Synthesis and Characterization of Quercetin Loaded Polycaprolactone microparticles

Dissertation submitted in partial fulfillment of the requirement for the degree of

MASTER OF TECHNOLOGY

IN

BIOTECHNOLOGY

By

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UNDER THE GUIDANCE OF

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SOLAN, H.P. (INDIA)

MAY 2018

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DECLARATION

I hereby declare that the work reported in this M.Tech thesis entitled **"Synthesis and Characterization of Quercetin Loaded Polycaprolactone Microparticles"** submitted at **Jaypee University of Information Technology, Waknaghat India,** is an authentic record of my work that was carried out under the supervision of **Dr. Gopal Singh Bisht**. I have not submitted this work elsewhere for any other degree or diploma.

……

Payal Gupta

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Department of Biotechnology and Bioinformatics

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Date: ……..

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the **M.Tech**. thesis entitled "**Synthesis and Characterization of Quercetin loaded Polycaprolactone microparticles**", submitted by **Payal Gupta (162552)** at **Jaypee University of Information Technology, Waknaghat India** is an authentic record of her original work carried out under my supervision. This work has not been submitted elsewhere.

……..

(Dr. Gopal Singh Bisht)

Associate Professor

Department of Biotechnology & Bioinformatics

Jaypee University of Information Technology

Date: …….

ACKNOWLEDGEMENT

I would like to thank everyone from the bottom of my heart who have helped me to complete my project on time either directly or indirectly.

I would like to give a sincere vote of thanks to my guide and mentor **Dr. Gopal Singh Bisht** without whose help, guidance and constant support I won‟t have been able to complete my project. His constant motivation helped me to complete my project.

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Payal Gupta

(162552)

LIST OF ACRONYMS & ABBREVIATIONS

PCL	Polycaprolactone
SEM	Scanning Electron Microscopy
FTIR	Fourier transform infrared spectroscopy
MP	Microparticles
UV-vis	ultra violet-visible
HPLC	High performance liquid chromatography

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ABSTRACT

Quercetin is flavonoid having various pharmacological activities such as anti-oxidative, anti-inflammatory, and anticancer. But the problem with quercetin is that it is having poor permeability, poor solubility, instability and low bioavailability. To improve the solubility of quercetin, we have incorporated quercetin in polycaprolactone polymer which is having high solubility and biodegradability. Quercetin loaded Polycaprolactone microparticles were synthesized by solvent evaporation method. These particles were characterized by SEM, FTIR, and size distribution analysis. The size of microparticles was found to be 700 nm. *In vitro* drug release studies of quercetin were carried out and 30% drug release was obtained in 24 hour of study. Percentage drug loading was observed through uv-spectrophotometer and drug loading was found to be 20%. SEM results show the spherical shape of microparticles. Overall study shows the sustained release of the drug which can be used for topical drug delivery system.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1 MICROPARTICLES

Microparticles are the particle having size less than 1μm. Microparticle drug delivery system provides sustained and controlled drug delivery [1]. Polymeric microsphere is used in oral drug delivery, therapeutic drugs and in sustained drug delivery. The first polymeric microsphere was prepared in 1960s for the better drug release. Polymers used were silicone rubber and polyethylene [2]. In drug delivery system these are consisting of natural and synthetic polymers, with the biotechnology advancement lots of therapeutic have been formed by using microparticles as drug delivery system [3]

1.2 HISTORY OF MICROENCAPSULATION

The concept of Microencapsulation systems started in 1932 and this concept was developed by Dutch Chemist H.G. Bungenberg de jong to describe droplets containing a colloid, rich in organic compound, surrounded by a tight layer of water, providing a locally segregated environment. These microparticles could have differentiated surfaces and thus be compared to cellular component such as membranes or vacuoles. The first industrial product from microencapsulation was carbonless copy paper [4]

Different type of microparticles used in drug delivery are:

Alginate based microparticles

PLGA based microparticle

Lipid based Microparticles

Polymeric Microparticles [5]

1.3 ADVANTAGES IN DRUG DELIVERY:

Microparticles are having various advantages in drug delivery system:

(i) These provides targeted drug delivery for pH-sensitive therapeutics [6]

(ii) They increase the therapeutic efficacy and reduce side effects.

(iii) Microparticles are easy to administer [7]

1.4 HISTORY OF MICROENCAPSULATION

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1.5 METHOD OF PREPARATION OF MICROPARTICLES:

1) Single emulsion method

2) Solvent Evaporation method

3) Double emulsion technique

4) Spray drying method

1) Single emulsion method

In this method the oil phase is drug and polymer which is dissolved in organic solvent. The emulsion of drug and polymer is then dispersed into aqueous phase which contain a dissolved emulsifier. Emulsifier enhances stability and inhibits flocculation of microparticles [8]

2) Solvent evaporation method

Solvent evaporation method is the one of the famous method to synthesize microparticles. This method is used for water-soluble drugs and proteins drugs which are

expensive. This method is flexible and easy to operate. High efficiency and reduced burst release is obtained by solvent evaporation method. [9]

3) Double emulsion technique

This method is based on oil-in-oil emulsification. This method is a new and efficient for encapsulation of hydrophilic drugs. This method is known for encapsulating hydrophilic drugs with high efficiency [10]

4) Spray drying method

Spray drying method is based on the drying of the content of polymer and drug in the air depending upon the cooling of solution and removal of solvent [11].

1.6 BENEFITS OF MICROENCAPSULATION

A substance may be microencapsulated for a number of reasons, which is described in detail as below:

1. For the development of modified release dosage form for targeted drug release purpose
2. Bitter taste of drugs can be enhanced by microencapsulation technique
3. This technique can protect drug from environmental hazard such as heat, light, oxygen and GI biodegradation.
4. Compatibility of drugs and excipients can be enhanced.
5. Volatile and oily Substance or extract can be converted into tablet dosage form to improve flow properties.
6. To prepare immobilized enzymes or cell. [12]

1.7 POLYCAPROLACTONE (PCL)

Polycaprolactone is a polyester based polymer. It has good solubility in dichloromethane, carbon benzene, acetone, ethyl acetate. The melting of PCL is 59 to 64^{o}C [13]. Advantages of PCL are mentioned in figure 1 [2,14]

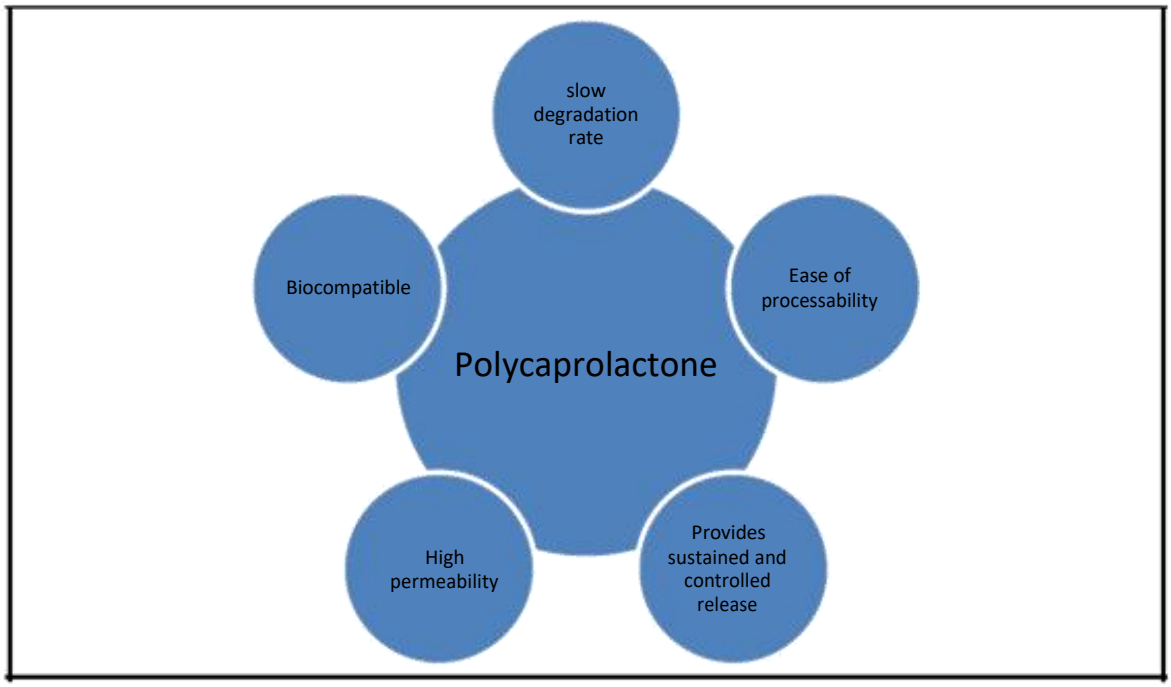


Figure 1.1: Advantages of PCL polymer

1.8 FORMATION OF POLYCAPROLACTONE:

The polymer is formed by ring opening polymerization of caprolactone with the use of low molecular weight alcohols to attain the desired molecular weight of PCL. Different catalysts are used for the preparation of PCL out of which ammonium decamolybdate is efficient to obtain aliphatic polyesters. The properties of polymer can be altered by cationic, anionic, coordination and radical mechanism. [15]

1.9 WOUND HEALING:

Wound healing mechanism is the process of healing injured cellular structures. Wound healing mechanism contains three phases: inflammation, proliferation and maturation. Various factors affect the wound healing process i.e. age, nutrition, health, infections and allergies and this cause impaired healing. There are two types of impaired healing: delayed acute wound and chronic wounds. Acute wounds have fast healing; chronic wounds have prolonged healing time. [16]

1.10 MICROPARTICLES IN WOUND DRESSING

Polymeric microparticles show promising result in wound dressing for homeostasis as well as rapidly removing exudates [17]

"The benefits of hollow over solid microparticles were found to be higher encapsulation efficiency and a more rapid drug release rate."

1.11 TOPICAL DRUG DELIVERY SYSTEM

Topical drug delivery system is beneficial in improving the effects of therapeutics and reduces the side effects of compounds which are administered. Biopolymeric system plays a role in developing new topical dosage and their applications. Topical drug delivery system offers drug delivery through rectal, vaginal, opthalmic and skin as topical routes [18].

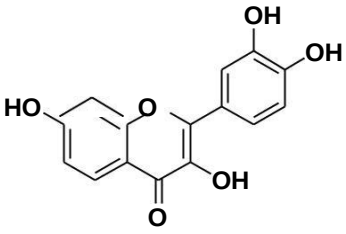
1.12 QUERCETIN

Quercetin belongs to flavonoid family of polyphenol derived from *quercetum.* This term is used since 1857. Quercetin is found in plant like apples, red wine, onions, broccoli which are good source of quercetin. The protective effects of quercetin against various diseases such as pulmonary diseases, cardiovascular diseases, cancer, and neurodegenerative disorders have been shown by many researchers. Quercetin is shown to modulate the intracellular signaling pathways. Quercetin also regulates the activity of kinases changing the phosphorylation state of target molecules, resulting in modulation of cellular function and gene expressing wound healing effect [19]

1.12.1 Occurrence

Quercetin is a flavonoid widely distributed in nature. It is a naturally occurring polar auxin transport inhibitor.

1.12.2 Structure of quercetin



1.12.3 IUPAC name

2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one

1.12.4 Solubility of quercetin

Very soluble in

- ether
- methanol

Soluble in

- ethanol
- acetone
- pyridine
- acetic acid

Insoluble in

- water

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Scalia S et al 2009, developed lipid microparticles loaded with quercetin for enhancing the stability in topical formulation. Tristearin was used as a lipid and phosphatidylcholine as used as an emulsifier. The particles were characterized by XRD, release study and scanning electron microscopy [20].

Paleco R et al 2014, developed lipid microparticles loaded with quercetin to improve the *in vitro* skin penetration of quercetin. These microparticles were prepared by emulsification and sonication. They described the enhanced intra-epidermal delivery of quercetin by the lipid microparticle strategy [21].

Baek Js et al 2017, prepared the polymeric drug loaded microparticles for the sustained drug release for cancer therapy. Polymeric microparticles were prepared for the delivery of chemotherapeutic agent. Hollow microparticles were synthesized by double-emulsion solvent evaporation technique using poly(D, L-lactic-co-glycolic acid). Hollow microparticles were co-loaded with doxorubicin and paclitaxel. By the addition of this the higher encapsulation efficiency of both of drugs and enhanced cumulative release of drug were achieved. These hollow dual-drug-loaded hollow microparticles with sustained releasing capabilities may have a potential for future application in cancer [22]

Mahmoudian M et al 2017, described the method for the problem like Distribution of antibiotic drug due to physiological barrier. In this study they synthesized the Vancomycin-loaded HPMC microparticles by spray drying method. SEM (scanning electron microscopy) results show the narrow size distribution and homogenous particle production. Their encapsulation efficiency was 72.6%. [23].

HirakuOnishi et al 2016, prepared method for oral drug delivery system for protein. Protein based substances suffer with the limitation of low absorption. To overcome this

problem they prepared microparticles using chitosan-4-thio-butylamidine conjugate, trimethyl-chitosan, eudragit and chitosan. Salmon calcitonin was used as a model protein drug. There result suggested that Eud- coated ch-TBAbased microparticles have potential as an oral drug delivery system of protein drugs [24].

Wang Z et al 2016, synthesized the nanocomposite microparticles for the delivery of Tacrolimus in the treatment of pulmonary arterial hypertension. Tacrolimus exhibits promising therapeutic potential in the treatment of pulmonary arterial hypertension but its application is affected because of its poor bioavailability, instability and poor solubility. To overcome this problem they synthesize these particles to provide targeted pulmonary delivery and controlled drug release. In this study the tacrolimus-loaded nanoparticles were synthesized by emulsion solvent evaporation and spray drying method [25].

Toniazzo T et al 2017, developed binary mixture containing microparticle of cornstarch and nanoparticles of quercetin. These mixtures were combined in this study to increase the stability of the food product by enhancing the agglomeration of corn starch. This not only increased nutritional quality but also added value to corn starch [26].

Karen M Doersch et al 2017, they demonstrated that the Quercetin is having wound healing activity and its wound healing activity can be improved by impacting integrin expression, which leads to lower extracellular matrix requirement to achieve healing. They studied the molecular mechanism by which quercetin, a naturally occurring antifibrotic agent, diminishes the scar formation. They used mice and fibroblast cell, and examined the quercetin impact on fibrosis and wound healing rate. It took 14 days for wound healing to occur in quercetin treated and controlled mice [27]

Rahvar M et al 2018, they demonstrated the effect of quercetin on gene expression of brain derived neurotrophic factor. The various *in-vivo* and *in-vitro* studies show the neuroprotective effects of quercetin. Brain derived neutrophic factor is responsible for the survival of neuronal cell. In this study they investigated the effects of quercetin on expression of BDNF mRNA in the hippocampus of rat brain [19].

Cheow WS et al 2013, has prepared the Levofloxacin loaded nanoparticles for ocular drug delivery with PLGA and PCL as polymer using nanoprecipitation and

Emulsification-solvent-evaporation method. The encapsulation and drug loading of the nanoparticles was found to be between 4-7% and the particle size was 200nm for both PLGA and PCL. The particles prepared using nanoprecipitation method exhibited the monophasic burst release of about 80% within first hour and the entire drug release after 6 hour. The antibacterial efficacy of the levofloxacin nanoparticles was studied using E.coli. and biofilm cells [28]

Chawla j.s. et al 2010 developed a nanoparticle formulation using Polycaprolactone as a polymer to increase the local concentration of the tamoxifen in the estrogen receptor positive breast cancer. solvent displacement method was used for the preparation of particles having size of 100-300 nm with spherical smooth surface. After carrying out the biodegradation study of the particles they found out that during in vivo studies PCL degraded at a faster pace in comparison to its in-vitro degradation. The drug loading of particle with tamoxifen was found to be 64% and further the drug release profile was studied which showed that at initial stage there was 68% of drug release during the first hour and maximum of the drug was released in next 24 hour. They studied that the particles gave targeted drug delivery and sustained drug release.[39]

Shokri N. et. al. 2011 prepared doxorubicin HCL loaded PCL nanoparticles using nanoparticles using nanoprecipitation and microemulsion poymerisation method. Different formulation were prepared using four different solvents i.e. acetone , methanol , DMSO and NVP (N-Vinyl pyrolidone) .The particle size varied accordingly with change in solvent and was in range 170-200 nm. The encapsulation efficiency and drug loading by 10% and 2.5% respectively. They finally concluded that by using PCL a sustained release of doxorubicin could be obtained due to slow degradation of the polymer which further was dependent upon the molecular weight of same.[40]

CHAPTER 3

AIM AND OBJECTIVES

3 AIM AND OBJECTIVES

3.1. AIM

The aim of present study was to synthesize and characterize quercetin loaded polycaprolactone microparticles.

Commercially, quercetin is available only as powdered form and in capsule form as a drug supplement. The problem with quercetin is that, it is having lower solubility. To solve problem of solubility it was incorporated in PCL microparticles.

3.2. OBJECTIVES

- The objective of present work was to synthesize and characterize quercetin loaded polymeric microparticles.

  Our specific objectives were:

- Preformulation studies, which included identification of drug and drug excipient compatibility studies.
- Synthesis of quercetin loaded polycaprolactone microparticle by using solvent evaporation method
- Characterization and evaluation of microparticles.

CHAPTER 4

MATERIALS AND METHODS

4. MATERIALS AND METHOD:

4.1 Materials

Table 4.1. Chemicals and reagent required

S.NO.	Name of chemicals	Name of company
1.	PCL(Polycaprolactone)	Sigma
2.	PVA(Polyvinyl Alcohol)	Lobachemie
3.	Quercetin	Sigma
4.	Methanol	Merck
5.	DCM(Dichloromethane)	Merck
6.	Phosphate buffer saline	Bio-Rad
7.	Acetonitrile	Merck
8.	TEA(tri-ethyl-amine)	Sigma
9.	Ethanol	Lobachemie

Table 4.2. APPARATUS AND EQUIPMENT USED

SR.NO.	Apparatus and equipments	Company
1.	Centrifuge	Thermofisher
2.	Lyophillizer	Allied Frost
3.	Uv-spectrophotometer	Sigma
6.	HPLC	Waters
7.	Sonicator	Citizen

4.2 Method

Quercetin loaded microparticles were prepared by solvent evaporation method, after certain modifications [29]. Methodology used is depicted in figure 4.2.1.

4.2.1Preparation of microparticles

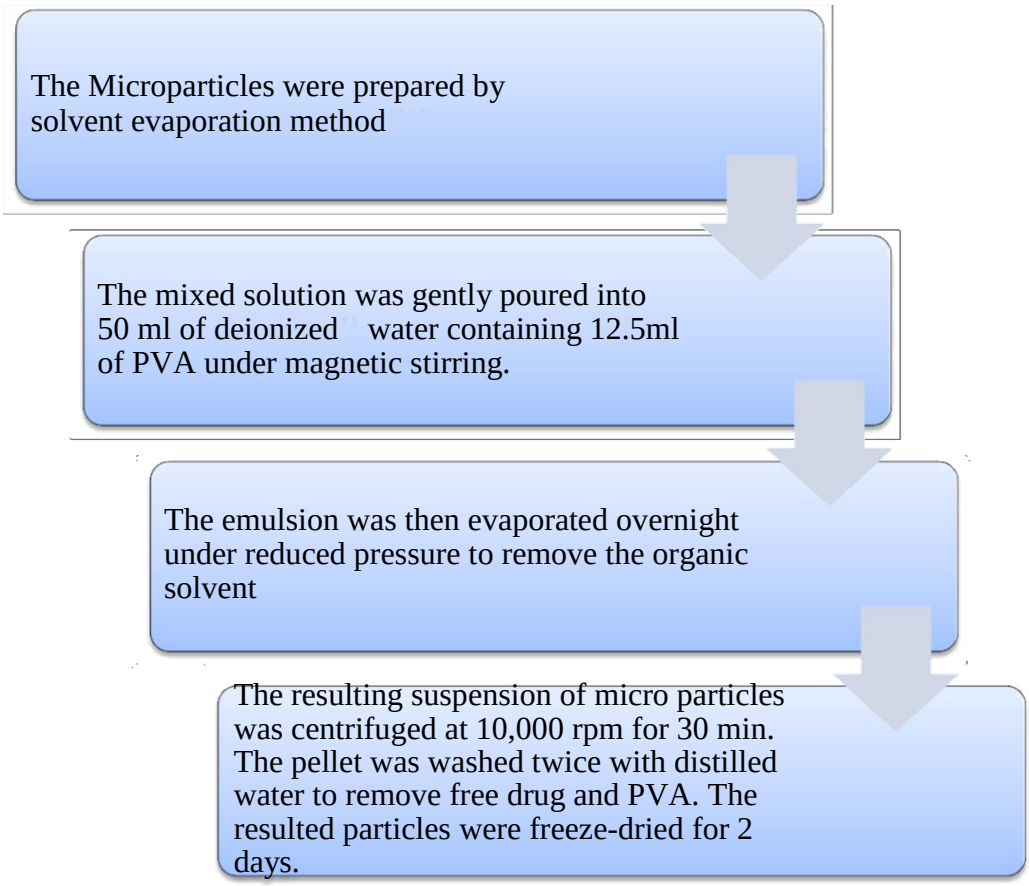


Figure 4.2.1: General methodology of preparation of quercetin loaded microparticles

4.3. Preformulation studies

Preformulation testing is done prior to the development of dosage for drug. Physical and chemical properties of drug are investigated by preformulation studies. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable dosage form [30].

Preformulation studies is done for

a) Identification of drug

b) To check drug excipients compatibilities

c) To determine stability of formulation [31]

Preformulation studies of obtained drug sample includes:

1. Identification of drug by:
    - Melting point
    - UV spectrophotometer
2. Compatibility study
    - FTIR

4.3.1 Melting point determination

Melting point of quercetin sample was determined by using melting point testing apparatus and method used was capillary fusion method. Capillary tube was taken and one end of this tube was sealed by gentle heating. Small amount of drug was filled in this capillary tube and the tube was then kept in the melting point testing apparatus. Melting point of the drug was noted at the point when it started melting and was compared with the literature value.

4.3.2 FTIR

FTIR Spectroscopy is used for the characterization of biological material. Specific chemical bond is absorbed by infra-red light which is visible by vibrational bands [32]

4.3.3 Identification of drug by IR Spectroscopy

The infrared spectrum of the pure quercetin sample was recorded and the spectral analysis was done. The FTIR spectra were recorded in the wavelength region between 1000 –4000 cm^{-1}. The spectra obtained for quercetin was compared with reference spectra of quercetin.

4.3.4 Compatibility studies by FTIR

The infrared spectrum of the drug polymer mixture was recorded and the spectral analysis was done. The spectra obtained for Quercetin and drug-polymer physical mixture were compared, in order to determine any sort of interactions between drug and polymer

4.3.5 Evaluation parameters

Evaluation parameters determined were size, zeta potential, shape, percent drug loading and *in vitro* drug release study

4.3.6 SEM Analysis

SEM analysis offers a very high magnification with very high-resolution capabilities and large depth of focus. SEM is indispensable tool for analysis of a wide class of conducting, semi-conducting and insulating material [33]. SEM was employed to determine shape and surface morphology of microparticles.

4.3.7 HPLC: (High performance liquid chromatography) is a technique that is widely used to determine the presence of drugs in a media [34]. The basic principle of High performance liquid chromatography is that molecules not only dissolve in liquids but can also get absorbed on to or interrelate with the solid surface. Now-a-days, High performance liquid chromatography [HPLC] is being used for the determination of immunosuppressive drugs in biological fluids with either fluorescence, ultraviolet (UV), diode-array, electrochemical or mass spectrometry (MS) detection [35]. With a suitable blending of solvent, column and detector, HPLC allows the separation of one type of molecule from others. Therefore, it is used to quickly establish purification or assay method. HPLC has an extensive range of applications in both routine clinical analysis and clinical research [36]. Genera conditions that were used to analyze quercetin are given in following table, Table 3.

Table 4.3: HPLC conditions used

HPLC method	Reverse phase (Gradient)
HPLC pump	Waters 515 HPLC pump
Column	C_{18} 5 μm Waters column (150 mm × 4.6 mm)
Detector	PDA detector
Detection wavelength	372 nm
Mobile phase A	2% acetic acid in milli Q water
Mobile phase B	Methanol:Acetonitrile (60:40)
Flow rate	1ml/min

4.3.8 Percent drug loading

Percent drug loading was determined by slight modification in method as described in literature [37]. 2.5 mg of freeze dried microparticles were taken and dissolved in 1 mL of methanol, this solution was further diluted 10 times and the absorbance was recorded using UV-spectrophotometer at 372 nm. Actual concentration of drug was calculated by slight modification in method as described in literature and % drug loading was calculated using the following formula.

$$\% \text{ drug loading} = \frac{\text{Amount of drug present in microparticles} \times 100}{\text{Amount of microparticles taken}}$$

4.3.9 *In vitro* release studies

The dialysis membrane was activated overnight in PBS. *In-vitro* release of quercetin microparticles was conducted by dialysis membrane with 75 ml of PBS (pH 7.4) at 37^0C. 2 mg of quercetin loaded microparticles were taken in a dialysis bag that was dipped in 75 ml of buffer solution in 100ml beaker. Beaker was kept on magnetic stirrer (250rpm at 37^0C temp). Sampling was done by withdrawing 2ml aliquots from a beaker after every hour. Immediately 2ml of fresh buffer was added to maintain sink conditions. UV absorbance of sample (diluted with methanol) was taken at 372 nm using UV vis spectrophotometer [38]

CHAPTER 5
RESULTS AND DISCUSSION

5. RESULTS

Quercetin loaded microparticles were synthesized by using polycaprolactone as polymer by Solvent evaporation method. The formulations were subjected to evaluation parameters like size distribution, SEM analysis, percent drug loading, *in vitro* release studies.

5.1. Preformulation Studies

5.1.1 Identification of drugs

A) <u>Confirmation of drug loading by HPLC</u>

Confirmation of drug loading confirmed by HPLC .The HPLC peak of quercetin loaded microparticles confirmed that quercetin has been loaded.

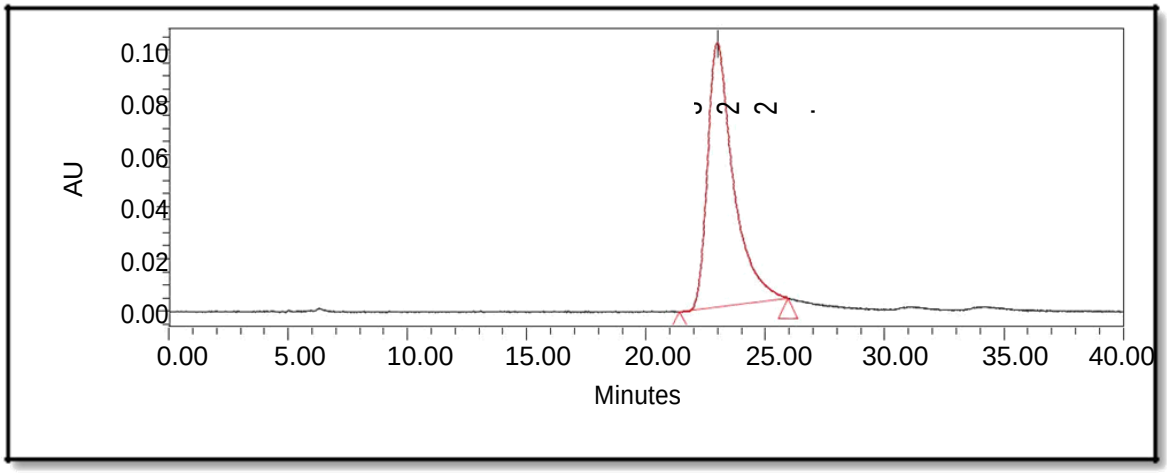


Fig5.1. HPLC spectrum of Quercetin

Table 5.1 HPLC spectrum details of Quercetin

Sr. No.	Retention Time	Area	%Area	Height	%Height
1.	22.993	7652457	100.00	101229	100

B). Melting point determination

Melting point of the drug was determined by capillary fusion method and the observed value was found to be comparable to literature value.

Table 5.2 Melting Point of Quercetin drug

Method Used	**Literature Value**	**Experimental value**
Capillary Fusion Method	300-316^{o}C	312^{o}C

C) Identification of drug by recording absorption maxima

Table5.3 Absorption maxima of drug

Method Used	**Literature value**	**Experimental Value**
UV Spectrophotometer	372nm	370nm

D) Identification of Polymer PCL by recording absorption maxima

Table 5.4 Absorption maxima of PCL

Method Used	**Absorption Maxima**	**Experimental Values**
UV Spectrophotometer	280nm	279nm

E) Identification of drug by FTIR studies

The confirmation of purity of drug sample was determined by FTIR spectroscopy. Results are shown in Fig.5.

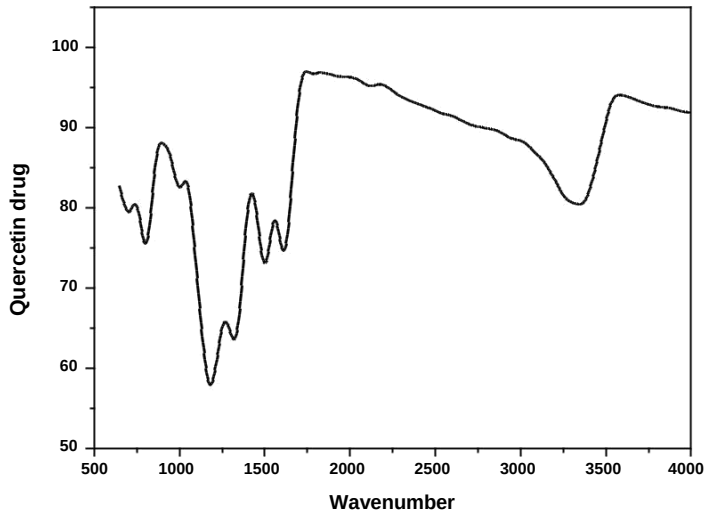


Figure.5.2. FTIR spectra of quercetin drug

Table.5.5. Interpretation of Spectral details of drug

GROUP	PEAK
C-O	1661cm^{-1}
C-C	1611 cm^{-1}
O-H	3406-3323 cm^{-1}
C-O-H	1319 cm^{-1}
C-O-C	1262 cm^{-1}

5.2. Compatibility studies

The infrared spectrums of the drug sample, drug-polymer mixture and prepared microparticles formulation were recorded and the spectral analysis was done. The FTIR spectrums of pure drug, drug -polymer combinations and prepared microparticles are shown in **Figure 5.3, 5.4, 5.5 and 5.6** respectively**.** There was no significant change in these peaks in drug-polymer mixture and prepared micro particles. This concluded that the drug was compatible with the polymer used.

Table.5.6.Interpretation of spectral details of pure drug, drug - polymer mixture and prepared microparticles

Group	Wave number (cm^{-1})		
	Drug	**Drug-polymer mixture**	**Microparticles**
C-O stretching	1161cm^{-1}	1164 cm^{-1}	1190cm^{-1}
C-C stretching	1161cm^{-1}	1164-1242 cm^{-1}	1172 cm^{-1}
O-H stretching	3406-3323 cm^{-1}	3560 cm^{-1}	3465cm^{-1}
C-H stretching	2944 cm^{-1}	2940 cm^{-1}	2948cm^{-1}

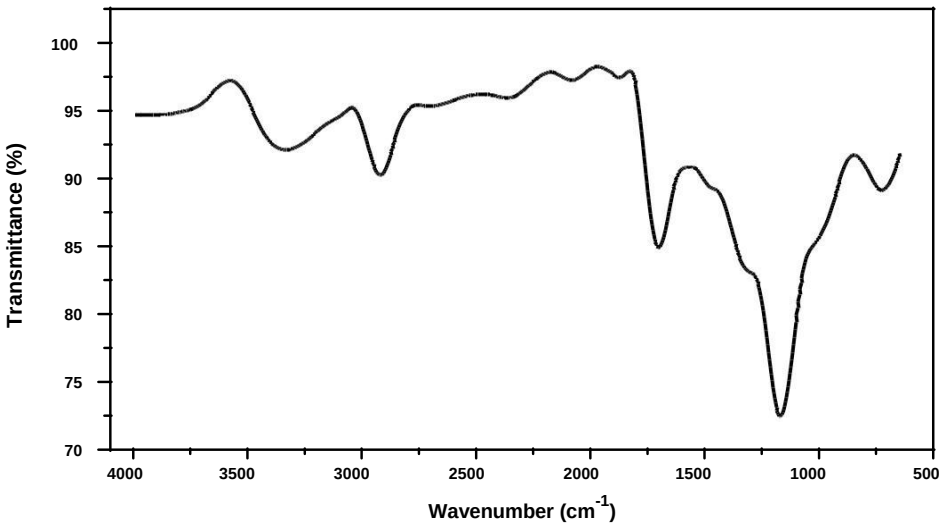


Fig.5.3. FTIR spectra of drug-polymer mixture

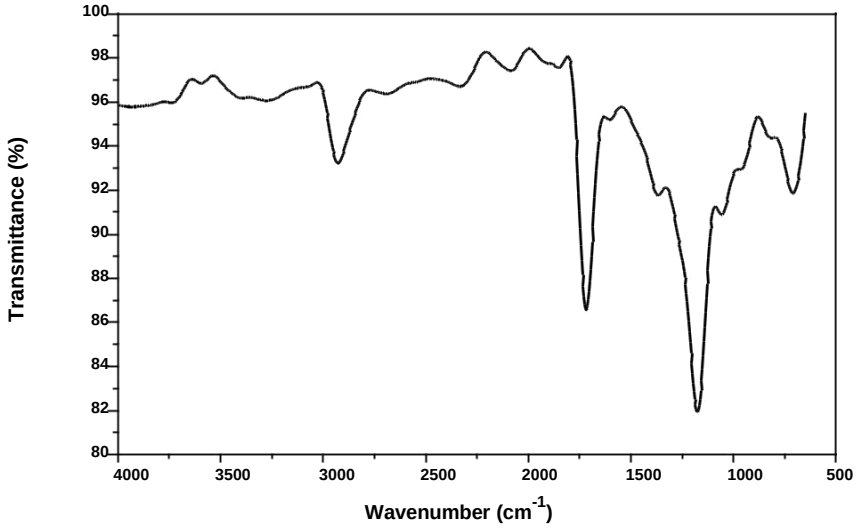


Fig.5.4. FTIR spectra of Drug loaded microparticles

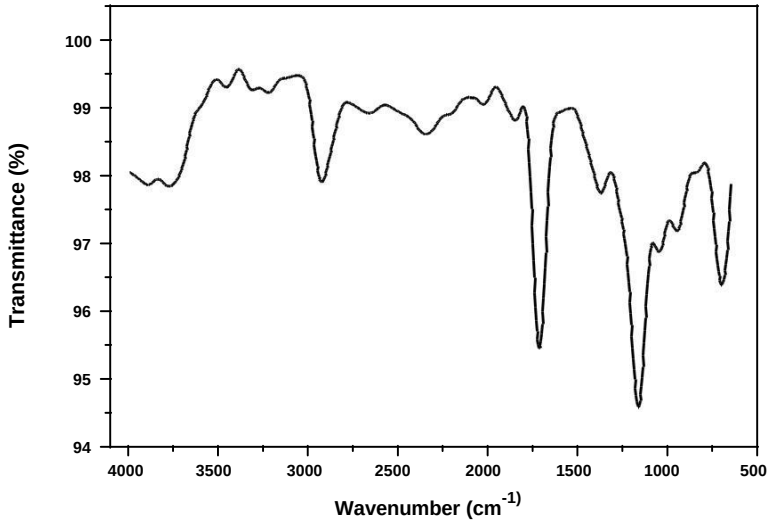


Fig.5.5. FTIR spectra of Polymer

5.3. Calibration curve

Calibration curve of Quercetin loaded microparticles was plotted in methanol and the results are shown in Fig 5.7**.** A graph was plotted between concentration and time. Regression coefficient was calculated as 0.988 and slope was 0.004

Table.5.7.Calibration curve of Quercetin loaded microparticles in methanol

Sr. No.	Conc. (µg/ml)	Absorbance
1	10	0.06
2	20	0.084
3	40	0.159
4	60	0.289
5	80	0.395
6	100	0.459

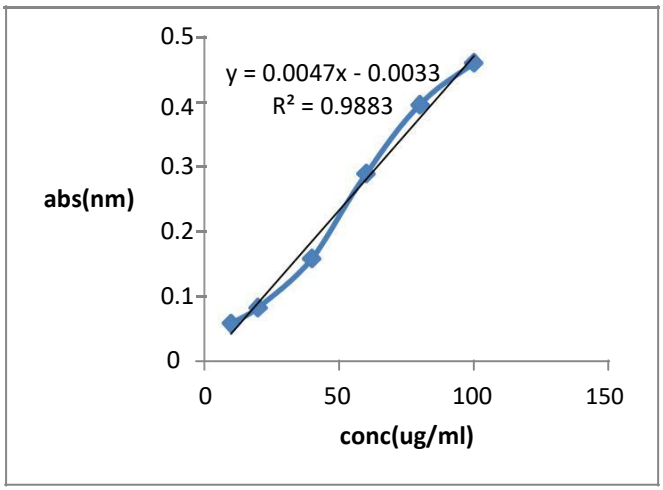


Fig.5.6.Calibration curve of Quercetin loaded microparticles in methanol

5.4. Evaluation Parameters

A). Size

Size distribution analysis of formulation

Size distribution analysis of formulation determined by recording intensity using size distribution .Size of microparticles was found to be 700nm.

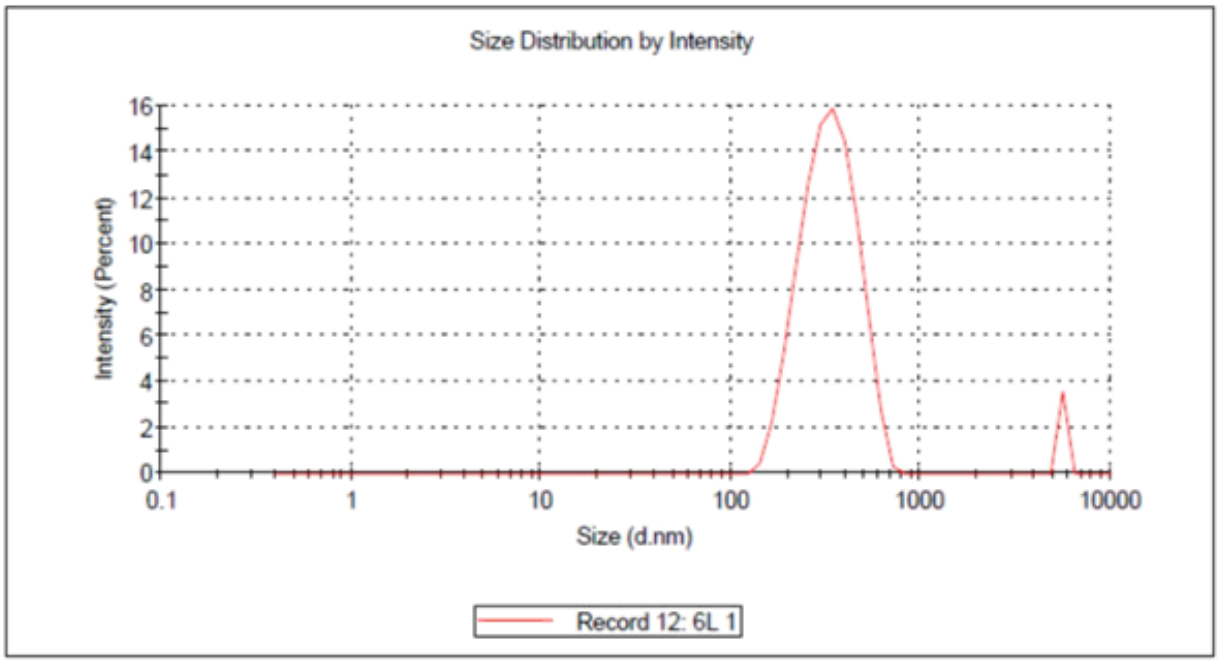


Fig.5.7 Size distribution by intensity

B). Shape

Surface characteristics of microparticles were characterized by Scanning Electron Microscopy. Quercetin loaded polymeric microparticles were found to be spherical in shape.

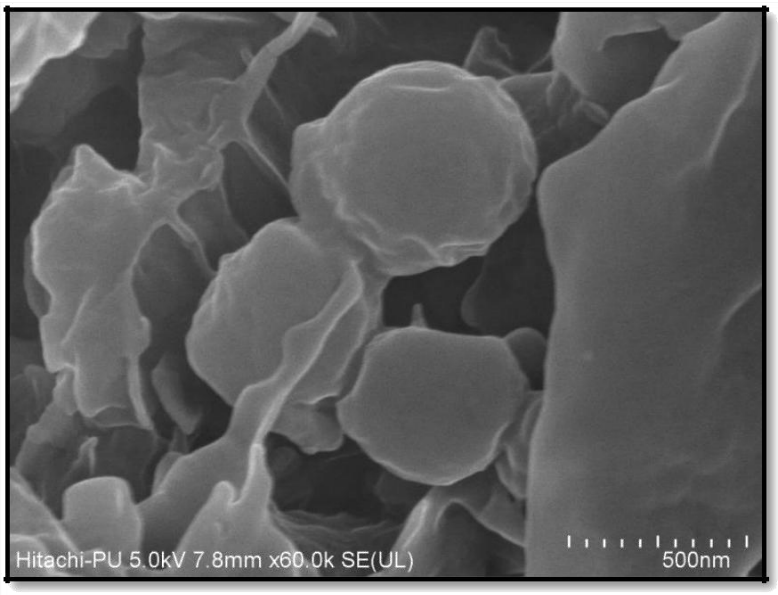


Fig.5.8.Scanning Electron Microscopy Image

C). Percent Drug loading

Percent drug loading of prepared formulation with respect to theoretical drug loading was found to be reasonable (20%).

D). *In vitro* release studies

The release profile of Quercetin loaded microparticles is shown in **Figure 5.10** and the data is given in **Table 5.7.** The formulations exhibited sustained release profile over the period of time. The cumulative drug release was calculated and graph was plotted with the help of MS Excel by using equations.

Table 5.8. In vitro drug release of microparticles

TIME	**PERCENTAGE RELEASE**	**CUMULATIVE RELEASE**
15 min	0.001	0.625
1 hour	0.002	0.78125
2 hour	0.004	1.094
3 Hour	0.007	1.563
4 Hour	0.009	1.876
5 Hour	0.042	22.657
6 Hour	0.153	24.38
12 Hour	0.181	28.75
24 Hour	0.215	34.063

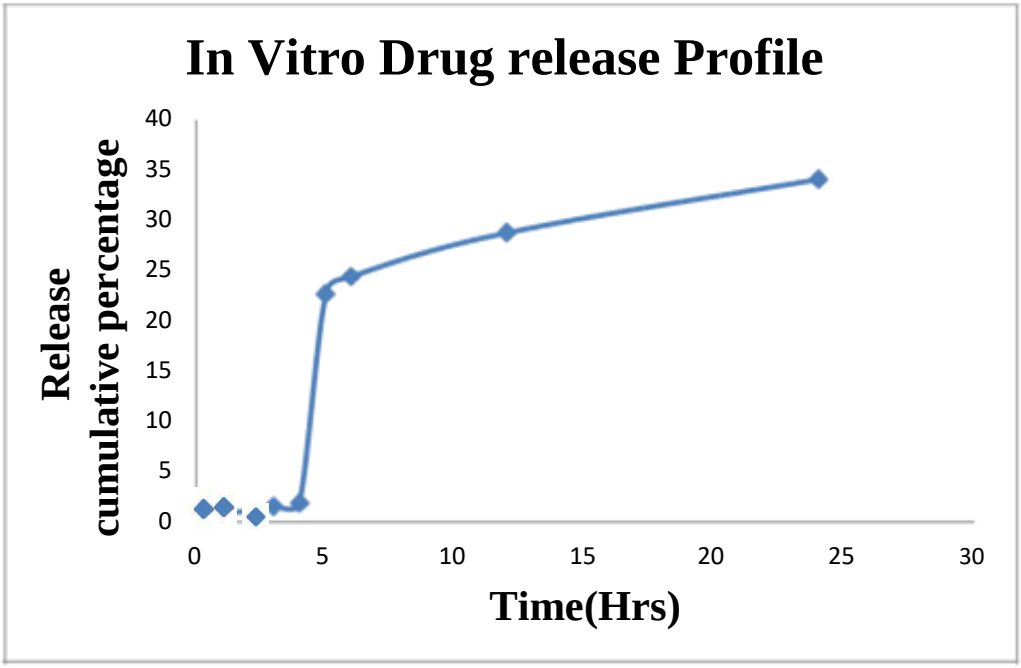


Fig.5.9. *In Vitro* drug release of Drug loaded Microparticles

Discussion

6.1. Preformulation studies

Melting point of the drug was determined by capillary fusion method. The melting point was found to be 312^{o}C, which was within the literature value range. This indicated that the drug sample obtained was pure. Interpretation of FTIR spectra of drug sample was done and compared to reference FTIR spectra Quercetin **(Figure 5.4, 5.5, 5.6, 5.7).** The spectrums were found to be comparable; this indicated that the drug sample obtained was of quercetin drug. The infrared spectrums of the Quercetin sample were recorded and the spectral analysis was done. **Figures (5.4, 5.5, 5.6)** It shows the FTIR spectra of Quercetin, mixture of drug-polymer and prepared microparticles .The data suggest that there was no chemical interaction between drug and excipients since no shifting of characteristic peaks of drug was noticed. So, the drug was found to be compatible with the polymer used in this formulation. The calibration curve was plotted in methanol at wavelength 372 nm. The calibration curve was found to be linear between 20-100 µg/ml concentration ranges. The regression coefficient (r^2) value was found to be 0.988. The tested concentration range obeyed Beer‟s Law.

6.2. Characterization of prepared microparticles

The prepared microparticles were characterized for size. The average size of micro particles was found to be 700 nm as shown in **Figure 5.8.** Distribution in size of nanoparticles was found to be wide in range; this might have occurred due to aggregation of particles. Shape of microparticles was characterized by SEM; microphotograph of microparticles formulation given in **Figure** 5.9.

6.3. Evaluation of microparticles

% drug loading of formulations was found to be 20%. Reason for less loading could be the poor solubility issue with quercetin. Percent drug release was found to be in the 34% at the end of 24 hours. Release behavior of quercetin from polycaprolactone microparticles demonstrated sustained release pattern up to 24 hours. At the initial stage micro particles showed remarkably small burst effect, due to drug entrapped near the surface of micro particles and it was followed by a very slow release stage. Percent drug release was found to be in the 34% at the end of 24 hours. Release behavior of quercetin from polycaprolactone microparticles demonstrated sustained release pattern up to 24 hours. At the initial stage micro particles showed remarkably small burst effect, due to drug entrapped near the surface of micro particles and it was followed by a very slow release stage.

CHAPTER7

CONCLUSION

Conclusion: The study was performed to prepare controlled drug delivery system of quercetin. Quercetin appears to have limited therapeutic window through topical delivery, therefore to increase the therapeutic effect of quercetin, we have developed a drug delivery system in which quercetin was incorporated in PCL. Solvent evaporation method was used to synthesize the quercetin drug loaded microparticles. SEM, FTIR studies were used to characterize the microparticles. SEM image showed the spherical shape of microparticles and FTIR study showed the compatibility between quercetin and PCL. 20% drug loading was found by the UV spectrophotometer analysis. *In vitro* drug release studies showed the sustained release of quercetin. Therefore the study concluded that PCL polymer is beneficial to prepare controlled drug delivery system of quercetin and the microparticles could be beneficial to deliver quercetin in topical formulation.

CHAPTER 8

REFERENCES

REFERENCES

1. M. Ahadon, S. Abdul Aziz, C. L. Wong, and C. F. Leong, "Plasma-derived microparticles in polycythaemia vera," *Malays J Pathol*, vol. 40, pp. 41-48, Apr 2018.
2. M. E. Aleman-Dominguez, E. Giusto, Z. Ortega, M. Tamaddon, A. N. Benitez, and C. Liu, "Three-dimensional printed polycaprolactone-microcrystalline cellulose scaffolds," *J Biomed Mater Res B Appl Biomater*, May 2 2018.
3. Nidhi, M. Rashid, V. Kaur, S. S. Hallan, S. Sharma, and N. Mishra, "Microparticles as controlled drug delivery carrier for the treatment of ulcerative colitis: A brief review," *Saudi Pharm J*, vol. 24, pp. 458-72, Jul 2016.
4. M. Auerbach and I. Macdougall, "The available intravenous iron formulations: History, efficacy, and toxicology," *Hemodial Int*, vol. 21 Suppl 1, pp. S83-S92, Jun 2017.
5. C. Y. Wong, H. Al-Salami, and C. R. Dass, "Microparticles, microcapsules and microspheres: A review of recent developments and prospects for oral delivery of insulin," *Int J Pharm*, vol. 537, pp. 223-244, Feb 15 2018.
6. A. Kumar, C. Montemagno, and H. J. Choi, "Smart Microparticles with a pH-responsive Macropore for Targeted Oral Drug Delivery," *Sci Rep*, vol. 7, p. 3059, Jun 8 2017.
7. M. V. Junqueira and M. L. Bruschi, "A Review About the Drug Delivery from Microsponges," *AAPS PharmSciTech*, vol. 19, pp. 1501-1511, May 2018.
8. K. Vasileiou, J. Vyslouzil, M. Pavelkova, J. Vyslouzil, and K. Kubova, "The size-reduced Eudragit(R) RS microparticles prepared by solvent evaporation method - monitoring the effect of selected variables on tested parameters," *Ceska Slov Farm*, vol. 66, pp. 274-280, Spring 2018.
9. S. Tian, J. Li, Q. Tao, Y. Zhao, Z. Lv, F. Yang, *et al.*, "Controlled drug delivery for glaucoma therapy using montmorillonite/Eudragit microspheres as an ion-exchange carrier," *Int J Nanomedicine*, vol. 13, pp. 415-428, 2018.
10. N. Pirooznia, S. Hasannia, A. S. Lotfi, and M. Ghanei, "Encapsulation of alpha-1 antitrypsin in PLGA nanoparticles: in vitro characterization as an effective aerosol formulation in pulmonary diseases," *J Nanobiotechnology*, vol. 10, p. 20, May 20 2012.

11. P. He, S. S. Davis, and L. Illum, "Sustained release chitosan microspheres prepared by novel spray drying methods," *J Microencapsul*, vol. 16, pp. 343-55, May-Jun 1999.

12. G. Murtaza, "Ethylcellulose microparticles: a review," *Acta Pol Pharm*, vol. 69, pp. 11-22, Jan-Feb 2012.

13. N. Kamaly, B. Yameen, J. Wu, and O. C. Farokhzad, "Degradable Controlled-Release Polymers and Polymeric Nanoparticles: Mechanisms of Controlling Drug Release," *Chem Rev*, vol. 116, pp. 2602-63, Feb 24 2016.

14. T. Potrc, S. Baumgartner, R. Roskar, O. Planinsek, Z. Lavric, J. Kristl, *et al.*, "Electrospun polycaprolactone nanofibers as a potential oromucosal delivery system for poorly water-soluble drugs," *Eur J Pharm Sci*, vol. 75, pp. 101-13, Jul 30 2015.

15. F. Wang, A. Mohammed, C. Li, P. Ge, L. Wang, and M. W. King, "Degradable/non-degradable polymer composites for in-situ tissue engineering small diameter vascular prosthesis application," *Biomed Mater Eng*, vol. 24, pp. 2127-33, 2014.

16. Y. Guo, C. Lin, P. Xu, S. Wu, X. Fu, W. Xia, *et al.*, "AGEs Induced Autophagy Impairs Cutaneous Wound Healing via Stimulating Macrophage Polarization to M1 in Diabetes," *Sci Rep*, vol. 6, p. 36416, Nov 2 2016.

17. Z. Tong, Y. Chen, Y. Liu, L. Tong, J. Chu, K. Xiao, *et al.*, "Preparation, Characterization and Properties of Alginate/Poly(gamma-glutamic acid) Composite Microparticles," *Mar Drugs*, vol. 15, Apr 11 2017.

18. A. D. Sezer and E. Cevher, "Topical drug delivery using chitosan nano- and microparticles," *Expert Opin Drug Deliv*, vol. 9, pp. 1129-46, Sep 2012.

19. M. Rahvar, A. A. Owji, and F. J. Mashayekhi, "Effect of quercetin on the brain-derived neurotrophic factor gene expression in the rat brain," *Bratisl Lek Listy*, vol. 119, pp. 28-31, 2018.

20. S. Scalia and M. Mezzena, "Incorporation of quercetin in lipid microparticles: effect on photo- and chemical-stability," *J Pharm Biomed Anal*, vol. 49, pp. 90-4, Jan 15 2009.

21. R. Paleco, S. R. Vucen, A. M. Crean, A. Moore, and S. Scalia, "Enhancement of the in vitro penetration of quercetin through pig skin by combined microneedles and lipid microparticles," *Int J Pharm*, vol. 472, pp. 206-13, Sep 10 2014.

22. J. S. Baek, C. C. Choo, N. S. Tan, and S. C. J. Loo, "Sustained-releasing hollow microparticles with dual-anticancer drugs elicit greater shrinkage of tumor spheroids,"

23. M. Mahmoudian and F. Ganji, "Vancomycin-loaded HPMC microparticles embedded within injectable thermosensitive chitosan hydrogels," *Prog Biomater,* vol. 6, pp. 49-56, May 2017.

24. H. Onishi and A. Tokuyasu, "Preparation and Evaluation of Enteric-Coated Chitosan Derivative-Based Microparticles Loaded with Salmon Calcitonin as an Oral Delivery System," *Int J Mol Sci,* vol. 17, Sep 13 2016.

25. Z. Wang, J. L. Cuddigan, S. K. Gupta, and S. A. Meenach, "Nanocomposite microparticles (nCmP) for the delivery of tacrolimus in the treatment of pulmonary arterial hypertension," *Int J Pharm,* vol. 512, pp. 305-313, Oct 15 2016.

26. T. Toniazzo, H. Galeskas, G. C. Dacanal, and S. C. Pinho, "Production of Cornstarch Granules Enriched with Quercetin Liposomes by Aggregation of Particulate Binary Mixtures Using High Shear Process," *J Food Sci,* vol. 82, pp. 2626-2633, Nov 2017.

27. K. M. Doersch and M. K. Newell-Rogers, "The impact of quercetin on wound healing relates to changes in alphaV and beta1 integrin expression," *Exp Biol Med (Maywood),* vol. 242, pp. 1424-1431, Aug 2017.

28. K. Hadinoto, A. Sundaresan, and W. S. Cheow, "Lipid-polymer hybrid nanoparticles as a new generation therapeutic delivery platform: a review," *Eur J Pharm Biopharm,* vol. 85, pp. 427-43, Nov 2013.

29. Mei, Y. Zhang, Y. Zheng, G. Tian, C. Song, D. Yang, *et al.*, "A Novel Docetaxel-Loaded Poly (epsilon-Caprolactone)/Pluronic F68 Nanoparticle Overcoming Multidrug Resistance for Breast Cancer Treatment," *Nanoscale Res Lett,* vol. 4, pp. 1530-9, Sep 16 2009.

30. S. M. Shah, A. S. Jain, R. Kaushik, M. S. Nagarsenker, and M. J. Nerurkar, "Preclinical formulations: insight, strategies, and practical considerations," *AAPS PharmSciTech,* vol. 15, pp. 1307-23, Oct 2014.

31. F. Chrzanowski, "Preformulation considerations for controlled release dosage forms. Part II. Selected candidate support," *AAPS PharmSciTech,* vol. 9, pp. 639-45, 2008.

32. H. Wagner, S. Dunker, Z. Liu, and C. Wilhelm, "Subcommunity FTIR-spectroscopy to determine physiological cell states," *Curr Opin Biotechnol,* vol. 24, pp. 88-94, Feb 2013.

33. R. D. Yadav, D. Raisingani, D. Jindal, and R. Mathur, "A Comparative Analysis of Different Finishing and Polishing Devices on Nanofilled, Microfilled, and Hybrid Composite: A Scanning Electron Microscopy and Profilometric Study," *Int J Clin Pediatr Dent,* vol. 9, pp. 201-208, Jul-Sep 2016.

34. M. Korecka and L. M. Shaw, "Review of the newest HPLC methods with mass spectrometry detection for determination of immunosuppressive drugs in clinical practice," *Ann Transplant,* vol. 14, pp. 61-72, Apr-Jun 2009.

35. K. Titier, N. Castaing, M. Le-Deodic, D. Le-Bars, N. Moore, and M. Molimard, "Quantification of tricyclic antidepressants and monoamine oxidase inhibitors by high-performance liquid chromatography-tandem mass spectrometry in whole blood," *J Anal Toxicol,* vol. 31, pp. 200-7, May 2007.

36. I. M. Bird, "High performance liquid chromatography: principles and clinical applications," *BMJ,* vol. 299, pp. 783-7, Sep 23 1989.

37. M. Hanif, H. U. Khan, S. Afzal, A. Mahmood, S. Maheen, K. Afzal, *et al.*, "Sustained release biodegradable solid lipid microparticles: Formulation, evaluation and statistical optimization by response surface methodology," *Acta Pharm,* vol. 67, pp. 441-461, Dec 20 2017.

38. K. Madhumathi, Y. Rubaiya, M. Doble, R. Venkateswari, and T. S. Sampath Kumar, "Antibacterial, anti-inflammatory, and bone-regenerative dual-drug-loaded calcium phosphate nanocarriers-in vitro and in vivo studies," *Drug Deliv Transl Res,* May 1 2018.

39. Chawla and M. Amiji, "Biodegradable poly(ε-caprolactone) nanoparticles for tumor- targeted delivery of tamoxifen", *International Journal of Pharmaceutics,* vol. 249, no. 1-2, pp. 127-138, 2002.

40. Shokri, N., et al. "Preparation and evaluation of poly (caprolactone fumarate) nanoparticles containing doxorubicin HCI." *Daru: journal of Faculty of Pharmacy, Tehran University of Medical Sciences* 19.1 Dec 22 2011

CONFERENCES ATTENDED

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