1 Introduction

1.1 Historical Perspective

"Chromatography" is the general term for a variety of physico-chemical separation techniques, all of which have in common the distribution of a component between a mobile phase and a stationary phase. The various chromatographic techniques are subdivided according to the physical state of these two phases.

The discovery of chromatography is attributed to Tswett [1, 2], who in 1903 was the first to separate leaf pigments on a polar solid phase and to interpret this process. In the following years, chromatographic applications were limited to the distribution between a solid stationary and a liquid mobile phase (**L**iquid **S**olid **C**hromatography, LSC). In 1938, Izmailov and Schraiber [3] laid the foundation for **T**hin **L**ayer **C**hromatography (TLC). Stahl [4, 5] refined this method in 1958 and developed it into the technique known today. In their noteworthy paper of 1941, Martin and Synge [6] proposed the concept of theoretical plates, which was adapted from the theory of distillation processes, as a formal measurement of the efficiency of the chromatographic process. This approach not only revolutionized the understanding of liquid chromatography, but also set the stage for the development of both gas chromatography (GC) and paper chromatography.

In 1952, Martin and James [7] published their first paper on gas chromatography, initiating the rapid development of this analytical technique.

High **P**erformance **L**iquid **C**hromatography (HPLC) was derived from the classical column chromatography and, besides gas chromatography, is one of the most important tools of analytical chemistry today. The technique of HPLC flourished after it became possible to produce columns with packing materials made of very small beads ($\approx 10\ \mu m$) and to operate them under high pressure. The development of HPLC and the theoretical understanding of the separation processes rest on the basic works of Horvath [8], Knox [9], Scott [10], Snyder [11], Guiochon [12], Möckel [13], and others.

Ion **C**hromatography (IC) was introduced in 1975 by Small, Stevens, and Bauman [14] as a new analytical method. Within a short period of time, ion chromatography evolved from a new detection scheme for a few selected inorganic anions and cations to a versatile analytical technique for ionic species in general. For a sensitive detection of ions via their electrical conductance, the separator

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column effluent was passed through a "suppressor" column. This suppressor column chemically reduces the eluant background conductance, while at the same time increasing the electrical conductance of the analyte ions.

In 1979, Fritz et al. [15] described an alternative separation and detection scheme for inorganic anions, in which the separator column is directly coupled to the conductivity cell. As a prerequisite for this chromatographic setup, low capacity ion-exchange resins must be employed, so that low ionic strength eluants can be used. In addition, the eluant ions should exhibit low equivalent conductances, thus enabling sensitive detection of the sample components.

At the end of the 1970s, ion chromatographic techniques were used to analyze organic ions for the first time. The requirement for a quantitative analysis of organic acids brought about an ion chromatographic method based on the ion-exclusion process that was first described by Wheaton and Bauman [16] in 1953.

The 1980s witnessed the development of high efficiency separator columns with particle diameters between 5 μm and 8 μm, which resulted in a significant reduction of analysis time. In addition, separation methods based on the ion-pair process were introduced as an alternative to ion-exchange chromatography, because they allow the separation and determination of both anions and cations.

Since the beginning of the 1990s column development has aimed to provide stationary phases with special selectivities. In inorganic anion analysis, stationary phases were developed that allow the separation of fluoride from the system void and the analysis of the most important mineral acids as well as oxyhalides such as chlorite, chlorate, and bromate in the same run [17]. Moreover, high-capacity anion exchangers are under development that will enable analysis of, for example, trace anionic impurities in concentrated acids and salinary samples. Problem solutions of this kind are especially important for the semiconductor industry, sea water analysis, and clinical chemistry. In inorganic cation analysis, simultaneous analysis of alkali- and alkaline-earth metals is of vital importance, and can only be realized within an acceptable time frame of 15 minutes by using weak acid cation exchangers [18]. Of increasing importance is the analysis of aliphatic amines, which can be carried out on similar stationary phases by adding organic solvents to the acid eluant.

The scope of ion chromatography was considerably enlarged by newly designed electrochemical and spectrophotometric detectors. A milestone of this development was the introduction of a pulsed amperometric detector in 1983, allowing a very sensitive detection of carbohydrates, amino acids, and divalent sulfur compounds [19, 20].

A growing number of applications utilizing post-column derivatization in combination with photometric detection opened the field of polyphosphate, polyphosphonate, and transition metal analysis for ion chromatography, thus providing a powerful extension to conventional titrimetric and atomic spectrometry methods.

These developments made ion chromatography an integral part of both modern inorganic and organic analysis.

Even though ion chromatography is still the preferred analytical method for inorganic and organic ions, meanwhile, ion analyses are also carried out with capillary electrophoresis (CE) [21], which offers certain advantages when analyzing samples with extremely complex matrices. In terms of detection, only spectrometric methods such as UV/Vis and fluorescence detection are commercially available. Because inorganic anions and cations as well as aliphatic carboxylic acids cannot be detected very sensitively or cannot be detected at all, applications of CE are rather limited as compared to IC, with the universal conductivity detection being employed in most cases.

Dasgupta et al. [22] as well as Avdalovic et al. [23] independently succeeded to miniaturize a conductivity cell and a suppressor device down to the scale required for CE. Since the sensitivity of conductivity detection does not suffer from miniaturization, detection limits achieved for totally dissociated anions and low molecular weight organics compete well with those of ion chromatography techniques. Thus, capillary electrophoresis with suppressed conductivity detection can be regarded as a complementary technique for analyzing small ions in simple and complex matrices.

1.2
Types of Ion Chromatography

This book only discusses separation methods which can be summarized under the general term *Ion Chromatography*. Modern ion chromatography as an element of liquid chromatography is based on three different separation mechanisms, which also provide the basis for the nomenclature in use.

Ion-Exchange Chromatography (HPIC)
(**H**igh **P**erformance **I**on **C**hromatography)

This separation method is based on ion-exchange processes occurring between the mobile phase and ion-exchange groups bonded to the support material. In highly polarizable ions, additional non-ionic adsorption processes contribute to the separation mechanism. The stationary phase consists of polystyrene, ethylvinylbenzene, or methacrylate resins co-polymerized with divinylbenzene and modified with ion-exchange groups. Ion-exchange chromatography is used for the separation of both inorganic and organic anions and cations. Separation of anions is accomplished with quaternary ammonium groups attached to the polymer, whereas sulfonate-, carboxyl-, or phosphonate groups are used as ion-exchange sites for the separation of cations. Chapters 3 and 4 deal with this type of separation method in greater detail.

Ion-Exclusion Chromatography (HPICE)

(**H**igh **P**erformance **I**on **C**hromatography **E**xclusion)

The separation mechanism in ion-exclusion chromatography is governed by Donnan exclusion, steric exclusion, sorption processes and, depending on the type of separator column, by hydrogen bonding. A high-capacity, totally sulfonated cation exchange material based on polystyrene/divinylbenzene is employed as the stationary phase. In case hydrogen bonding should determine selectivity, significant amounts of methacrylate are added to the styrene polymer. Ion-exclusion chromatography is particularly useful for the separation of weak inorganic and organic acids from completely dissociated acids which elute as one peak within the void volume of the column. In combination with suitable detection systems, this separation method is also useful for determining amino acids, aldehydes, and alcohols. A detailed description of this separation method is given in Chapter 5.

Ion-Pair Chromatography (MPIC)

(**M**obile **P**hase **I**on **C**hromatography)

The dominating separation mechanism in ion-pair chromatography is adsorption. The stationary phase consists of a neutral porous divinylbenzene resin of low polarity and high specific surface area. Alternatively, chemically bonded octadecyl silica phases with even lower polarity can be used. The selectivity of the separator column is determined by the mobile phase. Besides an organic modifier, an ion-pair reagent is added to the eluant (water, aqueous buffer solution, etc.) depending on the chemical nature of the analytes. Ion-pair chromatography is particularly suited for the separation of surface-active anions and cations, sulfur compounds, amines, and transition metal complexes. A detailed description of this separation method is given in Chapter 6.

Alternative Methods

In addition to the three classical separation methods mentioned above, reversed-phase liquid chromatography (RPLC) can also be used for the separation of highly polar and ionic species. Long-chain fatty acids, for example, are separated on a chemically bonded octadecyl phase after protonation in the mobile phase with a suitable aqueous buffer solution. This separation mode is known as ion suppression [24].

Chemically bonded aminopropyl phases have also been successfully employed for the separation of inorganic ions. Leuenberger et al. [25] described the separation of nitrate and bromide in foods on such a phase using a phosphate buffer solution as the eluant. Separations of this kind are limited in terms of their applicability, because they can only be applied to UV-absorbing species.

Moreover, applications of multidimensional ion chromatography utilizing multimode phases are very interesting, too. In those separations, ion-exchange and reversed-phase interactions equally contribute to the retention mechanism of ionic and polar species [26]. These alternative techniques are also described in Chapter 6.

1.3 The Ion Chromatographic System

The basic components of an ion chromatograph are shown schematically in Fig. 1-1. It resembles the setup of conventional HPLC systems.

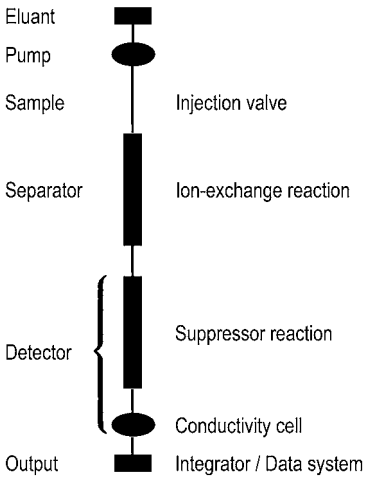

Figure 1-1. Basic components of an ion chromatograph.

A pump delivers the mobile phase through the chromatographic system. In general, either single-piston or dual-piston pumps are employed. A pulse-free flow of the eluant is necessary for employing sensitive UV/Vis and amperometric detectors. Therefore, pulse dampers are used with single-piston pumps and a sophisticated electronic circuitry with dual-piston pumps.

The sample is injected into the system via a loop injector, as schematically shown in Fig. 1-2. A three-way valve is required, with two ports being connected to the sample loop. The sample loading is carried out at atmospheric pressure. After switching the injection valve, the sample is transported to the separator column by the mobile phase. Typical injection volumes are between 5 µL and 100 µL.

The most important part of the chromatographic system is the separator column. The choice of a suitable stationary phase (see Section 1.5) and the chromatographic conditions determine the quality of the analysis. The column tubes are manufactured from inert material such as Tefzec, epoxy resins, or PEEK

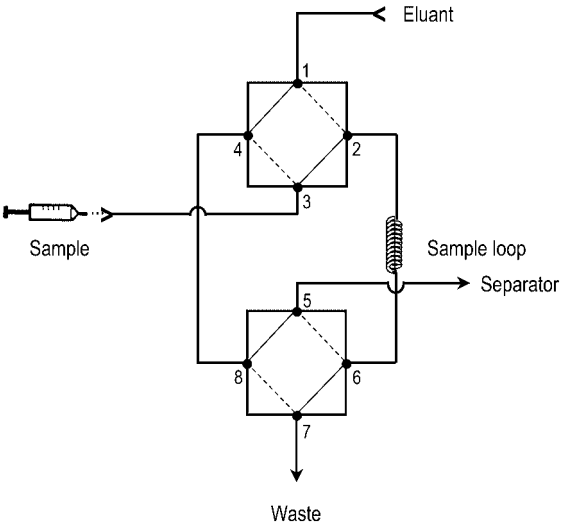

Figure 1-2. Schematic representation of a loop injector.

(polyether ether ketone). In general, separation is achieved at room temperature. Only in very few cases – for example for the analysis of long-chain fatty acids – an elevated temperature is required to improve analyte solubility. An elevated column temperature is also recommended for the analysis of polyamines in order to improve peak efficiencies.

The analytes are detected and quantified by a detection system. The performance of any detector is evaluated according to the following criteria:

- Sensitivity
- Linearity
- Resolution (detector cell volume)
- Noise (detection limit)

The most commonly employed detector in ion chromatography is the conductivity detector, which is used with or without a suppressor system. The main function of the suppressor system as part of the detection unit is to *chemically* reduce the high background conductivity of the electrolytes in the eluant, and to convert the sample ions into a more conductive form. In addition to conductivity detectors, UV/Vis, amperometric, and fluorescence detectors are used, all of which are described in detail in Chapter 7.

The chromatographic signals can be displayed on a recorder. Quantitative results are obtained by evaluating peak areas or peak heights, both of which are proportional to the analyte concentration over a wide range. This was traditionally performed using digital integrators which are connected directly to the analog signal output of the detector. Due to low computer prices and lack of GLP/GLAP conformity, digital integrators are hardly used anymore. Modern detectors

feature an additional parallel interface (e.g., RS-232-C), that enables the connection to a personal computer or a host computer with a suitable chromatography software. Computers also take over control functions, thus allowing a fully automated operation of the chromatographic system.

Because corrosive eluants such as diluted acids and bases are often used in ion chromatography, all parts of the chromatographic system being exposed to these liquids should be made of inert, metal-free materials. Conventional HPLC systems with tubings and pump heads made of stainless steel are only partially suited for ion chromatography, because even stainless steel is eventually corroded by aggressive eluants. Considerable contamination problems would result, because metal ions exhibit a high affinity towards the stationary phase of ion exchangers, leading to a significant loss of separation efficiency. Moreover, metal parts in the chromatographic fluid path would make the analysis of orthophosphate, complexing agents, and transition metals more difficult.

1.4 Advantages of Ion Chromatography

The determination of ionic species in solution is a classical analytical problem with a variety of solutions. Whereas in the field of cation analysis both fast and sensitive analytical methods (AAS, ICP, polarography, and others) have been available for a long time, the lack of corresponding, highly sensitive methods for anion analysis is noteworthy. Conventional wet-chemical methods such as titration, photometry, gravimetry, turbidimetry, and colorimetry are all labor-intensive, time-consuming, and occasionally troublesome. In contrast, ion chromatography offers the following advantages:

- Speed
- Sensitivity
- Selectivity
- Simultaneous detection
- Stability of the separator columns

Speed

The time necessary to perform an analysis becomes an increasingly important aspect, because enhanced manufacturing costs for high quality products and additional environmental efforts have lead to a significant increase in the number of samples to be analyzed.

With the introduction of high efficiency separator columns for ion-exchange, ion-exclusion, and ion-pair chromatography in recent years, the average analysis time could be reduced to about 10 minutes. Today, a baseline-resolved separation of the seven most important inorganic anions [27] requires only three minutes.

Therefore, quantitative results are obtained in a fraction of the time previously required for traditional wet-chemical methods, thus increasing the sample throughput.

Sensitivity

The introduction of microprocessor technology, in combination with modern high efficiency stationary phases, makes it a routine task to detect ions in the medium and lower μg/L concentration range without pre-concentration. The detection limit for simple inorganic anions and cations is about 10 μg/L based on an injection volume of 50 μL. The total amount of injected sample lies in the lower ng range. Even ultrapure water, required for the operation of power plants or for the production of semiconductors, may be analyzed for its anion and cation content after pre-concentration with respective concentrator columns. With these pre-concentration techniques, the detection limit could be lowered to the ng/L range. However, it should be emphasized that the instrumentation for measuring such incredibly low amounts is rather sophisticated. In addition, high demands have to be met in the creation of suitable environmental conditions. The limiting factor for further lowering the detection limits is the contamination by ubiquitous chloride and sodium ions.

High sensitivities down to the pmol range are also achieved in carbohydrate and amino acid analysis by using integrated pulsed amperometric detection.

Selectivity

The selectivity of ion chromatographic methods for analyzing inorganic and organic anions and cations is ensured by the selection of suitable separation and detection systems. Regarding conductivity detection, the suppression technique is of vital importance, because the respective counter ions of the analyte ions as a potential source of interferences are exchanged against hydronium and hydroxide ions, respectively. A high degree of selectivity is achieved by using solute-specific detectors such as a UV/Vis detector to analyze nitrite in the presence of high amounts of chloride. New developments in the field of post-column derivatization show that specific compound classes such as transition metals, alkaline-earth metals, polyvalent anions, silicate, etc. can be detected with high selectivity. Such examples explain why sample preparation for ion chromatographic analyses usually involves only a simple dilution and filtration of the sample. This high degree of selectivity facilitates the identification of unknown sample components.

Simultaneous Detection

A major advantage of ion chromatography – especially in contrast to other instrumental techniques such as photometry and AAS – is its ability to simultaneously detect multiple sample components. Anion and cation profiles may be obtained within a short time; such profiles provide information about the sample composition and help to avoid time-consuming tests. However, the ability of ion

chromatographic techniques for simultaneous quantitation is limited by extreme concentration differences between various sample components. For example, the major and minor components in a wastewater matrix may only be detected simultaneously if the concentration ratio is <1000:1. Otherwise, the sample must be diluted and analyzed in a separate chromatographic run.

Stability of the Separator Columns

The stability of separator columns very much depends on the type of the packing material being used. In contrast to silica-based separator columns commonly used in conventional HPLC, resin materials such as polystyrene/divinylbenzene copolymers prevail as support material in ion chromatography. The high pH stability of these resins allows the use of strong acids and bases as eluants, which is a prerequisite for the wide-spread applicability of this method. Strong acids and bases, on the other hand, can also be used for rinsing procedures. Meanwhile, most organic polymers are compatible with organic solvents such as methanol and acetonitrile, which can be used for the removal of organic contaminants (see also Chapter 9). Hence, polymer-based stationary phases exhibit a low sensitivity towards complex matrices such as wastewater, foods, or body fluids, so that a simple dilution of the sample with de-ionized water prior to filtration is often the only sample preparation procedure.

1.5 Selection of Separation and Detection Systems

As previously mentioned, a wealth of different separation techniques is summarized under the term "ion chromatography". Therefore, what follows is a survey of criteria for selecting stationary phases and detection modes being suitable for solving specific separation problems.

The analyst usually has some information regarding the nature of the ion to be analyzed (inorganic or organic), its surface activity, its valency, and its acidity or basicity, respectively. With this information and on the basis of the selection criteria outlined schematically in Table 1-1, it should not be difficult for the analytical chemist to select a suitable stationary phase and detection mode. In many cases, several procedures are feasible for solving a specific separation problem. In these cases, the choice of the analytical procedure is determined by the type of matrix, the simplicity of the procedure, and, increasingly, by financial aspects. Two examples illustrate this:

Various sulfur-containing species in the scrubber solution of a flue-gas desulfurization plant (see also Section 9.2) are to be analyzed. According to Table 1-1, non-polarizable ions such as sulfite, sulfate, and amidosulfonic acid with pK values below 7, are separated isocratically by HPIC using a conventional anion exchanger and are detected via electrical conductivity. A suppressor system may

be used to increase the sensitivity and specificity of the procedure. Often, scrubber solutions also contain thiocyanate and thiosulfate in small concentrations. However, due to their polarizability, these anions exhibit a high affinity towards the stationary phase of conventional anion exchangers. Three different approaches are feasible for the analysis of such anions. A conventional anion exchanger may be used with a high ionic strength mobile phase. Depending on the analyte concentration, difficulties with the sensitivity of the subsequent conductivity detection may arise. Alternatively, a special methacrylate-based anion exchanger with hydrophilic functional groups may be employed. Polarizable anions are not adsorbed as strongly on this kind of stationary phases and, therefore, elute together with non-polarizable anions. Taking into account that other sulfur-containing species such as dithionate may also have to be analyzed, a gradient elution technique has to be employed, which allows *all* compounds mentioned above to be separated in a single run utilizing a high efficiency separator column and conductivity detection. However, the required concentration gradient makes the use of a suppressor system inevitable. Concentration gradients on anion exchangers reach the limit when extremely polarizable anions such as nitrilotrisulfonic acid have to be analyzed. In this case, ion-pair chromatography (MPIC) is the better separation mode, because organic solvents added to the mobile phase determine analyte retention.

A second example is the determination of organic acids in soluble coffee. According to Table 1-1, aliphatic carboxylic acids are separated by HPICE on a totally sulfonated cation exchange resin with subsequent conductivity detection. While this procedure is characterized by a high selectivity for aliphatic monocarboxylic acids with a small number of carbon atoms, sufficient separation cannot be obtained for the aliphatic open-chain and cyclic hydroxy acids that are also present in coffee. Only after introducing a new stationary phase with specific selectivity for hydroxycarboxylic acids did it become possible to separate the most important representatives of this class of compounds in such a matrix. Ion-exclusion chromatography is not suited for the separation of aromatic carboxylic acids, which are present in coffee in large numbers. Examples are ferulic acid, caffeic acid, and the class of chlorogenic acids. Due to π-π-interactions with the aromatic rings of the organic polymers used as support material for the stationary phase, aromatic acids are strongly retained and, thus, cannot be analyzed. A good separation is achieved by reversed-phase chromatography using chemically bonded octadecyl phases with high chromatographic efficiencies. These compounds are then detected by measuring their light absorption at 254 nm.

Further details on the selection of separation and detection modes are given in Chapters 3 to 6.

Table 1-1. Schematic representation of selection criteria for separation and detection modes.

Anions	Non-polarizable ions Inorganic anions Carboxylic acids Sulfonic acids Fatty acids > C_6	$pK_a < 7$	Inorganic ions F^-, Cl^-, SO_4^{2-} etc.	HPIC	Conductivity detection or Amperometric detection
			Chelates Polyphosphates Polyphosphonates	HPIC	Conductivity detection UV/Vis detection with post-column reaction
			Organic ions > C_6 Formic acid Acetic acid Citric acid	HPIC MPIC	Conductivity detection or UV/Vis detection
			Metal complexes	HPIC MPIC	Conductivity detection or UV/Vis detection
		$pK_a > 7$	Electroactive ions Organic ions Mercaptanes	HPIC MPIC	Amperometric detection
			Weak inorganic acids HS^-, CN^-, CO_3^{2-}	HPIC	Conductivity detection or UV detection or Amperometric detection
			Alcohols Aldehydes	HPICE	Pulsed amperometry
			Carbohydrates	HPIC	Pulsed amperometry
	Polarizable ions Inorganic anions Carboxylic acids Sulfonic acids Fatty acids < C_{20}	$pK_a < 7$	Inorganic and organic ions Palmitic acid Stearic acid Anionic surfactants I^-, SCN^-, ClO_4^-	HPIC MPIC	Conductivity detection or UV detection
		$pK_a > 7$	Organic ions Phenols etc.	MPIC	Amperometric detection or UV/Vis detection
Cations	Non-polarizable ions Na^+, NH_4^+, K^+ Mg^{2+}, Ca^{2+} Transition metals Amines < C_6		Alkali metals Alkaline-earth metals	HPIC	Conductivity detection
			Amines < C_6	MPIC HPIC	Conductivity detection for $pK_b < 7$ or UV/Vis detection
			Transition metals	HPIC	UV/Vis detection with post-column raction
			Amino acids	HPIC	UV/Vis detection or Fluorescence detection with post-column raction
	Polarizable ions Aryl amines Alkyl amines < C_{20} Amino acids		Amines Pyrimidines Purines	MPIC HPIC	Conductivity detection for $pK_b < 7$ or UV/Vis detection Pulsed amperometry