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Protein Adsorption and Transport in Polymer-Functionalized Ion-Exchangers

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Abstract

A wide variety of stationary phases is available for use in preparative chromatography of proteins, covering different base matrices, pore structures and modes of chromatography. There has recently been significant growth in the number of such materials in which the base matrix is derivatized to add a covalently attached or grafted polymer layer or, in some cases, a hydrogel that fills the pore space. This review summarizes the main structural and functional features of ion exchangers of this kind, which represent the largest class of such materials. Although the adsorption and transport properties may generally be used operationally and modeled phenomenologically using the same methods as are used for proteins in conventional media, there are noteworthy mechanistic differences in protein behavior in these adsorbents. A fundamental difference in protein retention is that it may be portrayed as partitioning into a three-dimensional polymer phase rather than adsorption at an extended two-dimensional surface, as applies in more conventional media. Beyond this partitioning behavior, however, the polymer-functionalized media often display rapid intraparticle transport that, while qualitatively comparable to that in conventional media, is sufficiently rapid quantitatively under certain conditions that it can lead to clear benefits in key measures of performance such as the dynamic binding capacity. Although possible mechanistic bases for the retention and transport properties are discussed, appreciable areas of uncertainty make detailed mechanistic modeling very challenging, and more detailed experimental characterization is likely to be more productive.

1. Introduction

Chromatography remains the workhorse of downstream processing of biologics such as proteins, despite concerns about its efficiency and endeavors to develop non-chromatographic alternatives [1,2]. Even the enormous growth in production of monoclonal antibodies (mAbs), for which high titers and high-volume cell culture can produce batch sizes of order 100 kg, has been sustained by purification processes typically containing 2–3 chromatographic steps each [3–7]. Within the large realm of preparative chromatography applications in pharmaceutical and biotechnology manufacturing, ion-exchange chromatography (IEC) is especially widely used. A study in the late 1990s indicated an average of about three chromatographic steps in each protein production process, with about 40% of these being IEC [8].

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There are a large number of adsorbents available for bioprocessing applications, and their characteristics have been reviewed extensively [9–12]. A wide range of base matrices, including inorganic, natural polymer and synthetic polymer, are routinely used, with each having its own distinctive architecture and consequent functional properties. At a relatively coarse level of analysis the key properties can be categorized on different length scales, which are discussed here primarily for particulate adsorbents but can be related to other gross morphologies such as monoliths and membranes as well [13]. On the larger length scale, the particle diameter is the key dimension, with two competing phenomena central to optimization. To allow rapid mass transfer, controlled mainly by intraparticle diffusion, the particles should be smaller, but this can lead to unacceptably large pressure drops in packed columns. Typical preparative adsorbents are consequently of order tens of μm in diameter and therefore significantly larger than the particles used for fast analytical separations of small molecules.

On a shorter length scale, a key measure for an adsorbent is the pore size distribution (PSD). Here again there are competing considerations involved, namely the need for a high specific surface area (area per volume) and for rapid intraparticle diffusion. Since, for ideal cylindrical pores, the specific surface area is inversely proportional to the pore diameter, small pores favor high surface area, but reducing the pore diameter also reduces the size of the available path for pore diffusion, especially if pore constriction due to adsorbed solutes is taken into account. Furthermore, since uniformity of pore size is difficult to achieve, pore occlusion is also a possibility if the pore size is too small. A suggested optimum [14] is a pore size of order ten times the adsorbate size, although additional characteristics of the different adsorbent morphologies, including the porosity and the pore connectivity, are important considerations not discussed in detail in this simplistic analysis [15].

The smallest length scales of interest are those characterizing the topography and chemistry of the adsorbent surface. The topography is generally defined by the base matrix [12], leading to pronounced differences between such cases as the fibrous structure of agarose and the more extended sphere-like surface of polymethacrylates [15,16]. However, introduction of the functional ligands can alter the local topography and hence the adsorbate-adsorbent interaction. In particular, if a more extended spacer arm is used, it can not just change the topography but also interact directly with the adsorbate and therefore affect retention behavior [17,18].

The preceding discussion, especially that in the previous paragraph, is implicitly framed in terms of retention that occurs directly by adsorption on an extended surface, leading to formation of an adsorbed layer. Indeed, for a variety of conventional adsorbents the equilibrium adsorption capacity for proteins is consistent with that calculated for monolayer coverage [19–22]. However, this review concerns materials that have been modified so as to break this paradigm by extending the domain for protein retention into the third dimension [14] via modification of the base matrix in one of several ways with polymeric attachments. Several categories of such adsorbents have been introduced by various companies, especially over the past two decades, and there is now an extensive collection of data demonstrating the functional properties of these materials. Adsorbents are available for several modes of chromatography, including ion-exchange, hydrophobic interaction and mixed-mode. Only ion-exchange is considered here, partly because it is the mode for which most data are available but partly also because of the richness of the underlying scientific issues involved in understanding adsorption and transport effects within charged media of this kind.

The purpose of this review is to provide an overview of the structural and functional characteristics of the polymer-derivatized ion-exchange media. Reviews of chromatographic

stationary phases generally do not distinguish conventional and polymer-derivatized media, which is understandable given that they are used in a similar fashion operationally and their performance is analyzed within the same phenomenological framework. However, as noted in a key review cited above [14], the two classes of media are fundamentally different in how they function, specifically in the 2-D adsorption vs. 3-D partitioning that is the basis for retention; this is apparent in the transmission electron micrographs of protein-loaded underivatized and dextran-modified agarose media shown in Figure 1 [23]. The present review overlaps with the earlier one to only a limited extent. First, the structural information provided here emphasizes the physical structure of the media, with much less discussion of the synthesis chemistry presented before [14]. The earlier review also discussed some of the functional properties, but more detail is provided here and some recent studies have provided additional insights, both phenomenological and mechanistic. However, an additional focus of the presentation is on seeking mechanistic interpretations of the observed performance of the polymer-derivatized materials, definitive demonstrations of many aspects of which are still elusive. To this end the presentation discusses some of the numerous sources of complexity that present appreciable challenges to a more complete interpretation of experimentally observed behavior, and that therefore represent an obstacle to the more systematic design of materials with optimal properties.

2. Adsorbent Structure

A common characteristic of all the materials considered here is that they comprise a rigid base matrix within which the polymeric derivatization is performed. The ion-exchange ligands may be present only on the added polymer or also on the base matrix; this depends in part on whether they are included in the polymerization reagents [24,25] or added in a subsequent post-derivatization step [26]. A wide variety of synthetic schemes have been used, with concomitant differences in the chemical structures obtained; several of these are reviewed elsewhere [14], but many are accessible in greater detail in the archival literature and in patents. The polymer additives used are typically hydrophilic, and although additional reagents such as cross-linkers may be more hydrophobic, the effects of the final chemical structure on functional performance are likely to be relatively subtle. Therefore the emphasis here is less on the chemical structure than on the physical structure and its effect on relevant chromatographic properties.

The evolution of polymer-functionalized materials can be traced back [24] to the early days of protein chromatography on soft gels based on polymers such as dextran, poly(acrylamide) and agarose. While these were effective for laboratory scale separations, the media lacked the strength to allow column-based separations at the kinds of flow rates necessary for industrial bioprocessing. Suitable media for this purpose were therefore developed based on extensive cross-linking of the otherwise soft polymers or the introduction of new base matrices based on synthetic polymers such as poly(methacrylate) or poly(styrene-divinylbenzene). It was largely in a quest for higher capacities and/or faster solute transport that the new media emerged, but it is useful to note that the polymer inclusions have some of the same properties – and indeed in some cases are the same materials – as those associated with the early soft gel media. The composite nature of the new media, however, while intrinsic to obtaining the best of both worlds in mechanical and adsorptive/transport properties, can add a significant level of complexity to achieving a mechanistic understanding of observed performance metrics.

Three different classes of polymer-derivatized media can be identified (Figure 2), and are considered separately in the subsections that follow. Some reference is made to relevant commercial product lines, but in some cases proprietary materials and procedures have limited the availability of sufficient information to identify the class of media into which a

particular adsorbent falls. In addition to the types of materials described, there may be others synthesized following other routes that display similarities in their structural characteristics [27], particularly the kind of heterogeneity typical of the polymer-derivatized adsorbents.

2.1. Media Containing Covalently Attached Polymers

Structures that include covalently attached polymer inclusions represent the most widely used of the polymer-derivatized media and were also among the earliest. These adsorbents (Figure 2a) are typically made by infusing a polymer into pre-existing porous particles that provide sufficient mechanical strength for use in preparative chromatographic columns. The polymer is then covalently attached to the particle surface within the pore space, and if not already functionalized prior to infusion, it is derivatized by incorporation of ligands appropriate for the intended mode of chromatography. This approach dates back at least to the Spherodex™ media developed about 30 years ago [28] by adding dextran inclusions within silica particles. More recent materials of this kind include the Sepharose™ XL and Canto™ resins, based on dextran inclusions in highly cross-linked agarose base matrices.

The architecture of these adsorbents is intrinsically heterogeneous in comprising a composite of a rigid base matrix and a soft inclusion. However, there are additional levels of heterogeneity that are difficult to control and characterize; it is correspondingly difficult to assess their effect on different aspects of chromatographic behavior, although the functional properties may be acceptable or even superior. The types of heterogeneity involved are, for example, the distribution of the added polymer within the pore space and the distribution of ligand between the polymer and the base matrix. These issues can have an impact on several chromatographic mechanistic issues, such as the residual pore space available for diffusion of adsorbates, the accessibility of the base matrix to the adsorbates, and the relative amounts of adsorbate associated with the base matrix and the polymer. A key parameter is the effective size of the polymer molecules; molecular weights of order tens of thousands are typical [26], so depending on the properties of the polymer involved, random coils with effective diameters of 10 nm or more can be expected. This would then be a characteristic length scale for chain “packing” within the pore space.

2.2. Media Containing Grafted Polymers

Grafted polymer chains are typically introduced onto a base matrix by a surface activation or initiation procedure followed by polymerization of a monomer or copolymerization of multiple monomers; an alternative is block polymerization. An early class of polymer-functionalized media was the Fractogel™ EMD “tentacle” media [25], in which acrylamide derivatives were polymerized onto polymer base matrices, while the more recent media of this type include the Eshmuno™ product line [29]. The monomers included groups functionalized to produce different ion-exchange ligands, including strong and weak cation and anion exchangers. The tentacle groups were presented as accomplishing several functions [25]: decoupling the control of capacity from the surface area of the adsorbent, separating the adsorbates from the base matrix, and providing sufficient flexibility in the positioning of the ligands to allow more facile association with charges on the adsorbate (i.e., the tentacle functionality).

The media discussed in this section differ from those discussed in section 2.1 mainly in being attached via the chain ends rather than linked at one or more arbitrary points along the chain length (Figure 2b), and in fact the two classes are not always distinguished explicitly [14]. As was the case in section 2.1, the nature of grafted polymer media is determined to a large extent by the amount of polymer added, and for grafted polymer media in particular this can be decomposed into the distinct effects of the graft density and of the chain length, which affect the microstructure and consequently the polymer-adsorbate interaction. Chain

lengths in the range 5–50 monomer units were found in the original exploration to be effective [25], which would correspond to chain lengths of about 1–10 nm; given typical protein diameters of several nm, it is apparent that significant differences may be expected in the chain conformation and the nature of the interaction over the range of chain lengths explored. Shorter chains would be expected to form a brush (Figure 2c), while longer chains would be long enough to adopt a random coil conformation (Figure 2b). A fairly abrupt increase in binding capacity with increasing degree of grafting has been attributed to formation of a hydrogel in the pore space [30], but this was based on functional rather than structural information. Longer grafted chains may be long enough to occupy a significant fraction of the pore cross-section, as is the case for the media in section 2.1, and a transition from a brush to a coil conformation may also be expected with increasing length, so there remain significant questions about the structure of grafted polymer media.

2.3. “Gel-in-a-Shell” Media

The term “gel-in-a-shell” is applied to adsorbents in which the whole pore space is occupied by a cross-linked gel structure (Figure 2d). The best known of these materials are the HyperD™ group [24,31], the name of which reflects the rapid “hyperdiffusive” transport that is observed during protein uptake. These materials are made by infusing a monomer into particles of a porous inorganic or, more recently, a ceramic solid and polymerizing it *in situ*. The monomers may be chosen so as to incorporate the desired chromatographic functionality, or suitable ligands may be added by standard derivatization methods subsequently. The UNOsphere class of acrylamido-based materials appear to have a similar type of structure, although they are synthesized in a single step; their structural and functional properties were characterized extensively before they were commercialized under the UNOsphere name [27,32–34].

Gel-in-a-shell particles are nominally the simplest of the composite media because they comprise only two regions, namely the solid “shell”, which provides mechanical strength, and the uniformly gel-filled pore space. The gels should be somewhat similar to those used in early protein adsorbents or to those still used routinely for such applications as gel electrophoresis, drug delivery and tissue engineering [35,36]. The concentration of monomer used controls the mesh size of the gel within the pore space; the gel concentration in HyperD media has variously been reported to be 5% [37] and in the range 10–17%, compared to 5–7% in gel electrophoresis [24]. Whichever value is correct, these gels are clearly much more restrictive than open pores of order tens of nm. This is an important consideration in view of the need for protein transport through the gel in order to reach the particle interior; there is no parallel pathway through a more open pore route. This might suggest that protein transport would be slow, but that is in fact not the case, as indicated by the HyperD name; this topic is revisited in section 3.4.

3. Properties of Polymer-Functionalized Media

The performance of stationary phases in preparative chromatography of proteins is usually characterized in terms of measures relevant to the bind-elute mode of operation that is most widely employed in practice. Two quantities of interest are the dynamic binding capacity (DBC) during loading, in frontal elution (breakthrough) mode, and the resolution during elution, which for ion-exchange is typically via step or linear salt gradients. An alternative measure of performance during elution is the pool volume, which, like the resolution, is related to the peak width. Each of these measures represents a complex convolution of a number of more fundamental retention, transport and kinetic properties. A benchmark for loading capacity is the static or equilibrium capacity, which represents an upper bound that the DBC is limited from reaching by the finite rate of uptake and adsorption, with intraparticle diffusion the main rate-limiting step for high-capacity adsorbents. Resolution,

which is also an important measure of performance in analytical chromatography, is similarly controlled by a combination of adsorptive and transport properties. However, it is measured under conditions of decreasing affinity of the adsorbate for the stationary phase and there is a much wider range of conditions that can be employed, so DBC values are frequently reported by manufacturers whereas it is difficult to establish a meaningful basis of comparison for resolution or for a surrogate such as pool volume. In view of our interest here in mechanistic interpretations, the examination of chromatographic performance in this section covers both the measures of practical interest as well as more fundamental ones. These include sorptive behavior (isotherms, linear isocratic retention factors) and transport aspects, covering both loading and elution, as well as structural characterization.

There are of course a plethora of results available that are relevant to the present analysis, but those for individual proteins on individual stationary phases are much less informative than comparisons involving multiple proteins and media. The most comprehensive set of data is from a large set of studies involving five proteins and a wide variety of strong and weak cation and anion exchangers [38–43], augmented by additional results in a recent review [10]; these studies reported practical functional measures of performance such as dynamic capacities as well as more basic measures of retention and transport efficiency, such as plate heights. Although polymer-derivatized media represented only a fraction of the adsorbents studied, the trends were sometimes clear enough for the distinctive properties of this class of media to be identifiable. However, several other, albeit smaller, studies have included more controlled investigations in which the effects of the added polymer were explored by comparison with the base media or with media with differences in derivatization [23,26,29,30,44–49]. The phenomenology of many aspects of performance is therefore quite well established both qualitatively and quantitatively, and is summarized in the remainder of this section. However, clear improvements in different aspects of performance are not always apparent, which may indicate a role for one or more subtler effects, again emphasizing the importance of a more complete mechanistic understanding.

3.1. Structural Characterization

As discussed in the Introduction, relevant structures of interest for adsorbent characterization span length scales from the particle diameter and its distribution, through the pore size distribution, down to details of the surface topography and ligand structure. Since most of the adsorbents of interest are prepared on base matrices that are also used for more conventional resins, the distinctive features of the polymer-functionalized media are on smaller length scales, with the pore and polymer structure especially important for characterizing the internal architecture and inferring mechanistic information regarding retention and transport.

Several methods that are widely used to characterize porous media in general have been applied to characterization of polymer-functionalized media, but in all cases caution is in order in interpreting the results. Several of the best known methods, including mercury intrusion porosimetry [50] and nitrogen sorption [51], require the samples to be dried, which can cause reversible or irreversible changes in structure relative to that in the hydrated state in which the materials would be used in biochromatography. Inverse size exclusion chromatography (ISEC) [52,53] overcomes this handicap, but questions can arise regarding whether the full pore space of interest is accessible to the (usually neutral) molecular probes (e.g., dextrans) that are used. The results of most characterization methods, including those mentioned, also suffer in that their interpretation is based on physical models that introduce different levels of idealization. Direct comparisons of different methods [54,55] cannot be generalized because the different methods are most effective for pore sizes in different ranges. However, for bioseparations media it appears that a number of different methods produce quite consistent results for fairly rigid adsorbents [54]. However, for softer

materials methods such as mercury porosimetry can no longer be used, and even for the methods that can still be applied, the discrepancies become more severe [54].

In view of the complexities of dealing with softer materials, the polymer additions in the media of interest here introduce important complications, as is reflected in the behavior reported for several such adsorbents. Proteins as small as ribonuclease are completely excluded from HyperD under non-binding conditions, although smaller molecules such as NaCl and amino acids are able to enter [24]. For ISEC performed using dextrans covering a range of molecular weights, clear differences are seen between resins with and without polymer added [21,26,56] for dextran-loaded media such as Sepharose XL. However, for the smallest probes used, typically glucose, the total apparent porosity is the same independent of polymer derivatization [21,26], although ISEC with small probes appears to understate the total porosity [54]. Similar effects are seen for Fractogel EMD vs. Toyopearl 650 and for Spheredex vs. the silica base matrix [57]; furthermore, in both these cases there are clear differences in accessibility for the cation- and anion-exchange versions of the polymer-functionalized media, indicating differences in the degree of polymerization or in the polymer structure produced.

An important additional consideration is the effect of salt concentration on the ISEC results, the results of which can shed light on the dynamic nature of, yet differences among, the polymer additives. For example, if the standard ISEC analysis is valid under all conditions examined, the tentacle-containing Fractogel EMD and the dextran-containing Spheredex show opposite trends [57]. The tentacle adsorbent shows a loss of narrow-pore volume and an increase in mean pore size with increasing salt, suggesting a collapse of the tentacles due to screening of intra- and interchain electrostatic repulsion. For Spheredex, however, there is an apparent decrease in the mean pore size with increasing salt; this is more difficult to interpret, but it may reflect screening of repulsion within a cross-linked gel-like structure within the pores.

More recent measurements using dextran-functionalized agarose ion-exchange media show greater partitioning of both charged and uncharged dextran probes of intermediate size with increasing salt, but little effect of salt if both the immobilized and probe dextrans are uncharged [26,58]. These results were interpreted differently, however. A straightforward application of ISEC principles is that uncharged dextran probes are just steric measures of the size of the pore space. This leads to a conclusion that increasing salt screens intrachain repulsion that at low salt concentrations causes expansion of the charged dextran layer, reducing the effective pore space [26]. However, an alternative argument is that even a neutral probe can interact electrostatically with a charged surface due to its displacement of the electrical double layer, with the resulting analysis [59] leading to the conclusion that the thickness of the immobilized charged dextran layer is in fact relatively unaffected by the salt concentration [58]. A questionable element of this argument is that the theory on which it is based [59] assumes that the interacting probe and surface are electrically impermeable solids, whereas in this case they are in fact polymers filled with high-dielectric water and may also admit salts that can screen electrostatic interactions. Therefore the relative consistency of the data for these systems is offset by some controversy in the analysis.

While even relatively small neutral probes are excluded from the polymer or gel in many cases, this changes dramatically for proteins under binding conditions. Even fairly large proteins are readily taken up, showing the importance of an attractive driving force in overcoming an apparent steric exclusion effect. Such behavior is considered in the following sections in the context of binding, adsorption or partitioning.

3.2. Binding Capacity

As noted earlier, the static binding capacity represents an upper bound on the dynamic binding capacity under the same conditions, so the two quantities should be considered in juxtaposition. The static capacity is based on an equilibrium measure of adsorption that is most thoroughly captured in the adsorption isotherm, in which the amount of adsorbed protein is presented as a function of the protein concentration free in solution at equilibrium.

Full isotherms have been reported for several proteins of different sizes on different types of polymer-functionalized media, including dextran-containing Sepharose XL and Capto adsorbents [10,23,56] and custom-synthesized analogs [26,47], GigaCap materials [10,49] and HyperD gel-in-a-shell products [10,37,60]. Although lysozyme has been by far the most frequently used protein for such studies, there is sufficient breadth, reproducibility and variety to conclude that, despite the appreciable differences among these materials and between them and their unmodified counterparts, the isotherms follow the same kind of concave downward form to be indistinguishable in shape from isotherms on more conventional protein chromatography media. They also generally appear to be similarly close to rectangular, depending on the salt concentration, although there are some differences among results from different research groups. The isotherms can be fitted using standard isotherm equations such as the Langmuir and steric mass action models, although the Langmuir model is sometimes too “soft” to fit the transition to the plateau at low salt concentrations. Even if the quality of fit is satisfactory, however, the model fit must be regarded as purely descriptive, as the mechanism of partitioning in the polymer-functionalized media is likely to be quite different from the physical models assumed for the frequently used isotherm models (section 4.2).

The static capacity for a given system is the concentration of adsorbed material in equilibrium with the feed concentration. Because protein adsorption isotherms in ion exchangers at the low salt concentrations used for loading are near-rectangular, it is the plateau value of an isotherm that is of greatest interest for loading. For dextran-functionalized agarose-based ion exchangers, for which data are available [23,47,56] that provide the clearest basis of comparison to underivatized counterparts, there is some variability but a general tendency toward a higher capacity than for the dextran-free media at low salt, in some cases by as much as a factor of two [23]. Similarly dramatic differences are seen in comparing the static capacities of GigaCap to that of Toyopearl SP 650, a cation exchanger without added polymer prepared on the same base matrix [49], and clear differences are also seen with Fractogel EMD media, on the same base matrix [11]. Even without a direct basis for comparison with an underivatized counterpart, the results of isotherm measurements can be informative: comparisons for multiple proteins of isotherms for a variety of ion exchangers showed HyperD and GigaCap media to have the highest plateau values [10], with the static capacity values for Sepharose XL media also consistently at the high end [10,39,42]. The high values for HyperD media presumably reflect the fully pore-filling nature of the gel, allowing essentially the full volume to be occupied by protein. For GigaCap the high values are especially noteworthy given that the analog without polymer derivatization, Toyopearl SP 650, has a particularly low static capacity because of its low phase ratio [19]. That high static capacities are not seen more universally on polymer-modified media despite the 3-D partitioning capability discussed earlier presumably reflects the influence of additional mechanistic factors, some of which are discussed in section 4.2.

The sensitivity of the isotherms in general and the plateau values in particular to the salt concentration is important for both loading and elution. The general trend with increasing salt concentration on polymer-functionalized media [23,37,47,49,60] is towards softer isotherms and lower plateau values, similar to the behavior of conventional media. However,

direct comparisons of isotherms for adsorbents on the same base matrices with and without immobilized polymer [23,47,49] show important differences in the magnitude of the changes, particularly for larger proteins. For these systems the shifts in the isotherms towards lower adsorbed amounts with increasing salt concentration are significantly steeper for the polymer-functionalized media. This leads eventually to complete exclusion from the polymer at higher salt concentrations, a limit corresponding to that discussed for neutral probes or non-binding conditions in the previous section.

Complementary data showing similar trends are seen in comparisons of protein adsorption on different media measured using high-throughput screening methods [10]. These measurements were acquired via batch measurements that did not necessarily attain equilibrium, with the measured values reported as “binding capacity strength”. They also appear to show a higher sensitivity to salt concentration on polymer-functionalized media for most proteins studied.

Dynamic binding capacities are more widely reported than static capacities, especially by vendors and industrial researchers, but the differences in the conditions used (protein concentration, salt type and concentration, as well as flow rate) make direct comparisons among the results of different groups less meaningful. The very large data set of Staby and co-workers [10,38–43] allows many of these comparisons to be made, although comparisons among DBCs on different base matrices should be performed with some care since the “high” and “low” flow rates used for each resin were based on the maximum flow rate recommended by each manufacturer. Nevertheless, the conclusions involving polymer-functionalized media include that some of these media, such as representatives of the Sepharose XL, Ceramic HyperD and GigaCap families, tend to have among the highest absolute DBCs, at least for proteins up to about the size of BSA. Comparisons between Sepharose XL and the more conventional Sepharose FF on the same base matrix show clear excesses for the XL over the FF media. Similar conclusions can be reached for Capto S, GigaCap S, S HyperD and Fractogel SO₃⁻ in a separate study, especially for monoclonal antibodies [48].

The nature of the DBC as a compound measure combining the static capacity with transport limitations raises the question of whether the favorable DBC performance of some polymer-functionalized media is due to static capacity advantages or to rapid transport. The matching data of Staby et al. clearly show that these media with high DBCs also have high static capacities. A similar conclusion is indicated by DBC results on monoliths, where transport is predominantly convective and fast, and transport limitations are therefore often minimal. Polymer functionalization in such a system led to DBCs 2–3.5 times higher than for an unfunctionalized monolith for several proteins and plasmid DNA [61].

Although the DBC enhancement due to polymer functionalization appears to be attributable in some cases to the effects on the static capacity, DBC trends show the derivatized media to have very good transport properties as well. This is seen in that both the Sepharose XL and the HyperD media maintain high DBCs even at the higher flow rate studied [39–43]; the decline for Sepharose XL is smaller than for HyperD, but the flow rates used for HyperD were several-fold higher because of the much more rigid base matrix. Earlier results for S HyperD also showed impressive consistency of shape of full breakthrough curves for lysozyme even up to extremely high linear velocities [37,62].

The complex interplay between retention and transport in determining the DBC is seen in the effect of the salt concentration. Since the static capacity generally decreases with increasing salt, the DBC may be expected to do the same, but several reports [44,48,58,63–66] have demonstrated an initial increase in DBC with increasing salt concentration at low

salt, leading up to a well-defined maximum and a subsequent decrease. This form of response may be seen on both conventional and polymer-functionalized media for certain proteins under appropriate conditions, but where direct comparisons are available [44,48,64], the maxima appear higher and more pronounced for the polymer-functionalized media, although the DBCs drop off more rapidly with increasing salt beyond the maxima in some cases. The maxima on polymer-functionalized media also appear more likely to be observed for larger than for smaller proteins. The existence of a maximum has important practical implications in that it suggests a somewhat counterintuitive benefit to loading a column at an intermediate salt concentration rather than under very low salt conditions.

The existence of a maximum in DBC with increasing salt concentration despite a corresponding decrease in static capacity is consistent with faster transport with increasing salt at lower salt concentrations. This is discussed as part of the overview of intraparticle transport in section 3.4.

3.3. Linear Isocratic Retention

Isocratic retention data for proteins in ion exchange are rarely used in industrial practice because they are not directly relevant to the overloaded bind-elute mode of operation that is most widely employed. These measurements are also quite tedious even allowing for the ready automation of the measurements on a typical chromatographic workstation; simpler routes to estimating isocratic retention parameters are also possible by using a small set of gradient measurements [67,68]. Despite the lack of direct relevance of isocratic retention to industrial practice, such data can be useful in providing fundamental insight with practical implications, and they have been measured for a number of systems involving polymer-functionalized media.

The retention factor k' is determined experimentally from the incremental effect of adsorption on the retention time, normalized by that due simply to the transit time through the accessible volume of the column. Isocratic retention values measured under linear conditions provide a fundamental measure of the binding strength, i.e., the strength of the protein-adsorbent interaction. Such data have a practical use in that comparison of data for different proteins on the same adsorbent reflects the selectivity of adsorption and therefore provides information useful in inferring elution order and resolution. Furthermore, k' is essentially the initial slope of the full adsorption isotherm and so has proven useful in predicting isotherm behavior for conventional media [69].

The mechanistic origins of the retention factor can be seen via its decomposition $k' = K_{eq}\varphi$, where K_{eq} is the adsorption equilibrium constant and therefore captures the chemical contribution to retention and φ is the phase ratio and thus represents the physical contribution. This decomposition can be interpreted differently for conventional vs. polymer-functionalized adsorbents, being based on accessible adsorption area in the former and partitioning volume in the latter. Although phase ratios for typical conventional adsorbents for proteins may span an order of magnitude or so [53,57,70], the equilibrium constant is exponentially related to the interaction energy and so spans many orders of magnitude; similar behavior may be expected for polymer-functionalized systems. Therefore comparing plots of k' as a function of salt concentration for different systems provides a useful measure of the strength of protein-adsorbent interactions. Such plots are usually presented on semilog or log-log axes; the former form is suggested by the stoichiometric displacement model (SDM) [71,72], but it is also a convenient empirical representation even without invoking a particular retention model.

Extensive plots of k' against ionic strength for a number of proteins on a wide variety of ion-exchange stationary phases, both conventional and polymer-functionalized, have been

reported [10,38–43,73]; analogous data have been extracted from gradient-elution measurements [48,74]. These plots show the expected decrease in retention with increasing ionic strength, with the precise dependence captured quite well by straight-line empirical fits on log-log axes, consistent with the form suggested by the SDM [71,72]. A noteworthy feature of the plots is that the slope is approximately the same for a given protein at a fixed pH on different resins, suggesting some commonality in the binding mechanism, albeit not necessarily validating the SDM mechanism. This feature is seen for both strong and weak ion-exchange ligands and, more pertinent to the present context, for both conventional and polymer-functionalized media. Despite the similar slopes, there may be distinct differences in k' values on different materials, but a general similarity in the ordering of different resins for different proteins. In this approximate ordering [10] there is some bias of polymer-functionalized media toward stronger retention, but by no means a notable difference with conventional media.

Similar conclusions emerge from a direct comparison between retention behavior on agarose media with and without the presence of dextran modifiers [23]. However, there is a more subtle element that emerges, namely that the void volume measurements for larger proteins on the dextran-functionalized media indicate complete exclusion from the polymer at high salt concentrations. This effect has an operational consequence in that it complicates the calculation of k' on a consistent basis, but it also raises mechanistic questions regarding the protein-polymer interaction and its chromatographic interpretation.

Staby and co-workers [10,38–43] also report results on binding strength as a function of pH for numerous systems. These measurements were performed by gradient elution and therefore provide a more complex measure of retention, but they still reflect comparative trends as the protein and/or the stationary phase is titrated. As has been shown previously [72], net charge is not a definitive guide to retention, but the results generally show the expected trends due to the change in the net charge on the protein and, in some cases, titration of weak ion-exchange functional groups. Clear or striking systematic differences between the polymer-functionalized media and more conventional ones are not apparent, but in several individual cases notably strong retention is seen on some polymer-functionalized media, including Sepharose XL [39], HyperD [43] and UNO [10].

3.4. Interphase Transport

The rate at which a solute can be transferred from the mobile phase to the sorbed state within a porous stationary phase is a major factor determining the efficiency of a chromatographic operation. Whether this transfer is limiting is determined by the rate relative to that for flow along the length of the column: if transfer is fast, the chromatographic behavior approaches that for ideal equilibrium chromatography, while if transfer is slow it leads to broad peaks or diffuse breakthrough fronts that reflect low efficiency. The step that usually limits the rate of transfer is intraparticle diffusion, especially for macromolecules such as proteins, so measurement and interpretation of intraparticle transport are valuable routes to achieving systematic design of chromatographic operations.

The phenomenology of intraparticle diffusion is similar for conventional and polymer-functionalized materials, so the main features are described without distinction between the two classes; aspects peculiar to polymer-functionalized media are discussed subsequently. Intraparticle diffusion is usually described using one of two limiting transport models [75,76]. In the pore-diffusion limit only the free solute in the pore lumen is regarded as mobile, so the protein flux is calculated using a pore diffusivity and the gradient in the free protein concentration. The other limiting case, variously known as solid, gel, surface or homogeneous diffusion, treats the adsorbed solute as being mobile, so the flux is based on a

homogeneous diffusivity and the gradient in the adsorbed solute concentration. Homogeneous diffusion models may be ambiguous as to whether or not the free solute is included as well, but in the case of ion-exchange of proteins, the adsorbed concentrations are usually many times higher than the free concentrations, so the distinction is generally not significant. In other cases, however, the free and adsorbed solute may explicitly be treated separately, with overall transport accounted for using a parallel diffusion model [75,77–79].

Measurements of intraparticle transport [75] may be made macroscopically using column and batch methods or microscopically using confocal or optical microscopy. For macroscopic measurements the uptake mechanism is not necessarily known, so the data may be fitted to any of the available transport models; different models may produce comparable predicted uptake curves [19,60], making model discrimination via such data difficult. Microscopic measurements are well suited to facilitating model discrimination in view of the quite different uptake profiles predicted by the two limiting models. For pore diffusion coupled with adsorption via high-affinity (sharp) isotherms, the predicted uptake profile is sharp, approaching the form predicted by the shrinking-core model [80,81] in the limit of a perfectly rectangular isotherm. For homogeneous diffusion, on the other hand, the nature of the adsorption isotherm affects only the behavior at the particle surface, and the purely Fickian diffusion assumed in the particle interior is predicted to result in diffuse intraparticle concentration profiles.

The difference in the form of the concentration profiles can be distinguished to varying degrees experimentally using confocal or optical microscopy via any of several approaches [56,63–65,78,82–87]. Experimental observations of uptake patterns generally show sharp, shrinking-core-like behavior at lower salt concentrations and diffuse profiles consistent with a homogeneous diffusion mechanism at higher salt concentrations. The transition that is inferred from pore to homogeneous diffusion is accompanied by a notable increase in the apparent transport rate, as reflected in, for instance, the apparent pore diffusivity, that is generally also apparent within the domain where pore diffusion alone is observed. This rate increase is the likely explanation for the maximum in the DBC at intermediate salt concentrations, discussed in section 3.2, that is sometimes seen in breakthrough behavior.

Two mechanisms are most widely cited as likely explanations for the faster uptake with increasing salt. One is the electrostatic repulsion between adsorbed and diffusing protein molecules, which results in an electrostatic exclusion effect [58,63,64]. Given the nature and range of electrostatic repulsion, this effect is likely to be important especially for large molecules, narrow pores and low salt concentrations. However, it does not provide a clear rationale for a change in the form of the concentration profile with increasing salt. That transition is instead usually attributed to the weakening of the strength of adsorption with increasing salt concentration, leading to the onset of diffusion of the adsorbed protein [65,77,78,84]. The contribution of a homogeneous-diffusion component to the overall transport rate can not continue to increase monotonically because although the homogeneous diffusivity may increase at high salt, the equilibrium adsorbed concentration and hence the gradient in the adsorbed protein concentration decreases, eventually to zero.

For the specific case of polymer-functionalized adsorbents, the transition between the apparent shrinking-core and homogeneous-diffusion limiting cases is seen in some but not all cases as well, although some caution is needed because these systems have not been explored as extensively as have conventional media. Here, as in aspects of the discussion earlier, an informative system is that of dextran-functionalized agarose media, the transport properties of which have been compared with those of their dextran-free counterparts [26,44,47]. The general increase in apparent pore diffusivity with increasing ionic strength is seen in dextran-modified media especially for larger proteins, under conditions where both

confocal and optical microscopy indicate the presence of sharp intraparticle concentration profiles [44,47,88]. For lysozyme, however, a decline in the apparent pore diffusivity is reported for Na^+ concentrations from 20 mM upward [47], and the DBC trend is monotonically decreasing, tracking that of the equilibrium capacity [44]. Interpreting these transport rate trends in terms of microscopic data is problematic, however, since confocal [44] and optical microscopy [47] experiments were not performed on identical systems. Despite this difference, however, it appears that a transition to faster diffusion as a result of a homogeneous contribution occurs at lower salt concentrations in dextran-functionalized than in conventional agarose materials, contributing to the higher efficiency of the former.

An additional factor complicating interpretations of uptake in these media is that an apparent shrinking-core mechanism may not correspond to immediate local saturation of the adsorbent. The appearance of a slow uptake mode at long times in breakthrough measurements suggests a two-stage mechanism involving shrinking-core-like partial loading, limited by pore diffusion, followed by slower kinetically limited uptake to full saturation. This so-called partial shrinking-core mechanism [44] has been interpreted as resulting from a slow rearrangement of sorbed protein within the polymer layer to allow additional uptake to full saturation.

Other types of polymer-functionalized media have been studied as well, most notably the HyperD adsorbents, which display extremely fast uptake [24,37,60,62,89], to the extent that breakthrough fronts remain very sharp up to remarkably high linear velocities and extraparticle transport limitations become apparent at lower protein concentrations. These measurements were not accompanied by microscopic observations, but optical observations on charged acrylamide and agarose gels supported in capillary tubes [90–92] have generally shown diffuse protein concentration fronts even at very low ionic strengths, e.g., in 10 mM Na_2HPO_4 buffer. Sharper profiles are sometimes seen, though, and the form of the profiles appears to be related to the strength of binding [92], consistent with the discussion above.

The apparent homogeneous-diffusion contribution to transport in polymer-functionalized media is not surprising in view of the volumetric nature of partitioning in these media. At the same time, the uncertainties surrounding the partitioning mechanism(s) are compounded in the case of transport, given the evident roles of the polymer, the protein and the solution conditions. A particularly challenging complication is the dynamic nature of the polymer, which can undergo structural changes due to the presence of both the salt and the protein. Some of the issues concerned are discussed in the remainder of this review.

4. Molecular Mechanisms Affecting the Performance of Polymer-Functionalized Media

The functional properties of polymer-functionalized ion exchangers discussed in section 3 indicate a general qualitative similarity with those of conventional ion-exchange media. As a result it is feasible to use standard and well-established phenomenological models to describe these systems, including for process development and design purposes. There are, however, some noteworthy quantitative differences in performance between the two classes of media, and these are pronounced enough to have driven, and still to be driving, the development and marketing of new commercial resins. Further improvements and optimization of such new media would be aided by an improved mechanistic understanding of how these materials function. There are of course important similarities to conventional media, but the presence of the functionalizing polymer is a significant complicating factor that limits the development of more complete mechanistic models. The purpose of this section is to introduce and explore some of these complicating factors, at least qualitatively.

4.1. Structure of the Polymer Layer

Because of the quite different nature of gel-in-a-shell media from the other two classes of polymer-functionalized media discussed in section 2, these different classes of polymer are discussed separately. All of these kinds of polymer materials are also used in a variety of different applications, in some of which they have been characterized quite extensively, so there is the potential for chromatography research to benefit from insights developed in different contexts.

Covalently attached or grafted polymers at surfaces are used in such applications as steric stabilization of colloids [93] and promotion of biocompatibility by limiting adsorption [94]. The nature of the polymer layer depends on numerous factors [93]: the type of polymer and its interaction with the solvent, the molecular weight, the immobilization density, whether the polymer is adsorbed or grafted. Some possible situations are shown in Figure 3; they are discussed here only qualitatively, but the quantitative behavior can be estimated as well [93]. For polymer-functionalized media used for protein separations, the polymers are generally hydrophilic, so water is a good solvent and the coil swells when hydrated. The extent of swelling is also influenced by the intrinsic chain stiffness, which is determined by the chemical and physical structure of the polymer; for instance, branching, such as in dextran, increases the stiffness. Immobilization tends to bias the segment density towards having more segments near the surface, with adsorption or covalent attachment, where multiple regions of the molecule may be linked to the surface, showing a stronger such effect than grafting, where only one end is attached. The molecular weight plays the major role in determining the effective layer thickness, but the immobilization density can also play a role in that intra- and interchain interactions at high-density immobilization can force the chains out to a layer thickness that can significantly exceed the effective size of the polymer in free solution.

A major complication to especially a quantitative understanding of the structure of the polymer layer is the charge introduced by adding ion-exchange ligands, turning the chain into a polyelectrolyte. Electrostatic repulsion among immobilized charges causes the chain to swell well beyond the extent due simply to the solvent quality, and this swelling can be modulated by the addition of salt, resulting in electrostatic screening. The key issues for this phenomenon are well established, being based on the spacing between charges, determined by the ligand density on the polymer, relative to the Debye length [93], which characterizes the range of electrostatic interactions. The Debye length is about 1 nm at 0.1 M ionic strength and follows an inverse square root dependence on ionic strength, so ligand densities corresponding to charge spacings of order 1 nm are likely to result in polymer layers that are susceptible to changes in salt concentration.

Despite this simple conceptual structure, the physics of polyelectrolytes [95,96] remains incompletely understood even in free solution, and the additional complications of immobilization, possible ligand attachment to the base matrix and the finite pore structure only add to the challenge. The magnitude of the swelling possible can be seen for the polymers used in S- and Q-HyperD [24], where swelling ratios as high as about 14 were found in DI water; even in 1 M NaCl swelling ratios of about 3 were found. In the actual HyperD materials, confinement of the polymer prevents such swelling, but the resulting swelling pressure must be accounted for [97]. For the other kinds of polymer-functionalized materials, even the interpretation of experimental measurements of the polymer layer thickness in chromatographic media is controversial, as discussed in section 3.1.

4.2. Partitioning into the Polymer Layer

Partitioning of protein into the polymer layer is of course the goal of the chromatographic operation, so structure-function relations that can contribute to understanding of the mechanisms involved are highly desirable. As on conventional media, the driving force for sorption is electrostatic, but the space available for sorption may ultimately be limiting. On conventional media this space is the accessible surface area, whereas on polymer-derivatized media it would be expected to be the polymer volume. An analysis based on rough estimates suggests that the volume is often limiting on dextran-modified agarose, although for proteins with a high charge density, the available ligand charge may be limiting instead [23].

Beyond these simple concepts, however, are many potentially complicating features. Even for conventional media at low coverages, the mechanistic basis for retention, specifically the protein-surface interaction, has not yet reached the level of predictability. Simplistic stoichiometric models [71,72] have largely been supplanted by more mechanistic colloidal ones [70,98–105] that can often capture trends correctly but still lack quantitative predictive capabilities. The stoichiometric models can be applied to sorption on polymer-derivatized media as well, but again much of the essential physics is missing, and 3-D analogs to the colloidal models will require incorporation of several additional features discussed in the remainder of this section.

A less obvious issue that should be considered is that of partitioning of small ions, the concentrations of which are used to manipulate protein retention in ion-exchange systems. Since the ion concentrations within and outside the polymer layer may be different, the distribution of small ions should be considered in evaluating the mechanisms affecting protein partitioning. Ion exchange involving small ions was in widespread use before it became common for proteins, and the analysis of ion exchange of small ions has correspondingly been well established for many years, with the classic text of Helfferich [97] providing a thorough treatment. Some of the materials most commonly used for small-ion exchange were in fact polymer gels with some similarities to the polymers used to functionalize media for protein applications. The partitioning of small ions into charged gels is generally treated within the context of the Donnan [106] theory as applied to ion exchange, as can be understood using Helfferich's characterization of a cation exchanger [97]. The (negative) charge on the exchanger is balanced by the presence in the exchanger of an equal concentration of counterions (cations in this case), while free co-ions (anions in this case) are absent. If the charge density on the exchanger, in units of concentration, is much higher than the salt concentration in the outside solution, the cation concentration inside the exchanger is higher than that outside and the anion concentration lower. However, if free anions (co-ions) were to enter the exchanger, the disruption of electroneutrality would create a potential, the Donnan potential, that would tend to draw these co-ions back into the surrounding solution. Similarly, cations would be restricted from moving from the exchanger into the external solution despite the concentration driving force for such transport.

The net result of this situation is that the salt in the external solution is, to a large extent, excluded from the interior of the exchanger when the external solution is more dilute in salt than the effective ligand concentration within the exchanger; in this region the co-ion concentration within the exchanger varies approximately quadratically as a function of that outside, while still being much lower [97]. When the external salt concentration becomes similar to, or higher than, the effective ligand concentration, the magnitude of this exclusion effect becomes much less significant, and the external salt concentration then provides a more faithful indication of the effective salt concentration inside the exchanger.

As is the case for small ions, electrostatic effects also play a role in protein partitioning, but the much larger size of proteins adds steric interactions as a major issue determining protein behavior. Protein partitioning can be seen as a balance between enthalpic electrostatic attraction and entropic repulsion [107], but this analysis may be somewhat simplistic. For instance, the electrostatic attraction is mediated by the electrical double layer, so the electrostatic component may include an entropic contribution as well [108]. Furthermore, the salt concentration, which is used to control the strength and the extent of partitioning, may be different inside the exchanger compared to the surrounding solution, as discussed above. Indeed, in the absence of size effects the typically multivalent nature of protein charge can make the ion exclusion effects for a protein quite pronounced [97], and the coupling of electrostatics involving the polymer, small ions and protein requires balancing multiple competing effects.

Despite these features complicating the electrostatic analysis, the steric factors are arguably more challenging to analyze. A key consideration is the polymer mesh size, which plays a role similar to that of the pore size in more conventional materials but is similarly heterogeneous and arguably much more difficult to measure. In addition, the polymer structure is dynamic and the structure can change depending on the solution conditions, e.g., during polymer swelling. Partitioning of protein into the polymer can also change the polymer structure, effectively causing deswelling that can reduce the volume of a parent gel by as much as a factor of 10 [109]. That adsorption is typically performed at low salt, where swelling is most pronounced, means that the opposing effects of these two factors must be weighed against each other. Furthermore, for materials other than those of the gel-in-a-shell type, the swelling and deswelling occur under confinement within a larger pore space. That the gel-in-a-shell materials may be less complex to analyze may be borne out by the reasonable predictability of partitioning in HyperD media [24] using the Ogston model [110] of partitioning into a gel described as a medium of randomly oriented rods.

The key effects in protein partitioning into a dynamic charged layer in chromatography have been explored by simulation for a grafted polymer layer [107], but with some significant approximations made in the representation of the polymer structure, the protein structure and the electrostatics. Furthermore, the finite size of the pore space was not considered. As noted in the previous section, even the correct theoretical representation of the structure of a polyelectrolyte chain is not yet settled science, so a complete and accurate molecular simulation of protein partitioning in polymer-functionalized media appears unlikely in the near term. However, computational methods based on molecular theories can be effective at capturing the levels of complexity involved, and have been applied to systems with similarities to those of interest here [111], so they may be a promising approach for discerning mechanisms. A more realistic expectation for actual chromatographic modeling is the use of mesoscopic models in which some features of the behavior are represented phenomenologically, based on insights gleaned from experimental observations, but ultimately such models can be informed by results of molecular theory calculations as well.

4.3. Protein Transport in the Polymer Layer

As noted above, the penetration of proteins into the polymer layer can be seen as being loosely analogous to the penetration of proteins into the pore network of a conventional chromatographic particle. Similarly, protein transport in the polymer bears some similarity to that in the pore network. Indeed, protein transport in HyperD media has been analyzed [24] in terms of diffusion of the molecule through an effective (Brinkman) fluid [112], based on measured properties from which the model parameters were estimated.

More generally, however, there are also important differences from pore transport in the structural details involved in protein diffusion in polymer media that have direct

implications for functional comparisons. The polymer structure, as characterized especially by its mesh size, is often likely to be “tighter” than is normally the case for a pore network, so diffusive transport would be expected to be severely restricted by the factors normally retarding pore transport, namely the finite porosity, the tortuosity of the network and especially hindrance effects [113]. How well these concepts, normally defined for continuum systems, apply to molecular transport through a restricted polymer network is an open question in itself. The ability of a protein molecule to move through the polymer network is presumably due partly to the dynamic nature of the network and therefore likely to depend on the chain entropy generally and the polymer chain stiffness in particular, as is evidenced by the strong dependence on size of the partitioning ability of uncharged probe molecules, discussed earlier.

As is the case for partitioning, transport of proteins in this environment is evidently facilitated by the electrostatic attraction between the protein and the ion-exchange ligands. This introduces the possibility of such effects as an electrokinetic driving force [83] or the coupling of transport of the protein and small ions [47,114], although again a continuum description may be problematic in accounting for protein transport in such a restrictive environment.

5. Concluding Remarks

The qualitative similarities in performance between conventional and polymer-functionalized media conceal noteworthy differences in the mechanisms that underlie the chromatographic function. The central one is of course the volumetric partitioning character of the polymer-functionalized adsorbents as compared to the surface adsorptive character of the conventional ones. However, additional functional properties, most notably transport ones, which are presumably largely determined by the partitioning mechanism, are also distinctive. Similar phenomenological models can be used to describe the behavior of both kinds of media and may indeed be adequate for many applications, albeit with model parameters either fitted to experimental data or determined independently. However, a more complete mechanistic understanding of the polymer-functionalized media would permit more rational approaches to designing novel materials. Many commercial vendors are likely to use existing base matrices for such new products, so much of the novelty in the design is likely to lie specifically in the polymer attachments. As seen earlier there are several broad classes of these, but a more complete understanding could allow incorporation of more nuanced design features exploiting such parameters as the polymer stiffness or ligand distributions. As Section 4 suggests, mechanistic modeling of the effects of such variables is especially challenging, so a more likely route to obtaining novel insights is experimental exploration of the performance of materials in which key structural properties are varied systematically. Some such studies have been reported in the open literature [26,47,115,116], but it is likely that similar studies were undertaken by vendors in the course of developing new products, and a meta-analysis of such data may be informative.

In the longer term simulation approaches may be able to approach a more realistic characterization of these systems than is currently possible. However, it seems likely to require concerted progress in several distinct areas of research and then combining them within a unified framework. That molecular-level intraparticle structural information would also be needed suggests that such an approach is likely to be able to produce only a conceptual model, but even this may be informative when used in conjunction with experimental approaches.

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Figure 1.

Transmission electron micrographs of protein loaded on Q Sepharose FF (top) and on Q Sepharose XL at low (middle) and high (bottom) protein loadings. Dark regions show distribution of protein, which is localized to agarose base matrix on FF but partitioned into polymer volumes of increasing size as loading increases. Reprinted from ref. [23] with permission from Elsevier.

Figure 2. Schematic of different structures of polymer derivatives in pores of base matrix. (a) Covalently attached polymer, with multi-point attachment per chain; (b) grafted polymer, with single point of attachment per chain; (c) polymer brush, where grafted polymer chains are too short to form random coil; (d) “gel-in-a-shell” hydrogel.

Figure 3. Schematic of transitions in attached polymer layer. (a) Poor to good solvent, showing swelling of polymer; (b) low to high surface coverage, showing entropic expansion; (c) increase in salt concentration (charged polymer), showing deswelling.