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Research Article

Formulation Development and Evaluation of Spray Dried Sustained Release Microspheres of Gemcitabine Hydrochloride.

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ABSTRACT
The aim of this research was formulation; development and evaluation of spray dried sustained release microspheres of gemcitabine hydrochloride. Gemcitabine HCL Injectable microspheres were prepared by spray drying method reported by using combination of Chitosan and HPMC with some modifications. Gemcitabine HCL microspheres were evaluated for particle size, *in-vitro* release, FTIR, SEM, % Encapsulation Efficiency. All formulations showed good encapsulation efficiency i.e. 51.2 % to 86.8%. Amount of Chitosan and HPMC influenced on the properties of encapsulation efficiency and mean particle size of different formulations were studied. The optimized formulation showed the best results with 98.8% drug release in 48 hours. Chitosan and HPMC based injectable microspheres of Gemcitabine HCL can be effectively used for target specificity and sustained drug release for extended period of time in treatment of different types of cancers.

KEYWORDS
Spray drying, Injectable microsphere, Target specific delivery, Gemcitabine, Chitosan, and HPMC.

INTRODUCTION

Gemcitabine HCl (GEM) is an anticancer drug used in treatment of many types of cancer including lung, colon, pancreas, breast and ovarian cancers. Gemcitabine is a pyrimidine antimetabolite which is difluoro analogue of deoxycytidine (2′,2′difluorodeoxycytidine). For showing the pharmacological activity inside the body it has to be phosphorylated into its active metabolite, gemcitabine triphosphate and diphosphate. This happens by the deoxycytidine kinase in the body [1].These active metabolites get incorporated into the DNA strand and inhibit the DNA synthesis thereby inhibiting cell growth.

Presently the drug is available for parenteral administration but is rapidly metabolized as it is widely deaminated by cytidine deaminase in blood and other organs [2]. This causes the dosage frequency to be increased to get the therapeutic concentration for a longer duration which in-turn increases the total amount of GEM to be administered during the cancer therapy [3]. Hence there is a need to develop a delivery system which not only increases its stability but also has a sustained release of GEM for a longer duration.

Another reason for the drug to be incorporated into a novel drug delivery system is that it is low-molecular weight water soluble drug which does not have easy access to cells via cell membranes. It has been reported that novel drug delivery systems like nanoparticles or microparticles would not only provide efficient delivery of the anticancer drug like GEM to the cancer cells but also protect the drug from rapid metabolization [4,5].

Microspheres as drug delivery system not only protect the encapsulated drug but also provides sustained or controlled release of the drug. Microspheres made of biodegradable polymers have certain advantages like no need to remove from the body after implantation into the body, good biocompatible and are self-degradable. Among the many available biodegradable polymers, chitosan is one of the natural polysaccharides obtained from chitin used in formulation of microspheres [6]. Chitosan is cationic in nature; it is nontoxic and biocompatible. It has proven record of entrapping drugs to control the drug release for suitable periods. Anticancer drugs have been reported to have a sustained release profile with microspheres of small size (< 10µm) with the advantage of delivering the drug to the desired site of cancer [7]. In addition, chitosan based particulate delivery systems may be injected at the site of cancer for proficient delivery of anticancer drugs[8].

The present study was planned with an objective of formulating biodegradable microspheres of GEM to overcome the drawbacks associated with its conventional parenteral administration of repeated administration. Microspheres were prepared with spray drying to have uniform small sized particles to sustain the drug release.

2. MATERIALS AND METHODS

2.1. Materials

Gemcitabine HCl was procured as a gift sample form Shilpa Medicare Limited, Hyderabad. Chitosan (Medium MW) extra pure deacetylated degree 90% was procured form Sisco Research Laboratories, Taloja, MS, India. DCM was purchased from SD fine chem. Limited, Mumbai, India. The synthetic dialysis membrane (MWCO 12000) was procured from Himedia labs, Mumbai, India. HPMC, Di-sodiumhydrogen phosphate, potassium dihydrogen phosphate, were

obtained from Loba Chemicals, Mumbai. All other chemicals and reagents used were of analytical grade.

2.2. ATR-FTIR Characterization

ATR-FTIR characterization was done for pure drug (GEM), chitosan, HPMC and GEM loaded Chitosan Microspheres (GEM_Chito_MS). The attenuated total reflection (ATR) mode was used to get the FTIR spectra using the UATR Two model of Perkin Elmer. Samples were studied at room temperature with 50 scans and a resolution of 4 cm^{-1}. The spectra were detected over a range of 4000–400 cm−1.

2.3. Preparation of Microspheres by Spray drying

Microspheres were prepared by spray drying method reported by using combination of Chitosan and HPMC with some modifications [9]. Two solutions were prepared separately. Part 1 constituted of chitosan solution prepared by dissolving 250/500/750 mg of it in 100 ml of 1% (v/v) acetic acid solution by mechanical stirring. Part 2 was the drug solution in HPMC solution. Firstly 0.1/0.2/03 %w/v HPMC solution was prepared in 10 mL water to which 50 mg of GEM was added. Part 2 was then added to part 1 as per the nine batches shown in Table 1. The entire dispersion constituting of the drug-polymer mixture was then spray-dried in a minispray dryer. The Lab Ultima-222 Mini spray drier was used with the nozzle diameter of 0.7 mm. The variables used were as shown in Table 1A and 1B. In brief, the atomization pressure was set at 1.5 kg/cm2 with the feed rate of 5 mL/min. The inlet and outlet temperatures were set at 170± 5°C and 100± 5°C. The microspheres were then collected and stored in a desiccator until further use.

The same procedure was used for preparation of blank microspheres without GEM to set some of the initial factors. The process parameters that were set by preparation of blank microspheres were: (i) feed rate (5 ml/min); (ii) drying air inlet temperature (170◦C); and (iii) Atomization pressure (1.5 kg/cm^2) based on the mean particle size in the range of 5-15 microns and production yield above 75%.

Table 1. Gemcitabine Formulation Batches

Formulation Code	**X1**	**X2**
	Chitosan Conc. (mg)	**HPMC Conc. (w/v%)**
GEM-CH-MS1	250	0.1
GEM-CH-MS2	250	0.2
GEM-CH-MS3	250	0.3
GEM-CH-MS4	500	0.1
GEM-CH-MS5	500	0.2
GEM-CH-MS6	500	0.3
GEM-CH-MS7	750	0.1
GEM-CH-MS8	750	0.2
GEM-CH-MS9	750	0.3

Table 1A. Instrumental Parameters (Spray Drying).

Model of Spray Dryer	**Mini spray drier, Lab ultima-222**
Nozzle diameter	0.7 mm
Atomization pressure	1.5 kg/cm^2
Feed rate	5 mL/min
Inlet temperature	170± 5°C
Outlet temperature	100 ± 5°C
Aspirator speed	55%

Table 1B. Processing Parameters.

Batch Size	**110 mL**
Part 1-Chitosan Solution	100 mL
Part 2- Drug Solution with Polymer	10 mL
Drug Amount	50 mg
X1 (Chitosan Conc.)	250, 500 and 750 mg in 100 mL
X2 (HPMC Conc.)	0.1, 0.2 and 0.3 w/v in water

2.4. Factorial Design for optimization of MPS and EE

Factorial designs are routinely used in pharmaceutical development to optimize the dependent variables so as to get the desired outcome. Moreover, it saves time as minimum experimentation gives maximum output by the use of multiple regression equations [10]. A 3^2 Full factorial design with two factors (X1 and X2) were studied at three levels (-1,0 and +1) to perform the experimental design. The two factors selected were the concentration of Chitosan (X1) and concentration of HPMC (X2) as shown in Table 1.

The polynomial equation was generated using the Design Expert software (Design Expert ® v10 (DX10), Stat-Ease-Inc Minneapolis, USA) as shown in Equation 1.

$$Y=\beta_O+\beta_1X1+\beta_2X2+\beta_{11}X1^2+\beta_{22}X2^2+\beta_{12}X1X2 \text{ ---Equation. 1}$$

Where, Y is the dependent variable which is the response; there are two responses in this model design. First is the mean particle size denoted as Y1 and second is entrapment efficiency as Y2. X1 and X2 are the two independent variables set after carrying out the preliminary studies and β term denotes the model coefficients. The F-statistics was used with ANOVA to analyze the response using the interactive multiple regression statistics [11].

Table 2. A layout of 3^2 full factorial design.

Formulation Code	X1 (Chitosan Conc.)	X2 (HPMC Conc.)	X1X1	X2X2	X1X2
GEM-CH-MS1	-1	-1	1	1	1
GEM-CH-MS2	-1	0	1	0	0
GEM-CH-MS3	-1	1	1	1	-1
GEM-CH-MS4	0	-1	0	1	0
GEM-CH-MS5	0	0	0	0	0
GEM-CH-MS6	0	1	0	1	0
GEM-CH-MS7	1	-1	1	1	-1
GEM-CH-MS8	1	0	1	0	0
GEM-CH-MS9	1	1	1	1	1

2.5. Particle size analysis

The particle size of the spray-dried microspheres was checked by the dynamic light scattering (DLS) principle using the Malvern Zetasizer (Malvern Instruments, UK). For the measurements of particle size a dilute suspension of GEM_MS were prepared in Millipore water. All analyses were performed in triplicate.

2.6. Evaluation of drug Loading capacity and Entrapment Efficiency (EE)

The prepared dried microspheres were suspended in distilled water and centrifuged (Remi centrifuge, India) and both drug loading (DL) capacity and entrapment efficiency (EE) were measured indirectly by calculating the amount of present in the supernatant. Mass balancing was done to confirm the total amount of drug.

Drug Loading (DL)= Total GEM-Free GEM/Total MS weight

%EE= (calculated drug concentration/theoretical drug content) ×100

2.7. Scanning electron microscopy

To study the external morphology of the formulated microspheres scanning electron microscopy (SEM) was done on the spray dried particles. The sputter-coating with gold–palladium method was used wherein the microspheres were attached to the stubs by a double-sided tape[12]. Microspheres were then imaged with a JEOL JSM-840 scanning electron microscope (JEOL USA, Inc.) The accelerating voltage used was 5 kV at a distance of 10 mm.

2.8. Production Yield

The percentage of production yield (%) was calculated from the total weight of spray dried microparticles recovered after spray drying with respect to the sum of the total weight of the starting materials taken in dry form.

2.9. In Vitro Drug Release Studies

The cumulative amount of drug released (GEM) from the prepared microspheres was studied in vitro by dialysis bag method. The dialysis membrane with a molecular weight cut off of 12,000 (Himedia, Mumbai, India) was filled with microspheres corresponding to 100 mg of GEM. The dialysis bag was tied firmly at both ends by thread and immersed in a 50 mL glass beaker filled

with PBS pH 7.4. Aliquots of 1mL were withdrawn at predetermined time intervals from the dissolution medium and replaced with fresh medium. The drug samples were estimated by UV-spectrophotometer at a wavelength of λ max 268 nm.

3. RESULTS AND DISCUSSION

3.1. ATR-FTIR Characterization

The FTIR spectra of chitosan (Fig. 1A) were 3364, 1601, 1375 and 1026 which were similar to reported peaks of the polymer indicating purity and confirmation of the polymer [2].

FTIR spectra of Gem pure drug (Fig. 1B) revealed proper bands at approximately 3387, 3077, 1676 and 1061 cm^{-1}. These results matched with previously reported studies [13, 14]. FTIR spectra of pure GEM showed characteristic peaks of amine bands at 1676 cm−1 and 3387 cm−1 stretching vibration of (NH2) [3]. However, these major peaks were not present in the GEM_CG_MS (Fig. 1C) indicating that the drug was molecularly dispersed in the polymer matrix.

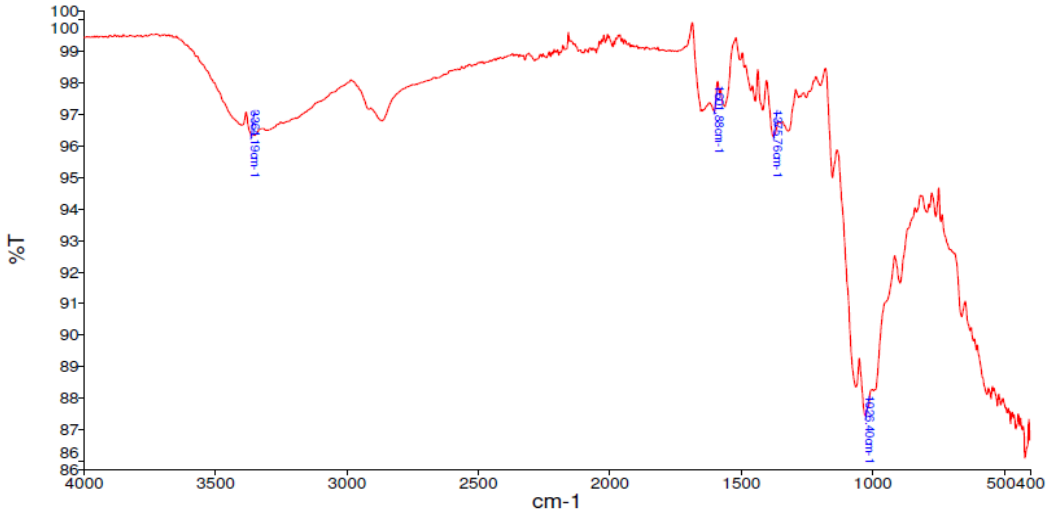

Fig. 1A. ATR-FTIR Spectrum of Chitosan.

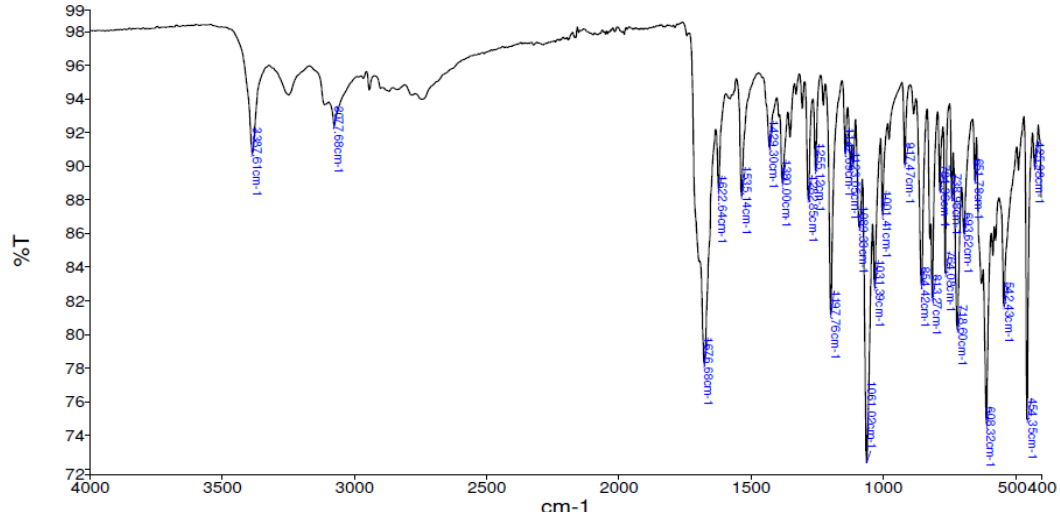

Fig. 1B. ATR-FTIR Spectrum of GEM pure drug.

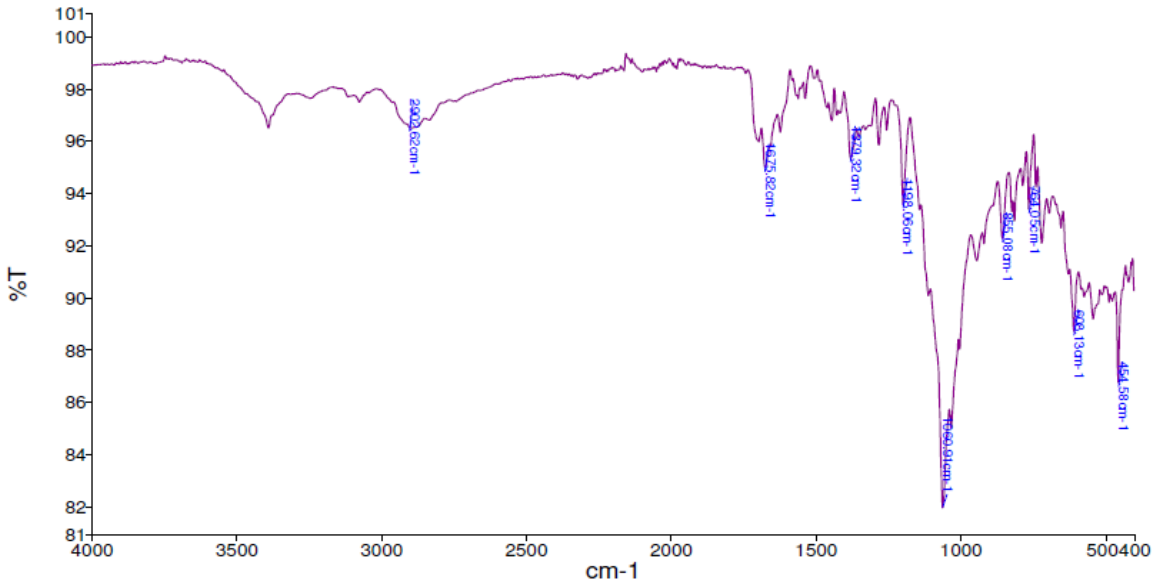

Fig. 1C. ATR-FTIR Spectrum of GEM_CH_MS.

3.2. Formulation of Microspheres by Spray drying

The spray drying is an efficient method where the dispersion/ solution is injected into the chamber through the nozzle and the flow is controlled to get the desired droplet size [15]. By the process of atomization produced by the compressed air; the droplet is broken down into much smaller droplets [16]. Once these small sized droplets are available in the drying chamber there is an immediate evaporation of the solvent of the droplet. The mechanisms like involvement of exchanging heats and blowing of hot air are responsible to remove the solvent [16]. Once the solvent from the droplet is evaporated only the dried particle/ powder is available which is then collected in a collecting chamber of the instrument.

In the present study water is the solvent which would definitely take more time to evaporate as compared to solvents like methanol or solvents. However, water-based solvents are much safer and more acceptable as per the regulatory guidelines.

The preliminary studies included to set the major conditions of the instrument such as the inlet temperature was varied from 150-180°C, outlet temperature was varied form 90-120°C. The solution feed rate was varied from 3 to 6mL/min. Accordingly the aspiration rate and compressed spray air flow were also varied. The blank microspheres were prepared to set these preliminary conditions to get the particles in the size range of 5-15 microns. Finally, the inlet and outlet temperatures were set at 170± 5°C and 100± 5°C respectively with atomization pressure of 1.5 kg/cm2 and the dispersion feed rate of 5 mL/min.

3.3. Factorial Design

During the process of formulation development there are many process variables which needs to be optimized to get the product with desired characteristics. Factorial design studies aim at utilizing a smaller number of trials with simultaneous estimation of a greater number of factors and understanding the interrelationship between them. Table 3 shows the actual values of both

the factors X1 (Chitosan concentration) and X2 (HPMC concentration) taken and the two responses obtained as MPS and EE.

Table 3. Outcome of the Factorial design values for MPS and EE.

Formulation Code	X1	X2	Y1		Y2	
	Chitosan Conc. (mg)	**HPMC Conc. (w/v%)**	**MPS (µm)**	**±SD**	**EE (%)**	**±SD**
GEM-CH-MS1	250	0.1	14.6	2.3	34.6	3.32
GEM-CH-MS2	250	0.2	13.4	1.8	46.2	3.81
GEM-CH-MS3	250	0.3	11.3	1.3	48.9	2.35
GEM-CH-MS4	500	0.1	8.1	0.9	37.5	1.95
GEM-CH-MS5	500	0.2	7.8	2.6	48.5	3.61
GEM-CH-MS6	500	0.3	6.2	1.6	51.6	3.63
GEM-CH-MS7	750	0.1	7.3	2.4	54.8	2.54
GEM-CH-MS8	750	0.2	6.4	1.1	65.3	2.13
GEM-CH-MS9	750	0.3	5.5	0.3	68.4	4.32

3.3.1. Response: MPS

The first parameter that was optimized was the mean particle size (MPS). The Mean Particle Size (MPS) varied from 14.6 to 5.5 µm. The desired mean particle size was below 10 microns. It has been reported that the microparticles of sizes 5-7 microns gets better accumulated in the lungs which may be beneficial for lung cancer [17].

Table 4A. Regression Statistics

Multiple R	**0.998358**
R Square	0.99672
Adjusted R Square	0.991252
Standard Error	0.310167
Observations	9

Table 4B. ANOVA output

	df	**SS**	**MS**	**F**	**Significance F**
Regression	5	87.69361	17.53872	182.3082	0.000636
Residual	3	0.288611	0.096204		
Total	8	87.98222			

Table 4C. Regression output showing t-stat and P-value of individual model terms.

Model term	Coefficients	Standard Error	t Stat	P-value
Intercept	7.611111	0.231185	32.92217	6.16E-05 #
X1	-3.35	0.126625	-26.456	0.000118 #
X2	-1.16667	0.126625	-9.21354	0.002704 #
X12	2.383333	0.219321	10.86686	0.001668 #
X22	-0.36667	0.219321	-1.67182	0.193152
X1X2	0.375	0.155084	2.418051	0.094334

 # significant (p-value is less than 0.05)

Table 4D. Residual output showing Observed, predicted and residual value.

Formulation Code	Observed Y1 Value (MPS)	Predicted Y1 Value (MPS)	Residuals
GEM-CH-MS1	14.6	14.51944	0.080556
GEM-CH-MS2	13.4	13.34444	0.055556
GEM-CH-MS3	11.3	11.43611	-0.13611
GEM-CH-MS4	8.1	8.411111	-0.31111
GEM-CH-MS5	7.8	7.611111	0.188889
GEM-CH-MS6	6.2	6.077778	0.122222
GEM-CH-MS7	7.3	7.069444	0.230556
GEM-CH-MS8	6.4	6.644444	-0.24444
GEM-CH-MS9	5.5	5.486111	0.013889

The Model F-value obtained (182.31) was high enough to imply that the model was significant. In such a situation a very small fraction of possibility as low as 0.06% could be due to noise. In addition, the value for the "Prob > F" is much less than 0.05 which clearly indicates that the entire model is significant [18].

In terms of the contribution of the individual model terms it was seen that not all the terms contributed to the significance as model terms whose p-values were more than 0.05 were known to be insignificant.

Hence only X1, X2 and X12 were known to be significant model terms. This means that the first factor i.e the concentration of Chitosan played a vital role in deciding the particle size of the microspheres. There was a good quadratic effect of chitosan on development of final equation. The model terms which did not contribute would hence be removed from the equation as there would be insignificant effect due to them. It is well known that only removal of such insignificant model terms would improve the model.

The final equation for the response MPS would be:

Y1 (MPS)= $7.611111-3.35X1-1.16667X2+2.383333X1^2$ ---Equation 2

The above equation when used in terms of actual factors can be used to make predictions about the response for each factor.

The Predicted R-Squared value (0.9643) was in close resemblance to the Adjusted R-Squared value (0.9913) as the difference between the two was less than 0.2. Another feature the Adequate Precision is a ratio which should be more than 4 to be acceptable and in this case it was much higher (35.670) which showed that the developed model was useful to navigate through the design space.

Table 4D gives the residual output showing the values obtained by performing the experimental runs in terms of the "Observed value". It depicts the "Predicted values" by the factorial design model used. The residual values are so less that the predicted values are in reasonable agreement with the observed values. This is also shown in the Fig. 2, Normal probability plot of the studentized residuals to check for normality of residuals.

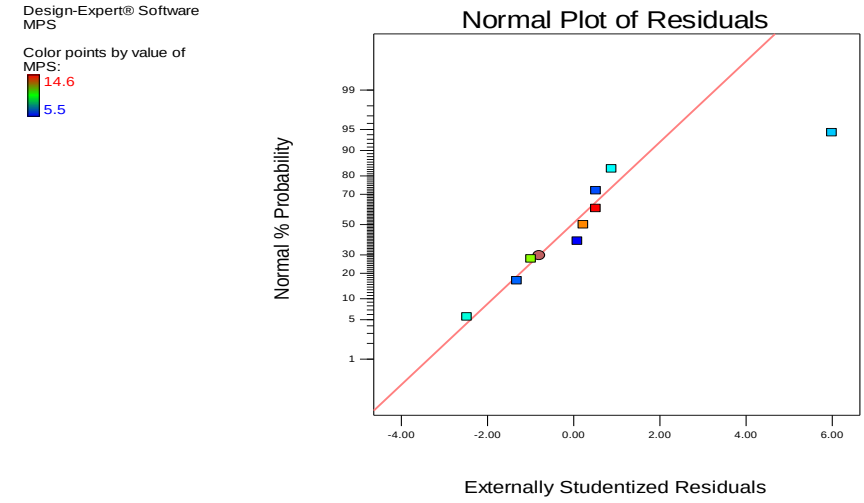

Fig. 2. Normal probability plot.

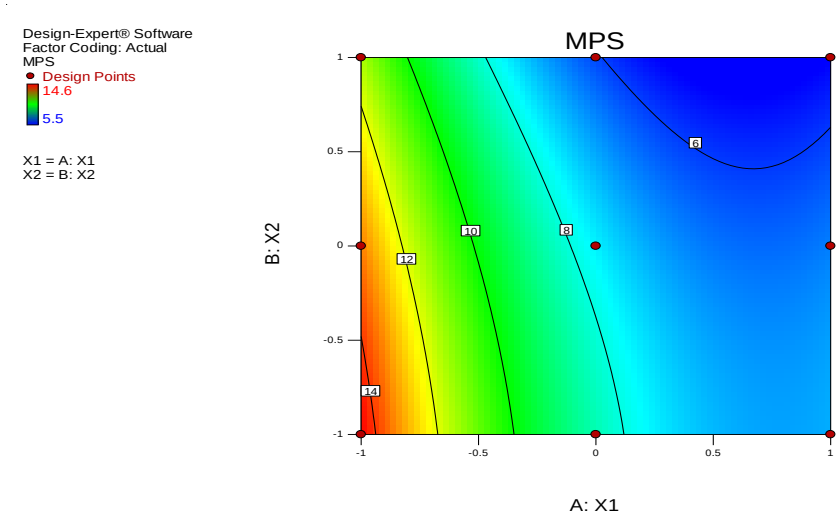

Fig. 3. Contour Plot of the response MPS.

The contour plots (Figure. 3) of the response MPS and response surface plots (Figure. 4) shows that the region of interest as marked in Blue colour (5.5). The said region could be obtained with an X1 value ranging from 0.2 to 1.0 and X2 value set in the range from 0.5 to +1.0.

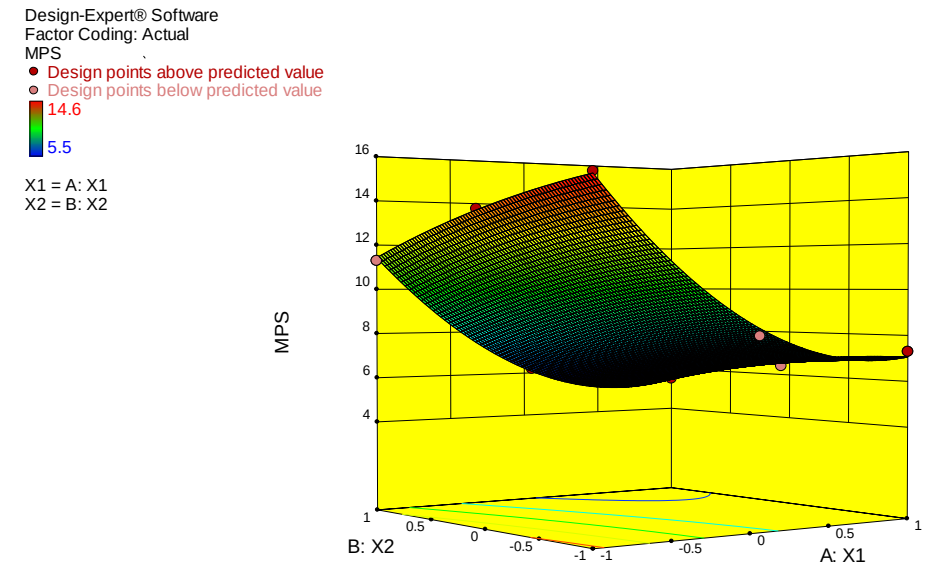

Fig. 4. Surface response plots of the response MPS.

Effect of Polymer concentration on MPS

From the factorial design regression equation both the terms X1 and X2 were negative, The minus sign of X1 and X2 implies a negative or inverse relationship in this case. Meaning as there is increase in concentration of Chitosan and HPMC there is a decrease in MPS. Similar to EE, the interactive term (X1X2) is non-significant. Quadratic term X2 is more powerful than the X1 term, meaning that is squared effect of HPMC concentration is more significant in decreasing the particle size.

3.3.2. Response: EE

A very high Model F-value of 2987.81 showed huge significance of the model, which was also supplemented with the information of much lower value of "Prob > F" which was much less than 0.05.

In this model all the model terms were significant except for the interactive model term X1X2. Hence this one model term needs to be removed from the

Sometimes including the insignificant model terms may be done if it supports hierarchy. However, in this case the model reduction would improve the model.

The "Pred R-Squared" value of 0.9976 was in reasonable agreement with the "Adj R-Squared" value of 0.9995 as the difference between the two values was less than 0.2.

The "Adeq Precision" was 158.610; a value high enough (more than 4) indicating adequacy of the model with acceptable signal. Hence, this model can be used to navigate the design space.

Table 5A. Regression Statistics.

Multiple R	**0.9999**
R Square	0.999799
Adjusted R Square	0.999465
Standard Error	0.259451
Observations	9

Table 5B. ANOVA output.

	df	**SS**	**MS**	**F**	**Significance F**
Regression	5	1005.62	201.1241	2987.813	9.66E-06
Residual	3	0.201944	0.067315		
Total	8	1005.822			

Table 5C. Regression output showing t-stat and P-value of individual model terms.

Model term	Coefficients	Standard Error	t Stat	P-value
Intercept	48.55556	0.193383	251.0845	1.39E-07 #
X1	9.8	0.10592	92.52229	2.78E-06 #
X2	7	0.10592	66.08735	7.63E-06 #
X12	7.166667	0.18346	39.06401	3.69E-05 #
X22	-4.03333	0.18346	-21.9849	0.000206 #
X1X2	-0.175	0.129725	-1.349	0.270123

significant (p-value is less than 0.05)

Table 5D. Residual output showing Observed, predicted and residual value.

Formulation Code	**Observed Y2 Value (%EE)**	**Predicted Y2 Value (%EE)**	**Residuals**
GEM-CH-MS1	34.6	34.71389	-0.11389
GEM-CH-MS2	46.2	45.92222	0.277778

GEM-CH-MS3	48.9	49.06389	-0.16389
GEM-CH-MS4	37.5	37.52222	-0.02222
GEM-CH-MS5	48.5	48.55556	-0.05556
GEM-CH-MS6	51.6	51.52222	0.077778
GEM-CH-MS7	54.8	54.66389	0.136111
GEM-CH-MS8	65.3	65.52222	-0.22222
GEM-CH-MS9	68.4	68.31389	0.086111

The polynomial regression analysis is used in predicting the significance of the mathematical models, With the help of multiple regression analysis in the factorial designs, the response surface methodology is useful in estimating the impact of independent variables on the responses (dependent variables).

The final equation for the response EE would be:

Y2 (EE)= $48.55556+9.8X1+7X2+7.166667X1^2-4.03333X2^2$ ---Equation 3

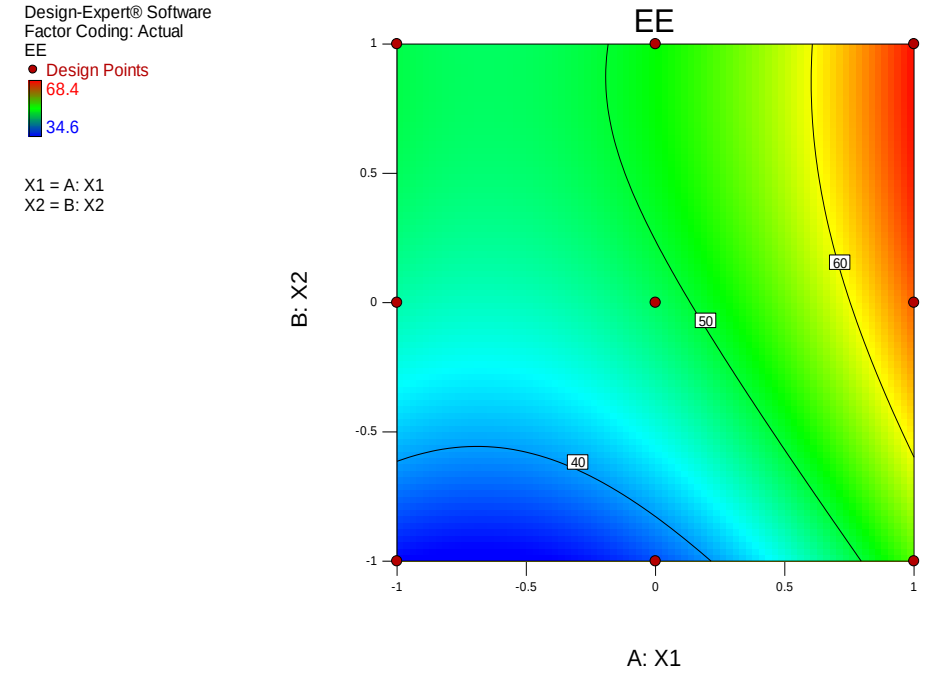

Fig. 5. Contour Plot of the response EE.

The contour plots (Figure 5) of the response EE and response surface plots (Figure 6) shows that the region of interest as marked in Red color (68.4%). The said region could be obtained with an X1 value set at the highest level of +1.0 and X2 value set in the range from 0.0 to +1.0.

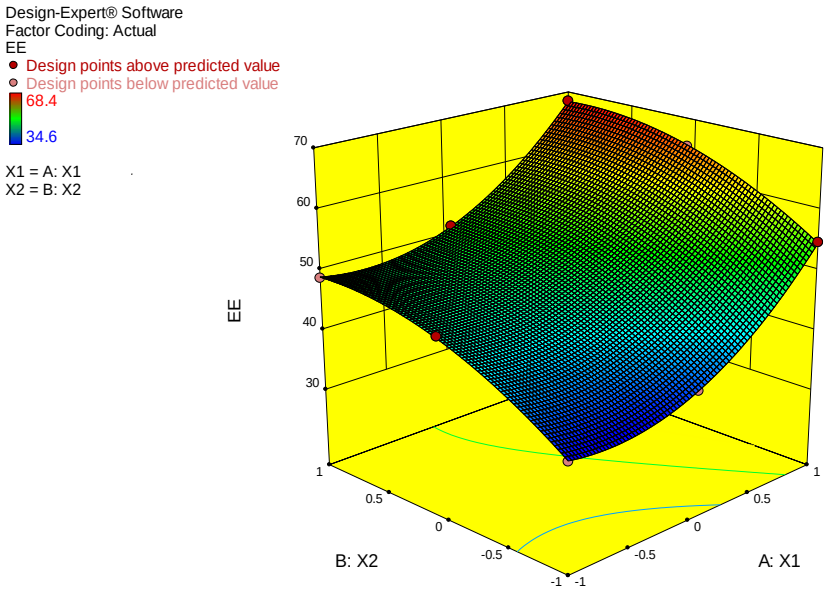

Fig. 6. Surface response plots of the response EE.

Effect of Polymer concentration on EE

It was concluded from the factorial design that X1 had a direct and a good quadratic effect on EE. A linear increase in X1 (Chitosan concentration) leads to a simultaneous increase in EE. However, for X2 (HPMC) there is a linear increase till the mid-point after that there is a plateau meaning any further increase in HPMC would not increase the EE of the drug. Individually as there is an increase in HPMC concentration from 0.1 to 0.3% w/v there is an increase in EE. However, increase from 0.1 to 0.2 is drastic but a lower increase is seen from 0.2 to 0.3.

There was a non-significant effect on interaction of X1 and X2, meaning the two polymers did not have an interactive effect to give the response.

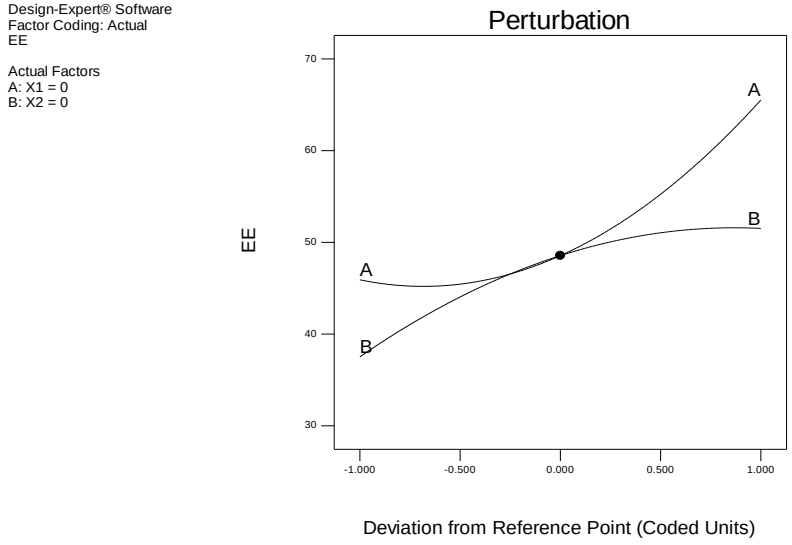

Fig. 7. Perturbation plot for EE.

In a design space the perturbation plot easily compares the effect of all the factors on a particular response. The *perturbation* plot shown in the Figure 7 gives an illustration to compare the cumulative effects of both the factors: A (X1) and B (X2). From the illustration it was clear that a constant increase in X1 (Chitosan concentration) leads to a simultaneous increase in EE. However, for X2 (HPMC) there is a linear increase till the mid-point after that there is a plateau meaning any further increase in HPMC would not increase the EE of the drug.

3.4. Particle size analysis

The Mean Particle Size (MPS) varied from 14.6 to 5.5 µm. The desired mean particle size was below 10 microns. It has been reported that the microparticles of sizes 5-7 microns gets better accumulated in the lungs which may be beneficial for lung cancer [17].

Table 6. Particle size of all formulations.

Formulation Code	**MPS (µm)**	**±SD**
GEM-CH-MS1	14.6	2.3
GEM-CH-MS2	13.4	1.8
GEM-CH-MS3	11.3	1.3
GEM-CH-MS4	8.1	0.9
GEM-CH-MS5	7.8	2.6
GEM-CH-MS6	6.2	1.6
GEM-CH-MS7	7.3	2.4
GEM-CH-MS8	6.4	1.1
GEM-CH-MS9	5.5	0.3

3.5. Evaluation of drug Loading capacity and Entrapment Efficiency (EE).

GEM being a hydrophilic drug has issues of lower drug loading in hydrophobic polymers. Hence the choice of polymers was hydrophilic. As the drug loading was decreased from 19.2% to 6.4% there was a parallel increase in EE from 34 to 68%. This is because the drug got greater chances of entrapping in the more available polymer.

Table 7. Drug loading Capacity and EE.

Batch No.	Theoretical DL(%)	EE (%)
GEM-CH-MS1	19.2	34.6
GEM-CH-MS2	18.5	46.2
GEM-CH-MS3	17.8	48.9
GEM-CH-MS4	9.8	37.5
GEM-CH-MS5	9.6	48.5
GEM-CH-MS6	9.4	51.6
GEM-CH-MS7	6.5	54.8
GEM-CH-MS8	6.4	65.3
GEM-CH-MS9	6.4	68.4

3.6. SEM

The SEM microphotograph shown in Fig. 8 clearly shows many discrete spherical particles having uniform size. The bar graph on the image represents 10 microns which means that the microspheres seen as white discrete particles against the dark background seen in the microphotograph are in the size range of 4 to 6 microns.

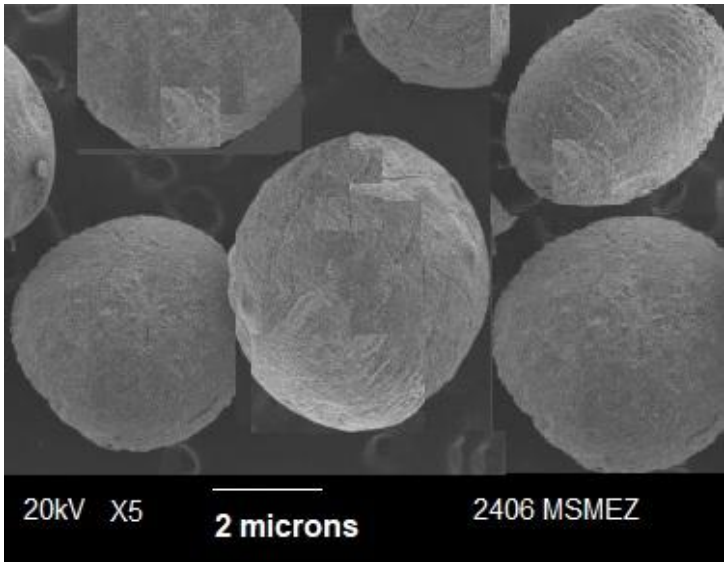

Fig. 8. SEM image of GEM_CH_MS.

3.7. Production Yield

It was seen that as there was an increase in the atomizing air flow there was an increase in the production yield. This was probably due to the fact that as the atomizing airflow increased the particles that were obtained were less sticky (Chitosan-HPMC combination tends to make the particles sticky).

Feed flow is one parameter for impacting the production yield. As the feed flow increased the particles were less dry and stickier in nature. It was noted that as the feed flow was increased beyond 6 mL/min there was a decrease in the production yield. An interesting result is that the yield did not depend on the total solid content in this case. The optimized total solid content in our case was ranging from 300 mg to 800 mg per 100mL.Production yield in spray drying can be an important constrain because not all the powder that is dried can be successfully recovered. It is because of the fact that not all the powder that is produced will easily fall on its own into the cyclone. Stickiness is one factor reducing the production yield. Collecting all the particles required a gentle tapping to be recovered at the collecting vessel. The average production yield for the batches was 75.8±5.3%. As the preliminary functions were set and were similar to the all batches there was no major difference in the individual batches.

3.7. In vitro drug release study.

The in vitro release of GEM from MS was studied using the dialysis method. The drug was released from the dialysis bags and dissolved into the release medium (PBS, pH 7.4). Fig. 6 shows drug release data of plain drug (GEM), GEM_CH_MS9 and GEM_CH_MS6. The plain drug GEM being a water-soluble drug did not take much time to be released across the membrane and was completely released in 2 hours. Whereas, from both the microsphere formulations the drug release was sustained for a comparable amount of time. This was due to sustaining capacity of the polymers used.

The drug releases of all nine batches were studied. GEM_CH_MS9, GEM_CH_MS6, GEM_CH_MS3 these three batches were selected as per the sustained drug release. The GEM_CH_MS9 batch showed 98.8% drug release in 48 hrs. and typically showed sustained drug release . Hence batch GEM_CH_MS9 was considered as an optimized batch. According to drug release pattern cumulative % drug release of GEM_CH_MS9, GEM_CH_MS6, and GEM (plain drug) was studied by plotting graph (Fig. 6).

Table 8. Cumulative % drug release of Optimized formulations.

TIME (H)	GEM		GEM-CH-MS9		GEM-CH-MS6		GEM-CH-MS3	
	CDR	**±SD**	**CDR**	**±SD**	**CDR**	**±SD**	**CDR**	**±SD**
0	0	0	0	0	0	0	0	0
0.5	35.7	3	9	1.5	21	3.5	22.0	3.5
1	64	3	19	2	35	2	37.1	4.2
2	99.1	2	32	3.5	51	2.5	62.2	5.2
4			43	2	62	3	81.7	3.6
6			57	2.5	78	4	98.2	2.8
12			72	3	88	4.5		
24			88	4	99.7	1.5		
36			96	4.5				
48			98.9	1.5				

CDR: Cumulative % drug released ; ±SD: ± standard deviation

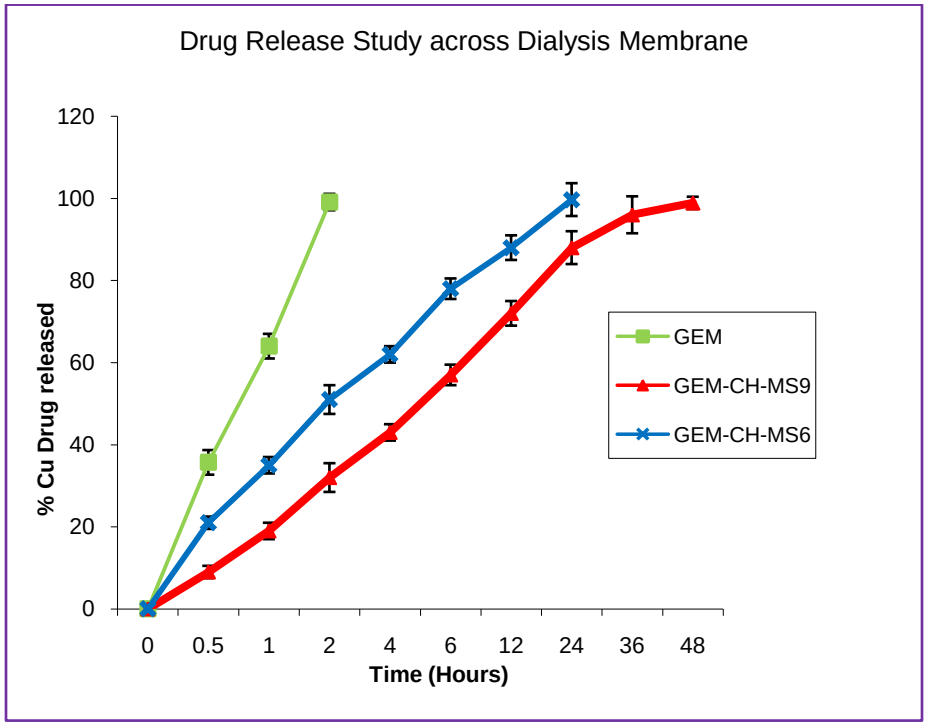

Fig. 9. Drug release study across dialysis membrane.

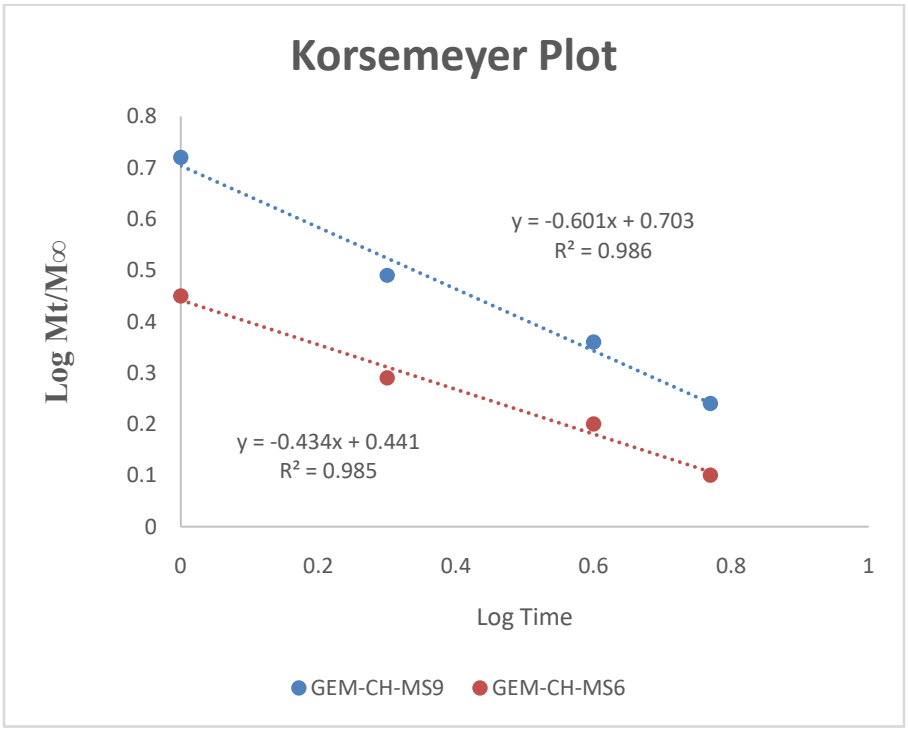

Fig. 10. Korsemeyer Plot.

Drug release Kinetics

The drug release data obtained from the in vitro drug dissolution profiles was fitted to different mathematical models as shown in Table 7. These models help in interpretation of the drug release mechanism. In due course of time these comprehensions can be useful as a tool to understand and modulate the drug release rate as per the therapeutic needs.

Different mathematical models have different coefficients and R^2 values which can be estimated to predict the release profiles. It can be seen that the highest R^2 values were seen for Zero-order plot indicating that the drug release best fitted to this model.

Table 9. Release coefficients for different mathematical models.

Mathematical Model	Formulation Code	Linearity Equation	R^2
Zero-order Plot	GEM-CH-MS6	y = 13.963x - 8.4964	0.9915
	GEM-CH-MS9	y = 11.988x - 14.447	0.9900
First Order Plot	GEM-CH-MS6	y = 20.547x + 13.5	0.9056
	GEM-CH-MS9	y = 14.985x + 8.3032	0.9401
Higuchi Plot	GEM-CH-MS6	y = 20.547x + 13.5	0.9056
	GEM-CH-MS9	y = 14.985x + 8.3032	0.9401
Korsemeyer Plot	GEM-CH-MS6	y = -0.4345x + 0.4414	0.9859
	GEM-CH-MS9	y = -0.6014x + 0.7036	0.9868

As per the need of the model, only 60% of the drug release data was fitted to the Korsemeyer Plot. With the Korsemeyer plot the diffusion exponent 'n value' describes the diffusion characteristics of the drug released from the polymeric system. It was seen that GEM-CH-MS6 had the n value of 0.43 which shows that the drug release was through Fickian diffusion. Whereas the GEM-CH-MS9 had n value more than 0.5 (0.601) indicating a perfect non-Fickian diffusion and a release closer to zero-order drug release.If the n value is between $0.45 < n < 0.89$ it is said to follow the Anomalous release in which there is diffusion of the drug with the simultaneous relaxation of the polymeric matrix [19]. In the GEM-CH-MS9 formulation the n value was 0.601 indicating a non-Fickian release which is also supported the release to be closer to zero-order. Such anomalous transport is known to have the drug release due to both the swelling of the polymer matrix and diffusion of the drug [20, 21].

4. CONCLUSION

Gemcitabine HCl (GEM) is an anticancer drug used in treatment of many types of cancer including lung, colon, pancreas, breast and ovarian cancers. Presently the drug is available for parenteral administration but is rapidly metabolized as it is widely deaminated by cytidine deaminase in blood and other organs. This causes the dosage frequency to be increased to get

the therapeutic concentration for a longer duration which in-turn increases the total amount of GEM to be administered during the cancer therapy. Hence there is a need to develop a delivery system which not only increases its stability but also has a sustained release of GEM for a longer duration.

Another reason for the drug to be incorporated into a novel drug delivery system is that it is low-molecular weight water soluble drug which does not have easy access to cells via cell membranes. It has been reported that novel drug delivery systems like nanoparticles or microparticles would not only provide efficient delivery of the anticancer drug like GEM to the cancer cells but also protect the drug from rapid metabolization.

Hence injectable microspheres of Gemcitabine were prepared to achieve the target specificity and sustain release action. Microsphere formulations prepared by using chitosan and HPMC polymers, were prepared by using Spray Drying method. The significant factors selected were concentration of chitosan and HPMC polymers. The dependent variables selected such as %entrapment efficiency, and mean particle size while independent variables are chitosan and HPMC. It was concluded from the factorial design that X1 had a direct and a good quadratic effect on EE. A linear increase in X1 (Chitosan concentration) leads to a simultaneous increase in EE. However, for X2 (HPMC) there is a linear increase till the mid-point after that there is a plateau meaning any further increase in HPMC would not increase the EE of the drug. Individually as there is an increase in HPMC concentration from 0.1 to 0.3% w/v there is an increase in EE. However, increase from 0.1 to 0.2 is drastic but a lower increase is seen from 0.2 to 0.3.

There was a non-significant effect on interaction of X1 and X2, meaning the two polymers did not have an interactive effect to give the response.

The GEM_CH_MS9 batch showed 98.8% drug release in 48 hrs. and typically showed sustained drug release . Hence, batch GEM_CH_MS9 was considered as an optimized batch.

5. ACKNOWLEDGEMENT

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6. ETHICAL ISSUES

Not applicable

7. CONFLICT OF INTEREST

None Declared.

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