OTR10. STUDY OF MONOCLONAL ANTIBODY CONFORMATIONAL BY INTRINSIC FLUORESCENCE SPECTROSCOPY.

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INTRODUCTION Monoclonal antibodies (mABs) are biomolecules commercially produced for therapy and diagnosis. The potential commercial use of mAbs is strongly linked to its stability. In this sense, accelerated thermal screening studies are useful to provide information about stability protein formulations at different conditions, such as pH, ionic strength, buffer composition and stabilizers. The intrinsic tryptophan (W) fluorescence spectroscopy (ITF) technique can be employed in an accelerated thermal screening protocol because it is fast, economical, robust and ready-to-use.

OBJECTIVE Evaluate the potential conformational changes of 4G2 monoclonal antibody by tryptophan emission fluorescence spectra analysis in different conditions.

METHODOLOGY Conformational changes of the monoclonal antibody 4G2 was monitored by tryptophan fluorescence emission spectrum (Jasco FP-6500 spectrofluorometer). In this sense, the anti-4G2 was incubated at different concentrations (0.5 mg/mL, 2mg/mL, 6 mg/mL, 10 mg/mL and 14 mg/mL) and pH (4.0 – 10.0). The kinetic thermal denaturation of the antibody was also determined in a temperature range (20°C - 85°C). The excitation wavelength used was 280 nm and emission wavelength was scanned from 295 nm to 415 nm. The light scattering analysis was performed in the wavelength range of 300-340nm using 320nm as excitation wavelength. The temperature of 65°C (inflaction point) was used to observe the behavior of three stabilizers (arginine 125mM, glycerol 10% and tween 20, 0.01%) using the same analysis parameters. Superdex 200 HR10/30 sieving exclusion chromatography (SEC) was used to evaluate molecule aggregation or degradation.

RESULTS The sieving exclusion chromatography peak homogeneity (91.3%) related to 4G2 monomer form showed a molecular weight of 150.000. The maximum fluorescence emission wavelength (λmax) was 331±1 nm and the mass center was

344.6nm at 20oC. The emission fluorescence ratio at 330nm and 350nm (F330/350) increased in the pH interval 5.0 to 9.0. Immunoglobulin aggregation forms in different pH measured by SEC analysis were: pH 4.0 (11.50%); pH 7.0 (8.72%) and pH 10 (10.23%). The fluorescence thermogram showed a progressive decay on F330/F350 with the increase of temperature. The mass center thermogram analysis showed a maximum shift of 3,23nm at 85°C. The gradual increase on protein aggregation was also showed by LS analysis in the temperature range (65°C-85°C) being this behavior also observed by SEC. The stabilizers used were not able to prevent aggregation/denaturation at 65°C, as observed by F330/F350 ratio and SEC.

CONCLUSION Our results point out that pH and temperature modifications induce conformational changes around tryptophan residues of 4G2mAB as demonstrated by ITF. Such analytical tool is suggested to be used in screening conditions that can prevent monoclonal antibody denaturation/aggregation process, important step in the immunoglobulin stability studies.

KEYWORDS mAB, stability, fluorescence.