

# Comparing the Safety of Synthetic and Biological Ligands Used for Purification of Therapeutic Proteins

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**In light of a recent FDA ruling to address concerns about communicable diseases, purification of both feedstock and processing media is essential to downstream processing of therapeutic proteins. A decision to use synthetic ligands for chromatography applications might be made on the basis of specificity, robustness in sanitization, and cost.**

**B**iotecnology has led to the emergence of a new category of drugs — those of biological origin — which require extensive purification. Suitable purification techniques and corresponding regulatory considerations need to be incorporated at the research and early process development stages. Recent industry forecasts suggest that biopharmaceuticals (particularly protein therapeutics) will play an increasing role in the human health care market (1).

Given the importance of biopharmaceuticals, regulatory authorities are emphasizing the requirements of well characterized biologicals (WCB) (2,3). This has focused particular attention on the composition not only of the final biological product, but also of the processing materials in contact with that product. In the downstream processing system, contaminants may be carried through to the final product either through the feedstock or through components of the processing media.

## CONTAMINANT SOURCES

As the products of living cells, biopharmaceuticals are typically generated in complex concentrated mixtures. Their sources may be animal or human tissue, eggs, blood plasma, mammalian or bacterial cell cultures, or transgenic organisms such as plants or animals. Downstream processing of feedstocks, from whatever source, usually involves three major chromatographic steps: capture, intermediate purification, and polishing using adsorbents of different selectivity. The selective adsorbents can themselves be of biological origin. Examples include immobilized protein A used in antibody purification and monoclonal antibodies (MAbs) used for coagulation factor purification processes. MAbs are used because they can be designed for high selectivity. However, the use of protein ligands in affinity chromatography media not only constitutes a significant portion of the cost of downstream processing; they also can introduce contaminants from the host cell products, from endogenous and adventitious viruses, and from protein components in the ligands themselves.

The combination of feedstock and protein ligand affinity materials presents several possible sources of contamination in final, biologically active biopharmaceuticals. Contaminating sources can be adventitious agents of fermentation and cell cultures or components of animal tissues and their contaminants in transgenic production. Ligands derived from animals or cells also can contaminate the final biopharmaceutical products.

## TYPE OF CONTAMINANT

Monoclonal antibodies are the major active components of many next-generation biopharmaceuticals. They therefore serve as good model systems for assessing possible interactions with contaminants. Commercial MAbs (used for therapeutics, diagnostics, or as ligands) are produced using one of three methods: in ascites



**Table 1. Potential contaminants in affinity chromatography products**

Affinity Media	Potential Contaminant
Protein-based ligands from a mammalian source on an agarose matrix	Endogenous and adventitious viruses, prions, phospholipids, DNA, RNA, protein impurities, host cell proteins and nucleic acids, carbohydrate impurities, carbohydrate–ligand moieties
Protein-based ligands from a mammalian source on a synthetic matrix	Endogenous and adventitious viruses, prions, phospholipids, DNA, RNA, protein impurities, host cell proteins and nucleic acids, nonbiological polymers, polymer–ligand moieties
Protein-based ligands from a bacterial source on an agarose matrix	Lipopolysaccharides (endotoxins), bacterial protein impurities, DNA, RNA, secondary DNA and RNA viral proteins, carbohydrates, carbohydrate–ligand moieties
Protein-based ligands from a bacterial source matrix	LPS (endotoxins), bacterial DNA on a synthetic and RNA protein impurities, secondary DNA and RNA viral proteins, nonbiological polymers, polymer–ligand moieties
Synthetic peptide ligands on an agarose matrix	Solvents from manufacturing, carbohydrates, carbohydrate–ligand moieties
Synthetic chemical ligands on an agarose matrix	Solvents from manufacturing, carbohydrates, carbohydrate–ligand moieties
Synthetic chemical ligands on a synthetic matrix	Solvents from manufacturing, nonbiological polymers, polymer–ligand moieties
Synthetic peptide ligands on a synthetic matrix	Solvents from manufacturing, nonbiological polymers, polymer–ligand moieties

or hollow-fiber reactors, in cell cultures, or in transgenic animals.

Regardless of how it is produced, the antibody makes contact with animal tissue. With the increasing importance that transmissible spongiform encephalopathies (TSEs) have assumed in recent years, culminating in the recent FDA ruling on the issue (see the “FDA Ruling” box), traceability of the animal tissue becomes very important (4–7). The need for traceability is justified by the resistance of prions to most chemical and biological inactivation methods. A biological active could be destroyed by the methods used to destroy a prion (8,9). TSE agents cannot be removed easily from dry process equipment, particularly stainless steel (10,11).

**Transgenics.** In the case of transgenic animals, prion-resistant herds and flocks can be selected from countries without scrapie or bovine spongiform encephalopathy (BSE) (12,13). In the past year, significant advances in prion detection assays have helped ensure the absence of prions in the feedstock from these herds (14,15).

**Cell culture.** Because cell culture media are often based on pooled animal derivatives (such as albumin, transferrin, and insulin), confirming traceability becomes that much more difficult. And of course, increasing traceability for components of the process inevitably increases costs. In bacterial cell cultures the contaminants can additionally be DNA or RNA vectors, bacterial

proteins, or lipopolysaccharides (LPSs) (including endotoxins). And the components of the growth media used for the propagation of bacterial cultures must be traceable also.

**Complexation.** Active biologicals, such as immunoglobulins, can form complexes with a number of contaminants, including viruses, proteins, endotoxins, phospholipids, DNA/RNA (vectors), and prions. Complexes can form as a result of hydrophobic interaction, metal bridging, or charge complexation (16).

The presence of contaminants has two main implications, both of which bear on the safety and quality of active biologicals: Contaminants can cause heterogeneity (in charge and size), leading to process variability, product loss, and impaired product quality; and biologically active contaminants (such as endotoxins, viruses, prions, active peptides, and small synthetic chemical ligands) can cause biological toxicity or infectivity.

## CHROMATOGRAPHY MEDIA CONTAMINANTS

Chromatographic media for downstream processing are among the most significant components with which biopharmaceutical active entities interact. The media employed in downstream processing are usually selective adsorbents. Adsorbents for affinity chromatography are gaining increasing acceptance in biopharmaceutical purification. Three types of affinity chromatography are being incorporated into the production of

## FDA Ruling

On 10 April 2000, FDA reopened for a 90-day comment period the "Suitability Determination for Donors of Human Cellular and Tissue-Based Products" ruling originally published 30 September 1999. The agency took that action in response to requests for an extension from several parties, including a professional association and state officials. The new comment period ended 17 July 2000. The rule requires manufacturers of human cellular and tissue-based products to screen and test the donors of cells and tissues used in those products for risk factors and clinical evidence of communicable disease. As part of this regulatory action, the agency proposed to amend the current good manufacturing practice regulations that apply to such products regulated as drugs, medical devices, and biological products to incorporate the new donor-suitability procedures.

The request for additional time was made so that an ad hoc group of experts could complete the collection and analysis of scientific data on transmissible spongiform encephalopathies and Creutzfeld-Jakob disease. FDA also learned that California and other states had enacted legislation governing tissue donor suitability and those laws might conflict with the provisions of the proposed rule. During the comment period, FDA stated it would appreciate comment on the need for uniform national standards for donor suitability to prevent communicable disease transmission through human cellular and tissue-based products, and any issues raised by this proposed rule affecting state laws.

[*Federal Register*, 18 April 2000 65(75), pp. 20774-20775.]

biopharmaceuticals: classical protein-based affinity chromatography media, peptide-based chromatography (derived from phage display and other technologies), and synthetic ligands (based on chemically synthesized small molecules).

Each type of affinity chromatography can potentially transfer contaminants. The nature of those contaminants depends on the type of adsorbent. If the ligand is biological (such as a protein ligand), the nature of the contaminant can vary depending on its source. If the affinity ligand is an antibody, problems include animal protein impurities (albumin, for example), host cell components (for example, proteins and nucleic acids), and traceability to ensure the absence of infectious agents (such as with prions) as well as DNA or RNA vector contamination. If a protein ligand comes from a bacterial source (such as protein A), it also can be contaminated by an LPS (such as endotoxins).

**Phage display.** An innovative way of producing specific peptides for ligands in affinity separation is phage display technology (17). Once a peptide of a known sequence is identified, it can be expressed in yeast or chemically synthesized. If it is expressed in yeast, biological contamination still will be a problem.

Phage display peptide sequences can be quite specific for a target protein. They are subject to chemical degradation, however, in the same way that protein ligands are, and they also are subject to loss of activity from unfolding due to pH changes. Because peptide and protein ligands share similar properties, they can both be attacked by microorganisms in storage. They also are subject to restrictions on sanitization. As a result, chromatographic media with protein and peptide ligands are further limited by the number of process cycles they can be put through for purification.

**Chemical sensitivity.** Because of the chemical sensitivity of both protein and peptide ligands, robust chemistries rarely can be used for coupling them to matrices. As a result, another source of contamination in the final product is leachate from protein and peptide ligand-matrices. Although that may not be a safety issue in itself, it can contribute to consistency and quality issues in a biopharmaceutical product. Leaching can occur during processing or during harsh, sodium hydroxide intercycle treatments.

**Synthetic Ligands.** In contrast, synthetic ligands can be specific and chemically robust. Chemical stability allows for the use of robust chemistries in the synthesis of affinity matrices. As a result, affinity matrices based on synthetic ligands have insignificantly low ligand leakage. Using

information taken from protein surfaces and protein-protein surface interactions, ligand design can be based on intelligent combinatorial libraries to enhance specificity (18-20). A significant advantage of synthetic ligands is that they can't be contaminated by DNA/RNA vectors or infectious agents such as prions at their source. Of course, that is also true of synthetic peptide ligands. Table 1 outlines potential contaminants for different affinity media.

## CONTAMINANT PREVENTION

Purification methods are usually unable to remove those contaminants that are in complexation with the target protein or that are introduced from the purification process itself. Other strategies need to be adopted to remove complexed contaminants and chemical processing entities.

Carry-over contaminants can be removed using several different strategies. For example, metal chelators, such as edetic acid, can be used to remove complexation based on metal ion interactions. Salt concentrations can be used to control hydrophobic or charged interactions. Ethylene glycol can dissociate hydrophobic complexes. Changes in pH can be used to control complexation levels by changing the charge topography of proteins (16).

For infectious contaminants such as TSE agents, strategies include species selection (goat and sheep from which there is no evidence of TSE transfection) and the introduction of TSE-resistant alleles into transgenic strains. Using both of those strategies would considerably reduce the risk of transfection by agents such as prions (12,13). Careful compliance with Good Agricultural Practices also reduces the risk of animal diseases (21).

Using specific protein-based ligands in affinity chromatography can induce contaminants from the source fermentation media and cell or through the source tissue from which the ligands are derived as well as from the protein ligands themselves. Because of the chemically sensitive nature of many proteins, excessively harsh contaminant removal processes can denature those highly specific (and often expensive) chromatographic media.

The use of synthetic ligands (peptide or other synthetic chemical entities) in downstream processing affinity chromatography presents a significant alternative to protein ligands for preventing contamination in biopharmaceutical manufacturing processes. They eliminate biological contamination from infective agents (such as prions), inflammatory agents (such as endotoxins), or other biological contaminants

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such as DNA and RNA normally associated with biological ligand source.

## CHOOSING SYNTHETICS

The choice of synthetic chemical rather than peptide ligands can be made based on their specificity, robustness in the sanitization process, and cost. Validating processes employing animal- or cell-derived products as ligand components is expensive, as is demonstrating acceptable levels of purity in ligands from those sources.

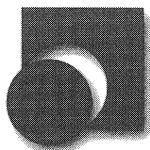
Contamination of a biopharmaceutical processed on affinity chromatography media employing protein or peptide ligands of biological origin can arise from a variety of sources. Those include media used to culture the cells expressing the ligand, contaminated cells, endogenous viruses, adventitious viruses and other infective agents, the ligand purification system, ligands as contaminants, the ligand-matrix complex, the matrix, chemicals used in processing the matrix, and downstream processing chemicals. Virtually all those sources are eliminated by the use of chemical entities that are synthesized in harsh acid/alkali and solvent environments.

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