Advancing GC×GC through integrated sample preparation methods and optimized column formats

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Link to publication

Citation for published version (APA):

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Chapter 1

General introduction

Summary

In this chapter the role of gas chromatography (GC) as a powerful tool for the identification and quantification of target compounds in complex samples is briefly introduced. In the first part of the chapter the importance of the other steps that form an integral part of the analytical process, namely the sample preparation and the detection, is addressed. The trigger that leads to the development of comprehensive two-dimensional gas chromatography (GC×GC) is also discussed. The main trends followed in the past and today in the development of novel sample preparation methods as well as in the GC×GC area are presented. The aim of this thesis is to provide significant technical advancements to tackle some of the fundamental limitations encountered in compositional analysis by GC. The second part of the chapter presents some insight in the strategies presented in the subsequent chapters. A detailed account is given in chapters 2-6.
1.1. Chromatography: a powerful analytical tool

The identification and quantification of target analytes in highly complex samples are two of the most common goals encountered daily by analytical chemists. In many cases reaching these goals with the right level of detail and accuracy at acceptable costs and delivery times is not trivial. On the contrary, it can be extremely challenging! A number of diverse analytical techniques has been developed over the years and is currently available for compositional analysis. In order to be successful, a method must satisfy the selectivity and sensitivity requirements, i.e. it needs to be able to (i) identify specifically the compounds of interest in the sample matrix and (ii) detect them at appropriate concentration levels, respectively.

Chromatography is one of the most powerful analytical tools available and likely the most widely applied method when a high selectivity is required. Since its inception chromatography has been subject of in-depth investigations and extensive developments. The variety of different approaches that have been introduced is very wide. Gaseous, liquid and supercritical eluents have been used. The column types range from wide to narrow, from columns packed with particles of various size or with a continuous bed to open columns coated with a thin layer of stationary phase. Nowadays a number of different, well-established chromatographic techniques are available; each characterized by a wide choice of different separation mechanisms based on chemical affinity, size and/or physical properties. With the instrument becoming increasingly more complex a more elevated flexibility in terms of operational parameters also became available. As a result, these days a remarkable selectivity can be achieved for a very wide range of applications, provided that the most suitable chromatographic method is chosen and the conditions applied are carefully optimized. Despite the high selectivity of the modern chromatographic systems it is very rare that all compounds present in a complex sample can be separated from each other and from all other species present. All too often the degree of complexity of the sample by far exceeds the resolving power at hand.
Although many researchers and users see the chromatographic separation as an independent process, a broader and more complete view shows that it is actually only one part of an integrated chain of interconnected steps that together form the analytical process. Detection and sample preparation are probably as important as the chromatographic separation for the final results in terms of selectivity and sensitivity [1]. Detection is a highly crucial step which requires a dedicated, careful choice and, in many cases, thorough optimization. Moreover, most samples require extensive sample preparation before they can even be injected onto the analytical column. Therefore the analysis, i.e. the procedure starting with the sampling and resulting in the qualitative and/or quantitative results, can be divided in three parts: the sample preparation, the chromatographic separation and the detection. These are distinct, yet complementary and strongly interdependent components of the analytical process and the ultimate analytical performance depends strongly on all three contributions and on their interactions. Clean-up steps, for instance, can be employed to remove many unwanted analytes, reducing the complexity of the sample and, hence, enhancing considerably the chance of separating the target compounds from the remaining interfering species [2]. A very selective detector can also be an extremely powerful tool [3]. In some cases it is in fact possible to monitor the target class of analytes without actually having to separate them from the rest of the matrix. Similarly, each step can contribute significantly to maximizing the sensitivity. These days this is a crucial aspect in analytical chemistry, as it is often required to quantify analytes at very low concentrations down to true trace levels. Many sample preparation methods that provide enrichment factors up to several hundreds have been developed for this purpose and are now widely applied [4]. Whenever narrow peaks and enrichment are not sufficient, simply choosing the right, i.e. the most sensitive, detector can solve the problem. The chromatographic separation can also be optimized to enhance the selectivity. For example chromatographic techniques and methods that generate narrower peaks give a higher signal-to-noise ratio and thus better detection limits.

In conclusion the analysis will be successful if the selectivity and sensitivity requirements are fulfilled. Often this will be accomplished only by combining and
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balancing the contributions of sample preparation, separation and detection. Sometimes a single step can provide the necessary performance. For example the separation resolves successfully the target analytes, and the other steps do not require extensive optimization. In many cases, however, the analytical scientist will have to exploit the strengths of each of the three steps and the proper combination to perform the analysis.

1.2. Gas chromatography (GC) and comprehensive two-dimensional gas chromatography (GC×GC)

Gas chromatography (GC) was suggested for the first time by Martin and Synge over 70 years ago [5], while the first GC chromatogram was presented more than one decade later [6]. Since these first steps GC has been the subject of thorough theoretical investigation and remarkable technical developments. Nowadays, GC is a well-established method and its use is widely accepted in numerous application fields. The main reasons for this widespread success are the high efficiency and the excellent sensitivity GC offers. The very high resolving power typical of GC can in fact hardly be matched by any other one-dimensional separation method. Additional (yet not secondary) advantages are its high reliability, the relatively low operational and maintenance costs, the ease of use and automation and the excellent characteristics for coupling to mass-spectrometric identification techniques. With the GC applications range becoming progressively broader and expanding towards a huge variety of extremely complex samples of various natures, the need for an even better efficiency and a higher resolving power became of increasingly critical importance. When the samples of interest contain thousands of peaks it is clear that GC, like any one-dimensional chromatographic technique, cannot provide the necessary peak capacity. Sample preparation can help, but will probably fail if the target analytes and the interfering species are physico-chemically similar.

The need to alleviate the resolving power limitation mentioned above triggered the development of heart-cutting multidimensional gas chromatography (MDGC) [7]. MDCG introduced the principle of having two different separations
performed in series in the same chromatographic run. One, or a few, narrow time-slices from a first column are directed into a secondary column where a second, independent separation takes place. In this way better resolution can be achieved for specific, complex fractions of interest selected from the initial GC chromatogram. Comprehensive two-dimensional gas chromatography (GC×GC) [8] was introduced several decades later as the natural development of MDCG. In GC×GC the principle of operation of MDCG is extended to the whole chromatogram. Instead of selecting only one or a few critical parts of the chromatogram for additional separation on a second column, in GC×GC a modulation device continuously cuts and re-injects the peaks eluting from the first column into the second one, where a very fast separation is performed. In this way a complete two-dimensional picture of the sample is generated, increasing dramatically the resolving power at hand. Thanks to the much higher peak capacity and the improved ease of use, GC×GC has surpassed MDGC. Compared to standard 1D-GC, GC×GC can provide a superior sensitivity and a 10-fold enhancement in peak capacity [9-11]. This became possible also thanks to the increasing variety of columns commercially available. With many stationary phases becoming accessible, each one characterized by a rather different separation mechanism, it is now possible to achieve high degrees of orthogonality and thus to exploit the incredible potential of GC×GC, achieving unprecedented peak capacities in the order of several thousands. Compared to the other comprehensive techniques so far developed, GC×GC saw a faster and more successful advancement. In two-dimensional liquid chromatography, for example, properly interfacing the two dimensions and performing an efficient modulation are still rather difficult tasks. As a consequence heart-cut liquid chromatography (LC-LC) and comprehensive two-dimensional liquid chromatography (LC×LC), although subjects of thorough investigation, are still far from being accepted for routine use. On the other hand, since its introduction GC×GC achieved very good efficiencies and instrumental robustness and is nowadays well-established and widely applied [12]. In fields such as petrochemical analysis, where samples are composed of several thousands of analytes, GC×GC has become one of the methods of choice.
1.3. **Trends in sample preparation**

In recent years analysis time, cost of analysis and the environmental impact of operating an analytical laboratory became of growing importance. Nowadays these are key factors that must be carefully considered when developing analytical methods. A lot of effort is in fact invested not only in developing novel applications, but also in performing those already existing in a better way, i.e. faster, cheaper, easier or with a reduced production of waste. The requirements set with regard to these aspects are already very demanding and yet they are becoming ever stricter.

Sample preparation is often a very time-consuming, laborious and expensive step. In fact its impact in terms of time, complexity and manual labor is often much more significant than that of the GC run itself. Moreover, if not carefully selected and performed it can have detrimental effects on the success of the analysis. Therefore it is not surprising that a lot of work has been performed to improve the sample preparation techniques available and to design new ones [1].

The need to improve or replace old-fashioned, manual techniques such as liquid–liquid extraction (LLE) has been recognized for decades, and still is, the main goal in the sample preparation field. Most of the attention has been paid to (i) reducing the time required, (ii) decreasing/eliminating the need for manual labor, (iii) minimizing the use of expensive and toxic organic solvents and (iv) maintaining the integrity of the sample, i.e. avoiding changes due to losses in extraction steps or transfer lines [13]. Thus, the most significant developments in sample preparation have been focused on automation, miniaturization and the development of on-line systems directly coupled to the GC.

The trends described above have led to the introduction of several miniaturized solvent-free extraction techniques, such as solid-phase microextraction (SPME) [14] and stir bar sorptive extraction (SBSE) [15]. These methods, and in particular SPME, encountered a fast acceptance and widespread success thanks to their high enrichment factors and the possibility to automate. A completely different approach to solve the problems typically encountered with the classic sample preparation methods is represented by continuous, automated liquid–liquid extraction systems coupled on-line to GC [16]. In this case the main
goal is not eliminating completely the use of solvents, but minimizing manual labor and maximizing sample throughput, while simultaneously reducing the exposure of laboratory personnel to toxic solvents. These set-ups received a lot of attention in the ’80s and ‘90s [17-19], but in spite of their merits they were not really successful. This was mostly due to their limited control and ruggedness and poor reproducibility, as they typically made use of a tubing manually interlaced to induce turbulent mixing. About a decade later a promising way to overcome these limitations became available with the introduction of microfluidic devices. The use of chips as extraction units has been investigated [20], the main merits of using microfluidic devices being the extreme degree of miniaturization, i.e. the low solvent consumption, and the precise process control. Different LLE systems based on chips have been proposed. In almost all cases the extraction is based on laminar flows and very small volumes down to a few nL can be handled. However, in spite of its great potential, the use of chip-based LLE set-ups is currently still restricted to research and very specific applications, for example when extremely small volumes need to be handled. The main reasons for this are their instrumental complexity and fragility, which make their on-line coupling to the GC and their use for routine purposes basically impossible. Yet, it is clear that miniaturization is a trend that will continue.

1.4. Past and current developments in GC×GC

If a GC×GC system is compared to a typical GC instrument, it is evident that no other modification than the addition of a (thermal) modulator is necessary. All other components remain essentially the same. This aspect has certainly had a great, positive influence in the rapid development of GC×GC. As a result, in the early years a great deal of attention has been dedicated to improving the modulation principles and/or design [21, 22]. The cryogenic modulators based on (i) the dual-jet and (ii) the dual-stage, quad-jet designs rapidly became the most successful options. These systems offer a very good efficiency, an excellent robustness and high reliability and are thus widely accepted.
Of course, interesting work is still done to develop new modulators, for example to eliminate the huge consumption of cryogens [23]. However, given the good performance achieved with the current systems the attention paid to technical developments gradually decreased. In fact the great majority of the recent publications in the GC×GC field focus on applications or method development. Countless articles are being presented to support the good results obtained with GC×GC in various new fields and with the most diverse classes of complex samples. On the other hand, the fraction of papers discussing the development of new hardware or suggesting new set-ups is minimal. This is a strong indication that these days the technique is mature and instrument development is of limited interest to the majority of the GC×GC users. On the other hand, the validity of this tendency is questionable. In spite of the excellent performance offered, GC×GC is in fact not free from fundamental drawbacks and limitations.

In the last years some authors finally put emphasis on an aspect that had not yet been addressed with the rightful attention: the limitations arising from the typical GC×GC column set. The standard column set consists of a long normal-bore first dimension (30 m×0.25-0.32 mm i.d.) followed by a short, narrow-bore second dimension (1-2 m×0.05-0.18 mm i.d.). Such a set-up definitely has its merits but it also has some fundamental limitations. The use of a narrow second column creates a very high speed in that dimension, allowing to finish the separation before the following modulation cut is injected, and at the same time generates a very high plate count per unit of time. However, this column combination does not allow the full exploitation of both dimensions at the same time as a result of the very different linear velocities [24]. Some interesting solutions have been presented in the last years to solve this flow mismatch. Stop-flow GC×GC introduced the possibility to decouple completely the two separations [25], whereas split-flow GC×GC adjusts the gas linear velocities in the columns [26]. Both these methods showed the benefits arising from fully exploiting both columns. However, neither of them encountered widespread acceptance nor managed to replace the standard 0.25-0.10 mm i.d. column combination. On the one hand, this may be due to the limitations of the approaches proposed. On the other hand, the fact that GC×GC users are used to consider the typical column sets as a given also plays an
important role. Whatever the reason may be, the result is that the actual GC×GC set-up is so well-established that the chance to change it has become rather remote. However, a few articles which aim at solving this fundamental matter are still occasionally presented. This indicates that the interest is still present as well as the room for more significant steps forward.

1.5. Scope of this thesis

The main goal of this work was the development of technical advancements, which could improve GC and GC×GC analysis in terms of automation, reliability and efficiency. We strongly believe that sample preparation and chromatographic analysis are deeply connected and cannot be considered separately. Their progress should proceed in parallel to their mutual benefit. Therefore our attention was dedicated to both aspects and the content of this thesis will focus partly on new sample preparation methods and partly on novel column formats and set-ups for a better exploitation of the GC×GC column set.

Chapter two of this thesis describes the development of a semi-automated chip-based set-up exploiting segmented flow and turbulent mixing for continuous LLE prior to GC and GC×GC analysis. A microfluidic device originally designed as microreactor for parallel reactions is used here as an extraction unit. The main objectives of the system developed are maximizing the extraction yield by exploiting the efficient mass-transfer characteristics while minimizing the manual labor required. Strong emphasis is also put on the instrumental robustness and the sample throughput. The influence of several operational parameters on the performance of the LLE is investigated and discussed. The set-up assembled is applied to different real-life samples.

Chapter three of this thesis investigates the use of hydrophobic polymer monoliths with very different affinities towards aqueous or organic phases to act as “selective solvent gates”. Macroporous monoliths with different chemistries and flow properties are prepared, characterized and evaluated through model experiments to assess their properties. The best-performing material is selected
and used to construct a prototype of a phase separator. This prototype is
implemented in the LLE system described in chapter two to perform continuous on-
line phase separation.

In Chapter four the development of divinylbenzene-based (DVB) monolithic
columns and their use in fast GC or as second dimension columns in GC×GC are
discussed. The preparation and characterization of the diverse monoliths are
described in detail. The effects of the composition of the polymerization mixture
and process parameters on the efficiency of the DVB columns in GC are also
investigated. A GC×GC system with a monolithic column installed as second
dimension and the two-dimensional separations obtained with it are illustrated.
The potential to solve the GC×GC flow mismatch using this column format is also
discussed.

Chapter five demonstrates the possibility to use multiple narrow-bore
capillary columns in parallel in the second dimension to achieve simultaneous
optimal operation of the two dimensions in GC×GC. GC×multi-GC systems with two
or three columns in parallel as second dimension are developed and tested in
terms of peak shape, peak width and efficiency. The van Deemter curves obtained
for both dimensions are compared with those obtained using a single second
dimension column to verify the beneficial effects achieved. Two-dimensional
separations of various samples are shown to demonstrate the feasibility of the new
column set-up.

In Chapter six another possible approach to adjust the carrier gas linear
velocity in the second dimension of the GC×GC set-up is presented. A restrictor is
added at the detector end of the column combination to increase the outlet
pressure of the system. The chromatograms obtained for a number of complex
petrol-derived samples with an elevated outlet pressure and under standard
conditions are shown and compared. The effect of this modification on the
efficiency and the speed of GC×GC is discussed.

Chapters 2 to 6 in this thesis are articles published in international scientific
journals or have been prepared for publication. Therefore they can be read
independently. Some overlap can occur.
List of references