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Applications of capillary electrophoresis in the eye-care pharmaceutical industry

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Abstract

The speed and resolution of capillary electrophoresis (CE), combined with its low cost and low volume requirements, make it a promising method of separating eye-care pharmaceuticals and analyzing the ionic composition of tears. Capillary zone electrophoresis (CZE) offers linearity, precision, and recovery comparable to high-performance liquid chromatography (HPLC) at a fraction of its cost. In addition, CZE separates and quantitates cocoamphocarboxyglycinate in the presence of a non-ionic fatty acid amide surfactant seven times faster than HPLC. CE easily detects such cations as sodium and potassium in human tears.

1. Introduction

The eye-care pharmaceutical industry has suffered from slow, expensive analyses and the generation of large volumes of solvent waste. Capillary electrophoresis (CE) offers inherent speed, low operating costs, high resolution, and low sample volume requirements. It is an attractive alternative for the separation problems of eye-care pharmaceuticals. CE is maturing as an analytical technique [1–3], as evidenced by improvement in its quantitative capabilities [4–6], and it is finding increased use in pharmaceutical analysis [7–9]. Capillary zone electrophoresis (CZE) is particularly attractive because it can perform separations with US$ 5 columns instead of the US$ 300–800 columns that high-performance liquid chromatography (HPLC) requires for analyzing multiple types of ionic analytes.

The present work applies CE to quantitative stability assays for the analysis of active ingredients in finished products, raw materials, and ionic components in tears. The contact lens care product segment of the eye-care pharmaceutical industry includes surfactant cleaners for removing protein and lipid deposits from contact lenses, proteolytic enzyme products for removing protein deposits, and disinfecting solutions for contact lenses. These pharmaceuticals require flexible and efficient separation techniques. Additionally, studies designed to assess their ocular effect require analytical tools capable of a detailed analysis of tear constituents.

Cocoamphocarboxyglycinate (see Fig. 1) is a zwitterionic surfactant used to clean lipid and

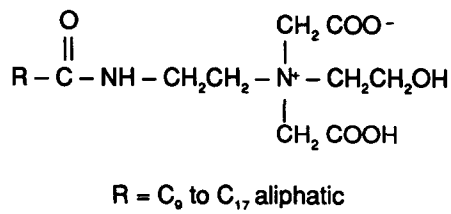

Fig. 1. The structure of cocoamphocarboxyglycinate.

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protein deposits from rigid gas permeable (RGP) contact lenses in the product RESOLVE/GP® Daily Cleaner. Presently, cocoamphocarboxyglycinate is effectively analyzed in this product formulation by ion-exclusion HPLC [10]. However, the method suffers from a long cycle time due to the late elution of the non-ionic fatty acid amide surfactant in the product formulation on the ion-exclusion column (76 min). The present work utilizes CZE to separate and quantitate cocoamphocarboxyglycinate in the presence of non-ionic fatty acid amide surfactant in under 12 min, seven times faster than the existing ion-exclusion HPLC method.

A CE method was developed for the quantitative analysis of the raw material N-acetylcysteine, which is used as a mucolytic agent for aiding in the removal of protein deposits from contact lenses. The current United States Pharmacopeia (USP) method for the analysis of this compendial raw material utilizes reversed-phase HPLC [11]. CE effectively separates N-acetylcysteine from its hydrolysis product cysteine and its oxidation products cystine and N-acetylcystine (see Fig. 2) 1.5 times faster than the USP method while providing comparable precision.

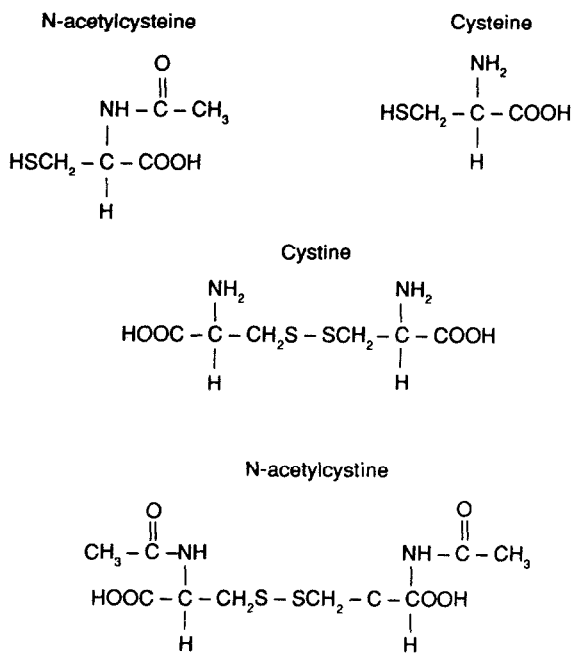

Fig. 2. The structure of N-acetylcysteine and its degradation products.

In ocular research, CE is a powerful technique for studying the composition of tears, including *in-vivo* measurements [12]. Due to the inherently low volume of the human tear, typically 6 μl [13], analysis by HPLC requires multiple tear collections to provide enough sample for analysis since a typical HPLC injection volume is 10 μl. The low sample injection volume required of CE (typically a few nl) makes this technique ideal for analyzing tear samples. Analyzing inorganic constituents of tears is important in establishing benchmarks for normal human corneal physiology. These benchmarks can help in designing products that are compatible with ocular physiology.

2. Experimental

2.1. Materials

Fused silica, polyimide coated capillary tubing (365 μm O.D. × 75 μm I.D.) was obtained from Polymicro Technologies (Phoenix, AZ, USA). ACS reagent grade sodium tetraborate decahydrate, boric acid, copper sulfate pentahydrate and formic acid (88%, w/w) were obtained from Mallinckrodt (Paris, KY, USA). ACS reagent grade sodium borate decahydrate was obtained from Aldrich (Milwaukee, WI, USA).

Cocoamphocarboxyglycinate was obtained from Rhone-Poulenc (Cranbury, NJ, USA). The non-ionic fatty acid amide surfactant was obtained from Stepan Chemical Comp. (Northfield, IL, USA). N-acetylcysteine was obtained from Diamalt/SST Corp. (Clifton, NJ, USA). Reagent grade cysteine, cystine, and benzoic acid were obtained from Sigma (St. Louis, MO, USA). HPLC grade methanol was obtained from Baxter Scientific Products (Irvine, CA, USA). Deionized water having resistivity greater than 17 MΩ · cm was obtained with a Millipore Milli-Q reagent water system (Bedford, MA, USA). N-acetylcystine was generated *in situ* from N-acetylcysteine by treatment with 3% hydrogen

peroxide. All reagents were used as received from the manufacturer.

2.2. Equipment

Electrophoresis experiments were conducted on a Dionex CES I capillary electrophoresis system obtained from Dionex (Sunnyvale, CA, USA). UV detection was employed by using 200 nm radiation for detection of cocoamphocarboxyglycinate and for N-acetylcysteine and its degradation products. For the detection of inorganic cations, indirect UV detection utilizing Cu^{2+} chromophore in the form of $CuSO_4$ at 215 nm was employed [14]. Electrophoretic data were acquired using a PE Nelson Analytical 941 analog-to-digital converter with subsequent storage on a VAX 6000 computer (Bedford, MA, USA).

2.3. Methods

Capillaries (52 cm long) were prepared by scoring the polyimide coating with a ceramic cutting tool and breaking at the scored mark. The on-column detection window was introduced onto the capillary 5 cm from the end by burning off the coating with a butane lighter. The capillary was prepared for use by flushing with 0.5 *M* NaOH under pressure of 5 p.s.i. (1 p.s.i. = 6894.76 Pa) for 3 min, followed by a 3-min rinse with deionized water. This cycle was repeated twice more. The capillary was then flushed for 15 min with the running buffer under pressure. Different running buffers were employed for each analysis and are indicated in the appropriate figures. All buffers were vacuum-filtered through a 0.45-μm Nylon-66 filter to remove particulates.

The 10 m*M* $Na_2B_4O_7$–50 m*M* H_3BO_3, pH 8.5 buffer was prepared from analytical reagent grade $Na_2B_4O_7 \cdot 10H_2O$ and H_3BO_3. The 50:50 (v/v) 10 m*M* $Na_2B_4O_7$–50 m*M* H_3BO_3/methanol buffer was prepared by mixing equal volumes of the 10 m*M* $Na_2B_4O_7$–50 m*M* H_3BO_3 buffer and the HPLC grade methanol. The 4.0 m*M* $CuSO_4$, pH 3.0 buffer was prepared from analytical reagent grade $CuSO_4 \cdot 5H_2O$ and 88% (w/w) formic acid. The pH of the buffer was adjusted to 3.0 with 1 *M* H_2SO_4.

All samples were injected with gravity injection by raising the capillary to a height of 5 cm for 10 s. For all analyses performed, the injection end of the capillary served as the anode. Different separation voltages were employed for the various separations employed. For the analysis of cocoamphocarboxyglycinate, an operating voltage of +30 kV was employed, +18 kV was used for the analysis of N-acetylcysteine, and +20 kV for the analysis of inorganic ions. Peak components were identified by comparing their migration times with those of the corresponding standard for each analyte.

3. Results and discussion

3.1. Separation and quantitation of cocoamphocarboxyglycinate

Cocoamphocarboxyglycinate is a zwitterionic surfactant possessing a quaternary amine nitrogen center and two carboxylate groups (Fig. 1). The RESOLVE/GP Daily Cleaner product formulation also contains a non-ionic fatty acid amide surfactant. These two surfactants may therefore be separated by using a buffer that imparts a non-zero electrical charge to the cocoamphocarboxyglycinate.

Due to the bifunctional nature of cocoamphocarboxyglycinate, one could either lower the pH to impart a positive charge or raise the pH above the pK_a of the cocoamphocarboxyglycinate carboxylate groups to provide a net negative charge to the cocoamphocarboxyglycinate molecule. A high pH buffer approach was adopted. This avoided electrostatic attraction of the cocoamphocarboxyglycinate molecules to the negatively charged silanol sites of the capillary and the associated band tailing problems that can result from such adsorptive interactions if cocoamphocarboxyglycinate carried a positive charge [15]. Additionally, since the electroosmotic flow increases substantially at basic pH, a high pH buffer gives a rapid separation [16].

Another consideration in selecting a separation buffer for the surfactants is the propensity for these materials to stick to glass in the absence of organic solvents. This is especially true with the non-ionic fatty acid amide surfactant, which has limited solubility in pure water. With the above considerations in mind, a buffer consisting of methanol/10 m*M* $Na_2B_4O_7$–50 m*M* H_3BO_3 (50:50) with a pH of 8.5 was employed.

Fig. 3 shows a representative electropherogram obtained by diluting the RESOLVE/GP Daily Cleaner product 1:50 (v/v) in the running buffer. The neutral non-ionic fatty acid amide surfactant homologues are shown to migrate as a single peak at 4.7 min. The cocoamphocarboxyglycinate homologues appear as a cluster of 6 peaks, corresponding to the different alkyl chain homologues centered near 7.0 min. The cocoamphocarboxyglycinate separation displays an efficiency of 54 500 theoretical plates for the main peak at 7.0 min.

Despite the good cocoamphocarboxyglycinate resolution shown in Fig. 3, multiple injections of the sample revealed a deterioration of the cocoamphocarboxyglycinate resolution, and the non-ionic fatty acid amide surfactant peak showed significant tailing (Fig. 4). Dilution of the product formulation in pure methanol reduced the non-ionic fatty acid amide surfactant tailing, recovered the good cocoamphocarboxyglycinate homologue resolution as seen in Fig. 4, and maintained this good resolution for multiple repeated injections of sample.

For quantitation and precision, an internal standard consisting of benzoic acid was added to the sample. Additionally, preliminary spiked recovery studies indicated that cocoamphocarboxyglycinate recovery was lower in the presence of the non-ionic fatty acid amide surfactant, probably due to co-adsorption of these surfactants on the capillary surface. Therefore, non-ionic fatty acid amide surfactant was added to the cocoamphocarboxyglycinate standard.

Fig. 5 illustrates the lack of interference of both the benzoic acid internal standard and non-ionic fatty acid amide surfactant in the detection of cocoamphocarboxyglycinate. Neither compound exhibits any response in the region of the cocoamphocarboxyglycinate peaks. The linearity was checked from 0.03% (w/v) to 0.15% (w/v), corresponding to a range of 25% to 150% of the

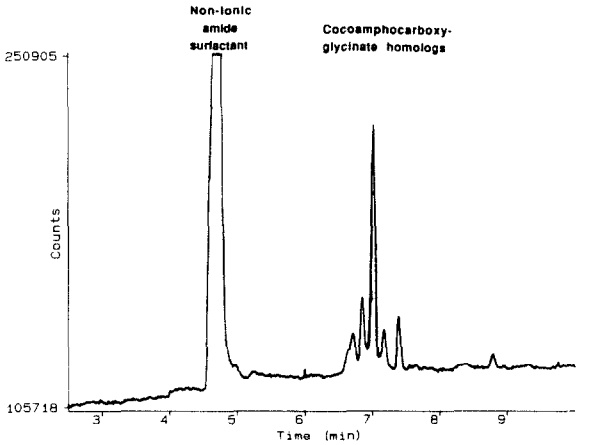

Fig. 3. Electropherogram of cocoamphocarboxyglycinate in RESOLVE/GP Daily Cleaner for contact lenses. Conditions: buffer, 50:50 (v/v) methanol/10 m*M* $Na_2B_4O_7$–50 m*M* H_3BO_3, pH 8.5; capillary, 52 cm (47 cm to detector) × 75 μm I.D.; applied voltage, +30 kV; temperature, ambient; detection, UV absorbance at 200 nm; injection, gravity, 50 mm height for 10 s. Sample concentration 0.4 mg/ml cocoamphocarboxyglycinate obtained by dilution of 1 ml of RESOLVE/GP Daily Cleaner in 50 ml methanol.

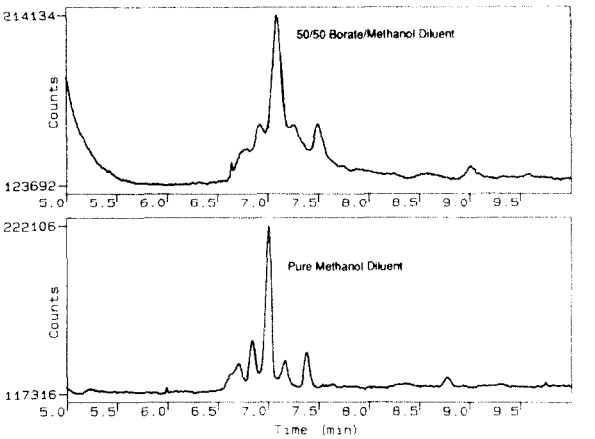

Fig. 4. Electropherogram showing the effect of sample diluent on cocoamphocarboxyglycinate resolution in RESOLVE/GP Daily Cleaner. All electrophoretic conditions as in Fig. 3. Top electropherogram: running buffer used as the diluent (1:50). Bottom electropherogram: pure methanol used as the sample diluent (1:50).

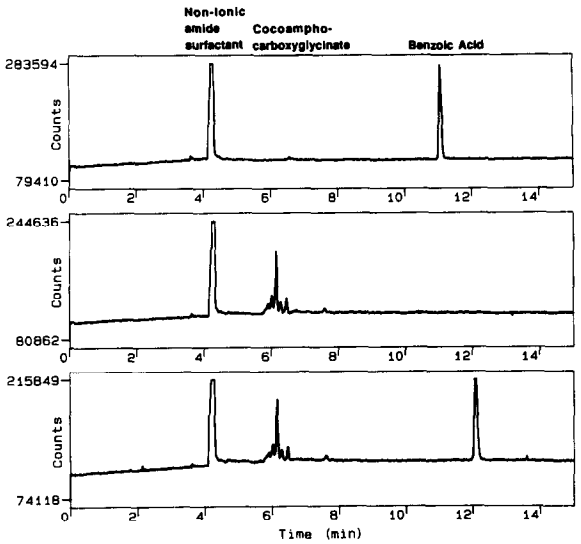

Fig. 5. Overlay of electropherograms showing lack of vehicle and internal standard interference in the analysis of cocoamphocarboxyglycinate. Top: electropherogram of product vehicle spiked with benzoic acid internal standard. Middle: full product formulation without benzoic acid internal standard added. Bottom: full product formulation spiked with benzoic acid internal standard.

label claim (2.00%, w/v) of cocoamphocarboxyglycinate in the surfactant cleaner product.

The cocoamphocarboxyglycinate quantitation data showed excellent linearity in this concentration range, as indicated by a linear correlation coefficient of 0.9995 and a *y*-intercept very close to zero. The precision at the target concentration of cocoamphocarboxyglycinate contained in the product is 1.5% relative standard deviation (R.S.D., ± 1 SD, $n = 4$).

The equivalence of the present CE method for the analysis of cocoamphocarboxyglycinate with the existing ion-exclusion HPLC method was demonstrated by analyzing a shared sample of RESOLVE/GP Daily Cleaner (see Table 1). Statistical *t*-testing of the mean cocoamphocarboxyglycinate assay values and *F*-testing of the standard deviations of these measurements at 95% confidence demonstrated the statistical equivalence of the data obtained by both methods.

Table 1 shows an overall performance comparison of the CE cocoamphocarboxyglycinate method and the ion-exclusion HPLC method. The CE method yields comparable linearity, precision, and accuracy to the ion-exclusion HPLC method. However, it is seven times faster and much more economical due to lower column costs and significantly lower consumption of chemicals.

3.2. Raw material analysis: N-acetylcysteine

N-acetylcysteine raw material analysis is currently performed by analytical methodology specified in the United States Pharmacopeia XXII [11], which utilizes reversed-phase HPLC. For analysis by CE, a pH 8.5 buffer was em-

Table 1
Comparison of cocoamphocarboxyglycinate analysis by CE and ion-exclusion HPLC

Parameter	CE	Ion-exclusion HPLC
Linearity, *r*	0.9995	0.9990
Precision, as % R.S.D. (± 1 SD, $n = 6$)	± 1.5%	± 1.6%
Recovery at 100% of product label claim (2.0% w/v corresponding to 0.04% w/v injected; ± 1 SD, $n = 6$)	100.0 ± 1.5%	100.6 ± 1.6%
Analysis time per sample	12 min	90 min
Volume mobile phase per sample	0.1 ml	50 ml
Column cost	US$ 5	US$ 800
Cocoamphocarboxyglycinate assay results for shared RESOLVE/GP Daily Cleaner sample	2.01 ± 0.03% (w/v) ± 1 SD, $n = 6$	2.03 ± 0.03% (w/v) ± 1 SD, $n = 6$

ployed. To analyze the purity of N-acetylcysteine raw material, the analytical method must be able to separate N-acetylcysteine from its degradation products: cysteine, cystine, and N-acetylcystine. Fig. 2 shows their structures and Fig. 6 displays the electropherogram of N-acetylcysteine and its degradation products. Cysteine and cystine are seen to comigrate at 3.9 min, ahead of the N-acetylcysteine peak seen at 4.6 min. The separation displays high efficiency, with approximately 41 000 theoretical plates seen for the N-acetylcysteine peak. N-acetylcystine (the disulfide dimer of N-acetylcysteine) is observed at 5.0 min.

For quantitative analysis, benzoic acid served as an internal standard and the area of the N-acetylcysteine peak was ratioed against the benzoic acid peak. Fig. 7 shows the electropherogram of N-acetylcysteine spiked with the benzoic acid internal standard. The linearity of the N-acetylcysteine CE method was tested in the concentration range of 0.3–1.6 mg/ml. Linear least-squares analysis yielded a correlation coefficient of 0.99995.

To assess the equivalence of the CE method to the USP HPLC method for N-acetylcysteine

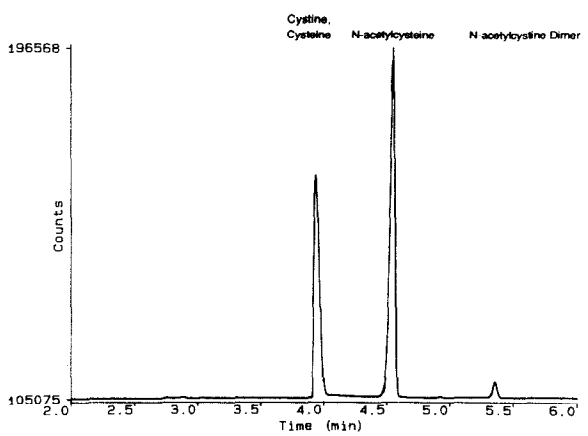

Fig. 6. Electropherogram of N-acetylcysteine and its degradation products. Conditions: buffer, 10 m*M* $Na_2B_4O_7$–50 m*M* H_3BO_3, pH 8.5. capillary, 52 cm (47 cm to detector) × 75 μm I.D.; applied voltage, +18 kV; temperature, ambient; detection, UV absorbance at 200 nm; injection, gravity, 50 mm height for 10 s. Sample concentration, 0.5 mg/ml of each component except N-acetylcystine, which appears at a concentration of approximately 0.1 mg/ml.

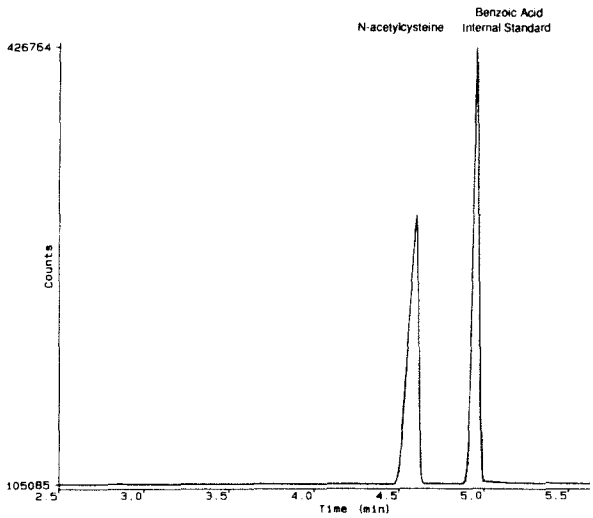

Fig. 7. Electropherogram of N-acetylcysteine with benzoic acid internal standard spiked in (all conditions as in Fig. 6).

analysis, a sample of N-acetylcysteine raw material was assayed by the two methods. Table 2 compares the assay data obtained by both methods. The mean N-acetylcysteine assay values were compared by *t*-testing at the 95% confidence interval and shown to be nearly equivalent. The CE method displayed a precision of 0.5% R.S.D. This is comparable to the HPLC assay data obtained with the USP method, and it is well within the USP guideline of $<2\%$ R.S.D.

The CE method for the analysis of N-acetylcysteine is extremely rapid and cost effective, as Table 2 shows. The CE method is three times faster and the column costs are approximately one sixtieth due to the low costs of fused silica capillaries compared to reversed-phase C_{18} HPLC columns.

3.3. CE analysis of inorganic cations in tears

Fig. 8 shows the electropherogram of a human tear sample diluted 1:24 (v/v) in deionized water. Comparison of the peaks observed for the tear electropherogram with the peaks for an inorganic cation standard containing potassium, sodium, calcium, and magnesium identifies the cation peaks in the tear electropherogram. Potassium migrates at 3.4 min in the tear sample,

Table 2
Comparison of CE USP reversed-phase HPLC methods for N-acetylcysteine raw material analysis

Parameter	CE	Reversed-phase HPLC
Precision, as % R.S.D. (±1 SD, $n = 6$)	±0.7%	±0.3%
Run time	6 min	15 min
Column cost	US$ 5	US$ 300
N-Acetylcysteine assay purity	99.6 ± 0.7% (w/w) ± 1 SD, $n = 6$	100.8 ± 0.3% (w/w) ± 1 SD, $n = 6$

followed by sodium at 5.0 min, calcium at 5.3 min, and magnesium at 5.8 min.

The efficiency of the separation as measured by the number of theoretical plates in the sodium peak displays 1200 theoretical plates for the tear sample. However, the separation has high enough efficiency to clearly separate sodium, potassium, calcium, and magnesium from one another. The relatively low number of theoretical plates seen for sodium is due mainly to sample overload.

Because the current study is preliminary, the method has not had rigorous linearity testing. However, a semi-quantitative estimate of the concentration of the various components was performed. It was based on peak area comparisons between the tear sample and the cation standard shown in Fig. 8. All the levels observed are in general agreement with values reported in the literature.

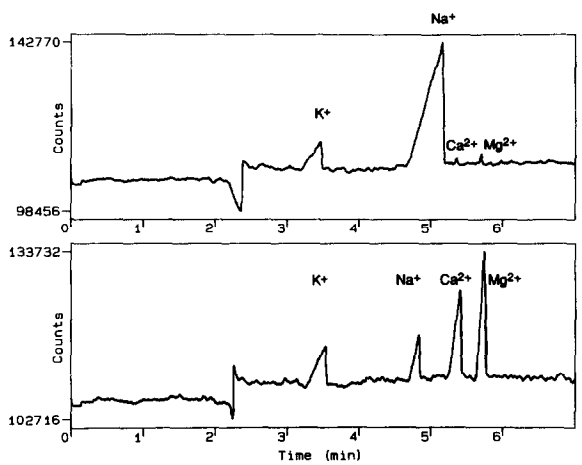

Fig. 8. Survey electropherogram of human tear sample diluted 1:20 in deionized water (top) and inorganic cation standard (bottom). Conditions: Buffer, 4 m*M* copper(II) sulfate–4 m*M* formic acid (3:6); capillary, 52 cm (47 cm to detector) × 75 μm I.D.; applied voltage, +20 kV; temperature, ambient; detection, indirect UV at 215 nm.

4. Conclusion

CE is an effective, flexible technique capable of analyzing components important to the eye-care pharmaceutical industry. The current project has demonstrated the applicability of CE to the analysis of active ingredients in product formulations, raw material analysis, and ocular research. The ability of CE to perform as a quantitative analytical technique was demonstrated by the analysis of cocoamphocarboxyglycinate in a surfactant cleaner product and by the analysis of N-acetylcysteine raw material. The precision, linearity, and accuracy were comparable to existing HPLC methods but analysis times and cost were significantly less.

CE was also shown to be a useful tool for probing the composition of inorganic cations in tears. The inherent speed, high resolution, and powerful separation mechanism of CE make it an excellent tool for separation challenges encountered by the eye-care pharmaceutical industry.

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