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Note

Soluble Prussian blue as a reagent for determining the void volume on Sephadex columns

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The experimental measurement of the void volume is usually effected by direct measurement of the elution volume of a standard substance, which is a macromolecular substance of extremely large particle size, showing little attractive interaction with the gel, soluble in water and visually detectable. Blue Dextran 2000 (Pharmacia Uppsala, Sweden, molecular weight *ca.* 2,000,000) has frequently been used as such a standard substance.

In this work, soluble Prussian blue, which is an intense blue inorganic macromolecular complex compound, was investigated with respect to its purification and use as a standard substance. Soluble Prussian blue, $KFe^{II}Fe^{III}(CN)_6$, can be prepared by several procedures. It was produced in this work by reaction of an iron(III) salt with potassium hexacyanoferrate(II) in equimolar proportions in aqueous solution.

The resulting blue solution usually contained substances with a variety of molecular sizes and also including unchanged reactants. The solution was purified on a Sephadex G-75 column and subsequent elution with water, the intense blue fraction eluting first being the required material. The substance thus obtained, free from substances with smaller molecular sizes, can be referred to as "macromolecularly purified soluble Prussian blue". It was considered to have a molecular weight above about 3,000,000 and was found to be very suitable as a standard substance for determining the void volume of a Sephadex column easily and accurately on elution with water. As the narrow band of this reagent descends through the gel column it retains a constant thickness, clear-cut outline and intense blue colour until it reaches the bottom of the column. In all these respects it is superior to Blue Dextran 2000.

EXPERIMENTAL AND RESULTS

The gradual addition with stirring of an aqueous solution containing 4.98 g (0.018 mole) of iron(III) chloride, $FeCl_3 \cdot 6H_2O$, dissolved in 400 ml of water to an aqueous solution containing 8.45 g (0.02 mole) of potassium hexacyanoferrate(II), $K_4Fe(CN)_6 \cdot 3H_2O$, dissolved in 400 ml of water resulted in an intense blue crude solution of soluble Prussian blue. The resulting blue solution was applied to a Sephadex G-75 column, and on elution with distilled water the intensely coloured fraction that was first eluted out was recovered.

The solution of the macromolecularly purified soluble Prussian blue thus obtained gave on chemical analysis, a molar ratio of Fe to CN of 1:3.04, and it was dispersed as molecules or particles of very large size in water, making a colloidal solution which on dialysis showed no permeation of the blue substance through the cellophane film.

A 0.8-ml volume of the aqueous solution of the purified soluble Prussian blue was tested for its behaviour on application to the columns of Sephadex G-25F and Sephadex G-100 and subsequent elution with water, in comparison with Blue Dextran 2000. The results of the elution from the Sephadex G-25F column were shown in Fig. 1. The purified soluble Prussian blue descended through the gel column as an intense blue layer of thickness about 1.5 cm, maintaining its clear-cut outline without increasing its thickness until it reached the bottom of the column, and showed no tendency for adsorption on to the gel. Its behaviour was superior to that of Blue Dextran 2000, the layer of which gradually increased in thickness and lost its clear outline as it descended through the gel column; at the bottom of the column its thickness had increased to more than about 3 cm.

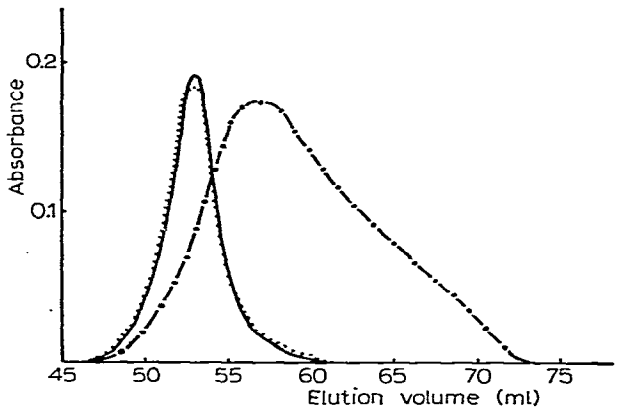

Fig. 1. Elution curve obtained on Sephadex G-25F column. Column dimensions: 2.5 × 28.5 cm. Sample volume: 0.8 ml. Eluent: water. Elution flow-rate: 131 ml/h; ———, Soluble Prussian blue; ·····, blue inorganic complex compound containing Cr; —·—, Blue Dextran 2000.

The elution volumes of the two reagents measured at the beginning of the elution of the blue layer were identical under the same conditions on the same column of Sephadex G-25F, but when measured at the mid-point of the blue layer they were different, a much higher value being obtained with the layer of Blue Dextran 2000.

The results of the comparison of the two reagents on the Sephadex G-100 column were similar to those on the Sephadex G-25F column.

DISCUSSION

The results demonstrate the advantages of macromolecularly purified soluble Prussian blue as a reagent for determining the void volume in Sephadex column chromatography with elution with water.

However, some disadvantages were also found. The purified Prussian blue was not suitable as a standard substance when using acrylamide gel, as it was adsorbed. Also, it was more easily salted-out from its aqueous solution than was Blue Dextran 2000 by the addition of potassium chloride, ammonium sulphate or phosphate salts. The former was not salted out by the first two salts up to concentrations of 0.1 *M* or by phosphate salts up to 0.2 *M*, at which concentration its decomposition occurred, whereas with Blue Dextran 2000 most of it always remained intact in the solution, accompanied by the formation of a very small extent of precipitation in the presence of these three salts at concentrations above 0.01 *M*.

The solution obtained by reaction of potassium hexacyanoferrate(II) with chrome alum in a molar ratio of 5:1 in aqueous solution gave a small amount of a blue inorganic complex compound[1], when the reaction solution was applied to a Sephadex G-25 and then a Sephadex G-50 column. This blue substance, containing K, Fe, Cr and CN, closely resembles the soluble Prussian blue in colour and properties and can also be employed to measure the void volume of Sephadex columns.

REFERENCE

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