NASAL NANOCARRIER DELIVERY SYSTEM FOR THE TREATMENT OF OSTEOPOROSIS
1.1. Intranasal drug delivery

In recent years the nasal drug delivery has received a great deal of attention as a convenient, promising and reliable method for the systemic administration of drugs, especially for those molecules which are ineffective orally and must be administered by injection. The nasal route of drug delivery has advantages over the other alternative systems of non-invasive drug administration. It is apparent that the nasal mucosa is permeable to more compounds than the gastrointestinal tract due to lack of pancreatic and gastric enzymatic activity, neutral pH of the nasal mucus, and less dilution by gastrointestinal contents. Above all, nasally administered drugs avoid hepatic first-pass effect, making this a favoured route for potentially degradable agents by the hepatic system (Hussain, 1998).

During the past several decades, the feasibility of drug delivery via the nasal route has received increasing attention from pharmaceutical scientists and clinicians. Drug candidates ranging from small metal ions to large macromolecular proteins have been tested in various animal models (Chien, 1989). It has been documented that nasal administration of certain hormones and steroids has resulted in a more complete absorption (Hussain et al., 1979; 1981). This indicated the potential value of the nasal route for administration of systemic medications as well as utilizing this route local effect. Many drugs, such as desmopressin acetate, narcotic antagonists (Hussain, 1984) male as well as female hormones (Hussain et al., 1981; Hussain et al., 1984; Bawarshi-Nassar et al., 1989) that are ineffective orally due to their metabolism in the gastrointestinal tract or by first-pass effect are absorbed nasally and some are being administered via the nasal route.

1.1.1. Possibilities for the use of the nasal cavity for drug delivery:

(i) Local delivery

Nasal delivery is a logical delivery choice for local (or topical) treatment. Prominent examples are decongestants for nasal cold symptoms, and antihistamines and corticosteroids for allergic rhinitis (Bloebaum, 2002). Examples of nasal products with widespread use in this area include the histamine H1-antagonist levocabastine (e.g., Janssens and Vanden-Bussche, 1991), the anti-cholinergic agent ipratropium
bromide (e.g., Milford et al., 1990), and steroidal anti-inflammatory agents such as budesonide (Stanaland, 2004), mometasone furoate (van Drunen et al., 2005), triamcinolone (Lumry et al., 2003), and beclomethasone (Lumry et al., 2003). As reviewed by Salib and Howarth (2003), nasal delivery of corticosteroids and antihistamines has minimal potential for systemic adverse effects (as opposed to oral therapy), due to relatively low doses, e.g., recommended therapeutic dosage of intranasal antihistamines does not cause significant sedation or impairment of psychomotor function, whereas these effects may be seen upon oral dosing (for which a much larger dose is required). Such factors make nasal delivery of antihistamines and corticosteroids an attractive and typically preferred route of administration, particularly if rapid symptom relief is required.

(ii) Systemic delivery

Nasal drug delivery for systemic effects has been practiced since ancient times. In modern pharmaceutics, the nose had been considered primarily as a route for local drug delivery. The last 2 decades heralded a number of advances in pharmaceutical biotechnology resulting in possibilities for large scale productions of biopharmaceuticals especially proteins and peptides. The inability to administer these drugs by routes other than parenteral injection motivated scientists to explore other possibilities such as pulmonary and nasal administration. Positive attributes of nasal systemic delivery include a relatively large surface area for drug absorption, rapid drug onset, no first-pass metabolism, and non-invasiveness to maximize patient comfort and compliance. Nasal systemic drug delivery is useful in crisis treatments where rapid onset is needed, long term treatment where daily administration and patient compliance is required, peptides and proteins which are degraded by the harsh condition of gastrointestinal tract. The relevance of gelatin microspheres, microcrystalline cellulose, chitosan, paloxamer 407 (Pluronic F-12) for systemic delivery of calcitonin, leuprolide (Suzuki and Makino, 1999; Morimoto et al., 2001; Sinswat and Tengamnuay, 2003), atrial natriuretic factor (Juhasz et al., 1989), desmopressin (Harris et al., 1989, Critchley et al., 1994) and tetracosactide (ACTH1-24) (Wuthrich et al., 1994) with respect to nasal administration have been reported.
(iii) Vaccine delivery

The nasal mucosa has received some attention as a vaccination route. This approach may be a particularly effective approach to achieve rapid mass immunization, like in children and/or in developing countries and disaster areas (Roth et al., 2003). Intranasal immunization may lead to development of local, as well as systemic, immunity. Furthermore, vaccination via the intranasal route does not require a sterile product or a sterile dosing technique (a distinct advantage in developing areas of the world). An example of an intranasal vaccine is Flu Mist®, a cold adapted live influenza virus (Kemble and Greenberg, 2003). This product is given as one or two doses over the influenza season via a syringe sprayer. Additional examples of human efficacy testing of intranasal vaccines includes those targeted against adenovirus-vectored influenza (Van Kampen et al., 2005), proteosome-influenza (Treasnor et al., 2006), influenza A (Treasnor et al., 1992), influenza B (Obrosova-Serova et al., 1990), meningococcal outer membrane vesicle (Oftung et al., 1999), and a combination respiratory syncytial virus (RSV) and parainfluenza 3 virus (PIV3) live, attenuated intranasal vaccine (Belshe et al., 2004). Effective nasal immunization requires an effective antigen and/or a potent mucosal adjuvant or carrier. Research in this area includes exploring various intranasal excipients such as chitosan (Read et al., 2005), chitin (Hasegawa et al., 2005), galactosamidase (Ko et al., 2005), and biodegradable polymers (Koping-Hoggard et al., 2005).

(iv) CNS delivery

Intranasal drug delivery for targeting the central nervous system (CNS) is currently an area of great interest (Illum, 2004; Vyas et al., 2005). Improved delivery to the brain via the intranasal route has been reported for some low-molecular weight drugs (Sakane et al., 1991, 1994, 1995; Kao et al., 2000; Chow et al., 2001; Barakat et al., 2006; Ali et al., 2010), as well as therapeutic peptides and proteins (Frey et al., 1997; Dufes et al., 2003; Banks et al., 2004; Lerner et al., 2004; Ross et al., 2004; Thorne et al., 2004). In addition to “nose to brain” delivery, intranasal drugs can enter via a “nose to systemic circulation to brain” pathway. In this case, it is necessary for the drug to readily permeate the blood–brain barrier (BBB) from the circulation and for this the drug (or prodrug) must exhibit satisfactory passive or active transport across the tight junction barriers of the BBB. For example, an insulin transporter across the BBB
1.1.2. Anatomical and physiological consideration for intranasal delivery

1.1.2.1. Anatomy

The human skull is composed of two functional sections that protect the delicate structures within them. The neurocranium surrounds and protects the brain while the viscerocranium surrounds and protects the eyes, the mouth and the nasal cavity (Ugwoke et al., 2001). Nasal cavity can anatomically be segregated into five different regions: nasal vestibule, atrium, respiratory area, olfactory region and the nasopharynx as shown in Fig.1.1. Nasal cavity is divided into two symmetrical halves by the nasal septum (comprised of bone and cartilage), each cavity has volume up to approximately 7.5 mL and a surface area around 75 cm² (Mygind and Anggard, 1984; Pomponi et al., 1990; Illum, 2000). Nasal cavity extends posteriorly to the nasopharynx. The most anterior part of nasal cavity, the nasal vestibule opens at the face via the nostrils. The atrium is an intermediate region between the vestibule and the respiratory region. The respiratory region occupies the major part of the nasal cavity which possesses lateral walls dividing it into 3 sections: inferior (C1), middle (C2) and superior (C4) nasal turbinates (Wuthrich and Buri, 1989). These folds provide the nasal cavity with a very high surface area of about 150 cm² in humans compared to its small volume. The respiratory region is richly supplied with blood, and receives the maximum amount of nasal secretions, rendering it most suitable for the permeation of compounds. The olfactory region situated above the superior nasal turbinate which possesses specialized ciliated olfactory nerve cells for smell perception. The total surface area of the olfactory epithelium is 200-400 mm² (Ugwoke et al., 2001).
Fig.1.1. Schematic representation of a sagittal section of human nasal cavity showing the nasal vestibule (A), atrium (B), respiratory region: inferior turbinate (C1), middle turbinate (C2) and the superior turbinate (C3), the olfactory region (D) and nasopharynx (E) (Ugwoke et al., 2001)

1.1.2.2. Morphology and physiology of the nose

The structural features of various regions and their impact on permeability of nasal cavity are listed in Table 1.1. The respiratory epithelium, covering the main part of the nasal cavity ~160 cm² in humans (Ugwoke et al., 2001), consists of four main cell types: ciliated and non-ciliated columnar cells, goblet cells and basal cells (Fig. 1.2). The epithelial cell layer is covered with mucus, which is produced by the goblet cells and cleared by the beating of the cilia, the so-called mucociliary clearance. This clearance mechanism protects the respiratory tract including the lungs from bacteria and other harmful exogenous compounds.
Table 1.1. Structural features of various regions and their impact on permeability of nasal cavity

<table>
<thead>
<tr>
<th>Region</th>
<th>Structural features</th>
<th>Permeability</th>
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| Nasal vestibule               | • Nasal hairs (vibrissae)  
• Epithelial cells are stratified, squamous and keratinized  
• Sebaceous glands present                                                                                                                   | Least permeable because of presence of keratinized cells, very resistant to hydration and can withstand insults from noxious substances of the environment. |
| Atrium                        | • Transepithelial region  
• Stratified squamous cells present anteriorly and pseudostratified cells with microvilli present posteriorly  
• Narrowest region of nasal cavity                                                                                                             | Less permeable as it has small surface area and stratified cells are present anteriorly |
| Respiratory region            | • Pseudostratified ciliated columnar cells with microvilli (300 per cell), large surface area. Receives maximum nasal secretions because of presence of seromucous glands, nasolacrimal duct and goblet cells  
• Richly supplied with blood for heating and humidification of inspired air, presence of paranasal sinuses                                                                 | Most permeable region because of large surface area and rich vasculature         |
| (Inferior turbinate)          |                                                                                                                                                                                                                 |                                                                            |
| (Middle turbinate)            |                                                                                                                                                                                                                 |                                                                            |
| (Superior turbinate)          |                                                                                                                                                                                                                 |                                                                            |
| Olfactory region              | • Specialized ciliated olfactory nerve cells for smell perception  
• Receives ophthalmic and maxillary divisions of trigeminal nerve                                                                                   | Direct access to cerebrospinal fluid                                           |
| Nasopharynx                   | • Upper part contains ciliated cells and lower part contains squamous epithelium                                                                                                                              | Receives nasal cavity drainage                                               |

Fig.1.2. Cell types of the nasal epithelium showing ciliated cell (A), non-ciliated cell (B), goblet cells (C), gel mucus layer (D), sol layer (E), basal cell (F) and basement membrane (G) (Ugwoke et al., 2001)

1.1.2.3. Nasal mucus secretion and mucociliary clearance

The respiratory part of nasal cavity is covered by viscoelastic fluid called mucus. Submucosal glands are responsible for secreting nasal mucus. These glands are composed of mucus cells and serous cells that produce mucus gel and watery fluids respectively (Lansley, 1993). Mucus is also released from the goblet cells, as mucus
granules. The nasal secretion is a complex mixture of many substances and consists of about 95% water, 2%mucin, 1% salts, 1% of other proteins such as albumin, immunoglobulins, lysozyme and lactoferrin, and <1% lipids (Kaliner et al., 1984). About 1.5–2 l of nasal mucus is produced daily (Marom et al., 1984). This mucus blanket, is about 5 µm thick, consists of two layers, a lower sol layer and an upper gel layer. The lower sol layer possesses low viscosity which bathes the cilia while upper gel layer has high fluid viscosity that rests on the cilia. The viscosity of both layers affects ciliary beating and the efficiency of transporting the overlying mucus-the mucociliary clearance (MCC). Mucin content of mucus greatly affects the mucus viscosity as small increase in mucin causes very large increase in mucus viscosity which results in prolongation of mucociliary clearance time (Rice, 1978). Mucin is a high molecular mass (2000 000-4 000 000 Da) glycoprotein crosslinked with disulphide bridges, ionic bond and physical entanglements. The carbohydrate side groups attached to the protein backbone include galactose, L-fucose, N-acetylglucoseamine, N-acetylgalactoseamine and N-acetylneuraminic acid (sialic acid). The carbohydrate side chains terminate with a sialic acid or L-fucose group, which make mucin an anionic polyelectrolyte at neutral pH. Due to the multiplicity of hydroxyl groups of the carbohydrate side chains, mucin easily forms hydrogen bonds with other suitable polymers (Kamath and Park, 1994). The nasal mucus performs a number of physiological functions (Chein, 1995). It covers the mucosa, and physically and enzymatically protects it. The mucus has water-holding capacity. It exhibits surface electrical activity. It permits efficient heat transfer. It acts as adhesive and transports particulate matter towards the nasopharynx.

1.1.3. Advantages of nasal drug delivery

- Avoids degradation of drug in gastrointestinal tract resulting from acidic or enzymatic degradation
- Avoids degradation of drug resulting from hepatic first pass metabolism
- Results in rapid absorption and onset of effect
- Results in higher bioavailability thus uses lower doses of drug
- Easily accessible, non-invasive route
- Self-medication is possible through this route
- Direct transport into systemic circulation and CNS is possible
• Offers lower risk of overdose
• Does not have any complex formulation requirement
• Improved patient compliance

1.1.4. Limitations of nasal drug delivery

• Volume that can be delivered into nasal cavity is restricted to 25–200 µL
• High molecular weight compounds cannot be delivered through this route (mass cut off ~1 kDa)
• Adversely affected by pathological conditions
• Large interspecies variability is observed in this route
• Normal defense mechanisms like mucociliary clearance and ciliary beating affects the permeability of drug
• Enzymatic barrier to permeability of drugs
• Irritation of nasal mucosa by drugs
• Limited understanding of mechanisms and less developed models at this stage

1.1.5. Factor affecting nasal drug absorption

The process of drug transport across the nasal membrane involves either the diffusion of drug molecules through the pores in the nasal mucosa or participation of some non-passive pathways before they reach the blood stream (Anandkumar et al., 1974). In addition, the olfactory epithelium is known to be a portal for substance to enter the central nervous system (CNS) and the peripheral circulation. However, the mechanism of transport still remains unknown. The rate and extent of nasal absorption of a drug is dependent upon various factors listed in Table 1.2. The factors that can influence drug absorption from then nasal cavity are very important in designing both formulation and the device used for intranasal administration.
1.2. Mucoadhesive drug delivery system

Intranasal route is the most feasible and frequently proposed delivery route alternative to parenteral route. Most of the time failure of delivery of drug via this route is mainly because of the rapid removal of mucus from the nasal cavity resulting in short residence time of the formulation within the nasal cavity. But this problem can be easily overcome by using mucoadhesive polymers. The application of mucoadhesive polymers in nasal drug delivery system has gained interest among pharmaceutical scientist as a means of promoting dosage form residence time in the nasal cavity as well as improving intimacy of contact with the absorptive membranes of the biological system. The mechanism of increasing drug absorption by mucoadhesive drug delivery systems may be done by several ways. Some polymers interact with the mucus and/or the epithelium in such a way as to increase epithelial permeability. This has been demonstrated in several studies with insulin (Illum et al., 2001; Dyer et al., 2002; Wang et al., 2002). Some mucoadhesive polymers like chitosan and polyacrylic acids also have enzyme inhibitory activities. Luessen and associates demonstrated that Carbopol 934P and polycarbophil can inhibit trypsin and increase absorption of...
co-administered peptides (Luessen et al., 1994). The mucoadhesive polymers form weak covalent, hydrogen and ionic bonds with the mucus and thereby decrease mucociliary clearance. The presence of mucoadhesive agents can alter the viscosity, rheology and the ciliary beating frequency. The ciliary beating frequency also depends on the presence of calcium in nasal environment. The mucoadhesive agents that can form complexes with calcium like polyacrylic acid decrease the ciliary beating frequency thus prolonging residence time. The most common way to achieve mucoadhesion is by addition of polymer to the formulation. Mucoadhesive materials are hydrophilic macromolecules containing numerous hydrogen bond-forming groups as in the cases of carbomers and chitosans. Mucoadhesive have been used to improve local and systemic delivery of therapeutic compounds. There are various examples in the literature indicating the specific applications of mucoadhesive compounds with respect to nasal administration of small organic molecules, antibiotics, vaccines, DNA, proteins and other macromolecules. Morphine when given orally, the bioavailability is approximately 20–32%. It was demonstrated by Illum and associates that chitosan-based mucoadhesive formulation of the morphine resulted in rapid absorption of the compound (T_{max} of 15 min) with a bioavailability of about 60% which was limited to 20-32% by oral route (Illum et al., 2002). The plasma profiles after nasal administration with chitosan were better than simple liquid formulations, but similar to those obtained after intravenous doses. Lim and associates prepared and evaluated mucoadhesive microspheres of hyaluronic acid and chitosan for nasal delivery of gentamicin and other drugs. The study showed that hyaluronic acid and hyaluronic acid/chitosan microspheres could adhere to the nasal mucus (Lim et al., 2000). Subsequently, the authors showed that hyaluronic acid and chitosan may be employed for nasal administration of antibiotics to obtain a high bioavailability and prolonged release (Lim et al., 2001). Successful nasal delivery of other antibiotics such as vancomycin and tobramycin with chitosan has been reported as well (Cerchiara et al., 2003). It was shown that the presence of chitosan salts slow down the release of vancomycin hydrochloride at pH 5.5 and pH 7.4, thus guaranteeing a sustained release at acidic and alkaline pH of drug in the nasal cavity.

1.2.1. Nanoparticles as a drug delivery approach

The development of nanoparticles for drug delivery began in the 1960s
Chapter -I

Introduction

Kreuter, 2007). Nanoparticles most commonly refer to solid colloidal particles made of macromolecular material ranging in size from 1 to 1000 nm (1 mm), although, depending on the context, the term sometimes identifies particles in the 1 to 200 nm range (Kreuter, 1991). The term nanoparticle is a collective name for both nanospheres and nanocapsules. Nanospheres have a matrix type of structure. Drugs may be absorbed at the sphere surface or encapsulated within the particle. Nanocapsules are vesicular systems in which the drug is confined to a cavity consisting of an inner liquid core surrounded by a polymeric membrane (Couvreur et al., 1995). In this case the active substances are usually dissolved in the inner core but may also be adsorbed to the capsule surface (Allemann et al., 1993). Incorporating therapeutics with polymeric nanoparticles offers additional degrees of manipulation for delivery systems, providing sustained release and the ability to target specific cells and organs. Therapeutically used polymeric nanoparticles are composed of biodegradable materials, for example synthetic polymers such as poly (lactide-co-glycolide) (PLGA), which degrade into biocompatible products in the body and are resorbable through natural pathways (Anderson and Shive, 1997).

1.2.1.1. Advantages of using nanoparticles as a drug delivery system

Nanoparticles drug delivery system has outstanding advantages:

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.

2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.

3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.

4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.

5. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.
6. They can pass through the smallest capillary vessels because of their ultra-tiny volume and avoid rapid clearance by phagocytes so that their duration in bloodstream is greatly prolonged.

7. They can penetrate cells and tissue gaps to arrive at target organs such as liver, spleen, lung, spinal cord and lymph.

8. They can improve the utility of drugs and reduce toxic side effects.

1.2.1.2. Limitations of nanoparticulate system

In spite of the above advantages, nanoparticles do have limitations. The major practical problems have to be overcome before nanoparticles can be used clinically or made commercially available. Following are the limitations associated with the nanoparticulate system:

1. Their small size and large surface area can lead to particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms.

2. Small particles size and large surface area readily result in limited drug loading and burst release.

1.2.1.3. Polymeric nanoparticles

Nanoparticles are defined as solid, submicron-sized drug carriers that may or may not be biodegradable (Couvreur, 1988; Couvreur et al., 1995). Nanoparticles are receiving considerable attention for the delivery of therapeutic drugs. The literature emphasizes the advantages of nanoparticles over microparticles (McClean et al., 1998) and liposomes (Soppimath et al., 2001).

As drug delivery system, nanoparticles can entrap drugs or biomolecules into their interior structures and/or absorb drugs or biomolecules onto their exterior surfaces. Presently, nanoparticles have been widely used to deliver drugs, polypeptides, proteins, vaccines, nucleic acids, genes and so on. Over the years, nanoparticles drug delivery systems have shown huge potential in biological, medical and pharmaceutical applications (Illum, 2007). Currently, the researches on nanoparticle drug delivery system focus on the selectness and combination of carrier materials to obtain suitable drug release speed; the surface modification of nanoparticles to improve their targeting ability; the optimization of the preparation of nanoparticles to increase their drug delivery capability, their application in clinics and the possibility of industrial
production; and the investigation of \textit{in vivo} dynamic process to disclose the interaction of nanoparticles with blood and targeting tissues and organs, etc.

Polymeric materials used for preparing nanoparticles for drug delivery must be biocompatible at least and biodegradable best. To this aim, many polymeric materials have been applied, including poly (lactic acid), poly (glycolic acid), polycaprolactone, polysaccharides (particularly chitosan), poly (acrylic acid) family, proteins or polypeptides (such as gelatin), etc. Among them, polysaccharides are the most popular polymeric materials to prepare nanoparticles for drug delivery.

Polysaccharides are the polymers of monosaccharides. In nature, polysaccharides have various resources from algal origin (e.g., alginate), plant origin (e.g., pectin, guar gum), microbial origin (e.g., dextran, xanthan gum), and animal origin (chitosan, chondroitin) (Sinha and Kumria, 2001).

Polysaccharides have a large number of reactive groups, a wide range of molecular weight (MW), varying chemical composition, which contribute to their diversity in structure and in property. Polysaccharides can be divided into polyelectrolytes and nonpolyelectrolytes, the former can be further divided into positively charged polysaccharides (chitosan) and negatively charged polysaccharides (alginate, heparin, hyaluronic acid, pectin, etc.).

Advantages of polysaccharides polymer in drug delivery system:

1. Due to the presence of various derivable groups on molecular chains, polysaccharides can be easily modified chemically and biochemically, resulting in many kinds of polysaccharide derivatives.

2. As natural biomaterials, polysaccharides are highly stable, safe, non-toxic, hydrophilic and biodegradable.

3. Polysaccharides have abundant resources in nature and low cost in their processing. Particularly, most of natural polysaccharides have hydrophilic groups such as hydroxyl, carboxyl and amino groups, which could form non-covalent bonds with biological tissues (mainly epithelia and mucous membranes), forming bioadhesion (Lee et al., 2000) e.g., chitosan, starch, alginate and so on are good bioadhesive materials.
4. Nanoparticle carriers made of bioadhesive polysaccharides could prolong the residence time and therefore increase the absorbance of loaded drugs.

All these merits endow polysaccharides a promising future as biomaterials. In recent years, a large number of studies have been conducted on polysaccharides and their derivatives for their potential application as nanoparticle drug delivery systems (Rubinstein, 2000; Sinha and Kumria, 2001; Vandamme et al., 2002; Lemarchand, 2004).

1.2.1.4. Natural biodegradable polymers used to prepare nanoparticles

(i) Agarose

Agarose nanoparticles were developed for the administration of therapeutic proteins and peptides (Wang and Wu, 1997). Agarose aqueous solution forms thermally reversible hydrogels while being cooled below the gelling temperature (318–368 °C). Thermal gelation results from the formation of helicoidal structures responsible for a three-dimensional network in which large amounts of water can be entrapped. The hydrogel, being hydrophilic, inert, and biocompatible, forms a suitable matrix for proteins and peptides that can be entrapped in the gel during formation (Vauthier and Couvreur, 2000). Agarose nanoparticles were produced using an emulsion-based technology. This methodology requires the preparation of an agarose solution in corn oil emulsion at 408 °C (Wang and Wu, 1997). Peptides and proteins to be encapsulated are initially added to the agarose solution. The small size of the dispersed aqueous nanodroplets is achieved by homogenization. Gelation of agarose is then induced by diluting the emulsion with cold corn oil under agitation at 58 °C. The liquid nanodroplets then gel to protein-containing agarose hydrogel nanoparticles.

(ii) Alginates

Alginates are linear, unbranched polysaccharides composed of random chains of guluronic and mannuronic acids. In calcium ion containing aqueous media, the sodium ions from salts of these anionic, heteropolymers exchange with divalent cations, to form water-insoluble gels (Rajaonarivony et al., 1993). Because of the favourable conditions during manufacture, alginates are ideal carriers for oligonucleotides (Gonzalez et al., 2002), peptides (Wee et al., 1998), proteins (Wee et al., 1998),
water-soluble drugs, or drugs that degrade in organic solvents. Alginates are non immunogenic and available in a wide range of molecular weights as characterized by their inherent viscosity. These cations cross-link the guluronic and mannuronic acids to form an egg-box structure that forms the core of the gel matrix. Therapeutic agents are released in vivo when the matrix re-dissolves due to the reversible exchange of divalent cations with monovalent ions, especially sodium present in physiological fluid. A disadvantage of the use of alginates is that this reversible ion exchange may result in the rapid release of the therapeutic agent (Tonnesen and Karlsen, 2002). However, an example of the use of alginate nanoparticles to sustain antibacterial drug levels above the minimum inhibitory concentration in the liver, lungs, and spleen after pulmonary administration was demonstrated using isoniazid, rifampicin, and pyrazinamide (Zahoor et al., 2005). One method to prolong release from alginate particles is to coat them with a cationic polymer, for example, poly-l-lysine or chitosan. In this application, the mass ratio of alginate to cationic polymer is critical in terms of release characteristics and particle size (De et al., 2003).

(iii) Pullulan

Similar to dextran and cellulose, the glucans in pullulan are water-soluble, linear polysaccharides that consist of three \( \alpha-1, 4 \)-linked glucose molecules polymerized by \( \alpha-1,6 \) linkages on the terminal glucose (Wolf et al., 2003). Pullulan is a fermentation product of the yeast *Aureobasidium pullulans*. When made hydrophobic by acetylation, these polymers will self-associate to form nanoparticles with a hydrophobic core that will encapsulate hydrophobic drugs. Pullulan nanoparticles have been prepared by dialysis of an organic solution against water. In one method, a reverse micellar solution of the anionic surfactant, Aerosol OT, in n-hexane is prepared and an aqueous solution of the drug and pullulan is added (Gupta and Gupta, 2004). The nanoparticles are stabilized by cross-linking with glutaraldehyde. These delivery systems have been used in delivering cytotoxic drugs, genes and as pH-sensitive delivery systems.

(iv) Chitosan

Chitosan is a linear copolymer of \( \beta-(1–4) \) linked 2-acetamido-2-deoxy-\( \beta \)-D-glucopyranose and 2- amino-2-deoxy- \( \beta \)-D-glycopyranose (Fig. 1.3). It is obtained by deacetylation of its parent polymer chitin, a polysaccharide widely
distributed in nature (e.g., crustaceans, insects and certain fungi) (Dash et al., 2011).
Due to chitin’s poor solubility in aqueous solution and organic solvents, it does not find
practical applications whereas chitosan which is an artificial variant of chitin is more
suitable for bio applications (Mima et al., 1983). Chitosan is readily soluble in dilute
acidic solutions below pH 6.0 due to the quaternisation of the amine groups that have a
pK$_a$ value of 6.3 making chitosan a water-soluble cationic polyelectrolyte. The
presence of the amino groups indicates that pH substantially alters the charged state
and properties of chitosan (Yi et al., 2005). At low pH, these amines get protonated
(Fig. 1.4) and become positively charged and that makes chitosan a water-soluble
cationic polyelectrolyte. On the other hand, as the pH increases above 6, chitosan’s
amines become deprotonated and the polymer loses its charge and becomes insoluble.
The soluble–insoluble transition occurs at its pK$_a$ value around pH between 6 and 6.5.

The positive facets of excellent biocompatibility and admirable biodegradability with
ecological safety and low toxicity with versatile biological activities such as
antimicrobial activity and low immunogenicity have provided ample opportunities for
its further development (Hirano, 1999; Yi et al., 2005; Jayakumar et al., 2007;
Kurita, 2008; Mourya and Inamdar, 2008; Rinaudo, 2008)

![Fig.1.3. (a) Structure of chitosan [poly (h1– 4-d-glucosamine)]. (b) Structure of
cross-linked chitosan (Agnihotri et al., 2004)](image)

Chitosan-based nano systems have shown a great potential for nasal drug delivery.
Two different nanostructures have been assayed for this modality of
administration: nanogels made of solely chitosan and nanosystems consisting of a
hydrophobic core and a chitosan coating. The formation of chitosan nanoparticles, also
called nanogels, is based on the ability of chitosan to gel upon contact with specific
polyanions (Calvo et al., 1997). Proteins such as tetanus toxoid and the peptide insulin
are examples of macromolecules that have been efficiently associated to them. The efficacy of chitosan nanoparticles for improving the nasal absorption of insulin was tested in rabbits. The results showed that these nanoparticles were able to increase significantly the hypoglycaemic response to nasally absorbed insulin (Fernandez-Urrusuno et al., 1999). The positive effect of chitosan in improving the nasal transport of macromolecules associated to nanosystems may be understood on the basis of the facilitated interaction and internalization of these nanosystems in the nasal epithelium, as it was shown by confocal fluorescence microscopy (Vila et al., 2004). The possible effect of chitosan in opening the tight junction between epithelial cells is currently under investigation. However, the results obtained until now suggest that this effect might be dependent on the physical presentation of chitosan in the nanosystem (as a solid nanomatrice or as a soluble coating).

1.2.1.5. Methods of Preparing Chitosan Micro/Nanoparticles

Different methods have been used to prepare CS particulate systems. Selection of any of the methods depends upon factors such as particle size requirement, thermal and chemical stability of the active agent, reproducibility of the release kinetic profiles, stability of the final product and residual toxicity associated with the final product. However, selection of any of these methods depends upon the nature of the active molecule as well as the type of the delivery device.
(i) Emulsion cross-linking

This method exploits the reactive functional amine group of chitosan to cross-link with the available reactive groups of the cross-linking agent. In this method, water-in-oil (w/o) emulsion is prepared by emulsifying the chitosan aqueous solution in the oil phase. A suitable surfactant is used to stabilize the aqueous droplets. Thereafter stable emulsion is cross-linked by using an appropriate cross-linking agent to harden the droplets. Microspheres are filtered and washed repeatedly with alcohol and then dried (Akbuga and Durmaz, 1994). This method is helpful in controlling the size of the particles by controlling the size of aqueous droplets. However, the particle size of final product is dependent on the extent of cross-linking agent used while hardening along with the speed of stirring. This method is schematically represented in Fig. 1.5. The emulsion cross-linking method has a few drawbacks. Besides being tedious it uses harsh cross-linking agents, which might possibly induce chemical reactions with the active agent. Moreover, complete removal of the unreacted cross-linking agent may be a challenge.

Sankar and associates used this method to prepare the chitosan based pentazocine microspheres for intranasal delivery. Formulation parameters such as drug loading, polymer concentration, stirring speed during cross-linking and oil phase were modified to develop microspheres having good in vivo performance. In vivo studies indicated a significantly improved bioavailability of pentazocine. In vitro release kinetic models indicated that these systems followed the diffusion controlled release kinetics (Sankar et al., 2001).

Fig. 1.5. Schematic representation of preparation of chitosan particulate systems by emulsion cross-linking method (Dash et al., 2011)
(ii) Coacervation/precipitation

The physicochemical property of chitosan is utilized in this method since it is insoluble in alkaline pH medium, but precipitates/coacervates when it comes in contact with alkaline solution. Chitosan solution is blown into an alkali solution like sodium hydroxide, methanol or ethanediamine using a compressed air nozzle to form coacervate droplets. Separation and purification of particles are performed by filtration/centrifugation followed by successive washing with hot and cold water. The method is schematically represented in Fig. 1.6. Variation in compressed air pressure or spray-nozzle diameter can be done to control the size of the particles. The drug release can be controlled by using appropriate cross-linking agent.

This technique has been used to prepare chitosan–DNA nanoparticles (Mao et al., 2001). Processing parameters such as concentrations of DNA, chitosan, sodium sulphate, temperature, pH of the buffer and molecular weights of chitosan and DNA have been investigated. The particle size was successfully optimized to 100-250 nm with a narrow distribution by keeping the amino to phosphate group ratio between 3 and 8 and chitosan concentration of 100 ng/mL. Surface charge of these particles was slightly positive with a zeta potential of 112–118 mV at pH lower than 6.0, and became nearly neutral at pH 7.2. Results indicated that the nanoparticles could partially protect the encapsulated plasmid DNA from nuclease degradation.

![Fig.1.6. Schematic representation of preparation of chitosan particulate systems by coacervation/precipitation method (Dash et al., 2011)](image)

(iii) Spray-drying

Spray-drying is a popular method to produce powders, granules or agglomerates from the mixture of drug and excipient solutions as well as suspensions. The method is
based on drying of atomized droplets in a stream of hot air. Briefly, chitosan is dissolved in aqueous acetic acid solution; drug is then dissolved or dispersed in the solution followed by the addition of a suitable cross-linking agent (Fig. 1.7). This solution or dispersion is then atomized in a stream of hot air which leads to the formation of small droplets, from which solvent evaporates instantaneously leading to the formation of free flowing particles (He et al., 1999). Various process parameters should be controlled to get the desired size of particles such as the size of nozzle, spray flow rate, atomization pressure, inlet air temperature and extent of cross-linking. This method is however more commonly used for the preparation of microparticles than for nanoparticles.

Huang and associates prepared betamethasone disodium phosphate chitosan microspheres by this method using type-A gelatin and ethylene oxide–propylene oxide block copolymer and poloxamer as modifiers (Huang et al., 2002). Investigation of the surface morphology and surface charges of the prepared microspheres were done which indicated that shape, size and surface morphology of the microspheres were significantly influenced by gelatin concentration. A good drug stability (less 1% hydrolysis product), high entrapment efficiency (95%) and positive surface charge (37.5 mV) was observed. *In vitro* drug release from the microspheres was related to gelatin content. The gelatin/chitosan ratio of 0.4–0.6 (w/w) showed a fairly prolonged release up to 12 h (Huang et al., 2002).
(iv) Emulsion-droplet coalescence method

This emulsion-droplet coalescence method, developed by Tokumitsu and associates, which combines the principles of both emulsion cross-linking and precipitation (Tokumitsu et al., 1999). Instead of cross-linking the stable droplets, precipitation is induced by allowing coalescence of chitosan droplets with sodium hydroxide droplets. Two separate emulsions are prepared, one containing aqueous solution of chitosan along with drug is produced in liquid paraffin oil, and another containing chitosan aqueous solution of sodium hydroxide is produced in the same manner. The emulsions are mixed under high-speed stirring; droplets of each emulsion collide at random and coalesce, forming small sized particles that precipitate (Fig. 1.8). The particle size increased with the decrease in degree of deacetylation of chitosan which in turn decreased the drug content. Completely deacetylated chitosan produced particle size of 452 nm with 45% drug loading. The efficiency of this method depends on the electrostatic interactions with the amino groups of chitosan, which could not have occurred if a cross-linking agent was used that blocked the free amino groups of chitosan. Thus, it was possible to achieve higher gadopentetic acid loading by using the emulsion-droplet coalescence method that did not involve the use of any cross-linking agent.

![Fig. 1.8. Schematic representation of preparation of chitosan particulate systems by emulsion-droplet coalescence method (Dash et al., 2011)](image-url)
(v) Ionic gelation

For the most part, complexation to prepare chitosan microspheres has attracted much attention since the process is very simple and mild (Polk et al., 1994; Liu et al., 1997). The reversible physical cross-linking by electrostatic interaction, instead of chemical cross-linking, decreases the potential toxicity impact of reagents and other undesirable effects. For example, the polyanion, tripolyphosphate (TPP), which interacts electrostatically with the cationic chitosan (Kawashima et al., 1985). After Bodmeier and associates (Bodmeier et al., 1989) reported the preparation of TPP–chitosan complex by dropping chitosan droplets into a TPP solution, many researchers have explored its potential pharmaceutical usage (Shiraishi et al., 1993; Sezer and Akbuga, 1995; Aydin and Akbuga, 1996; Calvo et al., 1997; Shu and Zhu, 2000). For ionic gelation, chitosan is dissolved in aqueous acidic solution which quaternizes the chitosan amino groups making it soluble; this solution is then added dropwise under constant stirring to polyanionic TPP solution. The complexation between oppositely charged species causes the chitosan to undergo ionic gelation and precipitate as spherical particles (Fig. 1.9).

Various formulations of chitosan nanoparticles produced by the ionic gelation of TPP and chitosan were studied by Xu and Du (Xu and Du, 2003). The spherical shaped particles, 20 - 200 nm in size, were observed by TEM. The factors that affected the release of bovine serum albumin (BSA) as a model protein have been studied which include molecular weight, degree of deacetylation and concentrations of chitosan and BSA, as well as the presence of polyethylene glycol (PEG) in the encapsulation medium.

Fig. 1.9. Schematic representation of preparation of chitosan particulate systems by ionic gelation method (Dash et al., 2011)
(iv) Reverse micellar method

Reverse micelles are thermodynamically stable liquid mixtures of water, oil and surfactant. These homogeneous and isotropic structure on microscopic scale into aqueous and oil microdomains are separated by a surfactant-rich film. The dynamic behavior of these reverse micelle systems provides very important characteristics.

The nanoparticles prepared by conventional emulsion polymerization methods are usually large (>200 nm), with a broad size range. Ultrafine polymeric nanoparticles with narrow size distribution could be achieved by using reverse micellar medium (Leong and Candau, 1982). Due to the Brownian motion of the micellar droplets, they undergo continuous coalescence followed by re-separation on a time scale that varies between millisecond and microsecond (Luisi et al., 1988). A rapid dynamic equilibrium maintains the size, polydispersity and thermodynamic stability of these droplets. To prepare reverse micelles, the surfactant is dissolved in an organic solvent followed by the addition of chitosan and drug under constant vortexing. To the transparent obtained solution, a cross-linking agent is added with constant stirring, continued overnight. The maximum amount of drug that can be dissolved in reverse micelles varies from drug to drug and has to be determined by gradually increasing the amount of drug until the clear microemulsion is transformed into a translucent solution.

This method is schematically represented in Fig. 1.10. Chitosan nanoparticles encapsulating doxorubicin–dextran conjugate was prepared by reverse micellar method (Mitra et al., 2001). The surfactant sodium bis (2-ethyl hexyl) sulfosuccinate (AOT) was dissolved in n-hexane.

![Fig.1.10. Schematic representation of preparation of chitosan particulate systems by reverse micellar method (Dash et al., 2011)](image-url)
(v) Sieving method

A simple, novel method to produce chitosan microparticles has been developed by Agnihotri and Aminabhavi (Agnihotri and Aminabhavi, 2004). This method, is devoid of tedious procedures and can be scaled up easily, microparticles are prepared by cross-linking a 4% acetic acid chitosan solution to form glassy hydrogels that are passed through a sieve (Fig. 1.11). Clozapine was incorporated into chitosan gel before cross-linking with 99% efficiency. Irregular shaped microparticles, ~540–700 nm, were formed on seiving and in vivo studies indicated a slow release of clozapine.

![Schematic representation of preparation of chitosan particulate systems by sieving method](Dash et al., 2011)

Fig.1.11. Schematic representation of preparation of chitosan particulate systems by sieving method (Dash et al., 2011)

1.3. Osteoporosis

Osteoporosis is a disease in which the density and quality of bone are reduced, leading to weakness of the skeleton and increased risk of fracture, particularly of the spine, wrist and hip. Osteoporosis and associated fractures are an important cause of mortality and morbidity. Osteoporosis is often called the "silent disease" because bone loss occurs without symptoms. In many cases, the first "symptom" is a broken bone. Patients with osteoporosis may not know that they have the disease until their bones become so weak that a sudden strain, bump, or fall causes a hip fracture or a vertebra to collapse. Collapsed vertebra may initially be felt or seen in the form of severe back pain, loss of height, or spinal deformities such as kyphosis, or severely stooped posture.

The World Health Organization defines osteoporosis on the basis of the number of standard deviations below the peak adult bone mass. Bone mineral density (BMD) that is less than 2.5 standard deviations below the mean for young people may be considered osteoporosis or established osteoporosis if the patient has sustained an
osteoporosis-related fracture.

<table>
<thead>
<tr>
<th>Category</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>BMD 1 SD below average peak bone mass or higher</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>BMD &lt;1 SD but &gt;2.5 SD below average peak bone mass</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>BMD &lt;2.5 SD below average peak bone mass</td>
</tr>
<tr>
<td>Severe osteoporosis</td>
<td>BMD &lt;2.5 SD below average peak bone mass with fragility fracture</td>
</tr>
</tbody>
</table>

BMD: Bone mineral density; SD: standard deviations

1.3.1. Pathophysiology

Osteoporosis is the most common metabolic bone disease, results due to a mismatch between bone resorption and bone formation. Bones are solid structures, but they are constantly being remodelled. Bone remodelling provides a mechanism for self-repair and adaptation to stress to the bone. This process has three primary functions i.e. to repair microdamage within the skeleton, maintain skeletal strength, and supply calcium from the skeleton to maintain serum calcium.

The process consists of the removal of bone by the osteoclasts, followed by the synthesis and mineralization of new bone matrix by the osteoblasts within the cavity created. This is a coordinated process orchestrated by basic multicellular units, which include groups of osteoblasts, osteoclasts and osteocytes along with supporting vascular, nervous, and connective tissue. Osteoclast degrades bone by attaching to a bone surface and secreting acids and enzymes into the mineralized bone surface (Suda et al., 1992). They are mobile cells and after eroding one pit in mineralized bone they may move to another site. Osteoblasts that descend from bone marrow-derived stem cells are the bone-forming cells which replace the removed bone. They synthesize collagen and other proteins and have an important role in the subsequent mineralization of the bone matrix (Owen, 1985). Osteocytes are mature osteoblasts that become trapped within calcified bone. They play an important role in the osteogenic response to mechanical stimuli, ‘sensing’ physical strains and initiating an appropriate modelling or remodelling response via the production of a cascade of chemical messengers (Lanyon, 1992). Under normal circumstances the sequence is always that of resorption followed by formation, and the amounts of bone resorbed and formed within individual
remodelling units are closely balanced.

With aging, less bone is formed by osteoblasts than is removed by osteoclasts. Estrogen deficiency increases osteoclast life span but decreases osteoblast life span, resulting in an increased rate of bone remodelling and a decreased rate of bone replacement by osteoblasts during menopause, which in turn increases the risk of osteoporosis in postmenopausal patients (Seeman, 2003).

The control of bone remodelling is complex, depending on both systemic and local factors and results from the interaction of mechanical stresses, systemic hormones and locally produced cytokines, prostaglandins and growth factors.

Mechanical stimuli are a major determinant of the size, shape and microarchitecture of bones during skeletal growth and they subsequently play an important role in the maintenance of bone mass. The systemic calcium regulating hormones that influence bone remodelling include parathyroid hormone (PTH), calcitonin and vitamin D metabolites. PTH is a hormone synthesized by the chief cells of the parathyroid gland and it has effects on both osteoclasts and osteoblasts. In addition, physiologically PTH is the most important regulator of extracellular calcium concentration and therefore some of its effects on bone are indirect. Calcitonin, C cells of the thyroid gland produce calcitonin. It directly inhibits osteoclastic bone resorption and does not increase the deposition of calcium in the skeleton unless bone turnover is high. Vitamin D is hydroxylated initially by the liver and its activity is subsequently regulated in the kidney where the active metabolite 1, 25-dihydroxyvitamin D is produced. It is this type of vitamin D that acts on the intestine and skeleton to maintain blood calcium supply. Other hormones that have impact on the remodelling cycle include the growth hormone and sex hormones. Growth hormone plays an important part in skeletal growth, increasing bone turnover, with a net increase in bone mass and in periosteal appositional growth. Sex hormones have marked effects on the skeleton and deficiencies are associated with low bone mass in both children and adults. Oestrogens act on the skeleton via the osteoblasts that contain nuclear receptors. Glucocorticoid receptors occur in bone cells and glucocorticoids have both direct and indirect effects on the skeleton. Whereas normal physiological concentrations play a positive role in osteoblastic function, high doses suppress osteoblastic bone formation, impair skeletal growth, decrease calcium absorption and decrease bone mass. Cytokines are locally
active factors formed by immunologically competent cells. Although the effects of cytokines are not clearly understood, they appear to act both directly and indirectly on the remodelling cycle.

1.3.2. Consequences of osteoporosis

Osteoporosis is a silent disease unless complicated by a bone fracture, which will then cause pain or deformity. After the age of 50 years, the risk of sustaining fractures due to this is 40% in women and 15% in men. This risk is termed as ‘lifetime fracture risk’. It greatly increases the risk of fracture, notably, forearm (Colle’s) fracture, hip fracture and vertebral fracture.

Osteoporosis then finally leads to increased mortality. The mortality is increased by 20% in the first year after a hip fracture. It is also increased after vertebral fracture, possibly as a result of diseases that increase the risk of fractures and death. The pain usually occurs in the early stages following vertebral fracture and subsides after 3 months. Prolonged pain may result from secondary osteoarthritis. Pain can also occur when the costal margin impinges on the pelvic brim. The deformities include kyphosis, loss of height and abdominal protrusion. The loss of independence has a considerable financial impact, because it may necessitate long-term community support or care in a nursing home after hip fracture.

1.3.3. Management and prevention of osteoporosis

Risk of osteoporotic fracture may be reducing by various non pharmacologic and pharmacologic (Table 1.4) interventions. These interventions slow or stop bone loss, maintain bone strength, increase bone strength, or minimize or eliminate factors that may result in fractures. Non pharmacologic measures are recommended for the population as a whole; pharmacologic interventions are usually reserved for patients at increased risk of fractures.

1.3.3.1. Non-pharmacologic interventions

There are numerous non pharmacologic interventions that can be implemented to reduce the risk of bone loss and fracture. It is generally recommended that these interventions be recommended for the entire population, not just patients with osteoporosis.
Non-pharmacologic interventions to reduce fracture risk are the following:

- Regular weight-bearing exercise,
- Muscle strengthening,
- Fall prevention,
- Avoidance of tobacco,
- Avoidance of excessive alcohol intake,
- Balanced diet, and
- Adequate calcium and vitamin D intake.

1.3.3.2. Pharmacologic interventions

The drugs which are used to treat osteoporosis increase BMD by inhibiting bone resorption while allowing bone formation to fill in pre-existing resorption cavities. These drugs prevent bone loss and, during a 3 to 5 year period, may increase BMD by up to 10% (estrogen, bisphosphonates, calcitonin, and selective estrogen receptor modulators). However, there is a potential of optimizing osteoporosis therapy by using bone anabolic agents that directly stimulate bone formation (intermittent injections of parathyroid hormone, sodium fluoride, and strontium ranelate).
Table 1.4. US Food and Drug Administration-approved pharmacologic agents for the treatment and/or prevention of osteoporosis (Levine, 2006)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Indication</th>
<th>Dosage form</th>
<th>Innovator</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bisphosphonates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Alendronate | • Treatment and prevention of osteoporosis in postmenopausal women  
• Treatment of men with osteoporosis  
• Treatment of glucocorticoid-induced osteoporosis  
• Treatment of Paget's disease | Tablets/ oral solution | Fosamax® (Merck & Co., Inc., Whitehouse Station, New Jersey) |
| Ibandronate | • Treatment and prevention of osteoporosis in postmenopausal women | Tablets/ intravenous | Boniva® (Roche Therapeutics Inc., Nutley, New Jersey)  
The intravenous formulation of ibandronate is approved only for treatment and prevention of osteoporosis in postmenopausal women |
| Risedronate | • Treatment and prevention of osteoporosis in postmenopausal women  
• Treatment of men with osteoporosis  
• Prevention and treatment of glucocorticoid-induced osteoporosis  
• Treatment of Paget's disease | Tablets | Actonel® (Proctor & Gamble Pharmaceuticals, Cincinnati, Ohio) |
| **Selective estrogen-receptor blocker** | | | |
| Raloxifene | • Treatment and prevention of osteoporosis in postmenopausal women | Tablets | Evista® (Eli Lilly and Company, Indianapolis, Indiana) |
| **Estrogens** | | | |
| Various products | • Prevention of osteoporosis in postmenopausal women | Tablets/ transdermal | Estrace® (Barr Laboratories, Inc. Pomona, NY)  
Climara (estradiol transdermal system, Bayer HealthCare Pharmaceuticals Inc. Wayne, NJ)  
Estraderm® (estradiol transdermal system, Novartis Pharmaceuticals Corporation, East Hanover, New Jersey) |
| **Calcitonin** | | | |
| Salmon calcitonin | • Treatment of osteoporosis in postmenopausal women  
• Treatment of symptomatic Paget's disease  
• Treatment of hypercalcemia | Intranasal/ subcutaneous/ intramuscular | Miacalcin® (Novartis Pharmaceuticals Corporation, East Hanover, New Jersey). The intranasal formulation of salmon calcitonin is approved only for treatment of osteoporosis in postmenopausal women |
| **Recombinant human parathyroid hormone** | | | |
| Teriparatide | • Treatment of osteoporosis in postmenopausal women  
• Treatment to increase bone mass in men with primary or hypogonadal osteoporosis | Subcutaneous | Forteo® (Eli Lilly and Company, Indianapolis, Indiana) |