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Evaluation of micellar electrokinetic capillary chromatography for the analysis of selected tobacco alkaloids

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Abstract

Micellar electrokinetic capillary chromatography was evaluated for simultaneous determination of several tobacco alkaloids with similar molecular structure. Satisfactory separation of nicotine, nornicotine, myosmine, anatabine and anabasine extracted from tobacco was achieved by introducing an ionic surfactant, sodium dodecyl sulfate, into the buffer system at a concentration of 100 m*M*. Sample preparation involved a single extraction with water containing 1% triethanolamine. Separation was performed on a 72 cm long, uncoated fused-silica capillary. Experimental parameters such as applied voltage, pH value, buffer composition and surfactant concentration were optimized. Enhancement of UV absorption of alkaloids in the presence of micelles was also studied.

Keywords: Tobacco; Alkaloids; Nicotine; Nornicotine; Myosmine; Anatabine; Anabasine

1. Introduction

The most common approaches for the analysis of tobacco alkaloids have been gas chromatography with nitrogen-specific detection or high-performance liquid chromatography with ultraviolet (UV) detection [1–4]. Previously we have demonstrated that capillary zone electrophoresis (CZE) can be used as an alternative tool for the determination of nicotine in tobacco [5]. Under CZE conditions, the ionic form, peak shape and UV absorbance of nicotine were pH dependent and were optimized by adjusting the pH of the buffer solution. The ionization of nicotine and the electroosmotic flow are key factors for obtaining desired resolution within a reasonable analysis time. A buffer solution with higher ionic strength and lower pH (e.g., 2.7) produce a slower electroosmotic flow and a better separation. At a pH of 2.7, nicotine predominately exists as a diprotonated form which favors its UV absorbance and thus, enhances the sensitivity. If nicotine accounts for more than 98% of the total alkaloids, which is often the case for a majority of the Nicotiania species, and matrix interferences are relatively insignificant, quick migration might replace resolution and sensitivity as the goal of optimization. By applying a combination of a weak buffer at a pH of 7 and a shorter capillary, the migration time of nicotine was successfully reduced to 60 s [5].

Simultaneous determination of structurally similar tobacco alkaloids such as nicotine, nornicotine, myosmine, anatabine and anabasine in the presence of a tobacco matrix, however, requires further modification of the reported CZE procedures [5]. Initial efforts of adding organic solvents in the buffer to enhance the effect of electrophoresis and the res-

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olution of the CZE analysis produced only limited success. Satisfactory separation of five common alkaloids from a tobacco sample was achieved after introducing an ionic surfactant, sodium dodecyl sulfate (SDS), into the CZE buffer system at a concentration above its critical micellar concentration.

2. Experimental

2.1. Chemicals

Nicotine, nornicotine, myosmine and anabasine were purchased from Sigma (St. Louis, MO, USA). Triethanolamine and sodium dodecyl sulfate (SDS, 70% purity, contains 25% tetradecylsulfate and 5% hexadecyl sulfate) were obtained from Aldrich (Milwaukee, WI, USA). Anatabine was a gift from a colleague, Dr. David Douglas (Philip Morris, Richmond, VA, USA). The water used was obtained from a Milli-Q water purification system.

2.2. Sample preparation

Ground tobacco (0.5 grams) was placed in a 30-ml glass vial. After adding 20 ml of aqueous solution with 1% triethanolamine, the vial was capped, placed in an ultrasonic bath containing water at ambient temperature and sonicated for 6 min. A portion of the extract was filtered through a 0.45 mm disposable filter into an autosampler vial for CE analysis.

2.3. Instrument and procedure

The instrument consisted of a Perkin Elmer 270A-HT capillary electrophoresis unit equipped with a UV detector, an autosampler and a Hewlett–Packard 3396 series II integrator. The wavelength monitored for quantitation was 262 nm. The fused-silica capillary was 72 cm length×50 μm I.D. with a window for on-column UV detection at a position 50 cm from the anode. The temperature was ambient. The hydrodynamic injection time was set at 1 s. The sample was run in a micellar solution containing 100 m*M* SDS, 6 m*M* sodium phosphate and 10 m*M* sodium borate at a pH of 9.5. The applied voltage was set at +30 kV unless noted otherwise. The above parameters are for the analysis of alkaloids in tobacco. Experimental parameters for various studies in this work are described in the text as appropriate.

UV absorbance of nicotine was measured using a Beckman DU-6 model UV–visible spectrophotometer. Nicotine solutions used were at a concentration of 30 μg/ml in buffers at various pH values, with or without surfactant. Spectral data were collected at wavelengths from 200 to 360 nm.

3. Results and discussion

3.1. CZE with organic modifiers

Initially the efforts focused on improving the separation of tobacco alkaloids using CZE. Since the differential migration of solutes in CZE is mainly determined by mass/charge ratio, it is not uncommon to encounter difficulties in the separation of compounds with similar structure and mass/charge ratio such as tobacco alkaloids. As shown in Fig. 1, anabasine and anatabine, as well as nornicotine and myosmine, differ only by a double bond. Poor resolution of structurally similar compounds under aqueous conditions in CZE analysis was suggested by Fijiwara and Honda [6] to be a result of hydration, because the increased size of hydrated solutes further decreased the difference in structure and mass/charge ratio, thus reducing the resolution. These authors proposed that the hydration problem

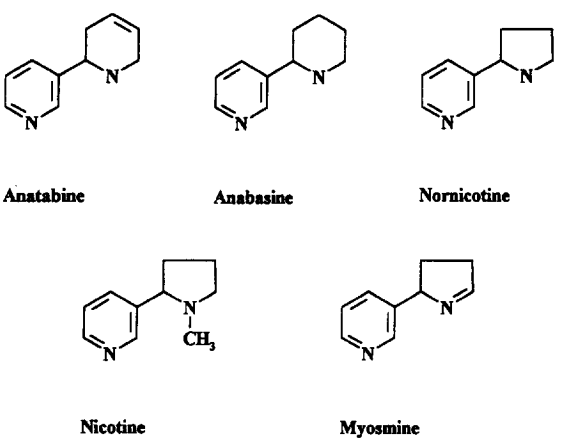

Fig. 1. Five alkaloids commonly found in tobacco; anatabine, anabasine, nicotine, nornicotine, myosmine.

could be reduced by mixing organic solvent into the buffer solution. They demonstrated that the separation of positional isomers of amino- and methyl benzoic acids by CZE were significantly improved after the hydrated solutes were desolvated by adding acetonitrile. In this study, organic solvents including methanol, 2-propanol and acetonitrile were tested as buffer modifiers. Anabasine and anatabine were mixed as test solutes. Methanol and isopropanol reduced the electroosmotic flow, slowed the migration of solutes, but provided little improvement in resolution. Adding acetonitrile in the buffer did not reduce the electroosmotic flow as much as adding alcohols, but the resolution of the two test solutes was somewhat enhanced. These results are consistent with the reported data [6]. As shown in Fig. 2, anabasine and anatabine were well resolved using a mixture of acetonitrile–phosphate buffer (50:50, v/v) at various pH values. In an extended test, baseline resolution for a mixture of four alkaloids (i.e., anabasine, anatabine, nicotine and nornicotine) was achieved using acetonitrile–phosphate at a ratio of 30:70 (v/v) at a pH of 6 (electropherogram not shown). However, in the presence of a tobacco matrix under those conditions, the achievement of baseline resolution for alkaloids was not possible. The volatility of acetonitrile also made it difficult to keep the buffer composition constant. Therefore, capillary electrophoresis under a different mode (i.e., micellar electrokinetic capillary chromatography, MEKC) was pursued for the analysis of tobacco alkaloids.

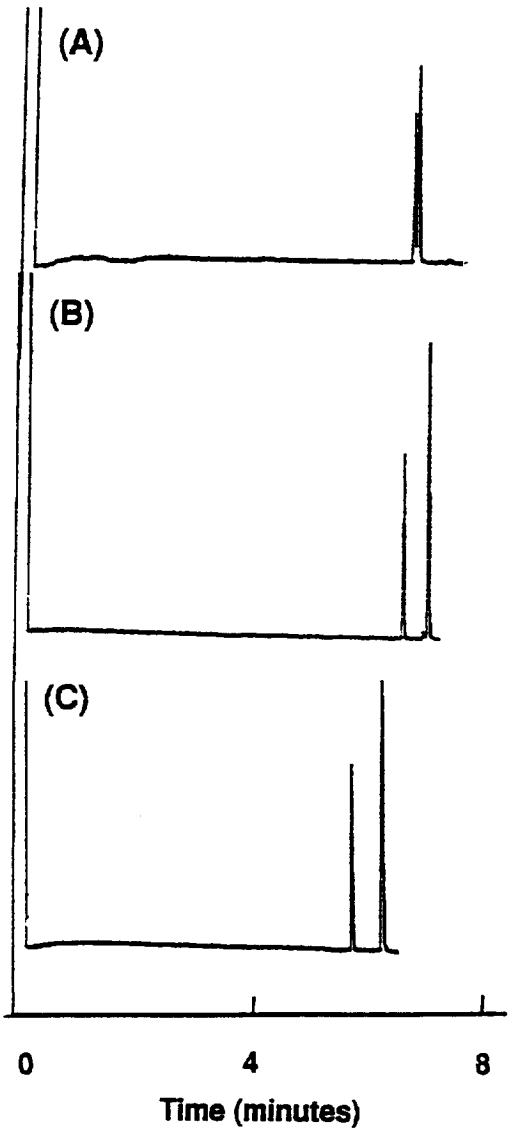

Fig. 2. Separation of anabasine (first peak) and anatabine (second peak) by CZE with the addition of 50% acetonitrile (v/v) into a buffer of 100 m*M* sodium phosphate at various pH values. (A) pH=2.7, (B) pH=6.0 and (C) pH=9.5. Other experimental conditions are described in the text.

3.2. pH effect in MEKC analysis

In MEKC analysis, differential partitioning of the solutes between the aqueous buffer and the micelles is mainly influenced by two factors; the nature of the solutes (i.e., ionization) and the hydrophobicity of the micellar system [7–9]. Because there are two pK_a values for tobacco alkaloids covered in this study (e.g., pK_a=3.12 and 8.02 for nicotine), these compounds can either exist in a mono-or di-protonated form or in a free form depending on the pH of aqueous solutions. The pH of a micellar system can affect the ionization of an alkaloid as well as its retention in the capillary. Usually a pH below 7 is not applicable for MEKC because of (i) the unfavorable partition of protonated alkaloids in the micellar phase which leads to a quick migration of solutes with poor separation and, (ii) slow electroosmotic flow which occurs in an acidic condition and is incapable of carrying the electrophoretically retarded micelles toward the detector within a reasonable time

period. Therefore, only the neutral and basic pH values (e.g., pH=7, 8, and 9) of the micellar system were tested for their influence on the separation of anabasine and anatabine. A micellar system consisting of 100 m*M* SDS and a mixed buffer of phosphate–borate was used. At pH 9, the migration times of anatabine and anabasine were 8.5 and 11.2 min, respectively. At pH 7, both solutes were retained in the capillary for longer than 15 min. Based on these results, a buffer solution consisting of 6 m*M* sodium phosphate and 10 m*M* borate with a pH value in the range of 8–10 was deemed appropriate for alkaloid analysis and was used for the rest of the work.

3.3. Selection of surfactant

The migration time and, to a lesser extent, the selectivity and the UV absorbance of tobacco alkaloids are influenced by the type and the concentration of surfactant used. Since alkaloids are relatively hydrophilic in nature, a less hydrophobic micellar system should give a more favorable partition coefficient for alkaloids and also allows more flexibility in the surfactant concentration [5], e.g., the surfactant could be at a lower concentration without losing resolution. For simplicity and easy access, surfactants with a linear alkyl chain (e.g., C_{12}, C_{14}, or C_{16}) were considered first. SDS was chosen for alkaloids analysis because of its higher electrophoretic mobility and lower hydrophobicity as compared to its longer chain analogs. The other properties of SDS such as high solubility in aqueous solution at room temperature, as well as the role of its counter ion (i.e., sodium ion) have been reported [10]. In order to optimize the concentration of SDS, buffer solutions containing SDS at various concentrations were used to separate a mixture of the five alkaloids commonly found in tobacco. The results, shown in Fig. 3, indicate that the migration time of each alkaloid increased with SDS concentration, e.g., the migration time of nicotine was 5.5, 7.8 and 8.8 min for SDS at 50, 100 and 150 m*M*, respectively. Only minor changes in the selectivity were observed with increasing surfactant concentration, and in all cases the five alkaloids were separated with baseline resolution. The effect of adding 2-propanol into the micellar system was also examined. The goal of adding propanol was to enhance the mass transfer of

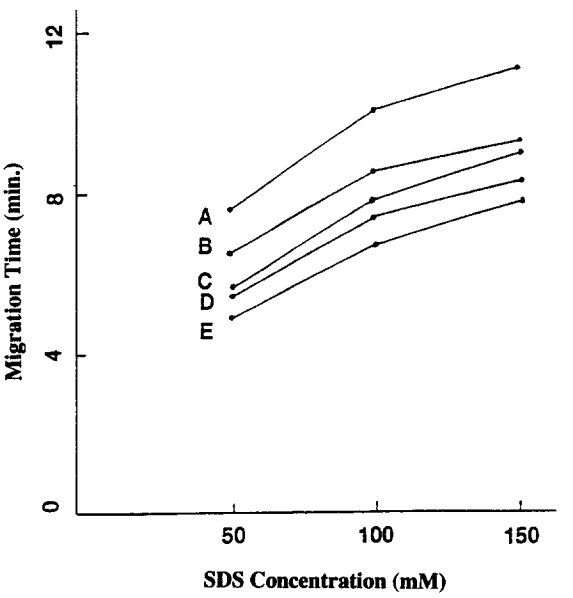

Fig. 3. The migration time of five alkaloids as a function of surfactant concentration. Micellar solution: a buffer solution of 6 m*M* sodium phosphate and 10 m*M* of sodium borate containing SDS at a concentration of 50, 100 and 150 m*M*, respectively. (A: anabasine, B: nornicotine, C: nicotine, D: anatabine, E: myosmine)

solutes between the aqueous and the micellar phases, which has been proved by Dorsey and coworkers [11] to be effective in improving separation efficiency in the micellar liquid chromatography. Balchunas and Sepaniak [12] reported the addition of 2-propanol to a micellar system when trying to extend the elution range in MEKC analysis by silanating a fused-silica capillary with trimethylchlorosilane. They were able to restore much of the column efficiency lost in a silanated fused-silica capillary by adding 10% of 2-propanol in the mobile phase. In this work, however, no significant improvement was observed in the separation of tobacco alkaloids after adding 3–10% of 2-propanol in to the micellar solution.

3.4. UV absorbance of nicotine in micelle

The UV absorbance of alkaloids was examined using nicotine as a model compound. It is known that the UV absorbance of nicotine measured at 262 nm varies with the ionic forms in the order of diprotonated>monoprotonated form=free base. No

significant shift was observed in the wavelength of absorption maximum as a function of pH [5]. The chromatographic peak area obtained for nicotine at pH above 7 is approximately one half of that obtained for nicotine at a pH of 2.5 due to the difference in UV extinction coefficient. Since buffer solutions with pH values in the range of 8–10 are used in the MEKC analysis of tobacco alkaloids to achieve an optimum separation, some sensitivity of detection is lost. However, the intensity of UV absorbance can be enhanced in the presence of micelles and the compromised sensitivity can be partially recovered. As shown in Fig. 4A, the UV absorbance of nicotine at pH 9.5 increased after the addition of 100 m*M* SDS, but was still lower than the absorbance obtained at pH 2.5. The enhancement of UV absorbance of nicotine in a micellar solution also increased as a function of the surfactant concentration (Fig. 4B).

3.5. MEKC for tobacco analysis

Alkaloids analysis by MEKC was conducted using Burley tobacco as a sample (Fig. 5). Alkaloids were extracted from the tobacco using water containing 1% triethanolamine with the aid of sonication for six min. The micellar system was 100 m*M* SDS in a buffer of 6 m*M* sodium phosphate and 10 m*M* sodium borate at a pH of 9.5. Based on the experimental results described above, optimal resolution and reasonable analysis time can be obtained within the concentration ranged from 50 m*M* to 150 m*M*. In order to avoid the viscosity and joule heat associated with high concentration, but to take the advantage of SDS enhanced UV absorbance, 100 m*M* was chosen as a result of balance. The high pH value and low ionic strength of the buffer generated sufficient speed for the electroosmotic flow, which then carried the micelles through the capillary. At

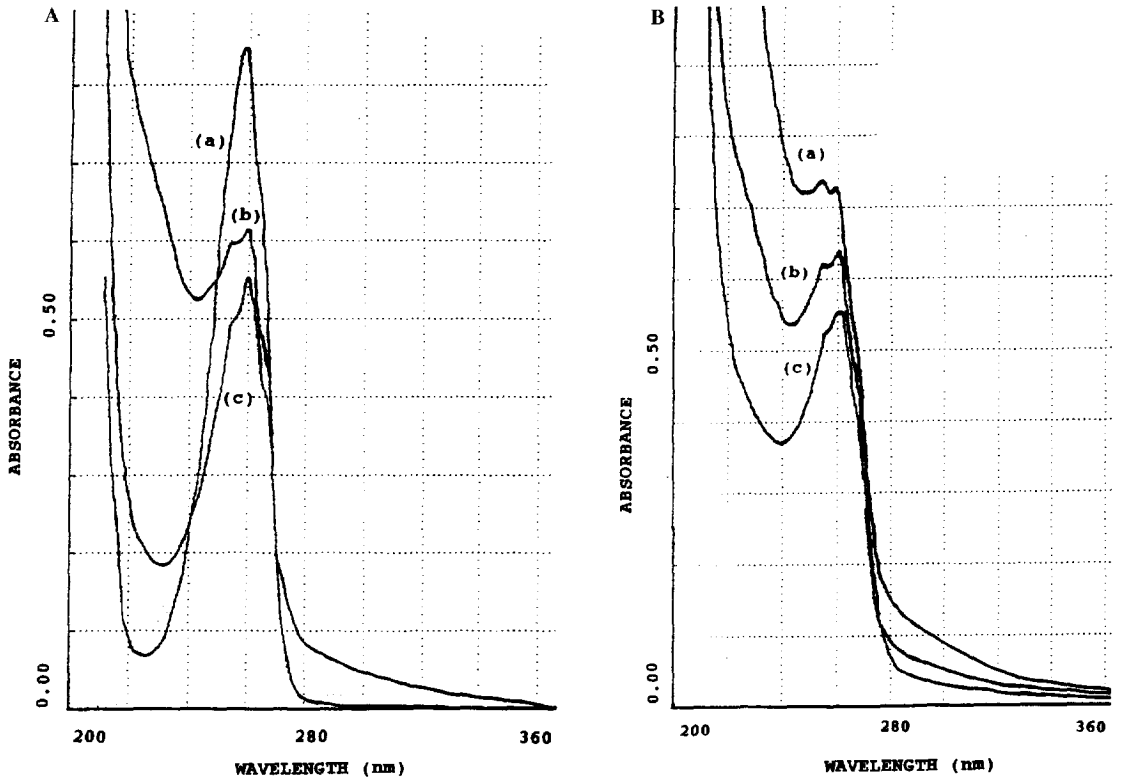

Fig. 4. (A) UV absorbance of nicotine in a sodium phosphate buffer at various pH and with or without micelles. (a) pH=2.5, (b) pH=9.5 with 100 m*M* SDS, and (c) pH=9.5 without SDS. (B) UV absorbance of nicotine as a function of surfactant concentration. (a) SDS=200 m*M*, (b) SDS=100 m*M*, and (c) SDS =50 m*M*.

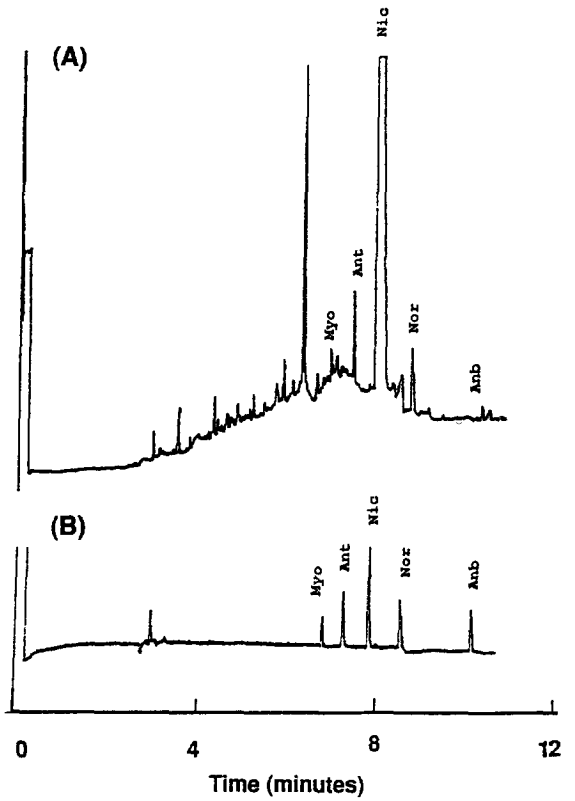

Fig. 5. Representative electropherogram for the determination of alkaloids in tobacco by MEKC. Tobacco sample was extracted by 1% triethanolamine in water. A 50 μm I.D. capillary with a total length of 72 cm (50 cm from anode to detector) was used. Micellar solution contained 100 m*M* SDS, 6 m*M* phosphate and 10 m*M* borate at a pH of 9.5. The applied voltage was set at +30 kV. Above: a tobacco sample. Below: a standard alkaloid solution. (Myo = myosmine, Ant = anatabine, Nic = nicotine, Nor = nornicotine, Anb = anabasine)

this basic pH, alkaloids mostly exist in an unprotonated form and have a higher partition coefficient in the SDS micelle. This makes possible the separation of solutes with small differences in molecular structure.

4. Conclusion

MEKC can be used for the analysis of alkaloids commonly found in tobacco. The separation was determined by the ionization of alkaloids, their partitioning into the micelles and the electroosmotic flow. Good separation was obtained using a micellar system containing SDS at 50–150 m*M* and a buffer at a pH of 8–10. Sensitivity of UV detection was enhanced in the presence of micelles as a function of surfactant concentration.

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