Multimodal Chromatography

How to use a single ligand carrying multiple interaction mechanisms to separate targets and impurities.

LT Biotech

Resource Series
No. 01

01 What MMC separates

The term multimodal describes a single column whose ligand operates through multiple mechanisms at once. Multimodal chromatography (MMC), also called mixed-mode chromatography, uses a stationary phase whose ligand interacts with biomolecules through more than one type of chemical interaction simultaneously. Depending on the ligand design and operating conditions, these interactions can include electrostatic attraction, hydrophobic interaction, hydrogen bonding, and other specific chemical contacts.

The scientific value of a multimodal resin is not that it binds more strongly than a conventional resin. It is that the combination of interaction types creates a selectivity profile that cannot be generated by any single-mode exchanger or hydrophobic interaction resin. A target and an impurity that share similar net charge, making them difficult to resolve on an IEX column, may still differ in hydrophobic character or hydrogen-bonding capacity. A mixed-mode ligand that responds to both dimensions simultaneously can exploit these differences to achieve resolution that a single-mode approach cannot.

Because the relative contribution of each interaction type depends on the specific ligand chemistry and the mobile-phase conditions, multimodal chromatography is highly resin-specific. Generalisations that hold for one mixed-mode medium do not necessarily apply to another, even when both are described as "mixed-mode cation exchangers" or similar. Conditions established on one multimodal resin must be re-developed rather than simply transferred when the resin changes.

02 How the separation works

A generalised multimodal process is:

What distinguishes multimodal method development from IEX or HIC development is that changes in pH, salt concentration, and buffer species affect multiple interactions simultaneously rather than one in isolation. Increasing salt in a conventional cation exchanger predictably weakens electrostatic binding. In a multimodal ligand with both electrostatic and hydrophobic character, increasing salt may weaken the electrostatic component while leaving or even strengthening the hydrophobic component, producing a net retention change that is not easily predicted from first principles.

This coupling between variables is both the defining challenge and the defining opportunity of multimodal chromatography. It means that the method-development space is larger and less intuitive than for single-mode resins. It also means that the selectivity can be tuned in directions unavailable to simpler chemistries: a particular combination of pH and conductivity may produce near-complete separation of a target and a co-eluting impurity that no IEX or HIC condition resolves.

Multimodal chromatography can be operated in bind/elute mode or flow-through mode. Which configuration is appropriate depends on the relative retention of the product and the key impurities under candidate operating conditions. In flow-through mode, conditions are selected so that the product passes through while impurities such as host-cell proteins that bind more strongly to the mixed-mode ligand are retained on the column.

03 Applications and method development

Multimodal chromatography is well established in biopharmaceutical manufacturing, most prominently as a polishing tool following affinity capture in monoclonal antibody processes. After Protein A capture, a mixed-mode cation exchanger can provide clearance of host-cell proteins, aggregates, leached Protein A, and viral particles at pH and conductivity conditions that are compatible with the antibody but exploitable for impurity retention. The selectivity advantages of mixed-mode chemistry are particularly evident in this application because many of the remaining impurities at the post-capture stage are difficult to remove by conventional IEX alone.

Mixed-mode resins are also increasingly applied when two proteins with similar net charge must be separated. Even when IEX fails to resolve them, the additional hydrophobic or hydrogen-bonding character of the mixed-mode ligand may create the differentiation needed. Similarly, product-related variants such as deamidated isoforms, glycoforms, and aggregated species that differ only subtly from the target may be better resolved by a multimodal resin than by any single-mechanism approach.

Method development should evaluate:

- Resin chemistry and the dominant interaction mechanisms it presents.
- pH, often the single most important variable for tuning multimodal selectivity.
- Conductivity and salt concentration.
- Buffer species, which can compete with or reinforce specific interaction types.
- Target and impurity retention as a function of pH and conductivity simultaneously.
- Loading and residence time.
- Gradient versus step elution.
- Recovery, aggregate clearance, and other product-quality attributes.

Because pH and conductivity interact, a design-of-experiment (DoE) approach or structured two-dimensional screen is particularly efficient for multimodal method development. Evaluating a matrix of pH and conductivity conditions in a single experiment can reveal regions of high selectivity that a univariate optimisation strategy would miss entirely. A successful multimodal process should be evaluated against its complete purification objective: product recovery, impurity clearance, pool volume, operating robustness, resin lifetime, and compatibility with adjacent steps, not retention behaviour alone.

04 Strengths, limitations, applications

Strengths

Selectivity — differs substantially, and often orthogonally, from conventional IEX or HIC. Particularly effective for difficult protein and impurity separations that single-mode chromatography cannot resolve.

Broad operating windows — some mixed-mode resin chemistries provide broad operating windows tolerant of moderate changes in pH and conductivity.

Established — in aggregate clearance, host-cell protein removal, and viral clearance for biopharmaceutical polishing.

Complementary — to affinity capture in multi-step bioprocessing workflows.

Limitations

Predictability — interaction mechanisms are ligand-specific and less intuitively predictable than single-mode chromatography.

Complicated optimisation — changes in pH, salt, and buffer composition alter multiple interactions simultaneously, complicating systematic optimisation.

Conditions do not transfer directly — between different mixed-mode resins, even superficially similar ones.

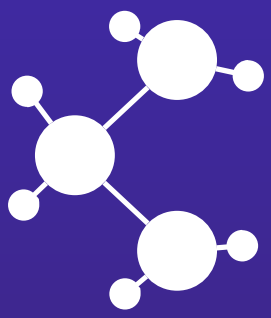
Screening is a prerequisite — retention cannot be reliably predicted from one or two molecular properties.

Product stability — constrains the usable operating window even when the resin chemistry offers a wider range.

Best-fit applications

Consider multimodal chromatography when conventional affinity, IEX, or HIC does not deliver adequate selectivity, particularly for polishing proteins, removing aggregates, or resolving closely related process impurities. It is most valuable when the target and principal impurities are difficult to separate by any single physicochemical property, and when the goal is to exploit a combination of interaction mechanisms that together create a separation unavailable from simpler chemistry.

Product-specific operating ranges, capacities, cleaning conditions, and regulatory claims should be taken from current LT Biotech resin documentation and product-specific validation data.



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