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Original Article

Development and characterization of albumin nanoparticles for pulmonary drug delivery

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ABSTRACT

Pulmonary drug delivery is a non-invasive, non-systemic delivery approach for both local and systemic drugs and a method to directly target disorders of lung. The albumin nanoparticles were prepared by modified desolvation method using different concentration of albumin as biodegradable polymer for lungs. The prepared albumin nanoparticles were evaluated for entrapment efficiency, particle size, polydispersity index, zeta potential, transmission electron microscopy, in-vitro drug release, stability study and in-vivo study. The entrapment efficiency of all albumin nanoparticles formulations were found to be in the range of 61.74 % - 73.45 %. The particle size of albumin nanoparticle formulations were found to be in range 81.6 nm - 148.4 nm. The zeta potential of albumin nanoparticles found to exhibit stability due to negative charge on the surface. The transmission electron microscopy indicated the spherical surface of the albumin nanoparticles. The in vitro release was found to follow higuchi plot as compared to zero order plot, first order plot and krosmeyer peppas plot. Stability studies showed that albumin nanoparticle formulation ANps5 was stable according to ICH guidelines for 6 months. In-vivo study using rat model indicated the localization of albumin nanoparticles in the lungs of rat.

Keywords: albumin nanoparticles, pulmonary drug delivery, terbutaline sulfate, particle size, entrapment efficiency, stability

INTRODUCTION

Lungs are an attractive target for the pulmonary administration of active pharmaceutical ingredients (APIs) in the form of various drug delivery systems [1–3]. Additionally, pulmonary route offers many advantages over conventional per oral administration, such as a high surface area with rapid absorption due to high vascularization and circumvention of the first pass effect [2]. Pulmonary drug delivery is a non-invasive, non-systemic delivery approach for both local and systemic drugs and a method to directly target disorders of lung such as asthma, chronic obstructive pulmonary diseases (COPD), emphysema ,cystic fibrosis, lung cancer, tuberculosis, pulmonary hypertension and diabetes. The principal of pulmonary drug delivery system is aerosolization which means reduction in the drug particle size delivered to alveoli and bronchioles.

The advantages of pulmonary drug delivery system[4,5] are

1. Allow efficient drug targeting to the lungs
2. It gives very fast onset of action comparable to the i.v. Route
3. Inhaling helps to avoid gastrointestinal tract problems such as poor solubility, low bioavailability, gut irritability, unwanted metabolites, food effects and dosing variability.
4. It requires low and fraction of oral dose
5. Pulmonary drug delivery having very negligible side effects since rest of body is not exposed to drug.

Nanoparticles are solid, colloidal particles which vary in size from 10 to 1000 nanometers. A drug can be dissolved, entrapped, adsorbed, attached or encapsulated into a nanoparticle matrix. Nanoparticles can protect the drug from degradation, enhance its transport and distribution

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and prolong its release; hence, the plasma half-life of the drug entrapped can be improved [6]. The advantage of using polymeric nanoparticles as colloidal carriers for advanced drug delivery is mainly their small size, which allows nanoparticles to penetrate even small capillaries and be taken up within cells, allowing efficient drug accumulation at targeted sites in the body. Biodegradable polymers used for nanoparticle preparation allow for sustained drug release at the targeted site over a period of days or even weeks after administration [7]. Nanocarrier systems can provide the advantage of sustained release in the lung tissue, resulting in reduced dosing frequency and improved patient compliance [8].

In the present research, albumin was used as biodegradable polymer to form albumin nanoparticles. Albumin nanoparticles were used to target lungs locally to treat various lung diseases such as asthma, chronic obstructive pulmonary diseases (COPD), cystic fibrosis, tuberculosis, lung cancer, pulmonary hypertension etc. Albumin nanoparticles administerd in lungs were inert to the surrounding tissue as they contain no irritating or toxic additives and degrade when applicable within an acceptable period of time without producing toxic by-products. Therefore, it was possible to locally target the lung tissue thereby reducing the dose as well as increasing the patient compliance by integration of nanotechnology and pulmonary drug delivery systems and provide a sustained effect of more than 12 hrs.

MATERIALS AND METHODS

Terbutaline sulfate obtained as gift sample from cipla, Baddi (H.P), Bovine serum Albumin obtained from the qualikem, Mumbai, acetone purchased from SD Fine chemical, chandigarh, glutaraldehyde purchased from the Qualikem, Mumbai, disodium hydrogen ortho Phosphate and Potassium dihydrogen ortho phosphate purchased from Central Drug House, New Delhi, Sodium Chloride is purchased from Qualikem, Mumbai. Sodium hydroxide is purchased from SD Fine Chemicals, Chandigarh.

Preparation of Albumin Nanoparticles[9]: Bovine serum albumin (BSA) nanoparticles were prepared by using a desolvation method with minor modifications. Bovine serum albumin (BSA) powder of required amount was added to distilled water to prepare aqueous solutions of different concentrations (1 - 5 % w/v), 100 mg of terbutaline sulfate was added to aqueous solutions and prepared aqueous solutions were adjusted to pH - 9 by using 0.1 N NaOH with continuous stirring at 500 rpm. The solutions were stirred overnight at 500 rpm using a magnetic stirrer for complete hydration. A desolvating agent, acetone, was added drop wise at a rate of 1 ml/min into the BSA solutions until the solutions became just turbid. Finally, 0.01 ml of a 4% glutaraldehyde–ethanol solution was mixed to induce intra-particle cross-linking. The solutions were stirred continuously at 500 rpm and kept for 3 hrs till albumin nanoparticles get formed. The composition of different albumin nanoparticles formulations are shown in Table -1.

Entrapment Efficiency [10]: The prepared nanoparticles suspension of 10 ml was centrifuged at 154350 g (approx. 35000 rpm) using Beckman Coulter Ultra Centrifuge for 30 mins. The pellet of nanoparticles was formed. The supernatant was removed and the supernatant was then observed under spectrophotometer at 276 nm for absorbance and the amount of the drug in the supernatant was estimated.

Particle size measurement [10]: The average particle size and the size distribution of the obtained nanoparticles was determined by photon correlation spectroscopy (PCS) using zetasizer. The analysis was performed at a temperature of 25 ºC using samples appropriately diluted with filtrated and double distilled water in order to avoid multiscattering events.

Zeta-Potential measurement [10]: The Zeta-Potential was measured by laser Doppler anemometry (LDA) using a Zetasizer. The analysis is performed at a temperature of 25ºC using samples appropriately diluted with 1.54 mM NaCl solution in order to maintain a constant ionic strength.

Transmission Electron Microscopy (TEM) of Gelatin Nanoparticles formulation: The morphology of gelatin nanoparticles of terbutalin sulfate was studied by transmission electron microscopy (TEM) on a Philips EM268D instrument (Philips, Netherlands) at SAIF Panjab University, Chandigarh. The aqueous dispersion of nanoparticles (one drop) was placed over a 400-mesh carbon-coated copper grid followed by negative staining with phosphotungstic acid solution (3% w/v, adjusted to pH 4.7 with KOH) and placed at the accelerating voltage of 95 kV for TEM.

In- vitro release study[10]: Drug release from known amount of Terbutaline sulfate loaded nanoparticles was determined using the dialysis tube diffusion method at 37 ± 1°C. Gelatin nanoparticulate formulation GNps3 (2 mL) and pure terbutaline sulfate were placed into the dialysis tube (Sigma Aldrich) tied at both the ends

and suspended in a beaker containing 50 mL of Phosphate Buffer Saline (pH-7.4) with 200 mg/mL ascorbic acid, an anti-oxidant to prevent oxidative degradation of Terbutaline Sulfate. The pure terbutaline sulfate of same amount as present in gelatin nanoparticle formulations were magnetically stirred at 50 rpm and the temperature was maintained at 37 ± 1°C throughout the procedure. Samples(1 ml) were withdrawn at definite time intervals and replaced with same volume of Phosphate Buffer Saline (pH-7.4). The samples were then analyzed spectrophotometrically (UV-1601 Shimadzu, Japan) for drug content.

RESULTS AND DISCUSSION

The prepared Albumin nanoparticle formulations were evaluated for entrapment efficiency. The results of the entrapment efficiency are shown in table – 2. The entrapment efficiency of different albumin nanoparticles formulations found to increase with increase in the concentration of albumin in different nanoparticle formulations. The entrapment efficiency found to be maximum for albumin nanoparticle formulation ANps5 and minimum for ANps1. The particle size and the polydispersity index of albumin nanoparticle formulations are shown in the Table -3. The particle size of the Albumin nanoparticle formulation ANps5 was found to be maximum and the particle size of the Albumin nanoparticle formulation ANps1 was found to be minimum. The effect of the albumin concentration on the particle size of Albumin nanoparticles observed that the particle size of the Albumin nanoparticle increase with increase in the albumin concentration. The sequence of particle size was found to be ANps5>ANps4>ANps3>ANps2>ANps1.

The Albumin nanoparticle formulation ANps5 was selected as a best formulation depending upon the particle size less then 500nm required to get entrapped in the alveoli of lungs and possess higher entrapment efficiency as compared to ANps1, ANps2, ANps3 and ANps4.

The zeta potential of albumin nanoparticle formulations are shown in the Table 4. The results of Zeta Potential of Albumin nanoparticles of all formulation showed slight negative charge on the surface. The albumin nanoparticle formulation were found to be stable depending upon the charges present on the surface of the albumin nanoparticle which prevent the aggregation of the particle suspended in suspension of albumin nanoparticle formulations. The selected albumin nanoparticles formulation (i.e ANps5) was also evaluated for Transmission Electron Microscopy(TEM). The results of the Transmission Electron Microscopy(TEM) of albumin nanoparticle formulation (ANps5) are shown in the figure 1 and figure 2.

TEM micrographs of prepared albumin nanoparticle formulation (ANps5) showed that the albumin nanoparticles were spherical in shape. The albumin nanoparticles loaded with terbutaline sulfate were found to be black in color. The Transmission Electron Microscopy(TEM) confimed the preparation of smooth and spherical nature of the albumin nanoparticles. The comparison of in-vitro release of free terbutaline sulfate (pure drug) and albumin nanoparticle formulations are shown in the graph below(figure 3).

In vitro release of terbutaline sulfate from all albumin nanoparticle formulation exhibited sustained release of the terbutaline sulfate as compared to the pure drug. Albumin nanoparticles formulations exhibited a biphasic pattern of drug release, an initial burst effect due to immediate release of the surface associated drug and prolonged release in the later stage due to the slow diffusion of drug from the matrix. The release of the terbutaline sulfate from the albumin nanoparticle formulations found to be best fit in higuchi plot as compared to zero order plot, first order plot and Krosmeyer peppas plot because the regression co-efficent in case of higuchi plot was found to higher as compared to the regression co-efficent in case of zero order plot, first order plot and Krosmeyer peppas plot.

Stability Study of Albumin Nanoparticles: The optimized formulation (ANps5) of Albumin Nanoparticles was selected for stability study to be performed at refrigerated condition (5º±3ºC), at room temperature (25º±2ºC/65%±5% RH) and at accelerated condition (40º±2ºC/75%±5% RH) according to ICH guidelines for 6 months(180 days). The samples were withdrawn after 0, 45, 90, 135 and 180 days and were checked for particle size, entrapment efficiency and *in vitro* drug release. The formulation at refrigerated condition serve as control and was used to compare the results of formulation kept at room temperature and at accelerated condition. The results of stability study of albumin Nanoparticle formulation (ANps5) are shown in Table 5.

The results of stability studies showed that the selected formulation of albumin nanoparticle formulation ANps5 was stable at refrigerated condition (5º±3ºC), at room temperature (25º±2ºC/65%±5% RH) and at accelerated condition (40º±2ºC/75%±5% RH) according to ICH guidelines for 6 months(180 days) because

there was negligible change in the values of particle size, entrapment efficiency and in-vitro drug release of albumin nanoparticle formulation ANps5.

In-Vivo Study Using Rat Model[11,12]: Direct Intratracheal administration technique was used in rats. In this technique, rats were anaesthised with Ketamine intraperitoneal injection (50 mg/kg). After anaesthesia, the animal was laid in a supine position, attached by its superior incisors to a board and titled at an angle of 45 degrees. The mouth was kept open to locate the vocal cords and for delivering selected nanoparticle formulation (ANps5) in trachea. The administration was performed by inserting a modified oral gavage needle No. – 16 (which had thin plastic tube at tip of needle and 1 ml of disposable syringe at the bottom of the needle) in the trachea, between the vocal cords. Nanoparticle formulations containing the drug (upto 25 – 30 µl) was delivered into the trachea as a liquid bolus by intratracheal instillation. For the deepest administration within the lung and for the highest bioavailability, the instillation was followed by the administration of 3 ml of air bolus by using microsyringe.The drug deposition was determined by taking blood samples 0.5 ml at different time intervals (i.e. 0, 1, 2, 4, 6, 12, 24, 36 and 48 hrs) from tail vein of the rats into heparinized Eppendrff tubes. The blood samples were centrifuged at 15000 rpm for 15 mins and obtained plasma was examined for Terbutaline Sulfate concentration using HPLC method and pharmacokinetic of Terbutaline Sulfate was studied from different selected nanoparticle formulations.

The results of the in-vivo studies indicated that the drug plasma concentration of Terbutaline sulfate found to be insignificant in the blood of the rat at different time interval of sampling which indicated that the albumin nanoparticles are localized in the lungs of the rat and no drug present in the blood of the rat upto 48 hrs of blood sampling and whole of the drug is releasing from albumin nanoparticles in lungs in sustained manner. The localization of gelatin nanoparticles are confirmed from the results of the in-vivo study as whole of the drug is present in the lung and no drug was present in the blood of the rat.

CONCLUSION

Pulmonary drug delivery is a attractive route to deliver the drug directly in the lungs which overcome various side effects of systemic delivery of the drugs. Exploiting pulmonary route for delivery of drugs indicated that the albumin nanoparticle with size less then 500 nm were localized effectively in the pulmonary route to treat the lung disease such as asthma, chronic obstructive pulmonary diseases (COPD) etc. The results indicated that the prepared albumin nanoparticles effectively sustain the release of the drug(terbutaline sulfate) as compared to the pure terbutaline sulfate. The prepared albumin nanoparticles were found to be stable when tested for entrapment efficiency, particle size and in-vitro release at different conditions temperature according to ICH guidelines for 6 months. The in-vivo data using rat lung model indicated that the albumin nanoparticles were effectively localized in the lungs and there were insignificant trace of drug in the blood samples of rat for upto 48 hrs which confirmed the presence of terbutaline sulfate loaded albumin nanoparticles in the lungs of rat.

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Table -1 : Composition of different Albumin Nanoparticle formulations

Sr.No.	Formulation code	Amount of Albumin used (mg)	Amount of terbutaline sulfate used(mg)	Concentration of albumin (w/v)
1	ANps1	200	100	1%
2	ANps2	400	100	2%
3	ANps3	600	100	3%
4	ANps4	800	100	4%
5	ANps5	1000	100	5%

Table - 2 : Entrapment Efficiencies of Albumin nanoparticle formulations

Sr. No.	Formulation code	Entrapment Efficiency(%)
1	ANps1	61.74 ± 0.65
2	ANps2	64.82 ± 0.54
3	ANps3	68.36 ± 0.94
4	ANps4	69.22 ± 1.08
5	ANps5	73.45 ± 0.77

Table-3: Particle size and polydispersity index of Albumin nanoparticle formulations

Sr. No.	Formulation code	Particle Size(nm)	Polydispersity Index
1	ANps1	81.6 ± 0.8	0.207 ± 0.011
2	ANps2	99.4 ± 0.3	0.247 ± 0.008
3	ANps3	116.5 ± 0.7	0.238 ± 0.024
4	ANps4	127.2 ± 0.5	0.165 ± 0.035
5	ANps5	148.4 ± 0.6	0.280 ± 0.018

Table-4 : Zeta Potential of Albumin nanoparticle formulations

Sr. No.	Formulation code	Zeta Potential
1	ANps1	- 4.64 ± 0.05
2	ANps2	- 31.03 ± 0.11
3	ANps3	- 21.81± 0.08
4	ANps4	- 15.21 ± 0.15
5	ANps5	- 18.44 ± 0.27

Table 5: Stability Study of Albumin Nanoparticles formulation (ANps5) at Refrigerated Condition (5º±3ºC), Room temperature (25º±2ºC/65%±5% RH) and Accelerated Condition (40º±2ºC/75%±5% RH)

Sr. No.	Parameters	Days	Refrigerated Condition (5º±3ºC)	Room Temperature (25º±2ºC/65%±5% RH)	Accelerated Condition (40º±2ºC/75%±5% RH)
1	Particle size (nm)	0	148.4 ± 0.6	148.6 ± 0.4	148.9 ± 1.3
		45	148.4 ± 0.6	148.8 ± 0.7	149.3 ± 0.5
		90	148.4 ± 0.6	149.1 ± 0.9	149.7 ± 0.8
		135	148.4 ± 0.6	149.3 ± 1.5	150.2 ± 1.1
		180	148.4 ± 0.6	149.6 ± 0.9	150.8 ± 0.5
2	Entrapment Efficiency (%)	0	73.45 ± 0.77	73.31 ± 0.47	73.09 ± 0.80
		45	73.45 ± 0.77	73.20 ± 0.75	72.65 ± 0.35
		90	73.45 ± 0.77	73.06 ± 0.32	72.30 ± 0.97
		135	73.45 ± 0.77	72.83 ± 0.45	71.96 ± 0.67
		180	73.45 ± 0.77	72.60 ± 0.37	71.57 ± 0.82
3	In vitro Drug Release (%)	0	68.26 ± 0.55	68.15 ± 1.51	68.04 ± 0.64
		45	68.26 ± 0.55	68.02 ± 0.45	67.57 ± 1.33
		90	68.26 ± 0.55	67.83 ± 1.26	67.24 ± 0.58
		135	68.26 ± 0.55	67.70 ± 1.17	66.91 ± 1.39
		180	68.26 ± 0.55	67.48 ± 0.83	66.57 ± 1.42

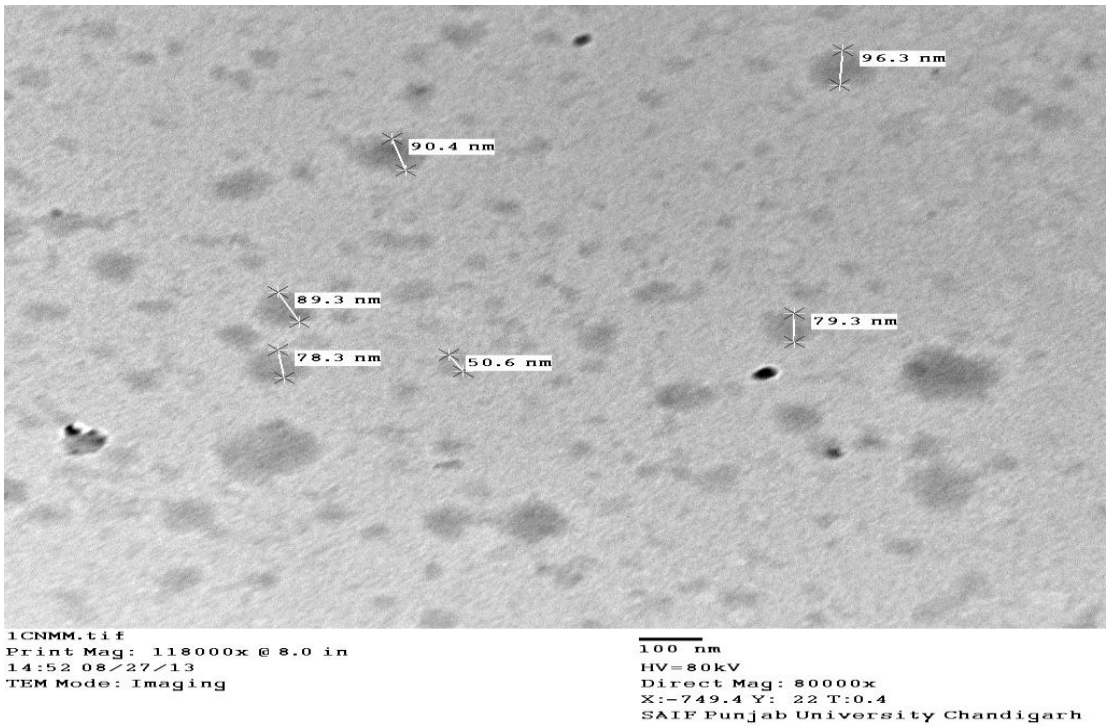

Figure 1 : TEM diagram of Albumin nanoparticle formulation, ANps5

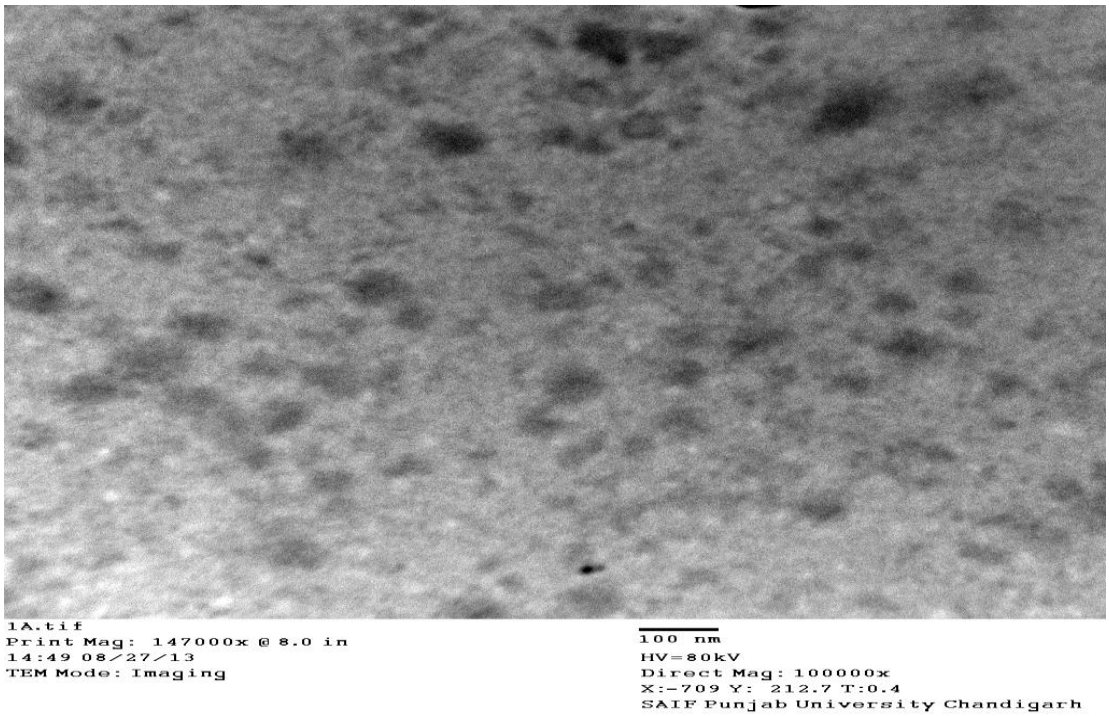

Figure 2 : TEM diagram of Albumin nanoparticle formulation, ANps5

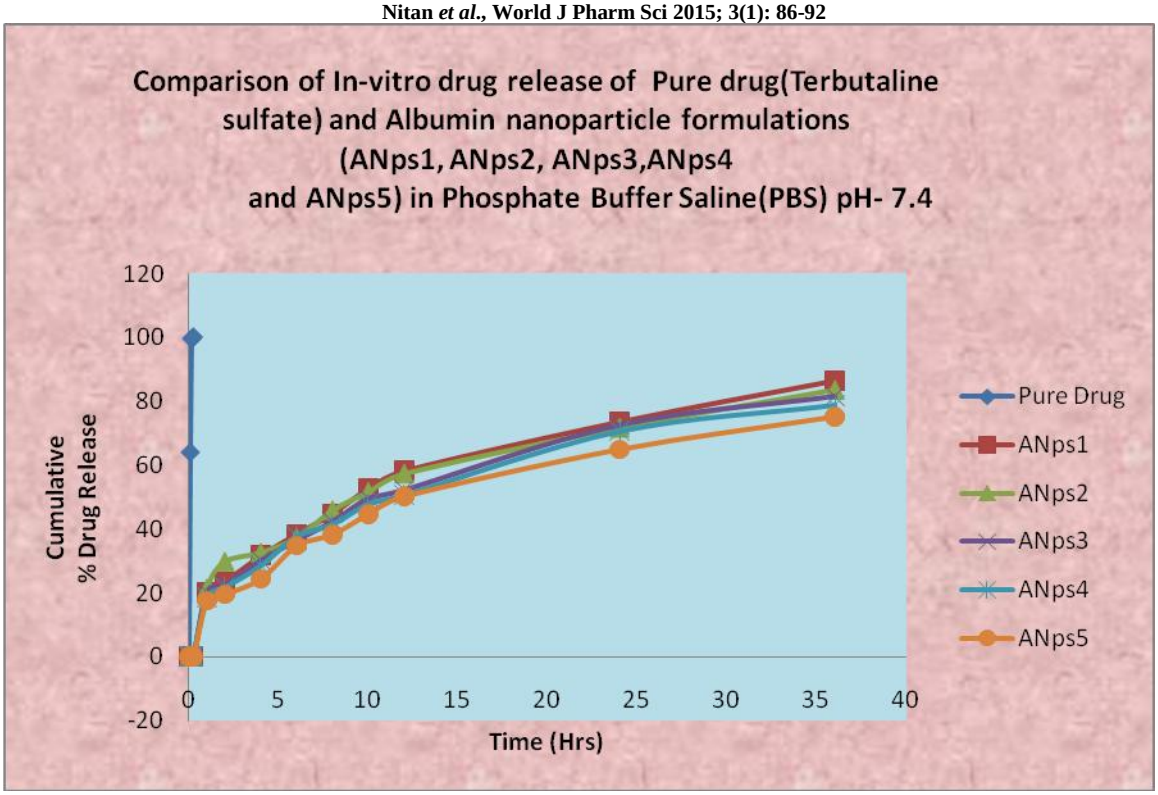

Figure 3: Comparison of In-vitro drug Release of Pure drug (Terbutaline sulfate) and Albumin Nanoparticle formulations (ANps1, ANps2, ANps3, ANps4 and ANps5) in Phosphate Buffer Saline (PBS) pH- 7.4

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