Oropharyngeal candidiasis is an opportunistic fungal infection which occurs whenever there is local or systemic immunosuppression. Systemic antimycotic therapy is undesirable because of potential side effects. Present local therapy by conventional dosage forms is inconvenient and comparatively ineffective. This may be due to their inefficiency in maintaining the salivary concentrations of the drug above the MIC for the prolonged period of time, which may in turn be due to the diluent effect of saliva secretions coupled with the cleansing action of the oral musculature. The present study is an attempt to develop mucoadhesive hydrogel films of drugs which could deliver the drugs locally in the oral cavity at concentration above MIC for a prolonged period of time.

The drugs, polymers and excipients were selected based on the preformulation studies. The drugs selected were Econazole nitrate and Nystatin. The polymer selected was PVA. The DSC and FTIR studies proved no any drug-polymer interactions (Figure 5.1 to 5.17 and Figure 5.70 to 5.84).

PVA forms hydrogels by various crosslinking methods. The PVA hydrogels were prepared by freezing/thawing as a crosslinking method and also by the copolymerization with gelatin method.

The optimization was done by $3^2$ full factorial design using Response Surface Methodology (RSM). The independent variables used for MHFs prepared by freezing/thawing method were concentration of PVA (% w/v of the initial gel) ($X_1$) and number of Freeze/Thaw cycles ($X_2$). For MHFs prepared by copolymerization with gelatin method the independent variables were concentration of PVA (% w/v of initial gel) ($X_1$) and the concentration of gelatin (% w/v of initial gel) ($X_2$). The dependent variables used in both the types of MHFs were, Time required for 50 % drug release ($T_{50\%}$) ($Y_1$), Percent of drug release at 8th hour ($Rel_{8hr}$) ($Y_2$), ‘k’ of Zero order equation ($Y_3$) and ‘n’ of Korsmeyer-Peppas equation ($Y_4$).

Based on the sequence of the plan of work described in the flowchart in Figure 3.1, the concentration of PVA, number of freeze/thaw cycles and concentration of gelatin were decided. The concentration of PVA decided were 10%, 15% and 20% w/v of initial gel. The numbers of freeze/thaw cycles
decided were 4, 6 and 8. And the concentrations of gelatin selected were 1.5, 2.5 and 3.5 % w/v of the initial gel. The factors considered for the selection of levels of variables were drug release and the mucoadhesive strength.

The hydrogels of Econazole Nitrate and Nystatin were prepared. The prepared hydrogels were studied for morphological evaluation, surface characterization by the Scanning Electron Microscopy, drug content uniformity, physical properties like microenvironment pH, MHF thickness, MHF weight, folding endurance and moisture absorption. In vitro mucoadhesive study, water uptake or swelling study, determination of the in vitro residence time and in vitro drug release studies were also carried out.

The colour of all the EF formulations observed by the naked eyes was white whereas of EG formulations was off white (Figure 5.18). The colour of all the MHF containing NYS (NF and NG formulations) were of dark yellow (Figure 5.85). The surface of MHFs prepared by freezing/thawing technique (EF and NF) was smoother compared to the MHFs prepared by the co-polymerization with gelatin method (EG and NG) (Figure 5.18 and 5.85). The crystals of the ECN were clearly observed on the surface of all the EF and EG MHFs (Figure 5.19 to 5.22). The surface of all the MHFs containing NYS was smooth without any crystals with some white patches seen in SEM images (Figure 5.86 to 5.89).

The drug content uniformity for all the MHFs was found in range (Table 5.1 and 5.65).

The microenvironment pH of all the MHFs was in the pH range of the oral cavity. So, there is minimum possibility of the irritation to the oral mucosa after application. The microenvironment pH of the MHFs prepared by freezing/thawing method was slightly higher than the MHFs prepared by copolymerization with gelatin method (Table 5.2 and 5.66).

The MHF thickness is unaffected by the number of freeze/thaw cycles and concentration of gelatin (Table 5.3 and 5.67).
The folding endurance was more than 300 numbers of folding for all the formulations. This indicates very high physical strength of the MHFs (Table 5.5 and 5.69).

The percent moisture absorption increased as the concentration of PVA increased. But there was no effect on moisture absorption in case of EF formulations by number of freezing/thawing cycles and in case of EG formulations by the concentration of gelatin. Comparatively the moisture absorption was high in the formulations prepared by freezing/thawing method than the formulations prepared by copolymerization by gelatin method. The difference in the moisture absorption from day 3 to day 7 is very less in all the formulations (Table 5.6 and 5.70).

As the concentration of PVA increased, the mucoadhesive strength increased. There was decrease in the mucoadhesive strength as the number of freeze/thaw cycle increased within the same concentration of PVA (Table 5.7 and Figure 5.23 and 5.24).

There was profound effect of the concentration of gelatin noticed on the mucoadhesive strength within the same concentration of PVA. As the concentration of gelatin increased the mucoadhesive strength increased (Table 5.71 and Figure 5.90 and 5.91).

The mucoadhesive strength of the MHFs prepared by Freezing/Thawing method was comparatively higher than the MHFs prepared by copolymerization with gelatin method (Table 5.7 and 5.71).

It was found that as the concentration of PVA increased, the percent swelling increased significantly (P<0.05) (Table 5.9, 5.10, 5.72, 5.73 and Figure 5.25, 5.26, 5.92, 5.93).

It was observed that within the same concentration of PVA, as the number of freeze/thaw cycles increased, the percent swelling was decreased significantly (P<0.05) (Table 5.9, 5.72 and Figure 5.25, 5.92).

In EG formulations, as the concentration of PVA increased from 10 % to 15 %, the percent swelling increased but surprisingly at 20 % the swelling
decreased. And within the same concentration of the PVA, as the concentration of gelatin increased, the percent swelling decreased (Table 5.10, 5.73 and Figure 5.26, 5.93).

The percent swelling data was subjected to Vergnaud swelling kinetics. The data fitted well ($R^2 > 0.99$) and in all cases ‘n’ lies in the range $0.51 < n < 0.95$, which is indicative of an anomalous mechanism of water uptake in which solvent diffusion, as well as polymer relaxation are of the same magnitude (Table 5.8 and 5.74).

The in vitro residence time increased as the concentration of the PVA increased in both EF and EG MHFs. It was observed that the residence time was decreased by increase in the number of freeze/thaw cycles within the same concentration of PVA (Table 5.11 and 5.75).

In EG formulations, as the concentration of gelatin increased, the in vitro residence time increased (Table 5.11 and 5.75).

The $R^2 > 0.999$ indicates a very good linearity of the standard calibration curve for both ECN and NYS (Table 5.12, 5.76 and Figure 5.27, 5.94). As the concentration of PVA increased, the ECN and NYS release decreased significantly (P<0.05) (Table 5.13, 5.15, 5.77, 5.79 and Figure 5.28, 5.29, 5.95, 5.96).

The release profiles were studied for zero order kinetics, Korsmeyer Peppas equation, Higuchi square root of time model and first order kinetics to determine the mechanism of drug release. Best fit was observed with Korsmeyer-Peppas equation with $R^2 > 0.99$ excluding MHFs with 10 % of PVA. The values $0.45 < n < 0.90$ of korsmeyer-peppas equation, indicates the anomalous behavior i.e. non-fickian kinetics corresponding to coupled diffusion/polymer relaxation (Table 5.14, 5.16, 5.78, 5.80).

As the number of freeze/thaw cycles increased the ECN and NYS release decreased significantly (P<0.05) (Table 5.14, 5.78).

As the concentration of gelatin increased, the ECN and NYS release decreased significantly (P<0.05) (Table 5.16, 5.80).
The significantly similar release kinetics show that the release mechanism was unaffected by the concentration of PVA, number of freeze/thaw cycles and concentration of gelatin (Table 5.14, 5.16, 5.78, 5.80).

The effect of independent variables i.e. concentration of PVA, number of freeze/thaw cycles and concentration of gelatin, and their levels on the dependent variables was studied by the 3-D curves and contour curves as well as by the values of the polynomial regression equations.

The four formulations (optimized formulations) were selected based on the required dependent variable values, two from each drug and one from each method of preparation (Table 5.129).

The optimized formulation selected among EF formulations was one with 15% PVA and 7 freeze/thaw cycles (OPT1), among NF formulations was one with 16% PVA and 7 freeze/thaw cycles (OPT2), among EG formulations was one with 15% PVA and 3.5 % Gelatin (OPT3) and among NG formulations was one with 16% PVA and 3.5 % Gelatin (OPT4) (Table 5.129).

The optimized formulations were prepared and evaluated for percent drug content, mucoadhesive strength, microenvironment pH, in vitro residence time, folding endurance, percent moisture absorbance (Table 130), swelling studies (Table 131 and Figure 5.137), in vitro drug release studies (Table 5.133 and Figure 5.138) and in vitro antifungal activity on Candida Albicans (Table 5.136). Results of all the above evaluation parameters of the optimized batches were satisfactory (Table 130, 131, 132, 133, 134 and 136).

The optimized MHFs demonstrated ample mucoadhesive strength with goat intestinal mucosa and gave a reasonable in vitro residence time (>12 hours) (Table 5.138), which is important for prolonging the contact time of the drug with the buccal mucosa, thus improving the overall therapy of Oropharyngeal Candidiasis.
The optimized MHFs of ECN and NYS prepared using PVA by freezing and thawing method as well as copolymerization with gelatin provide sustained release till 12 hour (Table 5.133 and Figure 5.138).

All the optimized formulations were fitted well in the Korsmeyer-Peppas equation and Zero order equation (R²>|0.99). The values for the ‘n’ of the Korsmeyer-Peppas equation for optimized MHFs were 0.89<n<0.94, indicate non-fickian anomalous mechanism includes simultaneous mechanism of diffusion and polymer relaxation. The good fit of the release data with zero order equation (R²>|0.99) suggests sustained drug release with the uniform release rate (Table 5.134).

The factorial optimization technique yields results with a high degree of prediction and fruition. The study can, therefore, enable the formulator to reach and quantify the optimum decreasing experimentation during formulation.

The drug released from the optimized MHFs was able to inhibit the growth of C. albicans for 12 hours (Table 5.136). This would be important for better patient compliance because of the decrease in the frequency of administration. From the standard calibration curve of ECN and NYS agar diffusion assay, the growth inhibition zone of 12 hour dissolution sample of optimized formulations correlated well with dissolution study data of the optimized formulations (Table 5.137). The diameter of zone of inhibition shown by the NYS containing MHFs was higher than the ECN MHFs (Table 136).

The optimized batches were subjected to accelerated stability study as per the ICH guidelines. The differences in the surface morphology by SEM, percent drug content, mucoadhesive strength (gm/cm²), microenvironment pH, in vitro residense time in minutes, folding endurance in number of folding and percent moisture absorption, release behavior and in vitro antifungal activity before and after stability study were compared.

The optimized formulations were stable in all the aspects after the accelerated stability testing as per ICH guidelines with P<0.05 (Table 5.138, 139, 141, 142
and Figure 5.142, 5.143, 5.144, 5.146) and the release profiles were significantly similar after the stability with $F_2 > 82.26$ (Table 5.140).

The hydrogel based mucoadhesive sustained release drug delivery system developed and evaluated for the delivery of ECN and NYS were formulated using PVA with freezing/thawing as a crosslinking method and also with copolymerization with gelatin method. They were better formulations for the local delivery in the oral cavity gave concentration above MIC for a period of more than 12 hrs.