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Studying the interaction between peptides and polymeric nanoparticles used as pseudostationary phase in capillary electrochromatography

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ABSTRACT

In this work, the interaction between synthetic bioactive peptides and polymeric nanoparticles (NPs) used as pseudostationary phase in capillary electrochromatography was studied. NPs were prepared from methacrylic acid and ethylenglycol dimethacrylate with benzoyl peroxide by utilizing a precipitation polymerization technique. The reaction was monitored by infrared spectroscopy. Polymer characterization was performed by dynamic light scattering and transmission electron microscopy. Experimental running conditions were tested, including organic solvent proportion in the background electrolyte, capillary conditioning, applied voltage, sample introduction amount, and how NPs were incorporated into the system. A continuous full filling technique in which the NPs were suspended in the entire electrolyte volume as well as a conventional partial filling technique were used. Results obtained at pH 7.0 suggest that the NPs have a very strong interaction with more basic peptides. The interaction between analytes and NPs was found to be predominantly ionic.

1. Introduction

Nanoparticles (NPs) have been used in separation science in the past decade with great success. Most applications are reported in academic research laboratories, where separation success has been greater than or equivalent to current state-of-the-art chromatographic and electrophoretic methods. In spite of this success, this technology has not penetrated industrial chemistry laboratories (e.g., pharmaceuticals and forensics) [1].

Particularly, the use of nano-sized materials in electro-driven separation systems has received special attention. Even though NPs have demonstrated their utility in separation science, they have not yet found regular application in pharmaceutical and other regulated chemistry environments, because complete understanding of the synthesis process along with size control and complete characterization has not yet been realized, so robustness and reliability are the limiting steps in this technology [1,2].

As we approach the nm range, the molecular physical and chemical properties can vary dramatically and sometimes in an unexpected manner. The potential of NPs seems to have no limits, and the area of separation science has been no exception. As novel stationary phases and dynamic coatings, NPs greatly enhance separation performance in terms of selectivity, efficiency, and resolution. They also fit well with the concept of the past decade, which has seen a major drive towards miniaturization of many analytical systems with the emergence of lab-on-a-chip technologies as the way forward. So far, NPs have found extensive use in capillary electrophoresis (CE), packed capillary electrochromatography (CEC) and open tubular CEC (OT-CEC) formats, and microchip CE (MCE) [3–10]. As stationary phases, NPs display a large surface area- to-volume ratio, which is ideal for low mass-transfer effects in chromatography. This feature is also invaluable in miniaturized electro-migration techniques (e.g., MCE where close electro chromatographic interactions are essential for good resolution and high efficiency) [11].

NPs are relatively easy to synthesize and can be functionalized by a wide range of different chemistries, with amino (NH2), thiol (SH), and carboxyl (COOH) functionalities, being the most popular currently [12–20]. Several kinds of NP and nanostructure have been applied to separation science, including fullerenes, nanotubes, silica, polymers, zeolite, lipids, latex, metaloxides, and silver NPs (AgNPs) and AuNPs [21, 22].

The NPs can be charged or uncharged to accommodate electroosmotic flow (EOF) in electrophoretic separations, the surface area-to-volume ratio is hugely increased compared to that of the bulk material. At this nm range, quantum-dot (QD) effects can be exploited, and there is huge scope for versatility regarding chemical functionality, while size and shape can be controlled during formation.

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In summary, the ideal NPs form stable suspensions in a variety of electrolytes, provide analyte selectivity, must be charged when moved concentrated in a plug into the capillary column in order to avoid elution with the EOF, show equal mobility to minimize overall band broadening, give minimal mass transfer effects, do not interfere with detection, and be small and porous with a large surface area. These considerations are important so as to achieve highly efficient separations [23].

NPs have mainly been used in OTCEC and to a limited extent in CE. In the OT formats, NPs are usually fixed on the inner wall of the capillary column [2] or used as pseudostationary phase (PSP)-CEC whereby NPs are added to the buffer [5].

CEC is habitually performed using packed, monolithic or open- tubular columns. However, the use of PSP has become an interesting alternative with the advantage that it does not require frits or packing. It also provides sites for analyte interaction and moves with or against the mobile phase. In this system, the stationary phase is continuously replaced and a renewed column is used for each analysis, which avoids the contamination associated with complex matrix samples and minimizes the need of column change [24]. In some respects, this approach is favored as column-packing technologies have not advanced at the same rate as particle technology, resulting in the absence of a robust method to pack NPs into narrow- bore capillary columns. Capillary packing has been somewhat of a black art in CEC and reproducibility can be quite difficult to achieve [25]. Therefore, more understanding and further advancement are required before there is a robust packing procedure for packing of NP stationary phases. Thus, NPs have found popular use in OTCEC as inner wall coatings or powerful additives to the background electrolyte.

The aim of this work was to evaluate the interaction between synthetic peptides and in-lab synthesized polymer particles acting as PSP in CEC. Different monomer concentrations were tested in order to modify the particle size and, thus, to study their influence on peptide electrochromatographic behavior. Synthesized NPs presented homogeneous size and shape. Experimental running conditions were tested, including organic solvent proportion in the background electrolyte (BGE), capillary conditioning, applied voltage, sample introduction amount, and how NPs were incorporated into the system. Results obtained at pH 7.0 suggest that the NPs have a very strong interaction with basic peptides.

2. Experimental

2.1. Chemicals

The bioactive synthetic peptides bradykinin, angiotensin I, luteinizing hormone releasing hormone (LHRH), oxytocin, methionine-enkephalin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Amino acid sequence, relative molecular mass, and isoelectric point of each peptide are listed in Table 1. Disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium hydroxide, phosphoric acid, acetonitrile (ACN), and triethanolamine were purchased from Merck (Darmstadt, Germany). Methacrylic acid (MAA), ethylenglycol dimethacrylate (EGDMA), and benzoyl peroxide were also obtained from Sigma-Aldrich. Deionized water was purified with an Easy Pure ™ UltraPure water system from Barnstead-Thermolyne (Dubuque, IA, USA). Nylon membrane filters (0.45 μm) were purchased from Microclar (Tigre, Buenos Aires, Argentina).

Table 1
Analyzed bioactive peptides and their amino acid sequences, relative molecular masses, M_r, and isoelectric points, p*I*.

Peptide	Amino acid sequence	M_r	p*I*
Angiotensin I	DRVYIHPFHL	1296.5	7.91
Oxytocin	CYIQNCPLG	1007.19	7.70
Bradykinin	RPPGFSPFR	1060.2	12.40
LHRH	Pyr-HWSYGLRPG	1182.3	7.30
Methionine-enkephaline	YGGFM	573.7	5.93

2.2. Peptide solutions and running conditions

Stock solutions of synthetic peptides (1 mg/mL) were prepared by dissolving each synthetic peptide in water, fractionated in aliquots, and frozen at −20 °C. Standard solutions were daily prepared (10 μg/mL each) by dilution with water or BGE, when indicated.

The BGE consisted of 25 mM sodium buffer phosphate, pH 7.0, unless otherwise indicated. Peptide solutions were introduced by 5 s at 0.7 psi, the separation voltage was varied between 5 and 20 kV, normal polarity. At the beginning of daily work, the capillary column was rinsed with 0.1 M NaOH, 2 min at 30 psi, washed with water, 5 min at 30 psi, and BGE, 15 min at 30 psi. Before each run, the capillary was rinsed with 0.1 M NaOH, 2 min at 30 psi, followed by water, 5 min at 30 psi, and BGE, 5 min at 30 psi. All solutions were filtered and degassed before use. When BGE contained ACN, that step included 1 h degassing under vacuum with stirring, alternating with 30 s ultrasonic degassing every 15 min.

2.3. Instrumentation

Separations were performed in a P/ACE MDQ (Beckman Coulter Inc., Brea, CA, USA), equipped with a UV–Vis photodiode array detector. Data were processed by 32 Karat™ software (Beckman Coulter). Fused-silica (FS) capillaries (Polymicro Technologies, Phoenix, AZ, USA) were 50 cm in length (75 μm id × 365 μm od). For all experiments, the CE system temperature was held at 25 °C. In all cases, UV-detection at 214 nm was performed. A stereomicroscope (Riechter, Vienna, Austria) was used for capillary examination.

2.4. Synthesis of NPs

Particles were prepared from MAA and EGDMA monomers (1:2, m/m) by utilizing a precipitation polymerization technique. Different total monomer concentrations were tested, from 2% to 8% v/v in ACN, in order to modify the size of the particles. Benzoyl peroxide was added to the mixture as radical initiator. The solution was carried out at 60 °C in a water bath, during 24 h. Particles were washed by successive centrifugation and re-suspension twice with ACN and three times with 25 mM phosphate buffer solution, pH 8.0 (the last, when necessary to change the solvent).

3. Results and discussion

3.1. NPs characteristics

The synthesis of polymeric NPs was performed by a precipitation polymerization technique from a monomer mixture containing two well-known monomers, MAA and EGDMA. The MAA provides the polymer carboxyl functional groups which can be charged depending on pH of the mobile phase. Thus, MAA gives the polymer an electrically negative character when mobile phase pH is set to a value above 5.0. Additionally, MAA could be chemically modified to couple a wide range of functional groups conferring the system different options for selectivity improvement. The EGDMA, a derivative of methacrylic acid and ethylene glycol that contains two double bounds, is responsible for the crosslinking degree of the polymer.

The utilized precipitation polymerization technique allows NPs to be prepared without the use of stabilizing surfactants, thus simplifying the washing step after NPs generation. A MAA:EGDMA 1:2, m/m, proportion was selected for NPs synthesis. That proportion has been demonstrated to be ideal for obtaining porous and stable particles, which additionally present an optimal surface area-to-volume ratio [17]. The monomer mixture was prepared in ACN at different concentrations in