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PREPARATION OF FLUTAMIDE NANOSPHERES: A NOVEL APPROACH FOR IMPROVED THERAPEUTIC EFFICACY

Sunam Sravani*, Purushothaman M

KLR Pharmacy College, Paloncha, Bhadradri Kothagudem, Telangana- 507511, India

ABSTRACT

In this study Flutamide-loaded nanospheres as a potential means for improving the therapeutic efficacy of Flutamide in treating prostate cancer. A nonsteroidal antiandrogen called flutamide is used in the treatment of prostate cancer. As a solution to these challenges, we created Flutamide nanospheres using a solvent evaporation technique, containing biodegradable polymers. Various polymer-to-drug ratios, surfactant concentrations, and solvent evaporation conditions were optimized in order to prepare nanospheres. In a cytotoxicity test performed on prostate cancer cells (PC-3) it was found that the nanospheres displayed enhanced anticancer activity as compared to free Flutamide, demonstrating the potential of nanospheres to improve uptake into cells and therapeutic efficacy. As a result of these findings, Flutamide-loaded nanospheres may be able to offer a promising delivery system for prostate cancer therapy, which features improved solubility, controlled drug release, and a reduced incidence of side effects, thus enhancing the quality of life of patients.

Key Words: Flutamide, Prostate, Cancer, Nanospheres.

INTRODUCTION

Nano drug delivery system (NDDS) is one of the fields of science that has been continuously developing due to the explosion of nanotechnology. Molecular structure is a study of how molecules, atoms, and compounds can be arranged into structures to produce materials with specific properties. Nanometer scale structures are studied because they have dimensions in the range of 1-100 nanometers. Using Nano DDS can ensure a long-term distribution of a drug, resulting in fewer fluctuations in plasma levels, and therefore, fewer side effects. As part of this Nano DDS, there are a wide range of Nano carriers including nanoparticles, micelles, nanogels, dendrimers, polymersomes, liposomes, carbon nanotubes, nanocrystals, silica nanoparticles, nanocapsules and nanospheres. (1).

There are several types of nanospheres, which are the subdivisions of polymeric nanoparticles. They are matrix-type structures with spherical particulate systems, characteristic of a size range of 10-200nm, that are commonly used as carriers in drug delivery systems in clinical settings.

Corresponding Author
Kagithoju Chaithanya
Email: sravanimaheshp@gmail.com

In essence, the drug dissolves, encapsulates, entraps, and attaches to the polymer matrix. As a result of uniform dispersion, a homogeneous structure was formed. It is possible that nanospheres can be amorphous or crystalline in nature, and that they can also protect drugs from degradation enzymatically and chemically. They can be biodegradable or non-degradable. There are two classes of biodegradable nanospheres: modified starch nanospheres and albumin nanospheres. In addition to these, there are polypropylene dextran nanospheres and polylactic acid nanospheres. System-based administration of medications offers several advantages, including the opportunity to swallow or inject them. The delivery of drugs to specific organs can be achieved with nanospheres. With nanospheres, established pharmacophores can have a new patent life that will allow them to be used in an inexhaustible variety of systems.

Nanosphere formulations aim to achieve the following objectives:

- Controltheparticlesize
- Doseregimen
- Therapeutically release the active agents to achieve

the site-specific action at the therapeutically optimal rate [1].

Polymeric Nanospheres

A polymeric nanosphere is a solid colloidal particle made up of dissolved, trapped, chemically bound, or adsorbed substances within a homogenous polymer matrix. Micelles made of polymeric materials have larger particles in comparison to those made of polymeric materials. Despite their similarity in morphology, nanospheres and nano capsules differ slightly in their structure. The liquid core of nano capsules is enclosed inside a hollow cavity and is covered by a polymer membrane. A nano reservoir is often described as a structure of this type. Nanospheres, which have a high loading capacity, are most commonly used in drug delivery systems to encapsulate hydrophilic drugs with small molecular weights. Nanospheres are not only extremely strong and provide excellent structural stability, but they can also be modified easily to change their porosity, allowing for finer control over cargo release patterns [2].

Preparation Method

There are generally two methods for preparing polymeric nanospheres. When the nanospheres themselves are formed, a direct polymerization process is required, and this is mainly accomplished by polymerizing them by emulsions (such as poly (methyl methacrylate) and poly (ethyl cyanoacrylate)) or by polymerizing them at the interface (such as poly (alkyl cyanoacrylate)) [3]. In a polymerized alkyl cyanoacrylate emulsion system, nanospheres form, for example. The construct polymer must first be polymerized before the nanospheres can be formed in the second method. After the polymers have been preformed, the macromolecules are self-assembled to form miniature nanospheres. For instance, polyelectrolyte complexes or neutral nanogels can be prepared through ionic gelation and polypolyelectrolyte complexation [4].

Electroluminescence Detection

In virus detection platforms, polymeric nanospheres have been employed to amplify the sensing signal. A signal amplification nanosphere can be constructed from polystyrene, which is considered the most versatile polymer in this context. In the facile polymerization method, polystyrene nanospheres can be synthesized by adding monomers, co-comonomers, and stabilizers to styrene. Wu and co-workers have successfully encapsulated quantum dots within polystyrene nanospheres for increased detection of the deadly Ebola virus. Figure 1a–c shows an example of how electron-luminescence activity of QDs can be exploited to produce signals. A polymeric nanosphere encapsulated QDs and preserved their ECL activity while improving their stability. ECL detection signals are significantly enhanced when hundreds of QDs are loaded into a nanosphere. Through a simple ultrasound technique, we embedded an abundance of CdSe/ZnS QDs into poly (styrene/acrylamide) copolymer nanospheres, which were then immobilized with antibodies [5, 6].

MATERIALANDMETHODS

MATERIALS

Materials Used

Flutamide, Chitosan, Acetic acid, Sodium tripolyphosphate (TPP), Tween80.

Instruments Used

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

METHODS

Preformulation studies

A preformulation is a phase in a research and development process where the physical, chemical, and mechanical properties of a new drug substance are characterized alone and in combination with excipients to ensure stability, safety, and efficacy.

Physicochemical properties may give insight into formulation design, support the need for molecular modification, or simply confirm there are no significant barriers to the development of a compound.

A. **Identification of pure drug**

Solubility Analysis

Various excipients, including solvent systems and excipients used for formulation of Nanospheres, were evaluated before formulation in order to ensure solubility.

Melting point

The fine powder of Flutamide was filled into a glass capillary tube (previously sealed at one end) and placed in a melting point apparatus. A melting point has been determined for Flutamide.

Spectroscopy

FT-IR spectra of the obtained sample were compared to the standard FT-IR spectra of the pure drug.

B. **Compatibility Studies**

Excipients (or) carriers must be compatible with pharmaceutical formulations in order to be selected effectively. The purpose of the present study was to verify the absence of chemical interactions between Flutamide and Chitosan using FT-IR spectrophotometers. Flutamade and Chitosan were examined separately and in mixtures by infrared spectroscopy using potassium bromide pellets. A hydraulic press compresses them

under 10 tonnes of pressure to form transparent pellets. A spectrophotometer was used to scan the pellet from 4000 to 400 cm-1.

Spectra of physical mixtures were compared with original spectra to identify potential molecular interactions. A FTIR analysis measures how light is selectively absorbed by specific chemical bonds in a sample based on their vibration modes. Vibration spectrum is used to evaluate the interaction between drug and polymer in encapsulated drugs.

Determination of λ max

Due to their aromatic properties or double bonds, many drugs absorb light at the ultraviolet light wavelength (200-400 nm).

Flutamide was measured using a balance and dissolved in 2% tween 80 in 100 ml. Stock solution-II was prepared by diluting 10 ml of stock solution-I into 100 ml with 2% tween 80. A further dilution was made to reach a concentration of 20 µg/ml. A UV scanner was used to scan the prepared solution between 200 and 400 nanometers. The λ max shown in the graph represents the pure drug.

Construction of Standard Curve of Flutamide

The tween 80 solution was prepared by taking 5 gm of the solution in a 250 ml volumetric flask, adding a little amount of distilled water to dissolve it, and filling the flask up with distilled water to obtain a volume of 250 ml with distilled water for 2% tween 80 solution. Flutamide drug was dissolved in 100ml of Tween 80 solution, resulting in a solution of 1 mg/ml concentration. A standard solution of 100 mg/ml was produced by diluting 5ml of stock solution to 50ml with Tween 80 solution. We transferred accurately measured aliquots of standard drug solution (100 mg/ml) to 10ml volumetric flasks and diluted up to the mark with Tween 80 solution. Thus the final concentration ranges from 10-50 mg/ml. Tween 80 was used as a blank for each solution and its absorbance was measured at 306 nm. Graph of drug concentration vs. absorbance was plotted.

Preparation of Flutamide Nanospheres

Ionic Gelation Method

A solution of chitosan was ionic cross linked with TPP anions in order to prepare nanospheres of chitosan. At various concentrations, chitosan was dissolved in aqueous acetic acid solutions (6%v/v) at 1.0, 2.0, 3.0, 4.0, and 5.0 mg/ml.

Evaluation of Flutamide Nanospheres

A. **Determination of Nanospheres Process Yield**

Nanosphere production yields were calculated by gravimetry. The suspensions of nanospheres were centrifuged (16,000 xg, 30 min, 15 °C) in fixed volumes, and the sediments were dried. Accordingly, the percentage process yield (% P.Y.) was calculated as follows:

$$\%P.Y. = \frac{Nanosphere sweight}{Totalsolids(CS + TPP + Flutamide)weight} x100$$

B. **Particle Size Analysis**

A scanning electron microscope (JOEL JSM-T330A) was used to measure particle size. A solvent paint was applied to the studs, and pellets were placed on the wet paint. After allowing the pellets to dry, the sample was photographed and examined under scanning electron microscopy. A diameter measurement of about 20 spheres was made manually from resultant photographs of each batch. A final average mean diameter was calculated.

C. **Determination of % Entrapment Efficiency**

A Nanosuspension containing 10 mg/20ml of drug was centrifuged at 5000 rpm for 15 minutes. It was separated from the supernatant solution. A UV spectrophotometer was used to measure the absorbance at 306 nm using 5ml of supernatant in 100ml of 2% w/v Tween 80 solutions. Using the supernatant, we calculated the amount of unentrapped drug. Drug unentrapped was used to determine the amount and percentage of drug entrapped. Three trials were analyzed to determine the standard deviation.

D. **Zetapotential**

It is common for nanospheres to be measured by the zeta potential to determine their surface charge property. A particle's electrical potential is influenced by the particle's composition as well as the medium in which it is dispersed. The zeta potential is also an important parameter to consider when evaluating and establishing optimum conditions for the stability of colloidal or dispersed systems. A microscope particle's surface charge produces a difference in electric potential between its surface and the bulk of the suspending liquid in millivolts. The difference between the two is known as the zeta potential.

The potential is easily measured because the charge moves as the suspension is placed between two electrodes with D.C. voltage across them, and the velocity will be proportional to the particle's zeta potential. This process is known as electrophoresis.

The zeta potential is used to measure electrostatic charges, which drive the repellence between adjacent spheres. Depending on the magnitude of both forces, the net result is either attraction or repulsion.

Generally, zeta potential determination responses of suspensions, particularly hydrophobic colloids, follow a thumb rule. A Malvern Zeta sizer (Malvern Zetapotential analyser) was used to determine

the zeta potential of the prepared nanosphere suspensions.

E. Invitro Drug Release Studies

A modified apparatus was used to examine in vitro drug diffusion from formulation 110 (cut off: 3500 Da). Freshly prepared 2% w/v tween 80 solution was used as the dissolution medium. A specially designed glass cylinder was used to hold an egg membrane - 110, which had previously been soaked overnight in the dissolution medium. This assembly was accurately filled with 5 ml of formulation. The cylinder was mounted on a stand and suspended in 50 ml of dissolution medium maintained at 37 ± 5ºC that the membrane just touched the surface of the receptor medium. Magnetic stirrers were used to stir the dissolution medium at low speed. Every hour, aliquots of 5 ml of receptor medium were withdrawn and replaced with equal volumes of aliquots. A UV-Vis spectrophotometer at 306 nm was used to analyze the aliquots after dilution with receptor medium. Diffusion studies were conducted using a quantity of drug equivalent to 10 mg of Flutamide.

F. Stability studies

Stability refers to a product's ability to remain within specified limits over time. Formulations are stable if they meet the following criteria.

- The active ingredient should constitute at least 90% of the product.
- If any preservatives are added, they should be in an effective concentration
- A discolored or precipitated product should not cause a foul odor or display any discoloration.

It should not cause irritation or toxicity [7 - 15].

RESULTS

Flutamide nanospheres were formulated using different drug polymer ratios, the composition of which was shown in Table 4.3. The formulations were evaluated for process yield, surface morphology, particle size, drug entrapment, zeta potential, and *invitro* drug release.

PREFORMULATIONSTUDY

Solubility

The solubility of pure drug in 10 mg/10 ml of solvent was carried out and found to be soluble in dichloromethane, acetone, and methanol, soluble in 2% tween 80 and 2%so Diu lauryl sulphate solution and completely insoluble in water.

Identification of pure drug

The purity of Flutamide was confirmed by comparing its I.R. Spectra with the standard I.R. Spectra of Flutamide. Functional groups of Flutamide and chitosan at their respective frequencies.

Table 1: IR Spectrum Values of Flutamide and Chitosan Combination.

Wavenumber(cm^{-1})	Vibrations
C-H Stretch	2790
C-O-C Stretch	1087
CH_3[C-H] Stretching	2963
N ═══ O Stretching	1548
C=O Stretch	1672
NH_2Stretch, N-H&O-H Stretch	3286
C-F_3Stretching	897
Aromatic C-H Stretching	3095

PREPARATION AND CHARACTERIZATION OF FLUTAMIDENANOSPHERES

Table 2: Process Yield% of Flutamide Nanospheres (Mean ± S.D, n=3)

Batch code	Drug: carrier Ratio	Process Yield %
F1	1:1	51.6±4.5
F2	1:2	62.4±5.4
F3	1:3	69.8±4.0
F4	1:4	77.5±5.7
F5	1:5	71.4±3.6

Table 3: Entrapment Efficiency Formulations with Drug and Chitosan Polymer.

Formulation Code	Drug: Poly mer Ratio	Absorbance	Drug content (mg)	% Entrapment
F1	1:1	0.542	6.24	62.34±0.48

F2	1:2	0.566	6.54	65.37± 0.89
F3	1:3	0.581	6.72	67.21± 0.95
F4	1:4	0.643	7.42	74.43± 0.56
F5	1:5	0.606	7.01	70.16± 0.95

Figure 1: Standard Curves for Flutamide by UV Method

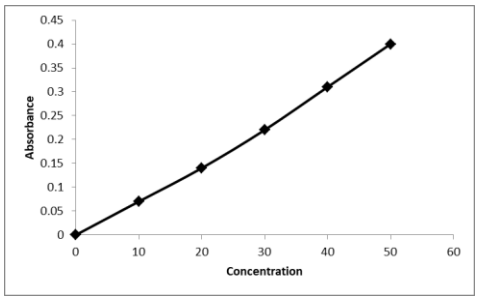


Figure 2: SEM offormulationF4

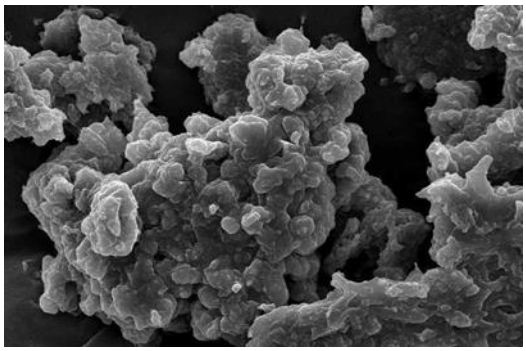


Figure3: Zeta Potential of Flutamide Nanospheres

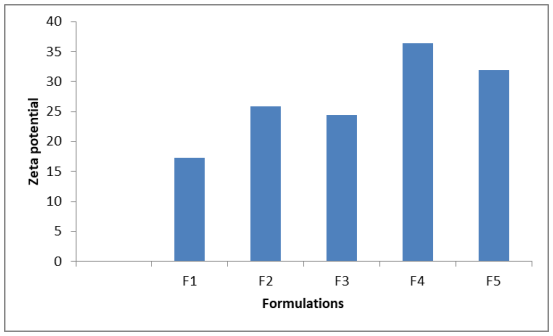


Figure 4: 5.8 Comparative In Vitro Release Profiles of Flutamide Nanospheres According to Zero Order Kinetics

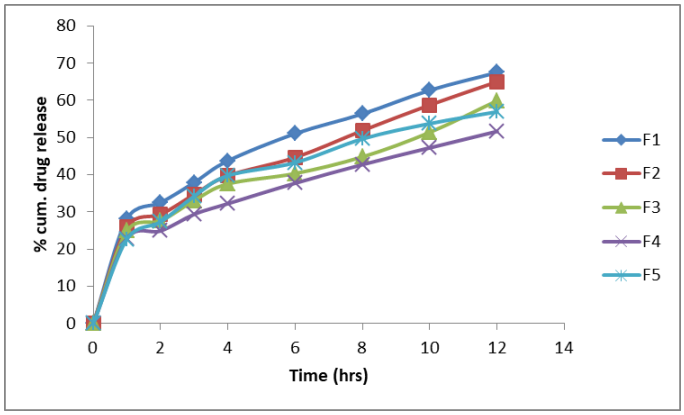


Figure 5: Comparative In Vitro Release Profiles of Flutamide Nanospheres According to First Order Kinetics

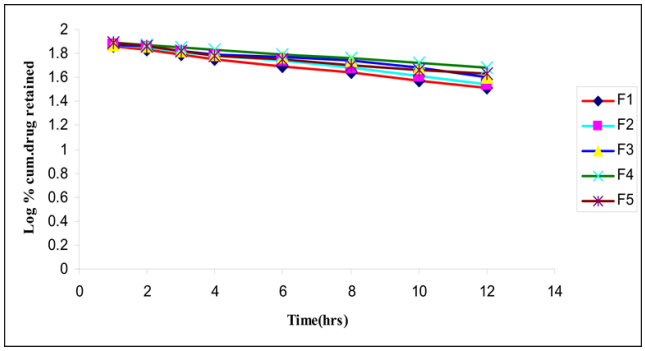


Figure 6: Comparative In Vitro Release Profiles of Flutamide Nanospheres According to Higuchi plot

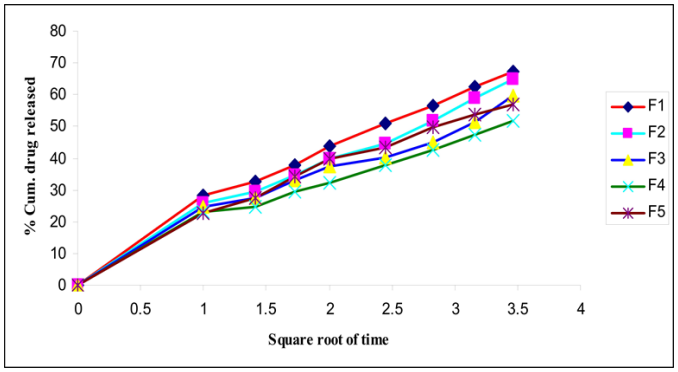


Figure 7: Comparative In Vitro Release Profiles of Flutamide Nanospheres According to Peppas plot

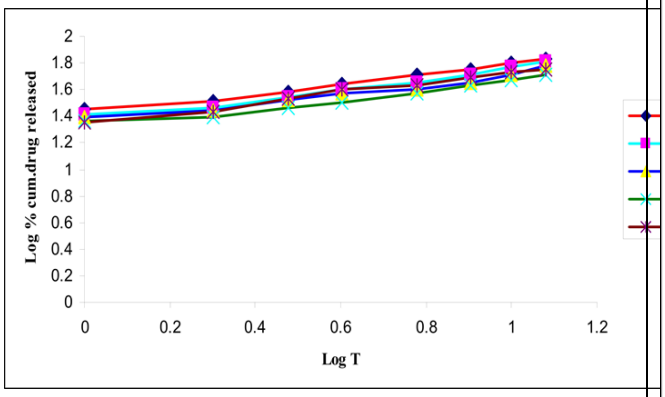


Figure 8: Stability Study: Comparison of %Drug Content of Formulation F4 at 4ºC, Room Temperature (29ºC) and 45ºC ± 2ºC / 75% RH

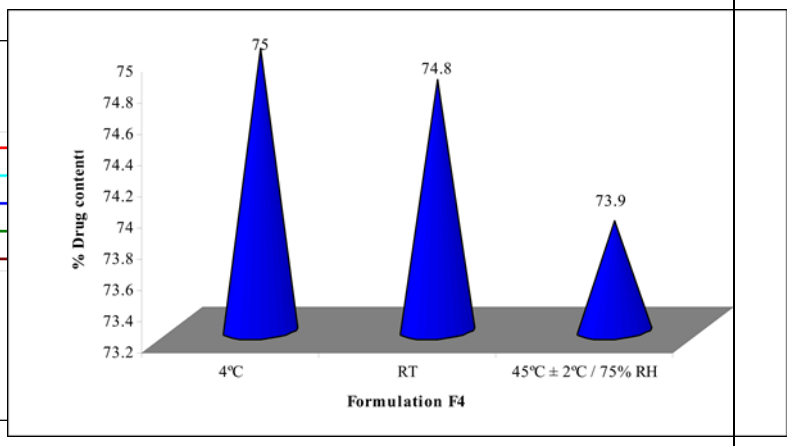
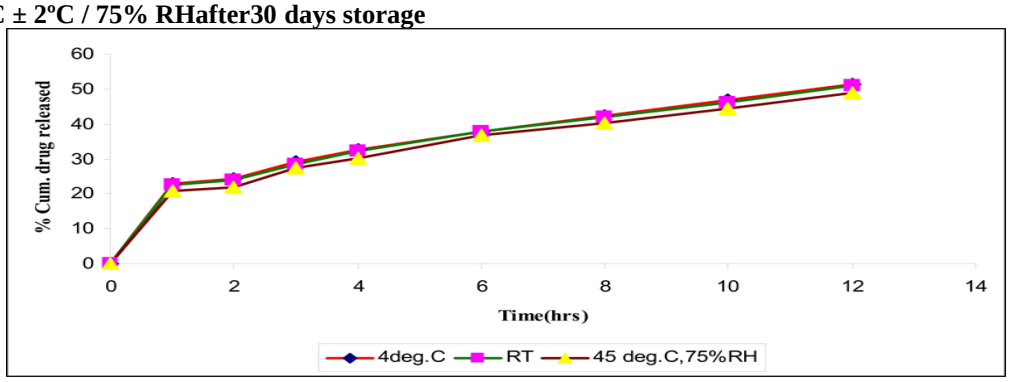


Figure 9: Stability Study: Comparison of In vitro drug release profile for Formulation F4 at 4ºC, Room Temperature (32ºC) and 45ºC ± 2ºC / 75% RHafter30 days storage



DISCUSSION

Various solvents were used to determine the solubility of the pure drug. The results of the study revealed that it is insoluble in water and soluble in 2% tween 80 solution, sodium lauryl sulphate solution, dichloromethane solution, acetone solution, and methanol solution. A comparison of Flutamide's I.R. Spectra with the standard I.R. Spectra of Flutamide. I.R. Spectral analysis studies were carried out to study the compatibility study of pure drug Flutamide with Chitosan prior to the preparation of Flutamide nanospheres. Spectrum 3 exhibited all the characteristic peaks of Flutamide, indicating drug-polymer compatibility.

A UV spectrophotometer was used to scan the absorption spectrum of pure drug at 20 µg/ml concentration in 2% Tween 80 solutions between 200 - 400 nm. It was found that the maximum peak could be obtained at 240 nm, which was taken as λ max. Flutamide standard solutions in 2% Tween 80 solution containing 10-50 g/ml of drug were analyzed for absorbances. With a regression value of 0.994, Figure 5.1 shows the standard calibration curve. A linear curve was found in the range of 10-50 mcg/ml at λ max 240 nm.

A room temperature mixture of two aqueous phases is necessary for the preparation of chitosan nanospheres based on an ionic gelation process. Both phases contain chitosan solutions and polyanion TPP solutions. Flutamide must be incorporated into the chitosan nanospheres under a number of conditions due to its hydrophobic nature. It was possible to successfully entrap the hydrophobic drug Flutamide by dissolving it in 2% tween 80 before incorporating it into the chitosan solution, followed by the addition of TPP. A preliminary study evaluated the effects of solvent volume and chitosan concentration on the physico-chemical characteristics of nanospheres to determine which formulation conditions were most suitable.

The percentage yields of nanospheres are shown batch F4 containing drug and polymer in the ratio 1:4 showed the highest percentage yield. As polymer concentrations increased, particle yields increased as well.

The Entrapment efficiency of the Flutamide 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. Formulation F1 with a 1:1 ratio of drug to polymer showed 63.3% entrapment. 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%, while formulation F5 with 1:5 ratios showed 71%. As the polymer concentration in the formulation increased, entrapment efficiency increased steadily. Among all formulations, formulation F4 achieved the highest level of entrapment with 75.2%. The scanning electron photomicrograph of formulation F4 is shown in Figure. Photographs were taken at 7,500 - 20,000 X magnification. A smooth surface was present on the surface of all formulations' spheres. Approximately 400 nanometers were the size of the particles. In order to evaluate the surface charge of the nanospheres, the zeta potential of the nanospheres was measured with a zeta meter ± 3M. We evaluated the results. The zeta potential of all formulated nanospheres ranged from 18.4 to 37.08 mV, indicating moderate stability.

The in vitro drug release profile of Flutamide nanospheres was plotted using various graphical models. A zero order graph was developed by plotting the percentage cumulative release of drugs against time in hours. First order graphs were prepared by plotting log percentage cumulative drug remaining against time.

A graph of Higuchi's graph was used to study diffusion patterns of the formulations using cumulative percentages of drug release versus square root of time.

Graph of cumulative percentage drug release against log time (Peppa's exponential equation)contain the release data obtained for formulations F1, F2, F3, and F4. Fig. All five formulations are plotted as functions of time all five formulations, plots of log cumulative percent drug retained were shown as a function of time. The Higuchi matrix is shown the Peppas kinetics of all formulations.

After 12 hours, the cumulative percentage of drug released for F1, F2, and F3 was greater than that for F4 and F5. F1, F2, F3, F4 and F5 cumulative percentages of drug release after 12 hours were 77.33 %, 73.86%, 64.78%, 52.68% and 58.76%. It was apparent that in vitro release of Flutamide showed a very rapid initial burst, and then followed by a very slow drug release. A fast initial release suggests that some drug is localized on the nanosphere's surface. The best formulation was considered to be F4, which demonstrated sustained release when compared to other formulations.

Various kinetic dissolution models were fitted to the dissolution data of all five formulations to describe their release kinetics, including zero order, first order, and Higuchi. A comparison was made between the model and the drug equation based on these values. All formulations release the drug according to a first-order release or Higuchi model, based on the higher R2 values. Higuchi's release mechanism was controlled by swelling and diffusion, since it was confirmed as a Higuchi model.

Compared to the previous data of F4, storage at 4oC and room temperature resulted in a slight decrease, but storage at 45oC resulted in a significant decrease in drug content. In vitro release studies showed that the formulation stored at 40C showed 51.19% release, the one stored at room temperature showed 50.93%, and the formulation stored at 45C + 2oC / 75% RH showed 48.74% release. It is possible that higher temperatures will cause drug degradation, which will decrease the release of the drug.

CONCLUSION

Chitosan is a suitable carrier for the preparation of Flutamide nanospheres. The F4 drug polymer ratio showed the highest 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. Therefore the chitosan polymer entraps more drugs by increasing organic phase viscosity, which increases diffusion resistance between organic and aqueous phases. 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. Following 12 hours of dissolution studies, formulation F1 showed maximum cumulative percent drug release and formulation F4 showed minimum cumulative percent drug release. Optimal formulation F4 was selected based on drug content, drug entrapment efficiency, particle size morphology, and zeta potential. There was an initial burst of rapid Flutamide release followed by a very slow release of the drug in vitro. Initially, the nanospheres released some drug rapidly, suggesting that some drug was localized on their surface. Generally, the in vitro releases of formulations were best fitted into first order mathematical models followed by Higuchi's model and zero order models. As the n values are less than 0.5, the drug release from nanospheres follows a Fickian diffusion controlled mechanism. A stability analysis showed that in vitro release and maximum drug content were found at 4 OC and room temperature for F4. The flutamide nanospheres could be stored at 4OC or room temperature, thus proving that they are suitable for storage.

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