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New Downstream Publication: Purification of IgM Monoclonal Antibodies

Manufacturing challenges surround the use of IgM monoclonal

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ABSTRACT
The affinity capture paradigm that dominates industrial IgG purification has proven unsuitable for IgMs because, in most cases, they are affected adversely by harsh elution conditions. The large size of IgMs is also a challenge because it limits the operating conditions and performance of traditional porous-particle-based chromatography media. This article describes how these challenges can be overcome with available technology to develop effective manufacturing procedures for IgM monoclonal antibodies.

Recent reports that IgM monoclonal antibodies offer promising anticancer activity have created a strong interest in their therapeutic potential.[1–4] IgMs occur naturally in a variety of forms, represented dominantly by cyclic pentamers with a molecular weight of about 960 KDa, and cyclic hexamers with a molecular weight of about 1.15 MDa.[5] IgMs are more heavily glycosylated than are IgGs, with a range of 8–12% carbohydrate. The extinction coefficient for polyclonal IgM is 1.18, but as with IgG, that value can vary from one monoclonal IgM to another.[6]

The primary challenge to purification process development is that IgMs tend to be soluble in a narrower range of conditions than IgGs, and they are more susceptible than IgGs to denaturation.[7–9] Turbidity is the usual consequence of exposure to unsuitable conditions. Light turbidity is often reversible on restoration of more moderate conditions, but heavy or persistent turbidity may precede aggregate formation or precipitation. The extreme pH values used to elute immunoaffinity and mixed-mode affinity columns sometimes appear tolerable at laboratory scale, but these frequently cause recovery problems at process scale.[7,10–11] Protein-based affinity ligands such as mannan-binding protein and C1q can be eluted under more moderate conditions, but they are susceptible to proteolysis and cannot withstand sanitization by sodium hydroxide.[12–14]

Low conductivity tends to compound the sensitivity of IgMs to pH. Conditions routinely used for ion exchange purification of IgG, such as pH 8.5 or 4.5, may result in precipitation. IgMs are generally tolerant of high salt concentrations but are sensitive to denaturation on exposure to strongly hydrophobic surfaces. Hydrophobic interaction chromatography (HIC) media commonly used for purification of IgG often denature IgMs.[7] Size exclusion chromatography (SEC) is gentle and provides good fractionation, but it is undesirable for manufacturing applications because of its low productivity.

Table 1. Diffusion constants for antibody classes and fragments

Protein	Mass	K^{diff} cm^2/sec
Light chain	23 kD	9.1×10^{-7}
Fab	50 kD	7.4×10^{-7}
IgG	150 kD	4.9×10^{-7}
IgA	335 kD	3.7×10^{-7}
IgM	960 kD	2.6×10^{-7}

(Data from Gagnon.[7])

Table 1. Diffusion constants for antibody classes and fragments

The large size of IgMs poses another challenge. Large size is associated with slow diffusion constants, and slow diffusion constants limit both capacity and resolution on traditional porous-particle-based chromatography media. Table 1 lists diffusion constants for several antibody classes and fragments. The diffusion constant for IgM is approximately twice as slow as for IgG; this means that the flow rate would have to be twice as slow to achieve similar capacity and separation performance, assuming that equivalent surface area was accessible. But equivalent surface area is not accessible; pore diameters for a given gel type span a characteristic distribution of values, and the larger the protein, the lower the proportion of pores accessible to it. This compounds the capacity limitation already imposed by the slow diffusion constant.

These limitations have collectively engendered the misconception that IgMs are difficult to purify. Indeed, their chemical sensitivities must be accommodated, but IgMs manifest a range of chemical characteristics that enable development of effective orthogonal purification procedures under conditions that avoid unnecessary stress.[15] Most IgM monoclonals are highly charged and retained strongly enough by ion exchangers to support high binding capacities at moderate pH values.[7,15] Hydroxyapatite binds IgM strongly at physiological pH and conductivity.[7,15–18] HIC on weakly hydrophobic ligands provides good retention without risk of denaturation.[7] Screening may reveal aggregate separation on any of these methods.