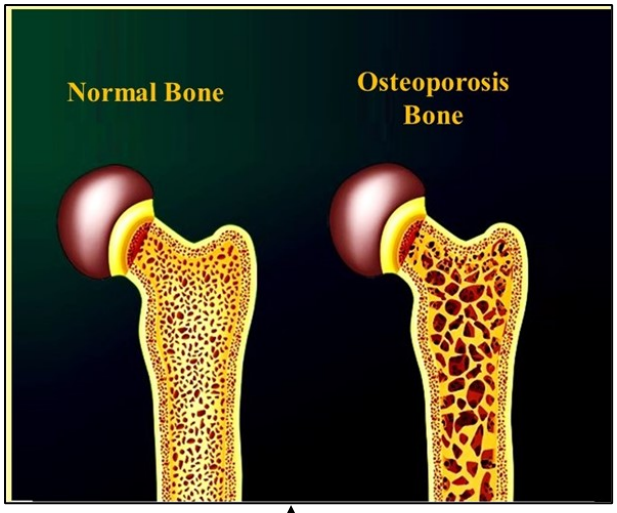
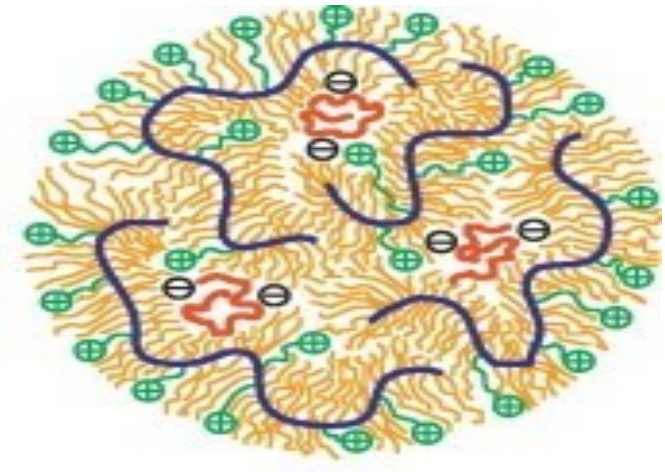
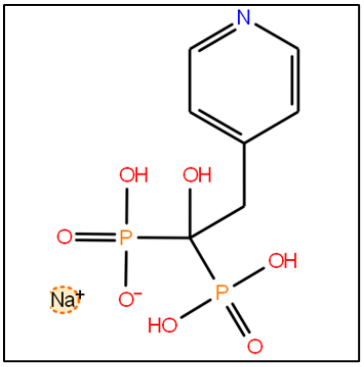


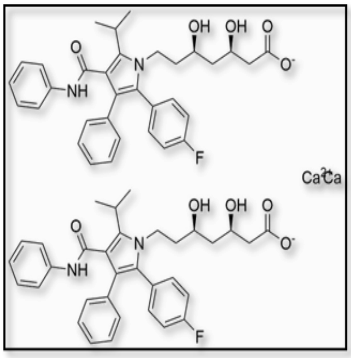


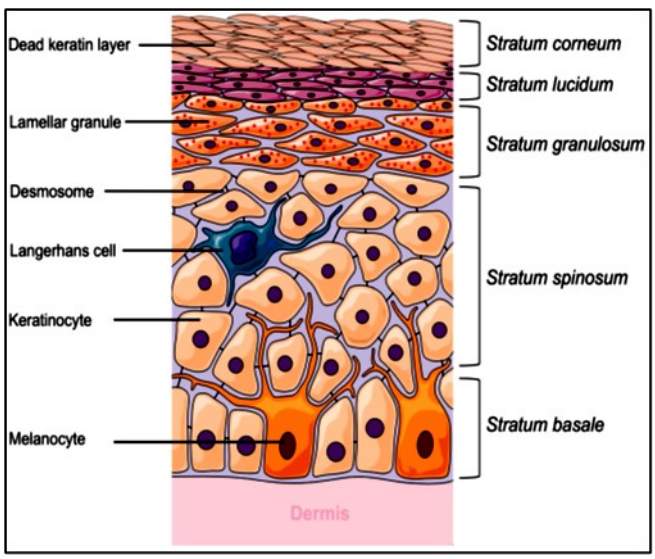
PECN

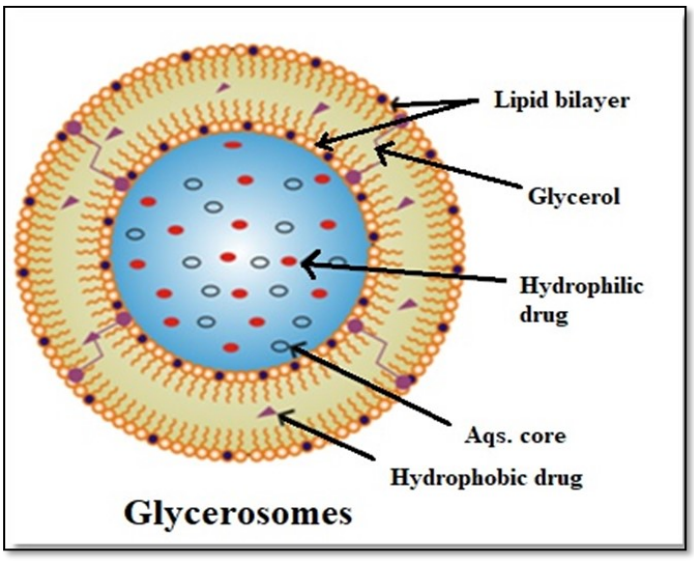
Chapter-2

Literature review









2.1. Osteoporosis:

Osteoporosis is a condition characterised by low bone density, bone tissue degeneration, and disturbance of bone microarchitecture, which may lead to decrease in bone strength with an increased risk of fractures [1-4]. Reduced bone mineral density (BMD) is a key consequence of weak, brittle, and broken bones [5]. Osteoporosis is a serious global public health issue that is expected to worsen during the next ten years in many developing countries [2, 6]. Osteoporotic fractures account for 0.83% of non-communicable disease burden worldwide [3, 7]. Osteoporosis is referred to as a "silent" disease because it often goes unnoticed until a bone is broken. Osteoporosis majorly causes fractures in postmenopausal women and older men. The incidence and burden of osteoporosis are expected to rise in the coming years, due mostly to population ageing [2, 8].

Over 50 million of people in India have osteoporosis or decreased bone density, yet barely 10 to 15 % are aware of the problem. Globally, postmenopausal osteoporosis in women has been recognised as a persistent health issue. The osteoporosis incidence among Indian women varies from ≈ 8% to 60%. In developed countries, the lifetime risk of osteoporotic fractures is ≈ 30% to 40%, nearly equivalent to the risk of coronary heart disease. Similar to Western populations, the incidence of fractures in India is ≈ 17.9% (≈ 18.8% in men and ≈ 17.1% in females). In the present Indian population, the annual number of hip fractures will be more than 440,000, with a female to male ratio of roughly 3:1, and a predicted incidence of more than 1 million in 2050 [9].

Fractures put an important burden on healthcare resources. By 2025, osteoporosis will account for approximately 3 million fractures and a $25.3 billion yearly expense in the United States [10-13]. Due to a lack of data, there is little information available on the direct economic impact of various osteoporotic fractures in the Indian population at risk.

2.1.1. Bone organization:

Bone is a porous mineralized structure composed of cells, vessels, and calcium compound crystals (hydroxyapatite) and their proportion varies from bone types and regions [14]. Genes control the processes of cellular differentiation that give rise to the skeleton, which first establish the pattern of skeletal structure in the form of

cartilage and mesenchyme and subsequently replace them with bone via osteoblast development [15, 16].

The structural components of bone consist of extracellular matrix (largely calcified), collagen, and cells [17]. Cortical bone and trabecular bone are the two types of bones in a human skeleton [18]. The structure of healthy bone is shown in figure-2.1.

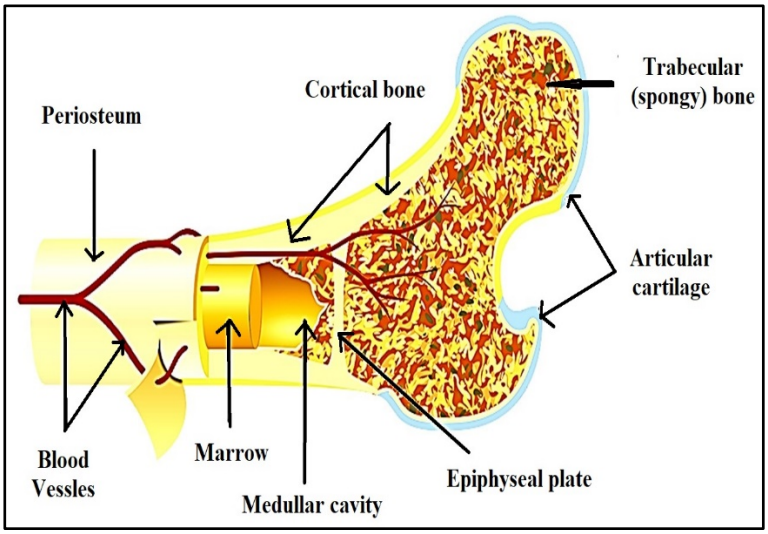


Figure-2.1. Structure of Healthy bone.

a. Cortical bone:

Cortical bone covers up 80% of the skeleton. It is dense and compact, has a slow turnover rate, and is resistant to bending and twisting. It is also the outer part of all skeletal structures. Most of the cortical bone is hardened, and its job is to provide mechanical strength and protection. It can also take part in metabolic responses, especially when there is a severe or long-term lack of minerals [18, 19].

b. Trabecular bone:

Trabecular bone covers up 20% of the total mass of the skeleton, but 80% of the bone surface is inside the long bones, like the vertebrae, the pelvis, and other large flat bones. Trabecular bone is less dense, more elastic, and has a higher turnover rate than cortical bone exhibiting a major metabolic function. Trabecular bone provides mechanical support, especially in bones like the vertebrae, and is the first source of mineral when there is an acute mineral deficiency [18, 19].

2.1.2. Bone matrix:

Around 90% of the total bone tissue is made up of type I collagen fibres, which are composed of two a1 chains and one a2 chain. Noncollagenous proteins also make up a significant portion of the bone matrix. Hydroxyapatite crystals [3Ca3(PO4)2(OH)2] are found on collagen fibres, inside them, and in the matrix, and are orientated in the same direction as the collagen fibres. The main noncollagenous protein produced includes osteocalcin (Gla protein), which is involved in calcium binding, matrix stability of hydroxyapatite, and bone formation control. Gla protein seems to be a negative regulator of bone development, inhibiting early or incorrect mineralization. Biglycan, a proteoglycan, on the other hand, is expressed in the bone matrix and positively promotes bone growth [20-22].

There are various cells presents in bone matrix as follows:

1. Osteocytes:

   Osteocytes are osteoblasts that have been confined in the osteoid. Microfilament-rich long cell processes that are organised during the formation of the matrix and prior to its calcification are found in osteocytes. The whole bone matrix is permeated by a network of very tiny canaliculi. Morphology and osteocyte functional activity varies with cell age. Newborn osteocytes have many of the same structural features as osteoblasts, but their cell volume and ability for protein synthesis are less. Further inside the calcified bone, an older osteocyte exhibits a further reduction in cell volume and a build-up of glycogen in the cytoplasm. When osteoclastic bone resorption occurs, the osteocytes are eventually phagocytosed and consumed. Despite the complexity of the osteocytic network's organisation, the precise function of these cells is unknown. It is likely that osteocytes respond to bone tissue strain and enhance bone remodelling activity by recruiting osteoclasts to sites where bone remodelling is required [17, 23-25].

2. Osteoblasts:

   Differentiate from mesenchymal stem cells, however they may also come from bone lining cells and perhaps chondrocytes. When they are active, they contain a large Golgi apparatus and endoplasmic reticulum, which are required for fast

osteoid formation. Osteoblasts may differentiate into three types of cells: bone lining cells, osteocytes, or apoptosis. The major role of these cells is secreting a matrix rich in type I collagen and control matrix mineralization [26, 27].

Bone formation involves three stages: the synthesis and maturation of osteoid matrix, followed by matrix mineralization. These processes run simultaneously in healthy adult bone, balancing matrix synthesis and mineralization at the same pace. The insulin-like growth factors (IGF), platelet-derived growth factors (PDGF), basic fibroblast growth factors (bFGF), transforming growth factor-beta (TGF-), and bone morphogenetic proteins (BMP) are only some number of the growth factors which osteoblasts release in response to different stimuli. Osteoblasts first produce osteoid through rapidly deposition of collagen. The rate of mineralization then rises to meet the rate of collagen production. The rate of collagen synthesis slows down and mineralization keeps on until the osteoid is entirely mineralized in the final stage. These growth factors, whose receptors have been identified on osteoblasts, influence osteoblast activity in an autocrine and paracrine way [17, 23, 27, 28].

3. Osteoclasts:

It is a multinucleated cell created by the fusion of monocyte/macrophage lineage precursors. Osteoclasts may more easily adhere to the surface of the bone thanks to podosomes, and the development of a sealing zone creates an enclosed, acidic milieu in which they can dissolve minerals and break down the bone matrix. These cells are more responsible for bone resorption. Integrins produced by osteoclasts bind to certain amino acid sequences in proteins on the surface of the bone matrix to form the attachment process between the osteoclast and the bone surface. Avb3 integrin binding triggers cytoskeletal remodelling inside the osteoclast after osteoclast attachment to the bone matrix. Podosomes, which are dynamic structures, are often used for attachment. They enable osteoclast mobility over the surface of the bone, which is when bone resorption takes place, by their ongoing assembly and disassembly. Several adhesion kinases, including as the proto-oncogene src, are required for integrin signalling and subsequent podosome formation. The major role of osteoclast is production of hydrochloric acid which

dissolves bone mineral, while proteolytic enzymes, notably cathepsin K, degrade bone matrix [17, 23, 29].

2.1.3. Pathophysiology of Osteoporosis

Skeletal fragility may occur because of: a. failure to generate a skeleton of adequate mass and strength during development; or b. excessive bone resorption, which results in reduced bone mass and microarchitectural degeneration of the skeleton. c. An insufficient response to increasing resorption during bone remodelling [30]. The structure of normal bone and osteoporotic bone is shown in figure-2.2.

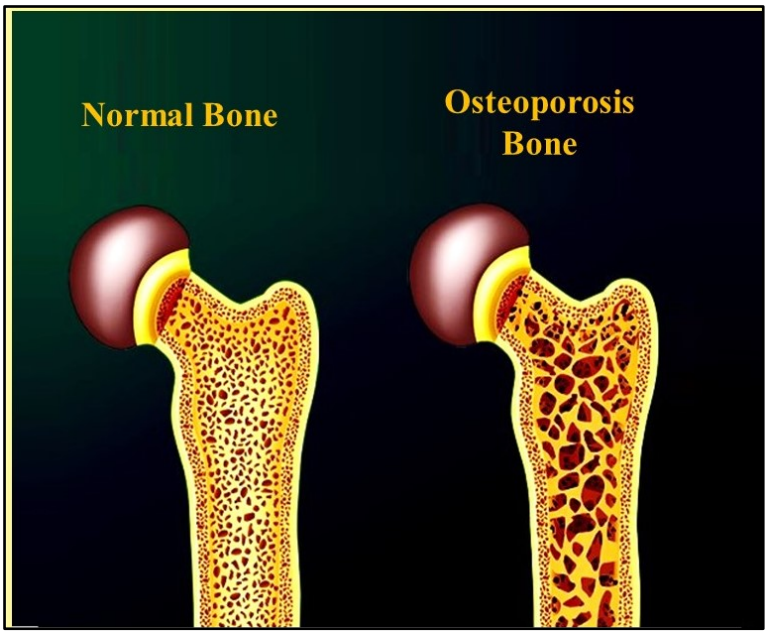


Figure- 2.2. Difference between structure of normal and osteoporosis bone

The bone remodelling process is based on the coordinated activity of bone-resorbing cells (osteoclasts) and bone-forming cells (osteoblasts). Osteoblasts develop from mesenchymal stem cells, as do many other cell types including adipocytes, chondrocytes, fibroblasts, and myoblasts. On the other hand, osteoclasts come from the hematopoietic mononuclear lineage [31, 32].

The two cytokines receptor activator of nuclear factor-kB ligand (RANKL), which is a member of the tumour necrosis factor (TNF) superfamily, and macrophage colony-stimulating factor (M-CSF), which is mostly generated by bone marrow stromal cells and osteoblasts, are crucial for osteoclastogenesis. The mononuclear osteoclast precursors express RANK, which is the RANKL receptor. Osteoprotegerin (OPG), a naturally occurring decoy receptor for RANKL that is also generated by stromal cells

and osteoblasts, counteracts the osteoclastogenic effects of RANKL. Considering their potential for treating osteoporosis, these interactions are actively being studied [32-34]. The pathophysiology of osteoporosis is shown in figure-2.3.

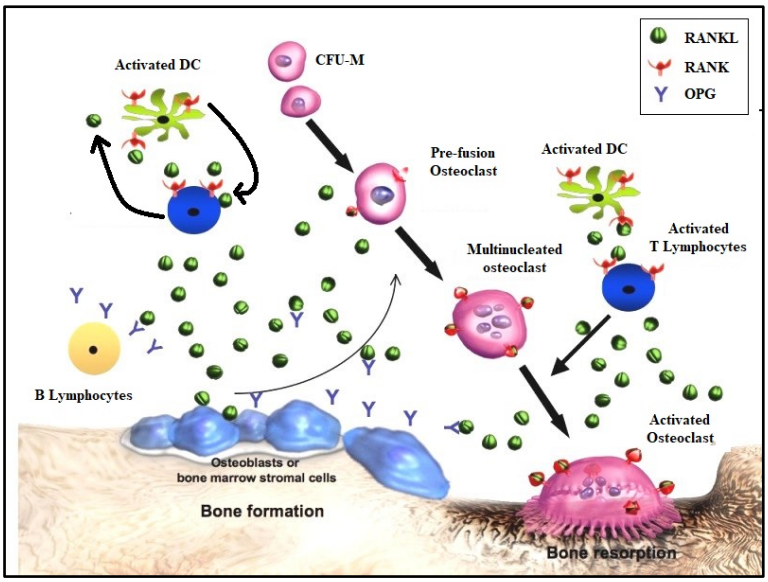


Figure- 2.3. Pathophysiology of osteoporosis

Bone loss in women occur due to decrease in serum concentration of estradiol. This deterioration is accompanied by a period of rapid and increasing bone loss. Menopause-related oestrogen deficiency causes an increase in both bone resorption and formation, with resorption exceeding formation. Yet, whereas bone resorption rises by 90% when compared to premenopausal levels, bone formation only increases by 45%, which is consistent with the net bone loss that occurs. An efflux of calcium from the resorbed bone mineral into the extracellular fluid is associated with this net effective increase in bone resorption, requiring physiologic compensation to avoid the development of hypercalcemia. Reduced renal calcium reclamation, decreased intestinal calcium absorption, and decreased parathyroid hormone release are compensatory strategies. These alterations result in a net negative whole-body calcium balance, resulting in skeletal demineralization. Moreover, oestrogen restoration to physiologic concentrations leads in the maintenance of renal calcium reabsorption and intestinal calcium uptake, indicating that these compensatory mechanisms seem to be a direct consequence of the substantial fall in circulating oestrogen concentrations [35, 36].

Unlike women, men undergo a natural, age associated and blunt decline in serum concentration of testosterone. In addition, since testosterone may be converted to

oestrogen via aromatization, bioavailable oestrogen concentrations decline by 50 % with age leading to bone resorption [35, 37, 38].

2.1.4. Risk factors/Causes of Osteoporosis:

Many risk factors that lead to osteoporosis are categorised as either non-modifiable or modifiable. Risk involved variables include gender, age, race, and genetics. The menopause-related oestrogen insufficiency significantly contributes to the development of osteoporosis. While the average age of menarche in Indian girls is 12.5 years, the average age at menopause is 46.2 years, which is younger than in non-Indian women [39]. This is a major risk factor for the development of osteoporosis in Indian women [40]. Moreover, genetic factors, race, and ethnicity have a significant impact on maximal bone mass attainment. There is evidence that Asian Indian women have 5–15% less bone mineral density (BMD) than non-Asian women. In addition, mutations in the vitamin D receptor gene have been hypothesised to contribute to the racial disparities in BMD [9]. Women have a smaller body frame size and, for cultural or secular reasons, are more likely to consume less calcium-rich foods and get insufficient sunlight exposure in developing countries like India [41]. In males, hypogonadism is a major cause of osteoporosis. According to a recent study, patients with the lowest amount of plasma bioavailable testosterone had a 2.5 times higher risk of non-spinal fractures than those with the highest concentration of testosterone [42, 43]. It's also important noting that oestrogen is essential for men's bone strength. Men lacking aromatase, the enzyme that turns testosterone into oestrogen. Low bone mass or osteoporosis treatment with oestrogen restores the abnormalities [44-46].

The modifiable risk factors include different nutritional factors like calcium and vitamin D, nutritional status as well as other lifestyle factors such as low physical activity, lack of exercise, etc. Calcium and vitamin D are two major nutritional contents for better bone strength and plays an important role in causing the risk of osteoporosis [9]. Calcium is deposited in bone matrix in the form of hydroxyapatite crystals which is responsible for bone hardness. Dairy products have a higher calcium content than non-dairy products. The requirement of daily intake for calcium through diet is 600 mg/day [47, 48]. According to most studies, Indian diets fail to nourish adult one with the recommended dietary limit of 600 mg/day of calcium [9, 40, 48, 49]. The human skin synthesizes vitamin D when exposed to sunshine. Among the

causal factors of deficiencies in vitamin D among Indians include indoor lifestyle, traditional clothing that reduces skin contact to sunlight (saris, salwar kameez, etc.), insufficient nutritional intake, inadequate vitamin D fortification of foods, darkly coloured skin, and air pollution. A deficiency in vitamin D inhibits calcium absorption from the intestines, which in turn impacts bone mineralization [9, 40, 50, 51].

In India, poor nutritional status is also major cause of risk of osteoporosis. Bodyweight less than 60 kg raises the risk of osteoporosis in women substantially [52]. Other lifestyle factors such as lack of exercise, low physical activity etc plays an important causal factor in risk of osteoporosis. A sedentary lifestyle and less exposure to sunshine (sometimes from traditional clothing use) in urban area leads to risk of osteoporosis. cigarette smoking and heavy alcohol consumption are also one of the causal factors for risk of osteoporosis [40, 53].

The long-term usage of over-the-counter glucocorticoids [54] and infrequent use of hormone replacement therapy (HRT) [12] are significant contributors to osteoporosis. Furthermore, osteoporosis and low BMD have also been linked to the usage of proton pump inhibitors [55] and anticonvulsants [40, 56].

2.1.5. Types of osteoporosis:

Osteoporosis can be divided into two major types based on factors affecting bone mineralization as follows:

a. Primary osteoporosis:

The primary osteoporosis is further divided into two subgroups as follows:

1. Involutional Osteoporosis Type I:

   It is brought on by a lack of estrogen. Because of this, it primarily affects the trabecular bone and affects women more than men, as shown by the men/women ratio of 4/5.7 [1, 57, 58].

2. Involutional Osteoporosis Type II:

   It is also known as senile osteoporosis occurring in senescent males and females (>70 years), and it is associated with bone mass loss owing to cortical and trabecular bone ageing [1, 57-59].

b. Secondary osteoporosis:

Many associated conditions and/or drugs may result in secondary osteoporosis. Osteoporosis-related diseases often include physiological functions including calcium, vitamin D, and sex hormones that are out of balance. For example, it has been discovered that Cushing's disease accelerates bone loss by generating too many glucocorticoids. Moreover, several inflammatory disorders, such as rheumatoid arthritis, may need long-term glucocorticoid therapy and have been linked to secondary osteoporosis. Interestingly, glucocorticoids are among the most often associated drugs to drug-induced osteoporosis. Secondary causes of osteoporosis were attributed to 32.4% of women, most often hypercalciuria, malabsorption of calcium, hyperparathyroidism, vitamin D insufficiency, hyperthyroidism, Cushing's disease, and hypocalciuric hypercalcemia. Notably, calcium metabolism problems and hyperparathyroidism accounted for 78% of the secondary causes [1, 60-62].

c. Idiopathic juvenile osteoporosis:

There are numerous kinds of idiopathic osteoporosis that can affect both children and adolescents, although it is uncommon. Juvenile osteoporosis affects previously healthy children between the ages of 8 and 14, impairing bone development over a period of many years. Patients with mild or moderate types of the condition may develop a curvature of the spine (kyphosis) and low height, whilst those with severe forms of the disease may be permanently disabled [63].

2.1.6. Bone remodelling Process:

Old bone is continually replaced by new tissue via the complicated process of bone remodelling, which is influenced by several biochemical and mechanical variables and necessitates interaction between various cell phenotypes [31]. The process of remodelling involves the replacement of bone, which maintains the skeleton's size, form, and quality. The skeletal system is made up of cartilage and bones and has two basic purposes. The first is a structural role that includes bone marrow support and protection, as well as the connection of muscles for motility. Second, by helping to buffer variations in hydrogen ion concentration, the skeleton serves as a store of calcium and phosphate required for the maintenance of serum homeostasis. In order to

do this, the structure is altered in response to stress and other biomechanical factors and microfractures are repaired [20, 23, 64].

Bone is a living organ that changes during its existence. Resorption and formation are maintained in a homeostatic equilibrium so that old bone is continually replaced by new tissue, allowing it adapt to mechanical stress and strain [65, 66]. Osteoclasts and osteoblasts work closely together in remodelling process and it is known as a basic multicellular unit (BMU). The BMUs are organised differently in cortical and trabecular bone and they differ morphologically rather than biologically [17, 67, 68]. The BMU burrows through cortical bone at a rate of 20-40 µm/day, forming a cylindrical canal approximately 2,000 µm long and 150-200 µm broad [64, 69]. Throughout a cycle, ten osteoclasts excavate a circular tunnel in the prevailing loading direction, which is eventually filled by thousands of osteoblasts. Because of its higher surface-to-volume ratio, trabecular bone is more actively remodelled than cortical bone [70]. Approximately, 2% to 5% of cortical bone is being remodelled every year. Osteoclasts move over the trabecular surface at a rate of around 25 µm/day, excavating a trench 40-60 µm deep [71, 72].

The remodelling cycle is divided into three stages: resorption, reversal, and formation. When partly differentiated mononuclear preosteoclasts move to the surface of the bone, they give rise to multinucleated osteoclasts, which signal the start of resorption. There is a reversal phase after the end of osteoclastic resorption, during which mononuclear cells develop on the surface of the bone. These cells indicate osteoblast differentiation and migration and prepare the surface for future osteoblasts to start bone formation. Osteoblasts then begin to lay down bone during the formation phase, replacing all of the resorbed bone with brand-new bone. After the completion of this stage, the surface is coated with flattened lining cells, and a lengthy period of rest lasts until a new remodelling cycle is started. The lengths of the various phases in the remodelling cycle vary. The duration of resorption is most likely 2 weeks, followed by a reversal phase that might take up to 4 or 5 weeks and then 4 months of formation until the new bone structure unit is fully formed [28, 64, 73].

The precise molecular mechanism for the interaction of osteoblastic and osteoclastic lineage cells been discovered. The activation phase of the bone remodelling cycle is mediated by cells of the osteoblast lineage. The osteocytes, lining cells, and

preosteoclasts' in the marrow may all be activated. There is still some uncertainty about the precise osteoblast lineage cells that are accountable. These cells undergo morphological changes, release enzymes that break down proteins on the surface of the bone, and produce receptor activator of NF-kappa B ligand, a 317 amino acid peptide that belongs to the tumour necrosis factor (TNF) superfamily (RANKL). The receptor RANK on the progenitors of osteoclasts interacts with RANKL. Hematopoietic cells of the osteoclast lineage are activated, differentiated, and fused by the RANKL/RANK interaction to start the resorption process. Additionally, by inhibiting apoptosis, it also extends osteoclast survival. This interaction shows that RANKL, among other things, is a link between bone resorption and bone formation [20, 28, 73, 74].

2.1.7. Treatment of osteoporosis:

While pharmacologic therapy is most often associated with osteoporosis, it is important to remember that nonpharmacologic treatment to reduce fracture risk are equally necessary for optimum osteoporosis treatment. Nonpharmacologic interventions include lowering the risk of falling, using proper lifting techniques, getting enough calcium, vitamin D, and protein, getting enough weight-bearing physical activity and exercise to maintain or improve balance and posture, and making appropriate lifestyle changes, such as quitting smoking and drinking less alcohol [4, 75]. In addition to pharmaceutical intervention, these therapeutic adjuncts should be considered with patients. There are various therapies/drugs available for treatment of osteoporosis as follows:

1. Selective Estrogen Receptor Modulators (SERM's):

   The use of pharmacologic oestrogen dosages in postmenopausal women was demonstrated to have antiresorptive effects on the skeleton and to prevent bone loss. According to bone histomorphometry, oestrogen may potentially have local anabolic effects. Raloxifene, lasofoxifene, bazedoxifene, tamoxifen etc. are approved selective oestrogen receptor modulators (SERM) for the treatment and prevention of postmenopausal osteoporosis. Notably, the US Food and Drug Administration (FDA) has approved the third-generation SERM bazedoxifene in

conjunction with conjugated oestrogens for the prevention of postmenopausal osteoporosis [76-79].

2. Bisphosphonates:

Bisphosphonates are chemically stable inorganic pyrophosphate equivalents with an extraordinarily high affinity for the mineral component of bone, hydroxyapatite [80]. Because of this feature, bisphosphonates may attain large local concentrations inside the skeleton. Due of this strong bone tropism, bisphosphonates have substantial pharmacologic effects on skeletal illnesses characterised by accelerated or unbalanced bone remodelling, as happens commonly with bone loss [80-82].

First generation non-nitrogen containing bisphosphonates such as clodronate, etidronate, tiludronate, and other function by becoming incorporated into nonhydrolyzable adenosine triphosphate (ATP) analogues. These bisphosphonate-containing ATP analogues become cytotoxic after osteoclast-mediated endocytosis from the bone surface, most likely due to inhibition of various ATP-dependent cellular processes, resulting in osteoclast apoptosis [83, 84].

Nitrogen-containing side chains have been found in all second- and third-generation bisphosphonates (alendronate, risedronate, ibandronate, and zoledronate). These second-generation bisphosphonates bind to hydroxyapatite mineral in bone even more tightly. Furthermore, after osteoclast endocytosis, they induce osteoclast apoptosis by inhibiting farnesyl pyrophosphate synthase, a mechanism distinct from that of first-generation bisphosphonates (FPPS). The inhibition of FPPS inhibits the post-translational lipid modification of small guanosine triphosphate-binding proteins within osteoclasts, resulting in osteoclast apoptosis. This inhibition of osteoclast function is most monitored clinically through longitudinal assessment of changes in BMD or more proximally through evidence of a decrease in serum or urine biochemical markers of bone resorption [84-91].

Because of their hydrophilicity, bisphosphonates are less than 1% bioavailable orally [92]. Bisphosphonates have variable hydroxyapatite mineral binding

potency, resulting in variable binding-site availability, bone turnover effect, and FPPS inhibition efficacy [93].

Different bisphosphonates used in the treatment of osteoporosis with their potencies are as shown in table- 2.1.:

Table 2.1 Bisphosphonates with their relative potency [94-97]

Bisphosphonate	Relative potency (to etidronate)
Non-Nitrogen containing bisphosphonates	
Etidronate	1
Clodronate	10
Tiludronate	10
Nitrogen containing bisphosphonates	
Pamidronate	100
Neridronate	100
Alendronate	100-500
Olpadronate	500
Ibandronate	500-1000
Risedronate	2000
Zoledronate	5000

Potential limitations associated with Bisphosphonate Therapy:

Short-term risks with oral bisphosphonates include upper gastrointestinal intolerance with indigestion, abdominal pain or nausea; acute-phase reactions with arthralgias, myalgias and fever after intravenous bisphosphonate infusion; severe chronic musculoskeletal pain; hypocalcaemia, which may occur primarily after intravenous bisphosphonate infusion; and ocular inflammation requiring ophthalmologic referral [35, 92].

3. Calcitonin:

Calcitonin is a peptide that inhibits bone resorption by reducing osteoclast activity by acting on the osteoclast calcitonin receptor. It is generated by thyroid C cells.

Calcitonin is weaker antiresorptive agent than other therapies. During the time of calcitonin treatment, calcium and vitamin D supplements are important. The USFDA has approved calcitonin preparations for Paget's disease, hypercalcemia, and osteoporosis in women over five years of menopausal. The European Medicines Agency (EMA) has revoked the osteoporosis indication for calcitonin owing to an increased risk of carcinomas [81, 98-101].

4. Strontium Ranelate:

   The first anti-osteoporotic drug that exhibits both an increase in bone formation and a decrease in bone resorption, leading to the development of new bone, is strontium ranelate. Strontium ranelate is recognised to be helpful in diverse patient profiles, from early postmenopausal women and osteopenic individuals to elderly women over the age of 80 years in lowering the risk of vertebral as well as non-vertebral fractures [1, 100-102].

5. Statins:

   Recently, several studies reported the use of statins in osteoporosis treatment, which is associated with increased bone formation. Statins are HMG-CoA reductase inhibitors and seem to generate their anabolic effect by boosting the synthesis of bone morphogenetic protein-2, hence enhancing bone growth. In studies on animals, statins administered locally or systemically increased bone mass. Like bisphosphonates, statins also inhibit osteoclast activity by blocking the farnesyldiphosphate synthase in the mevalonate pathway [103, 104]. In vitro research shows that statins simultaneously decrease osteoclast activity, prevent osteoblast apoptosis, and increase osteoblast activity. The resulting osteogenic stimulation is one of numerous pleiotropic effects that have been described with statin treatment. Statins are believed to be responsible for this combined antiresorptive/osteoanabolic effect. Commonly prescribed hydrophilic statins are Rosuvastatin, Pravastatin, Pitavastatin and Fluvastatin; while, commonly prescribed lipophilic statins are Atorvastatin, Lovastatin and Simvastatin [105-108].

2.2. Risedronate Sodium (RSNa):

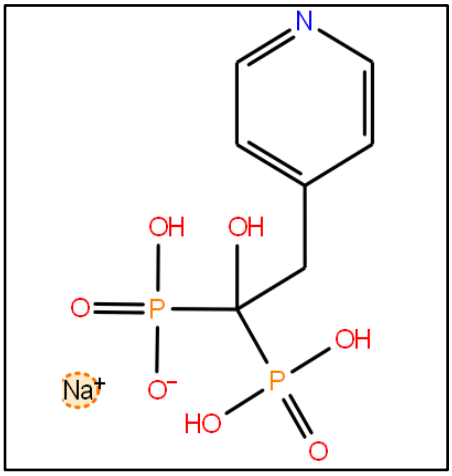
As a heterocyclic orally active nitrogen containing bisphosphonate (aminobisphosphonates), risedronate is a member of the bisphosphonate class of drugs [109]. Recent studies have shown that risedronate may reduce the resorption of both trabecular and cortical bone, affecting both the thickness and porosity of the cortical bone, factors that are major contributors to femoral fracture, particularly in older individuals [110]. RSNa is available in market with brand name of Actonel, STADA, Risofos, etc. in the dosage form of tablet. The dose of RSNa is 5 mg/day, 35 mg/week or 150 mg/month. But the oral bioavailability of RSNa is very low i.e., less than 1% with several GI related side effects [111, 112].

2.2.1. Risedronate sodium profile:

Risedronate sodium is fine white to off-white crystalline powder. The profile of risedronate sodium is shown in table-2.2.

Table- 2.2. Drug profile of risedronate sodium (RSNa)

Synonyms	- Risedronate sodium monohydrate - Risedronate sodium hydrate - Risedronic acid monosodium salt monohydrate
Molecular Formula	$C_7H_{12}NNaO_8P_2$
Molecular Weight	323.11 gm/mol
Log P	-3.3
Bioavailability (Oral)	0.6%
Melting point	252-262 °C [113]
Solubility	Highly soluble in water and insoluble in many organic solvents [114].



Structure of RSNa

2.2.2. Pharmacokinetics:

Absorption:

Peak absorption after an oral dosage occurs at 1 hour (Tmax) and occurs across the upper gastrointestinal system, according to simultaneous modelling of serum and urine data. Across the dosage range studied (single dose, 2.5 mg to 30 mg; repeated dose, 2.5 mg to 5 mg), the proportion of the dose absorbed is dose independent. Within 57 days of regular dosage, steady-state serum conditions are observed [90, 115].

Food Effect:

When administered 0.5 hours before breakfast, the extent of absorption of RSNa is reduced by 55% when compared to dose when fasting (no food or drink for 10 hours prior to or 4 hours after dosing). Dosing 1 hour before breakfast reduces absorption by 30% when compared to dosing when fasting. Dosing 0.5 hours before breakfast or 2 hours after dinner (evening meal) results in similar absorption [115, 116].

Distribution:

In humans, the mean steady-state volume of distribution for risedronate is 13.8 L/kg. Drug binding to human plasma proteins is around 24%. Preclinical studies in rats and dogs given single doses of [14C] risedronate intravenously show that approximately 60% of the dosage is delivered to bone. The balance of the dosage is eliminated through the urine. Risedronate absorption in soft tissues was in the range of 0.001% to 0.01% after repeated oral dosing in rats [90, 115].

Metabolism:

There is no evidence of risedronate systemic metabolism.

Excretion:

About half of the absorbed dosage of risedronate was eliminated in urine within 24 hours in young healthy volunteers, and 85% of an intravenous dose was recovered in urine over 28 days. According to simultaneous modelling of serum and urine

data, mean renal clearance was 105 mL/min (CV = 34%) and mean total clearance was 122 mL/min (CV = 19%), with the difference representing mostly nonrenal clearance or clearance attributable to bone adsorption. There is no concentration dependence in renal clearance, and there is a linear connection between renal clearance and creatinine clearance. Unabsorbed drugs are excreted unaltered. The terminal exponential half-life in osteopenic postmenopausal women was 561 hours, the mean renal clearance was 52 mL/min (CV=25%), and the mean total clearance was 73 mL/min (CV=15%) [90, 115].

2.2.3. Pharmacodynamics:

Risedronate sodium therapy reduces the elevated rate of bone turnover and balances bone resorption and bone formation. RSNa treatment helps to Improve Bone Mineral Density: [117, 118].

2.2.4. Drug Interaction:

There were no specific drug-drug interactions identified. Risedronate is not metabolised and has no effect on hepatic microsomal drug-metabolizing enzymes (e.g. Cytochrome P450). The absorption of RSNa is reduced by the co-administration of calcium, antacids, or oral drugs containing divalent cations [116].

2.2.5. Dose:

2.5 mg/day tablet orally (Actonel),
5 mg/day tablet orally (Actonel, Salost, Sedron, Cruzz etc.),
35 mg/week tablet orally (Actonel, Risofos, Gemfos, Risaldene etc.),
150 mg/month tablet orally (Actonel, Risedon etc.).

Table- 2.3. Literature Review on RSNa used in osteoporosis treatment

Author [Reference]	Formulation	Route of administration	Conclusion/observation
Ali et al. **[119]**	PLGA nanoparticles	Intranasal	The ex vivo study revealed enhancement of permeation of drug through nasal mucosa. An in vivo study revealed a significant change in the microstructure (trabeculae) of the bone internal environment. As compared to the non-treated group, biochemical estimation of the RSNa PLGA treated group revealed a significant recovery.
Jung et al. **[120]**	Chitosan coated liposomes	Oral	Comparable to the untreated medication, chitosan-coated liposomes significantly increased RSNa cellular uptake, resulting in a 2-fold increase in Caco-2 cells. In rats, chitosan-coated liposomes enhanced RSNa oral absorption by threefold.
Mukherjee et al. **[121]**	Thiolated chitosan-based mucoadhesive film	Buccal	The ex vivo permeation study's results revealed enhanced permeation of drug through buccal mucosa. The pharmacological investigation revealed that thiolated chitosan film containing RSNa reduced osteoclastic activity. The results indicate it is feasible to alter the bone microarchitectural characteristics associated with osteoporosis while also increasing the efficacy of treatment.

Rawat et al. **[122]**	Microcapsules	Oral	Authors prepared risedronate loaded three-ply walled microcapsules for enhancement of bioavailability. The prepared carriers sustained drug release upto 24 hrs. The results of in vivo study revealed that microcapsules enhanced oral bioavailability by 40 folds.
Cruz et al. **[123]**	Pullulan–Eudragit® S100 blend microparticles	Oral	Authors prepared risedronate loaded Pullulan–Eudragit® S100 blend microparticles by spray-drying technique. The results of in vitro release study showed prolonged drug release in intestinal fluid upto 480 min.
Nasr et al. **[124]**	Liposomes	Pulmonary	Authors reported that the risedronate sodium loaded liposomes can be successfully delivered to systemic circulation via pulmonary route. The results of in vivo bone deposition using radiolabelling technique revealed that 20% of administered dose was deposited in the rat bone.

2.3. Atorvastatin calcium (ATO):

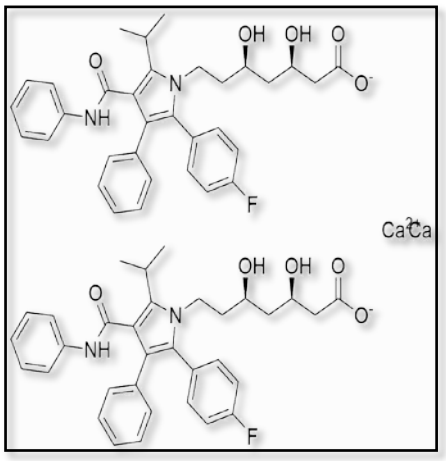
ATO is a specific inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, inhibited conversion of HMG-CoA to mevalonate in cholesterol synthesis [125]. The oral bioavailability of ATO is approximately 14 % due to presystemic clearance in the gastrointestinal mucosa and extensive hepatic first-pass metabolism [125].

2.3.1. Atorvastatin profile:

Atorvastatin calcium is white to off-white crystalline powder. The profile of atorvastatin is represented in table-2.4.

Table- 2.4. Drug profile of atorvastatin (ATO)

Synonyms	- Atorvastatin calcium trihydrate
Molecular Formula	$C_{33}H_{35}FN_2O_5$
Molecular Weight	1209.42 gm/mol
Log P	5.39
Bioavailability (Oral)	Approximately 14 %
Melting Point	159-161°C
Solubility	Very little soluble in distilled water, and phosphate buffer pH 7.4, somewhat soluble in ethanol, and freely soluble in methanol [126].



Structure of ATO

2.3.2. Pharmacokinetics:

Absorption:

After oral administration, atorvastatin is rapidly absorbed; peak plasma concentrations reach within 1 to 2 hours. The extent of absorption rises in direct proportion to the dosage of atorvastatin. The absolute bioavailability of atorvastatin (parent medication) is around 14%, but the systemic availability of HMG-CoA reductase inhibitory action is about 30%. Presystemic clearance in the gastrointestinal mucosa and/or hepatic first-pass metabolism are responsible for the poor systemic availability [127, 128].

Distribution:

The mean volume of distribution for atorvastatin is 381 litres. Approximately 98% of atorvastatin is bound to plasma proteins. A blood/plasma ratio of about 0.25 suggests that the drug is not getting into the red blood cells. According to rat studies, atorvastatin is probably found in human milk [127, 128].

Metabolism:

Atorvastatin undergoes extensive metabolism to produce a variety of beta-oxidation compounds as well as ortho- and parahydroxylated derivatives. Ortho- and parahydroxylated metabolites had the same inhibitory effect on HMG-CoA reductase in vitro as atorvastatin. Active metabolites are responsible for around 70% of the circulating HMG-CoA reductase inhibitory activity. A rise in plasma concentrations of atorvastatin in people following coadministration with erythromycin, a known inhibitor of this isozyme, is consistent with in vitro studies suggesting the significance of cytochrome P450 3A4 metabolism of atorvastatin [127, 128].

Excretion:

After hepatic and/or extra-hepatic metabolism, atorvastatin and its metabolites are predominantly removed in the bile; however, the drug does not appear to travel via enterohepatic recirculation. The half-life of atorvastatin's mean plasma elimination in humans is around 14 hours, but because of the presence of active metabolites, the inhibitory activity for HMG-CoA reductase has a half-life of 20 to 30 hours. After oral treatment, less than 2% of a dosage of atorvastatin is recovered in urine [127, 128].

2.3.3. Food interaction:

Blood levels of atorvastatin might increase after consuming grapefruit juice [129]. Food decreases the rate and extent of drug absorption by approximately 25% and 9%, respectively. Plasma atorvastatin concentrations are lower (approximately 30% for Cmax and AUC) following evening drug administration compared with morning [129, 130].

2.3.4. Dose:

10, 20, 40, and 80 mg/day tablet orally (Lipitor, Atorwel, Satvastin, Lipvas etc.).

Table- 2.5. Literature review on ATO used in treatment of osteoporosis

Author	Formulation	Route of administration	Conclusion/observation
Salem et al. **[131]**	Tablet	Oral	Authors performed pharmacodynamic study on ovariectomized model with dose of 20mg/kg of ATO. The results revealed that administration of atorvastatin resulted in a significant decrease in the levels of these bone metabolic markers. The results showed decrease in bone resorption and increase in bone formation rate.
Xie et al. **[132]**	Micelles with bone targeted ligands	Parenteral	Authors prepared ATO loaded tetracycline-poly (ethylene glycol)-poly (lactic-co-glycolic acid) (TC-PEG-PLGA/ATO) micelles for the targeted treatment of osteoporosis. The drug release study showed sustained release from nanocarriers for more than 48 hrs. Pharmacokinetic studies showed that TC-PEG-PLGA micelles may successfully prevent ATO leakage from micelles and extend their circulation duration. TC-PEG-PLGA/ATO micelles have been effective in pharmacodynamic studies to increase the mechanical strength and bone mineral density of osteoporotic rats.
Shokrollahi et al. **[133]**	PLGA/bioceramic composite micro-	Injectable	Authors prepared hydroxyapatite coated atorvastatin loaded PLGA

particles	microparticles for enhancement of osteogenic activity. The enhanced S Alizarin Red staining/calcium deposition measurements on PLGA-AT-HAp confirmed strong osteogenic differentiation.

2.4. Transdermal Drug Delivery Systems:

Transdermal drug delivery systems (TDDS) are self-contained discrete dosage forms that, when applied to intact skin, transport the drug(s) into the systemic circulation at a predefined and predicable rate over a prolonged period of time through the skin portal [134]. Conventional administration techniques, such as intravenous or oral administration, are unable to deliver sufficient drug molecules to the target location, whereas almost all drugs are excreted or accumulate in nonspecific sites where the drug exhibits adverse effects [134-136]. TDDS have some advantages for patients, such as being completely non-invasive (some methods are less invasive), avoiding first-pass metabolism, being easy to apply and administer, not requiring expert personnel, and having the potential to reduce frequency of administration [137]. Furthermore, this DDS has been used to deliver several drugs, including both hydrophilic and hydrophobic molecules. Transdermal drug delivery can alter or penetrate the stratum corneum to improve drug absorption through the skin.

The **skin** is used as the drug administration site in transdermal drug delivery systems [138]. The skin, being the biggest organ, protects the body from external disturbances such as physical, mechanical, and chemical attack [139]. The human skin is a multi-layered organ made of three major sections, which are: (i) epidermis, (ii) dermis, and (iii) hypodermis [138].

The **epidermis,** in general, is made up of five sublayers: the stratum basale, the stratum spinosum, the stratum granulosum, the stratum lucidum and the stratum corneum (non-viable epidermis). The stratum corneum (SC), the epidermis's most superficial layer, has a thickness of 10-20 μm and is made up of 15-30 corneocyte cell layers. Every four weeks, this layer regenerates. The SC is composed of lipids, including ceramides (30–40%), cholesterols, cholesterol esters, free fatty acids, squalene, wax esters, and

triglycerides. It also composed of keratin proteins, which are derived from dead keratinocyte cells. The stratum corneum, the major barrier to the entrance of substances into the skin, is formed by this ordered structure, which is known as the "bricks and mortar" model. The stratum lucidum is made up of 2-3 layers of keratinocyte cells and is exclusively present in the digits, palms, and soles. In the stratum granulosum, the cells' membrane is thicker than the first two layers. The stratum spinosum, an epidermal layer underneath the stratum granulosum, is made up of 8-10 keratinocyte layers. Because of the presence of cell connectors, mainly desmosomes, connecting the cells, this layer is known as the 'spiny' layer. Finally, the stratum basale is the epidermal skin's deepest layer, which has direct contact with the dermis via interconnected collagen fibres [136, 137].

The **dermis** is the second layer of skin in the integumentary system, located underneath the epidermis. The dermis is divided into two layers: papillary and reticular. The dermis is around 2-5 mm thick. It is mostly made of collagen and elastin fibres in a mucopolysaccharide gel, which provides skin with strength and elasticity. The dermis contains several structures, including nerve endings, Meissner corpuscles (touch receptors), pilosebaceous units (hair follicles and sebaceous glands), and eccrine and apocrine sweat glands. Due to the presence of phagocytes, fibroblasts, leucocytes, and mast cells, Due to the presence of phagocytes, fibroblasts, leucocytes, and mast cells, the dermal layer plays important role in immunological function. This layer is metabolically active, has a large vasculature, delivers nutrients and oxygen to tissue, and takes role in waste removal. The dermis-epidermis barrier is where the blood supply enters, and the majority of molecules may be delivered throughout the circulatory system from here [136, 137].

The **hypodermis** is the deepest skin layer underneath the dermis. Since it serves as a connective tissue between skin, muscle, and bones, the hypodermis, also known as the subcutaneous layer or the superficial fascia, is abundant in proteoglycans and glycosaminoglycans. The hypodermis is a layer with lymphatic and blood capillaries that is well vascularized. Its primary roles include insulating and shielding the body from physical harm as well as storing energy in the form of lipids [137]. The structure of human skin is shown in figure- 2.4.

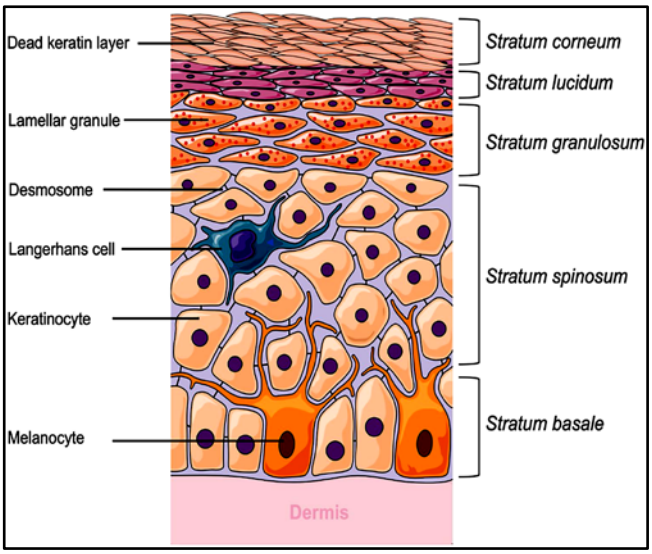


Figure- 2.4. Structure of human skin

2.4.1. Drug absorption via the skin:

Drug absorption from the skin via the SC can be divided into two routes: transepidermal and transappendageal. The preferred route will be determined by the physicochemical properties of the drug and the formulation type (e.g., nanoparticulate systems preferentially penetrate hair follicles). The primary and most important absorption route is known as transepidermal. Because of the SC's large surface area, drugs from transdermal patches can distribute over the skin's surface and permeate into cells (transcellular) or interspaces between cells (intercellular). Drugs diffuse through SC cells during absorption in the transcellular pathway. Drugs must thus cross the lipid bilayer–containing membranes. Because of the hydrophobic properties of the lipid complex in the cell membranes of the SC, hydrophobic drugs primarily adopt this route. The second pathway is intercellular, in which drugs must diffuse through the lipid matrix of residing keratinocytes in the SC's intercellular space. The intercellular route includes the permeant being partitioned inside the fibrous extracellular lipid matrix and then diffusing across it without passing through the corneocytes. This pathway transports hydrophilic drugs or small molecules to dermal vascular capillaries. Permeation through hair follicles (transfollicular route) or sweat ducts is included in the transappendageal or shunt route. This pathway is essential for the transportation of polar or ionisable substances and is helpful for the transportation of big macromolecules that have difficulty passing through epidermal cells because of their molecular size and

various partitioning characteristics. Nevertheless, due to the small absorption area (0.1% of the total skin surface) as compared to transepidermal route, the use of this route is relatively limited [136, 137]. The schematic representation of transdermal drug permeation mechanism is shown in figure- 2.5.

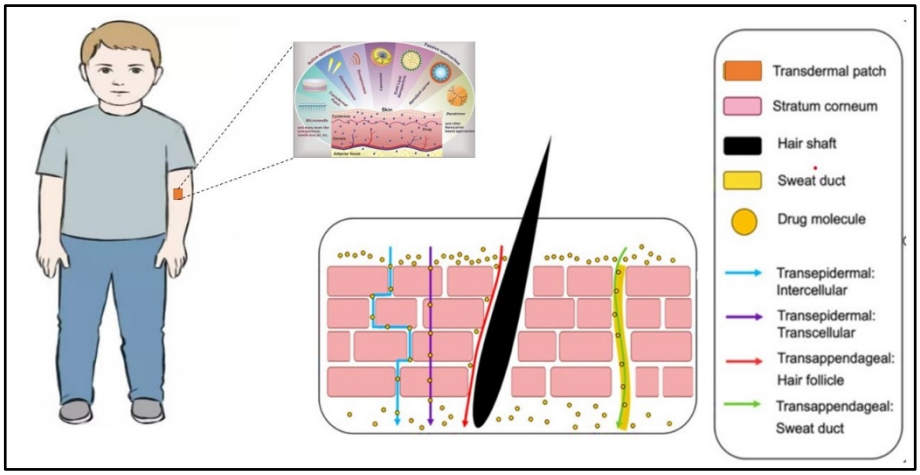


Figure- 2.5. Schematic diagram of pathways for drug absorption via the skin

2.4.2. Challenges for transdermal drug delivery:

Despite its attractiveness, the transdermal route for drug delivery poses several challenges that need to be overcome. Therapeutic agents must meet specific physicochemical criteria in order to enable passive transport through the skin such as log P, molecular weight, solubility, ionization, high pharmacological potency, and skin tolerability. Transdermal delivery is a suitable fit for molecules with an intermediate lipophilicity and a Log P value more than 2. Skin permeation and permeant size are inversely related; the optimal molecular weight must be less than 500 Da; bigger molecules, such as peptides and proteins, cannot cross the skin's barrier unless it is compromised. It is regarded as the overall driving force for permeation and is proportional to the tendency of molecules to escape from the vehicle. Because the stratum corneum is predominantly lipophilic, unionised compounds may have more drug penetration than ionised drugs. The drug's effective daily dosage should be about 10 mg or less. Because the skin, as an immunological barrier, can be irritated or sensitive to external stimuli such as drugs, excipients, or formulation components, irritation tests should be undertaken during formulation development [140-144].

As a consequence, investigators have found strategies to improve drug absorption across the skin by changing the structure of the SC chemically, physically, or by combining these methods.

2.4.3. Techniques for enhancing transdermal delivery:

Various enhancement approaches have been investigated in order to improve drug transdermal delivery described in detail below:

1. Chemical method:

a. Chemical penetration enhancers:

Modifying the barrier property of the stratum corneum using chemical penetration enhancers may result in better drugs penetration into the skin. An ideal chemical penetration enhancer should be pharmacologically inert, non-irritant, non-allergic, non-toxic, and cosmetically acceptable. Water, alcohols, amides, fatty acids, esters, ether alcohols, surfactants, sulphoxides and analogues, essential oils, terpenes, and their derivatives, pyrrolidones, oxazolidines, phospholipids, enzymes are some of the chemical compounds utilised as chemical penetration enhancers. Chemical penetration enhancers can act directly on the skin in three ways: (i) disorganisation of the intercellular lipid bilayer, (ii) interaction with intracellular proteins of the stratum corneum, and (iii) modification of the stratum corneum's solvent nature to improve drug partitioning into the tissue (iv) solvent penetration to the skin transporting the medication and (v) permeant solubilization in the donor [140, 145].

b. Vesicles:

The vesicular systems composed of water and amphiphilic molecules (phospholipids, non-ionic surfactants) that produce one or more concentric layers in which lipophilic or hydrophilic drugs can be encapsulated. Vesicles act as penetration enhancers following individual component penetration into the stratum corneum, creating a breakdown of the lipid bilayer and act as a depot to achieve sustained drug release. The different vesicular systems are liposomes, glycerosomes [146], niosomes, transferosomes, ethosomes, etc [140, 147].

c. Polymeric nanoparticles:

Polymeric nanoparticles are solid nanometric carriers composed of natural and synthetic polymers in a polymeric matrix. Some of the benefits of these systems include: simple and inexpensive fabrication, nontoxicity, higher stability than lipid nanocarriers, protection of unstable drugs, ability to release the therapeutic agent continuously to reduce side effects, and enhancement of transdermal drug permeation by increasing the concentration gradient. The drug permeation via nanoparticles either modifying skin permeability through hair follicles which allows drug permeation or positive charge nanoparticles can interact with negative charge surface resulting in an improved skin interaction [147, 148].

d. Other chemical methods:

Other colloidal systems such as solid lipid nanoparticles, nanostructured lipid carriers, microemulsions, use of cell permeation peptides and ion pair formation also enhance the skin permeation of drugs [140]. The SLNs are developed by mixing one or more solid lipids (at room temperature), stabilising them with a surfactant, and dispersing them in an aqueous phase [149]. On the other hand, NLC is composed of lipids which are both solid and liquid (oil), stabilised by a surfactant [150, 151]. The advantages of NLC and SLN include low toxicity, simple preparation method, and good biocompatibility [150, 151]. Due of their similarity to skin lipids, they have received extensive study as transdermal drugs carriers [150, 151]. A colloidal, optically isotropic, structured system comprised of surfactant, co-surfactant, oil, and water is referred to as a microemulsion [152]. They can spontaneously form, are thermodynamically stable, and have a low viscosity with Newtonian behaviour. Due to the large surface area and formation of small droplets, they may adhere to biological membranes and transport drugs [152, 153].

2. Physical methods:

The different physical methods, such as iontophoresis, the electroporation method, and radiofrequency ablation, help enhance the skin permeation of drugs. These methods also known as energy driven methods. Energy-driven or electrically assisted transdermal delivery techniques utilize electrical devices to

improve drug absorption via the skin [135, 145, 154]. The microneedle drug delivery system is a novel drug delivery technique that delivers drugs to the bloodstream through a needle. The strategy involves micron-sized needles to puncture the skin's outermost layer, causing drugs diffusion over the epidermal layer [155].

2.5. Transdermal drug delivery for treatment of osteoporosis:

Conventional administration methods for osteoporosis treatment have several disadvantages that can be overcome by delivering the therapeutic agents through the skin [156]. Several transdermal formulations are reported for treatment of osteoporosis as given in table- 2.4.

Table- 2.6. Strategies to enhance the transdermal permeation of drugs to treat osteoporosis

Drug	Formulation	Conclusion	Reference
Alendronate sodium	Self-dissolving microneedle arrays (MNs)	Bioavailability of ALN was enhanced 96% after administration by ALN loaded microneedles (ALN-MN). There was light erythema observed after 4 days application of ALN–MN. ALN(TIP)-MN exhibited rapid absorption of ALN as well as an effective osteoporosis preventive and therapeutic effect.	[157]
	Transdermal patch	The bioavailability of alendronate patch in rats was fivefold enhanced than oral administration. After the application of the alendronate patch, mild erythema of the rat skin was observed. In 1α-hydroxyvitamin D3-induced hypercalcemia model rats, the alendronate patch effectively reduced plasma calcium levels.	[158]
Risedronate sodium	Protransfersomal gel	Ex-vivo skin permeation study revealed that enhancement ratio was found to be 14.8 after Protransfersomal gel	[156]

		administration. The in-vivo study revealed that risedronate loaded protransfersome based formulation aided in restoring bone architecture and improving bone strength more efficiently in comparison to the marketed formulation.	
Raloxifene hydrochloride	Transfersomes	The in vivo pharmacokinetic study revealed that the relative bioavailability of raloxifene after application of transfersomes gel in Wistar rats was enhanced by 2.71 times as compared to the oral solution.	[159]
Fluvastatin	Nanoemulsion gel	Using the use of micro-CT scanning radiographs, in vivo anti-osteoporotic studies showed that new bone was being formed in the trabecular region of osteoporotic rat femurs. It also increased bone microarchitecture and load bearing capacity in osteoporotic rat femurs, showing its efficacy in osteoporosis treatment.	[107]
Lovastatin	Nanoemulsion gel	Application of NE gel formulations resulted in an increase in anabolic biomarker levels while significantly reducing resorptive biomarker levels. Micro-CT scans of the rat distal femur showed the development of new bone tissues in glucocorticoid-induced osteoporosis rats.	[108]

2.6. Nanocarriers:

The systemic availability of drugs can be improved by incorporating them into nanocarriers that can readily permeate and continuously release the drug near blood capillary networks. Glycerosomes and polyelectrolyte complex nanoparticles (PECN) were chosen as nanocarriers for this reason due to their excellent capability for transdermal delivery.

A. Glycerosomes:

Phospholipid nano-vesicular systems have sparked great attention, especially as a potential drug delivery system that can improve transdermal, dermal, and transmucosal absorption of various medicines and avoiding degradation in the GI tract and liver [160]. Glycerosomes, which are prepared from different phospholipids and high concentrations of glycerol, provide a novel strategy to improve liposome properties as dermal and transdermal drug delivery systems by altering liposomal bilayer fluidity [146]. Glycerosomes are flexible vesicular carriers that may contain various ingredients, such as cholesterol, that improve lipidic bilayer stability [161]. Glycerosomes have a great capacity to penetrate skin pores [162]. They can encapsulate both hydrophilic and hydrophobic drugs as they are made up of single or multiple lipid bilayers containing an aqueous compartment [160]. Glycerol incorporation in significant amounts improves the flexibility (deformability) of the liposomal lipid bilayer, improving vesicular penetration and absorption through skin [163]. Glycerol is a completely safe and acceptable short-chain alcohol that can enhance fluidity and deformability [146]. Glycerol appears to stabilise the vesicle dispersion, preventing aggregation or fusion, which occurs with liposome or other vesicular dispersions [162].

Table- 2.7. Literature review on glycerosomes.

Author [reference]	API used	Excipients used	Observations/conclusion
Zang et al. **[164]**	Paeoniflorin	Phospholipid, Cholesterol, Glycerol.	The in vitro transdermal flux of PF loaded in STO-glycerosomes was 1.4-fold, 1.6-fold, and 1.7-fold higher than that of glycerosomes, liposomes, and tinctures respectively. In vivo imaging studies confirmed this result, revealing that the fluorescence intensity of Cy5.5-loaded STO-glycerosomes in mice knee joints was 1.8-fold higher than that of glycerosomes 5 hours after administration.
Salem et al. **[161]**	Celecoxib and Cupferron	Soybean phosphatidyl choline, glycerol, Tween 80 and cholesterol.	The anti-inflammatory activity of CLX and CUP glycerosomal gel was evaluated using a carrageenan-induced rat oedema technique, which was followed by histological examinations. Celecoxib and Cupferron loaded glycerosomes achieved remarkable oedema inhibition as compared to the control group.
Manca et al. **[146]**	Diclofenac sodium	Dipalmitoylglycero-phosphatidylcholine, cholesterol, and glycerol.	Deformability index of both conventional liposomes and glycerosomes was evaluated and results showed that glycerol is able to act as edge activator for lipid bilayers when used in concentration higher than 10%.

Moolakkadath et al. **[165]**	Fisetin	Lipoid S 100, Cholesterol, and glycerol.	The confocal study confirmed that the glycerosomes incorporated Rhodamine B penetrated deeper layers of skin. The improved fisetin loaded glycerosomes gel formulation displayed zero-order release kinetics and a flux of 4.24 0.14 g/cm^2/h.
Badr-Eldin et al. **[163]**	Lacidipine	Phospholipon®90G, cholesterol and glycerol.	The ex-vivo skin permeation revealed that lacidipine loaded glycerosomes enhanced drug permeation by 3.65-fold compared to lacidipine suspension. A confocal laser scattering microscope revealed greater penetration depth in nasal mucosa (upto 60 µm). In comparison to oral drug solution, the optimised lacidipine glycerosomes significantly reduced methylprednisolone acetate-induced hypertension in rats for up to 24 hours.
Khallaf et al. **[160]**	Duloxetine HCl (DXH)	Phospholipon 90G, Tween 80, and glycerol.	As per pharmacokinetic studies, the DXH loaded glycerosomal in situ rectal gel enhanced DXH bioavailability by 2.24 times as compared to oral DXH solution. The DXH loaded glycerosomal rectal therapy significantly improved the behavioural analysis parameters and was more effective as an antidepressant than the oral DXH solution, according to the pharmacodynamic study.

Abd-Elsalam et al. **[166]**	Ganciclovir (GCV)	L-α-phosphatidylcholine, cholesterol, Sodium taurocholate, and glycerol.	A pharmacokinetic study in the rabbit's aqueous humour showed sustained release of GCV from optimal GCV-loaded glycerosomal occusert over a period of five days. The results of the confocal microscopy showed the higher penetrating ability of UFGs.
Maria et al. **[167]**	Rifampicin	Phospholipon®90G (P90G), cholesterol and glycerol with sodium hyaluronate (HY) or trimethyl chitosan chloride (TMC).	The in vitro cell line study's results revealed that rifampicin loaded glycerosomes reduced drug toxicity on A 549 cells and enhanced effectivity against *Staphylococcus aureus*. After intra-tracheal administration to rats, the in vivo biodistribution and accumulation were evaluated, and the result shows an improvement in rifampicin accumulation in the lungs.

B. Polyelectrolyte complex nanoparticles (PECN):

Nanoparticles are currently used extensively in delivery systems and have great potential in pharmacological, biological, and medicinal applications [168]. The self-assembly between positively and negatively charged polyelectrolytes that occurs between them without the use of an organic solvent, cross-linking agents, or high energy is known as polyelectrolyte complexation nanoparticles (PECN) [169]. The PEC nanoparticle formation innovation is popular because to its inexpensive cost, low energy requirements, and high drug loading capacity [168, 170]. PECNs promise for use as carriers of drugs has been significantly improved by the encapsulation of a drug during their formation [169]. In aqueous solutions, polyelectrolytes may partially or completely dissociate, making the polymers charged [170]. Most natural polysaccharides contain hydrophilic groups such as hydroxyl, carboxylic acid, and amino groups, which can form non-covalent

interactions with biological tissues resulting in bioadhesion. Chitosan (CS) a natural cationic polysaccharide, is an excellent bioadhesive polymer that has been widely investigated by the pharmaceutical and biomedical fields due to its strong biocompatibility, biodegradability, bioadhesive and cationic properties [171, 172]. Because of protonation of the amine functional group in the glucosamine unit, CS has a pKa of about 6.2-7.2 and is therefore readily soluble in an acidic environment [173]. The positive charge that emerges on CS allows for ionotropic gelation/coacervation with an anionic counterpart. The main principle of polyelectrolyte complex nanoparticles formation is based on ionic interaction between chitosan (as a polycation) with various natural polymers as polyanions, such as alginate, pectin, dextran sulphate (DS), hyaluronic acid (HA), chondroitin sulphate (CHON), and heparin. In present work, chondroitin sulfate (CHON) was used as polyanion for the preparation of PECN. CHON contains weak carboxylate and strong sulphate moieties linked to the major glycan backbone. CHON may form ionic compounds with cationic molecules because to its acidic nature [174]. In cartilage, bones, and connective tissues of mammals, chondroitin sulphate (CHON) is an unbranched polysaccharide that contains the alternating monosaccharides d-glucuronic acid and N-acetyl-d-galactosamine [175]. The primary glycan backbone of CHON has weak carboxylate and strong sulphate moieties linked to it [175]. It has biodegradability, low cost, high stability, flexibility to chemical change, and low toxicity. Additionally, because CHON is an anionic polyelectrolyte, it can form polyelectrolyte complexes through electrostatic interaction with materials that are oppositely charged [176] and gives it the advantage of being an ideal matrix for various biomedical applications due to their higher chemical and mechanical stabilities [177]. Because interpolymer complexed nanocarriers for chemotherapy readily form due to the attraction between (NH_3^+) of CS and ($-OSO_3^-$ and $-COO^-$) of CHON, a new drug delivery method was recently developed as PECs produced by self-assembling from natural polysaccharides, including CS and chondroitin sulphate (CHON) complexes. Additionally, these PECNs generate better carriers for ionised pharmaceuticals with excellent drug loading and entrapment efficiency, and they are biocompatible, bioadhesive and biodegradable [176, 178].

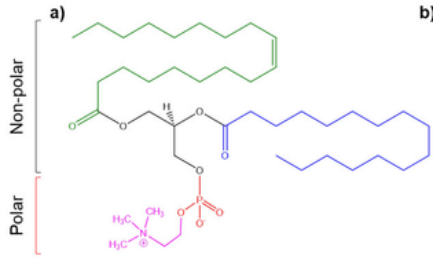
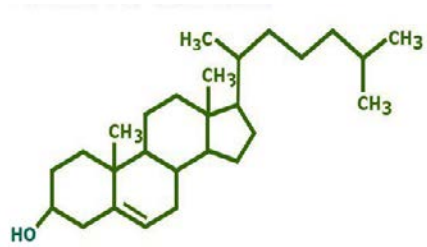
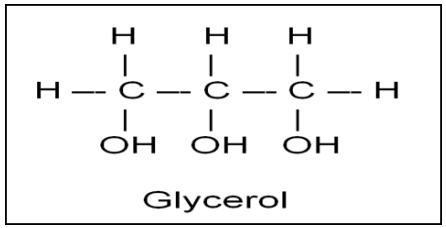
Table- 2.8. Literature review on PECN

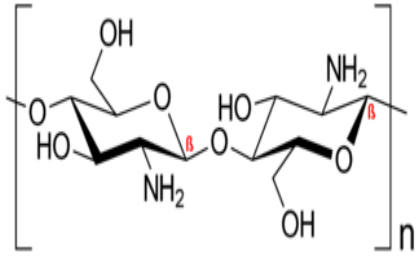
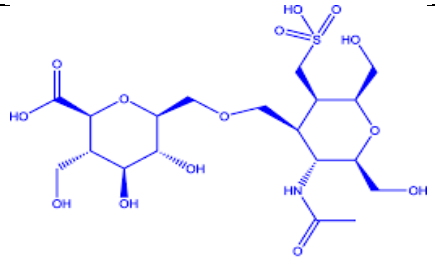
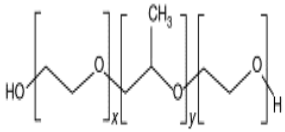
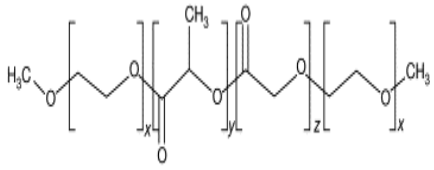
Author [Reference]	API used	Route of administration	Observation/conclusion
Rehman et al. **[179]**	Ketoprofen	Transdermal	Authors prepared PECN incorporated emulgel and it showed significantly enhanced skin permeation through mice skin as compared to marketed gel. Sustained release was observed in 72 hrs for drug loaded PECN-gel.
Talib et al. **[180]**	Artemether	Transdermal	Artemether loaded nanoparticles incorporated transdermal patch showed efficient and significant permeation drug through skin and reached to systemic circulation. The authors performed skin integrated study by FTIR and results revealed that nanocarriers loaded transdermal patch-maintained skin integrity.
Bijukumar et al. **[181]**	Indomethacin	Topical	Authors developed an inflammation sensitive PECN for arthritis by topical route. The chitosan- alginate - hyaluronic acid nanoparticulate system showed sustained drug release and effective skin permeation.
Barbosa et al. **[182]**	Methotrexate	Topical	Authors developed fucoidan/chitosan PEC nanoparticles for topical delivery. The prepared PECN showed significant inhibition of pro-inflammatory cytokines (IL-β, IL-6 and TNF-α). The in vitro skin permeation study revealed that drug-loaded PECN enhanced permeation by more than 2.5-fold as compared to

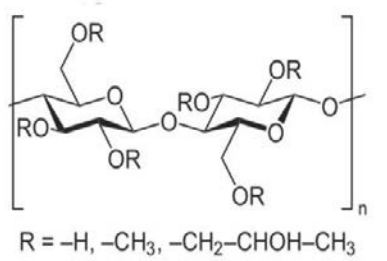
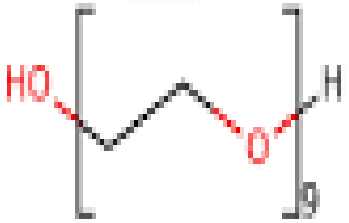
			free drug.
Wang et al. **[168]**	Curcumin	Oral	Authors formulated chitosan-cruciferin PECN using polyelectrolyte complexation method. The in vitro release study showed controlled release with simple diffusion mechanism. The in vitro cell line results revealed that PECN was non-toxic to Caco-2 cells and enhanced the cellular uptake of the curcumin loaded PECN as compared to free curcumin.
Akolade et al. **[183]**	Curcumin	Oral	The results of this research showed that chitosan-based polyelectrolyte complexes may be used to boost the effectiveness of phytoceuticals. Curcumin retention, bioactivities, and chemotherapeutic efficiency were increased by encapsulation in chitosan-based nanocomplexes.

2.7. Excipient Profile:

Table 2.9: Excipient Profile.

Name of excipients	Molecular weight	Melting/boiling point/TG	Structure	Solubility
Lipoid S-75	908 g/mol	DSC endotherm 40-300°C MP-40°C		Neutral *phospholipids* have in general an excellent *solubility* in ethanol or methanol: chloroform mixture.
Cholesterol	386.654 g/mol	148 °C		EtOH, MeOH, acetone, CHCl3, benzene, hexane
Glycerol	92.09382 g/mol	17.8 °C		Water

Name of excipients	Molecular weight	Melting/boiling point/TG	Structure	Solubility
Chitosan	*1526.5 g/mol*	102.5 °C		dilute aqueous acid (pH <6.5).: soluble acetic, citric, formic, hydrochloric, and lactic acid
Chondroitin sulphate	475.379 g/mol	*190-194ºC*		*highly soluble in water*
Poloxamer 407	12,600 g/mol	53–57 °C (127–135 °F; 326–330 K)	 Poloxamer or Pluronic® PEO-PPG-PEO  Triblock PEO-PLGA-PEO	Water

Name of excipients	Molecular weight	Melting/boiling point/TG	Structure	Solubility
HPMC K4M	13 000 - 200 000	225-230 °C	 R = –H, –CH_3, –CH_2–CHOH–CH_3	Water
PEG 400	380 - 420	4-8ºC		water, ethanol, acetone, glycols and chloroform

Chapter 2 – Literature Review

2.8. References:

1. Sözen, T., L. Özışık, and N.Ç.J.E.j.o.r. Başaran, *An overview and management of osteoporosis.* Eur J Rheumatol., 2017. **4**(1): p. 46.
2. Pouresmaeili, F., et al., *A comprehensive overview on osteoporosis and its risk factors.* Ther Clin Risk Manag., 2018: p. 2029-2049.
3. Harvey, N., E. Dennison, and C.J.N.R.R. Cooper, *Osteoporosis: impact on health and economics.* Nat Rev Rheumatol, 2010. **6**(2): p. 99-105.
4. LeBoff, M., et al., *The clinician's guide to prevention and treatment of osteoporosis.* Osteoporosis International, 2022. **33**(10): p. 2049-2102.
5. Cauley, J.A.J.J.o.G.S.A.B.S. and M. Sciences, *Public health impact of osteoporosis.* Public health impact of osteoporosis, 2013. **68**(10): p. 1243-1251.
6. Shen, Y., et al., *The global burden of osteoporosis, low bone mass, and its related fracture in 204 countries and territories, 1990-2019.* Frontiers in Endocrinology, 2022. **13**.
7. Kanis, O.J.A.J.O.i., *An estimate of the worldwide prevalence and disability associated with osteoporotic fractures.* Osteoporosis International, 2006. **17**(12): p. 1726.
8. Wilson, C.R. *Essentials of bone densitometry for the medical physicist*. in *American Association of Physicists in Medicine 2003 Annual Meeting*. 2003.
9. Bhadada, S.K., et al., *The Indian Society for Bone and Mineral Research (ISBMR) position statement for the diagnosis and treatment of osteoporosis in adults.* Archives of Osteoporosis, 2021. **16**(1): p. 102.
10. Boonen, S., A.J.J.C.m.r. Singer, and opinion, *Osteoporosis management: impact of fracture type on cost and quality of life in patients at risk for fracture I.* Current Medical Research and Opinion, 2008. **24**(6): p. 1781-1788.
11. Tran, O., et al., *Long-term direct and indirect economic burden associated with osteoporotic fracture in US postmenopausal women.* Osteoporos Int. , 2021. **32**: p. 1195-1205.

12. Kemmak, A.R., et al., *Economic burden of osteoporosis in the world: A systematic review.* Med J Islam Repub Iran. 2020. **34**: p. 154.

13. Bubshait, D. and M.J.C.t.i. Sadat-Ali, *Economic implications of osteoporosis-related femoral fractures in Saudi Arabian society.* Calcified Tissue International, 2007. **81**: p. 455-458.

14. Mohamed, A.M.J.T.M.j.o.m.s.M., *An overview of bone cells and their regulating factors of differentiation.* Malaysian Journal of Medical Sciences, 2008. **15**(1): p. 4.

15. Marín-Llera, J.C., D. Garciadiego-Cázares, and J.J.F.i.g. Chimal-Monroy, *Understanding the cellular and molecular mechanisms that control early cell fate decisions during appendicular skeletogenesis.* Frontiers in Genetics, 2019. **10**: p. 977.

16. Aghajanian, P. and S.J.B.r. Mohan, *The art of building bone: emerging role of chondrocyte-to-osteoblast transdifferentiation in endochondral ossification.* Bone Research, 2018. **6**(1): p. 19.

17. Florencio-Silva, R., et al., *Biology of bone tissue: structure, function, and factors that influence bone cells.* BioMed Research International, 2015. **2015**.

18. Bilgiç, E., et al., *Chapter 6 - Architecture of bone tissue and its adaptation to pathological conditions*, in *Comparative Kinesiology of the Human Body*, S. Angin and I.E. Şimşek, Editors. 2020, Academic Press. p. 71-90.

19. Clarke, B.J.C.j.o.t.A.S.o.N., *Normal bone anatomy and physiology.* Clin J Am Soc Nephrol., 2008. **3**(Supplement 3): p. S131-S139.

20. Zhou, H., S.S. Lu, and D.W. Dempster, *Chapter 2 - Bone Remodeling: Cellular Activities in Bone*, in *Osteoporosis in Men (Second Edition)*, E.S. Orwoll, J.P. Bilezikian, and D. Vanderschueren, Editors. 2010, Academic Press: San Diego. p. 15-24.

21. Hall, B.K., *Chapter 2 - Bone*, in *Bones and Cartilage (Second Edition)*, B.K. Hall, Editor. 2015, Academic Press: San Diego. p. 17-42.

22. Feng, X.J.C.c.b., *Chemical and biochemical basis of cell-bone matrix interaction in health and disease.* Curr Chem Biol. , 2009. **3**(2): p. 189-196.

23. Bolamperti, S., I. Villa, and A.J.B.R. Rubinacci, *Bone remodeling: An operational process ensuring survival and bone mechanical competence.* Bone Research, 2022. **10**(1): p. 48.

24. Schaffler, M.B. and O.D.J.C.o.r. Kennedy, *Osteocyte signaling in bone.* Curr Osteoporos Rep., 2012. **10**: p. 118-125.

25. Aarden, E.M., P.J. Nijweide, and E.H.J.J.o.c.b. Burger, *Function of osteocytes in bone.* Journal of Cellular Biochemistry, 1994. **55**(3): p. 287-299.

26. Ottewell, P.D.J.J.o.b.o., *The role of osteoblasts in bone metastasis.* J Bone Oncol. , 2016. **5**(3): p. 124-127.

27. Qiu, Z.-Y., Y. Cui, and X.-M. Wang, *Chapter 1 - Natural Bone Tissue and Its Biomimetic*, in *Mineralized Collagen Bone Graft Substitutes*, X.-M. Wang, Z.-Y. Qiu, and H. Cui, Editors. 2019, Woodhead Publishing. p. 1-22.

28. Bassi, A., et al., *5 - Bone tissue regeneration*, in *Electrospinning for Tissue Regeneration*, L.A. Bosworth and S. Downes, Editors. 2011, Woodhead Publishing. p. 93-110.

29. Boyce, B.F., et al., *Bone remodeling and the role of TRAF3 in osteoclastic bone resorption.* Frontiers in Immunology, 2018. **9**: p. 2263.

30. Rajan, R., et al., *Postmenopausal osteoporosis–An Indian perspective.* Current Medical Issues, 2020. **18**(2): p. 98.

31. Armas, L.A., R.R.J.E. Recker, and M. Clinics, *Pathophysiology of osteoporosis: new mechanistic insights.* Endocrinology and Metabolism Clinics of North America, 2012. **41**(3): p. 475-486.

32. Sipos, W., et al., *Pathophysiologie der Osteoporose.* EXCLI J. , 2009. **159**: p. 230-234.

33. Shams, R., et al., *The pathophysiology of osteoporosis after spinal cord injury.* Int J Mol Sci. 2021. **22**(6): p. 3057.

34. Rosen, C.J.J.E., *The epidemiology and pathogenesis of osteoporosis.* The epidemiology and pathogenesis of osteoporosis, 2020.

35. Drake, M.T., B.L. Clarke, and E.M.J.C.t. Lewiecki, *The pathophysiology and treatment of osteoporosis.* clinical therapeutics, 2015. **37**(8): p. 1837-1850.

36. Clarke, B.L. and S.J.R.C. Khosla, *Physiology of bone loss.* Radiol Clin North Am. 2010. **48**(3): p. 483-495.

37. Weinstock-Guttman, B., et al., *Risk of bone loss in men with multiple sclerosis.* Mult Scler. 2004. **10**(2): p. 170-175.

38. Russo, C.R., et al., *Structural adaptations to bone loss in aging men and women.* Bone, 2006. **38**(1): p. 112-118.

39. Ahuja, M.J.J.o.m.-l.h., *Age of menopause and determinants of menopause age: A PAN India survey by IMS.* Journal of Mid-life Health, 2016. **7**(3): p. 126.

40. Khadilkar, A.V. and R.M.J.I.j.o.w.s.h. Mandlik, *Epidemiology and treatment of osteoporosis in women: an Indian perspective.* International Journal of Women's Health 2015: p. 841-850.

41. Salari, N., et al., *The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis.* Journal of Orthopaedic Surgery and Research, 2021. **16**(1): p. 1-20.

42. Khosla, S., S. Amin, and E.J.E.r. Orwoll, *Osteoporosis in men.* Endocr Rev. 2008. **29**(4): p. 441-464.

43. Adler, R.A.J.B.r., *Osteoporosis in men: a review.* Bone Research, 2014. **2**(1): p. 1-8.

44. Almeida, M., et al., *Estrogens and Androgens in Skeletal Physiology and Pathophysiology.* Physiol Rev, 2017. **97**(1): p. 135-187.

45. Merlotti, D., et al., *Aromatase activity and bone loss in men.* J Osteoporos, 2011. **2011**: p. 230671.

46. Vanderschueren, D., et al., *Sex steroid actions in male bone.* Endocr Rev. , 2014. **35**(6): p. 906-960.

47. Allowances, R.D.J.I.-N.I.o.N.H., India, *Nutrient requirements and recommended dietary allowances for Indians.* ICMR, 2009.

48. Zhu, K. and R.L.J.C.b. Prince, *Calcium and bone.* nutrients, 2012. **45**(12): p. 936-942.

49. Heaney, R.P.J.A.B.d.E. and Metabologia, *Calcium intake and disease prevention.* Arq Bras Endocrinol Metabol. 2006. **50**: p. 685-693.

50. Agarwal, K., et al., *The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India.* Archives of Disease in Childhood, 2002. **87**(2): p. 111-113.

51. Mithal, A., et al., *The Asia-pacific regional audit-epidemiology, costs, and burden of osteoporosis in India 2013: a report of international osteoporosis foundation.* Iranian Journal of Endocrinology and Metabolism, 2014. **18**(4): p. 449.

52. Keramat, A., et al., *The assessment of osteoporosis risk factors in Iranian women compared with Indian women.* BMC Musculoskeletal Disorders, 2008. **9**(1): p. 1-10.

53. Dempster, D.W.J.A.J.o.M.C., *Osteoporosis and the burden of osteoporosis-related fractures.* Am J Manag Care. 2011. **17**(6): p. S164.

54. Staa, T.v., et al., *The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis.* Osteoporosis International, 2002. **13**: p. 777-787.

55. Wang, L., et al., *Proton pump inhibitors and the risk for fracture at specific sites: data mining of the FDA adverse event reporting system.* Sci Rep. 2017. **7**(1): p. 1-9.

56. Koshy, G., et al., *Derangements in bone mineral parameters and bone mineral density in south Indian subjects on antiepileptic medications.* Annals of Indian Academy of Neurology, 2014. **17**(3): p. 272.

57. Riggs, B.L., S. Khosla, and L.J. Melton, *Chapter 38 - The Type I/Type II Model for Involutional Osteoporosis: Update and Modification Based on New Observations*, in *Osteoporosis (Second Edition)*, R. Marcus, D. Feldman, and J. Kelsey, Editors. 2001, Academic Press: San Diego. p. 49-58.

58. Aibar-Almazán, A., et al., *Current status of the diagnosis and management of osteoporosis.* Int. J. Mol. Sci., 2022. **23**(16): p. 9465.

59. Peterson, J.A., *Osteoporosis overview.* Geriatric Nursing, 2001. **22**(1): p. 17-23.

60. Emkey, G.R., et al., *Secondary osteoporosis: pathophysiology & diagnosis.* Best Practice & Research Clinical Endocrinology & Metabolism, 2014. **28**(6): p. 911-935.

61. Brickley, M.B., R. Ives, and S. Mays, *7 - Secondary osteoporosis*, in *The Bioarchaeology of Metabolic Bone Disease (Second Edition)*, M.B. Brickley, R. Ives, and S. Mays, Editors. 2020, Academic Press: San Diego. p. 165-178.

62. Sobh, M.M., et al., *Secondary osteoporosis and metabolic bone diseases.* Journal of Clinical Medicine, 2022. **11**(9): p. 2382.

63. Imerci, A., et al., *Idiopathic juvenile osteoporosis: A case report and review of the literature.* International Journal of Surgery Case Reports, 2015. **9**: p. 127-129.

64. Hadjidakis, D.J. and I.I.J.A.o.t.N.Y.a.o.s. Androulakis, *Bone remodeling.* Encyclopedia Britannica, 2006. **1092**(1): p. 385-396.

65. Dunlop, J.W., et al., *New suggestions for the mechanical control of bone remodeling.* Calcified Tissue International 2009. **85**: p. 45-54.

66. Wang, L., et al., *Mechanical regulation of bone remodeling.* Bone Research, 2022. **10**(1): p. 16.

67. Owen, R., G.C.J.F.i.b. Reilly, and biotechnology, *In vitro models of bone remodelling and associated disorders.* Frontiers in Bioengineering and Biotechnology, 2018. **6**: p. 134.

68. Robling, A.G., A.B. Castillo, and C.H.J.A.R.B.E. Turner, *Biomechanical and molecular regulation of bone remodeling.* Annual Review of Biomedical Engineering, 2006. **8**: p. 455-498.

69. Oliveira, A.F.F., *The Biomechanical Analysis of Bone Callus using a Meshless Method*. 2021.

70. Gong, H., et al., *An adaptation model for trabecular bone at different mechanical levels.* Biomed Eng Online. 2010. **9**(1): p. 1-17.

71. Eriksen, E.F.J.R.i.E. and M. Disorders, *Cellular mechanisms of bone remodeling.* Journal of Biological Chemistry, 2010. **11**: p. 219-227.

72. Ruimerman, R., *Modeling and remodeling in bone tissue.* 2005: Technische Universiteit Eindhoven Eindhoven.

73. Siddiqui, J.A. and N.C.J.P. Partridge, *Physiological bone remodeling: systemic regulation and growth factor involvement.* Physiology, 2016. **31**(3): p. 233-245.

74. JS, K.J.A.C.B., *Bassett JHD. The bone remodelling cycle.* Annals of Clinical Biochemistry, 2018. **55**: p. 308-27.

75. Body, J.-J., et al., *Non-pharmacological management of osteoporosis: a consensus of the Belgian Bone Club.* Osteoporosis International 2011. **22**: p. 2769-2788.

76. Gennari, L., D. Merlotti, and R.J.C.i.i.a. Nuti, *Selective estrogen receptor modulator (SERM) for the treatment of osteoporosis in postmenopausal women: focus on lasofoxifene.* Clin Interv Aging., 2010: p. 19-29.

77. Lee, J., et al., *Effect of tamoxifen on the risk of osteoporosis and osteoporotic fracture in younger breast cancer survivors: a nationwide study.* Frontiers in Oncology, 2020. **10**: p. 366.

78. Hadji, P.J.C., *The evolution of selective estrogen receptor modulators in osteoporosis therapy*. climacteric. , 2012. **15**(6): p. 513-523.

79. Andersson, A., et al., *Selective oestrogen receptor modulators lasofoxifene and bazedoxifene inhibit joint inflammation and osteoporosis in ovariectomised mice with collagen-induced arthritis.* Rheumatology, 2016. **55**(3): p. 553-563.

80. Park, J., et al., *Phosphonate and bisphosphonate inhibitors of farnesyl pyrophosphate synthases: A structure-guided perspective.* Front Chem. 2021. **8**: p. 612728.

81. Tu, K.N., et al., *Osteoporosis: a review of treatment options.* Pharmacy & Therapeutics, 2018. **43**(2): p. 92.

82. Drake, M.T., B.L. Clarke, and S. Khosla. *Bisphosphonates: mechanism of action and role in clinical practice*. in *Mayo Clinic Proceedings*. 2008. Elsevier.

83. Russell, R.G.G.J.P., *Bisphosphonates: mode of action and pharmacology.* Pediatrics. 2007. **119**(Supplement_2): p. S150-S162.

84. Roelofs, A.J., et al., *Chapter 81 - Bisphosphonates: Mechanisms of Action*, in *Principles of Bone Biology (Third Edition)*, J.P. Bilezikian, L.G. Raisz, and T.J. Martin, Editors. 2008, Academic Press: San Diego. p. 1737-1767.

85. Compston, J.J.B., *Practical guidance for the use of bisphosphonates in osteoporosis.* Bone, 2020. **136**: p. 115330.

86. Oryan, A. and S. Sahvieh, *Effects of bisphosphonates on osteoporosis: Focus on zoledronate.* Life Sciences, 2021. **264**: p. 118681.

87. Khajuria, D.K., et al., *Risedronate/zinc-hydroxyapatite based nanomedicine for osteoporosis.* Materials Science and Engineering C, 2016. **63**: p. 78-87.

88. Lee, M.-S., et al., *Synthesis of composite magnetic nanoparticles Fe3O4 with alendronate for osteoporosis treatment.* International Journal of Nanomedicine, 2016. **11**: p. 4583.

89. Nikfar, Z., Z.J.J.o.M.G. Shariatinia, and Modelling, *Phosphate functionalized (4, 4)-armchair CNTs as novel drug delivery systems for alendronate and etidronate anti-osteoporosis drugs.* Journal of Molecular Graphics and Modelling, 2017. **76**: p. 86-105.

90. Mitchell, D.Y., et al., *The effect of dosing regimen on the pharmacokinetics of risedronate.* Br J Clin Pharmacol, 1999. **48**(4): p. 536-42.

91. Nuti, R., *Updates on mechanism of action and clinical efficacy of risedronate in osteoporosis.* Clin Cases Miner Bone Metab, 2014. **11**(3): p. 208-14.

92. Kennel, K.A. and M.T. Drake. *Adverse effects of bisphosphonates: implications for osteoporosis management*. in *Mayo Clinic Proceedings*. 2009. Elsevier.

93. Cremers, S., et al., *Pharmacology of bisphosphonates.* British Journal of Clinical Pharmacology, 2019. **85**(6): p. 1052-1062.

94. Dempster, D.W. and M.A.J.J.o.C.D. Bolognese, *Ibandronate: the evolution of a once-a-month oral therapy for postmenopausal osteoporosis.* Journal of Clinical Densitometry, 2006. **9**(1): p. 58-65.

95. Shaw, N. and N.J.A.o.D.i.C. Bishop, *Bisphosphonate treatment of bone disease.* Mayo Clin Proc., 2005. **90**(5): p. 494-499.

96. Zacharis, C.K., P.D.J.J.o.p. Tzanavaras, and b. analysis, *Determination of bisphosphonate active pharmaceutical ingredients in pharmaceuticals and biological material: a review of analytical methods.* Journal of Pharmaceutical and Biomedical Analysis, 2008. **48 3**: p. 483-96.

97. Sharma, D., et al., *Bisphosphonate-related osteonecrosis of jaw (BRONJ): diagnostic criteria and possible pathogenic mechanisms of an unexpected anti-angiogenic side effect.* Vascular Cell, 2013. **5**(1): p. 1-8.

98. Muñoz-Torres, M., G. Alonso, and P.J.T.i.e. Mezquita Raya, *Calcitonin therapy in osteoporosis.* Treatments in Endocrinology, 2004. **3**: p. 117-132.

99. Gass, M. and B.J.T.A.j.o.m. Dawson-Hughes, *Preventing osteoporosis-related fractures: an overview.* The American Journal of Medicine, 2006. **119**(4): p. S3-S11.

100. Chadha, M., et al., *Osteoporosis: Epidemiology, Pathogenesis, Evaluation and Treatment.* Open Journal of Orthopedics, 2022. **12**(4): p. 153-182.

101. Sandhu, S.K. and G.J.J.o.c.p. Hampson, *The pathogenesis, diagnosis, investigation and management of osteoporosis.* J Clin Pathol. 2011. **64**(12): p. 1042-1050.

102. Khan, A.-W., A.J.J.o.O. Khan, and G. Canada, *Anabolic agents: a new chapter in the management of osteoporosis.* Journal of Obstetrics and Gynaecology Canada, 2006. **28**(2): p. 136-141.

103. Oryan, A., A. Kamali, and A. Moshiri, *Potential mechanisms and applications of statins on osteogenesis: Current modalities, conflicts and future directions.* Journal of Controlled Release, 2015. **215**: p. 12-24.

104. Tang, Q.O., et al., *Statins: under investigation for increasing bone mineral density and augmenting fracture healing.* Expert Opin Investig Drugs., 2008. **17**(10): p. 1435-1463.

105. Morse, L.R., J. Coker, and R.A.J.A.e.o. Battaglino, *Statins and bone health: A mini review.* Actual osteol., 2018. **14**(1): p. 31.

106. Lai, S.-W.J.A.o.t.R.D., *Association between osteoporosis and statins therapy.* 2019.

107. Kaur, R. and M.J.E.j.o.p.s. Ajitha, *Transdermal delivery of fluvastatin loaded nanoemulsion gel: Preparation, characterization and in vivo anti-osteoporosis activity.* European Journal of Pharmaceutical Sciences, 2019. **136**: p. 104956.

108. Kaur, R., M.J.J.o.D.D.S. Ajitha, and Technology, *Formulation of transdermal nanoemulsion gel drug delivery system of lovastatin and its in vivo characterization in*

glucocorticoid induced osteoporosis rat model. Journal of Drug Delivery Science and Technology, 2019. **52**: p. 968-978.

109. Ebetino, F.H., et al., *Bisphosphonates: The role of chemistry in understanding their biological actions and structure-activity relationships, and new directions for their therapeutic use.* Bone, 2022. **156**: p. 116289.

110. Nuti, R.J.C.C.i.M. and B. Metabolism, *Updates on mechanism of action and clinical efficacy of risedronate in osteoporosis.* Clin Cases Miner Bone Metab, 2014. **11**(3): p. 208.

111. Guzman, M.L., et al., *Reduced food interaction and enhanced gastrointestinal tolerability of a new system based on risedronate complexed with Eudragit E100: Mechanistic approaches from in vitro and in vivo studies.* European Journal of Pharmaceutics and Biopharmaceutics, 2016. **107**: p. 263-272.

112. Fazil, M., et al., *Bisphosphonates: therapeutics potential and recent advances in drug delivery.* Drug Delivery, 2015. **22**(1): p. 1-9.

113. KD, P., et al., *Development of risedronate sodium-loaded nanosponges by experimental design: optimization and in vitro characterization.* Indian Journal of Pharmaceutical Sciences, 2019. **81**(2): p. 309-316.

114. Swami, A.S., et al., *Development and validation of stability indicating uv spectrophotometric method for the estimation of sodium risedronate.* International Journal of Pharmacy and Pharmaceutical Sciences, 2012. **4**(3): p. 4.

115. Colaco, S., et al., *Comparative pharmacokinetic study of risedronate 35 mg healthy male subjects under fed conditions.* Research Journal of Pharmacy and Technology 2020. **13**(12): p. 5876-5880.

116. Kleinermans, D., et al., *An open-label randomized study of the relative absorption of gastro-resistant risedronate taken fasted or with food versus immediate-release risedronate.* Pharmacology Research & Perspectives, 2022. **10**(3): p. e00957.

117. Elsayyad, N.M.E., et al., *Efficient lung-targeted delivery of risedronate sodium/vitamin D3 conjugated PAMAM-G5 dendrimers for managing osteoporosis: Pharmacodynamics, molecular pathways and metabolomics considerations.* Life Sciences, 2022. **309**: p. 121001.

118. Rawat, P., et al., *Design and development of bioceramic based functionalized PLGA nanoparticles of risedronate for bone targeting: in-vitro characterization and pharmacodynamic evaluation.* Pharmaceutical Research, 2015. **32**: p. 3149-3158.

119. Fazil, M., et al., *Biodegradable intranasal nanoparticulate drug delivery system of risedronate sodium for osteoporosis.* Drug Delivery, 2016. **23**(7): p. 2428-2438.

120. Jung, I.-W. and H.-K.J.I.J.o.N. Han, *Effective mucoadhesive liposomal delivery system for risedronate: Preparation and in vitro/in vivo characterization.* International Journal of Nanomedicine, 2014. **9**: p. 2299.

121. Mukherjee, D., et al., *Improvement of bone microarchitecture in methylprednisolone induced rat model of osteoporosis by using thiolated chitosan-based risedronate mucoadhesive film.* Drug Dev Ind Pharm. , 2018. **44**(11): p. 1845-1856.

122. Rawat, P., et al., *Three ply-walled microcapsules for enhanced pharmacokinetics of poorly absorbed risedronate sodium: novel stratagem toward osteoporosis.* Journal of Pharmaceutical Innovation, 2015. **10**: p. 130-139.

123. de Arce Velasquez, A., et al., *Novel Pullulan–Eudragit® S100 blend microparticles for oral delivery of risedronate: Formulation, in vitro evaluation and tableting of blend microparticles.* Mater Sci Eng C Mater Biol Appl. , 2014. **38**: p. 212-217.

124. Nasr, M., I. Taha, and R.M.J.D.d. Hathout, *Suitability of liposomal carriers for systemic delivery of risedronate using the pulmonary route.* Drug Delivery, 2013. **20**(8): p. 311-318.

125. Mahmoud, M.O., et al., *Transdermal delivery of atorvastatin calcium from novel nanovesicular systems using polyethylene glycol fatty acid esters: ameliorated effect without liver toxicity in poloxamer 407-induced hyperlipidemic rats.* Journal of Controlled Release, 2017. **254**: p. 10-22.

126. Shamsuddin, et al., *Atorvastatin solid dispersion for bioavailability enhancement.* J Adv Pharm Technol Res, 2016. **7**(1): p. 22-6.

127. Lennernäs, H.J.C.p., *Clinical pharmacokinetics of atorvastatin.* Clinical Pharmacokinetics, 2003. **42**: p. 1141-1160.

128. Sonje, V.M., et al., *Atorvastatin calcium,* in *Profiles of Drug Substances, Excipients and Related Methodology*. 2010, Elsevier. p. 1-70.

129. Spyrou, C. and M. Lange. *uc_FIDO: Unambiguous Characterization of Food Interactions with Drugs Ontology*. in *ICBO/BioCreative*. 2016.

130. USFDA, *Lipitor - (Atorvastatin Calcium) Tablets.* cited on (04-05-2023).

131. El-Nabarawi, N., et al., *Atorvastatin, a double weapon in osteoporosis treatment: an experimental and clinical study.* Drug Des Devel Ther. , 2017: p. 1383-1391.

132. Xie, Y., et al., *Atorvastatin-loaded micelles with bone-targeted ligand for the treatment of osteoporosis.* Drug Delivery, 2017. **24**(1): p. 1067-1076.

133. Shokrolahi, F., et al., *Atorvastatin loaded PLGA microspheres: Preparation, HAp coating, drug release and effect on osteogenic differentiation of ADMSCs.* International Journal of Pharmaceutics, 2019. **565**: p. 95-107.

134. Zaid Alkilani, A., M.T. McCrudden, and R.F.J.P. Donnelly, *Transdermal drug delivery: innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum.* Pharmaceutics 2015. **7**(4): p. 438-470.

135. Tomoda, K. and K. Makino, *Chapter 7 - Nanoparticles for transdermal drug delivery system (TDDS)*, in *Colloid and Interface Science in Pharmaceutical Research and Development*, H. Ohshima and K. Makino, Editors. 2014, Elsevier: Amsterdam. p. 131-147.

136. Yu, Y.-Q., et al., *Enhancing permeation of drug molecules across the skin via delivery in nanocarriers: novel strategies for effective transdermal applications.* Front Bioeng Biotechnol. 2021. **9**: p. 646554.

137. Ramadon, D., et al., *Enhancement strategies for transdermal drug delivery systems: Current trends and applications.* Drug Delivery and Translational Research, 2021: p. 1-34.

138. Alkilani, A.Z., et al., *Beneath the Skin: A Review of Current Trends and Future Prospects of Transdermal Drug Delivery Systems.* Pharmaceutics, 2022. **14**(6): p. 1152.

139. Nguyen, A.V. and A.M.J.I.j.o.m.s. Soulika, *The dynamics of the skin's immune system.* Int J Mol Sci., 2019. **20**(8): p. 1811.

140. Villanueva–Martínez, A., et al., *Transdermal formulations and strategies for the treatment of osteoporosis.* Journal of Drug Delivery Science and Technology, 2022: p. 103111.

141. Williams, A.C.J.t.A.s.P.t.D. and M.o.M.t.e.E. Elsevier, *Topical and transdermal drug delivery.* Curr Drug Deliv., 2018: p. 715-38.

142. Richard, C., S. Cassel, and M.J.R.a. Blanzat, *Vesicular systems for dermal and transdermal drug delivery.* RSC Advances, 2021. **11**(1): p. 442-451.

143. Wiedersberg, S. and R.H.J.J.o.c.r. Guy, *Transdermal drug delivery: 30+ years of war and still fighting!* Journal of Controlled Release, 2014. **190**: p. 150-156.

144. Mishra, D.K., et al., *Cutaneous and transdermal drug delivery: Techniques and delivery systems*, in *Basic Fundamentals of Drug Delivery*. 2019, Elsevier. p. 595-650.

145. Jeong, W.Y., et al., *Recent advances in transdermal drug delivery systems: A review.* Archives of Pharmacal Research, 2021. **25**: p. 1-15.

146. Manca, M.L., et al., *Glycerosomes: A new tool for effective dermal and transdermal drug delivery.* International Journal of Pharmaceutics, 2013. **455**(1-2): p. 66-74.

147. Bibi, N., N. Ahmed, and G.M. Khan, *Chapter 21 - Nanostructures in transdermal drug delivery systems*, in *Nanostructures for Drug Delivery*, E. Andronescu and A.M. Grumezescu, Editors. 2017, Elsevier. p. 639-668.

148. Dominguez, S., G.A. Mackert, and M.K. Dobke, *Chapter 29 - Nanotechnology to enhance transdermal delivery of hydrophilic humectants for improved skin care: a model for therapeutic applications*, in *Nanostructures for Drug Delivery*, E. Andronescu and A.M. Grumezescu, Editors. 2017, Elsevier. p. 919-939.

149. Nava-Arzaluz, M.G., E. Piñón-Segundo, and A. Ganem-Rondero, *Lipid nanocarriers as skin drug delivery systems*, in *Nanoparticles in Pharmacotherapy*. 2019, Elsevier. p. 311-390.

150. Sala, M., et al., *Lipid nanocarriers as skin drug delivery systems: Properties, mechanisms of skin interactions and medical applications.* International Journal of Pharmaceutics, 2018. **535**(1-2): p. 1-17.

151. Czajkowska-Kośnik, A., M. Szekalska, and K.J.P.R. Winnicka, *Nanostructured lipid carriers: A potential use for skin drug delivery systems.* Curr Pharm Des. 2019. **71**(1): p. 156-166.

152. Neubert, R.H.J.E.j.o.p. and biopharmaceutics, *Potentials of new nanocarriers for dermal and transdermal drug delivery.* European journal of pharmaceutics and biopharmaceutics, 2011. **77**(1): p. 1-2.

153. Kogan, A., N.J.A.i.c. Garti, and i. science, *Microemulsions as transdermal drug delivery vehicles.* Advances in Colloid and Interface Science, 2006. **123**: p. 369-385.

154. Alkilani, A.Z., M.T. McCrudden, and R.F. Donnelly, *Transdermal Drug Delivery: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the stratum corneum.* Pharmaceutics, 2015. **7**(4): p. 438-70.

155. Agrawal, S., et al., *Microneedles: An advancement to transdermal drug delivery system approach.* Journal of Applied Pharmaceutical Science, 2020. **10**(3): p. 149-159.

156. Gyanewali, S., et al., *Formulation development and in vitro–in vivo assessment of protransfersomal gel of anti-resorptive drug in osteoporosis treatment.* 2021. **608**: p. 121060.

157. Katsumi, H., et al., *Efficient Transdermal Delivery of Alendronate, a Nitrogen-Containing Bisphosphonate, Using Tip-Loaded Self-Dissolving Microneedle Arrays for the Treatment of Osteoporosis.* Pharmaceutics, 2017. **9**(3).

158. Kusamori, K., et al., *Development of a novel transdermal patch of alendronate, a nitrogen-containing bisphosphonate, for the treatment of osteoporosis.* Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research 2010. **25**(12): p. 2582-2591.

159. Waheed, A., et al., *Improved bioavailability of raloxifene hydrochloride using limonene containing transdermal nano-sized vesicles.* Journal of Drug Delivery Science and Technology, 2019. **52**: p. 468-476.

160. Salem, H.F., et al., *Glycerosomal thermosensitive in situ gel of duloxetine HCl as a novel nanoplatform for rectal delivery: in vitro optimization and in vivo appraisal.* Drug Delivery and Translational Research volume, 2022. **12**(12): p. 3083-3103.

161. Salem, H.F., et al., *Formulation design and optimization of novel soft glycerosomes for enhanced topical delivery of celecoxib and cupferron by Box–Behnken statistical design.* Drug Development and Industrial Pharmacy, 2018. **44**(11): p. 1871-1884.

162. Manca, M.L., et al., *Glycerosomes: Investigation of role of 1, 2-dimyristoyl-sn-glycero-3-phosphatidycholine (DMPC) on the assembling and skin delivery performances.* International Journal of Pharmaceutics, 2017. **532**(1): p. 401-407.

163. Naguib, M.J., et al., *Investigating the potential of utilizing glycerosomes as a novel vesicular platform for enhancing intranasal delivery of lacidipine.* International Journal of Pharmaceutics, 2020. **582**: p. 119302.

164. Zhang, K., et al., *Essential oil-mediated glycerosomes increase transdermal paeoniflorin delivery: Optimization, characterization, and evaluation in vitro and in vivo.* Int J Nanomedicine. 2017. **12**: p. 3521.

165. Moolakkadath, T., et al., *Preparation and optimization of fisetin loaded glycerol based soft nanovesicles by Box-Behnken design.* International Journal of Pharmaceutics, 2020. **578**: p. 119125.

166. Naguib, M.J., Y.R. Hassan, and W.H.J.I.J.o.P. Abd-Elsalam, *3D printed ocusert laden with ultra-fluidic glycerosomes of ganciclovir for the management of ocular cytomegalovirus retinitis.* International Journal of Pharmaceutics, 2021. **607**: p. 121010.

167. Melis, V., et al., *Inhalable polymer-glycerosomes as safe and effective carriers for rifampicin delivery to the lungs.* Colloids and Surfaces B: Biointerfaces, 2016. **143**: p. 301-308.

168. Wang, F., et al., *Polyelectrolyte complex nanoparticles from chitosan and acylated rapeseed cruciferin protein for curcumin delivery.* Journal of Agricultural and Food Chemistry, 2018. **66**(11): p. 2685-2693.

169. Ribeiro, T.G., et al., *Novel targeting using nanoparticles: an approach to the development of an effective anti-leishmanial drug-delivery system.* International Journal of Nanomedicine, 2014. **9**: p. 877.

170. Sarika, P. and N.R.J.C.p. James, *Polyelectrolyte complex nanoparticles from cationised gelatin and sodium alginate for curcumin delivery.* Carbohydrate Polymers, 2016. **148**: p. 354-361.

171. Wu, D., et al., *Chitosan-based colloidal polyelectrolyte complexes for drug delivery: a review.* Carbohydrate Polymers, 2020. **238**: p. 116126.

172. Ramasamy, T., et al., *Chitosan-based polyelectrolyte complexes as potential nanoparticulate carriers: physicochemical and biological characterization.* Pharmaceutical Research 2014. **31**: p. 1302-1314.

173. Jardim, K.V., et al., *Physico-chemical characterization and cytotoxicity evaluation of curcumin loaded in chitosan/chondroitin sulfate nanoparticles.* Drug Delivery, 2015. **56**: p. 294-304.

174. Umerska, A., O.I. Corrigan, and L.J.C.p. Tajber, *Design of chondroitin sulfate-based polyelectrolyte nanoplexes: Formation of nanocarriers with chitosan and a case study of salmon calcitonin.* Carbohydrate Polymers, 2017. **156**: p. 276-284.

175. Faris, T.M., et al., *Developed simvastatin chitosan nanoparticles co-crosslinked with tripolyphosphate and chondroitin sulfate for ASGPR-mediated targeted HCC delivery with enhanced oral bioavailability.* Saudi Pharmaceutical Journal, 2020. **28**(12): p. 1851-1867.

176. Umerska, A., O.I. Corrigan, and L. Tajber, *Design of chondroitin sulfate-based polyelectrolyte nanoplexes: Formation of nanocarriers with chitosan and a case study of salmon calcitonin.* Carbohydrate polymers, 2017. **156**: p. 276-284.

177. Sharma, S., K.L. Swetha, and A. Roy, *Chitosan-Chondroitin sulfate based polyelectrolyte complex for effective management of chronic wounds.* International journal of biological macromolecules, 2019. **132**: p. 97-108.

178. Rezazadeh, M., et al., *Incorporation of rosuvastatin-loaded chitosan/chondroitin sulfate nanoparticles into a thermosensitive hydrogel for bone tissue engineering: preparation, characterization, and cellular behavior.* Pharmaceutical Development and Technology, 2019. **24**(3): p. 357-367.

179. Gul, R., et al., *Biodegradable ingredient-based emulgel loaded with ketoprofen nanoparticles.* AAPS PharmSciTech, 2018. **19**: p. 1869-1881.

180. Talib, S., et al., *Chitosan-chondroitin based artemether loaded nanoparticles for transdermal drug delivery system.* Journal of Drug Delivery Science and Technology, 2021. **61**: p. 102281.

181. Bijukumar, D., et al., *Design of an inflammation-sensitive polyelectrolyte-based topical drug delivery system for arthritis.* AAPS PharmSciTech, 2016. **17**: p. 1075-1085.

182. Barbosa, A.I., S.A. Costa Lima, and S. Reis, *Development of methotrexate loaded fucoidan/chitosan nanoparticles with anti-inflammatory potential and enhanced skin permeation.* Int J Biol Macromol, 2019. **124**: p. 1115-1122.

183. Akolade, J.O., H.O.B. Oloyede, and P.C.J.J.o.f.f. Onyenekwe, *Encapsulation in chitosan-based polyelectrolyte complexes enhances antidiabetic activity of curcumin.* Journal of Functional Foods, 2017. **35**: p. 584-594.