



Formulation and Evaluation of Capsules of Ashwagandha Phytosomes

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ABSTRACT

Objective of the present study was to formulate and evaluate capsules of Ashwagandha phytosomes. There are many herbal extracts having excellent *in-vitro* activity but less *in-vivo* activity because of their macromolecular size and poor lipid solubility, which result in poor absorption and bioavailability problems. Many of these problems can be overcome by formulating novel drug delivery systems. Phytosomes provide better absorption and bioavailability than the conventional herbal extracts. This project aims in improving the drug release characteristics of Ashwagandha by formulating Ashwagandha phytosome capsules. Ashwagandha Phytosomes were produced by a process in which standardized plant extract was bound to phospholipids, producing a lipid compatible molecular complex. Ashwagandha phytosome complexes were characterized by particle size, zeta potential, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy and *in vitro* drug release. The results showed that the average particle size and zeta potential of optimized Ashwagandha phytosomes formulation were 98.4nm and -28.7 mV. *In vitro* drug release studies revealed that the cumulative % drug release of capsules of Ashwagandha phytosomes was found to be 76.8%. Antioxidant activity of Ashwagandha phytosomes was evaluated by reducing power method. The results showed that the Ashwagandha phytosome complex exhibited more antioxidant activity compared to the Ashwagandha extract. Hence it was concluded that Ashwagandha phytosomes serve as useful novel drug delivery system and provide more bioavailability than conventional formulations.

Keywords: Ashwagandha Extract, Ashwagandha phytosomes complex, Antioxidant activity, Soy lecithin.

INTRODUCTION

Plant Preparations or their parts have been widely used in medicine since ancient times and till today use of phytomedicines is wide spread. Most of the biologically active constituents of plants are polar or water-soluble. However, water-soluble phytoconstituents like flavonoids, tannins, glycosidal aglycones, etc. are poorly absorbed due to macromolecular size, which cannot be absorbed by passive diffusion or due to their poor lipid solubility, thus severely limiting their ability to transport across lipid-rich biological membranes, resulting in their poor bioavailability.¹

Phytosome is a newly introduced patented technology developed to incorporate the standardized plant extracts or water-soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes called phytosomes.²⁻⁴ Phytosomes provide better absorption and bioavailability than the conventional herbal extracts. When a stoichiometric amount of the phospholipid was made to react with purified herbal extract in an aprotic solvent, phytosomes were formed. This project aims in improving the drug release characteristics of ashwagandha by formulating Ashwagandha phytosome capsules.

Withania somnifera (L.) Dunal, commonly known as "Ashwagandha" (Family: solanaceae) is well known for its therapeutic use in the Ayurvedic system of traditional medicine. Chemical constituents include withanolides (steroidal lactones) and alkaloids. Steroidal lactones

include withanone, withaferin A, withanolides I, II, III, A, D, E, F, G, H, I, J, K, WS-I, P and S. Alkaloids include ashwagandhine, cuscohygrine, anahygrine, ropine. The withanolides are reported to show antioxidant properties, including the prevention of lipid peroxidation. It has been used as an antibacterial, antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent. In this study, a complex of Ashwagandha and soy lecithin was prepared and the physicochemical properties and antioxidant activities of the complex were investigated.⁵

MATERIALS AND METHODS

Materials: Ashwagandha extract was obtained as gift sample from Ayush herbs pvt ltd, Himachal Pradesh; Soy lecithin from Lobachemie, Potassium ferricyanide, ferric chloride and solvents (Dichloromethane, Ethanol, Trichloroacetic acid) used were of analytical grade.

Equipments: UV-Visible spectrophotometer (Labindia UV 3000⁺), centrifuge (Remi equipment pvt ltd), Particle size analyzer (Horibo scientific Nanopartica, UK), FTIR (Shimadzu), calorimeter (Digisun).

Methods

Characterization of Powdered Drug Ashwagandha

Organoleptic properties: Ashwagandha extract was observed for its organoleptic properties like color, solubility and wavelength maxima.

Solubility profile of Ashwagandha: Solubility of ashwagandha was determined in different solvents such



as water, ethanol, methanol, DMSO (Dimethyl sulfoxide), chloroform, acetone, phosphate buffer 6.8, phosphate buffered saline 7.4.

Determination of wavelength maxima: Concentration 100µg/ml of Ashwagandha extract dissolved in a mixture of ethanol and 7.4 pH phosphate buffer saline (2:8) scanned over a wavelength range of 200-400nm.

Compatibility Studies

FTIR spectroscopy can be used to investigate and predict any physicochemical interactions between Ashwagandha and soy lecithin in a formulation and therefore it can be applied to the selection of suitable chemically compatible excipients. The aim of the present study was to test, whether there is any interaction between the carriers and drug.

Preparation of Ashwagandha Phytosomes Complex (Apc)

Two methods have been attempted in preparing Ashwagandha phytosomes complex were Ethanol method and Reflux method.

Ashwagandha phytosomes complex in the ratios of (1:1, 1:2, 1:3, 1:4, and 1:5) were prepared using ethanol as a reaction medium. Soya lecithin was dissolved in dichloromethane and added drop by drop to the ethanol solution of Ashwagandha extract with continuous stirring and sonicated for 15 min. The resultant mixture was evaporated under vacuum at 40°C. After drying the residue is placed in desiccator for over-night. The dried residue is crushed in a mortar and sieved with a 100 mesh filter.⁶⁻⁸

Ashwagandha phytosomes complex in the ratios of (1:1, 1:2, 1:3, 1:4, and 1:5) were prepared by reflux method. Ashwagandha and soy lecithin were placed in a 100ml round bottom flask and refluxed in dichloromethane for 1hr not exceeding 40°C. The resultant clear solution was then evaporated and 15ml of n-hexane was added until precipitate was formed. The precipitate was collected and placed in desiccator.

Characterization of Ashwagandha Phytosomes Complex⁹⁻¹⁰

Microscopic view

Optical microscopy was used for characterization of the complex. The complex was suspended in buffer and a drop was placed on a slide and covered with a cover slip. Microscopic view of the complex was observed at a magnification of 10x10.

Measurement of particle size

The particle size of Ashwagandha phytosomes was measured by particle size analyzer (Horibo scientific nanopartica SZ100). For the measurement, 100 µl of the formulation was diluted with an appropriate volume of PBS pH 7.4 and the vesicle diameter was determined.

Measurement of Zeta potential

Zeta potential is the most important parameter for physical stability of phytosomes. The higher the electrostatic repulsion between the particles the greater is the stability. ZP value more than +20 mV or less than -20 mV predicts good physical stability of dispersion. Zeta potential measurement of the optimized phytosome suspension was done by using the (Horibo scientific Nanopartica SZ100). For the measurement, 1ml of the sample was diluted to 10ml with water, 5ml of this diluted sample was transferred to a cuvette and the zeta potential was measured.

Entrapment efficiency (EE)

Ashwagandha phytosomes were centrifuged at 12000rpm for 45 min using a Remi centrifuge to separate phytosomes from untrapped drug. Concentration of the free drug as the supernatant was determined by measuring absorbance at 226nm using UV-Visible spectrophotometer. The percentage drug entrapment was calculated by using the formula,

Entrapment efficiency (%) =

$$\frac{\text{Weight of total drug} - \text{weight of free drug} \times 100}{\text{Weight of total drug}}$$

In-vitro dissolution studies

The prepared Ashwagandha phytosomes complex was loaded in zero size capsules. *In-vitro* dissolution studies for all the prepared formulations was carried out using type-II apparatus at 50rpm in 900 ml of phosphate buffered saline 7.4 pH as a dissolution media, maintained at 37±5°C. 5ml aliquots were withdrawn at the specified time intervals and assayed spectrophotometrically. An equal volume of fresh media was replaced after each sampling to maintain the constant volume. The samples were analyzed at 226nm using UV-visible double beam spectrophotometer.

In-Vitro Antioxidant Activity

Reducing power method¹¹⁻¹³

This method is based on the principle that increase in the absorbance indicates an increase in the antioxidant activity. Various concentrations of phytosome complex were prepared (200-1000µg/ml). 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of K₃Fe (CN)₆ (1% w/v) are added to 1.0 mL of sample. The resulting mixture was incubated at 50 °C for 20 min, followed by the addition of 2.5 mL of trichloroacetic acid (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL), mixed with distilled water (2.5 mL) and 0.5 mL of FeCl₃ (0.1%, w/v). The absorbance was then measured at 700 nm against blank sample.



Stability Studies

The stability of phytosomes was carried out as per ICH guidelines. The optimized formulations were stored at different temperature ranges $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for a period of 3 months and studied for drug entrapment.

RESULTS AND DISCUSSION

Characterization of powdered drug Ashwagandha

Organoleptic properties: Ashwagandha extract was analyzed for their organoleptic properties like color, Solubility and wave length maxima of drug. From the results it was concluded that ashwagandha was found to be soluble in phosphate buffered saline (PBS pH 7.4) and dimethylsulphoxide. The concentration $100\mu\text{g/ml}$ of ashwagandha extract in phosphate buffered saline was found to be 226nm .

Standard calibration curve of Ashwagandha in UV spectrophotometer

The UV absorbance of Ashwagandha standard solution in the range of $20\text{-}120\mu\text{g/ml}$ of drug in phosphate buffered saline pH 7.4 showed linearity at λ_{max} 226nm . The linearity was plotted for absorbance against concentration with R^2 value 0.999 and with the slope equation $y = 0.006x + 0.012$.

Compatibility studies

The compatibility between the Ashwagandha and Soy lecithin was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug and lipid as shown in Figure 1 and 2.

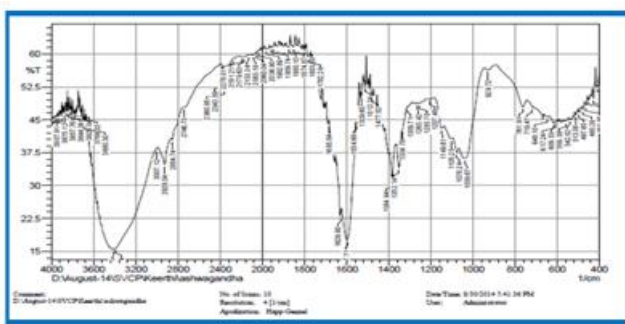


Figure 1: FTIR graph of ashwagandha

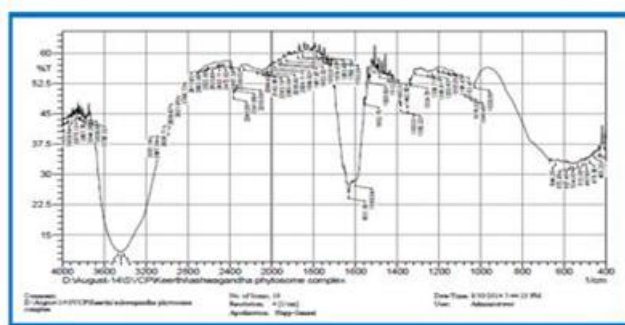


Figure 2: FTIR graph of ashwagandha phytosome complex

Preparation of Ashwagandha phytosomes complex

Ashwagandha phytosomes complex prepared by ethanol and reflux method in the ratios of (1:1, 1:2, 1:3, 1:4, and 1:5) with the respective percentage yields were given in the Table 1. Ashwagandha phytosomes were optimized based on effective concentration of drug and phospholipids ratio.

Characterization of Ashwagandha Phytosomes Complex

Microscopic view

The microscopic view of the Ashwagandha phytosomes complex indicated the presence of sphere shaped vesicles.

Particle size and Zeta potential of optimized Ashwagandha phytosomes

Optimized phytosomes were analyzed to determine their particle size distribution and zeta potential values. It was observed that the average particle size was found to be 98.4nm for optimized formulation (E3) and zeta potential value was found to be -28.7 indicating good stability of the formulation. The results were graphically represented in Figure 3 and Figure 4.



Figure 3: Particle size of prepared ashwagandha phytosomes



Figure 4: Zeta potential of prepared ashwagandha phytosomes



Table 1: Compositions of the formulations with the respective percentage yields

Preparation method	Formulation code	Ingredients					% yield
		Drug (mg)	Soylecithin (mg)	Dichloromethane	Ethanol	PBS7.4	
Ethanol method	E1	100	100	2ml	20ml	q.s	71.5%
	E2	100	200	2ml	20ml	q.s	80.3%
	E3	100	300	4ml	20ml	q.s	86.5%
	E4	100	400	6ml	20ml	q.s	85.5%
	E5	100	500	8ml	20ml	q.s	83.2%
Reflux method	R1	100	100	20ml	3ml	q.s	80%
	R2	100	200	20ml	6ml	q.s	76.6%

Entrapment efficiency (EE)

According to the drug entrapment study conducted the maximum drug entrapment was shown by E3. The entrapment efficiency of all formulations was graphically represented in figure 5. The Ashwagandha phytosomes prepared by Ethanol method has showed high entrapment efficiency compared to reflux method (R6, R7). The formulation E3 showed highest release entrapment efficiency of 90.1% indicating the optimum amount of lipid required for the formation of an Ashwagandha phytosomes. With further increase in the lipid concentration, the Entrapment efficiency decreased indicating that the lipid concentration did not help in entrapping the drug into the matrix.

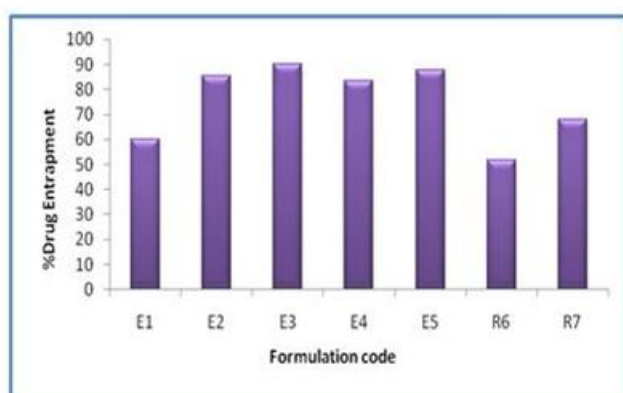


Figure 5: Graphical representation of encapsulation efficiency of ashwagandha phytosomes

In vitro Dissolution data

The prepared Ashwagandha phytosomes complex was loaded in zero size capsules and subjected to invitro dissolution studies using type-II apparatus at 50rpm in 900 ml of phosphate buffered saline 7.4 pH as a dissolution media, maintained at $37 \pm 5^\circ\text{C}$. For all the prepared formulations 100mg of dose was taken. It was observed that the formulation E3 (76.8%) has highest cumulative % drug release compared to other formulations.

In vitro drug release comparison between optimized (E3) and conventional formulation

Based on percentage yield, Entrapment efficiency and *in vitro* drug release study formulation E3 has been optimized. The optimized Ashwagandha phytosomes formulation (E3) were loaded as 250mg capsules and compared with conventional formulation and subjected to *in vitro* dissolution studies using type-II apparatus at 50rpm in 900 ml of phosphate buffered saline 7.4 pH as a dissolution media, maintained at $37 \pm 5^\circ\text{C}$. From the results it was observed that the formulation E3 (76.8%) has highest cumulative % drug release compared to conventional formulation (50.2%) and graphically represented in Figure 6.

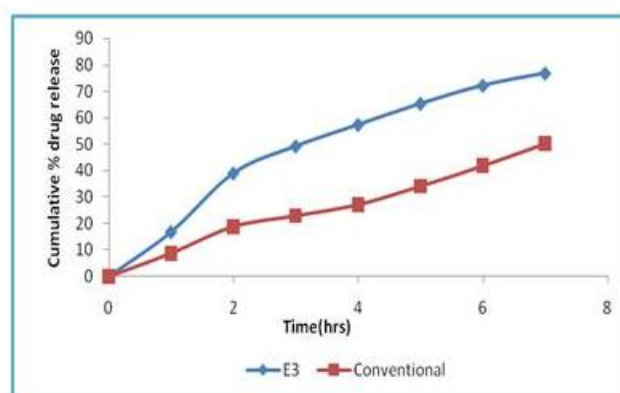


Figure 6: % Drug Release of E3 and Conventional Formulation

Antioxidant Activity

Reducing power method

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. As shown in Figure 7, at 0.2mg/ml and 1mg/ml the optical density of crude extract were 0.02, 0.09 and Ashwagandha phytosomes complex was 0.09 and 0.24. The results indicate that the activity of the Ashwagandha extract is very low compared to Ashwagandha phytosomes complex.

Stability Studies

Ashwagandha phytosomes were stored at room temperature and refrigerated temperature for 3 months and entrapment efficiency was determined. Stability studies were conducted for optimized formulation E3 which showed better entrapment efficiency. The results showed no significant changes. Thus we conclude that the drug does not undergo degradation on storage.

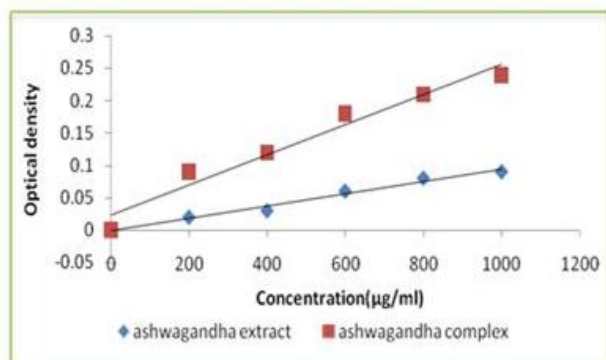


Figure 7: Reducing Power of Crude Plant Extract and Phytosomes Complex

CONCLUSION

Ashwagandha phytosomes capsules were successfully formulated using soy lecithin. The prepared phytosomes formulations were evaluated and optimized based upon their percentage yield, Entrapment efficiency, *In vitro* drug release, and *In vitro* antioxidant activity. By UV, FTIR it could be concluded that Ashwagandha and soy lecithin in the complex were joined by non-covalent-bonds, and did not form a new compound. The Antioxidant activity of Ashwagandha phytosomes complex is more compared to crude extract.

REFERENCES

1. Manach C, Scalbert A, Morand C, Polyphenols, food sources and bioavailability, American journal of clinical nutrition, 9, 2004, 727-747.
2. Prasanna Habbu, Smita Madagundi, Ramesh Kulkarni, Preparation and evaluation of Bacopae phospholipid complex for anti-amnesic activity in rodents, Drug invention today, 5, 2013, 13-21.
3. Pandey S, Patel K, Phytosomes: Technical revolution in phytomedicine, International journal of PharmTech Research, 6, 2010, 627-31.
4. Shalini Kushwaha, Agatha Betsy, Paramjit Chawla, Effect of Ashwagandha (*Withania somnifera*) Root Powder Supplementation in Treatment of Hypertension, Ethno Medical journal, 6, 2012, 111-115.
5. KeyongXu, Benguo Liu, Yuxiang Ma, Jiquan Du, Guanglei Li, Han Gao, Yuan Zhang, ZhengxiangNing, Physicochemical Properties and Antioxidant Activities of Luteolin-Phospholipid Complex molecules, 14, 2009, 3486-3493.
6. Qunyou Tan, Shan Liu, Xueliang Chen, Mingjun Wu, Hong Wang, Huafeng Yin, Dan He, Huarong Xiong, and Jingqing Zhang, Design and Evaluation of a Novel Evodiamine-Phospholipid Complex for Improved Oral Bioavailability, American Association of pharmaceutical scientists PharmSciTech, 13, 2012,534-547.
7. Maiti K, Kakali M, Arunava G, Bishnu PS, Pulok KM, Curcumine phospholipid complex: preparation, therapeutic evaluation and pharmacokinetic study in rats, International Journal of Pharmaceutics, 330, 2007, 155-163.
8. Prakash O, Gyanendra NS, Raman NS, Satish CM, Meenakshi B, Saroj Y, Determination of bacoside-A by HPTLC method in Bacopamonnieri extract, International Journal of Green Pharmacy, 2, 2008, 173-175.
9. Peng Q, Zhang ZR, Sun X, Zuo J, Zhao D, Gong T, Mechanisms of Phospholipid complex loaded Nanoparticles enhancing the oral bioavailability, Molecular Pharmacy, 7, 2010, 565–75.
10. Song Y, Zhuang J, Guo J, Xiao Y, Ping Q, Preparation and properties of a Silybin-phospholipid complex, Pharmazie, 63, 2008, 35–42.
11. Chanda S, Dave R, In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview, African Journal of Microbial Resistance, 3, 2009, 981–996.
12. Pathak G.P, Anubhav Khurana, Divesh Sood, Navjot Sharma, Amir, Vineet Sharma, In Vitro Study of Antioxidant Activity of *Withania Somnifera* Root, American Journal of PharmTech Research, 3, 2013, 465-471.
13. Dipankar chaudhuri, Nikhil baban ghate, Rhitajit sarkar, Nripendranath mandal, Phytochemical Analysis and Evaluation of Antioxidant and Free radical scavenging activity of *Withania somnifera* root, Asian Journal of Pharmaceutical and Clinical Research, 5, 2012, 193-199.

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