“SOLUBILITY ENHANCEMENT, SYSTEM DESIGN FOR TRANSBUCCAL DELIVERY, *IN-VITRO* CHARACTERIZATION AND *IN-VIVO* EVALUATION OF LOVASTATIN”

Thesis submitted to

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH (BELAGAVI)

(Deemed-to-be-University)

[Declared as Deemed-to-be-University u/s3 oftheUGC Act,1956videGovt.of IndiaNotificationNo.F.9-19/2000-U.3(A)]

Accredited‘A’Grade by NAAC (2nd Cycle)

Placed in Category ‘A’ by MHRD (GoI)

For the award of the degree of

Doctor of Philosophy
In the Faculty of Pharmacy

By

Bhuvaneshwari R. Sharannavar M. Pharm.

(Registration No: KLEU/Ph.D./10-11/DOUN10014)

Under the Guidance of

Prof. (Dr.) Sunil Satyappa Jalalpure Ph.D
Principal, KLE College of Pharmacy, Belagavi,
KLE academy of Higher Education and Research, Belagavi-590010,
Karnataka, India

2020

UNDERTAKING

I, Mrs. **Bhuvaneshwari R. Sharannavar** hereby declare that the information and the data mentioned in my thesis entitled **"Solubility Enhancement, System design for transbuccal delivery, *in-vitro* characterization and *in-vivo* evaluation of Lovastatin"** belongs to me and is original.

I am aware of definition of plagiarism as detailed below:

- An act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another's words, thoughts or ideas as one's own without attribution in connection with submission of academic work, whether graded or otherwise.

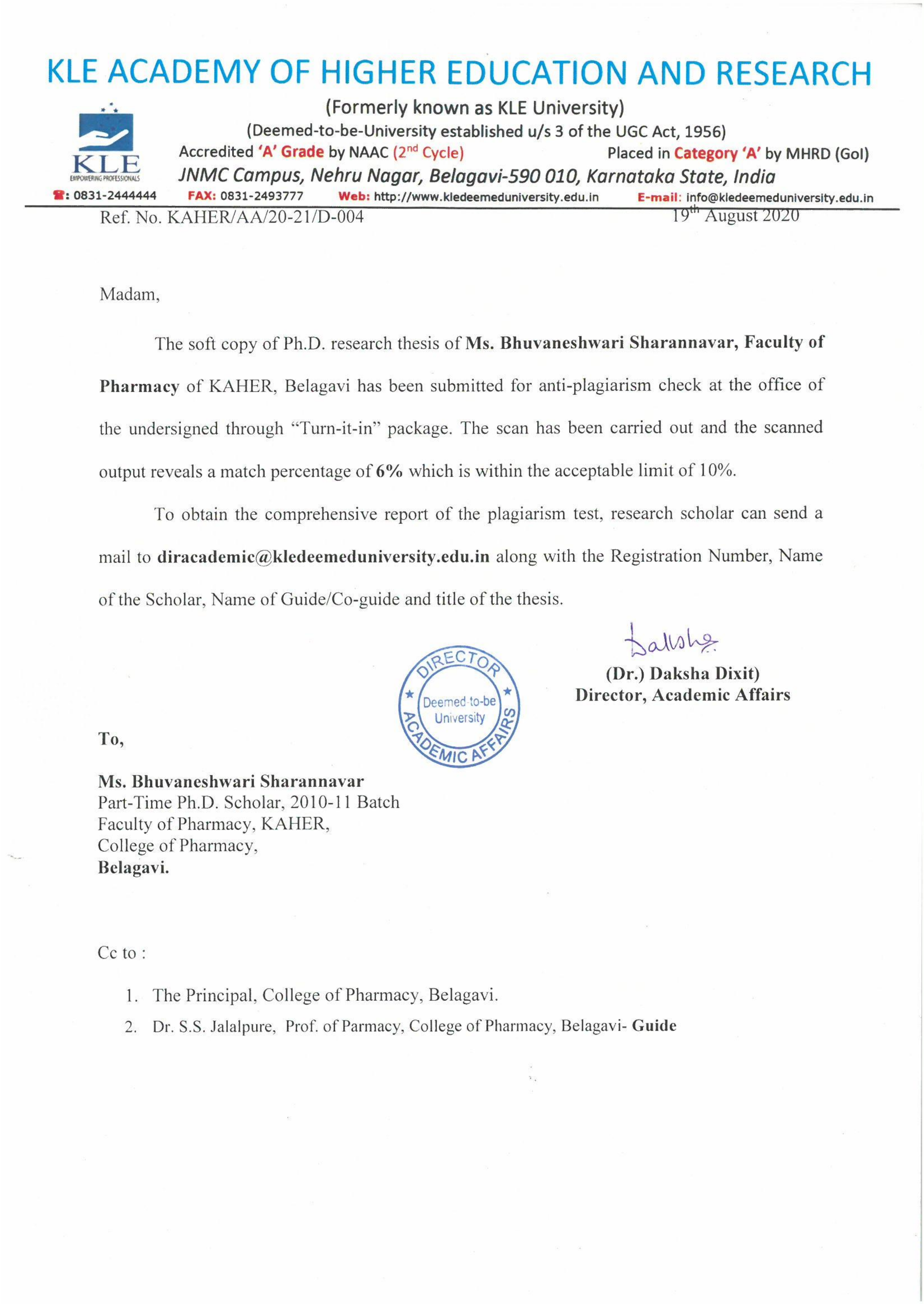
I hereby declare that the thesis prepared by me is original-one and does not involve plagiarism anywhere. In case at a later stage it is found that I have indulged in plagiarism, then I am solely responsible for the same and the Institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the University.

Mrs. Bhuvaneshwari R. Sharannavar
Part time Ph.D Research Scholar
Reg.No: DOUN10014
KAHER, Belagavi-590010.

Date:

Place: Belagavi

PLAGIARISM REPORT



KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Formerly known as KLE University)
(Deemed-to-be-University established u/s 3 of the UGC Act, 1956)
Accredited **'A' Grade** by NAAC (2nd Cycle) Placed in **Category 'A'** by MHRD (GoI)
JNMC Campus, Nehru Nagar, Belagavi-590 010, Karnataka State, India
☎: 0831-2444444 **FAX:** 0831-2493777 **Web:** http://www.kledeemeduniversity.edu.in **E-mail:** info@kledeemeduniversity.edu.in

Ref. No. KAHER/AA/20-21/D-004 19th August 2020

Madam,

The soft copy of Ph.D. research thesis of **Ms. Bhuvaneshwari Sharannavar, Faculty of Pharmacy** of KAHER, Belagavi has been submitted for anti-plagiarism check at the office of the undersigned through "Turn-it-in" package. The scan has been carried out and the scanned output reveals a match percentage of **6%** which is within the acceptable limit of 10%.

To obtain the comprehensive report of the plagiarism test, research scholar can send a mail to **diracademic@kledeemeduniversity.edu.in** along with the Registration Number, Name of the Scholar, Name of Guide/Co-guide and title of the thesis.

(Dr.) Daksha Dixit)
Director, Academic Affairs

To,

Ms. Bhuvaneshwari Sharannavar
Part-Time Ph.D. Scholar, 2010-11 Batch
Faculty of Pharmacy, KAHER,
College of Pharmacy,
Belagavi.

Cc to :

1. The Principal, College of Pharmacy, Belagavi.
2. Dr. S.S. Jalalpure, Prof. of Parmacy, College of Pharmacy, Belagavi- **Guide**

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

(Deemed-to-be-University)

[Declared as Deemed-to-be-University u/s3 of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]

Accredited 'A' Grade by NAAC (2ndCycle)

Placed in Category 'A' by MHRD (GoI)

Copyright Declaration

We hereby declare that **KLE ACADEMY OF HIGHER EDUCATIONAND RESEARCH, BELAGAVI, KARNATAKA,** *shall have the rights to preserve, use and disseminate this thesis in print or electronic format for academic/research purpose.*

Signature	**Signature Guide**
Mrs. Bhuvaneshwari R. Sharannavar	**Prof. (Dr.) Sunil Jalalpure**
Part time Ph.D Research Scholar Reg.No: DOUN10014 KAHER, Belagavi-590010.	Principal, KLE College of Pharmacy, KAHER, Belagavi-590010.
Place: Belagavi	**Place:** Belagavi
Date:	**Date:**

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

(Deemed-to-be-University)

[Declared as Deemed-to-be-University u/s3 of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]

Accredited ‘A’ Grade by NAAC (2nd Cycle)

Placed in Category ‘A’ by MHRD (GoI)

Declaration

I hereby declare that the thesis entitled **“Solubility Enhancement, System design for transbuccal delivery, *in-vitro* characterization and *in-vivo* evaluation of Lovastatin”** *is a bonafide and original research carried out by me under the guidance of* **Dr. Sunil Satyappa Jalalpure**, *Deputy Director, Dr. Prabhakar Kore Basic Science Research Center, Principal, KLE College of Pharmacy, Belagavi- 590010. The thesis or any part thereof has not formed the basis for the award of any degree/fellowship or similar title to any candidate of any University.*

Place: Belagavi

Date:

Signature

Mrs. Bhuvaneshwari R. Sharannavar
Part time Ph.D Research Scholar
Reg.No: DOUN10014
KAHER, Belagavi-590010.

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

(Deemed-to-be-University)

[Declared as Deemed-to-be-University u/s3 of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]

Accredited 'A' Grade by NAAC (2nd Cycle)

Placed in Category 'A' by MHRD (GoI)

Certificate

This is to certify that the thesis entitled **"Solubility Enhancement, System design for transbuccal delivery, *in-vitro* characterization and *in-vivo* evaluation of Lovastatin"** *is a bonafide record of original research carried out by* **Mrs. Bhuvaneshwari R. Sharannavar** *under the guidance of* **Prof. (Dr.) Sunil Satyappa Jalalpure,** *Deputy Director, Dr. Prabhakar Kore Basic Science Research Center, Principal, KLE College of Pharmacy, Belagavi-590010.*

Place: Belagavi

Date:

Signature

Prof. (Dr.) M. S. Ganachari
Dean, Faculty of Pharmacy
KAHER, Belagavi – 590010.

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

(Deemed-to-be-University)

[Declared as Deemed-to-be-University u/s3 of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]

Accredited ‘A’ Grade by NAAC (2nd Cycle)

Placed in Category ‘A’ by MHRD (GoI)

Certificate

This is to certify that the thesis entitled **“Solubility Enhancement, System design for transbuccal delivery, *in-vitro* characterization and *in-vivo* evaluation of Lovastatin”** *is a bonafide record of original research carried out by* **Mrs. Bhuvaneshwari R. Sharannavar** *under the guidance of* **Prof. (Dr.) Sunil Satyappa Jalalpure,** *Deputy Director, Dr. Prabhakar Kore Basic Science Research Center, Principal, KLE College of Pharmacy, Belagavi-590010.*

Place: Belagavi

Date:

Signature
Prof. (Dr.) Sunil Jalalpure
Principal,
KLE College of Pharmacy
KAHER, Belagavi - 590010

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

(Deemed-to-be-University)

[Declared as Deemed-to-be-University u/s3 of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]

Accredited 'A' Grade by NAAC (2ndCycle)

Placed in Category 'A' by MHRD (GoI)

Certificate

This is to certify that the thesis entitled **"Solubility Enhancement, System design for transbuccal delivery, *in-vitro* characterization and *in-vivo* evaluation of Lovastatin"** *is a bonafide record of original research carried out by* **Mrs. Bhuvaneshwari R. Sharannavar** *for the award of degree of DOCTOR OF PHILOSOPHY IN FACULTY OF PHARMACY under my supervision and guidance.*

Place: Belagavi
Date:

Signature Guide
Prof. (Dr.) Sunil Jalalpure
Principal, KLE College of Pharmacy,
Belagavi, KAHER, Belagavi-590010.

ACKNOWLEDGEMENT

The completion of this dissertation is not only the fulfillment of my dreams but also the dream of my parents and family members. I take this opportunity to express my gratefulness to each and every person who has been instrumental throughout the completion of my doctoral studies.

I would like to express my deepest sense of gratitude and appreciation to my esteemed research guide Prof. Dr. Sunil S Jalalpure; Principal KLECOP Belagavi, for his valuable guidance, constant inspiration, encouragement and persistent help throughout my dissertation work. I thank him for all his advice and for always guiding me in the right direction.

I would like to thank my previous guide Dr. A P Gadad, for his guidance and help throughout my dissertation work.

I sincerely thank Vice-chancellor Dr. Vivek Saoji. My special thanks to Deputy Registrar Prof. Dr. M S Ganachari; Dean, Faculty of Pharmacy and former Director of Academic affairs Dr. Daksha Dixit. I am highly grateful to Prof. Dr. B M Patil, for extending his help and kind co-operation.

I sincerely thank Vice Principal Prof. Dr. M B Patil, for his help and support. I am highly grateful to all HODS, Teaching and nonteaching staff of KLECOP Belagavi for their endless support during the course of my study.

I profusely thank Biocon Limited, Bangalore Karnataka for providing me gift sample of Lovastatin. I owe my special thanks to Sourashtra University Rajkot Gujarat and Shivaji University Kolhapur, Maharashtra for solid state characterization studies.

I express my immense gratitude and love to my greatest source of love and inspiration, my parents Mr. R I Sharannavar, Mrs. Shakuntala Sharannavar and my brothers Vishwanath and Satish for their endless encouragement throughout all these years.

I am ever thankful to my husband Mr. Rajshekhar Lokapur and my loving son master Advai Lokapur for their continual support, patience, and great help in everything I do.

Most importantly, I thank God for continually blessing me, and giving me the strength and wisdom in fulfilling all my endeavors.

Bhuvaneshwari Sharannavar

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

API: Active Pharmaceutical ingredient

BCS: Biopharmaceutical classification system

BBB: Blood brain barrier

BP: Boiling point

CS: Chitosan

CVD: Cardiovascular disease

DC: Drug content

DSC: Differential scanning calorimeter

FPM: First pass metabolism

FTIR: Fourier-transform infrared

GIT: Gastrointestinal tract

HDL: High density lipoproteins

HMG CoA: Hydroxy methyl glutaryl CoA

HPC: Hydroxy propyl cellulose

HPMC: Hidroxy propyl methyl cellulose

LDL: Low density lipoproteins

LD 50: Median lethal dose

LVS: Lovastain

MDT: Mean dissolution time

MP: melting point

PEG 4000: Polyethylene Glycol 4000

PMs: Physical mixtures

PVA: Polyvinyl alcohol

PPAR: Peroxisome proliferator-activated receptors

PVP K30: Polyvinyl pyrrolidone K30

PXRD: Powder X-Ray Diffraction

QbD: Quality by Design

RH: Relative humidity

RPM: Revolution per minute

SD: Spray drying

SE: Solvent evaporation

SEM: Scanning electron microscopy

SLS: Sodium lauryl sulphate

SMEDDS: Self micro emulsifying drug delivery systems

TEM: Transmission electron microscopy

TGA: Thermo gravimetric analysis

UV; Ultra-violet visible

VLDL: Very low density lipoproteins

XRD: X-ray diffraction

LIST OF TABLES

LIST OF FIGURES

Abstract:

Background: Lovastatin (LVS); HMG CoA inhibitor, is a drug of choice for hyperlipidemia. Its clinical efficacy is limited due to its low aqueous solubility, incomplete absorption, short-half life, first pass metabolism, low bioavailability, and adverse effects etc.

Objective: The objective of the present study was to improve the solubility of Lovastatin and develop mucoadhesive film of solid dispersions for buccal delivery to enhance bioavailability.

Methodology: Solid dispersions of Lovastatin were prepared by using two different polymers PEG 4000(solvent evaporation method) and PVPK30 (spray drying method). The best formulations were further subjected to FTIR, SEM, PXRD and DSC analysis. Further *in-vivo* lipid profile studies and stability studies were also carried out. Transbuccal drug delivery system of processed solid dispersions (SD4) were formulated by solvent casting technique using three different polymers such as HPMC K4M, HPMC E5LV and chitosan. The successful patches were evaluated.

Results: The aqueous solubility of Lovastatin was favored by the presence of polymers PEG 6000 and PVPK30. In contrast to the very slow dissolution rate of pure Lovastatin, solid dispersions considerably improved the dissolution rate. This can be attributed to the increased wettability and dispersibility of Lovastatin in polymeric matrix and increase in amorphicity which was confirmed by PXRD, DSC and SEM. IR studies also revealed that there is hydrogen bonding formation between Lovastatin and PVPK30. The formulation SD4 was considered as optimum formulation. Lipid profile study revealed that SD4 shows better therapeutic efficacy than pure Lovastatin. Stability study confirmed that there is no recrystallinity of drug. The thickness of all prepared patches ranged from 0.21±0.07 to 1.5± 0.39 mm, weight of the film 89.10±0.6 to 128.57±0.3 mg, drug content 85.47±0.87 to

97.33±0.31%, surface pH 5.6±0.67 to 7.6±0.98, swelling index 23.0±4.1 to 76.5±3.6%, folding endurance 165±1.9 to 350±2.5 respectively. *Ex-vivo* residence time ranged from 2.2± 0.08 to 8.2±0.17 hours and *ex-vivo* bioadhesive strength 30±0.64 to 66±0.43 gms. The percentage drug release and *ex-vivo* drug permeation was in the following descending order HPMC K4M> HPMC E5LV>chitosan. The kinetics data shows that drug release and permeation follows non-Fickian diffusion. Based on results of evaluation parameters, HPMCK4M formulations (F1-F6) were selected as the best formulations. The formulation F3 was selected for further stability and results indicate that there is no significant change in drug content, drug release and *ex-vivo* permeation.

Conclusion: In conclusion, the present study clearly demonstrated that overall therapeutic efficacy of Lovastain can be improved by enhancing solubility and bioavailability.

Keywords: Lovastatin, PEG 4000, PVPK30, Solid dispersion, HPMCK4M, HPMC E5LV, chitosan, *Ex-vivo* bioadhesion, *ex-vivo* permeation, bioavailability.

1. INTRODUCTION

1.1. BACKGROUND OF RESEARCH:

Cardiovascular disease (CVD) is the set of disorders of heart and blood vessels which includes hypertension, coronary cardiac disease, cerebro vascular disease, peripheral vascular disease, cardiac failure, rheumatic heart disease, and cardiomyopathies. By 2030, almost 23.6 million people will die due to CVDs mainly from stroke and heart diseases [1]. Death rates among men and women have been found to be inversely related to cardio respiratory fitness level, even in the presence of other predictors of cardiovascular mortality such as smoking, hypertension, and hyperlipidemia[2]. Hyperlipidemia is a major modifiable risk factor for cardiac disease and interventions to lower plasma cholesterol were shown to reduce cardiovascular risk. Hyperlipidemia is a metabolic abnormality which leads to a persistent raise in the plasma concentrations of cholesterol and tryglycerides[3]. One-third of ischemic cardiac diseases in the world are due to hyperlipidemia[4]. Globally, hyperlipidemia is estimated to cause 2.6 million mortality (4.5 % of total) and 29.7 % disability[5]. CVD patients need early detection and management using counseling and medicines. The cholesterol treatment trialists collaborators meta-analysis have consistently demonstrated that cholesterol lowering statin drugs are effective for the prevention of major vascular events[3]. Lovastatin (LVS) is the first clinically used statin. It inhibits HMG CoA to mevalonate, which is important step in cholesterol synthesis. Lovastatin is highly lipophilic, hence its absorption is incomplete and about 85 % of the administered dose is found in faeces. It has short half- life. It also undergoes first pass metabolism and major route of excretion is through bile. There are gastrointestinal side effects associated with Lovastatin namely flatulence, nausea, diarrhea, dyspepsia and constipation. The important adverse effect of Lovastatin is Myopathy or rhabdomyosis, which is dose dependent and due to its lipophilicity[6]. This necessitates the need to improve aqueous system solubility of a drug for improved dissolution which leads to better absorption and develop a new delivery system to prevent first pass metabolism and reduce adverse effects.

2. REVIEW OF LITERATURE:

2.1 HYPERLIPIDEMIA:

Hyperlipidemia is a metabolic abnormality leading to persistent increase in plasma cholesterol and triglyceride level[4].

Hypolipidaemic drugs:

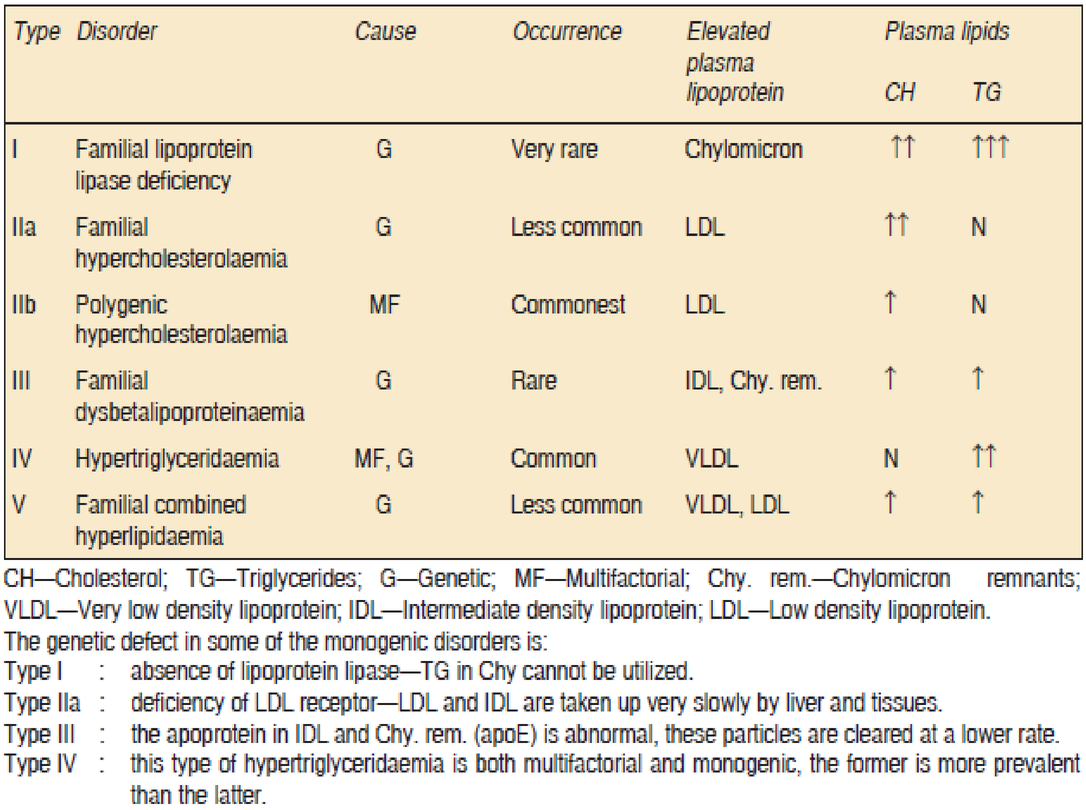
A Hypolipidemic drug lowers the level of lipids and lipoproteins in systemic circulation. They prevent the cardiovascular diseases by retarding the atherosclerosis in hyperlipideimic patients.

Hyperlipidemias are classified into following types.

1) **Secondary:** These are related with diabetes, nephrotic syndrome, myxoedema, chronic alcoholism, and drugs such as corticosteroids, oral contraceptives, and β blockers etc.
2) **Primary:** These are associated with,
    a) A single gene defect: It is a genetic
    b) Multiple genetic, dietary, and physical activity related that is polygenic or multifactorial.

Table 1 shows types of primary hyperlipidemias.Low density lipoprotein is the primary carrier of plasma cholesteryl esters and very low density lipoprotiens are the carriers of triglycerides.

Table 1: Types of Primary Hyperlipidemias



Type	*Disorder*	*Cause*	*Occurrence*	*Elevated plasma lipoprotein*	*Plasma lipids*	
					CH	*TG*
I	Familial lipoprotein lipase deficiency	G	Very rare	Chylomicron	↑↑	↑↑↑
IIa	Familial hypercholesterolaemia	G	Less common	LDL	↑↑	N
IIb	Polygenic hypercholesterolaemia	MF	Commonest	LDL	↑	N
III	Familial dysbetalipoproteinaemia	G	Rare	IDL, Chy. rem.	↑	↑
IV	Hypertriglyceridaemia	MF, G	Common	VLDL	N	↑↑
V	Familial combined hyperlipidaemia	G	Less common	VLDL, LDL	↑	↑

CH—Cholesterol; TG—Triglycerides; G—Genetic; MF—Multifactorial; Chy. rem.—Chylomicron remnants; VLDL—Very low density lipoprotein; IDL—Intermediate density lipoprotein; LDL—Low density lipoprotein.

The genetic defect in some of the monogenic disorders is:

Type I : absence of lipoprotein lipase—TG in Chy cannot be utilized.
Type IIa : deficiency of LDL receptor—LDL and IDL are taken up very slowly by liver and tissues.
Type III : the apoprotein in IDL and Chy. rem. (apoE) is abnormal, these particles are cleared at a lower rate.
Type IV : this type of hypertriglyceridaemia is both multifactorial and monogenic, the former is more prevalent than the latter.

Hypolipidemic drugs are classification is shown in Table 2 .

Table 2: Classification of Hypolipidemic drugs

Sl .no.	Class	Example
1.	HMG-CoA reductase inhibitors (Statins)	Lovastatin, Simvastatin, Pravastatin, Atorvastatin, Rosuvastatin.
2.	Bile acid sequestrants (Resins)	Cholestyramine, Colestipol.
3.	Lipoprotein lipase activators (PPAR α activators, Fibrates)	Clofibrate, Gemfibrozil, Bezafibrate.
4.	Lipolysis and triglyceride synthesis inhibitor	Nicotinic acid.
5.	Sterol absorption inhibitor	Ezetimibe.

2.2 STATINS:

Statins are the most efficacious drugs for both primary and secondary hypercholesteromias. They inhibit HMG CoA to mevalonate, which is important step in cholesterol synthesis. The enzyme activity is high at midnight; therefore these drugs are administered at bed time. Lovastatin is widely used statin. It is highly lipophilic. Statins show therapeutic effect in dose dependent manner. Lovastatin shows a mean reduction of LDL cholesterol by 25 % at a dose of 20 mg / day; 32 % at a dose of 40 mg/day and 40 % at a maximum daily dose 80 mg.

Limitations of Lovastatin:

- Absorption is incomplete
- First pass metabolism
- Half life is short
- Muscle aches or myopathy is the common side effect with rise in CPK levels. The risk of myopathy increases with increased dose of Lovastatin. A lower dose is advisable to reduce the myopathy[6].

2.3 SOLUBILITY ENHANCEMENT TECHNIQUES:

The enhancement of aqueous system solubility of lipophilic drugs is one of the challenges of drug development. The aqueous solubility less than 1 µg/mL creates bioavailability problem for drug[7].Amidon et al proposed a biopharmaceutics drug classification of drugs, which gives relationship between *in-vitro* drug dissolution and *in-vivo* bioavailability[8]. The biopharmaceutical classification system (BCS) helps in drug development for proper modification of physico chemical property of drug and selection of suitable dosage form. According to BCS, drugs are classified into four classes, as shown in the Figure 1.

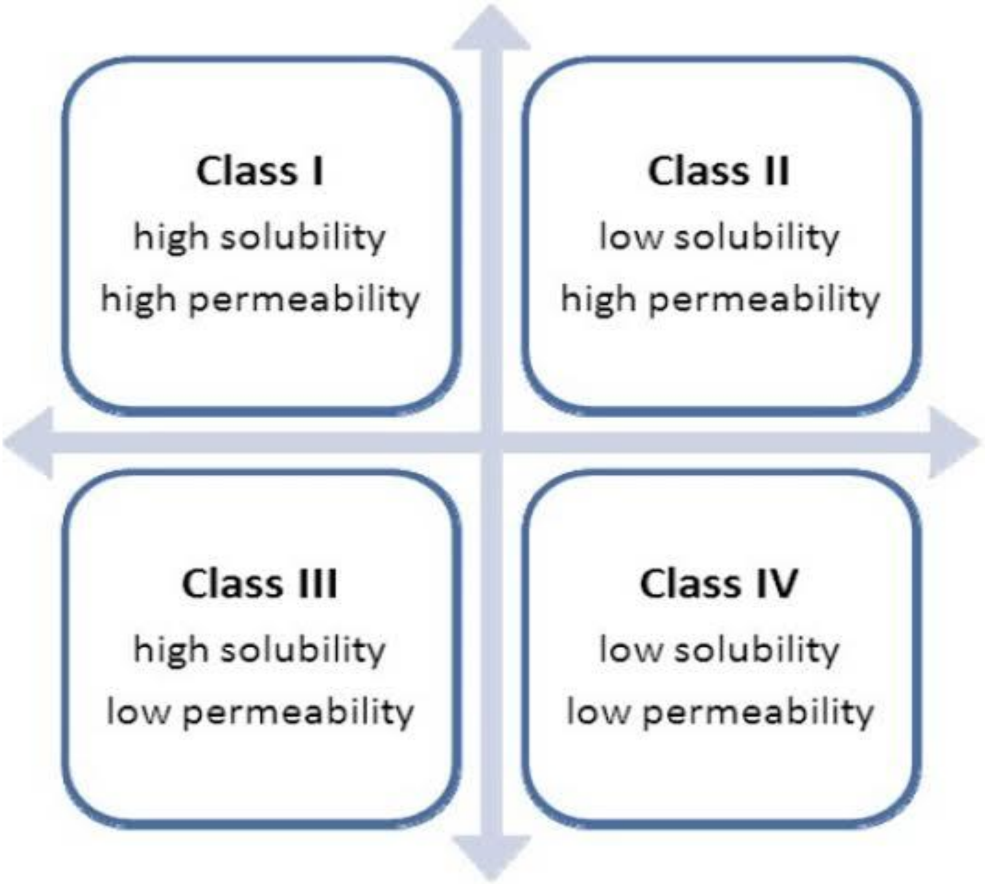


Figure 1: Biopharmaceutical Classification System

Drugs belonging to class II have unpredictable dissolution profile, which acts as rate limiting step in absorption and low bioavailability, which neccesitates increase in dose which in turn leads to increased adverse effects[9].

Modification of the solid state at molecular level of drug is the best method for improving solubility of drug. Different methods are employed to enhance the solubility are by milling; which increases particle surface area, use of surfactants; which improves wettability, decreasing crystallinity by preparing solid dispersions, preparation of inclusion complexes by cyclodextrins, use of polymorphic forms, use of salt forms of drugs and formulation of nanosuspensions. Solid dispersion is a most efficient technique. It is defined as the dispersion of active ingredient in an inert polymeric matrix which leads to reduction in drug particle, increasing surface area and improved dissolution[10].

Polyvinyl pyrrolidone K30 (PVP K30) and Poly ethylene Glycol 4000 (PEG 4000) polymers are preferred as carriers for drug because they show good aqueous solubility. These polymers are nontoxic, cost effective, and good physiological tollarance[11].

Methods:

Solvent evaporation method (SE):

This method utilizes organic solvent to dissolve drug and disperse in carrier molecule. Important factors to be considered are (i) Selection of suitable solvent and (ii) Complete removal of solvent; once the product is formed. Main advantage of this method is thermal decomposition of drug and polymer can be avoided[12].

Spray drying method (SD):

In this technique, a mixture of drug, polymer, and excipients in suitable solvent is atomized, sprayed, and dried by heated air stream. The advantages of this method are thermolabile drugs can be processed and product can be obtained as spherical particles with narrow size distribution. Inlet and outlet temperature, pump velocity, aspiration percentage, suitable solvent selections are important factors to be considered[13].

2.4 TRANSBUCCAL DRUG DELIVERY SYSTEMS:

Buccal delivery is an alternative option for oral route of drug administration. Transbuccal route consists of the mucosal linings of the oral cavity. Buccal administration is intended for delivering drug through the buccal mucosa to achieve a local or systemic effect[14].

Transbuccal drug delivery is classified as

A) Buccal delivery

B) Sublingual delivery, as shown in the figure 2.

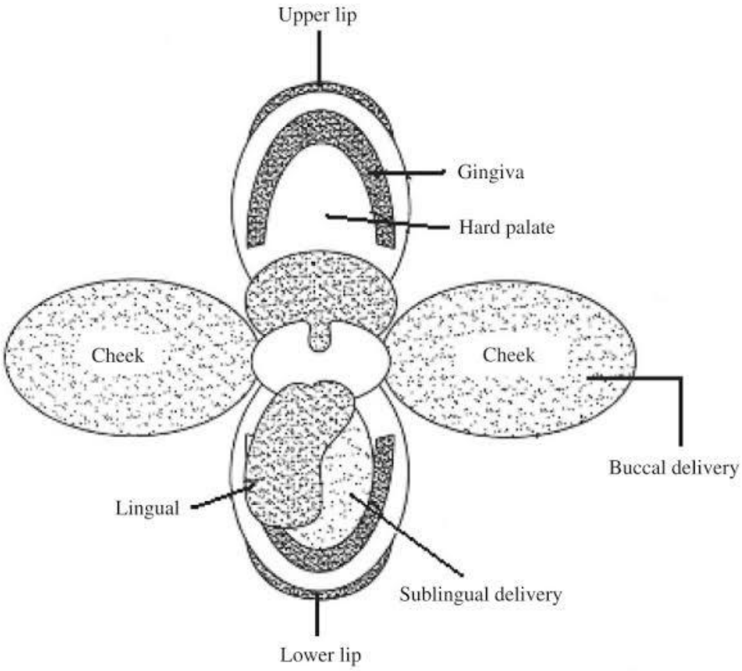


Figure 2: Transbuccal drug delivery routes

Buccal mucosa has smooth muscle which provides good accessibility and it is relatively immobile which is suitable for retentive type of dosage form.

Advantages of transbuccal drug delivery

1) Drug enters directly into systemic circulation, which prevents first pass metabolism leading to increased bioavailability.
2) Less enzyme activity
3) Highly vascularized tissue

4) Painless administration
5) Easy withdrawal of drug
6) Versatility in dosage form design
7) Improved patient complience[15].

Various transbuccal formulations include 1) Patches, 2) Tablets and 3) Gels. Buccal patches have overcome the drawbacks of the other dosage form. They show relatively faster onset of action and flexibility in therapy[16].

An ideal buccal patch should have sufficient strength to withstand stress and possess good elasticity and flexibility. Hence, mechanical and swelling properties of buccal patches are critical[17].

Preprogrammed drug delivery through transbuccal route, diminishes chance of over and under dosage, minimizes inter and intra patient variation. Transbuccal drug delivery permit lower dose, because first pass metabolism is avoided and reduces dosing frequency. It gives simplified dosage regimen by using transbuccal patch before going to bed which provides drug delivery for 6 h. It provides predictable and extended duration of action by maintaining steady-state drug concentration which increases therapeutic efficacy and reduces adverse effects[18].

If we formulate tablet dosage form for transbuccal administration, that dosage form will not able to assure therapeutic drug concentrations in the mucosa and systemic circulation because of the washing effect of the saliva and mechanical stress, which takes the formulation away from the mucosa, resulting in a very short exposure time. Therefore it is necessary to assure prolonged contact between the formulation and mucosa. Hence, transbuccal patches formulation is preferred, which comprises mucoadhesive polymers, plasticizers and penetration enhancers to overcome the above said problems[19].

Chitosan is a biodegradable, natural polymer. It is mucoadhesive, biocompatible, nontoxic cationic polymers. It has excellent film forming properties, so it is a good candidate for buccal delivery[20].Aboti P. et al., states that over hydration of chitosan may lead to slippery mucilage on surface, so to avoid mucoadhesion failure, other polymers added to the film[21]. PVP K30, hydrophilic polymers when used along with chitosan, produces pores in the matrix structure and thus promotes the diffusion of drug from the matrix[22].

Hydroxy propyl methyl cellulose (HPMC) is a bio compatible, non toxic and rate controlling polymer. It is stable at pH 3.0 to 11.0. It easily dissolves in cold water by forming colloidal solution. It is widely used film forming and viscosity modifying polymer[23].

Plasticizer is most important component of transbuccal film. It interposes between the polymer chain by interacting with the forces held together by extending and softening the matrix of polymer. Glycol derivatives are most widely used plasticizers[24].

Permeation enhancers are used to improve drug permeation. Different types of permeation enhancers are surfactants, bile salts and terpenes. Menthol is a monocyclic terpine with organoleptic properties. It is safe, nontoxic to buccal mucosa and also acts as a flavoring agent[25].

To ensure unidirectional drug delivery, backing layer is used for transbuccal patch. Ethyl cellulose polymer is used as backing layer. It is hydrophobic, less permeable to water, impermeable to drug and has got moderate flexibility, which assures the unidirectional drug delivery when used as backing layer[26].

Moor V et al., (2018) conducted cross-sectional study to determine the frequency of lipid abnormalities in patients with a cardiovascular risk and disease at the university teaching hospital of Yaounde. They involved 264 patients of which 119 were men and 145 were women. Their study concluded that the frequencies of lipid profile abnormalities were, LDL 44.3 %, high triglycerides 18.9 %, high cholesterol 3.8 % and total cholesterol 3.4 % and high triglycerides level was strongly associated with type 2 diabetes mellitus[27].

Taher Y et al., (2018) studied the prevalence and determinants of dyslipidaemia. In this analytic cross-sectional study, the result of lipid profile examination of 1541 clinic patients of king Faisal University were subjected to descriptive and analytical statistics. The prevalence was found to be, hypercholesterolemia 13.8 %, hypertriglyceridemia 17 %, hypo HDL cholesterolemia 40 %, hypo LDL cholesterolemia13 %. The study also concluded that females were 1.4 times more likely to have hypercholesterolemia[28].

Neduri K et al., (2012) studied the various techniques to enhance the solubility of Lovastatin. These methods are solid dispersions, superdisintegrants, and sublimation. The authors concluded that there was satisfactory dissolution enhancement from all three methods and could potentially lead to improvement in bioavailability[29].

Sharma M et al., (2013) studied the various techniques to enhance the solubility of lipophilic drug Atorvastatin calcium. Solid dispersions were prepared by using PEG 4000 in different ratios by normal fusion and microwave fusion method. The microwave induced fusion method shows the highest drug release when compared to pure drug[30].

Quereshi M et al., (2014) has prepared and evaluated a self micro emulsifying drug delivery systems (SMEDDS) of Lovastatin. The drug release profile of SMEDDS formulations was significantly higher than the pure drug.SMEDDS could be a promising technique to enhance the solubility[31].

Rodde M et al., (2014) studied the solubility enhancing effect of Neem gum as a hydrophilic carrier in solid dispersions. The study shows that, as the concentration of gum increases, solubility also increases. A significant reduction in HMG CoA reductase activity was found with solid dispersions of Atorvastatin. The drug release, *ex-vivo* and *in-vivo* studies confirmed that neem gum can enhance bioavailability of atorvastatin[32].

Aboti P et al., (2015) studied the various techniques to enhance the solubility of furosemide, by using bile salts and formation of cyclodextrin complex with β-cyclodextrin. Results suggested that the approach for permeation and solubility enhancement using transbuccal route can be further explored for systemic delivery of drug with poor solubility[33].

Karolewicz B et al., (2016) prepared and evaluated solid dispersions of Lovastatin by kneading method using Pluronic F127 as a polymer. Solid dispersion Lovastatin: pluronic acid 50/50 shown 100.0 %, 60/40 was 83.37 %, 70/30 was 98.95 % and 80/20 was 87.50 % respectively. These release rates were significantly higher than that of pure Lovastatin that is 16.73 % within 30 min[34].

Jagadale S et al., (2013) developed solid dispersions of chlorzoxazone with pluronic F127 to enhance its solubility and bioavailability. They selected the solid dispersion, which showed best *in-vitro* drug release, and further mucoadhesive buccal patches were developed and characterized. The *in-vitro* permeation study showed that patches could deliver drug to the oral mucosa for a period of 8 hours. The results indicate that suitable bioadhesive buccal patches with good permeability could be prepared[35].

Caro V et al., (2017) prepared sublingual buccal film solid dispersions of class II, highly lipophilic Furosemide to enhancing bioavailability. As a solid dispersion, Furosemide solubility is increased up to 28.36 mg/ml. The sublingual film produced good drug flux into the systemic compartment and thus produced rapid onset of action and bioavailability was enhanced[36].

Zolkiflee F et al., (2017) discussed importance of Lovastatin and problems associated with solubility and bioavailability. Authors also discussed the various approaches for solubility enhancement of Lovastatin. This review suggests current advanced technologies employed in order to provide effective and complete utilization of drug[37].

Khanfer M et al., (2017) prepared and evaluated solid dispersions of Lovastatin by using silica which has different surface area. From the results, they found that, Lovastatin and sylysia 350 in a ratio of 1:5 with incubation period 48 h gave best results and the dispersions were stable after 3 months at 75 % relative humidity and temperature 40 ^{o}C[38].

D’souza A et al,. (2016) focused on the use of PEG. PEG is one of the commonly used polymer.The authors have discussed and provided guidance to formulators on the appropriate PEG grade selection for particular application based on the available *in-vitro* and *in-vivo* literature data. This summarizes use of PEG in therapeutic applications, its clinical status, and commercial use as well. Toxicities related to different PEG grades were also discussed[39].

Patel R et al., (2018) studied the solubility enhancing effect of PEG 6000 and PVP K 30 polymers on class II drug Furosemide. The characterization is carried out by XRD, DSC and SEM revealed that Furosemide in crystal form is converted to amorphous form. The tablets prepared using solid dispersions shown improved wettability and increased cumulative drug release[10].

Wadher S et al., (2014) studied recent methodologies involved in solubility development of simvastatin. By achieving required concentration of drug, Pharmacological action of simvastatin was achieved in the systemic circulation. By formation of nanobiocomposites using microwave irradiation method, High pressure homogenization, and solvent evaporation method enhancement of solubility and dissolution of Simvastatin was achieved[40].

Pandya V et al., (2018) prepared Simvastatin nanosuspension by nanoprecipitation method which was assessed for *in-vitro* dissolution, particle size & characterized by SEM & DSC. A 2^3 factorial design was employed to study the effect of independent as well as dependent variables. Indpendent variables were amount of PVP K30(X1), amount of SLS (X2), organic to aqueous ratio (X3). Time required to release 80 % of drug, particle size were dependent variables. The dissolution rate of the augmented formulation was enhanced by 80 % in 20 minutes[41].

Biswal S etal., (2018) enhanced the dissolution rate of gliclazide by solid dispersions (SDs) with polyethylene glycol (PEG) 6000. Gibbs free energy values were all negative, indicating the spontaneous nature of gliclazide solubilization and they decreased with increase in the PEG 6000 concentration, demonstrating that the reaction conditions became more favorable as the concentration of PEG 6000 increased. The SDs of gliclazide with PEG 6000 were prepared at 1:1, 1:2 and 1:5 (gliclazide/PEG 6000) ratio by melting-solvent method and solvent evaporation method. Mean dissolution time (MDT) of gliclazide decreased significantly after preparation of SDs. The DSC and XRD studies indicated the microcrystalline or amorphous state of gliclazide in SDs of gliclazide with PEG 6000[42].

Ankita R et al., (2013) enhanced the oral bioavailability of Rosuvastatin calcium, by nanosuspension technology aiming to increase the dissolution rate. By Nanoprecipitation technique, Nanosuspensions were prepared in the presence of sodium lauryl sulphate and PVP K30 as surfactant and stabilizer respectively. Prepared nanosuspensions were evaluated for its *in-vitro* dissolution studies, particle size study. Sodium lauryl sulphate (SLS) and PVPK-30 were evaluated at different concentrations.[43]

Obaidat R et al,. (2019) investigated the effect of various polymers on solubility dissolution & stability of cefixime trihydrate. Different solid dispersions were prepared using SCF & conventional methods. Physico chemical properties of solid dispersions were evaluated using FTIR, DSC, TGA, XRD, and SEM. Prepared solid dispersions showed increased solubility except those prepared using soluplus & SCF without co-solvent. The best enhancement was found to be the one with soluplus based solid dispersions using conventional method[44].

***Dhore P et al,.* (2016)** formulated solid dispersion of Ritonavir using lyophilized milk to enhance the aqueous solubility of the drug.The prepared solid dispersions was characterized for FTIR, DSC, SEM, XRD which confirmed the formation of the solid dispersion. Solid dispersions were evaluated for saturation solublity, *in-vitro* drug release & *ex-vivo* permeation. It was seen that the optimized solid dispersions exhibited higher (30-fold) aqueous solubility (11.36±0.06µg/ml), as compared to the pure drug (0.37±0.03µg/ml). *In-vitro* dissolution released a higher (10 fold) efficacy of optimized formulation. *Ex-vivo* permeation was performed using everted intestine method, which showed that prepared solid dispersions had improved permeation than the pure drug.[45]

Ghosh I et al., (2013) used top down media milling technique to prepare nanosuspension by taking HPMC 3cp, PVPK-30, and HPC-EXF as stabilizers & Vitamin E as surface active agent. QbD approach was utilized to study the parameters such as drug content, agitation rate (RPM), size of grinding media. The significant process parameters were determined to be RPM of media milling through statistical analysis.[46]

Meher G et al., (2013) prepared HPMC and polymethacrylate polymers for buccal delivery of Carvediol. The evaluation studies of patches shows that, there is significant increase in *in-vitro* drug release up to 88% and *ex-vivo* permeation up to 80% and follows “Higuchi’s model and fickian type diffusion controlled mechanism”[47].

Castan H et al.,(2017) developed Doxepin mucoadhesive films for analgesic local effect in oral cavity. Results shown that all films were hydrated very quickly and permeation studies showed that HPMC films shown greater permeation of Doxepin[48].

Chandra sekhar K et al., (2018) developed mucoadhesive patches of chlorpheniramine maleate and characterized. *In-vivo* permeation studies were carried out in healthy humans. Results shown that bioavailability of optimized buccal patch was 1.46 times higher than the conventional dosage form and were statistically significant[49].

Parthasarathi G et al., (2017) studied the transbuccal permeation of Carbamazepine by using bile salts as permeation enhancers with 0.1 and 0.5 w/w concentrations by using HPMC K15M, carbopol 934 or chitosan in combination of PVA were subjected for permeation studies by using Franz diffusion cells. The flux and permeation coefficient confirmed that there was a significant increase in permeation amount of carbamazepine after the inclusion of permeation enhancer[50].

2.5 JUSTIFICATION FOR THE STUDY:

The poor drug absorption and low bioavailability reinforce the need for solubility enhancement and newer drug delivery system to beat these challenges. Lovastatin, a model drug selected in the present research is HMGCoA reductase inhibitor, is a drug of choice for both primary and secondary hyperlipidaemia. Its clinical efficacy is limited due to its low aqueous solubility; hence, its absorption is incomplete and about 85 % of the administered dose is found in faeces. It has short half-life. It undergoes first pass metabolism. Hence, there is low bioavailability. There are gastrointestinal side effects such as Flatulence, Nausea, Diarrhea, Dyspepsia and Constipation. Myopathy or rhabdomyosis is another potential side effect.

As per literature survey, transbuccal delivery system work has not been formulated by using Lovastatin. The research project proposed herein is based on modification of the solid state at molecular level of drug for enhancing aqueous system solubility by solvent evaporation and spray drying solid dispersion technique. Transbuccal drug delivery system of processed solid dispersions are formulated which offer advantages such as prevention of first pass metabolism, drug release control which will maintain desired plasma drug level, reduced gastrointestinal side effects, prevention of degradation of drug in GIT, enhanced therapeutic efficacy and achieving patient compliance.

The formulations of buccal patches were formulated using different polymers such as HPMC K4M, HPMC E5LV and chitosan. These polymers were finalized besed on a series of trails considering chemical compatibility and adhesive properties. Two different grades of HPMC polymer, which are HPMC K4M and HPMC E5 LV were selected to study the effect of viscosity parameter on adhesion of transbuccal film. Chitosan and HPMC polymers were compared to study the drug release pattern and duration of therapy.

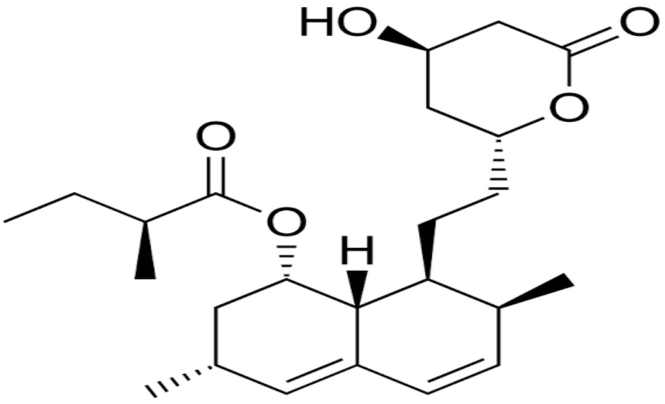
Thus, in the present research, efforts were made to improve the overall therapeutic efficacy of drug, by enhancing the solubility which provides therapeutic action at reduced dose which in turn reduces side effects of drug, especially myopathy. Literature survey reveals that myopathy of statins is due to its lipophilicity and dose dependent. Formulating transbuccal patches will prevent first pass metabolism and increase bioavailability and maintains constant drug levels throughout drug therapy period. Hence, present work ensures good patient compliance.

2.6 DRUG AND EXCIPIENT PROFILE:

2.6.1 DRUG PROFILE'[51,52,53,54]:

Drug selected for the study: Lovastatin

Structural formula:



Chemical name: [1S-[1 α(R*),3 α,7 β, 8β(2S*4S*),8a β]]-2-Methyl butanoic acid 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthalenyl ester;(1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[-2-(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]ethyl]-1-naphthalenyl(S)-2- methylbutyrate;1,2,6,7,8,8a-hexahydro- β, δ-dihydroxy-2,6-dimethyl-8-(2-methyl-1-oxobutoxy)-1-napthaleneheptanoic acid δ-lactone; 2 β,6 α-dimethyl-8 α-(2-methyl-1-oxybutoxy)-mevinic acid lactone.

Physical properties:

Appearance: White crystalline powder

Molecular Formula: $C_{24}H_{36}O_5$

Molecular Weight: 404.55 gm /mol

Meting Point: 174 ^{0}C

Boiing Point: 559.2^0C at 760 mmHg

Solubility: Lovastatin is insoluble in water and aqueous solubility at room temperature is 0.4 $X10^{-3}$mg/ml. It is sparingly soluble in the lower alcohols.

LogP: 4.26

Category: Antihyperlipidaemic agent, HMG CoA reductase inhibitor.

Stability:

1) Lovastatin (Crystalline form) stored at room temperature gives trace amounts of oxidation products. However non oxidative degradation products have not been found.
2) In aqueous solution, the lactone ring of Lovastatin undergoes hydrolysis under acidic or alkaline conditions. Hydrolysis under acidic condition is reversible which leads to a mixture of lactone and hydroxy acid. But in case of alkaline condition, it is irreversibly converted to the hydroxy acid, which has good stability.

Mechanism of action: "Lovastatin inhibit HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonate in cholesterol synthesis".

Pharmacokinetic parameters:

Absorption:

The absorption of Lovastatin following oral administration is approximately 30%.

Bioavailability:

The bioavailability of Lovastatin is nearly 5% of an oral conventional dosage form of Lovastatin.

Distribution: Lovastatin crosses the BBB and placenta.

Plasma protein binding: The plasma protein binding of Lovastatin is 95%.

Metabolism: Lovastatin undergoes first pass metabolism (FPM). It is metabolized to give "hydroxyl acid of Lovastatin and its 3-hydroxy, 3-hydroxy methyl and 3-exomethylene derivatives".

Excretion: Lovastatin is excreted in the bile and 85% of the administered dose has been recovered from the faeces and about 10% from the urine.

Plasma half-life: The hydroxy acid metabolite is rapidly cleared; plasma clearance is 300-1248 ml/min and half-life is 1.1-1.7 hrs.

Uses: Lovastatin is used in the treatment of Hyperlipidaemia, particularly in type IIa and IIb Hyperlipidaemias. Hyperlipidaemic patients with ischemic heart disease are given prophylactic dose.

Dose: Initial dose of 10 to 20 mg daily after dinner, which is increased, if needed at interval of 1 month or more to 80 mg daily in divided doses.

Adverse effects:

- Headache
- Gastrointestinal side effects.
  -Flatulence
  -Nausea
  -Diarrhea
  -Dyspepsia
  -Constipation.
- Myopathy or rhabdomyosis
- Sleep disturbances
- Rashes etc.

Drug interactions:

Lovastatin at doses more than 20 mg/day should not be used in along with gemfibrozil or any other fibrtates, niacin and cyclosporine. This will significantly increase the risk of rhabdomyosis

2.6.2 POLYMER PROFILE:

POLYETHYLENE GLYCOL 4000[55]:

Non proprietary names:

Polyethylene oxide, Polyoxy ethylene

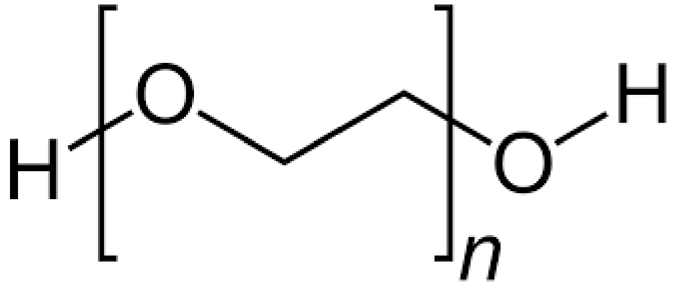
Chemical name and CAS registry number:

Polyethylene Glycol 4000 and 25322 -68 -3

Empirical Formula and Molecular Weight:

Polyethylene glycols are prepared by polymerization of ethylene oxide and are available in the range of molecular weights from 300 g/mol to 10,000,000 g/mol. These different polymeric chain lengths have different applications.

Structural Formula:



Functional Category:

Solubility enhancing agent, Plasticizer in films, Solvent, Surfactant, as a lubricant for Tablet and Capsule, Osmotic laxative and Denture fixing agent.

Description:

PEG 4000 appears as white to very pale yellow waxy crystalline flakes.

Typical Properties

These are relatively stable, hydrophilic polymer which are non irritant and do not penetrate skin

Acidity/alkalinity:	pH 5.5 -7.0 (25°c, 50 mg / ml in water)
Density:	1.2 g/ cm^3

Moisture content:

PEG adsorbs moisture from the atmosphere and the amount of water adsorbed depends on the initial moisture content and temperature/ relative humidity of the surrounding air.

Solubility:

It is soluble in water, ethyl alcohol, dichloromethane, and is insoluble in hexane and diethyl ether. It has tendency to couple with hydrophobic molecules to produce non-ionic surfactants.

Stability and Storage Conditions:

It is stable and it should be stored at 2-8° C in a tightly closed container.

Incompatibilities:

It is incompatible with oxidizing agents which is strong in nature.

Safety:

It is non-irritant and nontoxic polymer.

POLYVINYL PYRROLIDINE K-30[55]:

Non proprietary names:

PVP, Povidone, Polyvidone

Chemical name and CAS registry number:

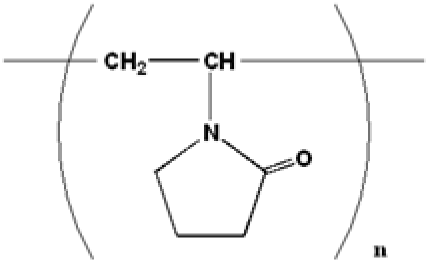
$(C_6H_9NO)_n$ and 9003 -39 -8.

Empirical Formula and Molecular Weight:

PVP (Polyvinylpyrrolidone) K30 is a synthetic water soluble polymer made from monomer N-vinylpyrrolidone. The molecular weight of the product is 40,000.

Molecular weight: 114.14 g/mol.

Structural Formula:



Functional Category:

Solubility enhancing agent, Adhesives, Fiber treatments, Cosmetics, Dye and pigment dispersants.

Description:

Polyvinylpyrrolidone is a white powder.

Typical Properties:

PVP (Polyvinylpyrrolidone) K30 polymer is a hygroscopic, amorphous polymer. They are linear nonionic polymers that are stable in water and organic solvent and pH stable. PVP K30 forms hard glossy transparent films and have adhesive, cohesive and dispersive properties. Hydrophilicity of PVPK30 is its dominant feature. The polymer is having high polarity and the resultant propensity to form complexes with hydrogen donors, such as phenols and carboxylic acids.

Acidity/alkalinity: pH = 3.0~7.0

Density: 1.2 g /cm^3

Moisture content:

When dry it is a light flaky hygroscopic powder, readily absorbing up to 40% of its weight in atmospheric water.

Solubility:

Soluble in water and other polar solvents. It is also soluble in alcohols like methanol and ethanol.

Viscosity (dynamic):

The K-Value of different grades of PVP polymer depends on molecular weight, its intrinsic viscosity, and degree of polymerization.

Stability and Storage Conditions:

It is stable and should be stored at room temperature in a tightly closed container in a dry place.

Incompatibilities:

It is incompatible with oxidizing agents which is strong in nature.

Safety:

It is non-irritant and nontoxic polymer.

LD50 (mouse): >40 mg/kg

LD50 (rat): >100 mg/kg

HYDROXYPROPYL METHYL CELLULOSE[55]:

Non proprietary names:

USP: Hydroxypropyl methyl cellulose

BPC: Hypromellose

Chemical name and CAS registry number:

Cellulose, 2-hydroxypropylmethylether

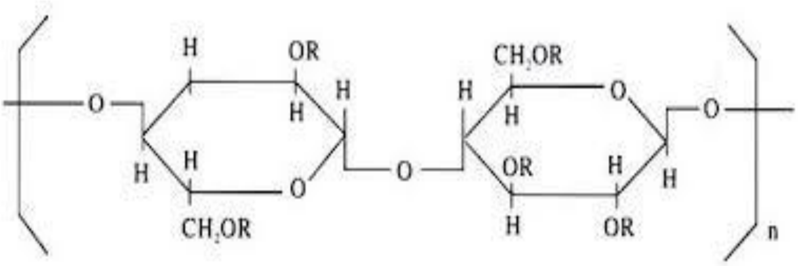
Cellulose hydroxypropylmethylether (9004 -65 -3)

Empirical Formula and Molecular Weight:

$C_8H_{15}O_6$ -$(C_{10}H_{18}O_6)n$ -$C_8H_{15}O_5$

Molecular weight: 86000

Structural Formula:



Functional Category:

Film forming agent in tablet coating, binding agent in tablet granulation, thickening agent, adhesive, emulsifier, suspending agent.

Description:

It is an odorless, tasteless, white, or creamy-white fibrous granular powder.

Typical Properties

It is relatively compatible polymer. It provides good viscosity and it is enzyme resistant. No ionic charge and it will not form complex with metallic salts and ionic organics.

Acidity/alkalinity: pH = 6.0 -8.0

Density: 0.25-0.70 g/cm^3

Solubility: soluble in cold water, insoluble in alcohol, ether, and chloroform.

Stability and Storage Conditions:

It is stable and should be stored in tight container in dry and cool place. Solutions are stable in the pH range from 3.0 to 11.0

Incompatibilities:

It is incompatible with oxidizing agents which are strong in nature and extreme pH conditions.

Safety:

It is generally considered as a nontoxic and non-irritant polymer.

CHITOSAN[55]:

Non proprietary names:

Chitosan hydrochloride

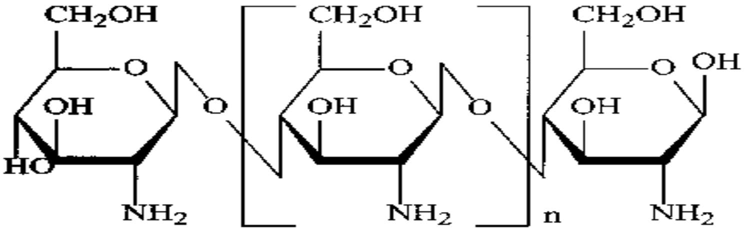
Chemical name and CAS registry number:

Poly –b -(1, 4) -2 –Amino -2-deoxy -D-glucose (9012-76-4)

Empirical Formula and Molecular Weight:

It is produced by deacetylation (partial) of chitin. It’s a polysaccharide containing copolymers of glucosamine and N-acetylglucosamine. It varies in composition, for different manufacturers. The degree of deacetylation should be more than 80-85% to form a soluble product. There are several types and grades of chitosan are available and they vary in molecular weight by 10 000–1 000 000, viscosity and degree of deacetylation.

Structural Formula:



Functional Category:

Tablet coating agent, mucoadhesive, disintegrant agent, film-forming agent, disintegrant agent, binding agent and viscosity-increasing agent.

Description:

Chitosan (CS) occurs as odorless, creamy-white or white flakes or powder. It forms fiber and it looks ‘cotton like’.

Typical Properties

It is a cationic polyamine and has high charge density at pH less than 6.0. Therefore it gets adhered to the negatively charged surfaces and forms chelate with metal ions.

Acidity/alkalinity: Aqueous solution of 1% w/v chitosan shows pH range between 4.0 to 6.0.

Density: 1.35 –1.40 g/cm^3

Glass transition temperature: 203 ^{0}C

Moisture content: Chitosan has tendency to adsorb moisture from the atmosphere and it depends on the initial moisture content, temperature, and relative humidity of the environment.

Particle size distribution: Particle size distribution is less than 30 mm

Solubility: Sparingly soluble in water and insoluble in ethanol. It dissolves readily with dilute and concentrated solutions of most organic acids

Viscosity (dynamic): Different varieties are available based on viscosity. It is very good viscosity enhancing agent in acidic pH. The viscosity increases with decreased temperature, increasing the chitosan concentration and increasing the degree of deacetylation.

Stability and Storage Conditions:

Chitosan is stable at room temperature. It should be stored in a tightly closed container in a dry place.

Incompatibilities:

It is incompatible with oxidizing agents which are strong in nature

Safety:

It is a non-irritant and nontoxic polymer. It is biocompatible with skin.

LD50 (mouse, oral): > 16 g/kg

2.7 AIM, OBJECTIVES, AND PLAN OF WORK:

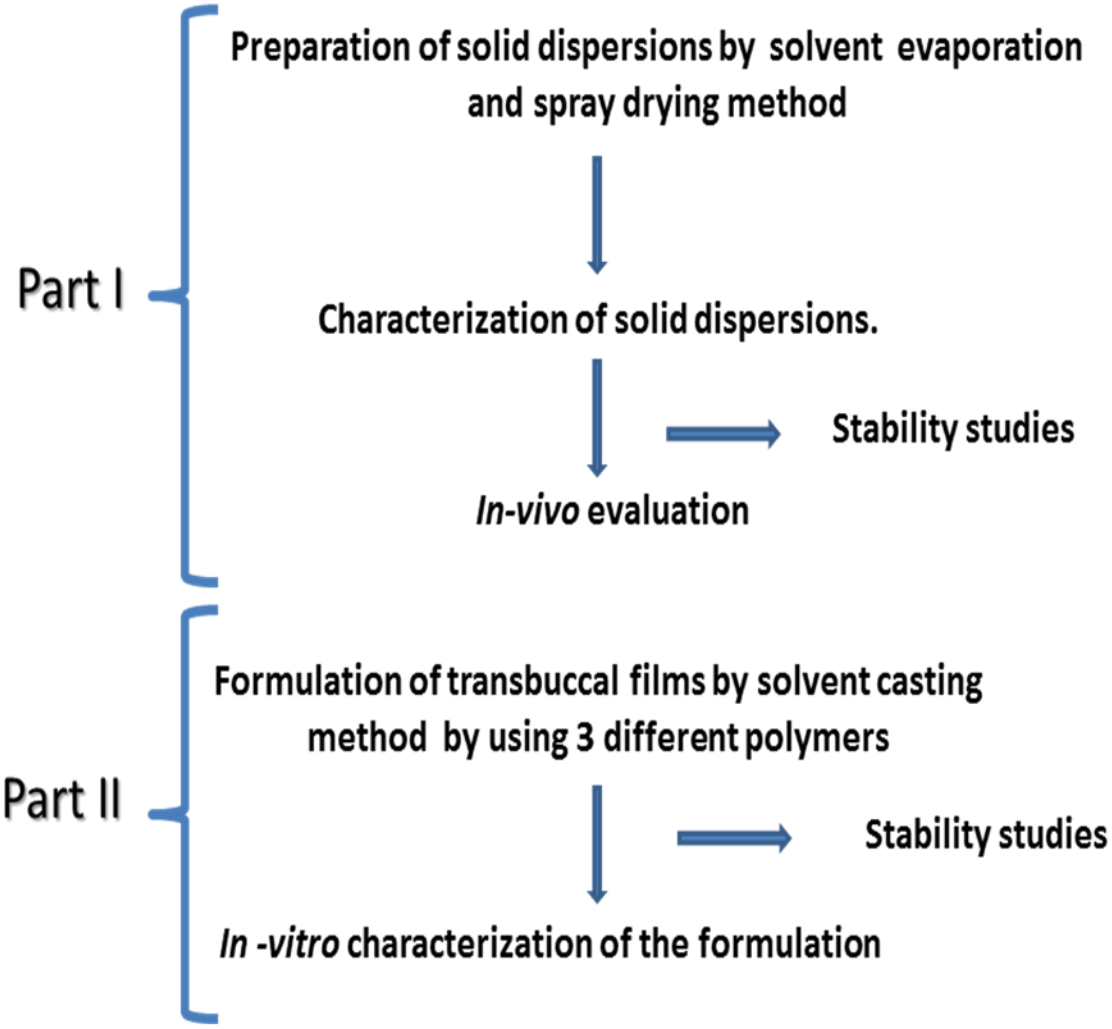
Aim:

Solubility enhancement of Lovastatin by solid dispersion technique, System design for transbuccal delivery, *in vitro* characterization and *in vivo* evaluation.

Objectives:

- Preformulation studies
- Preparation of solid dispersions by solvent evaporation and spray drying method
- Characterization of solid dispersions.
- *In-vivo* evaluation.
- Formulation of transbuccal films of solid dispersions by solvent casting method.
- *In-vitro* characterization of the formulation
- Stability studies

PLAN OF WORK


Part I
Preparation of solid dispersions by solvent evaporation and spray drying method
Characterization of solid dispersions.
Stability studies
In-vivo evaluation
Part II
Formulation of transbuccal films by solvent casting method by using 3 different polymers
Stability studies
In -vitro characterization of the formulation

3. MATERIALS AND METHODS

MATERIALS:

The following are the materials used and their manufacturers.

Table 3. List of chemicals used and supplier

Sr. No	Materials	Manufacturer
1.	Lovastatin	Biocon Limited (Bangalore Karnataka)
2.	Poly ethelyne glycol (PEG) 4000	SD Fine Chem. Ltd, Mumbai
3.	Polyvinyl pyrrolidone (PVP) K30	SD Fine Chem. Ltd, Mumbai
4.	Aerosil 200	Degussa, Germany
5.	Hydroxy propyl methyl cellulose (HPMC) K4M	Yarrow chemical products Mumbai Maharashtra India
6.	Hydroxy propyl methyl cellulose (HPMC) E5 LV	Lobachemie Mumbai
7.	Chitosan	Central Institute of fischeries technology Cochin, Kerala India.

Table 4. List of instruments used.

Sr.No	Equipment Name	Model	Make
1.	Electronic Balance	AA-2130	Accord
2.	Magnetic Stirrer	2-MLH	Remi
3.	FTIR	IRAffinity-1S	Shimadzu
4.	UV Spectrophotometer (UV Probe software)	Shimadzu-1800	Shimadzu
5.	Spray drier	LU-222	Labultima Mumbai
6.	Scanning Electron Microscopy	JEOL JSM-6360A	JEOL Tokyo
7.	Differential Scanning Calorimeter	DSC-60	Shimadzu
8.	X-ray powder Diffractometer	D2 Phaser	Bruker, Germany
9.	pH Meter	EQ-610	Equip-Tronics
10.	Dissolution apparatus	USP Type 2	Electrolab India
11.	Stability chamber	GMP model 400 Litres	Kesar Control Systems
12.	Screw gauge	Mitutoyo	Mitutoyo Corporation, Kavasaki, Japan

METHODS:

3.1. PREFORMULATION STUDIES:

This is the primary stage of dosage form development of a drug substance and is the study of a pure drug and excipients.

3.1.1. Characterization of Pure Drug

3.1.1.1. Identification of pure drug:

Lovastatin (drug) was identified by Fourier-transform infrared spectroscopy (FTIR). The pure Lovastatin supplied by Biocon Limited; Bangalore, Karnataka was further confirmed by FTIR.

3.1.1.2. Scanning of drug absorption (λ_{max}) by double beam UV-Visible spectrophotometer:

Accurately weigh 100 mg of Lovastatin and dissolve in 10 ml methanol. Make up the volume to 100 ml with phosphate buffer pH 6.8. Take 2.5 ml from the above stock solution and make up the volume to 50 ml with phosphate buffer to prepare standard solution having concentration of 50 µg /ml. The absorption maxima of the standard solution were scanned between 200-400 nm using UV Spectrophotometer against blank phosphate buffer pH 6.8.

3.1.1.3. Standard calibration curve of Lovastatin:

Preparation of standard stock solution:

Accurately weigh 100 mg of Lovastatin and dissolve in 10 ml methanol. Make up the volume to 100 ml with phosphate buffer pH 6.8. Take 2.5 ml from the above stock solution and make up the volume to 50 ml with phosphate buffer to prepare standard solution of concentration of 50 µg /ml.

Preparation of sample solutions:

From the above stock solution aliquots of 1.0, 2.0, 3.0, 4.0 and 5.0 ml were withdrawn and the volume was made up to 50 ml with phosphate buffer pH 6.8 to get a concentration of 5-25 µg /ml respectively. Absorbance of these solutions was measured against a blank phosphate buffer pH 6.8 at 238 nm for Lovastatin. The plot of absorbance vs concentration (µg/ml) was plotted and data was subjected to linear regression analysis. Average of triplicate readings was recorded.

3.1.1.4. Saturation Solubility determination[56]:

Known excess of pure Lovastatin was added to 10 ml of distilled water. The sample solution was kept with constant stirring at 20 rpm in water bath (25^0C) for 48 hours. The sample was then filtered, suitably diluted, and analyzed spectrophotometrically at 238 nm.

3.1.1.5. Melting point determination: Differential scanning calorimetry (DSC) analysis[57]

The thermal behavior of pure drug Lovastatin (LVS) was studied by DSC. DSC scan of the sample was recorded by using DSC Shimadzu-60. The sample was hermetically sealed in Aluminium pans processed under following conditions.

Heating rate: 10^oC/min

Dry Nitrogen flow: (100ml/min) up to 200^oC.

3.1.1.6. Crystallinity: Powder X-Ray Diffraction (PXRD) analysis[56,57]

PXR Diffractogram of pure Lovastatin was taken. The measurements were taken by using D2 Phaser Diffractometer under following conditions.

Temperature: Room temperature

Radiation: Monochromatic CuKa-radiation at 34 mA and at 38 Kv

2θ angle range: 5° to 90°with an angular increment of 0.05° /s.

3.1.1.7. Scanning Electron Microscopy (SEM) [56]

The microphotographs of pure Lovastatin were taken by scanning electron microscopy. Samples were mounted on double-faced adhesive tapes and coated with gold (200Å) under reduced pressure (0.001 torr) for 5 min using an ion sputtering device. The gold-coated samples were observed under the SEM and photomicrographs of suitable magnifications were obtained.

3.1.2. Drug-Excipient Compatibility Study:

The drug with other excipients was subjected to storage at normal room temperature for one month. The mixtures of drug and excipients were then evaluated by IR spectra by using FTIR spectroscopy

Compatibility studies were carried out for pure drug Lovastatin (LVS), (PEG 4000), (PVPK30)(Aerosil 200), physical mixtures of polymers used in solid dispersions for solvent evaporation (LVS+PEG4000) and spray drying method (LVS+PVPK30). FT-IR spectra of moisture free samples were obtained by using spectrophotometer (FT-IR Shimadzu Co., Japan) by potassium bromide (KBr) pellet method (2mg of sample in 200 mg of KBr). The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

3.2. PREPARATION OF SOLID DISPERSIONS AND PHYSICAL MIXTURE:

3.2.1. Solid dispersions prepared by solvent evaporation method[58]:

Solid dispersions of Lovastatin in PEG 4000 containing different ratios (1:1, 1:2, 1:4, and 1:8) were prepared by solvent evaporation method. Accurately weighed quantities of Lovastain and PEG 4000 were added to 25 ml of ethanol. The solvent was evaporated at 40^0 C, and the resulting residue was dried and stored in desiccator overnight. The product turned into fine powder using mortar and pestle and passed through sieve no.60. The products were stored in desiccator for further studies.

3.2.2. Solid dispersions prepared by spray drying method[56]:

Solid dispersions of Lovastatin in PVP K30 containing different ratios (1:1, 1:2, 1:4, and 1:8) were prepared by spray drying method. Lovastatin along with PVP in above said ratios was dissolved in sufficient amount of dichloromethane. To these clear solutions proposed quantity of Aerosil was added to obtain uniform suspensions. Spray drying was carried out using laboratory scale spray dryer (Labultima model LU 222 Mumbai India) under following set of conditions. Inlet temperature 35^0C; outlet temperature 26-28^0C; feed rate 4-6 ml per minute; atomization air pressure 2 kg/cm^2 and aspiration -250 mm WC. The resulting solid powder was placed in vacuum dryer for 24 hours to remove residual solvents if any. Then the samples were passed through sieve number 100. The samples were stored in desiccator for further studies.

3.2.3. Physical mixtures[59]:

Physical mixtures (PMs) having the same weight ratios were prepared by thoroughly mixing appropriate amounts of Lovastatin and PEG 4000 and PVP K30 in mortar until a homogeneous mixture was obtained. The resulting mixtures were sieved through a sieve number 60. The samples were stored in desiccator and further subjected for saturation solubility studies.

Table 5 shows, composition (drug: polymer ratio) of all Solid Dispersions (SE and SD methods) of Lovastatin.

Table 5: Composition of Solid Dispersions of Lovastatin (LVS)

Type of Formulation	**Composition (parts by weight)**	
	Solvent Evaporation Method	
	Lovastatin	**PEG 4000**
SE1	**1**	**1**
SE2	**1**	**2**
SE3	**1**	**4**
SE4	**1**	**8**
	Spray Drying method	
	Lovastatin	**PVPK30**
SD1	**1**	**1**
SD2	**1**	**2**
SD3	**1**	**4**
SD4	**1**	**8**

3.3. CHARACTERIZATION OF SOLID DISPERSIONS:

3.3.1. Saturation solubility[56]:

To evaluate increase in solubility of Lovastatin (as in solid dispersions) or only by the presence of hydrophilic polymer (as in PMs), saturation solubility measurements were carried out as follows: known excess of solid dispersions were added to 10 ml of phosphate buffer (6.8 pH). Samples were kept with constant stirring at 20 rpm in a water bath at 25^0C for 48 hours. Samples were then filtered, suitably diluted and analyzed spectrophotometrically at 238 nm.

3.3.2.1 Percentage yield:

Percentage yield can be calculated by dividing practical yield by theoretical yield and multiplying the value by 100.

3.3. 2.2 Drug content[56, 60]:

Solid dispersions equivalent to 10 mg of Lovastatin were weighed accurately and dissolved in suitable quantity of ethanol. The drug content was determined at 238 nm by UV spectrophotometer.

3.3.3. FTIR spectroscopy[61]:

Infrared spectroscopic analysis of solid dispersions by solvent evaporation method (SDSE 1:8) and spray drying method (SDSP1:8) was done. Fourier transform infrared spectra of moisture free powdered samples were obtained by using spectrophotometer (FT-IR Shimadzu Co., Japan) by potassium bromide (KBr) pellet method (2 mg of sample in 200 mg of KBr). The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

3.3.4. Scanning Electron Microscopy (SEM)[56,62]:

The microphotographs of selected formulations SE4 and SD4 are taken by scanning electron microscopy. Samples were mounted on double-faced adhesive tapes and coated with gold (200Å) under reduced pressure (0.001 torr) for 5 minutes using an ion sputtering device. The gold-coated samples were observed under the SEM and photomicrographs of suitable magnifications were obtained.

3.3.5. Powder X-Ray Diffraction (PXRD) analysis[56, 57,63]:

PXR Diffractograms of selected formulations SE4 and SD4 were taken by Powder X-Ray Diffractometer. X-Ray powder scattering measurements were carried out with a D2 Phaser Diffractometer at room temperature using the monochromatic

CuKa-radiation at 34 mA and at 38 Kv over a range of 2θ angles from 5° to 90° with an angular increment of 0.05° /s.

3.3.6. Differential scanning calorimetry (DSC) analysis[57,64,65]:

The thermal behavior of pure drug Lovastatin (LVS), PEG 4000, PVPK30, SE4 (best formulation prepared by solvent evaporation) and SD4 (best formulation prepared by spray drying) was studied by DSC. DSC scans of the samples were recorded by using DSC Shimadzu-60. The samples were hermetically sealed in aluminum pans and heated at constant rate of 10°C/min under dry nitrogen flow (100ml/min) up to 200°C.

3.3.7. Dissolution studies[57,66,67]:

The dissolution studies were performed using USP XXIII type 2 apparatus (electrolab India) for 3 hrs. Samples of pure Lovastatin and solid dispersions equivalent to 10 mg of the drugs were added to the 900 ml of phosphate buffer (pH 6.8) as dissolution medium maintained at 37±0.5°C, which was stirred at 100 rpm. At suitable intervals, 5 ml samples were withdrawn, filtered (0.22 µm), diluted, and analyzed at 238 nm using UV spectrophotometer. An equal volume of fresh medium at the same temperature was replaced into the dissolution medium after each sampling to maintain its constant volume (sink condition) throughout the test. Each reading was performed in triplicate (n=3) and calculated mean values of cumulative drug release were used for plotting graphs.

3.3.8. *In-vivo* study[56]

The hypolipdaemic activity of SD4 was compared with pure Lovastatin in healthy albino rats (wistar strains) of either sex weighing between 150-200 gms. The protocol of the study is approved by the institutional ethical committee and all the experiments are carried out as per CPCSEA guidelines. Animals had free access to food and water, which was made available ad libitum. The animals are divided into 3 groups of 6 animals each. The animals were treated for 14 days. Each group daily received 2ml of coconut oil orally. Reference group and test groups additionally received aqueous suspensions of pure Lovastatin and SD4 (equivalent to 10 mg/kg body weight) respectively, prepared using 2% w/v gum acacia as a suspending agent. Blood samples were collected under light ether anesthesia by retro orbital puncture; initially, after 7 days and after 14 days. The serum samples are analyzed for total cholesterol, triglycerides, HDL, and LDL levels by *in-vitro* diagnostic kit. The statistical analysis for the determination of differences in lipid profiles of different groups was done by one way ANNOVA and $p<0.05$ was taken as significant.

3.3.9. Accelerated stability study[56,68]

The accelerated stability of SE4 and SD4 were checked as per ICH guidelines at 40 ^{o}C/75% RH up to 3 months. Periodically (15days, 1 month and 3 months) samples were checked for *in-vitro* drug release and property of crystallinity using PXRD studies.

3.4. FORMULATIONS OF TRANSBUCCAL FILMS BY USING SOLID DISPERSIONS

The transbuccal film of solid dispersion is formulated to enhance the bioavailability of Lovastatin by preventing FPM in gastrointestinal tract. The solid dispersion SD4 was selected to formulate transbuccal films. SD4 shows high cumulative percentage drug release of 93 % as compared to SE4 which shows 89 %.

Further literature survey reveals that the hydrophilic polymer PVP K30 used in SD4 is used as plasticizer in formulating transbuccal films.

The formulations of buccal patches were formulated by using different polymers i.e., HPMC K4M, HPMC E5LV and chitosan. The placebo films were prepared in initial stage. The processing variables used while formulating the placebo patches were concentration of polymers and plasticizers. After results of placebo films were found to be satisfactory, the formulations containing different concentrations of polymers were prepared by using above said polymers.

3.4.1. Compatibility studies[69]:

Compatibility studies were carried out for SD4 and polymers used to prepare transbuccal film. SD4, HPMC K4M, HPMC E5LV, Chitosan, physical mixtures of SD4 and polymers of transbuccal films were subjected for Infrared spectroscopic analysis

Fourier transform infrared spectra of moisture free powdered samples were obtained by using spectrophotometer (FT-IR Shimadzu Co., Japan) by potassium bromide (KBr) pellet method (2 mg of sample in 200 mg of KBr). The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

3.4.2. Preparation of Backing Layer[70]

To prepare ethyl cellulose backing layer 300 mg of ethyl cellulose was dissolved in 10 ml acetone under constant stirring and to that 0.5 ml of glycerine was used as a plasticizer. The solution was poured in Petri dish.The solvent was allowed to be evaporated at room temperature for 4 hours.

3.4.3.1. Transbuccal film by using HPMC K4M[35,48, 70]

The polymer HPMC K4M is dissolved in 10 ml of mixture of ethanol and distilled water (3:2) and soaked for overnight. The polymeric solution was stirred on magnetic stirrer for 2 hours. The solid dispersion equivalent to 120 mg Lovastatin was added followed by addition of plasticizer 5 % glycerol and 20 mg of menthol as permeation enhancer. The mixture was stirred for 30 minutes and the mucoadhesive layer was cast on the pre formed ethyl cellulose backing layer in a petri dish, which was stored at 4°C to remove air bubbles entrapped and finally dried at 37 °C for 4 hrs. The dried films were cut into 1cm^2 and packed in Aluminium foil and stored. Composition of Transbuccal film by using HPMC K4M is shown in the Table 6

Table 6: Composition of Transbuccal film by using HPMC K4M

Formulation code	Solid dispersion equivalent to Lovastatin(mg)	HPMC K4M(mg)	Plasticizer Glycerol (%)	Permeation Enhancer Menthol (mg)
F1	120	100	5	20
F2	120	200	5	20
F3	120	300	5	20
F4	120	400	5	20
F5	120	500	5	20
F6	120	600	5	20

3.4.3.2. Transbuccal film by using HPMC E5[16, 48 ,69]

The polymer HPMC E5 is dissolved in 10 ml of mixture of dichloromethane and ethanol (1:1) and soaked for overnight. The polymeric solution was stirred on magnetic stirrer for 2 hours. The solid dispersion equivalent to 120 mg Lovastatin was added followed by addition of plasticizer 0.7 % glycerol and 20 mg of menthol as permeation enhancer. The mixture was stirred for 30 minutes and the mucoadhesive layer was cast on the pre formed ethyl cellulose backing layer in a Petri dish, which was stored at 4°C to remove air bubbles entrapped and finally dried at 37 °C for 4 hrs. The dried films were cut into $1cm^2$ and packed in Aluminium foil and stored. Composition of Transbuccal film by using HPMC E5 is shown in the Table 7.

Table 7: Composition of Transbuccal film by using HPMC E5

Formulation code	Solid dispersion equivalent to Lovastatin(mg)	HPMC E5 (mg)	Plasticizer Glycerol (%)	Permeation Enhancer Menthol (mg)
F7	**120**	**100**	**0.7**	**20**
F8	**120**	**200**	**0.7**	**20**
F9	**120**	**300**	**0.7**	**20**
F10	**120**	**400**	**0.7**	**20**
F11	**120**	**500**	**0.7**	**20**
F12	**120**	**600**	**0.7**	**20**

3.4.3.3. Transbuccal film by using Chitosan[48,70]

Citric acid 20 mg was dissolved in water. To this solution chitosan polymer was added and soaked for overnight. The polymeric solution was stirred on magnetic stirrer for 2 hours. The solid dispersion equivalent to 120 mg Lovastatin was added followed by addition of plasticizer 3 % glycerol. The mixture was stirred for 30 minutes and the mucoadhesive layer was cast on the pre formed ethyl cellulose backing layer in a Petri dish, which was stored at 4°C to remove air bubbles entrapped. The patches were finally dried at 25 °C for 24 hours. The dried films were cut into 1 cm^2 and packed in Aluminium foil and stored. Composition of Transbuccal film by using chitosan is shown in the Table 8.

Table 8: Composition of Transbuccal film by using Chitosan

Formulation code	Solid dispersion equivalent to Lovastatin(mg)	Chitosan (mg)	Plasticizer Glycerol (%)	Permeation Enhancer Citric acid (mg)
F13	120	100	3	20
F14	120	200	3	20
F15	120	300	3	20
F16	120	400	3	20
F17	120	500	3	20
F18	120	600	3	20

3.5. PHYSICOCHEMICAL CHARACTERIZATION OF TRANSBUCCAL PATCHES:

3.5.1. Film thickness and weight[71,72,73,74]

The thickness of all the formulations were measured by screw gauze (Mitutoyo Corporation, Kavasaki, Japan) and the weight of these films were determined by using electronic balance.

3.5.2. Content uniformity[71,75,76,77]

The film was dissolved in 100 ml isotonic phosphate buffer pH 6.8± 0.2, filtered (0.22 µm), suitably diluted and resultant solutions were analyzed by UV Spectrophotometer at 238 nm. The experiment was performed in triplicate.

3.5.3. Surface pH[71,78,79]

The microenvironmental pH of all the formulations was measured so as to predict its effect on buccal mucosa. The formulations were first wetted by adding distilled water to its surface. The surface pH was then recorded by bringing a glass electrode near the surface of the formulation and allowing it to equilibrate for 1min. The average pH ± SD was determined for all formulations.

3.5.4. Swelling index[71,80,81]

The buccal patches were weighed individually (designated as W_1) and placed separately in 2% agar gel plates, incubated at 37± 1 °C and examined for any physical changes. At regular 1 hour time intervals until 3 hours, films were removed from the gel plates and excess surface water was removed carefully using the filter paper. The swollen films were then reweighed (W_2) and the swelling index (SI) was calculated using the following formula:

$$SI = \frac{W_2 - W_1}{W_1} \times 100$$

The experiment was performed in triplicate and average ± SD values were recorded.

3.5.5. Folding endurance[71,81, 82]

Folding endurance of the films were determined by repeatedly folding and unfolding the films at the same place till it broke or for 300 times, which is considered to be a satisfactory value to reveal good folding endurance properties. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance.

3.5.6. *Ex-vivo* Residence Time/ Adherence time[71, 84,85]

The *ex-vivo* residence time is studied (n=3) to know the time required for complete erosion and /or detachment of the film from the mucosa surface .The fresh goat mucosa was fixed to the inner side of the beaker about 2.5 cm from the bottom. One side of the film was wetted with 1 drop of phosphate buffer pH 6.8 and it is adhered to the surface of the buccal mucosa by applying slight force with fingertips for 30 seconds. The beaker was filled with 500 ml of phosphate buffer pH 6.8 and was kept at 37± 0.5^{0}C. After 2 minutes, a 50 rpm stirring rate was applied to simulate the buccal cavity environment and film adherence is monitored for 8 hours.

3.5.7 *Ex -vivo* bioadhesion test[71,86,87,88]

Modified physical balance method was used to measure the *ex-vivo* mucoadhesive strength of prepared films. Fresh goat's buccal mucosa was taken and cut into piece and washed with phosphate buffer pH 6.8 and tied to the open mouth of glass vial which was tightly fitted into a glass beaker which is filled with phosphate buffer pH 6.8 in such way that, it just touched the buccal surface. The temperature of this beaker was maintained at 37^{0}C± 1^{0}C. The film was adhered to the lower side of a rubber stopper with cyano acrylate adhesive. Two pans of the balance are balanced with 5gm weight on the right side of the pan, which was lowered the pan along with the film over the mucosa. The balance was kept in this position for 5 minutes of contact time. The water drops were added slowly to the right-hand side pan, until the film detached from the mucosal surface. The weight required to detach the film from the mucosal membrane surface is a measure of mucoadhesive strength. All the experiments are performed in triplicates and mean ± SD are reported. The following formula was used to calculate detachment force.

"Force of adhesion (N)= (gm X 9.81)/ 1000"

"Detachment force (N/m^2) = Force of adhesion (N) / Surface area (m^2)"

3.5.8. *In-vitro* drug release study[71,,89,90,91]

In-vitro drug release was carried out by paddle over disc dissolution apparatus. The transbuccal patch was placed beneath the disc in the dissolution jar containing 900 ml phosphate buffer (pH 6.8) solution. The bath temperature was maintained at

37± 1^0C with 50 rpm speed. Aliquots of 5 ml were withdrawn at prespecified time intervals for 6 hours. Same volume of fresh buffer solution was replaced. The withdrawn sample solution was filtered through 0.4 µm membrane filter and the amount of drug was determined by measuring the absorbance of the aliquots at 238 nm using UV spectrophotometer. Each reading was performed in triplicate (n=3) and calculated mean values of percentage drug release were used for plotting graphs.

3.5.9. *Ex-vivo* drug permeation study[48, 71,92,93]

Franz diffusion cell was used to carry out *ex-vivo* drug permeation study. Fresh goat buccal mucosa was fixed on a diffusion cell between the donor and receptor compartment. The transbuccal patch is fixed on the mucosal membrane. Five ml of phosphate buffer pH 6.8 in the donor compartment and 25 ml of the phosphate buffer pH 6.8 in the receptor compartment is filled as dissolution fluid. The fluid is maintained at 37 ± 1^0C and stirred continuously at speed of 50±5 rpm. Aliquots of 1 ml of sample were withdrawn at pre-specified time interval. Same volume of fresh buffer solution was replaced. The withdrawn sample solution was filtered through 0.4 µm membrane filter and the amount of drug was determined by measuring the absorbance of the aliquots at 238 nm using UV spectrophotometer. Each reading was performed in triplicate (n=3) and calculated mean values of percentage drug permeated were used for plotting graphs.

3.5.10. Drug release kinetics[94,95]

To investigate the release kinetics of drug release from buccal films, the drug release and *ex-vivo* permeation data were subjected to fit various kinetic models using PCP Disso ver 2 software Pune India. The $\mathbf{r^2}$and **n** values are determined.

3.5.11. Stability study[35,,96,97,98,99,100]

The accelerated stability of optimized formulation F3 is conducted as per ICH guidelines at 40^{0}C /75 % RH up to 6 months. Periodically samples were removed (initial, 1 month, 2 months, 3 months) and analyzed for drug content, *in-vitro* release and *ex-vivo* permeation.

4. RESULTS

4.1. PREFORMULATION STUDIES:

4.1.1 Characterization of Pure Drug

4.1.1.1 Identification of pure drug:

The FTIR spectrum of Lovastatin was obtained (Fig 3) and interpreted for its structure. All the characteristic peaks of respective functional groups of drug were found in the spectrum and were matching with the structure of drug. Table 9 shows the interpretation of FTIR spectrum of drug along with its functional groups and corresponding peaks obtained in the spectrum.

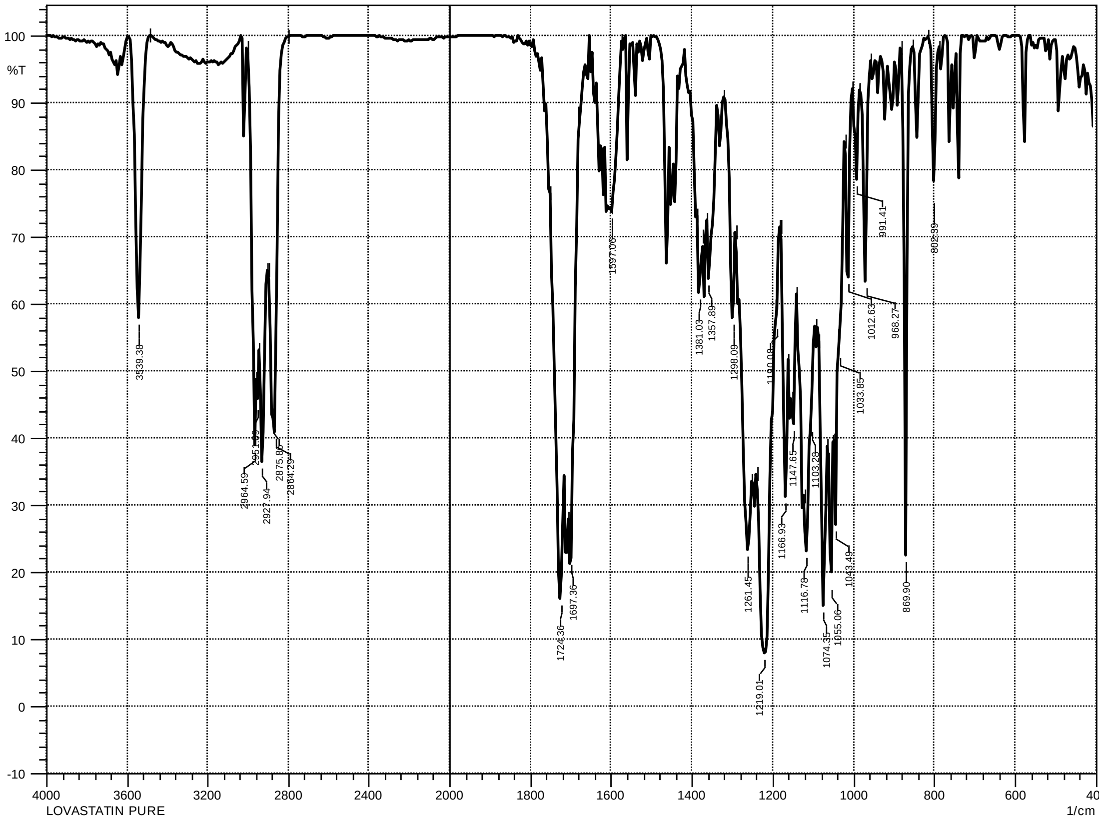


Fig. 3: FTIR Spectrum of Lovastatin

Table 9: Functional groups of Lovastatin and their corresponding peaks obtained in FTIR spectrum of drug.

Functional Groups	Wave number (cm^{-1})
Alcoholic O-H stretch	3539
Olefinic C-H stretch	3016
Methyl and methylene C-H stretch	2964 ,2927 ,2876
Lactone and ester carbonyl stretch	1725 ,1713 ,1690
Methyl and methylene bending vibration	1430 ,1381 ,1357
Lactone and ester C-O-C bending vibration	1282 ,1219 ,1075 ,1055
Alcoholic C –OH stretch	969
Olefinic C-H wagging	870

4.1.1.2. Scanning of drug absorption (λ_{max}) by UV-Visible spectrophotometer:

The λ_{max} of the standard solution of Lovastatin was found to be 238 nm as shown in Fig 4.

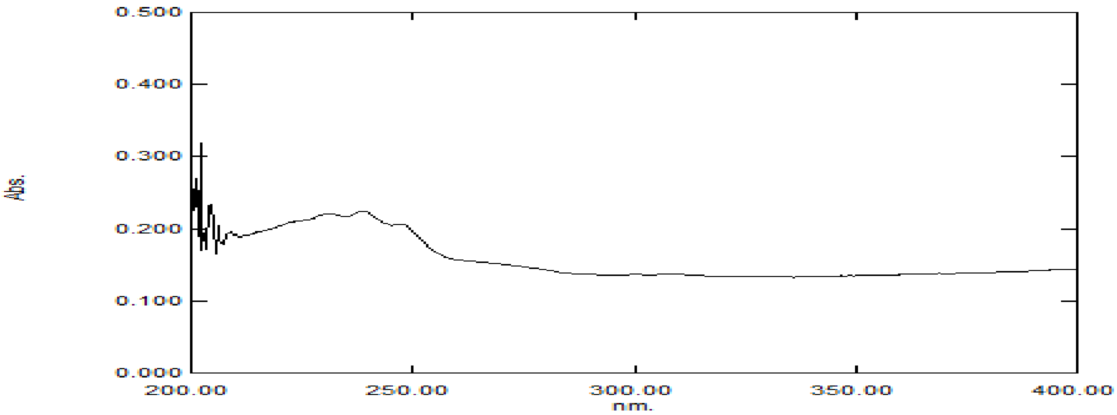


Fig. 4: Absorption maxima (λ max) of Lovastatin

4.1.1.3 Standard calibration curve of Lovastatin:

Standard calibration of Lovastatin is shown in the figure 5 and was found to be linear in the range of 5-25 µg /ml with regression coefficient value 0.993, slope value 0.009 and intercept value 0.01.

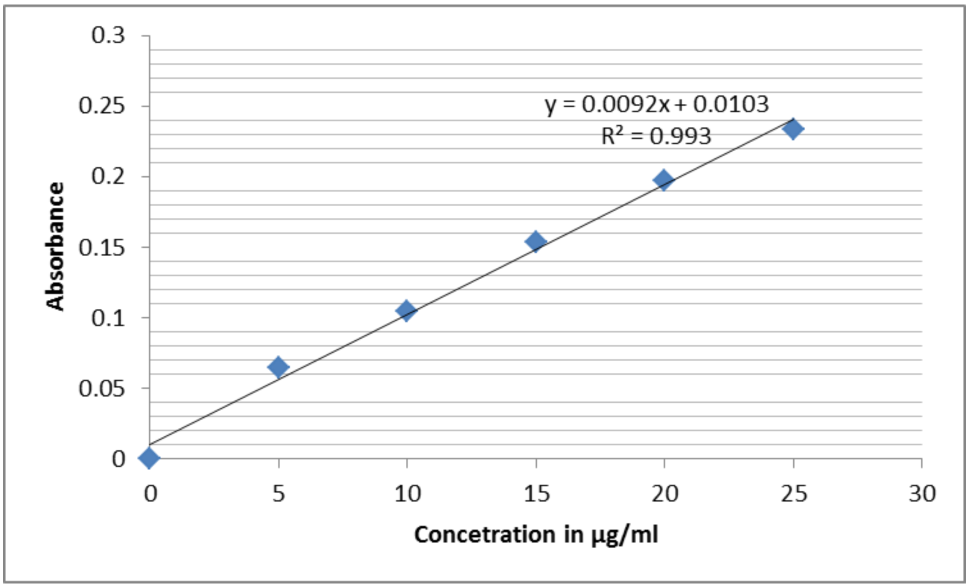


Fig. 5: Standard calibration curve of Lovastatin

4.1.1.4 Saturation Solubility determination:

Saturation solubility of Lovastatin was found to be 0.4 µg /ml in distilled water.

4 .1.1.5.Melting Point determination: DSC analysis

The DSC curve of Lovastain is shown in the figure 6. The pure Lovastatin shows melting endotherm at 170 ^{o}C with enthalpy of fusion (ΔH) of 163.15 mJ /g.

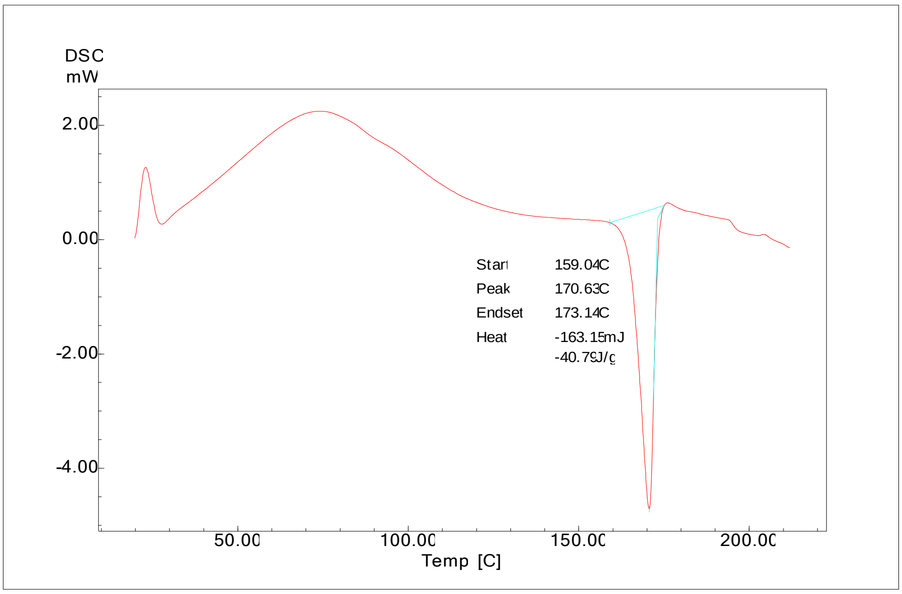


Fig. 6: DSC Thermogram of Lovastatin

4.1.1.6. Crystallinity: PXRD analysis

PXR Diffractogram of pure Lovastatin is shown in the figure 7. The presence of characteristic peaks (at 2 θ) in X-Ray diffraction suggests that Lovastatin is present in crystal form with respective diffraction peaks at a diffraction angle of 2θ at 10.69,12.61, 13.52, 14.74,15.45, 16.06, 17.11, 17.82, 19.56, 21.31, 22.57, 25.79, 26.96, 30.29, 32.01, 34.65, 35.86 etc.

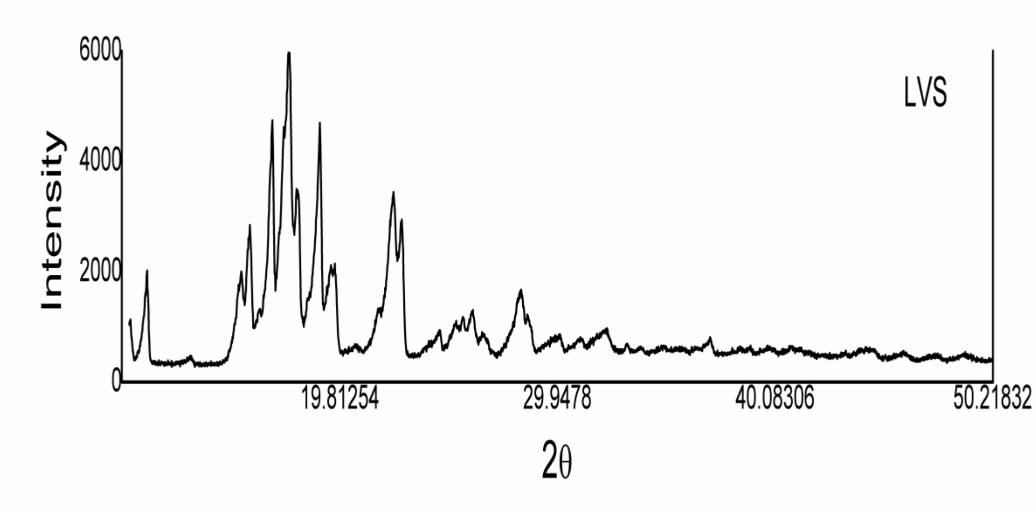


Fig.7: PXR Diffractogram of pure Lovastatin

4.1.1.7. SEM

The microphotograph of pure Lovastatin is shown in the figure 8. Pure drug is consisting of small and bigger elongated crystalline particles.

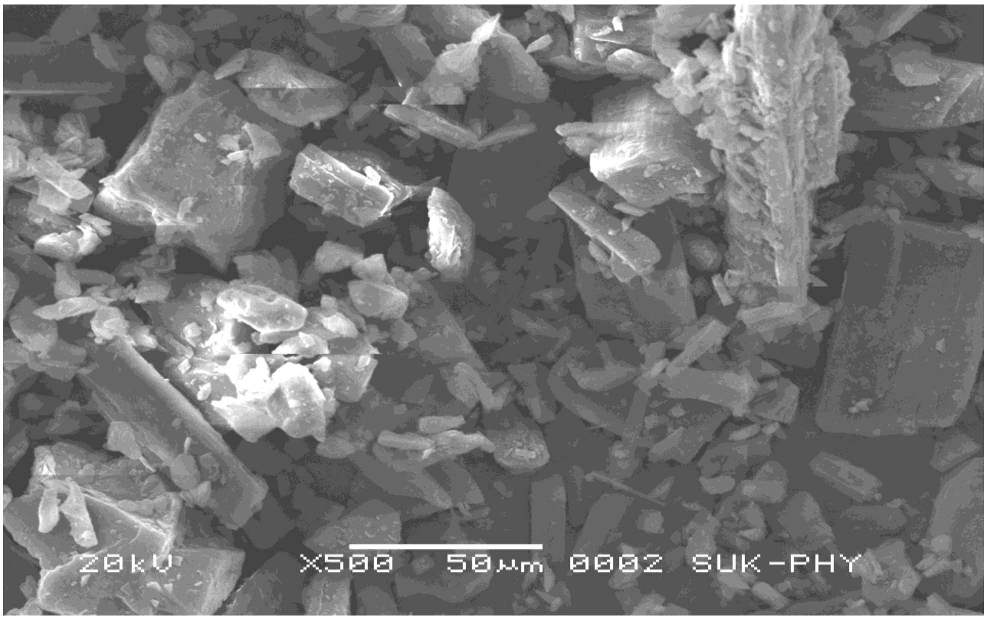


Fig. 8: SEM microphotograph of pure Lovastatin

4.1.2 Compatibility Study:

The spectra of all the samples are shown in the figure 3 and 9 to13. The spectrum of pure Lovastatin (figure 3) presented characteristic peaks at 3539 cm^{-1} (Alcohol O-H stretching vibrations), 3016 cm^{-1} (olefinic C-H stretching vibration), 2964 cm^{-1} ,2927 cm^{-1} ,2876 cm^{-1} (Methyl and methylene C-H stretching vibration),1725 cm^{-1},1713 cm^{-1} and 1690 cm^{-1} (Lactone and ester carbonyl stretch) 1430 cm^{-1} ,1381 cm^{-1} and 1357 cm^{-1} Methyl and methylene bending vibration), 1282 cm^{-1} ,1219 cm^{-1} ,1075 cm^{-1} 1055 cm^{-1} (Lactone and ester C-O-C bending vibration), 969 cm^{-1} Alcohol C –OH stretch), and 870 cm^{-1} (Trisubstituted olefinic C-H wagging) respectively.

Important vibrations detected in the PEG(figure 9) are the C-H stretching at 2884cm^{-1} and the C-O (ether) stretching at 1145 cm^{-1}. The spectrum of PVP(figure 10) showed important bands at 2925 cm^{-1} C-H stretch and 1653 cm^{-1} stretching vibration of the carbonyl group, which is most distinct peak in the IR spectrum of PVP K30. The broad peak at 3000-3300 cm^{-1} (OH stretching vibrations), which was attributed to the presence of water. The spectrum of Aerosil 200 (figure 11) showed the presence of broad prominent peak at 1076 cm^{-1} (strong Si-O linkage) is characteristic of Aerosil and also there is peak at 3549 cm^{-1} (O-H stretch).

The spectra of physical mixture of Lovastatin and PEG 4000 (figure 12) can be simply regarded as the superimposition of peaks of Lovastatin and PEG. The spectra of physical mixture of Lovastatin, PVP K30 and Aerosil (figure 13) shows all the charecterisitc peaks corresponding to drug, PVP K30 and aerosil suggesting there is no interaction between drug and polymer.

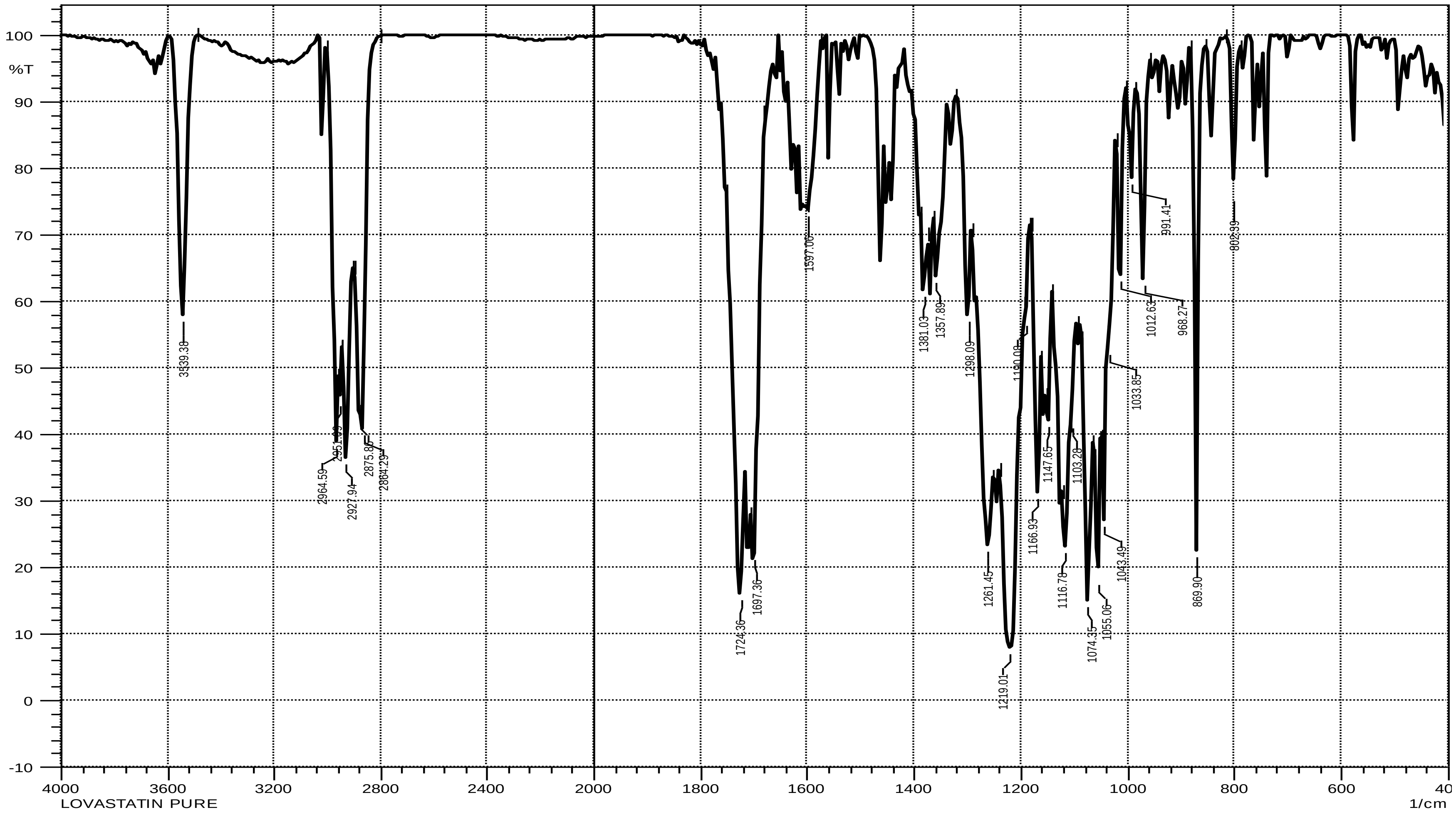


Fig. 3: FTIR Spectrum of Lovastatin

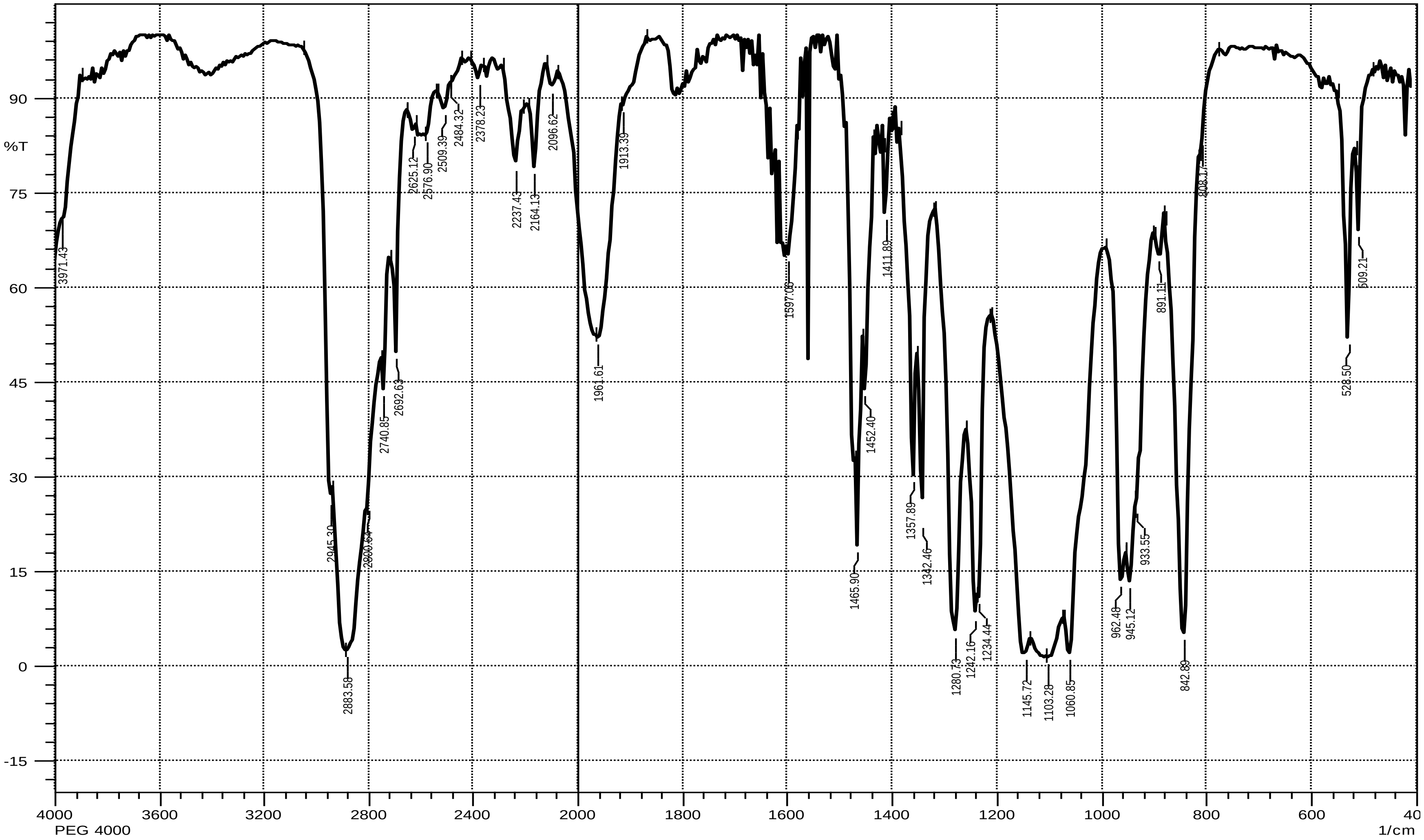


Fig .9: FTIR Spectrum of PEG 4000

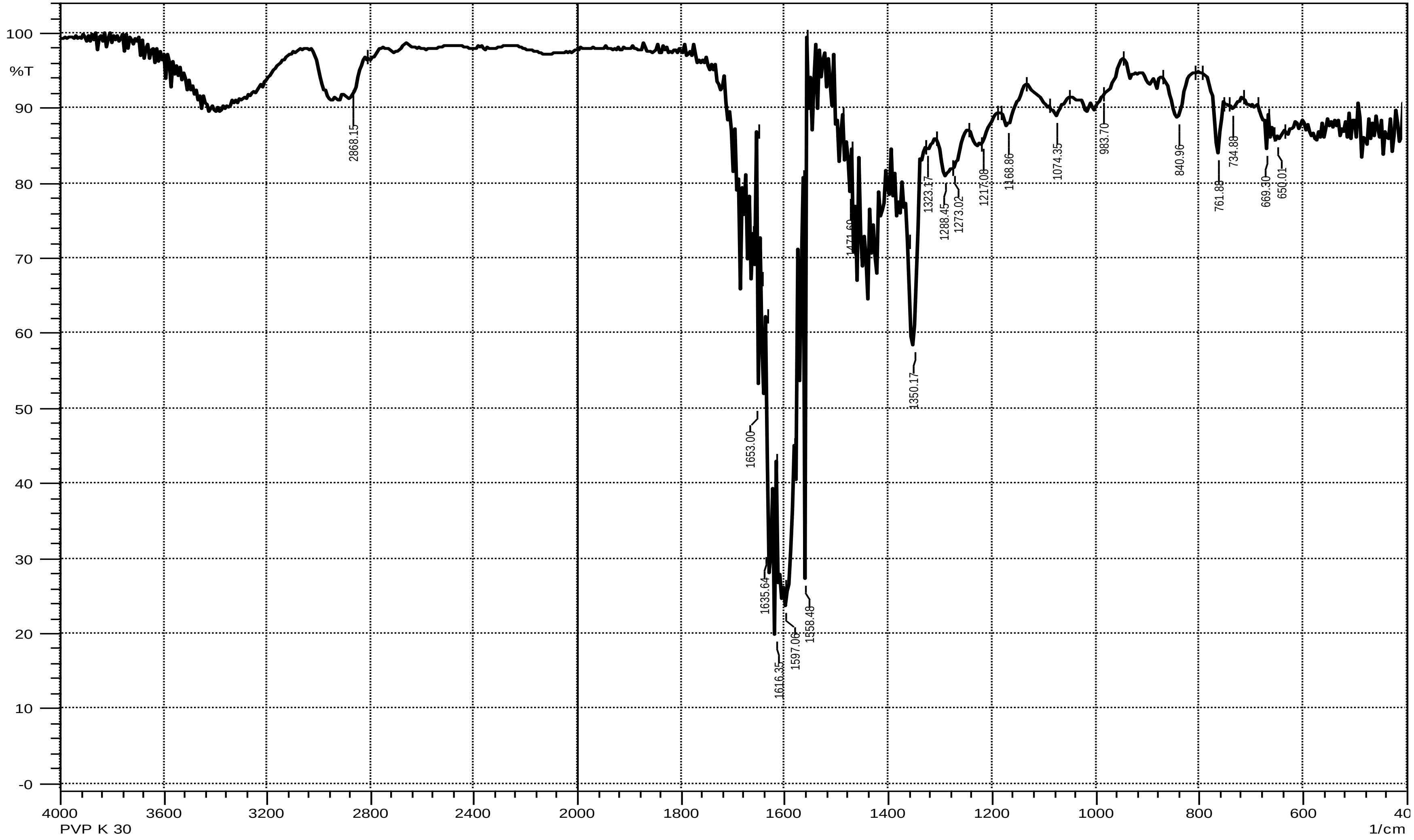


Fig. 10: FTIR Spectrum of PVP K30

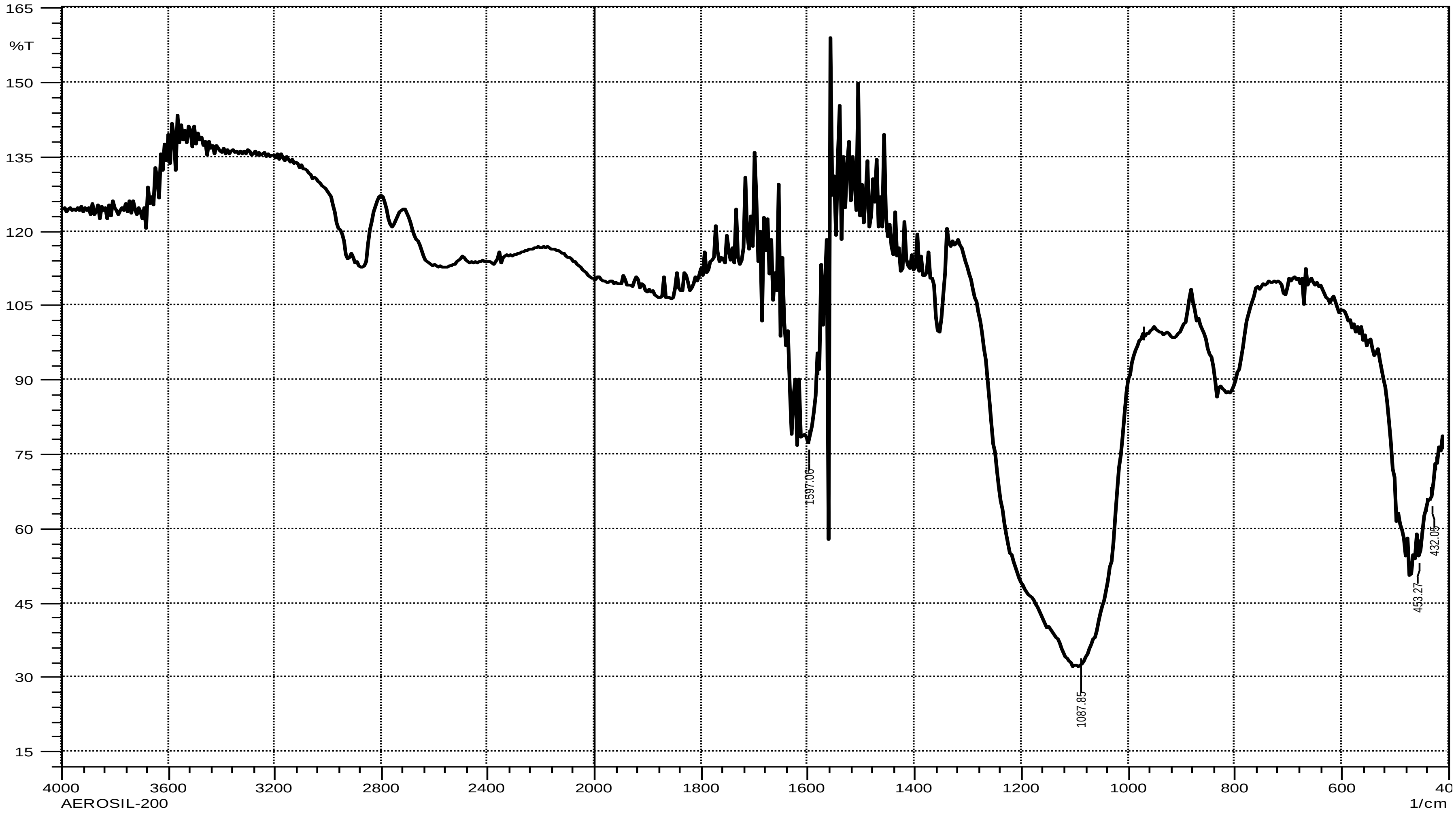


Fig. 11: FTIR Spectrum of Aerosil 200

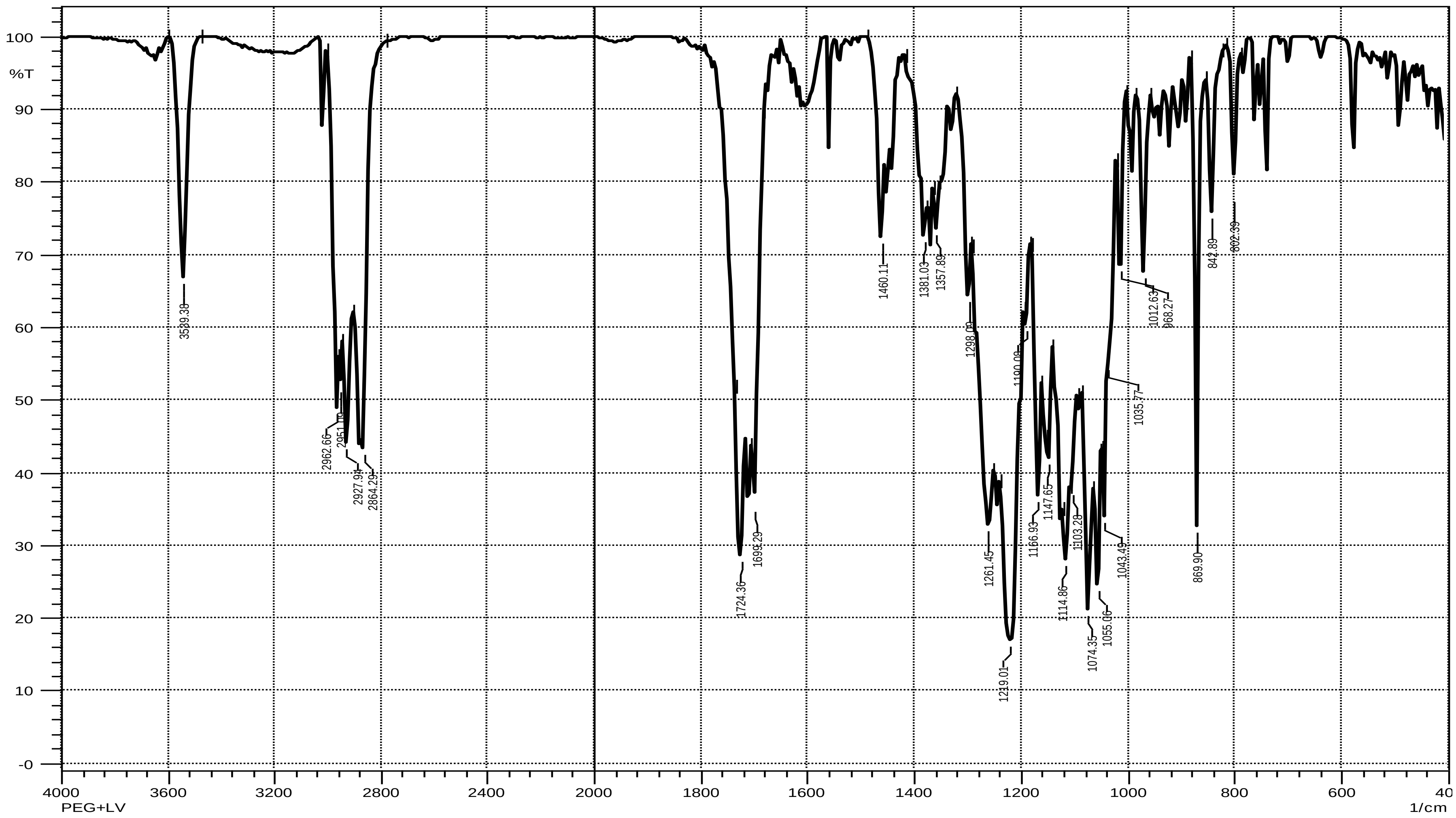


Fig. 12: FTIR Spectrum of physical mixtures of Lovastatin and PEG 4000

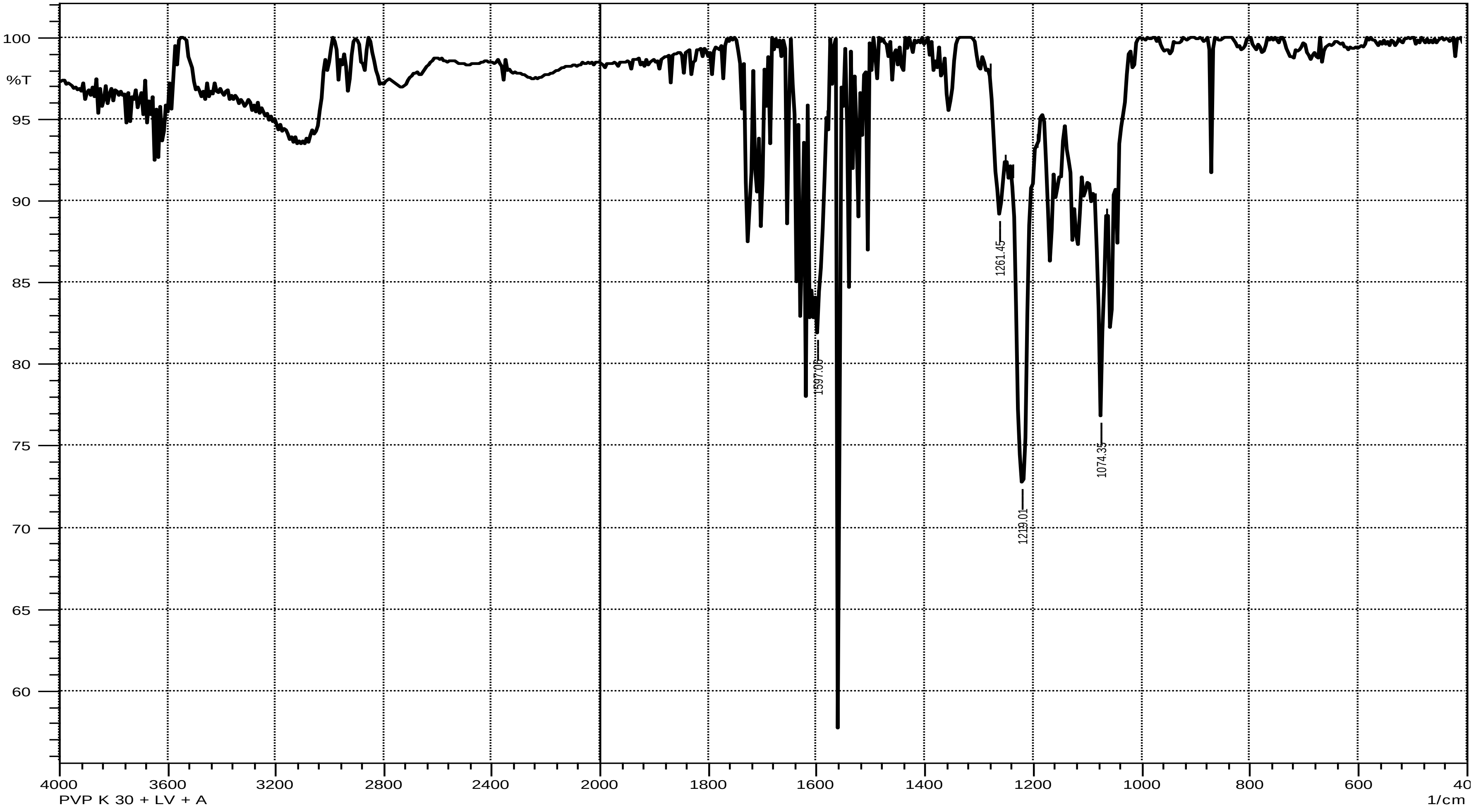


Fig. 13: FTIR Spectrum of physical mixtures of Lovastatin, PVP K 30 and Aerosil

4.2 REPARATION OF SOLID DISPERSIONS

Solid dispersions of Lovastatin were prepared by using two different polymers PEG 4000 (solvent evaporation) and PVPK30 (spray drying). The prepared dispersions were evaluated and compared. The best formulations were selected based on drug content, saturation solubility, and flow properties. These best formulations were further subjected to FTIR, SEM, PXRD and DSC analysis. Further *in-vivo* lipid profile studies and stability studies were also carried out.

4.3 CHARACTERIZATION OF SOLID DISPERSIONS

4.3.1 **Saturation solubility:**

Saturation solubility of physical mixtures and dispersions are shown in the Table 10 and 11. Saturation solubility studies shows that presence of polymers increases the solubility of drug in case of physical mixtures as well as formulations. Pure drug shows saturation solubility 0.4 µg /ml. As the concentration of polymer increases in case of physical mixtures, saturation solubility increases with almost 10 folds in case of PM4, whereas SE4 shows 176 folds increase in saturation solubility. In case of PVP K30 formulations, PM8 shows 8 fold increase and SD4 shows 216 fold increase in saturation solubility.

Table 10: Saturation Solubility of Physical mixtures and Formulations by solvent evaporation method

Type of formulation (Drug:Polymer)	Saturation solubility (µg /ml)(n=3)
Pure Lovastatin	**0.4 ± 0.03**
PM1	**0.8 ± 0.07**
PM2	**1.3 ± 0.05**
PM 3	**2.9 ± 0.04**
PM4	**4.1± 0.3**
SE1	**14.2 ± 0.5**
SE2	**26.3 ± 0.7**
SE3	**38.6± 0.4**
SE4	**69.5± 0.3**

Table 11: Saturation Solubility of Physical mixtures and Formulations by spray drying method

Type of formulation (Drug:Polymer)	Saturation solubility (µg /ml)(n=3)
Pure Lovastatin	**0.4 ± 0.03**
PM 5	**0.9 ± 0.05**
PM 6	**3.7 ± 0.04**
PM7	**6.3 ± 0.04**
PM8	**3.1± 0.2**
SD1	**28.6± 0.3**
SD2	**46.3± 0.2**
SD3	**73.4±0.5**
SD4	**87.3±0.4**

4.3.2. Percentage yield and Drug content:

The percentage yield and drug content of solid dispersions are shown in the Table 12 and 13 respectively. Percentage yield is high with solvent evaporation as compared to spray drying method.

Table 12: Percentage yield of solid dispersions

Formulation Code	Percentage yield
SE1	86
SE2	89
SE3	91
SE4	92
SD1	84
SD2	88
SD3	85
SD4	88

Table 13: Drug content of solid dispersions

Formulation Code	Drug content (w/w) (n=3)
SE1	69±0.03%
SE2	83±0.57%
SE3	88±0.04%
SE4	94±0.56%
SD1	68±0.10%
SD2	74±0.45%
SD3	85±0.08%
SD4	97±0.89%

The formulations SE4 and SD4 are considered to be optimum formulations based on the results of drug content and saturation solubility

4.3.3. FTIR spectroscopy

The FTIR spectra of SE4 and SD4 were recorded (Fig 14 and fig.15 respectively) and interpreted for its structure. All the characteristic peaks for the respective functional groups of drug and polymer were found in the spectrum. Table 14 shows the interpretation of FTIR spectrum of drug along with its functional groups and respective peaks obtained in the spectrum.

Table 14: Functional groups and their respective peaks obtained in FTIR spectra of SE4 and SD4.

Functional Groups	Wave number (cm^{-1})
SE4	
"Alcohol O-H stretching"	3535
"Olefinic C-H stretching vibration"	3016
"Methyl and methylene C-H stretching vibration"	2951 ,2926 ,2962
"C-H stretch"	2864,2875
"Lactone and ester carbonyl stretch"	1726 ,1713 ,1695
"Methyl and methylene bending vibration"	1429 ,1342 ,1351
"Lactone and ester C-O-C bending vibration"	1278 ,1232 ,1111 ,1060
"C-O (ether) stretch"	1147
"Alcohol C –OH stretch"	962
"Trisubstitutedolefinic C-H wagging"	842
SD4	
"Alcohol O-H stretching"	3535
"Olefinic C-H stretching vibration"	3016
"Methyl and methylene C-H stretching vibration"	2962 ,2926
"C-H stretch"	2875

“Lactone and ester carbonyl stretch”	1724 ,1713 ,1695
“carbonyl group”	1653
“Methyl and methylene bending vibration”	1423 ,1357 ,1342
“Lactone and ester C-O-C bending vibration”	1286 ,1215 ,1076 ,1060
“Alcohol C –OH stretch”	968
“Trisubstitutedolefinic C-H wagging”	869

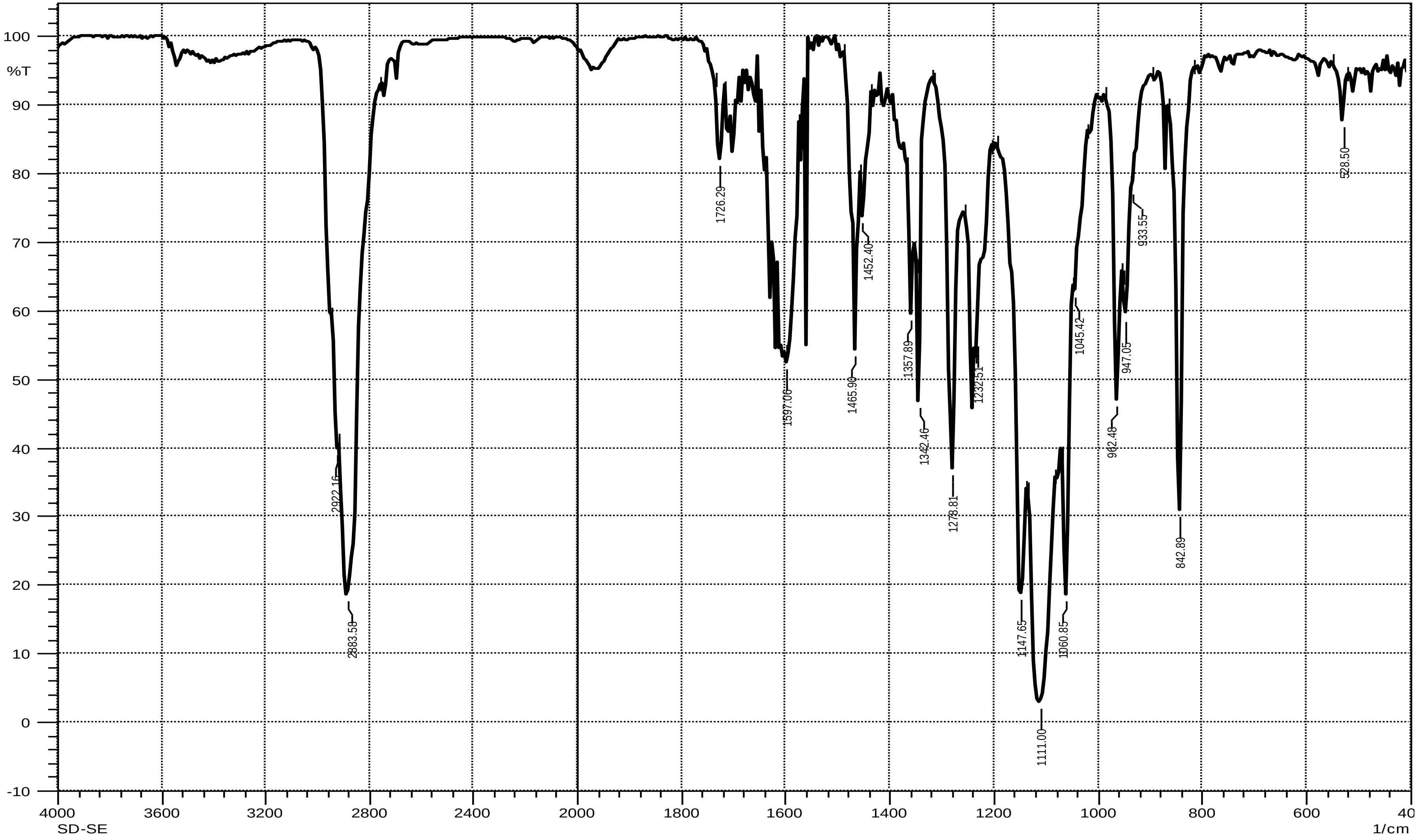


Fig. 14: FTIR Spectrum of SE4

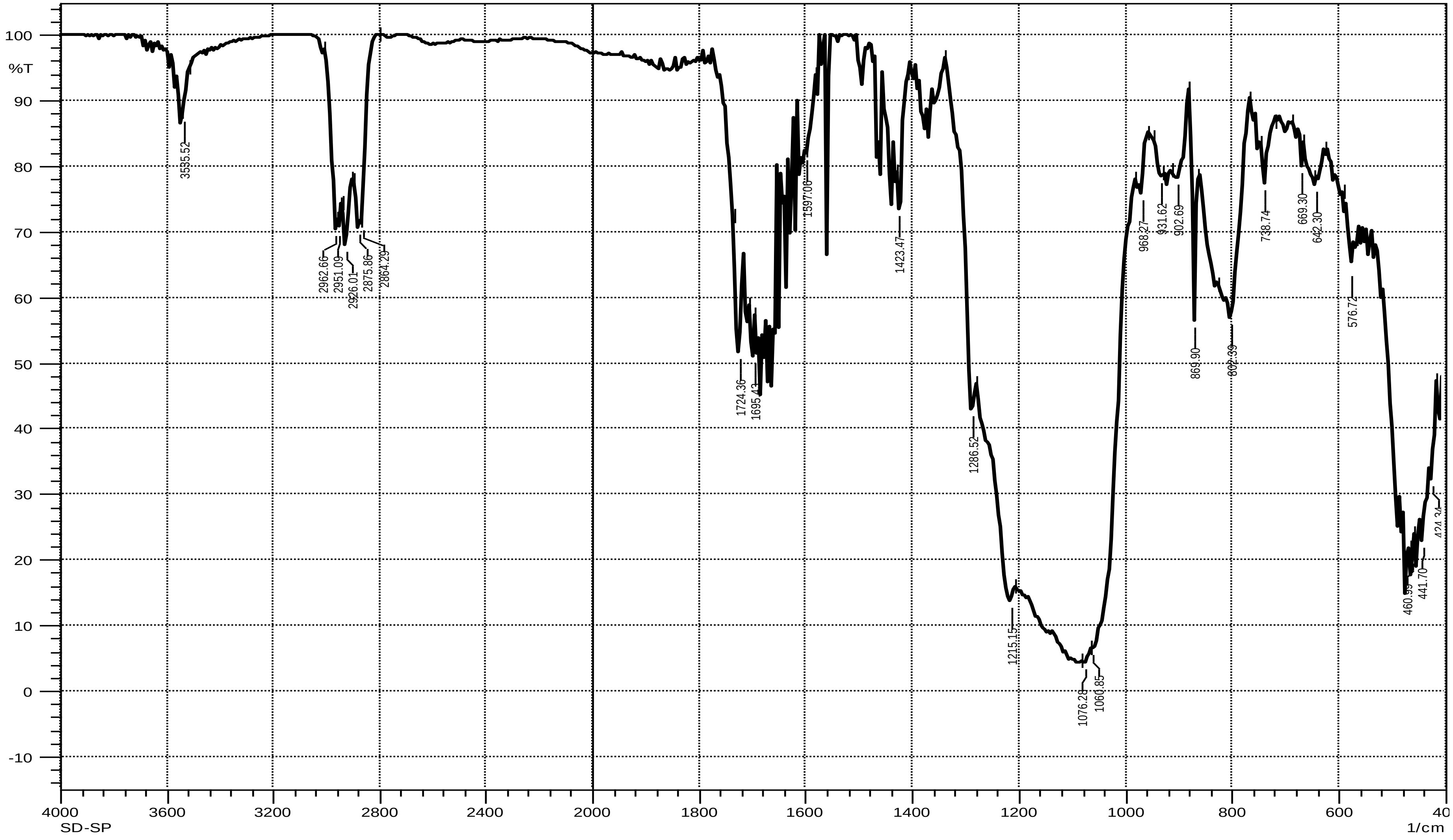


Fig. 15: FTIR Spectrum of SD4

4.3.4. SEM: The microphotographs of pure Lovastatin and optimized formulations SE4 and SD4 are shown in the figure 16.

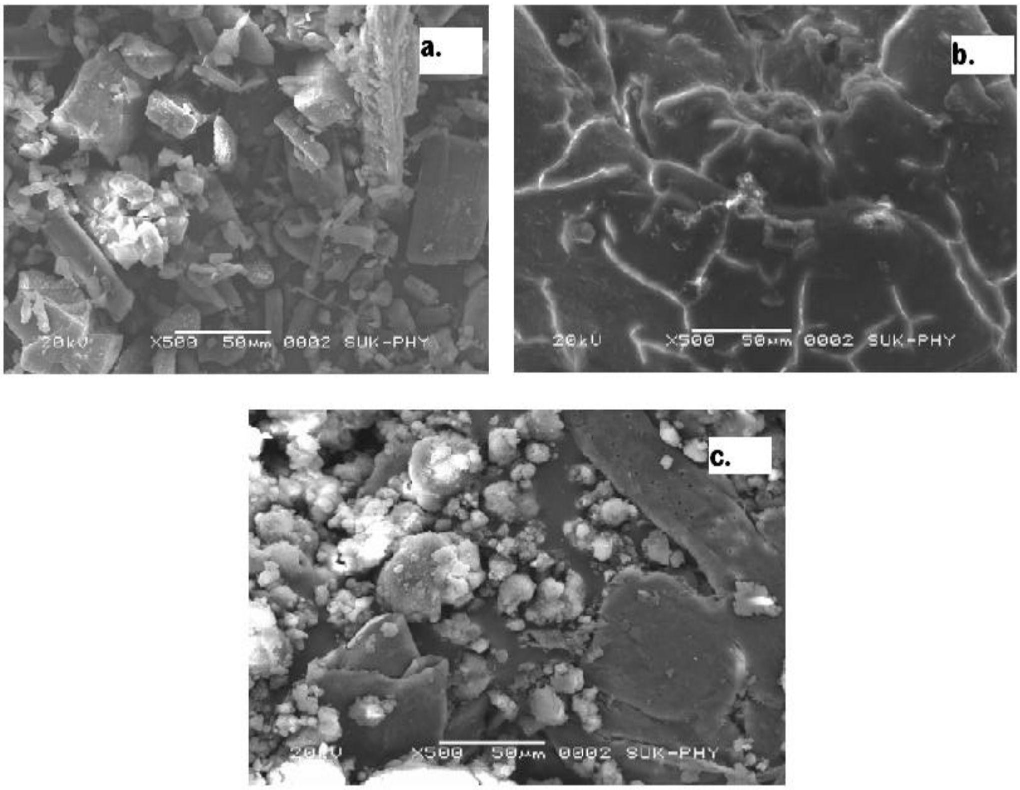


Fig. 16: SEM photomicrographs of a. Lovastatin Pure drug, b. SE4 and c. SD4 Formulation.

4.3.5. PXRD analysis

PXR Diffractograms of pure Lovastatin (LVS), SE4 and SD4 are shown in the figure 17.

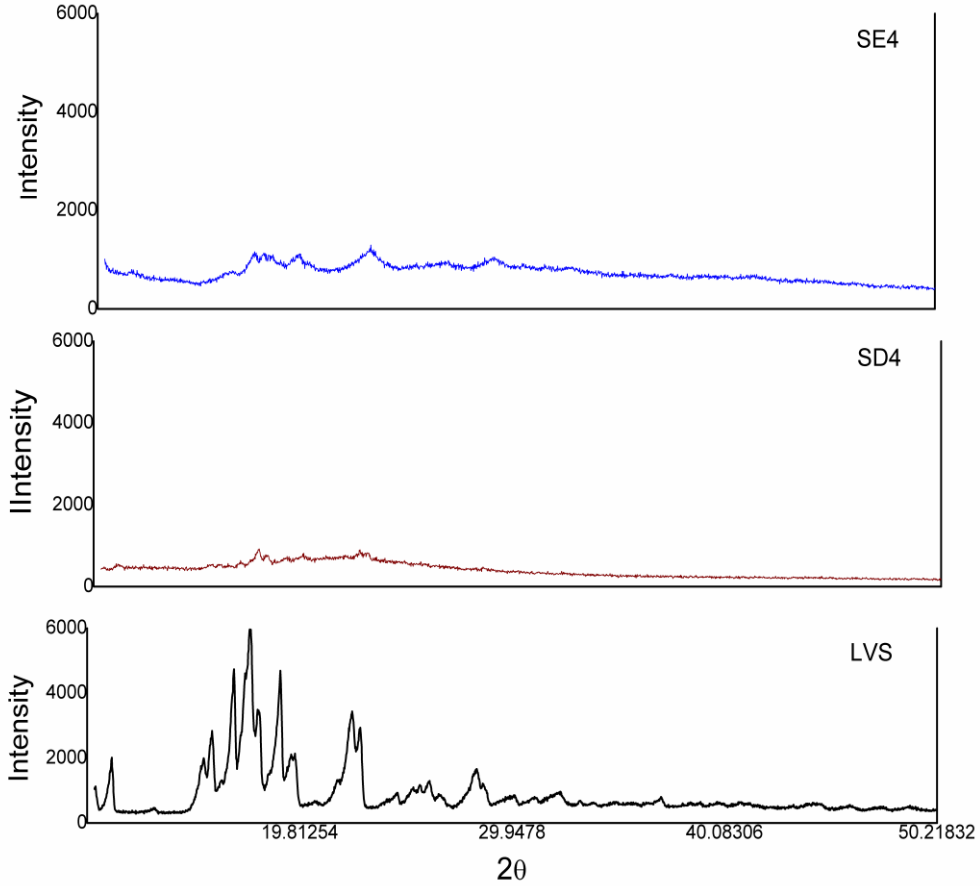


Fig. 17: PXR Diffractograms of LVS- Lovastatin, SE4 and SD4 formulations.

4.3.6. DSC:

The thermal behaviour of pure drug Lovastatin, polymers and solid dispersions was studied by DSC. The DSC therrmograms for pure Lovastatin, PEG4000, PVP K30, SE4 and SD4 are shown in the figure 18.

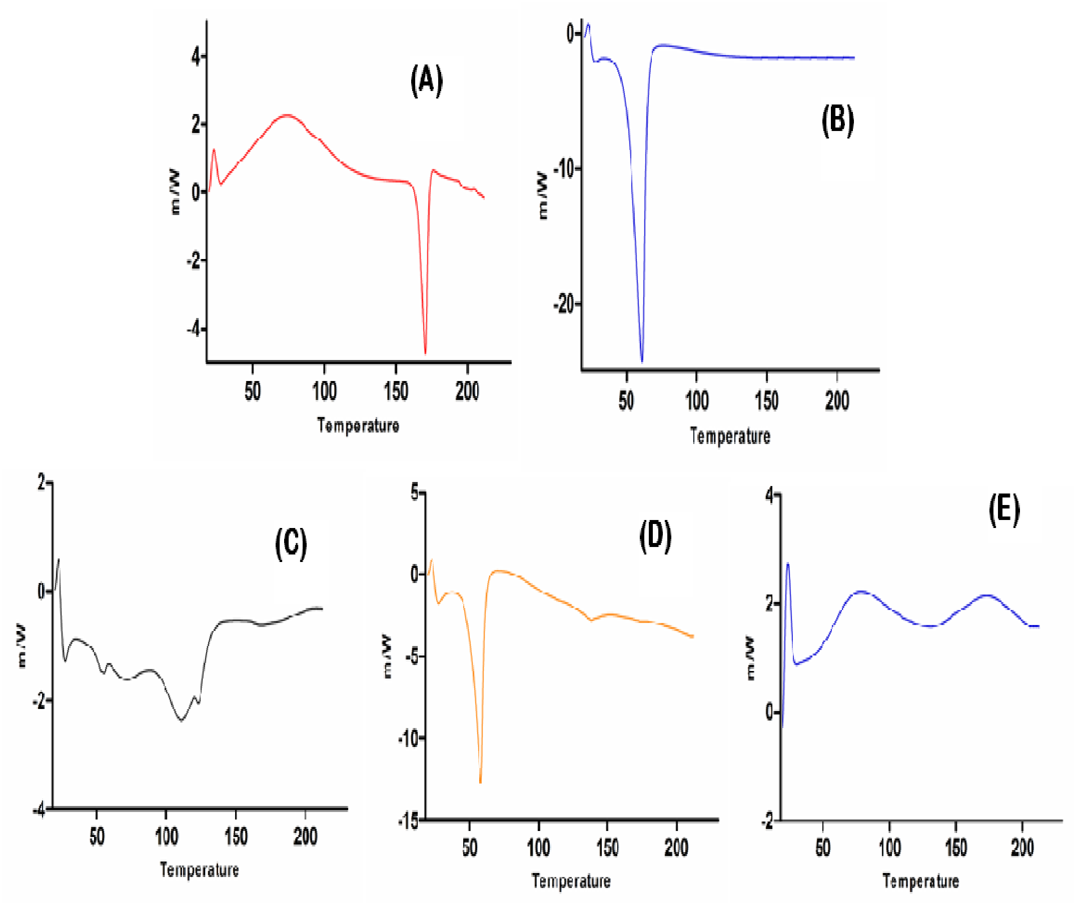


Fig. 18: DSC Diffractograms of (A) Lovastatin (B) PEG-4000 (C) PVP K30 (D) SE4 (E) SD4

4.3.7. Dissolution studies:

Dissolution profiles of pure Lovastatin and solid dispersions, both SE (Solvent evaporation) formulations and SD (spray drying) formulations over a period of 3 hours are shown in the Table 15. The results show that the in *vitro* dissolution rate of pure Lovastatin is less i.e., 23.63% is the cumulative percentage release in 3 hours. Whereas all the formulations (SE1 to SE4 and SD1 to SD4) shown better improvement in dissolution rate. When compared with pure API, formulation SD4 showing 93.8% and formulation SE4 showing 89.1% cumulative percent release which are depicted in Figure 19.

Table 15: Dissolution profiles of pure Lovastatin, SE (Solvent evaporation) and SD (Spray drying) formulations.

Time in minutes	Cumulative % drug release of formulations								
	Pure drug	(Solvent evaporation method)				(Spray drying method)			
		SE1	SE2	SE3	SE4	SD1	SD2	SD3	SD4
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	05.00±1.2	50.16±2.0	56.26±2.9	59.20±2.1	64.13±3.2	52.50±2.2	58.26±3.0	60.86±1.4	72.06±4.4
30	11.23±1.5	54.26±3.0	60.46±3.0	64.20±1.0	68.50±2.8	56.30±3.0	61.36±3.7	63.66±1.3	76.83±3.7
40	13.20±3.05	58.46±3.2	65.40±0.8	67.36±1.0	72.66±3.4	61.00±3.4	64.63±2.2	66.90±2.4	83.36±3.9
60	16.10±2.2	62.60±3.1	68.26±2.1	70.66±2.8	77.53±3.1	65.66±2.4	67.96±3.6	71.26±2.1	86.00±3.6
90	17.86±1.4	69.63±1.4	72.93±2.5	76.43±2.1	83.60±1.6	70.36±1.8	74.93±2.2	77.90±2.0	90.56±1.8
120	19.83±1.6	71.96±1.8	75.96±1.0	79.33±1.8	86.50±2.9	75.53±2.2	79.36±0.9	82.60±1.3	91.90±1.6
180	23.63±1.8	74.43±2.1	79.73±1.7	85.63±1.1	89.10±3.3	80.70±2.3	85.00±0.5	89.10±2.5	93.80±2.8

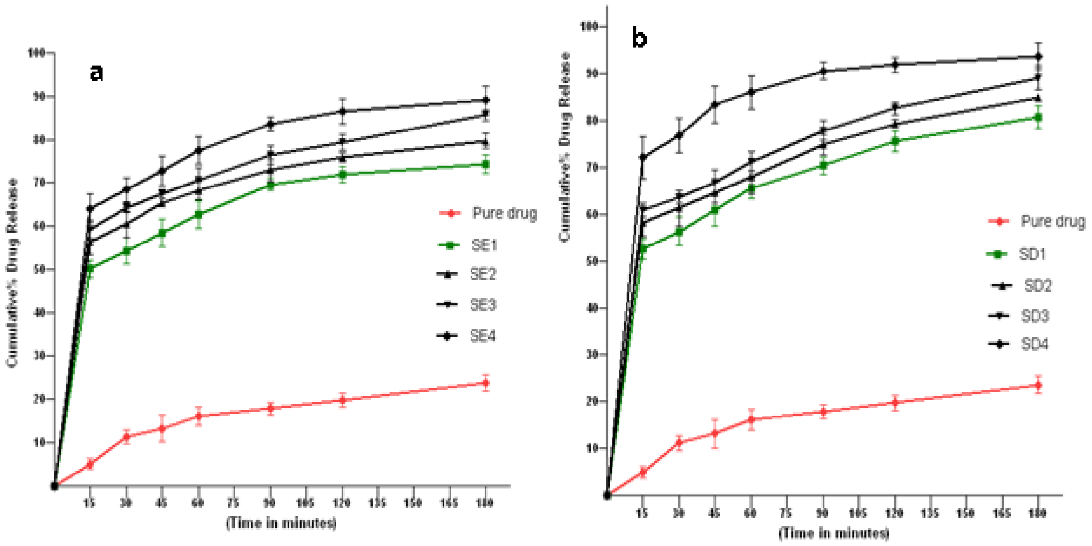


Figure 19: Dissolution profiles of solid dispersions of (a) Solvent evaporation method and (b) Spray drying method along with pure drug comparison

4.3.8. *In-vivo* study

The serum lipid levels of all the experimental groups at specific time intervals are shown in table 16. The change in total cholesterol, triglycerides, HDL and LDL along with their percentage change in respective lipids for all the groups are plotted in figure 20 to 23.

Table 16: The serum lipid levels of all experimental groups at specific time intervals

Experimental Groups	Time intervals	Total cholesterol (mg/dl)	TG(mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)
Control	**Initial**	**59.57 ±1. 5**	**66.38±0.9**	**28.35±0.8**	**17.95±0.7**
	7 days	**65.55±1.27**	**78.16±1.4**	**26.37±0.5**	**23.77±0.6**
	14 days	**83.2±1.1.19**	**89.47±1.2**	**24.3±1.0**	**41.2±1**
Reference	**Initial**	**54.6±1.60**	**60.6± 2.74**	**29.48±1.2**	**12.98±0.6**
	7 days	**51.2±1.0**	**61.3±2.3**	**30.4±1.3**	**8.53±0.8**
	14 days	**49.44±0.99**	**59.3±2.1**	**31.1±1.2**	**6.4±0.7**
Test	**Initial**	**55.3±1.3**	**60.37±1.5**	**27.75±0.9**	**15.85±0.8**
	7 days	**51.4±1.65**	**58.78±1.3**	**30.32±1.4**	**9.3±1.1**
	14 days	**46.97±0.8**	**53.3±0.94**	**32.25±0.99**	**4.01±0.4**

$n=6$, $p<0.05$

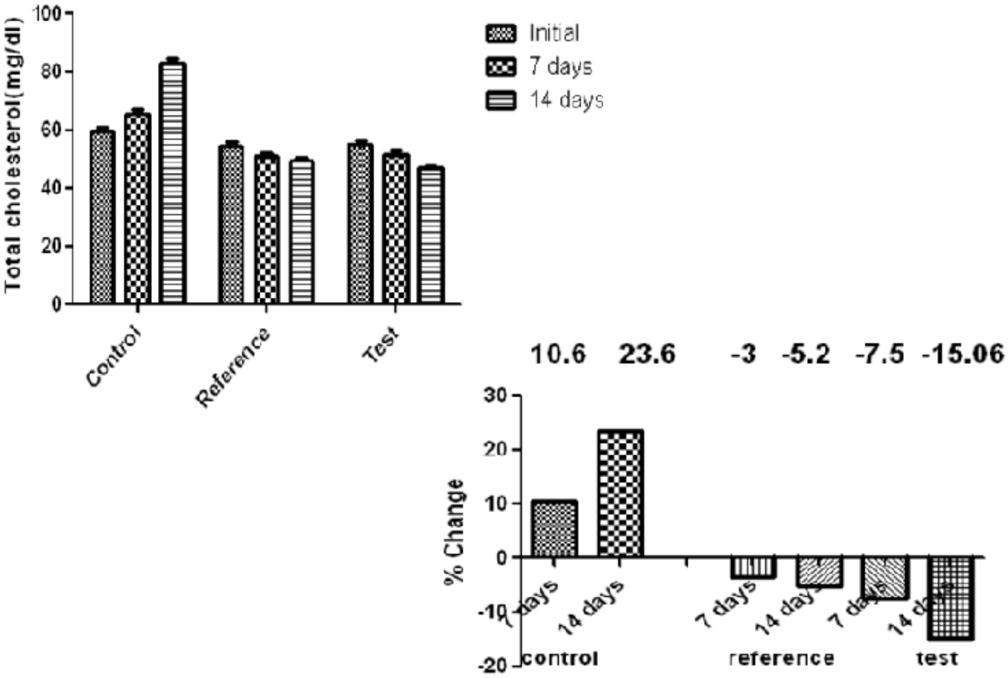


Fig.20: Serum total cholesterol levels of experimental groups

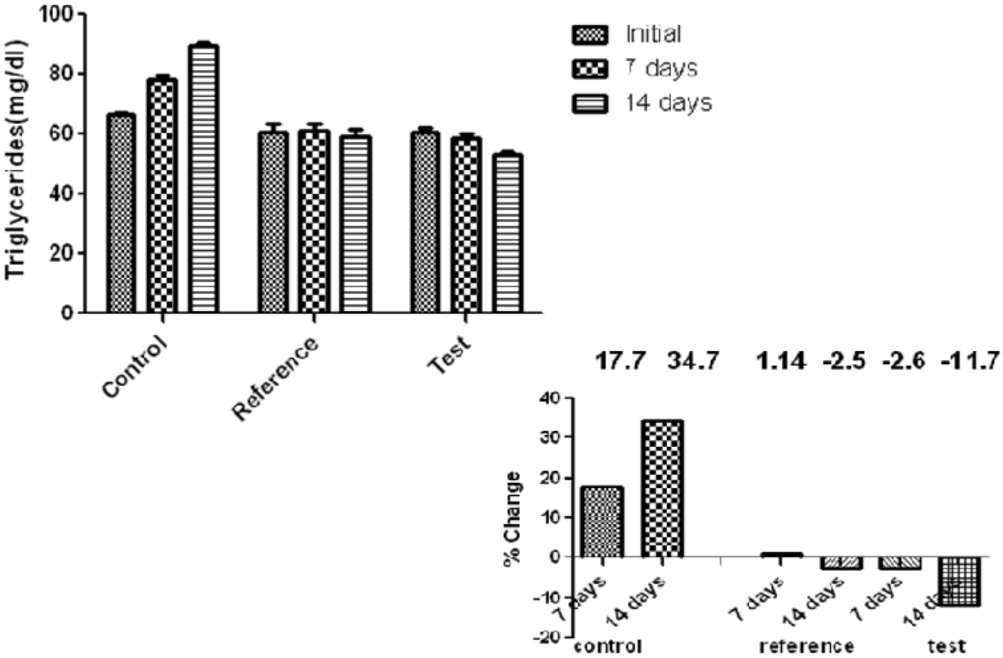


Fig. 21: Serum Triglycerides levels of experimental groups

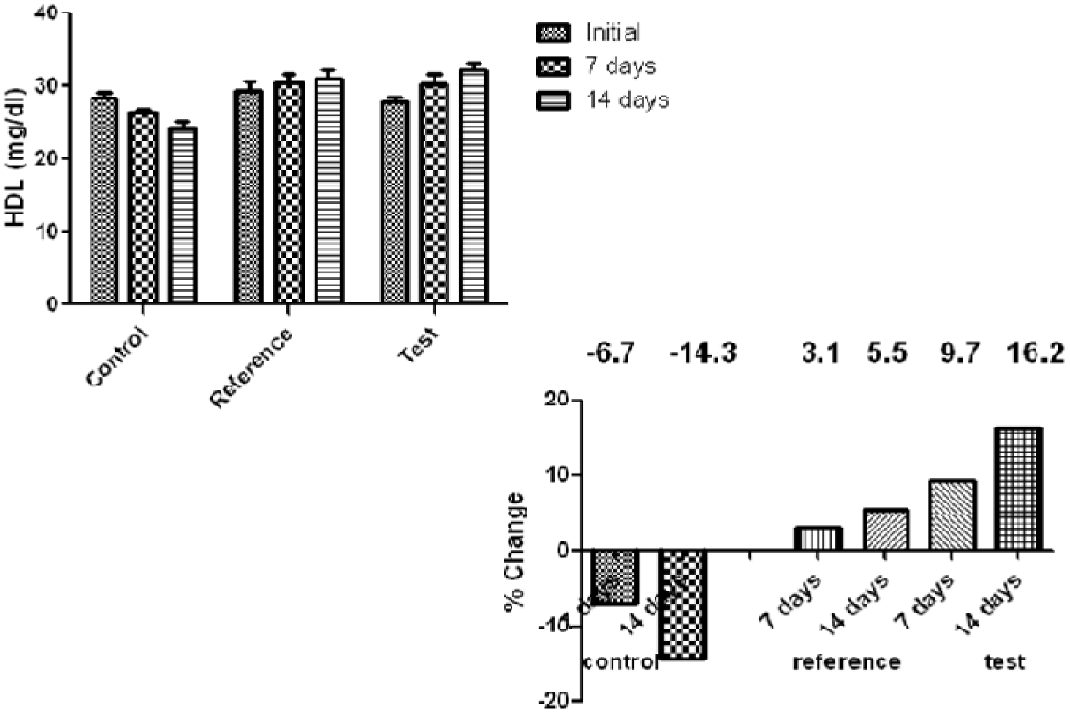


Fig. 22: Serum HDL cholesterol levels of experimental groups

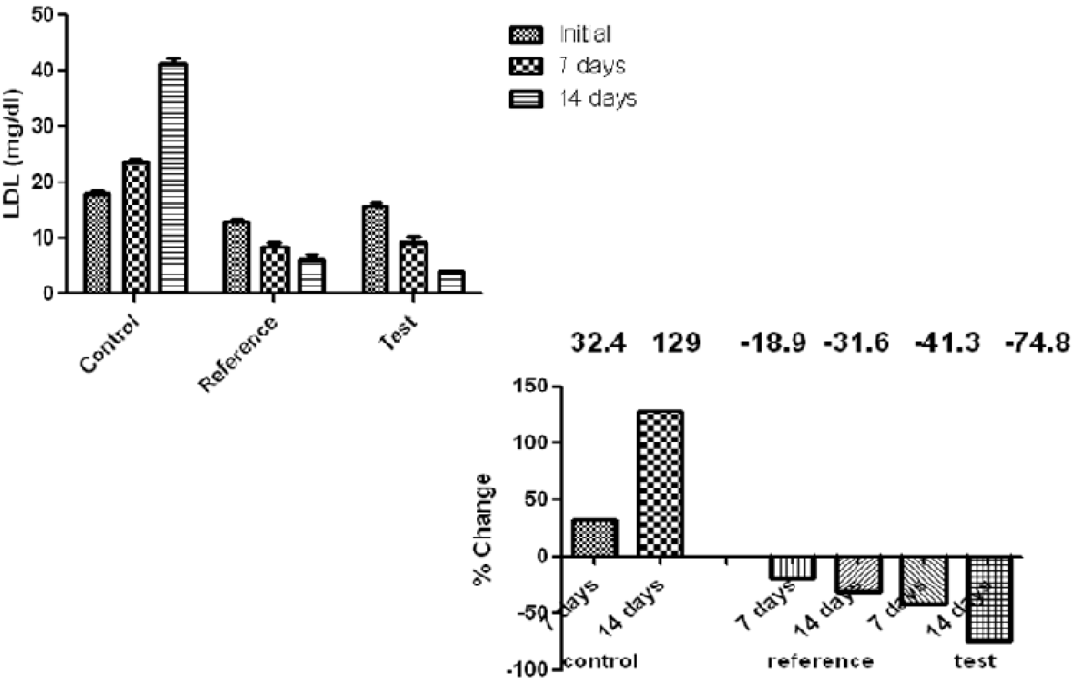


Fig. 23: Serum LDL cholesterol levels of experimental groups

4.3.9. Accelerated stability study

The solid dispersions, which were considered as best formulations i.e., SE4 and SD4 were subjected for accelerated stability study as per ICH guidelines at 40 ^{o}C /75% RH up to 3 months. Periodically (15days, 1 month and 3 months) samples were checked for *in vitro* drug release and property of recrystallinity using PXRD studies. The results are shown in the figure 24 and 25 respectively. The decrease in *in-vitro* drug release after each specified time interval was found to be insignificant. Additionally, there were no changes found in the powder X-ray diffractograms of SE4 and SD4, indicating that there were no peaks corresponding to the crystallinity of Lovastatin found in the diffractograms.

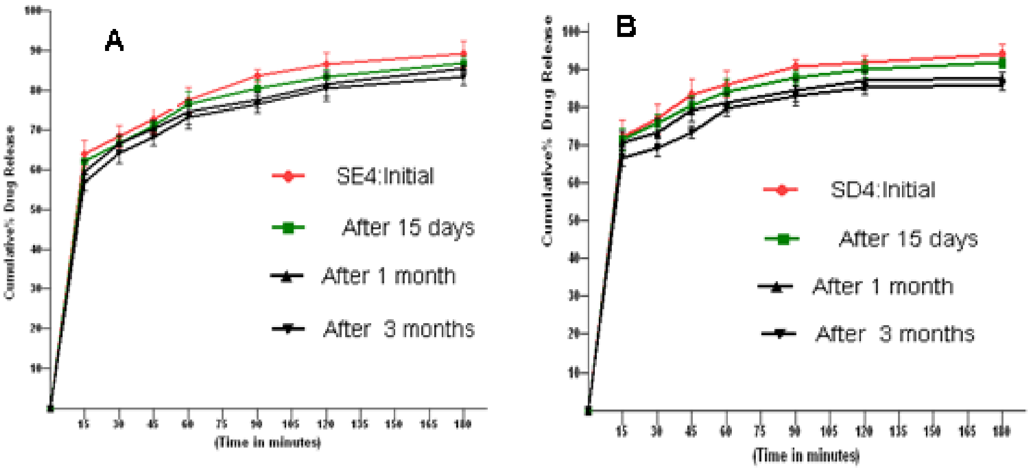


Fig. 24: Dissolution profiles of (A) SE4 and (B) SD4 (Stability studies).

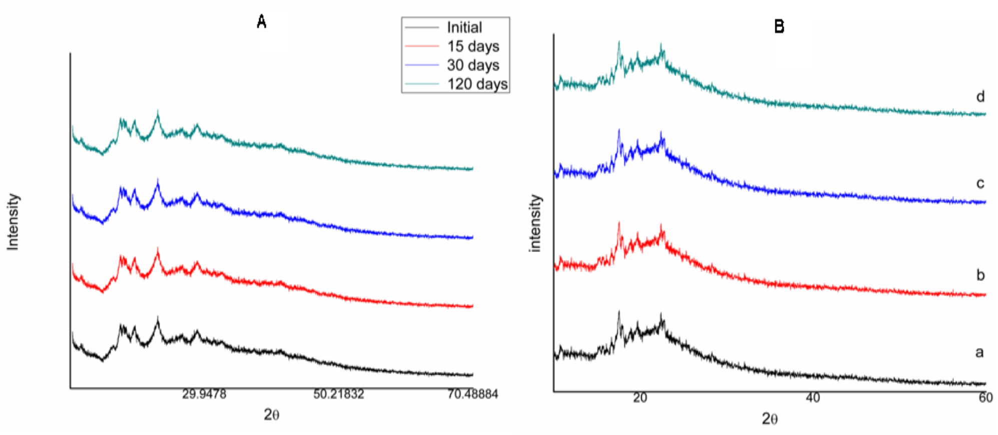


Fig. 25: Powder X-ray diffractograms of (A) SE4 and (B) SD4 (Stability studies).

4.4 FORMULATIONS OF TRANSBUCCAL FILMS BY USING SOLID DISPERSIONS

Transbuccal formulations of SD4 were prepared using three different polymers HPMCK4M, HPMC E5 LV and chitosan. The prepared transbuccal films were characterized and compared. The formulations containing different concentrations of polymers were prepared and their effects on physico-chemical characteristics were studied. However, the selection of plasticizer and permeation enhancer was finalized during placebo film preparation. Based on the results of physico-chemical and other characterization methods, the best formulations were selected.

4.4.1Compatibility studies:

Compatibility studies were conducted for SD4 and polymers used to prepare transbuccal film. SD4, HPMC K4M, HPMC E5LV, Chitosan, physical mixtures of SD4 and respective polymer of transbuccal films were subjected for Infrared spectroscopic analysis. The spectra of the samples are shown in the figure 26 to 32.

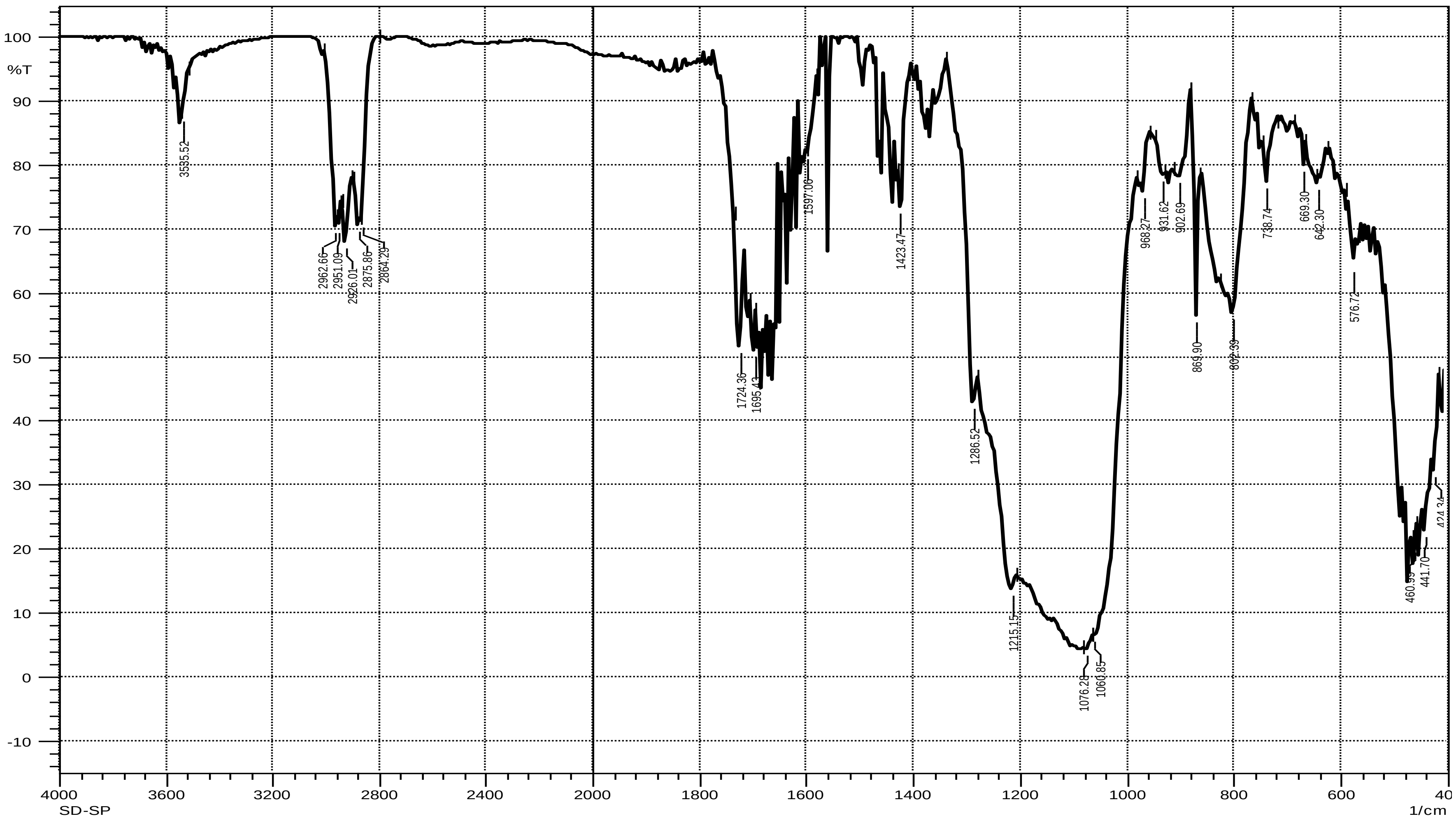


Fig. 26: FTIR Spectrum of SD4

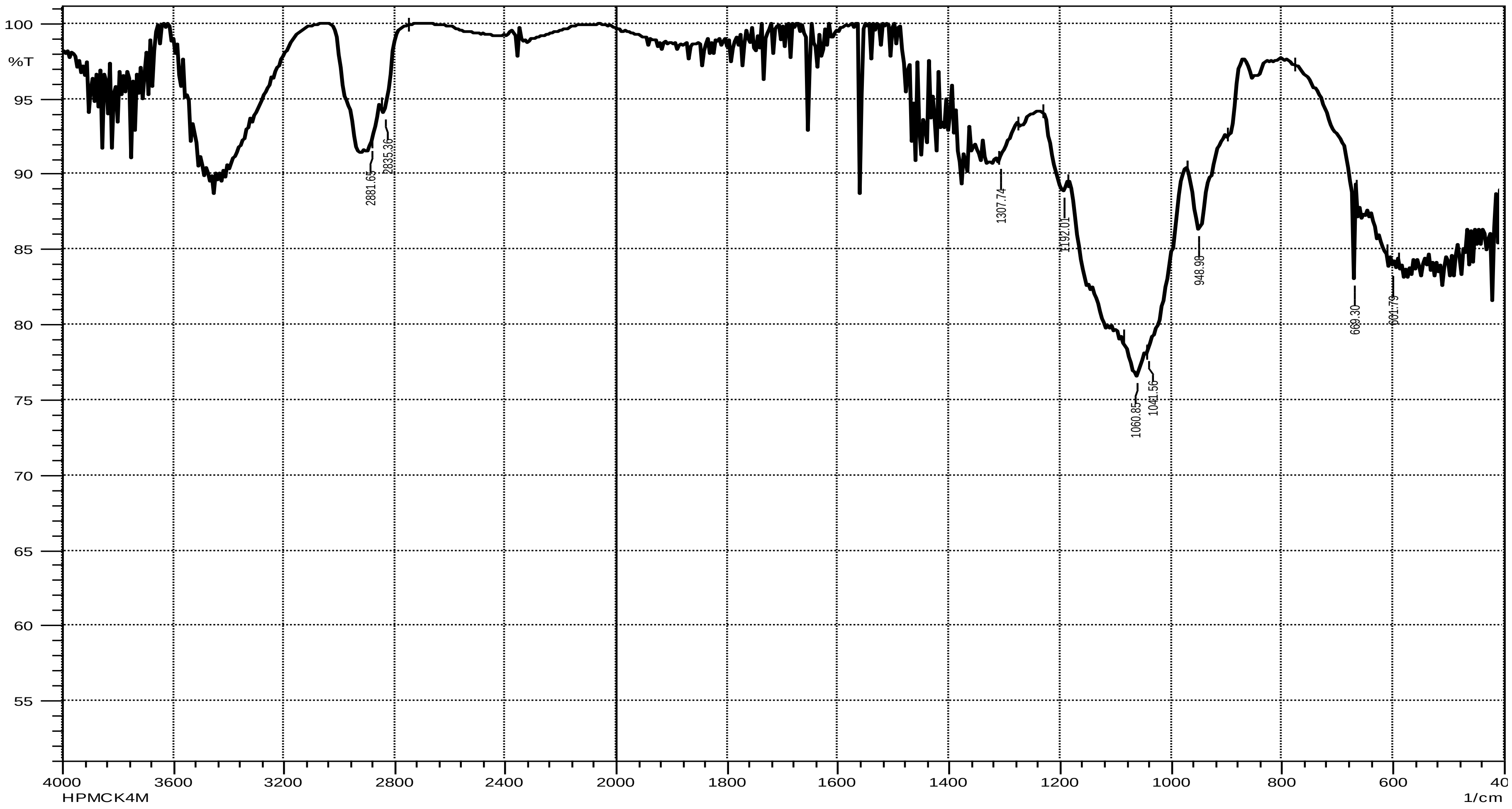


Fig. 27: FTIR Spectrum of HPMC K4M

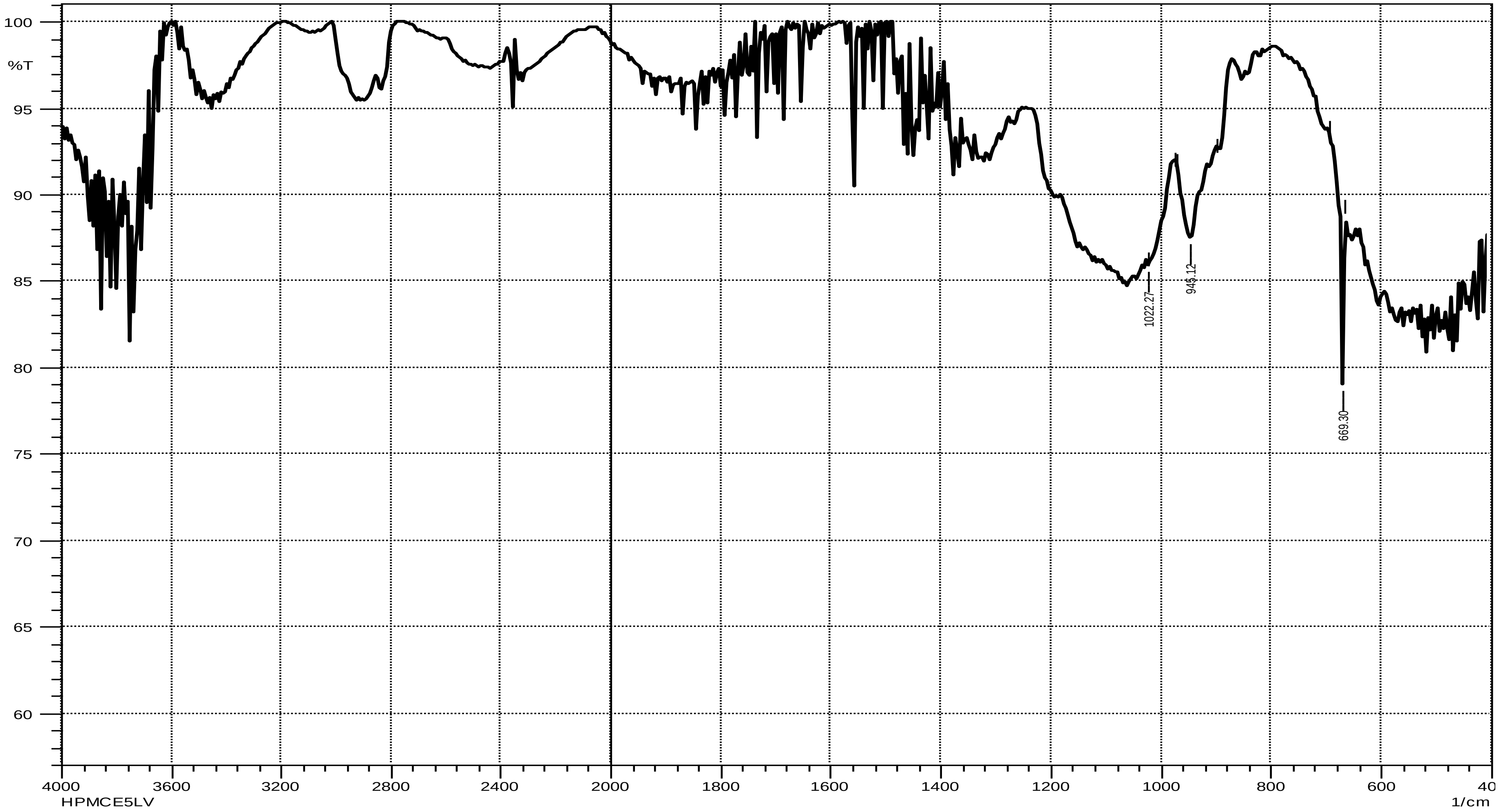


Fig. 28: FTIR Spectrum of HPMC E5LV

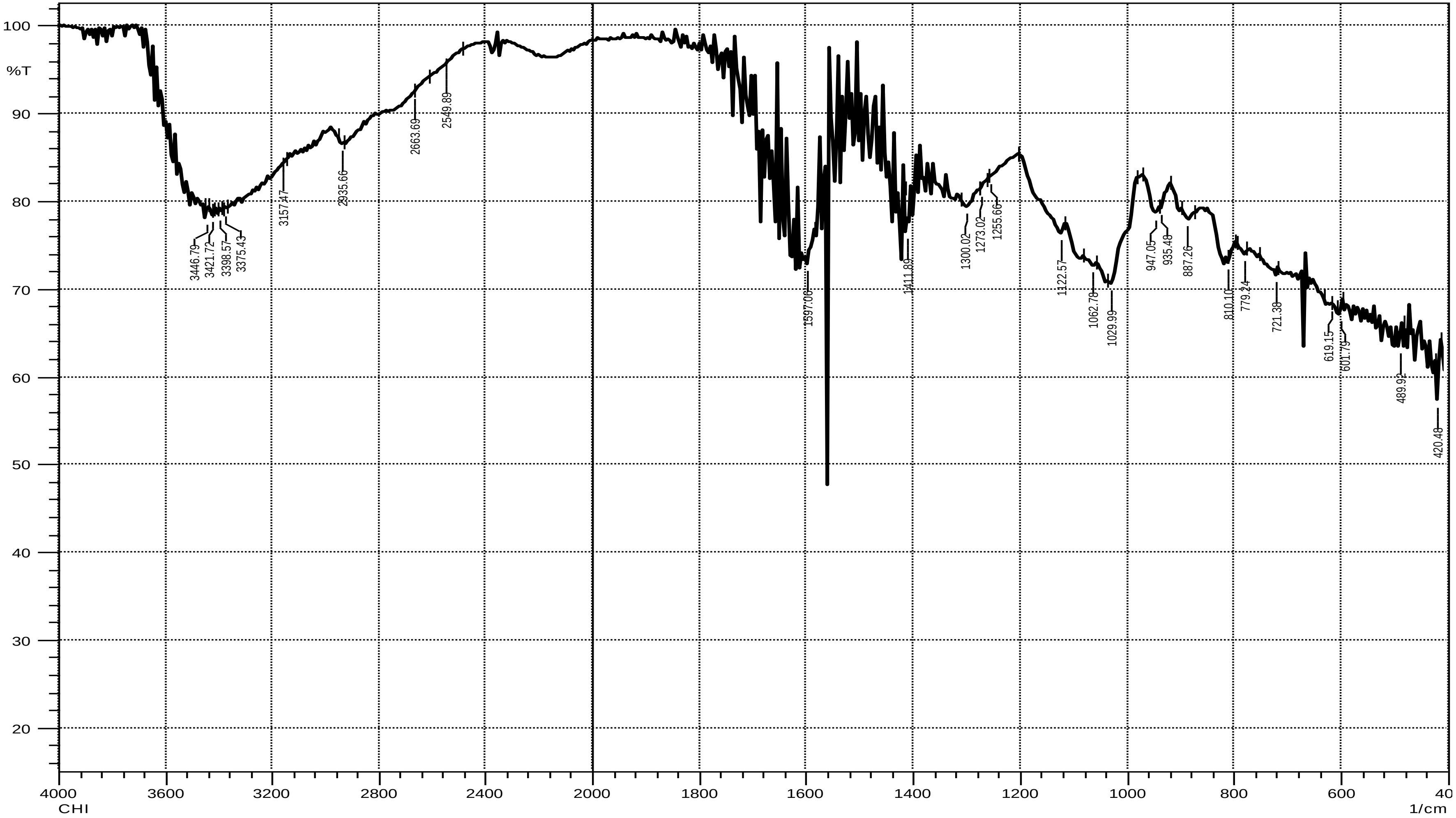


Fig. 29: FTIR Spectrum of Chitosan

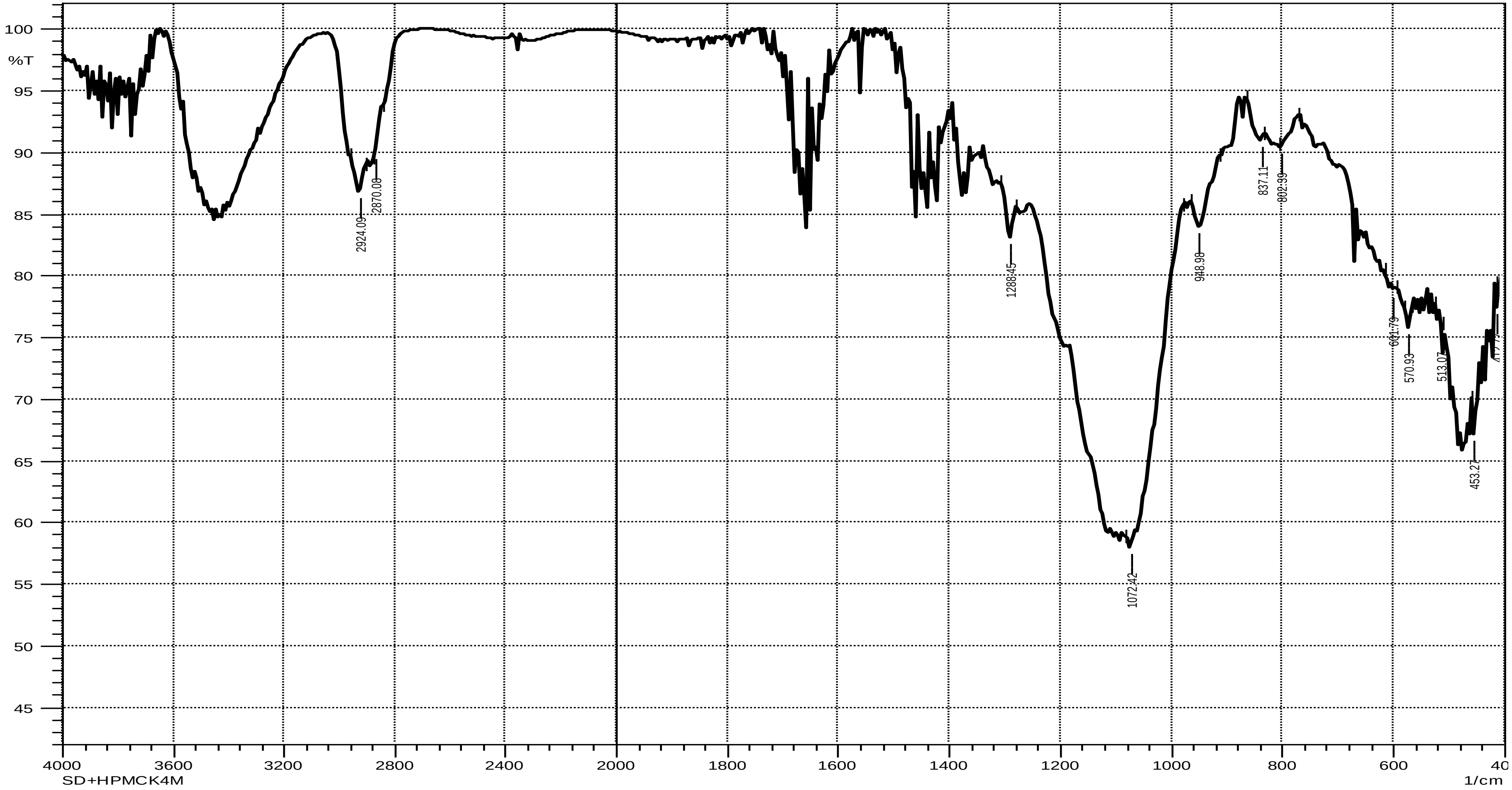


Fig. 30: FTIR Spectrum of physical mixtures of SD4 and HPMC K4M

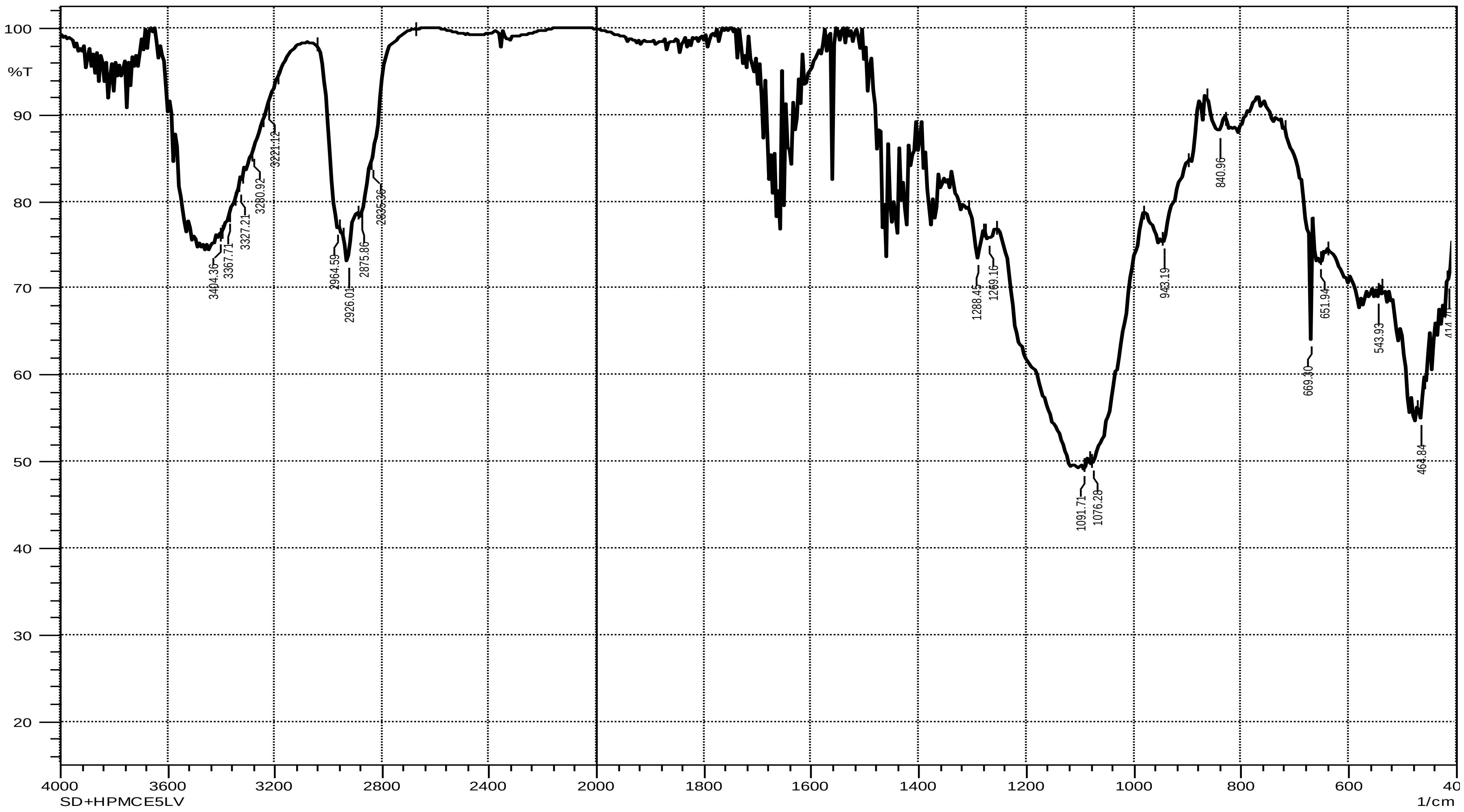


Fig. 31: FTIR Spectrum of physical mixtures of SD4 and HPMC E5LV

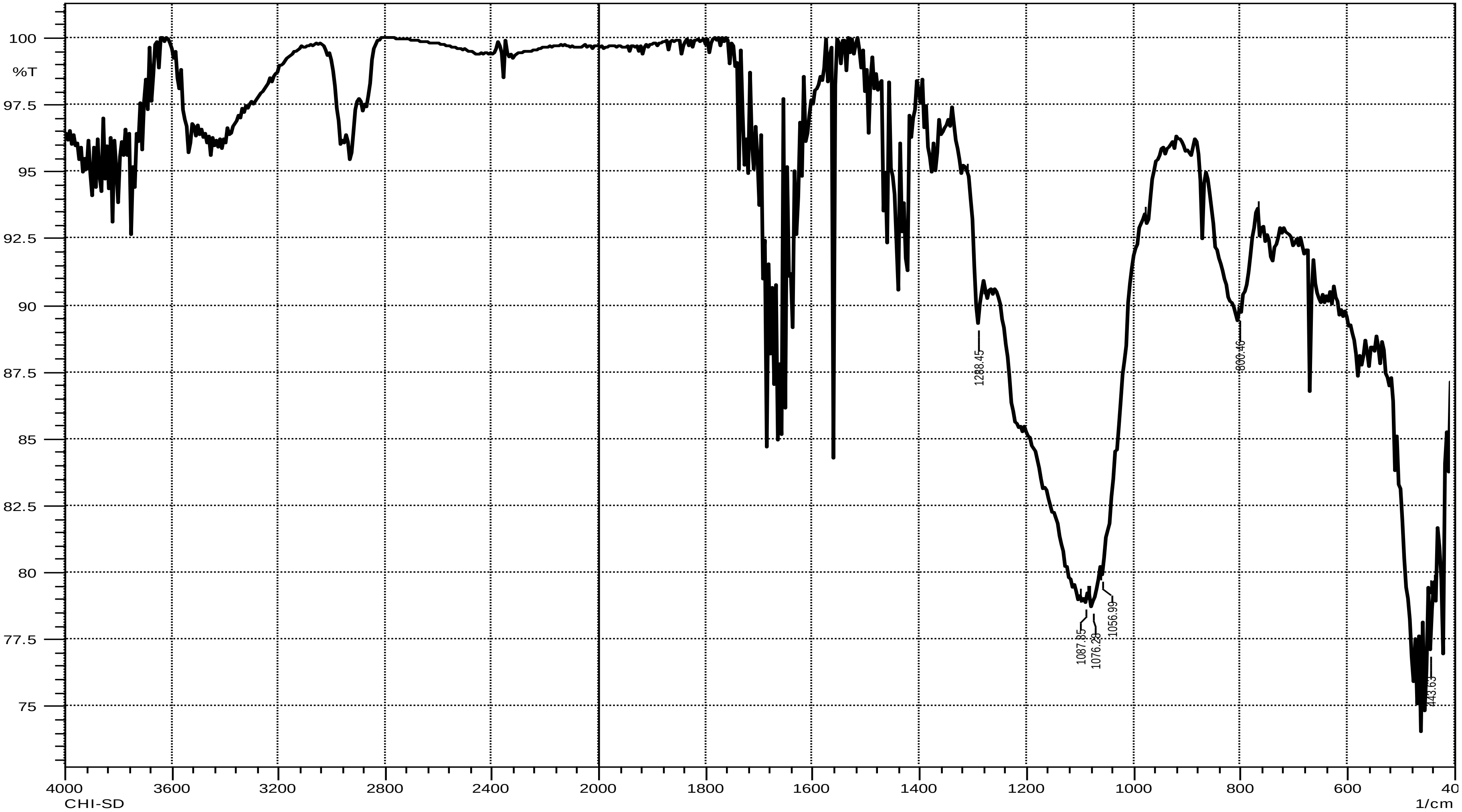


Fig. 32: FTIR Spectrum of physical mixtures of SD4 and Chitosan

4.5 PHYSICO-CHEMICAL CHARACTERIZATION OF TRANSBUCCAL PATCHES:

The physico-chemical characteristics data of transbuccal patches are shown in the tables 17-22.

Table 17: Physico-chemical characteristics of Transbuccal film by using HPMC K4M (F1-F6)

Formulation code	Thickness (mm)±SD	Weight of film (mg) ±SD	Drug Content (%) ± SD	Surface pH ± SD	Swelling Index (%) ±SD	Folding endurance ±SD
F1	0.27±0.05	91.91±0.2	91.71±0.33	6.2±0.07	31.7±3.5	315±7.5
F2	0.34±0.02	101.25±0.3	93.23±0.41	6.4±0.05	42.9±5.1	312±5.0
F3	0.51±0.04	108.32±0.4	95.75±0.56	6.7±0.04	56.0±2.1	350±2.5
F4	0.62±0.01	115.56±0.2	94.80±0.61	6.4±0.12	57.9±2.8	340±6.0
F5	0.68±0.03	125.46±0.6	91.68±0.23	6.3±1.23	49.4±1.8	324±4.5
F6	0.81±0.1	132.03±0.8	89.34±0.17	5.8±0.98	61.3±1.6	307±9.0

SD=Standard deviation, n=3

Table 18: Physico-chemical characteristics of Transbuccal film by using HPMC E5 (F7-F12)

Formulation code	Thickness (mm)±SD	Weight of film (mg) ±SD	Drug Content (%)±SD	Surface pH± SD	Swelling Index (%) ±SD	Folding endurance ±SD
F7	0.21±0.07	89.10±0.6	90.23±0.51	7.6±0.98	23.0±4.1	326±4.7
F8	0.27±0.04	98.91±0.5	95.27±0.47	6.7±0.07	27.0±3.6	319±5.1
F9	0.55±0.03	103.25±0.3	97.33±0.31	6.9±0.12	48.2±1.1	345±1.8
F10	0.82±0.02	112.16±0.2	93.82±0.11	6.3±0.83	52.0±2.6	337±3.1
F11	0.88±0.06	121.36±0.5	88.67±0.61	7.2±1.23	61.3±2.3	312±2.5
F12	0.91±0.1	128.57±0.3	85.47±0.87	6.4±0.03	56.4±5.2	336±4.5

SD=Standard deviation, n=3

Table 19: Physico-chemical characteristics of Transbuccal film by using Chitosan (F13-F18)

Formulation code	Thickness (mm)±SD	Weight of film (mg) ±SD	Drug Content (%)±SD	Surface pH±SD	Swelling Index(%) ±SD	Folding endurance ±SD
F13	0.92±0.03	91.91±0.4	93.51±0.43	5.6±0.67	54.4±5.2	193±1.1
F14	0.98±0.06	104.25±0.5	94.23±0.71	6.2±0.12	58.2±1.7	194±2.5
F15	1.15±0.07	103.32±0.2	93.75±0.76	6.5±0.27	63.3±1.6	199±3.7
F16	1.12±0.53	108.56±0.1	95.80±0.41	6.7±1.63	68.3±1.6	208±0.8
F17	1.5±0.39	122.46±0.7	92.68±0.73	7.2±0.28	76.5±3.6	176±3.8
F18	1.4±0.81	128.03±0.4	91.62±0.82	6.5±0.83	73.3±6.1	165±1.9

SD=Standard deviation, n=3

Table 20: Physico-chemical characterization, cumulative percentage release and percentage drug permeated of F1-F6.

Formulation code	*Ex-vivo* residence time	*Ex-vivo* bioadhesive strength(gm)	Detachment force (N/m^2)	% Drug released after 6 hours	% Drug permeated after 6 hours
F1	6.1± 0.12	40±1.41	3920	97.78±1.08	96.78±1.1
F2	6.2±0.08	42±0.86	4116	95.32±3.5	94.24±1.0
F3	6.5± 0.10	43±1.07	4214	94.17±0.62	93.27±2.6
F4	7.2± 0.15	44±1.43	4312	92.34±1.4	87.34±4.4
F5	7.3± 0.11	45±1.86	4410	89.24±2.0	77.43±3.0
F6	7.4 ± 0.13	47±0.88	4606	86.17±1.2	68.42±1.3

Table 21: Physico-chemical characterization, cumulative percentage release and percentage drug permeated of F7-F12.

Formulation code	*Ex-vivo* residence time	*Ex-vivo*bioadhesive strength(gm)	Detachment force (N/m^2)	% Drug released after 6 hours	% Drug permeated after 6 hours
F7	2.2± 0.08	30±0.64	2940	94.5±1.4	90.45±3.2
F8	2.3 ± 0.13	31±0.76	3038	92.7±1.1	89.57±3.9
F9	2.6± 0.18	32±0.63	3136	91.32±1.2	86.45±3.0
F10	2.7±0.16	34±0.35	3332	90.88±1.7	81.37±0.8
F11	2.7± 0.16	36±0.41	3528	89.88±1.7	78.73±1.3
F12	2.8± 0.17	37±0.36	3626	86.47±3.1	71.56±0.5

Table 22: Physico-chemical characterization, cumulative percentage release and percentage drug permeated of F13-F18.

Formulation code	*Ex-vivo* residence time	*Ex-vivo*bioadhesive strength(gm)	Detachment force (N/m^2)	% Drug released after 6 hours	% Drug permeated after 6 hours
F13	7.2± 0.17	54±0.52	5292	76.44±3.1	73.25±1.7
F14	7.5± 0.08	57±0.84	5586	74.15±1.0	69.83±4.8
F15	7.9± 0.12	60±0.57	5880	72.35±2.2	65.23±2.8
F16	8.1± 0.61	62±0.33	6076	71.83±3.6	63.12±3.6
F17	8.1± 0.91	65±0.57	6370	70.79±2.5	60.56±2.6
F18	8.2±0.17	66±0.43	6468	68.73±1.1	57.34±2.1

4.5.8. *In -vitro* drug release

The drug release profile of formulations is shown in the figure 33. In case of HPMC K4M formulations, at 6th hour, F1 shows the highest drug release of 97.78% and F6 is 86.17%. In case of HPMC E5 formulations, at 6th hour, F7 shows the highest drug release of 94.5% andF12 is 86.47%. In case of Chitosan formulations, at 6th h, F13 shows the highest drug release of 76.44% and F18 is 68.73%.

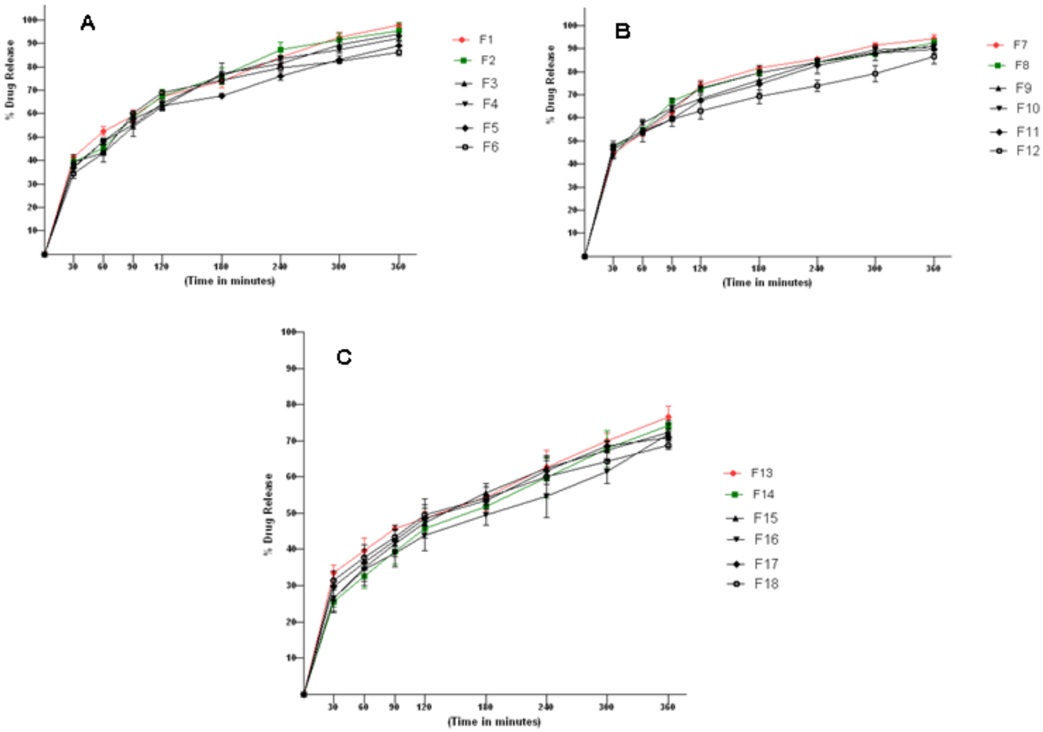


Fig.33: Percentage drug release profile of transbuccal films (A) F1-F6 (B) F7-F12 (C) F13-F18

4.5.9. *Ex-vivo* drug permeation study

Fig.34 shows the drug permeation profile of formulations. Drug permeated at the 6th hour of F1 is 96.78% and F6 is 68.42%. Similarly, F7 is 90.45 % and F12 is 71.56 %. Drug permeated at the 6th hour of F13 is 73.25 % and F18 is 57.34 %.

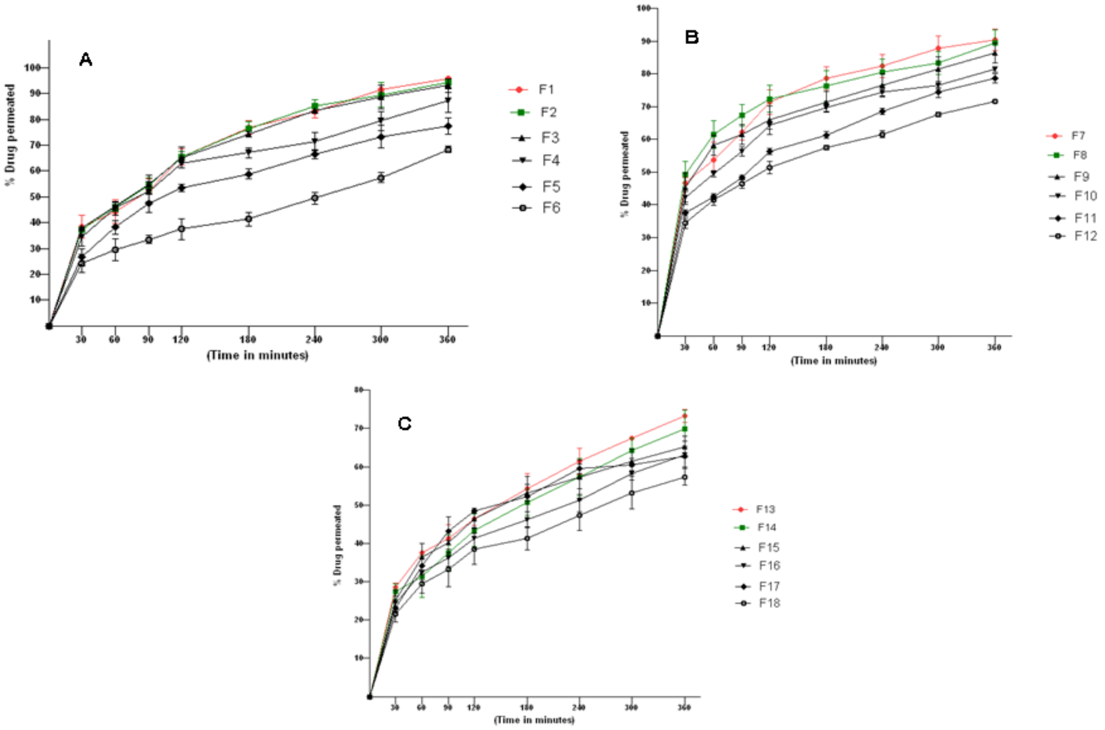


Fig. 34: Percentage drug permeation profile of transbuccal films (A) F1-F6 (B) F7-F12 (C) F13-F18

4.5.10. Drug release kinetics

To study the release and permeation kinetics from buccal films, the drug release and *ex-vivo* permeation data were subjected to fit various kinetic models using “PCP Disso. ver. 2 software Pune India”. The $r^2$and n values are determined. The $r^2$and n values, diffusion mechanism, and best fit model are shown in the table 23 and 24.

Table 23: Drug release kinetics of transbuccal films (n value and r^2), diffusion mechanism and best fit model

Formulation code	n value	r^2	Mechanism	Best fit model
F1	0.834	0.972	Non-Fickian diffusion	Higuchi
F2	0.865	0.975		
F3	0.860	0.978		
F4	0.856	0.975		
F5	0.822	0.963		
F6	0.853	0.940		
F7	0.811	0.939		
F8	0.776	0.914		
F9	0.772	0.927		
F10	0.792	0.919		
F11	0.769	0.929		
F12	0.734	0.905		
F13	0.785	0.969		
F14	0.863	0.995		
F15	0.848	0.989		
F16	0.811	0.980		
F17	0.806	0.978		
F18	0.769	0.957		

Table 24: Drug permeation kinetics of transbuccal films (n value and r^2), diffusion mechanism and best fit model

Formulation code	n value	r^2	Mechanism	Best fit model
F1	0.877	0.984	Non-fickian diffusion	Higuchi
F2	0.870	0.980		
F3	0.858	0.979		
F4	0.830	0.966		
F5	0.867	0.982		
F6	0.817	0.975		
F7	0.78	0.923		
F8	0.728	0.863		
F9	0.739	0.894		
F10	0.754	0.913		
F11	0.78	0.958		
F12	0.755	0.946		
F13	0.820	0.985		
F14	0.829	0.989		
F15	0.808	0.968		
F16	0.798	0.981		
F17	0.826	0.950		
F18	0.794	0.981		

4.5.11. Stability study

Accelerated stability data of the best formulation F3 is given in Table 25.

Table 25: Accelerated stability data of the best formulation F3.

Time	Drug content	% drug released	% drug permeated
Initial	93.80±0.71	93.13±2.15	92.34±1.14
1 month	93.21±0.37	92.17±3.17	92.23± 1.67
2 months	92.34± 0.54	92.45±1.13	91.45±2.44
3 months	91.46± 0.56	91.17±2.23	91.87±1.24

5. DISCUSSION:

PREFORMULATION STUDIES:

Characterization of pure drug:

The preformulation study was done for the identification of drug by its physical and chemical properties and to confirm its compatibility with other excipients used in the final formulation. The FTIR spectrum of obtained drug shows respective peaks of Lovastatin functional groups and also showed maximum absorbance (λ_{max}) at 238nm in the spectrum of drug obtained by UV-Visible spectrophotometer as per literature review, Analytical profiles of drug substances and excipients[52].Thus, it can be confirmed that the drug obtained from a company was Lovastatin.

"Standard calibration curve in analytical method is a linear relationship between concentration and absorbance". Standard calibration of Lovastatin was linear in the range of 5-25 µg/ml at 238 nm. The unknown sample concentrations can be calculated by using slope and intercept values of standard calibration curve and absorbance of suitable diluted unknown sample solution. Lovastatin is sparingly soluble in water and found to be 0.4 µg/ml. The DSC thermogram of Lovastatin shows a sharp endothermic peak at 170 ^{o}C which was a due to melting of drug at this temperature. The melting point range for Lovastatin mentioned in official books was 168-172^{0}C. The PXRD results show that Lovastatin is in crystalline form with its characteristic peaks and no polymorphs are found. The SEM Microphotographs supports the existence of Lovastatin in the crystalline form.

Drug-excipient compatibility study:

Drug-excipient compatibility is a very important parameter in preformulation studies before development of any dosage form. The possible physical and chemical interactions between drug and excipients may change the physical, chemical, as well as therapeutic properties of the developed dosage form. Drug-excipient incompatibility may also produce an unstable dosage form. To avoid such problems at the later stages of dosage form development, drug-excipient compatibility was confirmed at preformulation stage by FTIR studies.

In FTIR spectra, all the major respective peaks of the drug were present in the drug-polymers mixture. This indicates that the drug has not undergone any physical or chemical changes when stored with polymers. It confirmed the compatibility of drug with the excipients like PEG 4000, PVP K 30 and Aerosil. Thus, PEG 4000 and PVP K30 polymers can be used with Lovastatin to develop a stable dosage form.

PREPARATION OF SOLID DISPERSIONS

Optimization of the solid dispersions were carried out by varying different variables such as drug: polymer ratio, processing variables such as temperature; concentration of suspension/slurry; inlet and outlet temperature and feed rate etc. Dichloro methane was selected as solvent in spray drying method, because of the restriction on maintenance of outlet temperature below 35^0C and aerosil is added for uniform dispersion of Lovastatin in suspension before processing. Based on drug content, saturation solubility, and powder characteristics, the formulations were considered as optimum formulations.

CHARACTERIZATION OF SOLID DISPERSIONS

Saturation solubility:

Saturation solubility of pure drug is 0.4 µg/ml. Saturation solubility is increased in physical mixtures. This may be due to increased wettability. Solid dispersions with PEG 4000 shows significant increase in saturation solubility, which may be due to the formation of complex between drug and PEG 4000. Solid dispersions of PVPK30 also shows significant increase in saturation solubility, which could be due to 1) the strong hydrophilic characteristic of PVP K30 that improves the water penetration and the wettability of the hydrophobic Lovastatin and 2) optimal dispersion of drug Lovastatin in polymer matrix, which resulted in absence of crystallinity corresponding to low energy required for dissolving the drug. As per the literature study, the author R. P. Patel et al has shown the evidence that there is inter molecular hydrogen bond formation between drug Lovastatin and PVP K30[57] which can enhance the solubility is also confirmed by IR study.

Percentage yield and Drug content:

Percentage yield is less for spray drying method as compared to solvent evaporation method due to the loss of formulation during processing by spray drier. The low drug content could be possibly due to the loss of drug during processing.

FTIR spectroscopy:

When the spectra of solid dispersions by solvent evaporation (LV-PEG) and physical mixtures were compared, no difference was found in the position of absorption bands. The spectrum was the superimposition of spectra of Lovastatin and PEG 4000.

When the spectra of solid dispersions by spray drying (LV-PVPK30) and physical mixtures were compared; in contrast to the physical mixtures, solid dispersions showed hydrogen bonding between Lovastatin and PVPK30. Each pyrolidone moiety of PVP has two groups (=N- AND C=O) that can potentially form hydrogen bond with the drug at molecular level in solid dispersion formulation. Significant broad peaks at 3553 cm^{-1} and 1695 cm^{-1} suggested hydrogen bonding interaction between free O-H group of Lovastatin and carbonyl group of PVP K30.

The formulations SE4 and SD4 are considered to be optimum formulations, on the basis of results of drug content and saturation solubility. These formulations also shown good flow properties, further increase in polymer concentration will affect the flow properties, which will create problem during processing of solid dispersions.

Scanning Electron Microscopy

Pure drug Lovastatin is consisting of mixture of small and bigger crystalline particles. Whereas in SE4, the smaller drug particles are adsorbed on the polymer surface. The particles in SD4 show porous spherical amorphous particles with smaller size ranges due to processing by spray drying process. These micro photographs confirmed that the drug particles are entrapped within the polymeric matrix.

Powder X-Ray Diffraction (PXRD) analysis

The presence of characteristic peaks (at 2θ) in X-Ray diffraction indicates that Lovastatin is present in crystalline material with characteristic diffraction peaks appearing at a diffraction angle of 2θ at 10.69,12.61, 13.52, 14.74,15.45, 16.06, 17.11, 17.82, 19.56, 21.31, 22.57, 25.79, 26.96, 30.29, 32.01, 34.65, 35.86 etc. Whereas the diffraction patterns of the samples of SE4 and SD4 shows peaks due to PEG (at 2 θ) i.e. 15.00, 18.75, 23.17 and 29.35 and PVP (complete absence of any diffraction peak) and absence of major diffraction peaks corresponding to Lovastatin with most of the diffraction indicating Lovastatin was present as amorphous material inside the PEG or PVP matrix.

Differential scanning calorimetry:

DSC scan of the pure Lovastatin shows melting endotherm at 170^oC with enthalpy of fusion (ΔH) of 163.15 mJ/g. DSC scan of PEG 4000 showed single melting endotherm at 64^oC. The DSC scan of PVPK30 shows a broad endotherm between 90-130^oC (moisture residue).The DSC scan of SE4 showed peak at 58^oC, melting point of PEG 4000 and that of SD4 shows a peak at 90-140^oC due to loss of water and also complete absence of drug peak at 170^oC. This confirms the amorphicity of Lovastatin in solid dispersion or existence of drug as solid solution inside the polymer PEG and PVP matrix as indicated by the results of PXRD.

Dissolution studies:

The solid dispersion have increased the dissolution rate by the possible factors like 1) the strong hydrophilic characteristic of PVP K30 that improves the water penetration and the wettability of the hydrophobic Lovastatin and 2) embedded drug particles in polymer bed, which resulted in absence of crystallinity corresponding to low energy required for dissolution of the drug. As per the literature study, the author R. P. Patel et al has shown the evidence that there is inter molecular hydrogen bond formation between drug Lovastatin and PVPK30[57] which can enhance the dissolution rate is also confirmed by IR study.

***In-vivo* study**

In-vivo hypolipidaemic performance of pure Lovastatin and SD4 is compared in 14 days study. After 7 days of treatment with high fat diet, control group showed

10.6 % rise in total cholesterol, significant 17.7 % rise in triglycerides, 6.7 % decline in HDL and significant 32.4 % rise in LDL cholesterol. Whereas reference group showed 3 % decline in total cholesterol, slight 1.14 % rise in triglycerides, slight 3.1 % rise in HDL cholesterol and significant 18.9 % decline in LDL cholesterol. The test group showed 7.5 % decline in total cholesterol, slight 2.6 % decline triglycerides, rise in HDL cholesterol 9.7 % and significant decline in LDL 41.3 %.

After 14 days of same treatment, control group showed significant 23.6 % rise in total cholesterol, significant 34.7 % rise in triglycerides, significant 14.3 % decline in HDL levels and 129 % significant rise in LDL Cholesterol levels. Whereas reference group showed, 5.2 % decline in total cholesterol, 2.5 % decline in triglycerides, slight 5.5 % rise in HDL cholesterol and significant 31.6 % decline in LDL level. The test group showed 15.06 % significant decline in total cholesterol, non-significant 11.7 % decline in triglycerides, significant 16.2 % rise in HDL level and significant 74.8 % decline in LDL cholesterol. At the end of the study, SD4 performed better than pure Lovastatin in reducing total cholesterol, rise in HDL cholesterol and decline in LDL cholesterol significantly.

Accelerated stability study

To test the recrystallinity upon storage, Stability studies were conducted. The solid dispersions, which were considered as best formulations i.e., SE4 and SD4 were subjected for accelerated stability study. These formulations subjected for dissolution test; at specific interval (15days, 1 month and 3 months); over a period of 3 months, the change in drug release was insignificant, which is shown in the Figure 24 In addition, PXRD observations indicated that the presence of amorphicity at specific time intervals of 3 months period. There were no changes found in the powder X-ray difractograms of SE4 and SD4, indicating that there were no peaks corresponding to the crystallinity of Lovastatin found in the difractograms which is shown in the Figure 25. This may be due to the dispersion of drug molecules in the polymer bed, which prevents further recrystallization upon storage.

FORMULATIONS OF TRANSBUCCAL FILMS BY USING SOLID DISPERSIONS

Compatibility studies:

Upon comparison of IR Spectra of SD4 and transbuccal formulations, it was found that the characteristic peaks of Lovastatin in SD4 were 3535 cm^{-1}(“alcohol OH stretch”), 3016 cm^{-1} (“olefinic CH stretching vibrations”) 1725 cm^{-1} and 1695 cm^{-1}(“lactone and ester carbonyl stretch”) also found in transbuccal formulations along with characteristic peaks of respective polymers. It can be concluded by these observations that there are no possible interactions between the SD4 and polymers.

PHYSICOCHEMICAL CHARACTERIZATION OF TRANSBUCCAL PATCHES:

Thickness, Weight, Drug Content and Surface pH of the film

Film thickness and weight of the patches increases as the concentration of polymer increases in the film. Drug content was more than 90%, which ensures the uniform drug content. The surface pH was found to be 5.6 to 7.6 which ensure no mucosal irritation due to the formulation.

Swelling Index (%)

The swelling index study was carried out to find out the degree of swelling of buccal films in simulated saliva solutions. HPMC K4M and HPMC E5 films started swelling after 5 minutes and chitosan after 30 minutes when hydrated on agar medium due to the swellable hydrophilic polymers. The results show that as the polymer concentration increases the swelling index was also increased. As per the study carried out by the authors Pankaj kumar etal, swelling of the polymers makes strong secondary hydrogen bonding with buccal mucosa and thus results in good mucoadhesion, which provides unidirectional release of drug[71]. Chitosan buccal films (F13 to F 18) shown high values of swelling index as compared to HPMC films (F1 to F12). H.Castan etal stated that this might be due to chitosan when wetted be able to gain weight and size by 150 %. This high value of swelling index causes discomfort to the patient[48].

Folding endurance

All the developed formulations were flexible. However, HPMC K4M and HPMC E5 formulations (F1 to F12) shown good folding endurance but in case of chitosan containing formulations (F13 to F18), the films became brittle, lost elasticity as the polymer concentration increased. Hence, the folding endurance values decreased as the polymer concentration increased.

Ex-vivo Residence Time

Residence of film is directly related to the mucoadhesive strength, which is consequence of interaction between mucin and polymers. Further adequate hydration is required for the polymers to get charged and impart sufficient mucoresidence. *Ex-vivo* residence time ranged from 2.2± 0.08 to 8.2±0.17 hours. In the formulations with

HPMC K4M polymers (Table 20) containing films, the presence of hydroxyl groups which facilitate uptake of water into polymer matrix and enhance mucoadhesiveness. These polymers also form viscous liquids when hydrated with increasing their retention time over mucosal surfaces, which may lead to adhesive interactions. The formulations with HPMC E5 (Table 21) shown short period of residence time, which might be because of low viscosity of the polymer which resulted into weak adhesion. In case of formulations with chitosan (Table 22), there is interaction between positively charged amino group and negatively charged mucin of membrane and helps in bioadhesion for longer duration of time[71].

Ex -vivo bioadhesion test

Mucoadhesive strength plays important role in proper adherence of the film to the mucosal surface. Excessive adhesion may cause discomfort to the patient leading to patient noncompliance. Hence, optimum mucoadhesion is required for film for effective therapy.

The *Ex-vivo* bioadhesive strength and Detachment force value are shown in the above table (20-22). Transbuccal films with HPMC K4M (F1-F6); shows good mucoadhesion, which may be attributed to the hydration and liquid entrapment in the polymer network, after formation of viscous polymeric network, however HPMC E5 (F7-F12) polymer films shows weak force of adhesion. According to Magdy etal, this might be because of its low viscosity of the polymer. Apart from this, hydrophilicity of PVP K30 (from Solid dispersions which is incorporated in the transbuccal film) might further have weakened the force of adhesion[101]. The transbuccal films of chitosan (F13-F18) also show good mucoadhesion. According to Cui etal, the strong viscoelastic property and structure of chitosan might be attributed to its high mucoadhesive force which occurs because of interaction between amino group of chitosan and mucin of biomembrane[20].

In -vitro drug release

The drug release study was carried out over a period of 6 hours. The significant decrease in drug release is found as the polymer concentration increases in formulations from F1 to F6. Meher etal states that this could be related to the increase in thickness of the film with increase in HPMC concentration[47]. Thus, the time required for dissolution medium to penetrate into the polymer chain located through the depth of the film increases.

Similar observation is made with HPMC E5 formulations as well. However, initial drug release (30 minutes) is more as compared to other formulations. This might be because of low viscosity of HPMC E5 polymer as observed by Magdy etal[101].

In case of chitosan containing formulations drug release values after completion of 6th hour is lower as compared to other formulations. Similar observation is found by author Patel etal. Chitosan is a cationic polymer which might have formed complex with PVP K30 (polymer used to prepare solid dispersions which is incorporated in the transbuccal films), which is non-ionic polymer leading to extension of the drug release.

Ex-vivo drug permeation study

In case of HPMC polymers, formation of hydrogel occurs at the surface of biomembrane, which leads to hydration and swelling of mucoadhesive films which promotes diffusion of drug. Chitosan films when wetted can gain weight and size by

150% due to swelling of mucoadhesive films, formation of very thick hydrogen layer leads to the longer diffusion path length leading into delayed diffusion. Castan etal states that drug diffusion is delayed in the case of ionic polymers such as chitosan as compared to nonionic polymers (HPMC)[48].

Drug release kinetics

To investigate the release kinetics of drug release from buccal films, the drug release and *ex-vivo* permeation data are subjected to fit various kinetic models using PCP Disso ver 2 software Pune India. The $\mathbf{r^2}$and **n** values are determined. All the buccal films showed n values in the range of 0.73 to 0.87 indicating that the drug release followed by non-fickian diffusion and the best fit model observed to be Higuchi model.

Based on results of Drug content, Swelling index, Folding endurance, in-*vitro* bioadhesion, drug release and *ex-vivo* permeation, we can conclude that HPMC K4M formulations (F1-F6) are the best formulations. The formulation F3 is selected for further stability.

Stability study

Stability study results indicate that there is no significant change in *in-vitro* characterization studies such as drug content, drug release and *ex-vivo* permeation.

6. SUMMARY

The poor drug absorption and low bioavailability reinforce the need for solubility enhancement and newer drug delivery system. Lovastatin, a model drug selected in the present research is HMG CoA reductase inhibitor, is a drug of choice for both primary and secondary hyperlipidemia. Its clinical efficacy is limited due to its low aqueous solubility, hence its absorption is incomplete and about 85 % of the administered dose is found in faeces. It has short half-life. It undergoes first pass metabolism. Hence, there is low bioavailability. There are gastrointestinal side effects such as Flatulence, Nausea, Diarrhea, Dyspepsia and Constipation. Myopathy or rhabdomyosis is another potential side effect. The research project proposed herein is based on modification of the solid state at molecular level of drug for enhancing aqueous system solubility by solvent evaporation and spray drying solid dispersion technique. Solid dispersions of Lovastatin were prepared by using two different polymers PEG 4000 (solvent evaporation) and PVP K30 (spray drying). The prepared dispersions were evaluated and compared. The best formulations were selected based on drug content, saturation solubility, and flow properties. These best formulations were further subjected to FTIR, SEM, PXRD, and DSC analysis. Further *in-vivo* lipid profile studies and stability studies were also carried out. Optimization of the Solid dispersions were carried out by varying different variables such as drug: polymer ratio, processing variables such as temperature; concentration of suspension/slurry; inlet and outlet temperature and feed rate etc. Dichloro methane was selected as solvent in spray drying method, because of the restriction on maintenance of outlet temperature below 35^0C and aerosil is added for uniform dispersion of Lovastatin in suspension before processing.

The aqueous solubility of Lovastatin was favored by the presence of polymers PEG 4000 and PVPK30. In contrast to the very slow dissolution rate of pure Lovastatin, solid dispersions considerably improved the dissolution rate. This can be attributed to increased wettability and dispersibility of Lovastatin in polymeric matrix as well as decreased crystallinity and increased amorphicity which is confirmed by PXRD, DSC and SEM. IR studies also revealed that there is hydrogen bonding formation between drug Lovastatin and PVPK30. The formulation SD4 showed the highest solubility and dissolution rate and was considered as optimum formulation; based on drug content, saturation solubility, and powder characteristics. *In-vivo* Lipid profile study revealed SD4 had better therapeutic efficacy than pure Lovastatin. Stability study confirmed that there is no recrystallinity of drug from solid dispersions upon storage for specific period.

Transbuccal drug delivery system of processed solid dispersions (SD4) were formulated which offers advantages such as prevention of first pass metabolism, drug release control which maintains desired plasma drug level, reduced gastrointestinal side effects, prevention of degradation of drug in GIT, enhanced therapeutic efficacy and in turn helps in achieving patient compliance.

The formulations of buccal patches were formulated by solvent casting technique using three different polymers such as HPMC K4M, HPMC E5LV and chitosan. These polymers were finalized based on a series of trails considering chemical compatibility and adhesive properties. Two grades of HPMC polymer, which are HPMC K4M and HPMC E5 LV were selected to study the effect of viscosity parameter on adhesion of transbuccal film. Chitosan and HPMC polymers were compared to study the drug release pattern and duration of therapy.

The successful patches were evaluated for film thickness, weight, content uniformity, surface pH, swelling index, folding endurance, *ex-vivo* residence time, *ex -vivo* bioadhesion test, *in -vitro* drug release, *ex-vivo* drug permeation and stability study. The thickness of all prepared patches ranged from 0.21±0.07 to 1.5± 0.39 mm, weight of the film 89.10±0.6 to 128.57±0.3 mg, drug content 85.47±0.87 to 97.33±0.31%, surface pH 5.6±0.67 to 7.6±0.98, swelling index 23.0±4.1 to 76.5±3.6%, folding endurance 165±1.9 to 350±2.5 respectively. *Ex-vivo* residence time ranged from 2.2± 0.08 to 8.2±0.17 hours and *ex-vivo* bioadhesive strength 30±0.64 to 66±0.43 gms. The formulations with HPMC E5 shown short period of residence time and shows weak force of adhesion., which might be because of low viscosity of the polymer which resulted into weak adhesion. The percentage drug release and *ex-vivo* drug permeation was in the following descending order HPMC K4M> HPMC E5LV>chitosan. These results confirm the extension of drug release in case of ionic polymer chitosan. The kinetics data shows that drug release and permeation follows non-Fickian diffusion. Accelerated stability data revealed that there is no significant change in drug content, *in-vitro* drug release and *ex-vivo* permeation.

Based on results of Drug content, Swelling index, Folding endurance, in-*vitro* bioadhesion, drug release and *ex-vivo* permeation, we concluded that HPMCK4M formulations (F1-F6) are the best formulations. The formulation F3 is selected for further stability. Stability study results indicate that there is no remarkable change in *in-vitro* characterization studies such as drug content, release and *ex-vivo* permeation.

Thus, in the present research, efforts were made to improve the overall therapeutic efficacy of drug, by enhancing the solubility which provides therapeutic action at reduced dose which in turn reduces side effects of drug, especially myopathy. Formulated transbuccal patches increased increase bioavailability and maintained constant drug levels throughout drug therapy period.

7. CONCLUSION

Amorphous solid dispersions of Lovastatin were successfully prepared and both polymers PEG 4000 and PVP K30 showed solubilizing effect on Lovastatin. FT-IR spectra confirmed that there is inter molecular Hydrogen bonding between polymer PVP K30 and Lovastatin. XRD, SEM and DSC studies confirmed that solid dispersions converted the drug Lovastatin into amorphous form and its solubility is enhanced significantly, which was confirmed by dissolution studies. Solid dispersion SD4 WAS selected for *in-vivo* study. Lipid profile study revealed SD4 had better therapeutic efficacy than pure Lovastatin. Transbuccal formulations of SD4 using three different polymers successfully formulated and their *in-vitro* characterization were carried out successfully. Physicochemical characterization of formulations indicated that HPMC K4M formulations (F1-F6) were the best formulations. The formulation F3 was selected for further stability study. Stability study results indicate that there is no significant change in *in-vitro* characterization studies such as drug content, release and *ex-vivo* permeation.

8. LIMITATIONS AND FUTURE SCOPE OF THE STUDY:

Limitations:

Amorphous solid dispersions were prepared successfully by using two different polymers PVP K30 and PEG 4000. Both methods shown significant improvement in solubility, however, solid dispersions prepared using PEG 4000, posed problems of processing while development of transbuccal patches due to waxy texture of dispersions.

Future scope:

In order to achieve the clinical impact for the developed dosage form, further efforts should be directed to perform *in-vivo* permeation study, pharmacokinetic and bioavailability studies in human volunteers.

9. REFERENCES

1. Yusuf S, Rangarajan S, Teo K, Islam S, Li W, Liu L. Cardiovascular risk and events in 17 low-, middle- and high-income countries. N. Eng. J. Med.371; 2014:818-27.
2. Nystoriak M, Bhatnagar A. Cardiovascular effects and benefits of exercise. Front. Cardiovasc. Med.5; 2018:1-11.
3. Burkhardt R. Hyperlipidaemia and cardiovacluar disease: Reinforcement for ‘lower is better’. Curr. Opin. Lipidol. 26(5) 2015:468-9.
4. Robert N. Hyperlipidemia as a risk factor for cardiovascular disease. Prim care. 40(1)2013:195-211.
5. Nouh F, Omar M, Younis M. Risk factors and Management of hyperlipidemia. Asian. J. Cardiol Res. 2(1); 2019:1-10.
6. Tripati KD. Essentials of medical pharmacology.Jaypayee brothers medical publishers 7th ed.:2017: 634-46.
7. Monika Sharma, Rajeev Garg, G D Gupta. Formulation and evaluation of solid dispersion of Atorvastatin calcium: J. Pharm. Sci. Innov. 2(4); 2013: 73-81.
8. Amidon G, Lennernas H, Shah V, Crison J. A theoretical basis for a Biopharmaceutical drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm. Res. 12; 1995:413–20.

9. Sharma D K, Joshi S B. Solubility enhancement strategies for poorly water-soluble drugs in solid dispersions: A review. Asian. J. Pharmaceutics. 1(1); 2007:9-19.

10. Patel R, Patel D. Bhimani D, Patel J. Physicochemical characterization, and dissolution study of solid dispersions of Furosemide with PEG 4000 and Polyvinylpyrrolidone K30. Dissolut. Technol. 12; 2008: 17-25.

11. Kumar P, Kumar S, Kumar A, Chnder M. Physicochemical characterization of solid dispersions of cefdinir with PVPK30 and Peg 4000. Int. J. Pharm. Sci. Nanotech. 3 (2); 2010: 948-56.

12. Anujkumar. Review on solubility enhancement techniques for hydrophobic drugs. Int. J. Comprehen. Pharm.2 (3); 2011:1-7.

13. Mohac L, Licciardi M. Multicomponent solid dispersion: a new generation of solid dispersion produced by spray drying. J. Drug. Del. Sci. Tech. Article ID 101750.57 (6) 2020.

14. Gilhotra R, Ikram M, Srivastava S, Gilhotra N. A clinical prospective on mucoadhesive buccal drug delivery systems. J. Biomed. Res.28 (2); 2014:81-97.
15. Chandrashekhar K, Naidu K, Vishnu Y, Gannu R, Kishan V, Rao M. Transbuccal delivery of chlorpheniramine maleate from mucoadhesive buccal patches. Drug. Del.15; 2008: 185-191.
16. Hassan M, Barakat N, Badry M, Shehata S. Formulation, and *in vitro*/ *in vivo* evaluation of Naproxen mucoadhesive buccal patches for local effect.J. Drug Del. Sci. Tech. 21(5)2011; 423-31.
17. Patel R, Poddar S. Develpoment and charecterization of mucoadhesive buccal patches of salbutamol sulphate. Curr. Drug deliv.6; 2009:140-44.
18. Agarwal S. Development of transdermal and transbuccal drug delivery systems for cardioactive drugs with special reference to antihypertensive agents.

Atherosclerosis, Hypertension and diabetes.Kluwer academic publishers Boston. 8; 2011:229-46.

19. Reddy P, Chaitanya K, Rao M. A review on bioadhesive buccal drug delivery systems: current status of formulation and evaluation methods. DARU. J. Pharm. Sci. 19(6); 2011:385-403.
20. Cui F, Chunhai H, Miao H, Cui T, Yin L Qian F Yin C. Preperation, and evaluation of chitosan hydrogel films for the mucoadhesive transbuccal delivery of insulin. J. Biomed. Mat. Res. 89(4)2008.32071.
21. Aboti P, Parikh R, Samir P. Permeation enhancement of furosemide by using 2 level full factorial design: an *ex vivo* study.Der. Pharm. Lett. 2015, 7(6):146-65.
22. Patel V, Prajapati B, Patel M. Design and characterization of chitosan containing mucoadhesive buccal patches of propranolol HCl.Acta pharmaceut.57 (1);2007:61-72.
23. Chandra A, Chondkar A, Shirodkar R, Lewis S. Rapidly dissolving Lacidipine nanoparticle strips for transdermal administration. J. Drug Del.Sci. Tech. 47; 2018:259-67.
24. Ashwani M, Pathak A. Plasticizers: A vital excipients in novel pharmaceutical formulations. Curr. Res. Pharm. Sci. 07(01) ;2017:01-10.
25. Xiao Wu, Desai K, Mallary S, Holpuch, Phelps M, Schwendeman S. Mucoadhesive Fenretinide patches for site specific chemoprevention of oral cancer: enhancement of oral mucosal permeation of Fenretinide by coincorporation of propylene glycol and menthol. Mol. Pharm. 9(4) 2012: 937-45.
26. Shidhaye S, Saindane N, Sutar S, Kadam V. Mucoadhesive bilayered patches for administration of Sumatriptan succinate.AAPS Pharm. Sci. Tech. 9(3);2008:909-16.

27. Moor V, Amougou S, Ombatto S, Ntone F, Wouamba D, Nonga B. Dyslipidaemia in patients with cardiovascular risk and disease at the university teching hospital of Yaounde, Cameroon. Int. J. Vasc. Med. Article ID 6061306; 2017:1-5.
28. Taher Y, Eduardo L, Edric E, Atif M. Prevelance and determinants of Dyslipidaemia, data from a saudi university clinic. Open Public Health J. 11; 2018, 416-24.
29. Neduri K, Bontha V, Vemula S. Different techniques to enhance the dissolution rate of lovastatin: formulation and evaluation, Asian J. Pharm. Clin. Res. 6(1) 2013: 56-60.
30. Sharma M, Garg R, Gupta G. Formulation and evaluation of solid dispersion of Atorvastatin calcium: J. Pharm. Sci. Innov. 2(4) 2013: 73-81.
31. Quereshi M, Chitneni M, Kian W. Enhancement of solubility and therapeutic potential of poorly soluble Lovastatin by SMEDDS formulation adsorbed on directly compressed spray dried magnesium alumino metasilicate liquid loadable tablets: A study in diet induced hyperlipidaemic rabbits. Asian J. Pharm. Sci. 10(2015): 40-56.
32. Rodde M, Divase D, Devkar T, Tekade A. Solubility and biavailability enhancement of poorly aqueous soluble Atorvastatin:*In Vitro, ex Vivo* and *In vivo* studies. Biomed. Res. Int.10 .2014:1-10.
33. Parikh R, Samir P. Permeation enhancement of furosemide by using 2 level full factorial design: an *ex vivo* study.Der. Pharm. Lett. 2015, 7(6):146-65.
34. Karolewicz B, Gajda M, Pluta J, Górniak A. The effect of Pluronic F127 on the physicochemical properties and dissolution profile of lovastatin solid dispersions; J. Therm. Anal. Calorim; 123(3)2016: 2283-90.

35. Jagadale S, Hable A, Kuchekar B, Improvement of dissolution rate of Chlorzoxazone by Solid Dispersion technique and development of buccal patch. J. Appl. Sol. Chem. Model. 2.2013:145-57.
36. Caro V, Ajovalasit A, Maria F. Development amorphous solid dispersion of Furosemide in the form of a sublingual bioadhesive film to enhance bioavailability; Pharmaceutics; 9(22) 2017:1-15.
37. Zolkiflee F, Affandi M, Majeed A. Lovastatin: history, physicochemistry, pharmacokinetics and enhanced solubility; Int. J. Res. Pharm. Sci. 2017; 8(1), 90-102.
38. Khanfer M, Nimry S. Stabilization and Amorphization of Lovastatin using different types of Silica; AAPS Pharm. Sci. Tech. 18(6)2017: 2358–67.
39. D’Souza A, Shegokar R. Polyethylene glycol: A versatile polymer for pharmaceutical applications. 13(9) Expert Opin. Drug Del. 13(9)1257-75.
40. Wadher S, Gattani S. .Jadhav Y. Recent approaches in solubility enhancement of poorly water soluble drug simvastatin: A review. World J. Pharm. Pharm. Sci. 3(4); 2014:366-84.
41. Pandya V, Patel J. Formulation, Optimization, and characterization of Simvastatin Nanosuspension prepared by nanoprecipitation technique. Der Pharmacia Lettre. 3(2); 2011:129-40.
42. Biswal S, Saboo J, Murthy P. Enhancement of dissolution rate of Gliclazide using solid dispersions with PEG 6000.AAPS Pharm. Tech.9(2)2008.563-70.
43. Ankita R, Ganesh B, Preeti K. Formulation, and Evaluation of Nanosuspension of Rosuvastatin Calcium. Int. J. Drug. Regul. Aff. 1(3); 2013:14-8.

44. Obaidat R, Khanfar M, Ghanma R. A comparative solubility enhancement study of Cefixime Trihidrate using different dispersion techniques. AAPS Pharm. Sci. Tech. 20(5); 2019:194-201.

45. Dhore P, Dave V, Saoji S. Enhancement of the aqueous solubility and permeability of a poorly water soluble drug Ritonavir via lyophilized milk-based solid dispersion. Pharm. Dev. Technol. 22(1); 2017:90-102.

46. Ghosh I, Schenck D, Bose S, Liu F, Motto M. Identification of critical process parameters and its interplay with nanosuspension formulation prepared by top down media milling technology–a QbD perspective. Pharm. Dev. Technol. 18(3); 2013: 719-29.

47. Meher G, Tarai M, Yadav P, Pathak A, Mishra P, Yadav K. Development, and characterization of cellulose- polymethacrylate mucoadhesive film for buccal delivery of carvedilol. Carbohydr Polym. 96; 2017:172-80.

48. Castan H, Ruiz B, Morales M, Design, Development and charecterisation of buccal bioadhesive films of Doxepin for treatment of odontalgia.Drug Deliv. 22(6); 2017:869-76.

49. Chandrashekhar K, Naidu K, Vamshi V, Gannu R, Kishan V, Rao Y. Transbuccal delivery of Chlorpheniramine maleate from mucoadhesive buccal patches, Drug Deliv. 15(3);2008: 185-91.

50. Parthasarathi G, Reddy K, Jayaveera K, effect of bile salts on transbuccal permeation of carbamazepine through Porcine buccal mucosa. J. Pharm. Res. 16(2); 2017: 110-19.

51. Tripati KD. Essentials of medical pharmacology.Jaypayee brothers medical publishers 7th ed. 2017:634-46.

52. Britlain G. Analytical profiles of drug substances and excipients .Harcourt brace Jovanovich publisher.26th ed. 1999:278-305.

53. Parfitt K. Lipid regulating drugs. Lovastatin. Martindale The complete drug reference. Pharmaceutical press publisher.39nd ed. 2014:1275.

54. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. Fund. Clin. Pharmacol.19;2004:117-25.

55. Handbook of pharmaceutical excipients. The Pharmaceutical Society of America, London. 1986; 10th ed: 159-60.

56. Ambike A A, Mahadik K R, Paradkar A. Spray-dried amorphous solid dispersions of Simvastatin, a low tg drug: *in vitro* and *in vivo* evaluations. Pharm. Res. 2005; 22(6):990-98.

57. Patel R P, Patel M M. Physicochemical characterization and dissolution study of solid dispersions of Lovastatin with PEG 4000 and Polyvinylpyrrolidone K30; Pharm. Dev. Technol. 12; 2007:21-33.

58. Nagesh C, Shankaraiah M M, Attimarad S L, Patil A M, Vijay Kumar. Improving the solubility and dissolution of Ritonavir by solid dispersion. J Pharm. Sci. Innov.2013; 2(4) 2013: 30-35.

59. Girish C, PD Chaudhary, Sharma P. Solubility Enhancement of poorly water soluble drug Aceclofenac.Ind. J Pharm. Pharmacol. 3(3); 2016:139-45.

60. Ramana Reddy GV, Vidyadhara S, Ramesh Babu J, Sasidhar R, Ramu A. Formulation, and evaluation of Lovastatin solid dispersions with pregelatinised starch as newer superdisintegrant. J. Pharma Res. 11(2); 2012: 38-43.
61. Kumar P, Mohan C , Srinivasan M, Shankar U Physiochemical Characterization and Release Rate Studies of Solid Dispersions of Ketoconazole with Pluronic F127 and PVP K-30. Iran. J. Pharm. Res.10 (4); 2011: 685-94.
62. Chaudhari P. Melt sonocrystallisation: novel particle engineering technique for solubility enhancement. Int. J Pharmtech Res. 2009;1(1):1-12
63. Hyun-Jong C, Jun-Pil J, Kang J, Shin D. Cefdinir solid dispersion composed of hydrophilic polymers with enhanced solubility, dissolution, and bioavailability in Rats. Molecules. 22(280); 2017:1-14.
64. Mahmoud E, Gihan F, Mohamed Fathy. Improvement of solubility and dissolution rate of indomethacin by solid dispersions in Gelucire 50/13 and PEG4000. Saudi Pharm. J.17; 2009: 217–25.
65. Mokale V, Naik J, Wani D, Patil J, Yadava S, Verma U. Preparation and *in-vitro* Characterisation of Lovastatin Liquisolid Self-Emulsified Drug Delivery System Int. J Chem.Environ. Biol. Sci.2 (1);2014:17-23.
66. Shaikh K, Patwekar K, Payghan S, D’Souza J. Dissolution, and stability enhancement of poorly water soluble drug – Lovastatin solid dispersions. Asian Journal of Biomed. Pharmaceut. Sci. 1 (4); 2011:24-31.
67. Kurmi R, Mishra D, Dubey N, Jain D. Solubility Enhancement of poorly water soluble drug using solid dispersion Technique. J. Harmon. Res. Pharm. 4(4); 2016: 329-36.

68. Bhise D, Ternary solid dispersion of Fenofibrate with poloxomer188 and TGPS for enhancement of solubility and bioavailability. Int. J. Res. Pharm. Biomed. Sci. 2(2); 2011:583-95.
69. Chandra A, Chondkar A, Shirodkar R, Lewis S. Rapidly dissolving Lacidipine nanoparticle strips for transbuccal administration. J. Drug Del. Sci. Tech.47; 2018:256-67.
70. Boateng J, Okeke O.Chitosan based film for the sustained release of peptides: a new era in buccal delivery. Ther. Del. 5(5)2014. 497-500.
71. Pankaj Kumar, Gulshan Chhabra, Kamla Pathak. Development and statistical optimisation of bioadhesive films of Amiloride hydrochloride: *in vitro and exvivo* evaluation. Ind. J. Pharm. Edu. Res. 2012; 46:145-54.
72. Agarwal A. Development of transdermal and transbuccal drug delivery systems for cardioprotective drugs with special reference to antihypertensive drugs. Atherosclerosis. Hypertension. Diabetes. Progress in experimental cardiology.8; 2011: 229-46.
73. Kashinath P, Shivakumar S, Devprakash S, Prashant S. Design, and evaluation of transdermal film of Valsartan by using modified polymer.J. Pharm. Res. 5(5); 2012:2823-9.
74. Khate K, Khatpalia H. Film forming systems for topical and transdermal drug delivery. Asian J. Pharm. Sci.12; 2017: 487-97.
75. Pandit V, Khanum A, Bhaskharan S, Banu V. Formulation, and evaluation of transdermal films for the treatment of overactive bladder. Int. J. Pharm. Tech. Res. 1(3); 2009: 799-804.
76. Peddappalli H, Ganta R, Boggulla N. Formulation and evaluation of transdermal patches for Anti anxiety drug. Asian J. Pharmaceu. 12(2)2018.127-36.

77. Bhatia C, Sachdeva M, Bhajpai M. Formulation and evaluation of transdermal patches of Pregabalin. Int. J. Pharm. Sci. Res. 3(2)2012. 569-75.
78. Reddy S, Surekha C, Anusha G, Reddy M .A review on transbuccal delivery and its commercially available products. Res. J. Pharm. Tech. 6(1); 2013:22-28.
79. Ali E, Aboud H, Hassan A, Godah A. Transbuccal delivery of Betahistidine dihydrochloride from mucoadhesive tablets with unidirectional flow: *in-vitro, ex-vivo and in-vivo* evaluation. Drug. Des. Dev. Ther.10; 2016: 4031-45.
80. Singh A, Bali A, Formulation and characterisation of transdermal patches for controlled delivery of duloxetine HCl. J. Analytical Sci. Tech. 7(25); 2016: 1-13.
81. Rajurkar V, Zarekar S, Ghawate V, Pathan I. The development, evaluation, and invitro release study of the Terbinafine transdermal patch. Ind. Chem.1 (2) 2015. 1-6.
82. Reddy Y, Saraswat R, Reddy S. Design and characterization of buccal patches of Atomooxetine. Int. J. Pharm. Sci. Rev. Res.56 (1); 2019: 96-102.
83. Kriplani P, Sharma A, Pun P, Chopra B. Formulation and evaluation of transdermal patch of Diclofenac sodium. Glob. J. Pharmaceu. Sci. 1(5); 2018: 01-05.
84. Patel K, Patel H, Patel V. Formulation and characterization of drug in adhesive transdermal patches of Diclofenac sodium. Int J Pharm Investig. 4(1); 2012: 296-9.
85. Semalty M, Semalty A, Kumar G. Formulation and characterization of mucoadhesive buccal films of Glipizide. Ind. J. Pharm. Sci. 70(1); 2008: 43-8.
86. Chinna reddy P, Chaitanya K, Rao M. A review on bioadhesive buccal drug delivery systems: Current status of formulation and evaluation methods. DARU J. Pharm. Sci.19 (6)2011.385-403.

87. Govindaswami P, Kesavan B, Narasimha J. Formulation of unidirectional release buccal patches of Carbamazine and study of permeation through porcine buccal mucosa. Asian Pac J. Trop. Biomed. 3(12); 2013: 995-1002.
88. Shah S, Joshi R, Prabhu P. Formulation and evaluation of transdermal patches of Papavarine HCl. Asian J. Pharmaceu. 04(01) 2010.79-86.
89. Mortazavi S. Acomparative study between the strength of transdermal carbomer based aqueous gels. Iran. J. Pharm. Res.1; 2002:7-13.
90. Das P, Saha P. Design and characterization of transdermal patches of Phenformin HCl.9(6); 2017: 90-93.Int. J. Curr. Pharm. Res. 9 (6); 2017:90-3.
91. Yadav A, Urade M. Formulation and evaluation of Chitosan based transdermal patches of Lornoxicam for prolonged drug relase and to study the effect of permeation enhancer. Ind. J. Pharm. Edu. Res. 53(1); 2019: 88-96.
92. Cherkuri S, Batchu U, Mandava K, Cherkuri V. Formulation and evaluation of trandermal drug delivery system of Topirmate. Int. j. Pharma Investig. 7; 2017:10-17.
93. Colombo P, Colombo G, Buffini F, Cagnani S. Biological *in-vitro* models for absorption by nonoral routes.Comprehen. Med. Chem II.5; 2007:279-99.
94. Ying H, Wu Y, Xia X, Wu Z. Development of drug in adhesive transdermal patch for α-asarone and *in-vivo* pharmacokinetics and efficiency evaluation. Drug. Del. 18 (1); 2011: 84-9.
95. Sadashivaaiah R, Dinesh B, Patil U, Desai B, Raghu K. Design and *in vitro* evaluation of Haloperidol lactate transdermal patches containing ethyl cellulose povidone as film formers. Asian J. Pharmaceu. 2008; 43-9.
96. Prajapati S, Patel C. formulation and evaluation of transdermal patch of Repaglinide. ISRN. Pharmaceu.2011.651909.1-9.

97. Bharatkumar V, Ashokkumar A, Sudheer K, Sureshkumar K. Formulation design, *in vitro* evaluation, and stability studies on mucoadhesive buccal films of anti-anginal calcium channel blocker. J. Applied pharm. Sci. 1(6); 2011: 136-42.

98. Karthik A, Reddy S. Formulation and evaluation of Terbutaline sulphate buccal patches by using HPMC K4M, HPMC E15 polymers. World J. of Pharmaceut. Res. 7(2); 2018: 1075-113.

99. Khairnar A, Parridhi J, Dheeraj B, Dinesh J. Development of mucoadhesive buccal patch containing Acelofenac: *in-vitro* evaluation. Int. J. Pharm. Tech. Res. 1(4) ; 2009: 978-81.

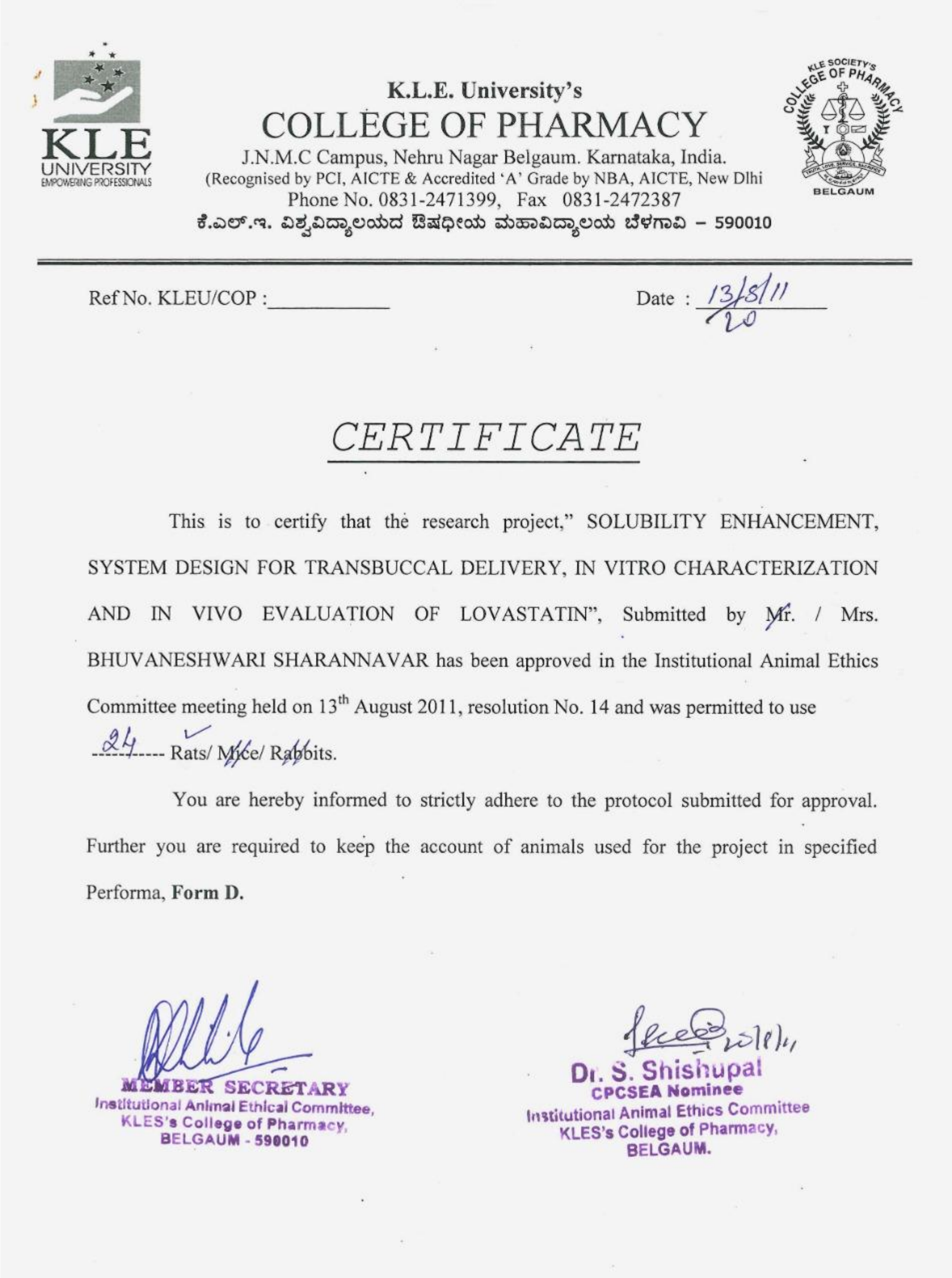
100. Subhas D, Madhuri C, Anil C, Umesh J, Kailash B. Chitosan based mucoadhesive patches containing Verapamil HCl. Int. J. Pharm. Pharmaceu. Sci. 1(1)2009.216-29.

101. Balagani, Pallapati K, Katamreddy J. P, Formulation, design and evaluation of mucoadhesive buccal tablets of Nitroglycerin. Int. J. Pharm. Pharm. Sci. 2014;6(7):251-9

102. Magdy M, Nadia S, Sarah R. Development of a novel ketorolac tromethamine sublingual film. J. Am. Sci. 2015; 11:27-37.

10. ANNEXURES

Animal ethical committee approval letter



K.L.E. University's
COLLEGE OF PHARMACY
J.N.M.C Campus, Nehru Nagar Belgaum. Karnataka, India.
(Recognised by PCI, AICTE & Accredited 'A' Grade by NBA, AICTE, New Dlhi
Phone No. 0831-2471399, Fax 0831-2472387
ಕೆ.ಎಲ್.ಇ. ವಿಶ್ವವಿದ್ಯಾಲಯದ ಔಷಧೀಯ ಮಹಾವಿದ್ಯಾಲಯ ಬೆಳಗಾವಿ – 590010

Ref No. KLEU/COP :____________ Date : 13/8/11 20

CERTIFICATE

This is to certify that the research project," SOLUBILITY ENHANCEMENT, SYSTEM DESIGN FOR TRANSBUCCAL DELIVERY, IN VITRO CHARACTERIZATION AND IN VIVO EVALUATION OF LOVASTATIN", Submitted by ~~Mr.~~ / Mrs. BHUVANESHWARI SHARANNAVAR has been approved in the Institutional Animal Ethics Committee meeting held on 13th August 2011, resolution No. 14 and was permitted to use 24 Rats/ ~~Mice~~/ ~~Rabbits~~.

You are hereby informed to strictly adhere to the protocol submitted for approval. Further you are required to keep the account of animals used for the project in specified Performa, **Form D.**

MEMBER SECRETARY
Institutional Animal Ethical Committee,
KLES's College of Pharmacy,
BELGAUM - 590010

20/8/11
Dr. S. Shishupal
CPCSEA Nominee
Institutional Animal Ethics Committee
KLES's College of Pharmacy,
BELGAUM.

RESEARCH GRANT

GOVERNMENT OF KARNATAKA

VISION GROUP ON SCIENCE AND TECHNOLOGY
Karnataka Science and Technology Promotion Society
Department of Information Technology, Biotechnology and Science & Technology

List of proposals recommended by VGST for Government Grant during the year 2019-20

Scheme: Research Grant for Scientist/Faculty (RGS/F)

Sl. No.	Name of the Principal Investigator	Title of the project	Subject	Funds to be released during the year 2019-20(Rs in Lakhs)
10	Mrs.Bhuvaneshwari Sharannavar Assistant Professor Pharmaceutical Quality Assurance, KLE College of Pharmacy, Nehru Nagar, Belagavi- 590 010	Solubility enhancement, *in-vitro* characterization and *in-vivo* evaluation of Rosuvastatin.	Pharmacy	3.00

***www.vgst.in (grantees selected for the FY 2019-20/RGSF)**

ORAL PRESENTATION AND PUBLICATIONS

ORAL PRESENTATION				
Sl. No.	Title of the paper	Conference/FDP	Venue	Date
1.	Importance and applications of solid state in pharmaceutical formulations	UGC Sponsored Faculty Development Program	UGC-SAP Approved Department of Pharmaceutical sciences; Sourashtra University; Rajkot, Gujrat.	23rd November 2013
2.	Physicochemical characterization and dissolution study of spray dried amorphous Lovastatin with Polyvinylpyrrolidone K30.	8th Asian Association of school of pharmacy conference	JSS University Mysuru	27th-29th October 2017

PUBLICATIONS				
Sl. No.	Title of the paper	Details of Journal (name, year, vol., issue, page no.)	Indexed/ UGC specified journal/	Impact Factor
1.	Physicochemical characterization and dissolution study of spray dried amorphous Lovastatin with Polyvinylpyrrolidone K30.	The Pharma Innovation. 2018; 7(3)498-502.	UGC Care List	-
2.	Transbuccal dellivery of spray dried lovastatin from mucoadhesive buccal patches and *in-vitro* charecterization.	International journal of applied pharmaceutics 2019; 5(11)181-87.	Scopus/ UGC Care List	0.22

CERTIFICATE

Re-Accredited
Grade B by NAAC
CGPA (2.93)

ज्ञान-विज्ञान विमुक्तये
University Grants Commission

This is to certify that

Mrs. Bhuvaneshwari R. Sharannavar

has delivered Oral Presentation under Faculty Development Program on

"Importance and Application of Solid State in Pharmaceutical Formulation"

at

UGC-SAP approved Department of Pharmaceutical Sciences, Saurashtra University, Rajkot
from 11th November 2013 to 23rd November 2013

Dr. Navin R. Sheth
Convener

Dr. Mihir K. Raval
Coordinator

Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India

Indian Association of Colleges of Pharmacy
for a better body of knowledge

JSS UNIVERSITY

The Asian Association of Schools of Pharmacy

in Association with

Indian Association of Colleges of Pharmacy

Presents this

Certificate of Oral Presentation

to

Mrs. Bhuvaneshwari. R. Sharannavar.

for the Topic

Physicochemical characterization and dissolution study of spray dried amorphous Lovastatin with PVP K30.

Presented in the 8th AASP Conference held during 27th - 29th October 2017 at

Jagadguru Sri Shivarathreeshwara University, Mysuru, India

Dr. Shu Chuen Li
President, AASP

Prof. K. Chinnaswamy
President, IACP

Dr. Gurumurthy Parthasarathi
Chairman, Local Organizing Committee

KLE
EMPOWERING PROFESSIONALS

BELAGAVI

KLE College of Pharmacy, Belagavi

A constituent unit of

KLE Academy of Higher Education & Research

[DEEMED-TO-BE-UNIVERSITY], REACREDITED AT THE 'A' LEVEL BY NAAC (SECOND CYCLE), PLACED IN 'A' CATEGORY BY MHRD (GoI)

Certificate

AICTE Sponsored Quality Improvement Programme

Bhuvaneshwari Sharannavar

KLES COLLEGE OF PHARMACY BELAGAVI

In recognition of his/her attendance as **Resource Person** at the two week Quality Improvement programme on "**Drug Development & Discovery: Natural & Synthetic perspective**" in virtual mode from 18th August to 2nd September 2020.

Prof. (Dr.) Shankar Alegaon	**Prof. (Dr.) Sunil Jalalpure**	**Prof. (Dr.) Kalpana Patil**
Program Coordinator	Chief Coordinator	Program Coordinator

nirf 37th
2020 Rank

B. Pharm Program Accredited by NBA for a period of Six Years (from July 2019 to June 2025)

klepharm_edu
www.facebook.com/klepharm.edu

www.klepharm.edu
YouTube

The Pharma Innovation Journal 2018; 7(3): 498-502

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(3): 498-502

www.thepharmajournal.com
Received: 13-01-2018
Accepted: 14-02-2018

Bhuvaneshwari R Sharannavar
Department of Pharmaceutics, KLE University College of Pharmacy Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India

Anand P Gadad
Department of Pharmaceutics, KLE University College of Pharmacy Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India

Correspondence
Bhuvaneshwari R Sharannavar
Department of Pharmaceutics, KLE University College of Pharmacy Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India

Physicochemical characterization and dissolution study of spray dried amorphous Lovastatin with Polyvinylpyrrolidone K30

Bhuvaneshwari R Sharannavar and Anand P Gadad

Abstract

The aim of the present study was to improve the solubility and dissolution of poorly water soluble drug Lovastatin (LVS). Solid dispersions were prepared by using PVPK30 and aerosil 200 by spray drying method, with different drug: polymer ratio (1:1, 1:2, 1:4 and 1:8). These formulations were charecterized by the saturation solublity, IR, DSC, PXRD and SEM. The aqueous solubility of LVS was favoured by the presence of polymer PVPK30. In contrast to the very slow dissolution rate of pure LVS, spray dried solid dispersions considerably improved the dissolution rate. This can be attributed to the increased wettability and dispersibility of LVS in polymeric matrix of PVPK30 as well as decreased crystallinity and increase in amorphicity which is confirmed by PXRD, DSC and SEM. IR studies also revealed that there is hydrogen bonding formation between drug LVS and PVPK30. The formulation SD4 showed highest solubility and dissolution rate. Stability study confirmed that there is no recrystallinity of drug over storage for specific period.

Keywords: Lovastatin, Spray drying, PVPK30, Aerosil 200, Solid dispersion

1. Introduction

Aqueous solubility of drug is a key property which governs dissolution, absorption and thus *in-vivo* efficacy [1]. In the current scenario, most of the drug molecules discovered are lipophilic and exhibits poor aqueous solubility which inturn results in low bioavailability and posses a chalenge in developing optimum oral solid dosage form [2]. Nearly 40% of the new chemical entities currently being discovered are poorly water soluble drugs [3]. If the drug is having good permeability then the rate limiting step in absorption is the drug dissolution. This is a charecteristic of compounds which belong to the biopharmaceutical classification system II (BCS class II) [4]. Drugs in this class are expected to have a variable dissolution profile due to the formulation and *in-vivo* variables. The aqueous solubility lesser than 1 µg/ml creates problem of bioavailability. Alteration of the solid state at the particle or molecular level involves a physical change in the drug can be used as an option to improve the drug solubility [5]. Physical modification of lipophilic drugs is done by using several carriers like cyclodextrins, carbohydrates, hydrotropes, dendrimers, polyglycolized glycerides and other methods by use of superdisintegrants, solid dispersions, surfactants melt granulation, particle size reduction [6]. Conversion of crystalline form of drug to amorphous is one of the strategies which improve the drug solubility.

Pharmaceutical materials that are processed by high energy processes such as spray drying, jet milling and melt extrusion are often rendered at least partially amorphous. This occurs by the virtue of the fact that these processes create conditions that can prevent crystallisation or mechanically disrupt the structure of an existing crystalline material. The high internal energy and specific surface area of the amorphous state relative to the crystalline state can lead to enhanced dissolution and bioavailability [7].

Lovastatin (LVS) is an antihyperlipidaemic drug. Its principal metabolite that is hydroxy acid is potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, which catalyses the conversion of hydroxy methyl gluterate to mevalonate which is an early and rate limiting step in biosynthesis of cholesterol [8]. The inhibition occurs due to the structural similarity between the hydroxyacid form of the statins and the HMGCoA intermediate formed. Reduced intracellular cholesterol synthesized induces the hepatic LDL-receptor, which results in increased extraction of LDL cholesterol from the blood and decreased circulating LDL cholesterol. It has been proven that LVS is effective as therapeutic and prophylactic agent in the management of major morbidities such as atherosclerosis, pheripheral artirial disease and cardiovascular disease. LVS however has low bioavailability, only a small fraction of the administered dose will reach the systemic circulation [9, 10]. It is a white crystalline powder which has low aqueous solubility, that is 0.4 µg/ml at room temperature [11]. An increase in aqueous solubility of LVS could be useful to help increase the efficacy of the drug by having it dissolved in the gastrointestinal fluid [12].

The hydrophillic polymer polyvinyl pyrolidone K30 (PVP K30) is used as carrier in the preperation of solid dispersions. It shows good water solubility, low toxicity, low melting point, rapid solidification rate, physiological tollarance and economic cost. These properties ensures that PVP K30 is good carrier polymer for preparing solid dispersion [13].

Various methods are available for determining the physical nature of solid dispersions. These charecterization methods include Infrared spectroscopy, Differential scanning calorimetry, Powder X-Ray Difraction etc., [7, 14].

In the present study, Solid dispersions are prepared by spray drying method. Spray drying of a drug LVS along with the water soluble carrier polyvinyl pyrrolidone K30 (PVP K30) was done. Collidal silicone dioxide (aerosil 200), a hydrophilic adsorbent is used for ease of processing and facilitating transport of the final product to the collection vessel. These solid dispersion formulations are prepared by different ratios of drug and polymer. These formulations were charecterized by solubility FTIR, DSC, PXRD and dissolution studies. The results are compared with the pure drug. Further accelerated stability studies were also carried out.

2. Materials and Method

2.1 Materials

Lovastatin is obtained as gift sample by Biocon Limited (Bangalore Karnataka). PVP K30 by (BASF) and Aerosil 200 (Degussa). All chemicals and solvents used in this study were of analytical grade reagents.

2.2Methods

2.2.1Solid dispersions prepared by spray drying method

Solid dispersions of LVS in PVP K30 containing different ratios (1:1, 1:2, 1:4 and 1:8) were prepared by spray drying method as follows. LVS along with PVP in above said ratios is dissolved in sufficient amount of dichloromethane. To these clear solutions proposed quantity of Aerosil 200 is added to obtain uniform suspensions. Spray drying was carried out using laboratory scale spray dryer (Labultima model LU 222 Mumbai India) under following set of conditions. Inlet temperature 35 ^{0}C; outlet temperature 26-28 ^{0}C; feed rate 4-6 ml per minute; atomization air pressure 2 kg/cm^2 and aspiration -250 mm WC. The resulting solid powder was placed in vacuum dryer for 24 hours to remove residual solvents if any. Then the samples are passed through sieve number 100. The samples were stored in dessicator for further studies [7].

2.2.2Physical mixtures

Physical mixtures (PMs) having the same weight ratios were prepared by thoroughly mixing appropriate amounts of LVS and PVP K30 in mortar until a homogeneous mixture was obtained. The resulting mixtures were sieved through a sieve number 100. The samples were stored in dessicator for further compatibility studies [7].

2.2.3 Compatibility studies by infrared spectroscopy

Compatibility studies were carried out for pure drug (LVS), (PVPK30), (Aerosil 200), physical mixtures of polymers (LVS+PVPK30) and solid dispersions prepared by spray drying method (SD4). Infrared spectroscopic analysis was done for the same. Fourier transform infrared spectra of moisture free powdered samples were obtained by using spectrophotometer (FT-IR Shimadzu Co., Japan) by potassium bromide (KBr) pellet method (2mg of sample in 200 mg of KBr). The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

2.2.4 Drug content

Solid dispersions equivalent to 10 mg of LVS were weighed accurately and dissolved in suitable quantity of ethanol. The drug content was determined at 238 nm by UV spectrophotometer [7].

2.2.5 Saturation solubility

To evaluate increase in solubility of Lovastatin (as in solid dispersions) or only by the presence of hydrophilic polymer (as in PMs), saturation solubility measurements were carried out. A known excess amount of solid dispersions were added to 10 ml of phosphate buffer (6.8 pH). Samples were rotated at 20 rpm in a water bath at 25 ^{0}C for 48 hours. Samples were then filtered, suitably diluted and analysed spectrophometrically at 238 nm [7].

2.2.6 Scanning Electron Microscopy (SEM)

Samples were mounted on double faced adhesive tapes and coated with gold (200Å) under reduced pressure (0.001 torr) for 5 minutes using an ion sputtering device. The gold coated samples were observed under the SEM and photomicrographes of suitable magnifications were obtained [7].

2.2.7 Differential scanning calorimetry (DSC) analysis

DSC scans of the samples were recorded by using DSC Shimadzu-60. The samples were hermatically sealed in alluminium pans and heated at constant rate of 10^{o}C/min under dry nitrogen flow (100ml/min) between 50 to 300 ^{o}C [9].

2.2.8 Powder X-Ray Diffraction (PXRD) analysis

X-Ray powder scattering measurements were carried out with a D2 Phaser Difractometer at room temperature using the monochromatic CuKa-radiation at 34 mA and at 38 Kv over a range of 2θ angles from 5^o to 90 ^{o} with an angular increment of 0.05 ^{o}/s [7, 9].

2.2.9 Dissolution studies

The dissolution studies were performed using USP XXIII type 2 apparatus (electrolab India) for 3hrs. Samples of pure LVP and solid dispersions equivalent to 10 mg of the drugs were added to the 900 ml of phosphate buffer (pH 6.8) as dissolution medium maintained at 37±0.5 ^{o}C, which was stirred at 100 rpm. At suitable intervals, 5ml samples were withdrawn, filtered (0.22µm), diluted and analysed at 238 nm using UV spectrophotometer. An equal volume of fresh medium at the same temperature was replaced into the dissolution medium after each sampling to maintain its constant volume throughout the test. Each test was performed in triplicate (n=3) and calculated mean values of cumulative drug release were used for plotting curves [9].

2.2.10 Stability studies

The accelerated stability of SD 4 was checked as per ICH guidelines at 40 ºC/75%RH upto 3 months. Periodically (15days, 1 month and 3 months) samples were removed and checked for *in vitro* drug release and presence of crystallinity using PXRD studies [7].

3. Result and Discussion

3.1 Compatibility studies

The spectra of all the samples are shown in figure 1. The spectrum of pure lovastain presented characteristic peaks at 3539 cm^{-1} (Alcohol O-H stretching vibrations), 3016 cm^{-1} (olefinic C-H stretching vibration), 2964 cm^{-1}, 2927 cm^{-1}, 2876 cm^{-1} (Methyl and methylene C-H stretch ing vibration),1725 cm^{-1},1713 cm^{-1} and 1690 cm^{-1} (Lactone and ester carbonyl stretch) 1430 cm^{-1}, 1381 cm^{-1} and 1357 cm^{-1} Methyl and methylene bending vibration), 1282 cm^{-1}, 1219 cm^{-1}, 1075 cm^{-1} 1055 cm^{-1} (Lactone and ester C-O-C bending vibration), 969 cm^{-1} Alcohol C –OH stretch), and 870 cm^{-1} (Trisubstituted olefinic C-H wagging) respectively.

The spectrum of PVP showed important bands at 2925 cm^{-1} C-H stretch and 1653 cm^{-1} stretching vibration of the carbonyl group, which is most distinct peak in the IR spectrum of PVPK30. The broad peak at 3000-3300 cm^{-1} (OH stretching vibrations), which was attributed to the presence of water. The spectrum of Aerosil 200 showed the presence of broad prominent peak at 1076 cm^{-1} (strong Si-O linkage) is characteristic of Aerosil and also there is peak at 3549 cm^{-1} (O-H stretch).

Upon comparison; the spectra of solid dispersions by spray drying (LV-PVPK30) and physical mixtures; in contrast to the physical mixtures, solid dispersions presented possibility of hydrogen bonding between Lovastatin and PVPK30. Each pyrolidone moiety of PVP has two groups (=N- AND C=O) that can potentially form hydrogen bond with the drug at molecular level in solid dispersion formulation. Significant broad peaks at 3553 cm^{-1} and 1695 cm^{-1} suggested hydrogen bonding interaction between free O-H group of Lovastatin and carbonyl group of PVPK30.

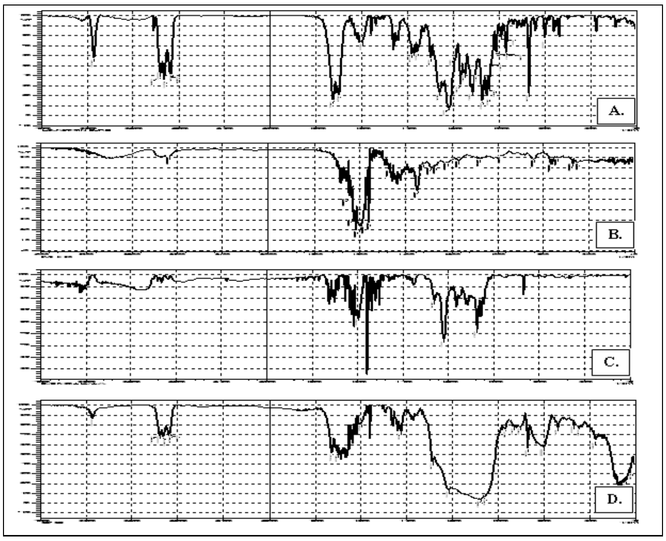


Fig 1: FTIR Spectra of A. Lovastatin B. PVPK30 C. Physical mixture D. SD4

3.2 Preparations of solid dispersions

Optimization of the solid dispersions were carried out by varying different variables such as drug: polymer ratio, processing variables such as temperature; concentration of suspension/slurry; inlet and outlet temperature and feed rate etc. Dichloro methane was selected as solvent in spray drying method, because of the restriction on maintenance of outlet temperature below 35 ^{0}C. Based on drug content, saturation solubility and powder characteristics, the formulations are considered as optimum formulations. Composition of Solid Dispersions (SD) and Physical Mixtures (PM) is shown in the Table 1.

Table 1: Composition of Solid Dispersions and Physical Mixtures of Lovastatin

Type of Formulation	Composition (parts by weight)	
	Lovastatin	PVPK30
SD1/PM1	1	1
SD2/PM2	1	2
SD3/PM3	1	4
SD4/PM4	1	8

The drug content values and Saturation solulibility for solid dispersions are given in the Table 2. The low drug content could be possibly due to the loss of drug during processing. Saturation solulibility of pure drug is 0.4µg/ml. Saturation solublity is increased in physical mixtures as compared to the pure drug. This could be due to increased wettability. The formulation SD4 is considered to be optimum formulation based on the results of drug content and saturation solubility of 97% and 87.3 respectively. The formulations also shown good flow properties, further increase in polymer concentration will affect the flow properties, which will create problem during processing of solid dispersions.

Table 2: Drug contents and saturation solubilities of solid dispersions

Formulation code	Drug content	Saturation solubility (Solid dispersions)	Saturation solubility (Physical mixture)
SD1	68±0.10%	28.6± 0.3	0.9 ± 0.05
SD2	74±0.45%	46.3± 0.2	3.7 ± 0.04
SD3	85±0.08%	73.4±0.5	6.3 ± 0.04
SD4	97±0.89%	87.3±0.4	3.1± 0.2

3.3 SEM

The microphotographs of pure LVS and optimized formulation SD4 are shown in the figure 2. Pure drug is consisting of mixture of small and bigger crystalline particles. Whereas the particles in SD4 shows porous spherical amorphous particles with smaller size ranges.

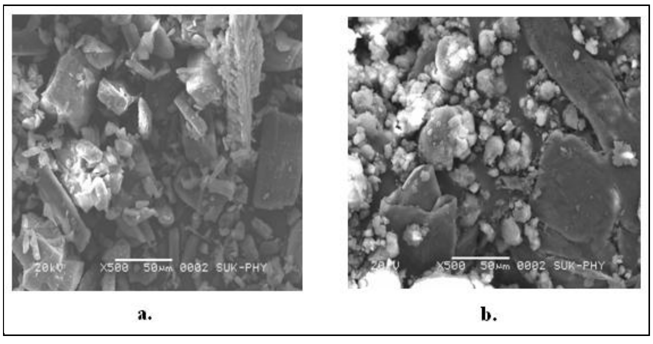


Fig 2: SEM Photomicrographs of a. Lovastatin b. SD4.

3.4 DSC

The thermal behaviour of pure drug LVS, PVPK30 and SD4 was studied by DSC. The DSC curves are shown in the figure 3. The pure LVS shows melting endotherm at 170 °C with enthalpy of fusion (ΔH) of 163.15 mJ/g. The DSC scan of PVPK30 shows a broad endotherm between 80-120 °C due to presence of moisture residue in PVP K30. The DSC scan of SD4 shows a peak at 80-130 °C due to loss of water and also complete absence of drug peak at 170 °C. This confirms the amorphicity of LVS in solid dispersion inside the PVP matrix as indecated by the results of PXRD.

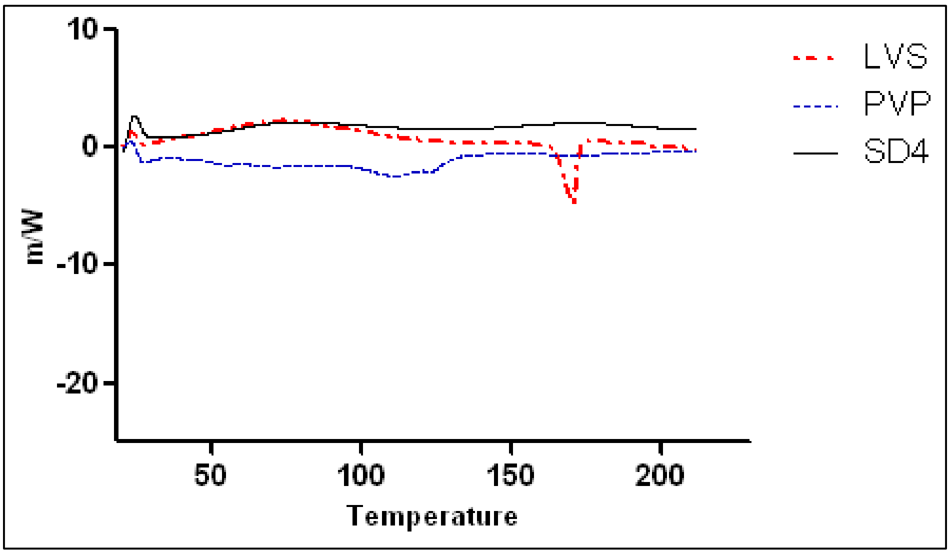


Fig 3: DSC Thermograms of (A) Lovastatin (B) PVP K30 (C) SD4.

3.5 PXRD

PXR Difractograms of pure LVS and SD4 are shown in the figure 4. The presence of charecteristic peaks (at 2 θ) in X-Ray diffraction indicates that LVS is present in crystalline material with charecteristic diffraction peaks appearing at a difraction angle of 2θ at 10.69,12.61, 13.52, 14.74,15.45, 16.06, 17.11, 17.82, 19.56, 21.31, 22.57, 25.79, 26.96, 30.29, 32.01, 34.65, 35.86 etc. Where as SD4 showed the absence of any diffraction peaks corrosponding to drug indecating the LVS was in the amorphous state.

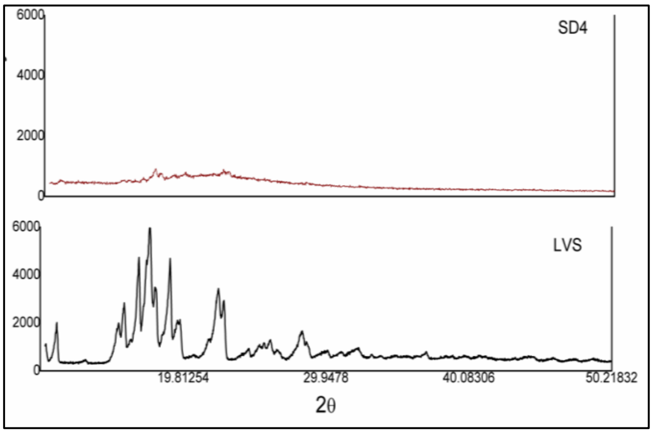


Fig 4: Powder X-ray difractograms of Lovastatin (LVS) and SD4

3.6 Dissolution studies

Dissolution profiles of pure LVS and solid dispersions over a period of 3 hours are shown in the figure 5. The dissolution rate of pure LVS is very low that is 23.63% drug is dissolved in 3 hours. Solid dispersions of LVS with PVPK30 significantly enhanced the dissolution rate of LVS. SD4 presented highest drug release 93.8 %. This improved drug release is due to the presence of amorphous form of LVS as confirmed by IR, SEM, DSC and PXRD studies.

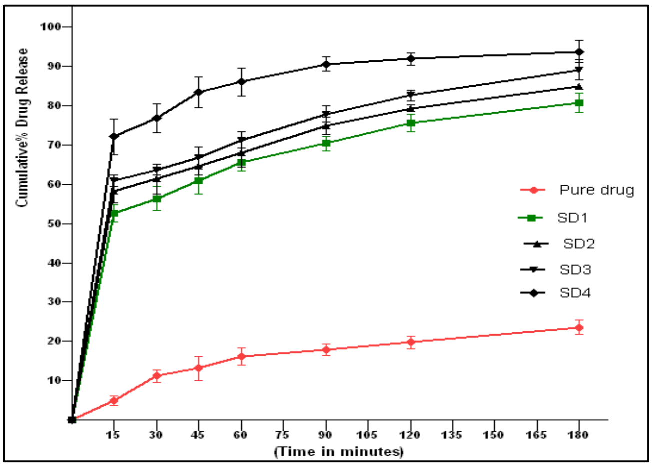


Fig 5: *In-vitro* dissolution profiles of Lovastatin pure drug and solid dispersions (SD1-SD4)

3.7 Stability studies

The evidences from the previous studies carried out say that, the solid dispersions prepared by different methods tend to recrystallize upon storage at high temperature and relative humidity. Hence stability studies were carried out to test the recrystallinity. When SD4 was subjected for dissolution test; at specific interval (15days, 1 month and 3 months); over a period of 3 months, the decrease in invitro drug release was found to be insignicant which is shown in the Figure 6. Also PXRD observations indicated that the presence of amorphicity at specific time intervals of 3 months period. There were no changes found in the powder X-ray difractograms of SD4, indecating that there were no peaks corresponding to the crystallinity of LVS found in the difractograms which is shown in the figure 7. This could be attributed to the entrapment of drug molecules in the polymer matrix, which prevents further recrystallization upon storage.

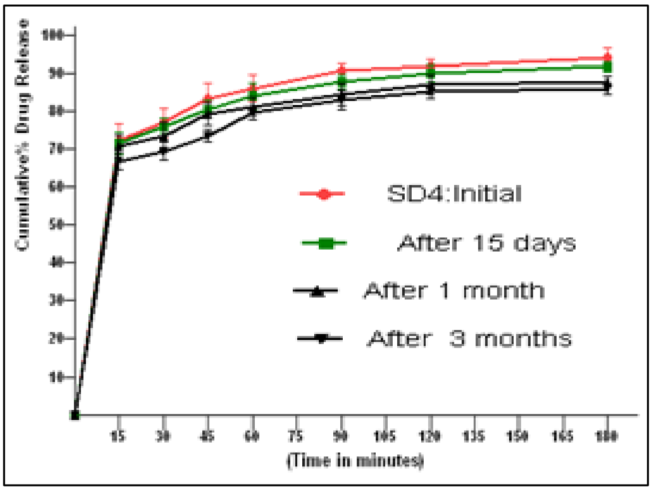


Fig 6: *In-vitro* Dissolution profiles of SD4 during stability study at different time intervals.

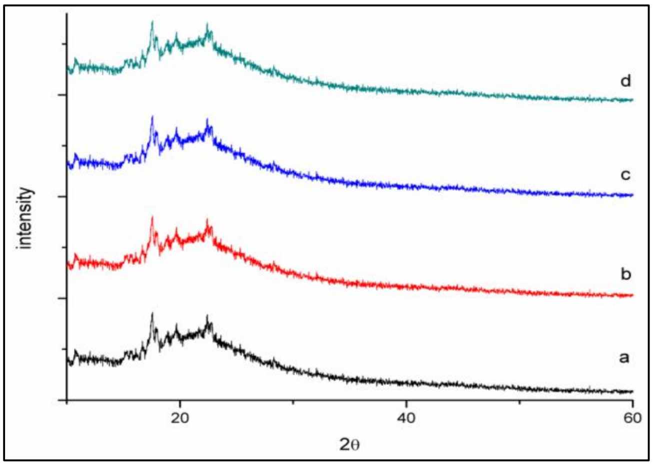


Fig 7: Powder X-ray Difractograms of SD4 during stability study at different time intervals. (a) Initial (b) 15 days (c) 1 month (d) 3 months.

4. Conclusion

The solubility and dissolution can be enhanced by the SDs of LVS with PVP K30 and aerosil 200. IR studies confirmed that there is hydrogen bonding between the drug LVS and polymer PVP K30 at molecular level in SDs, which could be the reason for enhanced solubility. Conversion of crystalline to amorphous form of LVS is confirmed by DSC, PXRD, SEM and dissolution studies. Stability study results confirm absence of recrystallinity upon storage over a period of 3 months. Hence present study suggests that spray drying method can be successfully used for the preperation of solid dispersions.

5. Acknowledgments

The authors are grateful to the Principal and management of KLE University College of Pharmacy, Belagavi, for providing all necessary facilities and infrastructure to carry out this study. The authors also acknowledge Biocon Limited, Bangalore, Karnataka, for providing gift sample of Lovastatin.

6. References

1. Rao M, Mandage Y, Thanki K, Bhise S. Dissolution improvement of Simvastatin by surface solid dispersion technology. Dissolution technologies. 2010; 17(2):27-34.
2. Quereshi MJ, Chitneni M, Kian WG. Enhancement of solubility and therapeutic potential of poorly soluble Lovastatin by SMEDDS formulation adsorbed on directly compressed spray dried magnesium alumino metasilicate liquid loadable tablets: A study in diet induced hyperlipidaemic rabbits. Asian Journal of Pharmaceutical Sciences. 2015; 10:40-56.
3. Yadav PS, Vikas Kumar, Singh UP, Bhat H, Mazumder B. Phsicochemical charecterization and *in-vitro* dissolution studies of solid dispersions of Ketoprofen with PVPK30 and D-M annitol. Saudi Pharmaceutical Journal. 2013; 21:77-84.
4. Sachan NK, Bhattacharya A, Pushkar S, Mishra S. Biopharmaceutical classification system: Astrategic tool for oral drug delivery technology. Asian Journal of pharmaceutics. 2009; 3(2):76-81.
5. Sharma M, Garg R, Gupta GD. Formulation and evaluation of solid dispersion of Atorvastatin calcium: Journal of Pharmaceutical and Scientific Innovation. 2013; 2(4):73-81.
6. Neduri K, Bontha V, Vemula S. Different techniques to enhance the dissolution rate of lovastatin: formulation and evaluation, Asian Journal of Pharmaceutical and Clinical Research. 2013; 6(1):56-60.
7. Ambike AA, Mahadik KR, Paradkar A. Spray-dried amorphous solid dispersions of Simvastatin, a low tg drug: *in vitro* and *in vivo* evaluations. Pharm Res. 2005; 22(6):990-98.
8. Tripathi KD. Essentials of Medical pharmacology. Ed 6; jaypee brothers medical, 2010, 612-623.
9. Patel RP, Patel MM. Physicochemical charecterization and dissolution study of solid dispersions of lovastatin with PEG 4000 and Polyvinylpyrrolidone K30. Pharmaceutical development and technology. 2007; 12:21-33.
10. Parfitt K. Lipid regulating drugs: Lovastatin, Martindale. Ed 32, The pharmaceutical press. 1999, 1275.
11. Gerald SB, Dean KE, Micheal JK. Lovastatin. Analytical profiles of drug substances and excipients. Harry G. B. Ed.; Academic press: Ed 1(25), Acadaemic press; 1998, 278-305.
12. Zolkiflee NF, Meor Mohd Affandi MMR, Majeed ABA. Lovastatin: history, physicochemistry, pharmacokinetics and enhanced solubility. International journal of research in pharmaceutical sciences. 2017; 8(1):90-102.
13. Patel RP, Patel DJ, Bhimani DB, Patel JK. Physicochemical charecterization and dissolution study of solid dispersions of Furosemide with PEG 4000 and Polyvinylpyrrolidone K30. Dissolution technologies. 2008; 8:17-25.
14. Nagesh C, Shankaraiah MM, Attimarad SL, Patil AM, Vijay Kumar. Improving the solubility and dissolution of Ritonavir by solid dispersion. Journal of Pharmaceutical and Scientific Innovation. 2013; 2(4):30-35.

International Journal of Applied Pharmaceutics

ISSN- 0975-7058

Vol 11, Issue 5, 2019

Original Article

TRANSBUCCAL DELIVERY OF SPRAY DRIED LOVASTATIN FROM MUCOADHESIVE BUCCAL PATCHES AND *IN VITRO* CHARACTERIZATION

BHUVANESHWARI R. SHARANNAVAR*, ANAND P. GADAD

Department of Pharmaceutics, Kles College of Pharmacy Belagavi, Kle Academy of Higher Education and Research, Belagavi 590010, Karnataka, India
Email: bhuvi_rs@yahoo.co.in

Received: 22 Jun 2019, Revised and Accepted: 25 Jul 2019

ABSTRACT

Objective: The aim of the present work was to develop and characterize mucoadhesive film of spray dried Lovastatin (LVS) for buccal delivery to enhance bioavailability.

Methods: Mucoadhesive films were prepared by solvent casting technique by using different polymers HPMCK4M, HPMC E5LV and chitosan. The successful patches were evaluated for film thickness, weight, content uniformity, surface pH, swelling index, folding endurance, *ex-vivo* residence time, *ex-vivo* bioadhesion test, *in vitro* drug release, *ex-vivo* drug permeation and stability study.

Results: The thickness of all prepared patches ranged from 0.21±0.07 to 1.5±0.39 mm, the weight of the film 89.10±0.6 to 128.57±0.3 mg, drug content 85.47±0.87 to 97.33±0.31%, surface pH 5.6±0.67 to 7.6±0.98, swelling index 23.0±4.1 to 76.5±3.6%, folding endurance 165±1.9 to 350±2.5 respectively. *Ex-vivo* residence time ranged from 2.2±0.08to 8.2±0.17 h and *ex-vivo* bioadhesive strength 30±0.64 to 66±0.43 g. The formulations with HPMC E5 shown short period of residence time and shows weak force of adhesion., which might be because of low viscosity of the polymer which resulted into weak adhesion. The percentage drug release and *ex-vivo* drug permeation was in the following descending order HPMC K4M>HPMC E5LV>chitosan. These results confirm the extension of drug release in case of ionic polymer chitosan. The kinetics data shows that drug release and permeation follows nonfiction diffusion. Accelerated stability data revealed that there is no significant change in drug content, *in vitro* drug release and *ex-vivo* permeation.

Conclusion: It can be concluded that mucoadhesive buccal patch is a promising dosage form to enhance the drug bioavailability by preventing first-pass metabolism thus providing better therapeutic efficacy.

Keywords: Lovastatin, HPMCK4M, HPMC E5LV, Chitosan, *Ex-vivo* bioadhesion, Swelling index, *Ex-vivo* permeation

DOI: http://dx.doi.org/10.22159/ijap.2019v11i5.34649

INTRODUCTION

The oral drug delivery is considered to be the most preferred route by majority of the patients amongst the various available routes of drug delivery. However, oral administration of drugs has certain disadvantages such as hepatic first-pass metabolism and enzymatic degradation within the GI tract that prohibits oral administration of various classes of drugs [1]. Since last three decades researchers have been focusing on buccal drug delivery, as it has shown ability to enhance the therapeutic efficacy of poorly effective oral drugs [2]. The direct entry of the drug into the systemic circulation avoids the first-pass hepatic metabolism leading to increase in bioavailability. Other advantages are low enzymatic activity, painless administration, easy drug withdrawal, facility to include permeation enhancers/enzyme inhibitors or pH modifiers in the formulation and versatility in designing as multidirectional or unidirectional release systems for local or systemic actions. Various mucoadhesive formulations were suggested for buccal delivery that includes buccal patches, adhesive tablets, and adhesive gels. However, buccal films are preferred over adhesive tablets in terms of flexibility and comfort [3]. Mucoadhesive formulations use polymers as adhesive components. These polymers form viscous liquids when hydrated, increasing their retention time over mucosal surfaces which may lead to interaction between polymers chain and the oral mucosa. Thus, the adequate selection of the polymer is crucial for the correct delivery of drugs in mucoadhesive formulations [4].

Lovastatin (LVS) is an antihyperlipidaemic drug. Its principal metabolite that is hydroxy acid is potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, which catalyses the conversion of hydroxy methyl gluterate to mevalonate which is an early and rate limiting step in biosynthesis of cholesterol [5]. It has been proven that LVS is effective as therapeutic and prophylactic agent in the management of major morbidities such as atherosclerosis, peripheral artirial disease and cardiovascular disease [6]. The drug lovastatin possesses some major limitations such as low solubility, less bioavailability (5%), short half-life (1.1-1.7 h), excretion in the bile (85%), gastrointestinal side effects [7]. It is a white crystalline powder which has low aqueous solubility, which is 0.4 µg/ml at room temperature [8].

To overcome the low aqueous solubility of model drug LVS an attempt has been made to enhance the aqueous system solubility of LVS by preparing solid dispersion by spray drying technique by using PVPK30 polymer. The result findings are published in our previous paper "Physicochemical characterization and dissolution study of spray-dried amorphous Lovastatin with PVPK30"; The Pharma Innovation journal; 7(3)2018:498-502.

In the current study, the solid dispersions (prepared by spray drying technique SD4) showing best in-vitro drug release profile is selected and further developed into transbuccal patches, which helps to facilitate drug absorption through transbuccal route and overcome the other problems such as first-pass metabolism, excretion in the bile and other side effects. Hence by formulating spray-dried lovastatin into transbuccal films, an attempt has been made to improve the bioavailability of the drug LVS.

MATERIALS AND METHODS

Materials

LVS is obtained as gift sample by Biocon Limited, Bangalore Karnataka India. HPMCK4M by Yarrow chemical products Mumbai Maharashtra India and chitosan from Central Institute of fischeries technology Cochin, Kerala India. All other chemicals and solvents used in this study were of analytical grade reagents.

Methods

Compatibility studies

Compatibility studies were carried out for SD4 and polymers used to prepare the transbuccal film. SD4, HPMC K4M, HPMC E5LV, chitosan polymers and transbuccal films were subjected for Infrared spectroscopic analysis.

Fourier transform infrared spectra of moisture-free powdered samples were obtained by using spectrophotometer (FT-IR Shimadzu Co., Japan) by potassium bromide (KBr) pellet method (2 mg of sample in 200 mg of KBr). The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

Formulation of transbuccal films

The solvent casting technique was used for the preparation of spray-dried LVS mucoadhesive buccal patches. The buccal patches were formulated by using different polymers i.e., HPMCK4M, HPMC E5LV and chitosan. These polymers were chosen from a series of trials on the base of the chemical compatibility, organoleptic properties and adhesiveness property. The placebo films were prepared. The processing variables used while formulating the placebo patches were the concentration of polymers and plasticizers. After results of placebo films were found to be satisfactory, the formulations containing different concentrations of polymers were prepared by using above-said polymers.

Transbuccal film by using HPMC K4M

The polymer HPMCK4M is dissolved in 10 ml of mixture of ethanol and distilled water (3:2) and soaked for overnight. The polymeric solution was stirred on magnetic stirrer for 2 h. The solid dispersion equivalent to 120 mg LVS was added followed by addition of plasticizer 4 % glycerol and 20 mg of menthol as permeation enhancer. The mixture was stirred for 30 min and then poured into Petri dish, which is stored at 4 °C to remove air bubbles entrapped and finally dried at 37 °C for 4 h. The dried films were cut into 1 cm^2 and packed in aluminum foil and stored [1, 4, 9].

Table 1: Composition of the transbuccal film by using HPMC K4M

Formulation code	Solid dispersion equivalent to LVS (mg)	HPMC K4M (mg)	Plasticizer Glycerol (%)	Permeation enhancer menthol (mg)
F1	120	100	5	20
F2	120	200	5	20
F3	120	300	5	20
F4	120	400	5	20
F5	120	500	5	20
F6	120	600	5	20

Transbuccal film by using HPMC E5

The polymer HPMC E5 is dissolved in 10 ml of a mixture of dichloromethane and ethanol (1:1) and soaked for overnight. The polymeric solution was stirred on magnetic stirrer for 2 h. The solid dispersion equivalent to 120 mg LVS was added followed by addition of plasticizer 0.7 % glycerol and 20 mg of menthol as permeation enhancer. The mixture was stirred for 30 min and then poured into Petri dish, which is stored at 4 °C to remove air bubbles entrapped and finally dried at 37 °C for 4 h. The dried films were cut into 1 cm^2 and packed in aluminum foil and stored [4, 10].

Table 2: Composition of transbuccal film by using HPMC E5

Formulation code	Solid dispersion equivalent to LVS (mg)	HPMC E5 (mg)	Plasticizer Glycerol (%)	Permeation enhancer menthol (mg)
F7	120	100	0.7	20
F8	120	200	0.7	20
F9	120	300	0.7	20
F10	120	400	0.7	20
F11	120	500	0.7	20
F12	120	600	0.7	20

Transbuccal film by using chitosan

Citric acid 20 mg was dissolved in water. To this solution, chitosan polymer was added and soaked for overnight. The polymeric solution was stirred on magnetic stirrer for 2 h. The solid dispersion equivalent to 120 mg LVS was added. The mixture was stirred for 30 min and then poured into petridish, which is stored at 4 °C to remove air bubbles entrapped. The patches were finally dried at 25 °C for 24 h. The dried films were cut into 1 cm^2 and packed in aluminium foil and stored [4].

Table 3: Composition of the transbuccal film by using chitosan

Formulation code	Solid dispersion equivalent to LVS (mg)	Chitosan (mg)	Plasticizer Glycerol (%)	Permeation enhancer citric acid (mg)
F13	120	100	3	20
F14	120	200	3	20
F15	120	300	3	20
F16	120	400	3	20
F17	120	500	3	20
F18	120	600	3	20

Physicochemical characteristics of transbuccal patches

Film thickness and weight

The thickness of all the formulations was measured by screw gauge (Mitutoyo Corporation, Kavasaki, Japan) and the weight of these films were determined by using electronic balance [1].

Content uniformity

The film was dissolved in 100 ml isotonic phosphate buffer pH 6.8±0.2, filtered (0.22 µm), and resultant solutions were analyzed by UV Spectrophotometer at 238 nm. The experiment was performed in triplicate [1].

Surface pH

The microenvironmental pH of all the formulations was measured so as to predict its effect on buccal mucosa. The formulations were first wetted by adding distilled water to its surface. The surface pH was then recorded by bringing a glass electrode near the surface of the formulation and allowing it to equilibrate for 1 min. The average pH±SD was determined for all formulations [1].

Swelling index

The buccal patches were weighed individually (designated as W_1) and placed seperately in 2% agar gel plates, incubated at 37±1 °C and examined for any physical changes. At regular 1 h time intervals until 3 h, films were removed from the gel plates and excess surface water was removed carefully using the filter-paper. The swollen films were then reweighed (W_2) and the swelling index (SI) was calculated using the following formula:

$$SI = \frac{W_2 - W_1}{W_1} \times 100$$

The experiment was performed in triplicate and average±SD values were recorded [1].

Folding endurance

Folding endurance of the films was determined by repeatedly folding and unfolding the films at the same place till it broke or for 300 times, which is considered to be a satisfactory value to reveal good folding-endurance properties. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance [1].

Ex-vivo residence time/adherence time

The *ex-vivo* residence time is studied (n=3) to know the time required for complete erosion and/or detachment of the film from the mucosa surface. The fresh goat mucosa is fixed to the inner side of the beaker about 2.5 cm from the bottom. One side of the film was wetted with 1 drop of phosphate buffer pH 6.8 and it is adhered to the surface of the buccal mucosa by applying slight force with fingertips for 30 s. The beaker was filled with 500 ml of phosphate buffer pH 6.8 and was kept at 37±0.5 °C. After 2 min, a 50 rpm stirring rate is applied to simulate the buccal cavity environment and film adherence is monitored for 8 h [1].

Ex-vivo bioadhesion test

Modified physical balance method is used to measure the *ex-vivo* mucoadhesive strength of prepared films. Fresh goat's buccal mucosa is taken and cut into piece and washed with phosphate buffer pH 6.8 and tied to the open mouth of glass vial which is tightly fitted into a glass beaker which is filled with phosphate buffer pH 6.8 in such way that, it just touched the buccal surface. The temperature of this beaker is maintained at 37 °C±1 °C. The film is adhered to the lower side of a rubber stopper with cyanoacrylate adhesive. Two pans of the balance are balanced with 5g weight on the right side of the pan, which is lowered the pan along with the film over the mucosa. The balance is kept in this position for 5 min of contact time. The water drops are added slowly to the right-hand side pan, until the film detached from the mucosal surface. The weight required to detach the film from the mucosal membrane surface is a measure of mucoadhesive strength. All the experiments are performed in triplicates and mean±SD are reported. The following formula is used to calculate the detachment force [1].

Force of adhesion (N) = (g X 9.81)/ 1000

Detachment force (N/m2) = Force of adhesion (N) / Surface area (m2)

In vitro drug release study

In vitro drug release is carried out by paddle over disc dissolution apparatus. The transbuccal patch is placed beneath the disc in the dissolution jar containing 900 ml phosphate buffer (pH 6.8) solution. The bath temperature is maintained at 37±1 °C with 50 rpm speed. Aliquots of 5 ml are withdrawn at prespecified time intervals for 6 h. same volume of fresh buffer solution is replaced. The withdrawn sample solution is filtered through 0.4 µm membrane filter and the amount of drug is determined by measuring the absorbance of the aliquots at 238 nm using UV spectrophotometer and percentage drug release were plotted [1].

Ex-vivo drug permeation study

Franz diffusion cell is used to carry out *ex-vivo* drug permeation study. Fresh goat buccal mucosa is fixed on a diffusion cell between the donor and receptor compartment. The transbuccal patch is fixed on the mucosal membrane. Five ml of phosphate buffer pH 6.8 in the donor compartment and 45 ml of the phosphate buffer pH 6.8 in the receptor compartment is filled as dissolution fluid. The fluids maintained at 37±1 °C and stirred continuously at speed of 50±5 rpm. Aliquots of 1 ml of the sample are withdrawn at prespecified time interval. Same volume of fresh buffer solution is replaced. The withdrawn sample solution is filtered through 0.4 µm membrane filter and the amount of drug is determined by measuring the absorbance of the aliquots at 238 nm using UV spectrophotometer and percentage drug permeated were plotted [1].

Stability study

The accelerated stability of optimized formulation F3 is conducted as per ICH guidelines at 40 °C/75% RH up to 6 mo. Periodically samples are removed (initial, 1 mo, 2 mo, 3 mo) and analyzed for drug content, *in vitro* release and *ex-vivo* permeation [9].

RESULTS AND DISCUSSION

Compatibility studies

The IR Spectra of SD4 (SDSP) and transbuccal formulations (SD-HPMCK4M, SD-HPMCE5LV and SD-chitosan) are shown in the fig. 1. Upon comparison of IR Spectra of SD4(SDSP) and transbuccal formulations(SD-HPMCK4M, SD-HPMCE5LV and SD-chitosan), it was found that the characteristic peaks of LVS in SD4 were 3535 cm^{-1}(alcohol OH stretch), 3016 cm^{-1} (olefinic CH stretching vibrations) 1725 $cm^{-1}$and 1695 cm^{-1} (lactone and ester carbonyl stretch) also found in transbuccal formulations along with characteristic peaks of respective polymers. It can be concluded by these observations that there are no possible interactions between the SD4 and polymers.

Formulation of transbuccal films

The formulations containing different concentrations of polymers were prepared and their effects on physicochemical characteristics were studied. However, the selections of plasticizer were finalized during placebo film preperation.

Physicochemical charecteristics of transbuccal patches

The physicochemical charecteristics data of transbuccal patches are shown in the tables (4-6) below.

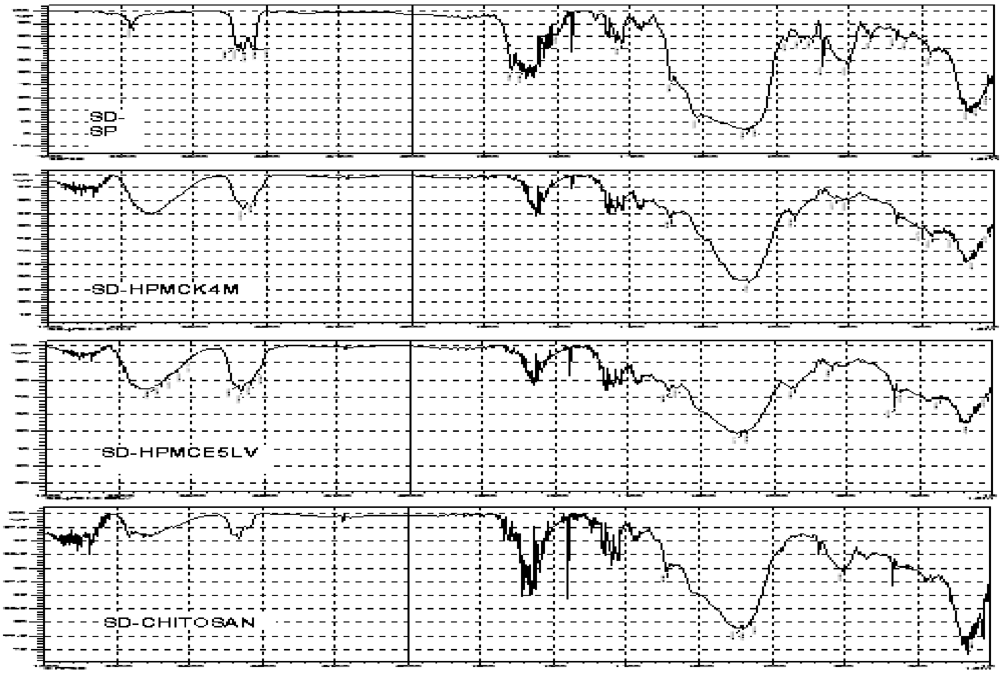


Fig. 1: FTIR spectra of SD4 (SDSP) and transbuccal formulations (SD-HPMCK4M, SD-HPMCE5LV and SD-Chitosan)

Table 4: Physicochemical characteristics of transbuccal film by using HPMC K4M (F1-F6)

Formulation code	Thickness (mm)*	Weight of film (mg)*	Drug content (%)*	Surface pH*	Swelling index (%)*	Folding endurance*
F1	0.27±0.05	91.91±0.2	91.71±0.33	6.2±0.07	31.7±3.5	315±7.5
F2	0.34±0.02	101.25±0.3	93.23±0.41	6.4±0.05	42.9±5.1	312±5.0
F3	0.51±0.04	108.32±0.4	95.75±0.56	6.7±0.04	56.0±2.1	350±2.5
F4	0.62±0.01	115.56±0.2	94.80±0.61	6.4±0.12	57.9±2.8	340±6.0
F5	0.68±0.03	125.46±0.6	91.68±0.23	6.3±1.23	49.4±1.8	324±4.5
F6	0.81±0.1	132.03±0.8	89.34±0.17	5.8±0.98	61.3±1.6	307±9.0

*Data are expressed as mean±standard deviation of the mean [SD], n=3.

Table 5: Physicochemical characteristics of transbuccal film by using HPMC E5 (F7-F12)

Formulation code	Thickness (mm)*	Weight of film (mg)*	Drug Content (%)*	Surface pH*	Swelling Index (%)*	Folding endurance*
F7	0.21±0.07	89.10±0.6	90.23±0.51	7.6±0.98	23.0±4.1	326±4.7
F8	0.27±0.04	98.91±0.5	95.27±0.47	6.7±0.07	27.0±3.6	319±5.1
F9	0.55±0.03	103.25±0.3	97.33±0.31	6.9±0.12	48.2±1.1	345±1.8
F10	0.82±0.02	112.16±0.2	93.82±0.11	6.3±0.83	52.0±2.6	337±3.1
F11	0.88±0.06	121.36±0.5	88.67±0.61	7.2±1.23	61.3±2.3	312±2.5
F12	0.91±0.1	128.57±0.3	85.47±0.87	6.4±0.03	56.4±5.2	336±4.5

*Data are expressed as mean±standard deviation of the mean [SD], n=3.

Table 6: Physicochemical characteristics of transbuccal film by using chitosan (F13-F18)

Formulation code	Thickness (mm)*	Weight of film (mg)*	Drug content (%)*	Surface pH*	Swelling index (%)*	Folding endurance*
F13	0.92±0.03	91.91±0.4	93.51±0.43	5.6±0.67	54.4±5.2	193±1.1
F14	0.98±0.06	104.25±0.5	94.23±0.71	6.2±0.12	58.2±1.7	194±2.5
F15	1.15±0.07	103.32±0.2	93.75±0.76	6.5±0.27	63.3±1.6	199±3.7
F16	1.12±0.53	108.56±0.1	95.80±0.41	6.7±1.63	68.3±1.6	208±0.8
F17	1.5±0.39	122.46±0.7	92.68±0.73	7.2±0.28	76.5±3.6	176±3.8
F18	1.4±0.81	128.03±0.4	91.62±0.82	6.5±0.83	73.3±6.1	165±1.9

*Data are expressed as mean±standard deviation of the mean [SD], n=3.

As the concentration of polymers increases, both film thickness and weight of the patches also increases. Weight of the film ranged from 89.10±0.6 to 132.03±0.8 mg. Weight and thickness are directly related to the accuracy of the dose distribution in the film. The thickness of the film ranged from 0.21±0.07 to 132.03±0.8 mm. the films of chitosan shown highest thickness. Drug content ranges from

85.47±0.87 to 97.33±0.31. Uniformity in drug content is important parameter in formulation which confirm reproducibility of the product. The assay of drug content at three different places in each film showed that the drug was uniformly distributed throughout the films. The surface pH ranged from 5.6 to 7.6 which ensure there is no mucosal irritation due to the formulation.

The swelling index study was carried out to find out the degree of swelling of buccal films in simulated saliva solutions. HPMCK4M and HPMC E5 films started swelling after 5 min and chitosan after 30 min when hydrated on agar medium due to the swellable hydrophillic polymers. The results shown that as the polymer concentration increases the swelling index was also increased. As per the study carried out by the authors Pankaj kumar *et al.*, [1] swelling of the polymers makes strong secondary hydrogen bonding with buccal mucosa and thus results in good mucoadhesion which provides unidirectional release of the drug. Chitosan buccal films (F13 to F 18) shown high values of swelling index as compared to HPMC films (F1 to F12). H. Castan *et al.*, [2] stated that this may be due to chitosan when wetted can gain weight and size by 150%. This high value of swelling index causes discomforts to the patient.

All the developed formulations were flexible and shown good folding endurance. However, HPMC K4M and HPMC E5 formulations (F1 to F12) shown good folding endurance but in case of chitosan containing formulations (F13 to F18), the films became brittle, lost elasticity as the polymer concentration increased. Hence the folding endurance values decreased as the polymer concentration increased.

Ex-vivo residence time

Residence of film is directly related to the mucoadhesive strength, which is consequence of interaction between mucin and polymers. Further adequate hydration is required for the polymers to get charged and impart sufficient mucoresidence. *Ex-vivo* residence time ranged from 2.2±0.08to 8.2±0.17 h. In the formulations with HPMC K4M polymers (table 7) containing films, the presence of hydroxyl groups which facilitate uptake of water into polymer matrix and enhance mucoadhesiveness. These polymers also form viscous liquids when hydrated with increasing their retention time over mucosal surfaces, which may lead to adhesive interactions. The formulations with HPMC E5 (table 8) shown short period of residence time, which might be because of low viscosity of the polymer which resulted into weak adhesion. In case of formulations with chitosan (table 9), there is interaction between positively charged amino group and negatively charged mucin of membrane and helps in bioadhesion for longer duration of time.

Table 7: Physicochemical characterization, cumulative percentage release and percentage drug permeated of F1-F6

Formulation code	*Ex-vivo* residence time*	*Ex-vivo* bioadhesive strength(g)*	Detachment force (N/m2)	% Drug released after 6 h*	% Drug permeated after 6 h*
F1	6.1±0.12	40±1.41	3920	97.78±1.08	96.78±1.1
F2	6.2±0.08	42±0.86	4116	95.32±3.5	94.24±1.0
F3	6.5±0.10	43±1.07	4214	94.17±0.62	93.27±2.6
F4	7.2±0.15	44±1.43	4312	92.34±1.4	87.34±4.4
F5	7.3±0.11	45±1.86	4410	89.24±2.0	77.43±3.0
F6	7.4±0.13	47±0.88	4606	86.17±1.2	68.42±1.3

*Data are expressed as mean±standard deviation of the mean [SD], n=3.

Table 8: Physicochemical characterization, cumulative percentage release and percentage drug permeated of F7-F12

Formulation code	*Ex-vivo* residence time*	*Ex-vivo* bioadhesive strength (g)*	Detachment force (N/m2)	% Drug released after 6 h*	% Drug permeated after 6 h*
F7	2.2±0.08	30±0.64	2940	94.5±1.4	90.45±3.2
F8	2.3±0.13	31±0.76	3038	92.7±1.1	89.57±3.9
F9	2.6±0.18	32±0.63	3136	91.32±1.2	86.45±3.0
F10	2.7±0.16	34±0.35	3332	90.88±1.7	81.37±0.8
F11	2.7±0.16	36±0.41	3528	89.88±1.7	78.73±1.3
F12	2.8±0.17	37±0.36	3626	86.47±3.1	71.56±0.5

*Data are expressed as mean±standard deviation of the mean [SD], n=3.

Table 9: Physicochemical characterization, cumulative percentage release and percentage drug permeated of F13-F18

Formulation code	*Ex-vivo* residence time*	*Ex-vivo* bioadhesive strength(g)*	Detachment force (N/m2)	% Drug released after 6 h*	% Drug permeated after 6 h*
F13	7.2±0.17	54±0.52	5292	76.44±3.1	73.25±1.7
F14	7.5±0.08	57±0.84	5586	74.15±1.0	69.83±4.8
F15	7.9±0.12	60±0.57	5880	72.35±2.2	65.23±2.8
F16	8.1±0.61	62±0.33	6076	71.83±3.6	63.12±3.6
F17	8.1±0.91	65±0.57	6370	70.79±2.5	60.56±2.6
F18	8.2±0.17	66±0.43	6468	68.73±1.1	57.34±2.1

***** Data are expressed as mean±standard deviation of the mean [SD], n=3.

Ex-vivo bioadhesion test

Mucoadhesive strength plays an important role in proper adherence of the film to the mucosal surface. Excessive adhesion may cause discomfort to the patient leading to the patient in compliance. Hence optimum mucoadhesion is required for film for effective therapy.

The *Ex-vivo* bioadhesive strength and Detachment force value are shown in the above table (7-9). Transbuccal films with HPMC K4M (F1-F6); shows good mucoadhesion, which may be attributed to the hydration and liquid entrapment in the polymer network, after formation of viscous polymeric network, however HPMC E5 (F7-F12) polymer films shows the weak force of adhesion. According to Magdy *et al.*, [12] this might be because of its low viscosity of the polymer. Apart from this, hydrophilicity PVP K30 (from Solid dispersions which is incorporated in the transbuccal film) might further have weakened the force of adhesion. The transbuccal films of chitosan (F13-F18) also show good mucoadhesion. According to Cui *et al.* [13] the strong viscoelastic property and structure of chitosan might be attributed to its high mucoadhesive force which occurs because of interaction between amino group of chitosan and mucin of biomembrane.

In vitro drug release

The drug release study was carried out over a period of no longer than 6 h given that formulations cannot remain for longer period s on the surface of the buccal membrane.

The drug release profile of formulations is shown in the fig. 2. At the end of the 6th h F_1 showed highest drug release of 97.78% and F_6 is 86.17%. In case of HPMC E5 formulations, at the end of the 6th h F7 showed highest drug release of 94.5% and F12 is 86.47%. The significant decrease in drug release is found as the polymer concentration increases in formulations from F1 to F6. Meher *et al.* [2] states that this could be related to the increase in thickness of the film with an increase in HPMC concentration. Thus the time requires for dissolution medium to penetrate into the polymer chain located through the depth of the film increases.

Similar observation is made with HPMC E5 formulations also. However initial drug release (30 min) is more as compared to other formulations. This might be because of low viscosity of HPMC E5 polymer as observed by Magdy *et al.*, [12]

At the end of the 6th h F13 showed highest drug release of 76.44% and F18 is 68.73%. In the case of chitosan containing formulations drug release values after completion of 6th h is lower as compared to other formulations. Similar observation is found by author Patel R P *et al.*, [1] chitosan is a cationic polymer which might have formed complex with PVP K30 (polymer used to prepare solid dispersions which is incorporated in the transbuccal films), which is non-ionic polymer leading to extension of the drug release.

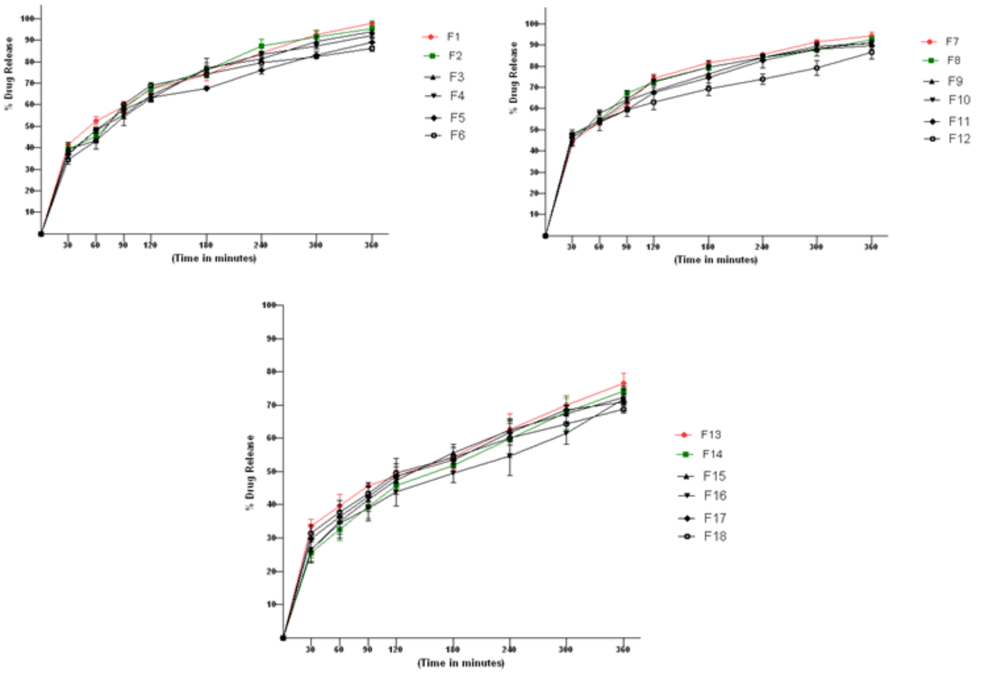


Fig. 2: Percentage drug release profile of transbuccal films (F1-F18) (Values are expressed as mean±SD, n=3)

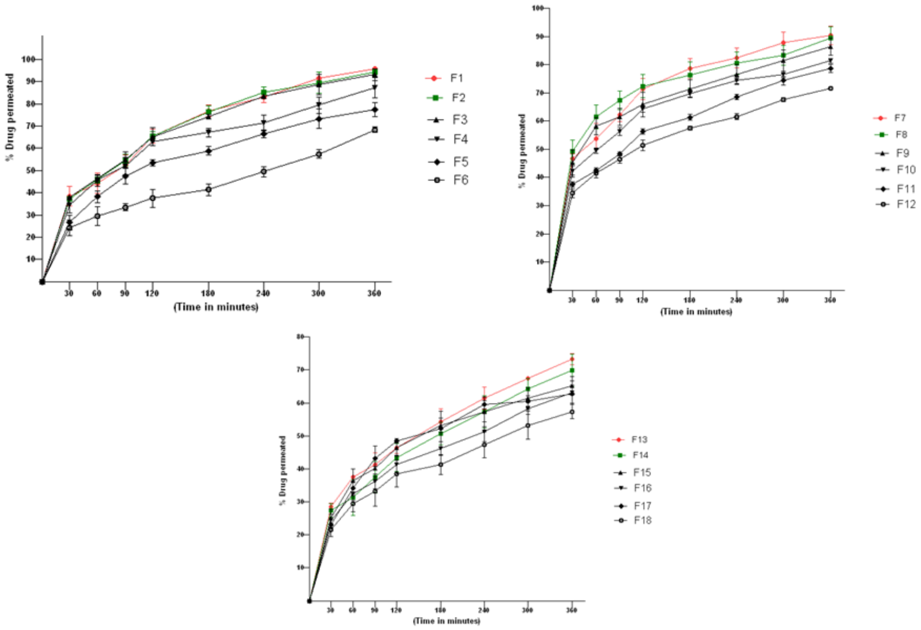


Fig. 3: Percentage drug permeation profile of transbuccal films (F1-F18) (Values are expressed as mean±SD, n=3)

Ex-vivo drug permeation study

The fig. 3 shows the drug permeation profile of formulations. Drug permeated at the 6th h of F_1 is 96.78% and F6 is 68.42%. Similarly, F7 is 90.45 % and F1271.56 %. In case of HPMC polymers, formation of hydrogel occurs at the surface of biomembrane, which leads to hydration and swelling of mucoadhesive films which promotes diffusion of drug. Drug permeated at the end of the 6th h of F13 is 73.25 % and F18 is 57.34 %. As discussed in the study of swelling index, chitosan films when wetted can gain weight and size by 150% due to swelling of mucoadhesive films, formation of very thick hydrogen layer leads to the longer diffusion path length leading into delayed diffusion. Castan *et al.* [4] states that drug diffusion is delayed in the case of ionic polymers such as chitosan as compared to nonionic polymers (HPMC).

To investigate the release kinetics of drug release from buccal films, the *in vitro* drug release and *ex-vivo* permeation data were subjected to fit various kinetic models using PCP Disso ver 2 software Pune India. The $\mathbf{r^2}$and **n** values are determined. All the buccal films showed n values in the range of 0.73 to 0.87, indicating that the drug release followed by nonfickian diffusion and the best fit model observed to be higuchi model.

Based on results of Drug content, swelling index, folding endurance *in vitro* bioadhesion, *in vitro* drug release and *ex-vivo* permeation, we can conclude that HPMCK4M formulations (F1-F6) are best formulations. The formulation F3 is selected for the further stability study.

Stability study

Accelerated stability data is given in table 10. The results of the stability study revealed that there is no significant change in drug content, *in vitro* drug release and *ex-vivo* permeation.

Table 10: Accelerated stability data of the best formulation F3

Time	Drug content*	% drug released*	% drug permeated*
Initial	93.80±0.71	93.13±2.15	92.34±1.14
1 mo	93.21±0.37	92.17±3.17	92.23±1.67
2 mo	92.34±0.54	92.45±1.13	91.45±2.44
3 mo	91.46±0.56	91.17±2.23	91.87±1.24

***** Data are expressed as mean±standard deviation of the mean [SD], n=3.

CONCLUSION

Mucoadhesive films of spraydried LVS were successfully developed by using 3 different polymers HPMCK4M, HPMCE5LV and chitosan. An *in vitro* charecterisation result shows that HPMCK4M formulations are best formulations. Drug release and permeation were found to be nonfickian diffusion and best fit model observed to be higuchi model. Stability study data revealed that there is no significant change in the drug content, *in vitro* drug release and *ex-vivo* permeation. By using transbuccal mucoadhesive films, it can enhance the drug bioavailability by preventing the first-pass metabolism thus providing better therapeutic efficacy.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Pankaj Kumar, Gulshan Chhabra, Kamla Pathak. Development and statistical optimization of bioadhesive films of Amiloride hydrochloride: *in vitro and ex-vivo* evaluation. Ind J Pharm Edu Res 2012;46:145-54.
2. Meher JG, Tarai M, Yadav NP, Pathak A, Mishra P, Yadav K. Development and characterization of cellulose-polymethacrylate mucoadhesive film for buccal delivery of carvedilol. Carbohydr Polym 2017;96:172-80.
3. K Chandra Sekhar, KVS Naidu, Y Vamshi Vishnu, Ramesh Gannu, V Kishan, Y Madhusudan Rao. Transbuccal delivery of chlorpheniramine maleate from mucoadhesive buccal patches. Drug Delivery 2008;15:185–91.
4. Castan H, Ruiz B, Morales M, Design. Development and characterisation of buccal bioadhesive films of doxepin for treatment of odontalgia. Drug Delivery 2017;22:869-76.
5. Tripathi KD. Essentials of medical pharmacology. 6th ed. Jaypee brothers medical publishers; 2010.
6. Patel RP, Patel MM. Physicochemical charecterization and dissolution study of solid dispersions of lovastatin with PEG 4000 and Polyvinylpyrrolidone K30. Pharm Dev Technol 2007;12:21-33.
7. Parfitt K. Lipid regulating drugs: lovastatin, Martindale. 32nd ed. The Pharmaceutical Press; 1999.
8. Gerald SB, KE Micheal, JK Lovastatin. Analytical profiles of drug substances and excipients.1st ed. Acadaemic Press; 1998.
9. Jagadale S, Hable A, Kuchekar B. Improvement of dissolution rate of chlorzoxazone by solid dispersion technique and development of buccal patch. J Appl Sol Chem Model 2013;2:145-57.
10. Hassan MA, Barakat NS, El-Badry M, Shehata SM. Formulation and *in vitro* and *in vivo* evaluation of naproxen mucoadhesive buccal patches for local effect. J Drug Delivery Sci Technol 2011;21:423-31.
11. Patel RP, Poddar SS. Deveopment and characterization of mucoadhesive buccal patches of salbutamol sulphate. Curr Drug Delivery 2009;6:140-4.
12. Magdy M, Nadia S, Sarah R. Development of a novel ketorolac tromethamine sublingual film. J Am Sci 2015;11:27-37.
13. Cui F, Chunbai H, Miao H, Cui Tang, Lichen Y, Feng Q. Preperation and evaluation of chitosan-ethylenediaminetetraacetic acid hydrogel films for the mucoadhesive transbuccal delivery of insulin. J Biomed Mater Res 2008;89:1063-71.

KLE
EMPOWERING PROFESSIONALS

KLE CENTENARY
OVER 100 YEARS OF SELFLESS SERVICE

KLE College of Pharmacy, Belagavi

A constituent unit of

KLE Academy of Higher Education & Research

[DEEMED-TO-BE-UNIVERSITY], REACREDITED AT THE 'A' LEVEL BY NAAC (SECOND CYCLE), PLACED IN 'A' CATEGORY BY MHRD (GoI)

Certificate

AICTE Sponsored Quality Improvement Programme

Bhuvaneshwari Sharannavar

KLES COLLEGE OF PHARMACY BELAGAVI

In recognition of his/her attendance as **Resource Person** at the two week Quality Improvement programme on "**Drug Development & Discovery: Natural & Synthetic perspective**" in virtual mode from 18th August to 2nd September 2020.

Prof. (Dr.) Shankar Alegaon
Program Coordinator

Prof. (Dr.) Sunil Jalalpure
Chief Coordinator

Prof. (Dr.) Kalpana Patil
Program Coordinator

nirf 37th
2020 Rank

B. Pharm Program Accredited by NBA for a period of Six Years (from July 2019 to June 2025)

klepharm_edu

www.facebook.com/klepharm.edu

www.klepharm.edu

YouTube
KLE College of Pharmacy, Belagavi

KLE
ACADEMY OF HIGHER EDUCATION AND RESEARCH
EMPOWERING PROFESSIONALS
Deemed-to-be-University u/s 3 of the UGC Act, 1956

KLE College of Pharmacy, Belagavi

Nehru Nagar, JNMC Campus, Belagavi - 590 010
Karnataka India

BELAGAVI

AICTE Sponsored Quality Improvement Programme

01st to 13th October 2018

Dr./Mr./[✓ Mrs]/ Ms ......B. A. SHARANNAVAR......

in recognition of ~~his~~ / [✓ her] attendance as [✓ Resource Person] / ~~Participant~~ at the Quality Improvement Programme on "TECHNOLOGICAL ADVANCES IN DRUG DELIVERY SYSTEM AND THEIR QUALITY MANAGEMENT"

Chief Co-ordinator
Dr. B.M. Patil

Seal

Co-ordinator
Dr. A. P Gadad

KLE
UNIVERSITY
EMPOWERING PROFESSIONALS

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

Accredited at 'A' Grade by NAAC (2nd Cycle)
Placed in Category 'A' by MHRD (GoI)

KLE UNIVERSITY'S COLLEGE OF PHARMACY, BELAGAVI

CERTIFICATE

This to Certify that

~~Dr.~~ / ~~Mr.~~ /Ms. B. R. Sharannavar

has participated in the

AICTE *Sponsored Two weeks Quality Improvement Programme On*

"Newer Trends in Pharmaceutical Technology and Research" from 30th October to 11th November 2017

organised by Department of Pharmaceutics, as a ~~Delegate~~ / Resource Person

Prof. (Dr.) B. M. Patil
Chief Co-ordinator

Dr. V. S. Mannur
Program Co-ordinator

PULLA REDDY INSTITUTE OF PHARMACY

विद्यया ऽ मृतमश्नुते

Near Dundigal Air Force Academy,
Annaram (V), Jinnaram (M), Hyderabad - 502313.

in association with

ISPOR India Telangana Chapter

CERTIFICATE FOR PRESENTATION

This is to certify that ~~Mr~~/Mrs/Ms/~~Dr~~. Bhuvaneshwari. R. Sharannavar

presented a Oral/Poster entitled" "PAT- A framework for innovative Pharmaceutical manufacturing." *"*

in the two day

"International Conference on the use of National Medicines Policy and Biostatistics in Pharmaceutical Sciences",

held on 28th & 29th September 2016.

Organized by

Pulla Reddy Institute of Pharmacy

Sri Ch. Sathi Reddy Chairman, PRIP.	**Mr. Ch. Aditya Reddy** Director, PRIP.	**Dr. V. Rama Mohan Gupta** Convener Innovate -16	**Mr. Subhas Sahoo** Scientific Committee Innovate -16

K.L.E. UNIVERSITY's

College of Pharmacy, Belagavi

(Accredited by NBA)

&

All India Council For Technical Education (New Delhi)

KLE UNIVERSITY
Accrediated 'A' by NAAC

AICTE

Grant this Certificate of Achievement to

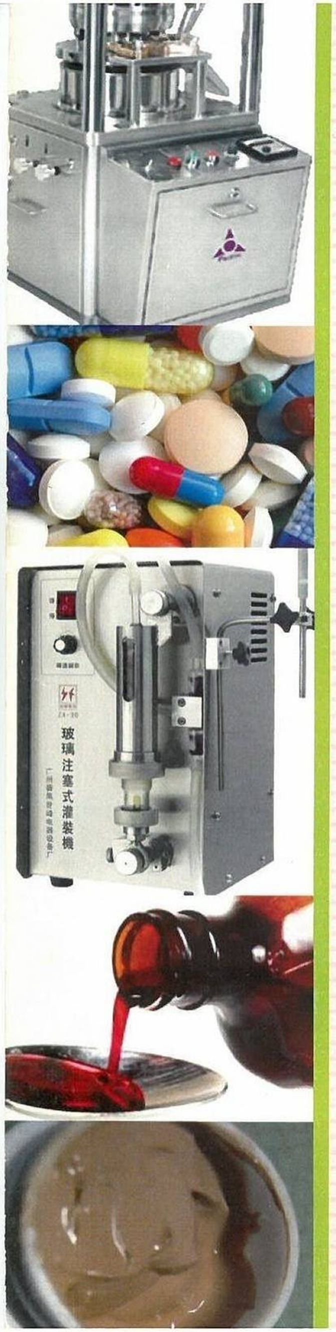
~~Dr.~~/~~Mr.~~/Mrs/ ~~Ms~~ BHUVANESHWARI R. SHARANNAVAR

in recognition of ~~his~~ / her attendance as Resource Person / ~~Participant~~ at the Quality Improvement Programme on "PHARMACEUTICAL TECHNOLOGY WITH CURRENT TRENDS & FUTURE SCOPE IN FORMULATION AND DEVELOPMENT" from 9th to 20th March 2015.

Chief Co-ordinator
Dr. V.P Rasal

Gadad
Co-ordinator
Dr. A. P Gadad

P. M. Dandagi
Programme Co-ordinator
Dr. P. M. Dandagi



K.L.E. UNIVERSITY'S
(Accrediated 'A' Grade by NAAC)

SHRI B. M. K. AYURVED MAHAVIDYALAYA

Post Graduate Studies and Research Center, Shahapur, Belgaum - 590003,
Ph : +91 831 2486286, Fax : +91 831 2424157
Web site : www.bmkayurveda.org, www.kleayurveda.org, ayurveda.kleuniversity.edu.in ; e-mail : bmkayurveda@rediffmail.com

Intensive Training Programme

BHESHAJA TECH - 2012

Organised by

P. G. Dept. of Bhaishajya Kalpana

Collaboration with
Dept. of Pharmaceutics, KLEU College of Pharmacy, Belgaum

CERTIFICATE

Dr./Mr./Mrs. ......Bhuvaneshwari R. Sharannavar.......................................

has participated as a Resource person / ~~Delegate~~ in the Intensive Training Programme "BHESHAJA TECH -2012" conducted on 11th, 12th & 13th January 2012.

Dr. R. S. Hiremath
Organising Secretary
P. G. Dept. of Bhaishajya Kalpana

Dr. K. S. Gudaganatti
Prof. & H. O. D.
P. G. Dept. of Bhaishajya Kalpana

Dr. A. D. Taranalli
Principal
KLEU College of Pharmacy, Belgaum

Dr. B. S. Prasad
Principal
KLEU Shri BMK Ayurved Mahavidyalaya

KLE
UNIVERSITY
EMPOWERING PROFESSIONALS

K.L.E.UNIVERSITY's

College of Pharmacy
&
All India Council For Technical Education
(New Delhi)

Grant this certificate of achievement to

Dr./Mr./Mrs./Ms. B. R. SHARANNAVAR

In recognition of ~~his~~ / her attendance as Resource Person / ~~Participant~~ at the Quality Improvement Programme on **"QUALITY ASSURANCE OF PHARMACEUTICALS MEETING A MAJOR PUBLIC HEALTH CHALLENGE"**. From Monday 5th to 16th October 2009

	P. M. Dandagi	
Chief Co-ordinator	Convenor	Co-ordinator
Dr. F. V. Manvi	Dr. P. M. Dandagi	Dr. V. S. Mastiholimath

NITTE
(Deemed to be University)

N G S M INSTITUTE OF PHARMACEUTICAL SCIENCES

Paneer, Deralakatte, Mangaluru-575018

Pharmacy Council of India
Sponsored

CONTINUING EDUCATION PROGRAMME FOR PHARMACY TEACHERS ON

EMERGING TRENDS IN PHARMACEUTICAL EDUCATION AND RESEARCH

CERTIFICATE

This is to certify that **Mrs. Bhuvaneshwari R Sharannavar** participated as Delegate in the PCI Sponsored CEP on "Emerging Trends in Pharmaceutical Education and Research" held on February 7th-9th, 2019 at NGSM Institute of Pharmaceutical Sciences.

Dr. M.D Karvekar	**Dr. C.S.Shastry**	**Dr. M.Vijay Kumar**
Member of Executive Council-PCI	Chairman, PCI-CEP	Coordinator, PCI-CEP

DEPARTMENT OF PHARMACEUTICS

KLE COLLEGE OF PHARMACY,

VIDYANAGAR, HUBBALLI

A constituent Unit of KLE Academy of Higher Education and Research, Belagavi

Accredited 'A' Grade by NAAC (2nd Cycle), Placed in category A by MHRD (GoI)

CERTIFICATE

This is to certify that

~~Dr~~./~~Mr~~/Ms. BHUNESHWARI . SHARANNAVAR.

has participated in the seminar entitled **"REGULATORY PROSPECTS IN DRUG PRODUCT DEVELOPMENT AND CLINICAL DATA MANAGEMENT"** on 1st October, 2019 organized by the **Department of Pharmaceutics,** KLE College of Pharmacy, Hubballi as a ~~Resource Person~~/Delegate.

CO-ORDINATOR
Dr. (Smt.) F. S. Dasankoppa

PRINCIPAL/CONVENER
Prof. (Dr.) V. G. Jamakandi

KLE
EMPOWERING PROFESSIONALS

KLE CENTENARY
OVER 100 YEARS OF SELFLESS SERVICE

Golden Jubilee Celebration
1968 – 2018

KLE COLLEGE OF PHARMACY, BELAGAVI

A Constituent Unit of KLE Academy of Higher Education and Research
[Deemed -To-Be-University]

Certificate

This is to certify that

~~Prof./Dr./Mr./Ms.~~ Mrs. BHUVANESHWARI S.

has participated as Organizing Committee Member and attended the scientific sessions of

National Conference on **"Advances in Drug Discovery and Development"**

held on the occasion of **Golden Jubilee Year Celebrations,**

26th & 27th October 2018 at KLE College of Pharmacy, Belagavi, Karnataka

Dr. B. M. Patil
Chairman - LOC

Dr. S. S. Jalalpure
Convener

KLE
ACADEMY OF HIGHER
EDUCATION AND RESEARCH
Deemed-to-be-University
EMPOWERING
PROFESSIONALS

KLE ACADEMY OF HIGHER EDUCATION & RESEARCH

(Formerly known as KLE University, Belagavi)

[Declared as Deemed-to-be-University u/s 3 of the UGC Act, 1956, vide Government of India Notification No. F.9-19/2000-U3(A)]
Placed in Category 'A' by MHRD, Govt. of India Accredited 'A' Grade by NAAC 2nd Cycle
Nehru Nagar, Belagavi - 590 010, Karnataka State, India
Ph. : 0831-2444444 FAX : 0831-2493777 **Web**: http://www.kledeemeduniversity.edu.in **E-mail**: info@kledeemeduniversity.edu.in

UNIVERSITY DEPARTMENT OF EDUCATION FOR HEALTH PROFESSIONALS

CERTIFICATE

This is to certify that

~~Dr.~~/~~Mr.~~/Mrs. B. R. SHARANNAVAR

has participated in the Workshop entitled

FOSTERING PUBLICATION INTEGRITY AND PLAGIARISM AWARENESS

on 21st AUGUST 2018 organised by KAHER'S - BSRC BELAGAVI as a Delegate ✓ / ~~Resource Person~~.

PRWalvekar
Dr. PADMAJA WALVEKAR
DIRECTOR, UDEHP

Dr. V. D. PATIL
REGISTRAR

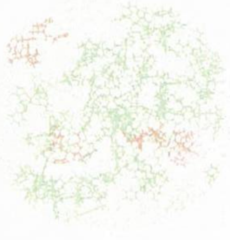
MANIPAL COLLEGE OF PHARMACEUTICAL SCIENCES

MANIPAL

(A constituent unit of MAHE, Manipal)

In association with

SCHRÖDINGER®



Certificate

This is to certify that Bhuvaneshwari R. Sharannavar

has participated as **Delegate** in the National Workshop on **"Computational Tools in the Design of Pharmaceutical Formulations"** held during 8-9 June, 2018 conducted by Centre for Drug Delivery Technologies, Department of Pharmaceutics, Manipal College of Pharmaceutical Sciences, MAHE, Manipal.

Number of credits awarded: 01

Dr C Mallikarjuna Rao
Principal

Dr K B Koteshwara
Head, Department of Pharmaceutics

Dr Srinivas Mutalik
Coordinator
Centre for Drug Delivery Technologies

KLE UNIVERSITY

(Formerly known as KLE Academy of Higher Education & Research, Belgaum)

[Declared as Deemed-to-be-University u/s 3 of the UGC Act, 1956, vide Government of India Notification No. F.9-19/2000-U3(A)]
Placed in Category 'A' by MHRD, Govt. of India Accredited 'A' Grade by NAAC
Nehru Nagar, Belgaum - 590 010, Karnataka State, India
Ph. : 0831-2444444 FAX : 0831-2493777 **Web**: http://www.kleuniversity.edu.in **E-mail**: info@kleuniversity.edu.in

UNIVERSITY DEPARTMENT OF EDUCATION FOR HEALTH PROFESSIONALS

CERTIFICATE

This is to certify that

~~Dr.~~/~~Mr.~~/Mrs. B. R. Sharannavar

has participated in the Workshop entitled

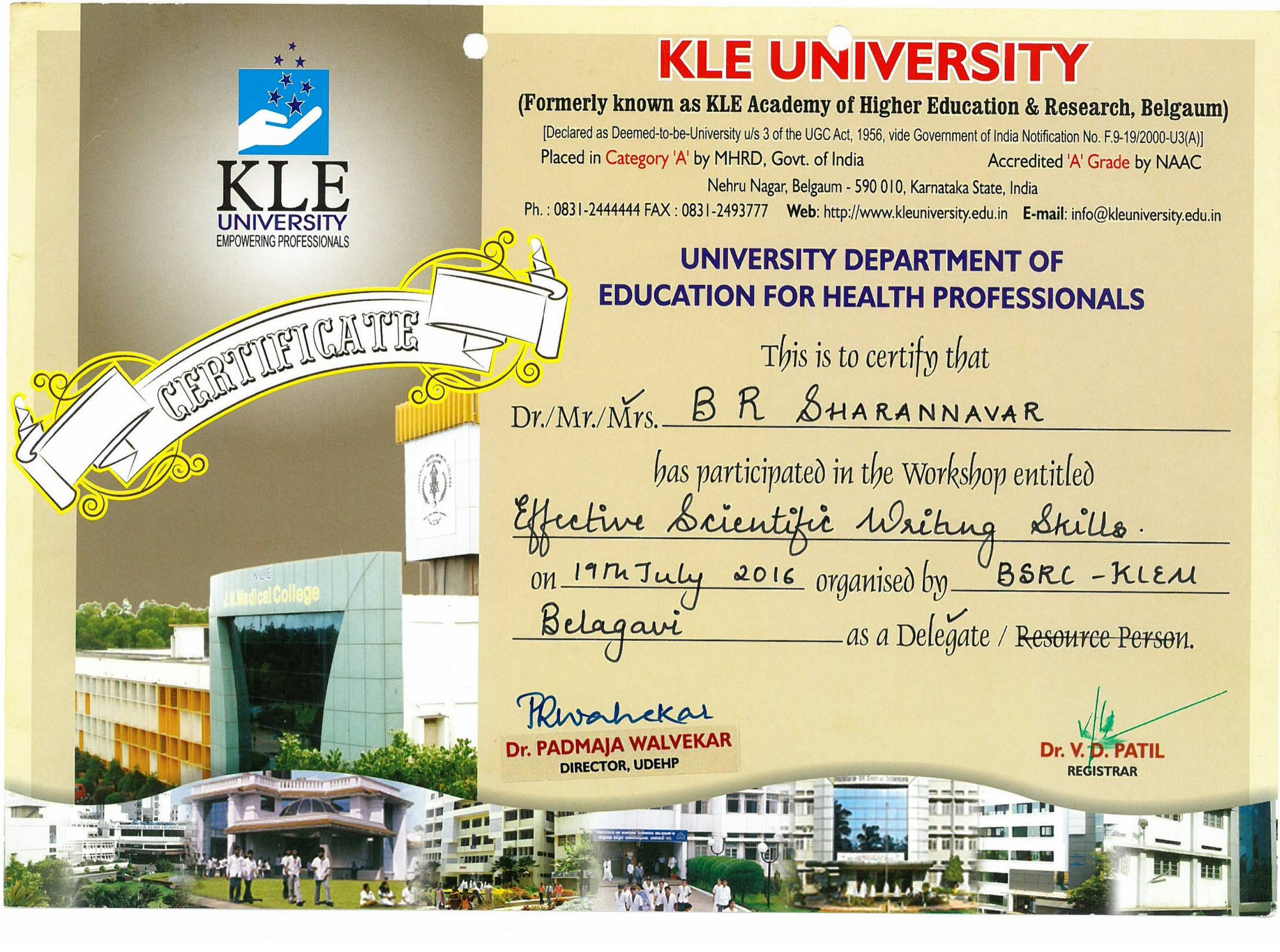
Recent Advances in Novel Drug Delivery System

on 11-11-2017 organised by KLE University's

Shri. BMK Ayurveda Mahavidyalaya as a Delegate ~~/Resource Person.~~

Dr. PADMAJA WALVEKAR
DIRECTOR, UDEHP

Dr. V. D. PATIL
REGISTRAR



KLE
UNIVERSITY
EMPOWERING PROFESSIONALS

CERTIFICATE

KLE UNIVERSITY

(Formerly known as KLE Academy of Higher Education & Research, Belgaum)

[Declared as Deemed-to-be-University u/s 3 of the UGC Act, 1956, vide Government of India Notification No. F.9-19/2000-U3(A)]

Placed in Category 'A' by MHRD, Govt. of India Accredited 'A' Grade by NAAC

Nehru Nagar, Belgaum - 590 010, Karnataka State, India

Ph. : 0831-2444444 FAX : 0831-2493777 **Web**: http://www.kleuniversity.edu.in **E-mail**: info@kleuniversity.edu.in

UNIVERSITY DEPARTMENT OF EDUCATION FOR HEALTH PROFESSIONALS

This is to certify that

Dr./Mr./Mrs. B R SHARANNAVAR

has participated in the Workshop entitled

Effective Scientific Writing Skills.

on 19th July 2016 organised by BSRC - KLEU

Belagavi as a Delegate / ~~Resource Person~~.

PRwalvekar
Dr. PADMAJA WALVEKAR
DIRECTOR, UDEHP

Dr. V. D. PATIL
REGISTRAR

Re-Accredited
Grade B by NAAC
CGPA (2.93)

CERTIFICATE

This is to certify that

Mrs. Bhuvaneshwari R. Sharannavar

has attended AICTE sponsored Faculty Development Program from date 11th November 2013 to 23rd November 2013 on

"Importance and Application of Solid State in Pharmaceutical Formulation"

at

Department of Pharmaceutical Sciences, Saurashtra University, Rajkot
Organized from 11th November 2013 to 23rd November 2013.

Dr. Navin R. Sheth
Convener

Dr. Mihir K. Raval
Coordinator

Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India

CERTIFICATE OF ATTENDANCE

This is to certify

Dr/Prof/Mr/Mrs **B R SHRANNAVAR**

attended the International Workshop on Bioavailability and Bioequivalence

8-9 August 2012, conducted by

KLE University , India

in association with

Louvain Drug Research Institute,

Catholic University of Louvain, Belgium

UCL
Université
catholique
de Louvain

KLE
UNIVERSITY
EMPOWERING PROFESSIONALS

R. Verbeeck

Chair: **Prof. Dr Roger K. Verbeeck**
Louvain Drug Research Institute

Coordinator: **Prof. Dr Srinivas Patnala**
Basic Sciences Research labs - KLE University

Prof. Dr P. F. Kotur
Registrar - KLE University

KLE
UNIVERSITY
EMPOWERING PROFESSIONALS

Certificate

KLE UNIVERSITY

[Established u/s 3 of the UGC Act, 1956 vide GOI. Notification No. F.9-19/2000-U.3(A)]
Belgaum - 590 010, Karnataka State, India, Website : http://www.kleuniversity.edu.in

UNIVERSITY DEPARTMENT OF EDUCATION FOR HEALTH PROFESSIONALS

This is to certify that

~~Dr.~~/~~Mr.~~/Mrs. Bhuvaneshwari

has participated in the Workshop / CME entitled

Qualitative Research and Bio-Ethics

on 10-11/5/2010 organised by UDEHP

as a Delegate / ~~Resource person.~~

Dr. JYOTI NAGMOTI
DIRECTOR, UDEHP

Dr. P. F. KOTUR
REGISTRAR

INDIAN PHARMACEUTICAL ASSOCIATION

Regulatory Affairs Division & Karnataka State Branch

CERTIFICATE

This is to certify that ~~Dr. / Mr.~~ / Ms / Bhuvaneshvari R. Sharanmma *has participated in the workshop "Challenges for Dissolution Testing in the 21st Century" held on 15th and 16th May 2009 at The Atria Hotel, Bangalore.*

Dr. Premnath Shenoy
Secretary (KSB)

Sri A. Krishnadev
Chairman - IPA (RAD)

Dr. B. R. Jagashetty
President (KSB)