

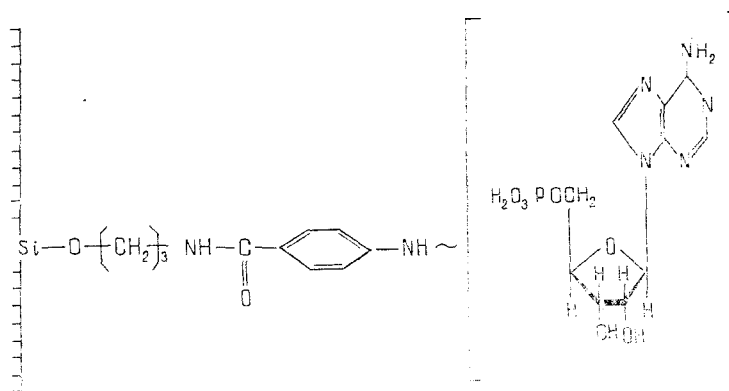
# DETERMINATION OF ADENOSINE 5'-MONOPHOSPHATE IMMOBILIZED ON SILOCHROME FROM THE PHOSPHORUS CONTENT

V. A. Karklinya, I. É. Vilks,  
and B. V. Alberta

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In the last decade, great interest in the field of biochemistry has been caused by the use of various biologically active compounds, including nucleosides and nucleotides, immobilized on water-insoluble supports [1]. This, for example, Silochrome with immobilized adenosine 5'-monophosphate (5'-AMP) is used as a group-specific sorbent for affinity chromatography in the isolation and purification of enzymes [2]. Macroporous modified silica with aminoaryl groups is used for the immobilization of 5'-AMP.

The immobilization product has the following structural formula



For the quantitative determination of immobilized 5'-AMP we have developed a method of analysis based on determining the phosphorus content, which is one of the criteria for characterizing the immobilization product. With this aim we have used a modification of the Fiske-Subbarow method [3] which permits phosphorus to be determined in the presence of silicon.

The quantitative determination was carried out in the following way. To construct a calibration graph, 0.2- to 1.5-ml portions of a standard solution of  $\text{KH}_2\text{PO}_4$  with a concentration of 100  $\mu\text{g}$  of P/ml were introduced into 50-ml measuring flasks, to each was added 1.0 ml of 10 N sulfuric acid, the volumes of the solutions were brought up to the marks with distilled water, and each was mixed. The standard working solutions prepared in this way contained 0.4-3.0  $\mu\text{g}$  of phosphorus.

Then, 2.0 ml of solution was taken from each flask and was transferred to a 20-ml test tube, to which 0.5 ml of 10 N sulfuric acid, 4.4 ml of distilled water, 0.2 ml of 5% ammonium molybdate solution, 0.2 ml of Eikonogen were added. The test tubes were placed in a boiling water bath for 7 min, and then they were removed and cooled to room temperature. The optical densities were measured in an SF-16 spectrophotometer at a wavelength of 830 nm with respect to a blank sample. The calibration graph was constructed in the coordinates of concentration of P,  $\mu\text{g}/\text{ml}$  (abscissas) versus optical density (ordinates).

To perform an analysis, 20-30 mg of the preparation was transferred to a 20-ml test tube, 1 ml of 10 N  $\text{H}_2\text{SO}_4$  was added, and the mixture was left in the thermostat at 150-160°C. After 5-6 h, the test tube was removed and cooled to room temperature, and 2-3 drops of perhydrol was added. After this, the test tubes were again placed in the thermostat and were kept there until decoloration was complete. After cooling, the contents of the test tubes were transferred quantitatively into a 25-ml measuring flask, made up to the mark with dis-

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TABLE 1. Comparative Results of the Determination of 5'-AMP Immobilized on Silochrome from the Phosphorus and the Ribose Contents

Experiment No.	From the phosphorus content by the proposed method		From the phosphorus content by the orcinol method		Difference between the methods, $\pm \mu\text{mole/g}$
	$\mu\text{mole/g}$	rel. stand. dev., $S_{\eta}$ , %	$\mu\text{mole/g}$	rel. stand. dev., $S_{\eta}$ , %	
1	28.8	3.9	26.7	2.7	2.1
2	23.3	4.8	20.4	2.9	2.9
3	31.8	4.7	28.7	2.5	2.9
4	26.5	2.8	27.7	1.8	1.2
5	20.0	2.0	21.1	2.4	1.1

tilled water, and mixed. An 8.0-ml portion taken from the flask with a pipette and the subsequent procedure was the same as in the construction of the calibration graph.

The amount of 5'-AMP (in  $\mu\text{mole/g}$ ) was calculated from the formula

$$X = \frac{C \cdot V \cdot 1000}{G \cdot M},$$

where C is the concentration of phosphorus found from the calibration graph at the corresponding optical density,  $\mu\text{g/ml}$ ; G is the weight of preparation analyzed, mg; V is the volume of the measuring flask taken for diluting the sample, ml; M is the molecular weight of phosphorus ( $M = 30.97$ ); and 1000 is the coefficient for converting milligrams into grams.

The results obtained were compared with the results of the determination of 5'-AMP from the amount of ribose by the orcinol method [4] modified for the determination of 5'-AMP in Silochrome. The results are given in Table 1.

The sensitivity of the proposed method is 0.4  $\mu\text{g P/ml}$  and the relative standard deviation 4-5%. The recommended lower limit of determination is 5  $\mu\text{mole}$  of 5'-AMP per 1 g of Silochrome. The method can be applied to the determination of the amounts of other nucleotides immobilized on Silochrome.

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