distillation may find a place in many other processes such as hydrolysis of methyl acetate, recovery of carboxylic acid from their aqueous solutions, hydrodesulfurization and purification of phenols.

See also: **II/Distillation:** Energy Management; Historical Development; Instrumentation and Control Systems; Theory of Distillation.

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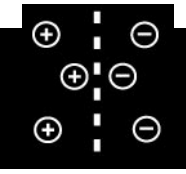
RESINS AS BIOSORBENTS: ION EXCHANGE



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Introduction

The term biosorbent is usually applied to solid polymeric media employed in the purification, separation or isolation of biotechnological products. To assure

efficient sorption, these materials must meet certain requirements: they must have a high sorption capacity combined with ease of regeneration, good kinetic properties and mechanical stability over many sorption–regeneration cycles. Both ion exchange resins and their precursors, the inert polymer matrices, are extensively used to isolate fermentation products, including low molecular weight compounds, such as acetic acid, and high molecular weight compounds, such as enzymes and proteins.

Ion exchange resins have been traditionally used in water treatment technologies, for example for desalination and softening and for wastewater treatment. Their first application to pharmaceuticals may be dated to the 1950s and 1960s, although the greatest surge in interest in terms of papers and patents published occurred in the period 1960–75.

Pharmaceuticals

Of the various pharmaceutical products processed by ion exchange technologies, antibiotics are probably the most important. Because antibiotics mostly consist of charged molecules, they lend themselves readily to isolation with ion exchange resins, and with cation exchangers in particular. In January 1945, Van Dolah, Christenson and Shelton filed a US patent application claiming the use of organic cation exchangers for the purification of streptomycin and streptothricin. First to be used for this purpose were the phenol-sulfonic acid-type cation exchangers (Amberlite IR-100, Ionac C-200, Dowex 30). These were followed by high capacity carboxylic acid exchangers for commercial applications (Amberlite IRC-50). Both groups are characterized by a gel structure. They have no open pores in the dry state, but when placed in contact with aqueous solutions they undergo swelling and acquire the ability to uptake large ions.

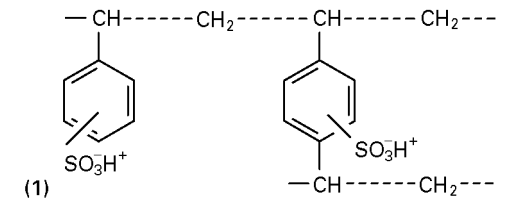
Commercialization of the macroporous sorbents of the Amberlite XAD series by Rohm and Haas in the 1960s was a revolutionary step in ion exchange technology and opened up new possiblities for the isolation of antibiotics. Macroporous sorbents had the necessary mechanical strength, provided large surface areas for sorption, and had appropriate pore sizes for rapid transport. Macroporous resin sorbents such as the polyaromatics Amberlite XAD-4,-16 and -1180, Diaion HP20, media consisting of aliphatic esters (Amberlite XAD-7) and nitrated aromatics (nitrated Amberlite XAD-16) were recommended for large scale application for antibiotics.

Vitamins constitute another class of pharmaceuticals that are purified by ion exchange resins. Vitamin B_{12}, for example, is produced by microbial fermentation and can be separated from the broth using a carboxylic acid exchanger.

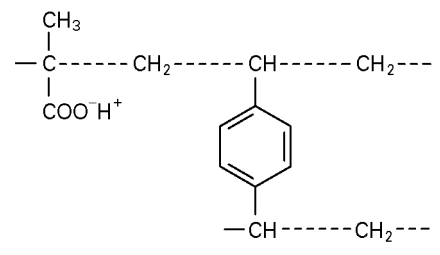
Proteins are based on copolymers of amino acids and may thus be regarded as polyionic materials. At a given pH they bear either a positive or a negative charge depending on their isoelectric point. Proteins are therefore eminently suitable for isolation by ion exchange technology. Exchangers based on matrices consisting of cross-linked polyacrylic and phenol-formaldehyde polymers have been used for large scale protein purification. However, the traditional ion exchangers are generally unsuitable for the adsorption of proteins due to their hydrophobicity, high charge density and high degree of cross-linking, which result in low protein capacities and a tendency towards denaturation of sorbed molecules. After the introduction in 1956 of the first ion exchanger specifically designed for proteins, a number of highly hydrophilic polysaccharide matrices have been proposed, all of them less rigid and more hydrophillic than the polystyrene type of biosorbents.

Synthesis of Resins

Today's ion exchange technology is based on organic polymer matrices. The typical spherical ion exchange beads are made by suspension polymerization of styrene with divinylbenzene to form an insoluble polymer gel. The mixture of monomers to which an initiator of radical polymerization has been added is stirred into an aqueous suspension under conditions designed to give the desired droplet size. This mixture is heated for several hours to yield solid spherical beads, which are then treated with concentrated sulfuric acid at about 80°C to obtain cation exchange resin. The final product is a sulfonated cross-linked polystyrene – the strong-acid cation exchanger most widely used commercially. It has a capacity of 5.25 mmol g^{-1} calculated for oven-dried resin. The structural formula of the resin is given below (Structure 1), together with the formula of a weak-acid cation exchanger based on acrylic acid copolymerized with DVB (Structure 2).

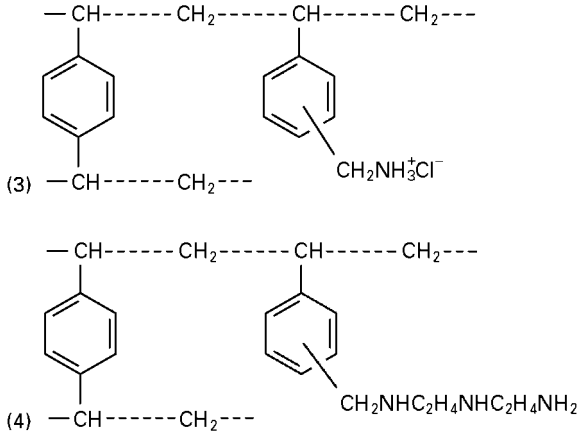


(1)



(2)

Anion exchange resins are produced by a two-step process. First, chloromethylation is applied to introduce chloromethyl groups. The second step is amination. When a tertiary amine such as trimethylamine is used, the product is a strong-base quaternary ammonium compound (Structure 3). This resin is the anionic equivalent of the sulfonic cation exchange materials. The capacity of a typical strong-base resin is 3.9–4.2 mmol g^{-1} of dry resin. The use of a secondary amine, such as dimethylamine or other multifunctional amine, gives various weakly basic resins, for example the one shown in Structure 4.



The resins mentioned above are among those most commonly used as ion exchangers. However, a wide range of resins tailored for specific needs is available; further information may be found in commercial catalogues as well as in relevant monographs. For illustration, a list of synthetic resins manufactured by Mitsubishi and designed for protein separation is given in **Table 1**, together with the relevant recommendations.

As an alternative to the highly hydrophobic organic polymeric matrices, ion exchange materials for biological compounds have also been developed from cross-linked dextran, agarose and beaded crystalline cellulose polymers. The functional groups typically added to such matrices are shown below.

Anionic functional groups

Aminoethyl (AE)	$-OCH_2CH_2NH_3^+$
Diethylaminoethyl (DEAE)	$-CH_2CH_2N(CH_2CH_3)_2$
Quaternary aminoethyl (QAE)	$-OCH_2CH_2N^+(C_2H_5)_2$-$CH_2CH(OH)CH_3$

Cationic functional groups

Carboxymethyl (CM)	$-OCH_2COO^-$
Phospho	$-PO_4H_2^-$
Sulfopropyl (SP)	$-CH_2CH_2CH_2SO_3^-$

DEAE-cellulose, an anion exchanger containing diethylaminoethyl groups attached to the cellulose, is applied extensively. An exchanger of this type having a content of basic groups of only 1 mmol g^{-1} adsorbs three-quarters of its own weight of bovine plasma albumin from 0.2% solution in 0.01 mol L^{-1} sodium phosphate at pH 7.0. CM-cellulose, a cation exchanger, which contains carboxymethyl groups, adsorbs its own weight of horse carbon monoxide haemoglobin from 0.2% solution in 0.01 mol L^{-1} sodium phosphate at pH 6.0. Cellulose ion exchangers with improved characteristics are now available, and numerous studies on their use in

Table 1 Sepabeads FP series product list[a]

Grade	*Functional group*	*Pore size*[b]			*Chromatography mode*				
		Small	*Medium*	*Large*	*GFC*	*CEC*	*AEC*	*HIC*	*AFC*
FP-HG	–OH	FP-HG20	FP-HG13	FP-HG05	◎				
FP-CM	$-CH_2COOH$		FO-CM13			◎			
FP-QA	$-N^+(CH_3)_2C_2H_4OH$		FP-QA13				◎		
FP-DA	$-N(C_2H_5)_2$	FP-DA20	FP-DA12	FP-DA05			◎		
			FP-DA13				◎	O	
FP-HA	$-NH(CH_2)_6NH_2$	FP-HA20	FP-HA13					◎	
FP-BU	$-O(CH_2)_3CH_3$		FP-BU13	FP-BU05				◎	
FP-OT	$-O(CH_2)_7CH_3$		FP-OT13					◎	
FP-PH	$-OC_6H_5$		FP-PH13			O	O		
FP-CL	$-N(CH_2COOH)_2$		FP-CL13						◎
FP-BL	Cibacron blue 3G-A		FP-BL13						◎

[a]Average particle size approximately 120 μm.
[b]The second digit in the product name refers to the pore size. GFC, gel filtration chromatography; CEC, cation exchange chromatography; AEC, anion exchange chromatography; HIC, hydrophobic interaction chromatography; AFC, affinity chromatography.
From Paion, Manual of Ion-Exchange Resins and Synthetic Absorbents.

the separation of biologicals have been reported in the last 5 years.

Characteristics of Resins

Selection of the exchange resin for a given application is a process of compromise based on examination of many factors, such as the polar nature of the sorbate, the size of the sorbate, resin capacity, equilibrium relationships, elution properties and flow characteristics.

Adsorption Isotherm

In order to design a purification process based on an ion exchange technique, it is essential to know something about the capacity of the exchanger. Equilibrium sorption capacity is commonly determined with the help of the sorption isotherm, which gives the sorption uptake (q) and the final equilibrium concentration of the residual solute in solution (c). Sorption isotherms are measured by placing solutions with different concentrations of solute in contact with a known weight of the resin at a constant temperature until equilibrium is attained. Calculation of the difference between the concentration of product before and after equilibrium, c^*, gives the sorbed protein mass q^*. Plotting q^* versus c^* yields the equilibrium sorption isotherm. Assuming that single-site interaction occurs between bioproduct and sorbent, and also that nonspecific interactions are absent, the apparent constant K_A and the maximum product-binding capacity q_m may be evaluated by fitting the experimental data to the well-known Langmuir model:

$$q^* = \frac{q_m \cdot K_A \cdot c^*}{1 + K_A \cdot c^*}$$

Non-Langmuirian behaviour may point to multiple interaction sites. In such cases, appropriate models may be worked out to fit the experimental data and used to determine whether this behaviour may be due to additional nonspecific interaction sites from the sorbent's surface, or to product–product interactions with the first adsorption layer.

Kinetics of Adsorption

Another important factor of sorption performance is the kinetics of the adsorption/desorption reactions. The rates of these reactions dictate the length of time that has to be allowed to attain equilibrium. For example, the adsorption of protein on to packed beds involves three processes.

First, the protein is transported from the bulk fluid to the outer surface of the adsorbent particles by film mass transport. Second, intraparticle transport occurs by diffusion. Finally, the protein binds to ligand attached to the inner surface of the particle. It is important to determine which of these processes is the rate-limiting step.

Process Design

Isolation of bioproducts by ion exchange processes can be carried out either batchwise or by traditional packed-bed techniques. In the former, the exchanger is added to the product solution in a vessel which is mixed until sorption has occurred.

Packed-Bed Column

In a packed-bed column the movement of liquid through the bed approximates to plug flow, resulting in a maximum number of theoretical equilibrium stages within the column and hence good adsorption and chromatographic performance. The overall flow performance is strongly related to the length and shape of the ion exchange zone evolving during sorption and regeneration. This zone appears between the section of column saturated with product and the section that still contains fresh sorbent. As loading or regeneration progresses, the zone moves along the column in the direction of the liquid flow. Breakthrough occurs when the zone approaches the end of the column and the concentration in the outlet stream increases sharply. Breakthrough profiles provide a measure of the performance of different ion exchangers in packed-bed operations. A sharp breakthrough profile is desirable in order to achieve efficient use of sorbent. **Figure 1** shows breakthrough profiles for two hypothetical adsorbents with identical equilibrium capacities. It can be seen that a greater proportion of bed capacity is used in the case of sharp breakthrough.

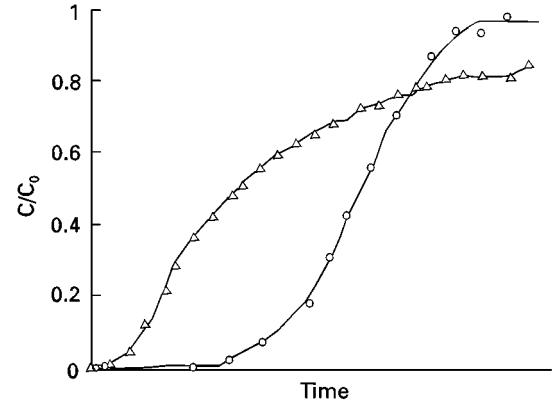


Figure 1 Hypothetical breakthrough curves for two sorbents. The unfavourable breakthrough curve (triangles) is flat and trailing, while the favourable breakthrough curve (circles) is sharp and steep.

Fluidized Bed Column

In a fluidized bed, liquid upflow through the column causes the resin particles to become separated from each other. This technology has attracted attention for biochemical separation processes because it enables direct treatment of crude feedstocks from fermentation reactors. There are two important criteria that must be met before fluidized bed sorption can be considered a viable method for separating products from unfiltered fermentation broths. First, broth solids must have a lower terminal settling velocity than the resin, and the terminal velocity of the resin must be sufficient to achieve reasonable time cycles. Terminal velocity is defined as the upflow velocity at which particles will not remain in the column. Second, the dynamic adsorptive capacity of the resin for the product must be of such a magnitude that optimal yield, purity and cycle time can be achieved.

Determining optimum resin terminal velocity and dynamic sorptive capacity for a specific product is a complex process. The breakthrough curves are usually obtained for a variety of design and operating conditions (column size, distributor, bed type, bed height, flow rate and number of stages). It is also essential to find an appropriate mathematical model for simulation and optimization of the processes. An extensive literature exists describing the mode of operation of fluidized beds with reference to bioproduct separation. A schematic representation of fluidized bed separation is given in **Figure 2**.

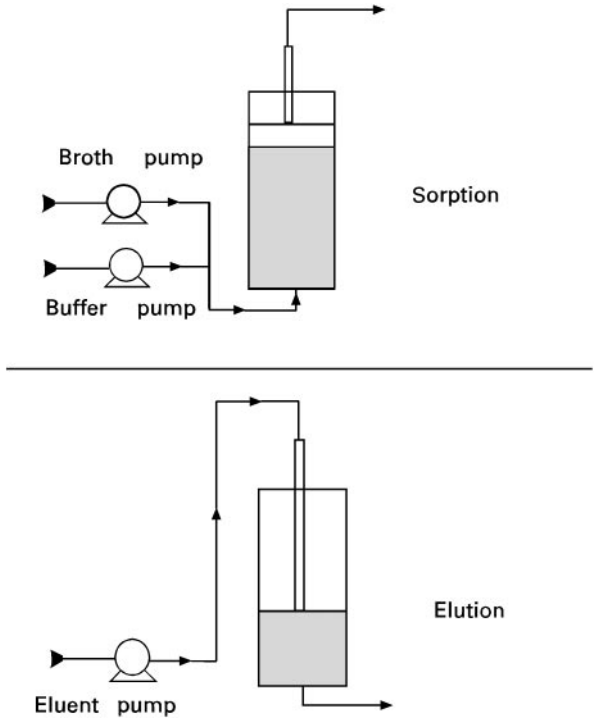


Figure 2 Schematic representation of fluidized bed separation.

New Developments

Although ion exchangers remain the most frequently used media for separation of biological mixtures, some novel approaches have emerged. Perfusion chromatography is one of them. This method exploits the fact that particle resins have very large pores (600–800 nm) that permit convective flow. A high surface area for sorption is provided by the presence of numerous small diffusive pores. Thus, convection rather than diffusion dominates the mass transport of the sample molecules. This makes the process 10 times faster than the usual separation process without much loss in capacity or resolution.

Another approach which has emerged as a powerful separation tool is immobilized metal affinity chromatography (IMAC). In this method, immobilized ligands, like iminodiacetic acid, produce chelates with transition metal ions (such as Ca^{2+}, Zn^{2+} and Fe^{3+}) which, when exposed to a protein, form a ternary complex on the protein surface. Further isolation is then accomplished with ease.

In conclusion, the latest developments in sorption media and separation technology provide a broad and varied basis for identification of appropriate sorbents and selection of contact mode between feedstock and sorbent.

See also: **II/Chromatography:** Protein Separation. **Ion Exchange:** Organic Ion Exchangers.

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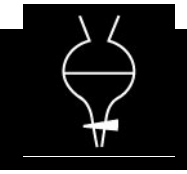
RESTRICTED-ACCESS MEDIA: SOLID-PHASE EXTRACTION



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Introduction

For the determination of drugs and their metabolites in serum or plasma by high performance liquid chromatography (HPLC), tedious and time-consuming pretreatment procedures such as liquid–liquid extraction, solid-phase extraction (SPE) or membrane-based extraction are often required. Among those pretreatment procedures, SPE is the most widely used for extraction of target compounds in biological fluids. However, direct injection of serum or plasma samples onto HPLC or SPE materials causes protein denaturation with accumulation of materials on the sorbent, resulting in undesired loss in the capacity and selectivity of the sorbent. Thus, it is essential to remove serum or plasma proteins before loading the samples onto the HPLC or SPE sorbents. Recently, restricted access media (RAM) materials were introduced for direct injection of proteinaceous samples onto the HPLC or SPE materials. With RAM materials large molecules such as proteins are eluted in the void volume without destructive accumulation because of restricted access to some surfaces, while allowing small molecules such as drugs and their metabolities to reach the hydrophobic, ion-exchange or affinity sites and be separated. One approach uses an internal-surface reversed-phase (ISRP) material, produced from porous silica gels, which has hydrophobic interior and hydrophilic exterior surfaces, as shown in **Figure 1**. The ISRP–GFF material (GFF = glycine-L-phenylalanine-L-phenylalanine) was prepared from covalently modified glycerylpropyl (i.e. diol) phases by attachment of the tripeptide GFF, bonded via the amino groups to the glycerylpropyl groups. The phenylalanine moieties were then cleaved from the external surface of the silica with carboxypeptidase A, which is too large to enter the small pores. After this enzymatic treatment, the glycerylpropyl moieties and glycine residues remain on the external surface. Because the ISRP concept was innovative for drug determinations in serum, many RAM materials were subsequently developed. Another RAM material based on silica gels is shielded hydrophobic phase (SHP), which consists of a

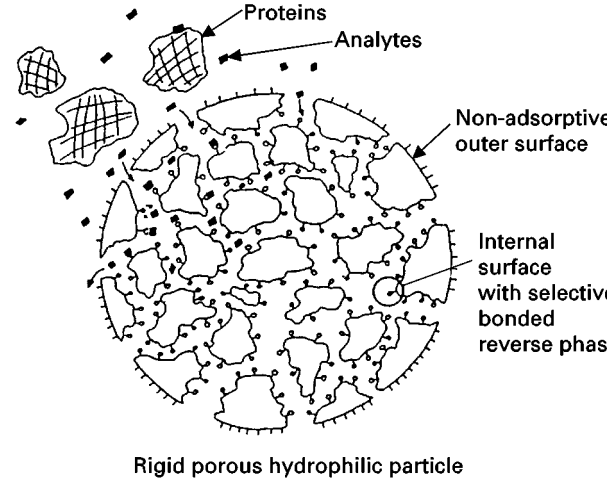

Figure 1 Schematic representation of an internal-surface reversed-phase (ISRP) material. Proteins do not adsorb on the hydrophilic exterior surfaces and do not penetrate into the hydrophobic interior surfaces, while analytes can reach the interior surfaces and be separated. (Reproduced with permission from Perry JA (1991) The internal surface reversed phase. Concept and applications. *Journal of Liquid Chromatography* 13: 1047–1074.)