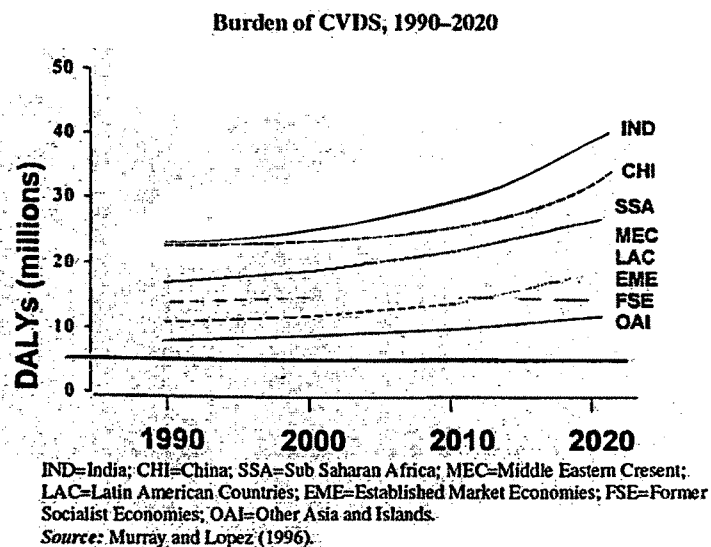


## **2.0 LITERATURE REVIEW**

## 2.1 Cardiovascular diseases:

Cardiovascular diseases occupy the number one position in the morbidity and mortality statistics in most industrialized countries of the world (WHO, 2005). The two leading causes of death, coronary heart disease and stroke are currently responsible for 12 million deaths (22% of the 55 million). Seven million deaths are due to coronary heart disease (CHD) and five million to stroke and other six million are due to other causes of cardiovascular diseases. Fig. 2.1 shows comparative forecasting of burden of cardiovascular diseases 1990-2020. The pattern will changed in 2020 with CHD and stroke remaining the two leading causes of death and together will be one of the leading causes of disability adjusted life years lost (DALY's) (Evans, 2000). By 2020, cardiovascular diseases are expected to account for 7 out of every 10 deaths in the developing countries compared with less than half this value today (Khor, 2001).

The prevalence of coronary artery disease has been increasing in India over the past few decades. WHO estimates that 60 % of the world's cardiac patients will be Indian by 2010. Nearly 50 per cent of cardiovascular diseases-related deaths in India occur below the age of 70, compared with just 22 % in the West (Pande, 2004).



DALYs- Disability Adjusted Life Years

**Fig 2.1 COMPARATIVE FORECASTING OF BURDEN OF CARDIOVASCULAR DISEASES 1990-2020 (Evans, 2000)**

### **2.1.1 Cardiac Heart Failure:**

Cardiac Heart Failure is also one of the most common causes of death and disability in industrialized nations and is among the syndromes most commonly encountered in clinical practice. The diagnosis of heart failure carries a risk of mortality comparable to that of the major malignancies. Patients with newly diagnosed heart failure have an average 5 year survival of only 35% (Goodmann and Gilmann, 2001).

Cardiac Heart Failure is a progressive syndrome resulting from the heart's inability to adequately perfuse and oxygenate peripheral tissues. This syndrome is manifested by symptoms of fatigue, dyspnea and congestion. Cardiac Heart Failure is associated with worsening ventricular dysfunction and pathologic ventricular remodeling, resulting in adverse hemodynamic changes (Eichhorn, 1994; Cohn, 1996).

Drugs used in Cardiac Heart Failure (Rang et al., 2003):

1.  $\beta$ -blockers e.g. Carvedilol.
2. Loop diuretics e.g. furosemide
3. Angiotensin-converting enzyme inhibitors e.g. captopril
4. Organic nitrates e.g. isosorbide mononitrate.

Out of above listed classes of drugs,  $\beta$ -blockers used to treat cardiac heart failure and their beneficial effects have been documented for several decades. Recent clinical trials showed that  $\beta$ -blockers markedly reduce mortality and reduce left ventricular function in cardiac heart failure patients (Sallach and Goldstein, 2003).

Carvedilol is indicated in the treatment of mild to moderate congestive heart failure, alone or in combination with other agents (e.g. digitalis, diuretics and ACE inhibitors). Carvedilol has also used for the management of essential hypertension (Vela, 1998). In studies that compared the acute haemodynamic effects of Carvedilol to baseline measurements in patients with congestive heart failure, there were significant reductions in systemic blood pressure, pulmonary artery pressure, pulmonary capillary wedge pressure and heart rate (Goodmann and Gilmann, 2001).

### **2.1.2 Atherosclerotic vascular disease:**

Atherosclerotic vascular disease is responsible for nearly 75% of all deaths from cardiovascular diseases, and it is the leading cause of death for both men and women in India. Elevated cholesterol, specifically cholesterol contained in low-density-lipoprotein

(LDL) particles, is an important risk factor for the development of atherosclerotic vascular disease ([www.americanheart.org/presenter](http://www.americanheart.org/presenter), 2004).

An elevated low-density lipoprotein (LDL) cholesterol level is a key risk factor for coronary heart disease (CHD). Despite multiple randomized trials showing that a reduction in an elevated LDL level lowers cardiovascular morbidity and mortality, most patients with high LDL levels remain unidentified or untreated (Sacks et al., 1996).

Statins have become the first-line agents for primary and secondary prevention of CHD in patients with elevated LDL levels because of their effectiveness, tolerability and safety. The statins work by blocking enzyme HMG-CoA reductase which assists in the manufacture of cholesterol. Upon blocking HMG-CoA reductase, there is reduction in cholesterol production. As a result of this reduction, greater number of LDL receptors is created thereby increasing the uptake of LDL-c. This reduction in cholesterol production results in reduced LDL-c, total cholesterol, and triglycerides and slightly increases high-density lipoprotein (HDL-c). Lipid-lowering drug therapy for primary CHD prevention is most clearly indicated when two or more CHD risk factors are present and the LDL remains higher than 160 mg per dL (4.15 mmol per L) after an adequate dietary trial (Shepherd et al., 1995).

**Table 2.1 Pharmacokinetic properties of statins (Chong et al., 2001)**

Parameter	Atorvastatin	Fluvastatin	Lovastatin	Pravastatin	Rosuvastatin	Simvastatin
Bioavailability	14%	24%	< 5%	17%	Unknown	< 5%
Excretion						
Renal	< 2%	5%	10%	20%	10%	13%
Feces	98%	90%	83%	70%	90%	60%
Metabolic enzymes	CYP 3A4	CYP 2C9	CYP 3A4	Sulfation	CYP 2C9, 2C19	CYP 3A4
Metabolites contributing to lipid lowering effect	Yes	No	Yes	No	No	Yes
Hepatic first pass effect	20-30%	40-70%	40-70%	50-70%	Unknown	50-80%
Lipophilicity	Lipophilic	Hydrophilic	Lipophilic	Hydrophilic	Hydrophilic	Lipophilic

The available statins (in order of labeling by the U.S. Food and Drug Administration) include lovastatin, Pravastatin sodium, simvastatin, fluvastatin, atorvastatin and rosuvastatin (Chong et al., 2001). Table 2.1 shows the pharmacokinetic properties of statins. The choice of statins is usually based on the clinician's judgment of the relative importance of three factors: evidence of beneficial clinical outcomes, efficacy for lowering LDL and cost.

Evidence for benefits in clinical outcome is strong for Pravastatin sodium (Crouch, 2001). A reduction in elevated LDL levels with Pravastatin sodium has been shown to significantly reduce coronary events in individuals without CHD.

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## **2.2 Oral Mucosal Drug Delivery:**

### **2.2.1 Introduction:**

Amongst the various routes of drug delivery, oral route is perhaps the most preferred to the patient and the clinician alike. However, peroral administration of a number of drugs suffers from disadvantages such as hepatic first pass metabolism and enzymatic degradation within the GIT, which may limit their oral administration. Consequently, other absorptive sites have to be considered for drug administration. Transmucosal routes of drug delivery (i.e., the mucosal linings of the nasal, rectal, vaginal, ocular, and oral cavity) offer distinct advantages over peroral administration for systemic drug delivery. These advantages include possible bypass of first pass effect, avoidance of presystemic elimination within the GIT, and, depending on the particular drug, a better enzymatic flora for drug absorption.

The nasal cavity as a site for systemic drug delivery has been investigated by many research groups (Rajinikanth et al., 2003; Preda and Leucuta, 2003; Tzachev et al, 2002) and the route has already reached commercial status with several drugs including LHRH (Behl et al., 1998) and calcitonin (Plosker and McTavish, 1996). However, the potential irritation and the irreversible damage to the ciliary action of the nasal cavity from chronic application of nasal dosage forms, as well as the large intra- and inter-subject variability in mucus secretion in the nasal mucosa, could significantly affect drug absorption from this site. Even though the rectal, vaginal, and ocular mucosae all offer certain advantages, but the poor patient acceptability associated with these sites renders them reserved for local applications rather than systemic drug administration. The oral cavity, on the other hand, is highly acceptable by patients, the mucosa is relatively permeable with a rich blood supply, it is robust and shows short recovery times after stress or damage (Rathbone et al., 1994; Vries et al., 1991). Also the virtual lack of Langerhans cells (Squier, 1991) makes the oral mucosa tolerant to potential allergens. Furthermore, oral transmucosal drug delivery bypasses first pass effect and avoids presystemic elimination in the GIT. These factors make the oral mucosal cavity a very attractive and feasible site for systemic drug delivery.

Within the oral mucosal cavity, delivery of drugs is classified into three categories: (i) sublingual delivery, which is systemic delivery of drugs through the mucosal membranes lining the floor of the mouth, (ii) buccal delivery, which is drug administration through the mucosal membranes lining the cheeks (buccal mucosa), and (iii) local delivery, which is drug delivery into the oral cavity.

**2.2.2 Advantages of mucoadhesive buccal delivery:** (Hoogstrate and Wertz, 1998; Junginger et al., 1999)

- 1) Bypasses the hepatic first pass metabolism.
- 2) Greater bioavailability and reduction in dosage.
- 3) Consistent and continuous supply of tissue, therefore rapid absorption.
- 4) Ability to readily manipulate experimental conditions.
- 5) Offers safe environment.
- 6) Virtual lack of langerhans cells makes it tolerant to potential allergens.
- 7) Facilitates intimate contact of the formulation with the underlying absorption surface.
- 8) Allows modification of tissue permeability for the absorption of macromolecules.
- 9) Decrease in overall use of medical resources.
- 10) Improved disease management.
- 11) Assurance of sustained release from the dosage forms and avoids dose dumping.
- 12) Offers passive system which does not require activation
- 13) Provide a means to confine and maintain high local concentrations of the drug and/or excipients to a defined, relatively small region of the mucosa in order to minimize loss to other regions and limit potential side effects.

**2.2.3 Disadvantages of mucoadhesive buccal delivery:** (Hoogstrate and Wertz, 1998; Junginger et al., 1999)

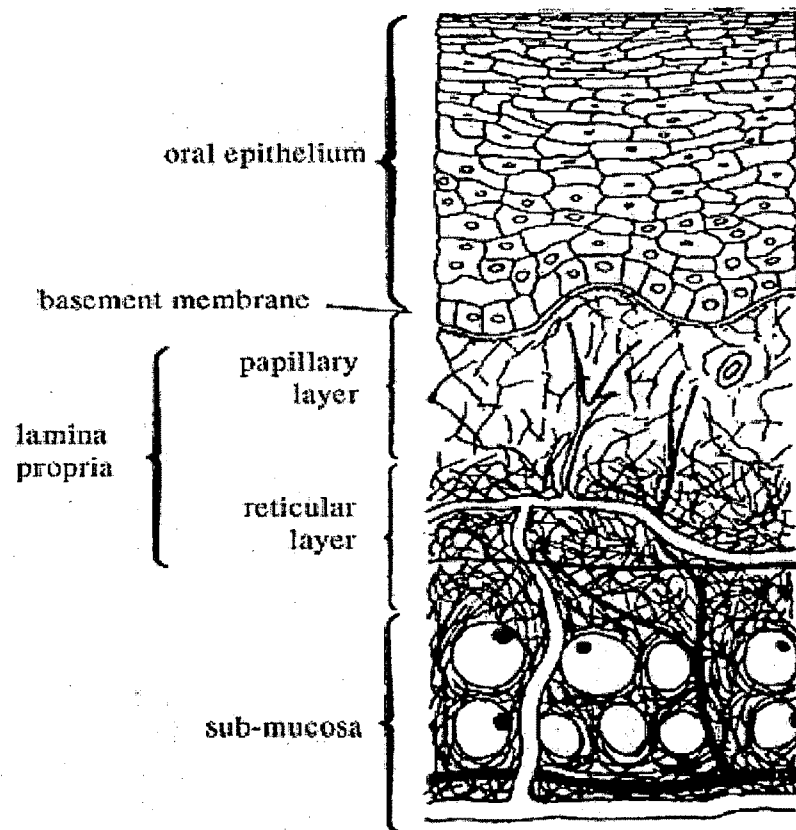
- 1) Barrier properties of the buccal mucosa make some compounds difficult to permeate rapidly.
- 2) Small absorption area.
- 3) Short residence time.

**2.2.4 Oral mucosa**

**2.2.4.1 Anatomy of the oral mucosa**

Light microscopy reveals several distinct patterns of maturation in the epithelium of the human oral mucosa based on various regions of the oral cavity. The oral cavity is lined with the epithelium, below which lies the supporting basement membrane. The basement membrane is, in turn, supported by connective tissues (Fig. 2.2).





**Fig 2.2 Anatomy of the oral mucosa**

The epithelium, as a protective layer for the tissues beneath, is divided into (a) non-keratinized surface in the mucosal lining of the soft palate, the ventral surface of the tongue, the floor of the mouth, alveolar mucosa, vestibule, lips, and cheeks, and (b) keratinized epithelium which is found in the hard palate and non-flexible regions of the oral cavity (Chen and Squier, 1984). The epithelial cells, originating from the basal cells, mature, change their shape, and increase in size while moving towards the surface. The thickness of buccal epithelium in humans, dogs, and rabbits has been determined to be approximately 500–800  $\mu\text{m}$  (Harris and Robinson, 1992). It provides the required adherence between the epithelium and the underlying connective tissues, and functions as a mechanical support for the epithelium. The underlying connective tissues provide mechanical properties to oral mucosa. The buccal epithelium is classified as a nonkeratinized tissue (Meyer and Gerson, 1964). It is penetrated by tall and conical-shaped connective tissues. These tissues, which are also referred to as the lamina propria, consist of collagen fibers, a supporting layer of connective tissues, blood vessels, and smooth muscles (Gandhi and Robinson, 1994). Membrane

coating granules (MCGs) are found in both keratinized and non-keratinized epithelia which are spherical or oval organelles that are 100–300 nm in diameter. The rich arterial blood supply to the oral mucosa is derived from the external carotid artery. The buccal artery, some terminal branches of the facial artery, the posterior alveolar artery, and the intraorbital artery are the major sources of blood supply to the lining of the cheek in the buccal cavity (Stablein M.J., 1984). A gel-like secretion known as mucus, which contains mostly water-insoluble glycoproteins, covers the entire oral cavity. Mucus is bound to the apical cell surface and acts as a protective layer to the cells below (Allen et al., 1984). It is also a visco-elastic hydrogel, and primarily consists of 1–5% of the above-mentioned water insoluble glycoproteins, 95–99% water, and several other components in small quantities, such as proteins, enzymes, electrolytes, and nucleic acids. This composition can vary based on the origin of the mucus secretion in the body (Lehr, 1996).

#### **2.2.4.2. Oral mucosa - Permeability barrier**

The effective permeability coefficient ( $P_{eff}$ ) values reported in the literature across the buccal mucosa for different molecules range from a lower limit of  $2.2 \times 10^{-9}$  cm/s for dextran 4000 across rabbit buccal membrane to an upper limit of  $1.5 \times 10^{-5}$  cm/s for both benzylamine and amphetamine across rabbit and dog buccal mucosa, respectively (Gandhi and Robinson, 1994). This range clearly demonstrates the presence of a permeability barrier in the oral mucosa, which is mostly imposed by the oral epithelium acting as a protective layer for the tissues beneath, and as a barrier to the entry of foreign material and microorganisms. However, this range is estimated to be 4–4000 times more permeable than that of skin (Galey et al., 1976).

The permeability barrier property of the oral mucosa is predominantly due to intercellular materials derived from the MCGs (Gandhi and Robinson, 1994). MCGs are spherical or oval organelles that are 100–300 nm in diameter and found in both keratinized and non-keratinized epithelia. These organelles have also been referred to as ‘small spherically shaped granules’, ‘corpusula’, ‘small dense granules’, ‘small lamellated bodies’, ‘lamellated dense bodies’, ‘keratinosomes’, ‘transitory dense bodies’, and ‘cementsomes’ (Hayward, 1979). However, most of these descriptive names have not fully defined the functions of this cellular species. MCGs were first named as such because it was believed that they were subject to exocytosis from the cytoplasm of the stratum spinosum of keratinized epithelia following thickening of these cells. Nonetheless, it is actually the contents of MCGs that are

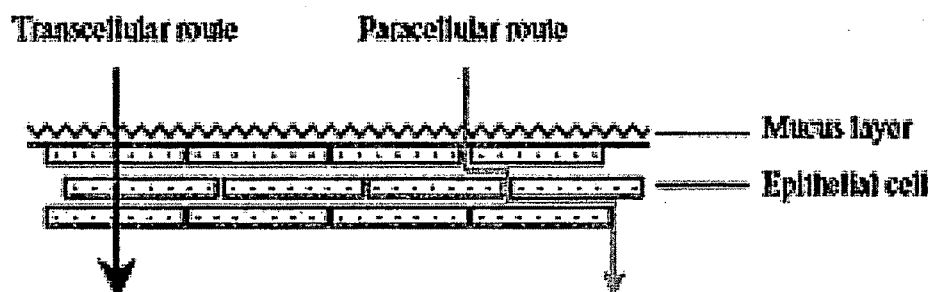
subject to exocytosis prior to the onset of membrane thickening. MCGs are found near the upper, distal, or superficial border of the cells, and a few occur near the opposite border. Several hypotheses have been suggested to describe the functions of MCGs, including a membrane thickening effect, cell adhesion, production of a cell surface coat, cell desquamation and permeability barrier.

The permeability barrier is most often attributed to MCGs (Hayward, 1979). They discharge their contents into the intercellular space to ensure epithelial cohesion in the superficial layers, and this discharge forms a barrier to the permeability of various compounds. Cultured oral epithelium devoid of MCGs has been shown to be permeable to compounds that do not typically penetrate oral epithelium (Squier et al., 1978). In addition, permeation studies conducted using tracers of different sizes have demonstrated that these tracer molecules did not penetrate any further than the top 1–3 cell layers. When the same tracer molecules were introduced sub-epithelially, they penetrated through the intercellular spaces. This limit of penetration coincides with the level where MCGs are observed. This same pattern is observed in both keratinized and nonkeratinized epithelia (Gandhi and Robinson, 1994), which indicates that keratinization of the epithelia itself, is not expected to play a major role as a barrier to permeation (Squier and Hall, 1984). Another barrier to drug permeability across buccal epithelium is enzymatic degradation. Saliva contains no proteases, but does contain moderate levels of esterases, carbohydrases, and phosphatases (Robinson and Yang, 2001). However, several proteolytic enzymes have been found in the buccal epithelium (Veuilleux et al., 2001).

#### **2.2.5 Buccal routes of drug absorption**

Fig. 2.3 shows the buccal routes of drug absorption. There are two permeation pathways for passive drug transport across the oral mucosa: paracellular and transcellular routes. Permeants can use these two routes simultaneously, but one route is usually preferred over the other depending on the physicochemical properties of the diffusant. Since the intercellular spaces and cytoplasm are hydrophilic in character, lipophilic compounds would have low solubilities in this environment. The cell membrane, however, is rather lipophilic in nature and hydrophilic solutes will have difficulty permeating through the cell membrane due to a low partition coefficient. Therefore, the intercellular spaces pose as the major barrier to permeation of lipophilic compounds and the cell membrane acts as the major transport barrier for hydrophilic compounds. Since the oral epithelium is stratified, solute

permeation may involve a combination of these two routes. The route that predominates, however, is generally the one that provides the least amount of hindrance to passage (Shojaei, 1998).



**Fig. 2.3 Buccal routes of drug absorption** (Shojaei, 1998)

#### **2.2.6 Mucoadhesion:**

Bioadhesion may be defined as the state in which two materials, at least one of which is biological in nature, are held together for extended periods of time by interfacial forces. In the pharmaceutical sciences, when the adhesive attachment is to mucus or a mucous membrane, the phenomenon is referred to as mucoadhesion (Gu et al., 1988).

##### **2.2.6.1 Significance of mucoadhesion**

Over the last two decades, mucoadhesion has gained significant interest for its potential to optimize drug delivery, by retaining a dosage form at the site of action. When mucoadhesion is utilized, the residence time of dosage forms on the mucosa can be significantly prolonged, allowing a sustained drug release at a given target site or sustained systemic delivery can be accomplished whereas in non-adhesive dosage forms localization of delivery system is not possible which fails to target the drug at a specified site.

Mucoadhesion can guarantee an intimate contact with the absorption membrane, providing the basis for a high concentration gradient as a driving force for passive drug uptake. Moreover, due to this intimate contact pre-systemic metabolism, such as the degradation of orally administered peptide drugs by lumenally secreted intestinal enzymes can be avoided. Non-adhesive systems do not allow intimate contact with absorption membrane and drug is directly exposed to variety of enzymes, undergoes enzymatic degradation and results in low bioavailability.

Also, interactions of the polymer with the epithelium, such as a permeation enhancing effect or the inhibition of brush border membrane-bound enzymes, become feasible but in non-adhesive system such effects are not possible (Bernkop, 2005).

#### **2.2.6.2 The mucoadhesive-mucosa theories**

A strong mucoadhesive-mucosa interaction is necessary for strong mucoadhesion which is important prerequisite for mucoadhesive drug delivery. These interactions can be classified as chemical bonds, electronic, wetting, diffusion, mechanical and fracture theory.

##### **2.2.6.2.1 Chemical bonds**

For adhesion to occur, molecules must bond across the interface. These bonds can arise in the following way.

- (1) Ionic bonds - where two oppositely charged ions attract each other via electrostatic interactions to form a strong bond (e.g. in a salt crystal).
- (2) Covalent bonds - where electrons are shared, in pairs, between the bonded atoms in order to 'fill' the orbitals in both. These are also strong bonds.
- (3) Hydrogen bonds - In this a hydrogen atom, when covalently bonded to electronegative atoms such as oxygen, fluorine or nitrogen, carries a slight positive charge and is therefore attracted to other electronegative atoms. The hydrogen can therefore be thought of as being shared, and the bond formed is generally weaker than ionic or covalent bonds.
- (4) Van-der-Waals bonds - These are some of the weakest forms of interaction that arise from dipole-dipole and dipole-induced dipole attractions in polar molecules, and dispersion forces with non-polar substances.
- (5) Hydrophobic bonds - These are indirect bonds (such groups only appear to be attracted to each other) that occur when non-polar groups are present in an aqueous solution. Water molecules adjacent to non-polar groups form hydrogen bonded structures, which lowers the system entropy. There is, therefore, an increase in the tendency of non-polar groups to associate with each other to minimise this effect.

There are six general theories of adhesion, which have been adapted for the investigation of mucoadhesion (Ahuja et al., 1997; Mathiowitz and Chikering, 1999; Peppas and Sahlin, 1996).

**2.2.6.2.2 Electronic theory:** It suggests that electron transfer occurs upon contact of adhering surfaces due to differences in their electronic structure. This is proposed to result in

the formation of an electrical double layer at the interface, with subsequent adhesion due to attractive forces.

**2.2.6.2.3 Wetting theory:** It is primarily applied to liquid systems and consider surface and interfacial energies. It involves the ability of a liquid to spread spontaneously onto a surface as a prerequisite for the development of adhesion. The affinity of a liquid for a surface can be found using techniques such as contact angle goniometry to measure the contact angle of the liquid on the surface, with the general rule being that the lower the contact angle, the greater the affinity of the liquid to the solid. The spreading coefficient ( $S_{AB}$ ) can be calculated from the surface energies of the solid and liquid using the equation:

$$S_{AB} = \gamma_B - \gamma_A - \gamma_{AB}$$

where  $\gamma_A$  is the surface tension (energy) of the liquid A,  $\gamma_B$  is the surface energy of the solid B and  $\gamma_{AB}$  is the interfacial energy between the solid and liquid.  $S_{AB}$  should be positive for the liquid to spread spontaneously over the solid. The work of adhesion ( $W_A$ ) represents the energy required to separate the two phases, and is given by:

$$W_A = \gamma_A + \gamma_B - \gamma_{AB}$$

The greater the individual surface energies of the solid and liquid relative to the interfacial energy, the greater the work of adhesion. The adsorption theory describes the attachment of adhesives on the basis of hydrogen bonding and van der Waals' forces. It has been proposed that these forces are the main contributors to the adhesive interaction. A subsection of this, the chemisorption theory, assumes that an interaction across the interface occurs as a result of strong covalent bonding.

**2.2.6.2.4 Diffusion theory:** It describes inter diffusion of polymer chains across an adhesive interface. This process is driven by concentration gradient and is affected by the available molecular chain lengths and their mobilities. The depth of interpenetration depends on the diffusion coefficient and the time of contact. Sufficient depth of penetration creates a semi-permanent adhesive bond.

**2.2.6.2.5 Mechanical theory:** It assumes that adhesion arises from an interlocking of a liquid adhesive into irregularities on a rough surface. However, rough surfaces also provide an increased surface area available for interaction along with an enhanced viscoelastic and plastic dissipation of energy during joint failure, which are thought to be more important in the adhesion process than a mechanical effect (Peppas and Sahlin, 1996).



**2.2.6.2.6 Fracture theory:** It differs a little from the other five in that it relates the adhesive strength to the forces required for the detachment of the two involved surfaces after adhesion. This assumes that the failure of the adhesive bond occurs at the interface. However, failure normally occurs at the weakest component, which is typically a cohesive failure within one of the adhering surfaces.

In the study of adhesion, generally two steps in the adhesive process have been identified (Wu S., 1982), which have been adapted to describe the interaction between mucoadhesive materials and a mucous membrane.

Step 1 —Contact stage: An intimate contact (wetting) occurs between the mucoadhesive and mucous membrane.

Step 2 —Consolidation stage: Various physicochemical interactions occur to consolidate and strengthen the adhesive joint, leading to prolonged adhesion.

#### **Step 1. The contact stage**

The mucoadhesive and the mucous membrane initially come together to form an intimate contact. In some cases, these two surfaces can be mechanically brought together, e.g. placing and holding a delivery system within the oral cavity. For smaller particles in suspension, adsorption onto the gastrointestinal mucosa would be an essential prerequisite for the adhesion process. Other examples where an adsorption step would be required would be the administration of nanoparticle suspensions to the precorneal region, or mouthwashes containing microparticles.

If a particle approaches a surface it will experience both repulsive and attractive forces. Repulsive forces arise from osmotic pressure effects as a result of the interpenetration of the electrical double layers, steric effects and also electrostatic interactions when the surface and particles carry the same charge. Attractive forces arise from van der Waals' interactions, surface energy effects and electrostatic interactions if the surface and particles carry opposite charges. The relative strength of these opposing forces will vary depending on the nature of the particles, the aqueous environment, and the distance between the particle and the surface.

For stronger adsorption to occur, particles have to overcome a repulsive barrier (the potential energy barrier) to get closer to the surface. If this barrier is sufficiently small or if the particle has sufficient energy, then adsorption into the primary minimum can occur. This type of adsorption would be required to allow a strong adhesive bond to form.

## Step 2. The consolidation stage

It has been proposed that if strong or prolonged adhesion is required, for example with larger formulations exposed to stresses such as blinking or mouth movements, then a second 'consolidation' stage is required. Mucoadhesive materials adhere most strongly to solid dry surfaces (Mortazavi S.A., 1995) as long as they are activated by the presence of moisture. Moisture will effectively plasticize the system allowing mucoadhesive molecules to become free, conform to the shape of the surface, and bond predominantly by weaker van der Waal forces and hydrogen bonding (Patel et al., 2003). In the case of cationic materials such as chitosan, electrostatic interactions with the negatively charged groups (such as carboxyl or sulphate) on the mucin or cell surfaces are also possible. The mucoadhesive bond is by nature very heterogenous, making it extremely difficult to use spectroscopic techniques to identify the type of bonds and groups involved although hydrogen bonds have been identified as being important. Polymer/mucosae interactions have been investigated by evaluating surface energies (Esposito et al., 1995; Rillosi and Buckton, 1995). It is also noticeable when undertaking tensiometer studies with these systems that the high affinity of materials like carbomers for water almost appears to have a 'suction-like' effect, helping to hold to formulation onto a solid surface. For surfaces with only limited amounts of mucus, a dry mucoadhesive polymer will almost certainly collapse the mucus layer by extracting the water component of the gel, allowing the polymer molecules the freedom to interact by hydrogen bonding with the epithelial surface (Smart, 1999).

However, when a substantial mucus layer is present, then the anti-adherent properties of mucus will need to be overcome if a strong adhesive joint is to be formed.

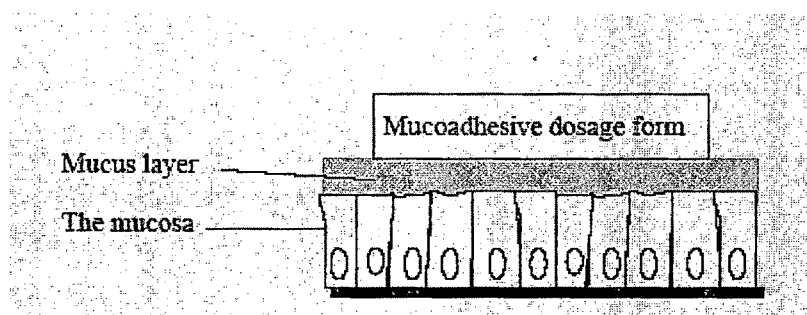
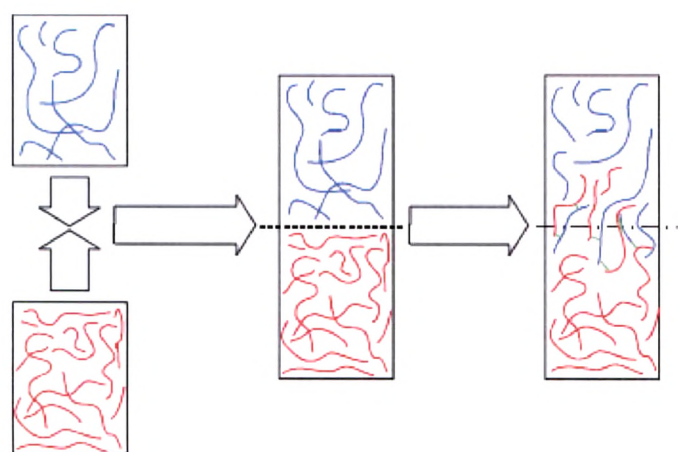


Fig 2.4 The three regions within mucoadhesive joint (Smart, 2005)

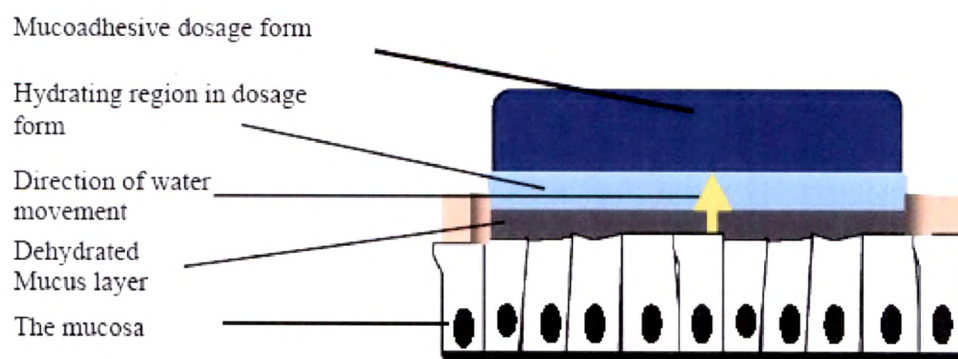


In this case the adhesive joint can be considered to contain three regions (Fig. 2.4), the mucoadhesive, the mucosa and an interfacial region, consisting at least initially of mucus. To achieve strong adhesion, a change in the physical properties of the mucus layer will be required, otherwise it will readily fail on application of a dislodging stress. There are essentially two theories as to how gel strengthening/consolidation occurs. One is based on a macromolecular interpenetration effect, which has been dealt with on a theoretical basis by Peppas and Sahlin (Peppas and Sahlin, 1996). In this theory, based largely on the diffusion theory for compatible polymeric systems, the mucoadhesive molecules interpenetrate and bond by secondary interactions with mucus glycoproteins (Fig. 2.5)



**Fig 2.5 The interpenetration theory; three stages in the interaction between a mucoadhesive polymer and mucin glycoprotein (Peppas and Sahlin, 1996).**

The second theory is the dehydration theory (Smart, 1999). When a material capable of rapid gelation in an aqueous environment is brought into contact with a second gel, water movement occurs between gels until equilibrium is achieved. A polyelectrolyte gel, such as a poly (acrylic acid) will have a strong affinity for water; therefore a high ‘osmotic pressure’ and large swelling force will develop (Silberberg-Bouhnik et al., 1995). When brought into contact with mucus gel, it will rapidly dehydrate that gel and force intermixing and consolidation of the mucus joint (Fig. 2.6) until equilibrium is reached. The movement of water from mucus into a poly (acrylic acid) film was observed by Jabbari et al. (Jabbari et al., 1993).



**Fig 2.6 The dehydration theory of mucoadhesion** (Smart, 1999).

A mucus gel, on dehydration, goes from having lubricant to the opposite adhesive properties, as observed in studies by Mortazavi and Smart (Mortazavi and Smart, 1995). The latter theory explains why mucoadhesion arises very quickly, within a matter of seconds, while the former requires two large macromolecules to interpenetrate several  $\mu\text{m}$  within a short time. The rheological synergy study suggests that as soon as mucus and mucoadhesive interpenetrate they are likely to interact and form a surface gel layer that will substantially inhibit any further interpenetration. However, the dehydration theory is limited to explaining the adhesion arising when a dry or partially hydrated formulation are brought into contact with a substantial mucus gel, and will not apply to the occasions where hydrated gels are involved.

#### **2.2.6.3 Factors affecting mucoadhesion**

Several factors have been identified as affecting the strength of mucoadhesion (Gu et al., 1988; Ahuja et al., 1997).

##### **2.2.6.3.1 Molecular weight:**

Many studies have indicated an optimum molecular weight for mucoadhesion, ranging from circa  $10^4$  Da to circa  $4 \times 10^6$  Da, although accurately characterizing the molecular weight of large hydrophilic polymers is very difficult. Larger molecular weight polymers will not hydrate readily to free the binding groups to interact with a substrate, while lower molecular weight polymers will form weak gels and readily dissolve.

##### **2.2.6.3.2 Flexibility of polymer chains:**

The flexibility of polymer chains is believed to be important for interpenetration and entanglement, allowing binding groups to come together. As the cross-linking of water-

soluble polymers increases, the mobility of the polymer chains decrease, although this could also have a positive effect in restricting over hydration.

#### **2.2.6.3.3 Presence of ionizable groups**

Mucoadhesive properties of polymers containing ionizable groups are affected by the pH of the surrounding media. For example, mucoadhesion of poly (acrylic acid) s is favored when the majority of the carboxylate groups are in the unionized form, which occurs at pH below the pKa. However, in systems with a high density of ionizable groups (e.g. carbomers or chitosan), the local pH within or at the surface of a formulation will differ significantly from that of the surrounding environment (Smart and Mortazavi, 1995).

#### **2.2.6.3.4 Miscellaneous**

The strength of adhesion has been found to change with the initial 'consolidation' force applied to the joint, or the length of contact time prior to testing. The presence of metal ions, which can interact with charged polymers, may also affect the adhesion process.

#### **2.2.6.4 Mucoadhesion Measurement**

Methods available for measuring mucoadhesion are limited and method selection depends on applicability and reproducibility. It is unnecessary to compare the absolute values obtained from different methods and is more meaningful to examine the relative bioadhesive performance using each technique. In addition, some factors, including saliva secretion, mastication, and mucus turnover that can markedly affect the adhesion strength and duration of adhesion *in vivo* are not present in *in vitro* testing (Jinsong and Paul, 2003).

##### **2.2.6.4.1 Duration of Mucoadhesion**

The duration of mucoadhesion *in vivo* can be measured by using gamma scintigraphy, electron paramagnetic resonance, or transit studies with fluorescent-coupled dosage forms. The measurement of residence time of adhesive at the application site provides quantitative information on *in situ/in vivo* bioadhesive properties. (Kockisch et al., 2001)

##### **2.2.6.4.2 Rheological Measurement**

Mucoadhesion can be indirectly inferred by changes in viscosity and other rheological properties. These measurements give certain information on the behavior of the polymer chain structures, particularly in terms of the rigidity, elasticity, and deformability of the systems. These indirectly indicate the desirable properties of the bioadhesive such as strong hydrogen bonding groups, strong anionic charges, high molecular weight, sufficient chain flexibility, and surface energy properties favoring spreading. The testing conditions need to

be carefully controlled for good reproducibility. However, these methods cannot give direct information about what actually occurs at the interface but provide greater predictability in screening potential mucoadhesive polymers when formulating buccal delivery systems.

#### **2.2.6.4.3 Tensile Test**

The tensile test is based on the measurement of detachment force of the polymer layer from the mucus substrate. Detachment force and adhesion work are indicative of mucoadhesion strength. The testing conditions are rather critical, and operation variables should be optimized and well-controlled in order to obtain reliable and reproducible results. Such tests cannot easily distinguish between bioadhesive and cohesive forces.

#### **2.2.6.4.4 Other Methods**

A **direct-staining method** (Kockisch et al., 2001) was established to evaluate the mucoadhesion of polymeric aqueous dispersion on buccal cells both *in vitro* and *in vivo* by employing *Alcian blue* to bind to anionic polymers and *Eosin* to bind to the amine groups in polymers. Unbound dye was removed by washing with 0.25 M sucrose. The extent of polymer adhesion was quantified by measuring the relative staining intensity of control and polymer-treated cells by image analysis. This method is only suitable for assessing the liquid dosage forms, which are widely employed to enhance oral hygiene and to treat local disease conditions of the mouth such as oral candidiasis and dental caries.

A **lectin-binding inhibition technique** (Lee et al., 2000) involving an avidin–biotin complex and a colorimetric detection system was developed to investigate the binding of bioadhesive polymers to buccal epithelial cells without having to alter their physicochemical properties by the addition of “marker” entities. The lectin from *Canavalia ensiformis* (Concanavalin A) has been shown to bind to sugar groups present on the surface of buccal cells. Therefore, if polymers bind to buccal cells, they would mask the surface glycoconjugates, thus reducing or inhibiting *Canavalia ensiformis* lectin binding.

**Atomic force microscopy** (Patel et al., 2000) was used to determine the mucoadhesion of polymer onto the buccal cell surfaces. Changes in surface topography were indicative of the presence of polymer bound onto buccal cell surfaces. Unbound cells showed relatively smooth surface characteristics with many small craterlike pits and indentations spread over cell surfaces, while polymer-bound cells lost the crater and indentation characteristics and gained a higher surface roughness.

## 2.2.7 Mucoadhesive polymers used in the oral cavity

### 2.2.7.1 Desired characteristics

Generally, some of the necessary structural characteristics for bioadhesive polymers include strong hydrogen bonding groups, strong anionic or cationic charges, high molecular weight, chain flexibility, and surface energy properties favoring spreading on a mucus layer (Lee et al., 2000).

### 2.2.7.2 Classification

In general, adhesive polymers can be classified as synthetic vs. natural, water-soluble vs. water insoluble, and charged vs. uncharged polymers. Examples of the recent polymers classified in these categories are listed in Table 2.2.

**Table 2.2 Mucoadhesive polymers in buccal delivery (Salamat-Miller et al., 2005).**

Criteria	Categories	Examples
Source	Semi-natural/natural	Agarose, chitosan, gelatin Hyaluronic acid Various gums (guar, hakea, xanthan, gellan, carrageenan, pectin, and sodium alginate)
	Synthetic	<i>Cellulose derivatives</i> [CMC, thiolated CMC, sodium CMC, HEC, HPC, HPMC, MC, methylhydroxyethylcellulose] <i>Poly(acrylic acid)-based polymers</i> [CP, PC, PAA, polyacrylates, poly(methylvinylether-co-methacrylic acid), poly(2-hydroxyethyl methacrylate), poly(acrylic acid-co-ethylhexylacrylate), poly(methacrylate), poly(alkylcyanoacrylate), poly(isohexylcyanoacrylate), poly(isobutylcyanoacrylate), copolymer of acrylic acid and PEG] <i>Others</i> Poly( <i>N</i> -2-hydroxypropyl methacrylamide) (PHPMAm), polyoxyethylene, PVA, PVP, thiolated polymers
Aqueous solubility	Water-soluble	CP, HEC, HPC (water <38 °C), HPMC (cold water), PAA, sodium CMC, sodium alginate
	Water-insoluble	Chitosan (soluble in dilute aqueous acids), EC, PC
Charge	Cationic	Aminodextran, chitosan, dimethylaminoethyl (DEAE)-dextran, trimethylated chitosan
	Anionic	Chitosan-EDTA, CP, CMC, pectin, PAA, PC, sodium alginate, sodium CMC, xanthan gum
	Non-ionic	Hydroxyethyl starch, HPC, poly(ethylene oxide), PVA, PVP, scleroglucan
Potential bioadhesive forces	Covalent	Cyanoacrylate
	Hydrogen bond	Acrylates [hydroxylated methacrylate, poly(methacrylic acid)], CP, PC, PVA
	Electrostatic interaction	Chitosan

Mucoadhesive polymers are generally linear polymers with high molecular weight, contain a substantial number of hydrophilic, negatively charged functional groups, and form three-dimensional expanded networks (Anders and Merkle, 1989). In the class of synthetic polymers, poly (acrylic acid), cellulose ester derivatives, and polymethacrylate derivatives are the current choices. Chitosan and examples of various gums, such as guar and hakea (from *Hakea gibbosa*), are classified as semi-natural/natural bioadhesive polymers. Poly (acrylic

acid), a linear or random polymer, and polycarbophil, a swellable polymer, represent water-soluble and water-insoluble polymers, respectively. The charged polymers are divided into cationic and anionic polymers, such as chitosan and polycarbophil, respectively, while hydroxypropylcellulose is an example of uncharged bioadhesive polymers (Gu et al, 1988).

## **2.2.8 Challenges and factors in dosage form design -**

### **2.2.8.1 Physiological aspects**

Constant flow of saliva and mobility of the involved tissues challenge mucosal drug delivery to the oral cavity. The residence time of drugs delivered to the oral cavity is typically short, in the range of <5–10 min (Lee et al., 2000). Buccal mucoadhesive formulations are expected to overcome this problem. The size of a buccal dosage form is restricted by the very limited area available for application of the delivery system. This size restriction, in turn, limits the amount of drug that can be incorporated in the dosage forms. The mucus layer covering the buccal mucosa is necessary for bioadhesive systems. Unfortunately, it not only forms a physical barrier to drug permeation, but also prevents long-term mucoadhesion and sustained drug release by its short turnover time. Interestingly, the presence of bioadhesive polymers on a mucous membrane might alter the turnover of mucin, since the residence time of mucoadhesives are usually longer than the reported mucin turnover time (Lee et al., 2000). Nevertheless, the maximum duration for buccal drug delivery is usually limited to approximately 4–6 h (Mitra et al., 2002).

### **2.2.8.2 Pathological aspects**

Many diseases can affect the thickness of the epithelium, resulting in alteration of the barrier property of the mucosa. Some diseases or treatments may also influence the secretion and properties of the mucus (Khanvilkar et al., 2001), as well as the saliva. Changes at the mucosal surface due to these pathological conditions may complicate the application and retention of a bioadhesive delivery device e. g. Cancer patients often show substantial decrease in salivary flow after irradiation treatment (Hao et al., 2003). Therefore, understanding the nature of the mucosa under relevant disease conditions is necessary for designing an effective buccal delivery system. In addition, drugs with the potential of changing the physiological conditions of the oral cavity may not be suitable for buccal delivery e.g. Drugs can interact with mucin through electrostatic attractions (e.g., tetracycline), hydrogen bonding (e.g., urea), or hydrophobic interaction (e.g., testosterone) and prevent their transport through the epithelia (Hao et al., 2003).

### 2.2.8.3 Pharmacological aspects

The intended application and target site of drug affect the selection of dosage form. For treatment of oral disease, the residence time and local concentration of the drug in the mucosa are important considerations. For a systemic effect, the amount of drug transported across the mucosa into the circulatory system is a determinant of dosage forms. A diagnostic agent can be delivered to assist the diagnosis of oral mucosal cancer. 5-Aminolevulinic acid and its esters in the form of rinse have been used in photodynamic diagnosis and photodynamic therapy. L-Cysteine was slowly released from buccal tablets for removing carcinogenic acetaldehyde, the first metabolite of ethanol, from saliva and thus prevented it from interacting with cellular proteins. Despite the type of dosage forms, the drug must be released from the dosage form and taken up by the oral mucosa. This can be optimized by a suitable formulation design.

Besides the physicochemical parameters such as solubility, permeability, and stability of drugs, organoleptic properties of drug or delivery device are important considerations for buccal administration. A bad tasting drug or rough textured device will result in poor patient compliance or acceptance.

### 2.2.8.4 Pharmaceutical aspects

#### 2.2.8.4.1 Factors and approaches:

**Factor 1:** Poor drug solubility in saliva could significantly retard drug release from the dosage form.

**Approach:** Cyclodextrin has been used to solubilize and increase the absorption of poorly water-soluble drugs delivered via the buccal mucosa (Jain et al., 2002).

**Factor 2:** Poor drug release and penetration through buccal mucosa will affect the therapeutic efficacy therefore it should be considered in the formulation design.

**Approach:** Some excipients such as release modifiers and permeation enhancers may be incorporated to enhance the effectiveness and acceptability of the dosage forms.

**Factor 3:** Selection of formulation excipients is yet another important consideration, since acidic compounds can stimulate the secretion of saliva, which enhances not only drug dissolution, but also drug loss by involuntary swallowing. Besides, addition of a separate additive for each function could complicate and enlarge the dosage form, which might be problematic for buccal applications.

**Approach:** Polymers with multiple functions seem promising and must be incorporated.



**Factor 4:** As the dosage form is to be resident in a highly developed taste-sensing organ, careful considerations for organoleptic factors, such as taste are needed.

**Approach:** Excipients enhancing palatal properties are often required to improve acceptability of dosage form or masking less desirable properties of the bioactive constituent.

#### **2.2.8.4.2 Permeation Enhancers:**

Buccal mucosa is considerably less permeable, and hence, does not provide rapid absorption and good bioavailability seen with sublingual administration. Permeability of the buccal mucosa can be increased by various penetration enhancers capable of increasing cell membrane fluidity, extracting the structural intercellular and/or intracellular lipids, altering cellular proteins, or altering mucus structure and rheology. At present, bile salts, fatty acids, and sodium lauryl sulfate are the most commonly investigated penetration enhancers. As one example, incorporation of unsaturated fatty acids into the mucoadhesive polymers has been shown to be effective in buccal delivery of drugs. The mechanism for the permeability enhancement by unsaturated fatty acids is through increasing the fluidity of the membrane phospholipids. This class of permeation enhancers reversibly alters the physical structure of the membrane by incorporating themselves into the phospholipid membrane. Unfortunately, penetration enhancers always raise concerns regarding their irritation and toxicity even though the oral mucosa is likely to be less sensitive to irreversible irritation or damage than other mucosal membranes, since it is routinely exposed to a multitude of foreign compounds (Murkle et al., 1986).

The significant enhancement in drug permeation across the buccal mucosa provided by chitosan renders this bioadhesive polymer a very attractive excipient (Senel et al., 2000). The pH-partitioning theory characteristic of passive diffusion also governs the transcellular permeability of ionizable drugs across the buccal mucosa, similar to other epithelial membranes. Maximal permeation occurs at the pH at which these drugs are predominantly in the unionized form. Control of pH is critical for successful buccal delivery of ionizable drugs. Saliva has a weak buffering capacity to maintain pH value within local regions. It might be desirable to include some pH modifiers in the formulation in order to temporarily modulate the microenvironment at the application site for better drug absorption. It is worth noting that pH can also influence the charge on the surface of the mucus, as well as certain ionizable groups of the polymers, which might affect the strength of mucoadhesion. In addition, it has been shown that the pH of the medium influences the degree of hydration of



cross-linked poly (acrylic acid), e.g. polycarbophil (Park and Robinson, 1985). Therefore, the pH needs to be carefully chosen to optimize both drug permeation and mucoadhesion. Unfortunately, buccal drug administration to animals is difficult, and only rabbits and pigs have a non-keratinized mucosal lining similar to that in humans. As a result, only a small number of absorption studies have been studied *in vivo*. However, it is very difficult to maintain the integrity and viability of the excised animal tissues. Although *in vitro* experiments can prove useful for predicting possible trends *in vivo*, caution must be exercised when extrapolating *in vitro* data to *in vivo* situations. As an example of an investigation aimed at assessing *in vitro/in vivo* correlation, Junginger et al. have evaluated the *in vitro* permeation of FITC-labeled, high-molecular-weight dextrans across excised sheep buccal mucosa, and compared these results with the *in vivo* administration of a buccal device to the oral cavity of pigs (Junginger et al., 1999). The results obtained demonstrated a less than optimal correlation between the *in vitro* and *in vivo* studies, even in the same species. However, it should be noted that similar trends were observed in both experiments, where FITC-dextran with a molecular weight of 4000 was easily permeable across both membranes, and the permeability of this compound increased in the presence of a permeation enhancer, sodium glycodeoxycholate.

### 2.2.9 Dosage Forms for Buccal Drug Delivery

Following dosage forms have been extensively researched for buccal application-

1. Mouthwash
2. Tablets
3. Chewing gums
4. Films/Patches

**Mouthwashes** (corticosteroids) have used in the treatment of oral lichen planus and other inflammatory conditions of the mouth. These allow only a short period of release, and reproducibility of drug absorption is poor (Epstein et al., 2003).

**Bioadhesive tablets** can adhere to the buccal mucosa, and the drug is released upon hydration of the device, forming a hydrogel. The device should be fabricated so that the swelling rate of bioadhesive polymer is optimized to ensure a prolonged period of bioadhesion as well as a controlled or sustained drug release. Single-layer buccal tablets of testosterone have a low bioavailability due to the lack of an impermeable backing layer on the tablet, causing a significant amount of the total dose to be swallowed. Tablets of

triamcinolone acetonide (Aftach®), developed for local treatment of aphthous ulcers, consist of a bioadhesive hydroxypropyl cellulose/polyacrylic acid layer and a lactose nonadhesive backing layer. Nifedipine/propranolol hydrochloride double-layer tablets for systemic delivery with prolonged drug release and adequate adhesiveness were developed (Remunan-Lopez et al., 1998). Nicotine replacement therapy requires a fast release of nicotine followed by a prolonged release of nicotine for maximal efficacy. A bilayer buccal adhesive nicotine tablet provided a drug release pattern combining fast release and prolonged release profiles and resulted in improved smoking cessation rates. A problem associated with the double-layer tablet was separation of the two layers. This may be overcome by modifying the device so that there is a gradient in hydrophilicity from one side to the other (Bologna et al., 2002). Bioadhesive tablets are usually prepared by direct compression. Drugs can also first be formulated in certain forms (e.g., microspheres) for achieving some desirable properties before direct compression to produce tablets. Chitosan was considered as a promising drug carrier for the buccal delivery of antimicrobial agents owing to its bioadhesive and antimicrobial properties as well as penetration enhancing effect. Chlorhexidine-chitosan microsphere-based buccal tablets have shown enhanced antimicrobial activity and prolonged drug release in the oral cavity. Recent invention can overcome the problem associated with application of semisolid dosage forms onto buccal mucosa. The bioadhesive tablet system of cationic ergotamine tartrate for treatment of migraine consisted of a reservoir of drug suspended in semisolid pharmaceutical bases in the central cavity and an adhesive region around the drug reservoir (Tsutsumi et al., 2002). This buccal delivery device has shown better drug absorption than homogenous polyvinyl alcohol hydrogel and oral capsules. Nevertheless, the disadvantage of bioadhesive tablets is lack of physical flexibility and poor patient compliance for long-term and repeated use.

**Chewing gums** have some advantages as drug delivery devices, particularly in the treatment of diseases in the oral cavity and in nicotine replacement therapy. Some commercial products are available in the market. Caffeine chewing gum, Stay Alert®, was developed recently for alleviation of sleepiness (Hao et al., 2003). It is absorbed at a significantly faster rate and its bioavailability was comparable to that in capsule formulation. Nicotine chewing gums (e.g., Nicorette® and Nicotinell®) have been marketed for smoking cessation. The permeability of nicotine across the buccal mucosa is faster than across the skin (Nielsen and Rassing, 2002). However, chewing gum slowly generates a steady plasma level of nicotine rather than

a sharp peak as experienced when smoking. Possible swallowing of considerable amount of nicotine during chewing may lead to decreased effectiveness of the chewing gum due to first-pass metabolism and gastrointestinal discomfort. It is a major challenge to optimize the dose-response relationship of nicotine administered in a chewing gum. It also poses difficulties in regulating the dose administered.

**Buccal films/patches** are the less developed type of dosage forms. These bioadhesive buccal films/patches were usually fabricated in different geometry. Type I is a single-layer device, from which drug can be released multidirectionally. Type II device has impermeable backing layer on top of the drug-loaded mucoadhesive layer, where unidirectional drug release is possible and drug loss into oral cavity can be greatly decreased. Flexible adhesive films and laminated patches are used as buccal delivery systems. These require (a) a bioadhesive to facilitate intimate contact with the mucosa and increase residence time, (b) a vehicle that releases the drug at an appropriate rate, and (c) additives such as penetration enhancers and/or enzyme inhibitors. An adhesive hydroxypropyl cellulose film containing lidocaine was studied for dental analgesia. Bioadhesive chitosan film of chlorhexidine gluconate showed characteristics of increased residence time of drug and prolonged antimicrobial action. A novel bilayer bioadhesive film of testosterone is composed of a pH-sensitive bioadhesive layer containing polycarbophil/Eudragit S-100 and a pharmaceutical wax as the impermeable backing layer (Jay S., 2002). The adhesion time of these films to rabbit buccal pouch was affected by the ratio of these two polymers. The presence of the wax-backing layer greatly enhanced the adhesion time of the bioadhesive layer and bioavailability by retarding the diffusion of saliva into the drug layer and drug loss into mouth. These bilayer bioadhesive buccal patches containing plasmid DNA were also explored for mucosal immunization in rabbits. The antigen-specific IgG titer with buccal films is comparable to that of subcutaneous protein injection, indicating that buccal immunization with these films is feasible.

Bioadhesive films/patches are commonly manufactured by solvent casting methods using adhesive coating machines, which involve dissolving a drug in a casting solution, casting film, and drying and laminating with a backing layer or a release liner. The processing technology is quite similar to pressure-sensitive adhesive-based patch manufacturing. Recently, a hot-melt extrusion method was reported to fabricate hot-melt extruded films for

buccal delivery, which overcomes the disadvantages associated with a solvent casting method such as environmental concerns, long processing times, and high costs (Repka et al., 2002).

Recently available drug products are,

1. Insulin –( Generex- Eli Lilly, 2003)
2. Testosterone – Striant™ (Schwartz, 2000)
3. Fast dissolving Fentanyl – Cephalon, 2006)
4. Fentanyl - (BioDelivery Sciences International, Inc., Phase III)

**Table 2.3 Available drug products for oral mucosal drug delivery (Hoogstrate and Wertz, 1998)**

Drug	MW	Therapeutic area	Product name	Manufacturer
Nitroglycerin	227	Angina pectoris	Suscard	Astra
Nitroglycerin	227	Angina pectoris	Cardilate	Burroughs Wellcome
Nitroglycerin	227	Angina pectoris	Nitrobid	Hoechst Marion Roussel
Nitroglycerin	227	Angina pectoris	Nitromex	Dumex Alpha
Nitroglycerin	227	Angina pectoris	Nitrong	Rhône-Poulenc Rorer
Isorbide mononitrate	236	Angina pectoris	Imdur	Key Pharmaceuticals
Isorbide mononitrate	236	Angina pectoris	Isordil	Wyeth
Isorbide mononitrate	236	Angina pectoris	ISMO	Wyeth, Boehringer Mannheim
Buprenorphine	504	Analgesia	Temgesic	Reckett Et Colman
Buprenorphine	504	Analgesia	Buprenex	Reckett Et Colman
Nicotine	162	Smoking cessation	Nicotinell	Fertin
Nicotine	162	Smoking cessation	Nicorette	Merrell Dow
Ergotamine	1313	Migraine	Ergostat	Parke Davis
Ergotamine	1313	Migraine	Ergomar	Fisons, Lotus Biochem
Methyl testosterone	302	Hypogonadism, delayed puberty	Oreton Methyl	Schering
Methyl testosterone	302	Hypogonadism, delayed puberty	Testred	ICN Pharmaceuticals
Methyl testosterone	302	Hypogonadism, delayed puberty	Virilon	Star Pharmaceuticals
Lorazepam	321	Anxiety, insomnia	Ativan	Wyeth

Abbreviation: MW, molecular weight

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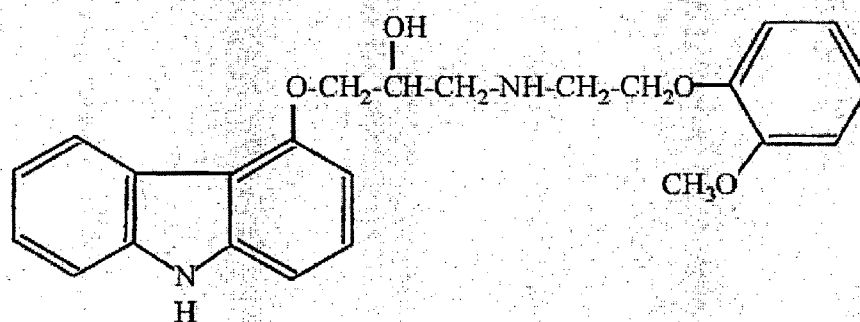
## 2.4. PROFILE OF CARVEDILOL

(Frishman, 1998; Martindale, 2005; Morgan, 1994; Rang et al., 2003; Weir and Darjie, 2005)

### 2.4.1 Physicochemical Properties:

2.4.1.1 Molecular formula:  $C_{24}H_{26}N_2O_4$

2.4.1.2 Structural Formula and Chemical Name:



(±) -1-(9 H-carbazol-4-yloxy)-3-{[2-(2-methoxyphenoxy) ethyl]amino}propan-2-ol.

2.4.1.3 Molecular Weight: 406.5

2.4.1.4 Appearance and colour: A white or almost white crystalline powder.

2.4.1.5 Solubility: Practically insoluble in water; slightly soluble in alcohol; practically insoluble in dilute acids.

2.4.1.6 Category: non-cardioselective beta blocker.

### 2.4.2 Mechanism of Action:

The mechanism for the beneficial effects of Carvedilol in congestive heart failure has not been established. Possible mechanisms includes, neurohormonal inhibition,  $\beta$ -blockade, balanced vasodilation (reduced preload and afterload), antioxidant activity, potent anti-ischaemic activity, and inhibition of neutrophil adhesion. Antioxidant activity and inhibition of neutrophil adhesion have been demonstrated in in-vitro and in vivo animal models. Carvedilol reduces the peripheral vascular resistance by vasodilation predominantly mediated through selective  $\alpha_1$  - antagonism and beta blockade prevents reflex tachycardia with the net result that heart rate is slightly decreased.

### 2.4.3 Pharmacokinetics:

#### 2.4.3.1 Absorption:

Carvedilol is rapidly and extensively absorbed following oral administration. The absolute bioavailability of Carvedilol is approximately 25%. Plasma levels peak approximately 1 hour after an oral dose. Carvedilol undergoes stereoselective first-pass metabolism with plasma levels of R (+)-Carvedilol approximately 2 to 4-fold higher than S (-)-Carvedilol following oral administration in healthy subjects. Plasma levels increase in a dose-proportional manner.

#### **2.4.3.2 Distribution:**

Carvedilol is highly lipophilic, therefore it is 98% bound to plasma proteins, primarily albumin. The volume of distribution is approximately 2 L/kg and is increased in patients with liver disease. When used as directed, Carvedilol is unlikely to accumulate during long-term treatment.

#### **2.4.3.3 Metabolism:**

In all animal species studies, and also in humans, Carvedilol is extensively metabolised into a variety of metabolites which are mainly excreted in the bile. The first-pass effect after oral administration amounts to about 60-75%; enterohepatic circulation of Carvedilol and/or its metabolites has been shown in animals. The major P450 enzymes responsible for the metabolism of both R(+) and S(-) Carvedilol in human liver microsomes were identified as CYP2D6 and CYP2C9, and to a lesser extent CYP3A4, CYP2C19 and CYP2E1.

#### **2.4.3.4 Elimination:**

After oral administration, the elimination half-life of Carvedilol is approximately 6 to 10 hours. Plasma clearance ranges from 500 to 700 mL/min. Elimination is mainly biliary, with the primary route of excretion being via the faeces. A minor portion is eliminated via the kidneys. The pharmacokinetics of Carvedilol is affected by age.

#### **2.4.4 Usage and Administration:**

Carvedilol is used in the management of hypertension and angina pectoris, and as an adjunct to standard therapy in symptomatic heart failure. It is also used to reduce mortality in patients with left ventricular dysfunction following myocardial infarction.

In **hypertension**, Carvedilol is given at an initial dose of 12.5 mg once daily by mouth, increased after two days to 25 mg once daily. Alternatively, an initial dose of 6.25 mg is given twice daily, increased after one to two weeks to 12.5 mg twice daily. The dose may be increased further, if necessary, at intervals of at least two weeks, to 50 mg once daily or in divided doses. A dose of 6.25/12.5 mg once daily may be adequate for elderly patients.

In **angina pectoris**, an initial dose of 12.5 mg is given twice daily by mouth, increased after two days to 25 mg twice daily.

In **heart failure**, the initial dose is 3.125 mg twice daily by mouth. It should be taken with food to reduce the risk of hypotension. If tolerated, the dose should be doubled after two weeks to 6.25 mg twice daily and then increased gradually, at intervals of not less than two weeks, to the maximum dose tolerated; this should not exceed 25 mg twice daily in patients with severe heart failure or in those weighing less than 85 kg, or 50 mg twice daily in patients with mild to moderate heart failure weighing more than 85 kg.

In patients with **left ventricular dysfunction following myocardial infarction**, the initial dose is 6.25 mg twice daily, increased after 3 to 10 days, if tolerated, to 12.5 mg twice daily and then to a target dose of 25 mg twice daily. A lower initial dose may be used in symptomatic patients.

#### **2.4.5 Adverse Effects:**

Hypotension is the common effect observed with the patients taking Carvedilol. Acute renal failure and renal abnormalities have been reported in patients with heart failure. Pruritus and elevated serum transaminase concentrations occurred.

#### **2.4.6 Contraindications:**

Carvedilol is contraindicated in patients with NYHA class IV decompensated cardiac failure requiring intravenous inotropic therapy, bronchial asthma or related bronchospastic conditions, second-or third-degree AV block, sick sinus syndrome (unless a permanent pacemaker is in place), cardiogenic shock or severe bradycardia. Use of Carvedilol in patients with clinically manifest hepatic impairment is not recommended. Carvedilol is contraindicated in patients with hypersensitivity to the drug.

#### **2.4.7 Drug Interactions:**

##### **Effects of other drugs on Carvedilol via the Cytochrome P450 System**

##### **2.4.7.1 Demonstrated Interactions:**

Since Carvedilol undergoes substantial oxidative metabolism, care may be required in patients receiving inducers (e.g. rifampicin) or inhibitors (eg cimetidine) of cytochrome P450, as plasma concentrations may be altered. Rifampicin reduced AUC and  $C_{max}$  of Carvedilol by about 70%. Cimetidine increased the AUC of Carvedilol by about 30% but caused no change in  $C_{max}$ . Simultaneous administration of a single dose of Carvedilol and 300mL of

grapefruit juice (an inhibitor of CYP3A4 and CYP1A2) increased the AUC of Carvedilol by approximately 16%.

#### **2.4.7.2 Theoretical Interactions:**

Interactions of Carvedilol with strong inhibitors of CYP2D6 (such as quinidine, fluoxetine, paroxetine and propafenone) have not been studied, but these drugs would be expected to increase blood levels of the R(+) enantiomer of Carvedilol. Retrospective analysis of side effects in clinical trials showed that poor 2D6 metabolisers had a higher rate of dizziness during up-titration, presumably resulting from vasodilating effects of the higher concentrations of the alpha-blocking R(+) enantiomer.

##### **2.4.7.2.1 Digoxin:**

Digoxin plasma concentrations are increased by about 15% when digoxin and Carvedilol are administered concomitantly. Both digoxin and Carvedilol slow AV conduction. Therefore, increased monitoring of digoxin is recommended when initiating, adjusting or discontinuing Carvedilol.

##### **2.4.7.2.2 Catecholamine Depleting Agents:**

Patients treated with both Carvedilol and a drug that can deplete catecholamines (e.g. reserpine and monoamine oxidase inhibitors) should be observed closely for signs of hypotension and/or severe bradycardia.

##### **2.4.7.2.3 Cyclosporin:**

A modest increase in mean cyclosporin concentration has been observed following initiation of Carvedilol treatment in renal transplant patients suffering from chronic vascular rejection. It is recommended that cyclosporin concentrations be monitored closely after initiation of Carvedilol therapy and that the dose of cyclosporin be adjusted as appropriate.

##### **2.4.7.2.4 Clonidine:**

Concomitant administration of clonidine with agents with beta-blocking properties may potentiate blood-pressure and heart-rate-lowering effects. When concomitant treatment with agents with beta-blocking properties and clonidine is to be terminated, the beta-blocking agent should be discontinued first. Clonidine therapy can then be discontinued several days later by gradually decreasing the dosage.

##### **2.4.7.2.5 Calcium channel blockers:**

Isolated cases of conduction disturbance (rarely with haemodynamic compromise) have been observed when Carvedilol and diltiazem were co-administered. As with other drugs with

beta-blocking activity, if Carvedilol is to be administered orally with calcium channel blockers of the verapamil or diltiazem type, it is recommended that ECG and blood pressure be monitored.

#### **2.4.8 Formulations Available:**

**Tablets:** White, oval film-coated tablets

3.125 mg in bottles of 100; 6.25 mg in bottles of 100; 12.5 mg in bottles of 100; 25 mg in bottles of 100.

#### **2.4.9 Analytical Techniques:**

##### **2.4.9.1 Colorimetric estimation: (Ajoy and Dilipkumar, 2001)**

A simple, accurate and sensitive spectrophotometric method for the estimation of Carvedilol in pharmaceuticals is developed by Ajoy and Dilipkumar. The method is based on the formation of a yellow colour with dilute HCl and sodium nitrite. This colour showed absorption maxima at 400 nm and obeys Beer's law up to 20 µg/ml. The colour was found to be stable for 1 to 3 hr.

##### **2.4.9.2 HPLC with fluorescence detector (Machado et al., 2001)**

This is an indirect method for the enantioselective analysis of Carvedilol in plasma and urine for application in clinical pharmacokinetic studies using (2)-methyl chloroformate as a chiral reagent. Plasma or urine samples (1 ml) were alkalized and extracted with chloroform for 3 min in a mechanical shaker. Derivatization was carried out by the addition of (2)-menthyl chloroformate in dichloro-methane (2%) in basic medium, followed by shaking for 2 min. The diastereoisomeric derivatives were extracted with chloroform after addition of water and analyzed by HPLC using a RP-8 column and a fluorescence detector. Method was applied in the investigation of the enantioselectivity in the kinetic disposition of oral multiple doses of racemic Carvedilol (3.125 mg/12 h) administered to a patient with chronic heart failure.

##### **2.4.9.3 Enantiomeric separation of Carvedilol using capillary electrophoresis: (Phuong et al., 2004)**

To investigate the stereoselective pharmacokinetics, the enantiomeric separation of Carvedilol in human plasma was undertaken using capillary electrophoresis (CE). Resolution of the enantiomers was achieved using 2-hydroxypropyl-β-cyclodextrin as the chiral selector. Phosphate buffer (50 mM, pH 4.0) containing 10 mM of 2-hydroxypropyl-β-cyclodextrin was used as electrolytic buffer. A chiral separation was carried out with the same electrolytic buffer without chiral selector.

The profiles of the plasma concentration of (R,S)-Carvedilol showed C<sub>max</sub> of 71.5, 72.2, and 73.5 ng/mL, as determined by the CE, HPLC/FD methods and calculations from the data of the chiral method, respectively.



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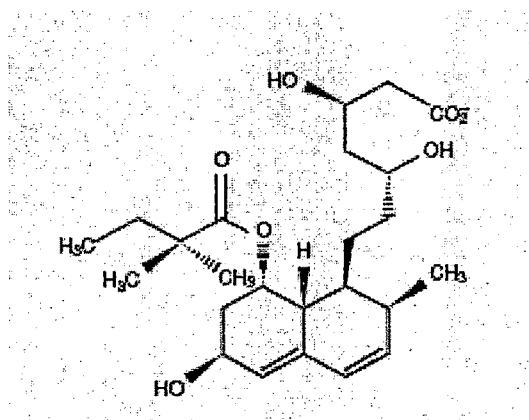
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## 2.5 PROFILE OF PRAVASTATIN SODIUM:

### 2.5.1 Physicochemical Properties:

2.5.1.1 Molecular formula:  $C_{23}H_{35}NaO_7$

### 2.5.1.2 Structural Formula and Chemical Name:



1-Naphthalene-heptanoic acid, 1,2,6,7,8,8a-hexahydro- $\beta$ , $\delta$ ,6-trihydroxy-2-methyl-8-(2-methyl-1-oxobutoxy), monosodium salt, [1S-[1 $\alpha$ ( $\beta$  S\*,  $\delta$  S\*), 2 $\alpha$ , 6 $\alpha$ , 8 $\beta$  (R\*), 8a $\alpha$ ]]-.

2.5.1.3 Molecular Weight: 446.5

2.5.1.4 Appearance and colour: White to yellowish white powder, or crystalline powder, hygroscopic.

2.5.1.5 Solubility: -

It is soluble in methanol and water (>300 mg/mL), slightly soluble in isopropanol, and practically insoluble in acetone, acetonitrile, chloroform, and ether.

2.5.1.6 Category: Lipid-lowering agent.

### 2.5.2 Mechanism of Action:

Pravastatin sodium produces its lipid-lowering effect in two ways. First, as a consequence of its reversible inhibition of HMG-CoA reductase activity, it effects modest reductions in intracellular pools of cholesterol. This results in an increase in the number of LDL-receptors on cell surfaces and enhanced receptor-mediated catabolism and clearance of circulating LDL. Second, Pravastatin sodium inhibits LDL production by inhibiting hepatic synthesis of VLDL, the LDL precursor.

2.5.3 Pharmacokinetics: (Hamelin and Turgeoun, 1998; Schachter, 2004)

### 2.5.3.1 Absorption:

Pravastatin sodium is administered orally in the active form. In clinical pharmacology studies in man, Pravastatin sodium is rapidly absorbed, with peak plasma levels of parent compound

attained 1 to 1.5 hours following ingestion. While the presence of food in the gastrointestinal tract reduces systemic bioavailability, the lipid-lowering effects of the drug are similar whether taken with, or 1 hour prior, to meals.

#### **2.5.3.2 Distribution:**

Protein binding of Pravastatin sodium is low. Circulating levels of unbound Pravastatin sodium are high relative to those of the other statins. Widespread tissue distribution is prevented by the hydrophilic nature of the drug.

#### **2.5.3.3 Metabolism:**

Pravastatin undergoes extensive first-pass extraction in the liver (extraction ratio 0.66). Oral bioavailability of Pravastatin sodium is 17%. Hepatic first pass effect accounts for 50-70%. *In vitro* studies demonstrated that Pravastatin sodium is transported into hepatocytes with substantially less uptake into other cells. In view of Pravastatin sodium's apparently extensive first-pass hepatic metabolism, plasma levels may not necessarily correlate perfectly with lipid-lowering efficacy. Pravastatin plasma concentrations [including: area under the concentration-time curve (AUC), peak ( $C_{max}$ ), and steady-state minimum ( $C_{min}$ )] are directly proportional to administered dose. Systemic bioavailability of Pravastatin sodium administered following a bedtime dose was decreased 60% compared to that following an AM dose. This finding of lower systemic bioavailability suggests greater hepatic extraction of the drug following the evening dose.

#### **2.5.3.4 Elimination:**

Approximately 20% of oral dose is excreted in urine and 70% in the feces. Since there are dual routes of elimination, the potential exists both for compensatory excretion by the alternate route as well as for accumulation of drug and/or metabolites in patients with renal or hepatic insufficiency.

#### **2.5.4 Usage and Administration:**

Therapy with Pravastatin sodium should be considered in those individuals at increased risk for atherosclerosis-related clinical events as a function of cholesterol level, the presence or absence of coronary heart disease, and other risk factors.

##### **2.5.4.1 Primary Prevention of Coronary Events:**

In hypercholesterolemic patients without clinically evident coronary heart disease, Pravastatin sodium is indicated to:

- Reduce the risk of myocardial infarction.

- Reduce the risk of undergoing myocardial revascularization procedures.

#### **2.5.4.2 Secondary Prevention of Cardiovascular Events:**

In patients with clinically evident coronary heart disease, Pravastatin sodium is indicated to reduce the risk of total mortality by reducing coronary death.

#### **2.5.4.3 Hyperlipidemia:**

Pravastatin sodium is indicated as an adjunct to diet to reduce elevated Total-C, LDL-C, Apoprotein B, and TG levels and to increase HDL-C in patients with primary hypercholesterolemia and mixed dyslipidemia (Frederickson Type IIa and IIb).

Pravastatin sodium is indicated as adjunctive therapy to diet for the treatment of patients with elevated serum triglyceride levels (Fredrickson Type IV).

Pravastatin sodium is indicated for the treatment of patients with primary dysbetalipoproteinemia (Fredrickson Type III) who do not respond adequately to diet.

The patient should be placed on a standard cholesterol-lowering diet before receiving Pravastatin sodium and should continue on this diet during treatment.

Pravastatin sodium can be administered orally as a single dose at any time of the day, with or without food. Since the maximal effect of a given dose is seen within 4 weeks, periodic lipid determinations should be performed at this time and dosage adjusted according to the patient's response to therapy and established treatment guidelines.

#### **2.5.5 Adverse Effects:**

Pravastatin is generally well tolerated; adverse reactions have usually been mild and transient. General adverse effects include nausea, vomiting, musculoskeletal pain, diarrhea, abdominal pain and chest pain.

#### **2.5.6 Contraindications:**

In active liver disease therapy should not be initiated.

Pregnancy and Lactation- Atherosclerosis is a chronic process and discontinuation of lipid-lowering drugs during pregnancy should have little impact on the outcome of long-term therapy of primary hypercholesterolemia. Cholesterol and other products of cholesterol biosynthesis are essential components for fetal development (including synthesis of steroids and cell membranes). Since HMG-CoA reductase inhibitors decrease cholesterol synthesis and possibly the synthesis of other biologically active substances derived from cholesterol, they are contraindicated during pregnancy and in nursing mothers. **Pravastatin should be administered to women of childbearing age only when such patients are highly**

unlikely to conceive and have been informed of the potential hazards. If the patient becomes pregnant while taking this class of drug, therapy should be discontinued immediately and the patient should be informed about the potential hazards to the fetus.

## **2.5.7 Drug Interactions:**

### **2.5.7.1 Itraconazole**

The mean AUC and  $C_{max}$  for Pravastatin sodium were increased, when given with itraconazole (a potent P450 3A4 inhibitor which also inhibits p-glycoprotein transport) as compared to placebo. The mean  $t_{1/2}$  was not affected by itraconazole, suggesting that the relatively small increases in  $C_{max}$  and AUC were due solely to increased bioavailability rather than a decrease in clearance, consistent with inhibition of p-glycoprotein transport by itraconazole.

### **2.5.7.2 Cholestyramine/Colestipol**

Concomitant administration resulted in an approximately 40 to 50% decrease in the mean AUC of Pravastatin sodium. However, when Pravastatin sodium was administered 1 hour before or 4 hours after cholestyramine or 1 hour before colestipol and a standard meal, there was no clinically significant decrease in bioavailability or therapeutic effect.

### **2.5.7.3 Cimetidine**

A significant difference was observed between the AUC's for Pravastatin sodium when given with cimetidine compared to when administered with antacid.

### **2.5.7.4 Digoxin**

The AUC of Pravastatin increase, but the overall bioavailability of Pravastatin plus its metabolites was not altered.

### **2.5.7.5 Cyclosporine**

In one single-dose study, Pravastatin sodium levels were found to be increased in cardiac transplant patients receiving cyclosporine.

### **2.5.7.6 Gemfibrozil**

In a crossover study in 20 healthy male volunteers given concomitant single doses of Pravastatin sodium and gemfibrozil, there was a significant decrease in urinary excretion and protein binding of Pravastatin sodium.

## **2.5.8 Formulations Available:**

Pravastatin sodium Tablets are supplied as:

10 mg, 20 mg, 40 mg and 80 mg tablets.

### **2.5.9 Ongoing Research:**

Kelley C. et al reviewed the efficacy, safety and administration of the 5 currently available hydroxymethylglutaryl-coenzyme A reductase inhibitors (statins) in the management of hypercholesterolemia.

The first tier studies provide consistent good-quality evidence that Pravastatin sodium, lovastatin, and simvastatin reduce cardiovascular events. For Pravastatin sodium and lovastatin there is fair-good and good-quality evidence for both primary and secondary prevention. For Pravastatin sodium and simvastatin there is good-quality evidence for secondary prevention. The latter two statins reduced deaths from cardiovascular and cerebrovascular disease as well. With regard to reduction in health outcomes, Pravastatin sodium have been demonstrated in good quality clinical trials to reduce cardiovascular health outcomes (Kelley et al., 2002)

Recently U.S. Food and Drug Administration (FDA) has approved a new indication for Pravastatin sodium for use in treating pediatric patients with heterozygous familial hypercholesterolemia (HeFH). The FDA approval for this new indication provides an additional treatment option for children ages 8 years and older who suffer from this condition and whose LDL cholesterol levels are above the indicated limits after an adequate trial of diet (Doctor's guide, 2005).

### **2.5.10 Analytical Techniques:**

#### **2.5.10.1 HPLC with UV detection:**

Mills and Roberson have analyzed Pravastatin sodium by HPLC with UV detection. They have used process parameters as follows, Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol, flow rate 0.5 mL/min. UV diode array detection. Retention time(s): Pravastatin sodium, 2.9 min. They found that developed method is accurate and reproducible (Mills and Roberson, 1993).

#### **2.5.10.2 HPLC with mass spectroscopy:**

Zhu and Neirinck has developed a new method, using high-performance liquid chromatography with (negative ion) mass spectrometry for the determination of a hydrophilic liver-specific inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, Pravastatin sodium in human plasma. In this method, plasma samples were prepared by a solid-phase extraction on C<sub>18</sub> Bond Elute cartridge. Chromatography was carried out with a Zorbax C<sub>8</sub> column. Simple isocratic chromatography conditions were

used. The method is simple and reliable with a total run time of less than 2 min. (Zhu and Neirinck, 2003)

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