OPTIMIZING STABILITY OF PLANT EXTRACTS USING LYOPHILIZATION

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Abstract: The growing need for bioactive metabolites necessitates an efficient method of extracting and processing diverse plant extracts. The majority of the existing literature focused on determining the influence of extraction procedure on plant extracts. However, the process used to dry plant extracts after extraction is also critical. The "gold standard" for drying plant extracts is lyophilization, also known as freeze drying, which is crucial for maintaining their quality and increasing their shelf life. A literature search using PubMed and Google Scholar was done, and a summary of all the particular issues associated with lyophilization was drawn up. Due to the low process temperature, freeze-drying is used as a microencapsulation technology and is especially ideal for the entrapment and protection of delicate bioactive chemicals. The zeta potential, poly dispersity index (PDI), and minimum particle size are typically used to optimize the stability of lyophilized extracts. In this review, the impact of lyophilization on the stability of plant extracts has been explored.

Keywords: Lyophilization, Stability, Plant extracts, Encapsulation, Zeta Potential, Poly dispersity Index

Introduction

Many drug discovery efforts have used plant extracts as a significant source of bioactive chemicals, and some significant medications have been extracted and discovered from plants [1]. Small biomolecules, biologics produced by plants, and a recently established third category of medications known as phytopharmaceuticals make up this class of medications [2]. Around 80% of the world's population, according to the World Health Organization (WHO), relied on medicinal plants as their main source of treatment in 1985 [3]. Various medicinal preparations, including powders, tinctures, tablets, etc., use plant extracts in crude form or standardized fractions [4]. Plant extracts contain a variety of bioactive substances with strong biological effects, such as polyphenols, alkaloids, and terpenes [5]. An adequate strategy is needed to effectively extract and process different plant extracts due to the growing demand for these phytochemicals [6]. Polar solvents (such as water and alcohols), intermediate polar solvents (such as acetone and dichloromethane), and nonpolar solvents (such as n-hexane, ether, and chloroform) are frequently used in the extraction of medicinal plants. Optimal extraction and the ability to maintain the stability of the chemical structure of desired substances are two qualities that define a good solvent [7]. The image illustrates how these bioactive chemicals are now subjected to a variety of thermal, nonthermal, and physicochemical

variables, including temperature, pH, pressure, sonication, radiation, electric field, humidity, oxygen, and light, as a result of advancements in extraction technology (Figure1) [8]. These processing and storage parameters have been thoroughly investigated by researchers, who have found that they have a considerable impact on the concentration, bioactivity, functionality, bioavailability, and overall stability of the active chemicals [9].

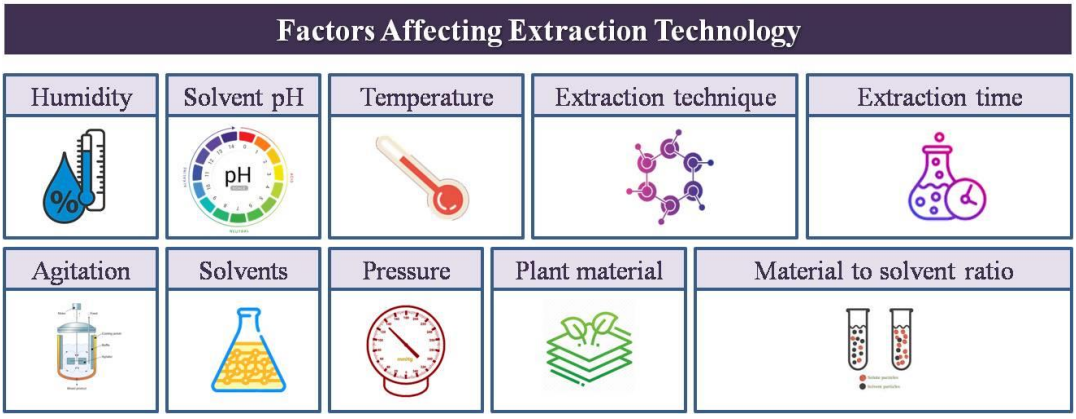

Figure2. Factors affecting extraction technology

Background

Enhancing the quality of plants and enhancing the safety, quality, and consistency of the finished products need the adoption of optimized high-quality agricultural and biomass processing processes [10]. The primary active substances utilized in the creation of natural products, which are often offered in the form of powders or tablets in order to ensure good stability and appropriate dosage, are dried plant extracts high in bioactive metabolites [11]. In order to manufacture these natural compounds, it is crucial to optimize the extraction protocol, which comprises the solvent choice, solvent to biomass ratio, extraction time, and extraction method choice [12]. The majority of the material that was published compared different extraction methods utilized for plant extracts. But it's also crucial to consider the process for drying plant extracts after extraction [13]. Especially because current research indicates that the drying technique used to prepare plant biomass prior to extraction influences their bioactivity and chemical makeup [14]. Similarly, the method of post-extraction drying used has an impact on the chemical and biological characteristics of plant extracts [15]. A mind map enumerating several drying techniques is represented as a figure (Figure2). Lyophilization is one of the more modern and inventive post-extraction techniques. The "gold standard" for drying plant extracts is lyophilization, also known as freeze drying, which is crucial for maintaining their quality and increasing their shelf life [16]. With the aforementioned context in mind, this review attempts to provide insight into the various ways that lyophilization can be used to maintain the stability of plant extracts.

Figure2. Methods of drying

Methodology

A PubMed and Google Scholar literature search was conducted using the key terms “lyophilization”, “freeze-drying”, “plant post-extraction methods”, “lyophilized plant extract”, “Stability studies” and “preserving bioactive compounds” to gather the information for this manuscript. An outline of all the special issues exclusive to lyophilization was prepared. All of the data cited has been written entirely in English.

State of the art: Lyophilization

Lyophilization in the pharmaceutical industry has undergone continuous growth and steady expansion as we approach a new century. In the freeze-drying process, water is sublimated directly from the solid state (ice) to the vapor state, omitting the liquid state, and is subsequently reabsorbed from the "dry" layer [17]. Although lyophilizers are a more recent invention than freeze-drying, they have only been around for about 100 years [18]. The procedure preserves the dried product's quality (including its biological, dietary, and olfactory qualities) [19]. Lyophilization is always used to increase the stability of a product that is sensitive to moisture or to make the product simpler to store or transport [20]. Three basic processes are involved in the pharmaceutical lyophilization, as depicted in the picture (figure3). The first step is to freeze the product in solution to create a matrix of ice along with additional crystallizable excipients while concentrating other solutes and the active pharmaceutical ingredient (API) inside the interstitial voids [21]. Aqueous medicinal formulations can be frozen at temperatures below -35°C [22]. The second phase is primary drying, which involves submerging ice in a vacuum at a low temperature. Traditionally, this process is performed at chamber pressures of 40-400 Torr at shelf temperatures ranging from -30° C to -10° C [23]. After the primary freeze-drying process is completed and all ice has sublimed, bound moisture

remains in the product. Although the product seems dry, the residual moisture level could be as high as 7-8% [24]. As a result, a last process called secondary drying at a warmer temperature is required to remove the residual moisture content to optimal levels. This is known as 'Isothermal Desorption' because the bound water is desorbed from the product [25]. By increasing the shelf temperature above ambient levels, this process is completed. The shelf temperature can be increased to 15–300°C to allow for vacuum-induced desorption of water molecules [26]. Four standard parts are typically included in freeze-dryers: a drying chamber, a vacuum pump, a heat source, and a condenser [27]. The tools and procedures are created to guarantee that the sterility of the product is preserved throughout the lyophilization process [28].

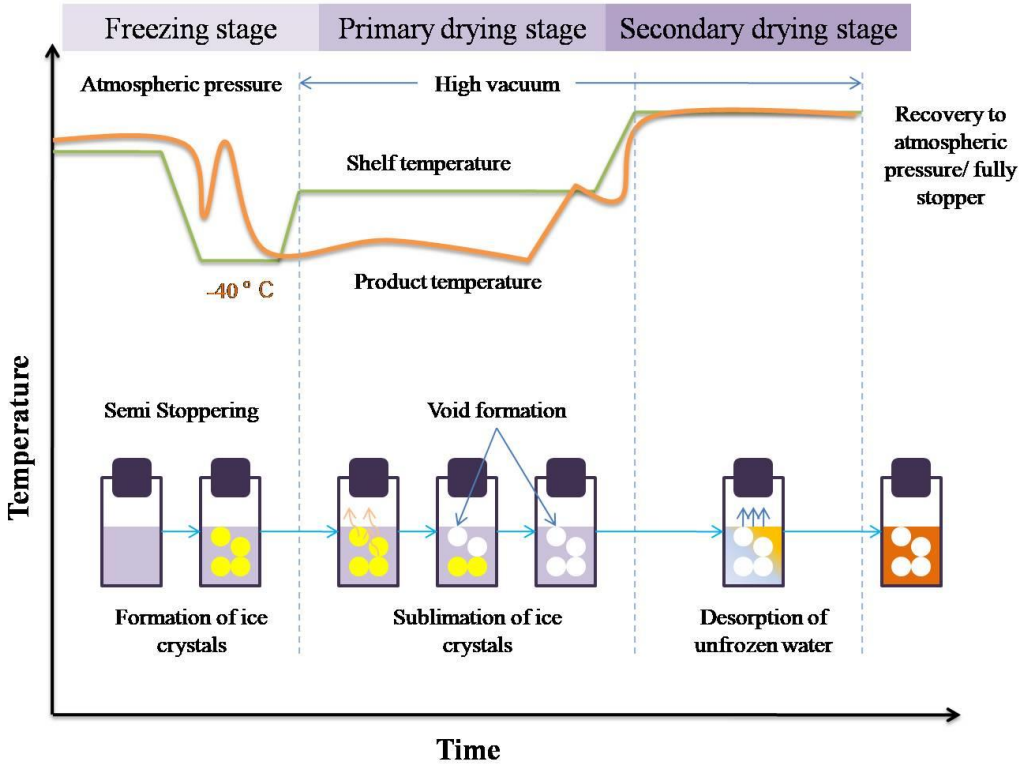

Figure3. Lyophilization principle and process

Lyophilization and its multidisciplinary application

Lyophilization is a common technique used to concentrate extracts or stabilize biological samples before storage [29]. On a laboratory and industrial scale, the freeze-drying method is mostly employed for delicate, temperature-sensitive plant materials [15, 30]. By using the freeze-drying method, plant samples are highly effective in removing water while retaining bioactive components, such as antioxidants [31]. The freeze-drying procedure is frequently employed to preserve and stabilize biological materials because of its benefits [32]. It guards against both the spread of microbes and the deterioration of biological materials linked to the activity of bacteria [33]. In order to prepare plant and food samples for the identification of physiologically active chemicals, the freeze-drying method

are also used [34]. It is used to preserve the chemical makeup of the plant material while stabilizing, enhancing, and/or extending its life [35]. Due to the low process temperature, freeze-drying is used as a microencapsulation technology, making it especially ideal for the entrapment and protection of delicate bioactive chemicals [36]. In freeze-drying-based encapsulation, the target component and the encapsulating ingredients create an emulsion solution, which is then used to create microcapsules using the freeze-drying method [37]. Additionally, due to the few stages required, the freeze-drying technique is simpler than other microencapsulation techniques [38].

Effect of lyophilization on stability of plant extracts

In order to maximize zeta potential and minimize polydispersity index (PDI), lyophilized extract stability is typically optimized [39]. The effects of lyophilization technique on stability of plant extracts are enlisted as table (Table1). The polydispersity index is a size-based indicator of a sample's heterogeneity [40]. Size distribution in a sample, as well as aggregation or agglomeration of the sample during isolation or analysis, can all lead to polydispersity [41]. Good homogeneity is indicated by a PDI value below 0.3 [42]. The zeta potential's size reveals the strength of electrostatic attraction between nearby, similarly charged particles in a dispersion [43]. For sufficiently small molecules and particles, a high zeta potential will impart stability, which means that the solution or dispersion will fend off aggregation. Zeta potential readings exceeding 30 mV are often indicative of high stability [44]. Plant extracts' zeta potential is influenced by the extraction process and, consequently, by the extracted chemicals [45]. The thickness of frozen material and its surface area are additional factors. The rate of lyophilization increases with the frozen material's surface area. In contrast, the rate of lyophilization is slower the thicker the frozen substance. The ability of a sample to absorb and transfer heat to the surface undergoing sublimation is influenced by sample thickness [46].

Table1. Effect of lyophilization on stability of plant extracts

S.No	**Plant Extract**	**Stability studies**	**Source**
1	*Serpylli herba*	Zeta potential at pH 6:-18.7±0.7 mV	[47]
2	*Allium cepa* L.	Particle size:177.73 nm PDI: 0.45	[48]
3	*Artemisia absinthium*	Zeta potential:−11.9 Particle size: 253.8 nm PDI:0.258	[49]
4	*Beta vulgaris* L. leaf hydroalcoholic extract nanogel	Zeta Potential : +28.8 mV Particle size:247 nm PDI: 0.259	[50]

5	*Anoectochillus burmannicus* Ethanolic Extract-Synthesized Selenium Nanoparticles	Zeta Potential : −24.5 ± 1.9 mV PDI: 0.366 ± 0.074	[51]
6	*Hippophaes rhamnoides* aqueous extract-containing nanoemulsion	Zeta Potential (mV): –30.11 ± 2.02 Droplet Size (nm): 183.07 ± 9.53 PDI: 0.295 ± 0.045	[52]
7	*Punica granatum* L. loaded nanophytosomes	Particle size: 166.70–144.40 nm PDI: <0.5	[53]
8	Chitosan-Coated- *Aloe perryi*- Solid Lipid Nanoparticles (C-ALP-SLNs)	Zeta Potential: 13.6 ± 1.1 mV Particle size: 173.6 ± 11.3 nm PDI: 0.21 ± 0.02	[54]
9	*Eupatorium adenophorum* leaf extract-Silver Nanoparticles	Zeta Potential:− 33.4 mV Particle size: 117.75 nm	[55]
10	*Calotropis gigantean*-loaded poliglusam-silver nanomatrices	Zeta Potential :42.42 mV Particle size: 178.5 nm PDI:2.97	[56]

Conclusion

Researchers have been inspired by the increased demand for plant extracts to create novel drying processes that preserve high concentrations of essential oils and other physiologically active substances without compromising the dried plant material's flavor, color, or texture. Lyophilization appears to be a useful approach for conserving the volatile profile of plants, although its effects depend on the species of plant (the location of volatile accumulation, the type of volatile components, etc.). The lyophilization process' technical specifications also have a big impact on the end product's quality. In order to more accurately examine the therapeutic benefits of plant extracts, additional systematic research is required on the effects of freeze-drying in comparison to alternative preparation methods, such as alcohol extractions of fresh plant material.

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