Evaluation of encapsulated catechin in chitosan-sodium tripolyphosphate nanoparticle

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Abstract
In present study assessment of suitable drug delivery system for catechin using polymer of chitosan and its in vitro antioxidant were studied. Chitosan-tripolyphosphate forms ionic cross linkage. Particle size analysis revealed blank chitosan nanoparticle of size 392 nm and catechin loaded chitosan nanoparticle of size 324.4 nm. Encapsulation efficiency 69.17% and drug loading capacity 43.46% was recorded. In vitro release study revealed 9.74% release in simulated gastric fluid (SGF) and 23.58% release of catechin in simulated intestinal fluid (SIF).

Keywords: Catechin, chitosan, nanoparticle, SGF, SIF

Introduction
Tea is an infusion of the leaves of the Camellia sinensis plant. First discovered in China, tea is grown in over 30 countries and is the most widely consumed beverage in the world, aside from water (Graham, 1992) [9]. Antioxidant properties of catechins are connected to the inhibition of free radical generation, free radical scavenging abilities, and metal ion chelating properties (Rice-Evans et al., 1996) [23]. Tea catechins exhibit antioxidant and neuroprotection activity, inhibit tumour angiogenesis, prevent atherosclerosis, prevention of autoimmune myocarditis (Suzuki et al., 2007) [27] and modulate cholesterol metabolism (Clement, 2009) [5]. However, catechins are highly unstable in alkaline solutions, such as those present in some biological fluids and has less bioavailability (Chen et al. 2001) [4]. The stability of catechin is influenced by pH, it is stable at pH < 4 and unstable at pH > 6 (Ananingsih et al., 2007) [9]. The polymeric nanoparticles (PNPs) are prepared from biocompatible and biodegradable polymers in size between 10-1000 nm where the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix (Tibbals, 2011) [28]. Depending upon the method of preparation nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed (Rao and Geckeler, 2011) [22]. Chitosan is a mucopolysaccharide closely related to cellulose. The primary unit in the chitin polymer is 2-deoxy-2- (acetyl amino) glucose. Chitosan is obtained by deacetylation of chitin. Chitin is the exoskeleton structure of arthropods such as crustaceans and insects. It was first described by Rouget in 1859 and in 1894 formally named by Hoppe-Seyler (Illum, 1998; Nagpal et al., 2010) [11, 20].

The naturally occurring chitosan polymers have a great potential in drug formulation due to their extensive application as food additives and their recognized lack of toxicity. As this group of polymers possesses a number of characteristics that render them useful as a formulation aid both as a conventional excipients and more specifically as a tool in polymeric-controlled drug delivery (Tonesen and Karlsen, 2002) [29]. Biodegradable nanoparticulate systems have received considerable attention as potential drug delivery vehicles (Kreuter, 1991) [15]. Considering the importance of drug delivery approach with nanoparticles the present study has been envisaged to assess suitable drug delivery system for catechin using polymer chitosan.

Material and method
Catechin, chitosan, sodium tripolyphosphate (Na-TPP) procured from Sigma Aldrich. 0.2% chitosan was dissolved in 50 ml of 0.35% acetic acid solution maintains pH 5.0 and stirred for 12 hours at 900 rpm. 0.2% Na-TPP was dissolve in distilled water after stirring 2 hours at 900 rpm. This Na-TPP solution was used as solvent for dissolving catechin. For preparation of blank chitosan nanoparticle, Na-TPP was added dropwise to chitosan solution while stirring on
magnetic stirrer and cured for one hour. After curing solution was centrifuged at 18000 rpm for one hour at 4 °C. Supernatant was discarded and sediment was freeze dried to obtain lyophilized product. For nanoencapsulation of catechin in chitosan polymer, 0.3% catechin was dissolved in Na-TPP solution by stirring at 900 rpm for one hour and added to chitosan solution dropwise and cured for one hour at 900 rpm. To get nanoparticle, solution was centrifuged at 18000 rpm for one hour maintaining at refrigerated condition of 4 °C. Supernatant was washed with deionized water and freeze dried to get lyophilized product of catechin encapsulated in chitosan nanoparticle (CCH-NP). The encapsulation efficiency of the nanoparticles was determined by analyzing the supernatant after removal of the nanoparticles by centrifugation. For the estimation of unbound catechin in the supernatant, the absorbance was measured spectrophotometrically at 425 nm and the amount of catechin hydrate was calculated from calibration curve of known concentrations of catechin hydrate. The amount of the percent encapsulation was calculated as under:

Encapsulation Efficiency (%) = \{(Total drug added - Unbound drug)/Total drug\} x 100C

Drug loading of the desired nanoparticles determined by the following formula

%DL = (Practical drug loading/theoretical drug loading) x 100

The release of catechin encapsulated chitosan nanosphere was investigated as per the method of Lee et al., 2009 [17] in two simulated digestive fluids: 0.05M aqueous sodium chloride solution adjusted to pH 1.5 with 1M HCl as simulated gastric fluid SGF and 0.05M sodium dihydrogen phosphate buffer solution adjusted to pH 6.8 with 1MNaOH as simulated intestinal fluid SIF. Accurately weighed amount of the lyophilized powder around 10 mg were placed in previously soaked dialysis bag (MWCO 12-14 kDa) in a beaker containing 20ml of enzyme free SGF and SIF separately and incubated at 37 °C. The drug released in simulated fluids was sampled predetermined time interval. The concentration of catechin in the simulated fluid was determined spectrophotometrically.

**Result and discussion**

For preparing polymeric nanoparticle of catechin hydrate 0.2% w/v chitosan, 0.2% w/v Na-TPP and 0.3% w/v catechin was used (Fig. 1). Blank chitosan nanoparticle without adding drug stirred at 900 rpm and subsequently analysed in particle size analyser (Malvern Instruments Ltd, Zetasizer Ver. 7.11) which revealed average particle size in 392nm range with 0.556 PDI (Fig. 2). The particle size analysis of chitosan coated catechin nanoparticle prepared with 900 rpm stirring speed and without incorporation of sonication which revealed 100% of particle population in the range of 324.4 nm (Fig. 3).

The encapsulation efficiency was determined by analyzing the supernatant after removal of the nanoparticles by centrifugation. For the estimation of unbound catechin hydrate in the supernatant, the absorbance was measured spectrophotometrically at 425 nm and the amount of catechin hydrate was calculated from calibration curve of known concentration of catechin hydrate (y=0.039x-0.014, R²=0.998). The encapsulation efficiency of prepared catechin encapsulated in chitosan nanoparticles were calculated to be 69.17%. The drug loading percentage of the obtained catechin encapsulated in chitosan nanoparticles through calculation was 43.46%.

There was very slow release (9.74%) of catechin hydrate in SGF even up to 48hrs of study. The critical analysis of result revealed constant slow release of catechin hydrate in the SGF with a maximum of 9.74% release by 96hrs of the coated catechin depicting better stability, decreased bioavailability and biodegradability in the stimulated gastric fluid. In SIF revealed property of slow release till 2nd hr of experiment followed by constant increasing release of the drug catechin up to the level of 23.58% by 96hrs of experiment.

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**Fig 1: Particle size analysis of blank chitosan nanoparticle**
Fig 2: Particle size analysis of catechin encapsulated in chitosan nanoparticle
Fig 3: Release pattern in in vitro release study

a) In-vitro drug release in simulated digestive fluids noted at 1hr
b) At 24hrs
c) At 72hrs
d) Minimum release noted in SGF
e) Consistent increased release noted in SIF

Fig 4: In vitro release of catechin hydrate from CCH-NPs in simulated fluids

Nanoparticle colloidal system using a non-toxic biopolymer material is expected to be able to protect functional properties of certain bioactive compound like catechin (Kallakua et al., 2010). Chitosan as hydrophilic polymer can easily cross-link with counter poly anions like sodium tripolyphosphate (Na-TPP) to control the release of the drug (Dudhani and Kosaraju, 2010). Chitosan is cationic polysachharide, mucoadhesive, biodegradable and biocompatible polymer which has been shown to enhance the penetration of peptide and protein across the intestinal mucosa (Illum et al., 1994). Physicochemical properties of the nanoparticles such as size, morphology and physical state will affect the functional performance of nanoparticles based delivery systems (Ahson et al. 2002; Galindo-Rodriguez et al., 2004 and Hector et al., 2012). Song et al. 2008 synthesized catechin nanoparticle using PLGA mean size up to 200 nm. Dube et al., 2010 prepared chitosan tripolyphosphate nanoparticles had a mean size of less than 200 nm. The encapsulation efficiency of prepared CCH-NP was calculated to be 64.17% which is in agreement with Jithin (2017) wherein encapsulation efficiency of 61.1±5% obtained in catechin loaded alginate nanoparticles. Hu et al. reported encapsulation efficiency of tea catechin in chitosan Na-TPP nanoparticles with a range between 24 to 53%. Quercetin- PLGA nanoparticles encapsulation efficiency of ≥ 95% was obtained by Kumari et al. (2010). The encapsulation efficiency of prepared pectin coated catechin nanoparticle was calculated to be 80.16% (Lekshman, 2017). The drug loading percentage of the obtained CCH-NPs through calculation was 43.46%. A similar drug loading 32.7% of alginate coated catechin nanoparticle was obtained by Jithin (2017). Drug loading percentage of 88±4% was obtained by Dudhani and Kosaraju (2010) from their catechin loaded chitosan nanoparticles. The loading capacity of Mesobuthus eupeus venom loaded chitosan nanoparticles was 76.3% (Mohammadpour et al. 2012) Samanta et al. (2016) who reported less than 40% release of catechin hydrate during 100 hrs from catechin loaded PLGA nanoparticles during in vitro release study. Behl et al. (2013) observed a peak release of gallic acid from nanogel after 20 hour period in alkaline pH. Sankar et al. (2014) found a release of curcumin from PLGA nanoparticles for 72 hours in alkaline pH. Nallamuthu et al. (2015) reported consistent release of 69 % release of chlorogenic acid (CGA), a phenolic compound from...
chlorogenic acid loaded chitosan nanoparticles for 100hrs release period. Sustained release of nanocapsulated drugs up to 3 days has proved its efficacy for better bioavailability and thus established its role as a suitable drug delivery vehicle (Samanta et al., 2016). [30] Catechin loaded chitosan showed slow release in the simulated intestinal fluid (SIF) up to 4 h and continued sustained release 32% up to 24 h (Dudhani and Kosaraju, 2010) [7].

**Conclusion**

Nanoencapsulated catechin at 0.2% concentration of chitosan, 0.2% Na-TTP concentration and 0.3% catechin concentration revealed size of 324.4 nm with 69.17% encapsulation and 43.46% drug loading capacity. In simulated gastric fluid encapsulated catechin nanoparticle depicted better stability and in simulated intestinal fluid depicted better stability and bioavailability.

**References**