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Original Article

Design and Evaluation of Chitosan Nanoparticles as Novel Drug Carriers for the Delivery of Donepezil

Dinesh Raja Azalea[a,*], Mazin Mohambed[a], Sandra Joji[a], C Sankar[a], B Mishra[b]

[a]*Department of Pharmaceutics, Kmch College of Pharmacy, Coimbatore, Tamilnadu, India*
[b]*Department of Pharmaceutics, IT (BHU) Varanasi, India*

Abstract

The present study deals with the formulation and evaluation of chitosan nanoparticles containing donepezil hydrochloride for the targeted delivery to the brain. Nanoparticles were prepared by ionic gelation method using sodium tripolyphosphate (TPP) as a cross linking agent followed by sonication. Nanoparticles were obtained in the average size ranging from 116.8 to 227.5 nm. Particle morphology was determined by scanning electron microscopy (SEM). The SEM image showed that each particle unit exhibited a nanostructure. Encapsulation efficiency of nanoparticles ranged between 46.66% and 70.41%. The prepared particles showed good drug-loading capacity. The *in vitro* release studies showed that after the initial burst, all of the drug-loaded batches provided a continuous and slow release of the drug. Drug released profile was found to be a non-Fickian analomous diffusion, and the drug release was followed by first order kinetics. The drug loaded batches showed a good stability when stored at room temperature for 60 days. FT-IR studies indicated that there was no chemical interaction between the drug and polymer. The present study revealed that ionic gelation technique followed by sonication can be used as an effective tool for preparation of donepezil nanoparticles, which may significantly improve its ability to cross BBB and enter CNS.

Keywords: Chitosan nanoparticles; Donepezil hydrochloride; Ionic gelation; Sodium tripolyphosphate.
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1.Introduction

Nanoparticles have been highly exploited for controlled drug release and site-specific drug targeting. They have shown promising results in the case of site-specific drug targeting for treating various diseases including cancer, human immunodeficiency virus infection, and central nervous system (CNS) disorders [1]. Drugs or other molecules may be dissolved entrapped, encapsulated, adsorbed or attached into nanoparticles ranging from 1 to 1000 nm. Nanoparticles have a higher surface-to-volume ratio compared to the bulk material, therefore, the dose and frequency of administration would be reduced, increasing patient compliance. Nanoparticles made of

*Corresponding author: Dinesh Raja Azalea, Department of Pharmaceutics, Kmch College of Pharmacy, Coimbatore, Tamilnadu, India.
E-mail:dinesh_azalea@yahoo.co.in

hydrophilic polymers have the added advantage of prolonged circulation in the blood, which would facilitate extravasation and passive targeting.

Chitosan is a biodegradable, biocompatible and bioadhesive polysaccharide. Chitosan has been shown to be soft tissue compatible and non-toxic in usable concentrations. It has been widely used in pharmaceutical research and in industry as a carrier for drug delivery and as biomedical material [2].

Alzheimer's disease (AD) is the most common brain disease of adulthood. It is 1.5 times more common than stroke or epilepsy and is as common as congestive heart failure. The worldwide prevalence of AD in 2006 was 26.6 million [3]. As of 2010, there are an estimated 35.6 million people with dementia worldwide. By 2050, it is projected that this figure will have increased to over 115 million. Much of the increase will be in developing countries. Already, 58% of people with dementia live in developing countries, but by 2050 this will rise to 71%. The fastest growth in the elderly population is taking place in China, India, south Asia and western Pacific.

AD is characterized by a marked atrophy of the cerebral cortex and loss of cortical neurons. Both genetic and environmental factors are believed to play an important role in the causation and progression of AD. Overproduction of Aβ, or failure to clear this peptide, leads to AD primarily through amyloid deposition, which produces neurofibrillary tangles. These lesions are associated with cell death, which is reflected in memory impairment, the hallmark of AD [4].

Donepezil is a specific and reversible inhibitor of the enzyme acetylcholinesterase. Acetylcholinesterase is an enzyme which breaks down acetylcholine. Donepezil may allow a greater concentration of acetylcholine in the brain, thereby improving cholinergic function. Acetylcholine, associated with memory and learning, is in short supply in subjects with AD. Donepezil is a hydrophilic drug. It is well absorbed with a relative oral bioavailability of 100%. Donepezil is approximately 96% bound to human plasma protein and its half life is 70 h. It takes 3 weeks to attain steady state when daily dose of 5 mg donepezil tablet is administered orally [5].

Based on these observations, donepezil was selected for formulation into nanoparticles which can pass through the blood brain barrier in order to target the drug to the brain and increase its concentration in the site of action which is expected to give a better patient care. So, different batches of nanoparticle based donepezil were prepared and evaluated.

2. Materials and methods

2.1. Materials

Donepezil hydrochloride was a gift sample from Actavis (Chennai, India). Chitosan was a gift from Central Marine Fisheries Research Institute (Cochin, India). Sodium tripolyphosphate was purchased from Loba Chemie (Mumbai, India). All other materials and reagents used in the study were of analytical grade.

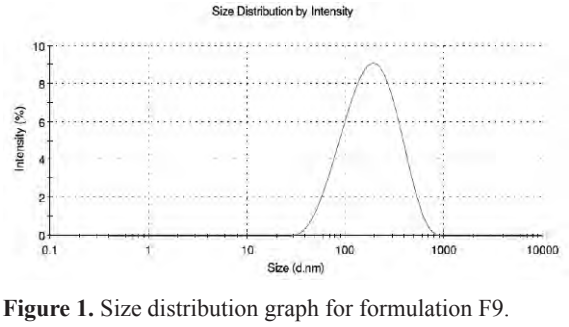

Figure 1. Size distribution graph for formulation F9.

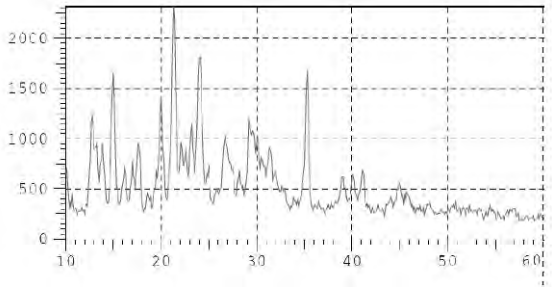

Figure 2. XRD pattern of pure donepezil hydrochloride.

Table 1. Formulation scheme of different batches.

FORMULATION	DRUG (mg)	CHITOSAN (mg)	TPP (mg)
1	50	50	50
2	50	75	50
3	50	100	50
4	50	50	25
5	50	75	25
6	50	100	25
7	50	50	75
8	50	75	75
9	50	100	75

2.2. Formulation of donzepezil nanoparticles

Nanoparticles were prepared according to the procedure reported by Calvo *et al*. [6] based on the ionic gelation of chitosan with sodium tripolyphosphate (TPP) anions. Chitosan nanoparticles were prepared in the presence of Tween80 (0.5%v/v) as a resuspending agent to prevent aggregation, at ambient temperature while stirring. Required quantity of chitosan, as shown in Table 1, was taken and dissolved in 5 ml of lactic acid under stirring at 1000 rpm for 10 min. Fifty mg of the drug was dissolved in 85 ml of 0.5% v/v Tween80 solution (0.5 ml Tween-80 in 100 ml of double distilled water). Drug solution was then added to chitosan solution and stirred for 20 min at 1000 rpm using magnetic stirrer.

Sodium TPP was dissolved in 10 ml of 0.5% Tween80 (v/v) solution and added drop wise using syringe under stirring. The suspension was then sonicated for 20 min at 80% amplitude and 1 second pulse for particle size reduction. The final suspension was then frozen and lyophilized at (-40 °C) for two days.

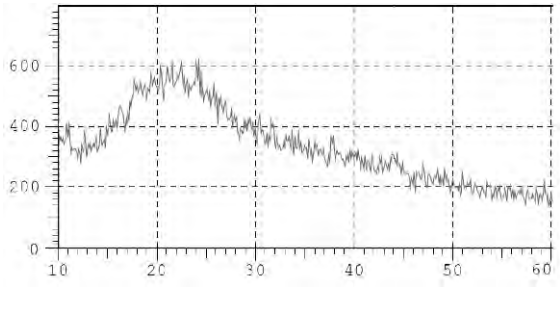

Figure 3. XRD pattern of donepezil nanoparticle (F9).

2.3. Characterization of nanoparticles

2.3.1. Particle size analysis

The size of nanoparticles was analyzed using a Zetasizer, Ver. 6.01 (Malvern Instrument Ltd) at CRL, Karunya University, Coimbatore. The suspension was placed in the sample holder and the particle size was measured as previously explained [7].

2.3.2. X-ray diffraction study

X-ray diffraction analysis was conducted using a XRD-6000 diffractometer (Shimadzu, Japan) at CRL, Karunya University, Coimbatore. X-ray diffraction analysis was used to detect the crystallinity of the pure drug and the formulation. The powder was placed in an aluminium sample holder. Cu radiation was generated at 30 mA and 40 kV. Samples were scanned at a range of 10° to 90° with scan speed of 10° min-1, as previously explained [8].

2.3.3. Scanning electron microscopy

The morphology of particle was observed using scanning electron microscopy (SEM), JSM-6390 (JEOL, Japan) at CRL, Karunya University, Coimbatore. The formulation was kept on an SEM stub using double-sided adhesive tape at 50 mA for 6 min through a sputter (KYKY SBC-12, Beijing, China). A scanning electron microscope with a secondary electron detector was used to obtain digital images of the nanoparticle [9].

2.4. Donzepezil entrapment efficiency of the nanoparticles

Prepared nanoparticle suspensions were centrifuged at 2000 rpm for 30 min. The supernatant was collected and the particles were washed with water and then subjected to another cycle of centrifugation. The amount of free donepezil in the supernatant was determined by the UV-Visible Spectrophotometer at 220 nm [10].

The entrapment efficiency of donepezil was calculated from the equation.

$$\text{Entrapment efficiency}(\%) = \frac{\text{Amount of donepezil added- Amount of free donepezil}}{\text{Amount of donepezil added}} \times 100$$

2.5. Determination of drug content

Twenty five mg of the prepared nanoparticles were weighed and dissolved in 5 ml of lactic acid and made up to 25 ml with phosphate buffer (pH 7.4). From the aliquots, 1 ml was taken and diluted to 10 ml with the buffer and the absorbance was measured in UV-Vis spectroscopy at 220 nm [10]. From the absorbance's total drug content in the batches were calculated.

2.6. In vitro drug diffusion studies using EGG membranes

Permeation study with egg membrane was done according to the method reported by Ansari *et al.* [11]. The egg shell was kept in concentrated HCl for 2 h. The separated membrane was attached to diffusion cell. Twenty mg of the drug was placed in the diffusion cell with 10 ml of phosphate buffer (pH 7.4). Fifty ml of phosphate buffer (pH 7.4) was placed in the receptor compartment in 100 ml beaker. The assembly was then attached to magnetic stirrer. Samples were withdrawn at specific time interval for 6 h and analyzed using UV-visible spectrophotometer at 220 nm.

2.7. Drug release kinetics

To study the release kinetics, data obtained from *in vitro* drug release studies were plotted in various kinetic models: zero order (Equation 1) as the cumulative amount of drug released vs. time; first order (Equation 2) as the log cumulative percentage of drug remaining vs. time; and Higuchi's model (Equation 3) as the cumulative percentage of drug released vs. square root of time [12].

$$C = K_0 t \quad (1)$$

Where, 'K_0' is the zero-order rate constant expressed in units of concentration/time; 't' is the time in hours.

A graph of concentration vs. time would yield a straight line with a slope equal to 'K_0' and intercept the origin of the axes.

$$\text{Log}C = \text{Log}C_0 - Kt_{1/2}.303 \quad (2)$$

Where, 'C_0' is the initial concentration of drug; 'K' is the first order constant, and t is the time.

$$Q = Kt_{1/2} \quad (3)$$

Where, 'K' is the constant reflecting the design variables of the system; 't' is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time.

Table 2. Average particle size of donepezil nanoparticles.

Formulation code	Z- Average (d.nm)
F1	126.7
F2	149.9
F3	165.9
F4	126.5
F5	227.5
F6	189.9
F7	116.8
F8	146.7
F9	146.9

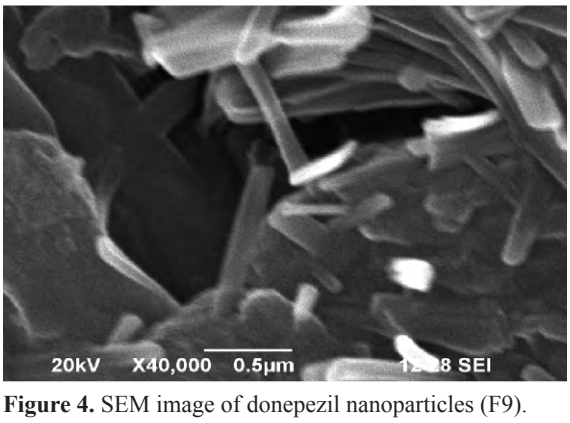

Figure 4. SEM image of donepezil nanoparticles (F9).

Table 3. Donepezil nanoparticle assay and encapsulation efficiency data.

Formulation code	Assayed drug content		Encapsulation efficiency (%)
	Amount (mg)	% of 25 mg nanoparticles	
F1	11.62	46.48	55.96
F2	13.45	53.81	67.21
F3	12.22	48.88	60.56
F4	9.96	39.84	50.37
F5	16.98	67.92	69.32
F6	10.74	42.96	47.66
F7	11.83	47.32	59.92
F8	12.65	50.66	62.77
F9	18.55	74.22	70.41

2.8. Mechanism of drug release

To evaluate the mechanism of drug release from donepezil nanoparticles, data of drug release were plotted in Korsmeyer Peppas equation (Equation 4) as the log cumulative percentage of drug released vs. log time, and the exponent 'n' was calculated through the slope of the straight line [13].

$$M_t - M_\infty = Kt^n \quad (4)$$

Where, M_t/M_∞ is the fractional solute release; 't' is the release time; 'K' is a kinetic constant characteristic of the drug/ polymer system, and n is an exponent that characterizes the mechanism of release of tracers.

If the exponent n = 0.45, then the drug release mechanism is Fickian diffusion, and if $0.45 \geq n \geq 0.89$, then it is non-Fickian or anomalous diffusion. An exponent value of 0.89 is indicative of Case-II Transport or typical zero-order release.

2.9. Stability studies

Stability of a drug in a dosage form at different environmental conditions is important, because it determines the expiry date of that particular formulation. Hence, the stability and chemical interaction of the drug in the nanoparticles were studied. The study was carried out to assess the stability of chitosan nanoparticles of the drug donepezil (F-9). This was carried out according to the procedure described by Zhang *et al.* [14]. For this purpose, the samples were taken in borosilicate glass vials and sealed, and the vials were stored in room temperature (25 to 30 °C) and refrigerator (3 to 5 °C) over a period of 3 months. Samples were evaluated at 0, 1, 2, and 3 months for their drug content as well as any changes in their physical appearance.

Chemical stability during the storage was checked by Fourier transform infrared (FTIR) studies after 3 months of storage. FTIR Emission Spectroscopy was used to record the

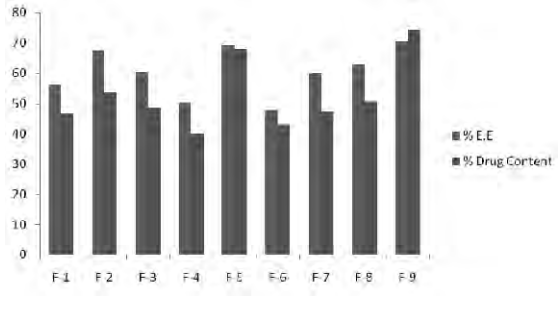

Figure 5. Comparision of % drug content and encapsulation efficiency (E.E).

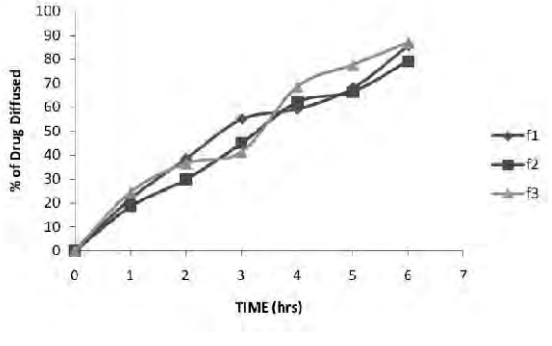

Figure 6. *In vitro* drug diffusion profiles of donepezil nanoparticles (F1-F3).

Table 4. *In vitro* drug diffusion studies data.

Time (hrs)	% of drug diffused								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
1	21.66	18.42	24.47	16.66	19.92	24.36	20.16	27.46	33.65
2	38.41	29.77	36.54	23.56	32.64	30.58	36.68	40.38	39.76
3	54.87	44.85	41.28	38.86	49.26	38.62	51.16	48.14	52.34
4	59.12	62.04	68.48	49.28	61.18	53.26	67.34	65.64	71.18
5	67.88	66.35	77.66	63.17	73.25	60.24	74.45	79.62	86.11
6	85.72	79.27	86.92	70.14	85.11	77.03	88.81	91.27	93.79

FTIR spectrum of the pure drug, polymer and nanoparticle formulation from 400 to 4000 cm^{-1} to evaluate the molecular states of the pure drug and the formulation. The sample was grounded with KBr and pressed to a suitable size disk for measurement.

3. Results

3.1. Particle size analysis

The particle size of chitosan nanoparticles containing donepezil was analyzed by Zetasizer. The Z-average particle size (d.nm) of chitosan nanoparticles formulations ranged from (116.8) to (227.5) as shown in Table 2. The ability of nanoparticles to alter the biodistribution and pharmacokinetics of drugs has important *in vivo* therapeutic applications. In this respect, the size and surface characteristics of nanoparticles are of prime importance. Nanoparticles of particle size 100 nm are easily captured by Kupffer cells or other phagocytic cell populations that restrict their biodistribution. Particles of 100 nm diameter with hydrophilic surfaces have a longer circulation in blood [15]. Such systems prolong the duration of drug activity and also increase the targeting efficiencies to specific sites [16].

Particle size distribution graph (size distribution by intensity) for formulation (F-9) is shown in Figure 1. All formulations showed uniform particle size distribution except formulations F-5 and F-6.

3.2. XRD analysis

XRD pattern of the pure drug and selected formulation are shown in Figures 2 and 3. Characteristic diffraction peaks were observed for donepezil. On the other hand, the nanoformulation was characterized by less intensity of the diffraction peak when compared to that of donepezil hydrochloride.

3.3. SEM analysis

Particle morphology was determined by scanning electron microscopy. The SEM image (Figure 4) showed that each particle unit exhibited a nanostructure.

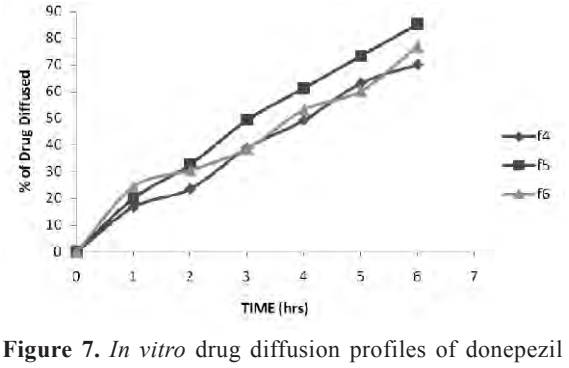

Figure 7. *In vitro* drug diffusion profiles of donepezil nanoparticles (F4-F6).

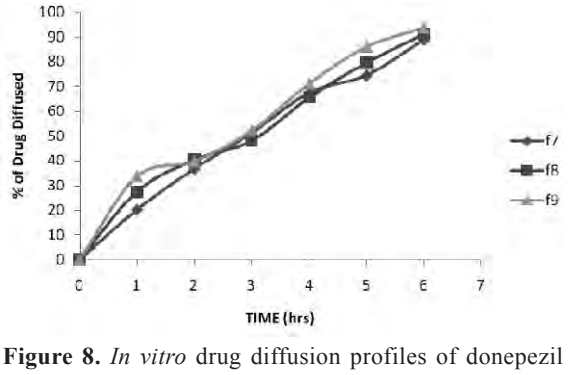

Figure 8. *In vitro* drug diffusion profiles of donepezil nanoparticles (F7-F9).

Table 5. *In vitro* drug release kinetics data.

Formulation code	Zero order	First order	Higuchi's	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	N
F1	0.966	0.900	0.974	0.984	0.730
F2	0.985	0.930	0.983	0.990	0.834
F3	0.972	0.952	0.943	0.951	0.733
F4	0.992	0.957	0.973	0.978	0.846
F5	0.992	0.941	0.991	0.997	0.822
F6	0.973	0.991	0.933	0.942	0.638
F7	0.986	0.918	0.993	0.997	0.824
F8	0.980	0.977	0.967	0.977	0.673
F9	0.966	0.977	0.950	0.938	0.612

3.4. Determination of drug content and encapsulation efficiency

Nanoparticles of donepezil were formulated by ionic gelation method followed by sonication. From the data shown in Table 3, it was clearly evident that the assayed drug content in the formulations was found to be within the range of 39.84% to 74.22% (w/w). The encapsulation efficiency of nanoparticles formulations were found to range from 47.66% to 70.41% (Figure 5).

3.5. In vitro drug diffusion studies

Permeation studies with natural membrane (egg membrane) were carried out according to the method reported by Ansari *et al.* [11]. The results of the study are given in Table 4. The drug release profiles from the nanoparticles are shown in Figures 6 to 8. The formulations showed good drug release from the polymer, and the percentage of drug released after 6 h ranged from 70.14% to 93.79%. The *in vitro* drug release profiles of all of the formulations showed an initial burst effect, and this was followed by a slow drug release. The burst effect occurred within 1 h, and the remaining amount of drug was released in a sustained manner over a period of 6 h. The burst release of drug was associated with those drug molecules dispersing close to the nanoparticle surface, which easily diffuse in the initial incubation time [17].

3.6. Release kinetics

The release kinetics was characterized by fitting the data obtained from *in vitro* drug release studies of nanoparticles to standard release equations (zero-order, first-order, Higuchi model, and Korsmeyer-Peppas model) [12]. The results obtained are presented in Table 5. The model that best fits the release data is selected based on the

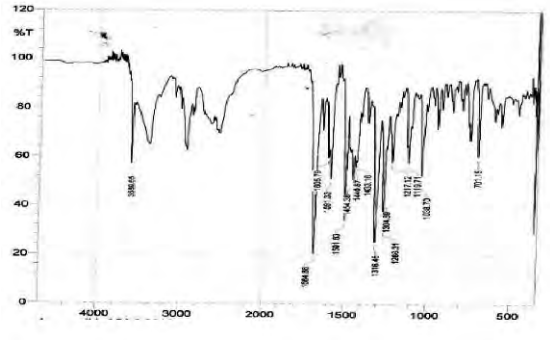

Figure 9. FT-IR spectrum of pure drug (donepezil hydrochloride).

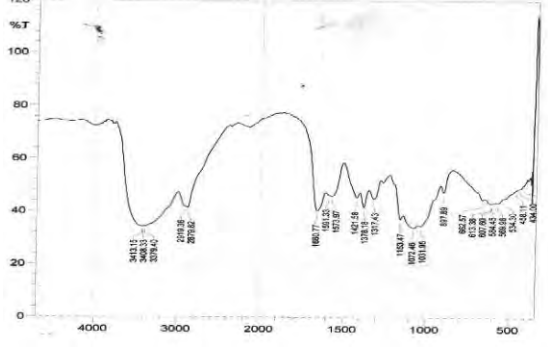

Figure 10. FT-IR spectrum of chitosan.

Table 6. Stability data of formulation F-6.

Temperature	Evaluation Parameter	Observation (months)			
		0	1	2	3
Room temperature (25-30°C)	Physical appearance		No change	No change	No change
	Drug content (%)	74.22	73.96	73.45	73.16
Refrigerator temperature (3-5°C)	Physical appearance		No change	No change	No change
	Drug content (%)	74.22	74.18	73.98	73.68

regression coefficient value of various models. The results indicated that release of drug from nanoparticles were diffusion-controlled as indicated by higher R^2 values in the Higuchi model. When the release data were analyzed using the Korsmeyer-Peppas equation, the 'n' values indicated that the mechanism of drug release from the chitosan nanoparticles was non-Fickian or anomalous diffusion [12].

3.7. Stability studies

The stability and chemical interaction of the drug in the nanoparticles were studied. Table 6 shows the stability studies results of formulation F-9. There was no change in the physical appearance. The total drug content in the formulation was determined at time 0 and after 1, 2, and 3 months of storage at room temperature (25 to 30 °C) and refrigerator (3 to 5 °C). It was observed that the initial drug content and the drug contents of the samples analyzed after 1, 2, and 3 months of storage at various conditions were similar, indicating there were no significant changes in the physical as well as chemical characteristics of the formulation.

Chemical interaction between the drug and polymer, if any, during the storage conditions was studied using FTIR (Figures 9-11). No significant changes were observed in the IR spectra of the pure drug and drug-loaded nanoparticles (F-9) after 3 months of storage at room temperature.

4. Discussion

The method used for the formulation of donepezil containing chitosan nanoparticles was ionic gelation method (using TPP as cross linking agent) followed by sonication to reduce the particle size.

The Z-average particle size of chitosan nanoparticles formulations ranged from 116.8 to 227.5 nm. All formulations showed uniform particle size distribution except formulations (F-5, F-6) which may be due to the lower concentration of the cross linking agent to the polymer; the (Chitosan: TPP) ratio used for formulations F-5 & F-6 were 3:1 and 4:1, respectively. XRD analysis confirmed the reduction in the crystallanity of donepezil in nanoparticles than the pure drug. Particle morphology was determined by scanning electron microscopy. The SEM image showed that each particle unit exhibited a nanostructure.

Nanoparticles formulations showed good results in terms of the assayed drug content and encapsulation efficiency. This indicates that the method used for the formulation produced good yield and it was suitable and reproducible in nature. Formulation (F-9) showed the highest encapsulation efficiency and drug content. It was found that as the concentration of chitosan and TPP increased, the % of encapsulation efficiency was also

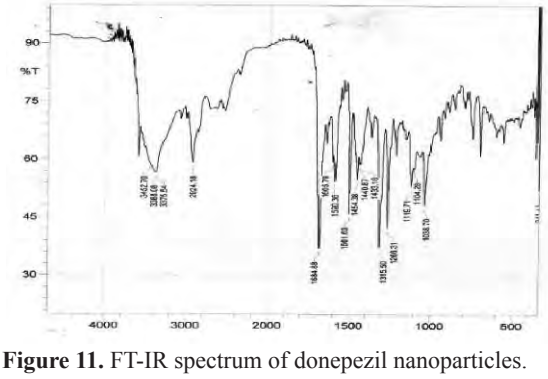

Figure 11. FT-IR spectrum of donepezil nanoparticles.

increased.

Permeation studies with natural membrane (egg membrane) were carried out as per the method reported. The formulations showed good drug release from the polymer, the *in vitro* drug release profiles of all the formulations showed an initial burst effect, and followed by a slow drug release. The burst release of drug is associated with those drug molecules dispersing close to the nanoparticle surface, which easily diffuse in the initial incubation time. The donepezil release was faster for those nanoparticles with higher drug content.

The release kinetics was characterized by fitting the data obtained from *in vitro* drug release studies of nanoparticles to standard release equations. The results indicated that release of drug from nanoparticles were diffusion-controlled as indicated by higher R^2 values in the Higuchi model. From the Korsmeyer-Peppas equation, the 'n' values indicated that the mechanism of drug release from the chitosan nanoparticles was non-Fickian or anomalous diffusion.

The stability and chemical interaction of the drug in the nanoparticles were also studied. There were no significant changes in the physical as well as chemical characteristics of the formulation. Chemical interaction between the drug and polymer, if any, during the storage conditions was studied using FT-IR. No significant changes were observed in the IR spectra of the pure drug and drug-loaded nanoparticles. These results indicated that the developed chitosan nanoparticles are physically and chemically stable and retain their pharmaceutical properties at various environmental conditions over a period of 3 months.

Despite enormous advances in brain research, CNS disorders remain the world's leading cause of disability, and account for more hospitalizations and prolonged care than almost all other diseases combined. This is because delivery of hydrophilic drugs to the brain is still a great challenge for the treatment of many brain-related diseases, in that hydrophilic drugs cannot cross the blood-brain barrier (BBB). The BBB represents an insurmountable obstacle for a large number of drugs, including anti-Alzheimer. Many studies have described the possibilities of using nanoparticles for targeting drugs in the CNS.

The present study revealed that ionic gelation technique followed by sonication can be used as an effective tool for preparation of donepezil nanoparticles, which may significantly improve its ability to cross BBB and targeting its delivery in the CNS. Further studies in animal models can be done to show the effectiveness of prepared nanoparticles *in vivo*.

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