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Note

Sensitive fluorescence monitoring of carbohydrates eluted by a borate mobile phase from an anion-exchange column

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An oxidative detector that relies on the reduction of reagent cerium(IV) to the fluorescent cerium(III) has recently been described for monitoring organic acids and other oxidizable compounds eluted from anion-exchange columns[1,2]. The general requirements for application of the cerate oxidative detector with separation systems are that the mobile phase transmits light at 260 nm and 350 nm, is stable in the presence of cerium(IV), and does not form interfering complexes with cerium(III) or (IV).

We have investigated the compatibility of the cerate oxidative detector with borate buffers as used for carbohydrate separation with anion-exchange columns[3] and found its use provided improved resolution and sensitivity. The existing detection methods using phenol–sulfuric acid[3], anthrone–sulfuric acid[4], orcinol–sulfuric acid[5], aniline[6], sulfuric acid[7], and others limit the usefulness of the carbohydrate separation system because of serious peak broadening and lack of relative sensitivity. A study of the carbohydrate separation system was therefore made with the cerate oxidative detector to determine the improvement in sensitivity, the extent to which the less specific detection might be a problem, and how well this detector in series with a UV detector might improve peak recognition.

Described here is the cerate oxidative monitor in its improved form as it has been adapted to the previously described carbohydrate separation system[3]. Representative chromatograms were obtained with reference compounds, blood serum and urine.

THE CERATE OXIDATIVE MONITOR

A schematic diagram of the cerate oxidative monitor as a component of a carbohydrate anion-exchange chromatograph, including a UV monitor in series, is shown in Fig. 1. The construction and operation of the separation components are essentially as described previously[2]. The UV photometer is that described by Thacker *et al.*[8]. The selection of the operating parameters for the cerate oxidative monitor are as discussed earlier[2] except for the following modifications.

* Operated for the U.S. Atomic Energy Commission by the Union Carbide Corporation.

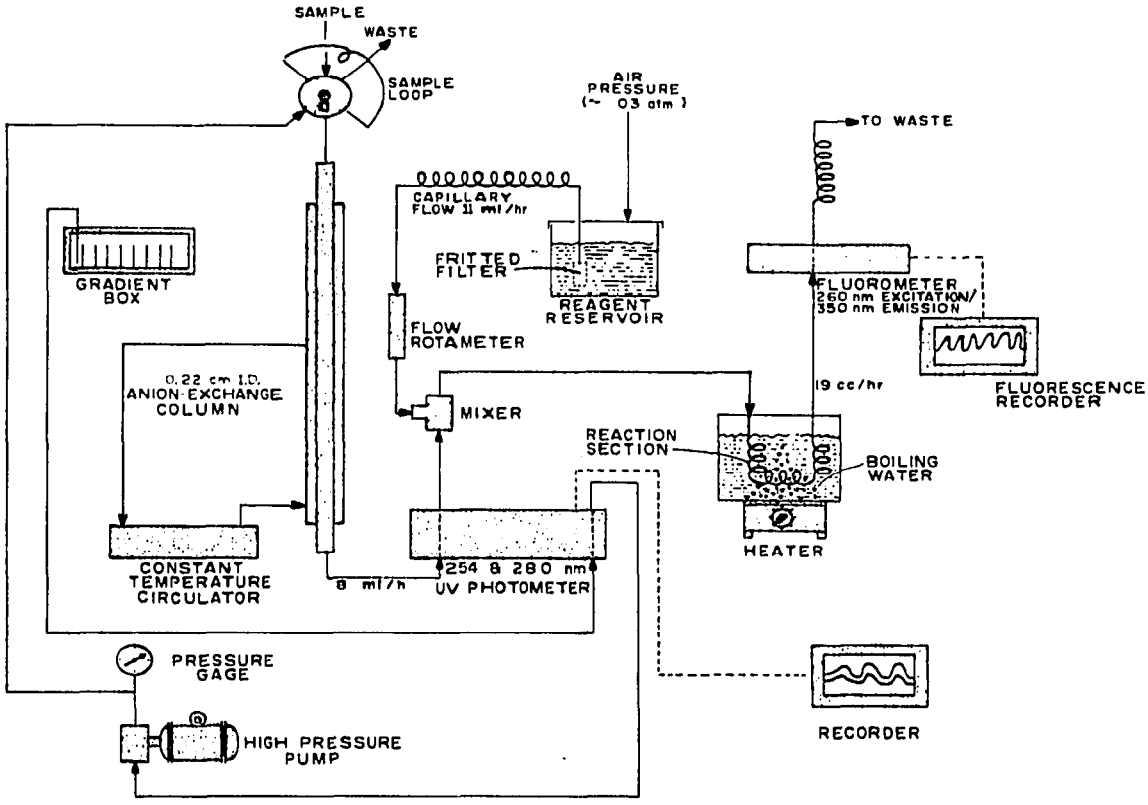


Fig. 1. Chromatographic system with UV and cerate oxidative monitors in series.

Fluorometer

The signal-to-noise ratio for the fluorometer described by Thacker[9] has been improved by reducing the internal diameter of the quartz flow cell from 3 to 2 mm, and by reducing the aperture to effect attenuation of about 100 in the excitation beam. The improvement with the smaller flow cell appears related to the interrelated optical and flow parameters. Undesirable photoreduction of the reagent cerium(IV) is avoided with the lower intensity excitation beam.

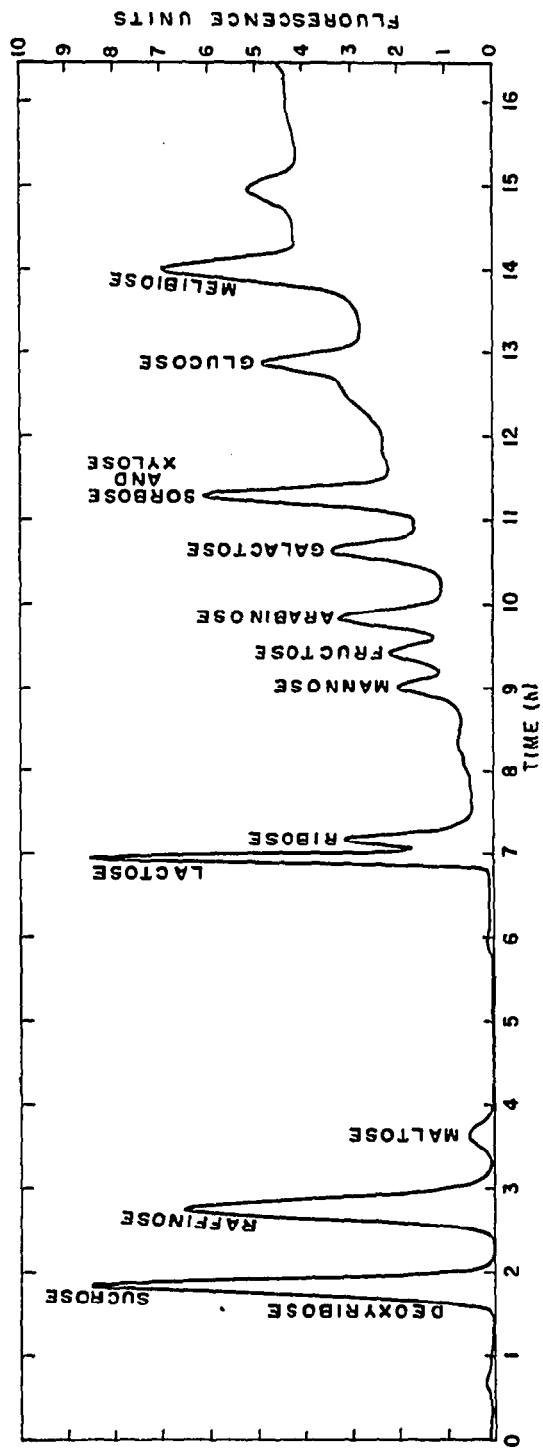
Cerium(IV) reagent solution

When very high concentration of an oxidizable compound is eluted, a signal "reversal" appearing as a flat or indented peak may occur. This peak distortion is reduced by increasing the cerium(IV) concentration to 5×10^{-4} *M*, and decreasing the sulfuric acid concentration to 1.5 mole/l. Additions of sodium bismuthate for reagent and reservoir stabilization can be in the 10 to 30 mg/l range without significant effect but larger quantities adversely affect sensitivity and peak distortion.

EXPERIMENTAL RESULTS AND DISCUSSION

Standard sample

The chromatogram for a reference standard containing 0.2 μmole/ml of each



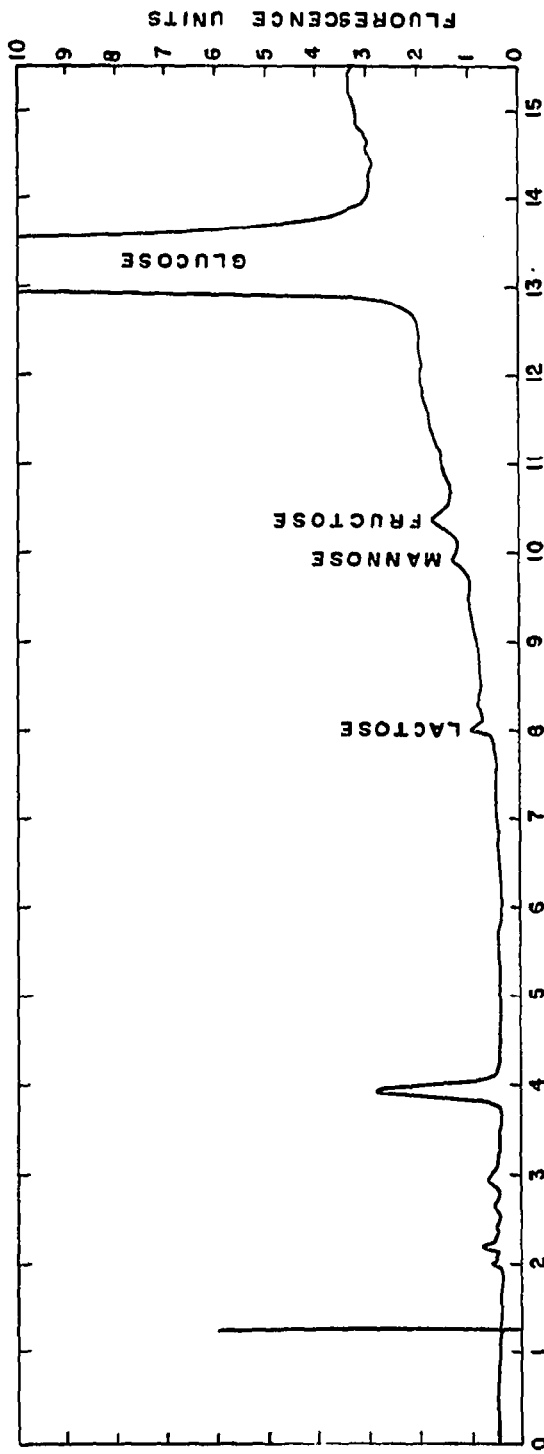


Fig. 2. Chromatogram of reference sugars (0.2 μmole/ml of each sugar in the 0.28-ml sample).

Fig. 3. Chromatogram of a pooled serum (0.28-ml sample).

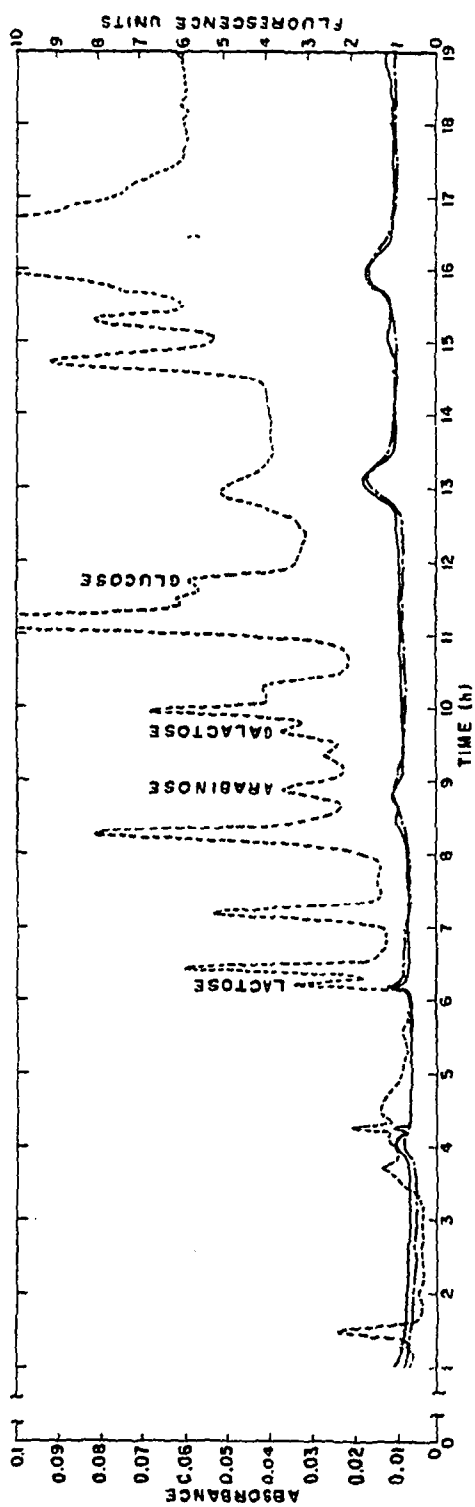


Fig. 4. Chromatogram of a standard reference urine before separation of interferences (0.28-ml sample). – – –, Fluorescence; ———, 254 nm absorbance; – · –, 280 nm absorbance.

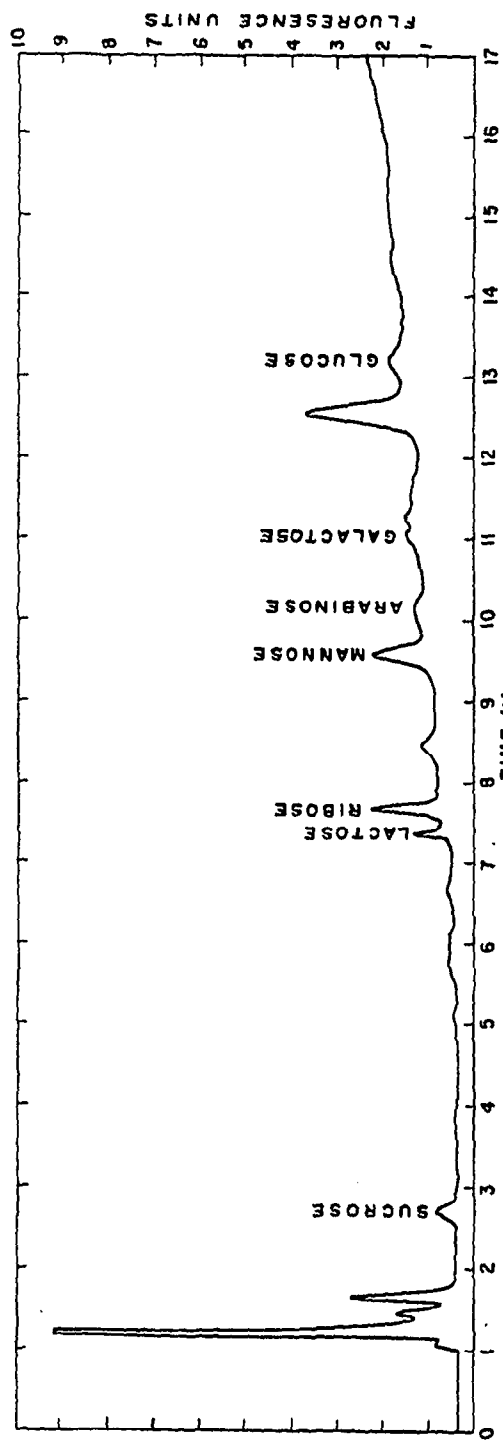


Fig. 5. Chromatogram of standard reference urine after separation of interferences by anion exchange (0.28-ml sample).

of sixteen sugars is shown in Fig. 2, using a 0.28-ml sample (0.056 μmole of each sugar). The sensitivity is improved by at least a factor of ten over that achieved in this laboratory by phenol–sulfuric acid[3] and the sulfuric acid[7] methods of monitoring. The resolution of peaks is also improved because of better mixing of the column eluate and reagent that is a result of the similarity of the density and viscosity. In addition, there is little heat of mixing as opposed to previous methods where the reagent was made up in relatively concentrated sulfuric acid and the mixing adversely affected resolution.

Blood serum

A chromatogram for a 0.28-ml sample of ultrafiltered pooled serum, shown in Fig. 3, is similar to that obtained earlier[3,7] with larger samples.

Urine

A chromatogram for a 0.28-ml sample of a standard pooled urine, shown in Fig. 4, is very complex and includes a large number of peaks, some of which show coincident UV adsorption. A chromatogram for the same size sample of this urine, in which the simple sugars and some basic and neutral constituents had been collected by anion-exchange, is shown in Fig. 5. The prior separation of the simple sugars by anion exchange can be made quantitative by the addition of an internal standard, such as melibiose, which is not present in urinary samples.

DISCUSSION AND CONCLUSIONS

The cerate oxidative detector provides better sensitivity and more effective peak resolution than other monitors for carbohydrates being eluted from chromatographic columns, but is less specific than most of the earlier detectors. The specificity is adequate for many types of samples such as the blood serums and water concentrates used as illustrations here. For very complex samples such as urine, a preliminary separation can easily be made.

ACKNOWLEDGEMENT

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REFERENCES

1 S. Katz and W. W. Pitt, Jr., *Anal. Lett.*, 5 (1972) 177.
2 S. Katz, W. W. Pitt, Jr. and G. Jones, Jr., *Clin. Chem.*, 19 (1973) 817.
3 S. Katz, S. R. Dinsmore and W. W. Pitt, Jr., *Clin. Chem.*, 17 (1971) 731.
4 L. H. Koehler, *Anal. Chem.*, 24 (1952) 1914.
5 O. Samuelson, in J. A. Marinsky (Editor), *Ion Exchange*, Vol. 2, Marcel Dekker, New York, 1969, Ch. 5.
6 E. F. Walborg, Jr. and L. E. Kondo, *Anal. Biochem.*, 37 (1970) 320.
7 S. Katz and L. H. Thacker, *J. Chromatogr.*, 64 (1972) 247.
8 L. H. Thacker, W. W. Pitt, Jr., S. Katz and C. D. Scott, *Clin. Chem.*, 16 (1970) 626.
9 L. H. Thacker, *J. Chromatogr.*, 73 (1972) 117.