

Dedicated to.....
My Family & Friends

KEYWORDS:

Low molecular weight gelator, Supramolecular Gels, Polymorphism, Multi-stimuli response, Gel phase crystallization, Concomitant crystallization, Gel screening, Conformational flexibility, In-situ gelation.

Declaration of Academic Integrity

I do hereby declare that this thesis titled "*Understanding Supramolecular Self-assembly of Bis-Urea Functional Low Molecular Weight Gel for Pharmaceutical Crystallization*" represents my ideas in my own words and where other's ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty, integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be caused for disciplinary action as per the rules and regulations of the Institute.

Due acknowledgement to all the related data used from different sources in order to support my research findings have been made wherever necessary. All funding agencies have been duly acknowledged for providing research grants to carry out my research work smoothly.

Date: 21-03-2025

Place: Tezpur University

Himanshu Sharma

(Himanshu Sharma)

Department of Chemical Sciences

Tezpur University



तेजपुर विश्वविद्यालय / TEZPUR UNIVERSITY
(संसद के अधिनियम द्वारा स्थापित केंद्रीय विश्वविद्यालय)
(A Central University established by an Act of Parliament)

Dr. Bipul Chandra Sarma
Assistant Professor
Department of Chemical Sciences

Mobile: 91-8399936229
Phone: 03712 275066
Email: bcsarma@tezu.ernet.in

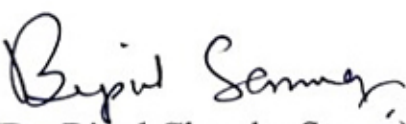
CERTIFICATE FROM SUPERVISOR

This is to certify that the thesis entitled "*Understanding Supramolecular Self-assembly of Bis-Urea Functional Low Molecular Weight Gel for Pharmaceutical Crystallization*" submitted to the School of Sciences, Tezpur University in partial fulfillment for the award of the degree of Doctor of Philosophy in Chemical Sciences is a record of research work carried out by Mr. Himanshu Sharma under my supervision and guidance at Department of Chemical Sciences, Tezpur University, Assam. He has successfully completed the work.

He has fulfilled all the requirements for submitting the thesis for award of the Degree of Doctor of Philosophy in Science. All help received by him from various sources have been duly acknowledged. No part of this thesis has been submitted elsewhere for award of any other degree.

Date: 21/03/2025

Place: Tezpur.


(Dr. Bipul Chandra Sarma)

Supervisor
School of Sciences
Department of Chemical Sciences
Tezpur University
Assam, India – 784028

ACKNOWLEDGEMENT

This journey is all about “One foot inside the boundary of the known, the other is just outside” in the world of gels. This work would not have been possible without the unwavering support and encouragement of numerous individuals. It is with great honour that I convey my profound gratitude to all those who have generously offered their assistance and steadfastly supported me throughout my doctoral journey.

I express my deepest appreciation to my esteemed supervisor, Dr. Bipul Chandra Sarma, for his invaluable suggestions, constant encouragement, and enlightening mentorship throughout my research tenure.

I remain obliged to my doctoral committee members, Prof. Ashim Jyoti Thakur and Dr. Sanjeev Pran Mahanta Sir for their valuable input and suggestions during the development of this thesis. Their thoughtful critiques and expert advice have significantly enriched the quality of my work. I take this opportunity to offer thanks to the Head, Department of Chemical Sciences, Tezpur University for providing access to the departmental facilities for my research work.

My sincere appreciation to all the technical and non-teaching staff of the department of Chemical Sciences, Food Engineering & Technology, and Sophisticated Analytical Instrumentation Centre (SAIC) Tezpur University for their constant help throughout this journey.

I extend my warm thanks to the past and present members of “Pharmaceutical Crystallization and Porous materials Laboratory” Dr. Rajiv Khatioda, Dr. Basanta Saikia, Dr. Pranita Bora, Dr. Nazima Sultana, Dr. Kasturi Sarmah, Dr. Manali Dutta, Dr. Rupa Pegu, Dr. Debabrat Pathak, Dr. Tamrat Yimenu Zeleke, Dr. Kankana Baruah, Bikash, Archita, Priya, Akankshya, Swashat and Neeha for their love and encouragement making this journey fun and beautiful.

I express my heartiest love to my friends Gunajit, Hirak Baishya, Nilutpal, Hirok Sarmah, Sanjay, Tapas, Partha (Barman), Sourav, Barasha, Nilutpol, JP, Priyanka, Suravi, Nitumani, Debanga Da, Nayab, Rituparna, Partha (Churi), Suranjana, Rashmi, Asfi, Sudhamoyee Ba, Sahtaz, Raju, Bikash, Raktim Da, Anup Da, Prantika Ba, Shubham, Veda, Niharika, Harsh Bhaiya for their good wishes, support, and motivation in all aspects of my research journey. I would like to express my gratitude towards Dr. Dhruba Jyoti Talukdar for the unconditional help in providing materials characterization.

I gratefully acknowledge DST-SERB (CRG/2019/004946), Tezpur University for the Research and Innovation Grant (RIG-2020) alongside the Institutional fellowship for providing financial assistance during my Ph.D tenure. I would also like to acknowledge INUP i2i program at CeNSE, IISc Bangalore and centre for Nanotechnology, IIT Guwahati for funding and training.

I am grateful to Prof. Parthasarathi Dastidar and his research group at IACS Kolkata for initial training in gel preparation methods and its characterization techniques. I would also like to acknowledge Dr. Akshai kumar A.S. and Prof. Pankaj Tiwari, IITG for mentorship and research collaboration.

Furthermore, I would like to extend my sincere appreciation and loving gratitude to my family for their tireless efforts in accompanying me on this arduous journey. Their constant prayers and moral support have served as a pillar of support for my professional aspirations. Their belief in my abilities has consistently given me the strength to overcome obstacles encountered during this pursuit.

Thank you everyone.

March, 2025

Tezpur University

Himanshu Sharma

ABBREVIATIONS AND SYMBOLS

%	percentage
°C	degree centigrade
μm	micrometer
Å	angstrom
π	pi
μL	microlitre
a.u.	arbitrary unit
cm	Centimeter
1D	One dimensional
3D	Three dimensional
ADA	Aminoacetaldehyde diethyl acetal
API	Active Pharmaceutical Ingredients
ASP	Aspirin
BFDH	Bravais-Fridel, Donnay–Harker
CAF	Crystallization of caffeine
CAQ	Cholesteryl anthraquinone-2-carboxylate
CBZ	Carbamazepine
CS	Clear solution
DBS	Di-benzylidene sorbitol
DEE	Diethyl ether
DMA	Dimethyl acetamide
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DSC	Differential scanning colorimetry
ETZ	Ethenzamide
FESEM	Field Emission Scanning Electron Microscopy
FG	Flexible Group

FLU	Flufenamic acid
FBRM	Focused Beam Reflectance Measurement
FT-IR	Fourier Transform Infrared spectroscopy
g	gram
G	Gel
GBH	Glycerol tertiary Butanol and Water
Gr	Grinding
HSP	Hansen solubility parameters
HC	Heat Cool
HRMS	High resolution mass spectrometry
H	hour
HCl	Hydrochloric acid
HSA	Hydroxy Stearic Acid
HH	Hyperhelical
HRXRD	High resolution X-ray diffractometer
IND	Indomethacin
IPE	Di-isopropyl ether
I	Insolubility
IG	Instant gelation
ISB	1,3-bis(2-isocyanato-2-propyl) benzene
K	Kelvin
Kcal/mol	Kilocalorie per mole
kV	Kilovolt
LC	Liquid crystalline
LVR	Linear Viscoelastic Regions
LMWG	low molecular weight gel
MFA	Mefenamic acid
MHz	Megahertz
mA	milliampere

mg	milligram
mm	millimetre
mmol	Milli mole
mW	milliwatt
MGC	Minimum Gelation Concentration
nm	nanometre
NCA	Niclosamide
NIF	Nifedipine
NMR	Nuclear Magnetic Resonance
ORTEP	Oak Ridge Thermal-Ellipsoid Plot
PVM	particle vision measurement
Pa	Pascal
pH	potential of hydrogen
PXRD	Powder X-Ray Diffraction
Rad	Radian
Rad/s	Radian per second
SEM	Scanning Electron Microscopy
SAM	Self Assembled Monolayer
Sh	Shaking
SCXRD	Single Crystal X-Ray Diffraction
SG	Slow gelation
SANS	Small Angle Neutron Scattering
SAXS	Small Angle X-Ray Scattering
S	Solubility
So	Sonication
THF	Tetrahydrofuran
TGA	Thermogravimetric analysis
TLC	Thin Layer Chromatography
TBA	Tolbutamide

TFA	Tolfenamic acid
UV-Vis	Ultraviolet-visible
w/v	Weight / Volume
WAXS	Wide angle X-Ray Scattering

LIST OF FIGURES

Figure No.	Figure Caption	Page No.
Chapter 1		
Figure 1.1	Trend of publication from 2001-2022 accessed from Web of Science with term ‘supramolecular gel’ or ‘low molecular weight organogels’ in the search field of ‘Topic’	3
Figure 1.2	A few representative applications of low molecular weight gels (LMWGs)	4
Figure 1.3	Classification of gels	5
Figure 1.4	A timeline of the significant events towards crystallization mediated in gel media	6
Figure 1.5	Dehydrobenzoannulenes (DBA) based gelators	7
Figure 1.6	Schematic representation of two different kinds of hydrogen bond networks (HBN)	7
Figure 1.7	Schematic representation of functionally modified gelator	8
Figure 1.8	Schematic representation of gel formation process	9
Figure 1.9	Hierarchy of gels across different length scales and associated characterization techniques	10
Figure 1.10	FESEM images of three dendritic gels (a), (b), and (c) and TEM images of the corresponding gels (d), (e), and (f)	11
Figure 1.11	(a) Height and (b) phase AFM images of 10% w/w of β -sitosterol: γ -oryzanol gel	12
Figure 1.12	(a) Two components (co-gel) of calixarene tosylate salt and bis(crown ether) gel, (b) host-guest mechanism of gel formation in 1,2,4 trichlorobenzene	19
Figure 1.13	Microscopic images of BAR crystals produced from (a) solution crystallization and (b) 14 gel phase crystallization in (i) 1-butanol, (ii) ethanol, (iii) nitromethane, and (iv) cyclohexanone, respectively	21
Figure 1.14	Schematic representation of the synergistic and antagonist effect of drug molecules on gelation	22
Figure 1.15	Comparison of crystals of metronidazole from solution and gel phase crystallization in nitrobenzene. (a) Solution	23

phase and (b) gel phase crystallization in 8, (c) 9, (d) 10, (e) 11, (f) 12, (g) 13, and (h) nonmemetic gelator, respectively

Figure 1.16	Face-indexed morphology of metronidazole crystals (a) grown from nitrobenzene solution, (b) wedge-shaped crystal from a nitrobenzene gel of metronidazole-mimetic gelator 10 expressing higher-order faces	24
Figure 1.17	Representative 3D plot of Hansen solubility: plotted for the various solvents where the axes represent the three Hansen solubility parameters (δD = dispersive, δP = polar, and δH = hydrogen-bonding interactions)	26
Figure 1.18	Zig-zag arrangement of gelator molecules forming a fibril: a) viewed along the b direction; b) viewed along the c direction; and c) water molecules connecting adjacent fibrils along the direction to form a 3D crystal; d) SEM images of xerogels and crystals	27
Figure 1.19	SEM image of the xerogels obtained by drying in the air the 1:2 AgNO_3 :PQ5U gel from EtOH (Left) and polymorphs crystallized directly from its gels (right)	28
Figure 1.20	Morphological evolution vs folic acid concentration of gel. SEM images, (a to e): samples with concentrations of 1 mM, 5 mM, 10 mM, 20 mM, 30 mM (v/v, 6/4) and b-1, d-1 e-1: enlarged images of (b), (d) and(e) respectively, (f): amplified images from images of 5 mM; insets: magnified images of vesicles (scale bar: 100 nm), twisted fibers and nanoparticles	29
Figure 1.21	Pictures of different stages CTX crystallization: (a) CTX solution before gelation; (b) gel formed in the crystallizer; (c) gel after the removal of nongelled solvent	31
Figure 1.22	Time scale of sol-gel (gel-crystal) transformation in Moxidectin solution	31
Figure 1.23	(a) PVM images of valnemulin hydrogen tartrate (b) Change of FBRM data during gel formation and subsequent transformation of Valnemulin hydrogen tartrate	33
Figure 1.24	Spontaneous breakdown of gel to form crystals. Small needle-like crystals initially grown within a 2% w/v I-TPI gel containing 2% w/v DTA (Left). Block-like single crystals formed after the breakdown of the same gel (Right)	34

Chapter 2

Figure 2.1	Different characterization results of G1: (a) ^1H NMR spectra, (b) ^{13}C NMR spectra, (c) HRMS, and (d) TGA thermogram	49
Figure 2.2	ORTEP diagram of G1 Microscopic images of G1 crystallized from different solvents, (a) Ethanol, (b) DMF, (c) DMSO, (d) DMSO- H_2O , (e) MeOH, (f) Ethylene glycerol, (g) Glycerol, (h) Nitromethane	50
Figure 2.3	Intermolecular interactions in G1 molecules in the crystal	50
Figure 2.4	BFDH morphology analysis of crystal of G1 Form III	52
Figure 2.5	Hirshfeld surface analysis of crystal of G1 Form III; (a) C–H, (b) N–H and (c) O–H interactions	53
Figure 2.6	Relative energy profile diagram with respect to varied dihedral angle (τ_1 & τ_2) of G1 (a) and 2D α -tape networks in crystal structure of G1 Form III (b)	53
Figure 2.7	Microscopic images of G1 crystallized from different solvents, (a) Ethanol, (b) DMF, (c) DMSO, (d) DMSO- H_2O , (e) MeOH, (f) Ethylene glycerol, (g) Glycerol, (h) Nitromethane	54
Figure 2.8	Overlay Powder X-Ray Diffraction (PXRD) patterns of the three polymorphs of G1	55
Figure 2.9	DSC endotherms and FT-IR spectra of G1 polymorphs	56
Figure 2.10	Transformation of polymorphs of G1: DSC heat-cool-heat cycle for G1 Form II (a), and III (b); IR spectra of polymorph before and after phase transformation of G1 Form II (c) and III (d)	56
Figure 2.11	Graphical representation of phase transformations among the G1 polymorphs	57
Figure 2.12	SEM images of all three polymorphs of G1 isolated by solution recrystallization	58
Figure 2.13	Graphical representation of solubility of the three polymorphs of G1 in toluene at room temperature (25°C)	58
Figure 2.14	Plausible molecular interactions between G1 molecules in two different phases: crystalline state and the gel state. Solvent entrapment during self-assembly of gelator resulted gel instead of crystal. FG in the figure denotes a flexible group (diethyl acetal) attached to the core structure	59

Figure 2.15	Pictures of the “vial inversion” test showing the gel formation in toluene: (a) G1 Form I using different stimuli, (b) all three polymorphs using heat-cool stimuli	59
Figure 2.16	Graphical representation of gel formation of all the polymorphs against four different stimuli namely heat-cool, sonication, shaking and grinding	61
Figure 2.17	DSC endotherms (a) and IR (b) of toluene xerogels prepared by different stimuli using Form I showing phase behaviour of G1 remained unaffected in the gel evolution process i.e. it remains in the G1 Form I	62
Figure 2.18	Tgel vs. Gelator concentration and (b) M.G.C vs. Stimuli graphs of gels (of G1 Form I in toluene) made from different stimuli: heat-cool (red), sonication (blue), shaking (black), and grinding (green)	63
Figure 2.19	Pictures of “vial inversion” test during measuring M.G.C. of the G1 Form I for gel preparation used all four stimuli. stimuli; (a) heat-cool, (b) sonication, (c) shaking, and (d) grinding	63
Figure 2.20	Amplitude sweep at constant frequency of 10 rad/s (a) and frequency sweep at a constant strain of 0.05 % strain within linear viscoelasticity (LVE) region (b) of G1 Form I. Represented as storage modulus (G'), loss modulus (G''), heat-cool (red), sonication (blue), shaking (black), and grinding (green)	64
Figure 2.21	FESEM images (at magnification of 15K X except shaking which is at 10K X) of xerogels of G1 Form I made from different stimuli heat-cool (HC), Sonication (So), shaking (Sh), and grinding (Gr)	65
Figure 2.22	(a) Amplitude sweep at constant frequency of 10 rad/s and (b) frequency sweep at constant strain of 0.05 % under heat-cool stimuli for all three polymorphs of G1. G' and G'' represent storage and loss modulus respectively	65
Figure 2.23	SEM images of precipitates of G1 Form II and III obtained during gel screening using different stimuli	66
Figure 2.24	Pictures showing stimuli responsiveness of G1 Form II after applying heating (Left: sonication, shaking and grinding; Right: grinding, shaking and sonication respectively)	66
Figure 2.25	Pictures showing stimuli responsiveness of G1 Form III before applying heating (No Gel; from left: sonication,	67

shaking and grinding); after applying heating (Gel formed; from right: grinding, shaking and sonication respectively)

Chapter 3

Figure 3.1	M.G.C. of G1 for diethyl ether (DEE) and di-isopropyl ether (IPE) gels for sonication and shaking as stimuli	80
Figure 3.2	Amplitude sweep (graphs a, and c) and frequency sweep (graphs b and d) graphs of DEE and IPE gels prepared by sonication and shaking as stimuli	82
Figure 3.3	FESEM images of xerogels of G1 in DEE and IPE (So: sonication, and Sh: shaking)	83
Figure 3.4	PXRD of xerogels of G1 in (a) DEE and (b) IPE So: sonication, and Sh: shaking)	84
Figure 3.5	Picture of ‘vial inversion’ test for gel screening using all three polymorphs of G1; (a) DEE and (b) IPE as solvents using sonication and shaking as stimuli	85

Chapter 4

Figure 4.1	Structure of Carbamazepine (CBZ)	91
Figure 4.2	Microscopic image of CBZ II (needle shape) and CBZ III (block shape) crystallized from toluene	93
Figure 4.3	(a) PXRD patterns and (b) DSC endotherms of CBZ polymorphs obtained using toluene	94
Figure 4.4	‘vial inversion’ test of G1 gel prepared by different stimuli containing CBZ crystals	94
Figure 4.5	Microscopic images of CBZ crystals in G1 (Form I) gels prepared by different stimuli	95
Figure 4.6	(a) PXRD patterns and (b) DSC endotherms of CBZ crystals obtained from G1 gels prepared by different stimuli	95
Figure 4.7	Amplitude sweep rheology of G1 gel prepared by different stimuli (a) with CBZ and (b) without CBZ under constant frequency of 10 Hz. G' and G'' denote storage and loss modulus. (HC, So, Gr and Sh represent stimuli heat-cool, sonication, grinding and shaking respectively)	96

Figure 4.8	Frequency sweep rheology of G1 gels prepared by different stimuli containing CBZ crystals. G' and G'' denote storage and loss modulus. HC, So, Gr and Sh represent stimuli heat-cool, sonication, grinding and shaking respectively	96
Figure 4.9	FESEM images of xerogels of G1 gels prepared by different stimuli used for CBZ crystallization	97
Figure 4.10	'vial inversion' test of G1 gels prepared from three polymorphs containing CBZ	98
Figure 4.11	(a) PXRD patterns and (b) DSC endotherms of CBZ crystals obtained from gel of G1 polymorphs	98
Figure 4.12	Amplitude sweep rheology of G1 gel prepared by three G1 polymorphs (a) with CBZ and (b) without CBZ under constant frequency of 10 Hz. G' and G'' denote storage and loss modulus. Form I, II, and III represent three polymorphs of G1	99
Figure 4.13	Frequency sweep rheology of G1 gels prepared by three G1 polymorphs containing CBZ crystals. G' and G'' denote storage and loss modulus. HC represents heat-cool stimuli	99

Chapter 5

Figure 5.1	¹ H (a) and ¹³ C (b) NMR spectra of G1 obtained from in-situ process	106
Figure 5.2	DSC endotherm of G1 (a) and PXRD pattern of G1 (b) obtained from in-situ approach	106
Figure 5.3	(a) Tgel vs. gelator concentration (wt. %), (b) FESEM image of G1 in-situ xerogel	107
Figure 5.4	Graphs of rheological experiments of G1 in-situ gel (3 wt. %); amplitude sweep (a), and frequency sweep (b). G' and G'' represent storage modulus and loss modulus	107
Figure 5.5	Pictorial representation of comparing the benefits between conventional and in-situ gel preparation process	108
Figure 5.6	Chemical structures of different drugs used in the crystallization experiments	111

Figure 5.7	Picture of “inverted-vial” test for drugs crystallized in G1 in-situ gel	111
Figure 5.8	PXRD pattern (a) and DSC (b) of NCA obtained from solution, in-situ gel (amine added first), and in-situ gel (isocyanate added first)	112
Figure 5.9	Niclosamide (NCA) crystallization in G1 in-situ gels following two different processes. From left to right: ‘vial inversion’ of NCA crystallized in G1 in-situ gel made by addition isocyanate first followed by amine; ‘vial inversion’ test of both gels made by both process and in-situ gel of G1 made by addition of amine first followed by isocyanate	112
Figure 5.10	Picture of “vial-inversion” test of in-situ gel containing TBA (a) and FLU (b)	112
Figure 5.11	Picture of “vial-inversion” test for convention gel phase crystallization (heat-cool) containing drugs (a)CBZ, NIF, TBA, ETZ and (b) NCA, FLU, TFA, MFA	113
Figure 5.12	Microscopic images of crystals extracted from solution, in-situ gel, and heat-cool gel phase crystallizations	113
Figure 5.13	SEM images of xerogels of G1 in-situ gel containing: (a) MFA and (b) NCA	115
Figure 5.14	PXRD of crystals of drug molecules obtained from (a) solution, and (b) in-situ gel	115

LIST OF SCHEMES

Scheme No.	Scheme Caption	Page No.
Chapter 2		
Scheme 2.1	Molecular structure of G1	48
Scheme 2.2	Synthesis of a bis-urea low molecular weight gelator G1	49
Scheme 2.3	Solid state polymorph screening of synthesized LMWG followed by gel screening leading to segregation of stimuli-responsive polymorphs yielding different gel performances in the prophecy of Gel-Sol transition temperature (T _{gel}), minimum gelator concentration (M.G.C.), morphology control, and allied rheological properties	67
Chapter 5		
Scheme 5.1	Pictorial representation of in-situ G1 gel preparation steps	104
Scheme 5.2	Pictorial representation of steps in crystallization of drug (here Nifedipine) in in-situ G1 gel	109
Chapter 6		
Scheme 6.1	Pictorial representation of different stages in gel phase crystallization process	119
Scheme 6.2	Important key stage in the gel making process for G1 gels	120
Scheme 6.3	Gel preparation strategy for low boiling solvents at ambient conditions	121
Scheme 6.4	Carbamazepine (CBZ) crystallization outputs from solution and gel matrix	122
Scheme 6.5	Pictorial representation of differences between conventional and in-situ gel phase crystallization. A and B represent reactants for synthesis of G1	123

LIST OF TABLES

Table No.	Table Title	Page No.
Chapter 1		
Table 1.1	Some of the LMWGs used for crystallizing drug molecules since 2010	15
Chapter 2		
Table 2.1	SCXRD Data parameter of G1	51
Table 2.2	Inter and Intra H-bonds of G1 in crystal structure	52
Table 2.3	Results of polymorph screening of G1 in solvent crystallization	57
Table 2.4	Gel screening for G1 (Form I) in different solvents with 2 % (w/v)	60
Table 2.5	Gel screening for all three polymorphs of G1 using different procedures in gelling solvents (2 weight %)	61
Chapter 3		
Table 3.1	Gel screening results of G1 (Form I) using sonication, shaking and grinding as stimuli	79
Table 3.2	Tgel values of DEE and IPE gels prepared through sonication and shaking	81
Chapter 4		
Table 4.1	Preparation techniques for different polymorphs of CBZ	92
Chapter 5		
Table 5.1	Gel screening result of in-situ preparation G1 in solvents	105