

Pharmacokinetics and biodistribution of negatively charged pectin nanoparticles encapsulating paclitaxel

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Abstract Pectin, a naturally occurring biopolymer has been found to have increasing applications in the pharmaceutical and biotechnology industry. Sugars with their three-dimensional structures are important for many biological functions. We report preparation of negatively charged pectin nanoparticles encapsulating paclitaxel, a broad-spectrum anti-cancer drug for possible therapeutic applications. The mean diameter of the nanoparticles was ~300–350 nm with an encapsulation efficiency ~17 %. The TEM studies indicated that the particles were spherical in shape and their sizes are in unison with the DLS size spectra. The surface charge of pectin polymer was -5 mV and of nanoparticles were ~ -32 mV. The enhanced surface charge shows greater stability. The high electrophoretic mobility of ~ 3.5 to 1.5 $\mu\text{mcm/Vs}$ confirmed the nano-size of particles. We compared the cytotoxic effect of paclitaxel (Pax) per se, pectin nanoparticles (PPN), and pectin chains on Hep G2, hepatic carcinoma cell line. Dose-dependent cytotoxicity was observed, wherein $\sim 21.7 \pm 3.2$ % cytotoxicity was observed by PPN, but Pax per se showed $\sim 55.6 \pm 3.5$ % cytotoxicity in a 72-h assay. The pharmacokinetics and biodistribution studies on Balb/c mice indicated that the nanoparticles had prolonged plasma retention of the drug with major accumulation in liver tissue after an i.v. tail vein injection of 20 mg/kg drug. The rank order of concentration are as follows, i.e., liver > kidney > lung > spleen for the PPN and spleen > liver > kidney \geq lung for Pax per se. The in vitro

studies clearly indicated that the efficacy of the drug was not compromised by encapsulation, making it a good candidate to deliver biopharmaceuticals. Nanoparticles produced free radicals in the free cell system and this ability caused oxidative stress, which may give rise to inflammation, cell destruction, and genotoxicity. Thus, the results obtained in this study holds great promise for pectin nanoparticles to be exploited for passive delivery of paclitaxel to tumor tissues, in particular, liver cancers.

Keywords Pectin nanoparticles · Paclitaxel · Pharmacokinetics · Biodistribution

1 Introduction

Pectin has gained wide acceptance as matrix materials because of their inherent biocompatibility, polyfunctionality, biodegradable nature, flexible structural networks, non-toxicity, and low production costs (May 1990). The physiological properties, functionality, and application of pectin are dependent on the degree of methoxylation (Thibault et al. 1993; Garnier et al. 1993). Pectin is used in food industry as a thickening agent, a gelling agent and a colloidal stabilizer, and a carrier and coating material in pharmaceutical sciences (Endress 1991). Pectin is highly hydrophilic in nature, and hydrophilic matrices are generally used for the oral drug delivery and for the preparation of modified release formulation (Sinha and Kumria 2003). However, the major challenge of using pectin for the development of modified drug formulation was to overcome its solubility in aqueous medium which can contribute to the undesirable premature and local release of the active principle from the polysaccharide matrix (Sande 2005; Wu et al. 2008).

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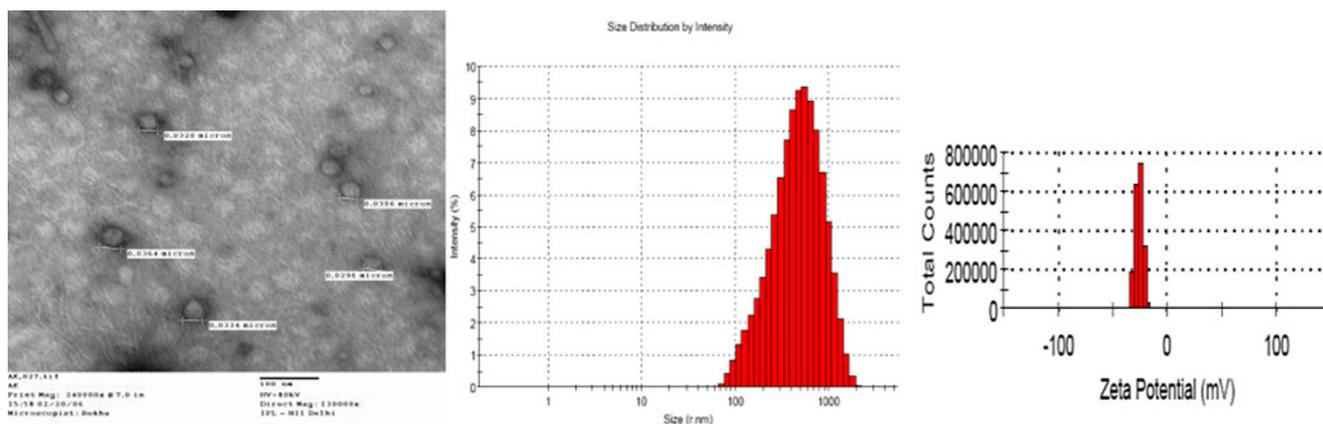


Fig. 1 TEM image of pectin nanoparticles. A representative graph of the size distribution spectra (~350 nm with PDI=0.30) and zeta potential (-26 mv)

We have previously reported that coordination bond between the platinum(II) atom and the carboxylic group in the galacturonate of pectin causes spontaneous folding to form nanoconjugates (Verma and Sachin 2008), as well as formation of pectin nanoparticles prepared by inducing the gelification and cross-linking of pectin solution with calcium chloride and L-asparagine at low pH (Verma et al. 2011). At higher pH values, the polycarboxylate groups that are present can be cross-linked by the addition of calcium ions which leads to macromolecular aggregates (Kohn 1975). It has been widely reported that thiolation of a polymer such as chitosan (Kast and Bernkop-Schnürch 2001) poly(acrylic acid) (Marschütz and Bernkop-Schnürch 2002), and alginates (Bernkop-Schnürch et al. 2001), enhances its cohesive properties leading to

oxidative cross-linking of thiol groups (Oosterveld et al. 2000). In continuation, our present investigation focuses on the *in vitro*, cytotoxicity, and *in vivo* pharmacokinetics and biodistribution of the negatively charged pectin nanoparticles.

2 Experimental

2.1 *In vitro* cytotoxicity

HepG2, hepatic carcinoma cell line was procured from American Type Culture Collection, (Rockville, MD) and maintained at 37 °C in 5 % CO₂ at 95 % relative humidity. Pectin nanoparticles (PPN) were used for cytotoxicity as per our

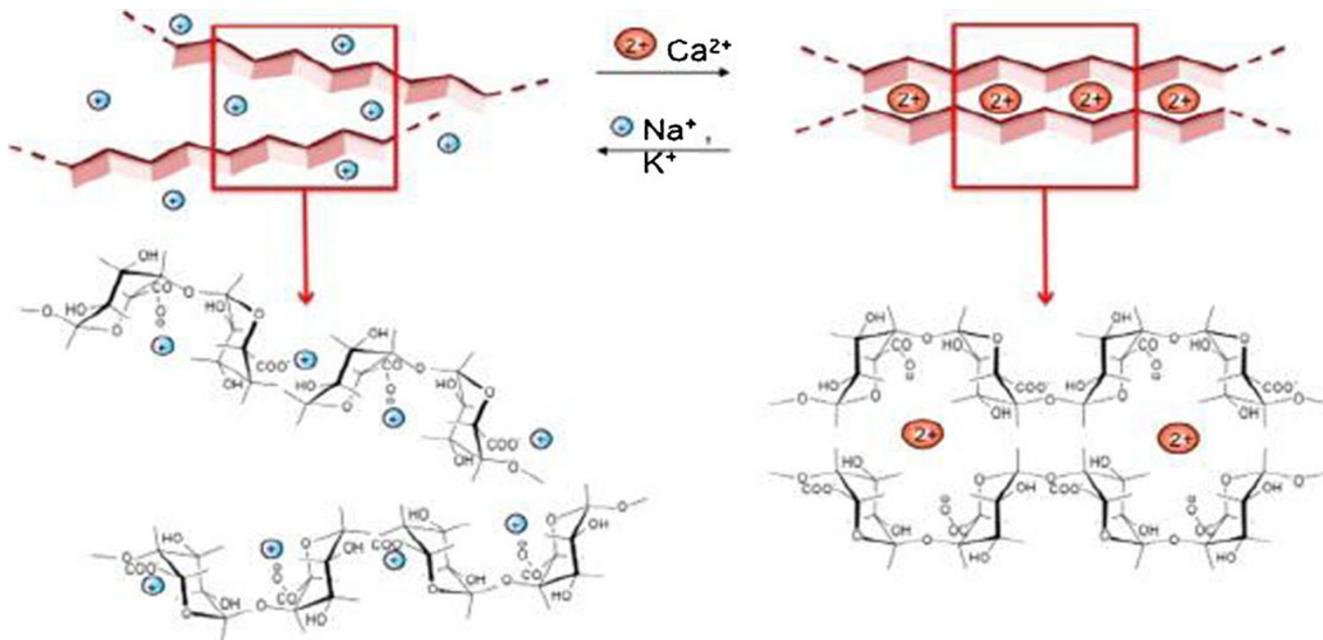


Fig. 2 Flow chart of preparation of pectin nanoparticles. Ca²⁺ cross-links in a co-operating binding fashion between carboxylate group of adjacent chains enabling nanoparticle formation

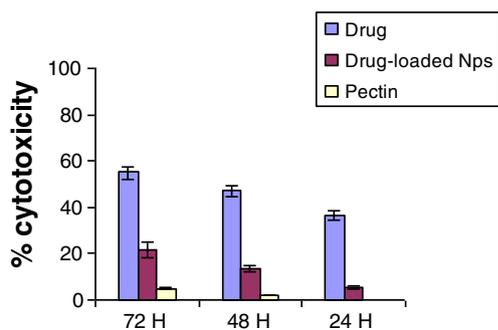


Fig. 3 Dose-dependant cytotoxicity of Pax and PPN on HepG2 (Conc., 0.02 μ M). Pure pectin (5 mg/ml)

previously published protocol (Verma et al. 2005) using a standard MTT assay in a 24-, 48-, and 72-h assay. MTT assay is a nonradioactive assay done routinely to assess the viability of the cell culture. Briefly, 5×10^3 cells/well were incubated in RPMI-1640 supplemented with 10 % FCS with paclitaxel (Pax) per se and paclitaxel encapsulated formazan crystals formed by the cellular reduction of MTT were dissolved in 150 μ l of DMSO and plates were read on an ELISA reader using 570-nm filter. The relative cell viability (percent) related to control wells containing cells without nanoparticles was calculated by: $[A]_{\text{control}} - [A]_{\text{test}} / [A]_{\text{control}} \times 100$

Where $[A]_{\text{test}}$ is absorbance of the test sample and $[A]_{\text{control}}$ is the absorbance of the control sample.

2.2 Animal studies: pharmacokinetics and biodistribution

Studies were conducted under standardized procedures on inbred, male 6–8-week-old Balb/c (18–20 g) mice (Verma et al. 2012). Destructive sampling method was adopted for acquisition of biological samples (blood and tissues) for analysis. The animals were anesthetized with ether inhalation and paclitaxel per se and re-dispersed PPN containing drug at a dose of 20 mg/kg body weight, and PBS as control were then administered intravenously (i.v.). Blood was drawn from tail vein at predetermined intervals at 5, 10, 15, 30, 60, 90, and 120 min. Mice were sacrificed by cervical dislocation and dissected to obtain various

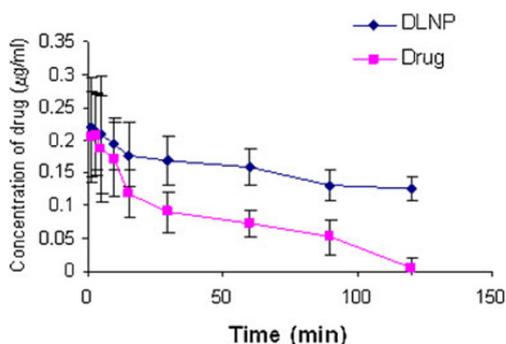


Fig. 4 Pharmacokinetic profile of Pax post-i.v. injection (20 mg/kg PPN) in Balb/c

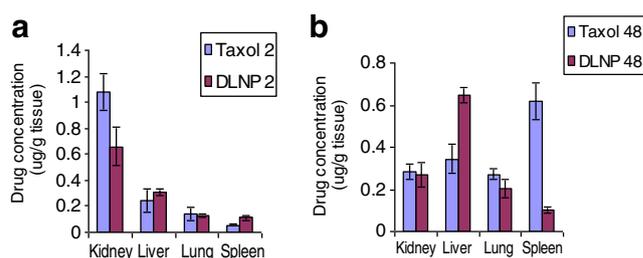


Fig. 5 Biodistribution profile of paclitaxel and PPN after a 2 h and 48 h of an i.v. infusion of 20 mg/kg in Balb/c mice. Mean \pm SD ($n=4$)

tissues (kidney, liver, lung, and spleen), each tissue was weighed and stored vial at -20 $^{\circ}$ C for further analysis. Frozen tissue samples were thawed for 45 min and homogenized.

2.3 Determination of paclitaxel

The drug was extracted from biological samples with *tert*-butyl methyl ether (Xu et al. 2005). The sample was mixed with the organic reagent at a 1:1 ratio on a vortex mixture. After centrifugation, the organic layer was transferred to a clean tube and evaporated. The residue was redissolved in ethanol and analyzed by HPLC.

3 Results and discussion

Pectin nanoparticles were prepared by ionotropic gelation of degraded pectin chains with calcium ions (Verma et al. 2011). The objective of this modification was to reduce the polarity of pectin by reducing the number of free hydroxyl groups. Pectin nanoparticles thus formed and assumed an oval shape with clear edges as can be clearly observed in TEM pictures.

The sizing of pectin nanoparticles was done by dynamic light scattering setup by Brookhaven 90 Plus Particle Size Analyzer using a 35-mW He-Ne laser source at a scattering angle of 90° . The zeta potential (ξ) value was of -32 mV.

The mean diameter (mean R_H) of the samples was in the range 350–500 nm with a polydispersity index of 0.3 (Fig. 1). ξ value of -32 mV at physiological pH was sufficient to keep the negatively charged particles under repelling effect thus bringing stability to the nanoparticle dispersion. Chains were cross-linked with Ca^{++} and folded into particulate shape in the presence of L-Asn (Fig 2), and the intrinsic viscosity $[\eta]$ that indicates the hydrodynamic volume of a polymer molecule was measured and found to be reduced more than six times (Verma et al. 2011). A reduced $[\eta]$ for nanoparticles as compared with that of pectin chains indicated that they can be administered at a much higher concentration in vivo. The nanoparticle solution was lyophilized to a white amorphous powder for further experiments.

We compared the cytotoxic effect of Pax per se, PPN, and pectin chains on Hep G2, hepatic carcinoma cell line. Dose-

dependent cytotoxicity was observed, wherein $\sim 21.7 \pm 3.2$ % cytotoxicity was observed by PPN (Fig. 3), but Pax per se showed $\sim 55.6 \pm 3.5$ % cytotoxicity in a 72-h assay. The slow release of the drug can be attributed to the cytotoxicity observed as a function of time. Pectin chains showed negligible toxicity even at a concentration of 5 mg/ml.

The plasma concentration–time curve of Pax and PPN at a dose of 20 mg/kg body weight of Balb/c mice has been shown in Fig. 4. A non-compartmental analysis of the pharmacokinetics graph indicates a biphasic pattern with initial rapid phase followed by a slower decay of Pax with the level falling beyond detection limit by 48 h. It was noticed that the blood profile remains much higher for PPN as compared to Pax per se.

Tissue biodistribution of Pax and PPN was also evaluated after an i.v. dose of 20 mg/kg PPN. Pax accumulates rapidly in various tissues with a maximum concentration found in the kidney. However, after 48 h, Pax accumulated in the lymphatic tissues, spleen, and liver. More than 90 % of Pax binds rapidly and extensively to plasma proteins (Bodmeier and Paeratakul 1991). On the contrary, the biodistribution was not altered as explicitly as with PPN at an early point of analysis, i.e., after 2 h, accumulation in kidney was markedly less. Forty-eight hours post-injection, tissue specificity changes (Fig. 5) from the difference in the rank order of concentration, i.e., liver > kidney > lung > spleen for the PPN and spleen > liver > kidney \geq lung for Pax per se. Higher accumulation of Pax in the spleen may be attributed to splenic filtration (Liu and Krishnan 1999).

4 Conclusion

The biomedical potential of the negatively charged pectin nanoparticles has been assessed. Paclitaxel was encapsulated in the aqueous nanoparticle dispersion with high efficiency. The in vivo studies reflected that the nanoparticles significantly delayed plasma clearance with detection of Pax possible up to 48 h. It was evident from the biodistribution studies that enhanced PPN accumulation was observed in the liver but a greater accumulation of Pax per se in the kidney. The reported pectin nanoparticles form a good candidate for passive delivery of paclitaxel to liver tissue and warrant further investigation to ascertain its efficacy to regress tumor implants in mice.

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