sschi SLOVAK SOCIETY OF CHEMICAL ENGINEERING

Slovak Society of Chemical Engineering
Institute of Chemical and Environmental Engineering
Slovak University of Technology in Bratislava

PROCEEDINGS

49th International Conference of the Slovak Society of Chemical Engineering SSCHE 2023

hotel SOREA HUTNIK I
Tatranské Matliare, High Tatras, Slovakia
May 15 - 18, 2023

Editors: Assoc. Prof. Mário Mihaľ, Dr. Ivan Červeňanský

ISBN: 978-808208-101-8, EAN: 9788082081018
Published by the Faculty of Chemical and Food Technology STU in Bratislava for the Institute of Chemical and Environmental Engineering in 2023

Kleová, K., Krosláková, S., Pätoprstý, V., Rajniak, P.: Design and optimization of formulations for freeze-drying of biological liquids and extracts of natural resources, Editors: Mihaľ, M., Červeňanský, I., In *49th International Conference of the Slovak Society of Chemical Engineering SSCHE 2023*, Tatranské Matliare, Slovakia, 2023.

Design and optimization of formulations for freeze-drying of biological liquids and extracts of natural resources

Kristína Klčová[2], Simona Krošláková[1], Vladimír Pätoprstý[3], Pavol Rajniak[1,2].

[1] *Sitno Pharma, Ltd., Rybné námestie 4135/1, 811 02 Bratislava, Slovakia*

[2]*Department of Chemical and Biochemical Engineering, Institute of Chemical and Environmental Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovakia*

[3]*Chemical Institute of the Slovak Academy of Sciences, Dúbravská cesta 5807/9 845 38 Bratislava, Slovakia*

Abstract

Lyophilization (or freeze-drying) is a method for drying and stabilization of thermolabile substances and chemically unstable entities. To prevent escape of the dissolved solids from formulations during drying at low pressures it is important to add some additional solids, so called excipients, to increase the solid concentrations. This work summarizes methodologies and workflow for design and optimization of solid concentrations of two kinds of liquid formulations: 1. Biological liquids (human proteines), 2. Extracts of natural resources (roots, leaves) of different plants. Three different excipients (sucrose, glycine, mannitol) were tested in different lyophilizers at different conditions. The key goals were a/ to find minimum necessary solid concentrations which already prevents the escape of solid phase, b/ to find excipients providing good properties of the final lyophilized solid cakes, c/ to find formulations giving fast and full reconstitution of the cakes.

Preliminary results and testing of reconstituted solutions show that lyophilization with the mannitol and glycine provide stable lyophilized products with very good reconstitution and without any degradation or modifications of chemical properties of original liquids.

Acknowledgment

This publication was created with the support of two projects:

1. Development of products by modification of natural substances and study of their multimodal effects on COVID-19, ITMS: 313011ATT2, co-financed by the European Regional Development Fund.
2. Study of structural changes of complex glycoconjugates in the process of inherited metabolic and civilization diseases (NFP313020Y920)

Design and optimization of formulations for freeze-drying of biological liquids and extracts of natural resources

Kristína Klčová[2], Simona Krošláková[1], Vladimír Pätoprstý[3], Pavol Rajniak[1,2]

[1]Sitno Pharma, Ltd., Rybné námestie 4135/1, 811 02 Bratislava, Slovakia
[2]Department of Chemical and Biochemical Engineering, Institute of Chemical and Environmental Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovakia
[3]Chemical Institute of the Slovak Academy of Sciences, Dúbravská cesta 5807/9 845 38 Bratislava, Slovakia

F C H P T

Introduction

Lyophilization is a method used for drying and stabilizing heat-sensitive substances and chemically unstable compounds. It generally consists of three steps: freezing (influences the entire lyophilization cycle and the quality of the resulting product), primary drying (sublimation phase), and secondary drying (desorption phase). During lyophilization, there can be a loss of dissolved solid materials from the samples due to reduced pressure, which is why it is necessary to add additional solid substances, known as excipients, to increase the concentration of solids.

Formulations for lyophilization of biological samples

Biological materials undergo degradation after being removed from their natural environment – storage samples at very low temperatures – alternative is lyophilization. Experiments: lyophilization of a biological product Apolipoprotein CIII (Apo CIII). Apo CIII is at very low concentration (caused a loss of the solid phase and a significant loss of the product → find a formulation that would prevent loss of the solid phase). Experiments with glycine solution in two different lyophilizers: Edwards (Faculty of Chemical and Food Technology) and Labconco (Institute of Chemistry, Slovak Academy of Sciences)

Experiments in Labconco lyophilizer

1. Experiment

Glycine solution concentrations: 0.25%, 0.5%, and 1%. Volume of samples - 1ml. Samples frozen at -20°C for 1 hour (freezer). **1-4** is a 0.25% glycine solution concentration, **5-8** is a 0.5% glycine solution concentration and **9-12** is a 1% glycine solution concentration.

Result: Loss of the solid phase (all concentrations) - increase the glycine concentration.

2. Experiment (asi maju byt vyssie koncentracie ?)

Glycine solution concentrations: 0.25%, 0.5%, and 1 %. Volume of samples - 1ml (2 samples) and 0.5 ml (2 samples). Samples frozen at -20°C for 1 hour (freezer). **1-4** is a 20% glycine solution concentration, **5-8** is a 10 % glycine solution concentration.

Result: No loss of the solid phase.

Experiments in Edwards lyophilizer

Same experiments as in the Labconco lyophilizer. Glycine solution concentration: 0.5%, 1%, 2.5% and 5%. Volume of samples 1ml (2 samples) and 0.5 ml (2 samples). The samples were frozen directly in the shelf lyophilizer for 1 hour. **17-20** is a 0.5% glycine solution concentration. **13-16** is a 1% glycine solution

Result: No loss of the solid phase - higher pressure in the Edwards lyophilizer compared to the Labconco lyophilizer.

Types of vials

The same experiments as in the Eppendorf tubes - using two types of vials - with a membrane and the traditional ones. Volume of samples 2ml.

Result: No loss of the solid phase.

1C-6C is a 0.25% glycine solution concentration. **1B-6B** is a 0.5% glycine solution concentration, **1A-6A** is a 1 % glycine solution concentration.

Storage and stabilization of Biological Material Samples

Stored biological samples conditions: warm storage at 37 °C, cold storage at -20 °C, ultra-cold storage at -80 °C, lyophilization of the sample, lyophilization of the sample with the addition of 2.5% glycine (lyophilized samples were stored at room temperature (22-25 °C)).

Result: Samples stored at 37°C completely degraded and lost their structure. Lyophilized samples showed similar results to those stored at -20°C and -80°C - lyophilization is indeed a suitable process for stabilizing such samples.

Formulations for lyophilization of extracts of natural resources

Glycine formulation:

Various concentrations of glycine solution. Freezing at -80°C (freezer). Volume of samples: 2ml of extract and 2ml of glycine solution. The optimal concentration of glycine is 12.5%.

Extract 2 Extract 4 Extract 5

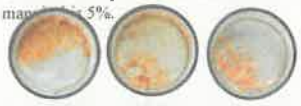
Extract 6 Extract 7

Extract 2 Extract 4 Extract 5

Extract 6 Extract 7

Mannitol formulation:

Various concentrations of glycine(asi ma byt mannitol ?) solution. Freezing at -80°C (freezer). Volume of samples: 2ml of extract and 2ml of mannitol solution. The optimal concentration of man[illegible]5%.



Extract 4 Extract 5 Extract 6

Sucrose formulation:

Same experiments as with glycine and mannitol. Sucrose is not a suitable excipient - it causes solid phase crystallization, resulting in a sticky product.

Reconstitution of natural resource extract products:

Analyse: before and after lyophilization. Determine if lyophilization leads to the loss of beneficial substances. The solvents used were a 50% ethanol solution and water. Reconstituted samples in the ethanol solution precipitated at the bottom of the Eppendorf tube.

Result:

The analysis confirmed that lyophilization stabilizes the sample and does not cause any loss of beneficial substances

Conclusions

The optimal formulation for lyophilization of biological samples is the addition of a glycine solution with a concentration of 2.5%. Storage at a temperature of 37 °C leads to sample degradation, while lyophilization showed similar results to storage at -20 °C and -80 °C (advantageous for sample transportation). The optimal formulation for lyophilization of natural source extracts is the addition of a glycine solution with a concentration of 12.5% or mannitol solution with a concentration of 5%. The analysis results confirmed that beneficial substances are not lost through lyophilization.

Acknowledgment:

1.Development of products by modification of natural substances and study of their multimodal effects on COVID-19, ITMS: 313011ATT2, co-financed by the European Regional Development Fund.

2.Study of structural changes of complex glycoconjugates in the process of inherited metabolic and civilization diseases (NFP313020Y920)