Methods xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Methods

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

Affinity capillary electrophoresis for studying interactions in life sciences

Mais Olabi[1], Matthias Stein[1], Hermann Wätzig*

Institute of Medicinal and Pharmaceutical Chemistry, TU Braunschweig, Beethovenstr. 55, 38106 Braunschweig, Germany

ARTICLE INFO

Keywords:
Affinity capillary electrophoresis
Frontal analysis affinity capillary electrophoresis
Immunoaffinity capillary electrophoresis
Mobility shift affinity capillary electrophoresis
Non-equilibrium capillary electrophoresis of equilibrium mixtures
Protein interactions

ABSTRACT

Affinity capillary electrophoresis (ACE) analyzes noncovalent interactions between ligands and analytes based on changes in their electrophoretic mobility. This technique has been widely used to investigate various biomolecules, mainly proteins, polysaccharides and hormones. ACE is becoming a technique of choice to validate high throughput screening results, since it is very predictively working in realistic and relevant media, e.g. in body fluids. It is highly recommended to incorporate ACE as a powerful analytical tool to properly prepare animal testing and preclinical studies. The interacting molecules can be found free in solution or can be immobilized to a solid support. Thus, ACE is classified in two modes, free solution ACE and immobilized ACE. Every ACE mode has advantages and disadvantages. Each can be used for a variety of applications.

This review covers literature of scopus and SciFinder data base in the period from 2016 until beginning 2018, including the keywords “affinity capillary electrophoresis”, “immunoaffinity capillary electrophoresis”, “immunoassay capillary electrophoresis” and “immunosorbent capillary electrophoresis”. More than 200 articles have been found and 112 have been selected and thoroughly discussed. During this period, the data processing and the underlying calculations in mobility shift ACE (ms ACE), frontal analysis ACE (FA ACE) and plug-plug kinetic capillary electrophoresis (ppKCE) as mostly applied free solution techniques have substantially improved. The range of applications in diverse free solution and immobilized ACE techniques has been considerably broadened.

1. Introduction

Biological interactions between proteins like receptors, enzymes or plasma proteins with other body compounds such as carbohydrates and nucleic acids are crucial for regulation physiological functions. The pathophysiology of various diseases, including cancer, often involves dysregulation of these. Biomolecules, like proteins, interact with pharmaceuticals and may influence them and their pharmaceutical effects. Describing and analyzing such complex interactions using specific and precise methods is vital for drug development, drug monitoring and

Abbreviations: Ab, antibody; Ab*, labeled antibody; ACE, affinity capillary electrophoresis; AFP, alpha-fetoprotein; Ag, antigen; ALP, alkaline phosphatase; AuNPs, gold nanoparticles; AuNRs, functionalized gold nanorods; BA MNPs, boronate affinity magnetic nanoparticles; BGE, background electrolyte; BTXs, Brevetoxins; β-CD, β-cyclodextrin; CACE, competitive affinity capillary electrophoresis; CD, circular dichroism; C^4D, contactless electric conductivity detector; CE, capillary electrophoresis; CSF, cerebrospinal fluid; DAD, diode array detector; ECEEM, equilibrium capillary electrophoresis of equilibrated mixtures; ED, equilibrium dialysis; EMD, electromigration dispersion; EOF, electroosmotic flow; Eq, equation; FA ACE (CE/FA, FACE), frontal analysis affinity capillary electrophoresis; FACCE (cNECEEM), capillary electrophoresis in frontal analysis mode (continuous capillary electrophoresis frontal analysis continuous non-equilibrated capillary electrophoresis of equilibrium mixtures); FITC, (fluorescein isothiocyanate)-labeled anti-TK1 antibody; FRET, Förster resonance energy transfer; FTIR, Fourier transform infrared spectroscopy; HD ACE, Hummel–Dreyer affinity capillary electrophoresis; HPAC, high performance affinity chromatography; HRP, horseradish peroxidase; IACE, immuno affinity capillary electrophoresis; ICP-MS, inductively coupled plasma mass spectrometry; IgE, Immunoglobulin E; IgG, Immunoglobulin G; ITC, isothermal titration calorimetry; K_A, association constant; k_a, association rate constant; K_D, dissociation constant; k_d, dissociation rate constant; LIF, laser-induced fluorescence detector; LODs, limits of detection; MASKE, macroscopic approach to studying kinetics at equilibrium; MBs, magnetic beads; MC, microchip technique; MC IACE, microchip immuno affinity capillary electrophoresis; MD, molecular dynamic; MIP, molecularly imprinted polymer; MIP NPS, modified molecularly imprinted nanoparticles; ms ACE, mobile shift affinity capillary electrophoresis; MS, mass spectrometry (mass spectrometer); MOE, molecular operating environment; NECEEM, non-equilibrium capillary electrophoresis of equilibrium mixtures; NIR, near infra red spectral region; NMR, nuclear magnetic resonance spectrometry spectroscopy; PAMPA, parallel artificial membrane permeability assay; PMMA, cellulose-based polymer dynamic coatings; POC, point of care; ppKCE (PF ACE), plug-plug kinetic capillary electrophoresis (partial-filling affinity capillary electrophoresis); PSA, prostate-specific-antigen; QDs, quantum dots; RNase B, Ribonuclease B; SPR, surface plasmon resonance; sSweepCE, short sweep capillary electrophoresis; sSweepCEEM, short sweep capillary electrophoresis of equilibrium mixtures; sweepCE, sweeping capillary electrophoresis; TK1, thymidine kinase 1; UAPA MBs, ultrarapid Agarose™ MBs; UAAF MBs, affiamino ultrarapid agarose™ MBs; UV, ultraviolet spectrophotometry; VACE, vacancy affinity capillary electrophoresis; VHH antibody fragments, single domain antibody fragments isolated from camelid animals; Vis, ultraviolet-visible spectral region; VP ACE, vacancy peak affinity capillary electrophoresis

* Corresponding author.
E-mail addresses: mais.olabi@tu-braunschweig.de (M. Olabi), matthias.stein@tu-braunschweig.de (M. Stein), h.waetzig@tu-braunschweig.de (H. Wätzig).
[1] Both authors equally contributed to this article.

https://doi.org/10.1016/j.ymeth.2018.05.006
Received 6 February 2018; Received in revised form 4 May 2018; Accepted 7 May 2018

for diagnostic purposes. Next to affinity chromatography and electrophoresis, surface plasmon resonance (SPR), circular dichroism (CD), ultraviolet spectrophotometry (UV), fluorescence, Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), equilibrium dialysis (ED), as well as, parallel artificial membrane permeability assay (PAMPA) and isothermal titration calorimetry (ITC) play an important role in studying biological interactions [1].

Chromatographic and electrophoretic techniques require minimal sample preparation. They are able to isolate an analyte from a liquid and to detect it through hyphenation with different detectors, like diode array detector (DAD), laser-induced fluorescence detector (LIF), mass spectrometer (MS) and contactless electric conductivity detector (C^4D). In conventional chromatographic methods, the separation occurs by nonspecific interactions between the analytes and the stationary phase. With electrophoretic methods, the analytes are separated based on differences in their electrophoretic mobilities. Further enhancements of these techniques include affinity chromatography and affinity electrophoresis, which achieve highly specific separation through the use of specific ligands, such as metal ions, proteins, lectins, aptamers or selective antibodies. An analyte's mobility is influenced by specific interactions between the analyte and a ligand. It is possible to isolate and to detect an analyte from complex biological samples, such as blood. With high-performance affinity chromatography (HPAC), the ligand must be immobilized in the stationary phase. In contrast, affinity capillary electrophoresis (ACE) allows for analysis in both the free solution and the immobilized mode.

ACE is a variation of capillary electrophoresis (CE) first reported in early nineties [2], which is increasingly used among modern analytical separation methods. The separation in ACE is based on modification the charge-to-size ratio through the associated reaction of an analyte with a specific ligand. By successively increasing the amount of ligand, the analyte's mobility gets shifted to the mobility of the formed complex. The usage of a specific interacting ligand specifically influences the mobility of the analyte and allows for separation of the analyte-ligand-complex from mixtures such as bodily fluids. ACE can be used both in free solution and immobilized modes [3]. With the free solution mode, analyte and ligand are dissolved in the buffer. Sections two to five of this review describe different free solution ACE methods, while some of their applications are described in section seven. With immobilized ACE, the ligand (selector) is fixed on the capillary wall or a certain solid surface and the analyte is solved in the sample solution [3,4]. This type is also called electrokinetic affinity chromatography [1]. Immobilized ACE techniques have disadvantages compared to free solution ACEs. Immobilization of the ligand on a solid surface influences its molecular structure and its binding affinity to the analyte, which could impair its separation efficiency [5]. Information about immobilized ACE techniques and their microchip form are presented in sections six and seven, with examples of recent applications.

Using ACE, the kinetic of both the association and the dissociation reaction between an analyte and a ligand can be determined [6]. This allows for measurement of the magnitude of interactions between small molecules and proteins [6,7], metals and proteins [6,8–11], proteins with each other [12], receptors with ligands [13,14], antibodies with antigens [15–17], as well aptamers [18–26] as lectins interactions [27]. Furthermore, ACE techniques could be used in clinical applications for isolation and detection of biomarkers. A miniaturization of this analytical method on a microchip reduces the required sample volume [28]. Microchip technique (MC) can potentially be used as point-of-care (POC) instrument for personalized medicine [4].

This review focuses on the latest developments in the use of ACE for studying biological interactions. Various ACE techniques will be discussed, including a juxtaposition of the advantages and disadvantages, their data processing characteristics, the benefits of combination with computational chemistry, as well as practical and laboratory applications.

This review covers literature identified through Scopus and the SciFinder database in the period from 2016 until early 2018. Inclusion criteria were the following keywords "affinity capillary electrophoresis" belonging (126 hits), "immunoaffinity capillary electrophoresis" (10 hits, covering 2015), "immunoassay capillary electrophoresis" (63 hits) and "immunosorbent capillary electrophoresis" (35 hits). Searching for both keywords of "immunoassay capillary electrophoresis" and "immunosorbent capillary electrophoresis" revealed many inconsequential hits, as, in some studies, immunoassay was performed without using a CE method, while, in others, capillary electrophoresis was used for purification purpose. In addition, double hits were found within the mentioned search terms of "immunoaffinity capillary electrophoresis", "immunoassay capillary electrophoresis" and "immunosorbent capillary electrophoresis".

All studies pertaining to "affinity capillary electrophoresis" and "immunoaffinity capillary electrophoresis" as well as a selection of studies belonging to the other terms were considered in this review.

The 126 reviewed articles matched with the keyword "affinity capillary electrophoresis" used the free solution ACE method. The 19 studies reviewed under the terms of "immunoaffinity capillary electrophoresis", "immunoassay capillary electrophoresis" and "immunosorbent capillary electrophoresis" were based on immobilized ACE methods. This reflects the dominance of free solution methods in ACE techniques, but also the tendency for using immobilized ACE methods, when utilizing immunoaffinity ACE (IACE). This tendency shows the increasing interest of using immobilized IACE as diagnostic tools for determination of biomarkers for various diseases.

2. Different free solution affinity capillary electrophoresis (ACE) methods and their main advantages and drawbacks

In most of the previous articles about free solution, several popular approaches for affinity capillary electrophoresis mentioned such as mobility shift affinity capillary electrophoresis (often referred simply as affinity capillary electrophoresis (ACE, ms ACE)), Hummel–Dreyer method (HD ACE), vacancy peak method (VP ACE), vacancy affinity capillary electrophoresis (VACE), capillary electrophoresis in frontal analysis mode (CE/FA, FA ACE or FACE), continuous capillary electrophoresis frontal analysis (FACCE, nowadays called continuous non-equilibrated capillary electrophoresis of equilibrium mixtures (cNECEEM)) and special capillary zone electrophoresis methods for slowly interacting binding partners [29]. Another, more detailed article contains a longer list, including methods for more sophisticated applications [30]. In addition to the above mentioned approaches, this article described non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM), equilibrium capillary electrophoresis of equilibrated mixtures (ECEEM), competitive affinity capillary electrophoresis (CACE), sweeping capillary electrophoresis (sweepCE), macroscopic approach to studying kinetics at equilibrium (MASKE), short sweep capillary electrophoresis (sSweepCE) and short sweep capillary electrophoresis of equilibrium mixtures (sSweepCEEM) [30]. Furthermore, there is a good working alternative to the classic ms ACE method, where just a part of the capillary is filled with the ligand-containing background electrolyte. This modification of the classical ms ACE method is called plug-plug kinetic capillary electrophoresis (ppKCE) (synonym term: partial-filling affinity capillary electrophoresis (PF ACE)) [3,31]. Reference [30] presents a comprehensive overview of all existing free solution affinity capillary electrophoresis methods. Ansorge et al. introduce the ppKCE in a very detailed manner, including a comparison with the classic ms ACE method [31].

Due to the general high complexity of ligand binding assays, all the methods discussed have specific benefits. The decision of which technique and method is best suited for a particular system is not an easy one [32]. For this reason, it is always better to have a larger pool of methods. For some of the ACE approaches, synonym terms are used in the literature. The authors recommend the use of the terms defined in