

**DEVELOPMENT AND EVALUATION OF SOME NOVEL  
FORMULATIONS FOR EFFICIENT DELIVERY OF  
CHOLECALCIFEROL**

**A  
THESIS  
SUBMITTED TO**



**MAHARAJA RANJIT SINGH  
PUNJAB TECHNICAL UNIVERSITY  
BATHINDA (PUNJAB)**

**IN FULFILLMENT OF THE REQUIREMENT  
FOR THE DEGREE OF**

**DOCTOR OF PHILOSOPHY  
IN  
PHARMACEUTICAL SCIENCES**

**By  
Neha Rawat  
Regd. No: 16211FPE04**

**Department of Pharmaceutical Sciences & Technology  
Maharaja Ranjit Singh Punjab Technical University  
Bathinda (Punjab), India  
2024**

## CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis, entitled **"Development and Evaluation of Some Novel Formulations for Efficient Delivery of Cholecalciferol"** in fulfilment of the requirements of the award of the degree of Doctor of Philosophy in Faculty of Pharmacy and submitted in Maharaja Ranjit Singh Punjab Technical University, Bathinda is an authentic record of my own work carried out during a period from 2017 to 2023 under the supervision of **Dr. Ashish Baldi**.

The matter embodied in this thesis has not been submitted by me for the award of any other degree of this or any other University/Institute.

  
(Neha Rawat)

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.



**Dr. Ashish Baldi**

(Supervisor)

Professor

Department of Pharmaceutical Sciences & Technology,  
Maharaja Ranjit Singh Punjab Technical University,  
Bathinda

The Ph.D. Viva-Voice examination of Neha Rawat, has been held on.....

Sign. of Supervisor

Sign. of External Examiner

## ACKNOWLEDGEMENT

Gathering of research work and materialization of thesis is not possible without the immeasurable help and suggestion from many people. I humbly acknowledge them who gave their support directly or indirectly in all kinds of way. I would like to show my gratitude and they are too many to thank individually.

First and foremost, I would like to praise and thank God, the Almighty, who has granted countless blessing, knowledge, and opportunity so that I have been finally able to accomplish the thesis.

I am heartily thankful to my supervisor, **Prof. (Dr.) Ashish Baldi**, Department of Pharmaceutical Sciences and Technology, Maharaja Ranjit Singh Punjab Technical University, Bathinda, for providing me this opportunity and whose encouragement, supervision and support from the preliminary to the concluding level enabled me to develop an understanding of the subject. I have sincerest sense of thankfulness for encouraging and providing freedom during the planning and execution of experiments. His critical evaluation and opinion thereafter enhanced my scientific curiosity.

I am thankful to the **Prof. (Dr.) Buta Singh Sidhu**, Vice Chancellor, Maharaja Ranjit Singh Punjab Technical University, Bathinda. I also extend my thanks to **Dr. Amit Bhatia**, Head of the Department, Department of Pharmaceutical Sciences and Technology, MRSPTU for all the administrative help and necessary facilities for the performance of this thesis work. I also thank other teaching staff of the university including Dr. Rahul Deshmukh for their constant support during my Ph.D.

I express my sincere thanks to **Dr. Shashank K Singh**, Senior Principal Scientist, CSIR-Indian Institute of Integrative Medicine, Jammu for their support and helping throughout the pursuit of this research project.

Many thanks to the non-teaching staff of the Department of Pharmaceutical Sciences and Technology, Maharaja Ranjit Singh Punjab Technical University, Bathinda, for their constant support and timely help towards completion of this project.

I place my special thanks to my friends and batch mates for their help and support.

I will do injustice if I fail to express my deep sense of gratitude and gratefulness towards my parents and in-laws. Special thanks to my parents, my brother and my sisters who are always an inspiration, supported me, encouraged me, and always stood next to me in the tough times.

Thank you, a lot, for your untiring love, care, support, and sacrifice. I fall short of words to express my feelings for all their deeds and concern.

I take this opportunity to express my heartfelt acknowledge to my husband and my kids (Ani and Aaru) for their love, affection, and cooperation during this period of my life. I would like to thank my whole family for the blessings they bestowed on me. Even if inadvertently some names have been left out, I can only say that their contributions will not go in vain, as this work of research is meant to benefit humankind in some form or the other.

Neha Rawat

## LIST OF TABLES

<b>Table No.</b>	<b>Title of Table</b>	<b>Page No.</b>
1.1	Concentration of 25(OH)D in blood with associated health impact	3
1.2	Dietary reference intakes for vitamin D and calcium	3
1.3	Study characteristics of ergocalciferol and cholecalciferol	8
1.4	Vitamin D analogs and their physicochemical properties	10
1.5	Solid dispersion based marketed products	16
1.6	Types of carriers used for solid dispersion.	21
4.1	Physicochemical parameter and other aspect of cholecalciferol	56
4.2	Properties of hydroxypropylmethylcellulose acetate succinate	57
4.3	Properties of polyvinylpyrrolidone	58
4.4	Approximate molecular weight of various PVP grades	59
4.5	Properties of poloxamer	60
4.6	Properties of Cremophor EL	61
4.7	Properties of Oleic acid	62
4.8	Properties of PEG 400	63
5.1	List of chemicals used in the experiments	64
5.2	List of equipment used in the experiments	65

5.3	Composition of cholecalciferol solid dispersions (CCF-SD-HPMCAS, CCF-SD-PVP)	69
5.4	Formulation composition of solid dispersion formulation for DRHCap-SD	74
5.5	Various composition for the selection of best micro-emulsion region	81
5.6	Composition of selected SEDDS formulations	81
6.1	Partition coefficient of cholecalciferol in n-octanol and PBS/water	87
6.2	Characterization of solid dispersion formulation for DRHCap-SD	109
6.3	Evaluation of flow properties of solid dispersion and physical mixture	119
6.4	Solubility of cholecalciferol in various excipients	122
6.5	Various composition of SEDDS for pseudo-ternary phase diagram	123
6.6	Grading system for assessing emulsification of formulation	125
6.7	Evaluation of self-emulsification	125

## LIST OF FIGURES

Figure No.	Legend	Page No.
1.1	Cardiovascular events on vitamin D deficiency	2
1.2	Risk factors associated with vitamin D deficiency	5
1.3	Structure of vitamin D analogues	11
1.4	Formulation approaches for vitamin D	13
1.5	Classification of solid dispersion	17
1.6	Mechanism of drug release in solid dispersion	23
1.7	Various methods of preparation of solid dispersion	25
1.8	The oral administration of lipid based formulations interact with gastric lipase which initiates its digestion. Simultaneously, propulsion, grinding and retropulsion in stomach facilitate crude emulsion formation while in small intestine pancreatic lipase with its cofactor digests the lipids. The products of lipolysis usually in the form of mixed micelles transports into the epithelial cells where chylomicrons and very low density lipoprotein (VLDL) furthers enhances its absorption while some of drug molecules usually precipitated out after lipid digestion (Kalepu et al., 2013). Vitamin D <sub>3</sub> in circulation bound to its binding proteins and transport to the liver, the site where it gets converted to calcifediol (25-hydroxycholecalciferol). Finally, calcifediol when reaches to the kidney with the help of hydroxylase produces an active form known as calcitriol. Calcitriol in blood circulation effect small intestine for increased absorption of calcium and phosphate.	35
4.1	Molecular structure of cholecalciferol	56
4.2	Molecular structure of hydroxypropylmethylcellulose acetate succinate	57
4.3	Molecular structure of polyvinylpyrrolidone	58

4.4	Molecular structure of poloxamer	60
4.5	Molecular structure of Oleic acid	62
4.6	Molecular structure of oleic acid	63
6.1	Standard curve of cholecalciferol in PBS (pH 7.4) at 265 nm	88
6.2	Standard curve of cholecalciferol in ethanol at 265 nm.	88
6.3	HPLC chromatogram of cholecalciferol in mobile phase comprising acetonitrile and water (99:1 v/v) at 265 nm.	89
6.4	IR spectroscopy of Cholecalciferol at scanning range was of 4000-550 $\text{cm}^{-1}$ using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.	92
6.5	IR spectroscopy of poloxamer at scanning range was of 4000-550 $\text{cm}^{-1}$ using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.	92
6.6	IR spectroscopy of PVP K-30 at scanning range was of 4000-550 $\text{cm}^{-1}$ using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.	93
6.7	IR spectroscopy of HPMCA's at scanning range was of 4000-550 $\text{cm}^{-1}$ using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.	93
6.8	IR spectroscopy of Physical mixture of PVP, poloxamer, cholecalciferol and HPMCA's at scanning range was of 4000-550 $\text{cm}^{-1}$ using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.	94
6.9	IR spectroscopy of solid dispersion formulation CCF-SD-PVP at scanning range was of 4000-550 $\text{cm}^{-1}$ using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.	94



6.10	IR spectroscopy of solid dispersion formulation CCF-SD-HPMCAS at scanning range was of 4000-550 cm <sup>-1</sup> using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.	95
6.11	IR spectroscopy of solid dispersion formulation, excipients and physical mixture at scanning range was of 4000-550cm <sup>-1</sup> : (A) Cholecalciferol, (B) Poloxamer, (C) PVP-K30, (D) HPMCAS, (E) Physical mixture of PVP, poloxamer, cholecalciferol and HPMCAS, (F) CCF-SD-PVP, (G) CCF-SD-HPMCAS.	95
6.12	Surface morphology of cholecalciferol using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10 kV.	96
6.13	Surface morphology of PVP K-30 using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10 kV.	97
6.14	Surface morphology of poloxamer using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10 kV.	97
6.15	Surface morphology of HPMCAS using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10kV.	98
6.16	Surface morphology of CCF-SD-PVP using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10kV.	98
6.17	Surface morphology of CCF-SD-PVP using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a	99

	brass stub and was sputter coated and image was captured at 10 kV.	
6.18	Differential scanning calorimetry analysis of (A) Cholecalciferol and (B) Solid dispersion formulation (CCF-SD-HPMCAS) at a scanning rate of 10°C min <sup>-1</sup> from 20 to 220°C.	100
6.19	X-ray diffraction analysis of powder samples of (A) Cholecalciferol and (B) Solid dispersion formulation (CCF-SD-HPMCAS) at scanning range from 5° to 50° (diffraction angle 2θ).	101
6.20	Evaluation of compatibility of the formulation with the mimics of intestinal cells. Various concentration of the cholecalciferol formulations (CCF-SD-HPMCAS and CCF-SD-PVP) were incubated with caco-2 cells and the viability of caco-2 cells was assessed by using MTT assay.	103
6.21	Dissolution profile of cholecalciferol (CCF), solid dispersion formulations (CCF-SD-PVP and CCF-SD-HPMCAS), and physical mixture (CCF-PM) in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Dissolution study was conducted for 90 min in SIF and 240 min in SIF using dissolution apparatus with paddle operated at 100 rpm and dissolution medium temperature was kept at 37±0.5°C.	104
6.22	The concentration time profile of cholecalciferol (CCF), PVP based solid dispersion (CCF-PVP-SD) and enteric solid dispersion CCF-SD-HPMCAS following oral administration in Wistar rats.	105
6.23	Evaluation of change in cholecalciferol content of solid dispersion-based formulation (CCF-SD-HPMCAS) after three months of storage at different conditions (4°C, 25°C/60%RH and 30°C/65%RH).	107
6.24	Evaluation of change in cholecalciferol content of solid dispersion-based formulation (CCF-SD-PVP) after three months of storage at different conditions (4°C, 25°C/60%RH and 30°C/65%RH).	107

6.25	IR spectroscopy of cholecalciferol at scanning range of 4000 - 400cm <sup>-1</sup> .	110
6.26	IR spectroscopy of solid dispersion formulation at scanning range of 4000-400 cm <sup>-1</sup> .	111
6.27	Scanning electron microscopic images of Cholecalciferol at accelerating voltage at 10kV.	111
6.28	Scanning electron microscopic images of PVP-K30 at accelerating voltage at 15kV.	112
6.29	Scanning electron microscopic images of solid dispersion formulation (CCF-SD-PVP) at accelerating voltage at 15kV.	112
6.30	Differential scanning calorimetry analysis of cholecalciferol at a scanning rate of 10°C min <sup>-1</sup> from 20 to 220°C.	113
6.31	Differential scanning calorimetry analysis of PVP K-30 at a scanning rate of 10°C min <sup>-1</sup> from 20 to 220°C.	114
6.32	Differential scanning calorimetry analysis of solid dispersion formulation (CCF-SD-PVP) at a scanning rate of 10°C min <sup>-1</sup> from 20 to 220°C.	114
6.33	X-ray diffraction analysis of cholecalciferol at scanning range from 5° to 80° (diffraction angle 2θ).	115
6.34	X-ray diffraction analysis of solid dispersion formulation (CCF-SD-PVP) at scanning range from 5° to 80° (diffraction angle 2θ).	116
6.35	Evaluation of compatibility of the formulation with caco-2 cells. Various concentration of the cholecalciferol solid dispersion-based formulations was incubated with caco-2 cells and the viability of caco-2 cells was assessed by using MTT assay.	117
6.36	Dissolution profile of cholecalciferol (CCF), physical mixture (PM), solid dispersion (SD), and solid dispersion in delayed release HPMC capsule (DRHCap-SD) in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Dissolution study was conducted for 90 min in SGF and 240 min in SIF using dissolution apparatus with paddle operated at 100 rpm	119

	and the dissolution medium temperature was kept at $37\pm0.5^{\circ}\text{C}$ .	
6.37	Evaluation of change in cholecalciferol content of solid dispersion-based formulation (DRHCap-SD) after three months of storage at different conditions ( $4^{\circ}\text{C}$ , $25^{\circ}\text{C}/60\% \text{ RH}$ and $30^{\circ}\text{C}/65\% \text{ RH}$ ).	121
6.37	Evaluation of change in cholecalciferol content of solid dispersion-based formulation (SD) after three months of storage at different conditions ( $4^{\circ}\text{C}$ , $25^{\circ}\text{C}/60\% \text{ RH}$ and $30^{\circ}\text{C}/65\% \text{ RH}$ ).	121
6.39	Pseudo-ternary phase diagram comprising oleic acid, Cremophor and polyethylene glycol	124
6.40	Photomicrograph of self-emulsifying formulation of cholecalciferol (SF-2) following dispersion in aqueous media.	126
6.41	Size distribution of self-emulsifying formulation of cholecalciferol (SF-2) following dispersion in aqueous media using dynamic light scattering technique.	127
6.42	Zeta potential of self-emulsifying formulation of cholecalciferol (SF-2) following dispersion in aqueous media.	127
6.43	Representative photomicrograph of SEDDS formulation (SF-2) in simulated gastric fluid (pH 1.2) at 100 times dilution (40X).	128
6.44	Representative photomicrograph of SEDDS formulation (SF-2) in simulated intestinal fluid (pH 6.8) at 100 times dilution (40X).	128
6.45	Dissolution profile of cholecalciferol SEDDS formulation and crystalline cholecalciferol. The study was performed utilizing a USP II paddle type dissolution apparatus (LABINDIA DS 8000). ( $P<0.001$ ; CCF-SEDDS Vs CCF).	129
6.46	Evaluation of biocompatibility of SEDDS with Caco-2 cells by using MTT assay. Caco-2 cells were seeded separately in 96-well flat bottom tissue culture plates at a density of $2\times10^4$ cells/well and allowed to grow in monolayer. Once the cells formed monolayer, treatment was given in 96-well plate with	132

	100µL of SEDDS formulation diluted in HBSS solution and kept for 2h incubation. After 2 h treatment, 20µL of MTT solution (2.5 mg/mL) was added in each well and the cells were again incubated for another 4 h to form formazan crystals. The formazon crystals were dissolved by adding 150µL of DMSO using an orbital shaker and absorbance was recorded at 570 nm using a microplate reader (TECAN Infinite Pro) and percent viability was calculated.	
6.47	Stability studies of cholecalciferol SEDDS. The formulations were placed in sealed containers and stability study was performed by storing the samples at 4°C (refrigerator), 25°C (60% RH), and 30°C (65% RH) for three months. The samples were removed from stability chamber after every month and analyzed for drug content.	133