Reprinted from
PHARMACEUTICAL ENGINEERING
THE OFFICIAL TECHNICAL MAGAZINE OF ISPE
JANUARY/FEBRUARY 2015, VOL 35, NO 1

www.PharmaceuticalEngineering.org

Expanded Bed Adsorption – Challenges and Advances in Column and Process Design

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The article presents a comparison of different column designs for Expanded Bed Adsorption (EBA).

The concept of chromatography in Expanded Bed Adsorption (EBA) was first proposed in early 90s.[1] The initial advantage of the idea was to be able to directly process particulate containing biological feedstocks for target products, while still maintaining sufficiently high separation efficiency that is usually enjoyed only by the traditional chromatography columns.

EBA could potentially replace several traditional unit operations combined, namely centrifugation, filtration, and capture chromatography. EBA can significantly shorten the overall processing time, increase the overall yield, and save both capital investment and operating cost for many biological purification processes - *Figure 1*.

EBA is different from the fluidized bed used in the traditional chemical industry.[2] The media beads are only intended to be fluidized in their local area, instead of a full mixing stage as in a fluidized bed, to provide reasonably high separation efficiency (plate count).

A typical EBA operation involves the following steps: bed stabilization/equilibration, sample loading, washing, elution, regeneration and cleaning, and re-equilibration as shown in Figure 2. The settled EBA bed is first expanded by applying upward flow that is sufficiently fast to fluidize the media beads. Particulate containing feedstock will be directly applied into the column after equilibration. The target proteins or smaller molecules will be binded to the EBA absorbent while other contaminants such as Nucleotide and lipids pass through. A wash will be applied using fresh buffer to remove the loosely bound contaminant molecules after the sample loading. Elution step is usually run as a packed bed by lowering the top adaptor and applying downward flow. The target proteins or molecules that were binding on the adsorbent will be eluted from the column and collected. The column will then be cleaned/regenerated using stronger agents such as sodium hydroxide and re-equilibrated using the starting buffer for the next loading cycle.

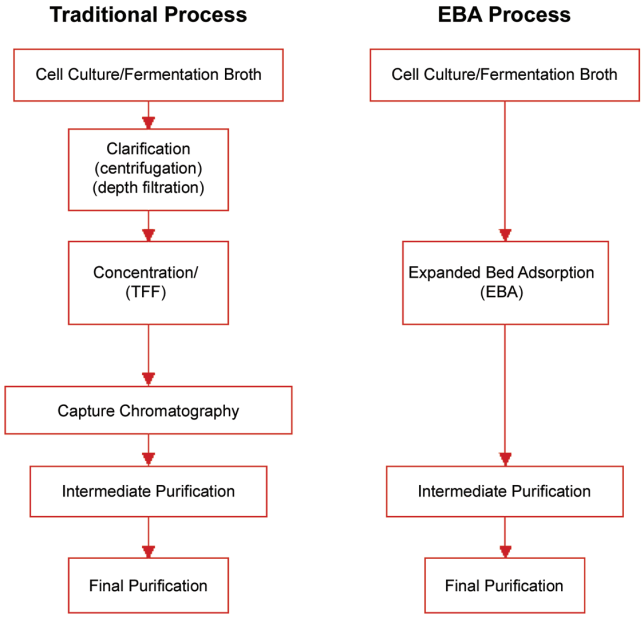


Figure 1. Comparison of traditional downstream purification process with one applying EBA.

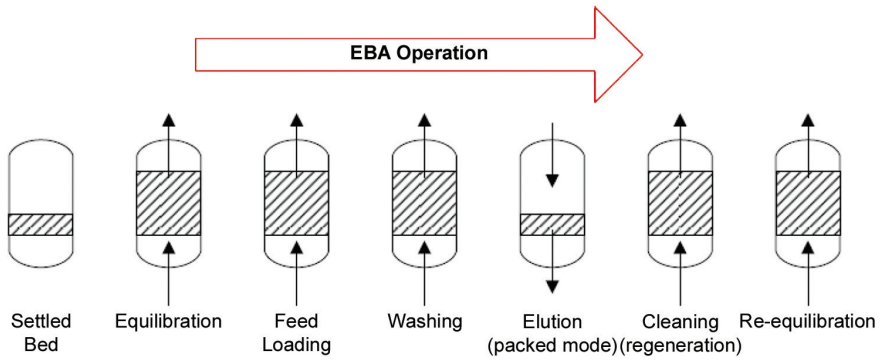


Figure 2. Concept of EBA operation.

The early EBA columns are susceptible to fouling and clogging in its distributor. The main drawbacks in EBA process were hygienic issue and bed stability. The issues were associated with the distributor, which is a critical component in providing even flow distribution in EBA technology. As some kind of high flow resistant components were necessary for distributing the flow, it turned out that it is difficult to clean the aggregates formed upstream of the high resistance component inside the distributor, which could happen as a result of aggregation of cells and biomass over time.[3] Aggregations of biomass and interactions between media and cell debris sometimes also causes bed stability and channeling problem. The first generation EBA column did not see wide application mostly owing to the hygienic issue involved with the distributor and bed stability with difficult feedstock from cell lysis.

The EBA technology was further developed into its second generation that features a patented distributor with moving arms to distribute the flow toward the bottom of the column. The distribution concept is illustrated in Figure 4. The second generation EBA column has an open flow design in the distributor and a bypass on the top adaptor and therefore has no cleaning issues. The distributor leads to significant back mixing in the lower part of the column. There are concerns regarding media grinding, operation reliability, and maintenance cost. In addition, elution can only be done in expanded bed mode. The concentration factor of the process is therefore much lower than the traditional EBA.

The second generation EBA column only had a dismal launch in the industry. In fact, some major players decided to stop manufacturing and marketing the product shortly after the second generation design was introduced to the market.

While EBA has obvious advantages, the challenges in developing EBA process are the hygienic design of the flow paths, stability of the expanded bed, and maintaining even flow distribution (to achieve sufficient separation efficiency).

A bed independent flow distributor with open cleanable flow paths is considered the critical challenge to make EBA a robust technology. A traditional distributor relying on high resistance components (such as the perforated plate or a thick mesh in the first generation of design) does not meet the hygienic requirement as the upstream of the high resistance component will be subjected to fouling and clogging and will not be cleanable by the flow itself. Aggregation formed in the bed and inside the distributor during the usually long time loading step cannot be effectively and reliably cleaned which is imperatively needed before elution starts.

On the other side most of the open channel distributors will not be able to distribute the flow without making significant disturbance to the EBA bed. It is therefore technically challenging to find a way to distribute even flow without high flow resistance components.

However, recent developments in improving distributor, column, and process have made it necessary to reevaluate the technology. A tangent flow pattern with feed recirculation was suggested to be used in the distributor. The concept was first seen in a poster in Prep in 2004. A similar concept was disclosed in a US patent application.[4] A 2010 patent revealed a design with radial tangent flow and more sophisticated flow paths of varying channel heights.[5-7] It was claimed that bed independent even flow distribution was achieved and flow paths are completely open and self cleanable.

Modern biological applications tend to be cell culture based and the target products are secreted from the cell. The feed stocks are relatively clean and not subjected to biomass aggregation and interaction between biomass and absorbents. Hygienic issue with the distributor and the mesh that existed in the old EBA design may be addressed by newly proposed ones that feature completely open and self cleanable flow paths and bed independent flow distribution.

The objective of this article is to analyze the technical challenges in EBA technology and evaluate the EBA design ideas for industrial applications. Bed independent flow distributor with open channels addresses the main technical challenge in EBA and may possibly make the key benefit of EBA technology realistic. The future of EBA looks promising and most of the modern biological applications are well fitted to it. In addition to possible applications in traditional biotech and biopharmaceutical industries, a particular exciting future for EBA could be the cell separation, which will be the next biggest separation challenge as biotechnology moves from proteins to cells.

EBA Technology Review

Operation Concept

Like fixed bed chromatography EBA operation involves

equilibration, loading, washing, elution, cleaning and regeneration as shown in Figure 2.

The settled EBA bed is first be expanded by applying upward flow that is sufficiently fast to fluidize the media beads. The beads are dynamically balancing by the gravity force and the dragging force of the upflow so that the beads will be fluidized in a local vicinity. The expanded bed is dynamically stabilized and chemically equilibrated after enough buffer passing through. The column is then ready for the next step, sample loading.

Particulate containing feedstock will be directly applied into the column. The cells, debris, and solid particulates will pass the bed though the void space in the bed. The target proteins or smaller molecules will be binded to the EBA absorbent while other contaminants such as Nucleotide and lipids should not.

A wash starts by applying fresh buffer after the sample loading is completed. During wash, the loosely bound contaminant molecules on the EBA absorbent will be eluted and any contaminants in the bulk flow will be washed away as well. The column is then ready for elution.

Elution is usually being performed using down flow in fixed bed mode. The up flow will first be stopped and the movable top adaptor is lowered to form a loosely packed fixed bed. The elution buffer will then be applied from the top down. The target proteins or molecules that were binding on the adsorbent will be eluted from the EBA column and collected. The column will then be cleaned/regenerated using stronger agents such as sodium hydroxide and re-equilibrated using the starting buffer for the next loading cycle.

EBA can replace several traditional processing steps by one simple unit operation and greatly reduce the overall processing time. In addition to the possibility of processing feedstock directly, EBA process allows much higher throughput without sacrificing much on binding capacity. The productivity, which is binding capacity times throughput, can be significantly increased. The usual long time loading step in chromatography process will not be the capacity bottleneck anymore, which also eliminates concerns associated with proteases during sample holding.

Evaluation of Separation Efficiency: Plate Count and HETP

A commonly used criterion for evaluating a chromatography column is total plate count and Height Equivalent To Plate (HETP), which are sometimes collectively called separation efficiency. Total plate count is a concept based on the theory of Residence Time Distribution (RTD).[8] According to RTD theory, a complete mixing stage such as a fully fluidized bed or fully stirred tank has total plate count of one while a plug flow with no back mixing would have a total plate count of infinitive. RTD theory was specifically built on the model of a series of Continuous Stirred Tanks (CSTR) and the number

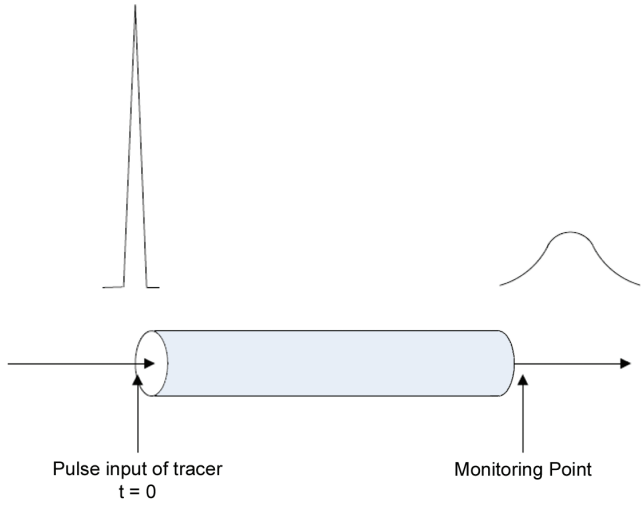


Figure 3. Residence Time Distribution (RTD) measurement using tracer material.

of tanks that allow the series of tanks to have the same kind of RTD as the actual flow system is theoretically the total plate count. Total plate counts in practice can be calculated from the actual RTD data of inert tracers using a pulse or step responding tests.

Figure 3 shows how a pulse injection is used to determine the total plate count of a flow system. A pulse injection of the tracer goes into the flow system from the inlet and the concentration of the tracer and the time elapsed at the outlet of the flow system are monitored.

According to RTD theory, N the total plate count, can be calculated based on the responding behavior of the trace material (pulse testing) going through the flow system.

$$N = \frac{\tau^2}{\sigma^2} \quad (1)$$

where

$$\tau = \int_0^\infty tE(t)dt \quad (2)$$

$$\sigma^2 = \int_0^\infty (t-\tau)^2 E(t)dt \quad (3)$$

$$E(t) = \frac{c(t)}{\int_0^\infty c(t)dt} \quad (4)$$

In other words, t is the mean residence time of the tracer and σ is the standard deviation of the residence time.

HETP is defined as the bed height of the column divided by the total plate count.

$$HETP = \frac{H}{N} \quad (5)$$

where H is the height of the bed and N is the total plate count.

Total plate count is one of the most important factors to separation performances in both adsorptive and distributive chromatography. Plug flow is desired in chromatography to achieve best performance. Increase of total plate count in EBA can dramatically increase the media binding capacity when the total plate count increases from one to several hundred. Total plate count however does not contribute much to separation performance in most adsorptive chromatography when the total plate count is greater than 1000 or the adsorption isotherm is ideal where the adsorption is independent of the target concentration in the bulk solution.

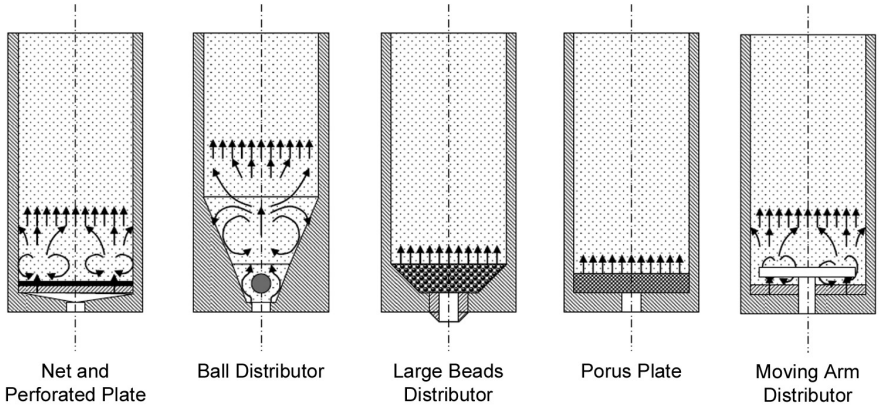


Figure 4. Comparison of different EBA distributors.

Alternatively, the non-ideal behavior of the flow system can be described using the axial dispersion model.[8] The governing equation in a dispersion model has the following form:

$$\frac{\partial C}{\partial t} + U\frac{\partial C}{\partial z} = D_a\frac{\partial^2 C}{\partial z^2} \quad (6)$$

where is concentration averaged over the radius, U is the superficial velocity.

In a close-close system, D_a is the characterizing parameter of the axial dispersion model. Sometimes Peclet number Pe_r is used instead.

$$Pe_r = \frac{Ul}{D_a} \quad (7)$$

where U is the superficial velocity in the column and l is the column length.

Pe_r can be calculated using RTD data of tracer material according to dispersion model

$$\frac{\sigma^2}{\tau^2} = \frac{2}{Pe_r} - \frac{2}{Pe_r^2}\left(1 - e^{-Pe_r}\right) \quad (8)$$

Total plate count is significantly reduced with increasing back mixing which could be caused by either undesired flow pattern or dilution. Column structure, operating condition, and bed condition have direct impact on total plate count and it is usually described by Van Deemter equation.[9]

$$HETP = A + \frac{B}{u} + Cu \quad (9)$$

where u is the superficial velocity of the fluid and A, B, and C are constants.

Although Van Deemter equation is an empirical equation, there is an analytical solution in similar form, which helps to give the physical meaning of A, B, and C in the Van Deemter equation.

The three terms in the equation, in short terms, A stands for the contributions from the basic bed structure, B/u, where B is proportional to tracer diffusivity, stands for diffusive mixing among different molecules, and C, where C is proportional to the sectional area of the beads, stands for back-mixing from convective flow. u is the linear velocity (superficial velocity) of the flow in the column.

The non-ideal behavior (anything between one and infinitive) can be described using total plate count or Peclet number. Total plate count N and $HETP$ are most popular in evaluating chromatography column performance for its simplicity and straightforward physical meaning.

One of the key benefits that the original idea of EBA was pursuing at was the relatively high plate count in comparison to a single stage of adsorption (such as in a fluidized bed). However, this has proven extremely difficult to achieve when aggregation happens in the feed and cleanability has to be taken into consideration.

EBA Column and Process Design

The flow distributor in the column is critically important to support EBA operation as the expanded bed lacks the kind of flow resistance required to help with evening the flow velocities along the radius. There has been recent progress both in the column design and the process configuration in achieving the appropriate flow distribution and cleanability.

Several early designs of EBA columns are shown in Figure 4. The first type uses a perforated plate underneath a mesh. There are usually vortex at where the perforated holes are and therefore the back mixing in the lower part of the column is still significant. The space between the mesh and the plate is considered non-hygienic and is problematic for pharmaceutical production. The second type uses a gradual opening shape with a ball check valve at the bottom of the

column. The flow path is completely open and cleanable, but the flow distribution is not ideal at all as shown in the diagram. The third type is called large bead distributor which provides a good even flow distribution, but hygienic aspect inside the beads bed is still a concern. The fourth type is thick porous plate. The problem is clogging and cleaning as well. The fifth type is the rotating arms with perforated holes. It is a cleanable design, but back mixing at the lower part of the column is significant.

The perforated plate and mesh went commercial and became the first generation EBA design. The other types never went to commercial except the rotating arms which is the second generation and showed up on the market only briefly.

Perforated Plates – First Generation Streamline

As shown in Figure 5 with more details, the first generation design of EBA column was introduced as an improved version of the traditional mesh support in fixed bed columns. Underneath the mesh, there is a flat or bowl shaped plate facing the mesh. On the bottom of the bowl, there are several symmetrically made holes to lead the flow to the mesh and the bed.

The earlier researchers realized that special distributor is required to pre-distribute the flow to provide the desired plug-like flow to the expanded bed. Extensive research was done to investigate different types of distributors.[10,11] Multiple outlets from four to eight and sophisticated channel shapes were explored in experiments. Bed stability and total plate count have been the main challenge. Until 2005, the only commercially available EBA column was the perforated plate design. The perforated plate distributor was chosen and researchers tend to agree that most of the open channel distributors did not even perform as well as the perforated plate in terms of the separation efficiency and bed stability.

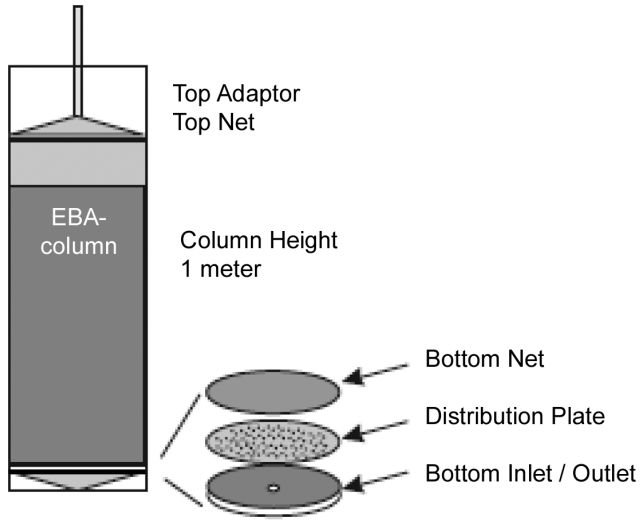


Figure 5. First generation EBA column design.[10]

A perforated plate inside the distributor however causes a largely enclosed space in the flow path. This space is not self-cleanable by flow in case aggregation happens inside it. Such aggregation is very likely during the long time loading step. The aggregation thus formed cannot be reliably cleaned before the elution and would therefore contaminate the product during the elution. Figure 6 shows the accumulated impact of aggregation inside a first generation distributor.[12] It was a challenge to clean such aggregates inside this enclosed space even during the CIP cycle where much stronger chemicals are used.

This drawback on the first generation distributor has significantly limited the possible applications of the EBA technology.

Movable Distributing Arms – Second Generation Distributor

The second generation of EBA was necessary as the first generation EBA columns suffered from serious cleanability issues. Cleanability became a top priority for EBA to be able to find realistic applications in the pharmaceutical industry.

As shown in Figure 7, the second generation EBA column does not have a bottom net.[13] Instead there is a movable device with multiple arms with perforated holes, placed at the center of the column bottom. Feed comes in from a tube at the center and flows into the perforated arms radially. The feed will then be distributed into the column through the perforated holes, which usually are pointing downward to the bottom of the column. These tubes will rotate or oscillate during operation to provide more even distribution performance. There is no net or porous media inside the entire flow path.

As the flow needs to turn from downward to upward and the moving arms keep disturbing the bed at the bottom portion of the bed, the back-mixing is particular an issue with the second generation designs as shown in Figure 4.

Elution in fixed bed using

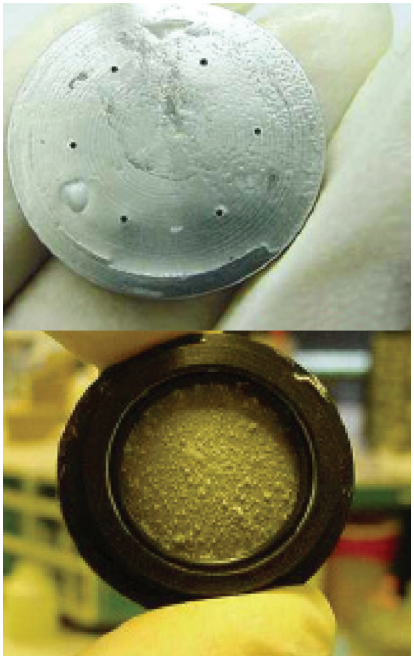
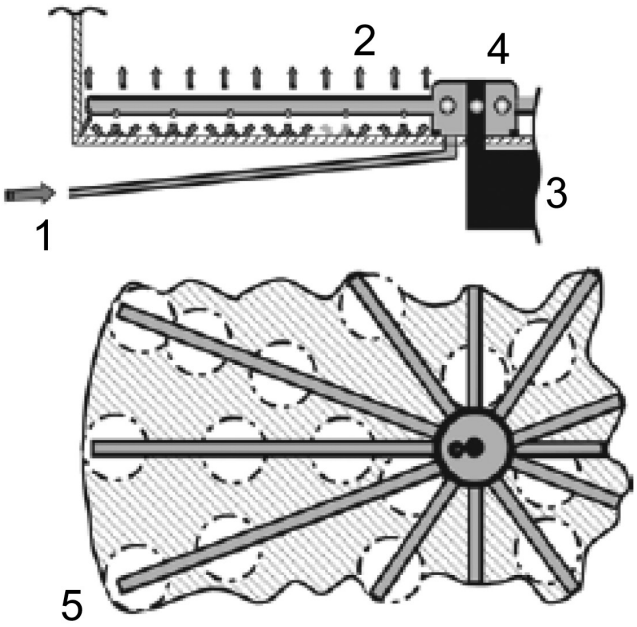


Figure 6. Fouling and aggregation in first generation distributor.[12]



1 – Incoming Flow to Inlet
2 – Upward Flow from Perforated Holes
3 – Axis
4 – Movable Distributor Assembly
5 – Distribution Arm

Figure 7. Second generation distributor: rotating/oscillating distribution arms.[13]

downward flow cannot be done in the second generation column because there is no bottom net. The concentration factor of the process is therefore reduced because of its less plate count compared to fixed bed as a result of having to do elution in expanded bed mode. The drawback of the design also may be the moving/oscillating arms that accidentally grind the absorbents. The moving and wearing parts in the column raise concerns on the reliability of the operation and increases maintenance requirement.

Distributor with Recirculation – Third Generation

The third generation of EBA column introduced a mechanism of flow recirculation under the mesh inside the distributor.[13] The initial idea was to have feed flow coming from one side of the distributor tangential to the mesh and out on the other side to avoid fouling and clean aggregates inside the distributor.[13] The original idea did not take into account of flow distribution and obviously does not apply to large diameter columns.

A radial flow pattern with sophisticated multi-layer of flow channels of varied channel height was proposed for a recirculation distributor as shown in Figure 8.[5] There is a nozzle with many holes pointing tangential to the net, placed at the center of the bottom adaptor right underneath the distributor mesh. Inside the distributor, there is a distributor core which separates the space inside the distributor into upper channels and lower channels. The feed flow goes into the distributor through the inlet at the center and injects into the upper distribution channels through the nozzle, and comes back out from the lower returning channels for recirculation.

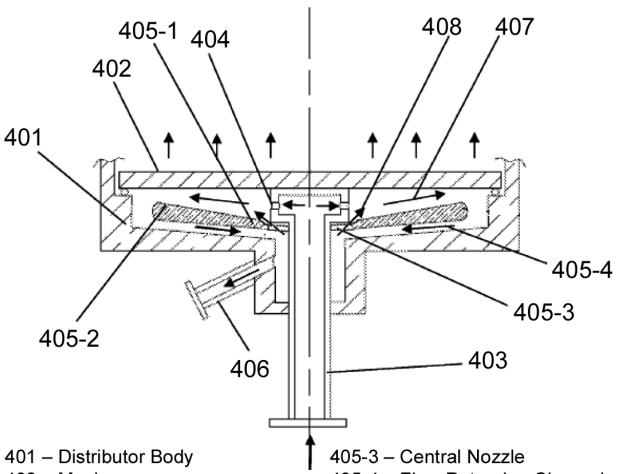
The distributor core is particularly important as it is shaped in such a way that it helps to even out pressure along the radius and assure cleanability in the channel. The even pressure will lead to an evenly distributed flow across radius into the bed.

In addition to the above external loop for recirculation, which is supposed to be driven by an external pump, an internal recirculation loop is also introduced between the upper channel and the lower channel to improve the homogeneity as shown in Figure 8. Such an internal recirculation loop could further improve the even flow distribution along the radius and reduce the recirculation flow rate required to achieve the even flow distribution.

Column Efficiency and Processing Capacity

Most of the fixed bed chromatography columns have plate count greater than 3000/m. High resolution columns may have 10,000 to 20,000/m plate counts. Performance of adsorption chromatography improves significantly when the column total plate count increases from one to several hundred, but becomes non-controlling factor when it is above 1000 for most of applications.

A traditional EBA column with perforated plate has a total plate count around 30 or 150/m.[11] Increasing the plate



401 – Distributor Body
402 – Mesh
403 – Inlet
404 – Injection Holes
405-1 – Distribution Inner Core
405-2 – Out Ring of Inner Core
405-3 – Central Nozzle
405-4 – Flow Returning Channel
406 – Outlet
407 – Flow Distribution Channel
408 – Internal Recirculation Channel

Figure 8. Third generation distributor: recirculation.[5]

count in the EBA column will therefore significantly bring up the dynamic binding capacity of the column.

Distributor Design

Deviation from ideal plug flow is the main reason the plate count goes down. In an EBA column, back mixing happens in both the distributor and the column. In a traditional perforated distributor, while the distributor was an improved version of a fixed bed, the distributor however causes significant back mixing in the lower part of the column.

The second generation of the EBA column with moving arms did not improve the plate count in the column as the result of the significant back mixing at the lower part of the column. The plate count was reported in the range of 5 to 20.[14] The downflow from the distributing arms need to turn around to back up and the moving arms cause constant disturbance to the bed. The highest was at the condition of moderate linear flow rate and medium oscillating frequency.

Dilution, either caused by convective flow or molecular diffusion, is another form of deviation from ideal plug flow. Over expanded bed and inappropriately designed distributor with large dead space could cause unnecessary dilution in sample concentration which in turn reduces total plate count and separation efficiency.

Traditional EBA columns, both the first generation and the second generation, need to have an expansion ratio (expanded bed height over sedimented bed height) of 2.5 to 3.0 to achieve the most total plate count.[14] One example of bed expansion in a second generation column is shown in Figure 9. The undesirable dilution in the bed is therefore quite significant.

It is important to note that such high expansion of bed is not required for cells, cell debris and particulates to pass through, but for flow to even out radially to overcome the back mixing unavoidably happening in the lower part to the column.

Bed expansion is characterized using expansion ratio which is defined as:

$$\frac{H}{H_0} = \frac{(1-\varepsilon_0)}{(1-\varepsilon)} \quad (10)$$

where H is expanded bed height, H_0 is settled bed height, ε is expanded bed voidage, ε_0 is settled bed voidage.

The third generation of the EBA column introduced a mechanism of flow recirculation under the mesh inside the distributor.[7] The idea was originally to address the clogging issue on the adaptor mesh, but it was later realized that the recirculation flow can greatly help with even flow distribution over the radius.

As described in the previous section, the distributor has a radial flow pattern and multi-layer of flow channels with sophisticated varied channel height. There is a distributor core inside the distributor which separates the space inside the distributor into the upper distribution channels and bottom returning channel. The shape of the distributor core allows additional possibility of achieving even pressure and cleanable path along the radius. An internal recirculation loop is also introduced between the upper channel and the lower channel to improve the pressure homogeneity. As the pressure underneath the mesh is mostly homogeneous the feed will flow through the mesh and into the bed in a uniform fashion similar to a plug flow.[7]

The third generation design is expected to have much higher total plate count and completely self-cleanable flow path as the result of the open recirculation paths. It was reported that impressive plug like flow has been demonstrated in experiments using tracer materials.[5] CFD modeling also supported the assumption that the radial flow pattern will lead to even flow distribution along the radius.[5] It was reported that the higher the recirculation flow, the more even the flow distribution will be and the bigger the column diameter is the more significant the improvement on even flow performance will be.

While the first and second generation columns require 2.5 to 3.0 expansion to achieve the most plate count, the third generation column expects to achieve much higher plate count (>50) at much smaller bed expansion (~1.5).

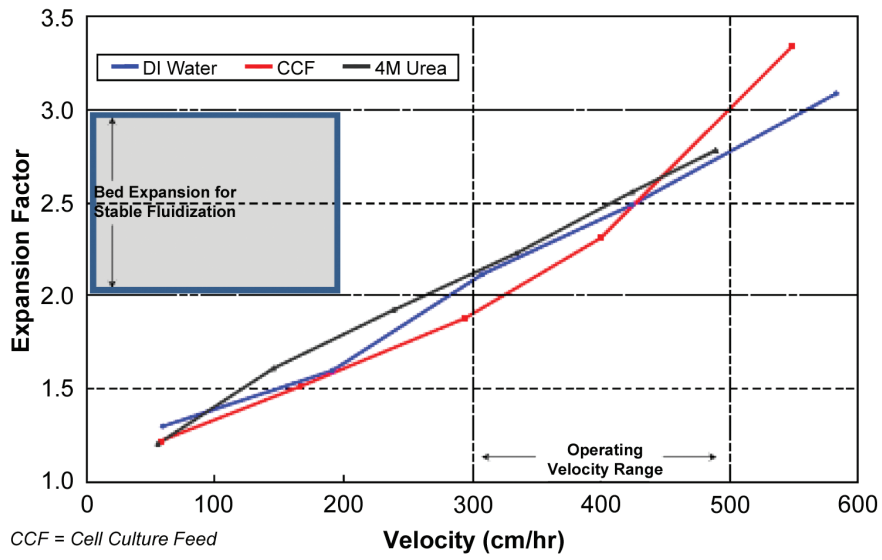


Figure 9. Operating range in a second generation EBA column.[14]

Absorbent Development

EBA absorbents need to have higher density than the media beads for normal

fixed bed chromatography. As the beads are balanced by gravity and dragging force by the flow in expanded beds, higher density of the beads will allow bigger throughput without the bed being over expanded or washed away.

Most of the EBA absorbents are made by porous matrix coated around a high density core. The most commonly used cores are made of quartz, Zirconium, Tungsten, and stainless steel.[15-18] See Table A for some of the commercially available EBA media and their physical properties.

The non-absorbing core does not take away the functional space of the beads. It has been long realized that the core of the media beads is barely accessible for effective binding because of the diffusional limitation for target molecules to reach there. Putting an inert core inside the media beads will therefore not reduce the beads' effective binding capacity. On the other side, heavier beads allow much higher processing throughput. As a result, the productivity of EBA process could be much higher than a traditional fixed bed chromatographic process. The additional advantage is that it also reduces holding time of samples during loading step, which is critical in avoiding target molecule deterioration and keeping up good yield. The development of heavier EBA adsorbents however had limited impact in improving column efficiency and bed stability.

It is worthwhile to note that improvement on the density of the absorbent does not have significant impact on the flow distribution or column efficiency. The main advantage is that the process would allow much higher throughput. As can be seen from Table A, the operating velocity was initially only 300 cm/hr when the agarose/quartz media was first developed by GE Healthcare, but the highest mean density of EBA media commercially available is now about 3.5 mg/ml (from UpFront) or 900 cm/hr. On the other side, the higher density does not help improve the expansion profile to achieve the best plate count. As evidenced by the work in Biogen Idec,[14] the column (e.g., a second generation column) with more dense media would need the same kind of expansion (2.5 to 3.0) to achieve the best total plate count as with less dense media.

Open Flow Path and Self-Cleanability

Flow distribution, the challenge of even flow in traditional chromatography column design, was mitigated by the relatively high flow resistance from the fixed bed itself. In expanded bed mode, the flow needs to be distributed into the bed almost independent of the flow resistance from the bed since the bed provides no resistance at all.

EBA bed does not provide much flow resistance and the open channel distributors (such as the second generation design) failed to provide the desired flow pattern and led to low separation efficiency. The lower part of the EBA bed in general has significant back mixing and is the main reason why the EBA column plate count is low.

A traditional EBA distributor relying on high resistance components (such as the perforated plate or a thick mesh in first generation) does not meet the hygienic requirement as the upstream of the high resistance component will be susceptible to fouling and clogging and is not cleanable by the flow itself.

On the other side most of the open channel distributors will not be able to distribute the flow without making significant disturbance to the EBA bed. In other words, most open channel distributors tested so far are not bed independent distributors as they have to perform with the bed in existence.

Both the first and the second generation of EBA columns provide very low total plate count. The second generation does improve the cleanability by using the moving distributing arms.

The third generation design as described in previous section may be on the verge of achieving both higher total plate count and complete cleanability. While it provides even flow distribution to the EBA bed, the aggregates underneath the mesh, in case it does happen, will be swept away from the mesh during the recirculation. It was suggested that coarse in-line filter be used to remove the aggregates before the feed returns to the recirculation tank.[5] The third generation design provides bed independent even flow distribution and

Company	Product	Functionality	Base Matrix/ Core	Particle Size (µm)	Mean Density (g/ml)	Operating Velocity (cm/hr)
GE Healthcare	Streamline	rProtein A, Q, SP, DEAE, Phenyl, IMAC	Agarose/ Quartz	100-300	1.2	200-400
GE Healthcare	Streamline Direct	Q, DEAE, MMC	Agarose/ Stainless Steel	80-165	1.8	400-800
Pall BioSepra	HyperZ	Q, CM	Zirconium Oxide (hydrogel filled)	45-101	3.2	300-450
UpFront	FastLine	rProtein A, MMC, PEI	Agarose/ Tungsten Carbide	20-200	2.5-3.5	600-900

Table A. Commercially available EBA media and their physical properties (company websites and product data sheets).

have flow paths that are completely open and self-cleanable.

To assure cleanability inside the column, in addition to distributor, both the second generation and third generation columns have a bypass opening on the top mesh to allow aggregates formed in the EBA bed to be removed. A more carefully designed version was disclosed using back flush instead of bypass for the third generation design.[5]

Recirculation Flow

The recirculation was initially proposed as a means to sweep away the aggregation underneath the mesh inside the distributor. The high recirculation flow will prevent fouling underneath the mesh from happening. It was later suggested that such recirculation could be a powerful instrument in helping with even flow distribution as well.

The ratio of recirculation flow to column operation flow could have desirable impact on the flow distribution into the bed. As mentioned previously, it was demonstrated in experiments using tracer material that the even flow distribution can be improved with increasing ratio of recirculation flow to column flow. The principle was proven in a CFD study as well.

In most of the cases, a radial flow with no recirculation has already shown satisfactory even flow distribution.[5] The larger the column diameter; the more significant the improvement. However, shear stress, which most of animal cells are sensitive to, may possibly be a concern.

Most animal cells are shear sensitive. CHO cells are relatively high shear tolerant. Shear stress was not considered as a problem in the previous designs of EBA column and distributor. Shear sensitive feedstocks such as animal cells has proven to be able to stand the perforated plates, the meshes, and the perforated holes in rotating arms. However, shear stress may need to be carefully considered in the recirculation design of the distributor.

High recirculation ratio will lead to unnecessary shear stress on the cells and cause cells to break, which will in turn deteriorate the adsorption performance because of the competitive binding from intracellular and cell membrane components.

Since cell lysis is a problem for EBA, it is a problem for all the chromatographic processes. Cell lysis also happens during centrifugation and TFF filtration. Such upper limits are usually controlled by the nature of different feedstocks. There have not been many reports about the impact of recirculation flow on cell lysis.

From the design point of view, one way to mitigate the issue is to maintain the shear stress in the flow path at the same level as the feedstock would go through the pipes. It is possible to maintain the same level of shear stress in the new design as in the previous designs by carefully arranging the size of the flow paths.

The flow distribution without recirculation, in the third generation radial flow design, has already shown great improvement over the previous generations. This essentially means that a bed independent flow distributor does not need to rely much on the recirculation flow. Without using recirculation flow, the radial flow multi-layer distributor plus occasional back flush from above the mesh and sweeping flow underneath the mesh may well be sufficient to prevent aggregation and fouling from happening inside the distributor.

The ratio of recirculation to column flow is therefore more of a problem of engineering design for specific applications. This leaves the space for equipment vendors to continue the study before any commercial product can be available.

Process Control and Media Handling

The third generation column will require slightly different system setup to operate.[4-7] A modified operation scheme was proposed to use the third generation columns, as shown in Figure 10.

The buffer will first be pumped into the column from the center inlet on the bottom adaptor. Most of the buffer goes into the EBA bed and the rest circulates back into a recirculation tank to be fed to the pump again. Fresh buffer will continue to be added to the recirculation tank. Plug-like flow goes through the EBA bed and prepares the bed physically and chemically as well.

After the EBA bed is stabilized and equilibrated with the equilibration buffer sample loading will start. Fresh feedstock is added to the recirculation tank instead of the buffer

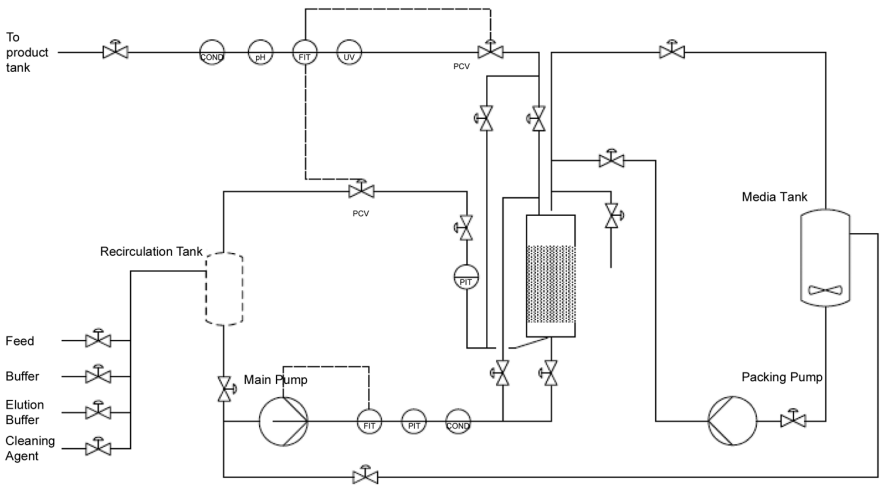


Figure 10. Process flowsheet of EBA with recirculation distributor.[6]

while the recirculation continues to run. The size of the tank is carefully chosen so that it is large enough to keep the recirculation, but not unnecessarily large to initially dilute the sample. Most of the feedstock will go into the bed in even flow fashion while the rest goes back to the recirculation pump. The recirculation ratio over column flow depends on specific applications. In many cases, only very small recirculation flow rate may need to be maintained. Occasional back flush from above the nets may be applied to remove any possible aggregates underneath the nets. Continue until all the feedstock is applied and washing will start.

Fresh buffer will again be added to the recirculation tank while the recirculation keeps running. Continue to wash until all loosely bound molecules and other contaminants in the bulk are washed out. The column is now ready for elution.

Elution buffer will either be added to the recirculation tank or be fed into the pump directly if recirculation is decided to be not needed. The elution can either be in down-flow or upflow mode. Since the expansion is low, either up flow in expanded mode or down flow in packed mode could give satisfactory elution profiles. The movable top adaptor in down flow mode was not considered necessary for the same reason, which is another plus for the third generation design.

When product is collected and elution is finished, the column will be cleaned and regenerated. Cleaning reagent is added to the recirculation tank and recirculation keeps running for sufficient amount of chemicals and time. The column now needs to be rinsed and re-equilibrated.

Fresh buffer is added to the recirculation tank while maintaining the recirculation. Continue until sufficient buffer has gone through the EBA bed and the column is re-equilibrated and ready for the next cycle.

The size of the recirculation tank needs to be appropriately chosen. It should be as small as possible, but large enough to keep the recirculation running without cavitation. The liquid level in the tank can be controlled using level sensor or load cell.

A new fashion of handling EBA media in a more automated fashion was also proposed as shown in Figure 10.[7] In the design EBA, media are being packed or unpacked into or out of the column using flow itself with flow rate greater than the particle terminal velocity. This is a great advantage in terms of the automation of the process particularly at industrial scale.

Applications

EBA feedstock may contain cells, cell agglomerates, cell debris, and contaminants from cell membrane and intracellular materials such as lipids and nucleic acids, which poses a tough challenge to the media and the column. A secretion cell system or an intra-cellular system is the first factor that will impact how a EBA process can be effective or not. It is important that the cells will not be broken and release membrane components and intracellular contaminants such as DNA, lipids and other proteins.[11] It has been well known that the large molecules that are associated with outer membrane of a bacterial cell tend to foul the chromatographic adsorbent. Charged particulates can act as ion exchangers and adsorb proteins. Biomass, cells, and cell debris tend to aggregate at low pH and may lead to bed stability problem or clogging the flow path during feed application/loading in EBA.

While it is possible that EBA can be applied to cell lysis from troublesome intra-cellular systems, such as plant extract, chicken egg white, etc., it is critical to choose the appropriate solution system and absorbent to avoid the interaction between the biomass and the absorbent during process development. The effectiveness of EBA is therefore case by case depending on the specific feedstock and the solution chemistry. It impacts on bed stability which has more to do with the chemistry between the biomass and the media beads rather than the column and distributor design. Selection of different buffer systems with different pH and conductivity, selection of different EBA media should be the key variables that need to be carefully optimized during process development.

EBA had limited commercial success. Very few EBA applications to intracellular cell system, which will require cell lysis before processing, has been seen in the industry probably because the cost of pre-treating the feedstock may have outweighed the benefit of applying EBA.

Even with relatively clean secretion cell systems, hygienic issue and operation reliability tempering the technology, EBA has never been widely accepted in biopharmaceutical industries. The road block was most likely the distributor and mesh fouling, the enclosed flow paths within the distributor and underneath the bottom and top meshes. Those issues may not be obvious in a short term operation, but could be a

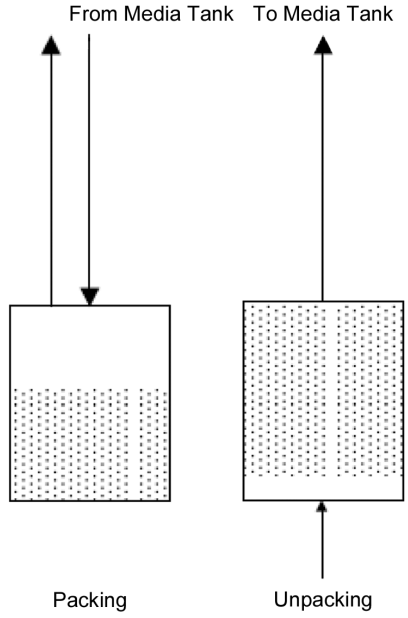


Figure 11. Media handling in EBA column.[6]

critical factor in repeated production operations.

Two exciting applications are with rHSA and Monoclonal antibodies. It has been reported that EBA was used in purification of rHSA from yeast fermentation media.[18,19] Monoclonal antibody (MAb) products from animal cell cultures were successfully captured using EBA at fairly large scale.[20-22]

Monoclonal antibodies (MAb) purification: MAb is becoming the biggest drivers in bio pharmaceuticals as the dosage usually leads to ton scale production. About 50% of top 15 blockbuster drugs are MAbs in 2012 and the percentage expects to further increase to 80% by year 2020. Few concerns regarding the interaction between the media and the cells or bed stability has been reported. MAbs are secreted from animal cells and cell culture is a great feed type that EBA can be effectively applied to.

Recombinant Human Serum Albumin (rHSA): rHSA estimated to have a global demand of 100 tons each year and is considered one of the strategically important pharmaceuticals. rHSA is secreted from yeast cells and EBA can be effectively applied for purification of the target rHSA.

Transgenic proteins from milk: transgenic drugs produced in animal such as cow may need to be purified from milk. As milk contains large amount of other proteins and lipids and is quite viscous fixed bed chromatography would often run into problems like bed clogging and over pressure. EBA could potentially be an ideal choice for processing such feedstock and simplify the entire process and reduce the cost.

Low value products are generally less incentives to drive this particular technology. One exception can be the purification of antibiotics from E.coli cells. Such process are usually at very large scale (greater than 10 tons a year) and downstream process capacity is critical in meeting its economical constraint. Many of the modern antibiotics require chromatography purification to bring up the purity of the drug to their specifications. Some of the processes were notoriously known for forming agglomerates even after microfiltration, which made it impossible for traditional fixed bed to be effective. EBA in such cases could be the perfect solution.

The last but not the least is the cell separation. As we all know that the next generation of biopharmaceuticals will evolve around cell products. Cell separation technology is becoming the most critical aspect in developing cell therapeutics.[23] Traditional chromatography using fixed bed will not be possible for cell separation because cells could easily clog the column and the cells would be stressed to break. EBA could however be very well adapted to cell separation.

Conclusion and Future Direction

EBA should not intend to be a universal solution for direct processing of all particulate containing stocks. Certain conditions such as heavy nuclei acid, lipids, and other biomass resulted from cell lysis for non-secreted protein products from cell lysis may be more economical to be pre-treated rather than being processed in EBA directly. However EBA is well fitted to most of the modern applications where the feed stocks are relatively clean and non-aggregating. Cleaning issue resulted from biomass or cell aggregation also may be effectively addressed by the new open path design for distributor and columns.

EBA brings in increased throughput without sacrificing binding capacity. As a result, the productivity of the process can be much higher than the traditional three steps. Particularly, there are technical advantages of reduced holding time of sample during the entire pre-capture stage.

It is not unreasonable to assume that the overall downstream process time using EBA may be 60% of the traditional three steps (A five day process may be done in three days). The overall yield also may increase by 6% as the result of combining three steps into one. If 80% of the production cost comes from time sensitive labor and overheads (as seen in most manufacturing process), it can be estimated that the Standard Production Cost (SPC) will roughly be reduced by 34% as far as the downstream SPC is concerned. This estimation does not even count the fact that there will be less consumables on operation cost and the initial capital investment may be one third of the traditional as there will be no expensive centrifugation and membrane filtration.

Depending on how expensive the raw materials are and the relative cost of the upstream process for the specific products this 35% could translate to significant cost reduction in the overall SPC.

EBA in technical sense may have been ready for industrial applications. However the drive for changing existing processes in pharmaceutical industry has been negligible because the risk involved with the regulatory control outweighs the economic benefit. Commercial driving force also ran out after GE Healthcare and Pall announced to pull out of the market. The main commercial activity about EBA has only been driven by UpFront at this moment. UpFront licensed its oscillating distributor technology to GE Healthcare, collaborated with DSM and Biogen Idec in commercializing its EBA technology.

Recirculation distributor has not seen any commercial activities probably because of the overall disappointing commercial environment around EBA. The main uncertainty regarding the recirculation distributor or the third generation EBA is around the shear sensitivity of the cells to the flow paths. Future researches need to focus on the application of the technology to real biological feedstocks and design details will be critical in bringing it to reality.

The economic benefit may still not be sufficient to drive a new wave of commercial efforts at this time, but considering the future of cell separation, which will be the next challenge for separation technology, the industry may start to see it differently.

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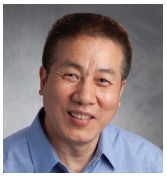
About the Author



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