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Fabrication and Characterization of chitosan based polymeric Escitalopram nanoparticles

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ABSTRACT

Objective: Escitalopram (ETP), an SSRI (selective serotonin reuptake inhibitor), and s-enantiomer of citalopram is exclusively used as an antidepressant. The drug shows extensive hepatic metabolism, reduced drug efficacy and potential side effects, which reduces its therapeutic index. The present study is focused on developing and characterizing chitosan based nanoparticles of Escitalopram oxalate (ETP).
Materials and Methods: The nanoparticles were prepared by ionic gelation method using chitosan and tripolyphosphate (TPP). The formulated nanoparticles were optimized and further characterized by various techniques like particle size, zeta potential analysis, TEM, SEM, EDX, rheological parameters and FT-IR techniques. Also, *in vitro* drug diffusion was studied to evaluate its pattern of drug release.
Result and Discussion: The optimized ETP loaded nanoparticles were made with chitosan: tripolyphosphate (1:1.5) ratio, showing particle size range of 60 – 115nm, with polydispersity index of 0.117, which was further confirmed by TEM analysis whereas; zeta potential was estimated to be -1.89mV. The SEM EDX scans showed almost smooth morphology of the same. The FT – IR results confirmed that there is no interaction between the polymers and drug molecules. The *in vitro* drug release study using dialysis membrane showed sustained drug release pattern of ETP nanoparticles.
Conclusion: ETP loaded chitosan nanoparticles were prepared successfully from ionic gelation method, suggesting a comparatively suitable option for treatment of disease with fewer side effects and increased affinity of drug.

INTRODUCTION

Depression and anxiety have been incorporated in our daily lifestyle now and are faced by people of all age groups, similarly it affects both men and women across the world, but various research studies showed higher occurrences in women than in men (Taylor *et al.*, 2008). It has various forms like general anxiety disorder (GAD), obsessive compulsive disorder (OCD), major depressive disorder (MDD), social anxiety disorder (SAD) etc. which prevails in ~5 - 7% of world population. Selective serotonin reuptake inhibitor (SSRI) class of drugs like Citalopram, Escitalopram, Paroxetine, Fluoxetine, Fluvoxamine, Sertraline etc are widely used to treat these anxiety and depressive disorders. Among these classes of drugs Escitalopram oxalate (ETP) is the most used form of SSRI category of drugs. It has been approved by FDA as an anti-depressant drug and is used in treatment of general anxiety disorder (GAD), major depressive disorder (MDD).

Escitalopram is an S- enantiomer (single isomer) of the racemic bicyclic phthalane derivative citalopram but it is highly metabolised in liver by enzymes like CYP2C19, CYP3A4 and CYP2D6 and forms metabolites like S-demethylcitalopram (S-DCT) and S-didemethylcitalopram (S-DDCT). It is said to be 7 and 27 times more effective than S-DCT and S-DDCT, respectively, in inhibiting the serotonin reuptake (Burke *et al.*, 2002; Montgomery *et al.*, 2008).

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Moreover, it has a bioavailability of 80% with plasma protein binding of 56% and is eliminated through renal path. But there arises the need for targeted nanosized formulation to further secure its therapeutic index and prevent it from chemical and enzymatic degradation.

Also, it has been reported that drug delivery through nanoparticles are more effective as it can easily cross the biological barriers as in case of lansoprazole nanoparticles whose pharmaceutical efficiency increased by encapsulating it in polymeric nanoparticles (Nagarajan *et al.,* 2015). The benefits of using the polymeric carriers are that they are biocompatible, biodegradable, non-immunogenic, non-toxic and water soluble (Agnihotri *et al.,* 2004). Thus, chitosan polymer remains an obvious choice for the same and aim of present study was to prepare chitosan nanoparticles by ionic gelation method for enhanced bioavailability and lesser side effects with lower hepatic metabolism (Llorca *et al.,* 2005).

MATERIALS AND METHODS

Chitosan, Tri Polyphosphate (TPP), Monosodium phosphate anhydrous and Disodium phosphate anhydrous were purchased from Himedia Laboratories, Mumbai, India. Dialysis Membrane (9777) was from Sigma Aldrich, USA. Escitalopram oxalate (ETP) was obtained as gift from Jubilant Life Sciences, India. Acetic acid, Methanol and all other chemical used were of analytical grade.

Preparation of ETP loaded chitosan nanoparticles

ETP loaded chitosan nanoparticles were prepared by ionic gelation method using chitosan and TPP polymers for cross linkages. The crosslinkage lattice is formed between two ionic polymers of opposite charge, (chitosan as cationic and TPP as anionic) bound to each other by ionic bonds (Baby *et al.,* 2012). Chitosan gel solution was prepared by dissolving chitosan(1-2.5mg/ml) in 5% (v/v) glacial acetic acid and stirred continuously at 600 rpm overnight to get a clear chitosan gel solution.

ETP drug (1.5-5mg/ml) was then added in chitosan solution and further stirred for 40 minutes. After this, sodium tripolyphosphate (TPP) solution (0.5-2.5mg/ml) was prepared in distilled water and added drop wise with constant speed of 0.8ml/minute to the chitosan solution under constant stirring for 40 minutes again. Further it was sonicated at frequency of 10,000MHz for 15 minutes. The prepared solution was centrifuged at 12750g for 40 minutes and the supernatant was discarded, this washing step was repeated thrice and then finally pellets were lyophilised for further characterization (Katas *et al.,* 2013).

Characterization of optimised nanoparticles

Drug encapsulation efficiency

The drug encapsulation efficiency (EE) of the prepared nanoparticles was determined by centrifuging the nanoparticles at 12750g for 40 min and analysing the supernatant at 238nm, (Katas *et al.,* 2013). Then encapsulation efficiency (EE) was calculated using the following equation:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total amount of drug loaded - free drug in supernatant}}{\text{Total amount of drug loaded}} \times 100$$

Particle size and zeta potential analysis

The particle size analysis was performed using dynamic light scattering (DLS) technique, which is based on brownian motion of molecules, dispersed in liquid and relates this to the size of the particles by illuminating the particles with a laser light and analyzing the intensity fluctuations in the scattered light (Gevariya *et al.,* 2011). The particle size along with zeta potential analysis of optimised nanoparticles was done on Malvern Zetasizer (Nano ZS). Zeta potential (ZP) shows the electro phoretic particle velocity in an electrical field where the particle obtains a charge due to the dissipation of the counter ions on the surface of molecule (Saha *et al.,* 2010).

Transmission electron microscopy (TEM)

Transmission electron microscopy (Morgagni 268D) analysis was done to find out the morphology of the nanoparticles and also to confirm the size range of the drug loaded nanoparticles. The optimised ETP loaded nanoparticle was further diluted (1: 50) by distilled water and ultrasonicated for 15 minutes. It was then stained with 2% phosphotungstic acid and a drop of sample was then fixed on 300 mesh carbon-coated copper grid. The images of representative areas were taken at suitable magnifications (200nm) (Saha *et al.,* 2010)

Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDX)

Scanning electron microscopy (ZEISS EVO 40) was done to study the topographical and compositional arrangements whereas, EDX (PANalytical epsilon 5) scan was performed to have elemental analysis of optimised nanoparticles. A drop of the nanoparticles suspension was placed on a metallic surface followed by air drying under vacuum, coated with gold sputtering (Verma and Ram, 2010).

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (IR-810, JASCO, Tokyo in SAIF, Panjab University) spectra of ETP drug, chitosan nanoparticles without drug and optimised ETP loaded chitosan nanoparticles were scanned. The samples were prepared by potassium bromide disc method and scanned for absorbance from the range of 400 – 4000 cm^{-1} (Gevariya *et al.,* 2011).

Rheological parameters

Different physico-chemical parameters like pH, conductivity and density of optimised nanoparticle were measured. pH and conductivity of the samples were measured using pH meter (Thermo Orion 420A+) whereas, density was measured using specific density bottle (Borosil).The viscosity was measured with a

Brookfield rotational viscometer (LVDV, Brookfield Inc., USA). The measurement was done at 30°C at 5 rpm viscosity (Lee *et al.*, 2004 & Li and Huang, 2011).

In vitro drug release studies

In vitro drug absorption and permeability analysis was performed by using Franz diffusion cell with pre treated dialysis membrane (Sigma9777). This was mounted between donor and receiver compartments held together on both sides with a clamp. The receiver compartment was completely filled with PBS buffer (pH 7.2) and kept under continuous stirring whereas, ETP loaded nanoparticle solution was filled in donor compartment to get diffused through the semi permeable dialysis membrane.

Diffused nanoparticle samples (1 ml) were collected at pre determined time intervals (0 – 24 hours) from the sampling port and reloaded it with equal volume of PBS (1 ml) again (Verma and Ram,2010). The collected samples were then analysed at 238nm.

RESULT AND DISCUSSION

Optimization of process parameters

After optimization of process parameters (Table1), most appropriate formulation A3 was selected as it showed the highest encapsulation efficiency of ~79% with polymer concentration of 1:1.5 (ratios of chitosan and TPP concentration) (Table2) and 2.5mg/ml of drug concentration.

Table 1: Volume optimization of polymeric solutions

Ratios	% Encapsulation efficiency				
	A1	A2	A3	A4	A5
1:1	13.4	16.4	34.7	53.2	42.57
1:1.5	24.7	47.2	49.42	49.42	52.7
1:2	31.2	45.4	48.8	53.4	57.4
1:2.5	50.86	52.71	79.14	60	60.02
1:3	28.6	35.4	52.14	43.8	48.8

It was concluded from the optimization data that as the amount of Chitosan and TPP concentration increased the encapsulation efficiency (EE) decreased proportionally (Figure1) which could be due to the reason that increased concentrations of polymers would have inhibited the stable covalent ionic bond formation whereas, in optimised ratio (1:1.5) there is a prevalence of higher degree of encapsulation efficiency due to the availability of one chitosan molecule with two molecules of tripolyphosphate to form a cross linking structure of nanoparticles. Similarly, in case of drug concentration the maximum EE was observed at dose of 2.5mg/ml. (Shu and Zhu, 2000).

Table 2: Optimization of process parameters (Polymer and drug concentration) with respect to encapsulation efficiency.

Composition	Entrapment efficiency (%) with Drug Concentration			
	1.5 mg/ml	2.5 mg/ml	3.5 mg/ml	5 mg/ml
Chitosan (1mg/ml) and varied TPP concentration				
A1	61.2	50.86	48.3	45.7
A2	65.7	52.708	49.2	46.1
A3	82.5	79.14	61.9	54.5
A4	69	60	46.9	57
A5	51.6	42.568	40.1	38.6
Chitosan (1.5mg/ml) and varied TPP concentration				
B1	46.8	23.428	41.6	41.7
B2	45.4	33.428	39.4	38.4
B3	43.3	34.288	43.3	42.8
B4	39.4	30.568	41.3	31.6
B5	35.1	34.288	32.9	31.7
Chitosan (2mg/ml) and varied TPP concentration				
C1	28.7	19.428	31.2	25.1
C2	23.1	16.428	30.7	20.8
C3	25.8	29.428	36.38	21.6
C4	25	24.428	26.1	24.2
C5	23.6	20.86	24.1	19.8
Chitosan (2.5mg/ml) and varied TPP concentration				
D1	25.4	22.708	18.2	15.9
D2	21.3	27.708	14.6	18.2
D3	31	30	24.1	28
D4	30.6	30.288	6.3	26
D5	24.9	30.14	10.3	19.1

Characterization of nanoparticles

Particle size (PS), Poly dispersibility index (PDI) and Zeta potential (ZP) analysis Average particle size (PS) of optimised ETP loaded chitosan nanoparticles (A3) was 93.63 ± 1.04 nm which was further confirmed by TEM. The PDI score of the same was recorded as 0.117 ± 0.06 showing good homogeneity and dispersibility of nanoparticles in the solution (Figure3a) (Gevariya *et al.*, 2011). Whereas, Zeta potential of A3 formulation was -1.89 ± 0.27 mV (Figure3b) indicating the surface electrical charge (negative) due to ionization or dissociation of surface groups (carboxyl and/or amino and phosphate groups) along with total molecular charge (positive or negative).Therefore, from the recorded observations, it can be suggested that the optimised formulation (A3) is highly stable with less ionic charge. Also, many earlier research studies had reported that there is non-aggregation of nanoparticles in the range of ± 30 mV (Saha *et al.*, 2010).

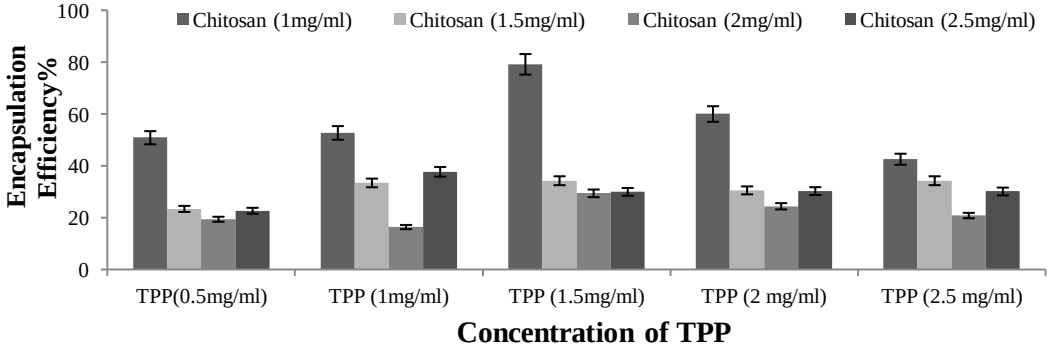


Fig. 1: Comparison between different chitosan-TPP formulations based on encapsulation efficiency.

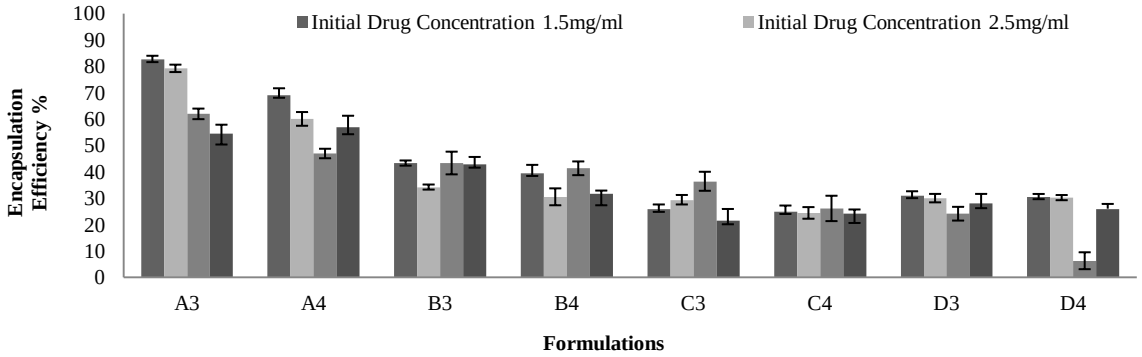
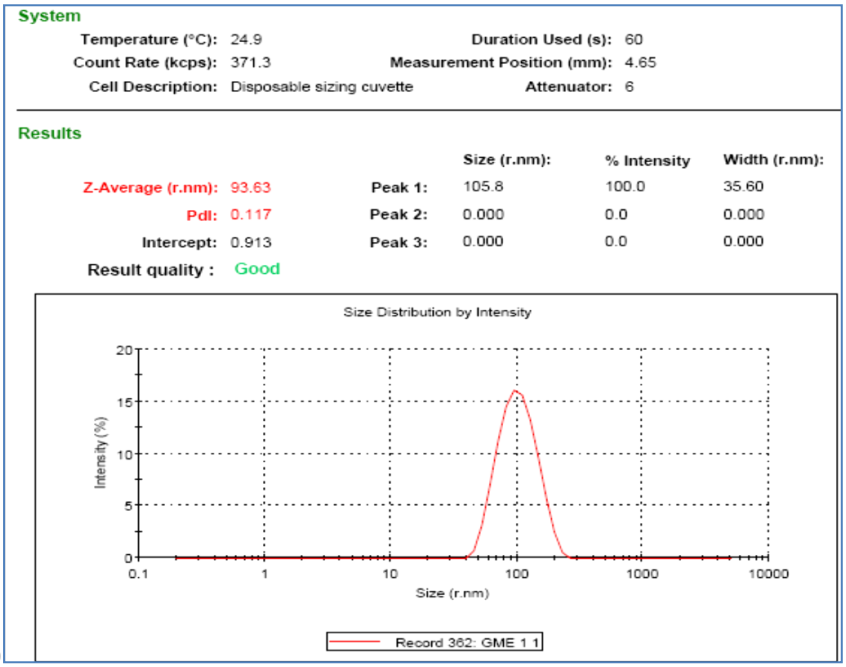
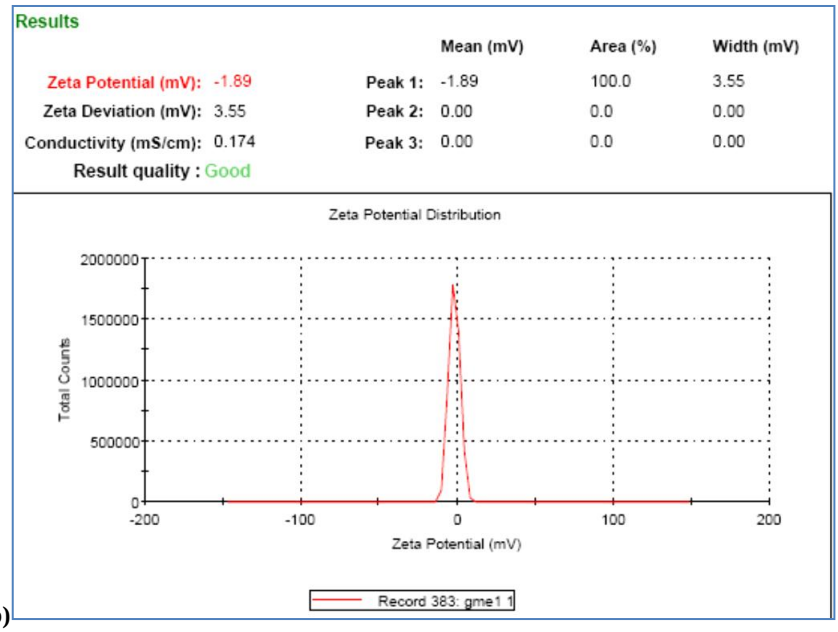


Fig. 2: Comparison between different drug concentrations based on encapsulation efficiency.



(a)



(b)

Fig. 3: (a)-Particle Size of optimized nanoparticles (A3) was 93.63ηm and and **(b)**-zeta potential of optimized nanoparticles (A3) was -1.89 mV with PDI 0.117 and conductivity 0.174 mS/cm.

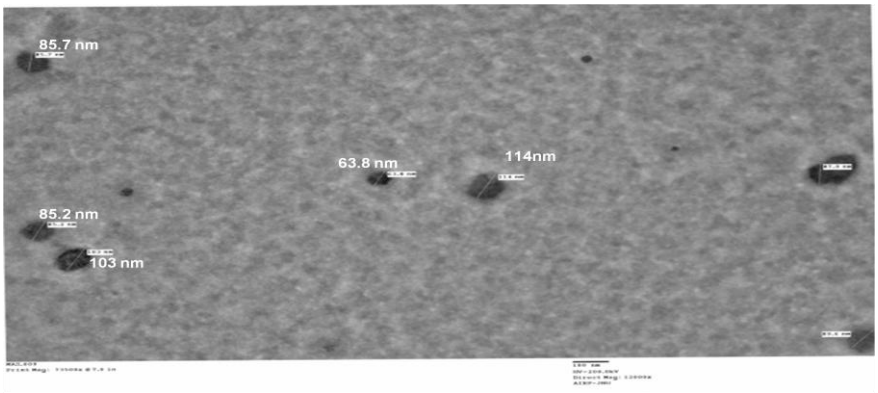


Fig. 4: Transmission electron spectroscopy (TEM) analysis done for A3 formulation.

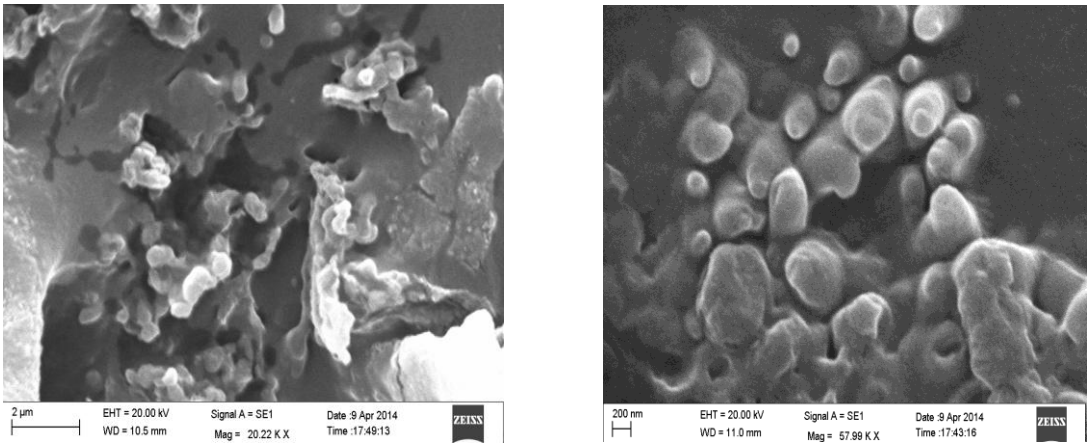


Fig. 5: (a)-Base image of SEM analysis and **(b)**-Magnified image of SEM analysis.

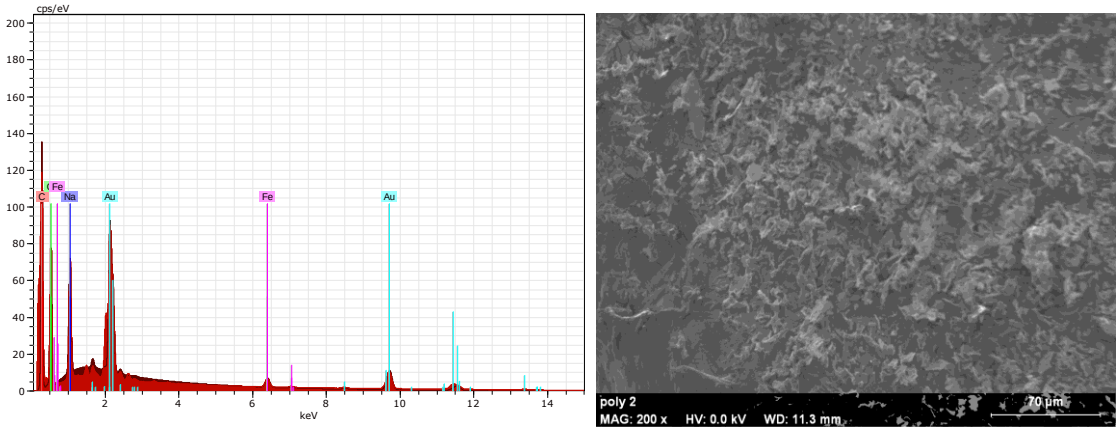


Fig. 6: EDX analysis showing localized chemical composition and topological image of nanoparticles.

Transmission electron microscopy (TEM)

The TEM micrograph obtained from the imaging showed that the droplet size of the samples were in nanometric range (60 - 115nm in diameter) (Figure 4).

These results were in accordance with the DLS findings, which recorded the average mean of PS as 93.63 ± 1.04 nm. The nanoparticle image appeared with almost spherical morphology of droplets, hence acquiring minimum surface area and indicating the strong possibility of permeating through any biological barriers (Saha *et al.*, 2010).

Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDX)

SEM technique is used for the morphological characterization of particles, thereby using a high energy electron beam to scan over the surface (Verma and Ram, 2010). The results obtained from SEM indicates the almost spherical and smooth morphology of nanoparticles when observed in the scale of 200 nm (Figure 5a) although it appears to be irregular when observed in the scale of 2 µm so it can be concluded that morphologically the particles are almost smooth and spherical (Figure 5b).

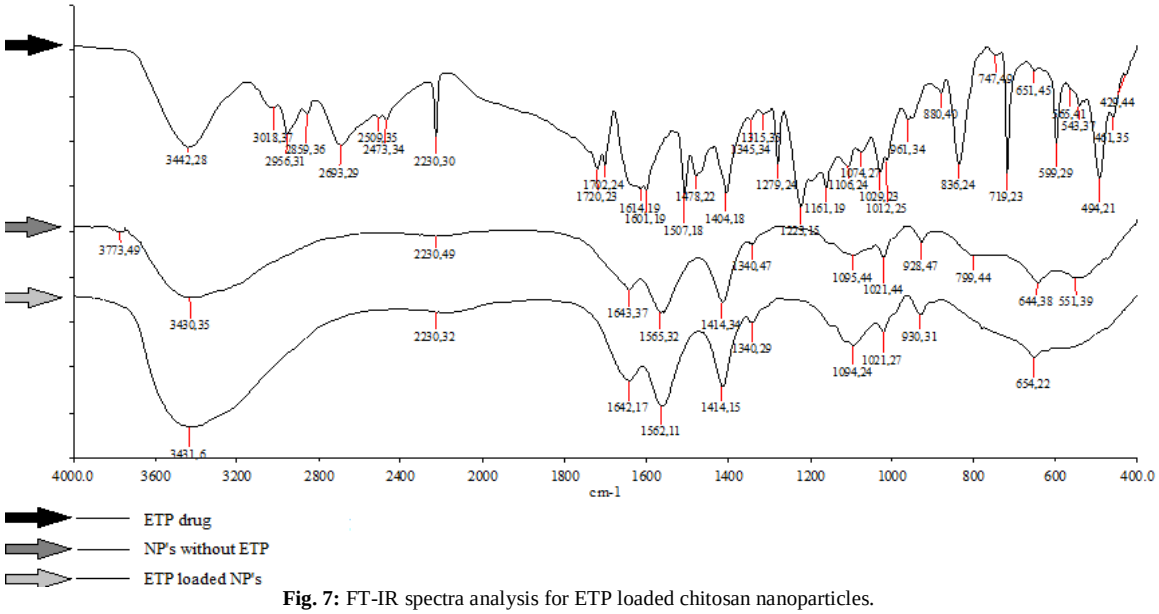


Fig. 7: FT-IR spectra analysis for ETP loaded chitosan nanoparticles.

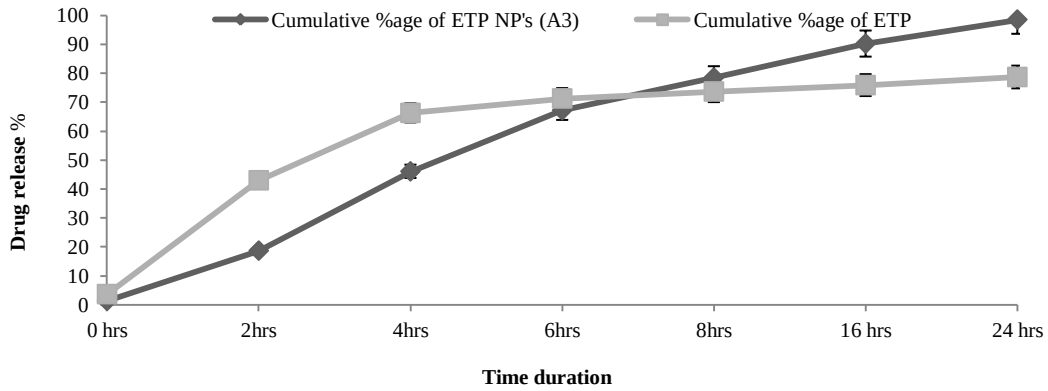


Fig. 8: Graph depicting release kinetics of drug in nanoparticle formulations as compared to normal drug.

Whereas, in EDX spectrometry scanning prominent peaks of C and Na indicates the presence of cross linkage between the chitosan and TPP and confirms the formation of chitosan nanoparticles whereas, Au appearance is due to gold sputtering done (Figure 6).

Fourier transform infrared spectroscopy (FTIR)

The graphical representation of FT-IR spectra of ETP drug, NP's without drug and ETP loaded NP's (A3) attributed to the linkage between phosphoric and ammonium ions concluding that the di polyphosphate groups of TPP are linked with ammonium groups of chitosan. Moreover it also showed non emergence of signature peaks of drug 3431, 2230 and 1414 representing OH (H bond), -C≡C-, and –C-C- stretch in ring (aromatic) groups in optimised ETP loaded NP's. In addition to the same, emergences of all the prominent peaks in ETP loaded NP's were quite similar with nanoparticles without drug, suggesting less possibility of ETP available on surface of NP's. (Figure 7) (Gevariya *et al.*, 2011).

Rheological parameters

Analysis of physicochemical properties is important for efficient drug release from nanoparticles through various biological and physical models (Table3).

Table 3: Physicochemical parameters of nanoparticles.

Sample Code	pH	Density (g/ml)	Conductivity (µS/cm)	Viscosity(cP)
C- 1mg/ml, TPP- 1.5mg/ml (A3)	6.7	0.976	174.8	0.887

Therefore, parameters like pH, density, conductivity and viscosity were measured. The pH of the formulation (A3) was recorded as 6.7 showing proximity with neutral (pH-7.3) (Katas *et al.*, 2013 & Fan *et al.*, 2012), similarly conductivity of the same was 0.174 mS/cm which is quite less than the conductivity of blood (blood plasma conductivity 12mS/cm) (Jaffrin and Fournier, 1999), hence allowing the nanoparticles to easily flow through blood vessels without any repulsion. The density observed was 0.976g/ml, equivalent to that of water (1g/ml) and is suitable for

administration through any of the delivery route whereas, the obtained viscosity of the sample (0.887cP) is reported to show good flowability and can easily pass through any biological barrier (Lee *et al.*, 2004; Li and Huang, 2011).

In vitro drug release studies

In vitro drug release pattern of Escitalopram (ETP) and ETP loaded was studied to check permeability of pure drug (ETP) and its optimized nanoparticles (A3) through dialysis membrane, it showed 78.6 ± 2.6% release (24 hours) of drug in receptor compartment whereas, for ETP loaded nanoparticles it was 98.4 ± 1.07% release in 24 hours (Figure8), proposing a typical linear diffusion profile of nanoparticles through the dialysis membrane (Verma and Ram, 2010). The expected characteristic of nanoparticles of sustained release was verified. However, diffusion equilibrium was attained after 6 hours with ETP drug, compared to nanoparticles (A3). Results further propose sustained release of drug molecules using nanoparticle systems.

CONCLUSION

In this study escitalopram nanoparticles were prepared to decrease the loss of drug entering in systemic circulation due to high hepatic metabolism of the drug by getting entrapped in nanoparticles, becoming more stable and shielded from enzymatic degradation. The optimized nanoparticle formulation of chitosan and tri polyphosphate (1:1.5) with 2.5mg drug (ETP) showed particle size in nanometric range (60-115 nm) and exhibited approximately 98.4 ± 1.07% of drug release from nanoparticles in 24 hours from the dialysis membrane. Also physicochemical study revealed that pH (6.7), conductivity (0.174 mS/cm), density (0.976g/ml) and viscosity (0.887cP) of optimised nanoparticles (A3) was equally suitable for all the route of drug administration including oral, systemic and intranasal drug delivery. Further FTIR and SEM analysis showed no bond formation between the drug molecules and polymers on the surface and almost smooth morphology of the nanoparticles respectively. Therefore, these nanoparticles being in nanometric size range can penetrate through various biological barriers and could serve as a potential delivery system for the treatment of anxiety disorders.

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