Editorial

LIQUID CHROMATOGRAPHY – QUO VADIS?

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Introduction

Liquid chromatography (LC) is even older that it seems at first sight. Although the birth certificate was legally issued in 1906 by M. Twsett [1] (separation of chloroplast pigments on calcium carbonate using petroleum ether as mobile phase), first attempts of doing separations through differentiate adsorption dated from 1834 by F.F. Runge [2] (use of unglazed paper and cloths for spot testing dye mixtures and plant extracts) and 1868 by F. Goppelsroeder [3] (introduction of paper strips for analysis of dyes, milk, beer, pigments of vegetal and animal origins). Its adolescence leads to findings of A. Tiselius in 1941 (frontal, elution, and displacement analyses) [4], A.J.P. Martin and R.L.M. Synge (model for describing column efficiency) [5], R.S. Alm in 1952 (gradient elution) [6], F. Alderweireldt in 1961 (UV spectroscopy used as detection system in LC separations) [7] and E.V. Piel in 1966 (high performance liquid chromatography)[8]. Maturity was certified in 1968 by V.L. Tal'roze [9] through hyphenation with mass spectrometry and in 1979 by E. Bayer trough LC coupling to nuclear magnetic resonance spectroscopy [10]. The first LC on a chip was reported in 1990, by A. Manz [11]. Such an historical approach is always necessary, as no present and future are allowed without past.

Discussions

Is anything new and promising for the future of liquid chromatography, when turning back to the basic separation principles? Few notable directions should briefly be discussed. First one is dealing with micellar electrokinetic chromatography (MEKC) [12,13], whose development was based on instrumentation designed for capillary zone electrophoresis in the late eighties of the past XX^{th} century. At this moment, the technique is somehow stagnating, after an initial enthusiastic growth, due to difficulties related to its hyphenation with mass spectrometry. The second approach refers to capillary electrochromatography (CEC) [14,15], with a high promise in terms of enhanced efficient separations due to reduced particle size of the packing and reduced column diameter. However, ultra high pressure liquid chromatography (UPLC) became rapidly a powerful challenger, additionally

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offering a classic and robust alternative to the capillary packed or open tubular columns used by CEC. Based on the observation that there are no theoretical boundaries between LC, gas chromatography (GC) and supercritical fluid chromatography (SFC) and on the introduction of open tubular capillary columns to LC and SFC, D. Ishii [16] proposed and demonstrated in practice the idea of unified chromatography (UC) [17,18]. However, when the open tubular columns (otherwise so successfully in GC) failed to face in LC and SFC routine applications, due to their intrinsic lack of robustness, the magic principle of an unified technique still remains at the moment a nice utopia.

Innovations in the field may be related to the general architecture of a LC instrument, as suggested in Fig. 1.

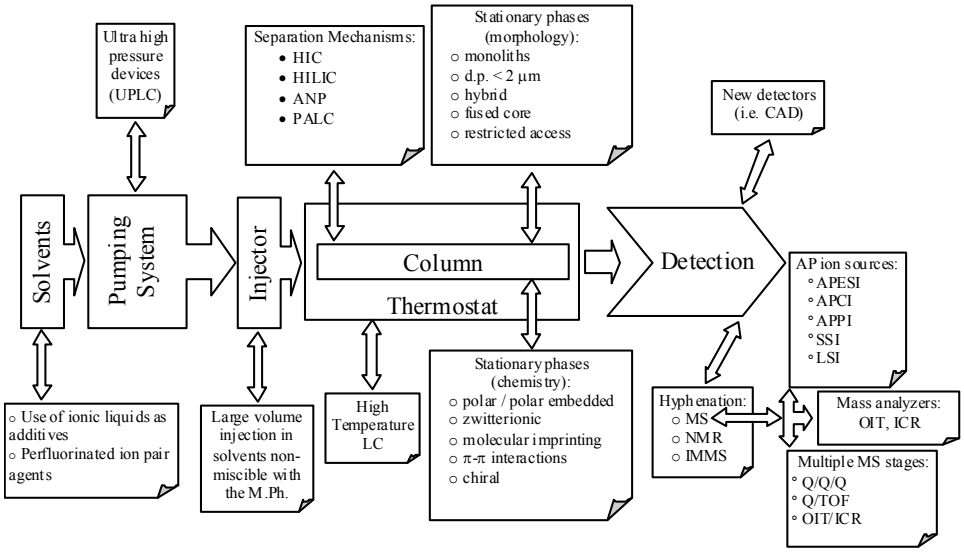

Fig. 1 *General architecture of a LC instrument*

When discussion about new directions in the field of the mobile phase components, at a first sight nothing seems new. However, additivation of mobile phases with ionic liquids for neutralizing the impact of residual silanol groups on peak shape (symmetry), especially for separation of compounds with basic moieties, should be mentioned [19-21]. Another topic refers on the introduction of per-fluorinated ion pair agents to allow MS hyphenation to LC separations driven in the ion-pairing separation mechanism [22,23]. The continuous search for environmental friendly chemistry solutions and the recent acetonitrile shortage generated by the economic crisis (producing at least a 6 fold increase of the unit price), lead to a specific interest for replacement of this solvent by ethanol, for reducing specific consumption through the use of narrow bore or capillary packed columns and sustains a revival of the SFC, commonly accepted as a “green” solution [24].

In terms of pumping instrumentation, the major finding relies to introduction of the ultra high pressure devices [25-27]. The ability of producing stable flow rates at a pressure regime up to 1200 bar is not simply a mechanical success, but should be also discussed in

relation with the new silicagel based materials used for production of the stationary phases and the new packing technologies.

Large volume injection in LC is more often conditioned by the nature of the sample solvent. In this respect, some interesting aspects were highlighted in references [28,29], dealing with injection of large volumes, the solvent of the sample being non-miscible with the mobile phase. This is not resulting only in an increased sensitivity, but also deeply refers to the adsorption model of the chromatographic separations.

Undoubtedly, a major attention is paid for innovation in the field of new separation mechanisms, new materials for stationary phases (surface chemistry) and enhanced physical properties of the supports (morphology). Shifting from the normal phase mechanism (NP) using apolar organic solvents to aqueous normal phase mechanism (ANP) on silica hydride phases [30,31] or bare silica ones [32] readily increased the reproducibility of retention and the robustness of the separations. The next step involved the proposal of the *per aqueous* liquid chromatography (PALC) [33], as a challenger of the classical reversed phase mechanism and a reversed concept for hydrophilic interaction liquid chromatography (HILIC)[34-36]. In HILIC, separation is primarily 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. This principle was also somehow mixed with ion-exchange mechanism due to the availability of zwitterionic phases (ZIC-HILIC) [37,38] for separation or ionic/highly polar analytes. The morphology of the materials used for building up stationary phases was also diversified. A continuous swing between monolithic materials [39,40] (characterized by reduced pressure drop, enhanced separation speed through high flow rates and obviously, high solvent consumption) and spherical, less than 2 μm particle sized phases [41-43] (characterized by high pressure drops maintained through the use of UPLC equipments and increased elution temperatures, high efficiencies, low solvent consumption and high separation speeds) has been observed in the last years. Increased high pressure rates applied to chromatographic columns fully contribute to the implementation of the fussed core technology for silicagel based materials used in tailoring stationary phases for LC [44,45]. The tendency of increasing elution temperatures for controlling pressure drop over the column imposed the identification of solutions for a sustained chemical stability. In this way, hybrid silicagel based materials were born [46]. But the use of high temperature water (above 100 ^{o}C) as a mobile phase has also led to new applications for chromatographic separations. The solvation properties of pressurized hot water change at high temperatures. Increasing temperature induces dramatic effects on the polarity of water. A gradual increase in temperature (when using pure water as a mobile phase) will produce effects similar to those achieved when using an elution gradient. This was the way taken for implementation in practice of the high temperature LC [47-49].

Combination of solute-stationary phase interaction types (mixed mechanisms) to produce increased selectivity represents an alternate policy designed for solving the never-ending variety of analytical experimental tasks. This resulted in specially tailored stationary phases, such as: restricted acces materials [50-54]; polar embedded hydrophobic phases [55,56]; mixed π-π, n-π and apolar interactions [57,58]; chiral phases involving inclusion and three points interactions mechanisms [59,60].

Last, but not least, interesting achievements have been done with respect to detection systems used in LC. Two directions have to be highlighted. First one refers to detection systems exhibiting universal response (the "supreme" dream consists in finding the way to produce identical response indifferently the structure of the analyte is). The second one refers to hyphenation, to produce both increased sensitivity and structural information or confirmation.

The corona charged aerosol detector (Corona CAD) is the last born among the universal detectors. It benefits from the experience gained to develop atmospheric pressure ion sources, and basically consist in a electrospray mass spectrometer without mass analyzer [61,62]. The main disadvantage still consists in its inability to accept gradient elution, but the problem may be fixed by using an additional pump, positioned post column, and delivering a "mirror" gradient profile compared to the main LC one.

The main achievement of the last two decades, in terms of hyphenation, was the routinely introduction of mass spectrometry (MS) or tandem mass spectrometry (MS/MS) as detectors for LC. Routine operation of such complicated devices was possible through introduction of the atmospheric pressure (AP) ion sources, tolerating elevated liquid flow rates. The following AP ion sources are now commercially available: a) electrospray (ESI) [63]; b) chemical ionization (APCI) [64]; c) multi photon ionization (APPI) [65]; d) sonic spray (APSSI) [66]; e) laser spray (APLSI) [67]. The continuous need to increase ionization yield for supporting the global sensitivity lead to special applications such electron capture negative ionization (ECNI - supported by the APCI instrumentation) [68] and coordination ion spray (CIS – supported by the ESI instrumentation) [69].

The tremendous reliability of the MS engines was also based on the development of new mass analysis principles, namely the orbital ion trap (OIT) [70], the linear ion trap (LIT) [71] and the ion cyclotron resonance (ICR) [72]. Additionally, the coupling of multiple mass analysis stages supported by different analyzers (i.e. triple quadrupole – QQQ; quadrupole / time of flight – QTOF; OIT/ICR) became largely available as commercial devices.

Without still being commercially available, platforms for supporting LC coupling to Fourier Transform nuclear magnetic resonance spectrometry (LC/FT-NMR) [73] and ion mobility mass spectrometry (LC/IMMS) [74] have been already experimented with excellent results.

Conclusions

After more than 150 years of liquid chromatography, nothing revolutionary is noticeable at the level of the fundamental principles. However, the field of applications has dramatically increased, requiring "subtle" solutions, more over achieved through technological improvements. LC is dealing to interdisciplinarity. Everything requires "border" solutions issuing from an extreme variety of scientific fields of knowledge. Hopefully, it is not time for boring. The lyrics from the poem "*Glossa*" of Eminescu are well fitting as a final conclusion: "Days go past and days come still, / All is old and all is new / What is well and what is ill / You remember and construe …"

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