Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 2240-2245
Received: 07-03-2019
Accepted: 12-04-2019

Shailja
Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India

Pawan Jalwal
Faculty of Pharmaceutical Sciences, Baba Mastnath University, Rohtak, Haryana, India

Priyanka Rani
Department of Pharmaceutical Sciences, Ch. Bansilal University, Bhiwani, Haryana, India

Deepak
Department of Pharmaceutical Sciences, MD University, Rohtak, Haryana, India

Jyoti
Department of Pharmaceutical Sciences, MD University, Rohtak, Haryana, India

Ajit
Janta College of Pharmacy, Butana, Sonepat, Haryana, India

Gourav
Janta College of Pharmacy, Butana, Sonepat, Haryana, India

Sunil Kumar
SDM College of Pharmacy, Rajound, Kaithal, Haryana, India

Gurdev Singh
SDM College of Pharmacy, Rajound, Kaithal, Haryana, India

Sharad Sardana
RKSD College of Pharmacy, Kaithal, Haryana, India

Correspondence
Shailja
Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India

Fabrication and evaluation of polymer loaded curcumin nanoparticles for Parkinson's disorder

Shailja, Pawan Jalwal, Priyanka Rani, Deepak, Jyoti, Ajit, Gourav, Sunil Kumar, Gurdev Singh and Sharad Sardana

Abstract
Parkinson's disease (PD) is a neurodegenerative disorder that affects predominately dopamine-producing (dopaminergic) neurons in a specific area of brain called substantia nigra and is marked by a combination of tremors, rigidity, impairment, slowness of movements, impaired balance, bradykinesia and shuffling gait. Curcumin being component of Curcuma longa is a natural polyphenol. Chemically, curcumin is a naturally polyphenol denominated (1E, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) which is extracted from the rhizomes of *Curcuma longa.* The objective was to formulate and evaluate curcumin loaded albumin nanoparticles. The studies done showed the spherical shape with smooth surface nanoparticles. The average particle size of F7 was found to be 226 nm and its Polydispersity index was 0.383.

Keywords: Albumin nanoparticles, curcumin nanoparticles, anti-Parkinson drug, Parkinson's disease

Introduction
Parkinson's disease (PD) is a neurodegenerative disorder that affects predominately dopamine-producing (dopaminergic) neurons in a specific area of brain called substantia nigra and is marked by a combination of tremors, rigidity, impairment, slowness of movements, impaired balance, bradykinesia and shuffling gait [1]. It also affects the speaking and writing ability of a person.Dopamine is involved in transferring the information to that part of brain which is responsible for coordination and movement. Parkinson disease is considered to be the second most common neurodegenerative disease spread worldwide [2].
Curcumin being component of Curcuma longa is a natural polyphenol. Chemically, curcumin is a naturally polyphenol denominated (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) which is extracted from the rhizomes of *C*urcuma. longa. Structurally it carries three chemical entities in the molecule: two aromatic ring system which containso-methoxy phenolic groups linked by a seven-carbon spacer consisting of an α,β-unsaturated β-diketone moiety.

Nanoparticulate-mediated Drug Delivery
Nanoparticlesas drug carriers present an innovative approach for the administration of therapeutic drugs. These carriers range between 10nm to 1000nm and can be of many types viz polymeric, lipoidal, metallic protein, etc. Nanoparticles prepared from Bovine serum albumin (BSA) are versatile carrier systems for drug delivery and can be prepared by an established desolvation process, ph-coacervation method under the aspect of the controllable particle size range between 100 to 300 nm with narrow size distribution [3].
Proteins represent good raw materials since they have the advantages of synthetic polymers together with the absorbability and low toxicity of the degradation end products. The protein nanotechnology holds a great promising material for the improvement in drug delivery systems, labeling agents and biosensors. Synthetic or natural polymers viz proteins, polysaccharide, lipids can be used for the preparation of nanoparticles [4].

Materials and Methods
Materials
The drug sample of curcumin was obtained from the Central Drug House (CDH), (P) Ltd.7/28 Vardaan House, Daryaganj, New Delhi. Albumin was obtained from the Central Drug House (CDH), (P) Ltd. 7/28 Vardaan House, Daryaganj, New Delhi. Ethanol, Glutaraldehyde and sodium chloride was obtained from Loba chemie Pvt. Ltd.107, Wodehouse Road, Mumbai.

Methods

Preparation of Nanoparticles

Nanoparticles were prepared by pH coacervation method. In this method, polymer (albumin) was dissolved in 2 ml of 10mM sodium chloride. The drug(curcumin) was added to the prepared solution. The pH of the solution was maintained between 7 to 8. The solution was kept on magnetic stirrer at speed of 500 rpm and ethanol was added at the rate of 1ml/min till the solution appeared turbid. In this, 0.1ml of 4% Glutaraldehyde was added for cross linking of the nanoparticles and kept for stirring for 2 hours. 1%anhydrous glucose was added to the nanoparticles suspension formed as a cryoprotectant and then freeze dried.

Optimization

Nanoparticles were formulated using an experimental design, based on providing model of response surface. This design is used for generating high order response surface and also needs less runs [5]. To get fit in second order of equation, box behnken design requires 12 middle edges and 3 centre nodes. The design places the points at the midpoint of the edges of the cubical region as well as in the centre. Box behnken design needs three levels per factor. This design helps in finding the values of the operating variables and also one of the most commonly used experimental design. Individual factor whether or the independent variable, is located to the equal spaced values. In case of 3 factors, 3 blocks are involved. A centre point is important at which all the points are placed.

Table 1: Formulation Tablefor Designing Albumin Nanoparticles

S. No.	Formulation No.	Polymer Conc. (X1, mg)	Cross linker conc. (X2,% v/v)	Stirring time (min)
1.	F1	70	7	80
2.	F2	60	6	100
3.	F3	60	7	120
4.	F4	65	8	120
5.	F5	65	6	120
6.	F6	65	7	100
7.	F7	65	6	80
8.	F8	60	7	80
9.	F9	70	8	100
10.	F10	70	6	100
11.	F11	65	8	80
12.	F12	70	7	120
13.	F13	60	8	100

Evaluation of Nanoparticles

Shape and Surface morphology

The prepared nanoparticles were checked for their shape and morphology by using scanning electron microscope (Scanning Electron Microscope (SEM) – Zeiss EVO40). The samples were sprinkled on a slide using double sided adhesive tape and gold plating was done under argon atmosphere with the help of gold sputter in a high vacuum evaporator. At different magnifications, samples were scanned and photomicrographs were taken [6].

Particle Size and Size Distribution

Accurate amount of 1ml of albumin nanoparticle suspension was diluted upto 10 ml with distilled water. Then DLS (Malvern instruments zetasizer, Nanoseries S-90) of the samples were done and their average particle size and Polydispersity index was measured.

Drug Entrapment Efficiency

10 ml of accurately weighed nanoparticle suspension equivalent to 10 mg of drug was taken out using a pipette and passed into a centrifuge tube and then centrifuged at 1000rpm for 15 min at 25 °C using refrigerated centrifuge (Remi Motors C-24 plus). The supernatant obtained was obtained and drug concentration present in supernatant was measured using spectrophotometer at 289 nm using calibration curve. Drug entrapment percentage was calculated using following formula

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Amount of drug in supernatant}}{\text{Amount of drug added}} \times 100$$

In-vitro Drug Release Study

In-vitro drug release study was done using dialysis membrane in phosphate buffer (PBS) at pH 6.8. Drug equivalent to 10 mg was kept in dialysis membrane. Dialysis membrane was used for characterising the drug release of the formulation. The chambers were filled with freshly prepared phosphate buffer ph 6.8. At regular time intervals aliquots of samples were taken out at different time intervals upto 24 hours and the sink condition was maintained by adding equal amount of phosphate buffer. The samples obtained were filtered using whatman filter paper and then scanned using UV-spectrophotometer. Percent cumulative drug release was calculated.

Ex-vivo Drug Release Studies

Using nasal mucosa of goat, Ex-vivo drug release of curcumin-albumin nanoparticles was investigated. The nasal mucosa of goat was taken from the local slaughter house within 15 minutes of sacrifice of goat. The skin was removed and the nose was stored in a cold phosphate buffer solution (pH 6.8). The nasal mucosa was removed carefully with the help of forceps and scissors. About 5 ml of the drug loaded nanoparticle suspension was kept on the freshly removed nasal mucosa. Aliquots of sample were withdrawn at different time intervals till 24 hours and sink condition was maintained by adding equal amount of buffer solution. The samples taken were scanned using UV-spectrophotometer at 289 nm.

Drug Release Kinetics Study

When the matrice is hydrophilic in nature, then polymers swells and gets eroded simultaneously and both these factors are responsible for the complete rate of drug release. It has been validated that release of drug in case of hydrophilic matrices exhibits time-independent profile which means release rate of drug is increased. This leads to zero order release kinetics.

In this study, the formulation i.e. albumin nanoparticles was prepared for sustained release of curcumin. The results of drug release kinetics were plotted using different kinetic models like zero order release (% cumulative drug release vs time), first order (log cumulative % of drug release vs time), Higuchi's kinetics (% cumulative drug release vs$\sqrt{time}$), and Korsmeyer and Peppas equation (log cumulative % of drug release vs log time).

Zero order equation: $C=K_0t$ where K_0 is the zero order rate constant which is expressed as concentration/time and 't' is time in hours.

First order equation: Log C=Log C_0 – kt/2.303 where C_0 is the initial concentration of drug, k is the first order constant, and t is the time.

Higuchi's equation: $Q= Kt^{1/2}$ where K is the constant about the variables and t is the time in hours. Therefore, rate of drug release is directly proportional to reciprocal of the square root time.

Korsmeyer and Peppas equation: $M_t/M\infty= Kt^n$ where t is the time of release, K is constant characteristic of drug or polymer system and n is an exponent which represents mechanism of release of traces.

Table 2: Release Mechanism with Variation in 'n' Values

'n' values	Mechanism	dM_t/d_t dependence
N<0.5	Quasi- Fickian Diffusion	$t^{0.5}$
0.5	Fickian Diffusion	$t^{0.5}$
0.5<n<1.0	Anomalous (non-fickian) Diffusion	t^{n-1}
1	Non- Fickian Case II	Zero Order
N>1.0	Non- Fickian Super case II	t^{n-1}

The prepared albumin nanoparticles were subjected to different parameters of evaluation to check the physicochemical properties, their efficacy and quality.

Stability Studies

The curcumin loaded albumin nanoparticles were assessed for the stability studies which was performed by storing the samples in glass vials and allowed to be kept at room temperature, in refrigerator at 5 °C and 45 °C in the stability chamber. The samples were then analysed at different intervals of time i.e. 0, 30, 45, 60 and 90 days for its drug content and the changes were checked in its physical appearance [7].

Results and Discussion

Shape and Surface Morphology

The prepared nanoparticles were checked for their shape and surface morphology with the help of scanning electron microscopy (Scanning Electron Microscope (SEM) - Zeiss EVO40) which indicated the spherical shaped particles with rough surface. The studies done showed the spherical shape with smooth surface nanoparticles.

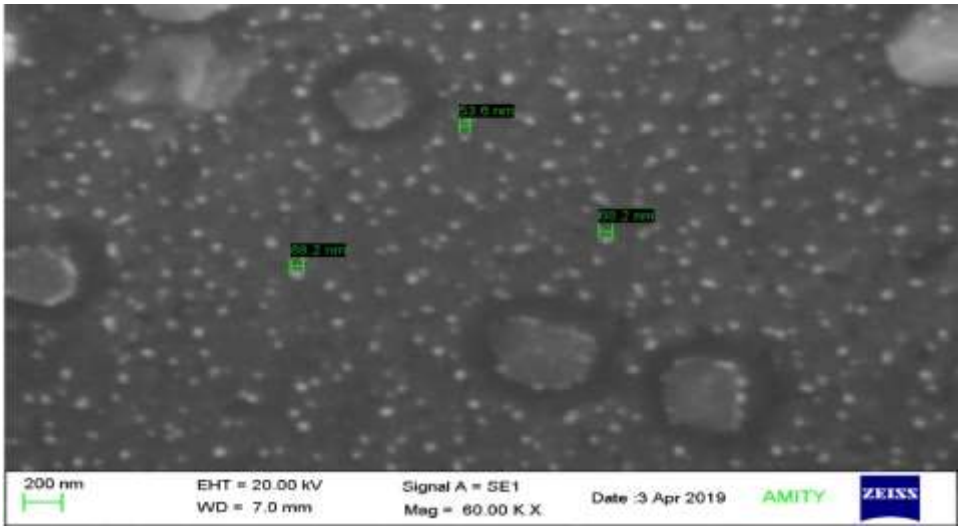

Fig 1: SEM Image of Albumin Nanoparticles of Curcumin

Particle Size and Size Distribution

Particle size can be analysed with the help of Dynamic Light Scattering method which gives information about average diameter of the particle size and distribution range of particles from 0.00 to 0.667 by Polydispersity index. Usually Polydispersity index should be ideally not greater than 0.50 as it shows that aggregation of particles occur. The average particle size of F7 was found to be 226 nm and its Polydispersity index was 0.383.

Drug Entrapment Efficiency

The drug entrapment efficiency is calculated using the following formula:

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Amount of drug in supernatant}}{\text{Amount of drug added}} \times 100$$

The percent drug entrapment efficiency was found to be 46.21% to 82.01%. The entrapment efficiency of the optimized batch (F7) was 81.57%.

Drug Content

The drug content was done to calculate the amount of drug present in 1 mg of the total formulation. The drug content was found to be 0.029 to 0.070 and that of optimized batch (F7) was 0.062.

Percentage yield

The total yield can be calculated using following formula:

$$\% \text{ Yield of Nanoparticles} = \frac{\text{Total weight of obtained nanoparticles}}{\text{Total weight of drug + polymer}}$$

The percentage yield was found to be 77.87%

***In-vitro* Drug Release Study**

In- vitro release study of curcumin loaded albumin nanoparticles were carried out. The results proved the fact that sustained release shown by the formulations is dependent on the concentration of the albumin (polymer). The formulation having less polymer concentration showed the drug release of 68.937% upto 24 hours and those having medium polymer concentration showed drug release of 84.712% upto 24 hours.

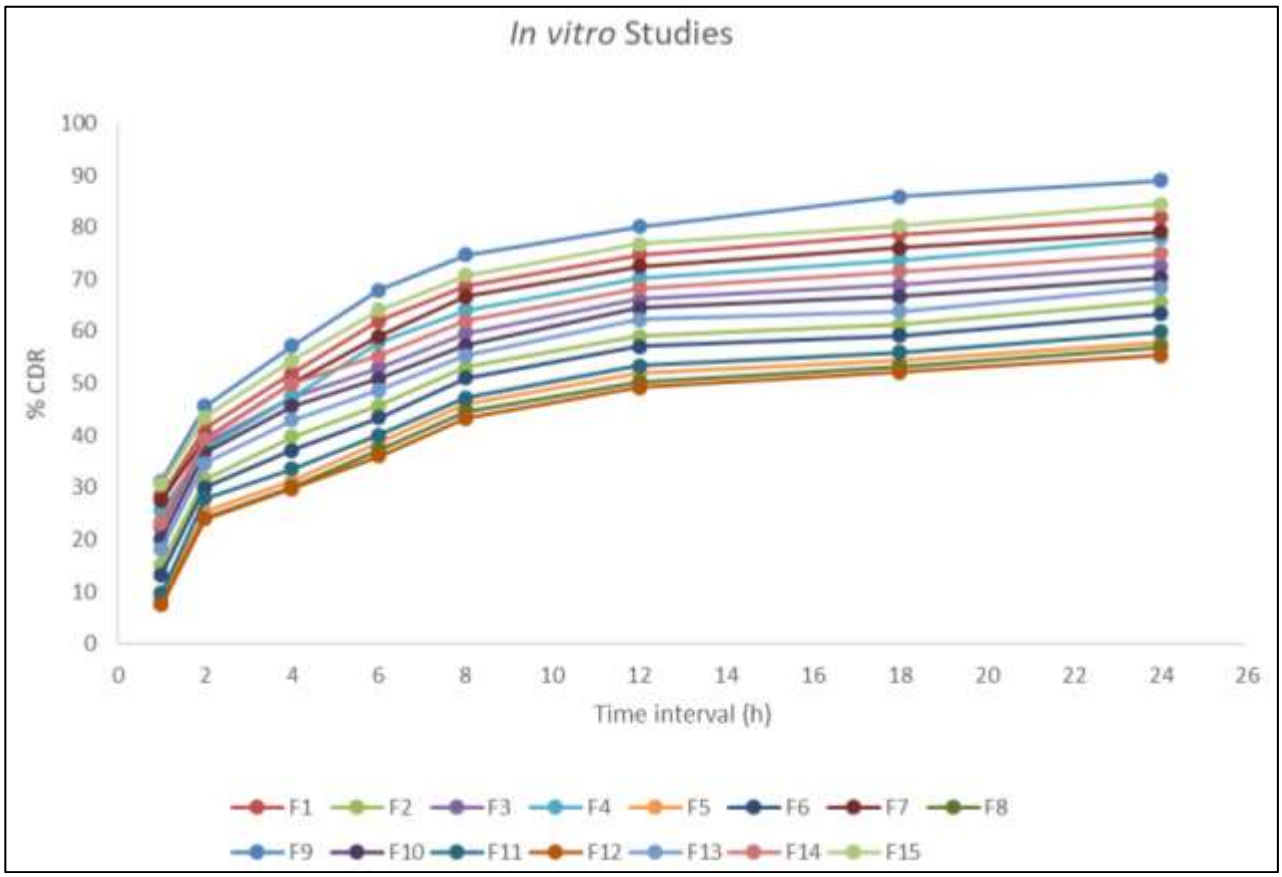

Fig 2: *In vitro* drug release of curcumin loaded albumin

***Ex vivo* Drug Release Study**

By using formulation F7, *ex vivo* release of drug behaviour of Curcumin loaded Albumin nanoparticles showed a release of 93.623%. The graph was plotted of time vs % CDR.

Table 4: *Ex vivo* drug release profile of F7 formulation

S.No.	Time (h)	Absorbance	Concentration	Amount	% CDR
1.	0	0	0	0	0
2.	0.083	0.033	6.391	1.597	28.989
3.	0.167	0.042	7.217	1.804	32.322
4.	0.25	0.050	7.960	1.990	36.556
5.	0.5	0.062	9.082	2.27	40.398
6.	0.75	0.075	10.27	2.567	46.438
7.	1	0.080	10.802	2.700	49.869
8.	1.5	0.094	12.027	3.006	56.674
9.	2	0.115	13.652	3.413	63.337
10.	4	0.136	15.955	3.988	72.899
11.	6	0.144	16.745	4.186	77.797
12.	8	0.164	18.602	4.658	85.656
13.	12	0.177	19.744	4.936	90.747
14.	24	0.185	20.533	5.133	93.623

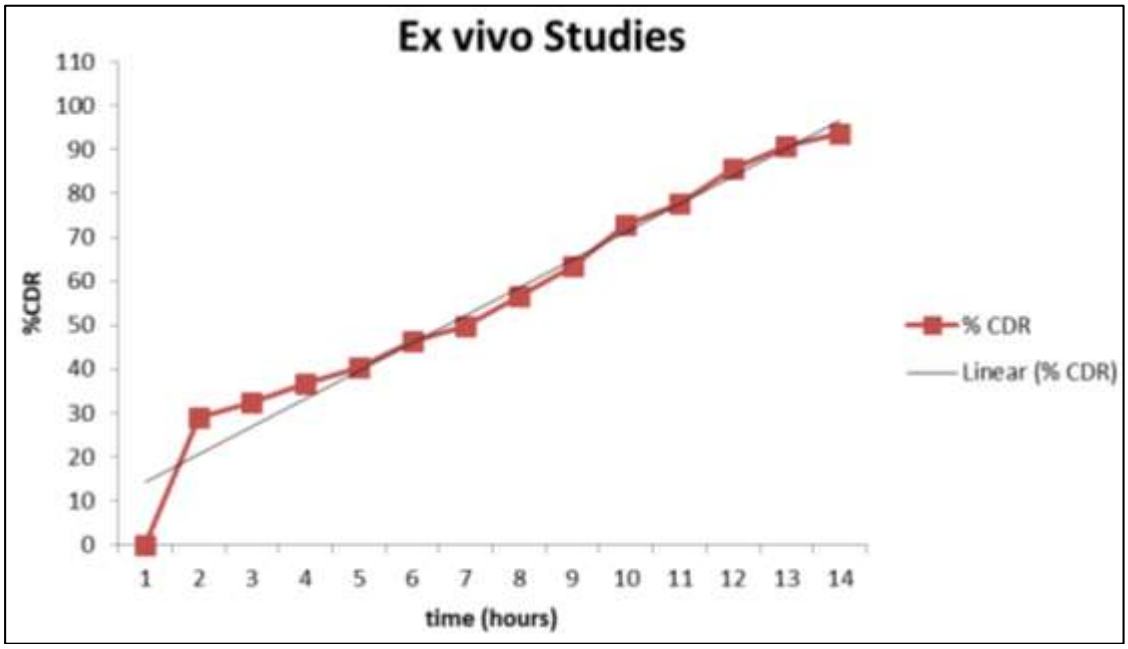

Fig 4: Graphical representation of *Ex vivo* drug release studies

Release kinetics study

In hydrophilic matrices, it has been observed that initially polymer swells up and finally the drug is released via diffusion. The *in vitro* drug release data of F7 formulation showed that the release of drug was explained by Korsmeyer-peppas model, which showed the highest linearity (R^2 = 0.969) which explained drug release through diffusion which occurs by swelling of polymer matrix and remained constant throughout the release of drug in body, followed by higuchi model (R^2 = 0.960) and zero order (R^2 = 0.952). The release exponent 'n' was found to be 0.209 which tells that Quasi fickian diffusion occurred showing that the drug was released partially through swelling of matrix of polymer of nanoparticles [8].

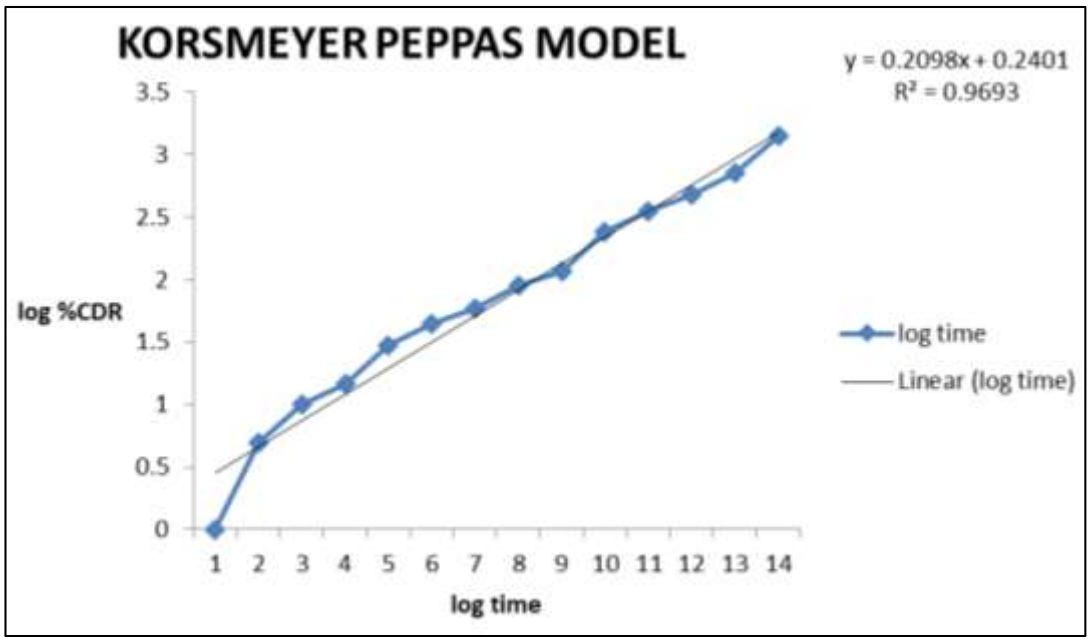

Fig 5: Korsmeyer and Peppas plot of F7

Stability Studies

The prepared formulation was subjected to stability studies to check the stability of Curcumin loaded albumin nanoparticles (F7). Initially the formulation appeared to be yellow coloured, odourless and in powdered form.

Table 4: Stability Data for Curcumin loaded Albumin Nanoparticles

S. No.	Days	Temperature and Relative Humidity Conditions for stability testing			
		5± 1 °C - 75% RH		45± 1 °C- 75% RH	
		Drug content (%)	Physical Appearance	Drug content (%)	Physical Appearance
1.	0	82.2	+	82.2	+
2.	30	82.2	+	81.98	+
3.	45	82.1	+	81.57	+

Conclusion

With the advancement in science and technology, different systems related to the nanotechnology are still developing to improvise the delivery of drug to cure the deadly diseases like HIV AIDs, cancer, diseases related to brain, tuberculosis etc. With the recent developments it has been found that the nanoparticles are more potential in treating a disorder as it is less toxic and has more capability of targeting. In the present study conducted, curcumin loaded albumin nanoparticles were found to be highly effective designed by pH coacervation method using Box Behnken design.

References

1. Antiparkinsonian Drugs. In: KD Tripathi, Essentials of Medical Pharmacology, 7th Edition. Jaypee Brothers Medical Publishers (P) Ltd. New Delhi; c2015. p. 425-434.
2. Rewar, Suresh. A systematic review on Parkinson's disease (PD). Indian Journal of Research in Pharmacy and Biotechnology. 2015;3:2321-5674
3. Dhuria SV, Hanson LR, Frey WH. Intranasal delivery to the central nervous system: mechanism and experimental consideration. J Pharm Sci. 2010;99:1654-1673
4. Targeted and controlled drug delivery by S.P Vyas and R.K Khar
5. Jain N.K. Advances in controlled and novel drug delivery, CBS Publisher and distributors, edition 1st; c2001. p. 408`
6. Rao JS, Kumar B. in 10th International Conference on Vibrations in Rotating Machinery; c2012
7. Gulati N, Nagaich U, Saraf SA. International Delivery of Chitosan Nanoparticles for Migraine Therapy. Sci Pharm. 2013;81:843-854.
8. Basu S, Bandyopadhyay AK. Development and characterization of mucoadhesive in situ nasal gel of midazolam prepared with ficuscarica mucilage. AAPS Pharm Sci Tech. 2010;11:1223-1231.