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Journal of Chromatography A, 1097 (2005) 171–178
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Design and evaluation of a coupled monolithic preconcentrator-capillary zone electrophoresis system for the extraction of immunoglobulin G from human serum

Jenny M. Armenta, Binghe Gu, Paul H. Humble, Craig D. Thulin, Milton L. Lee *

Department of Chemistry and Biochemistry, C267 Benson Science Building, Brigham Young University, P.O. Box 25700, Provo, UT 84602-5700, USA

Received 27 April 2005; received in revised form 1 August 2005; accepted 8 August 2005
Available online 9 September 2005

Abstract

The analysis of proteins in biological fluids by capillary electrophoresis (CE) is of interest in clinical chemistry. However, due to low analyte concentrations and poor concentration limits of detection (CLOD), protein analysis by this technique is frequently challenging. Coupling preconcentration techniques with CE greatly improves the CLOD. An on-line preconcentration-CE method that can selectively preconcentrate any protein for which an antibody is available would be very useful for the analysis of low abundance proteins and would establish CE as a major tool in biomarker discovery. To accomplish this, the development of an on-line protein G monolithic preconcentrator-CE device is proposed. To generate active groups for protein immobilization, glycidyl methacrylate (GMA) was used to prepare polymer monoliths. A 1.5–2 cm monolith was cast inside a 75 μm I.D. fused silica capillary that had previously been coated with alternating layers of negatively (dextran) and positively (polybrene) charged polymers. Protein G was covalently bound to GMA. Monoliths from different formulations were prepared and evaluated for binding capacity to optimize the monolith formulation for protein preconcentration. The physical properties of the column considered best for preconcentration were determined by mercury intrusion porosimetry. The total pore area was 4.8 m^2/g, the average pore diameter was 3.3 μm and the porosity was 82%. The monolith had a low flow resistance and was macroscopically homogeneous. The effectiveness of the monolith to rapidly preconcentrate proteins at flow rates as high as 10 μL/min was demonstrated using a 1.8 μM IgG solution. This system proved effective for on-line sample extraction, clean-up, preconcentration, and CE of IgG in human serum. IgG from diluted (500 and 65,000 times) human serum samples was successfully analyzed using this system. The approach can be applied to the on-line preconcentration and analysis of any protein for which an antibody is available.

Keywords: Proteins; Preconcentration; Monolith; Protein G; Capillary electrophoresis; IgG; Glycidyl methacrylate; Human serum

1. Introduction

Application of capillary electrophoresis (CE) in proteomics research continues to gain popularity [1]. However, in order to make CE more attractive to real bioanalytical applications, some drawbacks still need to be addressed. One of the most striking drawbacks is the restriction in sample volume that can be injected into the capillary to preserve high column efficiency. This coupled with the short path length for optical detection leads to poor concentration limits of detection [2]. For proteins, CE analysis is usually limited to the micromolar range when using UV absorption detection [3]. To compensate for this, different capillary geometries, novel optical designs, and sample preconcentration methods have been developed [2].

An approach to circumvent poor concentration detection limits in CE is to use a more sensitive detector, such as laser induced fluorescence (LIF), electrochemical or mass spectrometry (MS). Another approach is to increase the sample loadability by using techniques such as field-amplified stacking and transient isotachophoresis [4,5].

The typical approach to analyze components at low concentrations in complex matrices is to preconcentrate the analytes either on-line or off-line prior to separation. Even though they are more flexible, off-line preconcentration methods have the disadvantage that sample handing may lead to analyte losses on exposed surfaces (e.g., vials, tips, and pipets) [6]. Minimal

* Corresponding author. Tel.: +1 801 378 2135; fax: +1 801 378 9357.
E-mail address: milton_lee@byu.edu (M.L. Lee).

0021-9673/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.chroma.2005.08.050

sample handing can be achieved by the use of on-line preconcentration methods [5,7].

Much effort has been directed toward the development of on-line sample preconcentration in CE, and several papers can be found dealing with the preconcentration of trace components present in human specimens prior to separation [8–13]. Several on-line preconcentration systems for CE have been designed, in which a solid support (e.g., polymeric or silica based particles) is either positioned in a small section of the electrophoresis capillary or forms part of an external device that is coupled to the electrophoresis capillary [4,14–21]. Evidently, these systems have higher sample loadability compared to sophisticated sample injection techniques such as field-amplified stacking and transient isotachophoresis, since the loading capacity is not limited by the total capillary volume [19].

Preconcentration methods can be classified as non-selective and selective, depending on the affinity of the solid support for the analytes [8]. For selective analyte preconcentration, on-line immunoaffinity capillary electrophoresis has found widespread application. In immunoaffinity capillary electrophoresis, specific antibodies bound to the surface of a porous material (e.g., porous polymer, glass beads, silica beads, membrane, or the capillary wall itself) are used for the selective concentration of specific antigens [5,8,14,22]. Following capture, the antigens are eluted with a small plug of an elution buffer that disrupts the binding affinity. The desorbed antigens are then separated by CE.

Several groups have achieved on-line preconcentration CE using up to 1 cm solid packing material placed near the inlet of the separation capillary, and kept in position with frits. In addition to the formation of bubbles, a major disadvantage of this design is the increased back-pressure generated by the use of frits, which disrupts the electroosmotic flow (EOF) and eventually induces blockage of the capillary. To alleviate this problem, the use of a magnet instead of frits to hold the solid packing in place has been proposed [16]. Another approach is to replace the solid phase preconcentrator by an open tubular preconcentrator. Guzman [20] reported the use of antibodies immobilized on the wall of an array of open tubular capillaries attached to the separation capillary for the selective preconcentration of IgE. An advantage of this design is the absence of frits and packing materials.

More recently, in a very elegant approach, Guzman and Phillips [15,23] introduced an improved solid-phase microextraction system for use in on-line immunoaffinity CE. The system had a cross-shaped configuration, connecting the solid-phase extractor to two large-bore capillaries for sample and buffer transport, and to two small-bore capillaries for CE.

Polymeric materials have also been proposed as absorptive phases. Several groups have reported the use of membranes for preconcentration in CE [5,24–26]. This technique is termed “on-line membrane preconcentration-CE” and is based on the use of a polymeric membrane that is sandwiched in between two capillaries. An advantage of this approach is that because the preconcentration capillary can be separated from the separation capillary during sample loading, there is more flexibility in buffer selection. In addition, buffer and sample introduction becomes easy. A limitation, however, is the need to couple the preconcentration and separation capillaries. It is a fact that innovative designs must be found to alleviate the limitations of previously reported preconcentrators.

A versatile chromatographic support termed a monolith was first introduced in 1989 by Hjertén et al. [27]. Polymer monoliths [28,29] are typically prepared by in situ polymerization of monomer solutions composed of a monomer, crosslinker, porogen and initiator. Polymerization is initiated either thermally or by UV light. Because of the flexibility in monomer choice, monoliths with a variety of surface chemistries can be prepared [30]. An attractive feature that makes monoliths amenable as chromatographic supports is that no frits are required, since the rods are directly synthesized within the column [30]. The highly porous structures of monolithic columns give them high mechanical strength, low flow resistance and high rates of mass transfer. Diffusion in monoliths is much faster than in conventional supports and is no longer a limiting factor for analyte interaction. Consequently, the use of high flow rates is possible and rapid separations result [30].

The potential of monoliths as stationary phases for biochromatography has been extensively demonstrated. Acrylate, methacrylate and styrene based monoliths have been around since the early 1990s. The applicability of GMA monoliths in affinity chromatography for analytical and preparative purification of proteins has been demonstrated [30]. Polymeric monoliths molded within microfluidic devices have been used for on-chip solid-phase extraction of a standard peptide and green fluorescent protein [31]. More recently, the use of methacrylate based monoliths in capillary electrophoresis for the selective preconcentration of *S*-propanolol was demonstrated by Baryla and Tolt [18].

Here we propose the use of a polymeric monolith as the support for protein preconcentration prior to CE. To the best of our knowledge, only one system similar to the one proposed here has been reported [18]. However, it was not applied to the preconcentration of proteins, analysis of real samples was not demonstrated, and surface passivation of the fused silica capillary was not done. Furthermore, the porous properties of the monolith were not reported. Design, characterization and evaluation of an on-line protein G monolithic preconcentration-CE system for enrichment and separation of proteins is described in this paper.

2. Experimental

2.1. Chemicals

Anhydrous methanol, acetone and hexanes were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ, USA). Cyclohexanol was from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid was from Anachemia Canada (Montréal, Canada). Dextran sulfate sodium salt, hexadimethrin bromide (polybrene), glycidyl methacrylate (GMA) 97%, 3-(trimethoxysilyl)propyl methacrylate (γ-MPTS), trimethylolpropane trimethacrylate (TRIM) and 2,2-dimethoxy-2-phenylacetophenone (DMPA) 99% were supplied