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Fabrication of letrozole formulation using chitosan nanoparticles through ionic gelation method

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ABSTRACT

In this study, the anticancer drug letrozole (LTZ) was formulated using chitosan nanoparticles (CS-NPs) with the crosslinking agent sodium tripolyphosphate (TPP). The nano-formulation was optimized by varying the concentration of drug. The prepared particles were characterized using FTIR, TGA, XRD, SEM, TEM and DLS. From the FTIR results, the appearance of a new peak for ═C—H, C═C and C═N confirms the formation of LTZ loaded chitosan nanoparticles. TEM images shows that the average particle size was in the range of 60–80 nm and 20–40 mm air dried and freeze dried samples respectively. Also the prepared formulation had been evaluated *in vitro* for determining its hemocompatability, biodegradability and serum stability. The preliminary studies supported that the chitosan nanoparticles formulation has biocompatibility and hemocompatible properties and it can act as an effective pharmaceutical excipient for letrozole.

1. Introduction

Breast cancer is the most common type of cancer, especially in women, and, unfortunately, its incidence is increasing each year [1,2]. It is widely accepted that the majority of breast cancers are hormone-dependent and that estrogen is a key mediator in the progression and metastasis of breast tumors. Particularly, for post-menopausal women it has been reported that the concentration of 17b-estradiol (E2) in breast tumor can be tenfold higher than those in plasma [3]. The high concentration of E2 in breast tumors could be attributed to increased uptake from plasma or in situ aromatization of androgens to estrogens [4].

Letrozole is considered to be one of the most effective non-steroidal, third generation aromatase inhibitors (AIs) which inhibit excess estrogen bio-synthesis within the body [5–8]. Its use as an estrogen receptor positive breast cancer drug is well recognized [9,10]. In practice letrozole was given to treat breast cancers and it was administered orally. Letrozole prevents the aromatase from producing estrogens by competitive, reversible binding to the heme of its cytochrome P450 unit [11] by >99%. Therefore it is a highly potent drug [12,13].

Polymeric nanoparticles have recently been considered as promising carriers for anticancer agents [14,15]. Especially chitosan plays a vital role in drug delivery applications. The amino group in chitosan has a pKa value of ~6.5, thus, chitosan is positively charged and soluble in acidic to neutral solution. Chitosan is bioadhesive and readily binds to negatively charged surfaces such as mucosal membranes and enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and recyclable. Since in the conventional mode of administration, there is the need for the ideal drug delivery system owing to its adverse side effects caused by the non-specific targets. Therefore for designing ideal delivery systems, the parameters such as size and size distribution, drug loading capacity and stability are the important parameters to be taken into account [16,17]. The durg-polymer interactions can be proposed by increase drug loading capacity, non-covalent interactions including hydrogen-bonding and ionic interactions.

Generally, the development of new materials always leads to advances in technology and creates innovative solutions to old problems. Thus the nanotechnology has gained wide acceptance in contemporary life [18]. In oncology, nanomaterials can be engineered to serve as anti-cancer agents or as ideal drug delivery vehicles [19–21]. Hence the present work was aimed to synthesis the novel formulation using chitosan nanoparticles for the highly potent anticancer drug letrozole for sustained release and to over-

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come the problems of conventional method of administration of letrozole.

In the present work through ionic cosslinking method a novel drug carrier of letrozole was synthesized which overcomes the drawbacks of low loading efficiency, higher particle size etc. The formulations were characterized for its formation and tested for effective *in vitro* drug release, biocompatibility, stability and blood compatibility. Chitosan nanoparticles were used as a carrier for letrozole and sodium tripolypohosphate was used as a crosslinker.

2. Materials and methods

2.1. Materials

Chitosan (CS) with the degree of deacetylation 92% was procured from India Sea foods, Cochin, Kerala. Letrozole was synthesized as per the procedure given in US patent 7705159B2. Sodium tripolyphosphate (TPP) was purchased from finer chemicals and used without further purification. All other chemicals and reagents used are analytical grade.

2.2. Preparation of durg unloaded and loaded chitosan nanoparticles

50 mg of chitosan was dispersed in 5 mL of aqueous acetic acid (2% v/v) solution and continuously stirred for about 20 min at 600 rpm to obtain the homogeneous solution. 5 mL (0.8% w/v) of TPP solution was used as a crosslinker [22,23]. The resulting chitosan nanoparticle suspension was subsequently centrifuged for about 45 min at 12,000 rpm and re-suspended in water for washing followed by drying. Similarly for preparing letrozole loaded chitosan nanoparticles, the drug letrozole (5, 10 and 15 mg in 0.5 mL acetic acid) solution was added dropwise to the chitosan solution, under continuous stirring up to 30 min. TPP solution (38.00 mg in 5 mL deionised water) was added dropwise to the chitosan/Letrozole solution over a period of 60 min at a stirring speed in the range of 550- 600 rpm (Scheme 1). After the complete addition, the suspension was centrifuged at 12,000 rpm. The supernatant solution was subjected to determine the loading efficiency. The filtered solid was slurried in water and centrifuged, the centrifuged material was kept for drying.

2.3. Yield (% w/w)

Yield (% w/w) was calculated as weight of the dried nanoparticles recovered from each batch divided by the sum of the initial dry weight of the starting materials multiplied by a hundred, i.e.

$$Yield \quad \left(\%\frac{w}{w}\right) = \frac{weight \ \ of \ \ dried \ \ nanoparticles \ recovered}{drug + 50 \ \ mg \ chitosan + 38 \ mg \ crosslinker} \times 100$$

2.4. Characterization of nanoparticles

FTIR spectroscopy was measured for the determination of the types of bonds present in the nanoparticulate. FT-IR spectra of CS – NPs, LTZ and LTZ – CS – NPs were carried out using KBr tablets (1% w/w of product in KBr) with a resolution of 4 cm^{-1} and 100 scans per sample on a Thermo Nicolet AVATAR 330 spectrophotometer. Thermogravimetric analysis was conducted to measure the thermal weight loss of the samples on a TGA Q500 V20.10 Build 36 instruments at a heating rate of 20 °C per minute in nitrogen atmosphere. The weight losses at different stages were analysed. The scanning electron microscopy analysis (SEM, Leica, Cambridge, UK) was conducted to observe the size, shape and surface morphology of the nanoparticles. For the analysis, the samples are wiped with a thin gold – palladium layer by a sputter coater unit (VG – microtech, UCK field, UK), the shape and morphology were analysed with a Cambridge stereoscan 440 scanning electron microscope (SEM, Leica, Cambridge, UK). The transmission electron microscopy analysis (TEM) was conducted to observe the size, shape and surface morphology of the loaded and unloaded chitosan nanoparticles. The shape and morphology were analysed with a HITACHI-H-7650 transmission electron microscope. The particle size and size distribution of the nanoparticles were measured by Dynamic light scattering method (DLS, Zetasizer Nano-S, Malvern, England). Suitable amount of the dried nanoparticles from each formulation was suspended in deionised water and was sonicated for a suitable time period before the measurement. The volume mean diameter, size distribution and polydispersity of the resulting homogeneous suspension were determined using DLS technique. X-ray diffraction (XRD) patterns were obtained using an X-ray diffractometer (XRD-SHIMADZU XD-D1).

2.5. Letrozole loading measurements

The quantity of letrozole (LTZ) entrapped in the nanoparticulate system was determined indirectly, by measuring the quantity of letrozole remaining in the supernatant based on the absorbance of the samples at 250 nm [24]. The standard curve was obtained and the sample absorbance was measured in 3 mL quartz cuvettes using Shimadzu UV-1700 Pharma spec UV–vis spectrophotometer (Suzhow, Jiangsu, China). The measurement for quantifying the amount of drug loaded in the nanoparticles is drug entrapment efficiency (EE). Determining the EE allows for the optimization of the amount added, reducing wastage, and is defined as follows:

$$EE = \frac{Total \ letrozole \ \ (\mu g) - Free \ \ letrozole\,(\mu g)}{Total \ \ letrozole \ \ (\mu g)} * 100$$

Entrapment efficiency describes the quantity of the drug entrapped within the nanoparticle as it is related to the initial drug loading. 100% EE means that the entire drug quantity added has been incorporated into the nanoparticle.

2.6. In vitro *drug release studies*

In vitro drug release profiles were done by direct dispersion method as explained in literature [25,26]. *In vitro* drug release studies were done for a period of one week at pH 7.4. 10 mg of drug loaded CS – NPs were taken in 50 mL of 10 mM phosphate buffer solution (PBS) in a beaker under magnetic stirring at 100 rpm. The receptor phase was stirred and thermally controlled at 37 °C. The base absorbance of the release media was accounted by using the release medium as the solution in the UV-spectrophotometer reference cell, as well as the solution for zeroing the system. At fixed time intervals, 3 mL of the receptor phase was withdrawn, centrifuged to collect the supernatant and then substituted with fresh buffer. The drug release was assayed spectrophotometrically. The cumulative percentage amount of drug release was calculated and plotted against time.

2.7. Kinetics studies

To analyze the *in vitro* release data various kinetic models were used to describe the release kinetics. The zero order rate equation describes the systems where the drug release rate is independent of its concentration [27].

$$Q_t = Q_o + k_o t$$

where Q_o and Q_t are the initial amount of drug and cumulative amount of drug released at time t. K_o is the zero order rate constant.