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FORMULATION & EVALUATION OF GEMCITABINE HYDROCHLORIDE LOADED SOLID LIPID NANOPARTICLES

Dr. Prathima Srinivas*
K. Sai Preeti

Department of Pharmaceutics, Sri venkateshwara College of Pharmacy & Research Centre Madhapur, Hyderabad-081

ABSTRACT

Gemcitabine hydrochloride is an antimetabolite (pyrimidine analog) currently being used as drug of first choice in pancreatic metastatic cancer. GEM is a rapidly metabolizing water soluble drug (log p = -1.4) with a short half-life of 42 to 94 mins (short infusions) and 245 to 638 mins (long infusions) respectively. To protect the drug from rapid metabolism and achieve sustained drug release an attempt is made to formulate and evaluate GEM loaded solid lipid nanoparticles. GEM SLNs were prepared using stearic acid and GMS as lipid core, tween 80 as emulsifier and PVA as co-emulsifier by double emulsion method. Parameters investigated includes particle size, zeta potential, drug entrapment efficiency (EE %) and in vitro drug release of the SLNs. Optimized SLNs had particle size of 60.4 nm, zeta potential of -45.7 mV, EE of 78.84% and cumulative percent drug release of 54.72 % in 24 hrs and 63.12 % in 48 hrs. SEM images confirmed that the optimized formulation was smooth, uniformly distributed and roughly spherical in shape. Stability studies indicated that optimized formulations were stable at 2-4°C than at 25°C. It is concluded that GEM loaded SLNs were successfully formulated and evaluated to sustain the drug release by bypassing the first pass metabolism.

Keywords: Solid lipid nanoparticles, pancreatic cancer, double emulsion, stearic acid, GMS.

INTRODUCTION

Gemcitabine hydrochloride is an antimetabolite (pyrimidine analog) with poor oral bioavailability (9%) due to the first pass metabolism. GEM is a water soluble drug (log p = -1.4) with a short half-life of 42 to 94 minutes (short infusions) and 245 to 638 minutes (long infusions) respectively. Extensive first pass metabolism, low bioavailability, high dosing frequency and less half-life of GEM are evident through formulations of tablets and injections. These problems can be overcome by incorporating drug in a suitable delivery system such as SLN's.

Solid Lipid Nanoparticles are a new generation of submicron-sized lipid emulsions where the liquid lipid (oil) has been substituted by a solid lipid. Solid lipid nanoparticles are typically spherical with an average diameter between 1 nm to 1000 nm. SLNs have been proposed as an alternative drug carrier system to other novel delivery approaches such as emulsions, liposomes, and polymeric nanoparticles due to various advantages, including feasibility of incorporation of lipophilic and hydrophilic drugs, improved physical stability, low cost, ease of scale-up, and manufacturing.

Address for correspondence

Dr. Prathima. S*
Sri venkateshwara College of pharmacy & Research centre, Madhapur, Hyderabad-081
E-mail: drpssvcp@gmail.com

The objective of this work is to formulate and evaluate solid lipid nanoparticles loaded with Gemcitabine hydrochloride by double emulsion method using stearic acid & GMS as lipid core, with a view to protect the drug from rapid metabolism & sustain the drug release.

MATERIALS & METHODS

MATERIALS

Gemcitabine hydrochloride was obtained as a kind gift sample from Celon labs, Telangana, India. Glyceryl monostearate (GMS), Stearic acid, Poly vinyl alcohol (PVA), Polysorbate-80 was procured from SD-Fine chemicals limited. Dialysis membrane (mol wt. 12,000 – 14,000 Da) was obtained from Himedia Laboratories Pvt Ltd, Mumbai, India. All other reagents and chemicals used were of analytical grade.

METHOD

Optimization of process variables

Gemcitabine hydrochloride, a hydrophilic drug was used in the present investigation to determine the process variables effecting the incorporation of hydrophilic drugs in to SLN. In the present study, a simple approach for the fabrication of SLN of the basic molecule GEM was adopted. Two process variables namely sonication time and homogenization time were studied to optimize the formulation.

Optimization of homogenization time was carried out by varying the time from 5 to 20 minutes. Optimum particle size was observed when the homogenization was carried out for a period of 10 minutes. Further increase in homogenization time showed increase in particle size as shown in Table1.

As the ultrasonication time increased, the particle size in the formulation is further reduced to sub-micronized level. Optimum particle size was observed when the ultrasonication was carried out for a period of 10 minutes. Further increase in the ultrasonication time resulted in greater increase in the size of the particle.

PREPARATION OF SOLID LIPID NANOPARTICLES BY DOUBLE EMULSION TECHNIQUE[11]

Solid lipid nanoparticles of Gemcitabine hydrochloride were prepared using Stearic acid and GMS as lipid core, Tween 80 as emulsifier and PVA as co-emulsifier. In this method accurately weighed quantity of Gemcitabine hydrochloride previously dissolved in Tween 80 solution was taken into a beaker. In another beaker, lipid was melted and dissolved in solvent. Aqueous phase containing drug was added drop by drop to oil phase and sonicated (Sonics, Vibracell) for 10mins to get primary emulsion (o/w). The formed primary emulsion was added to PVA solution and homogenized (Remi motors KSL 2605) for 10mins to produce double emulsion (w/o/w). Organic solvent was removed using rota vapor (Superfit, Germany). Gemcitabine hydrochloride loaded solid lipid nanoparticles were obtained by allowing the nanoemulsion to cool to room temperature.

CHARACTERIZATION & EVALUATION OF GEMCITABINE LOADED SOLID LIPID NANOPARTICLES

The prepared SLN's were evaluated for various parameters such as invitro drug release, drug entrapment efficiency and characterized by FTIR, particle size, zetapotential & SEM.

Drug Excipient Compatibility Studies

FT-IR offers the possibility of chemical identification, provides information about the structure of molecule. The infrared analysis was carried out to find out the presence of drug-lipid interactions used in the preparation of SLNs. IR spectra were studied for the pure drug and the optimized formulation were studied in the range from 400-4000cm^{-1} and carbon black reference.

Particle Size

The z-average diameter of nanoparticles was determined by dynamic light scattering using a particle size analyzer (Horibo scientific nanopartica SZ 100). For the measurement, 100 µl of the formulation was diluted with an appropriate volume PBS, pH 7.4 and the nanoparticle diameter was determined.

Zeta Potential

Zeta potential measurements allow prediction about the storage stability of colloidal dispersions. Zeta potential of optimized formulation was carried out using zeta potential analyzer or zetameter (Horibo scientific nanopartica SZ 100) to determine its value.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was conducted to characterize the surface morphology of the SLNs. SEM uses electrons transmitted from the specimen to determine the overall shape and morphology i.e. both particle size and distribution.

Entrapment Efficiency

The percentage entrapment efficiency (EE %) corresponds to the percentage of GEM encapsulated within the nanoparticles, was determined by measuring the concentration of free GEM in the dispersion medium.

The EE % was calculated using the following equation:

$$\text{Entrapment efficiency} = \frac{\text{Weight of total drug-weight of free drug}}{\text{Weight of total drug}} * 100$$

In Vitro Drug Release[11]

The *in vitro* release of GEM-loaded SLNs was evaluated by Diffusion technique. Dialysis membrane with a molecular weight cut off of 12,000 (Himedia, Mumbai, India) were used in the study. 1 ml of SLN dispersion was loaded in the dialysis tube, and tied firmly at both ends was immersed in pH 7.4 phosphate buffer. Aliquots of 3mL samples were withdrawn from the medium and replaced with the same volume of fresh medium every time. The samples were estimated spectrophotometrically at λ_{max} 267 nm.

Stability Studies

The stability studies of the optimized SLN formulations were carried out at two different temperatures i.e., 2-4°C & 25°C for a period of 3 months. Samples were withdrawn at different time intervals and checked for physical appearance and drug content.

RESULTS & DISCUSSION

Formulation of slns using stearic acid & gms

Formulation of Gemcitabine hydrochloride loaded solid lipid nanoparticles using stearic acid and GMS are shown in Table 3 & 4

PARTICLE SIZE

Particle size of the optimized SLN G7 was determined and was found to be 60.4nm

ZETA POTENTIAL

Zeta potential of the optimized formulation G7 was determined using zetasizer and value was found to be -45.7mV.

SCANNING ELECTRON MICROSCOPY

The optimized formulation was subjected to SEM study. The shape and surface morphology of the optimized formulation (G7) was observed as smooth, uniformly distributed and roughly spherical as shown in Figure 6.

ENTRAPMENT EFFICIENCY

Entrapment efficiency of different GEM SLNs using Stearic acid

Formulations S1 to S12 were studied for entrapment efficiency. The formulation S7 showed highest release entrapment efficiency of 78.94% indicating the optimum amount of lipid required for the formation of a stable emulsion. With further increase in

the lipid concentration, the EE remained close to 76% indicating that the lipid concentration did not help in entrapping the drug into the matrix.

Entrapment efficiency of different GEM SLNs using GMS

Formulations G1 to G15 were studied for entrapment efficiency. Results indicated that formulation G7 was found to have highest entrapment efficiency of 79.89%. It is found that there is no direct relationship between the lipid concentration and the entrapment efficiency. Further increase in the lipid concentration could not influence the entrapment efficiency of the drug into the matrix.

IN VITRO DRUG RELEASE

Cumulative % drug release profiles of different SLNs using stearic acid

From the obtained diffusion data, it is observed that the release is biphasic in all the formulations i.e., initial burst release followed by sustained release. The presence of free GEM in the external phase and on the surface of the nanoparticles might be the reason for the burst release. The solubility of the GEM in the aqueous phase could be the reason for slow release of the drug from the lipid matrices after initial burst release.

Increase in the surfactant concentration causes increase in the burst release. This might be due to the fact that hydrophilic drugs have maximum tendency for rapid migration in to the external phase during the fabrication process because of their low affinity and weak interaction with the lipid.

Increase in the lipid concentration has significant effect on the drug release which prolongs the release of the drug from SLNs. It may be due to equal distribution of drug within the lipid matrix and good entrapment of drug in stearic acid.

Among all the formulations from S1-S12, S7 has shown the optimum drug release needed for the therapeutic action, hence it is optimized.

Cumulative % drug release profiles of different SLNs using GMS

From the obtained diffusion data, it is observed that the release is sustained in all the formulations and few formulations have shown the burst release.

Among all the formulations from G1-G15, G7 formulation is optimized because the release is biphasic hence it is possible to achieve the loading dose of the drug due to initial burst release followed by the maintenance of the drug due to sustained release.

STABILITY STUDIES

The optimized formulation G7 was stored at different temperatures i.e. 25°C and 4°C for 3 months and data is presented in Table 6 & Table 7. At 4°C no change in the physical appearance and colour was observed indicating the physical stability of drug in the formulation. There was negligible change in the drug content of optimized formulation after 3months indicating chemical stability. Hence it is concluded that GEM-SLNs are stable at 4°C than at 25°C.

CONCLUSION

Gemcitabine hydrochloride loaded SLNs for parenteral administration can be successfully formulated using simple lipids which are bio-compatible. GEM-SLNs are beneficial over the existing formulations formulations as they bypass the hepatic first pass metabolism, avoids repetitive dosing, reduces the toxic side effects, site targeting, sustains the drug release by entrapping the drug in to solid lipid. Therefore GEM-SLNs can be explored as potential candidates for the treatment of pancreatic metastatic cancer.

Table 1: Effect of homogenization time

Homogenization time (mins)	Observation
5	Particle size above 400nm
10	Particle size reduced to 200-300nm
15	No major changes were observed. Particle size ranged from 210nm to 300nm

Table 2: Effect of sonication time

Ultrasonication time (mins)	Observation
5	Particle size ranged from 100 to 200nm
10	Particle size ranged from 50 to 100nm
15	No major changes were observed. Particle size ranged from 60nm to 100nm

Table 3: Formulation of solid lipid nanoparticles with Stearic acid

Formulation code	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Drug	10	10	10	10	10	10	10	10	10	10	10	10
Stearic acid	100	100	200	200	300	300	400	400	500	500	600	600
Tween 80	10	20	10	20	10	20	10	20	10	20	10	20
PVA	1	1	1	1	1	1	1	1	1	1	1	1

Table 4: Formulation of solid lipid nanoparticles with Glyceryl monostearate

Formulation code	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15
Drug	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
GMS	100	100	100	200	200	200	300	300	300	400	400	400	500	500	500
Tween 80	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
PVA	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 5: Characteristic IR peaks of pure drug and optimized formulations

Functional group	Reported value	Observed value (drug)	Observed value (S7 Formulation)	Observed value (G7 Formulation)
N-H group	3500	3390	3365	3489
CONH group	1680-1630	1678	1633	1635

Table 6: Stability studies of the optimized formulation G7 at 2-4°C

Time point	G7			
	Initial	**1 month**	**2 months**	**3 months**
Physical appearance	Milky white & no phase seperation	Milky white & no phase seperation	Milky white & no phase seperation	Milky white & no phase seperation
Drug content	78.94	76.65	75.18	74.53

Table 7: Stability studies of the optimized formulation G7 at 25°C

Time point	G7			
	Initial	**1 month**	**2 months**	**3 months**
Physical appearance	Milky white & no phase separation	Phase separation	Phase separation	Phase separation
Drug content	78.94	75.37	71.81	68.48

Figure 1: FTIR Spectrum of pure Gemcitabine hydrochloride drug

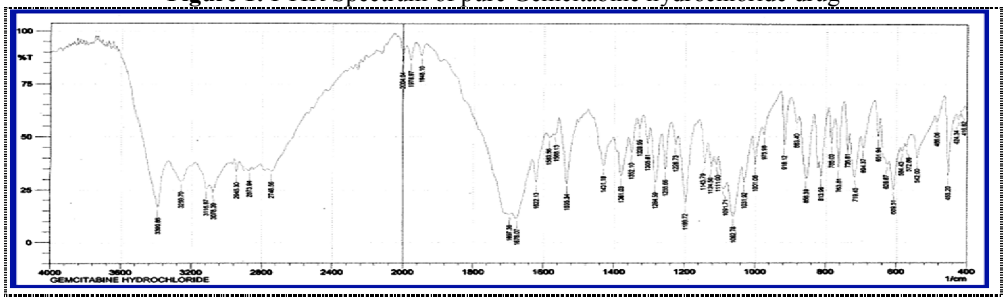


Figure 2: FTIR Spectrum of S7 Optimized Formulation

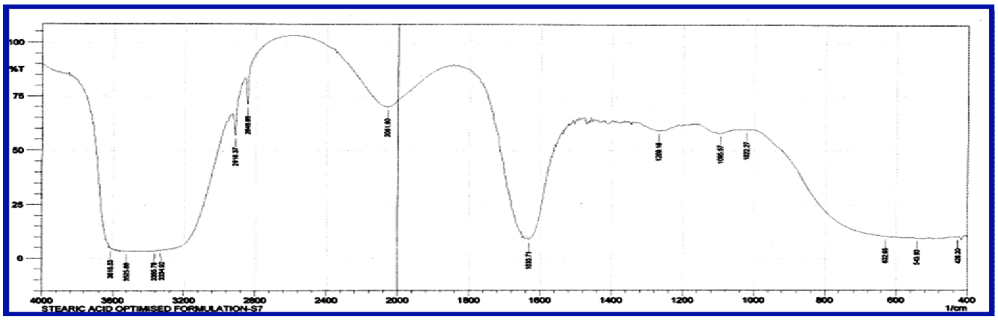


Figure 3: FTIR Spectrum of G7 Optimized Formulation

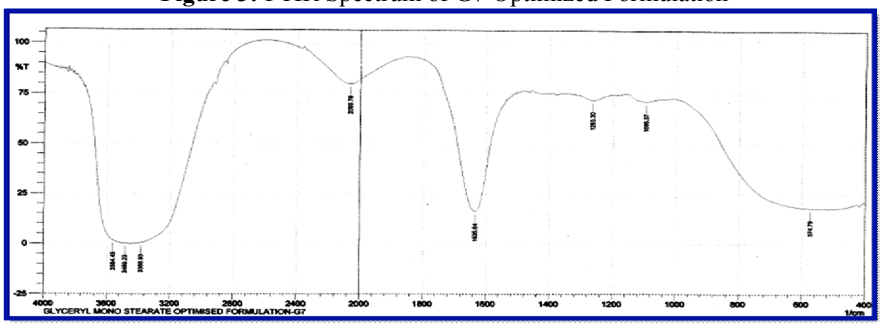


Figure 4: Particle size of optimized SLN G7

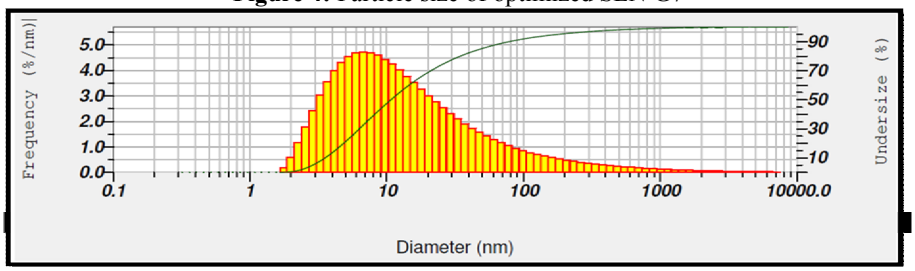


Figure 5: Zeta potential of optimized SLN G7

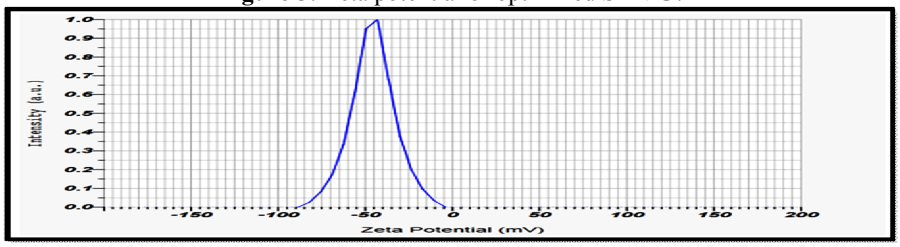


Figure 6: SEM image of optimized SLN G7

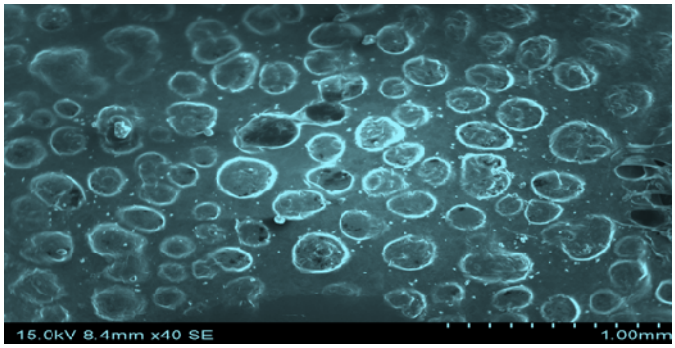


Figure 7: Graphical representation showing entrapment efficiency of different SLN formulations using Stearic acid

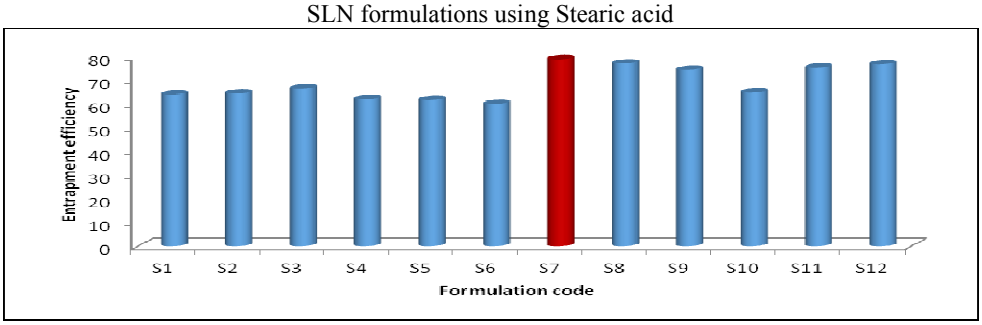


Figure 8: Graphical representation showing entrapment efficiency of Different SLN formulations using GMS

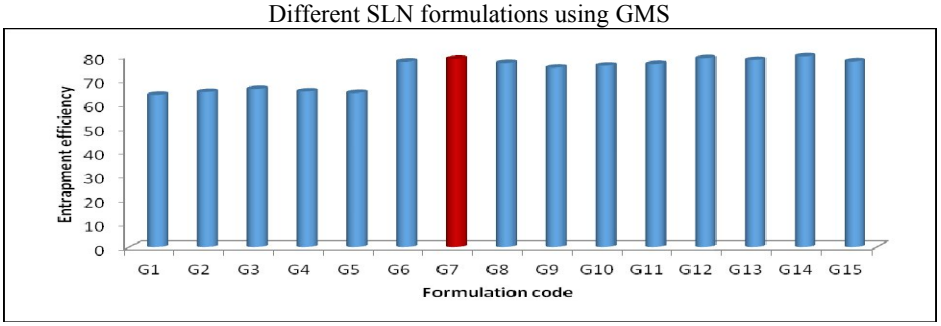


Figure 9: Cumulative % drug release from Gemcitabine hydrochloride loaded Stearic acid SLNs in 7.4 pH phosphate buffer from S1-S6

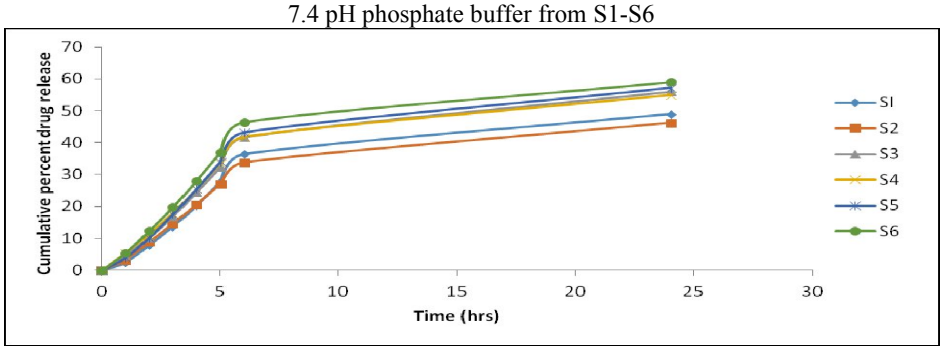


Figure 10: Cumulative % drug release from Gemcitabine hydrochloride loaded Stearic acid SLNs in 7.4 pH phosphate buffer from S7-S12

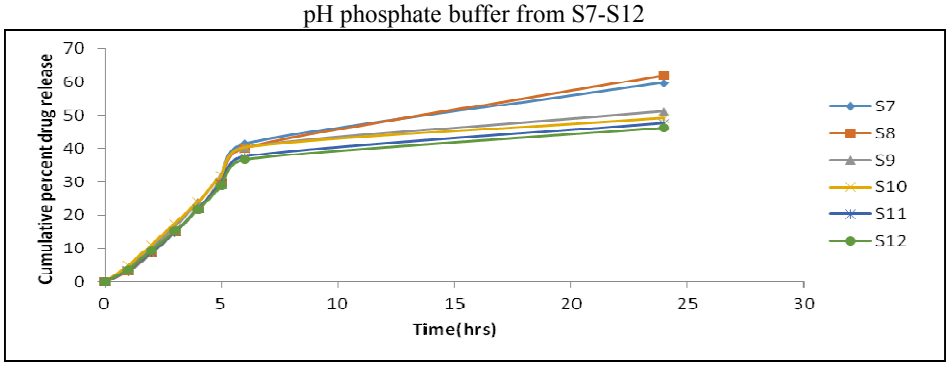


Figure 11: Cumulative % drug release from Gemcitabine hydrochloride loaded GMS SLNs in 7.4 pH phosphate buffer from G1-G5

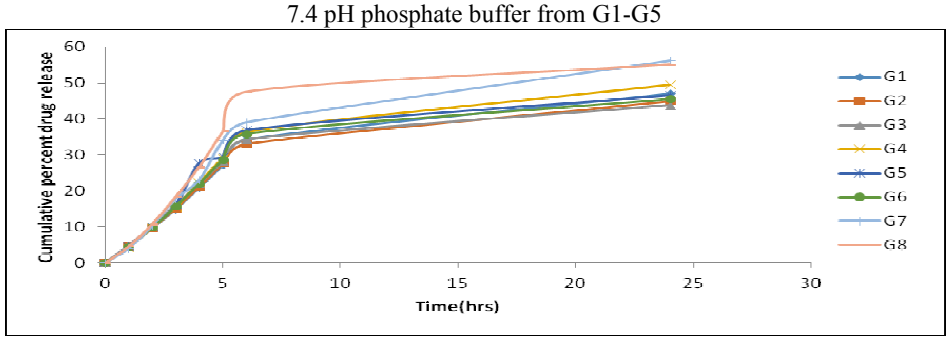
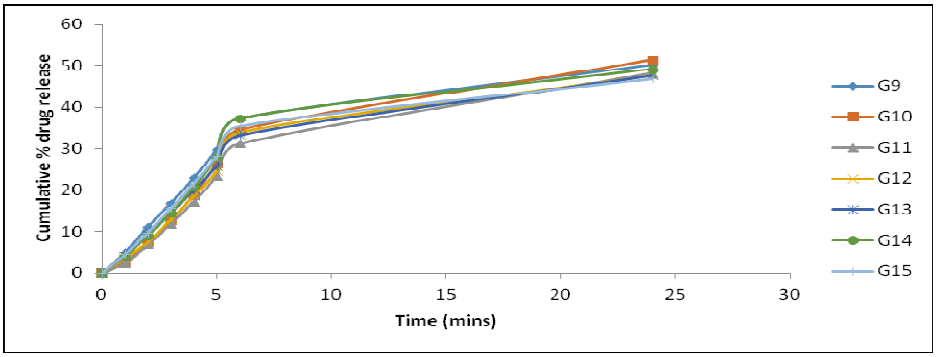


Figure 12: Cumulative % drug release from Gemcitabine hydrochloride loaded GMS SLNs in 7.4 pH phosphate buffer from G9-G15



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