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Determination of Glucosamine Content in Nutraceuticals by Capillary Electrophoresis Using In-Capillary OPA Labeling Techniques

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ABSTRACT

Capillary electrophoresis (CE) procedure was developed for determining glucosamine content in nutraceuticals. Glucosamine was labeled with *o*-phthalaldehyde (OPA) by mixing with the OPA reagent (4.5 mM OPA, 4.5 mM 2-mercaptoethanol dissolved in 20 mM borate buffer, pH 9.3). The fluorescent OPA-glucosamine adduct was separated and detected at A_{340} within 3 min. The pre-capillary labeling reaction can be performed by sequentially injecting OPA/sample/OPA segments into the separation capillary; the in-capillary labeling technique simplified the sample pretreatment and improved its reproducibility. The linear dynamic range of the in-capillary labeling CE method was 0.1~30 mM. No sample pretreatment procedure was required for tablets containing mainly starch or other sugar as the vehicles.

Key words: capillary electrophoresis, glucosamine, nutraceutical, in-capillary labeling, *o*-Phthalaldehyde

INTRODUCTION

Glucosamine is an important building block for cartilage, tendons and other connective tissues. This natural chemical has additional functions in inhibiting enzymes that destroy cartilage[1]. Oral administration of glucosamine has been reported to show clinical effects in re-growing cartilage and slowing down joint deterioration without significant adverse effects[2]. Therefore, the simple aminosugar has become the most popular ingredient in nutraceuticals for the treatment of osteoarthritis.

Simple and quick analytical methods for glucosamine are required along with the growing market demand, but the chemical species can hardly be analyzed optically or electrochemically without derivatization[3,4]. Reducing end quantification methods such as MBTH[5] are not suitable for most nutraceutical preparations since those methods usually contain sugars as the vehicles. Methods based on labeling the amino groups are considered for determining amino sugars[6,7].

o-Phthaladehyde (OPA) is a popular fluorescent labeling agent for amino group. The labeling reaction occurs rapidly in room temperature and is frequently applied as an on-line post-column derivatizing technique for HPLC analysis of amino acids and proteins[8]. Unfortunately, the reagent itself is unstable and reaction comes with unidentified side reactions. Pre-column labeling strategies are therefore practically difficult and intensive column clean-up procedures are need after the separation.

Capillary electrophoresis (CE)[9-12], a new analytical trend, is a rapid and ready-to-run technique that is especially suitable for routine and multi-sample analytical tasks. Easy column clean-up further improves the analytical speed and sample throughput.

In the present approach, pre-column OPA labeling technique was adapted for determining glucosamine by a rapid capillary zone electrophoresis procedure. To improve the reproducibility and ease the automatic sampling process, OPA reagent and the sample solution were sequentially injected into the capillary. The labeling reaction occurred within the capillary, and the fluorescent adduct was separated / detected in less than 3 min. Glucosamine content in the aqueous solutions of nutraceutical tablets were determined (A_{340}) with commercialized CE machine without sample pretreatment.

MATERIALS AND METHODS

I. *Chemicals*

o-Phthaladehyde (OPA), glucosamine hydrochloride (M. Wt = 215.63), and 2-mercaptoethanol were purchased from Wako Co. Sodium tertraborate (borax) was from Nacalai Tesque Co. Glycine was from Sigma Co. Other chemicals were of analytical grade and used without purification. Commercial glucosamine tablets were purchased from local drug stores.

Deionized water (< 1 μScm^{-1}) was used for preparing buffers, reagents and sample solutions.

II. *pH Buffer, OPA Reagent and Sample Solutions*

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Borate buffer was prepared by dissolving sodium tertraborate (borax) in deionized water as a 0.1 M stock solution. The unadjusted pH was 9.3. The stock buffer was stored at ambient temperature and diluted to suitable extent for later uses.

OPA reagent was prepared by dissolving *o*-phthalaldehyde solution (30 mg in 1 mL of ethanol) and 15 μL of 2-mercaptoethanol in 50 mL of 50 mM borate buffer (pH 9.3). The OPA reagent (4.5 mM) was prepared immediately before experiment.

Sample solutions were prepared by dissolving tablets of nutraceutical with 100 mL of deionized water. The solutions were filtered through a 0.22 μm membrane filter, Carrigtwohill Co.

III. *Instrumentation*

A thermo-controlled (25°C) capillary electrophoresis (CE) system (G1600A, Agilent) was used to obtain the CEgrams. Before the experiments, an uncoated fused-silica capillaries (length = 34 cm; effective length = 28.5 cm; O.D. = 375 μm; I.D. = 75 μm) were thoroughly rinsed with 1 N NaOH (950 mbar × 40 min) and then deionized water (950 mbar × 20 min).

The separation capillary was rinsed (950 mbar × 3 min) with the running buffer (20 mM borate buffer, pH 9.3) immediately before injection. The electrophoretic separation process was monitored with a built-in photodiode array (PDA) detector. After the electrophoresis, the capillary was cleaned up by sequentially rinsing with 1N NaOH (950 mbar × 3 min.) and deionized water (950 mbar × 3 min). The above procedures were controlled with Chemstation^{TM}, the software by Agilent.

RESULTS AND DISCUSSION

I. *Electrophoresis of the Reaction Mixture of OPA Reagent and Glucosamine*

Although OPA-amine adducts were usually measured fluorometrically, the sensitivity of photometric measurement (A_{340}) is acceptable for measuring the ingredient in pharmaceutical preparation. Acceptable electropherograms (similar to those in Figure 1) can be easily obtained by injecting (30 mbar × 3 sec) and separating (10 kV in 20 mM borate, pH 9.3) a reaction mixture containing 100 μL of 10 mM glucosamine, 100 μL of 5 mM glycine and 500 μL of OPA reagent. However, the reproducibility of quantification was poor.

As directly monitoring A_{340} of the OPA labeling reaction in a quartz cuvette, the reaction readily reached its maximum absorbance in approximately 2 min. The rapid reaction kinetics is suitable for analytical applications, but the decay in A_{340} after about 5 min implies unidentified problematic side reactions. It may be possible to improve the reproducibility by performing the reaction on-line within the separation capillary.

II. *In-capillary Labeling of Glucosamine and Separation of its OPA-derivatives*

In Figure 1, OPA-labeling was performed by sequentially injecting OPA reagent, sample solution and then OPA reagent into the capillary. The separation was not satisfactory when the reaction mixture was separated immediately after the mixing process (the lower trace of Figure 1). The separation was significantly improved by introducing a brief time interval (i.e. 6 sec, as shown by

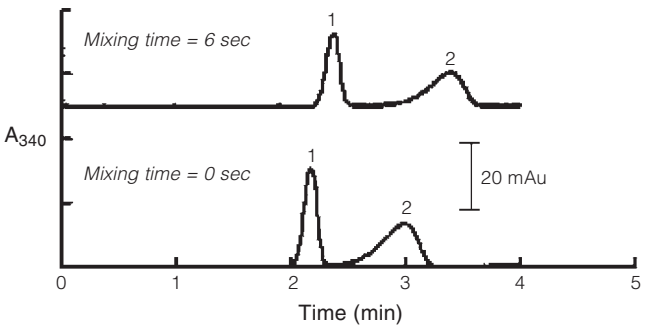

Figure 1. Effects of mixing time on electropherogram. Capillary were injected sequentially with OPA reagent (30 mbar × 5 sec), glucosamine solution (10 mM; 30 mbar × 3 sec) and OPA reagent (30 mbar × 5 sec), and then separated (10 kV) with the running buffer of 20 mM borate, pH 9.3. A_{340} was monitored. The glucosamine (Peak 1) solution contained 5 mM glycine (Peak 2). Other conditions are detailed in the experimental section.

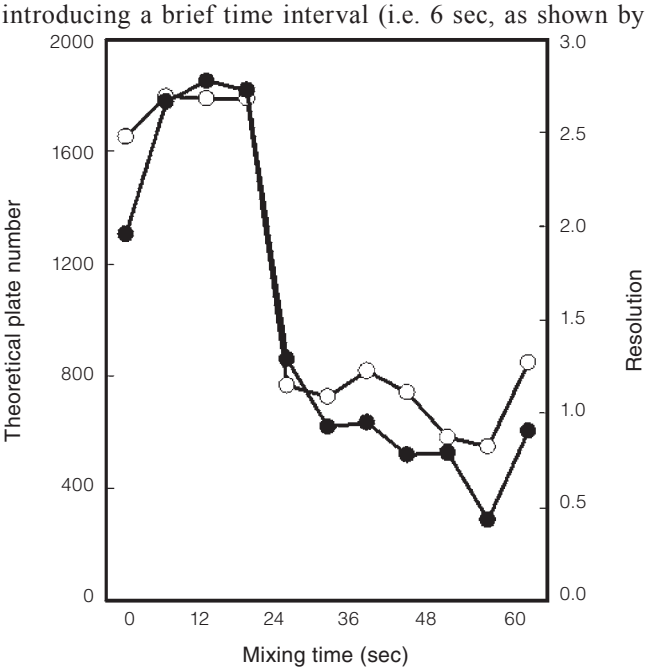

Figure 2. Effects of mixing time on the theoretical plate numbers and resolutions of electropherograms. The experimental conditions are shown in Figure 1. Open circles: theoretical plate numbers; solid circles: resolutions.

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the upper trace of Figure 1) before imposing separation voltage. However, further elongation of mixing time dispersed the sample plug and reduced both the theoretical plate number and the resolution (Figure 2). Twelve seconds was the proper time interval for the present experimental conditions and instrumentation.

The separation conditions were further optimized as in Figure 3. With the increase of buffer concentration (Figure 3 A), the increasing ionic strength shielded the surface charges of capillary wall, which resulted in lowering the zeta potential and the velocity of electroosmotic flow. The migration time was therefore reduced in low concentration buffers. By considering the separation speed and buffer capacity, 20 mM borate buffer was selected.

The separation voltage was selected to be 10 kV for shorter migration time (Figure 3 B), sufficient theoretical plate numbers (> 2000) and fewer joule-heating problem. Migration time was not significantly affected by the pH of running buffer (Figure 3 C). The unadjusted pH of borax solution (pH 9.3) was used throughout the study.

Linearity of the calibration curve of the optimized method (10 kV, 20 mM borate, pH 9.3, mixing time = 12 sec) was up to 30 mM (r^2 = 0.9615) with detection limit at 0.1 mM (S/N > 10). The CVs (n = 4) of peak area were less than 7%.

III. *Determination of Glucosamine in Nutraceuticals*

Three types of glucosamine tablets were measured for active ingredients, among which some tablets may contain other chemicals with primary amino groups such as amino acids and proteins (peak 3 of the upper trace in Figure 4). Judging from the spectra (Figure 5) obtained by the

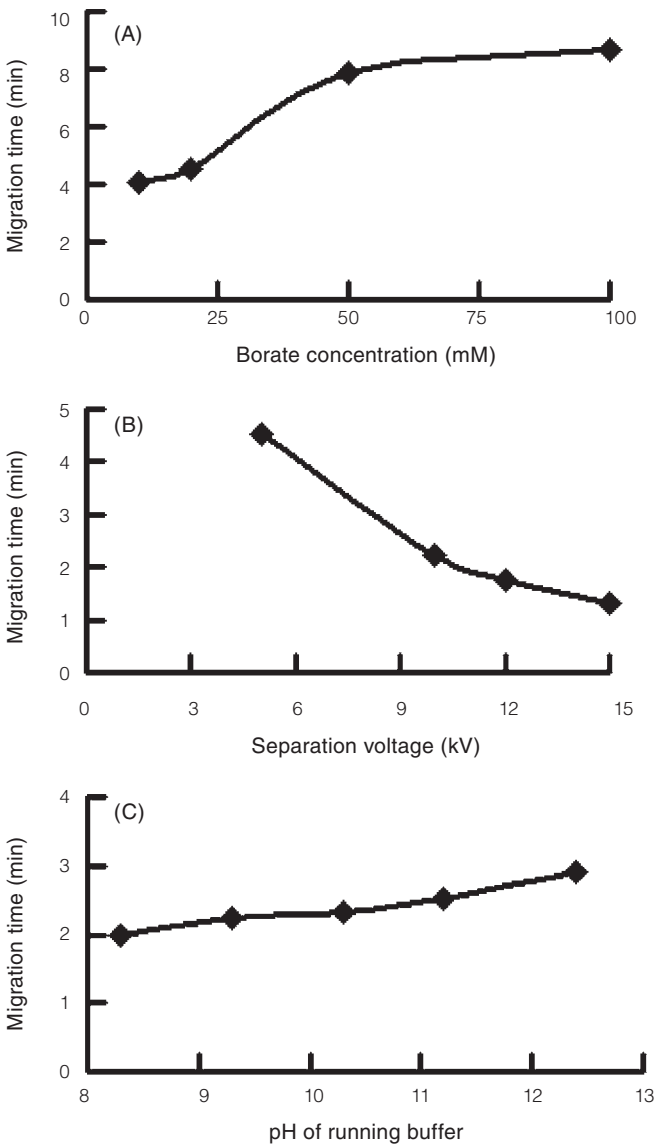

Figure 3. Effects of electrophoretic conditions on migration time of OPA-labeled glucosamine. (A) 5 kV, pH 9.3, (B) 20 mM borate buffer, pH 9.3, (C) 20 mM borate buffer, 10 kV. The mixing time was 12 sec; other conditions are as in Figure 1.

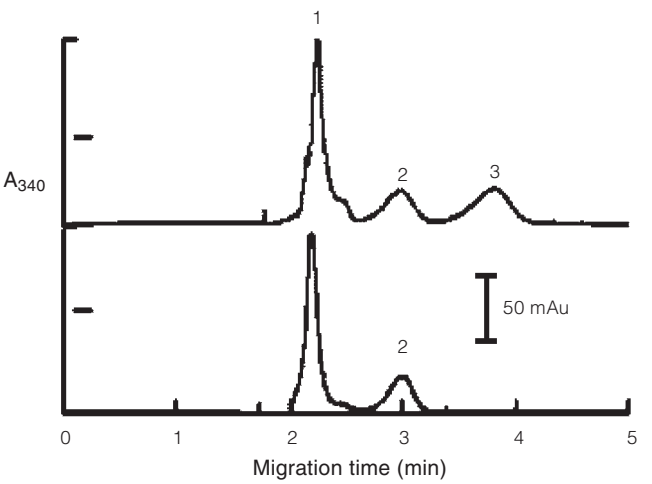

Figure 4. Electropherograms of a sample solution (upper trace) and glucosamine standard solution (lower trace). The mixing time was 12 sec; other conditions are as in Figure 1. Peaks 1, 2 and 3 are the signals of OPA-glucosamine, OPA-glycine and an unidentified chemical, respectively. The standard solution contained 20 mM glucosamine and 10 mM glycine.

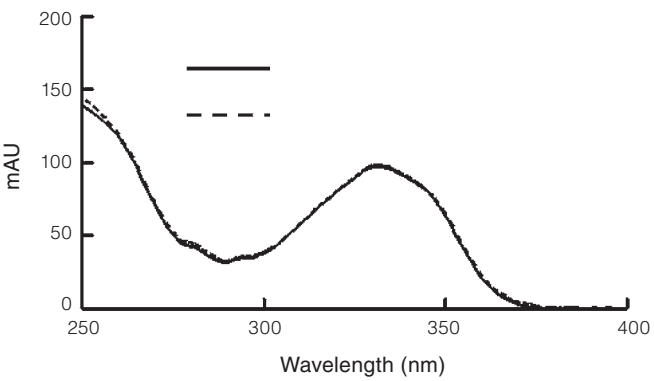

Figure 5. Comparison of the absorption spectra. Spectra of peak 1 (OPA-glucosamine) in Figure 4 were obtained with the built-in PDA detector of the CE machine. Solid line: glucosamine standard; dashed line: sample solution of a nutraceutical.

built-in PDA detector, the purities of OPA-glucosamine adducts (peak 1 in Figure 4) were nearly unity. Rapid and efficient separation was performed and confirmed by the purity check and the following data comparison. The glucosamine contents in tree tablets were determined (n = 4) to be 670 ± 31 mg, 447 ± 14 mg and 404 ± 29 mg, somewhat lower than the labeled contents (750 mg, 500 mg and 450 mg, respectively).

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