ORIGINAL RESEARCH ARTICLE

Design, Development, and Optimization of W/O/W Multiple Nanoemulsion for Anti-Cancer Drug, Gemcitabine Hydrochloride

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ABSTRACT

Purpose: The present study's objective was to develop, optimize, and evaluate water in oil in water (W/O/W) multiple nanoemulsion of Gemcitabine Hydrochloride for intravenous administration that would protect the drug in the internal aqueous phase thereby preventing its degradation when exposed in plasma.

Methods: The microemulsion method was used to prepare initial water in oil (W/O) nanoemulsion. Optimization of primary W/O nanoemulsion was done using 2-factor 3-level full factorial design. Optimized W/O primary emulsion was selected on the basis of particle size data and in-vitro drug release studies. The optimized W/O nanoemulsion was diluted with aqueous phase containing hydrophilic surfactants to get W/O/W multiple nanoemulsion.

Results: The optimized W/O/W Multiple emulsion had particle size 132.2nm with 13% drug release in 2hours in phosphate buffer pH 7.4 with 92.09% entrapment of drug within the multiple emulsion system.

Conclusion: A novel W/O/W multiple nanoemulsion of gemcitabine hydrochloride was successfully developed to meet the desired objectives of intravenous administration of drug with enhanced enzymatic stability in plasma.

Keywords: Factorial design, Gemcitabine Hydrochloride, Intravenous infusion, Optimization, W/O/W Multiple nanoemulsion.

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INTRODUCTION

Gemcitabine (2′-deoxy-2′,2′-difluorocytidine), BCS Class III drug, is a pyrimidine antimetabolite that depicts its anti-cancer activity by initiating S phase arrest and inhibiting DNA synthesis. It is used as the first-line therapy for treating pancreatic carcinoma. Gemcitabine HCl is marketed as Gemzar which is administered as an intravenous infusion of 1000-1250 mg/m^2.[1] The drug undergoes rapid deamination to form inactive uridine metabolite 2,2-difluorodeoxyuridine (dFdU) in the presence of deoxycytidine deaminase, which is present in higher amount in plasma and liver. Deamination occurs rapidly in plasma, demonstrating a $t_{1/2}$ of about 15 minutes.[2] Inspite of multiplex pharmacokinetic and pharmacological contour, a continuous IV infusion is required to attain the therapeutic levels as Gemcitabine HCl is clinically effective in numerous solid tumors.[3] Attempts have been made to develop Solid lipid Nanoparticles, Nanoemulsion, liposomes, microspheres etc. to improve its pharmacokinetic properties.[4-6]

Multiple emulsions are defined as novel, complex emulsion systems consisting of both W/O and O/W types of emulsion existing simultaneously in one system.[7] The system consists of oil phase sandwiched between two aqueous phases stabilized by at least two emulsifiers, the one emulsifier having low HLB value of 2 to 6 to stabilize the primary emulsion and another having higher HLB to stabilize secondary O/W emulsion.[8] Stability of multiple emulsions is an important aspect as it involves multiple submicron water droplets enclosed within the oil system.[9] These multiple emulsion colloidal carriers aim to deliver the drug throughout the body without exposing it to sensitive organs/tissues and then to concentrate the drug in higher amount at the target site. It also provides prolong action by preventing rapid drug release from this complex system.[10]

The present investigation focuses on developing W/O/W multiple nanoemulsion to protect Gemcitabine from rapid metabolism in plasma by incorporating the drug into W/O nanoemulsion and further diluting it with water to form double nanoemulsion. The system will be administered by IV injection with improved stability of drug in plasma, thus eliminating the need to administer the drug by continuous IV infusion for longer time period.

MATERIALS AND METHODS

Materials

Gemcitabine Hydrochloride used in the study was obtained as a gift sample from MAC-CHEM and Neon Labs. Labrafac

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lipophile, Transcutol HP, Plurol Olique were obtained from Gattefosse. Cremophor EL was obtained as gift sample from BASF. Methanol was purchased from Loba ChemiePvt Ltd. Design-Expert® 8 Application (Design-Expert version 8.0.6 Trial, Stat-Ease Inc., USA) was used for the design and optimization of the formulation.

METHODS

Pre-formulation Studies

Drug Release Target of W/O/W System

With the aim to retain the maximum amount of drug in the internal phase with minimum dose dumping in blood circulation, attempts were made to formulate W/O/W multiple nanoemulsion with longer retention time for the drug in the internal aqueous phase. Various excipients suitable for such delivery systems were screened.

Screening of Oils

Various synthetic oils approved for intravenous administration were screened to obtain oil in which the drug exhibited minimum solubility. Among the oils screened were Lauroglycol 90, Capryol 90, Labrafac PG, Labrafac lipophile WL1349, Labrafil M2125.

Solubility of a drug in oils was evaluated by the shaking flask method. The drug in excess was dissolved in 1 mL of the oils, and the vials were shaken for 48 hours. Then the contents from each vial were centrifuged, and the supernatant was analyzed by UV spectrophotometry at 269.2 nm for determination of the dissolved drug.[11]

Screening of Oil-soluble and water Soluble Surfactants

Various surfactants suitable for intravenous administration were screened, and desired surfactants were selected using %transmittance value. Oil and surfactants in the quantity of 300mg each were mixed and heated at 50°C. From each mixture of oil: surfactants, 50 mg was taken and diluted to 50 mL of water. It was allowed to stand for 3 hours. Then the % transmittance of the sample was recorded using UV spectrophotometry at λmax 638.2 nm against water as a blank solution.[12,13]

Formulation Studies

Multiple emulsion was prepared using the two-step emulsification method. The drug was dissolved in an internal aqueous phase emulsified with lipophilic surfactant and oil under mild vortexing to form primary W/O emulsion. The primary emulsion thus formed was further re-emulsified using hydrophilic surfactants and aqueous phase to form clear, transparent, stable W/O/W emulsion.[14]

Formulation of Primary W/O Nanoemulsion

The primary emulsion was prepared by using microemulsion based. The primary W/O nanoemulsions of gemcitabine hydrochloride were prepared by oil phase titration method using deionized water, Span 20, Plurol OliqueCC34 and Labrafac lipophile WL1349 as an aqueous phase, surfactant, cosurfactant and oil phase, respectively. Span 20 and Plurol OliqueCC349 (Smix) was taken in various weight ratios of 3:1, 2:1, 1:1, 1:2 and 1:0. Water and each of Smix were mixed in various ratios (from 1:9 to 9:1) followed by the slow addition of oil phase Labrafac lipophile WL1349 to form a clear, transparent, and easily flowable primary w/o. Amount of oil and surfactant required for the formulation of W/O nanoemulsion was decided based on initial trials. Further optimization was carried out using Factorial design to get stable system with an optimum amount of oil and Smix.[15,16]

Optimization of W/O Primary Emulsion by DoE

A factorial design was used for the optimization of the primary W/O nanoemulsion. The selected variable, factors and their levels are given in Table 1.[17-19]

The factorial design gave 13 trails in all. The systems were screened for particle size, polydispersibility index, and %drug release. The optimized W/O primary emulsion obtained by factorial design was further emulsified to form W/O/W Multiple nanoemulsion.

Formulation of W/O/W Multiple Nanoemulsion

Single point optimization method was used to develop W/O/W nanoemulsion as seen from Table 2. Keeping the amount of oil phase, i.e., W/O primary emulsion constant, external hydrophilic surfactants were varied to get the desired stable W/O/W multiple nanoemulsion.

Since multiple emulsions are complex, an unstable system wherein the drug is present in the dissolved form in the internal aqueous phase increases the drug leaching's risk towards the external aqueous phase. Hence, for improved stability, it was decided to store the formulation as premix of W/O nanoemulsion containing hydrophilic surfactant intended for a hundred times dilution at the time of administration to form a W/O/W multiple nanoemulsion.

Table 1: Design for Optimization of W/O Multiple Nanoemulsion

Types of Variables	*Levels*		
Independent Variables (CPP)	*High*	*Medium*	*Low*
X1-Amount of Oil	1	0	-1
X2-Amount of Surfactant	1	0	-1
Dependent Variables (CQA) Y1-Particle Size (nm) Y2-Drug release (%)			
Levels of factor studied			
Factors	*High*	*Medium*	*Low*
Amount of oil (mg)	600	400	200
Amount of Surfactant (mg)	500	300	100

Table 2: Formulation Trials for W/O Nanoemulsion

Trials	*W/O Primary emulsion (mg)*	*Hydrophilic Smix (mg)*
T-1	300	100
T-2	300	200
T-3	300	300
T-4	300	400
T-5	300	500

Evaluation of Optimized Formulation[20-22]

Particle Size Distribution

Globule size and PDI of the developed system were analyzed using Malvern ZS 90 globule size and zeta potential analyzer, which measured sizes between 10 and 5000 nm. The formulation measuring 1 mL was diluted 100 times for globule size and PDI analysis. All readings were repeated in triplicate.

In vitro Dissolution Profile

The drug release from premix of W/O nanoemulsion containing external hydrophilic surfactants was determined by dialysis method using dialysis membrane-60. Initially, the membrane was soaked in buffer solution for 24 hours. It was then washed thoroughly with distilled water. Typically, 2 mL of the formulation was taken in the dialysis bag tied at both ends by a thread and then placed in 250 mL beaker containing 100 mL phosphate buffer pH 7.4 dissolution medium. The beaker was then stirred at 100 rpm using a magnetic stirrer maintained at 37°C. Aliquots of 3 mL were collected at pre-defined time intervals and replaced by fresh dissolution media to maintain sink conditions. The amount of drug released was analyzed using UV spectrophotometer at 269.2 nm.

Permeation Studies[23-25]

Cock ileum was used as model membrane to evaluate tissue permeability. The drug permeation from premix of W/O nanoemulsion containing external hydrophilic surfactants was determined by dialysis method using cock ileum. Typically, 2 mL of the formulation was taken in the cock ileum sac tied at both ends by thread and then placed in 250 mL beaker containing 100 mL of phosphate buffer pH 6.5 dissolution medium. The beaker was then stirred at 100 rpm using magnetic stirrer maintained at 37°C. A sink condition was maintained and samples were withdrawn at different time intervals and replaced by fresh dissolution media.

The apparent permeability coefficient (Papp) of a drug in solution and drug in the formulation was calculated from the following equation:

$$Papp = (dQ / dt) / (C_0 \times A)$$

Where dQ/dt is the steady-state appearance rate on the acceptor solution,

A is the surface area of the intestinal sacs, C_0 is the initial concentration inside the sacs.

The sacs have a length of 5 cm and a volume of 1 mL; Assuming they have a cylindrical shape, their inner diameter is 0.50 cm and the surface area is 7.85 cm^2 per sac.

%Entrapment Efficiency[26]

W/O/W multiple emulsion measuring 2 mL was filled into dialysis bag and dialyzed to its own external aqueous phase solution for 6 hours.

The dialysis solution was then sampled, and a concentration of the unentrapped drug was measured using regression equation of calibration curve in water.

% drug entrapped was calculated using the formula:

$$\%EE = [Vi.Ci\text{-}Cd\ (Vd + Ve) \times 100]/Vi.Ci.$$

Where; Vi-vol. of an internal aqueous phase

Ci- Concentration of drug in the internal aqueous phase.

Cd- Concentration after dialysis

Vd- Volume of an external aqueous phase

Ve- Volume of total dialysis solution

% Drug Content

Drug content was analyzed using HPLC method developed on JASCO HPLC MD 4015, PDA detector using Spincotech C-18 column. The mobile phase consisted of phosphate buffer ph 2.5 and methanol in the ratio of 90:10 at a flow rate of 1.0mL/min. The formulation measuring 2 mL containing 10mg of drug was diluted with mobile phase to obtain 100 mL of stock solution. The solution was sonicated for 20 minutes. The solution was analyzed for drug content after suitable dilution with mobile phase to get final concentration as 10ppm.

% Drug content was analyzed using calibration curve equation.

Haemolysis test[27,28]

The premix of W/O nanoemulsion containing external hydrophilic surfactants was taken in saline. To 1.45 mL of each solution, 50 µL of human blood was added. Following incubation at 37°C for 60 minutes, the samples were centrifuged at 5000 rpm for 10 minutes and the amount of cell lysis or released hemoglobin was measured spectrophotometrically at a wavelength of 540 nm. Sodium carbonate and saline were taken as positive and negative controls.

Percent hemolysis = 100 × [Absorbance of test sample-Absorbance of negative control / Absorbance of difference of the controls].

Stability Studies

Stability studies were carried out by keeping the product at conditions as per ICH guidelines at 25° ± 2°/60%, RH ± 5% RH and 4°C for 1-month. The formulation was tested for Globule size, %drug release in pH 7.4, and entrapment efficiency.

RESULTS AND DISCUSSION

Pre-Formulation Studies

Screening of Oil

Since the aim was to restrict the drug in the internal aqueous phase, thereby preventing its diffusion through the oil phase into the external aqueous phase. The rationale for the selection of oil was to have the least solubility in the selected oil; as seen from Figure 1, the drug showed the least solubility of

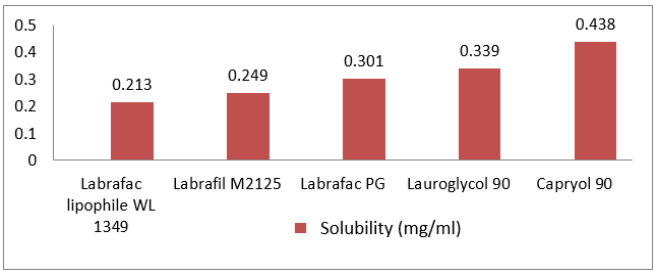

Figure 1: Solubility studies of drug in various oils

0.213 mg/mL in Labrafac lipophileWL1349; hence this oil was selected.

Screening of Surfactants

Oil-soluble surfactants: Oil soluble Surfactants like Span 20, Span 80, etc., were screened. Aqueous solution of surfactant showing least %transmittance value was selected for further formulation as less transmittance indicated less solubility. As seen from Table 3, aqueous solution containing Span 20 and PlurolOlique CC 497 showed the least transmittance and hence both were selected as surfactant and co-surfactant for oil phase.

Water-soluble Surfactants: Water Soluble Surfactants like transcutol HP, Tween 20, Tween 80, Cremophore EL were screened. An aqueous solution of surfactant showing highest %transmittance value was selected as high transmittance indicated more solubility. As seen from Table 3, an aqueous solution containing Cremophore EL and Transcutol HP showed the highest transmittance and hence both were selected as surfactant and co-surfactant for the external aqueous phase.

Formulation Studies

Intravenous W/O/W multiple nanoemulsion prepared was intended to protect the drug in the internal water phase surrounded by oil phase to prevent a drug's rapid metabolism. Enhanced enzymatic stability would eliminate need of continuous IV infusion and would improve its therapeutic efficacy.

Optimization of the W/O Nanoemulsion using 3^2 Factorial Design

Factorial Design was used for the optimization of W/O nanoemulsion, for screening of formulation and process parameters which may influence the critical quality attributes (CQAs) of W/O/W multiple nanoemulsion. Two factors, i.e., amount of oil and amount of surfactant, were tested at 3 levels, each with 13 runs. The factors were selected based on a preliminary study. Each experimental run was performed in triplicate and analyzed for the dependent variables, highlighted in Table 4.

It was seen from Table 4. that when the amount of oil was kept constant i.e. 600mg in case of T-1 to T-3, and the number of surfactants was increased from 100 mg to 500 mg ,the particle size of the system decreased from 187.23 nm to 137.19 nm. The drug released increased from 9.26 to 19.19 in the respective trials. The decrease in particle size increases the surface area of the particles thus the drug release from such system increases. Therefore systems giving lower particle size gave higher drug release.

The following responses were selected for statistical analysis

Particle Size

The particle size of w/o nanoemulsion significantly affects the effectiveness of the formulation. It was clear that particle size depends upon both amount of oil and surfactants used in preparation as seen from Table 4 and was found to be statistically significant at $p < 0.05$ as per Table 5. Figure 2 gives the contour plot of particle size responses.

The polynomial equation derived from the coefficients of estimate in term of coded factor is-

Particle Size = +125.73 +23.87 * A -29.60 * B +3.88 * A * B +9.13 * A2 +5.56 * B2

Table 3: Screening of oil and water-soluble surfactants

Oil soluble surfactant	*% Transmittance*	*Water soluble surfactant*	*% Transmittance*
Span 20	54	Tween 20	98.79
Span 80	79	Tween 80	99.02
PlurolOlique CC 497	52	Cremophor EL	99.29
		Transcutol HP	99.73

Table 4: Trials based on optimization design

Trials	*Coded*	*Actual amount of oil Mg*	*Coded*	*Actual Amount of surfactant*	*Particle size D90 (nm)*	*PDI*	*% Drug release in 1 hour*	*% Drug release in 2 hour*
1	-1	600	-1	100	187.23	0.129	4.12	9.26
2	0	600	-1	300	162.9	0.219	8.42	13.67
3	-1	600	0	500	137.19	0.204	6.95	19.19
4	1	400	0	100	164	0.233	10.03	13.13
5	0	400	1	300	125.12	0.342	9.06	21.91
6	-1	400	1	500	102	0.183	12.64	28.18
7	0	200	0	100	149.7	0.243	11.4	17.10
8	0	200	0	300	110.24	0.212	11.28	24.96
9	0	200	0	500	84.13	0.421	11.42	26.88
10	1	400	1	300	125.09	0.364	9.33	22.23
11	1	400	-1	300	124.83	0.334	9.52	21.54
12	0	400	0	300	125.1	0.316	9.4	22.24
13	0	400	0	300	125.08	0.149	9.18	21.73

Table 5: ANOVA table for particle size

Source	*Sum of squares*	*Df*	*Mean square*	*F-value*	*p-value Prob>F*	
Model	9232.05	5	1849.41	315.27	<0.0001	Significant
A Amount of oil	3420.09	1	3420.09	583.98	< 0.0001	
B Amount of surfactants	5257.55	1	5257.55	897.72	<0.0001	
Residual	41.00	7	5.86			
Lack of fit	40.94	3	13.65	939.15	0.0001	Significant
Pure error	0.058	4	0.015			
Cor. Total	9273.05	12				

Table 6: ANOVA table for particle size

Source	*Sum of squares*	*Df*	*Mean square*	*F-value*	*p-value Prob>F*	
Model	355.70	5	71.14	36.83	<0.0001	Significant
A Amount of oil	119.89	1	119.89	62.07	0.0001	
B Amount of surfactants	201.38	1	201.38	104.27	<0.0001	
Residual	13.52	7	1.93			
Lack of fit	13.14	3	4.38	46.28	0.0015	Significant
Pure error	0.38	4	0.095			
Cor. Total	369.22	12				

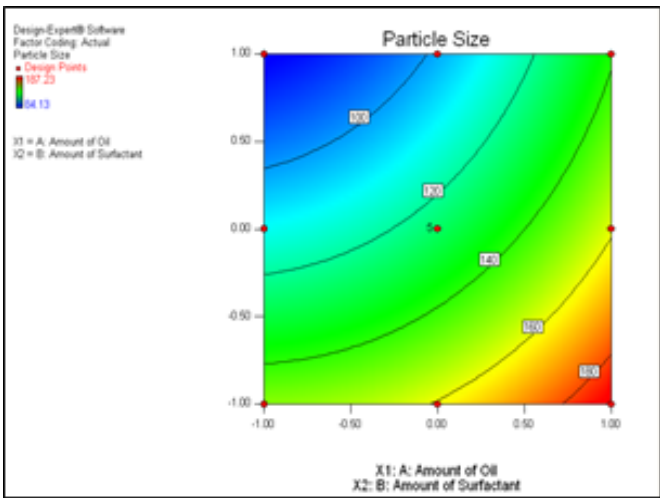

Figure 2: Contour plot for particle size

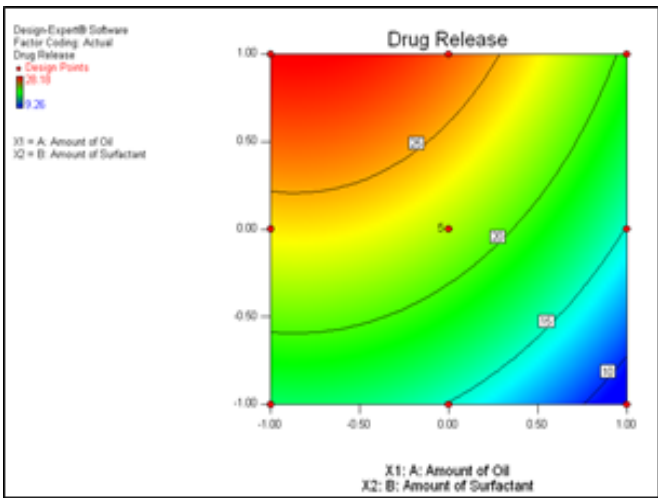

Figure 3: Contour plot for %drug release

% Drug Release

% Drug Release from w/o nanoemulsion significantly affects the effectiveness of the formulation. It was clear that % Drug Release from w/o nanoemulsion depended upon amount of oil and surfactants used in preparation as seen from Table 4 and was found to be statistically significant at $p < 0.05$ as stated in Table 6. Figure 3 gives the contour plot of % drug release.

The polynomial equation derived from the coefficients of estimate in term of coded factor is-

Drug Release =+21.92-4.47 * A+5.79 * B +0.037 * A * B-2.58 * A^2-1.24 * B^2

Figure 4 depicts the overlay plot of the two responses. We can conclude that an optimized formulation can be developed by considering the values in the working region. The formulation thus optimized showed particle size of less than 150 nm and less than 10% release in 1-hour and not more than 25% release in 2 hours.

Using 3^2 factorial design, W/O nanoemulsion was successfully prepared, which was clear, transparent and stable. Table 7. gives the optimized formula for W/O nanoemulsion.

W/O/W Multiple Nanoemulsion

For the development of W/O/W nanoemulsion, a fixed amount of primary nanoemulsion was mixed with a varying amount of

Table 7: Formula of optimized W/O nanoemulsion

Ingredients	*Quantity based on working range (mg)*	*Quantity taken (mg)*	*Remarks*
LabrafacLipophile WL 1349	325-365	347	Selected oil
Span 20: PlurolOlique CC 349	230-270	250	Hydrophobic surfactant and Co-surfactant in ration 3:1
Water	145-160	150	Containing 10mg of Gemcitabine Hydrochloride

Table 8: Results of optimization of W/O/W nanoemulsion

Trials	*W/O Primary emulsion (mg)*	*Hydrophilic Smix (mg)*	*Particle Size D90 (nm)*	*PDI*	*Drug release in 1 hr*	*Drug release in 2 hr*
T-1	300	100	139.4	0.241	4.73	11.66
T-2	300	200	132.3	0.224	6.32	13.14
T-3	300	300	120.2	0.294	9.06	16.30
T-4	300	400	105.9	0.203	11.380	18.31
T-5	300	500	97.9	0.208	13.40	23.45

Table 9: Optimised formulation of W/O/W multiple nanoemulsion

Ingredient	*Quantity (mg)*
Gemcitabine IP	10
Labrafaclipophile WL 1349	140
Span 20: PlurolOlique CC 349 (3:1)	100
Internal aqueous phase	60
Cremophor EL and Transcutol HP (1:1)	200

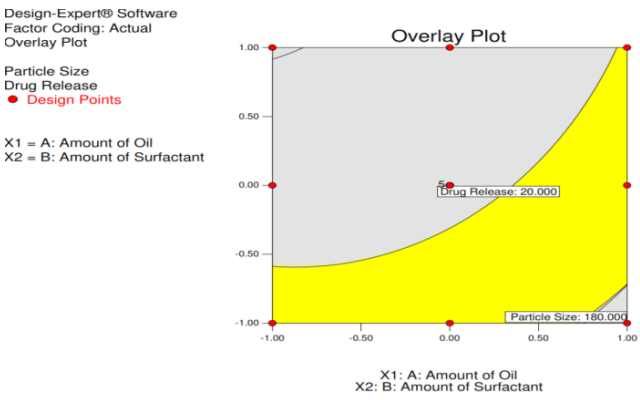

Figure 4: Overlay plot

external hydrophilic surfactants, particle size and drug release were evaluated (Table 8).

From the above trails, it can be seen that when the amount of external hydrophilic surfactants was increased, the system's particle size decreases because there was a decrease in interfacial tension between the two phases.But when the particle size of the system was reduced, there was an increase in the particles' surface area, which led to higher drug release.

Since W/O/W multiple nanoemulsion was developed for intravenous administration, it is desired to have less amount of surfactant. Hence the optimized formulation should contain a minimum amount of surfactants which gives a stable system with particle size less than 150 nm and minimum drug release from the system. Based on this, formulation T-2 was shortlisted as an optimized formulation and was subjected to further characterization.

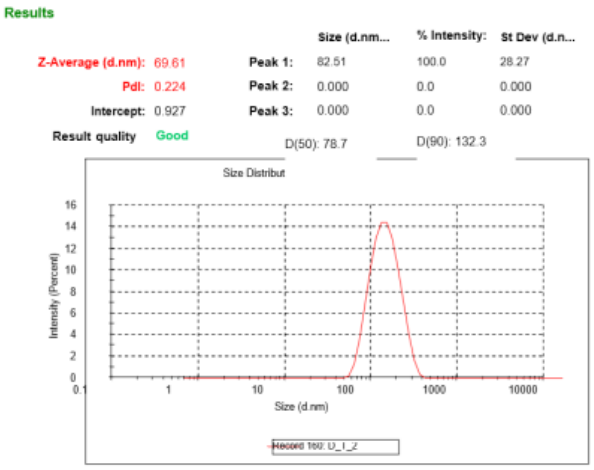

Figure 5: Particle size distribution of optimized formula

Evaluation of optimized formulation of Gemcitabine W/O/W Multiple nanoemulsion

Table 9 gives the optimized formula of W/O/W multiple nanoemulsion.

Determination of Droplet Size Distribution

Droplet size distribution following dilution to form w/o/w multiple nanoemulsion is a critical factor for intravenous administration. As shown in Figure 5 W/O/W multiple nanoemulsion after 100 times dilution showed globules with D90 of 132.3 nm with average particle size 69.61 and PDI 0.224. The result indicated that w/o/w multiple emulsion produced nanoemulsion of desired particle size and PDI.

In vitro Drug Release Studies

Drug release studies in pH 7.4 buffer solution were carried out to mimic blood pH. As seen from Figure 6 drug solution gave 103% release in 1-hour, whereas the optimized T-2 formula gave drug release of 13.14% in 2 hours. The results indicated protection of drug in the internal phase, thus stabilizing it from enzymatic degradation in plasma (Figure 6).

Table 10: Stability results for 3 months

Parameter	*Initial*	*4°C 3 months*	*25°C/60%RH 3 months*
Appearance	Clear transparent.	Nc	Nc
% Drug release phosphate buffer pH 7.4 for 2 hours	13.14%	16.20	17.24
Globule size	132.3 nm	135.2 nm	138.8 nm
%Entrapment Efficiency	92.28 %	90.99 %	89.84 %

*NC- No Change

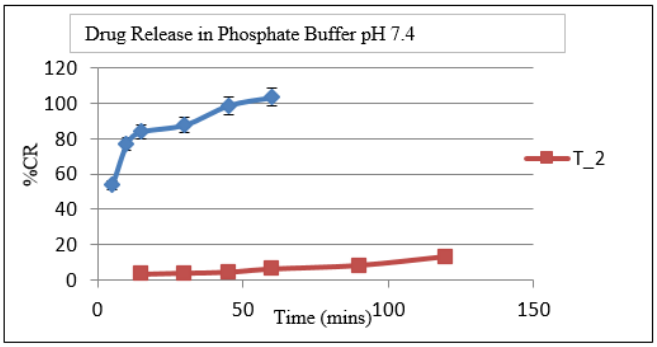

Figure 6: Drug release data for optimized formulation

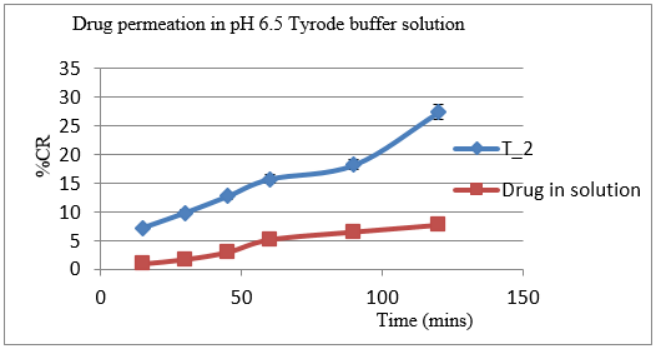

Figure 7: Drug permeated data

Permeation Studies

Gemcitabine is hydrophilic in nature, having restricted permeation through cellular membranes.The Apparent Permeability coefficient of plain drug in solution and drug in multiple emulsion was found to be 1.60×10^{-6} and $4.5\times10^{-5,}$ respectively. As shown in Figue 7, the % drug permeated for drug solution was 7.69%, while that in the formulation was 27.38%. The results indicated improvement in permeability of drug through a biological membrane in the form of W/O/W nanoemulsion. The improvement in permeability may be attributed to smaller globule size, the presence of sandwich oil phase and surfactants which can act as permeation enhancers.

%Entrapment Efficiency

The entrapment efficiency of drug was found to be 92.09% which was considered satisfactory.

% Drug content

% Drug Contents was calculated using calibration curve equation of drug using HPLC by considering the obtained peak areas of the injected formulation. %Drug content of optimized T-2 formulation was found to be 95.78% as desired.

Hemolysis Assay

Since the developed multiple nanoemulsion was designed for intravenous administration, it is was essential to check its hemolytic activity. The % hemolysis caused by the developed formulation was found to be 3.05% which was considered acceptable.

Stability Studies

The final optimized premix formulation of W/O nanoemulsion containing external hydrophilic surfactants was studied for stability as per ICH guidelines for 3 months. The optimized formulation was kept in a glass vial covered with parafilm and analyzed for % drug release in phosphate buffer pH 7.4 for 2 hours, entrapment efficiency and globule size. As seen from Table 10, the developed formulation was found to be stable over three months with no apparent sign of degradation or physical instability.

CONCLUSION

A novel drug delivery system of the anti-cancer drug gemcitabine was developed by incorporating the drug into W/O/W multiple nanoemulsion. The globule size of the formulated system was found to be less than 150 nm with possible potential to escape RES uptake and improved permeation of drug through leaky vasculature at the tumor site. The drug release from the multiple nanoemulsion system's internal aqueous phases was drastically decreased, minimizing the drug degradation in plasma and helping reduce the required dose. Stability studies data of gemcitabine premix W/O nanoemulsion containing external hydrophilic surfactants reveled adequate stability over three months period. The developed multiple emulsion approach can further serve as a platform technology for various drugs with similar drug delivery challenges.

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