

# Mucoadhesive Chitosan-Coated PLGA Nanoparticles of Ashwagandha Extract for Colon-Targeted Delivery

Abhilash Megaravalli Manjunath, Sneha Priya\*, Divya Jyothi

Department of Pharmaceutics, NITTE (Deemed to be University), NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore, Karnataka, INDIA.

## ABSTRACT

**Aim/Background:** *Withania somnifera* (Ashwagandha) belongs to the Solanaceae family, well known for its phyto-pharmacological properties such as anti-inflammatory, antioxidant, anti-stress, immunomodulatory, and anticancer properties. The study aimed to formulate and evaluate chitosan-coated PLGA nanoparticles of ashwagandha extract. The nanoparticle formulation technique was considered in order to rectify the various constraints associated with ashwagandhas, such as intestinal absorption, burst release of the drug, and bioavailability issues. **Materials and Methods:** The CS-PLGA NPs were prepared by single-emulsion solvent evaporation method and it was optimized by using the Box-Behnken design in Design-Expert Software to determine the influence of independent variables PLGA, chitosan concentration and sonication time on particle size, PDI and entrapment efficiency. **Results:** The particle size, PDI, and zeta potential of the optimized formulation were found to be 187.1nm, 0.148, and 31.3mV. Optical microscopy and SEM suggest that the particles were smooth, spherical, and uniform in size. The entrapment efficiency of the optimized formulation was found to be 84.28%. *In vitro* drug release study suggests that the enteric coating of the CS-PLGA NPs formulation prevented the drug release in SGF and showed sustained drug release than pure ashwagandha and the drug release kinetics followed the Korsmeyer-Peppas model. Furthermore, the CS-PLGA NPs were evaluated for *in vitro* antioxidant activity over DPPH, and *in vitro* cytotoxicity assay over CaCo-2 cell lines. The results showed enhanced antioxidant activity than pure ashwagandha and better cytotoxicity over CaCO-2 cells. **Conclusion:** The studies concluded that CS-PLGA NPs showed sustained drug release for a prolonged period. The formulation showed good antioxidant activity and better efficacy in cancer cells.

**Keywords:** Ashwagandha, Nanoparticles, Chitosan, PLGA, Anti-cancer activity.

## Correspondence:

**Dr. Sneha Priya**

Associate Professor, Department of Pharmaceutics, NITTE (Deemed to be University), NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangaluru-575018, Karnataka, INDIA.  
Email: snehpriya123@gmail.com

**Received:** 25-07-2022;

**Revised:** 20-04-2023;

**Accepted:** 14-08-2023.

## INTRODUCTION

Plants are often regarded as one of the most significant sources of medicinal substances. More than 80,000 plant species are utilized as medicines among the world's 2,50,000 higher plant species. Medicinal plants have been a source for many years of a wide range of bioactive compounds and are widely used as the whole extract or as isolated compounds for different diseases.<sup>1</sup> Plants have metabolites known as phytochemicals. These phytochemicals protect plants against microbial diseases and pest infestations. Phytochemicals are active compounds possessing pharmacological properties that are used as medicine or drug.<sup>2</sup> In the recent trend, herbal drugs and formulations gain more popularity; because of their possible therapeutic impact with less

cost as well as fewer side-effects than other allopathic medications, while also increasing the bioavailability of the medication.<sup>3</sup>

*Withania somnifera*, which is well known as Ashwagandha, is a plant in the Solanaceae family. Different plant sections have generally been used to treat a variety of diseases. The root is usually used as an aphrodisiac, hepatology, antifungal, antidepressant, astringent, neurasthenic, and low muscle tone. Ashwagandha is one of the medicinal plants that are well-known for their Phyto-pharmacological properties such as anti-inflammatory, antioxidant, anti-stress, and immunomodulatory properties. In addition to the above, ashwagandha extract also has anti-cancer properties. Ashwagandha leaves, stems, and roots contain a range of compounds known as withanolides responsible for most of the plant's pharmacology activities. In humans, withaferin A is primarily responsible for anticancer and antioxidant activity since it is an immunomodulator. Furthermore, withaferin A is an adaptogen and inhibitor of angiogenesis, with a radiosensitization effect on cancer cells which helps in the regeneration of neurons.<sup>4</sup>



DOI: 10.5530/ijper.57.4.119

### Copyright Information :

Copyright Author (s) 2023 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : EManuscript Tech. [www.emanuscript.in]

Colorectal Cancer (CRC) is a type of cancer that develops in the colon or rectum and is also known as colon cancer, bowel cancer, or rectal cancer. Colon cancer is one of the most frequent malignancies, having a significant morbidity and mortality rate. Surgery, cryosurgery, radiofrequency ablation, radiation therapy, and chemotherapy are some of the therapeutic options. Chemotherapy is the most convenient and popular therapeutic technique for improving patients' profiles and wellbeing. Conventional chemotherapy, although being a standard therapeutic technique, is unsuccessful or ineffective in many cases due to the low concentration of medication that reaches the tumor site. According to recent data, the introduction of nanotechnology represents an unprecedented chance to revolutionize the treatment of colorectal cancer.<sup>5</sup>

A targeted drug delivery system is a method of delivering a certain quantity of a medicament to a target diseased region within the body for an extended period of time. Colon-targeted Nano-drug delivery systems are important in the treatment of colon-specific diseases because nanoparticles may accumulate in diseased sections, enhance therapeutic efficiency, and facilitate specific therapies, which decrease systemic toxicity.<sup>6</sup>

Polymer nanoparticles are the colloidal carrier, 10 nm-1µm in size, consisting of synthetic or natural polymers. Polymeric nanoparticles are ideal for incorporating and delivering drugs to cancer cells. Poly(lactic-co-glycolide) is the biodegradable and biocompatible polymer used in drug delivery systems that have gained prominence due to its ability to provide sustained and controlled drug release while minimizing adverse effects. The polymeric substance PLGA has been shown to be a good medium for incorporating and delivering different chemotherapeutic agents to tumors in recent studies.<sup>7</sup> Nevertheless, one of the main disadvantages of PLGA nanoparticles is their inability to directly bind with cells or proteins, resulting in the inability to absorb drugs in specific tissues. Burst release of drugs is another limitation of PLGA nanoparticles, which can cause adverse effects.<sup>8</sup>

Chitosan (CS) is used for coating of PLGA nanoparticles to resolve these limitations. Chitosan (CS) is a polysaccharide that is safe, biodegradable, bio-compatible, and obtained from complete or partial deacetylation.<sup>9</sup> Chitosan (CS) is one of the natural polymers for the delivery of chemotherapeutic agents to the specified location as a nanocarrier. When it is positively charged because of its mucoadhesive property enables interactions with negatively charged mucosa and membranes in the pharmaceutical form that contains chitosan which promotes greater contact, adhesion, and retention near intestinal epithelium.<sup>10</sup>

## MATERIALS AND METHODS

### Materials

Ashwagandha roots were procured from Haridas bhandarkhar, Mangalore. Poly (lactic-co-glycolide), chitosan, poly vinyl alcohol, propylene Glycol, dichloromethane, acetic acid, methanol was procured from Loba Chemie, Mumbai.

### Methods

#### *Extraction of plant material - Soxhlet extraction method*

The roots of Ashwagandha were collected and dried for 48 hr under direct sunlight. The dried roots were then powdered in the mixer grinder. About 70 g of Ashwagandha powder is weighed and filled in a muslin cloth bag and it was placed inside thimble. Then 300 mL of methanol was added to the round bottom flask which is placed on the magnetic heater. Then the solution was heated at 50°C for 6-7 hr. The heated vapor then travels to the reflux condenser through the side arm. The vapour condenses and drops into the thimble which contains the plant material. The heated solvent trickles through the substance and the thimble's wall, and the extract gradually accumulates in the middle compartment.

When the extract's height reaches the top of the siphon, the entire solvent in the central compartment flows through it and back into the lower round-bottomed flask. The process is then repeated as necessary. The equipment was turned off once 50 cycles were finished. The concentrated extract was collected and dried at 90°C in a water bath for further concentration.<sup>11</sup>

#### **Preparation and Characterization of Chitosan-coated PLGA nanoparticles**

##### *Experimental Design*

For optimisation of the formulation, DOE was performed using Design Expert® software. A Box-Behnken design was applied for Chitosan-coated PLGA Nanoparticles (CS-PLGA-NPs), by considering PLGA (30, 60 and 90 mg), Chitosan (0.2, 0.45, and 0.7%) and sonication time (5, 10, and 15 min) as independent variables to find the optimized goal with minimum Particle size and PDI and maximum %EE. The responses obtained from the various runs of both the formulation were subjected to multiple regression analysis using "Design Expert" software (Stat-Ease, Inc., Minneapolis, MN) (Version 11).

##### **Preparation of chitosan-coated PLGA nanoparticles**

Chitosan-coated PLGA Nanoparticles (CS-PLGA-NPs) loaded with Ashwagandha extract (Table 1) were prepared by an oil-in-water single-emulsion solvent evaporation method with slight modifications. The organic phase was prepared by dissolving the required quantities of PLGA (50:50) and ashwagandha extract in dichloromethane (2 mL) and propylene glycol (0.5 mL)

respectively. Then, they were mixed. The aqueous phase consists of 10 mL of PVA solution and 10 mL of chitosan solution, both prepared in 2% acetic acid (v/v). The organic phase was then added to the aqueous phase drop by drop using a syringe with continuous stirring. Then the sample was sonicated for a specific time as per the experimental design using an ultra-probe sonicator at 40% power to obtain O/W emulsion. Subsequently, the organic solvent evaporates overnight on a magnetic stirrer at room temperature. The nanoparticles were precipitated by ultracentrifugation at 13000rpm for 30 min at 4°C. The precipitated nanoparticles were collected and washed with double distilled water to remove excess PVA. The precipitated nanoparticles were resuspended in double-distilled water and lyophilized using sucrose (5% w/v) as a cryoprotectant.<sup>12</sup>

### Characterization of Chitosan-coated PLGA nanoparticles

#### Particle size, Zeta Potential and PDI

PDI indicates the size distribution (polydispersity or monodispersity) of CS-PLGA NPs. Zeta potential is defined as the charged particle obtained when it is present in the medium. Dynamic light scattering was used to determine Particle size, and PDI and electrophoretic light scattering method was used to determine Zeta potential using Zeta sizer (Nano ZS, Malvern Instruments, UK).<sup>13</sup>

#### Percentage entrapment Efficiency

Take the required mL of the prepared nanoparticle suspension in the Tarsus centrifuge tube of 15 mL capacity and it is centrifuged by cold centrifugation at 10000 rpm for 30 min at

4°C. After centrifugation, the supernatant and the sediment are separated. The concentration of ashwagandha extract present in the supernatant was analyzed by UV-spectroscopic method at 224nm.<sup>13</sup> The percentage entrapment efficiency was calculated using the following formula,

$$\% \text{ Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Amount of free drug}}{\text{Total amount of Drug}} \times 100$$

### Optimization of Chitosan-coated PLGA nanoparticles

Based on the constraints given as minimum particle size, PDI and maximum entrapment efficiency, Design Expert® software provided a solution with high desirability which was considered as optimized formulation. The optimized formulation was prepared as per the solution that is 53.607 mg of PLGA and 0.448% w/v of chitosan and 15 min sonication time was used for the formulation.

#### FT-IR Study

A Shimadzu FT-IR 8300 Spectrophotometer was used for Fourier Transform Infrared (FT-IR) spectroscopy, and the spectrum was obtained from the 4000 to 400 cm<sup>-1</sup> range. The drug was disseminated in KBr (200-400 mg) and compressed in a hydraulic press for 5 min at a pressure of 5 tons to get the spectrum. The compatibility of the optimized CS-PLGA-NPs with the formulation ingredients was determined and compared with the FT-IR peak of pure ashwagandha extract.<sup>14</sup>

#### Percentage yield

The Ashwagandha CS-PLGA-NPs obtained after freeze-drying was weighed. Percentage yield value was calculated as follows:

**Table 1: Result of responses of CS-PLGA NPs as per Box Behnken design.**

Std	Factors			Responses			Percentage Yield (%)
	PLGA (mg)	Chitosan (% w/v)	Sonication Time (min)	Particle size (nm)	PDI	EE (%)	
F1	30	0.2	10	209.2±1.94	0.203±0.020	72.2±2.5	58.11
F2	90	0.2	10	210.8±1.28	0.288±0.014	80.2±3.8	65.76
F3	30	0.7	10	303.7±3.73	0.229±0.019	77.09±2.4	63.75
F4	90	0.7	10	438.7±2.95	0.287±0.029	83.7±3.1	69.23
F5	30	0.45	5	407.2±3.05	0.211±0.032	78.11±4.2	60.32
F6	90	0.45	5	436.3±1.01	0.241±0.011	88.6±2.7	66.28
F7	30	0.45	15	243.2±1.21	0.147±0.023	83.43±1.9	60.97
F8	90	0.45	15	231.4±1.72	0.212±0.025	85.5±2.9	66.87
F9	60	0.2	5	207.7±2.18	0.173±0.035	86.59±3.6	62.58
F10	60	0.7	5	486.7±2.38	0.225±0.029	88.17±3.9	67.82
F11	60	0.2	15	177.2±2.56	0.158±0.017	85.65±3.1	63.10
F12	60	0.7	15	205.7±1.79	0.206±0.021	87.99±4.2	67.23
F13	60	0.45	10	252.8±2.19	0.202±0.018	82.47±2.4	65.43
F14	60	0.45	10	247.1±1.07	0.211±0.030	82.47±2.8	64.95

$$\% \text{ Yield} = \frac{\text{Weight of CS - PLGA - NPs}}{\text{Total solid weight}} \times 100$$

### Scanning Electron Microscopy

SEM was used to study the surface morphology of the nanoparticles. Freeze-dried nanoparticles were used for SEM study. For this investigation, the nanoparticle samples were adhered to the metal surface with double-sided adhesive tape. All samples were gold palladium (AuPd) coated and examined using a SEM (ZEISS SIGMA VP Scanning Electron Microscope).<sup>12</sup>

### In vitro mucoadhesive test

The *in vitro* method was used to assess the mucoadhesive property of the nanoparticles. The turbidimetric measurement of nanoparticles was compared with mucin dispersion at 650 nm by UV-spectrophotometer. Precisely weighed amounts of nanoparticles were introduced to aqueous mucin dispersions stirred at 200 rpm. The turbidity of the dispersions was measured at different time intervals for 6 hr and compared to the mucin dispersion. The increase in turbidity of mucin nanoparticle dispersion indicates mucoadhesive property.<sup>15</sup>

### In vitro drug release study

*In vitro* drug release study was performed on the prepared chitosan-coated PLGA nanoparticles and the pure extract. The Pure Extract (PE) and Optimized Formulation (OF1) equivalent to 30mg were incorporated in gelatin capsules and coated with eudragit S 100 polymer solution. The drug release pattern of the Coated Capsules (CC) was compared with Uncoated Capsules (UC).

*In vitro* drug release study was performed using USP type 1 dissolution apparatus in simulated gastric fluid-SGF (pH 1.2) and simulated intestinal fluid-SIF (pH 7.4) for 2 and 8 hr respectively. Each vessel of the dissolution apparatus was filled with 900 mL of SGF buffer and maintain the temperature at 37°C. The Coated and Uncoated Capsules (CC and UC) are added to each vessel and the apparatus is operated at 50 rpm. After predetermined times, 5 mL of sample were taken from each vessel and filtered and diluted with the dissolving media. After 2 hr, the dissolution media was changed to SIF buffer, and the study was continued for another 8 hr. The absorbance of the sample was then determined using a UV-visible spectrophotometer at 224nm.<sup>16</sup>

### Drug release kinetics and mechanism of release

The data obtained from the dissolution study was subjected to kinetic analysis of first order (log cumulative % vs time) and zero order kinetics (cumulative amount of drug released vs time). Mechanism of drug release was determined by fitting the data to Higuchi's matrix model (cumulative % of drug release vs square root of time) and Korsmeyer-peppas model (log cumulative percentage of drug released vs log time).<sup>17</sup>

### In vitro antioxidant activity

The antioxidant activity of the formulations was assessed using the DPPH scavenging assay method. In 10 mL of methanol, 10mg of the extract was dissolved. From the stock solution, the various concentration of 5-160 µg/mL was taken in a test tube to which methanol and 3 mL of DPPH were added and kept for vortex. A blank was also prepared without extract. Ascorbic acid is used as standard at a different concentration range of 5-160 µg/mL. The samples were placed in the dark for about 30 min and later the absorbance was checked at 517 nm using the UV-visible spectrophotometer. By the following equation, we can calculate the capability to scavenge the DPPH radical. Similarly, the assay was conducted on the prepared formulation and the obtained % scavenging activity was compared with extract and standard ascorbic acid.<sup>18</sup>

$$\% \text{ Scavenging activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

### In vitro cytotoxicity assay

MTT assay of pure ashwagandha extract and Ashwagandha CS-PLGA-NPs was performed to evaluate cytotoxicity. The cells were seeded at a density of approximately 5×10<sup>3</sup> cells/well in a 96-well flat-bottom microplate and maintained at 37°C in 95% humidity and 5% CO<sub>2</sub> overnight. Different concentration (500, 400, 300, 200, 100 µg/mL) of samples was treated. The cells were incubated for another 48 hr. The cells in the well were washed twice with phosphate buffer solution, and 20 µL of the MTT staining solution (5 mg/mL in phosphate buffer solution) was added to each well, and the plate was incubated at 37°C. After 4 hr, 100 µL of Dimethyl Sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, and absorbance was recorded with 570 nm using a microplate reader.<sup>19</sup>

$$\% \text{ cell viability} = \frac{\text{Mean OD of test compound}}{\text{Mean OD of Negative control}} \times 100$$

### In vitro Stability studies

*In vitro* stability study was conducted for the prepared CS-PLGA NPs at pH 1.2 simulated gastric fluid and pH 7.4 simulated intestinal fluid. Dilute 1 mL of the prepared nanoparticle suspension in 10 mL of SGF and SIF and incubated for 2-6 hr. In a water bath shaker, samples were agitated at 50 rpm at 37°C. Particle size, zeta potential, and polydispersity index assays were then performed on the samples.<sup>20</sup>

## RESULTS

### Extraction of plant material

The percentage yield of methanolic extract of *Withania sonnifera* (Ashwagandha) by soxhlet extraction method was found to be 14.2%.

## Formulation and characterization of Chitosan-coated PLGA nanoparticles

### Particle Size, Polydispersity Index and Entrapment efficiency

The CS-PLGA NPs loaded with ashwagandha extract were successfully formulated using Box Behnken design to understand the effects of the nanoparticle constituents i.e. PLGA, Chitosan, and sonication time, on its attributes particle size, polydispersity index, and % Entrapment efficiency and result is shown in Tables 1, 2 and Figure 1.

### FT-IR study

The major peak of FT-IR spectra of the pure extract, physical mixture and optimized CS-PLGA NPs is shown in Table 3.

### Scanning electron microscopy

The surface morphology of the formulated nanoparticles was obtained using Scanning Electron Microscopy, as shown in Figure 2.

### In vitro drug release study

An *in vitro* drug release study was performed on the prepared PLGA nanoparticles and the pure drug. The Optimized

Formulation (OF1) and the Pure Extract (PE) were incorporated in gelatin capsules and coated with eudragit S 100 polymer solution. The drug release pattern of the Coated Capsules (CC) was compared with Uncoated Capsules (UC). The cumulative % drug release found is given in Figure 3.

### Drug release kinetics

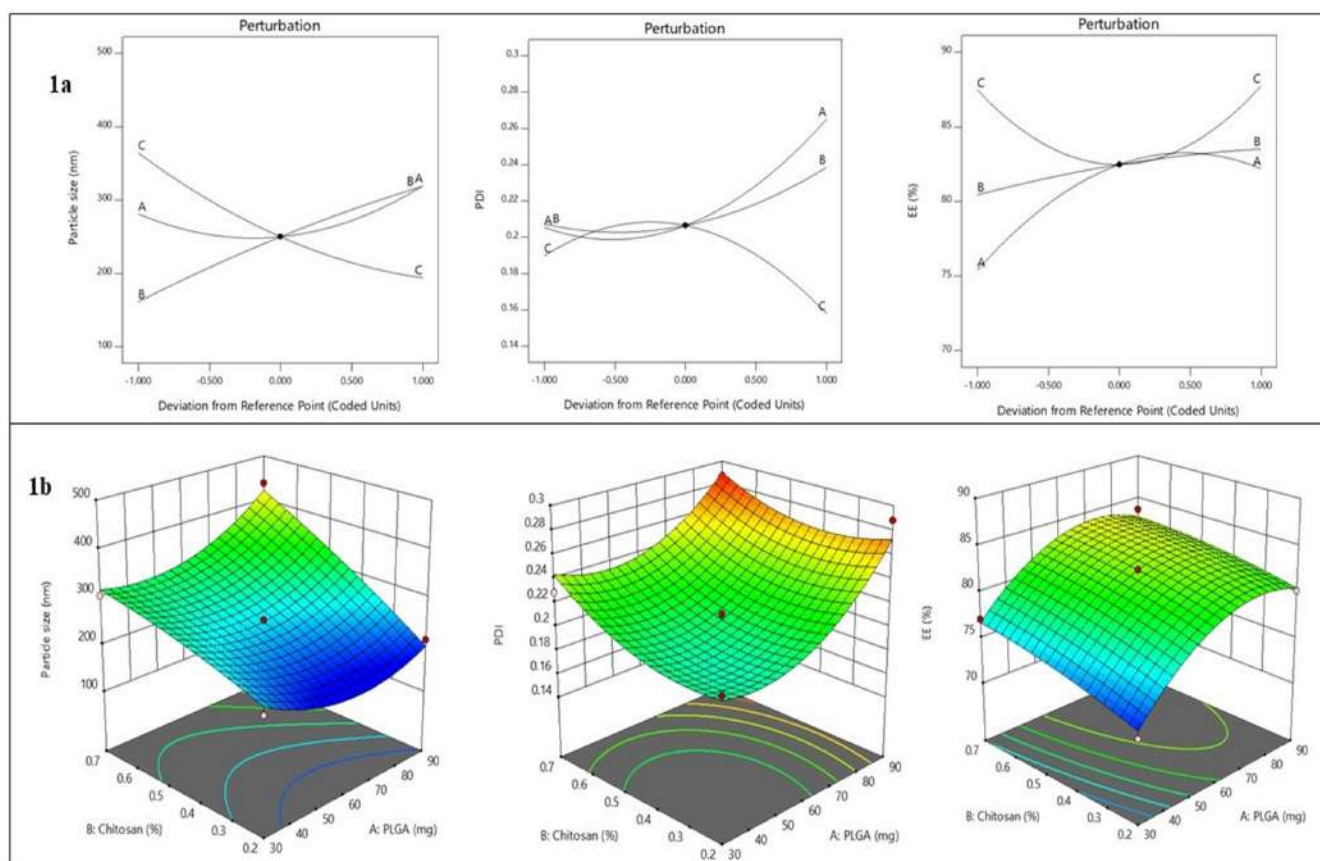
The data obtained from the *in vitro* drug release study is fitted to different kinetic models to determine drug release kinetics and the release mechanism is given in Table 4.

### In vitro antioxidant activity

DPPH free radical scavenging assay was conducted to determine the antioxidant activity of the ashwagandha extract and optimized Ashwagandha-loaded CS-PLGA NPs. The results obtained are shown in Figure 4.

### In vitro cytotoxicity assay

Cytotoxicity of various concentrations of pure extract and optimized CS-PLGA-NPs on CaCO-2 cells were evaluated by Methyl Thiazolyl Tetrazolium (MTT) assay. The results obtained are shown in Figure 5.



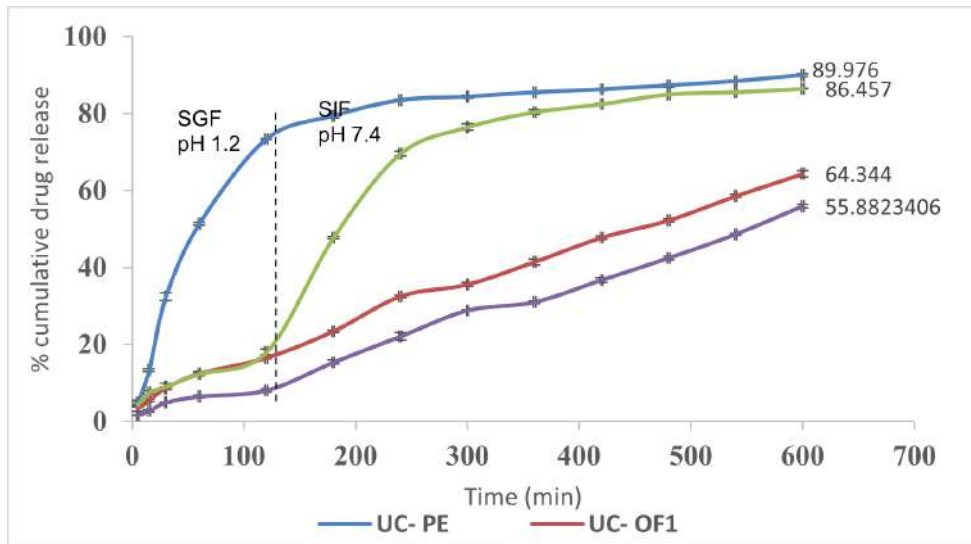
**Figure 1:** a. Perturbation plot; b. Response surface curve representing effects of different factors on (a) particle size (b) PDI (c) Sonication time.

**Table 2: Summary of regression analysis and ANOVA.**

Sl. No.	Factor	Particle size (Adjusted $R^2 = 0.9497$ )		PDI (Adjusted $R^2 = 0.7787$ )		% EE (Adjusted $R^2 = 0.9511$ )	
		Estimated beta coefficient	p-value	Estimated beta coefficient	p-value	Estimated beta coefficient	p-value
1.	Intercept	+249.95	0.0029	+0.2065	0.0488	+82.47	0.0027
2.	A- PLGA	+19.24	0.0828	+0.0297	0.0117*	+3.40	0.0008*
3.	B-Chitosan	+78.74	0.0007*	+0.0156	0.0822	+1.54	0.0144*
4.	C-sonication time	-85.05	0.0005*	-0.0159	0.0790	+0.1375	0.7301
5.	AB	+33.35	0.0477*	-0.0067	0.5197	-0.3475	0.5445
6.	AC	-10.23	0.4358	+0.0088	0.4125	-2.11	0.0160*
7.	BC	-62.63	0.0061*	-0.0010	0.9218	+0.1900	0.7359
8.	A <sup>2</sup>	+50.43	0.0188*	+0.0288	0.0549	-3.68	0.0033*
9.	B <sup>2</sup>	-9.77	0.5005	+0.0165	0.1981	-0.4912	0.4501
10.	C <sup>2</sup>	+29.15	0.0920	-0.0325	0.0386*	+5.12	0.0010*

**Table 3: Major IR peaks of ashwagandha root extract, Physical mixture, and CS-PLGA NPs.**

Samp	Composition	Major peaks (wave numbers $\text{cm}^{-1}$ )
A	Ashwagandha root extract	3343.75, 3120.31, 2944.53, 1737.77, 1643.30, 1036.46.
B	Physical mixture	3460.27, 3087.20, 2850.40, 1736.92, 1648.98, 1040.73.
C	Optimized CS-PLGA NPs	3460.60, 3090.68, 2840.70, 1739.19, 1650, 1046.84.



**Figure 3:** Comparison of *in vitro* drug release profiles of coated and uncoated capsules of pure ashwagandha extract and CS-PLGA NPs.

**Table 4: Comparison of *in vitro* drug release kinetics.**

Release models		Uncoated capsules		Coated capsules	
		PE	OF1	PE	OF1
Zero order	$R^2$	0.672	0.9928	0.8886	0.9942
	K	0.1319	0.1023	0.162	0.089
First order	$R^2$	0.8326	0.9899	0.9518	0.9846
	K	0.0016	-0.0007	-0.0017	-0.0005
Higuchi's model	$R^2$	0.8704	0.9501	0.9347	0.8794
	K	3.847	2.2699	4.2669	1.7886
Koresmeyer-peppas model	$R^2$	0.9969	0.9936	0.9477	0.9546
	K	0.0021	0.0004	0.0628	-0.2332
	n	0.9789	0.6316	0.6391	0.6645

## DISCUSSION

### Extraction of plant material

The *Withania somnifera* roots were collected and extracted by the soxhlet extraction method using methanol as a reagent. The colour and consistency of the methanolic extract were found to be brown and solid. The percentage yield of methanolic extract was found to be 14.2%.

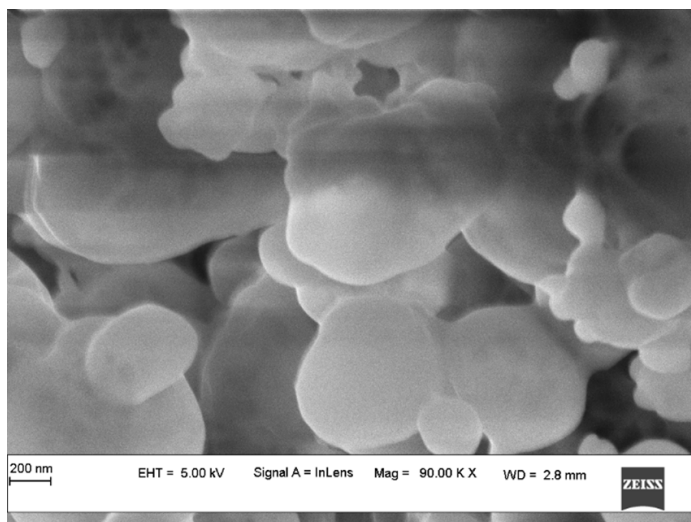
### Formulation and characterization of Chitosan-coated PLGA nanoparticles

#### Statistical analysis of design of experiment

The CS-PLGA NPs loaded with ashwagandha extract were prepared by a single emulsion solvent evaporation method. Box Behnken response surface design was employed for the optimization of the formulation. The influence of independent variables, i.e. PLGA, chitosan concentration, and sonication time on dependent variables particle size, PDI, and % entrapment efficiency was evaluated. The selected factors had a significant effect on all of the responses investigated. The responses obtained are given in Table 1, and the summary of regression analysis and ANOVA is shown in Table 2.

#### Particle Size

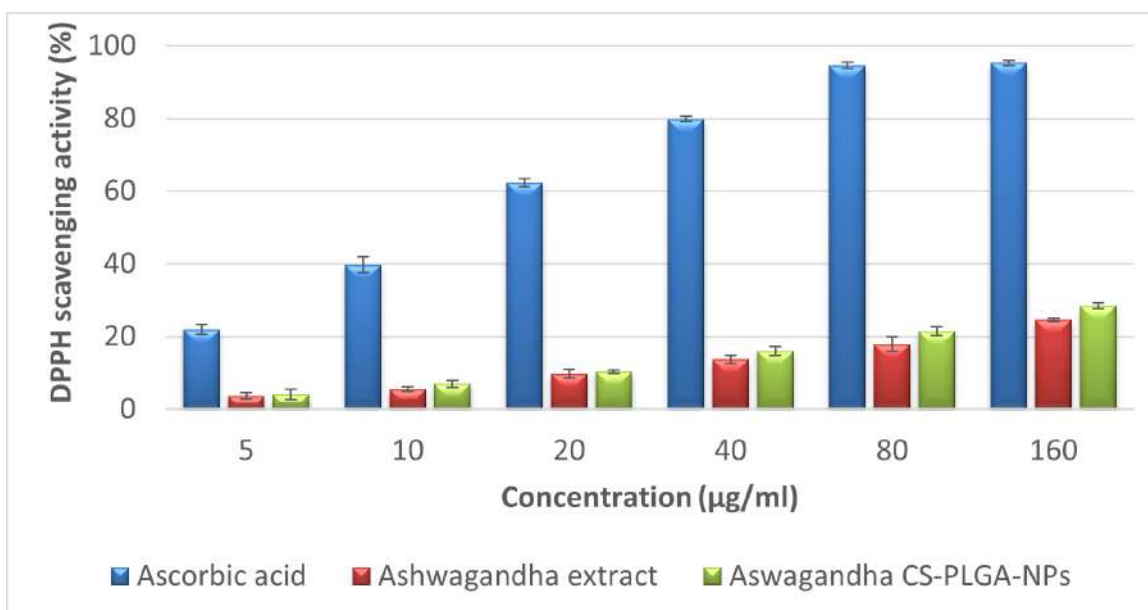
The particle size of CS-PLGA-NPs varied from  $177.2 \pm 2.56$  to  $486.7 \pm 2.38$  nm. The effect of independent variables i.e., PLGA concentration, chitosan concentration, and sonication time, on particle size is shown in the perturbation graph (Figure 1a) and 3D surface response curve (Figure 1b) and Table 1. According to the results, increasing the PLGA concentration causes an initial drop and, subsequently, a progressive increase in particle size. In a previous study, Madani *et al.*, also observed an increase in nanoparticle size with the increase in PLGA concentration.<sup>21</sup> The authors hypothesised that in an emulsion method, increasing the concentration of PLGA in organic solvent raises the viscosity of the dispersed media, resulting in lower shear stress and the generation of bigger nanoparticles.



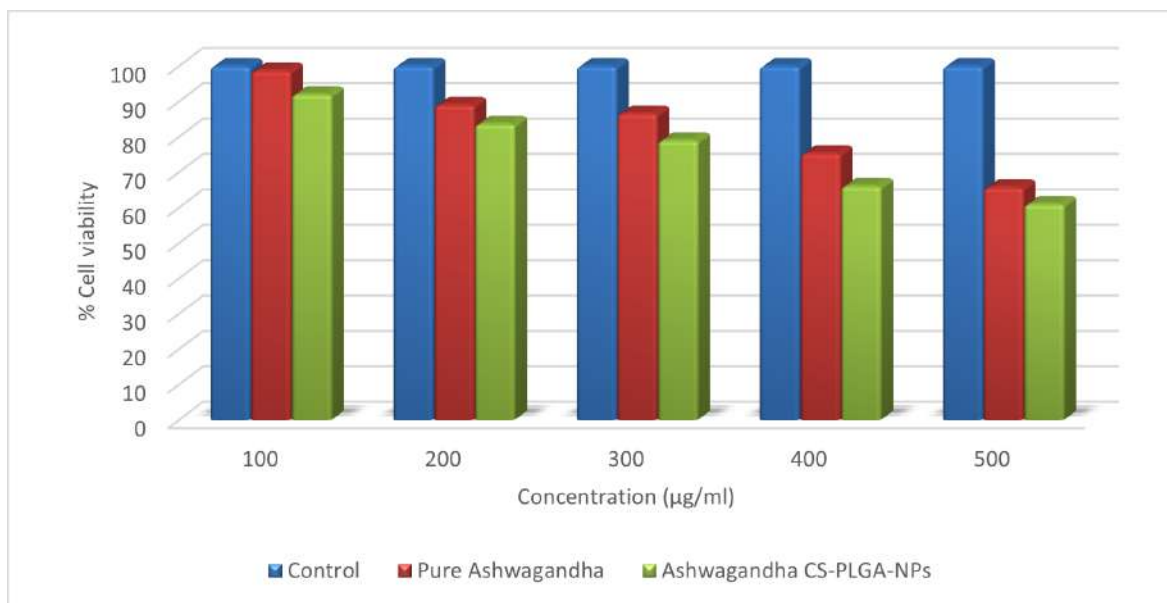
**Figure 2:** SEM image of optimized Chitosan-coated PLGA nanoparticles.

As the concentration of chitosan increased from 0.2 to 0.7% *w/v*, there was an increase in particle size. This might be due to chitosan adsorption on the porous surface of the PLGA NPs. Guo *et al.*, investigated the mechanism of chitosan adsorption onto PLGA NPs produced using an oil-in-water emulsion approach.<sup>22</sup> They discovered an increase in particle size of PLGA NPs with increased chitosan concentration in their study. The particle size decreased as the sonication time increased, owing to the fact that the sonication energy also increased the energy generated by emulsification and reduced the mean particle diameter.

The effect of formulation variables on particle size can be simultaneously studied by applying regression analysis shown in Table 2. The model generated for particle size had a *p*-value of  $< 0.05$  and an *F* value of 28.27, indicating the Quadratic model to be significant. There is only a 0.29% chance that an *F*-value this large could occur due to noise. The value of 45.52 indicates a non-significant lack of fit, implying the model is appropriate to calculate the particle size. The Predicted  $R^2$  of 0.7537 is in reasonable agreement with the Adjusted  $R^2$  of 0.9497; i.e. the



**Figure 4:** Percentage inhibition of DPPH by ascorbic acid, pure ashwagandha, and optimized ashwagandha loaded CS-PLGA NPs.



**Figure 5:** *In vitro* cytotoxicity of Pure Ashwagandha and ashwagandha CS-PLGA-NPs in CaCO-2 cells after 24 hr incubation.

difference is less than 0.2. The polynomial equation obtained from the results of the analysis:

$$\begin{aligned} \text{Particle size} = & +249.95 + 19.24(A) + 78.74(B^*) - 85.05(C^*) \\ & + 33.35(AB^*) - 10.23(AC) - 62.63(BC^*) + 50.43(A^{2*}) - 9.77(B^2) \\ & + 29.15(C^2) \end{aligned}$$

Where A is the concentration of PLGA and B is the concentration of Chitosan and C is sonication time, the co-efficient in this equation represents standardized beta co-efficient and the asterisk sign indicates the significance of variables.

### Polydispersity Index (PDI)

PDI determines the nanoparticle homogeneity. PDI < 0.5 considered optimal. The independent factors that have a significant effect on PDI are shown in the 3D graph (Figure 1b) and perturbation graph (Figure 1a). As the concentration of PLGA and chitosan increases, very little effect was observed till a certain concentration and then PDI increases. This is due to the creation of small droplets during emulsification, which stabilises the organic/aqueous interphase. As the sonication time increases, PDI also increases initially, and later it decreases. The increase in sonication time had a negative effect on PDI because high pressure was created for a longer period of time, resulting in agglomeration of particles with a wide dispersion in the



aqueous phase. Pandit J *et al.*, studied on Chitosan-coated PLGA nanoparticles of bevacizumab and similar results was achieved.<sup>23</sup>

The effect of formulation variables on PDI can be simultaneously studied by the application of regression analysis shown in Table 2. The polynomial equation obtained from the results of the analysis:

$$\text{PDI} = +0.2065 + 0.0297(\text{A}^*) + 0.0156(\text{B}) - 0.0159(\text{C}) - 0.0067(\text{AB}) + 0.0088(\text{AC}) - 0.0010(\text{BC}) + 0.0288(\text{A}^2) + 0.0165(\text{B}^2) - 0.0325(\text{C}^{2*})$$

The model generated for PDI had a *p*-value of < 0.05 and an *F* value of 6.08 indicating the Quadratic model to be significant. The value of 11.74 indicates a non-significant lack of fit. A negative Predicted *R*<sup>2</sup> implies that the overall mean may be a better predictor of the response.

### Entrapment efficiency

The 3D graph (Figure 1b) and perturbation graph (Figure 1a) show that an increase in PLGA concentration results in an initial increase in % EE till a certain concentration and then it decreases. When the PLGA concentration is raised, the viscosity of the organic phase increases, causing the drug molecules to be restricted from travelling in the aqueous phase, resulting in more drug being entrapped inside the polymeric system. Whereas % EE increases as chitosan concentration increases. This could be due to decreased leakage of the entrapped drug. The results achieved were found similar to the research work published by Khan N *et al.*<sup>24</sup>

As the sonication time increases % EE decreases to a certain concentration and later it increases. Smaller droplets occur as the stirring speed is increased, increasing the overall surface area of the nanoparticles. This gave the polymer matrix more room to incorporate more extract, improving encapsulation efficiency. These findings were consistent with those published by Tefas LR *et al.*<sup>25</sup>

The effect of formulation variables on %EE can simultaneously have studied by the application of regression analysis. The polynomial equation obtained from the results of the analysis:

$$\text{EE} = +82.47 + 3.40(\text{A}^*) + 1.54(\text{B}^*) + 0.1375(\text{C}) - 0.3475(\text{AB}) - 2.11(\text{AC}^*) + 0.1900(\text{BC}) - 3.68(\text{A}^{2*}) - 0.4912(\text{B}^2) + 5.12(\text{C}^{2*})$$

The model generated for %EE had a *p*-value of < 0.05 and an *F* value of 29.11 indicating the Quadratic model to be significant. The Predicted *R*<sup>2</sup> of 0.7594 is in reasonable agreement with the Adjusted *R*<sup>2</sup> of 0.9511; i.e., the difference is less than 0.2.

### Optimization of Chitosan-coated PLGA nanoparticles

CS-PLGA NPs were optimized based on constraints such as minimum particle size, PDI, and maximum entrapment efficiency, and desirability of more than 0.8. The optimized

formulation was prepared as per the suggestion given by the software, which contains 54.96 mg of PLGA, 0.4% w/v of chitosan and 15 min sonication time. The particle size, PDI, and % entrapment efficiency values given by the software were 191.96 nm, 0.15, and 87.1%, respectively. In contrast, the experimental value was found to be 187.1 nm, 0.148, and 84.28%, respectively. The observed values were found to be within ± 5% error of the predicted value, which is acceptable.

### Zeta potential

Zeta potential was determined by the electrophoretic light scattering method. The Zeta potential of the optimized CS-PLGA-NPs was found to be 31.3 mV, which indicates that the particles are stable.

### FT-IR study

The FT-IR analysis was carried out to analyze the compatibility of the extract with excipients using the Attenuated Total Reflection (ATR) technique. The principal peaks of the drug were present in both the physical mixture and final optimized formulations, as shown in Table 3, indicating no incompatibility between the drug and the excipient used.

### Scanning electron microscopy

The shape of the particle and the surface morphology of the prepared nanoparticle was determined by Scanning Electron Microscopy (SEM). The particles were found to be uniform and have a spherical shape. The particle surface was found to be smooth as shown in Figure 2.<sup>26</sup>

### In vitro mucoadhesive test

The turbidity of the optimized CS-PLGA NPs -mucin aqueous dispersion was examined to determine the mucoadhesiveness. The absorbance of the mucin dispersion did not show a significant deviation from 0.54. The changes in turbidity of CS-PLGA NPs-mucin dispersion demonstrated the interaction between mucoadhesive polymer chitosan of the nanoparticles and mucin, and not due to the movement of particles. The optimized nanoparticles-mucin dispersion showed higher turbidity compared to that of mucin dispersion. This confirmed the mucoadhesion of the nanoparticles. Chitosan exhibits cationic nature. The electrostatic interaction between the positively charged site of chitosan and negatively charged mucin dispersion is known to be responsible for Mucoadhesion.<sup>15</sup>

### In vitro drug release study

*In vitro* drug release profile of the PE and OF1 in coated and uncoated capsules is shown in Figure 3. From this, it was concluded that OF1 showed sustained drug release compared to pure extract. Eudragit S 100 is a pH-dependent enteric coating polymer soluble at a pH greater than 7. It has the highest entrapment ability compared to other polymers, protects the active ingredients from

gastric fluid, improves drug efficacy, and is suitable for sustained release of the formulation. Therefore, Eudragit S 100 is suited for colonic release targeting.<sup>27</sup> The uncoated capsules containing PE and OF1 showed drug release of 73.345% and 16.445%, respectively at the end of 2 hr in SGF. In SIF, PE and OF1 showed drug release of 89.976% and 64.344% at the end of 10 hr. whereas, the enteric-coated capsules containing PE and OF1 showed drug release of 17.987% and 8.037%, respectively at the end of 2 hr in SGF. In SIF, PE and OF1 showed drug release of 86.457% and 55.882% at the end of 10 hr. Compared with uncoated capsules, the enteric coated capsules showed very limited drug release in SGF due to eudragit S 100 coating.

The OF1 showed sustained drug release in 10 hr in both coated and uncoated capsules compared to PE. The drug encapsulated within the PLGA polymeric matrix that might be released by slow diffusion is called the sustained release phase. Chitosan-coated nanoparticles inhibited drug release during the sustained release phase, probably due to chitosan's protection against drug desorption and diffusion on PLGA nanoparticles. Abd El Hady *et al.*, achieved similar results showing that chitosan coating to PLGA NPs results in sustained release of the drug.<sup>28</sup> The amount of drug released from coated capsules containing OF1 is slower than from uncoated capsules.

### Drug release kinetics

The data obtained from the *in vitro* drug release study of the optimized formulation and pure extract were fitted to zero order, first order to predict the release kinetics. The drug release mechanism was studied by fitting the data to Higuchi model and Korsmeyer-Peppas exponential model as shown in Table 4. The data analysis was focused on the corresponding significance of the regression coefficients. The enteric-coated and uncoated capsules containing pure extract followed first-order kinetics and optimized CS-PLGA NPs followed zero-order kinetics based on high regression coefficient values. Korsmeyer-Peppas model showed good regression coefficients for pure extract and optimized formulation when compared to the Higuchi model. The release exponent (n) of enteric-coated and uncoated capsules of pure drug and the optimized formulation was found to be above 0.45 which indicates that the release can be defined by Non-Fickian diffusion, which may mean that more than one process regulates the release rates of drugs, i.e. diffusion coupled with erosion mechanism.

### *In vitro* antioxidant activity

The % inhibition of DPPH by pure extract and the optimized formulation was compared with standard ascorbic acid. Different concentrations, ranging from 5 µg/mL to 160 µg/mL were tested for antioxidant activity. The DPPH radical scavenging activity of standard ascorbic acid, Ashwagandha extract, and ashwagandha

CS-PLGA NPs was shown in Figure 4. As the concentration of ashwagandha increases, the antioxidant potential of pure drug and optimized formulation also increases statistically. The IC<sub>50</sub> values of ascorbic acid, pure extract, and the optimized formulation was found to be 14.52 µg/mL, 345.96 µg/mL, and 290.58 µg/mL respectively. As per the results, ashwagandha-loaded CS-PLGA NPs showed elevated antioxidant activity than pure extract. According to the literature, chitosan itself exhibits antioxidant activity and Pereira MC *et al.*, and Aldawsari HM *et al.*, reported enhancement of the antioxidant activity of PLGA entrapped drugs.<sup>13,29</sup> Therefore, the higher DPPH free radical scavenging capacity of Ashwagandha via ashwagandha-loaded CS-PLGA NPs could be justified by the enhanced delivery mechanism for phenolic compounds.

### *In vitro* cytotoxicity assay

Cytotoxicity of various concentrations of pure extract and optimized CS-PLGA-NPs on CaCO-2 cells were evaluated for 24 hr by Methyl Thiazolyl Tetrazolium (MTT) assay. Percentage cell viability is plotted against concentration (µg/mL) as shown in Figure 5. After 24 hr, Pure extract and CS-PLGA-NPs produced a concentration dependent reduction in cell viability. The percentage cell viability of Pure extract and CS-PLGA NPs at 500µg/mL was found to be 65.72±2.17% and 61.02±1.92% respectively. After 24 hr of treatment, the IC<sub>50</sub> of pure extract and CS-PLGA-NPs was found to be 716.74±4.25 µg/mL and 630.27±4.28 µg/mL respectively. As per the MTT assay results, a much better result was found in CS-PLGA-NP-treated cells. This can be explained by the fact that the CS-PLGA-NPs revealed significantly higher delayed drug release compared to pure extract, which produces an excellent cellular response. Our results are corroborated by a previously published report by Alshehri S *et al.*<sup>30</sup>

### Stability studies

Stability studies were carried out for CS-PLGA NPs in SGF (pH 1.2) and SIF (pH 7.4). The formulations were evaluated for particle size and PDI. The initial particle size was 187.1±2.05 in SGF, which reached a value of 195.3±1.76 in 2 hr, and particle size was increased from 232.8±2.43 to 265.6±1.63 in SIF within 8 hr. The particle size showed a slight and steady increase in SGF and SIF. When suspended in the buffer, the modest swelling of the Chitosan polymer is believed to increase particle size.

In SGF, the PDI values changed from 0.151±0.003 to 0.156±0.016 in 2 hr. In contrast, PDI values changed from 0.159±0.018 to 0.166±0.007 in SIF in 8 hr. No significant changes were observed in PDI, suggesting that there was no substantial particle aggregation, which would have converted the sample into a collection of particles with varying particle size ranges, leading to a high polydispersity.<sup>20</sup>

## CONCLUSION

Chitosan-coated PLGA nanoparticles of ashwagandha extract were successfully formulated by emulsion solvent evaporation method using different concentrations of PLGA, chitosan and varying sonication time by Box-behnken design. The optimized nanoparticles were smooth, spherical in shape, and uniform in size. The formulation showed better adhesion properties due to chitosan coating. *In vitro* drug release study showed that CS-PLGA-NPs exhibits prolonged drug release than pure Ashwagandha. After being encapsulated, antioxidant and cytotoxic properties of Ashwagandha were retained, suggesting that these properties may be the effect of combined action of chitosan and Ashwagandha. The nanoparticles also demonstrated resistance to pH fluctuations and prevented the premature release of Ashwagandha while maintaining their stability in simulated gastric and intestinal fluids. Hence, it can be concluded that chitosan-coated PLGA nanoparticles of Ashwagandha could be a promising formulation in oral drug delivery for the treatment of Colon cancer.

## ACKNOWLEDGEMENT

The authors are thankful to NGSM Institute of Pharmaceutical Sciences, Mangalore for providing the necessary facilities to carry out the research work.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**DoE:** Design of experiments; **µg:** Microgram; **FT-IR:** Fourier transform infrared spectroscopy; **PDI:** Poly dispersity index; **CS-PLGA-NPs:** Chitosan coated PLGA nanoparticles.

## SUMMARY

Chitosan-coated PLGA nanoparticles were prepared by emulsion solvent evaporation method. Box behnken design was utilized to examine the effect of independent variables such PLGA, chitosan concentration and sonication time on particle size, PDI and % entrapment efficiency. Statistical analysis revealed that independent variables had a significant effect on response. The prepared CS-PLGA-NPs were optimized based on constraints given as minimum particle size, PDI and maximum entrapment. The observed values for particle size, PDI, zeta potential and entrapment efficiency of the optimized CS-PLGA-NPs were found to be 187.1nm, 0.148, 31.3 mV and 84.28% respectively. The optimized CS-PLGA-NPs were smooth, spherical in shape, and uniform in size. The optimized formulation showed good mucoadhesive property. *In vitro* drug release study suggests that the enteric coating of the optimized formulation prevents the drug release in SGF and showed sustained release of the drug

than pure ashwagandha. The prepared CS-PLGA NPs showed enhanced antioxidant activity than pure ashwagandha and better cytotoxicity over CaCO-2 cells. The formulation was found to be stable in both simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4).

## REFERENCES

- Kunzelman JI, Durako MJ, Kenworthy WJ, Stapleton A, Wright JLC. Irradiance-induced changes in the photobiology of *Halophila johnsonii*. *Mar Biol.* 2005;148(2):241-50. doi: 10.1007/s00227-005-0070-x.
- Shakya AK. Medicinal plants: Future source of new drugs. *Int J Herb Med.* 2016;4(4):59-64.
- Goyal A, Kumar S, Nagpal M, Singh I, Arora S. Potential of novel drug delivery system for herbal system for herbal drugs. *Indian J Pharm Educ Res.* 2011;45:225-35.
- Madhuri S, Govind P. Anticancer activity of *Withania somnifera* Dunal (Ashwagandha). *Indian Drugs.* 2009;46(8):603-9.
- Liu K, Wang ZQ, Wang SJ, Liu P, Qin YH, Ma Y, et al. Hyaluronic acid-tagged silica nanoparticles in colon cancer therapy: Therapeutic efficacy evaluation. *Int J Nanomedicine.* 2015;10:6445-54. doi: 10.2147/IJN.S89476, PMID 26491300.
- Naem M, Awan UA, Subhan F, Cao J, Hlaing SP, Lee J, et al. Advances in colon-targeted nano-drug delivery systems: Challenges and solutions. *Arch Pharm Res.* 2020;43(1):153-69. doi: 10.1007/s12272-020-01219-0, PMID 31989477.
- Wang Y, Li P, Kong L. Chitosan-modified PLGA nanoparticles with versatile surface for improved drug delivery. *AAPS PharmSciTech.* 2013;14(2):585-92. doi: 10.1208/s12249-013-9943-3, PMID 23463262.
- Lu B, Lv X, Le Y. Chitosan-modified PLGA nanoparticles for control-released drug delivery. *Polymers.* 2019;11(2):304. doi: 10.3390/polym11020304, PMID 30960288.
- Wei Z, Wang C, Zou S, Liu H, Tong Z. Chitosan nanoparticles as particular emulsifier for preparation of novel pH-responsive Pickering emulsions and PLGA microcapsules. *Polymer.* 2012;53(6):1229-35. doi: 10.1016/j.polymer.2012.02.015.
- Chronopoulou L, Massimi M, Giardi MF, Cametti C, Devirgiliis LC, Dentini M, et al. Chitosan-coated PLGA nanoparticles: A sustained drug release strategy for cell cultures. *Colloids Surf B Biointerfaces.* 2013;103:310-7. doi: 10.1016/j.colsurfb.2012.10.063, PMID 23261553.
- Srivastav AK, Das P. Phytochemical extraction and characterization of roots of *Withania somnifera* for its anti-bacterial, antioxidant, anti-Inflammation and analgesic activity. *Int J Innov Res Develop.* 2014;3:22-33.
- Lima IA, Khalil NM, Tominaga TT, Lechanteur A, Sarmento B, Mainardes RM. Mucoadhesive chitosan-coated PLGA nanoparticles for oral delivery of ferulic acid. *Artif Cells Nanomed Biotechnol.* 2018;46(sup2):993-1002. doi: 10.1080/21691401.2018.1477788, PMID 29842790.
- Aldawsari HM, Alhakamy NA, Padder R, Husain M, Md S. Preparation and characterization of chitosan coated PLGA nanoparticles of resveratrol: improved stability, antioxidant and apoptotic activities in H1299 lung cancer cells. *Coatings.* 2020;10(5):439. doi: 10.3390/coatings10050439.
- Trivedi M, Branton A, Trivedi D, Nayak G, Balmer AJ, Anagnos D, et al. Evaluation of the energy of consciousness healing treated *Withania somnifera* (Ashwagandha) root extract using LC-MS, GC-MS, and NMR spectroscopy. *Am J Biomed Sci.* 2017;2(5):16-25.
- Hejjaji EMA, Smith AM, Morris GA. Evaluation of the mucoadhesive properties of chitosan nanoparticles prepared using different chitosan to tripolyphosphate (CS: TPP) ratios. *Int J Biol Macromol.* 2018;120(B):1610-7. doi: 10.1016/j.ijbiomac.2018.09.185, PMID 30282010.
- El-Badry M, Alanazi FK, Mahrous GM, Alsarra IA. Effects of Kollicoat IR® and hydroxypropyl-β-cyclodextrin on the dissolution rate of omeprazole from its microparticles and enteric-coated capsules. *Pharm Dev Technol.* 2010;15(5):500-10. doi: 10.3109/10837450903300171, PMID 19814584.
- Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol Pharm.* 2010;67(3):217-23. PMID 20524422.
- Mishra K, Ojha H, Chaudhury NK. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chem.* 2012;130(4):1036-43. doi: 10.1016/j.foodchem.2011.07.127.
- Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, et al. Cell viability assays. *Assay Guidance Manual.* 2016.
- Ravi GS, Charyulu RN, Dubey A, Prabhu P, Hebbar S, Mathias AC. Nano-lipid complex of rutin: Development, characterisation and *in vivo* investigation of hepatoprotective, antioxidant activity and bioavailability study in rats. *AAPS PharmSciTech.* 2018;19(8):3631-49. doi: 10.1208/s12249-018-1195-9, PMID 30280357.
- Madani F, Esnaashari SS, Mujokoro B, Dorkoosh F, Khosravani M, Adabi M. Investigation of effective parameters on size of paclitaxel loaded PLGA nanoparticles. *Adv Pharm Bull.* 2018;8(1):77-84. doi: 10.15171/apb.2018.010, PMID 29670842.
- Guo C, Gemeinhart RA. Understanding the adsorption mechanism of chitosan onto poly (lactide-co-glycolide) particles. *Eur J Pharm Biopharm.* 2008;70(2):597-604. doi: 10.1016/j.ejpb.2008.06.008, PMID 18602994.

23. Pandit J, Sultana Y, Aqil M. Chitosan-coated PLGA nanoparticles of bevacizumab as novel drug delivery to target retina: Optimization, characterization, and *in vitro* toxicity evaluation. *Artif Cells Nanomed Biotechnol.* 2017;45(7):1397-407. doi: 10.1080/21691401.2016.1243545, PMID 27855494.
24. Khan N, Ameeruzzafar, Khanna K, Bhatnagar A, Ahmad FJ, Ali A. Chitosan coated PLGA nanoparticles amplify the ocular hypotensive effect of forskolin: Statistical design, characterization and *in vivo* studies. *Int J Biol Macromol.* 2018;116:648-63. doi: 10.1016/j.ijbiomac.2018.04.122, PMID 29723623.
25. Tefas LR, Tomuță I, Achim M, Vlase L. Development and optimization of quercetin-loaded PLGA nanoparticles by experimental design. *Clujul Med.* 2015;88(2):214-23. doi: 10.15386/cjmed-418, PMID 26528074.
26. Ahmad N, Ahmad R, Alrasheed RA, Almatar HMA, Al-Ramadan AS, Buhezah TM, *et al.* A chitosan-PLGA based catechin hydrate nanoparticles used in targeting of lungs and cancer treatment. *Saudi J Biol Sci.* 2020;27(9):2344-57. doi: 10.1016/j.sjbs.2020.05.023, PMID 32884416.
27. Mehta R, Chawla A, Sharma P, Pawar P. Formulation and *in vitro* evaluation of Eudragit S-100 coated naproxen matrix tablets for colon-targeted drug delivery system. *J Adv Pharm Technol Res.* 2013;4(1):31-41. doi: 10.4103/2231-4040.107498, PMID 23662280.
28. Abd El Hady WE, Mohamed EA, Soliman OAE, El-Sabbagh HM. *In vitro-in vivo* evaluation of chitosan-PLGA nanoparticles for potentiated gastric retention and anti-ulcer activity of diosmin. *Int J Nanomedicine.* 2019;14:7191-213. doi: 10.2147/IJN.S213836, PMID 31564873.
29. Pereira MC, Oliveira DA, Hill LE, Zambiasi RC, Borges CD, Vizzotto M, *et al.* Effect of nanoencapsulation using PLGA on antioxidant and antimicrobial activities of guabiroba fruit phenolic extract. *Food Chem.* 2018;240:396-404. doi: 10.1016/j.foodchem.2017.07.144, PMID 28946289.
30. Alshehri S, Imam SS, Rizwanullah M, Fakhri KU, Rizvi MMA, Mahdi W, *et al.* Effect of chitosan coating on PLGA nanoparticles for oral delivery of thymoquinone: *in vitro*, *ex vivo*, and cancer cell line assessments. *Coatings.* 2020;11(1):6. doi: 10.3390/coatin11010006.

**Cite this article:** Abhilash MM, Priya S, Jyothi D. Mucoadhesive Chitosan-Coated PLGA Nanoparticles of Ashwagandha Extract for Colon-Targeted Delivery. *Indian J of Pharmaceutical Education and Research.* 2023;57(4):971-82.