Food Chemistry 157 (2014) 498–503

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Polyamidoamine dendrimers as off-column binding agent and in-column pseudostationary phase for efficient and sensitive capillary electrophoretic analysis of fluoroquinolones in chicken muscles

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ARTICLE INFO

Article history:
Received 12 June 2013
Received in revised form 26 January 2014
Accepted 17 February 2014
Available online 26 February 2014

Keywords:
Polyamidoamine
Fluoroquinolones
Binding agent
Capillary electrophoresis
Pseudostationary phase
Chicken muscle

ABSTRACT

A capillary electrophoresis method based on polyamidoamine dendrimers has been developed for quick and sensitive detection of moxifloxacin, gatifloxacin, lomefloxacin, enoxacin, ofloxacin and pazufloxacin. Increasing buffer alkalinity led to enhanced sensitivity, whereas the polyamidoamine–fluoroquinolone interactions, i.e., in-cavity hydrogen bonding/hydrophobic interaction and electrostatic interaction, contributed to the improvement in both separation efficiency and detection sensitivity. By using 1 mM polyamidoamine generation 0.5 as binding agent in sample solution and 0.1 μM polyamidoamine generation zero as pseudostationary phase to the running buffer of 5 mM sodium tetraborate at pH 9.3, the six fluoroquinolones were baseline separated in <10 min with detection limits at ng/mL level. Coupled with liquid–liquid extraction, the proposed method was successfully applied in the determination of fluoroquinolones in chicken muscle samples.

1. Introduction

Fluoroquinolones (FQs) are antibiotics that possess excellent activity against both pathogenic Gram-negative and Gram-positive bacteria through inhibition of bacterial DNA synthesis. Since the 1980s, they have been used in clinical medicine and food -producing animal husbandry for a variety of infections. However, the wide usage of FQs has drawn considerable and increasing concerns because the residues of these antibiotics in human body could be hazardous owing to the possible toxic effects, allergic reactions, and development of resistant strains of bacteria. To ensure human food safety, maximum residue limits (MRLs) for the commonly used FQs in animal products have been set by the European Union and the countries in other regions (Commission Regulation 1353/2007/EC, 2007; WHO, 1998). Therefore, determining FQs in various biological tissues and fluids is needed.

FQs are mainly analysed by liquid chromatography and capillary electrophoresis (CE), equipped with detectors based on UV–visible, fluorescence and mass spectrometry, etc. (Evaggelopoulou & Samanidou, 2013; Faria, de Souza, de Almeida, & de Oliveira, 2006; Gajda, Posyniak, Zmudzki, Gbylik, & Bladek, 2012). In recent years, CE has been increasingly employed in FQ separation on account of its short analysis time, high separation efficiency and low consumption of reagents relative to the liquid chromatography methods. Conventional CE separation of FQs is straightforward because most FQs are zwitterions with carboxylic acid groups being the proton donors and nitrogen atoms of the piperazine rings the proton acceptors (Lombardo-Agui, Gamiz-Gracia, Garcia-Campana, & Cruces-Blanco, 2010), and tuning the buffer to a pH value slightly higher than their isoelectric points (pI) can often offer acceptable separation. Nevertheless, empirically, sometimes the complete separation of all analytes cannot be achieved (Beltran, Jimenez-Lozano, Barron, & Barbosa, 2004) because some FQs have close charge-to-size ratios. Another problem should be considered regarding FQ analysis is the in-capillary wall adsorption, which can occur in acidic, neutral, and even alkaline buffers (Qin, Liu, & Fan, 2009), leading to deformed peak shapes and decreased detection sensitivity, making it more difficult for the CE methods of intrinsically low sensitivity to assay trace level FQs. A number of approaches have been employed to address this problem, such as in- or off-column preconcentration, surfactant or protein additives, and nonaqueous buffers (Herrera-Herrera et al., 2011; Qin et al., 2009). In addition to these strategies, increasing the buffer alkalinity might be an effective alternative to suppress the FQ adsorption, because protonation of the nitrogen atom on the piperazine ring of FQ molecule could be inhibited (please refer to the pK_{a2} values in Fig. 1). The tactic, however, is less frequently adopted because: (1) at high pH, most FQs are nearly fully deprotonated, and their mobility differ-

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http://dx.doi.org/10.1016/j.foodchem.2014.02.070
0308-8146/

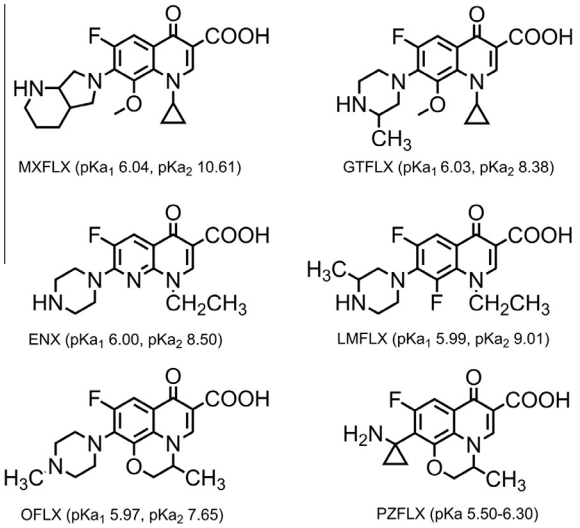

Fig. 1. Structures of the FQs studied. The pK_{a1} and pK_{a2} correspond to deprotonation constants of carboxylic acid group and protonated nitrogen atom on the piperazine ring, respectively.

ences are reduced, leading to deteriorated resolution; (2) the conventional additives do not have sufficient selectivity to resolve the overlapped peaks of the structurally similar FQs.

Polyamidoamine (PAMAM) dendrimers are highly branched, symmetrical macromolecules first synthesised by Tomalia et al. (1985). Their structures comprise cores, interior repeating units, and terminal groups. PAMAMs terminated with primary amine groups are full-generation dendrimers, whereas those terminated with methyl ester groups are half generations. To the best of our knowledge, the first application of PAMAM in CE is reported by Tanaka et al. (1992). To date, PAMAMs have been used as pseudostationary phases (PSPs) for separation of hydrocarbons, amino acids, parabens, proteins and positional isomers of neutral phenols (Castagnola et al., 1995; Liu et al., 2011). They offered better separation performances than the conventional PSPs mainly because of their uniform surface charge density and structural homogeneity (Castagnola et al., 1995; Tanaka et al., 1992). In recent years, the potential applications of PAMAM dendrimers have been also investigated using other analytical technologies, such as electrochemiluminescence detection of mercury in water (Ma et al., 2012) and amperometric detection of chlortetracycline in fortified milk and honey samples (Lian et al., 2012). In fact, PAMAMs can interact with a wider range of species because they have high densities of surface functional groups (Tomalia et al., 1985). It was reported that amine- and ester-terminated PAMAMs could interact with FQs, changing their aqueous solubility by means of electrostatic attraction, hydrogen bonding and encapsulation (Cheng et al., 2007). These findings implied the potential effectiveness of PAMAMs as modifiers in improving the FQ separation. Moreover, in liquid chromatography, amines were often employed to suppress the interaction between nitrogen-containing compounds and the silanols (Nahum & Horvath, 1981). Considering the structures of full-generation PAMAMs and the FQs, we think that the dendritic PAMAM molecules may be useful in suppressing the FQ adsorption and, therefore, improving separation and detection.

In this report, we employed half-generation PAMAM in sample solution as binding agent and full-generation dendrimer in running buffer as PSP to enhance the resolution and detection of six FQs, viz., moxifloxacin (MXFLX), gatifloxacin (GTFLX), lomefloxacin (LMFLX), enoxacin (ENX), ofloxacin (OFLX) and pazufloxacin (PZFLX) (structures shown in Fig. 1). The influences of the buffer pH, PAMAM generation and concentration were investigated and optimised. Finally, the CE method was combined with a liquid–liquid extraction (LLE) to determine FQs in fortified chicken muscle samples.

2. Experimental

2.1. Reagents and solutions

MXFLX, GTFLX, LMFLX, ENX, OFLX and PZFLX were bought from the Medicinal and Biological Research Institute (Beijing, China). Methanol (⩾99.5%), dichloromethane (⩾99.5%), *n*-hexane (>98%), sodium tetraborate (>96%), phosphoric acid (85%) and sodium hydroxide (⩾99%) were from Beijing Chemical Plant (Beijing, China). Ethylenediamine (⩾99.0%) and methyl acrylate (>95%) were products of Tianjin Fuchen Chemical Reagents (Tianjin, China). Tris(hydroxymethyl)aminomethane (Tris ⩾99.0%) was purchased from Genview (Houston, Texas, USA).

Solutions were prepared with triple distilled water, and filtered through a 0.45 μm filter (Peaksharp Tech., Beijing, China). Individual FQ stock solutions at 1000 μg/mL each were dissolved in 6 mM NaOH. The working standard solutions were prepared every day and stored at 4 °C.

2.2. Synthesis of PAMAM dendrimers

Four PAMAM dendrimers of different generations (Gs), namely, G 0, G 0.5, G 1.5 and G 2.5, were employed in this study; they were synthesised and purified according to the divergent method described elsewhere (Tomalia et al., 1985). Briefly, the tetravalent initiator core ethylenediamine reacted with excessive methyl acrylate via Michael addition for 24 h in methanol, followed by vacuum distillation to remove excess methyl acrylate and methanol at room temperature. Afterwards, the tetraester reacted with excessive ethylenediamine through amidation for 72 h, and the product was purified by vacuum distillation at 72 °C, yielding PAMAM G 0. Higher generation PAMAMs could be obtained by subsequent Michael addition/amidation cycles. The ^{1}H NMR results of PAMAMs G 0, G 0.5, G 1.5 and G 2.5 were given in Section 1 of the Supporting Information.

2.3. Capillary electrophoresis

The CE system consisted of a DW-P303-1AC high-voltage power supply (Dongwen, Tianjin, China) and a CE-10 UV detector (Johnsson Separation Science, Liaoning, China). The signal from the detector was collected by a desktop PC via a CT22 data acquisition unit (Qianpu, Jiangsu, China), processed with HW2000 chromatography station (Qianpu). Separations were carried out in a 50.0 cm (effective length = 40.0 cm) × 100 μm i.d. fused-silica capillary (Yongnian Photoconduction Fibre, Hebei, China). The fresh capillary was treated with 0.5 M NaOH, triple distilled water and running buffer (the optimal buffer was 5 mM $Na_2B_4O_7$ at pH 9.3) for 20 min, consecutively. Samples were hydrostatically injected by 20 s at a height of 18.0 cm. The separation voltage was set at 10 kV, and the analytes were monitored at 254 nm.

Resolution (R_s) was calculated according to $R_s = 2(t_2 - t_1)/(W_2 + W_1)$, where t_1, t_2 and W_1, W_2 are the migration times and the peak widths of two successive peaks, peak 1 being the first one and peak 2 the next one adjacent to it.

2.4. Treatment of chicken muscle sample

The chicken muscle samples (chicken breast, Arbor Acres broiler, Huadu Broiler Corporation, Beijing, China) were bought from a local supermarket. The products were claimed to meet the