Journal of Drug Delivery Science and Technology 82 (2023) 104379

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Oral lyophilizates obtained using aggressive drying conditions: Effect of excipients

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ARTICLE INFO

Keywords:
Patient-friendly dosage forms
Orodispersible dosage forms
Oral lyophilizates
Excipient selection
Hydrolyzed gelatin
Aggressive drying

ABSTRACT

Orodispersible drug formulations are a current trend in the pharmaceutical industry, mostly intended for pediatric and geriatric patients. Oral lyophilizates are solid forms, intended either to be placed in the mouth or to be dispersed (or dissolved) in water before administration. The correct excipient composition is a prerequisite to provide lyophilizates with the appropriate visual appearance and disintegration time. Typically, they are composed of binders, such as gelatin and polyvinylpyrrolidone, fillers such as sucrose, mannitol, or sorbitol, taste modifiers, colorants, sweeteners, and preservatives. The main purpose of this study was to determine the optimal excipient scaffold to ensure the proper appearance of lyophilizates that have undergone aggressive drying conditions and a disintegration time of less than 3 min. In addition to mannitol and gelatin, the most frequently used binders, PVP K25, PVP K90, glycine, croscarmellose, and hydrolyzed gelatin were investigated. The results obtained revealed that lyophilizates with only mannitol and gelatin have a disintegration time that is too long, and that replacement of gelatin with PVP K25 led to friable and cracked lyophilizates. Considering disintegration time and visual appearance, lyophilizates with a mixture of gelatin, PVP K25, and mannitol (1:2:5) formed from liquid formulations with 6% (w/w) excipients were determined to be the most suitable. As a binder, PVP K25 expresses more appropriate characteristics relating to PVP K90. Addition of croscarmellose provided lyophilizates with a shorter disintegration time, whereas glycine only had a positive effect on the elegant appearance of lyophilizate cakes. Hydrolyzed gelatin was introduced with the aim of obtaining an even shorter disintegration time and at the same time an acceptable visual appearance of lyophilizates. This was achieved by lyophilization of solutions with 15% (w/w) of excipients with a hydrolyzed gelatin:PVP K25:glycine/croscarmellose:mannitol ratio of 4:2:0.5:4.5. Such lyophilizates show the highest potential for incorporation of poorly soluble and low-dose drugs.

1. Introduction

Over the past 2 decades, the demand for the development of new dosage forms has increased. The pharmaceutical industry is constantly focused on researching and manufacturing new dosage forms that can maximize the therapeutic potential of an active pharmaceutical ingredient (API) [1]. Orodispersible drug formulations are a current trend in the pharmaceutical industry, especially with regard to pediatric and geriatric patients. The main advantage of orodispersible dosage forms is that they are suitable for patients with swallowing problems, children, and geriatric and psychiatric patients, leading to improvement in patient compliance [2]. In addition, water is not required for administration of orodispersible dosage forms, and so the drug can be used regardless of access to fluid. The dosage form will rapidly disperse or dissolve in the saliva and is swallowed easily. The faster the disintegration and dissolution occur, the quicker the absorption and onset of clinical effect. The drug can be absorbed via the buccal, sublingual, or oral route. Because of rapid drug absorption and associated increased bioavailability, orodispersible dosage forms are also very useful when rapid onset of action is needed; for example, for pain relief, fever, heartburn, diarrhea, migraine, anxiety, and insomnia [3,4]. Orodispersible dosage forms offer new opportunities to the pharmaceutical industry in terms of life cycle management, drug promotion exclusivity, new patents, and their extension [5]. In contrast, the development of such formulations is usually very expensive, the final products are often very hygroscopic and fragile, only a small amount of drug can be incorporated, and special

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https://doi.org/10.1016/j.jddst.2023.104379
Received 25 January 2023; Received in revised form 14 March 2023; Accepted 17 March 2023
Available online 20 March 2023

packaging is required [4]. A drawback of orodispersible dosage forms can be also the unpleasant taste of the drug substance, which is why researchers are focusing on various approaches to masking taste [6–8].

Orodispersible drug formulations include orodisperisble tablets (ODT), oral lyophilizates, orodispersible granules, mini-tablets, and orodispersible films, and some less common ones such as fast-disintegrating capsules, and electrospun fibers or webs [3]. According to the European Pharmacopoeia, orodispersible tablets and oral lyophilizates belong to the category of tablets, whereas orodispersible films fall under the monograph of oromucosal preparations. Oral lyophilizates are solid single-dose preparations, obtained by the lyophilization process, which are intended to be placed in the mouth, where the drug is released in saliva and swallowed, or alternatively intended to be dispersed or dissolved in water before oral administration [9].

Lyophilization is a process in which water is removed from the formulation by sublimation after it has been frozen [10]. In general, a lyophilization cycle consists of three main steps. The first step is freezing, which converts the water solution to ice and freeze-concentrated solution. The second step is primary drying, in which the frozen solvent is removed from the product by sublimation, and the third step (secondary drying) removes the unfrozen water by desorption. Lyophilization, specifically primary drying, is a very energy- and time-consuming process that is associated with high costs and represents the major bottleneck in the production of oral lyophilizates. To avoid unnecessary costs, lyophilization must be optimized through the selection of appropriate primary drying parameters; that is, maximum shelf temperature and chamber pressure that do not affect the quality of the product. During conventional primary drying, the product temperature (Tp) should be kept below the glass transition temperature of the maximally freeze-concentrated solution (Tg'), and so the contemporary trend is implementation of aggressive primary drying conditions (Tp above Tg') leading to reduction of the primary drying time [11,12].

After Claritin Reditabs (Schering Plough) became the first oral lyophilizate approved by the U.S. Food and Drug Administration in 1996, several research groups focused on studying the effects of various excipients and lyophilization conditions on the quality of this orodispersible dosage form [13–15]. Typically, oral lyophilizates consist of binders and fillers, taste modifiers, colorants, sweeteners, and preservatives. Binders are water-soluble polymeric matrix formers such as gelatin, sodium alginate, hypromellose or polyvinylpyrrolidone, which provide the shape and mechanical strength of the final product. Fillers are sugars and sugar alcohols such as sucrose, glucose, mannitol, or sorbitol, which serve as matrix-supporting agents (also improving the mechanical properties of lyophilizate) and disintegration-accelerating agents [16,17]. A review by Costa et al. (2019) shows that gelatin and mannitol are present in the majority of commercial oral lyophilizates [18]. A drug with suitable properties (i.e., low water solubility and particle size below 50 μm) is dissolved or dispersed in an aqueous solution of excipients. For water-insoluble or poorly water-soluble drugs, the maximum amount of drug is 400 mg, and for water-soluble drugs a lower dose of up to 60 mg is allowed [1,19]. This last limitation is due to the plasticizing effect of the drug molecules on the matrix, which lowers the collapse temperature, resulting in a longer lyophilization process [16]. The physicochemical properties of oral lyophilizates are closely related to the design of the manufacturing process used. The first and best-known technology is Zydis®, in which the main matrix-forming component is gelatin and mannitol is added as a filler. The prepared solution or dispersion of the drug in a mixture of excipients is filled into blisters, passed through a freezing tunnel with liquid nitrogen, where the samples are rapidly frozen, and then automatically transferred to the lyophilizer. The fast freezing ensures that ice crystals and subsequently pores of suitable size are formed during drying, which promote the disintegration of the lyophilizates [20]. Finally, the blisters are sealed without being exposed to ambient conditions [18,21]. Quicksolv® technology also uses gelatin as a matrix former and additional solvents (water as the first solvent and ethanol or acetone as the second solvent) to reduce the fragility of the product, but it is basically similar to Zydis®. In Lyoc® technology, the main matrix polymer is xanthan gum or, less commonly, polyvinylpyrrolidone, and the freezing step is performed in a lyophilizer [18].

The composition of oral lyophilizates determines their critical quality attributes. Studies have shown that the amount of gelatin in the formulation is a balance between the appropriate disintegration time and the friability of the lyophilizates. For example, Shoukri et al. (2009) showed that lyophilizates with 2% or 3% (w/v) gelatin were not cracked or broken, whereas 1% (w/v) gelatin resulted in friable products. Lyophilizates with 3% (w/v) gelatin had a longer disintegration time and higher hardness than lyophilizates with a lower gelatin content. The conclusion of the study was that the most suitable formulation composition consisted of 2% (w/v) gelatin, mannitol, and glycine below 1% (w/v), and additionally polyvinylpyrrolidone K90 at a concentration of 1% (w/v). The addition of polyvinylpyrrolidone K90 resulted in larger and more diffuse pores, leading to fast disintegration [13]. In a study by Iurian et al. (2017), the quality by design approach was used to determine the optimal formulation composition of oral lyophilizates containing meloxicam nanocrystals. It was found that the most suitable matrix agent was sodium alginate at a concentration of 1.2% (w/v), with the addition of mannitol (5%, w/v) and poloxamer 188 (1%, w/v) [14].

The aim of this work was to design and characterize formulations for preparation of oral lyophilizates with the special innovation of implementing the most aggressive primary drying conditions without adversely affecting the properties of oral lyophilizates, contributing to a smaller financial and environmental burden. The objectives of this research were: (i) to develop an optimized formulation composition for preparation of oral lyophilizates based on mannitol, gelatin, and polyvinylpyrrolidone K25; (ii) to examine the influence of PVP K90, glycine, and croscarmellose on the quality attributes of lyophilizates; and (iii) to discover new strategies to utilize the potential of hydrolyzed gelatin in oral lyophilizates.

2. Material and methods

2.1. Material

Gelatin, hydrolyzed gelatin, polyvinylpyrrolidone (PVP) K25, and K90 were obtained from Sigma-Aldrich, Germany, and glycine, mannitol, and croscarmellose were purchased from Merck, Germany. Ultra-pure water was obtained from a Milli-Q purification system (A10 Advantage; Millipore Corporation, Bedford, MA, USA).

2.2. Methods

2.2.1. Sample preparation

During the study, several different formulations were prepared. First, a half amount of water was weighed in a beaker and then the excipients (i.e., mannitol, glycine, PVP K25, PVP K90, croscarmellose, or gelatin) were gradually added during constant mixing. In the case of gelatin, the water was heated between 60 and 70 °C. At the end of the process, the rest of the water was added, and 2 ml of each sample was aliquoted in a 10 ml beaker and held at −20 °C for 24 h before lyophilization. Formulations were coded as: F1–F34.

2.2.2. Lyophilization procedure

Lyophilization was conducted in a laboratory freeze-dryer (Epsilon 2-6D; Christ, Osterode am Harz, Germany) equipped with a capacitance manometer (722B Baratron; MKS, USA). The beakers containing the formulations were placed in the middle of the shelf and surrounded by a row of placebo vials. The freezing procedure was kept constant throughout the cycles, with the shelf temperature ramped to −45 °C at −0.5 °C/min. During primary drying, the shelf temperature was set to 20 °C and chamber pressure to 0.10 mbar, and in secondary drying the shelf temperature was raised to 40 °C. The chamber pressure was the

same during the primary and secondary drying. Tp was monitored with a calibrated thermocouple probe positioned in the vials. Tp was determined at the beginning of primary drying as an average value of 10 consecutive measurements. The graphic display of the process parameters and Tp was provided by Christ LPC-32 (LSC) SCADA software. The criteria were based on the concept that, when Tp approached the shelf temperature, this indicated the end of the primary drying. For accurate determination of primary drying time, the recorded cyclical data were used. As an endpoint, the offset point was selected.

2.2.3. Product appearance

Oral lyophilizates were visually evaluated after the completion of each lyophilization cycle, according to whether they were "acceptable" or "collapsed." The height, shape, and adherence of the cake to the vial walls were evaluated.

2.2.4. Disintegration time

Disintegration time was measured according to the European Pharmacopoeia, 10th edition, which states that oral lyophilizates should be placed in 200 ml of water at a temperature of 15–25 °C. In our case, the disintegration tests were performed at a water temperature of 22 °C. The time required for disintegration was recorded. According to the European Pharmacopoeia, oral lyophilizates must disintegrate within 3 min.

2.2.5. Differential scanning calorimetry

Differential scanning calorimetry measurements (DSC 1; Mettler Toledo, Switzerland) were conducted in hermetically sealed aluminum pans. For Tg' evaluation, the samples were cooled from 25 °C to −80 °C at a rate of −1.5 °C/min, and then heated to 50 °C at a rate of 3.5 °C/min. To investigate the thermal properties of final lyophilizates (i.e., mannitol physical form), and each ingredient, the following procedure was used. First, the samples were cooled from 25 °C to 0 °C, and then instantly reheated to 300 °C with a heating and cooling rate of 10 °C/min. All of the measurements were performed under a nitrogen atmosphere, at a flow rate of 40 ml/min.

2.2.6. Scanning electron microscopy

The small amount of lyophilizates in a thin layer were fixed with double-sided adhesive tape (Oxon, Oxford Instruments, UK) onto the stubs for scanning electron microscopy (SEM; Supra 35 VP, Carl Zeiss, Oberkochen, Germany). The samples were analyzed with a secondary detector at an accelerating voltage of 1 kV. Images were collected at a magnification of 250 ×, which allowed visualization of the lyophilizate morphology at the microscopic level.

2.2.7. The Brunauer–Emmett–Teller method

The Brunauer–Emmett–Teller method (BET) based on adsorption theory was used to measure the specific surface area (SSA) of oral lyophilizates. For this, a Nova 2000 analyzer (Quantachrome, Germany) was used together with NovaWin software version 11.05. Five data points, in the P/P0 range from 0.050 to 0.300, were used to determine the SSA. Prior the measurement, the samples were degassed under a high vacuum at 50 °C for 20 h to remove any adsorbed species before nitrogen adsorption analysis. The sample mass was between 0.2 and 0.3 g.

3. Results and discussion

3.1. Development of oral lyophilizates containing gelatin, PVP, and mannitol

The role of excipients in oral lyophilizate formulations is very important. They serve as bulking agents (fillers), binders, disintegrants, lubricants, surfactants, flavors, sweeteners, and colorants. The most important among them are binders and fillers, which were the focus of our work. First, the effects of the total excipient concentration and the ratio of excipients in the pre-lyophilized formulations on the disintegration time of oral lyophilizates were examined. Formulations with a fixed gelatin (binder) to mannitol (filler) mass ratio of 1:5 were tested, with the total excipient concentration in the pre-lyophilized liquid formulation varying from 6% to 30% (w/w). All prepared liquid formulations were subjected to a lyophilization cycle, and lyophilizates were evaluated at the end of the cycle. The main decisive parameters in the selection of suitable excipient concentrations were disintegration time, visual appearance, and mechanical strength of the oral lyophilizates with respect to handling them. The aim was to prepare lyophilizates from pre-lyophilized liquid formulations with the highest concentration of excipients that still provides adequate hardness and consequently adequate handling. The results obtained showed that all oral lyophilizates with mannitol and gelatin, regardless of the total concentration of excipients in the pre-lyophilized formulations, had an acceptable appearance, which means that they are non-collapsed and have a flat surface (data not shown). However, the disintegration time of all lyophilizates was greater than 3 min and thus did not comply with European Pharmacopoeia. Therefore, we concluded that the gelatin to mannitol mass ratio of 1:5 formed a cake structure that was too coarse and lacked pores.

Next, the potential of PVP K25 as a gelatin replacement was investigated. The formulations tested again contained 6%–30% (w/w) of excipients in the pre-lyophilized liquid formulation in a fixed PVP K25 to mannitol mass ratio of 1:5. The disintegration time of oral lyophilizates prepared from liquid formulations with 30% and 25% (w/w) excipients was longer than 3 min, whereas the disintegration time for formulations with 20%, 15%, 10%, and 6% (w/w) excipients was 43, 31, 22, and 16 s, respectively. On the other hand, as the concentration of excipients in the pre-lyophilized formulations decreased, the oral lyophilizates tended to be more brittle and cracked. In the liquid formulation, which contained 6% (w/w) excipients, we further varied the PVP K25 to mannitol mass ratio (1:5, 2:5, and 3:5). All lyophilizates had a disintegration time around 15 s, but they were very friable and cracked. We concluded that PVP K25 alone is not suitable for forming oral lyophilizates that are appropriate for handling, and therefore the addition of gelatin as a third excipient in the formulation was tested. In a further study three-component lyophilizates formed from pre-lyophilized liquid formulations containing 6% (w/w) excipients (gelatin, PVP K25, mannitol) were evaluated.

The results in Table 1 (F1–F6) show that gelatin content is a key parameter affecting disintegration time. Namely, a higher content of gelatin resulted in prolongation of the disintegration time, from 12 s for the formulation F1 with 7.7% gelatin to 42 s for the formulation F3 with 14.3% gelatin in oral lyophilizate. The disintegration time of lyophilizates with 22.2% gelatin or more (F5, F6) was greater than 3 min and thus did not comply with European Pharmacopoeia. A higher gelatin content (more than 12.5%) results in an acceptable visual appearance of lyophilizates (a rugged enough cake without signs of collapse), but probably due to its inhibitory effect on mannitol crystallization (discussed below) such lyophilizates have a disintegration time that is too long. In contrast, a lower gelatin content results in an appropriate disintegration time but inappropriate appearance of lyophilizates (Fig. 1). These results suggest that increasing the gelatin concentration in the lyophilizates leads to the formation of more cohesive and stable gels that dissolve less readily in water [13]. In the case of a lower amount of gelatin, fewer crosslinks form between the gelatin strands, resulting in friable products. Increasing the gelatin concentration usually results in a more extended and rigid 3D network after lyophilization due to an increase in the number of gelatin fibers forming crosslinks and H-bonds between chains, which leads to an increase in the overall hardness of the lyophilizates. Considering the disintegration time and visual appearance, we determined that a formulation F4 with gelatin: PVP K25:mannitol in a ratio of 1:2:5 is the most suitable for further investigation.

Different PVPs are known to affect the physical behavior of

Table 1
The composition of tested formulations with different PVP and their disintegration times. The total concentration of excipients in the pre-lyophilized liquid formulation was 6% (w/w).

formulation code	% of gelatin in lyophilizate	ratio of excipients				disintegration time (s)
		gelatin	PVP K25	PVP K90	Mannitol	
F1	7.7	0.5	1	0	5	12
F2	6.7	0.5	2	0	5	9
F3	14.3	1	1	0	5	42
F4	12.5	1	2	0	5	45
F5	22.2	2	2	0	5	>180
F6	30.0	3	2	0	5	>180
F7	0	0	0	2	5	55
F8	6.7	0.5	0	2	5	90
F9	12.5	1	0	2	5	>180
F10	28.6	2	0	2	5	>180
F11	37.5	3	0	2	5	>180

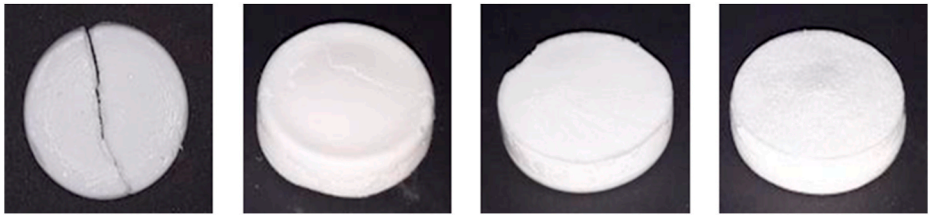

Fig. 1. Appearance of oral lyophilizates obtained from pre-lyophilized liquid formulation with 6% (w/w) of excipients in the mass ratios gelatin:PVP K25:mannitol = 0.5:1:5 (F1); 0.5:2:5 (F2); 1:1:5 (F3) and 1:2:5 (F4) (left to right).

formulations. For example, Shoukri et al. (2009) found that PVP can have an inhibitory effect on drug crystallization or crystallization of other excipients in formulations, which depends on the molecular weight of PVP [13]. Therefore, the effect of two different PVPs (K25 and K90) on disintegration time was compared. The results in Table 1 (F7–F11) again show that the disintegration time increases with increasing gelatin concentration in the formulation, and the shortest time was observed for the lyophilizate that did not contain gelatin. When comparing the lyophilizates with 6.7% (F8) and 12.5% (F9) gelatin, the disintegration time increased from 90 s to more than 180 s. Comparison of the disintegration times for formulations with PVP K25 and PVP K90 revealed that PVP K90 resulted in longer disintegration times, but the visual appearance of the oral lyophilizates was not affected (Fig. 2a). Whereas the formulation F4 with a gelatin:PVP K25: mannitol ratio of 1:2:5 disintegrated in an accepted time interval (45 s), similar formulation F9 (with PVP K90) required more than 3 min to disintegrate. Based on this, we can conclude that a combination of lower molecular weight PVP K25 and mannitol is a better choice in terms of the disintegration time of lyophilizates. We speculate that PVP K90 probably inhibits full crystallization of mannitol, resulting in a longer disintegration time. Therefore, PVP K25 was used in further studies.

Fig. 2. Appearance of oral lyophilizates obtained from pre-lyophilized liquid formulation with 6% (w/w) of excipients in the mass ratios: a) F8 (gelatin:PVP K90:mannitol = 0.5:2:5); b) F13 (gelatin:PVP K25:glycine:mannitol = 0.5:2:0.5:4.5); c) F18 (gelatin:PVP K25:croscarmellose:mannitol = 0.5:2:0.5:4.5).

3.2. Development of oral lyophilizates with addition of glycine or croscarmellose

In a further study, four-component formulations were prepared from gelatin, mannitol, PVP K25, and glycine or croscarmellose, and their effect on the quality attributes of lyophilizates were investigated. Glycine is a bulking agent and collapse protectant, and croscarmellose is a superdisintegrant. As shown in Table 2 (F12–F21), the disintegration time of the lyophilizates with either glycine or croscarmellose depended on the gelatin concentration. Whereas croscarmellose provided lyophilizates with shorter disintegration time and an appropriate visual appearance, glycine as a collapse protectant only had a positive effect on the elegant appearance of the lyophilizate cake (Fig. 2b and c). Lyophilizates without glycine (Fig. 1) exhibited cracking as an indicator of collapse, whereas no cracking was observed in lyophilizates with glycine at a comparable mass ratio of excipients.

The addition of croscarmellose resulted in lyophilizates with shorter disintegration times (Table 2); moreover, lyophilizates F19 containing 12.5% gelatin also disintegrated within 3 min. However, when the gelatin content exceeds 12.5% (F20, F21), its binding property outweighs the disintegration ability of croscarmellose, and the disintegration time of such lyophilizates exceeds 3 min.

3.3. Development of oral lyophilizates with hydrolyzed gelatin

Gelatin is a protein obtained by hydrolytic degradation of naturally occurring collagen. Chemical modification or enzymatic degradation can alter the protein-based composition of gelatin—for example, by making the protein hydrophobic, resulting in different functional properties of the gelatin obtained [22]. The addition of various enzymes, such as proteases, could decompose the gelatin and thus affect its solubility and gelling properties. Therefore, we decided to test the effect of hydrolyzed gelatin on the disintegration time and visual appearance of oral lyophilizates. According to the thermal characteristics, both gelatins used in this research exhibit comparable properties and show a rather flat curve in the DSC thermogram (Figs. SI–1 in Supplementary material), suggesting its amorphous nature, also observed by Mahor

Table 2
The composition of tested formulations with glycine and croscarmellose and their disintegration times. The total concentration of excipients in the pre-lyophilized liquid formulation was 6% (w/w).

formulation code	% of gelatin in lyophilizate	ratio of excipients					disintegration time (s)
		gelatin	PVP K25	glycine	croscarmellose	mannitol	
F12	0	0	2	0.5	0	4.5	22
F13	6.7	0.5	2	0.5	0	4.5	50
F14	12.5	1	2	0.5	0	4.5	>180
F15	22.2	2	2	0.5	0	4.5	>180
F16	30.0	3	2	0.5	0	4.5	>180
F17	0	0	2	0	0.5	4.5	10
F18	6.7	0.5	2	0	0.5	4.5	12
F19	12.5	1	2	0	0.5	4.5	15
F20	22.2	2	2	0	0.5	4.5	>180
F21	30.0	3	2	0	0.5	4.5	>180

et al. (2016). A disadvantage of hydrolyzed gelatin in the formulation is that it decomposes at lower temperatures and is therefore less stable than non-hydrolyzed gelatin [23].

In formulations that did not disintegrate in previous parts of the study, gelatin was replaced with hydrolyzed gelatin. All formulations (F22–F34) tested here are presented in Table 3. The hydrolyzed gelatin significantly shortened the disintegration time of the oral lyophilizates. All lyophilizates obtained from a liquid formulation with 6% (w/w) of excipients, i.e. F22–F31, disintegrated completely within 5 s, which corresponds to a very fast disintegration time. Hydrolyzed gelatin in lower concentrations is not capable of forming a compact structure (i.e., the lyophilizates obtained were extremely fragile), which made handling them impossible. However, despite the aggressive drying, they show no signs of collapse or cracking. Lyophilizates that contained 36.4% hydrolyzed gelatin in a hydrolyzed gelatin:PVP K25:mannitol ratio of 4:2:5 (F29) had a disintegration time of less than 5 s and were less friable than lyophilizates with a lower hydrolyzed gelatin content. A similar disintegration time was also seen in formulations containing glycine and croscarmellose (F30, F31). In all cases, aggressive drying was used, which did not adversely affect the visual appearance of the product. Considering that the lyophilizates disintegrated rapidly but still exhibited high friability, the concentration of excipients in the pre-lyophilized liquid formulation was increased in the next step, from 6% to 15% (w/w). The lyophilizates were not friable, and they handled well. Although they were aggressively dried, collapse or mechanical breakage was prevented. The hydrolyzed gelatin was strong enough to form a matrix, and its ability as a binder was confirmed. Lyophilizates obtained from the liquid formulation with 15% (w/w) excipients in hydrolyzed gelatin:PVP K25:mannitol ratio of 4:2:5 (F32) disintegrated in 50 s. Addition of croscarmellose (F34) as a superdisintegrant or glycine (F33) resulted in much shorter times: 8 and 10 s, respectively. The beneficial effect of glycine and croscarmellose in formulations containing hydrolyzed gelatin, PVP K25, and mannitol were confirmed. We concluded that formulations F33 and F34 with a hydrolized gelatin:PVP K25:glycine/croscarmellose:mannitol ratio of 4:2:0.5:4.5 have good potential for drug incorporation.

According to our study, the most promising scaffolds for drug incorporation are lyophilizates prepared from liquid formulations containing 6% (w/w) excipients in the following mass ratios: gelatin:PVP K25:mannitol = 1:2:5 (F4) and gelatin:PVP K25:glycine/croscarmellose:mannitol = 0.5:2:0.5:4.5 (F13 and F18). Among the lyophilizates obtained from pre-lyophilized liquid formulations containing 15% (w/w) excipients, the formulations with hydrolyzed gelatin:PVP K25:glycine/croscarmellose:mannitol mass ratio of 4:2:0.5:4.5 (F33 and F34) are the most promising. Pre-lyophilized liquid formulations that resulted in the most optimal lyophilizates were studied in detail from the viewpoint of the lyophilization process (Tp, primary drying time) and in terms of thermal characteristics (Tg'). The lyophilizates identified as the most promising were subjected to DSC analysis, SEM evaluation to take a look at the structural properties, and BET evaluation to compare the SSA of oral lyophilizates with various compositions.

3.4. Evaluation of lyophilization process and DSC results

The lyophilization process was monitored using thermocouples, and Tp was recorded. This allows detection of Tp during primary drying and consequent determination of primary drying time. In general, a higher Tp value during primary drying results in faster sublimation, thus requiring a shorter drying time. This fact is particularly important considering that primary drying is the most extensive phase of the lyophilization process, and so intensive research is being conducted to find ways to optimize it. As mentioned above, the contemporary approach to optimizing primary drying is aggressive drying; that is, drying at a Tp higher than Tg'.

Table 3
The composition of tested formulations with hydrolyzed gelatin (HG) and their disintegration times. The total concentration of excipients in the pre-lyophilized liquid formulation ($c_{pre\text{-}lyo}$) was 6% or 15% (w/w).

formulation code	$c_{pre\text{-}lyo}$ (% w/w)	% HG in lyophilizate	ratio of excipients					disintegration time (s)
			HG	PVP K25	glycine	Croscarmellose	mannitol	
F22	6	12.5	1	2	0	0	5	5
F23		22.2	2	2	0	0	5	5
F24		30.0	3	2	0	0	5	5
F25		22.2	2	2	0.5	0	4.5	4
F26		30.0	3	2	0.5	0	4.5	5
F27		22.2	2	2	0	0.5	4.5	3
F28		30.0	3	2	0	0.5	4.5	5
F29		36.4	4	2	0	0	5	2
F30		36.4	4	2	0.5	0	4.5	3
F31		36.4	4	2	0	0.5	4.5	4
F32	15	36.4	4	2	0	0	5	50
F33		36.4	4	2	0.5	0	4.5	8
F34		36.4	4	2	0	0.5	4.5	10

Our preliminary results showed that, by increasing the ratio of PVP K25 to mannitol (from 1:5 to 2:5 and 3:5), both Tp and Tg' decreased. The Tp of the pre-lyophilized liquid formulation with 6% (w/w) excipients and a PVP K25:mannitol ratio of 1:5 was −22.5 °C, whereas it was −23.1 °C and −25.4 °C for the 2:5 and 3:5 ratios, respectively. This resulted in a shorter primary drying time for the formulation with the lower proportion of PVP K25; namely, 9 h for the formulation with the PVP K25:mannitol ratio of 1:5 compared to 11 h for the formulation with the ratio of 3:5. The Tg' of the formulation with 6% (w/w) excipients and a PVP K25:mannitol ratio of 1:5 was −32.4 °C, whereas it was −32.8 °C and −34.6 °C for the ratios 2:5 and 3:5, respectively. A comparison between Tp and Tg' shows that all three formulations were aggressively dried because all Tp values were above the Tg' of the respective formulation. All formulations exhibit Tg', indicating their amorphous state during freezing. However, because no collapse occurred after aggressive drying, we hypothesized that mannitol at least partially crystallized during lyophilization. Such a crystalline matrix formed by mannitol provides mechanical support for the amorphous phase and prevents collapse even under aggressive conditions.

Tp and primary drying time were also determined for three-component pre-lyophilized liquid formulations containing 6% (w/w) excipients. We observed a trend that, as gelatin concentration increased, Tp also increased during primary drying (data not shown), but primary drying time was the same; namely, 14 h for all three-component formulations. Gelatin contributes to the mechanical strength of the crystalline mannitol matrix because lyophilizates with higher gelatin concentration did not crack despite aggressive drying. As noted by Seager (1998), gelatin is required to form a glassy structure, which affects the mechanical strength and friability of the lyophilized cake and allows handling and packaging [19]. In addition, the presence of a mannitol melting peak in the DSC heating thermogram (155–166.5 °C) reflects the fact that mannitol has at least partially crystallized during the lyophilization process (Fig. 3), with the intensity of the peak decreasing with increasing gelatin content in oral lyophilizates, resulting in an increase in the time required for disintegration.

Furthermore, we determined Tp and Tg' for the formulations previously shown to be the most suitable scaffolds for drug incorporation. As shown in Table 4, all pre-lyophilized liquid formulations have comparable Tg' values regardless of the excipients. With respect to the lyophilization process, an interesting effect was observed. Although significantly higher Tp values were observed for formulations F4 (gelatin:PVP K25:mannitol mass ratio of 1:2:5) and F13 (gelatin:PVP K25:glycine:mannitol mass ratio of 0.5:2:0.5:4.5), this did not lead to a reduction in primary drying time. Formulations F33, and F34, containing hydrolyzed gelatin had the lowest Tp values because they contained

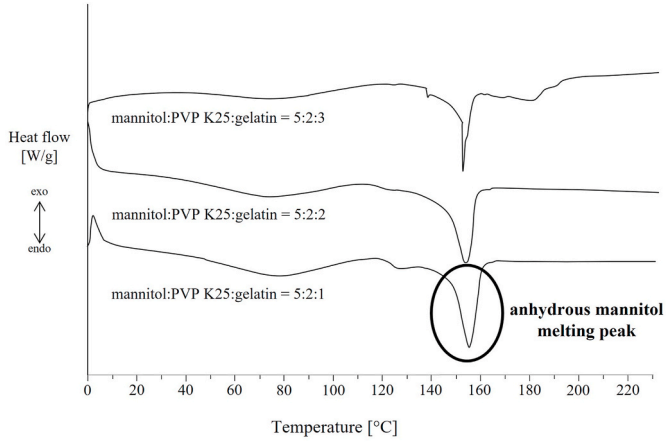

Fig. 3. DSC heating thermograms of oral lyophilizates (F4, F5, F6) with different mannitol:PVP K25:gelatin ratios with the focus on the intensity of the anhydrous mannitol melting peak.

Table 4
The most prospective scaffolds of lyophilizates for drug incorporation and their Tg', Tp, and primary drying (PD) time.

formulation code	composition	mass ratio	Tg' (°C)	Tp (°C)	PD time (h)
F4	gelatin:PVP K25: mannitol	1:2:5	−32.2	−6.3	13
F13	gelatin:PVP K25: glycine:mannitol	0.5:2:0.5:4.5	−32.3	−6.5	15
F18	gelatin:PVP K25: croscarmellose: mannitol	0.5:2:0.5:4.5	−36.3	−20.3	11
F33	hydrolyzed gelatin: PVP K25:glycine: mannitol	4:2:0.5:4.5	−33.0	−25.9	8
F34	hydrolyzed gelatin: PVP K25: croscarmellose: mannitol	4:2:0.5:4.5	−32.7	−24.1	7

a higher total excipient concentration (15%, w/w), but they were still dried in the shortest time (Table 4). We assume that they were able to form a structure with larger ice crystals during freezing, which accelerates the sublimation rate regardless of the Tp value at the beginning of primary drying, indicating a beneficial property of hydrolyzed gelatin [24]. However, from the lyophilization process graph, it is evident that all formulations reached the set shelf temperature within 15 h of aggressive primary drying, without any negative effect on the visual appearance of the cake. This was also justified by the presence of a mannitol melting peak, indicating its crystallization and thus mechanical support for the amorphous phase.

3.5. SEM and BET evaluation

Fast disintegration of oral lyophilizates is ensured by rapid uptake of water into the matrix, for which the presence of water-soluble excipients or even disintegrants is a prerequisite. From a morphological point of view, the accelerated penetration of water into the lyophilizates is ensured by an open network of larger globular pores [25]. In general, the SEM and BET methods are used in combination, where images of SEM can support an explanation for the SSA values obtained by BET.

As shown in Fig. 4d and e, oral lyophilizates containing hydrolyzed gelatin have higher SSA values than lyophilizates containing non-hydrolyzed gelatin. Specifically, the lyophilizate F33 containing hydrolyzed gelatin with the addition of glycine has the highest SSA value (5.3 m^2/g). This is probably a consequence of mannitol and glycine crystals forming a grainy structure of the lyophilizates, whereas the structure of the oral lyophilizates without glycine consists of smooth plates (Fig. 4e). The contribution of mannitol, and additionally glycine crystallization, to higher SSA was also demonstrated for lyophilizates F13 containing untreated gelatin, but, due to the lower total mass of dry matter (6% vs. 15% (w/w) of excipients in pre-lyophilized solution), crystals were less pronounced and SSA was lower (Fig. 4b). We also demonstrated that the incorporation of the superdisintegrant (croscarmellose) outweighed the impact of SSA value on the disintegration time because, despite the differences in SSA value between the lyophilizates with glycine (5.3 m^2/g) and those with croscarmellose (3.4 m^2/g), both showed practically the same disintegration time. The same trend was observed for oral lyophilizates containing untreated (non-hydrolyzed) gelatin, for which the SSA was higher for the lyophilizates F13 containing glycine (3.1 m^2/g) in comparison to the lyophilizates F18 containing croscarmellose (1.6 m^2/g), but the disintegration time was significantly shorter for the lyophilizates containing croscarmellose. We established that there is no correlation between SSA and disintegration time; namely, higher SSA does not inevitably result in faster disintegration of oral lyophilizates, as was also stated by AlHusban et al.

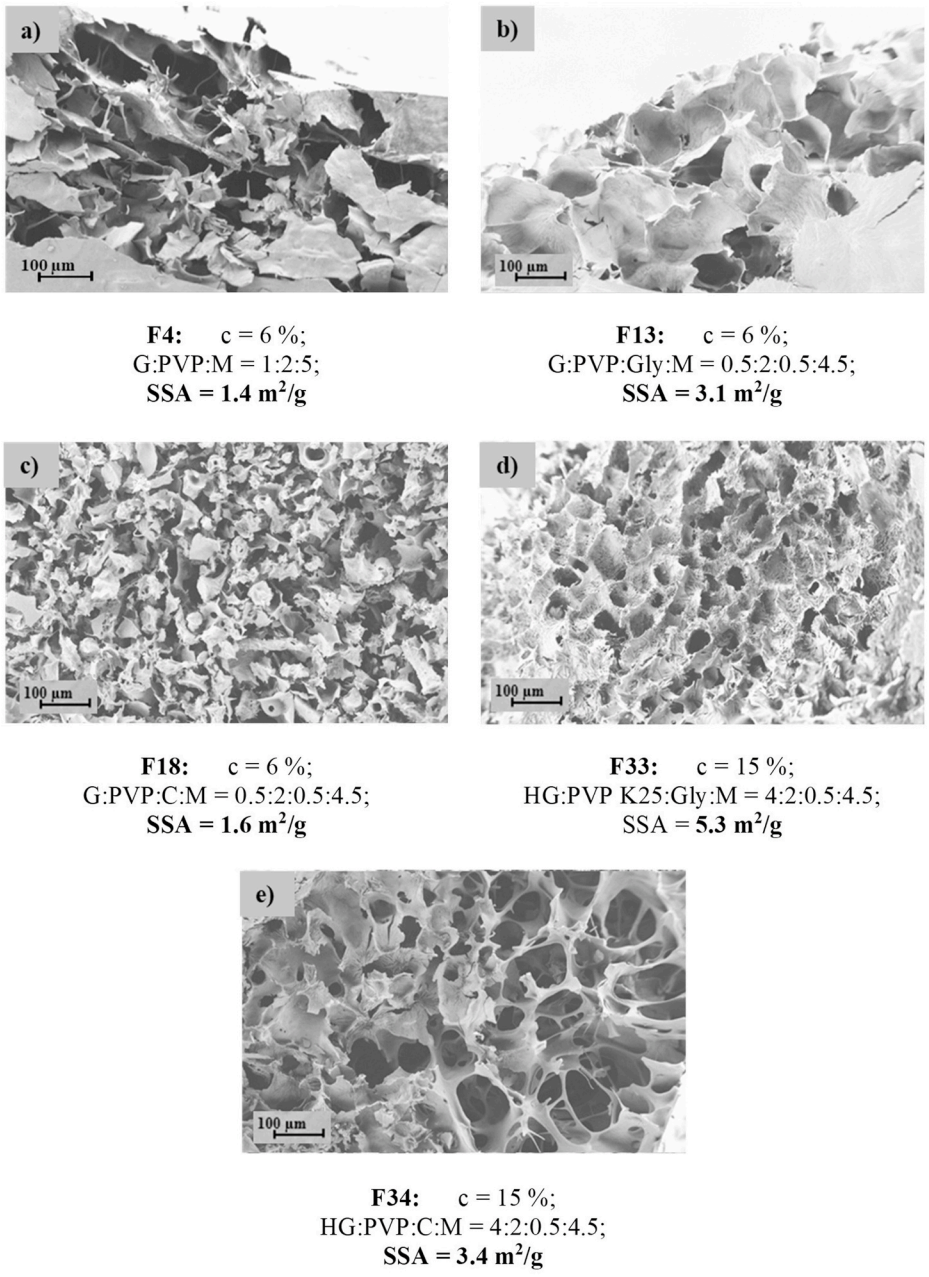

Fig. 4. Specific surface area (SSA) and SEM micrographs of the most promising oral lyophilizates.
Abbreviations: c = concentration of excipients (%, w/w) in the pre-lyophilized liquid formulation; C = croscarmellose; G = gelatin; HG = hydrolyzed gelatin; Gly = glycine; M = mannitol; PVP = PVP K25.

[25]. The size, geometry, and openness of pores are the main factors affecting the disintegration rate of lyophilizates.

4. Conclusion

This study demonstrates that the selection of appropriate excipients is of great importance to provide oral lyophilizates with acceptable critical quality attributes. In this study, lyophilizates were prepared from different mixtures of excipients. Lyophilizates with a gelatin concentration greater than 6.7% had disintegration times longer than 3 min, which is not in accordance with the regulation for these dosage forms. To address this issue, hydrolyzed gelatin was used. Formulations with this type of gelatin exhibited very short disintegration times even at higher concentrations. In summary, the most suitable formulations for drug incorporation were pre-lyophilized solutions containing 6% (w/w) of excipients in a ratio of 1:2:5 for gelatin:PVP K25:mannitol and a ratio of 0.5:2:0.5:4.5 for gelatin:PVP K25:glycine/croscarmellose:mannitol, and pre-lyophilized solutions with 15% (w/w) of excipients in a ratio of 4:2:0.5:4.5 for hydrolyzed gelatin:PVP K25:glycine/croscarmellose: mannitol. In addition, we have shown that aggressive drying conditions do not change the visual appearance of lyophilizates, which means faster and more economical manufacturing. We expect that the excipient scaffold proposed in this study is suitable for the addition of poorly soluble and low-dose drugs, but for high-dose drugs their effects on excipient composition still need to be studied in detail. Our further work will focus on the incorporation of API into the most promising excipient scaffolds presented in the study. In summary, oral lyophilizates represent a successful drug platform particularly for children and the elderly, and as such are increasingly important from the perspective of the pharmaceutical industry.

Author statement

Maja Bjelošević Žiberna: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Odon Planinšek**: Conceptualization, Writing – review & editing. **Pegi Ahlin Grabnar**: Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors acknowledge financial support by the Slovenian Research Agency, Slovenia, research core funding No. P1-0189 and grant L1-3160, and colleagues for their various contributions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2023.104379.

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