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Studies on drug-polymer interaction, *in vitro* release and cytotoxicity from chitosan particles excipient

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ABSTRACT

Nonobvious controlled polymeric pharmaceutical excipient, chitosan nanoparticles (CS-NPs) for lenalidomide encapsulation were geared up by the simple ionic cross linking method to get better bioavailability and to reduce under as well as overloading of hydrophobic and sparingly soluble drug lenalidomide towards cancer cells. Lenalidomide loaded chitosan nanoparticles (LND-CS-NPs) were in the size range of 220–295 nm and characterized by DLS, TEM, FT-IR, TGA and XRD. Encapsulation of lenalidomide over chitosan nanoparticles was observed about 99.35% using UV spectrophotometry method. *In vitro* release and the cytotoxic studies were performed using LND-CS-NPs. This study implies the new drug delivery route for lenalidomide and illustrates that the CS-NPs serves as the effective pharmaceutical carrier for sustained delivery of lenalidomide.

1. Introduction

Biopolymers with attractive characteristic properties acquire more attention in biomedical applications. Chitosan, the second most abundant biopolymer (cationic polysaccharide) is obtained by partial deacetylation of chitin. Chitosan is an most advantageous material with many promising applications. Biomedical application is one of them. Evidently, literature is galore with concern about their safety, toxicology and biodegradable consideration.

Chitosan and its derivatives have been used as gene carriers due to their less toxic nature (Lee et al., 1998) and carriers for directly compressed tablets (Kristmundsdottir et al., 1995). They have been used as blood anticoagulants as well as hypocholesterolemic agents (Agnihotri et al., 2004). Due to their good muco adhesive character, chitosan and its derivatives are used as pharmaceutical carriers for sustained drug release (Hirano, 1996). Chitosan derivatives are used in tissue engineering, wound healing (Jayakumar et al., 2005; Madhumathi et al., 2010) and to enhancing the bioavailability and dissolution rates of hydrophobic drugs (Miyazaki et al., 1981). Nowadays chitosan based micro and nanoparticles are playing a vital role in sustained and targeted drug delivery (Dev et al., 2010; Sanoj Rejinold et al., 2011a,b).

Lenalidomide, a thalidomide analog is an IMiDs immunomodulatory compound used for the treatment of myelodysplastic syndromes, with pleiotropic activities including induction of apoptosis, inhibition of angiogenesis and broad immunomodulatory effects (Kastritis and Dimopoulos, 2007; Richardson et al., 2006). The introduction of lenalidomide and other new anticancer agents, such as thalidomide and bortezomib, has a major impact on outcomes in patients with multiple myeloma (MM), significantly improving 5–10 years of survival rates (Brenner et al., 2009). Lenalidomide has dual mode action including tumoricidal and immunomodulatory effects (Morgan, 2010). Shaji Kumar and Vincent Rajkumar (2006), gave a detailed clinical response of

Abbreviations: LND, Lenalidomide; C-NPs, Chitosan nanoparticles; LND-CS-NPs, Lenalidomide loaded chitosan nanoparticles; DLS, Dynamic light scattering; PBS, Phosphate buffer solution; MTT, 3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide.

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thalidomide and lenalidomide in the treatment of multiple myeloma.

Lenalidomide is an off-white to pale-yellow solid powder. As lenalidomide is an effective derivative of thalidomide in medical oncology therapeutics, lower solubility may limit its effectiveness. In general, the formulation of a drug in soluble form is much essential and more challenging. Many researchers extended their research to increase drug solubility, where the solubility renders stability of the compound.

Nanoscience and nanotechnology are the focal points in recent years and propelled to the forefront by researchers from both academia and industry (Park, 2000; Hughes, 2005; Whitesides, 2005). In general the nanoparticulate acts as a promising drug delivery system, by bringing about substantial changes in drug biodistribution, minimizing toxicity and other adverse effects associated with important drugs. When the drug delivery device with a particle size about >5 μm is used, it accumulates in the lungs (Sato et al., 1996). Therefore, nanoencapsulation glitters with remediating advantages on these aspects; they also include the enhanced stability of labile drugs, enhanced drug bioavailability and controlled drug release owing to the fact that particles in the nanosize ranges are efficient in crossing permeability barriers (Sharma et al., 2004).

In recent days, biopolymeric nanoparticles have been focused as potential drug carriers which led to the development of chemotherapeutic oncology. From literature, as we have understood, chitosan nanoparticles play a vital role as good pharmaceutical excipients. Chitosan nanocarrier is a system, having the capacity to cross biological barriers, to protect macromolecules like proteins, oligonucleotides and genes from degradation in biological media, and to deliver drugs or macromolecules to a target site with controlled release (Lopez-Leon et al., 2005). Thus with indispensable interest, chitosan nanoparticulates were prepared and used as a vehicle for sustained release of lenalidomide, where chitosan nanocarriers enhance the water solubility of lenalidomide and the bioavailability by enhancing their permeability across physiological barriers.

Moreover, the chitosan nanoparticulate has high drug-loading capacity, a controlled release profile for the incorporated drug and good compatibility between the core-forming polymeric block and the incorporated drug. In the present study, an attempt was made to prepare the novel chitosan nanoparticles as carriers for the hydrophobic drug lenalidomide. Lenalidomide was synthesized, encapsulated in chitosan nanoparticles and characterized using FT-IR, TGA, XRD, TEM and DLS. *In vitro* sustained release study was also performed to see the effectiveness of the carrier.

2. Materials and methods

2.1. Materials

Chitosan (CS) with the degree of deacetylation of 92% was procured from India Sea Foods, Cochin, Kerala. Lenalidomide (LND) was in house synthesized (Kapoor et al., 2011). Sodium hexametaphosphate (SHMP) was purchased from Fischer Scientific, Mumbai and used without further purification. All other chemicals and reagents used are analytical grade.

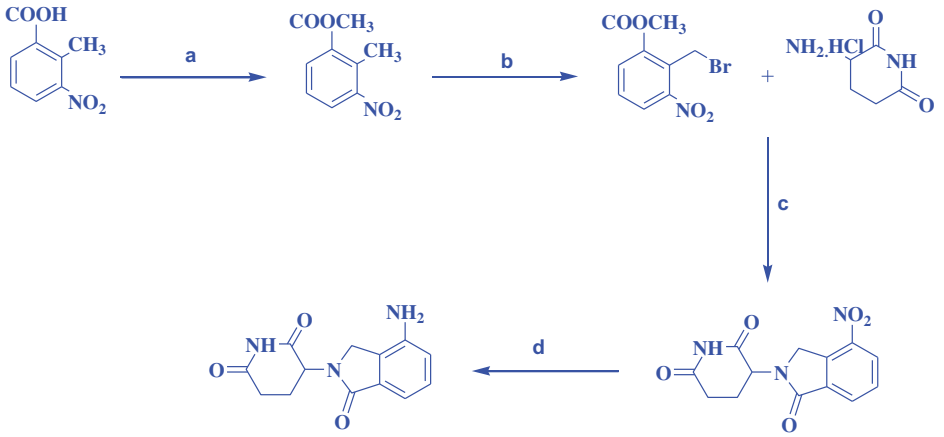
2.2. Preparation of lenalidomide

2-Methyl-3-nitro benzoic acid was esterified using thionyl chloride in the presence of methanol to give methyl ester of 2-methyl-3-nitro benzoic acid. Prepared methyl ester was brominated using *N*-bromo succinimide yielding bromo compound. This bromo compound was coupled with 3-amino piperidine-2,6-dione hydrochloride using triethylamine in the mixture of acetonitrile and dimethylformamide which formed 3-(4-nitro-1-oxoisoindoline-2-yl)piperidine-2,6-dione. Reduction of 3-amino piperidine-2,6-dione hydrochloride using triethylamine in the mixture of acetonitrile and dimethylformamide gave 3-(4-nitro-1-oxoisoindoline-2-yl)piperidine-2,6-dione. In the presence of palladium, 3-(4-nitro-1-oxoisoindoline-2-yl)piperidine-2,6-dione was reduced to get crude lenalidomide, and it was recrystalized using methanol to obtain a pure lenalidomide as shown in Scheme 1 (Kapoor et al., 2011).

HPLC purity: >99.00% and the structure was confirmed by ^{1}HNMR and IR spectroscopy.

2.3. Preparation of chitosan nanoparticles

50 mg of chitosan was dispersed in 10 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 SHMP solution was used as a crosslinker (Calvo et al., 1997). The



Reagents and conditions: (a) Thionyl chloride, Methanol, 4h, 65^{0} C; (b) N- Bromosuccinimide, Azoisobutyronitrile, Carbon tetrachloride, 3h, 80^{o}C; (c) Triethylamine, Dimethylformamide, Acetonitrile, 10h, 55^{o}C; (d) 10% Palladium on charcoal, Acetonitrile, Methanol, H_2, 1h, 25^{o}C.

Scheme 1. Synthesis of lenalidomide.