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Mass transfer effects in preparative chromatography of eremomycin on polymeric sorbents

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Abstract

This work is devoted to the study of the regularities of the sorption of the new antibacterial antibiotic eremomycin on carboxylic sorbents. The main sorption kinetic equilibrium and dynamic parameters for realization of one-act preparative chromatographic process were determined and the difference between gel-like and structurally segregated carboxylic cation exchangers was analyzed. The optimal conditions for sorption and complete desorption of eremomycin were found.

Keywords: Mass transfer; Preparative chromatography; Kinetic studies; Adsorption; Eremomycin; Antibiotics

1. Introduction

At present eremomycin is obtained biosynthetically. The initial preparation is purified by multistage extraction and then subjected to chemical modification. Production of eremomycin involves problems associated with the low yield of target product, toxicity of the by products, and detrimental environmental effect of the organic solvents [1].

The question of the behavior of the organic molecules (zwitterions) on the different types chromatographic carriers (especially on the carboxylic cation exchangers) has not been studied enough. Comparison of sorption characteristics of eremomycin zwitterion on sorbents of different types and structures is of interest from both scientific and practical viewpoints. Such a comparative study, on the one hand, extends the set of experimental data on sorption of such complicated substances as zwitterions, and on the other, allows the development of a technique for recovery of this antibiotic from the native solutions containing products of the microbial metabolism.

2. Experimental

2.1. Materials and methods

In this work we used crystalline samples of eremomycin sulfate (purity 95.6%) obtained from a culture broth of INA-238 actinomycetes in the Scientific Research Institute for New Antibiotics, Russian Academy of Medical Sciences, Moscow, Russia [2]. Eremomycin produces strong bactericidal effect on gram-positive microorganisms and it is 2–10-times more active than its analog vancomycin [3]. At the same time eremomycin does not influence the growth and development of animals and does not

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change the morphology of internal organs and it shows less toxic influence on mammals than vancomycin and its analogs [4].

Eremomycin is a zwitterion containing three amino groups ($pK^1_{\alpha1}$ 6.9, $pK^2_{\alpha1}$ 7.9, $pK^3_{\alpha1}$ 9.0), phenol groups ($pK^1_{\alpha2}$ 9.7, $pK^2_{\alpha2}$ 10.4, $pK^3_{\alpha2}$ 11.35) and a terminal carboxylic group (pK_α 3.1) (Fig. 1). The molecular mass of eremomycin ($C_{73}H_{89}N_{10}O_{20}Cl$) is 1540 [1].

The following sorbents were tested: carboxylic cation exchangers of BDM type produced at the Institute of Macromolecular Compounds, Russian Academy of Sciences, St. Petersburg, Russia by radical copolymerization of methacrylic acid (MAA) and the hydrophobic crosslinking agent ethylene glycol dimethacrylate (EGDM) [5]; sorbents elaborated by NIIPM ''Moskva'', and industrially produced at PO ''AOZT Kemerovo'': polysorb [nonionic macroporous sorbent with poly(styrene–divinylbenzene) matrix], polydextran T70 (nonionic sorbent), AV-17×8 [highly basic anion exchanger based on poly(styrene–divinylbenzene) copolymer] and MN-500 {isoporous sulfonated poly(styrene–divinylbenzene) copolymer prepared in the presence of cyclohexane as porophore [6]}.

2.1.1. Potentiometric measurement

The sorbent acidity is defined by the Henderson–Hasselbach empirical equation:

$$pK_\alpha = pH - n\log[\alpha/(1-\alpha)] \quad (1)$$

where α is the degree of ionization of the sorbent functional groups and is calculated as:

$$\alpha = C_{NaOH}V_{NaOH} \cdot 1000/Em_{sorb} \quad (2)$$

where C_{NaOH} and V_{NaOH} are the concentration (mg-equiv.$\cdot$ml^{-1}) and volume (ml) of the titrant, respec-

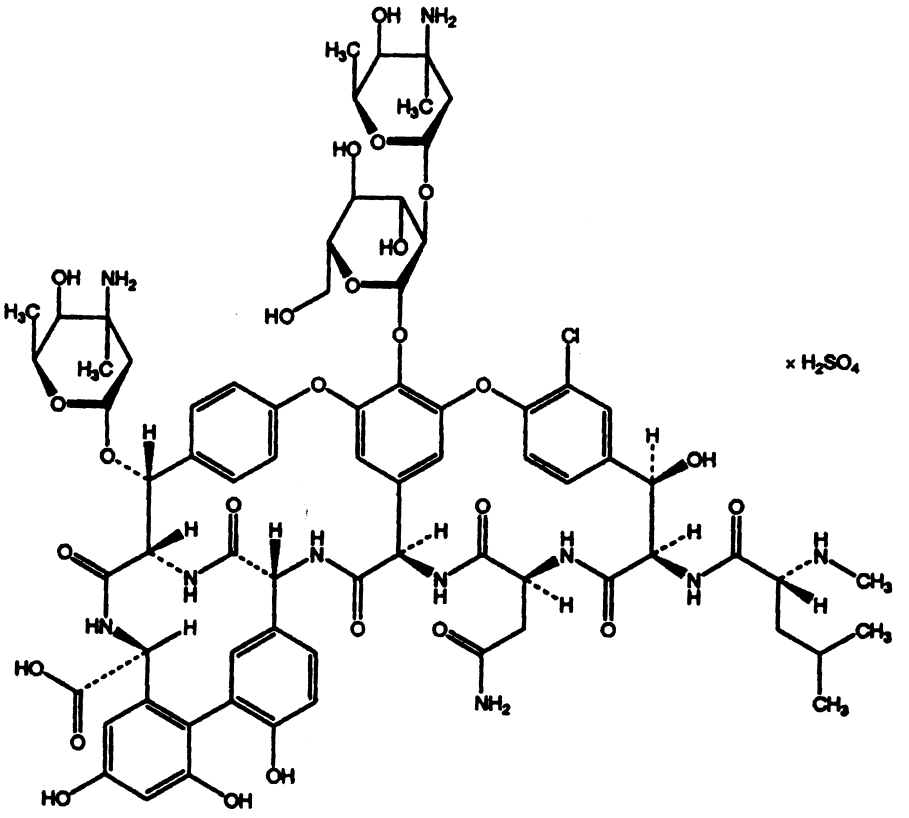

Fig. 1. Structural formula of eremomycin sulfate. Molecular mass ($C_{13}H_{89}N_{10}O_{26}Cl$) = 1540; terminal carboxylic group pK_α = 3.1; amino groups pK_α = 6.9, 7.9, 9.0; phenyl groups pK_α = 9.7, 10.4, 11.35.

tively; E, the total exchange capacity of sorbent (mg-equal g^{-}); m_{sorb} is sorbent mass (mg).

The sorbents acidity was studied by potentiometric titration, which was carried out by technique of separate weighed samples [7]. A 0.1–0.2-ml portion of distilled water was added to 50 mg of sorbent and stirred for 20 min to completion of swelling. To provide different degrees of ionization of the sorbent α, a calculated volume of a titrated NaOH solution was added.

After the attainment of equilibrium the pH of solution was measured and the curve of potentiometric titration in the Henderson–Hasselbach coordinates:

$$\mathrm{pH} = f(\log[(1-\alpha)/\alpha]) \quad (3)$$

was constructed to find pK_{α} and the parameter n as the tangent of the linear part of the plot slope.

The swelling coefficient, K_{swell} was determined as:

$$K_{swell} = V_{swoll}/V_{dry} \quad (4)$$

where V_{swoll} is the volume of the swollen sorbent; V_{dry}, the volume of the dry sorbent.

2.1.2. Equilibrium sorption of eremomycin

The experiments on sorption of eremomycin were carried out as follows [8]: 10 ml of eremomycin solution with defined initial eremomycin concentration was added to 10 mg of the swollen sorbent. Eremomycin initial concentration ranged from 0.2 to 1.5 mg ml^{-1}. To support a natural basic–acidic balance eremomycin solution was prepared in the 0.2 M ammonium acetate. The solution was stirred with sorbent for 24 h until equilibrium became settled. The eremomycin concentration in the equilibrium solution was determined spectrophotometrically at $\lambda = 280$ nm, using a calibration curve, $D^{280} = \mathrm{f}(C)$, where D^{280} is the optical density at $\lambda = 280$ nm; C, the initial concentration of eremomycin. The sorption capacity of sorbent, m (mg g^{-1}), was defined as:

$$m = (C - C_{eq})V \cdot 1000/m_{sorb} \quad (5)$$

where C is the initial concentration of eremomycin in solution, mg ml^{-1}; C_{eq}, equilibrium concentration of eremomycin, mg ml^{-1}; V, volume of solution, ml; m_{sorb} is mass of sorbent, g.

Since eremomycin displays great antibacterial activity when its concentration in cultural broth ranges from 1.0 to 1.5 mg ml^{-1} [1], the dependence of the sorbent sorption capacity on pH was realized in eremomycin solution with an eremomycin concentration 1.0 mg ml^{-1}. The pH of solution was adjusted with HCl (1 M) and NH_4OH (0.25%).

Studying the influence of the ion strength on the sorption capacity was realized by varying the molar concentration of the ammonium acetate water from 0 to 0.4 M.

Varying the initial concentration of eremomycin in solution from 0.2 to 1.5 mg ml^{-1} the dependence of the sorption capacity on eremomycin concentration was studied. All eremomycin solutions were prepared from the crystalline sample of antibiotic.

The contribution of hydrophobic connections to the sorption of eremomycin on the carboxylic sorbents was studied as the influence of the percent concentration of isopropilic spirit in the antibiotic water and spirit solution on the sorption capacity. The water and spirit solution was prepared as 10, 25, 50 and 70% of the isopropilic spirit in 0.2 M aqueous acetate ammonium, the concentration of eremomycin in this solution was 1.0 mg ml^{-1}.

2.1.3. Kinetic parameters of eremomycin sorption on the carboxylic cation exchangers

The sorption kinetics of eremomycin on the carboxylic cation exchangers was studied as follows. A 20-ml volume of eremomycin solution in 0.2 M ammonium acetate with eremomycin concentration of 1 mg·ml^{-1} was added to 50 mg of the swollen in the 0.2 ml of distillated water ion exchanger at the prescribed pH. In the course of the kinetic experiment performed with continuous stirring, the 0.2-ml samples were taken at regular intervals of time t (s). The eremomycin concentration in solution was determined spectrophotometrically at $\lambda = 280$ nm, using a calibrated curve. In preliminary experiments we found that the rate of eremomycin sorption is independent of the antibiotic concentration and stirring rate. These experiments also showed that the limiting stage of eremomycin sorption on the carboxylic cation exchangers is its diffusion into the sorbent granules. The intradiffusion mechanism was also confirmed in sorption experiments with intermittent separation of the cation exchanger and aqueous

phase. In these experiments, sorbent granules were separated from the solution, kept for 20 h, and then returned to the same solution. We found that, on repeated contact of the cation exchanger and solution, the sorption rate increases as compared to that before phase separation. This fact shows that the cation pore-diffusion is the limiting stage of sorption.

Mathematical interpretation of the kinetic parameters was realized by the Boyd equation [7]:

$$\overline{D} = 0.087\mathrm{tg}^2\beta R^2 \quad (6)$$

where $\overline{D}$ is the effective diffusion coefficient ($cm^2\ s^{-1}$); R is the radius of the swollen cation exchanger granule (μm); β is determined as the slope of the linear part of plot F vs. $t^{1/2}$, where t is time of regular interval when the 0.2-ml samples of eremomycin solution over sorbent were taken. The sorption capacity of sorbent m (mg g^{-1}) in time t was determined; $F = m_t/m_\infty$, the fraction of the total sorption capacity of the sorbent consumed by the sorbate; m_t is the sorption capacity in time t; and m_∞ is the equilibrium sorption capacity.

The half-saturation time ($t_{0.5}$) of the sorbent with antibiotic when $F = 0.5$ was calculated from the plot F vs. $t^{1/2}$.

For the Boyd model the average time of diffusion, $\overline{t}$, equals:

$$\overline{t} = R^2/15\overline{D} \quad (7)$$

Another model to interpret the experimental kinetic parameters was the ''shell and core'' model [9]:

$$F = \frac{1}{L}\cdot\frac{6}{\left[3-\frac{L}{R}\cdot\left(3-\frac{L}{R}\right)\right]}\cdot\sqrt{\frac{\overline{Dt}}{\pi}} \quad (8)$$

where L is the absorptive layer thickness (μm); $\overline{t}$, the average time of diffusion equals:

$$\overline{t} = L^2(1+3\rho+6\rho^2+5\rho^3)/15\overline{D}(1+\rho+\rho^2) \quad (9)$$

where ρ is the relative no absorbing radius of the ''core'' and it equals:

$$\rho = 1 - L/R \quad (10)$$

We experimentally determined the absorptive layer thickness L from the dependence of the sorption capacity m of sorbent on its granules size (radius R): $m = f(R)$. The different fractions of sorbent were selected as 4–8, 8–12, 12–18, 18–24, 24–54, 54–90, 90–150, 150–316, 316–400 μm. The average values of these fractions (R) were used for the plot $m = f(R)$. The sorption equilibrium experiment was made on BDM-5 and BDM-12. The fraction on which the maximal sorption capacity was achieved was assumed as absorptive layer L (μm).

2.1.4. Dynamic sorption

To study the dynamic sorption the sample of pure crystalline eremomycin sulfate was used. The dynamic sorption was realized on the column with carboxylic cation exchanger BDM-12 (16×10 mm). Sorbent was washed with 0.2 *M* ammonium acetate, pH 7.2. A 30-ml volume of eremomycin solution in 0.2 *M* ammonium acetate (pH 7.2) with an eremomycin concentration of 1 mg ml^{-1} was loaded to capacity. Concentration on the entrance from the column was controlled spectrophotometrically. Desorption of eremomycin was provided with the 0.2 *M* ammonium acetate by changing pH from neutral to basic (8.6; 9.5; 10.9).

3. Results and discussion

3.1. Acidity of the cation exchangers

3.1.1. Influencing the sorbents structure on the sorbents acidity

In our work, the main attention was paid to the cation exchangers of the BDM group because on one side they have a high volume concentration of carboxylic groups which provide the high sorption capacity. At the same time hydrophobic components (CH_3 radicals and EGDM) of their matrix promote a realization of strong ion-ionic, hydrophobic and another types of interactions between the organic molecules and chromatographic carriers.

The acidity and the ion-exchange capacities of the carboxylic cation exchangers of BDM group and the sulfocationit MN-500 were thoroughly studied from the potentiometric curves. The obvious influence of the matrix structure both on the sorbents acidity and their total ion-exchange capacity can be seen (Table 1, Fig. 2). A growth of the total ion-exchange capacity when the molar concentration of EGDM in

Table 1
The swelling coefficients and parameters of the Henderson–Hasselbach equation* for different cation exchangers

Sorbent**	K_{swell}	E (mg equiv. g^{-1})	pK_α	n
BDM-1	7.1	9.9	5.2	1.2
BDM-3	7.0	9.5	5.9	1.7
BDM-5	6.2	8.9	6.3	1.9
BDM-9	4.8	8.5	6.9	1.3
BDM-12	5.5	8.2	6.4	1.3
MN-500	1.4	2.0	4.6	3.8

* pK_α determined as intersection point of the linear plot of pH vs. $\log[(1-\alpha)/\alpha]$ with ordinate; n is tangent of the slope of this plot.
** The figure in the carboxylic sorbent brand refers to content of the cross-linking agent (mol %).

sorbents decreases can be seen. At the same time the acidity has an dependence on the swelling coefficient which bears an anomalous character for the cationits of the BDM group (see Table 1). The matrix strain enthropy of the cross-linked sorbents is limited with quantity of cross-linking agent [10,11]. The growth of the density of the polymeric net deduces the pK_α [12]. Thus when EGDM concentration increases the ion exchanger acidity must also increase. The sorbents lead themselves like gel-like structures. The structurally segregated cation exchangers in the H^+ form are characterized by an abnormally strong rise in swellability with increasing content of the cross-linking agent. The enhanced swelling may be due to the loose network structure formed because of isolation of the double bonds of the cross-linking agent [12,13]. In our case this factor is connected with the hydrophobic interactions between the molecules of the methacrylic acid and EGDM which promote the additional structural changes of the ‘‘microgel’’.

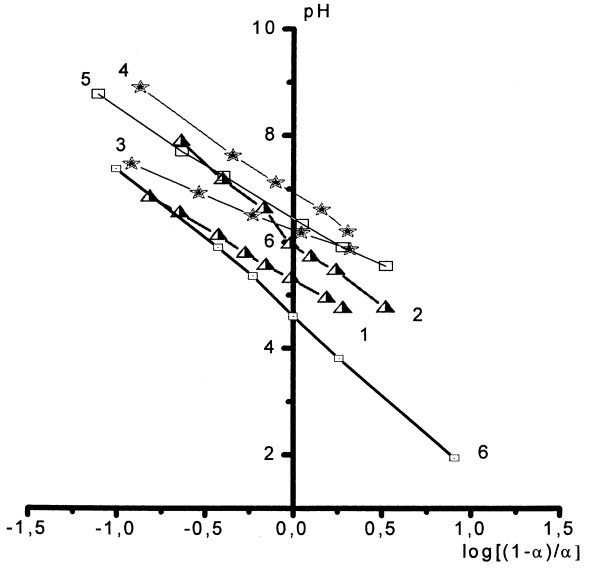

Fig. 2. Potentiometric titration of carboxylic ion exchangers. α, Degree of ion exchanger ionization. (1) BDM-1; (2) BDM-3; (3) BDM-5; (4) BDM-9; (5) BDM-12; and (6) MN-500.

3.1.2. Parameter of mutual interactions between functional groups

Any absorbed organic molecule interacts with the binding center of sorbent which can be presented by the functional group and its nearest neighbors. For carboxylic cation exchangers of the BDM group the binding (ion-exchange) center is represented by the carboxylic group and some matrix components near this group. On the polymeric chains of the sorbent matrix some electrostatic potential is accumulated which promotes the localization of the counterions. These make the mutual repulsion of the functional groups weaker and the swelling of the sorbent decrease also. Thus, parameter n characterises the mutual influence of the carboxylic groups for the first turn. The ideal ion-exchange process can be reached when there are no interactions between the ionized centers, then $n=1$ [7,14]. The greater the difference of the parameter n from unity the more interactions between centers. The structurally segregated sorbents with similar structures have close n. Also, it is of interest that parameter n of structurally segregated BDM-12 sorbent is virtually equal to that of the BDM-1 gel-like sorbent. That speaks about approximately equal distribution of the ion-exchange centers. But for chromatographic process the crosslinked sorbents are much better because they promote good reversible processes. The sulfonic cation exchanger MN-500 exhibits a higher acidity because of the connection energy of H-protons in sulfogroups is less then the same one is in the carboxylic groups. At the same time the parameter n of MN-500 is much bigger then those of structurally segregated carboxylic sorbents.

3.2. Study of the equilibrium sorption of the eremomycin on the sorbents with different polymeric structure

3.2.1. pH dependence of sorption capacity

The next very important step to find the proper conditions approaching the maximal equilibrium binding of eremomycin with sorbents is to study the dependence of the sorbent sorption capacities on pH. Since eremomycin is a dipole molecule it is possible to change its total effective charge by varying the solution pH. In Fig. 3 (curves 1, 2) the plots of the sorption capacity of carboxylic cation exchangers for eremomycin vs. pH show a sharp maximum at pH~ 6–7. Precisely in this pH interval eremomycin has a maximal positive charge (see pK_α of the eremomycin groups). At pH~2.5–3 amino groups of antibiotic are all charged and the sorbents carboxylic groups are all uncharged but eremomycim binds with sorbents. That fact allows us to suppose that eremomycin is able to hydrophobically interact with chromatographic carriers. The molecular sorbents Polysorb and polydextran T-70 (curves 4, 5) show the sorption capacities with respect to eremomycin ~200 mg g^{-1}.

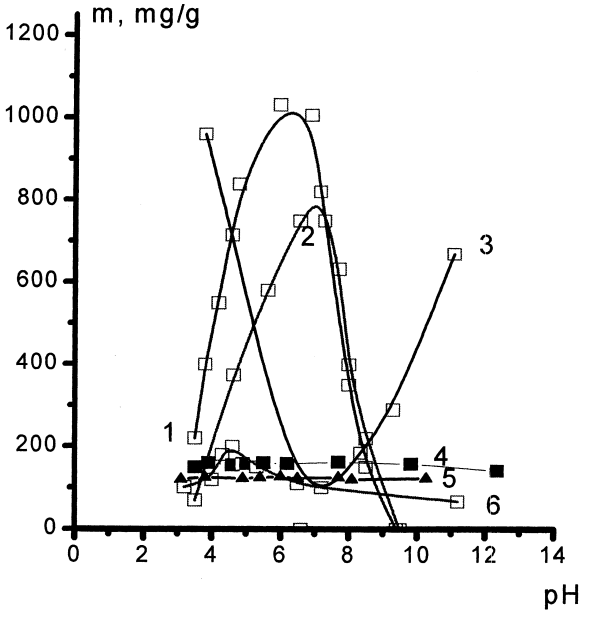

Fig. 3. Sorption capacity of eremomycin (*m*) vs. pH: (1) BDM-3; (2) BDM-12; (3) MN-500, (4) Polysorb, (5) dextran T-70, and (6) AV-17×8.

We can observe the same on the AV-17×8 anion exchanger. A very slight maximum at pH~4 (curve 6) correlates with anion exchange between the terminal carboxylic groups of eremomycin and sorbent functional groups.

Gradual decreases of eremomycin sorbability to zero with increasing solution pH to 9.5–10 are explained by the fact that in the alkaline pH range amino and phenol groups of eremomycin are fully unionized and the BDM carboxylic cation exchangers lose their ion-exchanging sorption power with respect to eremomycin. At the same time, the sorbents carboxylic groups and the antibiotic terminal carboxylic group are fully charged and the repulsion between them does not permit the hydrophobic interactions of antibiotic with sorbent to be realized.

Since the sulfonic groups of MN-500 are easy dissociated in the wide range of pH the maximal sorption binding is already observed at the high acidic pH (Fig. 3, curve 3). The eremomycin amino and phenol groups are all charged and there are the ideal conditions for the ion-exchange binding and hydrophobic interaction. The decrease of the MN-500 sorption capacity to zero in the neutral pH zone can be hypothetically explained by the growing of the screening effect of the fully charge sulfonic groups. The further growth of pH to basic value promotes the of the hydrophobic binding of eremomycin with MN-500.

3.2.2. Conditions of eremomycin binding on cation exchangers

The degree of hydrophobic and ion-exchange binding of eremomycin on carboxylic cation exchangers, as well as the possibility of eremomycin to form associations with molecules mostly depends on the ion strength, the dielectrical constant and the eremomycin concentration. The sorption capacity of sorbent changes with changing these factors (see Table 2). As one can see from the table when the ion strength of solution excessively increases the sorption capacity decreases. It is obvious that the most quantity of the antibiotic is sorbed from the water solution. Another factor, the electrolyte concentration, exercises a complex influence on the sorption system. On the one hand, when eremomycin concentration in solution is 0.2 mg ml^{-1} it must disso-

Table 2
Dependence of the sorption capacity of the BDM group carboxylic cation exchangers on different factors

Factor		BDM-5		BDM-12	
		m (mg g^{-1})	B (%)	m (mg g^{-1})	B (%)
I (M)	0	975	97.5	936	95.5
(C = 1 mg ml^{-1})	0.1	856	85.6	788	81.2
	0.2	460	60	392	62
	0.4	86	8.9	84	8.9
C (mg ml^{-1})	0.2	58	70	48	76
(I = 0.2 M)	0.3	130	57	70	76
	0.5	220	56	140	61
	1.0	420	58	370	61
	1.5	550	62	500	61
A (%)	0	458	52.5	390	50.7
(I = 0.2 M)	10	595	54.3	600	60.6
(C = 1 mg ml^{-1})	25	750	79.5	635	65.5
	50	156	16	618	61.2
	70	199	19	460	54

I, Ion strength of eremomycin solution measured in the mol concentration, M; C, concentration of eremomycin (mg ml^{-1}); A, concentration of isopropilic spirit (%); m, sorption capacity of sorbent (mg g^{-1}); B, quantity of absorbed eremomycin (%).

ciate fully and eremomycin ions must bind as possible completely. But the eremomycin ion concentration is too low to use full sorption capacity by ion exchange binding, in addition it is too low to overcome ion strength of 0.2 M ammonium acetate. The ammonium acetate competitively binds with ion exchange centers, and only 70% of eremomycin can bind on BDM-5 and 76% on BDM-12 (Table 2). The increasing electrolyte concentration decreases the dissociation and ion exchange decreases. One can observe the growth of the sorption capacity. That can be explained by the non exchange binding which often accompanies the sorption of the big organic molecules. Some of the basic reasons of the non exchange binding is the heterogeneous structure of sorbents and the increasing of the electrolyte concentration [15,16]. So eremomycin molecules penetrate the water medium in the sorbent. There they can dissociate and interact with sorption centers.

So eremomycin is a dipole molecule with a big aglyconic part (Fig. 1) and owing to such structure it can be sorbed by ion exchange and hydrophobic mechanisms. On a level with this, molecules of such nature can form associated complexes between themselves which can be sorbed like usual molecules. Moreover, the carboxylic groups of zwitterions which were sorbed like cations can be unionized or partly ionized [17,18]. These conditions promote the additional connections in the sorption system. In the experiment with eremomycin spirit and water solution we attempted to regulate these connections. As one can see from Table 2, dependence of the sorbents sorption capacity on isopropylic spirit percent concentration has an extremum. It is absolutely clear that isopropylic spirit must reduce both the electrolyte dissociation and the ions mobility. Together with that there is observed principle influence of EGDM agent which has a hydrophobic nature. The highest sorption capacity was established in the both gel structured and segregated sorbents when the concentration of spirit was 25%. This can be treated as obtaining the optimal conditions when, with help of spirit, the hydrophobic interactions were almost eliminated and high ion and molecular sorption maximally ran. The further growth of spirit concentration 50 to 70% promotes the full suppression of hydrophobic interactions between eremomycin and sorbents. In such spirit concentration the ion mobility comes considerably lower and the molecular sorption prevails over the ion exchanging. At the same time in the water medium of the swollen sorbents antibiotic molecules dissociate and interact by the ion-exchange mechanism, the hydrophobic interactions are suppressed. That fact is confirmed by

complete desorption of eremomycin from carboxylic sorbents by a basic solution (pH 10.5) in the presence of 0.2 *M* NH_4COOCH_3 during a short time. So such a mechanism of eremomycin sorption on carboxylic cation exchangers in presence of high concentrated spirit more completely proceeds on the structurally segregated sorbents because of their dissimilar porous medium. The gel-like sorbents structure is more homogeneous and more accessible for small molecules and ions, by this reason there is no observed a complete using of sorption capacity.

3.3. Kinetic parameters of eremomycin sorption

No dynamic process can be predicted and realized without knowing the sorption kinetic parameters. Traditionally the experimental kinetic parameters of small organic molecules are mathematically interpreted by the Boyd model which presupposes that the molecules distribute into the sorbent granules through all particle volume.

But the big organic molecules, especially enzymes, antibiotics and dyes, can cause some reversible structural changes of sorbent net, forming a dense sorbent core [19,20]. As result, the sorption capacity of sorbent is not fully used. The area from the sorbent surface to the sorption place can be reduced by grinding the sorbent particles. Thus the core size is minimized. Moreover the mathematical interpretation of the experimental kinetic data by the Boyd model showed some contradictions between experimental and the mathematically interpreted data. As one can see from Table 3 the experimental values $t_{0.5}$ and tg β, characterizing the rate of sorption, improve when the particles size decreases. This regularity is save for the kinetic data mathematically interpreted by the ''shell-core'' model [9].

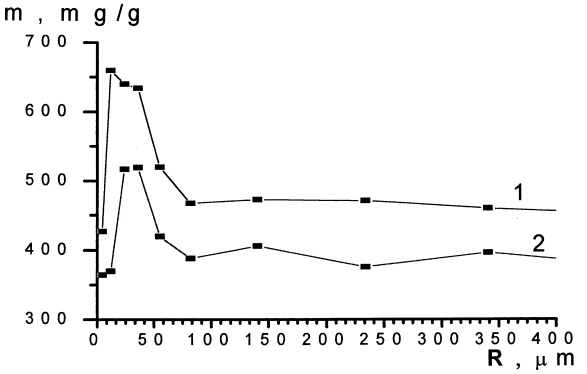

Fig. 4. The equilibrium sorption capacity of the carboxylic cation exchangers of BDM group: (1) BDM-5 (gel-like sorbent); BDM-12 (structurally segregated sorbent). Solution of eremomycin (C = 1 mg ml^{-1}) in the presence of 0.2 *M* ammonium acetate, pH 6.9.

Fig. 4 confirms the existence of the absorbing layer and no absorbing core. The plot $m = f(R)$

Table 3
Comparison of the kinetic parameters mathematically calculated by Boyd and ''core and shell'' models

Sorbent	K_{sw}	$R \cdot 10^4$ (cm)	tg β	$t_{0.5}$ (min)	$*\bar{D} \cdot 10^8$ ($cm^2\ s^{-1}$)	$*\bar{t}$ (min)	$\bar{D} \cdot 10^9$ ($cm^2\ s^{-1}$)	$\bar{t}$ (min)
BDM-3	7	358	0.028	6.02	31.99	16.24	3.02	9.36
		130	0.043	1.60	9.98	6.89	6.34	4.19
		80	0.085	0.27	14.72	1.76	21.92	1.38
BDM-5	6.2	238	0.026	10.83	11.31	18.84	2.34	11.01
		130	0.040	2.5	7.99	7.96	4.70	5.16
		80	0.072	1.07	9.80	2.46	14.60	1.92
BDM-9	4.8	238	0.036	3.04	18.3	9.82	3.77	5.74
		130	0.043	1.36	7.79	6.90	4.93	4.20
		80	0.178	0.15	50.35	0.40	74.98	0.31
BDM-12	5.5	238	0.034	2.80	17.81	11.00	3.64	6.44
		130	0.045	2.02	9.36	6.29	5.91	3.79
		80	0.227	0.10	89.67	0.25	133.5	0.19

$*\bar{D}$ and $*\bar{t}$ are calculated by the Boyd model; $\bar{D}$ and $\bar{t}$ are calculated by the ''core and shell'' model. tg β is tangent of the slope of the linear part of the plot F vs. $t_{0.5}$; $t_{0.5}$ is a half-saturation time; K_{sw} is the swelling coefficient.

demonstrates the clear increase of the sorption capacity both the gel-like sorbent BDM-5 and the structurally segregated sorbent BDM-12 when the average particle radius is from ~12 to ~36 μm. That refers to minimizing the non-absorbing core radius and the absorbing layer radius approximates the granules radius. When R is less than 12 μm, the absorptive layer, most likely, is destroyed and sorption capacity sharply reduces. The diffusion of eremomycin into the grain sharply decreases.

To confirm the established regularity between the kinetic experimental data we use Eq. (8). We have approximated absorptive layer thickness L to 20 μm. As a result, the mathematically calculated average coefficient of diffusion $\bar{D}$ and the average time of diffusion $\bar{t}$ improves when the size of the sorbent granules decrease and experimentally obtained parameters tg β and $t_{0.5}$ carroborate that regularity. The biggest improving of the kinetic parameters is observed when the sorbents granules radius maximally approaches the absorptive layer radius (Fig. 5). The linear parts of the plots F vs. $t^{1/2}$ once more demonstrates the intradiffusional sorption of eremomycin on the carboxylic cation exchangers.

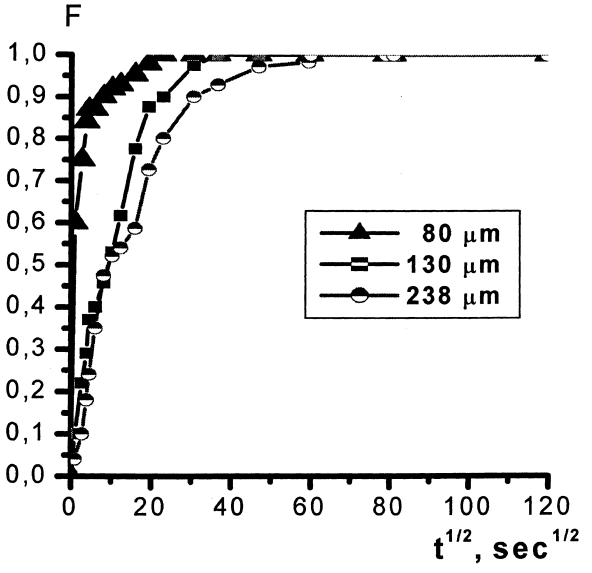

Fig. 5. The sorption kinetics of eremomycin on the BDM-12 in dependence on the sorbent particle size.

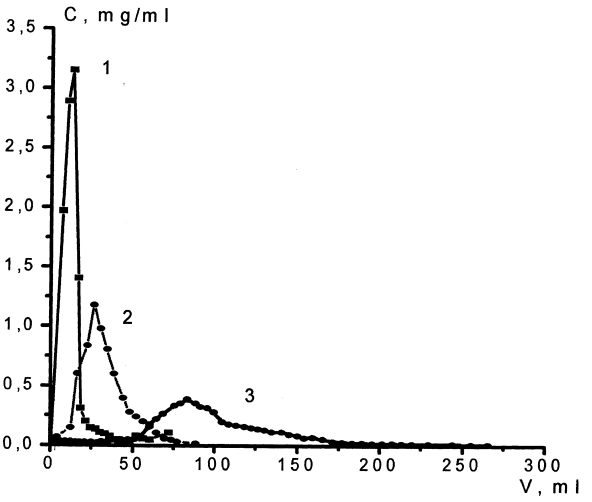

Fig. 6. Dynamic sorption of eremomycin on structurally segregated cation exchanger BDM-12. *C*, Eremomycin concentration in eluate (mg ml^{-1}); *V*, eluent volume, ml. Eluent, 0.2 *M* ammonium acetate with pH: (1) 10.5; (2) 9.5 and (3) 8.6.

3.4. Conditions of complete eremomycin desorption on the carboxylic cation exchanger

The detailed study of the equilibrium and kinetic parameters helped us to predict the conditions of dynamic maximal sorption and complete desorption. To study the conditions under which there can be observed the forming of the sharp elution zones we investigated the dynamic sorption of the pure sample of crystalline eremomycin. In Fig. 6 it is demonstrated that as far as the eluent becomes more alkaline the elution curve becomes sharper, and sorption of eremomycin becomes complete. Under pH 10.5 eremomycin is converted from zwitterionic to carboxylate anionic form and the ion strength of eluent is enough to overcome whatever interaction between the chromatographic carrier and eremomycin.

4. Conclusions

Combined analysis of data on potentiometric titration and swelling of carboxylic cation exchangers shows that the structural organization of the polymeric matrix considerably affects the sorbent total exchange capacity E (mg equiv. g^{-1}), the

ionization constant of the carboxylic groups pK_{α} and the parameter *n*, characterizing the mutual influence of the sorption centers. The growth of the molar concentration of the cross-linking agent ethylene glycol dimethacrylate (EGDM) in the carboxylic cation exchangers of the BDM group promotes the decrease of the total exchange capacity. The parameter *n* and acidity depend on the swelling coefficient K_{swell}. The strongest mutual effect of the ion exchange centers is observed in the gel-like carboxylic sorbents.

The opposite effects of ionization of carboxylic groups of cation exchangers, on one hand, and dissociation of amino, phenyl, and carboxylic groups of eremomycin, on the other, on the eremomycin sorption result in a peak in the plot of the eremomycin sorption vs. equilibrium pH of the sorbate solution. The experiment on the equilibrium sorption of eremomycin shows the high sorption capacity of the carboxylic cation exchangers of the BDM group (about 1000 mg g^{-1} for BDM-3 and about 750 mg g^{-1} for BDM-12).

The study of the influence of differ factors on the sorption capacity showed that: (1) the growth of the ion strength causes the decrease of the sorption capacity; (2) the best eremomycin proceeds from the water solution; (3) the antibiotic from the spirit solution bears extreme character (the best sorption is when the spirit concentration is 25%); (4) the growth of the initial eremomycin concentration on the one hand suppresses the molecules dissociation and decreases the ion-exchange binding, and on the other, provides the molecular sorption growth.

The equilibrium sorption on the carboxylic cation exchangers of the BDM group is reached sufficiently fast and has the intradiffusional character. At the same time eremomycin absorbed only into the absorptive layer of carboxylic sorbents. When the sorbents granule radius achieves approximately the absorptive layer thickness the mass transfer of eremomycin on carboxylic sorbents considerably improves.

Complete elution of eremomycin from carboxylic cation exchangers at high antibiotic concentration in the eluate can be reached by conversion of eremomycin from the zwitterion to the carboxylate anion.

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