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Note

Ion chromatographic determination of the azide ion in a prealbumin fraction from human serum*

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Quantitative assays for azide ion have been reported by spectrophotometric and oxidimetric procedures. Spectrophotometric methods[1–5] rely on the measurement of the absorbance of a metal–azide complex, while oxidimetric methods[6–9] are performed by direct measurement of the oxidation product, gaseous nitrogen, or by direct or back titration of the oxidant. Both procedures are destructive and are affected by the presence of interfering substances. Ion chromatography followed by conductimetric detection, originally described by Small *et al.*[10], has been applied to the separation and quantitation of a variety of cations[11–13] and anions[12–15]. However, its use for the determination of azide ion in aqueous solutions has not been reported. Azide ion is widely used as an antimicrobial agent for the stabilization of a variety of aqueous solutions.

In the present work, aqueous prealbumin fraction solutions, isolated from human serum in partially purified form, are stabilized for storage by the addition of sodium azide. After storage, azide ion is removed by a combination of chromatography, electrophoresis and dialysis. In this paper, we describe a sensitive chromatographic assay for residual azide in lyophilized prealbumin fractions.

Method development was initiated after attempts to assay residual azide, in the presence of protein, following the method of Brenna *et al.*[1], proved to be unacceptable for the following reasons: (a) some protein precipitated from solution on addition of ferric ammonium sulfate and had to be filtered off, thereby lengthening the procedure and increasing the likelihood of hydrazoic acid formation in the acidic solution; (b) the presence of protein in the assay solution caused an interfering absorbance background in the region from 440 to 500 nm. Consequently, a linear relationship between azide concentration and absorbance measured at 458 nm (the wavelength specified for the ferric–azide complex) could not be achieved.

EXPERIMENTAL

Equipment

The high-performance liquid chromatograph used was an SP8000B instrument (Spectra Physics, San Jose, CA, U.S.A.) comprised of a microprocessor controlled

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ternary solvent delivery system, an injection valve with 100-μl loop, column oven and data system. Chromatography was carried out on a 25 × 0.46 cm I.D. stainless-steel column packed with Vydac 302 ion chromatography packing, and ion detection was effected using a Vydac 213 conductivity detector (Separations Group, Hesperia, CA, U.S.A.).

Materials

The eluent was a solution of *o*-phthalic acid (10^{-3} *M*) in distilled deionized water (⩾ 2 MΩ resistivity) adjusted with pyridine to pH 3.5. This pH was chosen to achieve optimal resolution and peak shape of the ions of interest. Analytical reagent grade sodium azide, sodium chloride, sodium nitrite, sodium nitrate and potassium bromide were used to prepare standard solutions. The sodium azide standard solutions were prepared from a solution of 15.471 mg of sodium azide in 100 ml of water diluted to yield solutions containing 0.5, 2.0, 5.0 and 10.0 μg azide ion per 100 μl.

Procedure

Prealbumin protein mixtures (10 mg) were each dissolved in 1 ml of mobile phase buffer immediately prior to injection of 100-μl aliquots. Ion chromatography was performed at 35°C at a constant flow of 2.5 ml/min. Peak areas were determined by electronic integration and azide concentrations were determined by extrapolation from the standard calibration curve.

RESULTS AND DISCUSSION

All separations were performed following the procedures described above. A mixture containing azide ion and four potential contaminating ions, chloride, bromide, nitrite and nitrate, at concentrations of 3–6 μg/ml, was injected resulting in a chromatogram, as shown in Fig. 1. Adequate separation of azide ion from the other anions was achieved and the method is considered to be specific for azide ion

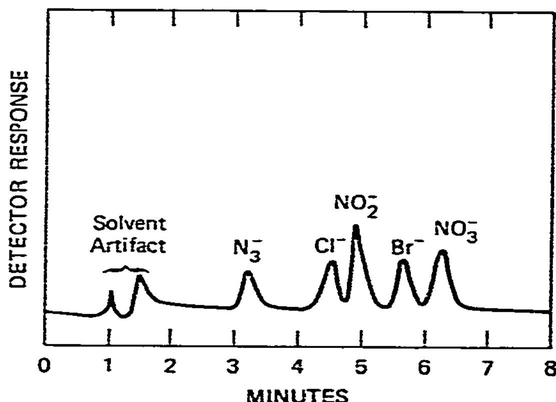

Fig. 1. Chromatogram of azide in the presence of chloride, nitrite, bromide and nitrate. HPLC conditions: column, Vydac 302 (15 μm), 25 × 0.46 cm I.D. at 35°C; eluent, 10^{-3} *M* *o*-phthalic acid in water, pH 3.5; flow-rate, 2.5 ml/min; conductivity detector, 1 $\mu\Omega^{-1}$.

in their presence. The detection limit for azide under these conditions was determined to be 0.2 μg per injection, equivalent to 2 ppm azide per 100-μl injection.

Using the azide standard solutions, a calibration curve was constructed over the range of 0.5–10 μg azide and is shown in Fig. 2. The correlation coefficient of the curve is 0.9996 with a standard error of estimate of 0.08 μg, demonstrating the linearity of the method.

The Vydac 302 ion chromatography packing consists of spherical silica particles of 15 μm mean diameter to which a quaternary amine is bonded. The lightly loaded quaternary amine bonded phase permits continuous operation without the need for regeneration of the column. Also, the low capacity of the column allows elution with low ionic strength buffers eliminating the requirement for a suppressor column. At pH 3.5, the proteins in the prealbumin fractions are protonated and are essentially unretained by the column, eluting as a single peak within one to two column volumes. Under these conditions, the proteins presented no interference and the lyophilized samples could be dissolved in buffer with no further treatment prior to injection onto the column. Fig. 3 shows a typical chromatogram of a prealbumin fraction containing both azide and chloride ions.

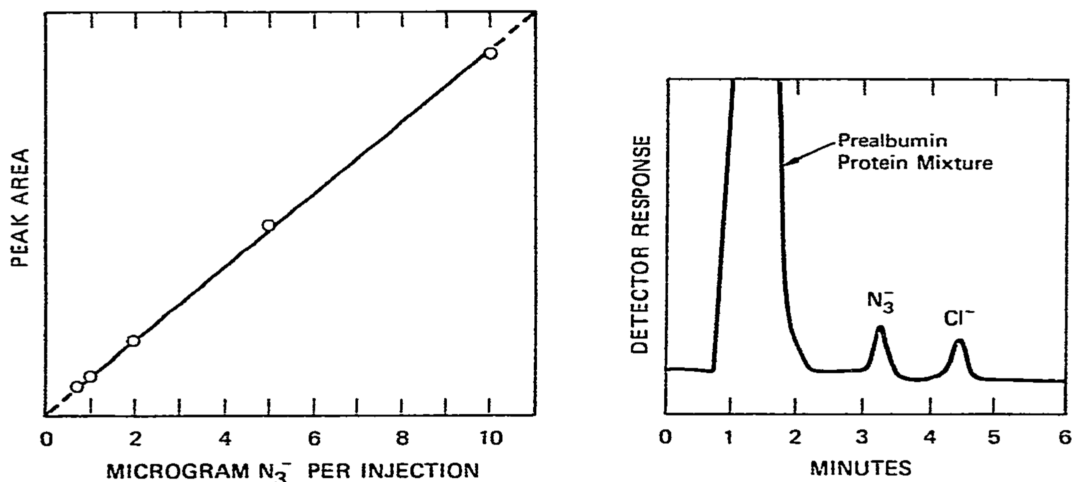

Fig. 2. Calibration curve for azide in HPLC; correlation between peak area and amount of azide as solute. HPLC conditions as in Fig. 1.

Fig. 3. Chromatogram of azide and chloride in the presence of a prealbumin fraction. HPLC conditions as in Fig. 1.

CONCLUSION

We have described a rapid and non-destructive procedure for the analysis of trace levels of azide ion in the presence of proteins. The assay procedure has been shown to be specific for azide ion and detection has been shown to be sensitive and linear. This ion chromatography procedure includes many of the advantages of high-performance liquid chromatography (HPLC): freedom from media interference, specificity in analyzing similar types of anions with high sensitivity and the potential for multiple ion determination in a single procedure.

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