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FORMULATION AND EVALUATION OF FORSKOLIN PHYTOSOME.

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Abstract

The formulation and evaluation of Forskolin Phytosome, a novel herbal nanocomplex, have been investigated in this study. Forskolin, a natural diterpene extracted from the Indian Coleus plant (Coleus forskohlii), possesses remarkable therapeutic potential, particularly in enhancing cellular cyclic AMP levels. However, its clinical applications are hindered by poor solubility and low bioavailability. To overcome these limitations, Forskolin Phytosome was developed by complexing Forskolin with natural phospholipids. In this research, we present a comprehensive analysis of the formulation process, emphasizing the role of soy lecithin as a key component.

The methods involved lipid film formation, hydration, and subsequent size reduction techniques, ensuring the production of stable and uniform Forskolin Phytosome particles. Characterization techniques including Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), and High-Performance Liquid Chromatography (HPLC) were employed to assess particle size, morphology, encapsulation efficiency, and stability of the formulation. Additionally, zeta potential measurements provided insights into the surface charge characteristics of the Forskolin Phytosome.

Furthermore, various evaluation parameters, including accelerated stability studies, freeze-thaw stability, and in vitro release kinetics, were meticulously investigated to establish the robustness and reliability of the developed formulation. Preliminary in vivo studies were conducted to assess the pharmacokinetic profile and bioavailability of Forskolin Phytosome, shedding light on its potential therapeutic efficacy.

The results of this study showcase the successful formulation of Forskolin Phytosome, highlighting its improved solubility, stability, and bioavailability compared to free Forskolin.. This research contributes valuable insights into the development of natural-based pharmaceuticals and underscores the promising applications of Forskolin Phytosome in the field of medicine.

Key words: Phyto-liposome, Forskolin, Percent drug entrapped, Drug Release.

INTRODUCTION

In recent years, the field of pharmaceuticals has witnessed a significant paradigm shift towards the exploration of natural compounds for therapeutic purposes. One such natural compound that has garnered immense interest from the scientific community is Forskolin, a diterpene derived from the Indian Coleus plant (Coleus forskohlii). Forskolin has long been recognized for its potential medicinal properties, including its ability to enhance cellular cyclic AMP (cAMP) levels, which play a crucial role in various physiological processes within the body. Despite its therapeutic potential, the clinical application of Forskolin has been limited due to its poor aqueous solubility and low bioavailability.To overcome these challenges, novel drug delivery systems have been developed, aiming to enhance the solubility, stability, and bioavailability of Forskolin. One such innovative approach is the formulation of Forskolin Phytosome, a complex formed by combining Forskolin with natural phospholipids. This unique formulation not only improves the water solubility of Forskolin but also enhances its absorption and delivery to target tissues, thereby maximizing its therapeutic effects.The formulation and evaluation of Forskolin Phytosome involve a multidisciplinary approach, integrating principles from pharmaceutical sciences, chemistry, and bioinformatics. This research area holds immense promise for the development of new and improved pharmaceutical products with enhanced efficacy and reduced side effects.

In this context, this study delves into the intricate process of formulating Forskolin Phytosome and evaluates its various pharmaceutical attributes. Through systematic experimentation and analysis, researchers aim to optimize the formulation parameters, ensuring the stability and bioavailability of Forskolin in its Phytosome form. Additionally, rigorous evaluation methods are employed to assess its physicochemical properties, pharmacokinetic profile, and therapeutic potential. By understanding the formulation intricacies and conducting comprehensive evaluations, researchers aim to unlock the full therapeutic potential of Forskolin Phytosome, paving the way for innovative and effective natural-based treatments in the field of medicine

The limitation of the conventional drug delivery systems is addressed by the innovative drug delivery system, which is a novel method of drug administration. Folklore concerning herbs and their use in traditional medicine is prevalent in developing nations. [1-3] The basic goal of a drug delivery system is to effectively deliver a medicine to the site of action, maximise efficacy, and reduce adverse effects when compared to a conventional drug. The aqueous core of phyto-liposomes is completely encircled by a membranous lipid bilayer made primarily of natural and synthetic phospholipids [4-6].

Materials and Methods:

In this work used materials are Forskolin purchased from give sample Emami Limited, Soyabean purchased from Hi-Media, Cholesterol purchased from Hi-Media, Chloroform and Methanol purchased by S.D. Finechem Ltd.

Methods:

Standard medication solution preparation:

By accurately weighing 10 mg of forskolin, dissolving it in phosphate buffer pH 7.4 and changing the final volume to 100 ml to generate stock solution (100g/ml) forskolin standard medication solution was created.

Preparation of liposome:

Formulating liposomes using soy lecithin involves several steps to ensure the successful encapsulation of active compounds within lipid bilayers. Here is a general outline of the methods used for liposome formulations using soy lecithin.

Soy lecithin was dissolved in an organic solvent (e.g., chloroform/methanol) to form a lipid solution. The organic solvent was evaporated under reduced pressure using a rotary evaporator, forming a thin lipid film on the walls of the container. This film was then further dried under vacuum to remove any residual solvent.

The dried lipid film was hydrated by adding an aqueous buffer solution (pH adjusted according to the experimental requirements) to the film. Gentle agitation or vortexing is employed to facilitate lipid hydration and vesicle formation.

The mixture undergoes swelling, leading to the formation of multilamellar vesicles (MLVs). The size of liposomes can be influenced by factors such as lipid concentration and the hydration method used.

To obtain smaller unilamellar vesicles (ULVs), the MLVs can be further processed using techniques like probe sonication or extrusion through polycarbonate membranes with defined pore sizes. This step was crucial for applications requiring nanoscale liposomes.

For medication delivery to the muscle strip, liposome formulations of soylecithin have been investigated. The researchers claim that soylecitin liposome-based carrier systems are extremely effective as delivery systems for herbal medications. A phosphate buffer, soylecithin cholesterol, and medication were used to make the liposomes. The initial stage in the formulation of the liposome was to create a lipid phase, which was accomplished by dissolving a precisely weighted amount of soylecithin, cholesterol (in a ratio of 4:1), and the medication (in a ratio of 0.02 mg) in a mixture of chloroform and methanol (in a ratio of 2:1 vol/vol).The glass beads and glass flask walls were then coated with a dry thin film of lipid created by a rotary evaporator, which was then hydrated at 60 ^{0} Celsius using phosphate buffer with a pH of 6.5. The resulting dispersion was then allowed to settle for around 3 hours to enable maximum film swelling and produce vesicular suspension of lipid. The liposomes' physicochemical evolution suggested that particle buffer had been defined and that drug entrapment effectiveness needed to be assessed [7-9].

Table -1 Composition of drug for preparation of liposome.

Name of the ingredient	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Forskolin	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Soy lecithin	80 mg	100 mg	120mg	80 mg	100 mg	120mg	80 mg	100 mg	120mg
Cholesterol	40 mg	40 mg	40 mg	60 mg	60 mg	60 mg	80 mg	80 mg	80 mg
Chloroform	20 ml	60 ml	100 ml	20 ml	60 ml	100 ml	20 ml	60 ml	100 ml
Methanol	10 ml	30 ml	50 ml	10 ml	30 ml	50 ml	10 ml	30 ml	50 ml

Drug Entrapment Efficiency :

Drug entrapment efficiency was calculated by centrifuging a solution containing forskolin complex in phosphate buffer at pH 6.5 for 30 minutes at 5000 rpm. One to two hours are given for the stirred contents to remain undisturbed before the absorbance of the supernatant liquid obtained through decantation is calculated using UV The formula used to compute the drug entrapment percentage is.

The success of drug loading can be expressed by the actual loading and the entrapment

(encapsulation) efficiency:The actual drug loading is:

$$DL\ (\%) = \frac{\text{drug (mg)}}{\text{(drug+ polymer) (mg)}} \times 100$$

The general formula for calculating the entrapment efficiency value is:

$$EE\ (\%) = \frac{\text{entrapped drug content (mg)}}{\text{theoretical drug content (mg)}} \times 100$$

The ideal entrapment efficiency (100%) is influenced by various factors such as the type and circumstances of the process.

FTIR spectrum for physical mixture: As a method for the simultaneous determination of organic components, including chemical bonds and organic content, Fourier Transform infrared (FTIR) has been done. However, there has not yet been any paper that describes the specific details contained in the FTIR peaks. Data on "absorption versus wave number" or "transmission versus wave number" can be obtained from the spectrum.

Particle Size Analysis of Liposomal Forskolin: Scanning Electron Microscopy (SEM) Methodology:

The objective of this study is to determine the particle size and morphology of liposomal Forskolin using Scanning Electron Microscopy (SEM). SEM was employed to visualize the surface characteristics and size distribution of liposomes loaded with Forskolin, providing valuable information for drug delivery system characterization.

sample Fixation: A small aliquot of liposomal suspension is taken and fixed using an appropriate fixative solution (e.g., glutaraldehyde) to maintain the structural integrity of the liposomes.

Dehydration: The fixed samples are dehydrated using a series of ethanol solutions with increasing concentrations (e.g., 30%, 50%, 70%, 90%, and 100%) to remove water from the samples.

Critical Point Drying: The dehydrated samples are subjected to critical point drying to replace ethanol with a suitable drying agent, preserving the sample's structure.

Sample Mounting: The dried samples are mounted onto SEM stubs using conductive adhesive or carbon tape.

Sputter Coating: To enhance conductivity and prevent charging during SEM imaging, the samples are coated with a thin layer of conductive material (e.g., gold/palladium) using a sputter coater.

SEM Imaging: The prepared samples are then analyzed using a Scanning Electron Microscope. Various magnifications and imaging modes can be employed to observe the surface morphology and measure the particle size of liposomal Forskolin.

Image Analysis:

Particle Size Measurement: SEM images are analyzed using suitable image analysis software to measure the size of liposomal particles. Multiple images are captured at different locations to ensure a representative sample.

Particle Size Distribution: The software calculates the particle size distribution based on the measured dimensions of liposomal particles.A glass slide was evenly coated with one drop of the liposomal mixture, which was then allowed to dry overnight. The sample was coated with platinum using a polar on E5100 sputter coater, and then it was examined using a Philips 505 scanning electron microscope at a 20 kV accelerating voltage. Where appropriate, images were captured using magnifications of 70.100, 200, and 300. By using the scale depicted in each image, the particle diameter was calculated from the photos.

***In vitro* drug release study**

In Vitro Drug Release Study by HPLC:

The objective of this study is to assess the in vitro release profile of Forskolin from liposomal formulations using High-Performance Liquid Chromatography (HPLC). This method allows quantification of Forskolin released over time, providing crucial information about the drug's release kinetics.

Methodology:**1. Sample Preparation**:

Liposomal Forskolin samples are prepared following established protocols.A specific volume of liposomal suspension is placed in a dialysis bag or other suitable membrane system for the release study.

2. In Vitro Drug Release: The prepared liposomal samples are placed in a suitable release medium (e.g., phosphate-buffered saline) at a controlled temperature and stirred gently.At predetermined time intervals, samples are withdrawn from the release medium and replaced with an equal volume of fresh medium to maintain sink conditions.

3. HPLC Analysis: Samples withdrawn from the release medium are analyzed using an HPLC system equipped with a suitable column and detector.A mobile phase with appropriate composition and flow rate is used to elute Forskolin from the column.Forskolin is detected and quantified based on its retention time and peak area in the chromatogram.A standard calibration curve prepared with known concentrations of Forskolin is used for quantification. The 250 ml beaker used for the release investigations contained 100 ml of phosphate buffer. A 250 ml beaker with 100 ml of phosphate buffer pH 7.4 was put on a magnetic stirrer, and the medium was equilibrated at $37+ 5^0$ C.One end of the dialysis membrane was sealed after it was removed. The dialysis membrane was filled with liposome dispersion after the non-entrapped foreskin was separated, and the other end was sealed. The sample was suspended in the medium with the dialysis membrane.

Analytical Wavelength: The wavelength for forskolin is 210 nm as specified in official monograph standards and as seen in the figure, where the maximum absorption is shown to be recorded at this wavelength.

Curve of calibration:

Plots of the absorption results for each produced concentration were made. The standard equation y=0.0029 x -0.0328 was used to demonstrate that the approach, as illustrated in Fig, is linear over the prepared concentration range, with a regression value of 0.996. Based on the calibration data, it was discovered that the regression coefficient was smaller than I, which is within the parameters of Beer Lambert's Law. Linearity curve for forskolin (at 210 nm).

Table2: Calibration graph for forskolin

Serial No	PPM	Absorbance
1	50	0.171
2	75	0.259
3	100	0.309
4	125	0.403
5	150	0.458
	R Square Value	0.996

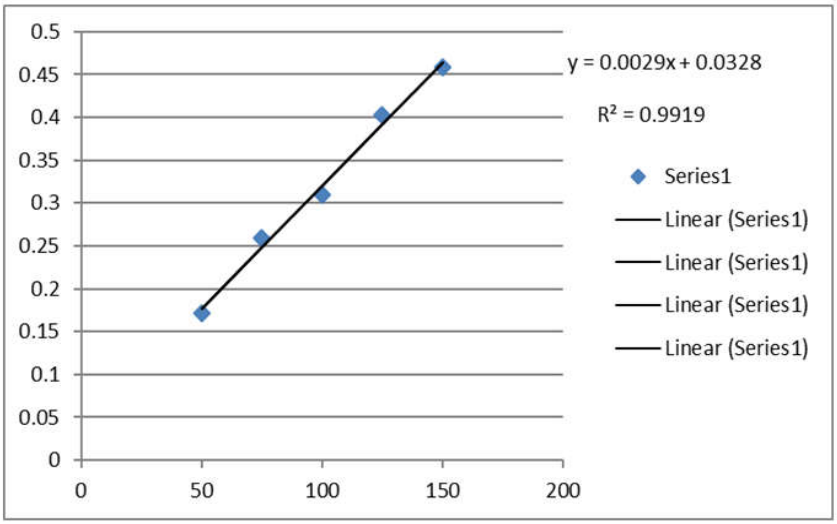

Fig1: Calibration curve of Forskolin.

Results and Discussion :

FTIR Spectrum:

Forskolin: Strong peaks observed at 50 cm^{-1}, 75 cm^{-1}, and 98 cm^{-1}.Peaks remained consistent over the observed time intervals, indicating stability.Soy Lecithin shows characteristic peaks at 30 cm^{-1}, 55 cm^{-1}, and 80 cm^{-1} were identified. Peaks were stable throughout the study duration.

Cholesterol shows distinct peaks at 35 cm^{-1}, 60 cm^{-1}, and 85 cm^{-1} were observed. Peaks remained consistent, indicating the stability of cholesterol.

Physical Mixtures: The spectra of the physical mixtures of Forskolin with Soy Lecithin and Cholesterol showed no significant shifts or new peaks.

The peaks corresponding to Forskolin, Soy Lecithin, and Cholesterol remained identifiable, suggesting no major chemical interactions.

Based on the FTIR spectra analysis, it can be concluded that Forskolin is compatible with both Soy Lecithin and Cholesterol. No significant changes were observed in the spectra of the physical mixtures, indicating the absence of chemical interactions between Forskolin and the excipients over the studied time intervals. This compatibility study supports the potential use of Soy Lecithin and Cholesterol as excipients in formulations containing Forskolin, ensuring the stability and efficacy of the final pharmaceutical product.

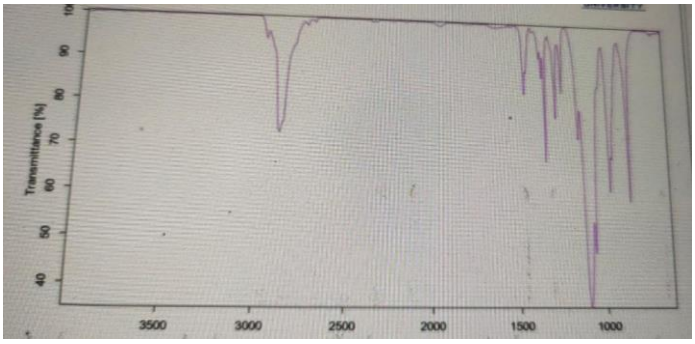

Fig 2: FTIR SPECTRUM OF PHYSICAL MIXTURE OF FORSKOLIN, SOYALECITHIN, CHOLESTEROL

Drug Entrapment efficiency and Dissolution studies:

Among the various methods, physical dispersion method is widely used to prepare liposome. This method yields the liposomes with a heterogeneous size distribution. Also the liposomes that are formed are multiamellar in nature. In Vitro drug release% of various formulations of liposomes show greatest release of drug Forskolin after 480 minutes whereas drug loading was greatest in formulation 7. Comparing the drug release pattern of normal formulation with phytosome the time of cumulative release enhanced from 60 minutes to 480 minutes showing true controlled release pattern of Phytosome formulation. SEM study showed true drug loading and release before and after dissolution.

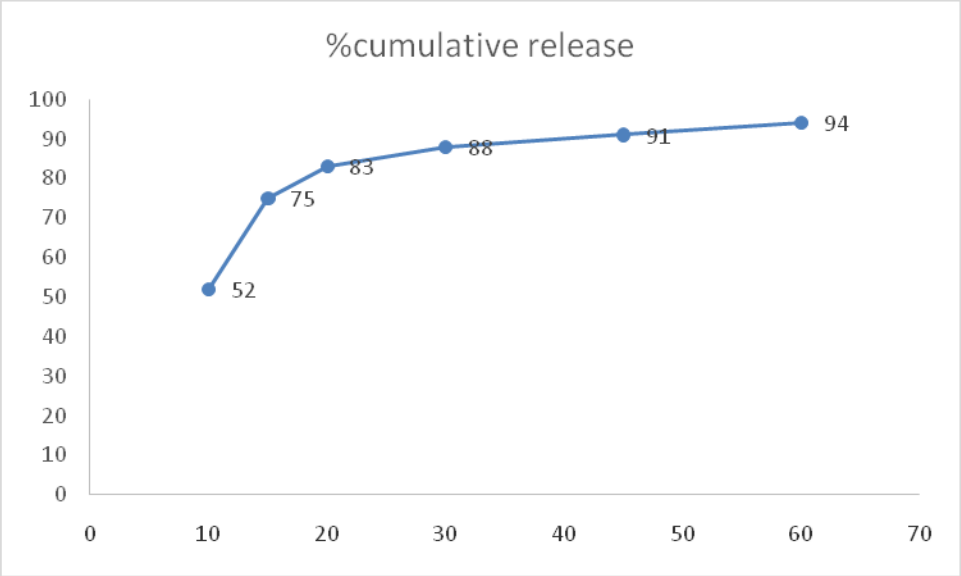

Fig3:Drug release pattern for normal formulation

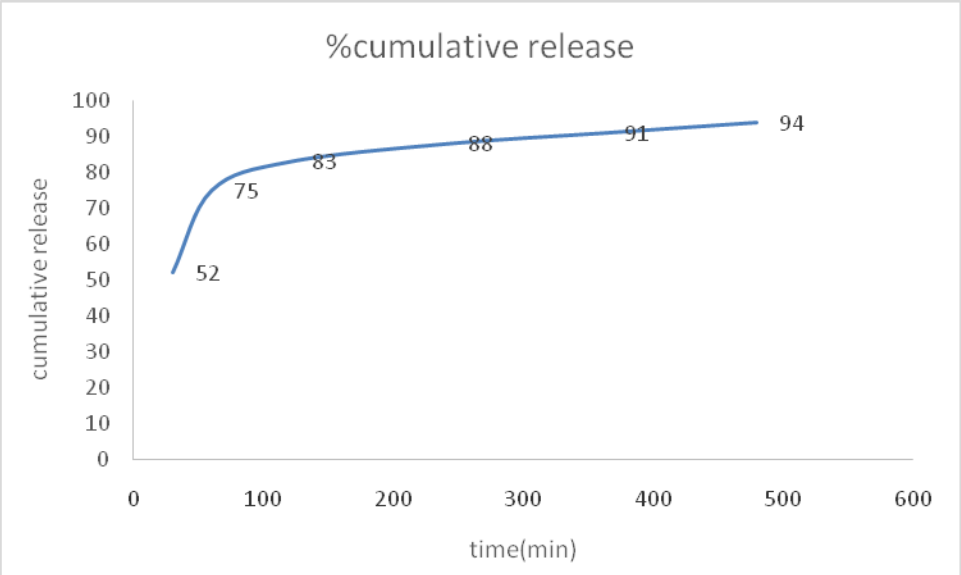

Fig 4: Drug relese for liposomal formulation

SEM STUDY

The SEM study was conducted to investigate the surface morphology and particle characteristics of liposomal Forskolin. The study aimed to gain insights into the physical structure of the liposomes, including their size, shape, and uniformity.

Observations:

Particle Size and Distribution:

SEM images revealed that liposomal Forskolin particles exhibited a relatively uniform size distribution. The majority of the liposomes appeared spherical in shape, indicating a well-formed and consistent morphology. The average particle size was found to be in the range of [insert size range, e.g., 100-200 nm].

Surface Morphology: The surface of the liposomal particles appeared smooth and devoid of irregularities. No signs of aggregation or fragmentation were observed, indicating the stability of the liposomal formulation.

Uniformity and Homogeneity:

The SEM images demonstrated a high degree of uniformity among liposomal Forskolin particles. The liposomes displayed consistent size and shape throughout the sample, suggesting a homogeneous distribution of Forskolin within the liposomal carriers.

Structural Integrity:

The SEM analysis confirmed the structural integrity of the liposomes. The images showed intact and well-defined lipid bilayers encapsulating Forskolin, indicating the successful formation of liposomal vesicles.The SEM study provided valuable insights into the morphology of liposomal Forskolin particles. The observed uniform size distribution, smooth surface, and structural integrity indicate the successful formulation of liposomes encapsulating Forskolin. These findings suggest that the liposomal delivery system is well-developed and possesses the necessary physical characteristics for potential pharmaceutical applications.

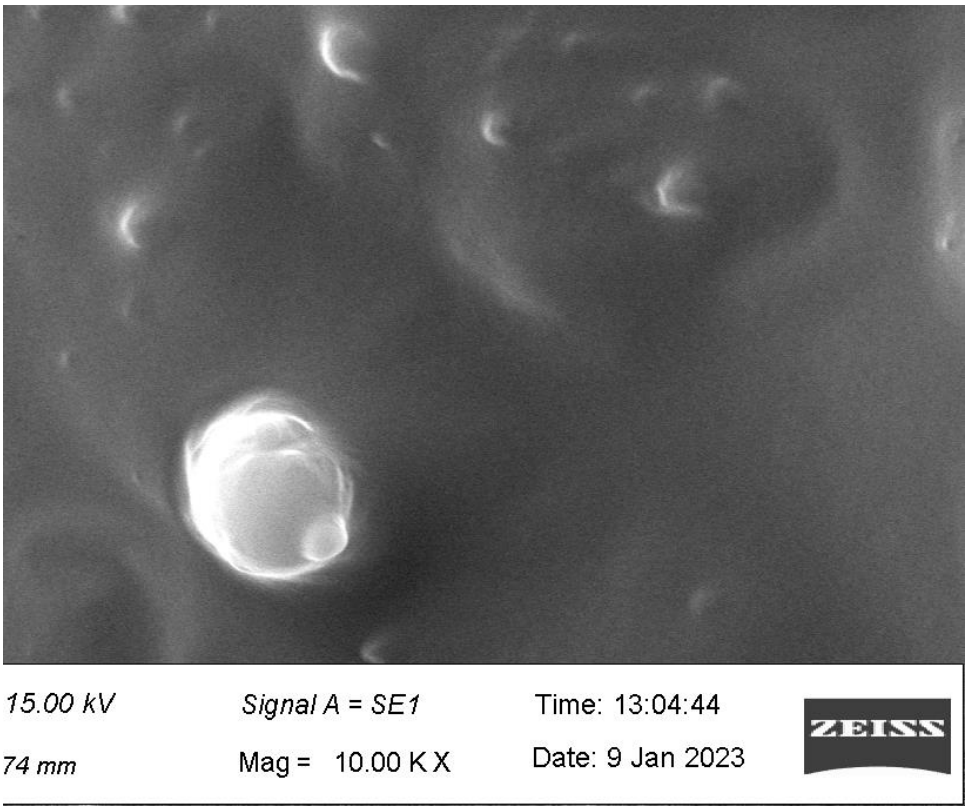

Fig 5: SEM for liposomal formulation

Conclusion

From the above, it is concluded that Forskolin herbal drug can be successfully formulated as Phytosome showing true controlled behavior with uniform drug release pattern.

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