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Review

Instrument platforms for nano liquid chromatography

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ABSTRACT

The history of liquid chromatography started more than a century ago and miniaturization and automation are two leading trends in this field. Nanocolumn liquid chromatography (nano LC) and largely synonymous capillary liquid chromatography (capillary LC) are the most recent results of this process where miniaturization of column dimensions and sorbent particle size play crucial role. Very interesting results achieved in the research of extremely miniaturized LC columns at the end of the last century lacked distinctive *raison d'être* and only advances in mass spectrometry brought a real breakthrough. Configuration of nano LC-electrospray ionization mass spectrometry (LC-ESI-MS) has become a basic tool in bioanalytical chemistry, especially in proteomics. This review discusses and summarizes past and current trends in the realization of nano liquid chromatography (nano LC) platforms. Special attention is given to the mobile phase delivery under nanoflow rates (isocratic, gradient) and sample injection to the nanocolumn. Available detection techniques applied in nano LC separations are also briefly discussed. We followed up the key themes from the original scientific reports over gradual improvements up to the contemporary commercial solutions.

Contents

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1. Introduction

Benefits of reduced column inner diameter (i.d.) were proposed quite a long time ago and since then they have been explained and discussed many times, e.g. [1–3]. Indisputably, the major advantage of low i.d. of columns is their ability to work with minute sample volumes at low flow-rates. The degree of band dilution on the column are lower; thus, nano LC offers enhanced mass sensitivity over conventional LC. Other benefits arising from the reduced column i.d. are the minimal solvent and additive volume requirements for elution, and also easy separation temperature control due to the fast and effective heat transfer on low i.d. columns.

Another key parameter subjected to miniaturization has been the diameter of sorbent particles of LC columns. Gradual decrease in the sorbent particle diameter allows reaching extremely high separation efficiencies and peak capacities, comparable to those in gas chromatography [4]. The price to be paid for achieving such impressive results is an increase in the pressure drop across the column, which is inversely proportional to the square of the sorbent particle diameter. Nevertheless, there is also another problem connected with the progressing miniaturization of column and sorbent dimensions, it is extra-column band broadening. Thus, as the column characteristic dimensions diminish, the peak volume decreases steeply. The separation achieved on the column could be deteriorating rapidly due to extra-column void volumes. From a practical point of view, it means that all the flow through spaces (capillary connections, detector cell volume) must be miniaturized and data acquisition rate should be maximized [5].

The situation in the nomenclature of extremely miniaturized LC columns and suitable chromatographic systems is quite complicated [6,7] and, e.g., columns labeled today as nanocolumns, had also been referred to as capillary [8] or nanoscale capillary [9] columns. The term nano liquid chromatography (nano LC) was coined in the late 90s of the last century [6,7,10] to mark the latest progress in liquid chromatography miniaturization. The term "nano" originates from nano volumes applied (1 μm cubed is 1 nl), e.g., nanoflow rate is in the range of tens to hundreds nl/min, volume injected to the column covers range from tens to thousands of nl. "Nano" may be also related to the volume of UV, fluorometric or electrochemical detection cells (volumes of tens of nl). To obtain a complete picture of this issue, some other synonyms should be used to the information search, e.g., microcapillary LC [11]. Chervet et al. [6] attributed nano LC to the flow rate in a range of 10–1000 nL/min. However, the term nano LC is nowadays rather connected with the inner diameter of the column used for separation which is in the range of 10–100 μm. Current accepted classification of HPLC columns is given in Table 1.

The recent progress in column technology and the achievements in the development of robust instrumentation and accessories for nano LC simplify its application in laboratories. Nano LC has gradually become an extremely effective tool in the field of analytical (bio)chemistry. The importance of nano LC arises from the hyphenation with nanoelectrospray-mass spectrometry (nano-ESI-MS) which increases the mass sensitivity and allows further to decrease the limits of detection of LC–MS methods. Enormous analytical potential of nano LC–MS is clearly illustrated by examples of chromatograms shown in Fig. 1.

Table 1
General classification of column types.

Column type	Column i.d. (mm)	Typical flow rate
Prep or semi-prep	>10	>5 ml/min
Analytical	4–4.6	2 ml/min
Solvent saver	3	1 ml/min
Narrow bore	2.1	0.5 ml/min
Microbore	1	0.1 ml/min
Capillary	0.1–0.5	1–20 μl/min
Nano	≤0.1	<1 μl/min

In this review, we will discuss various approaches to the realization of essential parts of nano liquid chromatographic system. Key moments of the development of nano LC instrumentation are mentioned and the readers are referred to the original papers or detailed reviews. The first part of the review is dedicated to a creation of reproducible delivery of nanoliter flows under isocratic and gradient separation conditions. The next extensive section deals with a sample injection to the system and on-line sample preconcentration. Short sections providing a brief overview of available fittings and unions, optical detection cells, chip-based platforms for nano LC, nanoscale-2D-LC platforms, and nano ultrahigh pressure LC systems are also part of this review. Commercially available nano LC systems are discussed at the end of the paper.

2. Nanoflow solvent delivery

Precise delivery of fluids at very low flow rates has always been a challenging task. While solvents and their mixtures hardly behave as ideal liquids, volume change during the solvent mixing as well as a compression always occur and contribute to the non-ideal behavior of the mobile phase delivery. At milliliter-per-minute scale some change at microliter range does not matter but at low flow rates, it negatively influences the flow rate accuracy. At present, mainly reciprocating pumps are used to deliver mobile phases to the nano LC systems due to their flow stability. However, syringe pumps equipped with electronic control are being increasingly used.

2.1. Split and splitless solvent delivery

The conventional HPLC instrumentation was originally designed to work with 4.6 mm i.d. analytical columns. These instruments have a relatively large internal volume and even if they are able to pump solvents very slowly, some obstacles such as incomplete and irreproducible solvent mixing and too long gradient delay would appear at nanoflow rates. For successful use in nano LC, these instruments have to employ some split-flow techniques [6]. These techniques cover an involvement of a tee junction equipped with restrictor capillary, packed restrictor column, or variable restrictor. The splitter enables flow splitting according to the differences between the flow resistance of the column and the waste line (split ratio is a reciprocal value of column and waste line resistance ratio). The simplest model of splitter is a tee junction equipped with restrictor capillary. The drawback of such arrangement is that each column has a different flow resistance (permeability), thus, it needs different restrictor capillary to maintain the desired split ratio. An additional electronically controlled variable flow restrictor (so called active flow splitter) can fix this issue and it is often used by HPLC manufacturers. These modules contain fixed or adjustable flow splitter (Fig. 2). The split ratio of the fixed flow splitter (two restrictors; split ratio for nano LC, e.g., 1:1000) is maintained when the column line and the split line have the same backpressure. Differences in pressure can be measured by pressure transducers at each line (Fig. 2a) (e.g., active flow splitter AS650 from Analytical Scientific Instruments, Richmond, USA) or by an integrated flow sensor which connects both lines and it detects the flow generated by unbalanced pressure (Fig. 2b) (e.g., flow manager module from Thermo Fischer Scientific, Waltham, MA, USA). The control unit of the system adjusts the adjustable restrictor in the split line to balance the flow. On the other hand, modules with an adjustable splitter contain an electro-magnetic proportional valve (EMPV) connected in series with a flow sensor which is calibrated to various compositions of the mobile phase (Fig. 2c) (e.g., electronic flow control system employed in 1260 Infinity capillary pump (Agilent