CHAPTER - 5

SUMMARY AND CONCLUSIONS
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5.1. SUMMARY:

One of the most important objectives in present day drug therapy is selective delivery of drugs and diagnostic agents to specific organs or target sites in the body. This would result in reduction of unwanted side effects and adverse reaction and allow the use of highly active but toxic chemicals that cannot be employed at present because of their unselective distribution in the body e.g. in cancer chemotherapy agents that show activity against neoplastic tissue, also show activity against the normal tissue. Hence greater selectivity and a consequent increase in therapeutic index would be of considerable benefit.

Drug targeting is a specific form of drug delivery where the pharmacological agent is directed selectively to its site of action i.e. organ or cell. Drug goes to the site where it is needed and other tissues are not exposed to possible harm, so it leads to reduction of side effects and adverse reactions. Cancer chemotherapy is often a quoted example of treatment where drug targeting would be of considerable benefit. Drug targeting can be achieved with the...
use of variety of different approaches including chemical modification, implants and by using specific carrier systems.

Various carriers such as microspheres, liposomes, nanospheres and emulsions have been investigated to be used for passive targeting or natural targeting to organs like liver, spleen, lungs and kidneys. This can take place due to interaction between colloidal particles and physiological processes. Following intravenous administration of these colloidal carriers, the drug targeting is influenced by various factors like particle size, surface charge, surface hydrophobicity and absorption of macromolecules to particle surface. Colloidal carriers have been reported to be used for first order targeting of drugs to liver, kidney and second order targeting to tumour bearing organs.

The present study involves the preparation of various colloidal drug delivery systems of 5-fluorouracil which could be used to achieve natural or passive targeting.

These colloidal drug delivery systems include:

(1) Niosomes of 5-fluorouracil using sorbitan ester group of surfactants.

(2) Microspheres prepared using acrylic polymers Eudragit RS 100 and Eudragit RL 100.

(3) Preparation of polyterephthalamide microspheres containing 5-fluorouracil.
These systems were optimized with respect to their formulation and method of preparation. They were characterized with respect to drug entrapment efficiency, particle size analysis, photomicrographic characterization and in vitro leaching rate study.

All the carriers were studied for in vivo organ distribution pattern in liver, lungs, kidneys and intestine following intravenous administration in rats.

5.1.1 Niosomes:

Niosomes containing 5-fluorouracil were prepared using a series of Span surfactants namely Span40, Span60, Span80 and Span85 by thin film hydration method. Niosomes were separated from unentrapped drug by gel filtration technique through Sephadex G-50 column. The effect of formulation additives like cholesterol and dicetylphosphate was studied by incorporating these additives in niosome formulation. Drug entrapment efficiency was determined after inclusion of cholesterol and dicetyl phosphate. It was found that inclusion of cholesterol increased the entrapment efficiency of drug in niosomes, from 10.36 to 20.49% in case of Span40, from 16.11 to 42.49% in case of Span60, from 3.39 to 7.78% in case of Span80 and from 1.901 to 8.551% in case of Span85. The inclusion of dicetyl phosphate did not have much significant effect on entrapment efficiency of drug within niosomes.
The drug entrapment efficiency of various formulations were compared. Based on this data it was found that niosomes prepared with Span60 gave maximum drug entrapment. Hence niosomes prepared from Span60 were studied for further characterization. The size analysis and size distribution of Span60 niosomes was determined using an Axiomat Zeiss microscope.

Vesicle size of two formulations of niosomes, one composed of Span60 and cholesterol and other composed of Span60, cholesterol and dicetyl phosphate was compared. The mean size of vesicles in these two formulations was found to be $3.10 \pm 1.29$ microns and $5.58 \pm 1.44$ microns respectively. This indicates that inclusion of dicetyl phosphate produces a reduction in mean size of vesicles. This effect may be attributable to the decrease in membrane curvature by inducing charge in niosomes through incorporation of dicetyl phosphate.

Photomicrographs of niosomes were taken and they indicated a spherical vesicles with a distinct boundary.

Niosomes were evaluated for stability with respect to drug leakage. Niosomes prepared from Span60 & cholesterol and Span60, cholesterol & dicetylphosphate were stored in aqueous suspension form at room temperature. The extent of drug leakage from these niosomes was determined upto a period of 60 days after removing aliquots at various time intervals. The aliquots
were gel filtered and amount of drug leakage was determined by analysing the eluent.

Comparison of the drug leakage of these two formulations indicate that there is a leakage of 48.19% of drug from niosomes prepared with Span60 & cholesterol as compared to a leakage of 42.58% from niosomes prepared with Span60, cholesterol & dicetyl phosphate at the end of 60 days. Thus, much difference in leakage was not observed between the two formulations.

The effect of freeze drying on leakage rate was studied after freeze drying of niosomes prepared from Span60, cholesterol and dicetyl phosphate. The extent of leakage was reduced from 42.58% to 12.46% after freeze drying during a time interval of 60 days.

In vivo organ distribution pattern of niosomes was determined by administration of niosomes in healthy rats (Wistar strain). The animals were sacrificed at the intervals of 2, 4 and 6 hours and organs like lungs, liver, kidneys and intestine were removed and homogenized. The amount of 5-fluorouracil in the organ homogenate was estimated by high performance liquid chromatography method after extraction of drug from the homogenate.

The in vivo organ distribution pattern of 5-fluorouracil from niosomes showed a slow release of drug as compared to free drug solution. Compared to free drug solution, niosomes showed detectable drug levels upto 6 hours. The maximum drug levels in four
organs, calculated as percentage of administered dose distributed were observed at 4 hours after administration of niosomes. A highly significant difference was found between total drug distributed in the four organs from niosomes as compared to free 5-fluorouracil solution \((p<0.001)\). The organ distribution pattern showed significant distribution in liver as compared to 5-fluorouracil solution \((p<0.001)\).

5.1.2 Microspheres containing 5-fluorouracil prepared using Eudragit RS 100 and Eudragit RL 100 polymers:

Microspheres of acrylic polymers Eudragit RS 100 and Eudragit RL 100 containing 5-fluorouracil were prepared using the quasi emulsification solvent diffusion technique. The microspheres of both polymers were separated from unentrapped drug by high speed centrifugation technique and subsequent washing of the microspheres with phosphate buffer saline. These microspheres were characterized with respect to drug entrapment efficiency.

The drug entrapment efficiency of Eudragit RS 100 microspheres was greater than that of Eudragit RL 100 microspheres.

Size analysis of Eudragit RS 100 and Eudragit RL 100 microspheres was carried out using an axiomatic microscope. The average size of Eudragit RS 100 microspheres was found to be \(4.02 \pm 2.66\) micron where as
that of Eudragit RL 100 microspheres was found to be 3.79 ± 2.55 micron.

The extent of drug leakage from Eudragit RS 100 and Eudragit RL 100 microspheres was determined by storing the microspheres in phosphate buffer saline at room temperature for a period of 60 days. At different time intervals aliquots were withdrawn and amount of drug leaked in phosphate buffer saline was determined. The amount of drug leached from Eudragit RS 100 microspheres was 8.04% as compared to 16.90% shown by Eudragit RL 100 microspheres, which may be due to low permeability of Eudragit RS 100 films as compared to that of Eudragit RL 100 films.

In vivo organ distribution pattern of 5-fluorouracil from both Eudragit RL 100 microspheres and Eudragit RS 100 microspheres showed a different pattern as compared to free 5-fluorouracil solution. Measurable drug levels were detected up to 24 hours after administration from both the systems. The maximum total drug levels, calculated as percentage of dose administered, were found at 6 hours from both the systems.

The maximum drug levels, calculated as percentage of dose administered, in the four organs were not significantly different (p>0.5) from Eudragit RL 100 microspheres as compared to free 5-fluorouracil. However a significant difference (p<0.001) was found in the drug levels in the liver from Eudragit RL 100.
microspheres as compared to free 5-fluorouracil.

A significant difference was found when total drug distributed in four organs from Eudragit RS 100 microspheres was compared with that of free 5-fluorouracil (p<0.001). With respect to distribution in the organs, a significant difference was seen in drug levels in intestine from Eudragit RS 100 microspheres as compared to free 5-fluorouracil solution (p<0.001).

5.1.3 Polyterephalamide microspheres:

Polyterephalamide microspheres were prepared by interfacial polymerization technique using histidine as the diamine and terephthaloyl chloride as acid dichloride. The formulation was optimized with respect to various parameters such as formulation ingredients and various physical conditions like time of emulsification, temperature and time of polymerization, stirring speed etc. The microspheres were separated from unentrapped drug by centrifugation followed by washing of microspheres with phosphate buffer saline.

The microspheres were then characterized with respect to drug entrapment efficiency. The drug entrapment efficiency of polyterephalamide microspheres was found to be 86.38 ± 4.4%. Size analysis of microspheres was carried out using Axiomat Zeiss microscope and photomicrographic characterization was also done using the same microscope. The average microsphere size was found to be 4.70 ± 2.11 micron.

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The microspheres were stored in phosphate buffer saline at room temperature and extent of drug leakage from microspheres was studied up to a period of 60 days. The leaching rate study indicated a 15.1% leaching of 5-fluorouracil from the microspheres during a period of 60 days.

In vivo organ distribution pattern of the drug from polyterephalamide microspheres showed significant levels up to 6 hours as compared with free 5-fluorouracil solution. A highly significant difference was found (p<0.001) when total drug levels in four organs were compared with that of free 5-fluorouracil solution.

The organ distribution pattern indicated significant levels in liver from polyterephalamide microspheres as compared to free 5-fluorouracil solution (p<0.001).

5.2 CONCLUSIONS:
Over the past few years several potential site specific drug delivery systems for parenteral administration such as liposomes, microspheres, nanoparticles and nanocapsules have been designed. Their aim is to target drugs to specific organs or cells, thereby preventing normal tissues from adverse toxic effects and leading to an improved therapeutic index.

The present study was carried out with a similar aim for site specific delivery of 5-fluorouracil by process of passive targeting (natural biodistribution) to specific
sites by preparing colloidal drug delivery systems like niosomes and microspheres.

Four different colloidal drug delivery systems containing 5-fluorouracil were prepared namely niosomes, microspheres using Eudragit RS 100 and Eudragit RL 100 polymers and polyterephthalamide microspheres. All systems were optimized with respect to formulation conditions and evaluated invitro for entrapment efficiencies, size distribution and leakage rate studies. All systems were evaluated for invivo organ distribution studies in rats.

Niosomes containing entrapped 5-fluorouracil were prepared by thin film hydration method using a combination of Spans with cholesterol and dicetyl phosphate. Incorporation of cholesterol an the formulation was found to increase the entrapment efficiency of 5-fluorouracil in niosomes. Incorporation of dicetyl phosphate in formulation resulted in reduction in vesicle size of niosomes. Aqueous dispersions of niosomes, like most vesicular systems, showed leakage of drug during storage, however a considerable reduction in leakage rate could be achieved by freeze drying of niosomes. The in vivo organ distribution study of 5-fluorouracil from niosomes showed a significant difference (p<0.001) in the amount of drug distributed in liver as compared to free drug solution.

Eudragit RL 100 microspheres and Eudragit RS 100 microspheres containing 5-fluorouracil were prepared by quasi-emulsification solvent diffusion method which gave discrete
microspheres. Both the systems were evaluated for size distribution study and in vitro leakage rate study. The leakage rate of drug from Eudragit RL 100 microspheres was found to be greater than that of RS 100 microspheres.

In vivo organ distribution pattern of 5-fluorouracil from Eudragit RL 100 microspheres showed a significant distribution in liver \((p<0.001)\) while that from Eudragit RS 100 microspheres showed significant distribution in intestine \((p < 0.001)\) as compared to free drug solution.

Polyterepthalamide microspheres containing 5-fluorouracil were prepared by interfacial polymerization technique by reacting amino acid histidine with terepthaloyl chloride using Span 85 as emulsifier. The microspheres of required size range and drug payload were obtained after optimization of formulation conditions.

In vivo organ distribution pattern of 5-fluorouracil from these microspheres showed a significant distribution in liver \((p<0.001)\) as compared to free 5-fluorouracil solution.

Based on the observations made by us we can conclude the following with respect to in vivo organ distribution:

The significant distribution of 5-fluorouracil from niosomes, Eudragit RL 100 microspheres and polyterephalamide microspheres into liver as compared to free 5-fluorouracil, could be due to process of passive targeting. All these systems had maximum number of particles in the range of 1 to 6 micron which explains their significant uptake in liver because of being engulfed by reticulo endothelial systems of this organ.
However with Eudragit RS 100 microspheres it was found that there was initial distribution of 5-fluorouracil in liver but subsequent distribution was observed in intestine. The initial uptake of drug in liver can be due to uptake of drug by reticulo endothelial systems of the liver. But subsequent distribution of drug in intestine is difficult to explain.

This observation does not confirm to phenomena of passive targeting. But besides particle size, there are several factors such as surface charge, surface hydrophobicity, and interaction of colloidal particles with blood components which influence drug distribution patterns. One or more of these factors may be responsible for significant distribution in intestine.

At present 5-fluorouracil is used for the treatment of gastro intestinal tract, ovary and breast cancers. However it exhibits a lot of tissue toxicities and hence its use is restricted because of its non specificity and non selectivity.

All the systems except Eudragit RS 100 microspheres, studied in the present work shows promising results with respect to site specific delivery of 5-fluorouracil to liver. However further work needs to be done with respect to long term stability of these products, scale up studies, invivo studies in tumour bearing animals, and clinical trials before they can be used in antineoplastic chemotherapy.