ION CHROMATOGRAPHY APPLICATIONS

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PREFACE

When ion chromatography was invented in 1978, it presented a significant advance in determining common inorganic anions such as chloride, nitrate, phosphate, and sulfate, in a variety of aqueous solutions. For the next 3 to 4 years, methods were developed to determine almost any inorganic anion with a pKa under seven. This was made possible by using a suppressor column to suppress the conductivity of the eluent and enhance the conductivity of the analytes. In addition, alkali and alkaline earth metals, ammonium, and some aliphatic amines could be determined by using cation chromatography and a cation suppressor. At least two books and several review articles have been written describing such aspects of Ion Chromatography. This book describes numerous advances that have been made since then. Today, ion chromatography implies much more than isocratic elution, suppressor columns, and conductivity detection. This is the first time that gradient ion chromatography, mobile phase ion chromatography, pulsed amperometric detection, post-column reaction, and other newer techniques have been covered in one review. Throughout the book, practical applications are emphasized, with the largest chapter devoted, exclusively, to industrial and biological applications. This book is intended to be both a quick reference to the analyst who is performing methods of development, and a guide to a more detailed understanding of the different separation and detection methods now available in the chromatography of ions.

Robert E. Smith

THE AUTHOR

Robert E. Smith, Ph.D., is an Associate Professor at the University of Missouri — Kansas City School of Pharmacy, and has been performing analytical methods of development, with special emphasis on ion chromatography.

Dr. Smith received his B.S. degree in Chemistry from the University of Missouri in 1973, and his Ph.D. in Biochemistry in 1978. He was a postdoctoral fellow at the Sinclair Comparative Medicine Research Farm in Columbia, Missouri, in 1979, and was awarded a research fellowship at the Federal Institute in Zurich, Switzerland, in 1980. Since then, he has been in Kansas City, with research interests in epoxy resin chemistry, electroplating, and analytical biochemistry. This work has resulted in over 30 scientific papers. His current efforts are in using ion chromatography to determine sugar phosphates, especially inositol phosphates, in the ischemic brain.

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I would like to gratefully acknowledge the support provided by Dionex in preparing this book. Dionex is the owner of many exclusive patents in the field of ion chromatography. All art work and figures in this book were kindly provided by Dionex. All ion chromatographic data reported in this book were obtained using equipment on which Dionex owns patents. I would especially like to thank the research scientists at Dionex, such as Rosanne Slingsby, Dennis Gillen, and Roy Rocklin for all the information that they provided.

This book is dedicated to the one person most responsible for my education. This book is dedicated to my Mother.

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Chapter 1

BASIC PRINCIPLES AND THEORY

I. INTRODUCTION

In 1974, just 1 year before the invention of ion chromatography, Horvath[1] described ion exchange chromatography (IEC) as the most prominent branch of liquid chromatography despite the lack of a universal detector for ions. Even in 1974, there were numerous applications for IEC. These included the analysis of organics, inorganics, and biopolymers. One application of IEC even helped win a Nobel Prize. Ion chromatography is simply a spin-off from these techniques. It is a modern method for ion analysis. It, too, can solve a number of very important analytical problems.

For example, consider the application which was responsible for a Nobel Prize. This application is amino acid analysis.[2] Using two different ion exchange columns, biochemists were able to separate and detect the 20 amino acids found in proteins and enzymes. The basic amino acids, i.e., those that have a net positive charge at pH 8, were separated on a cation exchange column that is relatively short. The neutral and acidic amino acids were separated on a longer anion exchange column. In both cases, the amino acids were detected by a post-column reaction with ninhydrin. The amino acids react with ninhydrin to form a colored complex which is detected by a UV-visible detector. This became an invaluable technique in studying the chemical properties of biologically significant polypeptides.

Amino acid analysis is not the only well-known application of ion chromatography. Another application which is commercially very successful, is water softening. Water softeners in the home contain ion exchange resin. The calcium and magnesium ions that contribute to the water hardness bind to the cation exchange resin in the water softener. As a result, the total calcium plus magnesium is significantly lowered. In each application, ions (such as an amino acid or calcium) in an aqueous solution, will bind to the ion exchange material under proper conditions of pH and ionic strength. These same ions may be eluted off the ion exchange material under different conditions of pH and ionic strength, thus, water softeners can be regenerated periodically. Often the analyst is required to take advantage of this difference in binding affinities to separate a mixture of ions into their individual components. Environmental chemists may want to perform an ion survey of different geographical sites and require multicomponent analysis. An enzymologist may want to separate a mixture of amino acids to determine the amino acid composition of a polypeptide. Both could use the same technique, ion chromatography, to accomplish their goals.

Ion chromatography is a subset of the broader field of IEC, but the two terms (ion chromatography and IEC) are not synonomous. Certainly there are numerous ion exchange methods for purifying a single enzyme from a tissue homogenate. However, at the time of writing, ion chromatographic methods for doing this, have not been described. However, the field of ion chromatography is growing rapidly and has been incorporated into Environmental Protection Agency (EPA) methods for water analysis.[3] Applications range from amino acid analysis to the determination of hydroxymethanesulfonate in fog water in Bakersfield, Calif.[4] Ion chromatography is used to help make the paper on which this book is printed, the microprocessors in hand-held calculators, and even in the food and beverages that we eat and drink.

The field of ion chromatography had its inception in 1975 with the landmark paper by Small et al.[5] They described an invention that made possible the simultaneous determination of inorganic anions and cations. The novelities of the invention were the use of low-capacity pellicular resins and chemical suppression of the conductivity of the eluent. A patent for

ion chromatography was awarded to Dow Chemical Co., under whose auspices the technique was first developed. This original technology was licensed to the Dionex Corp., which now owns patents and trademarks on numerous hardware used in modern ion chromatography. The bulk of the literature published at the time of writing, describes the use of columns and chromatography systems that have since been considerably upgraded. Because of the constant upgrades, many publications describe the use of different "separator" and "suppressor" columns. Not only conductivity detectors, but also UV-visible, fluorescence, and electrochemical detectors are used; the terminology rapidly becomes confusing. It is difficult for the biochemist, electroplater, environmental chemist, or even for the brewmaster to evaluate the data that are published on ion chromatographic analysis of carbohydrates, chromic acid, acid rain, or beer. By understanding the basic principles and terms used in ion chromatography, the different scientists can properly assess the importance of this relatively new area in analytical instrumentation.

Ion exchange can be compared to the binding of a metal to ethylenediamine tetraacetic acid (EDTA). EDTA can be compared to a column containing ion exchange sites. Both EDTA and a cation exchange column will bind metals at the proper pH. Both do so by exchanging loosely bound hydrogen ions for metal ions. EDTA at pH 6 to 9 contains two –COOH groups that act as "cation exchange sites" in this analogy. One calcium (Ca^{2+}) will exchange with two H^+ on the –COOH groups as illustrated below, where EDTA is abbreviated as H_2L^{2-}.

$$Ca^{2+} + H_2L^{2-} \rightarrow CaL^{2-} + 2^{H+}$$

Similarly, one calcium will exchange with two H^+ on the cation exchange resin. The difference here is that the two H^+ on the cation exchange resin are fixed on a solid support. The solid support is the ion exchange resin, which in many cases is a sulfonated polystyrene.

One can likewise compare the concentration of EDTA to the ion exchange capacity of the resin. For example, if there are 0.01 mol (2.42 g) of disodium EDTA, it would be able to bind 0.01 mol of calcium (or many other divalent metals). In the 0.01 mol of EDTA there are 0.02 mol of exchangeable H^+. If one mole of H^+ is defined as one ion exchange equivalent, the 0.01 mol of EDTA would represent 0.02 ion exchange equivalents. Similarly, one can measure the number of exchangeable H^+ on a cation exchange resin. This number of exchangeable H^+ can be expressed in terms of ion exchange equivalents. Suppose, then, that it takes 0.20 mg of ion exchange resin to have 0.02 ion exchange equivalents. The ion exchange capacity of the resin would then be 0.2/0.02 or 0.1 meq/g of resin. In fact, many of the cation exchange resins used in 1975 had ion exchange capacities of about this value.

Of course, there are some serious oversimplifications in this analogy of EDTA. Very different mechanisms are involved in ion exchange and in chelation with EDTA. More relevant to this discussion though, is the fact that the cation exchange resins are not small molecules with high mobility in solution, instead, they are polymers, with very limited mobility. The ion exchange sites on these polymeric resins are fixed on the solid, polymeric surface.

In order to attain 0.1 meq of ion exchange sites per gram of resin requires that the resin have a large surface area. This was accomplished by using polymeric resins that were relatively porous. These relatively high-capacity, porous, polymeric resins make it possible to do preparative-scale ion exchange chromatography. This is still quite useful in purifying large amounts of (ionic) biopolymers. Such porous resins do have their drawbacks, however. They compress when pressurized with an analytical pump. This makes it extremely difficult to incorporate porous resins into an analytical liquid chromatograph. This alone limits their reproducibility. In addition, porous ion exchange resins expand and contract as the ionic strength of the eluent changes. Unfortunately, changing the ionic strength of the eluent is

a very useful way to elute strongly retained ions. As a result, it would be ideal to have an ion exchange resin that could effectively separate the required ions and not be susceptible to compression under pressure and not change volume when the ionic strength changes. This was accomplished by the introduction of polymeric resins with much lower porosity and, therefore, much lower ion exchange capacity. The term "pellicular" is used to denote low porosity and only surface modification. Thus, the term "pellicular resin" is often used to describe the low porosity and surface sulfonation of ion exchange columns.

The development of these lower-capacity pellicular resins was instrumental in the success of ion chromatography as an analytical technique. The pellicular resins used to separate ions in the field of chemically suppressed ion chromatography are now used in ion separator columns. They are simply a specific example of an ion exchange resin.

The term "ion exchange" implies that the primary mechanism of separation is due to exchange of eluent ions on the column for analyte ions. The column is packed with a substrate that contains a permanent charge. For example, the substrate may be a copolymer of polystyrene/divinylbenzene. The fixed charge is obtained by reacting the substrate with hot sulfuric acid to produce a sulfonated polystyrene/divinylbenzene. The sulfonate ($-SO_3H$) groups are strongly acidic so that they exist as $-SO_3^-\ H^+$. The substrate now has a fixed negative charge from the $-SO_3^-$. Cations will be attracted to this negative charge and will exchange with the H^+. Thus, the sulfonated polystyrene/divinylbenzene is a cation exchange resin. The polystyrene/divinylbenzene resins used in 1974 were porous. This caused problems with compressibility under pressure, along with contraction and expansion as the eluent changed, as mentioned earlier. In addition, the analyte ions could diffuse into the pores of the resin. This permitted interactions between the analyte ions and the polystyrene/divinylbenzene. As a result, the separation mechanism was (and still is) a combination of ion exchange and nonionic interactions with the polymeric resin.

The porosity of the resin required slow flow rates and caused significant band spreading. This is typified by a standard experiment in quantitative analysis. Copper was determined by passing the unknown through a porous cation exchange resin in the H^+ form (i.e., the resin had $-SO_3H$ attached to it). The Cu^{2+} exchanged with two H^+ on the resin. The H^+ eluted off the column and was collected in a flask to be titrated with NaOH. Because the copper diffused into the pores of the high-capacity resin, significant "band spreading" occurred. If the copper was originally dissolved in 10 mℓ, it would spread into a "band" of about 50 mℓ. In addition, the porosity of the resin required that the eluent flow rate be limited. If one attempted to increase the flow rate by using a high-pressure pump, the porous resin would simply compress, severely restricting the flow of effluent. In addition, the porous resin would expand and contract as the ionic strength of the eluent changed. This made it difficult to perform different analyses with the same packed column. If a stronger, higher ionic strength eluent was required, the resin would contract, creating voids in the column. This would severely limit the reproducibility of the chromatography. In practice, the copper analysis described required a 1 to 2 mℓ/min flow rate and each determination lasted 1 hr.

Despite these limitations, porous ion exchange resins were quite popular. One of the most popular ion exchange methods in 1974 was amino acid analysis, which was developed by Moore and Stein.[2] Again, the porous resins used in amino acid analysis meant that the separation mechanism was a combination of ion exchange and nonionic interactions with the polystyrene/divinylbenzene substrate. Although methods did exist for separating acidic, neutral, and basic amino acids simultaneously on one column, it was very common to use two columns. Contaminants could interfere with the determination of basic amino acids on the single column method, encouraging the use of one column (the short column) for basic amino acids and a second column (the long column) for acidic amino acids.[6] The amino acids were detected by post-column reaction with a reagent such as ninhydrin that reacts to form a highly colored compound that absorbs visible light.

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There are several ions, though, that have no inherent physical properties (except conductivity) which permit their detection at parts per million (ppm) levels. Such anions include fluoride, chloride, nitrate, bromide, phosphate, and sulfate. Each of these could be detected individually by other methods, such as reaction with a color-generating reagent (i.e., molybdate for phosphate determination) or ion-selective electrodes. However, no such detection scheme could be used for all these anions if they were present in the same solution. The only detectable physical property that they have in common is conductivity. Thus, it was desirable to use a conductivity detector. The eluents used in ion exchange chromatography had high conductivity, forcing the analyst to detect low levels of conductivity (i.e., 3 μS) in the presence of a high background (i.e., 1000 μS).

Many of these problems (band spreading, expanding and contracting of resin, and high eluent conductivity) were overcome in the landmark paper by Small et al.[5] In this first paper describing the invention of ion chromatography, these three major problems were overcome. By using pellicular resins having low ion exchange capacity instead of high-capacity porous ion exchange resins, analyte diffusion and, therefore, band spreading, was minimized. The porous resins have ion exchange capacities as high as 1 meq/g whereas pellicular resins have ion exchange capacities as low as 0.02 meq/g. As mentioned earlier, the ion exchange capacity of a resin is simply a measure of the number of ion exchange sites per gram of resin.

The nonporous, or pellicular, nature of the resins of today also means that there is no expansion or contraction when different eluents are used. The resin does not compress when eluent is pumped through the column, permitting faster, controlled flow rates with limited back pressure. This permits the use of reproducibly packed analytical columns that can be used with an analytical pump to produce accurate eluent flow rates as high as 4 mℓ/min. Different analyses using different eluent ionic strengths could be used without creating column voids which appear with more porous columns.

This was certainly important in the original invention of ion chromatography. Perhaps more significantly, though, a second column was introduced which suppressed the conductivity of the eluent and enhanced the conductivity of the analyte ions, enabling the use of a conductivity detector. This second column was originally called a "stripper column" because it stripped the eluent of its conductivity. This column has also been described as a post-column reactor, because it does react with the eluent and analyte ions.[7] By reacting with the eluent, it suppressed its conductivity. This effectively lowered the background conductivity so that a conductivity detector became practical. It also helped that the second column reacted with the analyte ions, converting them to a highly conductive form. Thus, this second column, or suppressor column, suppresses the conductivity of the eluent and enhances the conductivity of the anlyte ions. As a result, the major problem plaguing analysts in 1974, the lack of a universal detector for ions, was overcome. The conductivity detector became the long-sought "universal" detector. It was useful only when the suppressor column was used. Thus, this form of ion chromatography is now known as "chemically suppressed ion chromatography".

Chemical suppression of conductivity can be best understood by describing the details of a real analytical situation. For example, in cation analysis, the eluent is HCl. The HCl elutes sodium, ammonium, and potassium, which then go to the second column. This stripper column was simply an anion exchange column in the $-OH$ form. The anions in the sample and eluent exchanged with the $-OH$ on the stripper column. Thus, the highly conductive HCl was converted to HOH or water, which has low conductivity. If the sample contained NaCl, NH_4Cl, and KCl, the Cl^- would exchange with the $-OH$, producing NaOH, NH_4OH, and KOH, which have higher conductivity than the corresponding chloride salts. Any cation in the sample would be converted to its hydroxide. This meant that transition metals would be converted to their insoluble hydroxides. Even though they eluted off the cation separator

column, the transition metals never reached the conductivity detector, since they were trapped as a precipitate on the stripper column. Transition metals are detected, instead, by post-column reaction with a colorimetric reagent, PAR. This will be discussed in more detail in Chapter 3 (Section III).

In anion analysis, the anion stripper was a cation exchange resin in the –H form, i.e., it was a sulfonated polystyrene/divinylbenzene. The –H on the $-SO_3H$ was exchanged for the cations in the sample and eluent. In the original paper by Small et al. NaOH was used as the eluent. Thus, the highly conductive NaOH was converted to water. If the sample contained NaF, NaCl, $NaNO_3$, Na_2HPO_4, $NaNO_3$, NaBr, and Na_2SO_4, they would be converted to HF, HCl, HNO_3, H_3PO_4, HNO_3, HBr, and H_2SO_4. The H^+ ion has the highest specific conductivity of any cation, so the acid forms of the analyte anions had an elevated conductivity after leaving the stripper column. Thus, both the cation and anion stripper columns suppressed the conductivity of the eluent and enhanced the conductivity of the analyte ions. For this reason, the stripper columns have been called "suppressor columns" ever since they became commercially available.

This invention was patented by Dow, licensed to Dionex, and given the name "ion chromatography". The pellicular ion exchange columns became known as "separator columns". The original anion exchange column became known as the anion separator one, or AS-1, and the original cation exchange column became the cation separator one, or CS-1. The stripper column used in anion analysis became the anion suppressor column, or ASC-1, and for cation analysis, the cation suppressor column became CSC-1. The first series of instruments called in chromatographs (the Model 10, 12, 14, and 16) contained an eluent delivery system with eluent reservoirs, valves for selecting eluents, and a pump to deliver eluent at a controlled flow rate. No great care was taken to ensure pulseless pumping, since the conductivity detector used is quite insensitive to small variations in pressure. The original ion chromatographs contained the eluent delivery system, separator, and suppressor columns, conductivity detectors, a load/inject valve, a valve for column selection, and a valve to control regeneration of the suppressor columns.

The suppressor columns required periodic regeneration because of the nature of the suppression reaction. A fresh suppressor column for anion analysis contained a highly sulfonated polystyrene/divinylbenzene resin. As Na^+ in the eluent exchanged with H^+ on the $-SO_3H$, the suppressor was gradually converted to $-SO^3Na$. After all, the $-SO_3H$ had been converted to $-SO_3Na$, and it was no longer capable of suppressing the conductivity of the eluent or enhancing the conductivity of the analyte ions. To overcome this, it was necessary to regenerate the suppressor by converting the $-SO_3Na$ back to $-SO_3H$. This was done by washing the suppressor with an acid solution such as 1 *N* H_2SO_4 for 12 to 15 min. In normal full-time use, a fully regenerated suppressor column would last 7 to 8 hr. before requiring regeneration. A single suppressor column could be regenerated several hundred times with no loss of performance.

Suppression for cation analysis was quite analogous. The cation suppressor column contained a polystyrene/divinylbenzene-based resin that contained quatenary ammonium hydroxide ($-NR_3OH$) sites. As HCl in the eluent and anions on the sample (i.e., Cl in NaCl, NH_4Cl, and KCl) eluted off the CS-1, the –OH of the $-NR_3OH$ was exchanged for the Cl. This is illustrated below:

Eluent

$$\text{Resin—}NR_3OH + HCl \rightarrow \text{Resin—}NR_3Cl + HOH$$

Analytes

$$\text{Resin—NR}_3\text{Oh} + \text{NaCl} \rightarrow \text{Resin—NR}_3\text{Cl} + \text{NaOH}$$

$$\text{Resin—NR}_3\text{OH} + \text{NH}_4\text{Cl} \rightarrow \text{Resin—NR}_3\text{Cl} + \text{NH}_4\text{OH}$$

$$\text{Resin—NR}_3\text{OH} + \text{KCl} \rightarrow \text{Resin—NR}_3\text{Cl} + \text{KOH}$$

Thus, the highly conductive eluent, HCl, is converted to the weakly conductive H_2O, and the moderately conductive NaCl, NH_4Cl, and KCl are converted to the highly conductive NaOH, NH_4OH, and KOH. At the same time, the suppressor column is gradually converted to the $-NR_3Cl$ form, rendering it temporarily useless. After all the $-NR_3OH$ sites are converted to $-NR_3Cl$, the suppressor column would need to be regenerated, or reconverted to the $-OH$ form. In the original cation suppressor columns, this was done by washing with 1 *N* NaOH for 12 to 15 min.

Thus, suppressor columns have two common features that are important to understand. Firstly, they suppress the conductivity of the eluent and enhance the conductivity of the analyte ions and secondly, they tend to be converted to an inactive form and need to be regenerated. This need for regeneration will be discussed later when describing further advances in suppressor column technology.

By introducing small particle size, reproducible, pellicular ion separator columns, and post-column chemical suppressor columns, Small et al.[5] effectively established a powerful new analytical technique for rapid, simultaneous determination of common inorganic anions and cations. After it became commercially available, ion chromatography became widely accepted for determining inorganic anions. Applications include analysis of chemical plant processes, airborne particulates and aerosols, and water samples (wastewater, plant effluents, drinking water, and precipitation). Thus, ion chromatography rapidly gained a reputation as a method for determining inorganic anions in dilute aqueous solutions. Early publications in this field were numerous and informative.[8-17] Two conferences were held in 1978 and 1979 and became the subject of two volumes.[16] Eventually, the Environmental Protection Agency (EPA), specified the ion chromatographic procedure as the method of choice for chloride, phosphate, nitrate, and sulfate determinations in precipitation samples in a recent quality assurance manual,[18] and has been incorporated in the American Society of Testing and Materials (ASTM) procedure for analysis of rain water.[19] During the first 5 to 8 years after its introduction, the vast majority of ion chromatographic methods were developed for inorganic ions, usually using ion exchange columns and isocratic eluents for separation and chemically suppressed conductivity for detection.

Although inorganic ion determinations are still quite numerous (and increasing daily), ion chromatography has developed much further. Ions need not be separated on ion exchange columns using isocratic elution. Gradient elution has recently been described.[20,21] Specialty ion separator columns have been developed for amino acid and carbohydrate analysis. Separations based on Donnan exclusion and ion pair chromatography are well known.[22,23] Chemically suppressed conductivity is no longer the sole method of detection or even the method of choice in many cases, instead, electrochemical detectors, post-column reactors with UV-visible or fluorescence detection are used.[24,25]

Ion chromatography has recently been defined as simply "the chromatography of ions". This definition implies that a variety of separation and detection modes can be mixed and matched to optimize the analysis of any given sample. This may also imply that the originally developed separator and suppressor columns are obsolete. However, the publications in the field of ion chromatography quite often report the use of these columns. Certainly, the results published in such reports are quite valid and reproducible. All that newer separator and

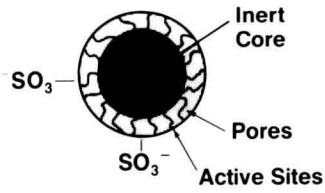
suppressor columns are capable of, is permitting faster determinations of more substances in a single analysis. They also enable some ions to be determined that were difficult to determine previously. The "obsolete" columns are still quite useful. If cared for properly, they can have a lifetime of several years, so undoubtedly many future publications will appear which will use these "obsolete" columns.

It is important for both the analyst and the applications chemist to understand the basic principles of ion chromatography. In this way, the methods published can be clearly interpreted. For example, an environmental chemist may wish to draw some conclusions about acid rain based on ion chromatographic analyses. It might be confusing to read some of the original reports that used the "standard" anion separator (the AS-1) and later publications that use other separators and suppressors. Most ions in rain samples are determined by chemically suppressed ion chromatography, but some by electrochemical detection. In evaluating the data, the environmental chemist should be able to distinguish between differences in experimental approaches to ion chromatography. Most such differences simply involve advances in hardware. One report may describe the use of an AS-1 column and 16 min are required to separate chloride, nitrate, phosphate, and sulfate, whereas another report may use an AS-4 column and only 7 min are required for the same analysis. The important issue is that both methods accurately determine the levels of pollution and can be used in evaluating environmental concerns.

Similarly, a biochemist studying the flow of carbons in photosynthesis may see one report that describes the separation of sugar phosphates using AS-1 and AS-2 columns.[26] Another report used as AS-5 column.[27] Both reports used an isocratic eluent, and neither used the suppressor column that is now recommended, the anion micromembrane suppressor. If this biochemist wanted to develop a method to determine subparts per million levels of sugar phosphates, an understanding of ion chromatography would help in selecting the best, most up-to-date hardware available.

Before the introduction of reproducible pellicular, low-capacity ion exchange separator columns, ion exchange columns were porous and had high ion exchange capacity. Not all the ion exchange substrates were based on a polystyrene/divinylbenzene resin. Some biochemical applications, such as enzyme purification, used cellulose or dextran substrates to which groups such as diethylaminoethane (for anion exchange) and carboxymethyl (for cation exchange) were attached. For separation of small ionic species, the polystyrene/divinylbenzene substrate was used. It took years of developmental work at Dow Chemical Co., for Stevens and Small to produce the advanced technology polystyrene/divinylbenzene-based ion chromatography separator columns. To understand the differences between ion chromatography separators and conventional ion exchange resins, it is important to better describe the conventional resins.

Conventional polystyrene/divinylbenzene resins were produced by suspension copolymerization of styrene and divinylbenzene. The divinylbenzene, being bifunctional, produces the cross links which give the polymer more rigidity. The percent cross link that is mentioned in the literature is simply the amount of divinylbenzene that is present in the original reaction mixture. Reaction conditions would be carefully controlled to produce the narrowest possible molecular weight and particle size distributions. After polymerization, the polystyrene/divinylbenzene becomes rigid, hydrophobic spheres. The next step, was to introduce ion exchange sites. For example, if the resin were reacted with hot (100°C) sulfuric acid for 10 to 15 min, the phenyl groups in the polystyrene/divinylbenzene would become sulfonated. The sulfonic acid ion exchange groups, having identical negative charges, repel each other, causing the once rigid polymer to swell. The hydrophilic nature of the sulfonic acid groups also meant that the resin would absorb considerable moisture and swell even more. Thus, the sulfonated polystyrene/divinylbenzene beads were usually preswollen with the desired eluent to minimize equilibration time. These conventional resins had ion exchange capacities



Surface Sulfonated

FIGURE 1. IC separator resins — cation separator. Inert core is polystyrene/divinylbenzene.

of about 4.5 to 5.5 meq/g of dry resin. Because the resins were porous, ions could readily diffuse into the resin. This caused band spreading and limited column efficiency. Moreover, the high-capacity, gel-type resins would swell and contract considerably as the ionic strength of the eluent changed. Thus, it would have been impossible to use a step gradient such as 0.036 *M* NaOH to 0.070 *M* NaOH to 0.175 *M* NaOH to 0.350 *M* NaOH, similar to that used with a modern ion chromatography separator for amino acid analysis. Moreover, the high ion exchange capacities of conventional ion exchange resins required high ion fluxes for good separations. These ion fluxes would have been much too high to have been suppressed by the first suppressor columns. These problems were largely overcome by detailed developmental work at Dow.

One of the most detailed discussions of the earliest separator columns was written by Stevens and Small in 1978.[28] The cation separator was prepared by surface sulfonation of a styrene/divinylbenzene copolymer (2% divinylbenzene). This produced a surface shell of sulfonic acid groups. Thus, two distinctly different regions were formed, an inner, pellicular polystyrene/divinylbenzene core particle and an outer surface sulfonated shell. The column efficiency was found to depend on the percent cross linking (percent divinylbenzene) and the size of the polystyrene/divinylbenzene beads. First, they kept the bead size constant at 50 μm and varied the percent divinylbenzene from 0.04 to 12%. They obtained optimum chromatographic resolution using 2% divinylbenzene. They then experimentally determined the ion exchange capacity and used this value to calculate the depth of the sulfonation layer. These experiments and calculations of the depth of sulfonation were repeated for other bead sizes ranging from 40 to 400 μm. Their results indicated that a sulfonation depth of 175 to 200 Å was optimum. It was shown to be best to use this polystyrene/divinylbenzene that was only superficially surface-sulfonated. They also calculated the rate of diffusion into the pellicular ion separator columns they prepared. The rate was 1×10^{-11} cm²/sec compared to 2×10^{-7} cm²/sec for conventional ion exchangers.[29] This indicates that there is less diffusion of ions into the pellicular resin and much less band spreading. The band spreading decreases sensitivity and resolution so the columns were more efficient than the previously used porous resins.

The inert, pellicular polystyrene/divinylbenzene core provides a structural rigidity that permits much higher flow rates than those obtainable from conventional totally sulfonated ion exchangers. The polystyrene/divinylbenzene is chemically quite stable. The separator resins do not swell when the ionic strength of the eluent changes. There is even very little swelling when organic solvents such as acetonitrile or methanol are added to the eluent. Eluents ranging from pH 0 to 14, consisting of 3 *M* nitric acid or 1.0 *M* NaOH, can be used.

The cation separator is illustrated in Figure 1. The inert core is polystyrene/divinylbenzene. The active cation exchange sites ($-SO_3$) are located at the surface. There are also pores into which uncharged solutes can fit.

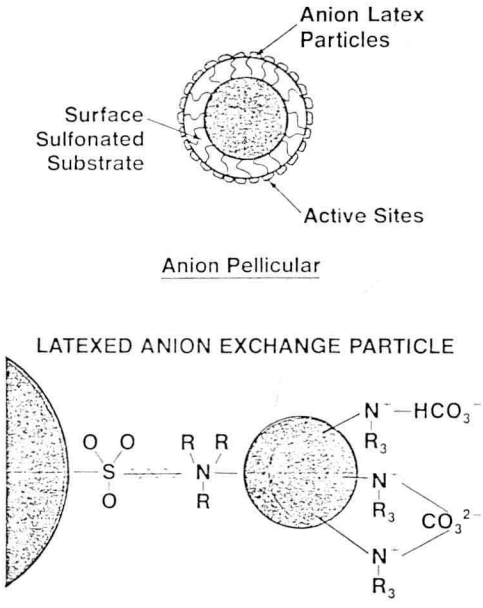


FIGURE 2. IC separator resins — anion separator. Active sites are quaternary amines.

The requirements of a good cation separator column as described by Pohl and Johnson[7] are as follows:

1. Low ion exchange capacity, i.e., 0.005 to 0.05 meq/g dry resin
2. Good pH stability
3. Wide range of selectivities

The low ion exchange capacity enables the use of low ion fluxes. The analyst is encouraged to analyze samples containing parts per million levels of cations and use relatively dilute eluents (i.e., 5 m*M* HCl). Thus, the suppressor column can effectively convert all the highly conductive eluent to a low conductive form. The good pH stability, due to the inert polystyrene/divinylbenzene core, permits the use of acidic and basic eluents and the analysis of highly corrosive samples, such as those found in the electronics industry. The wide range of selectivities was not available at first, since the only cation separator available was the CS-1. However, other cation separators with different polystyrene/divinylbenzene particle sizes, different crosslinking, and different levels of sulfonation (ion exchange capacity) are now used.

Similar factors are involved in preparing anion separator columns. From the beginning (AS-1 column), ion chromatographers took advantage of the very strong electrostatic bonds formed between the negatively charged surface sulfonated polystyrene/divinylbenzene and positively charged aminated latex beads. The latex beads are much smaller than the polystyrene/divinylbenzene core particles. The positive charges on the latex beads are due to chemically bonded quaternary amino groups. As shown in Figure 2, this produced a resin with three distinctly different regions: a large inert core, a surface sulfonation layer, and an outer layer of positively charged (aminated) latex beads. This electrostatic bond between the aminated latex beads and the surface sulfonated polystyrene/divinylbenzene is so strong because of the large number of ionic interaction sites between each aminated latex particle and the sulfonated layer. This produces an essentially irreversible bond, which is stable to some organic solvents and extremes of pH. The latex beads, as shown in Figure 2, are much

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