CHAPTER-9

RESULTS AND DISCUSSION

In the present study propranolol hydrochloride has been considered as the drug of choice and the polymers like ethyl cellulose (EC), poly vinyl pyrrolidone (PVP), hydroxypropyl methyl cellulose (HPMC) and acrycoat S100 has been verified for their suitability in sustaining the drug release from transdermal patch formulations. Percentage purity of propranolol hydrochloride as determined by potentiometric method was found to be 99.15 %. FT-IR spectrum of propranolol hydrochloride **(Figure 5.3, Chapter 5)** revealed the presence of peaks at 2964.69 cm^{-1} and 3281.99 cm^{-1} which may be due to the presence of a secondary amine group and secondary hydroxyl group respectively. The aryl alkyl ether has possibly displayed a stretching band at 1267.27 cm^{-1} and the presence of α-substituted naphthalene might have resulted in the peak at 797.59 cm^{-1}. The FT-IR spectra of propranolol hydrochloride granules along with different polymers (EC, PVP, HPMC and acrycoat S100) showed a broadening of peaks at 3283 cm^{-1}, which might be due to extensive hydrogen bonding **(Figure 5.4 to 5.7, Chapter 5)**. Major frequencies of functional groups of the pure drug found to remain intact when the FT-IR spectra of the drug- polymer combination were analyzed. From the FT-IR spectra analysis of drug alone and drug-polymer combination it is evident that there is no major interaction between the drug and the polymers used under study.

Initially transdermal patch formulations were prepared by using four polymers (EC, PVP, HPMC and acrycoat S100) in different combinations **(Table 5.3 to 5.6, Chapter 5)**. Formulated propranolol hydrochloride transdermal patches were found to show variable results when subjected to different evaluation parameters. Almost all the formulations prepared by aluminium foil method were satisfactory in respect to their apparent flexibility, smoothness and transparency. Formulations were even easier to remove from the glass block. Whereas formulations prepared by using mercury surface were neither fully transparent nor easy to remove from the petridishes. Tedious removal might be due to uneven thickness of the patches because of wavy surface of the mercury resulted in tampering of the patches. The physical appearances of the prepared patches are presented in **table 5.7, 5.8, 5.9 and 5.10 of chapter 5**.

The thickness of the patches prepared with EC and PVP were found in between 0.00114 cm to 0.00206 cm **(Table 5.11, Chapter 5)**. Patches containing EC and HPMC were found to show the thickness range in between 0.00129 cm to 0.00214 cm **(Table 5.13, Chapter 5)**. Low standard deviation values observed in the thickness profile of the patches of both the combination ensure uniformity of the formulated patches and less batch variation. On the contrary formulations containing acrycoat S100 and PVP (FVa and FVm) **(Table 5.14, Chapter 5)** and formulations containing HPMC and acrycoat S 100 (FYa and FYm) **(Table 5.12, Chapter 5)** were found to give variable thicknesses and higher values of standard deviation.

Weight variation study of the formulated patches revealed that the patches containing EC and PVP **(Table 5.15, Chapter 5)** have low standard deviation than the patches containing acrycoat S 100 and PVP **(Table 5.18, Chapter 5)**. Transdermal patches showed less weight variation as well as low standard deviation and the range was in between 0.122 gm to 0.141 gm for the patches composed of EC and PVP **(Table 5.15, Chapter 5)**. Formulations containing EC and HPMC **(Table 5.17, Chapter 5)** showed less weight variation than the formulations containing HPMC and acrycoat S100 **(Table 5.16, Chapter 5)**. It was found that the weight variation for the patches containing EC and HPMC was in between 0.126 gm to 0.143 gm **(Table 5.17, Chapter 5)**.

The folding endurance values of all the patches were found satisfactory which indicates that the dibutyl phthalate (30 % w/w of polymer) used as the plasticizer for the transdermal patches, rendered good flexibility and restricted brittleness **(Table 5.19 to 5.22, Chapter 5)**. Almost all the formulations prepared by using aluminium foil as the backing have shown optimum flatness and zero percent constriction whereas formulations prepared on mercury backing have shown little amount of constriction **(Table 5.23 to 5.26, Chapter 5)**.

From the results of various physical evaluations conducted on the initially formulated transdermal patches it is revealed that EC containing patches are better than the patches containing acrycoat S 100. So, for designing of final formulation and their *in vitro* and *in vivo* study EC, PVP and HPMC were selected as polymers to prepare matrix diffusion controlled

transdermal patches. Moreover the method in which aluminium foil was used to prepare backing membrane has been found more convenient and reproducible than the method where mercury was used as substrate. Hence, Approach 1 i.e. formulation of transdermal patches employing aluminium foil has been followed in the designing of final formulation.

The transdermal patches of propranolol hydrochloride were prepared as monolithic matrices by solvent casting technique employing glass moulds of known diameter into which polyvinyl alcohol (PVA) backing membrane was cast previously. Both side opened glass moulds were wrapped with aluminium foil at one end and PVA backing as well as drug-polymer matrix was prepared. Transdermal patches were formulated by using three polymers in two combinations and in different proportions like EC with PVP **(Table 6.1, Chapter 6)** and EC with HPMC **(Table 6.2, Chapter 6)**. Dibutyl phthalate (30 % w/w of polymer) and propranolol hydrochloride (20 % w/w of polymer) in ethanol (10 ml) along with the polymers in requisite ratios were prepared as the casting solution to formulate the transdermal patches.

The percent moisture content (% w/w) and percent moisture uptake (% w/w) of the patches prepared with different proportion of EC and PVP were found in between 0.9 to 6.25 (% w/w) and 1.12 to 5.34 (% w/w) **(Table 7.1, figure 7.1 and figure 7.2, Chapter 7)** respectively. Whereas it was found to be 1.64 to 5.79 (% w/w) and 1.45 to 5.03 (% w/w) for the formulations prepared with EC and HPMC in different ratios **(Table 7.2, figure 7.3 and figure 7.4, Chapter 7)**. It was observed that the moisture content increases gradually with the increase of hydrophilic polymer concentration. Moisture uptake profile of the transdermal patches was also found to be increased with the increase in hydrophilic polymer concentrations. Formulations containing higher proportions of PVP and HPMC blended with EC (TTS5 > TTS4 > TTS3 and TDS5 > TDS4 > TDS3) were thereby found to show higher moisture content and moisture uptake in comparison to the patches containing lesser proportions of PVP and HPMC (TTS6 < TTS7 < TTS8 < TTS9 < TTS10 and TDS6 < TDS7 < TDS8 < TDS9 < TDS10). Little moisture content of the formulations helps them not to become completely dried and brittle patches. Again low moisture uptake restricts the microbial contamination and bulkiness of the transdermal patches.

The tensile strength of the patches containing EC with PVP and EC with HPMC in different proportions were found to be in between 211.45 gm/cm^2 to 280.89 gm/cm^2 and 195.47 gm/cm^2 to 276.32 gm/cm^2 **(Table 7.3 and 7.4, Chapter 7)** respectively. It was observed that with the increase in PVP and HPMC concentration, the tensile strength of the patches gradually decreased.

The water vapour transmission was found to be least with the formulations containing less amount of hydrophilic polymer. Thus formulations TTS6, TTS7, TTS8, TTS9, TTS10 and TDS6, TDS7, TDS8, TDS9, TDS10 have shown lesser affinity towards water in comparison to the formulations TTS1, TTS2, TTS3, TTS4, TTS5 and TDS1, TDS2, TDS3, TDS4, TDS5; where proportions of PVP and HPMC were more **(Table 7.5 and 7.6, Chapter 7)**. Patches containing EC with PVP and EC with HPMC showed a water vapour transmission profile within the range of 0.51852×10^{-4} gm/cm/h to 3.09965×10^{-4} gm/cm/h and 0.90838×10^{-4} gm/cm/h to 2.95309×10^{-4} gm/cm/h respectively. Thus amongst all the formulations TTS6 and TDS6 have shown the least water vapour transmission profile may be due to having lesser concentrations of PVP and HPMC with highest concentration of hydrophobic polymer EC.

The drug content of the formulated patches was found to be less variable. Patches prepared with EC and PVP, the drug content was found to be in between 19.86 mg to 19.94 mg and it was in between 19.82 mg to 19.93 mg for the patches prepared with EC and HPMC **(Table 7.7 and 7.8, Chapter 7)** respectively.

In vitro permeation of drug from the transdermal patches were carried out in modified Keshary-Chein diffusion cell through dialysis membrane using 100 ml phosphate buffer of pH 7.4 as the diffusion media for a period of 48 hours. It was observed that the patches prepared with hydrophilic polymer in a higher concentration in the two series of formulations TTS1, TTS2, TTS3, TTS4, TTS5 and TDS1, TDS2, TDS3, TDS4, TDS5, where concentration of PVP and HPMC was used respectively in gradual increasing order, the release was very quick and the patches released more than 98 % of the loaded drug within 15 to 20 hours in case of formulations containing EC and PVP **(Table 8.1 to 8.5, Chapter 8)** and 14 to 20 hours in

case of formulations containing EC and HPMC **(Table 8.11 to 8.15, Chapter 8)** respectively. But the patches containing ethyl cellulose in a gradual increasing order of concentration in the formulations TTS6, TTS7, TTS8, TTS9, TTS10 and TDS6, TDS7, TDS8, TDS9, TDS10, where concentration of PVP and HPMC was minimum have showed a sustained release of the loaded drug over an extended period of 48 hours **(Table 8.6 to 8.10 and table 8.16 to 8.20, Chapter 8)**.

The data obtained from the *in vitro* permeation study of all the transdermal patches were fitted to various kinetic models (Zero order, Higuchi, First order, Korsmeyer-peppas) to determine the kinetics of drug release from the drug-polymer matrix. Formulations TTS6, TTS7, TTS8 and TDS6, TDS7, TDS8 have showed the R^2 value of 0.9967, 0.9969, 0.9952 and 0.9919, 0.9974, 0.9912 respectively, when the permeation data was fitted to Zero order model **(Figure 8.6, 8.7 and 8.8 and figure 8.16, 8.17 and 8.18, Chapter 8)**. The regression values suggest that the drug release from the transdermal patches was in a controlled fashion. It was evidenced that the formulations TTS6, TTS7 and TDS6, TDS7 exhibit the R^2 value of 0.9739, 0.9695 and 0.9861, 0.9667 **(Figure 8.6, figure 8.7 and figure 8.16, figure 8.17, Chapter 8)** respectively, when their *in vitro* permeation data was fitted to Higuchi kinetic model. This confirms the diffusional release pattern of the drug from these transdermal patches. R^2 values obtained from the Korsmeyer-peppas kinetic model for the formulations TTS6, TTS7 and TDS6, TDS7 was found to be 0.9822, 0.9813 and 0.9883, 0.9777 respectively **(Figure 8.6, figure 8.7 and figure 8.16, figure 8.17, Chapter 8)**, which again supports the fact that drug release from the matrix patches was diffusion controlled. Korsmeyer's exponent (n) value was found to be 0.557, 0.548 and 0.5728, 0.5475 for the formulations TTS6, TTS7 and TDS6, TDS7 **(Figure 8.6, figure 8.7 and figure 8.16, figure 8.17, Chapter 8)** respectively, which further suggest the Higuchi pattern or diffusion controlled drug release from these transdermal patches. From the formulations containing gradually less proportion of EC (TTS8, TTS9, TTS10 and TDS8, TDS9, TDS10), the drug release was found to be faster **(Table 8.8, 8.9 and 8.10 and table 8.18, 8.19 and 8.20, Chapter 8)**. Moreover the drug release was even quicker in the formulations containing higher proportions of PVP and HPMC in this fashion TTS5 > TTS4 > TTS3 > TTS2 > TTS1 and TDS5 > TDS4 > TDS3 > TDS2 > TDS1 **(Table 8.1, 8.2, 8.3, 8.4, and 8.5 and table 8.11,**

8.12, 8.13, 8.14 and 8.15, Chapter 8) respectively. *In vitro* permeation data of these formulations when fitted to various kinetic models, almost all the formulations showed regression values obeying Higuchi pattern or diffusion controlled drug release. For the formulations TTS5, TTS4, TTS3, TTS2 and TTS1 and TDS5, TDS4, TDS3, TDS2 and TDS1, the R^2 values were found to be in between 0.9874 to 0.9911 and 0.9937 to 0.9959 respectively **(Figure 8.1 to 8.5 and 8.11 to 8.15, Chapter 8)** when fitted to Higuchi kinetic model.

A comparative percentage cumulative drug release/cm^2 (% CDR/cm^2) versus time profile was plotted for all the transdermal patches composed of EC and PVP **(Table 8.21, Figure 8.41, Chapter 8)** and EC and HPMC **(Table 8.22, Figure 8.42, Chapter 8)**. It was evident that the drug release pattern from the transdermal patches was dependent on proportions of hydrophilic and lipophilic polymers present in the matrix. By increasing the quantity of PVP in the drug-polymer matrix the fact was evidenced that PVP produces crystallization-free polymeric patches leading to higher drug release. Also PVP has antinucleating effect that changes crystalline drugs into higher energy amorphous state with good solubility. Initial rapid release of the loaded drug observed may be due to immediate dissolution from the surface of the patches by rapid leaching of hydrophilic fraction of the film formers. Leaching results in the formation of pores and thereby leads to the decrease of diffusional path length for the drug molecules to travel to reach dissolution media. It was found that formulation TTS6 and TDS6 have shown the most extended % CDR/cm^2 of 101.44 and 100.8 in 48 hours amongst all the formulations **(Table 8.21, figure 8.41 and table 8.22, figure 8.42, Chapter 8)**.

All the formulations were subjected to *in vitro* skin permeation study of the loaded drug. Study was carried out using albino rat skin in modified Keshary-Chein diffusion cell taking 100 ml phosphate buffer of pH 7.4 as diffusion media. It was observed that the permeation of drug through the skin is little lesser than the drug permeation through dialysis sac. The data obtained from the *in vitro* permeation study of all the transdermal patches were fitted to various kinetic models (Zero order, Higuchi, First order, Korsmeyer-peppas) to determine the pattern of drug release from the drug-polymer matrix. Formulations TTS6, TTS7, TTS8 and TDS6, TDS7, TDS8 have showed the R^2 value of 0.9816, 0.9687, 0.9745

and 0.9942, 0.9932, 0.9816 **(Table 8.28 to 8.30 and table 8.38 to 8.40, Chapter 8) (Figure 8.26 to 8.28 and figure 8.36 to 8.38, Chapter 8)** respectively, when their *in vitro* skin permeation data was fitted to Higuchi kinetic model. It was observed that the release of the drug was followed by diffusion process. When the skin permeation data was fitted to Zero order model, the R^2 values were found to be in between 0.8864 to 0.9037. Which implies drug release pattern was less controlled in comparison to the results observed after *in vitro* dissolution study. R^2 values obtained after fitting the *in vitro* skin permeation data to the Korsmeyer-peppas kinetic model for the formulations TTS6, TTS7, TTS8 and TDS6, TDS7, TDS8 were found to be 0.9794, 0.9743, 0.9796 and 0.9959, 0.9901, 0.972 respectively, which supports the fact that drug release from the matrix patches was diffusion controlled. Again Korsmeyer's exponent (n) values were found to be 0.6986, 0.7276, 0.7133 and 0.5868, 0.5538, 0.5321 respectively for the formulations TTS6, TTS7, TTS8 and TDS6, TDS7, TDS8, which suggest diffusion controlled drug release from these transdermal patches **(Figure 8.26 to 8.28 and figure 8.36 to 8.38, Chapter 8)**. *In vitro* skin permeation data supports the findings from *in vitro* permeation study and confirmed the fact that increasing in the concentration of hydrophilic polymer in the transdermal patches would result in higher amount of drug release within a shorter period. It was evident from the % CDR/cm^2 data after skin permeation of the formulations containing gradually less proportion of EC (TTS8, TTS9, TTS10 and TDS8, TDS9, TDS10), the drug release was found to be faster **(Table 8.43, figure 8.43 and table 8.44, figure 8.44, Chapter 8)**. On the contrary the drug release was faster from the formulations containing higher proportions of PVP and HPMC in this fashion TTS5 > TTS4 > TTS3 > TTS2 > TTS1 and TDS5 > TDS4 > TDS3 > TDS2 > TDS1 **(Table 8.43, figure 8.43 and table 8.44, figure 8.44, Chapter 8)** respectively. It was observed that formulation TTS6 and TDS6 have shown the most extended % CDR/cm^2 of 101.47 and 100.28 in 54 hours and 53 hours respectively, amongst all the formulations **(Table 8.43, figure 8.43 and table 8.38, figure 8.44, Chapter 8)**.

The formulations TTS6 and TDS6 showing the best results in terms of *in vitro* permeation through dialysis membrane and *in vitro* skin permeation were taken for *in vivo* drug absorption study. Male healthy rabbits weighing 1.5 - 1.7 kg were used for this study. Transdermal patches were placed onto the clean dorsal surface of four rabbits constituting the

first set and immediately occluded with an adhesive tape. Four rabbits of the second set were administered 19 mg propranolol hydrochloride at every 6 hours interval for 48 hours. Data obtained after analyzing the samples by HPLC was plotted against plasma concentration (ng/ml) versus time **(Table 8.45 and 8.46, Chapter 8) (Figure 8.45 and 8.46, Chapter 8)**. From the graph it was evident that up to 24 - 25 hours there was increase in the release of the drug from the formulation TTS6, after which it maintained a constant. At 24 hours the concentration of the drug was 14.2793 ng/ml, which extends up to 48 hours giving a concentration of 14.5651 ng/ml of the formulation TTS6 **(Table 8.45, figure 8.45, Chapter 8)**. For formulation TDS6 drug concentration was found to be 13.2982 ng/ml after 24 hours after which there was slight increase in the drug concentration which was found to be 13.4757 ng/ml at 48 hours **(Table 8.46, figure 8.46, Chapter 8)**. A distinct trough and peak has been observed when the data was plotted against plasma concentration (ng/ml) versus time for orally administered propranolol hydrochloride **(Table 8.45 and 8.46, figure 8.45 and 8.46, Chapter 8)**. After every 6 - 7 hours there was declination in the drug plasma concentration. It was clearly observed that for both the transdermal formulations (TTS6 and TDS6), the drug concentrations in plasma were increased during the initial hours and remained consistent up to 48 hours.

The scanning electron microscopic (SEM) examination of the patches showed best performances by the formulation code TTS6 and TDS6. Transdermal patches at different conditions like blank patch without drug, drug loaded patch and patch after skin permeation were taken for the study. Blank patches **(Figure 7.5 and 7.6, Chapter 7)** and drug loaded patches **(Figure 7.7a and 7.7b and figure 7.8a and 7.8b, Chapter 7)** were seemed to be formed uniformly. The SEM photograph revealed the formation of a homogeneous layer of the drug-polymer matrix films. Patches after skin permeation study have appeared with the void spaces in the films, which indicate the release of the drug from the patches **(Figure 7.9a and 7.9b and figure 7.10a and 7.10b, Chapter 7)**.