**In vivo studies of formulated Bio/Mucoadhesive dosage forms**

Angiotensin-converting enzyme (ACE) inhibitors significantly reduce mortality in patients with chronic symptomatic heart failure and in survivors of myocardial infarction with left ventricular dysfunction (Pfeffer et al., 1992). Early trials attributed the survival benefit of ACE inhibitors predominantly to a retardation of hemodynamic deterioration (Cleland et al., 1993). Some recent trials have suggested a significant reduction in sudden, potentially arrhythmic death; however, sudden cardiac death still remains the most common mode of death in heart failure patients treated with ACE inhibitors (Waldo et al., 1996). The classification of the exact mode of death in heart failure patients is complex. Sudden death in heart failure patients often is preceded by a severe worsening in hemodynamic status, and in the absence of direct documentation, a clear association between ventricular arrhythmias and sudden death is not possible (Packer et al., 1992). Therefore, whether or not ACE inhibitors afford survival benefit via antiarrhythmic protection remains uncertain (Pahor et al., 1994). Further, clinical as well as preclinical in vivo studies have failed to demonstrate a consistent effect of ACE inhibitors on the frequency of ventricular arrhythmia (Visser et al., 1996).

Buccal drug delivery has lately become an important route of drug administration. The rich vascularization of the oral mucosa and its permeability to many drugs makes this route an attractive alternative to the oral and parenteral routes for systemic drug delivery. Absorption of therapeutic agents from the oral mucosa overcomes premature drug degradation due to enzyme activity and pH of the gastrointestinal tract, avoids active drug loss due to first-pass hepatic metabolism and therapeutic plasma concentration of the drug can be rapidly achieved (Jian Hwa et al., 1999). The buccal mucosa permits a prolonged retention of a dosage form especially with the use of mucoadhesive polymers without much interference in activities such as speech or mastication unlike the sublingual route (James Swarbrick et al., 2007). Various bioadhesive mucosal dosage forms have been developed which include tablets, gels, patches and films all of which make use of polymers such as carbopols, hydroxy propyl methyl cellulose etc. that prolong the residence time of the dosage form Mucoadhesive buccal films or patches are preferred in terms of flexibility, comfort, patient compliance and better adhesion of the system to the oral mucosa (Lewis et al., 2006). The antihypertensive, Losartan potassium is an angiotensin II receptor (type AT) antagonist, orally active and undergoes substantial first-pass metabolism by cytochrome P450 enzymes. The terminal half-life of losartan is about
2 hrs. The drug is orally administered as 25 mg tablets once or twice daily with total daily doses ranging from 25 to 100 mg. Following oral administration, losartan is well absorbed (based on absorption of radiolabeled losartan) and undergoes substantial first-pass metabolism; the systemic bioavailability of losartan is approximately 33%. In view of these facts, this drug can be considered as a suitable candidate for buccal delivery (Sang et al., 2000). In this study, an attempt is made to investigate the feasibility of mucoadhesive buccal films as a medium for the sustained delivery of losartan potassium with better bioavailability. Furthermore, in vivo anti-hypertensive tests were carried out aiming to assess higher efficient Los oral pharmaceutical composition.

There are 2 basic types of studies that use in vitro model systems: (1) Descriptive studies on the effect of a simulated human dose, and (2) Analytic studies to find the optimal association of a particular pharmacodynamic factor with antihypertensive action, define its magnitude, or test a hypothesis based on previous investigations.

*In vivo adhesion test of Losartan potassium tablets in human volunteers*

Male human volunteers selected for this test, 6 subjects were selected in the age between 23 to 35 years. The details of the test and drug were informed to the volunteers and consent was taken from them before the commencement of the work. Permission to carry out this work was obtained from the Institutional Ethics Committee. A tablet from F-8 containing 25 mg of Losartan potassium was used for the study was shown (Table 6.1.0 and figure 6.1.0). Before application of the tablet, the human volunteers were asked to rinse their mouth thoroughly with water. The tablets were applied to the buccal mucosa of human volunteers. After 90 min, the tablets were taken out and added to a beaker containing 10 ml of phosphate buffer solution (pH 6.6). The volunteers were directed to rinse their mouth with 10 ml of phosphate buffer solution (pH 6.6). The washing was added to the previous solution. After appropriate dilution, solutions were analyzed for drug content at 250 nm. The results represent the amount of drug remaining unabsorbed.
Table: 6.1.0 Release data of drug permeation of Losartan potassium tablet through buccal mucosa

<table>
<thead>
<tr>
<th>S.No</th>
<th>Time in Min</th>
<th>% Remained</th>
<th>% Absorbed</th>
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<tbody>
<tr>
<td>1.</td>
<td>2</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>4</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>3.</td>
<td>6</td>
<td>87</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>8</td>
<td>82</td>
<td>18</td>
</tr>
<tr>
<td>5.</td>
<td>10</td>
<td>78</td>
<td>22</td>
</tr>
<tr>
<td>6.</td>
<td>12</td>
<td>73</td>
<td>27</td>
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<tr>
<td>7.</td>
<td>14</td>
<td>70</td>
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<td>16</td>
<td>69</td>
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<tr>
<td>9.</td>
<td>18</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>10.</td>
<td>20</td>
<td>61</td>
<td>39</td>
</tr>
</tbody>
</table>

Figure: 6.1.0 Release Profiles of drug permeation of Losartan potassium tablet buccal mucosa
Results and Discussion of Losartan potassium tablets \textit{In vivo} studies

The study revealed that, the release of Losartan from the tablets was appreciable (Alur \textit{et al}., 1999). The kinetics of \textit{in vivo} drug release from buccal tablets in human volunteers indicated that about 50.9 to 65.5\% of the drug was released in 90 min from the tablets. Wetting has been reported essential for establishment of intimate contact between the mucoadhesive and mucin/tissue to develop strong adhesive bond (Wang \textit{et al}., 1983). Moreover, an increase degree of hydration may increase the chain segment mobility, which could lead to increase interdiffusion of polymer and mucin/tissue (Gupta \textit{et al}., 1990). In the measurement of \textit{in vitro} bioadhesive strength, the contact time between the tablet and tissue wetted with simulated saliva solution was relatively short (10 s). During \textit{in vivo} test, none of the tablets had to be removed due to irritation. The tablets did not cause any discomfort to the volunteers. No side effects like taste alteration, heaviness, dry mouth, or severe salivation were observed. The system claims the potential clinical usefulness in delivering the drug. Platelets were discovered in the 1880's (De Guetano \textit{et al}., 2001). Platelet function testing began with the application of the \textit{in vivo} bleeding time by Duke \textit{et al}., in 1910. The bleeding time was still regarded as the most useful screening test of platelet function until the early 1990's (Harker \textit{et al}., 1972). Recently, the widespread use of the bleeding time has rapidly declined because its limitations have been recognized and other, less invasive, screening tests have become available (Rodgers \textit{et al}., 1990).

Most platelet function tests have been traditionally utilized for the diagnosis and management of patients presenting with bleeding problems rather than thrombosis (Gawaz \textit{et al}., 2005). However, as platelets are now implicated in the development of atherothrombosis, which is the leading cause of mortality in the Western world, new and existing platelet function tests are increasingly being used for monitoring the efficacy of the antiplatelet drugs used to treat these conditions (Ruggeri \textit{et al}., 2002). This, coupled with the development of new, simpler tests and point-of-care (POC) instruments, has resulted in the increasing tendency of platelet function testing to be performed away from specialized hemostasis clinical or research laboratories, where the more traditional and complex tests are still performed (Harrison \textit{et al}., 2005).
In vivo buccal absorption study of Losartan Potassium films

In vivo buccal absorption of Losartan Potassium was studied to know about the amount of drug was absorbed, which is an evidence for the Losartan Potassium buccal evidence (Vamshi et al., 2007). Buccal absorption test for drug Losartan Potassium was carried out on six healthy male student volunteers aged between 23 to 25 years and shown in (Figure 6.1.1). Since this test indicates the evidence of buccal absorption of Losartan Potassium. Before the test, the volunteers were asked to moisten their mouth with a few ml of buffer solution. Twenty five ml of phosphate buffer (pH 6.6) containing 1 mg of the drug was placed in the volunteer’s mouth. The volunteers were asked to swirl the solution approximately at 60 swirling/min. for 20 min.

Figure: 6.1.1 In vivo residence of Losartan buccal films (A) at initial (B) After 3 hours of the study
Results and Discussion of Losartan potassium films In vivo studies

The release of Losartan from the film was appreciable and the kinetics of in vivo drug release from buccal film in human volunteers indicated that about 67.5 to 78.4 % of the drug was released in 90 min from the films. During in vivo film test, none of the films had to be removed due to irritation. The films did not cause any discomfort to the volunteers. No side effects like taste alteration, heaviness, dry mouth, or severe salivation were observed. The system claims the potential clinical usefulness in delivering the drug. The results were shown in Figure: 5.1.21. PEO, HPMC and Chitosan are hydrophilic swellable polymers and may have more affinity towards mucin which comprises of 95% water. It follows that since HPMC films hydrated at a faster rate and attained maximum swelling at a shorter period, it is thus anticipated that its in vitro bioadhesive strength should be greater than PEO films which hydrated at a slower rate. However, an optimum water concentration (Park et al., 1987; Nappinnai et al., 2008). For hydrocolloid particle to develop maximum adhesive strength and the adhesives would subsequently lose their adhesiveness if they became too susceptible to water since they would be eventually displaced by water. In the evaluation of residence time, the films were placed on the buccal mucosa and hence the contact time was sufficient for both films to reach maximum hydration (Semalty et al., 2010). As such, PEO films which achieved maximum swelling faster would then be expected to be displaced by water sooner than the HPMC films. These may help to explain the higher in vitro bioadhesive strength but shorter in vivo buccal residence time of the PEO films compared to that of HPMC films.

This may be the reason for longer residence time of films containing bioadhesive polymers. All films eroded completely except HPMC-Chitosan based films, which dislodged and detached from the buccal mucosa. These films remained intact without erosion.

In vivo radiographic study of the Clopidogrel microspheres

Clopidogrel is an antiplatelet compound that has recently been shown to be effective in the secondary prevention of cardiovascular complications of atherosclerosis. The thienopyridines clopidogrel and ticlopidine are specific inhibitors of ADP-induced platelet aggregation but not of ADP-induced shape change or Ca$^{2+}$ transients (Coukell et al., 1997). Although the exact mechanisms are not known, treatment with clopidogrel reduces the binding of ADP or stable ADP analogues to high-affinity binding sites on platelets (Mills et al., 1992). The ADP receptors that are modified by clopidogrel are also
believed to mediate the inhibition of prostaglandin I$_2$- or E$_1$-induced cyclic AMP formation by ADP. In platelet-rich plasma, clopidogrel ($\leq$100 μM) does not inhibit platelet aggregation \textit{in vitro}. In rats, the \textit{in vivo} activity of clopidogrel has been proposed to be dependent on hepatic biotransformation to an active metabolite (Defreyn \textit{et al.}, 1991). In these studies, clopidogrel (40 mg kg$^{-1}$) was less effective in Hepatectomized rats as compared to normal control rats. In addition, clopidogrel did inhibit platelet aggregation in isolated, blood-perfused rat livers. The bioactivation of clopidogrel has been suggested to be mediated by the hepatic cytochrome P450 system (Savi \textit{et al.}, 1992).

However, an active metabolite of clopidogrel has not been published so far and the need for hepatic activation of clopidogrel has not been demonstrated in humans. In this context, it is noteworthy that in animal studies much higher doses of clopidogrel were used as compared to the standard dosage for humans (75 mg d$^{-1}$). Thus, different mechanisms may account for platelet inhibition in humans as compared to animal studies.

Figure: 6.1.2 X-Ray images of mucoadhesive microspheres of Clopidogrel
(A) After 30 minutes (B) After 6 Hours
Results and discussion of *In vivo* radiographic study of the Clopidogrel microspheres

*In vivo* studies were conducted on 6 healthy male human volunteers to find the gastric residence time of the beads. The X ray images shows that the microspheres residence time in stomach for about 6 hours. After 8 hours the microspheres were disappeared clearly indicating the good mucoadhesive property up to 6 hours. The results were shown in figure: 6.1.2. *In vivo* radiographic studies revealed that microspheres remained in the stomach for more than 6 hrs (Peh *et al.*, 1999), which indicated that GRT was increased by the mucoadhesive principle and was considered desirable for improving bioavailability of the absorption window drugs. Thus, results of the current study clearly indicate, a promising potential of the clopidogrel mucoadhesive system as an alternative to the conventional dosage form (Rao *et al.*, 1998). However, further clinical studies are needed to assess the utility of this system for patients suffering from antiplatelet.

Oral controlled release dosage forms have been considered for their therapeutic advantages. However, these approaches not been suitable for certain drugs, are characterized by a narrow absorption window in the upper part of the gastrointestinal tract. This is due to the relatively short transit time of the dosage forms in the stomach. This results in a short absorption phase that is often accompanied by lesser bioavailability (Rajput *et al.*, 2010). However, the oral dosage forms for gastric retentions have drawn more attention for their therapeutic advantages in permitting control over the time and site of drug release. The challenge in the development of controlled drug delivery system is not just sustained drug release but also to prolong the presence of a dosage form in the stomach (Patil *et al.*, 2006).

The term mucoadhesive describes material that bind to biological substrate, such as mucosal layer. Adhesion of bioadhesive drug delivery devices to the mucosal tissue offers the possibility of creating an intimate and prolonged contact at the site of administration. This increased residence time can result in enhanced absorption and in combination with a controlled release of drug also improve patient compliance by reducing the frequency of administration (Uddhav *et al.*, 2009).

Clopidogrel is acid stable and completely absorbed in gastrointestinal tract and is rapidly hydrolyzed in the intestinal mucosa and blood to Clopidogrel, absorption is increased by the presence of food. Peak plasma concentration is approximately 2.2-2.5 ng/mL after a single 75mg oral dose. The biological half life is 2hrs and bioavailability is >50%. In the present study we tried to enhance the bioavailability of Clopidogrel.
formulating it in the form of gastro retentive mucoadhesive drug delivery system for controlled release which is expected to be a better dosage form when compared to conventional or immediate dosage form (William et al., 2006).

**In vivo radiographic study of the Clopidogrel mini tablets**

*In vivo* studies were conducted on 6 healthy male human volunteers to find the gastric residence time of the tablet (Guguloth et al., 2011). The institute’s human ethics committee approved the protocol for the study. The studies were based on X-ray radiography. The tablets prepared with PEO WSR 303 (F-5) were tested for the *in vivo* gastric residence time. The tablets were incorporated with 30 mg of BaSO$_4$ which was used in the various diagnostic tests. The tablets were compressed with same compression force. All the physicochemical properties were within the range.

**Figure: 6.1.3&6.1.4 X ray image of Clopidogrel mini tablets prepared with PEO WSR 303 at initial and after 4hours.**
Results & discussion of In vivo radiography study of the Clopidogrel mini tablets

*In vivo* studies were conducted on 6 healthy male human volunteers to find the gastric residence time of the tablet. X rays were taken at different time intervals such as initial, 4 hours and 8 hours. The X ray images show the tablet residence in stomach for about 8 hours clearly indicating the good mucoadhesive property (Peh *et al*., 1999). The results were shown in figure: 6.1.4 and 6.1.5. *In vivo* radiographic studies revealed that mini tablets remained in the stomach for more than 8 hrs, which indicated that GRT was increased by the floating principle and was considered desirable for improving bioavailability of the absorption window drugs. Thus, results of the current study clearly indicate, a promising potential of the Clopidogrel floating system as an alternative to the conventional dosage form (Nafee *et al*., 2004). However, further clinical studies are needed to assess the utility of this system for patients suffering from antiplatelet.