Monograph

Sample Preparation Techniques for Ion Chromatography

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Metrohm

Sample Preparation Techniques for Ion Chromatography

Based on a customer seminar at Deutsche Metrohm in 1999.

(Second Edition)

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Foreword of the first edition

In the past 30 years ion chromatography has experienced a turbulent development. Innovations in separation columns, suppressors and in instruments and application techniques have played a considerable part in this. In addition, the most important routine methods have been standardized; in this context we may mention the ISO 10304 series and ISO 15061 (anions), ISO 14911 (cations) and DIN 38413-8 (complexing agents).

Ion chromatography has, similar to classical (RP-)HPLC, reached a high stage of development; the only deficits still to be found are in the sample preparation techniques. These deficits have a negative effect on applications, which is particularly noticeable when attempts are made to automate the analytical procedures.

The aim of this seminar was to provide an overview of the sample preparation techniques for ion chromatography (Frenzel) and to present solid-phase extraction (SPE), the most widespread sample preparation technique, with all its advantages and disadvantages (Seubert).

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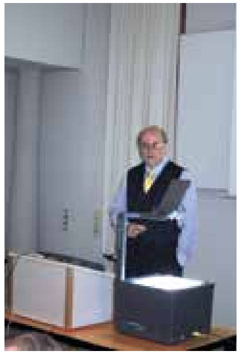

Dr. H. Schäfer,
Metrohm Ltd.

New developments in sample preparation for ion chromatography were presented. Filtration, ion exchange and matrix elimination, all of them performed inline (Helwig Schäfer) as well as inline dialysis (Frenzel) are techniques that are easy to automate. The possibilities of using conventional sample preparation techniques, extraction in particular (Bogenschütz), and combustion methods (Jochen Schäfer), were also treated within the context of ion chromatographic determinations.

Today, quality assurance occupies an important position in instrumental analysis. This is the reason why great attention was given to the contribution describing quality assurance measures in concentration analysis with sample preparation techniques (Seubert).

G. Bogenschutz, Deutsche Metrohm

J. Schafer, Deutsche Metrohm

Inline techniques for sample preparation also met with great interest; during the practical work in particular the seminar participants showed their desire for more information and lively discussions took place. Before the start of the seminar the participants were asked in writing about the number and type of experiments to be carried out. The discussion forum with the speakers also found a positive echo; the dialog between the speakers and the participants forms an important part of such seminars and will continue to do so in future.

Practical work in the IC application laboratory

Discussion forum with speakers

The assessment forms confirmed the impression of the speakers; the form and contents of the seminar were received very positively with some details being capable of improvement. This has persuaded us to offer the seminar again and to publish the seminar documents in a revised form as a monograph. In this way an increased number of interested parties can be given the opportunity of familiarizing themselves with the current situation concerning sample preparation techniques in ion chromatography.

We now hope that you, our readers, will have great pleasure in studying this monograph and that you will find it useful for your practical work.

Filderstadt, Germany, November 2000

Foreword for second edition

Even so that the first edition of this monograph is already 20 years old, it did not lose its actuality. Sample preparation is still one of the most important tasks analyzing samples with ion chromatography. Manual sample preparation still is critical in respect of accuracy and reproducibility of the results. Reduction of manual sample preparation reduces the workload of the lab personnel and increases the quality of results.

Although the full content of the first edition is still valid and even more important, the actual edition was improved by newer developments and updates of graphs and topical examples.

Herisau, Switzerland, 2021

A) Sample preparation techniques for ion chromatography - an overview

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1 Introduction

Since its introduction in the middle of the seventies, ion chromatography (IC) has undergone a tremendous development and can be regarded today as being one of the most versatile analytical methods for all kinds of ionic compounds. It offers an enormous range of possibilities for the selection of stationary and mobile phases and, in combination with different detection techniques, usually is able to solve even difficult separation problems. IC is eminently suitable for the rapid and simultaneous determination of numerous inorganic and organic anions and cations. The outstanding characteristics of IC are a very high selectivity, a broad dynamic measuring range and a sufficiently good sensitivity for most applications. Nevertheless, new problems in various scientific disciplines, increased requirements concerning the quality of the analytical results, altered administrative requirements, pressure to reduce costs or actual measuring problems continually lead to increasing demands being placed on IC performance. This is taken into account by continuous further developments in the scientific sector and by the instrument manufacturers.

Current scientific developments in the IC field chiefly concern the following topics:

- Improving the separating columns with regard to separation performance and analysis time
- Increasing sensitivity and selectivity by using other detection principles apart from the mainly used conductivity measurement
- Solving specific separation problems for the determination of individual ions (e.g. bromate) or for ion analysis in special matrices (e.g. electroplating baths)

On top of this, the instrument manufacturers are taking great effort to offer users a wide range of instruments which, with respect to their flexibility, robustness and cost, satisfy the widely different important fields of application requirements of the various fields of use.

In practice, IC applications vary greatly and there is virtually no scientific field in which this method cannot be used for the routine determination of ions or for solving special problems. The most are the environmental sciences, the food, beverages, the petrochemical, the tobacco industry and the clinical and pharmaceutical sectors. Further fields in which IC has established itself as a versatile method are the monitoring of power plants, the semiconductor and electroplating industries, mining and metal processing, paper manufacturing and biotechnology.
Ion determination in the wide range of samples occurring in the very different fields of application requires an adequate selection of separation conditions as well as the detection method. Aqueous samples with a moderately high total ion content that are not loaded with an organic matrix cause few problems in an IC analysis and can usually be injected directly. In the analysis of more complex matrices, where different types of interferences could occur, attempts should always be made to exhaust the separating performance of the chromatographic system and the selectivity of the detector used before a sample pretreatment step is considered. Uncharged small molecules usually do not interfere with the IC analysis as these sample constituents elute within the dead time.

It may be possible to solve problems related to overlapping signals caused by inadequate selectivity by optimizing the mobile phase or even by using a selective detector. However, often it is not possible to do without some form of sample preparation and even an exacting sample preparation procedure may be necessary.

It is therefore surprising that sample preparation for IC analysis is a subject of little interest, at least when the large number of scientific publications concerning IC is considered. At the same time sample preparation can be regarded as being the Achilles' heel of IC - as in many other instrumental analysis methods - as the working steps involved in it (together with sampling itself) represent the largest source of errors in the whole analytical procedure and usually cannot be easily automated; it is frequently also the limiting factor concerning sample throughput.

In this article a general overview of sample preparation methods is given and any peculiarities affecting IC analysis are discussed. Currently practiced working techniques (not always only proven ones) are presented and newer developments in sample preparation are mentioned.

2. When and why is sample preparation necessary?

The enormous performance of modern IC with respect to chromatographic resolution, simultaneity, sensitivity and speed can be demonstrated best by the analysis of standard solutions. However, in practice the user normally encounters real samples whose composition may vary considerably and which in many cases can only be analyzed by IC after a more or less complicated sample preparation procedure (see list below). This means, for example, that samples in which the analyte concentrations are too high must be diluted before injection. On the other hand, if the sensitivity of the detection system is insufficient, a quantitative determination may often only be possible after preconcentration of the analyte ions. Problems are also caused by samples in which the ratio of the concentrations of the ions to be determined (simultaneously if possible) differs by several orders of magnitude. This is, for example, the case in the determination of iodide in seawater or the simultaneous determination of chloride, nitrite, phosphate and other ions in strongly polluted wastewater. Greater difficulties are encountered in the analysis of samples containing substances that influence the chromatographic separation by causing overlapped signals, altered retention times or unstable baselines. This applies not only to strong acids and bases but also to nonionic (but strongly polar) organic substances that enter into interactions with the stationary phase that are similar to those of the ions to be separated. Even more serious are alterations to the column by matrix components, for example by irreversible sorption. This can often occur in the presence of humic material, lignin, organic dyes, fats, surfactants and proteins. Many (even supposedly «clean») samples contain undissolved fractions in the form of colloidal particles and microparticles that always need to be removed before injection.

Situations in which samples can only be analyzed by IC after sample preparation:

- Analyte concentration too high or too low
- Analyte concentrations differ greatly
- Presence of components that cause interference by overlapping signals
- Presence of substances that cause an irreversible change to the separation column
- Samples containing particles
- Solid samples
- Gaseous samples

In principle IC cannot be used directly for the analysis of solid samples. The sample must either be completely dissolved or at least the ions to be determined must be released. Very often the dissolution of solid samples or the release of the analyte cannot be carried out by water alone, so that the solution to be analyzed by IC also contains additional substances (acids, bases, salts) that are present as matrix components. A direct result of this is that, in the preparation of solid samples, initial consideration must be given to compatibility with the IC analysis and, after dissolution, extraction or digestion, further steps for matrix elimination or a pH adjustment may still be necessary.

Gases can also only be analyzed when the components to be determined have been transferred to an aqueous solution. This is done by either passing the sample gas through an absorption solution and then analyzing the pregnant solution by IC or by absorbing the gaseous analyte on a solid carrier and then eluting it with a liquid and carrying out the IC determination on the eluate. Additional components can also enter the measuring solution in this case as a result of the specific composition of the sorption or elution solution; it may then be necessary to eliminate them by post-treatment of the sample solution.

3. Sample preparation methods

The sample preparation methods for the wide range of problems to be solved are very numerous and the apparatus and time required for sample preparation differ enormously. The working steps involved range from relatively simple measures such as sample dilution, filtration or pH adjustment up to complicated and sometimes even multi-step procedures such as analyte preconcentration and matrix elimination. The list below gives a summary of the most important sample preparation methods (not only) for IC. The various techniques are arranged according to the preparation of liquid, solid and gaseous samples.

Important and frequently used sample preparation methods:

A - Liquid samples

- Filtration Dilution
- pH adjustment
- Standard addition
- Derivatization
- Liquid-liquid extraction
- Solid-phase extraction
- Distillation
- Microdiffusion
- Membrane separation (dialysis/ultrafiltration)
- Combustion

B - Solid samples

- Drying
- Homogenization
- Dissolution
- Extraction/leaching
- Digestion
- Ashing
- Combustion

C - Gaseous samples

- Absorption in liquids
- Adsorption on solid phases
- Membrane sampling
- Chemical conversion
- Combustion

The choice of the sample preparation method to be used depends on the one hand on the physical state and the composition of the sample; on the other hand, it depends to a considerable extent on the analyte ions to be determined. The criteria that are ultimately important, however, are the (possible) choice of the separation principle and separation conditions for the IC analysis and, for many users, the availability of particular instrument configurations (e.g. suppression techniques, gradient techniques, type of detector).

Sample pretreatment for the determination of anions frequently requires (or allows) different measures from those used for the determination of cations. It is also clearly much more difficult to carry out sample preparation when a large number of ions are to be determined simultaneously than when only a few or even a single ion is of interest. Under certain circumstances the actual concentrations of the ions to be determined may be critical for the choice of a particular sample preparation technique.

The quality of the separating column used with regard to its resolution, capacity, pH stability and compatibility to the presence of organic solvents as modifiers can also be decisive for the basic need for sample preparation or for the type of sample pretreatment, which finally is selected.

Apart from the unwanted increase in analysis time and the increased costs that sample preparation involves, as the number of procedures that need to be carried out increases, the risk of analytical errors also increases. Contamination, loss of analyte or conversion of the analyte may falsify the required qualitative and quantitative information or even cause its complete loss. This must be taken into account in the choice of the preparation steps and, if possible, avoided by taking suitable measures (see contribution G).

Sample preparation costs increase as the complexity of the sample composition increases. Measured against the large number of IC applications there are only a few types of sample that can be directly analyzed without any sample preparation at all. If samples are already present in aqueous form, then sample preparation may have various targets.

Sample preparation for liquid samples: aims

- Adaptation of the pH regarding eluent condition and separating column
- Isolation and preconcentration of the analyte ions
- Elimination of interfering matrix components and undissolved constituents
- Protection of the separating column against overloading and irreversible alteration
- Converting the analyte for better chromatographic separation and/or more sensitive detection

Adjustment to a particular pH value is only necessary on rare occasions, as many of the normal eluents possess a certain buffering effect; this means that interference to the chromatographic separation does not occur. In contrast, strong acids and bases cannot usually be analyzed directly; this means that the sample solution must at least be partially neutralized before injection. In anion chromatography in particular this causes problems as, after the addition of an acid (with alkaline samples), the acid anion can hinder the chromatographic separation and the addition of a base (in the analysis of strong acids) can lead to increased salt loading; this also causes interference. An often-used way of overcoming the problem described above is by the use of ion exchangers that, in the acidic H form or the basic OH form, can neutralize the sample without any further addition and without any dilution.

Apart from the relatively simple addition of substances for neutralization, matrix adaptation and/or modification of the chemical form of the species to be determined by derivatization, in many cases it is necessary to carry out further separation steps with or without phase transition before an interference-free IC analysis can be carried out. The basics and the possible applications of important separation methods and techniques are summarized and described in the following sections. Some of them are described in greater detail in other contributions to this monograph.

3.1 Filtration

Within the context of IC analysis, filtration is generally recommended so that blockages in the injection valve, in the capillary connection and, above all, in the column head frits can be avoided; filtration is indispensable for samples containing particles. Membrane filters with a pore size of 0.45 µm are normally used. For biologically active samples the use of sterile filters with a pore size of 0.2 µm is advisable as these also retain microorganisms and therefore prevent the conversion of certain analytes by microbial oxidation or reduction.

Carrying out membrane filtration is basically easy, particularly if commercially available disposable filters are used; however, the risk of contamination as well as the loss of analyte resulting from adsorption on the filter material or on the filter cake formed during the filtration process should not be underestimated. Therefore, it is recommended, to thoroughly rinse the filter at least with water before use and to reject the first milliliters of the sample filtrate as they could still contain the rinsing liquid.

There is a very wide range of filter materials that differ in their pore sizes, porosity, filtration speed, compatibility with acids, base and organic solvents as well as in their adsorption properties and blank values. This means that for special problems it is advisable to determine the most suitable material by carrying out preliminary tests. For samples with a very complex composition and heavily loaded with particles it may be helpful to carry out a coarse filtration before membrane filtration or to separate off the bulk of the solid substances by centrifugation.

3.2 Solid-phase extraction

For the isolation and preconcentration of analyte ions as well as the separation of interfering matrix components, the various versions of solid-phase extraction (contribution B) are particularly suitable. This normally involves passing the sample solution through a small column (solid-phase cartridge) that is filled with a sorbent material suitable for the separation, which either retains the analyte ions or the interfering matrix components. In the case of analyte preconcentration the analyte ions are eluted from the column in a subsequent step and the eluate is then analyzed by IC. If matrix components are retained on the solid phase, then the determination is carried out on the solution leaving the column.

For the practical performance of solid-phase extraction, many manufacturers offer ready-to-use cartridges filled with sorbent material as well as devices for passing the sample solution through the sorbent bed either manually, semi-automatically or fully automatically. A particularly elegant and efficient way of carrying out automated sample preparation by solid-phase extraction is provided by so-called column-switching techniques (contribution C). In this case matrix elimination occurs in a flow system functioning in the low-pressure range that is connected inline with the IC instrument.

In principle, when solid-phase extraction methods are used, care should be taken that the sorbents used have a sufficiently high degree of purity, as there is a serious contamination risk, particularly in trace determinations. Hydrophobic packing materials require conditioning with an organic solvent that is miscible with water in order to ensure the good wettability of the sorbent. The exchange or loading capacity of the sorbent used must be matched to the separation problem by the use of appropriately dimensioned cartridges. Finally, care should also be taken that the contact time between the components to be separated and the sorbent material is sufficiently long. Regardless of the type of sorbent used and the practical execution, an optimization of the solid-phase extraction taking into account the sample's matrix composition is absolutely essential. This includes the determination of the reproducibility and the recovery rate by spiking experiments as well as checking contamination and analyte loss.

The uses of solid-phase extraction for matrix modification and the separation of interfering substances are extremely versatile. The neutralization of acidic and alkaline samples can be carried out by means of ion exchanger resins in basic or acidic form. Ion exchangers can also be used for the selective separation of particular interfering ions. An example of this is the separation of a high chloride or sulfate content with cationic exchangers in the Ag and Ba forms respectively, or the exchange of multivalent cations (which could cause interference in anion chromatography) for alkali metal ions. Interfering concentrations of carbonate/bicarbonate can be eliminated by the use of H^+-form cation exchangers.

Non-specific sorption on inorganic and organic polymer phases has achieved great importance in the analysis of samples containing organic substances. Depending on their polarity and molecular size, these can adversely affect the IC analysis in various ways and, in the worst case, cause irreversible damage to the stationary phase. Hydrophobic packing materials such as polystyrenedivinylbenzene, polymethacrylate resins or chemically modified silica gels, which are used in HPLC as reverse phases, are particularly suitable for the separation of hydrocarbons, surfactants, aromatic compounds and long-chain organic (fatty) acids. Polyvinylpyrrolidone has been shown to be very suitable for use as the sorbent phase for the separation of humic materials, tannins, lignins as well as organic dye compounds, phenolic materials, aldehydes and aromatic acids. Other, more rarely used sorbents are carbon materials (charcoal, graphitic carbon), inorganic oxides and silicates (Al_2O_3, Mg silicate, zeolites) as well as a wide variety of other organic polymers that have proved to be advantageous for specific separating problems.

Nonpolar solid-phase extraction can also be used for the preconcentration of heavy metals as a complex compound and their subsequent determination by cation chromatography. Alternatively, so-called chelate-forming ion exchangers can be used for this. These are extraction reagents covalently bound to an organic polymer carrier that selectively bind individual metal ions or groups of metal ions. In a second step the metal cations are eluted with a small volume of mineral acid and can then be directly injected into the IC instrument.

3.3 Liquid-liquid extraction

Liquid-liquid extraction is of little importance in the context of IC analysis as the transfer of the analyte to an organic solvent phase means that either stripping or evaporation of the solvent is required before separation by ion chromatography can be carried out. The working steps associated with this require a great deal of expenditure on both work and time and include the great risks of contamination and loss of analyte. The few examples to be found in the literature mostly refer to the preconcentration of heavy metal ions after conversion with organic complexing agents and subsequent separation by cation chromatography. The analysis of ions in oil samples or solvent samples that are immiscible with water can be carried out after extraction with water or the chromatographic eluent. However, this involves a post-treatment step in which the solvent contained in the aqueous phase must be removed by solid-phase extraction before the sample can be analyzed by IC. This also means that only the extractable fraction can be determined. The total elemental content can only be determined after complete mineralization (e.g., with a combustion apparatus).

3.4 Gas phase separations

Separation via the gas phase is a method with a comparatively high degree of selectivity as only a few of the analytes that can be determined by IC can be converted to a volatile form. This also means that the attractive option of simultaneity offered by IC is lost to some extent. Nevertheless, there are numerous interesting applications involving this type of sample preparation in combination with IC analysis.

Gas phase separations can be carried out in very different ways as regards the apparatus that is required. Although classical distillation is easy to carry out, it usually requires large sample volumes, takes a long time and cannot be automated. A very elegant technique that has almost been completely forgotten is the so-called microdiffusion technique. It involves placing the sample solution and an absorption solution beside each other under a cover; under isothermal conditions there is a transfer of the volatile analyte (or a previously generated volatile form of it) from the liquid sample solution to the absorption solution via the gas phase. The separation process is relatively slow, but a very large number of samples can be prepared in parallel in this way.

A completely different possibility of utilizing gas separation exists in the use of hydrophobic microporous membranes. The methods known as gas diffusion or pervaporation are based on the fact that aqueous solutions cannot wet hydrophobic membranes. If the sample solution and the absorption solution are separated by such a membrane then volatile compounds diffuse through the air-filled pores of the membrane, and are enriched in the absorption solution. This type of gas phase separation is used in miniaturized batch procedures but can also be operated continuously in a flow system; this means that it can be automated.

Interesting examples of the use of gas phase separation by distillation, microdiffusion techniques or gas diffusion are the determination of cyanide, sulfide, sulfite and nitrite, which can be volatilized as gases (HCN, H_2S, SO_2, NO_x) from the matrix-containing sample and then converted back to their anionic forms in a suitable absorption solution for IC determination. In a similar way ammonium and some short-chain amines can be isolated via the gas phase and determined by cation chromatography.

3.5 Membrane separation

In recent years membrane separation techniques have achieved great importance in technical applications for separating substances. In the context of chemical analysis problems, the use of membranes for separation is still very limited - except for filtration. The variety of available membranes allows to take advantage of very different separation mechanisms; this means that the selectivity of the separation can be adjusted. This alone results in a great potential (not only) for sample preparation. A very important aspect is also the relatively simple automation of dialysis techniques by the use of continuously operating flow systems (contribution D).

Membrane techniques for analyte separation and matrix elimination

- Filtration, membrane filtration, ultrafiltration
- Passive dialysis, Donnan dialysis, electrodialysis
- Gas diffusion, pervaporation
- Membrane extraction (supported liquid membranes)

Properties that have an influence on the selectivity of membranes

- Sieve effect (chemically neutral molecular filter)
- Electrostatic effects (ion exchanger membrane)
- Solubility effects (organic solvent immobilized in the membrane)
- Gas permeability (microporous membranes)

3.5.1 Ultrafiltration

Classical filtration and microfiltration (paragraph 3.1) are not normally regarded as being membrane techniques but are directly linked to ultrafiltration as regards their aims and in their procedures. In all cases, a pressure gradient effects the transfer of the solvent and its dissolved fraction through the filter. Particulate constituents are retained and, depending on the pore size of the filter or the ultrafiltration membrane, colloidal fractions and dissolved molecules with a high molecular weight are also retained. Ultrafiltration is, however, regarded as being a filtration technique in which the membranes have pore sizes that are very much smaller than those used in membrane filtration. The characteristic value for the filtration effect is also no longer the pore size, but is now the molecular separation limit. This refers to the molecular weight of a substance that can just (or just not) pass through the membrane. The smaller pore sizes also require much higher pressure gradients.

As far as both the apparatus and time required are concerned, ultrafiltration is clearly more complicated and time-consuming than membrane filtration; the costs involved are also higher. In certain cases, ultrafiltration is also carried out with a series of membranes in order to achieve a fractionation according to molecular weight. The high pressure that is required is obtained either by suction with a vacuum pump or by an ultracentrifuge. Special miniature devices are commercially available, in which the membrane separates the sample vessel and the eluate collection vessel. These are placed in the centrifuge as a complete unit and make it much easier to carry out the ultrafiltration. It is also possible to carry out continuous ultrafiltration. In this case the sample solution is passed tangentially along the membrane (cross flow) and the eluate is removed continuously. As an alternative to this, hollow fiber modules can also be used; these have a much larger exchange area, which means a higher permeate flow combined with a longer working life.

Within the context of IC analysis, until now ultrafiltration has mostly been used for the determination of ions in samples containing large amounts of protein (blood, serum). However, in the light of its extraordinarily good matrix elimination a great potential can also be recognized for other applications. In combination with HPLC ultrafiltration has achieved great importance in the monitoring of fermentation processes.

3.5.2 Dialysis techniques

In principle, the term «dialysis» is used for very different membrane separation techniques. However, the same basic procedure applies for all dialysis techniques: a membrane separates the sample solution (donor) from an acceptor solution that takes up the substances that are transported through the membrane. There is a great variety and variability in the geometric arrangement of the dialysis cells, in the dimensions of the liquid compartments as well as in the operating procedures used in the various dialysis techniques. Besides the usually very tedious batch techniques, continuously operating flow-through dialyzers are now also being used. Dialysis modules exist in a sandwich configuration for flat membranes as well as hollow fiber dialyzers. Miniaturized disposable dialyzers are also commercially available. A detailed description can be found in contribution D.

In passive dialysis, ions or molecules are separated because of a concentration gradient across a neutral (non-reactive) membrane. The selectivity results from the pore size of the dialysis membrane used. This dialysis method is mainly used for the same purpose as ultrafiltration. However, its decisive advantage can be seen in the fact that it is far easier to carry out a continuous dialysis and it is therefore easier to automate. There is also no transfer of solvent through the dialysis membrane, which means that the membrane does not become blocked even when subjected to higher loading with particles and/or high-molecular sample constituents.

In Donnan dialysis ion exchanger membranes are used; depending on the charge on the membrane either anions or cations are transported. The driving force is the attempt to achieve an equal charge between the ions in the sample solution and the ions in the acceptor solution that diffuse in the opposite direction. There are two different Donnan dialysis applications used in combination with IC; these are matrix standardization and analyte preconcentration. Matrix standardization can be carried out by the selective addition or deliberate separation of ions with a particular charge. For example, in this way it is possible to achieve the neutralization of alkaline samples that are to be analyzed by anion chromatography by using a cation exchanger membrane and an acid as the acceptor solution. In this case the protons wander (in a very similar way to chemical conductivity suppression with membrane suppressors) from the acceptor into the sample solution and, in return, an equivalent number of cations wander from the alkaline sample into the acceptor flow. There is, however, a problem as ion exchanger membranes always have a certain permselectivity. This means that differently charged ions are transported through the membrane to some extent and, in the example given above, anions from the acceptor solution will also enter the sample solution; such ions can therefore not be determined.

The selective separation of the ions to be determined by Donnan dialysis has only found very limited applications as a difficulty exists in that the separated ions must be determined in the acceptor solution, which necessarily has a high ion concentration. The problems associated with this can be avoided to some extent by the use of a carbonate/hydrogen carbonate solution as the acceptor for separations by anion chromatography. Reports have also been made about the isolation and preconcentration of transition metal cations followed by their determination by cation chromatography.

By applying an electrical potential difference across an ion exchanger membrane, the transfer of ions can be realized without having to use a salt solution as the receiver flow. This is known as electrodialysis; it has hardly ever been used as a sample preparation technique because of the more complicated apparatus required and the long dialysis times, but it is used in IC as one of the possible ways of conductivity suppression.

4. Preparation of solid samples

In the case of solids, the determination of ionic substances or substances that can be converted into an ionic form is an important field of application in IC. This includes the analysis of soils, sediments, dusts, geological materials, various industrial products and materials as well as biological samples and all types of foodstuffs. Even oils, fats and similar substances can be included under the term «solids», as similar considerations must be applied to the necessary sample preparation steps.

The analysis of solids by IC requires either the transfer of the whole sample or at least the ions of interest into an aqueous phase. This can be carried out in very different ways, depending on the solubility of the substance to be analyzed and the ionic contents to be determined (contributions E, F). Important criteria for the selection of the possible sample preparation techniques are:

- The completeness of the ion transfer to the aqueous phase,
- The possible separation of interfering matrix components by selective extraction,
- The compatibility of the solvent or extraction solvent with the subsequent IC analysis. This applies to both the pH as well as to the presence of substances that could affect the separation by overloading, the production of additional signals or by causing an alteration in the separating column,
- The apparatus and time required to carry out the procedure,
- The danger of losses by adsorption, volatilization or the uncontrolled conversion of the ions to be determined into a different chemical form,
- The risk of contamination by auxiliary reagents used and/or vessel contact.

In principle, the sample preparation methods for solid substances can be classified according to whether treatment with a liquid, fusion or ashing or combustion of the dry sample is necessary. The use of liquids can include complete dissolution, extraction or leaching as well as a wet chemistry treatment with acids. It can be assumed that a mild treatment of the samples (i.e. dissolution and extraction) will provide information about the content of ions present in the sample as salts. Conversion of particularly easily oxidizable substances (e.g. nitrite, sulfite, sulfide) can also occur during a gentle extraction and must be taken into account when interpreting the results. The situation following drastic treatment with strong acids or thermal decomposition of the solid sample is completely different. In this case quantitative determination of the ions by IC only supplies information about the total content of all compounds that could be converted into the particular ion under the given digestion conditions. This means, for example, that the nitrate, phosphate or sulfate concentrations in the digestion solution can provide no unambiguous conclusions about the content of these ions in the solid sample, as other (particularly organic) nitrogen, phosphorus and sulfur compounds may be present in ionic form after ashing or combustion and absorption of the released gases.

4.1 Dissolution and extraction

Complete or partial dissolution is the simplest method of sample preparation for the determination of ionic constituents in solid samples by IC (contribution E). No special apparatus is required and virtually all common plastic or glass containers can be used as the vessels. Dissolution of the sample or extraction of the ions to be determined is normally carried out at room temperature, but can also be accelerated by gentle warming or by heating the solvent used to the boiling point. In practice long and vigorous shaking, stirring or the very efficient ultrasonic treatment are used.

Owing to its high polarity, water is often very suitable for use as the extraction solvent for ionic compounds in solid samples and should be used wherever possible. There are no problems concerning its compatibility with various IC separation methods and, as regards its purity, water also meets the highest requirements encountered in trace analysis problems. As an alternative, it may be possible to use the mobile phase of the particular chromatographic separating system. Salt solutions and acids can only be used with limitations for special applications. The determination of less soluble compounds such as condensed phosphates or sulfates, phosphates and carbonates of the alkaline earths may not always be quantitative in purely aqueous eluates. In such cases it may be Necessary, to use dilute acids for extraction and to make compromises with regard to the separation performance and possible interference to the detection of individual ions. The addition of an organic solvents improves the extractability of ions, particularly for samples of a vegetable or animal origin. However, interferences to the chromatographic separation in the form of system peaks are not uncommon; it must also be proven that the stationary phase is not damaged by the presence of the organic solvent.

4.2 Wet-chemical acid digestions

If an aqueous extraction is not capable of releasing the components to be determined then it may be necessary to consider a wet-chemical digestion. This consists of treating the solid sample with an acid or mixture of acids and is usually carried out in either an open system or a closed system; heat is normally applied. Open digestions can be carried out relatively simply in heat resistant vessels on a hotplate. Digestions under pressure require special vessels and the necessary safety precautions must be observed. In recent years, microwave ovens have proved to be outstandingly suitable for heating the samples.

A serious problem with treatment by strong acids is the incompatibility with the subsequent IC analysis. The low pH of the digestion solution prevents direct injection as this could damage the separating column. Very acidic samples also influence the equilibration on the separating column and can cause large retention time displacements, additional so-called system peaks or a very unstable baseline. In addition, the presence of the added acid anions means that any such ions present in the sample cannot be determined; the quantification of the other anions is also usually affected.

Determination is possible in some cases after diluting and, if necessary, buffering the digestion solution, but in such cases considerable restrictions regarding the sensitivity, the selectivity of the separation and the ability to detect the substances of interest must often be accepted. The problems encountered in the determination of cations are usually less critical, but the high proton content can also have serious effects, particularly in the determination of alkali ions. In principle, further treatment of the acidic digestion solution can solve the problems described above, but this requires additional working steps and analysis time, which makes it unattractive.

4.3 Fusion methods

An alternative to the wet chemistry treatment of solid samples with strong acids is fusion under alkaline conditions. The solid sample is mixed with a suitable fluxing agent and heated in a crucible until it melts. After cooling down the fused mass is treated with a suitable solvent and the ions are determined in this solution. Problems also exist here with the compatibility of the fusion solution and the IC analysis. However, the fluxing agents frequently used in the fusion process such as sodium hydroxide and sodium carbonate are also components of the mobile phase frequently used in anion chromatography. After simple dilution it is often possible to carry out an ion determination with little difficulty. Cation determination is strictly limited owing to the high alkali metal ion content (from the fluxing agent). Fusion is particularly used for the analysis of geological material as well as glasses. The procedure is usually very work-intensive, takes a long time and involves a high risk of error from contamination and loss of analyte.

4.4 Dry ashing and combustion methods

Other possible ways of preparing solid samples for IC analysis are dry ashing using air as the oxidizing agent or combustion in an atmosphere of pure oxygen (contribution F). These methods are particularly used for biological samples, pharmaceutical preparations, polymerized substances, coal and other (also liquid) fuels as well as foodstuffs and other samples with a very high organic matrix content. In ashing the dry and homogenized sample is mineralized in a crucible or combustion boat by heating it in a muffle furnace for several hours at a temperature of typically 300 to 800 °C; the ashes are then dissolved in water or, if necessary, in a dilute mineral acid. In dry ashing the elements nitrogen, sulfur and also any halogen compounds that are present in organic form are usually completely volatilized as gases and cannot be determined in this way. Ashing aids can accelerate the mineralization process and other additives are sometimes necessary in order to prevent the volatilization of particular components. The determination of iodine in biological material can be carried out loss-free in the presence of sodium carbonate. Dry ashing is particularly useful for the determination of cations, in particular those of the transition metals, as sample preparation is easy to carry out and the absence of acids and other additives means that no interference to the IC analysis occurs.

Combustion analysis can be carried out in various ways. The Schoeniger digestion method is widely used; the sample is burned in a closed glass or quartz flask in pure oxygen and the released combustion gases (e.g. SO_2, NO_x, HX) are taken up in an absorption solution contained in the flask. The advantages of this method are that it is simple, rapid and favorably priced. A disadvantage is that only relatively small amounts of the sample can be burned (approx. 0.1 g), which strictly limits the sensitivity of the method. As an alternative to the Schoeniger method combustion can also be carried out at high pressure in a «Parr oxygen bomb». Oxygen pressures of up to 40 bar allow sample weights of 1 g and more.

In other combustion analysis methods (e.g. the Wickbold method) a continuous gas stream is passed over the sample contained in a boat or crucible that is heated to a temperature of between 300 and 800 °C to induce thermal decomposition. The gas stream is then passed through an absorption solution to collect the decomposition products. In IC analysis this technique is used, for example, for differentiation of the organic halogen compounds in the AOX method and for the sulfur as sulfate. Combustion Ion Chromatography applies pyrohydrolysis for the analysis of organically bound halogens and sulfur. The combustion typically takes place at 1050 °C under a continuous flow of argon, oxygen and water.

With regard to the choice of absorption solution used in the methods mentioned above both easy solubility of the released gases and compatibility with the IC analysis are important. Water, weak buffer solutions as well as the mobile phase to be used in the subsequent chromatographic separation are basically suitable. As in some cases elements cannot be converted into a uniform oxidation stage during combustion (e.g. simultaneous formation of NO and NO_2 or SO_2 and SO_3) it is a good idea to modify the absorption solution so that conversion (by oxidation or reduction) to a single ionic form is achieved. This is why aqueous hydrogen peroxide solution is often used as the absorption solution for the determination of sulfur, nitrogen and phosphorus.

5. Analysis of gases

In general, the analysis of gases is not without its problems, because air and exhaust gas samples are usually heterogeneous multi-substance and multi-phase systems. Apart from the gas phase, aerosols may also be present in the form of solid microparticles (airborne dust) and liquid droplets (fog, rain). A selective determination of the ionic contents in the individual phases is complicated and requires highly specialized sampling strategies.

In many cases, dust constituents are determined by collecting the airborne dust on a filter, extracting it with water and then determining the ionic components by IC. In such cases the problems discussed for the analysis of solid samples can also occur. Dedicated instruments for catching aerosols in a liquid phase are available. Two major types are applied, the PILS (Paticle-into-liquid-sampler) and the SJAC (steam-jet aerosol collector). Connected to IC these units allow time-resolved measurement of ions in aerosol. The latter is part or the MARGA (Monitor for AeRosols and Gases in ambient Air).

The determination of substances present in gaseous form by IC is almost always preceded by preconcentration through absorption in a suitable liquid or by adsorption of the components on a solid sorbent material, on a reagent-impregnated filter, or applying the WRD (wet rotating denuder) where the gases are absorbed in a water film on rotating cylinders (MARGA). If adsorption on a solid phase is followed, as in the analysis of dust collected on a filter, by elution with an extraction solvent that is then analyzed by IC. In order to avoid the simultaneous collection of aerosols these are normally separated off by a prefilter. From the point of view of the apparatus required, absorption in washing or bottle impingers can be performed relatively simple, as can sorption on solid sorbents. However, the volume flow and the dimensions of the absorption bottles or adsorber tubes must be adapted to suit the particular collected samples. Absorption of gases by a WRD on the other hand does not require an aerosol filter, as the aerosol particles are not absorbed. They even can be absorbed subsequently by an SJAC or a PILS.

Gaseous substances are usually converted to ionic components during preconcentration; these are then determined by IC. The choice of the absorption solution used in the washing bottle is of great importance as its composition influences the selectivity of the conversion. For example, nitrogen dioxide is almost always collected as nitrite and nitrate, sulfur dioxide is collected in water as sulfite but as sulfate in a H_2O_2 solution. The composition of the absorption solution also plays an important role with regard to the completeness of the gas separation and conversion. Only in very few cases can the separation of several gaseous substances take place simultaneously, so that simultaneous determination by IC is of little importance. The same applies in principle to adsorptive sampling, except that in this case the additional elution step introduces further problems with respect to selectivity and contamination.

Other ways of gas sampling that differ in principle in permitting selective gas separation without particle filtration are based on a diffusion-controlled separation. This utilizes the fact that gases have a diffusion coefficient that is greater than that for particles by several orders of magnitude. In so-called passive sampling, which has found a very wide range of applications in workplace studies, diffusion collection takes place on an adsorber layer in free contact with the atmosphere without air having to be actively pumped. Such devices in the form of tubes (Palmes tubes) or placards supply integrative measurements of the pollutant content in the atmosphere with only minimal apparatus being required. For example, in some countries this technique is used for the determination of SO_2, NO_2, and O_3 as a screening method for monitoring the air quality.

Diffusion denuders are a further possible example of diffusion-controlled gas sampling; they can be configured in many different ways and can be used for numerous analytes. In the simplest case they consist of a tube whose inner walls are coated with a suitable adsorption material. As the gas being measured flows through the tube the gaseous air pollutants migrate, diffusion-controlled, to the wall, where they are separated off; in contrast the more inert particles pass unaltered through the tube and can be separated off on a filter placed downstream.

All the methods described above are batch methods involving many manual working steps and a relatively poor temporal resolution. By the use of membrane-supported gas sampling (permeation denuders, wet-effluent scrubbers) continuous sampling and determination can be realized; this is also easy to automate. In this case the gas being measured - in a similar way to the sample preparation techniques using membranes described above - flows past a gas-permeable membrane that has an absorption solution on its other side. This latter can be transferred, either continuously or after the gaseous analyte has been preconcentrated for a given period, to the injection valve of the IC instrument. This allows gases to be analyzed with a relatively high temporal resolution. This method has found its first interesting applications in the process gas monitoring sector.

6. Trace preconcentration for IC analysis

Trace analysis problems occur in many scientific disciplines and the demand for corresponding sensitive methods - including ion analysis - has led to various attempts to improve IC detection performance. With the currently available instrument configurations in IC (usually equipped with conductivity detection), numerous ions can be routinely determined without any great difficulty even in concentration ranges below 1 mg/L. An increase in the injection volume can increase the sensitivity by up to one to two orders of magnitude, but only for samples with a very low ionic strength.

A different way of using IC for trace analysis is an instrumental improvement using detectors with a lower noise level, less drift and therefore an improved signal-to-noise ratio. In this way, and also by the use of different detectors that are basically more sensitive than the conductivity detector (e.g., amperometry, fluorescence, ICP-MS), the detection limits for the determination of particular ions can be improved considerably. However, this is usually accompanied by a reduction in the spectrum of components that can be determined and/or an increase in the complexity of the necessary instrumentation (and usually an increase in the costs).

By preconcentration of the ions to be determined in a pre-chromatographic step, it is possible to achieve considerable improvements in the sensitivity and often, at the same time, better matrix elimination. This is of particular importance for the preconcentration of trace components in a difficult matrix. Whereas in individual cases as selective a preconcentration as possible of individual ions in the presence of very high concentrations of other ions may be asked for (e.g. iodide in seawater), in numerous other cases a multi-component preconcentration is required. This applies, for example, to the quality control of ultrapure water used in the semiconductor industry or to the monitoring of the boiler water in power plants.

In principle, the trace preconcentration methods are the same as those mentioned before in connection with sample preparation. The use of solid-phase extraction with appropriate sorbents is very versatile and is by far the most frequently used method for this purpose. The selectivity can be influenced by the type of sorbent so that particular ions (alone or together with other ones) can be preconcentrated and separated from the other matrix components. In principle, very high preconcentration factors can be achieved by loading the sorbent phase with relatively large volumes of the sample and eluting with a very small volume of the extraction solvent. However, there is also the risk that, even if the capacity of the sorbent is adequate with respect to the matrix, not all the ions to be determined will be retained quantitatively. For example, numerous anions in pure water can be preconcentrated simultaneously and without many problems on anion exchanger columns and then determined by anion IC. Whereas samples with a higher total ionic strength function as an eluent during preconcentration themselves; this means that only ions with a high affinity to the anion exchanger (e.g. sulfate) will be retained completely. A further general problem results from the fact that a sufficiently strong elution agent must be used for eluting the preconcentrated ions; this again represents a new matrix that must be compatible with the IC analysis.

From the aspect of the apparatus required, trace preconcentration can be carried out in a conventional offline procedure with ready-to-use cartridge systems. Although this can be realized instrumentally in a very simple way, the numerous possible sample contacts with the various vessels and the atmosphere mean that there is a high risk of contamination; it is also relatively tedious and is not easy to automate. Integration of a preconcentration step in the analytical procedure of an IC determination (inline preconcentration) is very easy to realize for certain problems by including a small preconcentration column in the injection loop of the IC system. The sample solution is then passed through the column for a certain time and, on switching the valve, the preconcentrated components elute and are separated on the column. A limitation is that the sorbent material must be stable at high pressures and the interaction between the analyte and the preconcentration column must not be too strong as the chromatographic eluent must be able to elute the analyte again. As an alternative to the position in the injection valve, the preconcentration column can also be included in a secondary low-pressure flow system and the eluate transferred to the injection loop by directly coupling it to the IC instrument (contribution C).

7. Conclusions

Sample preparation is often an indispensable, frequently a demanding but (highly) advisable step when determining ions by IC. The variety of sample preparation methods is great and it is not always easy to find a suitable method for the problem to be solved. For one and the same problem there are often several (also strategic) approaches so that the user has to make a decision based on different criteria.

Regardless of the choice of method, each of the working steps involved must be optimized again for each problem (with reference to the ions to be determined and the existing matrix). The risk of contamination and loss of analyte must always be kept in view as this is the only way in which the high quality of the analytical results can be ensured. The instrumental integration of sample preparation steps in the analytical procedure (inline operation) is not only interesting for automation reasons, but also opens up important analytical aspects concerning reliability, improved separation of traces, easier matrix elimination and reduced risk of contamination.

8. Literature references

[1] P.R. Haddad, P.E. Jackson, Ion chromatography - principles and applications, Chapter 14, Sample handling in ion chromatography, Elsevier, Amsterdam, 1990, 409-462.

[2] P.R. Haddad, Sample clean-up methods for ion chromatography, J. Chromatogr., 482 (1989) 267-278.

[3] S.K. Poole, T.A. Dean, J.W. Oudsema, C.F. Poole, Sample preparation for chromatographic separations: an overview, Anal. Chim. Acta, 236 (1990) 3-42.

[4] R.E. Majors, A comparative study of European and American trends in sample preparation, LC·GC, Vol. 6, No. 3 (1993) 130-140.

[5] N.C. van de Merbel, J.J. Hageman, A.A.Th. Brinkman, Membrane-based sample preparation for chromatography, J. Chromatogr., 634 (1993) 1-29.

B) Solid-phase extraction as a sample preparation technique

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Contents

1. Introduction to solid-phase extraction

In modern trace analysis, sample preparation is usually more important than the determination method itself for the accuracy and reproducibility of the results. This is even more the case as the concentration to be determined decreases and the complexity of the composition of the sample itself increases. Important examples are body fluids, tissues, foodstuffs, ultrapure substances, etc.

Solid-phase extraction (SPE) is currently one of the most powerful techniques used for rapid and selective sample preparation. SPE covers sample preparation techniques that are based on a solid sorbent and that permit the selective preconcentration of individual sample components from a complex matrix. The reverse process, the targeted removal of interfering sample components, also plays a great role.

The versatility of solid-phase extraction allows this technique to be used for purification, preconcentration, solvent exchange (e.g. transfer of the analyte from an organic sample to an aqueous medium), desalting, derivatization (the analyte is retained on the sorbent, derivatized and then eluted) and for prefractionation of the sample, e.g. into various classes of compounds.

Solid-phase extraction systems are available commercially both in column or cartridge form as well as in the form of extraction plates (well plates). Depending on the properties of the components to be preconcentrated (or purified) and the matrix, there is a large number of sorbents available.

As they function in a chromatographic manner, solid-phase extraction systems have many advantages when compared with conventional sample preparation techniques such as liquid-liquid extraction:

- high recovery rates
- preconcentration
- efficient purification
- easy to automate
- compatible with all determination methods for liquid samples
- minimization of solvent and sample consumption

As a result of these numerous advantages, solid-phase extraction has become widely used in the last years. A look at the monographs [1, 2, 3], which are appearing in ever greater numbers, shows that the number of published applications making use of this technique is still continuing to increase strongly, which is a proof of the state-of-the-art position of this technique. Examples of current arrangements for solid-phase extraction are shown in Figure 1.

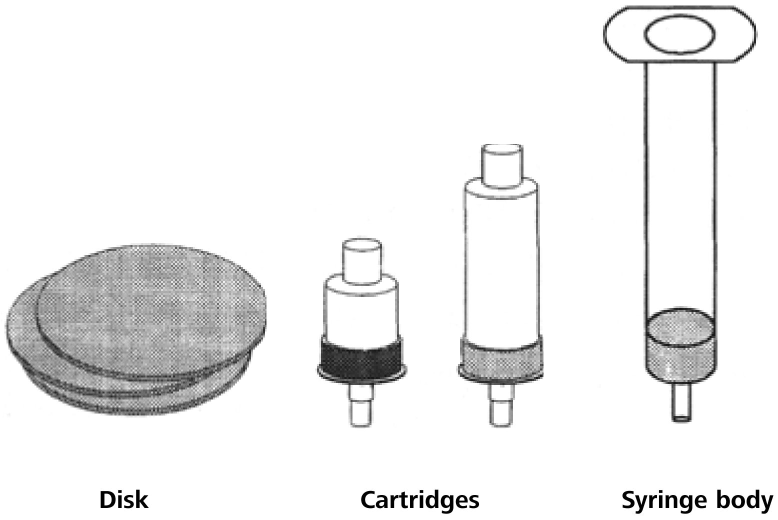


Figure 1: Most frequent types of solid phases used for solid-phase extraction (from [1J)

In the ion chromatography sector, solid-phase extraction is frequently used for the preconcentration of analytes from samples with an organic matrix such as solvents, fruit juices, body fluids, etc. A second important application is the removal of matrix ions that would interfere with the determination itself by overloading the column or by producing overlapping peaks. Examples of this are the removal of cations when analyzing mineral water for anions and the removal of anions such as chloride from water samples to permit interference-free bromate determination.

2. Basics of solid-phase extraction

The principle of solid-phase extraction can be regarded as being analogous to both liquid-liquid extraction as well as to chromatography. While the liquid sample flows through the sorbent bed, its sample constituents are «extracted» (because of the solid phase this should really be «adsorbed») and are therefore retained on the sorbent. This reversible process is repeated (during the passage through the sorbent bed), which is why solid-phase extraction can also be regarded as a chromatography variant. This means that interferences can be selectively removed by the selection of a suitable wash solution. In a final step, the compound to be isolated is selectively eluted in a suitable solvent to give as pure a fraction as possible. In chromatography language, this is an extreme step-function gradient with, for the analyte, a very weak starting and a very strong final eluent. In many cases, concentration can also be achieved at the same time as extraction/adsorption.

Figure 2 shows in diagram form a solid-phase extraction procedure based on an extraction column. As an alternative to the procedure shown here an extraction column can also be selected so that the sample contaminants are retained selectively while the analyte runs through the column without retention.

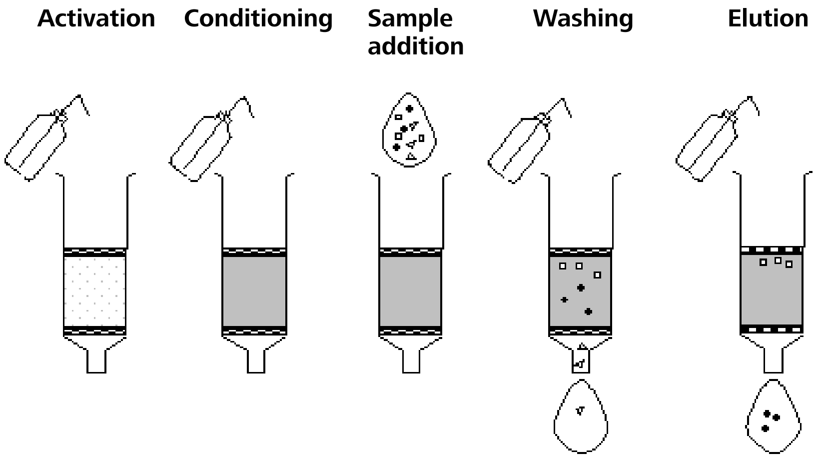


Figure 2: Solid-phase extraction procedure divided into five steps

For nonpolar sorbents, activation allows their use with polar media such as water. Without this step, which is usually carried out with methanol or acetone, the surface cannot be wetted and is therefore not sorption-active. The conditioning step is used for rinsing off solvent or for converting the extraction column into the required form, such as the conversion of ion exchangers to the counter- ion for precipitation operations or similar.

Sample addition is carried out at a controlled flow rate in order not to risk any breakthrough of the substance to be separated as a result of too slow mass transfer. In the rinsing procedure, which can consist of several steps, interfering substances are rinsed from the column. In the final elution step, the analyte is eluted from the column.

In the case of the reverse method, for example for the elimination of chloride from water samples, the procedure is as follows:

Activation and conditioning consist of loading the cation exchanger with $AgN0_3$ solution and then rinsing the column to remove the nitrate. The second part of conditioning is already the start of sample addition with a smooth transition to sample addition itself. The eluate of the sample addition, after a rinsing volume of 2 to 3 cartridge volumes, is collected as a sample for further processing. The rinsing and elution steps are used for the regeneration of the ion exchanger by re-dissolving the precipitated silver chloride, e.g. with a cyanide solution. The cartridge can then be loaded again and the cycle repeated. This regenerative working procedure is necessary for automated inline sample preparation instruments.

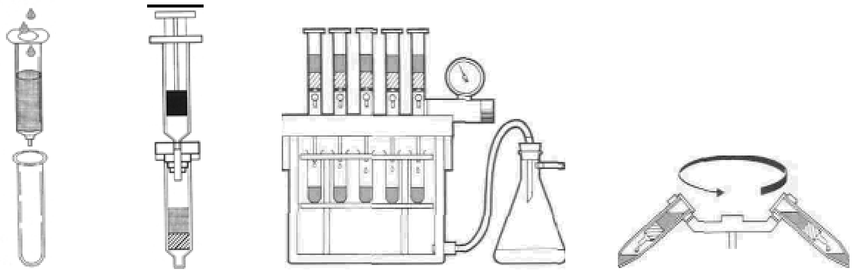
3. Practical procedures for solid-phase extraction

The extraction columns are packed with a sorbent with an average particle size of 63...125 µm. Water and most solvents flow through such columns by themselves owing to the force of gravity. One of the three following methods can be used for increasing the flow rate or for processing viscous solutions and samples:

- Pressure by using a syringe
- Vacuum by using a vacuum station
- Centrifugal force by using a centrifuge

The vacuum station and the syringe techniques are the ones most frequently used. The selection of the technique depends largely on the problem to be solved. For large series of samples with voluminous sample preparation protocols, a vacuum station is the ideal technique as numerous samples can be processed in parallel.

The application of a positive pressure by using a syringe is used for manually processing individual samples. In this case, the use of several extraction columns in series is also possible. Fully automatic solid-phase extraction systems also use positive pressure, which in this case is provided by a peristaltic pump or similar.



Gravity **Pressure** **Vacuum** **Centrifugal force**

Figure 3: Working techniques and the forces involved in solid-phase extraction (from [1J)

The successful application of solid-phase extraction requires the correct pretreatment of the extraction columns (and, of course, the initial sample) during each individual step involved in carrying out the extraction.

The individual liquids must not flow too quickly through the solid-phase columns. In particular, when carrying out extractions on ion exchangers, it must be remembered that a flow rate that is too high can lead to a rapid analyte breakthrough (i.e. the analyte will run through the extraction column without being retarded) or a poor equilibration. In chromatography language this means that work must be carried out far above the van-Deemter minimum and that selectivity is sacrificed unnecessarily. One to two milliliters per minute is an appropriate flow rate for a column filled with 100 mg sorbent. In practice, the dimensions of the column, the sorption mechanism and the particle size are decisive for the selection of the flow rate.

For sorption, it is essential that the analyte is in an uncombined state in the solution. With samples containing particles, it must be ensured that any substances that may possibly have been adsorbed are released from the suspended matter (solvent addition, pH change, heating, ultrasonics, etc.). The bonding of the analyte on macromolecules (e.g. protein bonding) must - if such bonding is not already broken down on passage through the column bed - be prevented.

Particles should first be removed by filtration or centrifugation. Although extraction columns themselves have a certain filter capacity, once this is exhausted the frits will become blocked and this means that there will be an inhomogeneous flow distribution in the column or even that the flow through the column is stopped completely. The properties of the extraction column will also be altered if it is loaded with particles that are possibly sorption-active.

4. Solid-phase extraction mechanisms

The retention of particular compounds or classes of compounds on the sorbents is made possible by various interactions between the analyte and the sorbent at the molecular level - a simplified term that is frequently used is retention mechanism. The most frequently occurring interactions are:

- Van der Waals forces («nonpolar» interactions),
- Dipole-dipole forces («polar, nonionic» interactions),
- Hydrogen-bridge bonding, and
- Electrostatic forces («ionic» interactions).

The combination of several of these interactions leads, together with the distribution and adsorption methods, to the separating mechanisms known from chromatography: reversed-phase, normal phase, ion exchange and ion exclusion chromatography.

Reversed phase

According to the solvophobia theory of Horvath-Sinanoglu, the retention behavior on nonpolar phases is based on the tendency of polar solvents to reduce their inner surface. With water this is particularly noticeable because of its large cohesive density and the high surface tension associated with this. Analytes with a high nonpolar fraction demonstrate the effect known from surfactant chemistry of the enrichment of such molecules at the solvent interface. The nonpolar fractions of the molecules are forced out of the polar molecule arrangement of the liquid phase. In RP-HPLC the phase boundary represents the transition to the carrier material with nonpolar surface ligands. The adsorption process itself, in contrast to ion chromatography, is based only on weak dispersion forces between the nonpolar surface of the column material and the analyte. Figure 4 shows these processes in schematic form.

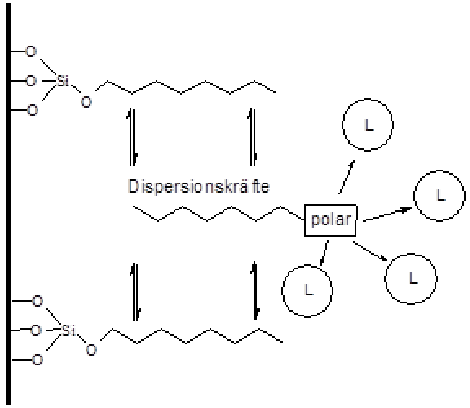


Figure 4: Schematic illustrating solvophobic interaction

The analytes with the largest nonpolar fraction are therefore retained most strongly by this type of stationary phase. These results, in a retention series of n-alkanes, i-alkanes, halogen alkanes, ethers, aldehydes, amines, alcohols and carboxylic acids, listed according to decreasing retention times.

With ionogenic substances such as aromatic or aliphatic amines a further factor is involved. Each RP column material still possesses silanol groups on its surface that could not be converted during esterification for steric reasons. An acid-base interaction occurs at the surface between alkaline substances and these relatively strongly acidic OH groups. The retention time of these substances is strongly increased and the corresponding peaks show marked tailing. An attempt is made to suppress this effect by special saturation of the silanol groups (end capping).

Normal bonded phase

Sorption on a normal bonded phase is based on polar mechanisms such as hydrogen-bridge bonding as well as on π- and dipole-dipole interactions. The relationship between the sorption force at the solid phase and the solubility in the mobile phase is decisive. The simplest normal bonded phase is unmodified silica gel, but polar modified silica gels such as aminopropyl, cyanopropyl and propyldiol phases are also suitable for normal bonded-phase sorption as shown in Figure 5.

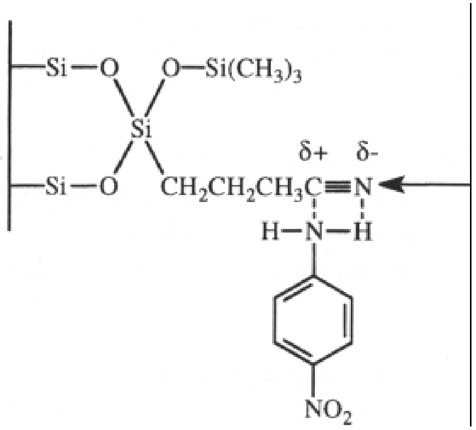


Figure 5: Mechanism of the normal bonded-phase retention of nitroaniline on a cyanopropyl phase (from [11])

The interactive forces are to be regarded as being moderate to strong and only play a subordinate role with ionic analytes owing to the absolute necessity for water to be absent.

Ion exchange

Ion exchange is based on a stoichiometric chemical reaction between ions in a solution and a normally solid substance carrying ionic functional groups that can fix ions by electrostatic forces. In cation exchangers these are, in the simplest case, carboxylic or sulfonic acid groups. In anion exchangers these are secondary to quaternary ammonium groups.

In theory, ions with the same charge can be exchanged completely reversibly between the two phases. The ion exchange process leads to a condition of equilibrium. The side on which the equilibrium lies depends on the affinity of the participating ions to the functional groups of the stationary phase. Figure 6 shows schematically the exchange processes for cations and anions. The analyte ions are marked with it A, the ions competing for the exchange places with E. In chromatography, this is the eluent anion; in the solid-phase extraction sector either further anions from the sample or anions from the wash or elution solutions.

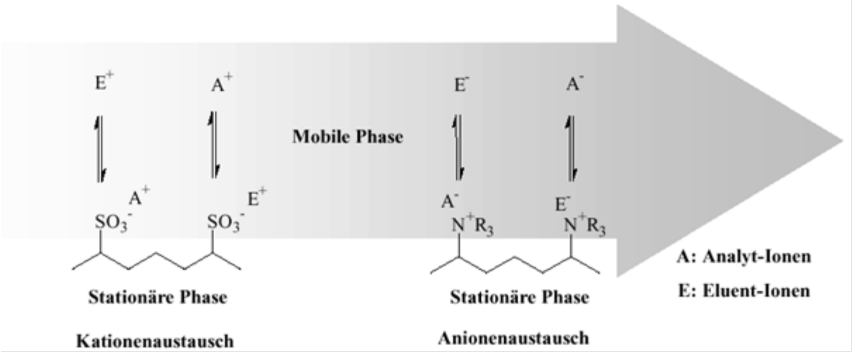


Figure 6: Reactions occurring during ion exchange

Ion exclusion

Ion exclusion is primarily used for the separation of weak acids or bases. It achieves its greatest importance in the separation of weak acids such as carboxylic acids, carbohydrates, phenols or amino acids. Figure 7 shows the ion exclusion principle using a carboxylic acid R-COOH as an example.

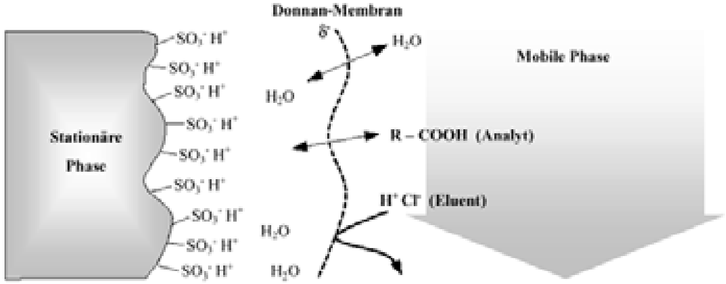


Figure 7: Donnan exclusion as the basis tor ion exclusion

In Figure 7 a completely sulfonated cation exchanger is used as the sorbent; its sulfonic acid groups are electrically neutral with protons as the counter-ions. In aqueous eluents the functional groups are hydrated. The hydration envelope is limited by an (imaginary) negatively charged membrane (Donnan membrane). It is only passable by uncharged, undissociated molecules such as water. Organic carboxylic acids can be separated if strong mineral acids such as hydrochloric acid are used as the solvent. Because of the low acid constants (pK_a values) of the carboxylic acids these are almost completely undissociated in the presence of strongly acidic eluents. They can pass through the Donnan membrane and adsorb at the stationary phase, whereas the chloride ions of the fully dissociated hydrochloric acid are excluded. The carboxylic acids can be separated on the basis of their different pK_a values.

The separation of weak bases, which is not very widespread in practice, takes place under exactly the opposite conditions with strongly basic anion exchangers and alkali hydroxides as the eluents.

5.Sorbents for solid-phase extraction

The large choice of sorbents with different functional groups allows the most important advantage of solid-phase extraction to be utilized: its selectivity.
In this context selectivity is understood to be the extent to which an extraction method is able to separate one compound from the other components in the original sample that interfere with the determination (see Fig. 2).

Table 1: Sorbents for solid-phase extraction

Sorbent	Structure	Typical capacity/load
	Reversed phase (nonpolar)	
Octadecyl (C"18)	$-(CH_2)_{"17}-CH_3$	"17% C
Octyl (C8)	$-(CH_2)_7-CH_3$	"14% C
Ethyl (C2)	$-CH_2-CH_3$	4.8% C
Cyclohexyl	$-CH_2-CH_2$-Cyclohexyl	"12% C
Phenyl	$-CH_2-CH_2-CH_2-(C_6H_5)$	"10.6% C
Graphite carbon	Pure carbon	
Copolymers	Styrene/divinylbenzene (PS/DVB)	
	Normal phase (polar)	
Cyano (CN)	$-(CH_2)_3-CN$	"10.5% C, 2.4% N
Amino (NH_2)	$-(CH_2)_3-NH_2$	6.4% C, 2.2% N
Diol	$-(CH_2)_3-OCH_2CH(OH)CH_2(OH)$	8.6% C
Silica gel	-SiOH	
Florisil	Mg_2SiO_3	
Alumina	Al_2O_3	
	Ion exchanger	
Amino (NH_2)	$-(CH_2)_3-NH^+Cl^-$	"1.6 meq/g
Quaternary amine	$-(CH_2)_3-N(CH_3)_3^+Cl^-$	0.7 meq/g
Carboxylic acid	$-(CH_2)_3-COO^-H^+$	0.4 meq/g
Sulfonic acid	$-(CH_2)_3-(C_6H_5)-SO_3^-H^+$	"1.0 meq/g
Cation exchanger	$PS/DVB-SO_3^-H^+$	5.2 meq/g
Anion exchanger	$PS/DVB-CH_2-N(CH_3)_3^+Cl^-$	"1.2 meq/g

The choice of sorbent is influenced by the composition of the sample matrix as this determines whether and how the analyte can interact with the stationary phase. Certain types of sample matrix only permit the use of certain types of sorbent.

An aqueous sample with a high salt content that contains a hydrophobic analyte should be extracted with a nonpolar phase. Attempting adsorption on a polar phase is bound to fail owing to the polar character of the matrix itself. In contrast, the extraction of ionic analytes from any matrix can only be carried out successfully with ion exchangers.

Reversed phase (C_{18}, C_8, C_2)

These nonpolar phases are prepared with the help of mono-functional to tri-functional silanes. In order to minimize the polar «secondary» interactions with silanol groups, these materials are often end-capped, i.e. the remaining silanol groups that are not sterically available for C_8 or C_{18} alkylsilanes are converted by treatment with a short-chain alkylsilane. These end-capped C_{18} materials are used for the extraction of molecules from aqueous samples over a wide range of polarities.

Materials that have not been deactivated («non-end-capped») allow the utilization of the polar «secondary» interaction of basic compounds with the remaining silanol groups. The non-end-capped C_{18} material is used to extract lipophilic molecules with differing polarities from aqueous samples.

With the C_8 materials the retention of nonpolar compounds is less than on C_{18} materials. However, this only applies as long as the retention is based exclusively on van der Waals' interactions. The C_2 phases offer an even lower retention for nonpolar substances and also allow a quicker elution with less use of solvent.

Phenyl

Phenyl phases are also nonpolar and are prepared with the help of tri-functional silanes. Endcapping is also widespread here. Compared with C_{18} and C_8 phases this phase exhibits a clear difference in selectivity in the extraction of aromatic and non-aromatic compounds. Owing to the lower carbon loading the retention force for nonpolar substances is lower than that of C_{18} materials.

Silica gel

Because of the silanol groups present on its surface, unmodified silica gel is suitable for use as a normal bonded phase. The extent of retention depends mainly on the water content of the silica gel and is therefore subject to large variations.

Acidic alumina

Highly active aluminas with average particle sizes of 50 to 200 µm are used for these extraction columns. The surface of this sorbent can retain molecules by interaction with the aluminum center, via hydrogen-bridge bonding with the hydroxyl groups or by ion exchange owing to its pH-dependent charged surface. The pH of the surface is controlled by washing the alumina with acidic, neutral, or basic solutions. In this way, it is possible to control the extent of the various interactions. Acidic pretreatment of this sorbent reduces its capacity for basic compounds. Certain compounds are retarded on the positively charged surface or as a result of a specific interaction with the central atom.

Cyano

This phase offers the possibility of both polar and nonpolar interactions. These sorbents are usually not end-capped so that the interactions of the remaining silanol groups can be used - for the selective isolation of basic compounds in particular. After suitable preconditioning the cyanopropyl phase can be used as a nonpolar («reversed-phase») sorbent for the extraction of polar and nonpolar compounds from aqueous samples. In addition, after correct (non-aqueous) preconditioning this sorbent is also able to retard polar compounds from relatively nonpolar solvents owing to dipole interactions between the functional group of the analyte and the nitrile functional group.

Amino

The amino functional group can act as a polar phase or as a weak ion exchanger. Endcapping is not normally carried out. The pK_a value of aminopropyl phases is 9.8. After conditioning with a nonpolar solvent such as hexane the NH_2 phase can form hydrogen-bridge bonds with compounds that possess OH, NH or SH groups. If the NH_2 phase is used under aqueous conditions, then it acts as a weak anion exchanger. At a pH below 7.8 all the amino groups are positively charged and are able to retard negatively charged molecules.

Diol

A polar phase that, depending on the pre-treatment of the sorbent and the sample, offers polar and weak nonpolar interactions. These sorbents are not end-capped and the remaining silanol groups are available for polar interactions. After appropriate preconditioning the diol phase can be used as a nonpolar («reversed phase») sorbent in order to isolate relatively nonpolar compounds from aqueous samples. However, this sorbent is more often used to extract polar compounds from nonpolar solvent mixtures owing to the formation of hydrogen-bridge bonds. These interactions are similar to those on unmodified silica gel, but the reproducibility on the diol phase is often better.

Propylsulfonic acid

This strongly acidic cation exchanger is used for the extraction of positively charged molecules from aqueous and non-aqueous samples. In comparison to classical strongly acidic ion exchanger, this material offers only a low degree of nonpolar interactions. This allows the use of a purely aqueous elution medium.

Carboxylic acids

As weakly acidic cation exchangers ($pK_a = 4.8$) the carboxypropyl functional groups permit the extraction of compounds that are positively charged at a pH of 4 and above.

Anion exchangers

Strongly basic anion exchangers are used for the extraction of compounds with a negative charge from both aqueous and organic solutions. These sorbents are prepared either on the basis of silica gel with the help of tri-functional silanes or on the basis of PS/DVB by chloromethylation. With anion exchangers it is possible to choose between two different amines; trimethylamine (type I) and dimethylethanolamine (type II).

Cation exchangers

Strongly acidic cation exchangers are used for the extraction of positively charged compounds from both aqueous and organic solutions. These PS/DVB-based cation exchangers also offer non- polar interactions, which in many cases increase the analyte recovery rate. In some applications these nonpolar secondary interactions make it necessary to add an organic solvent to the elution medium in order to achieve complete elution. A further use of these cation exchangers is the conversion of salts to other ionic forms. In the ion chromatography sector cation exchangers are used for the neutralization of basic solutions, for the precipitation of anions as insoluble salts and for the removal of metal cations.

6. Method development and criteria for selecting the solid phase

A typical solid-phase extraction method consists of five to six basic steps:

- Sample preparation
- Solvating the extraction column (only for nonpolar phases)
- Pre-equilibration of the extraction column
- Sample addition
- Washing the extraction column (elution of interfering substances)
- Elution of the analyte from the extraction column

Proper sample preparation means that the sample is prepared physically and chemically for the solid-phase extraction. Solid-phase extraction is a preparation technique for liquid samples. This means that solid samples must be dissolved, homogenized, and filtered (the chosen solvent must be able to dissolve the analyte from the sample). As an alternative, solid samples can also be extracted with solvents (e.g. Soxhlet); the extract obtained is then subjected to a solid-phase extraction. The preliminary chemical treatment of a sample includes dilution, (e.g. to reduce the viscosity of the sample or to reduce the elution strength of the sample), the addition of a buffer (to control the pH) or an initial extraction with a solvent. The aim of sample preparation is to bring the sample into such a condition that optimal retention of the analyte on the selected stationary phase is achieved.

If a modified silica gel is used in the extraction column then it is necessary to set up a suitable «mediator layer» on the stationary phase to permit interaction between the stationary phase and the analyte. This «wetting» (solvating) is the first working step to be carried out. With hydrophobic stationary phases such as C_{18}, C_8 or other RP phases, the stationary phase must be wetted in this first conditioning step by washing it with methanol, acetonitrile or another solvent that is miscible with water. Without this wetting process a very hydrophobic phase such as C_{18} would not retain anything from a purely aqueous sample. The minimum volume for the solvent to be used is regarded as being between 0.5...1 mL / 100 mg sorbent. In practice, the reservoir is normally filled once or twice with the appropriate solvent, which is then run through the column. With polar or ionic sorbents, this step is not necessary; in this case, the correct counter-ion is selected by conversion, for example.

In the second conditioning step, after the extraction column has been solvated and before the sample is added, the column must be equilibrated at the most optimal conditions for the extraction. This working step is carried out with a solvent that is as similar as possible to the sample. If, for example, a physiological fluid is to be extracted then work should be carried out with a buffer at the same pH and with a similar salt concentration. On the other hand, if an oil sample is to be extracted then conditioning should be carried out with a solvent with a similar polarity (e.g. hexane). Solvation and pre-equilibration should convert the stationary phase into a form that allows the optimal retention of the analyte or the sample constituents that are to be removed.

After the column has been solvated and pre-equilibrated the sample can be added. It is necessary to give the sample sufficient time for the interaction with the stationary phase to take place (this is necessary for retention). This also means that too rapid passage through the column will cause losses. The determination of the optimal flow rate when adding the sample plays an important part in the development work required for the method.

After sample addition, one or more washing steps are used to elute any interfering substances that are also retained on the column. At the same time, the retention of the analyte on the column must be sufficiently large to avoid its premature elution. In principle, the solvents that are suitable for this washing step are those in which the analyte is either insoluble or almost insoluble. In an extraction with nonpolar phases or ion exchangers it is often necessary to monitor the pH of the washing solution in order to achieve reproducible results. In this step, the correct choice of the flow rate is also crucial for the quality of the extract. Too high flow rates in the washing step may result in an inadequately purified extract or poor recovery rate reproducibility.

In the final solid-phase extraction step the required analyte is eluted from the extraction column with a suitable solvent; this must be a good solvent for the analyte and, at the same time, efficiently terminate the interactions between the stationary phase and the analyte. Several suitable elution media are often available. The one that is to be selected is the one that provides the purest extract in the smallest volume in a suitable form for the subsequent analysis. From the point of view of ion chromatography, this should be a buffer solution, that interferes as little as possible with the elution system in the subsequent ion chromatography determination.

In many cases, an easily volatile solvent is preferred so that the sample can be rapidly concentrated. Under optimal conditions elution can be carried out with only 250 µL elution medium per 100 mg sorbent used. Apart from a good elution medium, the choice of the flow rate is also important. If the flow rate is too high, this can result in reduced recovery rates or an eluate that is too dilute (an increased elution volume becomes necessary for complete elution).

7. Selected applications in the ion chromatography sector

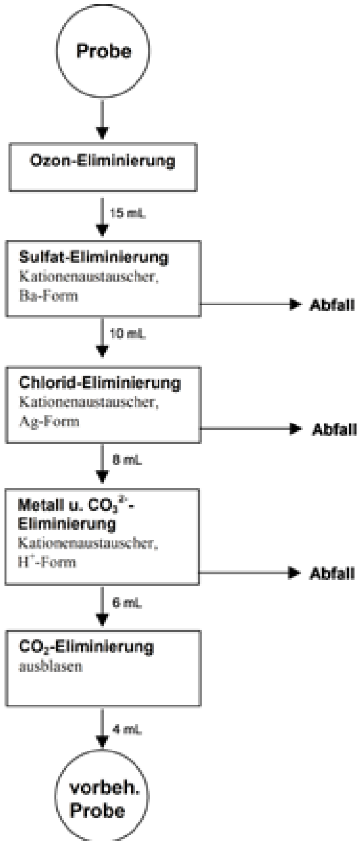


Figure 8: Example of a multi-step solid-phase extraction procedure: bromate determination by ion chromatography according to ISO 15061

A current problem in the ion chromatography sector is the determination of bromate in drinking water and recreational water. The enormous demands concerning the detection limit of the method are really beyond the capabilities of normal ion chromatography. The flow diagram according to ISO 15061 for sample preparation shown in Figure 8 includes a large part of the spectrum of the solid- phase extractions frequently used in ion chromatography.

In two steps (Ba^{2+}- and Ag^{+}-loaded cation exchangers) precipitation reactions are used for separating anions in the form of their insoluble salts. In principle, this method can also be used for preconcentration but great problems exist in redissolving the precipitates and in the solubility product, which often limits preconcentration at low concentrations.

A different type of example is the determination of nitrate and nitrite in meat products. In order to protect the column, proteins and fats must be separated off after the extraction; in this case RP cartridges or ion exchangers are used. The alternative method of using precipitants for proteins, e.g. perchloric acid, is often not possible in IC.
Anionic detergents such as lauryl sulfate (sodium dodecyl sulfate, SDS) are often separated by solid-phase extraction on reverse phases. Such detergents have a high affinity to polymer phases and are practically irreversibly bound (in-situ formation of an ion-pair chromatography column)

8. Literature references

[1] N.J.K. Simpson (ed.), «Solid-phase extraction: principles, techniques, and applications», Marcel Dekker, New York, 2000

[2] J. S. Fritz, «Analytical solid-phase extraction», Wiley-VCH, New York, 1999

[3] E.M. Thurman, M.S. Mills, «Solid-phase extraction: principles and practice», John Wiley, New York, 1998

[4] N. Simpson, C. Van Horne (editors), Sorbent Extraction Technology Handbook, Diane Publishing Co.; 1998, ISBN: 0788145517

C) Inline techniques for IC sample preparation

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Contents

1. Introduction

Since the middle of the eighties, ion chromatography has gradually become more automated, with PC control since the start of the nineties; this means that whole series of samples can now be processed automatically by using sample changers. However, until now far-reaching automation has been missing from the sample preparation sector. Apart from filtration and, in a few cases, the preparation of dilution series the work of the analytical chemist has hardly been made much easier in this sector by the use of sample changers in combination with sample preparation.

This is the reason why we have developed the concept of Inline Sample Preparation. To be accurate, each working step involving the sample from the point at which it enters the IC system to the injection valve belongs to inline sample preparation; this means that if a sample changer is used with filter caps on the sample vials then Inline Sample Preparation is already being used.

The first modules used for realizing this concept were the 754 Dialysis Unit for the automatic separation of particles and macromolecules and the 753 Suppressor Module for the neutralization of alkaline samples. Nowadays Inline Sample Preparation components are part of the respective IC system.

2. Inline Sample Preparation techniques

In principle, all the sample preparation techniques used in the laboratory can also be carried out in an inline sample preparation form.

Fig.1 shows the general arrangements and techniques used in inline sample preparation.

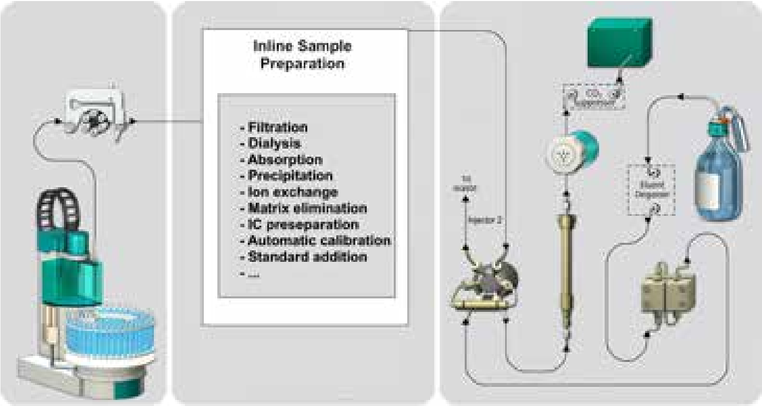

Figure 1: Schematic of an ion chromatograph with inline sample preparation: preparation techniques

Strictly speaking, techniques a) to d) are also matrix elimination techniques; however, in these cases matrix elimination is combined with filtration, dialysis, etc., whereas technique f) involves matrix elimination alone and is achieved solely by the use of a preconcentration column instead of the sample loop at the injection valve. In this case, the matrix that is not ionic (e.g. hydrogen peroxide or an organic solvent) passes through the preconcentration column and only the analyte ions are retained in it.

In IC preseparation (cutting techniques) two IC systems are linked to one another in such a way that it is possible to select sections from the first chromatogram and transfer them to the second chromatograph, in which a preconcentration column again replaces the sample loop in the injection valve. This method can be used when small fractions of other ions with the same charge are to be determined in an ionic matrix (e.g. anions in hydrochloric acid).

Automatic multi-point calibration with one standard solution can be regarded as being an inline sample preparation technique as only a single standard solution, which is more concentrated than the analyte ions in the sample by a factor of 100...1000, can be used to carry out a fully automatic multi-point calibration in the concentration range of the analyte. We use this technique primarily for trace determinations as it allows minimizing the errors that occur in preparing extremely dilute standard solutions.

The fully automatic standard addition technique can also be carried out with the same arrangement as is used for multi-point calibration. This technique has the advantage that matrix effects are minimized and that the content of the sample is determined by using a regression line. The reason why this method is only rarely used in common analytical techniques - not only in IC - lies in the time required for sample preparation, as each single sample must be prepared manually and individually with the appropriate standard addition. This is no longer the case when standard addition is carried out by an inline technique.

Two different modes are available for inline sample preparation:

- Rinsing a large amount of sample through the sample preparation part into the sample loop (direct method) and
- Transferring a small amount of sample with ultrapure water through the sample preparation part into a preconcentration column (transfer method).

Direct method

In this method, there must be a sufficiently large amount of sample available so that it is absolutely certain that the previous sample has been rinsed out of the system before the loop contents of the sample valve are injected into the chromatography flow. The use of large amounts of sample also means, of course, that the capacity of the sample preparation module is exhausted more quickly.

Transfer method

This method requires a second injection valve. A sample loop is mounted in the first valve and a preconcentration column in the second one. A flow diagram for this arrangement is shown in Fig. 2.

In the first step, Valve 1 and Valve 2 are both in the «Inject» position; this means that the sample preparation module is rinsed with water. In the second step both valves are set to «Fill» (displacement of the eluent in the preconcentration column by water and filling the sample loop with sample); in the third step Valve 1 is set to «Inject» (the sample is injected into the flow of water). In the fourth and final step, when the sample has passed through the sample preparation module and the preconcentration column, Valve 2 is set to «Inject» (start of the chromatogram).

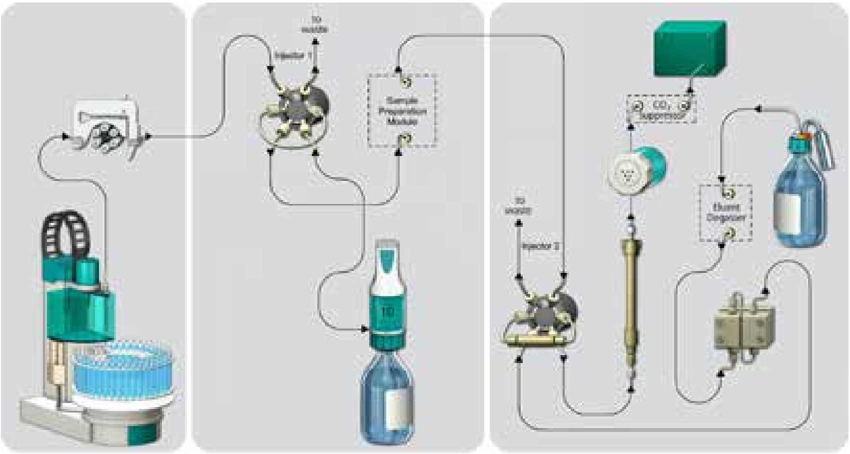

Figure 2: Flow diagram for sample preparation by the transfer method

This method has the advantage that only the amount of sample necessary for the chromatographic determination passes through the sample preparation module. Before and after the sample only the transfer solution (usually ultrapure water) passes through the module so that the system is always in a purified condition. If the transfer water becomes contaminated with analyte ions then it can be purified with a purification column inserted between the pump and Valve 1.

3. Filtration

Manual filtration of samples using syringe filters is labor-intensive and expensive, as every sample requires the use of a new filter. The use of filter caps is still expensive, but labor-cost is reduced. However, the filtration effect is compromised as the typical pore size (20 µm) of filter caps is much larger than the recommended 0.2 µm. Inline filtration can either be carried out on an autosampler by using filter caps on the sample vials or by Inline Ultrafiltration applying the external ultrafiltration cell. The arrangement of the two systems is shown in Fig. 3.

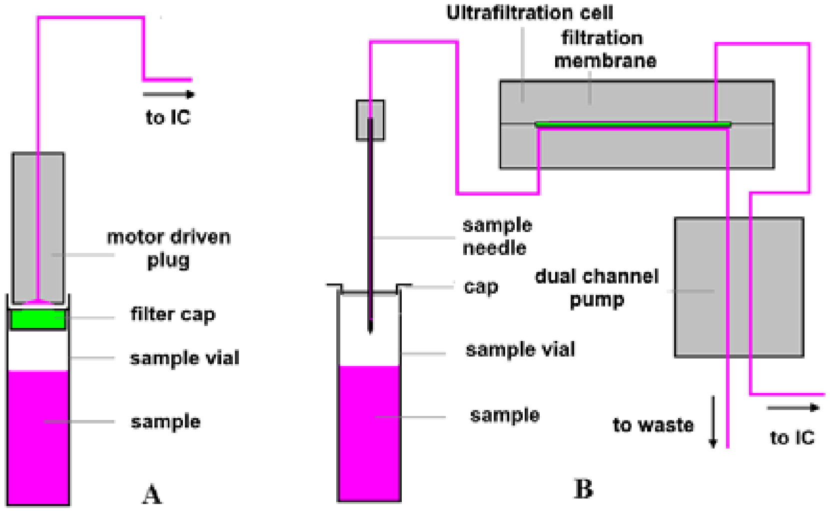

Figure 3: Working principle of a filtration changer with disposable filters (A) and with an ultrafiltration unit (B)

Metrohm Inline Ultrafiltration allows to use standard 0.45 or 0.2 µm membranes and therefore is a perfect replacement for syringe filters. Figure 3 shows the typical system setup of an IC system with Inline Ultrafiltration.

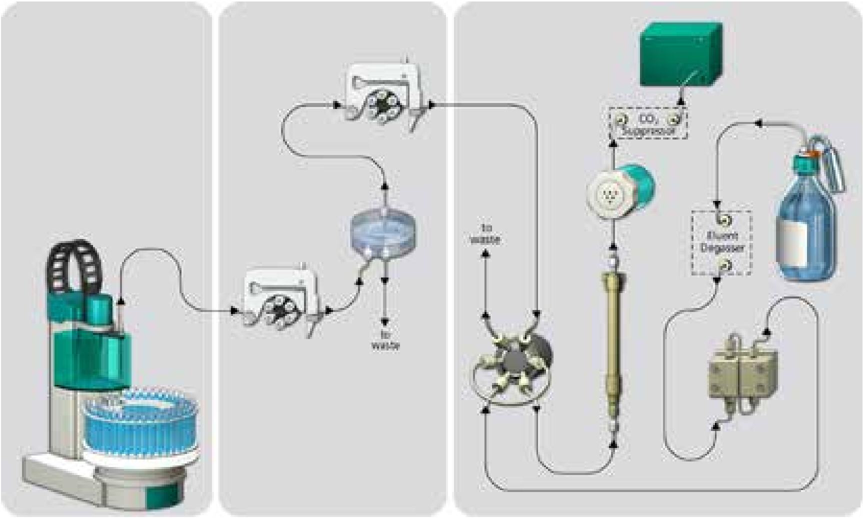

Figure 4: Flow diagram for Inline Ultrafiltration

The use of Inline Ultrafiltration is considerably more favorably priced, but care must be taken that, depending on the degree of sample contamination (number and size of particles), the filter must be changed from time to time. It is, however, an advantage that even filters with very fine pores can be used as the sample is not forced through the filter but only a fraction (20-30%) is drawn through the filter while the particles are carried along in the sample stream; this means that the filter is only loaded to a small extent. This is particularly important when the sample contains suspended particles with a size in the order of a few µm.

When the Inline Ultrafiltration is used for processing very different samples and there is danger of cross-contamination then an intermediate rinsing step with water can be included.

4. Dialysis

Dialysis is particularly well suited for inline sample preparation. Only the Metrohm inline version will be briefly treated here as dialysis is described in detail in the following section (Section D). The combination of dialysis cell, peristaltic pumps and/or Dosinos in an ion chromatograph allows this inline method to be carried out. The dialysis cell, which is shown in Fig. 5, is typically made of polymethylmethacrylate. The dialysis membrane has a standard diameter of 4.7 cm, so that different membranes can be used. The channels are arranged spirally. The sample flow is pumped in counterflow to the acceptor solution.

Figure 5: The Metrohm low volume dialysis cell

The flow diagram for inline sample preparation by dialysis is shown in Fig. 6. With the stopped-flow technique shown here a 100% dialysis rate is achieved as during dialysis (Fig. 6,: Dialysis left hand peristaltic pump is stopped) the acceptor circuit is closed, i.e. the acceptor solution is stationary on one side of the dialysis membrane while the sample solution flows past on the other side. This means that 100% of the analyte ions are always present on the sample solution side. The dialysis equilibrium is only achieved when the same concentrations are present on both sides of the membrane, i.e. 100% of the analyte ions. Only then is pumps are switched to the transfer position, the right-hand peristaltic pump is started while sample pump on the left-hand side is stopped. The acceptor solution is transferred into the sample loop. Then, as in all other, methods the ion chromatogram is recorded.

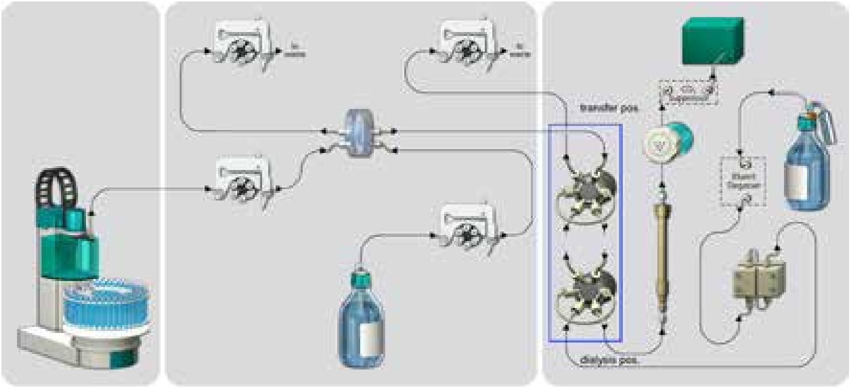

Figure 6: Flow diagram for inline dialysis (injector shown in dialysis (bottom) and transfer (top) position)

Fig. 7 shows an example of an ion chromatogram after inline dialysis. 0.5 g of a chocolate yoghurt was weighed in and homogenized in 50 mL of ultrapure water. The mixture was dialyzed prior to injection.

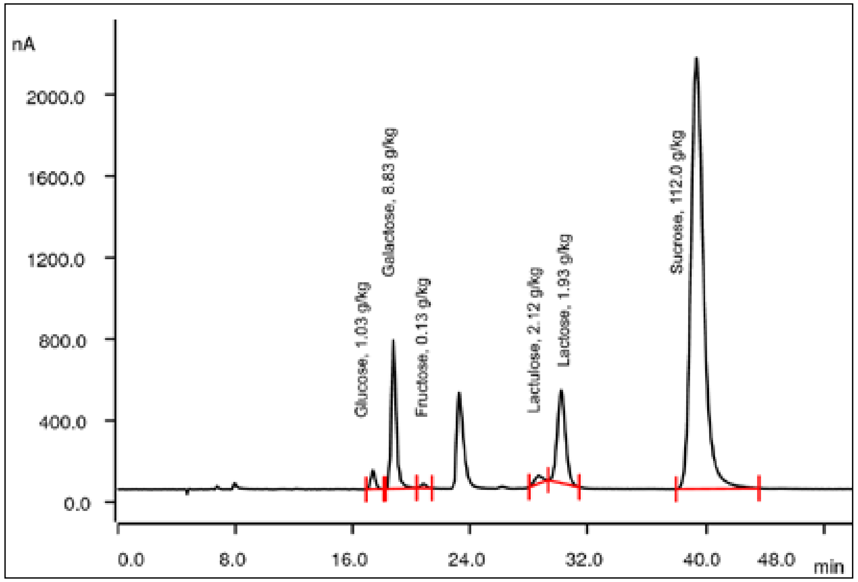

Figure 7: Ion chromatogram of a chocolate yoghurt sample after Inline Dialysis (detection mode is pulsed amperometric detection)

5. Ion exchange

Ion exchanger cartridges are frequently used for manual sample preparation in IC. This method is easy to integrate into an inline sample preparation procedure if the transfer method (see Fig. 8) is used. In this case the cartridge is always loaded with e.g. only 20 µL and rinsed additionally with water before and after the sample. If one considers that in a manual application normally at least 2 mL sample is rinsed through the cartridge then this corresponds to a capacity of 100 samples using the same cartridge in inline sample preparation. However, with this method care must be taken as some cartridges release ionic contaminants, which are then enriched on the preconcentration column.

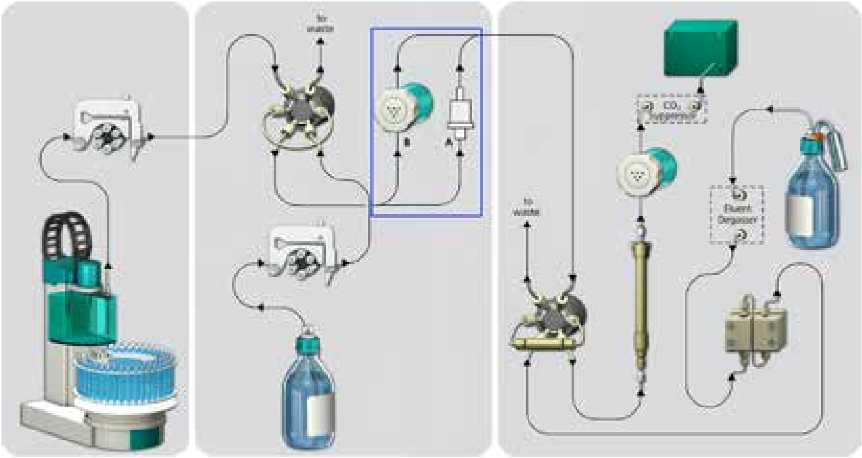

Figure 8: Arrangement for inline sample preparation with ion exchangers. A with the transfer of 20 µL sample and preconcentration column and C with the Sample Preparation Module

Even more convenient the ion exchanger cartridges is the use of the Sample Preparation Module (SPM). In this case, the regeneration of the ion exchanger including a rinsing step is carried out fully automatically within the system. As the rotors contain three chambers packed with an ion-exchanger material. One of these is always integrated into the analytical system while the second one is being regenerated and the third one rinsed with water. Before the next sample is injected the rotor moves on by one position and the sample then passes through a regenerated and rinsed chamber. In this way sample contamination by the previous sample is prevented and the same ion exchanger capacity is always available.

The flow diagrams for these different methods are shown in Fig. 8.

In the following two applications, the use of ion exchangers in sample preparation is explained:

a) Neutralization of bases with the SP module using 30% sodium hydroxide (see Figs. 9 and 10),

b) Neutralization of hydrochloric acid using an OH- cartridge (see Figs. 10 and 11)

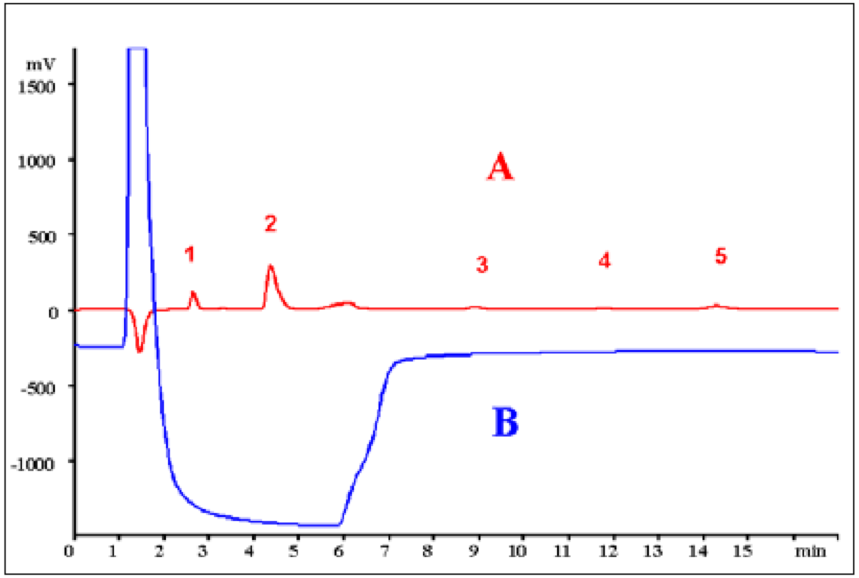

Figure 9: Chromatograms of 30% NaOH: A with and B without inline neutralization (sample volume 20 µL); 1 fluoride 0.74 mg/L, 2 chloride 3.6 mg/L, 3 nitrate 0.97 mg/L, 4 phosphate 0.98 mg/L, 5 sulfate 1.1 mg/L

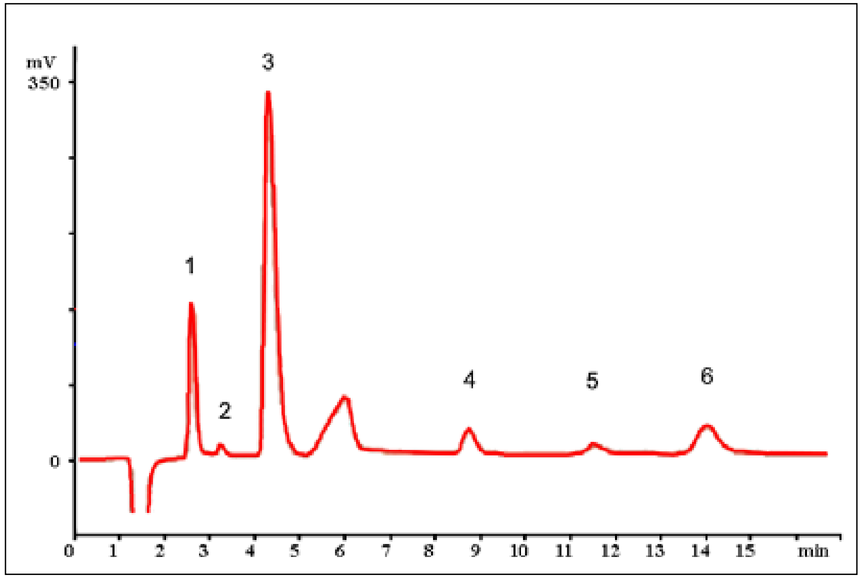

Figure 10: with Inline Neutralization chromatogram of 30% NaOH (sample volume 20 µL, Sample Preparation Module); 1 fluoride 0.74 mg/L, 2 formate <0.1 mg/L, 3 chloride 3.6 mg/L, 4 nitrate 0.97 mg/L, 5 phosphate 0.98 mg/L, 6 sulfate 1.1 mg/L

From Fig. 9, curve B, it can be clearly seen that the high concentration of OH- ions affects the baseline so that only the detection of nitrate can be expected. Nevertheless, even in this region the column equilibrium is so disturbed that interpretable values at higher concentrations can only be obtained for sulfate. In contrast, a completely «normal» ion chromatogram (see Fig. 9 A) is obtained by using the SP Module as in this case the sodium hydroxide is neutralized and the analyte ions are therefore present as the free acid in pure water. Fig. 10 shows the ion chromatogram of the 30% NaOH with the sensitivity increased by a factor of 10.

Instead of the SP Module, cartridges can also be used in the transfer mode. As these can only be loaded with 10 or 20 µL per sample and are then automatically rinsed with the transfer water, they only need to be replaced by a new cartridge, depending on their capacity, after numerous analyses have been carried out.

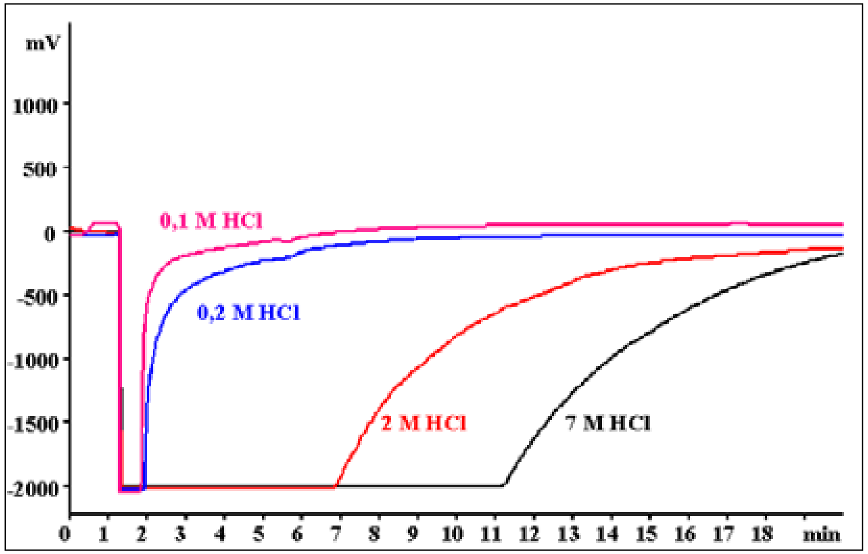

Figure 11: Ion chromatograms of HCl in a concentration range from 0.1 to 7 mol/L (sample volume 10 mL; direct injection without sample neutralization)

Fig. 11 shows the corresponding set of ion chromatograms of different concentrations of hydrochloric acid; these were recorded without inline neutralization. Even with 0.1 mol/L hydrochloric acid, the column equilibrium is so disturbed that no usable chromatogram is obtained.

In contrast, ideal chromatograms are obtained when inline sample neutralization is carried out with an OH- cartridge in the transfer mode, as can be seen in Fig. 12.

In this chromatogram numerous peaks can be observed that cannot be assigned to alkali metal or alkaline earth cations but correspond to a wide range of amines. From its retention time and shape, it is absolutely clear that the peak ahead of sodium does not correspond to the lithium ion.

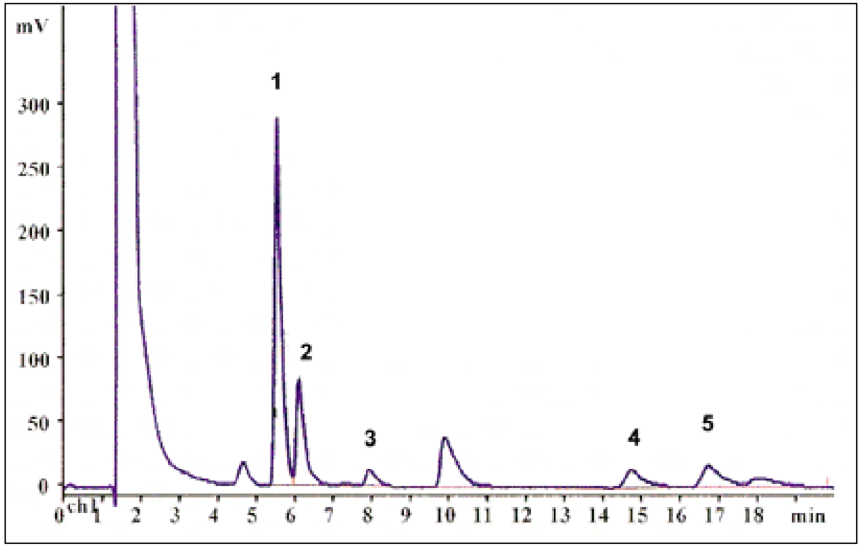

Figure 12: Ion chromatogram of 7 mol/l HCl with inline neutralization using an OH cartridge and the transfer technique (sample volume 10 µL); 1 Sodium 5.4 mg/L, 2 ammonium 1.3 mg/L, 3 potassium 0.8 mg/L, 4 magnesium 1.5 mg/L, 5 calcium 0.8 mg/L

6. Absorption reactions

Instead of ion exchanger cartridges, RP or active charcoal cartridges can also be used to separate interfering organic compounds. A typical field of application for this technique is the determination of anions or cations in

- Natural waters that are loaded, for example with humic acids
- Wastewater containing a high organic fraction
- Solutions of dyes

In all these cases, we are concerned with the removal of organic compounds that interfere with the separating column, whether by absorption in the filter, on the phase or by blocking the ion exchanger groups. In the absorption of very polar compounds further active centers may be formed in the column that change the properties of the column; however, in the author's experience a positive alteration has never been observed. In most cases these interferences can be recognized by peak broadening, fronting, tailing; sometimes even double peaks appear.

In all cases in which RP or active charcoal cartridges are used, care must be taken that these do not have a blank value or at least, if they do release ionic contaminants, that these do not interfere with the determination of the analyte ions.

7. Matrix elimination

Matix Elimination removes matrix components, which might disturb the ion chromatographic separation or may harm the column material from the sample prior to the injection.

A defined volume of sample solution is pumped through the preconcentration column. Only the analyte ions are retained on the column; the matrix is washed away. In the setup shown in Figure 13 the sample (e.g. 20 µL) is injected into a stream of transfer solution by use of an injector. The transfer stream loads the sample on to the preconcentration column and washes the matrix out.

Whenever traces of analyte ions have to be determined and/or variable sample volumes are required, Matrix Elimination and Preconcentration Technique can be combined.

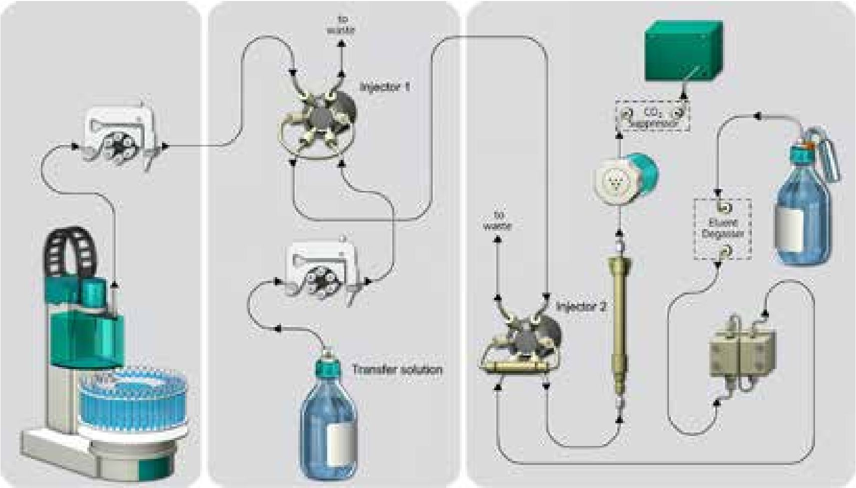

Figure 13: Flow chart for Matrix Elimination. The sample is loaded to the loop on Injector 1, injected in the transfer solution and transferred to the preconcentration column on Injector 2. Excess transfer solution washes off the matrix components prior to injection.

Typical examples of applications are the determinations of anions or cations

1. in 30% hydrogen peroxide (see Fig. 14), or
2. in organic solvents (see Figs. 15 and 16)

Hydrogen peroxide solutions of up to 50% can be analyzed directly in this way. This method has been used in the semiconductor industry for a long time. Even though interferences occurred - with the separating columns then available - in the sulfate determination (di- and tricarboxylic acids as well as an oxidation product of EDTA); this is not the case when high-performance columns such as the Metrosep A Supp 5 are used, as can be seen from Fig. 14.

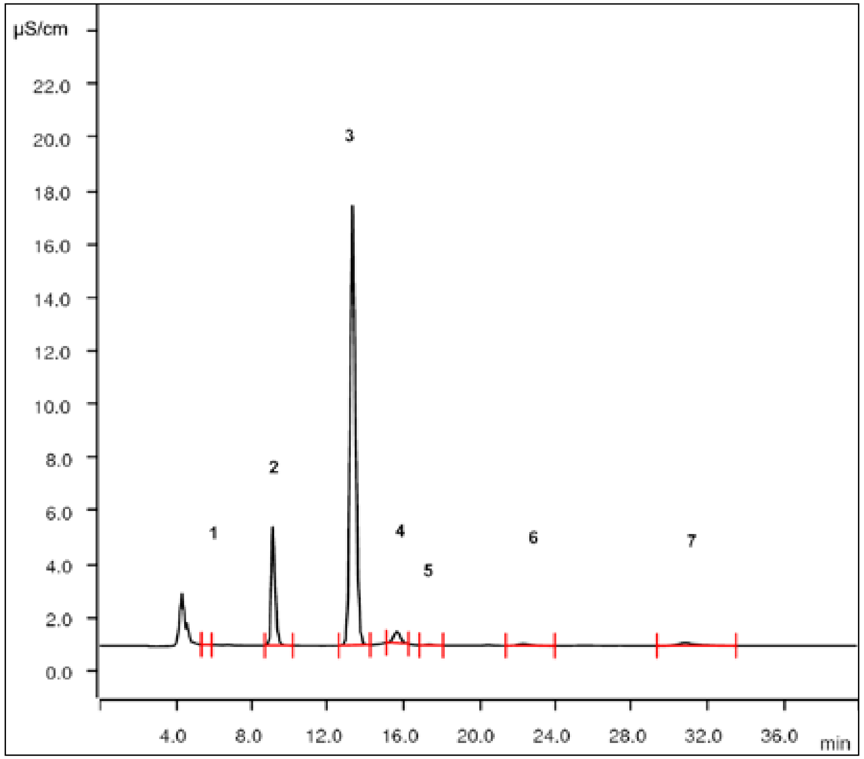

Figure 14: Ion chromatogram of a 70% hydrogen peroxide sample (1:5 dilution, 20 µL); chloride < 0.05 mg/L, nitrate 45 mg/L, phosphate 360 mg/L, sulfate 8.0 mg/L, oxalate 0.35 mg/L, 14 mg/L phthalate 6.2 mg/L, dipicolinate 4 mg/L

The determination of anions in organic solvents can also be carried out elegantly by the method according to Fig.12. In this case the ionic contaminants can be determined in polar solvents (acetone, methanol, ethanol), as only they are retained on the preconcentration column. Even aggressive solvents such as N,N-dimethylformamide can still be analyzed.

However, care must be taken that the capacity of the preconcentration column is sufficiently high, as otherwise there is the risk that some of the ions may pass through the preconcentration column owing to slow establishment of the equilibrium.

In the determination of traces of anions or cations in organic solvents the front peak interferes, which in this case is not caused by the water in the sample but by the solvent (see Fig. 15, Chromatograms of isopropanol with and without matrix elimination). This example shows clearly, that with this technique it is easily possible to carry out determinations in the lower ppb range with sample volumes of 50 µL. For larger injection volumes and flexible volume injection see Chapter 8, below.

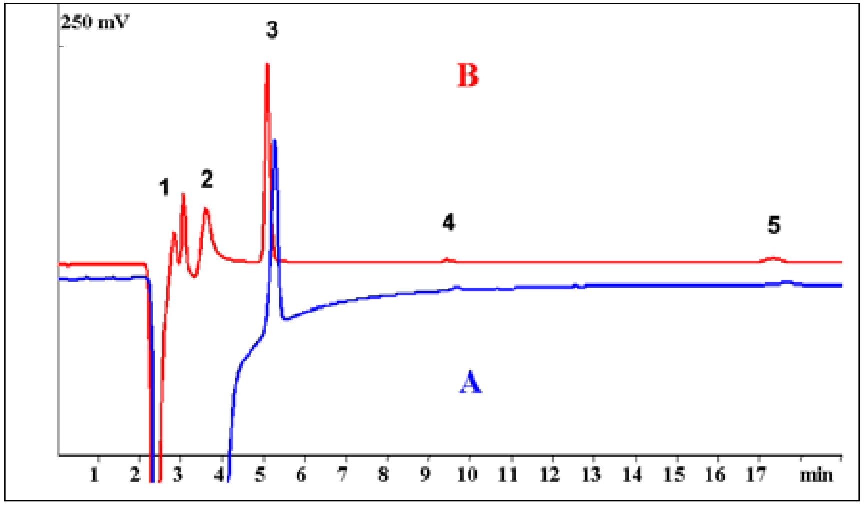

Figure 15: Ion chromatograms of an isopropanol sample; A without and B with matrix elimination (injection volume 50 µL); 1 fluoride and 2 acetate are not quantified, 3 chloride 443 µg/L, 4 nitrate 30 µg/L, sulfate 28 µg/L

8. Preconcentration

Sample preconcentration is necessary whenever the ionic analytes in very low concentration need to be determined.
In preconcentration, a typically higher volume of sample is loaded onto the preconcentration column on the sample valve. The Matrix Elimination setup (see Figure 7) may be used in combination with a large loop (e.g. 1000 µL).

Two flow charts of more flexible systems are shown in Figure 16 and Figure 17. These setups allow the preconcentration of varying volumes of sample from a few µL up to 2 and 4 mL respectively. The use of a Dosino for sample and ultrapure water handling yields in highly precise and accurate results. 2 mL preconcentration typically allows reaching single digit µg/L determinations.

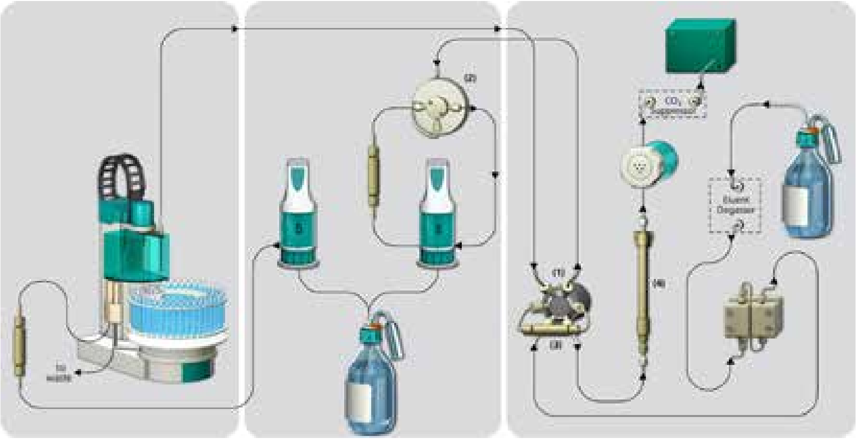

Figure 16: Flow chart for Inline Preconcentration

In this setup up the sample is pulled into the buffer tubing through the bypass of the sample valve. After switching to fill position, the Dosino pushes the exact volume onto the preconcentration column. After injection the sample path is rinsed with ultrapure water through the rinsing station. This setup allows to preconcentrate up to 2000 µL.

The setup shown in Figure 17 is slightly more complex. It is additionally using the rinsing station for rinsing as well as for a source of ultrapure water. This setup allows to combine Inline Precocentration with Inline Matrix Elimination.

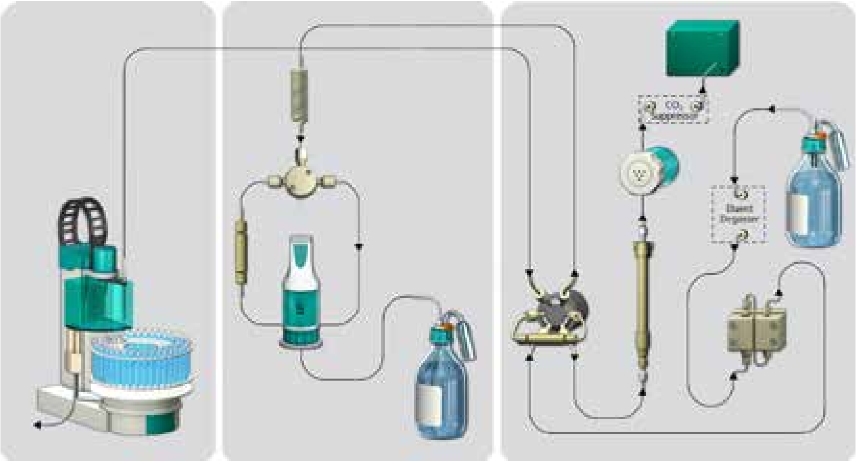

Figure 17: Flow chart for Inline Preconcentration with Inline Matrix Elimination.

The exact volume for preconcentration is pulled from the sample vial. The sample needle moves the ultrapure water supply (rinsing station) and the sample volume then is moved through the bypass of the sample valve (1) into the buffer tubing (2). The sample valve is switched to in fill position and the sample volume is loaded onto the preconcentration column (3). Flushing with additional ultrapure adds Matrix Elimination to preconcentration. This setup is mainly applied in power stations.

Please note that a total preconcentration of the ions depends on the total ion concentration of the sample. It needs to be low enough to have enough capacity on the preconcentration column. In higher concentration samples, the ions with high retention start eluting those with lower retention. This might lead to loss of analytes.

9. Cutting techniques

Cutting techniques are required when very small quantities of analytes besides high concentrations of one or more matrix ions have to be analyzed. Typical examples are concentrated acids. Two configurations may be applied.

1. Two-dimensional separation

In this cutting technique, the sample is preseparated on a first IC system. The injector of the second system is switched to the fill position when the ions of interest are eluted. This retains the ions of interest on the preconcentration column. On system 2 the target ions are now separated from the remaining (low amount) of the matrix ions (see Figure 18).

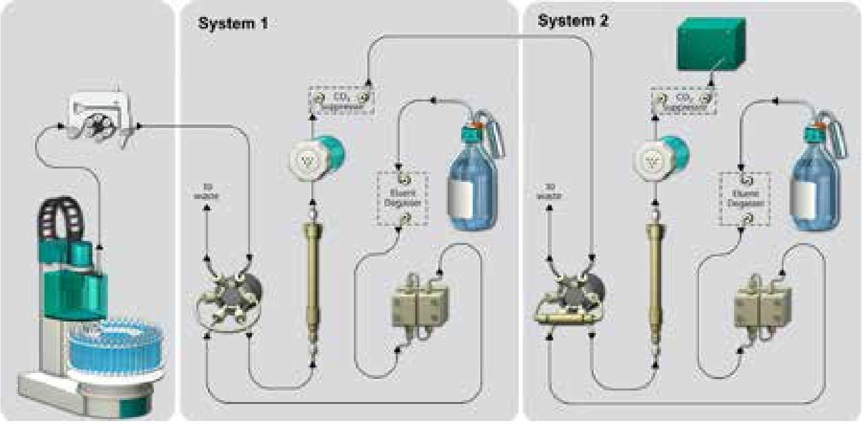

Figure 18: Flow chart of a two dimensional IC system.

Figure 19 shows an overlay of 10 chromatograms on system 1 applying a conductivity detector (optional, not shown in Figure 18). On the time axis, the time slot is marked. The final chromatogram of system 2 is shown in the foreground.

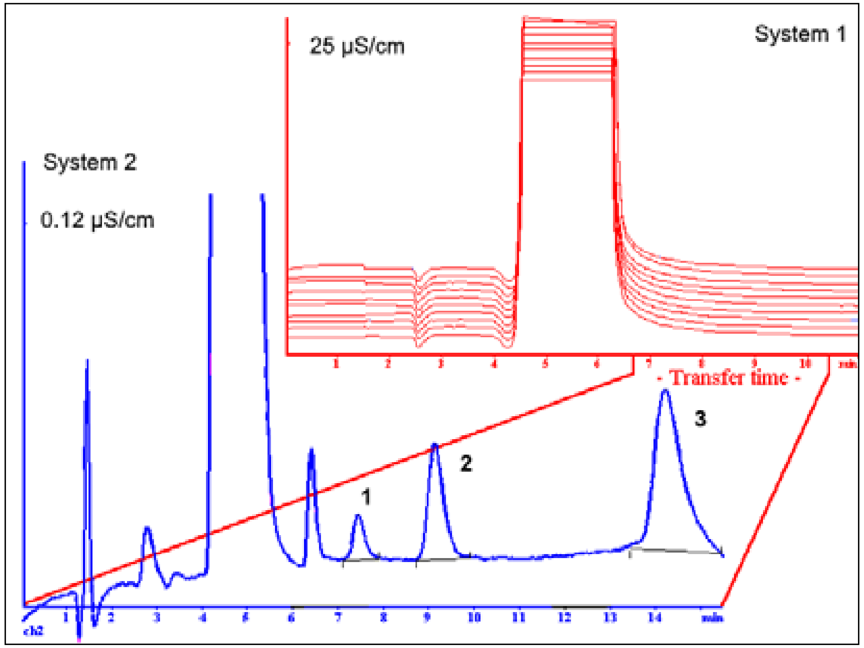

Figure 19: Ion chromatograms of the determination of trace anions in HCl applying two dimensional IC; 1 bromide 0.5 mg/L, 2 nitrate 1.2 mg/L, and 3 sulfate 3 mg/L.

b) Reinjection Analysis:

If the analyt is eluting at the end of the chromatogram, a reduced system setup may be applied. Instead of two combined IC systems only one additional valve is required. Figure 20 shows the respective flow chart.

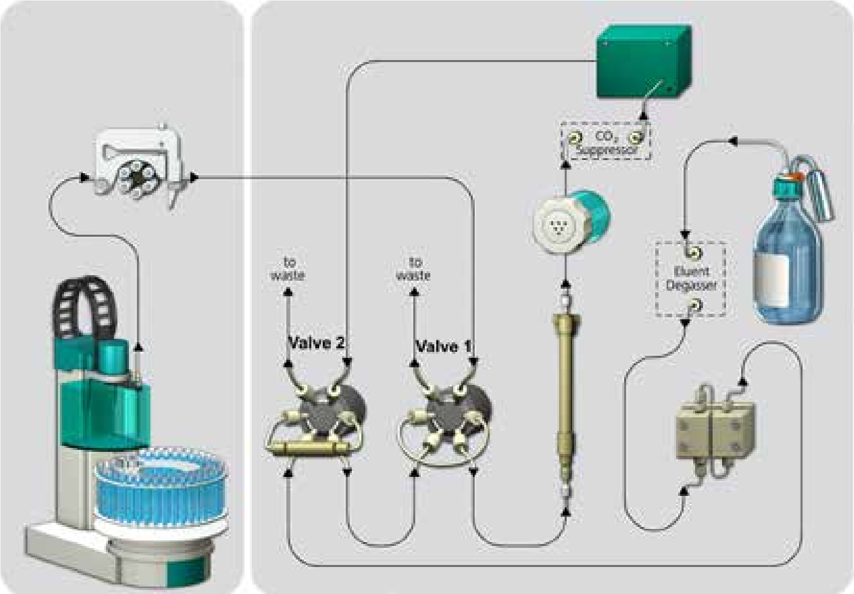

Figure 20: Flow chart for reinjection analysis

The sample is loaded to the loop of valve 1 and injected for the first separation. Valve 2 is in the "load" position. Therefore, the effluent from the conductivity detector is guided directly to waste. As soon as the matrix ions are eluted to waste and the analyt ion is eluted, valve 2 is switched to "fill" position. The detector effluent with the analyte ions now is guided on to the preconcentration column. The analyte ions are retained on the preconcentration column. Subsequently they are reinjected to the same analytical column for the second separation.

A typical application for the reinjection method is the determination of perchlorate in e.g. drinking water with a high anion content. A respective standard (ISO 19340) required the determination of 1 µg/L perchlorate besides 100 g/L chloride, hydrogen carbonate and sulfate each. The Metrohm Application Note AN-S-342 shows this application. Figure 18 shows the chromatogram of a water sample spiked with 1.0 µg/L perchlorate. Also shown is the chromatogram of the first injection as well as the trace of the unspiked water sample.

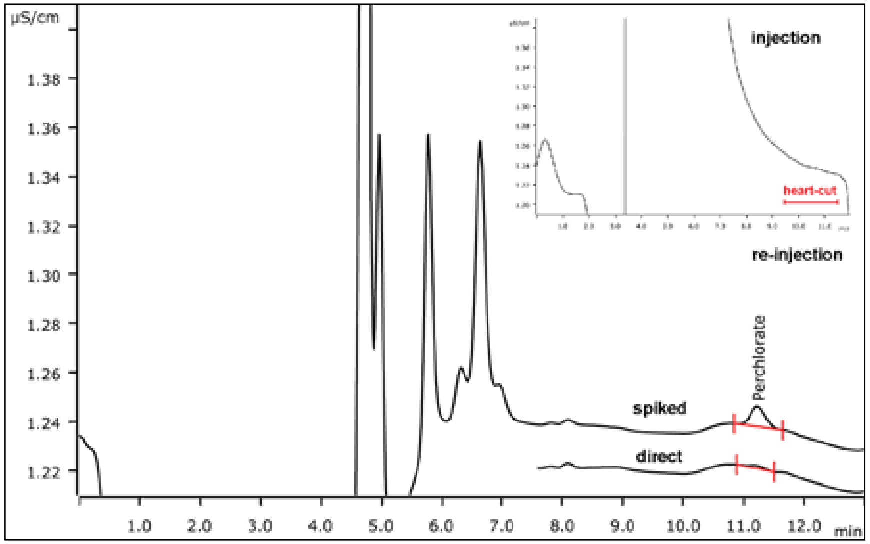

Figure 21: Chromatogram of a water sample achieved with re-injection. The spiked peak corresponds to 1.04 µg/L. The peak of the unspiked sample is below the calibration range, estimated < 0.1 µg/L.

10. Automatic calibration and injection techniques

a) Automatic calibration out of one standard solution

Automatic calibration out of one standard solution reduces the lab workload, as no manual dilutions need to be done. It also improves the quality of the calibration due to a very high reproducibility an accuracy. Especially for trace analysis it is often the only way to get suitable calibrations, as manual handling of low concentration standard solutions is very tricky and causes many problems. If standard solution in the low µg/l range (1...10 µg/L) are used, then they should be made up freshly each day. Despite taking the greatest care, the carried-over blank values, chloride in particular, can be recognized on the calibration curves as an axis intercept. If, however, calibration in the ng/L (ppt) range is to be carried out, manual standard preparation cannot be used any longer.

b) Inline dilution

For higher level concentration (µg/L and higher) Inline Dilution may be performed. Inline Dilution just automates this procedure applying a Dosino for liquid handling. Figure 22 shows the flow path of the automated method. A Dosino pulls the required volume of sample into a buffer tubing. This volume is then dispensed into the dilution vessel of the liquid handling station. The Dosino then dispenses the volume of diluent, the solution is mixed and subsequently injected to the IC.

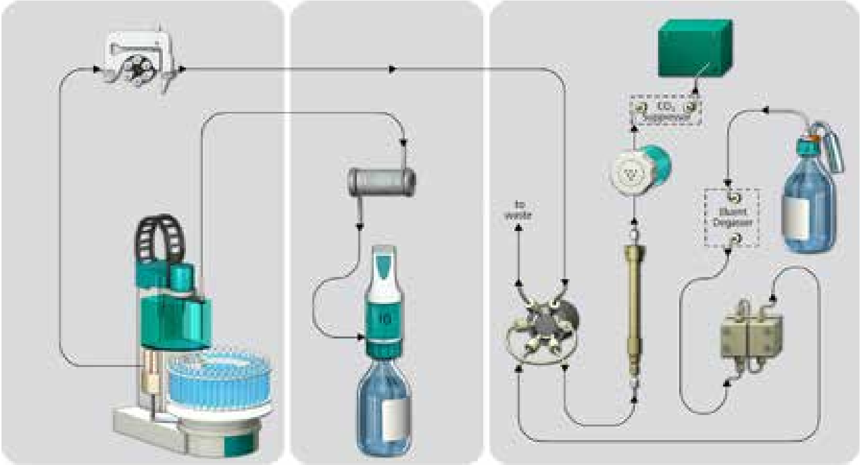

Figure 22: Flow chart for Inline Dilution

In case of unknown dilution factors, it often happens that samples have to be rediluted due to a result laying out of the calibration range. This requires additional work and rerunning the samples. To avoid this extra work MagIC net offers the additional feature of «logical dilution». MagIC Net runs an initial sample (diluted by the defined value). After the run MagIC Net checks whether all results lay within the calibration range. If one concentration is out of range, MagIC Net calculates a new dilution factor and injects that dilution again. This avoids samples to be rediluted and reinjected after a sample series. At the end of sample series, the final results are displayed from the analysis of the optimal dilution.

Traditional calibration means manual dilution of a standard stock solution to the respective calibration levels. This procedure is prone to artefacts. The automatic calibration with Inline Dilution just automates this procedure applying a Dosino for liquid handling. This automation step avoids artefacts and generates very precise and reproducible calibrations.

c) Inline Preconcentration and Matrix elimination

Automatic calibration from one single standard solution is applied as well to Inline Preconcentration techniques (Figure 16 and 17). With the preconcentration of different volumes of a standard stock solution, a calibration is performed. For this purpose, preconcentration volumes starting with 4 µL up to 4000 µL respectively can be used. This yields in maximum calibration range of three orders of magnitude. Figure 23 shows the result of a calibration of trace anion out of 10 µg/L multi-ion standard solution. The calibration range is set to 0.1 µg/L to 10 µg/L corresponding to preconcentration volumes from 40 µL...4000 µL.

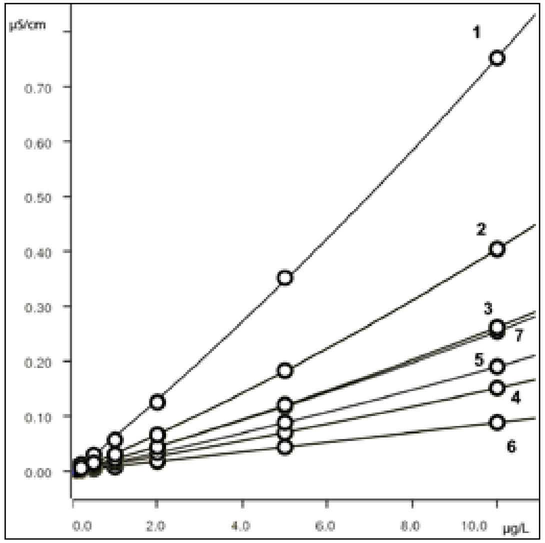

Figure 23: Calibration curves for a 0.1 to 10.0 µg/L range out of one standard solution (1 fluoride, 2 chloride, 3 nitrite, 4 bromide, 5 nitrate, 6 phosphate, 7 sulfate)

The calibration curves, especially for suppressed anion chromatography, are typically not fully linear (as it can be seen in Figure 23) it is recommended to split the full range into two or more sections. Each section is calibrated separately and the highest calibration level of the first section is the lowest calibration level of the next section. MagIC Net then decides for every result which calibration range to be applied. This can be applied for any large range calibration independent from the Injection technique.

The working range of such a calibration is doubled the calibration range. In extremis, this would allow a working range of 6 orders of magnitude with a calibration from 4 µL...4000 µL (see below).

Example: calibration with a 10 µg/L standard stock solution

Volume loaded	Corresponding Concentration	Injected amount
4000 µL	10 µg/L	**40.0 ng**
2000 µL	5.0 µg/L	**20.0 ng**
800 µL	2.0 µg/L	**8.0 ng**
400 µL	1.0 µg/L	**4.0 ng**
200 µL	0.5 µg/L	**2.0 ng**
80 µL	0.2 µg/L	**0.8 ng**
40 µL	0.1 µg/L	**0.4 ng**
20 µL	0.05 µg/L	**0.2 ng**
8 µL	0.02 µg/L	**0.08 ng**
4 µL	0.01 µg/L	**0.04 ng**

Calibration range is 40 pg...40 ng corresponding to **0.01 µg/L...10 µg/L** (3 orders of magnitude).
The lowest concentration fitting the calibration range correspond to the lowest calibration level and is 0.01 µg/L (0.04 ng injected).

The highest concentration fitting the calibration range is highest injected amount in the lowest applicable volume. This is 40 ng in 4 µL and corresponds to 10’000 µg/L or 10 mg/L.
The working range therefore is **10 ng/L...10 mg/L** (6 orders of Magnitude).

Such a large working range is not applicable as cross contamination would be very critical. However, lower working ranges with the respective lower calibration ranges are often applied.

d) Partial-loop Injection Technique

Full-loop injection is the mostly used injection technique in ion chromatography. It is easy and extremely reproducible. The absolute value of the loop volume is not important as the system is always calibrated injecting the identical volume.

Partial-loop Injection requires a very high precision and accuracy of the dosing device. The quality of the dosing devices influences the quality of the results directly. With the Dosino the required accuracy and reproducibility is given to reach perfect results. Figure 24 shows the flow chart of a Partial-loop Injection setup.

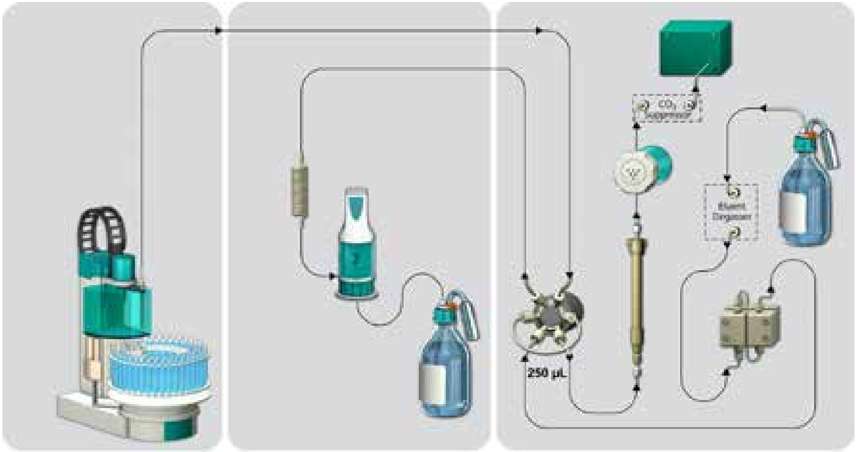

Figure 24: Flow chart for "Partial-loop" Injection

In Partial-loop Injection, a large loop (typical 250 µL) is partially filled with sample solution and subsequently injected to the IC. For the calculation of the final concentration, the injected volume is taken into account.

The Dosino pulls the sample solution (or standard solution) from the vial through the bypass of the sample valve into the buffer tubing. After switching the sample valve, the Dosino doses the exact sample volume into the loop. In case of the loop with 250 µL volume, the injected sample volume can be between 4 and 200 µL. Figure 25 shows the different steps of partial-loop injection.

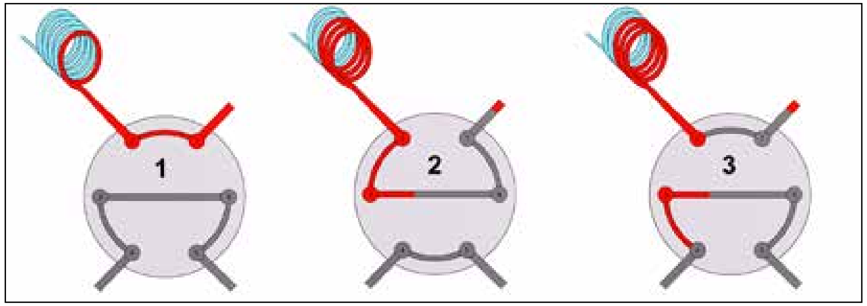

Figure 25: Partial-loop Injection; 1: sample transfer to the buffer tubing, 2: switch to «Fill» and loading the loop with the exact sample volume, 3: inject the sample volume for separation

Automatic calibration is achieved by injecting different volumes of the standard stock solution. As in preconcentration, also here higher concentrated samples may be injected with low volumes to enlarge the working range of a calibration.

Example: calibration with a 10 µg/L standard stock solution

Volume loaded	Corresponding Concentration	Injected amount
200 µL	10 µg/L	**2.0 ng**
80 µL	4.0 µg/L	**0.8 ng**
40 µL	2.0 µg/L	**0.4 ng**
20 µL	1.0 µg/L	**0.2 ng**
8 µL	0.4 µg/L	**0.08 ng**
4 µL	0.2 µg/L	**0.04 ng**

Calibration range is 40 pg...2 ng corresponding to **0.2 µg/L...10 µg/L** (1 : 50).

The lowest concentration fitting the calibration range correspond to the lowest calibration level and is 0.2 µg/L (0.04 ng injected).

The highest concentration fitting the calibration range is highest injected amount in the lowest applicable volume. This is 2 ng in 4 µL and corresponds to 0.5 mg/L.

The working range therefore is **0.2 µg/L...0.5 mg/L** (1 : 250).

This working range typically can be used without any problems of cross contamination.

e) "Pick-up" injection technique:

Full-loop and even partial-loop injection require a relatively large sample volume of a few hundreds of µL. It is required to flush the capillaries between sample and injector or buffer tubing.

If very low sample volumes are available, the "pick-up" injection is the method of choice. Figure 26 shows the flow chart for this method.

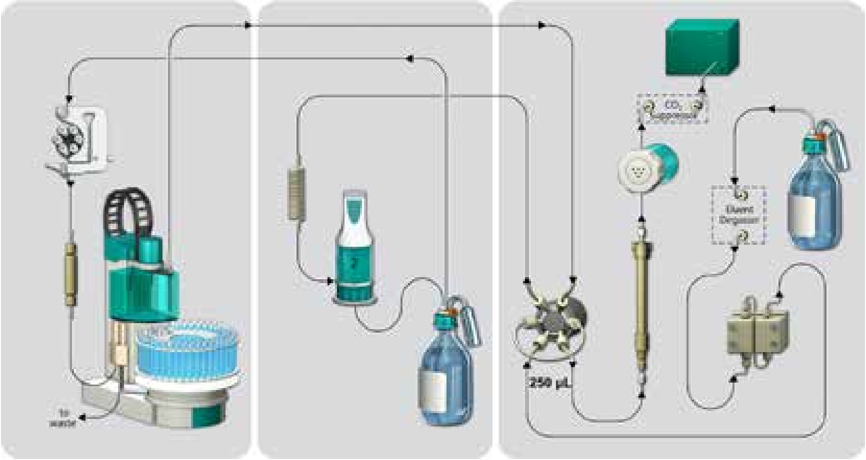

Figure 26: Flow chart for "Pick-up" injection

Similar to "partial-loop", the "pick-up" injection mode uses a 250 µL loop. Additionally a ultrapure water source is required on the liquid handling station. The required sample volume (4 to 60 µL) is pulled out of the vial by the Dosino. The sample needle then moves to the ultrapure water source on the liquid handling station. Now the Dosino pull the sample volume into the sample loop for injection. Also in this setup a variation of injected volumes creates a calibration.

d) Automatic spiking

In a preconcentration system (Matrix Elimination like setup) the standard solution is loaded on to a preconcentration column using an additional valve in a. The flow chart is shown in Figure 27. The concentrated standard solution (e.g. 0.1 mg/L) is injected into a ultrapure water flow via a 10 µL loop (valve 2) and transferred to the preconcentration column with 10 mL ultrapure water. As 10 µL 0.1 mg/L standard solution in 10 mL of water corresponds to a dilution, the analyte ion content corresponds to 100 ng/L.

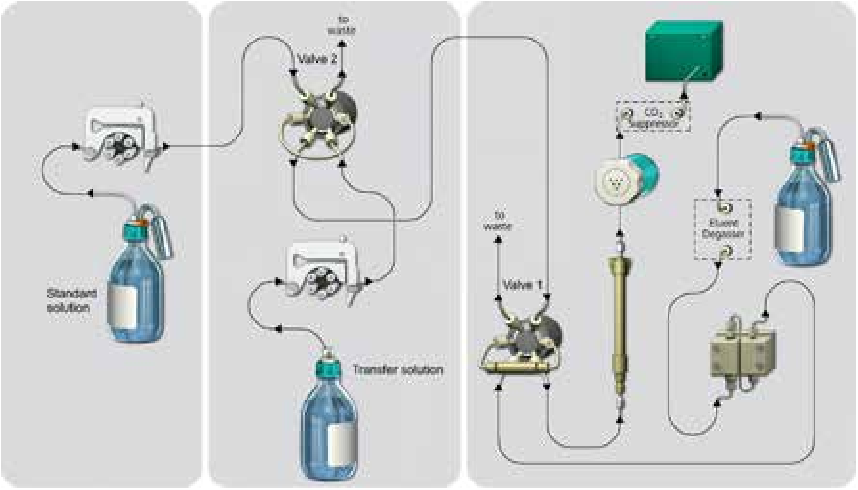

Figure 27: Flow chart for automatic calibration with multiple standard loading

By repeatedly switching valve 2 during the preconcentration cycle a wide range of concentrations can be prepared on the preconcentration column. This means that automatic multi-point calibration in the ng/L range can be carried out fully automatically out of one standard solution.

For Inline Spiking the same setup is applied, but now the standards solution is injected into the sample stream during the preconcentration cycle. Applying the example above, injecting 10 µL of a 0.1 mg/L standard solution into 10 mL of sample solution results in a spike concentration of 100 ng/L.

11. Standard addition

As in all analytical methods in which calibration curves are used to determine the analyte content in the sample, in complicated matrices the analytical results may be falsified by the matrix itself, as in most cases calibration must be carried out with pure standard solutions because no certified standards of the particular matrix exist.

In this case, the effect of the matrix can only be corrected by the standard addition technique. In normal cases, this procedure is quite complicated as, after the measurement itself, the sample must then be spiked with different amounts of the standard and reanalyzed each time. These solutions must all be made up individually and, in our case, analyzed chromatographically.

Such standard additions can be carried out any arrangement described in the previous sections; however, care must be taken that the preconcentration column is not overloaded, i.e. a high-capacity preconcentration column must be used and the matrix must not be strongly ionic.

Also very clean matrices require this standard addition technique, e.g., when the ultrapure water that is to be analyzed has to be used for calibration. In this case the calibration can be regarded as being a determination in which the analyte ions in this sample are determined by the standard addition technique. The determination of the analyte concentrations by this technique in such an ultrapure water sample from a power plant is shown in Figure 28.

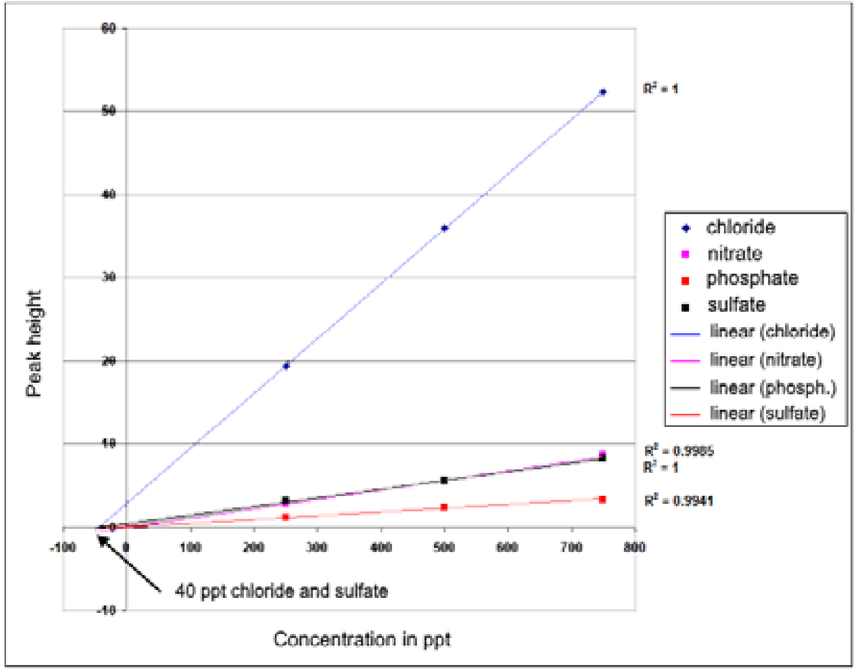

Figure 28: Results of a determination of anions in ultrapure water using the standard addition technique: chloride 40 ppt, nitrate < 20 ppt, phosphate < 40 ppt and sulfate 40 ppt

A 250 ppb standard solution and a 10 mL preconcentration column were used. Regression lines were used to determine the chloride and sulfate content of 40 ppt each. For phosphate and nitrate the regression lines pass through the origin.

12. Conclusion

As can be seen from the examples described above, the analyst can considerably improve the automation of the analytical procedure by using inline sample preparation. The basic principle of only passing as much sample as is absolutely necessary through the sample preparation module by using a suitable transfer solution can be used for most sample preparation problems. If a suit- able solution for preparing the sample for IC has been found by manual work in the laboratory, then the conversion of this solution to the inline sample preparation technique requires little additional work.

D) Dialysis as an automated sample preparation technique for ion chromatography

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Contents

1. Introduction

In many cases the analysis of samples with a complex composition by ion chromatography requires extensive sample preparation (see Section A). The steps associated with this are usually time-consuming and increase the risk of incorrect analyses owing to contamination or loss of analyte. This is why, particular attention is increasingly being given to sample preparation methods that can be combined directly (inline) with a chromatographic separation method in order to make the complete automation of the complete analytical procedure possible (Section C).

Up to now continuous flow-through dialysis as an inline sample preparation technique has only found comparatively little use within the context of IC, even though it offers a range of very attractive properties (not only) for chromatographic separating methods; these will be explained in this contribution.

2. Types of dialysis

The term «dialysis» covers separating methods that are based on the transport of molecules or ions through a semipermeable membrane. A differentiation is made between different types of dialysis (passive dialysis, Donnan dialysis and electrodialysis) according to the driving force and the type of separation membrane that is used. In an extended sense, both ultrafiltration and reverse osmosis can also be regarded as being among the dialytic separation methods (Section A).

In passive dialysis, the driving force is the concentration gradient of molecules or ions across the membrane. The membrane itself is not reactive and has basically the function of a molecular sieve whose pore size determines the selectivity of the separation. Donnan dialysis is based on the reciprocal transfer of ions through an ion exchange membrane between the ions of the sample solution and ions that are available on the other side of the membrane. Depending on the charge of the ions to be separated, anion or cation exchange membranes can be used. In this case the driving force is the simultaneously occurring attempt to achieve a concentration and charge equilibrium. In electrodialysis the driving force for the transfer of ions through the membrane is increased by a difference in electrical potential. In this case both inert (non- reactive) and ion exchange mem- branes can be used.

In contrast to ultrafiltration and reverse osmosis, one thing that the dialysis techniques mentioned above have in common is that the transfer of molecules or ions takes places by diffusion or migration and that the solvent itself is not transported through the membrane. This is of extraordinary importance as regards both continuous operation as well as the risk of altering the membrane properties by contact with the sample solution and its associated adsorption of matrix components.

3. Historical development of dialysis

A short historical review of the development of dialysis is given in Fig. 1. Dialysis in the form of passive dialysis was first described in 1861 by Graham [1]. It was only many years later that this method first found a practical application in the hemodialysis sector (artificial kidney) and, as a result of the enormous progress made in the membrane manufacturing sector, this has today developed into the most important application field for dialysis by a huge margin. In the industrial sector membrane separating methods, also including dialysis methods are used to an increasing extent for the purification of substances as well as for the separation of particular components from process flows.

The use of dialysis in analytical chemistry has, up to now, been chiefly in the field of automated flow analysis in the form of gas-bubble-segmented continuous flow analysis (CFA) and flow injection analysis (FIA) [2]. In addition, passive dialysis has also been used for desalting protein solutions as a step in post-chromatographic treatment. Dialysis was first mentioned as a sample preparation technique within the context of a chromatographic separation method in 1985. Since then this coupling technique has been the subject of a continuous (if slow) growth in interest, with the majority of the applications mentioned in published works referring to the separation of organic compounds by passive dialysis and their chromatographic separation by HPLC [3]. The combination of dialysis with ion chromatography was first published by Nordmeyer and Hansen [4]; however, only the development work by Frenzel et al. [5, 6] and its commercialization by Metrohm has established it as a very versatile, efficient, user-friendly and automatable sample preparation technique.

1861	**Graham**	**First attempt at dialysis with animal membranes**
1914	**Abel, Rowntree, Turner**	**First report on hemodialysis**
1944	**Kolff, Berk**	**Use of dialysis as artificial kidney**
1950	**Lonsdale**	**First commercially available dialysis membrane**
1957	**Skeggs**	**Analytical dialysis in combination with air-segmented flow analysis method (CFA)**
1965	**Kadish, Hall**	**Continuous dialysis without air segmentation**
1976	**Ruzicka, Hansen**	**Dialysis in combination with flow injection analysis (FIA)**
1982	**Nordmeyer, Hansen**	**First work on dialysis as a sample preparation method for ion chromatography**
1985	**Turnell, Cooper**	**Automation of the dialysis-chromatography coupling**
1992	**Frenzel**	**Work on dialysis as an online sample preparation technique for IC**
1997	**Metrohm**	**Commercial introduction of the dialysis-IC coupling**
2019	**Metrohm**	**Introduction of a small-volume dialysis cell to improve performance and lower required sample volume**

- **More than 200 publications on the subject of online dialysis for analytical chemistry problems**
- **More than 50 publications on Inline Dialysis combined with ion chromatography**
- **About 50 Metrohm Application Notes on Inline Dialysis combined with ion chromatography**

Figure 1: Historical review of the development of dialysis

4. Passive dialysis as a separation technique

In the further course of this article only the possible uses of passive dialysis will be discussed, as this method is particularly easy to carry out from a procedural point of view and requires comparatively little expenditure on apparatus. Passive dialysis is based on the selective transfer of molecules or ions from one liquid (donor or sample solution) to a second one (acceptor or receiver solution) via a semipermeable homogeneous membrane. The driving force for the transfer of substances is the concentration gradient across the membrane, so that the dialysis process is concluded when a concentration equilibrium in the two liquid phases has been achieved. The selectivity of the separation is determined by the membrane properties as well as to a certain extent by the kinetics of the transport of the different substances through the membrane. The substances are separated according to their size (which is related to their molecular weight) and mobility. Under ideal conditions, there is no interaction between the components of the liquid phase and the membrane, i.e. the membrane acts as a molecular sieve. Apart from dissolved substances with a high molecular weight, particles and colloidal fractions of the sample are also retained by the membrane and led off in the retentate flow.

5. Dialysis procedure and dialysis modules

In principle dialysis can be carried out in batch operation or in a continuous flow system. The batch technique is very simple as regards the apparatus required and can be carried out with dialysis tubes or bags, for example (Fig. 2). However, it requires not only comparatively large volumes of sample solution but also a very long time (usually several hours) before a concentration equilibrium is reached between the sample solution and the acceptor solution. In addition, the sample solution undergoes considerable dilution. The separation time and the sample volume required can be considerably reduced by miniaturizing the liquid chambers. However, all batch techniques require numerous manually performed steps and are not amenable to automation.

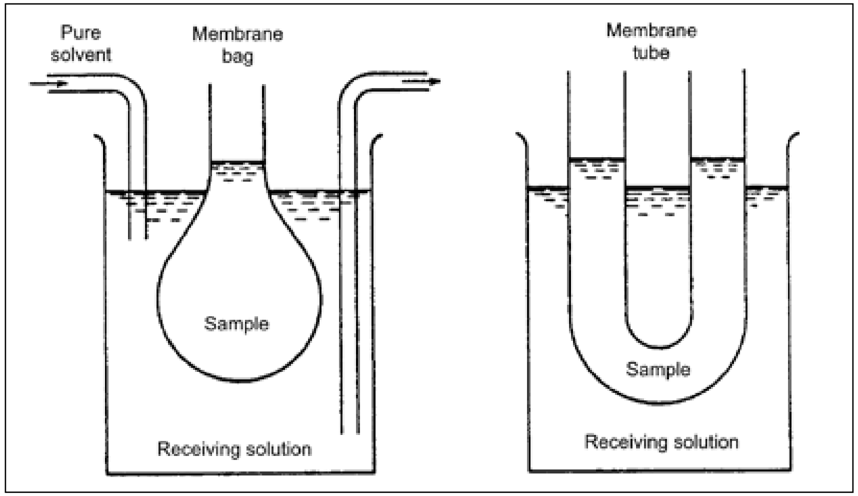

Figure 2: Basic types of laboratory dialysis cells for batch operation

For continuous flow dialysis, special modules with planar or tubular shapes are used (Fig. 3). The planar (sandwich) configuration with a flat membrane is preferred for analytical work, as a great variety of different membranes are available and, if necessary, the membrane can be easily exchanged by the user. The two half-blocks of the planar modules are usually made of chemically resistant plastics and generally have milled channels that are mirror images of each other and through which the sample and acceptor solutions are passed.

Planar («sandwich») configuration with flat membrane

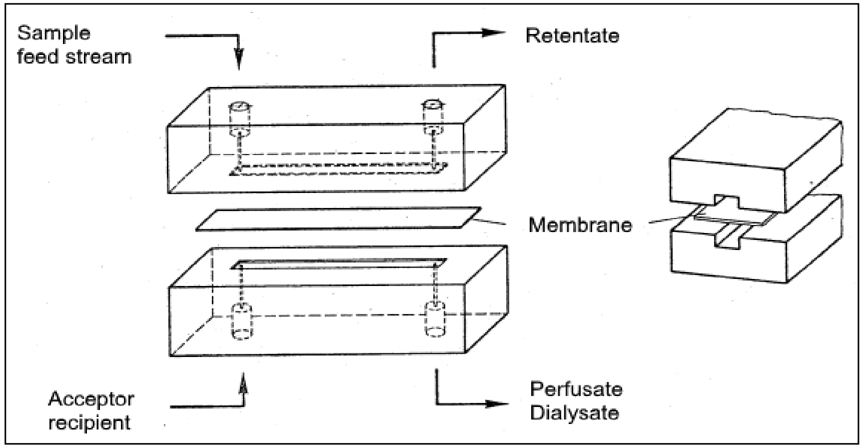

Figure 3a

Tubular cell with hollow fiber membrane

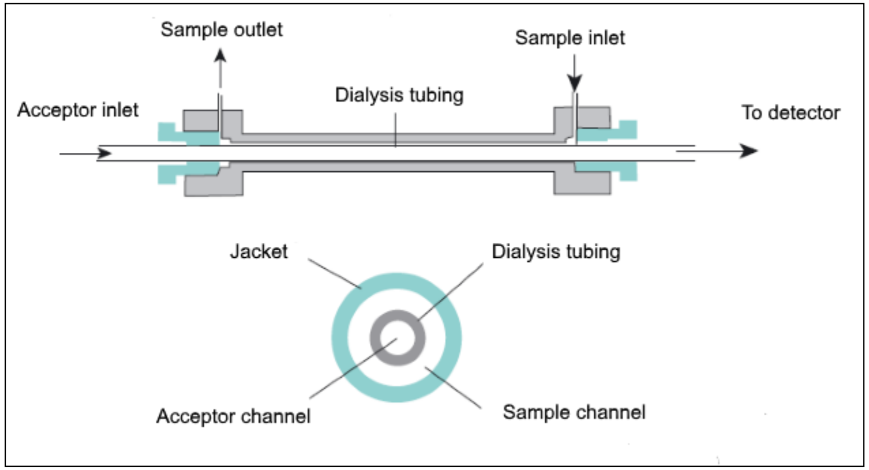

Figure 3b: Dialyzer arrangements for analytical applications

The arrangements and dimensions of the channels given in the literature and those that are commercially available differ (Fig. 4).

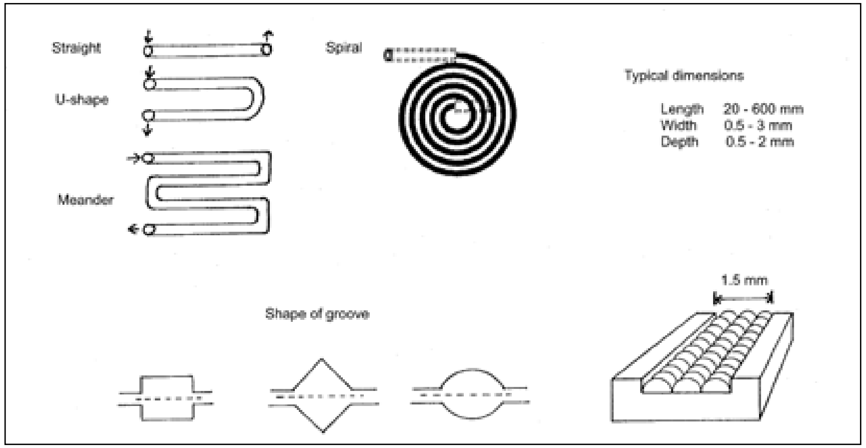

Figure 4: Arrangements and dimensions of flow channels in sandwich modules

Figure 5 shows Metrohm's dialysis cell (patent pending for the "low-volume" dialysis cell), an example of a cell with spiral channels. The typical dimensions of the channels of the various sandwich dialyzers used in practice are: length 20...600 mm, width 0.5...3 mm and depth 0.1...0.5 mm. The two blocks are pressed together by screws or clamps. The dialysis membrane separating the two blocks also acts as a seal. Tube modules with hollow fiber membranes have a larger membrane area to liquid volume ratio; this results in an increased transfer rate and therefore reduced dialysis times. However, their construction is more complicated and usually this type of module is offered as a molded disposable unit that is fairly expensive.

Figure 5: Metrohm spiral-flow low-volume dialysis cell

6. Principles of continuous flow-through dialysis and its coupling to IC

Continuous flow-through dialysis can be directly coupled to IC in a very simple way. As shown in Fig. 6 the acceptor flow outlet is simply connected to the injection valve of the IC instrument. The only requirement is that the counterpressure that is created by the solvent flow through the sample loop is not too high, as otherwise a pressure gradient would be formed within the dialysis module that would have a uncontrollable influence on the substance transfer or, in the worst case, could damage the dialysis membrane.

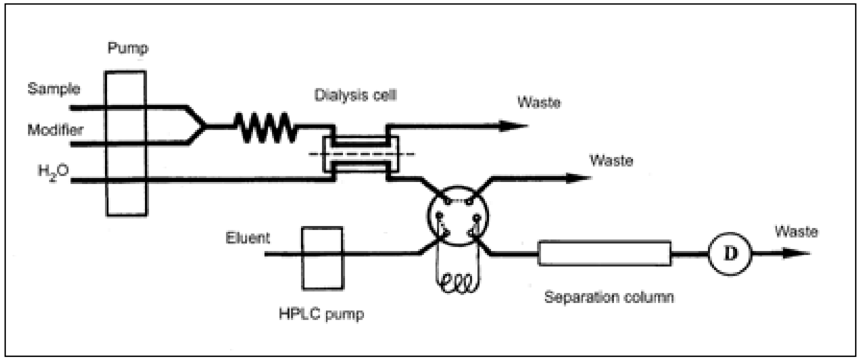

Figure 6: Schematic diagram showing the coupling of inline dialysis to IC

In principle, flow-through dialysis can be carried out in various ways depending on the intended purpose. In general, a differentiation is made between depletion, dynamic and equilibrium dialysis; the differences are explained below.

Depletion dialysis

In this case, a small volume (50–500 µL) of the sample solution is usually transferred to the donor channel of the dialysis module and kept there until the analytes to be separated have been completely removed in the constantly flowing acceptor solution. This results in an extremely high dilution of the analyte in the acceptor solution, so that it then usually needs to be enriched again by preconcentration column (which is placed on the injection valve of the IC instrument) [7]. The time required for complete preconcentration depends on the shape of the donor channel, the membrane properties and the type of analyte and is typically 10–30 min. If the dialysis and preconcentration parameters have been optimized then in principle a quantitative determination can be carried out using a single chromatographic calibration. However, in practice the dialysis process is also usually calibrated by treating the standard solution as a sample or by using an internal standard as a check.

Dynamic dialysis

In dynamic dialysis the sample and acceptor solution are passed continuously through the dialysis module. Under defined experimental conditions, the transfer rate for a particular analyte is constant but the transfer is not quantitative. The resulting concentration in the acceptor solution is therefore lower by a certain factor than that in the sample solution. This means that the analyte is diluted to an extent that depends on the experimental conditions (dimensions of the channels, absolute flow rates, ratio of the flow rates between donor solution and acceptor solution, membrane properties).

As a result of the different permeation speeds of the different substances, the ratio of the analyte concentrations in the acceptor solution differs from that in the original sample. This is the reason why it is essential that the calibration also include the separation process and be carried out under identical conditions to those used for the subsequent analysis of the samples. The limitation for particular applications is the fact that the permeation speed strongly depends on matrix and analyte concentration. A practical usage is possible for very similar samples.

Equilibrium dialysis

Equilibrium dialysis consists of the mass transport of the dialyzable components until an equilibrium concentration has been achieved. This can be carried out in two different ways. In one version both the sample and the acceptor solution are kept within the dialysis module until equilibrium has been achieved - in principle this is exactly the same as in the batch techniques. The resulting concentration in the acceptor solution then depends on the ratio between the volumes of the sample and acceptor channels. In the other version only the acceptor solution is temporarily stopped, and the sample solution is continuously passed along the membrane until the concentration in the acceptor channel is the same as that in the original sample. When equilibrium has been achieved the acceptor solution is transferred to the injection valve of the IC instrument and the quantitative determination of the ions is carried out. This technique, which is used by Metrohm, has the great advantage that calibration can be carried out easily by using external standards. However, quantitative transfer of the acceptor solution to the injection loop requires precise time processes. Fig. 7 shows the three-step sequence that consists of rinsing, the stopped-flow dialysis process and the transfer of the dialyzed sample to the injection loop. Applying Inline Dialysis, a Metrohm IC system consisting of two dual-channel peristaltic pumps is required.

1. Rinsing of sample and acceptor channel

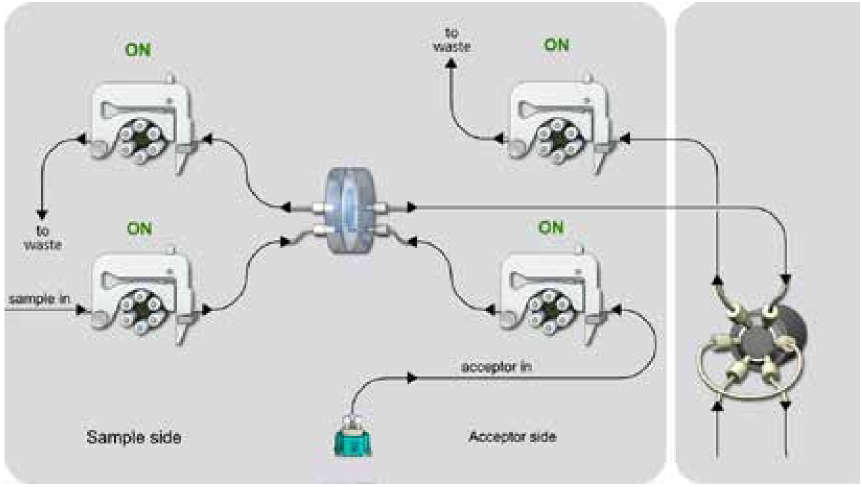

Figure 7a: All pumps are on. Sample and acceptor side are rinsed and equilibrated with new sample and acceptor solution respectively

2. Separation of analyte and matrix

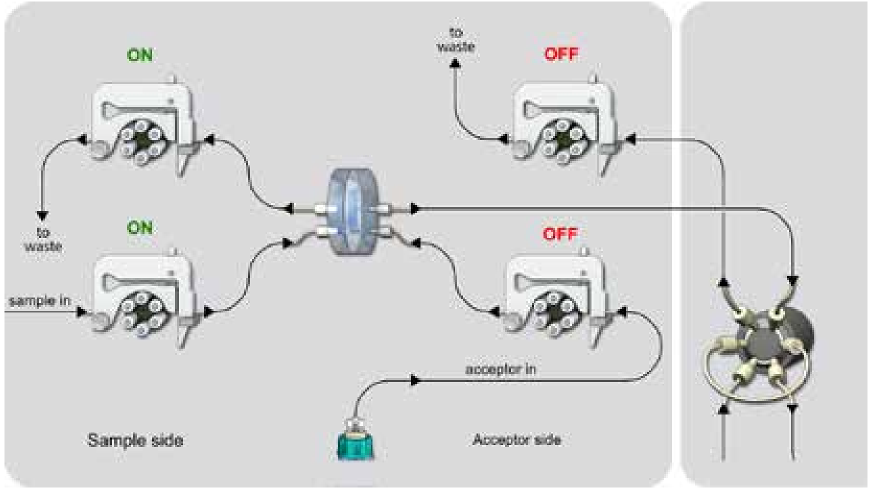

Figure 7b: The acceptor pumps are stopped. Dialysis is performed.

3. Transfer of analyte ions into the loop

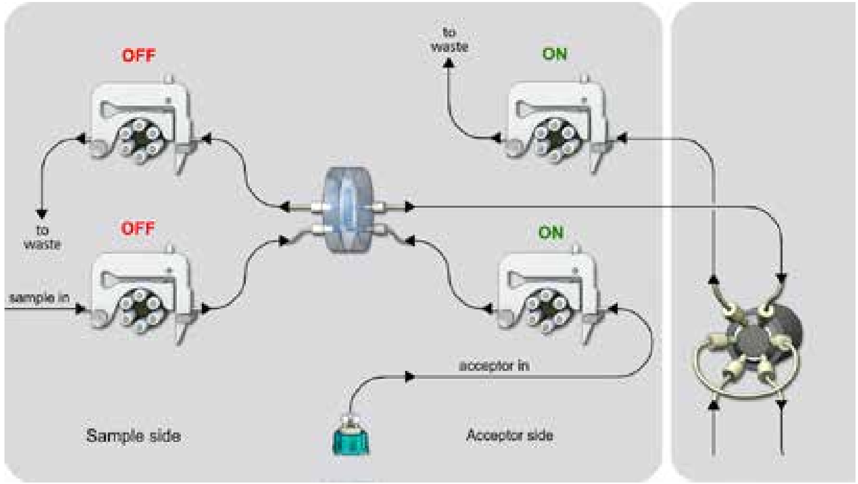

Figure 7c: The sample pumps are stopped. The dialyzed sample is moved to the injector.

7. Membrane properties and selectivity of the separation

Separation by dialysis requires homogeneous polymer materials with usually a very small pore size. Apart from the classical dialysis membranes made of regenerated cellulose or cellulose acetate, other organic polymers are being used to an increasing extent. Membranes with different thicknesses and pore sizes can be manufactured in a very well-defined way by targeted synthesis. The characteristic property used for the selectivity of the separation in passive dialysis is the molecular weight cut-off (MWCO), which is given in Dalton. It is a measure of the molecular weight at which transport through a membrane is just possible or just not possible. The separating limits given for various membranes are not always very sharp (see Fig. 8). Commercially available dialysis membranes normally have separating limit steps in the range of 100 Dalton to about 500 kDalton. However, the selectivity of a separation is not determined by the MWCO value alone but also depends, particularly when dialysis is carried out under dynamic conditions, on the mobility of the ions in the liquid phase as well as within the membrane. The transfer kinetics are again influenced by the molecular size (within the fraction that is able to pass through) and, for ions, by their charge.

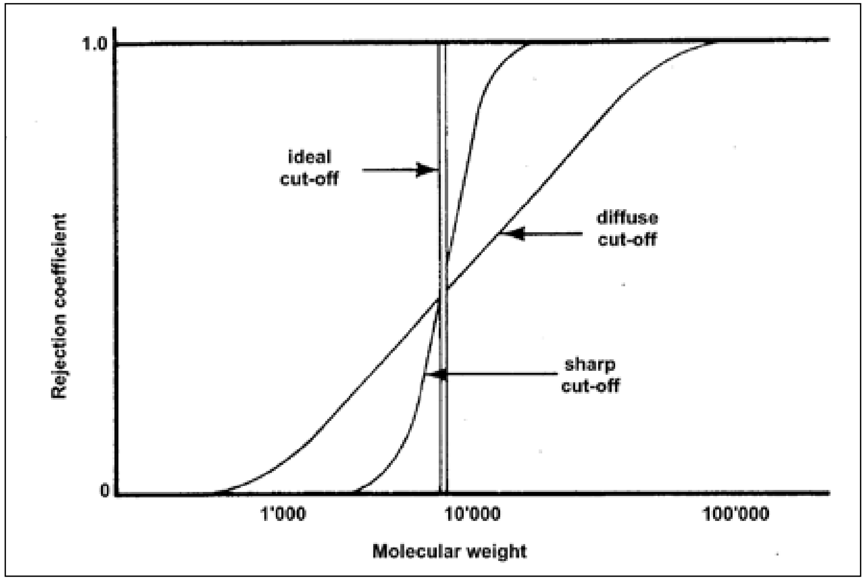

Figure 8: Typical separation characteristics of dialysis membranes with ideal, sharp and diffuse molecular weight limits

Apart from a separating limit that is as sharp as possible, additional requirements are placed on the dialysis membrane. A high transfer rate is preferable because of the shorter dialysis times. For a given MWCO this can be achieved by using particularly thin membranes. In addition, the mechanic strength and chemical resistance must be sufficiently high and the dialysis membranes should have the lowest possible tendency to adsorption, as all these are requirements for a constant transfer rate and a long working life.

Fig. 9 shows the range of dialysis in respect to the molecular weight cut-off (MWCO) and the pore size of membranes respectively. This is compared to the size and molecular weight of component groups that need to be separated from the analytes. According to their weight and size inorganic and small organic ions should pass most dialysis and filtration membranes easily. In practice, e.g. Metrohm Inline Dialysis, the transport of ions is slower than expected. Typically, here the time to reach equilibrium, typically 6...10 minutes are required with the standard 0.2 µm membranes. The reduction of the depth of the acceptor channel shortens the required dialysis time to 4...7 minutes [8]. Unfortunately, the pore size is only one parameter influencing the dialysis rate of the ions. Therefore, only a selection of membranes can be applied for Inline Dialysis.

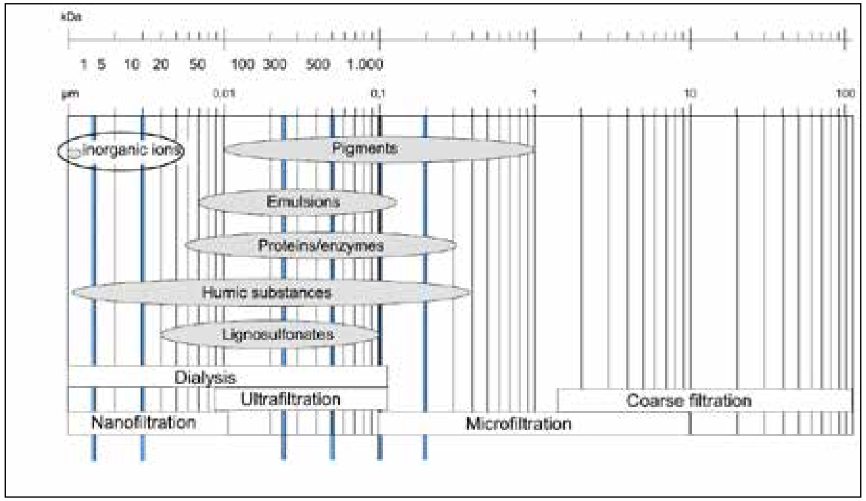

Figure 9: Separation areas of membranes according to pore size and MWCO [8]

8. Chemical influences on the dialysis process

Under given experimental conditions the substance transfer through the dialysis membrane is influenced by various factors. The diffusion coefficient of the analyte both in solution and within the membrane plays a part, and in the dialysis of ionic compounds the dialysis rate also depends on the mobility of the counterions. Owing to the very different mobilities of protons, alkali metal ions and multivalent cations, chloride ions from hydrochloric acid are dialyzed very much more quickly than from a sodium chloride solution, and these again are noticeably faster than from the chloride salt solutions of calcium, aluminum or the transitional metals, for example. Other chemical influences on the dialysis rate of ions are the pH value and the total ionic strength of the sample and acceptor solutions, as well as the presence of complexing agents or substances that form interionic associates. In principle the difficulties arising from this can be countered during the calibration in various ways. The first way is to attempt to match, as far as possible, the composition of the calibration solution to that of the sample to be analyzed. Another way is to harmonize the chemical surroundings as much as possible by a suitable modification of the sample solution so that the influences described above are to a large extent suppressed (matrix matching). For example, this can be done by adding a more concentrated solution of the chromatographic eluent, as the ions that it contains (which are also dialyzed) affect the chromatographic process either slightly or not at all. The third (in principle very much easier) possibility of not allowing the influences of the chemical composition of the sample to have any effect at all on the dialysis rate is to wait for a complete concentration equilibrium to be achieved, as is practiced in equilibrium dialysis.

The influence of sample constituents that are not dialyzed themselves as they have a molecular weight that is too high, has been investigated in numerous studies. In general, these have shown that there is either no effect at all on the dialysis rate of smaller, or that molecules or ions there is only a very small effect. Fig. 10 summarizes the results of some of these studies in which potentially interfering substances were added (some of them in very high concentrations) to the ionic standards and the results obtained were compared with pure standards. In general, exhaustive recovery rate determinations with spiked real samples of greatly differing composition (e.g. wastewater, fruit juice, soil extracts, dust and foodstuffs as well as biological samples) confirmed the minor effect of the particular matrices on the dialysis process.

Glucose, sucrose, lactose	**<10 g/L**	**Yeast extract**	**1% solution**
Starch	**saturated solution**	**Nonionic surfactants**	**1% solution**
Tannic acid	**1% solution**	**Albumin (from bovine serum)**	**<2%**
Humic acid	**0.5% solution**	**Glycerol**	**<5%**
Casein	**5% solution**		

Figure 10: Results of studies into matrix influences on the dialysis process. The simultaneous determination of a multi-ion standard was not affected in the presence of the given concentrations of potentially interfering substances.

9. Applications of Inline Dialysis - IC coupling

Inline sample preparation by dialysis offers very far-reaching application possibilities for the analysis of samples from various sectors.

Fig. 11 provides an overview.

• **Environment**	– Surface water and wastewater – Industrial effluents – Soil and filter eluates – Extracts of solid waste
• **Industrial processes**	– Electrolytic baths – Fermentation broths – Tablet dissolution studies
• **Agricultural science and production**	– Beverages – Food extracts – Plant extracts
• **Biomedicine**	– Blood, serum, plasma – Urine – Tissue extracts – Extracellular body fluids

Figure 11: Application areas in which inline dialysis coupled with IC can be applied

The retention of particles, colloidal fractions and the high-molecular fraction means that in all cases the dialysate can be directly analyzed by IC without any interference. Measures such as membrane filtration and solid-phase extraction are no longer necessary; this results in considerably less work and cost savings. Only solid samples and samples with a very high solid fraction require sample preparation steps in addition to dialysis alone (Fig. 12).

Surface water and normal wastewater	⇒	**direct introduction possible**
Strongly polluted wastewater	⇒	**coarse filtration or centrifugation**
Drinks (also with fruit pulp)	⇒	**direct introduction or centrifugation**
Dispersed foodstuffs	⇒	**centrifugation**
Extracts/eluates from soils, ashes, filters, plants, solid wastes	⇒	**sedimentation, centrifugation or coarse filtration**
Water-oil emulsions	⇒	**direct introduction possible**

Figure 12: Sample preparation requirements that cannot be met by dialysis alone

In the environmental sector most water samples can be dialyzed directly without any additional sample preparation. Only with wastewater containing large amounts of sludge it is occasionally required to carry out sedimentation, centrifugation or coarse filtration first to prevent the flow channels of the inline dialysis system from being blocked. The ionic contents of solid samples such as soils, sediments, waste and dust samples must first be released in a suitable manner (see Sections A, E, F). The resulting extracts, eluates or digestion solutions can then usually be dialyzed directly. A very impressive example of the capabilities of dialysis as a sample preparation technique is the analysis of drilling oil and lubricant oil emulsions, which can be analyzed directly without purification and phase separation. In the foodstuff analysis sector the ionic contents of beverages can usually also be determined by dialysis without any additional sample preparation being necessary. Even fruit juice containing fruit pulp can be dialyzed directly, provided that the pulp does not block the flow channels. The analysis of milk and dairy products presents no difficulties as the proteins they contain can be completely separated. Milk clarification (e.g. by Carrez precipitation), which is otherwise necessary, can be dispensed almost completely; this represents a considerable simplification of the analysis. The ions must be released from solid food samples by a suitable extraction process. The extracts can then usually be dialyzed without any further steps being needed. For example, determination of the nitrite and nitrate content in raw meat only requires the addition of water, homogenization with a rod stirrer and a brief centrifugation; the strongly colored and turbid supernatant can be dialyzed directly and the content of both ions can be quantified by IC without any interference. Plant materials can also be treated in a similar manner. In pharmaceutical and biomedical problems, the analysis of blood, serum, tissue extracts, and extracellular body fluids is important. Such samples can also usually be dialyzed directly, whereby the complete separation of colloidal fractions as well as interfering proteins is achieved. This also applies to the analysis of pharmaceutical and cosmetic preparations in which the ions only have to be released by dissolution or a suitable extraction process.

10. Conclusion and preview

From experience gained up to now in the use of inline dialysis as a sample preparation technique for IC, a very positive assessment can be made. It possesses a whole series of advantages (Fig. 13). With liquid samples, virtually no other sample preparation is required other than dialysis. Even samples with a very complex composition and a high content of microparticles and high-molecular substances can be dialyzed directly. With solid samples the preparation is reduced to homogenization and releasing the ions by extraction and, if necessary, carrying out a coarse filtration. The separation of high-molecular substances is very efficient, as these (in contrast to solid-phase extraction) are almost completely retained by the membrane regardless of their polarity.

Separation by dialysis is also characterized by its high degree of reliability. The method can be operated automatically for long periods without a change of membrane and without a significant change in the dialysis rate being observed. There is no alteration to the membrane by the adsorption of high-molecular substances or the deposition of microparticles, as the tangential flow has a self-cleaning effect. The high chemical resistance of the membranes allows even strong acids, bases and organic solvents to be dialyzed. A further advantage of inline dialysis is that the constant rinsing of the membrane and the high degree of automation virtually rule out contamination. If a short rinsing phase is included into the program then samples can be analyzed one after another without any mutual influences being observed, even when their ionic contents differ greatly.

• **High efficiency**	– No or only minimal sample preparation required – No breakthrough of high-molecular substances – Contamination-free
• **Extraordinary reliability**	– Excellent long-term stability – No membrane contamination – High chemical resistance – Biocompatible
• **Automation possible**	
• **No additional time required**	
• **Economical**	- Moderate investment, low running costs

Figure 13: Advantages of inline dialysis as a sample preparation technique

Dialysis is also very attractive from an economic point of view. The time required is extremely short and by coupling it with IC the chromatogram, running time can be used for the preparation of the following sample. The instruments needed for continuous dialysis are not very complicated and the acquisition costs for pump and dialyzer (which can also be made by any relatively well-equipped mechanical workshop) are rapidly repaid by the gain in time and the discontinuation of filtration and solid-phase extraction. The long working life of the dialysis membrane means that a change is often only necessary after hundreds of samples and the very moderate cost of the membranes keeps the operating costs very low (particularly when compared with those of the alternatives: membrane filtration and solid-phase extraction).

Apart from the separation of anions and cations mentioned in this contribution, continuous dialysis can also be used for the separation of uncharged low-molecular organic compounds and their subsequent determination, e.g. by ion exclusion chromatography. Of course, dialysis can also be used with principally different detection methods, e.g. photometry, electroanalysis. Current developments in the field of inline dialysis are aimed at the utilization of the separation kinetics for the preliminary separation of ions (e.g. determination of traces of fluoride, chloride and nitrate in sulfuric acid). As well as the use of dialysis as an integrated sampling technique [6] for the continuous monitoring of process flows, industrial processes or for monitoring surface waters and leachates.

11. Literature references

[1] T. Graham, Phil. Trans. R. Soc. London, 151 (1861) 1832

[2] J.F. van Staden, Fresenius J. Anal. Chem., 352 (1995) 271

[3] N.C. van de Merbel, L.A.Th. Brinkman, Trends Anal. Chem., 12 (1993) 249

[4] F.R. Nordmeyer, L.D. Hansen, Anal. Chem., 54 (1982) 2605

[5] W. Frenzel, H. Schafer, Europaisches Patent, Nr. EP 820804 (1998)

[6] W. Frenzel, GIT Labor-Fachzeitschrift, 41 (1997) 734

[7] D.H. Cooper, D.C. Turner, B. Green, F. Verillon, J. Chrom, 456 (1988) 53

[8] I. Markeviciute, Thesis, TU Berlin, 2018

E) Extraction as a sample preparation technique for ion chromatography

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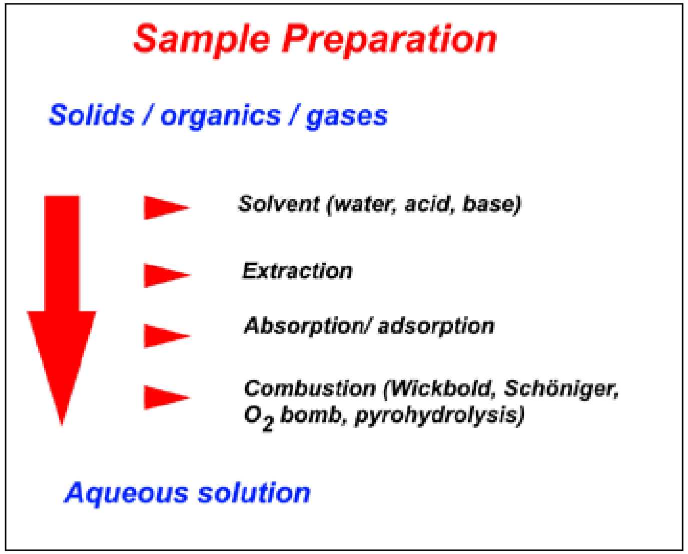
Contents

1. Introduction

Ion chromatography is an established method used in ion analysis. Today this method finds widespread use in a range of analytical sectors. In most cases, it is not the ions that are to be analyzed that cause problems but rather the matrices in which the ions are to be determined. The problems can be of different types, e.g. microparticles, various organic constituents, colored solutions or emulsions.



Sample Preparation

Solids / organics / gases

- Solvent (water, acid, base)
- Extraction
- Absorption/ adsorption
- Combustion (Wickbold, Schöniger, O_2 bomb, pyrohydrolysis)

Aqueous solution

Figure 0

This raises the question of how a sample solution can be obtained that can be injected into the ion chromatograph without causing any problems. One frequent difficulty is that the very high concentration of ions in the digestion solution overloads the separating column or even that the column may be damaged by organic compounds that have not been completely digested. The following examples show a range of possible extraction techniques that can be used for the sample preparation of solids, solutions or gases that cannot be determined directly by ion chromatography.

2. Solids

Matrix: soils, sludge, vines, filter dust, chemical products

Extraction methods

There are various extraction methods that differ in the extraction solvent, extraction time, type of extraction (Soxhlet, overhead shaking, simple stirring, ultrasonic bath) and the extraction temperature.

With extraction solvents, the solubility properties vary strongly with the pH. This is the reason why it is absolutely essential to strictly observe the procedures used and to describe them. Following are applicable solvents used for extraction: ultrapure water, Na formate solution, Ca lactate/chloride solution (CAL extract).

In the extraction of a soil sample, for example, the dry residue obtained according to DIN 38414 part 4 is extracted with water. The extraction solution is made up of 100 g soil and 1000 mL water. The extraction time is 24 hours. The solution is then filtered and, if necessary, injected via a solid phase (RP 18 phase) [1].

Metrohm Inline Extraction enables the automatic extraction in subsequent analysis of matrices like biodiesel, food, pharmaceuticals etc. reducing manual sample preparation. Typically, the extraction system is combined with Inline Dialysis to avoid contamination by traces of the sample matrix e.g. biodiesel. The schematic setup of an Inline Extraction system is shown in Figure 1. The Dosino transfers the sample into the liquid handling station and adds the extracting solution. After mixing and extraction, the aqueous phase is taken for Inline Dialysis with subsequent injection.

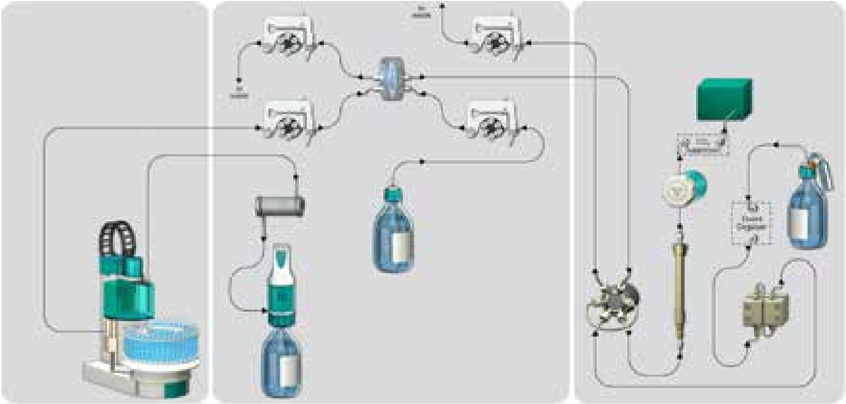


Figure 1: Schematic setup of Inline Extraction with subsequent Inline Dialysis.

Acid extraction

Dilute organic acids are generally used for acid extraction, e.g. dilute acetic acid is used for the extraction of vines. This is done by drying the vines, pulverizing them in a mill and then obtaining the extract with acetic acid.

In general, care should be taken when selecting the extraction solvent that no interference occurs to the ions that are to be determined by ion chromatography.

For acid extraction followed by the determination of the standard anions (Cl, NO_2, NO_3, o-PO_4, SO_4) by ion chromatography with **direct conductivity measurement** the following acids are suitable: tartaric acid, perchloric acid, formic acid.

For the **chemical suppression technique** acetic acid or formic acid can be used for determining the standard anions.

If mineral acids such as HCl, H_2SO_4 or HNO_3 have to be used then the difference in retention between the sample anion and the solvent anion should be as large as possible; it is better when the ion to be determined elutes before the solvent anion.

The use of alternative detectors for determination of the sample anions, e.g. a UV or an amperometric detector can also be of great advantage.

Matrix: foodstuffs (meat products, semolina, ashed samples)

Extraction methods

Another type of extraction is the exclusion of constituents by adsorption or precipitation (usually organic compounds). They not only interfere with the chromatography, but can also damage the separating column or render it unusable.

Simple examples of this type of extraction are SPE (Solid Phase Extraction) or SPME (Solid Phase Micro Extraction), dialysis or precipitation reactions with the interfering substances [2, 3].

In SPE both inorganic ions such as Cl, SO_4 or cations as well as organic components can be removed. RP, amino, diol, nitrilo or phenyl phases are normally used for adsorption of the organic components, whereas anion or cation exchangers are used for the elimination of inorganic ions.

An example of a precipitation reaction is the determination of anions in meat products (Official Collection of Methods for Analyzing Foodstuffs [in German]). The meat product is coarsely reduced in size, treated with borax solution and then homogenized with a high-frequency homogenizer. This solution is then heated and treated with zinc acetate and potassium hexacyanoferrate solutions to precipitate the proteins. The supernatant solution is then filtered through a 0.45 µm filter and injected [4].

Cation chromatography

In cation chromatography, in this case the determination of alkali metal and alkaline earth ions, dilute mineral acids are used as the extraction solvent.

The samples to be investigated are ashed and dissolved in the dilute mineral acid under heating. The solution is filtered through a membrane filter and then injected into the IC.

In the direct investigation of samples (e.g. wine or juices) the SPE is often carried out on RP cartridges.

3. Organic solvents

Matrix: polypropylene glycol, solutions immiscible with water

Extraction methods

Anions or cations in solutions that are not miscible with water cannot be analyzed directly by ion chromatography, as the separating column would be damaged by the matrix. Various digestion techniques such as the Schoeniger, Wickbold or combustion methods can be used to digest the sample [5, 6].

An alternative to these methods is aqueous extraction, the advantages of that are that thermally unstable ions are not destroyed and that no expensive digestion apparatus is necessary. In this case the extraction conditions such as temperature, type of extraction (overhead shaking, ultrasonic bath, stirring...) are decisive. Please note that the recovery rate of the ions to be analyzed must be checked.

For example, in the determination of potassium in propylene glycol approx. 0.1 g sample is stirred in 1 mmol/L HNO_3 in a plastic vessel for 1 hour. The solution is then injected directly into the IC via an RP cartridge [7].

4. Gases

Matrix: process gases, exhaust gases from firing plants, room air (monitoring the maximum allowable workplace concentration)

Absorption or adsorption is carried out by impinger wash bottles or solid phases by passing a known amount of gas through the absorption or adsorption medium.

XAD resins or charcoal tubes can be used as the solid phase in this case the compounds to be determined are usually analyzed by GC or HPLC.

Gases such as HF, HCl, NO_x, SO_2, SO_3 or NH_3 are absorbed in ultrapure water (possibly with the addition of H_2O_2 to oxidize sulfite to sulfate), dilute bases or sodium carbonate solutions and analyzed by ion chromatography. Online analysis of gases (and aerosols) are performed by The MARGA system.

Gases such as NH_3 are absorbed in acidic solutions, e.g. sulfuric acid. Depending on the concentration of H_2SO_4 these solutions must be neutralized by passing them through a cartridge before analysis by ion chromatography.

5. Examples of chromatograms

Determination of bromate in flour after Inline Extraction applying UV/VIS and conductivity detection [7]

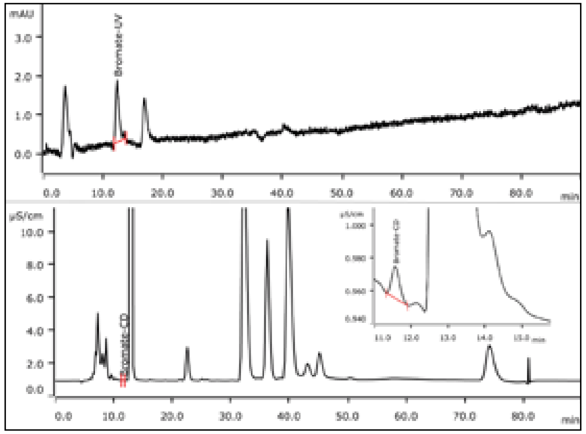


Figure 2: Bromate, with UV/VIS (top) and conductivity detection (below).

Column: 6.1006.630 Metrosep A Supp 7 - 250/4.0
Eluent: 3.6 mmol/L sodium carbonate
Detection: - Suppressed conductivity
- UV/VIS detection: after post-column derivatization
PCR reagent 1: 1 mol/L sulfuric acid
100 µmol/L ammonium heptamolybdate tetrahydrate
PCR reagent 2: 0.5 mol/L potassium iodide

Results: Conductivity detection: 41 µg/kg;
UV/VIS Detection: 55 µg/kg

Determination of cations in biodiesel with fully automated aqueous extraction and subsequent dialysis [8]

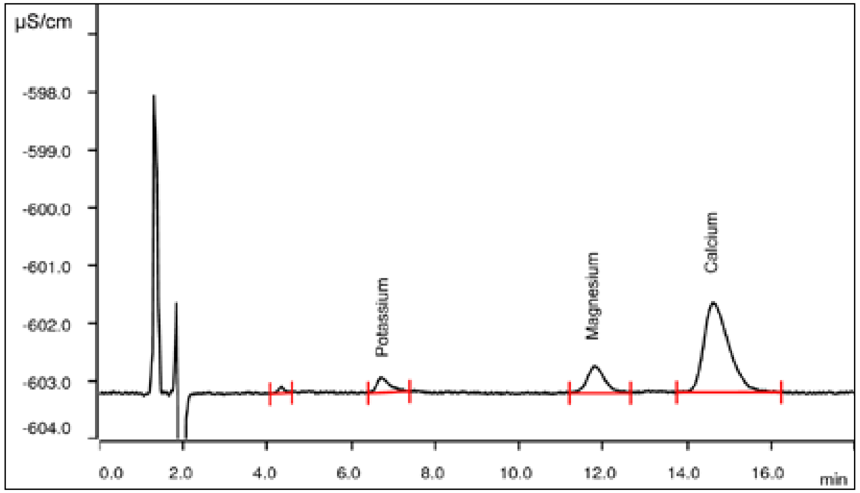


Figure 3

Column: 6.1050.220 Metrosep C 4 - 150/4.
Eluent: 2.0 mmol/L nitric acid
10% acetone
Detection: non-suppressed conductivity

Results: Sodium: not quantified
Potassium: 1.2 mg/L
Magnesium: 0.8 mg/L
Calcium: 5.0 mg/L

5. Literature references

[1] G. BogenschUtz, A. Wild, J. Schafer: Fortschritte in der Ionenanalytik mit IC, LaborPraxis 20 (1996) 38-46

[2] Application Bulletin 211, Metrohm AG, CH-9101 Herisau, Switzerland

[3] G. BogenschUtz, H. Schafer, K. H. Viehweger: Spurenanalytik in schwierigen Matrices, GIT Fachz. Lab. 44 (2000) 400-402

[4] Application Bulletin No. 8-169-1190 (1990) [in German], Deutsche Metrohm, Filderstadt, Germany

[5] G. BogenschUtz, U. Loyall, J. Schafer: Anorganische Ionenanalytik mit IC, LaborPraxis 14 (1990) 120-125M. Feige, J. Schafer, D. Frahne: Halogen- und Schwefelbestimung mit IC nach Verbrennung der Probe, GIT Fachz. Lab. 35 (1991) 443-447

[6] Application Note AN-U-063: Bromate in flour applying Inline Dialysis and conductivity as well as UV/VIS detection and PCR, Metrohm AG (2000)

[7] Application Note AN-C-101: Cations in biodiesel with fully automated aqueous extraction and subsequent dialysis, Metrohm AG (2007)

F) Conventional digestion techniques in ion analysis with ion chromatography

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Contents

1. Introduction

The classical digestion methods that are routinely used in the analysis of heavy metal ions and in elemental analysis are also suitable for use as digestion techniques for IC; however, they cannot be used so universally. This is partly owing to the high salt load occurring in fusions and wet-chemistry digestions - this normally requires dilution and/or causes large interference peaks - and partly because the chemical form of the anions is altered to some extent on combustion, so that the original ion concentration can no longer be determined.

Overview of conventional digestion methods for IC:

- Fusion
  - Alkaline with NaOH, KOH, Na_2CO_3, K_2CO_3
  - Acidic with $KHSO_4$, $K_2S_2O_7$
  - Fluorination, chlorination, sulfuration

- Combustion methods
  - Burning the sample in air or oxygen (crucible, Schoeniger, cold-plasma ashing)
  - Oxygen bomb (Berthelot), bomb calorimeter
  - Combustion in a stream of oxygen (AOX, EOX furnace)
  - Pyrohydrolytic combustion directly combined with IC (Combustion IC)
  - Combustion in an oxyhydrogen flame according to Wickbold

- Wet-chemical digestions
  - Open acid digestion with reflux (Kjeldahl, Digesdahl)
  - Pressure digestion (Teflon bomb, microwave, HPA asher)
  - UV photolysis

The above digestion methods are briefly described below and their advantages and disadvantages with respect to ion analysis by IC are explained.

2. Fusion

Fusion is frequently used for geological samples (ores, rocks) [1]. Alkaline fusion with Na_2CO_3 is the most suitable method for subsequent IC analysis, as the dilution harmonizes chemically to a large extent with the frequently used eluents $NaHCO_3/Na_2CO_3$. However, it is normally necessary to dilute the alkaline carbonate-containing fusion solution very strongly. Therefore, it is required to pay increased attention to the system and carbonate peaks as, depending on the eluent concentration, these may interfere.

The acidic, fluorinating, chlorinating and sulfurating fusions are only suitable under exceptional circumstances as the particular anion of the fusion agent massively overloads the column during the IC analysis.

3. Combustion methods

Burning the sample in air or oxygen (crucible, Schoeniger, cold-plasma ashing)

Open combustion in a platinum crucible in a muffle furnace is seldom used today. An exception is the determination of ash in foodstuffs; after dissolving the ash in HNO_3 the mineral substances Na, K, Ca and Mg can be determined by ion chromatography.

The Schoeniger digestion is suitable for solid organic substances (finely ground) as well as the elemental determination of halogens and sulfur in the percentage range. As a result of the small sample weight of 5...20 mg the determination limits are situated around 0.1%.

The combustion gases are absorbed in a neutral or weakly alkaline solution that may also contain H_2O_2 for the reduction of the Br and I as well as the oxidation of sulfite to sulfate formed. Chloride, bromide, iodide and sulfate can then be determined simultaneously by ion chromatography.

Cold-plasma ashing is a very gentle digestion method and is ideal for biological and vegetable matrices (low ash content, no fats).

Oxygen at 0.5...2 mbar is led into a process chamber and the plasma is generated by a high-frequency field. The reaction temperature is only 80...150 °C; this means that no elements are lost, although the formation of volatile compounds that could be drawn off by the vacuum pump is to be feared.

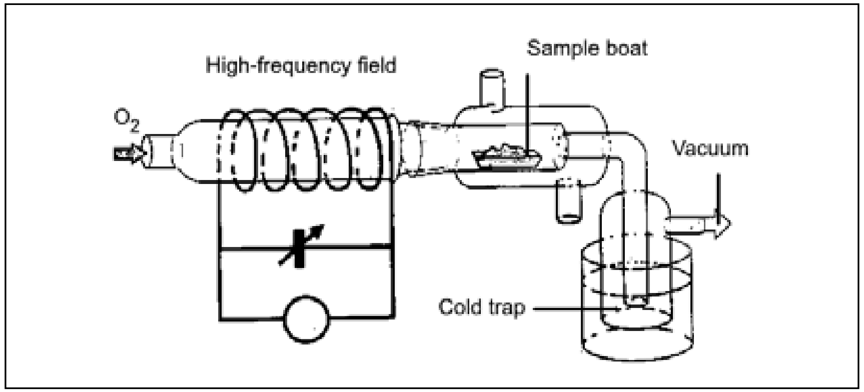

Figure 1: Cold-plasma-ashing apparatus (Technics Plasma).
Plasma reaction: e.g. $[CH_2\text{-}CH_2\text{-}]_n + 3n\ O_2^* \sim> 2n\ CO_2 + 2n\ H_2O$

As the digestion takes a long time and the apparatus is relatively expensive, this digestion technique is more likely to be used for ICP-AES and stripping voltammetry in trace metal analysis of the above-mentioned matrices. However, if this technology is available then halogens and sulfur can also be determined by ion chromatography.

Oxygen bomb (Berthelot), bomb calorimeter

The elegant Schoeniger digestion method can be considerably improved by applying oxygen pressures of up to 30 bar in a closed steel bomb with an ignition device and halogen-resistant coating:

- Higher sample weights of 50...1000 mg
- More variable sample consistency: liquid–paste-like–solid
- Calorific value can be determined simultaneously (bomb calorimeter)

Figure 2: ADD digestion system (/KA)

This digestion method is interesting in combination with ion chromatography for the differentiation of the cumulative parameters AOX / EOX, as well as for the operators of incinerators for evaluating the waste, etc. according to its calorific value and/or its halogen and sulfur content [4].

Combustion in a stream of oxygen (AOX, EOX furnace)

Higher sample weights and even liquid samples can be processed in a stream of oxygen according to the Schoeniger principle in a combustion apparatus like the one shown below:

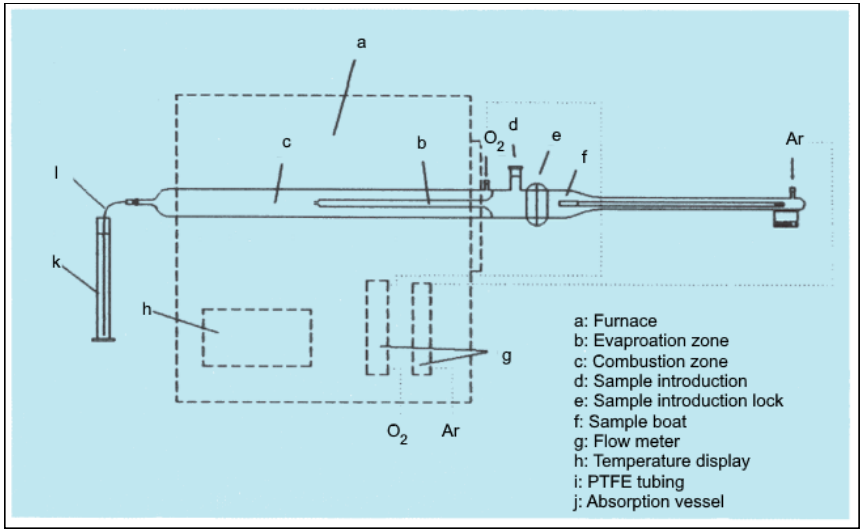

Figure 3: Diagram of a combustion apparatus with a Heraeus furnace (1200 °C)

In this case the arrangement for solids is the same as that used for the determination of the cumulative parameter AOX; the same applies for liquids and EOX.

With sample weights of up to 150 mg determination limits in the lower ppm range (µg/g) can now be reached. Absorption of the combustion gases takes place in water or weakly alkaline eluents, the reduction of the higher oxidation states of Br and I is carried out by H_2O_2. Apart from the anions mentioned, fluorine can also be determined; experience with phosphorus (and phosphate) with quartz at high temperatures has been negative [3].

For many years, universal combustion apparatuses have been commercially available. Apart from the well-known cumulative parameters AOX, EOX and POX, they also allow the combustion (in part the carbonization) of oils, solvents, waste and samples from contaminated industrial sites. If the absorption solution is chosen wisely then halogens and sulfur can generally be determined by ion chromatography.

Combustion in an oxyhydrogen flame according to Wickbold

If greater amounts of sample have to be burned for reasons of accuracy (elemental analysis) or homogeneity (waste) then the Wickbold digestion apparatus can be used. In this case 1.25 g sample material is burned in the oxyhydrogen flame; depending on the combustion period up to 200 mL water may be produced. This requires a dilution of the absorption solution so that despite the higher sample weight, the detection limits obtained are the same as for the oxygen bomb.

Combustion ion chromatography applying pyrohydrolysis

Metrohm Combustion IC is the direct coupling of a combustion oven system applying pyrohydrolysis with ion chromatography. Different to the above, in pyrohydrolysis ultrapure water is fed into the combustion tubing for easier reaction of halogens and sulfur. Figure 4 shows the respective schematic setup. The Absorber module as the connecting unit is able to do any liquid handling step required to directly inject the solution of the absorbed gases.

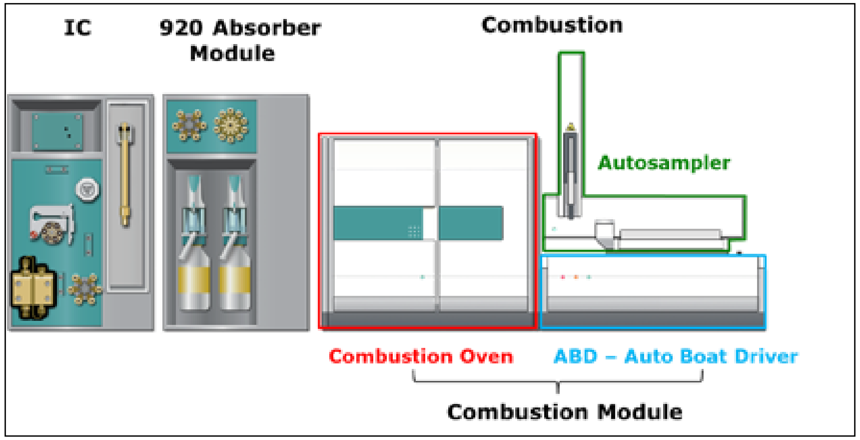

Figure 4: Diagram of a Metrohm Combustion IC system

4. Wet-chemistry digestions [4]

Open acid digestion with reflux (Kjeldahl, Digesdahl)

The Digesdahl digestion is a Kjeldahl digestion with reflux that can be carried out semi-automatically for samples in the gram range in 20...40 minutes. This digestion method has proven itself in practice, particularly in the foodstuffs sector (mostly for the analysis of heavy metal ions and the determination of nitrogen). Under certain circumstances, $CaSO_4$ may flocculate out; this leads to low-bias results!

In anion IC the acid anion of the digestion reagent limits the analytical possibilities regarding anions, with sulfuric acid only anions that elute before sulfate can still be determined. A step-function gradient is recommended for the rapid elution of the excess sulfate.

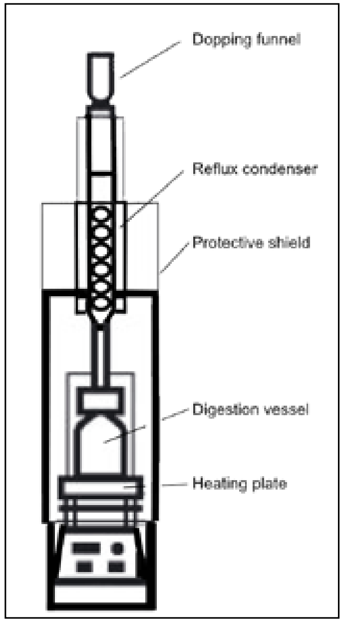

Figure 5: Digesdahl digestion system (Hach)

Procedure:

- Add 3.5 mL H_2S0_4 to 0.5.3 g sample
- Heat to 300 °C, add H202
- Repeat H_20_2 addition until solution is clear
- Heat to 500 °C, wait for secondary reaction
- Cool down and make up to defined volume
- Determine blank value daily!

Pressure digestion (Teflon bomb, microwave, HPA asher)

Acid digestion in a closed system is usually carried out in a plastic bomb at a high pressure and increased temperature. Microwaves are ideal for heating the sample and the digestion reagents as the vessel does not need to be heated as well.

Microwave digestion:

- Microwave energy is effective in the center of the sample
  - Quicker sample heating than conventional heating via the vessel walls
- In closed systems pressure build-up is possible
  - Increased reaction temperature
  - Increased oxidation potential
  - Shorter digestion times
- The use of different oxidation agents is possible
- Digestion of solid samples possible

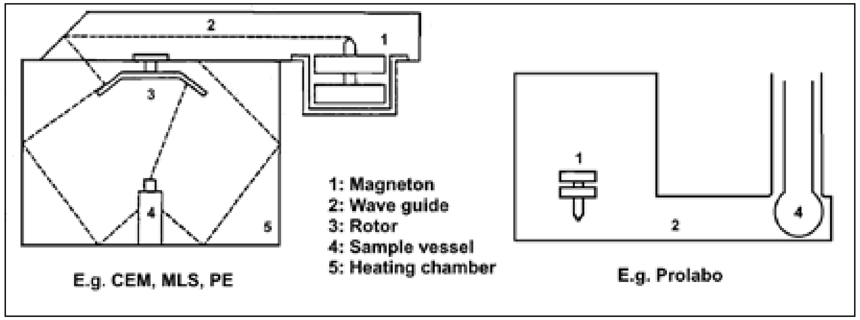

Figure 6: Schematic diagrams of microwave digestion systems

As hydrochloric and nitric acid are frequently used this means that an IC anion analysis is usually not possible, as the chloride and nitrate normally completely overload the ion exchange column, even though the digestion solution is diluted. This does not apply when appropriate dilutions can be made (approx. 1.10 g/L salt); in addition it is possible in some cases to carry out specific detection with UV or amperometry where, for example, the high chloride content does not interfere.

HPA high-pressure asher (according to Knapp)

The same applies in principle to the high-pressure asher, in which $HN0_3$ is also used as the acid, i.e. the extremely high nitrate content of the digestion solution limits anion IC.
HPA is characterized by the complete digestion of even the most difficult matrices such as fats and oils.

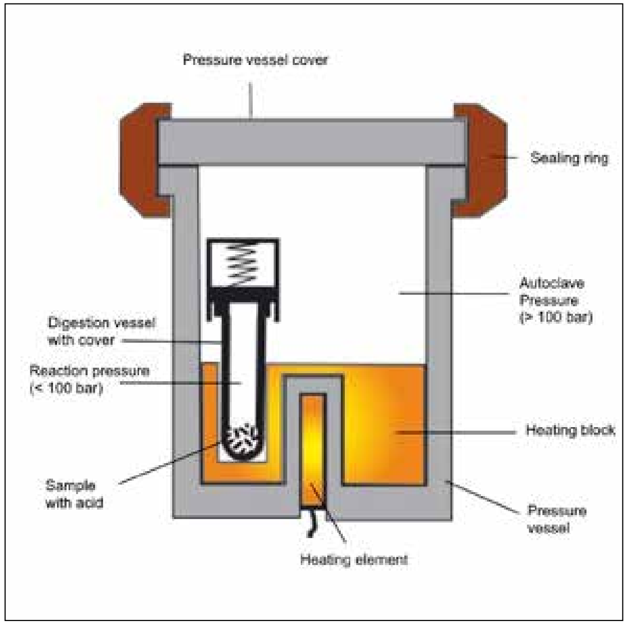

Figure 7: HPA digestion instrument (Perkin-Elmer)

5. UV digestion

UV digestion is a very mild digestion method that uses only small amounts of reagents, i.e. loss or contamination causes fewer problems. However, only clear liquid samples can be digested. Oxoanions are oxidized, i.e. only stable (such as Cl-) or high-valency compound forms such as ClO_3^- can be determined by IC, but not ClO^- or ClO_2^-

There is an interesting possibility of simply detecting coelutions of inorganic and short-chain organic ions by UV irradiation; this is done by recording the chromatograms twice, once before and once after UV digestion and superimposing the chromatograms on a PC.

The digestion of the organic matrix itself occurs by OH radicals; these are generated by a centrally arranged Hg high-pressure lamp as a wide-band UV irradiator.

- Radical-induced oxidation, by UV irradiation and addition of H_2O_2 as radical starter
- Penetration depth of UV irradiation is only a few mm and depends on the transparency of the sample
- Maximum temperature 100 °C, atmospheric pressure
- Different samples can be digested at the same time

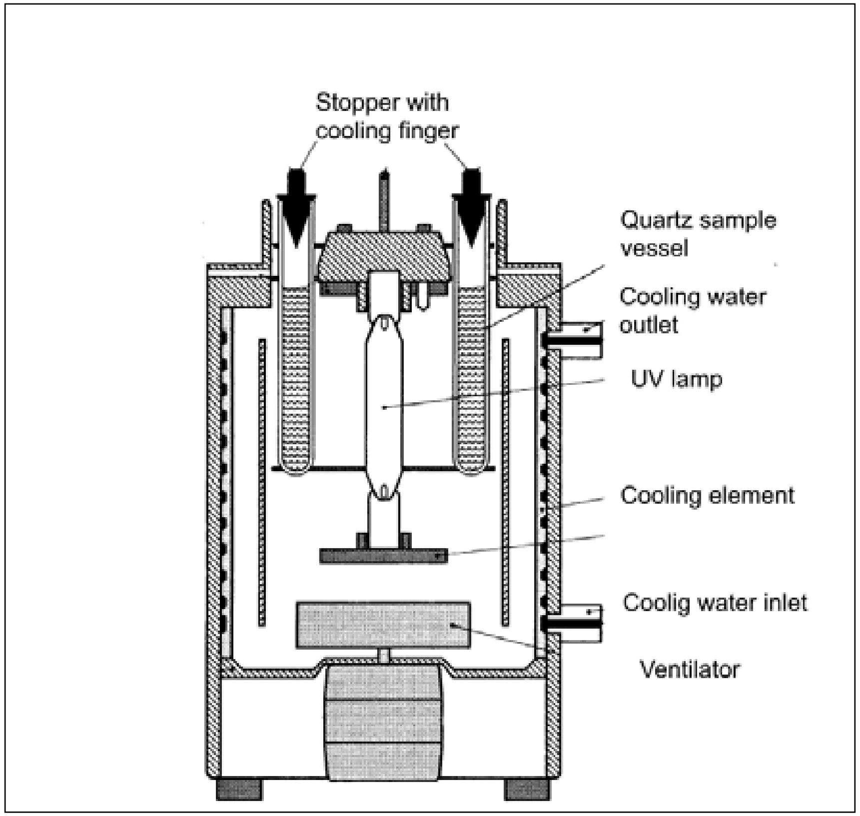

Figure 8: Basic arrangement of a UV digestion apparatus (909 UV Digestor, Metrohm)

Temperature control is by an external cooling-water controller with temperature sensor. method development it is a useful tool. In this way, interference by organic ions can be elegantly avoided.

6. Determination of Total Nitrogen, Total Kjeldahl Nitrogen by Calculation, and Total Phosphorus in Water, Wastewater by Ion Chromatography

In contrary to the Kjeldahl digestion where nitrogen is converted to ammonium which needs to be distilled for further analysis, the title method oxidizes nitrogen to nitrate and phosphorous to phosphate which directly are determined by IC. ASTM D8001 describes the method. While Kjeldahl method results directly in the Kjeldahl nitrogen content, ASTM 8001 directly gives the total nitrogen and total phosphorous content and a second run without the inorganic nitrate in the sample allows to calculate the Kjeldahl nitrogen.

7. Literature references

[1] P.R. Haddad, P.E. Jackson: Ion chromatography - principles and applications
Chapter 14: Sample handling in ion chromatography, Elsevier, Amsterdam
(1990) 409-462.

[2] H. Albrich, M. MOller: Simultane Halogen- und Schwefel-Bestimmung LaborPraxis 19 (1995) 62-69

[3] M. Feige, J. Schafer, D. Frahne:
Halogen- und Schwefelbestimung mit IC nach Verbrennung der Probe GIT Fachz. Lab. 35 (1991) 443-447

[4] P. Bruttel, J. Schafer (ed.):
Sample preparation techniques in voltammetric trace analysis Monograph, Metrohm AG, Herisau, Switzerland (1992)

G) Quality assurance measures in concentration determinations with sample preparation techniques

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Contents

1. Validation as the measure of all things?

In analytical chemistry the term «Validation» is understood to mean the checking of a method's suitability. This is expressed in a more complex form in ISO 8402 (1994), where the following is given for the term «Validation»:

«Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled».

«Objective evidence» means that demonstrable information based on facts is available; this can have been obtained by observation, measurement, a test or in a different manner. In the draft of DIN ISO/IEC 17025 (October 1998) the following citation for «Validation of methods» can be found:

«Validation is the confirmation by examination and the provision of effective evidence that the particular requirements for a specific intended use are fulfilled.

The laboratory shall validate non-standardized methods, laboratory- designed/developed methods, standardized methods used outside their intended range and amplifications of standardized methods to confirm that the methods are fit for the intended use. The validation shall be as extensive as is necessary to meet the needs in the given application or field of application. The laboratory shall record the results obtained, the procedure used for the validation, and a statement as to whether the method is fit for the intended use.

The range and accuracy of the values obtainable from validated methods (e.g. the uncertainty of the results, detection limit, selectivity of the method, linearity, limit of repeatability and/or reproducibility, robustness against external influences and/or cross-sensitivity against interference from the matrix of the sample/test object) as assessed for the intended use shall be relevant to the clients' needs.»

One of the following methods or a combination of them should be used to determine the features of the method:

- calibration with reference standards or reference materials,
- comparison of results that have been obtained by other methods,
- comparison between laboratories,
- systematic evaluation of factors that influence the result,
- evaluation of the degree of uncertainty of the result based on the scientific understanding of the theoretical principles of the method and practical experience.

2. Sources of error in analytical chemistry

Apart from numerous new terms, the decisive question still remains unanswered: Which practical points must be taken into consideration to avoid unnecessary errors? For designing a successful method that can then be validated, it is a great help to have an update of the most important sources of error in analytical chemistry. No claim is made that the following list is exhaustive:

1. Incomplete definition of the object under investigation (e.g. the exact form in which the selected analyte is present – mercury, organic mercury, etc.).
2. Sampling – the sample is not representative of the item under investigation.
3. Incomplete extraction and/or enrichment of the analyte, contamination of the measuring sample, interferences and matrix effects.
4. Inadequate knowledge about the influence of the ambient conditions on the analytical method or inadequate measurement of the ambient conditions.
5. Cross-contamination or contamination by reagents as well as blank values.
6. Analyte loss by volatilization and adsorption on vessel materials.
7. Individual, personal variations in reading off analog scales.
8. Uncertainties in the weights and volumes measured.
9. Resolving or separating capacity of the analytical method and the instrument used, i.e. possible overlapping peaks, dynamic range, etc.
10. Content of the measuring standard or the reference materials.
11. Data processing error (constants, integration limits, calculations).
12. Approximations and assumptions within the analytical method.
    For the development of a sample preparation technique points 3 to 6 and 9 are extremely important. Point 3 covers practically all the pitfalls present in sample preparation and point 9 all the demands placed on sample preparation.

3. Practical ways of characterizing analytical methods

Checking an analytical method and therefore the evaluation of the sample preparation steps can be carried out in various ways. These differ in the procedure to be used as well as the type of the uncertainty (random variations, systematic variations, total variations) and the degree of detection of the uncertainty.

Variation of all the factors influencing the result

In this method all the relevant parameters (influencing quantities) of the method are varied systematically and their influence on the result is quantified.

This method is always appropriate or may even be the only one possible when the operator has no reliable reference standards available. This is often the case when unknown samples have to be analyzed. The almost universal use of this method is countered by the fact that the results obtained in this manner are only estimated values for the random variations of the results. They are characteristic for the analysis method under investigation and for the operator and, if constant repeat conditions are maintained, a characteristic of the method. Information obtained in this way says nothing about the correctness of the results obtained with this analytical method, i.e. in this case the systematic variation from the correct result remains unknown. A favorite version of this method is the determination of the recovery by spiking experiments.

Calibration with reference materials

If a calibration is used at the same time for characterizing a measuring method, then it must be complemented by a systematic investigation of the influences on the calibration or measurement result in accordance with the method described above. The reference materials used for the calibration must correspond to the samples. As a result of the use of reference materials this method also allows a statement to be made about the systematic variation of the results obtained with this method, i.e. a check of the correctness. The uncertainty of this statement essentially depends only on the quality of the reference materials.

Ring tests and comparison tests between laboratories

A comparison of the results obtained by different laboratories using the same method and the same sample allows the estimation of the random variation of the uncertainty of the measured results for the given sample. If several samples with differing contents are measured in the comparison tests then a series of results is obtained from which the uncertainty of the results obtained under conditions of repeatability can be calculated as a characteristic quantity for the measuring method under investigation - the «standard uncertainty».

Characteristics obtained in this way say nothing about the correctness of the results obtained with this method, i.e. the systematic variation from the correct result continues to remain unknown.

4. Quality assurance specialties in the use of sample preparation techniques

Quality assurance has become an essential part of every analysis and every research laboratory. The quality of an analysis is - as in any sequential process - dependent on each individual step being checked and is, in the final analysis, dominated by that step that is least under control. An observation of the analytical process (Fig. 1) makes this chain clear. The influence of sampling, the measuring technique and the evaluation are often discussed and can be regarded as being well-recognized problems. However, the influence of sample preparation is frequently underestimated. Alone the necessity of having to carry out sample preparation already indicates a potential problem within the analytical strategy. If efficiency and costs are taken into consideration, then every laboratory will try to keep sample preparation at the lowest level possible. Unfortunately, this wish is subject to narrow limits, so that the most important quality assurance aspects of an (extensive) sample preparation procedure are discussed below.

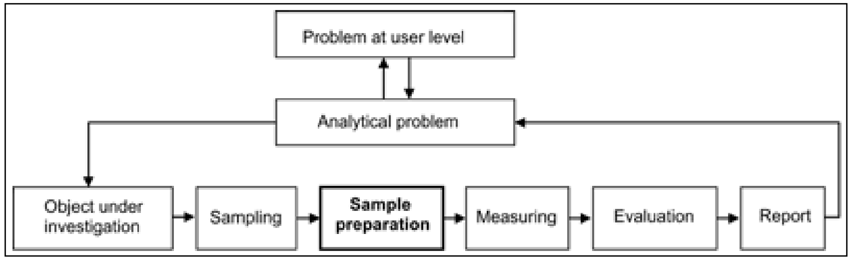

Figure 1: The analytical process with sample preparation at its center

- **How can contamination be recognized?**

In principle, contamination can be determined from the correctness of the result of an analysis, but how can the correctness be indicated in the absence of reference methods or reference materials? All that remains is a systematic search for the possible causes, e.g. by correlation studies. By checking the influences of reagent concentration, contact times, the sequence of measurements (carryover), choice of vessel, etc. it should be possible to clear up most cases of contamination. A greater problem remains with apparently non-correlated blank values caused by contamination or if a poorly controlled method is applied.

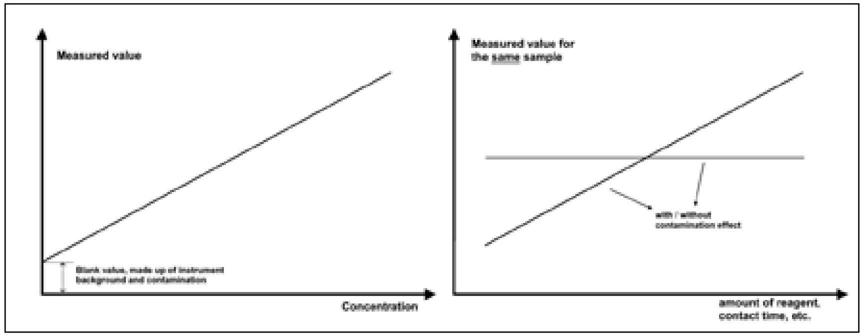

Figure 2: Basic procedure for identifying contamination

- **When do blank values cause problems and how should they be treated?**

An understood and controllable blank value is acceptable, provided that it does not unduly influence the detection limit or endanger the suitability of the method. In this case the most important quantities are the absolute value of the blank value and its standard deviation.

- **Matrix effects and their recognition**

The recovery rate is a good tool for discovering matrix effects. If the recovery varies systematically with the matrix concentration, then a matrix effect is clearly present.

- **Recognition of systematic errors**

This is always a question of correctness that is unfortunately difficult to prove. The best way is still to use a reference method or reference material; where these are not available, time-consuming detailed work as described in section 3 is necessary.

5. Determination and importance of the recovery rate

The recovery rate is the ratio of a particular determined analyte content to the correct (expected) value for the analyte in the sample under conditions of repeatability. Either spiking experiments or one or more reference materials can be used.

$$W_R = \frac{x_{Sample}}{x_{Reference}} \cdot 100$$

where:

W_R: Recovery rate in %
X_{Sample}: Measured mean value of analyte content in the sample
$X_{Reference}$: Expected or certified value

The ideal value for WR is 100%. By using the recovery rate, the whole method can be evaluatet. If the correct value $x_{Reference}$ is actually found, then the selectivity, correctness and robustness have been demonstrated - but, of course, only under the selected experimental conditions. If the analyte is present in the spike or reference material in a form different from that in the sample, then any statement about the recovery rate is not relevant for the actual sample.

A high recovery rate (almost 100%) means that no mentionable substance loss occurs during any of the steps of the method; this is an important requirement for trace methods. For example, if a recovery rate of 80% is found then the value obtained must be multiplied by the factor 1.25. If the loss of substance from the external or internal standard is also 20% then comparability is ensured and no mathematical correction is necessary.

Use of reference materials

If a certified reference material is available, then the certified value can be inserted in the above equation as the correct value. In the determination of the recovery rate by the analysis of certified reference materials, however, two problems remain. Even if reference materials are used, the correctness of a recovery rate still depends on the absence of interferences from other sources.

In addition, the number of suitable reference materials is limited, so that in doubtful cases no suitable reference material may be available. This results in the risk of an inadequate matrix agreement with the consequence of a possibly incorrect recovery rate being obtained.

Addition and spiking experiments

A known amount of a calibration substance is added to a blank sample (analyte-free sample with matrix) or a normal sample. This calibration substance is normally the analyte itself; if this is not possible then a substance that is as similar to the analyte as possible can be used. The value of the spiking method is limited to some extent as it can only determine the correctness of the steps of the method in which spiking is used. The added analyte must also be present in the same form as it is probably present in the sample.

The recovery rate can also depend on the analyte concentration. The best solution is the determination of the recovery at different concentrations. The data can be used to obtain a recovery factor. This reflects exactly the dependence of the recovery rate on the concentration.

In spiking experiments care must be taken that the linear range is not exceeded. The solvent must also cause no alteration in the matrix, nor should dilution errors occur (volume contraction). These problems are minimized when the calibration substance is added in as high a concentration as possible.

Recovery experiments should always be carried out at several concentration levels. Constant experimental conditions must be ensured during the recovery experiments as, for example, adsorption on surfaces is a very sensitive process and even minimal alterations could have a very strong influence on the kinetics.

If a recovery rate smaller than 100% is found, then the difference obtained can be evaluated as follows:

- If the expected value lies within the range of confidence of the measured value, then the recovery rate can be accepted as being adequate.
- If the recovery is below this then in individual cases a check should be made as to whether the method is fit for the particular purpose.

The most important sources of error in spiking experiments are listed below:

1. Repeatability of the recovery experiment
2. Uncertainty about the given content of the reference materials
3. Uncertainty about spiking
4. Inadequate agreement of the analyte in the sample and in the added spike
5. Poor or limited agreement between the experimental matrix (calibration solutions) and the range of the real sample matrices investigated with it
6. Influence of the analyte/spike concentration on the recovery and inadequate agreement between the analyte concentration and spiked amount or analyte concentration in the reference material.

6. Should the recovery rate be used for correcting the sample content?

There are a number of arguments both for and against the correction of the analytical results by using the recovery rate. The following individual points must be mentioned:

Arguments for a correction

- The aim of an analysis is to obtain as good an estimated value as possible for the true concentration of the analyte in the sample. The true concentration can only be obtained when significantly lower recovery rates are corrected (variation larger than the range of confidence of the measuring error).
- The uncorrected values cannot be compared with the values obtained by other methods.
- Practically all analytical methods contain mathematical corrections for inadequate measuring techniques (refer e.g. to the instructions for gravimetry). Exceptions are methods with an internal standardization and isotope dilution techniques.
- Even a faulty correction factor is better than no correction factor at all.

Arguments against a correction

- Recoveries that have been determined for an internal standard can be higher or lower than for the analyte itself. A correction introduces an additional error.
- Estimated correction factors are of doubtful value when they are used for different matrices and for different concentrations of the analyte.
- Small variations from the theoretical value (100%) are mainly caused by random scatter and less by systematic losses. This means that a correction of the result leads to a larger total error.

7. Literature

[1] W. Funk, V. Dammann, G. Donnevert Qualitatssicherung in der analytischen Chemie
Wiley VCH, Weinheim (1992)

[2] S. Kromidas
Validierung in der Analytik
Wiley VCH, Weinheim (1999)

[3] R. Kellner (ed.)
Analytical chemistry: The approved text to the FECS curriculum analytical chemistry
Wiley VCH, Weinheim (1998)

[4] G. Schwedt
Analytische Chemie: Grundlagen, Methoden und Praxis
Thieme, Stuttgart (1995)

[5] D. A. Skoog, F. J. Holler, T. A. Nieman
Principles of instrumental analysis
5th ed., Saunders College Publ., Philadelphia (1998)

www.metrohm.com