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DESIGN, PREPARE AND IN-VITRO EVALUATION OF PACLITAXEL MICROSPHERES

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ABSTRACT

Paclitaxel is an orally-administered chemotherapeutic agent used in the treatment of breast cancer. The present research was to formulate and optimize Paclitaxel-loaded microspheres targeting to enhance bioavailability, reduce dose, minimize side effect, and sustain drug release. The Paclitaxel chitosan microspheres were prepared by Ionotropic gelation technique method. The drug-excipient compatibility study of active drug (Paclitaxel) and polymer (chitosan) performed by Fourier transform-infrared spectroscopy and differential scanning calorimetry confirmed that there was no interaction. The resulting microspheres were evaluated for partial size, surface morphology, of Paclitaxel microspheres. Formulation F4 showed the maximum entrapment efficiency. Formulation F-4 showed percent entrapment efficiency of 94.60%. Percent yield value was found to be 80.42%. The particle size was found 145.21 µm A sustained release pattern was obtained from the microsphere and the drug's bioavailability was found to be enhanced. In vitro release study showed that Paclitaxel release from both kinds of microspheres was slow followed by an increase to reach a maximum of 95.30%

KEYWORDS: Paclitaxel, FTIR studies, sodium alginate, Ionotropic gelation technique, In vitro drug release studies.

INTRODUCTION

Microspheres are small spherical Particles, with a diameter in the micro meter Range (typically 1 micrometre to1000) micrometre Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials.[1] Microsphere have been extensively studied for use as drug delivery systems, where they have been shown to protect sensitive macromolecules from enzymatic and acid degradation, and allow controlled release and tissue targeting of the formulated drug. Paclitaxel is a major anticancer chemotherapeutic agent, extracted from the bark of the Pacific yew tree (Taxus brevifolia).[2] It is widely used against various types of solid tumours. Paclitaxel stops cell division in the late mitotic phase by preventing microtubules destruction and inhibits the proliferation of the cells.it has been clinically used in the treatment of various cancers especially breast and ovarian cancers.[3] The purpose of the present work was to develop Paclitaxel loaded microspheres and to determine the physicochemical characteristics (i.e. encapsulation efficiency, in vitro release, thermal profile, size distribution) of the developed microspheres.

MATERIALS

Paclitaxel was obtained from Hetero labs, HYD. Tragacanth and Sodium alginate procured from SD fine chemicals Mumbai. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY

Drug and Excipient compatibility studies[4]

Drug excipients compatibility studies were performed to know the compatibility of excipient with drug at accelerated conditions. The study was conducted by preparing homogenous mixture of excipients with drug and filled in HDPE bags and LDPE bags. Glass vials were exposed to 600 C and 400C/75 %RH for 4 weeks and LDPE bags were exposed to 400C±75 %RH for 4

weeks. Samples were observed periodically for any physical change.

Preparation and Evaluation of paclitaxel microspheres

Selection of polymers for preparation of microspheres[4]

Polymers were used as excipients for drug formulations, and cellulose derivatives are also used for the formulation of Microspheres. In the present study, for the preparation of Microspheres of Paclitaxel.

Preparation method of microspheres[5]

The microspheres of were prepared by using ionotropic gelation technique. In this method weighed quantity of drug and other polymers listed in Table 1. Polymeric solution (Sodium alginate and Tragacanth) and thoroughly mixed at 500 rpm. Resultant solution was extruded drop wise with the help of syringe and needle into 100 ml aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 10 minutes the obtained microspheres were washed with water and dried at 40 degrees - 3hours in a hot air oven and stored in desiccators.

Formulation table:

Table 1: Preparation of paclitaxel microspheres.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Drug	10	10	10	10	10	10	10	10
Tragacanth	100	200	300	400	-	-	-	-
Sodium alginate	-	-	-	-	100	200	300	400

Evaluation of microspheres[6,7,8]

The prepared Microspheres were evaluated for various parameters such as yield, particle size; drug entrapment efficiency, evaluation of in vitro drug and the effect of different formulation and process variables such as drug to polymer ratio, type of polymer, speed, and combination of polymers were studied.

Yield of microspheres

The yield of Microspheres was calculated from the amount of Microspheres obtained divided by the total amount of all non-volatile components

$$\% \text{ Yield } = \frac{Actual\ Weight\ of\ the\ Microspheres}{Total\ weight\ of\ all\ non-volatile\ components} \ x\ 100$$

Particle Size and Shape

The particle size of the Microspheres was measured by optical microscopy. The eyepiece micro meter was calibrated using a stage micrometer and the calibration factor was used further in the calculation of the size of Microspheres. The Microspheres were finely spread over a slide and visualized under an optical microscope using an eyepiece micrometer. About 50 readings were taken at random and the mean ± standard deviation was calculated. The shape of the Microspheres was visualized and the photographs were taken with the aid of a binocular microscope.

Surface morphology of the microspheres

The surface morphology of the Microspheres was studied with the aid of a Scanning Electron Microscope (SEM).

Drug Entrapment Efficiency (DEE)

The amount of drug entrapped was estimated by crushing 50 mg of Microspheres using mortar and pestle, and extracting drug with aliquots of 7.4 pH buffer repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using7.4 pH buffer. The solution was taken in a beaker and sonicated in a bath sonicator for 2 hours. The solution was filtered and absorbance was measured after suitable dilutions spectrophotometrically at 329 nm against an appropriate blank.

The amount of drug entrapped in the Microspheres was calculated using the following formula –

$$DEE = \frac{Amount\ of\ Drug\ Actually\ Present}{\text{Theoretical Drug Load Expected}} \ x\ 100$$

In vitro drug release study[9]

In vitro drug studies were carried out for all formulations in Franz diffusion cell. Microspheres equivalent to 10 mg of Paclitaxel were poured into1 ml aliquots were withdrawn at a predetermined intervals and equal volume of dissolution medium was replaced to maintain sink conditions. The necessary dilutions were made with 7.4 pH buffer and the solution was analyzed for the drug content spectrophotometrically using UV-Visible spectrophotometer (Lab India) at 329 nm against an appropriate blank. Three trials were carried out for all formulations. From this cumulative percentage drug was calculated and plotted against function of time to study the pattern of drug.

Drug release kinetics[10]

The models used were zero order (equation 1) First order (equation 2) and Higuchi model (equation 3) and Koresmeyer Peppas model (equation 4).

***i) Zero order kinetics*:**

$R = Ko\ t$ -- (1)

R = cumulative percent drug

Ko = zero order rate constant

ii) First order kinetics

$\log C = \log Co - K_1\ t / 2.303$ -- (2)

Where C = cumulative percent drug

K_1 = first order rate constant

iii) Higuchi model

$R = K_H\ t^{0.5}$ -- (3)

Where R = cumulative percent drug
K_{H} = higuchi model rate constant

iv) Korsermeyer Peppas Model:

$M t / M \alpha = K_k t^n$

$\log M t / M \alpha = \log K_{k+} n \log t$ -- (4)

Where K_k = Korsermeyer peppas rate constant

'M t / M α' is the fractional drug, n = diffusional exponent, which characterizes the mechanism of drug (Simon Benita, 2007).

Diffusional exponent (n) Drug Mechanism

0.43 -- Fickian diffusion
0.43- 0.85 -- Anamolous (non- fickian) transport
0.85- 1 -- Case II transport
> 1 -- Supercase II transport

The obtained regression co-efficient (which neared 0.999) was used to understand the pattern of the drug from the Microspheres.

Stability Studies[11]

The success of an effective formulation can be evaluated only through stability studies. The prepared Paclitaxel Microspheres placed on plastic tubes containing desiccant and stored at ambient conditions, such as at room temperature, 40±2°c and refrigerator 2-8°c for a period of 3 months.

RESULTS AND DISCUSSION

Drug - Excipient Compatibility Studies (FT-IR)

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals.

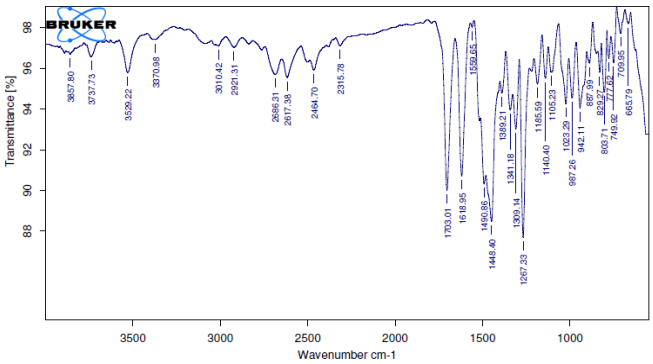

Fig. 1: FT-IR Sample for Paclitaxel.

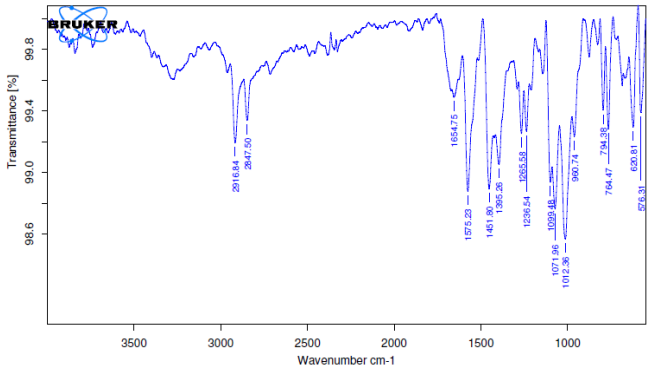

Fig. 2: FT-IR Sample for Optimized Formulation.

Formulation and Evaluation of sustained release microspheres of paclitaxel

Optimization of formulation variables

Therefore, the optimized conditions for the formulation of sustained release microspheres were:

Results of the evaluation parameters of formulated sustained release microspheres

The prepared sustained release microspheres were evaluated for various parameters such as yield, drug entrapment efficiency, particle size, and in vitro drug release. And effect of preparation and process variables such as drug polymer ratio, speed, type of polymer and combination of polymers on particle size, yield, entrapment efficiency, and *in-vitro* release of Paclitaxel from sustained microspheres were also studied.

Characterization of microspheres

Surface Topography by Scanning Electron Microscopy (SEM)

Figure shows SEM photograph of optimized microspheres at 100× magnification, at 1000× magnification. SEM photographs showed discrete, spherical microspheres. SEM photographs also showed the presence of drug crystal on the surface of

microspheres revealing that the microspheres were having some rough surface**.** The drug crystals on microspheres were may be due to the presence of un-entrapped drug in dispersion medium.

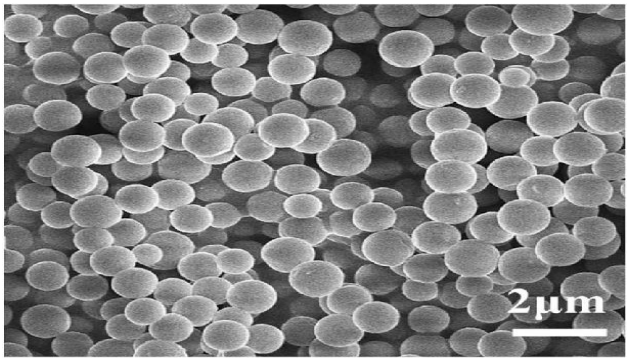

Fig. 3: SEM Analysis of Microspheres.

Table 2: Evaluation parameters of microspheres.

Formulation Code	% Yield	Particle Size	Drug Entrapment Efficiency
F1	70.22	142.30	86.45
F2	68.20	153.81	82.39
F3	70.32	140.15	86.46
F4	80.42	145.21	94.60
F5	75.75	138.20	82.36
F6	74.12	125.21	90.21
F7	69.35	129.55	79.30
F8	72.80	130.20	78.92

In vitro drug release studies

Table 3: Cumulative % drug release.

Time (hours)	F1	F 2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	13.59	14.53	14.25	15.25	12.34	13.30	12.62	13.12
2	26.38	24.69	26.98	36.88	28.55	32.25	29.23	28.66
3	36.25	39.86	45.86	47.55	31.78	40.87	37.68	39.12
4	49.85	47.96	52.68	58.75	50.27	52.39	50.36	48.73
5	58.35	59.45	67.85	69.38	65.28	63.96	61.85	60.64
6	68.56	70.25	71.25	75.86	72.85	70.83	69.94	71.19
7	80.95	81.26	80.45	84.40	84.64	83.60	83.86	80.68
8	92.51	92.18	94.59	95.30	90.98	91.85	91.42	90.80

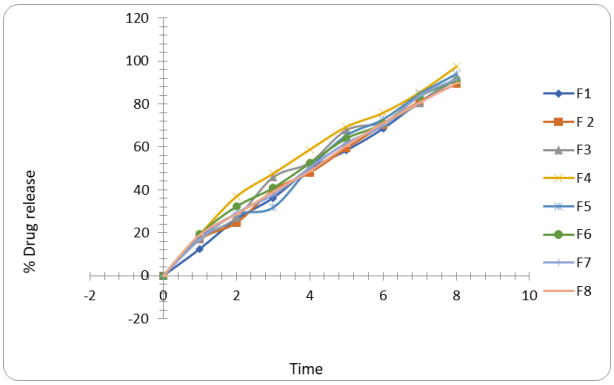

Fig. 4: Cumulative Percentage Drug Released Vs Time Curves of Microspheres F1–F 8 In pH 7.4 Buffer.

Here, keeping drug ratio constant and varied polymer ratio as the polymer concentration increases viscosity; this influences the interaction between disperse phase and dispersion medium that affects the size distribution

of particle. And F4 formulation shows good results when compared to other formulations.

CONCLUSION

Above graph indicates that %Drug release of F4 formulation shows better drug release when compared with other formulations

Release kinetics

The mechanism of Paclitaxel release from microspheres was studied by fitting the data obtained from *in-vitro* release studies into zero-order, first-order, Higuchi's, Korsermeyer peppas kinetic models. On application of different release kinetic models mentioned earlier, it was found that optimized formulations showed better fitting with the zero-order release and Korsermeyer peppas model.

Zero order kinetics:

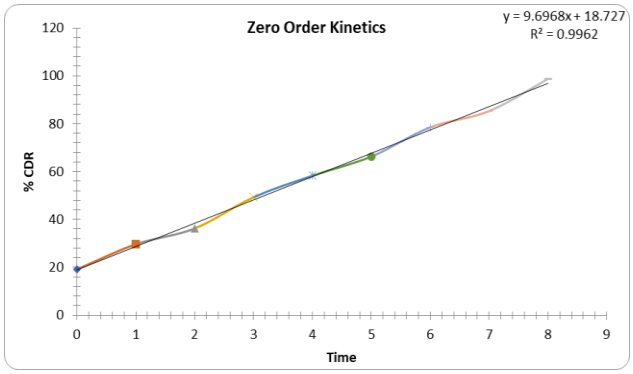

Fig. 5: Zero order plot for optimized formulation.

First order kinetics:

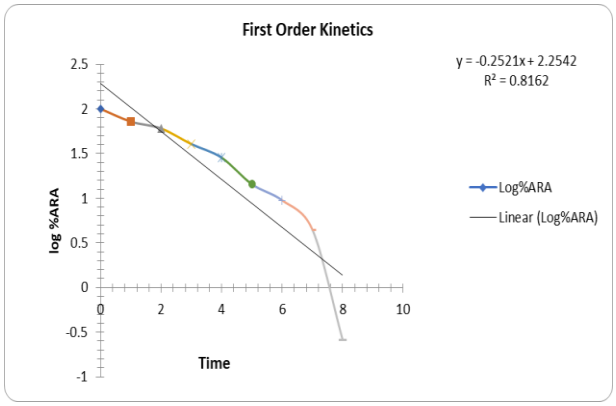

Fig. 6: First order kinetics for optimized formulation.

Higuchi model

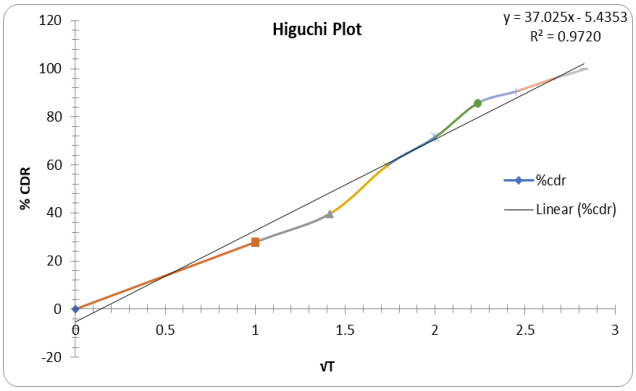

Fig. 7: Higuchi plot for optimized formulation.

Kross meyer peppas

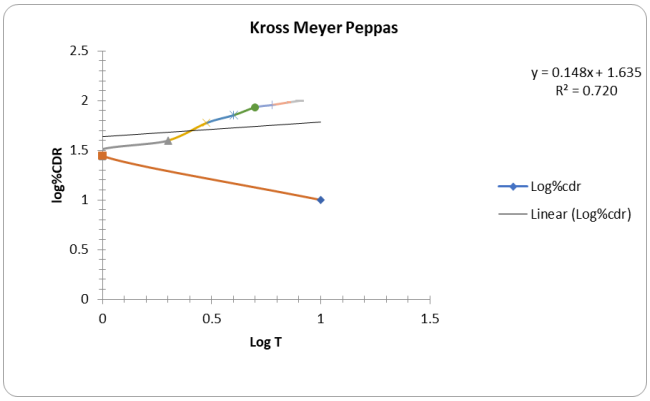

Fig. 8: Korsmeyer peppas plot for optimized formulation.

The drug release from the Paclitaxel microspheres was found to follow Zero order release based on the "r" value obtained for Zero order (0.996) and first order (0.816) for F4 formulation. Also, the drug release mechanism was found to be "Diffusion" based on the "r" value of 0.972 obtained for Higuchi's plot. Similarly, the drug release mechanism was found to be of Anomalous diffusion mechanism based on the "r ^{2}" value of 0.720 obtained for Peppa's equation.

Stability study

There was no significant change in physical and chemical properties of the formulation F-4 after 3 Months. Parameters quantified at various time intervals were shown.

Table 4: Results of stability studies of optimized formulation F-4.

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	25^0C/60%RH % Release	95.30	95.28	94.30	93.34	Not less than 85 %
F-4	30^0C/75% RH % Release	95.30	95.21	94.22	93.15	Not less than 85 %
F-4	40^0C/75% RH % Release	95.30	95.19	95.20	93.12	Not less than 85 %

CONCLUSION

As a novel drug delivery approach, Paclitaxel It is a cancer medicine that interferes with the growth of cancer cells and slows their spread in the body. It is loaded in microspheres prepared by sodium alginate and tragacanth as a polymer and to prepare polymeric microspheres which increases the bioavailability of the drug to the targeted area and in a controlled manner and reduces GI related side effects. Microspheres containing Paclitaxel as a core material were prepared by using Ionotropic gelation method. The yield and entrapment efficiency was high for microspheres were Particle size, entrapment efficiency and production yield were influenced by the type of polymer, polymer concentration, stirring speed and combination of polymers. *In vitro* dissolution of optimized formulations of various Polymers in pH 7.4 formulations are releasing the drug up to 8 hrs. F4 with high concentration of sodium alginate is considered as optimized formulation.

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