ORIGINAL ARTICLE

# Research of powders of the cryolyophilized xenoderm of porcine skin

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**Abstarct**

**Introduction:** Cryolyophilized xenoderm is widely used in the treatment of burn wounds in medical institutions of Ukraine. Therefore, the purpose of this work was to study the properties of the crushed substrate of the cryolyophilized xenoderm of porcine skin to substantiate the possibility and feasibility of using it in pharmaceutical technology for the development of new dosage forms. **Methods:** Exploring the crushed substrate of the cryolyophilized xenoderm of porcine skin studied fluidity, bulk density, tapped density, Carr index, angle of repose, shape, and particle size; elemental and amino acid composition was determined by atomic absorption spectroscopy and high-performance liquid chromatography, respectively. **Results:** The qualitative composition and the quantitative content of macro- and microelements and amino acids in the cryolyophilized xenoderm for epidermis and dermis layer have been defined using microscopic and pharmaco-technological tests for powders. **Conclusion:** It is determined that the pharmacotechnological properties of the dermis powder have better flowability characteristics than epidermis. The elemental composition of the epidermis and dermis (13 elements) has been studied. Quantitative content of 16 amino acids in the dermis and epidermis has been identified and determined.

**Key words:** Amino acids, cryolyophilized xenoderm of porcine skin, macroelements, microelements, pharmacotechnological properties

## INTRODUCTION

The cover system of the human body consists of skin, hair, nails, and exocrine glands. The skin is one of the largest organs that protect the body, creating a physical barrier between the outside world and inner tissues and consisting of the epidermis, dermis, and subcutaneous fat tissue.

Cellular elements have an important role in the skin, in particular, eosinophils, leukocytes, macrophages, plasma cells, lymphocytes, glycosaminoglycans, nerve cells, blood vessels, etc.

The epidermis of a healthy person contains dendritic epidermal T-cells that produce a number of cytokines (Interleukin [IL]-1 alpha, IL-2, IL-3, IL-7, IL-13, etc.).[1] Dendritic skin cells include Langerhans cells, Granstein cells, dermal dendrocytes, epidermal cells, and inflammatory dendritic epidermal cells.[2] Dendritic cells in the body are the most powerful antigen-presenting cells. Monocytes in the skin turn into macrophages, which are the factors of natural immunity. The fibroblasts in the cytoplasm contain fibrils (α- and β-smooth muscle actin) and are the main type of cells that actively synthesize the protein and mucopolysaccharides and on their surface are receptor proteins and glycolipids. Thanks to these cells, collagen is synthesized, from which further collagen fibers and extracellular matrix are formed.[3] Fibroblasts take an active part in the formation of connective tissue during healing of wounds. Myofibroblasts provide contraction of the edges of the wound. Glycosaminoglycan (except hyaluronic acid) with signaling molecule cells (growth factors and cytokines) is involved in the regulation of regeneration processes.[4]

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Growth factors may include proteins or small molecules such as peptides or steroids. In particular, polypeptide growth factors can be classified as several "super families" that combine substances that are close to the primary structure (for example, insulin-like, epidermal, transforming growth factors, and cytokines).[2-5]

Thus, the presence of a large number of cellular elements and growth factors in the skin proves that the epidermis and dermis are valuable and promising objects for further research.

Among natural sources of animal origin, the most similar in composition is cryolyophilized xenoderm of porcine skin, which is used to cover burn wounds.[6,7]

In Ukraine, a technology of cryolyophilized xenodermotransplants of porcine skin was developed, which consists of preserving the material in a liquid nitrogen medium, followed by drying at low pressure and a temperature of about 80°C. Sterilized xenodermotransplants can be stored for several years at temperatures from 4 to 6°C. The indicated technology is currently implemented in the industrial production of cryolyophilized xenodermotransplants in Ukraine (state registration No 1967/2003).

The cryolyophilized xenodermotransplants by Order No. 115 of the Ministry of Health of Ukraine dated May 11, 1998, are included in the State Register of Medical Products and are authorized for use in medical institutions of Ukraine. Introduction to the clinical practice of cryolyophilized xenodermotransplants as substitutes for the skin has allowed reducing the mortality of patients with severe burns by 30%, and the terms of staying patients at the departments of hospitals up to 18–20 days.

The Institute of Biomedical Technologies LLC (Ternopil, Ukraine) manufactures cryolyophilized xenodermotransplants as skin substitutes, which are used in treating burns (I-II-III-IV stages), donor and scalping wounds, and trophic ulcers. Sterile cryolyophilized xenodermotransplants with an area of 100–200–250–300 $cm^2$ and a thickness of 0.3–0.5 mm are packed in appropriate packages.[7]

Today, in Ukraine, there is a laboratory of cryo-vacuum preservation, bank of lyophilized xenoderm was created, Today, in Ukraine, there is a laboratory of cryo-vacuum preservation, was created bank of lyophilized xenoderm. The technique of collection, cryopreservation and lyophilization of xenodermotransplants was developed in the Institute of Biomedical Technologies LLC. Annually approximately 1 million 300 thousand $cm^2$ xenodermotransplants have been manufactured for the needs of all burn centers and the departments of hospitals in Ukraine.

The crushed substrate of cryolyophilized xenoderm of the porcine skin is a promising active pharmaceutical ingredients for the production of soft, solid, or liquid dosage forms with a wide range of pharmacological activity.[8]

## MATERIALS AND METHODS

All materials were ordered from Witec Industrial. The crushed substrate of cryolyophilized xenoderm of the porcine skin has been ordered from LCC Institute of Biomedical Technologies.

### Technology of Cryolyophilized Xenoderm of the Porcine Skin

Preparation of cryolophilized xenodermotransplants of the porcine consists of five stages: Skin preparation; cryopreservation of the porcine skin pieces; recleaning of the raw substrate of the porcine skin; lyophilization of the porcine skin pieces; and shredding of the cryolyophilized substrate of porcine skin.

The whole process of skin preparation is carried out under sterile conditions. With the help of electrodermatom, the skin is divided into layers (epidermis and dermis), and the thickness of the porcine skin pieces is from 0.2 to 0.3 mm.

Using sublimation chamber LZ-45.2 is carry out of the vacuum drying of the purified substrate in the form of porcine skin pieces.

According to the conditions of technical regulation, lyophilization lasts for 12–14 h.

The Institute of Biomedical Technologies LLC also worked out a technique for crushing cryolyophilized xenoderm of the porcine skin for the epidermis and dermis layer.[9]

All experimental studies with animals were conducted in compliance with the the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (18/3/1986, Strasbourg, France), Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes, Order of the Ministry of Health of Ukraine from February 13, 2006 No. 66, Law of Ukraine "On protection of animals from cruelty."

### Electron Microscopy

Investigation of particle forms was carried out by optical microscopy method using a light microscope MICROmed SEO SCAN and took pictures with the video camera Vision CCD Camera with an image output system on a computer monitor.

### Technological Properties

The pharmaco-technological properties such as bulk density, tapped density, Carr index, flowability, angle of repose and particle size for powders of epidermis and dermis layer of the cryolyophilized xenoderm of porcine skin have been defined.[10]

Bulk density of powders is the ratio of the mass of the non-compacted sample to its volume, including the contribution of the partially free volume. Bulk density of powder mixture was determined by pouring the powder into the graduated cylinder. The bulk volume (Vb) and weight of the blend (m) were determined.[10]

The tapped density is the density of the powder, which is achieved after consolidation.[10] Tapped density was calculated by determining the volume that holds set weight of powder after 1250 taps on tapped density tester ERWEKA SVM 202 (ERWEKA GmbH, Germany).

The Carr index (indicator of compressibility) was calculated according to the formula given in the State Pharmacopoeia of Ukraine: [11]

$$Ic=\frac{\rho_{o'\tilde{n}}-\rho_{i}}{\rho_{o'\tilde{n}}}X100$$

Carr index is an indication of the compressibility of a powder. The resulting index from 12 to 15 is considered to be an indication of good compressibility, 16–24 - fair to passable, 25–35 - poor, 36–39 - very poor, and more 40 - extremely poor.

To determined flowability, fixed funnel method was used. The mixture of powders (± 100 g) poured through the funnel. The time of the powder mixture fall down through the funnel was used to calculate flowability of the powder.[10] Flowability was tested by granulate flow tester ERWEKA GT (ERWEKA GmbH, Germany). This is the speed of rash powder through a funnel with an opening diameter of 10 mm. All experiments were conducted three times.

This device also allows measuring the angle of repose by a laser. This is a constant three-dimensional angle (relative to the horizontal surface), which is formed when the cone-shaped column of the material is formed.[10]

Particle size distribution was estimated by laser diffraction on particle analyzer Master Sizer 3000 (Malvern, UK), using automated dry powder dispersion unit (Aero S).[10]

### Determination of macro- and microelements

The study of macro- and microelement composition of dermis and epidermis of the cryolyophilized xenoderm of the porcine skin was carried out by atomic absorption spectroscopy method (AAS), using an atomic absorption spectrometer Analytik Jena Contr AA 300 (Germany). About 10 g (±0.0001 g) of homogenized products portions were weighed and transferred to quartz crucibles. Then, they were ashed in an electric furnace at 540°C. Mineralization was performed with 1.5 mL of HCl (Tracepure 36 %) and 2–3 drops of $HNO_3$ (Tracepure 63 %). AAS is a widely used technique for determining a large number of metals. In AAS, an aqueous sample containing the metal analyte of interest is aspirated into an air-acetylene flame, causing evaporation of the solvent as well as vaporization of the free metal atoms (atomization). For atomization of samples, a flame or a graphite furnace is used.

### Definition of amino acids

Determination of qualitative composition and quantitative content of amino acids in layers of the cryolyophilized xenoderm of the porcine skin was carried out by high-performance liquid chromatography with a pre-column derivatization 9-fluorenylmethoxycarbonyl chloride (FMOC) and o-phthalic aldehyde, which reacts with primary amines in the presence of a thiol compound (2-mercaptoethanol or N-acetyl-L-cysteine) to form a fluorescent product.[12,13] In this method, derivatization is best performed in an automatic mode using an autosampler and the mixer and combined with an urgent chromatography of the mixture on a reverse-phase column in a gradient elution mode. The method for determining amino acids is based on the extraction of free amino acids and acid hydrolysis of preparations with subsequent analysis of the resulting hydrolyzates. Reversed-phase high-performance liquid chromatography (HPLC) with pre-column derivatization is preferred because of the short time, simple instrumentation, and low cost required.

Chromatographic separation was carried out on a liquid chromatograph Agilent 1200 (Agilent technologies, USA). Column length Zorbax AAA – 150 mm, inner diameter - 4,6 mm, diameter of sorbent grain - 3 µ. Mobile phase A - 40 mm $Na_2HPO_4$, pH 7,8, and B - ACN:MeOH:water (45:45:10, v/v/v). The temperature of the thermostat column is 40°C. Gradient separation mode with a constant flow rate of 1.5 ml/min.

| Time, min | Moving phase A (%volume/volume) | Moving phase B (%volume/volume) |
|---|---|---|
| 0–2 | 100 | 0 |
| 2–17 | 100→44 | 0→56 |
| 17–20 | 44→0 | 56→100 |
| 20–22 | 0 | 100 |
| 22–24 | 0→100 | 100→0 |
| 24–26 | 100 | 0 |

| Chromatography detection was carried out in the next mode | | |
|---|---|---|
| Time, min | Excited wavelength, nm | Wavelength of radiation (emission/detection), nm |
| 0→15 | 340 | 450 |
| 15→26 | 266 | 305 |

For the purpose of extraction of free amino acids to the sample preparation, placed in vial, 0.1 mole/l aqueous solution of hydrochloric acid was added and kept on the water bath with ultrasound at 50°C for 3 h.

For extraction of the amount of free and bound amino acids up to the sample preparation, placed in vial, 6 mole/l aqueous solution of hydrochloric acid was added, placed in the thermostat at 110°C, and hydrolyzed for 24 h. 0.5 ml of centrifuged extract/hydrolysate was evaporated on a rotary evaporator and then rinse 3 times with purified water to remove hydrochloric acid. The fragmented products were resuspended in 0.5 ml of purified water and filtered through membrane filters from regenerated cellulose (pore size 0.2 μm). Before entering the samples into the chromatographic column in the automatic program mode, Before entering the samples into the chromatographic column in the automatic program mode, fluorescence derivative amino acids were obtained.[12,13]

For the identification of amino acids, the times of holding the peaks of amino acids on the chromatograms of the samples under study were compared of the cryolyophilized xenoderm of the porcine skin different layers with the retention time of the relevant substance amino acids on the chromatogram of the comparison solution.

The quantitative content of amino acids is calculated from the value of the height of the peaks of the amino acids studied on the corresponding chromatograms. The content of bound amino acids was determined by subtracting the content of free amino acids from their total content.

The calculation of the content of amino acids (X, μg/mg) was carried out according to the following formula:

$$X = \frac{CV_{solvent}}{m_{drug}}$$

C - concentration, obtained from the chromatogram by calculating the reference solution and the test solution, μg/ml;
$V_{solvent}$ - volume of solvent for extraction, ml;
$m_{drug}$ - weight of the drug, mg.[12,13]

### Statistical analysis

For all sets of the data F, testing is performed for homogeneity of variances. The variance for each set of samples was calculated and The variance for each set of samples and F-test was conducted (Microsoft Excel, 2010).

## RESULTS AND DISCUSSION

For the purpose of obtaining cryolyophilized xenoderm of the porcine skin, a specific method for obtaining and milling cryolyophilized xenoderm of the porcine skin was developed. A microscopic study of the resulting powder was performed, and the size of the particles and the pharmacotechnological properties of the powders were determined. The composition of macro- and microelements into the crushed substrates of dermis and epidermis has been investigated. The qualitative composition and quantitative content of amino acids in the dermis and epidermis of the cryolyophilized xenoderm of the porcine skin are determined.

At present, the method of obtaining and shredding the epidermal layer using a knife mills is worked out, and the technical parameters of which are determined in accordance with the specific tasks of shredding of bioorganic raw materials. The principle of action used in the work of the mill is to grind the shreds of the skin with a steel knife at its speed of rotation (3000 $min^{-1}$).[9]

As a result, we obtain an amorphous dry, coarse-grained, hygroscopic powder of gray color with separate yellow–brown particles of various shapes [Figures 1 and 2]. The presence of loosely loose lumps that crumble at a slight press may be allowed. Taste and smell are specific, characteristic of the product, without foreign flavors and smells, and without signs of fat.

Microscopic examination of the powder was studied using a light microscope MICROmed SEO SCAN with an image output system. Microphotographs of crushed powder of the first layer from the cryolyophilized xenoderm of the porcine skin (epidermis) are shown in Figures 1 and 2 increasing by 300 times (increase ×20; objective - ×20, eyepiece - ×15).

The external appearance and microphotographs of crushed powder of the second layer from the cryolyophilized xenoderm of porcine skin (dermis) is shown in Figures 3 and 4 increasing by 80 times (increase ×4; objective - ×10, eyepiece - ×8).

Light optical analysis of shredded substrates showed that they consist of fragments of tissues of different sizes. In appearance, the particles of the substrate are yellowish-white

**Figure 1:** Appearance of crushed powder of the first layer from the cryolyophilized xenoderm of porcine skin (epidermis)

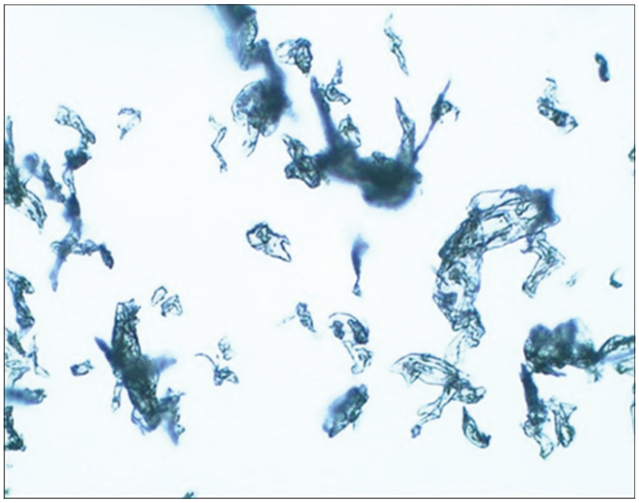

**Figure 2:** Photomicrography of powder of the first layer from the cryolyophilized xenoderm of porcine skin (increase by 300 times)

**Figure 3:** Appearance of crushed powder of the second layer from the cryolyophilized xenoderm of porcine skin (dermis)

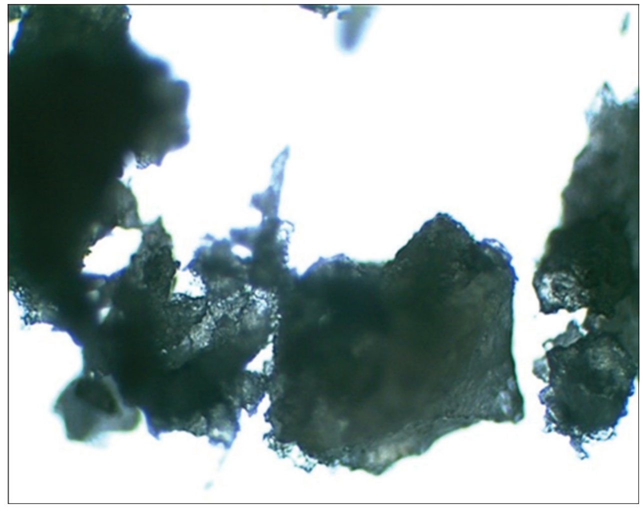

**Figure 4:** Microphotography of crushed powder of the second layer from the cryolyophilized xenoderm of porcine skin (dermis) (increase by 80 times)

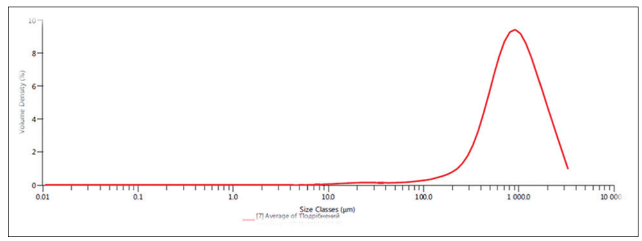


**Figure 5:** Distribution of the powder particles size of the first layer from the cryolyophilized xenoderm of porcine skin (epidermis)

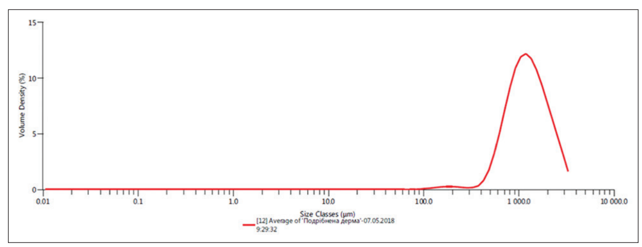


**Figure 6:** Distribution of the powder particles size of the second layer of cryolyophilized xenoderm of porcine skin (derma)

in colors and do not contain impurities and contaminants, since they are made from scraps of cryopreserved and freeze-dried skin under sterile conditions.

In powder of the first layer from the cryolyophilized xenoderm of porcine skin, the fragments are represented predominantly by the epidermis, which is a layer of epithelial cells and papillary layer of the dermis [Figures 1 and 2]. The crushed powders of the second layer are more massive connective tissue conglomerates and fragments [Figures 3 and 4].

The size of the cryolyophilized xenoderm of the porcine skin particles was studied by the laser diffraction method, and it was established that the powder of the cryolyophilized xenoderm of porcine skin first layer (epidermis) consists of particles, and 90% of which have a size up to 1970 μm, 50% to 885 μm, and 10% to 347 μm. The particle size distribution diagram is shown in Figure 5.

Particles of the second layer from the cryolyophilized xenoderm of porcine skin (dermis) are in the form of conglomerates of irregular shape in the size of 0.5–2.0 cm. Therefore, at the first stage of the study, the conglomerates of the dermal layer were crushed on the knife and screw mills.

The particles of the second layer of the cryolyophilized xenoderm of porcine skin are characterized by the following dimensions: D10 = 650 μm, D50 = 1200 μm, and D90 = 2270 μm [Figure 6].

The results of pharmacotechnological properties for powders of the epidermal and dermal layers of the cryolyophilized xenoderm of porcine skin are given in Table 1.

As shown from the result of the study, the powder of the first layer from the cryolyophilized xenoderm of porcine skin is characterized by poor flowability and a large amount of fine fraction.

These data confirm that the powder of the derma (second layer from the cryolyophilized xenoderm of porcine skin) is mainly composed of large particles that provide better flowability properties of the powder.

The characterization of powder flow properties is required for reliable design and proper operation in industrial processes. Pharmacotechnological properties of powders of the first and second layers of the cryolyophilized xenoderm of porcine skin allow better insight into the powder/process relationship.[14]

The results of the research of the pharmacotechnological indicators can actively influence the quality of the powder mass. The obtained results will be used for the development of solid dosage forms at the appropriate stages of the technological process.[15,16]

The method of atomic absorption spectrometry was used to study the qualitative composition and quantitative content of macro- and microelements in epidermis and derma of the cryolyophilized xenoderm of porcine skin and carried out a comparative analysis of the elemental composition in two layers [Table 2].

As a result of the conducted research, the quantitative content of 13 elements was identified and determined. It has been established that all identified elements are present in both the layers of the cryolyophilized xenoderm of porcine skin but are contained in different amounts. It was determined that the epidermis is dominated by the quantitative ratio of Mg and Ca and, in the dermis, contains more Fe, Cu, and Zn.

As know, amino acids are precursors of proteins that contain one or more characteristic amino acid sequences in their composition and play the role of growth factors that stimulate epithelization and cell regeneration, as well as have a wound healing effect. Determination of the composition and content of amino acids in biologically active materials has a great scientific and practical interest, due to their high biological activity.

Investigations of the qualitative composition and quantitative content of amino acids are carried out in the epidermis and dermis of the cryolyophilized xenoderm of porcine skin by the HPLC method, and the samples of the corresponding chromatograms are shown in Figures 7 and 8.

As a result of the studies, 16 amino acids were identified in the epidermis and dermal layers. In the dermis, all identified amino acids are in both free and bound states. In the epidermis, histidine is only in a bound state, and all the other 15 amino acids are represented in both forms. Among the identified amino acids, nine are irreplaceable, which confirms the high metabolic and oxidative-reduction potential of all layers of the cryolyophilized xenoderm of porcine skin, which promotes the improvement and acceleration of regenerative processes in the human body.

The results of the quantitative determination of amino acids in powders of the epidermal and dermal layers are given in Tables 3and 4.

According to the results of the analysis, it can be concluded that the epidermis of the cryolyophilized xenoderm of porcine skin contains more glutamic acid, glycine, proline, aspartic acid, arginine, and alanine.

Among the free amino acids, serine is most commonly found at 3.8 µg/mg and arginine 2.18 µg/mg. Among the bound amino acids, glutamic acid is predominantly 8.09 µg/mg.

In the dermal layer, the same amino acids are dominant in the quantitative content but in another ratio (glutamic acid, glycine, arginine, aspartic acid, and proline). In free state, glutamic acid (1.89 µg/mg), glycine (1.21 µg/mg), and alanine (0.88 µg/mg) predominate in quantitative content. Among the bound amino acids, glutamic acid (4.77 µg/mg) also predominates high levels of proline (3.89 µg/mg), glycine (3.71 µg/mg), and aspartic acid (3.49 µg/mg).

## CONCLUSION

As a result of the studies, the size and shape of the particles, fluidity, bulk density, tapped density, Carr index, and angle of repose were studied in the powder of the cryolyophilized xenoderm of porcine

**Table 1:** Pharmacotechnological properties of powders first and second layers of the cryolyophilized xenoderm of porcine skin

| Name of the indicator, unit of measurement | First layer (epidermis), $\bar{x}\pm\Delta\bar{x}$, $n$=3, $P$<0.05 | *Second* layer (*derma*), $\bar{x}\pm\Delta\bar{x}$, $n$=3, $P$<0.05 |
|---|---|---|
| Bulk density, g/ml | 0.294±0.004 | 0.347±0.005 |
| Tapped density, g/ml | 0.374±0.007 | 0.435±0.009 |
| Carr index, % | 21.322±0.415 | 20.155±0.315 |
| Flowability, s/100 g | 25.80±0.52 | 26.52±0.54 |
| Angle of repose, degrees | 44.76±0.86 | 48.52±0.92 |

**Table 2:** Results of the study of elemental composition of the cryolyophilized xenoderm of porcine skin

| Investigated element | Content of elements in the crushed powders of cryolyophilized xenoderm of porcine skin (mg/kg), $\bar{x}\pm\Delta\bar{x}$, n=5, $P<0.05$ | |
|---|---|---|
| | Epidermis | Derma |
| Potassium (K) | $2.45\cdot10^2\pm4.41$ | $2.52\cdot10^2\pm4.54$ |
| Calcium (Ca) | $3.32\cdot10^3\pm66.42$ | $2.78\cdot10^3\pm55.62$ |
| Magnesium (Mg) | $1.50\cdot10^2\pm2.63$ | $1.2010^2\pm2.10$ |
| Iron (Fe) | $5.30\cdot10^2\pm9.65$ | $6.70\cdot10^2\pm9.95$ |
| Zinc (Zn) | 82.00±1.28 | 98.00±1.43 |
| Copper (Cu) | 5.00±0.11 | 15.00±0.33 |
| Chromium (Cr) | 33.00±0.63 | 27.00±0.51 |
| Nickel (Ni) | 14.00±0.25 | 16.00±0.29 |
| Cadmium (Cd) | 0.11±0.002 | 0.09±0.001 |
| Lead (Pb) | $1.15\cdot10^2\pm1.78$ | $1.05\cdot10^2\pm1.63$ |
| Barium (Ba) | 3.60±0.06 | 3.20±0.06 |
| Silver (Ag) | 4.05±0.08 | 3.95±0.08 |
| Titanium (Ti) | 88.00±1.76 | 92.00±1.93 |

**Table 3:** The results of the determination of the amino acid composition in the epidermal layer of the cryolyophilized xenoderm of porcine skin

| RT | The name of the amino acid | Amount of amino acids in the epidermis, μg/mg | | |
|---|---|---|---|---|
| | | Free | Bound | Total |
| 1.616 | L-aspartic acid | 0.56 | 3.74 | 4.31 |
| 2.543 | L-glutamic acid | 1.08 | 8.09 | 9.17 |
| 5.745 | L-serine | 3.08 | 0.58 | 3.66 |
| 6.869 | L-hystidine | 0.00 | 0.86 | 0.86 |
| 7.126 | Glycine | 1.90 | 3.02 | 4.92 |
| 7.367 | L-treonin | 1.05 | 0.62 | 1.67 |
| 8.568 | L-arginine | 2.18 | 2.19 | 4.37 |
| 8.740 | L-alanine | 1.56 | 2.03 | 3.59 |
| 10.240 | L-tyrosine | 0.58 | 0.34 | 0.92 |
| 12.209 | L-valin | 0.54 | 1.70 | 2.24 |
| 12.469 | L-methionine | 0.33 | 0.35 | 0.68 |
| 13.854 | L-phenylalanine | 0.39 | 1.54 | 1.93 |
| 14.062 | L-isoleucine | 0.34 | 1.48 | 1.82 |
| 14.779 | L-leucine | 0.49 | 2.91 | 3.39 |
| 15.254 | L-lysine | 0.45 | 2.23 | 2.68 |
| 19.336 | L-proline | 0.61 | 3.37 | 3.98 |

skin in the epidermal and dermal layers. The elemental composition of the two layers of the substrate of the cryolyophilized xenoderm of porcine skin is determined by the AAS method.

**Table 4:** The results of the determination of the amino acid composition in the dermal layer of cryolyophilized xenoderm of porcine skin

| RT | The name of the amino acid | Amount of amino acids in the epidermis, μg/mg | | |
|---|---|---|---|---|
| | | Free | Bound | Total |
| 1.575 | L-aspartic acid | 0.33 | 3.49 | 3.82 |
| 2.519 | L-glutamic acid | 1.89 | 4.77 | 6.67 |
| 5.728 | L-serine | 0.42 | 1.06 | 1.48 |
| 6.854 | L-hystidine | 0.30 | 0.17 | 0.47 |
| 7.194 | Glycine | 1.21 | 3.71 | 4.92 |
| 7.351 | L-treonin | 0.35 | 0.67 | 1.02 |
| 8.581 | L-arginine | 0.46 | 3.29 | 3.75 |
| 8.720 | L-alanine | 0.88 | 2.86 | 3.74 |
| 10.187 | L-tyrosine | 0.54 | 0.01 | 0.55 |
| 12.191 | L-valin | 0.42 | 1.00 | 1.43 |
| 12.447 | L-methionine | 0.19 | 0.32 | 0.52 |
| 13.832 | L-phenylalanine | 0.24 | 0.94 | 1.18 |
| 14.040 | L-isoleucine | 0.30 | 0.58 | 0.87 |
| 14.760 | L-leucine | 0.29 | 1.52 | 1.80 |
| 15.230 | L-lysine | 0.34 | 1.61 | 1.95 |
| 19.310 | L-proline | 0.64 | 3.89 | 4.52 |

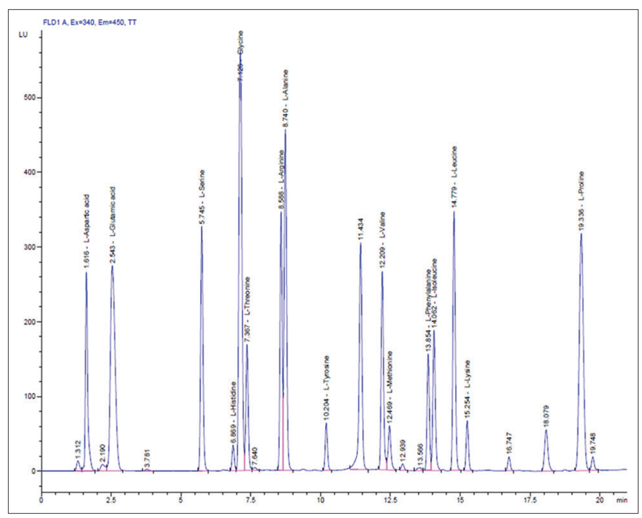

**Figure 7:** High-performance liquid chromatography obtained by determining the amino acids in the powder of the epidermal layer

The HPLC method was used to examine the amino acid composition in the epidermal and dermal layers from the cryolyophilized xenoderm of porcine skin. Five dominant amino acids (glycine, proline, alanine, asparagine, and glutamic acid) were identified, which prevail in each layer, but in different quantitative ratios.

A set of microelements and amino acids (especially, by histidine, arginine, valine, methionine, threonine,

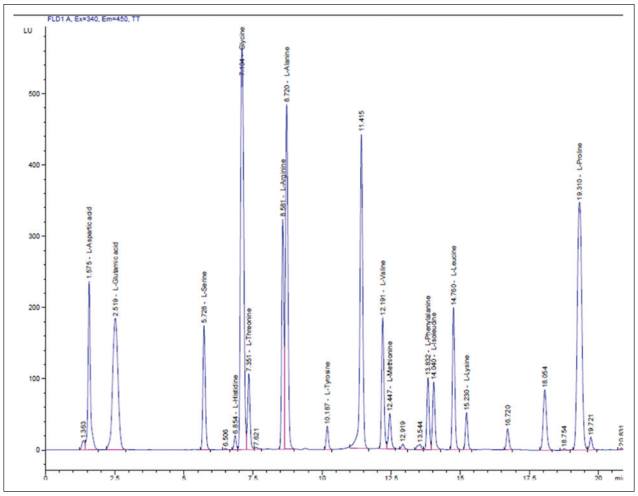


**Figure 8:** High-performance liquid chromatography obtained by determining the amino acids in the powder of the dermal layer

and lysine) found in the dermis and epidermis of the cryolyophilized xenoderm of porcine skin prove its high metabolic and oxidative-reduction potential, which is necessary for correction of regenerative processes.

Therefore, biologically active material obtained by the modern technology of cryopreservation, lyophilization, and grinding makes a special interest of pharmaceutical technology in the development of medicinal products in the different dosage forms, as a promising active pharmaceutical ingredient for the regeneration of pathologically damaged body structures and functions and can be used for external and internal use.

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