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The use of capillary electrophoresis to study the formation of carcinogenic aryl amines in azo dyes

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Abstract

A capillary zone electrophoresis (CZE) method for separating and detecting certain aromatic amines is described. In this study, the utility of this technique for monitoring dyes syntheses is also demonstrated. In addition to the analysis of amines, CZE can detect other impurities present in the starting materials used in dye synthesis. The influence of reaction conditions on the amount of carcinogenic amines liberated from azo dyes following sodium dithionite treatment is discussed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Capillary electrophoresis; Carcinogenic arylamines; Azo dyes; C.I. Acid Black 1

1. Introduction

Increasing concern about the environment had led government agency to restrict the use of toxic materials. The main aim of these restrictions is to protect human health and the environment.

A variety of consumer goods are dyed with azo dyes. Owing to its versatility this family of dyes are the most widely used [1]. Some of these azo compounds present a potential risk to man and his environment.

The adverse effects of certain azo dyes on human health were first apparent when cases of bladder cancer were detected in workers involved in the manufactory and use of these benzidine-based dyes [2].

Results of previous research [1] suggest that the toxicity of azo dyes arises from the reduction of the azo group, by the action of intestinal anaerobic bacteria or the hepatic azo reductases, which releases aromatic amines. Therefore, dye toxicity is often related to the toxicity of the amines employed in the synthesis steps.

Nowadays, dyes that produce carcinogenic aromatic amines upon reductive-cleavage of the azo bond(s) may not be used in many consumer products in Germany.

Because of this, some analytical methods for determining the existence of restricted dyes in consumer goods have been published. Many are based on techniques involving the reduction of the azo bond with sodium dithionite, extraction of the amines produced, and separating and quantifying the amines. HPTLC, HPLC, GC or HPCE [3] are the analytical tools normally used.

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Consumer goods dyed with azo dyes, which when subjected to these analytical methods generate more than 30 mg/L of one or more of 20 listed amines [3,4], cannot be sold in Germany.

Interestingly, there are also dyes that are not made from the 20 forbidden amines but contain impurities or undergo various leading to secondary reactions positive test results in amine determinations. This can lead to classification of the same dye with different grades of quality. A lower or higher grade depends on the quality of the starting material and the synthetic conditions used by the manufacturer.

This paper focuses on an azo dye (Acid Black 1) which, even though in its synthesis does not involve one of the 20 listed amines, gives positive results in amine determinations [1,5]. In this study, analysis of the starting materials and monitoring of the different stages of the synthesis reaction were carried out. Due to the possible influence of synthesis conditions on the formation of some of the amines, a study of the action of certain parameters on the quantity of amines liberated during the azo dye reductive-cleavage treatment was carried out. To achieve our objective, a CZE method for separating and determining some of the listed aromatic amines has been developed.

Capillary Electrophoresis (CE) offer extraordinary separation power, speed of analysis, and extreme sensitivity and requires little sample. This makes it attractive as an analytical tool. CE is a common technique used for the analysis of dyes in dye-manufacturing and dye application [6], in cotton and wool fibres [7], and food additives [8,9]. It has been used in the determination of aromatic amines in environmental samples [10]. Moreover, previous research carried out in our research group [11] has shown the use of capillary zone electrophoresis (CZE) in the determination of aromatic amines and the monitoring of dye synthesis.

2. Experimental

2.1. Instrumentation

A Waters Quanta 4000 capillary electrophoresis system equipped with a variable-wavelength spectrometric detector operating at 214 nm and 254 nm was used. Data manipulation was controlled by Maxima 820 chromatographic software (Waters Corporation).

A fused-silica capillary recovered with polyimide of 75 μm I.D., 60 cm total length and 52 cm effective length was provided by Waters Corporation. The capillary was conditioned by flushing with NaOH 1N for 15 min, then with milli-Q water for 15 min when used for the first time. Before each use, the capillary was conditioned by flushing with NaOH 1N for 10 min, then with Milli-Q water for 10 min and finally with the background electrolyte (BGE) solution for 10 min.

2.2. Chemicals

Benzidine, 4-chloro-o-toluidine, 2-naphthylamine, p-chloroaniline, 4,4'-diaminodiphenylmethane, 3,3'-dimethoxybenzidine, 3,3'-dimethylbenzidine, 4,4'-oxydianiline, o-toluidine, 2,4-toluilendiamine, 1-naphthylamine were supplied by Fluka; 4-aminodiphenyl, o-aminoazotoluene, 2-amino-4-nitrotoluene, 2,4-diaminoanisole, p-cresidine were supplied by Aldrich Chemical Company; aniline by Merck and 3,3'-dichlorobenzidine by Sigma Chemical Company. H acid, Chromotropic acid and Koch's acid were supplied by Robama, S.A. (Barcelona, Spain). Water was obtained from a Milli-Q water purification system (Millipore, USA).

Stock solutions were prepared dissolving the 18 amines in methanol at 500 ng/μL. The working solution was prepared by diluting the stock to 10 ng/μL with HCl (0.1 N). In the case of sulphonic acids, a stock solution was prepared dissolving the 3 acids in Milli-Q water at 50 ng/μL.

2.3. Experimental conditions

Two capillary zone electrophoresis methods were used. The first was developed to separate and determine certain aromatic amines [11,12], and it was also used to monitor the formation of diazonium salts. In this case, the BGE solution consisted of 50 mM phosphate buffer adjusted to pH 3.1 (with 0.1N NaOH), containing 10% methanol. The experimental parameters were: hydrostatic injection = 10 cm per 20 sec; analytical voltage = 22kV;

detection wavelength = 214 nm. The capillary was maintained near ambient temperature (20–21°C). In all experiments, the capillary was flushed with BGE solution for 2.0 min before each injection and the solution in the solvent reservoirs was renewed after every five injections to improve experimental reproducibility. The electropherograms of the amines analysed under these conditions are shown in Figs. 1 and 2.

The second method [11] was designed to separate and determine some of the components in intermediates commonly used in the synthesis of acid dyes. This included H acid, Koch's acid and Chromotropic acid. However, in the present work it was also used to analyse sulphonated compounds such as dyes and their impurities. The BGE solution consisted of 20 mM citrate buffer adjusted to pH 4.5 with NaOH 0.1N. The experimental parameters were: hydrostatic injection = 10 cm per 20 sec; analytical voltage = 20kV; detection wavelength = 254 nm. The capillary was maintained near ambient temperature (20–21°C). In all experiments, the capillary was flushed with BGE solution for 2.0 min before each injection and the solution in the solvent reservoirs was renewed after every five injections.

2.4. Sample preparation

Samples taken from diazotization and coupling reactions were diluted in Milli-Q water. It was necessary to dilute them to achieve an analytical response (peak area) in the linear regions.

3. Results and discussion

3.1. Analysis of CI Acid Black 1

CI Acid Black 1 is an acid azo dye. As is shown in Fig. 3, this dye is manufactured by coupling diazotized p-nitroaniline to H Acid at acidic pH, followed by an alkaline coupling of diazotized aniline to the monazo intermediate. The experimental details are described in [12].

Acid Black 1 is one of the major dyes manufactured. Apart from its use as a dye itself, it is commonly used as an intermediate in the manufacture of others azo dyes. However, as pointed out before, sometimes this dye, depending on the starting materials used in its synthesis and reactions conditions, can give positive results for the presence of carcinogenic amines. We found that

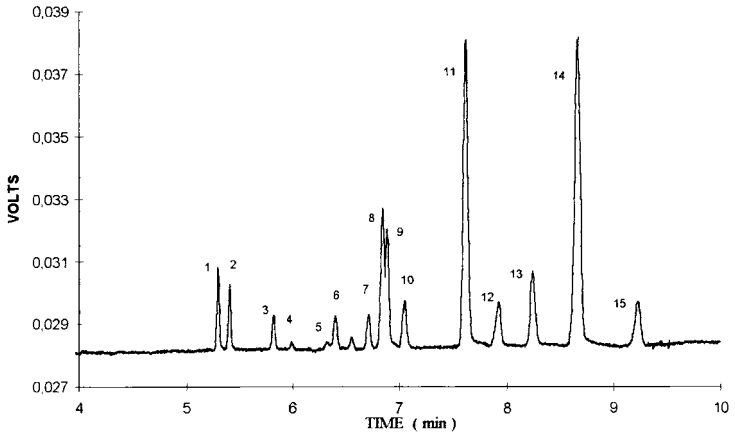

Fig. 1. Electropherogram for the working solutions of aromatic amines. Peaks: 1 = 4,4'-diaminodiphenylmethane, 2 = 4,4'-oxydianiline, 3 = benzidine, 4 = aniline, 5 = 2,4-diaminoanisole, 6 = 2,4'-toluilendiamine, 7 = o-toluidine, 8 = 3,3'-dimethylbenzidine, 9 = 3,3'-dimethoxybenzidine, 10 = p-cresidine, 11 = 2-naphthylamine, 12 = p-chloroaniline, 13 = 4-aminodiphenyl, 14 = 1-naphthylamine, 15 = 4-chloro-otoluidine. All at 10 ng/μL. Conditions Buffer = 50 mM phosphate 10% MeOH; pH = 3.1; fused-silica capillary recov-

some commercial Acid Black 1 dyes release 100–200 ng/μL of benzidine and 150–450 of 4-aminodiphenyl, when reduced with sodium dithionite. Fig. 4 shows the electropherogram for a commercial sample of Acid Black 1 following reduction with sodium dithionite.

3.1.1. Quality of starting materials

The use of methods develpoed in the present study showed that there were no aromatic amine impurities in our samples of p-nitroaniline, H acid and aniline at concentrations higher than 5 mg/Kg.

On the other hand, the use of the analytical method described in 2.3 designed to characterize dye intermediates, allowed us to detect and quantify the impurities (Chromotropic acid and Koch acid) present in H acid (Figs. 5 and 6). Although the impurities are not carcinogenic, they could affect the following synthesis stages.

The impurities present in our H acid sample were quantified. We found 0.6% Chromotropic acid and 0.09% Koch acid.

3.1.2. Monitoring the synthesis stages of Acid Blck 1

All 4 steps in ths synthesis of Acid Black 1 could be monitored using CZE. To achieve this, samples of each reaction mixture were collected at different times and analysed. The electropherograms corresponding to different stages of the reaction are shown in Figs. 7–10: p-Nitroaniline diazotation (Fig. 7), coupling between p-nitroaniline diazonium salt and H acid (Fig. 8), diazotation of aniline (Fig. 9), and coupling between aniline diazonium salt and the monoazo dye (Fig. 9).

Some impurities formed during dye synthesis were also detected by CZE. Due to the presence of small amounts of Chromotropic acid in the H acid used as starting material, an impurity was formed from a coupling between the p-nitroaniline diazonium ion and Chromotropic acid.

Another impurity was phenol, which comes from the hydrolysis of benzene diazonium ions. In this case, the impurity was detected as a consequence of maintaining the diazo solution at room temperature for more than two hours.

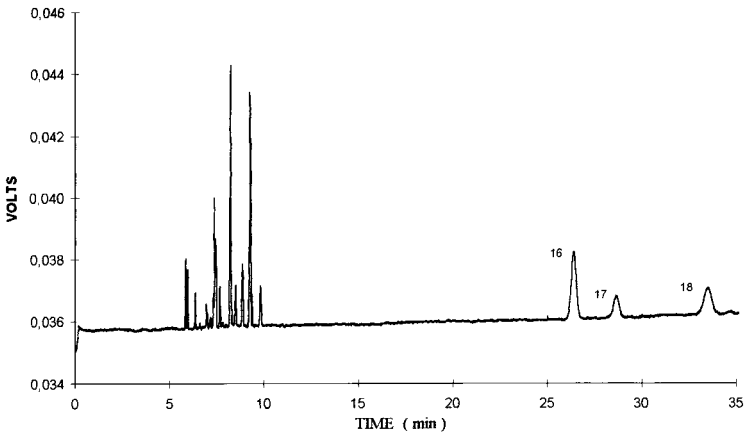

Fig. 2. Electropherogram for the working solutions of aromatic amines. Peaks: 16 = 3,3'-dichlorobenzidine 10 ng/μL 17 = 2-aminoazotoluene (10 ng/μL); 18 = 2-methyl-5-nitroaniline (10 ng/μL). Buffer = 50 mM phosphate 10% MeOH; pH = 3.1; fused-silica capillary recovered with polyimide, 52 cm×75 μm I.D.; applied potential = +22kV; UV detection at 214 nm.

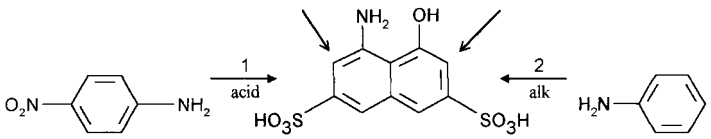

Fig. 3. Two step synthesis of Acid Black 1.

3.1.3. Influence of temperature

We found that the formation of 4-aminodiphenyl, a carcinogenic amine, occured during the diazotization of aniline. Some authors state that it was possibly due to the heterolitic dissociation of the diazonium salt at acidic pH [13]. It can be expected that this dissociation increases its rate with temperature and thus the amount of 4-aminobiphenyl would increase with increasing temperature. It can be seen from Fig. 11 that

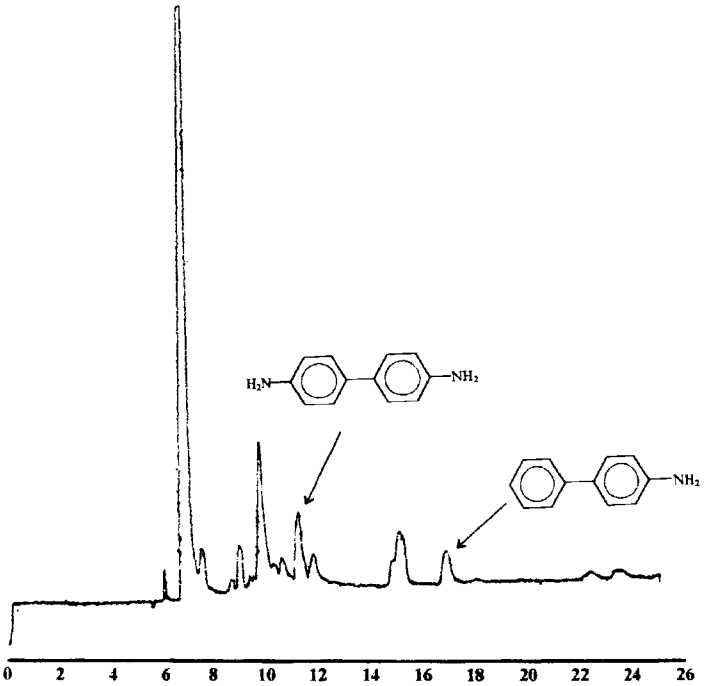

Fig. 4. Electropherogram for a reduced commercial sample of Acid Black 1 containing benzidine (190 mg/kg) and 4-aminodiphenyl (150 mg/kg).

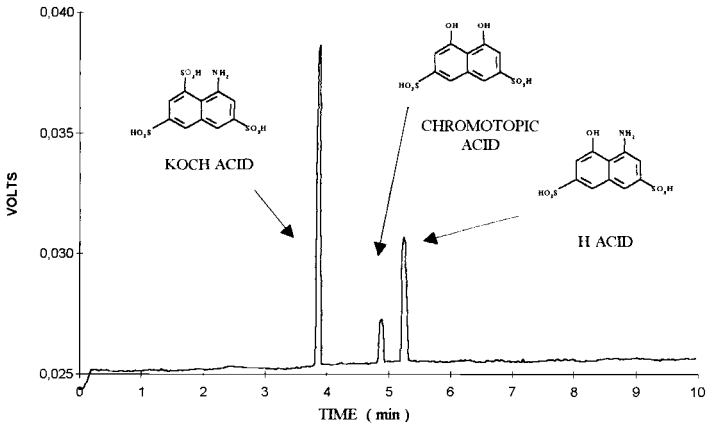

Fig. 5. Electropherogram from the analysis of the working solution of Koch acid, Chromotropic acid and H acid. All at 50 ng/μL using 20 mM citrate buffer, pH 4.5; fused-silica capillary recovered with polyimide, 52 cm×75 μm I.D.; −20kV potential; UV detection at 254 nm.

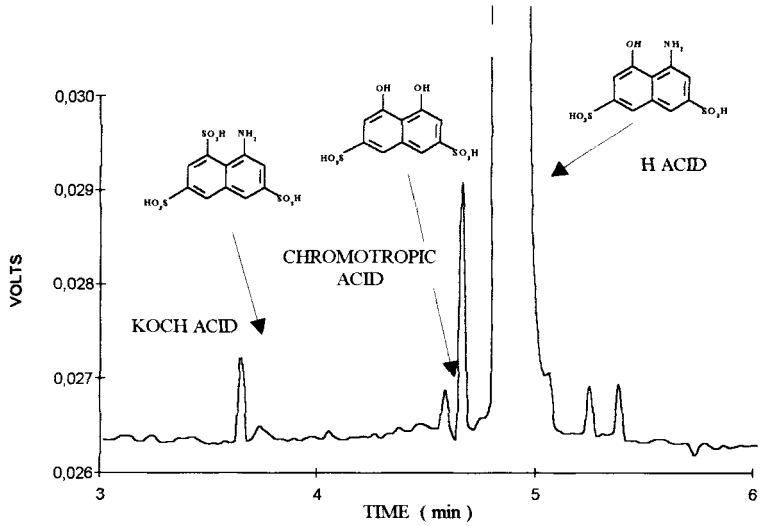

Fig. 6. Electropherogram from the analysis of a 1000 ng/L sample of H acid (81.5%), using 20 mM citrate buffer, pH 4.5; fused-silica capillary recovered with polyimide, 52 cm×75 μm I.D.; −20kV potential, UV detection at 254 nm.

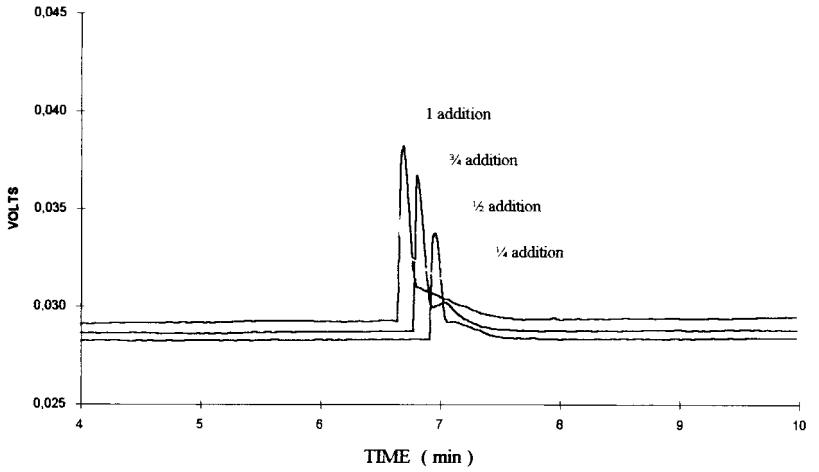

Fig. 7. Electropherogram from monitoring the diazotization of p-nitroaniline using 50 mM phosphate buffer, in 10% MeOH, pH 3.1; fused-silica capillary recovered with polyimide, 52 cm×75 μm I.D.; +22kV potential, UV detection, at 214 nm.

diazotizing aniline +5°C produced peaks that were not present at −5°C. One of these new peaks was identified as 4-aminodiphenyl.

The influence of reaction conditions on the quantity of carcinogenic amines released during Acid Black 1 reduction was studied. This was conductedwith the aid of a Plackett-Burman design, using the following factors: 1) pH of the coupling reaction between aniline and the monoazo dye, 2) , temperature of aniline diazotization, and 3) concentration of the aniline at the beginning of the diazotization. The effects of these factors on the amount of aniline, benzidine, and 4-aminodiphenyl liberated during reductive treatment were determined. The results show that:

1. The concentration of free aniline after dye reduction is smaller at higher pH

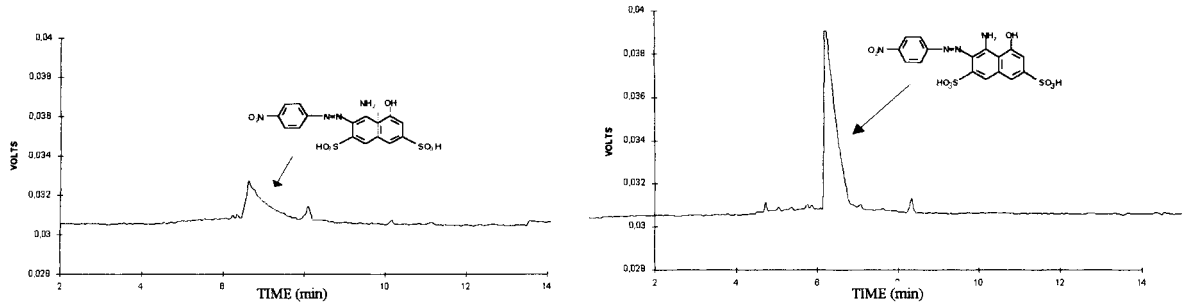

Fig. 8. Electropherograms from monitoring of coupling between p-nitroaniline and H acid at acidic pH, following the addition of 1/4 the amount of diazo component (left) and all of the diazo component (right). Conditions: 20 mM citrate buffer, pH 4.5, fused silica capillary recovered with polyimide, 52 cm×75 μm I.D.; −20kV potential; UV detection at 254 nm.

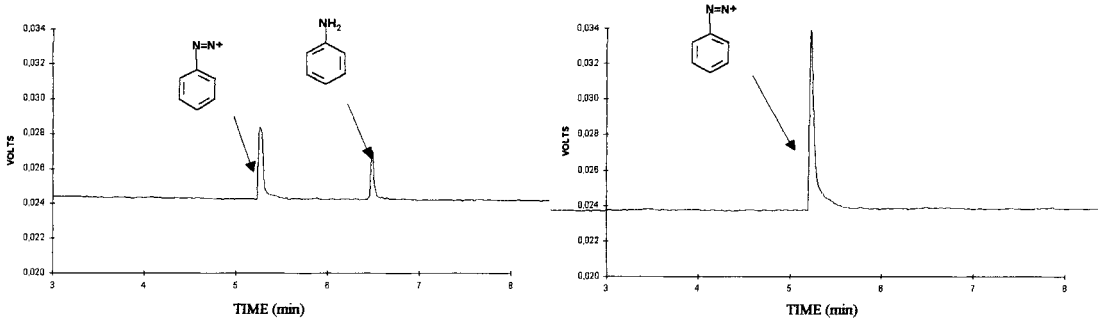

Fig. 9. Electropherograms from monitoring the diazotization of aniline, following 1/4 the added amount of HNO_2 (left) and the remaining HNO_2 (right). Conditions: 50 mM phosphate buffer, 10% MeOH, pH 3.1; fused-silica capillary recovered with polyimide, 52 cm×75 μm I.D.; +22kV potential, UV detection at 214 nm.

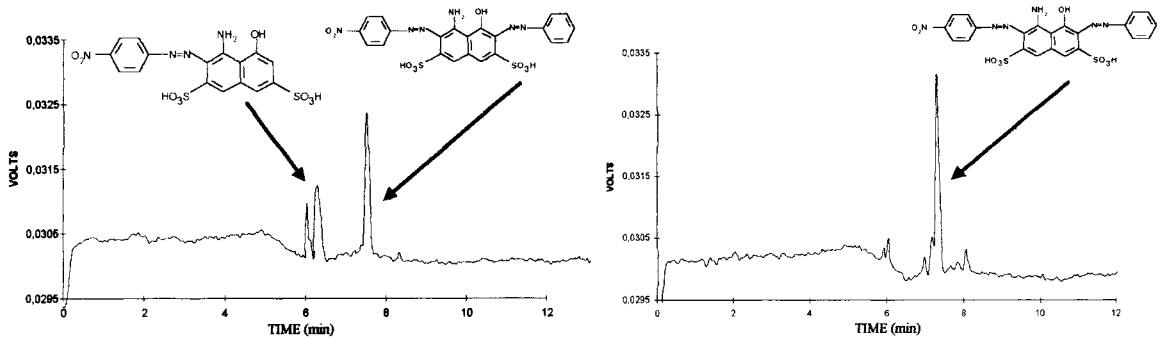

Fig. 10. Electropherograms from monitoring the coupling reacton between aniline and the monazo dye, following the addition of 1/2 the diazotized aniline (left) and all the diazotized aniline (right). Conditions: 20 mM citrate buffer, pH 4.5; fused-silica capillary recovered with polyimide, 52 cm×75 μm I.D.; −20kV potential, UV detection at 254 nm.

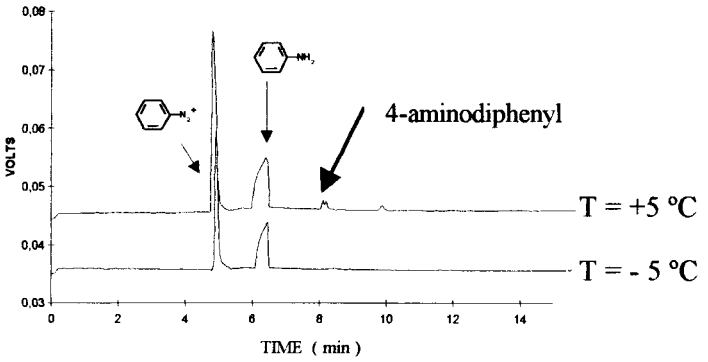

Fig. 11. Electropherograms from the diazotization of aniline at −5°C and +5°C. Conditions: 50 mM phosphate buffer, 10% MeOH, pH 3.1; fused-silica capillary recovered with polyimide, 52 cm×75 μm I.D.; +22kV potential, UV detection at 214 nm.

2. The amount of 4-aminodiphenyl produced following the reduction step increases with increasing aniline concentration

4. Conclusions

In this paper, capillary zone electrophoresis has been used successfully as a method to separate and determine some carcinogenic amines. Moreover, it has also been used as a dye synthesis quality control technique, in that it allows the characterization of the impurities presents in different starting materials. Additionally, this technique allows the detection of some impurities that have been formed during certain reactions which even though they are not carcinogenic, can affect the tone and purity of the dye produced. We also demonstrated that diazotization temperature influences the formation of unwanted by-products such as 4-aminodiphenyl, a carcinogenic amine.

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