CHAPTER-3

Objective & Plan of Study

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3.1. Objective

The overall aim of this study was to develop better drug delivery systems for the treatment of periodontitis which are more patient compliant in terms of comfort and cost. Natural polymers like chitosan, sodium alginates, and vanillin are of low cost, has good compatibility with the encapsulation of a wide range of drugs, and also minimal use of organic solvents. Furthermore, mucoadhesion, stability, safety and approval for human use by the US FDA are additional advantages. Chitosan based crosslinked microspheres and microspheres loaded gels for the treatment of periodontal infections were formulated and evaluated. Ornidazole (OZ) and doxycycline hyclate (DX) were incorporated as effective active pharmaceutical agents into microspheres formulated using various crosslinking agents.

The basic objectives of the study includes;

- To develop, optimize and characterize multiparticulate based biodegradable, mucoadhesive, injectable, intrapocket drug delivery systems containing OZ and DX, which could provide prolonged and sustained effect for the treatment of chronic periodontitis by eradicating broad range of anaerobic and aerobic microbes present in the subgingival ecosystem.
- To evaluate safety and efficacy of optimized formulation by preclinical and clinical studies.

3.2. Plan of work

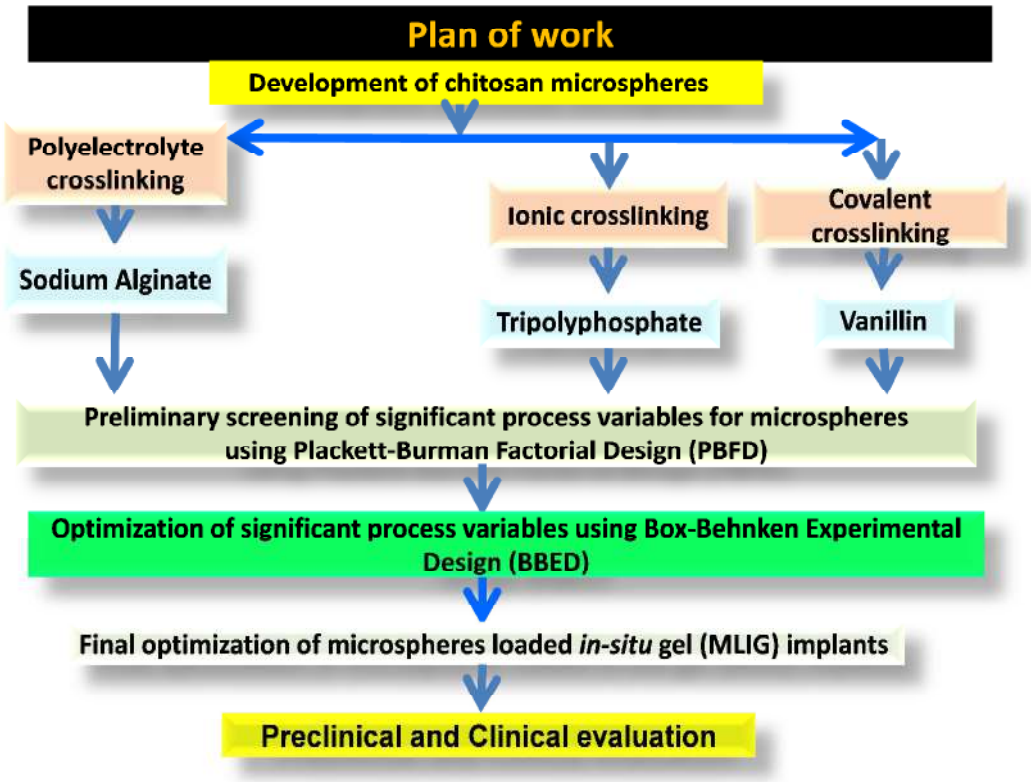

Figure 3.1. Schematic presentation of plan of work

The study was planned as outlined below:

(I) CHAPTER 4: PREFORMULATION STUDIES AND ULTRA-VIOLET (UV) METHOD DEVELOPMENT FOR SIMULTANEOUS ANALYSIS OF ORNIDAZOLE AND DOXYCYCLINE HYCLATE IN PHARMACEUTICAL DOSAGE FORM

- Compatibility study between drugs using Fourier-Transform Infrared (FTIR)
- Development of calibration curve for OZ and DX
- Development of Vierodt's method and Q-analysis method
- Validation of developed methods using ICH Q2R1 guidelines
- Determination of solubility of drugs by shake-flask method

(II) CHAPTER 5: FORMULATION, OPTIMIZATION AND CHARACTERIZATION OF CROSSLINKED CHITOSAN MICROSPHERES

❖ **Part A: Screening, optimization and characterization of polyelectrolyte complexed microspheres of chitosan and sodium alginate (CS-Ca-SA)**

- Identification of critical formulation variables using QbD approach.
- Formulation of CS-Ca-SA microspheres using w/o emulsion-ionotropic gelation method
- Screening of critical formulation variables and responses using RSM based Plackett-Burman Factorial Design (PBFD).
- Analysis of most significant formulation variables based on desired responses *viz.* particle size, entrapment efficiency, burst release and time for 80% drug release ($T_{80\%}$).
- Determination of optimized formulation based on desired responses.
- Solid state characterization of optimized formulations using FTIR, DSC, XRD, SEM and EDXA.
- Assessment of *in-vitro* release pattern and kinetics of both drugs from the optimized microspheres in simulated gingival fluid corresponding to phosphate buffer pH 6.8.
- Determination of swelling, erosion, mucoadhesion and pH of microspheres.
- Determination of antimicrobial activity of developed formulations on *Escherichia coli* and *Staphylococcus aureus*
- *In-vitro* cytocompatibility evaluation by sulphorhodamine (SRB) assay on cultured L929 cell lines.
- Assessment of stability of the optimized microspheres after 6 months of storage.

❖ **Part B: Design, optimization and characterization of chitosan-Tripolyphosphate (CSTPP) microspheres**

- Formulation of CSTPP microspheres by using ionic gelation and w/o emulsion technique
- Screening and optimization of formulations using PBFD

- Optimization of formulations for the desired responses such as percent yield, particle size, entrapment efficiency and $T_{80\%}$.
- Solid state characterization of CSTPP microspheres using FTIR, DSC, XRD, and SEM.
- Analysis of *in-vitro* drug release pattern and kinetics of both drugs
- Determination of swelling, erosion, mucoadhesion and pH of microspheres.
- Determination of antimicrobial activity of developed formulations on *Escherichia coli* and *Staphylococcus aureus*
- *In-vitro* cytotoxicity evaluation by SRB assays on cultured L929 cell lines.
- Assessment of stability of the optimized formulation under accelerated storage conditions.

❖ **Part C: Optimization and characterization of chitosan - vanillin (CSV) microspheres using dual design approach**

- ***Primary screening of significant formulation variables for crosslinked chitosan microspheres encapsulated with DX using PBFD***
  - Formulation of crosslinked microspheres by w/o emulsion technique
  - Application of PBFD for screening of most significant formulation factors
  - Selection of critical fabrication variables
  - Optimization of microspheres
  - Assessment of *in-vitro* release pattern and kinetics of both drugs from the optimized microspheres
  - Determination of swelling, erosion, mucoadhesion and pH of microspheres.
  - Solid state characterization of optimized formulations using FTIR, DSC, XRD, SEM and EDXA.
  - Determination of antimicrobial activity of developed formulations on *Escherichia coli* and *Staphylococcus aureus*
  - *In-vitro* cytotoxicity evaluation by SRB assays on cultured L929 cell lines.
  - Assessment of stability of the optimized microspheres after 6 months of storage

- ***Final optimization chitosan-vanillin crosslinked (CS-VAN) microspheres based on significant formulation variables using Box-Behnken Experimental Design (BBED)***
  - Formulation of chitosan-vanillin crosslinked microspheres using w/o emulsion technique
  - Design and optimization of microspheres using Box-Behnken Experimental Design (BBED)

(II) CHAPTER 6: FABRICATION, OPTIMIZATION AND EVALUATION OF MICROSPHERES LOADED IN-SITU GEL (MLIG) IMPLANTS

- Formulation of optimized microspheres loaded *in-situ* gel (MLIG) implants using cold method.
- Application of BBED for design and optimization of MLIG based on gelation temperature (G_{temp}) and Viscosity (ɳ) as responses
- Assessment of *In-vitro* drug release studies
- Solid state characterization using FTIR, DSC, XRD, and SEM.
- pH, mucoadhesion, rheology study of formulations
- Swelling and erosion studies
- Stability studies and shelf-life estimation of optimized formulation
- Determination of antimicrobial activity on *Escherichia coli* and *Staphylococcus aureus*
- Evaluation of *in-vivo* biocompatibility after subcutaneous injection to rats
- *In-vivo* gingival tissue regeneration studies in rats
- Evaluation of efficacy of formulations by calculating plaque Index (PI), gingival Index (GI), bleeding on probing (BoP), probing pocket depth (PPD) and clinical attachment level (CAL) during clinical trials.

3.3. List of materials

The plan of study was executed by employing following materials, equipments and softwares as listed in Table 3.1, 3.2 and 3.3.

Table 3.1: List of chemicals

Drugs	Source
Ornidazole (OZ)	Gift sample from ENDOC Lifecare Pvt. Ltd., Gujrat, India
Doxycycline hyclate (DX)	Gift sample from Ranbaxy industries, now known as Sunpharma Industries Ltd., Gurgaon, India
Chitosan (CS)	(Degree of deacetylation -80%) Sigma Aldrich, India
Sodium alginate (SA)	Sigma Aldrich, India
Vanillin (VAN)	Sigma Aldrich, India
Glutaraldehyde (GLU)	Sigma Aldrich, India
Pluronic F127 (P127)	Sigma Aldrich, India
Pluronic F68 (P68)	Sigma Aldrich, India
Ninhydrin	Sigma Aldrich, India
Glucosamine	Sigma Aldrich, India
Liquid paraffin (LP)	Himedia Labs
Soyabean oil (SB)	Himedia Labs
Muller-Hinton Agar (MHA) media	Himedia labs, India
Tween 80	Merck Ltd., Mumbai, India
Span 80	Merck Ltd., Mumbai, India
Acetic acid	Merck Ltd., Mumbai, India
Petroleum ether (40-60 °C)	SD Fine chemicals (Mumbai, India)
Dimethyl carbinol	SD Fine chemicals (Mumbai, India)

Table 3.2: List of equipments.

Equipments	Details
Agitator	Remi Instruments, India
Mechanical shaker	Remi Instruments, Mumbai, India
Bath sonicator	WUC-1.8L, Fisher Scientific, India
Brookfield DV-III Ultra programmable Rheometer	Brookfield Engineering Laboratories Inc., USA
Centrifuge	RC 4100 F, Eltek, Mumbai, India
Confocal Laser Scanning Microscope (CLSM)	Carl Zeiss, Germany
Deluxe pH meter	Perfit, India
Differential Scanning Calorimeter (DSC)	Mettler Toledo, Switzerland),
Fourier-Transform Infrared (FTIR) spectroscopy	Shimadzu 8400, Japan
Lyophilizer	Labconco, USA
Magnetic stirrer	IKA-C MAG HS, India
Optical microscope	Dewinter optical Inc. india
Scanning Electron Microscope (SEM)	FEI Quanta 200 F, Japan,
Ultra-Violet method	Shimadzu UV 1800, Japan
USP paddle apparatus	Campbell Electronics, India
X-ray diffractometer (XRD)	Rigaku, Japan

Table 3.3: List of software.

Software	Version
Design-Expert software®	8.0.6.1
GraphPad Prism® software	5.03
Microsoft office (Word, excel)	2007
Minitab®	17
Origin Pro	6.0