

Formulations and evaluation of oral delivery systems of poorly absorbed drugs used in osteoporosis

A. Abstract

Bisphosphonates (BPs) are drugs found useful in physiological regulation of calcification, bone resorption and currently have become drug of choice for the management of osteoporosis. The major drawback of the clinically utilized BPs is their poor oral absorption from the GI tract, typically less than 1% is absorbed which is due to their poor permeability. In addition, the BPs have been associated with undesirable gastrointestinal effects. Risedronate sodium (RIS) is a bisphosphonates popularly indicated for osteoporosis that belongs to BCS Class III and has poor oral bioavailability due to less permeability. The present work attempted to improve poor permeability of RIS through dual approaches, multiple emulsion and sublingual spray formulations which were found promising. In first approach Initially RIS w/o/w multiple emulsion formulations were prepared and evaluated for determining significant causative factors and their effect on formulation properties. Consequently determined causative factors were included in central composite design and total 18 formulations were prepared and evaluated for appearance, pH, globule size, viscosity, % creaming, pour ability, drug entrapment efficiency, *In vitro* drug release, *ex-vivo* permeation study. Numerically optimized F19 formulation was additionally subjected for zeta potential and stability test. Stability of F19 was improved by including surfactant blend, phase volume ratio and hydrophilic polymer in outer water phase and stabilized F19B was evaluated for *In vivo* absorption study. The second approach involved formulation of propellant free RIS sublingual spray using face centered central composite design in which independent variables considered were concentration of drug, co-solvent and spreading agent while responses as spray pattern, spray angle and drug permeation and optimized by numerical method to get optimized sublingual spray FO. Total 16 formulations of RIS sublingual spray were prepared. All formulations were evaluated for spray pattern, spray angle, leak test, prime test, drug delivery uniformity, drug content per spray, *In-vitro* drug release and *ex-vivo* drug permeation study data. It was concluded from the study that independent variables had not caused any significant effect on delivery characteristics but influenced the permeation. Higher level of independent variables showed higher drug permeation. The study concluded with satisfactory performance of the device and feasibility of the sublingual formulation of RIS. *In*

vivo absorption study was aimed to know the enhancement in permeability of optimized and stabilized RIS formulations FO and F19B after oral administration in rats and the extent of absorption was determined. Rats were divided in different four groups and blood samples were collected at regular interval for 8 hours. Various pharmacokinetic parameters like C_{max} , T_{max} and AUC were found 1300.10 ± 50 ng/ml, 4 hrs and 4425 ± 0.20 ng/ml for optimized multiple emulsion formulation F19B and 920 ± 0.045 ng/ml, 5 hrs and 3710 ± 0.18 ng/ml for sublingual formulations FO. The enhancement in permeability of RIS was evident from pharmacokinetic data when compared with drug solution and conventional preparation which were 420 ± 20 ng/ml, 8 hrs, $1630 \text{ ng/ml} \pm 80 \text{ ng/ml}$ and 460 ± 23 ng/ml, 6 hrs, 1855 ± 0.10 accordingly for C_{max} , T_{max} and AUC_{0-8} . Relative bioavailability of RIS multiple emulsion formulation was 3 fold and sublingual formulation was 2 fold than the plain drug solution and conventional preparation. The study suggested sublingual spray and multiple emulsion formulation may be an alternative way of administration of RIS, providing enhanced bioavailability and also opened future scope for incorporation of other bisphosphonates.

B. Brief description on the state of the art of the research topic

- The alleviation of pain of suffering population is topmost priority of the state and nation.
- Indians have poor bone health, and osteoporosis is common in India.
- Bisphosphonates, selective estrogen receptor modulator, Calcitonin, Parathyroid hormone and Denosumab are currently used medicines in osteoporosis.
- Bisphosphonates are primary agents in the current pharmacological arsenal against osteoclast-mediated bone loss due to osteoporosis, Paget disease of bone, malignancies metastatic to bone, multiple myeloma, and hypercalcemia of malignancy. Risedronate sodium is a member of Bisphosphonates family and widely prescribed as oral tablets for prevention and treatment of a variety of osteoporosis and other skeletal conditions, such as low bone density and osteogenesis imperfecta.¹⁵
- Poor absorption, gastric intolerance, administration constraints lead patient non-compliance to available bisphosphonates oral dosage forms necessitates to overcome these hurdles by designing and developing new oral delivery systems

Recent approaches attempted for oral bioavailability enhancement of Bisphosphonates:

Particulate adducts

Dissettea et al., (2010) prepared particulate adducts of sodium risedronate and titanium dioxide for the oral bioavailability enhancement. Nanocrystalline and colloidal TiO₂, was used to obtain adducts. In vivo studies indicate that after oral administration to male Wister rats, the micro particles of adduct were able to prolong the presence of risedronate in the bloodstream during an 8 h period, resulting in a relative bioavailability almost doubled with respect to the free drug. This behaviour allows envisioning an improvement of the risedronate therapeutic effects and/or a reduction of its frequency of administration with consequent reduction of gastro-oesophageal injuries typically induced by oral administration of bisphosphonates.¹

Floating matrices

Chauhan et al., (2004) prepared floating matrices of Risedronate sodium with an objective to avoid gastric irritancy by formulating it in lipid material and achieve sustained release. The matrix systems were prepared by melting Gelucire® 39/01 derived from the mixtures of mono-, di- and triglycerides with polyethylene glycol (PEG) esters of fatty acids and Caprol PGE 860 at 10 °C above the melting point of Gelucire®39/01. Results confirmed the use of Gelucire® 39/01 as sustained release carrier in gastro retentive drug delivery system.²

Mucoadhesive films

Dhrubojyoti Mukherjee et al (2014) prepared Risedronate sodium mucoadhesive films composing different polymers and characterised. Mucoadhesive films showed prolonged drug release with residence time.³

GIPET® (Merrion Pharmaceuticals, Dublin, Ireland)

Gastrointestinal Permeation Enhancement Technology (GIPET®) formulations (Merrion Pharmaceuticals, Dublin, Ireland) are a group of oral solid dosage forms designed to promote absorption of poorly permeable drugs. GIPET is based primarily on promoting drug absorption through the use of medium-chain fatty acids, medium-chain fatty acid derivatives and micro emulsion systems based on medium-chain fatty acid glycerides formulated in enteric-coated tablets or capsules. The typical GIPET®-I preparation contains a poorly-absorbed drug with Sodium caprate C10 as the promoter in an enteric-coated tablet. In a Phase I study, GIPET® also improved the oral F of the bisphosphonate, alendronate, 12-fold compared to alendronate sodium tablets (Fosamax®, Merck), to yield an oral F of 7.2 % based on urinary excretion data of the unchanged molecule.^{4,5}

Vit-B₆ conjugate technology (MBC Pharma, Inc)

MBC Pharma has patented a drug discovery technology based on the concept of chemically attaching established pharmacophores to bisphosphonates through specifically designed labile bonds. To improve the oral absorption of aminobisphosphonates, they chemically attach a vitamin B₆ molecule known to be actively absorbed in the GI tract to the bisphosphonate. Active absorption will enable lower doses and improved patient tolerance, which could be a critical improvement in bisphosphonate therapy. Once inside the cell, the bisphosphonates will be released by a specific enzyme (pyridoxine/pyridoxamine phosphate oxidase). Thus, the bisphosphonate will be both absorbed and delivered inside cells using vitamin B₆ metabolic pathways.⁶

Sustained release matrix tablets

P.Sunil et al (2014) studied the release pattern of the Risedronate sodium sustained release tablets composing different hydrophilic polymers and eudragit polymer. Sustained release tablets released the drug for prolonged period.⁷

W/O/W multiple emulsion

Kenjiro Koga et al (2010) investigated intestinal absorption of Calcein, a model BCS class III drug. Calcein incorporated w/o/w emulsion was administered in rats and compared with control calcein formulation. Analysis of samples showed C_{max} of calcein 118 ± 47 ng/ml and 13.7 ± 5.4 ng/ml after 90 mins for multiple emulsion and control formulation respectively suggesting enhanced absorption of calcein. Study concluded that w/o/w could be capable of enhancing absorption.⁸ Chong-Kook Kim et al (1995) studied Cytarabine-loaded w/o/w multiple emulsions using combination of Tween 20/80 and Span 20/80 as a hydrophilic and lipophilic surfactant system and produced most stable preparation. The release study showed that the multiple emulsion containing cytarabine in the internal aqueous phase was stable, exhibiting a prolonged release pattern.⁹ J.A. Omotosho (1990) studied the absorption of 5-fluoro[³H]uracil orally administered in a water/isopropyl myristate/water emulsion. w/o/w emulsions were prepared by two-stage emulsification procedure. In absorption study rats were given 1 ml emulsion orally and blood samples were collected. Study reported enhanced drug absorption and showed potential as lymphotropic carriers of water-soluble drugs to the mesenteric lymph nodes following oral administration.¹⁰

Sublingual spray

Thierry Buclin (2002) formulated Salmon Calcitonin (SCT) in new oral spray formulation along with penetration enhancers and was reliably absorbed from the oral cavity, with an absolute bioavailability of 0.5–1.4%, depending on the dose. This study showed that oral delivery of SCT would be feasible with reproducible absorption and systemic biological efficacy. Such an oral formulation could facilitate the use of SCT in the treatment of osteoporosis and other bone diseases.¹¹ Sam S et al. (2015) developed artemether sublingual spray formulation for children who could not tolerate oral antimalarial therapy. Sublingual artemether spray formulation was administered in children suffering from malaria and it was found that the formulation was as effective as oral with less incidents of discomfort associated with oral therapy and could be transferred to oral therapy after attaining effective blood concentration of drug.¹² Kalliopi Dodou(2012) in his review pointed the development of Ora-Lyn insulin preparation by Generax, a liquid formulation of human insulin combined with absorption enhancers that is sprayed in the buccal cavity, providing an alternative to injectable insulin formulations for the management of type 1 and type 2 diabetes. Oral spray formulations having sumatriptan for the treatment of migraine, ondasetron hydrochloride (Zensana) for chemotherapy- or radiotherapy-induced nausea and vomiting and, sildenafil (Duromist), for erectile dysfunction, are currently at preclinical stage or at phase I or II clinical trials.¹³ McInnes and co-workers evaluated radio labelled buprenorphine clearance from the buccal cavity and pharmacokinetic profiles of a sublingual spray formulation in the dog, spray formulation (400 µg/100 µl in 30% ethanol) was administered sublingually to four beagle dogs, and in comparison, absorption of buprenorphine was relatively slow, with a T_{max} of 0.56 ± 0.13 h. Good buccal absorption despite short residence time can be explained by lipophilicity of buprenorphine enabling rapid sequestration into the oral mucosa, prior to diffusion and absorption directly into systemic circulation.¹⁴

C. Definition of the problem

- Osteoporosis is evaluated by low bone mass with micro architectural deterioration of bone tissue leading to enhance bone fragility, thus increasing the susceptibility to fracture, an estimated 25 million Indians may be affected.¹⁵ Therapeutic intervention is costly and often found to be discontinued due to associated unwanted effects. This encouraged us to take up the problem and to find rational answer to it.
- The bisphosphonates make up a family of chemical compounds generally used by oral route to treat a variety of metabolic osteopathies.

- Oral bisphosphonates i.e. Risedronate sodium and alendronate products currently marketed are administered in the morning in the fasting state and patients must remain without food or drink for 30 – 60 minutes after administration.
- Low permeability and Interaction of these compounds with food present in the digestive medium hinders most their absorption to extent of 3% only.
- Poor bioavailability of oral bisphosphonates has necessitated the administration of higher than normally required oral doses which often leads to economic wastages, risk of toxicity, erratic and unpredictable responses.
- However, the clinical efficiency shown by the bisphosphonates is sufficient to justify the use of the oral route.
- Further, to obtain an effective antiresorptive effect in tumor osteolysis, a relatively high dose of drug must be given parenteral. The preferred mode of administration is by iv infusion, since im and sc injections are associated with local necrosis.
- Thus, if increased absorption could be attained, oral administration would be a viable delivery system in situations where higher doses are required. In addition bisphosphonates therapy in osteoporosis would improve significantly.
- The challenge over the years has been to design techniques that will allow oral administration of most drugs, irrespective of their properties, to achieve a therapeutic systemic availability. This will be a worthy achievement since over 90% of therapeutic compounds are known to possess oral bioavailability limitations.^{16,17}

D. Objective and scope of work

- The overall aim of this study was to formulate oral drug delivery systems for poorly absorbed drug RIS in order to increase permeation across mucous membrane there by improve the oral bioavailability and opening scope for other bisphosphonates. This study considers the possibility of designing a commercializable formulation and delivery system that will deliver drug to the systemic circulation with increased bioavailability. In order to achieve this aim, objective was to go with two approaches of oral drug delivery systems

W/O/W Multiple emulsion

- Multiple emulsion approach for increasing the intestinal membrane permeation of low-permeability drug RIS

- To formulate and evaluate RIS w/o/w multiple emulsion to select components and operation parameters
- To optimize and evaluate RIS multiple emulsion
- To carry out *ex vivo* permeation study
- To improve stability of optimized RIS multiple emulsion
- To investigate *in vivo* absorption study of stabilized formulation and compare with conventional formulation

Sublingual spray formulation

- To formulate sublingual formulation of RIS
- To evaluate and optimize sublingual spray formulation
- To carry out stability study of the formulation
- To investigate *ex-vivo* and *in-vivo* absorption study of optimized formulation and compare with conventional formulation
- Overall objective is to increase the permeability of selected drug and to increase patient compliance.

E. Original contribution by the thesis.

- One of the approach selected to enhance the permeation of RIS through the GIT was w/o/w multiple emulsion system. Enclosing the drug in inner oil phase may reduce direct contact of RIS with gastric lumen specifically stomach mucous membrane and increase lipophilicity to increase its permeability across mucous membrane may undergo lymphatic uptake. Because of the possible lower incidence of gastrointestinal problems associated with this dosage form, it is more advantageous than dosage forms such as tablets and capsules. In addition, w/o/w multiple emulsions possess many advantages as a low viscosity due to the aqueous external phase, which makes them more convenient to handle and use, especially for oral.
- Sublingual route of drug administration has been proven the alternate route for drugs which are poor permeable, having food interaction and unpredictable absorption. Further sublingual route offers ease of administration to patients, relatively rapid onset of action and large contact surface contributes to rapid and extensive drug absorption. Present work describes the formulation of Risedronate sodium sublingual spray without using any propellant.

F. Methodology of Research, Results / Comparisons

Preformulation studies

1. Melting Point

RIS was filled in glass capillary tube (closed at one end) and tied to thermometer such that closed end of the capillary was near the middle of the thermometer bulb and remaining procedure was followed as mention in Method I for Melting Range or Temperature in IP 1996.¹⁸

2. FT IR analysis

Identification of the drug was carried out by KBr pellet method¹⁹

3. UV Visible spectroscopy for λ_{\max} determination and linearity curve

Stock solution of RIS (0.6 mg/ml) was prepared in distilled water and 50 $\mu\text{g/ml}$ solution was scanned between 180 to 300 nm to determine λ_{\max} of. Appropriate dilutions (15 μg to 150 $\mu\text{g/ml}$) was made in 0.01mol HCL and 0.01mol NaoH and the absorbance difference (ΔA) was measured at λ_{\max} against the drug in sodium hydroxide as a blank.²⁰

4. Solubility

Solubility was determined by Common batch agitation method where excess amount of the RIS was dissolved in distilled water to form saturated solution and agitated for 48 hours. Solution was filtered and analyzed for RIS concentration to determine solubility.^{21,22}

5. Initial drug Stability studies

1% w/v solution of RIS was stored for 1 month at $40\pm 2^\circ\text{C}/75\pm 5\%$ RH and amount of RIS present was determined by UV spectroscopy.²³

a. W/O/W Multiple emulsion

1. Preparation of the emulsion

1. Trail batches

For first approach i.e., multiple emulsion, Different types of the oils like Arachis oil, olive oil, sunflower oil and Isopropyl myristate (IPM) along with hydrophilic and Lipophilic surfactants. Prepared batches were evaluated for selection of the components and operational parameters for further formulation and optimization.^{24,25}

2. Formulation and optimization.(Minitab 16, Design Expert 7 Trail version)

Face centered central composite experimental design was applied to prepare the model formulations. In this study, three factors such as Span 80 amount, homogenization speed and time of homogenization of the emulsification process were selected as the causal factors and selected responses as tabulated in table 1 and 2. W/O/E multiple emulsions were prepared by two stage

emulsification procedure. Drug and other excipients were first dissolved in purified water and was emulsified with oily phase with span 80 to form primary emulsion w/o. This primary emulsion w/o was further emulsified using Tween 80 as emulsifying agent by dispersing it in aqueous phase to form w/o/w multiple emulsion. Total 18 formulation batches of Multiple emulsions were prepared and characterized.

Table 1 : Selected independent variables and their levels

Name of variable	Unit
Span 80 (X1)	%
Homogenization speed (X2)	RPM
Time of homogenization (X3)	Min

Table 2: Selected Responses

Name of response	Unit
Globule size	Micron
Entrapment efficiency	%
Rate of creaming	ml/hr
Drug permeated Q 30	%

2. Evaluations-Characterization, *Ex-vivo* and *In vivo* permeability study

Characterization

1. Organoleptic properties

2. Identification of Emulsion type

2.1. Dilution test

Test was carried out to find the type of emulsion and performed by addition of water and oil to the preparation separately and state of the preparation was checked.

2.2. Conductivity test

The test confirms the type of emulsion and performed by addition of small fraction of an electrolyte in preparation under test and change in conductance was measured.²⁶

3. Microscopic analyses and Measurement (Carl Zeiss Trinocular microscope)

Small sample of emulsion with appropriate dilution was mounted on the slide and observed under microscope to determine average globule size and distribution.

4. Viscosity measurement (Brookfield viscometer DV-II+PRO)

Sufficient sample was filled in sample holder of the Brookfield viscometer previously stabilized and viscosity was determined and read on digital display (spindle no 63 at 50 rpm and RT)

5. Emulsion stability study

w/o/w emulsions were evaluated by visual observation for the creaming process. The emulsion samples were poured into 100 ml glass cylinders instantly after preparation. Multiple emulsions were observed for phase separation i.e., three phases; oil phase, unseparated emulsion and water phase from the top. The volume ratios of each separated phase were measured.²⁷

6. Entrapment efficiency

Small sample from the required quantity of test sample was withdrawn and analysed by spectroscopic method to find out the amount of drug present and subtracted from the total amount incorporated to know entrapment.²⁸

7. Osmotic behaviour

Osmotic behaviour of the formulations were determined by dispersing the formulation in distilled water having 1% sodium chloride in internal aqueous phase and percentage change in globule size was noted by microscopy.⁹

8. Zeta potential measurement

Zeta potential was measured to know stability of the of the dispersion as it indicates the kind of force acting between the nearby globules on [Malvern zetasizer nano].²⁹

9. *Ex-vivo* permeation study (Diffusion cell)- As described on page no.13

10. *In vivo* permeation study (approved by the IAEC proposal no. PhD/13-14/22)- As described on page no.14

b. Sublingual Spray formulation of Risedronate sodium

Experimental Design

Face centered composite experimental design was used to prepare the model formulations. In present study, concentration of Risedronate sodium (X_1) and Propylene glycol (X_2) and

Poloxamer 188 (X_3) were selected as the causal factors and their effects were seen on drug release(Y_1), drug permeated(Y_2) and apparent permeability(Y_3) (Table 1).

TABLE 1: EXPERIMENTAL DESIGN

FACTORS NAME	UNITS	TYPE
conc. of RIS (X_1)	%	Numeric
conc. Of PG (X_2)	%	Numeric
conc. of P-188(X_3)	%	Numeric

Formulation Composition

Preparation of sublingual spray formulation

In a clean beaker, measured quantity of propylene glycol, alcohol and small quantity of distilled water was poured and stirred to make solution. Required quantity of polymer and drug was added in this solution and sonicated. Finally volume was made with distilled water. Formulated solution was filled in pump spray container for evaluation. All the formulations were prepared accordingly.

Analytical method for estimation of RIS

Chromatographic technique was used as described in earlier work. HPLC system consisted of a Series I binary gradient system, a model 525 Dual-wavelength UV detector, Hypersil C18 reverse phase column (i.d. 5mm, 4.6mm×250 mm, ODS-2) and a Hypersil guard column (5 μ , 4.6mm×10 mm) at room temperature. The mobile phase for separation of Risedronate sodium in samples consisted of buffer (5mMTBABion-pair reagent, 11mMsodium phosphate and 1.5mM EDTA-2Na) – methanol (88:12,v/v), adjusted to pH 6.75with 0.2 M NaOH and was pumped at a flow rate of 1.0 ml min⁻¹. The injection volume was 10 μ l and the detection wavelength was 262 nm. Peak areas were used for quantitative analyses.³⁰

Drug container and component compatibility study

RIS and other excipients were added in distilled water to form solution. Prepared solution was filled in pump spray container and was kept for 1 month at 40±2°C/75±5% RH. The product was studied for the RIS content as well for container parameters.

Evaluation

Container specifications

Performance tests

1. Spray pattern

For the spray pattern test two third volume of each spray formulation was removed from the corresponding spray container and stored in labeled glass beaker for time being. One third volume of each Risedronate spray formulation was mixed with patent blue V dye and sprayed over Whatmann filter paper. Spray pattern was determined by Ovality ratio which was calculated using formula,

$$\text{Ovality Ratio} = D_{\max}/D_{\min}$$

Where, D_{\max} and D_{\min} are the maximum and minimum diameters of the spray pattern respectively

2. Prime test

Test for priming was done to support the number of actuations(priming actuations) that should be fired to waste solution prior using the product. Priming actuations was counted for container to release drug that would come out per actuation as per experimental design

3. Average weight per meter dose

This test was performed to find out the amount of solution delivered per spray. Initial weight of the container was recorded (W_i). Ten successive deliveries were sprayed from the container. Container was weighed again (W_f).

4. Content per spray= $(W_i - W_f) / n$

Where, W_f = final weight of the container,
 W_i = initial weight of the container and
 n = no. of deliveries.

5. Drug content per spray

Content per spray was determined by shots of two sprays in a beaker containing 0.1N HCl. This solution was shaken for 5 minutes and the drug content was determined at 262 nm by the UV-spectroscopy

6. Net content

Empty containers were weighed before filling and then reweighed after packed containers and the difference obtained was the net content

7. Density

Empty pycnometer was weighed and then filled with 25mL of the product and reweighed. Difference in the weight of filled pycnometer to empty pycnometer was divided by the volume filled to get the density of the product`

8. Spray profiling (Delivered dose uniformity)

Reproducibility of dosage was determined using this test as per USP. The average amount of active ingredient delivered through the actuator per spray was assayed. Uniformity of content was validated by performing the test at three different points i.e. starting, intermediate and ending point approximately.

9. Spray angle

The method of impingement of spray on a piece of paper was used for the study. Patent blue V (10 mg) was dissolved in formulation to facilitate visualization. The sprays were actuated in horizontal direction onto a white paper mounted at a distance of 1 cm from the nozzle. The radius of the circle, formed on the paper, was recorded for minimum and maximum diameters. Spray angle (θ) was calculated by equation.^{23,30}

$$\theta = \tan^{-1}(h/r)$$

θ = angle in degree, h= height of triangle and r radius of circle

c.Biological tests

10. *Ex-vivo* drug permeation study (Diffusion cell)

Ex-vivo permeation study was carried out using Franz diffusion cell. 20 ml phosphate buffer pH 7.4 was filled in receiver compartment containing magnetic stirring bar. Donor and receiver compartment were separated by treated goat sublingual mucosa which was stored at 2-8 °C in refrigerator in ringer's solution till its use to preserve its biological characteristic. Experiments were approved by the IAEC in accordance to proposal no. PhD/13-14/22. Entire assembly was set on magnetic stirrer and slowly temperature was raised and maintained at 37°C. Spray formulations containing Risedronate sodium were applied in donor compartment and 2 ml samples from receiver compartment were collected through sample withdrawal tube and equal volume of phosphate buffer was replaced at the interval of 10 minutes for one hour. Procedure was repeated for each spray formulation. Absorbance of Withdrawn samples was measured at 262 nm by UV spectroscopy.^{2,32}

11. Flux and apparent permeability determination

Flux and apparent permeability were calculated using following formulas.

$$\text{Flux (J}_{ss}) = \Delta Q_t / \Delta t \times S$$

Where, $\Delta Q_t/S$ is the cumulative drug permeation per unit of mucosal surface area ($\mu\text{g}/\text{cm}^2$), t is time expressed in h

$$\text{Apparent permeability (P}_{app}) = J_{ss} / C_d$$

Where, J_{ss} is the Flux and C_d is the concentration of drug in donor compartment.³²

12. Stability study of the optimized formulation

Short term stability study was carried out according to ICH guidelines Q1C at $40^\circ\text{C} \pm 2^\circ\text{C}/75 \pm 5\%$ RH for 3 month for optimized batch. The optimized formulation was kept at room temperature and humidity condition. At the end of studies, responses were measured which had been used to optimize the formulation.²³

13. Optimization and validation of model –Design experts software tool

14. *In vivo* permeation study (approved by the IAEC proposal no. PhD/13-14/22)

The study was carried out on healthy male Wister rats weighing 200-250 g. Rats were housed in polypropylene cages, maintained under standard condition (12 h light/dark cycle, 25°C , 35-55 % humidity) and allowed free access to diet. The animals were fasted at least 12 h prior to dose administrations and for 4 h after dosing with free access to water. Animals were divided into four groups each consisting of six animals. All animals were given different formulation group wise as described underneath.

1. Group I: Control group (Plain RIS solution, 5mg/kg, p.o.)
2. Group II: Optimized Multiple emulsion Formulation equivalent to RIS to 5mg/kg, p.o.)
3. Group III: Optimized sublingual spray formulation equivalent to RIS to 5mg/kg, p.o.)
4. Group IV: Marketed tablet formulation made in suspension form to RIS to 5mg/kg, p.o.)

Serial blood samples (0.5ml) were withdrawn through capillary inserted in to retro orbital plexus under mild ether anaesthesia at a time interval of predose 1, 2, 4, 8, 12 and 24 h post dose. Blood samples were collected in micro centrifuge tubes containing anticoagulant (1.2% w/v EDTA disodium). The plasma samples were collected immediately from aforementioned samples after centrifugation at 5,000 rpm at 4°C for 10 minutes and stored immediately at -20°C until further analysis.^{33,34}

G. Results / Comparisons

Identity of the drug sample was confirmed to RIS from the FTIR and UV visible spectroscopy. When 50 μ g/ml RIS solution was scanned in UV Visible spectrophotometer between 180-300 nm, maximum absorbance was at 262 nm (λ_{max}). Quantitative determination of RIS samples was performed by UV spectroscopy at 262 nm with linearity range 5 to 150 μ g/ml. FTIR study confirmed the presence of RIS as peaks near 933-910 cm^{-1} , 1100-900 cm^{-1} , 1130 cm^{-1} , 1207 cm^{-1} indicate presence of C-H, P-OH, C=N, P=O groups accordingly. RP-HPLC method was adopted to analyze specifically biological samples. Chromatograms of RIS 0.5 μ g/ml to 5 μ g/ml, were obtained and linearity curve was constructed and R^2 value was 0.998. Preformulation study was carried out to find out various physicochemical properties of RIS. Melting point and Solubility of the RIS was 255 $^{\circ}$ c and 11 mg/ml in water. Initial stability study was carried out to find out the feasibility and stability aspects of RIS in aqueous solution. Solution was analyzed after one month and the RIS content was 0.98% w/v indicating stability and absence of degradation reaction.

W/O/W Multiple emulsion

Various oils of Generally regarded as safe (GRAS) category were selected and emulsified by two stage method with aqueous phase varying operational parameters (homogenization speed and time) to form w/o/w multiple emulsion which were evaluated for appearance, Identity, viscosity, globule size, *In-vitro* diffusion study etc. From the study of different trial formulations Isopropyl myristate (IPM), span 80 and Tween 80 were selected as lipid phase, internal and external emulsifier accordingly. Operational parameters were also fixed in range and included in further study. Experimental design was used to formulate and optimize the formulation including variables and responses as mentioned earlier. Total 18 batches were prepared and evaluated for various parameters. The multiple emulsions were identified as w/o/w type based on dilution and conductivity (0.130-0.138 mS/cm). The multiple emulsions are kinetically stable but thermodynamically unstable hence spontaneously separated in three layers. Stability of the emulsions were determined from % creaming at the end of test period. Photomicrographs for multiple emulsions after preparation confirm the formation of double emulsion with the average globule diameter in submicron range and viscosity was found in 15-20 cp range. Change in average globule size after 90 days storage period was 10%. Osmotic behavior of the emulsion showed that as the concentration of electrolyte in external phase changes from lower to higher

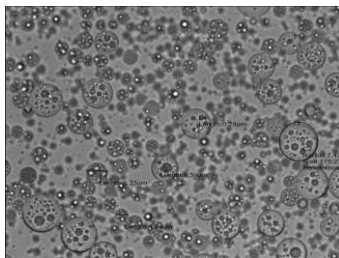


Fig.1. Photograph of RIS Multiple emulsion optimized formulation F19B

there was rapid change in the size of the oil globules. The entrapment efficiencies of RIS in the w/o/w multiple emulsions was found up to 70 %. Higher permeation i.e., Q 30 was 14 to 20.5 % observed for the formulation having small globule size in range 1.9 to 2.4 μm and 7.5 to 10 % span 80 as external emulsifier compare to other formulations in permeation study for the RIS multiple emulsion than the drug solution alone due to increase lipophilicity permeability across the membrane probably because the oil phase i.e., IPM has penetration enhancing property could have decreased the barrier to charged drug. Numerical method was used to optimize the formula, accordingly F19 an optimized formulation was prepared. F19 was evaluated for all the parameters additionally for Zeta potential test and was found -35mv. There was negligible phase separation observed in F19 and the rate of creaming was 10% in. In order to improve the stability of the optimized formula, 0.5 % xanthun gum as viscosity enhancer, Tween 80 and span 80 blend in 32.5/67.5 ratio and volume ratio 0.4 ml of w/o /ml w/o/w were found effective to get F19B stabilized emulsion. *In vivo* permeation study of F19B results are explained in later part.

Ovality ratio calculated for different formulations indicated circular to ellipsoid pattern with uniform spray pattern. Spray angle (θ) was found in the range of 60-69°. Direction of holding spray pack in upright, tilted and horizontal position may play role in assessment of the sealing characteristics of the container closure system. During the test there was no sign of leakage and weight of the entire container were unchanged indicating proper sealing of containers. Priming and repriming tests indicated that nearly 3 primes were required to deliver the desired amount of Risedronate sodium. Average amount of the product delivered from spray devices were 0.14 to 0.17 ml and the deviation could be due to formulation compositions. Drug content when determined by the RP-HPLC found within the specified limits i.e., 80 to 120% of theoretical dose as per experimental design for each formulation. In present study *Ex-Vivo* permeability study was carried out for 1 hour, highest % cumulative drug permeation was observed for the formulation F15, 50.1% (2.55 mg) and the least for formulation F 7, 15.4% (0.0662 mg) at the end of 1 hour.

Risedronate sodium spray formulation was optimised using numerical method depending on desirability 0.969 and bright yellow portion of the graph, which was evaluated and the predicted and experimental values. From the observation it was concluded that the model satisfied the selected level of variables and was able to predict 96.75% variation in the formulation. In present study for optimised formula (FO) 47.02% drug permeated though mucosa would enter in the blood without change. *Ex-Vivo* Permeation study findings proved that sublingual administration of Risedronate sodium can result in many folds than its oral bioavailability. Finally *In- vivo* study rat experiments were aimed to know the enhancement in permeability of Risedronate sodium after sublingual and oral administration of previously optimized Risedronate sodium formulations and the extent of absorption was determined, and this study was approved by the IAEC in accordance to proposal no. PhD/13-14/22. Animals were divided in four different groups and blood samples were collected at regular interval of 1 hour for 8 hours. Various pharmacokinetic parameters like C_{max} , T_{max} and AUC were found 1300 ± 80 ng/ml, 4 hrs and 4425 ± 200.64 for multiple emulsion formulation and 920 ± 45 ng/ml, 5 hrs and 2200 ± 50 ng/ml for sublingual formulations. The enhancement in permeability of Risedronate sodium was evident from pharmacokinetic data when compared with drug solution and marketed preparation. The study suggested sublingual route and multiple emulsion formulation may be an alternative way of administration of Risedronate sodium, providing enhanced bioavailability.

H. Achievements with respect to objectives

Objective was to formulate improved formulation that could increase the permeation of the poorly permeable drug Risedronate and respect to that following were achieved

- Stable and palatable Risedronate sodium w/o/w multiple emulsion F19B with average globule size 2.0 μ , drug entrapment 70%, 8 % creaming and % drug permeation (*ex-vivo*) 14.798.
- Stable and patient friendly sublingual spray formulation of Risedronate sodium FO with 50.1% drug permeation, flux (J_{ss}) 0.034 ($\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$) and permeability coefficient 6.89×10^{-05} K_p ($\text{cm}^{-2}\cdot\text{min}^{-1}$)
- Both formulation showed increased absorption which could be due to increased *In-vivo* permeation by >2 fold when compared to marketed and plain drug formulation
- Feasibility to present the other members of Bisphosphonate drug in these formulation to increase permeability and patient compliance

I. Conclusion

Overall study summarized with scope for formulation of hydrophilic and polar drug Risedronate sodium in oral drug delivery systems i.e., Multiple emulsion and Sublingual spray dosage forms. W/O/W multiple emulsions are difficult to stabilize for long period but phase separation can be minimized. Study concluded that permeability of poorly permeable drug can be increased by stable multiple emulsion formulation of water soluble drug with suitable oil phase, appropriate surfactant blend and addition of viscosity enhancer in external water phase with desired pourability and could be made palatable by incorporating appropriate flavoring and colouring agent, this feature may increase the patient compliance. The present research also successfully developed sublingual spray system for delivery of poorly permeable and water soluble drug which can lay down the foundation for future research to incorporate potential candidate for systemic delivery.

J. Copies of paper published and a list of all publications arising from the thesis

1. Thosar MM *et al.*, 2011, Recent Development In Oral Delivery Of Bisphosphonates – An Overview, American Journal of Pharmtech Research ,2011, 1,2,102-120.,2249-3387
2. M. Milind Thosar,2011, Intra oral sprays -An overview, International Journal Of Pharmacy & Life Sciences, 2,11,0976-7126
3. Thosar Milind M, Pancholi S S,2013, Permeability Enhancement Techniques for Poorly Permeable Drugs by Oral Route, Inventi Rapid: Pharmaceutical Process Development ,2013,1, 2320-1029
4. Milind M Thosar, S S Pancholi,2014, Water-in-Oil-in-Water Multiple Emulsions With Risedronate Sodium, Inventi Rapid: NDDS,2014,2, 0976-3791
5. Acceptance letter for Article entitled "In-Vivo Absorption Study Of Risedronate Sodium Dosage Forms In Rats" accepted in International Journal of Pharmacy and Technology ISSN no. 0975-766X

K. References

- 1 Dissetea V, Bozzia P, Bignozzia CA, Dalpiaz A, Ferraroc L, 2010, Particulate adducts based on sodium risedronate and titanium dioxide for the bioavailability enhancement of oral administered bisphosphonates, Eur J Pharm Sci, 41,328–336.,0928-0987
- 2 Chauhan B, Shimpi S, Mahadik KR, Paradkar A, 2004, Preparation and evaluation of floating risedronate sodium Gelucire® 39/01 matrices, Acta Pharm ,54,205–214.,1846-9558
- 3 Dhrubojyoti mukherjee, 2015, comparative evaluation of risedronate sodium mucoadhesive films using different mucoadhesive polymers, world journal of pharmacy and pharmaceutical sciences ,14,01,2278-4357
- 4 Leonard TW, Lynch J, McKenna MJ, Brayden DJ., 2006, Promoting absorption of drugs

- in humans using medium-chain fatty acid-based solid dosage forms: GIPET, *Expert Opin Drug Deliv*; 3,5,685-692., 1744-7593
- 5 Leonard TW, Adamczyk B, Lee A, O'Toole E, 2007,Eliminating the Bisphosphonate Dosing Ritual with an Improved Dosage Form of Alendronate, *MER-103, Menopause*; 4,6,1098, 1530-0374
- 6 Technology-MBCPharma, Available [online] <http://www.mbcpharma.com/html/technology.html> [Accessed 20 June 2011]
- 7 P. Sunil,2014,Formulation and evaluation of sustained release matrix tablets of Risedronate, *International Journal of Innovative Pharmaceutical Sciences and Research*, 2 ,10,2250-2260., 2347 - 2154
- 8 Kenjiro Koga, Nobuo Takarada, Kanji Takada,2010, Nano-sized water-in-oil-in-water emulsion enhances intestinal absorption of calcein, a high solubility and low permeability compound, *European Journal of Pharmaceutics and Biopharmaceutics*,74:223–232., 1873-3441
- 9 Chong-Kook Kim, Seong-Cheol Kim, Hee-Jong Shin,1995,Preparation and characterization of cytarabine-loaded w/o/w multiple emulsions, *International Journal of Pharmaceutics*, 124,61-67., 0378-5173
- 10 J.A. Omotosho et al.,1990,Absorption and lymphatic uptake of 5 fluorouracil in the rat following oral administration of w/o/w multiple emulsions , *International Journal of Pharmaceutics*, 61,51-56., 0378-5173
- 11 Thierry Buclin et al, 2002,Bioavailability and Biological Efficacy of a New Oral Formulation of Salmon Calcitonin in Healthy Volunteers, *Journal Of Bone And Mineral Research*, 17, 88, 1523-4681
- 12 Sam Salman, Daryl Bendel, Toong C. Lee, David Templeton and Timothy M. E. Davis, 2015,*Antimicrob. Agents Chemother*,59 ,6, 3208-3215.,
- 13 Kalliopi Dodou(2012) Research and developments in buccal and sublingual drug delivery systems, *The Pharmaceutical Journal* Available:[online] <http://www.pharmaceutical-journal.com/news-and-analysis/research-and-developments-in-buccal-and-sublingual-drug-delivery-systems/11098641.article>[accessed 20 June 2014]
- 14 V. Hearnden, et al.,2012, New developments and opportunities in oral mucosal drug delivery for local and systemic disease, *Adv. Drug Deliv. Rev.* 64,1,16-28., 0169409X
- 15 N. Malhotra et al., 2008,Osteoporosis in Indians, *Indian J Med Res*, 127,263-268., 0971-5916
- 16 Aviva Ezra et al, 2000,Administration routes and delivery systems of bisphosphonates for the treatment of bone resorption, *Advanced Drug Delivery Reviews*, 42,175–195., 0169409X
- 17 Elena M. et al,2001, Risedronate: A New Oral Bisphosphonate, *clinical therapeutics*. 23, 9, 0149-2918
- 18 Government of India. Ministry of health and family welfare(2007) *Indian Pharmacopoeia Vol. I*, p.135,The Controller of Publication, New Delhi.
- 19 Government of India. Ministry of health and family welfare(2007) *Indian Pharmacopoeia Vol. I*, p.108,The Controller of Publication, New Delhi.
- 20 Elham Anwer Taha et al., 2003, Spectrophotometric Determination of Some Drugs for Osteoporosis ,*Chem. Pharm. Bull.* 51,12,1444—1447., 1347-5223
- 21 Harry G. Brittain (1995) *Physical Characterization of Pharmaceutical Solids.* Taylor & Francis ,New jersey.
- 22 Mark Gibson (2000) *Pharmaceutical Preformulation and Formulation.* Taylor &

- Francis ,Florida.
- 23 FDA (2002), Guidance for Industry Nasal Spray and Inhalation Solution, suspension, and Spray Drug Products — Chemistry, Manufacturing, and Controls Documentation Available[online] <http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070575.pdf> [Accessed on 20 June 2014]
 - 24 Florence AT and Whitehill D, 1982, The formulation and stability of multiple emulsions, *Int J Pharm.*, 11, 277 - 308., 0378-5173
 - 25 Hideaki O and Masahiro N, 2000, Preparation and evaluation of W/O/W type emulsions containing vancomycin, *Adv Drug Del Rev*, 45, 5-26., 0169409X
 - 26 A. Silva-Cunha et al., 1997, W/O/W multiple emulsions of insulin containing a protease inhibitor and an absorption enhancer: preparation, characterization and determination of stability towards proteases in vitro, *International Journal of Pharmaceutics* , 158, 79-89., 0378-5173
 - 27 Dragana Vasiljevic et al., 2006, An investigation into the characteristics and drug release properties of multiple W/O/W emulsion systems containing low concentration of lipophilic polymeric emulsifier, *International Journal of Pharmaceutics*, 309, 171–177., 0378-5173
 - 28 Kai Lindenstruth, Bernd W. Muller , 2004, W/O/W multiple emulsions with diclofenac sodium, *European Journal of Pharmaceutics and Biopharmaceutics*, 58, 621–627., 1873-3441
 - 29 Pooja Sonakpuriya et al., 2013, Formulation And Evaluation Of Multiple Emulsion Of Valsartan, *International Journal of PharmTech Research*, 5, 1, 132-146., 0974-4304, 0378-5173.
 - 30 Hui-Juan Jia, Wei Li, Kang Zhao, 2006, Determination of Risedronate in rat plasma samples by ion-pair high-performance liquid chromatography with UV detector, *Analytica Chimica Acta*, 562, 2, 15, 171-175., 1873-4324
 - 31 Leon Lachman, H. A. Lieberman, J. L. Kanig (2009) *The Theory and Practice of Industrial Pharmacy*; Varghese Publishing House, Bombay, pp 589 – 618
 - 32 Hitesh Verma, et al, 2014, Sublingual Delivery of Flovatriptan: An Indication of Potential Alternative Route, *International Scholarly Research Notices* , 675868, 1-9., 2356-7872
 - 33 Hyo-Kyung Han, 2014, Effective mucoadhesive liposomal delivery system for risedronate: preparation and in vitro/in vivo characterization, *International Journal of Nanomedicine*, 9, 2299–2306., 1178-2013
 - 34 Michael F. Wempe et al., 2008, Pharmacokinetics of raloxifene in male Wistar–Hannover rats: Influence of complexation with hydroxybutenyl-beta-cyclodextrin, *International Journal of Pharmaceutics*, 346, 25–37., 0378-5173.