Radiosterilization of freeze dried biological products

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Sterilization

Sterilization is a kill, inactivation or removal process of microorganisms from a product or surface with the employ of physical or chemical agents.

Brock and Madigan, 1991

But the radiosterilization can not:

1. Remove other physic or chemical contaminants.
2. Remove killed or inactivated microorganisms or their toxins.

United States Patent 5817528

“Sterile and pyrogen-free columns containing coupled protein for binding and removal of substances from blood”

Sterilization of health care products- Radiation

ANSI/AAMI/ISO 2006

Reference number 111137

Part 1 Requirements for development, validation and routine control of a sterilization process for medical devices

Part 2 Establishing the sterilization dose

Part 3 Guidance on dosimetric aspects

Radiosterilization

- Applicable for products that can not be filtered
- Multipurpose Installations, easy to use
- Fast process that not requires quarantine storage
- Viable economically for high and low volumes
- Easy to repeat, ones the dose has been established
- Let the sterilization on the final container. The seal systems do not stressed
- Easy to control and validate, the only parameter to control is the absorbed dose

The sterilization marketing grown from 5% up to 50 %)

Requirements to apply the radiosterilization

- The radiation induces changes on the organoleptical and pharmacological product properties.
- Differentiation between irradiated and non-irradiated product.
- Assessment of product changes by specific techniques to detect radiolitical residues.
- The doses below 25 kGy requires radiosensitivity studies of the product and contaminant microorganisms.

Advantages

Aseptical conditions

Installation Specifications
Process controls
Adequate Materials
Low microbiological charge

Freeze dried

Low residual moisture
Low oxygen concentration
Freeze dryer Design –continuous cycles
Product stability
Additional handless

Radiosterilization

Approved by regulatory agency
It has been applied to medical devices
Validable, few parameters to control
Applicable to thermolabile products
Terminal sterilization (final package)
SAL: 10^{-6}

General purpose

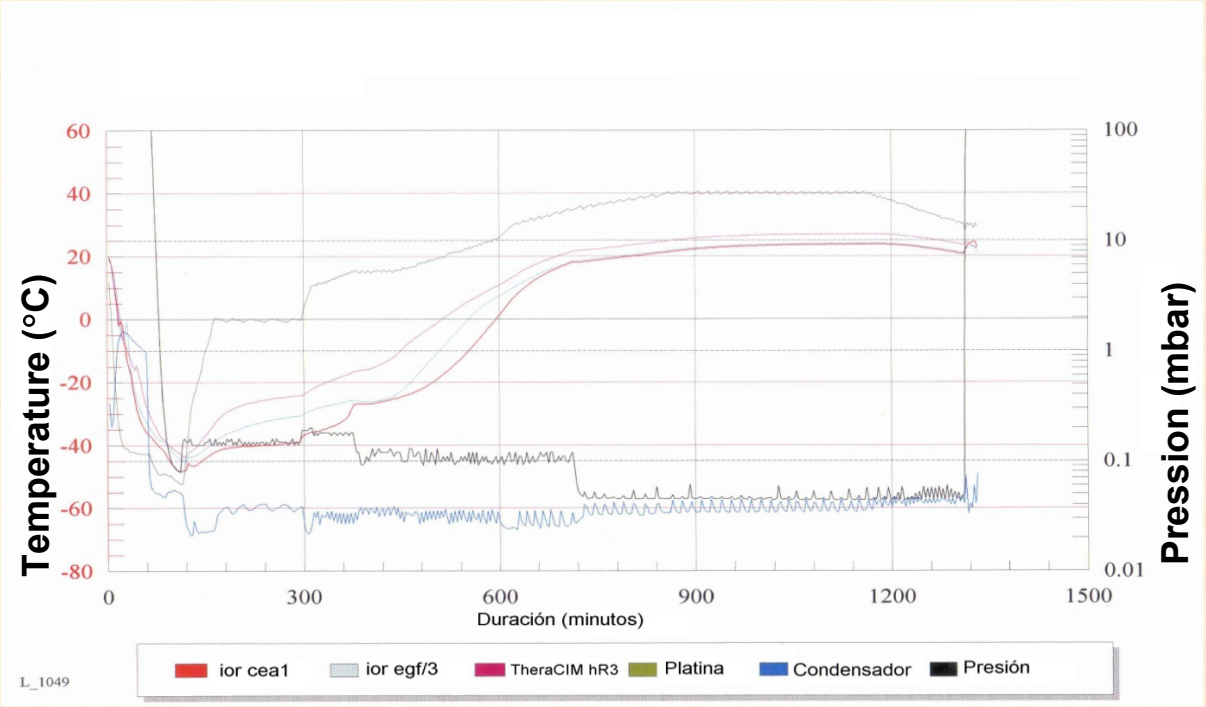
Obtain an adequate lyophilization cycle and select the minimal dose that let the sterilization of microbiological contaminants without change the pharmacological product specifications.

Experimental studies

- **Establish the lyophilization cycle**
  - Differential Thermal Analysis (DTA)
  - Differential Scanning Calorimetry (DSC)
  - Electrical Impedance (**Z)**
  - Residual Moisture
- **Assessment of product radiosensitivity**
  - Irradiated and no irradiated product differences
  - Specific techniques to detect radiolitical residues
  - Assessment of product properties (potency, purity, concentration,
- **Assessment of radio resistance of bioburden**
  - Environment conditions
  - Lyophilization
  - Radiation below 25 kGy

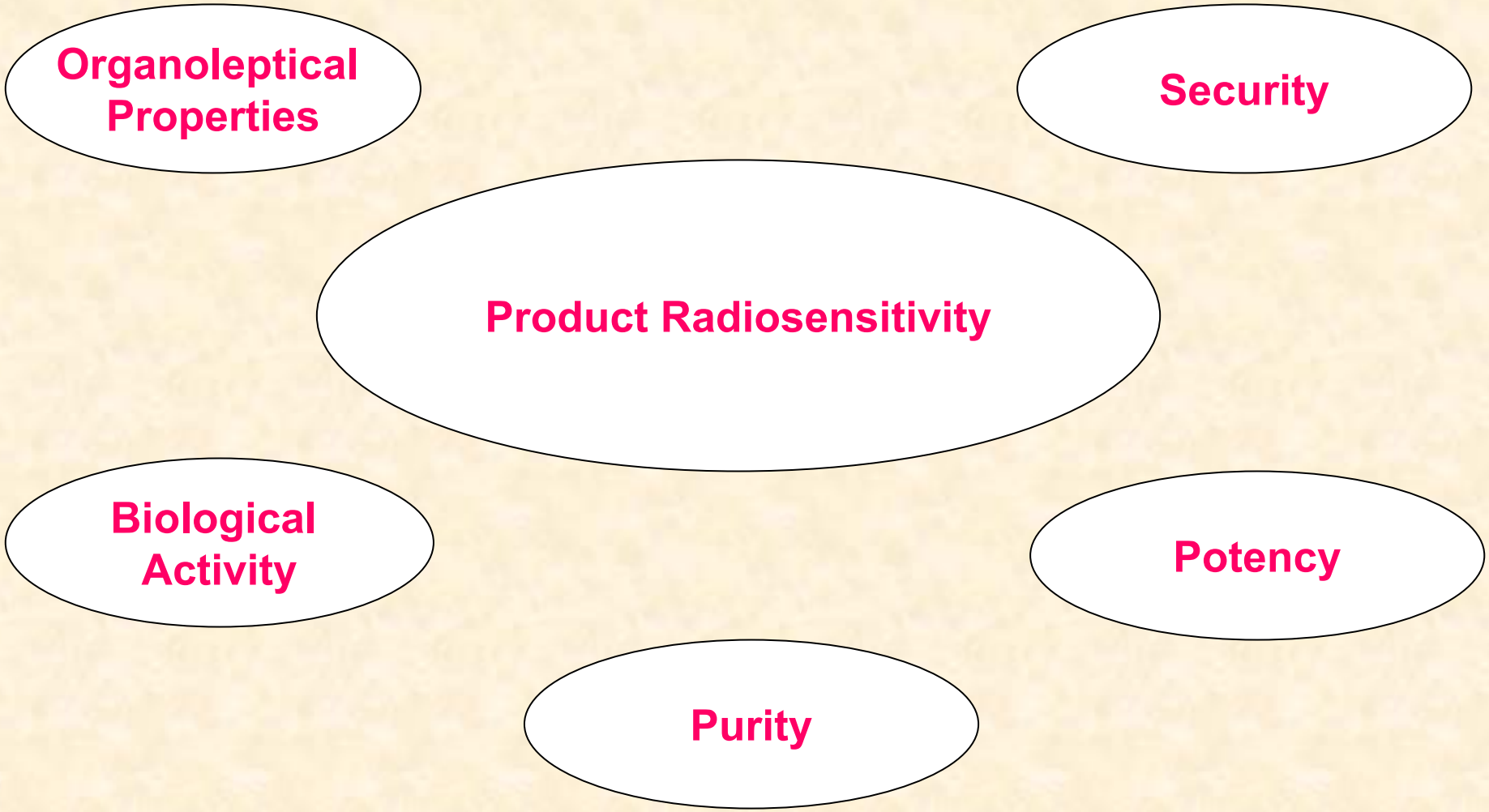
Lyophilization cycle

Analysis of product behavior at low and high temperatures (DTA/Z, DSC)



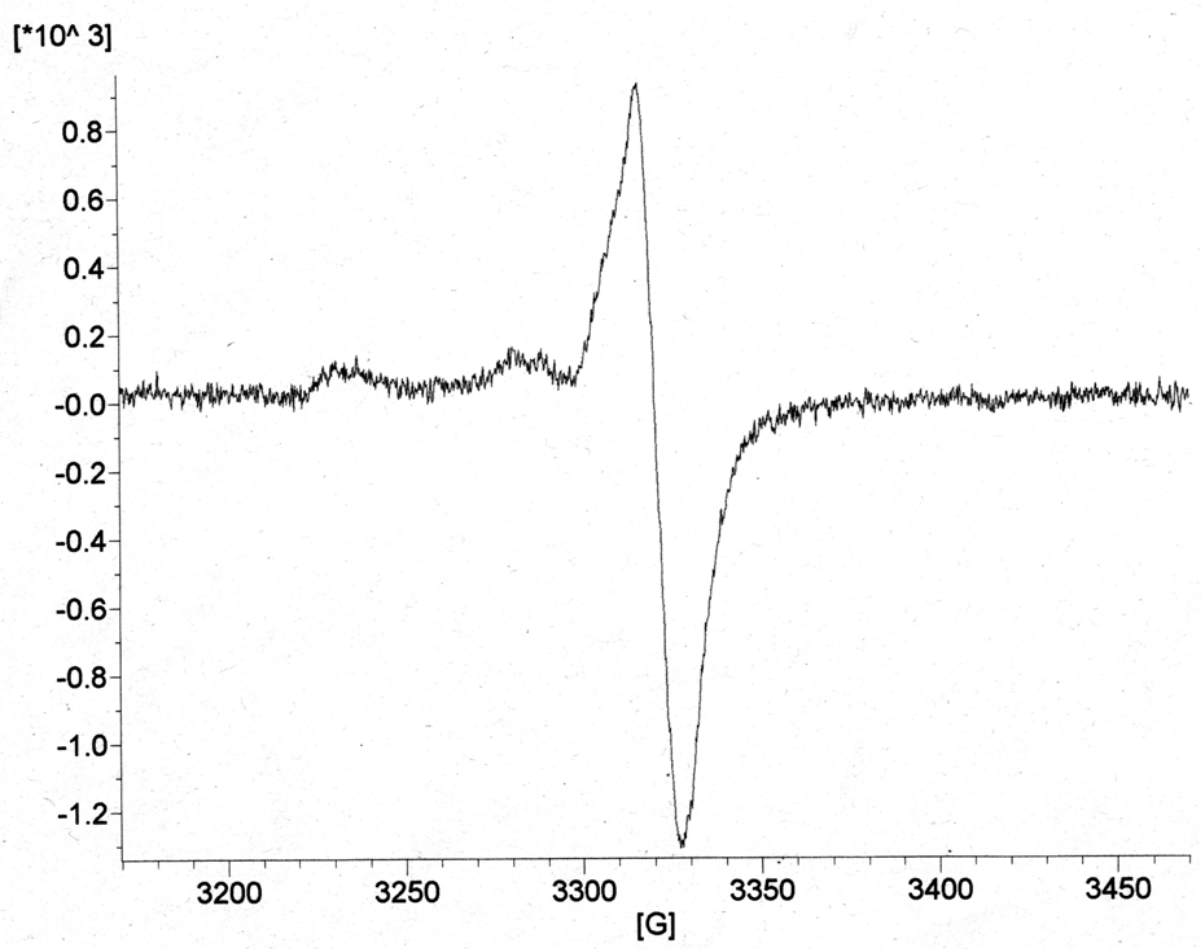
Low Residual Moisture, Shelf Homogeneity

Product Radiosensitivity



Organoleptical Properties

Other product properties
Electronparamagnetic Resonance



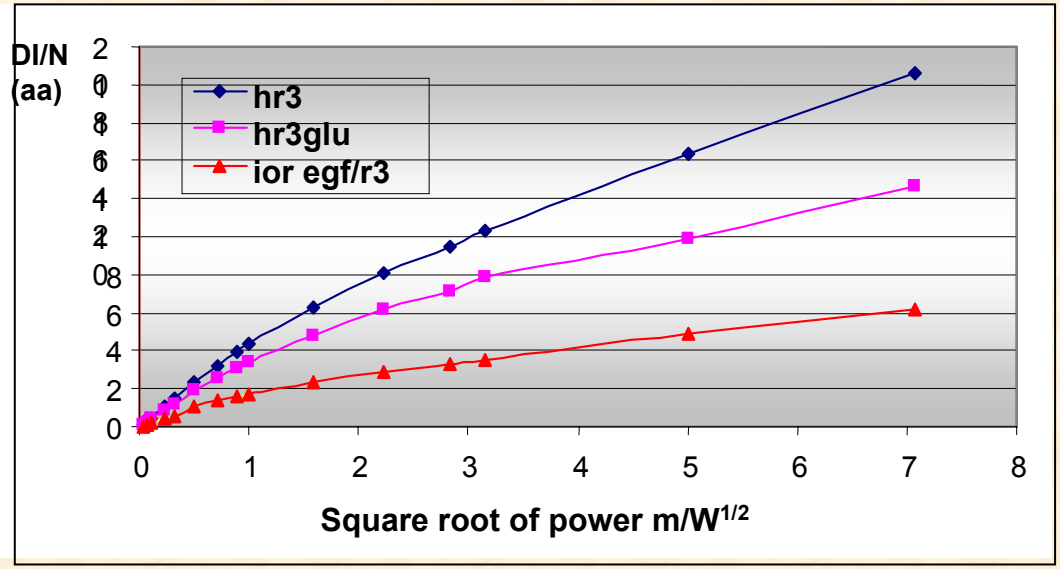
Reference
2,2 difenil-1-picrilidacil
(DPPH)
g= 2.0036

g-factor- a constant that depend of radical electronic configuration

$$g = h\,\nu / \beta e B$$

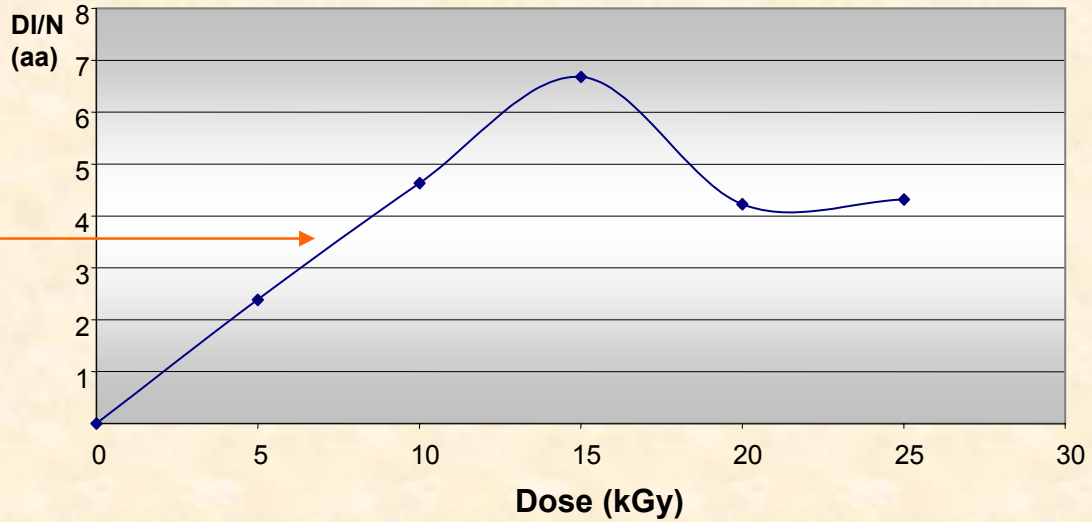
h: const. de Plank
v : frecuency
Be: Böhr's const.
B: magnetic field

Other product properties
Electronparamagnetic Resonance

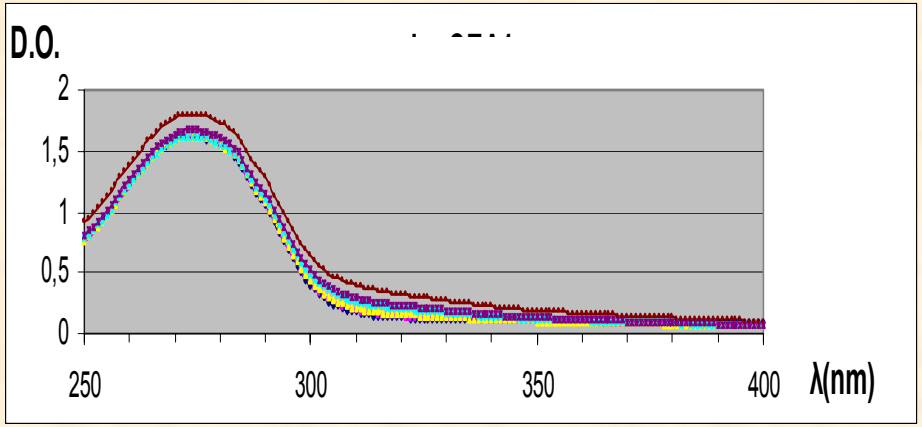


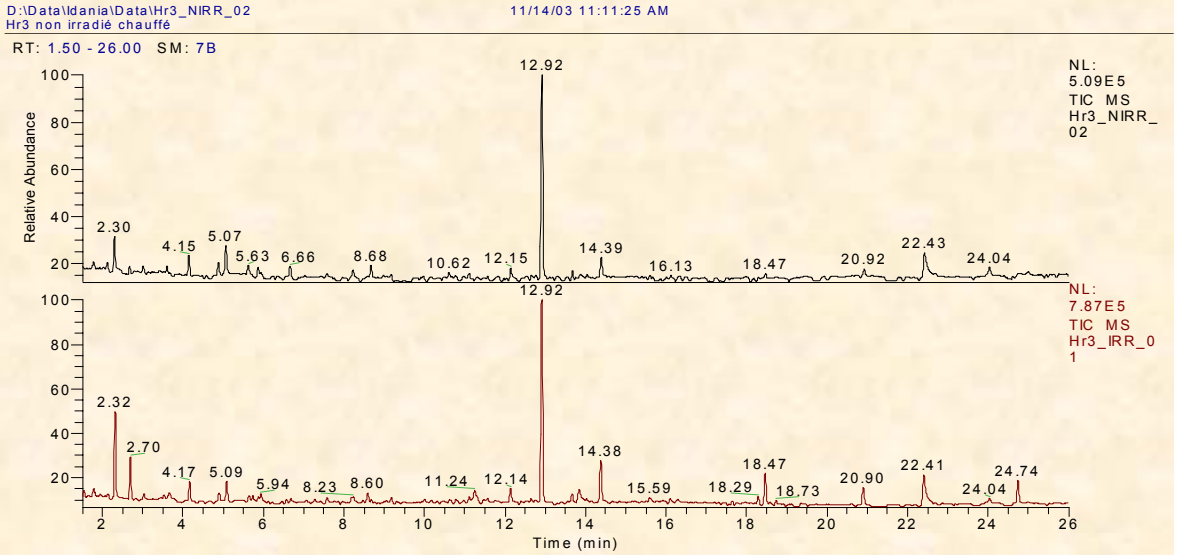
Signal area(DI/N):
Double integration of the derivate spectral curves

Useful in post-dosimetry

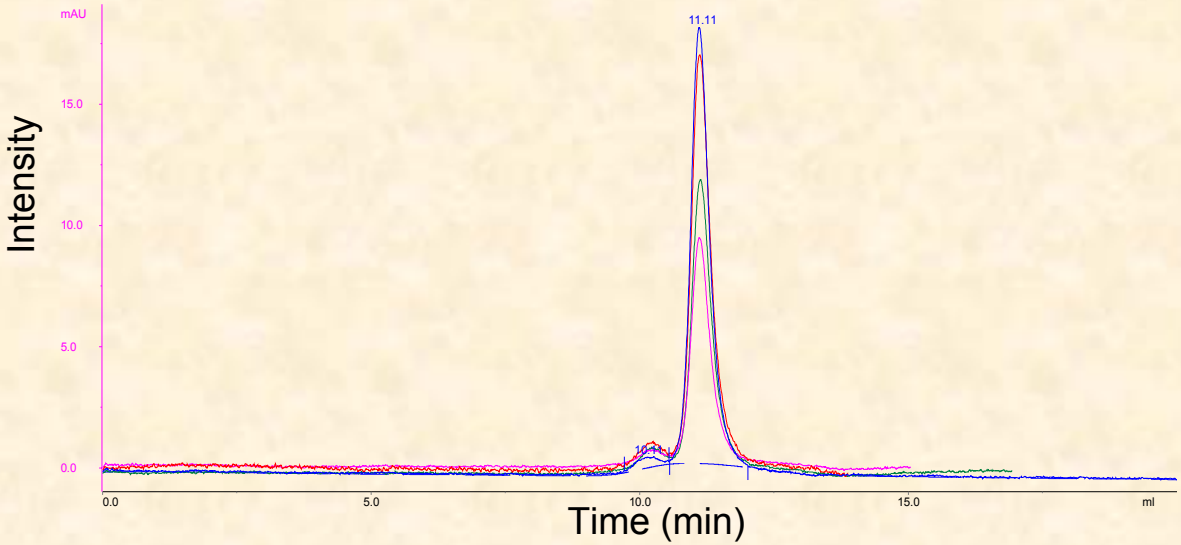


Other product properties
Luminescence, UV Scanning Spectroscopy and Gas Chromatography



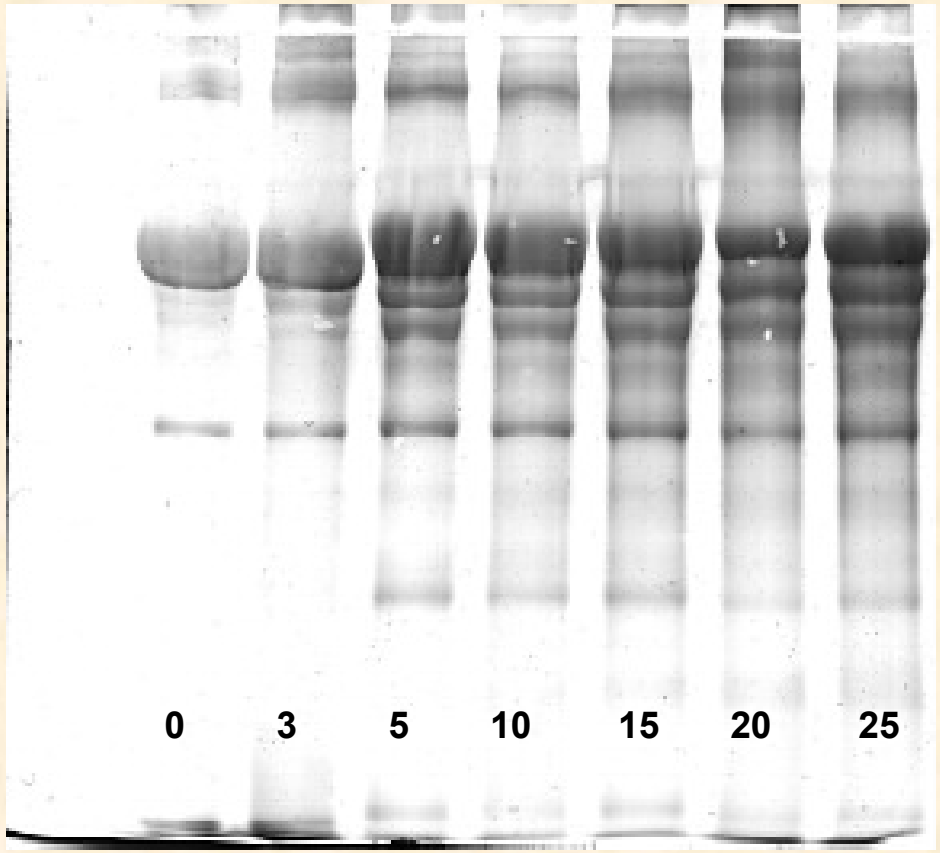


Other product properties
HPLC



Dosis (kGy)	Purity (%)		
	Without Additive	Glucose	Trehalose
0	97.3 ± 0.2	98.3 ± 0.1	98.7 ± 0.5
3	96.2 ± 0.0	97.9 ± 0.3	97.8 ± 0.4
5	95.7 ± 0.3	97.2 ± 0.3	96.4 ± 0.3
10	95.0 ± 0.2	96.8 ± 0.1	95.8 ± 0.3

Other product properties
Electrophoresis

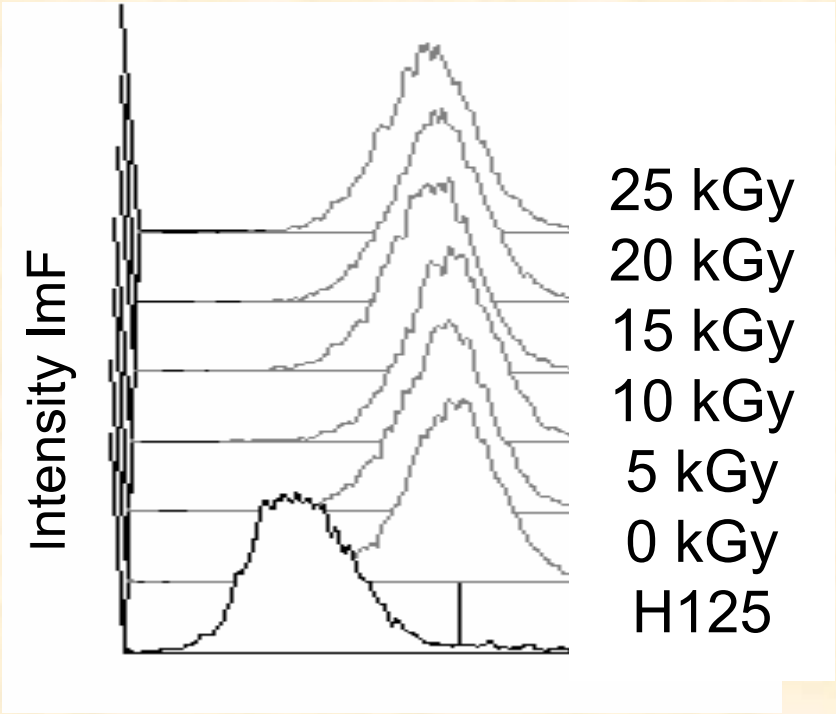


(SDS-PAGE) 12.5%

20µg total protein/
Comassie Blue (1 h)

Buffer
0.25M de Tris,
1.92M Glysine,
1% w/v SDS, pH 8.3

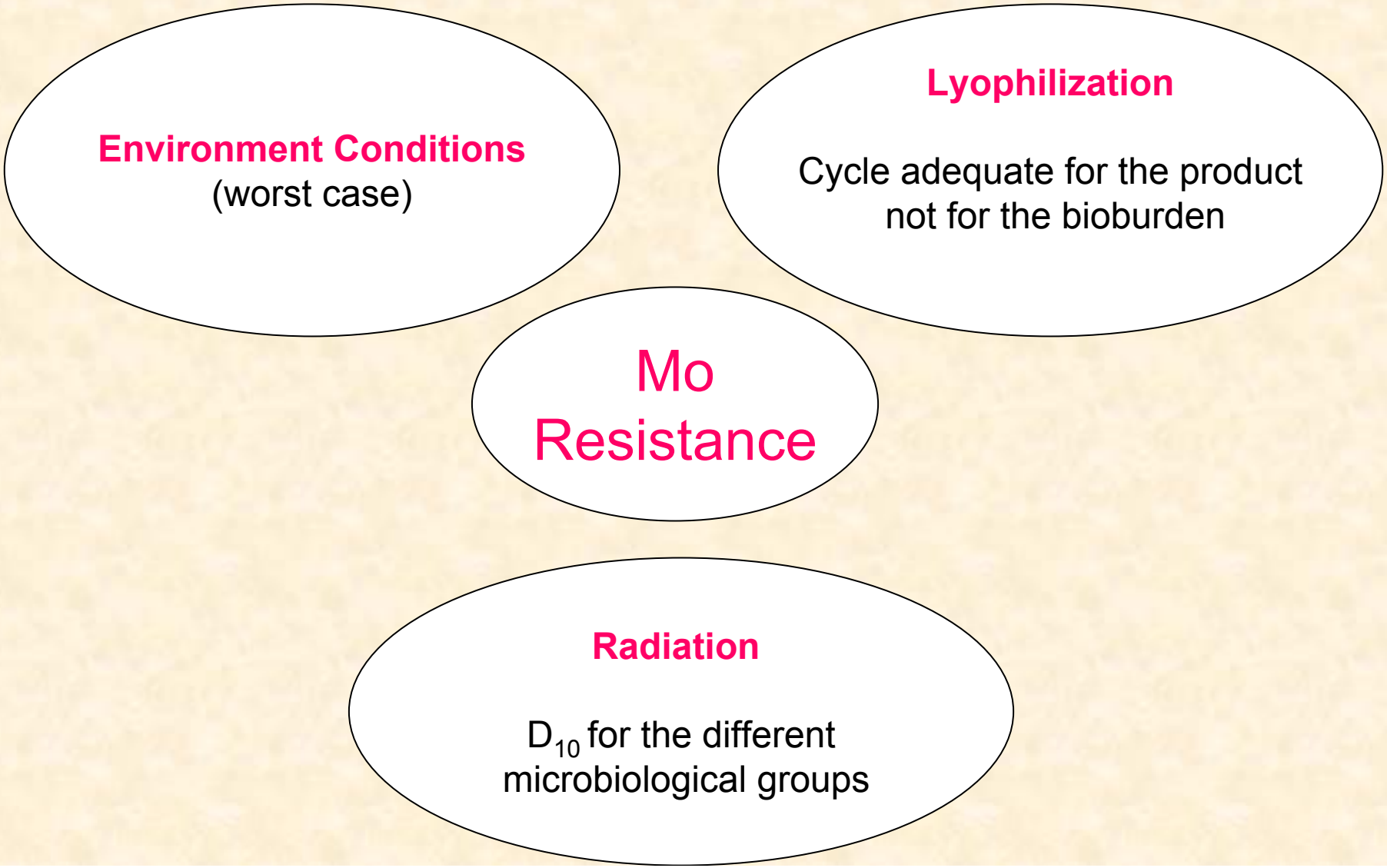
Other product properties
Flow Cytometry



H125	
Células	3.78
0 kGy	9.80
5 kGy	9.13
10 kGy	9.41
15 kGy	8.27
20 kGy	8.50
25 kGy	8.01

2 x 10^5 cells, tumoral line H125, Incubation 30 min, 4ºC
Product Concentration 10 µg, 5000 cells/dose

Microorganism Resistance



Resistance to environment conditions

For bacteria contamination

3 Batches- Isolated microorganisms from critical areas

Season
Charge
Specie

Staphylococcus spp, like Staphylococcus epidermidis
Micrococcus spp
Bacillus spp

Resistance to Lyophilization

Bacterial Group		Viable account (cfu/mL)					
		Lyophilization (10^7 cfu/mL)			Lyophilization (10^2cfu/mL)		
		Without additives	Glucose	Trehalose	Without additives	Glucose	Trehalose
C1	Coccus G +	10^5	10^7	10^7	0	10	10
C2	Coccus G +	10	10^4	10^4	0	1	5
C3	Bac. G+ spores	10^6	10^6	10^7	10^2	10^2	10^2
C4	Bac. G+ spores	10^6	10^6	10^6	10^2	10^2	10^2
C5	Coccus G –	10^2	10^4	10^4	0	6	1
C6	Coccus G –	10	10^3	10^3	0	0	0
C7	Bacilos G –	0	10	10	0	0	0
C8	Bacilos G –	10	10^3	10^4	0	1	1

Radiosensitivity of different bacterial groups

ED = D_{10} (log CM + log SAL), *(Kairiyama, 2003)*

MC = antilog[(ED/D_{10}) – logSAL]

Bacterial Group		D_{10} (kGy)	Charge (Maxima) (cfu/mL)
C1	**Coccus Gram +**	**0.38**	**2.1×10^{20}**
C2	**Coccus Gram +**	**0.22**	**2.84×10^{39}**
C3	**Bacillus Gram + spores**	**3.30**	**0.001**
C4	**Bacillus Gram + spores**	**2.74**	**0.004**
C5	**Coccus Gram –**	**0.48**	**6.8×10^{14}**

ED= 17 kGy Bacillus Gram + spores

ED= Sterilization dose

SAL= Sterility Assurance Level

MC= Microbiological charge

Radiosterilization of freeze dried antibodies produced in aseptic conditions

Doses (kGy)	Class	Requirement
10	Class A	Complain of laminar flow manufacturing specifications (less than 1 cfu)
17	Class B	Establish new specifications for radiolitical byproducts and perform pharmacological studies for the byproducts
25	Class B SAL 10^{-12} Class C	Establish new specifications for radiolitical byproducts and perform pharmacological studies for the byproducts

Conclusions

No significant loss of the active compound was detected in freeze dried antibodies, which confirms their radioresistant property. Moreover, no change in color or smell was observed.

The freeze dried products which have low water and oxygen contain and have high purity were identified as potential candidates for radiosterilization.