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Journal of Chromatography B, 714 (1998) 99–104

JOURNAL OF CHROMATOGRAPHY B

Short communication

Separation of a range of cations by nonaqueous capillary electrophoresis using indirect and direct detection

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Abstract

The use of nonaqueous media and indirect detection is reported for the separation and detection of a range of small cations. The novel applications involved separation of a range of metal ions, small nonchromophoric amines, cationic ion-pair reagents and cationic surfactants. Separations were achieved using acidified methanol containing imidazole as the UV co-ion for indirect detection. The methods produced different selectivity compared to aqueous methods using acidified aqueous imidazole solutions. Advantages of the methods include speed of analysis and prevention of sample micellerisation. The methods were shown to be quantitative and reproducible by their application to the determination of Tris content.

Keywords: Indirect detection; Cations

1. Introduction

Capillary electrophoresis (CE) is a rapid and efficient separation technique which is based on differences in electrophoretic mobilities between compounds. The majority of reports has focused on the use of aqueous buffers as background electrolytes. CE in nonaqueous media has previously been reported for a small number of basic drugs and anions [1,2].

The lower viscosity of some organic solvents allows higher mobilities and potentially faster separations [3] than with aqueous electrolytes. The migration order can be altered in nonaqueous CE, probably due to a change in the solvation of analytes resulting in solvation shell radia differing from the ones seen in aqueous media. Selectivity can be varied further by using different mixtures of two or more organic solvents.

Lower detection sensitivity is a disadvantage of many nonaqueous media as that they have higher background UV absorbances than water. This problem can be avoided by using indirect UV detection or an alternative detection type such as mass spectrometry. CE using nonaqueous electrolytes and indirect UV detection has been reported by Salimi-Moosavi and Cassidy in 1995 [2], for the separation of inorganic anions.

Since the currents are typically lower in nonaqueous media than in aqueous CE, less heat is produced inside the capillary, which has a positive impact on the separation efficiency. In these solvents it is often possible to achieve good resolution with a low ionic strength electrolyte.

Using organic solvents and low pH* (pH measurements in nonaqueous solvents are only indicative of pH and are denoted as pH*) makes it possible to

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0378-4347/98/$19.00
PII: S0378-4347(98)00040-1

analyse a number of cationic species such as metal ions, ion-pair reagents or surfactants. Some of the problems in aqueous CE including poor solubility of certain drugs and micelle formation are less likely to occur when using organic solvents. Other advantages are the possibility of using water-insoluble electrolyte additives for particular separations and compatibility of nonaqueous media with mass spectrometric detectors.

Separation of cations in aqueous CE using indirect detection has been reported by many workers using a range of different substances to provide the UV signal. A popular choice for the UV co-ion is imidazole which has a similar electrophoretic mobility as a range of metal ions. The use of imidazole as UV co-ion was reported by Beck and Engelhardt in 1992 [4]. If different selectivity for the metal ions is required additives such as small organic acids or crown ethers [5] can be added. For example crown ethers have a macrocyclic polyether ring and form stable inclusion complexes with metals and protonated amines which changes their mobility [6]. Because of the difference in affinity to the selector the mobility of the primary amines decreases to varying extents, resulting in different migration times and selectivities.

Metal ions and other small cations present in water-soluble pharmaceutical materials at trace levels have been determined by CE in aqueous electrolytes or by high-performance liquid chromatography (HPLC) methods [7,8]. Using nonaqueous CE these analytes can also be determined in water-insoluble pharmaceutical materials.

Analysis of simple organic amine compounds such as hydroxylamine and Tris (tromethamine) have important applications in many industries including the pharmaceutical. These compounds have limited UV absorbance and require use of elaborate detection methods. For example hydroxylamine has previously been analysed by CE with electrochemical detection [9] and Tris has been analysed by HPLC with conductivity detection [10].

Ion-pair reagents, such as tetrabutylammonium bromide, may be used as starting materials or catalysts in chemical reactions. There is a need to determine traces of these compounds in drug formulations and for quality control of raw materials before their use in manufacture. For example aqueous CE with indirect photometric detection has been used to determine alkyl quaternary ammonium compounds in a water-soluble drug substance [11]. These compounds have, however, a tendency to form micelles which must be controlled by addition of an organic modifier to the background electrolyte.

Cationic surfactants, such as benzyltrimethylammonium chloride and octadecyltrimethylammonium chloride, are used widely by the pharmaceutical industry in several different areas. Uses include emulsifiers in formulations, starting material for alkylation reactions and as softening agents in detergents. Surfactants have been determined in aqueous CE [12], but these compounds form micelles above a certain concentration in aqueous solutions which complicates determinations. Micelle formation is prevented in nonaqueous conditions which should improve analysis.

Tris(hydroxymethyl)aminomethane acetate (Tromethamine/Trizma/Tris) is commonly used as a buffering agent, alkalizer and emulsifying agent in pharmaceutical and cosmetic products. It can also be used as starting material in drug synthesis or as a counter-ion to acidic drug substances. A quantitative method using ion chromatography with conductivity detection has been reported by Hall et al. 1995 [10]. Although the method showed good linearity and precision, the problem with column degeneration, probably due to build-up of cationic compound on the column, demands extensive washing (90 min) to regenerate the column performance. This indicates the need for development of a new rapid and reproducible method to quantify Tris.

This report demonstrates that the use of indirect detection and nonaqueous CE electrolytes can provide a useful alternative to aqueous CE for separation of metal ions as a different selectivity is obtained. A standard set of operating conditions was devised which allowed separation and quantitation of a wide range of simple organic and inorganic cations. A number of novel nonaqueous CE separations of inorganic and organic cationic species of importance are reported. These solutes are raw materials or excipients in drug formulations; metal ions and small cations, cationic ion-pair reagents and cationic surfactants. A quantitative assay of Tris was

performed by using nonaqueous CE with indirect UV detection and validated in terms of linearity and precision.

2. Experimental

2.1. Apparatus

All experiments were performed on a Beckman P/ACE system 2200 instrument (High Wycombe, Berkshire, UK). The capillaries of fused-silica had an inner diameter of 50 μm and were 27 cm long (20 cm to the detection window). They were purchased from Composite Metal Services (Hallow, Worcestershire, UK). Capillary windows were burnt using a device purchased from Capital HPLC (Broxburn, Edinburgh, UK). Capillaries were rinsed with 0.1 *M* NaOH for 20 min before they were used the first time to activate the silanol groups at the inner surface. Between injections the capillary was rinsed with methanol (60 s) and the running buffer (60 s). All separations were run at a constant temperature of 30°C. Injections were performed using a hydrodynamic pressure of 34.45 kPa for 1 s, unless stated otherwise. To determine the limit of detection of Tris, an electrokinetic injection mode of 10 kV for 5 s was used.

Separation of cations is performed under acidic conditions, where the low pH makes basic positions in the analytes ionise. In these experiments 1 ml of concentrated acetic acid was added to every 100 ml of the methanolic electrolyte. Imidazole (5 m*M*) was used as the background UV co-ion and detection was performed at 214 nm, indirectly or directly.

The signal is converted by the software to produce positive peaks which are easier to automatically integrate.

2.2. Reagents

Reagents used were purchased from Sigma–Aldrich (Poole, Dorset, UK). The metal salts used were soluble in methanol. Acetates of sodium and lithium; sulphates of magnesium, copper and iron; nitrates of lead, silver and cobalt and zinc sulphide and potassium hydrogen phthalate was used.

All samples were dissolved in methanol with a concentration of 50 ppm (mg/l), except for the samples in the quantitation assay for Tris where a concentration of 100 ppm was used.

3. Results

3.1. Selection of the electrolyte

Pure methanol was determined to be the most suitable solvent since it offers good solvating properties for the analytes of interest. Different ratios of MeOH and ACN (acetonitrile) in the electrolyte were examined for the separation of small cations. The effect of the lower viscosity of ACN was clearly observed as shorter migration times and loss of resolution occurred when the buffer content of ACN was increased. The selected electrolyte was 5 m*M* imidazole in 99 ml MeOH and 1 ml glacial acetic acid. Fig. 1 shows the separation of seven small cations. In simple aqueous CE electrolytes [4] potassium and ammonium comigrate whereas they are well separated in the nonaqueous system. Also the mobility of magnesium is in this system lower than the mobility of both lithium and Tris, whereas in aqueous CE [4] magnesium would migrate faster than these two.

The resolution of potassium and ammonium can be achieved in aqueous CE by using weakly alkaline conditions [13] or by the addition of crown ether [5].

3.2. Applications

3.2.1. Metal ions

Separation of sodium, lithium, potassium, magnesium and cobalt was achieved with this method using a voltage of 10 kV and detected indirectly at 214 nm. The migration times showed good repeatability and the separation was completed within 5 min, see Fig. 1. Sodium and lithium have mobilities similar to the UV co-ion imidazole and are therefore seen as symmetrical peaks. Ions migrating faster or slower than the UV co-ion will give fronting or tailing peaks respectively.

Some metal ions including copper, silver and zinc absorb in the UV region. These ions can also be

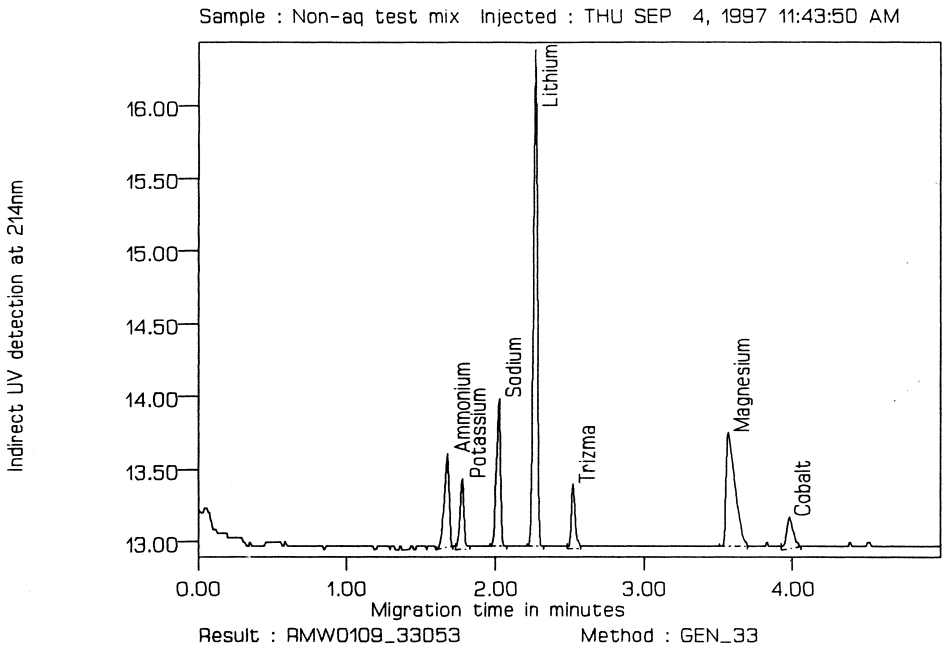

Fig. 1. Separations of seven small cations (concentrations in the range 1–10 ppm dissolved in methanol). Separation conditions: 5 m*M* imidazole in MeOH–acetic acid (99:1), 10 kV, 50 μm×27 cm long (20 cm to the detection window), indirect detection at 214 nm.

determined (Fig. 2) in the nonaqueous system using the same electrolyte containing imidazole, methanol and acetic acid. The detection at 214 nm is performed in the direct mode as the metal ions absorb more strongly than the background UV signal arising from imidazole. It may seem unnecessary to use a UV co-ion in the buffer when the mode of detection is direct, but removing the imidazole from the buffer causes problems as the current was severely reduced which caused voltage failures with the separations. As an alternative ammonium acetate can be used to increase the ionic strength.

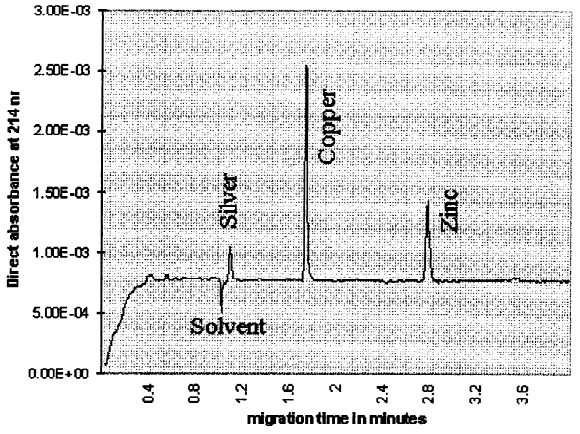

Fig. 2. Separation of UV-absorbing metal ions (17 ppm in methanol). Separation conditions: 5 m*M* imidazole in MeOH–acetic acid (99:1), 20 kV, 50 μm×27 cm long (20 cm to the detection window), direct detection at 214 nm.

3.2.2. Small amines

The small amines Tris and hydroxylamine comigrated under the separation conditions used for Fig. 1. Fig. 3 shows that addition of 2 m*M* of 18-crown-ether-6 to the electrolyte gave acceptable separation of Tris and hydroxylamine. Crown ether was added to the buffer to act as an chelator. Tris forms a more stable complex than hydroxylamine, therefore its migration time is more extended by the addition of crown ether.

3.2.3. Cationic ion-pair reagents

The ion-pair reagents tetramethylammonium, tetraethylammonium and tetrabutylammonium (hydroxide or bromide salts) were separated using the electrolyte employed for the separation of the metal

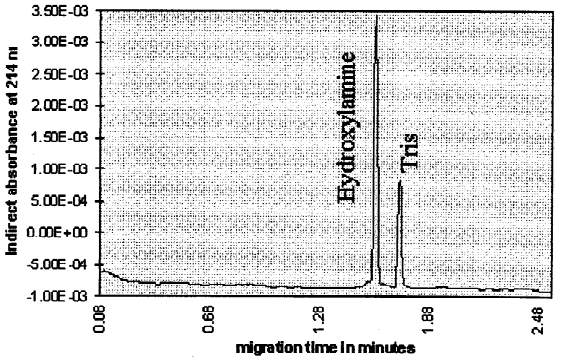

Fig. 3. Hydroxylamine (1.6 min) and Tris (1.8 min) (concentration 25 ppm in methanol). Separation conditions: 5 m*M* imidazole in MeOH–acetic acid (99:1) containing 2 m*M* 18-crown-ether-6, 20 kV, 50 μm×27 cm long (20 cm to the detection window), indirect detection at 214 nm.

ions in Fig. 1. An operating voltage of 2.5 kV gave migration times shorter than 8 min, see Fig. 4.

3.2.4. Cationic surfactants

Various salts of benzyltrimethylammonium, dodecyltrimethylammonium, tetradecylammonium, hexadecylammonium and octadecylammonium are frequently used surfactants. They can be separated in this nonaqueous system with good resolution within 2 min. Benzyltrimethylammonium has a chromophore and absorbs UV more strongly than imidazole and this therefore generated a negative peak at 1.3 min in Fig. 5. The other surfactants follow in order

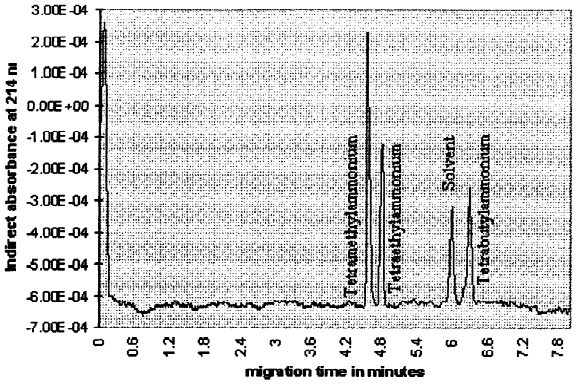

Fig. 4. Separation of cationic ion-pair reagents (concentration 17 ppm in methanol). Separation conditions: 5 m*M* imidazole in MeOH–acetic acid (99:1), 2.5 kV, 50 μm×27 cm long (20 cm to the detection window), indirect detection at 214 nm.

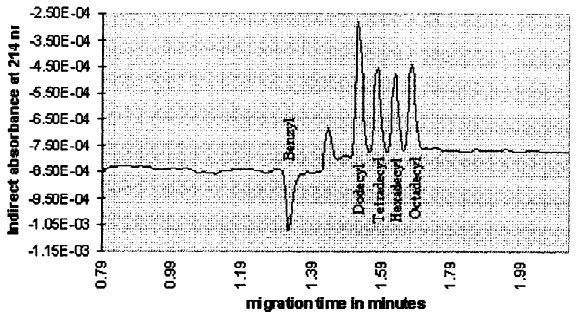

Fig. 5. Separation of cationic surfactants. Separation conditions: 5 m*M* imidazole in MeOH–acetic acid (99:1), 5 kV, 50 μm×27 cm long (20 cm to the detection window), indirect detection at 214 nm.

of size, with the heaviest, octadecylammonium, migrating at 1.7 min.

3.2.5. A quantitation assay for Tris

The nonaqueous method was used for a quantitative assay of Tris. At a voltage of 10 kV the separation of Tris and the internal standard (dodecyltrimethylammonium) was achieved with sufficient resolution and good precision of migration times. Ten injections of the same sample were made to validate the precision of the method. The results are given in Table 1.

An extra step was added to the method to assure a stable base-line throughout long injection. After the injection of each sample the capillary was dipped into a vial containing buffer for 10 s, before entering the running buffer vial. This was to avoid contamination of the buffer vial with sample solution. If this additional step was not included a gradually increasing step in the base-line occurred due to sample carryover into the electrolyte vial.

Table 1
Precision data for ten injections of a solution containing 100 ppm Tris and 100 ppm internal standard

	Mean	%R.S.D. (N=10)
Internal standard migration time	1.5531	0.30
Tris migration time	1.6934	0.31
Relative migration time	1.090335	0.02
Peak area internal standard	19.687	2.30
Peak area Tris	43.6932	2.21
Peak area ratio	2.219574	1.18

Detector response linearity was examined by preparing samples with 50, 75, 100, 125 and 150 ppm Tris dissolved in 100 ppm internal standard, and injecting them in the nonaqueous CE system. In this concentration range the detector response was linear ($R=0.9996$) and the intercept expressed as percentage of the peak area ratio for the 100 ppm sample was 1.32%.

To calibrate the method, five calibration samples were prepared by accurately weighing approximately 7 mg, which was dissolved in 50 ml of the internal standard solution and placed in an ultrasonic bath for 15 min. Duplicate 1 s injections of each calibration solution were run at 10 kV. The peak area ratio between Tris and the internal standard was divided by the amount of Tris in each sample to yield a response factor that ought to be constant for all samples. (This method has a relative standard deviation (R.S.D.) in the response factor of 1.26%).

For interest, a limit of detection of 33 ppb was determined for Tris with three times the noise of the baseline. To be able to detect such a low concentration it is necessary to use the electrokinetic injection mode. Further experimental work would be needed if electrokinetic injections were to be employed routinely including repeatability and linearity. The limit of detection was determined to 3.3 ppm using pressure injection.

An advantage of the method is that it shows no tendency to the gradual loss of performance as seen in the ion chromatography method [10]. The reconditioning required to obtain consistent capillary performance was 60 s rinsing with methanol and 60 s rinsing with the running buffer between each injection. This method is performed on standard CE equipment, while the HPLC method used a conductivity detector. Less time and expense is therefore required to carry out a large number of separations using this nonaqueous CE method.

4. Conclusions

CE with indirect UV detection and nonaqueous electrolytes can achieve rapid and efficient separations of a wide range of small cations. Different selectivity was achieved compared to that normally obtained with aqueous buffers, for example potassium and ammonium, which are normally unresolved in aqueous buffers, could be separated in acidified methanol.

A general set of operating conditions using imidazole as the UV co-ion allowed separation of metal ions, simple amines, cationic ion-pair reagents and cationic surfactants. Selectivity for primary amines could be altered by addition of crown ether. In the quantitative assay of Tris the method gave high repeatability, with R.S.D. values for the migration times of under 1%.

In the future nonaqueous CE is likely to become a useful tool in analysis of substances with poor aqueous solubility and for separations that cannot be achieved in water-based electrolytes. The combination of nonaqueous CE with indirect UV detection is a promising aspect that could facilitate separations of low aqueous solubility compounds or those with limited chromophores.

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