Abstract

The design and development of a new drug delivery system with a view to enhance the efficacy of existing drugs is an ongoing process in the pharmaceutical research.

Such delivery systems include solid lipid nanoparticles (SLN), liposomes, niosomes, polymeric nanoparticles, microparticles and many others. These delivery systems are considered to be promising systems considering drug targeting to specific sites, maximizing the therapeutic effect of some drugs and minimizing their side effects.

SLNs are colloidal lipid particles made of solid lipid materials that are stabilized by different types of surfactants and co-surfactants. They are used to deliver drugs via parenteral, ophthalmic and topical routes. They are used mainly in topical preparations as they are composed of non-irritant and non-toxic materials. They have an occlusive effect on the skin by decreasing water loss and hence increasing drug penetration through the stratum corneum.

Non-ionic surfactant based vesicles (niosomes) are formed from hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are usually used to incorporate hydrophilic drugs within their core; however hydrophobic drugs can be incorporated into niosomes lipophilic membrane.

Chemotherapeutic agents are cytotoxic drugs that are used to treat cancer because they are toxic to cancer cells that are characterized by rapid division and growth. These agents however are known for their profoundly toxic side effects because of which treatment failure still frequently encountered.

5-Fluorouracil (5-FU or 5-fluoro-2,4-pyrimidinedione) is one of the chemotherapeutic agents which is characterized by its activity against solid tumors like breast cancer, colon cancer and skin tumors. Nevertheless, 5-FU has many disadvantages including development of resistance to it by cancer cells and high rate of metabolism which leads to requirement of high doses of 5-FU in order to keep its

therapeutic serum concentration leading to sever side effects. The mentioned points make 5-FU a good candidate for loading on drug delivery systems which can accumulate such a drug in the requited action site leading to improvement in its therapeutic action and decreasing its serious side effects.

The work in this thesis is divided into

- **Chapter I: Formulation and evaluation of 5-flourouracil loaded solid lipid nanoparticles**

This chapter includes the preparation and characterization of 5-FU loaded SLN. Also the in vitro release and permeation studies of the prepared SLNs were performed.

In this chapter physicochemical properties of 5-FU (spectrophotometric scanning and saturated solubility in Sörensen's Phosphate Buffer (pH 5.5) and Phosphate Buffer Saline (pH 7.4)) were first evaluated.

Pre-formulation study was carried out to detect interaction between 5-FU and SLNs' components using Differential Scanning Colarimetry (DSC) and Fourier-transform Infrared spectroscopy (FT-IR).

SLNs preparation experiment was designed using 3^2 factorial design. SLNs formulae were prepared using stearic acid, different percentages of lecithin and different percentages of poloxamer188 using modified solvent diffusion-evaporation method. The concentration of 5-FU in each formula was 10 mg.

The prepared formulae were characterized with respect to encapsulation efficiency, particle size measurement by photo correlation spectroscopy (PCS), zeta potential, Transmission electron microscopy (TEM), scanning electron microscopy (SEM), in vitro permeation study and in vitro release study.

In vitro permeation study through lipophilic membrane was performed using double open-sided tube with diffusion area of 5 cm^2 and 25 ml receptor medium. The contents of the donor compartment (SLNs) and receptor compartment (PBS (ph 7.4)) were separated using cellulose nitrate membrane of pore size 0.2 µm which was immersed in

isopropyl myristate to simulate the lipophilic properties of stratum corneum. The receptor compartment was maintained at 37±0.5 ^{o}C while the donor compartment was left exposed to ambient temperature. The donor compartment's solution was stirred at 100 r.p.m by means of magnetic stirrer. Samples were withdrawn at specific time intervals and analyzed spectrophotometrically at 266 nm. The permeation parameters of different SLNs formulae along with plain drug were calculated.

Release experiments were carried out on SLNs formulae using reported method with modification. Certain volume of SLNs was inserted in dialysis bags that were then attached to the paddles of USP dissolution tester (apparatus II). The temperature of the medium (Sörensen's Phosphate Buffer (pH 5.5)) was set at 32±0.5^{o}C and speed of rotation was set at 50 r.p.m. samples were withdrawn at certain time intervals and diluted and the medium was compensated with equal volume of fresh medium. The linear regression analysis was applied to all in-vitro release results. The correlation coefficient (r^2) was determined in each case and accordingly, the orders of dissolution were determined.

Stability test was carried out on formula S8 by measuring encapsulation efficiency and particle size monthly for a period of six months.

The collected data was statistically analyzed using One-way factorial ANOVA for comparing between different SLNs formulae and One-way ANOVA for comparing between SLNs and plain 5-FU permeation data and in vitro release data.

From the obtained data in this chapter the following was concluded.

1- The saturated solubility of 5-FU in Sörensen's phosphate buffer (pH 5.5) and Phosphate Buffer Saline (PBS, pH 7.4) were found to be 0.01862 and 0.2488 % w/w respectively.

2- The results of DSC and FT-IR obtained showed no interaction between 5-FU and SLNs' components.

3-This investigation proved that 5-FU could be incorporated into SLNs' shell region successfully as illustrated in SEM examination of gold labeled SLN. Encapsulation efficiency of 5-FU reached up to 47.92±2.34, relatively small particle size with narrow particle size distribution ranging from 137±5.5 to 404±7 and zeta potential values from -8.44±2 to -19.7±0.4.

2- Formula S9 gave the highest encapsulation efficiency while formula S1 gave the lowest encapsulation efficiency. It was observed that formula S1 has the highest particle size while S8 gave the lowest particle size and formula S1 has the lowest zeta potential while formula S8 has the best zeta potential.

3- Zeta potential values where somewhat low which may affect the stability however this can be attributed to presence of poloxamer188 which participate in increasing particles' stability by forming a hydrophilic corona around nanoparticles.

4- TEM revealed that SLN have a spherical shape with particle size somewhat smaller than that obtained by PCS analysis, while SEM investigation of gold labeled SLN proved encapsulation of 5-FU in SLN shell region.

5- The produced SLN showed better diffusion and release of 5-FU compared to plain 5-FU.

6- Factorial analysis proved that the used excepients (lecithin and poloxamer188) exerted a significant effect on all the measured 5-FU loaded SLNs' characters, while One-way ANOVA proved that the prepared 5-FU loaded SLNs significantly improved the permeation parameters and release properties of 5-FU.

7- The formed 5-FU loaded SLNs showed particle size dependency in release and diffusion where the smaller particle size showed better diffusion and release than larger particle size, accordingly, formula S1 has the lowest permeation parameters and release

percentage of 5-FU, while formula S8 has the best permeation parameters and release percentage of 5-FU.

8- The formed SLN showed initial bursting effect which could be beneficial in providing a loading dose of drug, hence offering little side effects and encouraging patient compliance.

9- Most of SLN formulae had a release order of Higuchi-type release except formula S5, S8 and plain 5-FU solution that exhibited a first order release model. Formula S8 showed some changes in particle size and encapsulation efficiency after six month of storage, these changes were found to be insignificant.

From the obtained data in this chapter the formula S8 was chosen to be incorporated into different gel matrices for further studies.

- **Chapter II: Formulation and evaluation of 5-flourouracil loaded niosomes**

This chapter includes the preparation and characterization of 5-FU loaded niosomes. Also the in vitro release and permeation studies of the prepared niosomes were performed.

Pre-formulation study was carried out to detect interaction between 5-FU and niosomes' components using Differential Scanning Colarimetry (DSC) and Fourier-transform Infrared spectroscopy (FT-IR).

Niosomes' preparation experiment was designed using 2^3 factorial design. Niosomes formulae were prepared using cholesterol, sorbitan monostearate (span 60), sorbitan monolaurate (span 20), and sodium deoxycholate using thin film hydration method. The concentration of 5-FU in each formula was 10 mg.

The prepared formulae were characterized with respect to encapsulation efficiency, particle size, zeta potential, Transmission electron microscopy (TEM), in vitro permeation study and in vitro release study.

In vitro permeation study through lipophilic membrane was performed using double open-sided tube with diffusion area of 5 cm^2 and 50 ml receptor medium. The contents of the donor compartment (niosomes) and receptor compartment (PBS (pH 7.4)) were separated using cellulose nitrate membrane of pore size 0.2 µm which was immersed in isopropyl myristate to simulate the lipophilic properties of stratum corneum. A Procedure that is similar to that used in Chapter I was carried out.

Release experiments were carried out on niosomes' formulae using reported method. The used procedure was similar to that used in Chapter I. The linear regression analysis was applied to all in-vitro release results. The correlation coefficient (r^2) was determined in each case and accordingly, the orders of dissolution were determined.

Stability test was carried out on formula N8 by measuring encapsulation efficiency and particle size monthly for a period of six months.

The collected data was statistically analyzed using One-way factorial ANOVA for comparing between different niosomes' formulae and One-way ANOVA for comparing between niosomes and plain 5-FU permeation data and in vitro release data.

From the obtained data in this chapter the following was concluded.

1- The results obtained from DSC and FT-IR analysis showed no interaction between 5-FU and niosomes' components.

2-5-FU was successfully embedded in niosomes core with encapsulation efficiency of 5-FU reaching up to 67.08±2.53 % w/w, particle size of 5-FU loaded niosomes ranged from 138±2.3 to 274±1.99 nm and zeta potential ranged from -15±0.99 to -37.73±0.9 mV.

3-Factorial analysis showed that the studied factors (surfactant type surfactant concentration and presence of Na deoxycholate) had a significant effect on most of the

studied parameters. One-Way ANOVA revealed that the prepared niosomes had significantly improved the permeation parameters and release properties of 5-FU.

4- Formula **N3** gave the highest encapsulation efficiency, highest particle size and lowest zeta potential, while formula **N8** gave the lowest encapsulation efficiency, lowest particle size and highest zeta potential.

5-All the prepared 5-FU loaded niosomes formulae exhibited better permeation parameters of 5-FU compared to plain 5-FU and formula **N8** had the best permeation parameters while formula **N3** gave the lowest Q_{24} and largest lag time and formula **N4** gave the lowest Kp.

6- All the prepared niosomes formulae had better release profile of 5-FU compared to plain 5-FU solution, formula **N8** gave the best release percentage of 5-FU, while formula **N3** gave the lowest release percentage of 5-FU.

7-Formula **N8** was found to be stable after a storage period of three months, however after six month the decrease in encapsulation efficiency and increase in particle size were found to be significant using One-Way ANOVA at $p<0.05$.

Based on the data obtained in this chapter formula **N8** was chosen to be incorporated into three different gel matrices for further study.

- **Chapter III: Formulation and evaluation of 5-FU gel**

As a start in this chapter, pre-formulation studies to detect interaction between 5-FU and the polymers used to prepare the three gel bases were performed using Differential Scanning Colarimetry (DSC) and Fourier-transform Infrared spectroscopy (FT-IR).

The selected formula of the 5-FU loaded (**S8**), niosomes (**N8**) along with plain 5-FU were incorporated into three different polymers namely sodium carboxy methyl cellulose (Na CMC) (3% w/w), hydroxyl methyl propyl cellulose (HPMC) (2% w/w)

and chitosan (1.5%), all the prepared gel formulae had 5% (w/w) concentration of 5-FU. The gel formulae were prepared by sprinkling the polymers and the preservatives (mixture of 0.1% methylparaben and 0.01% propylparaben) in 20 ml SLN (S8), niosome's (N8) and plain 5-FU solution (concentration 50mg/ml), placed in 50 ml beaker, stirred with a magnetic stirrer until no lumps were observed and finally left to equilibrate in the refrigerator for 24 hours before use. In case of chitosan gel the matrix was made slightly acidic using 1% acetic acid.

Each gel base was prepared in 4 formulae;

- gel containing no drug
- Gel containing 5-FU
- Gel containing 5-FU loaded SLN
- Gel containing 5-FU loaded niosomes

The prepared gel formulae were characterized by visual inspection and measuring homogeneity percentage and spreadability.

The rheological properties of the prepared gel formulae were evaluated using a rotational Brookfield viscometer of cone and plate structure. About 0.5 g of the tested formula was applied to the plate and left until the temperature of the cone reached $25\pm1^{o}C$. Measures were taken on a range of shearing rates from 10 to 200 (1/sec) corresponding to 5 to 100 r.p.m

In vitro permeation study was performed on the gel formulae containing 5-FU either in the plain form or encapsulated in SLN or niosomes in the same way that was followed in chapter I and II.

In vitro release study of gel formulae was conducted using paddle method (USP, apparatus II). Certain weights of gel formulae were placed in plastic cups (2 cm in diameter, 0.5 cm in height), those cups were fitted to stainless steel wire screen and those cups were fitted to the bottom of dissolution flask. The temperature of the medium (Sörensen's Phosphate Buffer (pH 5.5) was set

at 32±0.5°C and speed of rotation was set at 50 r.p.m. Samples of three mls were withdrawn and analyzed spectrophotometrically at λmax 266 nm. The medium was compensated with equal volume of fresh medium. The linear regression analysis was applied to all in-vitro release results. The correlation coefficient (r^2) was determined in each case and accordingly, the orders of dissolution were determined

The collected data was statistically analyzed One-way ANOVA for comparing between different gel formulae.

From the obtained data in this chapters the following could be concluded

1- The results obtained from DSC and FT-IR analysis showed no interaction between 5-FU and gel bases.

2-All the prepared 5-FU gel formulae were elegant, transparent in case of plain 5-FU gel and opaque in case of 5-FU loaded niosomes and SLN gel. All formulae had good homogeneity and 5-FU loaded SLN and niosomes gels had higher spreadability than plain gels and gels containing plain 5-FU this increase in Spreadability was found to be significant using one way ANOVA at $p<0.05$.

3-All the tested 5-FU gel formulae exhibited pseudoplastic flow with thixotropy. Formula F8 (I.e. 5-FU loaded SLN in HPMC) exhibited the highest area of hysteresis loop and the highest Farrow's constant.

4-The permeation study showed that 5-FU loaded SLN and niosomes in NaCMC matrix (F7 and F10) had the best permeation parameters of 5-FU. The Q_{24} value of 5-FU from formula composed of plain 5-FU in Chitosan (F6) was higher than Q_{24} of 5-FU from 5-FU loaded SLN and niosomes in Chitosan.

5-SLN and niosomes in NaCMC (F7 and F10) had the highest release percentage of 5-FU. Release of 5-FU from 5-FU loaded SLN and niosomes in NaCMC and HPMC was higher than release of 5-FU from same matrices that contain plain 5-FU, while the

release of 5-FU from Chitosan containing plain 5-FU was higher than release of 5-FU from the formulae composed of 5-FU loaded SLN and niosomes in same matrix. All gel formulae exhibited Higuchi diffusion type of release.

5- The One-way ANOVA test showed that there's significant difference between most of the prepared gel formulae considering the permeation parameters and release percentage of 5-FU from the prepared 5-FU gel formulae.

From the previous study, the polymer NaCMC was chosen as a base for 5-FU loaded SLN and niosomes (F7 and F10) and plain 5-FU for further in vivo studies.

- **Chapter IV: In vivo evaluation of the selected 5-FU gel formulae**

The selected formulae from the previous study (F7 and F10) were studied for their anti tumoral effect using single cell suspension induced Ehrlich tumor in BALB/c mice. The cell suspension was inoculated in the right thigh of animals.

The anti tumoral effect of the gel containing 5-FU loaded SLN and niosomes was demonstrated against gel containing plain 5-FU as control.

The experiment was conducted on 24 mice that were divided into four groups as follows:

- **Group A:** negative control group (received no treatment).
- **Group B:** received NaCMC 5-FU gel twice daily.
- **Group C:** received NaCMC gel containing 5-FU loaded SLN twice daily.
- **Group D:** received NaCMC gel containing 5-FU loaded niosomes twice daily.

The anti tumeral effect of the used formulae was evaluated by measuring tumor size and survival period of each animal group. Biopsies were taken from each animal group and histopathological examination was performed on slides prepared from these biopsies.

One-way ANOVA test was carried out on the collected data to evaluate the difference between the anti tumeral effect of the used 5-FU gel formulae.

From the obtained data in this chapter the following can be concluded:

1-Animal group treated with 5-FU loaded SLN and niosomes in NaCMC (groups C and D respectively) survived for longer period than those treated with plain 5-FU in NaCMC and negative control group (groups B and A respectively).

2-animal groups C and D showed delay in tumor growth which was greater than that observed in case of groups A and B.

3-Histopathological examination revealed that groups C and D showed reduction in inflammation and hemorrhage observed in group A. There was some kind of reduction in inflammation and hemorrhage in case of group B, however this reduction was much lower than that observed in case of groups C and D.

4- Statistical analysis of the results using One-Way ANOVA revealed that there's significant difference between the anti tumeral effect of 5-FU loaded SLN and niosomes gel and plain 5-FU gel.

These results revealed that incorporation of 5-FU into SLN and niosomes had allowed deep penetration of 5-FU into deep layers of skin and delivered the drug to target tissue more accurately.