

Application of the principles of green chemistry in analytical chemistry*

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Abstract: The introduction of the dimension of green chemistry into the assessment of analytical methods should be a natural development trend in chemistry and should coincide with its general policy. Some of the principles of green chemistry—such as prevention of waste generation; safer solvents and auxiliaries; design for energy efficiency; safer chemistry to minimize the potential of chemical accidents; development of instrumental methods—are directly related to analytical chemistry.

Analytical chemistry is considered to be a small-scale activity, but this is not always true in the case of controlling and monitoring laboratories whose number of runs performed is high. This makes an analytical laboratory comparable with the fine chemicals or pharmaceutical industry. The use of instrumental methods instead of wet chemistry, automation, and minimization is a new trend in analytical chemistry, making this branch of chemistry more sustainable.

In this study, widespread separation methods are considered and an attempt is made to characterize them against the above-mentioned principles. Special attention is given to capillary electrophoresis (CE), which provides a very good opportunity to improve analytical chemistry by replacing many chromatographic methods that consume large volumes of solvents. The choice of different solvents and micronization in analytical chemistry is also discussed.

Keywords: green chemistry; electrophoresis; analytical chemistry; micronization; separation methods; supercritical fluids; ionic liquids.

INTRODUCTION

The main role of analytical chemistry is to be a controlling tool or set of tools in chemistry. It has supported the development of chemical engineering and technology for production of chemicals. As a tool, the analytical procedures give information about chemical substances, their occurrence in organisms, and the environment. The product life-cycle analysis is impossible without the chemical analysis of components and degradation products. The development of the standards and specifications to be used in industry and agriculture is strongly based on the evidence obtained by analytical chemistry and controlled by chemical methods. Analytical chemistry is the only way to justify the environmental friendliness of new methods, processes, and products.

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In cooperation with a producer and user, analytical chemistry has a stronger influence on socio-political decisions as well. The carefully and completely performed chemical analysis promotes using new technologies and logistic solutions not only for chemicals, but also for all other products. The results of chemical analysis are the main argument for establishing new laws and administrative prescriptions. The continuous development of analytical methods has made it possible to measure very small quantities of substances, which have been a basis for setting restrictions and establishing laws, like regulations on the emission of volatile organic compounds (VOCs): 1985—Vienna convention; 1987—Montreal protocol [1]; 1990—USA Clear Air Act; and the ban on persistent organic pollutants (POPs) 2001—Stockholm protocol [2].

First of all, to satisfy all these needs the main parameters of analytical procedures such as selectivity, sensitivity, reliability, and analysis time must be on a necessary level and assure their acceptable price.

ANALYTICAL SYSTEM

Every system of chemical analysis can be divided into several steps to which different approaches can be applied (Fig. 1). Analysis starts with treatment of the sample and its preparation for further separation into components. The components have to be detected in a way that allows the quantification of the separated components and gives necessary characteristics and possibly unique data for the identification of a substance.

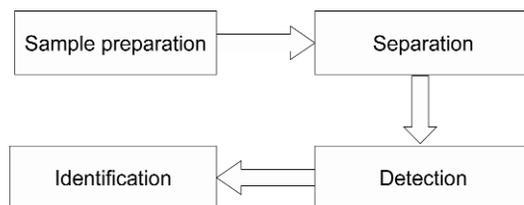


Fig. 1 The general scheme of an analytical system.

There is a wide choice of methods for sample pretreatment and preparation for further analysis. The same applies to methods of separation. The proper choice of the latter is much affected by the origin of the sample and the purpose of using the data to be obtained on the sample. Unfortunately, there are no universal methods for sample treatment or separation because of the existence of a huge variety of forms of samples for analysis. A sample is always a more or less complicated mixture of compounds in some sort of a matrix. Because of this complexity, analytical laboratories are usually specialized and there is a division of labor between them. Despite its importance, an analytical laboratory is in most cases considered to be a small-scale activity, but there are, for example, a lot of medical and state controlling and monitoring laboratories that have to analyze a huge number of samples daily. These analytical laboratories can be equalized with the fine chemicals industry. Their E-factor (the ratio of by-products to the desired products) is 25–100 [3], and concern about green chemistry in them is justified. The use of instrumental methods instead of wet chemistry, automation, and minimization is a new trend in analytical chemistry.

After the separation from the sample matrix and other interfering compounds, the detection and identification of an analyte are areas where the chemistry (in a classical meaning), which can be turned green, is not much involved anymore. The most chemical of them—the electrochemical detection—is very sensitive, which means a small amount of sample is needed and a small amount of waste is generated. Spectroscopic or laser-spectroscopic detection methods in a broad range of electromagnetic scale offer a wide selection of detection methods.

For identification, a comparison with the known physicochemical parameters and the known spectra is still a basic approach. The growing amount of data produced by automated instrumentation and high-throughput technologies forces the attention on the data processing where chemometrical methods are main tools for further development [4,5].

Green analytical chemistry

The relationship between green chemistry and analytical chemistry can be treated in two ways. Analytical chemistry is a subject to control and justify green chemistry. This is where analytical chemistry is an efficient tool for conformation of the green result of a chemical product or technology.

On the other hand, chemical analysis methods need solvents, reagents, and energy, and they generate waste. The principles of green chemistry, suggested by Anastas and Warner [6], are directly related to analytical chemistry as well, the most important of them being

- prevention of waste generation;
- safer solvents and auxiliaries;
- design for energy efficiency; and
- safer chemistry to minimize the potential of chemical accidents.

In this way, analytical chemistry becomes an object of application of the principles of green chemistry, and can be the target of a green chemistry approach similarly to other areas of chemistry and chemical technology. The term “green analytical chemistry” has been proposed by J. Namieśnik in [7,8] where several aspects of making analytical chemistry greener were discussed.

The development of instrumental methods to replace wet chemistry in sample preparation and treatment is a general trend in analytical chemistry. Here, the main analytical result is related to an increase of analysis reliability, higher precision, and time saving, which very positively combines with a substantial reduction of waste. In most cases, the result of instrumental methods in analysis is a decrease in sample volume needed for analysis. Special efforts to integrate microfluidics and processing in microscale can substantially decrease the sample amount and accompanying generation of waste.

In some cases, there is a choice of direct techniques of analysis (different laser-spectroscopic methods) or solventless processes of analysis, which are green processes. However, in most cases, the samples under study are very complicated mixtures with interfering matrices not allowing the use of wasteless methods.

The search for alternative solvents is an important step on the way of using greener methods. In this process, the main target should be not just the replacement, but introduction of an additional advantage from different properties of these solvents to improve the selectivity, sensitivity, and reliability of analysis, as well as reduce analysis time.

The development of instrumental methods in general leads to an efficient use of energy, especially when the method is highly automated and uses a minimal amount of sample. The hyphenation of several methods for sample treatment and separation of components or integration of separation and complicated methods of detection enables an efficient use of energy. Additional energy saving is possible when a microwave treatment or even just microwave heating is incorporated into the process. An ultrasonic irradiation may also have a strong effect on several sample treatments. The development of photochemical methods is a highly green way in analytical chemistry as well.

Most of the above-mentioned procedures also result in safer chemistry. In many cases of sample preparation and treatment, different chemical methods for derivatization and chemical modification of samples are still used. The search for less toxic compounds and processes with reduced waste generation should be an aim in the development of new methods.

SEPARATION METHODS IN ANALYTICAL CHEMISTRY

The increasing demand for faster, more cost-effective, and environmentally friendlier analytical methods is a major incentive to improve the classical procedures used for sample treatment in environmental analysis. In most classical procedures, the use of rapid and powerful instrumental techniques for the final separation and detection of analytes contrasts with the time-consuming and usually manual methods used for sample preparation, which slows down the total analytical process. The efforts made in this field in the past 10 years have led to the adaptation of the existing methods and development of new techniques to save time and chemicals, and improve overall performance. In these approaches, miniaturization has been a key factor in designing integrated analytical systems to provide higher sample throughput and/or unattended operation. The selected examples of novel developments in the field of miniaturized sample preparation for environmental analysis reviewed in paper [9] are used to evaluate the merits of the various techniques on the basis of published data on real-life analyses of trace-level organic pollutants. The list (adapted from [10]) of possible environmentally friendly sample-preparation techniques is long and includes the following more or less self-explanatory types:

- Solvent micro-extraction
 - In-vial liquid-liquid extraction (in-vial LLE)
 - Single-drop micro-extraction (SDME)
 - Liquid-phase micro-extraction (LPME)
 - Liquid-liquid-liquid micro-extraction (LLLME)
- Sorption micro-extraction and liquid desorption
 - Solid-phase extraction (SPE)
 - In-tube solid-phase micro-extraction (in-tube SPME)
 - Fiber-in-tube solid-phase extraction (fiber-in-tube SPE)
 - Single short column (SSC)
 - Solid-phase micro-extraction (SPME)
- Thermal desorption
 - Solid-phase micro-extraction (SPME)
 - Stir-bar-sorptive extraction (SBSE)
- Matrix solid-phase dispersion
 - Matrix solid-phase dispersion (MSPD)
- Enhanced fluid/solvent extraction
 - Supercritical-fluid extraction (SFE)
 - Pressurized-liquid extraction (PLE)
 - Subcritical-water extraction (SWE)
 - Microwave-assisted extraction (MAE)
 - Sonication-assisted solvent extraction (SASE)
- Thermal desorption from solids
 - Direct thermal desorption (DTD)

As regards liquid samples, it is probably true to say that SPE (in all of its modes and formats) is the preparation method of first choice, especially when more polar analytes are involved. An online (and automated) solid-phase extraction-liquid chromatography (SPE-HPLC) is a fully mature approach and is, moreover, the technique that can easily be miniaturized. As for most of the other sample-preparation techniques, many of these were introduced only very recently, and novel approaches keep emerging. SPME and SBSE are primarily useful for liquid samples. Although a wider range of analytes can be covered with the latter technique, less expensive automation and easy handling seem to be advantages over the former approach. Miniaturization and solvent-free operation are the shared advantages of the two techniques.

Electrophoresis

The “green” principles of analytical chemistry can be most easily achieved in electrically driven separation methods due to the low consumption of solvent and sample. A typical volume of the separation capillary in capillary zone electrophoresis (CZE) is about 5 μL , and such volume is also required for eluent. Although the low consumption of the eluent has been frequently pointed out as an advantage of CZE over high-pressure liquid chromatography (HPLC), this has never been discussed in the context of green chemistry.

The comparison of these two methods (Table 1) indicates directions in the development of separation methods—decrease of sample size, low consumption of solvent, higher selectivity, faster analysis time, mechanically simpler instrument. The situation is changing very fast in the CZE where growing number of new methods and instrumental solutions are published in journals and presented at conferences. During this time, CZE is overcoming the problems of instability of separation and lack of sensitivity of detectors, and more laboratories are starting to consider the capillary electrophoresis (CE) as a standard procedure for the separation of complex samples.

Table 1 Comparison of some parameters of liquid chromatography and CE.

	Liquid chromatography (HPLC)	Capillary electrophoresis (CZE)
Injected volume	1–100 μL	1–100 nL
Flow rate of the liquid phase	1–10 mL/min	1–100 nL/min
Flow profile	Parabolic	Plug
Number of peaks separated	20–30	20–100
Analysis time	10–60 min	1–20 min
Separation efficiency	>10 000 plates	>100 000 plates
Separation technique	High pressure with complicated pumping system	Electrical field with stable high voltage source, no back pressure
Solvents	Different solvents for different columns	Different solvents in the same column
Level of developments	Mature technique	Young, developing fast

MICRONIZATION IN SEPARATION METHODS

Micronization is an important approach to minimize the waste generated and is essential for analysis when the amount of sample available is very small (less than microliters). This is also influenced by combinatorial chemistry, which has stimulated the search for the alternative separation approaches. The key to a rapid and efficient synthesis is not only the parallel arrangement of reactions, but simple work-up procedures so as to circumvent time-consuming and laborious purification steps [10].

The similar reasoning applies even more to microfluidics in the case of which the amount of eluent consumed is even lower than in CZE. Miniaturized total analysis systems (μTAS) were first proposed as a novel concept for chemical sensing in 1990 [11], developing the field of microfluidics and leading to the vision of lab-on-a-chip. μTAS integrates all steps required in chemical analysis—sampling, pre-processing, and measurement—into a single device via miniaturization, resulting in an improved selectivity and detection limit compared to conventional sensors. Also, the dramatic downscaling and integration of chemical assays hold a considerable promise for a faster and simpler on-site monitoring of priority pollutants and make these analytical microsystems particularly attractive as “green analytical chemistry” screening tools. The amount of waste generated is reduced by ca. 4–5 orders of magnitude, in comparison, for example, to conventional liquid chromatographic assays (i.e., 10 μL vs. 1 L per daily use). A significant amount of research has been devoted to the development of microfluidics technology and applications of μTAS devices over the past decade [12–15]. Common

analytical assays, including polymerase chain reaction (PCR), DNA analyses and sequencing, protein separations, immunoassay, and intra- and inter-cellular analysis, have been reduced in size and fabricated in a centimeter-scale chip (for references, see, e.g., [16]).

Although there have been many successes, an important hurdle that still needs to be cleared is the connection between the microcomponents of a device and the macro-environment of the world. This part of the device is often referred to as the macro-to-micro interface [17], interconnect [18–21], or world-to-chip interface [22–26]. The difficulty results from the fact that samples and reagents are typically transferred in quantities of microliters to milliliters (or even liters) while microfluidic devices consume only nanoliters or picoliters of samples/reagents due to the size of reaction chambers and channels, which typically have dimensions of the order of microns. This challenge is often overlooked in research environments such as academic laboratories, but it reduces to nothing efforts to meet a requirement of green chemistry and cannot be ignored in routine applications. It must be addressed especially in high-throughput applications where manual manipulation is not economical and the macro-to-micro interface must be developed. In a review article [17], solutions that have appeared in the literature are presented and discussed.

Although designed lab-on-chip applications, these world-to-chip designs might be relevant in designs of similar interfaces for common CZE as well. The aim of such designs could well aim at achieving a computerized on-line sampling of small sample volumes without incurring complications of microfabrication. In a recent paper, two simple and economical techniques were described that are aimed at a computerized on-line sampling of the reacting small amount (a couple hundred microliters) reacting media into a common CZE capillary [27]. The first approach was similar to the “cross” injection device commonly used in lab-on-chip devices where sample and separation channels are located perpendicularly on a chip. However, instead of an electrokinetic loading, in this work the sample is loaded into a capillary by pressure pulse. This sampling technique has been used for microfabricated systems by Lin et al. [28]. In the second approach, the sample is delivered as droplets (10 μL volume) into a buffer situated in a pipette tip. The falling droplet sampler was first proposed by Liu and Dasgupta [29] and was later implemented for hyphenating flow injection analysis (FIA) to CZE [30–32]. Recently falling droplet sampling was adopted in the FIA-CZE system to avoid deterioration of separation due to hydrodynamic pressure created in the inlet, especially with higher flow rates in the FI system [33]. In this falling-drop design, a constant liquid head, equal to that at the capillary outlet, is maintained and the Poiseuille flow is avoided. In the design proposed in [28], the construction of the falling droplet sampler was further simplified by rejecting a specially designed FI-CZE interface body. The liquid was kept in the pipette tip by surface tension and if a new portion of liquid is delivered, it displaces an old portion that flows into a waste.

Alternative solvents

Most methods of sample treatment and further separation into components use solvents, and this is the main source of organic waste. The search for an alternative solvent is an important step in making an analysis “greener” and environmentally friendlier.

It appears that alternative solvents like supercritical fluids and ionic liquids (ILs) have one extra dimension which makes them even more attractive for researchers—tunability. The ability to fine-tune the properties of the solvent medium will allow this to be selected to replace specific solvents in a variety of different processes or create new methods for processing (analyzing) samples.

Supercritical fluids

The most popular substances used in supercritical fluid processing: water ($T_k = 374.1\text{ }^\circ\text{C}$; $p_k = 218.3\text{ atm}$) and carbon dioxide ($T_k = 31.1\text{ }^\circ\text{C}$; $p_k = 72.8\text{ atm}$). The supercritical CO_2 is nontoxic, easy to purify, and relatively inert.

Using supercritical fluids instead of organic solvents for extraction is becoming more popular for most liquid–solid extractions, especially when the supercritical CO₂ is used as a solvent [34]. There are already some official U.S. EPA methods where the supercritical CO₂ is replacing organic solvents in sample preparation to extract target compounds [35]. This replacement saves analysis time and minimizes the consumption of organic solvent. An additional green aspect of using gases in supercritical conditions allows saving of energy because of the absence of distillation and solvent evaporation.

An easy tuning of the solubility and other parameters of solvent by temperature and/or pressure is possible. In this way, the use of solvents in supercritical conditions means expanding the overall spectrum of solubility, polarity, and volatility properties of solvents and mobile phases. The same solvent can be used in different applications and procedures.

Despite all that is positive, the methods using supercritical fluids are not widespread, and there is a lack of standard procedures in controlling laboratories. This may be explained by the fact that methods are technically complicated and there is a need for a careful and precise control of processing (extraction) parameters because the solubility in the supercritical fluid is very sensitive to the changes of pressure and temperature [36]. Also, collection of the analyte after the extraction is a critical step for analysis performance, because of possible losses of analyte. Different systems have been developed, among them liquid trap, solid-phase trap, cold trap, etc. [37]. All this means that the process of extraction using supercritical fluids is not fully studied, and there is a lot of room for further development to establish reliable analysis methods based on supercritical fluids. One possible direction is online coupling of the extraction unit and the separation unit with suitable detector where possible losses of analytes when transferring from one instrument to another are minimized [38].

Using supercritical fluids in chromatography, it is possible to fill in the gap between gas and liquid, providing a continuum of mobile-phase properties and widening possibilities of one instrument [39].

Ionic liquids

ILs are salts with a melting point close or below room temperature. They form liquids composed of ions. This gives these materials, when used as solvents, the potential to behave very differently from conventional molecular liquids. Their physical properties are very promising for green chemistry applications: they are nonvolatile liquids and good solvents for many organic and inorganic materials. One of the advantages of ILs is thermal robustness. This means that the wide thermal operating range (typically, –40 to 200 °C) is possible that enables a wide range of kinetic control for reactions that proceed in ILs.

The most popular ILs are 1-alkyl-3-methyl imidazolium salts and 1-alkyl pyridinium salts with a multiple selection of anions. The search for applications is intensifying in each area of analytical chemistry—electrochemistry, chromatography, and electrophoresis, even mass spectrometry. The number of publications on ILs is increasing almost exponentially. The basis for this activity is an easy preparation of salts with different ion constituents. This ability might best be described as the “chemical tunability” of ILs—a class of solvents with members possessing similar physical properties but different chemical behavior. ILs can be applied not only in the existing methods whose sensitivity and selectivity of analysis need to be improved, but their different behavior and properties can offer original solutions in chemical analysis as well.

ILs have good solvating properties, which together with a large range of spectral transparency make them suitable solvents for spectroscopic measurements [40] of a wide range of species including organic, inorganic, and organometallic compounds. Notably, a variety of transition-metal complexes, which are unstable in other media, may be studied in room-temperature ILs. The use of room-temperature ILs as solvents for UV, visible, and IR spectroscopy for highly charged complex ions with high- or low-oxidation states like [MX_n]^{y-} complexes (M = transition metal; X = Cl, Br) circumvents the problems of solvation and solvolysis and permits reliable solution spectra to be recorded for these species. Spectroscopic measurements of solvatochromic and fluorescent probe molecules in room-

temperature ILs provide insights into solvent intermolecular interactions, although interpretation of the different and generally noncorrelated "polarity" scales is sometimes ambiguous [41].

It is demonstrated that task-specific ILs have advantages over common solvents used as separation media in an LLE process achieving high efficiencies and selectivities of separation. The development of simple systems for cleaning of fuels is of high practical importance. The extraction of sulfur and nitrogen compounds from gasoline and diesel oil by ILs indicates that such a process could be an alternative to common hydrodesulfurization for deep desulfurization down to values of 10 ppm S or even lower [42,43].

ILs' main advantage over organic solvents for other applications in analytical chemistry is their low volatility, which makes ILs useful as solvents for working at high temperature as well (GC stationary phases). The task-specific ILs have a high thermal stability up to 260 °C, provide symmetrical peak shapes, and because of their different for anions and cations range of solvation-type interactions, exhibit dual-nature behavior of selectivity [44,45]. This difference in selectivity between ILs and a common methylphenyl polysiloxane GC stationary phase is due to the unique solvation characteristics of the former and allows an optimistic conclusion to be made about them as useful dual-nature stationary phases.

It seems that ILs are not very good media for liquid separation, and in liquid chromatography and CE applications they are used as specific additives. It was found in [46] that ILs of the imidazolium tetrafluoroborate class, added to mobile phases at a concentration of 0.5–1.5 % (v/v), blocked silanols and provided excellent chromatographic separations of strongly basic drugs which were otherwise not eluted, even with neat acetonitrile as a mobile phase. Acetonitrile is a well-suited medium for non-aqueous capillary electroseparations and enables extending of the range of applications of CE techniques to more hydrophobic species. In many cases, salts, which are liquid at room temperature, show a better solubility in organic solvents and can be used in nonaqueous CZE as ionic additives for adjustment of analyte mobility and separation. The separation of different analytes in organic solvents is achieved because they become charged in the presence of ILs in the separation media. The first such kind of application of ILs in nonaqueous media was demonstrated in [47]. The electroosmotic flow of the bare silica capillary can be easily reversed by the covalently bonded IL coating [48], which works in both aqueous and nonaqueous buffers.

The low volatility of ILs makes them useful as solvent working in a high vacuum, and together with their more amorphous solid analogs they merit further study as MALDI matrixes. These ionic matrix systems allow a homogenous sample preparation with a thin IL layer having negligible vapor pressure. The vacuum-stable, liquid consistency of IL matrix sample preparations considerably enhanced MALDI-MS analysis in terms of shot-to-shot reproducibility, and this leads to a facilitated qualitative and quantitative measurement of analytes compared with classical solid matrixes [49,50].

Good electrolytes should have high conductivity, large electrochemical windows, excellent thermal and chemical stability, and negligible evaporation. Using an IL as an electrolyte medium, it is possible to achieve a wider range of operational temperatures and conditions, relative to other conventional electrolytic media, and make them promising materials in various electrochemical devices, such as batteries, fuel cells, sensors, and electrochromic windows [51].

The combination of ILs with supercritical carbon dioxide (scCO₂) as an extractant offers a potential for a chemical reaction and downstream separation in one system. Spectroscopic studies can offer reliable data on the properties of the media. The authors of [52] studied the solvent properties of mixtures of 1-butyl-3-methyl imidazolium hexafluorophosphate and CO₂ as functions of temperature (35–50 °C) and pressure of CO₂ (0–230 bar). The results are consistent with a picture of local enhancement of an IL around a chromophore, maintaining solvent strength even at fairly high loadings of CO₂, whereas the microviscosity in the vicinity of the solute is dramatically reduced, leading to enhanced mass transport and facilitated separation. They can be used together with organic cosolvents which "solvate" the constituent ions of the IL, resulting in a decrease in the aggregation of these ions (lower viscosity and higher conductivity).

At the moment, there are more opportunities than results. It can be said that there are wide horizons for studying different sides and applications of ILs. The need for data on the physical properties (density, viscosity, thermal conductivity, diffusion, solubility, reactivity) as well as toxicity (bioaccumulation, biodegradability) of ILs should be emphasized most. Lack of understanding of solvent/materials properties with regard to the structural features of the ILs (effect of anion choice and cation substitution) is substantial and a very promising area of study. ILs are commercially available, and intensive studies are underway in every area of chemistry to find a proper niche for them.

CONCLUSIONS

Emphasizing the importance of green chemistry and sustainable development for humans is not a good political slogan. We really must learn more and better about laws of nature and processes taking place in environment because, as Richard P. Feynman, says in his Lectures on Physics, "Nature does not care what we call it, she just keeps on doing it."

Analytical methods are developing rapidly, and there is a strong driving force that is concerned about the safety of the environment, water, and food. Also, the public needs confirmation that chemical products and processes are safe. Microfluidic technologies have enabled the miniaturization of established analytical techniques, enhancing achievable performance. Also, it can be exploited to develop completely new approaches to conducting chemical and biological processing.

On the other hand, waste prevention must become a part of the decision process when choosing the proper analytical method—analysis must be performed in a green way.

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