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Simultaneous detection and quantitation of sodium, potassium, calcium and magnesium in ocular lenses by high-performance capillary electrophoresis with indirect photometric detection

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Abstract

A high-performance capillary electrophoresis (HPCE) method which can be used to quantitatively determine Na^+, K^+, Ca^{2+} and Mg^{2+} simultaneously in ocular lenses has been developed. The proteins in the lens aqueous homogenates were precipitated by 10% trichloroacetic acid. The precipitated proteins were removed after a brief centrifugation, and the supernatant containing the cations was washed with ether and directly used for HPCE analysis. A 50 μm × 75 cm fused-silica capillary was used for separation and the detection wavelength was set at 214 nm. A 20-m*M* imidazole at pH 6.0 containing 0.1% hydroxypropyl methyl cellulose was used as background electrolyte. Sample solution was injected at 15 kV for 10 s, and the electrophoresis was carried out at 15 kV. All the cations can be separated and quantified from the peak areas within 9 min. The values obtained by this method were comparable with commonly used flame atomic absorption and flame atomic emission spectroscopy. It is demonstrated that this HPCE method can be used to quantify all the cation levels simultaneously within a short time even in a small single rat or mice lens.

1. Introduction

The mammalian eye lens is an important organ, with an unusual high protein content and unique arrangement, which provides the refractive index necessary to focus images on the retina. To achieve this the lens must be perfectly transparent. Loss of transparency or cataract is a common cause of blindness. Researchers have found that three inorganic cations K^+, Na^+ and Ca^{2+} show important metabolic fluxes in the normal lens, and especially calcium has been implicated in cataract and other lens pathology [1]. It has been proven that the Ca^{2+} level in cataract lens is much higher than that in the normal lens [2–6]. Increase in sodium and decrease in potassium levels have been reported in several types of experimental and hereditary cataract models [5–8]. The common technique used for measuring the calcium, sodium and potassium levels was flame atomic absorption (AAS) and flame atomic emission spectroscopy (AES). However, the major concerns are: (i) the AAS or AES technique can measure only one element at a time, therefore, large number of lenses are needed if the concentration of many cations such as K^+, Na^+ Ca^{2+} and Mg^{2+} needs

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to be determined; (ii) large errors could be possibly involved because the calcium level measured is close to the detection limit at least in the normal lens (if a AAS is used); (iii) the process of lens digestion and other treatment generally needs hours of time. Therefore, a simple and convenient method that can simultaneously determine all major cations in an ocular lens will be very useful for vision researchers.

High-performance capillary electrophoresis (HPCE) is the descendant of numerous electrophoresis and chromatography techniques, and has been growing rapidly since 1980s [9–12].

The separation of cations with indirect photometric detection has been demonstrated by quite a few investigators [13–17]. However, the simultaneous and quantitative analysis of these cations by HPCE in the biological samples has not been extensively investigated. Application of HPCE for quantitative determination of cations in ocular lens has never been reported.

In this paper, we report a HPCE method with indirect photometric detection being used for the simultaneous quantitation of K^+, Na^+, Ca^{2+} and Mg^{2+} in ocular lenses. The method is sensitive enough to detect all four cations in a single rat lens quantitatively. The main advantages of this method are: simple procedure and minimum sample requirement. It is much less time consuming than other available methods mentioned above. The results have been compared with AES or AAS, and they correlate with each other quite well. All the quantitative data are presented and the sources of errors are discussed.

2. Experimental

2.1. Reagents, standards and materials

All chemicals were of analytical-reagent or ACS grade unless stated otherwise. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA). Imidazole was purchased from Sigma (St. Louis, MO, USA), and hydroxypropyl methyl cellulose (HMC) [4000 C.P. (Centipoises)] was obtained from Aldrich (Milwaukee, WI, USA).

All the glasswares and plasticwares were washed carefully with 20% nitric acid and deionized water.

Standard stock solutions of cations were prepared by individually dissolving sodium chloride, potassium chloride, magnesium sulfate in deionized water. The concentration of stock solution for each was 80 m*M* K^+, 80 m*M* Na^+, 3 m*M* Mg^{2+}. The 25-m*M* stock calcium standard solution (calcium carbonate was dissolved into 2% nitric acid) was purchased from Fisher Scientific (Fairlawn, NJ, USA).

2.2. Preparation of background electrolyte

The background electrolyte (BGE) for HPCE was prepared as following: dissolve imidazole in deionized water, adjust to pH 7.2 with 5% H_2SO_4, then adjust to pH 6.0 with 1.0 *M* HCl. Dilute to the volume to make imidazole concentration 20 m*M*, then dissolve 0.1% HMC (w/v) by stirring at least for 30 min. The BGE was filtered and degassed before use.

2.3. Preparation of lens samples

Bovine, rabbit, dog and rat eyes were collected immediately after sacrificing the animals and the lenses were removed carefully, washed with 1 m*M* ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), briefly blotted on a filter paper and wet weights were recorded. Then the lenses were analyzed immediately or frozen stored at −70°C until analysis.

The U18666A [3-β-(2-diethylamino ethoxy)-androst-5-en-17-one hydrochloride] cataract in rats were developed as reported previously [6]. The steroid U18666A is a hypocholesterolemic drug and it inhibits the enzymatic conversion of desmosterol to cholesterol. A subcutaneous injection of the drug to rat pups every other day (beginning one day after birth) at a concentration of 12 mg per kg body weight can produce permanent nuclear cataracts in 2–4 weeks of age.

Bovine, dog and rabbit lenses were homogenized in deionized water (about 0.1 g wet tissue

per ml) with an all-glass Dounce homogenizer. The homogenization of rat lenses (1–3 lenses for each sample) was carried out in a 1.5-ml microcentrifuge tube with a tightly fitted pestle. A 100-μl aliquot of the homogenate was transferred into a 1.5-ml microcentrifuge tube, and the proteins were precipitated by adding 100 μl of 20% trichloroacetic acid (TCA). The mixtures were allowed to stay at room temperature for at least 30 min. Then the precipitate was removed by centrifugation, and the supernatant was washed with equal volumes of ether for four times to remove the extra TCA and to bring the pH of the final solution to about 6.0. The solution was then injected for HPCE analysis.

2.4. Equipment

The HPCE system with a UV detector was purchased from ISCO (Lincoln, NE, USA; Model 3850). A positive high voltage was applied to the capillary by maintaining the injection end at a positive high potential while the cathodic end was held at ground potential. The data were collected with a Datajet Computing Integrator (Spectra Physics, Mountain View, CA, USA; Model Datajet). The capillary columns used (Polymicro Technologies, Phoenix, AZ, USA) was 75 cm long with an inner diameter (I.D.) of 50 μm. The polymer coating was burned off at 25 cm from cathodic end of the capillary to form the detection window.

2.5. HPCE analysis

A new capillary column was pretreated as described previously [18]. Samples were injected electrokinetically at 15 kV for 10 s and electrophoresis was carried out at 15 kV. The wavelength of the detection was set at 214 nm. The data were collected and processed by the Datajet integrator. For the convenience of integration, the polarity of the integrator was reversed, so that the sample peaks shown in the following electropherograms appear as if they are absorption peaks.

2.6. Quantification

The standard addition method was used for quantitative determination of the four cations in the ocular lens samples. For each lens sample, after first injection, a 90-μl sample was mixed with 10 μl standard solution containing sodium, potassium, calcium and magnesium. The concentrations of the standard solution should be about 10 times higher than the corresponding concentration in the ocular lens sample. The calculation of the concentration for each cation was carried out by using following equation:

$$C_x = \frac{A_x \cdot V_s \cdot C_s}{A_{x+s} \cdot V_t - A_x \cdot V_x}$$

where C_x is the cation concentration in the sample; A_x is the peak area of cation in the ocular lens sample without standard addition; V_s is the volume of standard solution added; C_s is the concentration of standard solution; A_{x+s} is the peak area of cation in the ocular lens sample after standard addition; V_x is the sample volume before standard addition; V_t is the total volume of sample solution after standard addition.

2.7. Flame atomic absorption and flame atomic emission spectroscopy

To validate the HPCE method, some lens samples, in addition to the analysis by HPCE, were also analyzed by AAS or AES which are commonly used by lens researchers [6–7]. An aliquot of 1 ml of lens homogenate was taken and heated to dryness, and the dried sample was digested according to the procedures described by Bunce *et al.* [19]. After digestion, calcium and magnesium were analyzed by AAS, sodium and potassium were analyzed by AES. The instrument used was a AA-1475 series atomic absorption spectrophotometer (Varian Techtron, Mulgrave, Australia).

3. Results and discussion

Fig. 1 shows a representative electropherogram of standard mixtures of K^+, Na^+, Ca^{2+} and

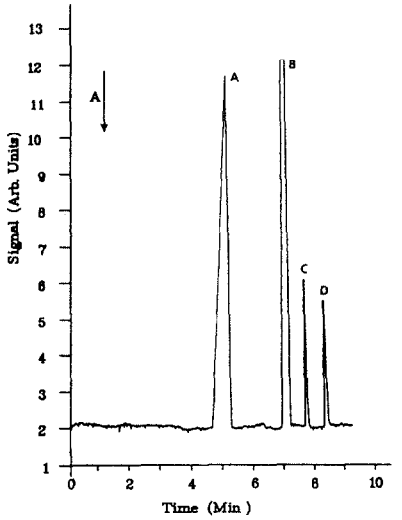

Fig. 1. The separation of four cation standards by HPCE with indirect photometric detection. A 10-s, 15-kV injection of standard solution containing K^+, Na^+, Ca^{2+} and Mg^{2+} was followed by electrophoresis at 15 kV on a 75 cm × 50 μm I.D. pretreated column (50 cm to detector). The detection wavelength was 214 nm. Running buffer: 20 m*M* imidazole–0.1% HMC with a pH of 6.0. Peaks: A = K^+; B = Na^+; C = Ca^{2+}; D = Mg^{2+}.

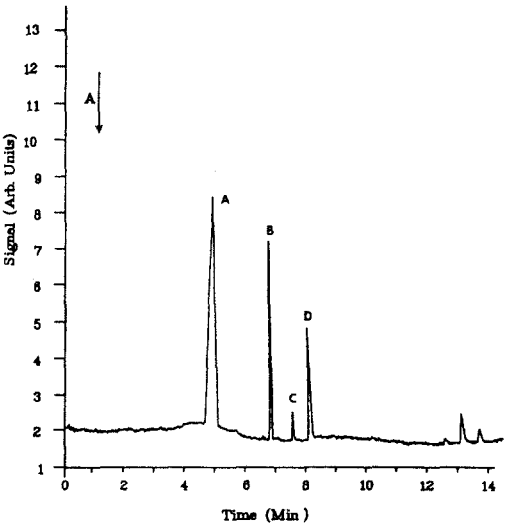

Fig. 2. The typical electrophoregram for a normal rat (20 days old) lens. The electrophoresis conditions and peak identifications are the same as those of Fig. 1.

Mg^{2+}. All four cations were separated within 9 min. The significant concentration differences of the cations in the standard mixture were made on purpose. It should be noted that in the ocular lens the concentrations of K^+ and Na^+ are much higher than those of Ca^{2+} and Mg^{2+}, therefore, the standard mixture with similar concentrations of all four cations is desirable for quantitative analysis of lens samples by the standard addition method we have employed in the present study.

A typical electropherogram for a normal rat lens sample is shown in Fig. 2. In addition to potassium, sodium, calcium and magnesium peaks, three other peaks were eluted at around 13 min migration time. Experiments have shown that the standard cations, such as Zn^{2+}, Cu^{2+}, Cd^{2+} and Pb^{2+}, were not eluted at this migration time. Identification of these peaks could not be established at this stage.

The reproducibility of the HPCE method for the quantitative analysis of these cations has been examined. The percent standard error of the mean was 2.39%, 5.15%, 2.05% and 2.49% for K^+, Na^+, Ca^{2+} and Mg^{2+}, respectively for five injections, which is good enough for quantitative analysis.

Table 1 shows the comparison of four cation levels in bovine, dog and rabbit lenses determined by HPCE and AAS or AES, and the data obtained were comparable. However, some differences in K^+ values could be noticed. These differences are possibly due to the large peak (*i.e.*, higher concentration) of K^+. In indirect detection, if the analyte concentration is too high or too low, the replacement ratio (analyte molecule to chromaphore) will change, which might influence the quantitation [20,21]. The results for K^+ should be much better if the sample were diluted further. Therefore, if the determination of the K^+ level is the major interest of study in the lens, the sample solution should be appropriately diluted for HPCE analysis.

For the drug developed cataract lenses, the calcium level was significantly elevated. A typical electropherogram for the cataract lenses is shown in Fig. 3. The levels of four cations in normal and U18666A drug developed cataract rat lens detected by HPCE are listed in Table 2. It is clear from the data that the levels of the cations in a single normal rat lens of 20 days age

Table 1
Comparison of cation levels (μmol per g wet weight) in bovine (B), Dog (D), and rabbit (R) lenses determined by HPCE, AES, and AAS ($n = 3$)

Lens sample	K^+		Na^+		Ca^{2+}		Mg^{2+}	
	AES	HPCE	AES	HPCE	AAS	HPCE	AAS	HPCE
B1	73.0	87.0	22.2	21.9	ND[a]	0.285	2.30	2.79
B2	52.9	41.6	22.1	22.9	0.235	0.162	1.83	2.73
B3	49.6	57.7	15.4	16.8	0.107	0.112	1.71	2.06
B4	61.9	62.3	15.2	19.0	0.120	0.159	2.32	2.47
B5	45.5	46.4	23.1	19.3	0.183	0.169	1.87	1.86
B6	44.8	43.3	23.4	21.7	0.184	0.197	2.13	2.80
B7	64.0	74.9	22.4	27.1	0.249	0.206	2.36	2.99
B8	64.6	66.6	22.3	23.9	0.114	0.094	2.47	1.96
R	56.0	64.3	13.8	14.9	0.610	0.490	1.73	1.70
D	60.0	60.5	36.0	30.1	0.543	0.577	3.17	2.78

[a]ND = Not determined.

(with an approximate wet weight of 18–20 mg) is very low (about 4 nmol for Ca^{2+}, 60 nmol for Mg^{2+}, 350 nmol for Na^+ and 1.2 μmol for K^+). Even with such low levels of Ca^{2+} and Mg^{2+} per lens, the HPCE method can determine all four cations simultaneously and all of them can be quantified in a single rat lens. With the AAS or AES method one can not quantitatively determine all cations by using a single rat lens. By comparing the data from normal lens with cataract lens, we can see that the calcium level was increased rapidly as the cataract developed; it is also clear that the sodium level was increased, and the potassium level was decreased. These observations are similar to those results reported earlier [6,7].

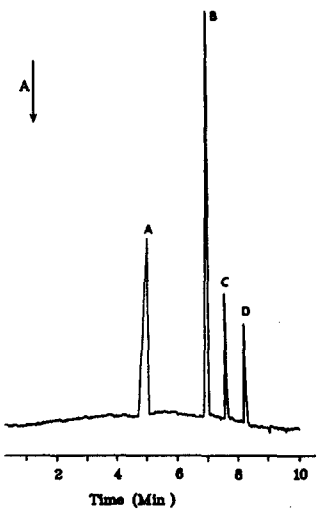

Fig. 3. The typical electrophoregram for the U18666A cataract rat lens (20 days old). The electrophoresis conditions and peak identifications are the same as those of Fig. 1.

4. Conclusion

The quantitative simultaneous determination of sodium, potassium, calcium and magnesium in an ocular lens has been demonstrated. The method is rapid, simple to use, and femtomole amounts of cations can be detected and quantified. The method should be highly suitable for lens researchers, since the cost involved in analysis is very low, time required is very short, and biological materials needed are significantly less, especially when dealing with small animal lenses like rat or mice, which are generally obtained at high cost.

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Table 2
Cation levels (μmol per g wet weight) in normal and U18666A cataract rat lens determined by HPCE ($n = 3$)

Lens sample	K^+	Na^+	Ca^{2+}	Mg^{2+}
20 Day normal	54.0	17.6	0.162	3.03
	71.5	16.8	0.247	2.78
12 Day U18666A cataract	54.1	16.7	1.90	3.93
	57.5	27.9	1.49	2.52
20 Day U18666A cataract	34.3	48.9	5.56	2.84
	42.46	63.63	3.58	2.50

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