Talanta 164 (2017) 148–153

Contents lists available at ScienceDirect

Talanta

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

Trapping magnetic nanoparticles for in-line capillary electrophoresis in a liquid based capillary coolant system

Pranov Ramana[a], Erwin Adams[a], Patrick Augustijns[b], Ann Van Schepdael[a,*]

[a] KU Leuven - University of Leuven, Department of Pharmaceutical and Pharmacological Sciences, Pharmaceutical Analysis, Herestraat 49, O & N2, PB 923, 3000 Leuven, Belgium

[b] KU Leuven - University of Leuven, Department of Pharmaceutical and Pharmacological Sciences, Drug delivery and disposition, Herestraat 49, O & N2, PB 921, 3000 Leuven, Belgium

ARTICLE INFO

Keywords:
IMER
Magnetic nanoparticles
Immobilization
In-line capillary electrophoresis

ABSTRACT

A method to trap magnetic nanoparticles inside the electrophoretic capillary has been developed without disabling and disturbing the coolant liquid recirculation. The complete optimization from setting up of the external magnetic field to injecting the magnetic nanoparticles in order to facilitate efficient trapping is described. In addition to this, the setup has been tested with 150 mM sodium phosphate buffer as the background electrolyte at different pH values (2, 4, 6 and 8). All these pH values have been investigated at 25 °C and 37 °C to mimic room temperature and physiological temperature, respectively. The capture of magnetic nanoparticles was examined via UV detection and also microscopically. To demonstrate the efficiency of the trapping, an in-line capillary electrophoresis enzymatic assay using hFMO3 was performed successfully. This method can be of further use for in-line capillary electrophoresis using magnetic nanoparticles as a solid support for enzyme/protein immobilization.

1. Introduction

Capillary electrophoresis (CE) is a separation technique that is increasingly being used in enzymatic assays. It is known for its separation efficiency, as well as low sample and solvent consumption. In-line capillary electrophoresis was first pioneered in 1992 by Bao and Regnier [1] who successfully performed an enzymatic reaction and separation of the products in a single capillary. Different techniques have been introduced since then to facilitate mixing of the reactants inside the capillary that is primarily based on electrophoresis or diffusion. In-line capillary electrophoresis consumes smaller quantities of sample, and to stop the enzymatic reaction, the substrate and the product can be separated by the application of voltage across the capillary ends, thus avoiding the use of organic solvents for quenching the reaction. However, in many cases, protein adsorption on bare fused silica capillary walls may occur [2]. It is possible to counter this phenomenon by coating the walls of the capillary with polymers to remove silanol groups on the surface of the capillary walls [2].

In a few cases, the inner capillary wall is modified to facilitate immobilization of enzymes or proteins; such systems have been termed immobilized microenzyme reactors (IMERs) [3,4]. Immobilization of enzyme/protein onto the capillary wall or insoluble supports such as gold and magnetic nanoparticles has been observed before to enhance the stability and activity of enzymes in certain cases [5]. More importantly, immobilization of enzyme/protein facilitates their multiple usage thereby reducing cost. Immobilized microenzyme reactors have predominantly been explored in the microfluidics or biosensor technologies and in in-line capillary electrophoresis [5]. With respect to CE, immobilization of proteins/enzymes can be either done on the capillary wall or on an insoluble support. Various techniques like adsorption, entrapment, crosslinking and covalent attachment have been explored to perform the immobilization based on the chemistry of the desired enzyme in use.

During recent years, the use of superparamagnetic beads has been extensively explored in the fields of biomedicine, biotechnology, pharmaceutical and food technology [6]. These particles are known for their high “surface area to volume ratio” and ease of surface modification. The main advantage of magnetic nanoparticles (MNs) is the ability to manipulate the location and movement by application of an external magnetic field. Magnetic nanoparticles have been used successfully in the field of drug delivery and protein/enzyme immobilization. The ability to incorporate functional groups on MNs allows

Abbreviations: **MNs**, magnetic nanoparticles; **IMER**, Immobilized microenzyme reactor; **EOF**, Electro-osmotic flow; **hFMO3**, human flavin-containing monooxygenase 3; **NADPH**, reduced nicotinamide adenine dinucleotide phosphate

* Corresponding author.
E-mail address: ann.vanschepdael@kuleuven.be (A. Van Schepdael).

http://dx.doi.org/10.1016/j.talanta.2016.11.028
Received 13 July 2016; Received in revised form 4 November 2016; Accepted 12 November 2016
Available online 17 November 2016

Table 1
IMERs based on magnetic nanoparticles for in-line CE.

Enzyme immobilized	Detector	Coolant	References
Alkaline phosphatase	UV	Liquid (capillary coated with polyvinyl alcohol)	9
Alkaline phosphatase	LIF	Air	10
Trypsin	MS	Air	11
Horseradish peroxidase	UV and MS	Air	12
CYP2C9	UV	Air	13

them to be used as drug delivery vehicles and/or insoluble supports for immobilization of proteins/enzymes. Magnetic nanoparticles have been used for in-line capillary electrophoresis for the development of IMERs (Table 1). This is particularly advantageous when compared to usage of frits in microfluidics or changing the chemistry of the capillary wall [7]. The capture can also be reversed by simply removing the external magnetic field to flush the magnetic particles. In this kind of setup, enzyme immobilized magnetic nanoparticles are injected into the capillary and their location is determined by the use of external magnets placed across the capillary. This allows for easy separation of substrate and products and repeated use of the immobilized enzyme thus enabling future automation. Enzyme immobilization on insoluble supports such as gold nanoparticles and magnetic nanoparticles has been applied in in-line capillary electrophoresis reactions. Capture of magnetic nanoparticles has been successfully attempted before in air based capillary cooling systems (Table 1) [9–13].

In capillary electrophoresis, heat dissipation and uniform temperature distribution across the capillary are important parameters to address. The temperature gradient between the internal capillary wall and the outside capillary wall increases with an increase in diameter. Hence, it can be seen that the larger the internal diameter the higher the current and heat generated across the capillary. This generation of heat can be easily detected by plotting Ohm's law (V=IR) [14]. If resistance is constant, a linear increase in generated current is expected upon increase of the voltage. Any deviation results in band broadening, joule heating and changes in reproducibility and reliability. To counter this, the outside wall of the capillary is usually either air cooled or liquid cooled. Liquid coolant generally has a higher heat capacity than an air cooled system. In present day CE, higher ionic strength buffers are regularly used and are expected to increase the temperature gradient across the capillary. However, the comparison between liquid cooled and air cooled capillary electrophoresis system is beyond the scope of this paper. In order to incorporate external neodymium magnets, in most cases, an air cooled capillary system is selected or the liquid coolant system is disabled [8]. In an earlier study, using magnetic nanoparticles in a CE system that was commercially available at that time, the particles were trapped under conditions where the inner wall of the capillary was coated with polyvinyl alcohol to prevent adsorption of proteins on the capillary wall. The polyvinyl alcohol coating also reduced EOF which facilitated trapping of the magnetic nanoparticles [9]. In this paper, we demonstrate a novel method to capture magnetic nanoparticles in the capillary cartridge of a current commercially available CE system, without disabling the liquid based coolant system.

2. Materials and methods

2.1. Reagents

Superparamagnetic silica particles (10 mg/mL) (600 nm mean diameter, amino functionalized) were obtained from MagnaMedics Diagnostics B.V. (Geleen, The Netherlands). Sodium dihydrogen phosphate (NaH_2PO_4), NADPH, glutaraldehyde 25% in water, sodium borohydride, clozapine, magnesium chloride anhydrous and disodium hydrogen phosphate (Na_2HPO_4) were acquired from Sigma-Aldrich (Steinheim, Germany). Human FMO3 Supersomes™ (hFMO3) with a concentration of 5 mg/mL were purchased from BD Biosciences (San Jose, CA, USA). Phosphoric acid was obtained from Chem-Lab (Zedelgem, Belgium). Mesityl oxide was purchased from Acros organics (New Jersey, USA). Sodium hydroxide was purchased from VWR chemicals (Radnor, PA, USA). Neodymium iron boron cube magnets (1×1 mm) were bought from Supermagnete (Gottmadingen, Germany).

2.2. Instrumentation

All experiments were carried out on a P/ACE™ MDQ capillary electrophoresis system (Beckman Coulter Instruments, Fullerton, CA, USA) equipped with a diode array detector. Bare fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with dimensions of 75 µm ID, 365 µm OD, 40 cm total length and 30 cm effective length was used. Detection was achieved at 191 nm with a bandwidth of 10 nm. Instrument control, data collection and evaluation were performed with 32 Karat™ software version 5.0 (Beckman Coulter Instruments, Fullerton, CA, USA). All the microscopic images were acquired with a motorized inverted IX-81 microscope connected to a CCD-FV2T digital camera (Olympus, Aartselaar, Belgium).

2.3. Solution preparations

Sodium phosphate buffer at a concentration of 150 mM was used to prepare background electrolyte at different pH conditions. To 150 mM H_3PO_4, 150 mM NaH_2PO_4 was added until pH 2 was obtained. To reach pH 4, 150 mM phosphoric acid was added to 150 mM NaH_2PO_4. To achieve pH 6, to 150 mM NaH_2PO_4 150 mM Na_2HPO_4 was added. For the basic pH 8, 150 mM NaH_2PO_4 was added to 150 mM Na_2HPO_4.

Magnetic nanoparticle solutions (1 mg/mL) were washed with incubation buffer (100 mM Na_2HPO_4 of which the pH was adjusted to 7.4 using 100 mM NaH_2PO_4) with the use of a separator (see Section 2.5. for details). In brief, the magnetic nanoparticles are dispersed in the incubation buffer and then subjected to the magnetic field of the separator. The magnetic nanoparticles move to one side of the eppendorf and then the incubation buffer can be removed by pipetting.

Mesityl oxide was used as a neutral marker and was prepared at a 0.086 M concentration in Milli-Q water (1 µL made up to 100 µL). All the stock solutions were prepared in Milli-Q water and filtered using 0.45 µm PTFE membrane filters.

For in-line enzymatic reaction, the background electrolyte (150 mM phosphate buffer pH 3.3) and incubation buffer of pH 7.4 were prepared in a similar manner as mentioned above with addition of 5 mM magnesium chloride in both the solutions. Clozapine stock (30 mM) was prepared in methanol and stored at −20 °C. NADPH solution (in incubation buffer) was freshly prepared each time before the start of the run.

2.4. CE conditions

A new capillary was rinsed with 1 M NaOH for 30 min. The capillary was maintained at 25 °C during the entire rinse sequence. At the beginning of the day, the capillary was flushed with 0.1 M NaOH, water and BGE for 10, 5 and 5 min, respectively. Before each run, the capillary was flushed with 0.1 M NaOH, water and BGE for 2.5, 1 and 3 min each, respectively. Sample injection was carried out at 0.5 psi (3.447 kPa) for 5 s. Before and after the injection, the capillary was dipped in water to prevent sample carry-over. At the end of the day, the capillary was rinsed with water for 20 min and with air for 4 min to dry the capillary. Each rinsing step was performed at 20.0 psi (137.895 kPa).