ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Special Issue 4, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

FORMULATION OF POLYMERIC NANOSUSPENSION CONTAINING PRAMIPEXOLE DIHYDROCHLORIDE AND HESPERIDIN FOR IMPROVED TREATMENT OF PARKINSON'S DISEASES

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Received: 10 October 2018, Revised and Accepted: 11 December 2018

ABSTRACT

Aims and Objectives: The present study is to formulate the nanosuspension containing a hydrophilic drug pramipexole dihydrochloride and hesperidin and to increase the drug entrapment efficiency.

Methods: Hesperidin and pramipexole dihydrochloride loaded in chitosan nanosuspension is prepared by ionic gelation method using chitosan and tripolyphosphate. There was no incompatibility observed between the drug and polymer through Fourier transform infrared and differential scanning calorimetric. Various other parameters such as particle size, zeta potential, scanning electron microscope, drug content, drug entrapment efficiency, and *in vitro* release have been utilized for the characterization of nanoparticles.

Results and Discussion: The average size of particle is 188 nm; zeta potential is 46.7 mV; drug content of 0.364±0.25 mg/ml; entrapment efficiency of 72.8% is obtained with HPN_3 formulation. The PHC1 shows the highest drug release followed by PHC2 due to low concentration of polymer and PHC4 and PHC5 show less drug release due to high concentration of polymer. The *in vitro* release of PHC3 is 85.2%, initial the burst release is shown which is approximately 60% in 8 h; then, slow release later on drastic reduction in release rate is shown in 24 h. The *in vivo* study histopathological report confers the effective protective against rotenone induces Parkinson's.

Conclusion: PHC3 was chosen as the best formulation due to its reduced particle size and controlled release at optimum polymer concentration which may be used to treat Parkinson's disease effectively.

Keywords: Parkinson's disease, Nanoparticles, Rotenone, Reactive oxygen species, Chitosan.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11s4.31721

INTRODUCTION

Nanoparticles are the front position of the fast-developing field with a potential interest in clinical medicine, delivery of drug and in research, as well as in different field of sciences [1]. It overcomes this problem due to its smaller particle size and may produce therapeutic effect in a particular site which offers the possibility to develop new therapeutics [2]. These may overcome many problems due to particle size, therapeutic effect in mainly due to the particular site which offers the possibility to develop new therapeutics [3]. Any drug delivery systems for brain disease have a lower efficacy due to insufficient bioavailability in the targeted site [4]. To overcome these problems, nanoparticles may be effective for its smaller particle size and may produce therapeutic effect in a particular site which offers the possibility to develop new therapeutics [3].

Is not sufficient only developing a new drug alone, to provide the progress in drug therapy and several research data give a disappointing results due to poor absorption, rapid metabolism and elimination lead to insufficient drug concentration at the targeted site, high fluctuation of the plasma levels due to unpredictable bioavailability after oral drug administration with poor drug solubility [5-7].

Oxidation is the main process in augmenting the energy production. However, oxidative stress is the predictable main cause which enhances the progression of disease. This condition arises when there is a reduction in antioxidant defenses or overproduction in reactive oxygen species (ROS). ROS is generated at the time of injury or inflammation. There are several other sites for ROS production such as mitochondrial electron transport. The Parkinson's disease is more prevalent in person with about 65 years of age concomitant with genetic influence, toxins, oxidative stress, mitochondrial abnormalities, free radicals, and alpha-synuclein aggregation in a higher rate, which is considered to be the main cause for Parkinson disease. A free radical is an atom or group with at least one unpaired electron. It is usually an antioxidant molecule that has lost and needs to stabilize itself by stealing an electron from a nearby molecule. Antioxidant can neutralize the free radical by accepting or donating an electron to eliminate the unpaired condition [8-12]. Antioxidant is effective in the treatment of cancer, coronary heart disease, and neurodegenerative diseases. It is also used in the treatment of fried Reich ataxia [13]. Bioactive derivatives acting as flavonoids, stilbenoids, and alkaloids possess potent antioxidative and anti-inflammatory properties are now being inducing in the treatment of Parkinson's disease [14]. Flavonoids like hesperidin have been proved to have an antioxidant effect against neurodegenerative disease [15].

Pramipexole is a well-known anti-parkinsonism drug which produces toxicity as a side effect with frequent administration in dose regimen only a minimal amount of the drug crosses the blood–brain barrier [16,17]. Pramipexole dihydrochloride is having manageable bioavailability due to its high first-pass metabolism and poor penetrability through blood–brain barrier due to its hydrophilic nature. Therefore, more amount of the drug is required to achieve the required therapeutic activity. However, in nanosuspension with small amount of drug which can achieve the better therapeutic activity. Nanosuspension is a novel drug delivery system which makes the drug lipophilic and also degradation of drug is protected [3,6]. Due to this, the drug can able to penetrate the

blood–brain barrier easily for targeting the brain disorder with increased bioavailability [7,18] capillary endothelial cells. A_2A adenosine receptor is a member of G protein-coupled receptor. Considering the properties of biodegradable nanoparticles essentially grand bioavailability, control release, less toxicity, and better encapsulation, they are frequently used as a vehicle in drug delivery. Apparently used biodegradable nanoparticle includes PLGA, PLA, chitosan, gelatin, polycaprolactone, and poly-alkyl-cyanoacrylates [19,20]. Chitosan is an amino polysaccharide which possesses the idiosyncratic features just as non-toxicity, hydrophilicity, biodegradability, and mechanical strength; biocompatibility and physical inertness are quotidianly used as a carrier for targeted drug delivery in neurodegenerative disorder. It possesses higher penetration capacity, absorption across the mucosal epithelia, and the potential of binding with various ligand molecules and helps in the formation of stable noncomplex [21,22]. It is cationic in nature which involves in the formation of stable ionic complexes with multivalent anionic ions or polymers in various forms such as gels and micro/nanoparticles [23,24]. It has an antioxidant activity which is used here as a polymer produced from the exoskeleton of crustaceans (e.g., crabs and shrimps).

The nanosuspension is a newer drug delivery system in the pharmaceutical field to overcome all these above problems. The present study is to prepare a polymeric nanosuspension containing a combination of pramipexole dihydrochloride and antioxidant hesperidin for treating Parkinson's disease.

METHODS

Hesperidin, pramipexole dihydrochloride, chitosan, and tripolyphosphate (TPP) are procured in Sigma-Aldrich. The chemicals which are used in the present work are of analytical grade.

Compatibility studies

Differential scanning calorimetric (DSC) analysis

DSC measurements are done by utilizing the instrument NETZSCH DSC 204. The samples are estimated at 0°C–300°C temperature where the heating rate is 5°C/min in the presence of nitrogen atmosphere. The aluminum pans are used for keeping the samples.

Fourier transform infrared (FTIR) analysis

FTIR analysis of pure hesperidin, chitosan, and mixture (pramipexole dihydrochloride, hesperidin, chitosan, and TPP) was performed and spectrum was obtained using FT-IR (Perkin Elmer 200 series). All spectra were recorded within a range of 2000–650 cm^{-1}.

Preparation of nanoparticles containing pramipexole dihydrochloride

Ionotropic gelation process is adopted for the preparation of nanoformulation.

1. Chitosan solution was prepared by dissolving in 100 ml of 1% v/v acetic acid and the resulting solution is stirred at 1500 rpm in magnetic stirrer for 30 min (different concentrations of chitosan solution such as 1%, 2%, 3%, 4%, and 5% were prepared).
2. TPP solution (1%w/v) is prepared in deionized water where 100 mg of TPP is dissolved in 100 ml.
3. Add 100 mg pramipexole dihydrochloride and hesperidin to the 1% TPP solution and mix to form a homogenous mixture by stirring with a glass rod.
4. Add the above mixture of TPP to pramipexole dihydrochloride and hesperidin solution drop by drop (10 ml) to the chitosan solution and kept stirring at 2500 rpm for 3 h on mechanical stirrer.

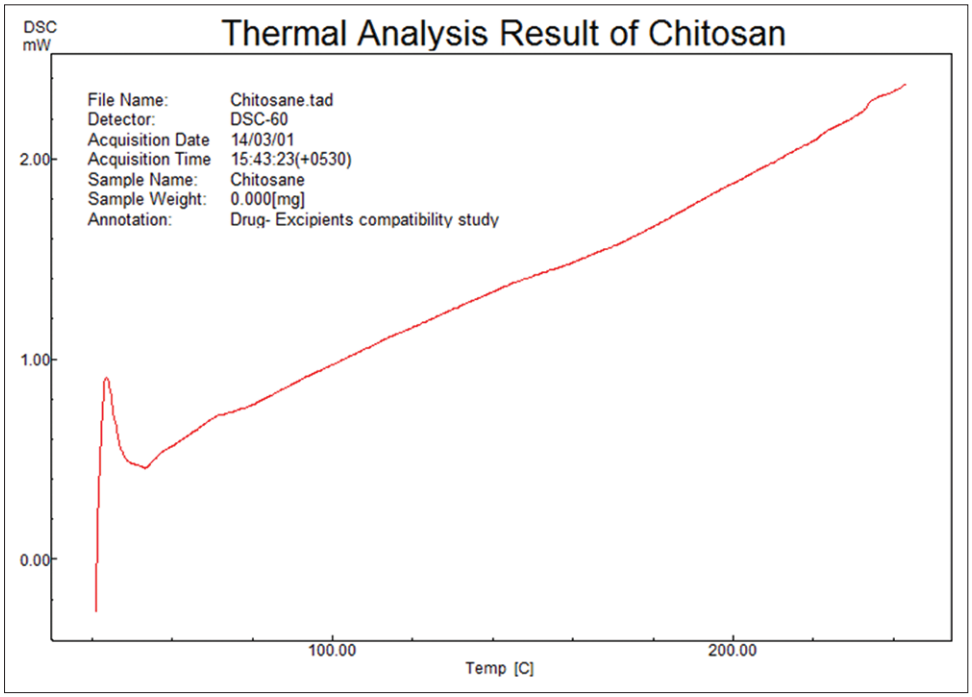

Fig. 1: Differential scanning calorimetric of chitosan

Table 1: Formula for preparation

S. No	Ingredients	PHC1	PHC2	PHC3	PHC4	PHC5
1	Pramipexole dihydrochloride (mg)	100	100	100	100	100
2	Hesperidin (mg)	100	100	100	100	100
3	Chitosan (%)	1	2	3	4	5
4	Tripolyphosphate (ml)	100	100	100	100	100
5	1% acetic acid solution (ml)	1	1	1	1	1

5. TPP solution is added to the aqueous solution containing pramipexole dihydrochloride and hesperidin with chitosan polymer, where the nanosuspension is formed.
6. The obtained nanosuspensions are centrifuged in high-speed centrifuge (Sigma) at 15,000 rpm for 10 min. Discard the sediment and preserve the supernatant.
7. The negative and positively charged groups in TPP as well as in chitosan interact themselves to form nanoparticle (Table 1).

Characterization of nanoparticles
The nanoformulation with pramipexole dihydrochloride and hesperidin was studied for different parameters.

Particle size
The size of particles in the nanoformulation is studied using photon correlation spectroscopy. The samples are diluted using ultra-purified water; then, it is seen in scattering at 90° angle at 25°C. The mean of hydrodynamic diameter for every sample is generated in triplicate.

Zeta potential
Zeta potential was characterized by utilizing an instrument as Zetasizer. The Zeta measurements were performed using an aqueous dip cell in an automatic mode by placing diluted samples in the capillary measurement cell and cell position is adjusted.

Surface morphology
The particles surface morphology is determined by utilizing scanning electron microscopy (SEM) where 200 kV is set for placing the nanoformulation samples in an air-dried condition on a copper grid.

Drug content
The total amount of drug present in nanoformulation is estimated by spectrophotometrically. An aliquot range from 0.50 ml of nanoformulation is evaporated under reduced pressure at 35°C. The resultant residue is then dissolved in water, which is filtered through a 0.45 µm size filter and assayed spectrophotometrically at 263 nm.

Drug entrapment efficiency
It is also known as association efficiency. The drug-loaded nanosuspension is centrifuged at 3500–4000 rpm for 30 min, where the supernatant is collected. Is assayed for non-bound drug concentration by UV spectrophotometer.

***In vitro* release studies**
In vitro diffusion studies (release studies) are determined using diffusion apparatus. A semipermeable membrane was supported on a ring of diffusion cell and the sample was kept on a membrane in such a way backing layer was phased toward donor compartment. The glass beaker was filled with 100 ml of phosphate buffer of pH: 6.8 at a temperature 37°C sample of 2 ml was withdrawn at regular intervals from glass beaker for analysis. 2 ml of phosphate buffer was replaced immediately after sampling to maintain volume equal to 100 ml. The absorbance of sampling was measured at 263 nm using UV spectrophotometer.

***In vivo* animal studies**
Animals weighing 150–200 g of male rats of Wistar strain are selected for the study where housed in a controlled room temperature with 12:12-h light/dark cycles is maintained, supplement of food and water is done. The care for in laboratory is given according to the Animal Ethical Committee which is formed for Control and Supervision of Experiments on Animals, Chennai, and Govt. of India. The protocol for the experimental is approved by the Institutional Ethical Committee. Rotenone to induce parkinsonism is purchased from Sigma-Aldrich Chemical Company (Bangalore, India).

Dosing and treatment
Animal was divided into five groups and each group containing six animals.
- Group A: Control rats received saline (2 ml/kg by per oral route) for 10 days.
- Group B: Animals received rotenone (2.5 mg/kg) dissolved in saline and administered intraperitoneally for 10 days.
- Group C: Animals received nanosuspension (PHC3) (drug equivalent to 0.8 mg/ml/day/kg) are given orally by gavage once daily 30 min before rotenone (2.5 mg/kg) dissolved in saline for 10 days.

Table 2: Particle size nanoformulation

S. No	Formulation	Ratio	Particle size
1	PHC1	1:1	646
2	PHC2	1:2	523
3	PHC3	1:3	188
4	PHC4	1:4	326
5	PHC5	1:5	452

Table 3: Zeta potential nanoformulation

S. No	Formulation	Ratio	Zeta potential
1	PHC1	1:1	62.6
2	PHC2	1:2	52.8
3	PHC3	1:3	46.7
4	PHC4	1:4	45.4
5	PHC5	1:5	44.7

Table 4: Drug content and entrapment efficiency of nanoparticle

Formulation	Average drug content (mg/ml)	Average entrapment efficiency (%)
PHC1	0.257	51.5
PHC2	0.335	67.1
PHC3	0.364	72.8
PHC4	0.452	90.5
PHC5	0.468	93.6

Table 5: *In vitro* release study of nanofrmulations

S. No	Time (h)	% cumulative drug release				
		PHC1	PHC2	PHC3	PHC4	PHC5
1	0	0	0	0	0	0
2	1	9.8±1.2	8.1±1.3	7.2±1.7	6.7±1.5	6.1±1.3
3	2	26.1±1.8	11.2±1.2	10.8±1.5	11.1±2.3	9.2±1.6
4	4	48.2±1.4	33.7±1.5	21.2±1.3	22.7±2.6	15.9±2.1
5	6	54±1.7	46.1±1.4	33.5±0.9	30±0.9	25.7±1.9
6	8	77.4±1.2	57.5±1.3	42.1±2.2	43.1±1.2	36.4±1.8
7	12	98.6±1.6	73.1±1.5	54.6±1.9	57.3±1.6	48.3±2.1
8	16	-	84.3±1.2	62.3±2.2	63.8±1.1	60.5±1.4
9	20	-	97.4±1.7	75.7±1.3	72.2±1.4	68.7±1.3
10	24	-	-	85.2±1.5	80.2±1.3	75.1±1.7

*±values indicate the triplicate trials

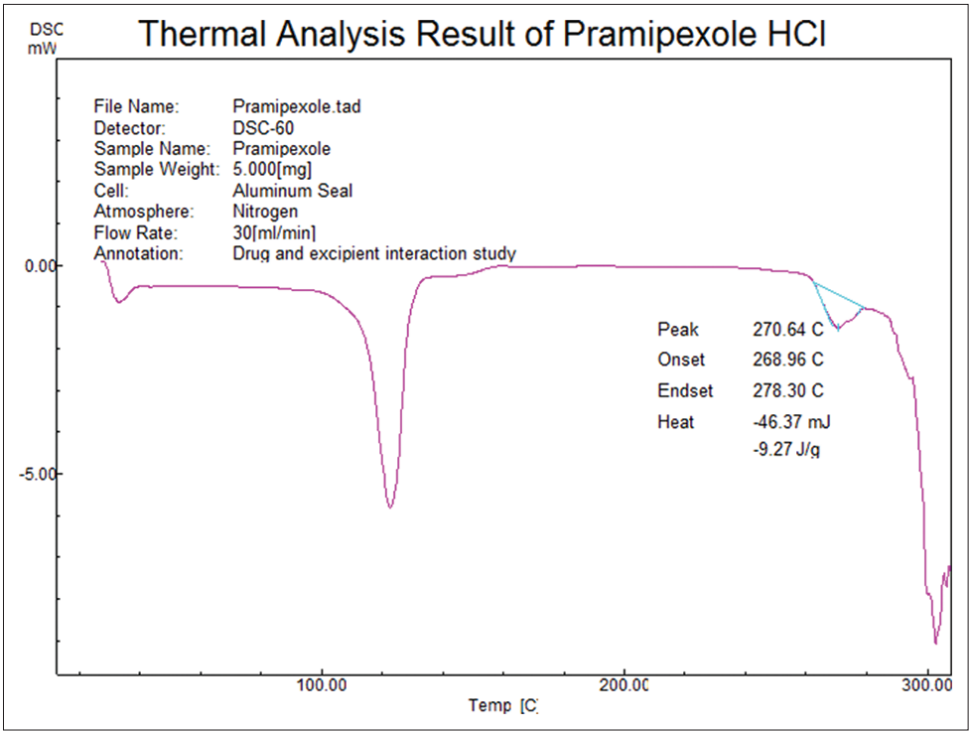

Fig. 2: Differential scanning calorimetric spectrum of pramipexole dihydrochloride

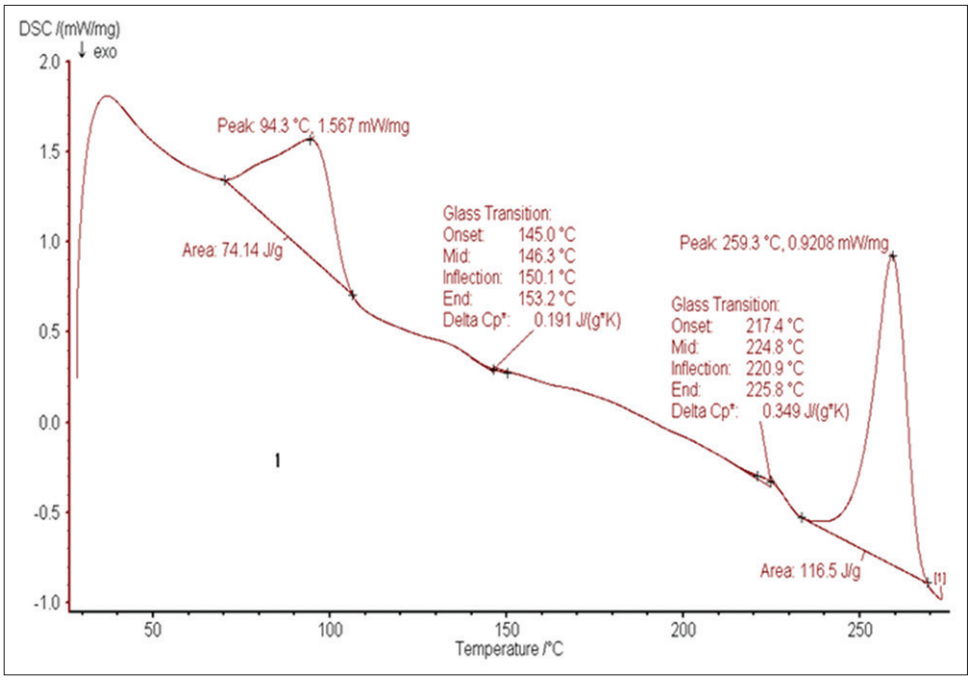

Fig. 3: Differential scanning calorimetric spectrum of hesperidin

- Group D: Animals received pramipexole dihydrochloride pure drug (1 mg/kg body weight/day) and hesperidin pure drug (1 mg/kg) were administered by oral gavage once daily 30 min before rotenone (2.5 mg/kg) dissolved in saline for 10 days.

RESULTS AND DISCUSSION

Compatibility studies

DSC

DSC studies were carried out for pramipexole dihydrochloride, hesperidin, chitosan, and mixtures of pramipexole dihydrochloride-chitosan-hesperidin by DSC. It was found that there was no interaction between drug and polymer (Figs. 1-4).

FTIR

FTIR spectroscopic studies were carried out for the standard pramipexole dihydrochloride, chitosan, TPP, and a mixture of pramipexole dihydrochloride-chitosan-TPP by KBr pellet technique using FTIR spectrophotometer. FTIR spectrum of standard is compared with that of mixture and found that there is no interference (Figs. 5-8).

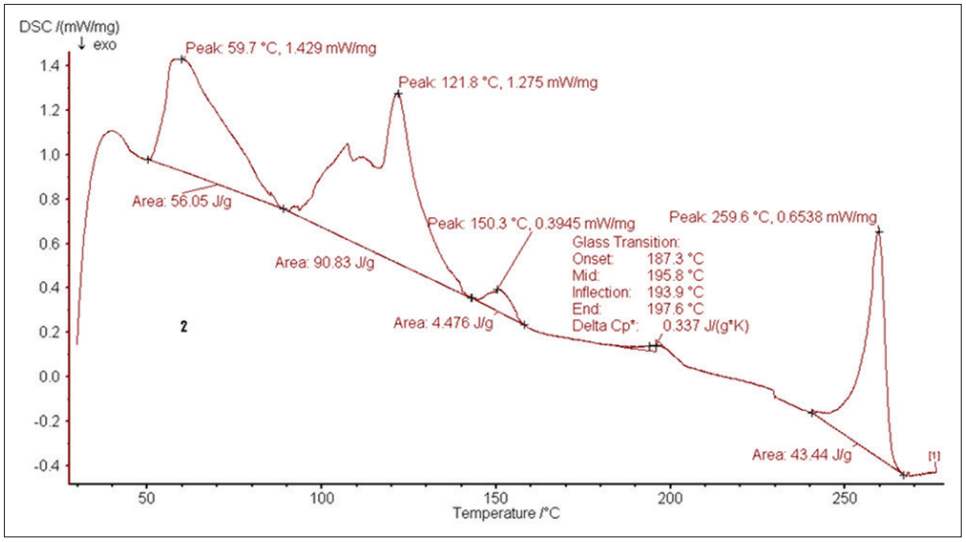

Fig. 4: Differential scanning calorimetric spectrum of mixture with drug and polymer

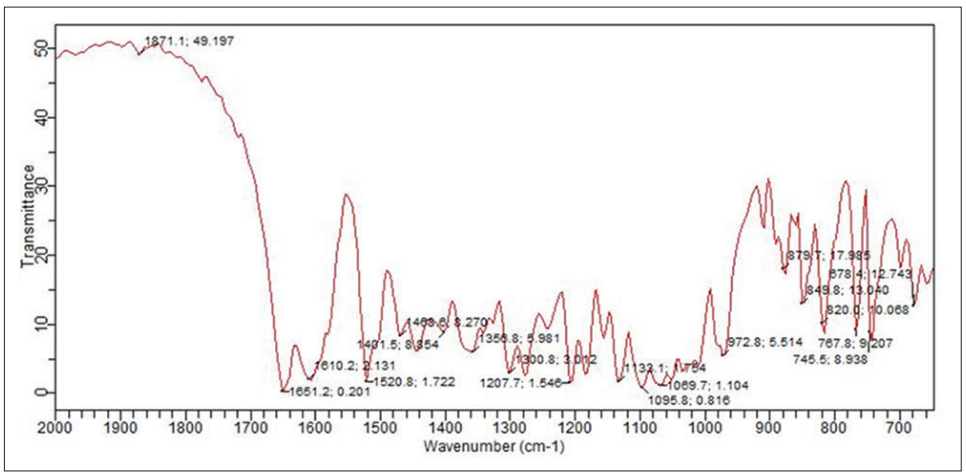

Fig. 5: Fourier transform infrared of pramipexole dihydrochloride

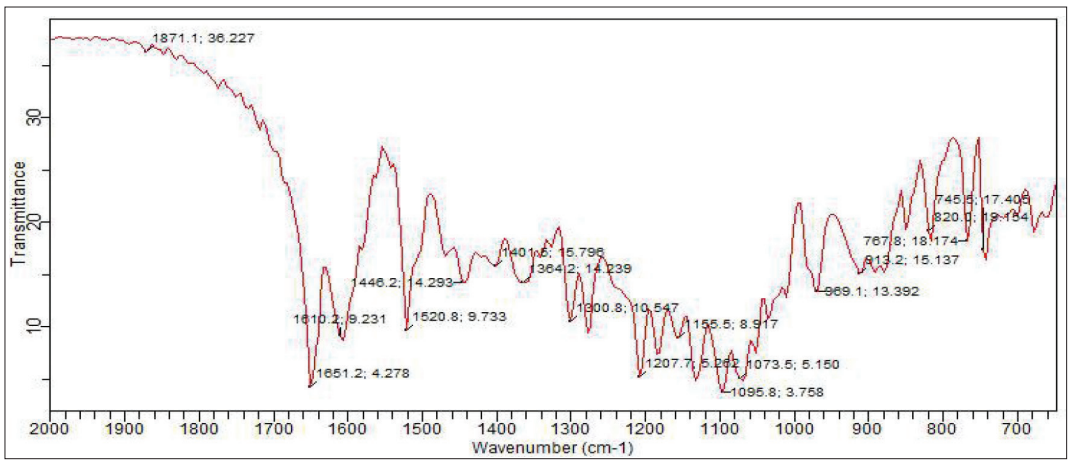

Fig. 6: Fourier transform infrared of chitosan

Preparation of pramipexole dihydrochloride and hesperidin nanoformulation

Pramipexole dihydrochloride and hesperidin-loaded chitosan nanoparticles were prepared by ionotropic gelation method. Two batches of process optimized formulations were prepared with varying concentrations of chitosan. The prepared nanoparticle formulations were found to be turbid and stable. Moreover, they were packed in air-tight containers and stored in a cool place and used for further studies.

Characterization of nanoparticles

Measurement of the particle size of nanoparticles

The particle sizes of prepared nanoparticles were measured from the microphotograph of 100 particles. The particle size ranged from 252 nm

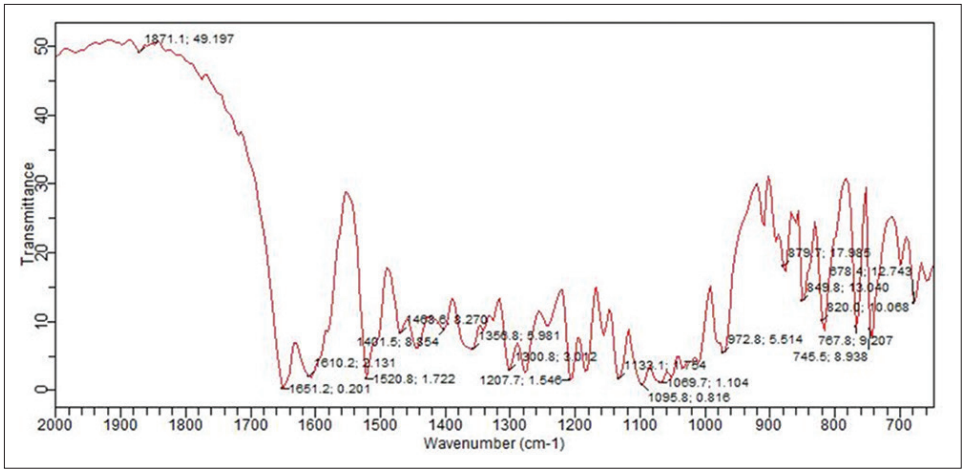

Fig. 7: Fourier transform infrared of hesperidin

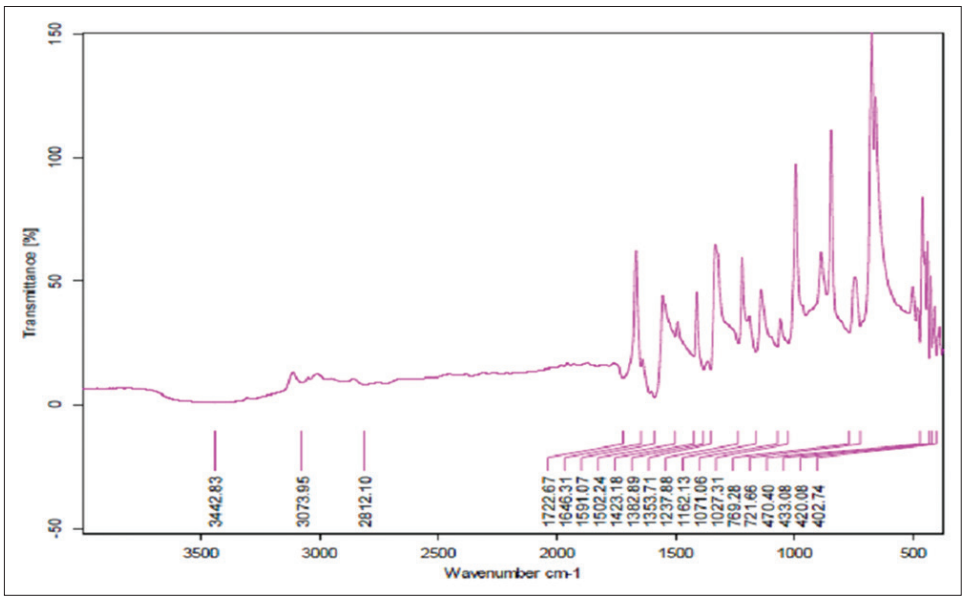

Fig. 8: Fourier transform infrared of pramipexole dihydrochloride, hesperidin, and chitosan

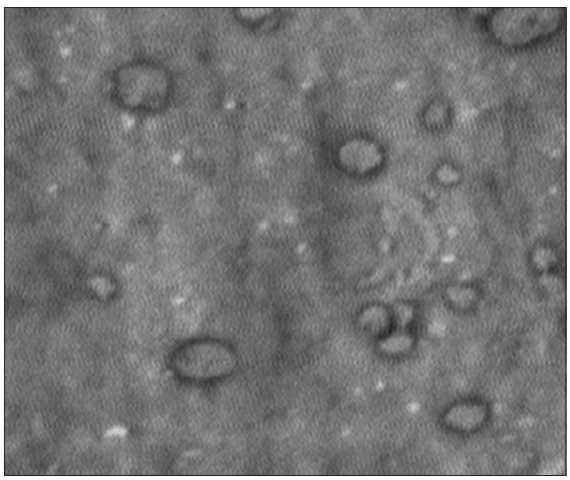

Fig. 9: Scanning electron microscopy images of pramipexole dihydrochloride and hesperidin in chitosan nanoparticles

to 432 nm for various batches. The increase in the concentration of the polymer ratio caused an increase in particle size (Table 2).

Zeta potential

The zeta potential of PHC3 is 46.7 having better particle, so this formulation may be the best formulation (Table 3).

Drug content and entrapment efficiency

The total drug content of PHC3 is having 0.364 with entrapment efficiency of 72.8 shows a better drug content with better entrapment efficiency (Table 4).

SEM

SEM analysis of the prepared formulation was carried out to understand the morphology of nanoparticles. In the SEM images indicate that the nanoparticles were discrete, uniform, and spherical with a smooth surface. Hence, the images show that proper expected shape has been achieved (Figs. 9 and 10).

***In vitro* release studies**

In vitro diffusion studies (drug release studies) were performed using diffusion apparatus. A semipermeable membrane was supported on a ring of diffusion cell and the sample was kept on a membrane in such a way backing layer was phased toward donor compartment. The glass beaker was filled with 100 ml of phosphate buffer of pH=6.8 at

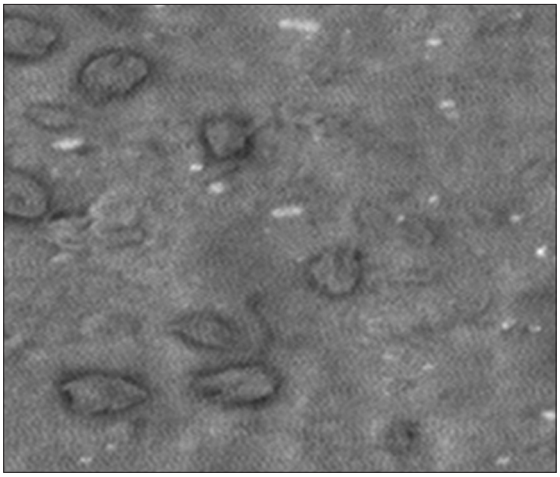

Fig. 10: Scanning electron microscopy images of pramipexole dihydrochloride and hesperidin in chitosan nanoparticles

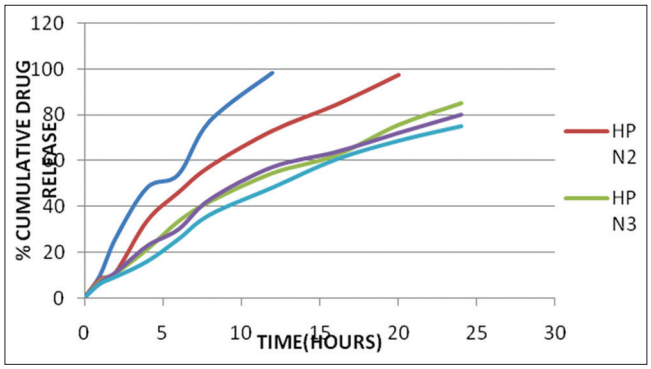

Fig. 11: *In vitro* drug release studies

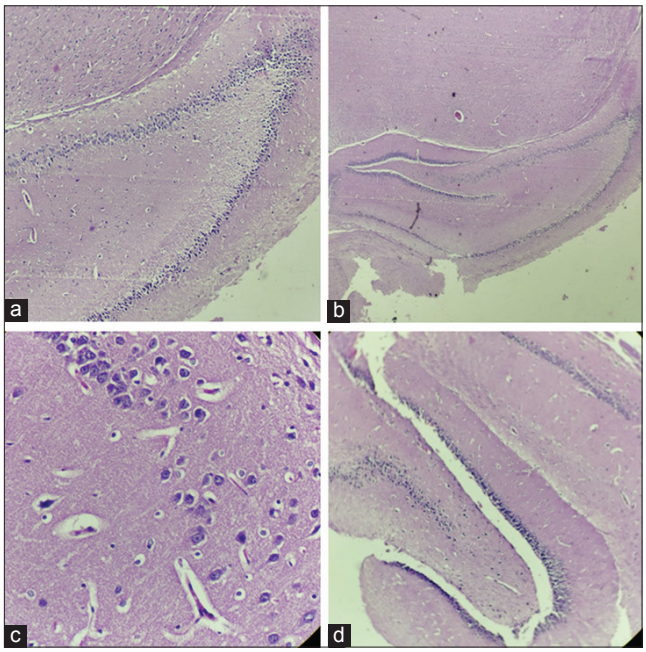

Fig. 12: Plates containing histopathological studies in experimental animals. (a) Control (×400) (normal saline), (b) Rotenone (2.5 mg/kg), (c) PHC3 (drug equivalent to 0.8 mg/ml)+rotenone (2.5mg/kg), (d) Pramipexole dihydrochloride (1 mg/kg body)+hesperidin pure drug (1 mg/kg)

a temperature 37°C sample of 2 ml was withdrawn at regular intervals from glass beaker for analysis. 2 ml of phosphate buffer was replaced immediately after sampling to maintain volume equal to 100 ml. The absorbance of sampling was measured at 263 nm using UV spectrophotometer (Table 5).

In this, PHC1 shows the highest drug release followed by PHC2 due to low polymer concentration. PHC4 and PHC5 show less drug release due to higher polymer concentration. The *in vitro* release of PHC3 is 85.2% showed an initial burst release of approximately 60% in the first 8 h, followed by a slow and much reduced additional release for about 24 h (Fig. 11).

In vivo studies

Histopathological evaluation of rat brain in experimental animal

Histopathology images of rat brain in experimental animal are in Fig. 12, it was observed that the rotenone-induced Group B rat shows degeneration of nucleus along with cell shrinkage due to the accumulation of more nucleus in one cell. This nanosuspension-treated Group C has been reversed when compared to induced Group B, whereas Group D shows same as control, which supports the nanoformulation treatment reveals the rotenone-induced degeneration of neural cells which further supports the nanosuspension is more effective in the treatment of parkinsonism diseases.

CONCLUSION

There was no incompatibility observed between drug and polymer. The particle size was found to be 188 nm for formulation PHC3. The entrapment efficiency was found to be 72.8% for formulation PHC3. More than 85% of drug release was observed in PHC3. Therefore, PHC3 was chosen as the best formulation as they have better release than other formulations. The result supports the biodegradable polymers which help in the production of more controlled release dosage form for the treatment in Parkinson's disease.

REFERENCES

1. Kreuter J, Alyautdin RN, Kharkevich DA, Ivanov AA. Passage of peptides through the blood-brain barrier with colloidal polymer particles (nanoparticles). Brain Res 1995;674:171-4.
2. Chang J, Jallouli Y, Kroubi M, Yuan XB, Feng W, Kang CS, *et al.* Characterization of endocytosis of transferrin-coated PLGA nanoparticles by the blood-brain barrier. Int J Pharm 2009;379:285-92.
3. Joshi SA, Chavhan SS, Sawant KK. Rivastigmine-loaded PLGA and PBCA nanoparticles: Preparation, optimization, characterization, *in vitro* and pharmacodynamic studies. Eur J Pharm Biopharm 2010;76:189-99.
4. Roney C, Kulkarni P, Arora V, Antich P, Bonte F, Wu A, *et al.* Targeted nanoparticles for drug delivery through the blood-brain barrier for Alzheimer's disease. J Control Release 2005;108:193-214.
5. Misra A, Ganesh S, Shahiwala A, Shah SP. Drug delivery to the central nervous system: A review. J Pharm Pharm Sci 2003;6:252-73.
6. Fazil M, Md S, Haque S, Kumar M, Baboota S, Sahni JK, *et al.* Development and evaluation of rivastigmine loaded chitosan nanoparticles for brain targeting. Eur J Pharm Sci 2012;47:6-15.
7. Trapani A, De Giglio E, Cafagna D, Denora N, Agrimi G, Cassano T, *et al.* Characterization and evaluation of chitosan nanoparticles for dopamine brain delivery. Int J Pharm 2011;419:296-307.
8. Anantharam V, Lehrmann E, Kanthasamy A, Yang Y, Banerjee P, Becker KG, *et al.* Microarray analysis of oxidative stress regulated genes in mesencephalic dopaminergic neuronal cells: Relevance to oxidative damage in Parkinson's disease. Neurochem Int 2007;50:834-47.
9. Lü JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. J Cell Mol Med 2010;14:840-60.
10. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. Cell 2005;120:483-95.
11. Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A. Antioxidants: Its medicinal and pharmacological applications. Afr J Pure Appl Chem 2010;4:142-51.
12. Kuppusamy T, Jagadhesan N, Udaiyappan J, Thamilarasan M,

Mohammed EM. Antioxidant and anti-inflammatory potential of hesperidin against 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced experimental Parkinson's disease in mice. Int J Nutr Pharmacol Neurol Dis 2013;3:294.
13. Essa MM, Vijayan RK, Castellano-Gonzalez G, Memon MA, Braidy N, Guillemin GJ, *et al.* Neuroprotective effect of natural products against Alzheimer's disease. Neurochem Res 2012;37:1829-42.
14. Jellinger K, Linert L, Kienzl E, Herlinger E, Youdim MB. Chemical evidence for 6-hydroxydopamine to be an endogenous toxic factor in the pathogenesis of Parkinson's disease. J Neural Transm Suppl 1995;46:297-314.
15. Gao X, Qian J, Zheng S, Changyi Y, Zhang J, Ju S, *et al.* Overcoming the blood-brain barrier for delivering drugs into the brain by using adenosine receptor nanoagonist. ACS Nano 2014;8:3678-89.
16. Orrego C, Salgado N, Valencia J, Giraldo G, Giraldo O. Novel chitosan membranes as support for lipases immobilization: Characterization aspects. Carbohydr Polym 2010;79:9-16.
17. Sarvaiya J, Agrawal YK. Chitosan as a suitable nanocarrier material for anti-alzheimer drug delivery. Int J Biol Macromol 2015;72:454-65.
18. Sailaja AK, Amareshwar P, Chakravarty P. Different techniques used for the preparation of nanoparticles using natural polymers and their application. Int J Pharm Pharm Sci 2011;3:45-50.
19. Madan J, Pandey RS, Jain V, Katare OP, Chandra R, Katyal A. Poly (ethylene)-glycol conjugated nanoparticles of noscapine improve biological half-life, brain delivery and efficacy in glioblastoma cells. Nanomedicine 2012;12:600-4.
20. Shakeel F, Ramadan W, Shafiq S. Solubility and dissolution improvement of aceclofenac using different nano carriers. J Bioequiv Availab 2009;1:39-43.
21. Rajan M, Raj V. Encapsulation, characterization and *in-vitro* release of anti-tuberculosis drug using chitosan-poly ethylene glycol nanoparticles. Int J Pharm Pharm Sci 2012;4:255-9.
22. Bilati U, Allemann E, Doelker E. Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. Eur J Pharm Sci 2005;24:67-75.
23. Yadav KS, Sawant KK. Modified nanoprecipitation method for preparation of cytarabine-loaded PLGA nanoparticles. AAPS PharmSciTech 2010;11:1456-65.
24. Rajan M, Raj V. Encapsulation, characterisation and *in-vitro* release of anti-tuberculosis drug using chitosan-poly ethylene glycol nanoparticles. Int J Pharm Pharm Sci 2012;4:255-9.