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Chapter 1

Ion Exchange Chromatography - An Overview

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1. Introduction

Chromatography is the separation of a mixture of compounds into its individual components based on their relative interactions with an inert matrix. However, chromatography is more than a simple technique, it is an important part of science encompassing chemistry, physical chemistry, chemical engineering, biochemistry and cutting through different fields. It is worth to be mentioned here that the IUPAC definition of chromatography is “separation of sample components after their distribution between two phases”.

1.1. Discovery and history of chromatography [1, 2]

M. Tswett (1872-1919), a Russian botanist, discovered chromatography in 1901 during his research on plant pigments. According to M. Tswett: “An essential condition for all fruitful research is to have at one’s disposal a satisfactory technique”. He discovered that he could separate colored leaf pigments by passing a solution through a column packed with adsorbent particles. Since the pigments separated into distinctly colored bands as represented in Figure 1, he named the new method “chromatography” (chroma – color, graphy –writing). Tswett emphasized later that colorless substances can also be separated using the same principle.

The separation results from the differential migration of the compounds contained in a mobile phase through a column uniformly packed with the stationary matrix. A mobile phase, usually a liquid or gas, is used to transport the analytes through the stationary phase while the matrix, or stationary phase, is generally an inert solid or gel and may be associated with various moieties, which interact with the analyte(s) of interest. Interactions between the analytes and stationary phase are non-covalent and can be either ionic or non-ionic in nature depending on the type of chromatography being used. Components exhibiting fewer interactions with the stationary phase pass through the column more quickly than those that interact to a greater degree.
Tswett’s initial experiments involved direct visual detection and did not require a means of quantitation. Nowadays, chromatography is not only a separation technique. In most versions, it is hyphenated analytical techniques combining the separation with the identification and quantitative determination of the separated components. In this form, chromatography has become the most widely used technique in the chemical analysis of complex mixtures.

Many versions of chromatography are used. The various chromatographic techniques are subdivided according to the physical state of these two phases, the mobile and the stationary phases. These are: liquid chromatography including high performance, ion, micellar, electrokinetic, thin-layer, gel-permeation, and countercurrent versions; gas chromatography and supercritical fluid chromatography. Various forms of chromatography can be used to separate a wide variety of compounds, from single elements to large molecular complexes. By altering the qualities of the stationary phase and/or the mobile phase, it is possible to separate compounds based on various physiochemical characteristics. Among these characteristics are size, polarity, ionic strength, and affinity to other compounds. Chromatography also permits a great flexibility in the technique itself. The flow of the mobile phase might be controlled by gravity, pressure, capillary action and electro-osmosis; the separation may be carried out over a wide temperature range and sample size can vary from a few atoms to many kilograms. Also, the shape of the system in which the separation takes place can be varied, using columns of various length and diameter or flat plates. Through all this, evaluation chromatography has been transformed from an essentially batch technique into an automated instrumental method. Through its continuous growth, chromatography became the most widely used analytical separation technique in chemistry and biochemistry. Thus, it is not exaggeration to call it the technique of the 20th Century.
2. Ion chromatography

Classical liquid chromatography based on adsorption-desorption was essentially a non-linear process where the time of retardation (retention time) and the quantitative response depend on the position on the adsorption isotherm. Essentially, it was a preparative technique: the aim was to obtain the components present in the sample in pure form which could then be submitted to further chemical or physical manipulations [3].

Ion exchange chromatography (or ion chromatography, IC) is a subset of liquid chromatography which is a process that allows the separation of ions and polar molecules based on their charge. Similar to liquid chromatography, ion chromatography utilizes a liquid mobile phase, a separation column and a detector to measure the species eluted from the column. Ion-exchange chromatography can be applied to the determination of ionic solutes, such as inorganic anions, cations, transition metals, and low molecular weight organic acids and bases. It can also be used for almost all kinds of charged molecule including large proteins, small nucleotides and amino acids. The IC technique is frequently used for the identification and quantification of ions in various matrices.

2.1. Ion chromatography process [4]

The basic process of chromatography using ion exchange can be represented in 5 steps (assuming a sample contains two analytes A & B): eluent loading, sample injection, separation of sample, elution of analyte A, and elution of analyte B, shown and explained below. Elution is the process where the compound of interest is moved through the column. This happens because the eluent, the solution used as the solvent in chromatography, is constantly pumped through the column. The representative schemes below are for an anion exchange process. (Eluent ion = ▲, Ion A= □, Ion B = ●)

**Step 1:** The eluent loaded onto the column displaces any anions bonded to the resin and saturates the resin surface with the eluent anion.

This process of the eluent ion (E) displacing an anion (X) bonded to the resin can be expressed by the following chemical interaction:

\[
\text{Resin}^-\text{X}^+ + \text{E}^- \leftrightarrow \text{Resin}^-\text{E}^+ + \text{X}^-
\]

**Step 2:** A sample containing anion A and anion B are injected onto the column. This sample could contain many different ions, but for simplicity this example uses just two different ions as analytes in the sample.
**Step 3:** After the sample has been injected, the continued addition of eluent causes a flow through the column. As the sample elutes (or moves through the column), anion A and anion B adhere to the column surface differently. The sample zones move through the column as eluent gradually displaces the analytes.

**Step 4:** As the eluent continues to be added, the anion A moves through the column in a band and ultimately is eluted first.

This process can be represented by the chemical interaction showing the displacement of the bound anion (A⁻) by the eluent anion (E⁻).

\[ \text{Resin}^-A^- + E^- \rightleftharpoons \text{Resin}^-E^- + A^- \]

**Step 5:** The eluent displaces anion B, and anion B is eluted off the column.

\[ \text{Resin}^-B^- + E^- \rightleftharpoons \text{Resin}^-E^- + B^- \]

The overall 5 step process can be represented pictorially as shown in Figure 2:
A typical ion chromatography consists of several components as shown in Figure 3. The eluent is delivered to the system using a high-pressure pump. The sample is introduced then flows through the guard and into the analytical ion-exchange columns where the ion-exchange separation occurs. After separation, the suppressor reduces the conductivity of the eluent and increases the conductivity of the analytes so they are delivered to the detector. A computer and software are used to control the system, acquire and process the data. Since the introduction of ion chromatography in 1975, many developments were carried out to improve suppressor technology to provide better sensitivity and consistency for the analysis of a wide variety of compounds [5].

3. Instrumentation [6-9]

Typical IC instrumentation includes: pump, injector, column, suppressor, detector and recorder or data system as represented in Figure 4.
3.1. Pump

The IC pump is considered to be one of the most important components in the system which has to provide a continuous constant flow of the eluent through the IC injector, column, and detector. The most practical system for the delivery of the mobile phase is that which can combine several liquids in different proportions at the command of the operator. This blending capability speeds the process of selecting the optimum eluent mixture required for isocratic analysis. There is a series of mobile phase reservoirs that can contain a range of different mobile phases that can be used individually, blended or for mobile phase programming purposes “gradient elution”. In general liquid chromatography, the reservoirs can be stainless steel but in ion chromatography where the mobile phases can have extreme pH values, the reservoirs need to be made of glass or preferably a suitable plastic such as PEEK (polyether-ether-ketone). The advantage of PEEK is that it is also inert to many organic solvents that may need to be used in the mobile phase. In fact, all components of an ion chromatograph that may come in contact with either phase of the distribution system should be constructed from appropriate inert material. This includes all mobile phase conduits, valves, pumps, sampling devices, columns, detector sensor cells, etc. The solvent reservoirs are connected to a solvent selection valve and a solvent programmer where a particular solvent or particular solvent program can be selected. The solvent then passes from the selector/programmer to a high pressure pump. The mobile phase passes from the pump to the sampling device, usually a simple rotating valve that on rotation places the sample in line with the mobile flow which then passes onto the column. The exit flow from the column passes either to an ion suppressor (which will be
discussed later) or directly to the detector. Gas may come out of the solution at the column exit or in the detector, resulting in sharp spikes. Spikes are created by microscopic bubbles which change the nature of the flowing stream making it heterogeneous. The drift may occur as these microscopic bubbles gradually collected and combined in the detector cell. The best results can be obtained by applying vacuum to each solvent for about 5 min. with subsequent helium purging and storing under helium atmosphere.

### 3.1.1. Pumps types

The constant-flow pumps is the most widely used in all common IC applications. Flow rate stability is an important pump feature that distinguishes pumps. For size exclusion chromatography, the flow rate has to be extremely stable. External electronic control is a very desirable feature when automation or electronically controlled gradients are to be run.

#### 3.1.2. Constant flow pumps

Constant-flow systems are generally of two basic types: reciprocating piston and positive displacement (syringe) pumps. Reciprocating piston pump can maintain a liquid flow for indefinitely long time.

#### 3.1.3. Reciprocating piston pumps

The pumping rate is controlled by piston retracts or by the cam rotating speed. The main drawback of this type of pump is sinusoidal pressure pulsations which lead to the necessity of using pulse dampers.

#### 3.1.4. Dual piston pumps

Provides a constant and almost pulse free flow. Both pump chambers are driven by the same motor through a common eccentric cam; this common drive allows one piston to pump while the other is refilling. As a result, the two flow-profiles overlap each other significantly reducing the pulsation downstream of the pump; this is visualized below.

Its advantages are: unlimited solvent reservoir allowing long-term unattended use; quick changeover and clean out capability; wide flow rate range (0.01 to 10 ml/min) is provided without gear change. While its drawbacks are: incompletely compensated pulsations might be observable at high refractive index detector sensitivities, especially at low flow rates; pump reliability depends on the cleanliness of the mobile phase and continued sealing capability of four check valves on each cycle (e.g. several times per minute).

Recent improvements include: A computer-designed camshaft is used to achieve maximum overlap of pump strokes, resulting in virtually undetectable pulsation or ripple and small-volume check valves are used to allow the pumps to function reliably at flow rates as low as 0.001 ml/min.
3.2. Injector

Sample introduction can be accomplished in various ways. The simplest method is to use an injection valve. In more sophisticated LC, automatic sampling devices are incorporated where sample introduction is done with the help of auto-samplers and microprocessors.

In liquid chromatography, liquid samples may be injected directly and solid samples need only to be dissolved in an appropriate solvent. The solvent need not to be the mobile phase, but frequently it is judiciously chosen to avoid detector interference, column/component interference or loss in efficiency. It is always best to remove particles from the sample by filtering, or centrifuging since continuous injection of particulate materials will eventually cause blockage of injection devices or columns.

Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 ml of volume with high reproducibility and under high pressure (up to the 4000 psi). They should also produce minimum band broadening and minimize possible flow disturbances. The most useful and widely used sampling device for modern LC is the microsampling injector valve. With these sampling valves, samples can be introduced reproducibly into pressurized columns without significant interruption of flow, even at elevated temperatures.

With commercially available automatic sampling devices, large numbers of samples can be routinely analyzed by LC without operator intervention. Such equipment is popular for the analysis of routine samples (e.g., quality control of drugs), particularly when coupled with automatic data-handling systems. Automatic injectors are indispensable in unattended searching (e.g., overnight) for chromatographic parameters such as solvent selectivity, flow rate, and temperature optimization.

Most of the autosamplers have a piston metering syringe type pump to suck the preestablished sample volume into a line and then transfer it to the relatively large loop (~100 ml) in a standard six-port valve. The simplest autosamplers utilize the special vials with pressurization caps. A special plunger with a needle, push the cap down to the vial and displace the sample through the needle into the valve loop. Most of the autosamplers are microprocessor controlled and can serve as a master controller for the whole instrument.

3.3. Columns

The principle of ion exchange chromatography is that, charged molecules bind electrostatically to oppositely charged groups that have been bound covalently on the matrix. Ion exchange chromatography is a type of adsorption chromatography so that, charged molecules adsorb to ion exchangers reversibly so, the molecules can be bounded or eluted by changing the ionic environment. Ion exchangers can be used in column chromatography to separate molecules according to charge; actually other features of the molecule are usually important so that the chromatographic behavior is sensitive to the charge density, charge distribution, and the size of the molecule. An ion exchanger is usually a three-dimensional network or matrix that contains covalently liked charged groups. If a group is negatively charged, it will exchange positive ions and is a cation exchanger. An example of a group used in cation exchanger is the carboxy-methyl group. However, if a group is positively charged, it will exchange negative
ions and is an anion exchanger. An example of a group used in anion exchanger is the di-ethylamino-ethyl group (DEAE). The matrix (stationary phase) can be made of various materials, commonly used materials are dextran, cellulose, and agarose.

The separation on an ion exchanger is usually accomplished in two stages: first, the substances to be separated are bound to the exchanger using conditions that give stable and tight binding; then the column is eluted with buffers of different pH, ionic strength or composition and the components of the buffer compete with the bound material for the binding sites. To choose whether the ion exchanger is to be anionic or cationic depend on the material to be separated. If the materials to be bound to the column have a single charge (i.e., either plus or minus), the choice is clear. However, many substances (e.g., proteins), carry both negative and positive charges and the net charge depends on the pH. In such cases, the primary factor is the stability of the substance at various pH values. Most proteins have a pH range of stability (i.e., in which they don’t denature) in which they are either positively or negatively charged. So, if a protein is stable at pH value above the isoelectric point, an anion exchanger should be used; but if stable at values below the isoelectric point, a cation exchanger is required. Ion exchange columns vary widely in size, packing material and material of construction. Depending on its ultimate use and area of application, the column material may be stainless steel, titanium, glass or an inert plastic such as PEEK. The column can vary in diameter from about 2mm to 5 cm and in length from 3 cm to 50 cm depending on whether it is to be used for normal analytical purposes, microanalysis, high speed analyses or preparative work. The life of a column will depend largely on the type of samples it is used to separate but the conditions under which the separations are carried out will also place limits on it useful lifetime.

**Guard column** is placed anterior to the separating column. This serves as a protective factor that prolongs the life and usefulness of the separation column. They are dependable columns designed to filter or remove particles that clog the separation column and compounds and ions that could ultimately cause "baseline drift", decreased resolution, decreased sensitivity or create false peaks.

### 3.4. Suppressor

The suppressor reduces the background conductivity of the chemicals used to elute samples from the ion-exchange column which improves the conductivity measurement of the ions being tested. IC suppressors are membrane-based devices which are designed to convert the ionic eluent to water as a means of enhancing the sensitivity. It can be used with universal detectors to act as a desalting device, thereby removing the interference resulting from the presence of ionic salts in the eluent. Suppressors are normally used with purely aqueous eluents, so there is a need to establish whether these suppressors can be used with the aqueous/organic eluents needed to elute organic analytes which are retained on the stationary phase during their interaction. Eluents using ionic gradients and containing organic solvents can be suppressed satisfactorily using either chemical suppression with a micromembrane suppressor or electrolytic suppression using a self-regenerating suppressor. For utilization in industry, the electrolytic suppressor is usually more appropriate since it can employ water as the suppressor regenerant and is fully automated in terms of response to changing eluent
conditions. Care needed to be taken with controlling the suppressor current in order to avoid
damage to the suppressor and also the generation of ionic components from oxidation of the
organic solvents (especially methanol) present in the eluent. Further potential problems,
arising when using suppressors as de-salting devices with organic analytes, are the possibility
of analytes loss in the suppressor as a result of adsorption or precipitation effects and disper‐
sion of the analyte band in the suppressor.

Weakly acidic analytes are anionic in the presence of the high pH eluents used with anion‐
exchange IC, but become protonated in the suppressor and are therefore prone to hydrophobic
adsorption or precipitation. Similarly, weakly basic analytes are separated as cations with low
pH eluents but are deprotonated in the suppressor to form neutral species. The micro‐
membrane suppressor consists of layered ion-exchange membranes and fibrous chamber
screens with the regenerant chamber screen modified to possess a high ion-exchange capacity
which serves as a reservoir for regenerant ions. There is also a possibility of losses of analytes
resulting from penetration of the analyte through the suppressor membrane into the regener‐
ant chamber. Theoretically, anionic analytes are not able to penetrate the cation-exchange
membranes of the anion suppressor due to the effects of Donnan exclusion.

Introduction of a suppression device between the column and the detector can be expected to
cause some degree of peak broadening due to diffusional effects. The shape of the analyte band
will also be influenced by hydrophobic adsorption effects, especially when the adsorption and
desorption processes are slow. Examination of peak shapes and analyte losses can therefore
provide important insight into the use of suppressors with organic analytes which are weakly
acidic or weakly basic. It can be expected that peak area recovery rates after suppression are
governed by a combination of hydrophobic interactions with the suppressor and permeation
through the membranes with the balance between these mechanisms being determined by
eluent composition, suppression conditions and analyte properties.

3.5. Detectors

Current LC detectors have a wide dynamic range normally allowing both analytical and
preparative scale runs on the same instrument.

An ideal detector should have the following properties: low drift and noise level (particularly
crucial in trace analysis), high sensitivity, fast response, wide linear dynamic range, low dead
volume (minimal peak broadening), cell design which eliminates remixing of the separated
bands, insensitivity to changes in type of the solvent, flow rate and temperature, operational
simplicity and reliability. It should be non-destructive.

Electrical conductivity detector is commonly use. The sensor of the electrical conductivity
detector is the simplest of all the detector sensors and consists of only two electrodes situated
in a suitable flow cell. The sensor consists of two electrodes sealed into a glass flow cell. In the
electric circuit, the two electrodes are arranged to be the impedance component in one arm of
a Wheatstone bridge. When ions move into the sensor cell, the electrical impedance between
the electrodes changes and the ‘out of balance signal’ from the bridge is fed to a suitable
electronic circuit. The ‘out of balance’ signal is not inherently linearly related to the ion
concentration in the cell. Thus, the electronic circuit modifies the response of the detector to provide an output that is linearly related to the ion concentration.

The amplifier output is then either digitized, and the binary number sent to a computer for storage and processing, or the output is passed directly to a potentiometric recorder. This would result in a false change in impedance due to the generation of gases at the electrode surfaces. The frequency of the AC potential that is applied across the electrodes is normally about 10 kHz. In its simplest form, it can consist of short lengths of stainless steel tube insulated from each other by PTFE connecting sleeves.

**Amperometric detection** is a very sensitive technique. In principle, voltammetric detectors can be used for all compounds which have functional groups which are easily reduced or oxidized. Apart from a few cations (Fe$^{3+}$, Co$^{2+}$), it is chiefly anions such as cyanide, sulfide and nitrite which can be determined in the ion analysis sector. The most important applications lie however in the analysis of sugars by anion chromatography and in clinical analysis using a form of amperometric detection know as Pulsed Amperometric Detection (PAD).

**Mass Spectrometry**: Mass to charge ratio (m/z) allows specific compound ID determination. Several types of ionization techniques: electrospray, atmospheric pressure chemical ionization, electron impact. The detector usually contains low volume cell through which the mobile phase passes carrying the sample components.

**Detector sensitivity** is one of the most important properties of the detector. The problem is to distinguish between the actual component and artifact caused by the pressure fluctuation, bubble, compositional fluctuation, etc. If the peaks are fairly large, one has no problem in distinguishing them however, the smaller the peaks, the more important that the baseline be smooth, free of noise and drift. Baseline noise is the short time variation of the baseline from a straight line. Noise is normally measured "peak-to-peak": i.e., the distance from the top of one such small peak to the bottom of the next. Noise is the factor which limits detector sensitivity. In trace analysis, the operator must be able to distinguish between noise spikes and component peaks. For qualitative purposes, signal/noise ratio is limited by 3. For quantitative purposes, signal/noise ratio should be at least 10. This ensures correct quantification of the trace amounts with less than 2% variance. The baseline should deviate as little as possible from a horizontal line. It is usually measured for a specified time, e.g., 1/2 hour or one hour and called drift. Drift usually associated to the detector heat-up in the first hour after power-on.

Sensitivity can be associated with the slope of the calibration curve. It is also dependent on the standard deviation of the measurements. The higher the slope of your calibration curve the higher the sensitivity of your detector for that particular component, but high fluctuations of your measurements will decrease the sensitivity. The more selective the detection, the lower is signal/noise and the higher the sensitivity. The detector response is linear if the difference in response for two concentrations of a given compound is proportional to the difference in concentration of the two samples.
3.6. Data system

The main goal in using electronic data systems is to increase analysis accuracy and precision, while reducing operator attention. In routine analysis, where no automation (in terms of data management or process control) is needed, a pre-programmed computing integrator may be sufficient. For higher control levels, a more intelligent device is necessary, such as a data station or minicomputer.

4. Advanced applications of ion chromatography

Ion chromatography is basically a chromatographic method that has become a routine analytical method. It is regarded as a versatile analytical technique for separating and quantifying ions. The concept of IC was successively widened with advancements of the rapid development in separation, column stationary phase, great variety of detectors, data analysis and hyphenated techniques. Moreover, it could include other separation methods (e.g., ion interaction and ion exclusion) for simultaneous separation of analyte components. IC analysis has matured to a well-established rugged, sensitive and reliable analysis technique for a wide variety of chemical compounds present in various matrices. On this manner, many papers have been published during the last few years dealing with new modalities in sample pretreatment, separation, detection, etc., for improving samples analysis. The following section deals with the recent development in instrumentations and applications to fit the desired fields of applications.

4.1. Qualitative and quantitative analysis of cations and anions

The demand for the determination of ionic species in various water samples is growing rapidly along with increasing environmental problems and it is clearly important to develop an appropriate analytical method for their determination. IC represents one of the most efficient methods that provide accurate and rapid determination of ionic species in water samples. Basically, anions and cations can be independently separated. Recent advances in ion chromatography (IC) make it a superior analytical method; it has been expanded for the simultaneous determination of inorganic anions and cations. Column switching has become a capable technique for the simultaneous determination of inorganic anions and cations in a single chromatographic run. Amin et al. [10] demonstrated a convenient and applicable method for various natural fresh water samples analysis (Figure 5). They proposed an ion chromatography (IC) method for the determination of seven common inorganic anions (F<sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, and SO<sub>4</sub><sup>2-</sup>) and/or five common inorganic cations (Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup>) using a single pump, a single eluent and a single detector. The system used cation-exchange and anion-exchange columns connected in series via a single 10-port switching valve. The 10-port valve was switched for the separation of either cations or anions in a single chromatographic run. Using a specific eluent, 1.0 mM trimellitic acid (pH 2.94), seven anions and the five cations could be separated on the anion-exchange column and the cation-exchange column, respectively. The elution order was found to be F<sup>-</sup> < H<sub>2</sub>PO<sub>4</sub><sup>-</sup> < NO<sub>2</sub><sup>-</sup> < Cl<sup>-</sup> < Br<sup>-</sup> < NO<sub>3</sub><sup>-</sup>
<SO₄>²⁻ for the anions and Na⁺ < NH₄⁺ < K⁺ < Mg²⁺ < Ca²⁺ for the cations. Complete separation of the above anions or cations was demonstrated within 35 min each. Detection limits calculated were 0.05–0.58 ppm for the anions and 0.05–0.38 ppm for the cations, whereas repeatability values were below 2.26, 2.76, and 2.90% for peak height, peak area and retention time, respectively.

Figure 5. Schematic diagram of the instruments used for simultaneous separation of anions and cations [10].

4.2. Qualitative and quantitative analysis of halides

4.2.1. Bromate

Bromate has been classified as a human carcinogen by both the IARC (International Agency for the Research on Cancer) and the USEPA (United States Environmental Protection Agency) and is known to be toxic to fish and other aquatic life [11, 12]. Bromate could be produced in aquatic systems upon the oxidation of aqueous bromide. Controlled ozonation has been considered as an effective disinfectant tool in aquatic systems [13] but when sea water is subjected to ozonation, oxy-bromide ozonation by-products (OBP) are produced and these are important both in terms of their disinfection ability and also in relation to their potential toxicity. When seawater is oxidized, aqueous bromide (Br⁻) is initially converted to hypobromite (OBr⁻) which can then either be reduced back to bromide or oxidized further to bromate (BrO₃⁻) which is known to be toxic to fish and other aquatic life and classified as a human carcinogen. There has been thus a considerable interest in bromate analysis so that trace analysis of bromate in water has received considerable attention in recent years.
Zakaria et al. [12] used a multi-dimensional matrix-elimination ion chromatography approach, two-dimensional and three-dimensional configurations as described in Figure 6, for the determination of bromate in seawater samples. The designed configurations were used effectively to eliminate the interference caused by the high concentration of ubiquitous ions present in seawater such as chloride and sulfate. A two-dimensional approach utilizing a high capacity second dimension separation, comprising two columns connected in series, was applied successfully and permitted the determination of bromate in undiluted seawater samples injected directly onto the ion chromatography system. A three-dimensional method utilizing two 10-port switching valves (Figure 6b) to allow sharing of the second suppressor and detector between the second and third dimension separations showed better resolution and detection for bromate and reduced the limit of detection to 60 µg/L for spiked seawater samples. Experimentally, the analyzed ozonated seawater samples exhibited a non-linear increase in bromate level on increasing ozonation time. A bromate concentration in excess of 1770 µg/L was observed following ozonation of the seawater sample for 120 min. The developed method provides the elimination of high concentration of interfering species, such as chloride and sulfate, by taking specific fractions from each separation column and re-injecting onto a subsequent column.

Using this approach, the limit of detection for bromate was 1050 µg/L using a 500 µL injection loop. Good linearity was obtained for bromate with correlation coefficients for the calibration curves of 0.9981 and 0.9996 based on peak height and area, respectively. The limit of detection achieved was more than sufficient to determine levels of bromate known to be toxic to aquatic species of interest in aquaculture applications. The developed method is therefore applicable to aquaculture, especially where water is recycled and repeatedly ozonated, leading to the probability of accumulation of bromate. Furthermore the described method is generally applicable to other high ionic strength samples, although re-optimization of cutting times would be required. The system is also potentially applicable for the analysis of other low concentration ionic species, including other oxyhalides such as chlorate.

4.2.2. Iodide and iodate

One of the problems of iodide estimation by conductivity detection is the expected interference from other ions and poor sensitivity of detection which rendered its estimation in complex samples difficult to apply. On the other hand, several methods have been developed for the estimation of iodate ion in water, however, one drawback of these methods is that it can give false estimation of iodate with oxidizing agents such as bleaching powder, which too can generate iodine from the reaction with I⁻. It is therefore necessary to devise a sensitive and selective precise test for the separation and detection of iodate species in different samples matrices. Kumar et al. [14] applied successfully an ion chromatographic method with conductivity detection for iodate estimation in common salt after sample pretreatment with on-guard silver cartridge for the removal of the large excess of chloride ion. Unfortunately, fresh Ag cartridge is required for each sample which would render the method expensive for routine use.
Figure 6. Schematic diagram of instrumentation used to perform the multi-dimensional IC. (a) 10-Port valve positions for detection of 2nd dimension separation (i.e. effluent from column 2 diverted through conductivity detector 2). (b) 10-Port valve positions for injection of 2nd dimension cut fractions onto 3rd dimension column with subsequent detection using conductivity detector 2 [12].
Ion chromatography employing anion-exchange column with amperometric detection is demonstrated to be well suited for quantitative estimation of iodide and iodate in iodised salt [15]. The success of the technique, which dispenses with the need for pre-treatment for chloride removal, hinges on the excellent resolution achieved and the high selectivity and sensitivity of detection of iodide by amperometry. The system consisted of a gradient pump with vacuum degas option, an electrochemical detector, liquid chromatography module, eluant organiser and a pH-Ag/AgCl combination reference electrode. The flow-through detection cell is made of a 1.0-mm diameter silver working electrode and a pH-Ag/AgCl combination reference electrode. The titanium body of the cell served as the counter electrode. Separations were accomplished on a 250 mm × 4 mm i.d. column coupled with a 50 mm × 4 mm i.d. guard column. Such a column contains a hydrophilic, anion-exchange resin that is well suited to the chromatography of the relatively hydrophobic iodide anion. Elution was carried out under isocratic condition using HNO$_3$ (50 mM) at a flow rate of 1.5 mL/min. The injection loop volume was fixed at 50 µL and the sample run time was 10 min.

This technique is easy to use and its most important merit is that it can readily indicate absence of iodate in case adulterants that give false positive iodometric test are used in its place. The method also enables trace quantities of iodide to be detected even in the presence of large excess of chloride ion. Interferences from impurities normally present in salt were insignificant.

### 4.2.3. Perchlorate

In chromatographic analysis, the highly retained species present a challenge for ion chromatographic analysis due to peak broadening which leads to low resolution between analytes of interest and to relatively poor detection limits. This problem is often more acute with monovalent anions than with monovalent cations because common anions are often large, and the greater radius to charge ratio facilitates partitioning to the hydrophobic stationary phase. The introduction of macrocycle-based ion chromatography has provided useful new techniques for analysis of both cations and anions. For example, capacity gradient ion chromatography [16] is beneficial in decreasing retention times and thus peak broadening for highly retained anions, making possible the analysis of a broad host of anions. Lamb et al. [17] focused on the introduction of macrocycles into ion chromatographic systems for increased versatility in the separation of both cations and anions. They described extensively the use of macrocycles based ion chromatography in the analysis of perchlorate ion.

As more information on the extent of the contamination and the dangerous effects of perchlorate consumption has become available, much concern has arisen over perchlorate contamination in public water systems. Furthermore, the US Environmental Protection Agency (USEPA) has periodically reduced the acceptable limit for safe consumption. Currently, the limit stands at 0.7 µg/kg/day, which corresponds to 24.5 µg/L for a 70 kg human drinking 2 L of water per day. The method described by Lamb et al. [17] provides effective perchlorate determinations (shown in Figure 7) using standard conductimetric detection by combining an
18-crown-6-based mobile phase with an underivatized reversed-phase mobile phase ion chromatography (MPIC) column. One unique feature of this method is the flexibility in column capacity that is achieved through simple variations in eluent concentrations of 18-crown-6 and KOH, facilitating the separation of target analyte anions; perchlorate. Using a standard anion exchange column as concentrator makes possible the determination of perchlorate as low as 0.2 µg/L in low ionic strength matrices. Determination of perchlorate at the sub-ug/L level in pure water and in hardwater samples with high background ion concentrations can be achieved this way. However, like other IC techniques, this method is challenged to achieve analyses at the µg/L level in the demanding high ionic strength matrix described by the United States Environmental Protection Agency (USEPA) (1000 mg/L chloride, sulfate and carbonate) [17]. This challenge was approached by use of the Cryptand C1 concentrator column to effectively preconcentrate perchlorate while reducing background ion concentrations in the high ionic strength matrix. The method makes possible the determination of perchlorate at the 5 µg/L level in the highest ionic strength matrix described by the EPA. In short, this method provides an alternative method for the analysis of perchlorate at concentration levels as low as 5 µg/L in high background samples and to well below 1 µg/L in pure water and low salt samples.

Figure 7. Optimal system configuration using AG4 guard column as the concentrator column. Five milliliters of Milli-Q water spiked with ClO₄⁻ was loaded onto the concentrator column at varying concentrations of perchlorate. Eluent: 0.5M 18-crown-6 and 5mM KOH. Injection: 5mL loaded onto concentrator column, flow rate: 1.0 mL/min, temperature: 20 °C [17].
4.3. Trace and ultra-trace analysis

There has been considerable interest in the determination of ions at trace levels as, for example, in applications need high-purity water as in semiconductor processing and the determination of trace anions in amine treated waters. For this investigation, we will define “trace” as determinations at or below 1 µg/l (ppb) levels. The Semiconductor Equipment and Materials International (SEMI) recommended the use of IC for tracking trace ionic contaminants from 0.025 to 0.5 µg/l [18]. In addition, the Electric Power Research Institute (EPRI) has established IC as the analytical technique for determining of trace level concentrations of sodium, chloride and sulfate down to 0.25 µg/l in power plant water [19].

To determine ions at mid µg/l to mg/l (ppb to ppm) levels with IC, a sample size of 10 to 50/µl is sufficient. To determine ions at lower levels, then a preconcentration or trace enrichment technique has typically to be utilized [20]. With this method, the analytes of interest are preconcentrated on another column in order to “strip” ions from a measured sample volume. This process concentrates the desired species resulting in lower detection limits. However, preconcentration has several disadvantages, compared with a direct method, additional hardware is required. A concentrator column is used to preconcentrate the ions of interest, a sample pump is needed for loading sample, an additional valve is often required for switching the concentrator column in and out-of line with the analytical column and extra time is required for the preconcentration step. It was of interest to explore the development of a high-volume direct-injection IC method that would facilitate trace ion determinations without a separate preconcentration step. This would represent a significantly simpler and more reliable means of trace analysis.

Kaiser et al. [21] described the evaluation of on-column preconcentration for enhancing sensitivity and enabling trace ion determination in high-purity water. They developed a high-volume direct-injection method for trace level determinations (low to sub µg/l) of anions and cations by ion chromatography as shown in Figure 8. The chromatographic signal was enhanced by increasing the sample volume up to 1300/µl with no significant loss in peak efficiency. Total analysis times were less than 30 min and the method detection limits for most ions ranged from 10 to 400 ng/l (ppt). The methods described exhibit increased sensitivity and greater reliability than methods using conventional preconcentration. Lower detection limits were achieved by increasing sample size with no significant loss neither in peak efficiency nor in peak resolution. Trace levels (low to sub µg/l) were determined without the added complexity of a concentrator column or loading pump and valve.

4.4. Heavy metals

Blazewicz et al. [22] investigated the basic validation parameters of determining the transition metal ions using ion chromatography. Moreover, they described the use of IC method together with the digestion conditions for the determination of heavy metals in different solid matrices. They designed an ion chromatography preceded by microwave-assisted acidic digestion of tissues samples in appropriate conditions for the determination of Co²⁺, Cu²⁺, Fe³⁺, Mn²⁺, and Zn²⁺ in human tissues (nodular goitre and healthy human thyroids). Microwave mineralization in a closed system, where the contamination problems are significantly reduced, is recom-
mended for such samples. The chromatogram of heavy metals separation is represented in Figure 9.

![Figure 8. IC system configuration for direct-injection sample loading [21].](image)

Figure 8. Chromatograms of blank sample (a), standard mixture (b), sample of thyroid from the control group (c), and sample of thyroid of a patient with diagnosed nodular goitre (d) [22].

![Figure 9.](image)

**Table 1.** Comparison of metal ions concentrations measured by IC and certified values [22].

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>LOD (LOD) * (μg/mL)</th>
<th>Measured value (μg/g) ± SD</th>
<th>Certified value (μg/g) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>0.022 (0.077)</td>
<td>3.75 ± 0.45</td>
<td>4.15 ± 0.38</td>
</tr>
<tr>
<td>Co</td>
<td>0.006 (0.007)</td>
<td>0.66 ± 0.15</td>
<td>0.77 ± 0.11</td>
</tr>
<tr>
<td>Cu</td>
<td>0.048 (0.10)</td>
<td>651 ± 59</td>
<td>663 ± 43</td>
</tr>
<tr>
<td>Fe</td>
<td>0.009 (0.03)</td>
<td>286 ± 17.2</td>
<td>339 ± 15</td>
</tr>
<tr>
<td>Mn</td>
<td>0.009 (0.02)</td>
<td>113 ± 4.21</td>
<td>123 ± 1.5</td>
</tr>
<tr>
<td>Ni</td>
<td>0.006 (0.02)</td>
<td>214 ± 0.59</td>
<td>223 ± 0.44</td>
</tr>
<tr>
<td>Zn</td>
<td>0.056 (0.19)</td>
<td>307 ± 2.45</td>
<td>380 ± 27</td>
</tr>
</tbody>
</table>

* LOD — limit of detection; LOQ — limit of quantitation; LOQ = 10/3 LOD.
An evaluation of the obtained data indicated that the mean values found for iron, copper, and zinc are within the values presented in literature. The main assets of the presented method lie in its simplicity and the practicality of determining analytes from samples of various origins. Suitability of the developed IC method was supported by validation results as shown in Table 1. Generally, very good results of precision (RSD below 5%) and recoveries (above 90%) were evaluated.

On the other hand, in the framework of long-term management and recycling of nuclear wastes, the transmutation process has been identified as a promising option to decrease the radiotoxicity of radionuclides. A sample containing around 5 mg of $^{109}$Ag metal powder is one of the fission product transmutation targets which were irradiated. This sample was initially enriched in $^{109}$Ag (>99%). After irradiation, the theoretical evolution scheme predicts respectively the formation of 366 µg of cadmium and 1µg of palladium compared to 4636 µg of silver. Determination of cadmium isotopic compositions is of prime interest to validate neutron calculation codes and to obtain the integral capture cross section of $^{109}$Ag. Isobaric interferences occur at mass 108 between cadmium and silver, and at mass 110 between cadmium, silver and palladium. The mass resolution required to overcome $^{109}$Cd/$^{109}$Ag/$^{109}$Pd interference is about 100,000 which is beyond actual possibilities of mass spectrometers. Thus, a chemical separation step must be completed to isolate cadmium in a purified fraction before offline isotopic measurements. In the case of radioactive materials, the chemical separations performed with gravity flow on ion exchange resins induce drawbacks for analysts, such as increased handling time on samples. Moreover, in most proposed procedures, cadmium is generally eluted after silver, which lowers the separation factor between silver and cadmium and decreases decontamination factors because of silver peak tailing in cadmium fraction. A powerful way to reduce analysis time and to improve selectivity is high performance ion chromatography. However, no detector classically associated with a HPIC system can measure Ag, Cd and Pd with high specificity and sensitivity. Ion chromatography-inductively coupled plasma mass spectrometry (IC-ICPMS) can tackle those specifications: it can be used to detect trace elements at the exit of the chromatographic column. Because of the fast mass scanning ability of the quadrupole in peak jumping mode, this kind of spectrometer enables an easy handling of transient signals associated with high sensitivity. The separation procedure was achieved with a carboxylate-functionalized cation exchange CS12 column using 0.5 M HNO$_3$ as eluent giving satisfactory results in terms of peak resolution and decontamination factors for example. The developed method demonstrates the possibility to obtain rapidly purified cadmium fractions which can be directly analyzed by multi collection inductively coupled plasma mass spectrometry (MC ICPMS). After the optimization of chromatographic conditions, the method was applied to the separation of non-radioactive solutions simulating the composition of the irradiated sample. This hyphenated technique minimizes sample pretreatment and shortens analysis time, which is of prime interest for nuclear applications. Moreover, the developed method displays a strong potential not only for nuclear issues but also for geological and cosmochemical applications where high accuracy and precision isotopic analyses are also needed [23].
4.5. Inorganic compounds

Hydrogen cyanide (HCN) is one of the major ciliatoxic components when tobacco products such as cigarettes are combusted and is thus classed to the “Hoffmann analytes”. It is formed in cigarette smoke in the burning zone mainly from pyrolysis of various nitrogenous compounds such as protein and nitrate in tobacco at oxygen-deficient conditions. The quantitative determination of HCN in cigarette smoke is integral to proper assessment due to its potential impact on public health. However, there are many challenges in accurately determining its amount in cigarette smoke; these include the need for developing an efficient and rapid smoke collecting method and the technique to analyze it in complex smoke mixture. Recently, extensive efforts have been done on determining cyanide by IC through the development and application of electrochemical detection (especially pulsed amperometric detection) which endow this kind of method with a high selectivity and improved accuracy, which eventually enable them to be widely applicable to the ion chromatography. Zhang et al. [24] focused on applying ion chromatography in the determination of hydrogen cyanide in cigarette mainstream smoke. Whole cigarette mainstream smoke was totally trapped by Cambridge filters, which are treated with sodium hydroxide/ethanol solution as shown in Figure 10.

The chromatographic analysis (Figure 11) has been achieved by developing an ion chromatography integrated with pulsed amperometric detection (PAD) and optimizing some factors include sample treatment, matrix interference, composition of eluents and so on. The method possesses the advantage of fast analysis time over the widespread used solution absorption method. The possible co-existing interferents are evaluated under the optimized detection conditions and excellent recoveries of cyanide. The optimization of composition of eluents and evaluation of possible interferents make this method selective and reliable so that the cyanide

Figure 10. Schematic diagram of solution absorption method [24].
content of absorption solution can be directly determined by the optimized IC-PAD method without any pretreatments. The linear range is 0.0147–2.45µg/mL with R² value of 0.9997. The limit of detection is 3µg/L for a 25µL injection loop. The overall relative standard deviation of the method is less than 5.20% and the recovery range from 94.3% to 101.0%. This developed method proves to be advantageous, due to expanded detection range with greater accuracy and is thus highly anticipated to find wide applications in cigarette smoke analysis.

![Typical chromatograms obtained under the following eluent composition: A: 0.2M NaOH, 0.2M NaAc; B: 0.4M NaOH, 0.2M NaAc; C: 0.6M NaOH; D: 0.6M NaOH, 0.3M NaAc; E: 0.6M NaOH, 0.2M NaAc. Flow rate: 1.0 mL/min, injection volume: 25 µL, column temperature 30°C. Peak 1: unidentified, peak 2: cyanide [24].](image)

4.6. Organic compounds

4.6.1. Hippuric acid

IC can also be used in detection of some acids. Zhao et al. [25] proposed a simple and eco-friendly ion chromatographic method for the determination of Hippuric acid (HA) in human urine (see Figure 12). Hippuric acid is a kind of metabolite of toluene in human body, therefore, HA is a physiological component of human urine if toluene was inhaled. The content of HA in human urine actually is confirmed as a diagnostic marker of exposure to toluene [26]. It has been reported that exposure to high concentrations of volatile organic compounds such as
toluene lead to a series of diseases such as acute and chronic respiratory effects, functional alterations of the central nervous system, mucous and dermal irritations, and chromosome aberrations. In order to diagnose patients who are suffering from a series of diseases caused by elevated HA levels, the determination of HA in human urine is necessary. Comparing with other chromatographic methods such as GC and HPLC, the proposed IC method used eco-friendly mobile phase (not containing organic solvent), and avoided complicated sample pretreatment. The separation was carried out on an anion exchange column with 2.0 mmol/L NaHCO$_3$ as mobile phase at the flow-rate 0.7mL/min. A suppressed conductivity detector was used and the detection limit was 1.0 µg/L (S/N = 3) for hippuric acid. The analysis time for one run was 30 min under the optimized IC condition. The recovery of hippuric acid was 93.2–98.0% while the relative standard deviation (RSD) was 1.4–2.3% by seven measurements. Furthermore the results shown that the proposed method has the advantages of easy operation, high sensitivity and accuracy. This method is suitable for routine clinical analysis of HA.

![Chromatogram of a standard solution of HA (10mg/L)](image)

4.6.2. Amines and its derivatives

Erupe et al. [27] developed an ion chromatography method with non-suppressed conductivity detection for the simultaneous determination of methylamines (methylamine, dimethylamine, trimethylamine) and trimethylamine-N-oxide (TMAO) in particulate matter air samples. The method can be used to detect, quantify and determine whether TMAO and methylamines are quantitatively significant components of organic nitrogen aerosol in the atmosphere. This was done using aerosol collected from smog chamber reactions of trimethylamine with ozone and/or nitrogen oxide. The method was tested using a solution of laboratory-generated aerosol containing a mixture of the analytes. The analytes were well separated by means of cation-exchange chromatography using a 3 mM nitric acid / 3.5% acetonitrile (v/v) eluent solution and a Metrosep C 2 250 (250mm×4mm
i.d.) separation column. The composition of the mobile phase was optimized and efficient separations between the analytes were achieved (Figure 13 and 14). Detection limits of methylamine, dimethylamine, trimethylamine, and trimethylamine-N-oxide were 43, 46, 76 and 72 µg/L, respectively. The method described is simple and has low detection limits suitable for analysis of aerosols generated in smog chamber experiments and in ambient air where the concentration of these species is expected to be high.

Figure 13. Separation of methylamines and methylamine-N-oxides from standard solutions. Analytes: 1-sodium, 2-ammonium, 3-methylamine (195 µg/L), 4-dimethylamine (390 µg/L), 5-trimethylamine-N-oxide (465 µg/L), and 6-trimethylamine (615 µg/L) [27].
Figure 14. Chromatogram of smog chamber filter analysis from reaction of trimethylamine with ozone. Analytes: 1-sodium, 2-ammonium, 3-potassium, 4-dimethylamine (1.72 µg/m$^3$), 5-trimethylamine-N-oxide (0.25 µg/m$^3$), 6-magnesium, and 7-trimethylamine (0.57 µg/m$^3$). The inset is a magnification of the trimethylamine-N-oxide peak (5) from the chromatogram [27].

4.6.3. Phenolic compounds

Phenolic compounds have attracted great concern in recent years due to their high toxicity and bio-recalcitrant effect in the ecosystem water cycling process. Numerous techniques have been studied and developed to determine phenols. However, most of these detection techniques focus on high performance liquid chromatography (HPLC) equipped with various kinds of detectors such as UV, electrochemical, fluorescence, and mass spectroscopy [28,29]. Among these detection techniques, fluorescence detector is a better choice in terms of selectivity and sensitivity. HPLC combined with fluorescence detector (HPLC/FD) have been used in numerous applications in trace analysis. However, some phenols have weak fluorescent property and post-column derivatization is often required to convert these compounds into strong fluorescent substances that can then be efficiently detected by the fluorescence detector [30]. Using on line electrochemical derivatization, Karst et al. [30] presented a method to determine mono-substituted phenols via HPLC equipped with fluorescence detector (HPLC/ED/FD). This method addressed the problems on phenols that could not be detected via fluorescence detector. However, the separation was performed by common silica-based C18 separation column. Unfortunately, the silica column works well only in the pH range of 2–8 (pH < 3), whereas the optimum pH for producing the fluorescence of oxidized phenols is basic (pH ~10). Obviously, the separation condition could not match well with that of downstream detection. Therefore, buffer solution of NH$_3$/NH$_4$Cl at pH 9.5 had to be added to the effluent.
from the column to perform the electrochemical conversion to enhance the fluorescence signal. Polymer-based stationary phases (e.g., divinylbenzene/ethylvinylbenzene, DVB/EVB) in IC dominate most of the applications due to their wide pH tolerance (0–14). Since the polymer-based column can work well in alkaline solution (e.g., pH ~10). The choice of alkaline eluent matching with the downstream fluorescence detection will not be a barrier if the phenols could be well separated by IC. Based on these considerations, a method to determine phenols, where their separation is performed using IC combined with online post-column, electrochemical derivatization and fluorescence detection (IC/ED/FD), has been developed [31] Six model phenols including 4-methylphenol (pMP), 2,4-dimethylphenol (DMP), 4-tert-butylphenol (TBP), 4-hydroxylphenolacetic acid (pHPA), 4-acetamidophenol (pAAP), and phenol (P) were well separated on an anion-exchange column under ion exchange mode using NaOH with small amount of acetonitrile added as eluent (as shown in Figure 15). The separation of phenols was carried out in the anion exchange column with basic eluent and the electro-oxidation of phenols is performed using a laboratory-made electrolytic cell (EC) consisting of porous titanium electrode and cation exchange membrane (CEM) which allows the oxidation products that are strongly fluorescent to be detected by the fluorescence detector. NaOH eluent used in the present method matches well with the maximal fluorescence intensity obtained at alkaline condition for oxidized phenols, thus the addition of specific buffer solution after oxidation could be eliminated. This method leads to a simplified procedure and eliminates the use of additional setup and greatly simplifies the operating procedures. The proposed method was sensitive to the limits of detection in the range of 0.4 µg/L and 3.8 µg/L and the limits of quantification between 1.2 µg/L and 13 µg/L due to the strong electro-oxidation capacity of porous titanium electrode, as well as the implementation of time-programmed potential over EC. The linear ranges were 2.0–1.0 × 10^4 µg/L for pAAP and DMP, and 10–1.0 × 10^4 µg/L for P, pMP, pHPA, and TBP, respectively. The relative standard deviations range from 0.9% to 4.8%. The utilization of the method was demonstrated by the analysis of real samples.
Figure 15. Chromatograms of phenolic compounds (~10 mg/L) at different potential [31].

5. Conclusion

This chapter deals with ion exchange chromatography, IC, as a subset of liquid chromatography. Due to the continuous growth, chromatography became one of the most widely used methods in different branches of science encompassing chemistry, physical chemistry, chemical engineering, biochemistry and cutting through different fields of analytical proposes.

Discovery and historical background on IC were mentioned. Steps of ion chromatography process were intensively discussed in addition to instrumental components of typical IC instrument including: pump, injector, column, suppressor, detector and recorder or data system.

The chapter emphasizes the superior analytical power of ion chromatography so that it can be used for qualitative and quantitative analysis of common cations, anions and halides in their different forms and matrices in trace and ultra-trace concentrations. Heavy metals separation and detection was also mentioned as well as hydrogen cyanide as an example of inorganic
compounds. As examples of organic acid separation and detection using ion chromatography, the analysis of hippuric acid, amines and its derivatives and phenolic compounds were mentioned.

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