



Solubility enhancement of selected anti-malarial drugs

A Louwrens



orcid.org / 0000-0002-2445-2904

Dissertation accepted in fulfilment of the requirements for
the degree Master of Science in Pharmaceutics at the
North-West University

Supervisor:	Dr L Badenhorst
Co-supervisor:	Prof A Wessels
Co-supervisor:	Prof W Liebenberg
Assistant-Supervisor:	Dr R Lemmer

Graduation: May/June 2024

This dissertation is dedicated to Henk and Claudette Louwrens

Acknowledgements

“The beautiful thing about learning is that nobody can take it away from you.”

– B. B. King

My Dad, Henki, I can never thank you enough for the opportunities you have granted me and for allowing me the freedom to choose my own path in life. I am grateful for every pep-talk every word of encouragement and your never wavering support. You are the most incredible father, and I could not have asked for anyone better to be my guiding light and sound of reason.

My sissie, Abby. Thank you for always believing in me and for showing me what persistence and hard work looks like. You are a true example of how passion drives success.

My baby sis, Smiley Miley. Mila, thank you for understanding why I had to be away from home for so long. You are such an inspiration in my life, and I am privileged to watch you grow into such a stunningly strong little girl.

My Auntie Ell, my heart is filled with appreciation for you, Uncle Jochen and the boys. You were my home away from home for many years. You were never too busy for a phone call or a quick check in. I am very grateful for every late-night talk around the kitchen counter over a cup of tea and all the years of comfort and support. You believed in me when I couldn't believe in myself.

Nanna and Pappa, thank you for every weekly phone call and for your constant support. I am forever grateful for having you both in my life for so many years. You are such an inspiration.

My Lallie, thank you for accompanying me on every late night in the lab, for being a listening ear through all the ups and downs. For supporting me through some of the toughest years of my academic career and for always motivating me to keep going. I love you.

The van Onselen's, thank you for the tightest support system I could have asked for. For every call, farm visit and word of encouragement. I am forever grateful for your input over the past 9 years.

Melinde, thank you for helping me build the foundation of my studies. Without your constant love, support, and unwavering friendship I would not have reached this point in my life. I am forever grateful to call you, my friend.

Thank you to the Language directorate of the NWU for providing me with a job for the past 7 years. Without that opportunity I would not have been able to further my studies with ease.

Thank you for allowing me to continuously build on my knowledge within the Pharmacy program.

To the NWU, thank you for the opportunity to further my studies and for providing me with all the tools needed to become this version of myself.

Dr B, thank you for seeing my potential and for taking the chance on me. It was a true honour to not only get to learn from you within the pharmaceutical scope but to also learn from you in life. You have helped shape me in more ways than I can thank you for. I salute you.

Prof Anita “HPLC” Wessels. Thank you for being our saving grace in the lab. If there is anybody who can run a HPLC with their eyes closed and hands tied behind their back, it would be you. Thank you for allowing me to learn so much from you, it has been some of the most enriching moments in my life.

Prof Wilna Liebenberg, I have never experienced somebody so full of knowledge and so willing to share said knowledge. It was a true privilege to get to spend this academic journey with you. I will forever look back at every moment spent with you with great fondness.

Dr Lemmer. Our true “Sheldon”. Only a lot smarter and miles funnier. I appreciate all your help throughout this journey. Your expertise regarding all the apparatus I worked on is commendable. I would be privileged to one day be half as smart as you are.

Prof Marius Brits, I want to thank you for playing such a supportive role in the past 3 years of my studies. It was an honour and a privilege to get to work alongside you. You have a true passion for your profession, and to be able to witness your work was inspiring. The role you played in my life during this time is something I will treasure for the rest of my life.

Pikkie, I would never have wanted to share a lab with anybody else but you. Thanks for being the best partner throughout this journey. For every moment of co-working in the office, lab mishap, lunch run and heart to heart. You are a true friend, and I thoroughly enjoyed this experience with you. Would not have had it any other way.

Mikal and Jealandri, I could never have dreamed of a better friendship and support team. Having you in my life has been a blessing.

Madelein, the TAM queen. Thank you for all your help.

Oom Neil, Safia and Claudia. Your help in the lab was crucial to this study, and all I can say is thank you.

To those who started this journey with me but are no longer a part of it. I can be grateful for the roles you played and thank you for it.

Abstract

Malaria remains a problem experienced globally, with 227 million cases being documented in 2019 by the “World Malaria Report”. Another noted statistic is the occurrence of about 54.7 million pregnancies in areas of continuous malaria transmission. The most prevalent malaria parasite species found in Sub-Saharan Africa is *Plasmodium falciparum* and infection by this species contributes significantly to neonatal and maternal death statistics. Development of resistance by *P. falciparum* to chloroquine and sulfadoxine–pyrimethamine has led to the recent adoption by The World Health Organization (WHO), of artemisinin-based combination therapies (ACTs) as the first line of treatment against uncomplicated malaria in pregnant woman. There is, however, a problem regarding the solubility profiles of sulfadoxine, pyrimethamine and artesunate, with the water solubility of these drugs referred to as ‘very slightly soluble’, ‘practically insoluble’ and ‘poorly soluble,’ respectively.

This study aims to address the limited aqueous solubility of these drugs, by means of the formulation of amorphous solid dispersions (ASDs) as a promising strategy to overcome the solubility challenges associated with the mentioned APIs.

The experimental methodology involved the systematic preparation of ASDs by means of combining the individual APIs with PVP30, PVPVA64 and HPMC. The ASDs were prepared by the solvent evaporation method whereafter physico-chemical characterisation was employed through techniques such as X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), polarised light microscopy (PLM), Fourier-transform infrared spectroscopy (FT-IR) and thermal analysis monitoring (TAM). This was done to ensure the successful formation of each ASD.

Solubility studies were performed on the ASDs and the profiles obtained were compared to that of the original individual APIs. Furthermore, the researcher integrated statistical analysis to evaluate the significance of various formulation parameters and their impact on the solubility enhancement of sulfadoxine, pyrimethamine and artesunate. The combination of physico-chemical characterisation and statistical analysis contributes valuable insights into the properties of each ASD. The outcomes of this research study hold significant implications for the pharmaceutical field, offering a promising avenue for improving the aqueous solubility of three essential APIs in the treatment of malaria.

Successful ASD formation was achieved in 7 out of the 9 formation attempts when reviewing the physico-chemical characterisation. Solubility studies were still performed on all attempted formations (9 in total). The following results were obtained regarding the solubility profiles of the ASDs. For sulfadoxine, the solubility was most improved when combined with HPMC,

followed by PVP64 and PVP30. Pyrimethamine produced two ASDs with PVP30 and PVP64 which were not fully amorphous, however, the solubility results were superior to that of the other ASD (pyrimethamine and HPMC) and pyrimethamine single component. Lastly artesunate delivered an unsuccessful pivot solubility test regarding the ASD containing PVP64. The solubility results ranked accordingly, Artesunate with PVP30 > Artesunate with HPMC > Artesunate single component. In all instances the individual API solubilities were improved upon combination with the various polymers in the formation of ASD (whether fully amorphous or semi-amorphous).

Key words: Malaria; Amorphous solid dispersions; Solubility; Sulfadoxine; Pyrimethamine; Artesunate.

Abbreviations

ACT - Artemisinin-based combination therapies
API - Active pharmaceutical ingredient
ART - Artesunate
ASD - Amorphous solid dispersion
BCS - Biopharmaceutical classification system
DSC - Differential scanning calorimetry
EDL/EML - Essential drug/medicines list
FTIR - Fourier transform infrared spectroscopy
HPLC - High-performance liquid chromatography
HPMCAS - Hydroxypropyl methylcellulose acetate succinate
IPTp - Intermittent preventive treatment in pregnancy
IPTp-SP - Intermittent preventive treatment in pregnancy utilising sulfadoxine-pyrimethamine
PABA - p-aminobenzoic acid
PCR - Polymerase chain reaction
PLM - Polarised light microscopy
PM - Physical mixture
PVP - Polyvinylpyrrolidone
PYRI - Pyrimethamine
RBC - Red blood cell
RDT - Rapid diagnostic tests
RM - Raw material
STA - Simultaneous thermal analysis
SULFA - Sulfadoxine
TAM - thermal activity monitor
UP - Ultrapure
WHO - World health organization
XRPD - X-Ray powder diffraction

Table of Contents

Acknowledgements	iii
Abstract	v
Abbreviations	vii
Chapter 1: Introduction	1
1.1 Introduction	1
1.2 Research Problem	2
1.3 Aim & Objectives	2
1.4 Chapter layout of dissertation	3
References	4
Chapter 2: Literature review	5
2 Introduction to Malaria	5
2.1 Epidemiology	6
2.1.1 Symptoms and Diagnosis	7
2.1.2 Treatment	9
2.1.2.1 Uncomplicated malaria	9
2.1.2.2 Complicated malaria	9
2.2 Sulfadoxine	10
2.2.1 Physico-chemical properties	10
2.2.2 Pharmacology	11
2.2.2.1 Indication	11
2.2.2.2 Mechanism of action	11
2.2.2.3 Dosage and administration	11
2.3 Pyrimethamine	12
2.3.1 Physico-chemical properties	12
2.3.2 Pharmacology	12
2.3.2.1 Indication	12
2.3.2.2 Mechanism of action	13
2.3.2.3 Dosage and administration	13
2.4 Artesunate	14
2.4.1 Physico-chemical properties	14
2.4.2 Pharmacology	15
2.4.2.1 Indication	15

2.4.2.2 Mechanism of action	15
2.4.2.3 Dosage and administration	15
2.5 API solubility, dissolution, and miscibility	16
2.5.1 Factors influencing the solubility of drugs	18
2.5.1.1 Temperature	18
2.5.1.2 pH	18
2.5.1.3 Solid-state properties	18
2.5.1.4 Solid dispersions	19
2.5.1.5 Co-crystals and salts of active pharmaceutical ingredients	20
Reference List	21
Chapter 3: Methods & Materials	28
3 Introduction	28
3.1 Materials	28
3.1.1 Polymers used as matrix formers in the ASDs	28
3.1.1.1 Polyvinylpyrrolidone (PVP)	28
3.1.1.2 Hydroxypropyl methylcellulose acetate succinate (HPMCAS)	29
3.2 Methods	29
3.2.1 Preparation of physical mixtures and amorphous solid dispersions	29
3.2.2 Physico-chemical characterisation	30
3.2.2.1 Simultaneous thermal analysis (STA)	30
3.2.2.2 Fourier transform infrared spectroscopy (FTIR)	31
3.2.2.3 X-ray powder diffraction (XRPD)	31
3.2.2.4 Polarised light microscopy (PLM)	31
3.2.2.5 Thermal activity monitor (TAM)	32
3.2.3 High-performance liquid chromatography (HPLC)	32
3.2.4 Solubility studies	33
3.2.4.1 APIs and ASDs	33
3.2.5 Statistical Analysis	34
Reference List	35
Chapter 4: Results & discussion	37
4 Introduction	37
4.1 Sulfadoxine and ASDs	38
4.1.1 Physico-chemical characterisation of sulfadoxine and ASDs	38
4.1.1.1 Differential scanning calorimetry	38

4.1.1.2 Fourier-transform infrared spectroscopy (FTIR)	40
4.1.1.3 X-ray powder diffraction (XRPD)	43
4.1.1.4 Polarised light microscopy	44
4.1.1.5 Thermal activity monitoring (TAM)	46
4.1.2 Validation of HPLC method for sulfadoxine and ASDs	48
4.1.3 Solubility studies of sulfadoxine and ASDs	50
4.1.3.1 Sulfadoxine preliminary solubility studies	50
4.1.3.1.1 SPVP30 ASD preliminary solubility studies	52
4.1.3.1.2 SPVP64 ASD preliminary solubility studies	53
4.1.3.1.3 SHPMC ASD preliminary solubility studies	55
4.1.3.2 Sulfadoxine API and ASD pivot solubility studies	57
4.2 Pyrimethamine and ASDs	59
4.2.1 Physico-chemical characterisation of pyrimethamine API and ASDs	59
4.2.1.1 Differential scanning calorimetry	59
4.2.1.2 Fourier-transform infrared spectroscopy (FTIR)	61
4.2.1.3 X-ray powder diffraction (XRPD)	64
4.2.1.4 Polarised light microscopy	65
4.2.1.5 Thermal activity monitoring	67
4.2.2 Validation of HPLC method for pyrimethamine and ASDs	69
4.2.3 Solubility studies of pyrimethamine and ASDs	71
4.2.3.1 Pyrimethamine preliminary solubility studies	71
4.2.3.1.1 PPVP30 ASD preliminary solubility studies	73
4.2.3.1.2 PPVP64 ASD preliminary solubility studies	75
4.2.3.1.3 PHPMC ASD preliminary solubility studies	77
4.2.3.2 Pyrimethamine API and ASD pivot solubility studies	78
4.3 Artesunate and ASDs	81
4.3.1 Physico-chemical characterisation of artesunate API and ASDs	81
4.3.1.1 Differential scanning calorimetry	81
4.3.1.2 Fourier-transform infrared spectroscopy (FTIR)	83
4.3.1.3 X-ray powder diffraction (XRPD)	85
4.3.1.4 Polarised light microscopy	86
4.3.1.5 Thermal activity monitoring	88
4.3.2 Validation of HPLC method for artesunate and ASDs	90
4.3.3 Solubility studies of artesunate and ASDs	92
4.3.3.1 Artesunate preliminary solubility studies	92
4.3.3.1.1 APVP30 ASD preliminary solubility studies	94
4.3.3.1.2 APVP64 ASD preliminary solubility studies	95

4.3.3.1.3 AHPMC ASD preliminary solubility studies	97
4.3.3.2 Artesunate API and ASD pivot solubility studies	98
4.4 Statistical analysis	100
4.4.1 ANCOVA analysis of preliminary data	100
4.4.2 Mann-Whitney U tests with Bonferroni adjustments of the resulting p-values for pivot solubility data	102
4.4.2.1 Sulfadoxine	102
4.4.2.2 Pyrimethamine	103
4.4.2.3 Artesunate	104
4.5 Conclusion	105
References	107
Chapter 5: Conclusion	109
5.1 Conclusion	109
5.2 Future recommendations	110

List of figures:

Figure 2.1: The chemical structure of sulfadoxine	10
Figure 2.2: The chemical structure of pyrimethamine	12
Figure 2.3: The chemical structure of artesunate	14
Figure 4.1: DSC thermogram of SULFA RM.	38
Figure 4.2: DSC thermogram of SULFA (blue), SPVP30 PM (red) and SPVP30 ASD (black).	39
Figure 4.3: DSC thermogram of SULFA (blue), SPVP64 PM (red) and SPVP64 ASD (black).	39
Figure 4.4: DSC thermogram of SULFA (blue), SHPMC PM (red) and SHPMC ASD (black).	39
Figure 4.5: FTIR spectrum of the SULFA RM sample	40
Figure 4.6: FTIR spectra of SULFA RM (black), SPVP30 ASD (green), and SPVP30 PM (red).	41
Figure 4.7: FTIR spectra of SULFA RM (black), SPVP64 ASD (green), and SPVP64 PM (red).	41
Figure 4.8: FTIR spectra of SULFA RM (black), SHPMC ASD (green), and SHPMC PM (red).	42
Figure 4.9: XRPD of SULFA RM sample.....	43
Figure 4.10: XRPD of SULFA (red), SPVP30 ASD (blue), SPVP64 ASD (green) and SHPMC ASD (black).....	43
Figure 4. 11: SULFA RM thermal activity monitoring.	46
Figure 4.12: SPVP30 ASD thermal activity monitoring.	46
Figure 4.13: SPVP64 ASD thermal activity monitoring.	47
Figure 4.14: SHPMC ASD thermal activity monitoring.....	47
Figure 4.15: Results of HPLC method linearity for SULFA RM.....	48
Figure 4.16: Results of HPLC method linearity for SPVP30, SPVP64 and SHPMC ASDs. .	49
Figure 4.17: Preliminary solubility results obtained for SULFA in ultrapure H ₂ O across 48 hours.....	51
Figure 4.18: Preliminary solubility results obtained for SPVP30 ASD in ultrapure H ₂ O across 48 hours.....	53
Figure 4.19: Preliminary solubility results obtained for SPVP64 ASD in ultrapure H ₂ O across 48 hours.....	54
Figure 4.20: SHPMC texture example.....	56
Figure 4.21: Preliminary solubility results obtained for SHPMC ASD in ultrapure H ₂ O across 48 hours.....	56

Figure 4.22: Pivot solubility results obtained for SULFA RM and ASDs across 2 hours.....	58
Figure 4.23: DSC thermogram of pyrimethamine raw material.	59
Figure 4.24: DSC thermogram of PYRI (blue), PPVP30 PM (red) and PPVP30 ASD (black).	60
Figure 4.25: DSC thermogram of PYRI (blue), PPVP64 PM (red) and PPVP64 ASD (black).	60
Figure 4.26: DSC thermogram of PYRI (blue), PHPMC PM (red) and PHPMC ASD (black).	60
Figure 4.27: FTIR spectra of the PYRI RM sample.....	61
Figure 4.28: FTIR spectra of PYRI RM (black), PPVP30 ASD (green), and PPVP30 PM (red).	62
Figure 4.29: FTIR spectra of PYRI RM (black), PPVP64 ASD (green), and PPVP64 PM (red).	62
Figure 4.30: FTIR spectra of PYRI RM (black), PHPMC ASD (green), and PHPMC PM (red).	63
Figure 4.31: XRPD of PYRI RM sample.....	64
Figure 4.32: XRPD of PYRI RM (red), PHPMC ASD (blue), PPVP30 ASD (green) and PPVP64 ASD (black).....	64
Figure 4.33: PYRI RM thermal activity monitoring.	67
Figure 4.34: PPVP30 ASD thermal activity monitoring.	67
Figure 4.35: PPVP64 ASD thermal activity monitoring.	68
Figure 4.36: PHPMC ASD thermal activity monitoring.....	68
Figure 4.37: Results of HPLC method linearity for PYRI raw material.	69
Figure 4.38: Results of HPLC method linearity for PPVP30, PPVP64 and PHPMC ASDs. .	70
Figure 4.39: Preliminary solubility results obtained for PYRI in ultrapure H ₂ O across 48 hours.	72
Figure 4.40: Preliminary solubility results obtained for PPVP30 ASD in ultrapure H ₂ O across 48 hours.....	74
Figure 4.41: Preliminary solubility results obtained for PPVP64 ASD in ultrapure H ₂ O across 48 hours.....	76
Figure 4.42: Preliminary solubility results obtained for PHPMC ASD in ultrapure H ₂ O across 48 hours.....	78
Figure 4.43: PHPMC texture example.....	79
Figure 4.45: DSC thermogram of artesunate raw material.	81
Figure 4.46: DSC thermogram of ART (blue), APVP30 PM (red) and APVP30 ASD (black).	82

Figure 4.47: DSC thermogram of ART (blue), APVP64 PM (red) and APVP64 ASD (black).	82
Figure 4.48: DSC thermogram of ART (blue), AHPMC PM (red) and AHPMC ASD (black).	82
Figure 4.49: FTIR spectrum of the ART raw material sample.	83
Figure 4.50: FTIR spectra of ART RM (black), APVP30 ASD (green), and APVP30 PM (red).	83
Figure 4.51 FTIR spectra of ART RM (black), APVP64 ASD (green), and APVP64 PM (red).	84
Figure 4.52: FTIR spectra of ART RM (black), AHPMC ASD (green), and AHPMC PM (red).	84
Figure 4.53: XRPD of ART raw material sample.	85
Figure 4.54: XRPD of ART (red), APVP64 ASD (blue), AHPMC ASD (green) and APVP30 ASD (black).....	85
Figure 4.55: ART RM thermal activity monitoring.	88
Figure 4.56: APVP30 ASD thermal activity monitoring.	88
Figure 4.57: APVP64 ASD thermal activity monitoring.	89
Figure 4.58: AHPMC ASD thermal activity monitoring.....	89
Figure 4.59: Results of HPLC method linearity for ART raw material.	90
Figure 4.60: Results of HPLC method linearity for APVP30, APVP64 and AHPMC ASDs. .	91
Figure 4.61: Preliminary solubility results obtained for ART in ultrapure H ₂ O across 12 hours.	93
Figure 4.65: Pivot solubility results obtained for ART API and ASDs.....	99
Figure 4.66: Boxplots and statistical significance indicators	102
Figure 4.67: Boxplots and statistical significance indicators	103
Figure 4.68: Boxplots and statistical significance indicators	104

List of Tables:

Table 2.1: Typical symptoms of uncomplicated malaria	7
Table 2.2: Typical symptoms of complicated malaria	8
Table 2.3: BCS of drug substances.....	16
Table 3.1: Prepared ASDs and PMs of the three APIs	29
Table 3.2: Weight of SULFA API and ASDs (mg) per test tube	33
Table 3.3: Weight of PYRI API and ASDs (mg) per test tube	34
Table 3.4: Weight of ART API and ASDs (mg) per test tube.....	34
Table 4.1: Light microscopy micrographs of SULFA raw material.....	44
Table 4.2: Polarised light microscopy micrographs of SULFA ASDs	45
Table 4.3 Preliminary solubility data of SULFA RM in ultrapure H ₂ O	50
Table 4.4 Preliminary solubility data of SPVP30 ASD in ultrapure H ₂ O across 48 hours	52
Table 4.5: Preliminary solubility data of SPVP64 ASD in ultrapure H ₂ O	53
Table 4.6: Preliminary solubility data of SHPMC ASD in ultrapure H ₂ O across 48 hours.....	55
Table 4.7: Pivot solubility data of SULFA API and ASDs after 2 hours	57
Table 4.8: Light microscopy micrographs of PYRI raw material.....	65
Table 4.9: Polarised light microscopy micrographs of PYRI ASDs	66
Table 4.10: Preliminary solubility data of PYRI in ultrapure H ₂ O across 48 hours.....	71
Table 4.11: Preliminary solubility data of PPVP30 ASD in ultrapure H ₂ O across 48 hours ..	73
Table 4.12: Preliminary solubility data of PPVP64 ASD in ultrapure H ₂ O across 48 hours ..	75
Table 4.13: Preliminary solubility data of PHPMC ASD in ultrapure H ₂ O across 48 hours...	77
Table 4.14: Pivot solubility data of PYRI API and ASDs after 2 hours	78
Table 4.15: Light microscopy micrographs of ART raw material.....	86
Table 4.16: Polarised light microscopy micrographs of ART ASDs.....	87
Table 4.17: Preliminary solubility data of ART in ultrapure H ₂ O across 12 hours.....	92
Table 4.18: Preliminary solubility data of APVP30 ASD in ultrapure H ₂ O across 24 hours ..	94
Table 4.19: Preliminary solubility data of APVP64 ASD in ultrapure H ₂ O across 48 hours ..	95
Table 4.20: Preliminary solubility data of AHPMC ASD in ultrapure H ₂ O across 48 hours...	97
Table 4.21: Pivot solubility data of ART API and ASDs after 2 hours	99
Table 4.22: ANCOVA Statistics regarding SULFA API and ASDs <i>F_{crit}</i> = 5.3177	100
Table 4.23: ANCOVA Statistics regarding PYRI API and ASDs	101
Table 4.24: ANCOVA Statistics regarding ART API and ASDs	101
Table 4.25: Mann-Whitney U tests regarding SULFA API and ASDs	102
Table 4.26: Mann-Whitney U tests regarding PYRI API and ASDs.....	103
Table 4.27: Mann-Whitney U tests regarding ART API and ASDs.....	104
Table 4.28: ASD formation	105

Chapter 1: Introduction

1.1 Introduction

Malaria continues to pose a significant societal challenge, as evident by the documentation of 234 million cases in the African region in 2021, with 593 000 resulting in fatalities ([WHO, 2023a](#)). Pregnant women within malaria-endemic areas face heightened transmission risks, with a concerning 50% fatality rate when infected. The causative vectors of malaria comprise five main species, which are *Plasmodium falciparum* (*P. falciparum*), *Plasmodium malariae* (*P. malariae*), *Plasmodium vivax* (*P. vivax*), *Plasmodium ovale* (*P. ovale*) and *Plasmodium knowlesi* (*P. knowlesi*). Notably, *P. falciparum*, the most prevalent strain, has developed significant resistance to conventional treatments such as chloroquine and sulfadoxine-pyrimethamine combination therapies. Consequently, artemisinin-based combination therapies (ACTs) have emerged as the primary treatment for uncomplicated malaria in pregnant women ([Eastman & Fidock, 2009](#)). The World Health Organization (WHO) has recommended the intermittent preventive treatment in pregnancy (IPTp) strategy in which a single dose of three tablets of single-pill combination (SPC) of sulfadoxine-pyrimethamine (SP) is administered integral to antenatal care service. This strategy mitigates or prevents malaria-related adverse maternal and fetal outcomes. The IPTp-SP is implemented in pregnant women starting as early as possible in the second trimester, with SP administered at monthly intervals up to the time of delivery ([Al Khaja & Sequeira, 2021](#)). Following this treatment, artemisinin-based combination therapy (ACT) can be completed parenterally.

Emphasising the malaria treatment challenge is the poor aqueous solubility profile of essential drugs, namely sulfadoxine, pyrimethamine, and artesunate. This limitation underscores the need for an innovative approach to enhance the solubility profiles of these drugs, potentially offering significant benefits in the effective treatment of malaria.

Various techniques exist to enhance the solubility of poorly soluble drugs. Amorphous solid dispersion formation has proved invaluable in this regard and numerous studies have been performed to aid in the poor aqueous solubility of BCS class II and IV drugs ([Fahr & Liu, 2007](#)). Amorphous compounds have no defined shape on a molecular level which enables the structure to have higher molecular mobility and thus better solubility. Another relevant factor is the absence of extended / long chains of molecules, in these structures. Amorphous materials are thermodynamically metastable and contain increased amounts of internal energy (Gibbs free energy) in comparison to crystalline structures of the same compound, which in effect can promote the solubility and dissolution thereof ([Hancock & Zografi, 1997](#);

[Byrn et al., 1999](#); [Cui, 2007](#)). It is estimated that around 70% of active pharmaceutical ingredients (APIs) exhibit inadequate solubility in aqueous solutions during development. This can be seen when referring to the APIs used within this study. As sulfadoxine is very slightly soluble, pyrimethamine is classified as a class II or IV drug according to the biopharmaceutical classification system (BCS) and artesunate as a class II drug indicating it has good permeability, but poor solubility. The successful formation of intimately blended dispersions necessitates miscibility between the drug and polymer components. This results in the creation of an amorphous system, wherein the apparent solubility and bioavailability of medications with low aqueous solubility can be significantly improved solubility ([Byrn et al., 1999](#); [Aucamp et al., 2016](#)).

There are numerous ways in which ASDs can be made namely, the fusion method, the hot-plate extrusion method, spray drying method and then the method to be used in this study, the solvent evaporation method. In this method the drug and the carrier are dissolved in a solvent which is then evaporated under a vacuum. A solid solution remains which is the dried in an oven ([Savjani et al., 2012](#)).

1.2 Research Problem

Malaria remains a global burden. Development of resistance by *P. falciparum* to chloroquine and sulfadoxine–pyrimethamine has led to the recent adoption by The World Health Organization (WHO) of artemisinin-based combination therapies (ACTs) as the first line of treatment against uncomplicated malaria in pregnant woman. However, according to the solubility profiles of these APIs, all three drugs present with poor aqueous solubility. Improving the solubility by means of solid dispersion formation might increase the bioavailability and thereby also the efficacy and therapeutic outcome of these drugs.

1.3 Aim & Objectives

The aim of this study is to improve the solubility profiles of three APIs namely, sulfadoxine, pyrimethamine and artesunate, by means of the formation of amorphous solid dispersions. To achieve this aim, the following objectives are set:

- To verify existing high-performance liquid chromatography (HPLC) methods for all three APIs in-house – HPLC was used for the analysis of all solubility samples.
- To develop amorphous solid-dispersions using the solvent evaporation method and three polymers HPMC, PVP30 and PVP64 individually.
- To establish new methods or confirm existing methods for high-performance liquid chromatography analysis of produced amorphous solid dispersions.

- To perform physico-chemical characterisation on all solid dispersions and APIs using X-Ray Powder Diffraction (XRPD), Simultaneous Thermal Analysis (STA), Polarised Light Microscopy (PLM), Fourier Transform Infrared Spectroscopy (FTIR) and Thermal Activity Monitor (TAM).
- To perform solubility studies on the APIs and amorphous solid dispersions in order to establish change in solubility.

1.4 Chapter layout of dissertation

In this dissertation, Chapter 1 provides an introduction and highlights the research problem as well as the aim and objectives of this study. Chapter 2 focuses on the literature overview. It refers to the background and epidemiology of malaria, a thorough discussion of the active pharmaceutical ingredients to be used throughout this study and an overview of solubility and miscibility. In Chapter 3 methods used throughout the study are clearly outlined, whereas in Chapter 4 all the results are clearly discussed and evaluated. Finally, Chapter 5 allows for conclusions and future recommendations to be made.

References

- AL KHAJA, K.A.J. & SEQUEIRA, R.P. 2021. Drug treatment and prevention of malaria in pregnancy: a critical review of the guidelines. *Malaria Journal*, 20 (1). doi:10.1186/s12936-020-03565-2.
- AUCAMP, M.E., LIEBENBERG, W. & STIEGER, N. 2015. Solvent-interactive transformations of pharmaceutical compounds. (In Mastai, Y. ed. Advanced topics in crystallisation). *InTech*, DOI: 10.5772/59586. <http://www.intechopen.com/books/advanced-topics-in-crystallization/solvent-interactive-transformations-of-pharmaceutical-compounds>. [Date of access: 22 September 2022].
- BYRN, S.R., PFEIFFER, R.R. & STOWELL, J.G. 1999. Drugs as molecular solids. (In Byrn, S.R., Pfeiffer, R.R. & Stowell, J.G., eds. Solid-state chemistry of drugs. 2nd ed. West Lafayette, IN: SSCI inc. p. 3-43.
- CAIRA, M.R. 2007. Sulfa drugs as model cocrystal formers. *Molecular Pharmaceutics*, 4 (3):310-316.
- CUI, Y. 2007. A material science perspective of pharmaceutical solids. *International Journal of Pharmaceutics*, 339 (1–2): 3-18.
- EASTMAN, R.T. & FIDOCK, D.A. 2009. Artemisinin-based combination therapies: A vital tool in efforts to eliminate malaria. *Nature Reviews Microbiology*, 7:867-874.
- FAHR, A. & LIU, X. 2007. Drug delivery strategies for poorly water-soluble drugs. *Expert Opinion on Drug Delivery*, 4(4): 403-416. doi:10.1517/17425247.4.4.403
- HANCOCK, B.C. & ZOGRAFI, G. 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. *Journal of Pharmaceutical Sciences*, 86(1).
- SAVJANI, K. T., GAJJAR, A. K. & SAVJANI, J. K. 2012. Drug Solubility: Importance and enhancement techniques. *International Scholarly Research Network Pharmaceutics*, 2012 (195727) 10p.

Chapter 2: Literature review

2 Introduction to Malaria

According to the latest “World malaria report” malaria remains a global problem with 234 million cases documented in the African region in 2021 ([WHO, 2023a](#)) and accounts for about 95% of the cases documented worldwide. The estimated number of deaths in the same year totalled 619 000 globally, with the African region accounting for 593 000 of those deaths ([WHO, 2023a](#)).

The focus remains on the five main species responsible for the cause and transmission of malaria, namely *Plasmodium falciparum* (*P. falciparum*), *Plasmodium malariae* (*P. malariae*), *Plasmodium vivax* (*P. vivax*), *Plasmodium ovale* (*P. ovale*) and *Plasmodium knowlesi* (*P. knowlesi*). The presence of malaria and its effects on human populations can be influenced by certain characteristics of the malaria parasite. There tends to be a higher prevalence of sickness and mortality in regions where *P. falciparum* predominates (such as Africa and south of the Sahara) than in areas where other species, which typically cause less severe symptoms, predominate ([CDC 2022](#)). The “hypnozoites” stages of *P. vivax* and *P. ovale* can lie latent in the liver cells for months or years before reactivating and infiltrating the bloodstream. Relapses of this nature may lead to the reappearance of malaria following seemingly effective control measures, or they may introduce the disease into previously malaria-free areas. *P. falciparum*, and to a lesser degree *P. vivax*, have evolved into drug-resistant forms. These strains are not dispersed equally. It is essential to continuously assess how susceptible these two parasite species are to locally prescribed medications in order to guarantee efficient treatment and fruitful control initiatives. Only medications that are known to be protective at the destination should be used as preventative measures by visitors to malaria-risk areas ([CDC, 2022](#)).

An estimate of 54.7 million pregnancies occur within areas of continuous malaria transmission caused by *P. falciparum*. Pregnant women in regions with unstable malaria transmission are more likely to contract severe falciparum malaria, which has a fatality rate of about 50%. Pregnant women experience higher rates of parasitaemia, severe anaemia, hypoglycaemia, and acute pulmonary oedema than non-pregnant women. Foetal discomfort, premature labour, and stillbirth is common in severe illness. There is a significant contribution to morbidity and mortality in pregnant women, estimating 10 000 maternal deaths and 100 000 neonatal deaths per year ([Kovacs et al., 2015](#)). According to the WHO intermittent preventive treatment in pregnancy (IPTp) guidelines, which went into effect on June 3, 2022, pregnant women of all gravidities should receive antimalarial medication at preset intervals in

malaria-endemic areas to lessen the risk of disease burden during pregnancy and unfavourable pregnancy and birth outcomes ([WHO 2023b](#)).

IPTp utilising sulfadoxine-pyrimethamine (IPTp-SP) should begin as early as possible in the second trimester and not before week 13 of pregnancy. SP has been used extensively for malaria chemoprevention during pregnancy and is still beneficial in improving important pregnancy outcomes ([WHO, 2023b](#)).

Development of resistance by *Plasmodium falciparum* to chloroquine and sulfadoxine–pyrimethamine has led to the adoption by The World Health Organization (WHO), of artemisinin-based combination therapies (ACTs) as the first line of treatment against uncomplicated malaria in pregnant woman ([Eastman & Fidock, 2009](#)). The addition of artesunate to the sulfadoxine-pyrimethamine combination may not only address the resistance but will also increase therapeutic efficacy, accelerate clinical recovery, and reduce malaria transmission ([Pritto et al., 2003](#)). The primary advancement in the management of uncomplicated *P. falciparum* malaria has been the substitution of the ineffective monotherapies chloroquine and sulfadoxine-pyrimethamine by artemisinin combination therapies (ACTs) specifically artesunate ([Hanboonkunupakarn & White, 2022](#)).

2.1 Epidemiology

Protozoan parasites belonging to the *Plasmodium* species are responsible for causing malaria. Individuals in numerous nations are still susceptible, and the quantity of cases of malaria, especially those caused by *P. falciparum*, that results in the most severe infection, continues to be elevated in nations with low economic standing, particularly in Africa ([Sato, 2021](#)). Infectiousness of malaria parasites in humans requires their prior development within mosquitoes. The duration of extrinsic incubation period necessary for the development of parasites in mosquitoes varies depending on the specific type of the parasite and the prevailing temperature conditions, with a minimum requirement of 9 days or longer. When a mosquito cannot survive longer than its extrinsic incubation period, it cannot transmit any malaria parasites ([CDC, 2022](#)).

The life cycle of the malaria parasite involves two primary hosts: humans and mosquitoes. The transmission of malaria is explained by the following steps:

Mosquito bite: Sporozoites are injected into a person's bloodstream the moment an infected *Anopheles* mosquito bites a human. These sporozoites, the infectious form of the malaria parasite, are transported via the bloodstream to the human liver which enables it to escape host immunity or drainage through the lymphatic system ([Tavares et al., 2013](#)).

Liver stage: The injected sporozoites travel to the liver, where they infect liver cells (hepatocytes). Inside the hepatocytes, the sporozoites undergo asexual replication, producing thousands of merozoites ([Sturm et al., 2006](#)).

Blood stage: After a period of development in the liver, the infected hepatocytes rupture, releasing the merozoites into the bloodstream. The merozoites then invade red blood cells (RBCs) and begin the blood stage of infection. Inside the RBCs, the parasites replicate asexually, leading to the release of more merozoites and the destruction of the infected RBCs ([Sologub et al., 2011](#)).

Transmission back to mosquitoes: When an infected person is bitten by another mosquito, the parasite can be taken up by the mosquito along with the blood meal. Inside the mosquito, the parasites undergo further development, ultimately forming sporozoites in the mosquito's salivary glands ([CDC, 2022](#)).

2.1.1 Symptoms and Diagnosis

In Table 2.1 are a few of the typical uncomplicated malaria signs and symptoms used to identify the illness.

Table 2.1: Typical symptoms of uncomplicated malaria ([EDL, 2020](#))

Severe headache	Shivering episodes
Fever >38 °C	Nausea and vomiting
Muscle and Joint pains	Flu-like symptoms
Diarrhoea	Dry cough

Malaria occasionally develops into a severe illness (complicated malaria) that can result in more extreme symptoms, listed in Table 2.2.

Table 2.2: Typical symptoms of complicated malaria ([EDL, 2020](#))

Severe general body weakness	Shock
Sleepiness, unconsciousness or coma, convulsions	Repeated vomiting
Respiratory distress and/or cyanosis	Hypoglycaemia
Jaundice	Severe anaemia (Hb < 7g/dl)
Renal failure	
Abnormal bleeding	

For the diagnosis of malaria, a blood sample from a patient presenting with symptoms, is examined under a microscope ([CDC, 2022](#)). The following additional testing procedures may be used to detect malaria:

1. Rapid diagnostic tests (RDTs): These are straightforward tests that can quickly diagnose a patient with malaria by identifying particular malaria antigens in their blood in approximately 15 minutes. In remote locations without access to laboratory equipment, RDTs are especially helpful ([WHO, 2023a](#)).
2. Polymerase chain reaction (PCR): A highly sensitive and accurate diagnosis of malaria can be made using the laboratory method known as PCR, which can identify the presence of malaria DNA in a patient's blood. PCR is especially helpful for identifying different parasite species and for detecting low-level malaria infections ([WHO, 2023a](#)).
3. Serological tests: examine a patient's blood for particular malarial antibodies, indicating previous infection. Since it can take several weeks for antibodies to form, serological tests are ineffective for identifying active malaria infections ([WHO, 2023a](#)).

In order to avoid complications and lower the chance of transmission to others, it is significant to observe that early diagnosis and treatment of malaria are essential. It's crucial to get tested for malaria if any malaria symptoms are present or if the individual has recently travelled to a region where the disease is widely transmitted ([WHO, 2023a](#))

2.1.2 Treatment

2.1.2.1 Uncomplicated malaria

Uncomplicated malaria is diagnosed in a patient with malaria symptoms and a positive parasitological test (microscopy or RDT) but without signs of severe malaria. For the management of uncomplicated malaria, the World Health Organization (WHO) suggests artemisinin-based combination therapy (ACT). ACT is an antimalarial drug combination that combines the quick-acting artemisinin with a longer-acting partner drug to assist the body in the clearance of any remaining parasites ([WHO, 2023c](#)).

The WHO recommends a variety of ACT regimens, including:

-  Artemether-lumefantrine
-  Dihydroartemisinin-piperaquine
-  Artesunate-mefloquine
-  Artesunate-amodiaquine
-  Artesunate-sulfadoxine-pyrimethamine

The selection of an ACT regimen is influenced by a number of variables, including the patient's age and weight, the frequency of antimalarial drug resistance in the area, to name a few ([WHO, 2023c](#)).

2.1.2.2 Complicated malaria

Malaria that is serious or that presents with extra complications such as anaemia, jaundice, kidney failure, or cerebral malaria and is thus classified as complicated malaria, a serious complication that can cause coma, seizures, or death ([CDC, 2022](#)). Malaria that is complicated needs to be treated immediately in a hospital environment, typically with injectable antimalarial medications ([CDC, 2022](#)).

Parenteral (injectable) artesunate is advised by the WHO as the initial treatment for both adults and infants who have severe malaria. In comparison to other injectable antimalarial medications such as quinine, artesunate has proven to be more successful and presents with fewer side effects ([WHO, 2023c](#)).

2.2 Sulfadoxine

Sulfonamides are among the oldest classes of antibacterial drugs known to science; they were discovered prior to penicillin. Since then, the development of resistance has reduced the efficacy of sulfonamides alone; however, in the 1970s, sulfonamides were once again used after being potentiated with trimethoprim or other potentiators, such as ormetoprim, pyrimethamine, or baquiloprim ([Maddison et al, 2008](#)). Sulfadoxine prevents the malaria parasite from producing folic acid, hence preventing it from growing and replicating. In particular, sulfadoxine works as a competitive inhibitor of the folic acid production enzyme dihydropteroate synthase ([Deck & Winston, 2015](#)). Sulfadoxine can kill the malaria parasite by blocking the enzyme that the parasite uses to synthesise folic acid. Adverse effects of sulfadoxine include skin rashes, headaches, vomiting, diarrhoea, and nausea. More severe side effects such as liver damage, blood disorders, and allergic responses are only sometimes experienced as a result of treatment ([Maddison et al, 2008](#)).

The WHO recommends the utilisation of sulfadoxine in conjunction with pyrimethamine for the purpose of intermittent prophylactic therapy of malaria in pregnant women ([WHO, 2023a](#)).

2.2.1 Physico-chemical properties

Sulfadoxine ($C_{12}H_{14}N_4O_4S$) (chemical structure shown in Figure 2.1) is an odourless, white, or creamy-white, crystalline powder known chemically as N' - (5,6-dimethoxy-4-pyrimidinyl) sulfanilamide ([Kapoor, 1988](#)). The molecular weight of sulfadoxine is 310.33 g/mol whilst a melting point of 195-200°C is documented ([BP, 2024](#)). Sulfadoxine is reported by the British Pharmacopoeia to be slightly soluble in alcohol and in methanol, and very slightly soluble in water (1 g per 100 – 1000 ml) ([BP, 2024](#)). It dissolves in dilute mineral acids and in alkali hydroxide solutions ([Kapoor, 1988](#)).

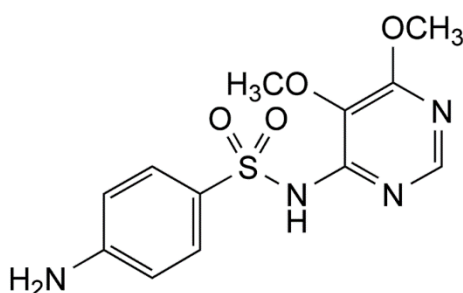


Figure 2.1: The chemical structure of sulfadoxine ([Ph. Int. 2020a](#)).

2.2.2 Pharmacology

2.2.2.1 Indication

The primary indication for the use of sulfadoxine is in the treatment of uncomplicated malaria caused by the malaria parasite *P. falciparum*. It is commonly used in combination with other antimalarial drugs, such as pyrimethamine or artesunate, to enhance its effectiveness and reduce the risk of drug resistance ([WHO, 2023a](#)).

2.2.2.2 Mechanism of action

Sulfadoxine is classified as a sulfonamide compound and shares a similar structure to p-aminobenzoic acid (PABA). It possesses the ability to impede the synthesis of dihydropteroate synthase and folate. Sulfadoxine functions as a competitive inhibitor of the bacterial enzyme dihydropteroate synthase. Its role involves facilitating the inclusion of PABA into the immediate precursor of folic acid, known as dihydropteroic acid ([Maddison et al, 2008](#)). Sulfonamides tend to have a higher affinity for an alkaline pH as opposed to an acidic pH. The concurrent use of sulfadoxine with a dihydrofolate reductase inhibitor, such as trimethoprim or pyrimethamine, can result in synergistic effects as a consequence of the sequential inhibition of folate production ([Deck & Winston, 2015](#)).

2.2.2.3 Dosage and administration

For the treatment of uncomplicated malaria, sulfadoxine is often administered orally in a single dose of 25 mg/kg (up to a maximum dose of 1.5 g) in combination with other antimalarial drugs such as pyrimethamine or artesunate. The actual dosage and duration of therapy will depend on the patient's age, weight, and level of disease ([WHO, 2023a](#)).

2.3 Pyrimethamine

The first of the 2, 4-diaminopyrimidines to be produced and evaluated for antibacterial activity was pyrimethamine. Its effectiveness against *Toxoplasma*, *Plasmodium*, and other protozoa species is its primary characteristic ([Scholar, 2009](#)).

By preventing the synthesis of nucleic acids, the folate antagonist pyrimethamine prevents the malaria parasite's growth and reproduction. Pyrimethamine permanently binds to the specific enzyme, dihydrofolate reductase, which is necessary for the parasite's synthesis of DNA and RNA. By attaching to the enzyme, it prevents the conversion of dihydrofolate to tetrahydrofolate (folinic acid), pyrimethamine prevents the parasite from synthesising folinic acid. Dihydrofolic acid synthetase inhibition stops para-amino benzoic acid from being converted to dihydrofolic acid ([Scholar & Pratt., 2000](#); [Kucers et al., 1997](#)).

2.3.1 Physico-chemical properties

Pyrimethamine (chemical structure shown in Figure 2.2) is a white, odourless, tasteless, crystalline powder also known chemically as 2,4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine ([Loutfy & Aboul-Enein, 1983](#)). Pyrimethamine is practically insoluble in water and slightly soluble in chloroform, acetone, dilute hydrochloric acid, and ethanol. It is classified as a class II or IV drug according to the biopharmaceutical classification system (BCS) ([Lindenberg et al., 2004](#)). It has a melting point that ranges between 233°C and 242°C ([Loutfy & Aboul-Enein, 1983](#)).

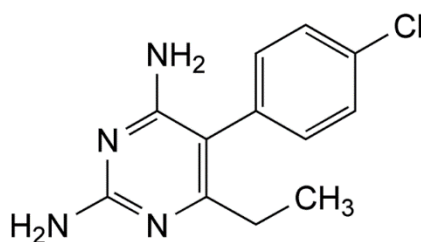


Figure 2.2: The chemical structure of pyrimethamine ([Ph. Int 2020b](#)).

2.3.2 Pharmacology

2.3.2.1 Indication

Pyrimethamine is an antiparasitic drug used in the prevention and treatment of toxoplasmosis and malaria. It is a folic acid antagonist that is used as an antimalarial or to treat toxoplasmosis ([Scholar, 2009](#)).

2.3.2.2 Mechanism of action

Pyrimethamine functions as an inhibitor of plasmodia's dihydrofolate reductase, effectively impeding the manufacture of purines and pyrimidines. These molecules play a critical role in DNA synthesis and cellular proliferation. This results in the inability of nuclear division to occur throughout the process of schizont formation in both erythrocytes and liver cells ([Scholar, 2009](#)).

2.3.2.3 Dosage and administration

Pyrimethamine dose and administration may change based on the patient's age, weight, and level of infection, amongst others. For the treatment of uncomplicated malaria, pyrimethamine is commonly administered orally at a dose of 1.25 mg/kg (up to a maximum dose of 75 mg) in conjunction with sulfadoxine ([EDL, 2022](#)).

2.4 Artesunate

Artesunate is mostly used as a first line treatment in malaria, especially when the disease is severe or complicated. It is a byproduct of artemisinin, a substance derived from the Chinese herb *Artemisia annua* that has been utilised for many years in traditional Chinese medicine ([Karunajeewa, 2011](#)). A group of scientists in China lead by pharmacologist Tu Youyou, who received the Nobel Prize in Medicine in 2015 for her work on the discovery and development of artemisinin-based antimalarial medications, discovered a means to extract the active ingredient, conducted the first clinical trial with the Artemisia extract in human patients, isolated the active ingredient for structural studies and discovered dihydroartemisinin ([Su & Miller, 2015](#)).

Due to its capacity to cause apoptosis (programmed cell death) in specific cell types, artesunate has not only been studied for its use in the treatment of malaria but also for its potential application in the treatment of other disorders, such as cancer and inflammatory diseases ([Khanal, 2021](#)).

2.4.1 Physico-chemical properties

Artesunate (ART) (chemical structure shown in Figure 2.3) is a fine, white crystalline powder. Artesunate is very slightly soluble in water estimated at 56.2 µg/ml at 25°C ([Pubchem, 2020](#)). Furthermore, ART has a pK_a of 4.35 and an estimate log K_{ow} (K_{ow} = Concentration in Octanol / Concentration in water) of 2.61. Therefore, artesunate is an ideal candidate for solubility enhancement studies. The BCS classifies ART as a class II drug indicating it has good permeability, but poor solubility.

ART raw material has a melting point of 146°C, and melts with decomposition ([Odendaal, 2019](#)).

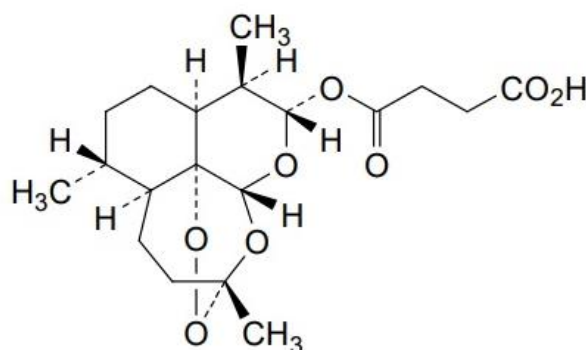


Figure 2.3: The chemical structure of artesunate ([Ph. Int. 2020c](#)).

2.4.2 Pharmacology

2.4.2.1 Indication

The anti-malarial drug which is used as the primary treatment in adults and children in cases of severe malaria, especially cases caused by *Plasmodium falciparum*, is artesunate ([WHO, 2023c](#)). Artesunate is an artemisinin derivative which can also be prescribed to women during their second and third trimesters of pregnancy. The ability to prescribe artesunate during pregnancy allows it to reign superior in comparison to various other anti-malarial drugs such as halofantrine and primaquine, which should be avoided during lactation and pregnancy. An increased risk in mortality cases due to severe malaria can be combatted by the administration of intravenous or intramuscular artesunate. In this instance, artesunate prove more effective than the administration of intravenous quinine ([Rossiter et al., 2016](#)).

2.4.2.2 Mechanism of action

The pharmacological action of artesunate is hypothesised to be associated with the generation of oxygen radicals by the iron-mediated schism of the endoperoxide bridge. Artesunate undergoes fast metabolism, resulting in the formation of dihydroartemisinin (DHA), which is its active form. The presence of oxygen radicals leads to the inhibition of parasite activity by their interaction with adjacent molecules, hence inducing oxidative stress ([Medscape, 2020](#)). Several other research endeavours have been undertaken, which demonstrate that artesunate possesses qualities that exhibit anti-inflammatory, anti-cancer, and anti-schistosomiasis effects ([Liu et al., 2010](#)).

2.4.2.3 Dosage and administration

Depending on the patient's age and weight, the severity of the malaria infection, and other considerations, different dosages and methods of administration of artesunate may be advised.

For uncomplicated malaria, artesunate can be taken orally once daily for three days at a dose of 4 mg/kg body weight, either alone or in conjunction with other antimalarial medications ([Borrmann et al, 2003](#)).

For cases of severe or complex malaria, an intramuscular injection of artesunate at a loading dose of 2.4 mg/kg body weight should be administered immediately, followed by a referral to a hospital (EDL, 2020). If referral is delayed another dose of 2.4 mg/kg is administered at least 12 hours later and again after 24 hours until the patient can take an oral treatment. Patients who weigh <20 kg will receive a dose of 3 mg/kg bodyweight until oral treatment is tolerable ([Blumberg, 2017](#)). In some circumstances, rectal artesunate suppositories may be used as an alternative.

When reviewing the oral administration of a 100 mg tablet of artesunate, it can be observed that the metabolite dihydroartemisinin achieves a plasma elimination half-life ranging from 40 to 95 minutes ([Batty et al., 1998a](#); [Batty et al., 1998b](#)). Intravenous delivery results in a shorter half-life of roughly 15 minutes, as reported by Cercioban *et al.* ([2020](#)). Artesunate is considered a very suitable treatment for cerebral malaria due to its rapid onset of action when delivered parenterally, leading to almost immediate therapeutic effects ([Barradell & Fitton, 1995](#)).

2.5 API solubility, dissolution, and miscibility

The Biopharmaceutics Classification System (BCS) is a tool used for the development of drugs and has been adopted by the FDA, European Medicines Agency (EMA) and the World Health Organization (WHO) ([Davitt et al., 2016](#)). The BCS has been utilised by health organisations and regulatory agencies globally since its introduction in 1995 ([Amidon et al., 2014](#)). Solubility and permeability are the major parameters used to determine the amount of drug that will be bioavailable ([Dahan, 2012](#)). The BCS places a drug in one of four categories depending on its oral dosing solubility and permeability (see Table 2.1) ([Papich & Martinez, 2015](#)). A drug is considered “highly soluble” when the highest possible dose strength is soluble in 250 ml or less of aqueous media over a pH range of 1.2 - 6.8 at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ([WHO, 2006](#)). A drug is considered “highly permeable” when the extent of the absorption is determined to be $\geq 90\%$ of an administered dose ([Benet et al., 2013](#)).

Table 2.3: BCS of drug substances ([Blume & Schug, 1999](#))

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

The improvement of the oral bioavailability of medicines with low aqueous solubility continues to be a major challenging aspect of drug development ([Serajuddin, 1999](#)). The increase in rate of dissolution and subsequent improvement in oral absorption and bioavailability of pharmaceuticals have often been achieved by the utilisation of solubilisation, salt formation, and particle size reduction techniques. However, it is important to acknowledge that there are practical limitations associated with the implementation of these methods which exist ([Serajuddin, 1999](#)).

The feasibility of synthesising suitable salt forms for medications that exhibit weak acidic or weak basic properties, as well as the creation of salts or neutral substances, can often be challenging. In several instances, the achievement of an enhanced dissolving rate in the gastrointestinal system may not be realised despite the preparation of salts, due to the reconversion of salts into aggregates including their corresponding acid or base forms ([Serajuddin, 1999](#)). The process of dissolving pharmaceuticals in organic solvents or aqueous solutions through the utilisation of surfactants and cosolvents results in liquid formulations that are typically unfavourable in terms of patient acceptance and commercial viability. [Sekiguchi and Obi \(1961\)](#) proposed a pragmatic approach to address certain constraints associated with the improvement of bioavailability for medicines with low water solubility, as discussed earlier. The approach, subsequently referred to as solid dispersion, entailed the creation of eutectic blends of pharmaceutical substances and water-soluble carriers by the fusion of their physical combinations. According to Sekiguchi and Obi, it was proposed that the medication existed in a microcrystalline condition within a eutectic mixture. Later, [Goldberg et al. \(1966\)](#) provided evidence indicating that the entirety of the pharmaceutical substance within a solid dispersion may not exclusively exist in a microcrystalline form. Instead, a portion of the medication might be disseminated at a molecular level within the surrounding matrix, resulting in the formation of a solid solution. In both scenarios, following exposure of the solid dispersion to aqueous fluid and subsequent dissolution of the carrier, the medication was released in the form of exceedingly small colloidal particles. The significantly increased surface area achieved by this method was anticipated to result in elevated dissolution rates and higher bioavailability of medicines with low water solubility.

The incorporation of organic compounds into polymeric matrices holds significant importance in several applications. The successful formation of intimately blended dispersions necessitates miscibility between the drug and polymer components. This results in the creation of an amorphous system, wherein the apparent solubility and bioavailability of medications with low aqueous solubility can be significantly improved. It is estimated that around 70% of active pharmaceutical ingredients (APIs) exhibit inadequate solubility in aqueous solutions during development. This impacts significantly on the administration of drugs via the oral route, leading to a decrease in the bioavailability of substances classified with poor solubility ([Coty et al., 2020](#)). As a result, this approach in formulation is gaining importance. Drug-polymer blends that exhibit miscibility also demonstrate enhanced resistance to drug crystallisation compared to the amorphous form of the drug alone. The amorphous state of a pharmaceutical compound has the potential to improve its dissolution rate, bioavailability, and solubility; but it will possibly crystallise over time. Miscible polymeric additives are employed in order to enhance physical stability ([Wang et al., 2021](#)).

2.5.1 Factors influencing the solubility of drugs

There are several factors or physico-chemical properties influencing solubility namely temperature, pH, solid state properties, solid dispersions, co-crystals, and salts of the API ([Aulton, 2005](#)).

2.5.1.1 Temperature

The prevalent influence of temperature on the solubility of drugs is clearly seen in the research done by [Mota et al. \(2009\)](#). The aqueous solubility of various APIs (i.e., budesonide, paracetamol, furosemide, and allopurinol) was determined at temperatures ranging from 25°C to 42°C. For all the above-mentioned APIs, an increase in solubility was seen when increasing the temperature of the dissolution medium. When there is a positive change in enthalpy during dissolution (ΔH), heat is usually absorbed, resulting in an endothermic reaction. Thus, an increase in a solid's solubility will be viewed with an increase in temperature, which is an example of Le Chatelier's principle. In systems that display exothermic dissolution, a decrease in solubility will be detected upon increasing the temperature; these systems occur less commonly ([Aulton, 2005](#)). [Murdande et al. 2011](#)) emphasises the importance of temperature control when solubility of compounds is measured.

2.5.1.2 pH

The influence of a change in pH within the different regions of the gastrointestinal tract on drug bioavailability is well researched. API absorption is largely dependent on diffusion, which is affected by the percentage of the API in the unionised form. The ratio of unionised to ionised drug molecules in solution varies within regional pH in the gastrointestinal tract and is dependent on the pK_a value of the API, the pH of the fluid in the specific region as well as the permeability coefficient of the API ([Sharma & Jain, 2010](#)).

2.5.1.3 Solid-state properties

APIs are known to occur in several solid-state forms, including polymorphs, solvates, and amorphous forms ([Byrn et al., 1999](#)). Polymorphism refers to the ability of a chemical substance to exist in various crystal forms ([Borka, 1990](#)). When a given substance presents with many crystal forms, the polymorph that is regarded as the most physically stable at a specific temperature would exhibit the lowest solubility at that temperature. Consequently, all other polymorphs would be classified as metastable. Every polymorph for a certain drug has a value for solubility under specific conditions such as solvent content, temperature, and pressure. According to [Byrn et al. \(1999\)](#), in cases where two or more polymorphs are generated, the system will favour the production of the least soluble form, which is typically the most stable form. Generally, crystalline forms have lower solubility compared to

amorphous forms. The amorphous state refers to the highest energy form of a given solid material, characterized by the absence of long-range molecular order. Amorphous materials exhibit increased molecular motion and improved thermodynamic properties in comparison to the crystalline state due to their elevated internal energy. This results in better apparent solubility and dissolution rate ([Hancock & Zografi, 1997](#); [Kaushal et al., 2004](#); [Yu, 2000](#)). However, it is important to note that these advantages are not without drawbacks. The increased chemical reactivity and propensity for crystallisation of amorphous materials, are results of their enhanced molecular mobility and high internal energy and can lead to potential challenges. These challenges may occur during various stages such as manufacturing, storage, or dissolution ([Aaltonen & Rades, 2009](#); [Hancock & Parks, 2000](#); [Hancock & Zografi, 1997](#); [Kaushal et al., 2004](#)).

2.5.1.4 Solid dispersions

According to [Habib et al.](#) (2001), a solid dispersion refers to the dispersion of one or more active ingredients inside a solid-state inert carrier or matrix. [Katariya and Patil](#) (2013) propose a classification system for solid dispersions, which encompasses various categories. These categories include eutectics, solid solutions, amorphous precipitations within a crystalline matrix, glass solutions, and glass suspensions. Solid dispersions originated in the year 1961, when Sekiguchi and Obi published a report on eutectic mixtures formation ([Habib et al., 2001](#)). Despite the initial promise of these approaches for the pharmaceutical sector, their commercial application ultimately proved to be highly limited. In recent times, there has been a resurgence of interest in the utilisation of solid dispersions. This increased attention can be attributed to the emergence of novel carrier types and advancements in manufacturing technology ([Katariya & Patil, 2013](#); [Vo et al., 2013](#)). According to [Katariya and Patil](#) (2013), the most recent advancements in solid dispersion system formulation include the dropping method, direct capsule filling method, electrostatic spinning method, and supercritical fluid technology. Solid dispersions are not always crystalline and may present as amorphous solid dispersions. Because amorphous or glass compounds lack a well-defined molecular shape, their structure can have greater molecular mobility and, thus, improved solubility. The absence of extensive or lengthy chains of molecules in these structures is another important consideration. When compared to crystalline forms of the same molecule, amorphous materials have more internal energy (Gibbs free energy) and are thermodynamically metastable, which can help the compound dissolve and become more soluble ([Hancock & Zografi, 1997](#); [Byrn et al., 1999](#); [Cui, 2007](#)).

Commonly used methods for preparing amorphous solid dispersions are the hot-melt or fusion method, solvent evaporation method, spray drying ([Paudel et al., 2013](#)), and melt agglomeration on process and surface-active carriers ([Katariya & Patil, 2013](#)).

2.5.1.5 Co-crystals and salts of active pharmaceutical ingredients

The impact of co-crystals on the physico-chemical properties of APIs has been illustrated, particularly in relation to dissolution and solubility. The crystalline nature of co-crystals offers the benefit of enhanced thermodynamic stability and solubility via solution phase chemistry. The utilisation of co-crystallisation is particularly beneficial in situations when the production of salts is impeded due to the limited presence of strong acidic and basic groups or sites in an active pharmaceutical ingredient (API), or when subjecting the API to harsh basic or acidic conditions may result in its degradation ([Thakuria et al., 2013](#)). Co-crystal formation occurred between trimethoprim and a sulfonamide (sulfadimidine), in a methanolic solution in a study conducted by [Caira](#) (2007).

The sulfonamides contain multiple hydrogen bond donor and acceptor functions ([Caira, 2007](#)) which allow for hydrogen bonding with APIs such as trimethoprim. Trimethoprim and sulfamethoxypyridazine formed a co-crystal when methanol was the crystallisation medium, but formed a hydrated salt when water was used as crystallisation medium ([Bettinetti et al., 2000](#); [Caira, 2007](#)).

Reference List

- AALTONEN, J. & RADES, T. 2009. Commentary: Towards Physico-Relevant Dissolution Testing: The Importance of Solid-State Analysis in Dissolution. *Dissolution Technologies*, 16, 47-54.
- AMIDON, G. L., LENNERNÄS, H., SHAH, V. P. & CRISON, J. R. 2014. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutics Research*, 12(3): 413–420.
- AULTON, M.E. 2005. Dissolution and solubility. (In Aulton, M.E., ed. Aulton's pharmaceutics: The science of dosage form design. 2nd ed. London: Churchill Livingstone. p. 15-32).
- BARRADELL, L.B. & FITTON, A. 1995. Artesunate. *Drugs*, 50(4):714-741.
- BATTY, K.T., ANH THU, L.T., DAVIS, T.M.E., ILETT, K.F., XUAN MAI, T., CANH HUNG, N., PHUC TIEN, N., POWELL, S.M., VAN THIEN, H., QUANG BINH, T. & KIM, N.V. 1998a. A pharmacokinetic and pharmacodynamic study of intravenous vs oral artesunate in uncomplicated falciparum malaria. *British Journal of Clinical Pharmacology*, 45(2):123-129.
- BATTY, K.T., TRUONG, X.M., DAVIS, T.M., NGUYEN, P.T., TRAN, Q.B., POWELL, S.M., NGUYEN, V.M., VUONG, V.C., HUYNH, V.T., LE, A.T., ILETT, K.F. & NGUYEN, C.H. 1998b. A pharmacokinetic and pharmacodynamic study of artesunate for vivax malaria. *The American Journal of Tropical Medicine and Hygiene*, 59(5):823-827.
- BENET, L. Z., AMIDON, G. L., BARENDT, D. M., LENNERNÄS, H., POLLI, J. E., SHAH, V. P., STAVCHANSKY, S. A., YU, L. X. 2013. The use of BDDCS in classifying the permeability of marketed drugs. *Pharmaceutics Research*, 25(3): 483-488.
- BETTINETTI, G., CAIRA, M.R., CALLEGARI, A., MERLI, M., SORRENTI, M. & TADINI, C. 2000. Structure and solid-state chemistry of anhydrous and hydrated crystal forms of trimethoprim-sulfamethoxypyridazine 1:1 molecular complex. *Journal of Pharmaceutical Sciences*, 89 (4):478-489.
- BLUMBERG, L. 2017. National guidelines for the treatment of malaria. *National Institute for Communicable Diseases of South Africa* <https://www.nicd.ac.za/wp-content/uploads/2017/03/National-Guidelines-for-Malaria-Treatment-SEPTEMBER-2019-Update-WITH-FRONT.pdf/> [Date of access: 19 Aug 2022].
- BLUME, H. & SCHUG B. 1999. The biopharmaceutics classification system (BCS): class III drugs — better candidates for BA/BE waiver? *European Journal of Pharmaceutical Sciences*, 9(2): 117-121.

BORKA, L. 1990. Crystal polymorphism of pharmaceuticals. *Acta Pharmaceutica Jugoslavica*, 40(1990):71-94.

BORRMANN, S., ADEGNIKA, A. A., MISSINOU, M. A., BINDER, R. K., ISSIFOU, S., SCHINDLER, A., MATSIEGUI, P. B., KUN, J. F., KRISHNA, S., LELL, B. & KREMSNER, P. G. 2003. Short-course artesunate treatment of uncomplicated *Plasmodium falciparum* malaria in Gabon. *Antimicrobial Agents in Chemotherapy*, 47, 901-4.

BP see British pharmacopoeia

BRITISH PHARMACOPOEIA. 2024. Sulfadoxine. <https://www.pharmacopoeia.com/bp-2024/monographs/sulfadoxine.html?date=2024-01-01&text=sulfadoxine> Date of access: 9 Oct. 2023.

BYRN, S.R., PFEIFFER, R.R. & STOWELL, J.G. 1999. Drugs as molecular solids. (*In* Byrn, S.R., Pfeiffer, R.R. & Stowell, J.G., eds. *Solid-state chemistry of drugs*. 2nd ed. West Lafayette, IN: SSCI inc. p. 3-43.

CAIRA, M.R. 2007. Sulfa drugs as model cocrystal formers. *Molecular Pharmaceutics*, 4 (3):310-316.

CDC see Centres for Disease Control and Prevention.

Centres for disease Prevention and Control. 2022. Malaria. <https://www.cdc.gov/parasites/malaria/index.html> Date of Access: 2 July 2023.

CIRCIOBAN, D., LEDETI, I., SUTA, L.-M., VLASE, G., LEDETI, A., VLASE, T., VARUT, R., SBARCEA, L., TRANDAFIRESCU, C. & DEHELEAN, C. 2020. Instrumental analysis and molecular modelling of inclusion complexes containing artesunate. *Journal of Thermal Analysis and Calorimetry*, 142(5):1951-1961.

COTY, J. B., MARTIN, C., TELO, I. & SPITZER, D. 2020. Use of Spray Flash Evaporation (SFE) technology to improve dissolution of poorly soluble drugs: Case study on furosemide nanocrystals. *International Journal of Pharmaceutics*, 589, 119827.

CUI, Y. 2007. A material science perspective of pharmaceutical solids. *International Journal of Pharmaceutics*, 339 (1–2): 3-18.

DAHAN, A. 2012. The solubility–permeability interplay and its implications in formulation design and development for poorly soluble drugs. *The AAPS Journal*, 14(2): 244-251.

DAVIT, B. M., KANFER, I., TSANG, Y. C. & CARDOT, J. M. 2016. BCS biowaivers: similarities and differences among EMA, FDA, and WHO requirements. *The AAPS Journal*, 18(3): 612- 618.

DECK, D. H. & WINSTON, L. G. 2015. Sulfonamides, trimethoprim & quimolones. *In*: Katzung, B. G. & Trevor, A. J., eds. Basic and Clinical Pharmacology. United States of America: McGraw-Hill Companies. pp. 807-809.

EASTMAN, R.T. & FIDOCK, D.A. 2009. Artemisinin-based combination therapies: A vital tool in efforts to eliminate malaria. *Nature Reviews Microbiology*, 7:867-874.

EDL see South Africa.

GOLDBERG, A. H., GIBALDI, M. & KANIG, J. L. 1966. Increasing dissolution rates and gastrointestinal absorption of drugs via solid solutions and eutectic mixtures II. Experimental evaluation of eutectic mixture: urea-acetaminophen system. *Journal of Pharmaceutical Sciences*, 55, 482-487.

HABIB, M.J., VENKATARAM, S. & HUSSAIN, M.D. 2001. Fundamentals of solid dispersions. (*In* Habib, M.J. ed. Pharmaceutical solid dispersion technology. Lancaster: Technomic. p. 7-35).

HANBOONKUNUPAKARN, B. & WHITE, N.J. 2022. Advances and roadblocks in the treatment of malaria. *British Journal of Clinical Pharmacology*, 88 (2), 374–382. Available from: doi:10.1111/bcp.14474.

HANCOCK, B.C. & PARKS, M. 2000. What is the true solubility advantage for amorphous pharmaceuticals. *Pharmaceutical Research*, 17:397–404.

HANCOCK, B.C. & ZOGRAFI, G. 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. *Journal of Pharmaceutical Sciences*, 86(1).

INTERNATIONAL PHARMACOPOEIA. 2020a. 19th ed. Sulfadoxine. <https://digicollections.net/phint/2020/index.html#d/b.6.1.342> Date of access: 4 May 2022.

INTERNATIONAL PHARMACOPOEIA. 2020b. 19th ed. Pyrimethamine. <https://digicollections.net/phint/2020/index.html#d/b.6.1.307> Date of access: 4 May 2022.

INTERNATIONAL PHARMACOPOEIA. 2020c. 19th ed. Artesunate. <https://digicollections.net/phint/2020/index.html#d/b.6.1.30> Date of access: 4 May 2022

KAPOOR, V. K. 1988. Sulfadoxine. *In*: Florey, K., ed. *Analytical profiles of drug substances*. New York: Academic Press.

KARUNAJEEWA, H. A. 2011. Artemisinins: Artemisinin, Dihydroartemisinin, Artemether and Artesunate. *In Treatment and Prevention of Malaria* (pp. 157-190). https://doi.org/10.1007/978-3-0346-0480-2_9

KATARIYA, V.R. & PATIL, S.B. 2013. Recent breakthroughs in solid dispersion: a review. *International Journal of Pharmaceutical Research and Allied Sciences*, 2(4):1-15.

- KAUSHAL, A.M., GUPTA, P., BANSAL, A.K. 2004. Amorphous drug delivery systems molecular aspects, design, and performance. *Critical Review in Therapeutic Drug Carrier Systems*, 21(3):133-193.
- KHANAL, P. 2021. Antimalarial and anticancer properties of artesunate and other artemisinins: current development. *Monatshefte für Chemie*, 152, 387-400.
- KOVACS, S.D., RIJKEN, M.J. & STERGACHIS, A. 2015. Treating severe malaria in pregnancy: A review of the evidence. *Drug safety*, 38:165-181.
- KUCERS, A., CROWE, S., GRAYSON, M.L. & HOY, J. 1997. Pyrimethamine. The Use of Antibiotics, 5, pp. 905–921, Butterworth-Heinemann, Oxford.
- LINDENBERG, M., KOPP, S. & DRESSMAN, J.B. 2004. Classification of orally administered on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics*, 58:265-278.
- LIU, W. M., GRAVETT, A. M. & DALGLEISH, A. G. 2010. The antimalarial agent artesunate possesses anticancer properties that can be enhanced by combination strategies. *International Journal of Cancer*, 128:1471-1480.
- LOUTFY, M.A. & ABOUL-ENEIN, H.Y. 1983. Pyrimethamine. (In Florey, K, ed. Analytical profiles of drug substances. Vol. 12. New York: Academic Press, p. 463-479).
- MADDISON, J. E., WATSON, A. D. J., & ELLIOTT, J. 2008. *Antibacterial drugs. Small Animal Clinical Pharmacology*, 148–185. doi:10.1016/b978-070202858-8.50010-5
- MEDSCAPE. 2020. Artesunate: pharmacology, mechanism of action. <https://reference.medscape.com/drug/artesunate-342684>. [Date of access: 21 Apr 2023].
- MOTA, F.L., CARNEIRO, A.P., QUEIMADA, A.J., PINHO, S.P. & MACEDO, E.A. 2009. Temperature and solvent effects in the solubility of some pharmaceutical compounds: Measurements and modelling. *European Journal of Pharmaceutical Sciences*, 37:499- 507.
- MURDANDE, S.B., PIKAL, M.J., SHANKER, R.M. & BOGNER, R.H. 2011. Aqueous solubility of crystalline and amorphous drugs: Challenges in measurement. *Pharmaceutical Development and Technology*, 16(3):187-200.
- ODENDAAL, S. 2019. Artesunate - An investigation into polymorphism. M.Sc. dissertation. NWU (Potchefstroom).
- PAPICH, M. G. & MARTINEZ, M. N. 2015. Applying biopharmaceutical classification system (BCS) criteria to predict oral absorption of drugs in dogs: challenges and pitfalls. *The AAPS Journal*, 17(4): 948-964.

- PAUDEL, A., WORKU, Z.A., MEEUS, J., GUNS, S. & VAN DEN MOOTER, G. 2013. Manufacturing of solid dispersion of poorly water-soluble drugs by spray drying: formulation and process considerations. *International Journal of Pharmaceutics*, 453:253-284.
- PRITTO, G., KABAKYENGA, J., PENOGES, L., RUIZ, A., ERIKSSON, T., COUSSEMENT, F., NGAMBE, T., TAYLOR, W.R.J., PEREA, W., GUTHMANN, JP., OLLIARO, P. & LEGROS, D. 2003. Artesunate and sulfadoxine-pyrimethamine combinations for the treatment of uncomplicated *Plasmodium falciparum* malaria in Uganda: a randomized, double-blind, placebo-controlled trial. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 97(3):325-330.
- PUBCHEM. 2020. Artesunate. <https://pubchem.ncbi.nlm.nih.gov/compound/6917864> [Date of access: 4 Apr 2023].
- ROSSITER, D., BLOCKMAN, M. & BARNES, K.I. 2016. South African Medicines Formulary (SAMF). 12th ed. South Africa: Health and Medical Publishing Group. pp. 515-518.
- SATO, S. 2021. Plasmodium-a brief introduction to the parasites causing human malaria and their basic biology. *Journal of Physiological Anthropology*, 40(1), 1. <https://doi.org/10.1186/s40101-020-00251-9>
- SCHOLAR, E. 2009. Pyrimethamine. xPharm: The Comprehensive Pharmacology Reference, 1–6. doi:10.1016/b978-008055232-3.62494-8
- SCHOLAR, E.M. & PRATT, W.B. 2000. Chemotherapy of Protozoal Diseases. The Antimicrobial Drugs, 2, pp. 419–457, Oxford Univ Press, NY, NY
- SEKIGUCHI, K. & OBI, N. 1961. Studies on Absorption of Eutectic Mixture. I. A Comparison of the Behavior of Eutectic Mixture of Sulfathiazole and that of Ordinary Sulfathiazole in Man. *Chemical and Pharmaceutical Bulletin*, 9(11):866-872.
- SERAJUDDIN, A.T.M. 1999. Solid dispersions of poorly water-soluble drugs: Early promises, subsequent problems, and recent breakthroughs. *Journal of Pharmaceutical Sciences*, 88(10):1058-1066.
- SHARMA, A. & JAIN, C.P. 2010. Techniques to enhance solubility of poorly soluble drugs: A review. *Journal of Global Pharma Technology*, 2(2):18-28.
- SOLOGUB, L., KUEHN, A., KERN, S., PRZYBORSKI, J., SCHILLIG, R. & PRADEL, G. 2011. Malaria proteases mediate inside-out egress of gametocytes from red blood cells following parasite transmission to the mosquito. *Cellular Microbiology*, 13: 897–912. <https://doi.org/10.1111/j.1462-5822.2011.01588.x>

South Africa. National Department of Health. 2020. Primary Healthcare Standard Treatment Guidelines and Essential Medicines List. 5th ed. Pretoria.

STURM, A. 2006. Manipulation of Host Hepatocytes by the Malaria Parasite for Delivery into Liver Sinusoids. *Science*, 313(5791), 1287–1290. doi:10.1126/science.1129720

SU, X. Z., & MILLER, L. H. 2015. The discovery of artemisinin and the Nobel Prize in Physiology or Medicine. *Science China Life Sciences*, 58(11): 1175-1179. <https://doi.org/10.1007/s11427-015-4948-7>

TAVARES, J., FORMAGLIO, P., THIBERGE, S., MORDELET, E., VAN ROOIJEN, N., MEDVINSKY, A., MÉNARD, R. & AMINO, R. 2013. Role of host cell traversal by the malaria sporozoite during liver infection. *Journal of Experimental Medicine*, 210: 905–915. <https://doi.org/10.1084/jem.20121130>

THAKURIA, R., DELORI, A., JONES, W., LIPERT, M.P., ROY, L. & RODRIGUEZ-HORNEDO, N. 2013. Pharmaceutical cocrystals and poorly soluble drugs. *International Journal of Pharmaceutics*, 453(2013):101-125.

VO, C. L., PARK, C. & LEE, B. J. 2013. Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. *European Journal of Pharmaceutics and Biopharmaceutics*, 85: 799-813.

WANG, Y., WANG, Y., CHENG, J., CHEN, H., XU, J., LIU, Z., Q., Shi, & C., Zhang. 2021. Recent Advances in the Application of Characterization Techniques for Studying Physical Stability of Amorphous Pharmaceutical Solids. *Crystals*, 11(12):1440.

WHO see World Health Organization

WORLD HEALTH ORGANIZATION. 2023a. World malaria report 2022. <https://www.who.int/publications/i/item/9789240064898> Date of access 31 July 2023.

WORLD HEALTH ORGANIZATION. 2023b. Technical consultation to assess evidence on community-based delivery of intermittent preventive treatment in pregnancy for malaria: report of a virtual meeting, 21–23 June 2022. World Health Organization. <https://iris.who.int/handle/10665/366329>. License: CC BY-NC-SA 3.0 IGO

WORLD HEALTH ORGANIZATION. 2023c. Guidelines for malaria, Geneva: World Health Organization; 16 October 2023 (WHO/UCN/GMP/ 2023.01 Rev.1). License: CC BY-NC-SA 3.0 IGO.

WORLD HEALTH ORGANIZATION. 2006. WHO Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. WHO Technical Report Series, 937: 379-380.

YU, L., REUTZEL-EDENS, S.M. & MITCHELL, C.A. 2000. Crystallization and polymorphism of conformationally flexible molecules: problems, patterns, and strategies. *Organic Process Research and Development*, 4:396–402.

Chapter 3: Methods & Materials

3 Introduction

To investigate if the addition of various polymers will have a positive influence on the solubility of anti-malaria drugs, namely sulfadoxine, pyrimethamine and artesunate, amorphous solid dispersions (ASDs) were prepared with the respective active pharmaceutical ingredients (APIs). Polymers, namely PVP30, PVPVA64 and HPMCAS were used in a 4:1 ratio with the APIs to form 9 ASDs, three for each API respectively. Solubility studies were performed on the individual APIs alone and also on their respective ASDs in HPLC grade water. Validation of the HPLC methods used for the APIs and ASDs were performed in the allocated solvent. Physico-chemical characterisation was also done on the APIs, polymers and ASDs to support the claim of amorphous solid dispersion formation. All methods used to reach the set objectives during this study are discussed in this chapter.

3.1 Materials

All APIs (artesunate, sulfadoxine and pyrimethamine) and other chemicals used were of analytical or HPLC grade and were purchased from reputable suppliers.

Pure and ultrapure water was obtained from a Rephile (Shanghai City, China) Direct-Pure UP Ultrapure & RO Lab Water System.

3.1.1 Polymers used as matrix formers in the ASDs

3.1.1.1 Polyvinylpyrrolidone (PVP)

PVP (Polyvinylpyrrolidone) is a non-toxic, bulky polymer. PVP consists of a variety of different components which exhibit different characteristic traits which influence its interactions with different liquids. The pyrrolidine moiety has strong hydrophilic characteristics, whereas its alkyl group presents with hydrophobic traits. Furthermore, the distinctly polar amide group in the pyrrolidine ring ensure that water and numerous other non-aqueous liquids are exceptional solvents for PVP ([Kurakula & Rao, 2020](#)). The characteristic traits of PVP enable it to act as a potent stabiliser due to its chain length, preventing aggregation by the steric hindrance effect via the repulsive forces that arise. Apart from exhibiting stabilising properties, PVP can be regarded a dispersant (used in solid dispersions) due to the extended obtained inter particle distances ([Regulska et al., 2019](#)). PVPVA64 is a vinylpyrrolidone-vinyl acetate which in turn is a co-polymer.

3.1.1.2 Hydroxypropyl methylcellulose acetate succinate (HPMCAS)

HPMCAS (hydroxypropyl methylcellulose acetate succinate) was originally developed as an aqueous enteric coating material. HPMCAS is regarded as an ester which bears acetyl and succinoyl groups. The dissolution rate of HPMCAS in different buffers can be modulated by adjusting the ratio of acetyl and succinoyl moieties ([Tanno et al., 2004](#)).

3.2 Methods

3.2.1 Preparation of physical mixtures and amorphous solid dispersions

Firstly, the respective API and polymers were mixed in mortar and pestle in a 1:1 and 1:4 ratio, producing a physical mixture for each API and respective polymer in the above-mentioned ratios. Both ratios underwent physico-chemical testing in order to indicate which ratio would deliver the most promising results with regards to ASD formation. The 1:4 proved to be the better option and was henceforth used to produce the physical mixtures for ASD formation. These physical mixtures were then dissolved in ethanol, where after the ethanol was removed under vacuum pressure at 80°C by means of rotary evaporation. The prepared amorphous solid dispersions were placed in an oven for 12 hours, at 60°C to remove any residual ethanol. The physical mixtures and ASDs prepared during this study are displayed in Table 3.1 below.

Table 3.1: Prepared ASDs and PMs of the three APIs

Physical mixture (1:4)	Amorphous solid dispersion (1:4)
Sulfadoxine and PVP30 (SPVP30 PM)	Sulfadoxine and PVP30 (SPVP30 ASD)
Sulfadoxine and PVP 64 (SPVP64 PM)	Sulfadoxine and PVP 64 (SPVP64 ASD)
Sulfadoxine and HPMC (SHPMC PM)	Sulfadoxine and HPMC (SHPMC ASD)
Pyrimethamine and PVP30 (PPVP30 PM)	Pyrimethamine and PVP30 (PPVP30 ASD)

Pyrimethamine and PVP64 (PPVP64 PM)	Pyrimethamine and PVP64 (PPVP64 ASD)
Pyrimethamine and HPMC (PHPMC PM)	Pyrimethamine and HPMC (PHPMC ASD)
Artesunate and PVP30 (APVP30 PM)	Artesunate and PVP30 (APVP30 ASD)
Artesunate and PVP64 (APVP64 PM)	Artesunate and PVP64 (APVP64 ASD)
Artesunate and HPMC (AHPMC PM)	Artesunate and HPMC (AHPMC ASD)

3.2.2 Physico-chemical characterisation

Physico-chemical characterisation was performed on the various APIs and their corresponding PMs and ASDs. The reason for physico-chemical characterisation of APIs, PMs and ASDs is to confirm the successful formation of the ASDs. This entire process will allow a comparison to be made between the various APIs, the PMs, and the ASDs.

3.2.2.1 Simultaneous thermal analysis (STA)

STA simultaneously measures the mass loss (TGA) and heat flow (DSC) of a sample during the heating process. With this combined technique, information about the mass loss, melting point, glass transition, solid-state transformation(s), loss of solvents and degradation of a sample can be obtained ([Brown, 2001](#)).

A Mettler DTG 3⁺ (Mettler Toledo, Greifensee, Switzerland) was used to record the DSC and TGA thermograms. Powder samples, weighing approximately 3 mg–5 mg, were placed in open aluminum cells, (100 µl) and heated to an end temperature dependent on the melting point of the API and/or polymer, at a heating rate of 10°C/min, with a nitrogen gas flow of 35 ml/min. The samples were then cooled and subjected to the same thermal program once more, to test for miscibility between the polymer and the API. A single glass transition temperature or melting point depression are mostly used to evaluate miscibility between the polymer and the API ([Liu et al., 2017](#)).

3.2.2.2 Fourier transform infrared spectroscopy (FTIR)

Fourier transformed infrared spectroscopy (FTIR) was used to obtain information on the structure and the molecular conformation of a sample by measuring the vibration modes of bonded atoms as well as to examine the intermolecular interactions between the drugs in combination within the solid-state ([Bernstein, 2002](#); [Rodriguez-Spong et al., 2004](#)). This method, together with numerous others is used to characterise solid dispersions ([Habib et al., 2001](#)).

All samples were examined using a Shimadzu IR Tracer-100 spectrophotometer (Shimadzu, Kyoto, Japan). The samples were placed on a QATR 10 accessory and scanned 48 times over a range of 4000 – 400 cm⁻¹. The data was then processed using LabSolutions version 1.108.

3.2.2.3 X-ray powder diffraction (XRPD)

X-ray powder diffraction (XRPD) produces a crystallographic diffractogram of an API which is unique to each crystal structure. It can be used to differentiate and identify different solid-state forms of the same API or to identify an API by unique peak positions ([Bhattacharya et al., 2009](#)). X-ray diffraction (XRD) is used to classify, identify, and characterise the crystalline or amorphous state of the drug. A crystalline sample will exhibit a distinctive fingerprint of the particular API, whereas with an amorphous material a broad, diffuse signal will be obtained ([Liu et al., 2017](#)).

Samples were evenly distributed on a zero-background sample holder and then placed into the PANalytical Empyrean diffractometer (PANalytical, Almelo, Netherlands) for analysis. The measurement conditions were as follows: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; antiscatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2° /min (step size, 0.025°; step time, 1.0 sec). The data was analysed using HighScore Plus software version 3.0a.

3.2.2.4 Polarised light microscopy (PLM)

Polarised light microscopy (PLM) can be used to detect crystalline material in an amorphous solid dispersion. Anisotropic materials can be birefringent, i.e., an optical property of a substance. Whenever birefringence is observed in a sample, it indicates crystallinity. A crystalline material showed interference or polarised colours. Amorphous solids are isotropic, has no long-range order in their molecular packing, and are non-birefringent and therefore exhibit no interference colours ([Liu et al., 2017](#)).

A polarised microscope is thus a useful instrument for the measurement of birefringence in materials. For this study the PLM was utilised as an instrument to test for birefringence. A Nikon Eclipse 50i microscope (Tokyo, Japan) was used fitted with a Nikon DS-Fil camera.

3.2.2.5 Thermal activity monitor (TAM)

Compatibility between the different APIs and polymers were established with a 2277 Thermal Activity Monitor (TAM III) (Thermometric AB, Sweden), equipped with an oil bath with a stability of $\pm 100 \mu\text{K}$ over 72 hours. The calorimeter's temperature was maintained at 40°C . During the compatibility studies the heat flow was measured for the single components (APIs and polymers individually), as well as for the ASDs. The TAM is a sensitive and accurate instrument for detecting potential incompatibilities and instabilities between APIs and/or excipients. Readings produced by the thermal activity monitor which exceed 2-3 Joules/gram indicate interaction and are thus unfavourable readings. Any readings below this range are desired to prove absence of interaction.

3.2.3 High-performance liquid chromatography (HPLC)

A Hitachi Chromaster (Tokyo, Japan) chromatographic system utilising a 5430-photodiode array detector, an auto-sampler (5260) with a sample temperature controller (5310) and a solvent delivery module (5160) was used during this study. Clarity VA software was used to evaluate the data. The most suitable method was validated in-house according to the pharmacopeia ([Ph. Int. 2020a](#); [Ph. Int. 2020b](#); [Ph. Int. 2020c](#)).

For sulfadoxine and pyrimethamine and their respective ASDs the same mobile phase and analytical method was used. 20 ml glacial acetic acid (100%) and 1ml triethylamine dissolved in approximately 1600 ml ultrapure (UP) HPLC grade water. The pH was adjusted to 4.2 by making use of sodium hydroxide solution (10 N). Thereafter HPLC grade water was added to total 2000 ml, 1600 ml of this solution was then measured out to which 400 ml of acetonitrile was added. The solution was then degassed and filtered. The flow rate on the HPLC was set at 1.5 ml/min, and the injection volume to 20 μl . The run time per sample was set at 15 minutes ([Ph. Int. 2020a](#); [Ph. Int. 2020b](#)).

For artesunate and its' respective ASDs the mobile phase consisted of 1.36 g of potassium dihydrogen orthophosphate dissolved in approximately 900 ml of HPLC grade water. The pH was adjusted to 3.0 by making use of an orthophosphoric acid solution (1440 g/L). Thereafter the solution was made to volume 1000 ml. From this solution, 560 ml was then taken, and 440 ml acetonitrile was added. The flow rate on the HPLC was set at 1 ml/min, and the injection volume to 20 μl . The run time per sample was set at 20 minutes ([Ph. Int. 2020c](#)).

3.2.4 Solubility studies

Solubility studies were completed at 37°C in a water bath with a rotating axis, spinning at approximately 54 rpm. Individual samples were weighed off into test tubes, to which the solvent (ultra purified H₂O), was added (see Tables 3.2-3.4 for details of all APIs and their respective ASDs). The test tubes were sealed with Parafilm® and a screw cap was fit onto each test tube to prevent leakage. Individually, the test tubes were attached to the rotating axis in the water bath and switched on to rotate for 48 hours. The motor of the rotating axis was switched off at various times (2, 4, 8, 12, 24 and 48 hours) throughout the 48 hours and the samples were left to settle. Samples were withdrawn at each time interval, filtered by a 0.45 µm pore size filter, and assayed by the HPLC using the corresponding analytical method per API. The preliminary solubility studies were employed to obtain solubility profiles for all APIs and ASDs to establish equilibrium. The pivot solubility experiments were commenced whereby the parameters remained constant, but the duration of the solubility studies were adjusted to a suitable time after equilibrium. This was done to ensure that exposure time of the drugs were minimised to produce optimal results after equilibrium with minimal possible degradation.

3.2.4.1 APIs and ASDs

The weight for the various studies were determined based on the weight needed to provide a super saturated solution of each individual API, which was tested in the beginning of the study. Then the 1:4 ratio was brought into consideration when deciding on the final weight.

For the preliminary and pivot solubility studies of sulfadoxine (SULFA) API, 20 mg was weighed off in each test tube (in triplicate). For the respective ASDs, SPVP30, SPVPVA64 and SHPMC 100 mg was weighed off in each test tube (in triplicate). A volume of 5 ml of solvent, which was ultrapure HPLC-grade water, was pipetted into each tube to produce super-saturated solutions. Table 3.2 represents the corresponding weights.

Table 3.2: Weight of SULFA API and ASDs (mg) per test tube

	Weight per test tube
SULFA RM	20 mg
SPVP30 ASD	100 mg
SPVP64 ASD	100 mg
SHPMC ASD	100 mg

For the preliminary and pivot solubility studies of pyrimethamine (PYRI) API, 25 mg was weighed off and for the respective ASDs, PPVP30, PPVPVA64 and PHPMC 25 mg was weighed off in each test tube (in triplicate). 5 ml of solvent, which was ultrapure HPLC-grade water, was pipetted into each tube to produce super-saturated solutions. Table 3.3 represents the corresponding weights.

Table 3.3: Weight of PYRI API and ASDs (mg) per test tube

	Weight per test tube
PYRI RM	25 mg
PPVP30 ASD	25 mg
PPVP64 ASD	25 mg
PHPMC ASD	25 mg

For the preliminary solubility studies of artesunate (ART) API, 10 mg was weighed off and for the pivot solubility 50 mg was weighed off to ensure a super saturated solution (as seen in Table 3.4). For the respective ASDs, APVP30, APVPVA64 and AHPMC 50 mg was weighed off in each test tube (in triplicate) and 5 ml of solvent, which was ultrapure HPLC-grade water, was pipetted into each tube to produce super-saturated solutions. Table 3.4 represents the corresponding weights.

Table 3.4: Weight of ART API and ASDs (mg) per test tube

	Weight per test tube
ART RM	50 mg
APVP30 ASD	50 mg
APVP64 ASD	50 mg
AHPMC ASD	50 mg

3.2.5 Statistical Analysis

To determine whether two solubility profiles differed from one another on a statistically significant level, analysis of covariance (ANCOVA) was used for the preliminary solubility studies. For the pivot solubility studies Mann-Whitney U tests were performed with Bonferroni adjustments of the resulting p-values in order to investigate the differences in solubility between the individual APIs and their ASDs.

Reference List

- BERNSTEIN, J. 2002. Analytical techniques for studying and characterizing polymorphs. *Polymorphism in molecular crystals*. Oxford: Clarendon Press. p410.
- BHATTACHARYA, S., BRITTAI, H.G. & SURYANARAYANAN, R. 2009. Thermoanalytical and crystallographic methods. (In Brittain, H.G., ed. *Polymorphism in pharmaceutical solids*. New York: Informa Healthcare. p. 318-346).
- BROWN, M.E. 2001. Differential thermal analysis (DTA) and differential scanning calorimetry (DSC). *Introduction to thermal analysis*. Netherlands: Kluwer Academic Publishers. p55.
- HABIB, M.J., VENKATARAM, S. & HUSSAIN, M.D. 2001. Fundamentals of solid dispersions. (In Habib, M.J. ed. *Pharmaceutical solid dispersion technology*. Lancaster: Technomic. p. 7-35).
- INTERNATIONAL PHARMACOPOEIA. 2020a. 19th ed. Sulfadoxine. <https://digicollections.net/phint/2020/index.html#d/b.6.1.342> Date of access: 4 May 2023.
- INTERNATIONAL PHARMACOPOEIA. 2020b. 19th ed. Pyrimethamine. <https://digicollections.net/phint/2020/index.html#d/b.6.1.307> Date of access: 4 May 2023.
- INTERNATIONAL PHARMACOPOEIA. 2020c. 19th ed. Artesunate. <https://digicollections.net/phint/2020/index.html#d/b.6.1.30> Date of access: 4 May 2023.
- KURAKULA, M. & RAO, G.S.N.K. 2020. Pharmaceutical assessment of polyvinylpyrrolidone (PVP): As excipient from conventional to controlled delivery systems with a spotlight on COVID-19 inhibition. *Journal of Drug Delivery Science and Technology*, 60, p.102046. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7462970/>. [Accessed 9 Oct. 2023].
- LIU, X., FENG, X., WILLIAMS III, R. O., ZHANG, F. 2017. Characterization of amorphous solid dispersions. *Journal of Pharmaceutical Investigation*, 48(2018):19-41.
- REGULSKA, K., REGULSKI, M., WZGARDA, A., KOTOWSKA, A., IGNASIAK, A., ĆWIERTNIA, B. AND STANISZ, B. 2019. Does Polyvinylpyrrolidone Improve the Chemical Stability of Cilazapril in Solid State. *Iranian Journal of Pharmaceutical Research*, 18(2):579–595. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6706751/>. [Accessed 2 Oct. 2023].
- RODRÍGUEZ-SPONG, B., PRICE, C.P., JAYASANKAR, A., MATZGER, A.J. & RODRÍGUEZ-HORNEDO, N. 2004. General principles of pharmaceutical solid polymorphism: A supramolecular perspective. *Advanced Drug Delivery Reviews*, 56:241-274.

TANNO, F., NISHIYAMA, Y., KOKUBO, H. AND OBARA, S. 2004. Evaluation of Hypromellose Acetate Succinate (HPMCAS) as a Carrier in Solid Dispersions. *Drug Development and Industrial Pharmacy*, 30(1):9–17.

Chapter 4: Results & discussion

4 Introduction

An approach to improve the poor solubility property of an API, is to produce an amorphous solid dispersion. Amorphous compounds have no defined shape on molecular level which enables the structure to have higher molecular mobility and hence a better solubility profile. Amorphous materials contain increased amounts of internal energy (Gibbs free energy) in comparison to crystalline structures of the same compound, which in effect can promote the solubility and dissolution thereof ([Hancock & Zografi, 1997](#); [Byrn *et al.*, 1999](#); [Cui, 2007](#)). The three APIs sulfadoxine, pyrimethamine and artesunate present with poor aqueous solubility. In an attempt to improve the aqueous solubility of these APIs, ASDs were prepared and evaluated through physico-chemical characterisation, HPLC analysis and solubility testing. In this chapter the results obtained during these tests are discussed.

4.1 Sulfadoxine and ASDs

4.1.1 Physico-chemical characterisation of sulfadoxine and ASDs

4.1.1.1 Differential scanning calorimetry

When performing a differential scanning calorimetry (DSC) on SULFA raw material (RM), an endothermic peak indicative of its melting point of 199.29°C was recorded (Figure 4.1) which correlates with literature ([Ph. Int. 2020a](#)).

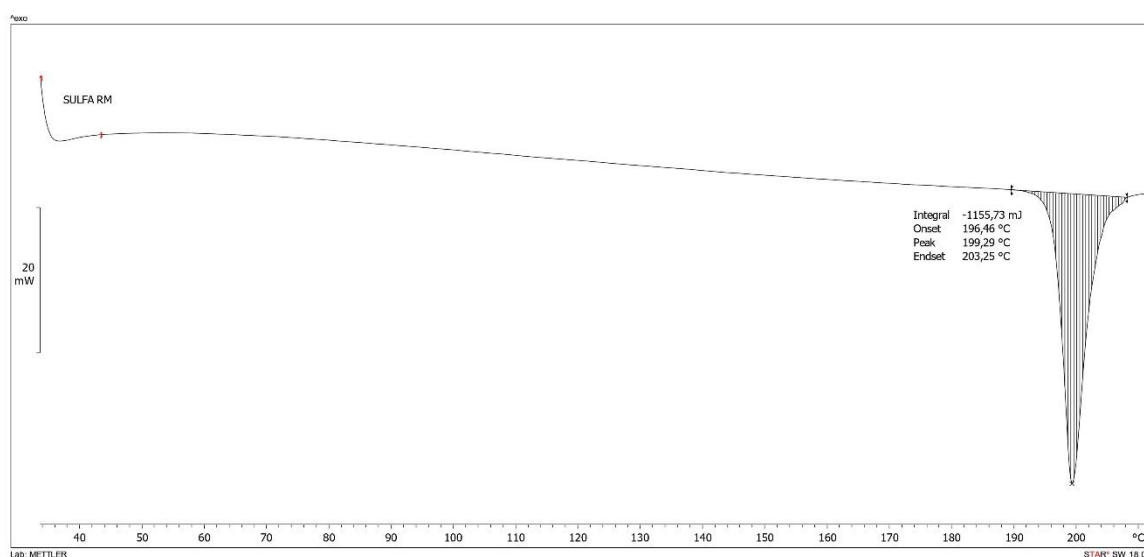


Figure 4.1: DSC thermogram of SULFA RM.

Miscibility tests are carried out to identify if the polymers were suitable in terms of miscibility with SULFA and which have adequate physico-chemical characteristics. The powder sample, weighing approximately 3 mg – 5 mg was placed in open aluminium 100 µl cells and heated to an end temperature dependent on the melting point of sulfadoxine, at a heating rate of 10°C /min, with a nitrogen gas flow rate of 35 ml/min. The sample was then cooled and subjected to the same thermal program once more. This will show whether a glass was formed if a glass transition temperature (T_g) can be observed and whether crystallisation occurs upon heating.

The miscibility tests were performed on physical mixtures which were prepared using SULFA and the respective polymer. Initially SULFA and PVP30, PVPVA64 and HPMCAS physical mixtures were prepared in ratios of 1:4 (weight to weight) respectively.

In order to produce ASDs of SULFA and the various polymers, the mixture had to be proven miscible. The DSC thermograms of SULFA and the various polymer physical mixtures and ASDs are illustrated in Figures 4.2-4.4.

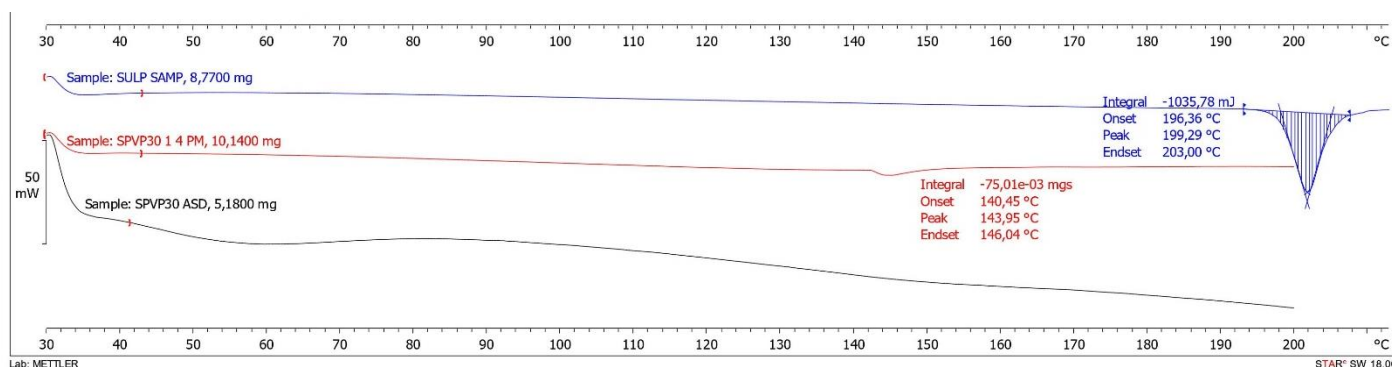


Figure 4.2: DSC thermogram of SULFA (blue), SPVP30 PM (red) and SPVP30 ASD (black).

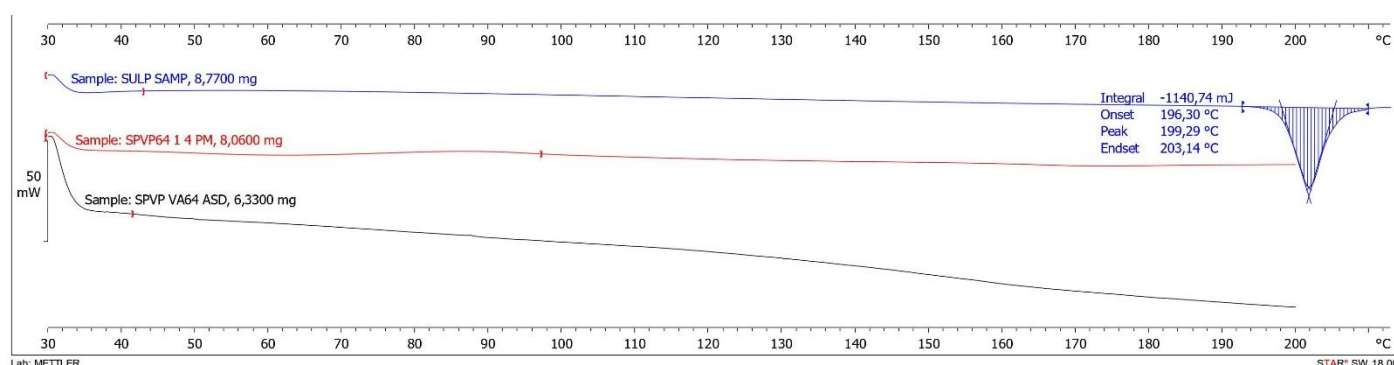


Figure 4.3: DSC thermogram of SULFA (blue), SPVP64 PM (red) and SPVP64 ASD (black).

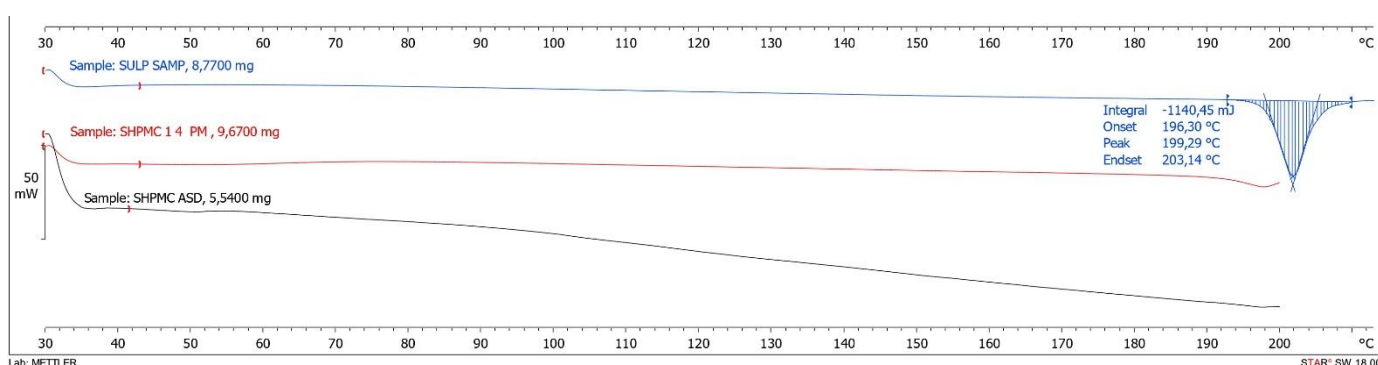


Figure 4.4: DSC thermogram of SULFA (blue), SHPMC PM (red) and SHPMC ASD (black).

Figures 4.2 to 4.4 exhibit only 1 endothermic event at 199°C, resembling the melting point of SULFA (blue). The physical mixture (red) showed no endothermic event, indicating a miscible system. The thermogram of the prepared ASD (black) showed also only a straight line, which suggests that an ASD was successfully prepared. The absence of a glass transition (T_g) may be due to the presence of the polymer within the ASD ([Newman & Zografli, 2015](#)).

4.1.1.2 Fourier-transform infrared spectroscopy (FTIR)

Fourier-transform infrared spectroscopy is a technique used to produce a characteristic molecular “fingerprint” of a substance as shown for SULFA raw material in Figure 4.5. By passing infrared radiation through a sample, the amount as well as the extent of absorption of the infrared radiation is dependent on the constituents of the sample (functional groups) and the frequencies of the vibrations between the different bonds and their respective angles. The relative absorption produces a spectrum with distinctive peaks indicating the different constituents of the sample ([NicDaéid, 2005](#)).

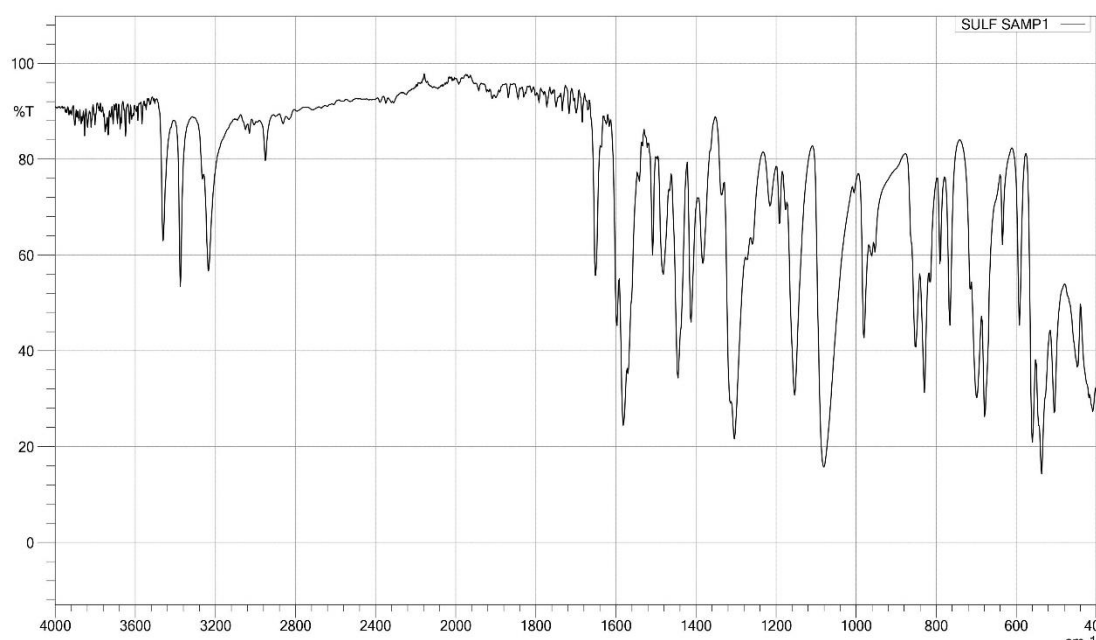


Figure 4.5: FTIR spectrum of the SULFA RM sample.

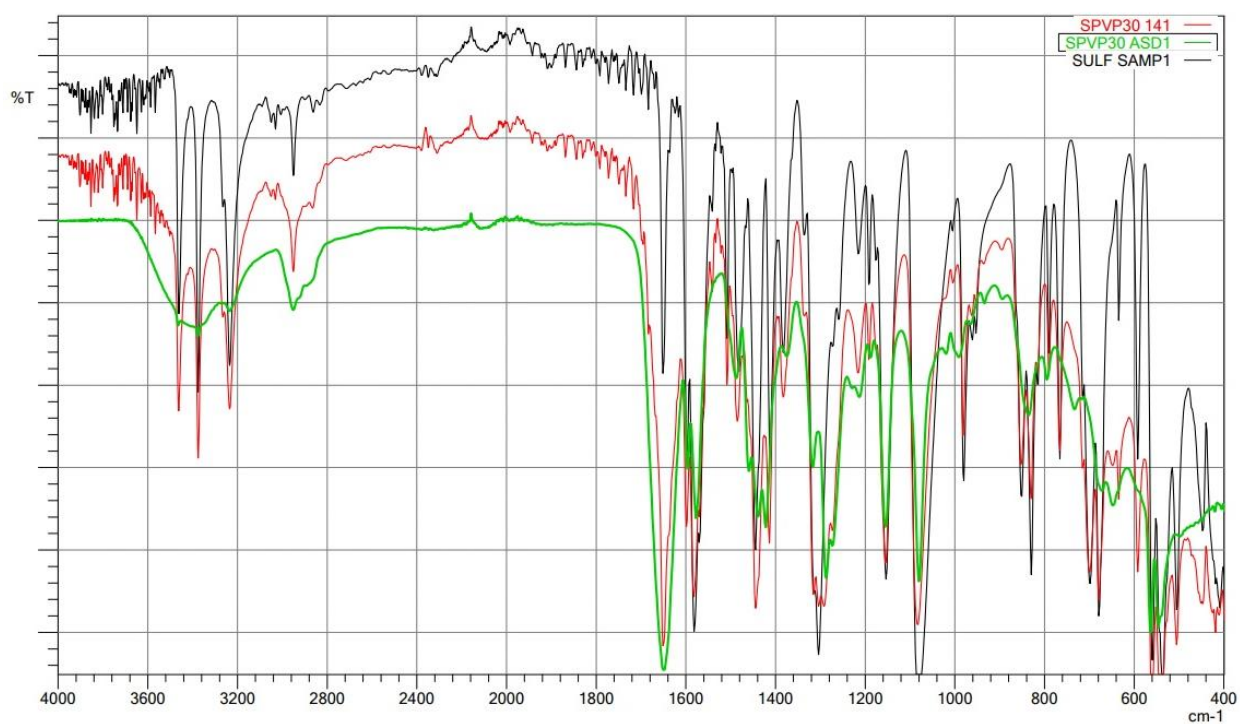


Figure 4.6: FTIR spectra of SULFA RM (black), SPVP30 ASD (green), and SPVP30 PM (red).

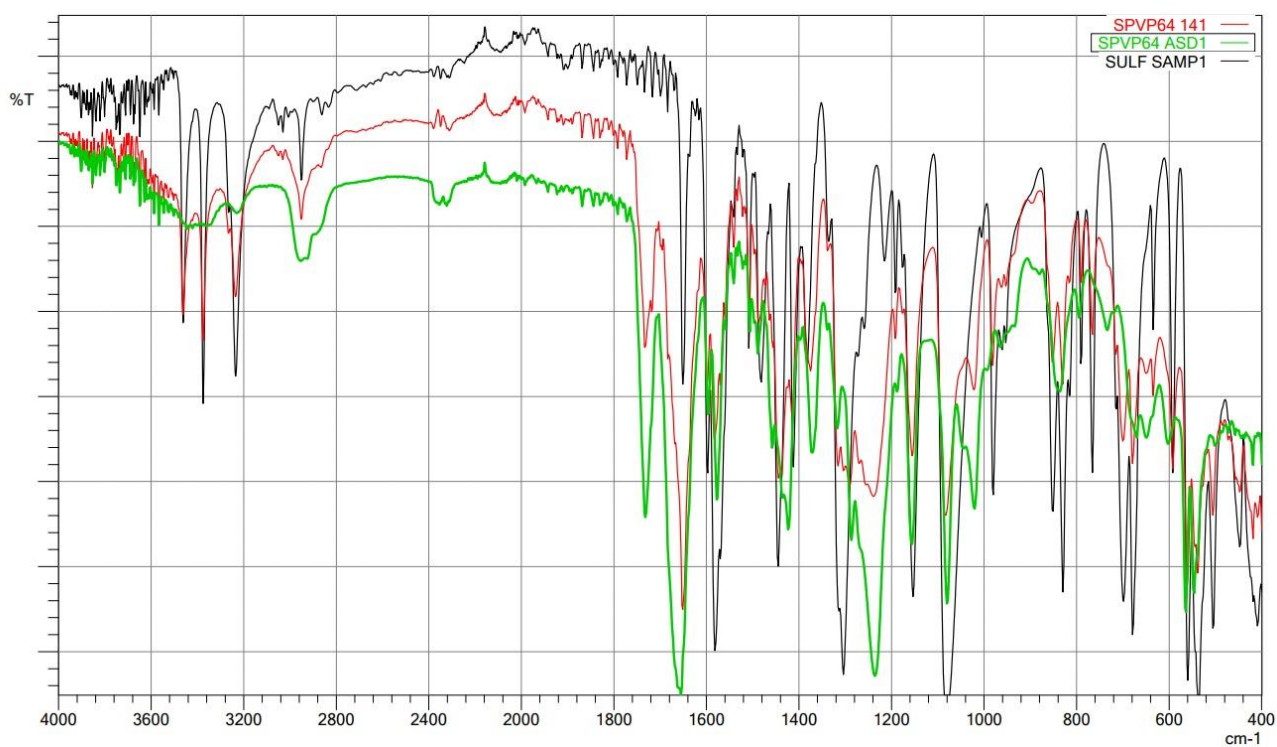


Figure 4.7: FTIR spectra of SULFA RM (black), SPVP64 ASD (green), and SPVP64 PM (red).

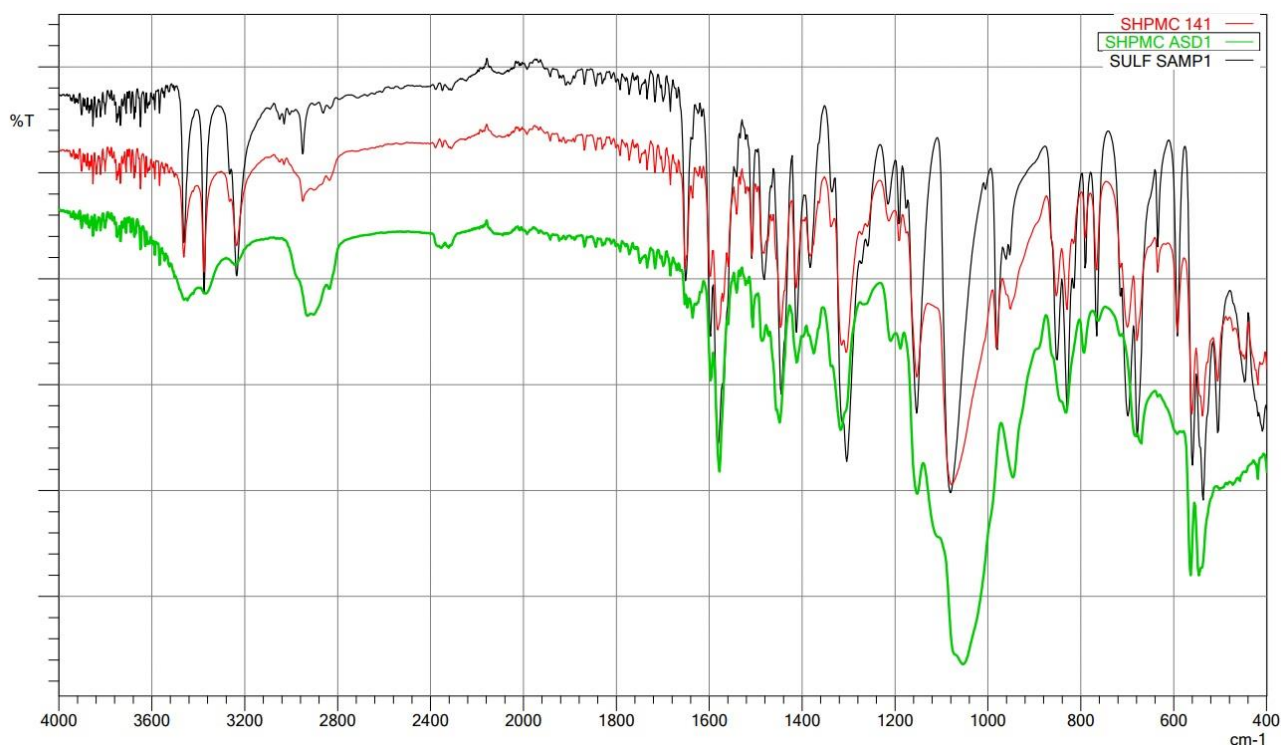


Figure 4.8: FTIR spectra of SULFA RM (black), SHPMC ASD (green), and SHPMC PM (red).

Figure 4.6: In the FTIR spectra of SPVP30 ASD (green), and SPVP30 PM (red) significant differences were observed at $3600 - 3200 \text{ cm}^{-1}$. The disappearance of peaks specific to SPVP30 ASD (green) is visible. This is indicative of a successful preparation of the ASD.

Figure 4.7: In the FTIR spectra of SPVP64 ASD (green), and SPVP64 PM (red), significant differences were observed at $3600 - 3200 \text{ cm}^{-1}$. The disappearance of peaks specific to SPVP64 ASD (green) is visible. Once again, an indication of a successful formation of an ASD.

Figure 4.8: In the FTIR spectra of SHPMC ASD (green), and SHPMC PM (red), significant differences were observed at $3600 - 3200 \text{ cm}^{-1}$, with the disappearance of peaks specific to SHPMC ASD (green) being visible, and also at $1200 - 1000 \text{ cm}^{-1}$ where the broadening of peaks specific to SHPMC ASD (green) can be seen. Once again, an indication of a successful formation of the ASD.

According to the FTIR spectra, all three polymers together with the API, were successful in the preparation of the various ASDs.

4.1.1.3 X-ray powder diffraction (XRPD)

Identifying the solid-state form of a compound is of significant pharmaceutical importance with regards to the physico-chemical properties of a compound, for example crystalline or amorphous. An XRPD analysis was carried out on SULFA raw material in order to establish whether SULFA is of amorphous or crystalline nature. The diffractogram illustrates the crystalline nature of SULFA (Figure 4.9).

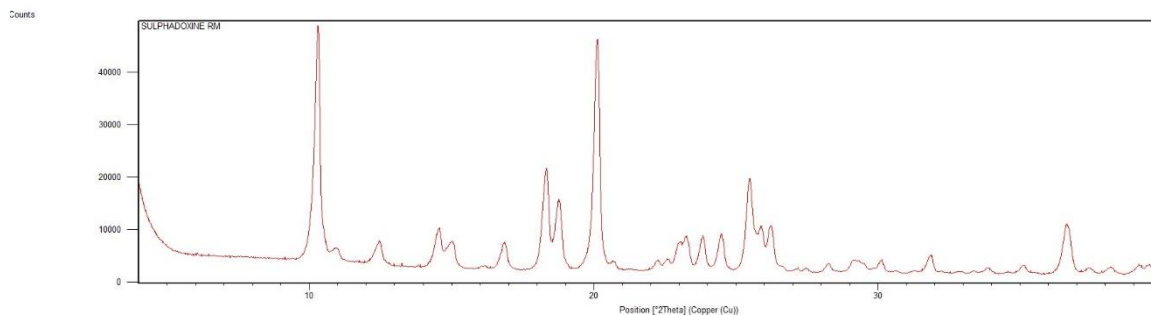


Figure 4.9: XRPD of SULFA RM sample.

Amorphous materials are characterised by a single optimum intermolecular bonding network, as these materials lack long-range translational, conformational and orientational symmetry. Crystalline materials on the other hand, exhibit a random distribution of bond angles and distances due to the specific packing of the molecular arrangement ([Bates et al., 2006](#)). Amorphous materials thus indicate a smoother halo type of pattern and in crystalline materials sharp peaks at different angles are displayed ([Lee et al., 2014](#)).

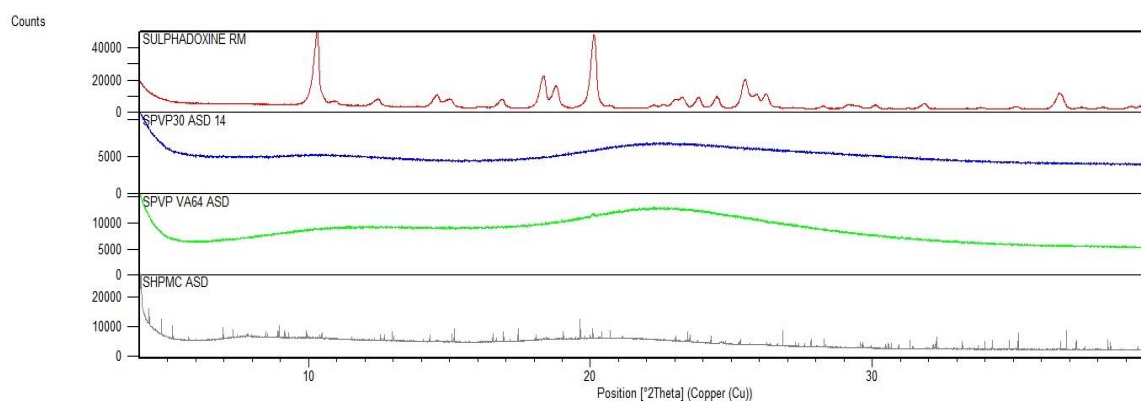


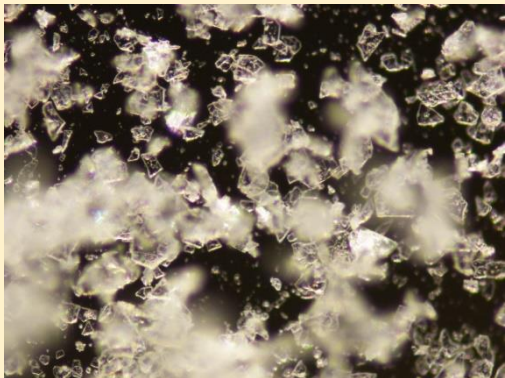
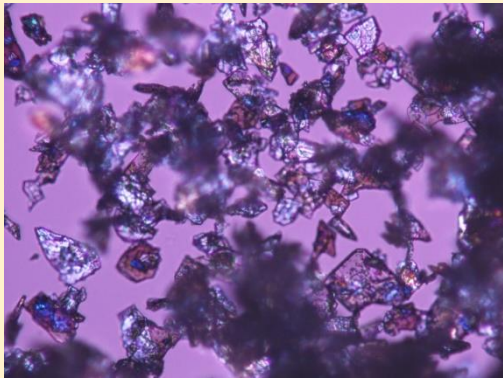
Figure 4.10: XRPD of SULFA (red), SPVP30 ASD (blue), SPVP64 ASD (green) and SHPMC ASD (black).

In Figure 4.9 and again in Figure 4.10 it can be seen that the XRPD of SULFA RM (red) exhibited crystalline peaks as expected. The SPVP30 ASD, SHPMC and SPVP64 ASDs all displayed amorphous diffractograms which are indicative of successful formation of ASDs.

4.1.1.4 Polarised light microscopy

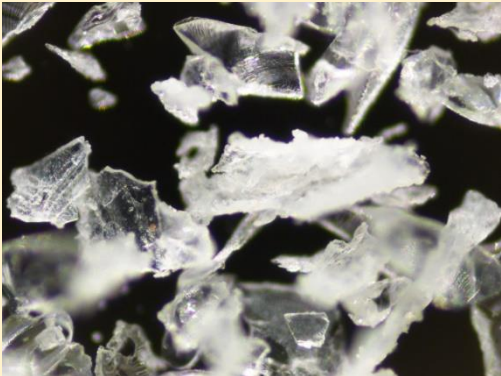
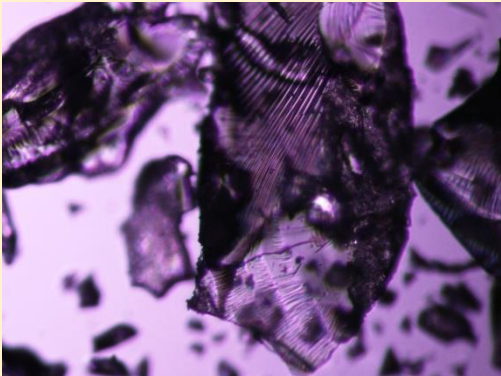
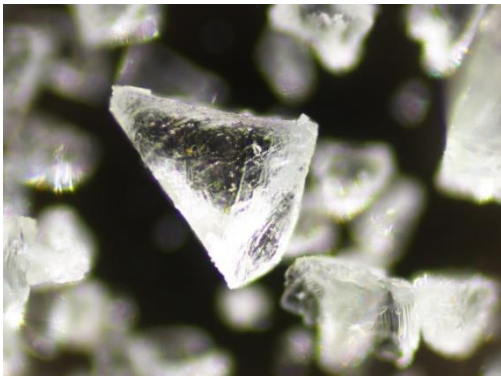
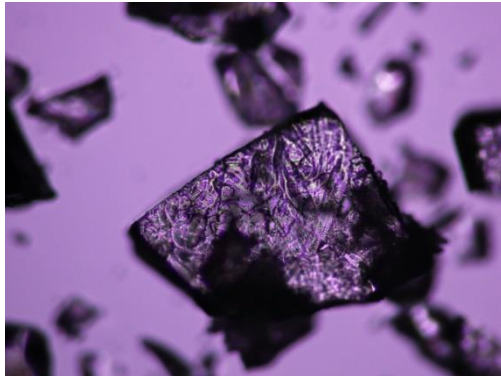
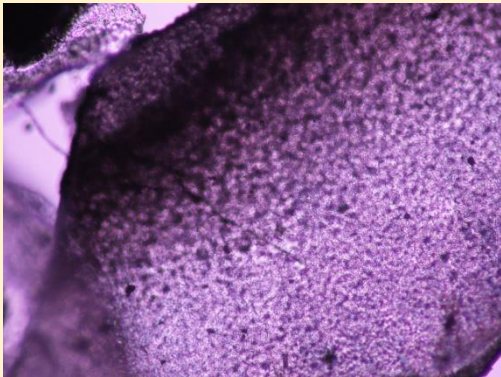
Polarised light microscopy is used to confirm whether a sample is in the crystalline or in the amorphous state. Birefringence is an optical property possessed by materials which has a refractive index and can be explained by double refraction. Double refraction occurs when a ray of light is split by polarisation upon contact with a birefringent material. A multi-colour, “rainbow-like” array of light is produced when the two rays of light proceed in exiguously different directions ([Liu et al., 2017](#)), as seen with the sulfadoxine raw material in Table 4.1.

Table 4.1: Light microscopy micrographs of SULFA raw material

SULFA RM Non-polarised (20x)	SULFA RM Polarised (20x)
	

The ASDs in fact exhibit no birefringence and thus no double refraction which acts as proof that crystalline properties are absent (Table 4.2).

Table 4.2: Polarised light microscopy micrographs of SULFA ASDs

	NON-POLARISED FILTER	POLARISED FILTER
SPVP30 ASD		
SPVP64 ASD		
SHPMC ASD		

4.1.1.5 Thermal activity monitoring (TAM)

From Figures 4.11-4.14 it can be concluded that the sulfadoxine API as well as the ASDs presented with no interactions as the interaction integrals for all the samples lie beneath the 2-3 joules/g range.

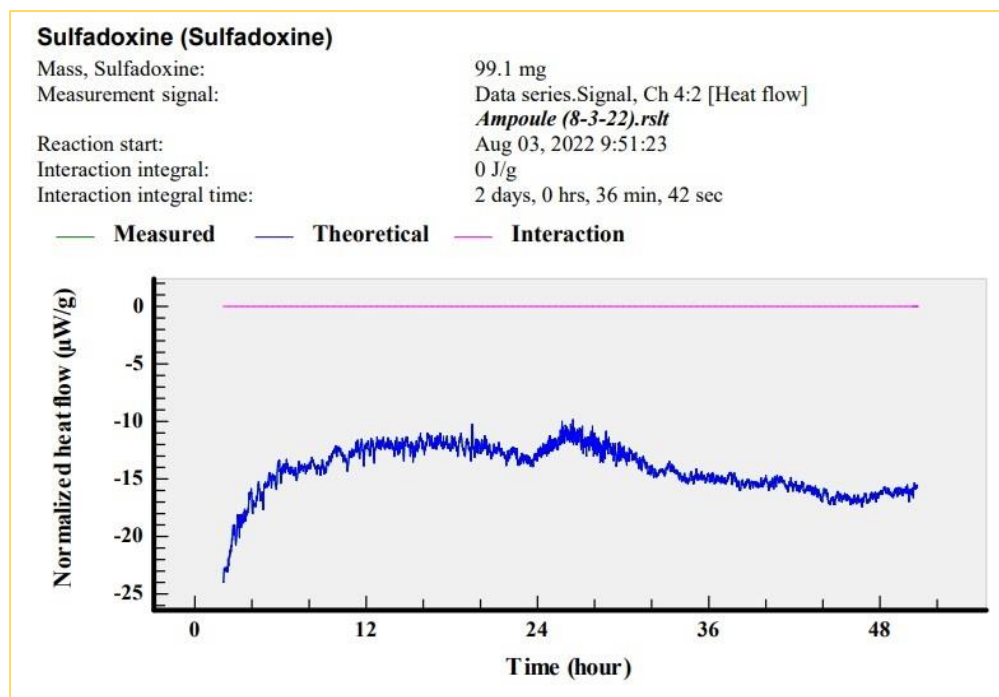


Figure 4. 11: SULFA RM thermal activity monitoring.

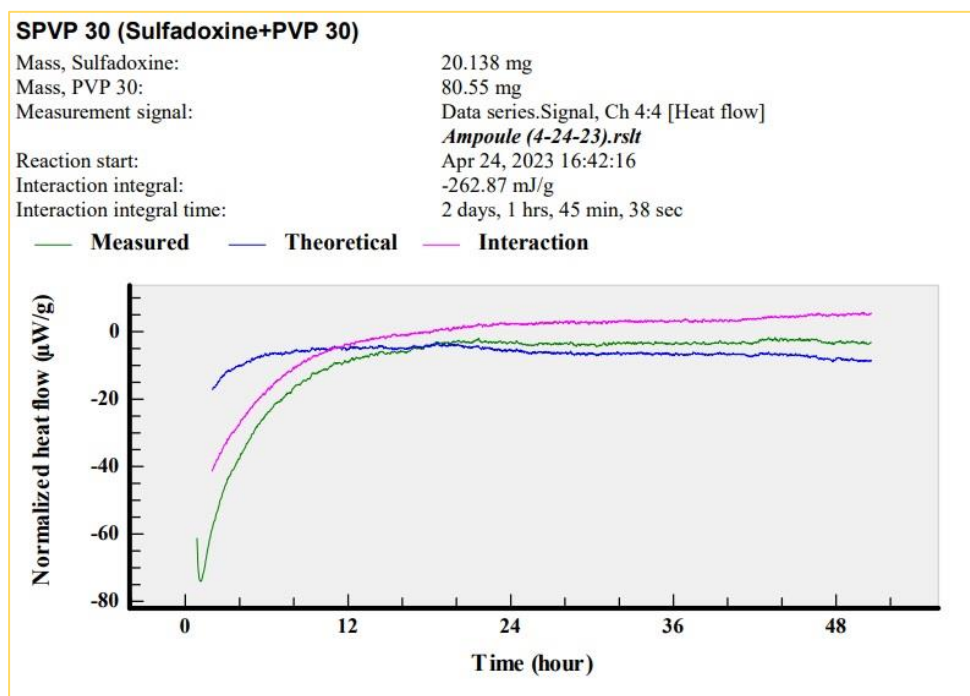


Figure 4.12: SPVP30 ASD thermal activity monitoring.

SPVP 64 (Sulfadoxine+PVP 64)

Mass, Sulfadoxine: 20.284 mg
Mass, PVP 64: 81.136 mg
Measurement signal: Data series.Signal, Ch 4:5 [Heat flow]
Ampoule (4-24-23).rslt
Reaction start: Apr 24, 2023 16:42:16
Interaction integral: -1.0836 J/g
Interaction integral time: 2 days, 1 hrs, 45 min, 38 sec

— Measured — Theoretical — Interaction

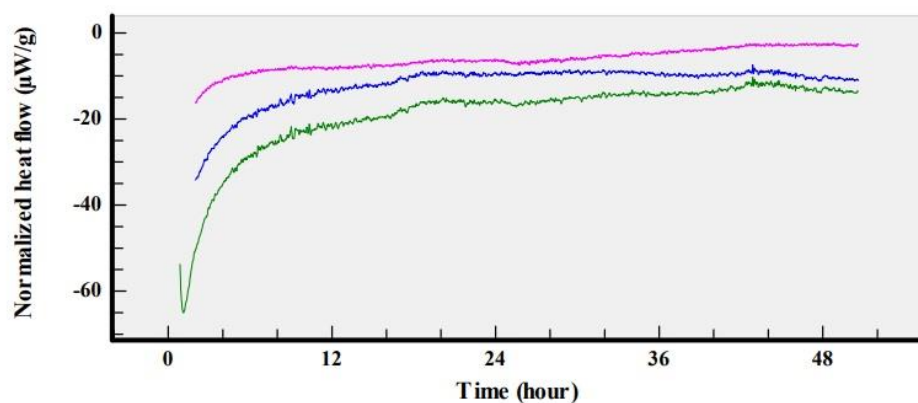


Figure 4.13: SPVP64 ASD thermal activity monitoring.

SHPMC (Sulfadoxine+HPMC)

Mass, Sulfadoxine: 20.268 mg
Mass, HPMC: 81.072 mg
Measurement signal: Data series.Signal, Ch 4:6 [Heat flow]
Ampoule (4-24-23).rslt
Reaction start: Apr 24, 2023 16:42:16
Interaction integral: 262.52 mJ/g
Interaction integral time: 2 days, 1 hrs, 45 min, 38 sec

— Measured — Theoretical — Interaction

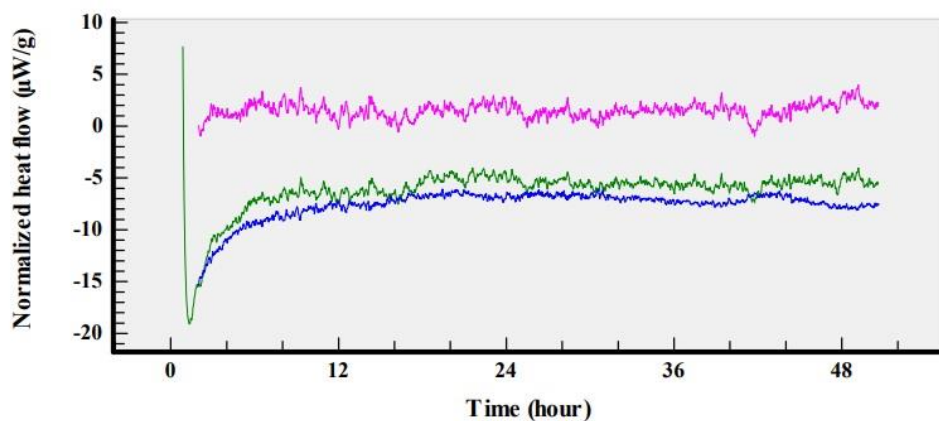


Figure 4.14: SHPMC ASD thermal activity monitoring.

4.1.2 Validation of HPLC method for sulfadoxine and ASDs

Validation of an HPLC method, entails conducting tests to confirm the technique as well as the procedure followed, and results acquired by an apparatus and specific method. In order to produce an outcome which is suited, and precise, specific requirements must be met by such a technique to ensure consistency ([Butler, 2007](#)). For the purposes of this study, limited parameters (e.g., linearity) were employed to ascertain the suitability.

Linearity plays an important role to prove a methods' sensitivity during the analysis of an analyte's concentration within specified limits. Linearity is an important validation parameter as most instruments have a limited linear range ([Van Loco et al., 2002](#)). The International Conference on Harmonization of Technical Requirements for registration of pharmaceuticals for humans (ICH) guidelines state that the linearity of a specific technique or procedure must be assessed using at least five different concentrations of the analyte ([ICH, 2020](#)). The requirements for a curve to be linear is that the regression is larger or equal to 0.99 ($R^2 \geq 0.99$) ([Singh, 2013](#)).

Linearity of SULFA raw material, as seen in Figure 4.15, was established by injecting a series of five different concentrations of SULFA using HPLC as analytical technique. These concentrations were as follow: 49 µg/ml, 198.8 µg/ml, 397.6 µg/ml, 596.4 µg/ml, and 700 µg/ml. The regression coefficient obtained was 0.9998 which thus verifies linearity ([Singh, 2013](#)).

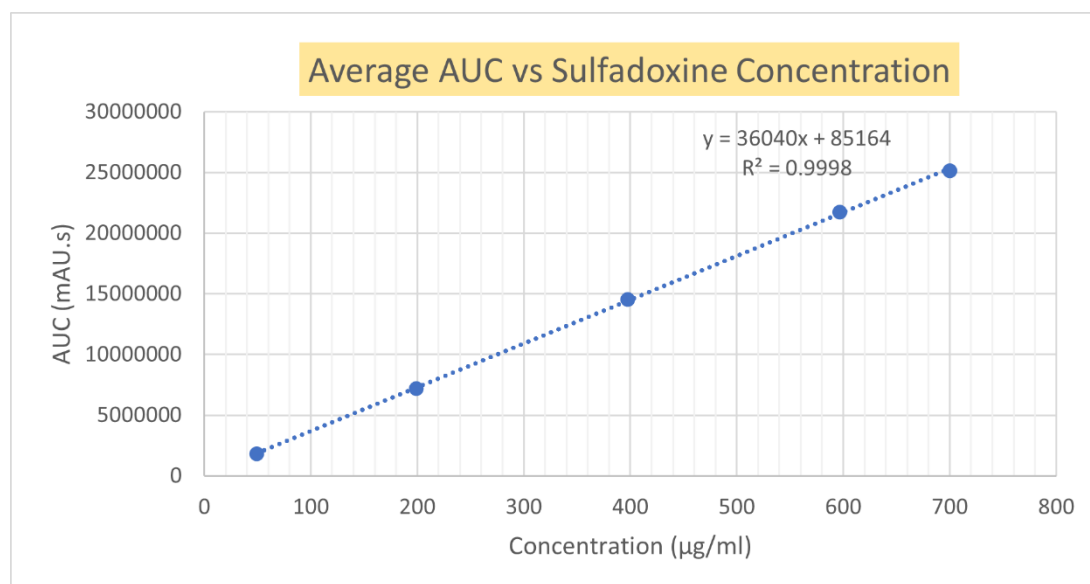


Figure 4.15: Results of HPLC method linearity for SULFA RM.

Calibration curves covering the appropriate analytical range for the respective ASDs are displayed in Figure 4.16, and according to the criteria mentioned above, all the calibration curves comply with regards to the correlation coefficient ($R^2 \geq 0.99$) (Singh, 2013). Thus, proving linearity was obtained across the applicable ranges for all of the ASDs.

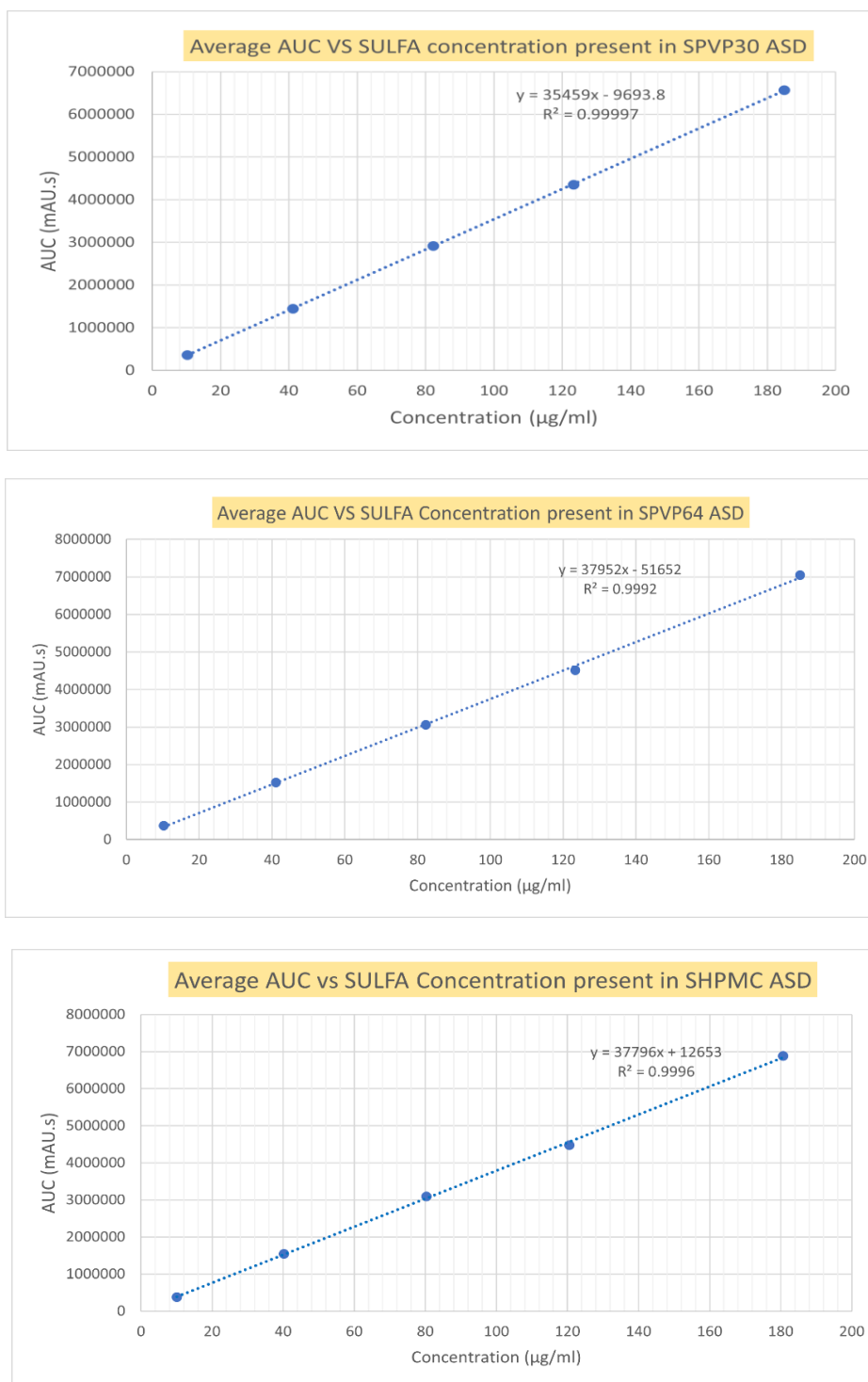


Figure 4.16: Results of HPLC method linearity for SPVP30, SPVP64 and SHPMC ASDs.

4.1.3 Solubility studies of sulfadoxine and ASDs

Solubility studies for the API and ASDs were performed using the parameters described in Chapter 3 (see Section 3.2.3). All samples were analysed using HPLC. In this section the results obtained for SULFA, SPVP30, SPVP64 and SHPMC (both preliminary and pivot studies) are discussed.

4.1.3.1 Sulfadoxine preliminary solubility studies

The results obtained for the preliminary solubility study of sulfadoxine RM in ultrapure H₂O are displayed in Table 4.3 and Figure 4.17.

Table 4.3 Preliminary solubility data of SULFA RM in ultrapure H₂O

Sulfadoxine Solubility Studies								
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	8554533	8472367	8513450	233.86	232.39	1.32	0.57
	2	8498662	8395563	8447113	232.02			
	3	8478617	8363659	8421138	231.30			
4hours	4	8565277	8512255	8538766	234.56	225.52	15.44	6.84
	5	8582406	8476288	8529347	234.30			
	6	7624608	7516447	7570528	207.69			
8hours	7	8664646	8612807	8638727	237.33	237.63	0.96	0.40
	8	8673901	8568819	8621360	236.85			
	9	8750182	8625576	8687879	238.70			
12hours	10	8736561	8681232	8708897	239.28	237.40	2.10	0.89
	11	8698535	8611339	8654937	237.78			
	12	8609316	8508910	8559113	235.12			
24hours	13	8822771	8700057	8761414	240.74	240.15	0.90	0.37
	14	8800414	8711729	8756072	240.59			
	15	8750421	8655553	8702987	239.12			
48hours	16	8957399	8901177	8929288	245.40	241.57	3.32	1.37
	17	8761457	8686764	8724111	239.70			
	18	8759890	8681134	8720512	239.60			

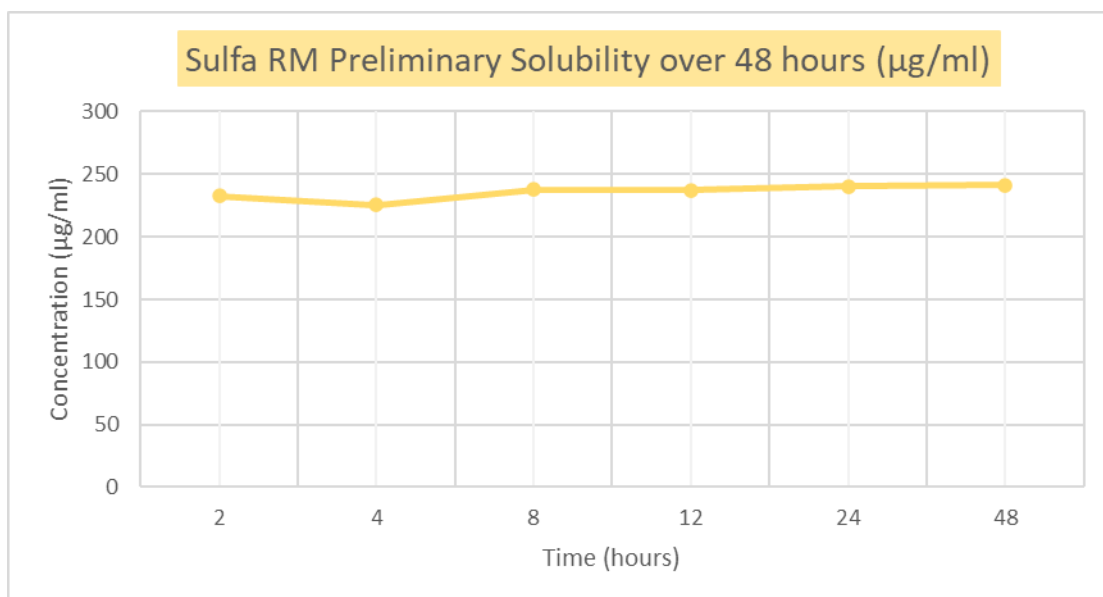


Figure 4.17: Preliminary solubility results obtained for SULFA in ultrapure H₂O across 48 hours.

It can be seen from Table 4.3 and Figure 4.17 that sulfadoxine remains stable over a 48-hour experiment and equilibrium is achieved within 2 hours. Average concentrations range from 225.52 µg/ml to 241.57 µg/ml. An initial weight of 20 mg API in 5 ml of HPLC H₂O was used per sample, ensuring a supersaturated solution. The %RSD showed that the variability for each time point was relatively low with all values presenting lower than 7%. A value below 5% is desired, this higher value can be attributed to a possible leakage in one of the test tubes leading to a difference in solubility concentration recorded, which in turn resulted in the higher %RSD value.

4.1.3.1.1 SPVP30 ASD preliminary solubility studies

The results obtained for the preliminary solubility study of SPVP30 ASD in ultrapure H₂O are displayed in Table 4.4 and Figure 4.18.

Table 4.4 Preliminary solubility data of SPVP30 ASD in ultrapure H₂O across 48 hours

SPVP30 ASD 1:10 Solubility Studies									
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	ADJUSTED Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	5213596	5238751	5226174	147.11	1471.11	1461.85	15.28	1.05
	2	5111667	5149893	5130780	144.42	1444.21			
	3	5193042	5214275	5203659	147.02	1470.23			
4hours	4	5055702	5075020	5065361	143.12	1431.23	1398.38	31.61	2.26
	5	4829988	4853546	4841767	136.82	1368.17			
	6	4923628	4955521	4939575	139.58	1395.75			
8hours	7	4802102	4836666	4819384	136.19	1361.86	1371.55	11.41	0.83
	8	4895198	4901501	4898350	138.41	1384.13			
	9	4835426	4851681	4843554	136.87	1368.67			
12hours	10	4732149	4765215	4748682	134.19	1341.92	1325.73	43.84	3.31
	11	4790759	4828968	4809864	135.92	1359.17			
	12	4498461	4532074	4515268	127.61	1276.09			
24hours	13	4631817	4575245	4603531	130.10	1300.98	1297.27	4.30	0.33
	14	4589308	4557975	4573642	129.26	1292.56			
	15	4615909	4571893	4593901	129.83	1298.27			
48hours	16	4523431	4578577	4551004	128.62	1286.17	1207.14	111.37	9.23
	17	3803259	3834982	3819121	107.98	1079.77			
	18	4423800	4460570	4442185	125.55	1255.48			

A 1:10 dilution was made during the preliminary solubility studies of SPVP30 ASD. These dilutions were employed to fit within the range of the validation of SPVP30 ASD. As seen in Table 4.4 and Figure 4.18 the maximum concentration (1461.85 µg/ml) of SULFA present in the ASD sample was reached after 2 hours after which a slight decline in concentration can be observed over 48 hours. It can be noted that at 48 hours an increase in the %RSD is detected. The outlier sample at 48 hours (sample 17), with the low AUC may be related to the fact that this specific sample possibly leaked during the study, causing a decrease in concentration recorded. An initial weight of 100 mg of SPVP30 ASD (20 mg SULFA present) in 5 ml HPLC ultrapure H₂O was used per sample, creating a supersaturated solution.

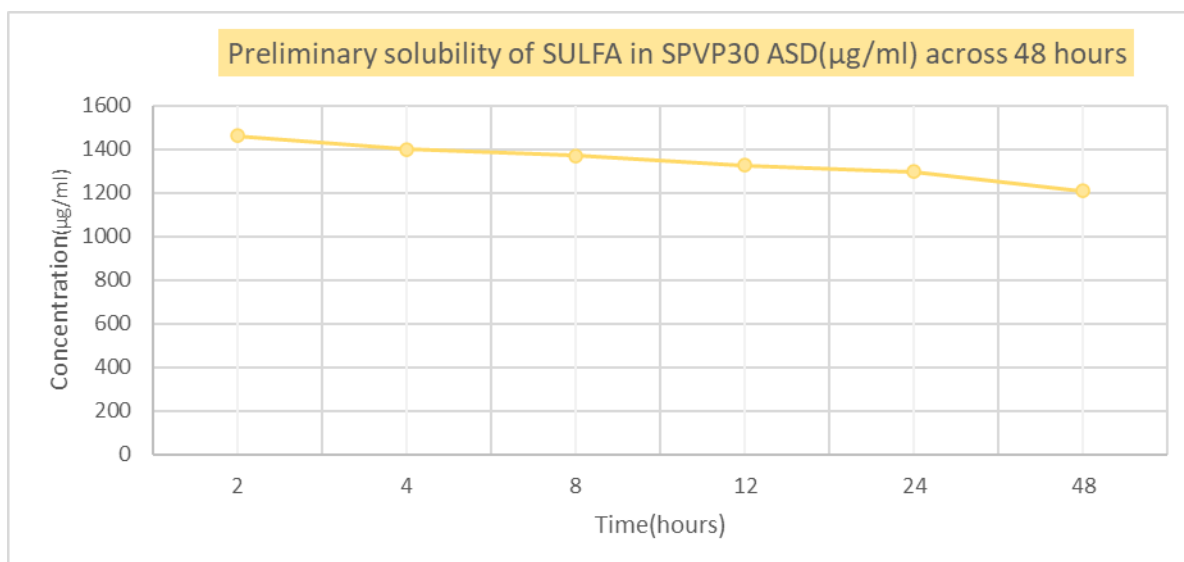


Figure 4.18: Preliminary solubility results obtained for SPVP30 ASD in ultrapure H₂O across 48 hours.

4.1.3.1.2 SPVP64 ASD preliminary solubility studies

The results obtained for the preliminary solubility study of SPVP64 ASD in H₂O are displayed in Table 4.5 and Figure 4.19.

Table 4.5: Preliminary solubility data of SPVP64 ASD in ultrapure H₂O

SPVP64 1:10 Solubility Studies									
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	ADJUSTED Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	5901569	5859979	5880774	153.59	1535.91	1545.16	25.26	1.63
	2	5856376	5828705	5842541	152.58	1525.83			
	3	6051242	5997459	6024351	157.37	1573.74			
4hours	4	5637029	5596345	5616687	146.63	1466.32	1439.17	53.82	3.74
	5	5664730	5626992	5645861	147.40	1474.01			
	6	5299058	5257661	5278360	137.72	1377.18			
8hours	7	5372530	5349192	5360861	139.89	1398.92	1388.89	10.94	0.79
	8	5293644	5263411	5278528	137.72	1377.22			
	9	5345753	5312368	5329061	139.05	1390.54			
12hours	10	5400779	5346986	5373883	140.23	1402.35	1376.23	221.41	16.09
	11	5190578	5160774	5175676	135.01	1350.12			
	12	3846517	3812475	3829496	99.54	995.42			
24hours	13	5067524	5070011	5068768	132.20	1321.95	1382.81	58.97	4.26
	14	5294966	5334659	5314813	138.68	1386.78			
	15	5500917	5530268	5515593	143.97	1439.69			
48hours	16	4953615	4886093	4919854	128.27	1282.72	1263.72	20.71	1.64
	17	4832455	4886502	4859479	126.68	1266.81			
	18	4742393	4785507	4763950	124.16	1241.64			

A 1:10 dilution was prepared during the preliminary solubility studies of SPVP64 ASD. These dilutions were made to fit within the range of the validation of SPVP64 ASD. As seen in Table 4.5 and Figure 4.19 the maximum concentration of 1545.16 µg/ml of SULFA present in the ASD sample was reached after the 2-hour withdrawal. Once again, a decline in

concentration is witnessed over time as displayed in Figure 4.19. Relatively low %RSD values are documented which may indicate that SPVP64 ASD had a more uniform distribution of API within the ASD. The outlier at 12 hours, which lead to an increased %RSD can be attributed to a leak in the sample. The initial weight of 100 mg of SPVP64 ASD (20 mg SULFA present) in 5 ml HPLC ultrapure H₂O was present in each sample, producing a supersaturated solution.

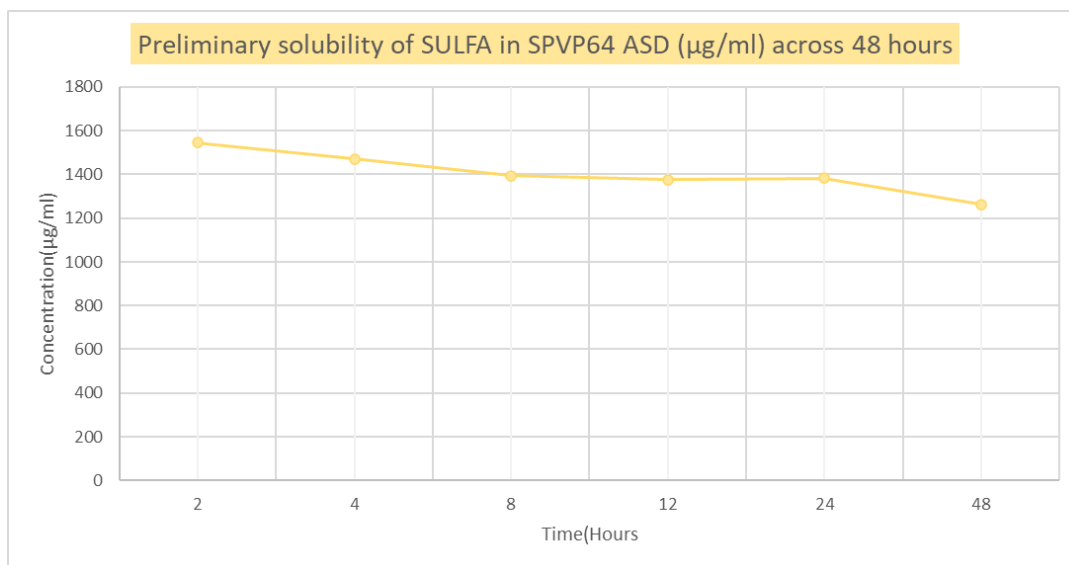


Figure 4.19: Preliminary solubility results obtained for SPVP64 ASD in ultrapure H₂O across 48 hours.

4.1.3.1.3 SHPMC ASD preliminary solubility studies

The results obtained for the preliminary solubility study of SHPMC ASD in ultrapure H₂O are displayed in Table 4.6 and Figure 4.21.

Table 4.6: Preliminary solubility data of SHPMC ASD in ultrapure H₂O across 48 hours

SHPMC 1:100 Solubility Studies									
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	ADJUSTED Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	2042698	2014849	2028774	53.34	5334.26	10110.08	4676.49	46.26
	2	3927730	3895203	3911467	103.15	10315.50			
	3	5572327	5550153	5561240	146.80	14680.47			
4hours	4	3163547	3144346	3153947	83.11	8311.25	9788.35	2067.22	21.12
	5	4622609	4587651	4605130	121.51	12150.79			
	6	3385447	3369775	3377611	89.03	8903.02			
8hours	7	4660227	4622555	4641391	122.47	12246.73	10595.79	2152.46	20.31
	8	4329171	4297915	4313543	113.79	11379.31			
	9	3114892	3079673	3097283	81.61	8161.33			
12hours	10	3774898	3741733	3758316	99.10	9910.29	10064.54	2166.20	21.52
	11	3047876	3009315	3028596	79.80	7979.59			
	12	4693538	4632340	4662939	123.04	12303.75			
24hours	13	3611228	3578688	3594958	94.78	9478.08	5991.46	3021.48	50.43
	14	1585590	1568233	1576912	41.39	4138.72			
	15	1663619	1655646	1659633	43.58	4357.58			
48hours	16	2107523	2127299	2117411	55.69	5568.78	4478.93	972.46	21.71
	17	1582342	1593753	1588048	41.68	4168.18			
	18	1404939	1417109	1411024	37.00	3699.82			

A 1:100 dilution was made during the preliminary solubility studies of SHPMC ASD. These dilutions were made to fit within the range of the validation of SHPMC ASD.

As seen in Table 4.6 and Figure 4.20 the maximum concentration of SULFA present in the ASD sample was reached after the 2-hour withdrawal at 10 110.08 µg/ml. Here very large %RSD values are observed, and it can possibly be attributed to the variation of API content within the ASD. SHPMC ASD presented with a rigid, plastic consistency which was unable to be refined with a pestle and mortar to deliver a uniform particle size. Thus, pieces of plastic were weighed off, instead of a powdered substance (Figure 4.21). A decline in the solubility profile across the 48 hours was noted, this may be due to degradation of the ASD, indicating that it is less stable than PYRI RM. Again, the initial weight of 100 mg of SHPMC ASD (20 mg SULFA present) in 5 ml HPLC ultrapure H₂O was used for each sample to ensure supersaturation.



Figure 4.20: SHPMC texture example.

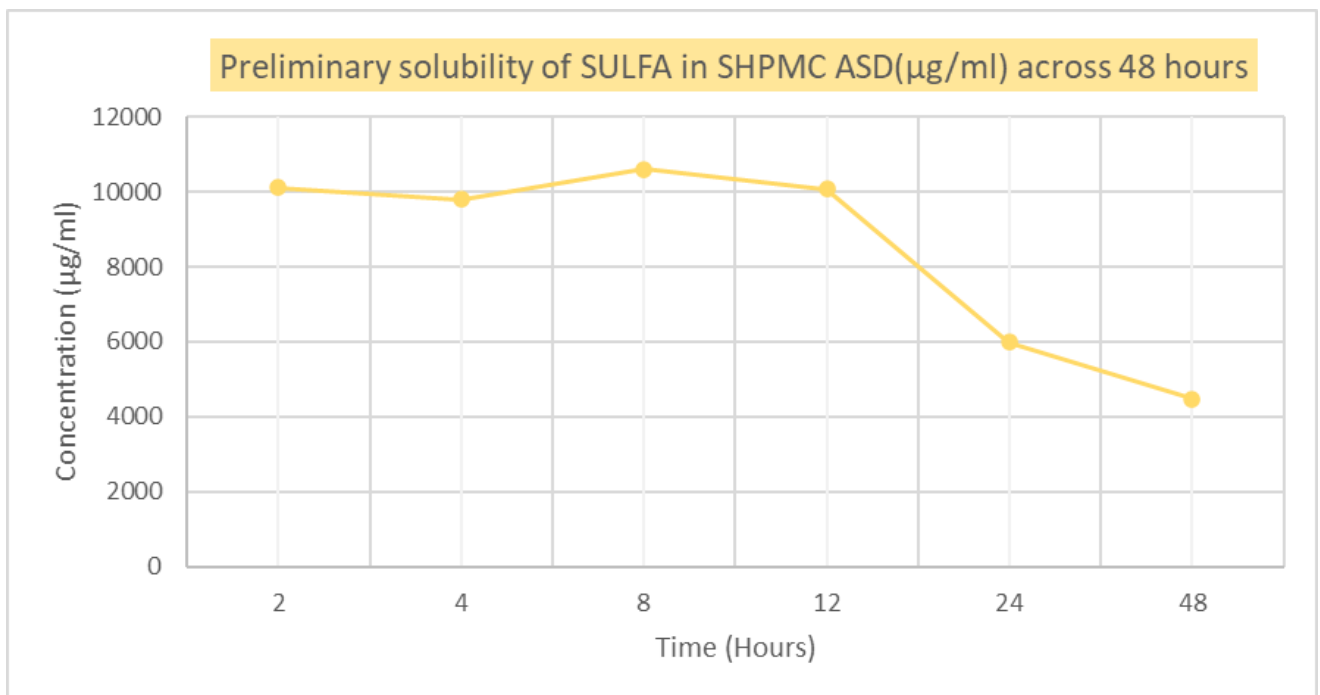


Figure 4.21: Preliminary solubility results obtained for SHPMC ASD in ultrapure H_2O across 48 hours.

4.1.3.2 Sulfadoxine API and ASD pivot solubility studies

The pivot solubility studies performed on SULFA and the various ASDs are discussed and displayed in Table 4.7 and Figure 4.22. The results obtained for the pivot solubility studies correlated with the data obtained from the preliminary solubility studies done for SULFA and the various ASDs.

Table 4.7: Pivot solubility data of SULFA API and ASDs after 2 hours

Sample Concentration (µg/ml)	Sulfadoxine RM	SPVP30 ASD	SPVP64 ASD	SHPMC ASD
SAMPLE 1	239	1489	1568	6332
SAMPLE 2	240	1533	1615	7904
SAMPLE 3	240	1431	1637	6262
SAMPLE 4	243	1515	1610	7344
SAMPLE 5	242	1513	1648	6213
SAMPLE 6	242	1531	1628	5831
Average Concentration (µg/ml)	241	1502	1618	6648
SD	1	38	28	796
% RSD	1	3	2	12

As seen in Table 4.7 SHPMC had the highest solubility of 6647.51 µg/ml. It was a significant 28-fold increase in solubility when compared to the SULFA RM. This ASD seems however to be unstable over the solubility period since considerable degradation was measurable (Figure 4.22). With the SPVP 30 ASD a substantial (6-fold) increase in solubility was measured when comparing to the SULFA RM. Comparably, the SPVPVA64 ASD produced a 7-fold increase in solubility. These values are purely based upon the values obtained within the study. Further statistical analysis on these results are discussed in section.4.4.

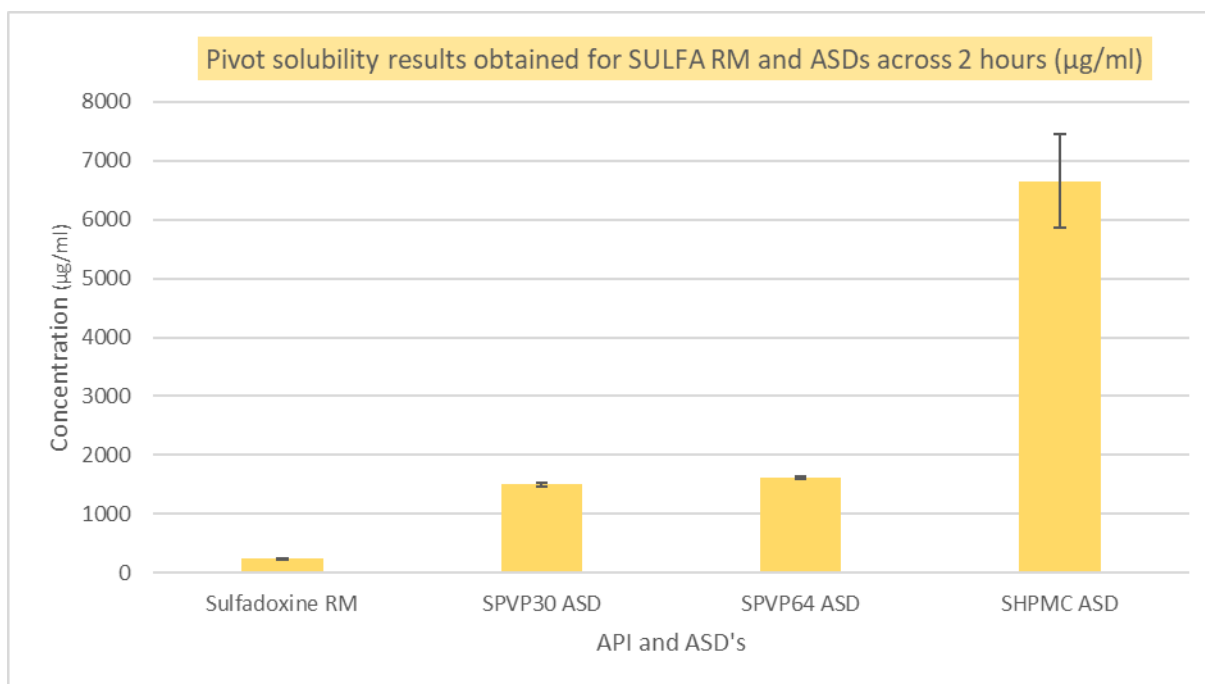


Figure 4.22: Pivot solubility results obtained for SULFA RM and ASDs across 2 hours.

4.2 Pyrimethamine and ASDs

4.2.1 Physico-chemical characterisation of pyrimethamine API and ASDs

4.2.1.1 Differential scanning calorimetry

When performing a differential scanning calorimetry thermal analysis (DSC) on PYRI raw material, an endothermic peak indication of its melting point of 239.27°C was recorded (Figure 4.23) which correlates with the literature ([Ph. Int. 2020b](#)).

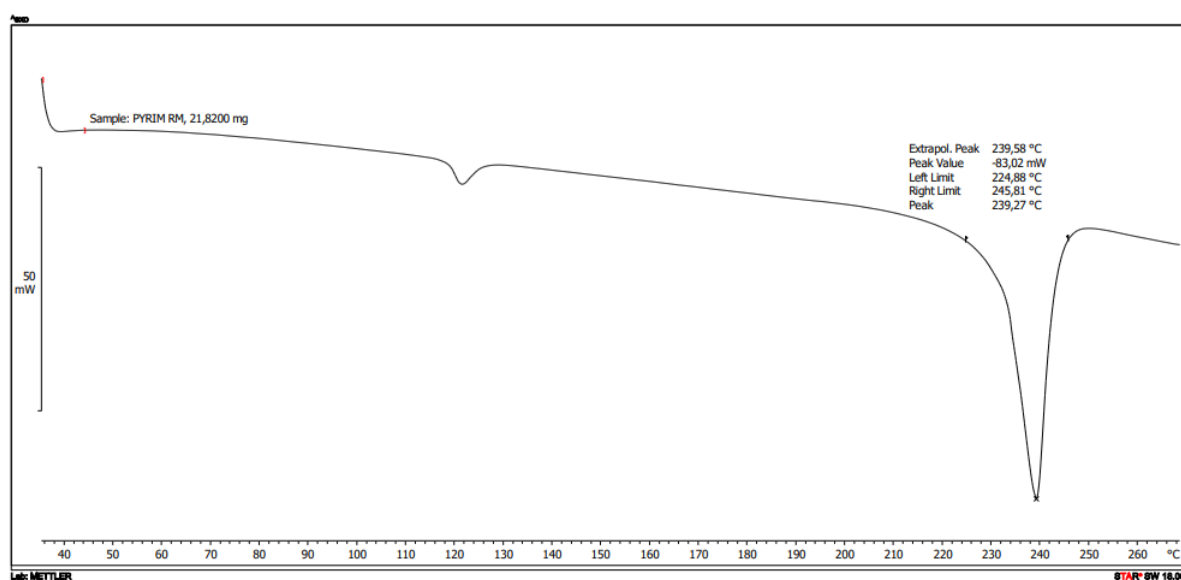


Figure 4.23: DSC thermogram of pyrimethamine raw material.

In order to produce ASDs of PYRI and the various polymers, the mixture had to be proven miscible. The DSC thermograms of PYRI and the various polymer physical mixtures and ASDs are illustrated in Figures 4.24-4.26.

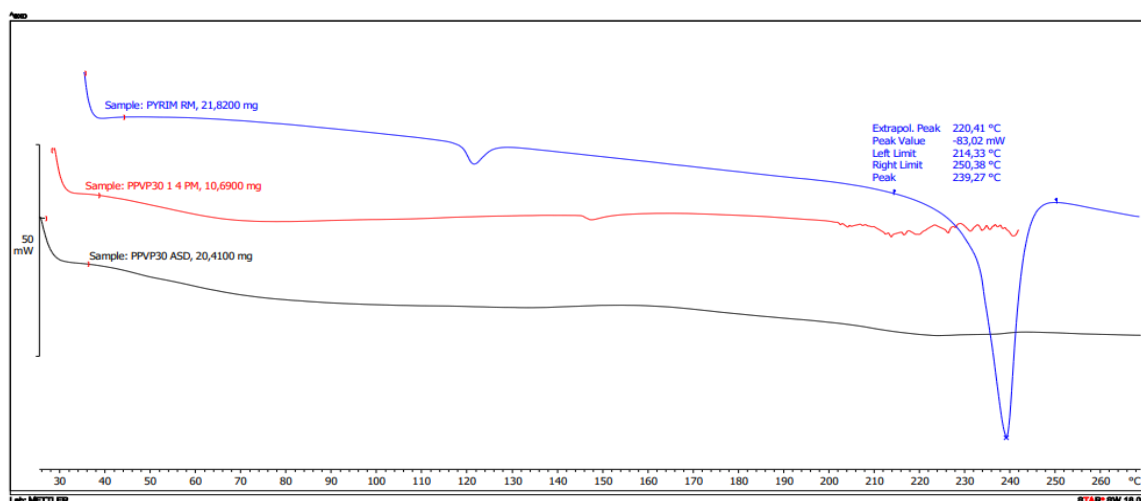


Figure 4.24: DSC thermogram of PYRI (blue), PPVP30 PM (red) and PPVP30 ASD (black).

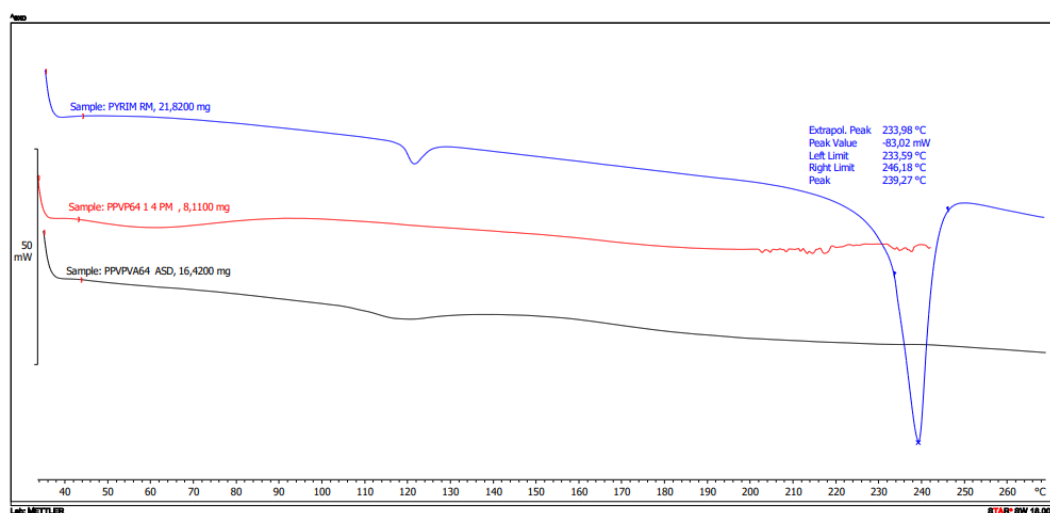


Figure 4.25: DSC thermogram of PYRI (blue), PPVP64 PM (red) and PPVP64 ASD (black).

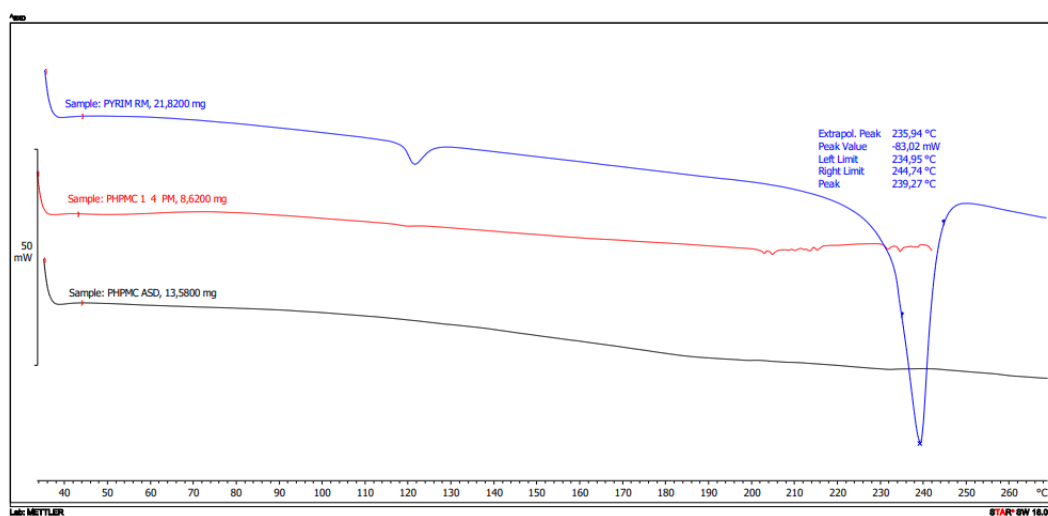


Figure 4.26: DSC thermogram of PYRI (blue), PHPMC PM (red) and PHPMC ASD (black).

Figures 4.24 to 4.26 exhibit only 1 endothermic event at 237°C, resembling the melting point of PYRI (black). The PM (blue) of the miscibility test showed no endothermic event, indicating a miscible system. The thermogram of the prepared ASD (red) also showed only a straight line, which suggest that an ASD was successfully prepared.

4.2.1.2 Fourier-transform infrared spectroscopy (FTIR)

Figure 4.27 represents the FTIR spectrum of the PYRI RM, which will be used in comparative nature to review the RM, PM and ASDs.

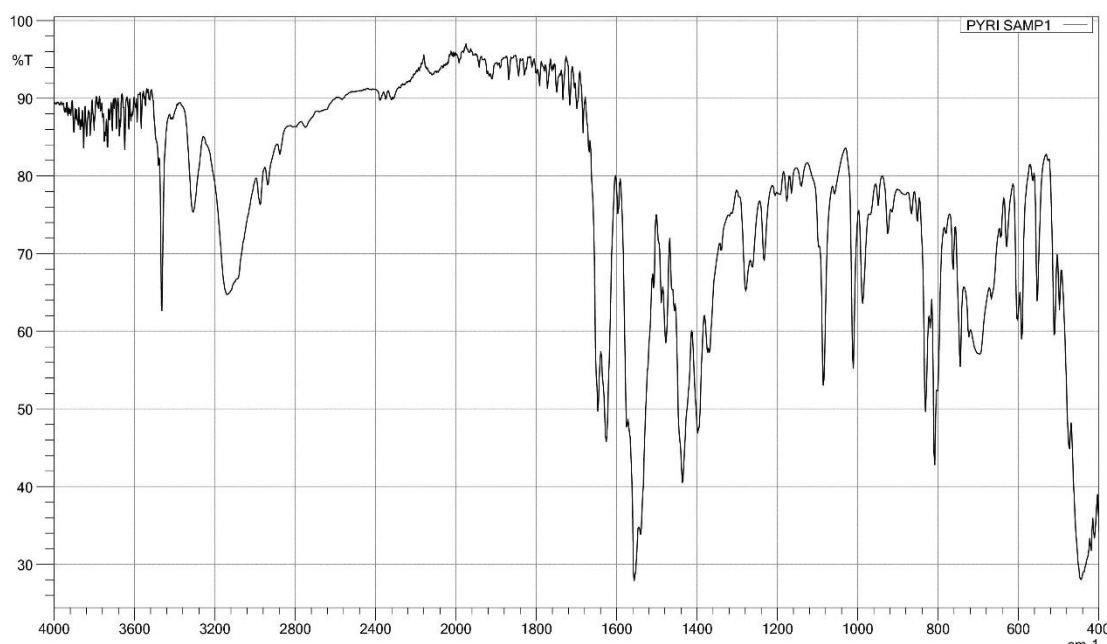


Figure 4.27: FTIR spectra of the PYRI RM sample.

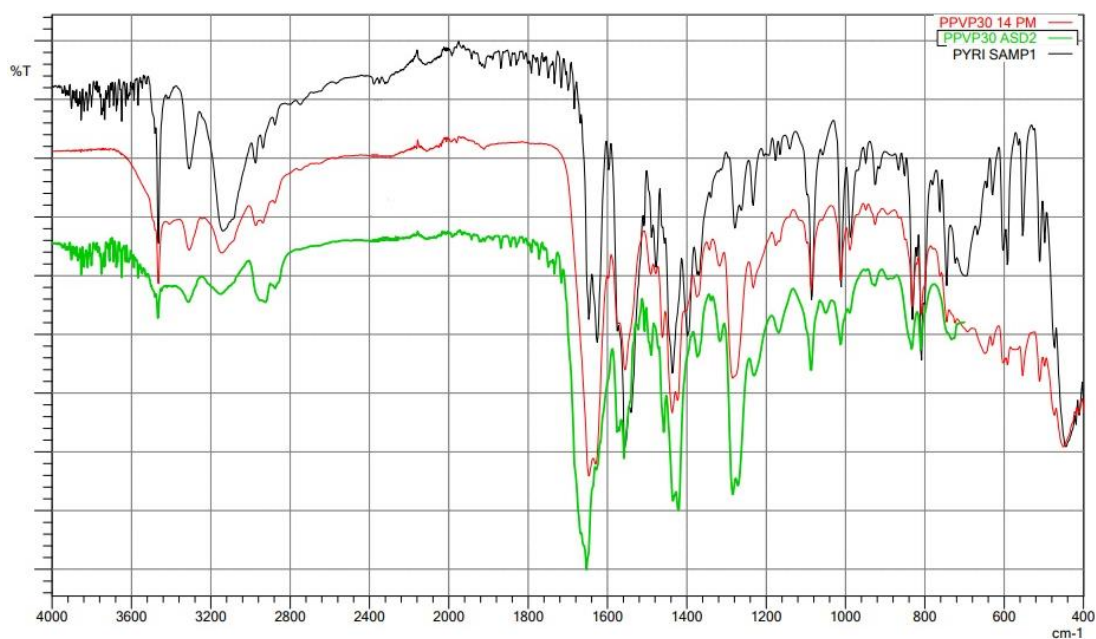


Figure 4.28: FTIR spectra of PYRI RM (black), PPVP30 ASD (green), and PPVP30 PM (red).

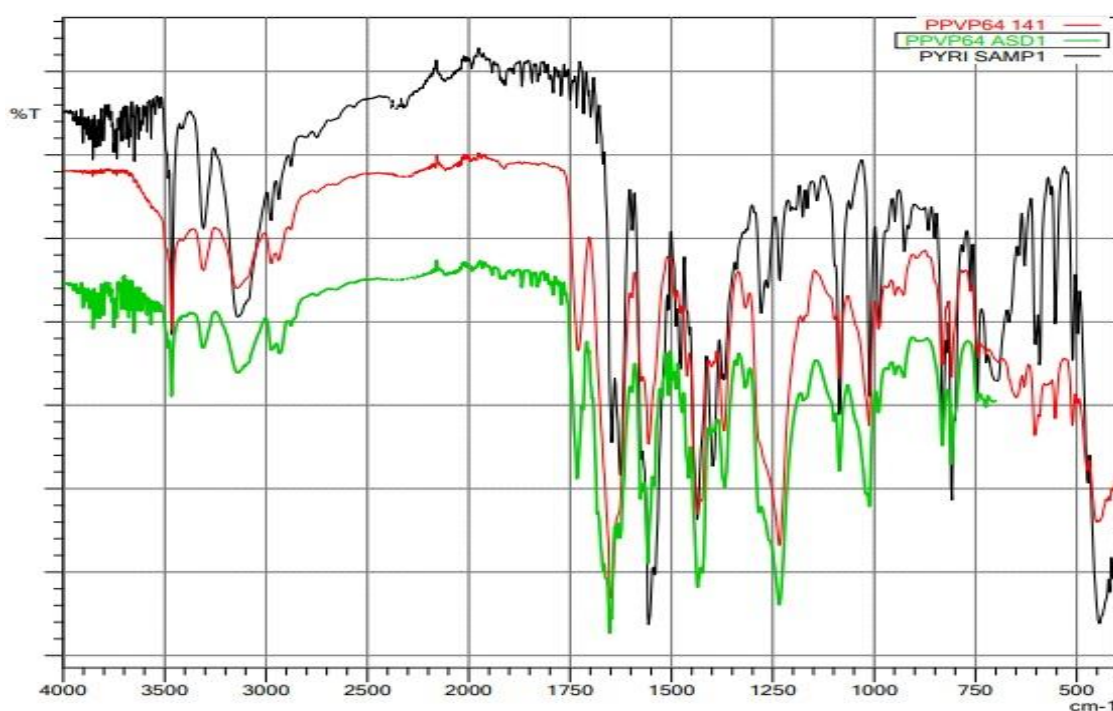


Figure 4.29: FTIR spectra of PYRI RM (black), PPVP64 ASD (green), and PPVP64 PM (red).

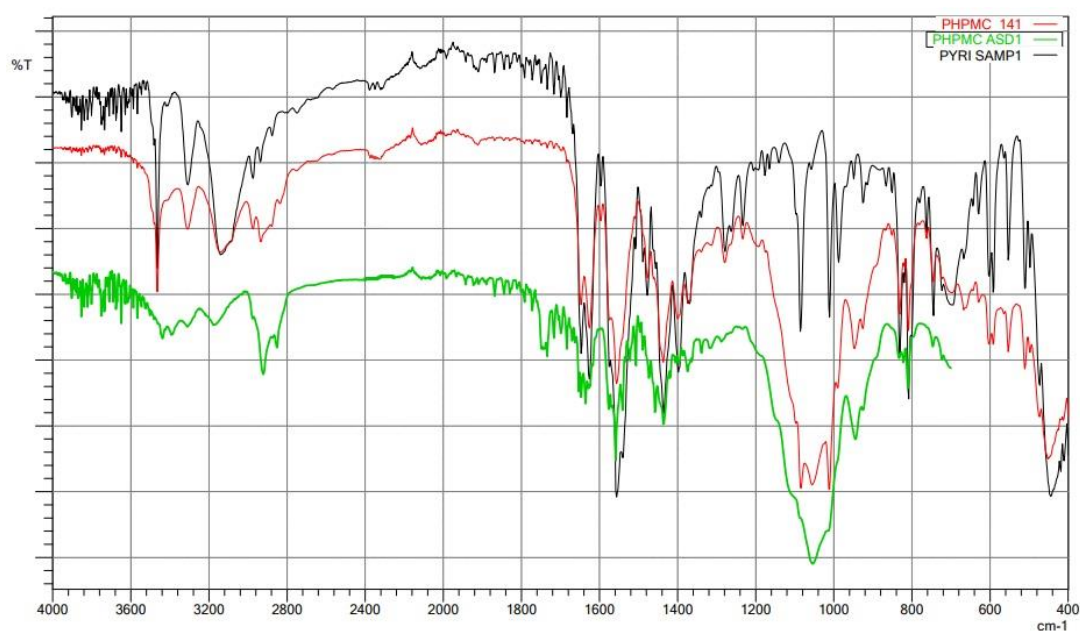


Figure 4.30: FTIR spectra of PYRI RM (black), PHPMC ASD (green), and PHPMC PM (red).

Overlays were made with the PYRI RM (black), PYRI PM (red) and ASD (green), as seen in Figures 4.28-4.30. No significant changes were observed between the PMs and the various ASDs of PVP30 and PVPVA64 polymers. Significant differences were observed in the overlay of the HPMC ASD and PM between 1400 – 1800 cm^{-1} , where the disappearance of peaks specific to PHPMC ASD (green) can be seen. Which is indicative of amorphous solid dispersion formation.

4.2.1.3 X-ray powder diffraction (XRPD)

In Figure 4.31 and again in Figure 4.32 it is shown that the XRPD of PYRI RM (red) exhibited crystalline peaks as expected. The PHPMC ASD displayed an amorphous diffractogram which is indicative of successful formation of the ASD. The PPVP64 ASD and PPVP30 ASD however do exhibit small percentages of crystalline peaks, which could suggest not a fully amorphous ASD formation.

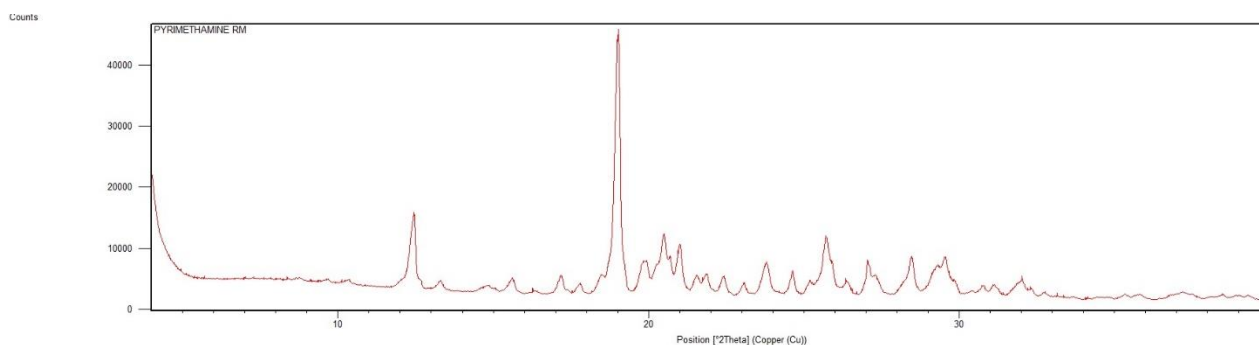


Figure 4.31: XRPD of PYRI RM sample.

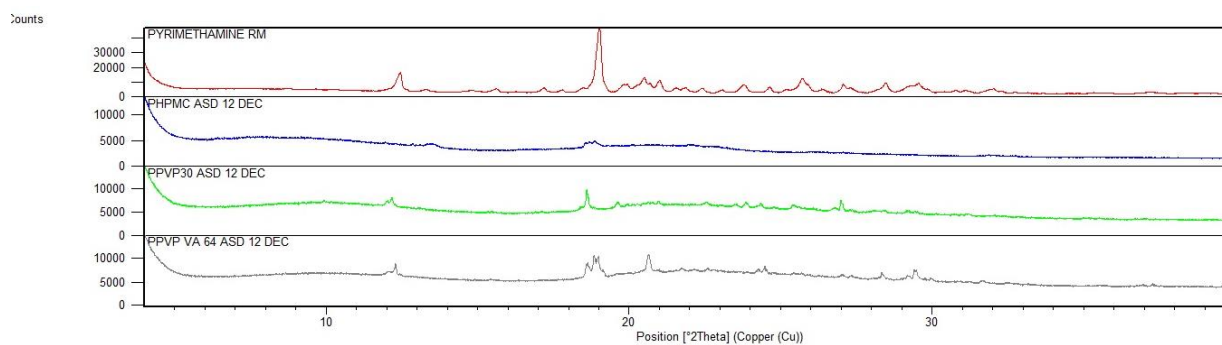
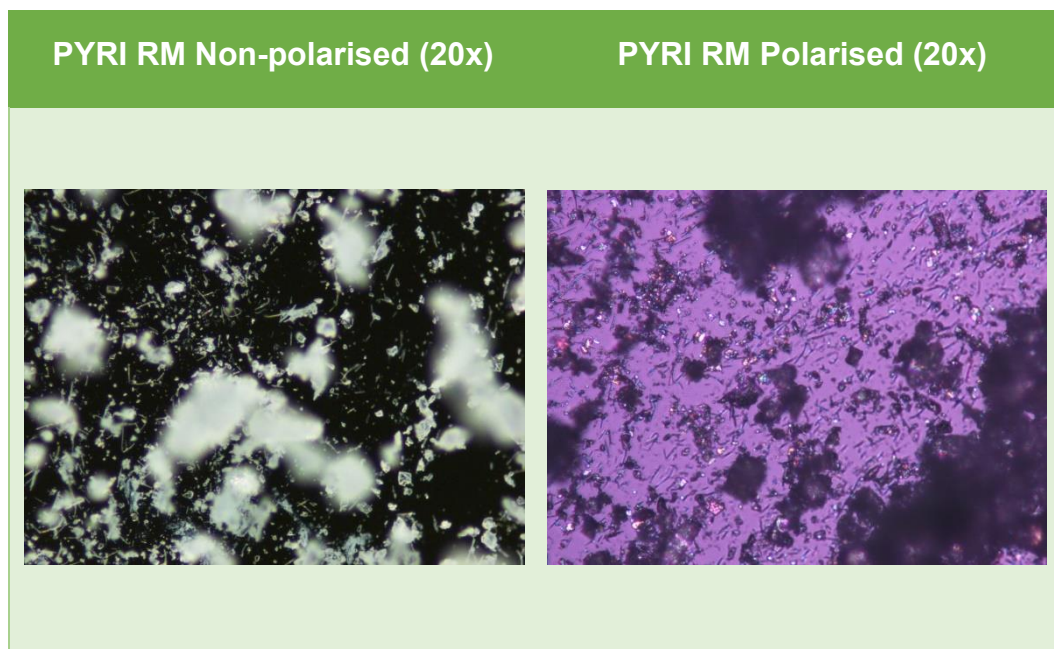


Figure 4.32: XRPD of PYRI RM (red), PHPMC ASD (blue), PPVP30 ASD (green) and PPVP64 ASD (black).

4.2.1.4 Polarised light microscopy

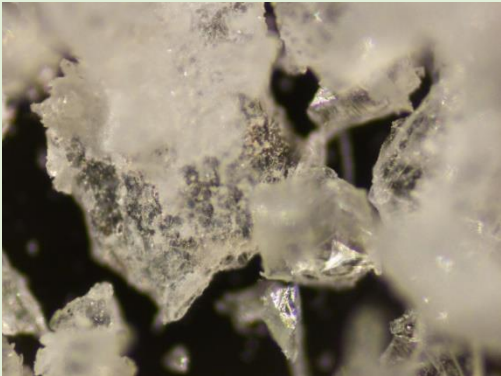
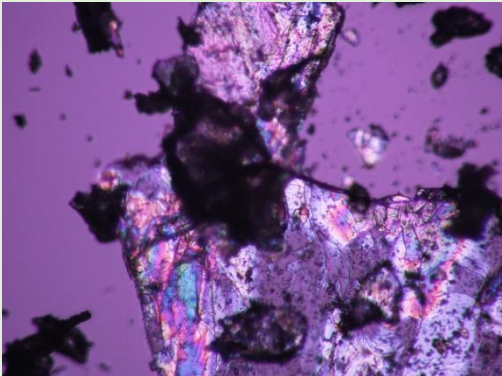
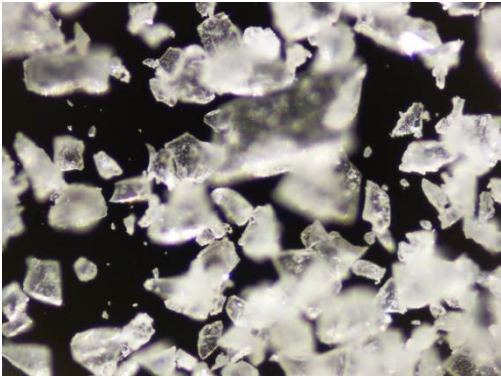
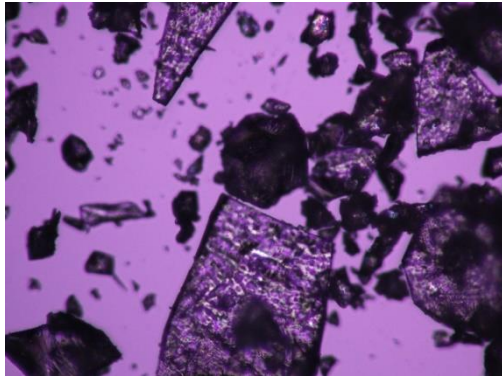
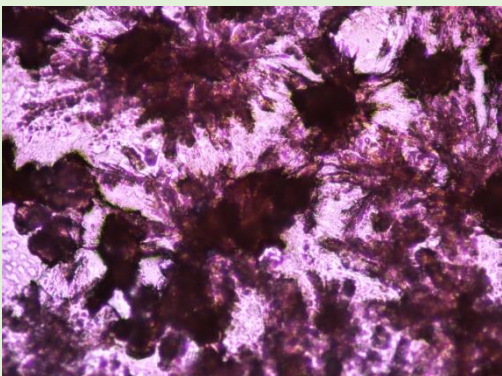
A multi-colour, “rainbow-like” array of light is produced, as seen with the pyrimethamine raw material in Table 4.8 below, an indication of a crystalline material.

Table 4.8: Light microscopy micrographs of PYRI raw material



In Table 4.9, the polarised micrographs of the PPVP30 and PPVP64 also showed a small percentage of birefringence. This observation is in line with the XRPDs of these two ASDs. The XRPDs of these two ASDs were not completely amorphous, with small crystalline peaks still visible. The polarised micrograph of PHPMC showed no birefringence, in agreement of the obtained XRPD.

Table 4.9: Polarised light microscopy micrographs of PYRI ASDs

	NON-POLARISED FILTER	POLARISED FILTER
PPVP30 ASD		
PPVP64 ASD		
PHPMC ASD		

4.2.1.5 Thermal activity monitoring

From Figures 4.33-4.36 it can be concluded that the pyrimethamine API as well as the ASDs presented with no interactions as the interaction integrals for all the samples lie beneath the 2-3 joules/g range.

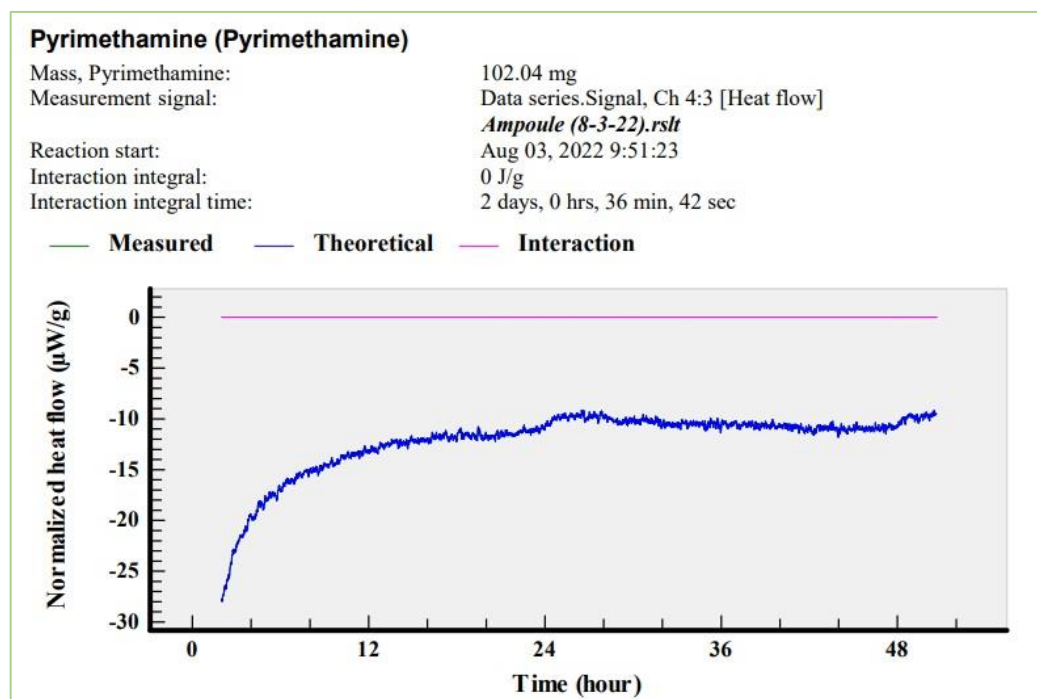


Figure 4.33: PYRI RM thermal activity monitoring.

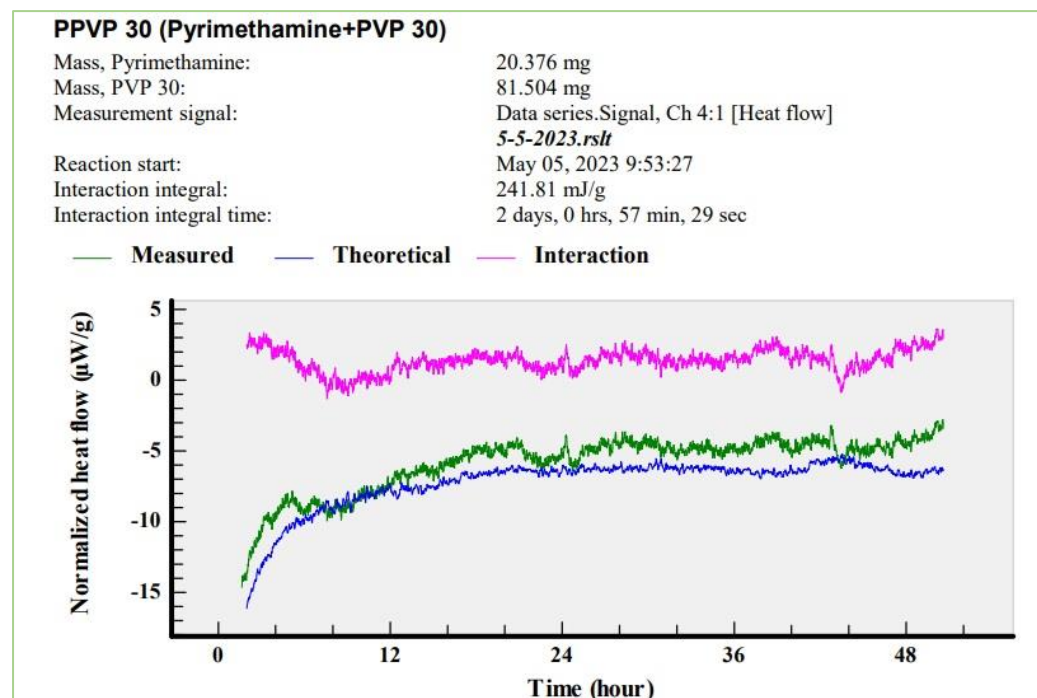


Figure 4.34: PPVP30 ASD thermal activity monitoring.

PPVP 64 (Pyrimethamine+PVP 64)

Mass, Pyrimethamine: 20.52 mg
Mass, PVP 64: 82.08 mg
Measurement signal: Data series.Signal, Ch 4:2 [Heat flow]
5-5-2023.rslt
Reaction start: May 05, 2023 9:53:27
Interaction integral: 1.3413 J/g
Interaction integral time: 2 days, 0 hrs, 57 min, 29 sec

— Measured — Theoretical — Interaction

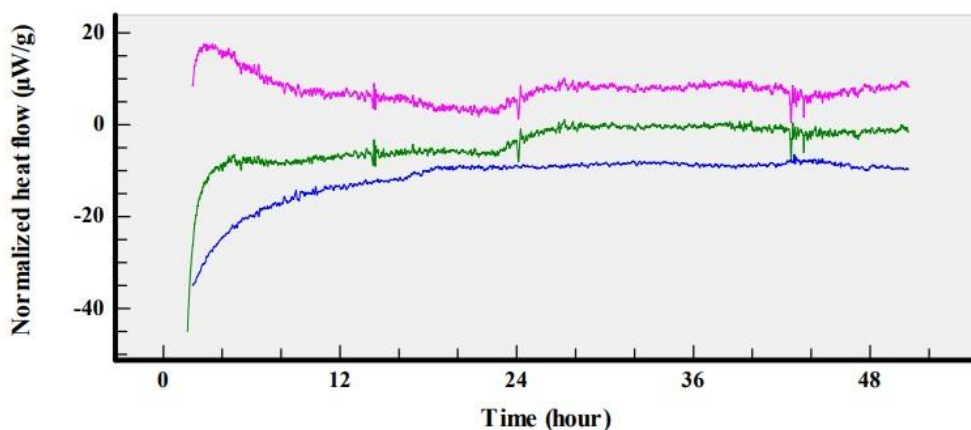


Figure 4.35: PPVP64 ASD thermal activity monitoring.

PHPMC (Pyrimethamine+HPMC)

Mass, Pyrimethamine: 20.55 mg
Mass, HPMC: 82.2 mg
Measurement signal: Data series.Signal, Ch 4:3 [Heat flow]
5-5-2023.rslt
Reaction start: May 05, 2023 9:53:27
Interaction integral: -319.43 mJ/g
Interaction integral time: 2 days, 0 hrs, 57 min, 29 sec

— Measured — Theoretical — Interaction

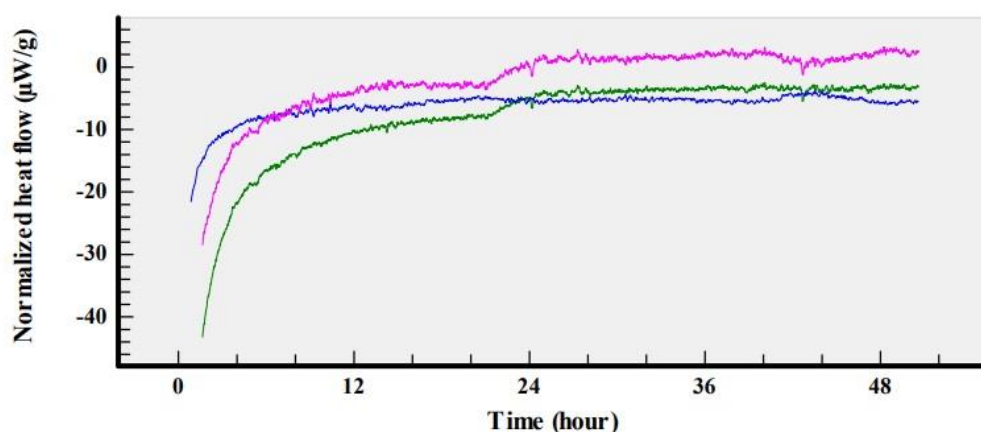


Figure 4.36: PHPMC ASD thermal activity monitoring.

4.2.2 Validation of HPLC method for pyrimethamine and ASDs

Linearity of PYRI raw material, as seen in Figure 4.37, was established by injecting a series of six different concentrations of PYRI into the HPLC. These concentrations were as follow: 2.53 µg/ml, 5.06 µg/ml, 12.65 µg/ml, 25.3 µg/ml, 37.95 µg/ml and 50 µg/ml. The regression coefficient obtained was 0.9995 which thus verifies linearity ([Singh, 2013](#)).

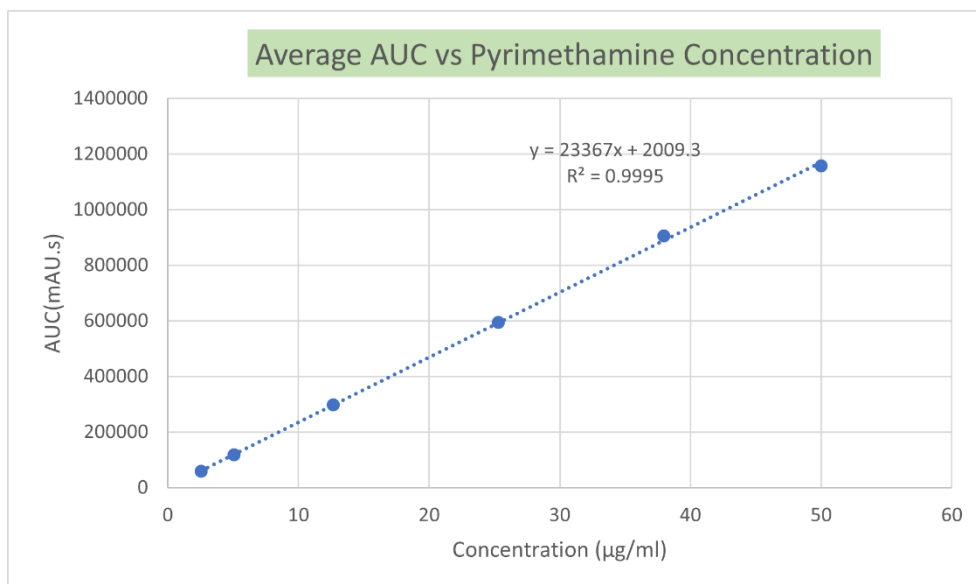


Figure 4.37: Results of HPLC method linearity for PYRI raw material.

Calibration curves covering the appropriate analytical range was set up for the respective ASDs. As seen in Figure 4.38, and according to the criteria mentioned above, all the calibration curves comply with regards to the correlation coefficient ($R^2 \geq 0.99$) thus proving linearity for all of the ASDs ([Singh, 2013](#)).

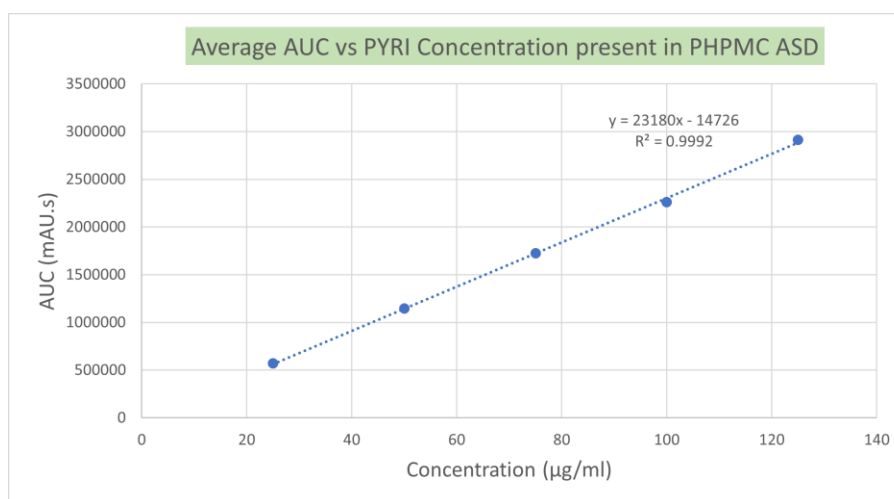
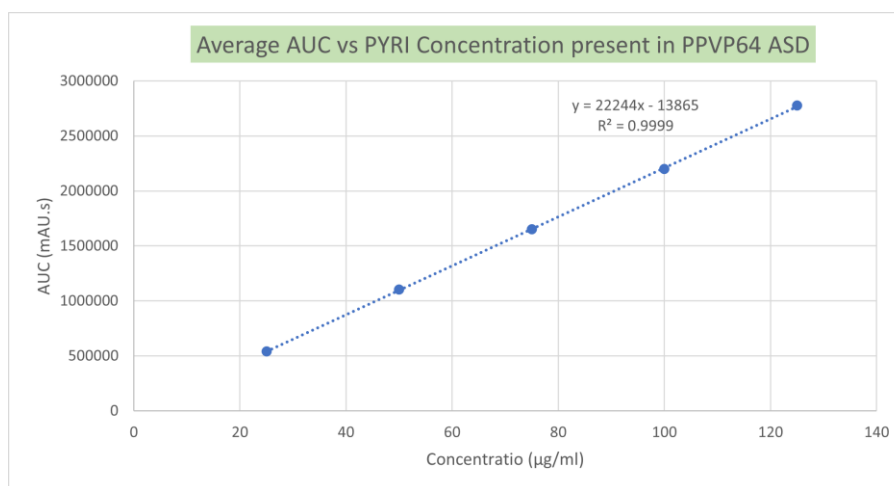
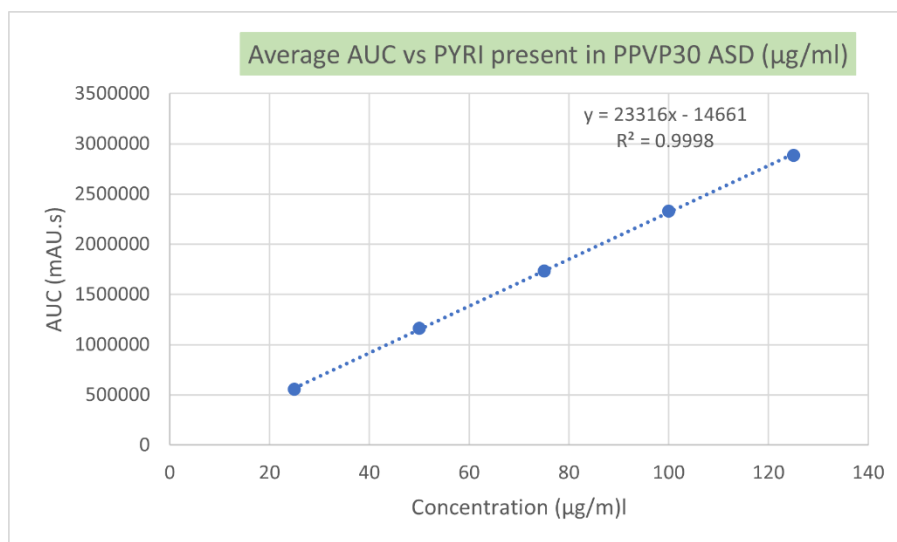


Figure 4.38: Results of HPLC method linearity for PPVP30, PPVP64 and PHPMC ASDs.

4.2.3 Solubility studies of pyrimethamine and ASDs

Solubility studies for the API and ASDs were performed using the parameters described in Chapter 3 (see Section 3.2.3). All samples were analysed using HPLC. In this section the results obtained for PYRI, PPVP30, PPVP64 and PHPMC (both preliminary and pivot studies) are discussed.

4.2.3.1 Pyrimethamine preliminary solubility studies

The results obtained for the preliminary solubility study of pyrimethamine in H₂O are displayed in Table 4.10 and Figure 4.39.

Table 4.10: Preliminary solubility data of PYRI in ultrapure H₂O across 48 hours

Pyrimethamine Solubility Studies								
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	518946	523267	521107	22.39	22.92	0.60	2.63
	2	546713	550874	548794	23.57			
	3	530031	531235	530633	22.80			
4hours	4	102371	103251	18302	0.87	*LEAK	1.44	6.86
	5	512194	514992	513593	22.07	21.05		
	6	465781	465996	465889	20.02			
8hours	7	484239	485132	484686	20.83	22.17	1.63	7.36
	8	557635	559220	558428	23.98			
	9	503532	506278	504905	21.69			
12hours	10	554534	556669	555602	23.86	21.57	1.99	9.21
	11	471843	472995	472419	20.30			
	12	478110	478264	478187	20.55			
24hours	13	414708	415424	415066	17.85	20.44	2.64	12.90
	14	536851	539691	538271	23.12			
	15	473058	474050	473554	20.35			
48hours	16	486730	485510	486120	20.89	21.09	0.44	2.09
	17	482161	484959	483560	20.78			
	18	501307	503751	502529	21.59			

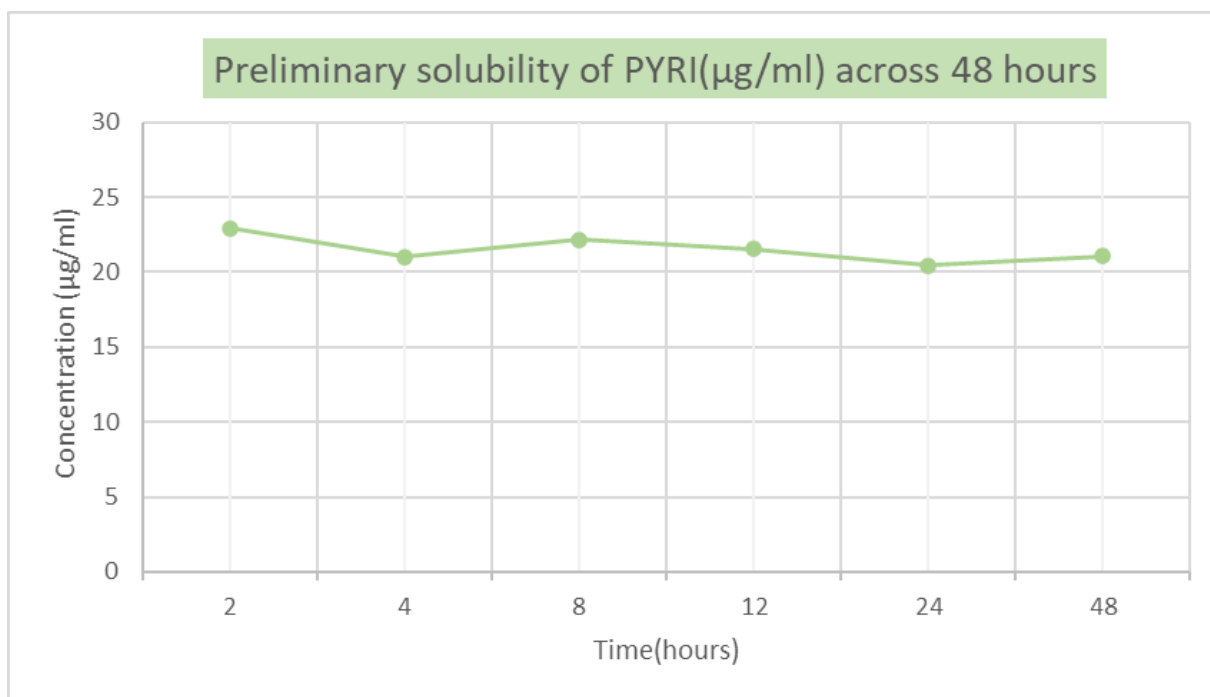


Figure 4.39: Preliminary solubility results obtained for PYRI in ultrapure H₂O across 48 hours.

It can be seen from Table 4.10 and Figure 4.39 that pyrimethamine remains stable across 48 hours and reaches equilibrium within 2 hours. An average concentration range of 20.44 µg/ml to 22.92 µg/ml was achieved. An initial weight of 25 mg API in 5 ml of HPLC ultrapure H₂O was introduced to each test tube to ensure supersaturation. The %RSD showed that the variability for each time point presented with values lower than 12%. This value remains high, and it may be attributed to the fact that PYRI is so poorly soluble that very little of the API goes into solution, and thus leads to these varying values.

4.2.3.1.1 PPVP30 ASD preliminary solubility studies

The results obtained for the preliminary solubility study of PPVP30 ASD in ultrapure H₂O are displayed in Table 4.11 and Figure 4.40.

Table 4.11: Preliminary solubility data of PPVP30 ASD in ultrapure H₂O across 48 hours

PPVP30 ASD Solubility Studies								
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	2347235	2347594	2347415	101.31	97.34	8.32	8.54
	2	2036216	2027914	2032065	87.78			
	3	2396342	2374091	2385217	102.93			
4hours	4	2237059	2211515	2224287	96.02	95.86	2.01	2.09
	5	2173931	2169696	2171814	93.77			
	6	2269936	2260348	2265142	97.78			
8hours	7	2309799	2301192	2305496	99.51	98.66	1.68	1.70
	8	2246022	2234961	2240492	96.72			
	9	2316562	2305210	2310886	99.74			
12hours	10	2241164	2229249	2235207	96.49	91.48	5.02	5.48
	11	2124354	2112517	2118436	91.48			
	12	2011012	1991487	2001250	86.46			
24hours	13	2214765	2194404	2204585	95.18	93.94	3.80	4.05
	14	2250658	2241552	2246105	96.96			
	15	2082388	2069781	2076085	89.67			
48hours	16	2113635	2102295	2107965	91.04	92.20	1.65	1.79
	17	2168901	2156046	2162474	93.37			
	18	1604758	1589749	1597254	69.13	*LEAK		

As seen in Table 4.11 and Figure 4.40 the maximum average concentration of PYRI present in the ASD sample, during preliminary solubility studies, was reached after 8 hours at 98.66 µg/ml. However, when referring to the single samples per time interval, at 2 hours the data presented with higher concentration values for samples 1 and 3. Thus, 2 hours proves sufficient time to produce optimal solubility values. The one outlier value at 2 hours may be attributed to the variation of API in ASD. The %RSD remains mostly within the 5% parameter with 2 hours being the exception. These values are based off an initial weight of 25 mg of PPVP30 ASD (5 mg PYRI present) in 5 ml ultrapure H₂O. The reason an equal amount of ASD is weighed off in relation to API, is due to limited resources. However, in this instance as with the PYRI RM, supersaturation was achieved for the API. Furthermore, it is evident that PPVP30 ASD remained stable in solution over 48 hours as seen in Figure 4.40.

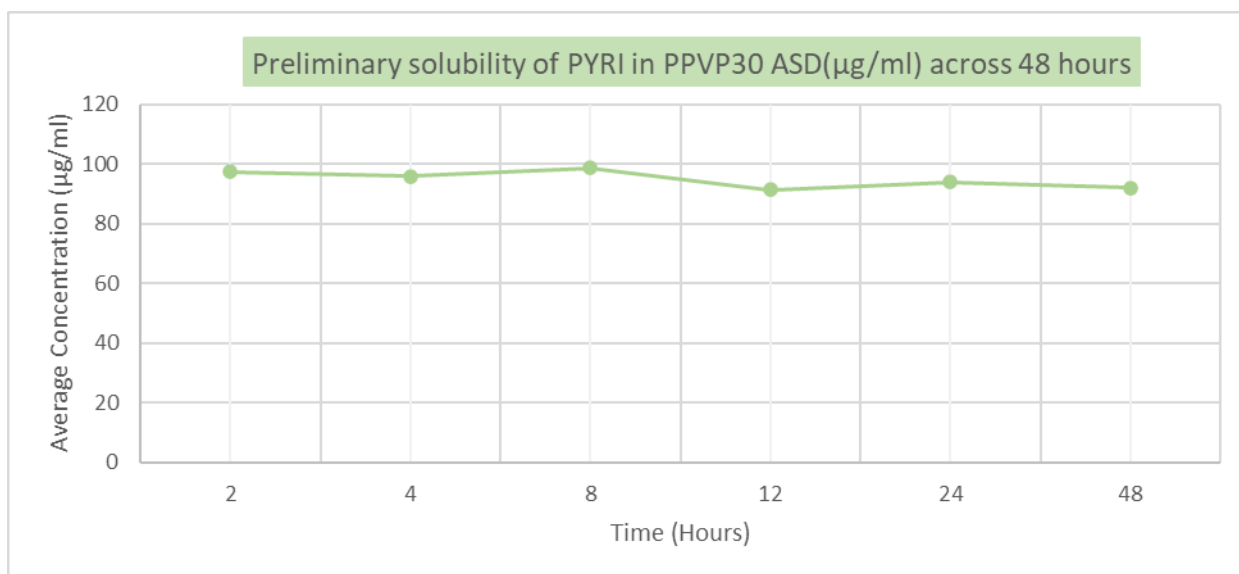


Figure 4.40: Preliminary solubility results obtained for PPVP30 ASD in ultrapure H₂O across 48 hours.

4.2.3.1.2 PPVP64 ASD preliminary solubility studies

The results obtained for the preliminary solubility study of PPVP64 ASD in ultrapure H₂O are displayed in Table 4.12 and Figure 4.41.

Table 4.12: Preliminary solubility data of PPVP64 ASD in ultrapure H₂O across 48 hours

PPVP64 ASD Solubility Studies								
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	2788870	2799710	2794290	125.00	127.84	4.80	3.75
	2	2794507	2800435	2797471	125.14			
	3	2985059	2976226	2980643	133.38			
4hours	4	2612873	2600313	2606593	116.56	120.23	5.77	4.80
	5	2875626	2796483	2836055	126.88			
	6	2631337	2612769	2622053	117.26			
8hours	7	2788674	2691371	2740023	122.56	118.23	6.12	5.17
	8	2558758	2536550	2547654	113.91			
	9	181758	178621	180190	7.48	*LEAK		
12hours	10	2857670	2744067	2800869	125.29	120.78	4.63	3.83
	11	2600390	2589754	2595072	116.04			
	12	2701815	2708523	2705169	120.99			
24hours	13	2404856	2381323	2393090	106.96	113.15	5.41	4.79
	14	2590299	2572882	2581591	115.44			
	15	2622311	2612166	2617239	117.04			
48hours	16	2369581	2388409	2378995	106.33	106.13	2.58	2.43
	17	2294693	2335655	2315174	103.46			
	18	2405685	2453658	2429672	108.61			

As seen in Table 4.12 and Figure 4.41 the maximum concentration of PYRI present in the ASD sample, during preliminary solubility studies, was reached after 2 hours at 127.84 µg/ml. The %RSD falls within the 5% limit, and thus shows a stable range of data. There is also a decline in concentration across 48 hours which shows the ASD to be slightly less stable than the PYRI RM, as shown in Figure 4.41. This concentration is based on an initial weight of 25 mg of PPVP64 ASD (5 mg PYRI present) in 5 ml ultrapure H₂O.

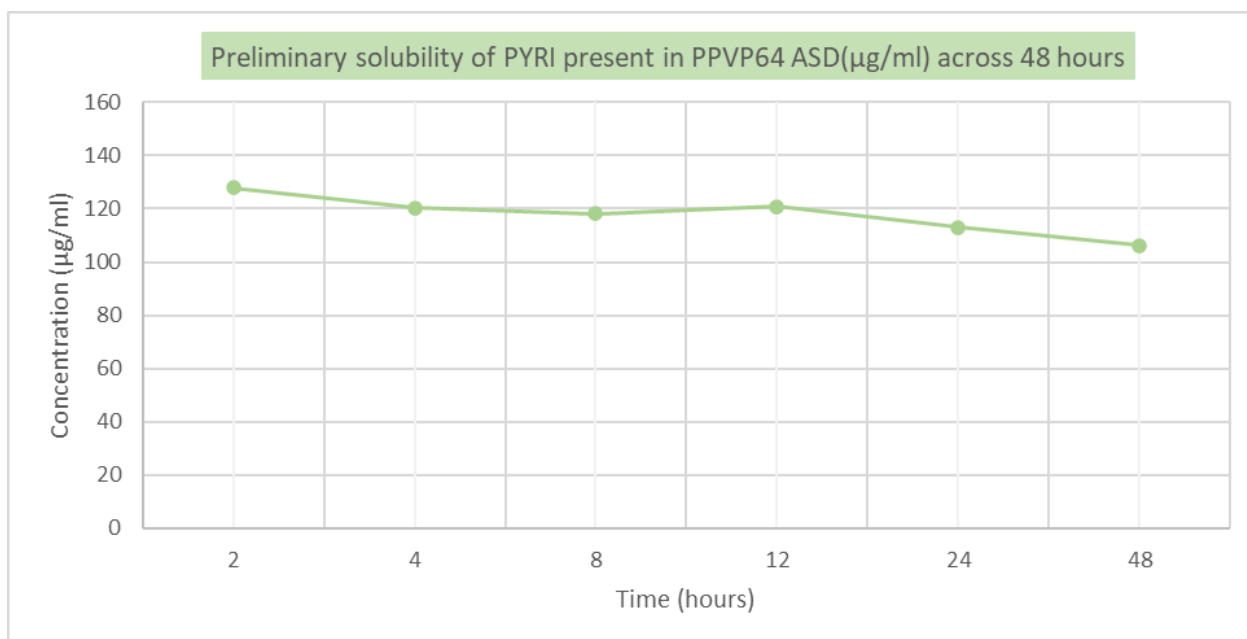


Figure 4.41: Preliminary solubility results obtained for PPVP64 ASD in ultrapure H_2O across 48 hours.

4.2.3.1.3 PHPMC ASD preliminary solubility studies

The results obtained for the preliminary solubility study of PHPMC ASD in ultrapure H₂O are displayed in Table 4.13 and Figure 4.42.

Table 4.13: Preliminary solubility data of PHPMC ASD in ultrapure H₂O across 48 hours

PHPMC ASD PRELIMINARY Solubility								
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	1790207	1778664	1784436	77.62	78.31	1.32	1.69
	2	1788882	1774088	1781485	77.49			
	3	1840072	1831726	1835899	79.84			
4hours	4	1664116	1676639	1670378	72.70	72.92	3.15	4.31
	5	1601599	1609455	1605527	69.90			
	6	1739678	1762541	1751110	76.18			
8hours	7	1684601	1703630	1694116	73.72	74.85	2.58	3.45
	8	1669830	1686586	1678208	73.03			
	9	1779965	1797835	1788900	77.81			
12hours	10	1829834	1840766	1835300	79.81	75.95	3.83	5.05
	11	1652674	1662426	1657550	72.14			
	12	1736159	1752758	1744459	75.89			
24hours	13	1814539	1823265	1818902	79.10	78.35	3.86	4.93
	14	1873917	1888212	1881065	81.79			
	15	1695992	1713086	1704539	74.17			
48hours	16	1853257	1806498	1829878	79.58	76.81	5.37	6.99
	17	1860149	1829600	1844875	80.22			
	18	1640860	1603456	1622158	70.62			

As seen in Table 4.13 and Figure 4.42 the maximum concentration of PYRI present in the ASD sample, during preliminary solubility studies, was reached after 2 hours at 78.31 µg/ml. The %RSD values vary around the 5% range, which still indicates a stable set of data. This concentration is based on an initial weight of 25 mg of PHPMC ASD (5 mg PYRI present) in 5 ml ultrapure H₂O. Furthermore, PHPMC ASD proved stable over 48 hours.

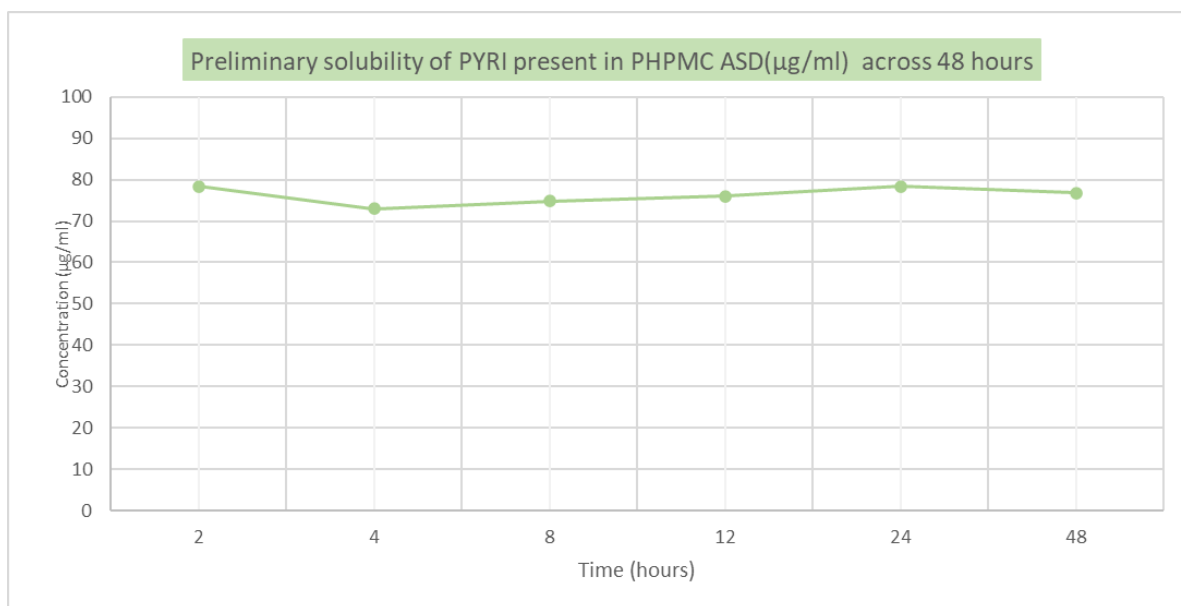


Figure 4.42: Preliminary solubility results obtained for PHPMC ASD in ultrapure H₂O across 48 hours.

4.2.3.2 Pyrimethamine API and ASD pivot solubility studies

The pivot solubility studies performed on PYRI and the various ASDs are discussed and displayed in Table 4.14 and Figure 4.44. The results obtained for the pivot solubility studies correlated with the data obtained from the preliminary solubility studies performed for PYRI and the various ASDs.

Table 4.14: Pivot solubility data of PYRI API and ASDs after 2 hours

Sample Concentration (µg/ml)	Pyrimethamine RM	PPVP30 ASD	PPVP64 ASD	PHPMC ASD
SAMPLE 1	28.43	126.14	124.96	71.84
SAMPLE 2	25.67	131.12	119.12	66.61
SAMPLE 3	25.02	139.94	130.87	72.74
SAMPLE 4	26.47	133.55	119.56	75.19
SAMPLE 5	26.92	139.24	125.38	76.24
SAMPLE 6	28.05	141.32	116.96	55.79
Average Concentration (µg/ml)	26.76	135.22	122.81	69.73
SD	1.32	5.96	5.19	7.61
% RSD	4.95	4.41	4.22	10.92

As seen in Table 4.14 PPVP30 ASD had the highest solubility with a 5-fold increase (135.22 µg/ml) compared to that of PYRI RM. A 4.6-fold increase in solubility was achieved with the PPVP64 ASD compared to the API. PPVP30 and PPVP64 ASDs were not fully confirmed as amorphous solid dispersions when undergoing physico-chemical characterisation but still performed well during the solubility studies, with PPVP30 showing the greatest improvement. PHPMC was confirmed as a successful ASD formation yet underperformed. PHPMCs lower solubility value may be attributed to the rigid plastic type of the product formed (Figure 4.43), which could be attributed to a content of uniformity problem, which consequently leads to a variation in the amount of API present within the ASD. This could possibly explain the variation in solubility concentrations and the higher %RSDs. PHPMC only showed a 2.6-fold solubility increase.

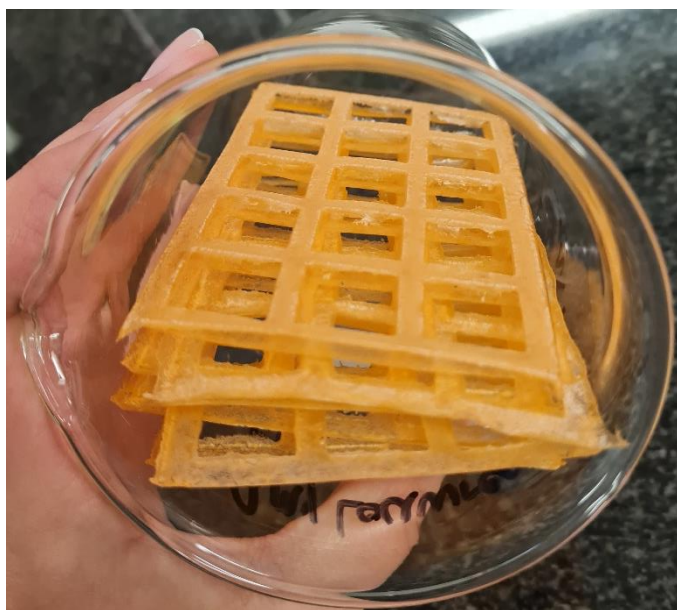


Figure 4.43: PHPMC texture example.

The amount of PRYI RM weighed off is 5 times that of the amount present in the ASD during the pivot studies and yet a significant increase in the solubility of pyrimethamine when in ASD form is witnessed. It can be concluded that PPVP30 ASD and PPVP64 ASD performed the best and delivered the highest improvement in solubility, with an average concentration of 135.22 µg/ml and 122.81 µg/ml respectively.

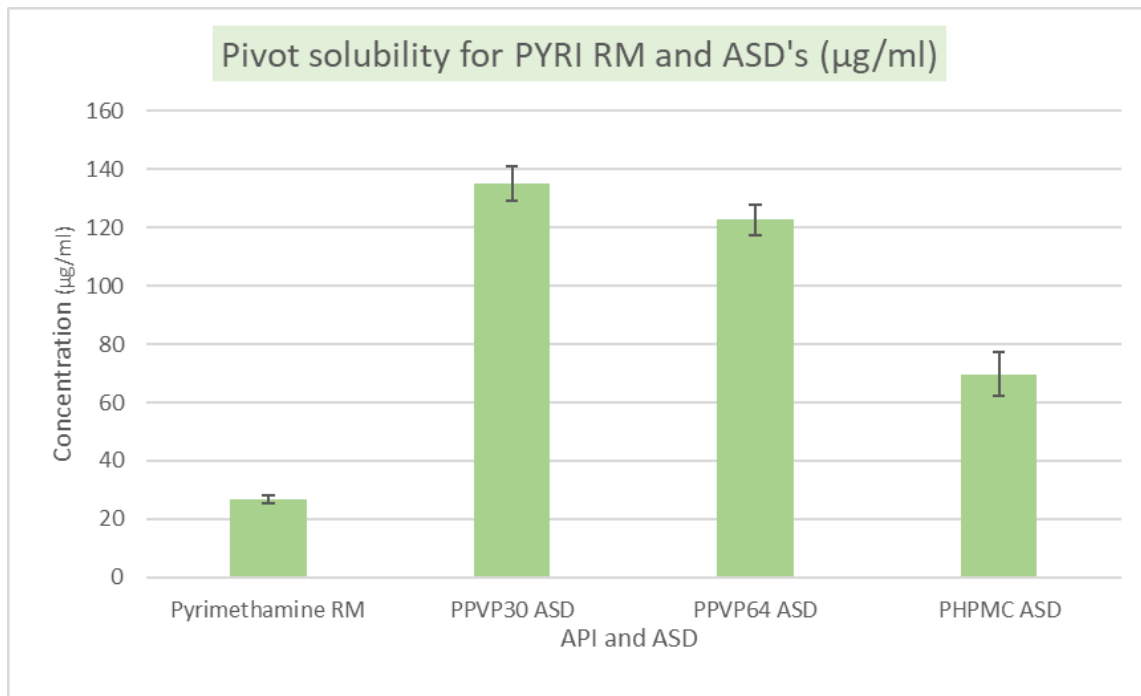


Figure 4.44: Pivot solubility results obtained for PYRI API and ASDs across 2 hours.

4.3 Artesunate and ASDs

4.3.1 Physico-chemical characterisation of artesunate API and ASDs

4.3.1.1 Differential scanning calorimetry

When performing a differential scanning calorimetry (DSC) on ART raw material, an endothermic peak indicative of its melting point of 147°C was recorded (Figure 4.45) which correlates with the literature ([Ph. Int. 2020c](#)).

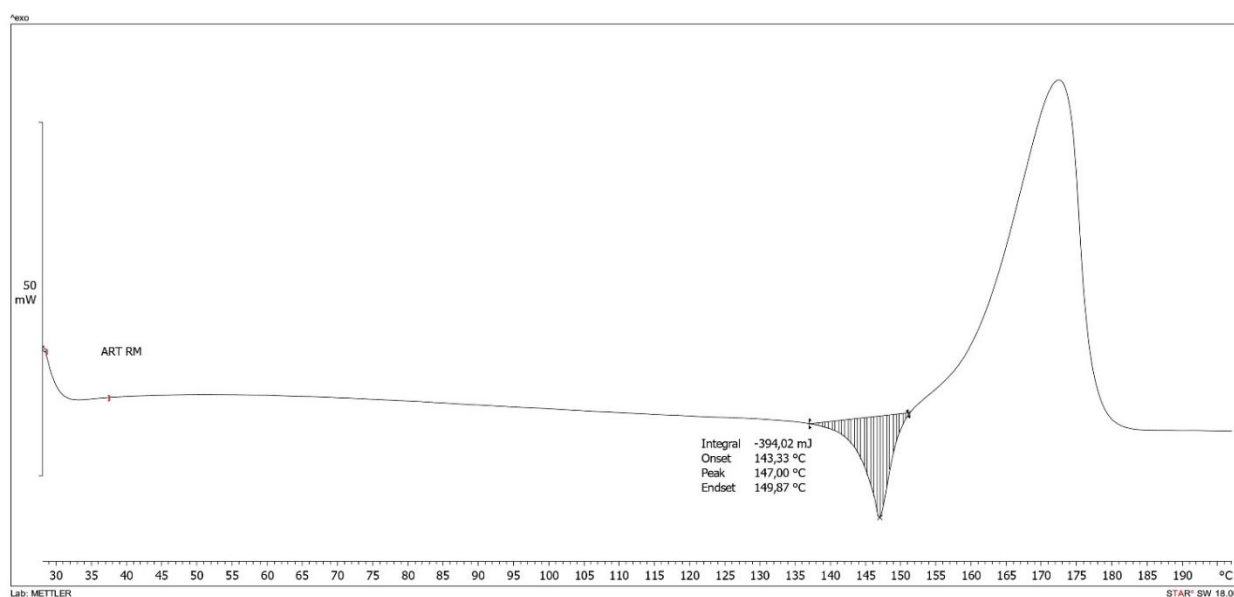


Figure 4.45: DSC thermogram of artesunate raw material.

To produce ASDs of ART and the various polymers, the mixture had to be proven miscible. The DSC thermograms of ART and the various polymer physical mixtures and ASDs are illustrated in Figures 4.46-4.48.

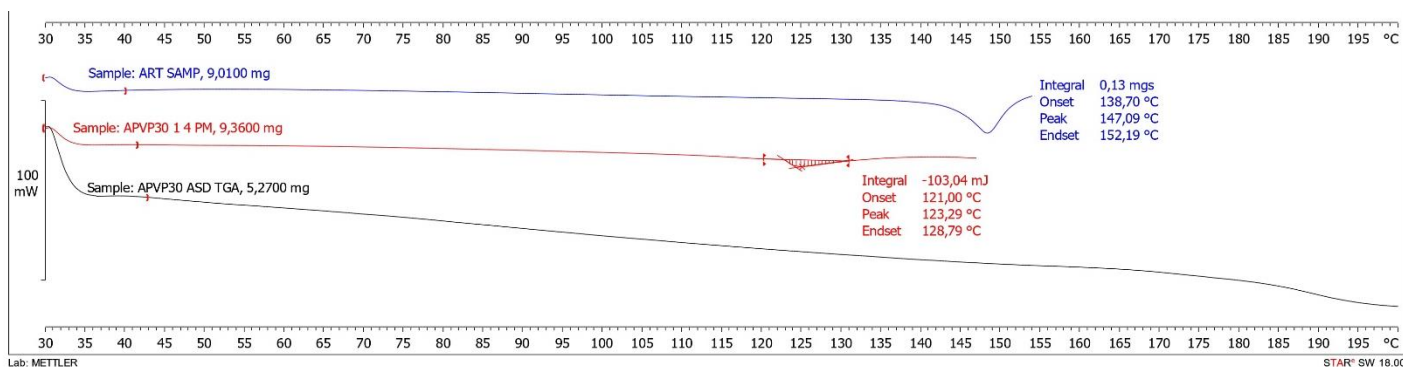


Figure 4.46: DSC thermogram of ART (blue), APVP30 PM (red) and APVP30 ASD (black).

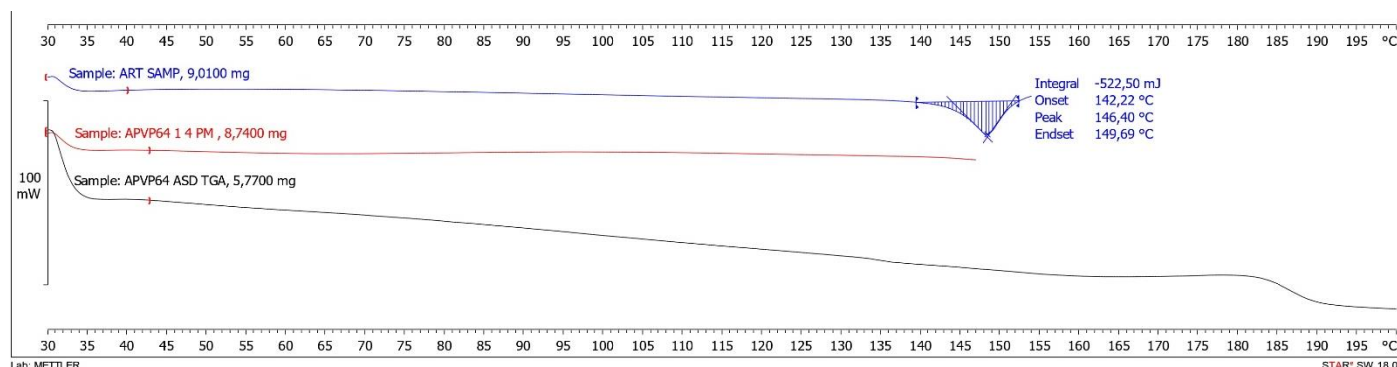


Figure 4.47: DSC thermogram of ART (blue), APVP64 PM (red) and APVP64 ASD (black).

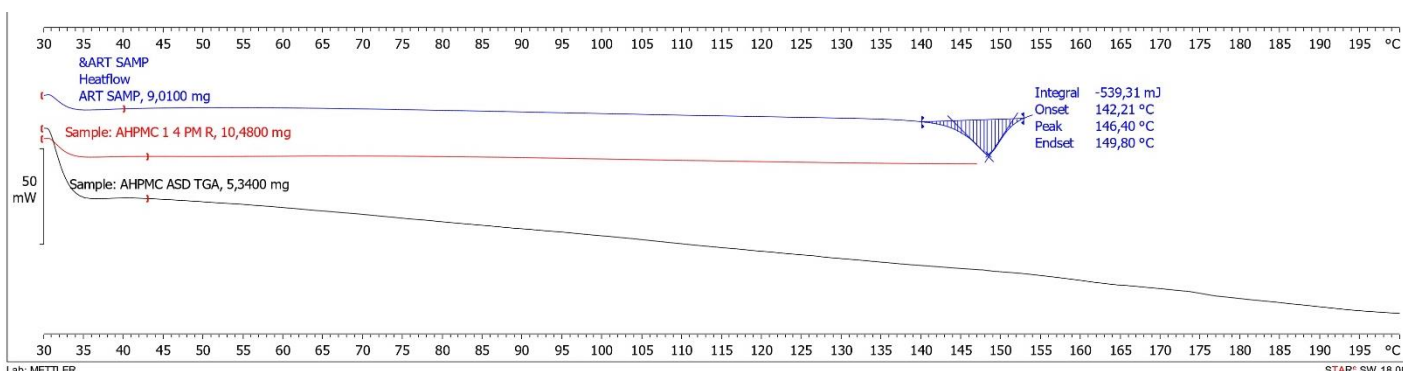


Figure 4.48: DSC thermogram of ART (blue), AHPMC PM (red) and AHPMC ASD (black).

Figures 4.46 to 4.48 exhibit only 1 endothermic event at 146.40°C, resembling the melting point of ART RM (blue). The PM (red) of the miscibility test showed no endothermic event, indicating a miscible system. The thermogram of the prepared ASD (black) a straight line, which suggest that an ASD was successfully prepared.

4.3.1.2 Fourier-transform infrared spectroscopy (FTIR)

In Figure 4.49 the FTIR spectrum of ART is displayed showing the characteristic absorption bands of ART RM.

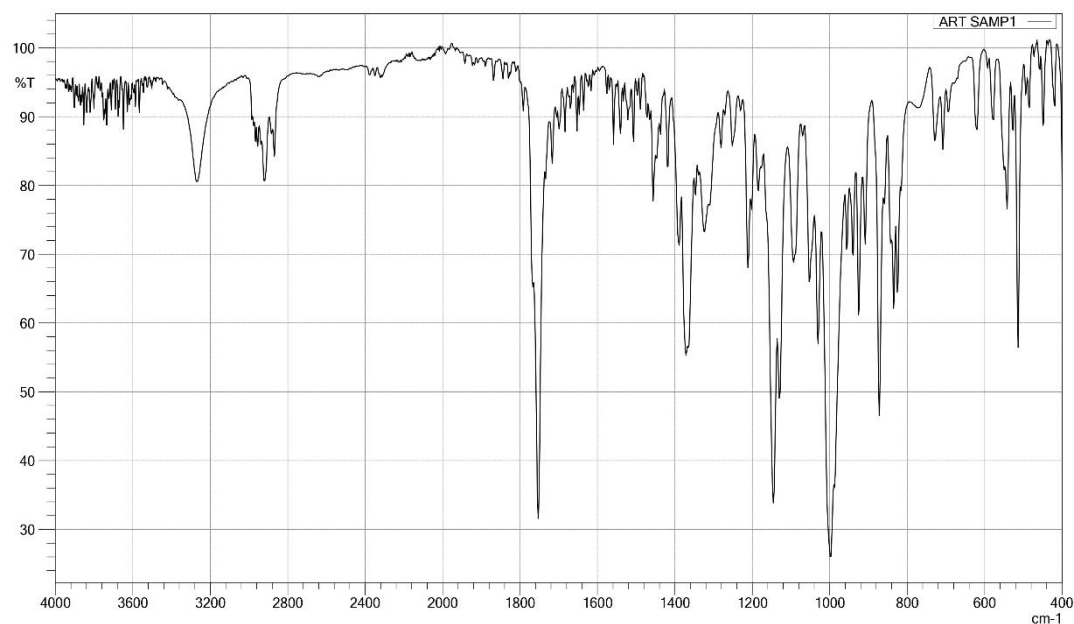


Figure 4.49: FTIR spectrum of the ART raw material sample.

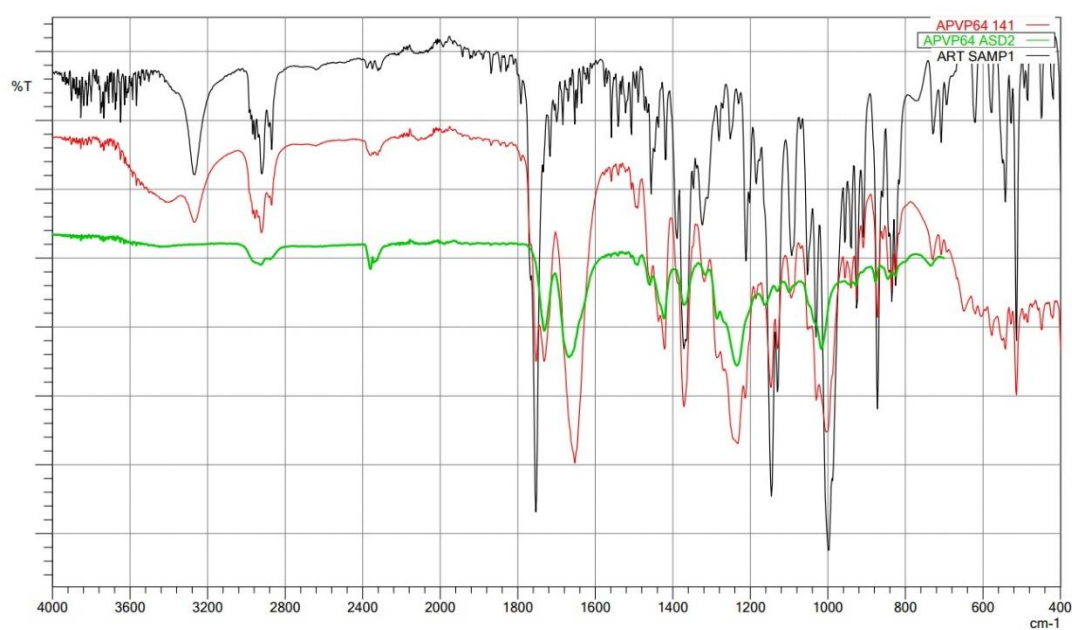


Figure 4.50: FTIR spectra of ART RM (black), APVP30 ASD (green), and APVP30 PM (red).

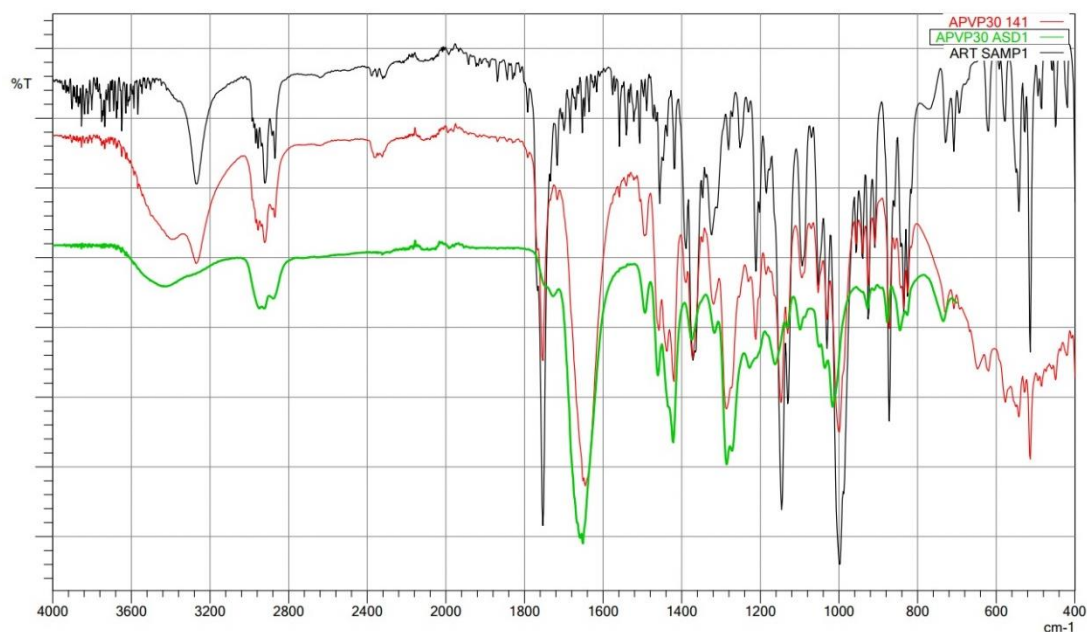


Figure 4.51 FTIR spectra of ART RM (black), APVP64 ASD (green), and APVP64 PM (red).

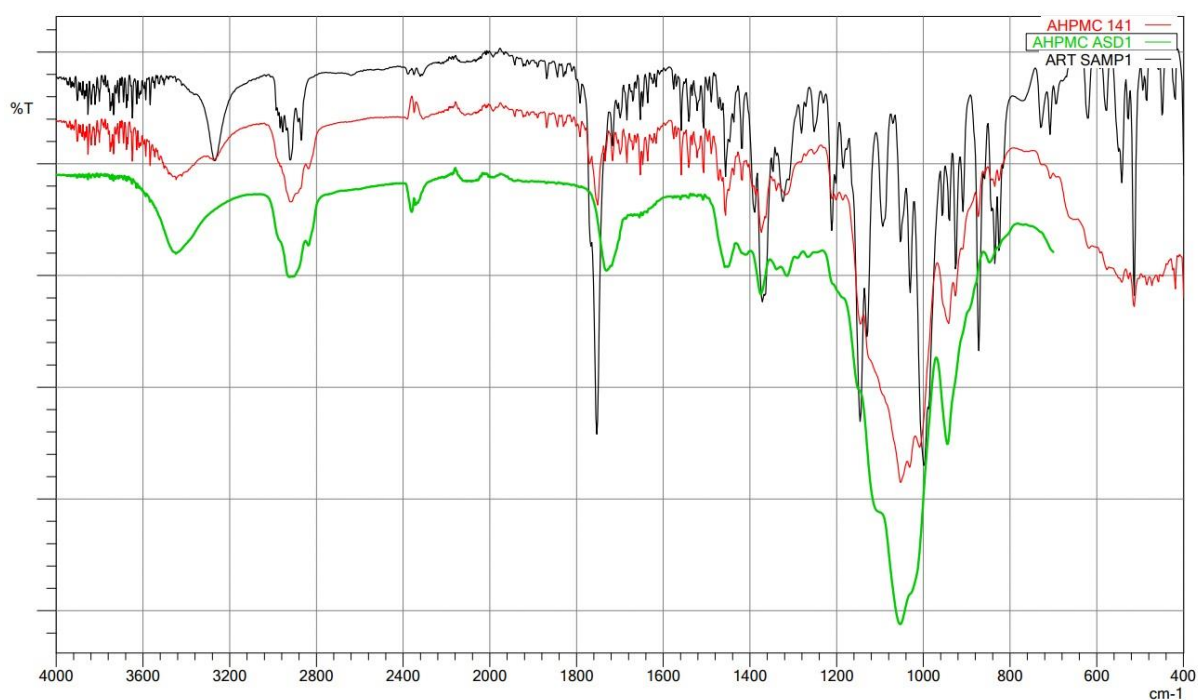


Figure 4.52: FTIR spectra of ART RM (black), AHPMC ASD (green), and AHPMC PM (red).

Figures 4.50 - 4.52 showed the FTIR spectra of the respective ASDs. In the region of 3600 – 3200 cm⁻¹, significant differences (disappearance of peaks) were observed between the PMs (red) and the PVP30 and PVPVA64 ASDs (green), indicating successful preparations of the ASDs. With the HPMC ASD, the differences between the physical mixture and the ASD were in the range of 1800 – 1000 cm⁻¹, with the disappearance of certain peaks unique to that of AHPMC ASD within the fingerprint region.

4.3.1.3 X-ray powder diffraction (XRPD)

In Figure 4.53 and 4.54 it is evident that the XRPD of ART RM (red) exhibited crystalline peaks as expected. The APVP30 ASD, APVP64 ASD and AHPMC ASD all displayed amorphous diffractograms which are indicative of successful formation of ASDs.

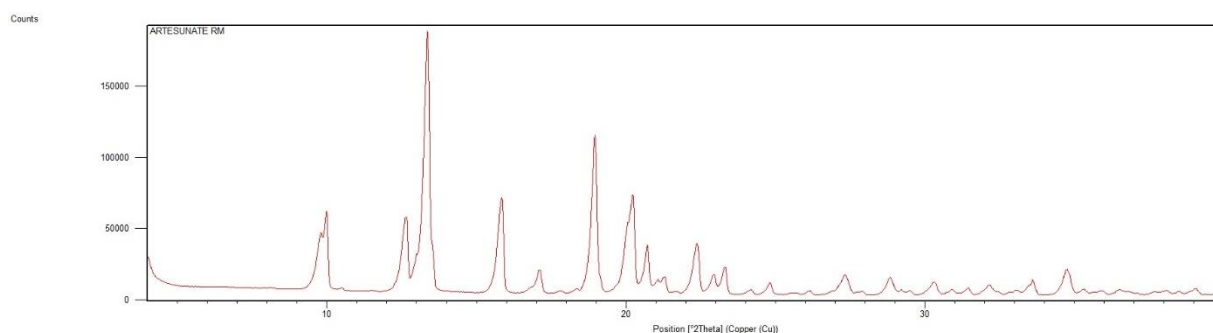


Figure 4.53: XRPD of ART raw material sample.

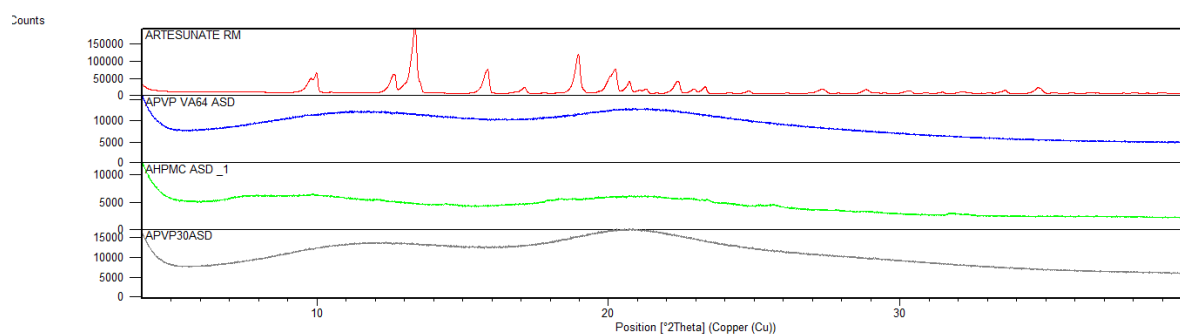


Figure 4.54: XRPD of ART (red), APVP64 ASD (blue), AHPMC ASD (green) and APVP30 ASD (black).

4.3.1.4 Polarised light microscopy

In Table 4.15 the birefringence effect of artesunate raw material can be seen, proving that the raw material is crystalline. In Table 4.16 the polarised micrographs of the prepared ASDs were without any birefringence, indicative of amorphous materials.

Table 4.15: Light microscopy micrographs of ART raw material

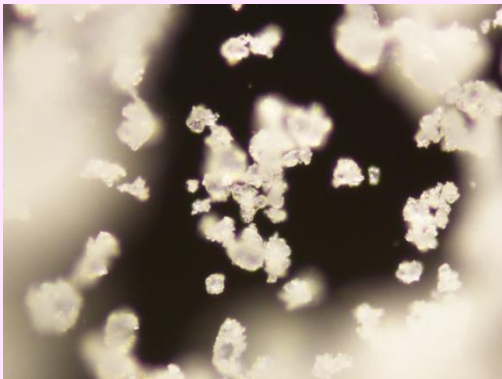
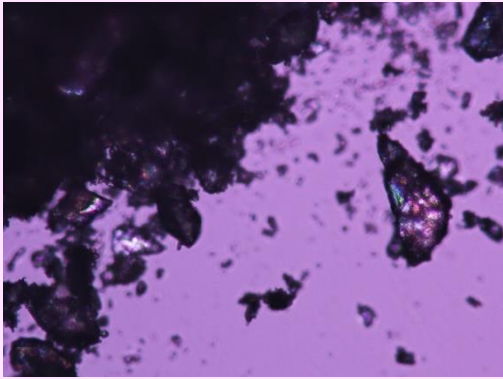
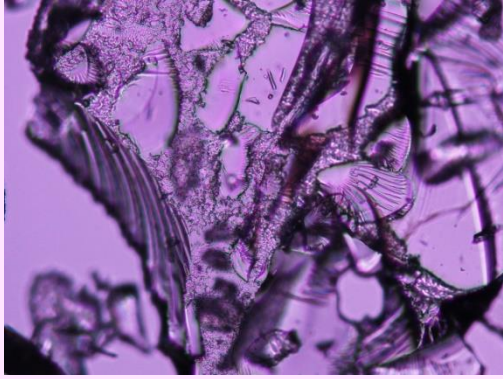
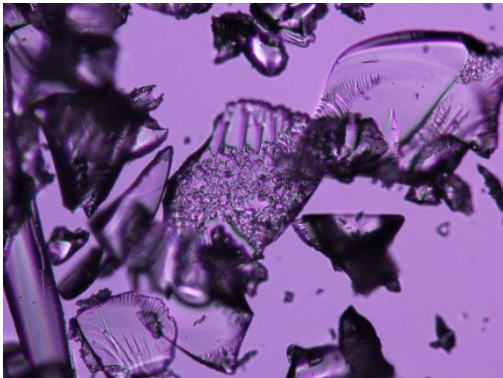
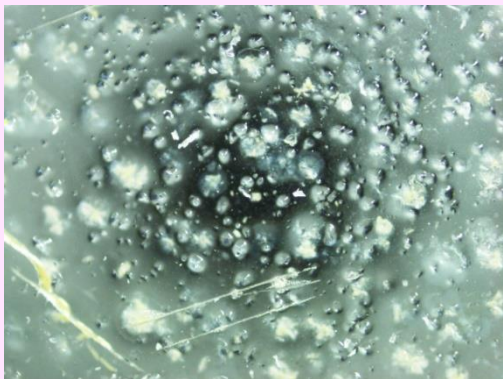
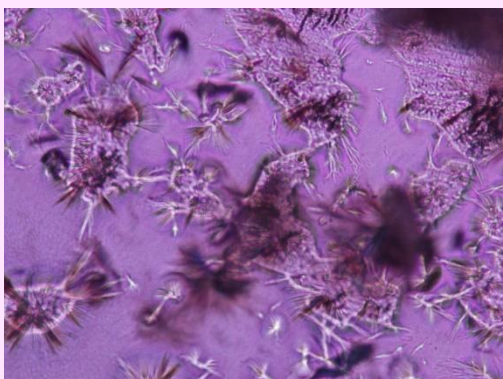
ART RM Non-polarised (20x)	ART RM Polarised (20x)
	

Table 4.16: Polarised light microscopy micrographs of ART ASDs

	NON-POLARISED FILTER	POLARISED FILTER
APVP30 ASD		
APVP64 ASD		
AHPMC ASD		

4.3.1.5 Thermal activity monitoring

From Figures 4.55 - 4.57 it can be concluded that the artesunate API as well as the ASDs present with no interactions as the interaction integrals are all lower than the 2-3 joules/g range. Figure 4.58, AHPMC, shows an interaction integral value above the 2-3 joules/g range and may thus present with minor interactions between the polymer and API.

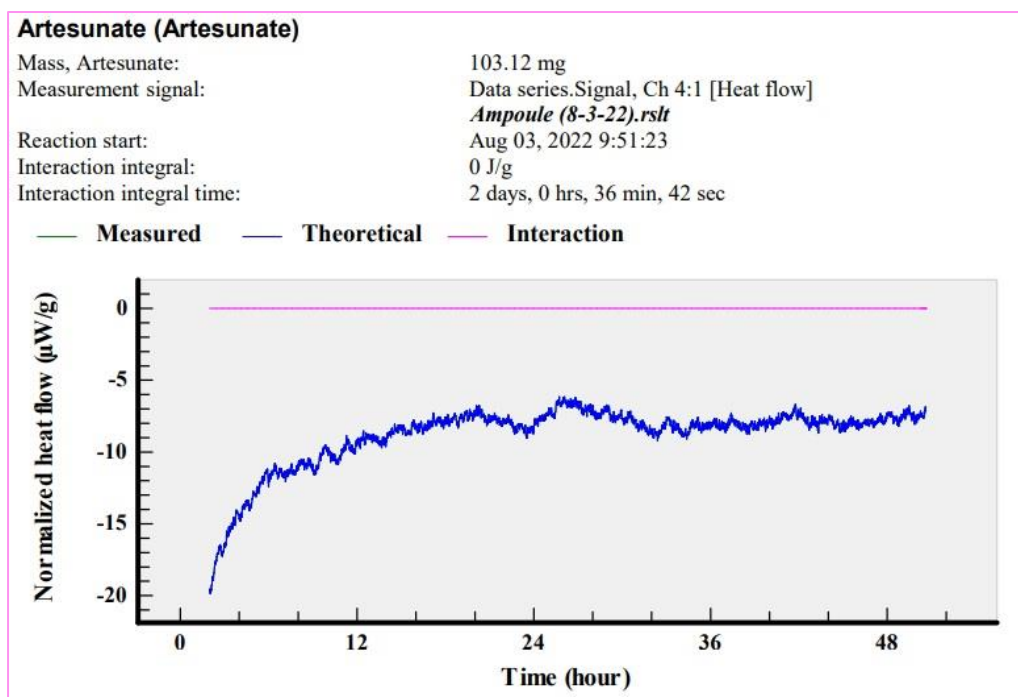


Figure 4.55: ART RM thermal activity monitoring.

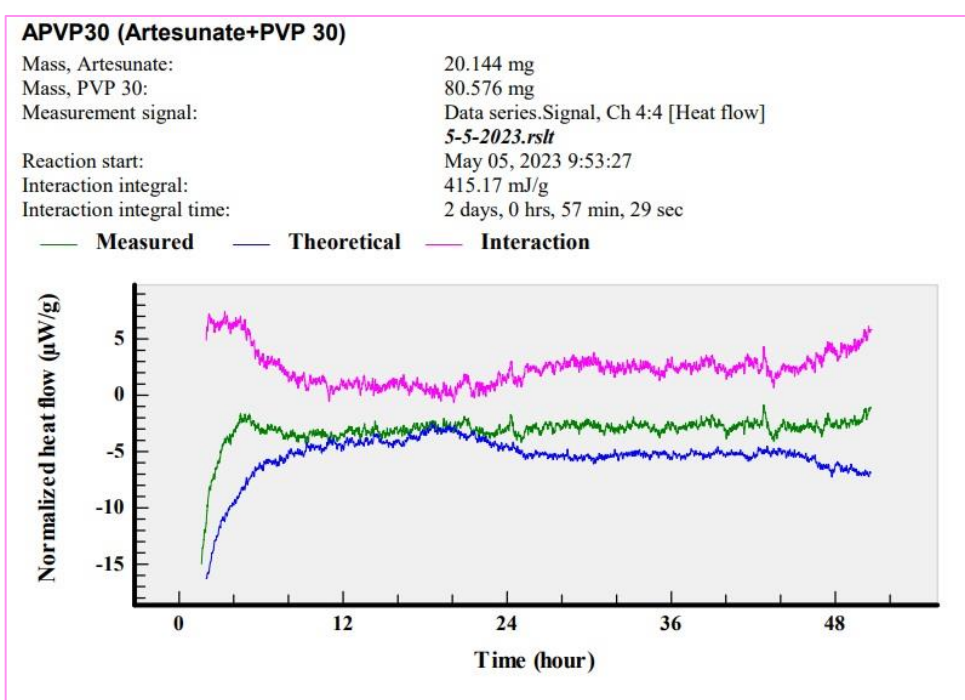


Figure 4.56: APVP30 ASD thermal activity monitoring.

APVP 64 (Artesunate+PVP 64)

Mass, Artesunate: 20.888 mg
Mass, PVP 64: 83.552 mg
Measurement signal: Data series.Signal, Ch 4:5 [Heat flow]
5-5-2023.rslt
Reaction start: May 05, 2023 9:53:27
Interaction integral: 1.5065 J/g
Interaction integral time: 2 days, 0 hrs, 57 min, 29 sec

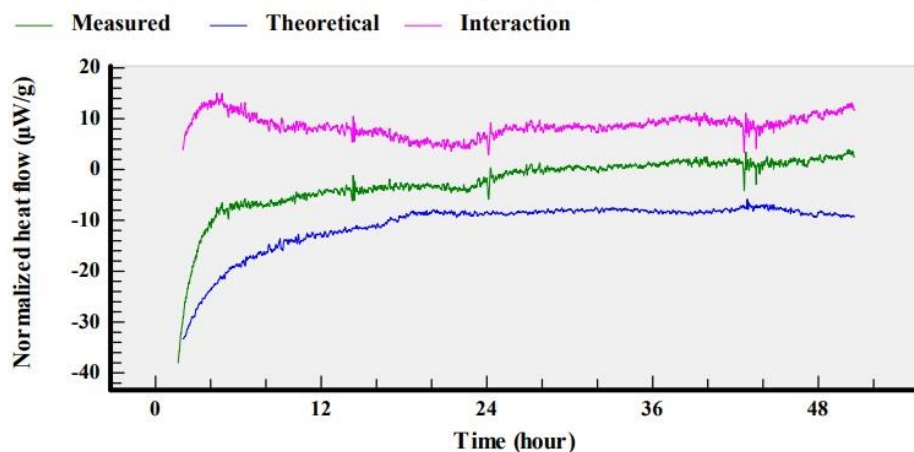


Figure 4.57: APVP64 ASD thermal activity monitoring.

AHPMC (Artesunate+HPMC)

Mass, Artesunate: 21.112 mg
Mass, HPMC: 84.448 mg
Measurement signal: Data series.Signal, Ch 4:6 [Heat flow]
5-5-2023.rslt
Reaction start: May 05, 2023 9:53:27
Interaction integral: 3.3923 J/g
Interaction integral time: 2 days, 0 hrs, 57 min, 29 sec

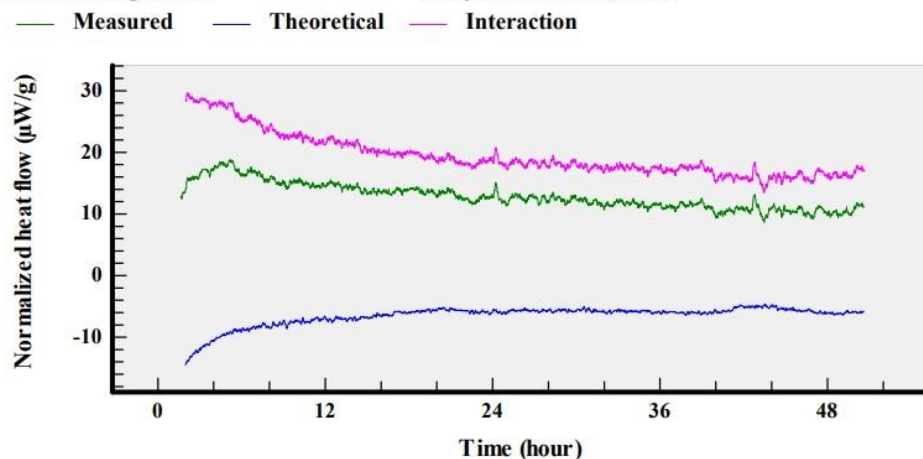


Figure 4.58: AHPMC ASD thermal activity monitoring.

4.3.2 Validation of HPLC method for artesunate and ASDs

Linearity of ART raw material, as seen in Figure 4.59, was established by injecting a series of five different concentrations of ART into the HPLC. These concentrations were as follow: 40 µg/ml, 80 µg/ml, 160 µg/ml, 200 µg/ml and 240 µg/ml. The regression coefficient obtained was 0.9983 which thus verifies linearity ([Singh, 2013](#)).

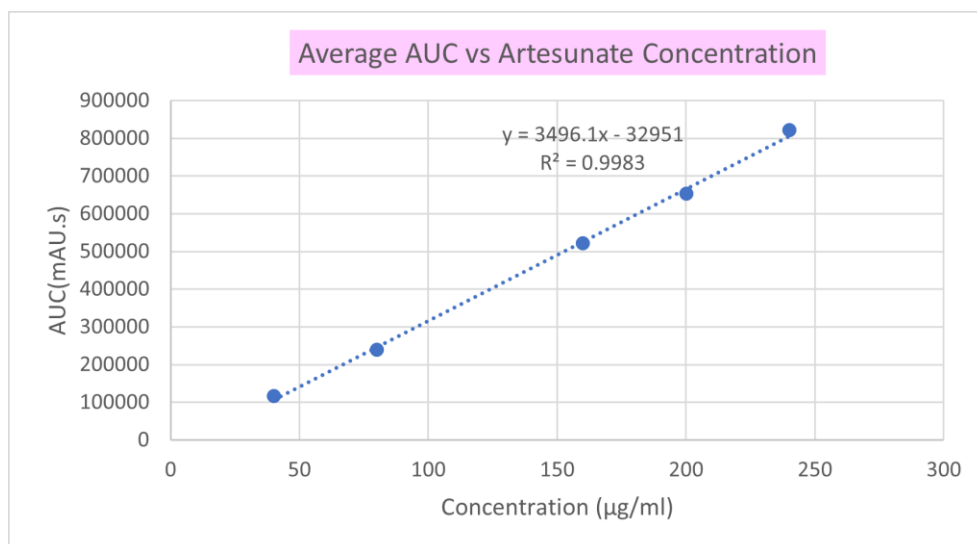


Figure 4.59: Results of HPLC method linearity for ART raw material.

Calibration curves covering the appropriate analytical range was set up for the respective ASDs. As seen in Figure 4.60, and according to the criteria mentioned above, all the calibration curves comply with regards to the correlation coefficient ($R^2 \geq 0.99$) ([Singh, 2013](#)). Thus, proving linearity was obtained across the applicable ranges for all of the ASDs.

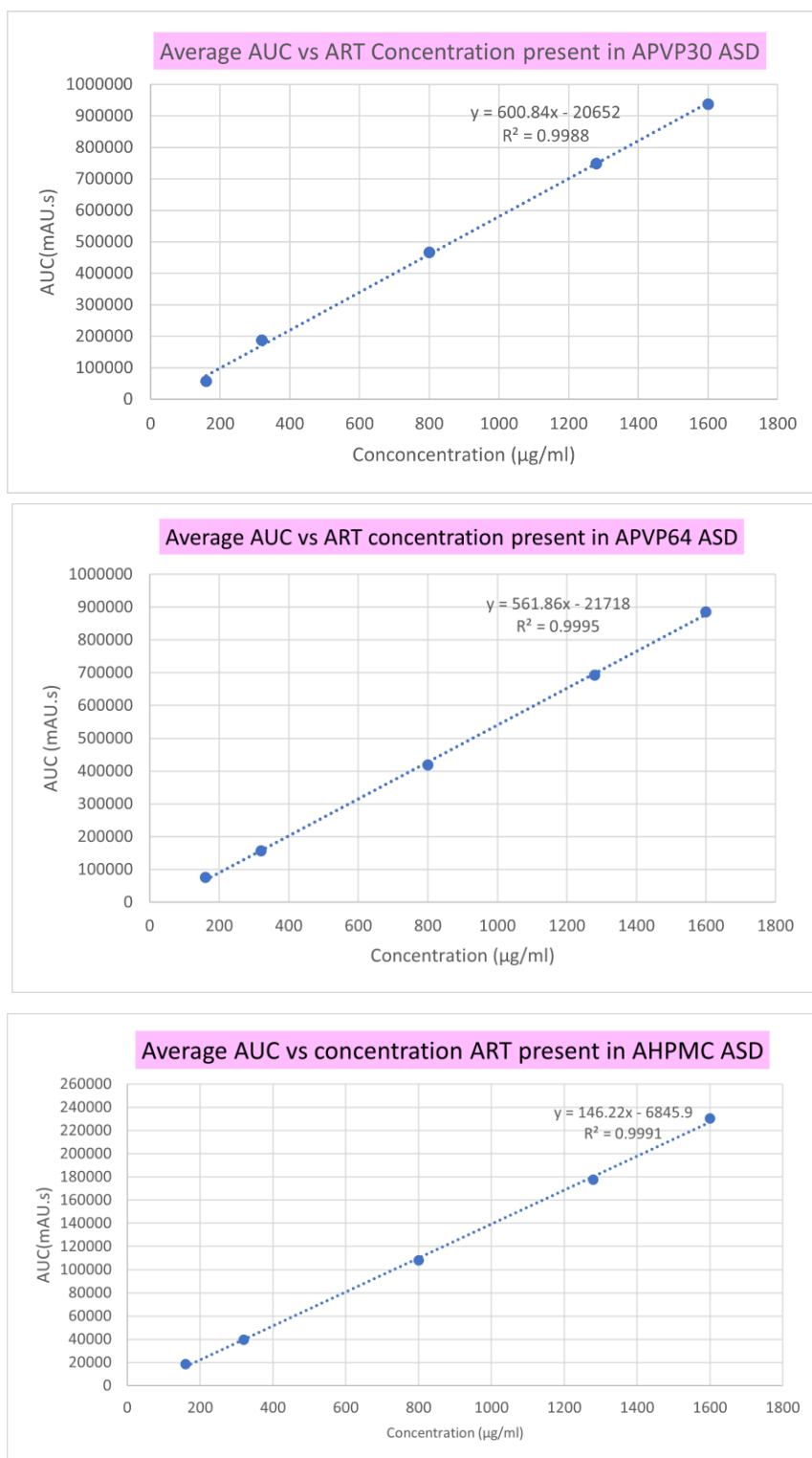


Figure 4.60: Results of HPLC method linearity for APVP30, APVP64 and AHPMC ASDs.

4.3.3 Solubility studies of artesunate and ASDs

Solubility studies for the API and ASDs were performed using the parameters described in Chapter 3 (see Section 3.2.3). All samples were analysed using HPLC. In this section the results obtained for ART, APVP30, APVP64 and AHPMC (both preliminary and pivot studies) are discussed.

4.3.3.1 Artesunate preliminary solubility studies

The results obtained for the preliminary solubility study of artesunate in H₂O are displayed in Table 4.17 and Figure 4.61.

Table 4.17: Preliminary solubility data of ART in ultrapure H₂O across 12 hours

Artesunate Solubility Studies								
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	171733	171835	171784	39.71	38.00	2.10	5.53
	2	168219	167916	168068	38.65			
	3	157944	157274	157609	35.66			
4hours	4	150276	151521	150899	33.74	32.65	1.13	3.47
	5	147902	146865	147384	32.73			
	6	143370	142621	142996	31.48			
8hours	7	132187	131166	131677	28.24	26.48	1.74	6.59
	8	125906	124929	125418	26.45			
	9	119927	119030	119479	24.75			
12hours	10	105200	105823	105512	20.75	20.80	3.02	14.53
	11	116277	116386	116332	23.85			
	12	94980	95427	95204	17.81			
24hours	13	Results obtained at 24 and 48 hours indicated complete degradation of ART and an increase in metabolites, degradation products.						
	14							
	15							
48hours	16							
	17							
	18							

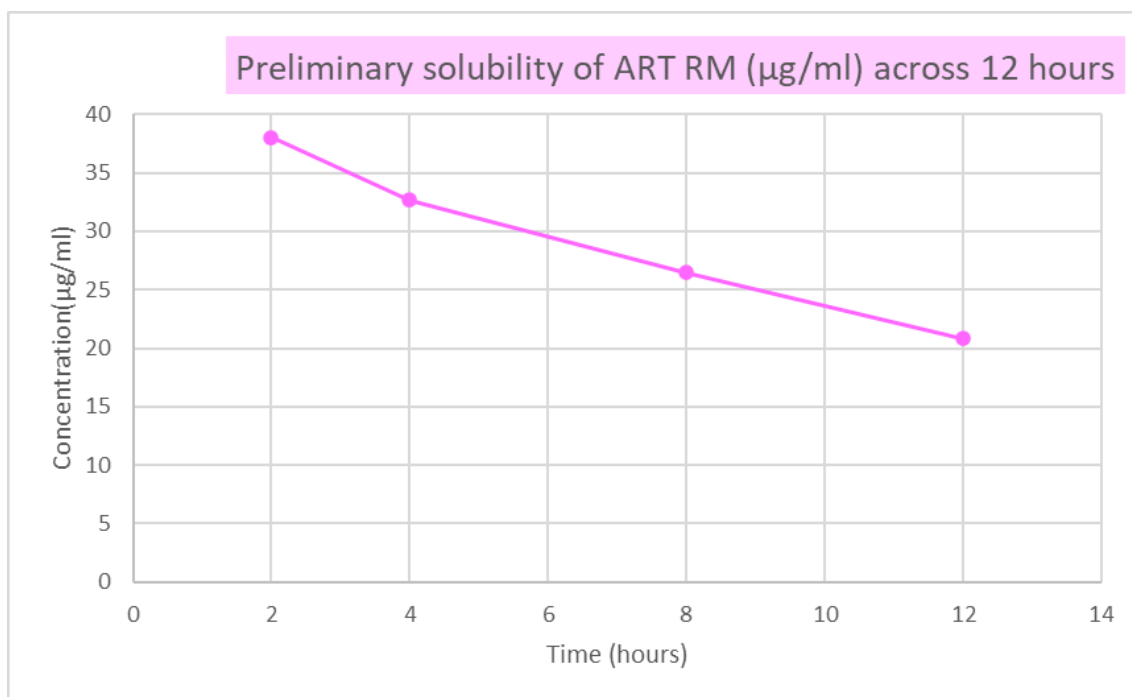


Figure 4.61: Preliminary solubility results obtained for ART in ultrapure H₂O across 12 hours.

It can be seen from Table 4.17 and Figure 4.61 that maximum solubility of artesunate is achieved at 2 hours (38.0 $\mu\text{g/ml}$). Here after, it is notable that artesunate undergoes degradation throughout the remainder of the experiment. After 12 hours the results were no longer quantifiable due to the formation of degradation products and metabolites. An initial weight of 10 mg API in 5 ml of HPLC H₂O was introduced to each test tube to ensure supersaturation. The %RSD showed that the variability for each time interval increased over time with the highest being 14.5 % at 12 hours. This is further indication that degradation occurred over time.

4.3.3.1.1 APVP30 ASD preliminary solubility studies

The results obtained for the preliminary solubility study of APVP30 ASD in H₂O are displayed in Table 4.18 and Figure 4.62.

Table 4.18: Preliminary solubility data of APVP30 ASD in ultrapure H₂O across 24 hours

APVP30 ASD Solubility Studies								
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	480365	428900	454633	791.04	934.62	277.35	29.67
	2	436219	433962	435091	758.51			
	3	732795	733198	732997	1254.33			
4hours	4	436954	499179	468067	813.39	909.04	84.01	9.24
	5	542525	549124	545825	942.81			
	6	562985	562420	562703	970.90			
8hours	7	520570	501874	511222	885.22	773.51	107.09	13.84
	8	440447	435820	438134	763.58			
	9	384263	381650	382957	671.74			
12hours	10	369525	369564	369545	649.42	611.76	119.97	19.61
	11	457100	352853	404977	708.39			
	12	412863	119610	266237	477.48			
24hours	13	23958	27523	25741	77.21	70.07	10.10	14.41
	14	17542	16780	17161	62.93			
	15	0	0	0	*LEAK			
48hours	16	Results obtained at 48 hours indicated complete degradation of ART and an increase in metabolites, degradation products.						
	17							
	18							

As seen in Table 4.18 and Figure 4.62 the maximum concentration of ART present in the ASD sample, during preliminary solubility studies, was reached after 2 hours at 934.62 µg/ml. It can be clearly seen that APVP30 undergoes significant degradation across the 48 hours, it does however provide solubility values for an extra 12 hours when compared to ART RM. It seems that the polymer delays the degradation process. There are also high %RSD values, which vary across the 48 hours. This may once again be a unique attribute held by ASDs, due to the variation of API present within the ASD. Each sample had an initial weight of 50 mg of APVP64 ASD (10 mg ART present) in 5 ml ultrapure H₂O.

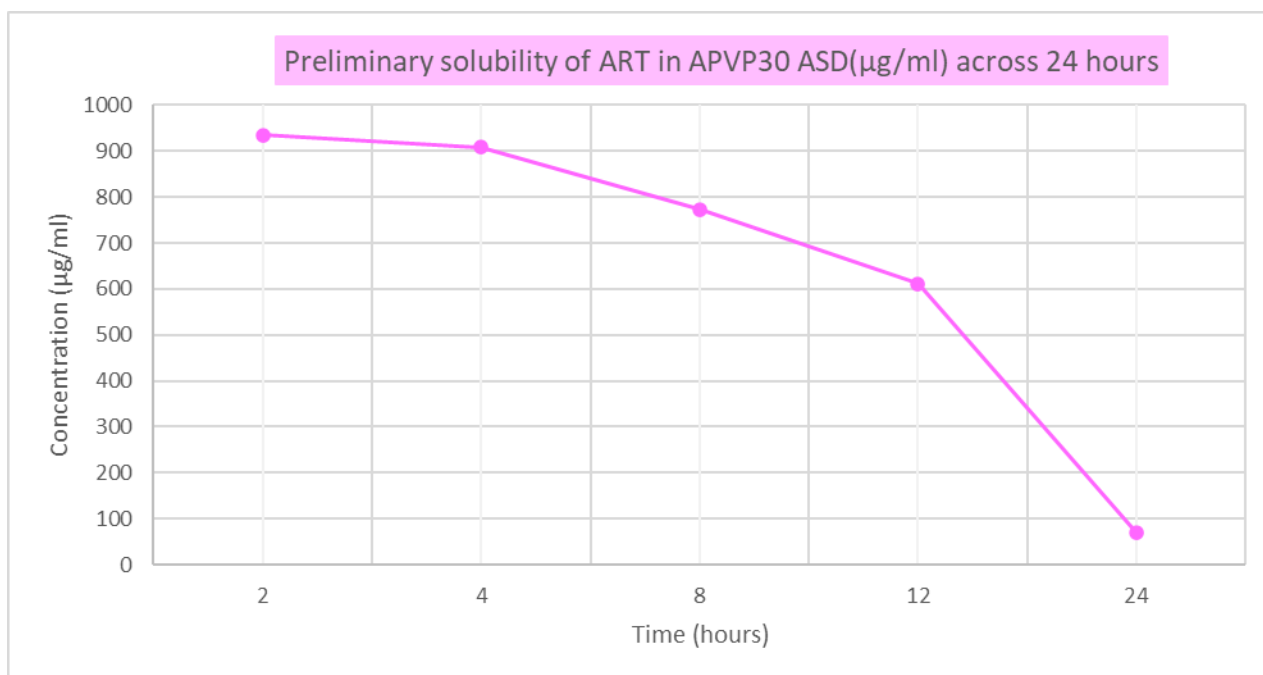


Figure 4.62: Preliminary solubility results obtained for APVP30 ASD in ultrapure H₂O across 24 hours.

4.3.3.1.2 APVP64 ASD preliminary solubility studies

The results obtained for the preliminary solubility study of APVP64 ASD in H₂O are displayed in Table 4.19 and Figure 4.63.

Table 4.19: Preliminary solubility data of APVP64 ASD in ultrapure H₂O across 48 hours

APVP64 ASD Solubility Studies								
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	584656	583992	584324	1001.33	977.86	29.15	2.98
	2	552661	552949	552805	945.23			
	3	578457	574113	576285	987.02			
4hours	4	447095	445256	446176	755.45	727.50	24.28	3.34
	5	420451	422643	421547	711.62			
	6	424297	423080	423689	715.43			
8hours	7	233233	234010	233622	377.15	376.51	5.06	1.34
	8	235675	236142	235909	381.22			
	9	229874	230638	230256	371.16			
12hours	10	152649	153554	153102	233.84	242.86	15.19	6.26
	11	169390	166661	168026	260.40			
	12	153021	153745	153383	234.34			
24hours	13	124561	124398	124480	182.90	180.57	15.09	8.36
	14	115947	112297	114122	164.46			
	15	131669	130181	130925	194.37			
48hours	16	148127	109964	129046	191.02	189.20	2.57	1.36
	17	6596	6226	6411	-27.24	*LEAK		
	18	129245	124756	127001	187.38			

As seen in Table 4.19 and Figure 4.63 the maximum concentration of ART present in the ASD sample, during preliminary solubility studies, was reached after 2 hours (977.86 µg/ml). Significant degradation is seen across the 48 hours, but APVP64 ASD produces solubility values for the entire 48 hours when compared to ART RM, which did not provide quantifiable data after 12 hours. The stability of the APVP64 ASD may prove to be better than that of the ART RM. Each sample had an initial weight of 50 mg of APVP64 ASD (10 mg ART present) in 5 ml ultrapure H₂O.

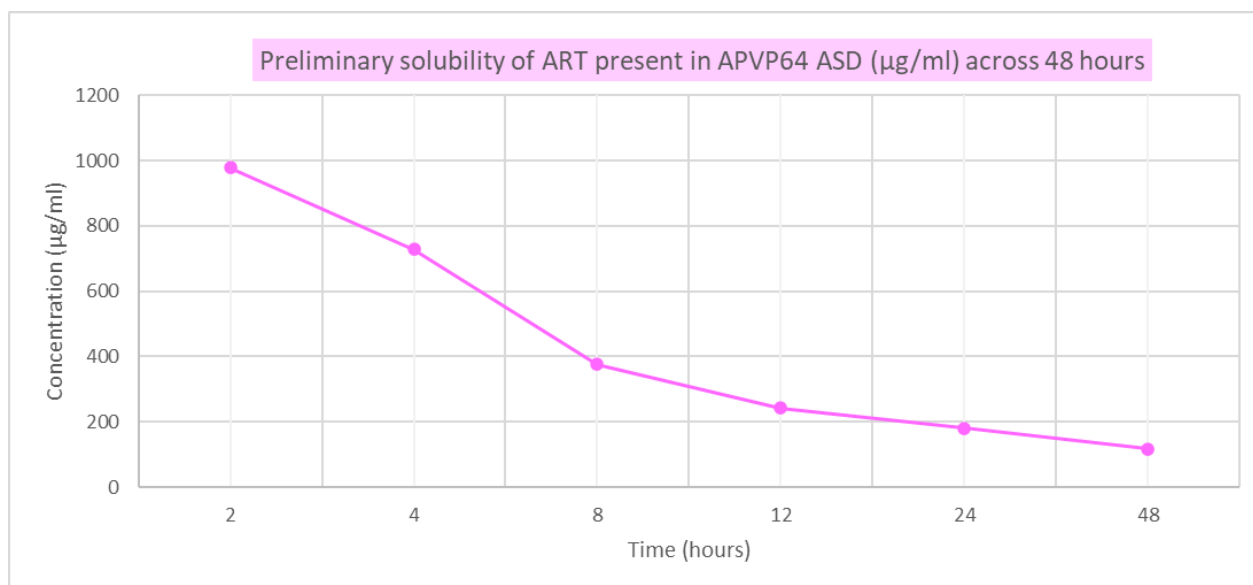


Figure 4.63: Preliminary solubility results obtained for APVP64 ASD in ultrapure H₂O across 48 hours.

4.3.3.1.3 AHPMC ASD preliminary solubility studies

The results obtained for the preliminary solubility study of AHPMC ASD in H₂O are displayed in Table 4.20 and Figure 4.64.

Table 4.20: Preliminary solubility data of AHPMC ASD in ultrapure H₂O across 48 hours

AHPMC ASD Solubility Studies								
Time	Vial	AUC1	AUC2	AVERAGE	Concentration (µg/ml)	Average Concentration (µg/ml)	SD	%RSD
2hours	1	143406	141081	142244	926.02	1006.24	97.05	9.64
	2	149244	150621	149933	978.60			
	3	170806	168687	169747	1114.12			
4hours	4	81157	80788	80973	506.97	626.31	103.49	16.52
	5	108007	107882	107945	691.44			
	6	106753	105942	106348	680.51			
8hours	7	58949	59130	59040	356.96	361.14	7.54	2.09
	8	61189	60655	60922	369.84			
	9	59661	58315	58988	356.61			
12hours	10	42057	40315	41186	234.86	264.06	31.60	11.97
	11	50214	50510	50362	297.62			
	12	44549	45090	44820	259.71			
24hours	13	37428	37932	37680	210.88	224.41	29.35	13.08
	14	38207	35215	36711	204.25			
	15	44560	44603	44582	258.08			
48hours	16	43248	43857	43553	251.05	223.42	39.07	17.49
	17	35611	35335	35473	195.79			
	18	0	0	0	0.00	*LEAK		

As seen in Table 4.20 and Figure 4.64 the maximum concentration of ART present in the ASD sample, during preliminary solubility studies, was reached after 2 hours (1006.245 µg/ml). Significant degradation is seen after 2 hours and continues throughout the 48 hours. This ASD still produces solubility values for a longer period of time when compared to the ART RM. Each sample had an initial weight of 50 mg of APVP64 ASD (10 mg ART present) in 5 ml ultrapure H₂O.

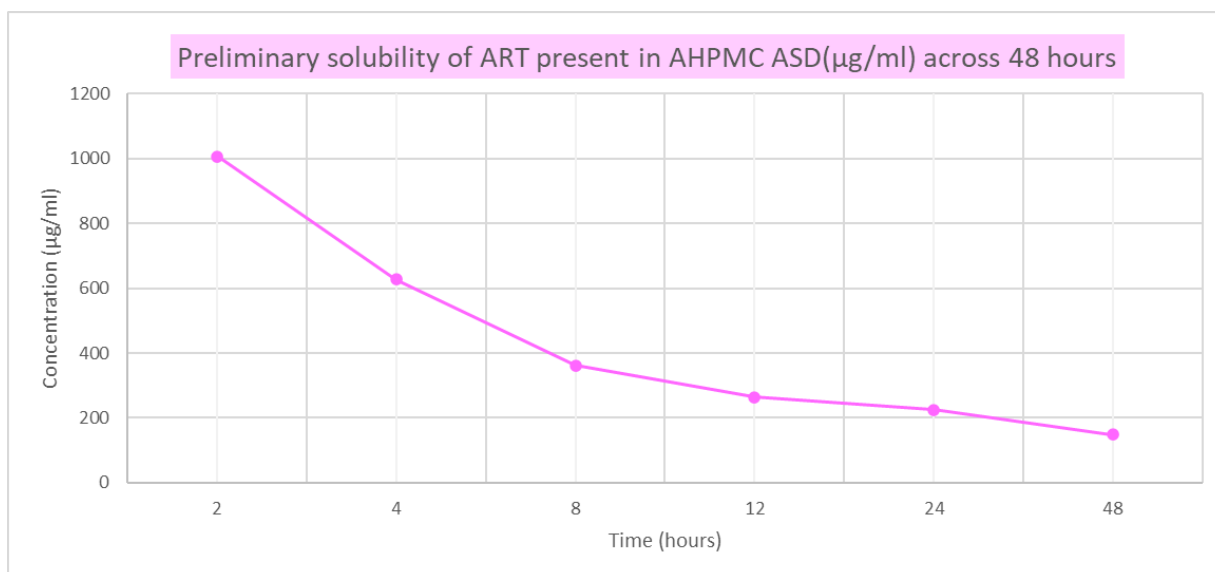


Figure 4.64: Preliminary solubility results obtained for AHPMC ASD in ultrapure H₂O across 48 hours.

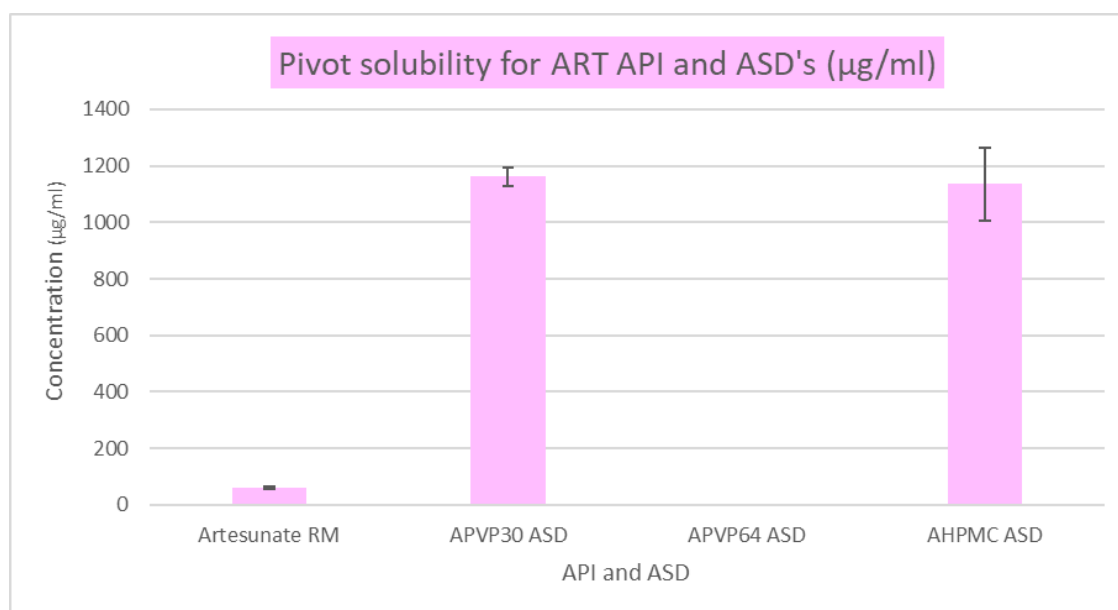
4.3.3.2 Artesunate API and ASD pivot solubility studies

The pivot solubility studies performed on ART and the various ASDs are displayed in Table 4.21 and Figure 4.65, below. During the preliminary solubility studies an uncertainty regarding the degree of the super saturated solution occurred. Thus, during the pivot studies the amount of ART RM used was increased, in order to ensure a super saturated solution. The increase seen in average concentration during the pivot studies may lead to the belief that a depot of ART is formed and as the ART in solution undergoes degradation, the depot ART then enters and shows a higher and prolonged solubility. APVP30 shows a 19-fold increase in solubility compared to that of ART RM and AHPMC shows an 18-fold increase. The results obtained for the pivot solubility studies correlated with the data obtained from the preliminary solubility studies done for ART and the various ASDs with the exception of APVP64.

Table 4.21: Pivot solubility data of ART API and ASDs after 2 hours

Sample Concentration (µg/ml)	Artesunate RM	APVP30 ASD	APVP64 ASD	AHPMC ASD
SAMPLE 1	66.90	1157.45	0.00	1261.94
SAMPLE 2	67.27	1222.87	0.00	1183.58
SAMPLE 3	59.58	1142.14	0.00	1263.38
SAMPLE 4	57.88	1151.36	0.00	1084.99
SAMPLE 5	57.98	1164.21	0.00	923.23
SAMPLE 6	56.57	1133.08	0.00	1101.08
Average Concentration (µg/ml)	61.03	1161.85	0.00	1136.37
SD	4.79	31.86		129.19
% RSD	7.85	2.74		11.37

As seen in Table 4.21 APVP30 and AHPMC ASDs had the highest solubility (1161.85 µg/ml and 1136.37 µg/ml respectively). It can also be seen that the solubility of ART RM is almost double of that of the preliminary solubility, this is due to an increase of RM added to the test tube during the pivots to ensure a super saturated solution (50 mg). Thus, upon investigation of the results of the pivot solubility studies it can be concluded that a huge increase is apparent in the solubility profiles of both APVP30 and AHPMC even though the amount of artesunate present in these ASDs were 5 times less than the amount of ART RM weighed off per sample. During the pivot solubility studies it was evident that the solubility study of APVP64 was unsuccessful, due to rapid degradation, which could possibly be due to an entrapment of moisture in the ASD while it was stored in preparation for the solubility studies. This necessitates further investigation to confirm these claims.

**Figure 4.65: Pivot solubility results obtained for ART API and ASDs.**

4.4 Statistical analysis

4.4.1 ANCOVA analysis of preliminary data

To determine whether two solubility profiles differed from one another on a statistically significant level, analysis of covariance (ANCOVA) was used. For this study, the assumption of parallel regression lines was foregone, and the following model (Eq. 1) was selected:

$$y_{ij} = \alpha_i + \beta_i x_{ij} + \epsilon_{ij} ; j = 1, \dots, n_i ; i = 1, 2 ; \quad \text{Eq. 1}$$

where ϵ_{ij} are independent $n(0; \sigma^2)$ variates.

In this model, we estimated (α_1, β_1) and (α_2, β_2) independently (Eq.'s 2 - 3), but σ^2 jointly as Eq. 4:

$$\hat{\beta}_i = \frac{\sum_j y_{ij}(x_{ij} - \bar{x}_i)}{\sum_j (x_{ij} - \bar{x}_i)^2} \quad \text{Eq. 2}$$

$$\hat{\alpha}_i = \bar{y}_i - \hat{\beta}_i \bar{x}_i \quad \text{Eq. 3}$$

$$\hat{\sigma}^2 = \frac{\sum_i \sum_j (y_{ij} - \hat{\alpha}_i - \hat{\beta}_i x_{ij})^2}{(n_1 + n_2 - 4)} \quad \text{Eq. 4}$$

For the solubility data, the responses at time zero and at time n_i , were tested to see if they were equal. Therefore, the hypothesis tested for the solubility data was $H_0: \alpha_1 + \beta_1 x = \alpha_2 + \beta_2 x$, compared to the test statistic:

$$f_x = (\hat{\alpha}_1 + \hat{\beta}_1 x - \hat{\alpha}_2 - \hat{\beta}_2 x)^2 / \hat{\sigma}^2 \left[\frac{1}{n_1} + \frac{(x - \bar{x}_1)^2}{\sum (x_{1j} - \bar{x}_1)^2} + \frac{1}{n_2} + \frac{(x - \bar{x}_2)^2}{\sum (x_{2j} - \bar{x}_2)^2} \right] \quad \text{Eq. 5}$$

with $F_{\alpha; 1; n_1 + n_2 - 4}$. Testing at $\alpha = 0.5$, any values of $f_x > F_{crit}$ will therefore be indicative of a statistically significant difference.

Table 4.22: ANCOVA Statistics regarding SULFA API and ASDs ($F_{crit} = 5.3177$)

	SULFA	SPVP30 ASD	SPVP64 ASD	SHPMC ASD
SULFA		4197.49	555.62	354.88
SPVP30 ASD	4197.5		24.79	248.84
SPVP64 ASD	555.62	24.79		287.57
SHPMC ASD	354.88	248.84	287.57	

Table 4.23: ANCOVA Statistics regarding PYRI API and ASDs ($F_{crit} = 5.3177$)

	PYRI	PPVP30 ASD	PPVP64 ASD	PHPMC ASD
PYRI		2690.00	3416.42	1673.30
PPVP30 ASD	2690		148.20	132.76
PPVP64 ASD	3416.42	148.20		553.29
PHPMC ASD	1673.30	132.76	553.29	

Table 4.24: ANCOVA Statistics regarding ART API and ASDs ($F_{crit} = 5.3177$)

	ART	APVP30 ASD	APVP64 ASD	AHPMC ASD
ART		139.66	48.29	30.66
APVP30 ASD	139.66		57.53	24.97
APVP64 ASD	48.29	57.53		19.40
AHPMC ASD	30.66	24.97	19.40	

From the ANCOVA analysis it can be concluded that a significant difference is seen between ASDs and the API. This leads us to the conclusion that the ASDs did in fact lead to an enhancement in the solubility of the raw material during the preliminary studies.

4.4.2 Mann-Whitney U tests with Bonferroni adjustments of the resulting p-values for pivot solubility data

To investigate the differences in solubility between the individual APIs and their ASDs, Mann-Whitney U tests were performed with Bonferroni adjustments of the resulting p-values.

4.4.2.1 Sulfadoxine

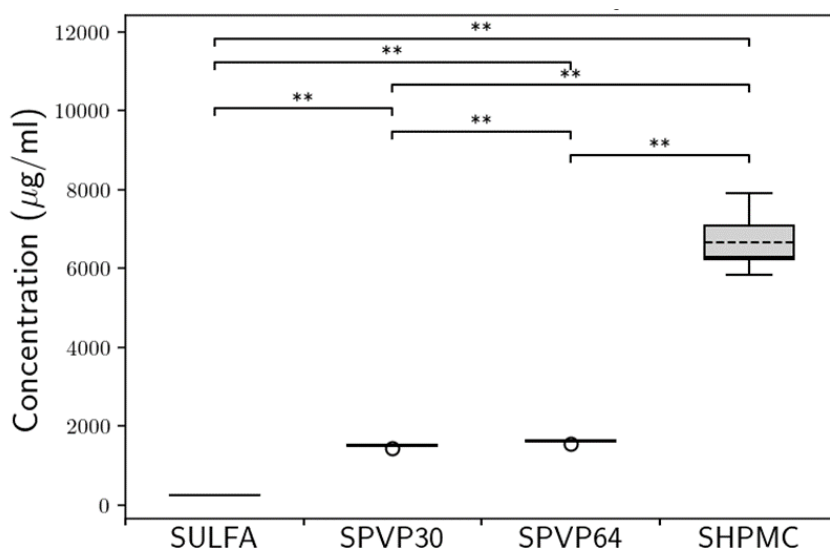


Figure 4.66: Boxplots and statistical significance indicators for the solubility data of sulfadoxine. Means indicated as dashed lines and medians as solid lines. For the significance indicators, * indicates significant differences ($p \leq 0.05$) and ** indicates very significant differences ($p \leq 0.01$).

The adjusted p-values are presented in Table 4.25.

Table 4.25: Mann-Whitney U tests regarding SULFA API and ASDs

	SULFA	SPVP30 ASD	SPVP64 ASD	SHPMC ASD
SULFA		0.00216	0.00216	0.00216
SPVP30 ASD	0.00216		0.00216	0.00216
SPVP64 ASD	0.00216	0.00216		0.00216
SHPMC ASD	0.00216	0.00216	0.00216	

The statistical analysis showed that sulfadoxine's solubility differed significantly from those of its ASDs, and that the solubility values obtained from all the ASDs differed significantly from each other.

4.4.2.2 Pyrimethamine

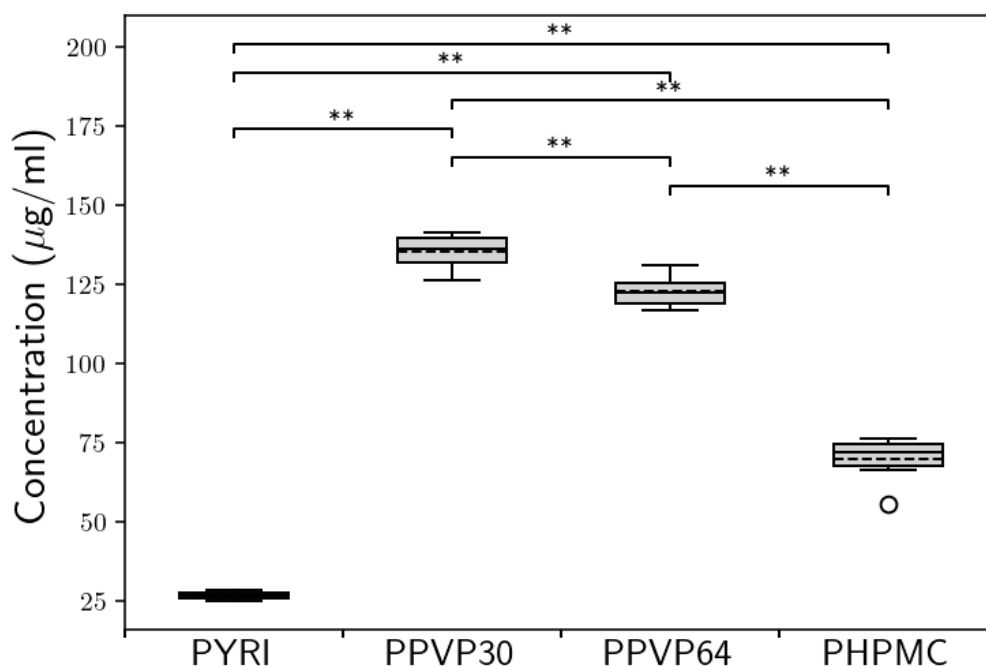


Figure 4.67: Boxplots and statistical significance indicators for the solubility data of pyrimethamine. Means indicated as dashed lines and medians as solid lines. For the significance indicators, * indicates significant differences ($p \leq 0.05$) and ** indicates very significant differences ($p \leq 0.01$).

The adjusted p-values are presented in Table 4.26.

Table 4.26: Mann-Whitney U tests regarding PYRI API and ASDs

	PYRI	PPVP30 ASD	PPVP64 ASD	PHPMC ASD
PYRI		0.00216	0.00216	0.00216
PPVP30 ASD	0.00216		0.00432	0.00216
PPVP64 ASD	0.00216	0.00432		0.00216
PHPMC ASD	0.00216	0.00216	0.00216	

The statistical analysis showed that pyrimethamine solubility differed significantly from those of its ASDs, and that the solubility values obtained from all the ASDs differed significantly from each other.

4.4.2.3 Artesunate

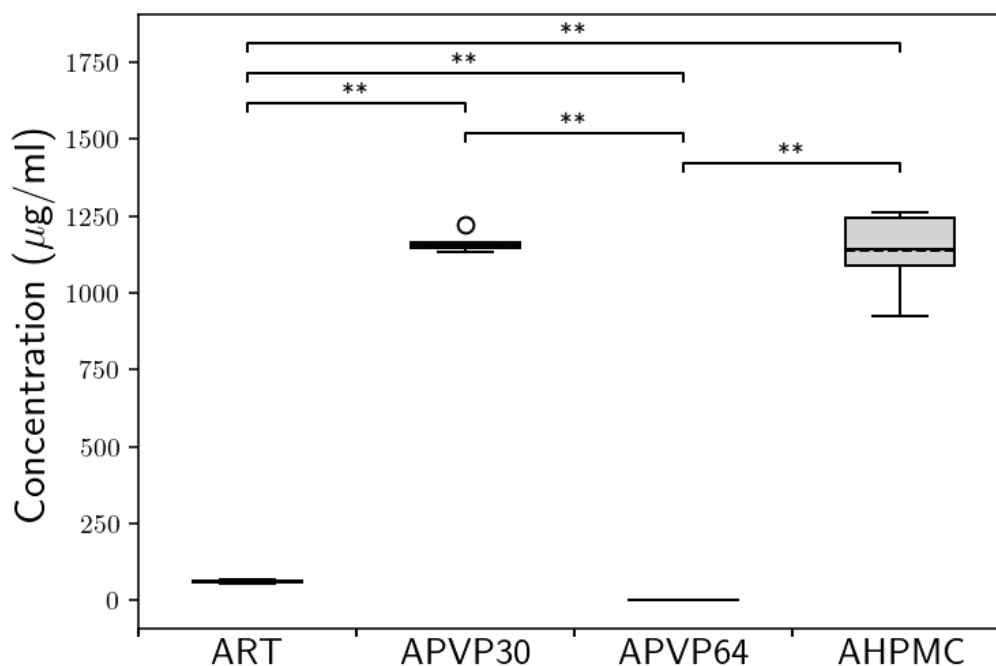


Figure 4.68: Boxplots and statistical significance indicators for the solubility data of artesunate. Means indicated as dashed lines and medians as solid lines. For the significance indicators, * indicates significant differences ($p \leq 0.05$) and ** indicates very significant differences ($p \leq 0.01$).

The adjusted p-values are presented in Table 4.27.

Table 4.27: Mann-Whitney U tests regarding ART API and ASDs

	ART	APVP30 ASD	APVP64 ASD	AHPMC ASD
ART		0.00216	0.00277	0.00216
APVP30 ASD	0.00216		0.00277	1.00
APVP64 ASD	0.00277	0.00277		0.00216
AHPMC ASD	0.00216	1.00	0.00277	

The statistical analysis showed that artesunate's solubility differed significantly from those of its ASDs. The only ASDs that did not differ significantly from each other in terms of solubility values were APVP30 and AHPMC.

From the statistical evaluation performed above it can be concluded that the ASDs presented a measurable difference with respect to the original API and from that it can be said that the formation of the ASD proved to be a suitable approach to the enhancement of the solubility of the various APIs.

4.5 Conclusion

It can be seen in Table 4.28 that the majority of the ASDs formed successfully upon preparation. The information gathered is based on the physico-chemical characterisation that was performed on all respective ASDs.

Table 4.28: ASD formation

Successful ASD formation	Semi-amorphous ASDs
SPVP30 ASD	PPVP30 ASD
SPVP64 ASD	PPVP64 ASD
SHPMC ASD	
PHPMC ASD	
APVP30 ASD	
APVP64 ASD	
AHPMC ASD	

When reviewing the semi-amorphous ASDs we can refer to the XRPD and PLM data presented in this chapter. It shows that the not completely amorphous ASDs exhibit small percentages of crystalline peaks on the XRPD results as well as a small percentage of birefringence on the polarised microscopy micrographs. These ASDs were still used in the solubility studies to see if any improvements occurred. Even though these ASDs were partly formed, the solubility of the respective APIs improved nonetheless, mostly because the polymers improve the wettability properties of the APIs.

The statistical data showed that the ASDs significantly improved the solubility of the respective APIs, which means that the objective of the study was achieved.

Pyrimethamine showed improvement in the solubility profile of the ASDs with the polymers PVP30 and PVP64. Although these 2 polymers indicated at not being fully amorphous, it still enhanced the solubility profile of the API. The concentration improving from PYRI RM (26.76 µg/ml) to 135.22 µg/ml with PPVP30 ASD and 122.81 µg/ml with PPVP64 ASD. PHPMC presented with a minor increase in solubility at 69.73 µg/ml and a much higher %RSD value, making it a very unpredictable choice.

During the solubility study performed on artesunate, improvements were seen amongst APVP30 ASD and AHPMC ASD with their concentrations at 1161.85 µg/ml and 1136.37 µg/ml respectively. When compared to the ART RM with a solubility value of 61.03 µg/ml, it can be

concluded that a substantial increase in solubility was seen. Based on the statistical analysis done by means of the Mann-Whitney U tests, it can be seen that APVP30 ASD and AHPMC ASD performed similarly in the enhancement of the solubility profile.

References

- BATES, S., ZOGRIFI, G., ENGERS, D., MORRIS, K., CROWLEY, K. AND NEWMAN, A. 2006. Analysis of Amorphous and Nanocrystalline Solids from their X-Ray Diffraction patterns. *Pharmaceutical Research*, 23(10):2333–2349.
- BUTLER, J. 2007. Forensic News Customer Corner Validation: What Is It, Why Does It Matter, and How Should It Be Done? [online] Available at: https://strbase.nist.gov/pub_pres/Butler_ForensicNews_Jan2007.pdf [Accessed 29 Sept. 2023].
- BYRN, S.R., PFEIFFER, R.R. & STOWELL, J.G. 1999. Solid-state chemistry of drugs. 2nd ed. West Lafayette, Ind.: SSCI. 574p.
- CUI, Y. 2007. A material science perspective of pharmaceutical solids. *International Journal of Pharmaceutics*, 339 (1–2): 3-18.
- HANCOCK, B.C. & ZOGRIFI, G. 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. *Journal of Pharmaceutical Sciences*, 86:1-12.
- INTERNATIONAL PHARMACOPOEIA. 2020a. 19th ed. Sulfadoxine. <https://digicollections.net/phint/2020/index.html#d/b.6.1.342> Date of access: 4 May 2023.
- INTERNATIONAL PHARMACOPOEIA. 2020b. 19th ed. Pyrimethamine. <https://digicollections.net/phint/2020/index.html#d/b.6.1.307> Date of access: 4 May 2023.
- INTERNATIONAL PHARMACOPOEIA. 2020c. 19th ed. Artesunate. <https://digicollections.net/phint/2020/index.html#d/b.6.1.30> Date of access: 4 May 2023.
- LIU, X., FENG, X., WILLIAMS III, R. O., ZHANG, F. 2017. Characterization of amorphous solid dispersions. *Journal of Pharmaceutical Investigation*, 48(2018):19-41.
- LEE, T.W.Y., BOERSEN, N.A., HUI, H.W., CHOW, S.F., WAN, K.Y. & CHOW, A.H.L. 2014. Delivery of poorly soluble compounds by amorphous solid dispersions. *Current Pharmaceutical Design*, 20(3):303-324.
- NEWMAN, A., ZOGRIFI, J. 2015. Introduction to amorphous solid dispersions. (In NEWMAN, A. *Pharmaceutical Amorphous Solid Dispersions*, Wiley. p. 1-41) <https://books.google.co.za/books?id=NPhQDwAAQBAJ>
- NICDAÉID, N. 2005. Forensic Sciences | Systematic Drug Identification. In A. T. Paul Worsfold, Colin Poole (Ed.), *Encyclopedia of Analytical Science* (2):471-480. United Kingdom: Elsevier.

SINGH, R. 2013. HPLC method development and validation- an overview. *Journal of Pharmaceutical Education & Research*, 4(1):26-33.

The International Council for Harmonisation of technical requirements for pharmaceuticals for human use. (ICH). 2020. <https://www.ich.org/> [Date of access: 23 April 2023].

VAN LOCO, J., ELSKENS, M., CROUX, C. & BEERNAERT, H. 2002. Linearity of calibration curves: use and misuse of the correlation coefficient. *Accreditation and Quality Assurance*, 7(7):281–285.

Chapter 5: Conclusion

5.1 Conclusion

According to the solubility profiles of sulfadoxine pyrimethamine and artesunate all three of these drugs present with poor aqueous solubility. Improving the solubility by means of solid dispersion formation was the main aim of this study. As stated in the conclusion of Chapter 4 not all of the attempts at forming ASDs were successful, however solubility was still evaluated on all of the ASDs. The combination of the API's with various polymers, which in turn produced an array of ASDs, allowed for further testing on their respective solubility profiles to indicate which polymer-API combination delivered the most promising results.

Ahead of ASD formation, standard samples of the respective APIs to be used were evaluated according to the USP monograph to validate the respective HPLC methods. The polymers were also evaluated with the same HPLC methods, this allowed for verification of substances being used and also predictions of possible interactions between the API and polymer when used in combination to form the respective ASDs. From the chromatograms it could be concluded that the "fingerprint" of the API produced by the HPLC method is accurate and that the polymer would have no effect or interference on future readings.

Thereafter, 1:4 ratios of API to polymer physical mixtures (PM) were produced and underwent physico-chemical characterisation. Once these results had been obtained, the various ASDs were prepared through the solvent evaporation process. Once again physico-chemical characterisation was performed on the resulting ASDs. These tests included X-ray powder diffraction (XRPD), simultaneous thermal analysis (STA), polarised light microscopy (PLM), Fourier transform infrared spectroscopy (FTIR) and thermal activity monitoring (TAM). The results obtained from these characterisation tests were used to evaluate whether an ASD was formed or not. The physico-chemical characterisation tests i.e., the XRPD and PLM were the most effective techniques in determining the amorphous nature of the ASDs. The ASDs, SHPMC; PPVP30 and PPVP64 were showing signs of not being completely amorphous. Solubility studies were however still performed on all ASDs irrespective of if they were fully amorphous or not.

Solubility studies of sulfadoxine, pyrimethamine, artesunate APIs and all 9 resulting ASDs were performed in a water bath with a set temperature of 37°C ($\pm 1^\circ\text{C}$) in ultrapure H₂O. The solvent was kept constant in order to be able to properly evaluate if there is a change in the solubility profile of the API once converted to an ASD. With sulfadoxine and the 3 resulting ASDs an increase within the solubility profile when compared to the API was noticeable. The

ASD showing the highest solubility was SHPMC, with a resulting 28-fold increase in solubility when comparing to that of the API. The SHPMC ASD, however, produced more of a plastic-like ASD which was difficult to work with and posed a problem when trying to maintain particle size uniformity.

When looking at pyrimethamine, all ASDs produced an enhancement in the solubility profile when comparing to the API. PPVP30 ASD and PPVP64 ASD delivered the most notable enhancement in solubility but also produced semi-amorphous results in the physico-chemical characterisation process.

Artesunate API posed to be a challenge regarding stability. Artesunate has a rate of degradation in the various polymer combinations which are a great deal faster than sulfadoxine and pyrimethamine. The degradation rate of the newly formed ASD remained very similar to that of the API. Due to artesunate's degradation products forming so close to the API 'fingerprint' on the HPLC chromatogram it was unclear if metabolites contributed to the AUC reading on the chromatogram. Peak purity will need to be investigated further to confirm the presence of artesunate API. APVP30 and AHPMC did result in a 19-fold increase in the solubility profile but once again it is unreliable to confidently say that those readings are representative of the artesunate API. APVP64 ASD proved to be unsuccessful in the pivot studies even though readings were present in the preliminary studies. This failure should be investigated further but it may be explained by moisture entrapment within the ASD during the storage process.

The focus of this study was to enhance solubility of poorly soluble anti-malaria drugs by means of the formation of amorphous solid dispersions. By analysing the results obtained throughout this study it can be concluded that the formation of ASD's does, in fact, have a positive effect on the solubility profiles of the respective APIs.

5.2 Future recommendations

- ✂ Perform peak purity analysis to confirm purity of ASD HPLC AUC readings.
- ✂ Perform permeability studies on the ASDs to identify if enhanced solubility would impact on the permeation of the APIs.
- ✂ Create fixed dose combinations (FDC) ASDs and evaluate solubility and permeability studies.
- ✂ Investigate the semi-amorphous ASDs and reasons why the solubility results were better when compared to the fully amorphous ASDs.