CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

# Determination of Glucose and Other Reducing Sugars

## Purpose

Determination of glucose and other reducing sugars at low concentrations in biological samples containing cations and anions.

This report outlines a procedure, as described recently (1,2), for determining glucose and other reducing sugars using an amperometric detector and copper bis(phenanthroline) (CBP) as a post-column mediator. The detection scheme is based on the reduction of the metal complex (by the reducing sugar) followed by its oxidation at the surface of a glassy carbon electrode:

$$Cu(phen)_2^{2+} \xrightarrow[OH^-,\Delta]{\text{reducing sugar}} Cu(phen)_2^{+} \xrightarrow[+e]{\text{electrochemical oxidation}} Cu(phen)_2^{2+}$$

Chemical reduction

## Existing Methods

Most liquid chromatographic (LC) determinations of carbohydrates rely on the separation of the compounds of interest on either amino-bonded silica or strongly acidic cation exchange resins followed by detection with a refractive index (RI) detector. RI detectors lack sensitivity and specificity, limiting this approach to the measurement of large concentrations of carbohydrates. To improve sensitivity, various pre-and post-column reactions have been used in combination with UV or fluorescence detectors.

## Reference

The experiments outlined below were carried out in the BAS research laboratories by Lee Elrod.

## Conditions

System: LC-154 with column temperature controller
Electrode: Glassy Carbon
Potential: 0.0 V vs. Ag/AgCl

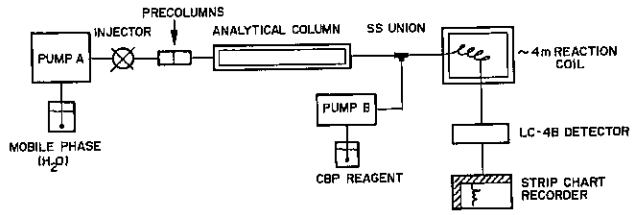


***Figure 1.*** Schematic drawing of LCEC system used for glucose determination.

Precolumns: Anion + cation (each 30 x 4.6 mm)*
Analytical Column: Strong cation exchange, calcium form (250 x 4.6 mm)*
Temperature: 85°C (analytical column)
Mobile Phase: Deionized water - Flow rate 0.25 mL/min (600 psi)
Injection Volume: 50 µL
Post Column Addition: The reagent solution consists of 2 mM CBP (prepared as the perchlorate salt, see reference 3) dissolved in 0.1 M phosphate buffer (pH 11.2). It is infused into the system at 0.85 mL/min. The mixture of column effluent and reagent solution reacts during passage through a 4-meter by 0.5 mm (ID) Teflon coil maintained at 100°C. The system is schematically presented in Figure 1. Both mobile phase and reagent solution are continuously sparged with helium for increased sensitivity.

## Comments

Typical chromatograms for reducing sugars are presented in Figure 2. In this report, pre-columns were used to eliminate cations and anions which were present in the (real world) biological samples. Pre-columns can be omitted when ions are not a problem. Using this procedure, glucose was quantitated at 50 ng/mL (2.5 ng injected) in a saline solu-

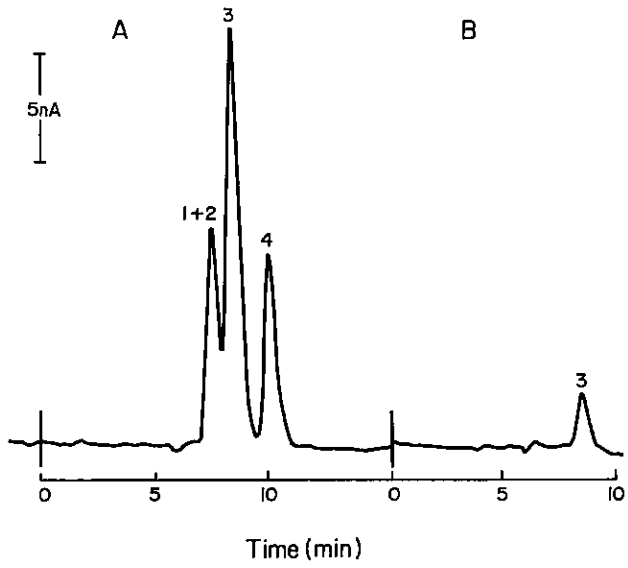


***Figure 2.*** Chromatograms of reducing sugars using the system outlined in Figure 1. A) mixed standard; 1,lactose; 2, maltose; 4, fructose (25 ng injected, each); 3, glucose (50 ng injected). B) glucose standard (5 ng, injected).

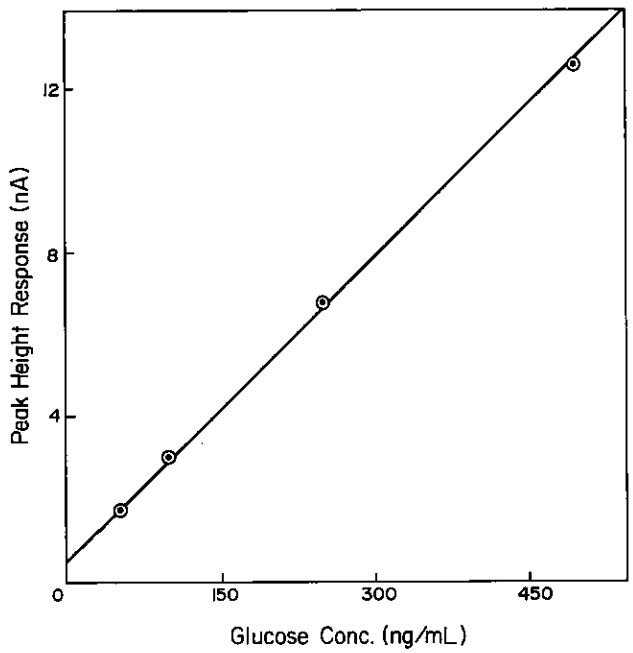


***Figure 3.*** Linearity curve for glucose. Slope, 2.41 x $10^{-2}$; Y-intercept, +0.58; correlation coefficient, 0.9997.

tion with precision (RSD of ± 13.5%; N = 10). Glucose peak-height response versus concentration is shown in Figure 3. This response is linear to at least 500 ng/mL.

* Contact BAS

**References**

*1. N. Watanabe and M. Inoue, Anal. Chem. 55(1983) 1016.*

*2. N. Watanabe , J. Chromatogr. 330(1985) 333. See Capsule 149 for a summary of this paper.*

*3. B.J. Hathaway, I.M. Procter, R.C. Slade, and A.A.G. Tomlinson, J. Chem. Soc. A, 2219 (1969).*

*4. P.G. Koski, Current Separations, 8 (1987) 26.*