Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(5): 335-339
Received: 04-07-2019
Accepted: 06-08-2019

Sonam Bhatt
Ph.D. Scholar, Division of Medicine, IVRI, Izatnagar, Uttar Pradesh, India

Ravi Shankar Kumar Mandal
Ph.D. Scholar, Division of Medicine, IVRI, Izatnagar, Uttar Pradesh, India

Jithin MV
Assistant Professor, Department of Veterinary Medicine, SVPUAT, Meerut, Uttar Pradesh, India

Narayani Yadav
M.V.Sc. Scholar, Division of Medicine, IVRI, Izatnagar, Uttar Pradesh, India

Raguvaran R
Scientist, Division of Medicine, IVRI, Izatnagar, Uttar Pradesh, India

DB Mondal
Principal Scientist, Division of Medicine, IVRI, Izatnagar, Uttar Pradesh, India

Correspondence
Sonam Bhatt
Ph.D. Scholar, Division of Medicine, IVRI, Izatnagar, Uttar Pradesh, India

Preparation and characterization of alginate polymer coated catechin nanoparticle

Sonam Bhatt, Ravi Shankar Kumar Mandal, Jithin MV, Narayani Yadav, Raguvaran R and DB Mondal

Abstract
In present study nano drug was prepared by using catechin and its characterization was done. 0.5% sodium alginate was used as delivery system for the preparation of the polymeric nanoparticles of catechin hydrate. Catechins are predominant form of flavanols present in plants and have attracted particular attention due to their relatively high antioxidant capacity in biological systems ionic cross linkage. Size of the prepared drug was analysed by using zeta sizer and its encapsulation efficiency and drug loading capacity were recorded.

Keywords: Catechin, alginate, zeta sizer, nanoparticle

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) [10]. 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) [24] 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) [3]. The stability of catechin is influenced by pH, it is stable at pH <4 and unstable at pH > 6 (Ananingsih *et al.,* 2013) [2]
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) [25]. Alginates are random anionic, linear, polymers consisting of varying ratio of glucuronic (G) and manuronic (M) acid unit. Salts of alginate are formed when metal ion react with glucuronic or manuronic acid residue (Rao and Geckeler, 2011) [21]. Alginate has been used in many biomedical applications, including drug delivery systems, as they are biodegradable, biocompatible and mucoadhesive. Alginates with a high glucuronic acid contents form more rigid, porous gel due to their orientation within the egg- box structure and conversely gel with low glucuronic content are more randomly packed and less porous.
Alginates are widely used in the pharmaceutical, cosmetic, and food industry (Espevik and Skja k-Bræk, 1996) [8]. This polymer is also accepted by European Pharmacopoeia. Compounds used as excipients will often find more than one application, and that is also the case for alginic acid and its salts. Their application generally depends on the thickening, gel-forming, and stabilizing properties. Transmittancy, swelling, and visco-elasticity of alginate gel membranes are highly affected by the M/G ratio (Draget *et al.*, 1996; Inukai *et al.*, 1999) [7, 12]. The calcium alginate gels are the most extensively studied. The ability of alginate to form two types of gel dependent on pH, i.e., an acid gel and an ionotropic gel, gives the polymer unique properties compared to neutral macromolecules. The physicochemical properties of the polymer system and the swelling process to activate the release of drugs will be dependent on the type of gel formed. Alginic acid and its sodium and calcium salts are regarded as generally non-toxic and biocompatible (King, 1983) [13]. Biodegradable nanoparticulate systems have received considerable attention as potential drug delivery vehicles (Kreuter, 1991) [14].
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 aliginate.

Materials and Methods

Catechin loaded alginate polymeric nanoparticles was prepared by the facile co-precipitation method of Wu *et al*. (2014) [26] was followed for preparation of the catechin-alginate polymeric nanoparticles with minor modification. Sodium alginate 0.5% prepared in deionized water at 30°C in which 10 ml of CaCl2 (0.02 M) was added slowly and stirred under 1200 rpm for 30 minutes followed by 10 ml of Na2CO3 (0.02 M) added into the mixture dropwise and stirred under 1200 rpm for 3 hours. The mixture was centrifuged at 18000 rpm for 20 minutes and washed by deionized water for several times, and freeze-dried to obtained alginate-CaCO3 hybrid nanoparticles. To prepare catechin hydrate loaded hybrid nanoparticle (CH-NPS), 50 mg catechin hydrate was dissolved in 10 ml deionized water, and then 10 mg of freeze dried nanoparticles was added in the solution and stirred for 12 h at 600 rpm. The catechin hydrate loaded nanoparticles were collected by centrifugation at 18000 rpm for 20 minutes, washed several times with deionized water, and freeze-dried. Different concentration of alginate (0.5 to 1%) with different curing time (1 to 3 hour) and incorporation of sonication (1 to 5 micron amplitude) was standardized during the course of study.

The size of the nanoparticles was determined by particle size analyzer (Microtrac Nanotrac Wave II, USA. 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 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 concentrations of catechin hydrate. The amount of the percent encapsulation was calculated as under:

Encapsulation Efficiency (%) = {(Total drug added – Unbound drug) / Total drug} ×100

Drug loading (%)

Drug loading of the nanoparticles was calculated as under:

Drug Loading (%) = {(Total drug added – Unbound drug) / Total nanoparticle weight} ×100

Result and Discussions

0.5% sodium alginate was used as delivery system for the preparation of the polymeric nanoparticles of catechin hydrate. The particle size analysis with 300 rpm stirring speed and without incorporation of sonication revealed 100% of particle population in the range of 1899 nm (Fig 1). Increasing stirring speed to 1200 rpm using 0.5%sodium alginate concentration provided 77.1% particles in 1968 nm and 22.9% population in 713nm (Fig 2). With the incorporation of sonication (5 micron amplitude) keeping same alginate concentration showed 46% particle population in 1983nm, 27.5% population in 374nm and 26.5% in 126nm (Fig 3). Development of alginate based drug delivery systems makes use of the ability of this polymer to undergo gelation in presence of divalent cations. However, a critical adjustment in the relative concentrations of alginate and the cation results in a pre-gel state (Ahmed *et al*., 2006). In the present study CH-NPs of desired morphology and size could also be obtained when a concentration of 0.5% alginate solution was used. Wu *et al.* (2014) [26] also highlighted that most important parameter that affects the particle size and size distribution is the concentration of sodium alginate for preparation of the nanoparticles and affirmed that on addition of $CaCl_2$ solution to aqueous solution of alginate, calcium ions were coordinated with G units in alginate chains. When Na_2CO_3 was added, Ca^{2+} ions tended to deprive the coordinated Ca^{2+} ions to form $CaCO_3$. However, alginate chains prevented the precipitation of CaCO3. To segregate the alginate $CaCO_3$ catechin particles, curing time and sonication is of prime importance in structurisation of polymeric nanoparticles in the present study. Wu *et al.* (2014) [26] also stated that factors like stirring speed may affect the particles size and size distribution and and size distribution and are not so critical however in our study we did find the importance of stirring speed and sonication important in providing particle of desired size. Functional performance of nanoparticles based delivery systems depends on the physicochemical properties of the nanoparticles, such as size, morphology and physical state (Ahsan *et al*., 2002; Galindo Rodriguez *et al*., 2004 and Hector *et al*., 2012) [1, 9, 11]. Olieveira *et al*. (2014) obtained the particle size in the range 223-399nm on using alginate/cashew gum nanoparticles for encapsulation of an essential oil. The encapsulation efficiency of prepared CH-NPs was calculated to be 61.1%. The finding was in agreement with Wu *et al*. (2014) [26] wherein encapsulation efficiency ranging from 24.6% to 87.7% was calculated in nanoparticles using alginate polymer. The use of chitosanalginate as encapsulation polymer by Motwani *et al.* (2008) [19] resulted in obtaining nanoparticles with encapsulation efficiency between 61% and 82%. Chopra *et al*. (2015) [4] obtained an encapsulation efficiency ranging from 68% to 98% keeping constant sodium alginate concentration and varying the cross linker and secondary polymer. Encapsulation efficiency of zinc oxide nanoparticles loaded into sodium alginate-gum acacia of 95.56% was obtained by Raguvaran *et al.* (2017a) [22]. The encapsulation efficiency of prepared pectin coated catechin nanoparticle was calculated to be 80.16% (Lekshman, 2017) [15]. The drug loading percentage of the obtained catechin nanodrug was 32.7%. Similar findings were also observed by Wu *et al*. (2014) [26] wherein 10 to 94% was the range of drug loading obtained from their prepared nanoparticles using alginate polymer. Liang *et al*. (2013) [16] reported drug loading in the range of 1.4 to 1.9%. Manuja (2014) [17] reported a drug loading percentage of 3.70% on using sodium alginate as polymer for encapsulating quinapyramine sulphate. A drug loading of 4.978% was obtained using polymer sodium alginate gum acacia by Raguvaran *et al*. (2017a) [22] for zinc oxide nanoparticles. Dudhani and Kosaraju (2010) [6] reported 88±4% of drug loading capacity of catechin loaded chitosan nanoparticles. The loading capacity of Mesobuthus eupeus venom-loaded chitosan nanoparticles was 76.3% (Mohammadpour *et al*. 2012) [18].

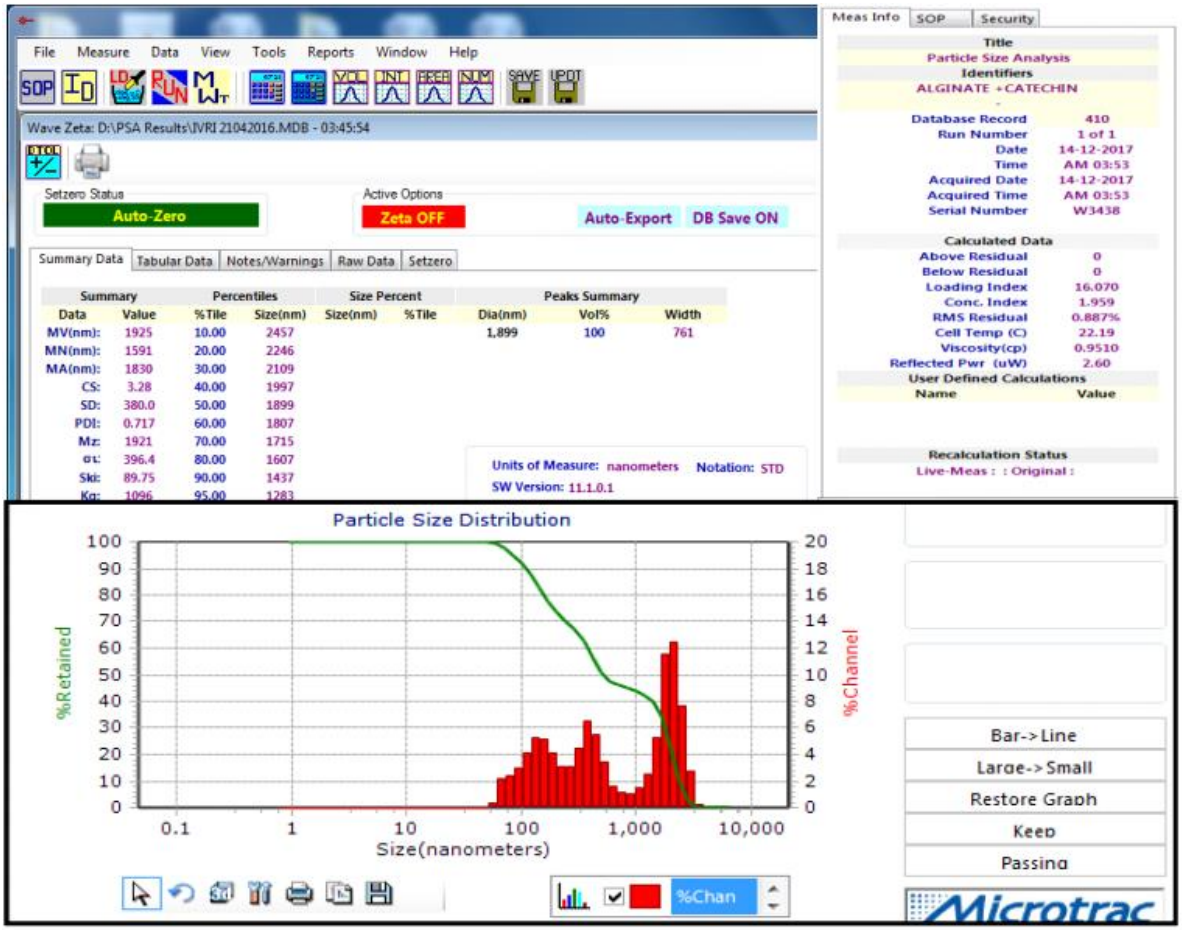

Fig 1: Particle size analysis of alginate loaded catechin nanoparticles by zeta analyzer without curing time

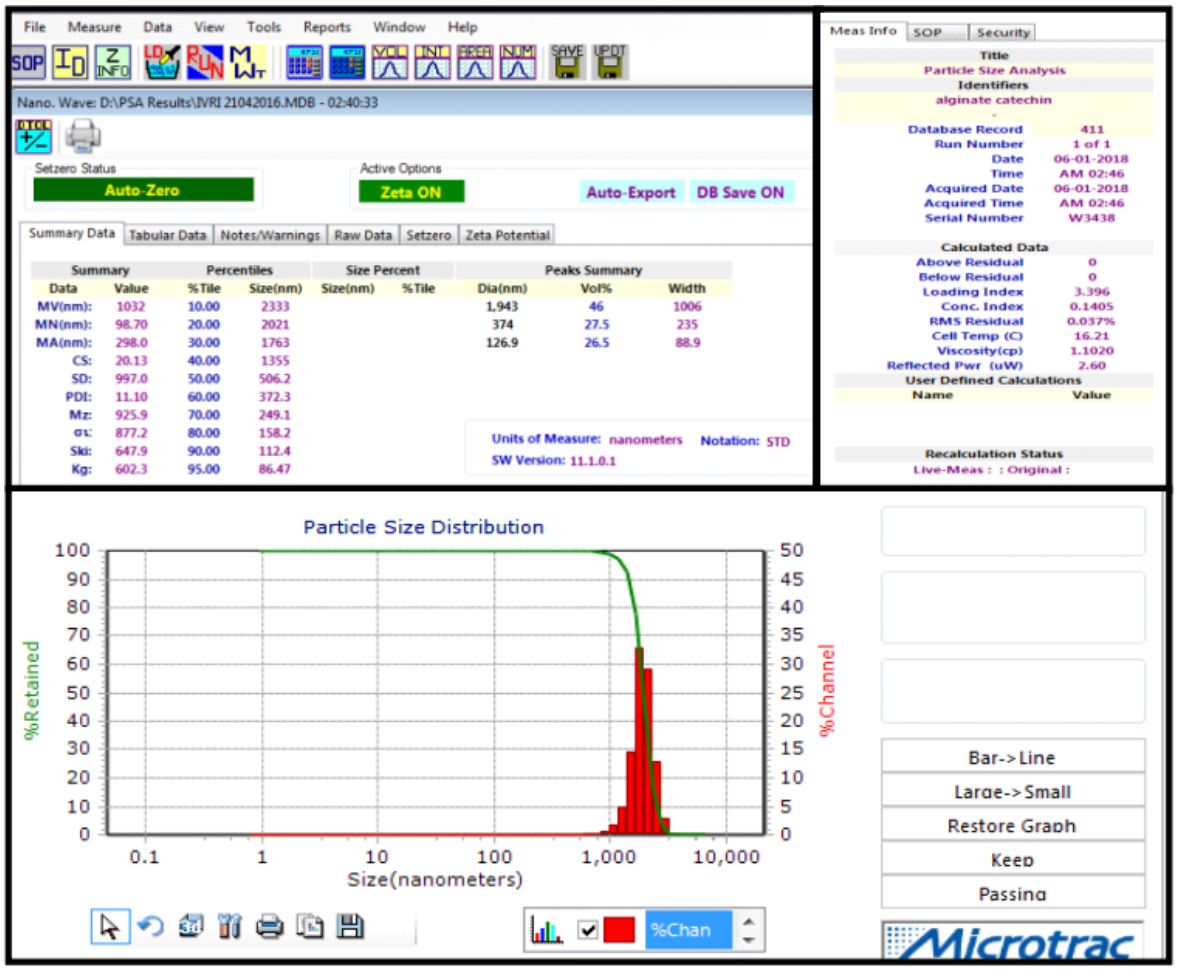

Fig 2: Particle size analysis of alginate loaded catechin nanoparticles with increased stirring speed (1200rpm) & curing time (3 hours)

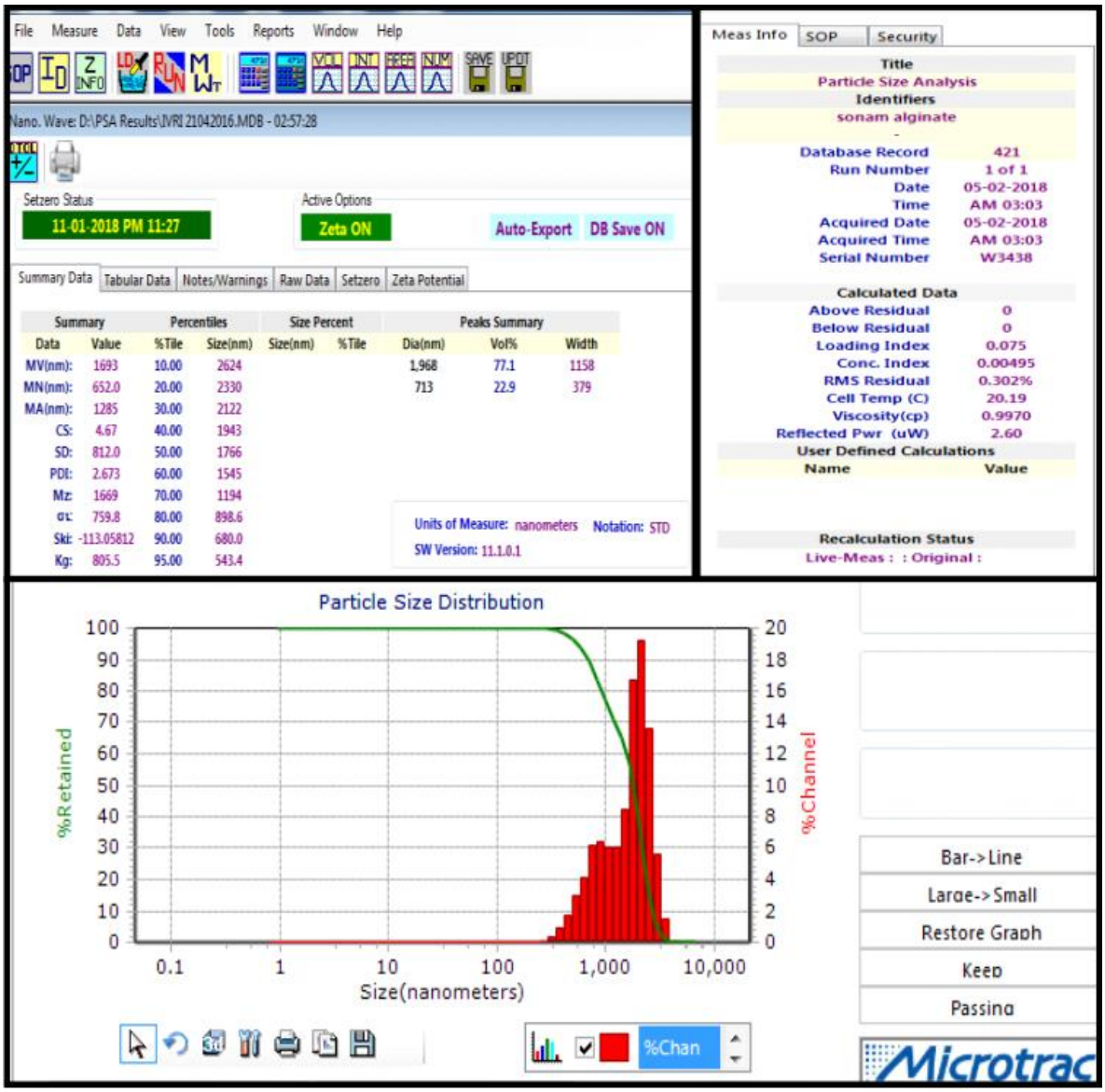

Fig 3: Particle size analysis of catechin nanoparticles after sonication (5micron amplitude) and increase curing time

Conclusion

Particle size analysis of catechin loaded polymer with 0.5% sodium alginate after inclusion of incorporation of both stirring speed (1200 rpm) and sonication (5 micron amplitude) revealed 46% particle population in 1983nm, 27.5% population in 374nm and 26.5% in 126 nm. Encapsulation efficiency of prepared catechin hydrate nanoparticles revealed 61.1% with 32.7% drug loading capacity.

References

1. Ahsan F, Rivas IP, Khan MA, Torres Su´arez AI. Targeting to macrophages: role of physicochemical properties of particulate carriers liposomes and microspheres on the phagocytosis by macrophages. J Cont. Rel. 2002; 79(1-3):29-40.
2. Ananingsih VK, Sharma A, Zhou W. Green tea catechins during food processing and storage: A review on stability and detection. Food Research International. 2013; 50:469-479.
3. Chen Z, Zhu QY, Tsang D, Huang Y. Degradation of green tea catechins in tea drinks. Journal of Agricultural and Food Chemistry. 2001; 49:477-482.
4. Chopra M, Manju B, Pawan K, Anju M, Balvinder K, Rajesh T. Alginate/gum acacia bipolymeric nanohydrogels-Promising carrier for Zinc oxide nanoparticles. Int. J Biol. Macromolecules. 2015; 72:827-833.
5. Clement Y. Can green tea do that literature review of the clinical evidence. Preventive Medicinr. 2009; 49:83-87.
6. Dudhani AR, Kosaraju SL. Bioadhesive chitosan nanoparticles: Preparation and characterization. Carbohydrate Polymers. 2010; 81:243.
7. Draget KI, Skjak-Bræk G, Christiansen BE, Gasrød O, Smidsrød O. Alginates from Algae Carbohydr. Polym. 1996; 29:209-215.
8. Espevik T, Skjak-Bræk G. Genetics and biosynthesis of alginates. Carbohydr. Eur. 1996; 14:19-25.
9. Galindo-Rodriguez S, All´emann E, Fessi H, Doelker E. Physicochemical parameters associated with nanoparticles formation in the salting-out, emulsification diffusion, and nano precipitation methods. Pharma. Res. 2004; 21(8):1428-1439.
10. Graham HN. Green tea composition, consumption, and polyphenol chemistry, Prev. Med. 1992; 21:334-350.
11. Hector P, David Q, Juan de Dios F, Camila MM, Etelvino HJ, Bechara Luis AG *et al*. Antioxidant Effects of Quercetin and Catechin Encapsulated into PLGA Nanoparticles. J of Nanomaterials, 2012. doi:10.1155/2012/145380.

12. Inukai M, Yonese M. Effects of Charge Density on Drug Permeability through Alginate Gel Membranes. Chem. Pharm. Bull. 1999; 47:1059-1063.
13. King AH. In Food Hydrocolloids; Glicksman, M., Ed.; CRC Press: Boca Raton, FL, 1983; II:115-154.
14. Kreuter J. Liposomes and nanoparticles as vehicles for antibiotics. Infection. 1991; 19(4):224-228.
15. Lekshman A. Evaluation of pectin coated catechin to produce polymeric nanoparticle and its hepatoprotective effect. Thesis, M.V.Sc., Demmed University, Indian Veterinary Research Institute, Izatnagar, India, 2017.
16. Liang HF, Yang TF, Huang CT, Chen MC, Sung HW. Preparation of nanoparticles composed of poly (gamma-glutamic acid)-poly(lactide) block copolymers and evaluation of their uptake by HepG2 cells. J Control Release. 2005; 105:213-225.
17. Manuja A, Sandeep K, Neeraj D, Gaurav B, Meenu C, Harmanmeet K *et al*. Quinapyramine sulfate-loaded sodium alginate nanoparticles show enhanced trypanocidal activity. Nanomedicine. 2014; 9(11):1625-1634.
18. Mohammadpour DN, Eskandari R, Avadi MR, Zolfagharian H, Mir M, Sadeghi A *et al*. Preparation and *in vitro* characterization of chitosan nanoparticles containing *Mesobuthus eupeus* scorpion venom as an antigen delivery system. Journal of Venomous Animals and Toxins including Tropical Diseases. 2012; 18(1):44-52.
19. Motwani SK, Shruti C, Sushma T, Kanchan K, Farhan JA, Roop KK. Chitosan–sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: Formulation, optimization and *in vitro* characterization. E. J Pharm. Biopharma. 2008; 68:513-525.
20. De Oliveira EF, Paula HC, de Paula RC. Alginate/cashew gum nanoparticles for essential oil encapsulation. Colloids and Surfaces B: Biointerfaces, 2014; 113:146-151.
21. Rao JP, Geckeler KE. Polymer nanoparticles: preparation techniques and sizecontrol parameters. Prog. Poly. Sci. 2011; 36(7):887-913
22. Raguvaran R, Anju M, Balvinder KM, Riyesha T, Sandeep S, Kesavan M *et al*. Sodium alginate and gum acacia hydrogels of zinc oxide nanoparticles reduce hemolytic and oxidative stress inflicted by zinc oxide nanoparticles on mammalian cells. Int. J Biol. Macromolecules. 2017a; 101:967-972.
23. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free radic Biol. Med. 1996; 20(7):933-956.
24. Suzuki JI, Ogawa M, Futamatsu H, Kosuge H, Sagesaka, YM, Isobe M. Tea catechins improve left ventricular dysfunction, suppress myocardial inflammation and fibrosis, and alter cytokine expression in rat autoimmune myocarditis. Eur. J heart Fail. 2007; 9(2):152-159.
25. Tibbals HF. Medical Nanotechnology and Nanomedicine- Crc Press, 2011, 75-116
26. Wu JL, Chao QW, Ren XZ, Si XC. Multi-drug delivery system based on alginate/calcium carbonatehybrid nanoparticles for combination chemotherapy. Coll. and Surf. B: Biointerfaces. 2014; 123:498-505.