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ACTIVATION OF ANGREN KAOLIN – AN ADSORBENT FOR SEPARATION OF AMINO ACIDS BY THIN-LAYER CHROMATOGRAPHY

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After heat treatment (at 800-850°C, 4 h) and acid activation (20% HCl, 4 h), Angren gray kaolin is not inferior in its chromatographic activity to adsorbents widely employed in TLC.

The results of the separation of a number of natural compounds in layers of silica gel–gypsum and of activated kaolin–starch show that the latter possesses a higher separating capacity and sensitivity.

To determine the activity of activated Angren kaolin we have determined the R_f values of standard azo dyes: azobenzene and Sudan I. For comparison, we give the R_f values of azobenzene and Sudan I on alumina (Brockmann activity II) and a fixed kaolin–starch layer (the R_f values on alumina are taken from the literature):

Adsorbent	Azobenzene	Sudan I
Alumina	0.59	0.01
Kaolin–starch	0.60	0.12

We have studied the chromatographic separation of amino acids in a thin layer of kaolin–starch. The methods of preparing the plates with a fixed layer corresponded to those given in the literature [1-3]. The most suitable solvent system for such separation is butan-1-ol–acetic acid–water (4:1:1). The revealing agent was a 1% solution of ninhydrin in acetone.

Below we give the R_f values of a number of amino acids in a layer of silica gel–gypsum and in a layer of kaolin–starch (the R_f values in the silica gel–gypsum layer have been taken from the literature [1, 2]; * denotes a hydrochloride):

Amino acid	Silica gel–gypsum*	Kaolin–starch
Alanine	0.27	0.54
Aspartic acid	0.21	0.32
Leucine	0.47	0.87
Valine	0.35	0.70
Threonine	0.25	0.45
Serine	0.22	0.17
Tryptophan	0.56	0.69

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Proline	0.19	0.27
Methionine	0.40	0.15
Glutamic acid	0.27	0.22
Phenylalanine	0.49	0.16
Isoleucine	0.46	0.63
Histidine*	0.06	0.08
Lysine*	0.09	0.06
Arginine*	0.08	0.12

A mixture of alanine, threonine, serine, and aspartic acid, which it is impossible to separate in a silica gel–gypsum layer, is clearly separated in a kaolin–starch layer. The separation of a mixture of leucine, methionine, and valine in a layer of silica gel–gypsum is difficult because of the close values of their R_f values, but in a kaolin–starch layer their separation takes place very clearly.

Thus, the readily available and cheap Angren kaolin can fully replace the industrial adsorbents used for the separation of amino acids by the TLC method, and this all the more because the activation of kaolin is not particularly difficult.

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USE OF BIOSPECIFIC ADSORBENTS WITH IMMOBILIZED POLYPEPTIDE FRAGMENTS OF THE COLLAGEN MOLECULE FOR AFFINITY CHROMATOGRAPHY OF FIBRONECTIN

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We have synthesized biospecific adsorbents with the α1- and α2-chains and β_{11}- and B_{12}-components of type I collagen and also with the α1CB7 and α1CB8 cyanogen bromide peptides of collagen immobilized on Sepharose. The study of the properties of this type of adsorbents is of interest both for increasing the efficiency of the affinity chromatography of fibronectin from the point of view of the capacity of the sorbents and the purity of the desired preparation and also for investigating the mechanisms of the biospecific interaction of collagen with biopolymers having an affinity for collagen (fibronectin, collagenase, etc.).

Collagen was separated into the α-chains and β-components by the known procedure [1] of the ion-exchange chromatography of previously denatured rat skin collagen on cellulose CM-52. To obtain the α1CB7 and α1CB8 cyanogen bromide peptides of collagen, fragmentation of the fraction containing the α1-chain and the β_{11}-fragment of collagen was performed by means of cyanogen bromide in 70% formic acid followed by ion-exchange chromatography on cellulose CM-52 [2]. The above-mentioned polypeptide fragments of the collagen molecule were immobilized on cyanogen–bromide-activated Sepharose by a standard procedure [3]. The amounts of immobilized peptides in the absorbents obtained were determined from the amounts of hydroxyproline in aliquots of the adsorbents hydrolyzed with 6 N HCl; they ranged between 0.6 and 1.7 mg/ml of gel.

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