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ISOTACHOPHORESIS OF QUATERNARY 4,4′-BIPYRIDYLIUM SALTS

ANALYTICAL CONTROL OF SYNTHESIS AND PURIFICATION PROCEDURES

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SUMMARY

Analytical capillary isotachophoresis (ITP) was employed to monitor the synthesis and subsequent purification of the quaternary bipyridylium salt 1-methyl-1′-[(3*S*)(−)-methylpinanyl]-4,4′-bipyridinium dichloride. The main synthetic product, intermediate and overreaction products as well as the starting material were analyzed in amounts of less than 1 μg in a single experiment. In addition to the qualitative approach, ITP allows an easy quantitative evaluation, thereby facilitating direct consideration of the reaction conditions used as well as enabling the optimization of purification steps.

INTRODUCTION

Quaternary 4,4′-bipyridylium salts are widely used as reversible redox systems, especially in agriculture as herbicides[1] and in laboratory systems as electron relays designed for solar energy conversion[2]. Starting from the simplest molecule of this class, 1,1′-dimethyl-4,4′-bipyridinium dichloride (methylviologen MV, Paraquat®), many chemically modified compounds have been tested in this respect, *e.g.*, viologen surfactants, substituted viologens and unsymmetrically substituted viologens[2,3].

The details of the transfer of an electron from a donor to an acceptor molecule in solution have been investigated[4], thereby utilizing the principle of diastereomerism in order to examine the relative importance of solvent and solute participation in modelling the transition state[5]. Such studies require the synthesis of optically active viologens in high purity. Owing to the complexity of the synthesis and the subsequent purification process, a proper analytical control of these procedures is a prerequisite to obtaining pure products in high yields.

In the present study, analytical isotachophoresis (ITP) was, for the first time, employed to monitor the synthesis and purification of 1-methyl-1′-[(3*S*)(−)-methylpinanyl]-4,4′-bipyridinium dichloride (PMV-Cl_2).

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MATERIALS AND METHODS

The starting materials for the synthesis were 4,4′-bipyridine (purified by sublimation), freshly distilled methyl iodide and (3*S*)(−)-iodo- or bromo-methylpinane. The latter compound was prepared from (3*S*)(−)-formylpinane by reduction with $NaBH_4$ and substitution of the hydroxy group by tetrabromomethane or by tosylation and substitution by iodide ion.

Butanol as a solvent was distilled before use. Nitromethane was dried over phosphorus pentoxide and distilled at about 100 mbar. Tetrahydrofuran was dried over potassium hydroxide, purified using alumina, (desiccated for 12 h at 450°C; 70 cm × 4.5 cm column), subsequently boiled with $LiAlH_4$ and distilled. Toluene was dried over $LiAlH_4$.

Following conventional techniques[6], the synthesis of PMV salts was carried out by two different routes.

Synthesis I

In the first step, 4,4′-bipyridine was quaternized with methyl iodide (concentration ratio 1.6:1.0) in benzene as a solvent at room temperature to yield MV iodide. Unreacted 4,4′-bipyridine and the side product 1,1′-dimethyl-4,4′-bipyridinium diiodine (M_2V diiodide) was extracted stepwise with dried toluene and acetonitrile, respectively.

In the second step, MV iodide was treated with (3*S*)(−)-methylpinanyl bromide in boiling *n*-butanol. The reaction product was converted into a dichloride by ion-exchange chromatography and subsequently freeze dried to give a light yellow flaky product which was moderately hygroscopic.

Synthesis II

Using dried nitromethane as a solvent, 4,4′-bipyridine was treated (48 h under reflux) with methylpinanyl iodide. The resulting extremely hygroscopic product 1-[(3*S*)(−)-methylpinanyl]-4-(4′-pyridyl)pyridinium bromide (PV bromide) was purified by gel chromatography on a glass column (45 cm × 3 cm) packed with Bio-Gel P-2 (200–400 mesh; Bio-Rad, Richmond, CA, U.S.A.) and using distilled water as the eluent. PV bromide was then converted into a tetrafluoroborate salt (PV-BF_4) by adding a 12.5% solution of ammonium tetrafluoroborate. Subsequently, a solution of PV-BF_4 in acetonitrile was stirred for 4 days at room temperature in the presence of an 1.5-fold excess of methyl iodide. The dark red crystalline product was converted into a tetrafluoroborate salt by ion-exchange chromatography (Dowex 50W 1-X8, 200–400 mesh, 30 cm × 1.5 cm, loaded with BF_4^-) and then freeze dried to give a pure white non-hygroscopic product.

The structures of all the target products synthesized were confirmed by mass spectrometry and nuclear magnetic resonance spectrometry[4]. Intermediate and end-products were analyzed isotachophoretically in the concentrations and amounts given in the figures.

Analytical isotachophoresis

Cationic isotachophoretic analysis were performed by using a 2127 LKB Tachophor (LKB, Bromma, Sweden) with an automatic driving control unit[7,8].

Separations were carried out in a PTFE capillary (230 mm × 0.55 mm I.D.). Conductivity and UV (254 nm) signals were monitored by employing a two-channel recorder (Kipp & Zonen, Delft, The Netherlands) with a chart speed of 6 cm/min. The separations required about 15 min and the current upon detection was 60 μA.

As the leading electrolyte, a 10 m*M* solution of potassium acetate containing 0.4% hydroxypropylmethylcellulose (HPMC; Dow Chemical, Midland, MI, U.S.A.) was used, titrated to pH 5.0 with concentrated acetic acid. The terminating electrolyte buffer contained 20 m*M* hydrochloric acid. These electrolyte solutions were prepared from analytical grade chemicals (E. Merck, Darmstadt, F.R.G.) using ultrapure water (> 15 MΩ/cm)[9].

RESULTS

Analysis of intermediate and end-products of synthesis I

Compared with the isotachophoretic pattern of the electrolyte system, the isotachopherogram of the intermediate product derived after the first synthesis step is illustrated in Fig. 1. Only one non-UV-adsorbing impurity from the chemicals used was present in the electrolyte system (Fig. 1A). For the intermediate product, one major UV-adsorbing zone corresponding to the main synthetic product MV^+ and two minor UV-absorbing zones, one of them not fully separated from the electrolyte impurity, were detected (Fig. 1B). These two minor zones were identified in doping experiments (Fig. 2). Addition of the starting material 4,4'-bipyridine resulted in a lengthening of zone 3 (Fig. 2A), whereas zone 1 corresponded to the overreaction product M_2V^{2+} (Fig. 2B).

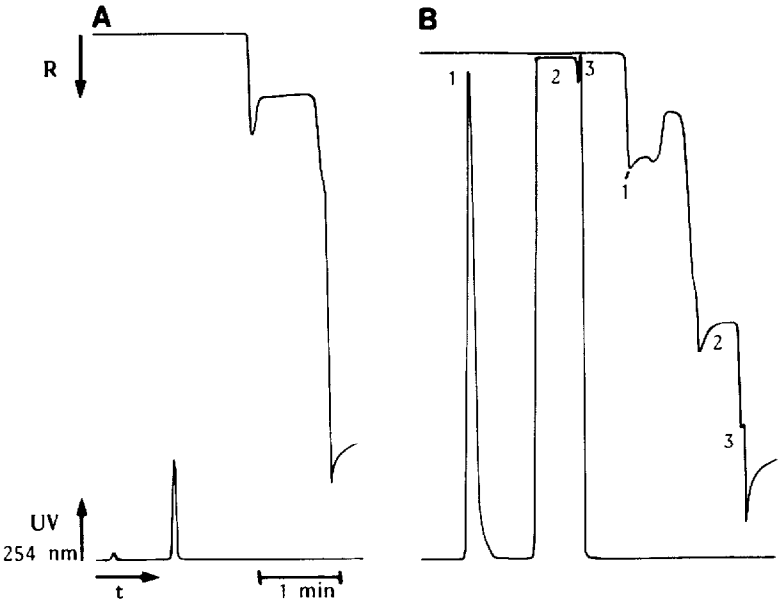

Fig. 1. Isotachophoretic analysis of the intermediate product (synthesis route I): (A) electrolyte system; (B) crude product (2.5 μl injected, corresponding to 1.25 μg material). Key: 1 = M_2V^{2+}; 2 = MV^+; 3 = 4,4'-bipyridine.

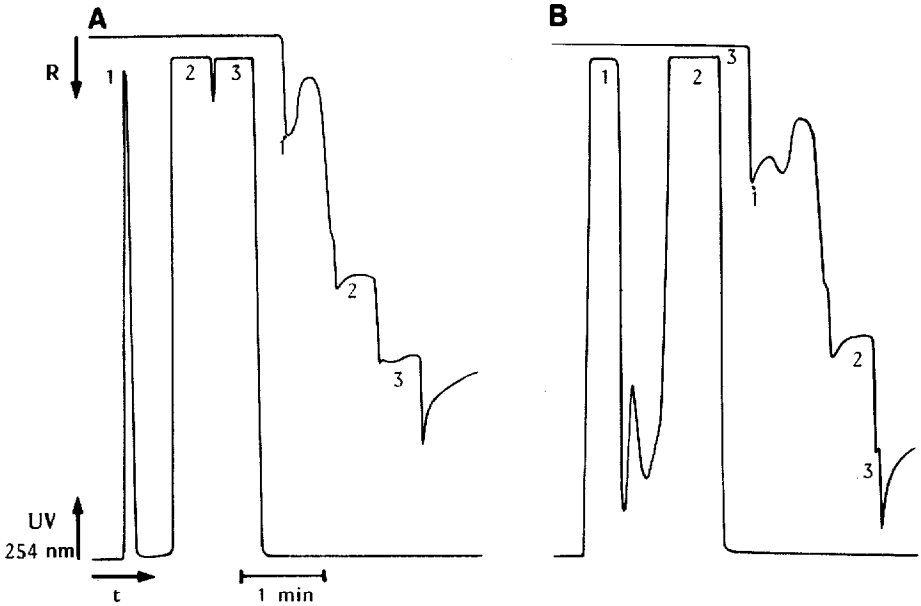

Fig. 2. Isotachophoretic analysis of the intermediate product (synthesis route I) with addition of 4,4′-bipyridine (A) and M_2V^{2+} (B), respectively. For the separations, 2.5 μl were injected, corresponding to 1.25 μg of each solute. Key as in Fig. 1.

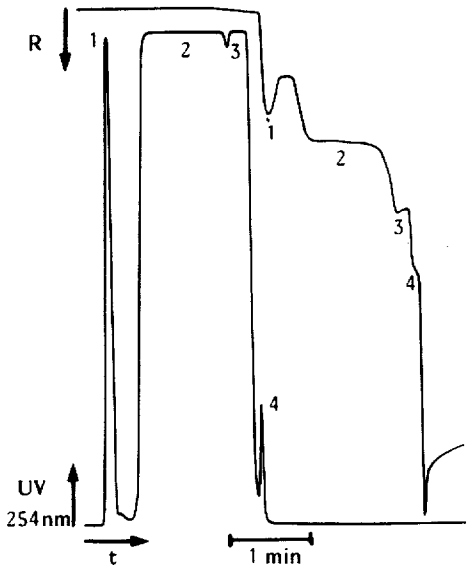

Fig. 3. Isotachophoretic analysis of the crude product derived after the second synthesis step (route I) (5 μl injected, corresponding to 2.5 μg material). Key: 1 = M_2V^{2+}; 2 = unknown; 3 = PMV^{2+}; 4 = MV^{+}.

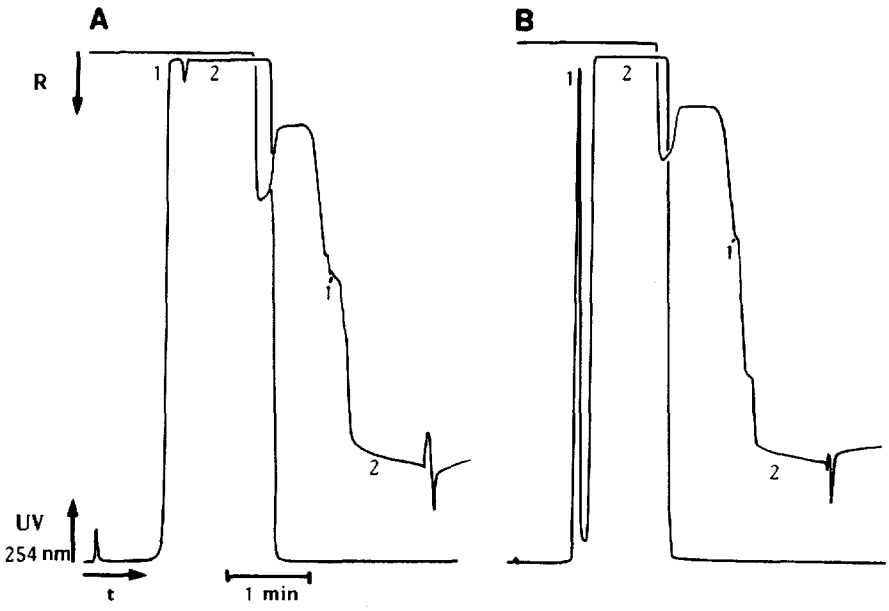

Fig. 4. Isotachophoretic analysis of the intermediate product (synthesis route II) before (A) and after (B) gel chromatographic purification. For each separation, 5 μl were injected, corresponding to 2.5 μg material. Key: 1 = P_2V^{2+}; 2 = PV^{+}.

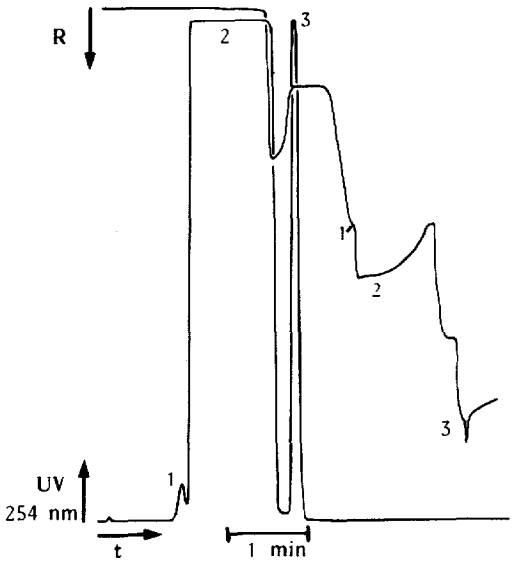

Fig. 5. Isotachophoretic analysis of the crude product derived after the second synthesis step (route II) (5 μl injected, corresponding to 2.5 μg material). Key: 1 = P_2V^{2+}; 2 = PMV^{2+}; 3 = PV^{+}.

Analysis of the final product after the second synthesis step revealed four UV-absorbing zones (Fig. 3). Evidently, two of the minor zones corresponded to the intermediate product MV^+ and the overreaction product M_2V^{2+}, respectively. Surprisingly, only small amounts of the desired product PMV^{2+} were detected. Owing to the lack of a suitable reference substance, the main synthetic compound could not be identified.

Analysis of intermediate and end-products of synthesis II

The isotachopherogram of the crude material derived after the first synthesis step as well as the isotachophoretic pattern of the purified product are illustrated in Fig. 4. Analysis of the crude material (Fig. 4A) revealed two UV-absorbing zones corresponding to the main synthetic product PV^+ and the overreaction product 1,1′-bis[(3*S*)(−)-methylpinanyl]-4,4′-bipyridinium dication (P_2V^{2+}). Repeated gel chromatographic purification of the crude material resulted in a significant decrease in the concentration of P_2V^{2+} (Fig. 4B).

At least three distinct UV-absorbing zones were detected after methylation of PV^+ (Fig. 5). In addition to the main synthetic product PMV^{2+}, small amounts of the starting compound PV^+ were detectable, indicating an incomplete reaction to PMV^{2+}. The third zone corresponded to the overreaction product P_2V^{2+} which could not be completely removed after the first synthesis step (Fig. 4).

DISCUSSION

The synthesis of unsymmetrical quaternary 4,4′-bipyridilium salts requires a two-step procedure in which the starting compound 4,4′-bipyridine is quaternized twice with two different alkyl halogenides. Owing to the strong influence of various factors, *e.g.*, solvent polarity and stereochemistry of the alkyl halogenides used, on the course and the yield of the reaction, a proper control of both the synthesis and subsequent purification procedures is necessary in order to obtain pure products in high amounts. Usually mass spectrometry, NMR and IR spectroscopy as well as elemental analysis are used for the identification and purity control of bipyridinium salts. Mass spectrometric analysis of salts, however, is extremely difficult due to the low volatility of such compounds. Furthermore, an adequate use of NMR spectroscopy requires the availability of pure products.

In the present study, ITP was used to monitor the synthesis and subsequent isolation of PMV^{2+}. Apparently, by applying a cationic electrolyte system at pH 5, it is feasible simultaneously to analyze the starting compound 4,4′-bipyridine, the end-product PMV^{2+} as well as intermediate and overreaction products (Figs. 1–5). In addition to this qualitative approach, ITP affords information about the composition of the samples analyzed without the necessity to analyze reference substances. Since the conductivity zone length is not influenced by substance-specific properties, *e.g.*, absorption coefficients, a relative quantification of sample ions with similar molecular weights and net charge is appropriate by simply measuring the corresponding zone length[7,10]. This combined qualitative and quantitative approach enables a direct evaluation of the course of the reaction and, when necessary, further optimization and/or change of the reaction conditions.

Obviously, only small amounts of the product PMV^{2+} are formed in synthesis

route I (Fig. 3). Since during the first synthesis step an almost complete reaction was observed (Fig. 1), the low yield of PMV^{2+} must be due to the formation of an undesired product during the second step (Fig. 3). Based on these results, a modified synthesis procedure (route II) with a reversed order of the quaternization steps was employed, enabling the synthesis of PMV^{2+} in high purity and acceptable yield (Figs. 4 and 5). In this connection it is of note that these differences in the purity of PMV^{2+}, derived from syntheses I and II, respectively, could not be revealed by mass spectrometry and NMR spectroscopy[4].

As illustrated in Fig. 4, it was feasible to analyze directly the reaction mixture after the first synthesis step (A) as well as to monitor the enrichment of the intermediate product PV^{+} during gel chromatographic purification (B). This easy possibility to determine rapidly the degree of purity of an intermediate product is an obvious advantage for the performance of the subsequent synthesis step.

In the present investigation, capillary ITP has been proven to be a sensitive, reliable and rapid method to monitor the synthesis and subsequent purification of PMV^{2+}. Thus, this electrophoretic technique may represent a valuable alternative to commonly used analytical methods in this field, enabling greater control of the synthesis of quaternary 4,4'-bipyridylium salts.

ACKNOWLEDGEMENT

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