CHAPTER V

Summary and Conclusions
Treatment of acute as well as chronic diseases have been attempted over the past several decades by delivering drugs to patients through various dosage forms such as tablets, capsules, liquids, creams, ointments, aerosols, suppositories and injectables.

The conventional products provide a prompt release of the drug. There is a quick rise and fall in blood levels of the drug (when elimination is fast) and frequent dosing to maintain therapeutic levels is accompanied with heavy fluctuations in drug levels. Recent technical advancements have led to the development of new drug delivery system which controls the rate of drug delivery giving sustained therapeutic activity and or targeting the delivery of the drug to a particular tissue controlled release dosage form (CDRF) and aims at providing sustained action through constant supply (zero order release) of drug to the absorption site and over a prolonged period of time.

Acyclovir is used for the treatment of virus infections. A major drawback in the therapeutic application of acyclovir is the drug is poorly water soluble, highly variable in absorption and has low bioavailability. Sustained release systems as in the case of microencapsulation were designed to achieve slow release of the drug acyclovir over an extended period of time.

Four polymers have been used to formulate microcapsules of acyclovir. Saccharomyces cerevisiae (Baker’s yeast) is surrounded by a true cell wall, aerobic, can tolerate acidic conditions and has got a mucoadhesive property. Guar gum swells to form a thixotropic sol and is a good encapsulating agent. Various grades of ethyl cellulose having higher viscosity tend to produce stronger and more durable films and by adding plasticizers we can alter the release of the drug. Egg albumin due to its high
viscosity is a good encapsulating agent. Thus, studies were carried out on the microcapsules of acyclovir so as to control the rate of release of the drug.

The first chapter of the thesis deals with a brief introduction to the conventional drug therapy, concept of sustained release, potential advantages of sustained release dosage forms, and techniques for preparing prolonged action dosage forms. Introduction to microencapsulation, various methods of preparation of microcapsules, characteristics and application of microcapsules in the pharmaceutical field. A brief description of virus, viral classification and antiviral drugs. Finally a description of pharmaceutical suspensions, properties, classification, additives used and the quality control tests for pharmaceutical suspensions.

The second part deals with the aim and objective of the work. This part explains the need for designing a suitable drug delivery system for the drug candidate selected for the present work.

The second chapter of the thesis deals with the literature review of the past work done using Saccharomyces cerevisiae, guar gum, ethyl cellulose and egg albumin.

The third chapter gives details of the drug acyclovir, its clinical use, mechanism of action, pharmacokinetics, adverse drug reaction, dosage forms and pattern of dosage. Literature review of polymers Saccharomyces cerevisiae, guar gum, ethyl cellulose and egg albumin giving details of their description, functional category, classification, stability and applications.

The second part describes the materials and equipments used, analytical and experimental methods employed in the present study. First part of this chapter explains the preparation of acyclovir microcapsule using Saccharomyces cerevisiae, (12 formulations); guar gum, (6 formulations); egg albumin, (6 formulations) and
ethyl cellulose (6 formulations). The acyclovir microcapsules using baker’s yeast were prepared by pre-treating baker’s yeast with 2% sodium azide, a respiratory inhibitor used to prevent the cells from performing any energy dependant process. The pre-treated yeast cells were sterilized by autoclaving to denature the carrier protein molecule. The pre-treated dead yeast cells with intact cell membrane were used for the encapsulation of acyclovir. Acyclovir, pre-treated yeast cells and distilled water were taken in the ratio of 1:2:4. The suspension was agitated for four hours using a magnetic stirrer and centrifuged for ten minutes at 2000rpm. The cells were washed five times with distilled water and dried in a lyophillizer for 48 hours. The effects of concentration at various temperatures using different stirring speed were studied. The acyclovir microcapsules using guar gum and ethyl cellulose were prepared by water-in-oil-oil (w/o/o) emulsion solvent diffusion method. The acyclovir microcapsules using egg albumin were prepared by water-in-oil (w/o) double emulsion (heat coagulation method). The four polymers were characterised by FT-IR, DSC and HPLC. The later part of this chapter deals with the characterisation of microcapsule namely particle size, bifocal microscopy, SEM, entrapment efficiency, FT-IR spectroscopy, DSC, drug content of acyclovir microcapsules by HPLC method, in vitro release study, mathematical modelling and stability studies as per ICH guidelines. Based on the data obtained from the acyclovir microcapsules it was observed that the microcapsules of acyclovir prepared with baker’s yeast (FY-IV, FY-VIII and FY-XII microcapsules) showed better results. Hence these microcapsules were formulated into oral suspension and compared with that of a marketed product. These acyclovir microcapsule suspensions were evaluated for physicochemical parameters, drug content, wt/ml, particle size and size distribution, sedimentation volume, rheology, in vitro release study and accelerated stability study.
The fourth chapter describes the results and discussions according to the plan of investigations carried out in the third chapter. The first section of this chapter that is 5.1 describes the solubility of acyclovir in various solvents. The drug acyclovir was very soluble in alcohol, slightly soluble in water, soluble in Dimethyl sulfoxide, freely soluble in Phosphate buffer pH 6.8, solution of mineral acids and alkali hydroxides. 5.2, 5.2.1 and 5.2.2 discusses about the calibration, preparation of the standard graph of the drug acyclovir in distilled water, 0.1N HCL of pH 1.2 and Phosphate buffer pH 6.8 respectively. The best linearity for concentrations was observed between 10µg/ml to 40µg/ml. The acyclovir microcapsules were successfully prepared using pre-treated Baker’s yeast, guar gum, egg albumin and ethyl cellulose. All the acyclovir microcapsules were characterised. The acyclovir microcapsules prepared with pre-treated Baker’s yeast showed better entrapment of the drug and release so only these microcapsules were taken for further formulation in the form of a suspension and were evaluated accordingly and the procedures were followed as given in chapter three.

Particle size determination of acyclovir microcapsules were done with various carriers using optical microscopic method. It was performed for 200 particles. The particle size of < 100µm diameter with hydrophilic surfaces have a longer circulation in the blood; such systems prolong the duration of drug activity and also increase the targeting efficiencies to specific sites. In the case of acyclovir microcapsules prepared with guar gum, egg albumin and ethyl cellulose the particle size were in the range of 100 to 180µm. The particle size in formulations of acyclovir prepared with Saccharomyces cerevisiae were found between 0 - 10µm especially in FY – VIII microcapsules there were 123 particles (62%), this optimum range is required to
prolong the duration of drug activity and also to increase the targeting efficiencies to specific sites.

Surface morphology of various acyclovir microcapsules prepared with carrier was examined by Bifocal microscopy. In all the formulations FY – IV, FY – VIII and FY- XII microcapsules it can be seen that the encapsulation with baker’s yeast is very clear. In a few formulations they are in the form of clusters and indicate a granular cytoplasm visible within each cell, surrounded with a thick cell wall. Although the cells were dyed with Nile blue, the lack of fluorescence on the adjacent confocal image demonstrated that the cells had absorbed very little fat soluble dye. However, located centrally within the cells, was a region that absorbed some dye. In the formulations FG, FA and FE microcapsules it can be seen that the shapes are vague in nature forming single agglomerate. In FG – III and FG – VI microcapsules the encapsulation in the cluster is visible.

The surface morphology of acyclovir microcapsules were observed using scanning electron microscopy and this was performed at various magnifications. From the SEM images it was observed that the formulations of acyclovir using pre-treated baker’s yeast showed a clear morphology and a rough and wavy surface and porous in character. The microcapsules were found to be discrete and spherical in shape especially in the case of FY – IV, FY – VIII and FY – XII acyclovir microcapsules. Though the magnification was done at a higher level for the formulations of acyclovir using guar gum, ethyl cellulose and egg albumin, the SEM image showed a wavy and was more porous and agglomerates were larger. In the case of FG –III, FE –III and FE –VI the microcapsules were discrete with a wavy outer surface.

The yield and % entrapment efficiency for all formulations of acyclovir microcapsules with various carriers was estimated. The percentage yield and %
entrapment efficiency of FY – IV was (70 & 96.66), FY – VIII (77 & 97.61), FY – XII (92 & 96.42); in the case of FG – III (96.42 & 92.77), FG – VI (94.64 & 92.55); FE – III (94.34 & 91.76), FE – VI (96.42 & 90.21); FA – III (89.28 & 90.61) and FA – VI microcapsules (92.85 & 89.28 ). So it can be seen that the maximum entrapment efficiency is seen in the formulations FY – IV, FY- VIII and FY – XII microcapsules which were done at a temperature of 40°C and at 2000 rpm. At 40°C a significant increase in the rate of encapsulation was noted and the process appeared more temperature dependant. This abrupt change in the encapsulation rate is likely to have been due to phase transition of the membrane phospholipids which occurs within a specific temperature range depending on the nature of the phospholipids. This observation suggested that the cell membrane of *Saccharomyces cerevisiae* is responsible for controlling the rate of entry of acyclovir into the cell.

The FT-IR spectroscopy of the twelve microcapsules FY-I to FY-XII were done. The IR spectra shows a strong absorption band at 1716.78 for C=O group. An intense peak is observed at 1635.1 cm\(^{-1}\) for – C= – aromatic nuclei. Two strong absorption band was seen at 1488.01 and 1541.68 cm\(^{-1}\) for 2 C=N, 8 C=N, stretching in ring. Absorption is seen at 1308.91 for C – N stretching for primary amino group. Absorption is seen at 902.01 for 5, 6 alkenes –CH=CH – group. Two strong absorption bands at 3441.87, 3480 cm\(^{-1}\) and this confirms primary amino group. Absorption at 3551 cm\(^{-1}\) for O-H at 1° alcohol. Absorption is seen at 3186.66 cm\(^{-1}\) for C-H stretching at alkenes. It was concluded that there was no interaction with acyclovir and baker’s yeast based on the above given data.

The FT-IR was done for pure drug and 18 microcapsules formulation (FG-I to FG – VI, FA – I to FA – VI and FE – I to FE – VI microcapsules) for drug identification. It indicates no chemical reaction between drug and polymers and also
confirmed the stability of the drug during micro encapsulation process. The characteristic peaks were due to pure acyclovir at 615 cm\(^{-1}\), 1215 cm\(^{-1}\), 1440 cm\(^{-1}\), 1515 cm\(^{-1}\), 1610 cm\(^{-1}\), 1627 cm\(^{-1}\) for Aromatic ring - NH\(_2\) Aromatic, ether, - OH binding secondary amine, primary amine, C=C ring aromatic stretching, C=O stretching have appeared in microcapsules spectra peaks, without any change in their position after successful encapsulation. The pure drug spectra peaks correlated with the microcapsules formulations peaks.

The DSC was done for acyclovir (pure drug), Baker’s yeast, and FY- IV, FY-VIII, FY-XII microcapsules and FG - III, FG - VI; FA- III, FA - VI and FE - III, FE - VI microcapsules.

The DSC thermogram of acyclovir showed the onset at 149.11°C and the peak was found to be 168.54°C, the second peak was found at 278.99°C. The thermogram has a linear melting curve, the melting point is defined by onset temperature and melting is characterised at peak maxima. It has a broad and asymmetric melting peak. The enthalpy of fusion of the first peak is (-171 J and -855.34 J/g) and for the second peak (-1.09J and -545.16 J/g). This indicated that the system was endothermic in nature characterised by melting and sublimation. The DSC thermogram of baker’s yeast showed three peaks. The first peak has the onset at 109.48°C, peak at 112.75°C and ended at 122.07°C; the second peak has the onset at 148.07°C, peak at 155.12°C and ended at 159.74°C; the third peak has the onset at 203.52°C, the peak at 211.59°C and ended at 227.73°C. It can be observed that the first peak has a linear melting curve, the second and third peak have a broad asymmetric melting peak. Melting is characterised at peak maxima. A eutectic melt was observed between the first and second peak. The enthalpy of fusion of the first peak is (-28.73 mJ and normalized – 7.48 Jg-1); for the second peak (-2.91 mJ and normalized – 0.76 Jg-1) and for the
third peak (-14.42 mJ and normalized -3.76 Jg-1). This indicates that the system is endothermic in nature characterised by melting and sublimation.

The DSC thermogram of FY – IV microcapsule formulation showed onset at 253.61°C reaching peak at 263.70°C and end set at 274.79°C. It has a linear melting curve characterised by peak maxima. The enthalpy of fusion observed is (- 196.95 mJ and -98.47J/g). The thermogram is endothermic in nature and matches that of baker’s yeast and that of acyclovir. The DSC thermogram of FY – VIII microcapsule formulation showed the onset at 148.38°C. The first peak obtained at 160.49°C and the end set at 176.18°C. The second onset was at 251.37°C with second peak at 260.77°C and end set at 272.21°C. The first peak was broad and asymmetric matched with acyclovir which matched with that of baker’s yeast and the second peak has a linear melting curve. The enthalpy of fusion of the first peak is (-305.18 mJ and -152.59J/g); for the second peak (-319.89 mJ and -159.95 J/g) this indicated that the thermogram is endothermic and characterized by melting and sublimation. The DSC thermogram of FY – XII microcapsule formulation showed onset at 254.50°C with the peak at 263.59°C and the end set at 274.27°C. The peak at 150°C matched with that of baker’s yeast which gave broad and asymmetric melting point peak. The enthalpy of fusion of the peak is (-160.29 mJ and -80.14J/g). The thermogram exhibits endothermic events like melting and sublimation.

The DSC thermogram of the FG – III microcapsule formulation showed the first onset at 133.57°C. The first peak obtained at 142.16°C and the endset at 159.60°C. The second onset started at 246.29°C with peak at 252.15°C and the end set at 257.87°C. A gradual change in enthalpy took place to produce an endothermic peak. The enthalpy of fusion is -131.99 mJ and -1.32Kj/g and the second peak is -232.83 mJ and -2.33Kj/g. The first peak is a broad asymmetric melting peak which
matches with that of guar gum and the second is a linear melting curve which matches with that of acyclovir. The DSC thermogram of FG – VI microcapsule formulation showed the first onset at 114.32°C. The first peak obtained at 121.06°C and the end set at 132.25°C. The second onset was at 256.17°C, the second peak at 260.46°C and the end set at 267.29°C. The enthalpy of fusion is -285.40 mJ and -2.85 KJ/g and for the second peak -232.56 mJ and -2.33 Kj/g. The first and second peaks are linear melting curve and they match with that of guar gum and of acyclovir.

The DSC thermogram of formulation FA-III microcapsule formulation showed the first onset at112.56°C, the first peak at 125.92°C and ended at 146.72°C. The second onset is seen at 229.85°C, the peak at 235.90°C and ended at 242.62°C. The enthalpy of fusion is – 463.53Mj and - 257J/g, for the second peak it is – 180.26 mJ and – 100.14 J/g. The first is a broader asymmetric peak and the second one is a linear melting curve. The DSC thermogram of FA – VI microcapsule formulation showed totally three peaks. The first onset started at 134.00°C, with the first peak at 151.90 °C and ended at 165.01°C. The peak obtained at 180.54°C and ended at 187.40°C. The third onset started at 243.26°C, the peak obtained at 255.66°C and ended at 264.09°C. The enthalpy of fusion is -496.15 mJ and -4.96 KJ/g, for the second peak it is -43.01 mJ and -430.10 J/g and for the third peak it is -861.89 mJ and-8.62 KJ/g. The first peak is a broader asymmetric peak, the second one is a phase transition peak and the third is a linear melting curve. The above peaks match that of egg albumin and of acyclovir.

The DSC thermogram of FE – III microcapsule formulation showed the first onset at 133.28°C with a first peak at 142.16 °C and the ended at 161.81 °C. The second onset started at 246.16 °C with a peak at 252.15 °C and the ended at 258.14 °C. The enthalpy of fusion is -154.39 mJ and -1.54 KJ/g ; for the second peak the heat
generated is $-224.28 \text{ mJ}$ and $-2.24 \text{ KJ/g}$. The first is a broad asymmetric melting peak and the second is a linear melting curve. The above peak matches with that of ethyl cellulose and that of acyclovir. The DSC thermogram of FE - VI formulation showed the first onset at 133.24 °C with a first peak at 144.77 °C and the ended at 163.06 °C. The second onset started at 262.33 °C with a peak at 267.20 °C and the ended at 274.43 °C. The enthalpy of fusion is $-249.96 \text{ mJ}$ and $-2.50 \text{ KJ/g}$ and for the second peak it is $-426.97 \text{ mJ}$ and $-4.27 \text{ KJ/g}$. The first is a broad asymmetric peak and the second is a linear melting curve. The above peaks match with that of ethyl cellulose and that of acyclovir.

The High Pressure Liquid Chromatography (HPLC) was done for standard solution of acyclovir, FY - IV, FY- VIII, FY – XII microcapsule formulation and FG - III, FG - VI; FA - III, FA - VI and FE - III, FE - VI microcapsules. The acyclovir standard has a peak value of 4462254, FY – IV 438039, FY – VIII, 4394564 and FY – XII microcapsules 4326804.

The peak value of FG – III was 4909.412, FG – VI microcapsules 6477.348; FE – III was 9572.457 and FE – VI was 7474.465. The peak value of FA – III microcapsules was 9924.944 and FA – VI 12955.628. The content uniformity was in the range of 86.2% (FY – VIII microcapsules) to 99.7% (FA – VI microcapsules).

The in vitro dissolution studies were done to characterize the acyclovir microcapsules using the various excipients. The in vitro dissolution studies were done in GI simulated condition for acyclovir microcapsules (FY – I to FY – XII); (FG – I to FG – VI); (FE – I to FE – VI) and FA – I to FA – VI. The in-vitro dissolution of acyclovir microcapsules prepared using pre-treated baker’s yeast (FY – I to FY – XII) was performed using a modified diffusion cell. Dialysis membrane was tied to one end of open ended glass tube which was immersed into 250 ml receptor fluid kept in
500 ml beaker. The apparatus was operated at 100 rpm over a magnetic stirrer maintained at 37 ± 0.5°C. The acyclovir microcapsules were taken into the dialysis membrane.

The in vitro release studies of acyclovir microcapsules prepared by using guar–gum, egg albumin and ethyl cellulose were performed in a modified diffusion cell as per USP specifications. Microcapsules equivalent to 100 mg of acyclovir was accurately weighed and the in vitro release studies were performed. The releases of acyclovir from various microcapsules were measured spectrophotometrically at 254 nm. There was a sustained release of acyclovir especially in the case of acyclovir microcapsules prepared using baker’s yeast (FY – IV, FY – VIII and FY – XII) when compared to the release of acyclovir from acyclovir microcapsules prepared using guar gum, egg albumin and ethyl cellulose. The dissolution profile was tabulated and analysed using various kinetic models. The release of acyclovir from the preparations followed super case II transport of drug release under Korsmeyer – peppa’s model.

Based on the best entrapment and release studies of acyclovir microcapsules prepared using baker’s yeast (FY - IV, FY - VIII and FY - XII); (FG - III, FG - VI); (FE - III, FE – VI) and (FA – III, FA – VI) were subjected to stability studies as per ICH guidelines for six months at 25 ± 2°C and 60 ± 5% RH and 40 ± 2°C and 75 ± 5% RH. The content uniformity and the appearance were quite stable and the morphological changes and agglomeration was not seen in the SEM taken after 180 days.

Based on the best entrapment, particle size analysis, bifocal microscopy, SEM, FT-IR spectroscopy, DSC, HPLC, dissolution studies and stability studies it is observed that acyclovir microcapsules prepared using Saccharomyces Cerevisiae (Baker’s yeast) (FY-IV, FY-VIII and FY-XII) showed excellent results compared to
the other acyclovir microcapsules that were prepared using guar gum, ethyl cellulose and egg albumin. Hence the formulations FY-IV, FY-VIII and FY-XII acyclovir microcapsules were taken into consideration to formulate as a suspension of acyclovir microcapsules and was compared with a marketed product.

The physicochemical parameters of acyclovir-baker’s yeast microcapsule suspension (FY-IV, FY-VIII, FY-XII) and the marketed suspension were done with respect to appearance, colour, odour, taste and pH. The appearance was opalescent turbid in all the formulations, colour observed was light pink in the marketed formulation (Zovirax) and off-white in all the other formulations. The odour was fruity in the marketed formulation and pleasant in all the other formulations. The pH of the marketed acyclovir formulation was 5.55, FY-IV suspension 5.58, FY-VIII suspension 6.02 and in FY-XII suspension it was 6.33. The details almost correlate with that of the marketed formulation. The drug content of the prepared formulations and the marketed suspension were done and was found to be 87.96% w/v in Zovirax suspension, 72.69% w/v in FY-IV, 84.98% w/v in FY-VIII and 77.26% w/v in FY-XII acyclovir microcapsule suspension. The drug content was comparatively higher in FY-VIII acyclovir microcapsule suspension and almost equals to that of the marketed suspension of acyclovir. The weight per ml of the prepared acyclovir formulations and the marketed suspension were done by pycnometer method and the marketed formulation was observed to be 1.1627 g/ml, FY-IV suspension was 1.008 g/ml, FY-VIII suspension was 1.077 g/ml and in FY-XII suspension was 1.0524 g/ml. The wt/ml in the case of the marketed suspension was found to be higher due to the added additives that must have been present but in the prepared formulation. The particle size analysis of the marketed suspension, FY-IV, FY-VIII and FY-XII acyclovir microcapsule suspension was done by optical microscopic method and was done for
200 particles. Various graphs were plotted with respect to size range versus number of particles, mean size range versus frequency, mean size range versus cumulative frequency undersize. In the case of the marketed preparation the maximum no of particles were found to be larger and were in the size range of 40-50 µm. In FY-IV, FY-VIII and FY-XII acyclovir microcapsule suspension the particles were smaller in size and the maximum no of particles were in the size range of 4-8 µm and this is considered as an optimum size range which denotes an increase in surface area which helps in better absorption and an increase in bioavailability. The sedimentation volume of the marketed formulation, FY-IV, FY-VIII and FY-XII was observed for ten days and the final sedimentation volume was almost the same as that of the marketed formulation. This shows that the prepared formulations are equally stable as that of the marketed suspension. The rheological studies in terms of the viscosity were done for the marketed suspension and the prepared acyclovir microcapsule suspension. The formulation FY-IV was 6420 cps, FY-VIII was 4740 cps, FY-XII was 2460 cps and the marketed suspension was 7620 cps. The viscosity of the marketed formulation was found to be higher due to the additives that must have been added. The calibration curve of acyclovir in Phosphate buffer pH 6.8 was plotted and the slope and intercept for acyclovir was found to be 0.024 and 0.038 respectively at 254 nm, with regression value of 0.995 for acyclovir it shows the best linearity for concentration 10µg/ml to 40µg/ml. The in vitro release was performed for the marketed acyclovir suspension, FY-IV, FY-VIII and FY-XII acyclovir microcapsule suspension. The mean cumulative drug released for the marketed formulation (zovirax) was 86.99%, FY –IV suspension was 77.46%, FY – VIII suspension was 76.11% and FY – XII suspension was 80.06%. There was a burst in release at the end of the second hour this may be due to erosion and diffusion. The kinetic modelling
was done and fitted in zero order, first order, Higuchi and Korsmeyer-peppa’s plot and as per the release exponent it was found to be case 2 relaxation or super case transport 2, it refers to the release mechanism by erosion. Stability studies of marketed suspension, FY –IV, FY– VIII and FY –XII suspensions at 40 ± 2 °C and 75 ± 5 % RH and 25 ± 2 °C and 60 ± 5 % RH were done. There were no physical changes and there was not much of change in the content uniformity. The stability of FY – VIII acyclovir-baker’s yeast suspension was found to be better than the other suspensions. The SEM photographic analysis also does not show any change in the morphological characteristics.

CONCLUSION:

Acyclovir microcapsules were prepared using *Saccharomyces cerevisiae* (Baker’s yeast), guar gum, ethyl cellulose and egg albumin. These microcapsules were characterised successfully. Acyclovir microcapsules (FY-IV, FY-VIII and FY-XII) were formulated in the form of a suspension and characterised.

*Saccharomyces cerevisiae* (Baker’s yeast) has been used to prepare acyclovir microcapsules using a novel technique. *Saccharomyces cerevisiae* is basically present in the flora of our stomach. Being a natural bio capsule it has got an excellent mucoadhesive property and has been proved to stick on to the gut wall for a longer time so thereby can sustain the action of the antiviral drug acyclovir. It does not have any side effects. In future In vivo and animal studies can be done on this acyclovir microcapsule using *Saccharomyces cerevisiae* so that it will be highly beneficial to humankind.