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Research Article

Design, Development and Characterization of Curcumin Loaded Albumin Nanoparticles for the Treatment of Parkinson's Disease

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ABSTRACT

Curcumin being component of *Curcuma longa* is a natural polyphenol, observing on a chemical level, curcumin is a natural polyphenol which is denominated (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene3,5-dione) which is usually extracted through the rhizomes of *Curcuma longa*. Structurally, it is composed of a trio of chemical identities on a molecular level: dual aromatic ring system.

The objective of the research was to design, development, and characterization of herbal drug-loaded albumin nanoparticles to cure Parkinson's disease for improving and increasing the therapeutic efficacy and also reducing the frequency of dose. The optimized formulations were obtained after applying the design of the experiment, which was the Box Behnken method, where three independent variables, polymer concentration, stirring time, and crosslinker concentration, were selected. Curcumin nanoparticles loaded with albumin were formulated by the ph coacervation method in which ethanol was used as a desolvating agent along with a cross-linking agent (Glutaraldehyde) and albumin as the polymer. The particle size and polydispersity index of curcumin loaded albumin nanoparticles was measured via a dynamic light scattering technique. Drug release research conducted using the *in vitro* method over the duration of 24 hours. *Ex vivo* drug release study of the albumin nanoparticles was performed using a nasal membrane of a goat. It has been shown that in case of hydrophilic matrices, swelling of the polymer occurs followed by the release of drug by diffusion which was best explained by Korsmeyer-Peppas equation, which indicates drug release through diffusion which occurs by swelling of a polymer matrix and remained constant throughout the release of drug in the body. By virtue of particle size, the designed nanoparticles effortlessly go into the nasal mucosa.

INTRODUCTION

"Parkinson's disease (PD) is a neurodegenerative disorder that affects dopamine-producing (dopaminergic) neurons predominately in a specific area of the 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 the brain, which is responsible for coordination and movement.[1-2] PD may occur due to viral infection of brain, cerebral ischaemia, mutations in gene or any other damage caused to the brain, or maybe drug-induced like with neuroleptics or on exposure to toxic elements like carbon monoxide, cyanid, etc.[3] PD leads to the destruction of dopamine-producing neurons within basal ganglia, which decreases the amount of striatal dopamine, i.e., inhibitory effects and increase in amount of acetylcholine which causes excitatory activities and hence difficulty in initiating body movements.[4-5] Curcumin being a component of *Curcuma longa*, is a natural polyphenol. Observing on a chemical level, curcumin is a natural polyphenol that is denominated (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene3,5-dione) which is usually extracted through the rhizomes of *Curcuma longa.* Structurally, it is composed of a trio of chemical identities on a molecular level: dual aromatic ring system.[6] Nasal drug delivery done in an intranasal manner offers a usable, non-obstructive methodology to bypass

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the blood-brain barrier (BBB), to provide therapeutic chemicals to the brain.[7-8] The non-invasive method gives novelty by directly and rapidly delivering the drug through the nasal route to the brain to cure CNS disease and simultaneously reduce exposure to systemic circulation. Transportation of drugs and chemicals to the brain occurs by crossing the olfactory lobe along with the trigeminal neural pathway and blood brain barrier via the intranasal route.[9-10] BBB constitutes a special vascular endothelium system having endothelial cells, pericytes, immune cells, astrocytes, and basement membrane, which is also known as a neurovascular system. The main function of BBB is to provide protection to the brain. This brain comprises of many complex pathways. In this study, nanoparticles were formulated, nanoparticles as drug carriers present an innovative approach for the administration of therapeutic drugs.[11] These are multipurpose carrier systems for delivering the drugs, and one of the methods to prepare these is an established desolvation process, ph-coacervation method case in point being a particle size range which can be controlled and lies between 100–300 nm.[12]

The main objective of the study was to improve the bioavailability of the drug to achieve the prolonged duration of action and sustained drug release and also reducing the dosing frequency.

Materials and Methods

Materials

The drug sample of curcumin was provided by Central Drug House, (P) Ltd (New Delhi). Albumin (Central Drug House, New Delhi). Ethanol, glutaraldehyde, and sodium chloride was obtained from Loba Chemie Pvt. Ltd., 107, Wodehouse Road, Mumbai.

Method-pH-Coacervation Method

The method was initiated by dissolving the polymer (albumin) in 2 mL of 10 mM sodium chloride.

The drug (curcumin) was solubilized into the solution of polymer, and 10mM sodium chloride prepared. The pH was maintained between 7–8. The solution was stirred at a speed of 500 using a magnetic stirrer, and ethanol was added at the rate of 1 mL/min till the solution appeared turbid. In this, 0.1 mL of 4% glutaraldehyde was added for cross-linking of the nanoparticles and kept for stirring for 2 hours. One percent anhydrous glucose was added to the nanoparticle suspension formed as a cryoprotectant and then freeze-dried.

Optimization is Done Using Box Behnken Design

Nanoparticles were formulated using an experimental design based on providing a model of the response surface, as shown in Table 1. This design is used for generating high order response surface and also needs less runs.[13-14] To get fit in the second order of equation, box behnken design requires 12 middle edges and three center nodes. The design places the points at the midpoint of the edges of the cubical region as well as in the center. 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 designs. Individual factor whether or the independent variable is located to the equal spaced values.[15] In the case of three factors, three blocks are involved. A center point is important at which all the points are placed.

Characterization of Albumin Nanoparticles

Shape and Surface Morphology

The prepared nanoparticles were checked for their shape and morphology by using a 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 using vacuum evaporator with the help of gold flake. At different magnifications, scanning of samples was done and photomicrographs were captured.[16-17]

Particle Size and Size Distribution

Accurate amount of 1 mL suspension of albumin nanoparticle was diluted with water till 10 mL. Then DLS (Malvern Instruments Zeta sizer, Nano series S-90) of the samples were done, and their average particle size and polydispersity index were measured.

Table 1: Formulation table for designing albumin nanoparticles

S. No.	*Formulation no.*	*Polymer concentration. (mg)*	*Crosslinker concentration. (% v/v)*	*Stirring time (min)*
1.	F1	57.5	6	90
2.	F2	57.5	8	90
3.	F3	57.5	7	105
4.	F4	50	7	120
5.	F5	57.5	7	120
6.	F6	65	7	120
7.	F7	65	6	105
8.	F8	65	7	90
9.	F9	50	6	105
10.	F10	50	7	90
11.	F11	65	8	105
12.	F12	57.5	8	120
13.	F13	50	8	105
14.	F14	57.5	7	105
15.	F15	57.5	7	105
16.	F16	57.5	7	105
17.	F17	57.5	6	120

Drug Entrapment Efficiency

A total of 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 1000 rpm for 15 minutes at 25°C using a refrigerated centrifuge (Remi Motors, C-24 plus). The supernatant obtained was obtained, and the drug concentration present in supernatant was measured using a spectrophotometer at 289 nm using a calibration curve. The percentage of drug entrapment efficiency was counted by following formula.[18]

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

In-vitro Drug Release Study

In-vitro drug release study was done using a dialysis membrane in phosphate buffer (PBS) at pH 6.4. Drug equal to 10 mg was kept in a dialysis membrane. The dialysis membrane was used for characterizing the drug release of the formulation. The prepared phosphate buffer was poured into the chambers, upto the given mark. At different time intervals, some amount of samples were taken out up to 24 hours, and the sink condition was maintained by adding an equal amount of phosphate buffer. The filtration of samples was done using the Whatman filter paper, and at last, the samples were scanned using UV-spectrophotometer. Percent of cumulative drug release was calculated.

Ex-vivo Drug Release Studies

Using goat's nasal membrane, ex-vivo drug release of curcumin-albumin nanoparticles was investigated. The nasal part of goat was obtained from the nearby butcher place within 15 minutes of the sacrifice of goat. The skin was removed, and the nose was stored in a cold phosphate buffer solution (pH 6.4). 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 the sample were withdrawn at different time intervals till 24 hours and sink condition was maintained by adding an equal amount of buffer solution. The samples taken were scanned using UV-spectrophotometer at 289 nm.

Release Kinetics Study

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

In this study, the formulation, i.e., albumin nanoparticles, was prepared for the 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), and "Korsmeyer and Peppas equation" (log cumulative % of drug release vs. log time).

The prepared albumin nanoparticles were subjected to different parameters of evaluation to check the physicochemical properties, 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 a refrigerator at 5 and 45°C in the stability chamber. The samples were then analyzed 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.[19]

Results and Discussion

Shape and Surface Morphology

The formulated albumin nanoparticles were checked for their shape and surface morphology using scanning electron microscopy (SEM - Zeiss EVO40), which indicated the spherical shaped particles with a rough surface. The studies done showed the spherical shape with smooth surface nanoparticles, as shown in Fig. 1.

Particle Size and Size Distribution

Particle size can be analyzed with the help of the dynamic light scattering method, which tells us about an average diameter of the particle size and distribution range of particles from 0.00–0.667 by polydispersity index. Usually, the polydispersity index should be ideally not greater than 0.50, as it shows that aggregation of particles occurs. The average particle size of F7 was found to be

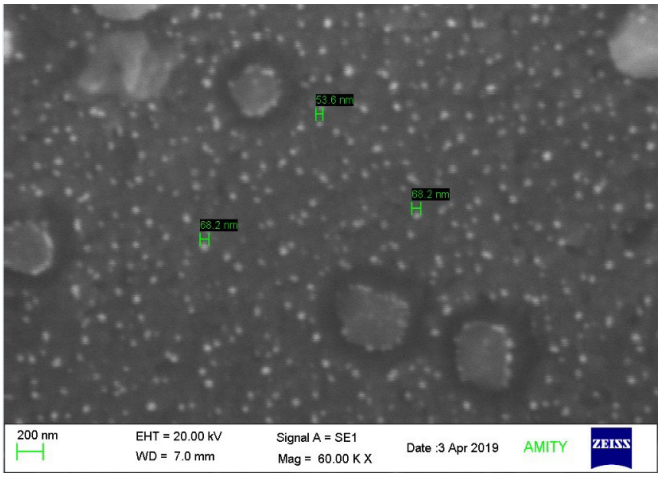


Fig. 1: Scanning electron microscopy image of albumin nanoparticles of curcumin

147.5 nm and its polydispersity index was 0.330, as shown in Fig. 2.

Drug Entrapment Efficiency

The drug entrapment efficiency is calculated, as shown in Table 2 using the following formula:

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

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%.

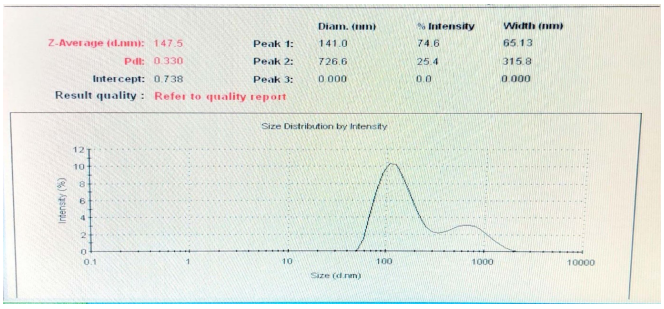


Fig. 2: DLS image of curcumin loaded albumin nanoparticles

Table 2: Entrapment efficiency of the curcumin loaded albumin nanoparticles

S. No.	*Formulation*	*Entrapment efficiency*
1.	F1	60.78% ± 0.85
2.	F2	54.08% ± 0.22
3.	F3	58.63% ± 0.96
4.	F4	46.21% ± 0.56
5.	F5	73.32% ± 0.49
6.	F6	78.51% ± 0.99
7.	F7	81.57% ± 0.15
8.	F8	72.24% ± 0.97
9.	F9	82.01% ± 0.26
10.	F10	64.67% ± 1.01
11.	F11	55.14% ± 0.67

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

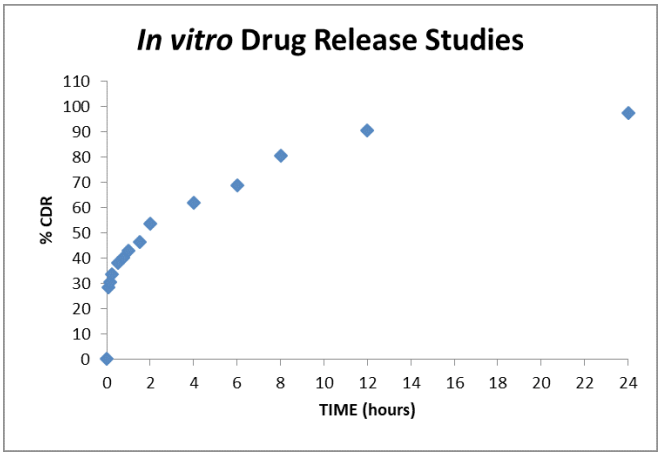
The total yield can be calculated using the following formula:

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

The percentage yield calculated was 77.87%

In-vitro Drug Release Study

In-vitro release study of curcumin loaded albumin nanoparticles were carried out and the data was summarized (shown in Table 3). The table shows clearly that the F7 formulation (as shown in Graph 1) have the best drug release amongst all the formulations followed by F6 and F8. The results proved the fact that sustained release shown by the formulations depended upon the amount of the albumin (polymer). The process of release of drugs initially started



Graph 1: *In vitro* drug release of curcumin loaded albumin.

Table 3: *In vitro* drug release profiles of different batches of albumin nanoparticles in Phosphate buffer solution at 6.4 pH

		Cumulative percent drug release of curcumin loaded albumin nanoparticles										
S. No.	*Time (h)*	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*	*F9*	*F10*	*F11*
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0.08	18.7	16.0	14.7	13.5	15.5	25.6	28.4	29.0	26.4	28.4	35.3
3	0.17	21.3	17.1	15.8	13.9	19.3	30.3	30.4	33.3	32.8	30.6	39.0
4	0.25	23.7	17.9	16.0	15.5	20.9	32.9	33.5	36.7	38.2	35.5	46.6
5	0.5	24.0	19.9	18.1	15.8	22.5	35.4	38.2	41.8	43.6	39.1	51.3
6	0.75	25.4	20.4	21.3	15.9	23.3	41.5	40.1	47.2	47.5	44.9	59.9
7	1	27.2	61.5	56.7	15.5	25.9	47.3	43.0	49.8	51.4	45.5	69.0
8	1.5	59.2	63.2	58.6	18.1	27.0	48.8	46.3	55.4	56.6	57.2	75.6
9	2	59.9	63.4	59.0	53.8	31.9	50.5	53.7	62.9	60.8	62.3	83.5
10	4	60.7	63.9	62.7	55.22	34.89	62.19	62.14	73.47	75.6	69.0	104.4
11	6	61.9	64.3	62.9	55.48	39.26	70.63	68.76	77.22	81.0	80.2	132.7
12	8	63.8	67.0	63.0	56.43	43.65	76.08	80.63	85.68	101.4	87.6	173.7
13	12	64.8	67.8	63.7	57.58	49.00	84.52	90.70	90.98	117.5	95.7	176.7
14	24	74.4	74.7	70.5	57.93	54.45	95.50	97.61	94.63	136.1	107.3	210.0

by polymer swelling followed by drug-releasing from the matrix of the polymer. The release of drugs varied with other formulations based on polymer concentration. The formulation of having less polymer concentration showed the drug release of 57.937% up to 24 hours, and those having medium polymer concentration showed a drug release of 74.712% up to 24 hours. F7 formulation had polymer concentration 65 mg, and the cross-linking agent was 6%.

In-vitro drug dissolution data of F7 was then subjected to different release kinetic models.

Ex-vivo Drug Release Study

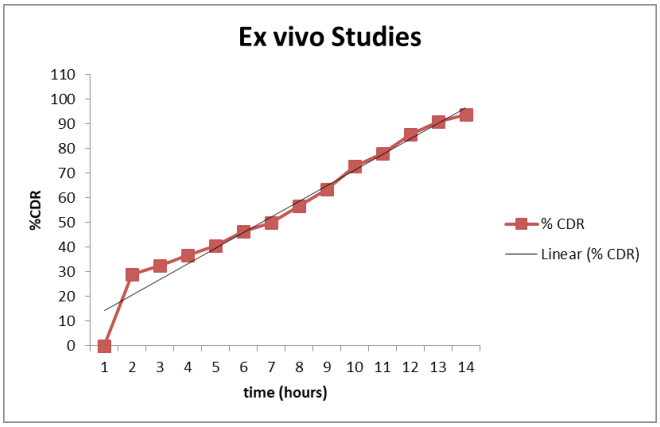
By using formulation F7, *ex vivo* release of drug behavior of curcumin loaded albumin nanoparticles showed a release of 93.623%, as shown in Table 4. The graph was plotted of time vs. % CDR, as shown in Graph 2.

Release Kinetics Study

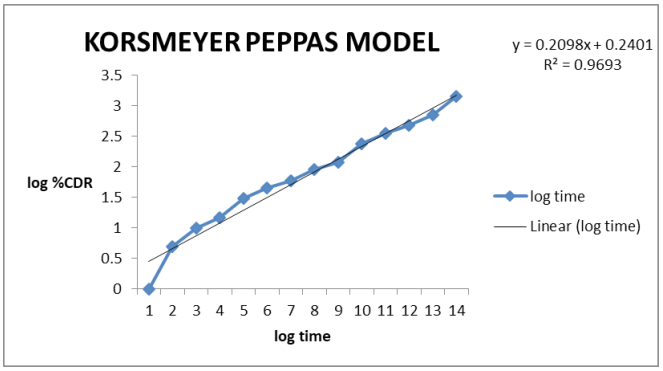
In hydrophilic matrices, it has been observed that initially polymer swells up, and finally, the drug is released via diffusion. The *in vitro* drug release data of F7 formulation showed that the release of drug was explained by Korsmeyer-peppas model as shown in graph 3, which showed the highest linearity (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 a matrix of the polymer of nanoparticles.

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



Graph 2: Graphical representation of *Ex vivo* drug release studies



Graph 3: Korsmeyer and Peppas plot of F7

Stability Studies

The prepared formulation was subjected to stability studies, as shown in Table 5, to check the stability of curcumin loaded albumin nanoparticles (F7). Initially the formulation appeared to be yellow-colored, odorless, and in powdered form.

Conclusion

In the present study conducted, curcumin loaded albumin nanoparticles were found to be highly effective designed by pH coacervation method using BBD. By virtue of particle size, the designed nanoparticles effortlessly goes into the nasal mucosa. The particle size of albumin nanoparticles was found to be 147.5 nm having 0.330 polydispersity index and entrapment efficiency of 81.57%. Drug release research conducted using *in vitro* method exhibits a release

Table 5: Stability data for curcumin loaded albumin nanoparticles

		Temperature and relative humidity conditions for stability testing			
		5± 1°C-75% RH		*45 ± 1°C-75% RH*	
S.No.	*Days*	*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	+

percentage of 97.614% over the duration of 24 hours. *Ex vivo* drug release study of the albumin nanoparticles showed a release of 93.623% in 24 hours. It has been shown that in case of hydrophilic matrices, swelling of polymer occurs followed by release of drug by diffusion which was best explained by Korsmeyer-peppas equation, which indicates drug release through diffusion which occurs by swelling of polymer matrix and remained constant throughout the release of drug in body. The prepared nanoparticles were more potential in treating the disease as they were found to be less toxic as compared to other dosage form. The results of the prepared nanoparticles showed sustained drug release and showed their action for a longer period of time.

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