

Table 1. Analytical data for HBP polysaccharide and subfractions.

	SO ₄ ²⁻ /aminosugar ^a molar ratio	glucosamine/ galactosamine ^b molar ratio	anticoagulant activity ^c units/mg
HBP polysaccharide	1.69	1.17	6
0.7 M subfraction	0.92	1.12	< 4
1.1 M »	2.20	0.60	< 2
1.4 M »	0.96	0.48	8
2.5 M »	1.39	4.83	12—20

Nucleotides were eluted at considerably lower salt concentrations. Adenosine 5'-phosphosulfate (APS)^{*} and adenosine 3'-phosphate-5'-phosphosulfate (PAPS)^{*} could be separated from adenosine 5'-phosphate and adenosine 5'-triphosphate at neutral pH. (Fig. 2). The recovery of nucleotides from the columns was close to 100%. Chromatography at acid pH caused some degradation of labile nucleotides but served the purpose of removing nucleotides from mucopolysaccharides.

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^{*} APS was synthesized by the carbodiimide method, Reichard, P. and Ringertz, N. R. *J. Am. Chem. Soc.* **81** (1959) 878. PAPS was synthesized enzymatically from APS + ATP with a crude rat liver enzyme. Brunngraber, E. G. *J. Biol. Chem.* **233** (1958) 472.

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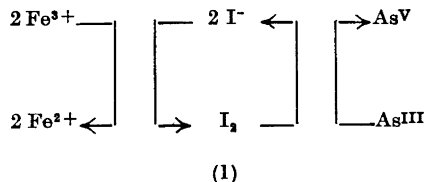
A Sensitive Colour Reaction for the Paper Chromatographic Detection of Iodide, Iodinated Tyrosines and Thyronines

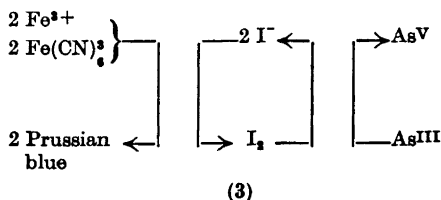
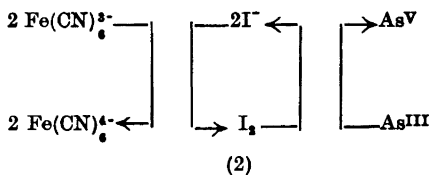
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The most sensitive chemical reaction for the detection of iodide, iodinated tyrosines and thyronines uses the catalytic action of traces of iodide upon the reduction of ceric sulphate by arsenic acid (Bowden *et al.*¹). It allows the detection of 0.1 μ g thyroxine, for instance. Modifications of the method have been proposed by Fletcher and Stanley², Dragunova and Langer³, Gawienowski⁴, and Mandl and Block⁵.

We have observed that in the presence of catalytical amounts of iodide also the systems $\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$ and $\text{Fe}(\text{CN})_6^{3-} \rightarrow \text{Fe}(\text{CN})_6^{4-}$ can be brought in reaction with the system $\text{As}^{\text{V}} \rightarrow \text{As}^{\text{III}}$ (Formulas 1 and 2). The reaction becomes especially sensitive when ferri- and ferricyanide-ions are reacting simultaneously, catalyzed by iodide, upon arsenic acid, since the formed ferro- and ferrocyanide-ions, respectively are fixed immediately, forming the insoluble pigment, prussian blue (Formula 3).





Iodide, iodinated tyrosines and thyronines appear in the ferrichloride-ferricyanide-arsenic acid (FFCA)-reaction as intense blue spots on a light blue background. The limit for the detection is 0.001 μg for iodide or 0.002 μg for thyroxine, triiodothyronine, diiodotyrosine, respectively. In practice it is advisable, however, to work with quantities between 0.01 and 0.1 μg per spot.

This means that the FFCA-reagent is about ten times more sensitive than the ceric sulphate-arsenic acid-reagent. Another advantage of the FFCA-reagent is that the paper chromatograms can be washed after the development of the spots and can be kept after drying indefinitely, while the paper chromatograms treated with the ceric sulphate-arsenic acid reagent will soon be destroyed under the corrosive influence of sulphuric acid.

Disadvantageous for the FFCA-reaction is the fact that also certain reducing substances (phenols, compounds with thiol groups, ascorbic acid, etc.) will respond to the reagent by formation of blue spots. Since these compounds are reacting, however, only in amounts above 1 μg per spot, and can be distinguished by their R_F -values, the usefulness of this new method is scarcely influenced by them.

Performance of the colour reaction. The carefully dried paper chromatograms are

sprayed evenly from both sides with freshly prepared reagent and are put cautiously and without pressure between two clean glass plates. After a developing time of 15 min the paper is thoroughly washed with distilled water, pressed between filter paper and dried in a current of warm air. The spraying and development of the paper chromatograms has to be carried out in dim light or better in a dark room.

The blue spots which have been formed by iodide, iodinated tyrosines and thyronines can be measured photometrically and can be used for quantitative determinations. Good and reproducible values could be obtained in the same range of concentrations between 0.01 and 0.07 μg per spot with the Zeiss-Extinktionsschreiber II (Filter FE 54).

Preparation of the FFCA-reagent. (All reagents used are analytical grade, Merck.)

Solution A. 2.7 g $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$ are dissolved in 100 ml 2 N HCl-solution.

Solution B. 3.5 g $\text{K}_3\text{Fe}(\text{CN})_6$ are dissolved in 100 ml distilled water.

Solution C. 5 g NaAsO_2 are dissolved under cooling in 30 ml N NaOH-solution. 65 ml 2 N HCl-solution are added under intense stirring.

Immediately before every use 5 parts of solution A, 5 parts of solution B, and 1 part of solution C are mixed. (Protection against light is important).

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Thermal Conductivity of Solid Solutions and the Measuring of Molecular Weights

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Investigations on the exact measuring of thermal conductivity of solids and certain liquids has resulted in a Report¹ and two publications²⁻³. The "hot wire resistance thermometer" method was chosen by Lars Rombén, and developed by him and H.-E. Nissen. Later, D. G. Gillam brought this method to a present accuracy of $\pm 0.3\%$. One of the goals of this investigation was to find out whether thermal resistivity could be used as a method for determining molecular weights. This seemed especially hopeful in the case of a solid solvent, owing to the high conductivity and the consequently greater and more well-defined effect of the introduction of new centres of scattering of the elastic waves through the solute molecules.

Accurate values have recently been reported by Gillam on the conductivity at 20°C of naphthalene and of four substances dissolved in naphthalene; diphenylmethane, triphenylmethane, diphenyl-*m*-tolylmethane, and diphenyl-*p*-tolylmethane⁴. The results clearly show that in all four solutions the conductivity decreases along one and the same function of the mole fraction. This function is a straight line up to a mole fraction of about 0.03. This is all what is needed for a *method of molecular weight determination* according to a relationship rather analogous to the ones valid for osmotic pressure and light scattering. The validity range of such a simple course will have to be settled by further experimental work. As in the other me-

thods, a more detailed theory will be needed in less ideal cases; a lack of ideality will express itself quite differently when going from one method to another. An essential factor is, in the case considered, the temperature dependency. As a whole, the use of rather low temperatures and solid solvents with high conductivity will give the simplest information. The field of liquid solutions also deserves increased attention.

The determination of molecular weights only represents one aspect of the many possible on the thermal conductivity of mixtures in general. With the exception of the well-known work by Eucken⁵ on solid solutions of KCl, KBr, the effects on solids seem to have been mainly referred to as a high sensitivity of the conductance to "impurities". However, the views expressed above, originating from the general ideas of Debye and Eucken, are related to recent, mainly low-temperature investigations on the scattering cross sections of isolated lattice points and on isotope effects, and by the recognition of resistivity to heat conduction as a very sensitive means of studying crystal imperfections, see Berman⁶, Klemens⁷, Slack⁸, and Cohen⁹.

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