Summary And Conclusions
SUMMARY

Biologic rhythms are implicated in cardiovascular events. Failure to recognize the circadian decline in blood pressure may result in undesired chronopathological events. Chronotherapeutics is a purposeful alteration of drug level to match circadian rhythms to optimize therapeutic outcomes and minimize side effects. The pathophysiological mechanisms behind the circadian phenomena of cardiovascular system are probably related to increased stress placed on the cardiovascular system by rapidly changing haemodynamic and haemostatic factors that occur during early morning. The rise in blood pressure in morning hours corresponds to increased secretion of catecholamine due to increased sympathetic tone and increased activities of renin-angiotensin-aldosterone as well as hypothalamic-pituitary-adrenal systems. Plasma rennin acts as the limiting factor for angiotensin II generation. Angiotensin II produces vasoconstriction directly as well as by enhancing adrenaline/noradrenaline release from adrenal medulla/adrenergic nerve endings and by increasing central sympathetic outflow which increases blood pressure sharply. The increased level of aldosterone acts on the distal tubule to promote $\text{Na}^+$ reabsorption and $\text{K}^+/\text{H}^+$ secretion which again contribute to raised blood pressure. Thus, vascular tone and total peripheral resistance increases in the morning hours and blood pressure rises as a result. In the late morning blood pressure reaches its peak. After that, blood pressure declines, falling 15 to 20 mm Hg between about 8 p.m. and 2 a.m., the time when blood pressure is usually lowest.

Hypertension is a widespread and a major risk factor for stroke and ischaemic heart diseases. Control of hypertension is therefore a major aspect of cardiovascular risk reduction. Most cardiovascular medicines are designed to achieve a constant or near constant effect throughout the day. In many cases the requirement for medication is not the same at night as it is during day time. The aim of chronotherapy is to deliver a drug at higher concentrations during the time of greatest need. In the case of cardiovascular diseases this represents the early morning post-awakening period. Because of changes in life style, stress and strain, systemic hypertension represents a significant risk factor for the development of atherosclerotic coronary artery disease and in myocardial infarction, cerebrovascular accidents and congestive heart failure. A major barrier to the management of hypertension is the extent to which patients
receive right amount of drug in the right time. Chronopharmaceutical drug delivery systems release a bioactive agent at a rhythm that ideally matches the biological requirement of diseases, their therapy and thereby providing minimum harm to the patient. In order to deal with circadian blood pressure changes in hypertension, timed release or delayed release systems of many drugs have been developed.

The purpose of the present studies were to develop 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.

Metoprolol tartrate is used in hypertension, angina pectoris and myocardial infarction. It works by blocking $\beta_1$-receptor sites on cardiac muscle with no intrinsic sympathomimetic activity. It has got an oral bioavailability of 50-60% with plasma half life of 3-7 h and plasma protein binding of only about 12%. Metoprolol is a basic drug with a 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.

Carvedilol is non selective $\beta$-adrenoreceptor and a $\alpha_1$-adrenoreceptor blocker and therapeutically indicated in conditions such as hypertension, angina and congestive heart failure. It belongs to BCS class II, has a low solubility-high permeability pattern throughout the GI tract and plasma half life of 4-7 h. Therefore based on above characteristics metoprolol and carvedilol were selected as the model drug for the present system design.

The main objective of the study was to develop a time-controlled release formulation based on a press coating technique. The target was that, if the tablet is taken just before going to bed, it should start to show its effect during early morning hours, when the blood pressure starts rising and then it maintains the effect during the period in which risk of cardiovascular events is maximum.

Firstly, a chronopharmaceutical drug delivery system of MT was developed. The drug sample (MT) was standardized as per IP standards by studying its physical appearance, melting point, solubility, UV spectrum and FTIR spectrum of Metoprolol tartrate which comply with the IP specifications. The observations were found to be
comparable with the standards prescribed, therefore it was inferred that the drug sample was authentic.
The polymers selected were hydrophilic in nature and were coated on the tablets by press coating. They swelled in water and could restrict the drug release for 5-6 h, causing release of drug thereafter. HPC-L and HPC-M, Methocel K4M, Methocel K15M were chosen as coat polymers.
The tablets were prepared by press-coating technique using punches of two different sizes. Initially two grades of HPC (HPC-L and HPC-M) were taken. Different timed release formulations (TMP1-TMP9) were prepared. The drug content uniformity was found to be in the range of 97.98-99.89% in all formulations. The average percentage weight deviation of all tablet formulation was in compliance with Pharmacopoeial indicating limits. The thickness of tablet was consistent in all formulations. Hardness and friability of all formulations (6-8 kg and <1%, respectively) conformed the standards. The % core erosion ratios of all formulations (TMP1 to TMP9) were 55.2, 60.5, 64.3, 62.4, 58.8, 63.3, 65.2, 59.2 and 63.5% respectively, all found to be greater than 55% thereby showing steady release of the drug after the lag time until complete dissolution. Further blending of HPC-M (100 mg) with 50 mg HPC-L provided desired lag period of 6 h and 99.02±0.82% drug release in 12 h (TMP9).
Further, a formulation has been developed by optimization of the different grades of hydrophilic polymer HPMC as a coat material in order to sustain the release of Metoprolol tartrate over a period of 24 h.
The drug content uniformity was found to be in the range of 99-102% in all formulations. The average percentage weight variation of all tablet formulation was in compliance with Pharmacopoeial limits. The thickness of tablet was consistent in all formulations. Hardness and friability of all formulation (6-8 kg and <1%, respectively) conformed the standards.
Tensile strength of blend of methocel (K4M and K15M) was found to be 13.26 ± 0.84 kg/cm² thereby showing practical applicability and good compressibility of HPMC. Water uptake study showed that there was little difference in initial rate of water uptake and after lapse of few hours, the rate of water uptake was decreased with an increase in the viscosity grades of polymers. Axial swelling was found to be higher than swelling in the radial direction probably due to the release of stress developed
during powder compaction along the axial direction. Core erosion ratios were found to be greater than 50% thereby showing steady release of the drug after the lag time until complete dissolution. Blending of polymers (100 mg of methocel K4M and 50 mg of methocel K15M) in the press coat (TM7) produced a lag phase of 6 h followed by a complete release of 98.66 ± 2.1% in 24 h. On this basis, tablet formulation TM7 was considered to be optimized wherein the penetration of dissolution media through the outer layer of the tablet resulted in hydration and/or swelling and gel formation at the interface of tablet and medium while diffusion of drug through the formed gel layer and erosion of gel layer resulted in drug release process. Also, it may be inferred that the performance of the present timed release system of MT is not largely influenced by pH of dissolution medium. There was reduction in lag period with increasing rotating speed of paddle, it could be because of initial fast diffusion of dissolved drug and easy erosion of outer layer of tablet followed by complete gelling of polymer for complete drug release. The n value in the present study was found to be 0.1229 that fails to indicate the exact mechanism of release as per the Korsemeyer model. These results were found to be in concurrence with literature that states absence of any correlation between n value and diffusion guided release for drug delivery systems based on HPMC.

In the in vivo studies, it was found that significantly higher values of peak time, $T_{\text{max}}$ (12 vs 0.5 h), mean residence time, $\text{MRT}_{0-24\text{ h}}$ (15.40 ± 0.01 vs 6.38±0.17 h) and half life, $t_{1/2}$ (19.13 ± 1.64 vs 5.03±0.29 h), were observed for MT press-coat tablets as compared to the same for reference solution, indicating extended absorption phase and presence of the drug for a longer time in the body. Significantly optimized values of peak concentration, $C_{\text{max}}$ (36.42 ± 1.96 vs 159.32 ± 2.64 ng/ml, $P< 0.05$) were exhibited by the test tablets. Area under the curve up to infinite time $\text{AUC}_{0-\text{inf}}$ (1158.65 ± 148.77 vs 1263.15±69.96 ng h/ml) for press-coat tablet and solution were not significantly different at $P<0.05$, indicating bioequivalence of the press-coat tablets to the reference MT solution. The relative bioavailability of the test tablets was found to be 89.88±6.83% indicates nearly the same bioavailability of the drug from both the formulations.

The stability studies of tablets were carried out according to WHO guidelines after storage them at 30±2°C/65±5%RH for two years and at 40±2°C/75±5%RH for 6
months. The formulations were evaluated for change in physical appearance, drug content, % drug release and interaction studies. There was no significant change in physical appearance, % drug content, % drug release and DSC thermogram and FTIR spectra. Thus it can be concluded that such formulations can be assigned a shelf life of 2 years.

Chronopharmaceutical drug delivery system of CRV was also developed. The polymers selected were HPMC (hydrophilic) and EC (hydrophobic) as coat polymers. The drug carvedilol was obtained as a gift samples from Ranbaxy Lab., Gurgaon (India). The drug sample was standardized as per IP standards by studying its physical appearance, melting point, solubility, UV spectrum and FTIR spectrum of the gift sample of CRV comply with the IP specifications. The observations were found to be comparable with the standards prescribed, therefore it was inferred that the drug sample was authentic. Observations during drug-excipients interaction study by DSC and FTIR precluded possible interactions between the drug and polymers. The absence of any significant change in thermal behavior endorsed the suitability of the drug with selected polymers. The drug content uniformity was found to be in the range of 98-102% in all formulations. The average percentage weight variation of all tablet formulation was in compliance with Pharmacopoeial limits. The thickness of tablet was consistent in all formulations. Hardness and friability of all formulations (7-8 kg and <1%, respectively) conformed the standards. Tensile strength of HPMC, EC and blending of HPMC and EC were found to be 12.41 ± 0.24, 18.18±0.51 and 20.37±0.64 kg/cm² respectively, thereby showing practical applicability and good compressibility of HPMC and EC. Lowest water uptake shown by EC might be due to its hydrophobic in nature while having hydrophilic nature of methocel K4M imparted highest water uptake characteristics. The maximum radial swelling of tablets containing both HPMC and EC were found to be 151.6 ± 7.2 and 67.2 ± 4.3 % in 0.1N HCl, pH 1.2 and phosphate buffer, pH 6.8 respectively while axial swellings were 233.3 ± 9.1 and 142.8 ± 6.8 % in 0.1N HCl, pH 1.2 and phosphate buffer, pH 6.8 respectively after 24 hours. Axial swelling was found to be higher than swelling in the radial direction probably due to the release of stress developed during powder compaction along the axial direction. The core erosion ratios of all formulations (TC1 to TC8) were 50.3, 40.8, 46.9, 44.3, 45.1, 43.9, 44.5 and 40.3% respectively, thereby
showing steady release of the drug after the lag time until complete dissolution. Addition of citric acid in the core of tablets may lower the pH of microenvironment since CRV is weak base (pKa 7.8) and showed pH dependent solubility while lowering the pH upto 4 increases solubility. The optimized formulation (TC8) contained 25% of methocel K4M in the press-coat. TC8 produced a lag period of 6 h followed by a complete release of 99.65±1.08% in 24 h. A probable reasoning for such observations could be that the lag time gets prolonged on increasing the proportion of hydrophilic polymer in press coat which in turn impeded rupturing of EC coated layer by releasing the osmotic pressure build up within core part through the pore formed in coat of polymer and delayed the process of dissolution and/or erosion because the hydrogel layer remained longer on the surface of core tablet and higher gelling capacity of HPMC retard the release of drug. The performance of the present timed release system of CRV is not largely influenced by pH of dissolution medium. The lag time decreased by 4 h in each case such that TC8 showed lag time of 6, 2 and 2 h at rotation speed of 50, 75 and 100 rpm respectively. The time taken for overall release of drug also reduced with increasing paddle speed. Such reduction in lag period with increasing rotating speed could be because of initial fast erosion and/or dissolution of outer polymeric layer as well as core part of tablet. Release data analysis provided ‘n’ value was 0.755 that indicated the coupled diffusion of hydrated matrix and polymer relaxation (erosion) of polymer commonly called anomalous transport as per the Korsemeyer model.

In the in vivo studies, significantly higher values of peak time, T_max (12 vs 0.5 h), mean residence time, MRT_0–24_h (15.05 ± 0.02 vs 6.32±0.09 h); and half life, t_{1/2} (14.25 ± 0.71 vs 5.10 ± 0.1 h), were observed for CRV test tablet as compared to the same for reference suspension, indicating extended absorption phase and presence of the drug for a longer time in the body. Significantly optimized values of peak concentration, C_max (52.30±1.86 vs 176.56 ± 2.89 ng/ml, P< 0.05) were exhibited by the press-coat tablets. Area under the curve up to infinite time AUC_0-inf (1285.67±100.21 vs 1419.23±56.69 ng h/ml) for test tablet and suspension were not significantly different at P<0.05, indicating bioequivalence of the CRV press-coat tablets to the reference CRV suspension.
The stability study of tablets was carried out according to WHO guidelines by storage them at 30±2°C/65±5%RH and at 40±2°C/75±5%RH for 6 months. The formulations were evaluated for change in physical appearance, drug content, % drug release and interaction studies. There was no significant change in physical appearance, % drug content, % drug release and DSC thermogram and FTIR spectra. Thus it can be concluded that such formulations can be stored for minimum six months without considerable change in its characteristics.

CONCLUSIONS

(1) Chronopharmaceutical drug delivery system of metoprolol tartrate has been developed which demonstrated extended delivery of drug upto 24 h after a lag time of 6 h in the onset of drug release. This was achieved through optimization of proportion of different grades of hydroxypropyl methylcellulose (HPMC) polymer in the outer coat of tablets.

(2) MT has not shown any incompatibility with polymers used viz. methocel K4M and methocel K15M.

(3) Press coated tablets consisting of methocel K4M and methocel K15M in the ratio of 2:1 in the outer coat (TM7) displayed a timed-function and produced a lag phase of 6 h followed by a complete release of 98.66 ± 2.1% in 24 h.

(4) Tablet formulation TM7 was considered to be optimized wherein the penetration of dissolution media through the outer layer of the tablet probably resulted in hydration and/or swelling and gel formation at the interface of tablet and medium while diffusion of drug through the formed gel layer and erosion of gel layer resulted in drug release process.

(5) Core erosion ratios were found to be greater than 50% thereby showing steady release of the drug after the lag time until complete dissolution.

(6) The performance of the present timed release system of MT was not influenced by the pH of the dissolution medium.

(7) There was reduction in lag period with increasing rotating speed of paddle, it could be because of initial fast diffusion of dissolved drug and easy erosion of outer layer of tablet followed by complete gelling of polymer for complete drug release.
(8) In the in vivo studies, significantly difference of $T_{\text{max}}$ (12 vs 0.5h) and $C_{\text{max}}$ (36.42 ± 1.96 vs 159.32 ± 2.64 ng/ml, P< 0.05) were exhibited by the press-coated MT tablets with reference to drug solution. AUC$_{0-\text{inf}}$ (1158.65 ± 148.77 vs 1263.15 ± 69.96 ng h/ml) for MT tablets and drug solution was not significantly different at P<0.05, indicating same extent of absorption of the press-coat tablets to the reference MT solution.

(9) Stability studies showed that such formulations can be stored for two years without considerable change in its characteristics.

(10) The present study demonstrated that metoprolol tartrate compression coated core tablets could be successfully formulated as a delayed onset extended release (DOER) system for obtaining timed-drug release over a period of 6-24 h.

(11) Chronopharmaceutical drug delivery system of carvedilolol has been developed. It was formulated through optimization of proportion of hydroxypropyl methylcellulose (HPMC) and ethyl cellulose (EC) polymers in the outer coat of tablets.

(12) Press coated tablets consisting of methocel K4M and EC in the ratio of 1:3 in the outer coat (TC8) displayed a timed-function and produced a lag phase of 6 h followed by a complete release of 99.65±1.08% in 24 h.

(13) Tablet formulation TC8 was considered to be optimized wherein lag time gets prolonged on increasing the proportion of hydrophilic polymer in press-coat which in turn impeded rupturing of EC coated layer by releasing the pressure build up within core part through the pore formed in coat of polymer and delays the process of dissolution and/or erosion because the hydrogel layer remained longer on the surface of core tablet and higher gelling capacity of HPMC retarded the release of drug.

(14) Core erosion ratios also indicated steady release of the drug after the lag time until complete dissolution.

(15) The performance of the present timed release system of CRV was not influenced by the pH of dissolution medium.
(16) There was reduction in lag period with increasing rotating speed of paddle, it could be because of initial fast erosion and/or dissolution of outer polymeric layer as well as core part of tablet.

(17) In the in vivo studies, significantly values of $T_{\text{max}}$ (12 vs 0.5h) and $C_{\text{max}}$ (52.30 ± 1.86 vs 176.56 ± 2.89 ng/ml, P< 0.05) were exhibited by the test tablets with reference to drug suspension. AUC$_{0-\text{inf}}$ (1285.67 ± 100.21 vs 1419.23 ± 56.69 ng h/ml) for test tablet and suspension were not significantly different at P<0.05, indicating same extent of absorption of the test tablets to the reference CRV suspension.

(18) Stability study showed that such formulations can be stored for six months without considerable change in its characteristics.

(19) The present study demonstrated that carvedilol compression coated core tablets could be successfully formulated as a DOER system for obtaining timed-drug release over a period of 6-24 h.

(20) Through this work we also propose to attain delayed onset extended release (DOER) for a hydrophilic drug by using hydrophilic swellable polymer and for a hydrophobic drug using blend of hydrophilic (HPMC) and hydrophobic polymers (EC) in the press-coat.

(21) Thus, this system may be beneficial for alleviating the symptoms of hypertension and related cardiovascular diseases. The results are not only encouraging but they also promise effective management of such diseases through this novel approach after suitable trials in human beings.