Academic Sciences **Asian Journal of Pharmaceutical and Clinical Research**
Vol 5, Suppl 1, 2012 ISSN - 0974-2441

Research Article

FORMULATION AND CHARACTERIZATION OF AN INTRAGASTRIC FLOATING DRUG DELIVERY SYSTEM OF DOXORUBICIN HYDROCHLORIDE: IN VITRO-IN VIVO RELEASE STUDY

RATHOR SHRUTI [1]*, RAM ALPANA [1]

[1]*School Of Pharmacy, Chouksey Engineering College, Lalkhadan, Masturi Road, Bilaspur, (Chhattisagarh)-495001, India,[1] S.L.T. Institute Of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur, (Chhatisgarh)-495001, India.Email: rathod.shruti@rediffmail.com.

Received: 14 November 2011, Revised and Accepted: 11 January 2012

ABSTRACT

The objective of the present study was to develop floating microspheres of doxorubicin in order to achieve an extended retention in upper gastrointestinal tract, which may result in enhanced absorption and thereby improved bioavaibility. Microspheres were prepared by emulsification extraction technique using pectin as polymer and casein as emulsifier. The 3^2 full factorial design was employed to evaluate contribution of independent variables, pectin-casein ratio (X1) and stirring speed (X2) on dependent variables, particle size, % drug entrapment, % buoyancy and time required for 80% drug release (t_{80} %). The microsphere exhibited buoyancy more than 8 hrs, high percentage drug entrapment efficiency. Microspheres were discrete, free flowing and spherical with rough surface. The polymer to emulsifier ratio had a more significant effect on the dependent variables. The optimum batch was F_6 which shown particle size 68.8 µm, 78% entrapment efficiency and $t_{80\%}$ is about 420 minutes. Infrared spectroscopy analysis revealed that there was no known chemical interaction between drug and polymer. Hence this investigation demonstrated that the polymer to emulsifier ratio had a more significant effect on the dependent variables. In vitro release study reveals that the drug release from microsphere is in Fickian pattern. Pure drug and Formulation (F6) both showed cytotoxicity against Kato III cells after in vitro cytotoxicity. In vivo gamma scintillography and antitumor activity indicated that the formulation F6 remained buoyant and uniformly distributed in the gastric contents throughout the study period of 8 hours and statistically significant (P <0.05) reduction in the number of tumors obtained.

Keywords: Porous microspheres, Doxorubicin, Emulsification extraction method.

INTRODUCTION

Oral drug delivery of drugs is by far the most preferable route of drug delivery due to ease of administration, patient compliance and flexibility in formulation etc. Oral sustained drug delivery formulations show some limitations connected with the gastric emptying time variables and too rapid gastrointestinal transit could result in incomplete drug release from the device into the absorption window leading to diminished efficacy of the administered dose. To overcome this problem several attempts have been made to develop oral dosage forms capable of having prolonged retention time in the stomach to extend the duration of drug delivery. It is evident from the recent research and patent literature that interest in novel dosage forms is unexpectedly increasing. Example of such systems are gastro retentive dosage forms, these dosage forms are based on different mechanism which include floatation, mucoadhesion, sedimentation, expansion, modified shape system or by simultaneous administration of pharmacologic agents that delay gastric emptying. Drug candidate suitable for this system are 1] which have site specific absorption in the stomach, or upper part of intestine, 2] drugs required to exert local action therapeutic action in stomach 3] drugs unstable in the lower part of gastrointestinal tract 4] drugs insoluble in intestinal fluids 6] drugs with variable bioavaibility[1,2].

Doxorubicin (Dox), an anthracycline antibiotic and one of the most widely used anticancer agents, shows high antitumor activity. However, its therapeutic effects are limited due to its dose dependent cardiotoxicity and myelosuppression. Indeed, nearly 2000 analogs were synthesized and evaluated; yet only few of them have reached the stage of clinical development and approval. Second generation analogs like mitoxantrone, epirubicin or idarubicin exhibited lower cardiotoxicity; but had lower efficacy compared to the parent molecule. Hence, doxorubicin becomes indispensable when it comes to cancer chemotherapy. Oral bioavailability of doxorubicin is less because it is eliminated by the first-pass extraction of the cytochrome P450-dependent metabolic process and the over expression of the multidrug efflux pump transporter P-glycoprotein (P-gp), which is rich in the intestine, liver and kidney, thus making it difficult to administer doxorubicin via oral route along with poor permeability. The general idea is to apply P-gp/P450 inhibitors such as cyclosporine A to suppress the elimination process. But these inhibitors suppress body's immune system and cause medical complications. Moreover, molecules like cyclosporine have their own side effects thus making it more difficult to incorporate them into drug delivery system along with anticancer agents. Advanced drug delivery strategies can offer alternatives which can circumvent the issues associated with drug's toxicity and on the other hand can lead to enhanced therapeutic performance by increasing the bioavailability of the drug [3, 4]. Another reason of low bioavaibility of doxorubicin is degradation of doxorubicin in the pH region 0.4-2.1; here cleavage of the amino sugar moiety occurs. Doxorubicin is stable at pH above than 3. Bioavaiblity of drug can be enhanced by increasing pH.

Therefore, porous microspheres (GRDFs) have emerged as an efficient means of prolonging gastric residence time, targeting stomach mucosa, and enhancing the bioavaibility. Porous microspheres remain buoyant for to having lower density than the gastric and intestinal fluids. They are not subjected to 'all or nothing' gastric emptying nature of single unit system. It releases the drug in controlled fashion. The pH of the stomach can be increased either by frequent administration of water or administration of alkali rich meal or by antacid. The present investigation describes the formulation development of an intragastric floating drug delivery system of doxorubicin hydrochloride and in vitro-in vivo release study at higher gastric pH [5].

MATERIALS

Doxorubicin was purchased from RPG Life sciences Limited, Navi Mumbai. Casein purchased from Loba chemicals and Pectin from Southern Citrus Products Pvt. Ltd, Gudur, AP (India). All other chemicals used were of analytical reagent grade.

METHODS

Analytical estimation of doxorubicin

Doxorubicin was estimated by UV- Vis spectrophotometric method (Shimadzu UV 1601, Kyato, Japan). Solution of drug was prepared in distilled water; the absorbance was measured at 495 nm spectrophotometrically from 2.0 to 20.0 µg concentration [6].

Experimental design

A 3^2 randomized full factorial design was adopted to optimize the variables. In the design two factors were evaluated, each at 3 levels, and experimental trials were performed at all nine possible combinations. In the present investigation, the ratio of polymer: emulsifier (X_1) and stirring speed (X_2) were selected as independent variables. The particle size, % drug entrapment, % Buoyancy and time required for 50% drug release were selected as dependent variables [7, 8].

Preparation of microsphere

Porous microspheres were prepared by emulsification extraction method which was previously described by Shruti et al [9]. In brief, 15% w/v solution of casein and pectin in different ratio were taken in 10 ml deionized water (60˚C) then added to 60 ml Soya oil preheated to same temperature. The dispersion was stirred to obtain emulsion and rapid cooling and 150ml previously cooled acetone was added to obtain solid microspheres that were filtered, washed with acetone and dried in dessicator and stored in well closed container.

Determination of particle size and its size distribution

The particle size of the microsphere was determined by using optical microscopy method. Approximately 500 particles were counted for particle size using a calibrated optical microscope [10, 11].

Morphological study of microsphere

The shape and surface morphology of the microsphere was investigated using scanning electron microscopy (photograph no 1). Photomicrographs were observed at 50x magnification operated with an acceleration voltage of 10kV and working distance 9.1mm was maintained [12, 13].

Determination of drug entrapment efficiency

Microspheres 200mg were crushed in a glass pestle and mortar, and the powdered mixed with distilled water solution then filtered with 0.2m membrane filter and aliquot of the filtrate was diluted with Buffer (pH-4.0). The filtrate was analyzed for drug content and absorbance was measured at 495nm using UV spectrophotometer. The drug entrapment efficiency was calculated by the following formula [14, 15] –

$$\text{Percentage drug entrapment} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Percentage buoyancy

Porous microspheres 200mg were spread over the surface of a USP XXIV paddle type dissolution apparatus filled with 900ml of acetate buffer pH 4.0 containing 0.02% v/v of tween 20. The mixture was stirred at 100rpm, particles was pipetted out and separated by filtration. Particles in sinking particulate layer were separated by filtration. Particles of both types were dried in dessicator until constant weight was obtained by both fractions of the microspheres. These were weighed and percentage buoyancy was determined by using following formula [16, 17].

$$\text{Buoyancy} = \{[wf/wf + ws] \times 100\}$$

Fourier transforms infrared spectroscopy

Drug polymer interaction was studied by FT-IR spectroscopy (Shimadzu Affinity I, FT-IR spectrophotometer). The spectrum was recorded for pure Dox, Dox loaded microspheres and unloaded microspheres (placebo). Samples were prepared by triturating 5% of drug or microsphere with 95% of KBr in glass pestle mortar. The scanning range was 4000 cm^{-1} to 400 cm^{-1} and resolution was 2 cm^{-1} [16, 18].

Thermo gravimetric and differential thermal analysis

The thermal behavior of Dox and its microsphere was examined with diamond TG/DT analyzer (Perkin-Elmer, USA). Argon was used as carrier gas and the analysis was carried out at a heating rate 8.00˚C/min and an argon flow rate of 35cc/min. The sample size was 3.857mg and 2.947mg for pure Dox and loaded microsphere, respectively. The curve was recorded at a temperature range 30.00˚C to 260˚C [3, 6, 9, 19, and 20].

Flow properties

Flow prope*rties* were determined in terms of Carr's index (I_c) and Hausner's ratio (H_R) using the following formula:

$$H_R = \rho_t / \rho_b$$

$$I_c = \rho_t - \rho_b / \rho_t$$

Where, ρ_t = tapped density
ρ_b = bulk density

The angle of repose (θ) of the microsphere, which measures the resistance to particle flow, was determined by the fixed funnel method, using the following equation:

$$\tan\theta = 2H/D$$

Where, H the height and D is the diameter of the heap that formed after making the microspheres flow from the glass funnel[21].

***In vitro* dissolution studies**

In Vitro dissolution studies were performed using US Pharmacopoeia XXIII Dissolution apparatus II (paddle type). An accurate weighed sample (40 mg) of optimized porous microsphere was dropped into 900 ml of acetate buffer pH 4.0 maintained at a temperature at 37˚C ± 0.5˚C and stirred at a speed of 50 rpm. At different time intervals, a 10ml aliquot of the sample was withdrawn and the volume was replaced with an equivalent amount of plain dissolution medium kept at 37˚C. The collected samples were filtered and analyzed at λ_{max} 496nm using a UV-Visible spectrophotometer against acetate buffer pH 4.0 taken as blank [(15, 16)]. Percentage drug dissolved at different time intervals was calculated using Lambert-Beer's equation. The drug release was calculated using various models. The average values of t_{80} for batches F1 to F9 are mentioned in Table 1.The percentage release of batch F6 is shown in Figure 4 [22].

***In vitro* cytotoxicity analysis of doxorubicin loaded microsphere on kato iii**

Human gastric cancer cell line

The KATO III human gastric cancer cell line were purchased from National Centre for Cell line Pune and cultured in Jawaharlal Nehru Cancer Research Centre and Hospital, Bhopal. MTT assay was performed, [(3 – (4, 5 – dimethylthiazol] 2] Y) 2, 5 – diphenyl tetra sodium bromide)] is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazon product. This process requires active mitochondria and even freshly dead cells do not cleave significant amount of MTT. Thus the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. This assay was performed using the standard operating procedures. To examine the effects of pure Dox and Dox loaded Porous microspheres, the cells were treated with 0.001, 0.01, 0.1, 1 and 10 mg/ml of Dox and similar concentrations of optimized microspheres (F6). Cell lines maintained in appropriate condition were seeded in 96 well plates is treated with different concentration of the test samples and in incubated at 37ºC, 5% CO2 for 1-5 days. MTT reagent was added to the wells and incubated for 4 hours, the dark blue formazon product formed by the cells as dissolved DMSO under a safety cabinet and read at 550nm in Elisa reader. Percentage inhibitions were calculated and plotted with the concentrations used to calculate the IC_{50} values [9, 18].

Gamma scintillography

The optimized formulation (F6) and nonfloating microspheres (NFM) of 500 mg each, loaded with $SnCl_2$ and Dox, were placed in a test tube and soaked in 10 mL of normal saline (0.9% NaCl) for 15 minutes. A small amount of sodium pertechnetate solution equivalent to radioactivity of 40 mBq obtained from a technetium generator and stored in a sterile vial was added to the tube. The suspension was mixed intermittently for 15 minutes using a shaker. The supernatant was removed and the labeled microspheres were recovered by filtration through a Whatman filter paper (No. 41) followed by washing thoroughly with distilled water. The microspheres were then allowed to dry in air for 15 minutes. Six healthy albino mice were used to monitor the in vivo transit behavior of the floating and nonfloating microspheres. These mice were divided into 2 groups (group A and group B). The location of the formulation in the stomach was monitored by keeping the subjects in front of E-CAM gamma camera with SPECT technology

(**Single-photon emission computed tomography**). The gamma camera has a field view of 40 cm and is fitted with a medium-energy parallel hole collimator. The 140 keV gamma rays emitted by ^{99m}Tc are imaged. Specific stomach site (anterior) was imaged by E-Cam gamma camera after definite time intervals and activity counts recorded for a 5-minute period to calculate the counts per minute (cpm). The gamma images were recorded using an online computer system, stored on magnetic disk, and analyzed to determine the distribution of activity in the oral cavity and stomach. In between the gamma scanning, the animals were freed and allowed to move and carry out normal activities and allowed to take water frequently until the formulation had emptied the stomach completely to increase the pH of stomach [16].

Antitumor activity

The in vivo study was performed as per the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The Institutional Animals Ethical Committee of School of Pharmacy, Chouksey Enginnering College (1275/ac/09/ CPCSEA/2010/07) approved the protocol for the study. Swiss albino male/female aged 8 to 9 weeks was used. The animals were kept under a standard 12/12 light/dark cycle and were given food and water ad libitum. The animals were administered 2 doses of 3 mg of benzo(a)pyrene (B(a)P) in 0.25ml of corn oil orally with 2 weeks between the doses i.e. on 1st and 15th day. The animals were sacrificed after 10 weeks using lethal chloroform anesthesia. Abdominal cavity was opened and the stomach and duodenum were isolated. Organs were flushed with PBS to remove the gastric contents. Organs were cut longitudinally to expose the lumen and were observed under stereo zoom microscope for the presence of tumors. Tumors were observed in the duodenal lumen. The B(a)P-treated mice were divided into 3 groups (n = 10): pure drug treatment, floating microsphere drug treatment, and no treatment (control group vehicle alone). During therapy frequent water was given to animals resulting in increased the pH of the stomach. The treatment groups were administered 30 mg/m^2 of the drug in a corn oil suspension or equivalent (in the case of FDF) orally. The dosage regimen was repeated till the end of the experiment. All the data were statistically analyzed by 1-way analysis of variance [5].

RESULTS AND DISCUSSION

Porous microspheres of doxorubicin were successfully prepared by emulsification extraction method. A statistical model incorporating interactive polynomial term was used to evaluate the response

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_{11} + b_{22}X_{22}$$

Where, Y is the dependent variable, b_0 is the arithmetic mean response of nine runs, bi is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average results of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the responses changes when two factors are simultaneously changed. The polynomial terms (X_{11} and X_{22}) are included to investigate nonlinearity.

Table 1: 3^2 Full factorial design layout.

Batch Code	Variable levels in coded form		Particle size analysis (µm)	Buoyancy (%)	Drug Entrapment (%)	$t_{80\%}$ (Min)
	X_1	X_2				
F1	-1	-1	96.1±0.23	87.0±0.45	38.0±0.89	230±2.78
F2	-1	0	87.6±0.45	84.0±0.34	40.0±0.48	249±2.02
F3	-1	1	80.0±0.35	80.0±0.67	42.0±0.87	256±1.86
F4	0	-1	88.9±0.26	78.0±0.63	69.0±0.88	390±2.48
F5	0	0	74.9±0.39	74.0±0.50	70.0±0.96	405±1.90
F6	0	1	68.8±0.98	2.0±0.59	78.0±0.99	420±1.99
F7	1	-1	88.4±0.23	68.0±1.23	73.0±0.93	395±1.23
F8	1	0	76.5±0.48	62.0±0.78	75.0±0.60	379±2.03
F9	1	1	69.3±0.95	58.0±1.30	76.0±0.98	398±2.79

Translation of coded levels in actual units.

Variable levels	Low (-1)	Medium (0)	High (+1)
Polymer to Emulsifier ratio (X_1)	2:1	1:1	1:2
Stirring speed (X_2) rpm	500	1000	1500

*** n = 3, all values ± standard deviation, statistically significant at 0.05 level**

Table 2: Angle of repose, carr's index and hausner,s ratio as an indication of flow properties.

Angle of repose (θ)	Carr's index	Hausner's ratio	Type of flow
>20	5-15	-	Excellent
20-30	12-16	<1.25	Good
30-40	18-21	-	Fair to passable
-	25-35	>1.25	Poor
-	33-38	1.25-1.5	Very poor
>40	>40	-	Extremely poor

Table 3: Micromeritic properties.

S.No.	Code	Mean Particle Size (µm	Angle of Repose (Θ)	Carr's Index (%)	Hausner's Ratio
1	F1	96.1±0.34	29.45±0.44	15.13±0.51	1.166±0.74
2	F2	87.6±0.65	27.34±0.63	14.98±0.48	1.159±0.85
3	F3	80.0±0.33	25.94±0.84	13.67±0.76	1.143±0.97
4	F4	88.9±0.28	26.62±0.76	13.46±0.63	1.158±0.58
5	F5	74.9±0.96	2.23±0.58	12.49±0.38	1.149±0.48
6	F6	68.8±0.52	23.67±0.38	11.63±0.54	1.139±0.85
7	F7	88.4±0.26	26.89±0.91	13.55±0.66	1.660±0.43
8	F8	76.5±0.75	25.45±0.65	12.23±0.38	1.154±0.71
9	F9	69.3±0.24	24.23±0.39	11.89±0.64	1.147±0.22

*** n = 3, all values ± standard deviation, statistically significant at 0.05 level**.

The statistical analysis of the factorial design batches was performed by multiple regression analysis using Microsoft Excel. To demonstrate graphically the influence of each factor on response, the response surface plots were generated using Sigma Plot Software version 11.0 (Jandel Scientific Software, San Rafael, CA). The particle size, % drug entrapment, % Buoyancy, and time required for 80% drug release for the nine batches (F1 to F9) showed a wide variation 68.8 – 96.1µm, 38.0-78.0%, 58.0 -87.0% and 230 - 485 min respectively (Table no.1). The data clearly depicts that the Particle size, % drug entrapment, % Buoyancy and time required for 80% drug release values are strongly dependent on the selected independent variables. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries.

Particle size

$$y = +76.03 - 4.91\, x_1 - 9.21\, x_2 + 5.45\, x_1^2 + 2.25\, x_2^2 - 0.75\, x_1 x_2$$

% Drug entrapment

$$y = +71.66 + 17.33\, x_1 + 2.66 x_2 - 1.5\, x_1^2 + 1.0\, x_2^2 - 0.25\, x_1 x_2$$

% Buoyancy

$$y = +74.33 - 10.5\, x_1 - 3.83\, x_2 - 1.5\, x_1^2 + 0.5\, x_2^2 - 0.75\, x_1 x_2$$

t_{80} %

$$y = +402.44 + 9.83\, x_1 + 9.83 x_2 - 87.16\, x_1^2 + 3.83\, x_2^2 - 5.75\, x_1 x_2$$

Three dimensional response surface plots for all response variables are presented in Figure 1(A), (B), (C) and (D) which are very useful to study the interaction effects of the factor (X_1X_2) on the responses. The response surface depicts effect of factor contribution at different levels on studied response.

Figure 1(A) depicts a decline in particle size with increase in stirring speed. At higher emulsifier to polymer ratio, particle size is more. The lowest particle size recorded at medium level of emulsifier and polymer ratio.

Figure 1(B) reveals increase in the value of % entrapment efficiency with increase in polymer content of the formulation, followed by an increase with decreasing value of emulsifier and again very much increase in % EE with lesser emulsifier content, the effect of emulsifier being significant.

Figure 1(C) reveals decrease in value of % buoyancy with increase in polymer content of the formulation. As the quantity of emulsifier is increased, it also increases the % buoyancy. Particle size slightly affects the buoyancy of formulation.

Figure 1(D) shows that a maximum of 80% drug release was obtained with medium level of emulsifier to polymer ratio. With increase in polymer content slight decrease in release was observed. High emulsifier content, results in a more significant decrease.

Figure 2 shows scanning electron microscopic photographs of microspheres which are spherical with rough surface.

Figure 3(A), (B) and (C) are the characteristic peaks of the pure drug, drug loaded microsphere and placebo microsphere. The drug remained unaltered in the FTIR spectra of doxorubicin microspheres. The peaks which are in pure drug spectra are also present in spectra of microsphere but doesnot exist in placebo microsphere. FTIR analysis reveals that there is no interaction between drug and drug loaded microsphere.

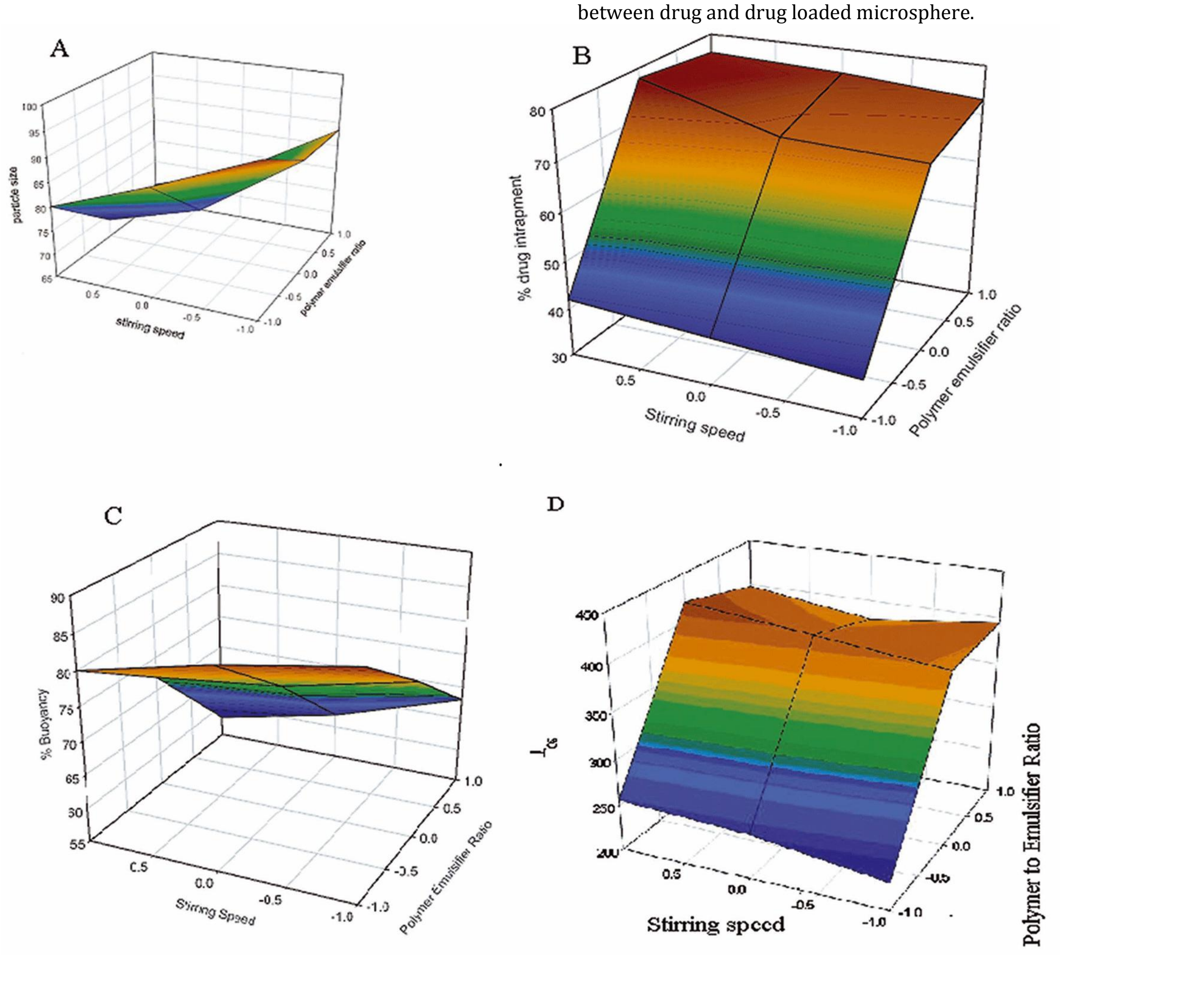

Figure 1:Three dimensional response surface plots for all response variables are (A) partcle size, (B) percentage drug entrapment, (C) percentage buoyancy and (D) percentage t_{80}.

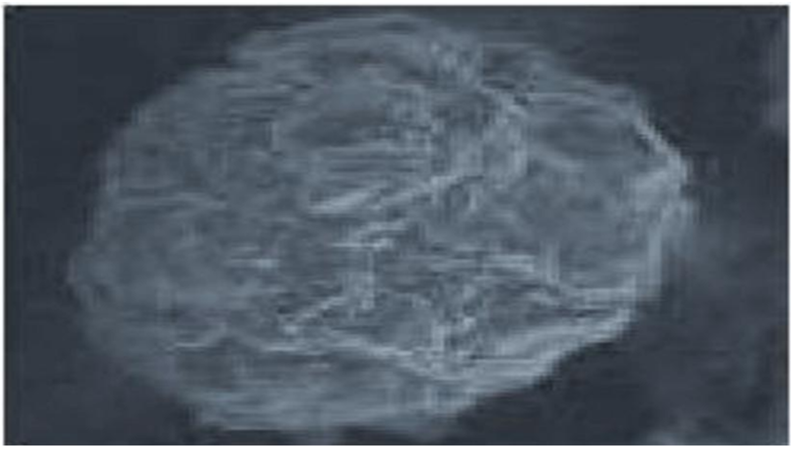

Figure 2: Scanning electron microscopic image of drug loaded microspheres (F6).

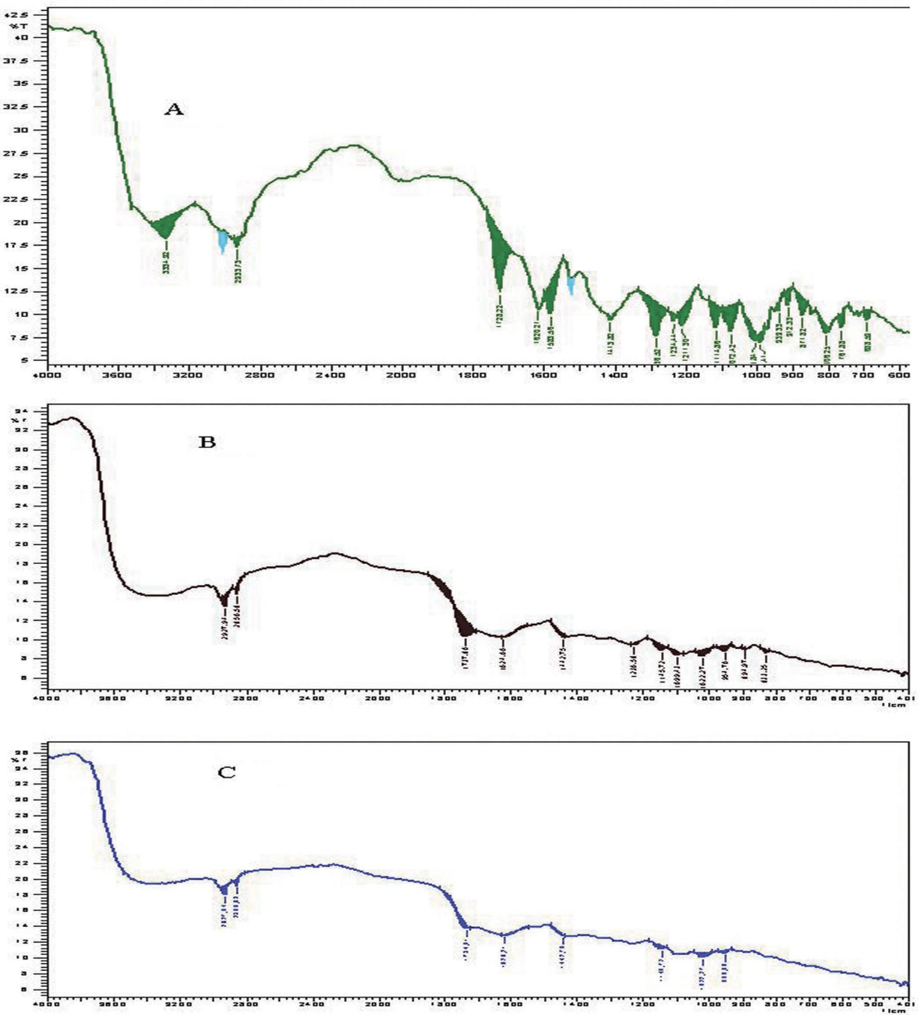

Figure 3: FTIR spectra of the (A) pure drug, (B) drug loaded microsphere and (C) placebo microsphere.

TG-DTA experiments were performed to investigate the physical state of the drug or polymer in the microspheres, because this aspect could influence the in vivo and in vitro release of the drug from the system. The specimen is continuously heated (or cooled) with a steady heating/cooling rate. Figure 4(A) illustrate a typical TG-DTA scan (heat flow vs. temperature), showing the melting of well-known drug doxorubicin at 223.25°C which is indicated by endothermic peak (heat absorption). The melting peak of the drug was totally disappeared in the thermogram of loaded microsphere evidencing the absence of crystalline drug in the microsphere sample. Loaded microspheres showed that exothermic melting transition started at 198.32°C (Figure 4(B)). The cross linking of polymer molecules is the exothermic process, resulting in a positive peak in the TG-DTA curve. Therefore, it could be concluded from the TG-DTA of optimized batch that doxorubicin in microspheres was in an amorphous or a solid solution state in the polymer matrix after the production. Size of microsphere greatly affects the flow properties. Particles or microspheres having a smaller size showed good flow properties.

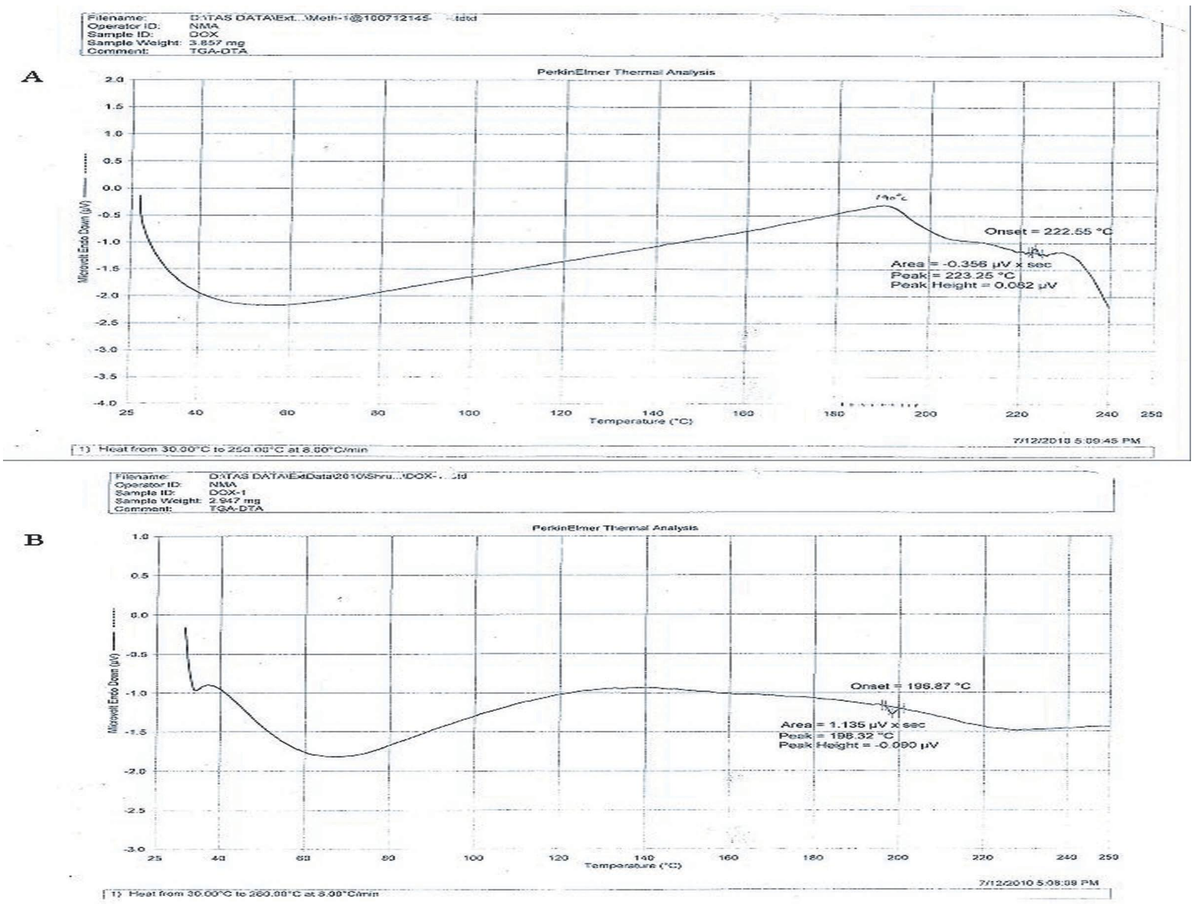

Figure 4: TG-DTA thermogram scan of (A) pure doxorubicin (B) drug loaded microsphere

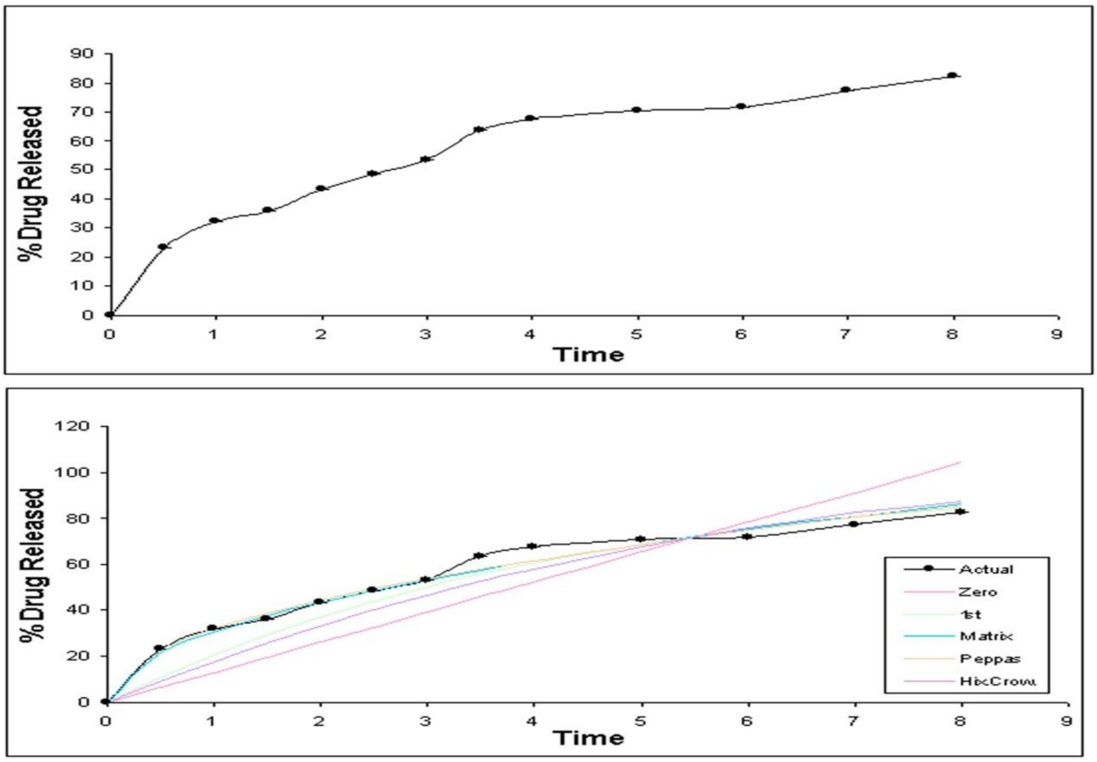

Figure 5: Percentage drug release data (a) without model fitted and (b) with model fitted.

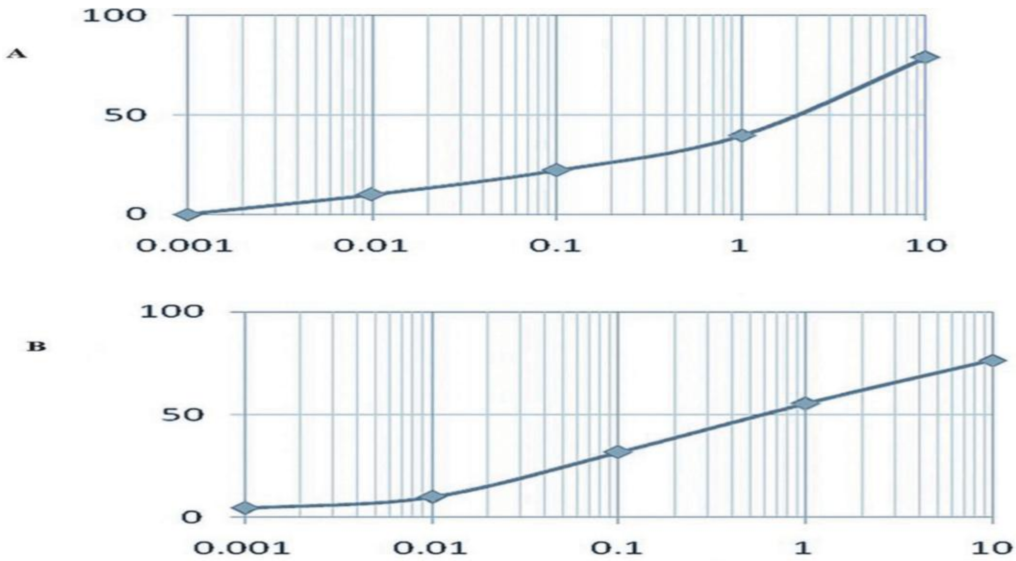

Figure 6: IC_{50} (A) pure drug (B) drug loaded microsphere.

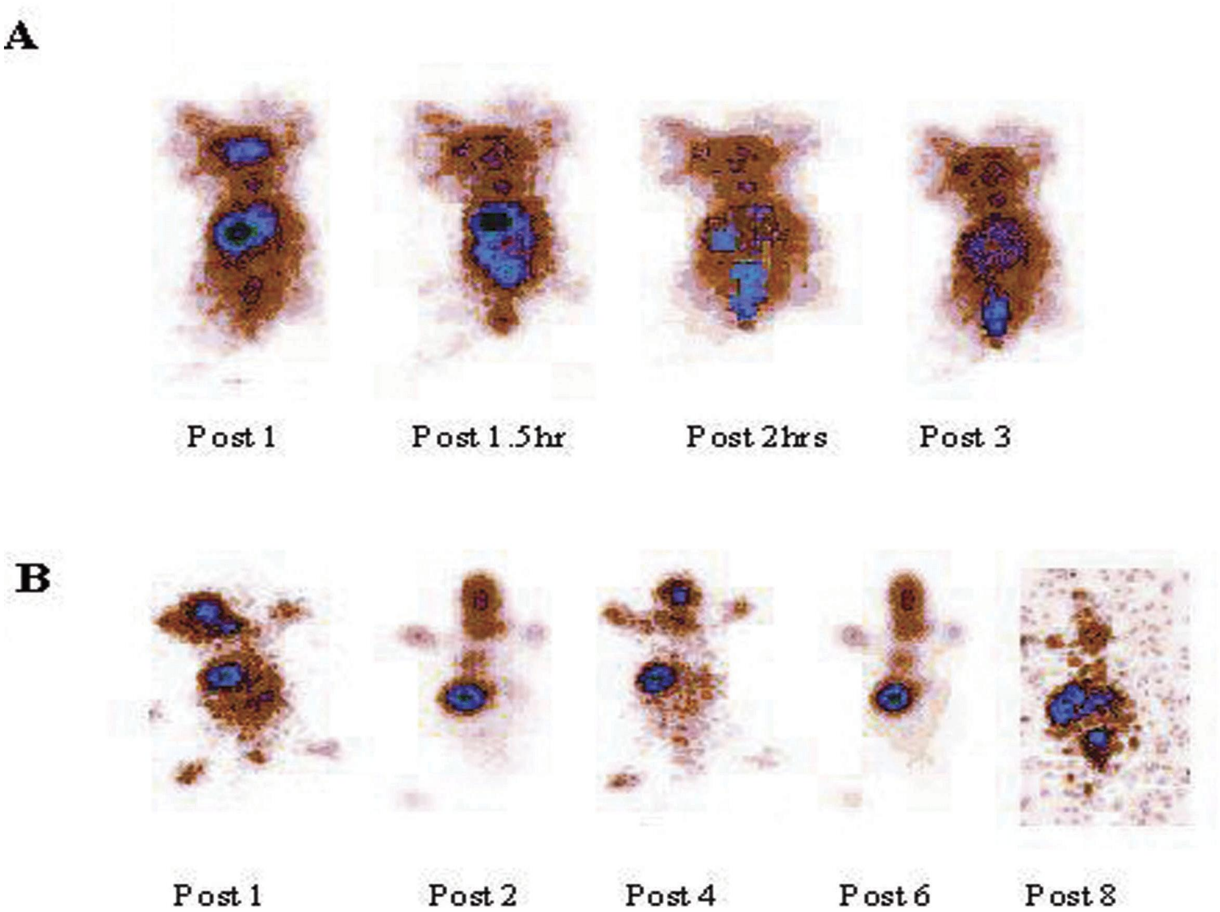

Figure 7: Gamma scintillography images of group a and group b.

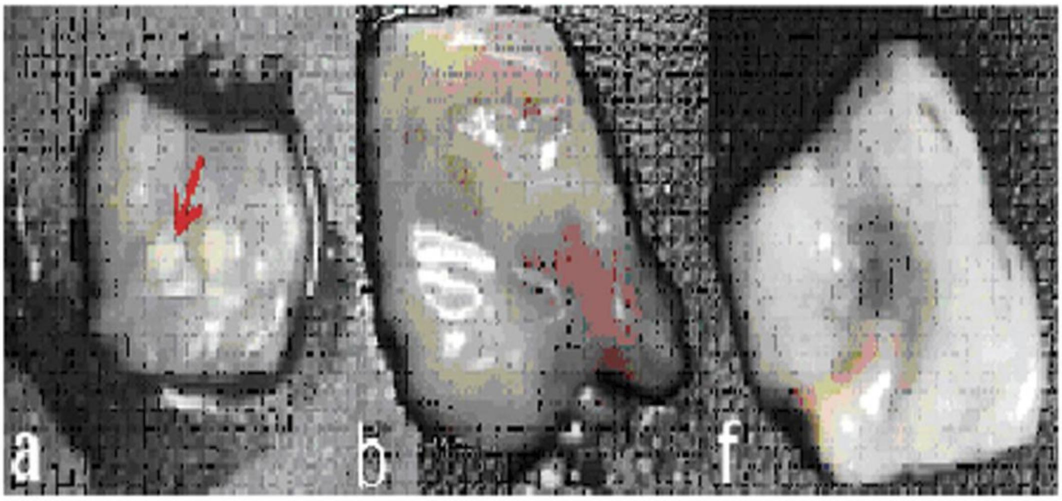

Figure 8: Tumor incidence after and before treatment

Microparticulate drug delivery systems are crucial where the sustained release of drug is desired for a longer time period and chronic illness like cancer forms no exception to this. One of the desired attributes of oral chemotherapy is reduced dosing frequency and accumulation of the dosed drug in the tumor tissues by enhanced permeation retention that can be attained using microparticles. In vitro release of doxorubicin mainly depends upon the polymer and emulsifier ratio. Figure 5(A) reveals that the drug release was sustained for more than 8 hrs and in controlled manner. To ascertain the drug release mechanism and release rate, data of formulation F6 were fitted by using PCP Diss V3 dissolution software. The model selected were zero order(r=0.7578, k=13.0591), first order (r=0.9664, k= -0.2319), Higuchi (r=0.9905, k=30.5968), korsemeyer peppas (r=0.9915, k=31.8977) and Hixon crowell (r=0.9247, k=-0626) as represented in figure 5(B). All models passes t test. The result suggests that for formulation F6 best fit model was found to be Korsemeyer Peppas model. The value of correlation coefficient was 0.9592. The value of slope and intercept were found to be 0.7714 and 10.1091. The n = 0.4734 value shows Fickian release pattern from formulation F6.

The inhibitory potency of the pure drug on the Kato III cell line was compared by using the IC_{50} value. The IC_{50} represents the concentration of the drug at which 50% of inhibition is produced. Pure drug sample showed good drug release at pH 4.0. Drug loaded microspheres showed very significant results compared with the pure drug. Figure 6(A) and (B) for pure drug and drug loaded microspheres (F6) both showed cytotoxicity against Kato III cells. Hence the formulation can be effectively tested for its anticancer activity.

The optimized formulation (F6)) had shown good in vitro buoyancy and controlled release behavior and hence was finally selected for in vivo study (i.e. gamma scintigraphy), and the results were compared with non-floating microsphere prepared using same polymer (sugar cross linked). Gamma images of the ^{99m}Tc-labeled F6 and non-floating microspheres are shown in Figure 7. Examination of the sequential gamma scintigraphic images during the study clearly indicated that the F6 remained buoyant and uniformly distributed in the gastric contents throughout the study period of 8 hours. Prolonged gastric retention time (GRT) of more than 6 hours was achieved in all the mice for the formulation F6, which remained buoyant in the stomach for the entire test period. In contrast, nonfloating microspheres showed gastro-retention of less than 2 hours. After swallowing, the floating microspheres adopted a floating position on top of the stomach content. This might be because of the presence of porous structure of microsphere due to emulsifier casein responsible for incorporation of air bubble inside the microspheres. A measurable number of counts of ^{99m}Tc-tagged F6 for the 8-hour study period showed very good gastro-retentive propensity as the administered microspheres remained floating and distributed in the stomach contents. In case of nonfloating microspheres, the radioactive counts decreased considerably after 2 hours in the stomach.

B(a)P resulted in 100% incidence of forestomach tumors after 10 weeks with an average of 2.19 tumors per mouse compared with corn oil–treated control animals as shown in Figure 8. Mice treated with pure drug and floating microspheres resulted in 30.0% and 55.66% reduction in tumor at first dose. At second dose 42.0% and 86.4 % reduction was seen as illustrated in Figure 9. The statistically significant (P <0.05) reduction in the number of tumors obtained with formulation F6 as compared with control and plain drug treatments indicates site-specific delivery of doxorubicin through floating dosage forms which results in maintenance of a local concentration of 5-FU for a longer period of time.

In the present study, the potential of porous microsphere as drug carriers for oral delivery was investigated. The method of preparation of microsphere of doxorubicin was found to be simple and reproducible. It was found that porous microspheres showed a desirable high drug content, good flow properties, buoyancy and adequate drug release at pH 4.0; hence, formulation prepared by this method is suitable for controlled and sustained drug delivery. This study shows that porous microsphere could be a useful carrier for doxorubicin.

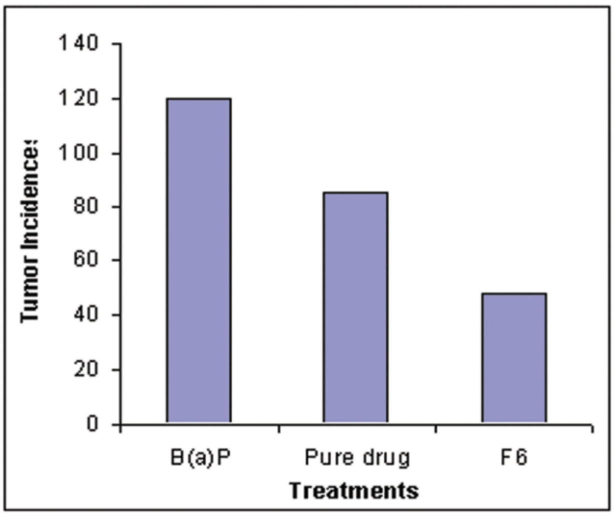

Figure 9: Comparison of tumor incidence for different treatment groups. benzo(a)pyrene, pure drug, and drug loaded microsphere (f6). *p < .05.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Bose Institute, Kolkata, India, and Dr. M. Mathur (Nuclear medicine department), Jawahar Lal Cancer Hospital and Research Institute, Bhopal, India, for providing scanning electron microscopic facility and gamma scintigraphy to us. We are also thankful to Principal, Poona College of Pharmacy for providing PCP Disso V3i software for research.

REFERENCES

1. Ojha G, Tanwar YS, Chouhan CS, and Naruka PS. Floating microspheres: development, characterization and applications. Pharmainfo.net 2006; 4(3).
2. Patel VF and Patel NM. Intragastric floating drug delivery system of cefuroxime axetil: in vitro evalution. AAPS Pharm Sci Tech 2006; 7(1): article 17.
3. Kalaria DR, Sharma G, Beniwal V, and Ravikumar MNV. Design of biodegradable nanoparticles for oral delivery of doxorubicin: In vivo pharmacokinetics and toxicity studies in rats. Pharm research 2009; 26(3):492-501.
4. Beijen JH, Wiese G, and Underberg JM. Aspects of chemical stability of doxorubicin and seven other anthracyclines in acidic solution. Vol 7. Pharmaceutisch Weekblad Scientific Edition; 1985.
5. Shishu, Gupta N, and Agrawal N. Stomach specific drug delivery of 5-FU using floating alginate beads. AAPS Pharm Sci Tech 2007; 8(2): article 48.
6. Chouhan R and Bajpai A. Real time in vitro studies of doxorubicin release from PHEMA nanoparticles. J of Nanobiotech 2009; 7(5).
7. Patel JK, and Patel RP. Formulation and evaluation of mucoadhasion glipizide microspheres. AAPS Pharm Sci Tech 2005; 06(01): E49-E55.
8. Patel DM and Patel MM. optimization of fast dissolving etoriocoxib tablets prepared by sublimation technique. Indian J of Pharm Sci 2006; 68(2): 222-226.
9. Rathor S and Ram A. porous microsphere of 5-FU: a tool for site specific drug delivery in gastric cancer. Inter J of Current Pharm Research 2011; 3(1): 38-42.
10. Murata Y, Sasaki N, Miyamoto E, Kawashima S. Use of floating alginate beads for stomach-specific drug delivery. Eur J Pharm Biopharm. 2000; 50: 221-226.
11. Choi BY, Park HJ, Hwang SJ, Park JB. Preparation of alginate beads for floating drug delivery system: effects of CO_2 gas-forming agents. Int J Pharm 2002; 239:81-91.
12. Paharia A, Yadav A, Rai G, Jain SK, Pancholi SS and Agrawal GP. Eudragit coated pectin microspheres of 5-FU for colon targeting. AAPS Pharm Sci Tech 2007; 8(1): article 12.
13. Deshpande AA, Rhodes CT, Shah NH, Malick AW. Controlled-release drug delivery systems for prolonged gastric residence: an overview. Drug Dev Ind Pharm 1996; 22: 531-540.
14. Ichikawa M, Watanabe S, Miyake Y. A new multiple-unit oral floating dosage form, I: preparation and evaluation of floating and sustained-release characteristics. J Pharm Sci 1991; 80: 1062-1066.
15. Singh BM, Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J Control Release 2000; 63: 235-239.
16. Jain SK, Agrawal GP, and Jain NK. Evaluation of porous carrier based floating orlistat microspheres for gastric delivery. AAPS Pharm Sci Tech 2006; 7(4): article 90.
17. Jain SK, Awasthi AM, Jain NK, Agrawal GP. Calcium silicate based microspheres of repaglinide for gastro-retentive floating drug delivery: preparation and in vitro characterization. J Control Release 2005; 107: 300-309.
18. Kaushik D, Sardana S, and Mishra D. In vitro characterization and cytotoxicity analysis of 5-FU loaded chitosan microsphere for targeting colon cancer. Int J Pharm Edu and Research 2010; 44(3): 267-273.
19. Baxter RA, Sswenker RF and Garn PD. Thermal Analysis. Vol 1. Academic Press, 1969: 65.
20. Kale RD, Tayade PT. A multiple unit floating drug delivery system of piroxicam using eudragit polymer. Int J of Pharmaceutical Sci 2007; 69(1): 120-123.
21. Jain AK, Jain CP, Tanwar YS and Naruka PS. Formulation, characterization and in vitro evaluation of floating microspheres of famotidine as a gastroretentive dosage form. Asian Journal of Pharmaceutics 2009; 3(3): 222-226.
22. Patel JK, Patel RP, Patel MP. Formulation and evaluation of mucoadhesive glipizide microspheres. AAPS Pharm Sci Tech 2005; 6(1): EE49-EE55.
23. Murata Y, Kofuji K, Kawashima S. Preparation of floating alginate beads for drug delivery to gastric mucosa. J Biomater Sci Polym Ed 2003; 14: 581-588.
24. Sato Y, Kawashima Y, Takeuchi H, Yamamoto H. In vivo evaluation of riboflavin containing microballons for floating controlled drug delivery system in healthy human volunteers. J Control Release 2003; 93:39-47.
25. Singh BM, Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J Control Release 2000; 63:235-259.
26. Bolten S. Pharmaceutical Stastics. 3rd ed. Marcel Dekker Inc, New York 1990: 326-354.
27. Hwang SJ, Park H, Park K. Gastric retentive drug-delivery systems. Crit Rev Ther Drug Carrier Syst 1998; 15:243-284.
28. Indian Pharmacopoeia, 4th Edn., Vol. II: Controller of Publications, Govt. of India, Ministry of Health & Family Welfare, New Delhi; 1996.