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Accelerated Stability Studies of *Mucuna prureins* Hydroalcoholic Extract Phytosome Formulation, and Evaluation of its Capsule Dosage Form

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ABSTRACT: *Mucuna prureins* extract (MPE) is useful in reducing depression symptoms. However, MPE's transit through biological membranes is restricted due to its large molecular weight and hydrophilic nature. Phytosomes could be promising carriers for improving the oral absorption of such encapsulated extracts. One of the major challenges was to get the desired EE and that was achieved by using soya phosphatidylcholine. In this work optimized batch of *Mucuna prureins* phytosomes (MPP) was subjected to accelerated stability studies and evaluated at regular time intervals for entrapment efficiency, particle size, polydispersity index, and zeta potential. Further, capsules of MPE and MPP were formulated and evaluated for quality control evaluation tests like weight variation, *in vitro* disintegration time, and *in vitro* dissolution testing. The accelerated stability studies revealed that the optimized phytosomeformulation was stable under the stability conditions of temperature and relative humidity. Moreover, MPE and MPP had weight variation, and *in vitro* disintegration time within permissible limits according to IP. The dissolution profile of MPP was found superior to MPE capsules. The findings demonstrated the ability of *Mucuna prureins* phytosomes to maintain quality control features under accelerated stability conditions. Furthermore, phytosomes could be given in a capsule dosage form, allowing for a prolonged release of encapsulated extracts with long-term health benefits.

Keywords: *Mucuna prureins* extract, phytosomes, accelerated stability studies, tablet, evaluation.

INTRODUCTION

According to the World Health Organization, approximately 80% of the population still relies on traditional medicine (such medicinal herbs) for basic health care in many third-world nations because of poverty and a lack of access to modern treatment. Because almost 80% of the world's 6.1 billion inhabitants live in developing nations, medicinal plants will most certainly be used regularly. The hunt for as many resources as feasible is required while looking at plants as potential sources of novel pharmaceuticals to treat cancer, AIDS, and CNS-related disorders. The discovery of phytochemical substances with antidepressant activity could lead to the development of new treatments for depression (Singh *et al*., 2019; Arora *et al*., 2019).

For mild conditions like coughs and colds, chronic problems like back pain, and major chronic diseases like asthma, cancer, depression, and diabetes, as well as for "improvement" of functions or processes, herbal medications continue to be a common healthcare option among the general people. In order to support the traditional use of herbal treatments with scientific evidence, much research has been done in recent years to identify the pharmacological basis of action and clinical use of herbal medications (Arora *et al*., 2019).

Additionally, comparable advancements in phytochemistry have been achieved in terms of figuring out how to extract, purify/isolate, and determine the molecular structure of the active components in herbal medicines (Husain *et al*., 2022; Shukla *et al*., 2022). Because of this, we are shifting away from "crude" herbal medicines and toward "purer forms of herbal therapeutics (purified and standardised extracts, single phytoconstituent)" (i.e., roots, leaves, etc.) Most biological plants' active components are polar, however, most phytoconstituents have poor water

solubility and thus bioavailability. This could be due to the phytoconstituents' complicated molecular composition and small size, which results in poor bioavailability (Bernardo *et al*., 2017; Karekar *et al*., 2022).

Mucuna pruriens, the velvet bean plant, grows in a variety of tropical and subtropical climates around the world (Lampariello *et al*., 2012). *Mucuna pruriens*, a member of the *Fabaceae* family, is a medicinal plant renowned for its impressive anti-oxidative and anti-inflammatory properties. With a longstanding history in Ayurvedic medicine, this plant has gained recognition as a potent remedy for various neurological disorders and male infertility. Its therapeutic effects have been cherished for centuries (Rai *et al.,* 2020). The extract derived from Mucuna pruriens seeds has demonstrated notable antioxidant properties while posing no harm to reproductive tissues. Furthermore, it has been observed to exert phytoestrogenic effects in females, and in males, it has been shown to enhance the expression of markers associated with testicular function and sperm quality, thereby promoting male fertility (amsaard *et al*., 2020).

According to some studies, the Mucuna species mainly contains levodopa, as well as certain phenols, tannins, and hallucinogenic tryptamines (Nweze *et al*., 2017). In addition, the seeds of *Mucuna prureins* extract consist of 5-indole chemicals, 5-hydroxytryptamine, and tryptamine (Tripathi *et al*., 2001).

Phytoconstituents are unable to be absorbed when taken orally despite the presence of different chemical ingredients as detailed above.

Thus, the cosmetic and pharmaceutical sectors are working on techniques to improve the solubility and permeability of plant-based compounds with biomedical applications (Chanchal *et al*., 2008; Djekic *et al*., 2015; Saraf 2010). One of the most promising solutions relies on the development of a specific chemical complex with phospholipids. According to the hypothesis, the resultant complex differs from the unmodified active component, the phospholipid itself, or their physical mixing in terms of its melting point, solubility, and oil-water partition coefficient (Semalty *et al*., 2010a; Semalty *et al*., 2010b; Tripathy *et al*., 2013). Utilizing the patented process Phytosome® (Indena, Italy), plant ingredient/phospholipid complexes that self-associate in aqueous fluids and form unilamellar vesicles were developed (phytosomes or herbosomes). Phytosomes have a larger loading capacity for active constituents than liposomes because the active ingredient is a natural component of the phospholipid bilayer in them (Das *et al*., 2014; Freag *et al*., 2013; More *et al*., 2012). The development of such complexes enhances the permeability of pure polyphenols across cellular membranes and boosts their effectiveness (Bhattacharyya *et al*., 2013; Bombardelli *et al*., 1994; Hush *et al*., 2013). Due to the hygroscopic and oxidizable nature of plant ingredient/phospholipid complexes, the development of stable formulations requires meticulous attention to formulation parameters, preparation techniques, packaging methods, and storage conditions. It is essential to carefully consider these factors to ensure the long-term stability and efficacy of the product (Khan *et al*., 2013; Maiti *et al*., 2007; Qin *et al*., 2010; Djekic *et al*., 2016).

As a result, the current study focused on phytosome complex formulation, accelerated stability testing of the optimized batch, and evaluation of phytosome complex tablets for sustained delivery of *Mucuna prureins* hydroalcoholic extract.

MATERIALS AND METHODS

MATERIALS. Amsar Goa Pvt. Ltd, Goa, India, provided the *Mucuna prureins* seeds extract as a gift sample. VAV Pvt. Ltd, Mumbai, India, provided soya phosphatidylcholine (SPC, LECIVA S-70) as a gift sample. Alkem Laboratories Pvt. Ltd., Mumbai, India, provided the drug levodopa.

METHODS. Indena's patented procedures (www.indena.com) were used to formulate phytosomes. The factorial design of 3^2 factorials was used. The complex was made with standardized *Mucuna prureins* extract and LECIVA S70 at various molar ratios and temperatures. In a 100 ml round bottom flask, a weighed amount of standardized *Mucuna prureins* extract and soya lecithin was combined with 20 ml acetone. The mixture was refluxed at temperature 40^0C for 1 hour followed by evaporation of the solution. n-hexane (20 mL) was added to the clear solution under continuous stirring. The precipitate was collected after filtration and stored in amber-colored glass bottleKarekar *et al*., 2022). As per our previous research paper, we found that the formulation with a 1:1 ratio and 60°C displayed the best results as compared to other formulations hence it was optimized.

Stability studies. This study was performed to determine the stability of the formulations by testing it in triplicate according to international norms (Medicines 2004). Stability studies should test those aspects of bioactive compounds that are vulnerable to alteration during storage and are anticipated to affect the quality, safety, and/or efficacy (WHO, 2009). Particle size, PI, entrapment efficiency, and zeta potential parameters were assessed during stability studies. The optimized phytosome formulation was stored at accelerated stability conditions of 40 ± 2◦C/75% RH ± 5% RH, over 6 months in the stability chamber (FOURTECH). The stability samples were examined at 0, 3, and 6M time points during accelerated stability storage conditions (WHO 2009; ICH 1993;ICH 2003).

Formulation of phytosome complex capsules

Formation of capsules. 200 mg *Mucuna prureins* hydroalcoholic extract and its phytosomes were filled in capsule number #0 shell and further tested for quality control testing like drug content, DT, weight variation, and drug release.

***In vitro* evaluation of capsules** (Indian Pharmacopoeia 1996; James *et al*., 1990)

***In vitro* DT:** The disintegration test medium was water. The device was run with discs for 15 minutes before the state of the capsules was inspected. The test was repeated with 6 capsules omitting the discs if the capsule failed to conform due to adherence to the discs.

***In vitro* dissolution study:** The formulated capsules were tested *in vitro* using a USP paddle dissolution apparatus (Electrolab TOT06L) rotating at 50rpm in 500ml of pH 6.8 phosphate buffer at 37.5ºC. The samples (5 ml) were withdrawn at predetermined time intervals for 12 hours and filtered through filter paper. The samples were analysed at 220 nm using UV spectrophotometer. To maintain the sink condition, equal volume of fresh buffer was added to release media.

RESULTS AND DISCUSSION

Stability studies: The optimized batch of phytosomes was subjected to stability studies. The particle size was increased from 216.3±14 to 346.4 ± 18 at the end of 6 months. The polydispersity index changed from0.457±0.016 to 0.472 ± 0.012 indicatingits monodisperse nature (Kolimi *et al*., 2023). The zeta potential was -37.45±0.20 initially and changed to about -21.36 ± 0.24. The entrapment efficiency values ranged from 99.76±1.24 initially to about 95.63± 0.98 at the end of the sixth month indicating that vesicles were intact and did not undergo leakage. The results showed that monodisperse phytosomes were effectively formed with size ranges of less than 300 nm for both formulations across time at all temperatures and RH conditions of the accelerated stability study (Table 1), which is important since smaller particle size is essential for oral absorption (Alshahrani, 2022; Mohammadi *et al*., 2021). The formulation was found stable for six months of storage at varying temperatures and RH. The size of the formulation remained constant during stability studies. Size is an important factor for oral absorption and formulation stability, which will result in a considerable improvement in bioavailability. Limiting the size of drug delivery devices enhances intestinal absorption (Hussain *et al*., 2001; Honary *et al*., 2013).

The negative zeta potential of the phytosomes employed in this study ranged from -20 to -40 mV (Puttipipatkhachorn *et al*., 2001). While particles with similar electric charges may also induce repulsion, preventing particle aggregation and facilitating easy re-dispersion, high positive or negative zeta potential values can produce considerable repulsive forces (Rani *et al*., 2007; Rabbani *et al*., 2021). In a scenario with coupled electrostatic and steric stabilisation, a minimum zeta potential of ±20 mV is preferred (Unger *et al*., 2007) whereas a high zeta potential (positive or negative) could give physical stability to the system. Negatively charged particles, on the other hand, are removed from the bloodstream more slowly than positively charged particles, staying in the bloodstream for longer periods (Khairnar *et al*., 2022). This shows that the formulation's potential was also linked to the calculated EE, which was near 100 percent. The results of entrapment efficiency also implicated that the phytosomes could maintain encapsulated drugs intact at the end of six months. Thus, the results of overall all the studies of particle size, entrapment efficiency, and zeta potential, claimed that phytosomes were in a good state of physical stability at the end of six months in the temperature and humidity conditions of accelerated stability studies.

Capsule evaluation:

***In vitro* DT:** The quality control parameters of MPE and MPP capsules were studied (Khairnar *et al*., 2022). The *Mucuna prureins* extract capsules, as well as phytosome capsules, had necessary physicochemical evaluation parameters like weight variation and *in vitro* disintegration time. The *in vitro* DT for MPE tablets was in the range of 7.42 ±1.68 to 7.64 ±1.23 while for MPP tablets it was 7.22 ±1.64 to 7.32 ±1.66.

***In-vitro* drug release study:** The drug release pattern differed in extract capsules and phytosome complex capsules. The cumulative % drug release was in the range of 7.58 ±1.46 to 55.53 ±2.62 for MPE capsules and MPP capsules it was 13.15 ±3.46 to 90.366 ±4.42 **(**Fig. 1). It can be observed from the above values that drug release from MPE capsules did not reach 60% while those for MPP capsules it was observed to sustain releasing almost 90 % of the drug.

Table 1: Characterization of phytosomes

Months	Parameters	Optimized batch of phytosomes
0	Average particle size ± SD (nm)	216.3±14
	Polydispersity Index (PI)	0.457±0.016
	Zeta potential (mV)	-37.45±0.20
	Entrapment efficiency	99.76±1.24
3	Average particle size ± SD (nm)	256 ± 16
	Polydispersity Index (PI)	0.859 ± 0.014
	Zeta potential (mV)	-35.42±0.26
	Entrapment efficiency	97.56±1.36
6	Average particle size ± SD (nm)	346.4 ± 18
	Polydispersity Index (PI)	0.472 ± 0.012
	Zeta potential (mV)	-21.36 ± 0.24
	Entrapment efficiency	95.63± 0.98

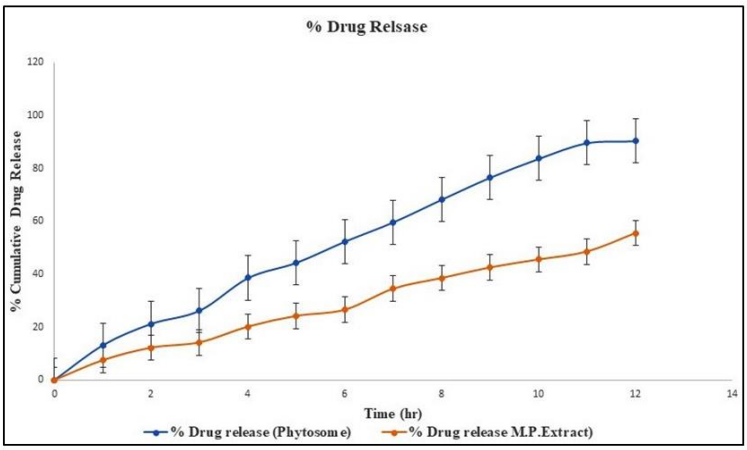

Fig. 1. % Drug release of MPE capsules and MPP capsules.

CONCLUSIONS

The standardized extract of *Mucuna prureins* and phospholipids was used to make the phytosome complex. The optimized batch from the previous results was subjected to accelerated stability studies and the sample was evaluated for different quality control tests. These phytosomes also encapsulated 95.63 % of total phenolics, shielding them from the hostile environment of heat and humidity. The optimized batch of phytosomes was found stable at the end of 6 months from the readings of particle size, zeta potential, and polydispersity index. The phytosome capsules possessed the required quality attributes. However, the MPE and MMP capsules differed in the dissolution profiles. The results highlighted the potential of *Mucuna prureins* phytosomes to retain the quality control attributes in adverse conditions of accelerated stability conditions. Moreover, phytosomes could be delivered in the form of tablet dosage form, offering sustained release of encapsulated extracts that could lead to long-term health benefits. Thus, the authors of this study propose that phytosome drug delivery systems could be considered attractive options for the delivery of bioactive substances in the future.

FUTURE SCOPE

The promising *Mucuna Prureins* phytosomes will have improved stability over the *Mucuna prureins* hydroalcoholic extract.

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Conflict of Interest. None.

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