17th International Symposium and Summer School on Bioanalysis

ASSESSMENT OF CHANGES IN FREEZE-DRIED PROTEIN PHARMACEUTICALS

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- Growing number of potential peptide and protein drugs valuable in treatment of various diseases

- Freeze drying is often employed in formulation of protein pharmaceuticals

Challenges in formulation of protein pharmaceuticals

- Careful selection of containers (glass and plastic surfaces adsorb proteins and peptides)
- Careful selection of excipients
  - High risk of aggregation (can be prevented using various substances)
  - Potential loss of activity
- Need of sterility for parenterals
- Stability and shelf-life

Experience

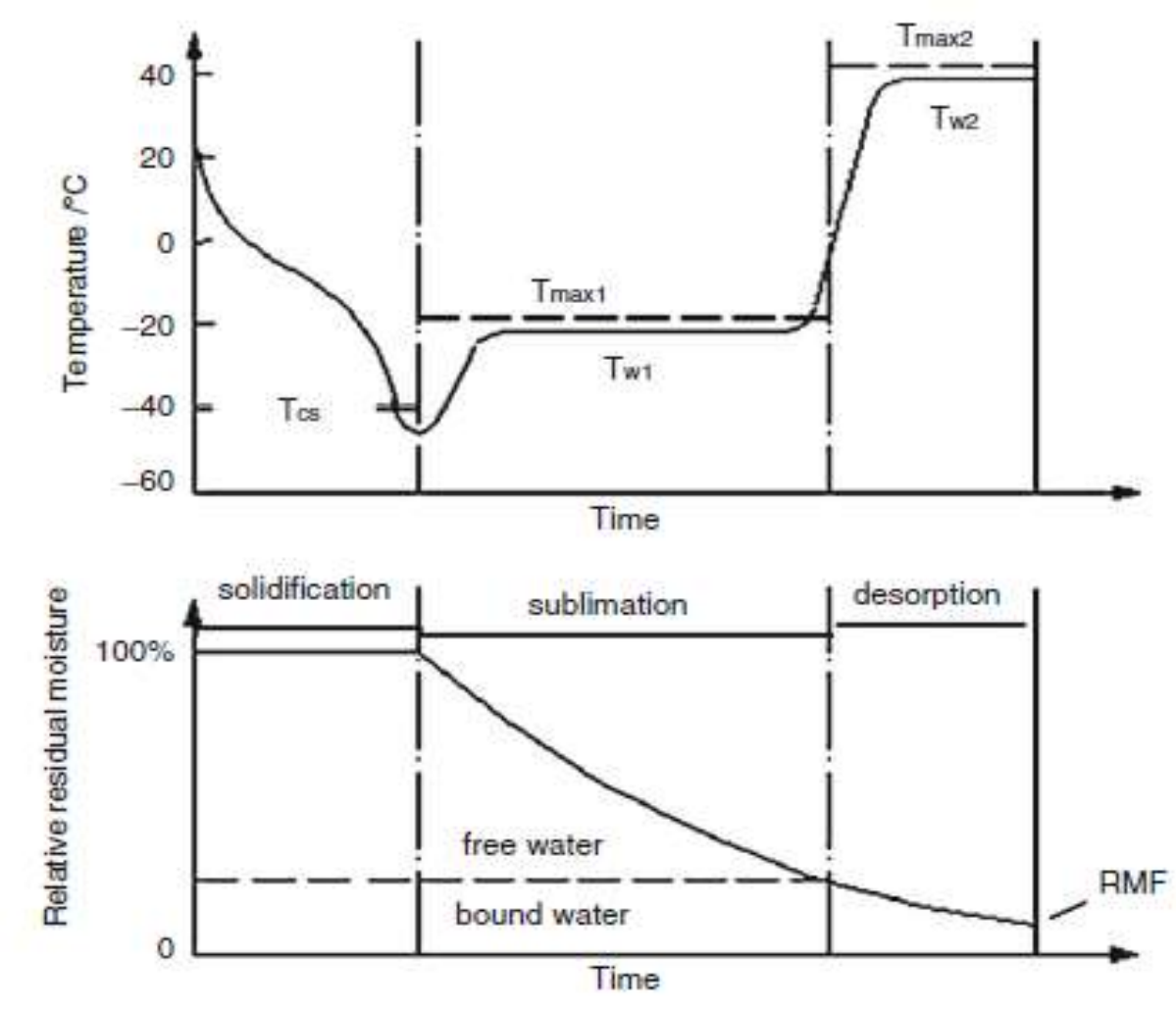
- Formulation of freeze-dried, conjugated mAb kit, ready-to-label with radioisotopes (Lu-177)

Freeze-drying

1. • Freezing
2. • Primary drying
3. • Secondary drying

http://www.zlpharmapkg.com/pharmaceutical-vials/34516554.html

Freeze-drying



Baolin, L. & Xinli, Z. (2015). Cryopreservation and Freeze-Drying Protocols, Third Edition. Freeze-Drying of Proteins, 23, 459-476.

- Many tests are required for different types of assessment of protein formulations

identity, purity, potency and stability of formulation

➢ Proteins can be assayed both in vitro and in vivo ...

Thermal analysis

- Differential scanning calorimetry (DSC) transitions of confirmation as a function of temperature
- Very helpful in designing a suitable freeze-drying cycle,

- designing the step of freezing

- effect of added excipients

- The apex of the endothermic peak is the transition temperature between native and partially unfolded conformations.

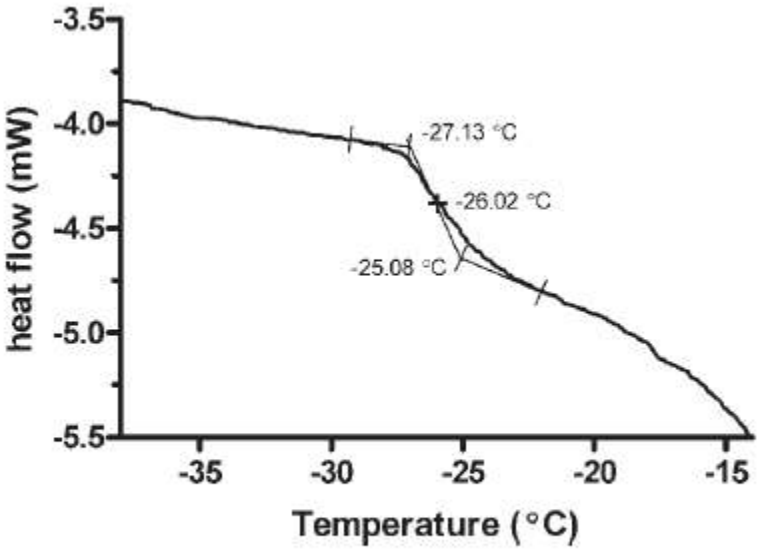


Figure 1. T_g' measurement by DSC of mAB solution at 80 mg/mL.

Journal of Pharmaceutical Sciences
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Lyophilization cycle development for a high-concentration monoclonal antibody formulation lacking a crystalline bulking agent

James D. Colandene ✉, Linda M. Maldonado, Alma T. Creagh, John S. Vrettos, Kenneth G. Goad, Thomas M. Spitznagel

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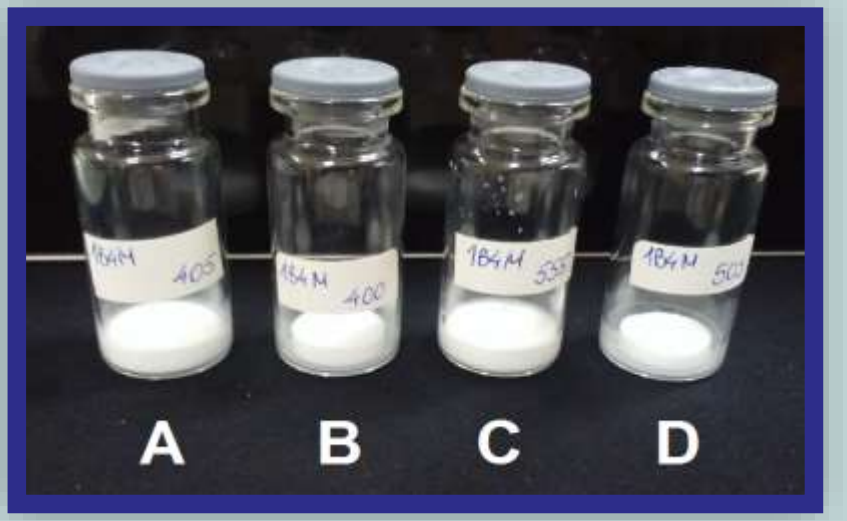
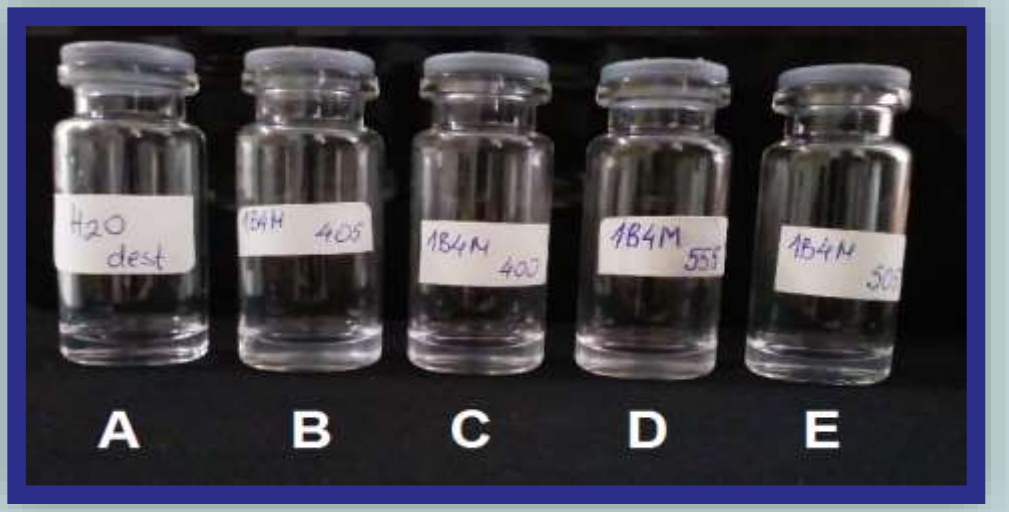
Table 2. DSC and Freeze-Dry Microscopy Results for mAB Formulated at Different Protein Concentrations in Formulation Buffer

Concentration (mg/mL)	DSC	Freeze-Dry Microscopy	
	$T_g' \pm$SD (°C)	$T_{c,on}$ (°C)	$T_{c,com}$ (°C)
0	−32.6 ± 0.7	−33	−31
10	−31.4 ± 0.4	−32	−30
20	−30.5 ± 0.4	−29	−24
40	−28.7 ± 0.2	−27	−23
60	−26.9 ± 0.1	−27	−17
80	−25.9 ± 0.2	−19	−12
100	−25.9 ± 0.3	−21	−13

Other techniques in design of freeze-drying process

- Visual inspection / particle size / light obscuration
- Moisture content





UV/VIS spectroscopy

- Protein aggregates scatter UV light and absorbance increases – can be used to monitor protein aggregation.
- UV/VIS A_{280nm}/A_{410nm}

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Impact of controlled ice nucleation on process performance and quality attributes of a lyophilized monoclonal antibody☆

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VIS spectroscopy

Determination of the concentration of total protein

BRADFORD ASSAY :

- Binding of protein molecules to Coomassie blue dye under acidic conditions results in a color change from brown to blue with absorption maximum change from 465nm to 595nm

BIURET PROTEIN TEST :

- Under alkaline conditions, substances containing two or more peptide bonds form a purple complex with copper salts, measurable at 520-570 nm

FTIR, Raman spectroscopy

- Methods of choice in assesment of changes that happen during freeze-drying

- Vibrational spectra can be used to estimate the secondary structure of proteins by inspection of the frequencies at which the amide bonds absorb infrared radiation
- Evaluation of the conformation changes, protein–protein interactions, protein aggregation

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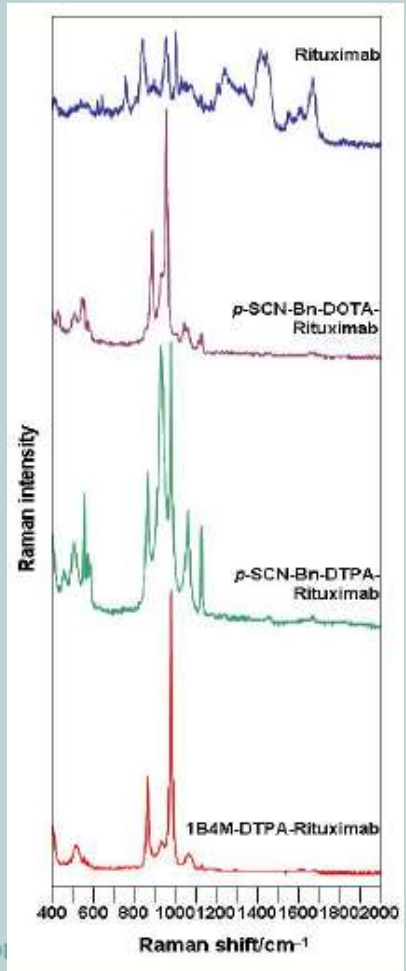
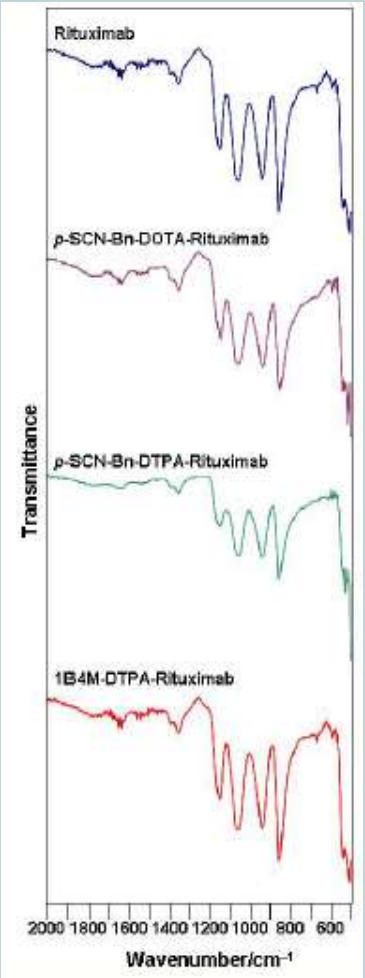
EVALUATION OF NON-RADIOACTIVE LUTETIUM- AND YTTRIUM-LABELED IMMUNOCONJUGATES OF RITUXIMAB – A VIBRATIONAL SPECTROSCOPY STUDY

Darinka Gjorgieva Ackova[1,*], Katarina Smilkov[1], Emilija Janevik-Ivanovska[1], Trajče Stafilov[2], Zorica Arsova-Sarafinovska[1,3], Petre Makreski[2,*]





The obtained spectra can be compared to aqueous reference spectrum, thus examining the degree of retention of native secondary structure

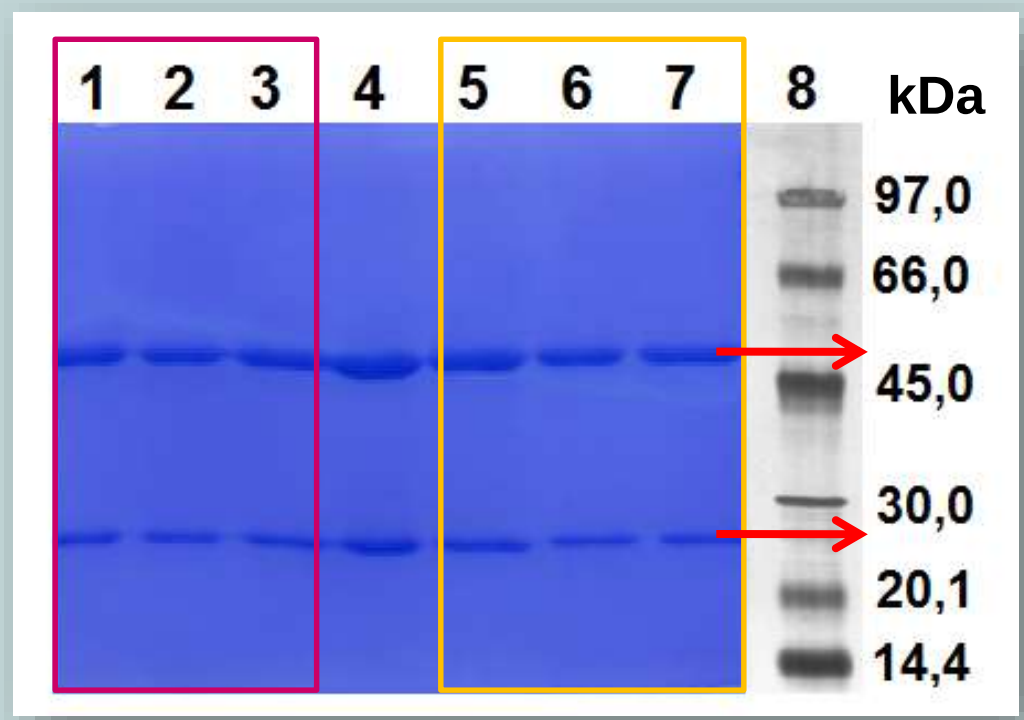
Electrophoresis

- SDS-PAGE is frequently used in protein analysis, especially valuable in determining species with various Mw
- Visualization is made with Coomassie blue or silver nitrate

Rituximab – DTPA-SCN 1,5

Rituximab – DOTA-SCN 2,4

Rituximab – DTPA-1B4M 3,7



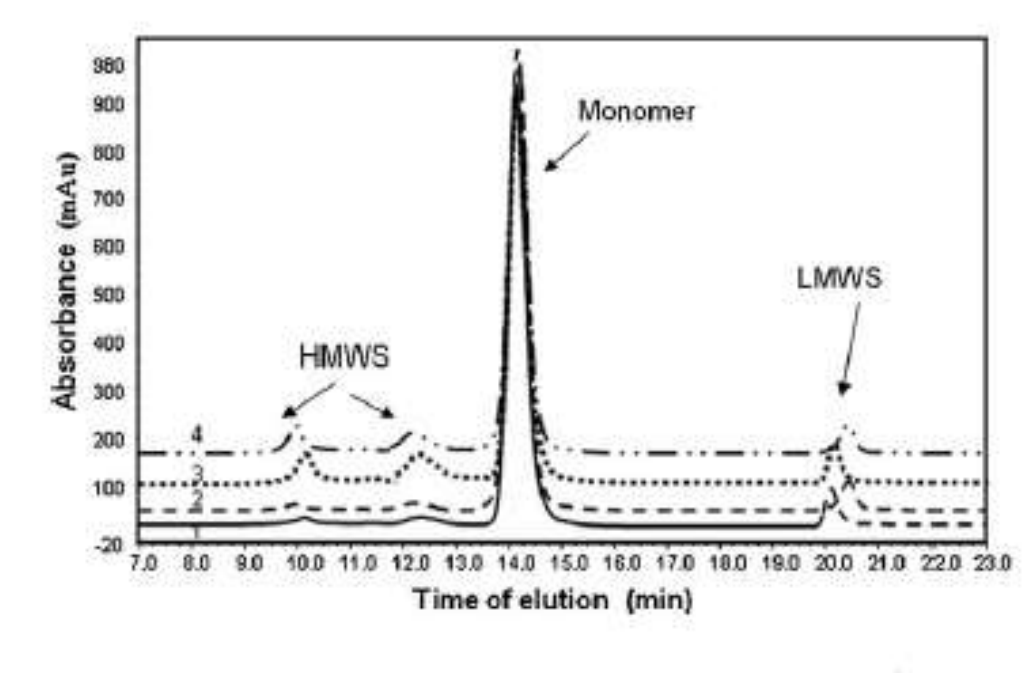
Chromatography

- Affinity chromatography (Protein A HPLC)
- SE - HPLC
- Ion Exchange Chromatography

RESEARCH PAPER

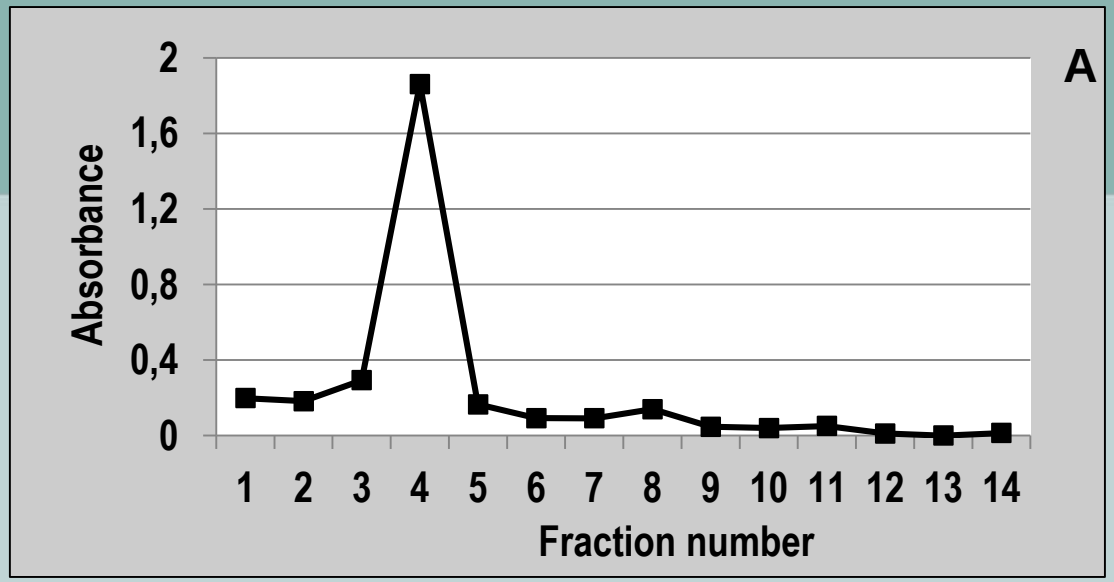
Effect of pH and Excipients on Structure, Dynamics, and Long-Term Stability of a Model IgG1 Monoclonal Antibody upon Freeze-Drying

Jihea Park • Karthik Nagapudi • Camille Vergara • Ranjini Ramachander • Jennifer S. Laurence • Sampathkumar Krishnan



SEC chromatogram of anti-streptavidin IgG1 monoclonal antibody showing the different species of monomer, HMWS and LMWS.

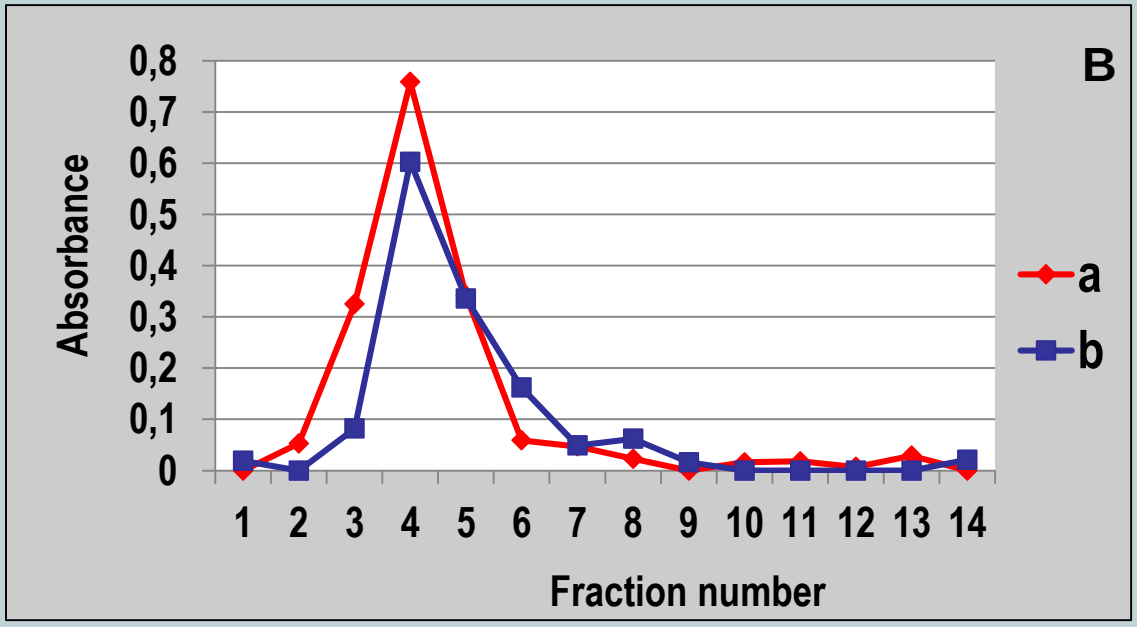
(1) C5MSu 6 months at 25°C
(2) C5MSu 0 months at 4°C
(3) C5M 6 months 25°C
(4) C5M 0 months 4°C



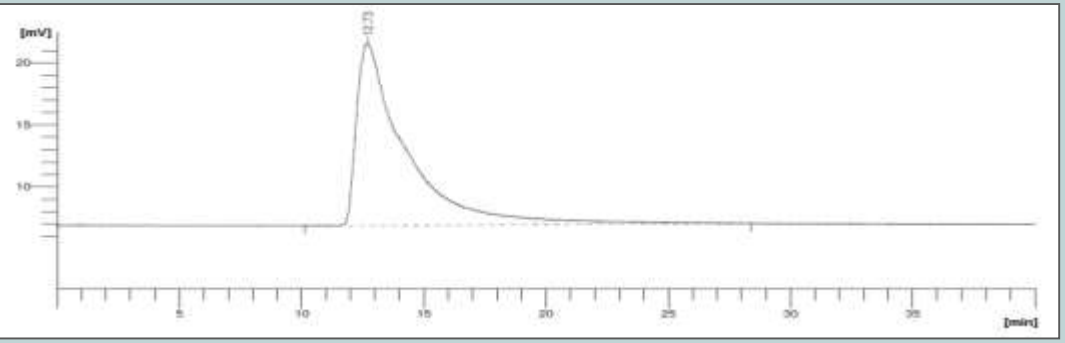
(A) Chromatographic profile of liquid, purified rituximab at 280nm

Sephadex G25 column

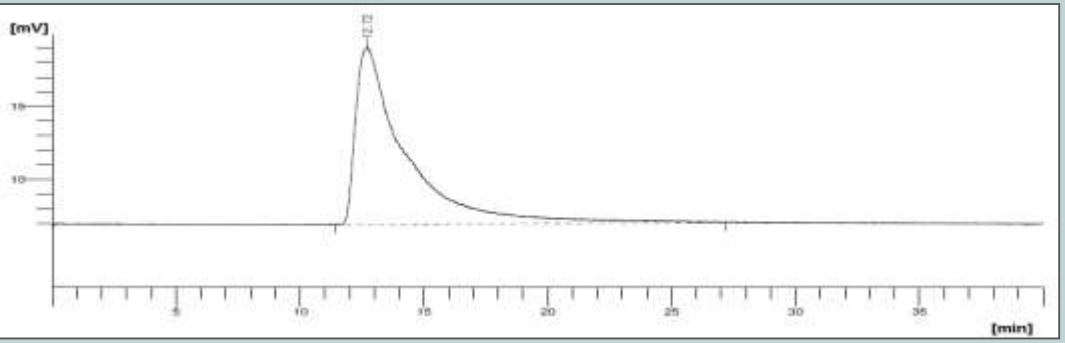
(B) Chromatographic profile of the eluted fractions at 280nm of the freeze-dried rituximab-1B4M-DTPA immunoconjugate after reconstitution: a) sample without mannitol, b) sample with 10 mg/mL mannitol.



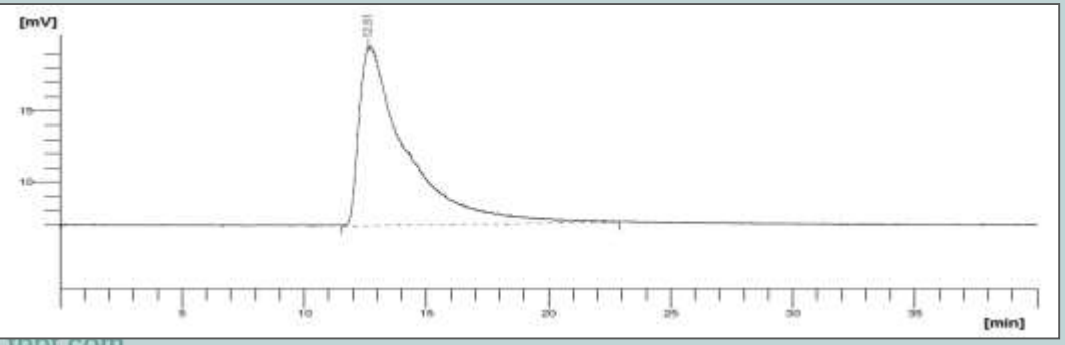
ITLC -radiodetection



^{177}Lu-DOTA-rituximab



^{1177}Lu-DTPA-rituximab



^{177}Lu-1B4M-DTPA-rituximab

Thank you

This research has been performed in the frames

IAEA
International Atomic Energy Agency