Special issue: Biochemical Engineering Sciences
This Special Issue is a collection of the latest research in biochemical engineering science presented at the 9th ESBES Conference in Istanbul, Turkey, in 2012. The cover illustrates the development in biochemical engineering science by showing symbols for several biochemical engineering sub-disciplines, such as process engineering, strain and drug design, and material science, linked by covalent bonds in a hypothetical biological molecule.

Review

Functional monolithic platforms:
Chromatographic tools for antibody purification

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Polymer monoliths are an efficient platform for antibody purification. The use of monoclonal antibodies (mAbs) and engineered antibody structures as therapeutics has increased exponentially over the past few decades. Several approaches use polymer monoliths to purify large quantities of antibody with defined clinical and performance requirements. Functional monolithic supports have attracted a great deal of attention as they offer practical advantages for antibody purification, such as more rapid analysis, smaller sample volume requirements and the opportunity for a greater target molecule enrichment. This review focuses on the development of synthetic and natural polymer-based monoliths for antibody purification. The materials and methods employed in monolith production are discussed, highlighting the properties of each system. We also review the structural characterization techniques available using monolithic systems and their performance under different chromatographic approaches to antibody capture and release. Finally, a summary of monolithic platforms developed for antibody separation is presented, as well as expected trends in research to solve current and future challenges in this field. This review comprises a comprehensive analysis of proposed solutions highlighting the remarkable potential of monolithic platforms.

Keywords: Antibody purification · Downstream processing · Monolithic platforms · Polymeric monoliths.

1 Introduction

In recent decades, the use of antibodies, monoclonal antibodies (mAbs) and engineered antibody structures for cancer, autoimmune, inflammation and infectious disease therapy has increased exponentially, with an overall annual market worth tens of billions of US dollars [1–3]. Therefore, innovative platforms for large scale antibody production and purification are required [4]. Current research is aimed at developing more selective isolation methods for antibody purification, rather than relying on traditional chromatographic techniques [1, 3]. A chromatographic process can be defined as a separation process which allows the isolation of a target molecule from a complex mixture. This is enabled through the different chemical interactions between a specific ligand immobilized on a chromatographic support and the target molecule. Presently, chromatographic methods such as hydrophobic interaction chromatography (HIC), ion exchange chromatography (IEC) and affinity chromatography (AC) dominate the manufacturing of biopharmaceuticals [5, 6]. Numerous biological (antibodies, peptides, proteins, lectins) and non-biological (synthetic dyes, ion exchangers, metal chelates) ligands, materials and geometries (agarose beads, polymeric membranes, monoliths) may be incorporated into chromatographic separation matrices. The plethora of options available...
make chromatography the most commonly used technique for antibody purification [5, 7].

Thus, the ideal bioseparation matrix must fulfill the following criteria: (i) high selectivity and high binding capacity for the target molecule; (ii) good mechanical, morphological and chemical stability; (iii) inhibition of non-specific molecular adsorption; (iv) high stability under cleaning in place (CIP) and sterilization in place (SIP) conditions; and (v) facilitation of short processing times for high volumes [5]. To date, materials typically employed in chromatographic processes are beads or gels manufactured from such raw materials as agarose and polymeric membranes [1]. These materials, while readily available, present certain shortcomings such as limitations with the mass transfer, gel compressibility and poor pore diffusion leading to high pressure drops and low flow rates – all of which incur process time and cost. These weaknesses have led to an investment on alternative chromatographic supports which maintain the efficiency of the established processes while improving their associated limitations. One of these new generation of alternatives are monolithic supports, herein referred to as monoliths [8], which were introduced in the early 1990’s [5].

2 Monolithic platforms

We define monolith here as a porous, single-unit material introduced into a chromatographic device [9]. Individual monoliths are characterized by a network of large interconnected pores (or channels) which allow high operational fluxes and consequently lead to rapid processing times [1]. Due to their excellent morphological and mechanical properties, monoliths have attracted attention for use in antibody purification [10] (Fig. 1A) both at research and industrial scale (Fig. 1B). Incorporation of monoliths into chromatography stationary phase also avoids a high-shear fractionation atmosphere, which is crucial for optimal recovery of shear-sensitive molecules such as viruses, sensitive proteins, DNA and cells [8]. A vital requirement for implementation within the pharmaceutical industry is translation to large-scale operation. High-throughput processing must be enabled at moderate pressure drops without sacrificing the product purity. In this respect, the main advantages associated with monoliths (convention dominated mass transport, high porosity, low cost preparation and simple column filling) has encouraged several manufacturers to examine monoliths as potential supports [11]. Nowadays there are sev-

Table 1. Overview of commercially available monoliths for applications in bioseparation [11]

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Manufacturer</th>
<th>Material</th>
<th>Separation modes</th>
<th>Macro pore size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIM</td>
<td>BIA Separations</td>
<td>Polymethacrylate</td>
<td>Ion exchange</td>
<td>0.03–1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hydrophobic interaction</td>
<td>Reversed phase</td>
</tr>
<tr>
<td>UNO</td>
<td>Bio-Rad</td>
<td>Polyacrylamide</td>
<td>Ion exchange</td>
<td>1</td>
</tr>
<tr>
<td>SWIFT</td>
<td>Isco</td>
<td>Polymethacrylate</td>
<td>Ion exchange</td>
<td>1.5</td>
</tr>
<tr>
<td>SepraSorb</td>
<td>Sepragen</td>
<td>Modified cellulose</td>
<td>Ion exchange</td>
<td>50–300</td>
</tr>
<tr>
<td>Chromolith</td>
<td>Merck</td>
<td>Silica</td>
<td>Reverse phase</td>
<td>≥ 2</td>
</tr>
</tbody>
</table>
eral commercially available polymeric monolith based supports, for both small scale and analytical purposes, offering a wide range of pore diameters. These allow the purification of a large number of biomolecules ranging in size and features in a simple and effective way (Fig. 1 and Table 1).

Monoliths employed in antibody purification have been prepared using inorganic materials as well as natural and synthetic polymers. Recently, Arrua et al. [12] reviewed current developments and future possibilities for polymeric monolithic structures. Depending on the material, different manufacturing routes can be followed, including polymerization initiated by different stimuli [13], sol-gel [14] and cryogelation [15], creating porous networks with distinguishing structural properties. Since these polymers and materials adopt the format of the mold used, monolithic materials can be prepared in different formats, such as large rod polymers (used in standard HPLC/capillary columns), monolithic disks, cylinders and flat sheet polymers [12]. A classification according to the morphological features of different monolithic supports is indicated in Table 2 according to the commonly defined literature criteria [16].

Since the optimal performance of monoliths depends on the balance between morphological, mechanical and physicochemical properties, it is difficult to single out any specific parameter range to be set as the “gold standard”. It is crucial to establish first whether the monolith will be for analytical or large scale applications. For analytical purposes, pore size diameter can be designed according to the target antibody. In contrast, at large scale pore size must be considered in light of the contaminants also residing in the load solution, so that all components are able to permeate freely through the support. Hence, in general, a monolith for antibody purification must have a range of pore size diameter between 1 and 50 µm, a porosity of around 60 ± 10%, a surface area within 1000–350 m² cm⁻³ with a permeability and a binding capacity of up to 100 L m⁻² h⁻¹ atm⁻¹ and 50 mg mL⁻¹, respectively [17, 18]. This range of values can be tuned according to the components of the load solution by the methods selected to prepare the monoliths. Thus, different types of monoliths can be generated and customized to ensure maximum efficiency in the capture of the target antibody.

### Table 2. Morphological features of different types of porous structures [16].

<table>
<thead>
<tr>
<th>Feature</th>
<th>Micropore</th>
<th>Mesopore</th>
<th>Macropore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore Diameter (nm)</td>
<td>&lt; 2</td>
<td>2–50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>≤ 25</td>
<td>25–65</td>
<td>≥ 65</td>
</tr>
<tr>
<td>Surface Area (m² cm⁻³)</td>
<td>≥ 1000</td>
<td>1000–350</td>
<td>≤ 350</td>
</tr>
</tbody>
</table>

### 2.1 Synthetic polymer monoliths

Polymer monoliths produced by organic synthesis were first used in chromatography columns in the late 1980’s and the early 1990s [10–21]. Monolithic columns were prepared by radical polymerization of monovinyl monomer in the presence of a crosslinker, radical initiator and porogen (responsible for pore formation) (Fig. 2A). Inspired by this straightforward strategy for monolith production, different monomers such as acrylamide (AAm), methacrylate and styrene were then employed to create rigid monoliths with desired morphological properties and dimensions [22–28]. In particular, glycidyl methacrylate (GMA) and ethylene glycol dimethacrylate (EDMA) have become the...
most commonly employed monomers for the preparation of synthesized monoliths [13, 29]. The great advantages of using these monomers is that GMA, which carries the very reactive epoxy group, facilitates further functionalization for target molecule capture, while EDMA, as an excellent crosslinker, confers mechanical stability to the final monolith. As an example, Hahn et al. [30] developed an affinity poly(GMA-co-EDMA) monolith using a simple strategy for ligand immobilization. The model peptide (or ligand) was directly immobilized by reaction with the epoxy groups on the GMA chains incorporated into the matrix of monolith. Spacer arms can be introduced between the ligand and the support to promote the accessibility of the ligand functional groups to interact with the target biomolecule. As an example, reactive macroporous monoliths of poly(GMA-co-EDMA) were prepared by in situ copolymerization of GMA and EDMA in the presence of porogenic agents, followed by Protein A and L-histidine linkage to the monoliths either directly or through the use of a spacer arm. The IgG adsorption capacity of the monolith functionalized with Protein A was greatly increased with the introduction of the spacer [31]. Poly(GMA-co-EDMA) monoliths have also been functionalized with Protein L and Protein G with promising results [32-34], sufficient to justify their commercialization [35, 36].

To evaluate the application of synthetic monoliths to antibody purification, several studies have been performed. Lokman et al. [37] developed a novel porous monolithic system for effective IgG purification from human plasma based on the preparation of porous monoliths through the bulk polymerization of (hydroxyethyl) methacrylate HEMA and N-methacryloyl-(L)-histidine-methylester (MAH). An upper adsorption value (>96.5 mg g⁻¹) was achieved from human plasma with an associated purity value of 95.3%. Moreover, the authors verified that IgG could be reversibly adsorbed using poly(HEMA-MAH) monolith. Another strategy from the same group [38] involved the preparation of imprinted poly(hydroxyethyl methacrylate-N-methacryloyl-L-tyrosine methyl ester) particles using hepatitis B antibody as the surface template. These particles demonstrated spectacular binding specificity, adsorbing an amount of hepatitis B antibody 18.3 times greater than anti-hepatitis A antibody and 2-fold greater than immunoglobulin E. The self-polymerization of poly(glycerol polyglycidyl ether) (PG-PGE) using methyl tert-butyl ether as a porogenic agent resulted in the formation of a particularly rigid monolith where the epoxy groups of the poly(glycerol polyglycidyl ether) served a dual purpose: firstly to provide functional groups for the polymerization reaction, and secondly to allow direct binding of Protein A to the monolith surface. Capillary columns loaded with this monolith allowed the isolation of IgG (5.3 ± 0.9 µg) and presented a capacity of 0.44 ± 0.08 mg mL⁻¹ within a capillary volume of 12 µL [34].

In addition to affinity-based monoliths, anion-exchange methacrylate monolithic systems constituted by a monolithic macroporous convective interaction media (CIM) were tested and proved to be effective in the isolation of anti-glycophorin-A IgG1 mouse mAbs from cell culture supernatant. Also, CIM-iminodiacetate (IDA) disks with four different metal ions (Zn²⁺, Cu²⁺, Co²⁺ and Ni²⁺) immobilized were employed for mAb isolation from cell culture supernatant, achieving a maximum recovery of 85.4% of purified antibody [39].

Synthetic polymer monoliths have also been employed in complex IgM purification systems. Recently, an epoxy-activated monolith CIM disc functionalized with an affinity peptide [40] was developed for IgM, IgG and mAb isolation from embryonic stem cells. With this approach, it was possible to recover 67%, 83% and 95% of IgG, IgM and mAb, respectively. In addition, the binding capacity was reproducible over two thousand cycles. Recently, ammonium quaternized monolith CIM disks (CIM-QA (quaternary amine) and –EDA from Bia Separations) have also been used to purify IgM from mammalian cell cultures, with recovery yields up to 85% [41, 42]. CIM-QA and CIM-EDA discs can separate IgM from human plasma and can fractionate low abundance plasma proteins [43]. Other approaches regarding IgM isolation have also been developed [13, 44, 45] using synthetic polymer monoliths.

2.1.1 Hydrogels and cryogels

Cryogels and hydrogels are synthetic polymer monoliths which can be defined as supermacroporous gels. In cryogels, networks are formed by the cryogelation of monomers (e.g. GMA, allyl glycidyl ether [AGE]) at sub-zero temperatures using ammonium persulfate (APS) as an initiator and N,N,N',N'-tetramethylene diamine (TEMED) as the catalyst (Fig. 2B). Hydrogels are formed by the polymerization of AAm, N,N'-methylenebisacrylamide (MBA) and AGE in an aqueous buffer which works as a porogen, just as in the formation of acrylamide gels for gel electrophoresis assays [5]. The use of GMA and AGE allows a direct introduction of epoxy groups enabling further functionalization with ligands or other synthetic and natural species.

The macroporous network of hydrogels and cryogels makes them very attractive for cell and antibody separation [46], due to their higher porosity (up to 90%) and larger pore size (0.1–200 µm) [5, 46–48]. Unlike methacrylate or silica monoliths, cryogels and hydrogels have poor mechanical behavior. Low material rigidity can be minimized through physical blends or the addition of stiff polymers to the initial casting solution [12].

Over the past decade, different polymeric cryogel systems such as AAm and MBA grafted with N,N-dimethylaminoethyl methacrylate (DMAEMA) and poly(methacrylic acid (MAA))-co-polyethylene glycol diacrylate) embedded with polystyrene or poly(EDMA) nanoparticles have been prepared at sub-zero temperatures. Due to the large porous network, efficient separation of highly puri-
fied antibody from fermentation broth was achieved using affinity supermacroporous monolithic cryogels functionalized with Protein A [46, 49, 50]

Poly(AAm-AGE) cryogels functionalized with concanavalin A (Con A) were able to capture IgG from pure aqueous solutions and human plasma, with high capacity (up to 25.6 mg g⁻¹) and eluant purity (85%) [51]. Similar approaches were developed to purify IgM using polyHEMA cryogels activated with cyanogen bromide for further functionalization with Protein A. Due to their hemocompatibility, these systems enabled IgM and IgG isolation from human plasma with high reproducibility over repeated cycles [52, 53]. In related work, Cibacron Blue F3GA and (IDA)-Cu²⁺ covering PGMA particles incorporated into the polyHEMA cryogel allowed IgG and albumin isolation from human serum with efficiency of 93.6 and 89.4%, respectively [55]. N-methacryloyl-(L)-histidine methyl ester (MAH) was selected to function as a pseudospecific ligand and as co-monomer simultaneously to prepare polyhydroxyethyl methacrylate-N-methacryloyl-(L)-histidinemethylester cryogel [56]. The MAH incorporation into the support elevated the specific surface area up to one hundred times and allowed the highest registered quantity of IgG adsorbed from human plasma (97.3 mg g⁻¹ of cryogel) with an associated purity of 94.6%.

2.2 Monoliths based on naturally occurring polymers

Societal, environmental, and regulatory drivers are pressing industry to design engineered products from “cradle to grave” [56]. This has been a driving force for the use of natural and biodegradable polymers at an industrial level. The most widespread natural polymers are polysaccharides, such as cellulose, chitosan and agarose [56]. Cross-linked agarose beads possess insufficient mechanical support for chromatography use under high flow rates [5]. The popularity of agarose beads as first-choice supports for traditional affinity chromatography stems from bead hydrophilicity and good chemical stability, even under extremes of pH [7]. Thus, it is not surprising that agarose has been used for monolith preparation. Unfortunately, agarose based monolith supports exhibit poor mechanical properties, and at the time of writing they are only known as porous particles confined in a mold or as a macroporous gel [57].

Chitosan is also a natural polymer obtained by deacetylation of chitin originated from the exoskeleton of crustaceans [58]. Chitosan has been extensively investigated in diverse fields of work [59, 60] due to its nontoxic, antimicrobial, biocompatible, and biodegradable properties and sensitivity towards changes in pH [61]. Due to its high molecular weight, chitosan yields viscous solutions which can be utilized to produce porous gels and structures through methodologies such as freeze drying and supercritical fluid technology [62–67]. Sun et al. [68] prepared chitosan-agarose cryogels in situ through cryopolymerization and linked 2-mercaptopyridine onto divinylsulfone-activated matrix, producing cryogels used to purify IgG. Cryogels presented interconnected pores of 10-100 μm size, a specific surface area of 350 m² g⁻¹ and a high adsorption and elution capacity for IgG of 71.4 mg g⁻¹ and 90%, respectively. These supports proved to be stable and reusable for more 10 cycles without substantial loss in their performance. More recently, Barroso et al. [69] prepared chitosan-based monoliths for IgG purification by combining freezing and lyophilization
methods. The authors were able to improve the mechanical properties of chitosan through blending with polyvinyl alcohol (PVA) and by cryopolymerizing with GMA at sub-zero temperatures (Fig. 3). The supports were functionalized with a Protein A biomimetic ligand, through plasma technology, a free solvent technique. This sustainable and faster approach allowed high binding capacities (150 ± 10 mg IgG g⁻¹ support), and 90 ± 5% recovery of the bound protein with 98% purity directly from cell-culture extracts.

Cellulose has also been employed in chromatographic procedures using cellulose derivatives in the form of discs/membranes retaining the possibility for further functionalization with different type of molecules for protein separation and evaluation of affinity interactions [70–74]. Recently, Barroso et al. [75] prepared cellulose membranes/discs using an alternative approach to generate cellulose porous structures for different applications, namely that of human IgG purification.

Presently, the use of natural polymers for the preparation of chromatographic supports is still low, but this trend needs to be reversed in view of stricter chemical legislation regarding health and safety, thus pushing the industry towards greener and more sustainable processes. Table 3 provides a summary of the key supports, targets and separation criteria for the processes discussed above.

3 Structural characterization of monoliths

Ensuring optimal performance of a monolith based chromatographic medium requires accurate characterization to determine whether the monolith’s morphological properties fall within the range of desired values. Thus, depending on the application, the best balance between porosity, pore size and surface area must be attained [8]. Larger pores decrease the available surface area and reduce the mechanical strength of the support. Conversely, smaller pores allow for a larger surface area and impart better mechanical integrity, albeit at the expense of lower fluxes and slower processes. One of the most critical issues is the pore size distribution. Various authors allude to the difficulty in producing monoliths with an acceptable degree of homogeneity [76, 77]. Therefore, a number of methods have been described for evaluating the porosity within monolithic networks. These include scanning and transmission electron microscopy (SEM/TEM), mercury intrusion porosimetry (MIP), adsorption or desorption of nitrogen (AN/DN), and inverse size inclusion chromatography (ISEC) [12, 78, 79]. However, these techniques all require a significant quantity (of the order of milligrams) of monolith sample to obtain representative results. Additionally, these analyses are often destructive. Electron microscopy samples require heavy metal sputter coating for analysis, while MIP requires samples to be impregnated with mercury. In case of studies evaluating adsorption or desorption of nitrogen, the samples may be destroyed through the degasification procedures and pressures employed during the analysis.

Developing non-invasive methods for characterizing monolith morphology has become a great challenge for some researchers. Petter et al. [80] utilized near-infrared spectroscopy to determine pore size, pore volume, total porosity and surface area in a single analysis. Although this technique is not destructive, it still does not provide comprehensive morphological detail such as potential wall defects and the degree of radial heterogeneity, both of which are particularly important in evaluating monoliths as chromatographic devices.

The introduction of other recent techniques, such as scanning coupled contactless conductivity (sC4D) methods, confocal laser scanning microscopy, magnetic resonance imaging and small angle neutron scattering, offers innovative options to complement the aforementioned techniques in order to attain a thorough understanding of monolith structural features [81–83].

4 Performance evaluation of monolithic platforms

Important key parameters that must be studied when developing new monoliths include static and dynamic binding capacity, scale up potential and resistance to cleaning and sterilization procedures.

An adsorption isotherm is a useful tool for estimating the maximum binding capacity to the target molecule as well as evaluating the level of non-specific adsorption [84]. By taking into account parameters describing the porous network, material and monolith surface area, different adsorption isotherms can be applied to achieve the best fit to the experimental data obtained through static studies.

To assess the monolith dynamic binding features and mass transfer, breakthrough curves obtained by frontal analysis are usually estimated. If breakthrough curves do not alter with feed concentration, or molecular dimension and velocity, it indicates that the adsorption is not restricted by mass transfer phenomena [6]. To obtain an effective mass transfer, the pores have to be sufficiently wide. For this reason, monoliths are ideal for the separation of antibodies and other biomolecules with diameters above ~5 nm, since it is technically difficult to produce particles with a pore size wide enough to allow permeation of these larger molecules [4, 8, 85].

Regarding the cleaning and regeneration issues of monoliths, different protocols can be adopted according to the stability of the immobilized ligand and of the polymeric composition. Thus, cleaning and regeneration regimes need to be optimized for individual situations. However, the most common procedures employed involve
### Table 3. Monolithic materials for antibody purification

<table>
<thead>
<tr>
<th>Material</th>
<th>Mode</th>
<th>Ligand</th>
<th>Target</th>
<th>Surface area (m² g⁻¹)</th>
<th>Flow rate (mL min⁻¹)</th>
<th>Capacity (mg g⁻¹)</th>
<th>Recovery (%)</th>
<th>Purity</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMA-EDMA</td>
<td>Affinity</td>
<td>Protein A</td>
<td>IgG, IgM, IgA</td>
<td>89.1</td>
<td>0.05; 1.0</td>
<td>20 (IgG)</td>
<td>99</td>
<td>High</td>
<td>[31, 33]</td>
</tr>
<tr>
<td>Protein L</td>
<td></td>
<td>IgG</td>
<td>n.a.</td>
<td>n.a.</td>
<td>1 × 10⁻⁵</td>
<td>n.a.</td>
<td>n.a.</td>
<td>High</td>
<td>[29]</td>
</tr>
<tr>
<td>Protein G</td>
<td></td>
<td>IgG</td>
<td>n.a.</td>
<td>0.05; 2.5</td>
<td>20 mg g⁻¹</td>
<td>n.a.</td>
<td>n.a.</td>
<td>High</td>
<td>[33, 34]</td>
</tr>
<tr>
<td>L-histidine</td>
<td></td>
<td>IgG</td>
<td>89.1</td>
<td>1.0</td>
<td>22.0 mg g⁻¹</td>
<td>n.a.</td>
<td>n.a.</td>
<td>High</td>
<td>[31]</td>
</tr>
<tr>
<td>Anion Exchange</td>
<td></td>
<td>DEAE</td>
<td>mAs</td>
<td>n.a.</td>
<td>1.0</td>
<td>n.a.</td>
<td>95.0</td>
<td>High</td>
<td>[35]</td>
</tr>
<tr>
<td>EDA</td>
<td></td>
<td>n.a.</td>
<td>1.0</td>
<td>n.a.</td>
<td>91.4</td>
<td>High</td>
<td>[35]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion Exchange</td>
<td></td>
<td>MAA</td>
<td>IgG</td>
<td>57.1</td>
<td>1.0–2.0</td>
<td>n.a.</td>
<td>98.8</td>
<td>Good</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG-PGE</td>
<td>Affinity</td>
<td>Protein A</td>
<td>IgG from rabbit serum</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0.44 mg mL⁻¹</td>
<td>n.a.</td>
<td>n.a.</td>
<td>[33]</td>
</tr>
<tr>
<td>HEMA-MAH</td>
<td>Pseudo-affinity</td>
<td>(MAH)</td>
<td>IgG</td>
<td>145.8</td>
<td>1.0</td>
<td>96.5 mg g⁻¹</td>
<td>n.a.</td>
<td>95.3%</td>
<td>[37]</td>
</tr>
<tr>
<td>CIM-IDA</td>
<td>IMAC</td>
<td>Cu²⁺/Ni²⁺/Zn²⁺/Co²⁺</td>
<td>IgG, mAb</td>
<td>n.a.</td>
<td>2.0</td>
<td>n.a.</td>
<td>63/41/85/40</td>
<td>n.a.</td>
<td>[39]</td>
</tr>
<tr>
<td>IMAC</td>
<td>Cu²⁺/Ni²⁺/Zn²⁺</td>
<td>IgG, Ma</td>
<td>n.a.</td>
<td>3.0</td>
<td>0.5 mg mL⁻¹</td>
<td>82.4</td>
<td>n.a.</td>
<td>[92]</td>
<td></td>
</tr>
<tr>
<td>CIM</td>
<td>Pseudo-affinity</td>
<td>Peptide</td>
<td>IgM, IgG, mAb</td>
<td>n.a.</td>
<td>1.0–10</td>
<td>n.a.</td>
<td>83/67/95</td>
<td>n.a.</td>
<td>[40]</td>
</tr>
<tr>
<td>CIM</td>
<td>Ion Exchange</td>
<td>QA/DEAE/EDA</td>
<td>IgM, IgG</td>
<td>n.a.</td>
<td>1.0–2.0</td>
<td>20 mg g⁻¹</td>
<td>n.a.</td>
<td>93%</td>
<td>[93]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgM</td>
<td>n.a.</td>
<td>n.a.</td>
<td>16–36 mg g⁻¹</td>
<td>n.a.</td>
<td>[94]</td>
<td></td>
</tr>
<tr>
<td>DMAA-AGE cryogel</td>
<td>IMAC</td>
<td>IDA-Cu²⁺</td>
<td>Fv antibody fragments</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>84–96</td>
<td>High</td>
<td>[94]</td>
</tr>
<tr>
<td></td>
<td>Affinity</td>
<td>Protein A</td>
<td>from E. Coli cell culture</td>
<td>n.a.</td>
<td>20.2</td>
<td>1.6 × 10⁸ cells mL⁻¹ adsorbent</td>
<td>60–70</td>
<td>High</td>
<td>[52]</td>
</tr>
<tr>
<td>AAm-AGE cryogel</td>
<td>Affinity</td>
<td>Con A</td>
<td>IgG</td>
<td>n.a.</td>
<td>1.0</td>
<td>25.6 mg g⁻¹</td>
<td>94</td>
<td>85%</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>Affinity</td>
<td>Protein A</td>
<td>IgG labeled inclusion bodies</td>
<td>n.a.</td>
<td>0.5</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>[52]</td>
</tr>
<tr>
<td>HEMA-cryogel</td>
<td>Affinity</td>
<td>Protein A</td>
<td>IgM</td>
<td>20.2</td>
<td>0.5</td>
<td>42.7 mg g⁻¹</td>
<td>90</td>
<td>–</td>
<td>[52]</td>
</tr>
<tr>
<td>IMAC</td>
<td>PGMA-IDA-Cu²⁺</td>
<td>n.a.</td>
<td>0.5–2.0</td>
<td>257 mg g⁻¹</td>
<td>89.4</td>
<td>–</td>
<td>[54]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affinity</td>
<td>PGMA-Cibracron Blue F3GA</td>
<td>IgG</td>
<td>n.a.</td>
<td>0.5–2.0</td>
<td>342 mg g⁻¹</td>
<td>93.6</td>
<td>–</td>
<td>[54]</td>
<td></td>
</tr>
<tr>
<td>Affinity</td>
<td>Protein A</td>
<td>n.a.</td>
<td>0.5–3.0</td>
<td>83.2 mg g⁻¹</td>
<td>85</td>
<td>85%</td>
<td>[53]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEMA-MAH cryogel</td>
<td>Pseudo-affinity</td>
<td>(MAH)</td>
<td>IgG</td>
<td>n.a.</td>
<td>0.5–3.0</td>
<td>97.3 mg g⁻¹</td>
<td>80.7</td>
<td>94.6%</td>
<td>[53]</td>
</tr>
<tr>
<td>Chitosan-agarose</td>
<td>Affinity</td>
<td>2-mercaptopyridine</td>
<td>IgG</td>
<td>350</td>
<td>1.0</td>
<td>71.4 mg g⁻¹</td>
<td>90</td>
<td>High</td>
<td>[68]</td>
</tr>
<tr>
<td>Chitosan-PVA</td>
<td>Affinity</td>
<td>Artificial Protein A</td>
<td>IgG, mAb</td>
<td>2.3</td>
<td>1.0</td>
<td>150 mg g⁻¹</td>
<td>90</td>
<td>98%</td>
<td>[69]</td>
</tr>
</tbody>
</table>

a) n.a. data not available in the literature
the use of alkaline (0.1–1 M NaOH) and salt solutions (1–2M NaCl) which contain competitor agents that force the removal of antibody and proteins from the monolithic supports. Alcohol solutions such as ethanol (up to 20%) and isopropyl alcohol (up to 30%) can also be used [17, 86]. Moreover, the use of detergents (e.g. Tween 80) or organic solvents (acetone, ethanol, and isopropanol) may be required for sanitization of chromatographic media after use with particular feedstocks.

The pressure drop across monolith based media is typically lower than traditional beads or membranes. Monoliths used in biomolecules separation field should have porosity higher than 50% allowing a pressure drop reduction of 50% compared with beads or membranes [8].

An additional and also fundamental concern associated with monolith-based media is its scale up capability. This issue still needs to be addressed; however the preparation of monolithic devices capable of operating over multiple cycles without capacity loss is within the grasp of existing manufacturing processes [87]. Attachment of monolith to the column wall can also be challenging. Monoliths can be attached to a column with a flexible wall, though this set-up would prove cumbersome when working with high pressure gradients and high flow rates [6, 87]. Concerns over column attachment may explain why silica monoliths are not yet available as industrial scale chromatography media. In marked contrast, the scale-up of CIM disks and tubes made from polymethacrylate has been widespread, since the preparation of these supports results in superior mechanical behavior and resilience to aggressive regeneration conditions (e.g. 1 M NaOH). Thus, scale up is straightforward and amenable to biopharmaceutical process development strategies [88].

At present, intermediate scale purification has been performed by linking monolith columns in parallel [89], creating an array system with a volume capacity up to 1000 L. Effective scale-up from a 0.34 mL disk to 8 L radial columns and tubes is well established [6]. However, the incorporation of monoliths in industrial processes is still a challenge that deserves attention. In the near future it is expected that monoliths could increase processing capacity to directly compete with traditional chromatographic resins that are able to process hundreds of liters with high resolution. At the time of writing, 8 mL of a CIM monolith functionalized with Protein A is able to purify 10 mg IgG g⁻¹ wet support, while 2 mL of Protein A agarose resin purifies 20 mg IgG g⁻¹ wet support. Thus, an improvement in monolith purification capacity is still required before large scale comparisons are attempted.

5  Summary, concluding remarks, and future trends

Interest in high-value biomolecules in medicine, pharmacology, biochemistry and diagnostics has resulted in the development of alternative systems for antibody isolation and purification. Monolithic support technology, though nascent, requires further maturation before its full potential can be exploited. The advantages and disadvantages of synthetic, cryogel and natural polymer monoliths are summarized in Table 4.

Up to now, silica-based monoliths have not been extended to antibody purification and therefore were not included in this review. Silica-based monoliths have been applied to drug and chiral separations and for immunochromatography [90, 91]. The technologic transition in purification processes has already begun. There is a substantial amount of literature highlighting the virtues of monolithic supports. Commercially available monolith based chromatography media have demonstrated efficient biomolecule separation across a number of applications. Particularly, the suc-

| Table 4. Summary of monolithic platforms in antibody separation: pros and cons |
|-------------------------------|-------------------------------------|-----------------------------|
| Monolith Base | Pros | Cons |
| Organic | High mechanical stability, Easy preparation, Numerous monomers available, Easy scale up, Easy ligand attachment | Some difficulties in processing biomolecules with high molecular weight (≥ 100 kDa) |
| Cryogels | High performance to process viscous fluids (e.g. blood and cells), Easy preparation | Low purification efficiencies for biomolecules with low molecular weight (< 100 kDa), Low mechanical properties, Low surface area |
| Natural polymers | Easy preparation, Tunable mechanical properties, Biocompatible, Biodegradable | Lack of processing methods |
Successful purification of large target molecules indicates that the technology may find a niche in the purification of antibodies of various formats. Additional work is needed to expand the range of ligands available, fine tune their immobilization and optimize the scale up of monolithic platforms.

Protein A was known to biochemists long before its true potential as an antibody purification ligand was fully realized. This recognition resulted in a paradigm shift in biomolecular separations technologies. The recent advances in proteomics have led to the identification and classification of multitudes of new proteins with vital roles in living organisms. The call for technology to assist the efficient purification of large proteins from complex mixtures has never been greater. To meet this burgeoning demand, the time is ripe for the next leap in affinity supports. Monoliths may well prove to be the ideal bespoke chromatographic medium that takes complex bioseparations from the research bench to sustainable large-scale industrial processes.


The authors declare no conflict of interest.

References


