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Macrosorb Kieselguhr-Agarose Composite Adsorbents

New Tools for Downstream Process Design and Scale Up

Scientific Note

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ABSTRACT

Incompressible Macrosorb composite adsorbents, while retaining all the desirable properties of traditional agarose-based hydrogel media, overcome the operational limitations imposed by the use of soft hydrogels: They permit useful application of fast flow rates without restrictions on bed depth and they can be used in fluidized bed mode. Considerations which are important when contemplating scaled-up processing are discussed. A comparative cost estimate for a production process for extracting albumin from bovine serum in column equipment illustrates the various advantages which may be exploited when using a composite adsorbent in place of a conventional soft gel equivalent.

Index Entries: Composite adsorbents; downstream processing; large-scale; fast-flow.

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INTRODUCTION

Recent developments in biotechnology have given rise to a multitude of potentially commercial products, all of which require suitable isolation procedures. Ion-exchange and affinity adsorption are probably the methods of choice when the aim is to provide a high purity protein from a heterogenous feedstock (*1,2*). In comparison with other protein extraction techniques, these operations are relatively simple and efficient, and are especially suited for dealing with dilute solutions. However, various problems can arise when it is desired to increase throughputs from a laboratory-scale operation to a production unit for handling larger feedstock volumes. Most of the difficulties encountered are a consequence of the physical properties of the adsorbents used.

Beaded hydrogel materials are commonly selected when designing specific adsorption processes for protein recovery, and suitably derivatized agarose hydrogels are most often chosen because of their regular, open hydrophilic structure. However, agarose, acrylamide, and cellulose hydrogels, even when strengthened by extensive crosslinking, exhibit considerable compressibility that limits the operating conditions under which they can be used.

Consequently, there exists a large performance gap between flow rates under which currently available hydrogen adsorbents are able to operate and the much higher flow rates that can be used before adsorption efficiency begins to suffer under the limitations of the kinetics of the adsorption mechanisms on a molecular level. Thus, the full potential of hydrogel adsorbents, in their current form, cannot be realized.

It can be argued that high flow rates are of little use if chromatographic efficiency is impaired. However, column washing, scrubbing and re-equilibration steps also take their time, but do not require slow flow rates. Even if a particular separation requires loading or elution at lowered flow rates to achieve maximum performance, the ability to perform the column conditioning steps at considerably increased fluxes has a significant effect on the whole chromatographic process by decreasing the total cycle time.

When compressible gels are to be utilized, scale-up is generally achieved by the use of specially engineered equipment for the operation of shallow, large surface-area beds. The usefulness of adsorption and chromatography in downstream processing is reflected in the fact that these techniques can sustain the costs of the special engineering requirement, albeit only in the case of the production of high value products. It is clear that there is a need for considerable improvement, so that these recovery methods are made more economically accessible to permit the commercial production of low value materials.

There have been various attempts at producing alternative media which would overcome the operational restrictions of soft hydrogels, e.g., silicas with derivatized pellicular layers (*3*) and spirally wound ra-

dial flow cartridges (*4,5*). In contrast, Macrosorb composites (*6*) retain all the features of the original hydrogel. This is achieved by inserting the gel into a rigid, macroporous support granule.

Generally speaking, for a given pressure drop per unit length of packed bed, the flow rate achievable across a rigid composite medium is of an order of magnitude greater than for heavily crosslinked semirigid media. The semirigid media, in turn, exhibit a similar performance advantage over pure gel media (Fig. 1).

Macrosorb Composite Adsorbents

Macrosorb composite adsorbents suitable for large-scale protein extraction are manufactured using a three-stage process.

In the first step, purified kieselguhr (diatomaceous earth) is fabricated into Macrosorb-K (*7*). The physical form of Macrosorb-K is spheroidal granules that are typically in the particle size range 100–500 μm and the process provides them with macropores which have an average diameter of 15 μm. These macropores are several orders of magnitude larger than the pores found in other available porous silicas, and offer a large interconnected pore volume which can be space-filled with a hydrogel.

The low surface area of Macrosorb-K (0.5–1.0 m^2/g) ensures minimal nonspecific adsorption (nsa) by the support matrix and makes the support effectively "transparent" to proteins.

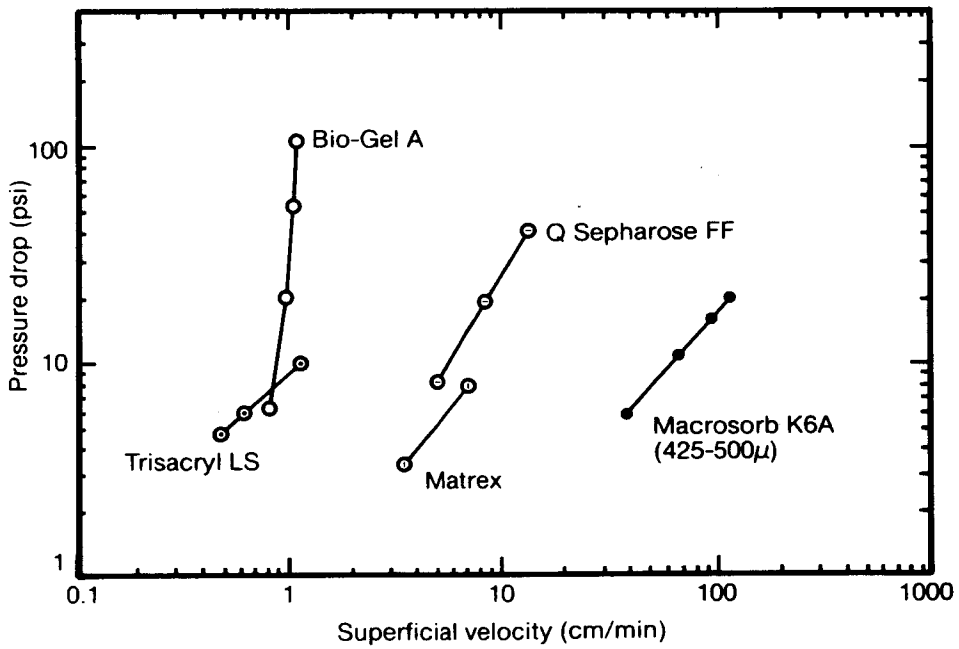

Fig. 1. Comparative flow characteristics of some adsorbent types.

In the second step, a hydrogel is introduced into the interconnected internal pore volume of the granule. The pore volume of Macrosorb-K is approximately 0.45 mL/mL solid. Thus, insertion of a 6% agarose hydrogel yields Macrosorb-K6A, which is the parent composite from which the currently available adsorbent range is manufactured. The third step involves the chemical derivatization of the agarose content to provide the desired adsorbent. Macrosorb-KAX adsorbents contain chemically crosslinked agarose. The types of adsorbents which can be produced range from ion-exchangers to any of the affinity adsorption which are based on traditional agarose "backbones."

When making ion-exchangers based on agarose, some degree of crosslinking is required to prevent the dissociation of the hydrogen-bonded gel as a result of forces exerted by the mutually repelling ion-exchanger groups present on the agarose strands. However, the degree of crosslinking needed to prevent this is substantially less than that required to produce the "mechanically improved" hydrogels widely used in current chromatographic practice. As a result, the composite ion-exchanger possesses a much lower level of nsa, which is expressed in practice as more specific adsorptions and significantly reduced levels of cumulative residue build-up. This has a great impact on product purity and column scrubbing requirements.

Macrosorb composite adsorbents are manufactured in the nominal size range 75–300 μm. The particle size of a column packing medium is a factor that becomes increasingly important as flow rates increase. We have shown that columns packed with composite adsorbents having particle diameters of less than 350 μm operate quite satisfactorily when flow rates of 5–10 cm/min are used. With larger particles, adsorption efficiency does not necessarily suffer, but elution profiles broaden significantly. The volume of a packed bed of Macrosorb adsorbent remains constant when it is subjected to changes in pH and ionic strength. The back pressure exerted by the composite medium has been demonstrated to be negligible, the major contributor to the back pressure being the equipment itself (Fig. 2).

Macrosorb composite ion-exchangers perform well under continuous cyclic operation. Lifetime studies show that subjecting Macrosorb-KAX.DEAE to 100 cycles of 25 m*M* tris-HCl pH 8 (10 vol), 25 m*M* tris-HCL pH 8 containing 1*M* NaCl (5 vol) and 0.2*M* NaOH (2 vol) at a linear flow rate of 2.5 cm/min (at 25°C) has no effect on the capacity or the resolving power of the column. After 600 further cycles of similar treatment, the capacity of the column appears to be unaffected, and there is only a barely perceptible worsening of the resolving power. Similarly, continuous pumping of 0.2*M* NaOH for 80 h at 2.5 cm/min was also shown to have no effect on column volume or capacity. This particular stability of the adsorbent is a highly desirable property because these NaOH solutions are commonly used for in-place scrubbing and depyrogenation of process columns and their packings.

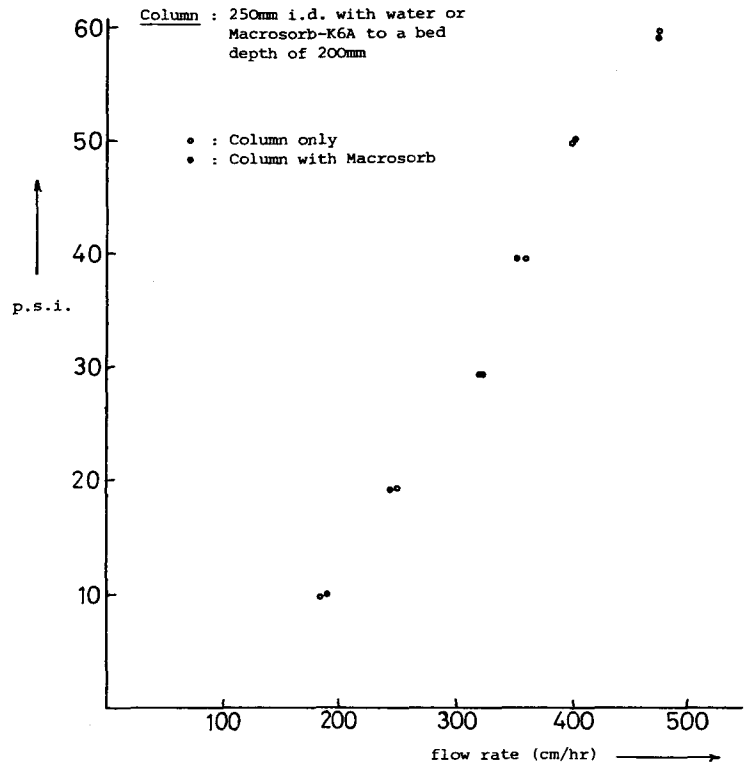

Fig. 2. Pressure drop vs flow rate for Macrosorb-K6A.

The additional density conferred to the composite by the inorganic support enables the adsorbents to be used in fluidized bed environments, an operational configuration impossible with conventional hydrogels.

Macrosorb composites are manufactured in compliance with current good manufacturing practice (cGMP) on an FDA approved site, and drug master files (DMFs) are being submitted for approval by the appropriate authorities. This will ensure that processes which utilize Macrosorb adsorbents for the production of pharmaceutical products comply with current regulatory requirements for the quality control of chromatography media which are to be used for that purpose.

The Use of High Flow Rates in Scaled Up Processing

Small-scale separations are normally performed with a view to isolating small quantities of a very pure material, with throughput being a minor consideration. The approach is akin to analytical chromatography, where maximum resolution is required.

On the other hand, in large-scale preparative separations where the process economics of continuous production is the factor that determines the viability of the whole operation, throughputs became the major con-

cern. Thus, the cost performance characteristics of an adsorbent and the hardware requirements for operating large-scale preparative separations are completely different from those that are ideal for small laboratory isolations. Additionally, most downstream "work up" schemes consist of more than one extraction and purification step. Thus, it is not possible to generate a universal formula with which to calculate the economics of all possible processes. In addition to throughput, the useful lifetime of the column packings and the degree of sterility required in the final product can all influence the cost of processing.

One of the primary objectives in using a composite adsorbent is to enable high flow rates to be used. The aim is to reduce cycle times and to increase throughput.

Typically, soft hydrogel column processes operate at linear flow rates of the order of 0.5–2 cm/min, whereas Macrosorb processes can easily be performed at 5–10 cm/min. This applies to all aspects of column processes: adsorption, washing, desorption, re-equilibration, and scrubbing. Normally, soft hydrogels are operated in special columns designed to limit the bed height, with scale-up being achieved by increasing surface area. By contrast, Macrosorb adsorbents do not have bed-depth restrictions and are in fact limited by the backpressures created in the pipework and the valves of currently available column designs.

In practice, a column's performance is determined by its adsorption efficiency and its peak elution profiles under given operating conditions. Adsorption efficiency can be measured by comparing the sample breakthrough profiles with equilibrium capacity of the medium. The column's resolving power is estimated by eluting multicomponent mixtures under various conditions. As the operational flow rate increases, the column's chromatographic performance deteriorates. Thus, the rate at which a process is actually performed is a compromise, but one that is no longer determined by the physical characteristics of the adsorbent. When operating composite adsorbents at limits imposed by the kinetics of the adsorption mechanism itself, capture efficiency can be optimized by adjusting the bed volume and its aspect ratio to allow maximum flow rate capability to be exploited. It should be noted that, for a given flow rate, maximizing surface area permits maximum volumetric throughput, minimizes the elution volume, and that solute breakthrough will depend on bed depth.

Large-Scale Processing: A Case Study

In this section, a simplified comparative per kg production cost estimate for the extraction of albumin from bovine serum serves as a basic illustration of the overall advantages that may be gained when exploiting the fast flow capability of composite adsorbents (*8*). Macrosorb-KAX.DEAE is compared directly with DEAE-Sepharose in a Pharmacia KS 370 "Stack" column unit.

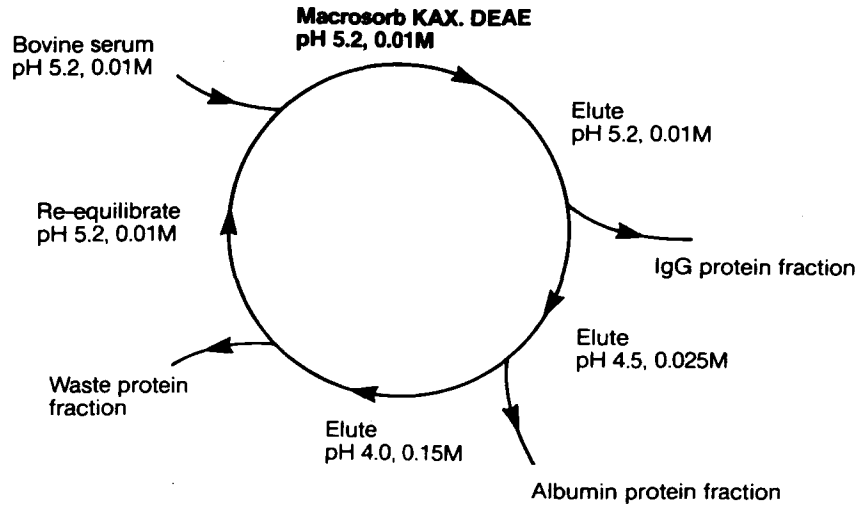

Fig. 3. Albumin separation from bovine serum: the process cycle.

Under the conditions described, desalted bovine serum is applied to the column. IgG and other proteins are not adsorbed and pass through, with albumin being retained on the column. The flow rates used in the separation were determined using small scale (30 × 1.6 cm) columns. For this particular separation (Fig. 3), both gels are loaded to 30% of their specific capacity for albumin under the operating conditions to be used, and the capacity of DEAE-Sepharose for albumin was found to be a factor of 2.66 higher than that of Macrosorb-KAX.DEAE when the gels were operated on 0.4 and 5 L/min, respectively. The quality of albumin produced using either gel is the same when using the conditions described. The disparity in the flow rates used is a reflection of the back pressures generated when using soft adsorbents: The pressure drops exerted by Macrosorb adsorbents in columns of all sizes are negligible.

For the DEAE-Sepharose column, 0.5 kg albumin was applied as a 25 L sample of desalted serum. The total cycle, including re-equilibration, was 612 min and yielded 0:45 kg albumin in 23 L of eluate. By contrast, the Macrosorb column was loaded with 0.19 kg albumin in 9.4 L of sample, and in a 42 min cycle yielded 0.17 kg albumin in 25 L of eluate. Time for time, the throughput of albumin in the Macrosorb column was ca.2 kg albumin in 300 L (using 12 cycles).

The cost is derived in Table 1, and it can be seen that when applying desalted serum as the sample, the Macrosorb column produces albumin for approximately one-third the cost at more than four times the throughput. The lower adsorption capacity of the Macrosorb column is easily compensated for by the rapid cycle times possible, this being a consequence of the faster useful flow rate which can be used. In addition, when using the Macrosorb column, serum can be *diluted* to the correct ionic strength as an alternative to desalting. This requires the application

Table 1
Albumin Separation from Desalted Bovine Serum-
A Comparative Cost Estimate

Column[a]	DEAE Sepharose	Macrosorb-KAX.DEAE
Flow rate	0.4 L/min	5 L/min
Albumin loading	500 g	187 g
In sample volume	25 L	9.4 L (33L)[c]
Buffer vol/cycle	220 L	200 L
Cycle time	612 min	42 min (47 min)
Vol. of product peak	23 L	25 L
Albumin yield/cycle	0.45 kg	0.168 kg
Cycles p/d	1	12 (12)
Daily buffer usage	220 L	2400 L
Buffer cost/d[b]	£1.630[d]	£17.78
Labor @ £25.00/d	£25.00	£25.00
Total costs/d	£26.63	£42.78
Albumin yield/d	0.45 kg in 23 L	2.016 kg in 300 L
Cost albumin/kg	£59.18	£21.22

[a]Pharmacia KS370 Stack Unit (16L bed volume)
[b]Average buffer cost at £0.00741/L
[c]Figures in parentheses in the Macrosorb column show values obtained when applying *diluted* serum in place of *desalted* serum.
[d]The exchange rate of the British pound to the American dollar, as of publication, was $1.69.

of a sample with three times the original volume, but only increases the process cycle time from 42 to 47 min. The daily throughput using the Macrosorb column is unaffected, but the desalting step is completely eliminated. The albumin eluted from the Macrosorb column using either sample application method is three times as dilute as that obtained from the soft gel column. However, product concentration is automated and incurs no additional labor costs and only a marginally increased energy expenditure.

One aspect of the comparative costing that has not been considered is the column scrubbing frequency requirement. The soft gel process normally requires scrubbing every three cycles to prevent gross performance loss caused by cumulative buildup of nonspecifically bound residues. However, when Macrosorb-KAX.DEAE is used as the adsorbent, it has been shown that the residual hold-back after 12 cycles is negligible. The scrubbing frequency, when finally determined, will have a considerable improving effect on the cost, in addition to the already demonstrated advantage obtained when using Macrosorb for this particular process.

The albumin separation has also been successfully performed using an adjustable bed-depth Amicon G300 × 500 glass column containing 21 L of Macrosorb-KAX.DEAE, with loading and elution flow rates of 4 cm/min and the re-equilibrations being run at 10 cm/min. Operation on this column showed an increase in specific throughput (per liter of column

packing) of 30% over that achieved in the Macrosorb column described previously.

The process is not yet fully optimized in terms of economic operation. Small-scale experiments showed that it is possible to load the Macrosorb-KAX.DEAE column to 60% albumin capacity (compared to 30% capacity as described earlier), while suffering only a 10% breakthrough loss of albumin (which could be recovered by appropriate recycling). This would increase throughput by another factor of two. The throughput of the Amicon G300 × 500 column was further improved without raw material loss by using appropriately adjusted flow rates for the various steps in the process cycle. Thus, reduction of flow rate from 5 to 2 L/min during the sample application step (when using desalted serum) also enabled the column to be loaded to 60% of its equilibrium capacity. Re-equilibrations may, if required, be run at flow rates of up to 10 L/min.

Utilization of Macrosorb Adsorbents in Fluidized Beds

The stability of proteins in fermentation broths and cell lysates is not infinite. Indeed, it is often required to process feedstocks as rapidly as is possible in order to maximize product recovery. With highly diluted and unstable feedstocks, any process that permits rapid processing of large volumes is useful. An adsorbent configuration that permits speedy processing, with recycling to compensate for incomplete adsorption on a first pass, is a fluidized bed.

Macrosorb-KAX.CM has been used to recover asparaginase from alkaline cell lysate, both in packed and fluidized bed mode (*9*). In particular, experiments show that with a sample recycled three times, it is possible to operate fluidized beds at flow rates in excess of 1000 cm/h (16.6 cm/min) before capture efficiency is impaired. This is approximately equivalent to the performance of a single pass column operating at one-third of the flow rate, so there appears to be no advantage in using a fluidizing bed under such circumstances. However, fluidized bed processes are particularly appropriate in cases where the feedstock applied exhibits a tendency to blind the adsorbent when used in the packed bed mode (*10*). Thus, fluidized bed configurations may be, in appropriate cases, suitable for extractions directly from unclarified fermentation broths, and experiments on the extraction of aryl acyl amidase from *Pseudomonas putida* culture demonstrate the soundness of this approach (*9*).

Researchers at the Oak Ridge National Laboratory are developing a process in which Macrosorb-KAX.DEAE is used to recover soluble cellulase from aqueous process liquors and undigested solid residues after the hydrolysis step is completed. Cellulase bound to Macrosorb can be easily separated from the broth and the residual cellulosic solids due to the higher density of the composite adsorbent (*11*). This work is of significance because, in this particular process, the cost of the cellulase repre-

sents approximately 60% of the total production costs if the enzyme is used on a zero recovery basis.

CONCLUSION

The favorable handling and operational properties of composite adsorbents enable their use both in laboratory and large industrial scale uses.

Composite media permit the downstream process designer to scale up from laboratory scale to large volume processing by eliminating the compressibility problem, but without losing the otherwise highly desirable characteristics of agarose-based adsorbents. Thus, Macrosorb composite adsorbents enable the use of fast flow rates in low pressure column equipment. This allows the use of dilution of sample to the correct ionic strength as an alternative to desalting. In appropriate cases, fluidized bed operation enables extractions directly from unclarified broths.

These improvements are expected to have a significant impact on the realization of economically viable downstream processing schemes for the production of intermediate and lower value proteins.

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