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Research article

Formulation and Evaluation of Leflunomide Loaded Magnetic Solid-Lipid Nanoparticles for The Targeted Therapy of Breast Cancer

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ABSTRACT

The present research work focuses on the targeted delivery of anti-cancer drug Leflunomide. This drug belongs to BCS class II which implies that it has lipophilic nature with poor water solubility. Hence an attempt was made to reduce the particle size to nano dimensions using microemulsion technique. In this technique, stearic acid was used as the lipid and tween 80 was used as a surfactant. For the tumor targeted formulation, the Magnetic nanoparticle was prepared using precipitation technique. The Solid Lipid Nanoparticles was coated on the Magnetic Nanoparticles. The resultant nanoparticles were optimized using 3^2 full factorial design and characterized for their entrapment efficiency, percentage yield, in-vitro diffusion studies. The particle size and zeta potential were found out and surface morphology was studied using Scanning electron microscopy. The histopathology study clearly showed a significant concentration of iron in test (magnetically targeted) kidney compared to control kidney.

Keywords: Leflunomide, microemulsion, Magnetic nanoparticles, Histopathology

INTRODUCTION

Cancer is an illness portrayed by uncontrolled duplication and spread of irregular manifestations of particular cells of the body's own.[1] It is one of the significant reasons for death in the developed countries. Malignancy is a sickness portrayed by uncontrolled increase and expansion of irregular types of body's cells. Tumor is class of illness in which a gathering of cells showcases uncontrolled development (division past as far as possible), attack (interruption on and destruction of nearby tissues) and in some cases metastasis (spread to different areas in the body by means of lymph or blood). The extension of drug concerned with the study, finding, treatment and anticipation of malignancy is Oncology. Growth may influence individuals at all ages, even fetuses, yet the danger of most mixtures increment with age. The conventional Cancer treatments, including surgery, radiation therapy, chemotherapies are limited by lack of target specificity, risk of operating on vital organ, high rate of drug metabolism, cytotoxicity, high dose requirement, poor patient compliance and spread of cancer cells throughout the body. The disease-modifying antirheumatic drugs (DMARDs) provide novel approaches to the endocrine treatment of Breast Cancer[2]. Leflunomide is disease-modifying antirheumatic drug which can inhibit pyrimidine nucleotide synthesis through directly blocking the activity of DHODH. Some reports have shown that DHODH inhibition through leflunomide was effective for treatment of some cancers including glioma stoma. Long term administration of leflunomide may lead to systemic toxicity. Leflunomide is a lipophilic drug with poor water solubility[3]. A novel formulation approach is needed for minimizing systemic toxicity of drug without compromising its therapeutic potential. Due to the higher toxicity of leflunomide, it is very important in cancer treatment to localized drugs to specific disease site so that there will be interaction of drugs with only diseased tissue. The Solid lipid Nanoparticles (SLNs) have numerous advantages over routine routes of administration[4]. By incorporating leflunomide with SLNs, the solubility of drug can be increased and the side effects can be minimized with improved anti- cancer action which in turn could improve patient compliance. So, by preparing SLNs, it can be easily pass through into the leaky vascular endothelium of cancer tissues through the Enhanced Permeability and Retention (EPR) effect and are consequently able to target passively to tumor cells. Magnetically targeted particles are viewed to have fabulous potential as drug targeting carrier because of its non-intrusive character and high

focusing ability in presence of magnetic field. By applying external magnetic field, drug loaded magnetite nanoparticles could be able to attain extremely high concentrations of the drugs, very close to the target site for a given period of time with no significant toxic effects to normal tissue or to the entire body. Magnetic nanoparticles in uneven magnetic field are able to generate heat. This heat (42-45°C) has more discriminatory effect on fast dividing tumor cells than the normal tissues/cells[5]. Thus, by formulating Leflunomide loaded Magnetic Solid Lipid Nanoparticles may be targeted to the specific affected tissue/organ by applying suitable strength of external magnetic field. In addition to this, synergistic effect may obtain due to hyperthermia as it is induced by magnetite nanoparticles. As a result, reduction in dose of the drug may also possible with much minimized side effects[6].

MATERIALS AND METHODOLOGY

Materials

Leflunomide was obtained as a gift sample from Torrent Pharmaceuticals Limited, Ahmedabad, Neodymium magnet was procured from Patel Magnets Vadodara, Stearic acid, Tween 80 and n- butanol were obtained from sulab laboratory, Vadodara whereas ferrous chloride and ferric chloride were obtained from Loba chemie Pvt Ltd. Mumbai.

Methodology

The magnetite nanoparticles were prepared by precipitation method. 100 ml of 0.1M $FeCl_3$ and 100 ml of 0.2M $FeCl_2$ solution were mixed together in to a 250 ml beaker. The stirring was started by mechanic stirrer. With continued stirring, sufficient volume of 25% v/v aqueous NH_3 solution was added slowly by syringe. After an initial brown precipitate, a black precipitate was formed (magnetite). After one hour of stirring the stirrer was turned off and the magnetite nanoparticles allowed to settle down. Then decanted and discarded the clear liquid without losing a substantial amount of solid. The settling process can be speed up by putting a magnet under the container. Neodymium magnet was used to attract the ferrofluid to the bottom of the beaker. Clear liquid was poured off and discarded as much as possible. The rinsing was repeated thrice and solid particles were collected and dried[7, 8].

Calibration curves of drug in various solvents

10 mg of Leflunomide was first dissolved in 10 ml of methanol and remaining diluted with water upto 100 ml to obtain a stock solution of 1000 μg/ml concentration. Then from the stock solution 1.2 ml , 1.4 ml , 1.6 ml , 1.8 ml , 2.0 ml of solution diluted with 100ml of water to obtain 12 to 20μg/ml respectively. The absorption of all the prepared solutions was then measured at the absorbance maxima, 247 nm against the reagent blank. The readings were recorded in triplicate. Mean value (n=3) along with the standard deviation (SD) are recorded. The average values of absorption were plotted graphically against the concentrations. Similar procedure was followed for taking calibration curve in phosphate buffer pH 7.4 and in methanol

Compatibility study of drug with excipients

This study was performed using FTIR spectroscopic analysis by using FTIR instrument. In this study first the spectra of drug was taken. Then after the drug and other excipients were mixed in specific ratios and then spectras were taken for combination. All spectras were compared to know that excipients affect the drug molecule or not. The spectrum was recorded in the wavelength region of 4000-400 cm^{-1}. The procedure consisted of dispersing very small amount of sample in KBr by maintaining 1:300 ratio for the sample to KBr and gently triturated, thus avoiding solid transition possibly inducing by extended grinding and compressed softly on disc of sample holder. The spectrum would be scanned at a resolution of 0.15 cm^{-1} and scan speed 20 scan/sec.

FORMULATION OF MAGNETITE LOADED LEFLUNOMIDE SLNS (MSLNS)

Magnetite loaded NP were prepared by Warm microemulsion dispersion technique. In this method first of all, accurately weighed lipid was melted in a beaker on water-bath at about 60°C. The accurately weighed amount of drug was dispersed in lipid melt. Simultaneously, mixture of distilled water and tween 80 was warmed to the same temperature. Under mild mixing the aqueous phase was added to the melted lipophilic suspension. Finally, an aqueous suspension of colloidal particles was obtained by dispersing the warm transparent phase in cold water (7°C) at 1:5 ratio (microemulsion: water, v/v). This particle suspension was washed with distilled water in order to eliminate the large proportion of surfactants used to obtain the transparent phase. The suspension was filtered and lyophilized. Warm microemulsion dispersion technique was selected for further study

CHARACTERIZATION OF MAGNETITE LOADED LEFLUNOMIDE SOLID LIPID NANOPARTICLES (MSLNS)

Particle Size and Zeta Potential

The average diameter and zeta potential of magnetite nanoparticles was determined by photon correlation spectroscopy (PCS). Nanosuspension in distilled water was added to the sample dispersion unit (deionized water) and

stirred at 2000 rpm with magnet in order to reduce the inter-particulate aggregation. The samples were adequately diluted with deionized water and placed in an electrophoretic cell. The average particle size was measured after performing the experiment in triplicates.

Effect of magnetic field on MSLNs

The influence of magnetic field on MSLNs was done by applying the fixed external magnetic field to the outer side of the beaker containing MSLNs dispersion in distilled water. Movement of the MSLNs in the dispersion medium towards the applied external magnetic field(with Neodymium magnet) was observed physically.

% Drug Entrapment Efficiency (%EE)

In this method, analysis of leflunomide from MSLNs was done by centrifugation, taking supernatant (diluted, if necessary) with distilled water and absorbance was taken against distilled water as a blank on UV-Visible Spectrophotometer (at 247 nm wavelength of absorption maxima).[9]

% entrapment efficiency was determined by using the following equation;

$$\% \text{ EE} = \frac{W_{\text{total drug}} - W_{\text{free drug}}}{W_{\text{total drug}}} \text{ X } 100$$

% Drug content

The drug content in the MSLNs was determined by dissolving 50 mg of lyophilized MSLNs in few ml methanol and make up the volume up to 20 ml with distilled water. The magnetite nanoparticles were separated by applying the magnetic field and absorbance of the resulting solution was measured spectrophotometrically at 247 nm after filtration and appropriate dilution with distilled water. Drug content in the MSLNs was determined by using following equation;[9]

$$\% \text{ Drug content} = \frac{\text{Practical drug content}}{\text{Weight of MSLNs taken}} \text{ X } 100$$

In-vitro release study

In vitro release studies were performed in phosphate buffer pH 7.4 by dialysis bag method using dialysis membrane having molecular weight of 12000-14000 Da. 5 ml of nanoparticulate dispersion (strength 20 mg/ml) was placed inside the dialysis bag, tied at both ends and dipped in the dissolution medium. Stirring of the medium was maintained at 100 rpm using magnetic bead and the temperature at 37 ± 0.2 °C. 5 ml aliquots were withdrawn at pre-set time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 48 and 72 h) and replaced by an equal volume of fresh dissolution medium. After suitable dilution (if necessary), the samples were analyzed spectrophotometrically at 247 nm. The concentration of leflunomide in test samples was calculated by using the regression equation of the calibration curve[10].

Optimization of formulation

Most formulation studies involve variation of one factor at a time, keeping other factor constant. Factorial design enables all factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them. In order to optimize the preparation of formulations in the present study, various computations were done by using RSM, carried out by employing Design-Expert software (Version:10). A three factor, three level full factorial design was used to explore and optimize the main effects, and effects of the formulation ingredients on the performance of the SLNs. At 3^3 full factorial design requires 13 experimental runs to determine the experimental error and the precision of the design. From the trail batch formulation, the ranges for each component were selected as follows: Amount of lipid (Stearic acid) - 1400 mg to 1800 mg and amount of tween 80 – 800mg to 1000mg with respect to amount of water phase taken. Percentage entrapment efficiency was considered as the significant response factor used to assess the quality of the MSLN formulation. The models were validated by analysis of variance (ANOVA) and multiple correlation coefficients (R^2) tests.

Two dimensional and three-dimensional contour plots were established using reduced polynomial equation and percentage entrapment efficiency was taken as response using Design-Expert software.

To find the compositions of optimized formulation over the whole experimental region, validation of RSM results were conducted. Three optimum checkpoint formulations were selected to validate the chosen experimental domain and polynomial equation. The optimized checkpoint formulations were prepared and evaluated for response properties. The consequent experimental value of the response was quantitatively compared with that of the predicted values.

Kinetics of drug release

In order to investigate the mechanism of drug release from MSLNs, the release data obtained from diffusion studies was fitted to various kinetic equations. The kinetics models used were a zero order equation ($Q_t = Q_0 - K_0t$), first order equation ($\ln Q_t = \ln Q_0 - Kt$), Higuchi's equation ($Q_t = K_h\, t^{1/2}$). Where Q_t is the percent of drug released at time t, Q_0 is the initial amount of drug present in the MSLNs and K_0, K and K_h are the constant of the equations of zero order, first order and Higuchi model respectively[10].

Particle Size and Zeta Potential

The average diameter and zeta potential of optimized magnetite loaded leflunomide SLNs was determined by photon correlation spectroscopy (PCS). It was carried out at Department of Pharmacy, Parul University, Vadodara. Nanosuspension in distilled water was added to the sample dispersion unit (deionized water) and stirred at 2000 rpm with magnet in order to reduce the inter-particulate aggregation. The samples were adequately diluted with deionized water and placed in an electrophoretic cell. The average particle size was measured after performing the experiment in triplicates[9].

Scanning Electron Microscopy (SEM)

The surface morphology of optimized batch MSLNs was studied using scanning electron microscopy. Prior to examination, the samples were mounted on to metal stubs using a double sided adhesive tape under vacuum. The scanning electron microscope was operated at an acceleration voltage of 25 kV[9].

Stability Study

The stability study for optimized formulation MSLNs was carried out as per ICH guidelines. Various ICH storage conditions are available as: at subfreezing temperature 2 - 8 °C, at room temperature 25 °C ± 2 °C (60 % ± 5 %RH) and at an elevated temperature 40 °C ± 2 °C (75 % ± 5 %RH). The optimized formulation magnetite loaded leflunomide SLNs were placed in screw capped glass container and stored at various ICH storage conditions for a period of 60 days. The samples were analyzed for physical appearance, drug content and for in vitro drug release at regular interval of 15 days[11].

Histopathology study

Histopathological study entails the study of pathological changes that occur in the given tissue sample. In this study we aimed to look at the tissue level changes which occur after the administration of the test drug. The administration of the test drug was done via iv route. Before the drug was administered the rat was anaesthetized with 0.5ml Xylazine and Ketamine (1:1) for ease of experimentation. The animal was given unrestricted access to water and feed and post 24 hrs it was sacrificed and both the kidneys were isolated and stored for further investigations in 10% formalin solution. The samples were then processed for histopathological sectioning and pearl Prussian blue staining was done[12].

Table 1: 3^2 full factorial design for MSLNs

Factor level	Level		
	Low (-1)	Medium (0)	High (+1)
A	1400	1600	1800
B	600	800	1000

A = Concentration of stearic acid B = Lecithin concentration

Table 2: % yield and % EE of MSLNs batches LFM1 to LFM13

BATCH	Drug(mg)	Stearic acid(mg)	Tween 80(mg)	%Yield	%Entrapment
LFM1	20	1600	1000	56.32 ± 0.28	57.41 ± 0.14
LFM2	20	1600	800	63.74 ± 0.14	42.36 ± 0.24
LFM3	20	1600	800	61.85 ± 0.13	49.61 ± 0.12
LFM4	20	1400	800	66.81 ± 0.21	37.05 ± 0.32
LFM5	20	1600	600	68.18 ± 0.27	40.4 ± 0.15
LFM6	20	1400	1000	58.33 ± 0.13	42.75 ± 0.25
LFM7	20	1600	800	64.11 ± 0.30	39.67 ± 0.30
LFM8	20	1400	600	75.11 ± 0.29	25.6 ± 0.24
LFM9	20	1600	800	62.48 ± 0.27	46.21 ± 0.15
LFM10	20	1800	800	56.51 ± 0.18	59.31 ± 0.13
LFM11	20	1800	600	60.52 ± 0.37	51.23 ± 0.24
LFM12	20	1800	1000	47.62 ± 0.34	67.23 ± 0.26
LFM13	20	1600	800	62.21 ± 0.17	48.23 ± 0.11

Table 3: check point analysis

Batch No.	A	B	Observed		Predicted		Residual		%Error	
			% yield	% Entrapment	% yield	% Entrapment	% yield	% Entrapment	% yield	% Entrapment
LFM12	1800	1000	47.62	67.23	48.61	67.73	-1	-0.49	2.09	0.73
LFM14	1798	1000	49.25	67.10	48.69	67.61	0.56	-0.51	1.14	0.76
LFM15	1799	994	49.47	66.58	48.83	67.44	0.64	-0.86	1.29	1.29

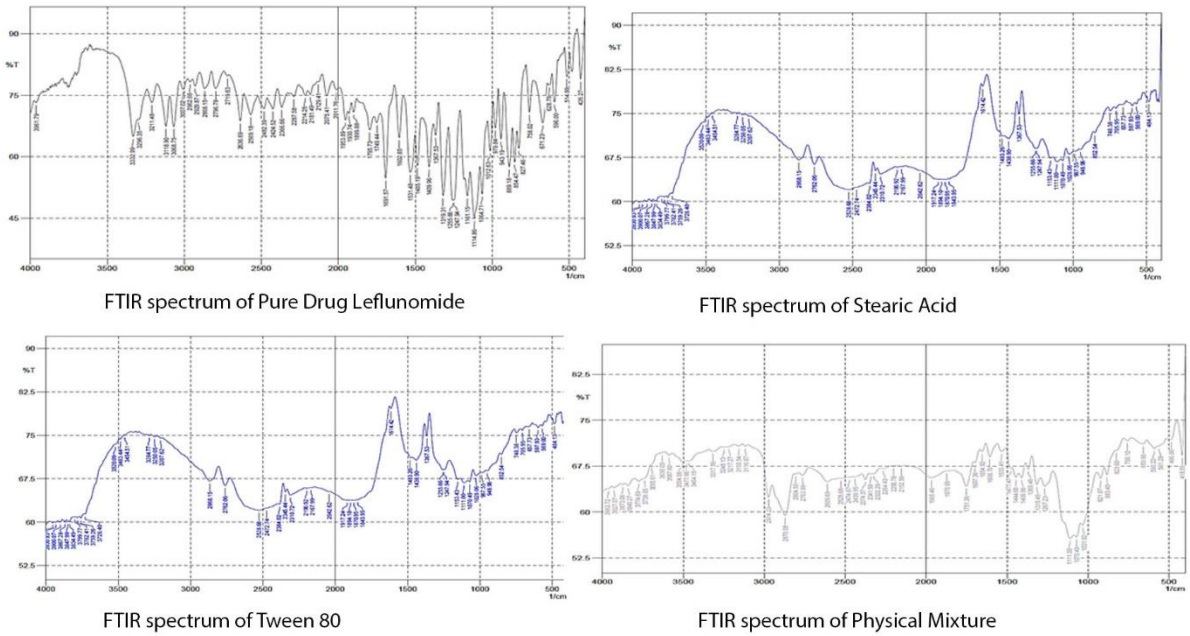

Figure 1: FTIR spectra

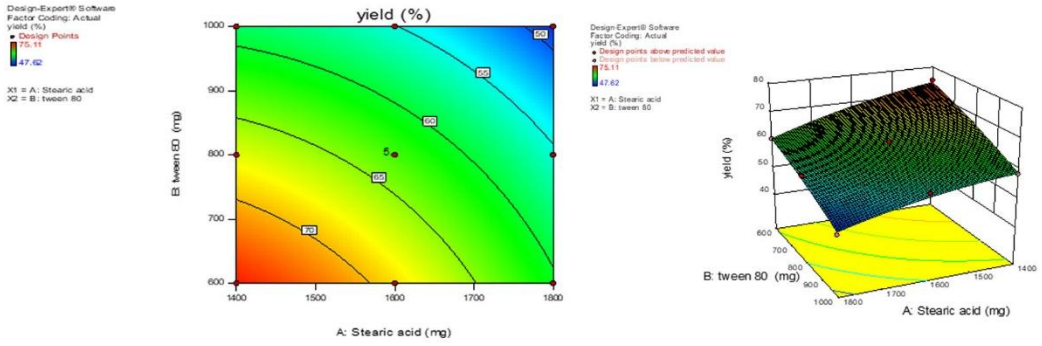

Figure2: Two dimensional contour plot and Three dimensional contour plot showing the effect of amount of Stearic acid(A) and tween 80(B) on yield

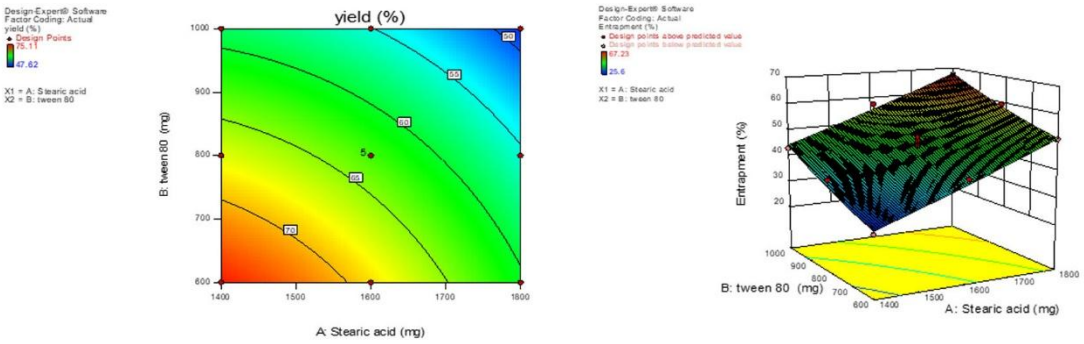

Figure 3: Two dimensional contour plot andThree dimensional contour plot showing the effect of amount of Stearic acid(A) and tween 80(B) on entrapment

Checkpoint analysis

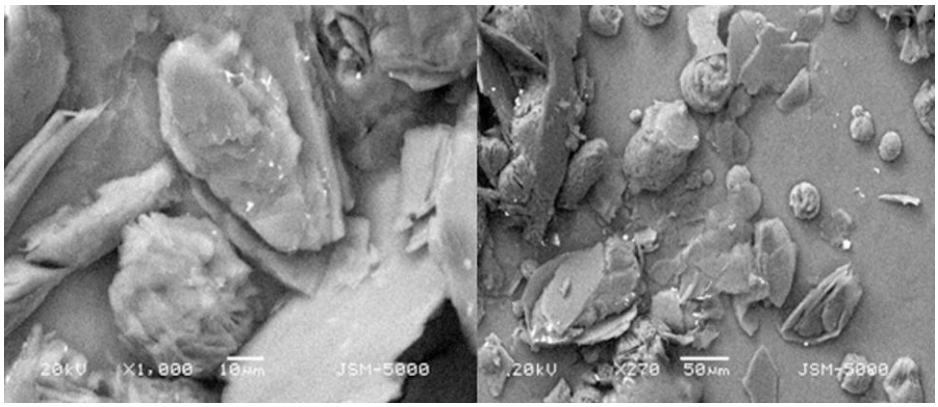

Figure 4 : The SEM image on 270 times magnification

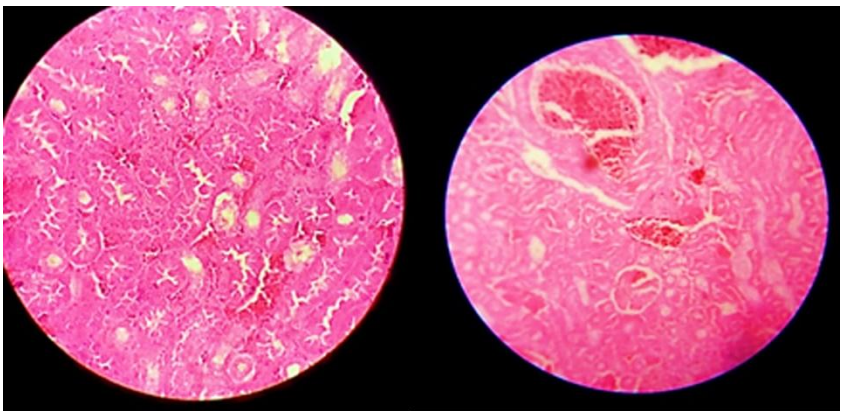

Figure 5 : Histology of control kidney and Test Kidney

RESULT AND DISCUSSION

The absorption maxima, λmax of drug leflunomide was found to be 247 nm in different solvents used like distilled water and methanol by screening of 10 μg/ml drug solution in appropriate media over entire UV range

The regression value (R^2) for distilled water, phosphate buffer pH 7.4 and in methanol was found to be 0.995, 0.999 and 0.996 respectively. It indicates that the calibration curve obtained in each solvent was linear in the range of prepared concentrations of drug.

All spectra's were compared with the spectrum of drug to check any interaction was there or not in

between drug molecule and excipients. No major changes were observed in the functional groups peak for leflunomide in any spectra when compared it with spectrum of pure drug. So, it was considered that there was no any interaction takes place in between drug and excipients used for the formulation development and they were compatible with drug.

All the spectra's of drug and excipients were found as follows in Figure no 1.

Warm microemulsion dispersion technique is based on dilution of oil-in-water microemulsion in cold water. For formulation of microemulsion with solid lipid at room temperature, it must be heated above the melting point of the lipid. Surfactants and co-surfactants include lecithin and tween 80 were used to form microemulsion. Subsequent addition of the microemulsion to cold water leads to precipitation of the lipid phase forming fine particles. This technique also appears feasible at large scale production. During the trial batch formulation of MSLN, lipid and surfactant concentrations were observed to influences the entrapment of drug in formulation and production yield. When lipid was used under the strength of 3 % w/w, production yield and entrapment efficiency was very less and when the concentration of lipid was increased beyond the 7 % w/w, larger particle size was obtained. Henceforth, in order to optimize the lipid amount, different concentrations of lipid were used in the range from 3 % w/w to 7 % w/w. More stable emulsions were obtained when the lecithin concentration was varied from 1 % w/w to 2 % w/w. By visual observation, coarse emulsion was obtained with high coalescence rate of the solvent droplets, when lecithin was used less than 1 % w/w, when lecithin was used above 2 % w/w, it increases the time for melting of lipid. Generally, it was found that the emulsifying agents are used to slow down the inevitable separation; in some rare cases a single emulsifier can yield the desired formulation. More often, though, in the case of oil-in-water emulsions, combinations of emulsifiers have been used to have synergistic effect on emulsion stability in term of coalescence rate. The combined use of two or more surfactants appears to produce mixed surfactant film at the interface having high surfactant coverage as well as sufficient viscosity to promote stability. Due to this fact, another surfactant i.e. Tween 80 were chosen in between the range of 2 % w/w to 6 % w/w as it was found to affect the microemulsion formulation. Further increase in tween 80 concentrations did not show any significant change in the formulation. The volume of aqueous solvent for microemulsion, microemulsion: cold water ratio, melting point temperature and strength of cryoprotectant for lyophilization of MSLN were kept constant. In the present work, for optimization of formulation, lipid concentration and tween 80 concentrations were studied as independent variables with three different levels. According to that 3^2 full factorial statistical design was applied which demands 13 batches to formulate and evaluate.

MLSNs loaded with 1% w/w magnetite nanoparticles were attracted towards the applied external magnetic field.

The percentage yield for each formulated batch was found to be in the range of 47.62 ± 0.34 to 75.11 ± 0.29. It was observed that production yield was not shows significant variations with change in concentration of lipid, surfactant.

The percentage entrapment for each formulation was found to be in the range of 25.6 ± 0.24 to 67.23 ± 0.26. Among the SLN formulations highest entrapment efficiency was observed with formulation LFM12, whereas formulation LFM8 showed lowest entrapment efficiency. As the lipid concentration decreased there was a decline in entrapment efficiency of MSLN formulations. The possible reason of this might be that the optimized concentration of surfactant provides sufficient covering to the lipid core so as to minimize the possible leaching of the drug. However, tween - 80 concentrations also shown significant change in the result of percentage entrapment. As the concentration of tween 80 concentration increased the entrapment efficiency was increased as well.

Two dimensional and three-dimensional contour plots were prepared for the response percentage entrapment efficiency. Entrapment efficiency is considered to be one of the most crucial factors for assessing the quality of MSLNs. The contour plot shows that at the higher level of A and B, % EE was higher, whereas at low level of A and higher level of B % EE was low. Shows the concentration of A affects the %EE but there was not any significant effect of B on % EE. Contour plots for MSLNs formulation are represented in Figure no. 2 and 3.

Check point analysis was done for three batches and the results are depicted in table 3.

In order to investigate the mechanism of drug release from MSLNs of an optimized formulation, the release data obtained from in-vitro release studies were fitted to various kinetics equations. As indicated by higher R^2 values, the drug release from optimized formulation follows first order release and Higuchi model. Since it was confirmed as Higuchi model (R^2 = 0.7506), the release mechanism was matrix-based diffusion controlled. SEM images of optimized formulation is shown in Figure 4.

The surface morphology of optimized formulation was studied using scanning electron microscopy at two different magnifications i.e. at 1000X and 270X.

The average diameter and zeta potential of optimized formulation was determined by photon correlation spectroscopy (PCS) at room temperature. The particle size of magnetic nanoparticles was found to be 528.8 nm and particle size of the optimized formulation was found to be 541.9 nm. It indicates the layer of solid lipid nanoparticles on the surface of magnetic particles have been very thin and uniform due to increased particle size from 528.8 nm to 541.9 nm.. Zeta potential was found to be -6.19 mV for optimized formulation, suggest fairly stable formulation and negative charge distribution at the surface of SLNs

The optimized formulation was subjected to stability studies at various ICH storage conditions i.e. 25 ºC ± 2 °C/60 ± 5 %RH and 40 ºC ± 2°C/75 ± 5 %RH for a period of 60 days. The formulation was evaluated for physical appearance, drug content and in-vitro drug release study at regular interval of 15 days. No major changes were observed in physical appearance, drug content and In-vitro drug release profile when stored at room temperature. Also, at 40 ºC ± 2 °C/75 ± 5 %RH storage condition, no major changes were observed in physical appearance as well as in drug content, while very minor decline were observed in in-vitro release data at the end of 60 days study. It indicates that the formulation was stable at various ICH storage condition for longer period.

The microscopic observations of excised rat kidney in control and stacked magnet treated with optimized batch of MSLNs after 24 hours indicate that the control kidney has less iron moiety present in comparison to that of test kidney (under magnetic field) which is indicated by the stark difference in the number of strongly stained iron moiety which is seen as a blue dot. Histology of control kidney and test kidney is shown in Figure 5.

The anti-cancer activity of leflunomide has been previously reported but weather the prepared MSLNs has a site specific targetability was not yet confirmed. So, from our study we were able to successfully demonstrate that the prepared formulation had magnetic characteristics and when subjected to magnetic field the drug which was in circulation reached the target site and this was proved by the high iron content staining seen in the test kidney histopathology. This study therefore enables us to confirm the site specificity, magnetic nature and targetability of the prepared MSLNs. Further investigations in various animal models are needed to establish reproducibility of the results. The present study also throws light on the ability of MSLNs to decrease systemic side effects as it shows specificity and targetability.

CONCLUSION

This study concluded that, the conventional cancer treatments, including surgery, radiation, chemotherapy and biologic therapies are limited by the lack of target specificity, risk of operating on vital organ, high rate of drug metabolism, cytotoxicity, high dose requirement and poor patient compliance etc., which can be overcome by developing novel drug delivery systems that can target the drug at the specific site in a controlled manner. An effort was made to design magnetite nanoparticles of leflunomide to target the drug to the cancerous cells of breast. For that purpose, magnetic nanoparticles have been prepared. By trial and error method it was concluded that the 0.1M Ferric chloride and 0.2M Ferrous chloride along with 25% aq. Ammonia solution gave uniform magnetic nanoparticles. Solid lipid nanoparticles have been designed and prepared. From the results, it was concluded that concentration of stearic acid showed crucial role in the entrapment of the drug. It was found that the 1800 mg of stearic acid and 1000 mg of tween 80 was appropriate to obtain maximum drug entrapment (67.23 %) of leflunomide, with controlled release of 89.36 % for 72 hr. As a result, Magnetite loaded SLN formulation containing leflunomide (MSLNs formulation), was successfully prepared and optimized. In order to target the leflunomide to specific site of the body, the histopathology study was performed to target one kidney in presence of fixed external magnetic field. From the results, it was concluded that the MSLNs have ability to give action on targeted area. Thus, the present work concluded that a novel technique of developing magnetically targeted drug delivery is an excellent targeted therapeutic system in order to prevent the toxic effect of anticancer drugs to the healthy cells.

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