Vol 5|Issue 4| 2015 |338-344

International Journal
of
Pharmacy Review & Research
www.ijprr.com

e-ISSN: 2248 – 9207
Print ISSN: 2248 – 9193

DEVELOPMENT AND INVITRO CHARACTERIZATION OF PACLITAXEL SUSTAINED RELEASE MICROSPHERS

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ABSTRACT

The objective of the present work was to formulate and evaluate sustained release paclitaxel microspheres for treating breast, ovarian and colon cancer effectively. Sustained release paclitaxel microspheres for minimum five days depot which had been designed based on disease condition were prepared in the form of microspheres for subcutaneous administration for sustained release. Different formulations were prepared by using Solvent Evaporation technique and ionic gelation method using synthetic polymer like HPMC K 100, Eudragit RS 100, and Ethyl cellulose. The formulations were evaluated for percentage yield, entrapment efficiency, particle size analysis, and *In-vitro* drug release. The optimized formulation of the microspheres containing polymer and drug was found to be compatible from FTIR studies. The *In-vitro* release of drug from the formulations were studied in pH 7.4 phosphate buffer solution, and it was found that the prepared microspheres (F10) were able to sustained the release of the drug 48 hrs of about 42.28%. The release of the drug from the microspheres was found to follow zero order kinetics.

Key words: Paclitaxel microspheres, Sustained release, HPMC K 100, Eudragit RS 100, Ethyl cellulose.

INTRODUCTION

Cancer introduction

Cancer refers to any one of a large number of diseases characterized by the development of abnormal cells that divide uncontrollably and have the ability to infiltrate and destroy normal body tissue. Cancer also has the ability to spread throughout your body. Not all tumors are cancerous; tumors can be benign or malignant [1].

Introduction to breast cancer

Breast cancers are potentially life-threatening malignancies that develop in one or both breasts. The structure of the female breast is important in understanding this cancer:

- The interior of the female breast consists mostly of fatty and fibrous connective tissues.
- It is divided into about 20 sections called lobes [1]
- Each lobe is further subdivided into a collection of lobules, structures that contain small milk-producing glands.
- These glands secrete milk into a complex system of tiny ducts. The ducts carry the milk through the breast and converge in a collecting chamber located just below the nipple.
- Breast cancer is either noninvasive (referred to as *in situ*, confined to the site of origin) or invasive (spreading) [2].

Introduction of microspheres

There is growing interest in the development of homogenous monolithic drug release systems for various routes of administration. One very attractive type of such dosage from is micro spheres.

- Flexibility in design and development.
- Attractive in appearance.
- Better, improve the safety and efficiency of bio-active agents.
- Desired release pattern can be engineered [1, 2].

MATERIAL AND METHOD

Material

Paciltaxel, HPMC K15M,HPMC K100M**,** Eudragit RS100, Sodium Alginate, Ethylcellulose., PvP, Methanol, Isopropyl alcohol, Potassium di- hydrogen Ortho phosphate, Sodium Hydroxide, Calcium chloride, DMSO, Chloroform [2].

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Preparation of paclitaxel microspheres

Orifice ionic gelation method

Sodium alginate and polymers were dissolved in purified water separately. Paclitaxel is dissolved in DMSO. The drug was added to the polymer solution and mixed thoroughly with help of stirrer to form viscous dispersion. The resulting dispersion was added drop wise into 10% w/v calcium chloride solution through a syringe with needle (size no 22) with continues stirring at 500 rpm. The added droplets were retained in the calcium chloride solution for 15 minutes to produce spherical rigid microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45^0C for 12 hours and stored in desiccators [3].

Preparation of Microspheres by solvent evaporation method:

The microspheres were prepared by (o/w) solvent evaporation method, since paclitaxel is a water insoluble drug. Drug is dissolved in 3ml of DMSO. These polymers and drug are mixed vigorously. Then this mixture is dispersed with 10ml of chloroform and methanol in ratio of 7:3 mixed vigorously to form a clear solution. Then the above solution was emulsified by adding drop by drop into the aqueous solution containing 2% w/v of PVA as an emulsifier. Chloroform and methanol was removed at 35°C by evaporation. As the solvent was being removed, the emulsifier continued to maintain the oil droplets in their spherical configuration and prevented from aggregating until the solvent was completely removed, and the microspheres were hardened as discrete particles [4].

Preformulation studies

Preformulation studies are the first step in the rational development of dosage form of drug substance. Preformulation can be defined as investigation of physical and chemical properties of drug substance alone when combined with excipients. The following preformulation studies were performed for paclitaxel

- Solubility
- Melting point Determination.
- Density
- Carr's Index
- Hauser's Ratio
- Angle of repose
- FT-IR Spectral studies
- Stability Studie.

Solubility

The solubility of drug is an important physicochemical property because it affects the bioavailability of the drug, the rate of drug release into dissolution medium and consequently, the therapeutic efficiency of the pharmaceutical product. The solubility of the molecules in various solvents is determined as a first step. Solubility is usually determined in variety of commonly used solvents and some oils if the molecules are lipophilic. The solubility of material is usually determined by the equilibrium solubility method, which employs a saturated solution of the material, obtained by stirring an excess of material in the solvent for a prolonged until equilibrium is achieved. A known quantity of solute was dispersed in the solvent and based on following table the solubility was determined.

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Melting Point Determination

A characteristic of a pure substance is a defined melting point or melting range. If not pure, the substance will exhibit a change in melting point. This phenomenon is commonly used to determine the purity of a drug substance and in some cases the compatibility of various substances before inclusion in the same dosage form. it is determined by capillary tube method [5].

Bulk Density (Db)

It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder and the volume was noted. It is expressed in gm/ml and is given by

$$D_b = \frac{M}{Vb}$$

Where, M = is the mass of powder.
Vb =is the bulk volume of the powder.

Tapped Density (Dt)

It is the ratio of total mass of powder to the tapped volume of powder. The tapped volume was measured by tapping the powder for 750 times and the tapped volume was noted if difference between these two volumes is less than 2%. If it is more than 2%,tapping is continued for 1250 times and tapped volume was noted. Tapping was continued until the difference between successive volumes is less than 2% (in a bulk density apparatus). It is expressed in gm/ml and is given by

$$D_t = \frac{M}{Vt}$$

M= is the mass of powder.
$V_{t=is}$thetapped volume of the powder

Carr's Index (I): (or) % compressibility

It indicates the ease with which a material can be induced to flow. It is expressed in percentage and is given by

$$I=[Dt-Db/Dt] X100$$

Where,

D_t=is the tapped density of the powder.

D_b=is the bulk density of the powder.

Angle of Repose (θ)

The friction forces in a loose powder can be measured by the angle of repose θ. It is defined as maximum angle possible between the surface of a pile of powder and the horizontal plane. The powder mixture was allowed to flow through the funnel fixed to a stand at

definite height (h). The angle of repose was the calculated by measuring the height and radius of the heap of powder formed.

tan θ = (h/r)

$\theta = \tan^{-1}(h/r)$

Where, θ is the Angle of repose,
h is height of pile,
r is radius of pile,

Hauser's ratio

Hausner ratio is an indirect index of ease of powder flow. It is calculated by the following formula

Hausner's ratio = Dt/Db

Where, DT_{IS} the tapped density.

D_b is the bulk volume.

FT-IR Spectral studies

The IR spectra for the formulation excipients and pure drugs were recorded on BRUKER FT-Infrared spectrophotometer using ATR technique at the resolution rate of 2-2.5µm Spectrum was integrated in transmittance mode at the wave number range 400-4000 cm^{-1}

STABILITY STUDIES

Acid degradation studies

Solutions for acid degradation studies were prepared in 0.1N hydrochloric acid and the resultant solutions refluxed for 30 min.

Alkali degradation studies

Solutions for alkali degradation studies were prepared in 2 M Sodium hydroxide and the resultant solutions refluxed for 30 min.

Neutral degradation studies

Solutions for neutral degradation studies were prepared in water and the resultant solutions refluxed for 3 h.

Temperature stress studies

API powder was exposed to different temperature in an oven for 24 h.

Photo stability

Paclitaxel API, contents and solutions of paclitaxel were prepared and exposed to light to determine the effects of light irradiation on the stability of paclitaxel in solution and in the solid state. Approximately 50 mg of paclitaxel API powder was spread on a glass dish in a layer that was less than 2 mm thick. A solution of API (1 mg/ml) was prepared in Water. Tablets were prepared in the same way. All samples for photo stability testing were placed in a light and exposed to light. Control samples which were protected with aluminum foil were also placed in the light cabinet and exposed concurrently [5-6].

Analytical method Development by UV/Visible Spectrophotometer

Standard calibration with 7.4 pH phosphate buffer

Preparation of Stock solution

100 mg of paclitaxel was solubilized in methanol/ethanol then dissolved in 100 ml of 7.4 pH phosphate buffer in a 100 ml volumetric flask and made up to the volume with 7.4 pH phosphate buffer. From this 1 ml of solution was taken and made to 100 ml with 7.4 pH phosphate buffer.

Method

For the estimation of paclitaxel in 7.4 pH phosphate buffer the stock solution has to be diluted subsequently with 7.4 pH phosphate buffer to get a series of dilutions containing 2,4,6,8 &10 µg/ml of solution. The absorbances of these solutions were measured at 229 nm against blank [6].

Evaluation of microspheres

- Determination of percentage yield
- Drug entrapment efficiency
- Particle size analysis
- In-vitro drug release
- In-vitro release kinetics

Determination of percentage yield

Microspheres dried at room temperature were weighed and the yield of microspheres was calculated using the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield (gm)}}{\text{Theoretical yield}} \times 100$$

Drug entrapment efficiency

The amount of drug entrapped was estimated by dissolving the 100mg of microspheres in DCM and water in 3:1 ratio ,under vigorous shaking for 1hr, the resultant solution is centrifuged, both layers were separated, paclitaxel was soluble in water but not in DCM. The drug content in aqueous solution was analyzed by using UV at 229nm with further dilutions against appropriate blank.

The amount of the drug entrapped in the microspheres was calculated using the formula [6].

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

Particle size analysis

Determination of average particle size of paclitaxel microspheres with carrier was very important characteristic. It was measured by using Malvern Instruments, Startech Labs Pvt. LTD.

***In vitro* dissolution studies**

The *in vitro* release of drug from the micro particles was carried out in basket type dissolution tester USP XXIII, TDT-08L, with auto sampler containing 900 ml of 7.4 pH phosphate buffer for 24 hrs. The volume of the dissolution media was maintained at 900 ml with constant stirring (50 rpm) and temperature of bath was maintained at 37 ± 0.5°C. Aliquots (10 ml) of dissolution media were sampled at specified time intervals and replaced with fresh media immediately after sampling.

Samples were analyzed for drug content by UV visible spectroscopy (Shimadzu UV 1601).The release data obtained were fitted into various mathematical models. Dissolution studies were carried out for all the batches of the prepared formulations i.e. F1, F2, F3, F4, F5, F6,F7, F8, F9, F10, F11, F12, F13, F14, F15, F16, F17, F18. (18 batches) and compare.

***In-vitro* release kinetics**

To analyze the *In-vitro* release data various kinetic models were used to describe the release kinetics. The zero order rate Equation describes the systems where the drug release rate is independent of its concentration. The first order Equation describes the release from system where release rate is concentration dependent.

Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion.

The results of *In-vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows:

- Zero - order kinetic model – Cumulative % drug released versus time.
- First – order kinetic model – Log cumulative percent drug remaining versus time.
- Higuchi's model – Cumulative percent drug released versus square root of time.
- Korsmeyer equation / Peppa's model – Log cumulative percent drug released versus log time.

Zero order kinetics

Zero order release would be predicted by the following equation:

$$At = A_0 - K_{0t}$$

Where,

A_t = Drug release at time't'

A_0 = Initial drug concentration.

K_0 = Zero- order rate constant (hr-1)

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero – order kinetics and its slope is equal to Zero order release constant K_0**.**

First order kinetics

First - order release could be predicted by the following equation:

$$\text{Log } C = \log C_0 - K_t / 2.303$$

Where,

C = Amount of drug remained at time't'

C_0 = Initial amount of drug.

K = First - order rate constant (hr^{-1}).

When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant 'K_1' can be obtained by multiplying 2.303 with the slope value.

Higuchi's model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

$$Q = [D\epsilon / \tau (2A - \epsilon Cs) Cst] 1/$$

Where,

Q = Amount of drug release at time't'

D = Diffusion coefficient of the drug in the matrix.

A = Total amount of drug in unit volume of matrix.

Cs = Solubility of drug in the matrix.

ϵ = Porosity of the matrix.

τ = Tortuosity.

t = Time (hrs at which q amount of drug is released.

Above equation can be simplified as if we assume that 'D', 'Cs' and 'A' are constant. Then equation becomes:

$$Q = Kt_{1/2}$$

When the data is splitted according to equation i.e cumulative drug release versus square root of time yields a staright line, indicating that the drug was released by diffusion mechanism.the slope is equal to 'k'.ss

Korsmeyer equation / Peppa's model

To study the mechanism of drug release from the liposomal solution, the release data was also fitted to the well-known exponential equation (Korsmeyer equation/ Peppa's law equation), which is often used to describe the drug release behavior from polymeric systems.

$$Mt / M\alpha = Ktn$$

Where,

Mt / Mα = the fraction of drug released at time't'.

K = Constant incorporating the structural and geometrical characteristics of the drug / polymer system.

n = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified as follows by applying log on both sides,

$$\text{LogMt/M}\alpha = \text{LogK} + n\text{Logt}$$

Evaluation of microspheres

Percentage Yield, Entrapment Efficiency and Mean Particle size of Paclitaxel microspheres:

Table 1. Formulation of Microspheres Ionic gellation method (F1-F9)

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug	30 mg	30 mg	30mg	30mg	30mg	30mg	30mg	30mg	30mg
Hpmc k100	30mg	20 mg	20mg	_	10mg	_	_	10mg	_
Eudragit RS100	_	10 mg	_	30mg	20mg	20mg	_	_	10mg
Ethyle cellulose	_	_	10mg	_	_	10mg	30mg	20mg	20mg
DMSO	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml
Water	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml
Sodium alginate	60	60	60	60	60	60	60	60	60

Table 2. Formulation of Microspheres Solvent Evaporation method (F10-F18)

Ingredients	F10	F11	F12	F13	F14	F15	F16	F17	F18
Drug	30mg	30mg	30mg	30mg	30mg	30mg	30mg	30mg	30mg
Hpmc k100	30mg	20mg	20mg	_	10mg	_	_	10mg	_
Eudragit rs 100	_	10mg	_	30mg	20mg	20mg	_	_	10mg
Ethyle cellulose	_	_	10mg	_	_	10mg	30mg	20mg	20mg
Dmso	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml
Chloroform and methanol	7:3	7:3	7:3	7:3	7:3	7:3	7:3	7:3	7:3

Table 3. Calibration curve values for estimation paclitaxel in 7.4 PH phosphate buffer: Standard calibration values (Absorbance values) of paclitaxel

Sno	Concentration (µg/ml)	Absorbance
1	2	0.072
2	4	0.132
3	6	0.205
4	8	0.270
5	10	0.334

Table 4. Percentage Yield, Entrapment Efficiency and Mean Particle size of Paclitaxel microspheres from formulation F1 to F9

Batch code	Percentage yield (%)	Entrapment efficiency	Rounded mean particle size
F1	82.87	67.38	794
F2	78.53	71.47	752
F3	81.85	75.61	776
F4	76.56	76.91	609
F5	86.03	73.01	875
F6	79.47	77.32	748
F7	85.73	86.19	836
F8	83.14	81.23	632
F9	80.92	84.59	812

Table 5. Percentage Yield, Entrapment Efficiency and Mean Particle size of Paclitaxel microspheres from formulation F10 to F18

Batch code	Percentage yield (%)	Entrapment efficiency	Rounded mean particle size
F10	81.46	71.61	272
F11	79.52	69.47	265
F12	80.73	76.32	294
F13	78.35	77.42	191
F14	82.37	72.37	284
F15	81.16	76.91	235
F16	84.93	84.95	303
F17	84.23	82.13	229
F18	81.62	85.19	241

***Invitro* drug release studies**

Table 6. Percentage drug release of F1 to F9

Time(hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2.86	2.90	2.92	2.77	2.90	3.36	4.10	2.92	2.81
4	6.55	6.72	7.08	6.71	7.17	7.73	7.99	7.73	7.41
8	10.28	10.77	11.61	10.94	12.06	12.14	14.32	13.70	12.74
12	12.42	13.19	14.54	13.56	15.33	15.64	19.04	18.06	16.47
16	14.16	15.32	17.37	15.75	18.22	18.78	23.36	22.09	19.81
20	16.78	18.42	21.16	19.43	22.54	23.48	29.84	27.69	24.82
24	18.43	20.26	23.71	21.53	25.42	26.64	34.16	31.92	28.16
28	21.63	25.25	27.64	25.96	29.52	30.91	40.25	35.65	32.75
32	24.71	28.74	31.59	29.55	34.06	35.35	46.97	41.07	37.44

36	27.75	31.73	35.08	33.27	37.65	39.87	53.03	46.98	42.13
40	30.73	34.94	38.92	37.21	41.90	44.83	58.02	52.59	46.82
44	33.85	38.36	42.87	40.33	46.01	48.84	63.23	58.01	51.54
48	36.92	41.73	46.98	43.92	50.24	53.28	68.96	63.24	56.23

Table 7. Percentage drug release of F10 to F18

Time in(hrs)	F10	F11	F12	F13	F14	F15	F16	F17	F18
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	6.42	6.84	7.22	6.71	7.11	7.27	7.26	6.69	7.65
4	10.15	10.73	11.36	10.71	11.42	11.13	12.01	11.89	12.33
8	13.73	14.66	15.88	14.85	16.12	16.10	18.90	18.43	17.83
12	15.60	16.73	18.60	17.19	19.06	19.46	24.08	23.17	21.55
16	17.47	18.85	21.32	19.60	22.01	22.82	29.26	27.93	25.27
20	19.31	21.01	24.10	22.08	24.97	26.18	34.44	32.67	28.99
24	21.15	23.17	26.88	24.56	27.93	29.54	39.62	37.45	32.71
28	24.31	27.25	31.76	28.29	31.29	34.55	45.72	43.42	36.97
32	28.42	31.20	36.24	32.74	36.85	39.47	52.56	49.82	42.42
36	32.14	35.06	40.56	36.82	41.01	44.39	59.28	56.06	48.04
40	35.13	39.05	44.67	41.35	46.49	49.25	66.27	62.60	53.92
44	38.75	42.57	49.19	45.23	51.21	54.19	72.82	68.58	59.79
48	42.28	46.43	53.67	49.32	55.86	59.11	79.42	74.82	65.24

Fig 1. Cross section of breast

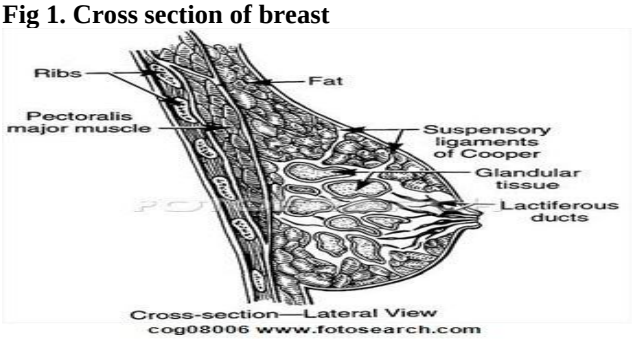


Fig 3. Standard calibration curve of paclitaxel

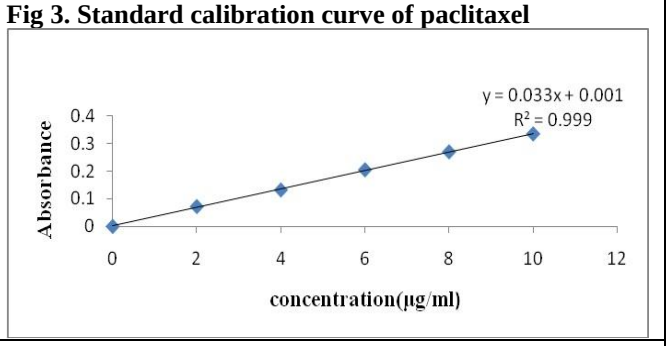


Fig 4. Percentage drug release of F1 to F9

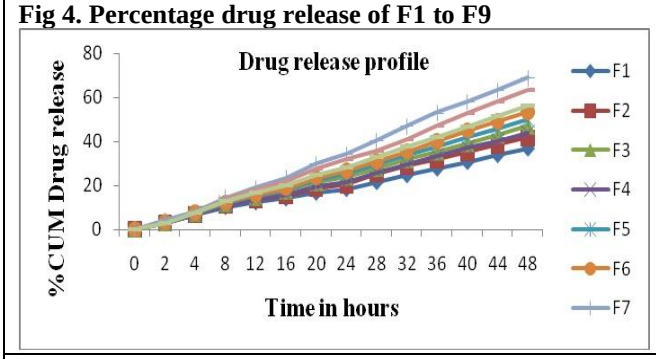


Fig 5. Percentage drug release of F10 to F18

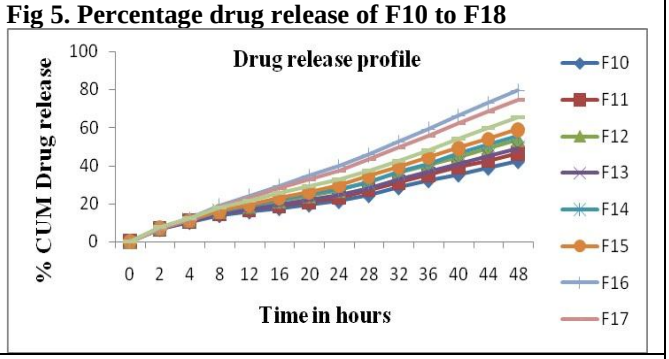


Fig 6. Comparison of drug percentage drug release of F1,F2,F10 and F11

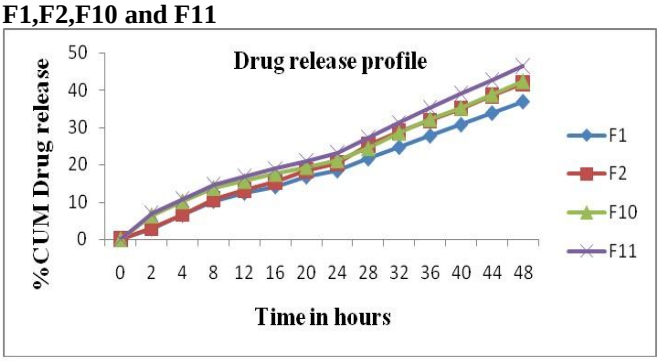


Fig 7. Zero order comparison graph of F1, F2, F10 and F11

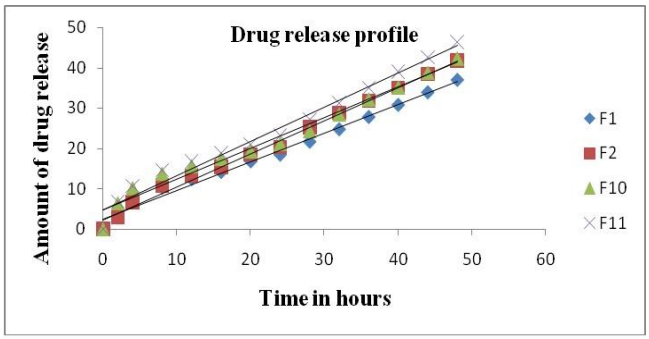
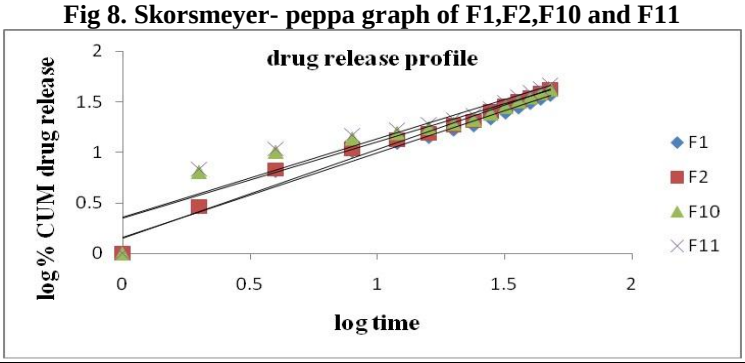


Fig 8. Skorsmeyer- peppa graph of F1,F2,F10 and F11



CONCLUSION

The present investigations were studied paclitaxel sustained release microspheres stable dosage form was developed to prolong action of drug to compare with its general profile. The summary and conclusions of investigations is as follows

- The present study was carried out to design the sustained release microspheres paclitaxel for the treatment of breast cancer, colon cancer.
- The use of HPMC k100 polymer makes possible a sustained release of paclitaxel microspheres with dissolution mechanism.
- These concept is explained the application of fixed dose dosage form which results in cost effectiveness and reduce multiple of dosage forms.
- From the above observations it is concluded that by ionic gelation technique F1 formulation was found 36.92% of paclitaxel at 48 hrs, and solvent evaporation method of formulation F10 release paclitaxel 42.28% at 48 hrs.
- The microspheres were formulated for sustained release by using different polymers like HPMC K100, eudragit Rs 100, ethyl cellulose in different ratios was found to be 48hrs control and stable drug release.
- The release characteristics of the formulation appear as to follow (F10) shows near zero order drug release and korsmeyer- peppas mechanism..
- Among these 2 techniques the best method was ionic gelation is proved to control stability of paclitaxel, but the particle size is too large to compare with solvent evaporation. So the formulation F10 is best compared to remaining formulations

ACKNOWLEDGEMENTS

The authors are thankful to Mr. A. Anil kumar for providing their guidance and support in completion of this research work. We are also thank full to the Vikas College of Pharmacy for providing the facilities for the research.

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