



# ADVANCED BIOSCIENCES

Enhancing biopharmaceutical production and purification

## Decolorization

- AMBERLITE™ FP  
Ion Exchange Resins



For decolorization, separation and purification

# Pharmaceutical Processing

## AMBERLITE™ FP RESINS

### FOR DECOLORIZATION

#### Introduction

Within the pharmaceutical industry, there are many process streams which require purification by some means or another. Typically, this requires that a specific impurity is removed or that a range of impurities are eliminated. In the latter case, these impurities are never fully characterized and are generally given the name "color species". In all cases, the "color" is regarded as an impurity which must be removed in order to avoid any adverse quality problems with the final pharmaceutical product.

"Color" in a process stream can be derived from a variety of different sources but the two major sources are:

- The fermentation from which the product, or a bulk intermediate, is derived. Those "colors" which are derived from the fermentation tend to be similar to those encountered during the processing of sugar and tend to be large molecules as outlined below.
- Those derived from degradation products which tend to be different according to the type of product being studied. The molecular weight of these degradation products vary greatly but tend to have a carboxylic functionality which is commonly present in products such as antibiotics etc.

Despite the fact that a particular solution can be quite colored, the concentration of the species responsible for the color can be quite low. The most common method of measurement is by UV absorbance at a defined wavelength although HPLC and other techniques can sometimes be used.

#### Traditional Treatments

In most modern pharmaceutical processes, specific "color" is removed by some form of chromatography while more general "color" tends to be removed by either the use of granulated activated carbon or during the crystallization stage in the mother liquors.

The use of activated carbon, while widespread, is not without some problems as listed below:

- Absorption of active product,
- Environmental issues (e.g. handling of fine powders),
- Regeneration of the spent carbon (external),
- Colloidal carbon which requires filtration downstream,
- Low flowrate or filtration system,
- Possible contamination with metals.

In terms of crystallization, a highly colored solution can cause some of this "color" to be included into the crystal structure and makes any mother liquor recovery more problematical.



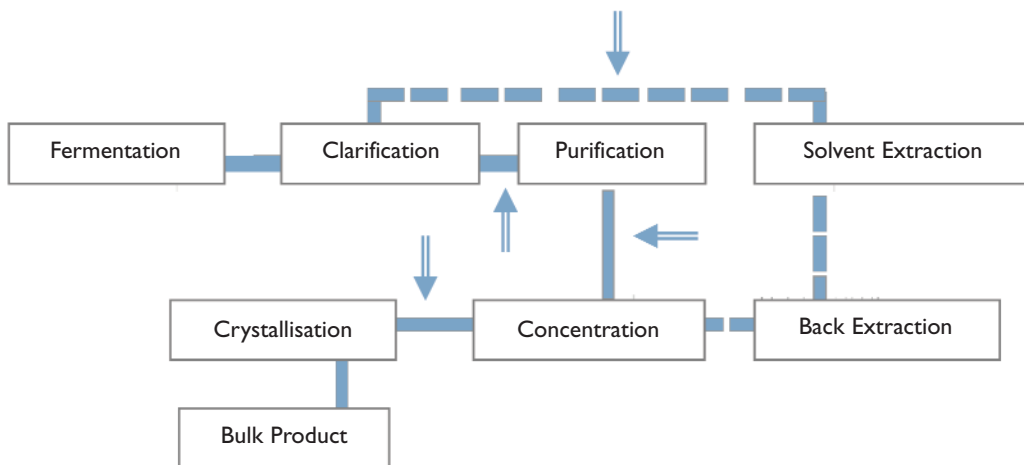
## Ion Exchange Resins

In the food and beverage industry, the use of ion exchange resins has either partially or wholly replaced the traditional carbon treatments by demonstrating good efficiency and cost effectiveness. In many cases, the use of ion exchange resins can significantly reduce the amount of activated carbon required such that it is only used as a "polishing" step which involves a low amount of carbon with a longer cycle time. Ion exchange resins have also shown the following advantages when used to decolorize process streams in other areas:

- Reduction of active product losses by optimisation of the resin type and process conditions,
- Fully automatic installation with cleaning/regeneration in place,
- No fines or colloids,
- High flowrates - 50 m/h linear velocity,
- No metal leachables.



Within the pharmaceutical industry, there is the possibility to use ion exchange resins both in aqueous streams and in solvent streams. A typical process scheme for the recovery of a bulk fermentation product (e.g. antibiotic) is given below and shows both a possible solvent extraction and aqueous extraction route.



*The arrows indicate possible points within the downstream process where a decolorization using ion exchange resins could be applied. However there are different selection criteria depending on whether the process stream is an aqueous media or solvent.*

## Aqueous Process Streams

In general, the colors which are present in typical fermentation processes are similar to those seen in the food industry. This is hardly surprising as the carbon source for the fermentation tend to be molasses or some other form of natural products such as rape seed oil, etc. Within the sugar industry, these "colors" have been studied and classified into four groups; caramels, melanoids, sugar degradation products (Maillard reactions) and phenolic-iron complexes.

As mentioned earlier, most degradation products tend to be polymeric species containing one or several carboxylic acid groups. These "colors" tend to be large organic molecules which have a weakly negative charge. The types of resins which are typically used in these cases are ion exchange resins which have large pores (macroreticular or MR) and/or a positive charge derived from either a quaternary amine or tertiary amine function. As an initial screening, the following ion exchange resins should be studied:

Name	Type	Matrix	Comments
AMBERLITE™ FPA40 CI	SBA	Gel - Styrenic	High moisture gel resin
AMBERLITE™ FPA53	WBA	Gel - Acrylic	Resin of choice of Ceph-C
AMBERLITE™ FPA90 CI	SBA	MR - Styrenic	Used extensively in Food & Pharma
AMBERLITE™ FPA98 CI	SBA	MR - Acrylic	Used extensively in Food & Pharma
AMBERLITE™ FPX66	ADS	MR - Styrenic	Recovery of natural products
AMBERLITE™ XAD™7HP	ADS	MR - Acrylic	Pharmaceutical applications
AMBERLITE™ XAD™76I	ADS	MR - Phenolic	Food applications

SBA : Strong Base Anion Exchanger    WBA : Weak Base Anion Exchanger    ADS : Adsorbent

When studying the use of ion exchange resins to decolorize an aqueous process stream, the objective is to find a resin which shows a difference in selectivity between the "color species" and the active product. This selectivity is then used to separate the two products with the "color species" typically showing a higher affinity for the resin as compared to the active product. Due to this, it is recommended that the initial screening be performed in small laboratory columns rather than simply stirring in a beaker.

As mentioned above, there are a number of different possibilities as to where ion exchange resins could be used within a particular downstream process. This choice of position depends on a number of factors as follows:

### After Clarification

This position is used in those cases where the majority of the color is derived from the fermentation itself and from the degradation of the active product during the fermentation which can last up to 10 days. If there is any degradation during the course of the downstream processing then this position will not be the most effective. At this stage, the color species tend to be dilute and the volumes to be treated are important.

### During Purification

Some color species will be removed during the purification stages of the process. Here, the volumes tend to be smaller and the color more concentrated. Operations at these later stages allow more selective color removal and can significantly improve the final product quality. Highly selective resins, such as Amberchrom™ adsorbents offer excellent potential at this stage.

### After Concentration and After Back Extraction

Primarily used in those applications where the colors derived from the degradation of the active product during the downstream processing. The solutions can be very concentrated and attention must be given potential yield losses of the active product on the resin. Careful choice of resin and/or conditions such as flowrate can reduce this to a minimum.

### Solvent Extraction

Rarely used unless very specific for a particular color derived from the fermentation. At this stage, the volume of solvent to be treated is often significant.

## Decolorization in Solvents

In general, the color species which are present in solvent streams tend to have a relatively low molecular weight and are polar in nature. In non-polar solvents such as acetone, MIBK etc., which are commonly used in the pharmaceutical industry, the choice of resin tends to be directed towards either ion exchange resins or the more polar adsorbents such as AMBERLITE™ XAD™7HP. This one area which activated carbon, either in granular or powdered form, is used extensively. As an initial screening, the following ion exchange resins should be studied:

Name	Type	Matrix	Comments
AMBERLITE™ FPA53	WBA	Gel - Acrylic	
AMBERLITE™ FPA98 CI	SBA	MR - Acrylic	Used extensively in Food & Pharma
AMBERLITE™ XAD™7HP	ADS	MR - Acrylic	Pharmaceutical applications
AMBERLITE™ FPC22	SAC	MR - Styrenic	Na+ and H+ form
AMBERLITE™ FPA51	WBA	MR - Styrenic	

SBA : Strong Base Anion Exchanger   WBA : Weak Base Anion Exchanger   ADS : Adsorbent

The use of the cationic resin is interesting in that it is probably the water of hydration around the sodium or hydrogen ion which plays a key role. The approach in the use of ion exchange resins in solvent media is for the polar color species to partition between the solvent phase and the water inside the resin matrix. Thereafter, the ionic groups on the resin can adsorb the color effectively removing it from the solvent stream.

While the use of ion exchange resins in solvent stream may not completely replace the use of activated carbon, it could both significantly reduce the volumes required and give the carbon columns a longer operating lifetime.

#### ATTENTION :

**Ion exchange and adsorbent resins both swell when passing from an aqueous to a solvent solution. The degree of swelling depends on the solvent used but the pressure generated is sufficient to shatter glass columns.**

**Nitric Acid should never be used with ion exchange resins or adsorbents since an explosive situation could ensue.**

When conditioning ion exchange and adsorbent resins in glass columns for use in a solvent solution, the following steps should be performed in the laboratory.

Follow the steps outlined earlier under "Charging resins to columns" and then the following additional steps :

1. Pump a water miscible solvent such as methanol or IPA UPFLOW slowly through the column allowing the resin bed to expand to around 30-50%.
2. Pass at least 3 BV of the water miscible solvent upflow through the column.
3. Change to the solvent which will be used in the process stream and pass 2 BV upflow through the column.
4. Stop the pump and allow the resin to settle in the column.
5. Drain down to just above the top of the resin bed.
6. Start to load process stream downflow.

The same procedure can also be carried out in a beaker with the two solvents and then charge the resin to the column following the same procedure as before but using solvent.

Once the loading cycle has been completed, the solvent remaining in the resin should be displaced by water as it is been observed that regeneration with caustic solution is an excellent way of removing the polar color species. Care should be taken with regards to any heat generated within the column due to either the rehydration of the resin or a change in pH. In most cases, the resin bed will shrink as it is gradually rehydrated. This technique is also known as "sweetening on and sweetening off" in the sugar industry. In general, ion exchange resins are considered as insoluble in solvent and over a wide range of pH's (1-14).

## Lab & Pilot Scale trials

Both lab and pilot trials follow the same general principles as indicated earlier for aqueous streams. A good understanding of the behavior of the ion exchange and adsorbent resins in solvents is important for the efficient use of these resins in an industrial application. There are many such examples of both ion exchange and adsorbent resins being used in solvent stream.

## Practical Work Resin Screening & Applications

### Equipment Requirements:

- Laboratory columns (100 ml) - dia. 25 mm, height 200 mm min.
- Gravity feed via a separatory funnel or laboratory pump and beaker.
- UV spectrophotometer,
- HPLC system (for active product)
- Other common laboratory equipment (beakers, stirrer etc.).

### Charging Resins to Columns:

#### a) Ion Exchange Resins

Deionized water should be used to transfer ion exchange resins to the column. The column itself should contain some water at the start of the operation, and the ion exchange resin should be poured into the column as a water slurry using a beaker or other vessel. Dry ion exchange resins should always be fully hydrated (typically requires 30 minutes in a beaker) before introduction into the column, since the swelling pressure created when the resin imbibes water may shatter the glass column.

The following steps should be followed:

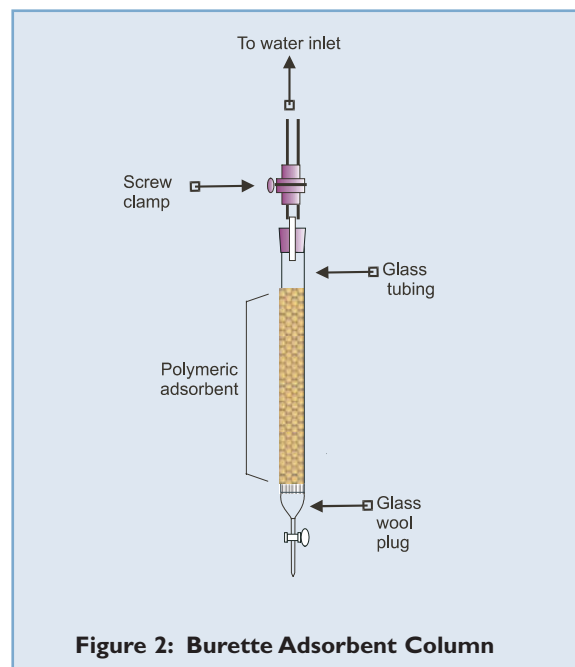
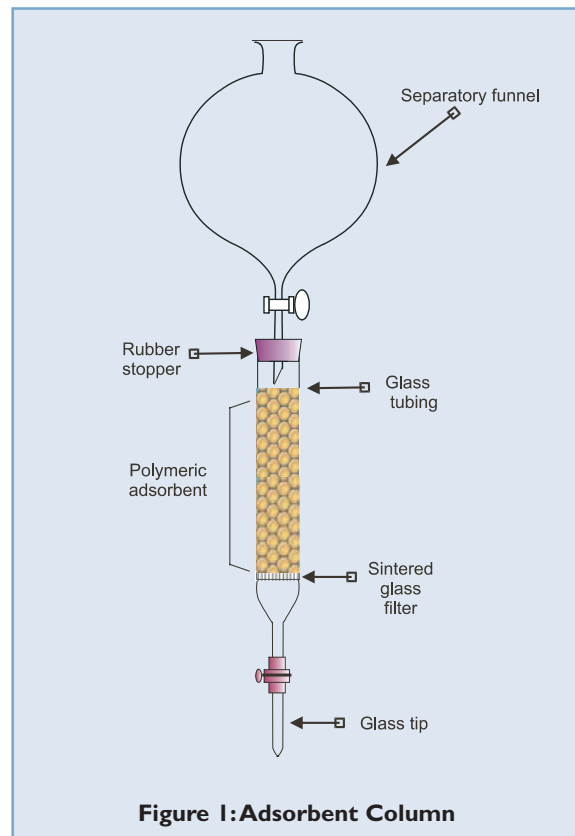
1. Add resin-water slurry as described above to a column containing some water.
2. Occasionally drain excess water from the bottom of the column
3. Do not allow the liquid level to fall below the resin level
4. Continue adding in this manner until all of the resin is transferred.
5. Do not load columns to more than about half of their height.

Prior to use, the resin should be backwashed as follows in order to ensure that the column is free of air pockets and that the resin is properly classified.

1. Attached a water line to the bottom of the column.
2. Introduce deionized water upflow very slowly,
3. Increase the flow slowly until the bed of resin expands near to the top end of the column
4. Maintain this flow until all of the air pockets are removed and all the particles have achieved mobility. This typically is done for ~ 30 minutes. This step is most important and, if done correctly, will results in good particle size classification with the smaller particles at the top and the larger ones at the bottom of the column.
5. Stop the flow of water and allow the resin to settle by gravity.

#### b) AMBERLITE™ XAD™ Adsorbent Resins

The above method equally applies to the AMBERLITE™ XAD™ adsorbent resins although a water wash with 10 bed volumes of water is recommended prior to use in order that all of the imbibed salt, which is used to inhibit bacterial growth, is removed.



## Screening Studies

Once the columns have been prepared, the screening can be performed as follows on as many columns as is practical to control within the laboratory.

**Loading** The solution to be decolorized should be loaded downflow at a rate of 2 to 5 Bed Volumes (BV)/h. Care should be taken that the liquid level does not fall below the level of the resin during this stage. The first ½ BV of effluent should be discarded

**Breakthru** The end-point is reached when either the top half of the resin is saturated with color or when the color passes through the column

Once the results are available then they should be discussed with the Rohm and Haas Representative.

The objective of these trials is to determine which type of resin has the best selectivity for the color species.

## Laboratory Trials

Laboratory trials are based on the results of the screening trials and have the following objectives :-

- Determination of mass balance,
- Optimization of pH, temperature, flowrate, regeneration, ionic form and capacity,
- Type of final installation required (number of columns etc.)

The reason for performing these trials is similar to that indicated above for the screening studies but on a slightly larger scale. The screening trials are designed such that for these larger scale trials, one or two resins at most, can be studied further in order to assess which is the best to move forward into pilot trials. In the case of the laboratory trials, the volume of resin used can be up to 1 liter although 500 ml is more common.

## Choice of Ionic Form

The AMBERLITE™ XAD™ adsorbent resins are obviously non-ionic but in the case of the ion exchange resins, there is a choice as to which counter ion (Cl<sup>-</sup>, OH<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, acetate etc.) can be used. In general terms, the strongly basic anionic resins such as AMBERLITE™ FPA40 Cl, AMBERLITE™ FPA90 Cl etc. are supplied as the chloride form. This is the form in which the resins are generally used in most applications and is generally considered to be their most stable form with regards to temperature. The weakly basic anionic resins such as AMBERLITE™ FPA53 are supplied as the free base form.

The choice of ionic form will be dictated by the process requirements. The OH<sup>-</sup> should not be used if the product of interest in the process stream is sensitive to changes in pH, particularly high pH, or is susceptible to nucleophilic attack (as in the case of Cephalosporins or Pencillins). The most common forms will be either the chloride form or a weak organic acid form such as acetate, etc. The advantage of using an acetate form is that the adsorption of any product containing a carboxylic group will be retarded due to competition while the removal of color will be preferred. A good example of this is the use of AMBERLITE™ FPA53 (acetate) in the Cephalosporin C recovery process. The one disadvantage of using an acetate form resin is that an additional regeneration step will be required to convert the resin back into the acetate form after regeneration either with NaOH or NaCl.

As an initial starting point, it is recommended that the chloride form should be studied.

## Regeneration of the Ion Exchange Resins

The objective of any regeneration is to return the resin to the same state as it was in the previous cycle. The efficiency of regeneration is directly linked to the lifetime of the resin in a particular process. In general, it is recommended to perform a regeneration after each cycle as a build up of foulants, particularly polymeric degradation products, may cause problems. Suggestions of how to regenerate the resin after loading and washing are given in the product data sheets. Please contact your Rohm and Haas representative for specific regeneration details for your process.

## Regulatory Information

The AMBERLITE™ FP product line is being developed from resins which have been used successfully for many years in the Food processing market. These resins are supported by a high level of quality and manufacturing systems specific to the evolving FDA, European, and other national regulations designed to support the safe use of these materials to the various health authorities.

With the creation of the AMBERLITE™ FP product line, Rohm and Haas can continue to offer exceptional technical and regulatory support to both our existing and new customers in addition to the leading technology providers to the globally food and bioprocessing markets.

Rohm and Haas is actively working on adding new products to the product line focussed on specific applications in cooperation with the market leaders.

## Ordering Information

10235527	AMBERLITE FPA40 CI	1 L
10235548	AMBERLITE FPA51	1 L
10235545	AMBERLITE FPA53	1 L
10235465	AMBERLITE FPA55	1 L
10235533	AMBERLITE FPA90 CI	1 L
10235537	AMBERLITE FPA91 CI	1 L
10235523	AMBERLITE FPC22 H	1 L
10235470	AMBERLITE FPC3500	1 L
10235550	AMBERLITE XAD1180	1 L
10235530	AMBERLITE XAD16	1 L
10285467	AMBERLITE XAD18	1 L
10235540	AMBERLITE XAD7HP	1 L

For all others, please inquire at:

<https://www.rohmhaas.com/wcm/contact.page>



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**CAUTION**

Ion exchange resins and polymeric adsorbents, as produced, contain by-products resulting from the manufacturing process. The user must determine the extent to which organic by-products must be removed for any particular use and establish techniques to assure that the appropriate level of purity is achieved for that use. The user must ensure compliance with all prudent safety standards and regulatory requirements governing the application. Except where specifically otherwise stated, Rohm and Haas Company does not recommend its ion exchange resins or polymeric adsorbents, as supplied, as being suitable or appropriately pure for any particular use. Consult your Rohm and Haas technical representative for further information.

Acidic and basic regenerant solutions are corrosive and should be handled in a manner that will prevent eye and skin contact. Nitric acid and other strong oxidising agents can cause explosive type reactions when mixed with Ion Exchange resins.

Proper design of process equipment to prevent rapid buildup of pressure is necessary if use of an oxidising agent such as nitric acid is contemplated. Before using strong oxidising agents in contact with Ion Exchange Resins, consult sources knowledgeable in the handling of these materials.

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