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Research Article

Development and Evaluation of Paclitaxel Loaded Nanoparticles.

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ABSTRACT
The present formulation study of Paclitaxel was performed as an attempt to prepare BSA nanoparticulate drug delivery system using desolvation method with spray drying and performance of these formulations was evaluated. From the experimental results it can be concluded that: Bovine serum albumin (BSA) is an ideal carrier for preparing nanoparticles of paclitaxel. The use of acetone consistently gave smaller particles as compared to ethanol for all BSA concentrations. Desolvated BSA nanoparticles have the stability problem hence glutaraldehyde (GTA) is used as cross-linking agent for the production of stable nanoparticles. GTA also affect the entrapment efficiency and release rate but had no influence on particle size. Tween 80 is non-ionic surfactant used as dispersing agent to form stable nanoparticles.

KEYWORDS
Nanoparticulate drug delivery system, Paclitaxel.

1. INTRODUCTION

Nano-medicine is having application in delivery and targeting of pharmaceutical, therapeutic and diagnostic agents. These involve the identification of precise targets (cells and receptors) related to specific clinical conditions and the choice of the appropriate nanocarriers to achieve the required responses while minimizing the side effects. Nanoparticles are a type of colloidal drug delivery system[1]. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticles matrix. Depending upon the method of preparation nanoparticles, nanospheres or nanocapsules can be obtained. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly-(ethylene glycol) known as long circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time to target a particular organ and their ability to deliver proteins, peptides and genes. The major goals in designing nanoparticles as a delivery system is to control particle size, surface properties, enhance solubility and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen[2].

1.1. Advantages of Nanoparticles

- **a)** Passive and active drug targeting both can be easily achieved by manipulating particle size and surface characteristics of nanoparticles after parental administration.
- **b)** They can be made to achieve both control and sustain release of drug during the transportation and at the site of localization by controlling polymer characteristics and surface chemistry.
- **c)** Increase in drug therapeutic efficacy and reduction in side effects of the drug.
- **d)** Drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity and drug loading is relatively high.

1.2. Disadvantages of Nanoparticles

- **a)** Their small particle size and large surface area can lead to particle-particle aggregation.
- **b)** Making physical handling of nanoparticles difficult in liquid and dry forms.
- **c)** Small particles size and large surface area results in limited drug loading and burst release(53)

1.3. Need and Objective

Development and evaluation of paclitaxel (PTX) loaded bovine serum albumin (BSA) nanoparticals using 2^4 factorial designs. Prior to achieving this goal, several issues needed to be addressed. First, understanding how processing variables control particle size and cross-link density was necessary since they control nanoparticles properties and potentially drug release characteristics. Second, studies to maximize drug loading and loading efficiency were needed to develop an efficient drug delivery system. Cancer is the life threatening disease in which there is uncontrollable growth of abnormal tissues. As we know chemotherapy is having various side effects and toxicities, to overcome these problems nanoparticles are formulated. Nanoparticles

accumulate in the tumor cells due to enhanced permeation and retention effect. Paclitaxel (PTX) is a naturally occurring diterpenoid extracted from the Pacific Yew tree, Taxus brevifolia, is one of the best antineoplastic drugs used in treatment of breast cancer, ovarian cancer, lung cancer, head and neck carcinomas. PTX promotes and stabilizes microtubule assembly by non-covalent interaction with tubulin, thereby blocking cell replication in the late G2 mitotic phase of the cell cycle.

2. MATERIALS AND METHODS

2.1. Spray Drying of Nanoparticles

Nanoparticles may subject to a series of stability problems such as aggregation, fusion, and leakage of the encapsulated drugs in to the storage medium. One of the approaches to resolve this kind of problems is spray drying of the nanoparticles. Spray drying is a process which forces fluid through a nozzle, producing a mist that is dried to produce a fine powder. Spray drying of nanosuspensions used to produce easily redispersible dry powders.

2.2. Procedure

All formulations in the study were prepared via spray drying on the LABULTIMA (LU222) spray dryer. All solutions were filtered through a 0.45 µ filter (Millipore, Bedford, MA, USA) prior to spray-drying to minimize blockage due to any undissolved particles at the spray mesh. Table 1 shows the operating conditions for spray drying. The dried powder was collected from the cyclone using a particle scraper and then stored in desiccators at room temperature for further characterization.

Table 1. The Operating Conditions for Spray Drying

Sr. No.	Parameters	Set Value
1	Inlet temp.	50 ˚C
2	Outlet temp.	35 ˚C
3	Flow rate	2 ml/min
4	Aspirator	35 Nm^3/hr

Table 2. Formulations of Trial Batches.

Sr. No.	BSA (%w/v)	Tween 80 (%v/v)	GTA (%v/v)	Speed (r.p.m.)	Solvent Used	% Yield	% Drug Entrapment
1	10	0.5	5	5000	Ethanol	12.74	19.07
2	20	2	25	16000	Ethanol	17.65	21.83
3	20	2	25	16000	Acetone	46.37	43.99
4	40	2	10	5000	Acetone	50.50	35.29
5	10	5	25	16000	Acetone	25.33	22.45
6	20	5	5	5000	Acetone	49.56	20.21
7	40	5	25	5000	Acetone	55.28	40.67
8	40	0.5	25	16000	Acetone	36.71	43.74

Trial batches were formulated by using above procedure that is desolvation method and particles were dried by spray drying method. When ethanol was used as solvent, practical yield and drug entrapment were obtained was very low, that's why acetone was used as solvent for remaining batches. When concentration of BSA (10%) was lower in batch no.5, practical yield (25.33%) and drug entrapment (22.45) was also low. It was found that in batch no.3, 7 and 8 shows high % yield that is 46.37%, 55.28%, 36.71% respectively and entrapment efficiency is 43.99%, 40.67% and 43.74% respectively. Hence considering above parameter following sixteen batches was prepared. Sixteen batches were prepared and labeled as F-1, F-2, F-3, F-4, F-6, F-7, F-8, F-9, F-10 , F-11, F-12, F-13, F-14, F-15 and F-16 as mentioned in Table 1 and Table 2.

Table 3. Investigating Range of Variables.

Factors → / Level ↓	Factor A BSA (%w/v)	Factor B Tween 80 (%v/v)	Factor C GTA (%v/v)	Factor D Speed (r.p.m.)
Low level	20	2	10	5000
High level	40	5	25	16000

Table 4. Formulations for PTX-BSA Nanoparticles.

Formulation Code No.	**Drug (PTX) mg**	**BSA (%w/v) X1**	**Tween 80 (%v/v) X2**	**GTA (%v/v) X3**	**Speed (r.p.m.) X4**
F-1	100	40	2	25	**5000**
F-2	100	20	2	25	**5000**
F-3	100	40	5	25	**5000**
F-4	100	40	2	10	**16000**
F-5	100	20	2	25	**16000**
F-6	100	40	5	10	**5000**
F-7	100	40	2	10	**5000**
F-8	100	20	2	10	**5000**
F-9	100	20	5	25	**5000**
F-10	100	40	5	25	**16000**

F-11	100	20	5	25	**16000**
F-12	100	20	2	10	**16000**
F-13	100	40	5	10	**16000**
F-14	100	40	2	25	**16000**
F-15	100	20	5	10	**5000**
F-16	**100**	**20**	**5**	**10**	**16000**

3. RESULTS AND DISCUSSION

3.1. Solubility Analysis

PTX was found to be practically insoluble in water, freely soluble in acetone, methanol, DMSO and ethanol. BSA was freely soluble in water.

3.2. Drug Excipients Compatibility Study

Preformulation studies were carried out to study the compatibility of pure drug paclitaxel with bovine serum albumin prior to the preparation of nanoparticles of paclitaxel. After 2 weeks, it was observed that paclitaxel and BSA without moisture were stable in vial A & C. But vial-B and D containing moisture had shown caking formation and vial-B shown odour due to gas formation as shown in Table 5.

Table 5. Compatibility Study

Sr. No.	Code	Caking	Liquefaction	Odour or Gas Formation	Discoloration
1	**A**	No	No	No	No
2	**B**	Yes	No	Yes	No
3	**C**	No	No	No	No
4	**D**	Yes	No	No	No

3.3. Particle Size

Particle size of nanoparticles was determined by dynamic light scattering method using the particle size analyzer (Nanophax, NX0080, cross correlation). The particle size of nanoparticles varied somewhat among the formulation due to variation in the composition of formulations. The mean particle size of nanoparticles formulation was in the range of nm. Formulation F14 showed relatively large size i.e. 9280.8 nm and formulation F6 showed relatively small size i.e. 7.1 nm of nanoparticles. The Table 17 shows mean particle size of various batches. Nanoparticles size can be affected by amount of desolvating agent (acetone), BSA concentration, pH, ratio of

acetone/BSA and Tween 80 as surfactant. Stirring speed and cross-linking agent do not have significant effect on particle size.

3.4. Drug Entrapment Efficiency

The entrapment efficiency of 16 batches of PTX nanoparticles was studied. The drug entrapment efficiency of different batches of nanoparticles was found in the range of 19.807 % to 60.36 %. The result for entrapment efficiency is shown in Table 6. It was observed that the entrapment efficiency increases with the increase in concentration of GTA in the formulations and also it decreases with increase in the Tween 80 concentration and speed. The maximum entrapment was found in F-1, F-2 and F-6 i.e. 57.29 %, 60.36 % and 55.95 % and low was found in F-13 and F-16 i.e. 23.18 % and 19.80 % respectively. BSA did not have any effect on entrapment efficiency. Many factors may affect the entrapment efficiency of the drug in nanoparticles. E.g. Nature of the drug, drug-polymer ratio, stirring speed cross-linking agent, etc.

Table 6. Mean Particle Size, Entrapment Efficiency and % Yield of 16 Batches of NPS.

Sr. No.	Formulation Code	Levels of Factors	Particle Size (nm)	Entrapment Efficiency (%)	% Yield
1	F1	+ - + -	1046.44	57.29	69.12
2	F2	- - + -	18.13	60.36	58.18
3	F3	+ + + -	403.55	42.42	72.48
4	F4	+ - - +	9174.64	40.74	39
5	F5	- - + +	1063.88	42.59	46.3
6	F6	+ + - -	7.1	55.95	34.65
7	F7	+ - - -	1122.96	30.89	55.07
8	F8	- - - -	469.55	26.09	49.08
9	F9	- + + -	818.47	36.09	33.2
10	F10	+ + + +	50.49	28.21	50.50
11	F11	- + + +	470.07	47.32	54.31
12	F12	- - - +	833.13	42.83	31.09
13	F13	+ + - +	1786.55	23.18	56.36
14	F14	+ - + +	9280.8	43.29	55.29
15	F15	- + - -	37.44	30.04	56.01
16	F16	- + - +	1390.06	19.80	36.66

[(+) indicates high level and (-) indicates low level]

3.5. Scanning Electron Microscopy (SEM)

Morphological analysis of the PTX-BSA nanoparticles was carried out by scanning electron microscopy and showed regular and isolated particles showed in Fig. 1and 2.

SEM is done at STIC, Cochin University of Sciences & Technology, Cochin by scanning electron microscope (JEOL Model JSM - 6390LV). SEM analysis of the samples revealed that all nanoparticles prepared were spherical in shape. The photographs of SEM are given in Fig. 1 and 2. These photographs showed that in the samples with high polymer concentration the particles are spherical possessing smooth surfaces. On the other hand the low concentration caused a coarse covering, likely due to drug's residue that has not been surrounded by polymer, thoroughly. The surface roughness decreased with increasing GTA concentration.

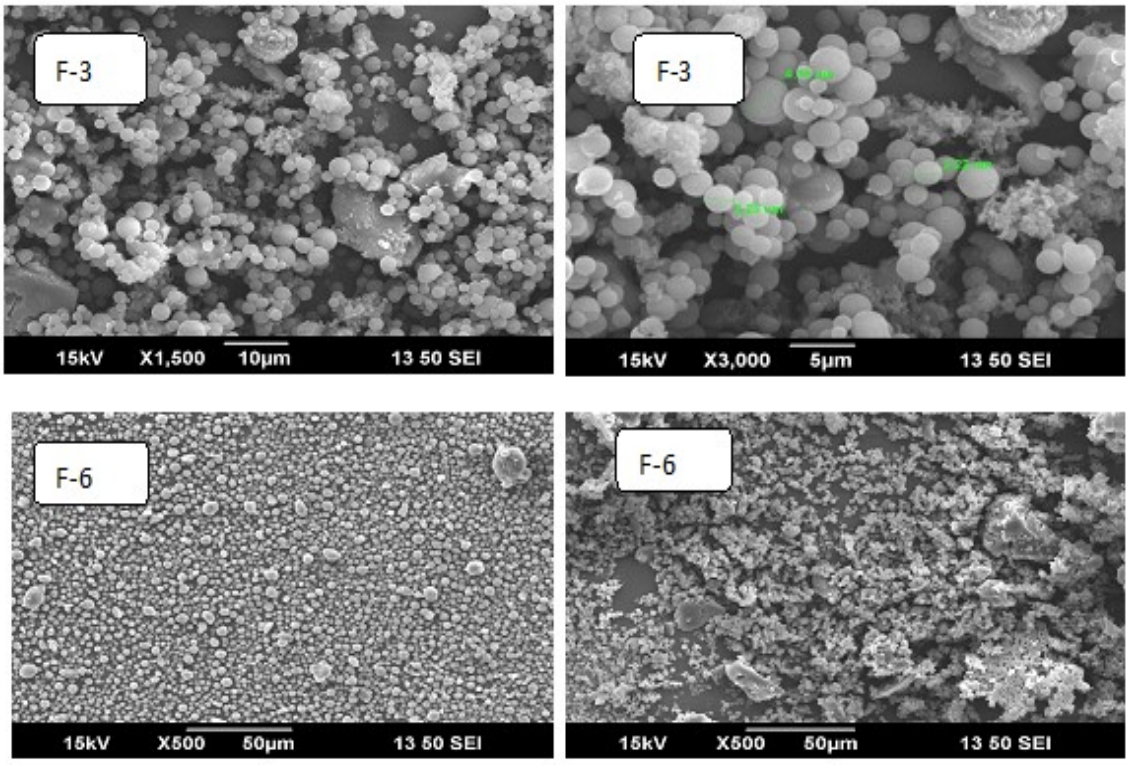

Fig. 1. Typical SEM images of the PTX-loaded BSA Nanoparticles.

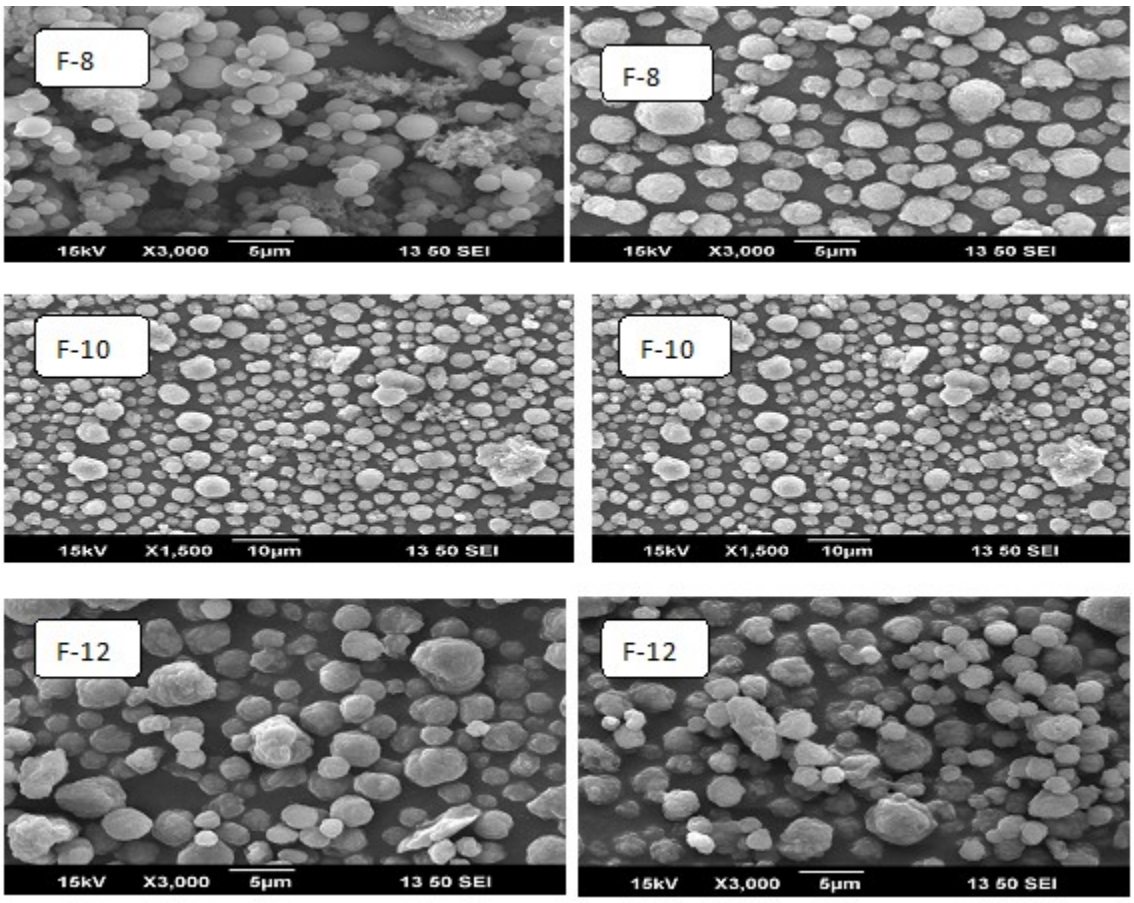

Fig. 2. Typical SEM images of the PTX-loaded BSA Nanoparticles.

3.6. In-Vitro Drug Release

To determine whether the availability of PTX is in controlled manner by formulating the polymeric nanoparticles, *in-vitro* drug dissolution studies were carried out phosphate buffer pH 7.4 using 0.22 µm cellophane membranes. The results were tabulated in Table 7 and Table 8 and combine release graph of 16 formulations showed in Fig. 3, 4 and Fig. 5. Drug release occurs mainly due to diffusion and erosion mechanism.

Table 7. **Percentages Cumulative Drug Release of 1-8 Batches**

Time (min.)	F-1 Mean ±SD	F-2 Mean± SD	F-3 Mean ±SD	F-4 Mean ±SD	F-5 Mean ±SD	F-6 Mean ±SD	F-7 Mean ±SD	F-8 Mean ±SD
0	0	0	0	0	0	0	0	0
15	21.15 ±0.67	11.35 ±0.33	14.79 ±0.13	17.18 ±0.66	38.99 ±0.55	35.64 ±0.37	17.01 ±0.49	7.76 ±0.55
30	26.61 ±0.43	17.45 ±0.19	23.43 ±0.49	23.7 ±0.05	46.86 ±0.52	52.95 ±0.61	24.12 ±0.58	13.28 ±0.33

60	31.11 ±0.46	26.06 ±0.38	30.43 ±0.12	32.48 ±0.37	60.0 ±0.41	66.02 ±0.86	27.08 ±0.59	17.37 ±0.8
120	39.45 ±0.14	32.27 ±0.47	39.41 ±0.66	43.62 ±0.54	68.28 ±0.5	79.44 ±0.88	36.04 ±0.34	21.88 ±0.32
240	47.67 ±0.17	37.53 ±0.26	49.49 ±0.29	59.54 ±0.32	70.75 ±0.7	88.63 ±0.93	46.95 ±0.6	25.27 ±0.36
360	54.23 ±0.15	47.56 ±0.40	59.56 ±0.35	70.81 ±0.13	88.47 ±0.33	93.46 ±0.39	60.24 ±0.45	32.36 ±0.49
480	68.68 ±0.26	64.29 ±0.69	73.50 ±0.10	86.66 ±0.31	96.00 ±0.79	98.13 ±0.25	80.63 ±0.37	43.35 ±0.89
720	81.7 ±0.18	85.47 ±0.39	82.63 ±0.27	100 ±0.08	100 ±0.43	100 ±0.2	95.50 ±0.08	53.24 ±0.85
1440	92.30 ±0.10	98.32 ±0.29	99.41 ±0.25	100 ±0.46	100 ±0.47	100 ±0.4	98.68 ±0.56	70.37 ±0.32

Table 8. Percentages Cumulative Drug Release of 9-16 Batches.

Time (min.)	F-9 Mean± SD	F-10 Mean± SD	F-11 Mean± SD	F-12 Mean± SD	F-13 Mean± SD	F-14 Mean± SD	F-15 Mean± SD	F-16 Mean± SD
0	0	0	0	0	0	0	0	0
15	22.71 ±0.42	15.52 ±0.37	2.15 ±0.68	5.24 ±0.67	6.23 ±0.77	4.65 ±0.51	14.46 ±0.66	12.48 ±0.06
30	30.05 ±0.19	19.51 ±0.74	4.89 ±0.93	15.93 ±0.92	13.11 ±0.25	9.89 ±0.83	21.42 ±0.95	21.52 ±0.36
60	35.87 ±0.32	25.41 ±0.58	10.15 ±0.89	25.35 ±0.65	24.85 ±0.98	18.27 ±0.34	32.76 ±0.45	28.54 ±0.24
120	43.04 ±0.92	30.61 ±0.31	16.46 ±0.61	37.93 ±0.96	34.43 ±0.73	30.68 ±0.78	43.69 ±0.67	43.59 ±0.41

240	51.3 ±0.38	35.56 ±0.84	24.64 ±0.19	47.37 ±0.85	41.39 ±0.37	43.06 ±0.5	53.11 ±0.47	57.48 ±0.34
360	60.27 ±0.94	44.52 ±0.73	34.57 ±0.87	60.15 ±0.56	53.69 ±0.34	59.00 ±0.47	66.38 ±0.89	85.99 ±0.85
480	69.82 ±0.71	58.36 ±0.33	43.21 ±0.54	72.00 ±0.61	68.08 ±0.55	70.31 ±0.75	84.92 ±0.8	96.63 ±0.36
720	84.99 ±0.85	72.55 ±0.3	57.63 ±0.6	85.13 ±0.55	86.52 ±0.31	88.24 ±0.21	99.17 ±0.55	100 ±0.77
1440	99.40 ±0.65	91.02 ±0.68	78.83 ±0.8	98.63 ±0.32	99.36 ±0.21	97.43 ±0.3	100 ±0.31	100 ±0.51

(Mean ± SD, n = 3)

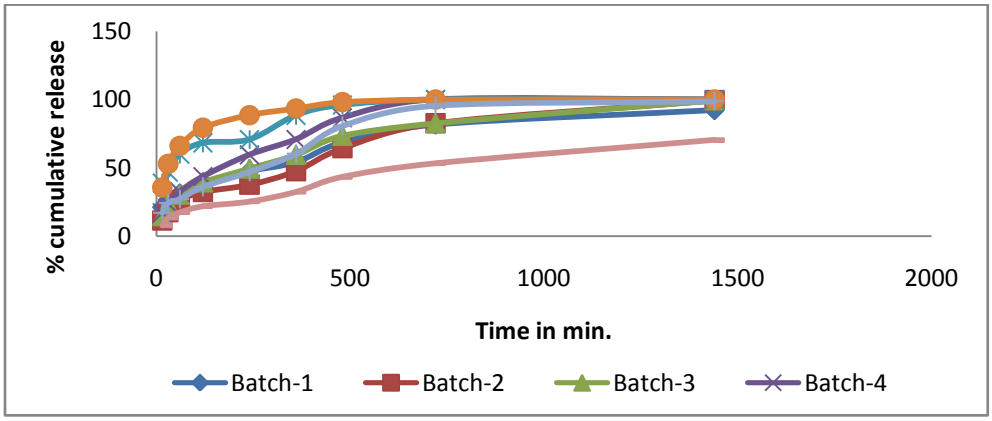

Fig. 3 Graphical representation of comparative drug release profile of 1-8 formulations.

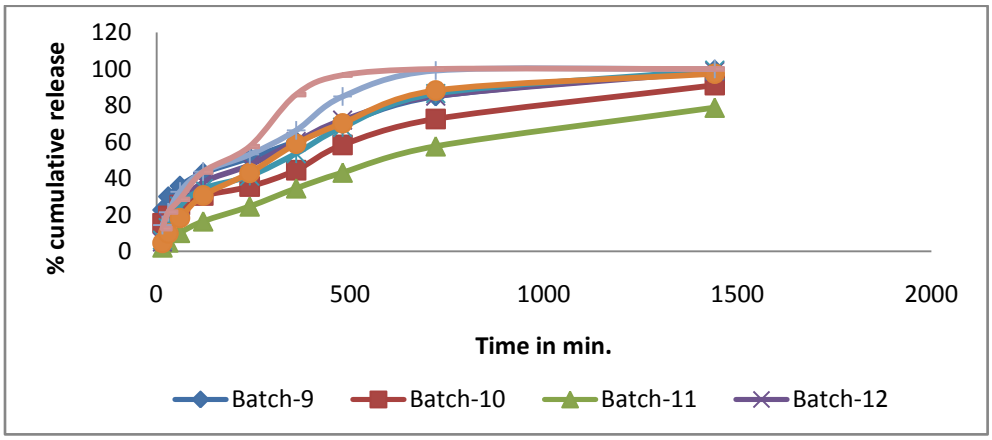

Fig. 4 Graphical representation of comparative drug release profile of 9-16 formulations

Table 9. R^2value for Model Fitting Analysis of *In-Vitro* Release Data.

Formulation	Zero Order	First Order	Korsemayer –Peppas	Higuchi Matrix	Best Fit Model
F1	0.848	0.965	0.974	0.955	Peppas
F2	0.886	0.975	0.966	0.957	First order
F3	0.867	0.951	0.993	0.977	Peppas
F4	0.806	0.841	0.984	0.942	Peppas
F5	0.820	0.806	0.967	0.945	Peppas
F6	0.630	0.617	0.953	0.835	Peppas
F7	0.771	0.934	0.961	0.910	Peppas
F8	0.931	0.975	0.971	0.978	Higuchi
F9	0.917	0.971	0.982	0.988	Higuchi
F10	0.927	0.992	0.957	0.972	First order
F11	0.928	0.995	0.988	0.993	First order
F12	0.831	0.985	0.981	0.963	First order
F13	0.866	0.981	0.971	0.968	First order
F14	0.821	0.983	0.969	0.953	First order
F15	0.788	0.692	0.973	0.930	Peppas
F16	0.679	0.701	0.948	0.853	Peppas

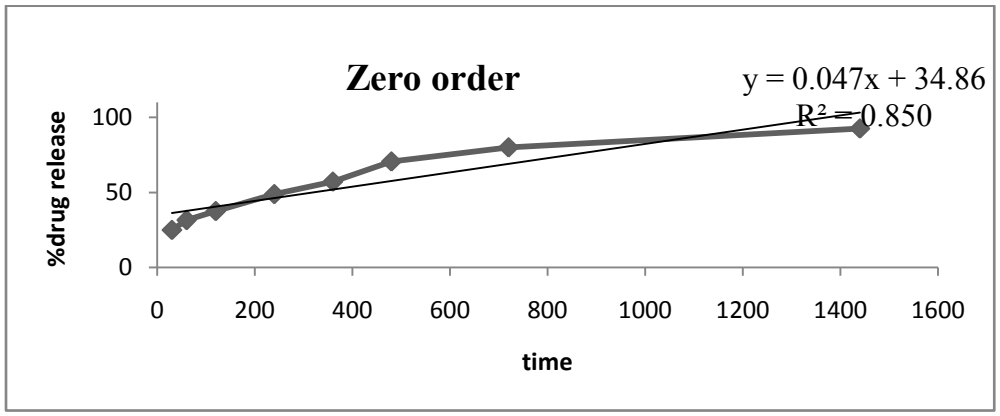

Fig. 5a. Plot of (a) Zero order release.

Fig. 5b. Plot of (b) First order

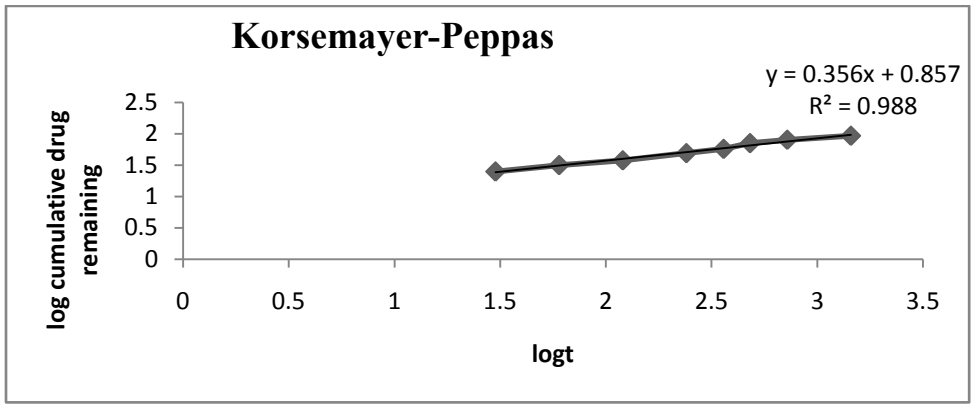

Fig. 5c. Plot of (c) Korsmeyer-peppas

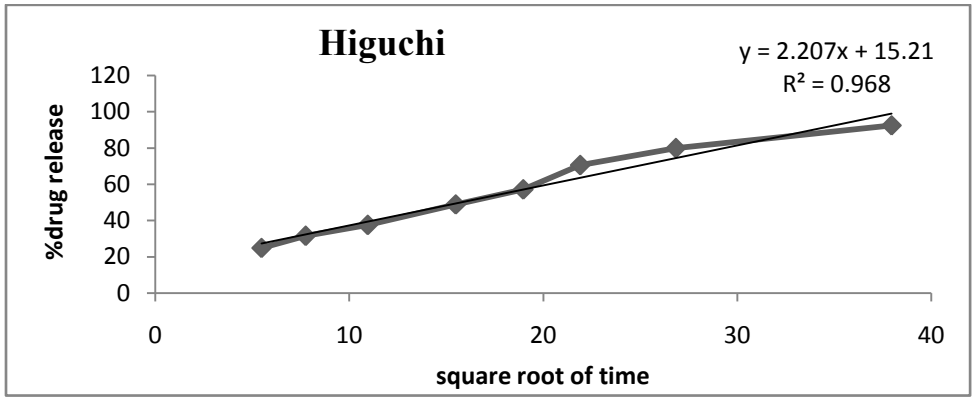

Fig. 5d. Plot of (d) Higuchi release.

Table 10. R^2 Value for Model Fitting Analysis of *In-Vitro* Release Data for Optimized Batch.

Models	R^2 Value	Constant
Zero order release	0.850	34.86
First order release	0.988	1.881
Korsmeyer-peppas release	0.988	0.857
Higuchi release	0.968	15.21

An *in-vitro* drug release study was carried out in phosphate buffer pH 7.4 using 0.22 µm cellophane membranes. The results were tabulated in Table 9 and release graph of formulation shown in fig. 5. The model value obtained for formulation indicated in Table 10. Among the model tested for formulation was fitted to Korsmeyer-peppas model. Drug release occurs due to diffusion and erosion of polymer.

3.7. Differential Scanning Calorimetry (DSC) study

DSC thermograph of PTX, BSA and PTX-loaded BSA nanoparticles are shown in diagram. A physical change gives the endothermic peak and chemical changes give rise exothermic peak. The pure drug PTX (Fig. 6) gives rise to a sharp endothermic peak that corresponds to melting at 213.32˚C with an onset at 211.91℃, indicating its crystalline nature. A broad peak is observed due to the dehydration reaction of the drug. The pure BSA polymer also gives rise to sharp endothermic peak that corresponds to melting point at 55.34˚C with an onset at 53.33˚C (Fig. 7). No distinct melting point was observed because BSA is amorphous in nature. The two peaks at 55.34˚C and 213.32˚C are related to the thermal decomposition of the polymer and drug. The DSC curves of optimized batch are observed at 68.58˚C and 238.54˚C, it showed that the shifting of melting endotherm of PTX and BSA, which could indicate the amorphous nature of the drug as well as loss of crystalline, indicates change in melting point, which releases kinetics and bioavailability. DSC of optimised formulation is given in fig. 8.

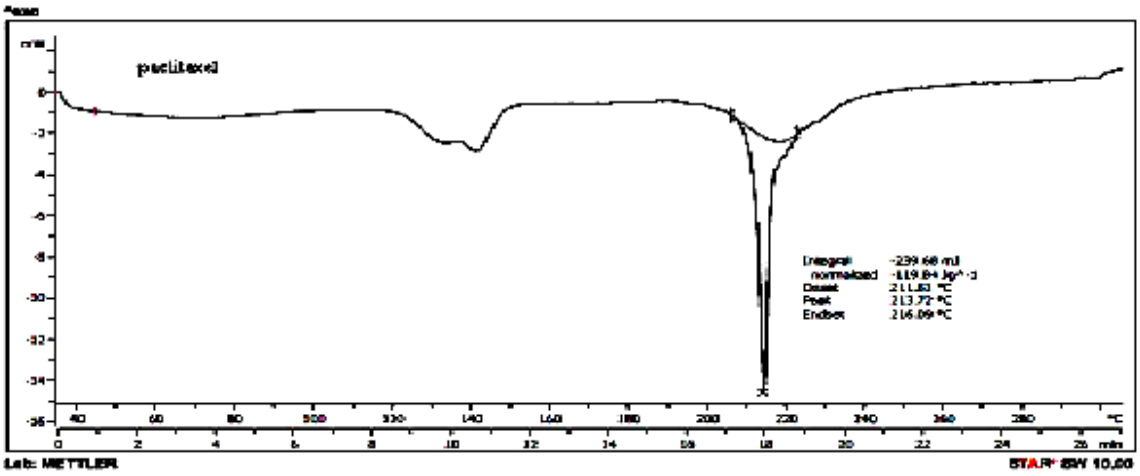

Fig. 6. DSC thermogram of PTX

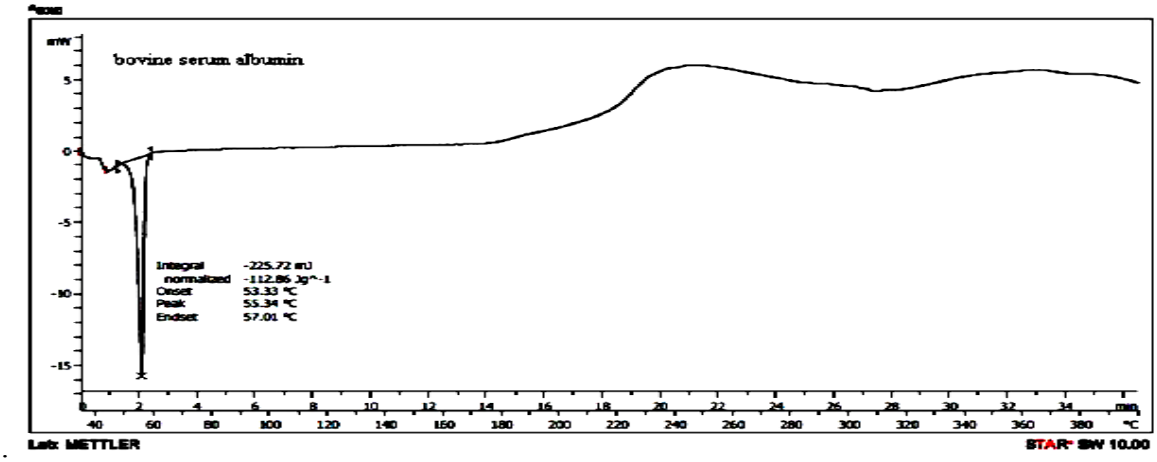

Fig. 7. DSC thermogram of BSA.

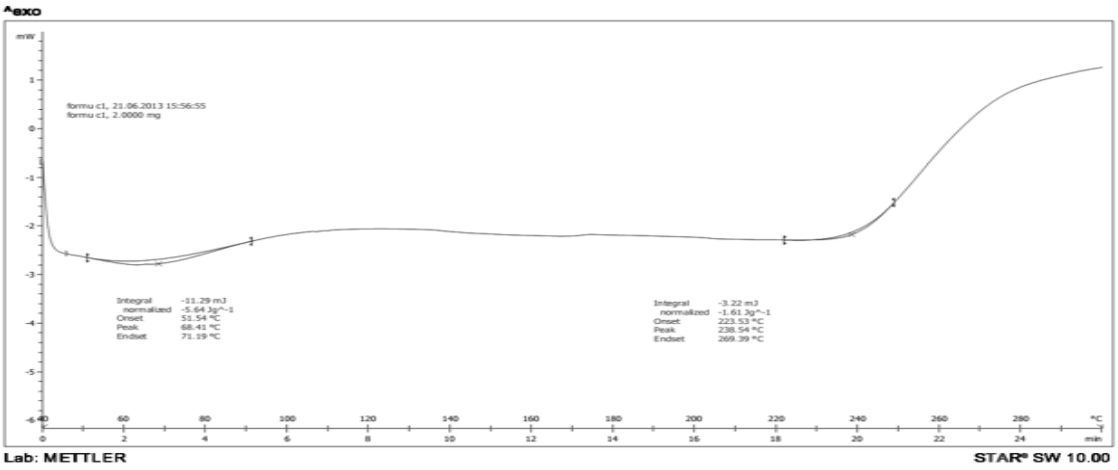

Fig. 8. DSC thermogram of optimized formulation.

3.8. *Zeta Potential*

Zeta potential of PTX loaded BSA nanoparticles for optimized batch was determined and it was found – 10.4 mV, showed in Fig. 9 which indicates moderate stability with no agglomeration. The negative surface charge originates from free carboxylic acid groups at the chain ends of the BSA polymer. The possible effects of surface charge may affect the *in-vivo* life span of the natural drug delivery system.

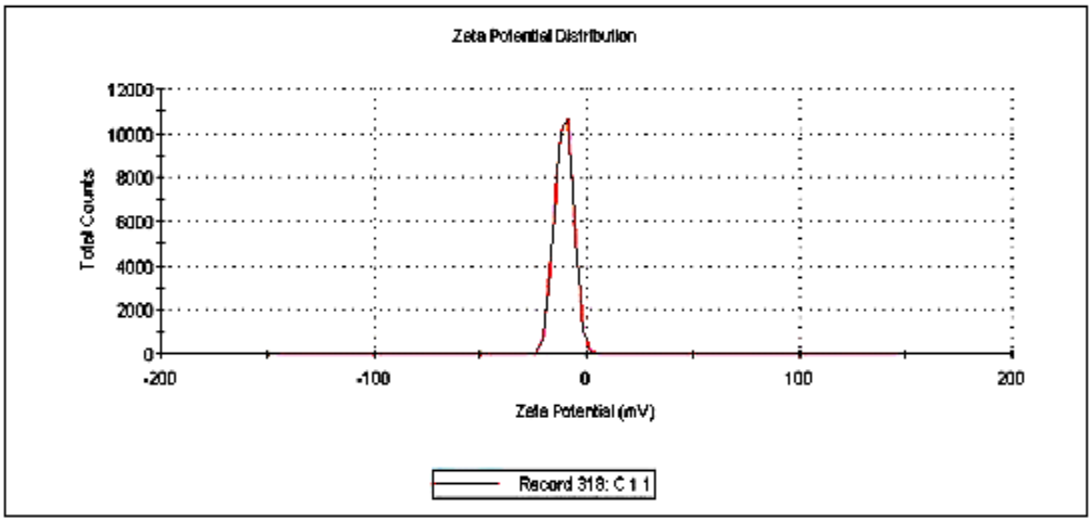

Fig. 9. Zeta potential graph of optimized formulation.

3.9. Scanning *Electron Microscopy (SEM)*

Morphology of the nanoparticles was studied by SEM. The Fig. 10 shows image of the optimized batch. It shows the smooth surface with spherical shape, may be due to optimum polymer and GTA concentration.

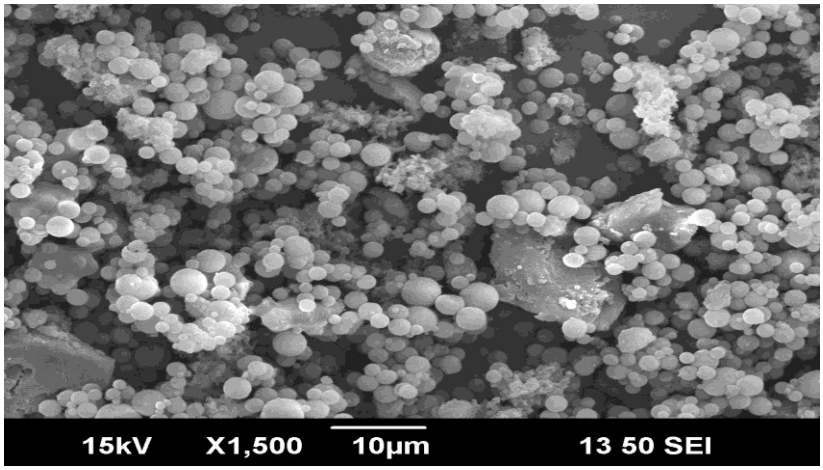

Fig. 10. SEM image of optimized batch.

3.10. Effect of Temperature and Humidity

Effect of temperature and humidity of the prepared nanoparticles were carried out, by storing optimized formulation at 4 ± 2 °C, and at room temperature 45 % RH for 30 days in stability chamber. Two parameters namely residual entrapment efficiency and *in-vitro* release studies were carried out. It is found that nanoparticles stored at room temperature are not stable where as stored at 4°C is stable given in Table 11. NPs at room temperature showed decrease in the residual entrapment efficiency and different release pattern.

Table 11. Effect of Temperature on Optimized Batch.

Temperature Condition	Parameters	Days			
		0	7	14	28
4 ± 2 ˚C	Entrapment efficiency (%)	60.35	60.20	59.98	59.73
	Release (%)	92.48	92.24 in 24^{th} hr	91.83 in 24^{th} hr	91.66 in 24^{th} hr
Room temp.	Entrapment efficiency (%)	60.35	56.68	50.74	42.23
	Release (%)	92.48	98.15 in 24^{th} hr	99.87 in 24^{th} hr	100 in 24^{th} hr

4. CONCLUSION

Bovine serum albumin (BSA) is an ideal carrier for preparing nanoparticles of paclitaxel. The use of acetone consistently gave smaller particles as compared to ethanol for all BSA concentrations. Desolvated BSA nanoparticles have the stability problem hence glutaraldehyde (GTA) is used as cross-linking agent for the production of stable nanoparticles. GTA also affect the entrapment efficiency and release rate but had no influence on particle size. Tween 80 is non-ionic surfactant used as dispersing agent to form stable nanoparticles. Speeds do not have significant effect on the particle size of nanoparticles. The results revealed that larger particles

have higher drug content, because very few drug molecules get sufficient time to diffuse into aqueous phase.

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