FORMULATION AND DEVELOPMENT OF NANOCRYSTALS BY USING NATURAL AMINO ACID

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ABSTRACT:

Objective: The primary target of this study was to formulate and invitro study of BSA loaded nanocrystal containing paclitaxel as a novel method by desolvation technique. The impact of various test on the particle size, entrapment efficiency, percent drug released etc. was assessed**.** The 3^2 full factorial designs was employed to investigate the influence of formulation variables on nanocrystals characteristics.

Method: BSA loaded nanocrystals were prepared by using desolvation technique. Nanocrystals were prepared by using BSA and PVP K 30; these were characterized for various methods such as shape, size and, entrapment efficiency and Zeta Potential, Drug excipients compatibility which is determined by FTIR.
Result: FTIR shows there was no interaction between formulation ingredients. The average particle size was found251.3 nm. The size of nanoparticles increases with BSA-PVP polymer concentration. Prepared nanocrystal shows drug content 96.04% and Entrapment Efficiency 92.05% with particle size 251.3 nm. In-vitro drug releases shows maximum 96.07%.

Conclusion: By using desolvation method BSA loaded nanocrystals was successfully prepared and evaluated, containing good particle size, EE% and zeta potential % drug release so by doing further in vivo study it could be good choice for conventional drug delivery system.

KEYWORDS: Nanocrystals, Bovine Serum Albumin, Desolvation.

INTRODUCTION:
Nanoparticles are solid colloidal particles ranging in size from about 10 nm to 1000 nm. The major goal in designing nanoparticles as a delivery system is to control particle size, surface properties, and release of pharmacologically active agents in order to achieve the site-specific action of drugs at a therapeutically optimal rate and dosage regimen [1,2]
Nanocrystals are considered as a formulation choice for drug showing poor solubility and dissolution rate[3] . Nanocrystals consists of stabilized submicron sized crystalline drug particles in liquid medium, usually water [4,5].They can be produced either by precipitation technique (bottom-up approach) or by size reduction (top-down approach)[6,7]. Drug nanocrystals are nanoparticles being composed of 100% drug without any matrix material and mean particles size is below 1µm. The term drug nanocrystal implies a crystalline state of the discrete particles but depending on the production method, they can be partial or completely amorphous [8].

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The development of nanoparticle-based drug delivery systems is rapidly growing due to their great therapeutic potential. Various types of materials including polymers, lipids, polysaccharides, and proteins have been explored as drug delivery carriers. The selection of nanoparticle materials is dependent on many factors including the size of nanoparticles needed, inherent properties of the drug such as aqueous solubility and stability, drug release profile desired, surface charge and hydrophobicity of nanoparticles, biocompatibility and biodegradability of nanomaterials, and antigenicity and toxicity of the product[9].

The fundamental distinction lies in the number of tryptophans (Trps). BSA has two Trps, while HSA has just a single. This distinction is valuable with regards to its examination by spectrofluorimetry since this amino acid is the liable one in charge of the characteristic fluorescence of proteins[10,11]Albumin nanoparticles are considerable in nanotechnology due to their high solubility in aqueous media, their ability to interact with various compound.

The arrangements of nanostructures in Bovine serum albumin have many desirable properties that provide biodegradability, biocompatibility, and improvement in the water solubility for poorly water-soluble drugs and informal surface adaptation for the drug delivery system on tumor cells. Also, it has the possibility for drug targeting ligands on covalent derivatization. Because of its surface modification mechanisms and various preparation methods exploited the specific targeting sites for DDS[12].

MATERIAL AND METHOD:

Materials

Paclitaxel was obtained from B.M.R. Chemicals Hyderabad, India. PVP K30 was obtained from Green Pharma, Hyderabad, India. Poloxamer was obtained from Global Vision, Mumbai. BSA, Bovine Serum Albumin was obtained from B.M.R. Chemicals Hyderabad, India. Chloroform, methanol, ethanol were of HPLC grade (Merck, India).

Solubility studies:

Solubility of Paclitaxel was determined in Methanol, Ethanol, pH 1.2, pH 6.8 and pH 7.4 phosphate buffers. Solubility studies were performed by taking excess amount of Paclitaxel in different beakers containing different solvents. The mixtures were shaken for 48 hrs in rotary shaker. The solutions were centrifuged for 10mins at 1000rpm and supernatant were analyzed at 236 nm by using UV Spectrophotometry.[13]

Determination of absorption maximum (λ max):

10mg of Paclitaxel was dissolved in 2mL of ethanol and volume was make upto 10ml with buffer (7.4pH buffer) so as to get a stock solution of 1000 µg/ml concentration. From the above stock solution pipette out 1ml of the solution and makeup the volume to 10ml using buffer to get the concentration of 100µg/ml concentration. From this stock solution pipette out 2.5 ml of the solution and makeup the volume to 10ml using buffer to get the concentration of 25µg/ml concentration, this solution was scanned under UV Spectroscopy using 200-400nm.Determination of Paclitaxel λ-max was done in pH 7.4 buffer medium for accurate quantitative assessment of drug dissolution rate.

Construction of Calibration Curve of Paclitaxel:

10 mg of drug was dissolved in 2mL of ethanol and volume was make upto 10ml with buffer 7.4 pH buffer so as to get a stock solution of 1000 µg/ml concentration. 1 ml of this solution was taken and made up to 10 ml with 7.4pH buffer, which gives 100 µg/ml concentration (stock solution).From the stock solution, concentrations of 5, 10, 15, 20, 25 & 30 µg/ml in 7.4pH buffer were prepared. The absorbance of diluted solutions was measured at 236nm and a standard plot was drawn using the data obtained.

Drug-Excipient Compatibility Studies:

There is always possibility of drug- excipient interaction in any formulation due to their intimate contact. The technique employed in this study is IR spectroscopy.

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IR spectroscopy is one of the most powerful analytical techniques, which offers possibility of chemical identification. The IR spectra was obtained by KBr pellet method. (Perkin-Elmer series 1615 FTIR Spectrometer).[14]

Formulation of Paclitaxel Nano suspension:

Weigh accurately 6mg of paclitaxel was added to 10ml of ethanol to prepare the drug solution. The above drug solution was added drop wise into required quantity of water containing stabilizers (PVP K30 & Poloxamer) with continuous stirring for 1hr on homogenizer at 1000rpm. Further stirring was done for the removal of organic solution.

Preparation of BSA- Paclitaxel Nanocrystals:

BSA-NPs were prepared by the desolvation method. Briefly described, different concentrations of BSA was dissolved in 1 ml of sodium chloride solution (10 mM) individually. Then, 8.0 ml of ethanol was added drop wise into the BSA solution under magnetic stirring (400 rpm) at room temperature. Subsequently, the as-prepared BSA-NPs were cross-linked with 0.2% glutaraldehyde (GA) for 24 h or denatured at 70°C for 30 min. BSA-NPs were incubated with paclitaxel Nano suspension for 2 h in the preparation of Paclitaxel-BSA-Nanocrystals. The particles were centrifuged at 10,000rpm for 10min at 4°C and washed with ultrapure water.

Initially nine batches of Different concentration of Polymer and BSA was prepared. Based on Particle size, Drug content and entrapment efficiency for optimization of batch 3^2 factorial design was applied.

Table 1: Experimental designing by 3^2 factorial

Formulation Variables	Levels coded			Dependent Variable
	-1	0	+1	
X1=Polymer Concentration	5	10	15	Y1=Particle size(nm)
X2=BSA Concentration	0.25	0.50	0.75	Y2=Drug Content (%) Y3=Zeta potential

Table 2: A 3^2 Full factorial Experimental Design Layout

Formulation Code	Coded Factor Levels	
	X1	X2
F1	-1	-1
F2	0	-1
F3	+1	-1
F4	-1	0
F5	0	0
F6	+1	0
F7	-1	+1
F8	0	+1
F9	+1	+1

Statistical Design Study:
To study the influence of the different variables used for preparation, as a characteristic of formulated Nanocrystal Central composite experimental design Design-Expert software was used. (Version 12) In this design and within 9 runs, two factors were evaluated. The independent variables were polymer concentration (X1) and BSA concentration (X2). The dependent variables are particle size (Y1: PS), Drug Content (Y2: EE) and Zeta potential (Y3: ZP) were selected.

EVALUATION OF BSA- PACLITAXEL NANOCRYSTALS:
Particle size and shape
Average particle size and shape of the formulated nanocrystals was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size.

Entrapment efficiency:
 The freshly prepared nanocrystals was centrifuged at 20,000 rpm for 20 min at 5°C temperature using cool ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 5 ml of supernatant solution at 236nmusing UV spectrophotometer against blank/control nanocrystal. % EE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. [15-18]
The entrapment efficiency (EE %) could be achieved by the following equation:

$$\text{Entrapment efficiency} \% = \frac{\text{Total drug} - \text{free drug}}{\text{Total drug}} X100$$

Drug Content:
Weighed amount of Paclitaxel Nanocrystals was dissolved in 100mlof 7.4pH buffer and sonicated for 30min. This solution was filtered and further diluted to make aconc.of10µg/ml solution. The absorbance of the solutions was measured at 236nm using UV-Visible spectrophotometer against 7.4 pH buffer solution as blank and calculated for the percentage of drug present in the sample.

Zeta potential:
As the concentration of electrolyte increases in the medium, the zeta potential falls off rapidly due to the screening effect of the counter ions. The zeta potential cannot be measured directly; however, it can be calculated using theTheoreticalmodelsandfromexperimentallydeterminedelectrophoreticmobilitydata. The theory is based on electrophoresis and can be expressed as:

$$\mu = \zeta\varepsilon/\eta$$

Where (μ) is the electrophoretic mobility, (ε) is the electric permittivity of the liquid,
(η) Is the viscosity and (ζ) us the zeta potential.

***In vitro* drug release study:**
In vitro dissolution study was performed by USP dissolution apparatus-type I using 900 ml of 7.4pH buffer as a dissolution medium maintained at 37 ± 0.5°C and stirring speed (50 rpm). Weight equivalent to 6mg of paclitaxel were placed in the dissolution medium, five-milliliter samples were withdrawn at specific intervals of time, then filtered through a 0.45 µm filter paper and analyzed for their drug concentrations by measuring at 236nm wavelength.

RESULT AND DISCUSSION:
Solubility studies:

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The solubility of Paclitaxel in 0.1N HCL was found to be equal to 0.239mg/ml, this indicated that the drug is very slightly soluble in 0.1 N HCL and it shows good solubility in ethanol and Methanol as 2.641 mg/ml and 1.352 mg/ml respectively..

Solubility data of drug Paclitaxel in various liquid vehicles is shown in table 4.

Table 3: Solubility of drug in different solvents

Solvent	Solubility (mg/ml)
Ethanol	2.641
Methanol	1.352
0.1N HCL	0.239
pH 6.8 phosphate buffer	0.389
pH 7.4 phosphate buffer	0.561

Values represent mean±SD (n =3)
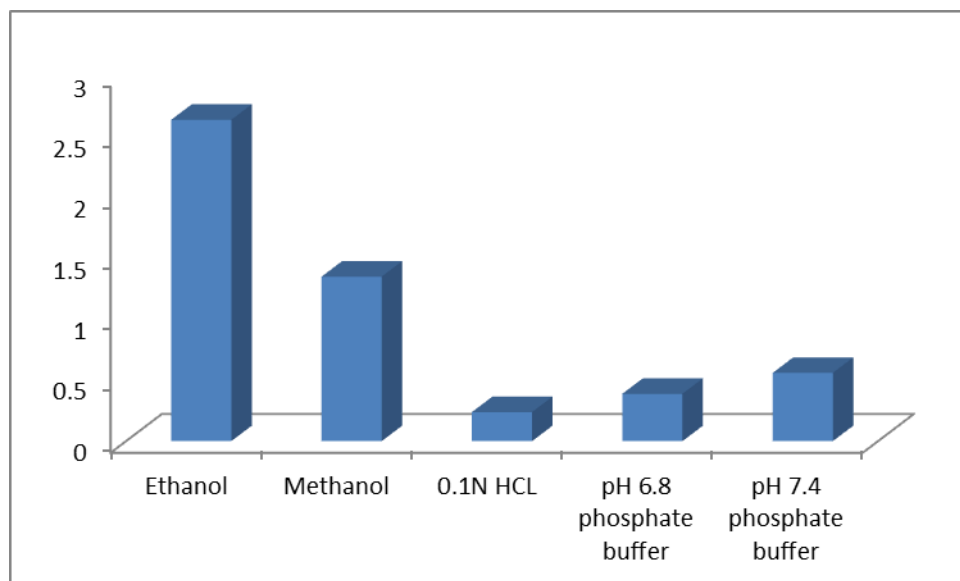
Fig 1: Solubility studies of Paclitaxel

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Determination of absorption maximum (λ max):
Spectrum of Paclitaxel was obtained at 236nm in Phosphate buffer with pH7.4
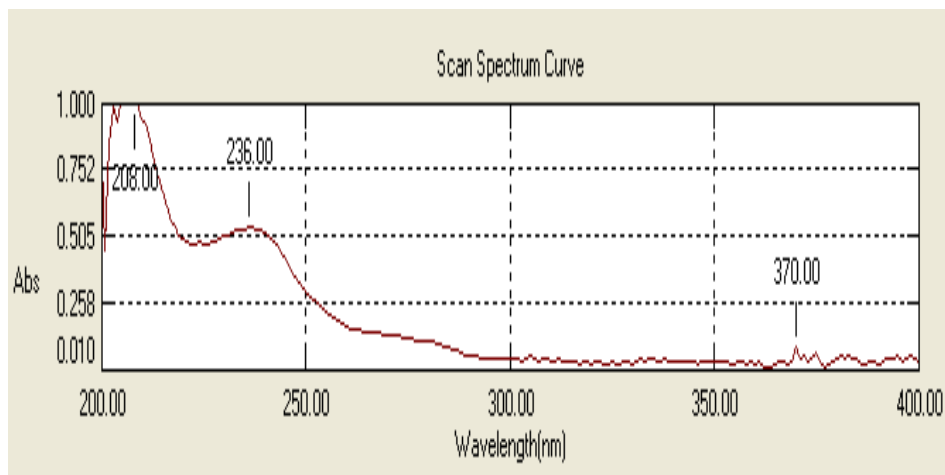
Fig 2: UV spectrum of Paclitaxel

Construction of Standard Calibration Curve for Paclitaxel:
Calibration curves of Paclitaxel in pH 7.4 is represented in fig. 3. The curves were linear at the concentration range of 5-30 μg/ml with regression values of 0.999.The high regression values indicate that the calibration curves follow Beer's law.

Table 4: Standard readings of Paclitaxel in pH 7.4 (λ max 236 nm)

| Concentration(μg/ml)	Absorbance at 236nm
0	0
5	0.112
10	0.221
15	0.311
20	0.409
25	0.512
30	0.624
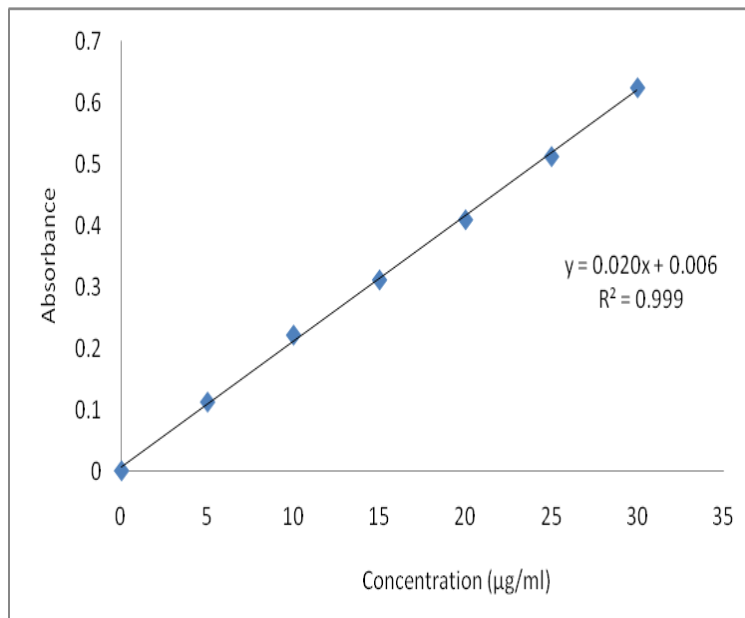
Fig.3: Standard calibration curve of Paclitaxel in pH 7.4

Drug and Excipients Compatibility studies by FTIR spectrophotometer:
Drug-excipients interaction was studied using Fourier transform infrared (FTIR) spectroscopy, and the results are presented in fig 4,5. Paclitaxel demonstrates characteristic peaks in FTIR at 3500-3200 cm−1 (due to its NH stretching) and 1274 cm−1 (due to C-N stretching) , 1735-1720cm−1 (due to C=O Stretching) 3300-2500cm−1 (due to O-H Stretching).In this study both Drug Paclitaxel and Mixture of Drug With Excipients (BSA) showed the characteristic peak about at same wavelength.

From this study and the graphs based on peaks and wave numbers that specific functional group, no additional peaks were obtained which indicates that there is no significant interaction between drug and excipients.
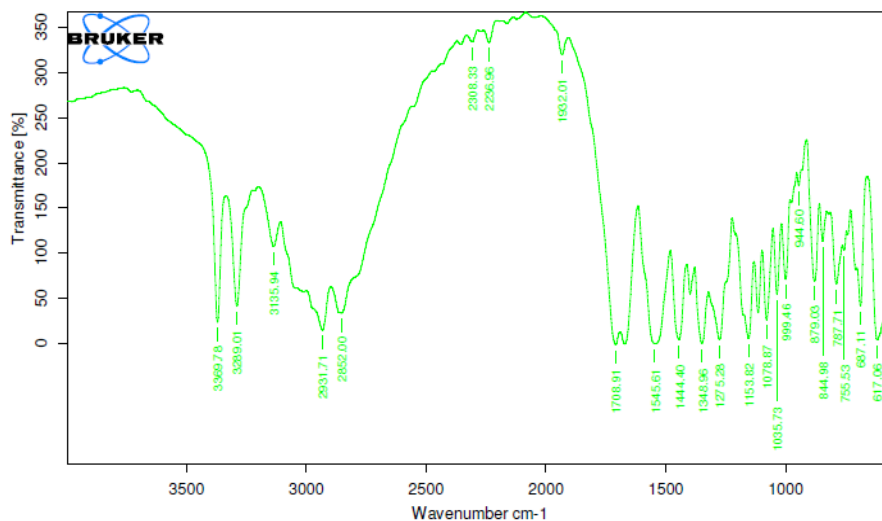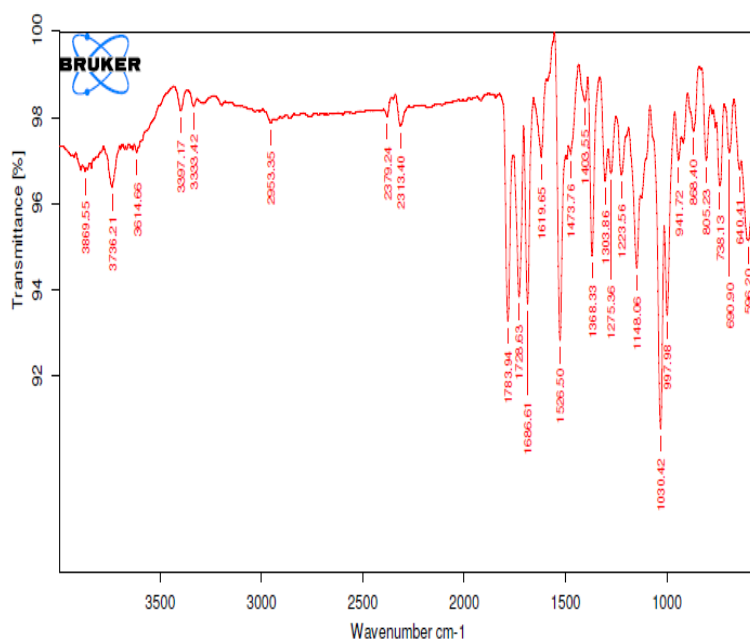

Fig 4: FTIR of Paclitaxel

Fig 5: FTIR of Drug + Excipients

Characterization of prepared Nanocrystals:
Analysis of particle size, Drug Content, Entrapment efficiency and zeta potential:
Particle Size:
By using different ratio of Polymer and BSA, Paclitaxel Nanocrystal was prepared and particle size of Nanocrystal varies from 251.3 nm to 636.6nm

Entrapment Efficiency:
EE of all Paclitaxel Nanocrystal prepared formulations were within the range of 69.35% to 92.05%, as shown in Table 6

Drug Content:
Drug Content of all Paclitaxel Nanocrystal prepared formulations were within the range of 86.36% to 96.04%, as shown in Table 5
Zeta Potential:
ZP values of the prepared formulations between –21.1and –27.7 mV.

Table 5: Particle size and entrapment efficiency of prepared Nanocrystal

Formulations	Polymer	Drug (mg)	BSA	Particle size (nm)	Drug Content (%)	Entrapment efficiency (%)
F1	5	6	0.25	475.3±3.7	89.34±2.11	90.25±3.21
F2	10	6	0.5	577.2 ±3.6	90.25±4.16	91.75±5.19
F3	15	6	0.75	636.6 ±5.5	92.05±3.12	86.24±4.13
F4	5	6	0.25	318.1 ±6.1	95.21±1.96	87.34±5.40
F5	10	6	0.5	369.2 ±11.4	91.07±4.26	91.05±3.32
F6	15	6	0.75	273.2 ±10.3	90.65±3.96	90.72±5.45

F7	5	6	0.25	251.3 ±1.32	96.04±2.84	92.05±3.41
F8	10	6	0.5	259.3 ±10.2	86.36±4.12	75.02±4.52
F9	15	6	0.75	294.3 ±0.93	92.04±1.329	69.35±4.96

Values represent mean±SD (n =3)

Invitro Drug release studies:
The Paclitaxel Nanocrystal was released in vitro in phosphate buffer pH 7.4 using the dialysis bag diffusion technique. Figure 10 depicts the in vitro drug release profile of Paclitaxel NC formulations. The drug release from the formulations was observed to decrease slightly as the particle size of the formulation decreased, and all eight formulations demonstrated continuous drug release from the first hour. The drug release was discovered to be dependent on the concentrations of Polymer and BSA used.

Table 6: Invitro drug release studies (F1-F9)

Time (min)	Percentage Cumulative Drug Release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	**0**	0	0
5	39.62	23.34	10.24	36.24	26.31	12.05	**42.05**	22.31	16.32
15	45.06	30.52	19.63	45.02	36.24	19.35	**49.63**	36.12	29.34
30	49.82	36.35	26.34	53.16	42.02	26.31	**62.05**	46.37	35.82
45	56.37	41.05	32.02	59.32	49.64	32.53	**69.24**	59.62	41.27
60	62.69	49.36	38.71	65.18	58.23	38.42	**73.16**	63.57	50.23
90	69.21	55.05	43.16	70.36	65.31	46.21	**82.36**	69.82	59.32
120	73.65	59.31	49.66	76.28	72.13	51.26	**89.62**	76.32	65.04
150	75.02	65.24	55.04	83.14	78.36	59.38	**92.04**	79.24	72.96
180	79.35	70.25	59.24	89.35	83.12	65.41	**96.07**	83.62	81.35

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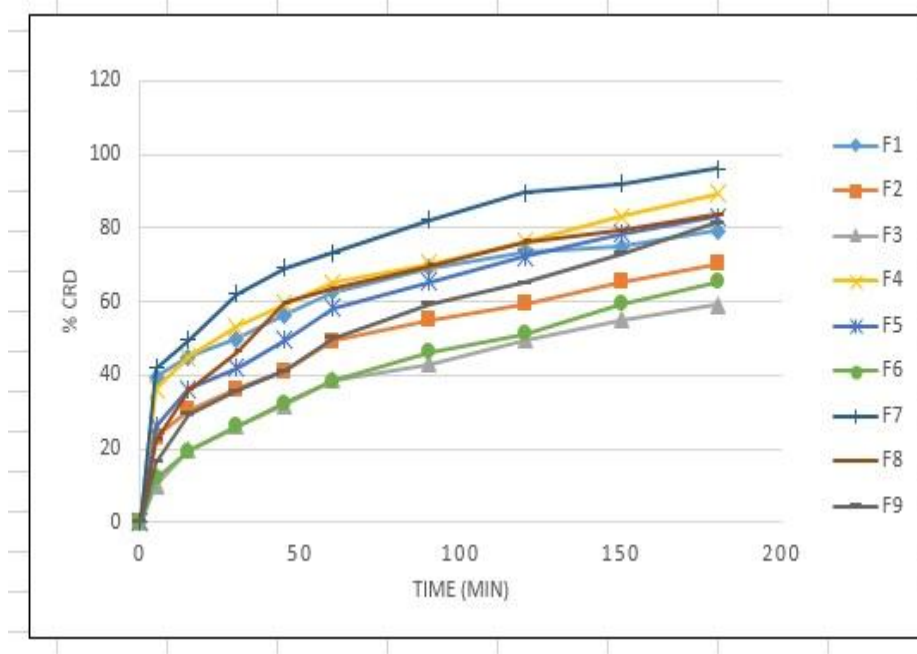
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Fig 6: Invitro drug release studies of F1-F9

CONCLUSION:

In this Study paclitaxel loaded Nanocrystal Prepared successfully by Desolvation Method by using natural Amino acid BSA.FTIR studies confirms there is no interaction between drug and Polymers. Prepared nanocrystal shows drug content 96.04% and Entrapment Efficiency 92.05% with particle size 251.3 nm. Invitro drug release shows maximum 96.07% .this suggest that formulated nanocrystal by using BSA natural amino acid may be good choice for anticancer treatment.

Nanocrystal prepared by desolvation method using polymer and BSA showed promising results in delivering the drug, and there exists a scope for in-vivo Evaluation.

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