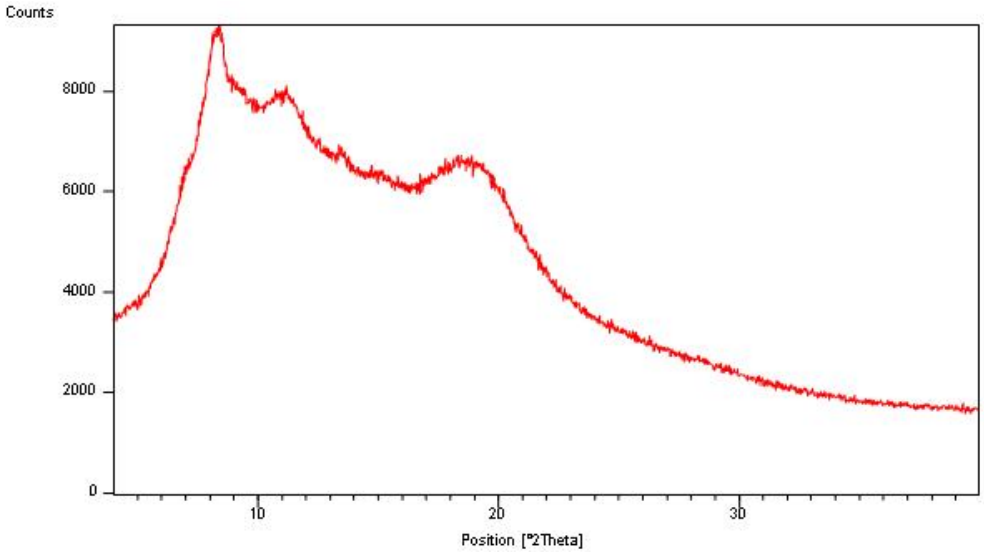


Figure 4.19: X-ray powder diffractogram of roxithromycin chloroform desolvate.

4.6.4 Infrared spectroscopy (IR)

There were no significant differences between the IR spectra of the chloroform solvate and the desolvate. Figure 4.20 illustrates the IR spectrum of the roxithromycin chloroform amorphous desolvate. No absorption peaks at 3742 cm^{-1} were identified. The absorption peak at approximately 3500 cm^{-1} seemed to be characteristic of anhydrous roxithromycin, since it correlated well with the absorption peaks present in the IR spectra of the amorphous chloroform solvate (figure 4.16), roxithromycin glass (figure 4.9) and roxithromycin glass powder (figure 4.12).

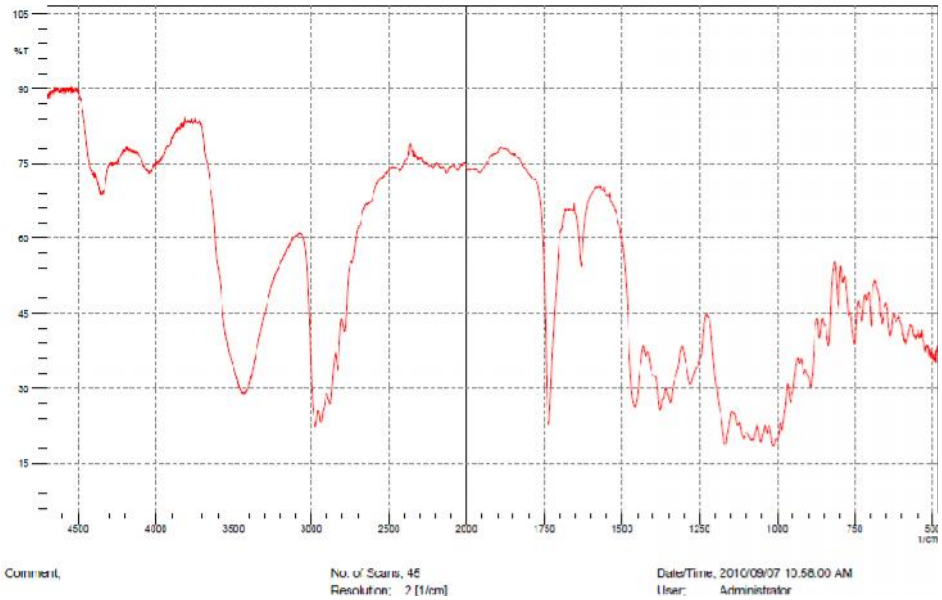


Figure 4.20: IR spectrum of amorphous roxithromycin chloroform desolvate.

4.7 Roxithromycin ethyl acetate solvate

As described in paragraph 3.4, a solvate was formed through ethyl acetate recrystallisation. According to literature, roxithromycin exhibits solvent exchange when being recrystallised in organic solvents. It was especially true during an acetonitrile recrystallising study performed by Mallet *et al.* (2003),

where acetonitrile and water exchanged within the crystal structure of roxithromycin. It may be possible that the same could occur with ethyl acetate.

4.7.1 Differential scanning calorimetry (DSC)

Figure 4.21 illustrates the DSC thermogram obtained for roxithromycin ethyl acetate solvate. A single melting and desolvation endotherm was observed at 88.2°C.

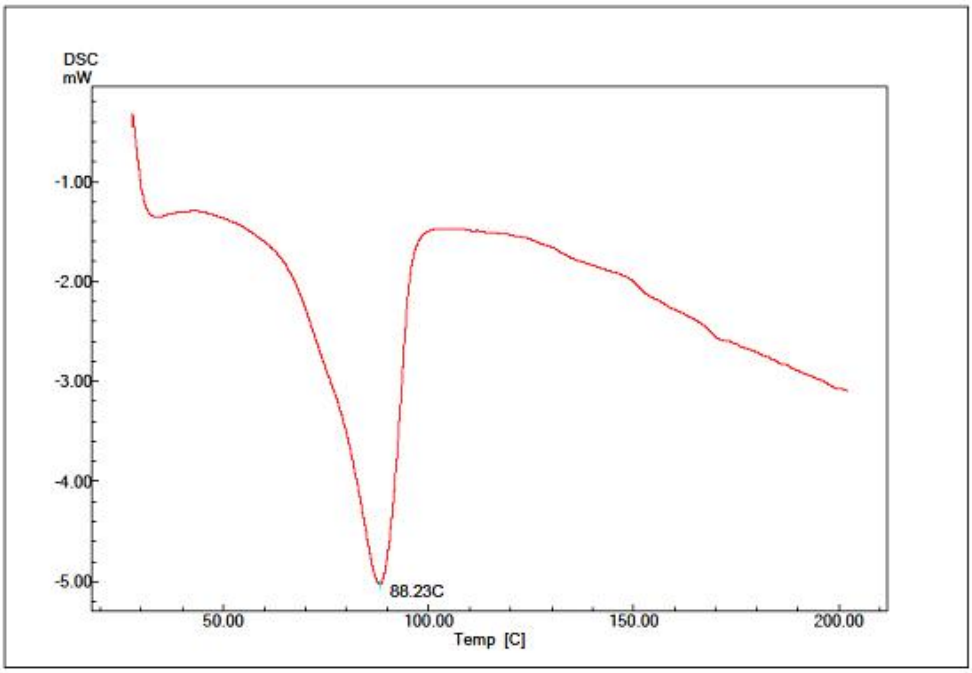


Figure 4.21: DSC thermogram of roxithromycin ethyl acetate solvate.

4.7.2 Thermogravimetric analysis (TGA)

As illustrated in figure 4.22 the thermogravimetric analysis showed a total weight loss of 6.5%. By calculating the weight loss over both thermal events, a theoretical 1:1 weight loss for an ethyl acetate solvate would result in 9.5%. According to Aucamp (2009), it seemed that desolvation / dehydration and melting occurred simultaneously, which explained the lower thermogravimetric weight loss value that had been obtained. The heating rate was maintained at 10°C/min. According to Aucamp (2009), the percentage weight loss being calculated for the first thermal

event was 2.0%. Karl Fischer analyses performed by Aucamp (2009), resulted in a water percentage of 1.8%, indicative of the presence of a monohydrate.

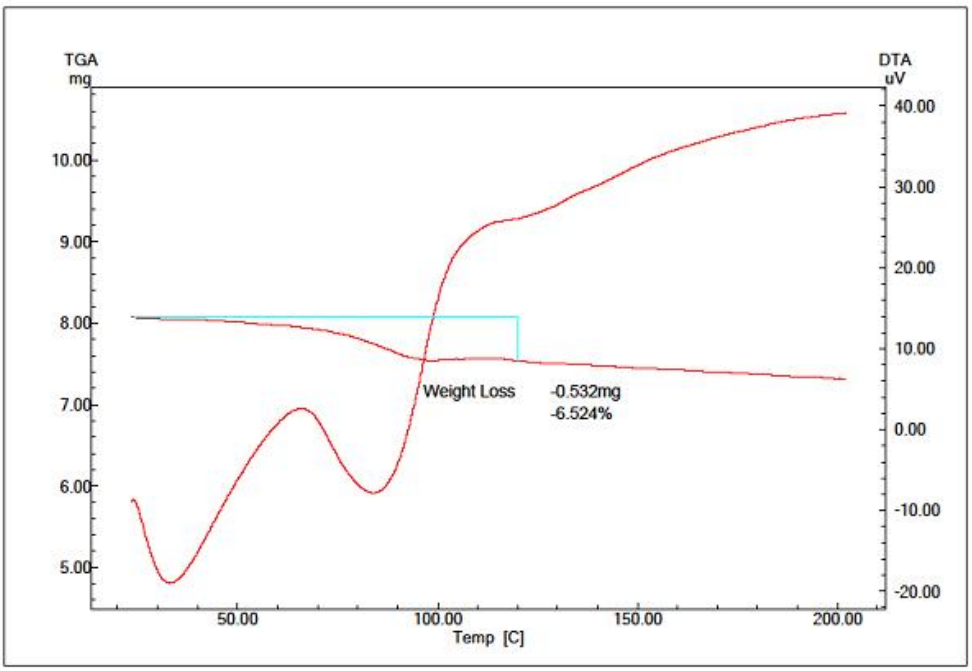
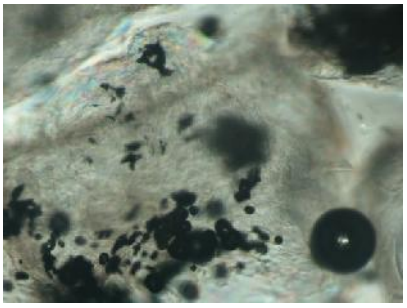
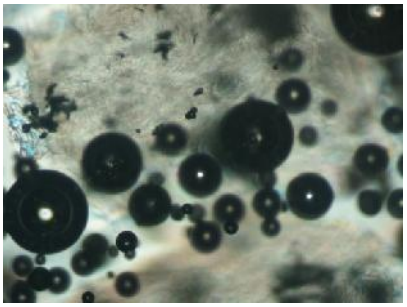


Figure 4.22: TGA thermogram for roxithromycin ethyl acetate solvate.

4.7.3 Thermal microscopy (TM)

Thermal microscopy (table 4.6) showed the evolution of air bubbles at a temperature of 85.0°C, indicative of desolvation or dehydration. This correlated well with the fact that the TG analysis also exhibited desolvation or dehydration (figure 4.22). The micrograph generated at 88.0°C confirmed the DSC trace, as shown in figure 4.21.

Table 4.6: TM micrographs of roxithromycin ethyl acetate solvate

	Amorphous ethyl acetate solvate at 25.0°C
	Dehydration / desolvation at 85.0°C
	Melting at 88.0°C

4.7.4 X-ray powder diffraction (XRPD)

XRPD traces were generated, as described in paragraph 3.7. Although the roxithromycin ethyl acetate solvate had a distinct pattern, it was not as crystalline as the raw material. The diffractogram being obtained for roxithromycin monohydrate showed an intensity count of more than 150 000, while the diffractogram of the roxithromycin ethyl acetate solvate showed an intensity count of only 20 000. Thus, although this form was a solvate, it was still more amorphous than the roxithromycin monohydrate. The diffractogram is shown in figure 4.23, whereas the peak angles are listed in table 4.6.

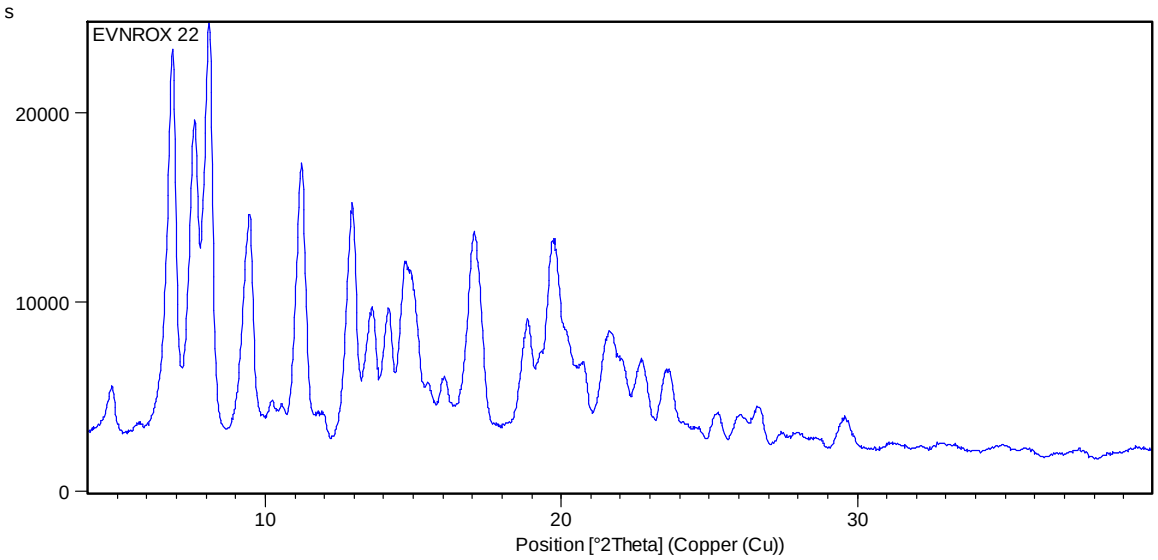


Figure 4.23: X-ray powder diffractogram of roxithromycin ethyl acetate solvate.

Table 4.7: XRPD data for roxithromycin ethyl acetate solvate

Position [°Th]	Intensity [%]
4.8	11.9
6.9	84.9
7.6	77.3
8.1	100.0
9.3	44.7
9.5	51.4
11.3	65.1
12.9	56.2
13.6	27.7
14.1	25.6
14.7	35.6
14.9	33.5
17.0	47.4
17.3	31.3
18.9	25.0
19.9	38.9
20.8	11.8
21.5	15.2
21.7	19.7

4.7.5 Infrared spectroscopy (IR)

The infrared spectrum of the roxithromycin ethyl acetate solvate differed from that of the monohydrate. The unique peak at 3742 cm^{-1}, indicative of the roxithromycin monohydrate, was absent. The spectrum is shown in figure 4.24.

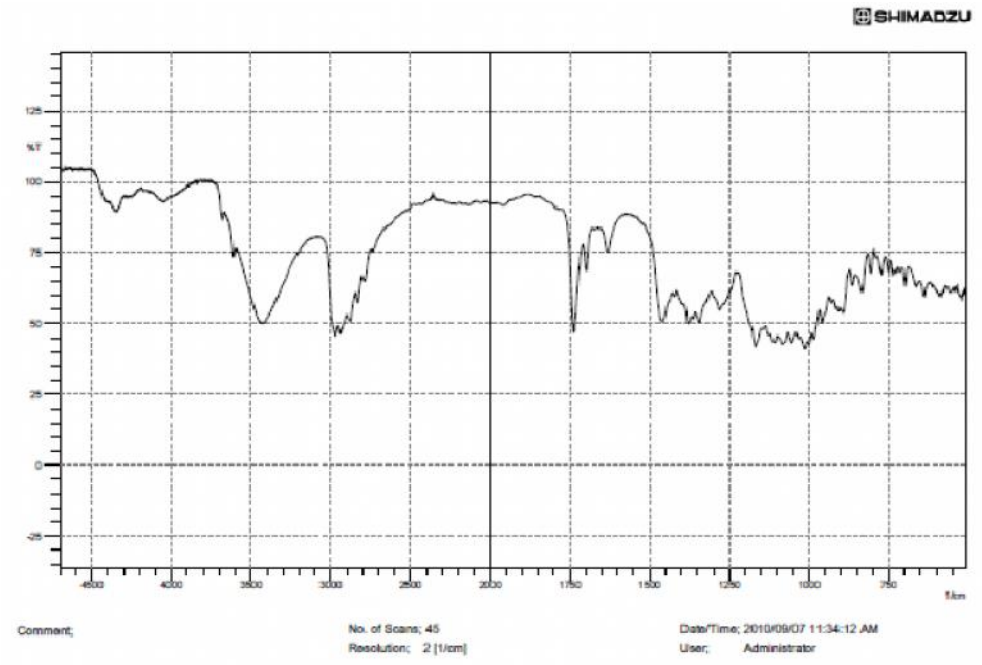


Figure 4.24: IR spectrum of roxithromycin ethyl acetate solvate.

4.8 Roxithromycin ethyl acetate desolvate

By placing the ethyl acetate solvate in a Binder® (GmBH, Germany) oven at 50°C for five days, desolvation of the ethyl acetate solvate took place to form a desolvated solvate. A desolvated solvate refers to a compound that is crystallised as a solvate, by undergoing desolvation prior to analysis. The structure of the solvate has small changes with regards to parameters and atomic coordinates, but contains no solvent. Desolvated solvates tend to be less crystalline than their crystalline counterparts. This occurrence can be identified by X-ray powder diffraction (Byrn *et al.,* 1995).

4.8.1 Differential scanning calorimetry (DSC)

The DSC overlay of the roxithromycin ethyl acetate solvate and desolvate (figure 4.25) showed that the melting point shifted from 88.2°C to 95.6°C. The endotherm of the desolvate was also sharper than that of the solvate.

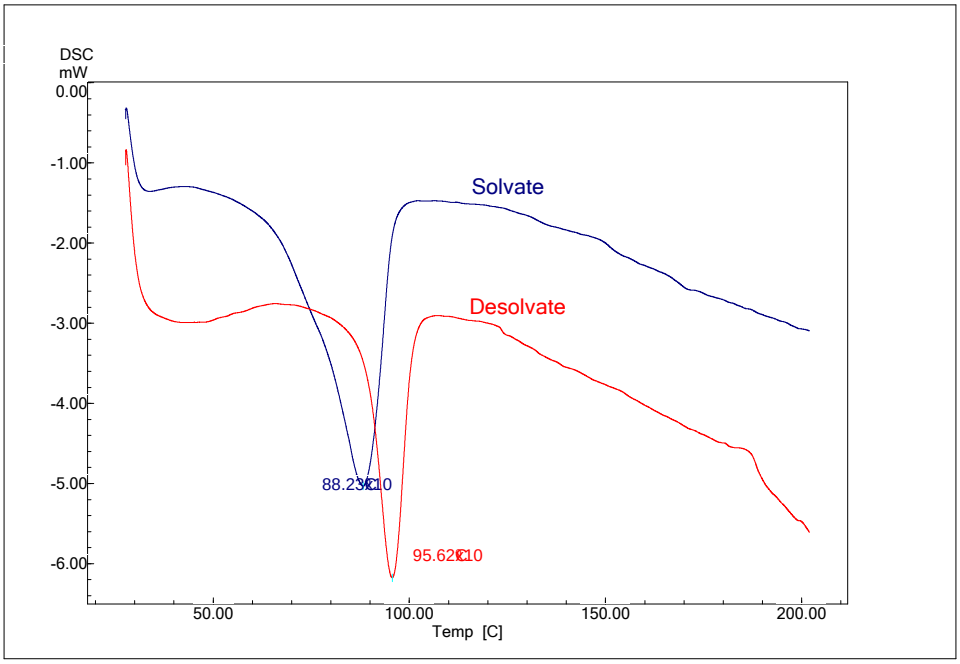


Figure 4.25: Overlay of DSC thermograms of the roxithromycin ethyl acetate solvate (blue) and roxithromycin desolvate (red).

4.8.2 Thermogravimetric analysis (TGA)

The TGA trace (figure 4.26) showed that complete desolvation was achieved. The weight loss being observed was ±1.0%.

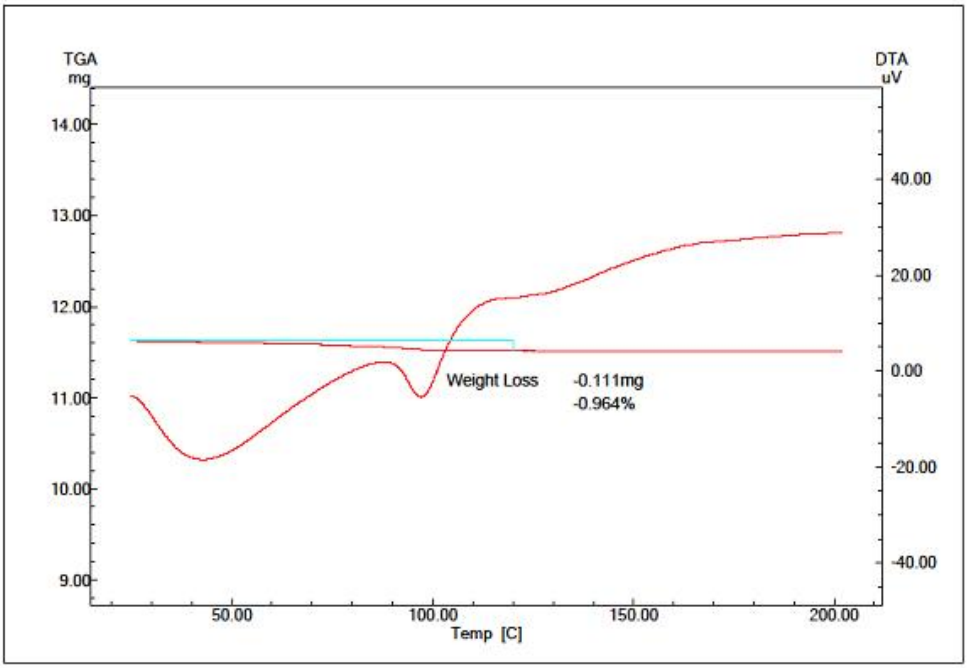


Figure 4.26: TGA thermogram of roxithromycin ethyl acetate desolvate.

4.8.3 X-ray powder diffraction (XRPD)

The X-ray powder diffractogram of the ethyl acetate desolvate is illustrated in figure 4.27, whereas the diffraction angles with relative intensities are listed in table 4.7. A more amorphous content was visible in this diffractogram, with the intensity of the peaks being lower, whilst in comparison with the solvate, some peaks also disappeared. An overlay of the ethyl acetate solvate and desolvate diffractograms is shown in figure 4.28. Byrn (1995) reported that desolvated solvates tended to be less crystalline than their crystalline counterparts, as was confirmed by thus study with regards to the desolvation of the solvate.

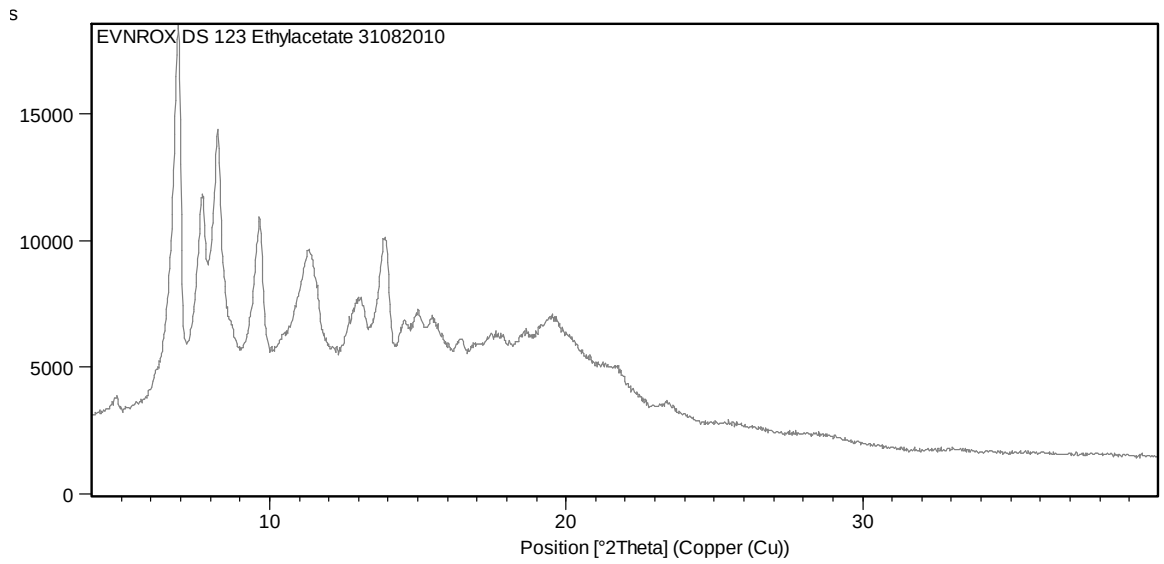


Figure 4.27: X-ray powder diffractogram of roxithromycin ethyl acetate desolvate.

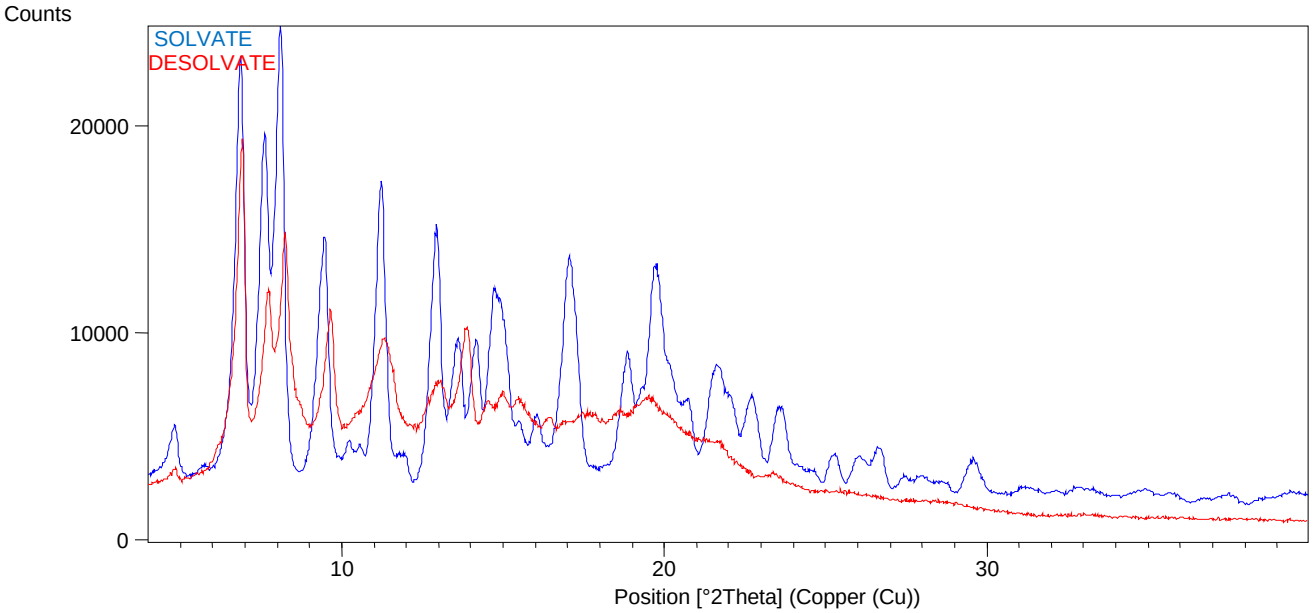


Figure 4.28: Overlay of X-ray powder diffractograms of roxithromycin ethyl acetate solvate and desolvate.

Table 4.8: XRPD data for roxithromycin ethyl acetate desolvate

Position [°Th]	Intensity [%]
6.9	100.00
7.7	49.05
8.2	67.19
9.6	38.97
11.3	28.78
13.0	14.62
13.8	28.22
13.9	31.98

4.8.4 Infrared spectroscopy (IR)

The IR spectrum of the ethyl acetate desolvate is illustrated in figure 4.29. An overlay of the IR spectra of the roxithromycin ethyl acetate solvate and desolvate, as shown in figure 4.30, showed no significant differences.

An overlay of all the solid forms being prepared and discussed in this chapter is illustrated in figure 4.31. The differences and similarities are clearly evident. The IR spectrum of the raw material had the unique characteristic peak at 3742 cm^{-1}, indicative of a monohydrate. The IR spectra of the roxithromycin glass, chloroform desolvate and ethyl acetate desolvate were almost identical, and represented an IR spectrum, typical of an amorphous, anhydrous roxithromycin.

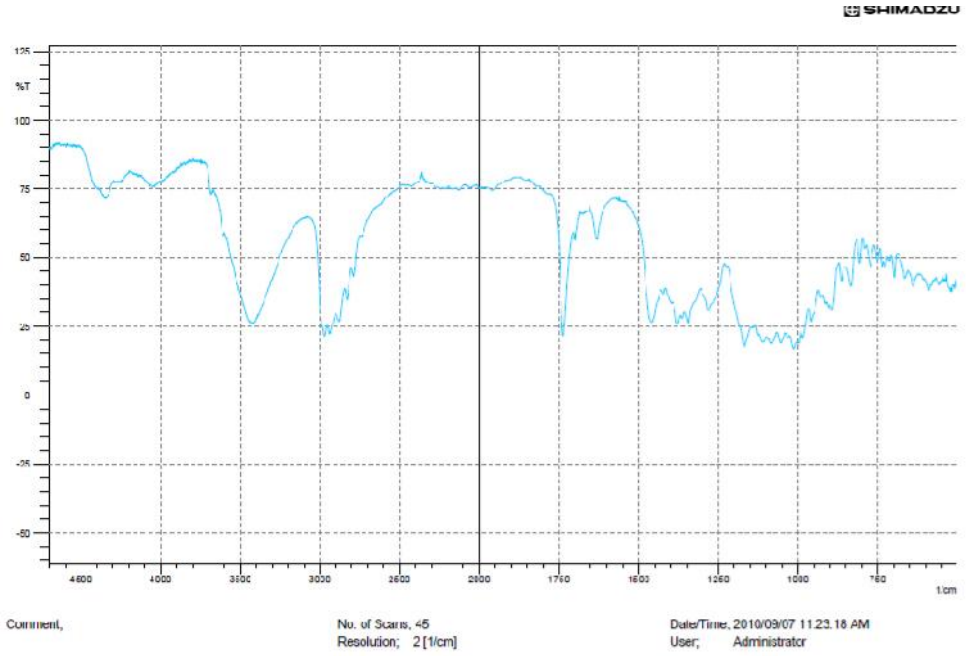


Figure 4.29: IR spectrum of roxithromycin ethyl acetate desolvate.

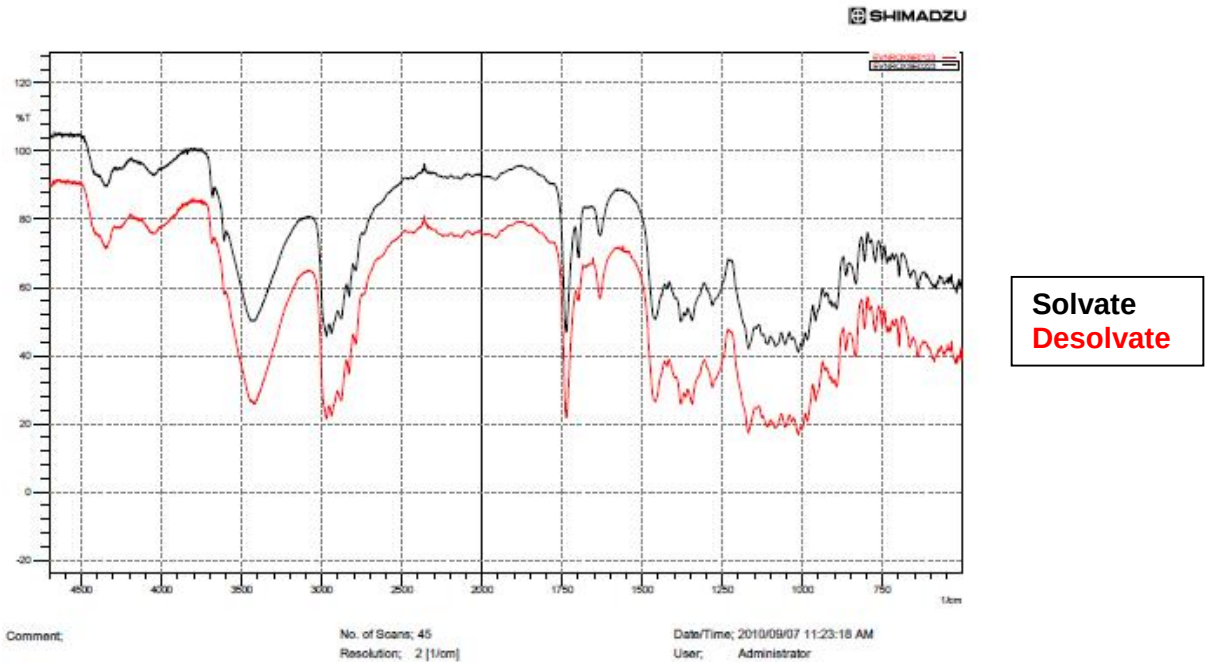


Figure 4.30: Overlay of the IR spectra of roxithromycin ethyl acetate solvate and desolvate.

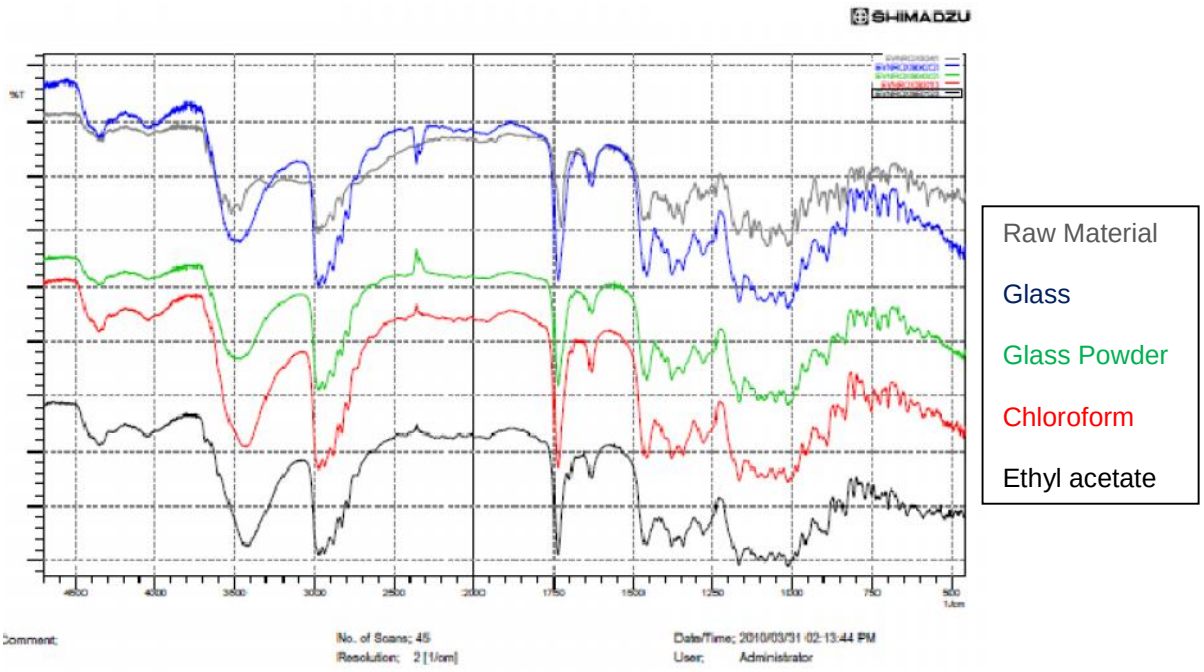


Figure 4.31: Overlay of IR spectra of the roxithromycin monohydrate, glass, glass powder, chloroform desolvate and ethyl acetate desolvate.

4.9 Conclusion

In this chapter, the different forms of roxithromycin that were prepared and characterised, were discussed. Most of the forms were found to be amorphous.

Two methods were used to prepare the three amorphous forms of roxithromycin. The first method was through quench cooling from a melt to obtain the amorphous glass form. This glass form was then ground to prepare a consistent, amorphous glass powder.

Secondly, roxithromycin chloroform and ethyl acetate solvates were prepared through recrystallisation. These solvates were then desolvated by placing the roxithromycin ethyl acetate and chloroform solvates in an oven for a few days. Complete desolvation was achieved and solid amorphous masses with no single crystals were obtained. These roxithromycin ethyl acetate solvate and desolvate were, however, more crystalline than the recrystallisation and desolvation products from chloroform.

In this chapter, the distinct differences between the roxithromycin monohydrate and the amorphous forms being prepared, were thus discussed. The solvates were more crystalline than their desolvated counterparts.

The observed differences in physico-chemical properties between the roxithromycin monohydrate and the glass forms could impact on their stability and solubility properties. In the next chapter, the outcomes from the stability and solubility investigations being performed on the different roxithromycin forms, prepared during this study, are discussed.

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Chapter 5

Stability studies of roxithromycin amorphous forms

5.1 Introduction

All things made by man have the potential to decompose with time and this includes pharmaceutical products (Rhodes, 2007). This statement is especially relevant to the quality and safety of pharmaceutical products. It is thus imperative that each drug undergoes some form of stability testing to assure its safety, efficacy and quality over a given time. Since the decomposition rate of every product differs, drug stability and shelf life studies are essential.

Some of the adverse effects which may occur if instability of a drug exists include loss / degradation of the active, variance in the content uniformity and appearance, rising concentration of the active and changes in bioavailability of the API. Stability testing is therefore important for patient safety, for maintaining a database for the formulation of future products and for protecting the manufacturer (Rhodes, 2007).

The importance of this chapter was to determine whether the amorphous forms being prepared during this study were thermodynamically stable. Bhugra and Pikal (2008) claim that, normally, amorphous forms are found to be thermodynamically unstable and tend to revert to the more stable, crystalline state.

5.2 Stability study at 40°C / 75% RH

As described in chapter 3, samples of the roxithromycin monohydrate, the roxithromycin glass and glass powder were placed in a Binder® oven at 40°C and 75% relative humidity (RH). The stability tests were performed over a one-month period (four weeks). The samples were analysed at onset (initial) and thereafter at weekly intervals in order to detect any changes caused by exposure to elevated temperature and humidity. Whilst TGA is the most widely used method to determine

moisture content, Karl Fischer (KF) was used in conjunction. Due to the small sample sizes, TGA analyses appeared to be more accurate than the Karl Fischer analyses in this study.

Functional terms used in this chapter:

1. Roxithromycin raw material is referred to as *monohydrate*;
2. Amorphous (glassy) roxithromycin is referred to as *glass*;
3. Amorphous (glassy) ground roxithromycin is referred to as *glass powder*;
4. The term *moisture* indicates water;
5. *Sorption* indicates spontaneous acquisition of water (Hollenbeck, 2007);
6. *Adsorption* is sorption confined to the surface of the solid (Hollenbeck, 2007);
7. *Absorption* is characterised by penetration of the sorbed component into the bulk structure of the solid (Hollenbeck, 2007); and
8. *Desorption* is the spontaneous loss of water to the atmosphere (Hollenbeck, 2007).

5.2.1 Thermal analysis (DSC and TGA)

a) Roxithromycin monohydrate

The DSC results being generated over the four weeks are shown in figure 5.1. The thermograms were almost identical from initial until week 4. No peak shifts, or any disappearances of peaks were observed. It could thus be concluded that the monohydrate remained stable over the period of testing. A very slight endotherm was observed after week 4 at about 100°C. The TGA results, however, did not show a significant increase in adsorbed moisture.

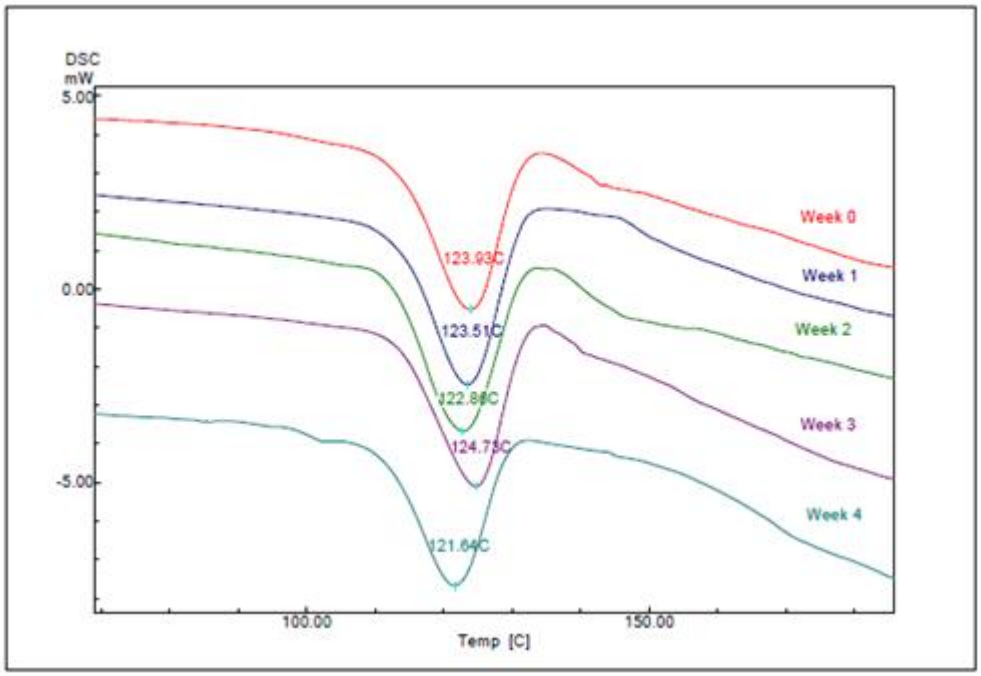

Figure 5.1: DSC thermograms of roxithromycin monohydrate.

b) Roxithromycin glass

Figure 5.2 illustrates the DSC traces of roxithromycin glass over the four weeks. Week 1 showed a significantly lower glass transition at 80.1°C, compared with those at initial and at weeks 2 - 4 of approximately 89.0°C. According to Craig *et al.* (1999), the amorphous state tends to take up considerably more water than the crystalline state, due to an increase in void space, free energy and increased surface area (Burnett *et al*., 2004). With an increase in the concentration of water in the solid, the T_g decreases according to the Gordon-Taylor equation. Furthermore, below the T_g, the water sorption is limited to surface activities (Burnett *et al.,* 2004). According to the obtained TGA values (table 5.1), there was an increase in the sorption values from 0.5% to 1.8% from initial to week 1, which could explain the observed lower glass transition temperature. After week 4 the adsorbed moisture was also above 1.8%, but the glass transition did not change from the initial value obtained. This could probably be due to a very small degree of recrystallisation that took place (small endothermic peak at 113.4°C).

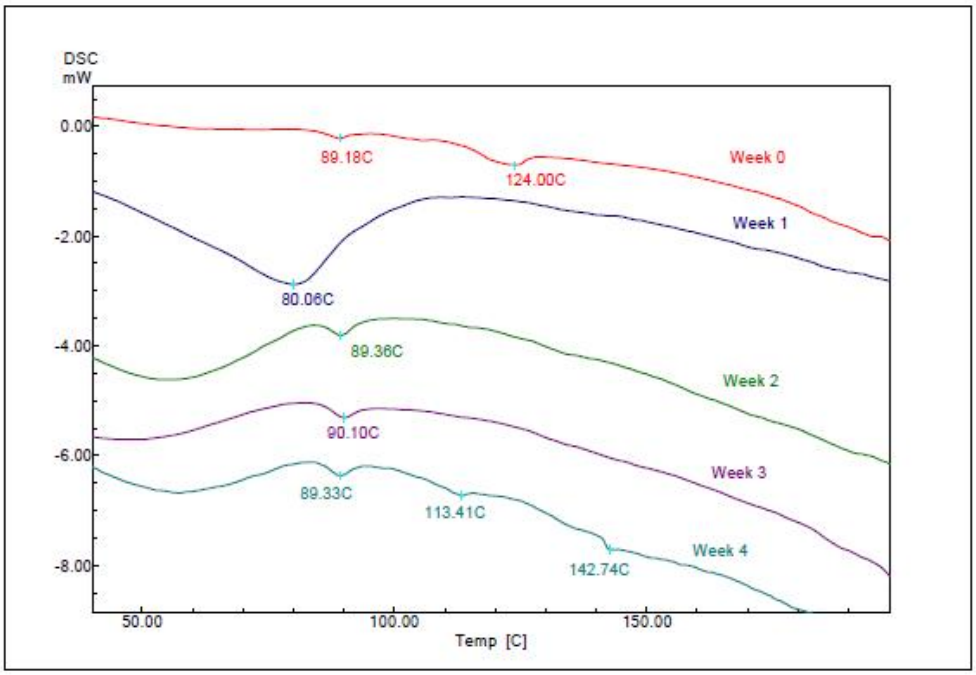

Figure 5.2: DSC thermograms of roxithromycin glass.

c) Roxithromycin glass powder

Figure 5.3 illustrates the DSC results of roxithromycin glass powder over the four weeks. The glass powder (probably due to an increased surface area) displayed a larger and more significant endothermic event at 110.9°C at week 4. Further experimental work would be required to determine the critical relative humidity for moisture-induced phase transitions, since this experimental aim was only to determine the stability of the three forms at a pre-determined, fixed RH value.

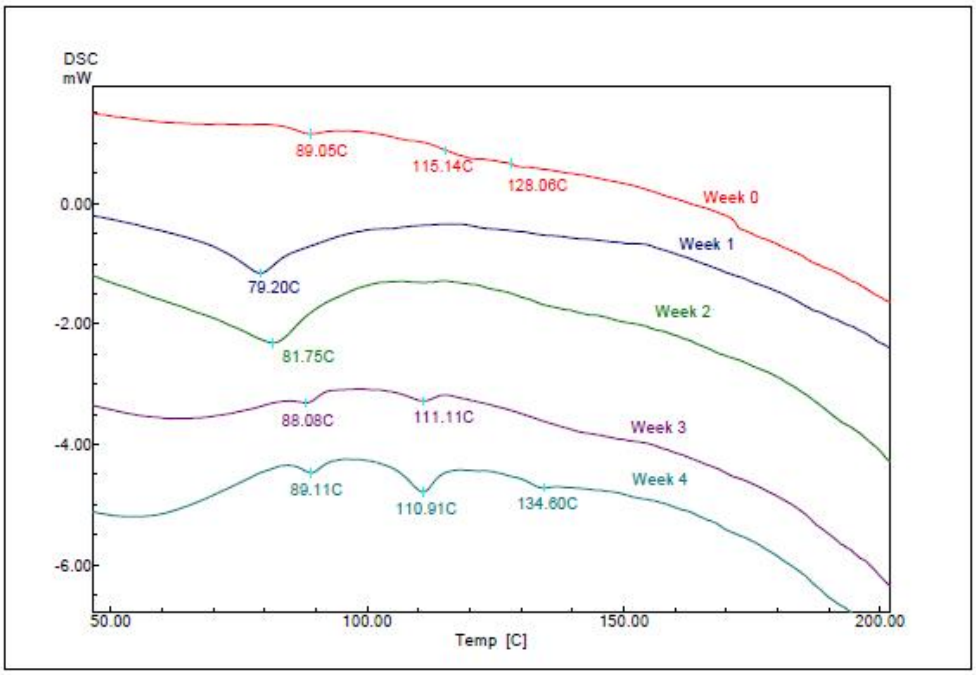

Figure 5.3: DSC thermograms of roxithromycin glass powder.

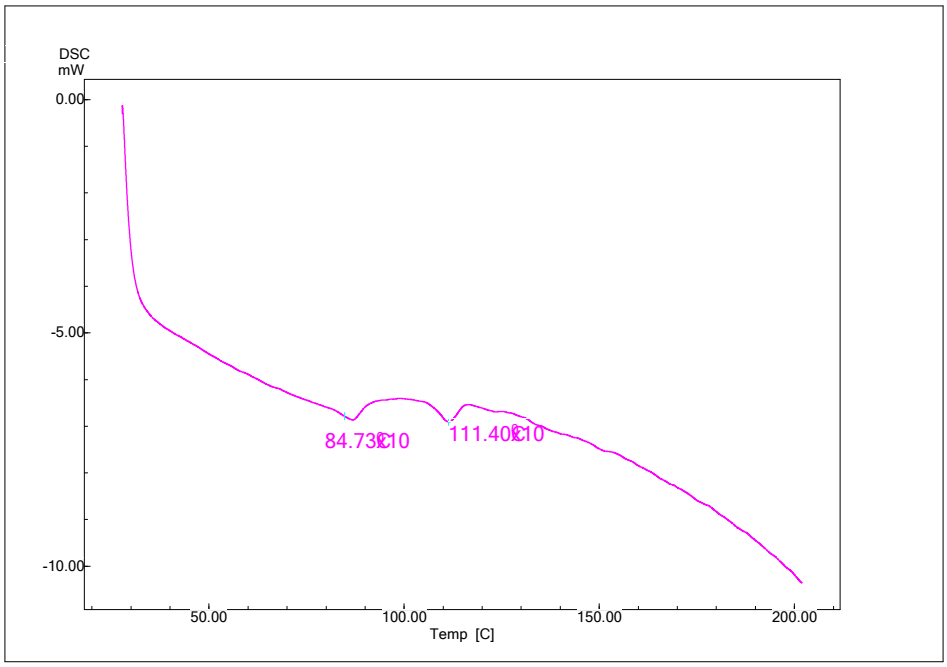

Figure 5.4: DSC thermogram of roxithromycin glass powder at week 5.

Table 5.1: TGA results of roxithromycin monohydrate, roxithromycin glass and glass powder

Roxithromycin	% weight loss					
	Initial	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
Monohydrate	2.8	2.4	2.4	2.8	2.5	*N/A
Glass	0.5	1.8	1.2	1.4	1.9	*N/A
Glass powder	0.2	0.2	0.6	0.7	0.7	2.0

**N/A = Not analysed*

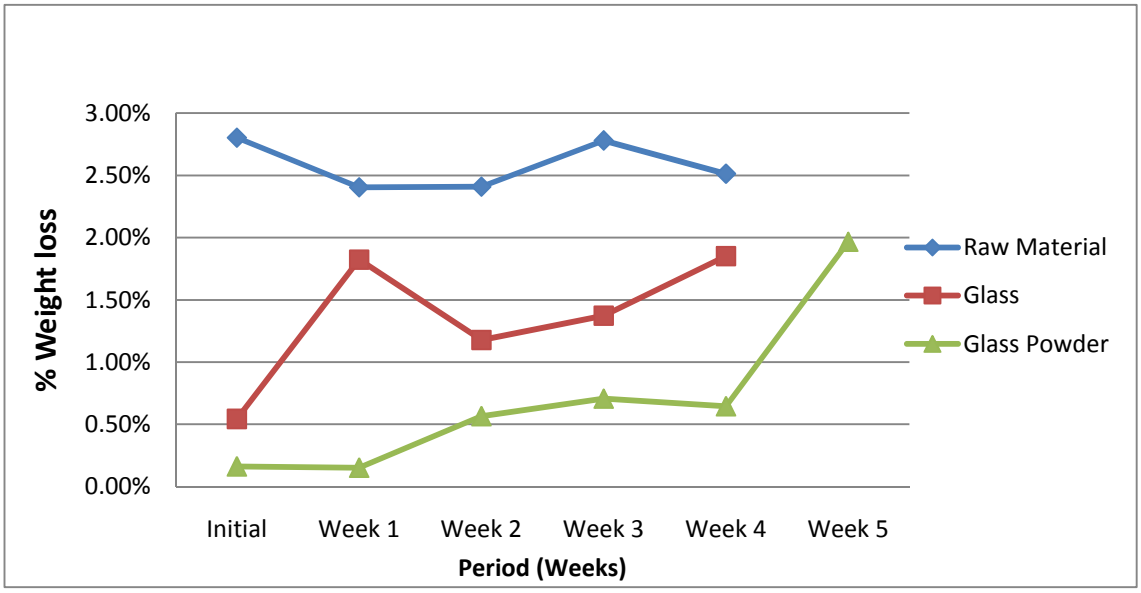

Figure 5.5: TGA % weight loss of roxithromycin monohydrate (raw material), roxithromycin glass and glass powder, over a period of 4 – 5 weeks.

Figure 5.5 is an overlay of the TGA results obtained for roxithromycin monohydrate, the roxithromycin glass and glass powder over the duration of the stability study. As per table 5.1, the TGA results of the glass powder showed a weight loss of almost 2.0% after week 5, which was equal to that of a monohydrate.

5.2.2 Karl Fischer analyses (KF)

Table 5.2 and Figure 5.6 illustrate the KF results of roxithromycin monohydrate, and the roxithromycin glass and glass powder over a period of one month. The KF analysis showed that the absorbed moisture of the monohydrate remained within the 2.6 - 2.7% margin. For both the glass and the glass powder, there was a significant increase in water content, compared to the initial value.

Table 5.2: KF results of roxithromycin monohydrate, glass and glass powder

Roxithromycin	% weight loss					
	Initial	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
Monohydrate	2.6	2.7	2.8	2.7	2.7	*N/A
Glass	0.9	2.5	2.5	2.6	2.6	*N/A
Glass powder	0.9	2.4	2.1	2.0	2.4	2.3

**N/A = Not analysed*

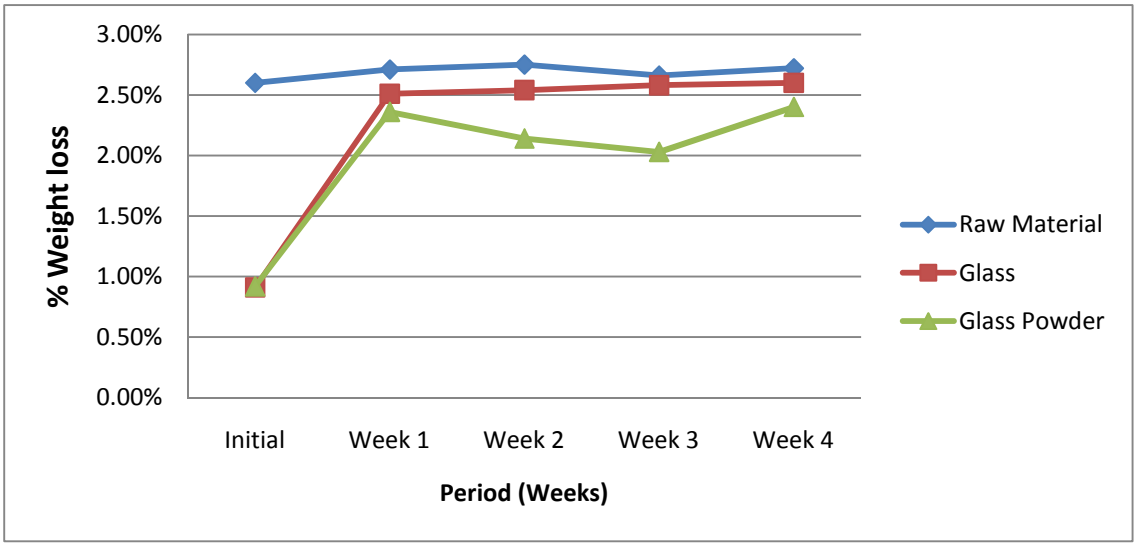

Figure 5.6: KF results of roxithromycin monohydrate (raw material), roxithromycin glass and glass powder over a period of 4 weeks.

For the monohydrate, the KF results correlated well with those of the TGA. However, the KF results showed that both the glass and glass powder sorbed, within a week, more than 2.0% of moisture, which contradicted the TG results.

5.2.3 Infrared analyses (IR)

The IR spectra of the monohydrate did not show any significant differences over the four-week period of stability testing (figure 5.7).

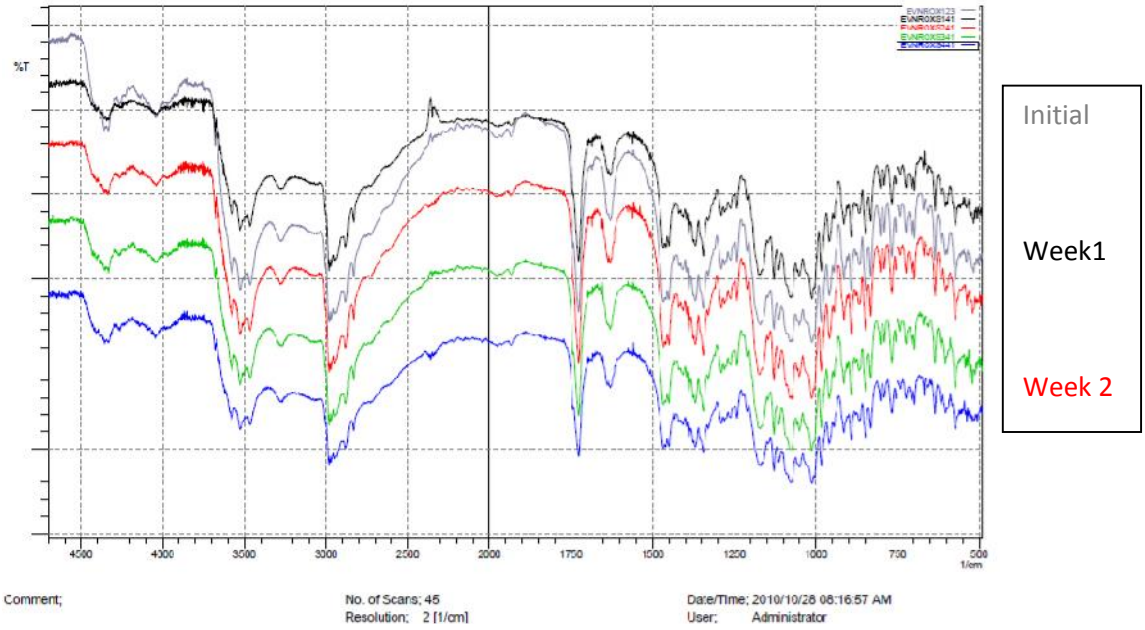

Figure 5.7: IR spectra of roxithromycin monohydrate.

Figure 5.8 shows the IR spectra of roxithromycin glass over the four weeks. No significant changes were observed over the one-month testing period.

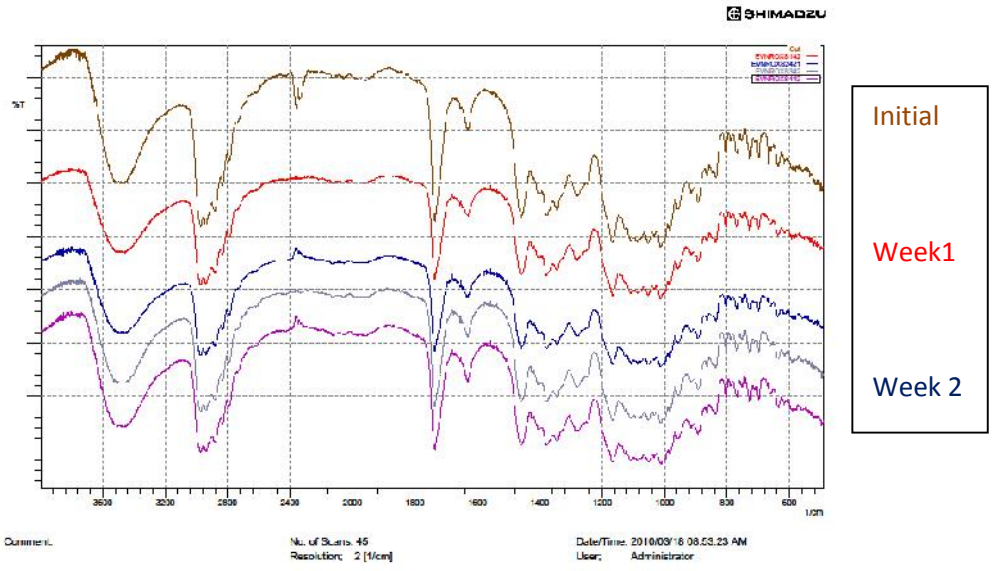

Figure 5.8: IR spectra of roxithromycin glass.

Figures 5.9a and 5.9b illustrate the IR spectra of roxithromycin glass powder, gathered over the four weeks. At week 4, small changes of the peak at 3472 cm^{-1} was observed, which could have been an indication of the conversion to the roxithromycin monohydrate, or a more crystalline state.

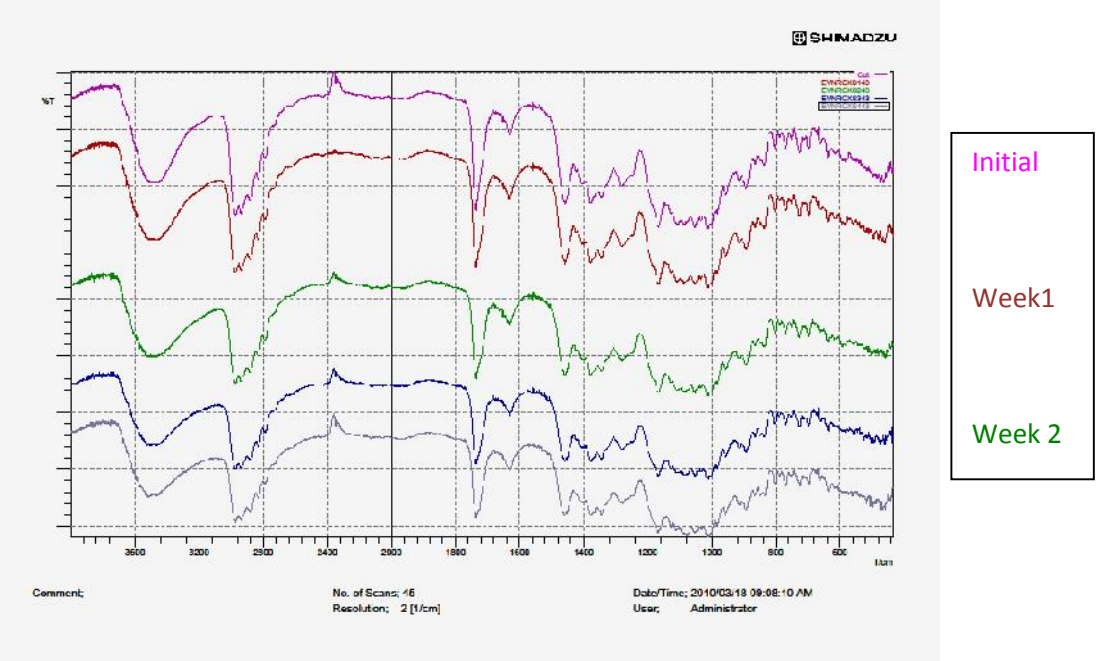

Figure 5.9a: IR spectra of roxithromycin glass powder over a period of 4 weeks.

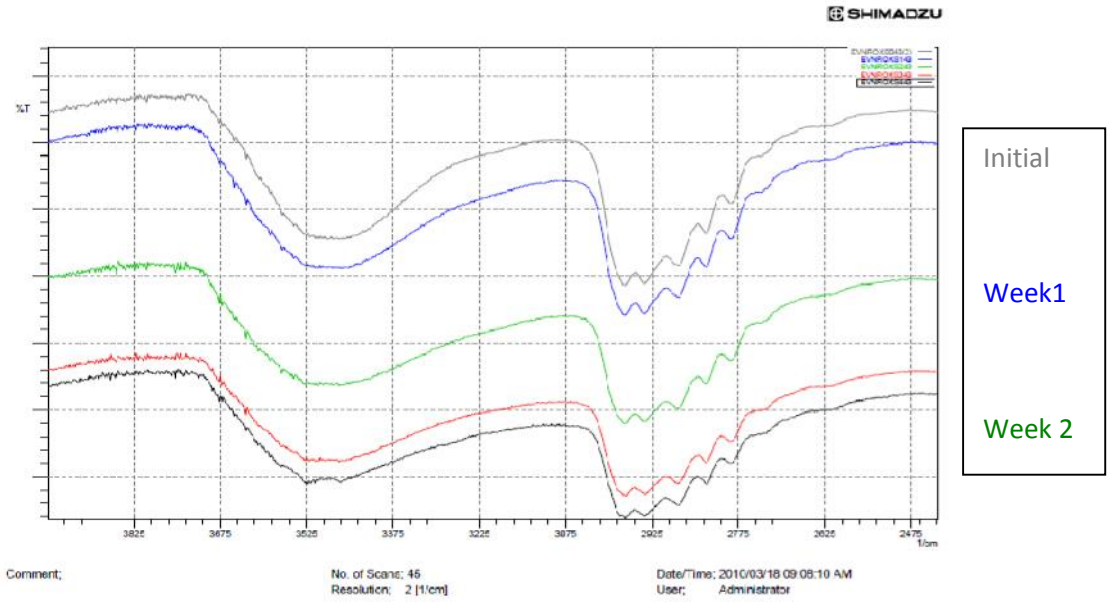

Figure 5.9b: IR spectra (3472 cm^{-1}) of roxithromycin glass powder over a period of 4 weeks (enlarged).

5.2.4 X-ray powder diffraction (XRPD)

Figures 5.10 and 5.11 illustrate the XRPD diffractograms obtained for roxithromycin monohydrate over the four-week stability testing period. No significant changes were observed.

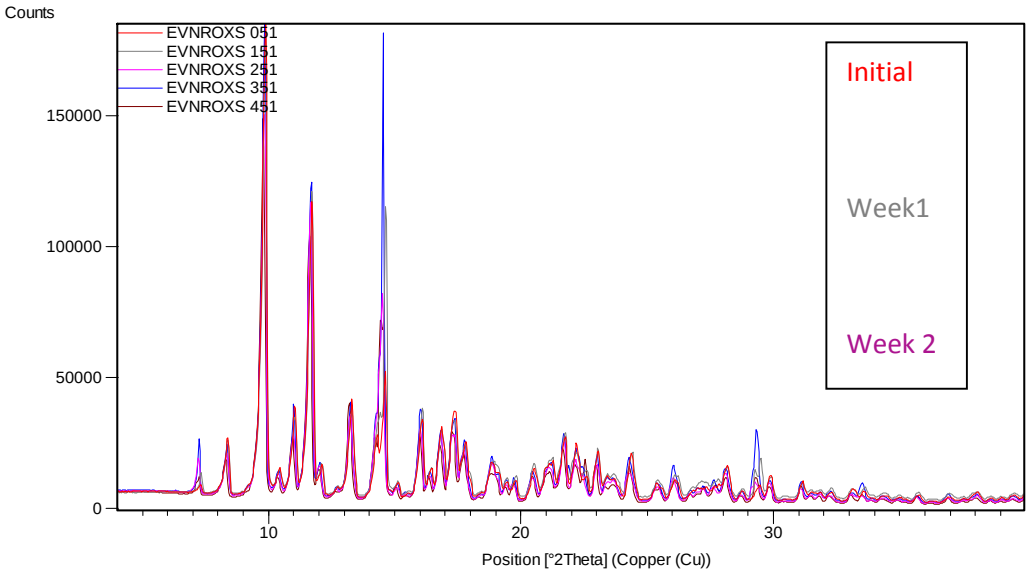

Figure 5.10: Overlay of the X-ray powder diffractograms of roxithromycin monohydrate over a period of 4 weeks.

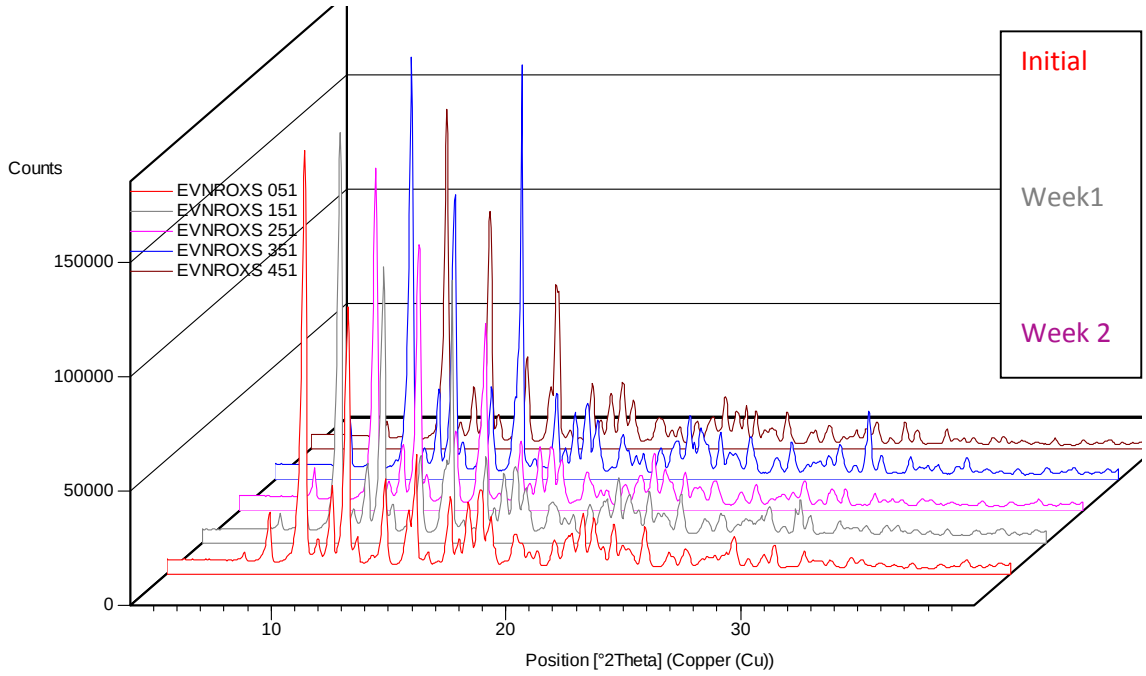

Figure 5.11: X-ray powder diffractograms (staggered) of roxithromycin monohydrate over a period of 4 weeks.

Figures 5.12 and 5.13 illustrate the XRPD results of roxithromycin glass, gathered over the four weeks. It was evident that the roxithromycin glass remained intact over the duration of the test period. An amorphous halo, typical of an amorphous solid, was displayed.

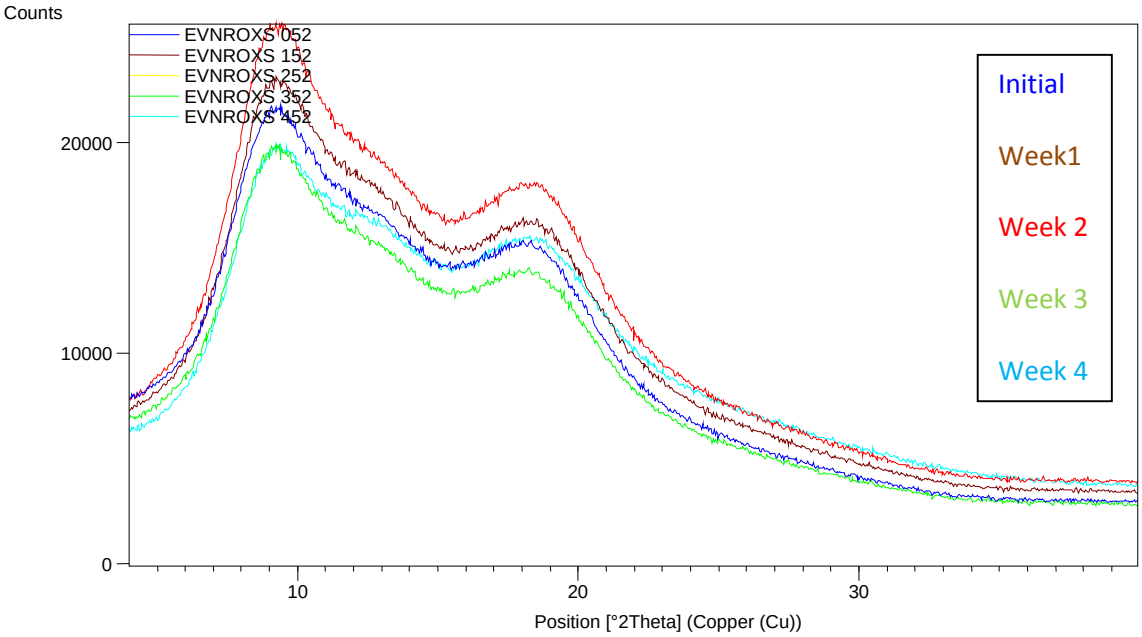

Figure 5.12: Overlay of the X-ray powder diffractograms of roxithromycin glass over a period of 4 weeks.

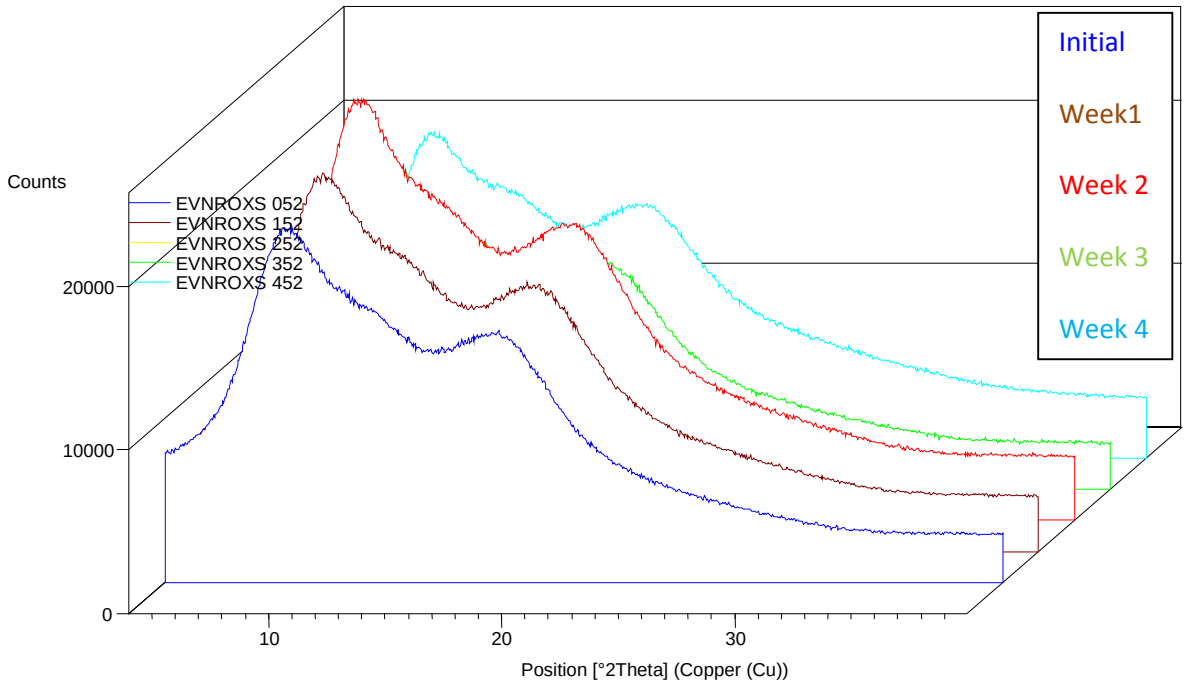

Figure 5.13: X-ray powder diffractograms (staggered) of roxithromycin glass over a period of 4 weeks.

Figures 5.14 and 5.15 illustrate the XRPD results of roxithromycin glass powder, gathered over five weeks. From week 3 onwards, changes occurred and roxithromycin glass powder started to change to the more crystalline state. The diffractogram at week 5 showed more crystalline peaks.

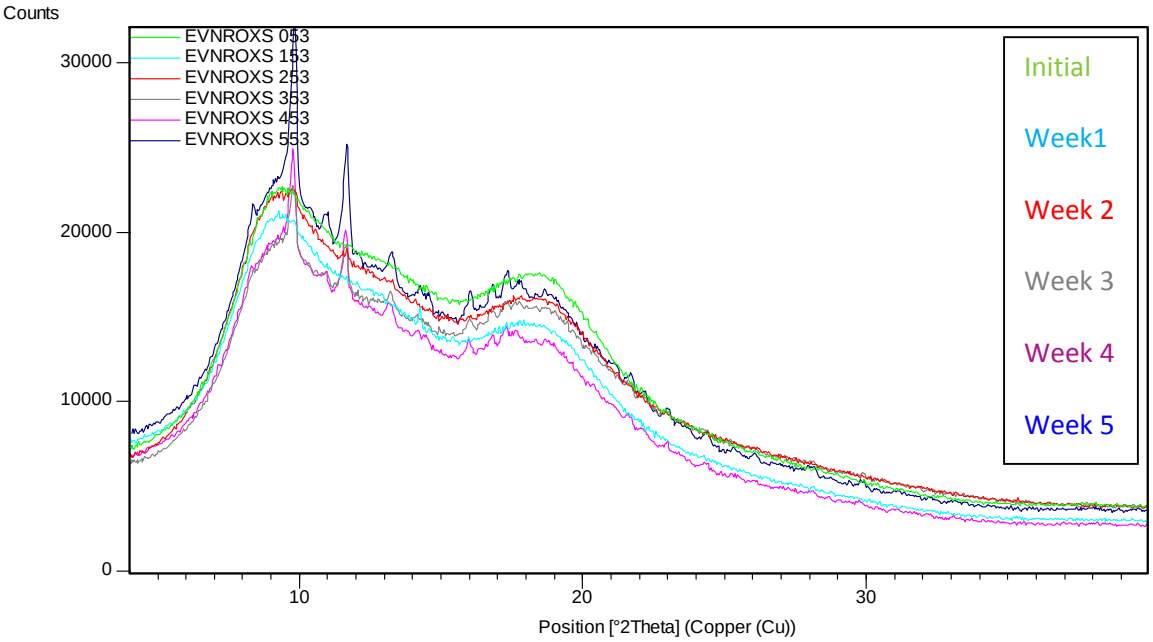

Figure 5.14: Overlay of the X-ray powder diffractograms of roxithromycin glass powder over a period of 5 weeks.

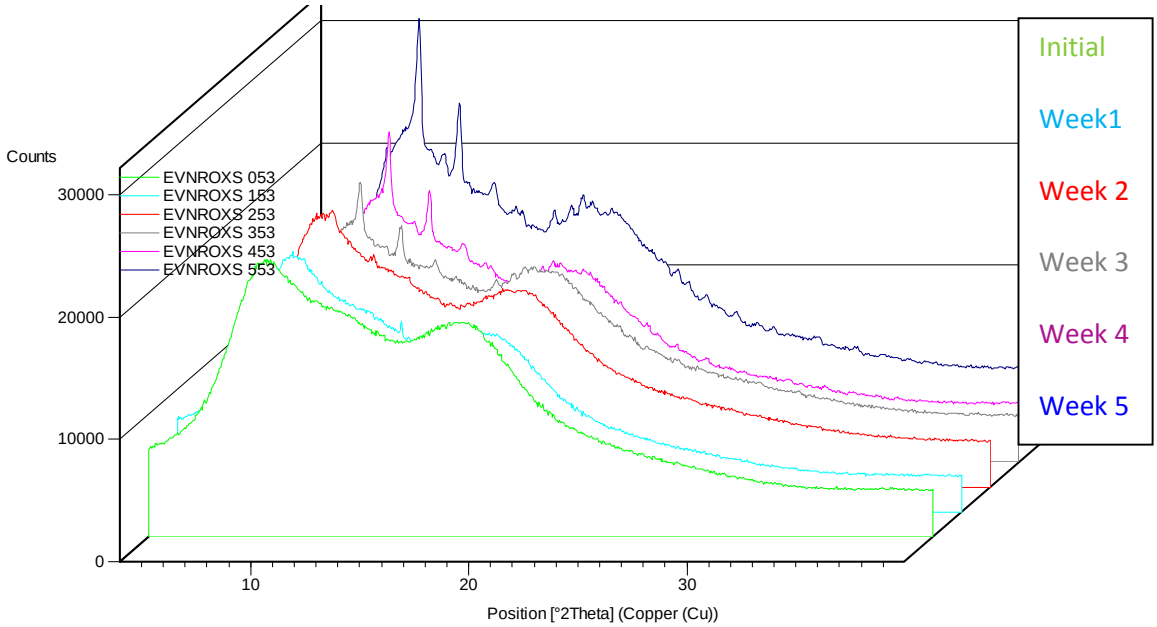

Figure 5.15: X-ray powder diffractograms (staggered) of roxithromycin glass powder over a period of 5 weeks.

An increase in crystallinity was observed after week 3, which meant that the amorphous glass powder partially reverted to the more crystalline state, with exposure to a higher temperature and relative humidity.

5.2.5 Discussion

Table 5.1 summarises the TGA results of roxithromycin monohydrate, the roxithromycin glass and glass powder initially and over a period of four weeks (five weeks for the glass powder). The XRPD and IR results showed significant differences after week 4 in the glass powder. In figure 5.9, the IR trace at week 4 displayed IR absorption peaks at 3472 cm^{-1}, which were indicative of transformation into the monohydrate. From the X-ray powder diffractograms (figure 5.14) it was evident that the amorphous glass powder transformed to become more crystalline. The DSC thermogram at week 5 (figure 5.4) showed a definite melting endotherm at 111.4°C. Neither the monohydrate, nor the glass showed any significant differences with regards to crystallinity changes after week 4. For this reason, the stability study of the roxithromycin glass powder was extended with another week.

5.3 Stability studies at 25°C / 75% RH and 30°C / 75% RH

In the above stability study, the roxithromycin glass powder showed instability over the four-week period of exposure to 40°C / 75% RH. In the following two studies, the glass powder was exposed to lower temperatures of 25°C and 30°C, at the same relative humidity of 75%. This was done in order to determine whether the glass powder would be more stable at these lower temperatures, or whether it would again revert to the more stable monohydrate.

Samples were also taken at onset and thereafter weekly over a one-month period and analysed by DSC, TGA, IR and XRPD.

5.3.1 Thermal analysis (DSC and TGA)

Figure 5.16 is an overlay of the DSC traces of the roxithromycin glass powder after four weeks for both the 25°C and 30°C samples. The T_g did not change significantly over this period for both samples.

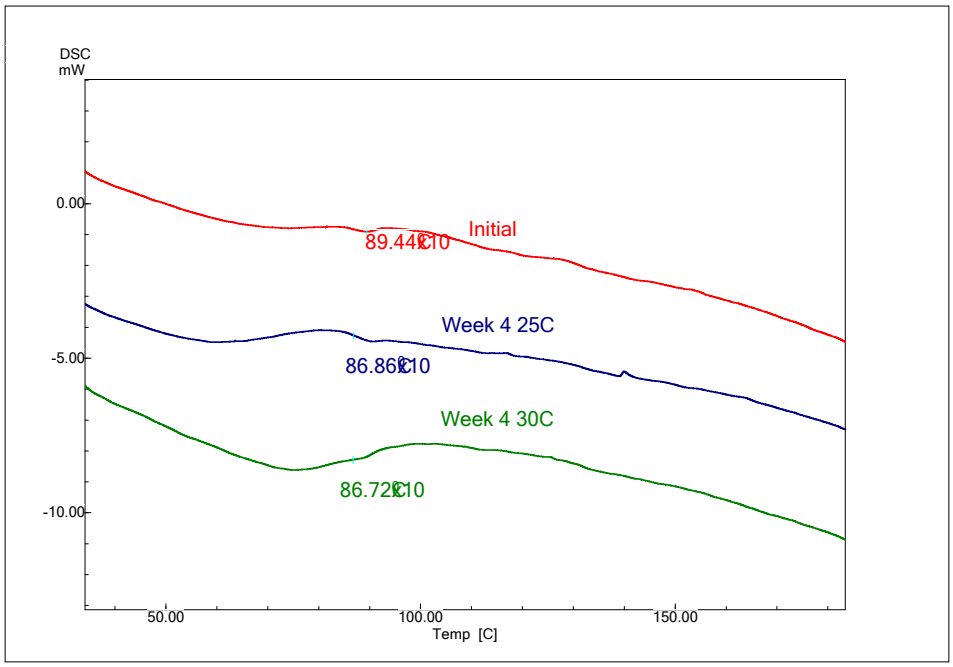

Figure 5.16: Overlay of the DSC thermograms of roxithromycin glass powder at initial and at week 4 (25°C and 30°C, at 75% RH).

Table 5.3 summarises the TGA results gathered weekly over a period of one month, whilst figure 5.17 illustrates the results graphically. With storage at 25°C / 75% RH, the adsorbed moisture gradually increased from 0.8% to an average of 2.0% after weeks 3 and 4. The adsorption was significantly faster with storage at 30°C / 75% RH. From initial to week 1, the adsorbed value increased from 0.8% to 1.9% within 7 days. For comparison, the values obtained from storage at 40°C / 75% RH during the initial stability study, were also included in this table. The initial value of the sample stored at 40°C / 75% RH was significantly lower than that of the two samples being tested a month later. Also, it took five weeks to reach a sorption value of approximately 2.0%. An interesting observation was that, although with the lower temperatures, more sorption was measured at an earlier stage, no transformation to

the more crystalline form was visible prior to week 4. With the 40°C sample, the absorbed value was 0.7% after week 3, when the crystalline transformation started.

Table 5.3: TGA results of stability studies performed at 25°C, 30°C and 40°C and at 75% RH

	Glass powder - % weight loss at 75% RH					
	Initial	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
25°C	0.8	0.9	1.2	2.3	2.0	*N/A
Habit / form	Amorphous	Amorphous	Amorphous	Amorphous	Crystalline transformation starts	-
30°C	0.8	1.9	2.3	2.0	2.1	*N/A
Habit / form	Amorphous	Amorphous	Amorphous	Amorphous	Crystalline transformation starts	-
40°C	0.2	0.2	0.6	0.7	0.7	2.0
Habit / form	Amorphous	Amorphous	Amorphous	Crystalline transformation starts	More crystalline	More crystalline

**N/A = Not analysed*

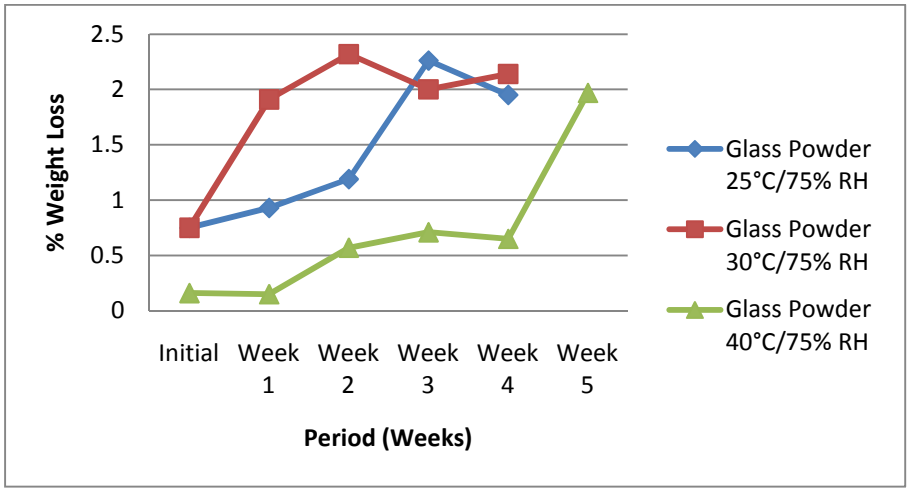

Figure 5.17: TGA % weight loss of roxithromycin glass powder at 25°C, 30°C and 40°C and at 75% RH.

5.3.2 Infrared spectroscopy (IR)

Figure 5.18 shows the IR spectra of roxithromycin glass powder. No changes in the IR absorption peak in the region of 3472 cm^{-1} were observed, which meant that the IR results were not indicative of any conversion to the more stable monohydrate.

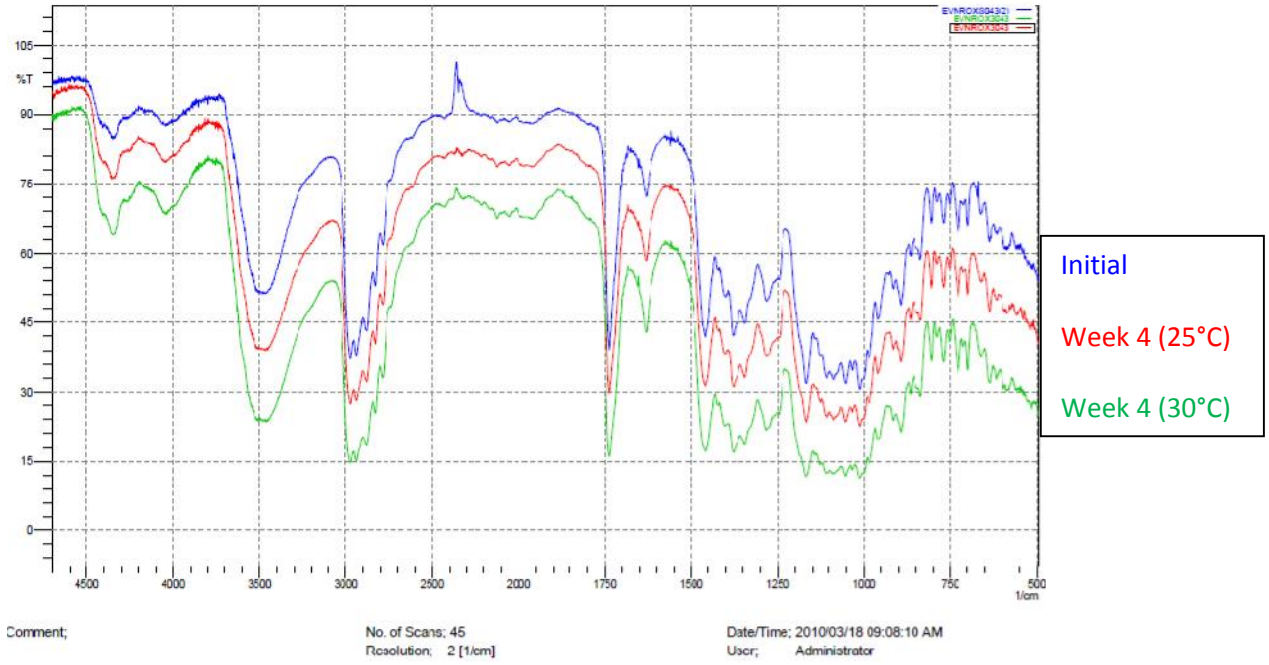

Figure 5.18: IR spectra of roxithromycin glass powder at week 4 at 25°C and 30°C and at 75% RH.

5.3.3 X-ray powder diffraction (XRPD)

Figure 5.19 displays the XRPD traces of the roxithromycin glass powder after week 4. Small crystalline peaks were observed, but the transformation was not as significant as in the 40°C / 75% RH study.

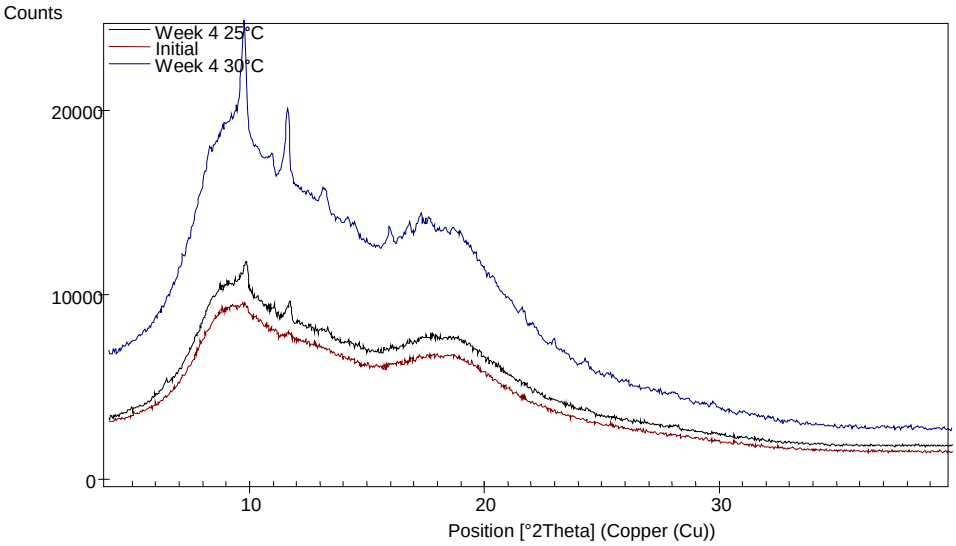

Figure 5.19: Overlay of the X-ray powder diffractograms of the roxithromycin glass powder at week 4 at 25°C (black) and 30°C (blue) at 75% RH, compared with the initial diffractogram (red).

5.3.4 Conclusion

From the results being generated over the four weeks, it appeared that storage at the lower temperatures (25°C and 30°C) did not induce any significant changes over the first three weeks of the study. The XRPD results showed transformation of both samples at week 4. It was therefore concluded that the phase transformation of the roxithromycin glass powder to the more crystalline form was temperature dependant, irrespective of the moisture being adsorbed over the stability period, due to exposure to the high RH.

5.4 Stability studies of the chloroform- and ethyl acetate desolvates at 40°C / 75% RH

As discussed in chapter 3, two desolvated forms of roxithromycin were placed in a Binder ® oven and exposed to 40°C / 75% RH. Samples were taken at onset and thereafter weekly for one month and analysed by means of DSC, TGA, IR and XRPD, in order to determine any changes over time, due to exposure to the elevated temperature and humidity.

5.4.1 Roxithromycin chloroform desolvate stability study

5.4.1.1 Thermal analysis (DSC and TGA)

Figure 5.20 illustrates the DSC results being generated over the four weeks of testing. The only significant change being observed over the period was the change in melting point from 79.3°C to 90.1°C. The higher melting point could have been indicative of a partial transformation to the more stable monohydrate.

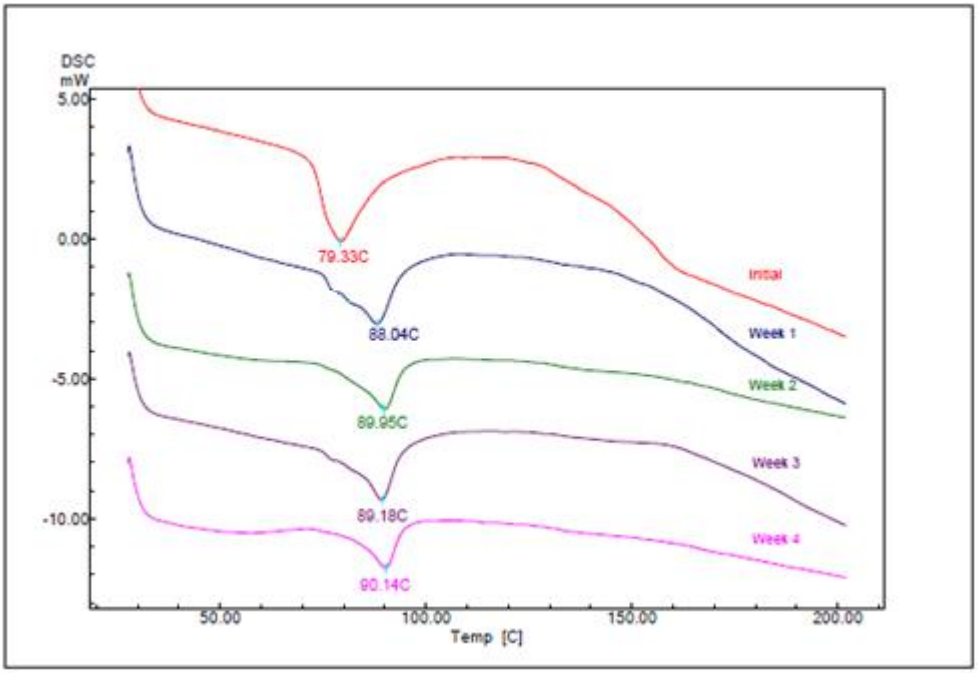

Figure 5.20: An overlay of the DSC thermograms of the roxithromycin chloroform desolvate.

Table 5.4 summarises the TG results of the roxithromycin chloroform desolvate. The data clearly demonstrated good stability, since no significant increase in the weight loss percentage occurred.

Table 5.4: TGA results of roxithromycin chloroform desolvate

	% weight loss					
	Initial	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
Chloroform desolvate	1.5	1.5	1.6	1.6	1.9	*N/A

**N/A = Not analysed*

5.4.1.2 Infrared analyses (IR)

Figure 5.21 shows the IR results gathered over the four-week period. Since no changes were observed at the IR absorption peak at 3472 cm^{-1}, it could be concluded that the roxithromycin chloroform desolvate was stable over the four weeks of exposure to 40°C / 75% RH.

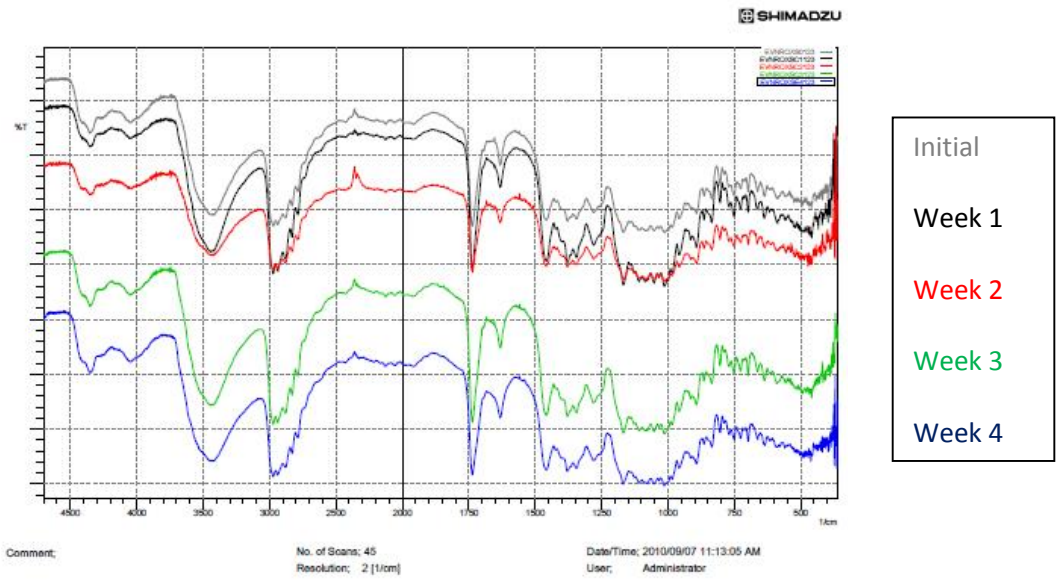

Figure 5.21: IR spectra of roxithromycin chloroform desolvate over a period of 4 weeks.

5.4.1.3 X-ray powder diffraction (XRPD)

Figure 5.22 illustrates the XRPD traces of roxithromycin chloroform desolvate initially and at week 4 of the stability study. No change into the more stable crystalline monohydrate took place.

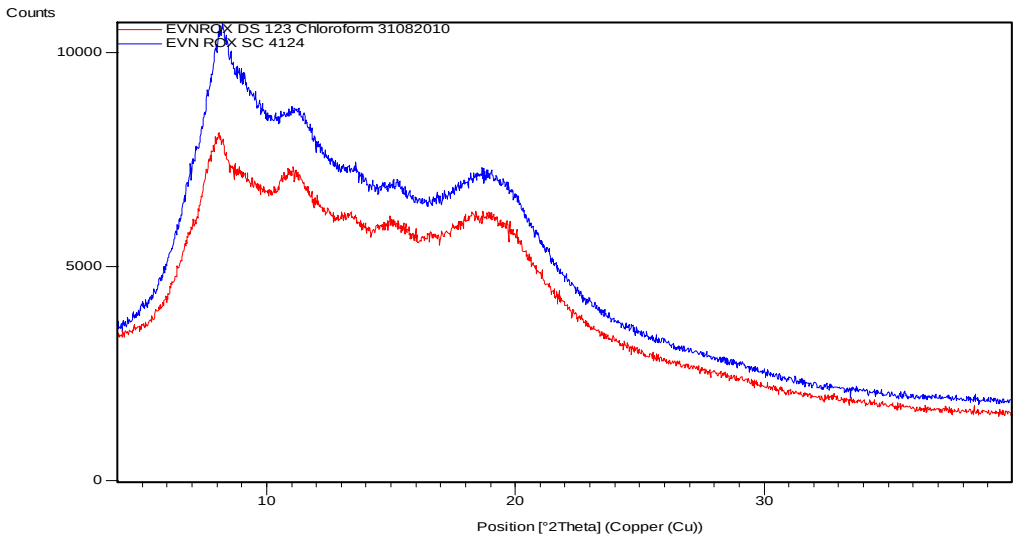

Figure 5.22: XRPD overlay of roxithromycin chloroform desolvate at initial (red) and at week 4 (blue).

5.4.2 Roxithromycin ethyl acetate desolvate stability study

During previous studies by Du Plessis (2004) and Bawa (2007), the form obtained from recrystallisation with ethyl acetate was described as an amorphous, low melting point form and a hemi-solvated form, respectively. Aucamp (2009) characterised this form as a stable, amorphous monohydrate. It was thus decided to perform a stability study on this form for comparison against the amorphous chloroform desolvated form, as well as the glassy form of roxithromycin.

5.4.2.1 Thermal analysis (DSC and TGA)

Figure 5.23 shows the DSC results of roxithromycin ethyl acetate over a one-month period. It appeared stable over the four weeks, with no changes being observed.

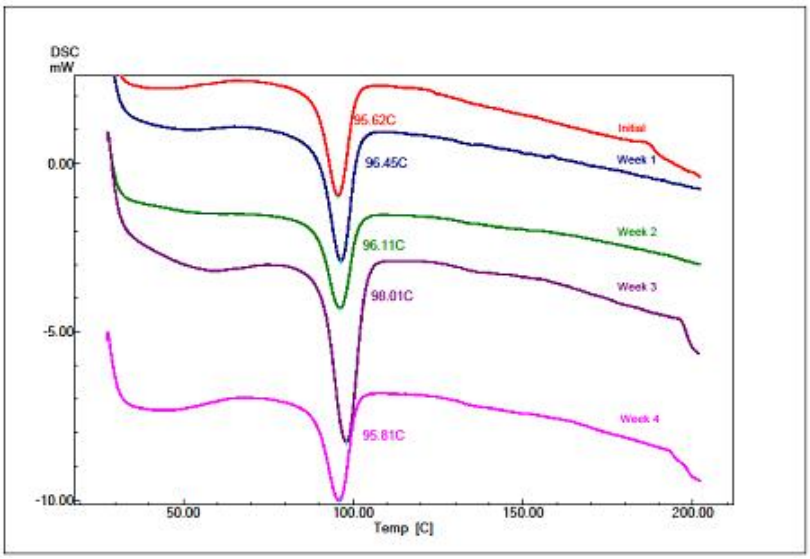

Figure 5.23: An overlay of the DSC thermograms of roxithromycin ethyl acetate desolvate over a period of 4 weeks.

Table 5.5 summarises the TG results of the roxithromycin ethyl acetate desolvated form gathered over the one-month period. No significant increase in the weight loss percentage was observed.

Table 5.5: TGA results of roxithromycin ethyl acetate desolvate

	% weight loss					
	Initial	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
Ethyl acetate desolvate	1.5	1.5	1.6	1.8	2.3	*N/A

**N/A = Not analysed*

5.4.2.2 Infrared analyses (IR)

Figure 5.24 illustrates the IR spectra being gathered over a four-week period. Since no changes were observed at the IR absorption peak at 3472 cm^{-1}, it could be concluded that this form remained stable over the duration of the stability period at 40°C / 75% RH.

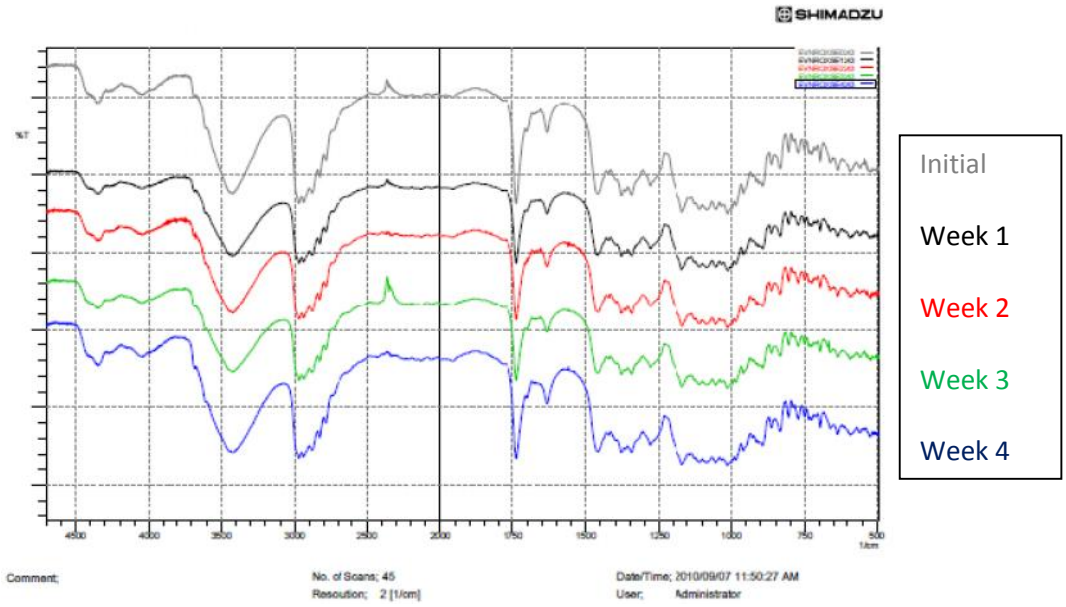

Figure 5.24: An overlay of the IR spectra of the roxithromycin ethyl acetate desolvate over a period of 4 weeks.

5.4.2.3 X-ray powder diffraction (XRPD)

Figure 5.25 illustrates the XRPD traces of roxithromycin ethyl acetate desolvate initially and at week 4 of the stability study. No significant changes in the diffraction pattern were observed.

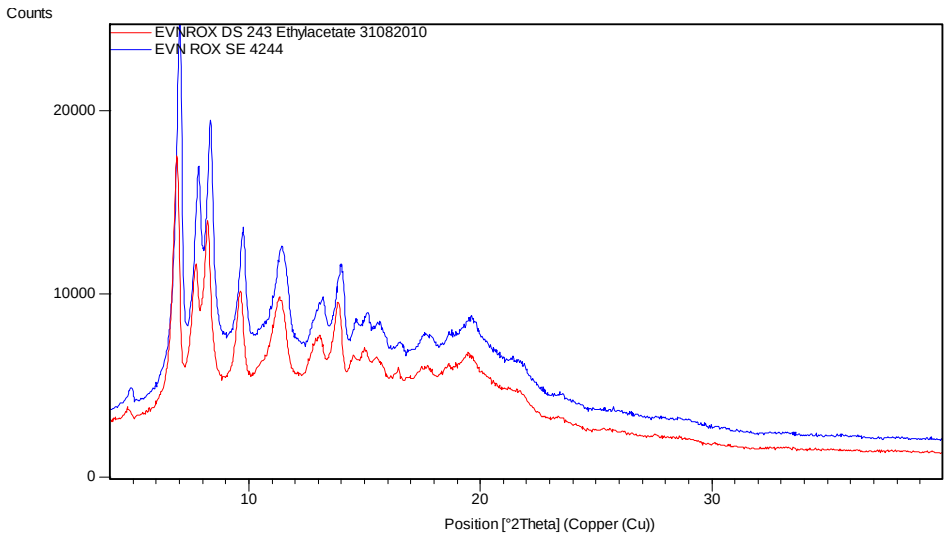

Figure 5.25: Overlay of the X-ray powder diffractograms of roxithromycin ethyl acetate desolvate at initial (red) and at week 4 (blue).

5.5 The effect of moisture on amorphous roxithromycin

As described in paragraph 3.13, different concentrations of water were added to roxithromycin amorphous form in order to determine whether moisture would affect its glass transition temperature. The concentrations of water added to each roxithromycin glass sample ranged from 1% to 100% w/w.

Figure 5.26 shows the DSC traces obtained with this test. The first indication of a significant change was observed at a water content of 70% w/w and beyond. The glass transition point disappeared, whilst a small melting endotherm appeared. This test, together with the data obtained from the different solubility studies would, however, require further investigation in order to determine the precise point of transformation from amorphous to crystalline forms.

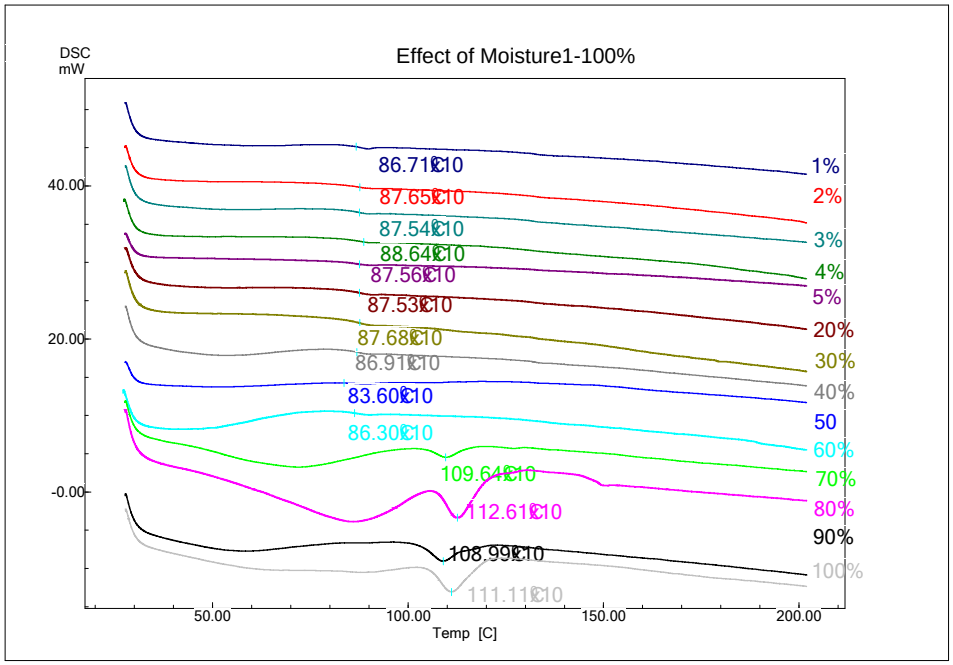

Figure 5.26: Overlay of the DSC thermograms of roxithromycin glass exposed to different weight fractions of water.

5.6 Conclusion

Stability studies were performed on roxithromycin monohydrate, the roxithromycin glass and glass powder, in order to determine whether exposure to elevated temperatures and humidity of the prepared forms would have any effect on their stability.

It could be concluded that the roxithromycin glass showed stability over the period of a month (four weeks) of exposure to conditions of 40°C / 75% RH.

Contrary, the roxithromycin glass powder tended to revert to the more stable crystalline form (monohydrate) after week 3 of the stability period. The roxithromycin glass powder at lower temperatures of 25°C and 30°C at 75% RH tended to transform to the more crystalline form during week 4 of the study. The changes were, however, not as significant as during the 40°C / 75% RH study.

Since the sorbed moisture among the three stability trials varied significantly, it was concluded that humidity alone was not the only catalyst for crystallisation to occur, but that the transformation from amorphous to crystalline forms was more evident at a higher temperature i.e. 40°C.

Stability studies on the two roxithromycin desolvates (recrystallised from chloroform and ethyl acetate) were also performed in order to determine whether these amorphous forms that had been prepared from organic solvents, would differ with regards to their stability from the roxithromycin glass, prepared through heating and cooling. It was observed that these amorphous forms appeared to be more stable than the other roxithromycin glass forms.

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Chapter 6

Solubility study of roxithromycin monohydrate and amorphous forms

6.1 Introduction

Roxithromycin has a poor solubility profile and therefore an oral bioavailability of only 50% (Biradar *et al.,* 2006). This may negatively influence this active's oral performance, whilst Zhang *et al*. (2004) also reported the degradation of roxithromycin in acidic conditions. It is known that by preparing an amorphous form of a drug, its free energy increases and not only does this lead to a higher solubility, but also a faster dissolution rate, with a subsequent higher oral bioavailability. Amorphous forms of drugs have become of much interest to pharmaceutical scientists, as the characteristics of amorphous solids show very promising potential for future usage in pharmaceutical applications (Bhugra & Pikal, 2008; Gao, 2008).

This chapter focuses on the solubility of the amorphous forms of roxithromycin, prepared during this study and as discussed in the previous chapters. The solubility and dissolution rates of the prepared forms will be compared with those of the roxithromycin reference material.

6.2 Solubility study A

As described in paragraph 3.10.1, a solubility study was done by preparing saturated solutions in distilled water of the different roxithromycin forms to be tested, in amber glass test tubes. These tubes were placed in a water bath (37°C ±2°C) and left to rotate for 24 hours for equilibrium to be reached. The solubility profiles of the three amorphous roxithromycin forms, namely the glass, glass powder and the chloroform desolvate were compared with the roxithromycin monohydrate. High performance liquid chromatography (HPLC)

analysis was performed to determine the concentrations of the filtrates. The mobile phase used was 30 g/L ammonium dihydrogen phosphate buffer (pH adjusted to 5.3 with sodium hydroxide) and acetonitrile. The ratio of buffer to acetonitrile was 600:400. A Luna C_{18}, 150 mm x 4.6 mm column was used. The flow rate was 1.0 ml/min and the wavelength 205 nm. Validation of this method provided a linear regression of $R^2 = 0.998$.

The measured solubility of the roxithromycin reference material was 3.9 µg/ml, the roxithromycin glass 6.4 µg/ml, the roxithromycin glass powder 7.4 µg/ml and the amorphous chloroform desolvate 10.0 µg/ml (figure 6.1).

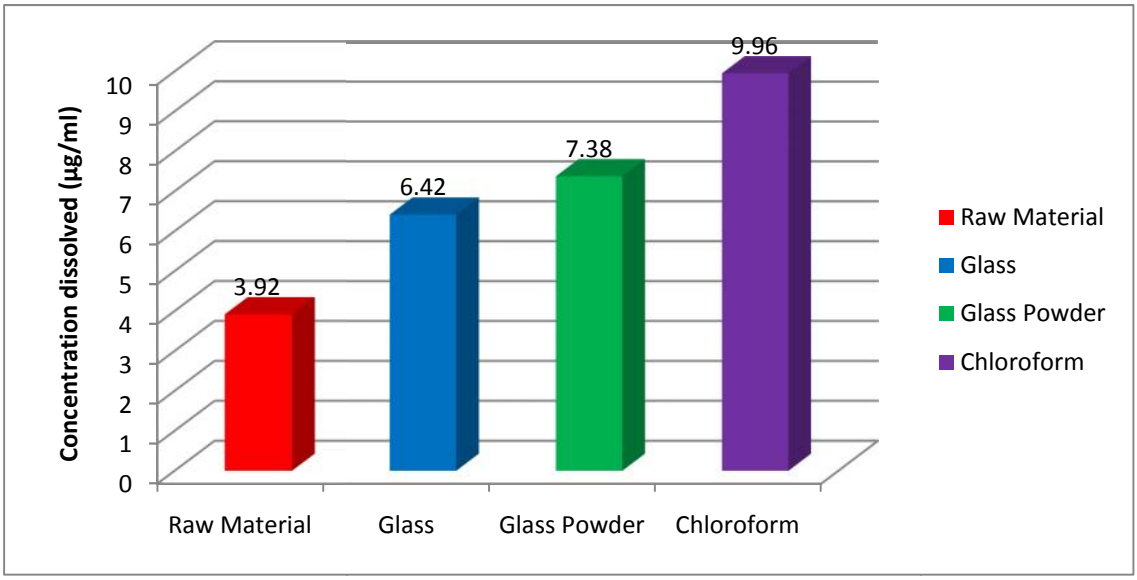

Figure 6.1: Comparison of the solubility profiles of roxithromycin monohydrate (raw material) with the amorphous roxithromycin forms (glass, glass powder and chloroform desolvate).

According to a study done by Aucamp (2009), the solubility profile of roxithromycin glass was much higher than that being obtained during this study. This may be due to the tendency of these glass forms to revert to the more stable monohydrate with exposure to moisture. The rate of transformation could differ between the different forms, due to habit differences. The solubility

of the monohydrate, however, was much lower than those of the amorphous forms.

The results from this study showed that the roxithromycin glass was 1.6 times more soluble than the monohydrate and therefore it could be concluded that the preparation of the roxithromycin glass enhanced the solubility of the roxithromycin active. Grinding of the glass reduced the particle size, leading to a larger surface for exposure to solvent and thus an improved solubility. The ground glass was 1.9 times more soluble than the roxithromycin monohydrate. The unexpectedly high solubility values of the amorphous chloroform desolvated form were unclear, because the morphology of this amorphous form did not differ much from that of the other amorphous forms. The amorphous chloroform desolvate was 2.5 times more soluble than the roxithromycin monohydrate. The morphologies of the monohydrate, the chloroform desolvate and the glass are illustrated in figure 6.2 (scanned electron microscope (SEM) images).

The solubility improved in the order of roxithromycin monohydrate < glass < glass powder < chloroform desolvate.

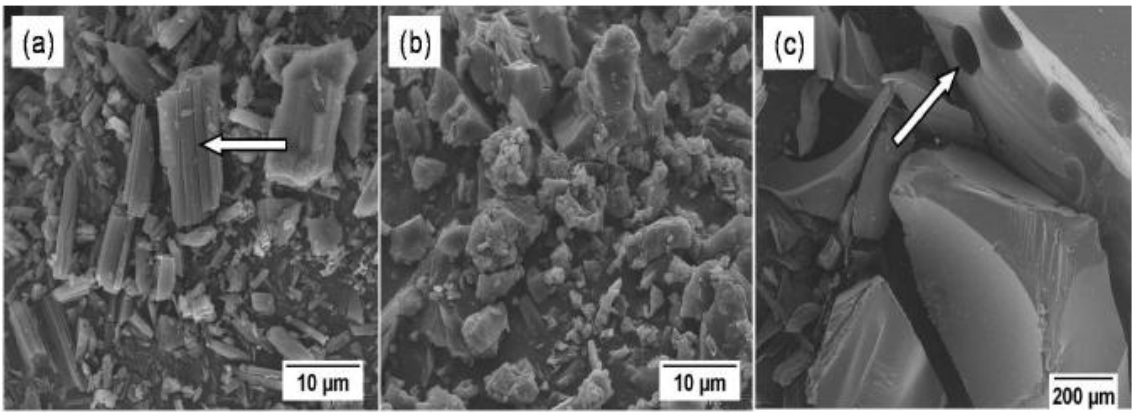

Figure 6.2: SEM images of (a) roxithromycin monohydrate, (b) roxithromycin chloroform desolvate and (c) roxithromycin glass.

In order to verify the occurrence of any phase transformation during the solubility study, such as the conversion into the monohydrate, a series of DSC and TGA analyses were done after each solubility test.

6.2.1 Roxithromycin monohydrate

Figure 6.3 illustrates the DSC results of the roxithromycin monohydrate (raw material) before and after the solubility study in water. As expected, no changes were observed for this stable monohydrate and melting points showed to be identical before and after the solubility test.

Figure 6.4 illustrates the TG results of the roxithromycin monohydrate after the solubility study. The theoretical weight loss for a 1:1 monohydrate is 2.1%, whilst the measured experimental value was 2.0%. The monohydrate therefore remained stable during the solubility study and did not change into a dihydrate.

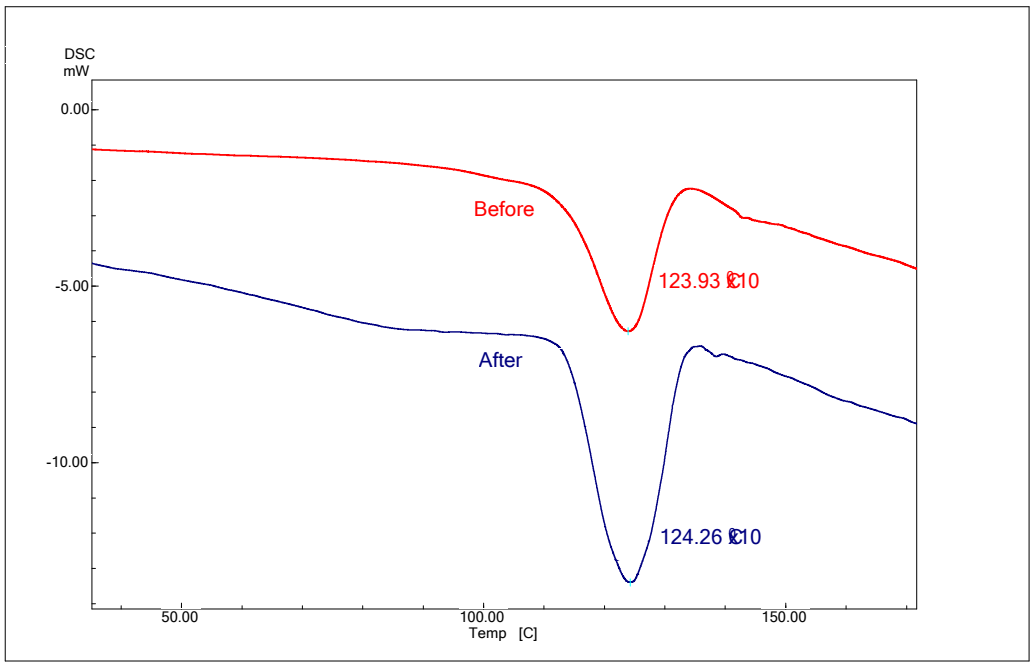

Figure 6.3: Overlay of the DSC thermograms of the roxithromycin monohydrate before and after the solubility test.

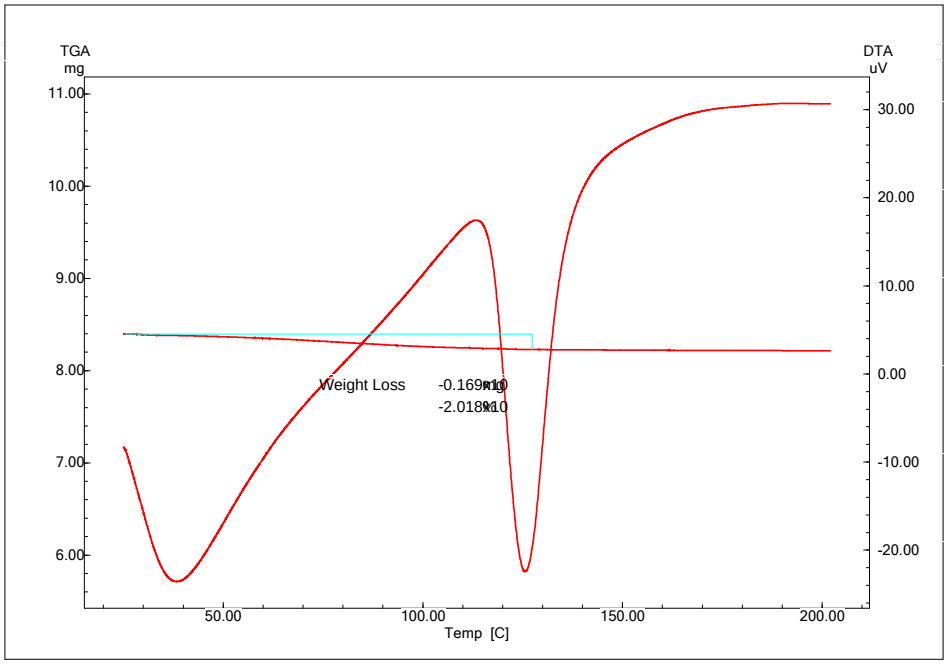

Figure 6.4: TGA thermogram of roxithromycin monohydrate after the solubility test.

6.2.2 Glass

The roxithromycin glass transformed into the monohydrate during the 24-hour solubility study. The DSC trace clearly illustrated the transformation (figure 6.5). The glass transition disappeared and a melting endotherm was observed at 109.3°C. The TGA (figure 6.6) results showed a weight loss of 3.4%, which was higher than the theoretical value for a 1:1 monohydrate. This value could include the 2.0% of the monohydrate, as well as adsorbed moisture on the surface of the glass.

6.2.3 Glass powder

The glass powder also transformed into the roxithromycin monohydrate during the solubility test. This transformation from glass to the monohydrate is clearly illustrated in figure 6.7. The percentage weight loss, as determined by TGA (figure 6.8), indicated a significant weight loss of about 27.8%, but it should be

noted that with the sample preparation for the TGA analysis, it was difficult to "dry" the glass powder completely. It was observed that this fine powder and water formed a finely dispersed, paste-like sediment, which made it difficult to dry with filter paper. It could therefore be concluded that the 27.8% weight loss was an inaccurate measurement.

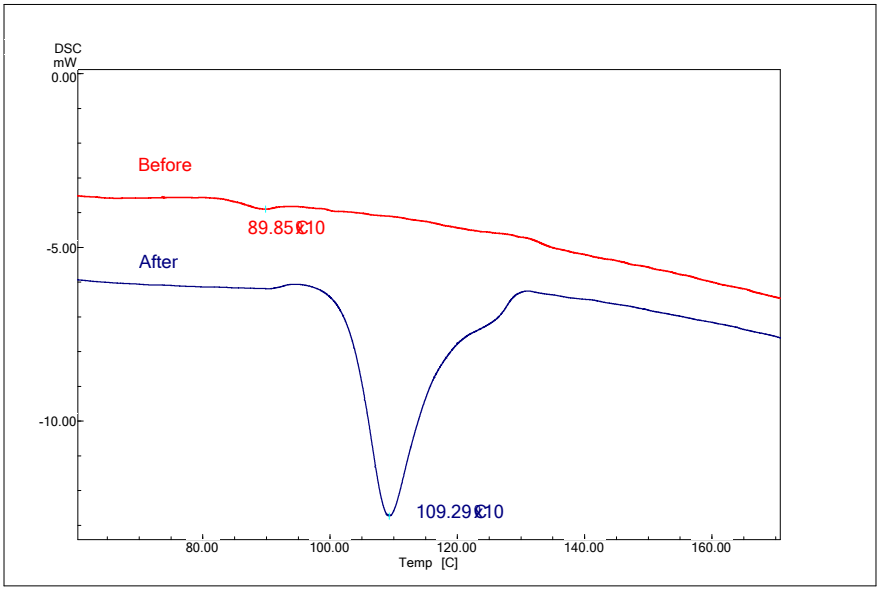

Figure 6.5: Overlay of the DSC traces of the roxithromycin glass before and after the solubility test.

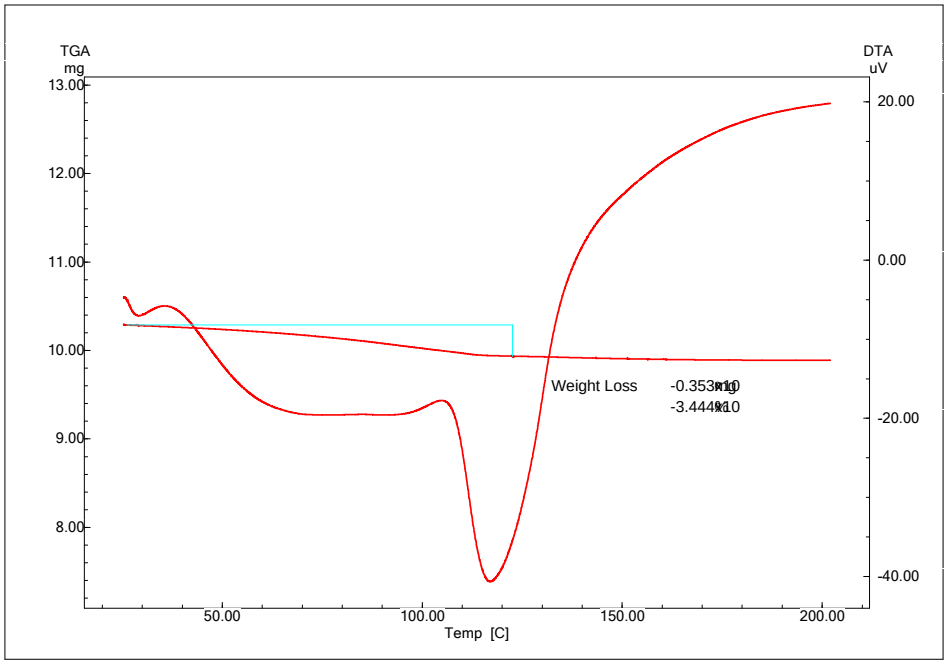

Figure 6.6: TGA thermogram of the roxithromycin glass after the solubility test.

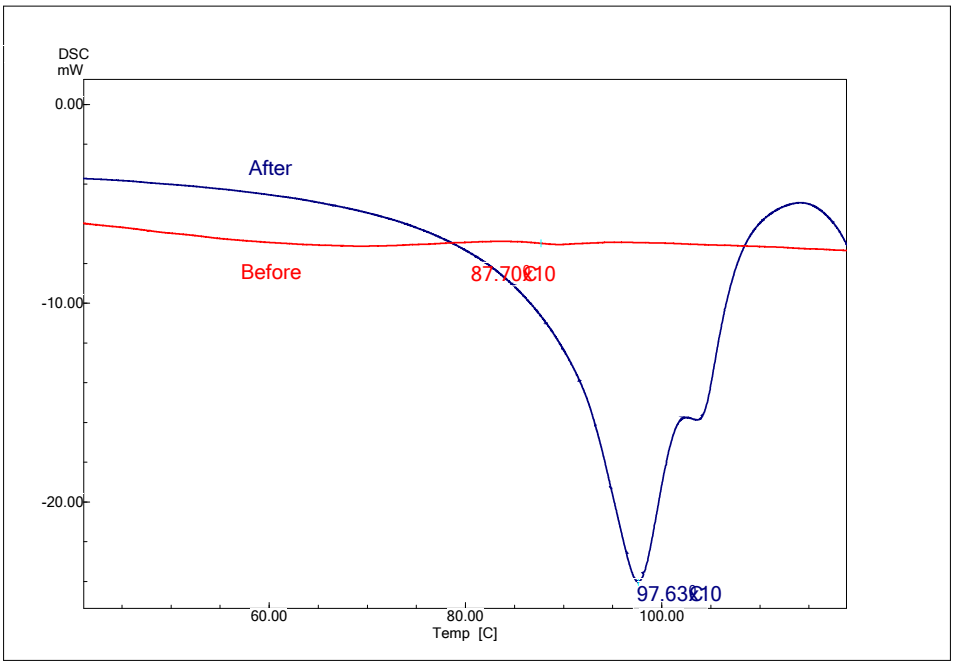

Figure 6.7: Overlay of the DSC thermograms of the roxithromycin glass powder before and after the solubility test.

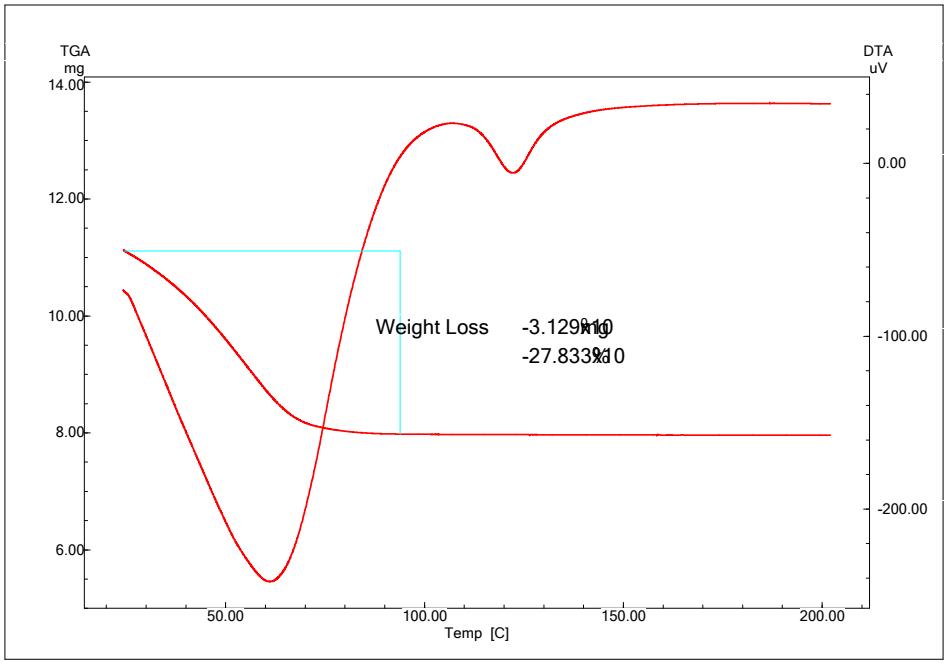

Figure 6.8: TGA thermogram of roxithromycin glass powder after the solubility test.

6.2.4 Chloroform desolvate

During the stability studies, as discussed in chapter 5, it was found that the chloroform desolvate remained stable over a four-week period at 40°C / 75% RH. After the 24-hour solubility test in water it was, however, found that the desolvate transformed into the monohydrate. The DSC thermograms showed that the melting point of the amorphous desolvate shifted from 78.7°C to 113.8°C, indicative of a roxithromycin monohydrate (figure 6.9). The TGA results (figure 6.10) showed a weight loss of about 6.1%. As this value was too high for a monohydrate, it would require further investigation. No dihydrated or trihydrated forms of roxithromycin are described in literature. Single X-ray studies would thus be required to describe these forms. Table 6.1 is a summary of the TGA results of the four samples tested.

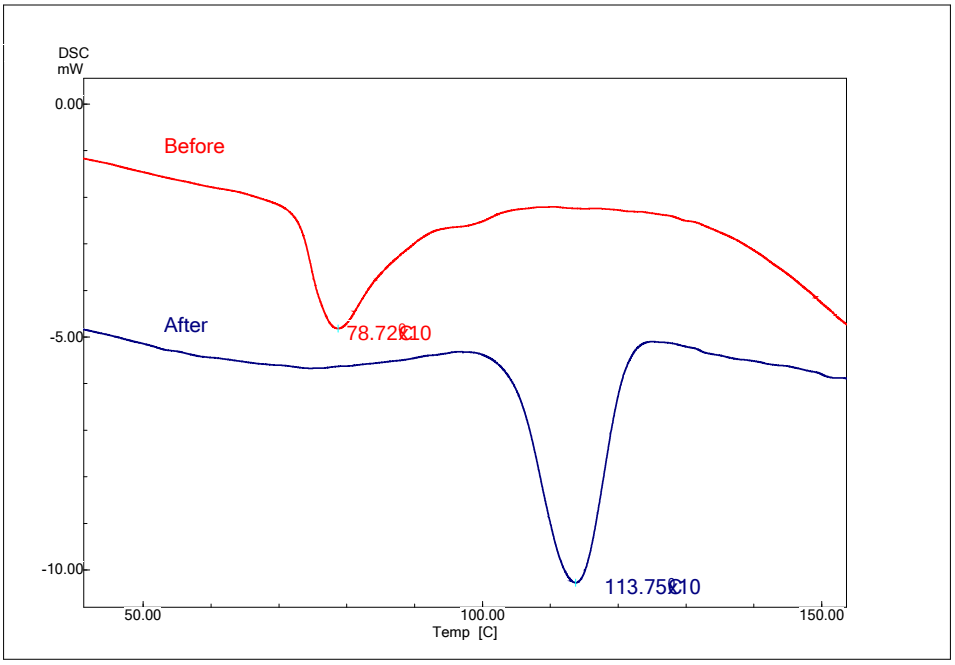

Figure 6.9: Overlay of the DSC traces of the amorphous roxithromycin chloroform desolvate after the solubility test.

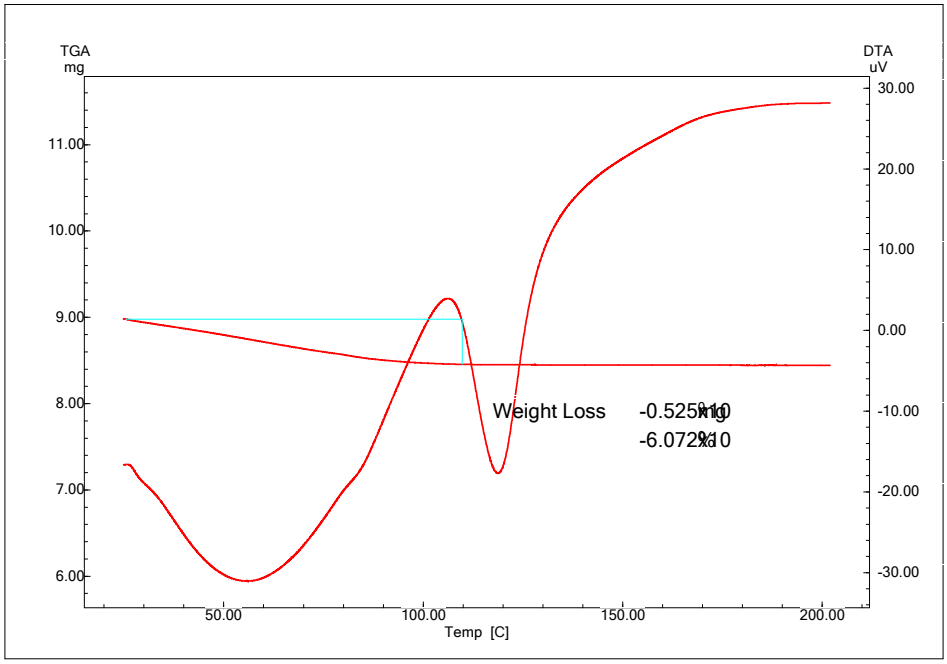

Figure 6.10: TGA thermogram of the amorphous roxithromycin chloroform desolvate after the solubility test.

Table 6.1: TGA results before and after the solubility study of the different roxithromycin forms

Roxithromycin form	Percentage weight loss (%) determined by TGA	
	Before solubility test	**After solubility test**
Monohydrate	2.0	2.0
Glass	0.6	3.4
Glass powder	0.3	27.8
Chloroform desolvate	1.8	6.1

6.3 Solubility study B

6.3.1 Solubility results

As described in paragraph 3.10.2, a solubility study was performed by adding 900 ml of distilled water to a 1,000 ml beaker, which was placed in a water bath at a temperature of 37°C ±2°C and stirred at 200 rpm. Approximately 500 mg of roxithromycin glass was added to the solvent and 1 ml of solution was extracted every 5 minutes. This test lasted for 105 minutes. Each sample taken was filtered through a 0.45 µm Millipore® filter and the concentration determined by HPLC analysis. The results were compared with the reference material. The aim of this investigation was to determine at which time the transformation into the monohydrate form occurred and whether such transformation was stable or not. It is known that roxithromycin exhibits solvent exchange within its crystal structure (Mallet *et al.,* 2003). It is also known that a monohydrate is less soluble in water and that a transformation into the stable monohydrate could negatively affect the solubility of the active. Tables 6.2 and 6.3 illustrate the solubility results of the monohydrate and glass obtained during this study.

Table 6.2: Solubility study results of roxithromycin monohydrate in water

Sample	Withdrawal time (min)	Sample area	Mean STD area	% Dissolved
RM 1	5	7366	1662958	0.7949
RM 2	10	7284	1662958	0.7852
RM 3	15	7542	1662958	0.8121
RM 4	20	7198	1662958	0.7742
RM 5	25	7306	1662958	0.7849
RM 6	30	7352	1662958	0.7889
RM 7	35	8611	1662958	0.9231
RM 8	40	9684	1662958	1.0369
RM 9	45	8493	1662958	0.9084
RM 10	50	12697	1662958	1.3565
RM 11	55	9033	1662958	0.9639
RM 12	60	9036	1662958	0.9632
RM 13	65	11666	1662958	1.2422
RM 14	70	9639	1662958	1.0252
RM 15	75	12346	1662958	1.3116
RM 16	80	13252	1662958	1.4063
RM 17	85	15294	1662958	1.6211
RM 18	90	11673	1662958	1.2359
RM 19	95	15507	1662958	1.6399
RM 20	100	11987	1662958	1.2663
RM 21	105	13501	1662958	1.4246

Table 6.3: Solubility study results of roxithromycin glass in water

Sample	Withdrawal time (min)	Sample area	Mean STD area	% Dissolved
Glass 1	5	14029	1662958	1.5139
Glass 2	10	6940	1662958	0.7481
Glass 3	15	8989	1662958	0.9679
Glass 4	20	7788	1662958	0.8376
Glass 5	25	8129	1662958	0.8733
Glass 6	30	9992	1662958	1.0723
Glass 7	35	10198	1662958	1.0931
Glass 8	40	9208	1662958	0.9859
Glass 9	45	11035	1662958	1.1803
Glass 10	50	14591	1662958	1.5588
Glass 11	55	23004	1662958	2.4549
Glass 12	60	13274	1662958	1.4149
Glass 13	65	8856	1662958	0.9429
Glass 14	70	8736	1662958	0.9291
Glass 15	75	11506	1662958	1.2224
Glass 16	80	11341	1662958	1.2035
Glass 17	85	13698	1662958	1.4519
Glass 18	90	16590	1662958	1.7565
Glass 19	95	21336	1662958	2.2564
Glass 20	100	32682	1662958	3.4524
Glass 21	105	33473	1662958	3.5319

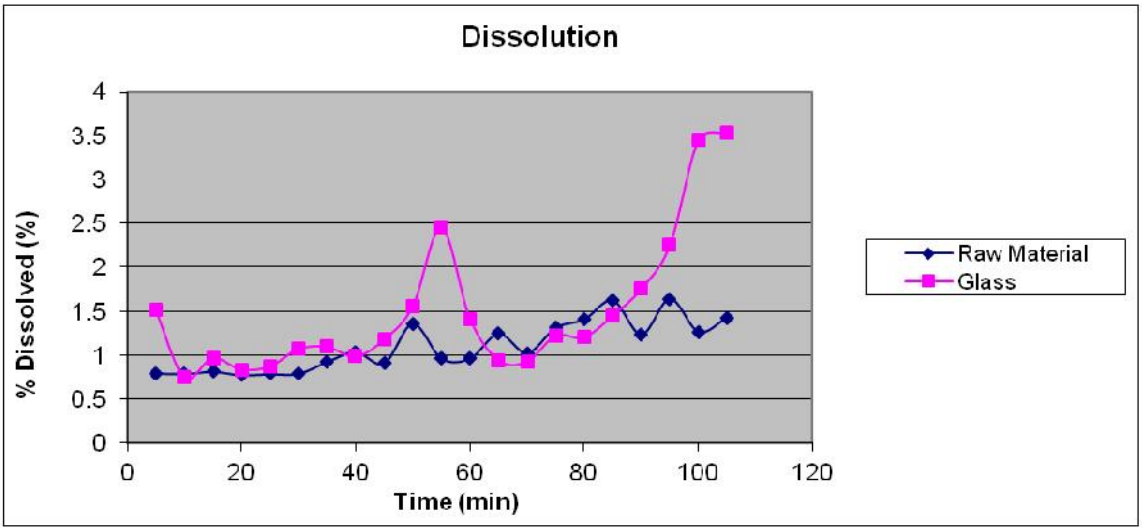

Figure 6.11: Solubility study results of roxithromycin monohydrate (raw material) and roxithromycin glass in water.

Figure 6.11 graphically illustrates the solubility study results obtained for the roxithromycin monohydrate *versus* the roxithromycin glass. A significant increase in solubility was observed for both the monohydrate and the glass for the period of 50 - 55 minutes, with the increase being more significant for the glass. The reason for this notable increase would require further investigation with a more sophisticated instrument, like the Thermal Activity Monitor (TAM), in order to determine a more accurate energy activity at a specific time interval. From these solubility profiles obtained, it was evident that the solubility values differed significantly at each time interval. As each 1 ml sample being withdrawn was not subsequently replaced, the calculation used to determine the dissolved percentage was adjusted to compensate for the decreasing volumes to obtain accurate results.

The dissolution profiles of the roxithromycin monohydrate and the glass were mostly comparable, with the largest differences at ± 55 minutes and from ± 95 - 105 minutes. The solubility of the glass was the highest at 100 – 105 minutes. The solubility values of the monohydrate remained relatively constant throughout the 105 minute test period (varying between ± 0.7 – 1.7%), with the highest solubility measured towards the end of the solubility test. The solubility

profiles of the monohydrate and the glass were very similar over the first 50 minutes.

6.3.2 Infrared analysis (IR) of dissolved roxithromycin glass

IR analysis was performed on each sample taken of the dissolved solid glass to determine whether any conversion into the roxithromycin monohydrate had occurred. Whilst the above solubility test was performed over 105 minutes, the test tubes were left in the water bath for up to 345 minutes.

The IR results illustrated that exposure of roxithromycin glass to water for 180 minutes or more resulted in the conversion into the stable roxithromycin monohydrate. This conversion would thus largely impact on the dissolution and solubility rates of this roxithromycin form. Figure 6.12 is an overlay of the IR spectra at 55, 105 and 180 minutes, whilst figure 6.13 illustrates the enlarged IR results over the 4100 – 3100 cm^{-1} range. The IR spectrum of the sample taken after 180 minutes showed the transformation into the monohydrate (figure 6.13).

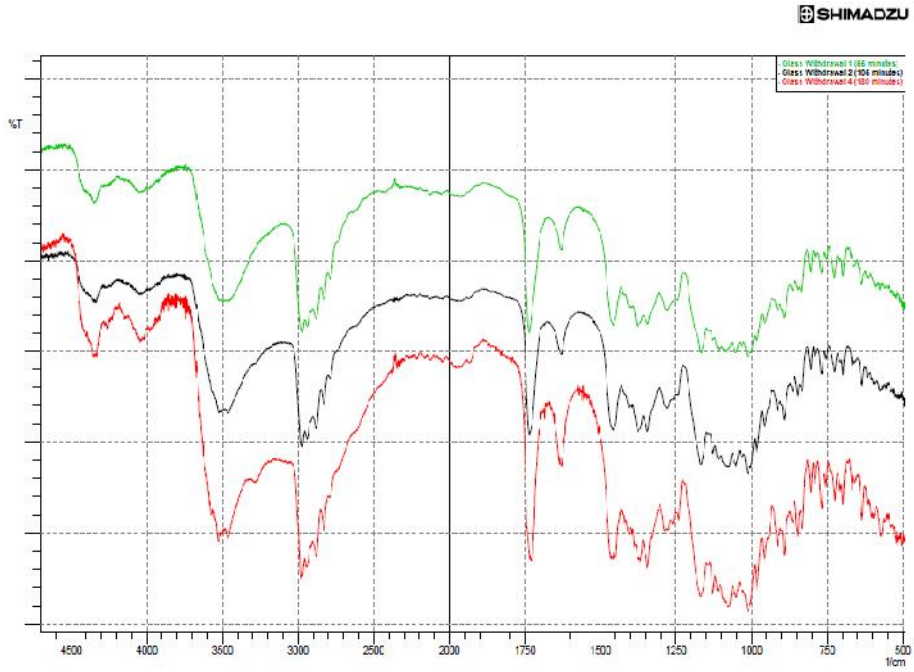

Figure 6.12: Overlay of the IR spectra of undissolved roxithromycin glass at 55 minutes (green), 105 minutes (black) and 180 minutes (red).

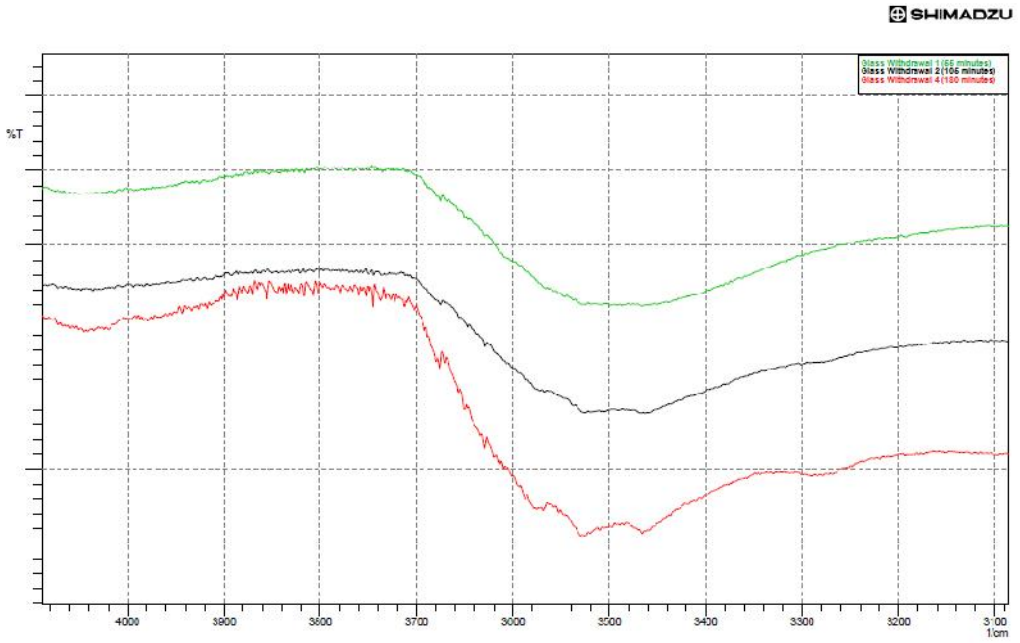

Figure 6.13: Overlay of the enlarged IR spectra of roxithromycin glass over the 4100 – 3100 cm^{-1} range, illustrating the differences in the spectra at 55 minutes (green), 105 minutes (black) and 180 minutes (red).

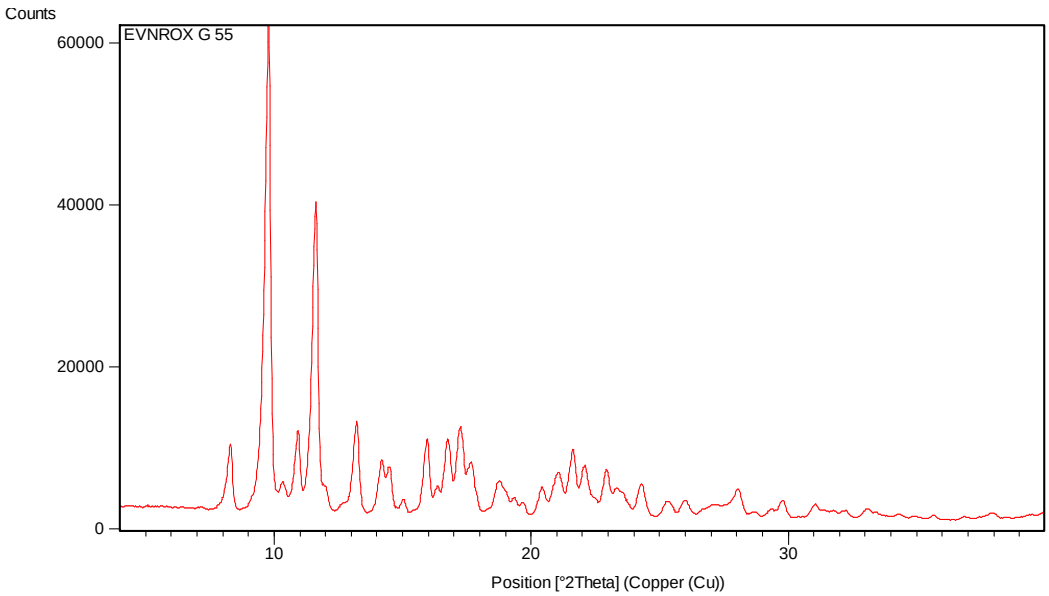

Figure 6.14: X-ray powder diffractogram of the roxithromycin glass after 55 minutes of the solubility study in water.

Figures 6.14 and 6.15 illustrate the XRPD results of the roxithromycin glass after exposure to water for 55 and 180 minutes. A clear transformation from the amorphous halo to the more crystalline state can be observed.

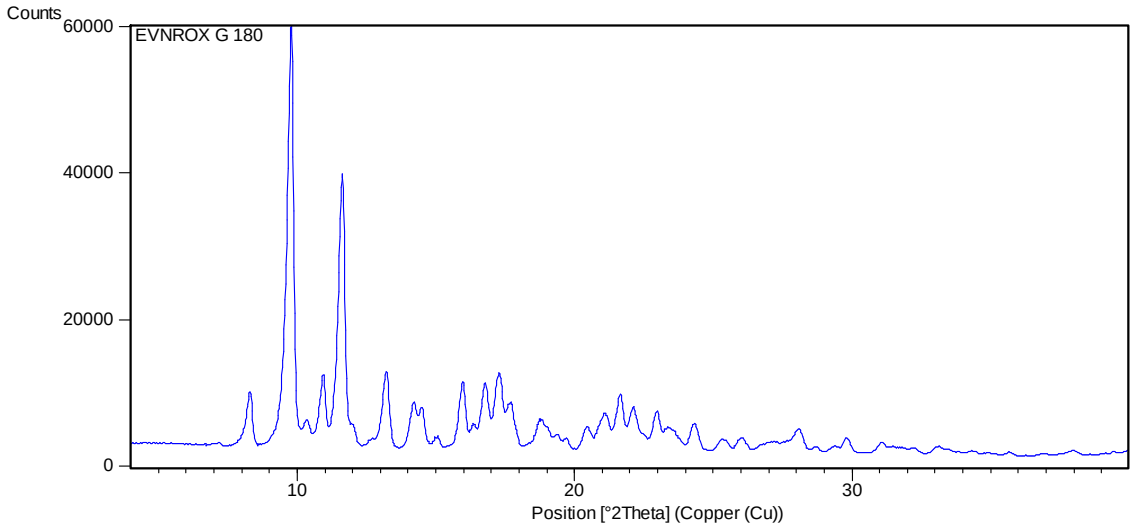

Figure 6.15: XRPD trace of roxithromycin glass after 180 minutes of the solubility study in water.

6.4 Powder dissolution

A powder dissolution was performed on the roxithromycin monohydrate (raw material) and the glass powder, according to the method being described in paragraph 3.11. 200 mg of each sample and 100 mg of glass beads were accurately weighed into test tubes and vortexed for 120 seconds. Each study was performed in triplicate.

This powder dissolution was performed in order to determine whether the dissolution would follow the same trend as the solubility studies. Figure 6.16 shows that the glass powder dissolved faster than the raw material. In both cases the dissolution was still incomplete after 1440 minutes.

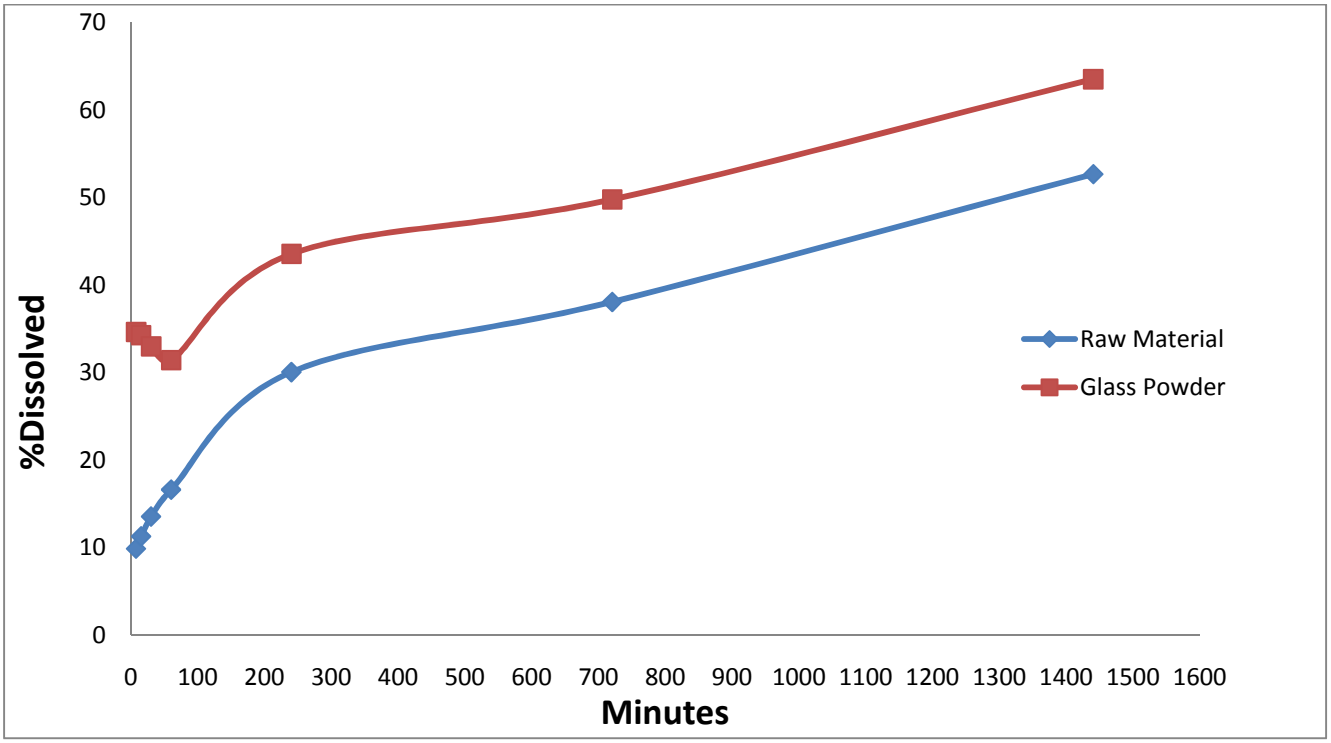

Figure 6.16: Powder dissolution results of the roxithromycin monohydrate (raw material) and the roxithromycin glass powder.

Between 0 – 60 minutes, a decrease in the dissolved percentage of roxithromycin glass powder occurred. This could have been as a result of a conversion from the amorphous to the crystalline state. At 240 minutes, a

significant increase in solubility occurred, which may have been because of the larger surface area available for dissolution. After 240 minutes, the difference between the solubility / dissolution of the roxithromycin monohydrate and the glass remained constant. Whilst the solubility studies resulted in a more complex profile for both the roxithromycin monohydrate and the glass powder, the dissolution study showed a gradual increase in solubility.

6.5 Conclusion

The three types of solubility studies that were performed during this study were discussed in this chapter. Furthermore, the effects of moisture on the roxithromycin reference material and its amorphous forms were investigated. The outcomes from the solubility studies illustrated the complexity of roxithromycin and its amorphous forms with regards to their interactions with water. Solubility study B showed that the solubility the roxithromycin amorphous forms were greatly influenced by the presence of water and that the complete transformation to the stable monohydrate occurs within approximately 180 minutes. Furthermore it was proven that the transformation to the stable monohydrate form could cause mentionable concentration differences even between samples taken 5 minutes from each other.

These studies confirmed the superior solubility of the glass and amorphous forms over the roxithromycin monohydrate (reference material). The kinetics and phase transitions that had occurred were identified as subjects for further investigation and were not within the scope of this study.

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Chapter 7

Conclusion

Many of the active pharmaceutical ingredients (APIs) being developed and used by the pharmaceutical industry in dosage forms, are poorly water soluble. With orally administered pharmaceutical actives, this may result in inadequate bioavailability and thus in ineffective treatment regimes. Similarly, roxithromycin also has a poor solubility profile and the form available on the market is mostly the stable, crystalline monohydrate.

Three amorphous, or glass-like forms of roxithromycin were prepared during this study from roxithromycin raw material (monohydrate) through quench cooling, recrystallisation from chloroform and ethyl acetate with subsequent desolvation of the solvates. It was found that the ethyl acetate desolvated form was not as amorphous as the chloroform desolvate.

Although amorphous materials tend to be more soluble than their crystalline counterparts, they are also more likely to be unstable. During this study, accelerated stability trials over a period of four weeks, together with solubility studies were performed on the roxithromycin monohydrate and the prepared amorphous forms. The influence of water on the roxithromycin monohydrate, compared with its amorphous forms, was also investigated. This investigation has led to various unanswered questions regarding the interactions between water and the amorphous forms.

Stability studies on the roxithromycin monohydrate, the roxithromycin glass and glass powder were performed with the aim of determining whether exposure of the prepared forms to elevated temperatures and relative humidity (RH) would have any effect on the stability of these forms. It was concluded that the roxithromycin glass showed stability over a one-month period of exposure to 40°C / 75% RH. After week 1 it seemed that the glass adsorbed about 2% of

moisture, but it was confined to the surface of the solid, as the X-ray powder diffraction (XRPD) pattern showed no change in the amorphous nature of the glass. Contrary, the roxithromycin glass powder tended to revert to the more stable, crystalline monohydrate after week 3 of the study. The roxithromycin glass powder at lower temperatures of 25°C and 30°C, both at 75% RH, tended to transform into the more crystalline form at week 4 of the study. These transformations were, however, not as significant as in the 40°C / 75% RH study. The sorbed moisture over the three test periods varied significantly and it was concluded that moisture was not the only catalyst for crystallisation to occur, but that the transformation from amorphous to crystalline forms was more temperature - than moisture dependant.

Stability studies on two amorphous roxithromycin desolvates (recrystallised from chloroform and ethyl acetate, followed by subsequent desolvation) were also performed in order to determine whether these amorphous forms, prepared from organic solvents, would differ with regards to the stability of the glass, prepared through heating and cooling. These amorphous forms appeared more stable than the other glass forms. No changes were observed in the IR spectra and X-ray powder diffractograms.

The three types of solubility studies being performed during this study illustrated the complexity of roxithromycin and its amorphous forms with regards to their interactions with water. Solubility study B showed that the solubility the roxithromycin amorphous forms were greatly influenced by the presence of water and that the complete transformation to the stable monohydrate occurs within approximately 180 minutes. Furthermore it was proven that the transformation to the stable monohydrate form could cause mentionable concentration differences even between samples taken 5 minutes from each other.

These studies confirmed the superior solubility of the roxithromycin glass and the amorphous roxithromycin forms over the roxithromycin monohydrate (raw material). The kinetics and phase transitions that had occurred were identified as subjects for further investigation and were not within the scope of this study.

To conclude, a stable, amorphous roxithromycin form was prepared during this study, with superior solubility over the roxithromycin raw material that is currently available on the market.

Future prospects regarding this investigation would be to formulate a dosage form for possible commercialisation purposes. Furthermore, future studies would be required to examine the energy changes that occur during the dissolution of the amorphous forms, in order to explain the significant solubility value differences obtained during the dissolution studies of this study.

Annexure

Roxithromycin Recrystallisation with Ethanol:Water mixtures

Introduction

In the previous chapters, various methods were utilised to prepare amorphous forms of roxithromycin. Two organic solvents were used during recrystallisation of roxithromycin from solvents, i.e. chloroform and ethyl acetate.

Due to the poor water solubility of roxithromycin, its solubility in a binary system of ethanol and water in various concentrations was investigated. As this investigation was performed in addition to the main objectives of this study, i.e. the investigation of amorphous forms of roxithromycin, only a summary of these results are presented in this Annexure.

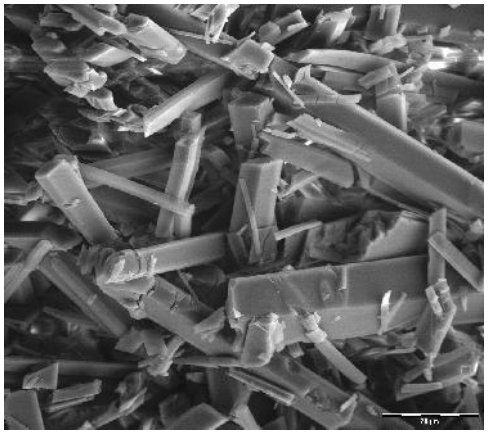
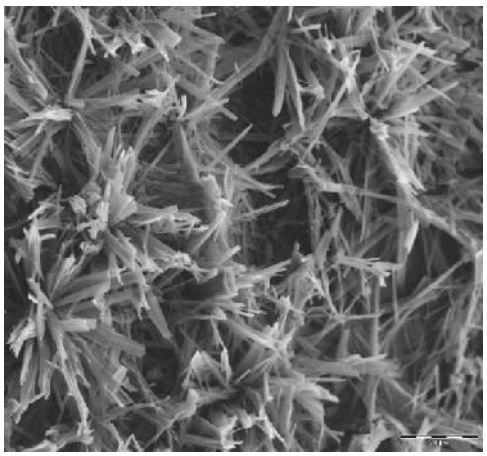
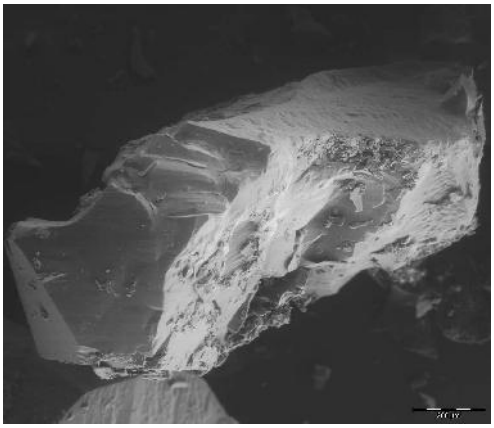
All the recrystallisation yields were well formed and crystalline, with differences only in habits. Crystals were obtained by slow evaporation of the solvent.

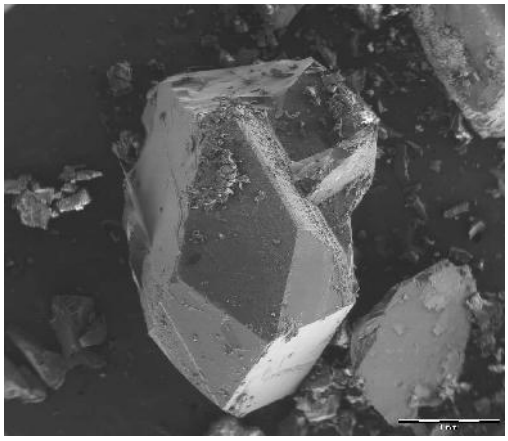
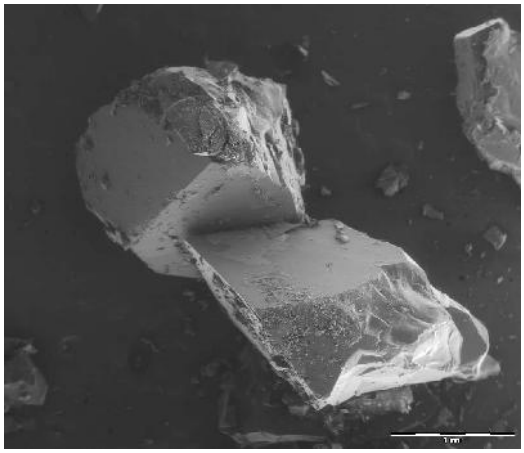
The binary systems used for these recrystallisations were 90:10 (ethanol:water), 80:20 (ethanol:water), 70:30 (ethanol:water), 60:40 (ethanol:water) and 50:50 (ethanol:water). A 100% ethanol recrystallisation was also performed. A 100% water recrystallisation was not attempted, as it would serve no purpose, due to the known poor solubility of roxithromycin in water.

The recrystallisation products of all the mixtures showed complex thermal analysis (TA) data. Most of the results suggested the possibility of a hydrate, or a solvate, or a mixture thereof. Since TG-GC analyses, together with KF analyses would be able to clarify whether the crystal form is an ethanol solvate, a hydrate or a mixture, such future investigation is recommended.

Table A1 illustrates the differences in habits obtained during this solubility study.

Table A1: Recrystallisation yields from varying water and ethanol mixtures

	100% ethanol
	90:10 Ethanol:Water
	70:30 Ethanol:Water

	60:40 Ethanol:Water
	50:50 Ethanol:Water

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Title: Stability study of amorphous roxithromycin

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Abstract

It is well known that active pharmaceutical ingredients (APIs) may exist in numerous solid states. Differences in the solid state significantly influence the physical and chemical properties of an API. The *in vivo* performance of a dosage form is also influenced by the solid state properties of a given pharmaceutical active. The physical transformation of an API into a more soluble, but metastable amorphous form is one approach towards the improvement of the bioavailability of a given drug (Ambike *et al.,* 2004). During this study, the preparation of roxithromycin glass was performed by utilising the method of quench cooling. Due to the fact that amorphous forms tend to revert to their more stable, crystalline counterparts, the glassy roxithromycin was subjected to elevated temperature and humidity during an accelerated stability study of a month. This study proved that the recrystallization of roxithromycin into the stable monohydrate was more dependent upon exposure to the elevated storage temperature, than to the relative humidity (% RH). This study showed potential for the use of glassy roxithromycin in future product development.

Keywords

roxithromycin, glassy, amorphous, stability, elevated temperatures, relative humidity

1. Introduction

Dosage form design is mainly influenced by the manufacturability, stability and bioavailability of drugs, as well as of the excipients. The majority of active pharmaceutical ingredients (APIs) are in the crystalline form (Hancock & Zografi, 1997). However, over the past decade there has been a significant increase in the number of poorly soluble pharmaceutical actives, which has heightened the interest in methods to increase their water solubility. Amorphous drugs are known to be more soluble and therefore show better bioavailability (Bahl & Bogner, 2006).

Techniques, such as quench cooling of the melt and recrystallization from a solvent could often result in the transformation of an API into an amorphous or 'glassy' phase of the active (Ambike *et al.,* 2004). Amorphous forms are more energetic than their crystalline counterparts, due to their disordered structures. Subsequently, amorphous forms tend to be more soluble with higher dissolution rates. However, amorphous forms also tend to be thermodynamically unstable and easily revert to the more stable, crystalline form (Hancock & Parks, 1999).

The stability of amorphous solids could be maintained by using the correct storage conditions. Generally, the storage conditions of amorphous forms are considered to be well below its glass transition temperature (T_g), together with protection against water vapor (Hancock & Zografi, 1997).

Roxithromycin is a 14-membered-ring, macrolide antibiotic, which is semi-synthetically derived from erythromycin A. The chemical structure of roxithromycin is shown in figure 1. Roxithromycin is a white, crystalline powder, which is very slightly soluble in water (BP, 2011). This drug has an oral

bioavailability of only 50%, due to its poor aqueous solubility. Its poor solubility thus is an obstacle in formulation development (Biradar *et al.*, 2006).

During this study, the techniques of quench cooling from the melt and recrystallization from solvents were utilized in preparing two amorphous forms of roxithromycin. Both amorphous forms were characterized initially through comparison with the pure drug (roxithromycin reference material). These forms were then subjected to stability testing at variable temperatures and a fixed relative humidity for a period of one month (four weeks).

2. Materials and methods

2.1 Materials

Roxithromycin was purchased from DB Fine Chemicals (South Africa).

2.2 Preparation of quenched cooled amorphous roxithromycin

A glassy form of roxithromycin was prepared through the process of quench cooling. The raw material was placed in a petri dish and heated to just above its melting point in a Binder® E28 oven (GmBH –Germany), with the temperature set at 125°C. After removal of the melt form the oven, it was placed on a cool surface and allowed to cool down. The glassy form being prepared through this method was subsequently powdered into a homogenous powder, using a mortar and pestle.

2.3 Analysis techniques

2.3.1 Differential scanning calorimetry (DSC)

DSC studies were performed using a Shimadzu DSC-60 (Shimadzu, Japan) system. Indium and zinc standards were used for the calibration of the DSC temperature. Approximately 6 - 9 mg of sample was sealed in aluminium cells. For the purpose of this study the sample cell lids were not pierced. The

samples were heated at a constant rate of 10°C/min over a range of 25 – 200°C. An inert atmosphere was maintained by using nitrogen as purge gas at a flow rate of 35 ml/min.

2.3.2 Thermogravimetric analysis (TGA)

A Shimadzu DTG-60 (Shimadzu, Japan) system was used during this study. Indium and zinc standards were used for the calibration of the TGA temperature. Approximately 6 - 9 mg of sample was used during analysis. The samples were heated at a constant rate of 10°C/min over a range of 25 – 200°C. An inert atmosphere was maintained by using nitrogen as purge gas at a flow rate of 35 ml/min.

2.3.3 X-ray powder diffraction (XRPD)

The XRPD diffractograms were recorded on a Philips XPert-Pro (Netherlands) diffractometer. The samples were irradiated with monochromatized CuKα radiation. The voltage and current were set at 40 kV and 45 mA, respectively. The monochromator scanning speed was 2°/min.

2.3.4 Karl Fischer titration (KF)

A Metrohm 870 KF Titrino Plus (Metrohm, Switzerland), equipped with a Metrohm 803 Ti Stand (Metrohm, Switzerland), was calibrated with purified water, whilst sodium tartrate dihydrate was used in the water determinations during this study.

2.3.5 Stability studies

Both the roxithromycin raw material (reference material) and the amorphous forms were subjected to elevated temperatures and relative humidity conditions of 25°C / 75% RH, 30°C / 75% RH and 40°C / 75% RH. The stability was

monitored frequently over the four-week period. Sampling occurred at onset (initial) and thereafter weekly, with subsequent analysis of the samples by means of DSC, TGA, XRPD, and KF.

3. Results and discussion

3.1. Differential scanning calorimetry (DSC)

Roxithromycin raw material showed a melting endotherm at 123.9°C (figure 2). The glassy amorphous form of roxithromycin was confirmed, due to the presence of a clear glass transition (T_g) at 89.6°C (figure 3). The method of quench cooling of the melt therefore proved an appropriate method for the preparation of the glassy form of roxithromycin.

Figure 4 illustrates the DSC traces obtained for the roxithromycin reference material over the period of four weeks. From these traces it was evident that no transformation had occurred during the storage period of four weeks at 40°C / 75% RH, nor during storage at 25°C / 75% RH or 30°C / 75% RH. However, the DSC traces of the glassy roxithromycin showed significant changes, indicative of a transformation into the monohydrated form of roxithromycin, after a period of 4 weeks at 40°C / 75% RH (figure 5). A shift in the glass transition (T_g) was visible through the development of an endotherm at approximately 111.4°C. This change suggested that recrystallization into the more stable monohydrate of roxithromycin had occurred. The small endotherm at 111.4°C also suggested that a partial recrystallization may have occurred and that complete reversion to the crystalline state was time dependent.

The results from those samples stored at 25°C / 75% RH and 30°C / 75% RH did not show the same transformation after four weeks (figure 6). Although a shift in the glass transition temperature (T_g) was observed for these samples, no melting endotherm developed, and therefore no transformation into the crystalline monohydrate of roxithromycin had occurred.

3.2 Thermogravimetric analysis (TGA)

A theoretically calculated weight loss of 2.1% is indicative of a monohydrated form of roxithromycin. The weight loss percentage of 2.0% obtained for the roxithromycin raw material during this study thus correlated well with the theoretical value. TG analysis of the glassy form of roxithromycin showed a percentage weight loss of 0.6%, which was indicative of the anhydrous character of the amorphous roxithromycin glassy form.

The average percentage weight loss of the roxithromycin monohydrate raw material, over the four-week period at all the storage conditions, did not differ significantly and was it evident that no transformation of the raw material had occurred during the stability study. The roxithromycin raw material showed an average percentage weight loss over the four weeks of 2.6%. This confirmed the theoretical weight loss of a roxithromycin monohydrate, with the excess of 0.5% probably being due to surface moisture.

Table 1 summarizes the weight loss percentages of roxithromycin glass powder. This data was obtained from the weekly TG analysis over a period of a month. With the test conditions at 25°C / 75% RH, the moisture being adsorbed gradually, increased from 0.7% to an average of 2.0% after weeks 3 and 4. Adsorption occurred significantly faster at the 30°C / 75% RH conditions. From initial to week 1, the adsorbed value increased from 0.7% to 1.9%. The initial value of the 40°C / 75% RH study was significantly lower than those of the other two samples, stored at the lower temperatures. At the lower temperatures also, it took four weeks to reach a sorption value of approximately 2.0%. This increase confirmed the fact that amorphous solids tend to absorb a substantial amount of water vapors, due to higher free energy (Crowley & Zografi, 2002).

3.3 Karl Fisher analyses (KF)

Table 2 summarizes the percentage of water content obtained for roxithromycin raw material and for the roxithromycin glass powder. The results differed

substantially from those obtained through TG analyses. The reason for these notable differences could be attributed to the fact that dehydration and melting of roxithromycin occurred almost simultaneously (figure 6). An accurate differentiation between dehydration and melting was not possible, given the broad endotherm. Therefore, it was suggested that the analysis of the stability samples of roxithromycin should rather be performed by means of Karl Fischer.

The anhydrous state of the amorphous roxithromycin glass form was confirmed by the water content being measured as 0.92%. The transformation of roxithromycin from the amorphous form to the monohydrate was evident, due to the increase in water content to 2.40%. This correlated well with the water percentage obtained for roxithromycin raw material when subjected to the same storage conditions over the same period of time (four weeks). The transformation from a complete amorphous form into a partially crystalline form was thus confirmed.

3.4 X-ray powder diffraction (XRPD)

XRPD analyses of roxithromycin monohydrate raw material did not show any significant changes during the stability study period of four weeks (figures 8 and 9). However, the conversion into the more crystalline monohydrated form of roxithromycin was clearly evident from the XRPD results obtained for the powdered glassy roxithromycin, stored at 40°C / 75% RH for four weeks. Figure 10 is an overlay of the X-ray powder diffractograms of roxithromycin glass powder, stored at 25°C / 75% and 30°C / 75% RH for four weeks. Both figures 9 and 10 show the developing diffraction peaks at approximately 9.9°2θ, for the two higher storage temperatures.

4. Conclusion

The manufacture of amorphous forms of active pharmaceutical ingredients, in order to improve the solubility and dissolution characteristics of poorly water soluble drugs, currently attracts much interest among pharmaceutical scientist.

During this study, amorphous (glassy) roxithromycin was successfully prepared through quench cooling. During the stability study at 40°C / 75% RH over four weeks, a gradual reversion of amorphous roxithromycin into the roxithromycin monohydrate was observed. Although at the lower storage temperatures (25°C and 30°C, both at 75% RH) more sorption was measured at an earlier stage, no transformation into the more crystalline form was observed prior to week 4. For the samples being stored at 40°C / 75% RH, the absorbed value was 0.7% after week 3, when the crystalline transformation started.

It seemed that the lower temperatures (25°C and 30°C) did not induce any significant changes over the first 3 weeks of the stability study. The XRPD showed transformation at week 4 at both these temperatures. It therefore seemed evident that the phase transformation of the glass powder was temperature dependant, irrespective of the moisture adsorbed over the duration of the stability period. It could hence be concluded that the transformation into the more crystalline form was rather temperature – than moisture dependant.

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List of figures

Figure 1: **Molecular structure of roxithromycin raw material.**

Figure 2: **DSC thermogram of roxithromycin raw material.**

Figure 3: **DSC thermogram of glassy roxithromycin prepared through quench cooling.**

Figure 4: **Overlay of DSC thermograms of roxithromycin monohydrate raw material over a period of four weeks stored at 40°C / 75% RH (weekly sampling interval).**

Figure 5: **Overlay of DSC thermograms of glassy roxithromycin over a period of four weeks stored at 40C / 75% RH (weekly sampling interval).**

Figure 6: **Overlay of DSC thermograms of glassy roxithromycin over a period of four weeks stored at 25°C / 75% RH and 30°C / 75% RH (weekly sampling interval).**

Figure 7: **TG analysis of roxithromycin raw material indicating a weight loss percentage of 2.0%.**

Figure 8: **X-ray powder diffractograms of roxithromycin monohydrate raw material over a period of four weeks (weekly sampling interval).**

Figure 9: **X-ray powder diffractograms of roxithromycin glass powder over a period of four weeks, (a) initial, (b) week 1, (c) week 2, (d) week 3, (e) week 4.**

Figure 10: **Overlay of X-ray powder diffractograms of glassy roxithromycin powder over a period four weeks stored at 25°C / 75% RH and 30°C / 75% RH (weekly sampling interval).**

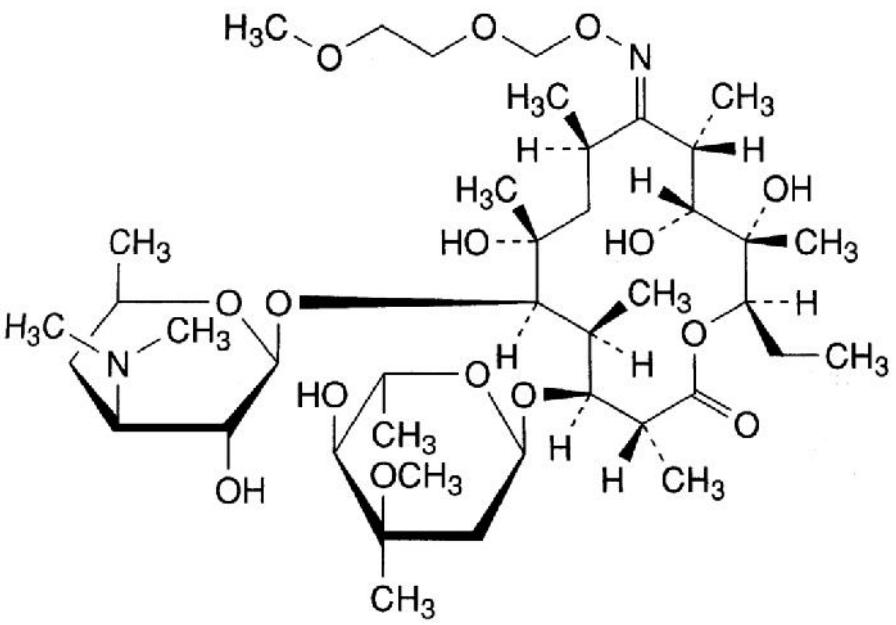
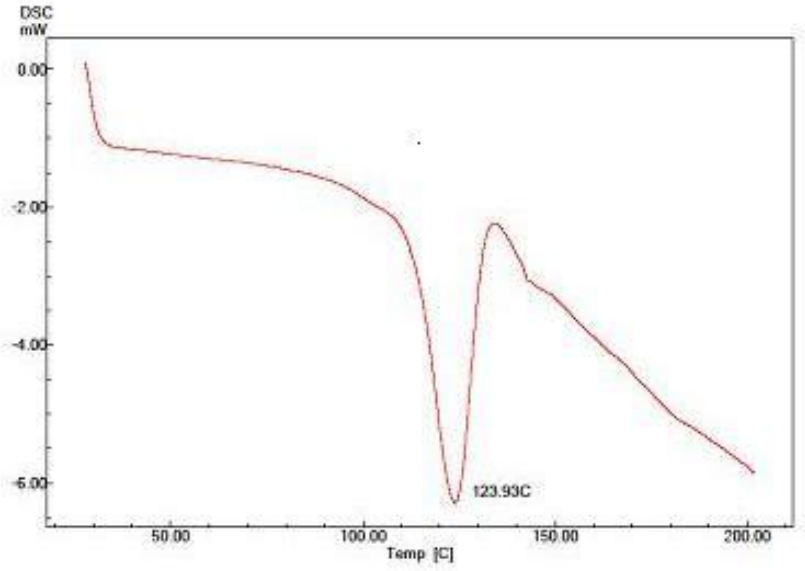


Figure 1



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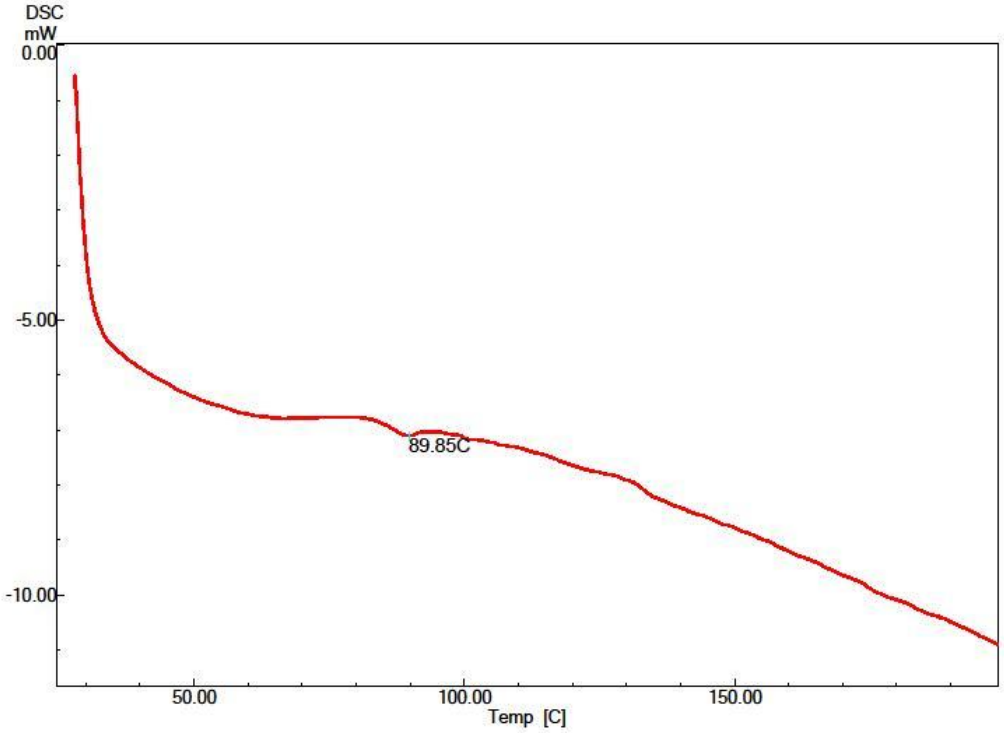

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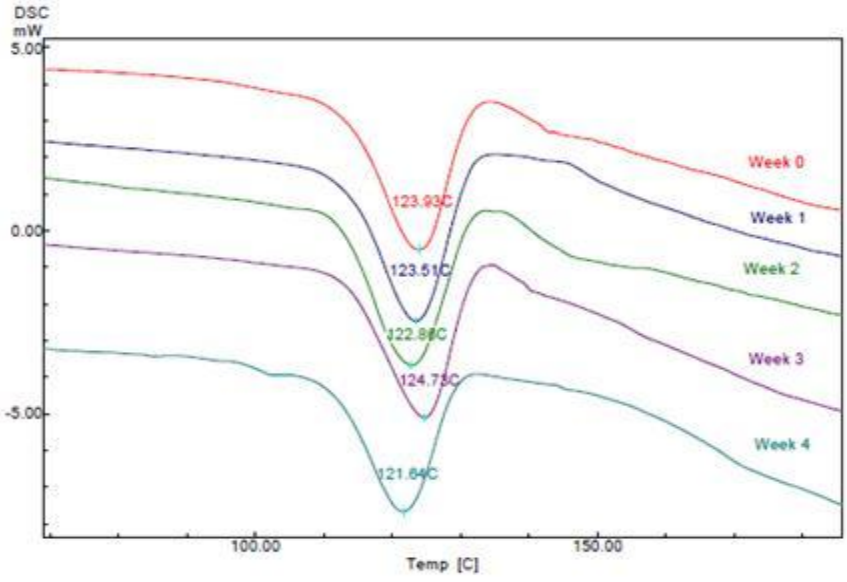

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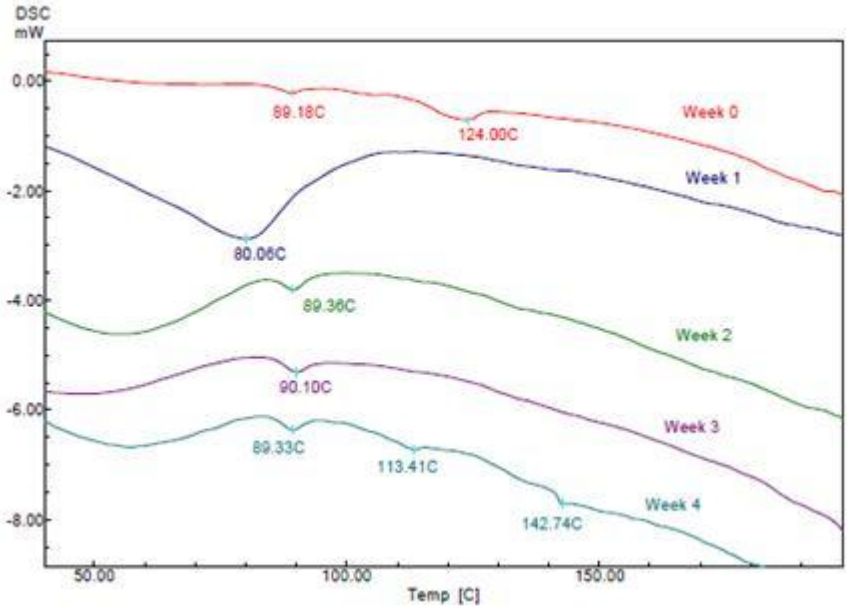

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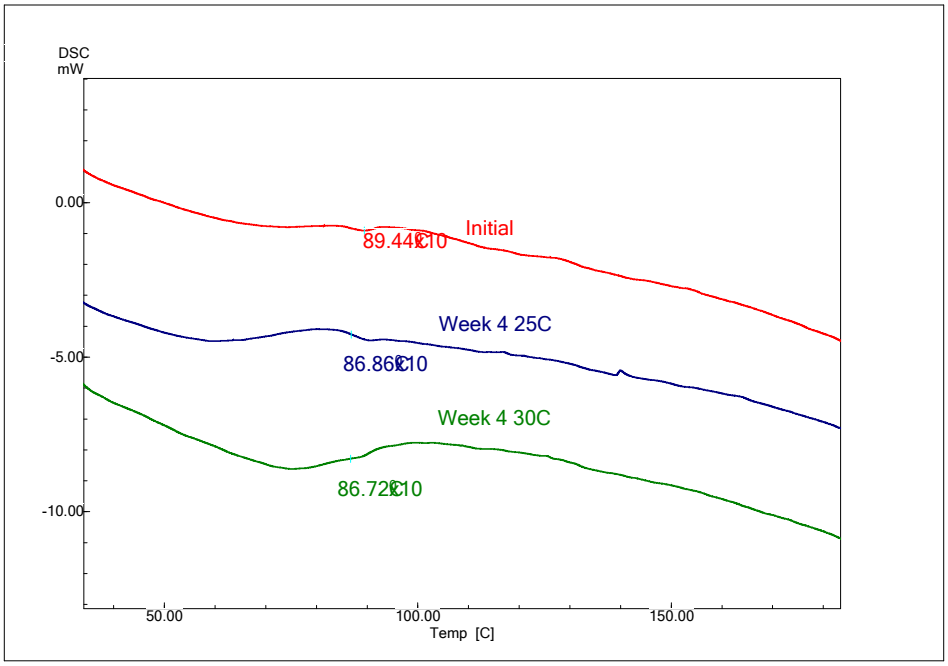

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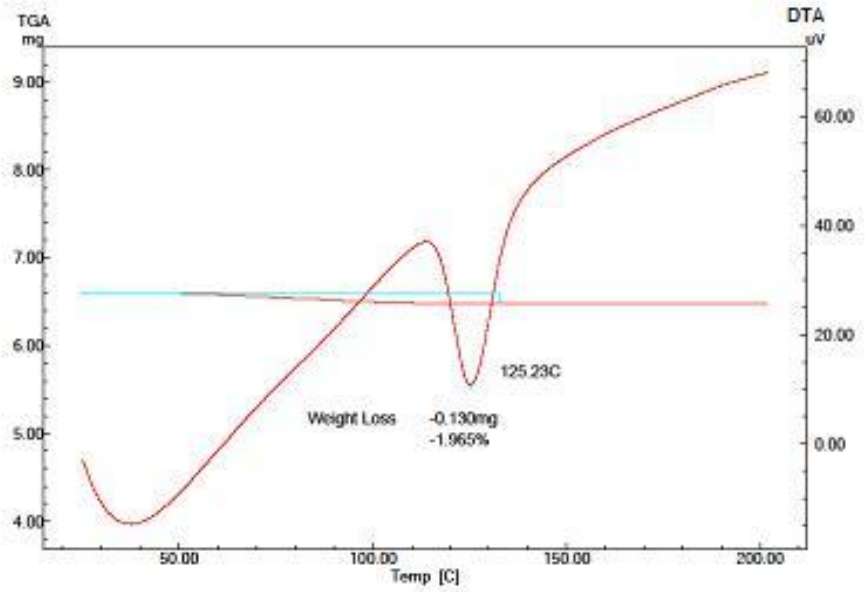

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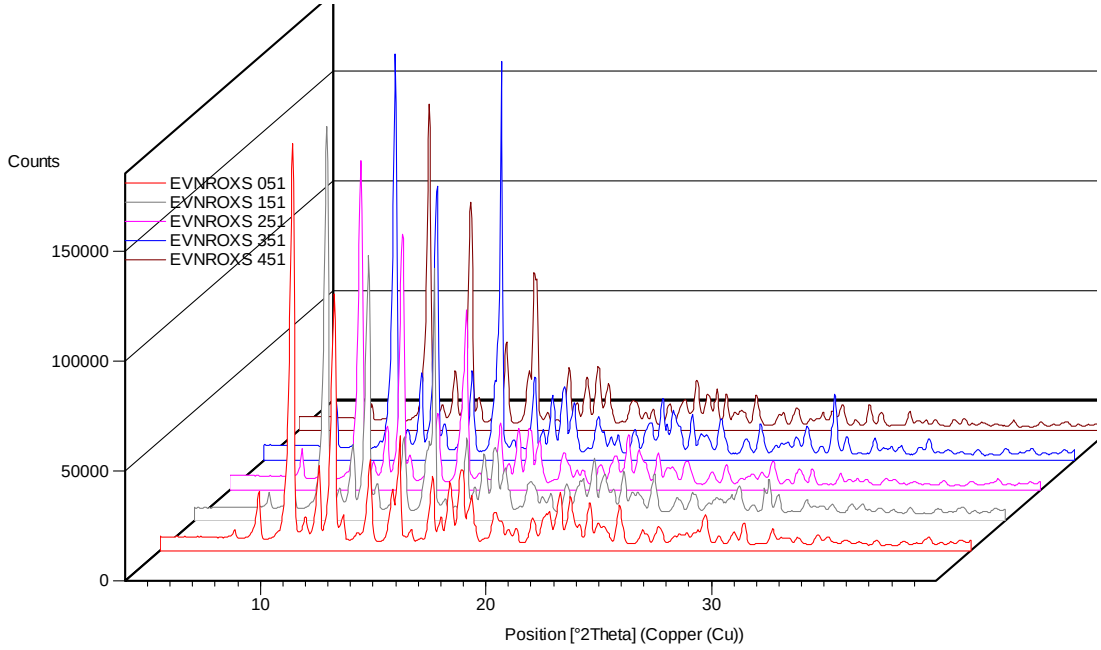

Figure 8

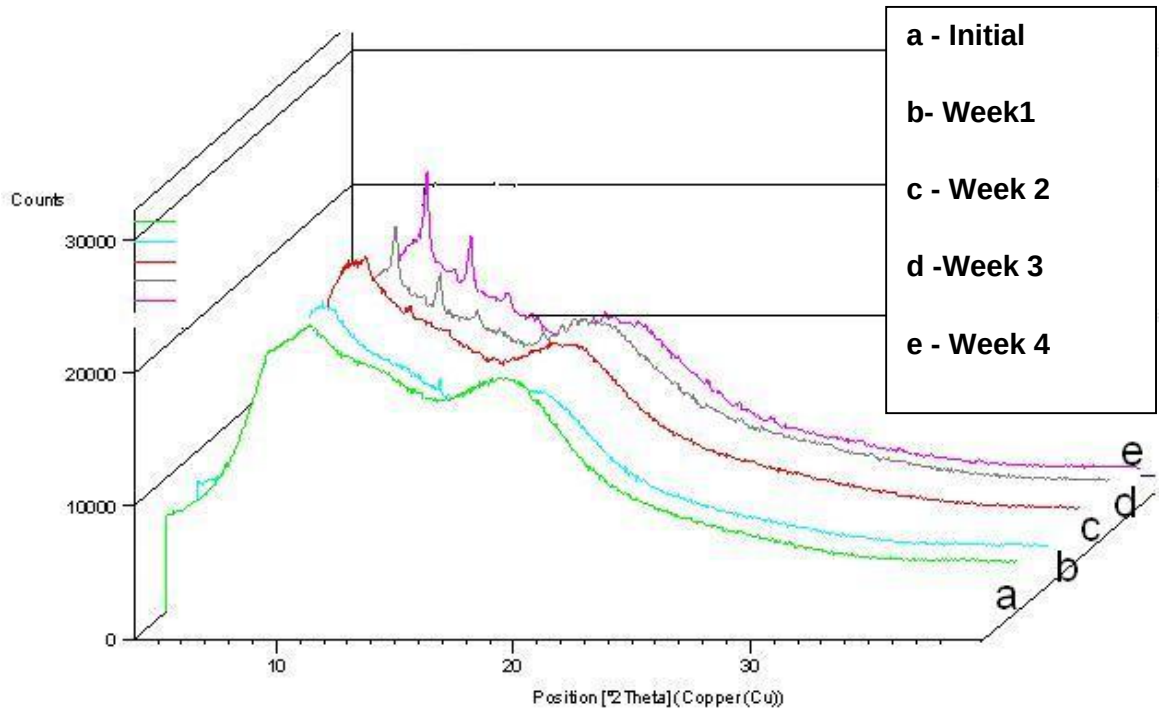

Figure 9

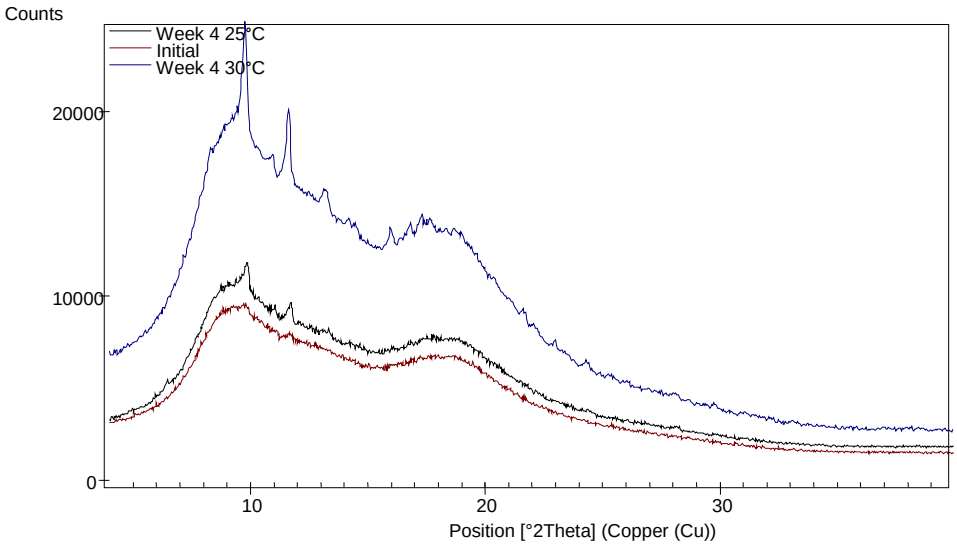

Figure 10

List of tables

Table 1: **TG analysis results of roxithromycin glass powder over a period of four weeks stored at 25°C, 30°C and 40°C at 75% RH (weekly sampling interval).**

Table 2: **TG analysis results of roxithromycin raw material and roxithromycin glass powder over a period of four weeks stored at 40°C / 75% RH.**

Table 1

	Glass powder - % weight loss at 75% RH					
	Initial	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
25°C	0.75	0.93	1.19	2.26	1.95	*N / A
Habit / form	Amorphous	Amorphous	Amorphous	Amorphous	Crystalline transformation starts	-
30°C	0.75	1.91	2.32	2.00	2.14	*N / A
Habit / form	Amorphous	Amorphous	Amorphous	Amorphous	Crystalline transformation starts	-
40°C	0.16	0.15	0.57	0.71	0.65	1.97
Habit / form	Amorphous	Amorphous	Amorphous	Crystalline transformation starts	More crystalline	More crystalline

Table 2

	% weight loss				
Roxithromycin	**Initial**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Raw material	2.60	2.71	2.75	2.66	2.72
Glass	0.91	2.51	2.54	2.58	2.60
Glass powder	0.92	2.36	2.14	2.03	2.40