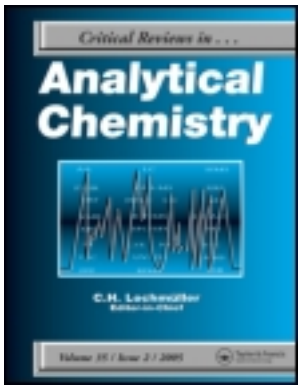

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Recent Advancements on Greening Analytical Separation

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Recent Advancements on Greening Analytical Separation

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60–80% of the analysis time for a significant number of analytical methods is taken up by the preparation, treatment, and separation of individual sample components, and most of the chemicals and solvents involved in the analysis are consumed in this step. We will demonstrate that many emerging methods of analytical separation science can meet the requirements of green chemistry by reducing the use of solvents and other reagents, lowering energy consumption by increasing the speed of analysis, and by miniaturizing and making equipment portable. Although recent efforts to make high performance liquid chromatography greener are praiseworthy, capillary electrophoresis, which comprises a group of separation methods that use narrow-bore fused-silica capillaries, is especially promising. It is highly competitive with liquid chromatography, which is the biggest consumer of organic solvents in analytical chemistry. However, capillary electrophoresis has not received the recognition it deserves as a green separation method that consumes microscopic volumes of solvent. It is a technique that is sufficiently mature to promote the greening of analytical chemistry via miniaturization—the most auspicious development in contemporary analytical chemistry. In this review, we will discuss recent developments in greening chromatography, and demonstrate the potential of electrophoresis and microfluidics in this regard.

Keywords chromatography, supercritical fluids, electrophoresis, microfluidics, green chemistry

INTRODUCTION

A significant number of analytical methods relate to the preparation, treatment, and separation of individual components of samples [by some estimates up to 80% of analysis time (Hyötyläinen, 2007)] and most of the chemicals and solvents involved in analysis are consumed in this step. For one run, the amounts may be minimal. But in the long-term, for example, the amount of acetonitrile (ACN) consumed per year by an individual high-performance liquid chromatograph (HPLC) in an average laboratory, assuming that 2,000 runs is a typical instrument load, could exceed 250 L. If more than one chromatograph is operating, then the ACN consumption calculations transform an academic exercise into an economic and environmental problem. From here green chemistry comes in.

ACN is one of the most important and popular solvents in analytical chemistry. It has minimal chemical reactivity, low acidity, and a reasonably low boiling point. Its miscibility with water, wide range of achievable polarities with water mixtures, low viscosity compared to other organic solvents (resulting in low pressure drop, even with water binary systems), and low ultraviolet cut-off (down to 192 nm) make it ideal for reversed-phase (RP-HPLC) applications. The unique properties of ACN make it the solvent of choice in separations of pharmaceuticals. These physical and chemical properties, as well as its former abundance, led to the validation of many HPLC methods using ACN as an eluent. In addition to the uses described above, ACN is also a highly functional solvent for sample preparation techniques for dilution and protein precipitation in biological fluids, and as an eluent for solid-phase extraction. ACN is used in synthetic biochemistry for solid-phase oligonucleotide–DNA synthesis, for peptide synthesis, and drug manufacturing.

However, ACN is toxic to humans and slightly persistent in water, with a half-life of between 2 and 20 days. ACN has acute and chronic toxicity to aquatic life. Therefore, reducing the use of ACN in the laboratory is sensible from a green perspective. Academic research related to green chromatography has provided many ACN-free separation protocols and methodologies which do accomplish the task; however, they might not seem as efficient or effective as ACN-based solutions. Analysts are neither instantly nor universally convinced of the benefits of green chromatography and have always lagged behind in the

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application of green chemistry principles because their first priority has always been analytical performance.

Very often to support green ideas people need economical pressure. Until the well-known ACN crisis in 2009, precipitated by the downturn in the global economy, [the cost and availability of ACN were influenced by decreasing automobile parts production, of which ACN is a by-product (Tullo, 2008)], no one seriously thought about replacing ACN as an HPLC eluent. The labs and corporations who are involved in preparative- and process-scale chromatographic purification and use ACN as an eluent have been hit particularly hard. Now, in light of the ACN crisis, corporations are considering green chromatographic solutions seriously.

In this paper we would like to demonstrate that separation science can meet the requirements of green chemistry with regard to the reduction of the use of solvents and other reagents lowering energy consumption by increasing the speed of analysis, and through portability and miniaturization of equipment. We will describe advances of recently reported green gas chromatograpgy (GC) and HPLC techniques. If the green chemistry is about the solvent and energy economy the microfluidics fits the best demands of it. The process of miniaturization started in the 1950s in electronics and had transformed society since then. At the beginning it was intended just for automation of computations but new applications rapidly emerged transforming society into a "global village" where each member has its personal means of information processing. Large scale investigation of the possibilities of miniaturization in analytical chemistry and especially in separation science started in the 1980s by introducing capillary electrophoresis (CE) and in the 1990s by introducing lab-on-a-chip. Thus, the miniaturization in chemical analysis lacks behind in electronics about 40 years. Still, miniaturization has already demonstrated that analytical chemistry is part of the informational science and as such need not to be resourceful. It is difficult to foresee, but eventually the downsizing of analytical procedures might have an influence on society which could be comparable to that of electronics. Perhaps the breakthrough will come in the field of personal medicine. In this paper some new solutions provided by CE and especially in non-instrumental and paper microfluidics to make analytical separation science more environmentally benign are described.

GREEN CHROMATOGRAPHY

Gas Phase Separations

GC is a relatively green separation method because its eluents are usually helium and hydrogen, which are gases harmless to the atmosphere. However, GC sample preparation methods should be revisited in the light of green chemistry principles. Eliminating or minimizing the amount of solvent used in sample preparation techniques before the final chromatographic analysis is highly recommended. Various approaches to the implementation of green chemistry principles in GC, so-called direct chromatographic analysis, are pre-eminent (Namiesnik and Wardencki, 2000; Wardencki and Namiesnik, 2002) because they permit the determination of analytes in a sample without any pre-treatment or sample preparation.

There are two truly solventless approaches in gas phase separation: gas extraction and membrane-based techniques. Supercritical fluid extraction could be considered as a solventless extraction method when CO_2, which is a gas at normal conditions, is used, and if the extraction unit is directly connected to a gas chromatograph.

The U.S. Environmental Protection Agency recommends another solventless extraction method: dynamic gas extraction, i.e., purging inert gas through the solution under investigation and trapping extracted analytes on a suitable sorbent (i.e., Tenax). This is the widely accepted method for routine analysis of volatile organic compounds (VOCs) in water.

Static headspace micro-extraction is also becoming very popular, mainly because it does not require such sophisticated instruments as the purge technique. Gas extraction is the most widely used method for isolating volatile pollutants from different matrices, primarily because it is a pro-ecological (solvent-free) means of isolation and enrichment. Gas extraction provides the required sensitivity (up to the ppt level) and can be automated by combining it with GC. Thermal desorption of pollutants collected on a sorbent is a standard method for the measurement of VOCs in workplace or environmental air (Harper, 2000). The advantages of thermal desorption over conventional solvent extraction include an improvement in detection limits (by three orders), no chromatographic interference from solvents or solvent impurities, enhanced sample throughput, and lower cost-per-analysis. Another advantage is that thermal desorption is a straightforward gas extraction process. In light of these considerations, thermal desorption meets all the requirements of green chemistry for chromatographic analysis.

Membrane-based separation can be considered a solventless method when the analytes are volatile and the accepting media is a gas phase. In this technique, the separation of analytes is performed by a membrane: a stream of gas flushes the external side of a membrane and the gas flow from the internal side of the membrane is used to dispense the analytes into the inlet of the gas chromatograph. Another option is the collection of analytes from a stream of gas or liquid flushing the external side of a membrane, on the internal trap side of the membrane, and after the collection cycle, desorption of the analytes for GC analysis.

In addition to sample preparation, fast GC is gaining attention for greening gas phase separations. Fast GC reduces overall analysis time leading to significant savings in time and energy (Cramers et al., 1999; Sacks et al., 1998). Fast chromatography can be especially attractive for laboratories where many routine samples are analyzed every day. It can also be advantageous in situations where quick results are needed. However, increasing the speed of analysis requires modification of commercially available instrumentation. Faster separation can be achieved by decreasing the inner diameter of the capillary columns, reducing the thermal mass of the column thermostat for fast temperature

programming, applying shorter columns, or working at turbulent flow either by using a vacuum outlet operation or working above optimal carrier gas velocities.

Liquid Phase Separations

Green HPLC Methods

Liquid chromatography (LC) is one of the biggest consumers of organic solvents in analytical chemistry; therefore, greening this method could start with reducing the use of solvents and replacement with environmentally benign ones. The ACN crisis led to a consensus that reducing the use of toxic solvents via miniaturization, column temperature programming, and the use of less toxic solvents than ACN could provide the solution. The early efforts to make HPLC greener have been thoroughly documented in recent reviews (Koel and Kaljurand, 2010). The recent publications indicate that the established trend continues. R. Mayors (2009), P. Sandra et al. (2010) and Welch (2010) have recently discussed possible options of greening HPLC at length. The merits of these emerging technologies are described, tested experimentally, and the factors influencing the decision to move from conventional analytical separation methodologies to greener alternatives are discussed. These authors have identified the following opportunities:

Shorter columns with the same internal diameter. This is the obvious option that is available without any mofification of HPLC instrument. However, the loss of resolution must be tolerated.

Shorter columns with smaller particles. The new submicrometer-sized particles are particularly attractive in this situation because separation times and solvent usage can be reduced up to one order of magnitude compared with longer columns packed with larger particles. A major effect of smaller particles is the column-pressure increase. When the particle size is reduced twice it requires, a pressure increase by a factor of four if one wants to keep the same flow rate. A variety of ultra-high pressure LC (UHPLC) systems are currently on the market and are gradually replacing more conventional LC instrumentation (Sandra et al., 2010).

Reducing the internal column diameter. Nano-LC instruments that operate with a few hundred nanoliters per minute are now available commercially; these are being applied to proteomics, where samples are mass-limited and mass spectrometry is used for detection. These types of systems yield the ultimate solvent savings. With chip-based systems, a liter of ACN could last a month or more!

The green character of these scale changes is only valid when micro- or nanoliter pumps are used. Pumps operating in the split-flow mode are wasting the mobile phase.

Using other organic modifiers in RP-HPLC. The most popular replacement for ACN is methanol. Other solvents that have been considered are tetrahydrofuran, isopropanol, ethanol, and n-propanol. Another way to reduce the use of ACN might be to employ a different washing solvent to remove unwanted compounds from the HPLC column. Ethanol should be an especially green solvent which is available in the large quantities and is non-toxic. Welch et al. (2010) demonstrated the use vodka as an HPLC eluent (Figure 1).

Disadvantages of the use of ethanol are the high viscosity of ethanol/water solutions and ethanol control regulations rendering the routine use of ethanol complicated and problematic (Sandra et al., 2010). However, with the appearance of UHPLC instrumentation with pressures up to 1200 bar, viscosity issues are no longer of concern.

Changing the type or amount of the stationary phase. Using a less hydrophobic stationary phase or the same stationary phase with lower surface coverage decreases the retention of organic analytes.

One example of that kind approach is hydrophilic interaction chromatography (HILIC), where separation is achieved by partitioning between a water-enriched layer on the surface of a polar stationary phase and a mobile phase that contains a high percentage of organic solvent (mostly ACN) (Alpert, 1990). HILIC is used for the separation of highly polar ionizable solutes. In recent research on the use of HILIC, dos Santos Pereira et al. (2009) demonstrated that the HILIC mechanism could be reversed. (A dos Santos Pereira et al., 2009) The features of the method they named "per aqueous LC" were illustrated in the analysis of catecholamines, nucleobases, acids, and amino acids. In keeping with the principles of green chromatography, the more environmentally friendly ethanol was used.

Changing the pH of the eluent for ionizable compounds. Lowering or raising the pH below/above the pK_a of ionizable compounds might elute them from the column more rapidly than using an organic solvent, thereby saving it. The authors are unaware of any examples where this method has been used as a green alternative to common HPLC with the aim of reducing organic solvent consumption.

Using increased column temperatur. Chromatographers frequently overlook temperature as an optimization parameter in HPLC, but it can play an important role in selectivity because nearly all of the physical parameters of LC separation depend on it. Moreover, using only pure water as the eluent and increasing the temperature from 25 to 250°C decreases value of parameters such as polarity, surface tension, and viscosity, and results in solvation behavior that resembles that of an organic solvent like methanol or ACN. Consequently, instead of using a water–ACN gradient, one can use temperature programming as in GC. With the introduction of new solid phases capable of operating up to 200°C and higher, the use of pure water as a mobile phase produces a truly "green" analytical technique. An extremely attractive feature of temperature as a separation parameter is the fact that it can be set instrumentally. It is much easier to adjust temperature than eluent composition or the buffer pH. If the column temperature is higher, the authors prefer to use the term "elevated temperature", because many applications of interest to chromatographers take place at temperatures in the 40–200°C range, which are certainly elevated with respect

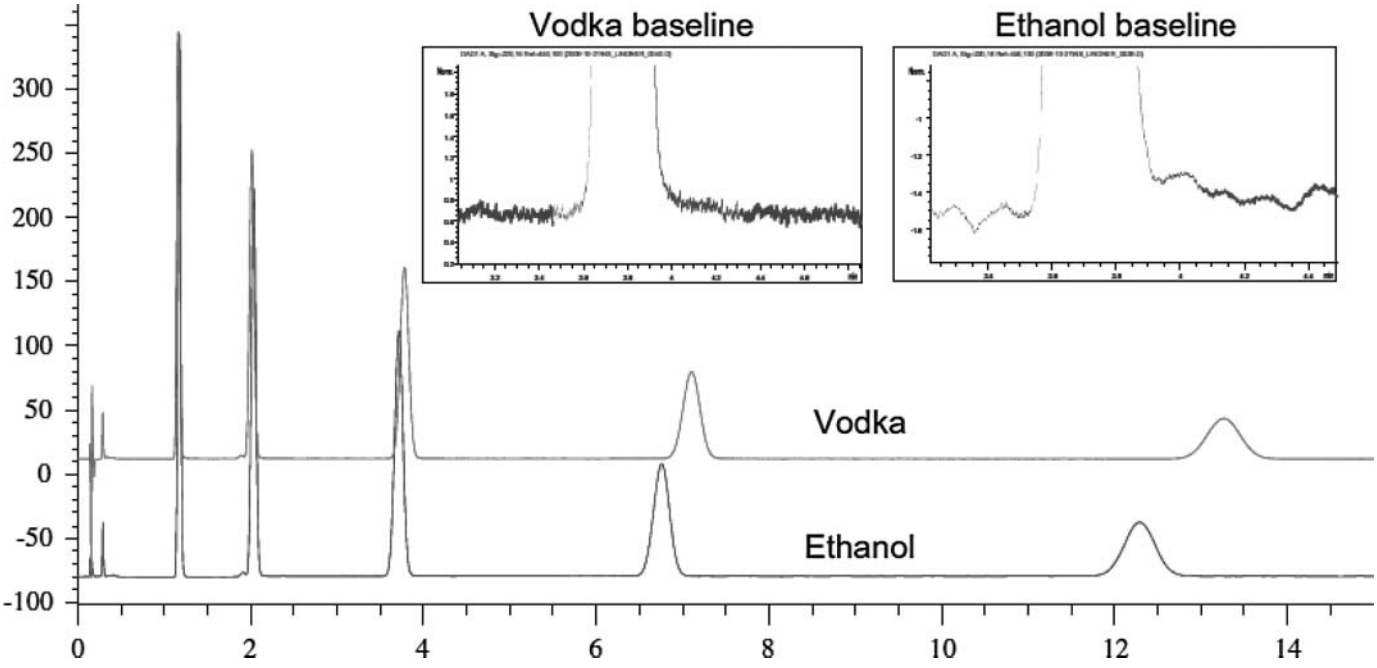

FIG. 1. HPLC separation of alkylbenzene test mixture using inexpensive vodka as a mobile phase. Conditions: column = Ascentis C18 4.6 mm × 50 mm, 2.7 micron; mobile phase = either 40% EtOH/water or vodka; flow rate = 3 mL/min; column temperature = 60°C; UV detection at 220 nm. [From (Welch et al., 2010) with permission].

to ambient temperature but would not normally be considered high. When planning to use elevated temperature chromatography, one must keep in mind an important pre-requisite: the stability of the analytes at those temperatures. Obviously, this is a vital consideration for biologically significant analytes.

Reviews summarizing the development and applications of elevated temperature HPLC have been published recently (Greibrokk and Andersen, 2003; Hartonen and Riekkola, 2008; Jandera et al., 2004; Jones, 2004; Teutenberg, 2009; Zhu et al., 2004). A special issue of the 2001 Journal of Separation Science was dedicated to the role of temperature in LC (J. Sep. Sci., 2001). To illustrate the effect of temperature, Fig. 2 shows a separation of a mixture of ten triazine and ten phenylurea pesticides (Vanhoenacker and Sandra, 2006). A temperature gradient was combined with a solvent gradient to enhance the selectivity because it is impossible to reach the complete resolution of all pairs of peaks under isothermal conditions; however, the use of a moderate temperature gradient made it possible to achieve higher resolution between solute pairs.

Even more sophisticated use of temperature can be made. There are available temperature-dependent stationary phases, where, for example, retention can be modulated by a temperature-responsive polymer with reversible hydrophilic-hydrophobic conformation (Kanazawa, 2007; Kobayashi et al., 2001). The temperature-responsive properties of the coupled phase were demonstrated using only water as a mobile phase: an increase in retention was observed with rising temperature. Kikuchi and Okano (2002) describe several applications using surfaces and interfaces modified with stimuli-responsive polymers for stimuli-responsive surface property alteration and posit their application in the separation sciences for affinity separation of proteins (Kikuchi and Okano, 2002). Particular attention is directed to the temperature-responsive polymer, poly(N-isopropylacrylamide) (PIPAAm) and its derivatives, as surface modifiers for green chromatography, in which only an aqueous mobile phase was used for separating bioactive compounds. This polymer exhibits hydrophilic properties below 32°C and becomes hydrophobic above that temperature. Several effects of bioactive compounds on separation were investigated and discussed, including the effects of temperature-responsive hydrophilic-hydrophobic changes, copolymer composition, and graft polymer molecular architecture. Compounds covering a wide polarity range including phenones, alkylbenzenes, phenols, alkylated benzoic acids, anilines, sulfonamides, and carbamates were analyzed, and the retention, peak shapes, and plate counts were compared under identical conditions. Interest in these materials is growing and a new stationary phase for temperature responsive LC the poly(N-vinylcaprolactam) (PVCL) connected to aminopropyl silica, was synthesised and the temperature responsive characteristic of the polymer was illustrated by Miserez et al. (2010). PVCL demonstrates a transition from hydrophilic to hydrophobic interaction between 30 and 40°C.

Supercritical Fluid Chromatography

An important direction in greening separation methods is to seek replacements for existing organic solvents, as discussed above. A second approach is to use common gases and solvents

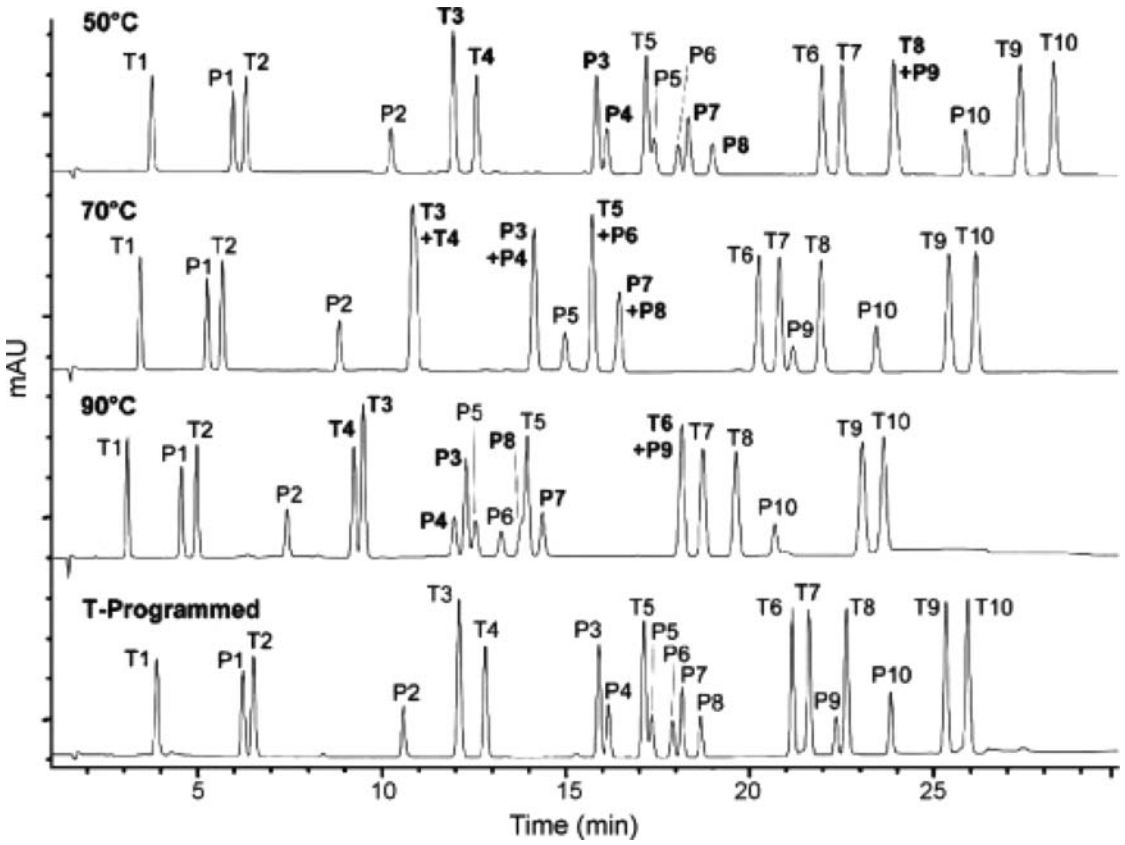

FIG. 2. Separation of a mixture of ten triazine and ten phenylurea pesticides on a Zorbax StableBond-C18 column (150 mm × 4.6 mm I.D., 1.8 μm d_p). Flow-rate: 1 mL/min, gradient: water/ACN 80:20 to 45/55 in 30 min. Temperature program (lower chromatogram): initial 40°C hold 0–1 min, ramp 40–60°C at 1.3°C/min, and ramp 60–90°C at 10°C/min. [From (Vanhoenacker and Sandra, 2006) with permission].

under different conditions—pressure and temperature. The use of solvents above their normal temperature and pressure conditions will continue to expand the range of analytical methods and should be seen as part of an overall spectrum of solubility, polarity, and volatility properties of solvents and mobile phases.

Supercritical fluids have served to link gases and liquids, providing a continuum of mobile phase properties to the analyst. The possibility of making seamless changes using supercritical fluids is very attractive for researchers. Slight changes in temperature and/or pressure around the critical point of supercritical fluids cause significant changes in density and other physical properties that make it possible to tune the solubility and other parameters of the solvent. With supercritical fluids, a greater range of solvent properties can be achieved with a single solvent, through careful manipulation of temperature and pressure. The ability to fine-tune the properties of the solvent medium allows it to replace specific solvents in a variety of different processes, or to create new methods for processing (analyzing) samples. However, although the multitude of supercritical fluid parameters lends flexibility to the method, the lack of fundamental knowledge about how these parameters affect the process makes straightforward method development difficult, and the technique remains largely empirical. Nevertheless, using supercritical fluids—especially CO_2—instead of organic solvents for chromatography is becoming more popular. They can act as substance carriers like the mobile phases in GC, and also dissolve these substances like solvents in HPLC (Klesper et al., 1962).

During the development of packed column sub-critical fluid chromatography (SFC), researchers realized that the characteristics of chromatographic separation are present irrespective of whether the fluid is defined as a liquid, a dense gas, or a supercritical fluid. In some instances, the initial pressure used in SFC is actually below the critical pressure. The differences between SFC, enhanced fluidity chromatography, and HPLC have been overstated in the past. When the outlet pressure is elevated and the pressure and temperature are controlled for the mobile phase, the resulting techniques are similar and the behaviors of conventional GC and HPLC are completely and seamlessly bridged (Chester and Pinkston, 2004). Each type of chromatography represents part of a continuum of increasing mobile phase solvating power coupled with increasing mobile

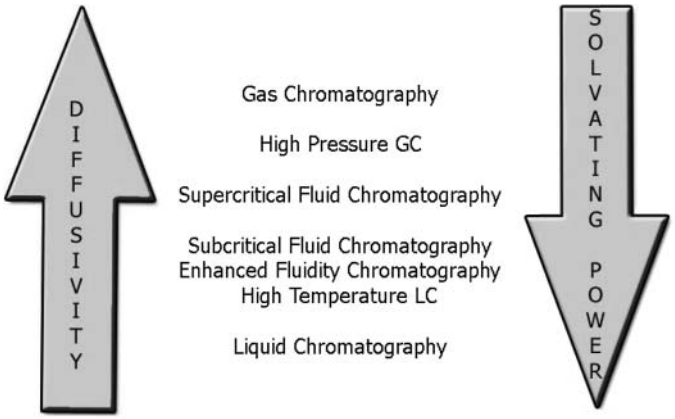

FIG. 3. The chromatography continuum.

phase viscosity and decreasing mobile phase diffusivity. In principle, with a supercritical carbon dioxide carrier it is possible to perform GC, SFC, and HPLC in the same chromatograph (Fig. 3).

The SFC technique has been in existence for several decades and recently has been applied effectively to chiral separations, especially for preparative work (Sandra et al., 2010). SFC significantly reduces organic solvent usage and waste by using CO_2 as the mobile phase; therefore, it has been labelled a "green" technology. Commercial instrumentation is available, including accessories that can convert a conventional HPLC system to one that is SFC-capable. Supercritical CO_2 exhibits liquid-like densities and can be pumped like a liquid as long as the system is kept at the proper pressure and temperature to maintain the supercritical state. Supercritical CO_2 has very low viscosity and is highly diffuse, enabling fast and highly efficient separation, often exceeding those of HPLC. SFC could be considered a subset of normal-phase HPLC because CO_2 is a non-polar eluent, even at high density. To increase its polarity, the supercritical fluid must be doped with an organic solvent such as methanol, but usually at less than 30%. Columns used in SFC are similar to those used in normal-phase HPLC such as cyanopropylsilica and aminopropylsilica, but SFC-specific stationary phases such as 2-ethylpyridylsilica have been developed that provide even better selectivity. Although traditionally regarded as a technique only for non-polar compounds, SFC has been applied to cationic, anionic, and, as mentioned previously, chiral compounds, as well as to proteins and drugs. Increased interest in the petrochemical and food industries, the determination of environmental air quality, biodisel quality control, etc. can be expected in the future (Taylor, 2010).

SFC can be interfaced with most detection systems including mass spectrometers, with the only qualification being the requirements of a high-pressure flow cell or back-pressure regulator and flow-splitter at the exit of the column. Of course, an existing HPLC method would have to be completely redesigned. Nevertheless, SFC could be a strong competitor with HPLC for certain applications, and the reduction of organic solvent used in SFC is significant. Also, the compounds of interest can be isolated in a relatively small amount of solvent because CO_2 vaporizes away. It must be emphasized that using CO_2 - based techniques (extraction or chromatography) are not generating the gas the causes global warming, because the CO_2 used is reclaimed from the atmosphere. Moreover, for preparative processes, the CO_2 is recycled.

An important component of laboratory procedure is solvent recycling and purification for reuse. Pure ACN is rarely used as a mobile phase in RP-HPLC. The solvent is generally used in a binary system with water or buffered water; therefore, the column effluent contains a mixture of solvents. Some ingenious solutions can be devised, such as collecting solvent when there are no chromatographic zones eluting from the column (Mayors, 2009). This can be performed by an intelligent system controlled by a detector signal that directs the solvent to the collection bottle by means of a switching valve at the end of the chromatographic column. ACN/H_2O azeotrope can also be distilled as a purified binary mixture containing 14% water, which could be collected and used as material for eluent preparation.

The Greening of Chromatography is Not Black and White

It is obvious that many of the suggestions outlined above could reduce ACN usage and make HPLC greener. With the exception of SFC, none of the techniques require the purchase of new instruments, although the use of high-temperature ovens for HPLC might pose a technical problem for laboratories performing routine work. However, the more difficult problem for those laboratories would be changing approved methods because—as stated above—regulating agencies have strict directives against altering column dimensions and experimental conditions without a full revalidation.

One should not take a simplistic view that green chromatography is merely a matter of using aqueous rather than organic eluents. The whole process should be considered when evaluating the greenness of a particular form of chromatography. The most promising green separation method—elevated temperature chromatography—consumes more energy. At the time this paper was being written (early in 2010), the energy aspects of green chromatography were mostly being ignored. A remarkable exception is the work of van der Vorst et al. (2009) who performed an *exergetic* life cycle analysis of a chromatographic separation of enantiomers of a racemic mixture of phenyl acetic acid derivatives in order to compare preparative HPLC with preparative SFC. The exergy of a system is the maximum work that is required to bring the system into equilibrium in a process (Perrot, 1998). Their conclusions are as follows: if one considers instrumentation alone, the exergy consumption related to the preparative HPLC technique is about 25% higher than for preparative SFC due to its inherently higher use of organic solvents. Considering the whole undertaking (plant, lab, etc.), one must take into account exergy calculations for the physical

boundaries of the production site and the resources crossing those boundaries. Resources have to be purchased including the cooling and industrial water, the cooling and heating medium, and steam. Storing the product entails costs. From this perspective, preparative SFC is more favorable because it consumes about 30% less resources than preparative HPLC as quantified in exergy. However, an analysis of the cumulative exergy extracted from the environment to deliver the mass and energy to the plant and a chromatograph "boundary" via the industrial network reveals that preparative SFC requires about 34% more resources than preparative HPLC. The conclusion of the work (van der Vorst et al., 2009) is astonishing: the most sustainable process in terms of integral resource consumption is preparative HPLC (using ACN as the eluent modifier). The authors reason that the requirement for electricity for heating and cooling and the production of liquid CO_2 argues against the use of preparative SFC.

Although the study (van der Vorst et al., 2009) is limited to the preparative separation of a particular sample, its findings could be far-reaching. Therefore, the actual greenness of elevated temperature chromatography, which avoids the use of harmful solvents by employing temperature, remains unproven until a thorough exergy life cycle analysis has been performed. The exergy calculations should also be applied to recycling and disposal processes on a case-by-case basis. Moreover, solvents that are considered innately green (such as ionic liquids) may not be completely green. Even 100% aqueous waste effluent cannot be flushed into municipal sewers without cleaning, which consumes energy and has an environmental impact. When energy consumption is taken into account, a completely green chromatography is probably impossible, in the sense that it is not sustainable as the term is defined in green chemistry (Anastas and Warner, 1998). Even GC, the greenest mode of chromatography, consumes purified carrier gases, the extraction of which from the atmosphere results in a measurable carbon footprint.

Finally, two lesser-known chromatographic preparative techniques also deserve mention because they qualify as green separation methods. These are steady-state recycle chromatography (Lee and Wankat, 2009) and simulated moving-bed chromatography (Lee and Wankat, 2009). Steady-state recycle chromatography is a discontinuous, single-column separation technique that involves recycling unresolved fractions back into the column. It combines high throughput and low solvent consumption. Simulated moving-bed chromatography uses the countercurrent flow of the stationary and mobile phases in continuous mode. Continuous chromatography provides two significant benefits: higher throughput (due to smaller runs and columns) and up to a hundredfold reduction in solvent consumption.

At present, green chromatography is being developed by a small group of proponents. Opposition to its application on a wider scale has been based not on chromatographers' ignorance of the principles of green chemistry, but rather on the fact that green chromatography cannot solve some important separation problems faced by the chemical industry and regulatory agencies. If green HPLC makes further progress and becomes widely accepted, its exergy calculations will transform an academic exercise into organizational policy issues. One can envision conflicting scenarios in which green chromatography is environmentally acceptable and economically attractive in local laboratories and institutions but not sufficiently benign for the environment and society as a whole.[1] Cynically speaking, green laboratory practices might involve the transportation of wastes to regions where regulations are not as stringent as they are where the pollution is being produced.

MINIATURIZATION OF ANALYTICAL SEPARATIONS

Miniaturization in analytical chemistry and separation science is an old trend. As said above, analytical chemistry is about processing specific information about chemical species in our environment. Obviously the reliable concentration estimations should be obtained in the most optimal way, the "optimal" being a rather vague (but still easily understood in different situations) term here. So the search has been intensive for faster and cheaper alternatives to laboratory-based analysis on expensive instruments. First lab-on-a-chip (a GC instrument) was proposed by Terry and Jerman (1984) but serious interest in microfabricated separation systems was received when Manz proposed a micro total analysis system, a μTAS (Manz et al., 1990), based on electrophoresis phenomenon. It is particularly suitable for microfabrication because there are no mechanically movable parts (neither valves nor pumps are needed). When electrophoresis phenomenon is frequently implemented as a force moving separation media, "classical" electrophoresis in capillaries has its own benefits which we would like to de monstrate below.

Miniaturization and Portability

Portable instruments are commonly believed to be more economical than their stationary counterparts. It is generally understood that portable instruments are designed to be taken to an analysis site, i.e., a point-of-care (POC). The consumption of resources (either power or chemical) and the generation of waste are limited in the case of portable instruments which makes them greener. A POC can be any place, such as a hospital, home, or crime scene. In fact, many field analyses could be accomplished without traditional sampling. Field analytical chemistry

[1]To view green chromatography from a wider green philosophical perspective it is interesting to consider the work of K. Rebane, an Estonian physicist. He writes that history indicates that the species and societies that act more quickly and consume more high-quality energy and materials are the "winners": in other words, those that cause more pollution and promote the faster growth of entropy. This could be a reason why protection of the environment is inherently difficult and why it is almost impossible to significantly reduce man's consumption of energy and materials in a competitive world. To escape this inevitably fatal evolutionary outcome, fundamentally different thinking is needed—thinking which makes the survival of mankind the foremost value. A completely green process could be one in which pollution is transported away from Earth as infrared radiation. Karl K. Rebane, Energy, entropy, environment: why is protection of the environment objectively difficult?", Ecological Economics, 13, 2, 89–92, (Rebane, 1995).

(FAC) is a growing trend that promises to liberate the analyst from tedious and inconvenient sample manipulations. Analytical methods typically involve sample preparation; a time- and labor-consuming collection step usually needs to take place before samples can be transported to a laboratory for analysis. Sample collection can often be problematic. For instance, when analyzing polluted soil, samples have to be collected in many locations according to a time-consuming and laborious sampling plan to avoid missing the "hot spot." Samples must usually be treated with specific reagents and stored in containers under certain conditions to maintain their integrity before analysis. Furthermore, in many cases, there are difficulties in sampling, such as at hazardous polluted sites or with precious cultural relics and archaeological objects. FAC can not only eliminate the need for sample transportation but also greatly shorten the analysis time or even provide real-time results, thereby providing rapid warning and accurate feedback. Thus, the logic and nature of FAC are such that analysis is done with little or no sample collection and preparation. Transportation of the sample to a laboratory is eliminated. He et al. (2007) summarize the green characteristics of FAC instruments. In order to perform field analyses, the ideal analytical instruments should meet several requirements: they should (a) have a fast response time to be able to acquire the necessary information on a real-time or near real-time basis; (b) be capable of *in-situ*/at-site rather than just on-site[2] analysis and need little or no sample preparation; (c) be portable for field use with a minimum requirement for energy (battery-powered is desirable), consumables (gases /solvents), or clean space for handling samples; and (d) perform a cost-effective analysis. Turl and Wood (2008) are even more specific with regard to the characteristics of an FAC instrument. A step out of the lab into the field requires a giant leap from technology to capability because the instrument, which size and weight enables portability and is consuming very little power, must perform adequately even in a harsh environments, must be easy to operate and maintain, and ensure the security of classified data. In addition operator training must be simple and health and safety risks must be minimized. Appropriate sampling methods must be developed for these kind of instruments.

Capillary Electrophoresis as an Unrecognized Green Alternative to HPLC

CE is a liquid-based separation method that can offer substantial competition to LC. CE comprises a family of separation methods that use narrow-bore fused-silica capillaries to separate mixtures of large and small molecules. Sample introduction is accomplished by immersing the end of the capillary into a sample vial and applying pressure, vacuum, or voltage. Depending on the types of capillary and electrolyte used, CE technology can be segmented into several separation techniques. CE typically uses high electric fields to separate analytes, which may be based on their charge, size, or hydrophobicity. CE is a powerful separation technique that involves minimal solvent consumption. The typical CE column is a capillary with an i.d. of 50 μm and a length of 50 cm having a volume of about 5 μL. The analysis runtime is typically 10 minutes and electro-osmosis, the eluent driving force in CE, has a rate of sub-μL/min. This means that eluent consumption during a CE run is almost nonexistent with a buffer/sample volume of approximately 100 μL. ACN and other harmful solvents are rarely used as eluents in CE because most of the separations are conducted in ordinary aqueous buffer systems (see Fig. 4).

Capillary electrophoresis was very actively studied at the beginning of the 1990s, but its development has stagnated in recent years. More than 2000 papers in fundamental studies in CE have been published each year during the last decade. Nevertheless, acceptance of CE by the industry has been slow. CE has struggled to replace HPLC for the analysis of conventional small molecule pharmaceuticals. The lack of familiarity with CE can lead to methods being developed that are not robust, perform poorly, and give CE a negative reputation. Analytical chemists have learned that CE is highly sensitive to parameter changes and that it is not a very reproducible technique, but modern instrumentation has eliminated most of the early variability issues. As robust interfaces with mass spectrometers are developed, the obstacle of CE not being compatible with MS will soon be overcome (Maxwell and Chen, 2008).

Surprisingly, only recently CE has received recognition as a genuinely green separation method (Pena-Pereira et al., 2010; Xiea and He, 2010). The authors of this paper have pointed out that its greenness is due to the microscopic volumes of solvent it consumes[3] (Koel and Kaljurand, 2006). Moreover, the solvents used are usually harmless aqueous buffers. As the ACN shortage continues to influence the field of analytical separations, CE is gathering increased attention as confirmed in the report from one panel discussion (http://chromatographyonline.findanalytichem.com/lcgc/Technology-Forum-Capillary-Electrophoresis/ArticleStandard/Article/detail/618413). Suppliers of CE equipment have responded to the need for improved robustness and reliability. They

[2]He *et al.*, 2007, provide a definition of the terms "*in-situ*" and "on site." In this chapter, on-site analysis is understood to be a common analysis procedure that involves sample collection/preparation using a field-portable instrument. *In-situ* analysis leaves the sample site virtually undisturbed. *In-situ* analysis could be done with an x-ray spectrometer but it is difficult to imagine how a chromatographic analysis of art or soil samples would be possible.

[3]Although arguments in favor of the benefits to society may not move a chromatographer who is wrestling with everyday problems of maintaining his/her research in a competitive level, the political-economic situation in a particular country may persuade scientists to accept green solutions. The authors have personal experience in this regard. The rise of CE at the beginning of the 1990s coincided with the political changes in Eastern Europe that caused a dramatic reduction in funding for fundamental research. In this situation it was almost impossible to use HPLC due to the lack of supplies and the requirement for large amounts of solvents and spare parts. On the other hand, it was relatively easy to assemble CE instruments from old color television sets (which contained a high voltage power supply) and modify the cells of discarded optical HPLC detectors and thereby carry on research at a reasonably competitive level.

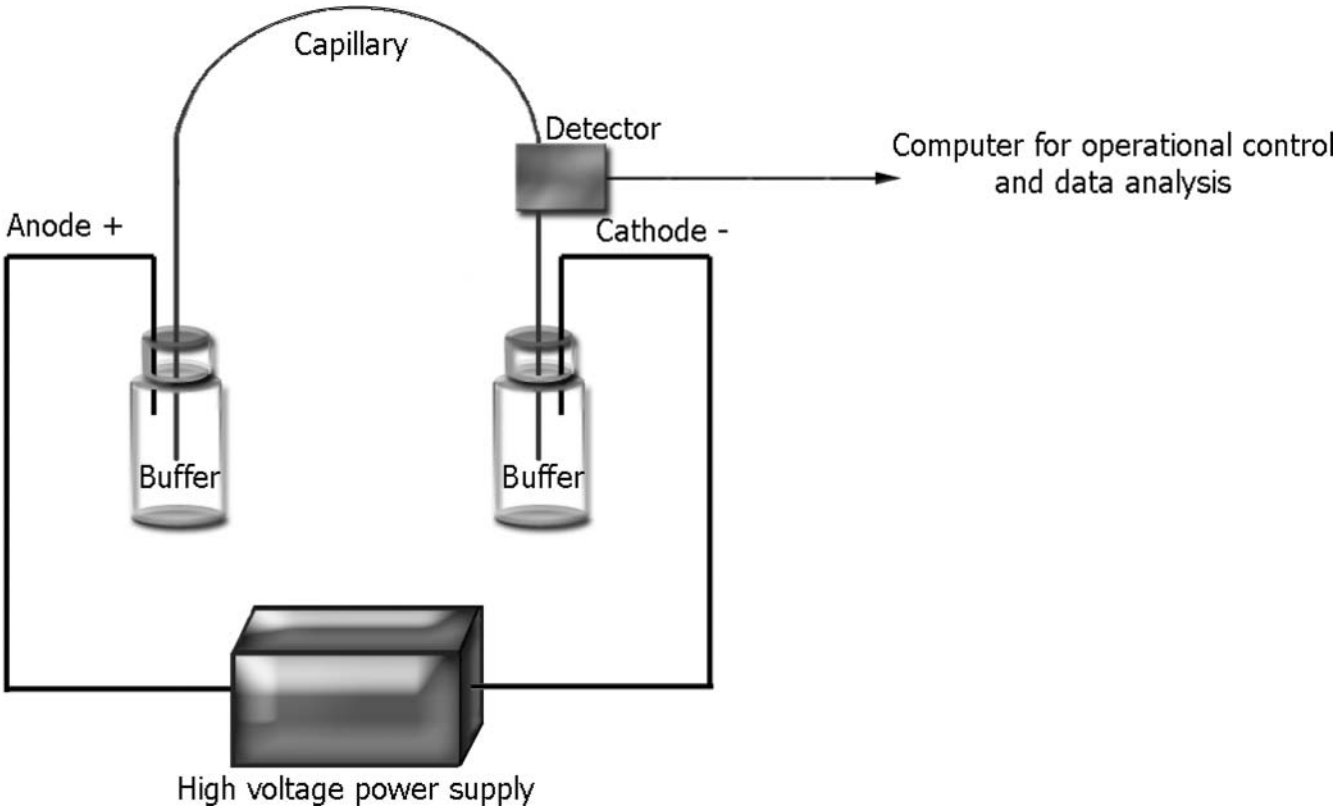

FIG. 4. CE equipment. For sampling, the buffer reservoir is replaced by a sample reservoir.

have made improvements in recently upgraded equipment. There is also a trend towards well-controlled and validated chemistry/capillary kits to improve performance, for example, in inorganic anions and metal ion determinations. The emergence of more powerful, automated instruments makes this technique more accessible than ever. There are some signs towards the better future of CE. It is expected (http://chromatographyonline.findanalytichem.com/lcgc/Technology-Forum-Capillary-Electrophoresis/ArticleStandard/Article/detail/618413) that the use of CE for small inorganic anion and metal ion analyses will replace ion-exchange chromatography. Chiral analysis by CE is well established. CE is used for screening and characterization (determination of pKa, solubility, etc.) of compounds. Dedicated equipment and related kits/capillaries will be applied to specific protein characterization/assays. CE is far superior to either sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) or IEF gels. However, CE should not replace HPLC. It is a technique that is complementary/orthogonal to HPLC. Proponents of CE believe that outdated thinking among the part of separation scientists is the biggest obstacle to acceptance of the technique. The method still needs the support of instrument manufacturers (to build better and more robust instrumentation), consumables vendors (to continue to devise kits and reagents), and scientists themselves (to develop novel methodologies and applications) (http://chromatographyonline.findanalytichem.com/lcgc/Technology-Forum-Capillary-Electrophoresis/ArticleStandard/Article/detail/618413). Without this type of investment, the technique cannot expand, but without expansion, no one wants to invest. Wider acceptance of green chemistry by analysts may help to break this impasse.

CE as a Method of Choice for Portable Instruments

It is difficult to imagine a portable gas or liquid chromatograph meeting all these conditions, although it might be technically possible (portable gas chromatographs are used successfully in space applications). Nevertheless, chromatography is not well suited to portability because eluent is required for analysis. There are, however, publications on field-portable gas (Contreras et al., 2008; Lin et al., 2008) and liquid chromatographs (Nelson et al., 2004). On the other hand, CE is a promising technology for field instruments. The main thrust of portable CE systems development has been towards chip-based miniaturized CE. However, capillary-based (non-chip) portable CE systems have certain unmatched advantages as recently described by Ryvolová et al. (2010). These include the relatively simple cylindrical geometry of the CE capillary, maximum volume-to-surface ratio, no requirement to design and fabricate a chip, the low costs of capillary compared to chip, and better performance with some detection techniques. On the other hand, microfluidic chips—the key components of LOC devices—have frequently been designed for very specific applications. They are relatively

expensive and unique. Compared to portable field analyzers based on "classical" CE, in which a capillary can easily be discarded if a problem occurs, custom-made microfluidic chips are definitely not yet disposal products. This condition restricts their use in field applications. Portable non-chip CE instruments can be constructed easily because the power consumption of the electrophoresis process in a capillary is small, so small-sized high voltage power supplies can be used. Optical detection is not well suited for portable instruments; however, the emergence of small-sized light-emitting diodes will soon change that situation. da Silva and do Lago (1998) and Zemann et al. (1998) have developed a useful detection device: a contactless conductivity detector (CCD) that measures the conductance of a small cap between tubular electrodes laid on the separation capillary. The device is intrinsically small in size, and most analytes can be detected with a CCD. Several groups have developed new portable CE instrument designs. Hauser's group (Kuban et al., 2007) has developed and optimized a portable CE instrument with CCD for the sensitive field measurement of ionic compounds in environmental samples. Inorganic cations and anions, including ions of heavy metals and arsenates, can be determined with detection limits of approximately 0.2 to 1 mM. Nitrite and ammonium were determined on-site at concentrations as low as 10 ppb in the presence of other common inorganic ions at concentrations two to three orders of magnitude higher. Another CE instrument based on a sequential injection analysis (SIA) manifold was reported by the same group (Mai et al., 2010). Hydrodynamic injection was implemented by using a split-injection device based on a needle valve for precise adjustment. The system was successfully tested in the field for the determination of the concentration levels of major inorganic cations and anions in a creek over a period of 5 days.

In another publication, Haddad's group demonstrated the use of CE for the detection of explosives in the environment (Hutchinson et al., 2007). Instead of CCD, Haddad's team used indirect photometric detection. They proved that it is possible to analyze blast residues at a crime scene, where they can be sampled simply by wiping hard surfaces with a wet cloth, rather than by transporting the residues back to the laboratory. They found that they could separate and detect the 12 cations at concentrations as low as 0.11mg/L and separate and detect the 15 anions at concentrations as low as 0.24 mg/L. In both cases, the analyses took less than 10 minutes. However, they found that CCD performed better than indirect photometric detection (Hutchinson et al., 2008). Optical detection was used also in a portable CE system developed for the identification of cattle breeds (Lee et al., 2010). The identification of Korean cattle and Holstein was based on the difference in the DNA mobility of the microsatellite and single nucleotide polymorfism (SNP) markers using a diode-pumped solid-state laser induced flourescence (LIF) detector. The system had a weight of only about 8kg. The specific sizes of genes were quickly separated, detected and precisely identified as the model cattle breeds within 32 and 3.5min, respectively.

Seiman et al. (2009) developed a robust sampling procedure for on-site analysis. In this project, the CE analyzer consisted of two pieces of capillary that were separated by a narrow gap (30 μm). To introduce the sample, a plastic syringe was inserted into a socket connected to the gap, and the background electrolyte (BGE) in the cross-section of the sampler was flushed out by the sample stream injected by the syringe. Then the sample between the capillaries was carried into the separation channel by electroosmotic flow (EOF), and BGE filled the junction between the two capillaries as soon as high voltage was applied. By this method, the manipulation of buffer vials is reduced. The method developed for this instrument has an LOD of 4–8 μM for phosphonic acids and 0.3–0.5 μM for cations, and an RSD (internal standard) of 8%.

The complexity of construction can be reduced even further. A possible design for what is conceivably the simplest portable instrument is demonstrated in Fig. 5. An analysis compartment with buffer vials, sampling syringe and a CCD sensor is located in the front part of the instrument and small lap-top computer. A typical electropherogram of the phosphonic acids recorded with this instrument is shown in Fig. 6.

As we will see in the next paragraphs, electrophoresis is a key technology for micronizing analytical separation methods even further by making use of an advanced concept based on lab-on-a-chip platforms. It is believed that this will open the way to many inexpensive point-of-care medical diagnostic devices. Many reports on portable CE analyzers based on microfluidics platforms have been published in recent years [see the following publications for examples (Kaigala et al., 2009; Kaigala et al., 2008)]. CE-based microfluidic devices will be described in the following paragraphs.

Micronization of Separation Methods

Analytical chemistry deals with acquiring and processing information about chemicals in our environment. It should consume no more resources than are needed to obtain information about the sample. Contemporary analytical chemistry must consume fewer resources, and the most acute problem is the usage of toxic compounds and solvents. This is the driving force behind the miniaturization of analytical chemistry. As we saw when discussing "black and white" issues in green chromatography (p 6), the greenness of elevated temperature chromatography remains ambiguous because the consumption of energy is not taken into account. Miniaturization of various analytical methods is on the "cutting edge" of research at the moment because it can provide the solution to energy economy as well. It is not surprising that many miniaturized analytical methods and technologies were inspired by developments in information technology (such as computer chips) where the miniaturization process has been underway for many decades. Frequently miniaturized analytical instruments copy the architecture of computer components. Miniaturization, known as "microfluidics", has influenced separation science in general and electrophoresis in particular. Microfluidics handles volumes of fluid on the order

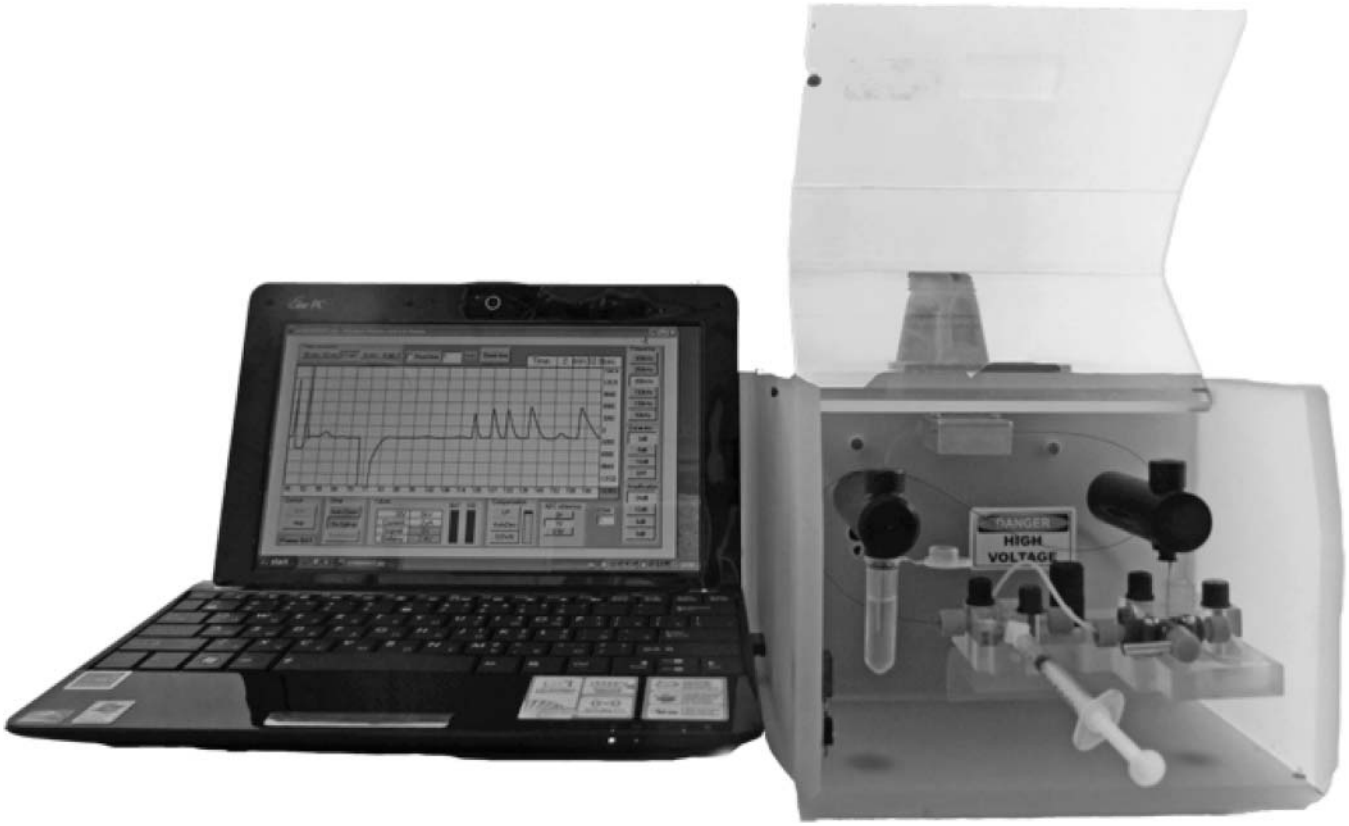

FIG. 5. Portable CE instrument controlled via a labtop computer. The electronics and high voltage power supply is located on the back part of the box. In the front, there is the CE compartment with sample/buffer vials, sampling syringe and CCD bloc. High voltage is applied to vials via electrodes located inside of the two plastic insulators (black rods). (Photograph of the working instrument from the authors laboratory).

of nanoliters and picoliters. Recent trends in microfluidiscs have been described thoroughly by Mark et al. (2010). They point out the expectations connected with the microfluidics saying that microfluidic platforms have to compete against these established systems by offering new opportunities. Expectations often quoted in this context are:

- Portability/wearability,
- Higher sensitivity,
- Lower cost per test,
- Shorter time-to-result,
- Less laboratory space consumption.

This list definitely points to the green features of microfluidics. The activity in this field is indeed enormous. A microfluidic platform in general can be characterized as continuous flow (lateral flow, linear actuated, pressure driven laminar flow, centrifugal microfluidics, and electrokinetic devices) and digital based (electrowetting and segmented flow microfluidics).

Continuous-Flow Microfluidics

Continuous-flow microfluidics deals with the precise control and manipulation of fluids that are geometrically constrained to small (typically sub-millimeter) channels. "Micro" in this context means the following features: small volumes (nL, pL, fL), small size, low energy consumption, and other effects of the micro scale on fluids. The flow of liquid is actuated either by external pressure sources (external pumps or integrated micropumps) or by electrokinetic mechanisms. Microfluidic devices are well suited for many simple biochemical applications and for chemical separations. Continuous-flow operation is the mainstream approach to microfluidics.

Methods of fabricating microfluidic devices have been inspired by photolithography, a technology for manufacturing computer chips. Initially, most systems were made of silicon, but because of demand for such features as specific optical characteristics, bio- or chemical compatibility, lower production costs, and faster prototyping, various new substrates such as glass, ceramics and metal etching, deposition, and bonding have been proposed. Soft lithography, used for fabricating microfluidic channels in polydimentylsiloxsane (PDMS), is especially popular. Typically, a "negative" template is etched on silicon or glass, which is then coated with "Slygard," a commercially available product, and cured to make a PDMS polymeric substrate for the device (Xia and Whitesides, 1998). The channels are covered

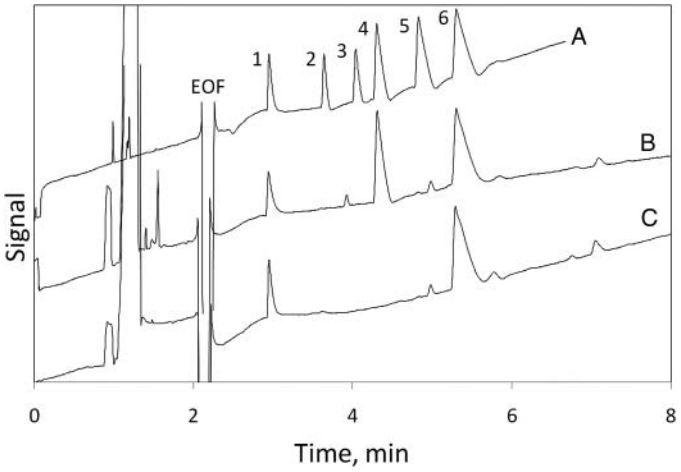

FIG. 6. An example of on-site analysis of phosphonic acids by a portable CE instrument. Sample collection: wiped with DI water pre-moistened Ghost Wipe (Environmental Express, Mt. Pleasant, South Carolina, USA) tissue. (A) Standard mixture of phosphonic acids; (B) Acids sample collected from concrete matrix. Peaks: EOF - neutral marker (H_2O), 1 - 2-aminoethyldihydrogenphosphonate (AEDHP), 2 - pinacolylmethylphosphonate (PMPA), 3 – isopropylmethylphosphonic acid (IMPA), 4 - ethylmethylphosphonate (EMPA), 5 - methylphosphonic acid (MPA), 6 - salicylic acid; (C) blank of concrete matrix. Separation condition: 7.5 mM MES/His buffer, 16kV separation voltage, capillary length 33/38 cm, inner diameter 0.75 μm. (From the authors' laboratory).

with a glass or polymer plate after curing, and detectors and other supporting pieces of equipment, such as electrodes for carrying voltage to the microchip, are attached. Microfluidics has increasingly more in common with lithography-based microsystem technology, nano technology, and precision engineering.

One disadvantage of continuous flow microfluidic systems is that they are less suitable for tasks requiring complicated fluid manipulations or a high degree of flexibility. The fluid flow at any location in the channels is dependent on the properties of the entire system. Permanently etched microstructures also lead to limited reconfigurability and poor fault tolerance.

Droplet and Digital Microfluidics

Droplets are used and manipulated in continuous microfluidics as a separate liquid phase differentiated from the carrier by chemical composition. This approach is referred to as "droplet" microfluidics. Novel alternatives to the closed-channel continuous-flow systems described above are open structures, in which discrete, independently controllable droplets are manipulated on a planar substrate. Using an analogy to digital microelectronics, this approach is referred as "digital" microfluidics (DMF), and was pioneered at Duke University (Pollack et al., 2000). It should not be confused with droplet microfluidics. In droplet microfluidics, individual droplets are held in channels and the droplets are not controlled independently. DMF uses discrete unit-volume droplets, and one unit of fluid is moved over one unit of distance. This facilitates the use of microfluidic biochip design approaches such as cell-based assays. Because each droplet can be controlled independently, these systems can be reconfigured (reprogrammed) to change their functionality during the concurrent execution of a set of bioassays.

In droplet microfluidics, two of the most common methods of forming droplet streams in channels are T-channel geometry and flow-focusing (Chiu et al., 2009). In T-channel geometry, a perpendicular flow of continuous oil phase meets the inlet of the aqueous phase, where droplets are generated. Separating and analyzing the content of droplets is highly desirable; CE is particularly suited for separating and analyzing ultra-small-volume droplets because of its high separation efficiency, speed, and sensitivity. The content of droplets can be analyzed by directing them fluidically to the CE separation channel via sophisticated microchannel manifolds (Edgar et al., 2006; Roman et al., 2008). The flow-focusing method relies on three

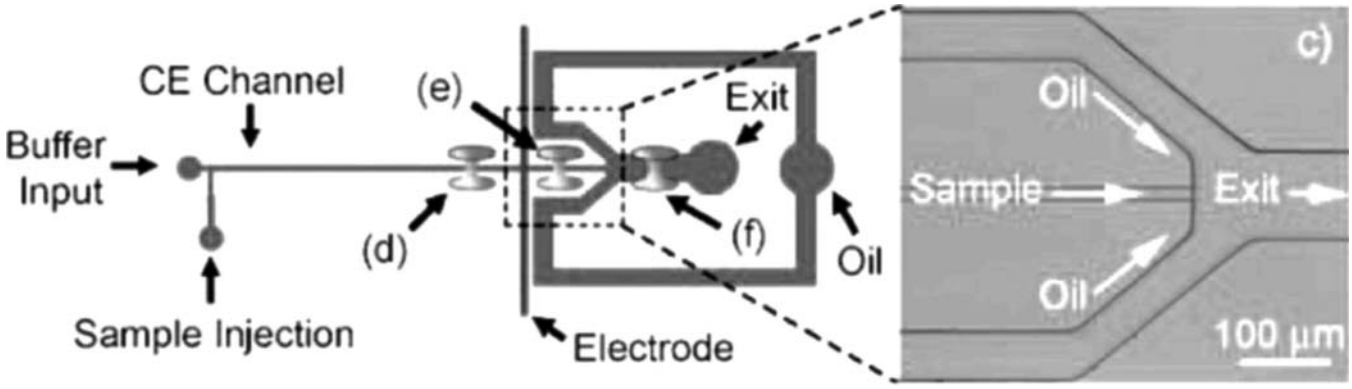

FIG. 7. Schematic representation of the fluidic design used to integrate CE output with droplet compartmentalization. c) Droplet-formation region shown in detail. d), e), and f) are the locations of detection spots. Oil channels: 50 × 50 mm; exit channel: 50 × 100 mm; CE channel 10 × 10 mm. [From (Edgar et al., 2006) with permission].

parallel flows. The aqueous phase is located between two oil-phase flows. These three flows converge on an orifice to break the aqueous flow into droplets. In this way, eluted CE zones can be converted into individual droplets, thus preventing their dilution and loss of the separated components, and facilitating their downstream manipulation and analysis. Edgar et al. (2009) performed the integration of CE with droplet generation driven by electro-osmotic flow, which enabled the compartmentalization of molecular components separated by CE into a series of droplets (see Fig. 7). The droplet-confined bands can be docked for further study.

Digital microfluidics devices are not yet very common; therefore, we will devote a few words to their preparation. One common actuation method for digital microfluidics is the electrowetting-on-dielectric (EWOD) phenomenon. (Other techniques for droplet manipulation using surface acoustic waves, optoelectrowetting, etc. have also been demonstrated recently). Briefly, EWOD is the phenomenon by which an electric field changes the contact angle and thus the wetting behavior of a polarizable and/or conductive liquid droplet in contact with a hydrophobic, insulated electrode. The insulating film is frequently made of two layers of Paralyene C and Teflon-AF. The application of voltage to a series of adjacent electrodes (that can be turned on or off) can be used to manipulate droplets because the semi-spherical shape of the droplet becomes asymmetrical and the internal tension of the droplet actuates its movement (Berthier, 2008). This effect is illustrated in Fig. 8. Droplets can be sandwiched between two parallel plates to prevent evaporation, or an open format can be used without an upper glass plate, enabling easier access to the droplets for further study.

Digital microfluidics is an attractive platform for biological applications, which often require the use of expensive or rare reagents and small amounts of samples.

Digital microfluidics has been applied for solving various biological problems such as enzymatic- and immunoassays, proteomics, and DNA analysis. Applications such as cell-sorting and cell-based assays, polymerase chain reaction (PCR), and pyrosequencing have been reported. These and several others are described by Miller and Wheeler (2009).

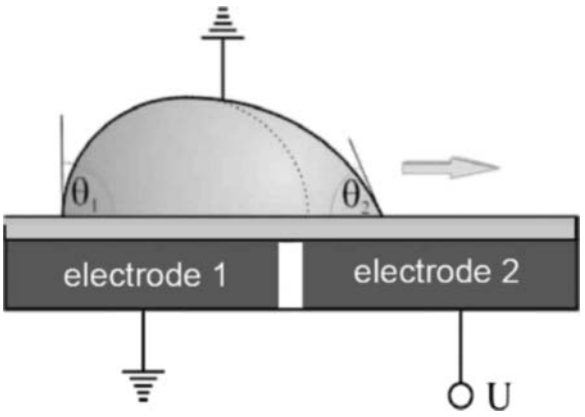

FIG. 8. Droplet deformation due to the application of electric potential to one of the electrodes in an open setup. Droplet movement results from a change in geometrical shape of the droplet.

The number and variety of analyses being performed on chip has increased with the need to perform multiple sample manipulations. As in the case of droplet microfluidics, components that produce a signal of interest must be isolated in order to be detected. Although DMF could be a good platform for miniaturizing separation techniques, mass separation methods such as CE are not currently an established part of the digital microfluidic toolkit and the integration of separation methods seems to present a significant challenge. There have been very few attempts to perform molecular separation on a digital microfluidic platform. In one example, Shah and Kim (2009) achieved high-purity separation using EWOD-based droplet microfluidics by introducing a "fluidic conduit" between a sample droplet and a buffer droplet. The long, slender fluidic path minimizes the diffusion and fluidic mixing of the two droplets, which eliminates non-specific mass transport but provides a channel between them for actively transporting particles (thus allowing specific transport). The effectiveness of the technique was demonstrated by eliminating approximately 97% of non-magnetic beads in one purification step, while maintaining high collection efficiency (>99%) of the magnetic beads (Shah and "CJ" Kim, 2009). A recent publication by Abdelgawad et al. (2009) described a hybrid microfluidic device in which a sample was delivered to the separation chip channel by a DMF device. In another study, a common CE device with a contactless conductivity detector was interfaced to DMF platforms (Gorbatsova et al., 2009). In this research, to pursue "the spirit of low-cost digital microfluidics" advanced by Abdelgawad and Wheeler (2008), it was demonstrated that the actuation of droplets can be achieved using an electrode system prepared from the copper substrate of a common printed circuit coated only with food wrap (without the hydrophobic layer). The DMF sample injection was performed by transporting sample and buffer droplets in succession under the end of the CE capillary inlet, immersing the capillary in the sample/buffer droplet, and performing CE separation by applying a high voltage between the (grounded) buffer droplet and the CE outlet reservoir. Using a DMF sampler, CE separation of a mixture of vitamins was achieved (Gorbatsova et al., 2009).

World-to-Chip Interfacing and the Quest for a "killer" Application in Microfluidics

The connection between the components of a microfluidic device and the macro-environment of the world is often referred to as a macro-to-micro interface (Jesson et al., 2003), interconnect (Chen et al., 2003; Gonzalez et al., 1998; Nittis et al., 2001; Puntambekar and Ahn, 2002), or world-to-chip interface (Attiya et al., 2001; Bings et al., 1999; Liu et al., 2003; Ramsey, 1999; Yang and Maeda, 2002). This is a problem that jeopardizes the greenness of microfluidic devices. The difficulty results from

the fact that samples and reagents are typically transferred in quantities of microliters to milliliters (or even liters), but microfluidic devices deal only with nanoliters or picoliters of samples/reagents due to reaction chambers and channels that typically have dimensions on the order of microns. This challenge can be overlooked in research environments such as academic laboratories, but it erodes the founding pillar of green chemistry: minimal use of organic solvents. The "world-to-chip" problem cannot be ignored in routine applications.

The so-called world-to-chip interface problem has plagued microfluidics since its inception. Microfluidics must integrate all components of the system on the same chip to ensure the portability and minimum energy consumption required by the principles of green analytical chemistry. Pumps, valves, mixers, etc. must be miniaturized in order to achieve an integrated system, which forces a choice between active methods—efficient, but requiring energy sources and difficult to miniaturize—and the passive methods provided by non-instrumental microfluidics—easier to integrate, but less efficient. This is a huge challenge, especially in biotechnology, where the volume of the targets of study located in the macroscopic environment must be reduced to accommodate the microscopic environment of the microfluidic device. Finally, the huge surface/volume ratio of miniaturized systems could modify the physical behavior of the system, giving rise to new problems, such as adherence of target molecules to the solid walls, or the effect of capillary forces that may prevent the fluid from entering the microchannels.

According to some researchers, the dilemma of the world-to-chip interface is one of the bottle-necks in the development of μTAS (Zhong et al., 2008). It is critical for high-throughput applications where manual manipulation is not economical and a macro-to-micro interface must be developed. The solutions described above—droplet and digital microfluidics for sample processing and injection, continuous flow microfluidics or CE for molecular separation, and CCD and LED for detection—could well be combined into a portable instrument consuming little energy and material. The main breakthrough could well come from such integration.

Almost 10,000 papers have been published over the last 10 years on the topic of microfluidics and the annual numbers of new publications are increasing continuously. Still, there is a rising concern that regardless of the 10,000 available publications, offering solutions for almost every problem that might occur, the development of a lab-on-a-chip product is still a risky adventure. The lab-on-a-chip has frequently been designed for a very specific application. Academic institutions and corporations have developed a plethora of lab-on-a-chip devices for different applications, but what is really needed is a universal application that could trigger widespread use of microchips in biomedicine. There is a general consensus that miniaturization should have advanced more quickly than it has. A few years ago, many analytical chemists were enthusiastic about chip-based analyses, but now one can sense disappointment and disillusionment. If there is no widely used application, then the development of microfluidic devices is not economically profitable. This phenomenon is known as the absence of a "killer" application. What is a killer application? The term ". . . is commonly used to describe a product which has such highly desirable properties that it generates very large revenues with attractive margins in a comparatively short amount of time. In addition to this purely economic description it also helps to promote the underlying technology, thus helping typically "disruptive technologies" (a technology that enables products which dramatically change markets due to their (often unexpected) performance and which are not achievable by simple linear extrapolation of existing products or technologies" (Becker, 2009). Examples of killer applications are digital photography and large flat panel television screens. Let us hope that a killer application will soon be found, that the world-to-chip problem will be solved as quickly as possible, and that obstacles to developing the ultimate green analysis method will be surmounted.

Non-Instrumental Microfluidic Devices

As we saw above, the typical setup for a microfluidic experiment consists of a small custom-made chip that is surrounded by desktop-sized analysis instruments and power supplies. The microminiaturization of the main analysis process is only part of the story, and to make analytical methods environmentally friendly, one must solve this world-to-chip interface problem. Therefore, any rapid prototyping technique which is flexible and robust to accommodate different aspects of microfluidic integrations is of interest. There is an active search for the simple methods of preparation of microfluidics chips. Recently a rapid prototyping technique of microfluidic devices was reported using adhesive transfer tapes (Nath et al., 2010). Prototypes demonstrating microfluidic mixing, dielectrophoretic trapping, complex microchannel networks and biologically relevant high temperature reactions were fabricated in less than 30 minutes. A novel ready to use world-to-chip interface was also developed using the same fabrication platform. All components (e.g., tapes, electrodes, acoustic sources or heaters) were obtained as finished products alleviating any chemical or clean-room specific processing. Using a desktop digital craft cutter is another method for rapid prototyping of flexible microfluidic devices. This method can achieve microchannels as thin as 200 μm in width and can be used to fabricate three-dimensional microfluidic devices using only double-sided pressure sensitive adhesive tape and laser printer transparency film (Yuen and Goral, 2010). A craft cutter was also proposed for shrink film patterning for producing microfluidics chips with high resolution/high-aspect ratio channels (Taylor et al., 2010).

One way to miniaturize supporting instruments is to replace complex elements in analyzers with passive components that operate without external power by manipulating fluids using gravity, air pressure, or simple manual actions. The need to develop such simple and possibly disposable devices is motivated primarily by the need for simple POC tests in developing countries, where non-instrumental analytical devices could be put to

good use in medical diagnostics. In a recent report on the top ten biotechnologies for improving health in developing countries, "modified molecular technologies for affordable, simple diagnosis of infectious diseases" were ranked as the number one priority (Chin et al., 2007). On the other hand, such devices can also find application in developed countries where, although most medical diagnostics are performed in centralized, well-equipped hospital laboratories, home tests have a place as well. Two of those applications are home glucose and pregnancy testing and detection by first responders of natural or man-made bio-emergencies (bioterrorism).

On test strips (e.g., pregnancy test strip), the liquids are driven by capillary forces. Liquid movement is controlled by the wettability and by the microstructure of a substrate. All required chemicals are pre-stored within the strip. The readout of a test is typically by the naked eye as a color change in the detection area. Based on this technique, the commonly known "over-the-counter pregnancy test" was introduced into the market in the middle of the 1980s. Today, this microfluidics platform is commonly designated as a "lateral flow test" (ICS). Other terms are "test strip", "immunochromatographic strip", "immunocapillary tests" or "sol particle immunoassay" (Mark et al., 2010). Urine, saliva, serum, plasma, or whole blood can be used as specimens. To perform the test, a sample is placed on a pad at one end of the strip. The signal reagent is solubilized and binds to the antigen or antibody in the sample and moves through the membrane by capillary action. If a specific analyte is present, the signal reagent binds to it. The complex proceeds to the second antibody or antigen, and is immobilized at a test line on the strip, which captures the complex. If the test is positive, a pink/purple line develops. Moving further along the strip, the signal reagent encounters a second set of antibodies and forms an antibody-analyte-antibody sandwich matrix with a visible (control) signal. Because ICS relies on inexpensive reagents and components, the cost is less than $2 US to the end user in many cases. ICS strips require little or no sample processing, and they do not require an external instrument.

For these applications, paper is the best prospective material. It has potential as an inexpensive, biodegradable, renewable, flexible polymer substrate for designing lab-on-a-chip prototypes. Paper-based, three-dimensional microfluidic devices can be constructed with capabilities that are difficult to achieve using conventional open-channel microsystems made from glass or polymers. In particular, paper-based devices wick fluids and they can distribute microliter volumes of samples from single inlets to arrays of detection points. This capability opens the way to carrying out a variety of new analytical protocols simply and inexpensively on a piece of paper without external pumps. Much of this line of study has been pursued by Whitesides and his colleagues at Harvard University. They have achieved the ultimate operational robustness and cost reduction with microfluidic devices to date. They demonstrated that three-dimensional microfluidic devices could be made from stacked layers of ordinary paper and sticky tape (Cheng et al., 2010). Because of paper's wicking ability, the devices don't require external pumps to drive the liquids through. To define the pathways of the liquids in such a paper-based microfluidic device, the team impregnated each layer of paper with a common photoresist, and patterned them with UV light. With channels thus established on a sheet of paper, layers of paper were alternated with layers of double-sided tape; holes cut in the tape connected the channels in adjacent layers of paper. The complex routing that can be achieved can wick liquid horizontally and vertically to an array of 1024 detection zones underneath. With reagents or antibodies placed in detection zones prior to assembly, such devices could provide highly parallel, independent assays. The results could be transmitted to a central hospital using camera phones (Martinez et al., 2008). Wax printing simplifies the fabrication of paper-based microfluidics even further. The fabrication of paper-based microfluidic devices in nitrocellulose membrane for protein immobilization was reported in (Lu et al., 2010). The fabrication process, which can be finished within 10 minutes, is comprised mainly of printing and baking. Other methods are reported for patterning paper like paper sizing for fabrication of paper-based microfluidic sensors (Li et al., 2010). Patterned paper sizing uses paper sizing agents to selectively hydrophobize certain area of a sheet.

The success of this approach depends on the absorption of test fluids into hydrophilic areas of porous paper and the use of capillary forces for fluid actuation. The products of reactions occurring inside such labs-on-paper (LOP) cannot easily be extracted for further biochemical analysis. If wicking is undesirable, one approach would be to develop an LOP device capable of storage, transfer, mixing, and sampling of liquid drops by making the surface of the paper superhydrophobic (by oxygen plasma etching and fluorocarbon film deposition) and marking it with high surface energy ink patterns (lines and dots) using simple software similar to that used for word processing (Balu et al., 2009). Surface energy and gravitational force can then be used to manipulate and transfer drops, thus eliminating the need for an external power source.

Paper-based microfluidic devices are especially appropriate for use in distributed healthcare in the developing world and in environmental monitoring and water analysis (Martinez et al., 2010). It is obvious that components of non-instrumental, microfluidics-based disposable diagnostic devices must function on simple physical-chemical phenomena like capillary action, evaporation, endo/exothermic reaction, gravity, and laminar flow in microchannels. Some of the energy would be supplied by the analyst because a power supply would likely not be available. In their review, Weigl et al. (2008) give many examples of components designed to perform unit operations:

- Liquid transport (pumps) can be achieved by wicking and capillary action, gravity, or finger-operated bellows fabricated from polydimethylsiloxane (PDMS).
- Mixing reagents and samples in microfluidic structures is challenging because of the laminar flow of liquids in

microchannels. Spiral microchannels, expansion vortices, channel obstacles, lamination splits, and recombining have been designed for passive methods such as transverse mixing wells.
- Flow switching can be implemented by means of microvalves, but the complexity of manufacturing has largely limited the application of this method.
- Molecular separation can be conducted by diffusion. An H-filter is a device based on the parallel laminar flow of two or more miscible streams in contact with each other. Because of the laminarity of the flow, the streams do not mix, but chemicals can diffuse from one stream to the other. Smaller molecules diffuse faster than larger ones and this phenomenon can be exploited to extract targeted components from one stream to another.
- A sample can be concentrated by evaporation.
- Localized heating and cooling can be effected by positioning the endothermic (e.g., evaporation of acetone) and exothermic (e.g., dissolution of concentrated sulfuric acid in water) processes in microreaction chambers near the reactant flow interface (Guijt et al., 2003).
- Detection in non-instrumental microfluidics relies solely on the physical senses and therefore can only be based on colorimetry.

In summary, the eventual goal of this line of research is to develop methods that do not require electronics, such as a piece of paper that can detect disease markers or pathogens, like litmus paper detects pH. Sample preparation and, if needed, even target amplification, are possible without the use of instrumentation, but is difficult to imagine how very low signal intensities could be observed without electronic signal amplification. Various colorimetric tests have been proposed for paper microfluidics devices (Dungchai et al., 2010). In other words, detection will become a bottleneck for POC design. This may limit the applicability of this type of assay where extremely high sensitivity is required. Therefore, the incorporation of simple instruments in POC devices could be tolerated. The successful integration of paper-based microfluidics and electrochemical detection has been demonstrated (Dungchai et al., 2009). Whitesides and others. developed an inexpensive handheld colorimeter that generates quantitative data in a point-of-care analytical system (Ellerbee et al., 2009). The aluminium-cased colorimeter has a tricolor light-emitting diode (LED) for illuminating colored spots on paper, and a manifold to hold the paper device in proper alignment with the LED for measurements. The LED light is modulated and detected by a narrow-bandpass detector; therefore, measurements can be performed in any lighting conditions. Whitesides speculates that there is ample scope for ingenious chemistry, because many colorimetric tests are potentially relevant to this type of analytical problem. Developing new dyes that have more pronounced color changes and are more stable would be very useful for POC diagnostics. Another example of an extremely simple non-instrumental microfluidics device is a chip fabricated by Grudpan et al. (2009). They made a simple chip by drilling channels in a piece of acrylic plastic. The chip was tilted to actuate sample and reagent flows by gravity. Different uses of the chip were demonstrated by reactions involving color detection. To eliminate the need for instruments such as spectrophotometers, detection was done visually, based on the migration time of the reaction zone, using a simple stopwatch.

Consumer electronics-based analytics could support non-instrumental microfluidics. Filippini et al. (2003) demonstrated how a combination of a computer monitor and an inexpensive webcam could be used as a spectrophotometer. This computer screen photo-assisted technique (CSPT) is based on the fact that a computer screen can easily be programmed to display millions of colors that combine three narrow band emission profiles. The light emitted from a computer screen is not monochromatic, but a combination of three polychromatic primary colors that excite the human perception of red, green, and blue. Personal computers are inexpensive and widely available. In this approach, the computer monitor acts as a light source and the camera as a detector. It is possible to envision the application of this type of system to non-instrumental microfluidics devices with colorimetric output. No reports of such applications are available, so we don't know what the detection limits of such devices would be, but the premise seems reasonable.

CONCLUDING REMARKS

Greening separation science is possible, but it might involve increasing the overall energy consumption of the separation process. One cannot overlook the possibility that even if green analytical laboratory practices are locally feasible, global green analytical chemistry might be regarded with scepticism. When energy consumption is taken into account, a completely green chromatography is probably impossible. Even GC, the greenest mode of chromatography, consumes purified carrier gases, the extraction of which from the atmosphere produces a measurable carbon footprint. One way to reduce energy consumption is to miniaturize the entire analytical process. As we have previously pointed out, analytical chemistry is an information science that does not consume more resources than are required to support the process of analysis. This should be borne in mind by anyone who is tempted to acquire a new model of an instrument that is based on well known phenomenon. The greening of analytical chemistry via miniaturization seems to be possible in principle, and the development of greener instrumentation is only limited by the creativity of the analyst. Research and development of "smart," miniaturized POC instrumentation would be highly advantageous. The most pressing issue seems to be solving the world-to-chip problem. In this respect an emerging new science—non-instrumental microfluidics—looks very promising because it is a genuinely green separation method. Paper microfluidics devices use simple colorimetric tests for detection. It might not be too presumptuous to say that because

the small number of assays would limit the potential usefulness of a class of methods based solely on the color perception of the operator, gradient elution RP-HPLC-ESI-Q-TOF-MS/MS might be the only viable option for solving an analytical problem. As stated by de la Guardia and Armenta (2011), "The incorporation of green parameters in the evaluation of existing and new methods, the economic balance of their applicability from both economical and environmental view points, together with the miniaturization and automation of methods. . . [are] . . . the basis for a change of mentality of method developers and users in order to move from pollutant practices to clean ones."

REFERENCES

Abdelgawad, M.; Watson, M. W. L.; Wheeler, A. R. Hybrid microfluidics: A digital-to-channel interface for in-line sample processing and chemical separations. *Lab Chip* **2009**, *9*, 1046–1051.

Abdelgawad, M.; Wheeler, A. R. Low-cost, rapid-prototyping of digital microfluidics devices. *Microfluid Nanofluid.* **2008**, *4*, 349–355.

Alpert, A. J. Hydrophilic- interaction chromatography for the separation of peptides, nucleic-acids and other polar compounds. *J. Chromatogr.* **1990**, *499*, 177–196

Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, 1998.

Attiya, S.; Jemere, A.; Tang, T.; Fitzpatrick, G.; Seiler, K.; Chiem, N.; Harrison, D. J. Design of an interface to allow microfluidic electrophoresis chips to drink from the fire hose of the external environment. *Electrophoresis* **2001**, *22*, 318–327.

Balu, B.; Berry, A. D.; Hess, D. W.; Breedveld, V. Patterning of superhydrophobic paper to control the mobility of micro-liter drops for two-dimensional lab-on-paper applications. *Lab Chip* **2009**, *9*, 3066–3075.

Becker, H. Hype, hope and hubris: the quest for the killer application in microfluidics. *Lab. Chip* **2009**, *9*, 2119–2122.

Berthier, J. *Microdrops and Digital Microfluidics*; William Andrew Inc.; Norwich, NY, 2008.

Bings, N. H.; Wang, C.; Skinner, C. D.; Colyer, C. L.; Thibault, P.; Harrison, D. J. Microfluidic devices connected to fused-silica capillaries with minimal dead volume. *Anal. Chem*. **1999**, *71*, 3292–3296.

Chen, H.; Acharya, D.; Gajraj, A.; Melners, J.-C. Robust interconnects and packaging for microfluidic elastomeric chips. *Anal. Chem*. **2003**, *75*, 5287–5291.

Cheng, C. M.; Martinez, A. W.; Gong, J. L.; Mace, C. R.; Phillips, S. T.; Carrilho, E.; Mirica, K. A.; Whitesides, G. M. Paper-Based ELISA. A*ngew. Chem. Int. Ed.* **2010**, *49*, 4771–4774.

Chester, T. L.; Pinkston, J. D. Supercritical fluid and unified chromatography. *Anal. Chem.* **2004**, *76*, 4606–4613.

Chin, C. D.; Linder, V.; Sia, S. K. Lab-on-a-chip devices for global health: Past studies and future opportunities. *Lab. Chip.* **2007**, *7*, 41–57.

Chiu, D. T.; Lorenz, R. M.; Jeffries, G. D. M. Droplets for Ultrasmall-Volume Analysis. *Anal. Chem*. **2009**, *81*, 5111–5118.

Contreras, J. A.; Murray, J. A.; Tolley, S. E.; Oliphant, J. L.; Tolley, H. D.; Lammert, S. A.; Lee, E. D.; Later, D. W.; Lee, M. L. Hand-Portable Gas Chromatograph-Toroidal Ion Trap Mass Spectrometer (GC-TMS) for Detection of Hazardous Compounds. *J. Am. Soc. Mass Spect.* **2008**, *19*, 1425–1434.

Cramers, C. A.; Jannssen, H.-G.; Van Deurse, M. M.; Leclerc, P. A. High-speed gas chromatography: an overview of various concepts. *J. Chromatogr. A* **1999**, *856*, 315–329.

da Silva, J. A. F.; do Lago, C. L. An oscillometric detector for capillary electrophoresis. *Anal. Chem*. **1998**, *70*, 4339–4343.

de la Guardia, M.; Armenta, S. Ideas for a Change of Mentality and Practices, in *Comprehensive Analytical Chemistry Green Analytical Chemistry* **2011**, *57*, 207–218.

dos Santos Pereira, A.; David, F.; Vanhoenacker, G.; Sandra, P. The acetonitrile shortage: Is reversed HILIC with water an alternative for the analysis of highly polar ionizable solutes? *J. Sep. Sci.* **2009**, *32*, 2001–2007.

Dungchai, W.; Chailapakul, O.; Henry, C. S. Electrochemical Detection for Paper-Based Microfluidics. *Anal. Chem*. **2009**, *81*, 5821–5826.

Dungchai, W.; Chailapakul, O.; Henry, C. S. Use of multiple colorimetric indicators for paper-based microfluidic devices. *Anal. Chim. Acta* **2010**, *674*, 227–233.

Edgar, J. S.; Milne, G.; Zhao, Y. Q.; Pabbati, C. P.; Lim, D. S. W.; Chiu, D. T. Droplet Compartmentalization of Chemical Separations. *Angew. Chem. Int. Ed*. **2009**, *48*, 2719–2722.

Edgar, J. S.; Pabbati, C. P.; Lorenz, R. M.; He, M. Y.; Fiorini, G. S.; Chiu, D. T. Capillary electrophoresis separation in the presence of an immiscible boundary for droplet analysis. *Anal. Chem*. **2006**, *78*, 6948–6954.

Ellerbee, A. K.; Phillips, S. T.; Siegel, A. C.; Mirica, K. A.; Martinez, A. W.; Striehl, P.; Jain, N.; Prentiss, M.; Whitesides, G. M. Quantifying Colorimetric Assays in Paper-Based Microfluidic Devices by Measuring the Transmission of Light through Paper. *Anal. Chem*. **2009**, *81*, 8447–8452.

Filippini, D.; Svensson, S.; Lundström, I. Computer screen as a programmable light source for visible absorption characterization of (bio)chemical assays. *Chem. Commun*. **2003**, *2*, 240–243.

Gonzalez, C.; Collins, S. D.; Smith, R. L. Fluidic interconnects for modular assembly of chemical microsystems. *Sens. Actuators, B* **1998**, *49*, 40–45.

Gorbatsova, J.; Jaanus, M.; Kaljurand, M. Digital Microfluidic Sampler for a Portable Capillary Electropherograph. *Anal. Chem*. **2009**, *81*, 8590–8595.

Greibrokk, T.; Andersen, T. High-temperature liquid chromatography. J. *Chromatogr. A* **2003**, *1000*, 743–755.

Grudpan, K.; Lapanantnoppakhun, S.; Kradtap Hartwell, S.; Watla-iad, K.; Wongwilai, W.; Siriangkhawut, W.; Jangbai, W.; Kumutanat, W.; Nuntaboon, P.; Tontrong, S. Simple lab-on-chip approach with time-based detection. *Talanta* **2009**, *79*, 990–994.

Guijt, R. M.; Dodge, A.; van Dedem, G. W.; de Rooij, N. F.; Verpoorte, E. Chemical and physical processes for integrated temperature control in microfluidic devices. *Lab Chip* **2003**, *3*, 1–4.

Harper, M. Sorbent trapping of volatile organic compounds from air. *J. Chromatogr. A* **2000**, *885*, 129–151.

Hartonen, K.; Riekkola, M.-L. Liquid chromatography at elevated temperatures with pure water as the mobile phase. *TrAC Trends in Analytical Chemistry* **2008**, *27*, 1–14.

He, Y.; Tang, L.; Wu, X.; Hou, X. Spectroscopy: The best way toward Green Analytical Chemistry? *Applied Spectroscopy Reviews* **2007**, *42*, 119–138.

Technology Forum—Capillary electrophoresis. **2009**, <http://chromatographyonline . findanalytichem . com / lcgc / Technology - Forum - Capillary - Electrophoresis / ArticleStandard / Article / detail / 618413>, last accessed December 2010.

Hutchinson, J. P.; Evenhuis, C. J.; Johns, C.; Kazarian, A. A.; Breadmore, M. C.; Macka, M.; Hilder, E. F.; Guijt, R. M.; Dicinoski, G. W.; Haddad, P. R. Identification of inorganic improvised explosive devices by analysis of postblast residues using portable capillary electrophoresis instrumentation and indirect photometric detection with a light-emitting diode. *Anal. Chem*. **2007**, *79*, 7005–7013.

Hutchinson, J. P.; Johns, C.; Breadmore, M. C.; Hilder, E. F.; Guijt, R. M.; Lennard, C.; Dicinoski, G.; Haddad, P. R. Identification of inorganic ions in post-blast explosive residues using portable CE instrumentation and capacitively coupled contactless conductivity detection. *Electrophoresis* **2008**, *29*, 4593–4602.

Hyötyläinen, T. Principles, developments and applications of on-line coupling of extraction with chromatography. *J. Chromatogr. A* **2007**, *1153*, 14–28.

J. Sep. Sci. **2001**, *24*, 899–959.

Jandera, P.; Blomberg, L.; Lundanes, E. Controlling the retention in capillary LC with solvents, temperature, and electric fields. *J. Sep. Sci*. **2004**, *27*, 1402–1418.

Jesson, G.; Kylberg, G.; Andersson, P. A versatile micro-to-macro dispensing system, in *Micro Total Analysis Systems 2003*, M. A. Nothrup; K. F. Jensen; D. J. Harrison, eds. (Transducers Research Foundation, San Diego CA, 2003, 155–158.

Jones, B. A. Temperature programmed liquid chromatography. *J. Liq. Chromatogr. Relat. Technol*. **2004**, *27*, 1331–1352.

Kaigala, G. V.; Behnam, M.; Bliss, C.; Khorasani, M.; Ho, S.; McMullin, J. N.; Elliott, D. G.; Backhouse, C. J. Inexpensive, universal serial bus-powered and fully portable lab-on-a-chip-based capillary electrophoresis instrument. *IET Nanobiotechnol*. **2009**, *3*, 1–7.

Kaigala, G. V.; Hoang, V. N.; Stickel, A.; Lauzon, J.; Manage, D.; Pilarski, L. M.; Backhouse, C. J. An inexpensive and portable microchip-based platform for integrated RT–PCR and capillary electrophoresis. *Analyst* **2008**, *133*, 331–338.

Kanazawa, H. Thermally responsive chromatographic materials using functional polymers. *J. Sep. Sci*. **2007**, *30*, 1646–1656.

Kikuchi, A.; Okano, T. Intelligent thermoresponsive polymeric stationary phases for aqueous chromatography of biological compounds. *Prog. Polymer Sci.* **2002**, *27*, 165–193.

Klesper, E.; Corwin, A. H.; Turner, D. A. High pressure gas chromatography above critical temperatures. *J. Org. Chem.* **1962**, *27*, 700–701.

Kobayashi, J.; Kikuchi, A.; Sakai, K.; Okano, T. Aqueous chromatography utilizing pH-/temperature responsive polymer stationary phases to separate ionic bioactive compounds. *Anal. Chem*. **2001**, *73*, 2027–2033.

Koel, M.; Kaljurand, M. Applications of the principles of green chemistry in analytical chemistry. *Pure Appl. Chem.* **2006**, *78*, 1993–2002.

Koel, M.; Kaljurand, M. Green Analytical Chemistry, *Royal Society of Chemistry* **2010**, 332p.

Kuban, P.; Nguyen, H. T. A., Macka, M.; Haddad, P. R.; Hauser, P. C. New fully portable instrument for the versatile determination of cations and anions by capillary electrophoresis with contactless conductivity detection. *Electroanalysis* **2007**, *19*, 2059–2065.

Lee, J. W.; Wankat, P. C. Comparison of Recycle Chromatography and Simulated Moving Bed for Pseudobinary Separations. *Ind. & Eng. Chem. Res*. **2009**, *48*, 7724–7732.

Lee, J. W.; Wankat, P. C. Optimized Design of Recycle Chromatography for Separation of a Single Component from a Ternary Mixture. *Ind. & Eng. Chem. Res.* **2008**, *47*, 9601–9610.

Lee, M.; Cho, K.; Yoon, D.; Yoo, D. J.; Kang, S. H. Portable capillary electrophoresis system for identification of cattle breeds based on DNA mobility. *Electrophoresis* **2010**, *31*, 2787–2795.

Li, X.; Tian, J. F.; Shen, W. Progress in patterned paper sizing for fabrication of paper-based microfluidic sensors. *Cellulose* **2010**, *17*, 649–659.

Lin, H. Q.; Ye, Q.; Deng, C. H.; Zhang, X. M. Field analysis of acetaldehyde in mainstream tobacco smoke using solid-phase microextraction and a portable gas chromatograph. *J. Chromatogr. A* **2008**, *1198*, 34–37.

Liu, J.; Hansen, C.; Quake, S. R. Solving the "world-to-chip" interface problem with a microfluidic matrix. *Anal. Chem*. **2003**, *75*, 4718–4723.

Lu, Y.; Shi, W.; Qin, J.; Lin, B. Fabrication and Characterization of Paper-Based Microfluidics Prepared in Nitrocellulose Membrane By Wax Printing. *Anal Chem*. **2010**, *82*, 329–335.

Mai, T. D.; Schmid, S.; Müller, B.; Hauser, P. C. Capillary electrophoresis with contactless conductivity detection coupled to a sequential injection analysis manifold for extended automated monitoring applications. *Anal. Chim. Acta* **2010**, *665*, 1–6.

Manz, A.; Graber, N.; Widmer, H. M. Miniaturized total chemical analysis systems: A novel concept for chemical sensing. *Sensors and Actuators B: Chemical* **1990**, *1*, 244–248.

Mark, D.; Haeberle, S.; Roth, G.; von Stetten, F.; Zengerle, R. Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications. *Chem. Soc. Rev*. **2010**, *39*, 1153–1182.

Martinez, A. W.; Phillips, S. T.; Carrilho, E.; Thomas, S. W.; Sindi, H.; Whitesides, G. M. Simple telemedicine for developing regions: Camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. *Anal. Chem*. **2008**, *80*, 3699–3707.

Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E. Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices. *Anal. Chem*. **2010**, *82*, 3–10.

Maxwell, E. J.; Chen, D. D. Y. Twenty years of interface development for capillary electrophoresis-electrospray ionization-mass spectrometry. *Anal. Chim. Acta* **2008**, *627*, 25–33.

Mayors, R. The Continuing Acetonitrile Shortage: How to Combat it or Live with it. *Chromatography Online. com* June 1, 2009.

Miller, E. M.; Wheeler, A. R. Digital bioanalysis. *Anal Bioanal Chem.* **2009**, *393*, 419–426.

Miserez, B.; Lynen, F.; Wright, A.; Euerby, M.; Sandra, P. Thermoresponsive Poly(N-vinylcaprolactam) as Stationary Phase for Aqueous and Green Liquid Chromatography. *Chromatographia* **2010**, *71*, 1–6.

Namiesnik, J.; Wardencki, W. Solventless sample preparation techniques in environmental analysis. *J. High Resol. Chromatogr* **2000**, *23*, 297–303.

Nath, P.; Fung, D.; Kunde, Y. A.; Zeytun, A.; Branch, B.; Goddard, G. Rapid prototyping of robust and versatile microfluidic components using adhesive transfer tapes. *Lab Chip* **2010**, *10*, 2286–2291.

Nelson, M. A.; Gates, A.; Dodlinger, M.; Hage, D. S. Development of a portable immunoextraction-reversed-phase liquid chromatography system for field studies of herbicide residues. *Anal. Chem*. **2004**, *76*, 805–813.

Nittis, V.; Fortt, R.; Legge, C. H.; de Mello, A. J. A high-pressure interconnect for chemical microsystem applications. *Lab. Chip* **2001**, *1*, 148–152.

Pena-Pereira, F.; Lavilla, I.; Bendicho, C. Liquid-phase microextraction techniques within the framework of green chemistry. *TrAC Trends in Analytical Chemistry* **2010**, *29*, 617–628.

Perrot, P. A *to Z of Thermodynamics* (Oxford University Press, 1998).

Pollack, M. G.; Fair, R. B.; Shenderov, A. D. Electrowetting-based actuation of liquid droplets for microfluidic applications. *Appl. Phys. Let.* **2000**, *77*, 1725–1726.

Puntambekar, A.; Ahn, C. H. Self-aligning microfluidic interconnects for glass- and plastic-based microfluidic systems. *J. Micromech. Microeng*. **2002**, *12*, 35–40.

Ramsey, J. M. The burgeoning power of the shrinking laboratory. *Nat. Biotechnol*. **1999**, *17*, 1061–1062.

Roman, G. T.; Wang, M.; Shultz, K. N.; Jennings, C.; Kennedy, R. T. Sampling and electrophoretic analysis of segmented flow streams using virtual walls in a microfluidic device. *Anal. Chem*. **2008**, *80*, 8231–8238.

Ryvolová, M.; Preisler, J.; Brabazon, D.; Macka, M. Portable capillary-based (non-chip) capillary electrophoresis. *TrAC Trends in Analytical Chemistry* **2010**, *29*, 339–353.

Sacks, R.; Smith, H.; Nowak, M. High-speed gas chromatography. *Anal. Chem*. **1998**, *70*, 29A–37A.

Sandra, P.; Pereira, A.; David, F.; Dunkle, M.; Brunelli, C. Green Chromatography (Part 2): The Role of GC and SFC, *LCGC Eur.* **2010**, *23*(Issue 9).

Sandra, P.; Vanhoenacker, G.; David, F.; Sandra, K.; Pereira, A. Green Chromatography (Part 1): Introduction and Liquid Chromatography, *LCGC Eur.* **2010**, *23*(Issue 5).

Seiman, A.; Martin, J.; Vaher, M.; Kaljurand, M. A portable capillary electropherograph equipped with a cross-sampler and a contactless-conductivity detector for the detection of the degradation products of chemical warfare agents in soil extracts. *Electrophoresis* **2009**, *30*, 507–514.

Shah, G. J.; "CJ" Kim, C.-J. Fluidic conduits for highly efficient purification of target species in EWOD-driven droplet microfluidics. *Lab Chip* **2009**, *9*, 2402–2405.

Taylor, D.; Dyer, D.; Lew, V.; Khine, M. Shrink film patterning by craft cutter: complete plastic chips with high resolution/high-aspect ratio channel. *Lab Chip* **2010**, *10*, 2472–2475.

Taylor, L. T. Supercritical Fluid Chromatography. *Anal. Chem.* **2010**, *82*, 4925–4935.

Terry, S. C.; Jerman, J. H. Miniature Gas Chromatograph Apparatus, US Patent, 4,474,889, Oct. 2,1984.

Teutenberg, T. Potential of high temperature liquid chromatography for the improvement of separation efficiency-A review. *Anal. Chim. Acta* **2009**, *643*, 1–12.

Tullo, A. A solvent dries up. *Chem. Eng. News* **2008**, *86*, 27.

Turl, D. E. P.; Wood, D. R. W. A step out of the lab and into the field: a giant leap from technology to capability. *Analyst* **2008**, *133*, 558–562.

van der Vorst, G.; van Langenhove, H.; de Paep, F.; Aelterman, W.; Dingenen, J.; Dewulf, J. Exergetic life cycle analysis for the selection of chromatographic separation processes in the pharmaceutical industry: preparative HPLC versus preparative SFC. *Green Chem.* **2009**, *11*, 1007–1013.

Vanhoenacker, G.; Sandra, P. Elevated temperature and temperature programming in conventional liquid chromatography - fundamentals and applications. *J. Sep. Sci.* **2006**, *29*, 1822–1835.

Wardencki, W.; Namieśnik, J. Some remarks on gas chromatographic challenges in the context of green analytical chemistry. *Polish J. Environ. Studies* **2002**, *11*, 185–187.

Weigl, B.; Domingo, G.; LaBarre, P.; Gerlach, J. Towards non- and minimally instrumented, microfluidics-based diagnostic devices. *Lab. Chip* **2008**, *8*, 1999–2014.

Welch, C. J.; Wu, N.; Biba, M.; Hartman, R.; Brkovic, T.; Gong, X.; Helmy, R.; Schafer, W.; Cuff, J.; Pirzada, Z.; Zhou, L. Greening analytical chromatography. *TrAC Trends in Analytical Chemistry* **2010**, *29*, 667–680.

Xia, Y. N.; Whitesides, G. M. Soft lithography. *Ann. Rev. Mat. Sci.* **1998**, *28*, 53–184.

Xiea, H.-Y.; He, Y.-Z. Green analytical methodologies combining liquid-phase microextraction with capillary electrophoresis. *TrAC Trends in Analytical Chemistry* **2010**, *29*, 629–635.

Yang, Z.; Maeda, R. A world-to-chip socket for microfluidic prototype development. *Electrophoresis* **2002**, *23*, 3474–3478.

Yuen, P. K.; Goral, V. N. Low-cost rapid prototyping of flexible microfluidic devices using a desktop digital craft cutter. *Lab Chip* **2010**, *10*, 384–387.

Zemann, A. J.; Schnell, E.; Volgger, D.; Bonn, G. K. Contactless conductivity detection for capillary electrophoresis. *Anal. Chem*. **1998**, *70*, 563–567.

Zhong, F.; Chen, Y.; Marcus, J. S.; Scherer, A. L.; Quake, S. R.; Taylor, C. R.; Weiner, L. P. A microfluidic processor for gene expression profiling of single human embryonic stem cells. *Lab. Chip.* **2008**, *8*, 68–74.

Zhu, C. R.; Goodall, D. M.; Wren, S. A. C. Elevated temperature HPLC: Principles and applications to small molecules and biomolecules. *LC-GC Eur*. **2004**, *17*, 530–535.