Introduction
1.1 INTRODUCTION

**Homeostasis** is an important factor in the field of biology and medical sciences. According to it, our body system maintains the relative consistency of the inner biological environment. But recent studies in the field of chronobiology have challenged this concept, as biological processes and functions are not constant but highly variable in a predictable time manner as expressed by prominent period, this stipulate that most of the biological processes follow a circadian pattern.

**Chronobiology** is the study of biological rhythms and the mechanisms of biological time keeping. Chronobiology is highly relevant to the fields of medicine, pharmacology, and drug delivery. Clinical studies showed that the magnitude of the rhythmic differences can be so great that it can be a strong determinant during the 24 h severe morbid and mortal events and when the symptoms and signs of many chronic medical conditions flare. Accumulating evidence also suggests that the day of week and month of the year that diagnostic tests are applied or certain medications are administered can affect responses (Reinberg *et al.*, 1983; Haus *et al.*, 1992; Dalton *et al.*, 1964; Case *et al.*, 1998; Smolensky *et al.*, 1988).

The **circadian rhythm** (CR) is an approximate daily periodicity, a roughly daily 24 h cycle in the biochemical, physiological or behavioural processes of living being. Almost all physiological functions are organized according to circadian rhythm. Circadian rhythms are controlled by an inherited master clock network composed of the paired suprachiasmatic nuclei (SCN) that are situated in the hypothalamus and the pineal gland (Kalsbeek *et al.*, 2006; Maronde and Stehle, 2007). The rhythmic activities of specific clock genes like per1, per2, per3, bmal, clock, and CRY and the cyclic (nocturnal) secretion of melatonin from the pineal gland comprise the central timekeeping mechanism. This master clock network orchestrates the period and phase of the multitude of peripheral circadian clocks located in cells, tissues and organ-systems. Thus, the temporal organization of biological processes and functions during the 24 h period ensures peak functioning of the diurnal human species during daytime activity and restoration and repair during nocturnal rest. It also ensures fertility during the menstrual
cycle and during the year it ensures a biological adjustment to predictable-in-time changes associated with the different seasons of the year. The results of numerous biological rhythm studies define the temporal organization of human beings. Incidence of several cardiovascular events like myocardial infarction, stroke and sudden cardiac death also exhibit circadian rhythm (Fox et al., 1989; Argentino et al., 1990; Willich et al., 1987). Patients with cardiovascular diseases are more prone to suffer from these events during early morning (Fox and Mulcahy, 1991; Muller et al., 1989) as the blood pressure rises significantly just before waking hours (Millar-Crag et al., 1978; Staessen et al., 1992). This rise in blood pressure corresponds to increased secretion of catecholamines and increased plasma renin activity. Thus vascular tone and total peripheral resistance increases in morning hours and blood pressure rises as a result. At the same time heart rate increases.

The increase in cardiovascular events in the early morning hours is a function of three pathophysiologic factors, which act together to increase the risk of acute cardiovascular events (Tofler et al., 1987). The first, activation of the sympathetic nervous system, causes an early morning rise in catecholamine levels. This results into vasoconstriction, which increases intra-arterial pressures. The second is the increase in blood pressure itself, which creates an increase in cardiac stroke work. The combination of vasoconstriction and increased cardiac work substantially increases shear stress on blood vessels, increasing the risk for plaque rupture. The third factor is a morning state of hypercoaguability, induced by an increase in platelet aggregation and reduced functioning of the body's fibrinolytic system. Together, these three factors lower the threshold for myocardial ischemia in susceptible individuals with coronary plaques and significant vessel stenosis, early morning plaque rupture can lead to vessel occlusion and infarction (Anwar and White, 1998).

A major objective of chronotherapy for hypertension is to deliver the drug in higher concentrations during the early morning post-awakening period, when blood pressure is highest and in lesser concentrations during the middle of a sleep cycle, when blood pressure is low (Hermida et al., 2007).
From the viewpoint of therapeutic optimization, maintaining a constant blood level for a drug in the human body is questionable. Long term constant drug concentrations exposed in blood and tissues may induce many problems such as tolerance developed with the drug and activation of physiological system. For example, a study on Japanese patients suggests that excessive reduction of systolic blood pressure during sleep may result in a higher incidence of white matter lesions in the brain resulting from small cerebral vessel hypoperfusion (Drayer et al., 1985).

Recently chronotherapy has been extensively applied in clinical therapy by modulating the dosage regimen of drug administration according to physiological needs. Optimum therapy is more likely to result when the right amount of drug is delivered to the correct target organ at the most appropriate time. This drug delivery aims to improve the therapeutic efficacy and safety by varying drug release in accordance with patient need (i.e. the ideal drug delivery system should also involve a non delivery period rather than a continuous delivery period).

FDA approved chronopharmaceutical drug delivery systems (ChrDDS) for hypertension (Youan, 2004)

<table>
<thead>
<tr>
<th>ChrDDS</th>
<th>Drug</th>
<th>Year of approval</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>COER-verapamil</td>
<td>Verapamil</td>
<td>1996</td>
<td>G.D. Searle (a division of Pfizer) NY, USA</td>
</tr>
<tr>
<td>CODAS-verapamil</td>
<td>Verapamil</td>
<td>1998</td>
<td>Schwars Pharma, Monheim, Germany</td>
</tr>
<tr>
<td>Cardizem LA</td>
<td>Diltiazem</td>
<td>2003</td>
<td>Biovail Corporation Mississauga, Canada</td>
</tr>
<tr>
<td>Innopran XL</td>
<td>Propranolol</td>
<td>2003</td>
<td>GlaxoSmithKline, USA</td>
</tr>
</tbody>
</table>

Chronotherapeutic strategy for the hypertension could be met by designing compression coated or press-coated core tablet formulations consisting of core containing the drug and surrounded by polymers that dissolve and/or erode to produce a desired release profile. In
the past this technique has been applied to many drugs requiring modification of drug release. Though, the technique has advantage in terms of minimal requirement of coating solvents or coating equipments, the reliable and reproducible central position of core tablet within press coated tablet is a requirement. Nevertheless, many timed release tablets have been developed in recent years by press-coating technique using various polymers such as hydroxypropyl methylcellulose (HPMC) (Zou et al., 2008), ethylcellulose (EC) (Lin et al., 2004), hydroxyethyl cellulose (HEC) (Matsuo et al., 1995), hydroxypropyl cellulose (HPC) (Fukui et al., 2000), polyethylene oxide (PEO) and polyethylene glycol (PEG) (Sawada et al., 2004), low substituted hydroxypropyl cellulose (L-HPC) (Ghimire et al., 2007) and sodium alginate (Gutsche et al., 2008). Since β blockers specifically block the sympathetic drive, they may be particularly effective for suppressing the morning blood pressure surge which occurs when sympathetic activity exceeds parasympathetic activity in the course of the circadian rhythm. Thus β blocking agents reduce ischaemic events during day time hours and are also of therapeutic value in the morning hours (Lemmer, 1998; Hanes and Weir, 2001).

Metoprolol Tartrate (MT) is therapeutically indicated in conditions such as hypertension, angina and myocardial infarction. It belongs to BCS class I, has a high solubility-high permeability pattern throughout the GI tract, half life of 3-7 h and plasma protein binding of 12%. Metoprolol is a basic drug with pKa of about 9.6 and can attain higher tissue concentrations. Studies have shown that negligible amount is absorbed in stomach but the duodenum, jejunum, ileum and colon have more capacities for absorption (Godbillon et al., 1985).

Carvedilol (CRV) is non selective β-adrenoreceptor and a α1-adrenoreceptor blocker and therapeutically indicated in conditions such as hypertension, angina and cardiac heart failure. It belongs to BCS class II, has a low solubility-high permeability pattern throughout the GI tract and half life of 4-7 h. Therefore, based on above characteristics metoprolol and carvedilol were selected as the model drug for the system design.

Both slow release and immediate release tablets of metoprolol and immediate release tablets of carvedilol are already available in the market. But the design of these
conventional tablets does not take into consideration the biological rhythm of blood pressure. So the body is confronted with drugs even when it is not needed.

1.2 RESEARCH ENVISAGED

Patients with cardiovascular diseases like myocardial infarction, stroke and sudden cardiac death are more prone to suffer from these events during early morning as the blood pressure rises significantly just before waking hours. This rise in blood pressure corresponds to increased secretion of catecholamines and increased plasma renin activity. So, chronotherapy for hypertension is required to deliver the drug during the early morning post-awakening period, when blood pressure is highest after giving a suitable lag period during the middle of a sleep cycle, when blood pressure is at lowest level.

Such strategy could be met by designing compression coated or press-coated core tablet formulations consisting of core containing the drug and surrounded by polymers that dissolve and/or erode to produce a desired release profile. Since, β blockers specifically block the sympathetic drive, they may be particularly effective for suppressing the morning blood pressure surge which occurs when sympathetic activity exceeds parasympathetic activity in the course of the circadian rhythm. Thus, β blocking agents reduce ischaemic events during day time hours and are also of therapeutic value in the morning hours. The chronopharmaceutical drug delivery system presented as timed-release press-coated tablet would provide desired lag time of about 6 h in onset of drug release followed by a sustained release over a period of 24 h. Therefore, if tablet is administered between 9:00-10:00 p.m., it is expected that the tablet will start releasing drug in between and around 3:00-4:00 a.m. and the peak plasma level should be achieved during 6:00 a.m.-12:00 noon. It is also necessary that the dosage form releases drug in sustained pattern upto 24 h. Therefore, this system will offer characteristic advantage over conventional dosage forms by avoidance of unnecessary load of drug to the body, hence reducing the frequency and intensity of side effects.
1.3 OBJECTIVES AND APPROACHES OF PRESENT WORK

The purpose of present studies was to prepare two chronopharmaceutical drug delivery systems of MT and CRV by preparing compression coated core tablets for once a day administration that would provide desired lag time of about 6 h followed by a sustained release over a period of 24 h. Apart from physical evaluation, \textit{in vitro} dissolution test, \textit{in vitro} core erosion test, effect of rotation speed of paddle, effect of pH of dissolution media, swelling studies, water uptake test, \textit{in vivo} and stability studies have also been carried out.

\textbf{Approaches}

\begin{itemize}
  \item \textbf{Preformulation studies of metoprolol tartrate}
    \begin{itemize}
      \item Drug identification
      \item Physical appearance and melting point
      \item Determination of $\lambda_{\text{max}}$
      \item Infrared spectroscopy
      \item Differential scanning calorimetry (DSC)
      \item Solubility studies
      \item Partition coefficient
      \item Preparation of standard curves
      \item Drug-excipient interaction studies
    \end{itemize}
  \item \textbf{Preparation and evaluation of chronopharmaceutical drug delivery system of metoprolol tartrate}
    \begin{itemize}
      \item Preparation of press coated core tablets
      \item Determination of drug content in tablets
      \item Test for compressibility of tablets
      \item Water uptake study
      \item Swelling of tablets
      \item \textit{In vitro} core erosion test
      \item \textit{In vitro} drug dissolution test
      \item Effect of dissolution medium and rotation speed on release profile
    \end{itemize}
\end{itemize}
Kinetics of drug dissolution
Comparison of in vitro release profile of MT against market brand
In vivo evaluation
Statistical analysis
Stability studies

✓ Preformulation studies of carvedilol
  Drug identification
  Physical appearance and melting point
  Determination of $\lambda_{\text{max}}$
  Infrared spectroscopy
  Differential scanning calorimetry
  Solubility studies
  Partition coefficient
  Preparation of standard curves
  Drug-excipient interaction studies

✓ Preparation and evaluation of chronopharmaceutical drug delivery system of carvedilol
  Preparation of press coated core tablets
  Determination of drug content in tablets
  Test for compressibility of tablets
  Water uptake study
  Swelling of tablets
  In vitro core erosion test
  In vitro drug dissolution test
  Effect of pH and rotation speed on release profile
  Kinetic assessment of drug dissolution
  In vivo evaluation
  Statistical analysis
  Stability studies