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FORMULATION AND *IN-VITRO* EVALUATION OF IRINOTECAN LOADED MUCOADHESIVE MICROSPHERES MADE OF CHITOSAN-ALGINATE MIXTURE BY USING IONOTROPIC GELATION TECHNIQUE

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ABSTRACT

In this study, we prepared irinotecan microparticles using Chitosan and alginate as a polyelectrolyte complex by ionotropic gelation method for sustained release of the drug. Compatibility studies of drug with various excipients were performed by FT-IR and DSC studies between 1:1 physical mixtures of drug and excipients. The developed microspheres were evaluated for particle size, entrapment efficiency, *in-vitro* drug release and surface morphology by scanning electron microscopy (SEM). The results of FT-IR and DSC studies confirmed the compatibility of the drug with excipients. The average particle size of formulations was found to be 410±12.54µm, the entrapment efficiency of drug in formulation was found to be 84.56±2.150%, and the *in-vitro* drug release studies of F5 formation in phosphate buffer pH 6.8 showed 87.21±2.514 % release of drug at the end of 8 h. Thus the study concludes development of sustained release micro particular delivery of irinotecan using simple inotropic gelation method.

Keywords: Irinotecan, Sodium alginate, Chitosan, Microsphere, Ionotropic gelation technique, *In-vitro* release.

INTRODUCTION

Novel drug delivery systems present an opportunity to overcome many challenges associate with anticancer drug therapy. A lot of drugs bear some significant draw backs such as low bioavailability, poor permeability, short half-life and undesirable adverse effects. Microsphere formulation is an important part of these particulate drug delivery system on account of their small particle size which ranges from 1-1000µm.[1] Microspheres are characteristically free flowing powders consisting of protein or synthetic polymers which are biodegradable in nature.[2] Irinotecan is a synthetic analogue of the natural alkaloid, camptothecin. It is achemotherapeutic agent, a topoisomerase 1 inhibitor and mainly used as a drug of choice in colon cancer. It is a prodrug which is decarboxylated in liver to the active metabolite. Cholinergic effects are produced in some patients because it inhibits AchE. These effects can be suppressed by prior atropinisation. Irinotecan is indicated in metastatic advanced colorectal carcinoma, cancer lungs/cervix/ovary etc.[3] The other adverse effects are dose limiting toxicity in diarrhoea, neutropenia, thrombocytopenia, haemorrhage etc. Irinotecan is chemically 4 (s) - 4, 11 – diethyl -3 ,4, 12, 14- tetrahydro - 4 hydroxy -3, 14 - dioxo-1H pyrano (3,4:6,7] indolizino[1, 2b] quinolone-9-ylester, [1,4-bipiperidine] 1-carboxylic acid, monohydro-chloride. After intravenous infusion of irinotecan in humans, irinotecan plasma concentrations decline in a multiexponential manner, with a mean terminal elimination half-life of about 6 to 12 hours. The mean terminal elimination half-life of the active metabolite SN-38 is about 10 to 20 hours. The half-life of the lactone (active) forms of irinotecan and SN-38 are similar to those of

total irinotecan and SN-38, as the lactone and hydroxy acid forms are in equilibrium. Over the recommended dose range of 50 to 350 mg/m^2, the AUC of irinotecan increases linearly with dose; the AUC of SN-38 increases less than proportionally with dose. SN-38 is highly bound to human plasma proteins (approximately 95% bound). The plasma protein to which irinotecan and SN-38 predominantly binds is albumin.[4]

In this study, we prepared irinotecan loaded Chitosan-alginate microspheres to improve the therapeutic effect of the drug. The usefulness of Irinotecan loaded chitosan-alginate microspheres was evaluated by measuring its particle size and surface morphology, loading efficiency, *in-vitro* drug release to characterize the system.

MATERIALS AND METHOD

Irinotecan was obtained as a gift sample from EMCURE pharmaceuticals limited, chitosan was obtained from Himedia, Mumbai, sodium alginate was obtained from Balaji Drugs, Mumbai, calcium chloride, potassium dihydrogen phosphate and sodium dihydrogen phosphate were obtained from MERC specialist Pvt. Ltd., and all other chemicals used were of analytical grade.

Drug-Excipient Compatibility Study

The drug compatibility study of the physical mixture was performed by FT-IR spectroscopy and Differential Scanning Calorimetry (DSC).

FT-IR Spectroscopy

Drug compatibility with other excipients was studied using FT-IR spectrophotometer (Bruker 10059736, model alpha, Germany). Each drug-excipient samples were scanned from 400 cm^{-1} to 4000 cm^{-1} to evaluate the physical stability and compatibility of the drugs with the excipients. The spectra obtained were compared with the pure drug spectra.[5,6]

Differential Scanning Calorimetry (DSC) Study

DSC thermograms of drug and drug-excipients mixtures were weighed directly in the pierced DSC aluminium pan and scanned in the temperature range of 20–300°C under an atmosphere of dry nitrogen. Heating rate of 10ºC/min was used and thermograms obtained were observed for any interaction.[7]

Preparation of Microspheres

Chitosan-alginate microspheres were prepared by ionotropic gelation technique as per the composition shown in Table 1. Chitosan was dissolved in aqueous solution of acetic acid and sodium alginate was dissolved in distilled water. Both these solutions were mixed under magnetic stirrer. The required amount of drug was then added to the polymeric solution. Then this solution was kept under homogenizer for 15 minutes. Calcium chloride solution was also prepared by dissolving calcium chloride in distilled water. Calcium chloride solution was added drop wise to Chitosan-alginate solution of irinotecan under a magnetic stirrer. The stirring was continued for about 20 minutes. Microspheres formed immediately and were left into the original solution for 1 hour. Then these were filtered, washed with alcohol and dried at room temperature.[8]

Particle Size Analysis

Particle size of the prepared micro particles was determined using optical microscopy technique. A microscope is fitted with a stage micrometer and desired magnification was adjusted to calculate the particle size of the prepared micro particles.[9]

Drug Entrapment Efficiency

Microspheres equivalent to 10 mg of pure drug were crushed and then dissolved in distilled water with the help of ultrasonic stirrer for 3 hours and filtered and analyzed spectrophotometrically using UV spectrophotometer. Entrapment efficiency was calculated as follows.[10]

$$\% \text{ Entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

In-Vitro Drug Release Study

The *in-vitro* release study of the formulation was carried out by using dissolution apparatus of rotating paddle type. The dissolution fluid for this purpose was phosphate buffer p^H 6.8. The samples were placed in each compartment containing the dissolution medium which was maintained at 37º C and was stirred at 100 rpm.

After certain time intervals, samples were drawn out and fresh fluid was added in order to maintain the sink condition. Finally the samples were filtered and dilutions ifnecessary was made and the amount of drug was measured spectrophotometrically against the specified blank solution.[11]

Scanning Electron Microscopy

The surface morphology and size distribution of prepared micro particles were determined by using Scanning Electron Microscopy (JSM-848, ZEISS, Germany) under high resolution electron microscope with an acceleration voltage of 80 KV. For the observation of morphology a drop of the sample was transferred into the copper mesh grids. After the sample was adsorbed (about 15-20 min), the staining dye (Potassium Phosphotungstate) was dripped onto the film. The staining time was about 1-2 min. After drying the copper mesh grids, the surface topography was then visualized using a Scanning Electron Microscope.[12]

In-Vitro Mucoadhesion Test

A 1cm×1cm piece of goat intestine is tied onto a glass slide (3 inch×1 inch) using a thread. Microsphere was spread on to the wet, rinsed, tissue specimen and the prepared slide was hung onto one of the groves of the USP tablet disintegrating test apparatus. The disintegrating test apparatus is operated such that the tissue specimen regular up and down movements in a beaker containing the stimulated gastric fluid. At the end of every time interval, the number of microsphere still adhering onto the tissue was counted and there adhesive strength is determined.[13]

Drug Release Kinetics

The *in vitro* release data obtained from optimized formulation (F5) in the dissolution medium Phosphate buffer pH 6.8 was fitted to various kinetic models. Data obtained from drug release studies was analyzed according to the equations given in the given Table 3.[14]

RESULTS AND DISCUSSION

FT-IR Spectroscopy

Drug-excipients compatibility studies were carried out using FT-IR. The spectra of pure drug irinotecan, chitosan and sodium alginate and their physical mixtures were obtained from FT-IR spectroscopy at wavelength from 400 to 4000 cm^{-1}. In Figure 1 characteristic peaks showed at 1746 $cm^{-1,}$ 1450 cm^{-1}, 1231 cm^{-1}, 744 cm^{-1} due to the presence of C=O, C-H, C-O, C-Cl functional groups. In Figure 2, characteristic peaks showed at 1404 cm^{-1}, 1297 cm^{-1}, 2882 cm^{-1} due to presence of C-H, C-C, and C-H functional groups. In Figure 3, characteristic peaks showed at 1608 cm^{-1}, 1433 cm^{-1}, 953 cm^{-1} and 1746 cm^{-1} due to presence of C=C, C-H, C-C, C=O functional groups respectively. The results showed that all the drug peaks were retained in the physical mixture of drug-excipients. Thus it revealed the compatibility of drug with the excipients used in the formulations.

DSC Study

DSC scans of pure irinotecan showed an endothermic peak at 271° C, corresponding to the melting point of the compound. Physical mixture of the drug and polymer in a 1:1 ratio, also exhibited the endothermic peak associated with irinotecan. It suggests that there is no any interaction of the drug along with the polymers used in preparing the formulations.

Physico-Chemical Characteristics

The Physico-chemical characteristics of irinotecan loaded chitosan-alginate microparticles were showed in Table 2. The results showed that the particle size of the prepared microparticles varied from 354±27 to 694±10 µm. The maximum entrapment efficiency 84.56±2.150 % was observed at 1:4 (chitosan and sodium alginate ratio) in formulation F5. The maximum percentage of drug loading was also observed at 1:4 (chitosan and sodium alginate ratio) in formulation F5.

In-Vitro Drug Release Study

In-vitro release study of irinotecan from various formulations was conducted for 8 hours by using USP paddle type dissolution test apparatus. Cumulative % drug release was plotted against time and was showed in figure below. All the

formulations showed more than 30% release in the first 1 hour. Formulation F5 was able to sustain the release of drug for 8 hours with 87.22%. Overall, *in-vitro* drug release study showed sustaining of drug release for long periods with increase in concentration of Chitosan-Sodium alginate.

SEM Analysis

The prepared microparticles were subjected for SEM analysis in order to determine the surface morphology. Scanning Electron Microscopy is a prominent tool used for determining the surface topography. From the SEM analysis it was found that the obtained micro particles exhibit good spherical nature.

Mucoadhesion Study by Texture Analyzer

The percentage mucoadhesion was found to be good in almost all the batches of the formulated microspheres. The in vitro Mucoadhesion test gives satisfactory result and from the result the promising mucoadhesive strength of both sodium alginate and chitosan polymers can be established.

Drug Release Kinetic Studies

The *in-vitro* release data obtained from optimized formulation (F5) was fitted to various kinetic models. The release rate kinetic models are shown in following figures. The drug release kinetic study was studied by using different models like Zero order, First order, Higuchi models, Korsmeyer models. The regression coefficient was found out in each model and the results were tabulated in following table. The value of resulting regression coefficient for each model is calculated. The data was also fitted into Korsemeyer-Peppas model in order to obtain the 'n' value to describe the mechanism of drug release. The 'n' value of 0.6786 in Phosphate buffer pH 6.8 indicates that the drug release follows anomalous (non-fickian) diffusion mechanism which signifies that the drug release is both diffusion-controlled and swelling-controlled. From these results, it can be revealed that the release of Irinotecan from the microparticles follows first order and diffusion and swelling mechanism in case of phosphate buffer pH 6.8. The best formulation F5 showed high entrapment efficiency (84.56 ± 2.150%), particle size (694 ± 17 µm) and drug release (87.22 ± 3.786 %) over 8 hours. Hence, the results of the study are expected to improve the therapeutic effect of the drug with lesser dose related side effects. FT-IR and DSC studies revealed absence of incompatibility of drug with excipients. Different formulations have been formulated by changing drug and polymer ratio and performed their evaluation parameters.

CONCLUSION

In conclusion, irinotecan microspheres were prepared successfully using blends of Chitosan-sodium alginate by ionotropic gelation method for sustain release of the drug. The developed microspheres were evaluated for particle size, entrapment efficiency, *in-vitro* drug release and surface morphology by scanning electron microscopy (SEM). Thus the study concludes development of sustained release micro particular delivery of irinotecan using simple ionotropic gelation method.

ACKNOWLEDGEMENT

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Table 1: Composition of formulation batches of microspheres

F. Code	Drug (mg)	Sodium alginate (%w/v)	Chitosan (%w/v)	Calcium chloride (%w/v)	Curing time (min)
F1	50	2	-	3	30
F2	50	2	0.5	3	30
F3	50	2	1	3	30
F4	50	2	1.5	3	30
F5	50	2	1.5	3	45
F6	50	2	1.5	3	15

Table 2: Physico-chemical characterization of Irinotecan loaded chitosan-alginate micro particles

F. Code	Particle size (µm ±SD)	% Entrapment efficiency (% ± SD)	% Drug loading (%± SD)	% Yield (% ± SD)
F1	354 ± 27	61.34 ± 1.894	26.66 ± 2.56	86.62 ± 2.894
F2	367 ± 25	70.74 ± 2.031	27.33 ± 2.89	83.65 ± 3.452
F3	456 ± 13	74.03 ± 2.150	25.13 ± 1.67	72.21 ± 3.675
F4	589 ± 10	79.44 ± 3.010	26.63 ± 2.21	88.34 ± 2.480
F5	694 ± 17	84.56 ± 2.150	33.33 ± 2.53	91.07 ± 2.083
F6	389 ± 17	69.32 ± 2.093	23.33 ± 3.09	68.56 ± 2.995

Table 3: Different models of release mechanism

Model	Equation
Zero-order	$Q_t = Q_o - K_o t$
First-order	$\ln Q_t = \ln Q_o - K_1 t$
Higuchi matrix	$Q_t = K_H t^{1/2}$
Korsmeyer-Peppas	$\text{Log}(Q_t/Q_o) = \text{Log}K + n\text{Log}t$

Where,

Q_t: Cumulative amount of drug released at any specific time (t).

Q_o: Amount of drug remaining in the formulation

K_o: Rate constant of Zero-order

K_1: Rate constant of First-order

K_H: Rate constant of Higuchi-matrix model

K: Release rate constant which considers structural and geometric characteristics of the nanoparticles.

n: The diffusion exponent; indicative of the mechanism of drug release. The 'n' value could be used to characterize different release mechanisms as mentioned in Table 3.

Table 4: % of Mucoadhesion of formulated batches

Formulation Code	Absolute force (gm)	Positive Force (gm.s)	Separation Distance (mm)	% of Mucoadhesion
F1	177.3	12.1	3.4	52
F2	124.4	25.1	5.6	61
F3	127.3	14.1	3.4	74
F4	188	31.3	3.9	81
F5	100.8	10.3	1.7	86
F6	141.1	20.1	6.2	72

Table 5: Data for Release kinetic study of optimized formulation in Phosphate buffer pH 6.8

Time (min)	Square root of time (hr)	Log time (hr)	% Cumulative Drug Release(Q_t) (%± SD)*	Log Q_t ± SD *	Log Q_o ± SD *
30	5.4	1.477	42.22±0.45	1.62±0.56	2.29 ± 0.53
60	7.74	1.77	47.74±0.39	1.67±0.43	2.17 ± 0.45
120	10.95	2.07	53.74±0.37	1.73±0.39	2.11 ± 0.34
180	13.41	2.25	59.73±0.48	1.77±0.56	1.99 ± 0.63
240	15.49	2.38	66.97±0.54	1.82±0.67	1.95 ± 0.32
300	17.32	2.47	72.26±0.36	1.85±0.39	1.92 ± 0.30
360	18.97	2.55	77.15±0.42	1.88±0.45	1.89 ± 0.42
420	20.49	2.623	83.27±0.63	1.92±0.53	1.87 ± 0.65
480	21.90	2.68	87.22±0.19	1.94±0.28	1.84 ± 0.27

Table 6: The release rate constant and correlation coefficient value of different kinetic models of in-vitro release data of the formulations

Formulation code	Zero order		First order		Higuchi model		Korsmeyer and Peppas model	
	K_0	R_0	K_F	R_F	K_H	R_H	K_P	R_P
F1	0.0759	0.9942	0.0004	0.9804	1.6626	0.9827	0.1363	0.6783
F2	0.0646	0.9503	0.0004	0.9212	1.828	0.9828	0.1413	0.6612
F3	0.0759	0.9669	0.0005	0.9038	2.1421	0.9948	0.1854	0.7577
F4	0.0764	0.9788	0.0005	0.9538	2.1365	0.9905	0.1504	0.6519
F5	0.0991	0.9937	0.0007	0.9626	2.7487	0.9893	0.2184	0.6985
F6	0.0699	0.9828	0.0005	0.9774	1.9465	0.9853	0.1348	0.6188

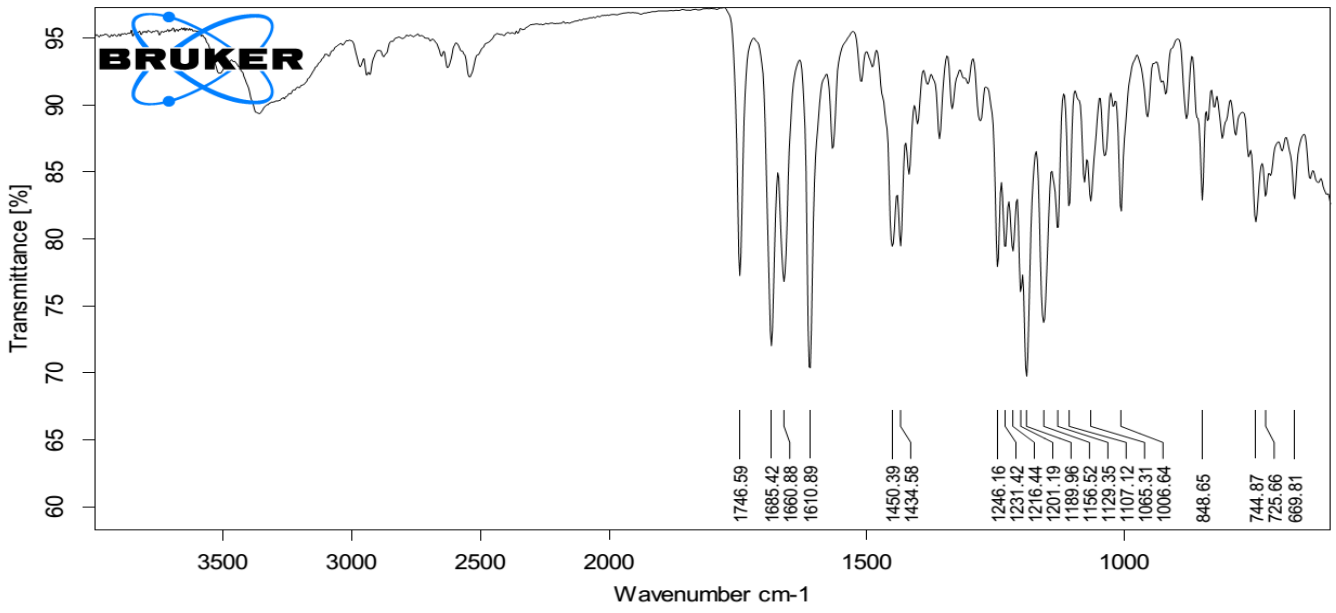

Figure 1: FT-IR spectra of Irinotecan

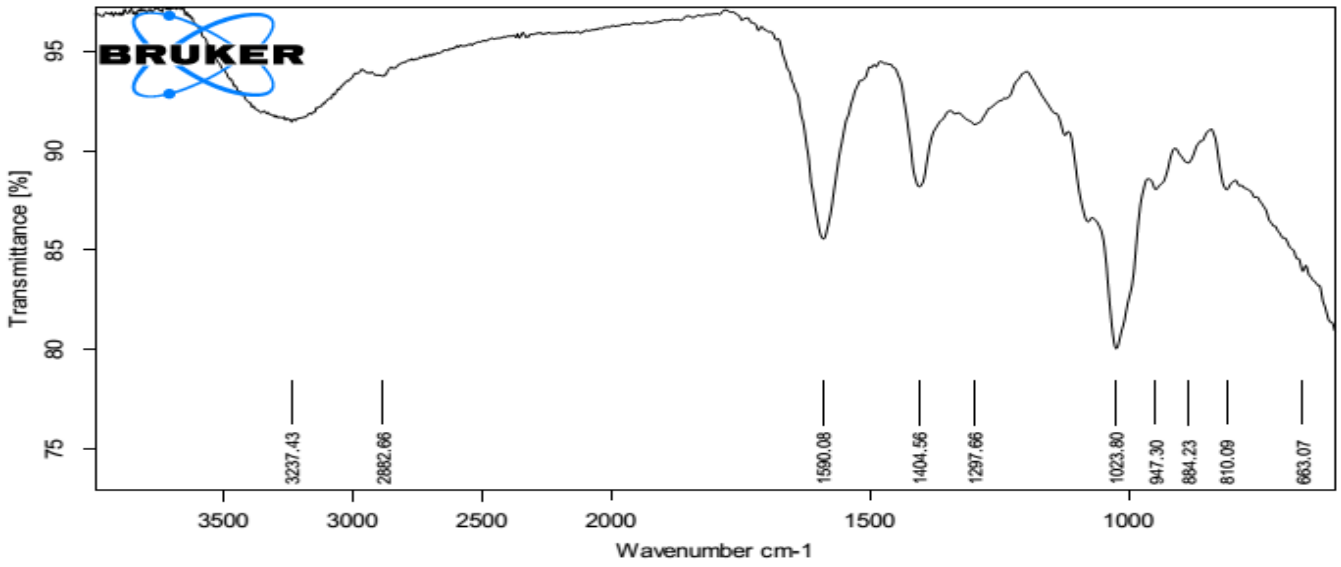

Figure 2: FT-IR spectra of Chitosan-sodium alginate mixture

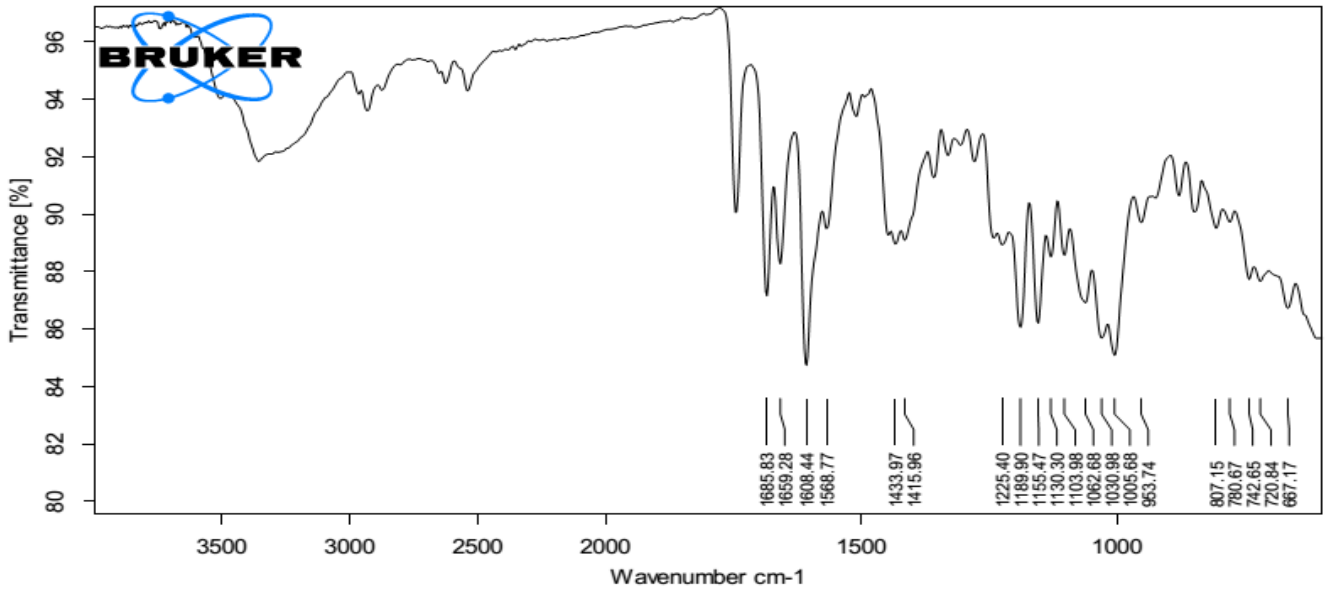

Figure 3: FT-IR spectra of Irinotecan + Chitosan-alginate mixture

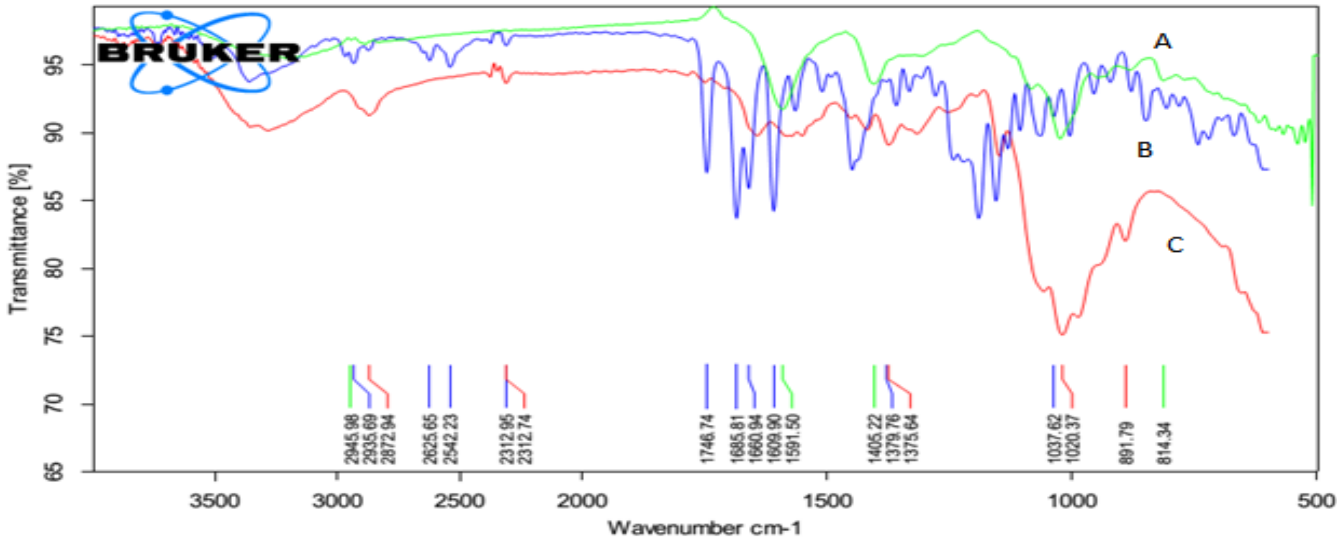

Figure 4: Comparative FT-IR spectra of Irinotecan (A), Sodium alginate-Chitosan Mixture (B) and irinotecan + Sodium alginate-Chitosan mixture (C)

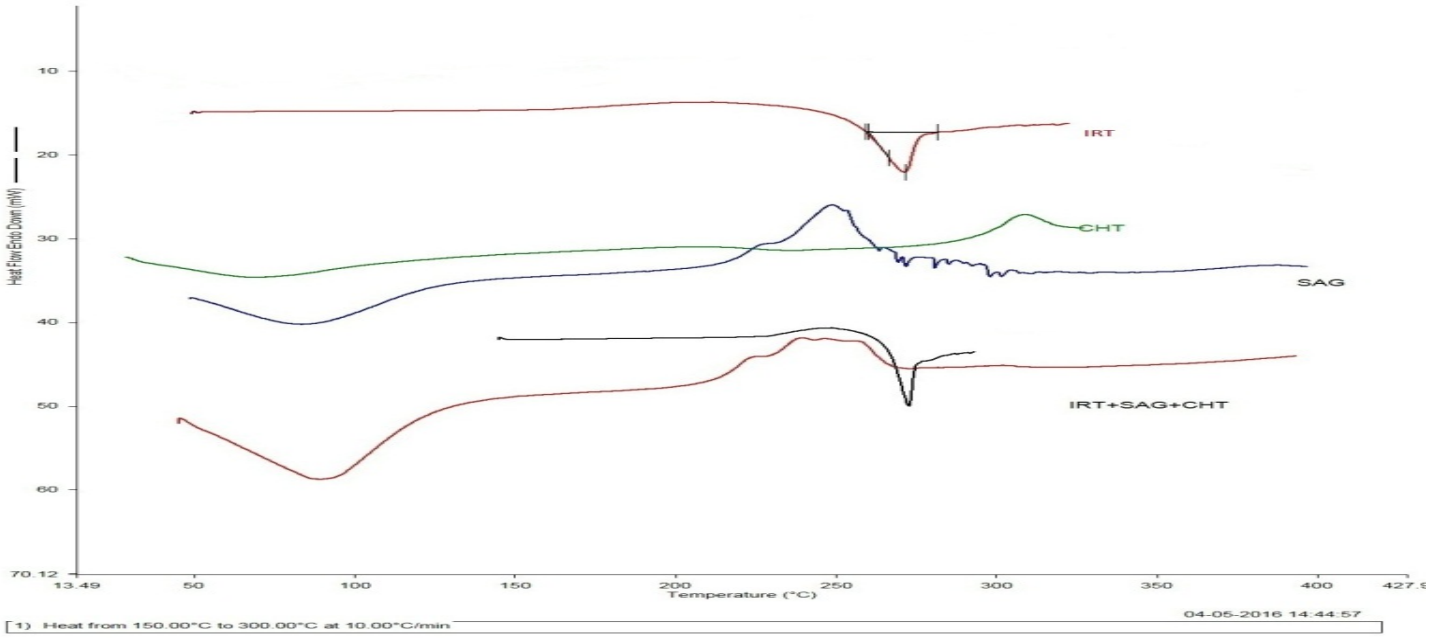

Figure 5: DSC study of pure Irinotecan, Chitosan - Sodium alginate mixture and Irinotecan + Chitosan-Sodium alginate mixture

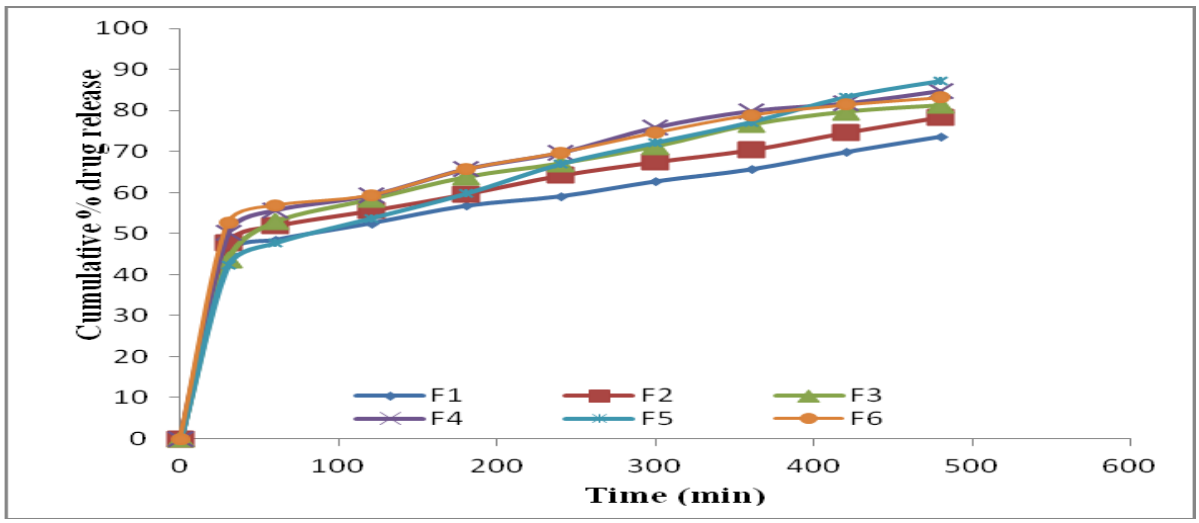

Figure 6: % Cumulative drug release of Irinotecan

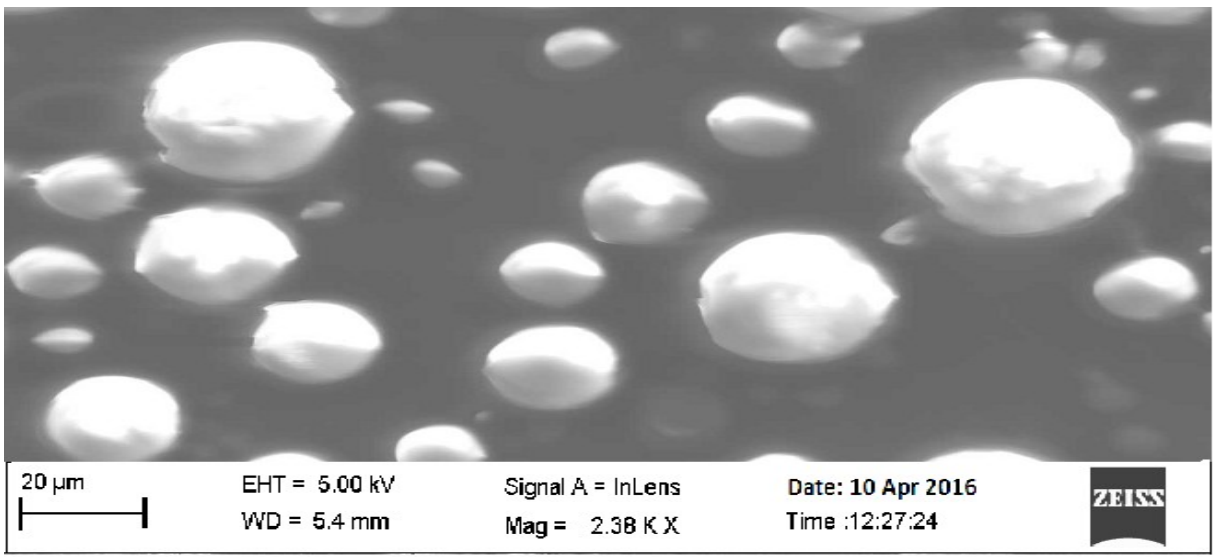

Figure 7: SEM image of drug loaded microparticles

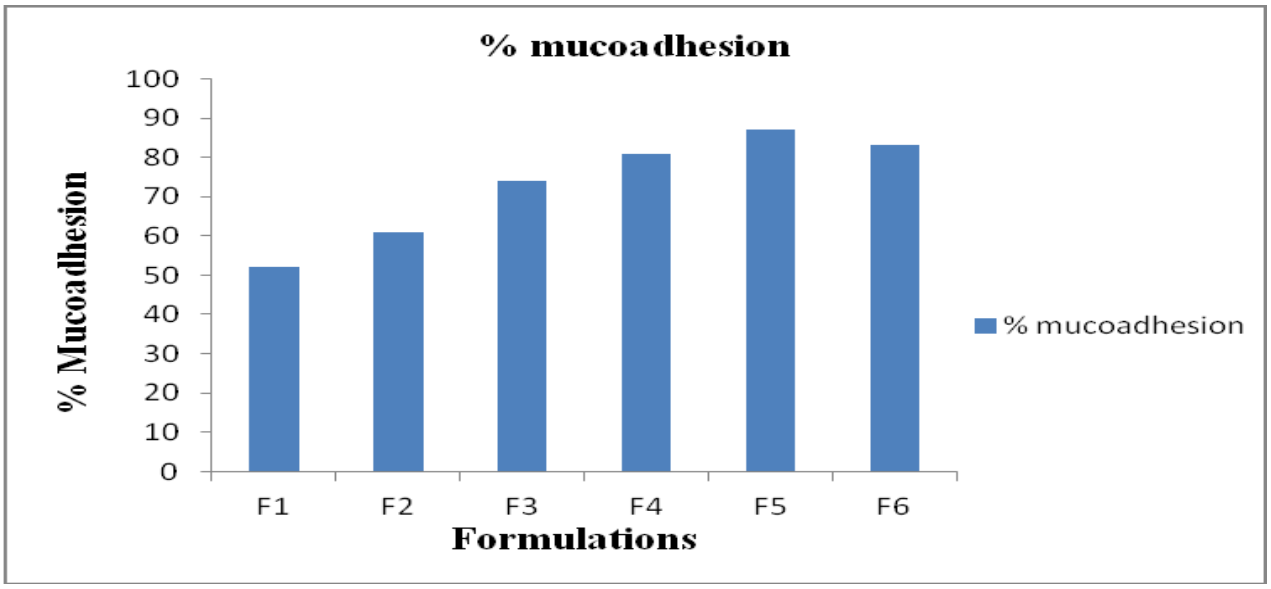

Figure 8: Percentage of Mucoadhesion of different formulated batches

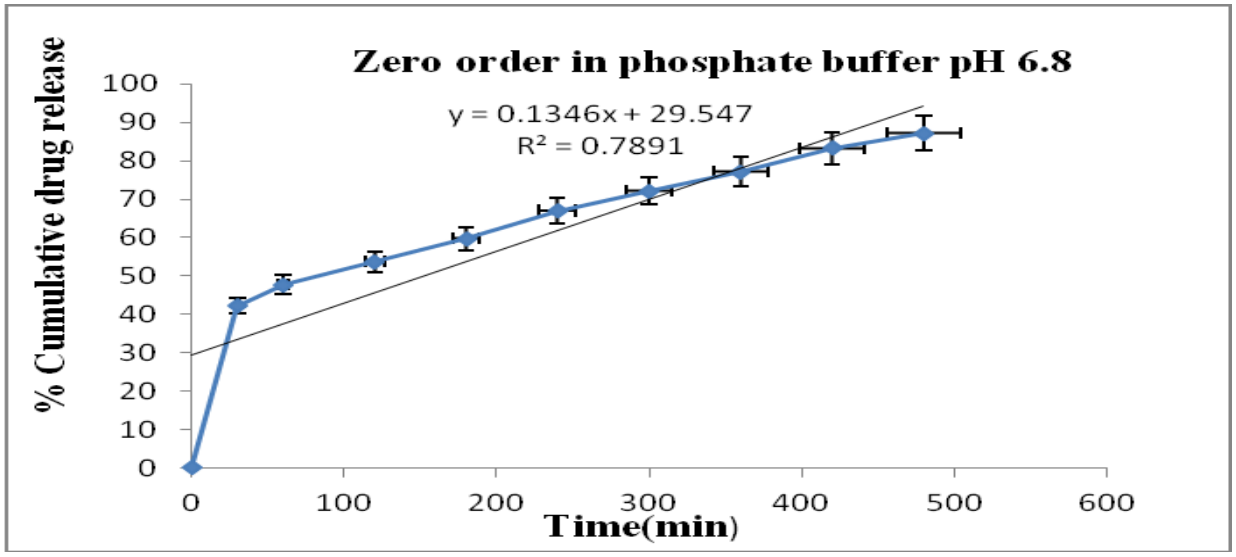

Figure 9: Zero order release kinetic study of optimized formulation in phosphate buffer pH 6.8

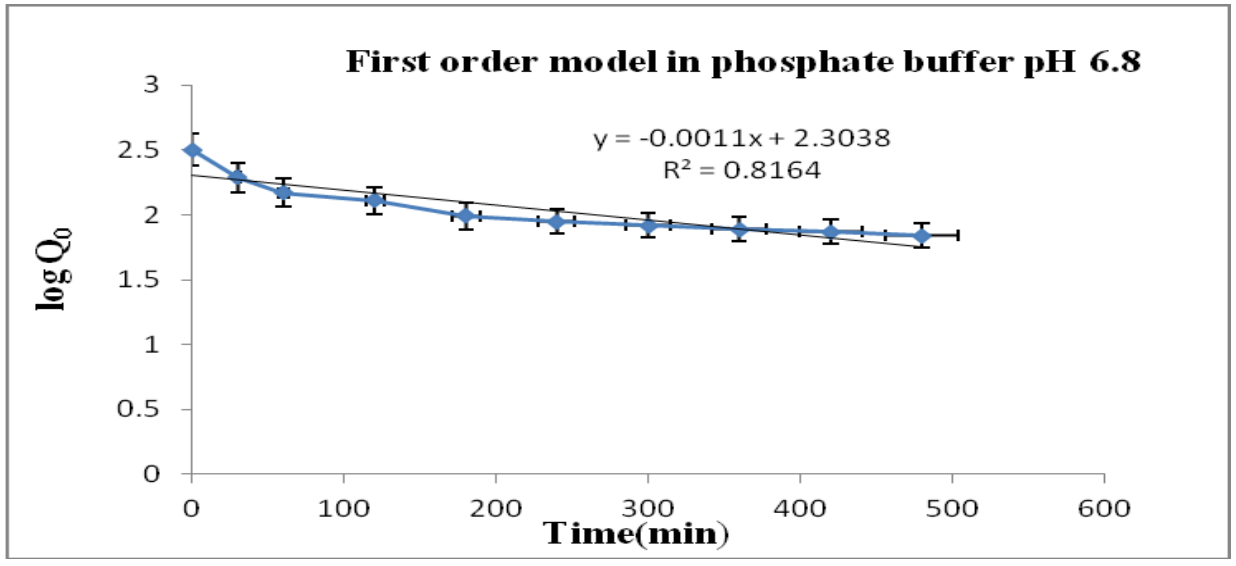

Figure 10: First order release kinetic study of optimized formulation in phosphate buffer pH 6.8

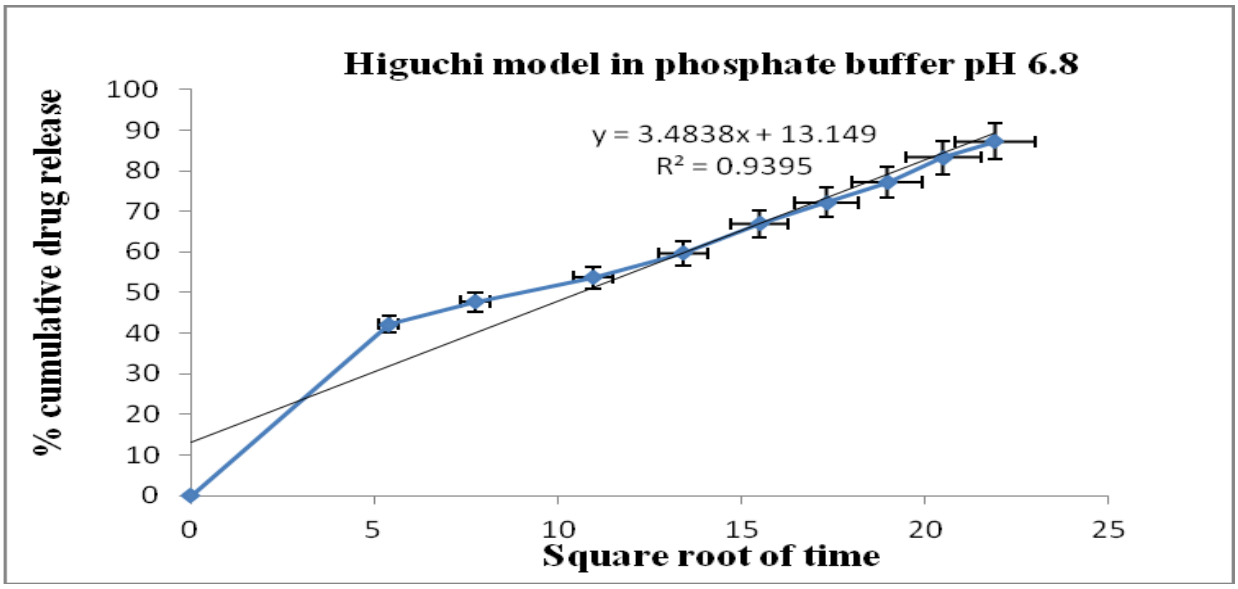

Figure 11: Higuchi model of optimized formulation in phosphate buffer pH 6.8

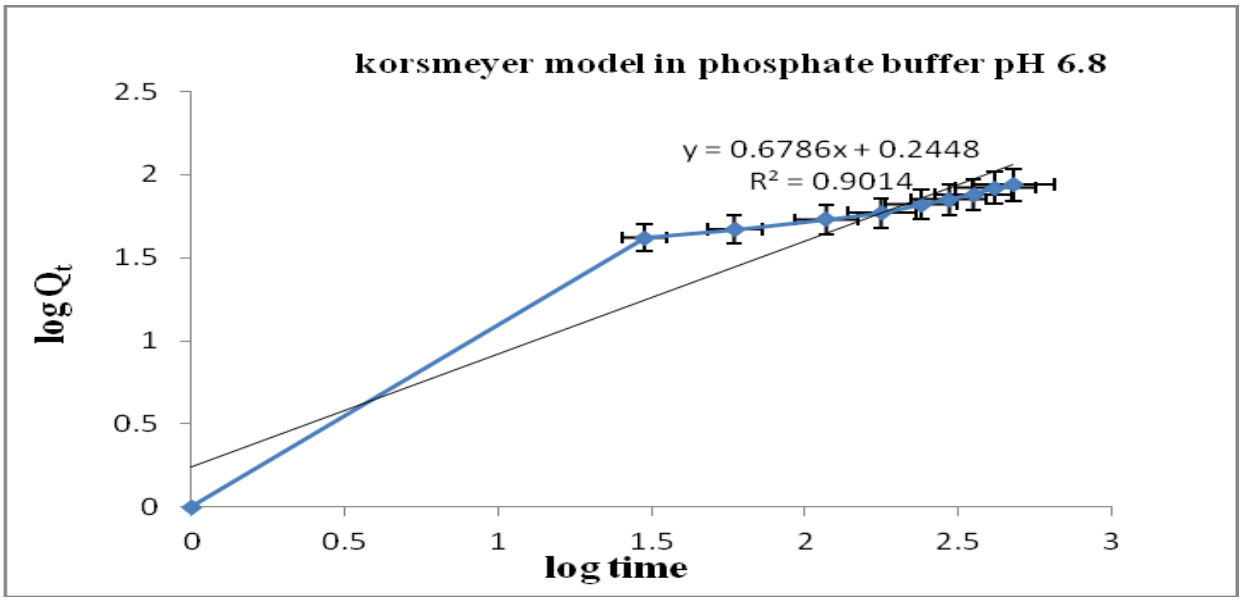

Figure: Korsmeyer model of optimized formulation in phosphate buffer pH 6.8

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