

**EFFECT OF THE CONCENTRATION OF
5,5'-DITHIOBIS(2-NITROBENZOIC ACID) ON PARAMETERS
OF THE KINETICS OF ITS CHEMISORPTION ON
THIOL DERIVATIVES OF CELLULOSE**

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Equations describing the dependence of parameters of sorption kinetics on the sorbate concentration have been determined. The validity of the equations has been verified for the chemisorption of 5,5'-dithiobis(2-nitrobenzoic acid) on bead O-(2-mercaptoethyl)-, O-(3-mercapto-2-hydroxypropyl)- and O-[2-(4-mercaptophenylsulfonyl)ethyl]cellulose. Isothermic constants obtained from the equations can be calculated also under experimental conditions unfavourable for their determination. These constants may be utilized for characterizing relations between the chemical structure of cellulose derivatives and the sorption process. The equation which provides a complete time-concentration description of sorption is suggested.

In our preceding paper¹ the validity of empirical equations (1a,b) which originally characterized the chemisorption kinetics of thiols on formylphenyl derivatives of cellulose²,

$$B = B_e t (t + t_{0.5})^{-1}, \quad (1a)$$

$$B^{-1} = B_e^{-1} + t_{0.5} B_e^{-1} t^{-1}, \quad (1b)$$

where B , B_e are the amounts of the compound sorbed at the times t and $t \rightarrow \infty$ respectively, and $t_{0.5}$ is the half-time of sorption, was verified by numerous examples. For the rate of sorption, the equation

$$v = dB/dt = B_e t_{0.5} (t + t_{0.5})^{-2} \quad (2)$$

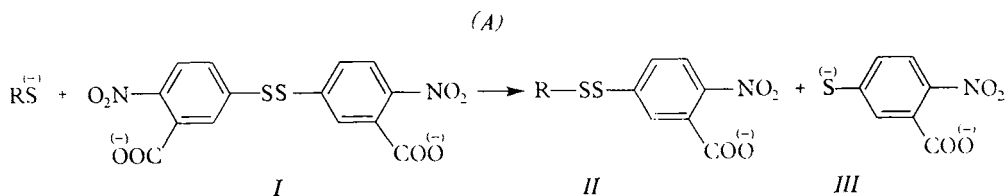
can be derived. For the initial rate v_0 we have

$$v_0 = B_e t_{0.5}^{-1}. \quad (3)$$

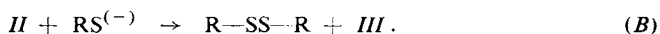
In the isothermic arrangement of the experiment the parameters B_e , v_0 , $t_{0.5}$ depend only on the sorbate concentration.

The validity of Eqs (1–3) was verified also for other types of sorption¹, such as physical, ionic, and biospecific. Recently, rate equations have been derived describing the adsorption of proteins on the solid phase^{3,4}, but their validity is limited to the initial state of sorption. Although Eqs (1a) and (2) characterize sorption at any time of the process¹, the latter can be described exactly only using a complete time-concentration model. For this purpose it is of course necessary to characterize dependences of the parameters of sorption kinetics¹ on the initial concentration of the sorbate.

The chemisorption of an aromatic disulfide, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), on thiol derivatives of cellulose appeared to be a suitable model for the determination of such dependence, because it does not undergo cooperative interactions and has a sufficiently defined physical background. The interaction of DTNB with thiols in solution (Scheme A) is a very sensitive, selective and fast reaction



involving a complete shift of the equilibrium towards the formation of product *II* (refs^{5–7}). In the presence of thiol in excess, the latter is oxidized to disulfide⁷,



Under the chemisorption conditions where thiol is covalently bound to the insoluble matrix the thiol-disulfide exchange (Scheme A) proceeds more slowly⁸, but no subsequent oxidation⁹ according to Scheme (B) takes place. The reaction of thiol groups of thiolated cellulose⁸ and thiolated agarose¹⁰ with DTNB is quantitative.

In this study we have tried to determine dependences of the parameters of chemisorption kinetics B_e , v_0 , $t_{0.5}$ on the concentration of DTNB using the chemisorption of DTNB on thiol derivatives of bead cellulose as an example.

THEORETICAL

Let it be assumed that the dependence of the amount of bound sorbate on its concentration at a constant time of the sorbent-sorbate interaction may be described by the Langmuir adsorption isotherm. In such case the parameters of sorption

kinetics B_e , v_0 , and $t_{0.5}$ depend on the initial concentration of the sorbate (c) in the following way:

a) The equilibrium sorption B_e may be expressed by a rearranged form of the Langmuir isotherm

$$B_e = B_e^+ c(c + c_B)^{-1} \quad (4)$$

in which B_e^+ is sorption attained after an infinitely long time at an infinitely high concentration of the sorbate, c_B is the concentration of the sorbate needed for obtaining $B_e = 0.5 B_e^+$.

b) From Eq. (2) we have that at the beginning of sorption, when $t \ll t_{0.5}$, the rate of sorption is constant. If the initial rate of sorption is regarded as sorption reached after a very short time, the dependence of the initial rate of sorption on the sorbate concentration may be described by means of the Langmuir isotherm in the form

$$v_0 = v_0^+ c(c + c_v)^{-1}, \quad (5)$$

where v_0^+ is the initial rate of sorption from a solution of the infinite concentration of the sorbate, c_v is the concentration of the sorbate needed for $v_0 = 0.5 v_0^+$.

c) By combining Eqs (3–5), we obtain a relation characterizing the half-time of sorption as a function of the sorbate concentration

$$t_{0.5} = B_e^+ (c + c_v) [v_0^+ (c + c_B)]^{-1}. \quad (6)$$

The ratio B_e^+ / v_0^+ is the half-time of sorption $t_{0.5}^+$ within which the sorption $B_e = 0.5 B_e^+$ is reached from a solution of the infinite sorbate concentration.

EXPERIMENTAL

Sorbate and sorbents

5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB, puriss. 99%) was supplied by Fluka. Bead porous cellulose (particle size 90–315 μm , water regain (W. R. 5.1) was prepared by thermal solidification of a chlorobenzene suspension of viscose^{11,12}. 2-(4-Aminophenylsulfonyl)ethylcellulose was supplied by O. Tokar (United Chemical and Metallurgical Works, Ústí nad Labem). Reduction of O-(3-thiosulfato-2-hydroxypropyl)cellulose¹³ (5.19% S; 19.25% dry matter) (IV) with 2-mercaptoethanol in a buffer aqueous solution pH 9.0 in the presence of tributylphosphine (1% v/v)⁸ gave O-(3-mercapto-2-hydroxypropyl)cellulose, 3.79% S (V).

Preparation of O-(2-mercaptoethyl)cellulose (VIII)

4-Toluenesulfonate of bead O-(2-hydroxyethyl)cellulose^{14,15} (VI) sucked off from ethanol (13.0 g; 1.1 mmol TsO^- per one gram of dry product; 6.5 mmol TsO^-) was stirred in a 10%

(w/v) ethanolic solution of thiourea (10 ml) at 80°C 16 h. O-[2-(2-isothioureido)ethylcellulose]-4-toluenesulfonate (*VII*) was washed with ethanol and water. The compound contained 44.15% dry matter, 1.65% nitrogen and 5.11% sulfur. The sucked-off *VII* in an amount of 3.4 g was resuspended in 16 ml of 4M ammonium hydroxide and stirred at room temperature 24 (*VIIIa*) or 48 (*VIIIb*) h. The solid phase was washed repeatedly with water, 1M HCl, water and 0.05M sodium tetraborate, and filtered with suction. The washed product was resuspended in 0.05M sodium tetraborate (15 ml), 1.25 ml of 2-mercaptoethanol was added, and pH of the suspension was adjusted to 9.0. 150 μ l tributylphosphine was also added. The mixture was stirred at room temperature 30 min. The product *VIII* (1.28 g) was washed with an EDTA solution (1 mmol l⁻¹) to a negative reaction to thiols and was freed from water by equilibration with acetone and ether. The final product contained 3.69% S (*VIIIa*) or 3.62% S (*VIIIb*).

Preparation of O-[2-(4-mercaptophenylsulfonyl)ethyl]cellulose (*XII*)

2-(4-Aminophenylsulfonyl)ethylcellulose (*IX*) sucked off from water (10.0 g; 1.75% N in the dry matter; 4 mmol -NH₂) was stirred in 10 ml of 1M HCl at 0°C for 15 min. 1 ml of a 30% solution of sodium nitrite was added dropwise within 5 min and the stirring continued for another 30 min. The diazonium chloride (*X*) sucked off by filtration was washed with ice water and resuspended in 7 ml water at 0°C. A solution of potassium xanthogenate (0.75 g in 1.6 ml water) was then added dropwise. The suspension was heated to 40°C within 30 min and was stirred at this temperature for 1 h and then for another 1 h at 60°C. O-[2-(4-Ethoxythiocarbonylthiophenylsulfonyl)ethyl]cellulose (*XI*) was washed with water and (at W.R. 1.9) contained 40.4% dry matter and in the latter, 6.27% S and 0.56% N. Compound *XI* (2.475) filtered with suction was resuspended in 13.5 ml NH₄OH (3 mmol l⁻¹); the suspension was stirred at room temperature 48 h. The solid was washed on the glass filter with water, 0.1M HCl, aqueous solution of NaH₂PO₄ (50 mmol l⁻¹), and sucked off. After that, the solid phase was resuspended in 10 ml of an aqueous solution of NaH₂PO₄ (50 mmol l⁻¹), 1 ml of 2-mercaptoethanol was added, pH was adjusted to 9.0, and 100 μ l tributylphosphine was added. The suspension was stirred at room temperature for 60 min, pH was maintained at 9.0, the product *XII* was treated further as celluloses *VIIIa* and *VIIIb*; 0.93 of dry compound *XII* contained 6.75% S.

The sorption of DTNB on thiol derivatives of bead cellulose


Thiol cellulose (2 mg) was left at 25.0 \pm 0.1°C to swell 1 h in 2.0 ml (50 mmol l⁻¹) of phosphate buffer, pH 7.6. The sorption was started by adding 20 μ l of a methanolic solution of DTNB; it was stopped after mild stirring at 25°C by filtering the suspension, and the absorbancy of the filtrate at 412 nm was measured. The amount of sorbed DTNB was calculated from the concentration of 4-nitro-3-carboxythiophenoxide anion *III* (Scheme *A*) using $\epsilon_{412} = 13\,600\text{ l mol}^{-1}\text{ cm}^{-1}$ (ref.⁵). A similar procedure was used in controlling the chemisorption of DTNB on cellulose derivatives *IV*, *VII*, and *XI*, as the last intermediates of the preparation of thiol derivatives of cellulose. The spontaneous formation of 4-nitro-3-carboxythiophenoxide anion from DTNB in the reaction medium was checked under identical conditions in the absence of the sorbent. The values thus obtained were used to correct the originally determined ones.

Treatment of results

The validity of Eqs (4–6) was verified after the experimental data had been transformed into such system of coordinates which guaranteed their linearization. The straight lines were obtained by linear regression, the correlation coefficients were tested by a one-sided significance test.

RESULTS AND DISCUSSION

Preparation of thiol derivatives of cellulose involved also reactions on cellulose in many cases with not very high conversions. The last intermediates of these reactions, *i.e.* O-(3-thiosulfato-2-hydroxypropyl)cellulose (IV), 4-toluenesulfonate O-[2-(2-isothioureide)ethyl]cellulose (VII) and O-[2-(4-(ethoxythiocarbonylthiophenylsulfonyl)ethyl)]cellulose (XI), did not sorb DTNB. This is in good agreement with the time-concentration dependence of the chemisorption of DTNB on O-(2-mercaptoethyl)cellulose (VIIIa) according to which no cooperativity took place (Fig. 1). This is also one of the reasons of the high rate of statistical reliability proved for the validity of Eqs (1a) and (1b). Parameters of the kinetics of chemisorption of DTNB on bead O-(2-mercaptoethyl)cellulose (VIIIa) are given in Table I. After substitution of the experimental results into Eqs (4-6) it was possible to determine the isothermic constants B_e^+ , v_o^+ , and $t_{0.5}^+$ of the kinetics of chemisorption of DTNB on VIIIa. The plots are given in Figs 2-4 which at the same time show the linearized forms of these equations. The straight lines in Figs 2-4 were obtained by linear regression analysis, the correlation coefficients (Table II) satisfy the significance level $P < 0.1\%$. The isothermic constants B_e^+ , v_o^+ , and $t_{0.5}^+$ of the kinetics of chemisorption of DTNB on thiolated celluloses, *i.e.* O-(3-mercapto-2-hydroxypropyl)cellulose (V), 2-mercaptoethylcellulose (VIIIa, VIIIb) and O-[2-(4-mercaptophenylsulfonyl)ethyl]cellulose (XII), are given in Table II. In most cases the isothermic constants were determined with the highest measure of statistical significance.

The validity of Eqs (4-6) was verified on thiol derivatives of cellulose with small changes in structure. These changes mean a change in the degree of substitution of O-(2-mercaptoethyl)cellulose (VIIIa vs. VIIIb, *cf.* Experimental), extension of the aliphatic spacer by the —CH(OH)— segment (V) and by the —SO₂—— segment (XII), in the latter case accompanied by a change in nucleophilicity of the —S⁽⁻⁾ group leading to a change in its reactivity^{6,7}. In order to investigate the effect of changes in the structure of sorbents on the sorption process, it was necessary to introduce the equilibrium factor — (E) or the rate factor — (R). Factor E is the molar ratio of the amount of DTNB needed for obtaining $B_e = 0.5 \cdot B_e^+$; factor R is the molar ratio of the amount of DTNB to —SH groups, needed for reaching $v_o = 0.5 \cdot v_o^+$.

Compound:	V	VIIIa	VIIIb	XII
E:	0.818	0.463	0.618	0.525
R:	2.288	2.632	0.962	0.440

From the values of factor E we can see that sorbents VIIIa and XII operate very economically in the range of theoretical stoichiometry ($E = 0.5$; Scheme A), with

a complete shift of equilibrium towards the product. Sorbent *XII* is moreover the most reactive of all ($R = 0.44$), which may be related to the similarity between the chemical structure of the reactive groups of the sorbent (4-substituted thiophenol)

TABLE I

Parameters of the chemisorption kinetics (symbols *cf.* text) of DTNB on O-(2-mercaptoethyl)cellulose *VIIIa*. Symbol r denotes the correlation coefficient of a linear regression of the double reciprocal plot of experimental data according to Eq. (1b)

c mmol l^{-1}	B_e mmol g^{-1}	v_0 $\text{mmol g}^{-1} \text{h}^{-1}$	$t_{0.5}$ h	r
0.05	0.071	0.147	0.480	0.995
0.10	0.099	0.275	0.340	0.998
0.25	0.125	0.477	0.254	0.996
0.50	0.150	0.816	0.184	0.996
0.75	0.161	0.925	0.174	0.996
1.00	0.164	1.089	0.151	0.998

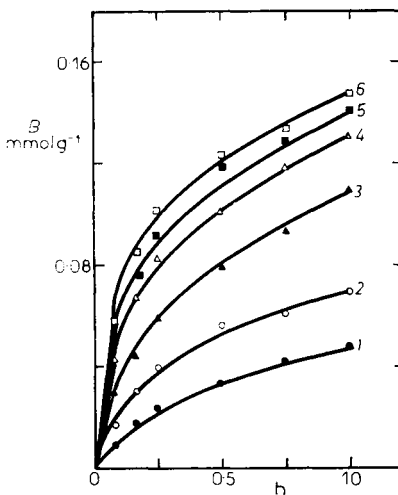


FIG. 1

Chemisorption of DTNB on thiolated cellulose *VIIIa*. Initial concentrations of DTNB (c): 1 0.05, 2 0.10, 3 0.25, 4 0.50, 5 0.75, 6 1.00 mmol l^{-1}

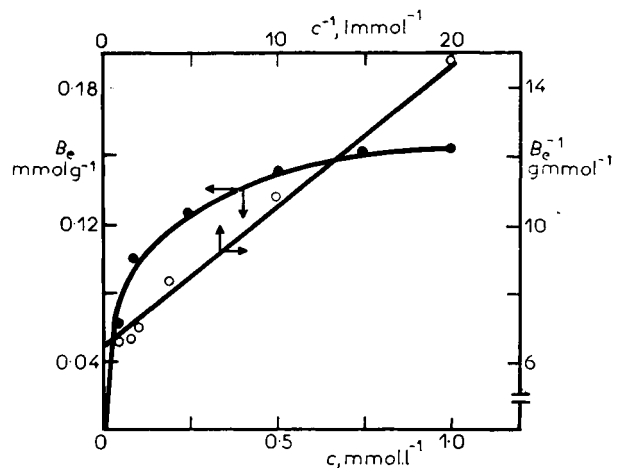


FIG. 2

Isotherm of the chemisorption of DTNB on thiolated cellulose *VIIIa*. B_e values summarized in Table I were used. Parameters B_e^+ and c_B calculated from the double reciprocal plot are given in Table II

and of the sorbate (3,4-disubstituted dithiophenol). An increase in the degree of substitution of the sorbent with thiol groups has raised its reactivity; extension of the aliphatic spacer by the $-\text{CH}(\text{OH})-$ segment shifts the equilibrium towards the reactants and at the same time slows down the rate at which it is established.

TABLE II

Isothermic constants of the chemisorption of DTNB on thiol derivatives of bead cellulose. Symbols are defined in the text; r denotes the corresponding correlation coefficients of the double reciprocal plot of dependences according to Eq. (4) or (5)

Cellulose derivative	B_e^+ mmol g^{-1}	c_B mmol l^{-1}	r	v_0^+ $\text{mmol g}^{-1} \text{h}^{-1}$	c_v mmol l^{-1}	r	$t_{0.5}^+$ h
V	0.244	0.200	0.967	0.833	0.558	0.993	0.293
VIIIa	0.170	0.078	0.992	1.466	0.447	0.998	0.116
VIIIb	0.390	0.241	0.998	1.233	0.375	0.997	0.316
XII	0.343	0.180	0.988	0.607	0.151	0.967	0.565

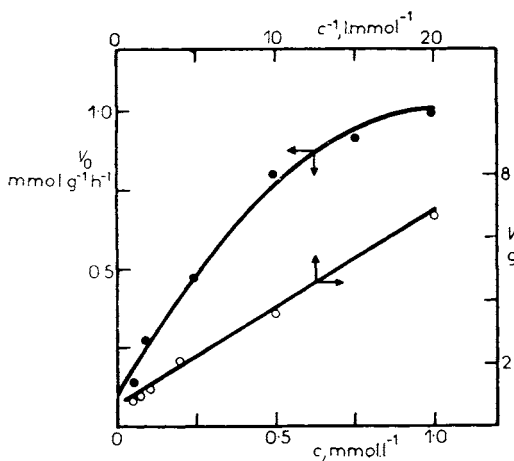


FIG. 3

Dependence of the initial rate of chemisorption, v_0 , on thiolated cellulose VIIIa on the concentration of DTNB. Constants v_0^+ and c_v calculated from the double reciprocal plot are given in Table II

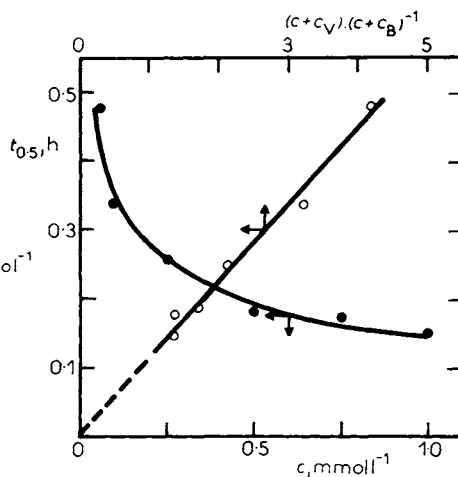


FIG. 4

Dependence of the half-time of chemisorption, $t_{0.5}$, on thiolated cellulose VIIIa on the concentration of DTNB. Constant $t_{0.5}^+$ calculated from the equation of the straight line, $t_{0.5} = 0.113 (c + c_B)/(c + c_v)$, is given in Table II

It may be stated, in conclusion, that the dependence of parameters of the chemisorption kinetics of DTNB on thiol derivatives of cellulose from aqueous buffer solutions on the concentration of DTNB is adequately described by Eqs (4-6). The values of the isothermic constants B_e^+ , v_o^+ , and of $t_{0.5}^+$ defined by these constants reflect changes in the chemical structure of the sorbent. By combining Eqs (4-6) with the equation of sorption kinetics (1a), the equation

$$B = ct[(1/B_e^+) ct + (c_B/B_e^+) t + (1/v_o^+) c + (c_B/v_o^+)]^{-1} \quad (7)$$

is obtained which characterizes the total process of sorption as a function of time and concentration. In the verification of Eq. (7) by the reported experimental material, the highest deviation found between the calculated and measured values was 7% (ref.¹⁶).

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