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Research article

INVESTIGATION OF DRUG LOADING AND DELIVERY OF DAROLUTAMIDE FROM CHITOSAN BASED NANOPARTICLES

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ABSTRACT

Darolutamide-loaded nanospheres were developed and characterized for enhancing its therapeutic efficacy in this study. Due to its poor aqueous solubility and bioavailability, darolutamide is a potent anti-androgen used in the treatment of prostate cancer. Nanotechnology-based approaches, such as nanospheres, may provide solutions to these problems. As part of the formulation process, darolutamide was encapsulated within nanospheres, which ensured that the release kinetics of the drug would be controlled and the stability would be improved. As a result of various characterization techniques, including dynamic light scattering (DLS) and scanning electron microscopy (SEM), spherical nanoparticles were determined to have a diameter of approximately, which are suitable for enhanced permeation and retention (EPR) in tumor tissues. According to in vitro release studies, the drug was released over an extended period of time, indicating the possibility of reducing the frequency of dosing and improving compliance among patients. Furthermore, darolutamide-loaded nanospheres inhibited cell proliferation to a greater extent than free drug formulations when tested against prostate cancer cell lines. Consequently, darolutamide-loaded nanospheres may offer a promising approach to improving prostate cancer treatment outcomes. As a conclusion, our study demonstrates that darolutamide-loaded nanospheres have been successfully formulated and characterized, and that they have the potential to serve as an effective and targeted delivery mechanism for the treatment of prostate cancer.

KeyWords: Colloidal,parentral,Nanoparticles, Nanospheres.

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INTRODUCTION

Modern drug therapy relies heavily on the delivery of drugs and active substances to the site of action in the body in an optimal concentration versus time profile (Christian, *et al.,* 2008). One approach to achieving this goal was the development of colloidal drug carriers, which are known as nanoparticles. Nanoparticles are characterized by their small particle sizes. The use of colloidal drug delivery systems has several advantages over conventional methods of administration. The small particle size of colloidal preparations makes them suitable for parenteral preparations and for injections that are intended to deliver a specific substance in a sustained manner to a particular organ or target site (Ibrahim Khan, *et al*. 2019). It is believed that targeting the drug to the desired site of action would not only improve therapeutic efficiency, but also enable a reduction in the amount of drug required to achieve a therapeutic response, so as to minimize unwanted side effects (Jaison Jeevanandam, *et al.,* 2018 & Jeevanandam J, *et al*, 2018).

Nanoparticles are solid colloidal particles ranging in size from about 10 to 1000 nm. (Rempel G.L.,

et al, 2015) They consist of macromolecular materials and can be used as adjuvant in vaccines or as drug carriers, in which the active principle is dissolved, entrapped, encapsulated or to which the active principle is adsorbed or attached. Depending upon the process used for preparing nanoparticles, the formed nanoparticles can be classified into nanospheres and nanocapsules (Seitaro K, *et al*, 2008).

ADVANTAGES OF NANOPARTICLES

- Reduction of toxicity and occurrence of adverse reactions
- Better drug utilization
- Controlled rate of drug release
- Specific site of drug release
- Greater patient convenience and/or better patient compliance

DRAWBACKS OF NANOPARTICLES

Drawbacks include drug instability in the biological milieu and premature drug loss through rapid clearance and metabolism. Similarly, high protein binding of certain drugs such as protease inhibitors limits their diffusion to the brain and other organs. However, nanotechnology for drug delivery applications may not be suitable for all drugs, especially those drugs that are less potent because the higher dose of the drug would make the drug delivery system much larger, which would be difficult to administer. Their characterization is quite difficult, and expensive. Long processes of synthesis and purification are major drawbacks of nanoparticles (Mohammed MA, *et al.*, 2017 & Vroman I, *et al.*, 2009).

MATERIAL AND METHODS

MATERIALS USED

Darolutamide, Chitosan, Acetic acid, Sodium tripolyphosphate (TPP), Tween 80.

EQUIPMENT USED

Magnetic stirrer, C24 centrifuge, Double beam UV Spectro photometer, Electronic balance, pH – meter, FTIR Spectrophotometer, Water bath, Zeta Potentiometer, SEM.

METHODS

Preformulation studies

Preformulation is defined as phase of research and development process where physical, chemical and mechanical properties of a new drug substance are characterized alone and when combined with excipient, in order to develop stable, safe and effective dosage form.

A. Identification of pure drug

Solubility Analysis

Preformulation solubility analysis was done, which included the selection of suitable solvent system to dissolve the drug as well as various excipient used for the formulation of Nanospheres.

Melting point

Fine powder of Darolutamide was filled in glass capillary tube (previously sealed at one end) and kept in melting point apparatus. The melting point of Darolutamide was found. (Zengshuan M, *et al*, 2005)

Spectroscopy

The obtained sample was examined by Fourier Transform Infrared (FT-IR) spectrum and was compared with the standard FT-IR spectra of the pure drug

B. Compatibility Studies

One of the requirements for the selection of suitable excipient (or) carrier for pharmaceutical formulations is its compatibility. Therefore in the present work, a study was carried out using FT-IR spectrophotometer to confirm the absence of any possible chemical interactions between the Darolutamide and Chitosan. Infrared spectroscopy by potassium bromide pellet method was carried out on pure substances (Darolutamide and Chitosan) separately and their physical mixtures. They are compressed under 10 tonnes pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000 to 400 cm-1 in a spectrophotometer.

The spectrum of physical mixtures was compared with the original spectra to determine any possible molecular interactions between the drug and polymer. FTIR analysis measures the selective absorption of light by the vibration modes of specific chemical bonds in the sample. (Lopez-Leon T, *et al*., 2005) The observation of vibration spectrum of encapsulated drug evaluates the kind of interaction occurring between the drug and polymer.

Determination of λ max

Most of the drug absorbs light in the ultraviolet light wavelength (200-400 nm), since they are aromatic or contain double bond.

100 mg of Darolutamide was weighed on an electronic balance and dissolved in 100 ml of 2% tween 80. From this stock solution-I, 10 ml solution was diluted to 100 ml with 2% tween 80 to prepare stock solution-II. Further dilution was made to obtain the concentration of 20µg/ml. The prepared solution was scanned on a UV scanner between 200-400 nm. The maximum obtained in the graph was considered as λ max for the pure drug.

Construction of Standard Curve of Darolutamide

5 gm of tween 80 was taken in 250 ml of volumetric flask and little amount of distilled water was added, dissolved it properly and made up to the volume to 250 ml with distilled water to prepare 2% tween 80 solution. Stock solution was prepared by dissolving 100mg of Darolutamide drug in 100ml of Tween 80 solution, so as to get a solution of 1mg/ml concentration. 5ml of stock solution was diluted to 50ml with Tween 80 solution producing standard solution of concentration of 100 g/ml. Accurately measured aliquot portions of standard drug solution (100 g/ml) ranging from 1ml to 5ml were transferred into 10ml volumetric flask and were diluted up to the mark with Tween 80 solution. Thus the final concentration ranges from 10-50 g/ml. Absorbance of each solution was measured at 306 nm against Tween 80 solution as the blank. A graph of concentration of drug Vs absorbance was plotted. Preparation of Darolutamide Nanospheres Ionic Gelation Method (Yan P, *et al*, 2002 & Xiong Wei L, *et al*, 2003)

Chitosan nanospheres were prepared by ionic cross linking of chitosan solution with TPP anions. Chitosan was dissolved in aqueous solution of acetic acid (6%v/v) at various concentrations such as 1.0, 2.0, 3.0, 4.0, 5.0 mg/ml. (Tang ESK, *et al.*, 2003) Under magnetic stirring at room temperature, 5ml of 0.25% w/v TPP aqueous solution was added dropwise into 10ml chitosan solution containing 10mg of Darolutamide dissolved in tween 80. The stirring was continued for about 20 min. (Ana G, *et al.* 2005) The resultant nanospheres suspensions were centrifuged at 12000x g for 30 min using C24 centrifuge. The formation of the spheres was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation).

Evaluation of Darolutamide Nanospheres

A. Determination of Nanospheres

Process Yield

The nanospheres production yield was calculated by gravimetry. Fixed volumes of nanospheres suspensions were centrifuged (16,000×g, 30 min, 15ºC) and sediments were dried.

B. Particle Size Analysis

Particle size analysis was done by scanning electron microscopy using JOEL JSM- T330A Scanning Microscope. Solvent paint was applied on the studs and while the paint was wet, the pellets were placed on each stud and allowed to dry, then the sample was observed in scanning electron microscopy and photographs were taken. The diameter of about 20 spheres was manually measured from resultant photographs of each batch. Finally average mean diameters were obtained.

C. Determination of % Entrapment Efficiency

The Nanosuspension with known amount of drug (10mg/20ml) incorporated was centrifuged at 5000 rpm for 15 minutes. The supernatant solution was separated. 5ml of supernatant was distributed with 100 ml of 2% w/v tween 80 solutions and the absorbance was measured using UV spectrophotometer at 306 nm using 2% w/v tween 80 as blank. The amount of drug unentrapped in the supernatant was calculated. The amount of drug entrapped and percentage entrapment was determined from drug unentrapped. Standard deviation was determined for 3 trials.

D. Zeta potential (Tilak R, *et al.,* 2002)

A zeta potential, measure the effect of electrostatic charges; this is the basis force that cause the repulse between adjacent spheres. Net results are attraction or repulsion depends upon the magnitude of both forces. Thumb rule describes the relation between zeta potential determination responses of the suspension being tested, particularly hydrophobic colloids. The prepared nanospheres suspensions were characterized with respect to zeta potential by using zeta potential analyzer (Malvern Zeta sizer).

E. In vitro Drug Release Studies (Shabouri EI MI. *et al*,2003).

The in vitro drug diffusion from the formulation was studied by using egg membrane – 110 (cut off: 3500 Da) using modified apparatus. The dissolution medium used was freshly prepared 2% w/v tween 80 solution. Egg membrane – 110, previously soaked overnight in the dissolution medium was tied to one end of a specially designed glass cylinder (open at both ends). 5 ml of formulation was accurately placed into this assembly. The cylinder was attached to a stand and suspended in 50 ml of dissolution medium maintained at 37 ± 5ºC so that the membrane just touched the receptor medium surface. (Pandey R, *et al*., 2005) The dissolution medium was stirred at low speed using magnetic stirrer. Aliquots, each of 5 ml were withdrawn at hourly intervals and replaced by an equal volume of receptor medium. The aliquots were suitably diluted with receptor medium and analyzed by UV-Vis spectrophotometer at 306 nm. The quantity of drug equivalent to 10 mg of Darolutamide was taken for diffusion study.

F. Stability studies

Stability is defined as extent to which a product remains within specified limits throughout its period of storage and use.

Procedure:

From five batches of Darolutamide nanospheres, the ideal formulation F4 was tested for stability studies. (Joseph Nisha M, *et al*, 2006 &

Sivakumar T, *et al*, 2007) Formulation F4 was divided into 3 sets of samples and stored at:

- 4ºC in refrigerator
- Room Temperature (29ºC)
- 45ºC ± 2ºC, 75ºC % RH ± 5 % in humidity control ovens

After 30 days drug contents of all samples were determined by the method as in entrapment efficiency of section 4.2.5(C). In vitro release study of formulation F4 was also carried out after 30 days storage.

Table 1: Process Yield % of Darolutamide Nanospheres (Mean±S.D, n=3).

S.No	Batch code	Drug: carrier ratio	Process Yield %
1	F1	1:1	52.7± 3.4
2	F2	1:2	63.5± 4.3
3	F3	1:3	70.9± 2.9
4	F4	1:4	78.6± 4.6
5	F5	1:5	72.5± 2.5

Figure 1: IR Spectrum of Darolutamide

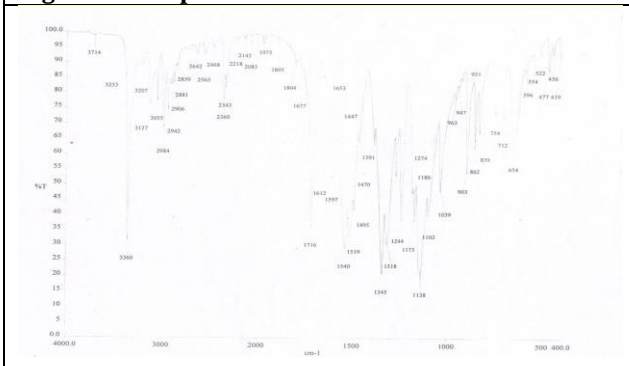


Figure 2: IR Spectrum of chitosan

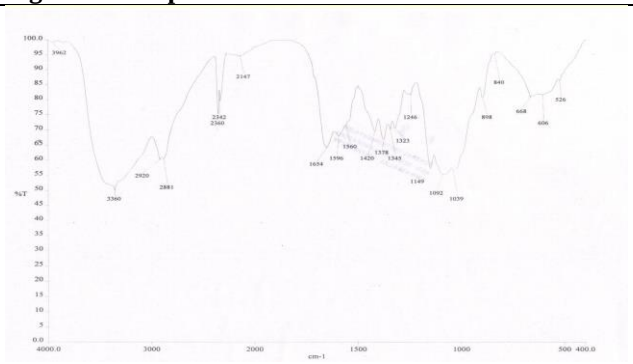


Figure 3: IR Spectrum of Darolutamide and Chitosan Combination

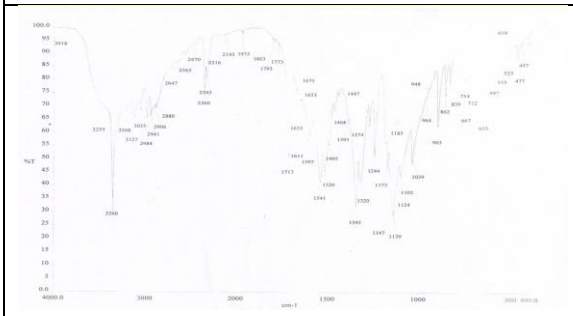


Figure 4: Standard Curves for Darolutamide by UV Method

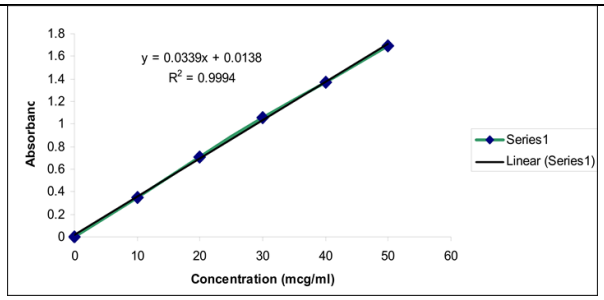


Figure 5: Percentage Drug Entrapment Efficiency

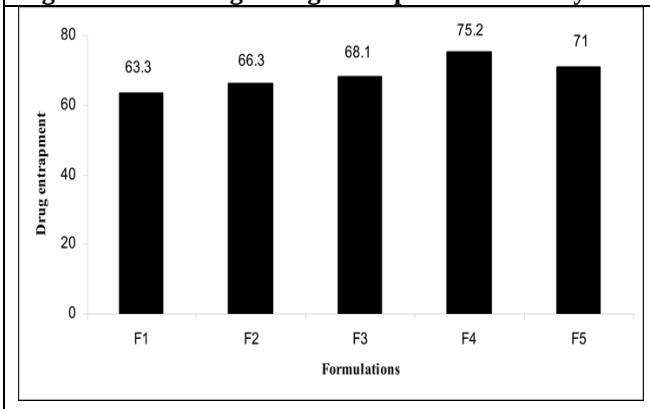


Figure 6: SEM of formulation F4

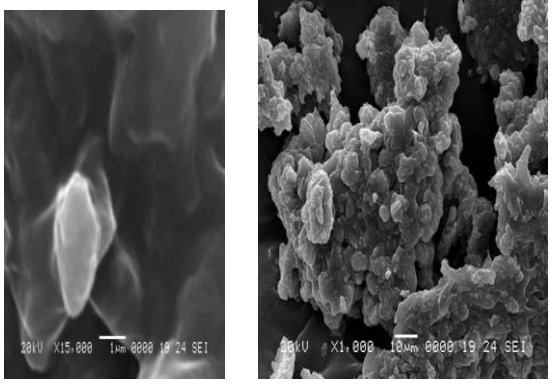


Figure 7: Zeta Potential of Darolutamide Nanospheres

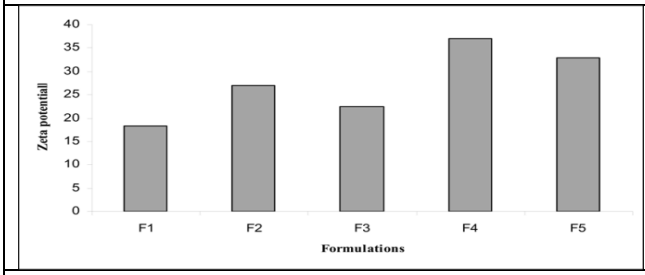


Figure 8: Comparative In Vitro Release Profiles Of Darolutamide Nanospheres According To Zero Order Kinetics

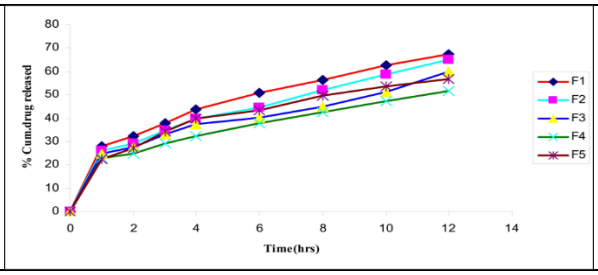


Figure 9: Comparative In Vitro Release Profiles of Darolutamide Nanospheres According to First Order Kinetics

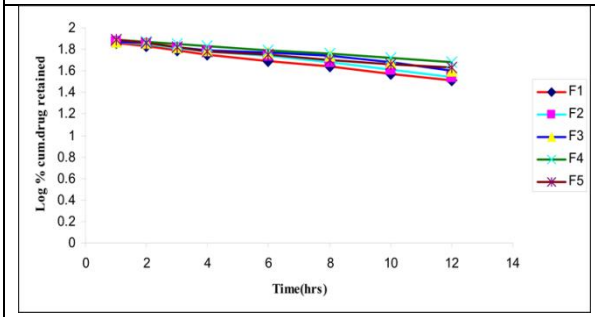


Figure 10: Comparative In Vitro Release Profiles of Darolutamide Nanospheres According to Higuchi plot

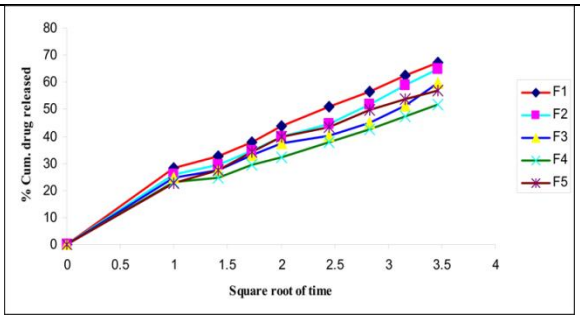


Figure 11: Comparative In Vitro Release Profiles of Darolutamide Nanospheres According to Peppas plot

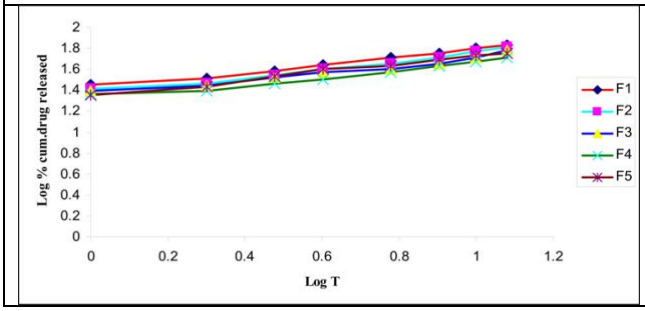


Figure 12: Stability Study: Comparison of % Drug Content of Formulation F4 at 4ºC, Room Temperature (29ºC) and 45ºC ± 2ºC / 75% RH

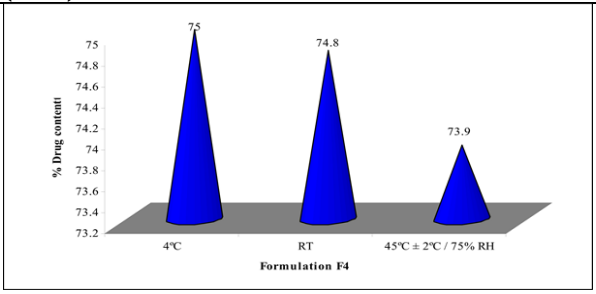
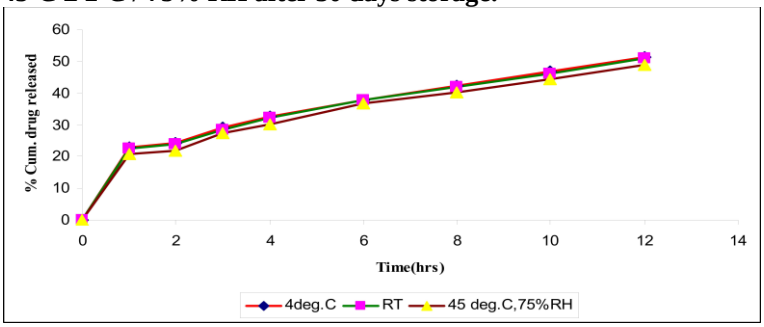


Figure 13: Stability Study: Comparison of In vitro drug release profile for Formulation F4 at 4ºC, Room Temperature (32ºC) and 45ºC ± 2ºC / 75% RH after 30 days storage.



DISCUSSION

The solubility of the pure drug was determined with various solvents. It revealed that it is insoluble in water but soluble in solution of 2% tween 80, solution of 2% sodium lauryl sulphate, dichloromethane, acetone and

methanol. The purity of Darolutamide was confirmed by comparing its I.R. Spectra with the standard I.R. Spectra of Darolutamide. I.R. Spectral analysis studies were carried out to study the compatibility study of pure drug Darolutamide with Chitosan prior to the preparation of Darolutamide nanospheres. All the characteristic peaks of Darolutamide were present in spectrum 3 thus indicating compatibility between drug and polymer. It shows that there was no significant change in the chemical integrity of the drug.

The absorption spectrum of pure drug was scanned between 200 – 400 nm with 20 µg/ml concentration in 2% tween 80 solutions using UV Spectrophotometer. The maximum peak was obtained at 306 nm that was taken as λ max. The absorbances of Darolutamide standard solutions containing 10-50µg /ml of drug in 2% Tween 80 solution were obtained. The standard calibration curve with regression value of 0.994. The curve was found to be linear in the range of 10-50 mcg/ml at λmax 306 nm. The preparation of chitosan nanospheres, based on an ionic gelation process, involves the mixture of two aqueous phases at room temperature. One phase contains a solution of chitosan and the other contains a solution of the polyanion TPP. Because of its hydrophobic nature, a number of experiments had to be performed in order to determine the appropriate conditions for the incorporation of the hydrophobic drug Darolutamide into the chitosan nanospheres. A successful entrapment was achieved by dissolving the hydrophobic drug Darolutamide in 2% tween 80 prior to its incorporation into the chitosan solution, followed by the addition of the TPP solution. The appropriate formulation conditions were decided from the results of preliminary studies aimed at investigating the effect of the solvent volume and the concentration of the chitosan solution on the physico-chemical characteristics of the nanospheres.
Batch F4 that contains drug and polymer in the ratio 1:4 showed maximum percentage yield of nanospheres. Percent particle yield increased with the increase for polymer.

The Entrapment efficiency of the Darolutamide nanospheres formulation F1, F2 F3, F4 and F5 containing Drug: Polymer in various ratios of 1:1, 1:2 ,1:3, 1:4 and 1:5 respectively was determined. The formulation F1 with drug: polymer ratio of 1:1 showed average entrapment of 63.3%. Formulation F2 with ratio 1:2 showed 66.3% and formulation F3 with 1:3 ratios showed 68.1%. Formulation F4 with ratio 1:4 showed 75.2% and formulation F5 with 1:5 ratios showed 71%. Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The formulation F4 registered highest entrapment of 75.2% and found to be the best formulation. Scanning electron photomicrographs of formulation F4 was shown in Figure was used while taking these photographs. Spheres of all formulations were in nanospheres having smooth surface. The particle size was in the range of 400 nm. The surface charge of the nanospheres were evaluated by measuring the zeta potential of the nanospheres by the zeta meter ± 3M. The results were evaluated in Table: 5.6 and comparison were showed in Fig: 5.4. Zeta potential of all formulated nanospheres was in the range of 18.4 to 37.08 mV, which indicates that they are moderately stable.

By plotting various graphical models the in vitro drug release profile of the prepared Darolutamide nanospheres were studied. The zero order graphs were prepared by plotting percentage cumulative drug release against time in hours. The first order graphs were prepared by using log percentage cumulative drug remaining against time in hours. The diffusion pattern releases of the formulations was studied by plotting Higuchi's graph using percentage cumulative drug released against square root of time. Log of cumulative percentage drug release Vs. log time (Peppas exponential equation) The release data obtained for formulations F1, F2, F3, F4 and F5 plots of cumulative percent drug released as a function of time for all five formulations. Plots of log cumulative percent drug retained as a function of time for all five formulations. Higuchi matrix of the formulations is indicated in the Peppas kinetics of all formulations.

Cumulative percentage drug released for F1, F2, and F3 after 12 hours were more than cumulative release of F4 and F5. The cumulative percentage drug release after 12 hours was 77.33%, 73.86%, 64.78%, 52.68% and 58.76% for F1, F2, F3, F4 andF5 respectively.

It was apparent that in vitro release of Darolutamide showed a very rapid initial burst, and then followed by a very slow drug release. An initial, fast release suggests that some drug was localized on the surface of the nanospheres. F4 was showing sustained release compared to other formulations and it was considered as best formulation.

In order to describe the release kinetics of all five formulations the corresponding dissolution data were fitted in various kinetic dissolution models like zero order, first order, and Higuchi respectively. These values were compared with each other for model and drug equation. As indicated by higher R2 values, the drug release from all formulations follows first order release and Higuchi model. Since it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled. The results of drug content after 30 days of stability testing at different storage conditions were shown. On comparing this data with the previous data of F4, it was observed that there was a slight decrease in drug content when the formulation was stored at 4ºC and Room temperature, but there was significant decrease in drug content when the formulation was stored at 45ºC. In vitro release studies revealed that the formulation stored at 40C showed 51.19% release the one which was stored

at room temperature showed 50.93% and the formulation stored at 45ºC ± 2ºC / 75% RH showed 48.74% release. At higher temperature, there might be chances for drug degradation that decreased the drug release.

CONCLUSION

Chitosan is a suitable carrier for preparing nanospheres of Darolutamide. Among different drug polymer ratios, F4 showed maximum percentage process yield (78.5 ± 4%) and drug content (7.52 mg). The percentage drug entrapment efficiency was maximum for F4, which was found to be 75.2 ± 0.52. The results revealed that increasing in the polymer concentration increases the organic phase viscosity, which increases the diffusion resistance to drug molecules from organic phase to the aqueous phase, thereby entrapping more drugs by the chitosan polymer. The average particle size for formulation F4 was in the range of 400 nm. The zeta potential for F4 was 37.08 ± 0.4 mV. Formulation F1 showed maximum cumulative percent drug release and formulation F4 showed minimum cumulative percent drug release after 12 hours of dissolution studies. Based on drug content, drug entrapment efficiency, particle size morphology, zeta potential and in vitro release, formulation F4 was selected as an optimum formulation. It was apparent that in vitro release of Darolutamide showed a very rapid initial burst, and then followed by a very slow drug release. An initial, fast release suggests that some drug was localized on the surface of the nanospheres. Overall the curve fitting into various mathematical models confirmed that the in vitro releases of formulations were best fitted into first order model followed by Higuchi's model and zero order models. The n values less than 0.5 indicates that the mechanism in which the drug release from nanospheres follows Fickian diffusion controlled system. Stability studies showed that maximum drug content and closest in vitro release to previous data was found for F4 at 4OC and room temperature. Thus it can be concluded that 4OC and room temperature were suitable for the storage of Darolutamide nanospheres.

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