# EFFECTS OF PEGYLATION IN PROTEIN A AFFINITY CHROMATOGRAPHY

José González-Valdez[a,b]; Jorge Benavides[a]; Marco Rito-Palomares[a]; Todd M. Przybycien[b]
[a]*Centro de Biotecnología-FEMSA, Tecnológico de Monterrey, Campus Monterrey, Ave. Eugenio Garza Sada 2501 Sur, Monterrey, NL, 64849, México;* [b]*Chemical Engineering, Carnegie Mellon University, 5000 Forbes Ave. Pittsburgh, PA, 15213, USA;* E-mail: jose_gonzalez@itesm.mx

*Key words: PEGylation, Protein A, Affinity Chromatography*

**Introduction.** Affinity chromatography with Protein A has for years been one of the methods of choice in purifying monoclonal antibodies (MAbs) from complex mammalian cell culture media due to its high physiochemical stability (1). Improvements in specificity may translate into a reduction in the number of downstream polishing steps required to produce MAbs (2).

Our working hypothesis is that covalent modification of macromolecular affinity chromatography ligands by attachment of polyethylene glycol chains, or "PEGylation" (3), improves selectivity by decreasing non-specific binding interactions with the ligand without sacrificing binding capacity.

**Methods.** Protein A affinity media was PEGylated *in situ using* aldehyde-activated mono-methoxy polyethylene glycols with molecular weights of 5 and 20 kDa. The extent of PEGylation was determined via a PEG solution depletion assay. The rabbit IgG binding capacities, selectivities and overall performance of the modified media were compared with those of unmodified protein A media using both a biomolecular interaction screening platform technology and column-based measurements using a standard MAb bind/wash/elute protocol. Non-specific binding studies of rabbit IgG were conducted in yeast extract (YE) and bovine fetal serum (BFS) mixtures. Experiments were conducted at least by triplicate.

**Results.** Performance of unmodified and PEGylated media was compared. Results suggest that PEGylated media are capable of eliminating an amount up to 5% larger of contaminant proteins during the binding step without affecting IgG specificity or increasing the contaminant levels in the IgG elution pool. In fact, an increment of up to 15% on the average recovery yields was observed while using the PEGylated media, being the 20 kDa PEGylated column the one that presented the best results with IgG recovery yields of 98%.

**Table 1.** Average recovery yields of IgG and contaminant proteins at the washing step in chromatographic runs using unmodified, 5 kDa and 20 kDa mPEG PEGylated columns. Yeast Extract (YE) and Bovine Fetal Serum (FBS) were used in the samples as model contaminants.

| | | Recovery Yields | |
|---|---|---|---|
| | **Column Type** | **Contaminants at Washing Step** | **IgG** |
| **YE** | Unmodified | 85.25 ± 2.52 | 83.17 ± 0.58 |
| | PEGylated (5 kDa) | 90.63 ± 0.16 | 91.90 ± 0.49 |
| | PEGylated (20 kDa) | 90.51 ± 0.28 | 98.35 ± 0.47 |
| **BFS** | Unmodified | 94.92 ± 1.42 | 95.18 ± 0.45 |
| | PEGylated (5 kDa) | 96.98 ± 0.45 | 96.13 ± 0.71 |
| | PEGylated (20 kDa) | 94.96 ± 1.26 | 98.11 ± 0.41 |

**Conclusions.** PEGylation enhanced the ability of rSPA media to reject the non–specific binding of yeast extract and fetal bovine serum components. The amount of non–specifically bound YE species was reduced on average by a third, over the range of concentrations studied. PEGylated media provided increases of up to 15% in IgG recoveries relative to the unmodified media. With this work, it was demonstrated that the use of PEGylated rSPA affinity chromatography columns allows a better throughput and general performance of this unitary operation in antibody purification strategies.

**Acknowledgements**. The authors would like to thank CONACyT (Grant 53654 and Joint Fellowship 212025) and the ITESM Research Chair (Grant CAT161) in Mexico. They would also like to acknowledge the Chemical Engineering Department and the Mellon Institute from Carnegie Mellon University.

**References.**

(1) Jungbauer A., Hahn R. (2004). *Curr. Opin. Drug Discov. Devel.* 7(2):248–256.
(2) Hober S., Nord K., Linhult M. (2007). *J. Chrom. B.* 848(1):40–47.
(3) Veronese F., Pasut G. (2005). *Drug Discov. Today*. 10(21):1451–1458.