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In-capillary derivatization with *o*-phthalaldehyde in the presence of 3-mercaptopropionic acid for the simultaneous determination of monosodium glutamate, benzoic acid, and sorbic acid in food samples via capillary electrophoresis with ultraviolet detection

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ABSTRACT

For the rapid simultaneous determination of monosodium glutamate (MSG), benzoic acid (BA), and sorbic acid (SA) in canned food and other processed food samples, we developed a method that combines in-capillary derivatization with separation by capillary electrophoresis. This method employs the rapid derivatization of MSG with *o*-phthalaldehyde (OPA) in the presence of 3-mercaptopropionic acid (3-MPA) and enables the detection of the resulting OPA-MSG derivative and of non-derivatized BA and SA at 230 nm. The composition of the background electrolyte and the parameters of derivatization and separation are as follows: 25 mM borax containing 5 mM OPA and 6 mM 3-MPA, separation voltage 25 mV, injection at 30 mbar for 20 s, and column temperature 25 °C. Because of the high reaction rate and suitably adapted effective electrophoretic mobilities, band broadening due to the derivatization reaction at the start of the separation process is kept to a minimum. The optimized method is validated with respect to LOD, LOQ, linearity, recovery, and precision. This method can be applied to real samples such as soy, fish, oyster and sweet and sour chili sauces after application of appropriate clean-up steps. Mechanisms of zone broadening and zone focusing are discussed showing the validity of the employed theoretical approach regarding the dependence of the peak shape for OPA-MSG on the concentration of MSG in the sample.

1. Introduction

Monosodium glutamate (MSG, flavor enhancer), sodium benzoate and potassium sorbate (food preservatives) are often added together in processed food. MSG, benzoic acid (BA) and sorbic acid (SA) are generally recognized as safe (GRAS) when used as a salt substituent which is produced in agreement with good manufacturing practice (GMP) established by FDA [1,2]. Glutamate itself has a very low toxicity under most circumstances. The oral lethal dose LD_{50} in rats and mice is 15,000–18,000 mg/kg body weight, respectively [3]. Glutamate is an effective excitatory neurotransmitter in the human brain. If the glutamate receptor inactivation is not balanced with glutamate reuptake in the synaptic cleft, glutamate may accumulate and becomes neurotoxic [4], affecting the memory formation, learning, and regulation processes. As reported by FASEB in 1995, exposure to MSG in dosage from 0.5 to 2.4 g may result in a transient MSG syndrome ("Chinese restaurant syndrome") characterized by flushing, headache, numbness in the mouth and other symptoms. Hence, there are controversial views on the safety of MSG consumption. An acceptable daily intake of BA and SA is 5 mg/kg (JECFA, 1997) and 25 mg/kg body weight per day (JECFA, 1974) [5,6]. BA is relatively non-toxic but it can also be transformed to benzene, a known carcinogen, by reacting with ascorbic acid in the presence of a transition-metal catalyst [7]. Sorbic acid has conjugated double bonds that render it susceptible to nucleophilic attack by species such as thiols, amines and nitrite, sometimes producing mutagenic products [8].

To date, various methods have been developed for the determination of MSG in food including the Soerensen formol titration

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method [9], paper and TLC chromatography [10,11], reversed phase HPLC after pre-column derivatization [12–15] and methods based on biosensors employing different enzymes [16,17]. The quantitation of glutamate can also be carried out by capillary electrophoresis (CE) after pre-column derivatization with different fluorescence labeling agents [18–20]. In principle, CE can be combined with pre-, in- and post-capillary derivatization [21], while the advantage of in-capillary derivatization (derivatization is performed within the separation capillary, derivatization agent is component of the separation electrolyte) is not only the easy automation of the derivatization procedure but also the fact that the analyte is separated from matrix constituents before the derivatization takes place. In-capillary derivatization of amino acids and aliphatic amines (combined with CE) has been reported by several authors. They employ different derivatization agents such as naphthalene-2,3-dicarboxaldehyde in the presence of NaCN for histamine [22] or for amino acid neurotransmitters from brain microdialysis samples [23], 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate for amino acids in dietary supplements and wine [24], and *o*-phthalaldehyde in the presence of 3-mercaptopropionic acid for glucosamine in nutritional supplements [25]. BA and SA in food are determined by spectrophotometric methods [26], by HPLC [27,28], gas chromatography [29] and CE [30], with HPLC being the most commonly employed method.

There is yet no report on in-capillary derivatization of MSG for CE analysis of food samples. We, therefore, intended to develop a method for the simultaneous identification and quantification of MSG, BA and SA in processed foods employing CE with UV detection together with in-capillary derivatization. According to the method developed by Akamatsu and Mitsuhashi [25] for the determination of glucosamine in nutritional supplements, we selected the in-capillary derivatization of MSG with *o*-phthalaldehyde (OPA) in the presence of 3-mercaptopropionic acid (3-MPA) as derivatization procedure. Introducing a mercaptopropionyl moiety on or near the isoindole ring lessens the liability of the formed isoindole to oxidative degradation due to its bulky size (Fig. 1) [31–33]. The most similar approach to this study is the determination of MSG in various foodstuffs by HPLC using OPA as derivatization agent.

This is the first study that combines the determination of in-column derivatized MSG together with the determination of UV-active underivatized BA and SA within one CE method (using the same background electrolyte) at a fixed UV-wavelength. Method development is accompanied by a fundamental investigation of parameters to be optimized for minimization of reaction band broadening. We also examine whether this method can be applied to real-world samples such as soy, fish, oyster and sweet and sour chili sauces.

2. Theoretical considerations

2.1. Reaction band broadening

We assume the following simplified reaction scheme (the role of 3-MPA is neglected for simplification purposes):

$$A + B \rightarrow P \quad (1)$$

where A = MSG, B = OPA, P = product (OPA-MSG).

Here MSG and OPA-MSG denote the negatively charged deprotonated species being present at the pH of the background electrolyte. We assume that at the beginning of the separation (and the reaction) the total amount of substance of MSG is contained in a very thin slide at the beginning of the capillary (Fig. 2A). We also assume that slide broadening by diffusion can be neglected. Hence, the volume V_{ini}, in which MSG is dissolved, is kept constant. Because MSG rapidly migrates from the injection volume into the background electrolyte compartment, we neglect initial mixing effects (quasi-instantaneous mixing) and assume (at $t \geq 0$) that the molar concentration of OPA (and 3-MPA) in the injected slide (the volume V_{ini}) is constant and identical to the concentration of this reagent in the background electrolyte. Because of electrophoretic mixing, it is also reasonable to assume that the electric conductivity in the transferred injected slide (the transferred volume V_{ini}) is identical to the electric conductivity of the background electrolyte. It is important to note that under the defined conditions the start time of the separation process and the start time of the derivatization reaction are identical. We now consider the reaction rate of this reaction having a pseudo-first order kinetics (high excess of B):

$$\frac{dc_p}{dt} = kc_A c_B = k' c_A \quad (2)$$

where c_p = molar concentration of product (OPA-MSG), t = reaction time = migration time, k = reaction rate constant, c_A = molar concentration of A (MSG), c_B = molar concentration of B (OPA). Solving this equation by integration results in (refer to Fig. 2B):

$$n_P = n_{P,tot}\left(1 - e^{-k't}\right) \quad (3)$$

with n_P = amount of substance of P, $n_{p,tot}$ = total amount of substance of P after complete conversion of A. Because the derivatization reaction is accompanied by the application of a constant electric voltage (generation of a constant and homogeneous electric field strength), the volume V_{ini} becomes the source of a flux $\vec{J}_P$ of ions of type P ($\vec{J}_P = dn_P/dt$) migrating at constant velocity (due to the effective electrophoretic mobility of P) versus the detection end or versus the injection end (as will be discussed later). Simultaneously, the flux density $\vec{j}_P$ is defined as the flux $\vec{J}_P$ per area A ($\vec{j}_P = \vec{J}_P/A$). Area A corresponds to the capillary cross sectional area at the "outlet" boundary of V_{ini}. As the "particle production rate" dn_P/dt in the source is decreasing, also the flux $\vec{J}_P$ and the flux density $\vec{j}_P$ are decreasing with time:

$$\vec{j}_{(P,t)} = \frac{n_{P,tot}}{A} k' e^{-k't} = \vec{j}_{(P,t=0)} e^{-k't} \quad (4)$$

where $\vec{j}_{(P,t)}$ = flux density of ions of type P leaving V_{ini} at time t and $\vec{j}_{(P,t)}$ = flux density of ions of type P leaving V_{ini} at time t = 0. Simultaneously, the produced zone of P is migrating due to electroosmosis and the effective electrophoretic mobility of P, while V_{ini} is migrating due to electroosmosis and the effective electrophoretic mobility of A. We can also say V_{ini} is migrating relative to the produced zone of P. Because the whole process can be described as a one-dimensional problem, we now define a moving coordinate system having the velocity of the produced zone of OPA-MSG (Fig. 2C) with the origin at the maximum (or start value) of this zone. At $t \rightarrow \infty$ a zone is formed, which can be described with the following equation after employing the transfer equations $\vec{j}_P = c \times v_{rel}$ and $t = x/v_{rel}$ (for the definition of x refer to Fig. 2C):

$$c_{(P,x)} = c_{(P,x=0)} \times e^{-\frac{k'x}{v_{rel}}} \quad (5)$$

where $c_{(P,x)}$ = molar concentration of P at distance x, $c_{(P,x=0)}$ molar concentration of P at origin of moving coordination system, v_{rel} = velocity of the zone containing P relative to V_{ini}. We now define: μ_{eo} = electroosmotic mobility, μ_A = observed mobility of A = $\mu_{eo} + \mu_{eff,A}$ (with $\mu_{eff,A}$ = effective electrophoretic mobility of A), μ_P = observed mobility of P = $\mu_{eo} + \mu_{eff,P}$ (with $\mu_{eff,P}$ = effective electrophoretic mobility of P), E_0 = electric field strength under homogeneous conditions = U/L_T (with U = applied voltage, L_T = total length of capillary). It follows for v_{rel}:

$$v_{rel} = \left(\mu_{eff,P} - \mu_{eff,A}\right) E_0 \quad (6)$$