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Synthesis, characterization and evaluation of antioxidant properties of catechin hydrate nanoparticles

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Abstract

Context: Catechin hydrate (CH), is an important phyto compound, reported to have potential therapeutic activity for prevention and treatment of various central nervous system (CNS) disorders. However, its therapeutic action is limited by their low oral bioavailability, poor stability and intestinal absorption, therefore, development of a targeted nanoparticle based carrier system which can overcome its physicochemical limitations and can enhance its biological activity is required. The objective of the present study was to formulate nanoparticle based formulation by ionic gelation method for catechin hydrate. Result and conclusion: After optimising the formulation by statistical tool, further, characterization results showed zeta average particle size of 68.76 ± 1.72 nm along with polydispersibility index of 0.174 ± 0.81 and zeta potential of -5.32 mV. Moreover, TEM analysis also confirmed its nanometric size range (range of 61. 8- 128nm) and FT – IR scan showed no bond formation between polymers and loaded extract (CH). The *in vitro* compound release kinetics showed a typical linear diffusion profile and cytotoxicity analysis done on NB41A3 cell lines results exhibited the cell viability of 89.5 ± 0.25% in catechin loaded nanoparticles (CH NP's) whereas, it is 82.7 ± 0.34% in CH indicating negligible toxicity in nanoparticle based formulation. The stability testing was done for CH NP's after 8 weeks, and results revealed minimal degradation of catechin. Lastly, the antioxidant activities estimated through DPPH (2, 2 – Diphenyl-1-picrylhydrazyl-hydrate), Nitric oxide (NO) and Hydrogen peroxide (H_2O_2) scavenging assays revealed that CH NP's have higher and prolonged antioxidant activity in comparison with CH.

Keywords: Ionic gelation; encapsulation efficiency; statistical modelling; release kinetics; antioxidant activity.

Introduction

Phytopharmaceuticals have recently become an area of great interest owing to their diverse applications. Medicinal plants are the richest resource of bioactive compounds that have a broad application in modern medicines and chemical entities for synthetic drugs [1]. These bioactive compounds have been known to relieve various diseases and hence, in recent decades more emphasis is given on evaluation and characterization of various plants and its constituents for their potential therapeutic role [2]. Catechin hydrate (CH), one such important natural flavonoid derived as plants secondary metabolite has gained considerable attention due to its potential therapeutic activity like antioxidative and anti-inflammatory properties, apart from having its immense role in prevention and treatment of diseases caused due to oxidative damage. It also exhibits certain potential biological effects including cardio protective, neuroprotective and anti-cancer effects [3].

But the therapeutic efficiency of CH depends on its bioavailability and stability via oral route of administration and it is highly prone to lose its potency due to low solubility, degradation at different pH of gastrointestinal tract and enzymatic activity, poor intestinal absorption, , instability in highly acidic pH and excretion etc.[4]. Consequently, there is a need of formulating CH so as to surpass the mentioned problems of reduced absorption and rapid metabolism. Therefore, developing a novel drug delivery system for CH can help in enhancing the shelf life, improved absorption and minimal degradation [5].

Nanoparticle formulation can be of potential use as this carrier system[6] has an advantage such as stability, protects drug against chemical and enzymatic degradation, sustained drug release at targeted site and easy penetration into small capillaries [7], thus crossing the biological barriers easily. In the present study attempt was made to prepare CH loaded nanoparticles by ionic gelation method, where cross linkage was formed between two ionic

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polymers of opposite charge (anionic and cationic) bound to each other by ionic bond. The polymers used were chitosan and tri-poly-phosphate [8]as they were biocompatible, biodegradable, non-immunogenic, non-toxic and water soluble [9]. This formulation can enhance the bioavailability and stability of CH.

Material and Methods

Catechin hydrate and dialysis membrane (9777) of cut size 12000 Daltons were purchased from Sigma Aldrich, USA. Chitosan, tri polyphosphate, monosodium phosphate anhydrous, disodium phosphate anhydrous were obtained from Himedia, Mumbai, India. Phosphoric acid, hydrogen peroxide and all other chemicals used in experiments were of analytical grade.

Preparation of Chitosan Nanoparticles by Ionic Gelation Method

Catechin loaded chitosan nanoparticles (CH NP's) [10] were prepared by ionic gelation method, where chitosan solution (CS) (1 - 2mg/ml) and catechin hydrate (1 – 2.5 mg/ml) of different concentrations were prepared by dissolving it in glacial acetic acid (AA) (1 – 5 % (v/v)) and stirred for overnight, continuously to obtain a clear chitosan solution[11]. Sodium tripolyphosphate (TPP) solution (1 - 2% w/v) was prepared in distilled water and was added drop wise with a syringe, to the chitosan solution (Figure 1) at constant stirring for 40 minutes, this was added with sonication of the same sample for 15minutes. This solution was then centrifuged 20,000 g for 30 minutes and after discarding supernatant, pellet was again dispersed in distilled water. This washing step was further repeated twice and then nanoparticles were lyophilised and stored at 4 C for analysis.

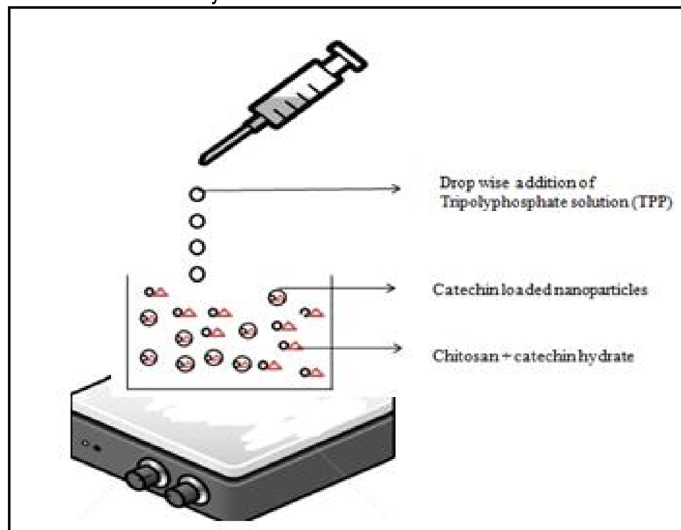


Figure 1: Schematic diagram representing chitosan loaded nanoparticles preparation by ionic gelation method.

Abbreviations: CH (Catechin Hydrate), CH NP's (Catechin loaded chitosan nanoparticles), TPP (Tri polyphosphate), CS (Catechin Solution)

Optimization of prepared nanoparticles

The optimization of different process parameters like – chitosan to tri polyphosphate weight ratio, acetic acid concentration, catechin hydrate concentration was done by mathematical modelling using Design-Expert® software with two level full factorial design[12]. Variations in chitosan to tri-poly phosphate weight ratio and acetic acid concentration during nanoparticle formation were evaluated for their effects on entrapment efficiency (EE) of drug.

The EE was determined by separating the nanoparticles from the aqueous medium containing free catechin [13]by centrifugation at 10,285g, at 25 C for 40 min. The amount of free catechin in supernatant was quantified by measuring absorbance at 280 nm using Shimadzu UV spectrophotometer. The EE was calculated using the following equation:

$$EE (\%) = (DL_{NP} - DL_{SUP}) / DP_{NP}$$

Where, EE is entrapment efficiency, DL_{NP} is the total amount of drug loaded, DL_{SUP} is the free drug in supernatant.

Characterization of optimised nanoparticles

Particle size (PS) and zeta potential (ZP) analysis

The size measurements using dynamic light scattering (DLS) on Malvern Zetasizer (Nano ZS) was done. Dynamic light scattering (also known as PCS - photon correlation spectroscopy) measures brownian motion and relates this to the size of the particles (PS) by illuminating the particles with a laser and analysing the intensity fluctuations in the scattered light. Whereas, the zeta potential (ZP) describes the surface charge developed over the particle, dispersed in a liquid. It is the magnitude index of the electrostatic repulsive interaction between particles. Particle's ZP was done to predict dispersion stability [14] of the nanoparticles. The samples were diluted (1:100), filtered (syringe filter membrane -0.40μm) and then subjected for PS and ZP analysis by zeta sizer.

Transmission Electron Microscopy (TEM)

TEM analysis (Morgagni 268D, AIRF, JNU, Delhi) was done to reconfirm the size range and morphology of the optimised nanoparticles (A1). The optimized formulation was diluted 50 times by 5% acetic acid and sonicated for 15 minutes [15]. Then sonicated sample was fixed on carbon-coated copper grid (300 meshes). The images of representative areas were taken at suitable magnifications (200 and 500 nm).

Fourier transforms infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (IR-810, JASCO, Tokyo in SAIF, Punjab University) measurement was done to investigate the bonding interactions in catechin nanoparticles (CH NP's). It is used to obtain an infrared spectrum of absorption, emission, and photoconductivity of a solid, liquid or gas. Also, it can be utilized to do the quantifying analysis of an unknown mixture and assumes the intensities of the peaks are directly related to the amount of sample present[16]. Potassium bromide (KBr) disc method was used to prepare the samples and scanned for absorbance from the range of 400-4000 cm^{-1}.

Stability studies

The optimised nanoparticles were divided in to 3 batches. Each of them were kept in small air tight glass vials and stored at different temperatures - 4 C, room temperature (37 C) and 45 C. The CH content was estimated from each batch at different time interval (1 – 8 weeks) at 280nm by UV – vis spectrophotometer.

Physicochemical measurement

Different physico-chemical parameters like pH, conductivity, viscosity and density of optimised nanoparticles were measured. pH and conductivity of the samples were measured using pH meter (Thermo Orion 420A+). Specific density was calculated using pycnometer i.e., specific gravity bottle (Borosil) and viscosity was estimated by viscometer (LVDV, Brookfield Inc., USA)[17].

Evaluating free radical scavenging and antioxidant properties of optimised catechin hydrate nanoparticles

DPPH assay

DPPH (2, 2 – Diphenyl-1-picrylhydrazyl-hydrate) is a free radical, which when scavenged by antioxidant molecule, produces colourless ethanol solution, which is detected spectrophotometrically. Catechin (2mg/ml) and catechin loaded nanoparticles (A1) were taken and ethanol solution of DPPH radical (0.1mM) was added. Vigorous vortexing was done [18].The reaction mixture was incubated in dark at room temperature for 30 minutes and the absorbance was recorded at 517nm on a micro plate reader (Thermo, Varioskan Flash). The ability to inhibit DPPH radical is expressed as:

% Scavenging = $[1 – (A_{CH} / A_{AC})]$ 100.

Where, A_{CH} is the optical density of the Catechin hydrate, A_{AC} is the absorbance of positive control (Ascorbic acid).

Nitric oxide scavenging assay

Sodium nitroprusside is converted into NO at physiological pH 7.2, which in coming contact with oxygen produces nitrates and nitrites [19]. These are quantified by the griess reagent. Compounds which have antioxidant property scavenge nitric oxide by competing with oxygen and results in decrease in production of nitrite ions. The CH (2mg/ml) and catechin loaded nanoparticles (A1) were taken; sodium nitroprusside (5mM) prepared in phosphate buffer was added. The reaction mixture was incubated at room temperature for 30 minutes and griess reagent was added. Then, the absorbance was recorded at 530nm on a micro plate reader. The ability to inhibit NO radical is expressed as

% Inhibition= $[1 – (A_{TS}/A_{AC})]$ 100.

Where, A_{TS} is the optical density of the test sample, $A_{AC}$is the absorbance of positive control.

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a free radical which is normally produced under stressful conditions and when oxidized by an antioxidant compound, absorbance gets reduced, which is detected spectrophotometrically. Hydrogen peroxide solution (20 mM) was prepared in 50 mM phosphate buffer (pH 7.4). 100 ul catechin and catechin loaded NPs (2mg/ml) were transferred into the test tubes and their volumes were made up to 400 ul with 50 mM phosphate buffer. 600 ul hydrogen peroxide solution was added [20] followed by vortexing. Absorbance was determined at 230 nm after 10 min, on a micro plate reader. The ability to scavenge the hydrogen peroxide is expressed as

% Inhibition= $[1 – (A_S/A_{AC})]$ 100.

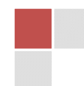
Where, AS is the optical density of the test sample, $A_{AC}$is the absorbance of positive control.

In vitro compound (catechin hydrate) release kinetics

The release kinetic study was primarily done to find out the permeation of required concentration of drug/component being permeated. This *in vitro* permeation study was carried out by using Franz diffusion cell with activated dialysis membrane (Sigma 9777), which was mounted between cell compartments (receptor and donor compartment) held together with a clamp. The receptor compartment was completely filled with 10 ml distilled water [21]. Optimised Catechin nanoparticle formulation (A1) was filled in donor compartment. The receptor solution was continuously stirred for 12 hours at room temperature. Samples were collected every half an hour time from the sampling port [22] and fresh solution was added to maintain the equilibrated condition. Absorbance was taken at 280 nm for all the samples.

Cytotoxicity analysis of formulated nanoparticles

The *in vitro* cytotoxic effect of catechin hydrate nanoparticles was evaluated on NB41A3 cell lines (Mouse neuroblastoma) by MTT (3-



(4, 5- Dimethylthiazol-2-Yl)-2, 5- Diphenyltetrazolium Bromide) assay. It is a quantitative assay which gives a direct relation between cell viability and absorbance obtained. Viable cells reduce MTT to MTT formazan which is then dissolved in DMSO to give a single peak at 570nm. The absorbance is proportional to the number of viable cells. It is a monotetrazolium salt, the reduction of which is one of the most frequently used methods for measuring cell cytotoxicity. The tertrazolium dye, 3-(4,5-dimethlthiazol)-2,5 diphenyltetrazolium bromide (MTT) is reduced by viable cells to MTT formazan [23]. NB41A3 cell lines were propagated in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% (v/v) fetal bovine serum (FBS) at 37ºC in a humidified atmosphere of 5% CO_2. Cells were then seeded at 1 10^5 cells/ml concentration in 96 well plates for 24 hours. Thereafter, cells were treated with different concentration of catechin nanoparticles for 24 hours.

After treatment, MTT solution (20 µl of 5mg/ml) was added to the test samples, the viable cells converted the yellow dye 3-(4, 5-dimethyltiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) to purple (insoluble formazan crystals). These crystals were further dissolved in 200 µl of DMSO followed by vigorous mixing. The optical density was determined by micro plate reader at 570 nm.

Results and Discussion

Optimization of process parameters

Optimization of polymeric and catechin concentration

Variations in chitosan to tri-poly phosphate w/v ratio, acetic acid concentration and CH concentration during nanoparticle formation were optimised statistically for their effects on entrapment efficiency of CH. At 1:1 w/v ratio of chitosan to tri-poly phosphate with 5% acetic acid and 2mg/ml CH concentration, maximum encapsulation efficiency of 60.42% was obtained (Table 1) as there was a stable ionic bond formation between the two polymers with the favourable extract concentration in suitable environment. The statistical evaluation of the process parameters was also showing (Table 2) the p value < 0.5 confirming that the model is significant and it was estimated that the percentage contribution of chitosan concentration was higher for stable nanoparticle formulation. Also, it was seen that at catechin extract concentration of 2.5mg/ml with chitosan and TPP ratio (2: 1.5) was showing least encapsulation efficiency which can be due to unstable cross linkage between chitosan and TPP polymers and higher catechin hydrate concentration[24].

Table 1: Statistical analysis; Showing optimisation parameters for the formulation against entrapment efficiency as a response.

Std	Run	A: Chitosan (mg/ml)	B: TPP conc.(mg/ml)	C: Acetic acid conc. (mg/ml)	D: Drug conc. (mg/ml)	Response: Entrapment Efficiency %
14	1	2	1	5	2.5	19.93
10	2	2	1	1	2.5	18.45
16	3	2	1.5	5	2.5	17.48
2	4	2	1	1	2	21.05
9	5	1	1	1	2.5	45.26
12	6	2	1.5	1	2.5	13.65
15	7	1	1.5	5	2.5	30.51
7	8	1	1.5	5	2	33.65
1	9	1	1	1	2	57.42
8	10	2	1.5	5	2	18.44
13	11	1	1	5	2.5	47.6
6	12	2	1	5	2	21.18
5	13	1	1	5	2	60.42
3	14	1	1.5	1	2	51.52
11	15	1	1.5	1	2.5	40.21
4	16	2	1.5	1	2	15.68

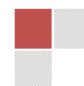


Table 2: Analysis of process parameters; Showing level of significance of various process parameters for optimised nanoparticles formulation.

Source	Sum of Squares	df	Mean Square	F value	p-value prob>F	
Model	3783.508	10	378.3508	17.68859	0.0027	significant
A-Chitosan	3045.108	1	3045.108	142.3643	< 0.0001	
B-TPP	307.7393	1	307.7393	14.38737	0.0127	
C-Acetic Acid conc.	12.30256	1	12.30256	0.575167	0.4824	
D-Drug Conc.	133.8071	1	133.8071	6.255722	0.0544	
AB	97.26891	1	97.26891	4.547497	0.0861	
AC	57.87406	1	57.87406	2.705717	0.1609	
AD	66.38176	1	66.38176	3.103467	0.1384	
BC	48.75531	1	48.75531	2.279399	0.1915	
BD	8.108256	1	8.108256	0.379076	0.5650	
CD	6.162806	1	6.162806	0.288122	0.6144	

Entrapment Efficiency (%) = + 32.03 – 13.80(A) – 4.39(B) – 0.88(C) – 2.89(D) + 2.47(A*B) + 1.90(A*C) + 2.04(A*D) – 1.75(B*C) + 0.71(B*D) + 0.62(C*D)

Characterization of nanoparticles

Particle size analysis (PS) and zeta potential (ZP) analysis

Zeta average particle size (PS) and poly dispersity index (PDI) score of CH loaded optimized formulation (A1) was reported to be 68.76 ± 1.72 nm and 0.174 ± 0.81 respectively (Figure 2). A poly dispersity index highlights the homogeneity of formulation and in the present study it was quite low (0.174 ± 0.81) suggesting that most of the nanoparticles present in the solution exist in the nanometric range below 100 nm. Whereas, Zeta potential is the measure of overall charge present on the particles in a medium and shows the stability of the colloidal system. It has also been reported earlier that particles suspended in solution usually repel each other if the system has high positive or negative value of zeta potential till 30mV and hence leading to less aggregation with enhanced stability while higher than 30mV will lead to increased agglomeration. The CH NP's exhibited the zeta potential of -5.32 mV (Figure 3), indicating a good stability and less agglomeration [25].

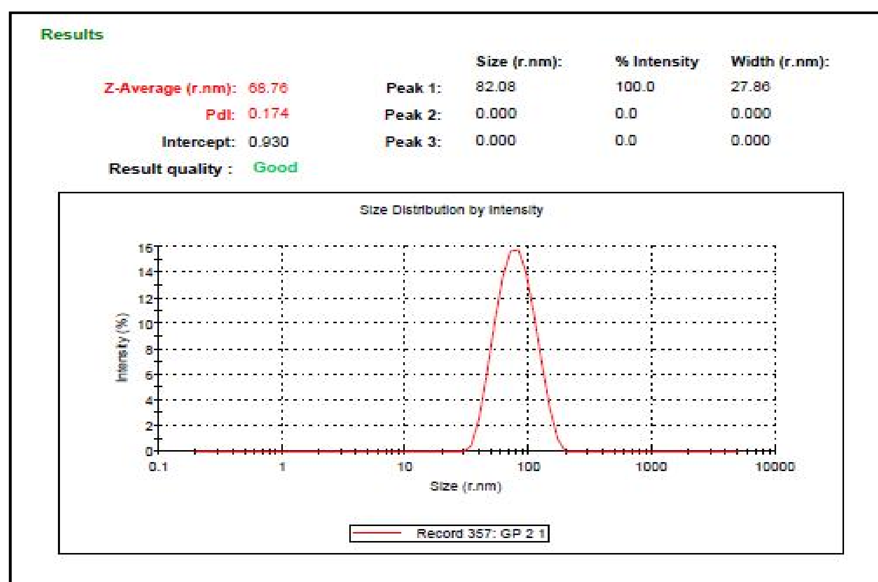


Figure 2: Showing particle size of optimized formulation of catechin loaded chitosan nanoparticles. Abbreviations: CH NP's (Catechin loaded chitosan nanoparticles), PS (Particle size), PDI (Poly dispersibility Index)

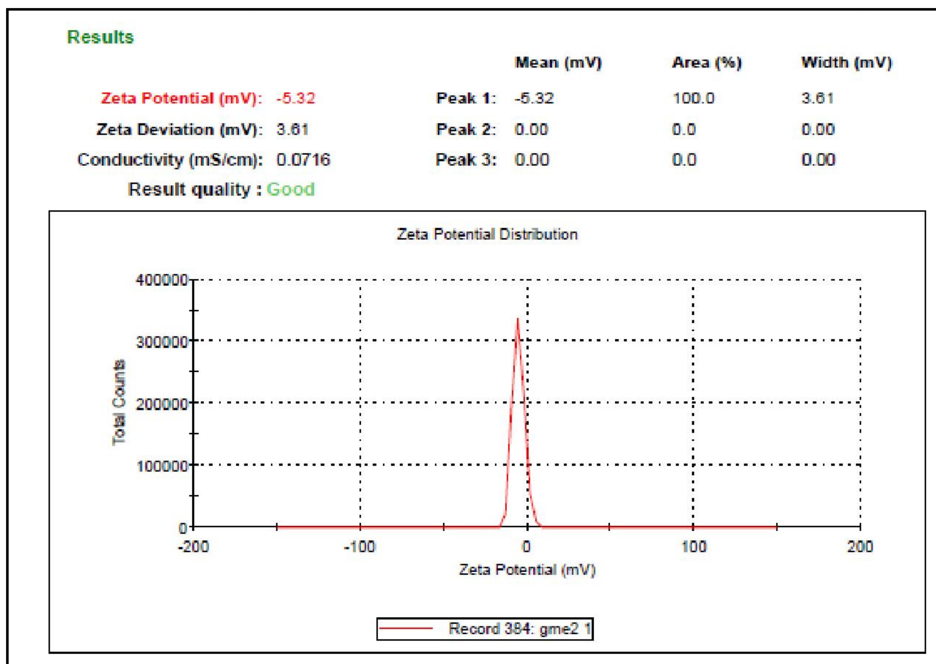


Figure 3: Showing Zeta-potential of optimized formulation of catechin loaded chitosan nanoparticles. Abbreviations:CH NP's (Catechin loaded chitosan nanoparticles), ZP (Zeta potential).

Transmission Electron Microscopy (TEM)

The TEM micrograph showed spherical morphology of optimized nanoparticles (CH NP's) with particles size range of 61.8 nm to 168nm in diameters (Figure 4) which validates the average particle size of 68.76 ± 1.72 nm obtained from zeta sizer. This size range of CH NP's will further enable the nanoparticles to easily permeate through any biological barrier.

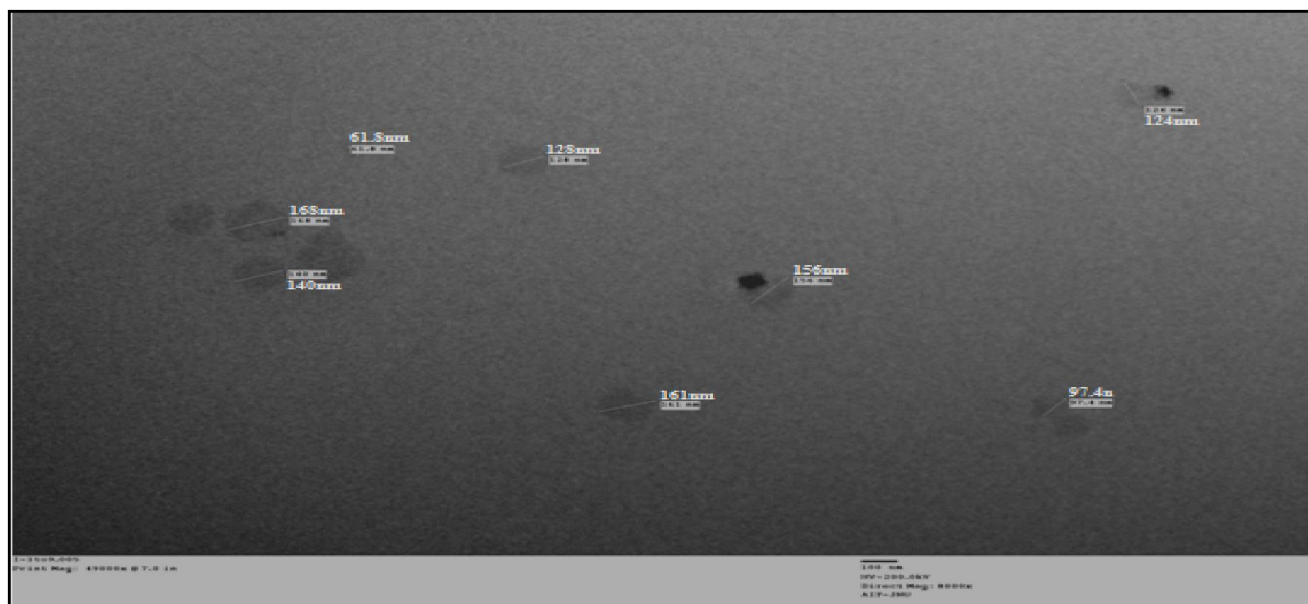
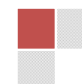


Figure 4: Depicting the scan of Transmission Electron Spectroscopy (TEM) analysis for CH NP's at the scale of 100 nm. Abbreviations:CH NP's (Catechin loaded chitosan nanoparticles), TEM (Transmission Electron Spectroscopy)



Fourier transforms infrared analysis (FTIR)

The FTIR spectra obtained for catechin hydrate (pure), NP's without catechin hydrate and NP's with catechin hydrate (Figure 5) highlight the broad peak at 3380 cm^{-1} due to OH stretch. Also the peaks 1652 cm^{-1}, 1259 cm^{-1}, 1165 cm^{-1} and 1072 cm^{-1} shows the presence of aromatic ring quadrant, OH deformation of aromatic alcohol, CO stretching of aromatic alcohol and aliphatic secondary alcohol, CO stretch respectively. The presence of similar peaks in case of NPs with CH and NPs without CH depicts the encapsulation of catechin hydrate in chitosan nanoparticles without any bond formation between chitosan and catechin hydrate.

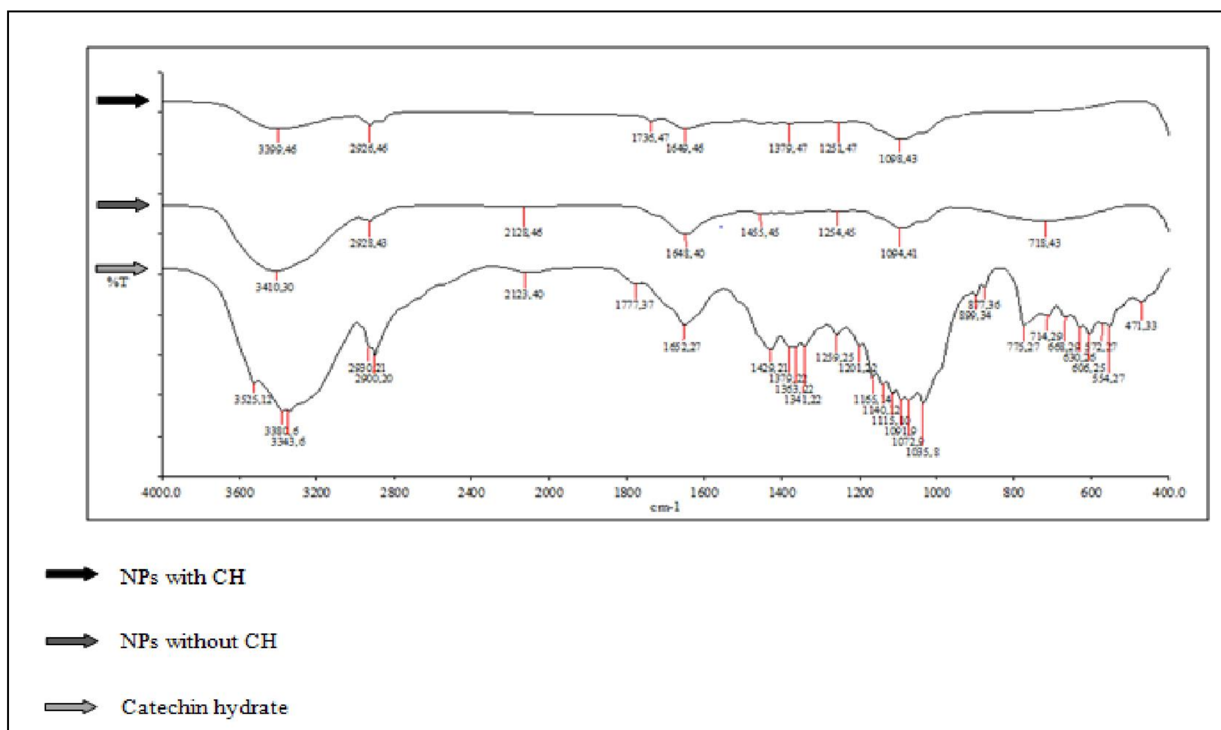


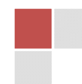
Figure 5: Graph showing functional group analysis by FT - IR spectra of catechin loaded chitosan nanoparticles from 400 – 4000 cm^{-1}
Abbreviations:CH NP's (Catechin loaded chitosan nanoparticles),FT – IR(Fourier transforms infrared)

Stability studies

Stability testing of the optimised formulation showed that the catechin loaded nanoparticles (CH NP's) remained stable for 2 weeks at almost all temperatures (4 C, 37 C and 45 C) but minor degradation was observed at 45 C (92.51%) similarly, CHNP's after the completion of 6th week (83.60%), whereas at room temperature the amount of drug remained was 90.11%. At the end of 8 weeks, 81.03% of the drug remained in the CHNP's at 45 C, whereas, at room temperature 88.76% was observed (Table 3). Hence, it was observed from the result that there was a decrease in stability of the CHNP's with the increase in the storage temperature (45 C) and time duration (6th - 8th week).

Table 3: Stability studies of CH NP's at different temperatures and time intervals (up to 8 weeks)

Amount of drug remained (%)			
Batch	Temperature		
Time	4 C	37 C	45 C
Initial	100	100	100
2nd week	97.35	96.20	92.51
4th week	94.51	92.48	87.39
6th week	92.06	90.11	83.60
8th week	88.76	85.24	81.03



Physicochemical measurement

Various physicochemical parameters of prepared nanoparticles like pH, density, conductivity and viscosity were measured (Table 4). The pH of the formulation approached to neutral (7.1) and the densities of samples came out to be equivalent to that of water (0.968 g/ml) as it was prepared in aqueous medium, making it more suitable for administration through any of the delivery route. The conductivity for the same was recorded as 0.0716 mS/cm which was lesser than the conductivity of human blood which allow nanoparticles not to coagulate and block the permeation site further, consequently allowing the easy permeation of NP's through blood capillaries. Measured viscosity of the sample (0.917cP) shows good flowability and can easily pass through any biological barrier.

Table 4: Depicting results of physicochemical parameters for CH NP's

Optimized sample	pH	Density (g/ml)	Conductivity (μS/cm)	Viscosity (cP)
C- 1mg/ml TPP- 1mg/ml	6.82	0.968	0.0943	0.917

Evaluation of ROS scavenging and antioxidant properties of optimised catechin hydrate nanoparticles

DPPH Assay

For analyzing the anti-oxidant activity, DPPH assay was performed. It was observed that the scavenging activity of Catechin loaded nanoparticles (CH NP's) was slightly higher (67.01 ± 0.15%) than the pure Catechin hydrate compound (65.69 ± 0.34%) as shown in Figure 6. This may be due the presence of chitosan's already existing scavenging ability againstanti-radical units formed as it is a well known natural biopolymer with extensive antioxidant properties.

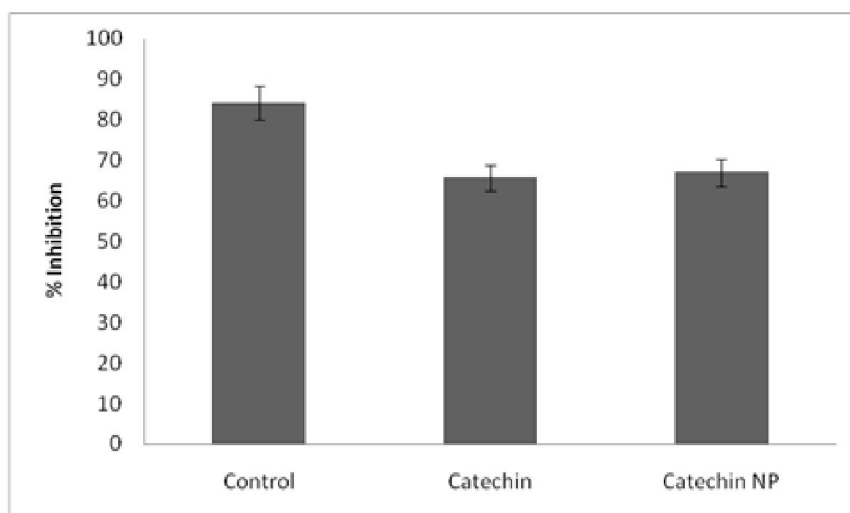


Figure 6: Antioxidant analysis; Comparative analysis of antioxidant activity of Catechin loaded NP and pure Catechin using DPPH assay.

Nitric oxide scavenging assay

Nitric oxide (NO) assay was also performed to evaluate the anti-radical activity. The scavenging activity of CH NP's was higher as compared to pure CH. For pure catechin the percentage inhibition came to be 42.31±0.14% whereas, catechin nanoparticles it was 46.12±0.11% as shown in Figure 7. The increase in the scavenging activity of CHNP's may be attributed to the antioxidant properties possessed by chitosan.

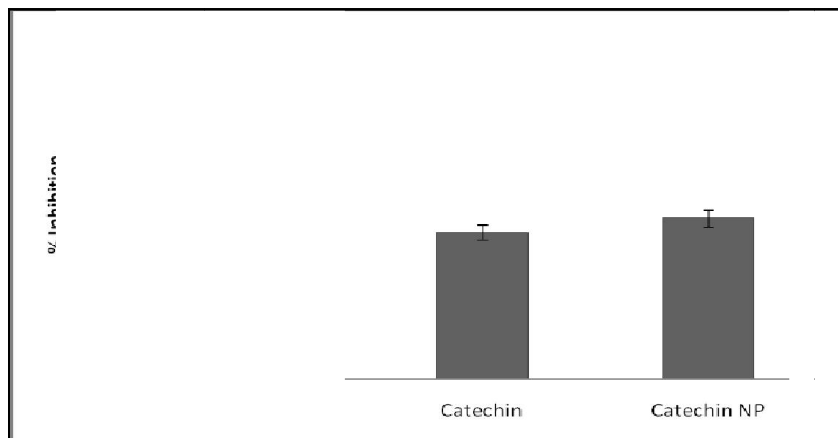


Figure 7: Antioxidant analysis; Comparative analysis of antioxidant activity of Catechin loaded NP and pure Catechin using Nitric Oxide scavenging Assay.

Hydrogen peroxide scavenging activity

Another antioxidant scavenging activity i.e., Hydrogen peroxide scavenging assay was performed. Hydrogen peroxide, a free radical is produced under the stressful conditions and thus, the scavenging activity of the catechin extract and CHNP's was checked The scavenging activity of Catechin loaded nanoparticles was 36.31±0.31% whereas, for pure catechin hydrate it was 38.12±0.11% (Figure 8), which was consistent in results to DPPH and nitric oxide assays.

Thus, all the three antioxidant assays concludes that the CHNP's have more antioxidant activity as compared to the pure catechin hydrate which is because of the antioxidant property possessed by chitosan.

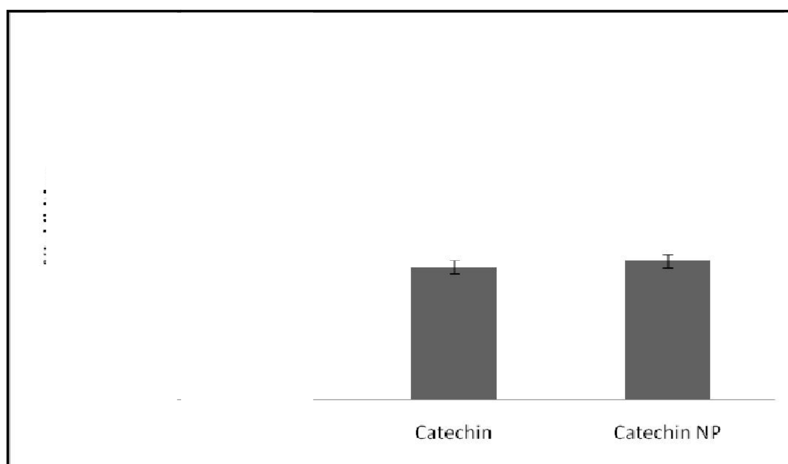
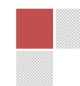


Figure 8: Antioxidant analysis; Comparative analysis of antioxidant activity of Catechin loaded NP and pure Catechin using Hydrogen peroxide scavenging Assay

In vitro compound (catechin hydrate) release kinetics

In vitro release kinetics of Catechin hydrate and catechin loaded nanoparticle (A1) was studied to determine the permeability through the dialysis membrane. It was observed that 96.34±0.22% release of catechin hydrate after 12 hours whereas, in the case of catechin loaded nanoparticle 82.56±0.34% release was observed (Figure 9), indicating a typical linear diffusion profile through the dialysis membrane. The expected characteristic of nanoparticles of sustained release was verified.



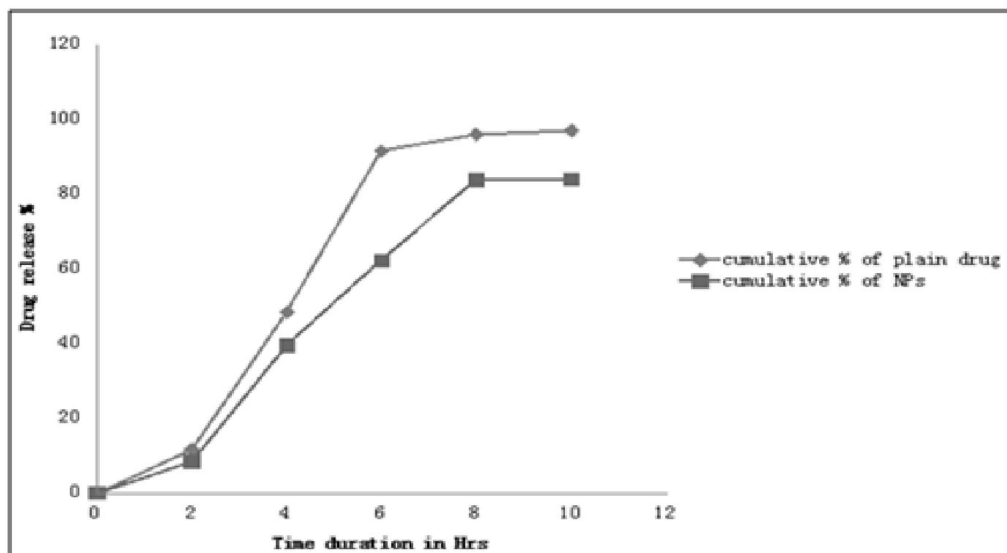


Figure 9: *In vitro* release kinetics analysis of catechin hydrate loaded nanoparticle pure catechin hydrate.

Cytotoxicity analysis of formulated nanoparticles

After analysing the cytotoxic effect of formulated nanoparticles it was revealed that as the concentration of catechin hydrate increases, percentage cell viability decreases. The results also showed that there is no significant decrease in cell viability when they were exposed to NP's with different concentration of catechin hydrate and less toxic than catechin hydrate (pure) at optimized catechin hydrate concentration with cell viability of 89.5±0.25% and 82±0.76% respectively (Figure 10). The cell viability of NP's without CH was 91.2±0.23%.

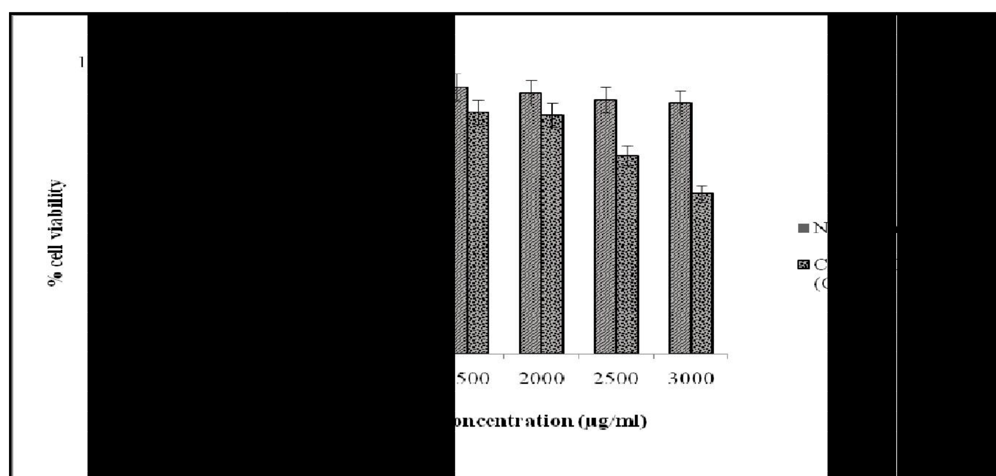


Figure 10: MTT assay; Cytotoxicity analysis of formulated nanoparticles at different concentration of Catechin Hydrate by MTT assay.

Conclusion

The nanoparticles were prepared and suitable combination of polymers were selected after optimizing process parameters like ratio of TPP solution: chitosan solution, formulations of chitosan and TPP and drug concentration and then finally, A1 formulation (chitosan 1mg/ml, tri polyphosphate 1mg/ml and catechin 2mg/ml) was selected showing entrapment efficiency of around 60%. Further they were subjected for characterization, exhibiting the particle size of 68.76 ± 1.72nm with a PDI score and zeta potential of 0.174 ± 0.81 and -5.32mV respectively. The nanometric size of the formulation was further confirmed by TEM analysis which displayed the size range from 61.8-128 nm. Further, FTIR scan showed no bond formation between the polymers and loaded extract, confirming the same by *in vitro* release kinetics studies,

proposing the peculiar characteristic of CH release pattern by showing linear diffusion of nanoparticles through dialysis membrane. Various physicochemical properties of optimized nanoparticles were measured: pH (7.1), density (0.968 g/ml), conductivity (0.0943 μS/cm) and viscosity (0.917 cP) which indicated the suitability of formulation to be administered through all delivery routes. Moreover, enhanced antioxidant activity presented by CH NP's in comparison with pure Catechin hydrate reflects the increased efficacy and therapeutic index of CH when encapsulated in nanoparticle structure, also nanoparticles being in the size range of nanometer can cross the biological membrane and can be used in the treatment of various CNS disorder.

Author's Contribution

RK: have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data.

RR: have been involved in drafting the manuscript or revising it critically for important intellectual content.

PN: participated in the design of the study and performed the statistical analysis.

R: Drafting the manuscript.

MS: Initiated the conceptualization of the experimental work, analysis, drafting of the manuscript and have given final approval of the version to be published and

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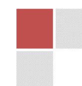

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