L. Sasikala et al /J. Pharm. Sci. & Res. Vol. 14(8), 2022, 873-882

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Formulation, Characterization and Evaluation of Eplerenone Drug Loaded Nanoparticles

L. Sasikala[1], V. Koteswara Rao[1]*, R. Gangadhara[2], M. Ramakrishna Reddy[3] and T. Shobha Rani[3]*

[1]Department of Chemistry, Gandhi Institute of Technology and Management, Hyderabad, Telangana, India.
[2]Department of Chemistry, Jawaharlal Nehru Technological University, ATP, Ananthapuramu, A. P., India.
[3]Department of Chemistry, Dravidian University, Kuppam-517425, Chittoor (DT), A. P., India.

Abstract:

Formulation, characterization and evaluation of nanoparticles loading with epleronone by using ionotropic gelation method employing chitosan, sodium TPP and Tween 80 was the main aspire of current investigation. Total eleven formulations were prepared by using different drug: polymer ratios for knowing the evaluation of epleronone nanoparticles. Herein, the solubility, calibration of drug, drug content and drug entrapment efficiency of the drug loaded nanoparticles were investigated. The prepared nanoparticles with epleronone drug were characterized using Fourier-transform infrared spectroscopy, Scanning electron microscopy, Zeta potential and *In-vitro* drug release studies. Information from the FT-IR studies has revealed that drug and excipients used have no interactions.

Among them 0.5% chitosan containing F5 formulation shows maximum drug release than the other trails. So F5 formulation was considered as the optimized formulation. The drug content of the formulated nanoparticles was found in the range of 72.31 to 87.22 %. The entrapment efficacy of the formulated nanoparticles was found to be in the range of 75.48% - 91.22% respectively. Scanning electron microscopy image indicated that nanoparticles were in granules form. Based on the outcomes, it was concluded that the optimized formulation F5 follows Zero order drug release with Non fickian diffusion mechanism.

Keywords: Chitosan, Epleronone, FT-IR and Sodium TPP.

INTRODUCTION

The nanoparticles (NPs) are defined as particulate dispersions or solid particles of drug carrier that may or may not be biodegradable. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticles matrix. The term nanoparticles are a combined name for both nano spheres and nano capsules[1]. Drug is confined to a cavity surrounded by a unique poly mermembrane called nano capsules, whereas nano spheres are mould systems in which the drug is actually and consistently diffused. On the way to utilizing with conservative techniques if reach their limits, nanotechnology supplies opportunities for the medical applications[2]. In drug delivery system, there were abundant benefits recommended by nanoparticles. These advantages take account of, but are not edged. Nanoparticles enhance the aqueous solubility of poorly soluble drug, which improves bioavailability of drug. As targeted drug carrier of nanoparticles reduce drug toxicity and enhance efficient drug distribution[3]. The modified polymers would be used for drug release which makes polymeric nanoparticles an ideal drug liberation system for cancer treatment, vaccines, contraceptives and antibiotics[4].

To improve survival of patients with indicative heart stoppage and to lesser blood pressure, Eplerenone was used an aldosterone receptor contender. Eplerenone binds to the mineral corticoid receptor and thereby blocks the binding of aldosterone (component of the rennin-angiotensin-aldosterone-system, or RAAS)[5]. Aldosterone synthesis, which occurs primarily in the adrenal gland, is modulated by multiple factors, including angiotensin II and non-RAAS mediators such as adreno cortico tropic hormone (ACTH) and potassium. The therapeutic use of epleronone is limited by the rapidity of the onset of its action and its short biological half-life[6]. In order to produce a form devoid of these disadvantages we made nanoparticles of epleronone by applying ionotropic gelation method using chitosan.

Nanoparticulate carriers may provide a better therapeutic output by targeting drugs specifically to their site of action and by improving the pharmacokinetic profile of effective drugs having poor water solubility, low bioavailability and high toxicity[7]. They have been assumed to be a good strategy for drug delivery in the treatment of liver diseases. In present investigation, Ionotropic gelation method was used for preparation of nanoparticles. Currently, nanoparticles have gained importance since the ease of manufacturing and diversified applications which are assured the promising objected and controlled release dosage form[8]. Due to availability biodegradable polymers and of its low toxicity which usage was more in the pharmaceutical research of present trend[9].

Nanoparticles containing drug was prepared by ionotropic gelation method by using chitosan and sodium TPP as a cross-linking agent and quantity sufficient water. Estimation of Epleronone was carried out spectrophotometerically at 244 nm. The Nanoparticles were evaluated for parameters such as drug content uniformity, entrapment efficiency, scanning electron microscopy, particle size analysis, zeta potential, in-vitro release, drug excipients interactions (FTIR). The stability data was also subjected to statistical analysis.

MATERIALS AND METHODS

Materials:

As a gift sample i.e., Epleronone was received from B.M.R. Chemicals, Hyderabad, Chitosan was obtained from Oxford laboratories, Mumbai, Tween 80 was procured from Rankem, Mumbai, and Sodium tripoly phosphate was attained from Lobo chemicals, India. All other solvents and reagents were get hold of from local supplier, Hyderabad, India were of analytical grade and used as received.

Pre-formulation studies of Epleronone drug:

The preceding to build up drug as dosage form, it is essential that determined the drug for certain fundamental physical and chemical properties of the drug molecule alone and when combined with excipients. This first phase of learning is known as pre-formulation. The overall objective of the pre-formulation is to generate information useful to the formulator in developing stable and bioavailable dosage forms which can be mass produced[10]. The objectives of pre-formulation studies are: Analytical evaluation of the drug substance and determine its necessary characteristics and to establish its compatibility with different excipients.

Melting point:

To know the drug in the formation of nanoparticles, melting point of the substance has studied. The temperature at which the first particle starts to melt or of the substance completely melts or otherwise last particle completely melts is regarded as melting point of the substance[11]. The melting point of drug was determined by capillary method.

Solubility studies:

The solubility of Eplerenone 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 Eplerenone in different beakers containing the solvents. The mixtures were shaken for 24 h at regular intervals. The solutions were filtered by using Whitman's filter paper grade no.41. The filtered solutions were analyzed spectrophotometerically at 244 nm.

Drug-excipients interaction studies:

There is always possibility of drug- excipients interaction in any formulation due to the irintimate contact. The technique employed in this study is IR spectroscopy. IR spectroscopy is one of the most powerful analytical techniques, which offers possibility of chemical identification[12]. The IR (FTIR Spectrometer-Perkin-Elmer series 1615) spectra were obtained by KBr pellet method.

Procedure for standard curve of Epleronone in 0.1N HCl:

In 10 ml of 0.1N HCl, the 10 mg of Epleronone was dissolved by slight shaking (1000 µg/ml). 1 ml of this solution was taken and made up to 10 ml with pH 0.1N HCl, which gives 100 µg/ml concentration (stock solution). From the stock solution, concentrations of 0, 2, 4, 6, 8, 10 and 12 µg/ml in pH 0.1N HCl were prepared. The absorbance of diluted solutions was measured at 244 nm and a standard plot was drawn using the data obtained.

Preparation of nanoparticles - Ionic gelation method:

As Calvo et al. (1997b) reports, chitosan nanoparticles with drug loading were prepared with some modifications based on the ionic gelation of Epleronone with TPP anions[13]. In different concentrations such as 1.0, 2.0, 3.0, 4.0, 5.0 and 0.6 mg/ml, Chitosan was dissolved into aqueous acetic solution (2 % v/v). 25 mg of drug (Epleronone) was dissolved in methanol and added to 5 ml of 2 % w/v tween 80 solutions, which was added to the chitosan solution. At room temperature, under magnetic stirring aqueous solution of 5 ml 0.25% sodium tri polyphosphate (TPP) was added drop wise into drug and polymeric mixture respectively. The stirring was continued for about 30 min. At 6000 rpm for a period of 45 min. the obtained nanoparticles suspension was centrifuged. As a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation) the drug loaded nanoparticles was formed. The data of different formulations based on polymer and cross-linking agent concentration and stirring time was given in table 1.

Evaluation parameters of Epleronone nanoparticles:

The nanoparticles was evaluated for various parameters

Drug content uniformity:

Drug content uniformity of prepared nanoparticles was carried out using the spectrophotometric method. 5ml of each formulation was taken and dissolved in 7.4 pH buffer solution and kept overnight. 10mg (similar as in formulation) of drug was taken and dilution was made to 10 µg/ml. The dilutions were filtered and analyzed using UV for their content uniformity. The absorbances of the formulations were read using on cellina UV-Vis spectrophotometer. The instrument was set at 244nm. The drug content in each formulation was calculated based on the absorbance values of known standard solutions.

Entrapment efficacy:

The brightly prepared nanoparticles were centrifuged for 20 min by 20,000 rpm at 5^0C temperature using cool ultracentrifuge. The quantity of unincorporated drug was assessed by obtaining the absorbance of the appropriately weakened 5 ml of supernatant solution at 244 nm using UV spectrophotometer alongside blank/control nanoparticles. Drug entrapment efficiency was determined by subtracting the amount of free drug in the supernatant from the initial amount of drug taken[14].

From the following equation, the entrapment efficiency (EE %) could be achieved.

% Entrapment efficiency = Drug content X 100/Drug added in each formulation

Particle size and shape:

Average particle size and shape of the formulated nanoparticles was determined by using Malvern Zetasizer ZS using water as scattering medium. For determination of particle size, the sample was scanned 10 times after which, the average value was used for further calculations.

Scanning electron microscopy:

With this scanning electron microscopy at various magnifications, the morphological facets of Epleronone nanoparticles were monitored and photomicrographs were taken at good resolution. From the resulting image, the particle size and shape was determined.

Zeta potential measurement:

Zeta potential is an important parameter to evaluate and establish an optimum condition for stability of colloidal or dispersed systems[15]. Zeta-potential measurements were analyzed by zeta sizer Nano ZS (Malvern Instruments, version 2.1). For the analysis, the nanoparticles sample of the desired concentration was flushed through a folded capillary cell (DTS1060), and the measurement was carried out on the second filling; a sufficient sample volume was used to cover the electrodes of the cell

completely. The sample was injected slowly, and analysis was carried out if there were no visible air bubble inclusions present. After inspection, the cell was placed into the zeta sizer and equilibrated for 2 min prior to the particle size measurements of which there were six replicates.

***In- vitro* drug release study:**

The dialysis bag diffusion technique was utilized to study the *in-vitro* release studies. The dialysis bag (molecular weight cut off: 12,000–14,000 Da) was filled with prepared SLN dispersion of definite volume. A bottle holding 100 mL of phosphate buffer, i.e., pH 6.8 was used for immersing the filled dialysis bag in it. With help of a thermostatic horizontal shaker (GFL; Gesell's chatt Laboratories, Berlin, Germany) set upped at $37^0C \pm 0.5^0C$ and 100 rpm the release was carried. Through 1, 2, 4, 6 and 8 h time intervals for a period of 15 and 30 min aliquot samples were withdrawn and analyzed. At 244 nm with UV/VIS spectrophotometer (UV-1800) the drug concentration was evaluated spectrophotometerically. The outcomes were articulated as the mean values ± SD and all experiments were conceded out three times repeation. The end results of *in-vitro* release outlines obtained for the NDDS formulations were fitted into four models of data treatment as follows.

To know the release rate of the drug from matrix systems, various release kinetic equations (zero-order, first-order, Higuchi's equation and Korsmeyer-Peppas equation) were applied for the optimized formulation. The best fit with higher correlation (r^2) was estimated.

Here Mt / M∞ < 0.6 could be used for calculation of exponent of n^{th} portion of the release curve[16]. For study of release kinetics, the graph was drawn between log cumulative percentage drug release *and log* time which obtained data from *in- vitro* drug release studies.

RESULTS AND DISCUSSION

Melting point determination

The Epleronone melting point was found to be in range of 240^0C which was determined by capillary method.

Saturation solubility

Using 0.1N HCL, purified water, 6.8 and 7.4 phosphate buffers and the saturation solubility was carried out at 25^0C. The results were shown in table 3 and Figure 1 is as follows. From the above conducted solubility studies in various buffers we can say that pH 0.1N HCl has more solubility when compared to other buffer solutions. So 0.1N HCL was used as dissolution medium, based upon the solubility studies was used in the nanoparticles formulation. It was observed that in table 3 & figure 1, more solubility is found in methanol i.e., 23.93 mg/ml while the lowest solubility can be seen in 7.4 buffers (6.13mg/ml) and in ethanol also find good solubility. From the above, it is understood that the solubility in 6.8 pH and 7.4 pH buffer are more or less equal. Among all the buffers, methanol and 0.1N HCL were suitable for Epleronone drug soluble and acquiring calibration.

Determination of absorption maximum (λ max)

For precise quantitative measurement of drug closure rate at 0.1N HCl medium λ-max of Epleronone was determined. The UV spectrum of epleronone was depicted in the following figure 2. The spectrum was plotted between wavelength and absorbance. From the figure 2, it was evident that the λ-max of Epleronone at 244 nm was optimized for calibration.

Table 3: Saturation solubility data of Epleronone.

S.No	Buffers	Solubility (µg/ml)
1	0.1N HCL	10.30
2	6.8 buffer	7.21
3	7.4 buffer	6.13
4	Methanol	23.93
5	Ethanol	18.55

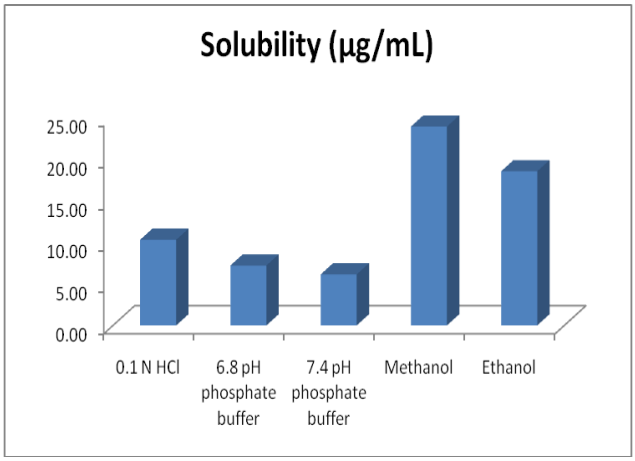

Figure 1: Solubility studies of Epleronone.

Table 4: Standard calibration curve of Eplerenone in 0.1N HCl at 244 nm.

Concentration (µg/ml)	Absorbance
0	0
2	0.162
4	0.329
6	0.468
8	0.641
10	0.789
12	0.963

Table 5: Standard calibration curve of Eplerenone at 244 nm in 6.8 pH buffer.

Concentration (µg/ml)	Absorbance
0	0
2	0.132
4	0.259
6	0.391
8	0.519
10	0.641
12	0.763

Table 6: Standard calibration curve of Eplerenone at 244 nm in 7.4 pH buffer.

Concentration (µg/ml)	Absorbance
0	0
2	0.138
4	0.275
6	0.419
8	0.558
10	0.698
12	0.822

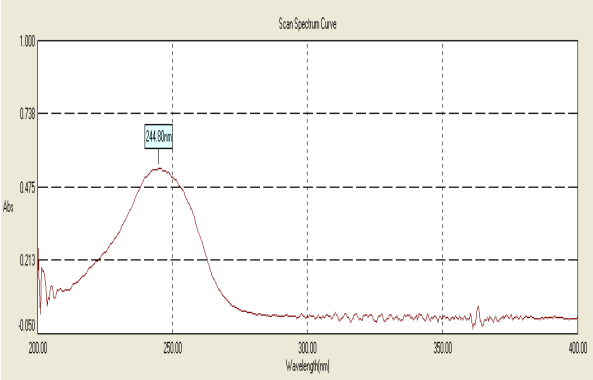

Figure 2: UV spectrum of Epleronone.

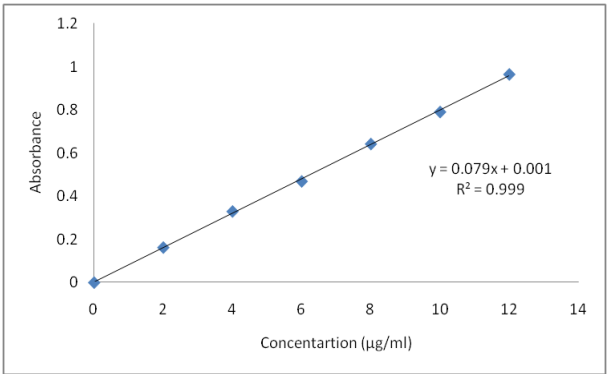

Figure 3: Eplerenone standard calibration curve at 244 nm in 0.1N HCl.

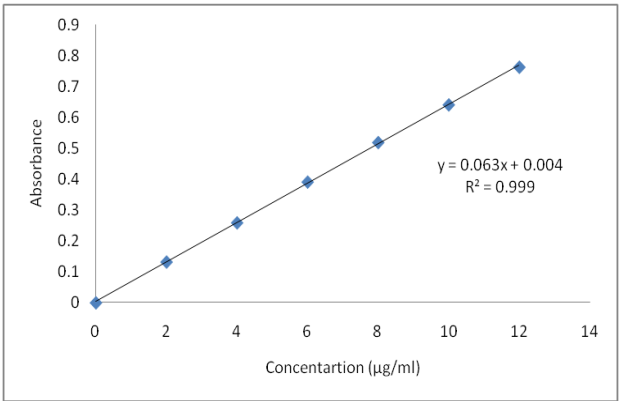

Figure 4: Standard calibration curve of Eplerenone at 244 nm in 6.8 pH buffer.

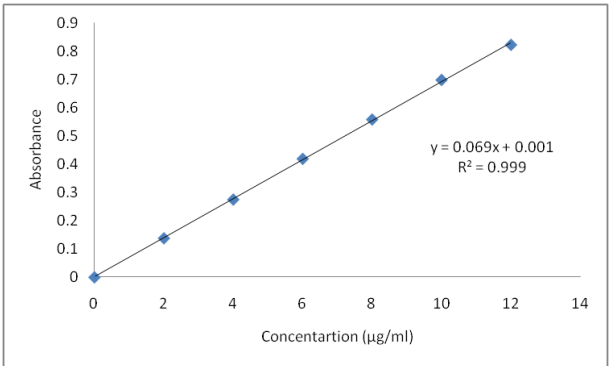

Figure 5: Standard calibration curve of Eplerenone at 244 nm in 7.4 pH buffer.

Table 7: Drug content of formulated nanoparticles.

Formulation code	Mean % of Drug content
F1	78.23
F2	80.14
F3	82.24
F4	84.22
F5	86.64
F6	72.31
F7	76.42
F8	80.76
F9	74.52
F10	82.54
F11	87.22

Table 8: Entrapment efficiency of formulated nanoparticles.

Formulation code	Mean % entrapment efficiency
F1	79.26
F2	81.54
F3	82.26
F4	85.27
F5	86.29
F6	75.48
F7	79.38
F8	82.92
F9	79.28
F10	83.56
F11	91.22

Preparation of standard calibration curve of Eplerenone in 0.1N HCl

In the range of 0-12 µg/ml into 0.1N HCl the linearity was established. The regression value was closer to 1 designating the method obeyed Beer-lamberts' law. The data of Eplerenone calibration in 0.1 N HCl was presented in table 4 and figure 3. In the figure 3 it was known that a linear relationship was obtained with an R^2 value of 0.999 at 244 nm and line equation, y=0.079x+0.001.

Preparation of standard calibration curve of Eplerenone in pH 6.8 phosphate buffer

The 10mg of Eplerenone was accurately weighed and transferred into10ml volumetric flask. It was dissolved in 2-3 ml of methanol and reduced to volume with pH 6.8 phosphate buffer to provide reserve solution containing1000µg/ml. From the standard stock, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml was pippeted out and volume was adjusted to10ml with same buffer solution to get 2, 4, 6, 8, 10 & 12µg/ml of Eplerenone. The absorbance of the solution was measured against pH 6.8 phosphate buffer as blank at 244 nm using UV visible spectrophotometer. The absorbance values were plotted against concentration (µg/ml) to obtain the standard calibration curve. The concentration of the drug and corresponding absorbance values are mentioned in table 5. The results/curve on U.V. Spectrum of Eplerenone (λ-max) in 6.8 phosphate buffer as shown in the figure 4. A linear relationship was obtained between concentration (0-12 µg/ml) and the absorbance of the drug in 6.8 pH buffer with an R^2 value of 0.999 at 244 nm and line equation, y=0.063x+0.004. The regression value was closer to 1 indicating the method obeyed Beer-lamberts' law.

Preparation of standard calibration curve of Eplerenone in pH 7.4 phosphate buffer

10mg of Eplerenone was accurately weighed and transferred into10ml volumetric flask. It was dissolved in 2-3mL of methanol and diluted to volume with pH 7.4 phosphate buffers to give stock solution containing1000µg/ml. From the standard stock, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml was pippeted out and volume was adjusted to10ml with same buffer solution to get 2, 4, 6, 8, 10 & 12µg/ml of Eplerenone and results were represented in table 6. The absorbance of the solution was measured against pH 7.4 phosphate buffer as blank at 244 nm using UV visible spectrophotometer. The absorbance values were plotted against concentration (µg/ml) to obtain the standard calibration curve. Beer Lambert's plot of drug sample was prepared in 7.4 pH buffer. A linearity was acquired between concentration (0-12 µg/ml), and the absorbance of the drug in phosphate buffer (pH 7.4) with an R^2 value of 0.999 at 244 nm is shown in the calibration curve of Figure 5 and line equation, y= 0.069x+0.001. Based on these results, a phosphate buffer pH 7.4 was chosen and calibrated for dissolution of the drug.

Compatibility studies of drug & excipients

The FT-IR analysis was carried out for pure drug with that of various excipients used in the formulation. This analysis was used for confirmation of compatibility of drug and excipients by comparing their spectra's. The FT-IR spectrums of neat drug and Epleronone optimised formulation were depicted in figures 6 & 7. Form the drug and its excipients compatibility studies, it was cleared that there are no interactions between the pure drug (Epleronone) and optimized formulation (Epleronone + excipients) which indicates there are no physical changes and which were confirmed by above figures.

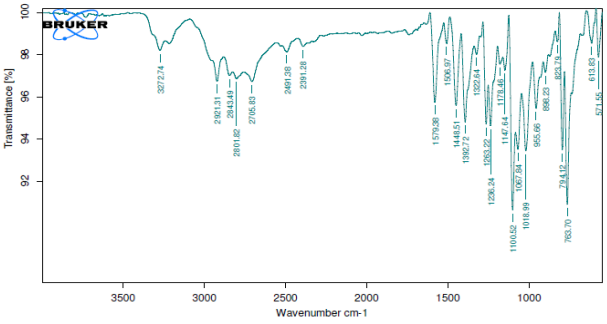

Figure 6: IR spectrum of Epleronone.

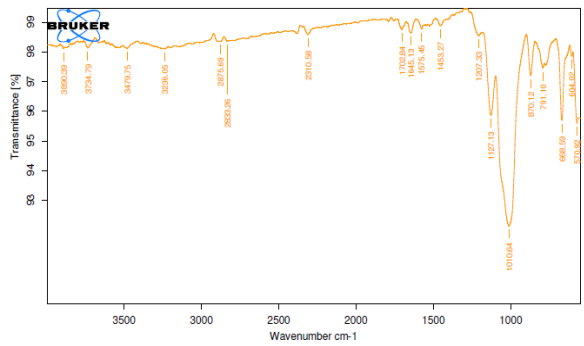

Figure 7: IR spectrum of Epleronone optimised formulation (F5).

Drug content of Epleronone nanoparticles

The drug content of the formulated nanoparticles was found in the range of 72.31 to 87.22 % respectively. The drug content (Mean %) results are given in table 7 and as follows. From the table 7, it was understand that lowest drug content percentage was observed for F6 formulation and higher content was found in formulation F11. For formulations F3, F4 and F5 having gradual increased percentage of drug content. These are considered an acceptable range for topical formulation.

Entrapment efficacy

The **%** of entrapment efficiency was calculated for F1-F11 formulations using the above mentioned formula and the results acquired are presented in table 8. The entrapment efficiency was measured for formulations F1-F11 and the amount of drug entrapped was found to be in the range of 75.48% - 91.22% respectively. It was seen from table 8 and the percentage of entrapment efficiency was found to be more for F11 than other formulations which is 91.22% and the less entrapment efficiency was found for F6 formulation (Chitosan:TPP, ratio 0.6:5). The entrapment efficiency was affected for the drug and the variation in entrapment efficiency was due to the changes in the polymer/cross-linker concentration and difference in the degree of cross-linking. For formulations F2, F3, F4 and F5 having gradual increased entrapment efficiency was noticed. The higher efficiency for formulation means a good amount of drug was encapsulated.

Scanning electron microscopy

In the current study, the drug-loaded nanoparticles were prepared by ionic gelation method and their surface morphology was determined by SEM analysis. The representative SEM photographs of the nanoparticles were shown in figure 8. It was observed that the appearance of nanoparticles in scanning electron microscopy in granules form, which indicates a thin and uniform coating over the drug. The shape of the formulated nanoparticles affects the surface area and surface area per unit weight of spherical nanoparticles. The irregular shape of the particles may affect the dissolution rate present in the dissolution environment. The size of the Epleronone nanoparticles was ranged from 66 -160 nm can be seen in figure 8.

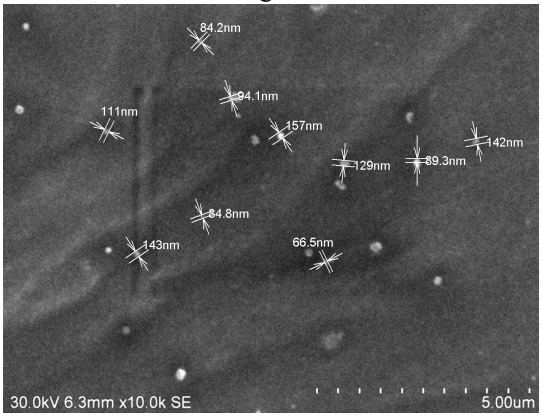

Figure 8: Scanning electron microscopy for optimized formulation.

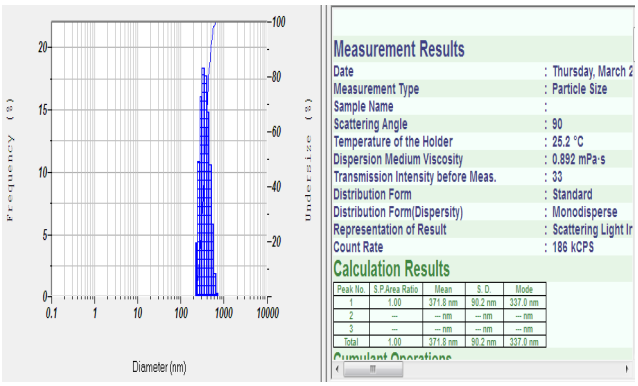

Figure 9: Particle size analysis of optimized formulation.

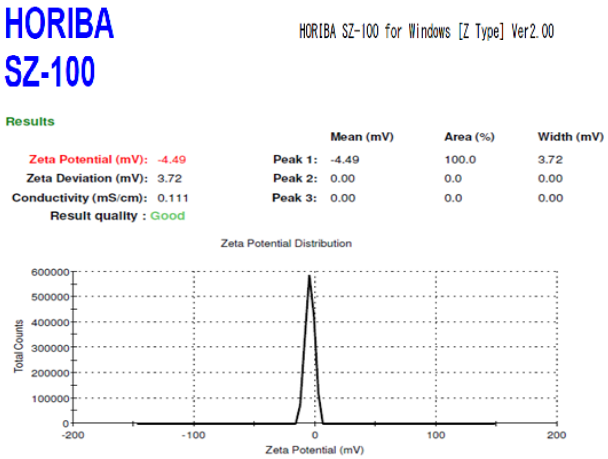

Figure 10: Zeta potential distributions analysis of optimized formulation.

Zeta potential and particle size analysis

Mean particle size, PI, and zeta potential values of the Epleronone nanoparticles are presented in figures 9 & 10. The particle size and size distribution of nanoparticles play a key role in their adhesion and interaction with cells. Average particle size of nanoparticles of optimized formulations (F5) was found to be having maximum particles at a range of 244 nm. PI values were used to define the particle size distribution. All prepared nanoparticles were nanometer sized and the size distributions were relatively in mono disperses form. All nanoparticles prepared in the present study had PI values lower than 1.0; therefore, particle size distribution was uniform. Formulations show a relatively narrow particle size distribution which may be attributed to the positive charges on polymeric matrices, indicating adequate physical stability. Considering negatively charged cell membranes, cationic nanoparticles have great potential in the enhancement of internalization with the cells and also on their uptake [17]. The measurement itself is a particle electrophoresis, the particle velocity is determined via the Doppler shift of the laser light scattered by the moving particles. The field strength applied was 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV using the Helmholtz-Smoluchowski equation. At standard measuring conditions (room temperature of 25^0C, water) this equation can be simplified to the multiplication of the measured electrophoretic mobility (µm/cm per V/cm) by a factor of 12.8, yielding the ZP in mV.

Formulation Tables 1 of Epleronone nanoparticles.

Trail No	Drug (mg)	Chitosan (%)	TPP (ml)	Stirring time (mins)	Centrifugation (RPM) 6000
Formulations based on polymer concentration					
1	25	0.1	5	30	45
2	25	0.2	5	30	45
3	25	0.3	5	30	45
4	25	0.4	5	30	45
5	25	0.5	5	30	45
Formulations based on cross- linking agent concentration					
6	25	0.6	5	30	45
7	25	0.5	7.5	30	45
8	25	0.5	10	30	45
Formulations based on stirring time					
9	25	0.5	5	15	45
10	25	0.5	5	45	45
Optimized formulation concentration					
11	60	1.0	10	30	45

Table 2: Drug transport mechanisms suggested based on 'n' value.

S. No.	Release exponent	Drug transport mechanism	Rate as a function of time
1.	0.5	Fickian diffusion	$t^{-0.5}$
2.	0.45 < n = 0.89	Non -Fickian transport	t^{n-1}
3.	0.89	Case II transport	Zero order release
4.	Higher than 0.89	Super case II transport	t^{n-1}

Table 9: *In-vitro* drug release data of formulation F1to F11.

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
0	0	0	0	0	0	0	0	0	0	0	0
1	56.75	26.29	26.67	23.74	25.95	44.03	10.32	15.17	25.69	20.84	19.52
2	63.83	44.18	48.39	31.84	35.17	52.93	16.34	22.54	39.39	31.62	28.26
4	75.96	59.36	56.28	40.67	44.03	61.07	25.16	36.87	51.68	40.84	37.54
6	87.11	74.94	63.32	48.39	52.93	69.26	33.69	44.82	63.85	49.62	46.22
8	97.18	82.68	74.29	56.28	61.07	76.19	47.39	51.03	78.68	57.29	53.10
10		90.29	81.57	63.32	69.26	87.93	55.68	69.93	87.09	65.42	61.86
12		98.69	89.12	74.29	76.19	98.73	61.85	78.07	93.18	72.42	70.29
18			92.16	84.26	83.29		75.68	89.26	98.59	79.56	79.16
24					92.54		88.09	81.19		84.22	89.52

The zeta potential of the nanoparticles formulation with Horiba SZ-100 which present in the formulation are de-aggregated and remain same and more stable in the suspension. In this regard, zeta potential (mV) is -4.49 and zeta deviation (Mv) is 3.72 and conductivity (Ms/CM) is 0.111. So polymer is more suitable for nanoparticles preparation and the result shows smooth surface character repelled action and it decrease the opsonisation. The zeta potential of the nanoparticles was found to be negative, and the results are shown in Figure 10. The zeta potential values of Epleronone nanoparticles were in the negatives. Hence from these studies, formulation F9 was considered as optimum. The results indicated that Epleronone nanoparticles possessed good stability. All optimized EPL-NPs formulations had a zeta potential in the range of −4. 49 mV which indicating good stability of the optimized systems. The zeta potential value for the optimized formulation (F9) was found to be within the acceptable limits.

***In-vitro* drug release studies of nanoparticles**

The *in-vitro* diffusion data of all the designed formulations are shown and dissolution profiles depicted in figures 11-13. *In-vitro* drug release data of all the nanoparticles formulations of Epleronone was subjected to goodness of fittest by linear regression analysis according to zero order, first order kinetics and concurrence to equations of drug release. The results obtained in *in-vitro* release studies were plotted as percent cumulative drug Vs time and shown in figures 14-17 and the results were given in table 9. As of the above *in-vitro* diffusion learning's it was observed that at low polymer concentrations i.e., at 0.1, 0.5% of chitosan, the drug release was rapid and it didn't maintain constant drug release.

While the chitosan concentration was increased to 0.2%, 3% and 4%, it was detected that the drug discharge time was enhanced. So the concentrations were further increased to decrease the drug release time from the nanoparticles was decreased rapidly due to high polymer concentration. By comparing the all diffusion profiles, it was finish off that the drug discharge from the F3 formulation containing 0.2% chitosan, as it releases 92.16% of drug at the end of 24 h. To optimize the best formulation, the F3 formulation was further modified by using different ratios of cross-linking agent and TPP concentration from F6-F9 formulations.

Among them F6 and F7 were formulated by changing the TPP concentrations from lower to higher ratios, and F8 and F9 formulations were prepared by changing the calcium chloride concentration. Among the F6-F11 trails it was found to be the formulation containing 0.6-0.5% chitosan with changing cross-linking concentration and stirring time, by comparing all the 10 trails it was observed that the effect of concentration of cross-linking agent and the stirring time plays a vital role in the formation of nanoparticles[18] .

Further F11 trail was formulated due to maintain the dose of Epleronone as the prior trails didn't get sufficient percentage yield. So for maintaining the dose concentration 10 F11 trail twice the concentration of Epleronone was taken for taking weight equivalent of 25 mg of Epleronone. But the F11 trail didn't show higher drug release at the end of 24 h when contrasted with the F5 trail. So F5 trail was considered as the optimized formulation. So further drug release kinetics were performed for the F5 formulation.

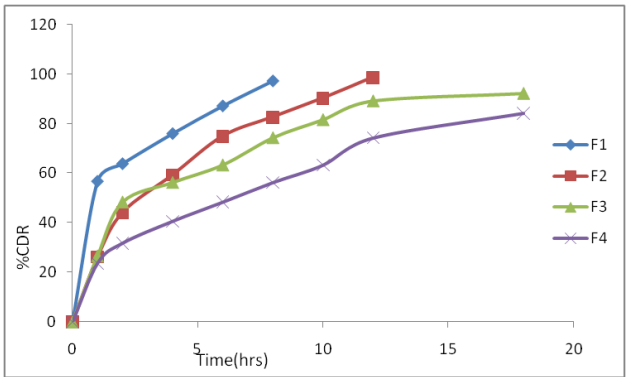

Figure 11: *In-vitro* diffusion studies of formulation F1 to F4.

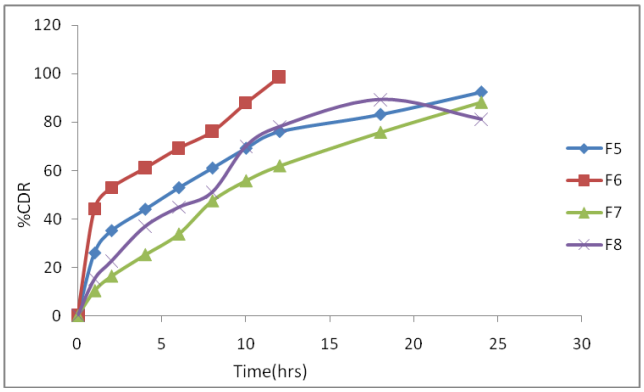

Figure 12: *In-vitro* diffusion studies of formulation F5 to F8.

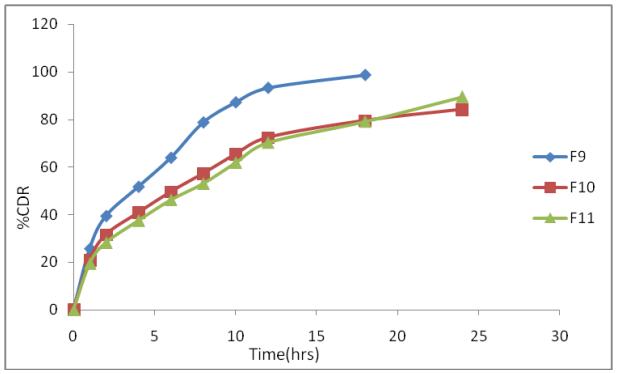

Figure 13: *In-vitro* diffusion studies of formulation F9 to F11.

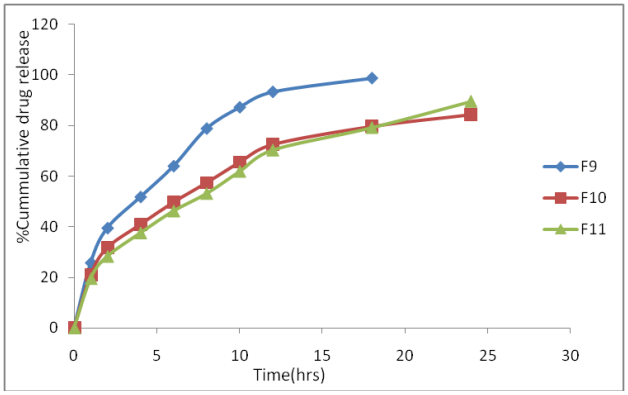

Figure 17: *In- vitro* drug release of formulations F9-F11.

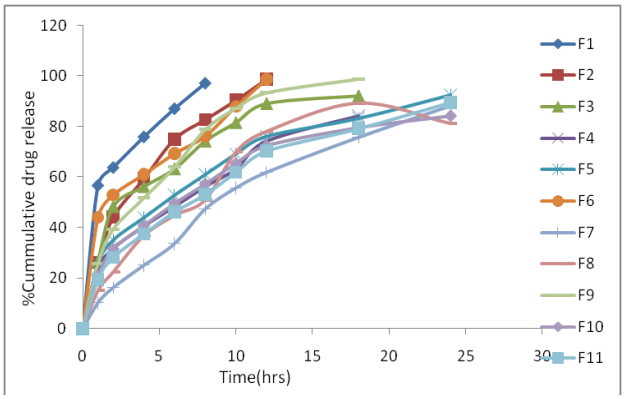

Figure 14: *in-vitro drug releases* of formulations F1-F9.

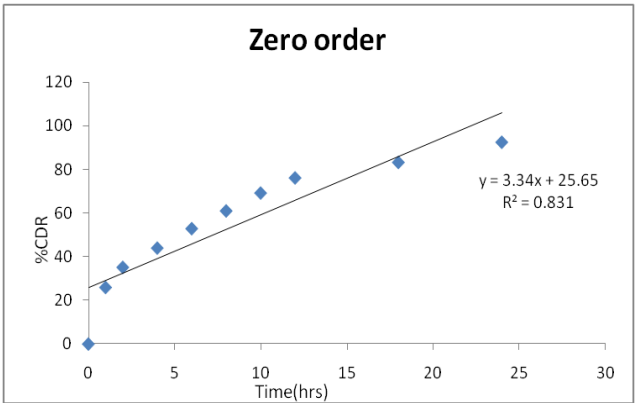

Figure 18: Zero order release profile of formulation F5.

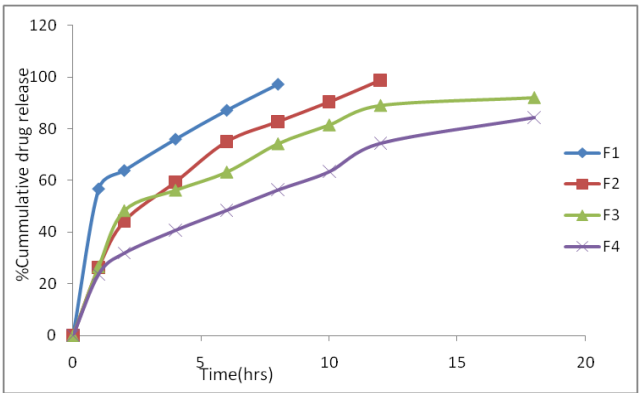

Figure 15: *In- vitro* drug release of formulations F1-F4.

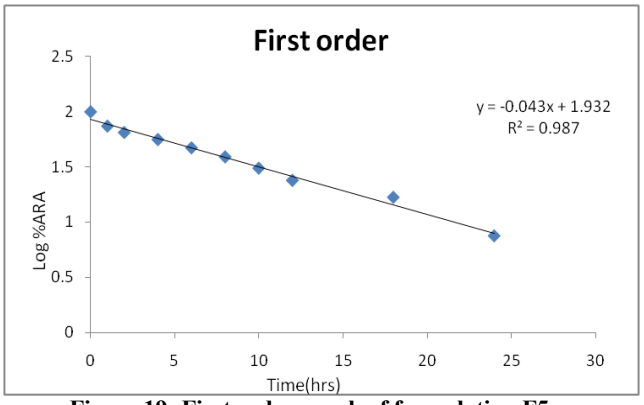

Figure 19: First order graph of formulation F5.

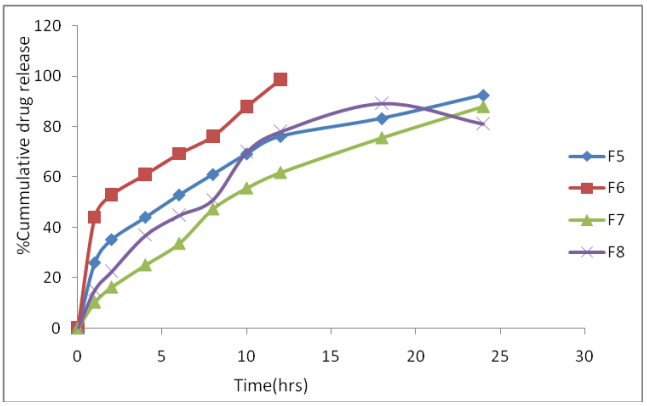

Figure 16: *In- vitro* drug release of formulations F5-F8.

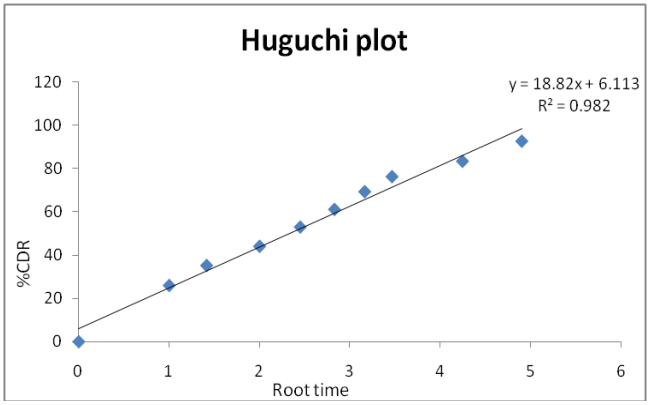

Figure 20: Higuchi graph of formulation F5.

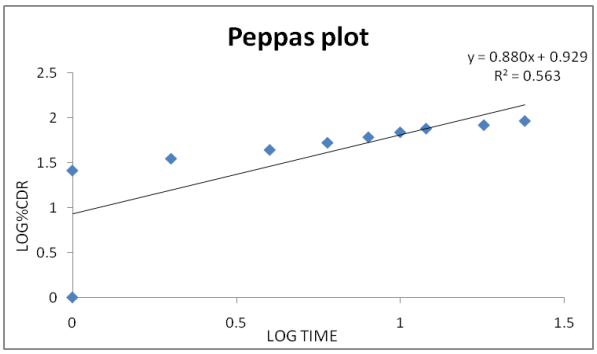

Figure 21: Peppas graph of formulation F5.

Drug release kinetic studies of best formulation F5 [Eplerenone nanoparticles]

The *in-vitro* drug release data of all the Eplerenone loaded nanoparticles formulations were subjected to the goodness of fit test by linear regression analysis according to zero-order and first orders kinetic equations, Higuchi's and Korsmeyer–Peppas models to ascertain the mechanism of drug release. The plot/profiles were obtained between points at time in hrs on X-axis Vs cumulative % drug release on Y-axis of selected formulation as shown in figures. 18-21. The slopes and regression coefficient values (R^2) of various mathematical models for the optimized formulation, 0.831, 0.987, 0.982, 0.563 for zero-order, first order, Higuchi and Peppas model respectively. Good linearity was observed with the zero-order and regression coefficient value close to 1. Hence this formulation is best fitted into the zero-order release kinetics model and the regression coefficient value shows linearity as shown in figure 18. Based on the regression values it was concluded that the optimized formulation F5 follows Zero order kinetics with Non fickian diffusion mechanism.

CONCLUSIONS

From the present study, the following conclusions can be drawn. Oral Nanoparticles of Epleronone were prepared by employing ionotropic gelation method using chitosan, Sodium TPP, and Tween 80. In view of calibration results, the 6.8 pH buffer has opted for Epleronone drug dissolution. The melting tip of Epleronone was bringing into being in range of 240^0C which was determined by capillary method. Saturation solubility was carried out at 25^0C using 0.1N HCL, 6.8 phosphate buffer, and purified water. It was observed that as of the drug excipients compatibility studies, there are no interactions between the pure drug (Epleronone) and optimized formulation (Epleronone + excipients) which indicates there are no physical changes.

All the prepared formulations were found to be having drug content within acceptable limits. As the polymer concentration increases, the drug release rate decreases, whereas nanoparticles strength increases. IR spectroscopic studies indicated that there were no drug-excipients interactions. From the drug release studies, it was clearly observed that the chitosan concentration and sodium TPP concentration plays a vital role in nanoparticles formation and strength. From the studies, it was concluded that the Epleronone nanoparticles were successfully formulated by using chitosan (0.5%), and sodium TPP (5 ml) for sustained drug delivery of Epleronone. When compared to other all the formulations F5 is the best formulation which showed 92.59% of drug released respectively at the end of 24 h. Zeta potential value for the optimized formulation (F5) was found to be within the adequate limits. Average particle size of nanoparticles of optimized formulations (F5) was found to be 244 nm. Based on the regression values, it was concluded that the optimized formulation F5 follows Zero order drug release with Non fickian diffusion mechanism.

Acknowledgement

The authors give thanks to authorities of Gandhi Institute of Technology and Management, Hyderabad, Telangana, India for providing needful requirements. The authors also extend their sincere thanks to Dr. Sridhar, Dr. G. Venkata Reddy, Mr. Waseem, and Mr. Gangadhara of Spectrum Pharma labs for permitting us to conduct the study and research.

Financial support and sponsorship

There was no financial support and sponsorship to carry out this entire work.

Conflicts of interest

We declare that we have no conflict of interest**.**

Human and animal rights

The research work of this paper has not involved any human participants and animals.

REFERENCES

1. Sovan Lal Pal., Utpal Jana., Manna P. K., Mohanta G. P., Manavalan R., Nanoparticles: An overview of preparation and characterization. *J of App Pharm Sci.* 2011, 1(6), 228-234.
2. Gayatri Khosla., Lakshmi Goswami., Preeti Kothiyal., Sayantan Mukhopadhyay., Nanoparticles: A Novelistic Approach for CNS disorders. *J Adv Pharm Sci.* 2012, 2, 220-259.
3. Abhilash M., Potential applications of Nanoparticles. *Int Jou Phar and Bio Sci.,* 2010, 1, 1-12.
4. Nagavarma B. V. N., Hemant K., Yadav S., Ayuz A., Vasudha L.S., Shiva Kumar H.G., Different techniques for preparation of polymeric nanoparticles – A Review, *Asia J Pharm & Cli Res.* 2012, 5, 1-8.
5. Mullaicharam A. R., Nanoparticles in drug delivery system. *Int. J of Nut Phar Neu Dis.* 2011, 1, 103-121.
6. Joachim Allouche., Synthesis of Organic and Bioorganic Nanoparticles: An Overview of the Preparation Methods. Springer-Verlag London, 2013, 27-30.
7. Tamizhrasi S., Shukla A., Shivkumar T., Rathi V., Rathi J. C., Formulation and evaluation of Lamivudine loaded poly methacrylic acid nanoparticles. *Int J Pharm Tech Res.* 2009, 3, 411-415.
8. Partha S., Amit K.G., Goutam R., Formulation and Evaluation of Chitosan-Based Ampicillin Trihydrate Nanoparticles. *Tro J Pharm Res Sci.* 2010, 9(5), 483-88.
9. Lakshmana Prabu S., Shirwaikar A. A., Shirwaikar A., Kumar A., Formulation and evaluation of sustained release microspheres of rosin containing Aceclofenac. *Ars Pharm.* 2009, 50(2), 51-62.
10. Nidhi Gupta., Rampal Rajera., Manju Nagpal., Sandeep Arora., Primaquine Loaded Chitosan Nanoparticles for Liver Targeting. *Pharm Nano tech.* 2013, 14, 561-573.
11. Ji J., Hao S., Wu D., Huang R., Xu Y., Preparation, characterization and *in vitro* release of chitosan nanoparticles loaded with gentamicin and salicylic acid. *Carbo hyd Polym.* 2011, 85, 803-808.
12. Xu Y., Du Y., Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles. *Int J Pharm.* 2003, 250, 215-226.

13. Calvo P., Remunan-Lopez C., Vila-Jato J. L., Alonso M. J., Novel hydrophilic chitosan- polyethylene oxide nanoparticles as protein carriers. *J Appl Poly Sci.* 1997, 63(1), 125-132.
14. Aktas Y., Andrieux K., Alonso MJ., *et al*. Preparation and *in-vitro* evaluation of chitosan nanoparticles containing a caspase inhibitor. *Int J Pharm.* 2005, 298, 378-383.
15. Sayantan Mukhopadhyay N.V., Satheesh Madhav and Kumud Upadhyaya., Formulation and evaluation of bio-nano particulated drug delivery of Rivastigmine. *Worl J of Phar Sci.* 2016, 4:5, 264-272.
16. Motwani SK., Chopra S., Talegaonkar S., Kohli K., Ahmad FJ., Khar RK., Chitosan–sodium alginate nanoparticles as sub microscopic reservoirs for ocular delivery: formulation, optimisation and *in- vitro* characterisation. *Eur J Pharm Bio pharm.* 2008, 68, 513-525.
17. Oyarzun-Ampuero FA., Brea J., Loza MI., Torres D., Alonso MJ., Chitosan-hyaluronic acid nanoparticles loaded with heparin for the treatment of asthma. *Int J Pharm.* 2009, 381(2), 122-129.
18. Dudhani AR., Kosaraju SL., Bio adhesive chitosan nanoparticles: preparation and characterization. *Carb Polym.* 2010, 81, 243-251.