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Analytical Methods

# Capillary electrophoresis method with UV-detection for analysis of free amino acids concentrations in food

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ABSTRACT

Simple and inexpensive capillary electrophoresis with UV-detection method (CE-UV) was optimized and validated for determination of six amino acids namely (alanine, asparagine, glutamine, proline, serine and valine) for Sudanese food. Amino acids in the samples were derivatized with 4-chloro-7-nitro-2,1,3-ben zoxadiazole (NBD-Cl) prior to CE-UV analysis. Labeling reaction conditions (100 mM borate buffer at pH 8.5, labeling reaction time 60 min, temperature 70 °C and NBD-Cl concentration 40 mM) were systematically investigated. The optimal conditions for the separation were 100 mM borate buffer at pH 9.7 and detected at 475 nm. The method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability) (RSD%) and accuracy (recovery). Good linearity was achieved for all amino acids ($r^2 > 0.9981$) in the concentration range of 2.5–40 mg/L. The LODs in the range of 0.32–0.56 mg/L were obtained. Recoveries of amino acids ranging from 85% to 108%, ($n = 3$) were obtained. The validated method was successfully applied for the determination of amino acids for Sudanese food samples.



## 1. Introduction

Amino acids are important for life because they are the basic components of proteins and serve as a source of energy (Cui et al., 2014; Veledo, de Frutos, & Diez-Masa, 2005). The nutritional value of proteins depends mainly on their amino acids composition (González-Castro, López-Hernández, Simal-Lozano, & Oruna-Concha, 1997; Veledo et al., 2005). Rapid and efficient determination of amino acids concentration in complex matrices is of broad interest in food chemistry and industry. Determination of free amino acids levels play a major role for the quality and safety of many foods (Veledo et al., 2005).

Determination of amino acids in complex samples is a challenge because they are not sufficiently volatile, most of them are highly polar and do not have a chromophore (Elbashir, Aboul-Enein, & Suliman, 2011; Lorenzo, Navarrete, Balderas, & Garcia, 2013).

Several techniques have been described for the determination of free amino acids, including high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE) (Cui et al., 2014; Mustafa, Åman, Andersson, & Kamal-Eldin, 2007; Song, Funatsu, & Tsunoda, 2013). The traditional analytical technique used to measure free amino acids is cation-exchange chromatography with post-column derivatization with ninhydrin (Lorenzo et al., 2013). However, these methods have drawbacks, such as they require expensive equipment, long analysis time and extensive sample preparation and cleanup (Mustafa et al., 2007; Soga & Heiger, 2000; Warren, 2008). Reverse phase HPLC (RP-HPLC) methods for amino acids analysis suffered from poorly retained of polar amino acids on the RP columns and difficult to separate from the solvent peak (Warren, 2008). CE has been considered as a powerful separation technique for amino acids and peptides in complex samples (Poinsot, Bayle, & Couderc, 2003; Akamatsu & Mitsuhashi, 2013; Hirayama, Igarashi, Tomita, & Soga, 2014; Simionato, Moraes, Carrilho, Tavares, & Kenndler, 2008).

UV–vis detector is the most used detector in commercially available CE system because of its general applicability (Zacharis et al., 2006). Since most of amino acids have no chromophore, a derivatization step is often essential in order to enhance their detectability using optical detection (Neda et al., 2012). As it has been reviewed in literature the commonly used pre-column derivatizing reagents for amino acids include 6-aminoquinoly-*N*-hydroxysuccimidyl carbamate (AQC), *o*-phthalaldehyde (OPA), naphthalene dicarboxaldehyde (NDA), 9-fluorenylmethyl

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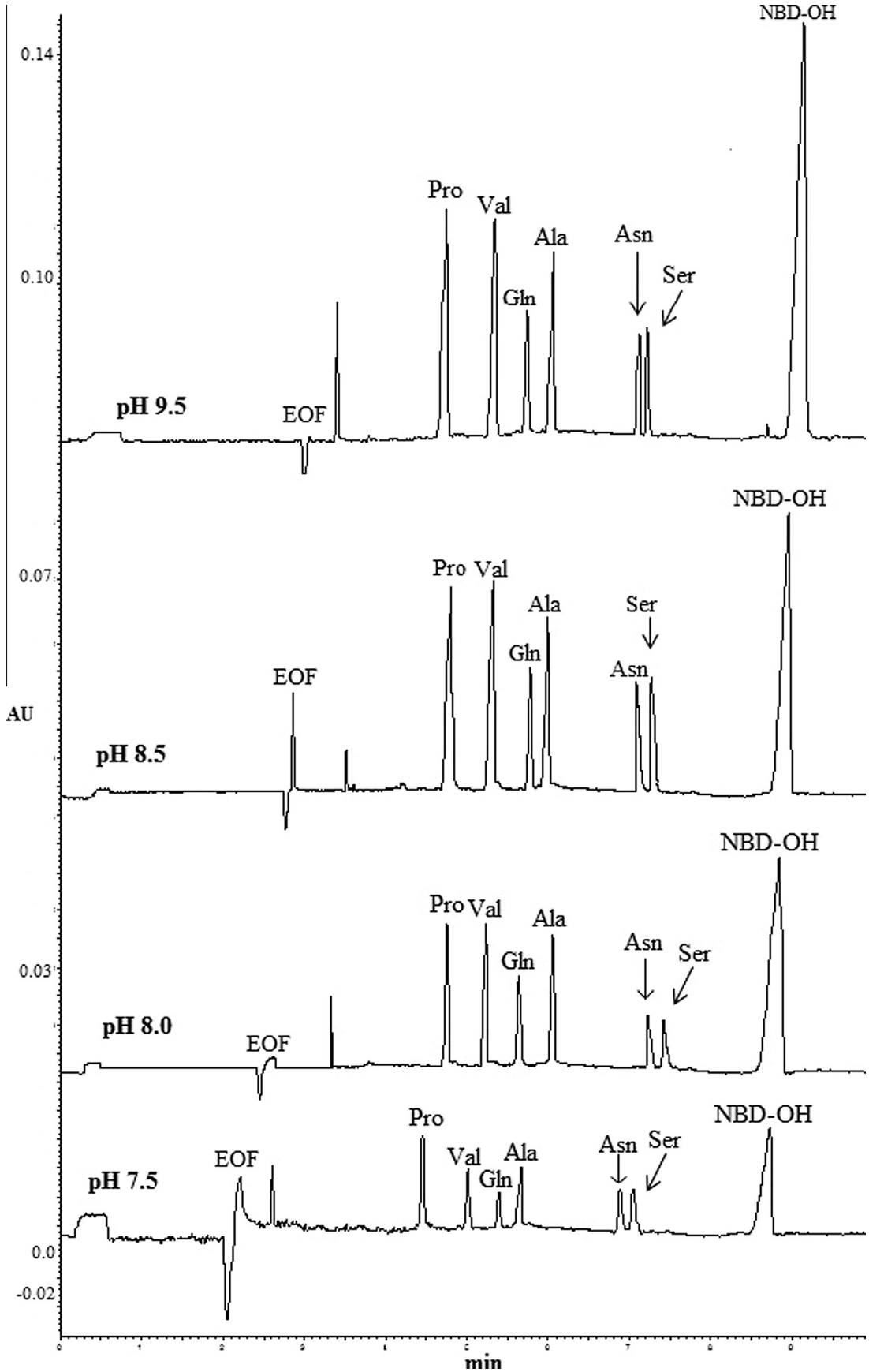


**Fig. 1.** Electropherograms obtained at different derivatization pH. Amino acids concentration 10 mg/L each. Derivatization conditions: NBD-Cl concentration 30 mM, reaction time 40 min, 100 mM borate buffer, reaction temperature 60 °C. Peaks: Pro, proline; Val, valine; Gln, glutamine; Ala, alanine; Asn, asparagine; Ser, serine.