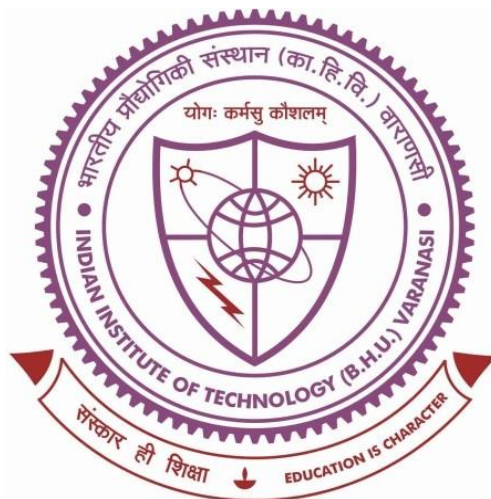


DIMETHYL FUMARATE AND ITS COCRYSTALS: POTENTIAL BIOLOGICAL APPLICATIONS



Thesis submitted in partial fulfillment for the
Award of Degree

Doctor of Philosophy

By

Qadir Alam

DEPARTMENT OF PHARMACEUTICAL ENGINEERING & TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI- 221005
INDIA

Roll No. 16161007

2023



Certificate

It is certified that the work contained in the thesis titled “**Dimethyl fumarate and its Cocrystals: Potential biological applications**” by **Mr. Qadir Alam** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy and SOTA for the award of Ph.D. Degree.

Date: 04-04-24

Place: IIT (BHU), Varanasi

Qadir Alam

Mr. Qadir Alam



DECLARATION BY THE CANDIDATE

I, **Mr. Qadir Alam**, certify that the work embodied in this Ph.D. thesis is my own bonafide work and carried out by me under the supervision of **Prof. Sairam Krishnamurthy** from **July, 2016 to July, 2023** at the **Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi**. The matter embodied in this Ph.D. thesis has not been submitted for the award of any other degree/diploma.

I declare that I have faithfully acknowledged and given credit to the research workers wherever their works have been cited in my work in this thesis. I further declare that, I have not willfully copied any other's work, paragraphs, text, data, results, etc. reported in the journals, books, magazines, reports, dissertations, theses, etc., or available at websites and have not included them in this Ph.D. thesis and have not cited as my own work.

Date:

Place: IIT (BHU), Varanasi

Qadir Alam

Mr. Qadir Alam

CERTIFICATE BY THE SUPERVISOR AND HEAD OF THE DEPARTMENT

It is certified that the above statement made by the student is correct to the best of our knowledge.

S/ 4/4/24

Prof. Sairam Krishnamurthy

(Supervisor)
DR. SAIRAM KRISHNAMURTHY
Dept. of Pharm. Engg. & Tech.
Indian Institute of Technology
(Banaras Hindu University)
Varanasi-221005 (U.P.)

S. Hemalatha

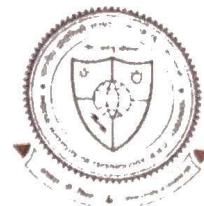
Prof. Siva Hemalatha

(Head of the Department)

विभागाध्यक्ष / Head
भैषजकीय अभियांत्रिकी एवं प्रौद्योगिकी विभाग /
Department of Pharmaceutical Engineering & Technology
भारतीय प्रौद्योगिकी संस्थान / INDIAN INSTITUTE OF TECHNOLOGY
(बनारस हिन्दू विश्वविद्यालय) / (BANARAS HINDU UNIVERSITY)
वाराणसी-221005 / Varanasi-221005

Department of Pharmaceutical Engineering & Technology

Indian Institute of Technology
(Banaras Hindu University)
Varanasi-221005



COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis: Dimethyl fumarate and its Cocrystals: Potential biological applications

Candidate's Name: Mr. Qadir Alam

Copyright Transfer

The undersigned hereby assigns to the Indian Institute of Technology (Banaras Hindu University), Varanasi all rights under copyright that may exist in and for the above thesis submitted for the award of the "*Doctor of Philosophy*".

Date:

Place: IIT (BHU), Varanasi

A handwritten signature in black ink that reads "Qadir Alam".

Mr. Qadir Alam

Note: However, the author may reproduce or authorize others to reproduce material extracted verbatim from the thesis or derivative of the thesis for author's personal use provided that the source and University's copyright notice are indicated.

ACKNOWLEDGEMENTS

Joys on successful completion are always cherishing and everlasting. It gives the feeling of completeness on looking back over the journey and remembering all those friends and family who have helped and supported me along this long but fulfilling path. I owe my gratitude to the almighty 'Allah' for blessings and for making things all right in place. **Bharat Ratna Mahamana Pandit Madan Mohan Malaviya Ji**, Founder of BHU, for providing me with such a divine platform.

Words cannot express my gratitude to my family: my Father, **Late Shri Abdul Jalil Ahmed**, who always stood by my side, My mother, **Bibi Zenab Khatoon**, and my siblings- **Mehar-un-Nisha, Mohammad Daude Alam, Hazra, Mahmood Alam, Noorjahan** and **Phooljahan**, for supporting me spiritually throughout completing this thesis and my life in general.

At this moment of accomplishment, firstly of all, I pay homage to my Ph.D. supervisor **Prof. Sairam Krishnamurthy** (Professor of Pharmacology), for giving me an opportunity to work in the field of neuropharmacology, the research area I love to work with; I am also grateful to him for grooming me not only to conduct independent research but also for acquainting me with other areas affiliated with scientific pursuits. His wide knowledge and logical way of thinking have been a great value to me. This work would not have been possible without his guidance, support and encouragement. Under his guidance, I successfully overcame many difficulties and learned a lot. His unflinching courage and conviction will always inspire me, and I hope to continue to work with his noble thoughts.

I thank my research progress evaluation committee members, **Dr. P.K. Nayak**, Department of Pharmaceutical Engineering and Technology, and **Dr. Jeyakumar Kandasamy**, Department of Chemistry, for their valuable suggestions and comments during my Ph.D. tenure.

I warmly thank **Dr. Munendra Tomar**, Banaras Hindu University, for supporting in FACS analysis. I would also like to express my thanks to BHU's Interdisciplinary School of Life Sciences for providing microtome for tissue sectioning.

I thank Head of the Department (HOD) *Prof. Siva Hemlatha* and former HOD, *Prof. B. Mishra, Prof. S.K. Singh, Prof. Sanjay Singh* and *Prof. S. K. Shrivastava* for their support.

It is my extreme privilege to gratuitously convey my special thanks to all the faculty members of the Department, *Dr. A.K. Srivastava, Dr. Senthil Raja, Dr. A.N. Sahu, Dr. S.K. Mishra, Dr. Ruchi Chawla, Dr. Ashok Kumar, Dr. M.S. Muthu, Dr. G.P. Modi, Dr. P.K. Nayak, Dr. A.K. Agrawal, Dr. S.K. Jain, Dr. Vinod Tiwari, Dr. Deepak Kumar, Dr. Rajnish, Dr. Dinesh Kumar* and *Dr. Jairam Meena* for their kind cooperation and valuable suggestions throughout the research work.

My sincere thanks to *Mr. Ram Jiyawan, Mr. Yashwant Singh, Mr. Chotelal, Mr. Virendra Kumar, Mr. Nandlal, Mr. Madanlal, Mr. Ram Hriday Pathak, Mr. Akhila Nand Upadhyay, Mr. Arun Kumar, Mr. Md. Jameel, Mr. Sunil Kumar Singh, Mr. Anand, Mr. Atul* and all other non-teaching staff of the department who had provided me all the necessary support while needed.

I am thankful to my labmates, *Dr. Dhananjay, Sukesh, Pankaj, Akanksha, Ramakrishna, Prabha, Pratigya, Shreyasi, Smriti, Neha, Gajendra, Aquib, Vishal, and Asha* for stimulating discussions, around the clock experiments, and all the fun we have had in the last five years. I would also like to express my gratitude to all those who supported me directly or indirectly for the study. I would also like to thank *Ankit Ganeshpurkar, Ravi Singh, Kaushik Neogi, Kanchan Bharti, and Rayala Swetha* for their support during my Ph.D. period.

Last but not the least my special thanks to my beloved wife *Sajda Yasmine* for bearing with my late-night thesis and manuscript preparations and also to my lucky charm daughter *Sanaa* for bringing happiness into my life.

Finally, I am highly indebted to the almighty for showering immense blessing in gifting me my beloved parents, family, and friends.

Date:

Place: IIT(BHU), Varanasi

Mr. Qadir Alam

Table of Contents

List of Figures	XII
List of Tables	XVI
List of Abbreviation	XVII
Preface	XXII
1 Introduction	2
1.1 Epidemiology	3
1.2 Causes of multiple sclerosis	4
1.3 Clinical symptoms and diagnosis of MS	5
1.4 Animal models of multiple sclerosis	6
1.4.1 Mouse models of EAE.....	7
1.4.2 Rat models of EAE.....	8
1.4.3 Clinical comparison with EAE.....	8
1.4.4 Choice of autoantigen.....	9
1.4.5 Role of adjuvants.....	10
1.5 Treatment and prevention of multiple sclerosis	11
1.6 Limitations of current treatments of multiple sclerosis.....	11
1.7 Dimethyl fumarate.....	13
1.7.1 Limitations of Dimethyl fumarate	14
1.7.2 Cathepsin C as a novel target in multiple sclerosis	15
1.8 Cocrystals	15
1.9 Rationale.....	17
1.10 Objectives.....	18
2 Immunological mechanism of dimethyl fumarate in EAE model of multiple sclerosis	20
2.1 Introduction	20

2.2	Materials and methods	23
2.2.1	Chemicals.....	23
2.2.2	<i>In-vitro</i> experiment	23
2.2.3	Time dependent/ Irreversible enzyme kinetics and calculation of IC ₅₀ :.....	24
2.2.4	Reversibility Inhibition Studies	24
2.2.5	Experimental animals and drug treatment.....	24
2.2.6	Preparation of MOG ₃₅₋₅₅ emulsion in CFA.....	25
2.2.7	Preparation of Pertussis Toxin	26
2.2.8	EAE induction.....	26
2.2.9	Histopathology to assess demyelination:	27
2.2.10	Mononuclear cells isolation and preparation:	27
2.2.11	FACS analysis.....	28
2.2.12	Cathepsin C Activity	29
2.2.13	Granzyme B activity	29
2.2.14	Statistical Analysis.....	29
2.3	Results.....	30
2.3.1	Time dependent and irreversible enzyme kinetics and calculation of IC ₅₀	30
2.3.2	Reversibility inhibition studies of DMF and MMF with cathepsin C enzyme:	33
2.3.3	Effect of DMF on clinical scoring in MOG ₃₅₋₅₅ induced EAE mice	35
2.3.4	Effect of DMF on the migration of CD4 ⁺ and CD8 ⁺ T cells migration into CNS of MOG ₃₅₋₅₅ induced EAE mice.....	36
2.3.5	Effect of DMF on cathepsin C activity in MOG ₃₅₋₅₅ induced EAE mice	39
2.3.6	Effect of DMF on Granzyme B activity in MOG ₃₅₋₅₅ induced EAE mice.....	40
2.3.7	Effect of DMF on demyelination in MOG ₃₅₋₅₅ induced EAE mice	42
2.4	Summary	43
3	Preparation, characterization, <i>in-vitro</i> and <i>in-vivo</i> pharmacokinetic evaluation of dimethyl fumarate cocrystal with basic co-former (Nicotinamide)	45
3.1	Introduction.....	45

3.2	Materials and Methods	48
3.2.1	<i>In-silico</i> work.....	48
3.2.2	Molecular docking.....	48
3.2.3	Molecular dynamics	49
3.2.4	Experimental Animals	49
3.2.5	Materials	49
3.2.6	Method of Preparation.....	50
3.2.7	Formulation	50
3.3	Characterization of cocrystals	51
3.3.1	Attenuated total reflectance FTIR (ATR- FTIR) spectroscopy	51
3.3.2	Powder X-ray Diffraction (PXRD).....	51
3.3.3	Thermal stability evaluation of cocrystals.....	51
3.3.4	Differential Scanning Calorimetry (DSC).....	52
3.3.5	Thermogravimetric Analysis (TGA)	52
3.3.6	Evaluation of Sublimation behavior	52
3.3.7	Dissolution.....	53
3.3.8	HPLC analysis	53
3.4	<i>In vitro</i> evaluation of biological activity of cocrystals	54
3.4.1	Isolation of peripheral blood mononuclear cells (PBMCs)	54
3.4.2	PBMC counting via Trypan blue exclusion (TBE) assay.....	54
3.4.3	Cytotoxicity assay	54
3.4.4	Cytokine analysis.....	55
3.4.5	Measurement of intracellular ROS (iROS)	55
3.4.6	Pharmacokinetic study.....	56
3.4.7	Acute ulcer model of rat	57
3.4.8	Acetic acid-induced chronic gastric ulcers in rats	57
3.4.9	Evaluation of gross lesions on the gastric mucosa	58

3.4.10	Determination of related biochemical indexes in gastric tissues	58
3.4.11	Histopathology	59
3.4.12	Statistical analysis	59
3.5	Results.....	59
3.5.1	Molecular docking	59
3.5.2	Molecular dynamics.....	60
3.5.3	Characterization of DMF-NIC cocrystal by attenuated total reflectance FTIR (ATR-FTIR) 61	61
3.5.4	Characterization of DMF-NIC cocrystal by TGA.....	62
3.5.5	Characterization of DMF-NIC cocrystal by DSC.....	63
3.5.6	Characterization of DMF-NIC cocrystal using PXRD	64
3.5.7	Effect of cocrystallization on sublimation behaviour of DMF	65
3.5.8	Effect of cocrystallization on dissolution profile:	66
3.5.9	Pharmacokinetics of DMF and DMF-NIC cocrystal:	67
3.5.10	Cell viability assay	69
3.5.11	Effect of DMF-NIC cocrystal on oxidative stress.....	69
3.5.12	Effect of cocrystals on IL-6 activity.....	70
3.5.13	Effect of Cocrystals on TNF- α activity.....	71
3.5.14	Acute gastric acid model.....	72
3.5.15	Acetic acid induced gastric healing.....	73
3.5.16	Acetic acid induced gastric healing by MDA activity	74
3.5.17	Total Nitrite level.....	76
3.6	Summary.....	77
4	Preparation, characterization, in-vitro and in-vivo pharmacokinetic evaluation of dimethyl fumarate cocrystals with acid-based cofomers Preparation, characterization, in-vitro and in-vivo pharmacokinetic evaluation of dimethyl fumarate cocrystals with acid-based cofomers	79
4.1	Introduction.....	80
4.2	Materials and methods	83

4.2.1	Molecular docking.....	83
4.2.2	Molecular dynamics	83
4.2.3	Experimental animals	84
4.3	Materials.....	84
4.3.1	Method of preparation	84
4.3.2	Attenuated total reflectance FTIR (ATR- FTIR) spectroscopy.....	85
4.3.3	Powder X-ray Diffraction (PXRD).....	85
4.3.4	Thermal analysis.....	85
4.3.5	Differential Scanning Calorimetry (DSC).....	86
4.3.6	Thermogravimetric Analysis (TGA)	86
4.3.7	Evaluation of Sublimation behavior	86
4.3.8	<i>In-vitro</i> dissolution study.....	87
4.3.9	Quantification of the drug in prepared cocrystals.....	87
4.3.10	Isolation and counting of peripheral blood mononuclear cells (PBMCs)	88
4.3.11	Cytotoxicity assay	88
4.3.12	Cytokine analysis.....	89
4.3.13	Measurement of intracellular ROS (i-ROS)	89
4.3.14	Pharmacokinetic study.....	90
4.3.15	Statistical analysis	91
4.4	Result and discussion	91
4.4.1	Molecular docking.....	91
4.4.2	Molecular dynamics	92
4.4.3	Formulation of cocrystals.....	94
4.4.4	Attenuated total reflectance FTIR (ATR- FTIR) spectroscopy.....	94
4.4.5	Characterization of cocrystals by TGA	96
4.4.6	Characterization of cocrystals by DSC.....	97
4.4.7	Characterization of cocrystals by PXRD.....	99

4.4.8	Effect of cocrystallization on sublimation behavior of DMF	101
4.4.9	Dissolution	102
4.4.10	Pharmacokinetics of DMF and its cocrystals.....	103
4.4.11	Cell viability assay	105
4.4.12	ROS activity: DCF Assay	106
4.4.13	Effect of cocrystals on IL-6 activity.....	107
4.4.14	Effect of cocrystals on TNF- α activity.....	109
4.5	Discussion	110
4.6	Summary	113
5	Summary & Conclusion.....	115
5.1	Important outcomes	117
5.2	Future studies	117
5.3	Impact on the treatment of multiple sclerosis	118
6	References.....	120

List of Figures

Figure 1-1 Succination of cysteine amino acid by DMF.	17
Figure 1-2 Proposed hypothesis.....	17
Figure 2-1 Experimental design.....	25
Figure 2-2 Percentage enzyme activity of cathepsin C with different concentrations of MMF	31
Figure 2-3 Kitz-Wilson Plot for MMF with cathepsin C.....	31
Figure 2-4 Percentage enzyme activity of cathepsin C with different concentrations of DMF.	32
Figure 2-5 Kitz-Wilson Plot for DMF with cathepsin C.	33
Figure 2-6 Reversibility inhibition studies of DMF with cathepsin C.....	34
Figure 2-7 Reversibility inhibition studies of MMF with cathepsin C.....	34
Figure 2-8 Clinical scoring of Control group, EAE group and EAE+DMF group.....	35
Figure 2-9 Flow cytometry data of CNS-derived immune cells isolated from MOG35– 55immunized C57BL/6 mice.....	37
Figure 2-10 % CD8+ T cells count in the CNS.	38
Figure 2-11 % CD4+ T cells count in the CNS.	39
Figure 2-12 Cathepsin C activity.	40
Figure 2-13 Granzyme B activity.	41
Figure 2-14 Demyelination in the spinal cord.	42
Figure 3-1 The proposed hypothesis.....	48
Figure 3-2 (a) 3D interaction between DMF and NIC, (b) RMSD deviation of the system, (c) RMSF deviation of the atoms of DMF and NIC (d) Number of hydrogen bonds between DMF and NIC w.r.t. time.....	61
Figure 3-3 IR spectra of DMF and NIC, DMF-NIC Physical mixture, and DMF-NIC cocrystal.	62
Figure 3-4 TGA thermogram of DMF and NIC, DMF-NIC Physical mixture, and DMF-NIC cocrystal	63
Figure 3-5 TGA thermogram of DMF, Nicotinamide their physical mixture and cocrystal respectively	64
Figure 3-6 PXRD pattern of DMF-Nicotinamide cocrystal.	65
Figure 3-7 Sublimation of DMF, its physical mixture and DMF-NIC cocrystals over 20 days.	66

Figure 3-8 Percentage cumulative drug release with time for DMF and its cocrystals.	67
Figure 3-9 Plasma concentration of MMF over 24 hr for DMF and DMF-NIC cocrystal.	68
Figure 3-10 Showing PBMC viability of in terms of % control.	69
Figure 3-11 Showing ROS activity upon LPS treatment of PBMC.	70
Figure 11 Figure 3-12 Showing IL-6 activity upon LPS assault.	71
Figure 3-13 Showing TNF- α activity on treatment with DMF and DMF-NIC after LPS induction in PBMC.	72
Figure 3-14 Showing ulcer area (cm ²) on treatment with DMF and DMF-NIC cocrystal. ...	73
<i>Figure 3-15</i> Showing ulcer index on treatment with DMF and DMF-NIC cocrystal after acetic acid induced ulcer.	74
Figure 3-16 Showing lipid peroxidation on treatment with DMF and DMF-NIC after acetic acid induced ulcer.	75
Figure 3-17 Showing ulcer area (cm ²) on treatment with DMF and DMF-NIC cocrystal.	76
Figure 4-1 Graphical outline	81
Figure 4-2 (a) 3D interaction between dimethyl fumarate and citric acid, (b) RMSD deviation of the system, (c) RMSF deviation of the atoms of dimethyl fumarate and citric acid, (d) Number of hydrogen bonds between dimethyl fumarate and citric acid w.r.t. time.	91
Figure 4-3 (a) 3D interaction between dimethyl fumarate and succinic acid, (b) RMSD deviation of the system, (c) RMSF deviation of the atoms of dimethyl fumarate and succinic acid, (d) Number of hydrogen bonds between dimethyl fumarate and succinic acid w.r.t. time.	92
Figure 4-4 Showing IR spectra of DMF, citric acid, DMF-CIT physical mixture and DMF-CIT cocrystal.	93
Figure 4-5 showing IR spectra of DMF, succinic acid, DMF-SUCC physical mixture and DMF-SUCC cocrystal.	94
Figure 4-6 TGA thermogram of DMF, citric acid, DMF-CIT mixture and DMF-CIT cocrystal.	95
Figure 4-7 TGA thermogram of DMF, succinic acid, DMF-SUCC physical mixture and DMF-SUCC cocrystal.	96
Figure 4-8 DSC thermogram of DMF, citric acid, DMF-CIT physical mixture and DMF-CIT cocrystal.	97
Figure 4-9 DSC thermogram of DMF, succinic acid, DMF-SUCC physical mixture and DMF-SUCC cocrystal.	98
Figure 4-10 PXRD pattern of DMF, citric acid, DMF-CIT physical mixture and DMF-CIT cocrystal.	99
Figure 4-11 PXRD pattern of DMF, citric acid, DMF-CIT physical mixture and DMF-CIT cocrystal.	100

Figure 4-12 Showing sublimation of DMF, DMF-CIT cocrystal, and DMF-SUCC cocrystal over 20 days.	101
Figure 4-13 Showing % cumulative drug release with time for DMF, DMF-CIT cocrystal, and DMF-SUCC cocrystal.	102
Figure 4-14 Showing plasma concentration of MMF over 24 hr after treatment of rats with DMF, DMF-CIT, and DMF-SUCC.	103
Figure 4-15 Showing cell viability assay of PBMC upon treatment with DMF, DMF-CIT cocrystal, and DMF-SUCC cocrystal.	105
Figure 4-16 showing fluorescence intensity due to i-ROS generation upon LPS induction and treatment with DMF, DMF-CIT, and DMF-SUCC in PBMC.	106
Figure 4-17 showing IL-6 activity upon LPS induction and treatment with DMF, DMF-CIT, and DMF-SUCC upon LPS induction in PBMC.	107
Figure 4-18 showing TNF- α activity on treatment with DMF, DMF-CIT, and DMF-SUCC upon LPS induction in PBMC.	108

List of Tables

Table 1 : Protective and risk factors associated with MS, adapted from [21].	5
Table 2 Limitations of current treatments of multiple sclerosis	11
Table 3 Showing pharmacokinetic parameters of DMF, DMF- NIC cocystal.....	68
Table 4 Showing pharmacokinetic parameters of DMF, DMF- CIT and DMF-SUCC cocystals.	104

List of Abbreviations

ACN	: Acetonitrile
ADP	: Adenosine diphosphate
Amber	: Assisted model building with energy refinement)
AMP	: Adenosine monophosphate
AM-BCC1	: Austin model with bond and charge correction
AMPK	: Adenosine monophosphate-activated protein kinase
ANOVA	: Analysis of variance
API	: Active pharmaceutical ingredient
ATP	: Adenosine triphosphate
ATR	: Attenuated total reflectance
AUC	: Area under the curve
BBB	: Blood-brain barrier
CAT	: Catalase
CD	: Cluster of differentiation
CIT	: Citric acid
CSD	: Cambridge structural database
CSF	: Cerebrospinal fluid
CT	: Computed tomography
CL	: Clearance
C_{max}	: Maximum plasma concentration
CNS	: Central nervous system
Cyt. C	: Cytochrome C enzyme
DCFH-DA	: Dichlorodihydrofluorescein diacetate

DMSO	: Dimethyl sulfoxide
DMF	: Dimethyl fumarate
DNA	: Deoxy-ribonucleic-acid
DPP1	: Dipeptidyl peptidase
DSC	: Differential scanning calorimetry
DTA	: Differential thermal analysis
EB	: Evans blue
EAE	: Experimental autoimmune encephalitis
EDTA	: Ethylenediamine tetra acetic acid
ELISA	: Enzyme linked immunosorbent assay
FACS	: Fluorescence assorted cell sorting
FDA	: Food and Drug Administration
FTIR	: Fourie transform infrared
GAFF2	: General AMBER force field
GAPDH	: Glyceraldehyde 3-phosphate dehydrogenase
GSH	: Glutathione
GPSCH	: Guinea pig spinal cord homogenate
GrB	: Granzyme B
HPLC	: High-performance liquid chromatography
H ₂ O ₂	: Hydrogen peroxide
IAEC	: Institutional Animal Ethical Committee
ICH	: International Council for Harmonisation
IL-6	: Interleukin-6
IL-10	: Interleukin-10
L-1 β	: Interleukin -1 β

I.V.	: Intravenous
KCl	: Potassium chloride
K_{inact}	: Inactivation rate constant
K_i	: Inhibition rate constant
K_{obs}	: Observed rate constant
LLOD	: Lower limit of detection
LLOQ	: Lower limit of quantification
LGA	: Lamarckian Genetic Algorithm
LPS	: Lipopolysaccharide
MAPK	: Mitogen activated protein kinase
MD	: Molecular dynamics
MDA	: Malonaldehyde
$MgCl_2$: Magnesium chloride
MS	: Multiple sclerosis
MMF	: Monomethyl fumarate
MRT	: Mean residence time
MTT	: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
MOG	: Myelin oligodendrocyte glycoprotein
NaCl	: Sodium chloride
$NaHCO_3$: Sodium bicarbonate
$NaHPO_4$: Sodium monohydrate phosphate
NADH	: Nicotine adenine dinucleotide phosphate
NBT	: Nitroblue tetrazolium
$NF\kappa B$: Nuclear factor- κB
NIC	: Nicotinamide

p-AMPK	: Phosphorylated-adenosine monophosphate-activated protein kinase
PBS	: Phosphate buffer saline
PBMC	: Peripheral blood mononuclear cells
PGC1- α	: Peroxisome proliferative activated receptor- γ co-activator 1- α
PDA	: Photodiode array
PK	: Pharmacokinetics
PD	: Pharmacodynamics
PGE2	: Prostaglandin E2
PDB	: Protein data bank
PLP	: Proteolipoprotein
PT	: Pertussis toxin
PXRD	: Powder X-ray diffraction
RH	: Relative humidity
ROS	: Reactive oxygen species
RMSF	: Root mean square fluctuation
RMSD	: Root mean square deviation
RRMS	: Relapsing-remitting multiple sclerosis
RSD	: Relative standard deviation
SD	: Standard deviation
SOD	: Superoxide dismutase
SUCC	: Succinic acid
TGA	: Thermogravimetric analysis
TMRM	: Tetramethyl rhodamine methyl ester
TLR	: Toll like receptor
T _{max}	: Time to reach the maximum plasma concentration
TNF- α	: Tumor necrosis factor- α

$T_{1/2}$: Half-life
UV : Ultra violet
 V_d : Volume of distribution
2SC : S-(2-succinyl) cysteine

Preface

The research work of the thesis entitled “**Dimethyl fumarate and its Cocrystals: Potential biological applications**” assessed the novel mechanism of action of dimethyl fumarate (DMF), including the effect of DMF on MOG₃₅₋₅₅ induced EAE model and enzyme kinetics of DMF/MMF on cathepsin C. Also, we have prepared cocrystals of DMF which overcome its sublimation problem and provide safety against its gastric-related adverse effects. The whole work has been compiled into six chapters: **Chapter 1** describes the introduction and significance of the present study. **Chapter 2** contains the immunological mechanism of DMF as a covalent inhibitor of cathepsin C; the novel mechanism of action has been explored in both *in-vitro* and *in-vivo*. **Chapter 3** discusses the preparation and characterisation of cocrystals to overcome their gastric-related adverse effects. **Chapter 4** includes the preparation, characterization, *in-vitro* and *in-vivo* pharmacokinetic evaluation of thermostable dimethyl fumarate cocrystals. **Chapter 5** summarizes all the experiments with their essential outcomes.