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Phytosomes in Nanovesicle Herbal Formulation: A Contemporary Advancement in Natural Medicine

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ABSTRACT: In the contemporary era, herbal remedies and their associated phytochemical compounds have emerged as highly promising alternatives for treating a wide array of medical conditions. However, the practical application of these compounds in clinical settings faces substantial challenges due to their limited bioavailability and specificity. As a result, addressing the issues related to bioavailability has become a central concern, essential for enhancing the effectiveness of dietary phytochemicals. Various strategies have been proposed to develop efficient carrier systems aimed at improving the bioavailability of these phytochemicals. Among these strategies, nano-vesicles have garnered significant attention as promising candidates for delivering insoluble phytochemicals. Their straightforward preparation methods and adaptability have gained widespread acceptance and utilization, as evidenced by their substantial presence in scientific discourse. The initial portion of this comprehensive review is dedicated to introducing the concept of phytosome technology, alongside its diverse applications. This section places specific emphasis on the fundamental principles underlying formulations and the techniques employed for characterization. The subsequent section provides a comprehensive overview of the varied biological activities exhibited by both commercially available and non-commercial phytosomes. This classification is further organized based on systems and their corresponding pathological contexts. The cumulative findings consistently underscore the superior efficacy of phytosomes, demonstrated through increased biological activity and the potential for dose reduction. Notably, compounds such as curcumin and silymarin frequently feature as key components in this context. In conclusion, the review delves into the promising results arising from both clinical trials and experimental investigations, shedding light on the manifold applications of phytosomes. The insights gleaned from this study serve as a resounding call to action, encouraging researchers to translate their laboratory-derived knowledge into tangible, market-ready products. This transition is seen as a pivotal step toward advancing the development of these innovative products, ultimately benefiting a broader spectrum of individuals seeking improved health solutions.

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INTRODUCTION:

Over many decades, the healing properties of medicinal herbs and their active constituents have been employed to effectively manage and treat a broad range of medical conditions and illnesses [1-5]. The increased adoption of herbal remedies can be attributed to several significant factors. Firstly, modern medical interventions do not consistently provide comprehensive solutions to all

human health issues. Secondly, concerns and considerations about the safety and reliability of synthetic drugs have grown, prompting a shift towards natural alternatives. Lastly, the demonstrable efficacy of many natural products compared to synthetic drugs, coupled with their tendency to cause fewer adverse effects, has contributed to their rising popularity [6]. However, a persistent challenge in the clinical use of active compounds from plants is their inadequate oral bioavailability [7,8]. This limited absorption can be attributed to various factors, including low lipid solubility, intricate polyphenolic structures with multiple rings, and high molecular weights [9,10]. In response to these limitations, diverse strategies have been proposed to tackle these issues. These strategies encompass the creation of emulsions, liposomes, nano-formulations, modifications in molecular structure, and the administration of prodrugs [11-15]. Within this spectrum of approaches, the utilization of phyto-phospholipid complexes, termed phytosomes, emerges as a particularly promising method to enhance the bioavailability of these plant-derived compounds. This innovative technique directly addresses the challenges posed by poor absorption, offering a potent solution to amplify the effectiveness of bioactive constituents sourced from medicinal plants. The term "Phyto" is related to plants, while "some" indicates cell-like structures. Phytosomes, also referred to as herbosomes, constitute a specialized drug delivery system designed to improve the absorption and bioavailability of drugs with low solubility. These phytosomes are complex formations composed of phospholipids and naturally occurring active phytochemicals, intricately intertwined within their composition. They are created through the interaction between phosphatidylcholine (or other hydrophilic polar head groups) and plant extracts in a solvent that lacks protons. In comparison to conventional preparations, these formulations show significant enhancements in both pharmacological and pharmacokinetic properties. The lipid-soluble phosphatidyl component surrounds the hydrophilic phytoconstituent-choline complexes, providing comprehensive coverage. Phytosomes offer notable advantages, including a high capacity for encapsulating drugs, increased stability (attributed to the formation of chemical bonds between the polar head of the amphiphile molecule and phytoconstituent), and significantly improved bioavailability. Furthermore, the accelerated absorption rate leads to a reduced need for active constituents to produce a biological effect, even in the case of polar phytoconstituents.

PREPARATION METHOD:

Phytosomes are specialized drug delivery systems designed to enhance the bioavailability of phytoconstituents by forming complexes with phospholipids. Below, we provide concise descriptions of four different methods commonly used for preparing phytosomes.

Solvent evaporation method:

This method begins by combining phytoconstituents and phospholipids (PC) within an organic solvent. The resulting mixture is maintained at an optimal temperature, typically around 40 °C, for a specific duration, often an hour, to facilitate the formation of phytosomes. Afterward, the phytosomes are separated and stored in desiccators [16,17].

Mechanical dispersion method:

In the mechanical dispersion method, lipids dissolved in an organic solvent are mixed with an aqueous phase containing the drug. The subsequent removal of the organic solvent under reduced pressure leads to the creation of phytosomes. Modern variations of this technique incorporate supercritical fluid methods [18].

Salting out technique:

The salting-out technique involves dissolving both phospholipids and plant extracts within a suitable organic solvent. The addition of n-Hexane induces precipitation, resulting in the formation of an extract-phospholipid complex [19].

Lyophilization Method:

For the lyophilization method, the drug (DSN) is initially dissolved in DMSO. This DSN solution (typically 2.5 % w/v) is then added to an SPC (phospholipid) solution in t-butyl alcohol (1.5 % w/v). Complex formation is achieved through stirring for an extended period, followed by isolation via lyophilization (freeze-drying). Various factors, including the type of phospholipid, the ratio of drug to phospholipid, and the choice of co-solvent, can be modified in this method [20,21]. These techniques serve as valuable tools for crafting phytosome complexes, which, in turn, enhance the solubility, stability, and overall bioavailability of phytoconstituents derived from natural sources. The selection of a specific method depends on the unique characteristics of the

phytoconstituents and the desired attributes of the resulting phytosome formulation.

Anti-solvent precipitation method:

In the anti-solvent precipitation process, a specific quantity of herbal extract and phospholipids are subjected to reflux in the presence of 20 ml of an organic solvent, such as acetone. This reaction is conducted under carefully controlled experimental conditions, maintaining a temperature below 50 °C for 2 to 3 h. Afterwards, the reaction mixture is concentrated to reduce its volume to approximately 10 ml. To induce precipitation, a low-polarity solvent, such as n-hexane, is added while continuously stirring the mixture. The resulting precipitates are separated by filtration and then stored in desiccators. Once dried, the precipitates are finely powdered and stored in dark amber-coloured glass bottles at a specified temperature [22].

Rotary evaporation process:

In the rotary evaporation process, a specific quantity of herbal extract and phospholipids are combined within a round-bottom glass container, using 30 ml of an organic solvent that is miscible with water, such as acetone. The mixture is stirred for two hours at a temperature not exceeding 50 °C, utilizing a rotary evaporator. Optionally, an anti-solvent like n-hexane can be added to the resulting thin film formed during continuous stirring. The phytosome precipitates obtained through this process are stored in amber-coloured glass containers under controlled temperature and humidity conditions as prescribed [23].

Phospholipids, when solubilized in ether, are carefully introduced drop by drop into a solution containing the phytoconstituents earmarked for encapsulation. This gradual addition triggers the formation of cellular vesicles. As the solvent is subsequently removed, these vesicles transform intricate structures, ultimately giving rise to the phytosome formulation [24]. The specific configuration of these phytosomes hinges on the concentration of amphiphiles used. Lower concentrations typically yield monomeric phytosomes. However, with an increase in concentration, a diverse array of structures can emerge, including round, cylindrical, disc-shaped, cubic, or hexagonal vesicles [25].

Common stages involved in the preparation of phytosomes:

The commonly employed method for producing phytosomes is called the thin-film hydration technique. The process of phytosome preparation involves a series of well-defined steps. It commences with the interaction of 3 to 2 mole of either natural or synthetic phospholipids with one mole of an herbal extract. This chemical reaction takes place in an aprotic solvent, such as dioxane or acetone, which facilitates the subsequent isolation of the resulting complex. Isolation can be accomplished through either precipitation using non-solvents like aliphatic hydrocarbons or by employing methods like lyophilization or spray drying. The interaction between these two components during phytosome formation follows a mole ratio ranging from 0.5 to 2.0 moles. It's worth noting that to achieve optimal results; the preferred phospholipid-to-flavonoid ratio is 1:1. Subsequently, the process involves drying, the creation of a thin film, and hydration, resulting in the formation of a suspension. Further isolation is attained by precipitating the mixture with a non-solvent, typically an aliphatic hydrocarbon, followed by additional drying using either lyophilization or spray drying methods [26].

CHARACTERIZATION OF PHYTOSOMES:

Average Size and Shape:

The assessment of both size and morphology is a critical aspect of phytosome analysis, offering valuable insights into the composition and characteristics of a given sample. Multiple techniques are available for characterizing the size of phytosomes, including methods like Dynamic Light Scattering (DLS), various microscopic observations such as Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), optical microscopy, Atomic Force Microscopy (AFM), fluorescence microscopy, Field Flow Fractionation, Nanoparticle Tracking Analysis, Scanning Ion Occlusion Sensing, Flow Cytometry, Size-Exclusion Chromatography, Centrifugal Sedimentation, and Differential Scanning Calorimetry (DSC). Furthermore, flow analysis and size-exclusion chromatography are also applicable methods for assessing phytosome size. Electron microscopy serves as a widely adopted technique for visualizing phytosomes, with Cryogenic Transmission Electron Microscopy (Cryo-TEM) and Freeze-fracture Transmission Electron Microscopy being among the most frequently utilized approaches. Notably, Cryo-TEM stands out as it enables direct observation of phytosomes in their frozen state, preventing any disruption or alteration of their structure during the examination [27-35].

Surface Charge:
Zeta potential, which quantifies the overall charge within the surrounding medium, serves as a crucial factor in assessing the charge and stability of phytosomes within emulsions. Phytosome emulsions are considered stable when they exhibit a zeta potential exceeding or falling below the threshold of 30 mV [36]. Various techniques, such as Dynamic Light Scattering (DLS), free-flow electrophoresis, and laser Doppler velocimetry, are commonly employed to determine this parameter.

Chemical Composition:
The analysis of the chemical composition and the interactions among vesicle components and phytochemicals is commonly explored through various analytical methods. These methods include NMR (Nuclear Magnetic Resonance) [37,38], FTIR (Fourier-Transform Infrared Spectroscopy) [39], and mass spectrometry [40]. Moreover, the quantification of phospholipids within phytosomes can be achieved by reacting them with an appropriate reagent, followed by spectrophotometric quantification [41]. Mass spectrometry stands out due to its high signal-to-noise ratio, sensitivity, and selectivity, making it one of the most robust techniques for assessing the phytochemical composition of plant extracts and phospholipids [42]. Additionally, thin-layer chromatography is another viable analytical method in this context.

Lamellarity and Stability:
The term "lamellarity" refers to the number of lipid bilayers present in phytosomes [43]. Determining lamellarity commonly involves the use of electron microscopy methods [44]. Additionally, various other techniques utilized for this purpose include Small-angle X-ray scattering, DSC (Differential Scanning Calorimetry), TGA (Thermogravimetric Analysis), DLS (Dynamic Light Scattering), and UV-Vis (Ultraviolet-Visible Spectroscopy).

Encapsulation Efficiency and Release Behavior:
Extensive research efforts have been dedicated to studying the drug release behavior of vesicle carriers in recent years. This focus is driven by the recognition that the *in vitro* release profile can serve as an indicator of the carrier's efficacy *in vivo* [45]. Commonly employed methods for evaluating the release rate of active agents include traditional approaches like membrane diffusion strategies (such as dialysis, micro-dialysis, fractional dialysis, and reverse dialysis), the sample and separate strategy, in situ processes, and continuous flow techniques [46–51]. Various analytical techniques, including Mini-column centrifugation, HPLC (High-Performance Liquid Chromatography), UPLC (Ultra-Performance Liquid Chromatography), UV-Vis (Ultraviolet-Visible Spectroscopy), dialysis, enzymatic assays, gel electrophoresis, field flow fractionation, and the sample-and-separate approach, are employed to determine drug release characteristics in these investigations.

Surface tension activity measurement:
The evaluation of the drug's surface tension activity in an aqueous solution will be carried out using a Du Nouy ring tensiometer [52].

BIOLOGICAL ACTIVITIES OF PHYTOSOMES CATEGORIZED BY BODILY SYSTEMS:

Research papers focused on phytosomes and their physiological effects are classified based on the specific physiological system under investigation. Notably, the gastrointestinal, nervous, genitourinary, and musculoskeletal systems collectively constitute nearly 75 % of the published works. It's important to clarify that systems associated with metabolic syndrome were not included in this analysis.

PRIMARY NATURAL COMPONENTS IN PHYTOSOMES:

Research studies on phytosomes are further grouped based on the primary natural ingredients employed. Specifically, botanicals or active principles with three or more publications are individually highlighted, while constituents with two or fewer studies are grouped under the category "Others." The data underscores a greater prevalence of phytosomes loaded with pure compounds as opposed to natural extracts, with curcumin being a particularly noteworthy example. Comprehensive references corresponding to the specific studies considered are available throughout the manuscript.

CHALLENGES ON THE COMMERCIAL LEVEL:

Phytosomes have gained acclaim as efficient nanocarrier delivery systems with significant potential to revolutionize drug formulation practices [53]. However, the journey from initial product development to successful commercialization is fraught with challenges. Despite their manifold advantages, the number of phytosomal products that have made it to market remains relatively limited [54]. One of the most significant barriers to market entry, even after

formulating effective products, is the need to establish their safety. Thanks to their biologically neutral structures, introducing phytosomes into the human body is generally well-accepted, with minimal concerns regarding safety or the triggering of immunological responses [55]. However, given their nanoparticle size, a thorough investigation of aspects like bioaccumulation, biocompatibility, metabolism, and excretion is essential before considering them market-ready [56]. In notable instances, researchers have successfully designed phytosomes, such as curcumin phytosomes, for intravenous administration in animal models. These designs demonstrated substantial accumulations in specific tissues, like bone marrow and spleen, highlighting their potential effectiveness [57]. Furthermore, phytosomes' ability to seamlessly integrate with biological membranes, facilitating passive targeting of normal cells, underscores the need for comprehensive evaluation through well-designed animal studies and rigorous clinical trials to confirm their actual biological effects [58,59].

CONCLUSION:

Numerous studies support the notion that phytosomes are biologically safe. Beyond safety considerations, an essential step in phytosome development involves the meticulous assessment of pharmacokinetic and pharmacodynamic parameters in both animal models and humans to substantiate their superiority over their pure phytoconstituent counterparts. A pivotal factor for successful market penetration is determining the optimal dosage form to ensure improved absorption and efficacy. The process of upscaling the production of phytosomes comes with its own set of unique challenges. It is imperative to maintain the distinctive characteristics of phytosomes when moving from laboratory-scale procedures to full-scale industrial manufacturing. It's important to note that while manufacturing processes for many types of phytosomes are typically straightforward, those involving pH-sensitive phytosomes can be intricate due to their inherent low physicochemical stability. Ensuring quality control and consistent product quality over time are paramount, aligning with the stringent standards applied to other pharmaceutical products. In the current landscape, the growing preference for natural therapies underscores the increasing popularity of biocompatible, cost-effective, and safe natural products. Furthermore, the swift adoption of phytosome technology in commercial applications can be attributed to its uncomplicated manufacturing process and the relative ease with which it can be transitioned to an industrial scale. Numerous pharmaceutical industries have embarked on exploring the enhanced bioavailability, advantages, and biological activities associated with phytosome formulations. For a comprehensive overview of marketed phytosomes, including details about active constituents, industry participants, and specific medical applications. In conclusion, phytosomes hold significant promise in the field of drug delivery, bridging the gap between innovation and seamless integration into the market.

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