

Topical delivery of clofazimine, artemisone and decoquinate utilizing vesicles as carrier system

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If we knew what we were
doing, it would not be called
research, would it?

-Albert Einstein

Table of contents

TABLE OF CONTENTS	i
TABLE OF TABLES	xi
TABLE OF FIGURES	xv
ACKNOWLEDGEMENTS	xxiii
ABSTRACT	xxiv
References	xxvi
UITTREKSEL	xxvii
Bronnelys	xxix

PREFACE

1

CHAPTER 1

Introduction and problem statement

1.1.	Introduction and problem statement	2
1.2.	Research aim and objectives	3
	References	4

CHAPTER 2

Review article published in Tuberculosis

1.	Introduction	8
2.	Classification of cutaneous tuberculosis	9
2.1.	Inoculation of tuberculosis from an exogenous source	9
2.2.	Tuberculosis from an endogenous source	9
2.2.1.	Haematogenous tuberculosis	10

2.2.1.1.	Tuberculids	11
3.	Atypical mycobacterium infections of the skin	12
4.	Current treatment regimens of cutaneous tuberculosis	13
4.1.	True cutaneous tuberculosis and tuberculids	14
4.2.	Atypical mycobacterium infections	14
5.	Summary	15
	Acknowledgements	15
	Funding	15
	Competing interests	15
	Ethical approval	15
	References	15

CHAPTER 3

Article on the validation of the analytical method accepted for publication in DIE Pharmazie

	Abstract	19
1.	Introduction	19
2.	Investigations, results and discussion	20
3.	Experimental	21
	Acknowledgements	22
	Disclaimer	22
	References	22

CHAPTER 4

Manuscript to be submitted to the Journal of Pharmaceutical and Biomedical Analysis on the topical delivery of artemisone, clofazimine and decoquinat encapsulated in vesicles and their in vitro efficacy against a tuberculosis cell line

Abstract	29
Keywords	29
Highlights	30
1. Introduction	31
2. Materials and methods	32
2.1. Materials	32
2.2. Methods	32
2.2.1. Preparation of vesicles	32
2.2.2. Pre-formulation and characterisation	33
2.2.2.1. Isothermal calorimetry	33
2.2.2.2. Encapsulation efficiency	33
2.2.2.3. Zeta-potential, size distribution and vesicle size	33
2.2.2.4. pH and viscosity	33
2.2.3. Topical delivery	34
2.2.3.1. Skin preparation	34
2.2.3.2. Skin diffusion studies	34
2.2.3.3. Tape stripping	34
2.2.4. Efficacy against tuberculosis	35
3. Results and discussion	35
3.1. Pre-formulation and characterisation	35
3.1.1. Isothermal calorimetry	35

3.1.2.	Encapsulation efficiency	37
3.1.3.	Zeta-potential, size distribution and vesicle size	37
3.1.4.	pH and viscosity	39
3.2.	Skin diffusion studies	40
3.3.	Efficacy against tuberculosis	41
4.	Conclusions	41
	Acknowledgements	43
	References	44

CHAPTER 5

Final conclusion and future prospects

5.1.	Final conclusion	47
5.2.	Future prospects	50
	References	51

ANNEXURE A

Analytical method validation for the concurrent determination of decoquinat, artemisone and clofazimine by means of HPLC

A.1.	Introduction	53
A.2.	High performance liquid chromatography method validation for decoquinat, artemisone and clofazimine	53
A.2.1.	Chromatographic conditions	53
A.2.2.	Reference standard and sample preparation	54
A.2.3.	Analytical validation of test procedure and acceptance criteria	55
A.2.3.1.	Linearity	55
A.2.3.2.	Limit of detection and quantitation	59
A.2.3.3.	Accuracy	60

A.2.3.4.	Precision	62
A.2.3.4.1.	Repeatability (Intra-day assay variation)	63
A.2.3.4.2.	Intermediate precision (Inter-day assay variation)	65
A.2.3.4.3.	Reproducibility	67
A.2.3.5.	Ruggedness (Stability)	70
A.2.3.6.	System suitability	74
A.2.3.7.	Conclusion	76
	References	77

ANNEXURE B

Full compatibility report of clofazimine, artemisone and decoquinatate with vesicle components

1.	Introduction	79
2.	Method of analysis for compatibility	79
3.	Results	80
3.1.	Combination of artemisone, clofazimine and decoquinatate	80
3.2.	Artemisone in combination with phosphatidylcholine	81
3.3.	Clofazimine and phosphatidylcholine	82
3.4.	Decoquinatate and phosphatidylcholine	82
3.5.	Artemisone, clofazimine and decoquinatate in combination with phosphatidylcholine	83
3.6.	Artemisone in combination with cholesterol	84
3.7.	Clofazimine in combination with cholesterol	85
3.8.	Decoquinatate and cholesterol	86
3.9.	Artemisone, decoquinatate and clofazimine in combination with cholesterol	87

3.10.	Phosphatidylcholine and cholesterol	87
3.11.	Artemisone and Tween®20	88
3.12.	Clofazimine and Tween®20	89
3.13.	Decoquinat and Tween®20	89
3.14.	Phosphatidylcholine and Tween®20	90
3.15.	Combination of Tween®20 and cholesterol	91
3.16.	Decoquinat, artemisone and Tween®20	91
3.17.	Decoquinat, clofazimine and Tween®20	92
3.18.	Artemisone, clofazimine and Tween®20	93
3.19.	Artemisone, clofazimine, decoquinat and Tween®20	94
3.20.	Liposomes containing artemisone	94
3.21.	Liposomes containing clofazimine	95
3.22.	Liposomes containing decoquinat	96
3.23.	Liposomes containing artemisone, decoquinat and clofazimine	97
3.24.	Transferosomes containing artemisone	98
3.25.	Transferosomes containing clofazimine	99
3.26.	Transferosomes containing decoquinat	100
3.27.	Transferosomes containing artemisone, decoquinat and clofazimine	101
3.28.	Niosomes containing artemisone	102
3.29.	Niosomes containing clofazimine	103
3.30.	Niosomes containing decoquinat	104
3.31.	Niosomes containing artemisone, clofazimine and decoquinat	105
3.32.	Conclusion	106

ANNEXURE C

Liposomes, niosomes and transferosomes utilised for topical drug delivery

C.1.	Introduction	107
C.2.	Background	107
C.2.1.	Liposomes	108
C.2.2.	Niosomes	110
C.2.3.	Transferosomes	111
C.3.	Preparation of vesicles	112
C.3.1.	Materials	112
C.3.2.	Method of preparation	112
C.4.	Pre-formulation of vesicles	116
C.4.1.	Differential scanning calorimetry	116
C.4.2.	Isothermal calorimetry	117
C.4.3.	Hot stage microscopy	120
C.5.	Characterisation	123
C.5.1.	Transmission electron microscopy	123
C.5.2.	Encapsulation efficiency	125
C.5.3.	Zeta-potential, size and size distribution	127
C.5.4.	pH	144
C.5.5.	Viscosity	146
C.6.	Efficacy against tuberculosis	149
C.6.1.	Effect of empty vesicles on tuberculosis cells	151
C.6.2.	Effectivity of a combination of APIs against tuberculosis	151

C.6.3.	Effect of the type of vesicle used to encapsulate the APIs on tuberculosis cells	151
C.7.	Summary	152
References		154

ANNEXURE D

Transdermal diffusion studies of different vesicle dispersions

D.1.	Introduction	158
D.2.	Methods and materials	159
D.2.1.	Preparation of phosphate buffer solution	159
D.2.2.	Skin preparation	159
D.2.3.	Skin diffusion studies	161
D.2.4.	Tape stripping	162
D.2.5.	HPLC analysis	162
D.3.	Results and discussion	162
D.3.1.	Skin diffusion studies and tape stripping	162
D.4.	Conclusion	169
References		170

ANNEXURE E

Author's guide for ^{DIE}Pharmazie

E.1.	Aim	171
E.2.	Conditions	171
E.3.	Ethical considerations	172
E.3.1.	Conflicts of interest	173
E.3.2.	Informed consent	173
E.3.3.	Human and animal rights	173

E.4.	Preparation of manuscripts	173
------	----------------------------	-----

ANNEXURE F

Author's guide to the Journal of Pharmaceutical and Biomedical Analysis

F.1.	Introduction	177
F.1.1.	Types of paper	178
F.1.2.	Submission checklist	178
F.2.	Before you begin	179
F.2.1.	Ethics in publishing	179
F.2.2.	Declaration of interest	179
F.2.3.	Submission declaration and verification	179
F.2.4.	Changes to authorship	180
F.2.5.	Copyright	180
F.2.6.	Role of the funding source	181
F.2.7.	Open access	181
F.2.8.	Submission	183
F.3.	Preparation	183
F.3.1.	Peer review	183
F.3.2.	Article structure	184
F.3.3.	Essential title page information	185
F.3.4.	Abstract	186
F.3.5.	Keywords	186
F.3.6.	Artwork	188
F.3.7.	Tables	189
F.3.8.	References	189

F.3.9.	Video	192
F.3.10.	Supplementary material	193
F.3.11.	AudioSlides	194
F.3.12.	Interactive plots	194
F.4.	After acceptance	194
F.4.1.	Online proof correction	194
F.4.2.	Offprints	195
F.5.	Author inquiries	195

ANNEXURE G	197
Certificate of language editing	

TABLE OF TABLES

CHAPTER 1

Introduction and problem statement

Table 1.1:	Physicochemical properties of the three chosen APIs	2
------------	---	---

CHAPTER 2

Review article published in Tuberculosis

Table 1:	Atypical mycobacterium species responsible for cutaneous infections	9
Table 2:	The classification of established leprosy	14

CHAPTER 3

Article on the validation of the analytical method accepted for publication in **DIE Pharmazie**

Table 1:	Solubility ($\mu\text{g/ml}$) (37°C) determined for artemisone, clofazimine and decoquinatone in nine different solvents	24
Table 2:	Obtained validation parameters for the three compounds	24
Table 3:	Precision data for artemisone, clofazimine and decoquinatone	24

CHAPTER 4

Manuscript to be submitted to the Journal of Pharmaceutical and Biomedical Analysis on the topical delivery of artemisone, clofazimine and decoquinatone encapsulated in vesicles and their in vitro efficacy against a tuberculosis cell line

Table 1:	Encapsulation efficiency (%) of vesicle dispersions containing 1% API(s)	37
Table 2:	Zeta-potential, size and size distribution (PDI) of the different dispersions	38
Table 3:	The pH and viscosity of 1% API dispersions at 25±1.0°C	39

Table 4:	Growth inhibition (%) of the APIs in solid form, as well as in, the different dispersions	41
----------	---	----

ANNEXURE A

Analytical method validation for the concurrent determination of decoquinate, artemisone and clofazimine by means of HPLC

Table A.1:	Linear regression data obtained for artemisone	56
Table A.2:	Linearity data for clofazimine	57
Table A.3:	Linearity data obtained for decoquinate	58
Table A.4:	Limit of detection (LOD) determined for artemisone, clofazimine and decoquinate	59
Table A.5:	Accuracy of artemisone	60
Table A.6:	Accuracy of clofazimine	61
Table A.7:	Accuracy of decoquinate	62
Table A.8:	Artemisone repeatability	63
Table A.9:	Clofazimine repeatability	64
Table A.10:	Decoquinate repeatability	64
Table A.11:	Intermediate precision of artemisone	65
Table A.12:	Intermediate precision of clofazimine	66
Table A.13:	Intermediate precision of decoquinate	66
Table A.14:	Reproducibility of artemisone	67
Table A.15:	Reproducibility of clofazimine	68
Table A.16:	Reproducibility of decoquinate	68
Table A.17:	Precision of artemisone between three days	69
Table A.18:	Precision of clofazimine between three days	69
Table A.19:	Precision of decoquinate between three days	70

Table A.20:	Stability of artemisone	71
Table A.21:	Stability of clofazimine	72
Table A.22:	Stability of decoquinat	73
Table A.23:	System suitability for artemisone	74
Table A.24:	System suitability for clofazimine	75
Table A.25:	System suitability for decoquinat	75

ANNEXURE C

Liposomes, niosomes and transferosomes utilised for topical drug delivery

Table C.1:	Liposome vesicles (5%)	115
Table C.2:	Transferosome vesicles (5%)	116
Table C.3:	Niosome vesicles (5%)	116
Table C.4:	Compatibility report of different ingredients used for vesicle preparation	119
Table C.5:	Encapsulation efficiency (%EE) of the vesicles in the different dispersions	126
Table C.6:	Amount (mg) of API entrapped in the vesicles for the different dispersions with the initial amount added to dispersion in brackets	127
Table C.7:	The average zeta-potentials for the different dispersions	128
Table C.8:	Average sizes (n=3) of the niosomes with 0.2% API and 4 min sonication	129
Table C.9:	Average sizes (n=3) of the vesicles of different dispersions	131
Table C.10:	Average pH measurements (n=3) of the different dispersions at room temperature (25±1.0°C)	145
Table C.11:	The average viscosity (mPa.s, n=12) for the different dispersions at 25±1.0°C	148
Table C.12:	Dispersions prepared for efficacy against tuberculosis	149
Table C.13:	Efficacy against <i>Mycobacterium tuberculosis</i> H37Rv	150

Table C.14: Percentage inhibition of the three APIs, combination of the APIs and also when encapsulated into selected vesicles

151

TABLE OF FIGURES

CHAPTER 2 Review article published in Tuberculosis

Figure 1:	Inoculation tuberculosis in a child	9
Figure 2:	Tuberculosis verrucosa cutis	9
Figure 3:	Scrofuloderma	10
Figure 4:	Scrofuloderma in a male patient showing lymph gland involvement	10
Figure 5:	Orifacial tuberculosis	10
Figure 6:	Tuberculous gamma on the dorsum of the right foot of an eight-year old boy	10
Figure 7:	Lupus vulgaris plaque of the face, neck and chest	11
Figure 8:	Deforming, ulcerative lupus vulgaris in a caucasian male	11
Figure 9:	Cutaneous miliary TB before rupture of papules and crust formation	11
Figure 10:	Lichen scrofulosorum of the forearm and abdomen	11
Figure 11:	Papulonecrotic tuberculid	12
Figure 12:	Erythema induratum of Bazin showing prevalence in the lower extremities	12
Figure 13:	Infection with <i>Mycobacterium marinum</i> in the upper extremities	12
Figure 14:	Buruli ulcer in an eleven-year old boy from Australia	12
Figure 15:	Cervicofacial <i>Mycobacterium haemophilum</i> lymphadenitis in a child, A: presenting as a red swelling of the skin, B: after skin breakdown, and C: ulcerating open wound	13
Figure 16:	A fresh tattoo infected with <i>Mycobacterium chelonae</i>	13

Figure 17:	Lesions caused by <i>Mycobacterium abscessus</i>	13
Figure 18:	Established leprosy in order from A: tuberculoid leprosy, B: borderline leprosy, to C: lepromatous leprosy	14

CHAPTER 3

Article on the validation of the analytical method accepted for publication in DIE Pharmazie

Fig. 1:	Molecular structures of A) clofazimine, B) artemisone and C) decoquinatone.	25
Fig. 2:	Chromatographs of a standard solution containing clofazimine, artemisone and decoquinatone, respectively. The top chromatogram signifying detection obtained at 284 nm and the bottom chromatogram showing detection at 210 nm.	25
Fig. 3:	Chromatographs obtained with a solution containing typical excipients used in formulation of solid oral dosage forms, observing clofazimine, artemisone and decoquinatone, respectively. The top chromatogram signifying detection obtained at 284 nm and the bottom chromatogram showing detection at 210 nm.	26
Fig. 4:	Chromatographs of excipient solution for transdermal/topical delivery systems showing clofazimine, artemisone, and decoquinatone, respectively. The top chromatogram signifying detection obtained at 284 nm and the bottom chromatogram showing detection at 210 nm.	26

CHAPTER 4

Manuscript to be submitted to the Journal of Pharmaceutical and Biomedical Analysis on the topical delivery of artemisone, clofazimine and decoquinatone encapsulated in vesicles and their in vitro efficacy against a tuberculosis cell line

Figure 1:	Heat flow versus time graph obtained for a combination of ART, CLF and DQ	36
Figure 2:	Heat flow data obtained for ART, CLF, DQ and Tween®20	36
Figure 3:	TEM imaging illustrating: A. Liposomes, B. Niosomes and C. Transferosomes prepared with pure water	38

Figure 4:	Average concentrations of APIs present in the stratum corneum-epidermis (SCE) and epidermis-dermis (ED) after tape stripping	40
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ANNEXURE A

Analytical method validation for the concurrent determination of decoquinat, artemisone and clofazimine by means of HPLC

Figure A.1:	Chromatogram of a reference standard injected into HPLC and the retention times of the three APIs	55
Figure A.2:	Linear regression curve of artemisone	56
Figure A.3:	Linear regression curve for clofazimine	57
Figure A.4:	Linear regression curve for decoquinat	58

ANNEXURE B

Full compatibility report of clofazimine, artemisone and decoquinat with vesicle components

Figure 1:	Heat flow <i>versus</i> time graph obtained for a combination of artemisone, clofazimine and decoquinat.	80
Figure 2:	Graph depicting the heat flow data of artemisone combined with phosphatidylcholine.	81
Figure 3:	Heat flow <i>versus</i> time graph obtained for a combination of clofazimine and phosphatidylcholine.	82
Figure 4:	Heat flow data obtained for decoquinat and phosphatidylcholine.	83
Figure 5:	Heat flow data obtained for artemisone, clofazimine, decoquinat and phosphatidylcholine.	84
Figure 6:	Heat flow <i>versus</i> time graph obtained for a combination of artemisone and cholesterol.	85
Figure 7:	Heat flow <i>versus</i> time graph obtained for a combination of clofazimine and cholesterol.	86
Figure 8:	Heat flow data obtained for decoquinat and cholesterol.	86

Figure 9:	Heat flow data obtained for artemisone, decoquinatate, clofazimine and cholesterol.	87
Figure 10:	Heat flow data obtained for phosphatidylcholine and cholesterol.	88
Figure 11:	Heat flow data obtained for artemisone and Tween® 20.	88
Figure 12:	Heat flow data obtained for clofazimine and Tween® 20.	89
Figure 13:	Heat flow data obtained for decoquinatate and Tween® 20.	90
Figure 14:	Heat flow data obtained for phosphatidylcholine and Tween® 20.	90
Figure 15:	Heat flow data obtained for Tween®20 and cholesterol.	91
Figure 16:	Heat flow data obtained for decoquinatate, artemisone and Tween®20.	92
Figure 17:	Heat flow data obtained for decoquinatate, clofazimine and Tween®20.	93
Figure 18:	Heat flow data obtained for artemisone, clofazimine and Tween®20.	93
Figure 19:	Heat flow data obtained for artemisone, clofazimine, decoquinatate and Tween®20.	94
Figure 20:	Heat flow <i>versus</i> time graph obtained for a combination of artemisone, phosphatidylcholine and cholesterol.	95
Figure 21:	Heat flow <i>versus</i> time graph obtained for a combination of clofazimine, phosphatidylcholine and cholesterol.	96
Figure 22:	Heat flow <i>versus</i> time graph obtained for a combination of decoquinatate, phosphatidylcholine and cholesterol.	97
Figure 23:	Heat flow <i>versus</i> time graph obtained for a combination of decoquinatate, phosphatidylcholine and cholesterol when formulated as liposomes.	98
Figure 24:	Heat flow data obtained for transferosomes containing artemisone and phosphatidylcholine.	99
Figure 25:	Heat flow data obtained for transferosomes containing clofazimine and phosphatidylcholine.	100
Figure 26:	Heat flow data obtained for transferosomes containing decoquinatate and phosphatidylcholine.	101

Figure 27:	Heat flow data obtained for transferosomes containing artemisone, clofazimine, decoquinatate and phosphatidylcholine.	102
Figure 28:	Heat flow data obtained for niosomes containing artemisone.	103
Figure 29:	Heat flow data obtained for niosomes containing clofazimine.	104
Figure 30:	Heat flow data obtained for niosomes containing decoquinatate.	105
Figure 31:	Heat flow data obtained for niosomes containing artemisone, clofazimine and decoquinatate.	105

ANNEXURE C

Liposomes, niosomes and transferosomes utilised for topical drug delivery

Figure C.1:	Diagrammatical presentation of a unilamellar vesicle	108
Figure C.2:	Labcon® hotplate and stirrer	113
Figure C.3:	Transsonic® TS540 ultrasonicator bath	113
Figure C.4:	Hielscher® ultrasonic processor UP200St at 200 W and 26 kHz	114
Figure C.5:	Lipid film containing clofazimine in a beaker	115
Figure C.6:	DSC thermogram of the three APIs and their combination	117
Figure C.7:	Hot stage microscopy micrographs of artemisone during continuous heating	121
Figure C.8:	Hot stage microscopy micrograph of clofazimine during continuous heating	121
Figure C.9:	Hot stage microscopy micrograph of decoquinatate during continuous heating	122
Figure C.10:	Hot stage microscopy micrograph of artemisone, clofazimine and decoquinatate during continuous heating	123
Figure C.11:	TEM imaging illustrating: A. Liposomes, B. Niosomes and C. Transferosomes prepared with PBS as the aqueous phase	124
Figure C.12:	TEM imaging illustrating: A. Liposomes, B. Niosomes and C. Transferosomes prepared with pure water	124

Figure C.13:	Dispersions after centrifugation showing the formation of pellets	125
Figure C.14:	Size distribution of niosomes containing 0.2% artemisone (A), clofazimine (B), decoquinatate (C), and a combination of all three APIs (D), sonicated 4 min	130
Figure C.15:	Size distribution of liposomes with no APIs	132
Figure C.16:	Size distribution of the liposomes containing 0.2% artemisone (A), clofazimine (B), decoquinatate (C), and all three APIs (D)	133
Figure C.17:	Size distribution of the liposomes in the dispersion containing 0.4% of all three APIs	134
Figure C.18:	Size distribution of the liposomes containing 1% artemisone (A), clofazimine (B), decoquinatate (C), and all three APIs (D)	135
Figure C.19:	Size distribution of transferosomes in the blank dispersion	136
Figure C.20:	Size distribution of the transferosomes containing 0.2% artemisone (A), clofazimine (B), decoquinatate (C), and a combination of all three APIs (D)	137
Figure C.21:	Size distribution of the transferosomes in the dispersion containing 0.4% of all three APIs	138
Figure C.22:	Size distribution of the transferosomes containing 1% artemisone (A), clofazimine (B), decoquinatate (C), and a combination of all three APIs (D)	139
Figure C.23:	Size distribution of niosomes in the blank dispersion	140
Figure C.24:	Size distribution of the niosomes containing 0.2% artemisone (A), clofazimine (B), decoquinatate (C), and a combination of all three APIs (D)	141
Figure C.25:	Size distribution of the niosomes in the dispersion containing 0.4% of all three APIs	142

Figure C.26: Size distribution of the niosomes containing 1% artemisone (A), clofazimine (B), decoquinatate (C), and a combination of all three APIs (D)	143
Figure C.27: Mettler® Toledo pH meter	144
Figure C.28: A Brookfield® Viscometer used for measuring viscosity	147

ANNEXURE D

Transdermal diffusion studies of different vesicle dispersions

Figure D.1: Vertical Franz diffusion cell components and assembly	159
Figure D.2: Full thickness black skin as received from donor	160
Figure D.3: Zimmer® electric dermatome model 8821	160
Figure D.4: A Grant® JB series water bath equipped with a magnetic stirrer plate	161
Figure D.5: Average concentration of clofazimine for the liposome dispersion in the individual Franz cells	163
Figure D.6: Average concentration of decoquinatate for the liposome dispersion in the individual Franz cells	164
Figure D.7: Average concentration of clofazimine for the transferosome dispersion in the individual Franz cells	165
Figure D.8: Average concentration of decoquinatate for the transferosome dispersion in the individual Franz cells	165
Figure D.9: Average concentration of clofazimine for the niosome dispersion in the individual Franz cells	166
Figure D.10: Average concentration of decoquinatate for the niosome dispersion in the individual Franz cells	166
Figure D.11: Average concentration of clofazimine for the no vesicles dispersion in the individual Franz cells	167
Figure D.12: Average concentration of decoquinatate for the no vesicles dispersion in the individual Franz cells	167

Figure D.13: Average concentrations APIs present in the SCE and ED for the different dispersions investigated

168

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ABSTRACT

Artemisone, clofazimine and decoquinatone are part of the MALTBRex MRC South African University Flagship Projects, which focus on oxidant-redox drug combinations for the treatment of tuberculosis and a few other diseases. These active pharmaceutical ingredients (APIs) were chosen as a possible treatment of cutaneous tuberculosis (CTB), an uncommon and undefined disease that is often misdiagnosed (Abdelmalek *et al.*, 2013; Baig *et al.*, 2014; Fader *et al.*, 2010). Currently CTB is only treated with regular oral anti-tuberculous medication, with occasional invasive procedures such as skin grafts (Yates, 2010).

Artemisone, clofazimine and decoquinatone have a log P of 2.49, 7.7 and 7.8, respectively (Biamonte *et al.*, 2013; Dunay *et al.*, 2009; Nagelschmitz *et al.*, 2008; Steyn *et al.*, 2011). A high log P-value indicates that the API is highly lipophilic and therefore a delivery system, namely vesicles, was chosen to improve skin permeation. Many vesicles are currently being investigated all over the world as carriers for APIs in topical delivery, though for this study liposomes, niosomes and transferosomes were selected.

Dispersions containing a single API, a combination of all three APIs, as well as no API, were prepared for all three types of vesicles. Characterisation of dispersions containing 0.2%, 0.4% and 1% API was performed. Isothermal calorimetry indicated that no incompatibility occurred in the 1% API combination dispersions, except the niosome dispersion, which indicated a probable incompatibility. Encapsulation efficiency was above 85% for all 1% API dispersions. The empty vesicles depicted an average size of 154 nm, 167.5 nm and 106.3 nm for liposomes, niosomes and transferosomes, respectively. Vesicle sizes increased with increase in API concentration, whereas stability decreased. Clofazimine was found to have the most significant impact on vesicle size and stability when added as 1%, increasing the average niosome size to 2 461 nm. Viscosity was below 2 mPa.s for all 1% API dispersions, ensuring even spreadability when applied to the skin. The pH of all the dispersions were between 5–6, thus limiting skin irritation.

In vitro transdermal diffusion studies were conducted on black skin, using dispersions containing 1% of all three APIs. No APIs could be detected in the receptor phase. Artemisone was not detected in the skin by means of HPLC analysis, which might be due to the fact that the concentration was below the limit of detection (LOD). The LOD for artemisone was determined at 4.42 µg/ml, whereas it was 0.042 µg/ml for clofazimine and 0.703 µg/ml for decoquinatone. Higher API concentrations were present in the stratum corneum-epidermis (SCE), compared to in the epidermis-dermis (ED) for all the dispersions. Transferosomes delivered the highest

concentration clofazimine into the SCE and ED, as well as the highest concentration decoquinatate into the ED. The highest concentration decoquinatate in the SCE, however, was obtained by the niosome dispersion.

Efficacy against tuberculosis of the APIs (1%) encapsulated in vesicles was tested on strain H37Rv. All dispersions were found to be effective to some degree against the tuberculosis strain tested, with clofazimine in niosomes being the most effective with 52% growth inhibition. The least effective was decoquinatate in niosomes, with only 8% inhibition. The combination dispersions delivered inhibitions of 42%, 38% and 12% for liposomes, niosomes and transferosomes, respectively. Surprisingly, it was found that the vesicle dispersions containing no APIs also presented some efficacy against the tuberculosis strain tested.

New knowledge contributed to pharmaceuticals by this study includes encapsulating the three APIs in liposomes, niosomes and transferosomes and successfully delivering them into the skin as proved by transdermal diffusion studies. Developing an HPLC method for the concurrent analysis of the three APIs and determining the activity of the vesicle dispersion against the specific tuberculosis strain tested also contributed new knowledge. Results indicated that decoquinatate, an API never before considered for tuberculosis, does have anti-tuberculous activity. No significant increase in efficacy against the tuberculosis strain was noted when combining the three APIs in a vesicle dispersion, compared to when the APIs were incorporated separately into the vesicles, though the blank vesicles had surprisingly high activity against the specific tuberculosis strain tested.

Keywords: Clofazimine, artemisone, decoquinatate, liposomes, niosomes, transferosomes, transdermal

REFERENCES

- ABDELMALEK, R., MEBAZAA, A., BERRICHE, A., KILANI, B., OSMAN, A.B., MOKNI, M. & BENAÏSSA, H.T. 2013. Cutaneous tuberculosis in Tunisia. *Médecine et maladies infectieuses*, 43(9):374-378.
- BAIG, I.A., MOON, J.Y., KIM, M.S., KOO, B.S. & YOON, M.Y. 2014. Structural and functional significance of the highly-conserved residues in *Mycobacterium tuberculosis* acetohydroxyacid synthase. *Enzyme and microbial technology*, 58-59:52-59.
- BIAMONTE, M.A., WANNER, J. & LE ROCH, K.G. 2013. Recent advances in malaria drug discovery. *Bioorganic & medicinal chemistry letters*, 23(10):2829-2843.
- DUNAY, I.R., CHI CHAN, W., HAYNES, R.K. & SIBLEY, L.D. 2009. Artemisone and artemiside control acute and reactivated toxoplasmosis in a murine model. *Journal of antimicrobial agents and chemotherapy*, 53(10):4450-4456.
- FADER, T., PARKS, J., KHAN, N.U., MANNING, R., STOKES, S. & NASIR, N.A. 2010. Extrapulmonary tuberculosis in Kabul, Afghanistan: a hospital-based retrospective review. *International journal of infectious diseases*, 14(2):e102-e110.
- NAGELSCHMITZ, J., VOITH, B., WENSING, G., ROEMER, A., FUGMANN, B., HAYNES, R.K., KOTECKA, B.M., RIECKMANN, K.H. & EDSTEIN, M.D. 2008. First assessment in humans of the safety, tolerability, pharmacokinetics, and ex vivo pharmacodynamics antimalarial activity of the new artemisinin derivative artemisone. *Antimicrobial agents and chemotherapy*, 52(9):3085-3091.
- STEYN, J.D., WIESNER, L., DU PLESSIS, L.H., GROBLER, A.F., SMITH, P.J., CHAN, W.C., HAYNES, R.K. & KOTZÉ, A.F. 2011. Absorption of the novel artemisinin derivatives artemisone and artemiside: potential application of Pheroid™ technology. *International journal of pharmaceutics*, 414(1-2):260-266.
- YATES, V.M. 2010. Mycobacterial infections. (In Burns, T., Breathnach, S., Cox, N. & Griffiths, C., eds. *Rook's textbook of dermatology*. 8th ed. Vol 2. West Sussex, United Kingdom: Blackwell Publishing Ltd. p. 31.1-31.41.)

UITTREKSEL

Artemisoon, klofasimien en dekokwinaat is deel van die MALTBRédox MRC Suid-Afrikaanse Universiteit Flagship Projekte wat fokus op oksidasie-reduksie geneesmiddelkombinasies vir die behandeling van tuberkulose en 'n paar ander siektes. Hierdie geneesmiddels is gekies vir moontlike behandeling van kutaneuse tuberkulose (KTB), 'n ongewone en ongedefinieerde siekte wat dikwels verkeerd gediagnoseer word (Abdelmalek *et al.*, 2013; Baig *et al.*, 2014; Fader *et al.*, 2010). Tans word KTB slegs behandel met gewone orale anti-tuberkulose-medisyne, en soms met indringende prosedures soos veloorplantings (Yates, 210).

Artemisoon, klofasimien en dekokwinaat besit 'n log P van 2.49, 7.7 en 7.8, onderskeidelik (Biamonte *et al.*, 2013; Dunay *et al.*, 2009; Nagelschmitz *et al.*, 2008; Steyn *et al.*, 2011). 'n Hoë log P dui op 'n sterk lipofiliese geneesmiddel en om hierdie rede is 'n afleweringstelsel, naamlik vesikels, gekies om veldeurlaatbaarheid te verbeter. Baie vesikels word tans reg oor die wêreld ondersoek as draers van geneesmiddels vir topikale aflewering, maar vir hierdie studie is liposome, niosome en transferosome geselekteer.

Dispersies met 'n enkele geneesmiddel, 'n kombinasie van al drie geneesmiddels, sowel as geen geneesmiddel, is voorberei vir al drie tipes vesikels. Karakterisering van dispersies wat 0.2%, 0.4% en 1% geneesmiddel bevat, is uitgevoer. Isotermiese kalorimetrie-resultate het aangetoon dat geen onverenigbaarhede voorkom in die 1% geneesmiddeldispersie nie. Resultate verkry vanaf die niosoomdispersie het egter op 'n moontlikheid van onverenigbaarheid gedui. Enkapsuleringsdoeltreffendheid was bo 85% vir alle 1% geneesmiddeldispersies. Die leë vesikels het 'n gemiddelde grootte van 154 nm, 167.5 nm en 106.3 nm gehad vir liposome, niosome en transferosomes, onderskeidelik. Vesikelgrootte het toegeneem met 'n toename in geneesmiddelkonsentrasie, terwyl stabiliteit afgeneem het. Dit is gevind dat klofasimien die grootste impak gehad het op vesikelgrootte en stabiliteit wanneer dit bygevoeg is in 'n 1% konsentrasie, met 'n gemiddelde vesikelvergroting tot 2 461 nm. Viskositeit was onder 2 mPa.s vir alle 1% geneesmiddeldispersies, wat eweredige spreikbaarheid sal verseker tydens aanwending op die vel. Die pH van al die dispersies was tussen 5–6, wat vel-irritasie beperk.

In vitro transdermale-afleweringstudies is uitgevoer op swart vel, deur van dispersies gebruik te maak wat 1% van al drie geneesmiddels bevat. Geen geneesmiddel is waargeneem in die reseptorfase nie. Artemisoon kon nie in die vel opgespoor word met behulp van die HPLC-metode nie, wat moontlik verduidelik kan word deur die feit dat die konsentrasie onder die opsporingslimiet was. Die opsporingslimiet van artemisoon is bepaal as 4.42 µg/ml, terwyl dit

0.042 µg/ml vir klofasimien en 0.703 µg/ml vir dekokwinaat is. Hoër konsentrasies van die geneesmiddels was wel teenwoordig in die stratum korneum-epidermis (SKE) in vergelyking met die epidermis-dermis (ED) vir alle dispersies. Transferosome het die hoogste konsentrasie klofasimien afgelewer in die SKE en ED, sowel as die hoogste konsentrasie dekokwinaat in die ED. Die hoogste konsentrasie dekokwinaat in die SKE is egter verkry deur die niosoomdispersie.

Effektiwiteit van die geneesmiddels (1%) ingesluit in vesikels is getoets teen die spesifieke bakteriële stam van tuberkulose teen die H37RV variasie. Daar is gevind dat al die dispersies effektiwiteit toon, hoewel in 'n klein mate; met klofasimien in niosome die effektiwiefste met 52% groei-onderdrukking. Die laagste effektiwiteit teen die spesifieke tuberkulose-stam is getoon deur dekokwinaat in niosome met 8% onderdrukking. Die kombinasie-dispersies het onderdrukkings van 42%, 38% en 12% gelever vir liposome, niosome en transferosomes, onderskeidelik. Verbasend is daar gevind dat die vesikeldispersies wat geen geneesmiddels bevat het nie, ook 'n mate van effektiwiteit getoon het.

Nuwe kennis wat bydra tot Farmaseutika deur hierdie studie, sluit in die enkapsulering van die drie geneesmiddels in liposome, niosome en transferosome, asook die suksesvolle aflewering daarvan in die vel soos bepaal deur transdermale afleweringstudies. Ontwikkeling van 'n HPLC-metode vir die gesamentlike analise van die drie geneesmiddels, asook die getoetste aktiwiteit van die vesikeldispersies teen die spesifieke tuberkulose-stam, dra ook by tot nuwe kennis. Resultate het aangedui dat dekokwinaat, 'n geneesmiddel wat nooit voorheen oorweeg is teen tuberkulose nie, wel anti-tuberkulose-aktiwiteit besit. Geen merkwaardige toename in effektiwiteit teen tuberkulose is waargeneem wanneer die drie geneesmiddels gekombineer is in 'n vesikeldispersie, teenoor wanneer die geneesmiddels apart ingesluit is in die vesikels nie, alhoewel die blanko-vesikels verbasend hoë aktiwiteit teen die spesifieke tuberkulose-stam getoon het.

Sleutelwoorde: Klofasimien, artemisoon, dekokwinaat, liposome, niosome, transferosome, transdermaal

BRONNELYS

ABDELMALEK, R., MEBAZAA, A., BERRICHE, A., KILANI, B., OSMAN, A.B., MOKNI, M. & BENAÏSSA, H.T. 2013. Cutaneous tuberculosis in Tunisia. *Médecine et maladies infectieuses*, 43(9):374-378.

BAIG, I.A., MOON, J.Y., KIM, M.S., KOO, B.S. & YOON, M.Y. 2014. Structural and functional significance of the highly-conserved residues in *Mycobacterium tuberculosis* acetohydroxyacid synthase. *Enzyme and microbial technology*, 58-59:52-59.

BIAMONTE, M.A., WANNER, J. & LE ROCH, K.G. 2013. Recent advances in malaria drug discovery. *Bioorganic & medicinal chemistry letters*, 23(10):2829-2843.

DUNAY, I.R., CHI CHAN, W., HAYNES, R.K. & SIBLEY, L.D. 2009. Artemisone and artemiside control acute and reactivated toxoplasmosis in a murine model. *Journal of antimicrobial agents and chemotherapy*, 53(10):4450-4456.

FADER, T., PARKS, J., KHAN, N.U., MANNING, R., STOKES, S. & NASIR, N.A. 2010. Extrapulmonary tuberculosis in Kabul, Afghanistan: a hospital-based retrospective review. *International journal of infectious diseases*, 14(2):e102-e110.

NAGELSCHMITZ, J., VOITH, B., WENSING, G., ROEMER, A., FUGMANN, B., HAYNES, R.K., KOTECKA, B.M., RIECKMANN, K.H. & EDSTEIN, M.D. 2008. First assessment in humans of the safety, tolerability, pharmacokinetics, and ex vivo pharmacodynamics antimalarial activity of the new artemisinin derivative artemisone. *Antimicrobial agents and chemotherapy*, 52(9):3085-3091.

STEYN, J.D., WIESNER, L., DU PLESSIS, L.H., GROBLER, A.F., SMITH, P.J., CHAN, W.C., HAYNES, R.K. & KOTZÉ, A.F. 2011. Absorption of the novel artemisinin derivatives artemisone and artemiside: potential application of Pheroid™ technology. *International journal of pharmaceutics*, 414(1-2):260-266.

YATES, V.M. 2010. Mycobacterial infections. (In Burns, T., Breathnach, S., Cox, N. & Griffiths, C., eds. *Rook's textbook of dermatology*. 8th ed. Vol 2. West Sussex, United Kingdom: Blackwell Publishing Ltd. p. 31.1-31.41.)