Note

Determination of cations in tear fluid samples by non-suppressed ion chromatography with indirect UV detection

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The determination of electrolytes (such as sodium, potassium, magnesium, calcium, chloride and hydrogencarbonate) in tear fluid is of considerable clinical importance. Increased tear osmolarity is responsible for "dry eye" symptoms (ceratoconjunctivitis sicca)[1,2], which may result from a disturbed equilibrium between tear secretion and evaporation. Increased tear osmolarity has also been demonstrated in wearers of contact lenses[3] and might explain several complaints associated with contact lens wear. Further, ions of the tear film play an important role in the protection of the epithelium of the cornea[4] and corneal transparency is strongly dependent on transport processes of electrolytes between the tear film and the cornea[5].

The determination of normal values of electrolytes in the tear fluid is difficult, because during sampling the normal physiological secretion of tear fluid should be maintained and any stimulation should be avoided. These requirements can only be fulfilled if the amount to be sampled is reduced to 1–2 μl. Therefore, reliable microanalytical techniques are necessary to obtain clinically relevant data about the composition of tear fluid.

In this paper, the application of ion chromatography to the determination of cations in tear fluid is described. This technique shows some advantages over spectroscopic methods with limited amounts of sample and could therefore be the method of choice for routine work and diagnostic purposes. Further, as it is a non-destructive method, it allows the determination of anions from the same sample after cation chromatography.

EXPERIMENTAL

Instrumentation and reagents
The chromatographic instrumentation consisted of a Waters Assoc. M510 high-performance liquid chromatographic pump, a Rheodyne 7125 injection valve with a 20-μl loop and a Waters Assoc. Model 481 UV detector. An Apple IIe computer with Chromatochart software (Interactive Microware) was used for recording the chromatograms and determining peak areas.

Cations were separated on a 100 × 3.2 mm I.D. Polyspher IC CA column (Merck) with a mobile phase of 0.05 mM cerium(III) sulphate solution, prepared from cerium(III) sulphate octahydrate (Aldrich). The flow-rate was 1 ml/min. Indirect UV detection at 254 nm was used.

Chloride determination by anion chromatography was carried out on a 50×6 mm I.D. Polyspher IC AN column. The mobile phase was 0.5 mM potassium hydrogenphthalate (containing 53 mM ethylene glycol)–2-propanol (1000:27). The flowrate was 1 ml/min. Indirect UV detection at 260 nm was used.

Atomic absorption spectrometric (AAS) experiments were carried out with a Perkin-Elmer Model 2380 instrument. AAS signals were recorded by a BBC SE120 chart recorder and evaluated from their peak heights.

Determination of cations and chloride in tear fluid

Tear samples were collected by placing a disposable 2-μl glass capillary on the lower conjunctival sac, where it was kept until filled with tear fluid. After collection, the capillary was blown out into a polypropylene micro-tube containing 30 μl of 0.05 mM cerium(III) sulphate solution. The tube was centrifuged at 800 g 2 μl of this solution were further diluted with 50 μl of 0.05 mM cerium(III) sulphate solution and used for determination of sodium and potassium by cation chromatography. The remainder was used for the determination of calcium and magnesium by cation chromatography; 10 s after injection of this solution, the eluate was collected for 30 s (corresponding to a volume of 0.5 ml) and 20 μl of this fraction were injected on to the anion-exchange column for determination of chloride.

AAS analysis was carried out with 2 μl of tear fluid diluted to 250 μl (measurement of calcium and magnesium) and 20 μl further diluted to 1000 μl (for measurement of sodium) or 100 μl (for measurement of potassium). All dilutions were made with a solution containing 11.4 mM strontium chloride and 7.5 mM caesium chloride. In each instance 90 μl of the dilution were injected into the flame and wavelengths of 422.7 nm (calcium), 285.2 nm (magnesium), 589.0 nm (sodium) or 766.5 nm (potassium) were used.

RESULTS AND DISCUSSION

From the practical point of view, it was desirable to carry out the separation of alkali and alkaline earth metal ions in one run under isocratic conditions. This can be achieved with different types of cation exchangers: a silica-based polymer-coated weakly acidic cation-exchange column[6], with tartaric acid or other organic acids as the mobile phase, or a polymer-based strongly acidic cation-exchange column with cerium(III) sulphate solution as the mobile phase[7]. The latter column, with its inherently greater sensitivity than non-suppressed conductimetric detection, also allows indirect photometric detection[8]. Therefore, we chose this column for cation analysis of tear samples.

Sodium ions are present in a large excess over calcium and magnesium ions in tear fluid. Therefore, a simultaneous analysis of all ions from one sample dilution suitable for calcium and magnesium determination was not possible, because owing to overloading effects the resolution between sodium and potassium was poor. Therefore, these two ions were analysed in a second run after further dilution of the sample (with all chromatographic parameters unchanged).

Typical chromatograms of a standard and a tear fluid sample are shown in Figs. 1 and 2. As can be seen, ammonium could also be detected in the tear fluid, but this ion was not determined because it was of only minor interest for these clinical investigations.
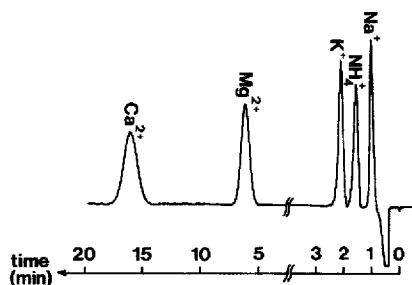
Fig. 1. Separation of a standard mixture of cations. Column, Polyspher IC CA (100 × 3.2 mm I.D.); eluent, 0.05 mM cerium(III) sulphate solution; flow-rate, 1 ml/min; indirect UV detection at 254 nm. Attenuation: 0.02 a.u.f.s.

The limits of detection (signal-to-noise ratio = 3) were about 0.07 mg/l for sodium, 0.12 mg/l for potassium, 0.25 mg/l for magnesium and 0.60 mg/l for calcium (injection volume 20 μl). For all ions linearity was found over two orders of magnitude of concentration, beginning at the detection limits (r better than 0.9995, $n = 7$); at higher concentrations peak asymmetry due to overloading of the column was observed.

Evaluation of the reproducibility, carried out with a pooled tear fluid sample, gave a relative standard deviation ($n = 5$) of 3–4% for sodium (2.62 g/l), potassium (206 mg/l) and magnesium (12.5 mg/l) and 7% for calcium (25 mg/l).

As can be seen from Fig. 2, the calcium levels in tear fluid are low, so that the analysis had to be carried out near the detection limit of the method. This might be overcome by using a narrow-bore column (1–2 mm I.D.); sensitivity should be inversely proportional to the square of the diameter, provided that the same amount of sample can be injected (which can be expected to be practicable in this instance).
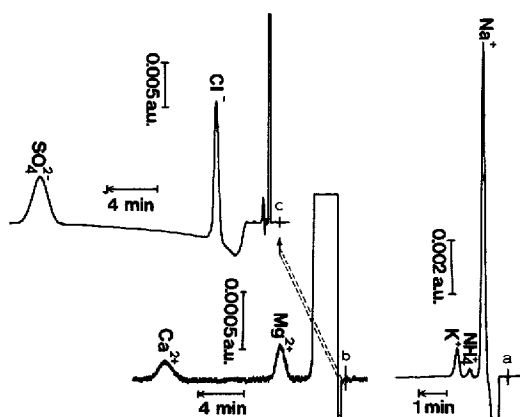

Fig. 2. Chromatograms of a tear fluid sample containing 2.62 g/l of sodium, 206 mg/l of potassium, 12.5 mg/l of magnesium, 25 mg/l of calcium and 3.94 g/l of chloride. (a) Sample diluted 1:416; (b) sample diluted 1:16; (c) anion chromatogram of the fraction collected from cation chromatography. Conditions for cation chromatography as in Fig. 1. Conditions for anion chromatography: column, Polyspher IC AN (50 × 6 mm I.D.); eluent, 0.5 mM potassium hydrogenphthalate (containing 53 mM ethylene glycol)–2-propanol (1000:27); flow-rate, 1 ml/min; indirect UV detection at 260 nm.

Prepacked narrow-bore columns were not commercially available, so work is in progress to prepare and test such narrow-bore columns.

On the other hand, the detection limits reported in the literature[7] are about one order in magnitude lower than those found in our investigations. By careful selection of a highly stable type of UV detector, the necessary improvements in detection might therefore easily be achieved [to avoid confusion, it should be mentioned that in the paper cited[7] there are erroneous data on detection limits (amounts injected) of magnesium and calcium, which should be 1 and 2 ng rather than 0.1 and 0.2 ng].

Indirect fluorescence detection is also applicable when a cerium(III) solution is used as the mobile phase. Unfortunately, the detection limits reported in the literature[9] are similar to those for indirect UV detection, so that fluorescence was not further investigated in this work.

In addition to ion chromatography, flame AAS was tested for its applicability to the analysis of electrolytes in tear fluid. In continuation of previous work[10], a flame injection technique was used after appropriate dilution of the sample. The reproducibility of this method was approximately the same as that of ion chromatography. Likewise, the sensitivity for calcium was unsatisfactory, so that in general AAS did not exhibit major advantages over the chromatographic method.

On the other hand, a disadvantage of AAS is the risk of contamination by the addition of caesium and strontium chloride, which is necessary to avoid ionization or chemical interferences in the flame. Further, AAS measures the total amount of an element, but ion chromatography allows the determination of the free ions; similarly to blood, in tear fluid calcium will be present only partly in the ionized form. In principle, speciation analysis can be done by ion chromatography, although investigations are still necessary to check how far the equilibrium between the free and protein- or complex-bound ion is maintained during chromatography. In addition, AAS is a destructive method, so that sample is lost after analysis, whereas in ion chromatography fractions of the eluate can be collected and used for anion chromatography. This is shown by the chromatogram in Fig. 2 for the determination of chloride, which elutes in the void volume of the cation-exchange column (the sulphate peak is caused by the mobile phase in cation chromatography).

The relative standard deviation of the determination of chloride in a pooled tear fluid sample containing 3.94 g/l of chloride was 1.4% ($n = 5$). The amount of chloride in the sample is high, so that chloride might be determined just as well by injecting 20 μl of the dilution used for sodium and potassium. Nevertheless, the determination of chloride in fractions collected from cation chromatography should demonstrate the compatibility of both the cation and the anion chromatographic systems. This is important for on-line column-coupling techniques for the determination of other anions present in low concentrations. Such anions must be determined in the same injection as calcium and magnesium, as the sample size is limited. In this instance on-line transfer of the whole fraction containing the anion of interest to the anion-exchange column is advisable.

In conclusion, these investigations have shown that ion chromatography is a useful microanalytical technique for the diagnosis of ceratoconjunctivitis sicca. Other routine diagnostic methods for detecting changes in tear-film osmolarity, such as the "tear mucus ferning test"[11], can be supplemented and confirmed by this chromatographic method.

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