



Tutorial

Sampling frequency, response times and embedded signal filtration in fast, high efficiency liquid chromatography: A tutorial

M. Farooq Wahab^{**}, Purnendu K. Dasgupta, Akinde F. Kadjo, Daniel W. Armstrong^{*}

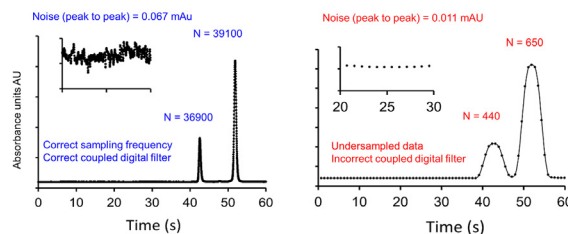
Department of Chemistry and Biochemistry, University of Texas at Arlington, 700 Planetarium Place, Arlington, TX 76019-0065, United States

HIGHLIGHTS

- Concept of sampling frequency relevant to chromatographic peaks is revisited.
- Association of response time with digital filters is described.
- Qualitative identification of embedded filters with pulsed LED experiments.
- Practical considerations for choosing sampling frequency and response times.
- Effect of coupled sampling frequency and response time is shown.

GRAPHICAL ABSTRACT

Consequences of Detector Settings on Ultrafast and Ultrahigh Efficiency Chromatography



ARTICLE INFO

Article history:

Received 2 September 2015

Received in revised form

24 November 2015

Accepted 25 November 2015

Available online 17 December 2015

Keywords:

High-efficiency chromatography

Fast separations

Sampling theorem

Response times

Digital filters

ABSTRACT

With increasingly efficient columns, elute peaks are increasingly narrower. To take full advantage of this, choice of the detector response time and the data acquisition rate a.k.a. detector sampling frequency, have become increasingly important. In this work, we revisit the concept of data sampling from the theorem variously attributed to Whittaker, Nyquist, Kotelnikov, and Shannon. Focusing on time scales relevant to the current practice of high performance liquid chromatography (HPLC) and optical absorbance detection (the most commonly used method), even for very narrow simulated peaks Fourier transformation shows that theoretical minimum sampling frequency is still relatively low (<10 Hz). However, this consideration alone may not be adequate for real chromatograms when an appreciable amount of noise is present. Further, depending on the instrument, the manufacturer's choice of a particular data bunching/integration/response time condition may be integrally coupled to the sampling frequency. In any case, the exact nature of signal filtration often occurs in a manner neither transparent to nor controllable by the user. Using fast chromatography on a state-of-the-art column (38,000 plates), we evaluate the responses produced by different present generation instruments, each with their unique black box digital filters. We show that the common wisdom of sampling 20 points per peak can be inadequate for high efficiency columns and that the sampling frequency and response choices do affect the peak shape. If the sampling frequency is too low or response time is too large, the observed peak shapes will not remain as narrow as they really are – this is especially true for high efficiency and high speed separations. It is shown that both sampling frequency and digital filtering affect the retention time, noise amplitude, peak shape and width in a complex fashion. We show how a square-wave driven light emitting diode source can reveal the nature of the embedded filter. We discuss time uncertainties related

* Corresponding author.

** Corresponding author.

E-mail addresses: mwahab@ualberta.ca (M.F. Wahab), sec4dwa@uta.edu (D.W. Armstrong).

to the choice of sampling frequency. Finally, we suggest steps to obtain optimum results from a given system.

© 2015 Elsevier B.V. All rights reserved.

Contents

1. Introduction	32
2. Theory	33
2.1. Sampling frequency	33
2.2. Noise filtering by time constants and response times	34
2.3. Association of response or rise times with digital filters	34
3. Materials and methods	35
3.1. Simulations and calculation software	35
3.2. Chemicals	35
3.3. Instrumentation and chromatographic conditions	35
3.4. Detector response time/time constant experiments	35
4. Results and discussion	35
4.1. Revisiting the sampling frequency concept	36
4.2. Real chromatograms	37
4.3. Effect of noise filters on the signal	38
4.4. Effect of filtering parameters on fast chromatography	39
4.5. Effects on best case performance	41
4.6. Suggestions for practitioners and manufacturers	41
Acknowledgments	43
Supplementary data	43
References	43

1. Introduction

The goal in analytical chromatography is to obtain baseline separations with maximum peak capacities per unit time without sacrificing detection sensitivities (and hence detection limits) and reproducibility. While the last parameter is largely governed by the fluid handling system, all the foregoing benefit from better chromatographic efficiencies. Achieving high efficiencies may be dependent on the column technology but actually visualizing this is also demanding of the detection and the data acquisition system. Efficient columns generally require smaller sample volumes. Ever smaller analyte amounts passing through the detector at ever faster flow rates creates a challenge for the detection system as well.

In the context of HPLC, the terms “fast”, “ultrafast”, etc. are yet undefined. However, a survey of the current literature suggests that both these terms imply sub-minute separations [1–5]. The efficiencies attainable by present separation media can only be realized if the rest of the system does not pose limitations. History suggests that the development of the rest of the instrumentation often lags behind advancements in column technology [2–4,6,7]. As early as the 1970s, Halász showed that while a baseline resolution of two components on a well-packed 5 μm silica column could be achieved in under 2 min, the existing HPLC hardware had to be modified to reduce extra-column effects [6].

In the last two decades, rapid developments have taken place in liquid chromatography. Smaller particles have enabled faster analyses by allowing shorter columns with the same efficiency as previous 25 cm benchmarks. The early 2000's saw a rapid increase in separation speed. For example, 7 analytes were separated in 24 s in reversed phase liquid chromatography [8], whereas 8 anions were separated under 30 s on monoliths in the ion-pairing mode [7]. The performance bottleneck was often the detection technology. With present well-packed core-shell silica columns, reduced plate heights (h) ≤ 2 are becoming common, even in columns of

5 cm length [3,9]. Such separations are characterized by very narrow peak widths, very fast elution times and relatively low backpressures. The core-shell morphology is often the preferred choice for HPLC/UHPLC instruments since it allows column efficiencies comparable to sub-2 μm fully porous particles while permitting the use of conventional HPLCs and column hardware. Recently, enantiomeric separations were reported on 3–5 cm long columns packed with 2.7 μm core-shell bonded chiral selectors in 4–15 s ($h = 1.6$ –2.0) on a modified state-of-the-art UHPLC [3].

With high-speed separations and sub-2 h values, detector/data system parameters can significantly affect the observed results. Even today's state of the art high performance instruments can potentially limit the “true” performance of a chromatography column. In our experience as well as that of others [3,5,10–13], even current generation instrumentation can contribute as much as 30–60% to peak variance for short, narrow bore columns. Remedial focus is often on injection ports and connection tubings as these are easily changed. The influence of the choice of detector/data system parameters such as response time/time constant and data sampling/acquisition rate is often ignored. Few chromatographic publications discuss these and the chosen parameters are often not even stated despite the well-recognized importance of sampling frequencies and time constants in signal processing over the years. However, there is no consensus on mathematical approaches and/or goals. Table 1 summarizes the various suggestions/opinions of different individual investigators for selecting the proper sampling frequency for chromatographic separations. It should be noted that many of the suggestions differ because the focus of the investigations differed. For example the goal some workers was to accurately render peak heights [14] or while others considered peaks areas [15,16] and some focussed on the minimum number of points needed to describe a Gaussian function [17,18]. Heien et al. suggested oversampling of data during signal acquisition up to MHz frequency followed by signal averaging [19]. Several texts suggest

Download English Version:

<https://daneshyari.com/en/article/1163102>

Download Persian Version:

<https://daneshyari.com/article/1163102>

[Daneshyari.com](https://daneshyari.com)