DESIGN AND DEVELOPMENT OF PACLITAXEL - LOADED MICROSPHERES FOR TARGETED DRUG DELIVERY TO THE COLON

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ABSTRACT

The purpose of this investigation was to prepare and evaluate the colon-specific microspheres of Paclitaxel for the treatment of colon cancer. Core microspheres of alginate were prepared by the modified emulsification method in liquid paraffin and by cross-linking with calcium chloride. The core microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach and small intestine. The microspheres were characterized by shape, size, surface morphology, size distribution, incorporation efficiency, and in vitro drug release studies. The outer surfaces of the core and coated microspheres, which were spherical in shape, were rough and smooth, respectively. The size of the core microspheres ranged from 20 to 52 µm, and the size of the coated microspheres ranged from 107 to 178 µm. The core microspheres sustained the drug release for 10 hours. The release studies of coated microspheres were performed in a pH progression medium mimicking the conditions of the gastrointestinal tract. Release was sustained for up to 20 hours in formulations with core microspheres to a Eudragit S-100 coat ratio of 1:7, and there were no changes in the size, shape, drug content, differential scanning calorimetry thermogram, and in vitro drug release after storage at 40°C/75% relative humidity for 6 months.

KEY WORDS: Paclitaxel, colon-specific, microspheres, alginate, Eudragit S-100, DSC, HPLC

INTRODUCTION

Colorectal cancer is a very common malignancy in industrialized nations and a major cause of mortality and morbidity. Surgery, radiation therapy, and chemotherapy are the modalities commonly employed in an attempt to cure colorectal malignancy. Since its introduction by Heidelberger et al in 1957,[1] The advantages and necessity of colon targeting to provide more effective therapy for colon related diseases, such as colon cancer, irritable. [2] The colon, as a site for drug delivery, offers distinct advantages on account of a near neutral pH, a much longer transit time, relatively low proteolytic enzyme activity, and a much greater responsiveness to absorption enhancers[3].

Paclitaxel is a natural plant product extracted from bark of western *Taxus brevifolia*, effective against tumors

including breast and colon cancer. It blocks the G-2 M phase of the cell cycle of proliferating cell[4] and stabilizes tubulin polymer formation by promoting microtubule assembly.[5] Paclitaxel is a very potent anticancer agent, but its efficacy is limited because of low solubility and oral bioavailability.[6] Some cancer cells have a high level of efflux pump, P-glycoprotein (Pgp), which results in removal of Paclitaxel from the cell during their transport through the cell.[7] Paclitaxel is administered by IV infusion, and to enhance its solubility, cremphor EL is used as a solvent. This solvent causes severe hypersensitivity reactions and cytotoxicity and has shown incompatibility with polyvinyl chloride (PVC), commonly used in IV dosage forms.[8] Even though Paclitaxel is a highly effective anticancer agent, it cannot differentiate between cancer and normal cells, resulting in major toxicity to normal tissues. This toxicity can be fatal if not prevented. To minimize the cytotoxicity and adverse side effects associated with Paclitaxel, a targeted drug delivery system needs to be developed for colon cancer.

The approaches to achieving colonic delivery of drugs include use of prodrugs, pH-sensitive polymer coatings, time-dependent formulations, bacterial degradable coatings, time/pH-controlled deliveries, and intestinal luminal pressure-controlled colon delivery capsules. In addition, the use of biodegradable polymers such as azopolymers and polysaccharides for colon targeting has been described in the literature.[9] Alginates are linear polymers that have 1-4' linked β-D-mannuronic acid and α-L-guluronic acid residue arranged as blocks of either type of unit or as a random distribution of each type. (Alginate building block units are β-D-mannuronic acid and α-L-guluronic acid.) Alginates have many advantages as colonic drug carriers, including nontoxicity, biocompatibility, and biodegradability by colonic flora, availability, and cheapness. A Eudragit L-30D–coated calcium alginates bead for colonic delivery of 5-aminosalicylic acid has been reported.[10]

A colon-specific guar gum–based tablet also been reported.[11] However, because of variations in transit throughout the colon, the drug release can be impaired when the colon-specific tablet matrix is not readily disintegrated, and treatment will remain ineffective.[12-14] This problem could be circumvented by reducing the size of the delivery carrier, since it has been reported that gastrointestinal retention depends upon the size of the carrier,[15] meaning that smaller carriers will lead to longer residence in the colon. The present investigation involves developing and characterizing a colon-specific microsphere delivery system of Paclitaxel using alginate and Eudragit S-100 as a carrier.

MATERIALS AND METHODS

Materials

The Paclitaxel was a gift from Dabur Research Foundation (Ghaziabad, India). Sodium alginate (viscosity of 1% solution is 54 mPa at 25°C) was purchased from SD Fine Chemicals (Mumbai, India). Eudragit S-100 was obtained from Ranbaxy Laboratory Ltd (Haryana, India). Liquid paraffin was from SD Fine Chemicals; and calcium chlorides, potassium dihydrogen phosphate, cyclohexane, Span 80, Span 85, Tween 80, methanol, and dichloromethane were purchased from Merck Limited

(Mumbai). All other reagents were of analytical grade or better.

Methods

Preparation of Core Alginate Microspheres

Different formulations of Paclitaxel microspheres were prepared as shown in Table 1, using the method of Calis et al[16] with some modifications. The Paclitaxel was dispersed in an aqueous solution of 5% wt/vol sodium alginate. The solution was emulsified in liquid paraffin containing 2% vol/vol Span 80 using a mechanical stirrer (Remi Instruments Ltd, Mumbai, India) at 1200 to 1500 rpm for 1 hour. A calcium chloride solution (5% wt/vol in isopropanol) was added to the emulsion at the rate of 2 mL/min. The emulsion was stirred for 10 more minutes. Microspheres were collected by filtration and washed 3 times with cyclohexane to remove liquid paraffin (Figure 1). Microspheres were deep-frozen at –50°C for 12 hours (Premium U410, New Brunswick Scientific Co Inc, Edison, NJ) and freeze-dried at –100°C (Heto Dry Winner 10–110, Jouan Nordic A/S, Allerod, Denmark) for 10 hours.

Encapsulation of Core Microspheres

Various formulations of coated microspheres were prepared by varying the core-to-coat ratio (Table 1) by the solvent evaporation technique. Core microspheres (A1) were dispersed in the Eudragit S-100 solution (10% wt/vol) in the methanol and dichloromethane mixture (1:4). The Eudragit S-100 and core microspheres dispersions were emulsified in liquid paraffin containing 1% vol/vol Span 85 and 0.1% vol/vol antifoam A, respectively, by a mechanical stirrer at 1200 to 1500 rpm. Stirring was continued for 2 to 3 hours to ensure that all the solvent was evaporated (Figure 1). Encapsulated microspheres were obtained by filtration and washed 3 to 4 times with cyclohexane to remove liquid paraffin, then vacuum-dried in desiccators for 48 hours.

Incorporation Efficiency

Drug-loaded core microspheres (25 mg) were washed with 10 mL of 0.2M monobasic potassium phosphate buffer of pH 6.8 to remove the surface-associated drug. Then microspheres were kept in phosphate buffer for digestion for 24 hours and sonicated for 1 hour at room temperature. The samples were centrifuged at 1000*g* for 10 minutes to remove any insoluble solids, the supernatant layer was removed, the membrane was filtered, and the drug content was determined using the reverse phase high-performance liquid chromatography (RP-HPLC) method. Incorporation efficiency was calculated using the following formula:

$$\text{Incorporation efficiency} = b\,a \times 100 \quad (1)$$

where a is the theoretical drug content and b is the drug entrapped. The incorporation efficiency of coated microspheres was determined as described above after removing the Eudragit S-100 coating by washing with methanol.

Surface Morphology

The shape and surface characteristics of the microspheres were observed by scanning electron microscopy (Leo 435 VP, Carl Zeiss NTS GmbH, Oberkochen,

Germany). The freeze-dried microspheres were coated with gold using a sputter coater (Agar sputter coater, Agar Scientific, Stansted, UK) under high vacuum (100 mTorr) and high voltage (1.2 kV and 50 mA) to achieve a film thickness of 30 nm. The samples were imaged using a 15-kV electron beam.

Determination of Particle Size and Particle Size Distribution

The particle size distribution of core and coated microspheres was measured by (Malvern Zetasizer, France). Weighed microspheres (50 mg) were suspended in triple-distilled water (2 mL) and vortexed before measurement. The obtained homogenous dispersion was examined to determine particle size distribution.

X-Ray Diffraction (XRD) :

X-ray diffraction analysis was carried out using Nonius PDS 120 diffracttometer. The monochromatic radiation of wavelength, 1.488A was supplied by a Nonius FR 590 generator equipped with a monochromator. Paclitaxel, core microsphers and coated microsphers recorded by Nonius CPS 120 detector.

Micromeritic Properties of Coated Microspheres

The flow properties of coated microspheres were investigated by determining the angle of repose, bulk density, and tapped density. The angle of repose was determined by the fixed-base cone method. Bulk and tapped densities were measured in 10 mL of a graduated cylinder. The sample contained in the cylinder was tapped mechanically by means of a constant-velocity rotating cam. The tapped volume was noted down when it showed no change in its value and bulk density and tapped density was calculated. Each experiment was performed 3 times.

In vitro Release Studies From Alginate Paclitaxel Microspheres

The horizontal shaker method was used to study *in vitro* release profile of core alginate microspheres.[17] Core microspheres equivalent to 2 mg of Paclitaxel were suspended in 10 mL of phosphate buffer of pH 7.4 containing 0.02% wt/vol Tween 80 at 37 ± 0.2°C and 60 rpm. Various replicates were placed in a biological shaker. Samples were withdrawn at specified time intervals (1, 2, 3, 4, 6, 8, and 10 hours) and centrifuged at 1000*g* for 10 minutes; then supernatant was membrane filtered and assayed for drug release by the RP-HPLC method. For each formulation, determination was performed 3 times.

In vitro Release Studies From Coated Microspheres

Coated microspheres equivalent to 2 mg of Paclitaxel were placed in 10 mL of pH progression medium at 37 ± 0.2°C and 60 rpm in a biological shaker (to simulate gastrointestinal tract conditions) containing 0.02% wt/vol Tween 80 to improve the wettability of microspheres. The pH of the medium was gradually increased: 5.8 during the first 2 hours, 6.8 during the next 2 hours, and 7.4 until the end of the experiment. At specific time intervals (2, 4, 6, 8, 10, 12, 14, 16, 20, and 24 hours), samples were withdrawn and centrifuged at 1000*g* for 10 minutes; then supernatant was membrane filtered and assayed for drug release by the RP-HPLC method. Drug release studies were also performed in 0.01N HCl (pH 2.0) for 2

hours. For each formulation, determination was performed 3 times.

HPLC Analysis

The HPLC system (Shimadzu, Japan) consisted of 2 LC 10AT VP pumps, a variable wavelength programmable UV-Vis detector SPD-10A VP, a system controller SCL-10AVP, and an Waosil 5C18 RS column (250 mm X 2.0 mm). It was equipped with the software Class VP series version 5.0. A manual injection valve was equipped with a 20-µL sample loop injector. Quantitation was performed according to an earlier reported method, with slight modification.[18] All HPLC assays were performed isocratically at ambient temperature. The mobile phase was 0.05M phosphate buffer (monobasic potassium phosphate) at pH 3.0, filtered through a 0.45-µm membrane filter and degassed prior to use. The flow rate was 0.25 mL/min. The eluent was detected by UV detector at 230 nm. The standard curve was constructed for Paclitaxel in the concentration range of 1 to 10 µg/mL. A good linear relationship was observed between the concentration of Paclitaxel and the peak area (R^2 = 0.9999). The detection limit was found to be 0.1 µg/mL. The retention time was found to be 7 minutes. The required studies were performed to estimate the precision and accuracy of this HPLC method for analysis of Paclitaxel. The HPLC method used in the study was found to be precise and accurate, as indicated by less than 1.3% coefficient of variation (CV) (intra- and interday) and high recovery of 99.6% to 100.2% of Paclitaxel. The standard curve constructed as described above was used for estimating Paclitaxel in entrapment efficiency.

Thermal Studies

Thermograms of the samples were obtained by a Perkin-Elmer differential scanning calorimeter (Switzerland). Samples of 3 mg were accurately weighed into aluminum pans and then hermetically sealed with aluminum lids. The thermograms of samples were obtained at a scanning rate of 10°C/min over a temperature range of 50 to 300°C. All tests were performed twice.

Stability Studies

To assess long-term stability,[19] the core-coated microsphere formulations (E3) was put in hard gelatin capsules and sealed in aluminum packaging coated inside with polyethylene. The studies were performed at 40°C/75% relative humidity (RH) in the stability chamber (Stability Oven, Nirmal Instruments, Delhi, India) for 6 months. At the end of the storage period, the formulation was observed for physical appearance, size, shape, surface morphology, drug content, *in vitro* drug release, and differential scanning calorimetry (DSC) studies.

Data Analysis

Statistical evaluation of data was performed using an analysis of variance (ANOVA) and, depending on the outcome of the ANOVA Student-Newman-Keuls multiple comparison test, the evaluation data was used to assess the significance of differences. To compare the significance of the difference between the means of 2 groups, the Student *t* test was performed; in all cases, a value of $P < .05$ was accepted as significant.

RESULTS AND DISCUSSION

Incorporation Efficiency

The incorporation efficiency of various formulations is given in Table 2. The incorporation efficiency was higher for the formulations with a polymer to cross-linking agent ratio of 1:1.5 (% wt/wt) and a cross-linking time of 10 minutes (formulations A1, A2, A3, B1, B2, B3, and B4). The incorporation efficiency decreased progressively with increases in drug concentration (formulations A1, A2, and A3), suggesting that an insufficient amount of alginate was available to entrap the drug. The incorporation efficiency was also found to be proportional to the sodium alginate concentration (formulations B1, B2, B3, and B4). An inverse relationship was found between the incorporation efficiency and the time of cross-linking and concentration (formulations C1, C2, C3, D1, and D2). The decrease in the incorporation efficiency with an increase in the cross-linking time and concentration could be attributed to incomplete emulsification as a result of higher viscosity of the external oil phase, as the cross-linking agent was present in the external phase. In the present study, the low concentration of calcium used was found sufficient to decrease the porosity of alginate matrixes, as shown by the higher incorporation efficiency of Paclitaxel. The higher incorporation efficiency of coated microspheres (formulations E1, E2, and E3) was due to the fact that the drug was inside the core microspheres and that the solvents methanol and dichloromethane dissolved the Eudragit S-100 while maintaining the integrity of the core microspheres.

Morphology, Size of Microspheres, and Micromeritic Properties of Microspheres

Scanning electron microscopy revealed that alginate microspheres were discrete and spherical in shape with a rough outer surface because of the surface-associated crystals of the drug (Figure 2). Table 2 indicates that a higher ratio of drug and polymer is associated with increase microsphere size. A decrease in the alginate concentration (2% wt/vol) resulted in the clumping of microspheres, whereas a higher sodium alginate concentration (6% wt/vol) resulted in the formation of discrete microspheres with an average diameter of 78 µm. This could be due to higher viscosity at a higher concentration and formation of larger microspheres. Interestingly, an increase in the mean diameter of microspheres was observed with an increase in calcium chloride concentration and time of cross-linking (formulation C1, C2, C3, D1, and D2). This could be explained by the fact that more of the calcium ions became available for cross-linking guluronic acid units of sodium alginate, resulting in the formation of more cross-linked alginate, which in turn could increase the viscosity of the formulation, leading to the formation of larger microspheres. The diameter of the core microspheres was in the range of 20 to 52 µm. A scanning electron microscopy photograph of coated alginate microspheres showed that the microspheres were discrete and spherical in shape, with a smooth outer surface (Figure 3). The size of coated microspheres ranged from 107 to 178 µm. X-ray diffraction analysis was performed to determine whether the entrapped paclitaxel existed in the less water-soluble crystalline state or the more soluble amorphous state. Sharp peaks in X-ray

diffraction spectra indicate a crystalline structure. Results in (Figure 4) show sharp peaks for paclitaxel and the core microsphers and, but not for coated microsphers . This indicates that the paclitaxel entrapped in the nanoparticles existed in an amorphous All formulations showed excellent flowability, as represented in terms of angle of repose (<40°)[20] (Table 2). The angle of repose of coated microspheres (formulations E1, E2, and E3) was smaller than that of core microspheres (formulations A1, A2, A3, B1, B2, B3, B4, C1, C2, C3, D1, and D2), possibly because the core microspheres had a rough surface. The bulk and tapped densities indicate that microspheres have good packability. The improvements of micromeritic properties suggest that microspheres can be easily handled.

In vitro Release Studies

The *in vitro* release profile of different core alginate microsphere formulations is shown in Figure5 and Figure 6. There was no significant difference in rate and extent of drug release from formulations A1, A2, and A3 ($P > .05$). The effect of polymer on the drug release is shown in Figure 4. A significant ($P < .05$) difference in the rate and extent of drug release was observed in formulation A1 compared to formulations B1, B2, B3, and B4. This could be attributed to an increase in the density of the polymer matrix and the diffusional path length that the drug has to traverse. The release of Paclitaxel was characterized by a burst release followed by a moderate, slow release. The biphasic pattern of drug release is characteristic of matrix diffusion kinetics.[21] The burst release can be reduced by increasing the polymer concentration, resulting in better incorporation efficiency, as discussed earlier, and a decrease in surface-associated drug. The effect of cross-linking agent concentration and cross-linking time is shown in Figure 6. The results indicate that rate and extent of drug release decreased significantly ($P < .05$). Sodium alginate is a linear copolymer consisting of β (1→4) mannuronic acid and α (1→4) L guluronic acid residues; a tight junction is formed between the residues of alginate with calcium ions. An increase in cross-linking time from 10 to 30 minutes ($P < .05$) significantly decreased the drug release. For an optimized formulation, the cross-linking time and the polymer to cross-linking agent ratio chosen were 10 minutes and 1:1.5 (wt/wt).

The second part of the formulation focused on the microencapsulation of the alginate core microspheres. The cores were microencapsulated by the solvent evaporation technique. The coating polymer, Eudragit S-100, dissolves above pH 7.0, thereby protecting the drug from releasing from the alginate core before reaching the colonic region. Once the enteric coating dissolves, it is expected that drug release would then be controlled by alginate in the target area. The *in vitro* release behavior of encapsulated microspheres was very dramatic (formulations E1, E2, and E3). As expected, no drug release occurred at gastric pH 2.0 for 2 hours. As shown in Figure 7, no drug release occurred below the pH of polymer solubility. After this lag time, drug release and the time for the total drug varied depending on the core-to-coat ratio. The release of Paclitaxel slowed down as the concentration of coating polymer increased ($P < .05$).

The *in vitro* release studies data were fitted into various release equations to explain the kinetics of drug release from

these microspheres. The kinetic models used were first-order,[22] zero-order, and Higuchi release[23] models. Linear regressions are summarized in Table 3. The examination of the determination R^2 coefficient indicated that drug release followed the diffusion control mechanism from the core and coated microspheres. To explore the kinetic behavior, *in vitro* release results were further fitted into the following Korsmeyer and Peppas equation[24]:

$$M t M \infty = K t n \quad (2)$$

where M_t/M_∞ is the fraction of drug released after time t, K is a kinetic constant, and n is a release exponent that characterizes the drug transport and was in the range of 0.3923 to 0.5028, indicating the Fickian drug diffusion (Table 4).

Thermal Characterization of Microspheres

DSC is very useful in the investigation of the thermal properties of microspheres, providing both qualitative and quantitative information about the physicochemical state of drug inside the microspheres.[25] There is no detectable endotherm if the drug is present in a molecular dispersion or solid solution state in the polymeric microspheres loaded with drug.[26] In the present investigation, DSC thermograms of pure drug, blank Eudragit S-100–coated core microspheres (formulation E3), drug-loaded Eudragit S-100–coated core microspheres (formulation E3), and drug and polymer physical mixtures in the same ratio as in formulation E3 were taken. As shown in Figure 8, prominent melting endotherms of pure Paclitaxel and a physical mixture of drug and polymer were found at 218°C and 262.8°C. Drug-loaded Eudragit S-100–coated core microspheres showed a broad small peak at 263.5°C, indicating the presence of drug in crystalline form. The reduction of height and sharpness of the endotherm peak is due to the presence of polymers in the microspheres.

Stability Studies

In view of the potential utility of formulation E3 for targeting Paclitaxel to the colon, the stability studies were performed at 40°C/75% RH for 6 months (climatic zone IV conditions for accelerating testing) to assess their long-term stability (2 years). The protocol conformed to the recommended World Health Organization document for stability testing of products intended for the global market.[17] After storage, formulation E3 was observed for physical appearance, particle size, particle shape (Figure 9), drug content, *in vitro* drug release, and DSC studies. Before and after storage at 40°C/75% RH for 6 months, *in vitro* release data (Table 5) were analyzed for dissolution efficiency.[27] No significant difference ($P > .05$) was found, and similarity factor f2 and dissimilarity factor f1[28] were found to be 97.5 and 0.9, respectively. There was an insignificant change in the particle size distribution and shape (Figure 9), indicating that formulation E3 could provide a minimum shelf life of 2 years. Similarly, there was no change in the DSC thermograms before and after storage of the formulation (Figure 8).

CONCLUSION

The results of our study clearly indicate that there is great potential in delivery of Paclitaxel to the colonic region as an

alternative to the conventional dosage form. However, more extensive pharmacokinetic and pharmacodynamic studies are needed before establishing colonic delivery of Paclitaxel as an alternative. Biocompatibility studies of the formulation additives must also be done. Sodium alginate is a biocompatible polymer; we expect it to cause no harmful effects if used for prolonged periods.

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Table 1- Various Formulations of Alginate Microspheres

F Code	Drug to Polymer Ratio (wt/wt)	Polymer to Cross Linking Agent Ratio (wt/wt)	Time of Cross Linking (min)
A1	1:1	1:1.5	10
A2	1.5:1	1:1.5	10
A3	2:1	1:1.5	10
B1	1:2	1:1.5	10
B2	1:3	1:1.5	10
B3	1:4	1:1.5	10
B4	1:5	1:1.5	10
C1	1:1	1:1	10
C2	1:1	1:2	10
C3	1:1	1:3	10
D1	1:1	1:1.5	20
D2	1:1	1:1.5	30
E1	1:5*	—	—
E2	1:6*	—	—
E3	1:7*	—	—

*Core microspheres to coating polymer ratio.

Table 2- Physical Characteristics of Core and Coated Microspheres*

F Code	Average Particle Size (µm)	Incorporation Efficiency (%)	Yield (%)	Angle of Repose	Bulk Density (g/cc)	Tapped Density (g/cc)
A1	34.67 ± 3.02	82.31 ± 1.13	92.19 ± 1.65	33.01 ± 2.09	0.668 ± 0.040	0.622 ± 0.031
A2	36.09 ± 2.45	74.44 ± 1.20	83.35 ± 2.98	32.78 ± 1.96	0.661 ± 0.032	0.631 ± 0.067
A3	44.98 ± 1.98	68.97 ± 1.87	77.05 ± 2.89	34.53 ± 2.98	0.664 ± 0.091	0.624 ± 0.056
B1	39.26 ± 5.36	91.72 ± 0.40	94.34 ± 3.67	33.33 ± 1.45	0.677 ± 0.076	0.657 ± 0.101
B2	35.28 ± 4.39	96.00 ± 1.89	95.87 ± 2.19	31.90 ± 0.78	0.656 ± 0.043	0.645 ± 0.072
B3	26.35 ± 1.09	97.19 ± 1.00	96.37 ± 2.90	30.75 ± 1.05	0.682 ± 0.051	0.658 ± 0.100
B4	27.99 ± 3.56	98.68 ± 3.52	99.00 ± 1.80	33.54 ± 2.22	0.693 ± 0.053	0.642 ± 0.098
C1	22.08 ± 3.11	70.16 ± 1.57	88.76 ± 0.98	33.39 ± 3.01	0.675 ± 0.086	0.651 ± 0.039
C2	33.27 ± 6.89	61.12 ± 1.72	79.87 ± 1.64	31.69 ± 0.45	0.669 ± 0.066	0.653 ± 0.099
C3	49.56 ± 2.57	53.93 ± 2.00	63.23 ± 2.22	32.78 ± 2.64	0.652 ± 0.103	0.633 ± 0.048
D1	50.97 ± 1.11	62.01 ± 1.98	77.32 ± 3.27	32.87 ± 1.03	0.659 ± 0.097	0.655 ± 0.084
D2	52.67 ± 2.06	45.52 ± 1.87	78.87 ± 0.78	32.23 ± 0.67	0.669 ± 0.085	0.661 ± 0.059
E1	122.47 ± 5.60	99.45 ± 1.34	92.87 ± 2.34	29.34 ± 1.97	0.550 ± 0.076	0.571 ± 0.034
E2	123.34 ± 6.64	98.00 ± 0.34	93.98 ± 1.23	29.56 ± 2.06	0.558 ± 0.033	0.589 ± 0.045
E3	141.09 ± 1.35	97.76 ± 0.34	93.45 ± 0.98	28.98 ± 1.89	0.554 ± 0.026	0.581 ± 0.019

*Results shown are the mean ± SD. n = 6 for particle size and n = 3 for yield, incorporation efficiency, angle of repose, bulk density, and tapped density.

Table 3- *In vitro* Release Kinetic Parameters of Microspheres

F Code	Zero Order			First Order			Higuchi		
	K(mg/h)	*R*	R^2	K(h^{-1})	*R*	R^2	K($mg/h^{1/2}$)	*R*	R^2
A1	0.1412	0.9479	0.8985	0.2797	–0.9754	0.9515	0.2656	0.9980	0.9960
A2	0.1327	0.9406	0.8848	0.2674	–0.9783	0.9572	0.2436	0.9881	0.9764
A3	0.1281	0.9466	0.8961	0.2885	–0.9832	0.9666	0.2519	0.9880	0.9860
B1	0.1201	0.9414	0.8863	0.1745	–0.9821	0.9646	0.2290	0.9917	0.9834
B2	0.1093	0.9597	0.9211	0.1325	–0.9913	0.9827	0.2064	0.9926	0.9854
B3	0.0867	0.9424	0.8882	0.0858	–0.9675	0.9357	0.1650	0.9812	0.9628
B4	0.0889	0.9601	0.9218	0.0825	–0.9802	0.9608	0.1677	0.9900	0.9801
C1	0.1291	0.9304	0.8656	0.4655	–0.9754	0.9515	0.2474	0.9892	0.9785
C2	0.1212	0.9461	0.8952	0.1773	–0.9846	0.9695	0.2306	0.9941	0.9883
C3	0.1090	0.9717	0.9443	0.1345	–0.9949	0.9899	0.2039	0.9955	0.9911
D1	0.1314	0.9398	0.8833	0.2048	–0.9768	0.9541	0.2501	0.9783	0.9570
D2	0.1321	0.9531	0.9084	0.1794	–0.9831	0.9666	0.2497	0.9854	0.9711
E1	0.1161	0.9418	0.8870	0.3780	–0.9679	0.9393	0.3084	0.9692	0.9394
E2	0.1183	0.9745	0.9497	0.2743	–0.9421	0.8875	0.3075	0.9802	0.9609
E3	0.1186	0.9777	0.9558	0.2305	–0.9300	0.8649	0.3048	0.9835	0.9673

Table 4- Result of Korsmeyer-Peppas Equation Treatment of *In vitro* Drug Release Data

F Code	K	n	*R*	R^2
A1	0.3351	0.4887	0.9904	0.9909
A2	0.3725	0.4359	0.9867	0.9736
A3	0.3648	0.4500	0.9914	0.9829
B1	0.7291	0.4778	0.9865	0.9732
B2	0.2849	0.4622	0.9945	0.9890
B3	0.2718	0.4152	0.9910	0.9822
B4	0.2290	0.4614	0.9951	0.9903
C1	0.4200	0.4123	0.9863	0.9727
C2	0.3066	0.4798	0.9890	0.9781
C3	0.2954	0.4398	0.9974	0.9948
D1	0.3046	0.5028	0.9887	0.9775
D2	0.3580	0.3923	0.9122	0.8321
E1	0.0432	0.5157	0.9815	0.9633
E2	0.0311	0.4275	0.9925	0.9850
E3	0.0179	0.4173	0.9948	0.9807

Table 5- Percentage of Paclitaxel Released From the E3 Microsphere Formulation Before and After Storage at 40°C/75% Relative Humidity for 6 Months*

pH	Time (h)	Percentage Released	
		Before Storage	After Storage
5.8	2	0	0
6.8	4	0	0
7.4	6	4.2675 ± 0.4224	4.1567 ± 0.4563
	10	23.3474 ± 0.6384	23.1198 ± 1.234
	16	64.2717 ± 0.4855	62.8762 ± 2.098
	24	98.2834 ± 0.4855	99.0145 ± 1.7652

*Values indicate mean ± SD (n = 3).

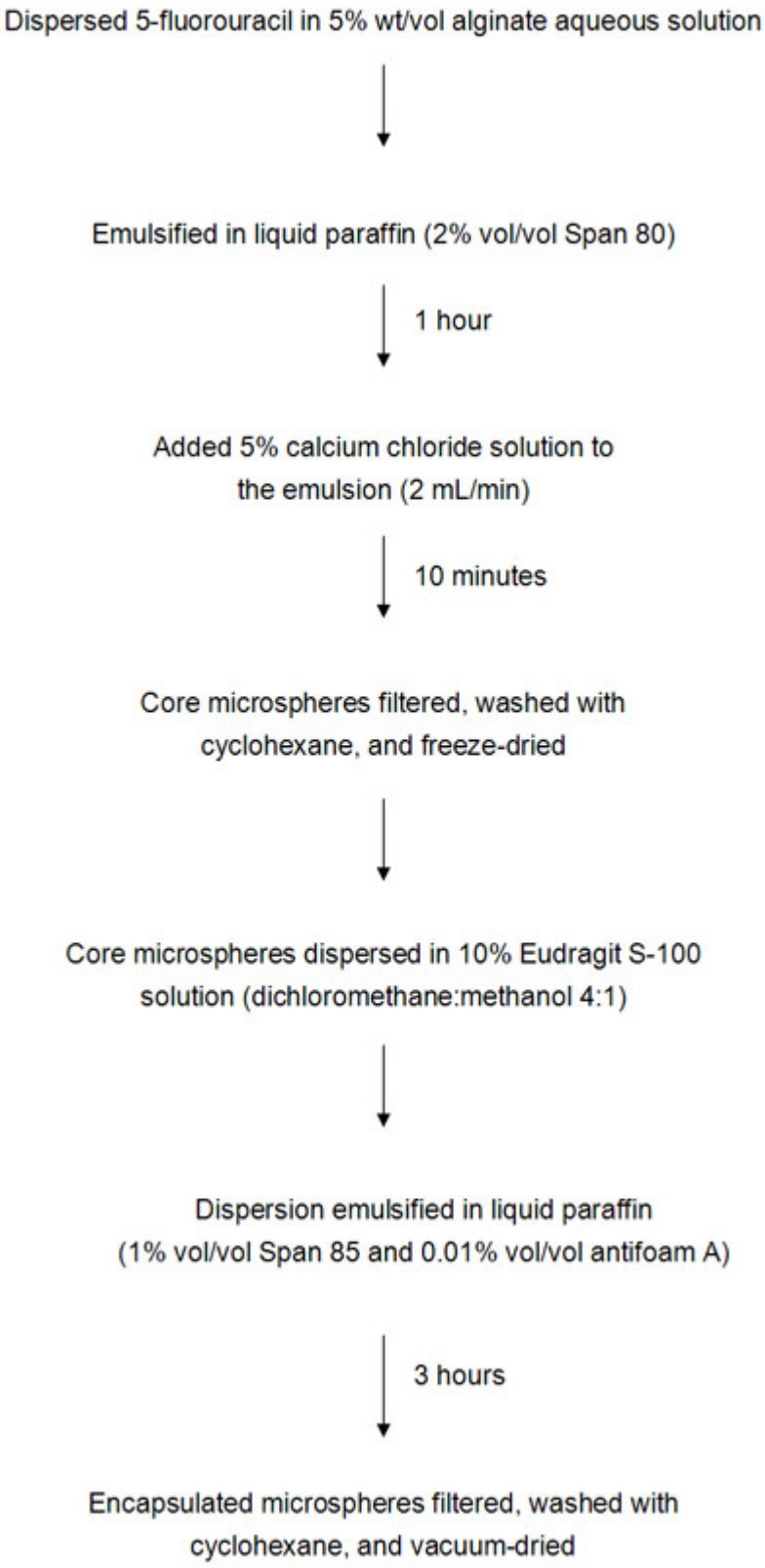

Figure 1- Schematic Illustration of Microsphere Preparation

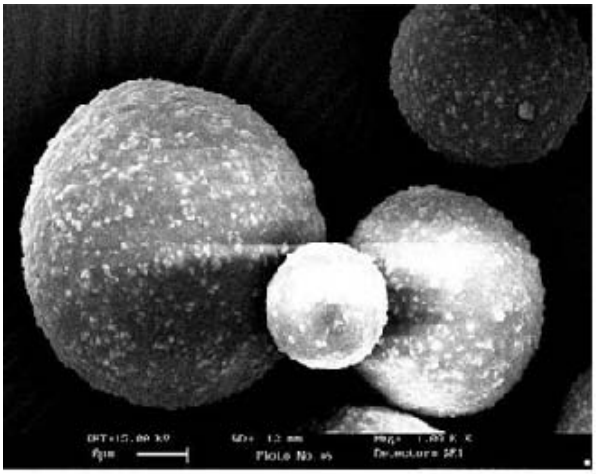

Figure 2. SEM Photograph of Core Alginate Microspheres, Formulation A1.

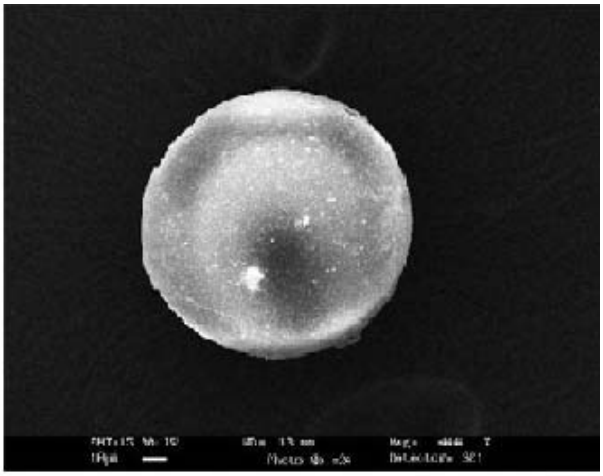

Figure 3. SEM Photograph of Eudragit S-100 Coated Core Microspheres, Formulation E3.

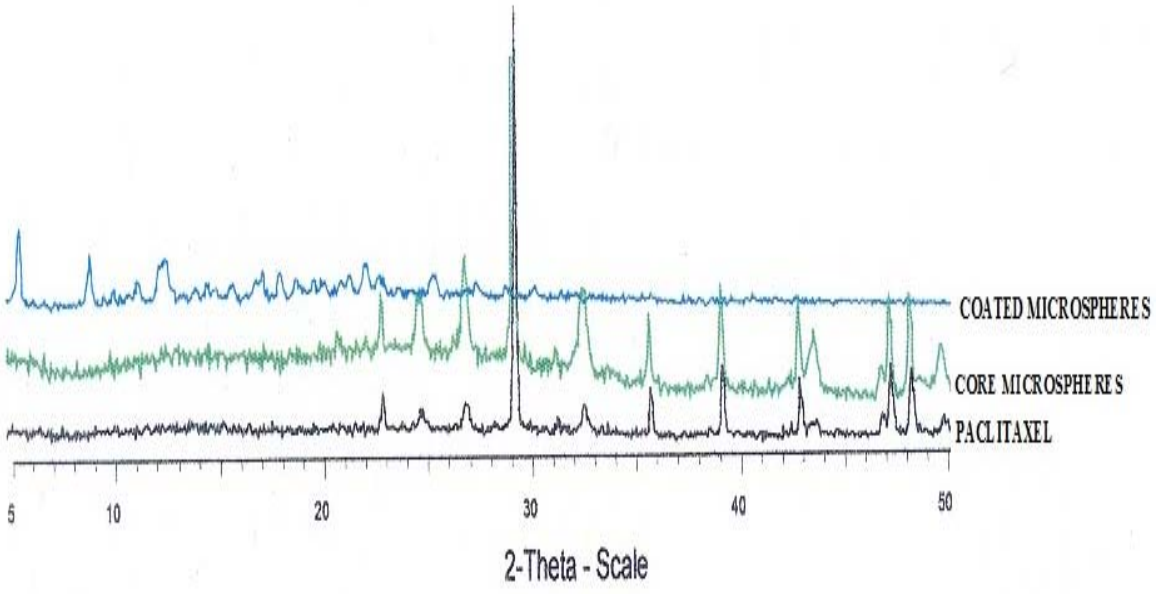

Figure 4- X-ray diffraction analysis of paclitaxel and the core microsphers and, coated microsphers.

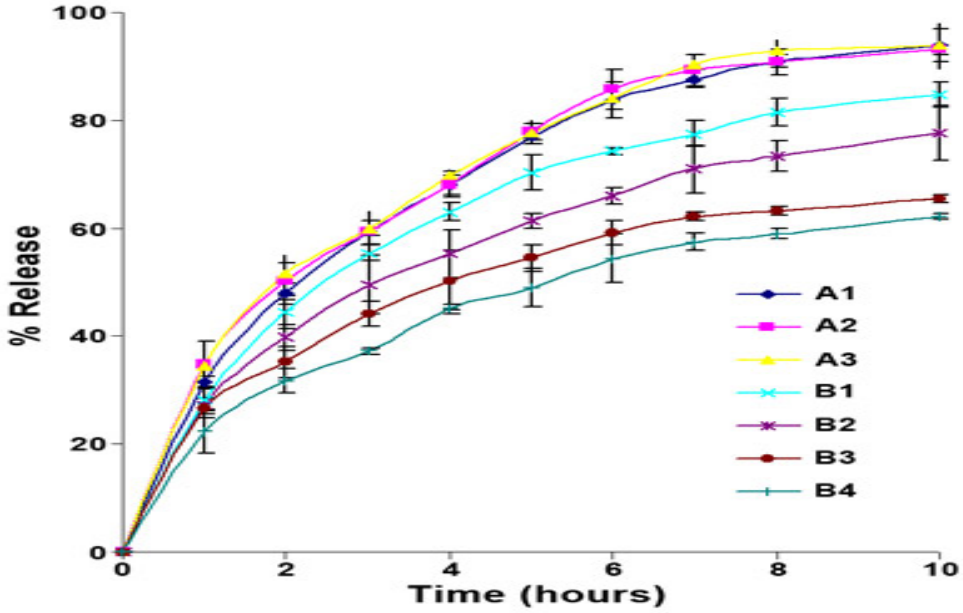

Figure 5- *In vitro* release profile showing the effect of drug and polymer on drug release from core alginate microspheres. Results indicate mean ± SD (n =3)

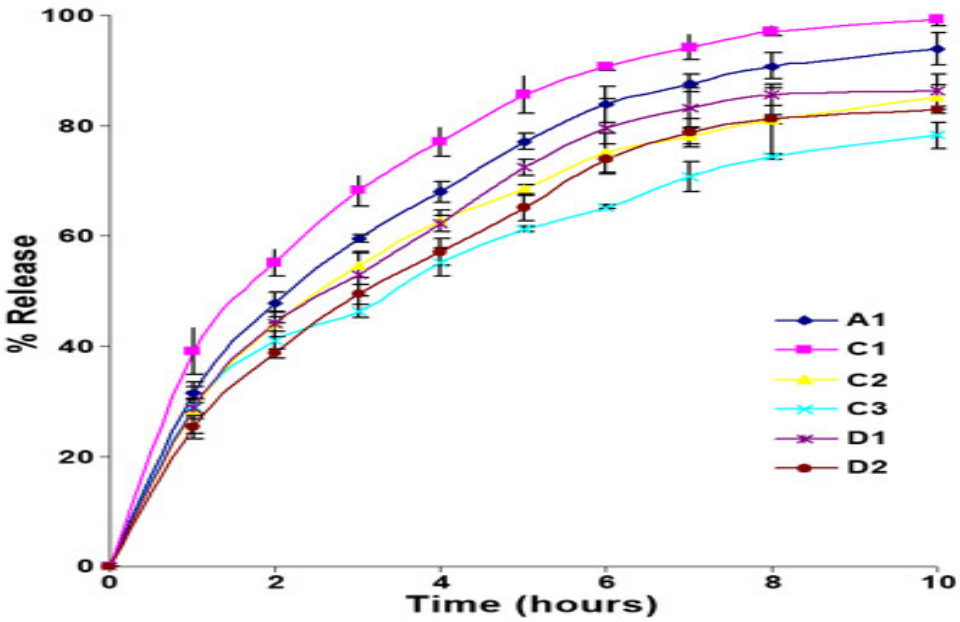

Figure 6. *In vitro* release profile showing the effect of cross-linking time and cross-linking agent concentration. Results indicate mean ± SD (n = 3).

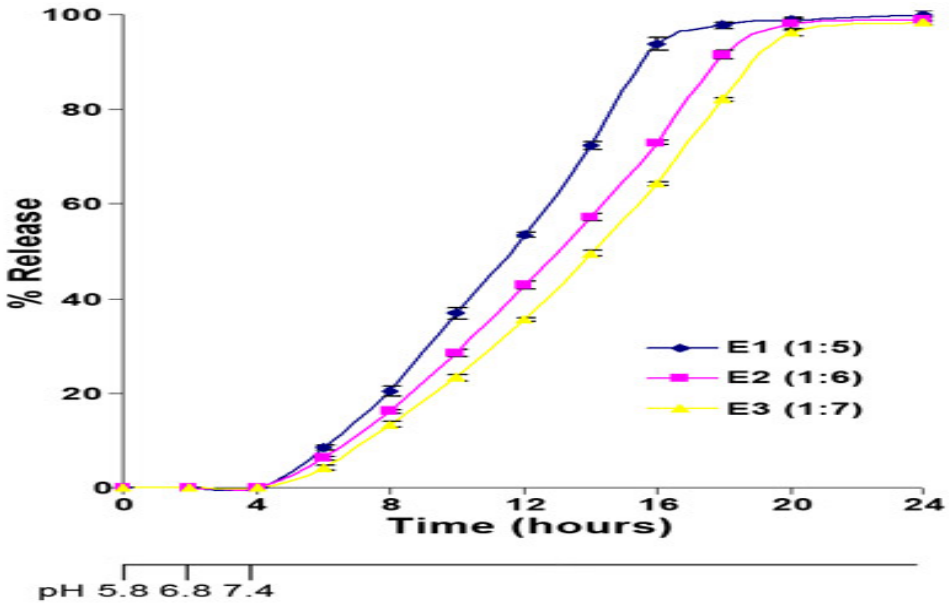

Figure 7- *In vitro* release profile of coated microspheres.Results indicate mean ± SD (n = 3).

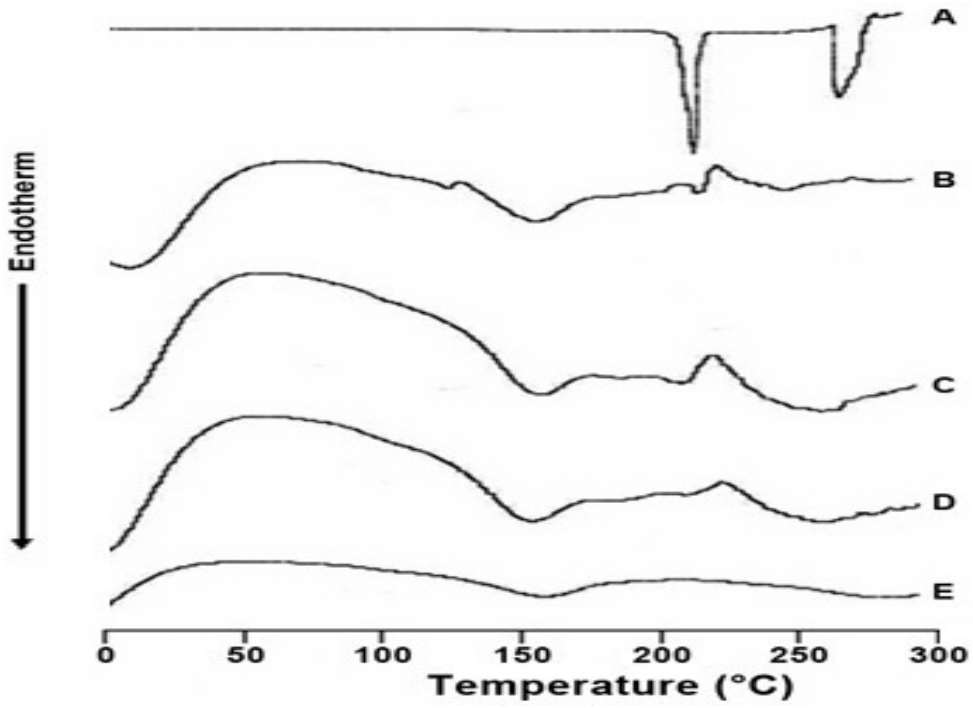

Figure 8- Differential scanning calorimetry thermogram of (A) Paclitaxel, (B) physical mixture of drug and polymer, (C) formulation E3, (D) formulation E3 after storage at 40°C/75% relative humidity for 6 months, and (E) blank microspheres, formulation E3.

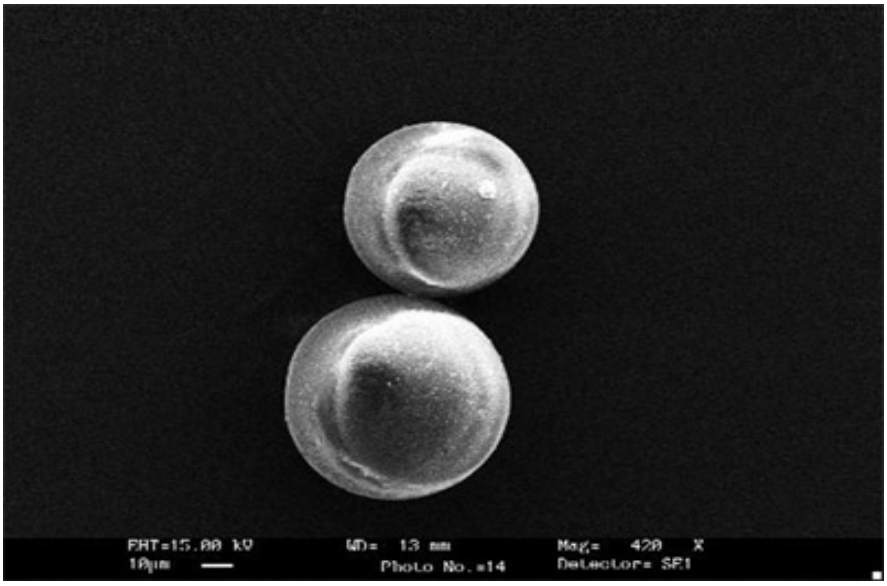

Figure 9- Scanning electron microscopy photograph of formulation E3 after storage at 40°C/75% relative humidity for 6 months