

LYOPHILIZED BOVINE PERICARDIUM TREATED WITH PHENETHYLAMINE-DIEPOXIDE AS AN ALTERNATIVE FOR CARDIOVASCULAR BIOPROSTHESIS

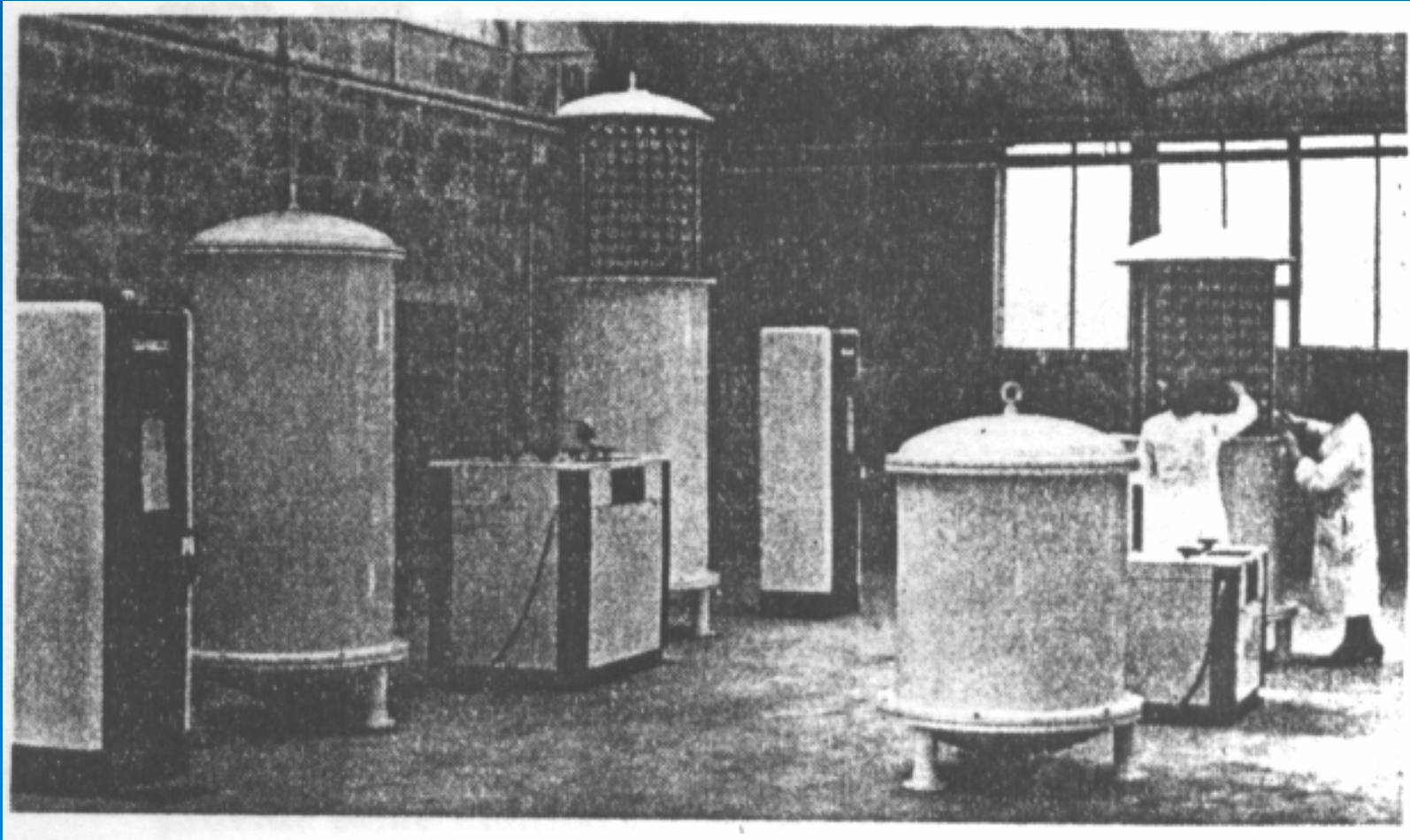
RONALDO N. M. PITOMBO

Pharmaceutical Sciences School of Sao Paulo University
Department of Biochemical and Pharmaceutical
Technology

INTRODUCTION

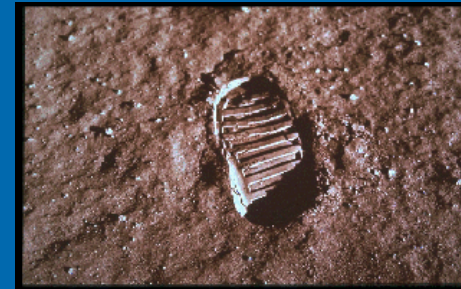
- **Freeze-drying or lyophilization** is a process in which water or any other solvent is transferred directly from the solid phase, into the vapor phase
- Multistage operation that **stabilizes biomaterials** through the four main operation:
 - **Freezing**
 - sublimation or **primary drying**
 - desorption stage or **secondary drying**
 - **storage**
- Products of **superior quality**, which are **easily reconstituted** and have **long shelf-life**.

One of the first medicinal uses of the lyophilization process on a large scale was by Greaves in 1944



Structural changes during freeze drying of foods materials

- Apollo Project -1960s



Telemetric medicine

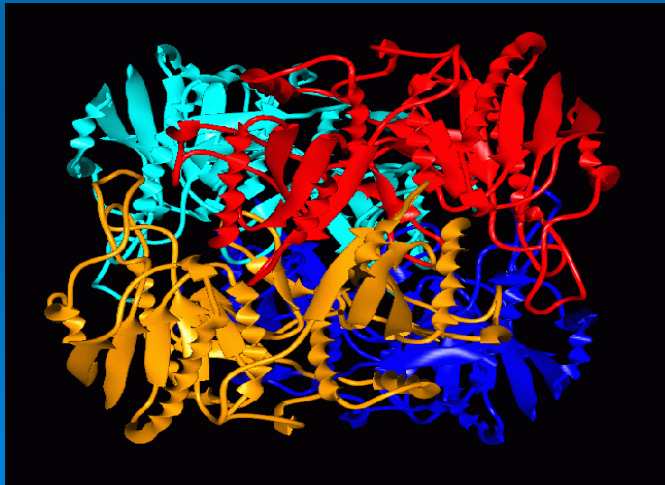


Space food

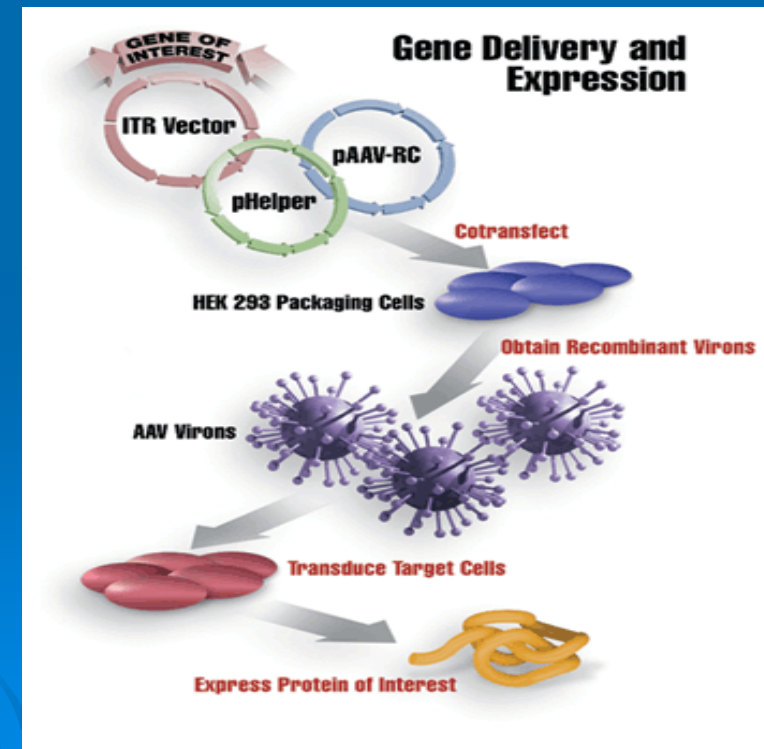
Some products lyophilized today

Delicate **biochemical** as well as highly sophisticated **biotechnological products**.

Living **microorganisms** or products that come from molecular biology



tetrameric structure of L-asparaginase II

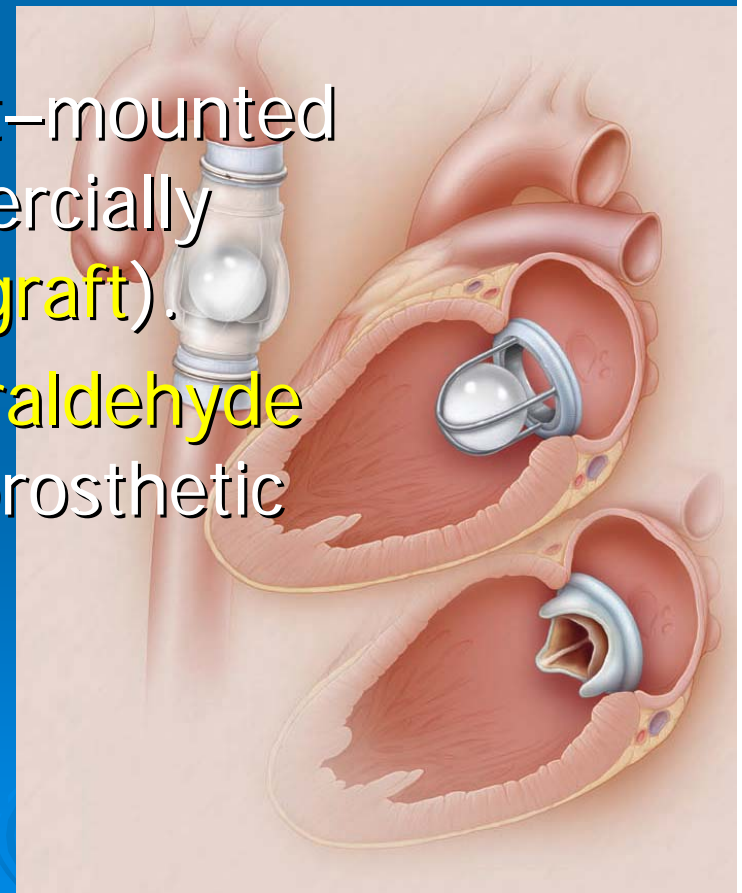


Development of biomaterial Regeneration medicine

- Based on thorough understanding of the **structure to be substituted**
- Properties depending on the **site of application** and **the function** it has to restore.
- The material should **mimic** the living tissue from **mechanical, biological, and functional** point of view.

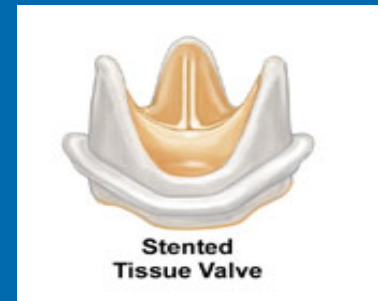
The Development of Prosthetic Heart Tissue valves

- Tissue valves have been used since the early 1960s (**homograft**)
- 1970s **chemically preserved** stent-mounted tissue bioprostheses were commercially produced and implanted (**heterograft**).
- Today, mechanical and **stentglutaraldehyde** preserved bovine pericardial bioprosthetic valves are used widely.



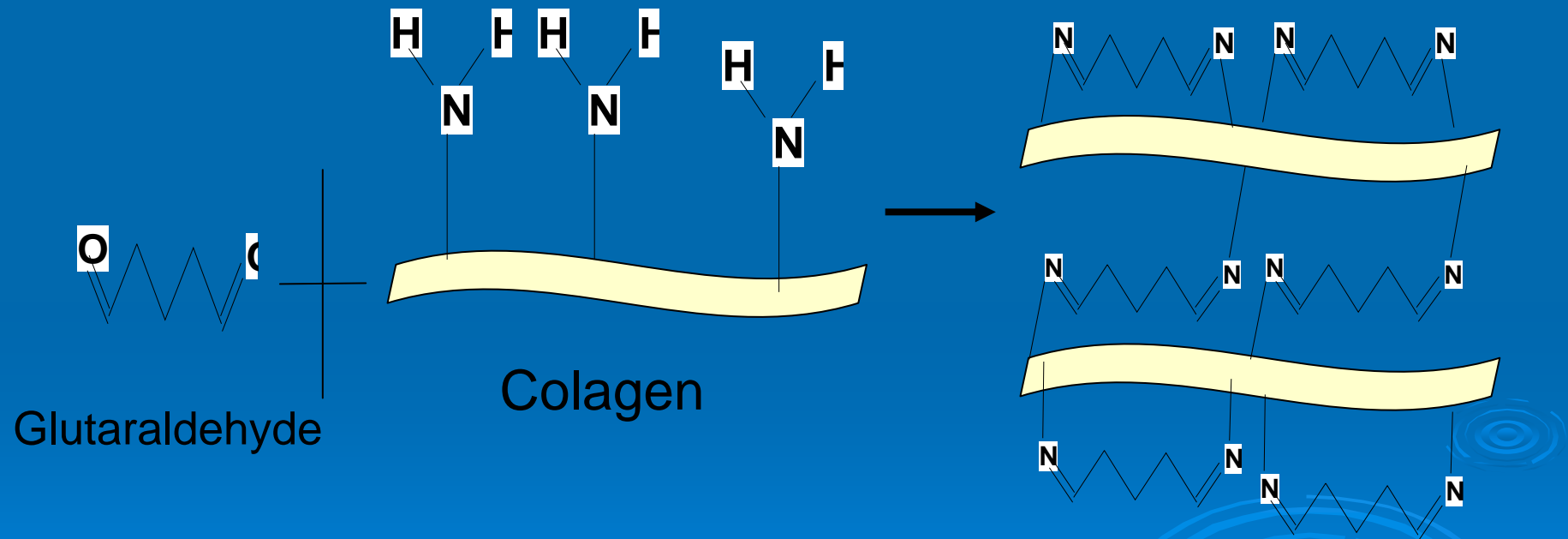
Tissue valves

- From the **social and economic point of view** their advantages are numberless:
- The raw material **costs** are minimum
- The utilization of bioprosthesis **dispense the use of anticoagulants**
- **Reduction** of the incidence of **hemorrhagic episodes**
- **Reduces** the necessity of laboratorial tests of **protrombine time**



Glutaraldehyde-treatment

Improve mechanical and immunogenic properties of bovine pericardium




Approximately 85,000 substitute valves are implanted in the United States and 275,000 worldwide each year, of which we presently estimate that approximately half are mechanical and half are tissue, suggesting a shift toward increasingly greater usage of tissue valves over the last decade.

Premises

When applied to **biological human or animal tissues**, the lyophilization process allows them to be **preserved dried**, making manipulation and storage easier. The lyophilization process also allows **gamma rays sterilization** and other ways to guarantee an effective sterilization of the material, especially to **eliminate slow viruses** when the material is destined to medical or surgical applications (prosthesis, grafts, etc).

Lyophilization is a **sophisticated technology**, demands a **multi-disciplinary approach**, combining researchers of different areas of knowledge as biological, biochemical, medical and physics.



Ronaldo N. M. Pitombo & Bronislaw Polakiewicz
Departamento de Tecnologia Bioquímico-Farmacêutica,
Faculdade de Ciências Farmacêuticas da USP, São Paulo
(SP), Brasil.

Adolfo A. Leirner & Marina J. S. Maizato
Divisão de Bioengenharia do Instituto do Coração (InCor)
Faculdade de Medicina da USP, São Paulo (SP) Brasil.

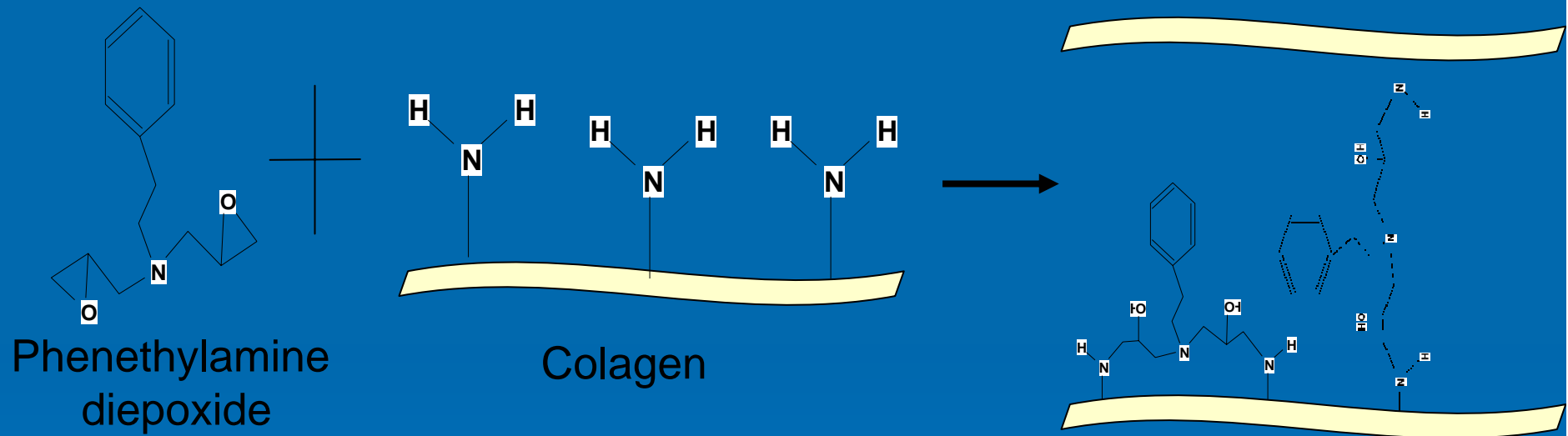
Marisa M. Beppu
Faculdade de Engenharia Química da UNICAMP, Campinas,
(SP) Brasil.

Olga Z. Higa
Instituto de Pesquisas Energéticas e Nucleares (IPEN), São
Paulo (SP), Brasil.

The recognition that glutaraldehyde promotes calcification has led to new fixation strategies that limit mineralization and optimally preserve molecular structure. Low thrombogenicity has been maintained and durability improved, but the risk of late structural failure remains.

CHAIKOF, E.L. THE DEVELOPMENT OF PROSTHETIC HEART VALVES —
LESSONS IN FORM AND FUNCTION. *New Engl. J. Med.* 357; 14
www.nejm.org october 4, 2007

➤ Phenethylamine-diepoxide treatment



OBJECTIVES

To study **the effect of lyophilization** on biological tissue from a biomaterial developing perspective:

In vitro calcification studies

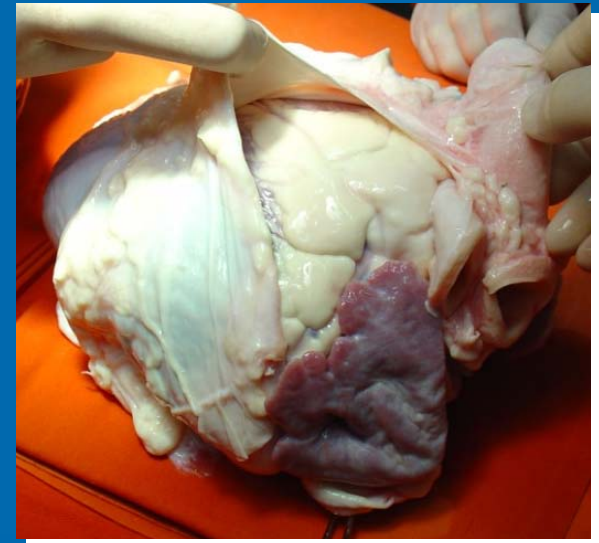


MATERIAL AND METHODS



BOVINE PERICARDIUM MEMBRANE OBTAINMENT

- collected in the slaughter houses
- cleaned
- chemically treated
- stored at - 4 ° C



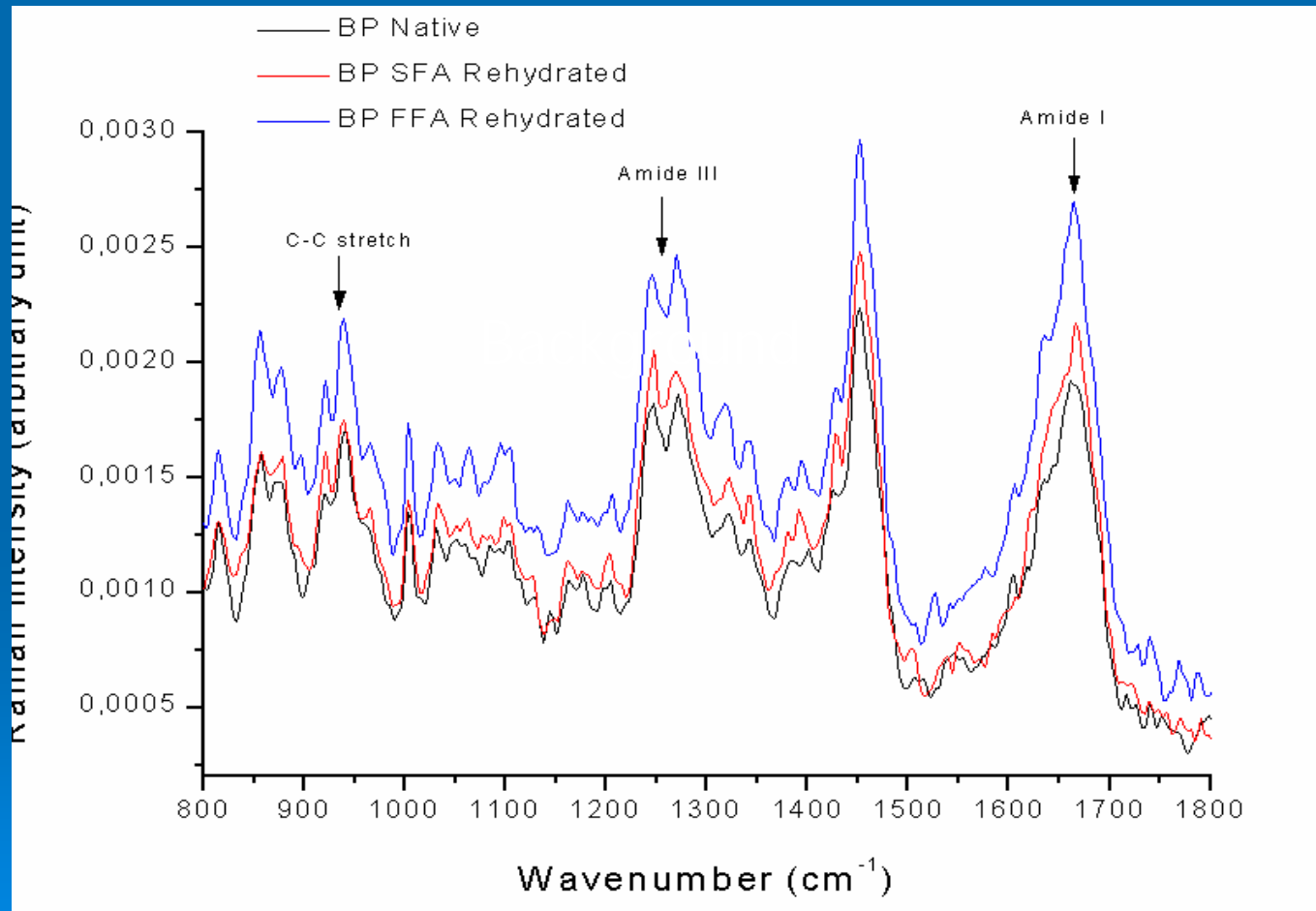
Phenethylamine-diepoxyde

Lyophilization

The bovine pericardium tissue was placed in Petri dishes, frozen at -70°C , and then lyophilized for 24 h at 140 mTorr pressure and condenser temperature at -90°C

(TDS 00209-A model, FTS Systems, Stone Ridge, NY, USA)


Secondary structure of freeze-dried bovine pericardium were determined by **FT-RAMAN spectroscopy**



Raman spectrum of rehydrated freeze-dried bovine pericardium under two different freezing protocols.

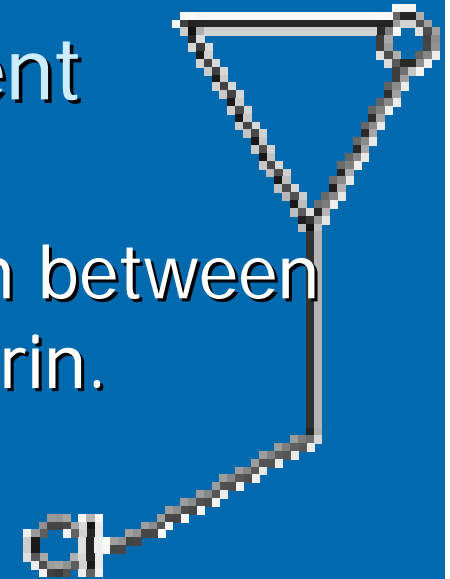
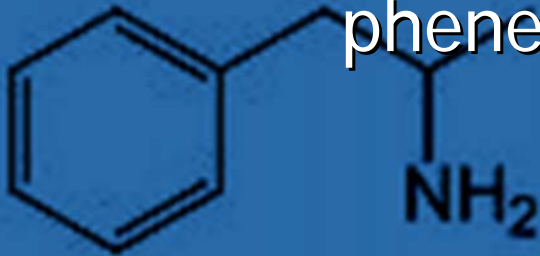
It was **not observed structural changes** on the bovine pericardium tissue after freeze-drying using different freezing protocols

Bovine pericardium tissue has enough physical and chemical support **to avoid structural changes** after freeze-drying.



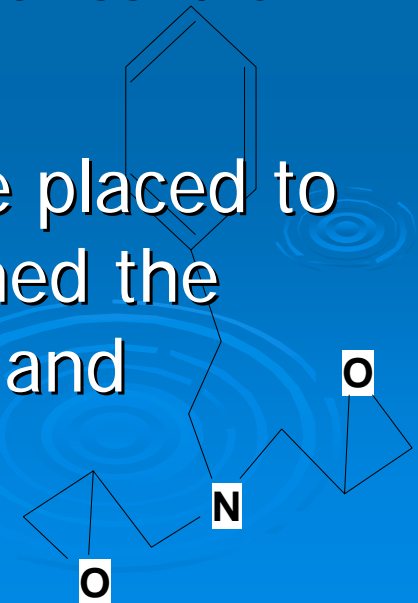
Phenethylamine-diepoxide treatment

Epoxide was derived from the reaction between phenethylamine and epichlorohydrin.



The **epoxide** was mixed to a phosphate buffer and water solution and then spread over the pericardium.

In the second procedure, the reagents were placed to react in a Petri dish, which already contained the pericardium soaked in a phosphate buffer and **acetone** solution.



Calcification test was performed by **soaking the samples in simulated body fluid (SBF)**

The substrates (2 x 1 cm bovine pericardium membrane pieces) were soaked in the 1x SBF solution for 7 days, changing the solutions for fresh ones in intervals of 2 days.

Similar procedure using an SBF solution with 50% higher salt concentration (1.5x SBF) was used **to accelerate the calcification process.**

The experiments were carried out in polyethylene flasks at 36.5 °C (using a Shaking Water Bath-Line Orbit [Lab-Line Instruments Inc., Melrose Park, IL, USA] at 50 rpm agitation).

The influence of different chemical treatments on bovine pericardium calcification

Scanning electron microscopy (SEM) was used to analyze the morphology of bovine pericardium samples.

Energy Dispersive Spectroscopy (EDS) was used to evaluate the characteristics of calcium phosphate



RESULTS AND DISCUSSION



The chemically treated pericardium samples were:

- **Lyophilized** phenethylamine-diepoxyde
- **Nonlyophilized** phenethylamine-diepoxyde
- **Lyophilized** phenethylamine-diepoxyde-acetone
- **Nonlyophilized** phenethylamine-diepoxyde-acetone

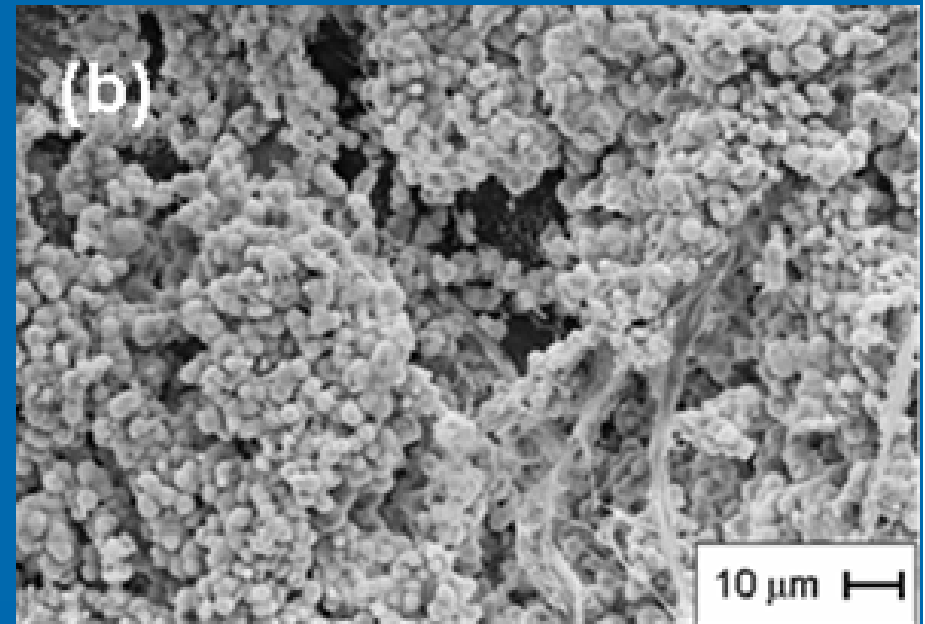
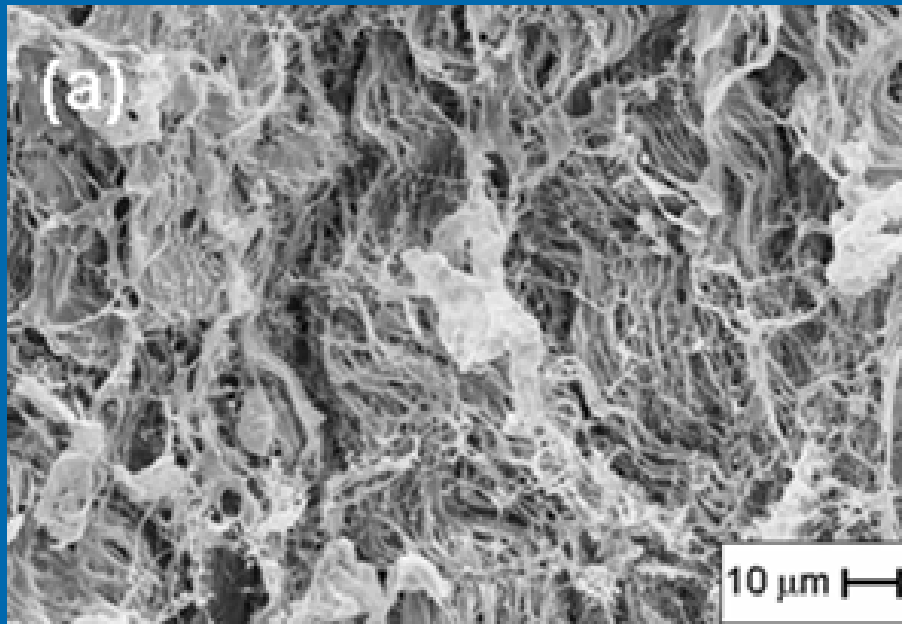
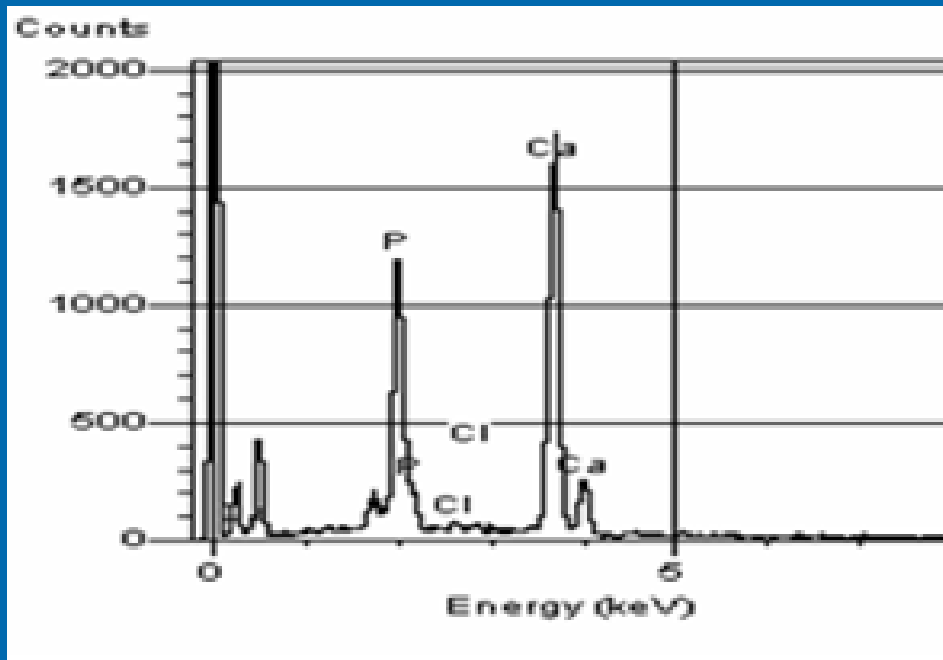
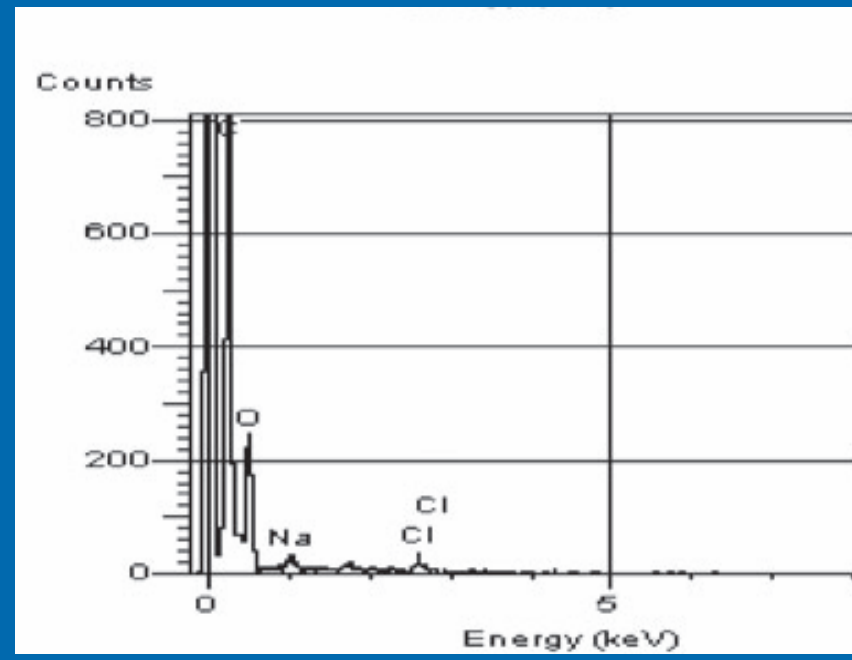


Figure 1. SEM micrographs of **nonlyophilized** bovine pericardium treated with phenethylamine-diepoxide after calcification test in (a) SBF-1x and (b) SBF-1.5x



(a)



(b)

Figure 2. (a) EDS spectrum for **nonlyophilized** bovine pericardium treated with phenethylamine-diepoide after calcification test in 1.5 x SBF.

(b) Typical EDS spectrum for noncalcified samples after contact with SBF.

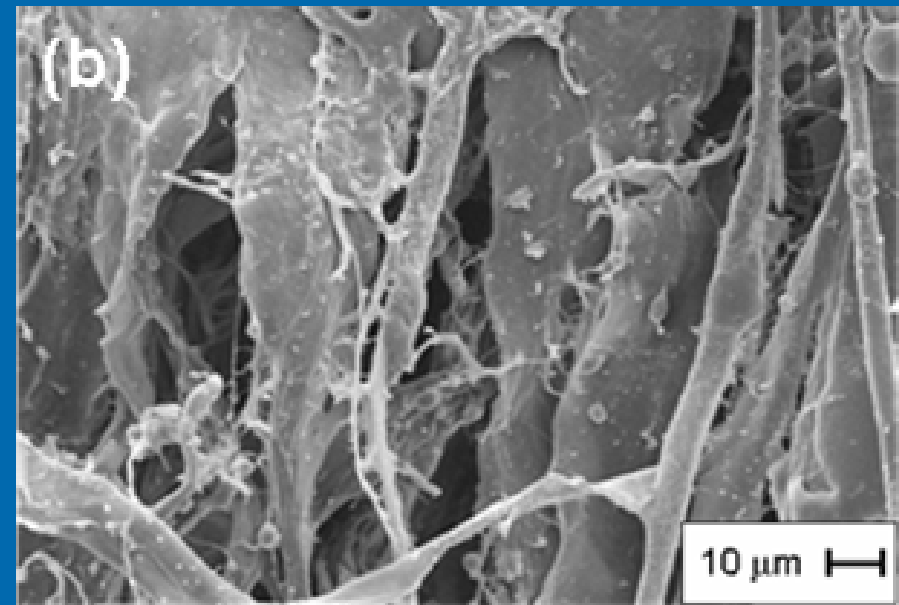
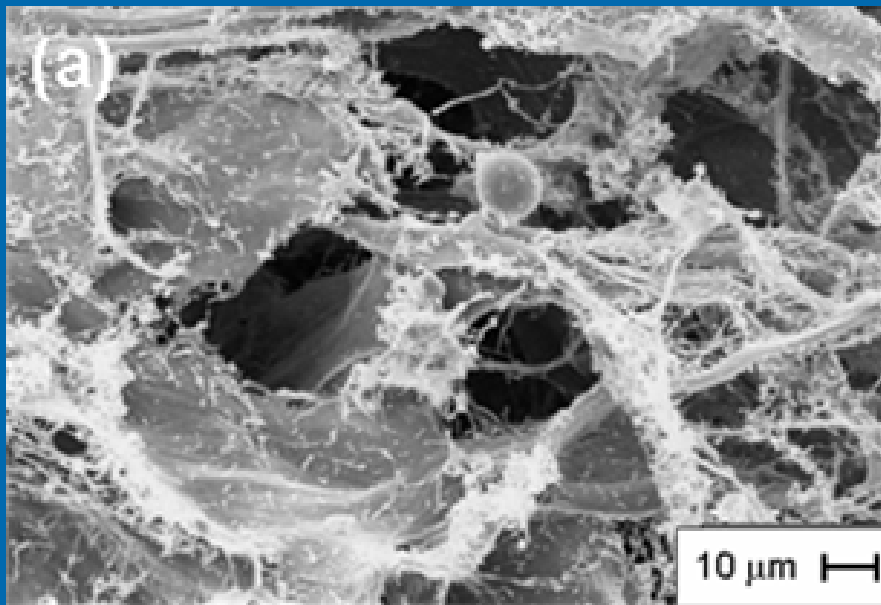


Figure 3. SEM micrographs of **lyophilized** bovine pericardium treated with phenethylamine-diepoxide after calcification test in (a) SBF-1x and (b) SBF-1.5x

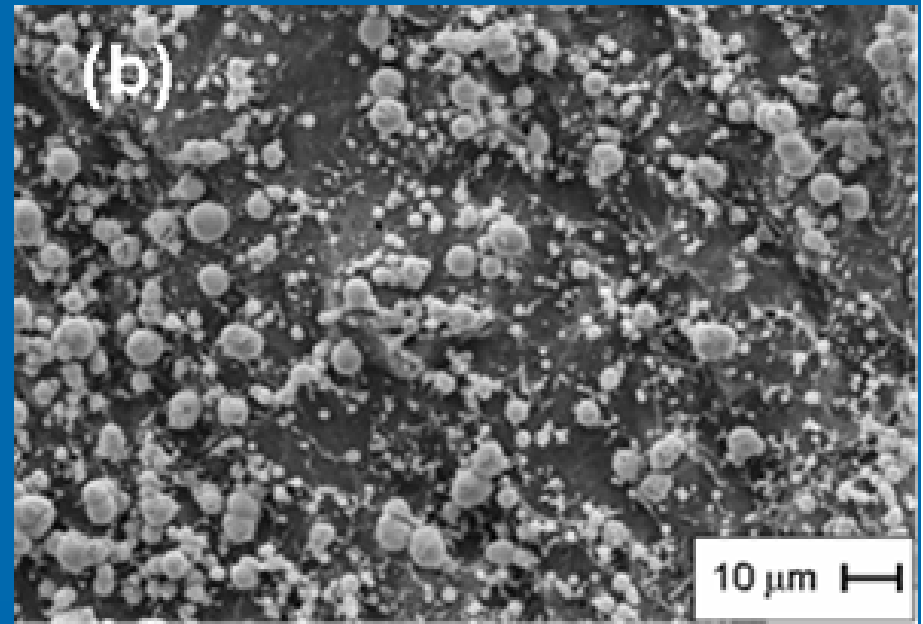
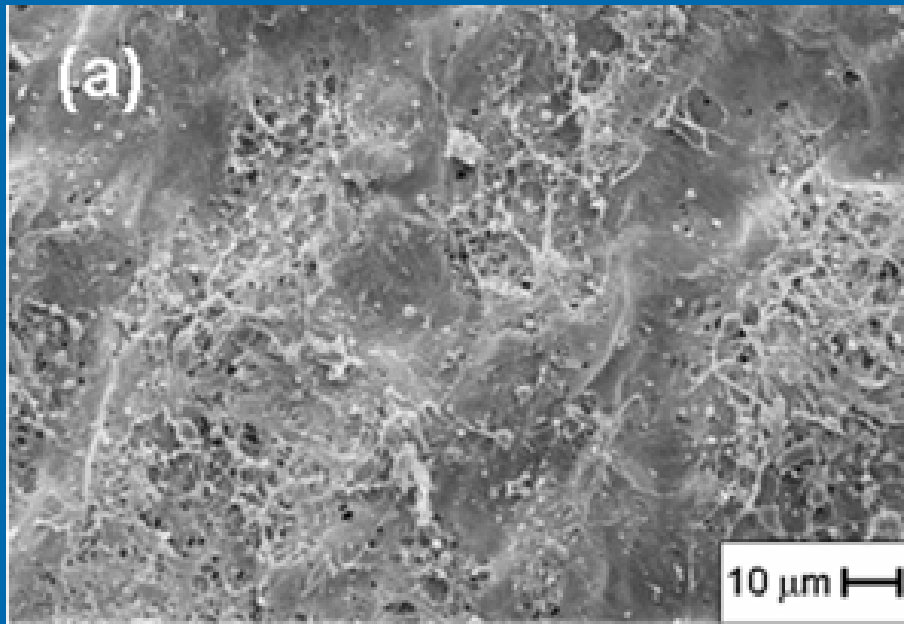


Figure 4 SEM micrographs of **nonlyophilized** bovine pericardium treated with phenethylamine-diepoxyde-acetone after calcification test in (a) 1x SBF and (b) 1.5x SBF.

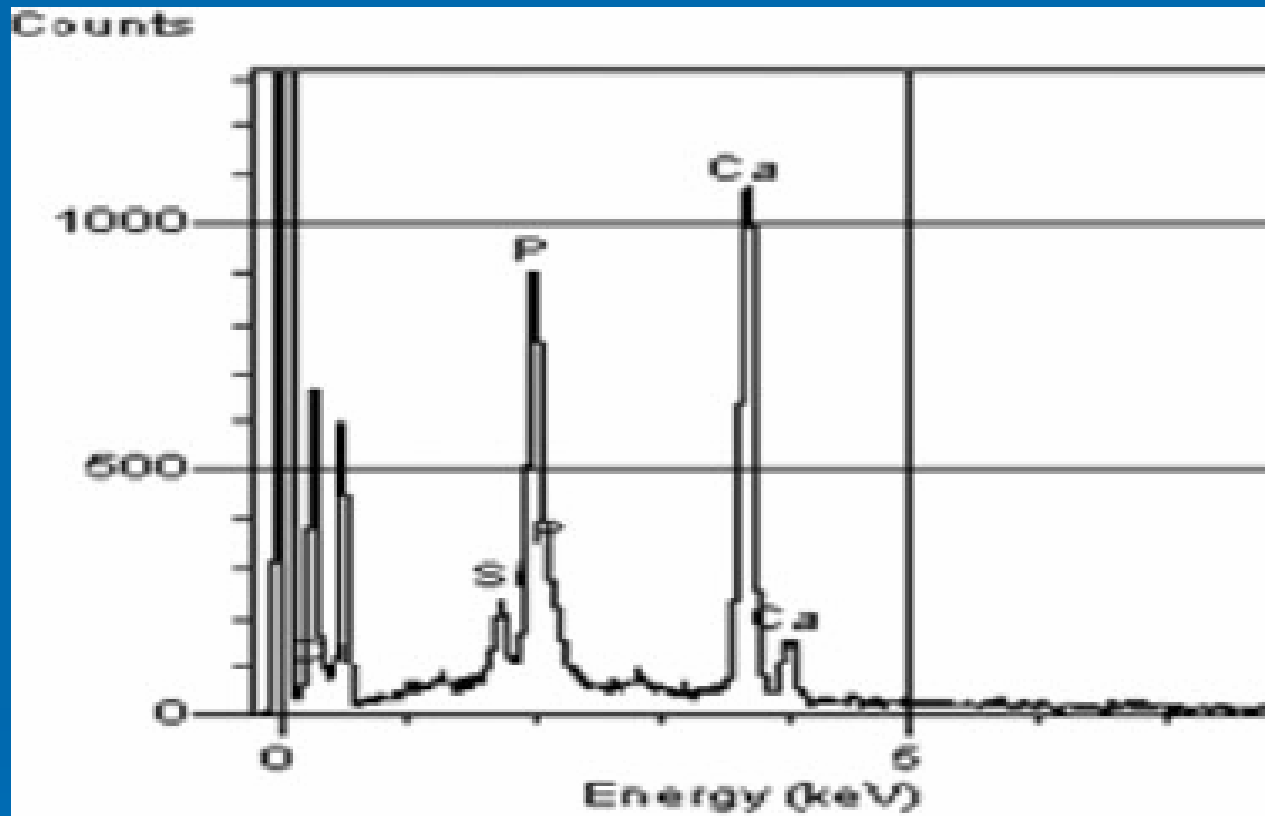


Figure 5. EDS spectrum for **nonlyophilized** bovine pericardium treated with phenethylamine-diepoide-acetone after calcification test in 1.5x SBF.

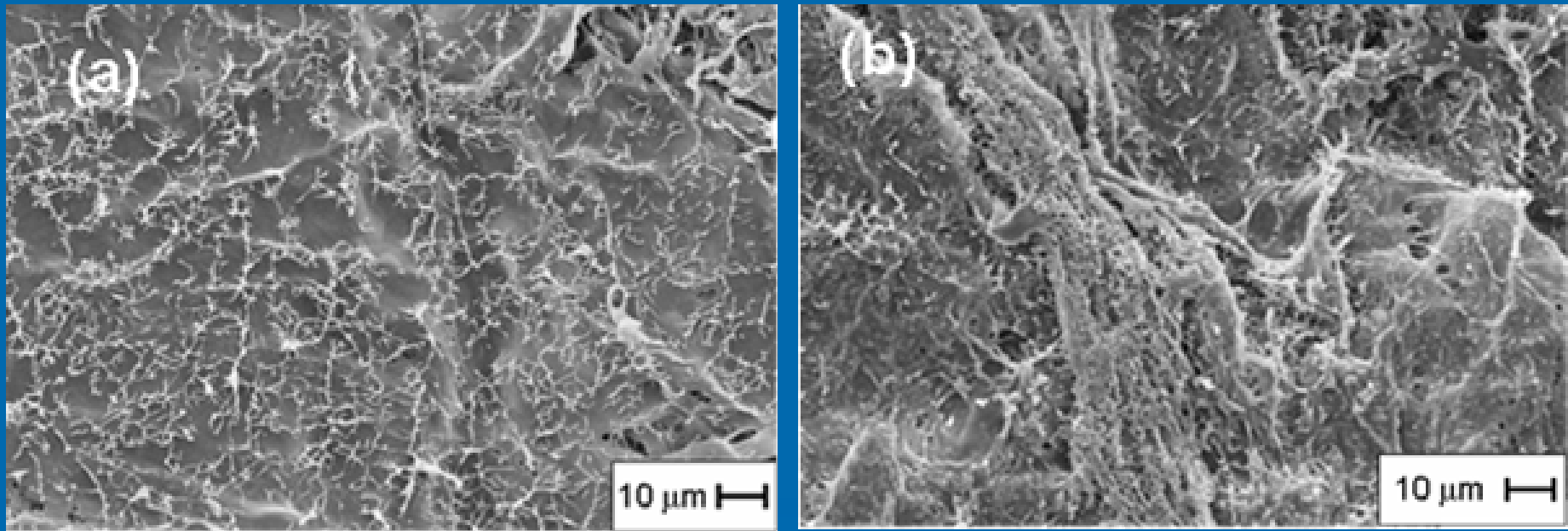
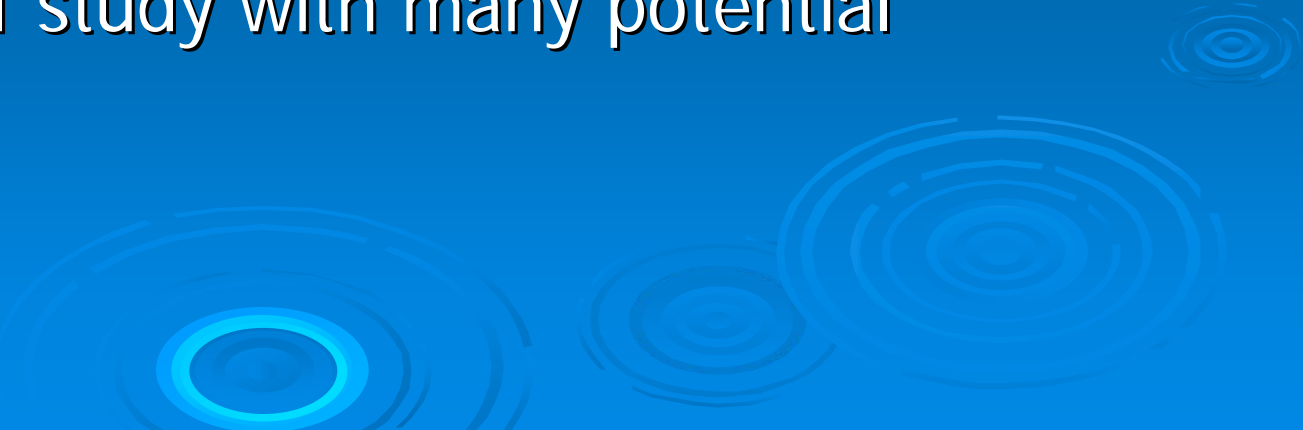


Figure 6. SEM micrographs of **lyophilized** bovine pericardium treated with phenethylamine-diepoxyde-acetone after calcification test in (a) 1x SBF and (b) 1.5x SBF

- **Lyophilization** associated with the epoxide treatment is a potential route of bovine pericardium bioprosthetic heart valves preparation.
 - This hypothesis is sustained by the fact that nonlyophilized samples showed calcium phosphate deposits, in contrast with the lyophilized ones.
 - This is a field of study with many potential applications.
- 


CONCLUSIONS

The presented treatment using the two different phenethylamine-diepoxy solutions showed no effects on calcification experiments as showed by the tests.

The lyophilized bovine pericardium samples, treated with both solutions, did not show any detectable phosphate deposits.

The lyophilization of bovine pericardium before chemical treatments with cross-link agents such as epoxy compounds may be an alternative to the conventional calcification prevention methods, but further investigations are recommended to check if the same behavior is found in all lyophilized systems.

CONCLUSIONS

- These results can be specific to this system and the chemical treatments that were used.
 - Further studies applying larger number of replicates and chemical routes should be performed to check if the effect of lyophilization would be the same in all systems.
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Thank you