

Development and evaluation of bio-nanoparticles as novel drug carriers for the delivery of Donepezil

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Abstract

The purpose of the present study was to formulate and evaluate donepezil loaded bio-nanoparticles for effective treatment of Alzheimer's disease. For the preparation of bio-nanoparticles biomaterial was isolated from fruits of *Carica papaya* by an economic method. The biomaterial recovered from the concentrate was subjected for various physicochemical properties like color, solubility, color changing point and chemical test. Bio-nanoparticles were prepared by modified nanoprecipitation method in different batches with variable drug/biomaterial ratio. Prepared batches were subjected for various evaluation studies like particle size, zeta potential, scanning electron microscopic studies, transmission electron microscopy, surface entrapment, *in-vitro* diffusion, differential scanning calorimetry and stability. Particle size and zeta potential result revealed that all nanoformulation were within range of 1.808 to 995.1 with slight negative in charge. Scanning electron microscopy and transmission electron microscopy report indicate that formulations were spherical in shape with less or no aggregation. Less surface entrapment leads to better drug entrapped inside nanomatrix. Bio-nanoformulations were capable of releasing the drug in a slow sustained manner. From the present investigation, it may be concluded that biomaterial isolated from fruits of *Carica papaya* used in the preparation of bio-nanoparticle act as an efficient carriers for deliver donepezil at a controlled rate and may significantly improve the ability to cross blood-brain barrier.

Keywords: Bio-nanoparticle; Diffusion study; Donepezil; Modified nanoprecipitation method; Stability study.

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INTRODUCTION

Alzheimer's (AHLZ-high-merz) is an irreversible, progressive brain disease that slowly destroys memory and thinking skills, and eventually even the ability to carry out the simplest tasks [1]. Alzheimer's disease (AD) is rapidly becoming a major public health concern. An estimated 20% of individuals aged >80 years are believed to be affected [2]. The annual incidence of AD increases with age, from about 1% in those aged 65 to 75 years to more than 8% in those aged >85 years [3, 4]. A sharp increase in the number of persons afflicted by this debilitating disease is anticipated as the proportion of the population >65 years continues to rise in Western countries [5]. Diseases of the Central Nervous System (CNS) such as

Alzheimer's disease require delivery of the drug to the brain for treatment. However such transport remains problematic, especially for hydrophilic drugs and large molecular weight drugs, due to the impervious nature of the endothelial membrane separating the systemic circulation and central interstitial fluid, the Blood-Brain Barrier (BBB) [6]. It is estimated that more than 98% of the small new molecules do not cross the BBB, and hence fail to achieve the therapeutic concentration within the brain parenchyma cells [7]. Many approaches have been developed to circumvent this problem using liposome [8] magnetic nanoparticles [9] solid lipid nanoparticles [10] and polymeric nanoparticles. Among these, polymeric nanoparticles are advantageous when compared

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with the aforementioned drug delivery systems in terms of obtaining a sustained release and better stability during storage [11]. Donepezil is a specific and reversible inhibitor of the enzyme acetyl cholinesterase. Acetyl cholinesterase 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 [12]. In this present study donepezil loaded bio-nanoparticles were prepared by using biomaterial isolated from fruits of *Carica papaya* belongs to family *Caricaceae* which may effectively cross BBB and increase drug concentration at its site of action and serve as an effective tool to treat AD.

EXPERIMENTAL

Materials required

Donepezil hydrochloride was a gift sample from Actavis (Chennai, India). The fresh *C. maxima* fruits were collected from plants growing in local area of Dehradun, India. Acetone was procured from SD fine chemicals (Mumbai, India). All other chemicals used were of analytical grade.

Isolation and characterization of biomaterial

Initially fruit pulp of *Carica papaya* was separated, skin was removed and minced with double distilled water and filtered and the filtrate was centrifuged at 4000 rpm for 5 min. the supernatant was then treated with acetone and set for refrigeration at 4°C for 4-6 hours. The bio-material was collected by centrifugation and kept in desiccators for 24 hours. After that the biomaterial was dried and passed through the sieve 100. The isolated biomaterial was subjected

for various physicochemical tests like color, odor, solubility, color changing point etc.

Preparation of bio-nanoparticles by modified nanoprecipitation method

Accurately weighed drug was dissolved in 70%v/v aqueous ethanolic solution. Weighed quantity of Biomaterial and propylene glycol were dissolved in specified ratio of Water: Acetone solution (10ml) and sonicated for 3 cycles 1 minute each. The drug solution was added to biomaterial solution drop wise with continuous stirring. After 5 minute mixing, the organic solvent was evaporated at 35°C temperature under normal pressure until the particles started to precipitate. Then the solution was sonicated for 5 minutes. Nanoparticles were separated by using cooling centrifuge (12,000rpm for 20 min) and dried in desiccators [13]. Five different batches of bio-nanoparticle were prepared by varying the concentration of biomaterial. Different composition batches were shown in Table 1.

Characterization of bio-nanoparticles

Particle Size distribution and Surface charge determination

The particle size and size distribution of the drug loaded nanoparticles was characterized by photon correlation spectroscopy (PCS) using a Zetasizer 2000 Malvern Instruments, UK. Nanopreparation was diluted with filtered (0.22 µm) ultra pure water and analyzed using Zetasizer. The surface charge determination was performed using an aqueous dip cell in an automatic mode by placing diluted samples (with ultra-purified water) in the capillary measurement cell and cell position was adjusted [14].

Table 1: Composition table of prepared drug loaded bio-nanoparticles using *Carica papaya*.

Sl.No.	Ingredient	Quantity Used				
		FCR1	FCR2	FCR3	FCR4	FCR5
1	Donepezil	10 mg	10 mg	10 mg	10 mg	10 mg
2	Biomaterial	10 mg	20 mg	30 mg	40 mg	50 mg
3	propylene glycol	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
4	Aqueous ethanolic solution (70%)	10 ml	10 ml	10 ml	10 ml	10 ml
5	Acetone	4 ml	4 ml	4 ml	4 ml	4 ml
6	Water	6 ml	6 ml	6 ml	6 ml	6 ml

Scanning electron microscopic studies (SEM)

The external morphology of nanoparticles was analyzed by scanning electron microscopy (SEM). The nanoparticles were fixed on supports, and coated with gold under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the scanning electron microscope at 200 kV [15].

Transmission electron microscopy (TEM)

A drop of nanosuspension was placed on a carbon film coated copper grid for Transmission electron microscopy (TEM). And the studies were performed at 80kV using JOEL JEM 2100, Japan equipped with a selected area electron diffraction pattern (SAED) [15].

Surface entrapment and %drug entrapped

Surface entrapment and %drug entrapped study was performed by centrifugation method. Accurate quantity of nanoparticles was taken in centrifuge tube with 10 ml of phosphate buffer pH 7.4. The amount of drug presented in clear supernatant after centrifugation for 30 min at 12,500 rpm was determined by UV spectroscopy. The amount of drug in supernatant was then subtracted from the total amount of drug added during preparation of nanoparticle (W). Effectively (W-w) will give the amount of drug entrapped [16].

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was performed by Perkin Elmer DSC (Model: JADE) instrument. Differential scanning calorimetry scan for drug and drug with bio-material was recorded at heating rate of 10°C/min in temperature range 30°-300°C under flow rate of nitrogen at 20 ml / min [17].

In vitro drug diffusion studies

Dialysis membrane diffusion technique was used to study *in-vitro* diffusion of drug from the prepared bio-nanoparticle formulations. The receptor medium used was freshly prepared phosphate buffer pH 7.4. Dialysis membrane (Molecular weight cut off > 12, 000, Hi media) previously soaked overnight in the receptor medium was on the Franz's Diffusion cell assembly. Prepared formulation was placed in the donor compartment and the assembly was kept on the multi station diffusion study apparatus (make Orchid Scientific) at 37 °C± 2 °C and stirred

at 700 rpm. Aliquots of 1 ml were withdrawn at pre-determined time intervals and immediately replaced by same volume of the fresh medium. The aliquots were suitably diluted with the dissolution medium and analyzed by UV-Vis Spectrophotometer at appropriate wavelength. The data obtained from the *in vitro* diffusion studies were fitted to various kinetic equations to find out the mechanism of drug release from the bio-nano formulation.

Stability Studies

Stability studies of prepared nanoparticles were carried out, by storing optimized formulation at 4 °C ± 1 °C and 30 °C ± 2 °C in stability chamber for 90 days. The samples were analyzed at 0, 1, 2, and 3 months for their drug content, drug release rate ($t_{50\%}$) as well as any changes in their physical appearance (ICH Q1A (R2) 2003)[18].

RESULTS AND DISCUSSION

Physicochemical evaluation of isolated biomaterial

Isolated bio-material from fruits of *Carica papaya* was subjected for various evaluation parameters. Our experimental results revealed that the isolated biomaterial was light yellow powder with a color changing point of 235 °C and percentage yield 25%. It was soluble in water, slightly soluble in chloroform and insoluble in alcohol.

Bio-nanoparticles were prepared successfully by modified nanoprecipitation method. The yield of formulated nanoparticles was observed between 22.59 to 50%. As the concentration of bio-material was increased the yield of nanoparticle was also increased.

Particle size distribution and surface charge

The particlesize of drug loaded bio-nanoparticles was analyzed by Zetasizer. The Z-particle size (r.nm) of bio-nanoparticles formulations ranged from 1.808 to 995.1 as shown in Table 2. The ability of nanoparticles to alter the bio-distribution 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 bio-distribution. Particles of 100 nm diameter with hydrophilic surfaces have a longer circulation in blood [19]. Such systems prolong the duration of drug activity and also increase the targeting

efficiencies to specific sites [20]. Particle size distribution graph (size distribution by intensity) for formulation (FCR4 and FCR5) is shown in Figs. 1 and 2. All formulations showed uniform particle size distribution except formulations FCR1 and FCR3. The electric charge present on the nanoparticles was evaluated by measuring the zeta potential. Zeta potential of all formulated nanoparticles was in the range of -2.86 to -6.16

mV (Table 2) which indicates moderate stability with no agglomeration.

Scanning electron microscopic study

Scanning electron microscopy micrograph of optimized drug loaded bio-nanoparticles showed that the particles have uniform loose aggregates in spherical shape with a smooth surface and they are uniformly distributed (Fig. 3).

Table 2: Evaluation of particle size and zeta potential.

Formulation Code	Particle Size		Zeta Potential (mV)
	Size (r.nm)	Mean Intensity (%)	
FCR1	265.6	0.2	-2.86
	307.6	1.4	
	356.2	4.8	
	412.5	10.6	
	477.7	17.2	
	553.2	21.7	
	640.7	21.2	
	741.9	15.4	
	859.2	6.9	
995.1	0.7		
FCR2	127.5	34	-6.16
	147.7	66	
FCR3	265.6	7.8	-4.83
	307.6	20.3	
	356.2	28.2	
	412.4	25.7	
	477.7	14.6	
FCR4	1.808	49.9	-4.77
	2.093	50.1	
FCR5	9.083	26.1	-5.05
	10.52	73.9	

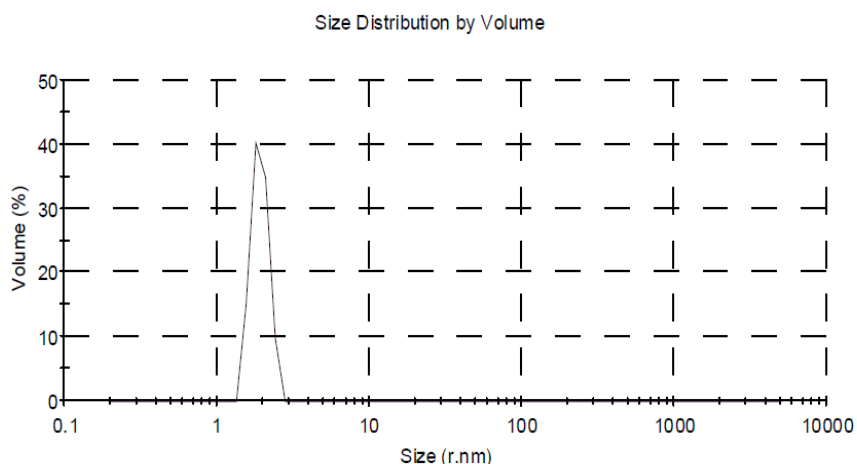


Fig. 1: Particle size distribution curve for Formulation FCR4.

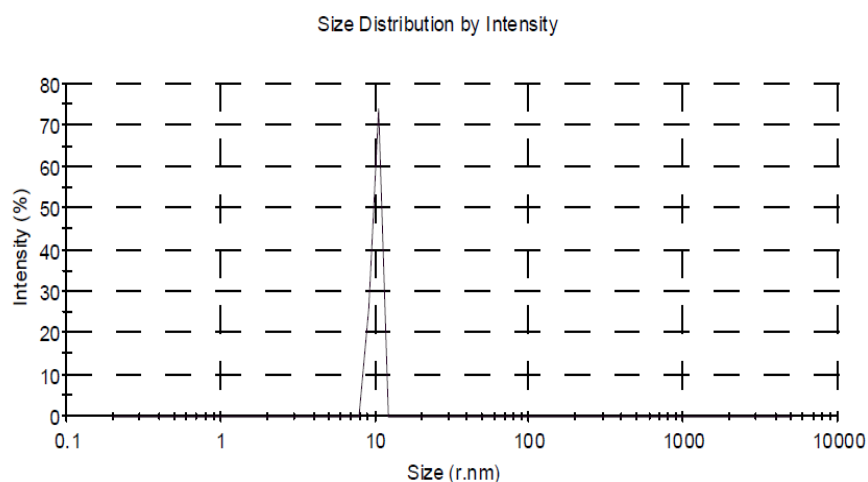


Fig. 2: Particle size distribution curve for Formulation FCR5.

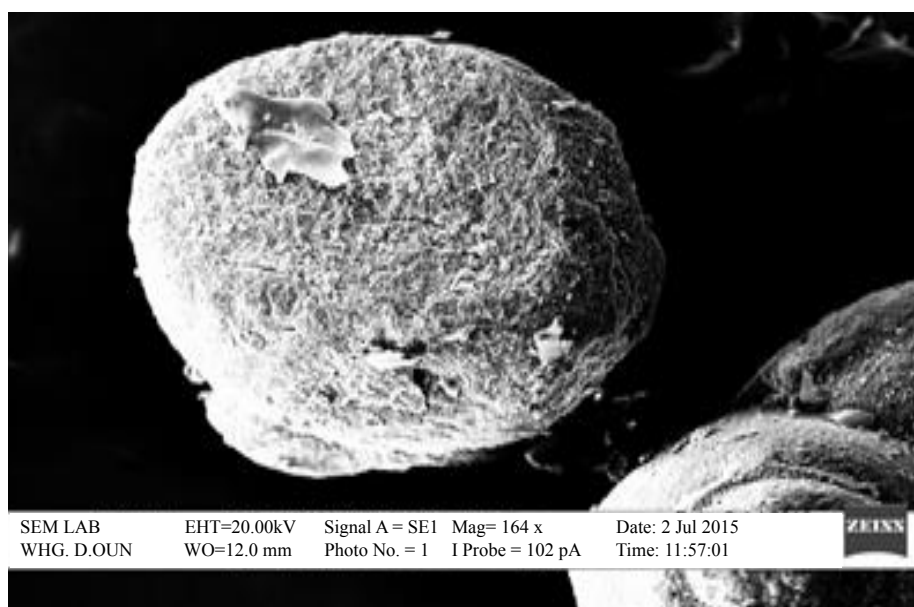


Fig. 3: SEM image of Formulation FCR4.

Transmission electron microscopic study

Transmission electron microscopic study of nanoparticulate dispersion (Fig. 4) further confirms the spherical shape of nanoparticle with less or no aggregation.

Surface entrapment and %drug entrapped

Surface entrapment and %drug entrapped study result clearly revealed (Table 3) that less amount drug was present on surface that means more amount of drug was entrapped inside the polymeric matrix. The drug entrapment

values increase with increase in biomaterial concentration.

Differential scanning calorimetry (DSC)

Thermal analysis is an important evaluation technique to find any possible interaction between the drug and used biomaterial. Any of such interaction may reduce the drug entrapment efficiency of the polymer and may also alter the efficacy of the drug. Such interaction can be identified by any change in thermo gram. The thermo grams of donepezil and drug loaded bio-

nanoparticle were studied. Sharp endotherm was obtained with donepezil at 136.82 °C. Drug loaded bio-nanoparticle showed peak at 127.72 °C (Figs. 5 and 6). All these endothermic peaks were obtained in same temperature range. This result suggests that no interaction had been taken place in bio-nanoparticles.

In vitro drug diffusion and kinetic study

In-vitro diffusion study of all different five batches of drug loaded bio-nanoparticles was performed using Franz's Diffusion cell assembly. The drug release profiles from the bio-nanoparticles are shown in Figs. 7. There was a sustained release of drug from all prepared nanoparticulated formulation with no burst release. Burst effect is generally associated with surface entrapment. Experimental result showed that all formulations release more than 75% of drug during 24 hours study period. Biomaterial concentration does not affect greatly on *in-vitro* diffusion profile of drug. Kinetic models describe drug release from immediate and modified release dosage forms. Thus the model fitting

analysis (Zero Order, Higuchi, Hixon Crowell, First Order and Korsmeyer–Peppas Model) were done by comparing the coefficient of regression (R^2) values and corresponding N value of all the kinetic equation. The correlation coefficient (R) values were used as criteria to choose the best model for the drug release from the bio-nanoparticulated formulation. From Table 4 it was observed that the individual formulation having different R^2 value for different model. On the basis of higher value of R^2 we select the best fit model. Now Korsmeyer–Peppas Model poses great importance to know the release mechanism of the drug from the formulation [21]. Result indicated that 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 bio-nanoparticles was followed super case 2 transports. On the basis of experimental result formulation FCR4 was selected as optimized formulation and subjected for stability study.

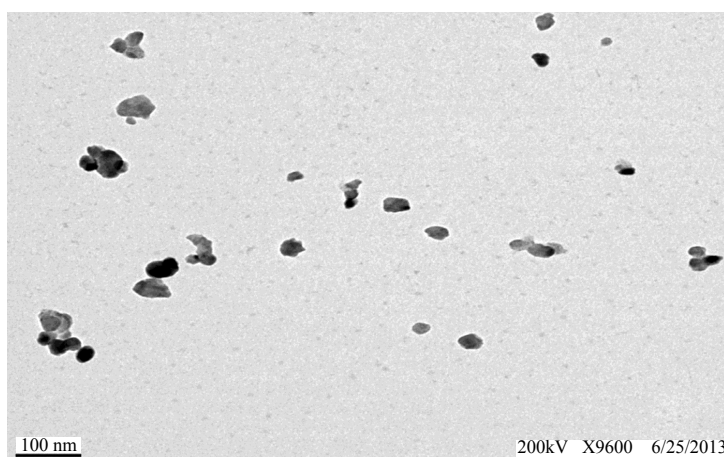


Fig. 4: TEM image of Formulation FCR4.

Table 3: Surface Entrapment and Drug Content study.

Sl.No.	Formulation code	Surface entrapment (mg)	Drug Entrapped (%)
1.	FCR1	1.736	82.64
2.	FCR2	1.552	84.48
3.	FCR3	1.341	86.59
4.	FCR4	1.005	89.95
5.	FCR5	1.001	89.99

Stability study

Stability studies of FCR4 bio-nanoparticles were carried out, by storing optimized formulation at 4 °C ± 1 °C and 30 °C ± 2 °C in stability chamber for 90 days. The samples were analyzed at 0, 1, 2, and 3 months for their drug release rate ($t_{50\%}$), drug content as well as any changes in their physical

appearance. As the experiment result revealed (Table 5) no significant changes were observed in the physical appearance, drug content as well as drug release rate. These results indicated that the developed drug loaded bio-nanoparticles are physically and chemically stable and retain their pharmaceutical properties at various environmental conditions over a period of 3 months.

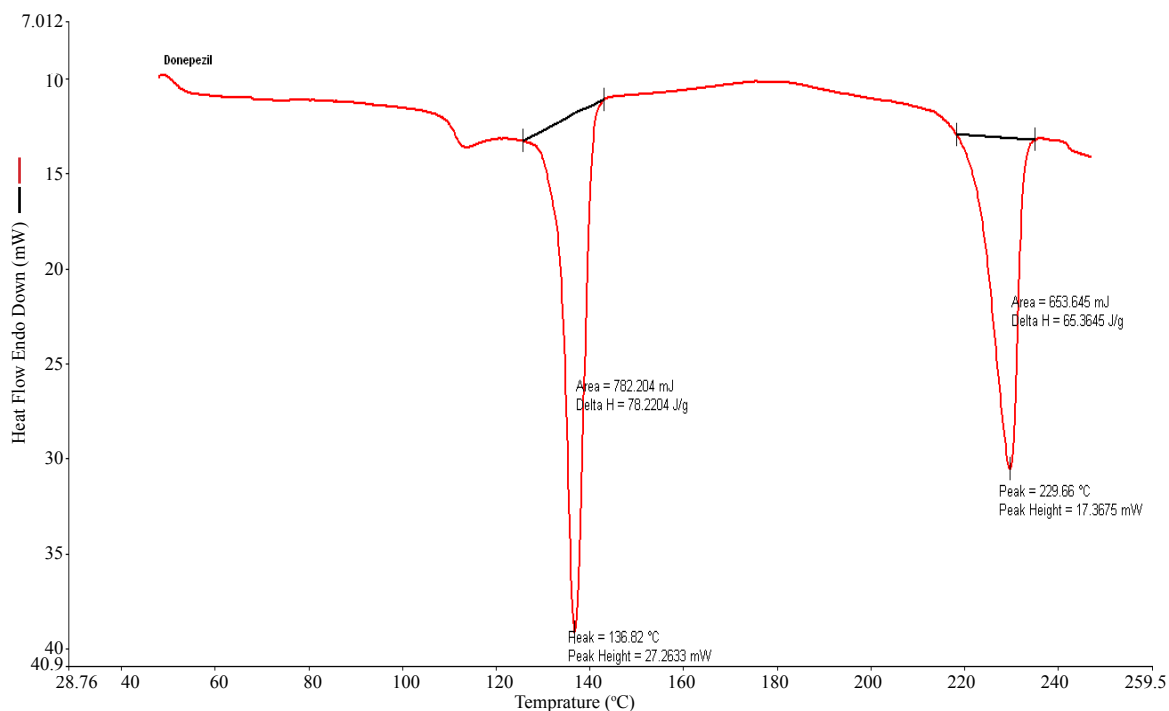


Fig. 5: DSC thermo grams of Donepezil.

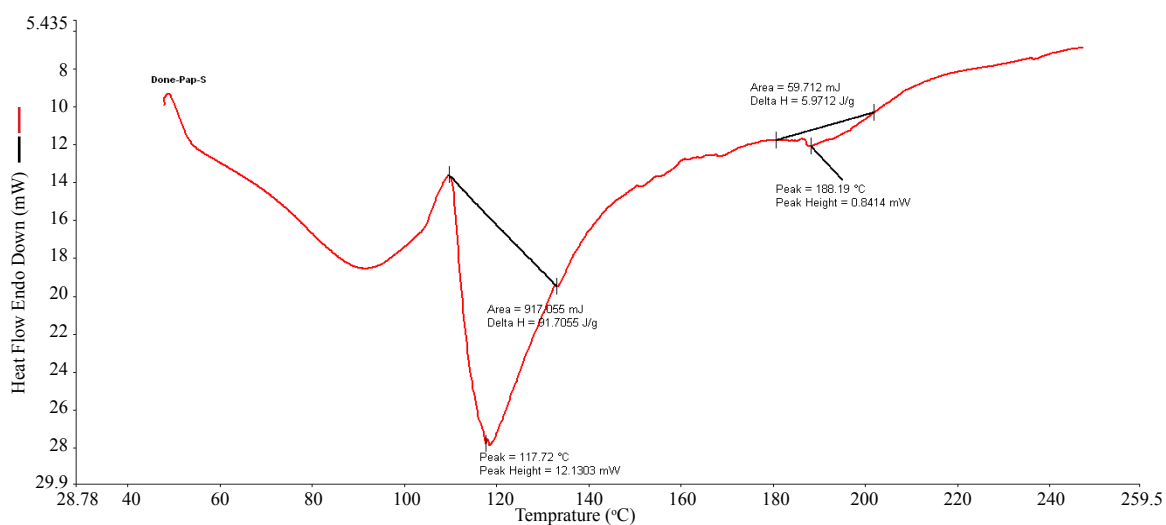


Fig. 6: DSC thermo grams of Donepezil loaded bio-nanoparticle formulation.

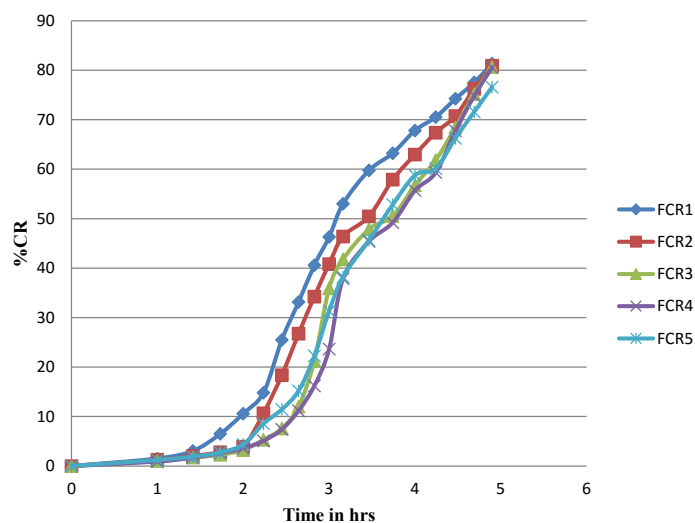


Fig. 7: In-vitro release profile study.

Table 4: In-vitro kinetic study.

Formulation code	Zero order R ²	First order R ²	Higuchi matrix R ²	Hixson crowell R ²	Peppas R ²	N	Mechanism of release
FCR1	0.9302	0.9858	0.9492	0.9744	0.9509	1.3292	Supercase 2 Transport
FCR2	0.9554	0.9849	0.9508	0.9840	0.9337	1.5159	Supercase 2 Transport
FCR3	0.9588	0.9593	0.9552	0.9685	0.9442	1.6204	Supercase 2 Transport
FCR4	0.9674	0.9483	0.9520	0.9637	0.9644	1.6150	Supercase 2 Transport
FCR5	0.9698	0.9751	0.9498	0.9795	0.9630	1.5049	Supercase 2 Transport

Table 5: Stability study data of formulation FCR4.

Temperature	Evaluation Parameters	Observation (Months)			
		0	1	2	3
30°C ± 2°C	Physical appearance	---	No change	No change	No change
	%Drug content	72.65	71.54	71.12	70.76
	t _{50%} (hrs)	14.03	13.68	13.57	13.43
4°C ± 1°C	Physical appearance	---	No change	No change	No change
	%Drug content	72.65	72.47	71.91	70.29
	t _{50%} (hrs)	14.03	14.01	13.76	13.81

CONCLUSION

Donepezil loaded bio-nanoparticles were successfully prepared by modified nanoprecipitation method. The concentration of biomaterial used for formulating these batches of nanoparticles showed significant effect on its efficiency to entrap donepezil molecule. Donepezil loaded bio nanoparticles with a small size and narrow size distribution were obtained. *In-vitro* release study revealed that donepezil loaded bio-

nanoparticles were capable of releasing the drug in a slow sustained manner. From the present investigation, it may be concluded that biomaterial isolated from fruits of *carica papaya* used in the preparation of bio-nanoparticle act as an efficient carriers for deliver donepezil at a controlled rate. It may significantly improve the ability to cross blood-brain barrier and serve as an effective tool to treat Alzheimer's disease.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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