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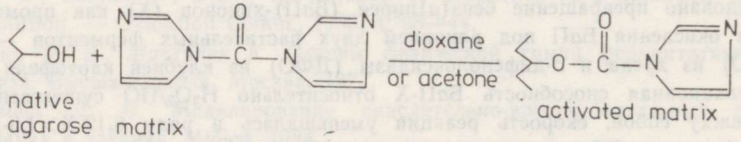
Merike VAHER, Reet KOLJAK*, and Kalle TRUUS**

ACTIVATION OF AGAROSE MATRIX WITH 1,1'-CARBONYLDIIMIDAZOLE IN VARIOUS CONDITIONS

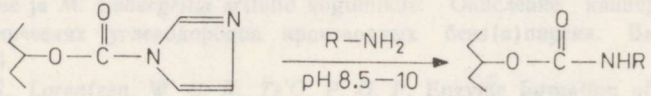
Merike VAHER, Reet KOLJAK, Kalle TRUUS. AGAROOSMAATRIKSI AKTIVEERIMINE 1,1'-KARBONÜÜLDIIMIDASOOLIGA ERINEVATES TINGIMUSTES

Мерике ВАХЕР, Реэт КОЛЬЯК, Калле ТРУУС. АКТИВАЦИЯ МАТРИЦЫ АГАРОЗЫ 1,1'-КАРБОНИЛДИИМИДАЗОЛОМ В РАЗНЫХ УСЛОВИЯХ

The extensive application of affinity chromatography to the separation of biopolymers has been due greatly to the cyanogen bromide activation method of the agarose chain [1] as a key process for producing most widely spread bioselective sorbents. But the method has serious drawbacks: the matrix receives additional charges from basic isourea groups which can affect the biological specificity of the column [2, 3]. Nowadays there are many other activation methods (with N-hydroxy succinimide, epoxides, active dyes, etc.), but all of them are inferior to the 1,1'-carbonyldiimidazole (CDI) treatment which gives a high-capacity matrix free of interfering charge effects [4]:



In addition, the CDI-agarose couples in the aqueous as well as the organic phase and forms (in mild conditions for proteins) a leak-resistant N-alkyl carbamate linkage with N-nucleophiles [5]:



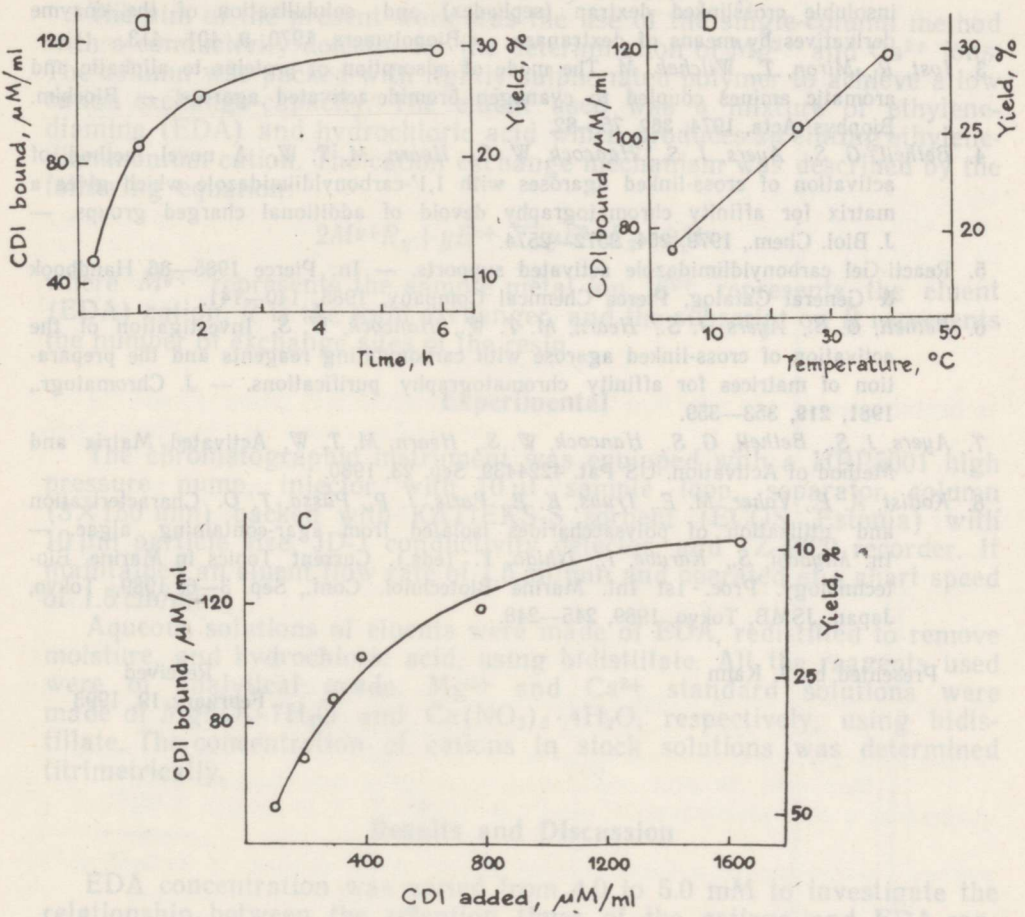
Although other analogous carbonylating reagents for agarose activation are available [6, 7], the possibilities and limits of obtaining suitable imidazolylcarbamate ligand density of the matrix have not been elucidated. These problems are discussed in the present paper using the agarose matrix from *Ahnfeltia tobuchiensis* (the Sea of Japan, the Pacific) [8].

* Eesti Teaduste Akadeemia Keemia Instituut (Institute of Chemistry, Estonian Academy of Sciences). Akadeemia tee 15, EE-0026 Tallinn. Estonia.

Experimental

Materials. Beaded agarose, 4%, and cross-linked, 6%, was a product of the Pilot Production Plant of the Institute of Chemistry (Tallinn, Estonia). 1,1'-Carbonyldiimidazole (m. p. 118—120 °C) was purchased from Aldrich Chemical Co. (WI, USA). 1,4-Dioxane (Reakhim, CIS) was redistilled twice: first over KOH and then over a Na wire.

Activation procedure. Beaded agarose (3 g of moist cake) was washed sequentially with water; dioxane/water, 3:7; 5:5; 7:3; and dried dioxane (50 ml of each) and suspended in dried dioxane (5 ml). CDI was added and the suspension was mixed using a Heto end-over-end mixer (Denmark). The quantity of CDI, time and temperature were independently varied (Figure). Activated gel was thoroughly washed with dried dioxane (100 ml). The content of the bound groups was determined by elemental analysis using a Hewlett Packard 185 CHN analyser (USA).



The CDI binding capability of agarose matrix. Dependence on time (a), temperature (b), and the quantity of reagent added (c):

a — 396 μM CDI per 1 ml of gel added, reaction temperature 25 °C;
b — 395 μM/ml CDI added, reaction time 2 h;
c — binding of CDI (various quantities) to matrix. Reaction time 2 h at 25 °C.

Results and Discussion

The capability of activation of a 4% agarose gel is about 140 μM/ml (Fig., c), while the yield is very low (8.5%). In case of a 6% cross-linked gel this value is 227 μM/ml (2 hours at 40 °C, yield 21%). As may be supposed, the steric conditions will improve at higher temperatures: the yield increases linearly at 4—40 °C (Fig., b), which are the limits for operating with agarose gels.

According to Fig., a, the high activation level (and good yield) at 25 °C was achieved very slowly: the maximum of activation was obtained after 6 hours only.

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