FORMULATION AND EVALUATION OF POLYMERIC MICROSPHERES LOADED WITH ASHWAGANDHA EXTRACT

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***ABSTRACT:** The present research was to formulate and optimize Ashwagandha-loaded microspheres targeting to enhance bioavailability, reduce dose, minimize side effects, and sustain drug release. The Ashwagandha polymeric microspheres were prepared by Ionotropic gelation technique method. The drug-excipient compatibility study of the active drug (Ashwagandha) and polymer performed by Fourier transform-infrared spectroscopy confirmed that there was no interaction. The resulting microspheres were evaluated for partial size, and surface morphology, of Paclitaxel microspheres. Formulation F2 showed the maximum entrapment efficiency. Formulation F-2 showed percent entrapment efficiency of 92.80 %. The percent yield value was found to be 88.92 %. The particle size was found 146.82µm. A sustained release pattern was obtained from the microsphere and the drug's bioavailability was found to be enhanced. In vitro release study showed that Ashwagandha release from both kinds of microspheres was slow followed by an increase to reach a maximum of 92.80%*

***Key words:** Ashwagandha, FTIR studies, sodium alginate, Ionotropic gelation technique, In vitro drug release studies.*

I. INTRODUCTION

The goal of controlled-release medication delivery systems is to maximize therapeutic efficacy and bioavailability while minimizing local and systemic negative effects. [1] Since no organic solvents are used in the formulation, ionotropic gelation is one of the most environmentally benign methods for encapsulating the required medicine.[2] When cross-linked by different methods, naturally occurring polysaccharides have demonstrated considerable potential for several pharmaceutical applications, including the construction of controlled-release drug delivery systems.[3] Ashwagandha is a small, woody shrub, that grows about 2 feet and found in Africa, the Mediterranean and India.[4] Ashwagandha or Withania somnifera is a popular herb in the Ayurvedic system of medicine. It is a small shrub that belongs to the family Solanaceae. [5] It might be useful for different diseases and mostly as a nervine tonic (has a soothing effect on nerves). Ashwagandha is commonly called Indian Ginseng or Indian winter cherry. Ashwagandha is used to relieve Stress and Anxiety. Ashwagandha is perhaps best known for its stress-relieving properties.[6] Microspheres have been promising approaches for achieving oral sustained release. As a coating material or carrier, the microsphere requires a polymeric component.[7] A number of various substances both biodegradable and non-biodegradable have been demonstrated for the preparation of microsphere. It not only reduces the drug's dose by rapidly reaching the active biological sites, but it also lowers toxicity.[8] Biodegradable polymer microspheres for controlled drug delivery have got much attention due to their outstanding clinical benefits: lowering dose frequency, improving patient convenience and acceptance for patients, as well as drug targeting to particular region.[9]

In the present study, Ashwagandha microspheres were prepared by encapsulating with natural polymers respectively with sodium alginate by ionic gelation technique with an aim to prepare and improve its delivery characteristics.

II. MATERIALS

Ashwagandha was obtained from Hetero Labs, HYD. Sodium alginate, Tragacanth were procured from Synpharma Research Labs, Hyderabad, and other chemicals and other reagents used were of analytical grade.

Methodology

Drug and excipient compatibility studies[10]

Drug excipients compatibility studies were performed to know the compatibility of excipients with drug at accelerated conditions. The study was conducted by preparing a homogenous mixture of excipients with drug and filled in HDPE bags and LDPE bags. Glass vials were exposed to 600 C and 400C/75 %RH for 4 weeks and LDPE bags were exposed to 400C±75 %RH for 4 weeks. Samples were observed periodically for any physical change.

Preparation of plant extracts[11]

The roots of ashwagandha were procured from the floral garden of the Government College of Indian medicine, Mysuru, Karnataka. The roots were washed with water shade dried. The shade-dried roots were coarsely powdered and subjected to sequential extraction with non-polar to polar solvents (Petroleum ether, benzene, chloroform, alcohol, water and NaOH) using soxhlet apparatus. In the present study, alcoholic extracts of ashwagandha were selected for further studies .The solvents were evaporated and the powder was stored in 37°C till further use.

Preparation and evaluation of Ashwagandha Microspheres[12, 13]

Selection of polymers for preparation of Microspheres

Polymers were used as excipients for extract formulations, and polymer derivatives are also used for the formulation of Microspheres. In the present study, for the preparation of Microspheres of Ashwagandha.

Preparation method of Microspheres

Microspheres containing Ashwagandha as a core material were prepared by Ionotropic gelation technique method.

Formulation table:

Table-1: Preparation of Ashwagandha Microspheres

Ingredients	F1	F2	F3	F4
Ashwagandha	50	50	50	50
Sodium alginate	500	1000	-	-
Tragacanth	-	-	500	1000
Calcium chloride	2%	2%	2%	2%

Preparation of Ashwagandha extract loaded microspheres [14]

The microspheres of extract were prepared by using an ionic gelation technique. The polymers used in the formulation were sodium alginate and tragacanth. The polymeric solution was prepared by dissolving sodium alginate and tragacanth in 10 ml of distilled water and sonicated for 20 min to remove air bubbles. The drug was dissolved in distilled water. The prepared polymer solution was added dropwise through a 26-gauge hypodermic needle into 50 ml of 2 % w/v of calcium chloride solution which is used as a cross-linking agent, with stirring at 600 rpm. The prepared microsphere was allowed to stir with the crosslinking agent for one hour. The prepared microspheres were filtered and washed 2-3 times with distilled water to remove the traces of calcium chloride solution. The microsphere was then dried at room temperature for 12 hrs.

Evaluation of Microspheres

The prepared Microspheres were evaluated for various parameters such as yield, particle size, drug entrapment efficiency, evaluation of in vitro drug, and the effect of different formulation and process variables such as drug-to-polymer ratio, type of polymer, speed, and combination of polymers were studied.

Yield of Microspheres[15]

The yield of Microspheres was calculated from the amount of Microspheres obtained divided by the total amount of all non-volatile components.

$$\% \text{ Yield} = \frac{\text{Actual weight of the Microspheres}}{\text{Total weight of all non-volatile components}} \times 100$$

Particle size and shape[16]

The particle size of the Microspheres was measured by optical microscopy. The eyepiece micrometer was calibrated using a stage micrometer and the calibration factor was used further in the calculation of the size of Microspheres. The Microspheres were finely spread over a slide and visualized under an optical microscope using an eyepiece micrometer. About 50 readings were taken at random and the mean ± standard deviation was calculated. The shape of the Microspheres was visualized and the photographs were taken with the aid of a binocular microscope.

Surface morphology of the Microspheres[17]

The surface morphology of the Microspheres was studied with the aid of a Scanning Electron Microscope (SEM).

Drug entrapment efficiency (DEE)[18]

The amount of drug entrapped was estimated by crushing 50 mg of Microspheres using mortar and pestle, and

extracting the drug with aliquots of 7.4 pH buffer repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using a 7.4 pH buffer. The solution was taken in a beaker and sonicated in a bath sonicator for 2 hours. The solution was filtered and absorbance was measured after suitable dilutions spectrophotometrically at 329 nm against an appropriate blank.

The amount of drug entrapped in the Microspheres was calculated using the following formula –

$$DEE = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

In vitro drug release study[19]

In vitro drug studies were carried out for all formulations in Franz diffusion cell. Microspheres equivalent to 10 mg of Ashwagandha were poured into1 ml aliquots were withdrawn at a predetermined intervals and equal volume of dissolution medium was replaced to maintain sink conditions. The necessary dilutions were made with 7.4 pH buffer and the solution was analyzed for the drug content spectrophotometrically using a UV-Visible spectrophotometer (Lab India) at 286 nm against an appropriate blank. Three trials were carried out for all formulations. From this cumulative percentage drug was calculated and plotted against the function of time to study the pattern of drug.

Stability studies[20]

The success of an effective formulation can be evaluated only through stability studies. The prepared Ashwagandha Microspheres placed on plastic tubes containing desiccant and stored at ambient conditions, such as at room temperature, 40±2°c and refrigerator 2-8°c for a period of 3 months.

III. RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)

The compatibility between the Ashwagandha and polymers was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug- polymers mixture, which confirmed the absence of any chemical interaction between the drug and polymers.

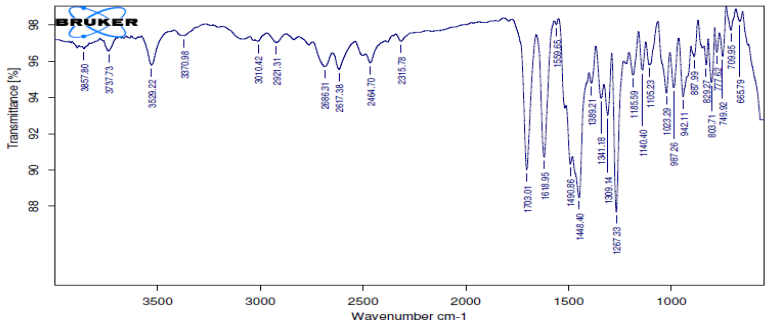

Fig.1. FT-IR Sample for Ashwagandha

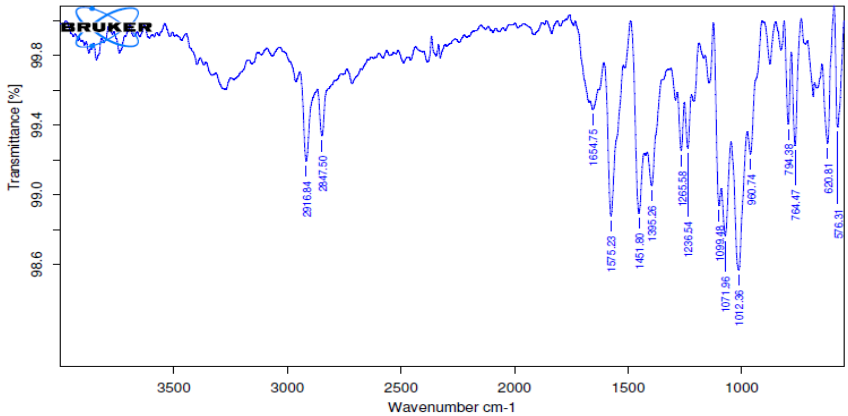

Fig.2. FT-IR Sample for Optimized Formulation

Formulation and Evaluation of Sustained Release Microspheres of Ashwagandha

Results of the evaluation parameters of formulated sustained-release microspheres

The prepared sustained-release microspheres were evaluated for various parameters such as yield, drug entrapment efficiency, particle size, and in vitro drug release. The effect of preparation and process variables such as drug-polymer ratio, speed, type of polymer, and combination of polymers on particle size, yield, entrapment efficiency, and *in-vitro* release of Diltiazem from sustained microspheres were also studied.

Characterization of microspheres

Surface topography by scanning electron microscopy (SEM)

Figure shows SEM photograph of optimized microspheres at 100 × magnification, at 1000× magnification. SEM photographs showed discrete, spherical microspheres. SEM photographs also showed the presence of drug crystal on the surface of microspheres revealing that the microspheres were having some rough surface**.** The drug crystals on microspheres were may be due to the presence of unentrapped drug in dispersion medium.

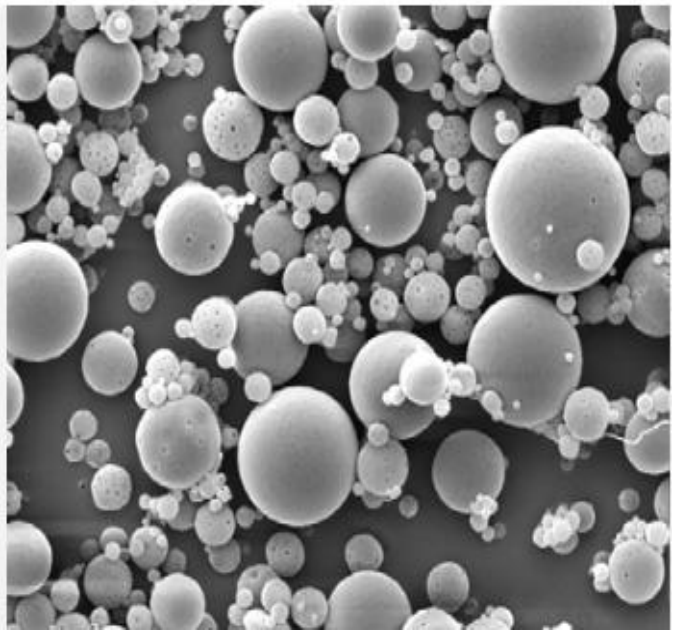

Fig-3: SEM Analysis of Microspheres

Table-2: Evaluation parameters of microspheres

F. no	% Yield	Particle size	Drug entrapment efficiency
F1	75.96	145.9	85.94
F2	73.27	146.82	88.92
F3	70.15	148.86	82.47
F4	73.58	149.7	83.36

In vitro drug release studies

Table-3: Cumulative %drug release

TIME (hours)	F1	F 2	F3	F4
0	0	0	0	0
1	13.86	14.58	11.88	12.86
2	25.80	26.93	28.90	27.24
3	36.92	38.75	37.82	35.89
4	43.57	45.85	44.78	42.37
5	59.81	60.23	62.81	60.87
6	63.48	72.52	74.72	73.89
7	75.82	85.96	82.90	80.57
8	90.25	92.80	90.87	91.89

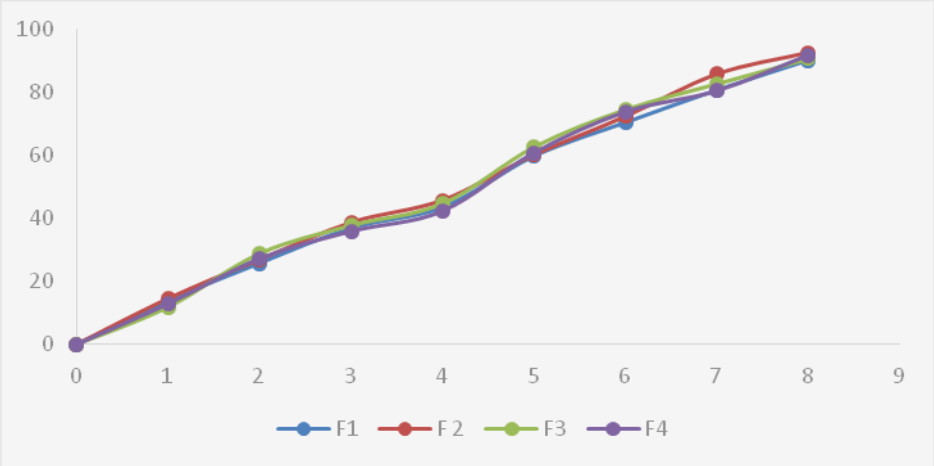

Fig.4. Cumulative percentage drug released vs. time Curves of microspheres F1–F 4 in P^H 7.4 buffer.

Here, keeping drug ratio constant and varied polymer ratio as the polymer concentration increases viscosity; this influences the interaction between disperse phase and dispersion medium that affects the size distribution of particle. And F2 formulation shows good results when compared to other formulations. Above graph indicates that %Drug release of F2 formulation shows better drug release when compared with other formulations

Stability Study

There was no significant change in physical and chemical properties of the formulation F-2 after 3 Months. Parameters quantified at various time intervals were shown;

Table-4: Results of stability studies of optimized formulation F-2

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-2	25^0C/60%RH % Release	92.80	92.50	91.58	90.58	Not less than 85 %
F-2	30^0C/75% RH % Release	92.80	92.32	91.17	90.42	Not less than 85 %
F-2	40^0C/75% RH % Release	92.80	92.20	91.15	90.30	Not less than 85 %

IV. CONCLUSION

Ashwagandhaloaded in microspheres prepared by sodium alginate and tragacanth as a polymer and to prepare polymeric microspheres which increases the bioavailability of the drug to the targeted area and in a controlled manner and reduces GI related side effects.Polymeric Microspheres containing Ashwagandha as a core material were prepared by using Ionotropic gelation technique. The yield and entrapment efficiency was high for microspheres were Particle size, entrapment efficiency and production yield were influenced by the type of polymer, polymer concentration, stirring speed and combination of polymers. *In vitro* dissolution of optimized formulations of various Polymerin pH 7.4 formulations are releasing the drug up to 8 hrs. F2 with high concentration of sodium alginate is considered as optimized formulation.

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