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Chromatographic purification and super-purification of biologically active compounds using heteroreticular and composite ion exchange resins at low pressure

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Abstract

The proposed methods of isolation, separation, and fine purification of biologically active compounds (BAC) are based on a theoretical analysis of sorption dynamics and chromatography and the use of a new type of biosorbents (heteroreticular polymer ion exchangers and composite sorbents of the Cellosorb type). Fine separation is carried out by a directed parallel shift in the thermodynamic and kinetic parameters in the sorbent-sorbate system with a stepwise change in the properties of the eluting solutions.

FUNDAMENTAL PRINCIPLES OF SHARP BOUNDARY FORMATION

The analysis of selective column sorption and selective elution by our methodology is based on the formation of sharp boundaries of chromatographic zones of the compounds (Refs. 1,2,3). In order to determine the conditions of the formation of sharp boundaries, it is convenient to use the dimensionless criteria Λ, Λ, Λ_1, and Λ_2, which predict the experimentally confirmed conditions of complete saturation of the column or its part and complete elution when Λ (Λ, Λ_1, Λ_2) = 1 + 4. This range depends on the type of the isotherms of interphase substance distribution: highly selective sorption, linear or intermediate type isotherms. A dimensionless parameter λ is introduced for sorbent grains that sorb substances throughout the mass at linear or rectangular isotherms under the conditions of gel (internal) limitation due to the kinetics of heterogeneous mass exchange:

$$\lambda = 3(1-\varepsilon)\frac{\mathcal{D}}{R^2 V} K d L \qquad (1)$$

For surface-layer (or pellicular) sorbents under similar conditions the value of Λ is used:

$$\Lambda = \frac{1+\varsigma+\varsigma^2}{1+3\varsigma+6\varsigma^2+5\varsigma^3}\lambda \qquad (2)$$

In these two equations ε is the sorbent porosity (free volume fraction), $\mathcal{D}$ is the effective quasidiffusion coefficient (in the sorption or desorption processes) in sorbent grains, R is the sorbent grain radius, V is the flow rate of the solvent through the column, L is the height of the sorbent layer, and $\varsigma = 1 - \ell/R$ where ℓ is the thickness of the sorbing layer. For the mixed diffusion kinetics (limiting role is determined by the intradiffusion process in grains and by surface diffusion) a generalized parameter Λ_1 is introduced. In contrast to the values of λ and Λ, it is difficult to calculate from the kinetics thermodynamics and geometry of the sorbentliquid phase system under investigation, but it is easy to estimate est: mate Λ_1 from the dynamic parameters of the column process taking into account the same factors in column experiments:

$$\Lambda_1 = \frac{t_{eq} - t_0}{5\bar{t}_1} \qquad (3)$$

where t_{eq} is the equilibrium elution time of the sharp boundary from the column, t_o is the elution time of the free volume of the column, the mean sorption time $\bar{t}_1 = \int t dF$ (F is the degree of saturation of the sorbent with the sorbate). Finally if the longitudinal diffusion coefficient $\mathcal{D}_\ell$ is taken into account, the generalized criterion Λ_2 is given by:

$$\Lambda_2 = \frac{2(t_{eq}-t_o)}{5\bar{t}_t^2} \qquad (4)$$

where $\bar{t}_t^2 = 2\left[\Sigma \bar{t}_1^{(s)} + \alpha \mathcal{D}_\ell \left(\sqrt{(1-\alpha)\bar{C}_\infty/\alpha C_\infty} + 1/\sqrt{\alpha C_\infty/(1-\alpha)\bar{C}_\infty}\right)^2/v^2\right](t_{eq}-t_o)$ (5)

Here $\bar{t}_1^{(s)}$ is the average sorption time, C_∞ and $\bar{C}_\infty$ are the limiting equilibrium substance concentrations of the substance in solution and in sorbent grains, respectively. Experimental investigations (ref.3) have shown that for the effective chromatographic processes that occur on sorbents on which the sorption and desorption rate is high, it is sufficient to use the value of Λ_1 and only in some cases to take into account the value of Λ_2. Thus, Fig.1 shows elution curves for the chromatographic separation of thiamine mono- and diphosphate on a highly permeable CS KU-2 cellosorbent. Each component is eluted from the column in the form of a highly concentrated zone only at $\Lambda_1(\Lambda_2) > 1$. At $\Lambda_1(\Lambda_2) < 1$ the component virtually does not move along the column (only slight spreading of the zone is observed). For complete separation of the components the column should be filled on the level of 25-75%.

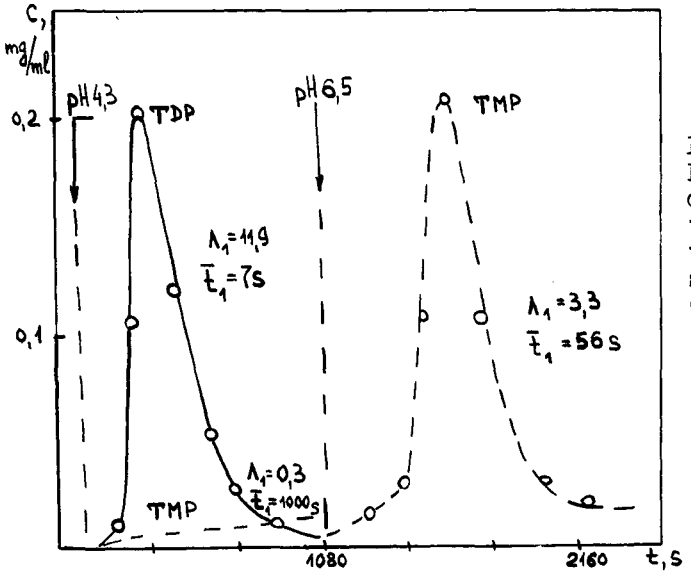


Fig. 1.
Elution curves of thiamin-diphosphate (TDP) and thiaminmonophosphate (TMP) using composite type sulfostyrin ion exchanger CS-KU-2.

PREPARATIVE CHROMATOGRAPHY USING HETERORETICULAR BIOSORBENTS

The relationship between the sorption capacity and diffusion coefficients as factors that simultaneously favour the effective and economically advantageous process of organic ion isolation is illustrated in Fig. 2 and Table 1. This figure shows the effect of the amount of the crossagent introduced on the sorption properties of the antitumoral antibiotic daunorubimycine. Heteroreticular inhomogeneous structures of biosorbents appear in the process of precipitation copolymerization upon the introduction of a considerable amount of the crossagent (Refs. 4,5). It is the right-hand part of the curve in Fig. 2 that corresponds to heteroreticular biosorbents which can absorb organic ions not only reversible but also at a high sorption capacity. On the heteronetworks the degree of desorption of daunorubimycin approaches 100%, whereas on gel ion exchangers with the same composition and exhibiting the same sorption capacity (left-hand branch of curve in Fig.2) the degree of desorption of the antibiotic does not exceed 10-15%. We observe here the non-traditional increase in sorption capacity Fig.2, the right hand branch with increasing degree of crosslinking.

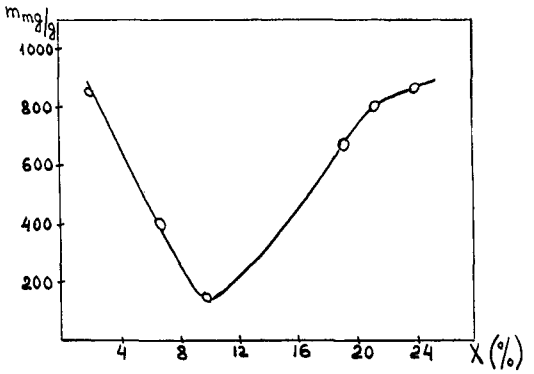


Fig.2. Dependence of sorption capacity (m) of daunorubomycin on percentage (x) of crossagent using biosorbent BD-M-A (copolymer of methacrylic, acrylic acids and ethylene glycol dimethacrylate).

TABLE 1. Diffusion coefficients as a function of percentage (X) of the crossagent Biosorbent BD-MA grains. (Sorption on daunorubomycine)

% X	$\mathcal{D}_{cm^2s^{-1}}$
2	7.2
6	3.6
10	2.2
15	2.3
21	6.3
24	7.1

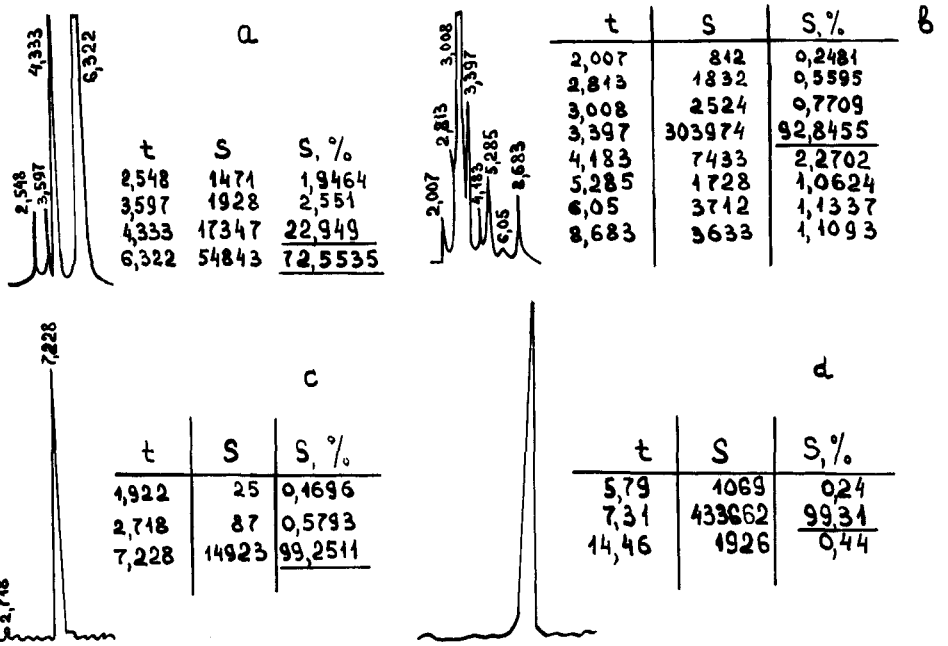


Fig.3. HPLC of daunorubomycine (a,c) and doxorybomycine (b,d) after the first (a,b) and second (c,d) stages parification and superparification using heteroreticular biosorbent.

The isolation of an antitumoral antibiotic, daunorubicine from the native solution and that of doxorubicin from the reaction mass on a heteroreticular sorbent Biosorbent BD-M-A Anthrasorb (9) during preparative up-scaled industrial chromatography is very effective and gives very cheap products (Ref.6). Fig.3 shows the chromatograms of antibiotics after the first stage of (3a and 3b) and after the second stage of chromatographic superpurification on the same biosorbent (3c and 3d). The second stage is distinguished by small shifts in the parameters of the eluting solvents, which leads to the formation of preparations with a high degree of purity that attains 99.2-99.5%. The loading of the column is on the level of 500 mg of antibiotics per gram of sorbent.

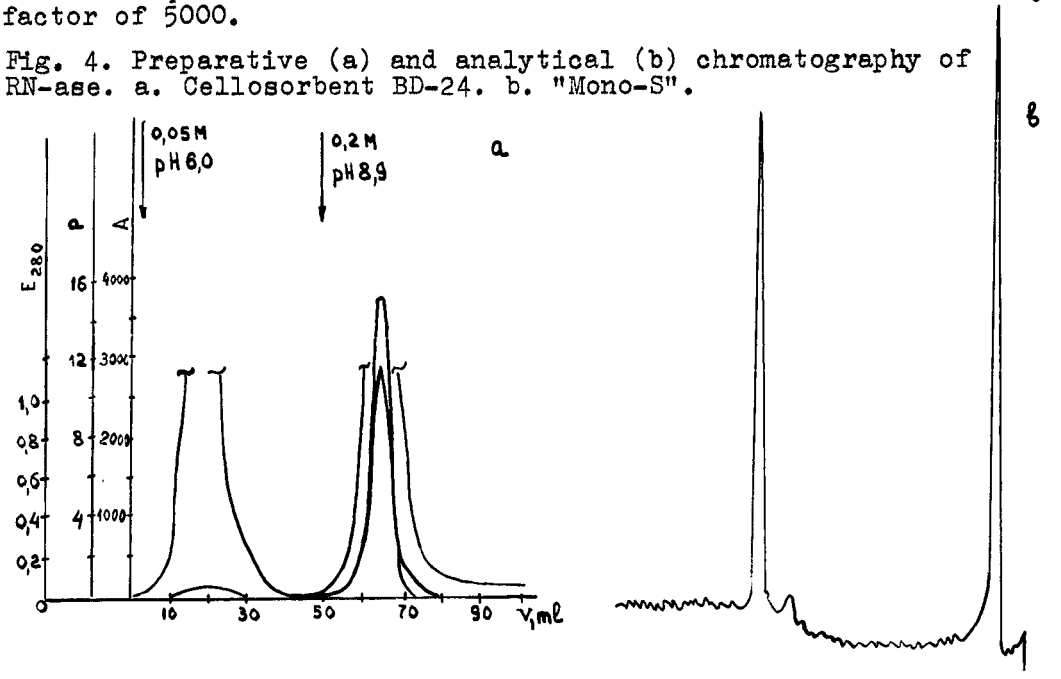
PREPARATIVE CHROMATOGRAPHY USING COMPOSITE BIOSORBENTS

One of preparative separation processes with composite biosorbent is demonstrated in Fig.1.

It should be emphasized that the transition to selective desorption on selectively sorbing ion exchangers in accordance with the above analysis (eqs. 1-5) takes place only at a high rate of heterogeneous mass-exchange. High values of coefficients of gel (internal) diffusion for the sorption of organic ions have been obtained on composite sorbents (cellosorbents the grains of which are formed by inserting the micrograins of the exchanger into an inert cellulose matrix during synthesis (Refs. 7,8,9). In this case the effective diffusion coefficient for organic ions increases 10-50 times as compared to that for initial exchangers introduced into the composite in the form of micrograins. The effective separation of standard RN ase preparations (manufactured for agricultural purposes) on CS BD-24 cellosorbent is shown in Fig. 4. Cellosorb CS BD-24 is a composite including cellulose matrix and micrograins of a copolymer of methacrylic acid and ethylene glycoldimethacrylate.

The comparison of Figs. 4a and 4b is particularly interesting because at a virtually completely similar component separation the load on columns of equal volumes in the former case exceeds that in HPLC by a factor of 5000.

Fig. 4. Preparative (a) and analytical (b) chromatography of RN-ase. a. Cellosorbent BD-24. b. "Mono-S".



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