Journal of Chromatography A, 1204 (2008) 157–170

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Recent developments in capillary isoelectric focusing

L.H.H. Silvertand*, J. Sastre Toraño, W.P. van Bennekom, G.J. de Jong

Division of Biomedical Analysis, Department of Pharmaceutical Sciences, Faculty of Science, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands

ARTICLE INFO

Article history:
Available online 27 May 2008

Keywords:
Capillary isoelectric focusing
Review
Detection
Applications
Methodology
Microfluidic devices
Multidimensional platforms
Recent developments

ABSTRACT

The developments in capillary isoelectric focusing (cIEF) over the period 2003–2007 are reviewed. With the focus on technological aspects, cIEF papers published in the fields of methodology, new techniques, detection, multidimensional systems, miniaturization and applications are summarized. The methodology section covers recent research in ampholytes composition, detergents and other additives, carrier ampholyte free cIEF, coatings and other capillary modifications. In the section on new systems adjustments to the technique (e.g. dynamic IEF), different applications of cIEF (e.g. as injection system) and new devices are reported. Systems focusing on whole column imaging, fluorescence and chemiluminescence detection and coupling to mass spectrometers are discussed in the section on detection. Interfacing cIEF with MS via RPLC systems and hyphenation of cIEF with capillary electrochromatography and other capillary electrophoresis modes are also summarized. Papers focusing on miniaturization are reviewed in the section on microfluidic devices. The section on applications will show analysis of biopharmaceutical compounds and isolated proteins for metabolomic studies. For the analysis of complex biological matrices, generally multidimensional systems are needed, which are mentioned throughout this review.

Contents

Abbreviations: AGP, α-1-acid glycoprotein; CA, carrier ampholyte; CAF-IEF, carrier ampholyte free isoelectric focusing; CCD, charge coupled device; CEC, capillary electrochromatography; CZE, capillary zone electrophoresis; CGE, capillary gel electrophoresis; cIEF, capillary isoelectric focusing; cNGSE, capillary non-gel sieving electrophoresis; dIEF, dynamic isoelectric focusing; 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; EOF, electroosmotic flow; ESI, electrospray ionization; FFIEF, free flow isoelectric focusing; FTICR, fourier transform ion cyclotron resonance; gIEF, gel isoelectric focusing; HDMS, hexa(dimethylsiloxane); HFF-FFF, hollow fiber flow field-flow fractionation; HPC, hydroxypropylcellulose; HRP, horseradish peroxidase; IEF, isoelectric focusing; IET, isoelectric trapping; IPG, immobilized pH gradient; ITP, isotachophoresis; LIF, laser-induced fluorescence; MALDI, matrix-assisted laser desorption ionisation; MEKC, micellar electrokinetic chromatography; M-IPG, monolithic immobilized pH gradient; OGE, off-gel electrophoresis; OLED, organic light emiting diode; PAA, polyacrylamide; PB-PEG, pyrenebutanoate poly(ethylene glycol); PDMS, poly(dimethylsiloxane); PEG, poly(ethylene glycol); PEHA, pentaethylenehexamine; PEO, polyethyleneoxide; PGDE, pH gradient driven electrophoresis; PMMA, poly(methyl methacrylate); PMT, photon multiplier tube; PVA, polyvinylalcohol; PVP, poly(vinylpyrrolidone); RPLC, reversed-phase liquid chromatography; SDS, sodium dodecylsulphate; SEC, size-exclusion chromatography; SPME, solid-phase microextraction; TOF, time-of-flight; WCI, whole column imaging.

* Corresponding author at: Research Group Pharmaceutical Analysis, Division of Biomedical Analysis, Department of Pharmaceutical Sciences, Faculty of Science, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands. Tel.: +31 30 2537678; fax: +31 30 2535180.
E-mail address: L.H.H.Silvertand@uu.nl (L.H.H. Silvertand).

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.05.057

1. Introduction

The moment of conception for isoelectric focusing (IEF) was in the early sixties, when Vesterberg, under the supervision of Svensson, found a procedure to synthesize a wide range of carrier ampholytes [1–6]. With several ups and downs, IEF developed into a more preparative technique. Labour started in the early seventies with the first descendant: the polyacrylamide gel-based IEF (gIEF). Still, it took nearly 15 years before a second sibling saw daylight. The capillary format of isoelectric focusing (cIEF) was born around 1985 when Hjertén and Zhu decided to "adapt the equipment for high-performance electrophoresis to isoelectric focusing" [7]. And while gIEF in combination with mass spectrometry (MS) developed into one of the principal techniques used for protein analysis and later on in proteomics, cIEF has always lived in the shadow, but is now gradually coming of age. In the early days the focus was on figuring out the principles and solving the childhood diseases and shifted via new methodologies and one-dimensional applications to integration in multidimensional platforms and miniaturized systems. The majority of problems in cIEF has been overcome and the (bio)analytical world is now seeing the benefits of cIEF for protein analysis: high resolution, speed, concentrating factor and the possibility of integration in automated (miniaturized) multidimensional platforms. Next to its utilization for the analysis of pharmaceutical proteins, the emerging 'proteomics' platform as an application field has contributed to the popularity of cIEF as well. This gradual shift is also observed in the time-line of publications. In cIEF-specific reviews the focus has shifted from the principles of cIEF via technological aspects towards proteomic and biopharmaceutical applications [8–19]. The last review dedicated to cIEF was published by Kilár [16] in 2003, but a number of cIEF papers from 2003 on has also been discussed in other reviews on various subjects like capillary electrophoresis (CE) in general [20–23], the role of CE in proteomics [24–36] and peptidomics [29,30,37–39], CE-MS coupling [25,29,31,32,34] and papers on specific clinical applications like hemoglobin [40], transferrin [41] or somatropin analysis [42].

Gel IEF and chromatofocusing are beyond the scope of this review, as well as two fairly new non-capillary (non)-gel isoelectric focusing techniques, i.e. free flow IEF (FFIEF) and off-gel electrophoresis (OGE). This review focuses on papers published in the last 4 years (2003–2007) dealing with the capillary format of isoelectric focusing and will mainly discuss technological aspects: methodology, new techniques, detection systems, multidimensional platforms, miniaturization and applications.

2. Methodology

2.1. Carrier ampholytes

Although in the past much attention has been paid to the conductivity and buffering power of carrier ampholytes (CAs), the group of Righetti recently investigated various properties of different narrow-cut CA blends in a series of papers in Electrophoresis [43–48], including an overview [48]. Narrow-cut (2–3 pH units) CA blends of the four major brands (Pharmalytes, Servalyte, Ampholine and Bio-Lyte) were fractionated into 20 fractions with the Rotofor [49] and injected onto a capillary zone electrophoresis-electrospray ionization-ion-trap-MS (CZE-ESI-ion-trap MS) system. Linearity (pH of the fractions), mass distribution, polydispersity and focusing properties were investigated. Their findings were as much surprising as they were clarifying. In the acidic range (up to pH 7) all blends contained high numbers of amphoteric compounds with good focusing properties (compounds present in only 1–3 Rotofor fractions were denoted as 'good' CAs) as can be seen in Table 1. In the alkaline range, however, the majority of the CAs does not only fail to focus, but the number of species per pH unit was low, due a low variety of pK_a values of the aminogroups. These factors lead to a decline in the quality of the pH gradient in that range and are possible explanations for the problems encountered when analyzing proteins in this pH region. Furthermore, the measured pH range covers usually more than the claimed cut. Exception is Ampholine 3.5–5, where measured pH values start at a pH of 3.8. Servalyte generally contains in all pH cuts the highest number of isoforms. This also explains that Servalytes form the pH gradient with the best linearity approach. Pharmalytes possess the highest percentage of 'good' CAs. The authors of this series of papers make a few remarks about their results (e.g. not all Rotofor fractions were analyzed), and they correctly state that the alkaline region of CA pH gradients can be improved by new synthesis routes.

Table 1
Composition of different brands of ampholytes [43–48]

	Ampholine	Servalyte	Bio-lyte	Pharmalytes
Claimed pH cut	3.5–5	2–4	3–5	2.5–5
pH range	3.8–7	2–6	3.8–7	2–6
No. Mr species	105	277	84	245
No. isoforms	446	1201	383	857
Mr range	205–965	204–929	216–965	203–857
% 'good' CA	~70	~65	~55	~72
Claimed pH cut	4–6.5	4–6	4.6	4–6.5
pH range	3.8–7.8	3.6–6.9	4.0–6.4	3.6–7.5
No. Mr species	80	199	66	217
No. isoforms	325	1302	436	812
Mr range	203–893	204–907	388–835	150–1179
% 'good' CA	~50	~34	~20	~66
Claimed pH cut	6–8	6–8	6–8	5–8
pH range	5–8	5.3–9.5	4.8–8	5.4–8
No. Mr species	80	126	62	123
No. isoforms	326	703	237	476
Mr range	216–979	240–785	341–1048	221–992
% 'good' CA	~46	~26	~17	~45
Claimed pH cut	7–9	7–9	8–10	8–10.5
pH range	6.1–9.0	4.7–8.8	8.5–12.2	7.5–10.3
No. Mr species	29	65	43	58
No. isoforms	85	306	136	102
Mr range	210–870	290–760	205–495	200–725
% 'good' CA	~25	~35	~25	~50